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Improvement of soybean through plant tissue culture and genetic transformation: a review

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Abstract

Tissue culture and genetic transformation in combination with molecular biology play an imperative role in the improvement of cultivated soybean for developing tolerant/ resistant transgenics against various biotic and abiotic factors and enhancement of various beneficial qualitative traits. For development of transgenic soybean, efficient and reproducible plant regeneration system from explant cultures is pre-requisite. In a direct approach of tissue culture techniques such as embryo rescue, homozygous lines developed from haploid technology, somatic hybridization and cybridization, and in vitro selection in conjunction with somaclonal variations for screening of tolerant/ resistant mutant lines are proving advantageous for crop improvement. Numerous studies conducted so far describe different nutritional and hormonal requirements of a culture system for eliciting efficient in vitro regeneration. Several factors, which influence the speed of in vitro response, are growing conditions and genotype of donor plants, age and size of explant, composition of culture medium (especially relative concentrations and proportions of growth regulators), culture techniques and culture conditions. For genetic transformation, various disarmed Agrobacterium strains and particle bombardment method with different markers as well as character genes have been used with various degree of success. In this review paper, an effort has been made to describe work already done in India and abroad with relation to advancement in tissue culture and genetic transformation in soybean, major constraints and future strategies to improve soybean via biotechnological means.

Keywords: Soybean, Organogenesis, Somatic embryogenesis, *Agrobacterium* mediated transformation, Transgenics.

Soybean [*Glycine max* (L.) Merrill] is one of the world's most important agronomic crops. Soybeans have dominated world oilseed production among the eight major oilseeds traded in international markets. Both soybean oil and protein are used extensively in food products for human and animal consumption. There are numerous prospective applications of biotechnological tools towards the improvement of soybean production. At present, emphasis is on developing profitable soybean lines with improved seed quality, either with very high oil or with very high protein content. Additionally, it is desirable to develop lines with improved protein quality relative to amino acid content or with elevated levels of selected amino acid. It is also highly desirable to improve high yielding soybean lines tolerant/resistant to herbicide and/or pesticide, biotic and abiotic stresses.

In 2010, biotech soybean accounted for 50% of all the biotech crop hectarage in the world. The global hectarage of herbicide tolerant soybean in 2010 was 73.3 million hectares, up by 4.1 million hectares, or 6% from 2009 at 69.2 million hectares. The increase resulted from the following significant changes at the country level. The largest increase in biotech soybean was in Brazil with an increase of 10%, equivalent to 1.6 million hectares followed by the USA with an increase of 0.9 million hectares and Argentina at 0.8 million; more modest increases were recorded in Uruguay, Canada, South Africa and Bolivia. The top three countries, growing by far the largest hectarage of herbicide tolerant soybean, were the USA (30.0 million hectares), Argentina (19.5 million ha) and Brazil (17.8 million ha). The other eight countries growing biotech soybean in decreasing order of hectarage included Paraguay, Canada, Uruguay, Bolivia, South Africa, Mexico, Chile and Costa Rica. Of the global hectarage of 90 million hectares of soybean grown in 2010, an impressive 81% or 73.3 million hectares were biotech soybean (James 2010).

Genetic improvement of soybean crop has largely depended upon the two primary instruments of genetic manipulation *i.e.* sexual hybridization and selection. Sexual hybridization used to create genetic variability, and to acquire and deploy specific genes. Selection, based on measurement of the phenotype identifies superior recombinant inbred lines or their progeny. In spite of its

Table 1. Summary of experiments conducted by various investigators on embryo culture of soybean

Nature of explant	Culture media	Results	References
Late heart and older embryos	B_5 + 0.1 mg.l^{-1} IBA	Root and shoot formation	Tilton and Russell (1983)
Globular, heart stage and cotyledonary embryo	B_5 salts + vitamins + sucrose + IBA + coconut milk	Heart stage embryos and plantlet proliferation	Hammatt and Davey (1987)
Immature embryos	MS + NAA, 2,4-D, BA and IBA alone or in combinations	Somatic embryogenesis and Plant regeneration	Lazzeri et al. (1987a)
Immature embryos	MS + B_5 vitamins + NAA + sucrose	Somatic embryogenesis increased with decrease in sugar concentration	Lazzeri et al. (1987b)
Embryo culture	B5 + 6-8 µM BA	Multiple shoot formation within 30 days	Boghar et al. (1988)
Immature zygotic embryo	Basal medium + 40.0 mg.l^{-1} 2,4–D and 6% sucrose	Somatic embryo initiated after 4 weeks of culture	Finer (1988)
2-10 mm long immature embryos	MS basal medium + different growth regulators in various combinations	Callus initiation, formation of embryoid	Li et al. (1988)
Immature embryos (3 to 30 days old)	MS+ 0.1mg.l -NAA B5 + 0.1mg.l^{-1} BA	Somatic embryogenesis followed by plantlet regeneration	Yeh (1989)
Immature embryo	MS + 10.0 mg.l^{-1} NAA + 3 % sucrose	Somatic embryoid formation followed by plantlet regeneration	Komatsuda and Kao (1990)
Immature zygotic embryo	L_2 medium with various growth regulators	Somatic embryogenesis	Sellars et al. (1990)
Immature embryonic axes	Basal medium fortified with different levels of sucrose	Culture response significantly affected by genotypes, sucrose concentrations and genotype x sucrose interaction	Komatsuda et al. (1991)
Immature embryonic axes	½MS + BA	Multiple shoot proliferation	Yeh and Chyuan (1991)
Immature embryo	½MS salts + 0.203 mg.l^{-1} IBA + 20 g.l^{-1} sucrose + 1 g.l^{-1} KNO_3	Shoot initiation in higher frequencies Root initiation (2.0 mg.l^{-1}NAA)	Amer (1992)
Embryonic axis from immature seeds	MS + B5 vitamins + 3.0 mg.l^{-1} BA + 0.4 mg.l-1 NAA	Regeneration	Nawracala et al. (1996)
Embryogenic callus cultures	Finer and Nagasawa liquid medium	Transformable cultures with cytoplasm rich cells in outermost layers	Hazel et al. (1998)
Immature embryonic axes	MS basal medium supplemented with 30.0 mg.l^{-1} 2,4-D, 3.0 mg. l^{-1} BAP + 0.5 mg l^{-1} NAA and 3.0mg.l^{-1} PCPA + 2.5 mg.l^{-1} BAP	Morphogenesis followed by plantlet regeneration in higher frequencies in culture medium containing higher proportion of BAP to NAA	Tripathi and Tiwari (2004b)

tremendous achievements, the strategies of traditional plant breeding remain same as to create genetic variability, evaluate variants and select superior phenotypes. There are three major developments in crop biotechnology, which could facilitate the task of plant breeders by emancipating from the above constraints. The initial development in the field of plant tissue culture *i.e.* the ability to recover plants, not only from micropropagated meristematic tissues, but also from *in vitro* cultures of protoplasts, cells, pollen, ovules, embryos, cotyledons and other tissues and organs took quite a long time for its development. Another biotechnique *i.e.* genetic transformation- the ability to integrate desired DNA sequences in to the genomes of regenerable plant cells, so that the regenerated plants or their progeny express the phenotypes encoded by the introduced DNA, in Mendelian fashion.

Several methods of plant regeneration from cells of cultivated and various related species of soybean developed throughout the world. The foremost impediment to the pursuit of these applications is the lack of routine in facsimile manners for recovering plants from cultured cells and tissues of the locally adapted cultivars. Interests in using *in vitro* means to facilitate these goals in soybean improvement are varified by enormous work carried out on various aspects. In this review, biotechnological applications that have been or, can be, used in the soybean improvement program have been discussed.

Tissue Culture

Rapid multiplication through *in vitro* propagation

Tissue culture techniques are becoming increasingly popular as alternative means of plant vegetative propagation. There is potential for viral disease eliminations for elite soybean clones and/or rapid

micropropagation of soybean lines as pertinent techniques have been developed and refined during the past several years (Cheng et al. 1980; Kameya and Widholm 1981; Li et al. 1989; Christea and Cachita-cosma 1992; Kothari et al. 1991; Droste et al. 1993; Shan et al. 2005).

Hypocotyl and epicotyl culture

Apart from multiplying plants in large number, hypocotyl and epicotyl cultures have been considered potentially useful for maintaining distinct genetic material, *in vitro* selection and transformation experiments. First report on soybean hypocotyl culture came from Kimball and Bingham (1973) however, without shoot initiation. Later, efficient plant regeneration from hypocotyl and epicotyl sections of *Glycine species* was reported time-to-time (Wright et al. 1987b; Shu and Yeh 1988; Li et al. 1989; Kadlec et al. 1991; Christea and Cachita-Cosma 1992; Dan and Reichert 1998; Yoshida 2002; Tripathi and Tiwari 2003a; Amarasinghe and Yang 2006).

Leaf disc culture

Plant regeneration from leaf explants proved an important tool where *Agrobacterium* mediated genetic transformation is prime objective. Several workers (Hymowitz et al. 1986; Hammatt et al. 1987; Wright et al. 1987a; Kollipara and Hymowitz 1989; Rajasekran and Pellow 1997; Tripathi and Tiwari 2004a) achieved plant regeneration from cultured leaf segments.

Embryo culture

Embryo culture is now a well-established branch of plant tissue culture. In soybean, regeneration has been reported using intact isolated zygotic, immature and mature embryos and its sections for efficient plant regeneration via either shoot morphogenesis or somatic embryogenesis. Work carried out on embryo culture of soybean by several investigators throughout the world with varying degree of success is presented in Table 1.

Cotyledon culture

In soybean, immature and mature cotyledons have been used very competently for raising embryogenic callus cultures, cell suspension cultures, somatic embryos and isolation of totiopotent protoplasts. Culture system raised from cotyledons is widely adopted for soybean improvement through *in vitro* selection, somatic hybridization and genetic transformation. Plant regeneration in soybean reported by using isolated cotyledon and its cut portions by various workers with different results are presented in Table 2.

Embryo rescue for interspecific and intergeneric crosses

In wide crosses, post fertilization barriers hinder or retard the development of the zygote after fertilization and normal development of the seed. This frequently results from the failure of the hybrid endosperm to develop properly, leading to starvation and abortion of the hybrid embryo or embryo-endosperm incompatibility, where the endosperm produces toxins that kill the embryo. Endosperm failure generally results in abnormal embryo development and eventual starvation. Thus, isolation and culture of hybrid embryos prior to abortion may circumvent these strong post-zygotic barriers to interspecific and intergeneric hybridization. The production of interspecific and intergeneric hybrids is the most conspicuous and impressive applications of embryo rescue technique, particularly for subsequent valuable gene transfer from wild species. The embryo of different developmental stages, formed within the female gametophyte through sexual process, can be isolated aseptically from bulk of maternal tissues of ovule, seed or capsule and cultured *in vitro* under aseptic and controlled physical conditions. Embryo culture was used for the recovery of interspecific hybrids between *G. tomentella* x *G. canescens* (amphiploid) (Broue et al. 1982) and *G. max* x *G. tomentella* (Coble and Schapaugh 1990) in soybean.

Somatic embryogenesis

In somatic embryogenesis, the embryos regenerate from somatic cells, tissues or organs either *de novo* or directly from the tissues (adventive origin), without involving sexual processes. Somatic embryogenesis differs from organogenesis in the embryo being a bipolar structure with a closed radicular end rather than a monopolar structure. The embryo arises from a single cell and has no vascular connection with the maternal callus tissue or the cultured explants. Somatic embryos or embryoids are small, bipolar embryo like structure developed at the callus surface. They have a number of structural similarities to the sexual or zygotic embryo found in the developing seed.

In soybean, encouraging report on somatic embryogenesis came from Phillips and Collins (1981) where embryo-like structures obtained from cell suspension cultures of *Glycine soja*. Gamborg et al.

Table 2. Summary of experiments conducted by various investigators on cotyledon culture of soybean

Nature of explants	Culture medium	Results	Reference
Cotyledons	MS + 2% sucrose + Dicamba (4.5, 9 and 18 µM)	Maximum response with 4.5 µM Dicamba 77% cotyledons initiated embryoids	Kim and LaMotte (1987)
Immature cotyledons	NAA (6.25, 12.5, 25.0 or 50.0 $mg.l^{-1}$ and sucrose (0.5, 1, 2 or 4%)	Better somatic embryo formation with low to moderate levels of sucrose (1 to 2%) and NAA (6.25 to 12.5 $mg.l^{-1}$)	Lazzari et al. (1988)
Cotyledons	MS + 8-10 $mg.l^{-1}$, 2, 4-D MS + 0.15 $mg.l^{-1}$ NAA + 0.33 $mg.l^{-1}$ kinetin	Plantlet regeneration	Ancelet et al. (1988)
Immature cotyledons	MS + 5.0 $mg.l^{-1}$ 2, 4 D MS + 10 $mg.l^{-1}$ NAA	Frequencies of somatic embryo induction were 94% and 85% respectively	Feng et al. (1989)
Immature cotyledons	MS + B_5 vitamins + 3% sucrose + various growth regulators	2,4-D induced callusing and frequency of somatic embryogenesis increased with addition of myo-inositol and casein hydrolysate	Tran et al. (1989)
Cotyledons	MS + 90.4 µM 2,4-D MS + 0.64 µM IBA + 1.0 µM ABA B5 + 0.6 µM IBA + 0.3 6 µM GA	Embryoid formation Maturation of embryoid Plantlet regeneration	Ferreira et al. (1990)
Cotyledonary node and shoot apices	B5 + 0.5-1.0 $mg.l^{-1}$ IBA B5 + 6% sucrose + no growth regulators	Shoot formation in higher frequency with lower callusing For most genotypes in the second passage best shoot elongation and rooting	Kothari et al. (1991)
Cotyledonary tissue of *G. max*, *G. soja* and their hybrids	Media fortified with 2,4,5-T, PCPA, MCPA and 2, 4-D (20-40 ppm each)	Somatic embryo formation	Ranch and Buchhein (1991)
	Maturation medium with 6-12% sucrose	Fertile plants with seed formation	
Immature cotyledon	MS + 20.0 $mg.l^{-1}$ 2, 4-D + 3-6% sucrose	Somatic embryogenesis	Lamseejan et al. (1993)
	MS +20.0-30.0 $mg.l^{-1}$ 2,4-D	Fertile plants with seed formation	
Immature zygotic cotyledons	Induction medium- MS +30.0 $mg.l^{-1}$ 2, 4 -D + 3% sucrose + 6.0 $g.l^{-1}$ agar	Embryoid formation followed by plantlet regeneration	Bodanse-Zanettini et al. (1993)
Cotyledonary node explants	MS/B_5 medium + BAP (0.15/1.5 $mg.l^{-1}$) + 0.04 $mg.l^{-1}$ NAA + 3 % sucrose	Adventitious buds from cotyledonary node explants	Thome et al. (1995)
	MS/B_5 + 0.038 $mg.l^{-1}$ BA + 0.05 $mg.l^{-1}$ IBA	Formation of bud clumps	
Mature cotyledons with node	MS + 3.0 $mg.l^{-1}$ BAP + 0.4 $mg.l^{-1}$ NAA	Somatic embryogenesis followed by plantlet regeneration	Fu et al. (1995)
Immature cotyledons	MS + 100 $mg.l^{-1}$ myo-inositol + 30 $g.l^{-1}$ sucrose with: L_1 -5.0 $mg.l^{-1}$ 2, 4-D L_2 -9.3 $mg.l^{-1}$ NAA L_3 -10.0 $mg.l^{-1}$ NAA+ 0.1 $mg.l^{-1}$ BA	L_1 was most effective for development of embryogenic structures and somatic embryos	Nawracala and Konieczny (1996)
Cotyledons	MS basal medium with NAA and IBA	IBA was found highly effective in promoting germination of somatic embryos	Fu et al. (1997)
Mature cotyledons and hypocotyls from 5-6 days old seedlings	Induction medium- MS + 0.25 $mg.l^{-1}$ TDZ Germination medium- MS + 1.5 $mg.l^{-1}$ BA + 0.5 $mg.l^{-1}$ kinetin	Mature cotyledon explants induced more embryoid than hypocotyl explants. 63%-83% embryoids germinated	Gai et al. (1997)
Immature cotyledons	MS salt + B_5 vitamins + 60% sucrose + 40 $mg.l^{-1}$ 2,4-D	Highest embryo induction from abaxial side, the addition of 15 µM $AgNO_3$ resulted in faster production of secondary embryos	Santarem et al. (1997)
Cotyledons	Basal medium supplemented with ethylene inhibitors	Use of ethylene inhibitors might facilitate plant recovery from soybean genotypes	Santos et al. (1997)
Immature cotyledons	MS30D –MS+30.0 $mg.l^{-1}$ 2,4-D MS3BN-MS+ 3.0 $mg.l^{-1}$ BAP + 0.5 $mg.l^{-1}$ NAA MS PB-MS+3.0 $mg.l^{-1}$ PCPA + 2.5 $mg.l^{-1}$ BAP	Somatic embryo induction depending upon genotype and the explants inoculation medium	Tripathi and Tiwari (2004c)
Mature cotyledons	MS basal medium supplemented with -5.0 mg. l^{-1} PCPA. 3.0 mg. l^{-1} PCPA + 0.5 mg. l^{-1} BAP 3.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA	High frequency *in vitro* morphogenesis followed by plantlet regeneration depending upon genotype and inoculation medium	Tripathi and Tiwari (2003b)
Mature and immature cotyledons	MS medium containing 0.29 ?M gibberellic acid and 2.69 ?M NAA	Synergistic effect due to BAP and TDZ for plant regeneration	Franklin et al. (2004)
Cotyledonary node	MS medium with 1.7 $mg.l^{-1}$ BAP + 0.5 $mg.l^{-1}$ IBA	Up to 81% shoot bud and root induction and regeneration	Li et al. (2005)
Half seed	B_5 medium fortified with different concentrations of BAP, 2,4-D, KIN, IBA and NAA	Callus induction BAP (13.3 ?M) + 2,4–D (13.5 ?M) and plant regeneration 13.3 ?M BAP	Radhakrishnan and Ranjithakumari (2007)

(1983) developed procedures that produced somatic embryos in cell culture of *Glycine species*. Kerns et al. (1986) observed genotype specific response in suspension culture system where the number and stages of somatic embryos varied with genotype. Nevertheless, in these experiments the embryoids did not develop further to form complete plant. Kameya and Widholm (1981) were able to obtain shoots directly on the young seedling explant of *G. canescens*. Further, shoots produced at a high frequency from *G. canescens* callus and suspension culture, without rooting (Widholm and Rick 1983). Grant (1984) regenerated whole plants from *G. canescens* callus via embryogenesis. Various other investigators throughout the world, presented in Table 3, have reported somatic embryogenesis in soybean.

Apart from these, several aspects related to somatic embryogenesis in soybean viz., influence of media components and pH on somatic embryo induction in three genotypes of soybean and mutagenesis of embryogenic cultures and detection of polymorphisms using RAPD markers (Hofmann et al. 2004), factors affecting somatic embryogenesis from immature cotyledon of soybean (Kim et al. 2004), culture methods and screening of high number of somatic embryos from soybean varieties (Amarasinghe and Yang 2005), soybean somatic embryo conversion using carbon sources and polyethylene glycol (Korbes and Droste 2005), studies on callus and somatic embryogenesis followed by regeneration (Sharifi et al. 2006; DosSantos et al. 2006; Ranjithakumari et al. 2006) have been thoroughly investigated.

Somaclonal variations

Genetic variability is an essential component of any breeding program intended to improve the crop plants. In recent years, plant cell culture hailed as one of the potential sources of useful genetic variation. The variability generated by the use of tissue culture cycle has been termed somaclonal variation by Larkin and Scowcroft (1981). They defined a tissue culture cycle as a process that involves the establishment of a dedifferentiated cell or tissue cultured under defined conditions, proliferation from a number of generations and the subsequent regeneration of plants. Somaclonal variation is obviously highly undesirable in situations where cells and tissue culture used to produce plants with genetic identity. On the other hand, possibility exists to use as means of expanding the pool of desirable genetic variability for crop improvement. Two schemes, with and without *in vitro* selection, have been generally followed for screening somaclonal variants in crop plants, which can be utilized further, by selection of novel variants against various biotic and abiotic stresses. Barwale and Widholm (1987), Graybosch et al. (1987), Hildebrand et al. (1989) and Freytag et al. (1989) have reported somaclonal variation in soybean for various agronomic traits including protein and oil content.

In vitro selection for biotic and abiotic factors

It is established fact that *in vitro* cultures of higher plants could be used for selection of desirable mutants. Protoplast, cell suspension and callus cultures are handled like microorganisms to search for biochemical mutants. Selection for resistance/tolerance is the most straightforward method for mutant selection, whereby resistant cells in a large population can be selected by their ability to grow in the presence of inhibitors like antibiotics, amino acid analogues, pathotoxins etc., while the sensitive cells do not.

In soybean, there are limited reports on *in vitro* selection technique. Zhou et al. (1981) isolated sodium chloride tolerant variants. Gray et al. (1986) demonstrated that an apparently host specific toxin found in *Phialophora gregata* culture filtrate could be used to differentiate between callus from BSR sensitive and resistant genotypes. *In vitro* selection was practiced for herbicide resistance with a view to increase methionine and proline content in soybean (Amer et al. 1988).

Haploid technology

The term haploid refers to those plants, which possess a gametophytic number of chromosomes (single set) in their sporophytes. The interest in haploid plants came from their considerable potential in plant breeding, especially for the production of homozygous plants and in the studies on the detection of mutants/mutations.

Ivers et al. (1974) initiated culture of soybean anthers in an unsuccessful attempt to obtain haploid plants. Guangchu et al. (1984) and Hu et al. (1995) reported haploid callus formation from pollen, which later produced plantlets. However, the plant regeneration frequencies were very low in these studies. Encouraging reports have come out from the studies of Jian et al. (1986), Zhuang et al. (1991), Sunciu et al. (1991), Ye and Wang (1995), Kaltchuk-Santos et al. (1997), Tiwari et al. (2004) and Cardoso et al. (2004). Effects of light conditions and 2,4-D concentration in soybean anther culture, histology of embryogenic responses in soybean anther culture, and isolation and culture of soybean microspores and pollen grains were further investigated (Rodrigues et al. 2004, 2005ab, 2006). Increased symmetrical and extra

Table 3. List of experiments conducted by various investigators on somatic embryogenesis of soybean

Nature of explants	Culture medium	Results	References
Immature cotyledons	MS+10 mg.l^{-1} 2,4 D; For regeneration MS + 0.15 mg.l^{-1} NAA + 0.033 mg.l^{-1} KIN, zeatin and BAP	Induction of somatic embryos	Ancelet et al. (1988)
Immature cotyledons (3-5 mm in length)	MS + 2, 4-D (0.25-1.0 mg.l^{-1}), 10.0 mg.l^{-1} NAA + multiple levels of sucrose	Maximum embryos initiated from central region of abaxially oriented explants	Hartweck et al. (1988)
Immature cotyledons	Basal medium with different levels of auxins and sucrose	Maximum somatic embryos with 2% sucrose and 12.5 mg.l^{-1} NAA	Lazzeri et al. (1988)
Various Explants	N_6 +2.0 mg.l^{-1} Dicamba	Fertile plants obtained via somatic embryogenesis	Christou and Yang (1989)
Immature cotyledons	MS medium + 5.0 mg.l^{-1} 2,4-D. MS medium + 10.0 mg.l^{-1} NAA	Better somatic embryogenesis with 2,4-D than NAA	Feng et al. (1989)
Cotyledons	Basal medium supplemented with BA, NAA and 2,4-D	Frequency of embryoids increased with addition of 2,4-D instead of 10.0 mg.l^{-1} NAA	Komatsuda (1989)
Immature cotyledons	MS basal medium + B_5 vitamins+ 30.0 g.l^{-1} sucrose + different growth regulators	Callus formation improved by 2, 4-D. Myo–inositol and casein hydrolysate improved somatic embryogenesis IAA improved plantlet regeneration	Tran et al. (1989)
Immature embryos of 3 to 30 DAP	MS+ 0.1 mg.l^{-1} NAA B5 + 0.1 mg.l^{-1} IBA	Callus, shoot, root and somatic embryos formed	Yeh (1989)
Immature cotyledons (2-4 mm in length)	MS medium with 2,4-D, IBA, ABA and GA_3	Genotype dependent somatic embryogenesis and plantlet regeneration	Ferreira et al. (1990)
Immature cotyledons	MS basal medium + 10 mg.l^{-1} NAA + 3% sucrose	Genotype dependent embryogenesis and plant regeneration	Komatsuda (1990)
Immature zygotic embryos	Basal medium with various hormonal treatments	Somatic embryogenesis	Sellars et al. (1990)
Immature embryonic axis	Basal medium fortified with different levels of sucrose	Highly significant effect of genotypes, sucrose and genotype x sucrose interaction	Komatsuda et al. (1991)
Cotyledonary tissue explants	Basal medium supplemented with 20-40 ppm auxin	6-12% sucrose led to faster maturation of somatic embryos	Ranch and Buchhein (1991)
Immature embryonic axes	½ MS + BA + IBA	Multiple shoot	Yeh and Chyuan (1991)
Anthers	B_5 basal medium + 0.5 mg.l^{-1} BAP + 0.5 mg.l^{-1} 2,4-D	Embryoid formation	Zhuang et al. (1991)
Mature cotyledons from 2 days old seedlings	Basal medium + 0.1 mg.l^{-1} 2, 4, 5-T or 1.0 mg.l^{-1} 2, 4-D Basal medium+ 1.15 mg.l^{-1} BA + 0.05 mg.l^{-1} picloram	Embryogenic callus initiation Induction of somatic embryogenesis	Cho et al. (1992)
Embryos	Basal medium with different concentrations of sucrose	Highly significant effect of genotype, sucrose and genotype x sucrose interaction Maturation of embryo required sucrose up to 60 mg.l^{-1}	Komatsuda (1992)
Immature embryonic axes and immature cotyledons	MS + 2.5 mg.l^{-1} 2, 4-D + 30 g.l^{-1} sucrose + 8.0 g.l^{-1} bacto agar	Gelrite® and Casein hydrolysate promoted callus and somatic embryos. Higher *in vitro* response from immature embryonic axes	Yeh and Chyuan (1992)
Cotyledons	Basal medium+2,4-D	Genotype and auxin were proved important factors	Griga (1993)
Immature cotyledons	MS fortified with different growth regulators	Higher embryo initiation with genotypic differences	Mauro et al. (1994)
Cotyledons	MS + 2,4-D+B_5 vitamins+sucrose	Somatic embryogenesis affected by genotype	Tian et al. (1994)
Mature cotyledons	½ MS+ 3 mg.l^{-1} BAP + 0.04 mg.l^{-1} NAA	Somatic embryogenesis	Fu et al. (1995)
Immature cotyledons, mature cotyledons and hypocotyls	For induction MS + 0-40 mg.l^{-1} 2, 4-D For regeneration - MS + 0.15 mg.l^{-1} NAA + 0.33 mg.l^{-1} Kinetin + 0.33 mg.l^{-1} BA	Immature cotyledons produced embryogenic calli and plantlets with 30.0 mg.l^{-1} 2, 4-D	Melchiorre et al. (1995)
Immature cotyledons	MS + 100 mg.l^{-1} myo-inositol + 30.0 g.l^{-1} sucrose with: L_1 -5.0 mg.l^{-1} 2,4-D L_2 -9.3 mg.l^{-1} NAA L_3 -10.0 mg.l^{-1} NAA+0.1 mg.l^{-1} BA	$L_1$was found to be most effective for development of somatic embryos	Nawaracala and Konieczny (1996)

Cotyledons	MS basal with NAA, organic nitrogen supplements and IBA	Higher IBA enhances frequency of somatic embryogenesis	Fu et al. (1997)
Mature cotyledons and hypocotyls obtained from 5-6 days old seedlings	Induction medium-MS + 0.25 $mg.l^{-1}$ TDZ Germination medium-MS + 1.5 $mg.l^{-1}$ BA + 0.5 $mg.l^{-1}$ kinetin	Genotypic differences Mature cotyledons induced more embryoid than hypocotyls 63%-83% embryoids germinated	Gai et al. (1997)
Young inflorescence	B_5 + 2.0 $mg.l^{-1}$ 2,4-D+0.5 $mg.l^{-1}$ BA	Somatic embryos similar to zygotic embryos were found	Kaltchuk-Santos et al. (1997)
Immature cotyledons	MS salt + B_5 vitamins + 60 % sucrose + 40.0 mg.l-1 2,4-D	Higher embryo induced from abaxial side of explant in contact with culture medium Addition of 15 µM $AgNO_3$ enhanced production of secondary embryos	Santarem et al. (1997)
Cotyledons	Basal medium supplemented with ethylene inhibitors	Plantlet regeneration	Santos et al. (1997)
Epicotyls and primary leaves	MS + 0.5$mg.l^{-1}$ 2,4-D	Fertile plants from individual somatic embryos and adventitious shoot bud cultures	Rajasekran and Pellow (1997)
Embryogenic callus cultures	Finer & Nagasawa liquid medium	Transformed and regenerable calli	Hazel et al. (1998)
Mature cotyledons and mature embryonic axis	MS + 26.00 - 36.00 $g.l^{-1}$ sucrose + 3.50 - 4.0 $mg.l^{-1}$ BAP + 5.00 - 5.50 $mg.l^{-1}$ NAA	Maximum somatic embryos obtained in cultivar JS 90-41 followed by JS 80-21 and JS 335 for both explants cultures	Tripathi (2004)

nuclei frequencies in soybean pollen grains have also been reported by using cold treatment of floral buds and anther culture (Rodrigues et al. 2005b).

Cell suspension culture

Haberlandt (1902) made the first attempt to isolate and culture single cell from leaves of flowering plants. Although, he failed to achieve the division of free cells, his work stimulated scientists to pursue investigation on this procession. To date, the progress in this field is so fantastic that it is possible not only to culture free cells but also to induce divisions in a cell and to raise whole plant from it. Cell suspension culture consists of cell aggregates dispersed and growing in moving liquid media. Suspension cultures have also been initiated from sterile seedlings, imbibed embryos or leaves and used for obtaining single cell clones by plating cell suspension on agar medium. Plants regenerated from such clones routs through embryogenesis. Cell culture may be used for whole or partial synthesis of secondary plant products, such as alkaloids, glycosides etc. Cell suspension cultures facilitated the mutagenesis studies to produce mutant cell lines through *in vitro* selection. It can also be used as an object for direct and indirect gene transfer.

Gamborg et al. (1968) developed protocols to produce somatic embryos in cell cultures of *Glycine* species with prominent genotypic differences. In *G. soja* somatic embryo-like structures obtained from suspension cultures but did not germinate to produce whole plants (Phillips and Collins 1981). Christianson et al. (1983) were the first to describe a morphogenically competent soybean suspension culture. In another suspension culture system, the number and stages of somatic embryos varied from genotype to genotype (Kerns et al. 1986). Widholm and Rick (1983) reported shoot proliferation at high frequency from *G. canescens* callus and suspension cultures but without root initiation. Proliferative embryogenic suspension cultures followed by complete plantlet regeneration (shoot with root) have also been reported in various *Glycine* species (Finer and Nagasawa 1988; Lamseejan et al. 1992; Bailey et al. 1993).

Protoplast culture

The protoplast, also known as naked plant cell, refers to all the components of a plant cell excluding the cell wall. Protoplasts have been used extensively for fusion and physiological studies, genetic manipulations and direct uptake of foreign DNA, cell organelles, bacteria or virus particles through their naked plasma membrane. Plant protoplasts offer exciting possibilities in the field of somatic cell genetics and crop improvement. The technique of hybrid production through the fusion of isolated somatic protoplasts under *in vitro* conditions and subsequent development of their product (heterokaryon) to a hybrid plant is known as somatic hybridization. This procedure eliminates sexual processes altogether in hybridization. Thus, protoplast fusion technique may be useful to overcome the barriers of incompatibility and acts as a method for the genetic manipulations of plant cells.

Since the first report of Kao et al. (1970), various laboratories throughout the world reported callus formation from soybean protoplast without efficient plant regeneration (Xu et al. 1982; Lu et al. 1983; Choudhury and Widholm 1985; Tricoli et al. 1986). At present, there are reports of reproducible and efficient plant regeneration from soybean protoplasts. Protoplasts have been isolated

either from cell suspension culture (Kao et al. 1970; Choudhury and Widholm 1985; Myers et al. 1989) or from various explants viz. immature pods (Zieg and Outka 1980), root tips (Xu *et al*. 1982), leaves (Gamborg et al. 1983; Oeick et al. 1983; Tricoli et al. 1986), hypocotyls (Newell and Lu 1985; Hammatt and Davey, 1988), immature cotyledons (Lu et al. 1983; Oeick et al. 1983; Luo et al. 1990; Wei and Xu 1990; Dhir et al. 1991; Xiao and Wang 1994), mature cotyledons (Kim and Chae 1989; Hammatt et al. 1989), seedlings (Jones and Davey 1991) and embryos (Zao et al. 1995). Baldes et al. (1987) transformed soybean protoplasts from permanent suspension cultures by cocultivation with cells of *Agrobacterium tumefaciens*.

There is great potential for achieving wide hybridization in soybean through fusion of protoplasts. Regeneration protocol for hybrid plant from protoplasts derived calli is well reported in *G. max* x *G. canescens* (Newell and Lu 1985; Hammatt and Davey 1988; Hammatt et al. 1989) and *G. max* x *G. clandestina* (Hammatt et al. 1987; Jones et al. 1989).

In vitro response of different explants

To establish successful plant tissue cultures and to select suitable explants, it is essential to have full knowledge of natural propagation systems of plants (Hartman and Kester 1986). It is more likely that the sections of leaves will be more suitable as explants in cases where plants normally regenerate from leaves and sections of roots, stems, flowers and their parts, nucellus, cotyledons and other structures will have more applicability in certain other cases. Explants from juvenile plants regenerate more readily than adult plants. The suitable explants for tissue culture are meristematic tissue, which consist of cells that possess the ability of expressing totipotency. The occurrence of embryogenesis in somatic cells found to be associated with cultures initiated from embryo explants rather than non-embryogenic and matured tissues.

Differences in *in vitro* response for various explant cultures of soybean were reported by several investigators (Ranch et al. 1985; Barwale et al. 1986; Ghazi et al. 1986; Komatsuda and Ohyama 1986; Shu and Yeh 1988; Feng et al. 1989; Kien et al. 1989; Yeh and Chyuan 1992; Nadolska-Orczyk and Orczyk 1994; Gai et al. 1997; Tripathi and Tiwari 2004d).

Factors affecting various explant culture in soybean

To attain specific goals employing *in vitro* technology, it is always important to have a highly efficient and reproductive culture system. Several factors, which influence the tissue culture, are growing conditions and genotype of donor plants, age and size of explant, composition of culture medium, culture techniques and culture conditions. Sairam et al. (2003) studied the effect of genotypes, plant growth regulators and sugars in promoting plant regeneration via organogenesis from soybean cotyledonary nodal callus.

Genotypic differences

There is considerable evidence that variation exists among the different genotypes for the *in vitro* response. Genotypic differences in the initiation of callus culture, growth rate, colour, and callus texture as well as morphogenic response indicate genetic conditioning of these characters. There is a wide variation in regeneration competence in the plant kingdom. As certain genotypes lend themselves more than others do to vegetative propagation, the response of different genotypes to a given set of *in vitro* cultural conditions is different. Dicotyledonous plants can generally, regenerate better than monocotyledonous. There are great differences in cell division and regenerative capacity between plants within a single species and similarly *in vitro* response of quite closely related genotypes found to be variable.

In various investigations on soybean tissue culture, genotypic differences were observed for embryo culture (Hammatt and Davey 1987; Komatsuda and Ohyama 1988; Nawracala et al. 1996; Tripathi and Tiwari 2004d), cotyledon culture (Parrott et al. 1989; Ferreira et al. 1990; Komatsuda 1990; Kothari et al. 1991; Komatsuda et al. 1991; Bailey et al. 1993; Bodanse-Zanettini et al. 1993; Thome et al. 1995; Li and Grabau 1996; Nawracala and Konieczny 1996; Tripathi and Tiwari 2004 b, c), hypocotyl culture (Tripathi and Tiwari 2003a) and leaf culture (Kollipara and Hymowitz 1989; Tripathi and Tiwari 2004a).

Composition of culture medium

Regeneration of whole plant from undifferentiated cells in culture is necessary before any of the *in vitro* techniques applied to plant improvement. Gamborg et al. (1968) developed a medium (B_5) fortified with high nitrate to ammonium ratio that induced and maintained soybean callus and suspension cultures. In the early and late 1970s, several reports emphasized the importance of nitrogen source and proper nutritional balance for growth and maintenance of soybean cultures (Gamborg and Shyluk 1970; Bailey et al. 1972 a, b; Ohira et al. 1975; Ojima and Ohira 1977). Mott et al. (1984) reported that ammonium stunted the growth of shoots unless a relatively high concentration of nitrate was also supplied

to soybean *in vitro* cultures. However, with the information available on the nutritional requirement for soybean callus cultures, plantlet formation still not attained. Media modification by Beversdorf and Bingham (1977) and Oswald et al. (1977) resulted in some progress, since structures, resembling somatic embryos obtained but these did not form plantlets. Kaneda et al. (1997) increased the frequency of shoot organogenesis in soybeans with addition of thidiazuron in basal medium.

Till date, for soybean tissue culture experiments, a wide range of basal medium have been employed such as MS (Lazzeri et al. 1987ab; Kim and LaMotte 1987; Ancelet et al. 1988; Hartweck et al. 1988; Feng et al. 1989; Shoemaker et al. 1989; Tran et al. 1989; Yeh 1989; Ferreira et al. 1990; Komatsuda 1990; Komatsuda and Kao 1990; Yeh and Chyuan 1991, 1992; Amer 1992; Lamseejan et al. 1993; Mauro et al. 1994; Tian et al. 1994; Fu et al. 1995; Melchiorre et al. 1995; Thome et al. 1995; Nawracala et al. 1996; Nawracala and Konieczny 1996; Fu et al. 1997; Gai et al. 1997; Santarem et al. 1997; Rajasekran and Pellow 1997; Tripathi and Tiwari 2003b, 2004b, c), B_5 medium (Tilton and Russell 1983; Boghar et al. 1988; Yeh 1989; Ferreira et al. 1990; Kothari et al. 1991; Zhuang et al. 1991; Thome et al. 1995; Kaltchuk-Santos et al. 1997), N_6 medium (Christou and Yang 1989) and Finer and Nagasawa medium (Hazel et al. 1998). Various components of culture medium exert their influence on developmental pathways in a culture. The culture medium has two major functions; the first is to supply the basic macro and micronutrients, vitamins and amino acids essential for continuous growth of excised explant and developing propagules. The second function is to direct the growth and development pattern of the explants-the hormonal control. The type and relative proportion of growth regulators present in the culture medium largely decide the growth pattern and regeneration potential of the culture system. Hormonal control of growth and developments influenced with the kind, their concentrations and the sequence in which they have supplied.

Given the use of a single culture medium, varying the growth regulator levels and type often determines the route of *in vitro* morphogenesis. Generally, medium containing high auxin levels will induce callus formation. Inclusion of cytokinins with auxins may be beneficial for promotion of callus formation in some species. Lowering the auxin concentrations and increasing the cytokinin concentration induces shoot organogenesis from callus. In addition, the ratio of auxin to cytokinin is important for the production of direct shoots from cultured explants. For somatic embryogenesis, transfer of callus to medium devoid of growth regulators is usually sufficient to stimulate the development in later stages and subsequent germination of somatic embryoid. Meristem shoot tips and nodal sections cultured on medium containing low levels of auxins and cytokinins at various ratios leads to induce auxiliary bud growths. Addition of other growth regulators such as ABA or Gibberellic acid into the culture medium is not usual but in some cases be advantageous to promote rooting, shoot elongation and plantlet development.

Beside the level and type of growth regulator in culture medium, other constituents like carbon, potassium, phosphate and nitrogen sources and the pH of culture medium also, influence the *in vitro* performance.

The physiological status of the donor/ mother plant and explant

The material growing under natural day light conditions responds differently than the one growing in the green house. The light should include a mixture of fluorescent and incandescent lights or contain lamps designed to provide balanced wavelengths of light for plant growth and photosynthesis. Generally, the explants collected from the green house grown plants (more elongated and etiolated) regenerated more readily *in vitro* than that from out side (Kumar and Kumar 1996). In soybean, mostly the tissue culture experiments conducted on young leaves, hypocotyls, epicotyls and cotyledons obtained from germinating seeds or seedlings grown under controlled conditions. On the other hand, immature embryos and immature cotyledons collected from the plants grown under field or green house conditions exhibit culture responses without much difference. However, growing conditions of donor plants for *in vitro* culture affect the plant regeneration efficiency up to a great extent.

Culture environment

Plant tissue cultures grow differently depending upon the type of culture environment wherein they nurture. The intensity, type and duration of light, temperature, humidity, oxygen/carbon dioxide ratio and concentrations of other gases, and physiological conditions of the culture medium, all play a vital role in the induction of morphogenesis from the cultured explants. Generally, callus proliferation occurs in dark since light tends to promote embryogenesis, shooting and greening of the callus. Novak et al. (1987) observed 100% callus proliferation with less necrosis in dark incubation for 4 week, whereas, a period of 10 h light and 8 h darkness promoted 90.9% callus proliferation with more necrosis. Lazzeri et al. (1987b) reported improved somatic embryo development by higher illumination whereas, desiccation in an

atmosphere of 85% RH resulted in higher germination frequencies (Bailey et al. 1993). Thome et al. (1995) registered higher bud formation in light than in darkness from cultured cotyledonary node explants.

Genetic Transformation

Plants that carry additional stably integrated and expressed foreign genes (transgenes) transferred from other genetic sources referred to as transgenic plants. The development of transgenic plant is the result of integrated application of recombinant DNA technology, gene transfer methods and tissue culture techniques. These techniques have enabled the production of transgenic plants in food, fiber, vegetable, fruit and forest crops. In recent times, plant biotechnology has become a source of agricultural innovation, providing new solutions to the age-old problems. Plant genes are being cloned, genetic regulatory signals deciphered and genes transferred from entirely unrelated organisms (notably bacteria and virus) to confer new agriculturally important traits in crop plants.

The list of plant species that can be transformed by *Agrobacterium*-mediated and other methods has been growing continuously and at present transformation capability has been extended to more than 120 species in at least 35 families. The first generation application of genetic engineering to agricultural crops targeted towards the generation of transgenic plants expressing foreign genes that confer resistance to viruses, insects, herbicides or post harvest deterioration and accumulation of useful modified storage products. Transgenic plants containing such genes have already been developed in potato, tomato, tobacco, cotton, maize, sorghum, oilseed rape and in soybean.

Agrobacteruim mediated genetic transformation in soybean

Transformation of dicots is usually carried out using the bacterium, *Agrobacterium tumefaciens.* Desired gene sequences are introduced into plants with the help of a disarmed Ti plasmid whose virulence gene products allow the genes to be transferred to the plant nucleus where they are integrated into the genome.

The introduction and expression of diverse group of genes in plants by *Agrobacterium*-mediated method has been described firstly for tobacco (Horsch et al. 1984; DeBlock et al. 1984). The establishment of stable soybean cell lines (after 61 generation of successive suspension culture) containing a *nopaline synthase* gene transferred by *A. tumefaciens* was shown by Shao et al. 1985. Hypocotyls and embryonic culture of soybean were transformed with PNOH 9749 and PTV 100 as vector and transgenic plants were obtained (Simpson and Herrea-Estrella 1989). Transgenic soybean plant accumulating a methionine-rich Brazil nut protein in seeds was obtained through transformation of seed protein gene from *Bertholletia excelsa* via *Agrobacterium*-mediated method (Townsend et al. 1992). Similarly, transgenic soybean plants resistant to soybean mosaic virus (SMV) were obtained by transforming with the coat protein gene and the 3'-UTR from PMV (Wang et al. 2001). An improved *Agrobacterium*-mediated transformation of soybean using cotyledonary node system in "Jack-Mervick" or "Williams 82" genotype of soybean has also been reported (Zeng et al. 2004). *A. tumefaciens* strain KYRT 1 harboring the virulence helper plasmid pKYRT showed high frequency of transformation in somatic embryos (Ko et al. 2006). Experiment to evaluate transformation efficiency of four soybean cultivars (Nannong 88-1, Nannong 18-6, Yu 23 and Nannong 87C-38) by infecting cotyledonary-node with *A. tumefaciens* EHA 105 harbouring pBI121 containing GFP reporter gene was carried out by Xinping & Deyue 2006. They observed that the addition of thiol compounds, L-cysteine, dithiothreitol and sodium thiosulphate in co-cultivation medium increased the transformation efficiency of all the four soybean cultivars.

Microprojectile bombardment method for development of transgenic soybean

Stewart et al. (1996) used somatic embryos of jack, a soybean cultivar, which were transformed using microprojectile bombardment with a synthetic *Bacillus thuringiensis* insecticidal crystal protein gene (Bt crylA) driven by the 35S promoter and linked to the *hph* gene. In particle-mediated transformation of soybean, based on gene delivery into the growing meristem of a soybean embryo, it has been found that a post bombardment culture with glyphosate selection can dramatically increase the efficiency of transformation leading to the development of "Round up ready" soybean (Martinell et al. 1999). Maughan et al. (1999) cloned a 630-bp DNA fragment encoding a bovine milk protein, b-casein, into a seed-specific lectin promoter expression cassette and introduced into soybean somatic embryos via particle bombardment.

Standardization of bombardment parameters such as particle size, target distance, acceleration pressure, amount of DNA per bombardment and number of bombardment to increase the efficiency of soybean transformation by gene gun were made (Khalafalla et al. 2005). Application of these optimized conditions proved

effective for the generation of stable transgenic soybean plants. Soybean plants resistant to soybean dwarf virus (SbDV) were developed through transformation with a construct containing inverted repeat SbDV coat protein into soybean somatic embryos via microprojectile bombardment (Tougou et al. 2006). Soybean plant exhibiting high expression of a synthetic *cry*1AC transgene that confers a high degree of resistance to lepidopteran pest was also generated through this technique (Miklos et al. 2007).

Integrated transformation system for soybean

Droste et al. (2000) described a new method for soybean transformation, based on microwounding of embryonic clumps by particle bombardment prior to inoculation with an *Agrobacterium* suspension. This method combines the advantages of somatic embryogenesis and gene transfer through an integrated transformation system. This was the first report of application of this technique to transformation of soybean.

Sonication Assisted *Agrobacterium*-mediated Transformation (SAAT)

SAAT is currently the most important use of ultrasound in plant tissue culture. This method consists of subjecting the target plant tissue to brief periods of ultrasound while immersed in an *Agrobacterium* suspension. Plant tissue damaged by sonication permits the tissue to be more easily transformed by *Agrobacterium*. SAAT overcomes barriers such as the host specificity and the inability of *Agrobacterium* to reach proper cells in the target tissues. It also enhances DNA transfer in diverse plant groups including dicots, monocots and gymnosperm. It is likely that the enhanced transformation rates using SAAT result from micro wounding both on the surface and deep within the target tissue. Therefore, unlike other transformation methods, this system also has the potential to transform meristemetic tissue buried under several cell layers.

SAAT was first reported by Trick and Finer (1997), who used this technique for the production of transgenic soybean and Ohio buckeye (*Aesculus glabra*) plants and for transient expression in Maize (*Zea mays*), Cowpea (*Vigna unguiculata*), Spruce (*Picea glauca*) and wheat (*Triticum aestivum*). SAAT enabled transient and permanent transformation of plant cells leading to the recovery of transgenic plants through surface damage and creation of microwounds permitting *A. tumefaciens* cells to enter and colonize surface and interior cells that did not occur in controls. SAAT tremendously improved the efficiency of *Agrobacterium* infection by introducing large numbers of microwounds into the target explants of soybean (Santarem et al. 1998; Solis et al. 2003). A summary of experiments conducted by various investigators resulting in transgenic soybean plants has been presented in Table 4.

Overview

In soybean, one tissue culture system, which has not been extensively evaluated in transformation studies, is immature cotyledon culture for somatic embryogenesis. Although, proliferative embryogenic cultures provide a suitable target tissue for transformation, the time and labour required for establishment of these cultures can be great. Induced embryos, on the other hand, directly form from the explant within a few weeks and may be suitable for direct transformation using either *Agrobacterium* or the particle gun. The current difficulty with the use of somatic embryo initiation for soybean transformation work is the inefficiency of its early induction process. Early-staged embryogenesis is desirable for two reasons: 1) It is the best starting material for establishing embryogenic suspensions and 2) embryo development can better controlled with proliferation of early-staged embryogenic tissue on one medium and development of these embryos on another medium.

As regards to genetic transformation, efficient protocols are not readily available in soybean since, regeneration is genotype specific. Therefore, further research is essential for both regeneration as well as genetic transformation of this crop especially in locally adapted cultivars. With the developments in the gene transfer techniques, it was assumed that transformation of soybean would become routine within a few years. Although, first regenerated transgenic soybean was reported long back in 1988 and genetically engineered soybean continued to be the principal biotech crop, unfortunately, soybean like many other crops have provided some of the greatest challenges to transformation efforts.

उत्तक संवर्धन एवं आनुवांशिक रुपांतरण का आण्विक जीव विज्ञान के साथ समायोजन सोयाबीन फसल सुधार में जैविक एवं अजैव कारको के प्रति सहनशील/प्रतिरोधी ट्रांसजेनिक (पराजीनी) किस्मों के विकास तथा विभिन्न लाभकारी गुणात्मक लक्षणों की वृद्धि हेतु महत्वपूर्ण भूमिका निभाता है। ट्रांसजेनिक सोयाबीन के विकास हेतु कर्तोत्तकों से एक सक्षम एवं पुनरावृत्ति योग्य पादप पुनर्जनन प्रणाली का होना पूर्वापेक्षित है। प्रत्यक्षतः उत्तक संवर्धन तकनीके यथा भ्रूण रक्षा, अगुणित तकनीक द्वारा समयुग्मनज किस्मों का विकास, कायिक संकरण एवं कोशिकाद्रव्यी संकरण तथा कायिक क्लोनीय विविधता-सह-पात्रे चयन द्धारा सहनशील/प्रतिरोधी उत्परिवर्ती किस्मों का चयन फसल सुधार के लिये लाभप्रद

साबित हो रहे है। सक्षम पात्रे पुनर्जनन हेतु विभिन्न पोषण एवं वृद्धि नियंत्रको की आवश्यकता का वर्णन असंख्य अध्ययनों में किया गया है। पात्रे पुनर्जनन की गति को प्रभावित करने वाले कई कारक है जैसे कि दाता पौधो का जीनोटाइप एवं वृद्धि दशा, कर्तोत्तकों का उम्र एवं आकार, संवर्धन माध्यम की संरचना (विशेषकर वृद्धि नियंत्रको की सापेक्ष सान्द्रता एवं अनुपात), संवर्धन तकनीक एवं हालात। आनुवांशिक रुपांतरण हेतु विभिन्न मार्करों एवं लक्षित जीनो के साथ कई निरस्त्र एग्रोबैक्टेरियम उपभेदों तथा कण बमबारी विधि का प्रयोग सफलता पूर्वक किया गया है। इस समीक्षा.पत्र में भारत और विदशों में सोयाबीन के उत्तक संवर्धन एवं आनुवांशिक रुपांतरण में प्रगति हेतु किए गए कार्यो एवं जैव प्रौद्योगिकीय उपायो द्वारा सोयाबीन फसल सुधार में प्रमुख बाधाओं तथा भविष्य की रणनीतियों के वर्णन का प्रयास किया गया है।

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Measures for enhancing crop productivity and resource use efficiency in rainfed Vertisols of central India

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Abstract

Major options for enhancing crop productivity and resource use efficiency on sustainable basis discussed with special reference to soil and water management. The results of the studies are based upon the long term experimentations. Conclusions are drawn on the basis of long-term experiments, conducted in Vertisols of JNKVV, Jabalpur. It is concluded that the productivity under rainfed production system can be sustained on long terms basis, if the measures suggested are implemented. Conservation tillage is sustainable practice for rice–wheat-cropping sequence of central India. Low till farming strategy can be adopted for increasing soybean production in place of intensive tillage. Results indicate that by the use of organics, the tillage intensity can be reduced that will help in increasing soil quality and reducing energy consumption without sacrificing the crop yields. Use of organics and green manure holds the key to enhanced productivity and resource use efficiency in rice based and soybean based production systems. Soil and water management practices, viz. Raised – Sunken based system, ridge and furrow system, Broad bed and Furrow system ensure better drainage, safe disposal of runoff which were found effective in sustainable crop production and also for enhancing resource use efficiency. In rice-wheat cropping system zero tillage or minimum tillage produced higher yield of crops as compared to deep tillage. It is recommended that the technologies should be demonstrated on lage scale and popularized as an integral part of package of practices for upland kharif crops. The *in-situ* conservation of rainwater, storage in the surface ponds, dug wells and recycling of harvested water for supplemental irrigation, help in increasing productivity of crops. The overall strategy, calls, for rational use of available technologies for soil, water and fertility management for maximizing productivity of soil-water and crops without detriment to the environment.

The objectives for improving productivity of rainfed/dryland agriculture are to enhance *in situ* moisture conservation and retain rainfall by using agronomic, biological and engineering measures in an integrated way and to make complete use of resources like light, heat, soil, fertilizer, water and improved seeds for increasing yield and agriculture productivity in the rainfed areas. In future Indian agriculture will face with declining availability of natural resources. The global climate change and its impact in the form of abiotic and biotic stresses are likely to further aggravate the situation. There would be growing pressure on technological needs to make agriculture sustainable, competitive in terms of cost and quality under varying agriculture situations. An ideal farming approach should, therefore, aim at increasing the agriculture production, conservation of soils and water, ensuring livelihood and generating employment. In this endeavour, developing technologies that supports for enhancing resource use efficiency, improves soil and environmental health and over all returns to the producers. Hence, research efforts are to be seen in system's perspective on a fast changing agriculture environment (Rai 2009). At JNKVV, Jabalpur a information has been generated specifically, for rainfed soybean based and rice based production systems for Madhya Pradesh on various aspects of crop production. These include the cost effective management practices related to crops and cropping systems, rain water management, soil fertility and nutrient management, conservation of natural resources and their efficient management and conclusions have been drawn for direct use by the beneficiaries. The long term results of these technologies have been discussed in following sub-heads.

Rainfed Agro-Ecosystem

Rainfed Agro-Ecosystem covers about 66% of the net cultivated area supporting 40% of the India's population and contributed about 44% to the national food basket. The rainfed agro-ecosystem also supports two third of India's livestock population. The ecosystem as a whole

is characterized by instability in biological productivity caused by aberrant weather. Farmers are resource poor with inadequate infrastructure and credit support. The rainfed farming system suffers from low and erratic rainfall. The major risk and hazards associated with rainfed crops in central part of India are related to weather and associated soil problems. This paper discusses the potential of various technologies developed and tested in the region for sustainable crop production and for maintaining soil fertility in long run under rainfed/dry land condition of Vertisols region of central India. Key issues of rainfed agriculture are : Continued resources degradation and increased weather related risks, stagnant productivity in major growing districts, loss of soil fertility, stagnant/ falling cropping intensity, poor extension of watershed approach and poor technology adoption uptake over stakeholder.

Rice based production system

According to an estimate (Subbaiah et al. 2006) with increasing population and improving standard of living, additional 2.0 mt of rice is to be produced every year, to maintain the self-sufficiency in rice. In view of little or almost no scope for horizontal expansion, the increased demand has to be met through vertical growth. The area, productivity and production of wheat have increased 119%, 236% and 634% respectively, by the year 1999-2000, as compared with 1965-66 (base year). During 1999-2000, the wheat production in the country touched the highest peak of 76.37 mt. In wheat production since last seven years, India is maintaining its second position in the world next to China and at present produces more wheat than the USA.

The target of 80 mt appears to be achievable since the yield gap between research fields and farmer's field is around 1.0-1.5 t/ha, the gap being more prominent in the states of Madhya Pradesh, Bihar, Rajasthan and Uttar Pradesh (Mishra 2006). The 80mt wheat production target by the end of the next five-year plan could be realistic one only if we concentrate on the short term strategies which give more emphasis to the effective transfer of available technologies to the farmers. So that the gap between farmer's practice and FLD yield can be filled up and also to increase resource use efficiency on sustainable basis.

Soybean based production system

Soybean with coverage of 6 mha and production of 6 mt has occupied an important place in agricultural and oil economy of India. It has revolutionized rural economy and has uplifted socio-economic status of farmers of M.P.. Occupying third place in area, production and share in national basket for edible oils, soybean is the only cost effective alternative to ameliorate pulse protein deficiency in the country. While there had been unprecedented increase in area and production (75% and 155% respectively, during 1986-87 to 1993- 94), the increase in productivity had been slow (91%). It is gaining more coverage in adjacent areas to Madhya Pradesh and Maharashtra and even in tribal districts in Adilabad Andhra Pradesh. The present yield levels in these non traditional areas are higher than the Madhya Pradesh. Therefore, it is necessary to identify the areas that are more suitable for growing soybean. The major concern is that the yield at national level has stagnated around at 1 t/ha as against 2-3 to 2.5 t/ha in Front Line Demonstrations and 2.5 to 3.0 t/ha achieved in research experiments. For the sustainability of the crop in India, creation of domestic market is inevitable, which in turn calls for development of products based on soya meal and consumer awareness. Soybean is grown more than 9.67 mha in 202 districts. About 85% of the area (8.22 mha) is in 21 districts. The major soybean based production system that are adopted in promising for dryland production system are Soybean-gram/wheat/safflower etc. Recent figures indicate that in M.P. soybean area is about 5.30 mha which is increasing every year.

Series of field investigations were conducted in deep Vertisols, to generate the technological options for sustainable crop production under rice-wheat and soybean-based cropping systems, at JNKVV, Jabalpur, Madhya Pradesh under AICRP on Tillage requirement of major Indian soils and AICRP for Dryland Agriculture, College of Agriculture, Indore.

A good deal of information has been generated on various aspects of rice - based and soybean-based production systems mainly related to cost effective management of crops and cropping systems, rain water management, soil fertility and nutrient management, conservation of natural resources and their efficient management and conclusions have been drawn for direct use by the beneficiaries. Major options for enhancing crop productivity and resource use efficiency includes:

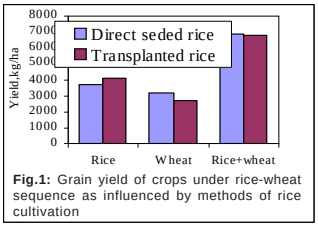

Fig.1: Grain yield of crops under rice-wheat sequence as influenced by methods of rice cultivation

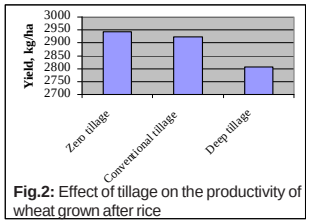

Fig.2: Effect of tillage on the productivity of wheat grown after rice

Table 1. Rice yield (kgha^{-1}) as influenced by methods of cultivation

Year	Method of rice cultivation		CD (5%)
	Transplanted paddy	Direct seeded paddy	
1991-92	4913	4209	417
1992-93	2715	3502	353
1993-94	5560	4788	562
1994-95	3036	2871	NS
1995-96	3940	2898	116
1996-97	3482	3043	279
1997-98	4540	4208	NS
1998-99	4302	3726	NS
1999-00	3573	2922	521
2000-01	4938	4097	384
Average	4093	3711	-

Source : Tomar and Sharma (2009)

Rice based production system

Reduced tillage option for Rice-wheat system

In a long-term study conducted (1991 to 2000) under rice wheat cropping system in Vertisols farmers of Typic Haplusters at JNKVV, Jabalpur, MP, India, the results showed that puddling deteriorates the soil physical environment for subsequent wheat crop as compared to under direct seeded plots. It is concluded that in Vertisols paddy should be grown as direct seeded crop, which provides better physical environment for subsequent wheat crop. Significantly higher yield of wheat was recorded in direct seeded paddy plots as compared to transplanted plots, and the total productivity of the system was higher under reduced tillage conditions i.e. direct seeded paddy followed by zero till wheat (Fig1 and 2).

The mean yield of transplanted rice (TP) was higher than the yield of direct seeded rice (DS) (Table 1 & Fig. 1). Out of ten years the grain yield of TP rice was significantly higher in six years than the DS rice, while in other years the yield levels were statistically at par except during 1992 where the DS paddy gave significantly higher yield than that of transplanted paddy. A comparison of method of rice cultivation on root growth showed that total root length upto 45 cm depth was significantly higher in Ds rice as compared to that in TP rice (Fig 3).The soil organic carbon content was significantly higher under Ds rice than the TP rice (Fig. 4). Lal (1993) also reported 2.2 and 1.7 per cent organic carbon in unpuddled and puddled plots, respectively.

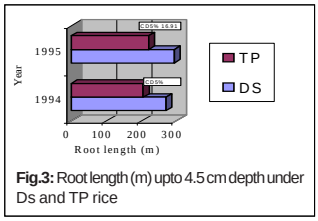

Fig.3: Root length (m) upto 4.5 cm depth under Ds and TP rice

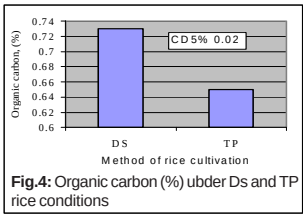

Fig.4: Organic carbon (%) ubder Ds and TP rice conditions

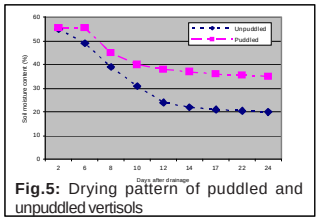

Fig.5: Drying pattern of puddled and unpuddled vertisols

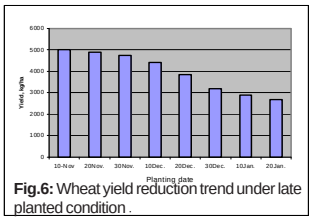

Fig.6: Wheat yield reduction trend under late planted condition .

Moisture content profile depicted in Fig.5 at harvest were similar on puddled and unpuddled soils. But drying pattern and the rate of drying pattern were greater in nonpuddled soils. Cultivation of puddled paddy produced cloddy seedbed, resulted in poor seed soil contact, rapid drying of surface soil and reduces germination of wheat. Having to wait for a puddled soil to arrive at the optimum moisture content for tillage has another disadvantage. A longer 'turnaround' time resulted in low wheat yields.

Table 2. Influence of tillage on the grain yield of paddy and wheat under rice-wheat system

Year	Rice yield (kgha^{-1})				Wheat yield (kgha^{-1})				Wheat
	No tillage	Conventional tillage	Deep tillage	CD (5%)	No tillage	Conventional tillage	Deep tillage	CD (5%)	Planting date
1991	4311	4182	4092	NS	3873	3807	3275	251	Nov.13
1992	3042	2922	2919	NS	3545	3137	3646	418	Dec.13
1993	5219	5413	5165	NS	4431	4302	4095	194	Nov.25
1994	2930	2931	2885	NS	4221	3582	3195	101	Nov.11
1995	3378	3049	2670	139	4112	3270	3005	266	Nov.20
1996	3321	3343	3122	NS	2423	2731	2605	NS	Dec.4
1997	4583	4254	4286	268	781	739	730	NS	Jan1
1998	4109	4110	3823	NS	1516	2083	2064	459	Dec.15
1999	3508	3011	3210	NS	2338	2859	2769	270	Nov.25
2000	4722	4558	4372	NS	2195	2674	2669	455	Dec.2
Av.	3912	3773	3654	-	2943	2918	2805	-	-

Source: Tomar and Sharma (2009)

Results of regional experiments showed that delay in wheat planting decreased the yield potential of wheat in the range of 1 to 1.5 percent ha/day when planting of wheat was done beyond last week of November (Tomar and Verma 1985). Late planting not only results in lower yield but also reduces the efficiency of applied inputs. Fig 6 shows the wheat yield reduction pattern when it s planted beyond November (Tomar and Verma 1985).

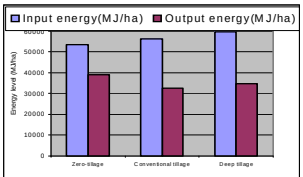

Fig.7: Input-output energy requirement in wheat production under different tillage treatments

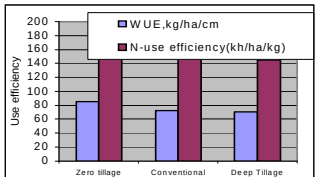

Fig.8: Effect of tillage on resource use efficiency

The long term results of rice-wheat cropping system in Vertisols (Typic Haplusters) at JNKVV, Jabalpur, revealed the significance of zero tillage as a technological option for timely establishment of wheat. In this technology, direct drilling of wheat using Pantnagar Zero till fertilizer seed drill can be done without any tillage operations immediately after harvest of rice on residual moisture. In rice –wheat system, paddy followed by no till wheat resulted in significantly increased productivity of rice wheat system as compared to the productivity of paddy followed by shallow till or deep till wheat (Fig.1&2). Zero tillage has yield advantage particularly in early sown crop i.e. November planted wheat (Table 2). No till system of wheat and reduced tillage in paddy (avoiding puddling and direct seeding of paddy) helped to reduce the turnaround time and get the wheat planted closure to the optimum date. This system offers the advantages of timely wheat sowing, favouring crop productivity, saving energy (Fig.7) and turnaround time. The system increases productivity of the system and reduces the cost of cultivation, fuel cost, wear and tear of tractors and increases input use efficiency. The farmers of rice wheat sequence are resource poor and the savings on tillage operations can be used to buy other inputs like weedicides, fertilizer, irrigation which will further enhance productivity. The twin advantage of enhancement of productivity and its profitability under zero till system, will go a long way in enhancing the sustainability of rice-wheat system.

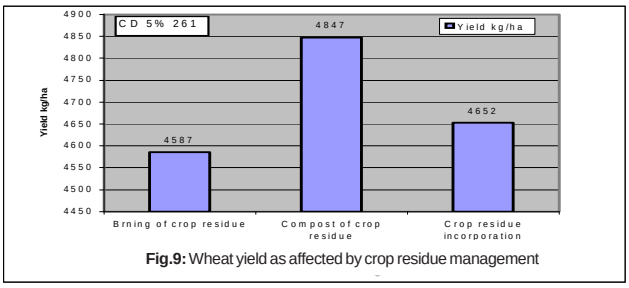

Fig.9: Wheat yield as affected by crop residue management

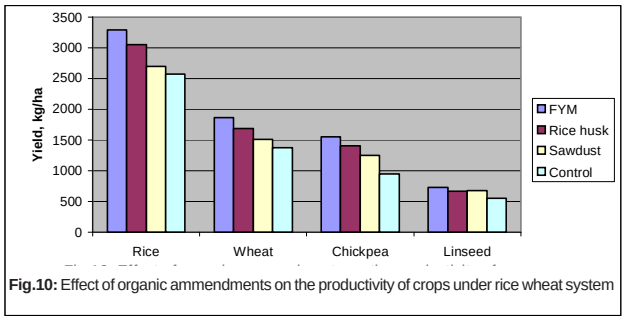

Fig.10: Effect of organic ammendments on the productivity of crops under rice wheat system

Table 3. Effect of organic amendments and forms of urea on the grain yield of crops in rice based cropping system (average of two years) (Tomar et al. 2009)

Treatment	Yield, kgha^{-1}			
	Paddy	Wheat	Linseed	Chickpea
Prilled Urea (PU)	3848	3561	798	1923
FYM+PU	4909	4450	1312	2568
Paddy husk + PU	4079	4112	1123	2314
Paddy Straw + PU	3818	3762	1014	2260
Sawdust + PU	3950	3935	810	2046
Urea Super granules (USG)	3928	3621	835	2141
FYM+USG	5085	4597	756	1850
Paddy husk + USG	4241	4290	1250	2507
Paddy Straw + USG	4171	3816	1068	2314
Sawdust + USG	4025	4025	949	2200
Control	2214	1966	375	1012
CD(5%)	489	109	361	644

After six years of experimentation there is decrease in yield of wheat under zero till plots. Poor yield of wheat recorded during 1997-98 was due to continuous heavy rains received in the month of November and December months which delayed the planting of wheat (Table 2).

Crop residue management in rice-wheat soybean

Field experiment was conducted in Vertisols at Jabalpur by incorporating of rice and wheat crop residue both *in situ* or as compost. Results of this experiment indicated that incorporation of crop residue increased the soil organic carbon content and improved soil health and also improve the total productivity of the rice-wheat system (Fig. 9). The return of carbon to soil and yield of crops was maximum, when crop residue was added along with fertilizer N @ 120 kg N ha^{-1}. Similar results are reported by Tomar and Sharma (2009) in Vertisols of Jabalpur under rice-wheat system.

Table 4. Effect of watering and compaction on the productivity of rainfed rabi crops (Average of five years) grown after puddle rice

Treatment	Yield, kgha^{-1}		
	Wheat	Chickpea	Linseed
Watering in seeded rows	1598 (40%)	1438 (25%)	724 (28%)
Compaction in seeded rows	1436 (26%)	1355 (13%)	639 (13%)
Control	1144	1148	566

Source: Tomar and Sharma (2009)

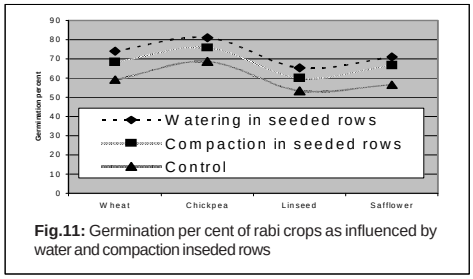

Fig.11: Germination per cent of rabi crops as influenced by water and compaction inseded rows

Nitrogen management in rice - wheat system

Low N use efficiency in rice – wheat system, especially in rice is a major concerned, recommendation has been evolved for application of FYM along with urea super granules/ prilled urea deep placement for rice crop. Significantly higher yield of paddy was recorded when N was applied through Urea Super granule (USG) along with FYM @ 5tha^{-1} (Table 3). All the amendments in combination with USG gave higher grain yield of paddy in comparison to prilled urea combinations. The grain yield of rabi crops was also significantly superior in amendment treated plots irrespective of forms of urea used. Application of organic amendments improved the soil physical environment, which has favourable effect on the grain yield of both paddy and subsequent rabi crops (Fig.10).

Improving seed soil contact in rice based cropping system

After rice harvest, the seedbed became quite cloddy, that affects adversely the germination of post rainy season crops. Vertisols are having a high water storage capacity, which can be very well exploited if we can have a good germination of rabi crops, specifically under rainfed situation. Highest germination of the entire crop was recorded in watering treatment followed by compaction (Fig. 11). This was due to the better moisture condition and good seed soil contact in this treatment as compared to control. For better stand of rainfed rabi crops in rice based cropping system either watering in seeded rows

Table 5. Low till farming strategy for sustainable of soybean productivity

Treatment	Mean	B:C	SI
T_1:CT + RF (-OT) + HW.	1376	1.45	0.53
T_2:CT + RF (+OT) + HW.	1653	1.52	0.63
T_3:LT + 4t /ha straw + HW.	1493	2.27	0.55
T_4:LT + 4t /ha straw + Hb.	1178	1.67	0.43
T_5:LT + 4t/ha Compost + HW	1462	1.97	0.57
T_6: LT + 4t/ha Compost + Hb.	1177	1.48	0.46
T_7:LT+2 t/ha Gliricidia green leaves + Hb	1099	1.57	0.42

Source: Sharma and Sharma (2005)

or rollering with 20-30 kg roller was found effective in improving seed soil contact, which resulted in water conservation and increased germination and over all productivity of rabi crops grown after rice. About 40% and 26% higher wheat yield, 25 and 18% higher chickpea yield, 28 and 13 percent higher yield of linseed were obtained due to watering and compaction, respectively, as compared to control (Table 4).

Soybean based production system

Low till farming strategy for improving soybean crop productivity and soil quality in long run.

With the objective of exploring potential of conservation swell-shrink soils in tillage in rainfed soybean based production system for enhancing productivity and soil quality, a long term field experiment was initiated in the year 1999 at JNKVV, College of Agriculture Indore for evolving low till farming strategies for rainfed soybean production system. On the basis of sustainability index it is recommended that the conjoint use of organics with reduced level of tillage and chemicals which are not only cost effective but also enhance the productivity of soybean

Table 6. Soybean yield and sustainability index under Long term manurial trail in Vertisols of Indore, M.P.

Treatments	Mean yield (16 Years)	Sustainability Index
Control (N0P0)	1261	0.22
N 20 P 13 to each crop	1620	0.33
N 30 P 20 to each crop	1781	0.37
N 40 P 26 to each crop	1886	0.41
N 60 P3 5 to each crop	1994	0.45
FYM @ 6t/ha+ N 20 P 13	2095	0.50
Residue @ 6t/ha + N 20 P 13	1790	0.35
FYM @ 6t/ha	1863	0.39
Residue@ 6t/ha	1629	0.31

Source: Sharma and Sharma (2005)

(Table 5) and build up soil health in the long run. These results revealed that under rainfed conditions soybean should be grown with the addition of organics such as compost, straw as a mulch and addition of toppling of gliricidia. With the help of these additives low till farming strategy is highly recommended with hand weeding and the application of herbicide was not found effective and low sustainability index was obtained in the treatments involving herbicide application. Energy requirement of various treatments revealed that conservation tillage requires lesser input energy and gave higher energy use efficiency as compared to conventional tillage and intensive tillage options.

The major recommendation emerged from this experiment is that low till farming strategy (minimum tillage + use of organics i.e. Compost @ 5t/ha, Gliricidia leaves @ 2t/ha) is recommended for enhancing productivity and soil quality in long run under rainfed conditions**.** Low till farming strategy can be adopted in place of intensive cultivation which includes low tillage + use of organics like compost, straw and gliricidia toppling. The SI > 0.50 revealed that this is one of the most promising technologies for rainfed soybean in Vertisols. The over all results of this study indicated that by the use of organics the tillage intensity can be reduced which will help in improving soil quality and reducing energy consumption without sacrificing crop yields.

Integrated Nutrient Management practices for soybean based cropping system

Maintaining soil health is an important consideration so that productivity potential of a soil is not diminished. Integrated nutrient management envisages the combined application of chemical fertilizers along with organic manures, green manures, biofertilizers and other recycling materials for crop production. Studies on permanent manorial trails at Indore (1993-08) showed that the conjoint use of FYM along with chemical fertilizers helps in increasing soybean yields on sustainable basis (Table 6). Application of FYM has been found to increase water use efficiency, moisture availability to rainfed soybean-safflower sequences and recovery of N, P, K and S from black clay soils. The data presented in the Table 1 indicated that the productivity of soybean can be increased with application of FYM @ 6 t/ha + 50% of the recommended dose of N and P. The highest sustainability index of 0.50 was obtained in this treatment as against 0.22 of recommended dose of chemical fertilizers.

Table 7. Safflower seed yield and sustainability index as influenced by different fertility treatments

Treatment	Mean seed yield, kgha^{-1} (11 years av.)	Sustainability index
T_1:N0P0 (Control)	628	0.13
T_2:N20P13	735	0.12
T_3:N30 P20	1042	0.26
T_4:N40P26	1166	0.30
T_5:N60P35	1182	0.29
T_6:FYM 6t/ha + T_2	1408	0.41
T_7:Residue 5t/ha + T_2	1194	0.32
T_8:FYM 6t/ha	1211	0.33
T_9:Residue 5t/ha	1022	0.25

Source: Sharma et al. (2009)

The highest safflower seed yield of 1408 kg ha^{-1} was recorded due to the treatment FYM 6 t + N20P13 which was statistically superior to the rest of the treatments. Thus, a combination of 6 t FYM, 20 kg N and 13 kg P is optimum for realizing higher yield of safflower. Decline in levels of fertilizer N and P down to 60 kg N and 35 kg P ha^{-1} resulted in gradual reduction in seed yield of safflower Table 7. Application of FYM at the rate, of 6 t ha^{-1} along with reduced level of fertilizer (FYM + N20P13) resulted 35% additional seed yield of safflower as compared to the treatment having RDF. Crop residues application as surface mulch at the rate of 5 t ha^{-1} along with reduced level of fertilizer N and P (Residues 5 t + N20P13) enhanced the seed yield in the range of about 14.6%. The highest sustainable yield index (SYI) of 0.41 was estimated due to conjunctive use of FYM at the rate of 6 t ha^{-1} along with reduced levels of fertilizer N and P at the rate of 20 and 13 kg ha^{-1}. Chemical fertilizer application 60 kg N and 35 kg P ha^{-1} gave sustainable yield index of 0.30 which, emphasized that integrated application of nutrient enhances crop productivity of safflower on sustainable basis when applied in Vertisols under rainfed

Table 8. Water use and water use efficiency of safflower as influenced by fertility treatments

Treatments	Water use, mm	Water use efficiency, kgha^{-1}mm^{-1}
N0P0	105	5.02
N20P13	108.2	7.11
N30P20	105	7.94
N40P26	122	7.83
N60P35	152.5	8.88
FYM 6t + N20P13	112.1	9.34
Residues 5t+ N20P13	128	8.45
FYM 6t	131.2	7.85
Residues 5t	123.8	7.34

Source: Sharma et al. (2009)

conditions. Similar results were also reported by Sharma and Dixit (1987), Sharma (1992) and Sharma and Sharma (2005).

Water use and water use efficiency

Water use and water use efficiency of safflower was worked out taking into account all the components of filed water balance namely rainfall, surface runoff, deep percolation, upwards flux of water and profile moisture content changes during the cropping season. Data presented in Table 8 revealed that water use by safflower crop varied from 105 to 152.5 mm, while water use efficiency ranged in between 5.02 to 9.34 kg ha^{-1} mm^{-1}. The highest water use efficiency of 9.34 $kgha^{-1}$ mm^{-1} was recorded from the treatment of FYM + N20P13 followed by 8.88, 7.94, 7.85, 7.83. 7.34, 7.11 and 5.02 kg ha^{-1} mm-1 due to treatment N60P35, N30P20, FYM alone, N40P26 Crop residue N20P13 and N0P0 treatments, respectively. The water use efficiency increases with increased levels of N and P. The highest water use efficiency was observed in N60P35\ (Table 8) Application of FYM at the rate of 6 t ha^{-1} resulted in an increase of water use efficiency of safflower by 54% as compared with control, which was further increased by 30% due to conjunctive use of FYM and N20P13. Similarly application of crop residues as surface mulch at the rate of 5 t ha^{-1} led to an increase in water use efficiency of safflower 44% compared with control which was further enhanced up to 24% when crop residues was applied in conjunction with N20P13.

Overall results suggest that in-situ mulching with different materials for weed control and moisture conservation increase the seed yield of soybean by 11 to 24% as compared to no mulch treatment. The percent increase in the seed yield of subsequent *Rabi* crop i.e. safflower due to mulching was 52-110% (Table 9). Polythin mulch (ordinary grade), straw mulch, weed biomass mulch and glyricidia green leaves mulch have been found effective in conserving soil moisture and enhancing productivity of soybean–safflower sequence in medium to deep Vertisols under dry land conditions. Mulching has been found economically viable.

Table 9. Effect of Mulch on the productivity of soybean – safflower cropping sequence

Treatments	Yield, kg ha^{-1}		B:C ratio
	Soybean	Safflower	(soybean+ safflower)
No mulching	1605	594	2.20
Gliricidia mulch, 2 tha^{-1}	1934	1117	3.00
	(20)	(88)	
Straw mulch, 4 tha^{-1}	1905	1248	3.03
	(19)	(110)	
Weed biomass mulch, 5 t/ha	1972	1097	3.02
	(23)	(85)	
Soil mulching	1779	905	2.63
	(11)	(52)	
Polythene mulch	1990	1142	2.91
	(24)	(92)	

Source: Anonymous (2009)

Table 10. Effect of land configuration on the yield (Kg/ha) of crops in Vertisols of high rainfall area

Year	Soybean seed yield		Paddy grain yield	Seasonal
	Improved system (Raised bed 6 m wide)	Farmer practice (Flat bed)	Improved system (Sunken bed 6 m wide)	rainfall (mm)
1979	1373	1000	-	472
1980	2962	2229	5100	1432
1981	1830	1050	-	700
1982	2230	1054	2030	1236
1983	2340	817	1400	1445
1985	2253	1000	2500	1380
1986	2131	647	4220	1034
1987	1840	715	1716	1216
1988	3260	1731	1877	1010
1989	2779	144	1520	627
1990	2425	581	3001	1624
1991	2523	125	2200	1215
1992	3107	581	3001	1055
1993	1733	1050	4758	1391
1994	1969	1469	3922	1090
1995	1755	1113	4473	1153

Source: Tomar et al. (1996)

Effect of land configurations on the productivity of soybean

Madhya Pradesh is endowed with well distributed rains ranging from 700 to 1200 mm. Vertisols with good moisture holding capacity can be used to grow short-duration soybean by adopting sound land management practices such as broad bed and furrow system, Ridge and furrow system otherwise about 2.02 m ha accounting for 6.57% of the total area of the state were under fallowing. The adoption of proper land configuration system will help increase income to the farmers besides preventing land degradation due to runoff erosion.

The small changes through land configuration in flat field conditions may help in improving the productivity of Vertisols of Malwa region as at present extensively the flat –land cultivation system is more popular, which faces the problem of water logging and poor aeration thereby affecting crop productivity adversely. Using light machinery like bund former and *desi hal* with minor modification may improve the physical conditions and drainability. It is

Table 11. Effect of land configuration on the yield (Kg ha-1) of soybean and pigeon pea grown as sole and intercrop in a Vertisols of high rainfall area

Land Configuration	Cropping system	1990-91	1991-92	1992-93	1993-94	1994-95	1995-96
Improved system	Soybean sole	2425	2523	3107	1733	1969	1755
(Raised bed 6 m wide)	Pigeon pea sole	2012	2004	2532	1825	1626	1174
	Soybean +	1805	1977	2310	1433	1631	1344
	Pigeon pea	1805	1608	2394	1436	1426	900
Farmers practice	Soybean sole	849	125	581	1050	1469	1113
(Flat bed)	Pigeon pea sole	128	167	Failed	Failed	Failed	Failed
	Soybean +	650	95	581	1050	1469	1113
	Pigeon pea	125	151	Failed	Failed	Failed	Failed
Seasonal rainfall, mm		1631	1215	1055	1391	1090	1132

Source: Painuli et al. (2002)

assumed that land treatments will help to improve soil physical conditions, root development and over all productivity in Vertisols. Shrinking seed size is resulting in over plant population therefore reduced seed rate may help in maintaining optimum plant population with this hypothesis a comparative study with 100% seed rate and reduced seed rate was carried out. The results of the study are presented here:

Growing of crops on Raised sunken bed system

To improve the productivity of rainfed Vertisols in such area, there is a need to provide to the resource poor farmers such a system, which will provide adequate means of surface drainage at a cheaper cost than sub-surface drainage and will also assure adequate root zone moisture recharge for *Kharif* (Rainy season) crops. Jabalpur center of the AICRP on Soil Physical Constraints and their amelioration for sustainable crop production has conducted field experiments over more than 16 years to develop and validate Raised-Sunken Bed Technology. This technology provides: an adequate drainage during heavy rains, favorable moisture regime during dry spell in *Kharif* season to upland crops, sufficient pounding of water for paddy and channelizes the excess runoff water safely to a farm pond for recycling to provide supplemental irrigation to rainy and post rainy season crops. The technology has been demonstrated successfully at the farmer's fields in Madhya Pradesh and also found beneficial by the Parbhani (Maharashtra) center of the project. In Maharashtra, approximately 0.53 m ha land experiences temporary water logging during rainy season.

Table 12. Performance of sole and intercrop on Vertisols of different depth of soil (mean of 2001 to 2003 seasons)

Cropping System	Shallow soils		Medium Soils		Deep Soils	
	Yield*	SI	Yield*	SI	Yield*	SI
Sole soybean (JS 335)	1908	0.50	2082	0.37	2138	0.41
Soybean (JS 335) + Pigeonpea (JA4)	2655	0.71	3668	0.73	3785	0.78

Source: Sharma and Sharma (2005)
* Soybean Equivalent Yield

Based on long-term trials at Jabalpur (M.P.) involving various crops the recommended raised-sunken bed system consists of 6 to 9 m wide and 30 to 35 m high raised bed along which runs the sunken bed of 6m width. The sunken beds are connected for example with the pipes to maintain water level at a desired height viz. 15 to 20 cm in all the sunken beds. The excess water is safely carried away and collected in a farm pond for recycling to provide supplemental irrigation to rainy season and post rainy season crops.

Raised-Sunken Bed system has also been found successful for intercropping upland crops on raised bed. Two rows of soybean or black gram followed by one row of pigeon pea (medium duration) produced 3, 12 & 4 times higher yield for soybean sole, pigeon pea sole and soybean + pigeon pea intercrop (Table 11) under improved system compared to farmers practice. It was observed that in most of the years pigeon pea and black gram could not survive in flat bed (farmers practice) but performed satisfactorily under the improved system. The total productivity under improved system of soybean + pigeonpea cropping was more than 3.3 t/ha which from a view point of yield under rainfed condition in Vertisols of the region was a good yield. The main advantage of the intercropping was that even if the rains ceased earlier the pigeonpea survived on residual moisture left over in the profile. The intercropping results suggest that by incorporating it in Raised-Sunken Bed Technology crop productivity can be increased in high rainfall areas of Vertisols regions.

Table 13. Performance of post rainy season crops on Raised and Sunken bed

Land configuration	Crop	86-87	87-88	88-89	89-90	90-91	Geometric mean ±SE
Raised bed 6m width	Chickpea	2146	1866	1842	1722	1579	1822±84
	Safflower	1412	1424	1240	1488	1348	1380±38
Sunken bed 6m width	Linseed	1170	949	1416	938	1048	1091±79
	Wheat	989	1972	1218	1047	1162	1236±159

Source: Painuli et al. (2002)

Table 14. Economic viability of raised-sunken bed technology

Treatment combinations	Operational cost (Rs/ha)	Yield (q/ha)	Gross Return (Rs/ha)	Net Return (Rs/ha)	B: C ratio
S: Rice -wheat	9869	27.92 to 18.00	21147	11278	1.14:1
R: Soybean-Chickpea		24.47 to 18.31			
S: Rice - Chickpea	9269	27.92 to 14.49	21894	12625	1.36:1
R: Soybean- Chickpea		27.47 to 18.31			
S: Rice -wheat	7359	27.92 to 18.00	15462	8103	1.10:1
R: Pigeon pea		20.93			
S: Rice - Chickpea	7769	27.92 to 14.49	16401	8632	1.11:1
R: Soybean fallow		24.47			
S: Rice -wheat	8519	27.92 to 18.00	20917	12398	1.46:1
R:Pigeonpea+ soybean (Intercrop)		18.81+18.11			
S: Rice -wheat	7919	27.92 to 14.49	21664	13745	1.74:1
R:Pigeonpea + soybean (Intercrop)		18.81+18.11			
S: Rice - Chickpea	6759	27.92-14.49	16209	9450	1.40:1
R: Pigeon pea		20.93			

Source: Tomar et al. (1996)

Evaluation of intercropping in Vertisols

To evaluate the suitable cropping system for shallow, medium and deep Vertisols the sole soybean and Intercropping of soybean and pigeon pea were tried on shallow, medium and deep Vertisols. The data on soybean equivalent yield and Sustainability Index are presented in Table 12. Data clearly emphasized that intercropping of soybean and pigeon pea is more sustainable and therefore, recommended in place of sole soybean under all the three soil depths. Incase of intercropping the sustainability index ranged from 0.65 to 0.83 while in case of sole soybean it ranged from 0.37 to 0.50 only.

Performance of post rainy season crops grown on raised bed and sunken bed was evaluated against farmer's practice of flat bed sowing. As compared to farmers practice i.e. flat bed sowing under improved system the yield of each test crop was nearly two times more (Table 13). This could be attributed to more favorable moisture regime in the seed zone under improved system at the harvest of rainy season crop resulting in better establishment and improved stand of post rainy season crop.

Benefit-cost ratio based on the prices prevailing in the year 1997 for various operations and yields have revealed that the Raised-Sunken Bed System was economically viable under all the major cropping systems of the region (Table 14). The benefit-cost ratio showed that the land shaping cost could be recovered within 1 to 2 year. Economics also revealed that in case of failure of post rainy season crops or even rice during *Kharif* (due to scanty rains as was the case in 1979 and 1981) improved system gave higher returns compared to the farmers practice. Highest benefit-cost ratio was observed in soybean-pigeon pea intercropping on raised bed and paddy-chickpea in sunken bed.

Economic viability analysis

Recycling of Rainwater for use in Rabi crops

Harvesting of rainwater and its recycling enhances crop productivity, specifically in Vertisols because Vertisols have very high water storage capacity and have sufficient profile stored water for growing of rabi crops, but surface soils dries out very fast, which affects germination and stand of post rainy season crops adversely. Therefore,

one come-up irrigation through rain water harvesting can enhance crop productivity tremendously. Data presented in Table 15 revealed that by applying one come up irrigation one can obtained a very good crop yields. Results also emphasized that by means of mulch application, proper tillage operations and nutrient management further increase in crop productivity can be achieved (Table 16) to enhance resource use efficiency and farmer's profitability.

Conclusion

Studies carry in Vertisols of high rainfall areas (>1000mm) and medium rainfall area (700 to 1000 mm) of Madhya Pradesh revealed that for rice based cropping system and soybean based production system reduced tillage/ zero tillage helps in enhancing crop productivity, improves soil quality and enhances resource use efficiency. Integrated nutrient management has proved its potential for sustaining soil and crop productivity in sustainable manner. Addition of organics supports in improving soil organic carbon, soil microbiological activities, soil quality as well as crop productivity. Rain water harvesting and its recycling will help in increasing crop productivity in Vertisols tremendously. Application of mulch helps in improving crop productivity under rainfed cultivation. On the basis of long term studies it is concluded that reduced tillage, integrated nutrient management, rain water harvesting, conservation of soil moisture, intercropping, application of deficient nutrients such as application of S holds the key for sustainable rainfed crop production in Vertisols of Central India.

मृदा एवं जल प्रबन्धन के द्वारा फसल उत्पादकता एवं संसाधन उपयोग क्षमता बढ़ाने हेतु प्रमुख आयामों की इस अनुसंधान प्रपत्र में चर्चा की गई है । जवाहरलाल नेहरू कृषि विश्व विद्यालय जबलपुर में लम्बी अवधि के प्रयोगों के परिणामों के आधार पर जो तकनीकी का विकसित कर अनुशंसाएं की गई हैं वो कृषि के स्थायी विकास का आधार हो सकती है । इस तकनीकों एवं अनुशंसाओं का पालन करने पर वर्षा आधारित कृषि में स्थिरता प्रदान की जा सकती है । मध्य भारत में धान–गेहूं फसल चक्र हेतु कनर्सवेशन टिलेज तकनीक उत्तम पाई गई है । धान आधारित फसल पद्धति में बिना जुताई (जीजो टिलेज)/कम जुताई (मिनीमम) के माध्यम से गहरी जुताई की अपेक्षा अधिक उत्पादन प्राप्त किया जा सकता हे । सोयाबीन उत्पादन हेतु कम जुताई (मिनीमम) तीव्र जुताई (इन्टेन्सिव टिलेज) की अपेक्षा उत्तम पाई गई । अनुसंधान के परिणाम दर्शाते हैं कि कार्बनिक खादों के उपयोग द्वारा सोयाबीन उत्पादन हेतु जुताई की तीव्रता को कम किया जा सकता है, मृदा गुणवत्ता को बढ़ाया जा सकता है, ऊर्जा खपत को घटाया जा सकता है एवं सोयाबीन उत्पादकता को बढ़ाया भी जा सकता है । हरी एवं कार्बनिक खाद का उपयोग धान–गेहूं एवं सोयाबीन–गेहूं आधारित ुसल चक्रों की उतपादकता बढ़ाने की कुंजी है । मृदा एवं जल प्रबन्धन की विधियां जैसे ऊंची–नीची क्यारी तकनीक, पृष्ठ एवं कुड पऋति, चौड़ी क्यारी एवं कूड पद्धति के माध्यम से जल निकास एवं बिना ह्रास के प्रवाह जल का संरक्षण कर काली मृदाओं में फसलोत्पादन एवं संशाधन उपयोग क्षमता को बढ़ाया जा सकता है । अतः इन तकनीकों का प्रदर्शन कषकों के खेतों पर वृहद् रूप से करने की अनुशंसा की जाती है तथा इस बात की आवश्यकता महसूस की जाती है कि इन तकनीकों का समावेश कषि की उन्नत पैकेज एवं प्रेक्टिसेज़ में यिा जाना चाहिये । खेत में ही जल संरक्षण, वर्षा जल का जल तालाबों में संग्रहण एवं उसका सिंचाई हेतु पुर्नउयोग कर उत्पादकता को बढ़ाया जा सकता है ।

इस प्रपत्र में वर्णित उन्नत तकनीकों का उपयोग कर स्थायी उत्पादन एवं प्राकृतिक संसाधनों की उपयोग क्षमता बढ़ाने की रणनीति इस तरह तैयार की जा सकती है । इन उन्नत तकनीकों का उपयोग कर प्राकृतिक संसाधनों को प्रदूषित किये बिना मृदा, जल एवं पोषक तत्वों का उचित प्रबंधन कर प्रमुख फसलों की अधिकतम उत्पादकता प्राप्त की सकती है ।

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Organic cotton cultivation in Madhya Pradesh: Prospects and Problems

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Traditionally, Indian farmers have been following organic cultivation till the middle of the last century, as they had no other choice. The advent of high yielding varieties and hybrids of cotton during the last three decades has resulted in large scale dependence on chemical fertilizers and specially toxic pesticides. Cotton, cultivated on 5% of India's cultivable land consumes 54% of the total pesticides used in Indian agriculture (Mehta 1996). This has not only increased cost of cultivation but also caused environmental pollution. Some of the chemicals marketed, particularly, for plant protection are highly dangerous to human health and also leave poisonous residues in soil after application lasting over a few years.

Global awareness of health and environmental issues is spreading fast in the recent years, especially in the developed countries. Sustainability in production has become the prime concern in agriculture development. Organic method of farming is the best option to ensure air, water and soil around us unpolluted, leaving the environment safe for the present and future generations.

A new trend is being developed in India now to produce some of the crops organically not only on account of the love for protection of nature but also because of the need for having safe products for human consumption.

Cotton enjoys an extremely positive image due to its naturalness and gentleness to human skin. Life cycle of cotton products from cultivation through production to disposal is embedded with harmful chemicals, which seems to impair human health and develop allergic illness. Realizing that millions of tones of chemicals are used to produce and process cotton and the likely effects these chemicals will have on human health and environment in the long run, it becomes imperative that organic cotton production is enhanced.

General Principles

Organic agriculture is a process for developing a viable and sustainable agro-ecosystem. The time between the start of organic management and certification of crops is known as the conversion period. When traditional agricultural methods fulfill the principles of the organic standards, no conversion period is required. When also claiming virgin land for organic agriculture, no conversion period is required. The whole farm, including livestock, should be converted according to the standards over a period of time. The certification programme defines this period as conversion period. In the case of field crops or annuals, normally the conversion period is two years and for perennial crops three years. If a farm is not converted all at once, it should be done on a field-by-field basis, whereby full standards are followed from the start of conversion on the relevant fields. The area of land being managed and meeting the certification standards will therefore progressively increase. It is not recommended to separate the conversion of individual fields unless this is on the basis of imposed public restrictions or can be justified according to local conditions. The compositions of a farm unit can vary widely according to geographical conditions, ownership structure, time span required etc. In the case of farms having both crop production and livestock, the entire operation should be seen as a whole.

Organic management prevents degradation of common biotic and abiotic resources, including areas used for rangeland, fisheries, forests, forage for bees as well as neighbouring land, air and water. So organic agriculture follows viable and sustainable agro-ecosystem by working compatibly with natural living systems and cycles. Species and varieties selected and cultivated in organic agriculture should be adaptable to the local soil and climatic conditions and tolerant to pests and

diseases. Biological and cultural means are followed to prevent unacceptable losses from pests and diseases. Crops and varieties should be suitable to the environment and there should be a balanced fertility programme to maintain required nutrient levels in the soil with high biological activity, locally adapted rotations, companion planting, green manure production and other recognized organic practices.

Organic cultivation improves structure and fertility of the soil through balanced choice of crops and implementation of diversified cropping system. Biological processes are strengthened without re-coursing to chemical remedies, such as, synthetic fertilizers and chemical pesticides. In this system control of pests, diseases and weeds are primarily preventive, and if required, adopting organic products, which will not adversely affect the environment. Organic matter of various kinds, nitrogen fixing plants, bio-fertilizers, pests and disease resistant varieties, bio-control agents, soil improvement practices, such as, mulching, fallowing, crop rotation, multiple cropping, mixed farming etc. are freely adopted. In brief, organic farming merges traditional and respectable views on nature with modern insights.

Organic livestock husbandry is based on harmonious relationship among land, plants and livestock; respect for the physiological and behavioral needs of livestock and feeding of good-quality organically grown foodstuffs. Organic management practices promote and maintain health and well being of the animals through balanced organic nutrition, stress-free living conditions and breed selection for resistance to diseases, parasites and infections. The conversion period from the conventional livestock husbandry depends upon the period to develop natural behavior, immunity and desirable metabolic functions. Organic animals are those born and raised on organic holdings. Breeds should be adapted to the local conditions. Such animals should receive their nutritional needs from organic forage and feed of good quality. Their transport and slaughter should be with minimum stress.

For organic bee keeping hives should be made of natural materials, feed given to overcome temporary shortages should be of organic origin and bees when placed in wild areas should be able to ensure the integrity and safety of indigenous insect population and pollination requirements of the native plants. When colonies are moved to new areas bees should be safe and capable of adapting to the local conditions. Honey temperature should be maintained as low as possible during extraction and storage. Destruction of bees in the combs as method of harvesting bee products is prohibited. Mutilations, such as clipping of the wings of queen bees are prohibited. But, artificial insemination of queen bee is permitted. Use of smoke for handling bees should be kept to the minimum requirement.

Organic aquaculture promotes biodiversity, biological cycles, biological activity and sustainability. Use of organic feed, adoption of proactive health management and provision of desirable living conditions are part of it. Standards generally do not allow the use of synthetic chemicals for biocides, colourants, antibiotics and growth hormones. For piscivorous (fish-eating) species, fishmeal and fish oil can be included as part of their diet. Net cage operations that degrade ambient environmental conditions with direct release of effluents containing metabolic wastes, uneaten feed other debris, such as, dying or dead animals to an open public body of water are not permitted. Use of destructive fishing methods like, cynamide fishing are not allowed as they cause for dwindling of natural population of many species.

Genetic engineering is not allowed in organic production and processing. Accordingly Genetically Modified Organisms (GMOs) and their derivatives are excluded from organic production, processing and handling.

Organic Standards

The most significant factors distinguishing organic farming from other methods of sustainable agriculture are the existence of production and processing standards and certification procedures. The standards are developed by private associations, companies and certification bodies or by the State itself. Universally accepted standards have been prepared by the IFOAM. A number of regional, national and international standards have been developed worldwide.

Several countries have formulated and adopted or are formulating rules and regulations on organic farming, processing and certification requirements considering the national situations. The European Union developed their standards as early as 1991 and enacted under EEC Regulation No. 2092/91. The standards of the European Union are improved periodically by amending the regulation. The United States published their standards under the name, United States Department of Agriculture National Organic Programme (USDA NOP) and made effective from 21st October 2002 under the Organic Food Production Act of 1990, as a part of the 1990 Farm Bill. Japan introduced their organic standards under the title, Japanese Agricultural Standards (JAS) as per the Notification No. 59 of the Ministry of Agriculture, Forestry and Fisheries, Certain countries like Switzerland,

Argentina have also made their regulations for organic production and processing.

In India the organic standards came into existence when the Department of Commerce, Ministry of Commerce and Industries, Govt. of India notified them in March 2000 considering the export potential of organic agricultural products. These standards are put under the National Programme for Organic Production (NPOP). A logo has also been adopted for the organic products certified under NPOP called Indian Organic Logo. The National Steering Committee has been constituted with representatives from the Ministries of Agriculture, Food Processing Industries, Forest and Environment, Science and Technology, Rural Development and Commerce and Industries to act as the apex advisory body for assisting the Govt. of India in shaping the growth and development of organic production. The National Steering Committee will meet as often as necessary but not less than twice a year. An accreditation committee has also been set up with membership to Tea Board, Coffee Board, Spices Board, APEDA and other agencies as the executive arm of the NPOP for given accreditation to inspection and certification agencies.

Future prospects

India has a great potential for organic cotton. Nearly 70% of the Indian cotton area (6.4 m ha) is managed by resource poor farmers and they grow cotton under risky rain fed condition and farmers who cultivate cotton in general avoid application of costly chemical inputs like pesticides and fertilizers (Venugopal et al., 1996). Out of total rain fed area nearly 45% is under desi cotton which do not requires either knowingly or unknowingly follow the organic method of cotton cultivation. If these potential organic farmers are enlightened with the technology of organic cotton cultivation and premium price is ensured. Thus these traditionally cultivated desi cotton farmers can be channeled into a organic farming system

The 68th Plenary Meeting of the International Cotton Advisory Committee (ICAC) suggested that organic cotton has the potential to provide new idea that can influence and support wider sustainability in the sector. The organic cotton sector continues to grow, albeit more slowly in the global economic slowdown, reaching over 180,000 metric tons of lint in 2008/09 (Narayanan 2010). Organic cotton is one of the option to meet the challenges of land use, food security and water security. Small growers exposed in the variable rainfall and problems from debt produce most organic cotton in India. Many farmers view organic cotton as a risk reduction tool, but the use of fewer purchased inputs involves a tradeoff with productivity. Often the smallest and most resource poor farmers come to organic, and the fact organic works better in marginal or tribal areas. Certification of organic cotton is expensive and in some states of India the government is supporting certification costs. Organic cotton production, processing, spinning and marketing at retail level involves a long and complex chain requiring traceability and communication links between producers and consumers. The future of organic cotton production may involve contract farming with direct link to retailers.

Madhya Pradesh is an important cotton growing state having acreage of more than 6 lakh ha with a productivity of 415 kg lint /ha. The important cotton growing zones in the state are Nimar and Malwa, each having peculiar cotton growing situations. In these areas predominant cotton is grown as rain fed and there are production imbalances between the well-endowed or poorly endowed areas.

In Madhya Pradesh despite cotton cultivation being highly dependent on intensive chemical uses, there are certain pockets like part of Khandwa , Jhabua and Burhanpur (tribal area) where cotton is grown more or less organically , that is without chemical inputs . Thus there is an enormous opportunity of organic cotton cultivation over a vast area of around 1 lakh hectares in general and in pockets like tribal belt of Nimar and Malwa regions in particular, where the traditional agriculture has not been exposed to modern cultivation practices. In these area farmers are not using pesticides to raise the crop. Though organic agriculture is traditionally linked with low yield , the productivity levels of these areas are encouraging under rainfed condition . This is despite the area having the problem of water scarcity. The farmers of these area , changing varieties every year , it is because of non availability of seed of suitable variety for these areas. The important recommended varieties for these areas are Jawahar Tapti, JK-5 and sarvottam in G arboreum category and Khandwa-2 and JK-4 in G hirsutum category. Despite several factors in favour of organic cultuvation in the state, the organic cotton production is still negligible in the national pool. This is mainly because of

1. Lack of awareness among the farmers for organic cultivation.
2. Conventional farmers have psychological make up that organic production is risky. This is despite un-intentionally their growing cotton nearly organically.
3. Organic cotton production is subject to strict test of regulations in order to be certified by the certifying agencies
4. Farmers have no exposure for the requisite procedures and norms adopted in organic agriculture.

5. Organic market itself is in fluid state with consumers having little knowledge on organic values while making purchase decisions.
6. Lack of clear cut wok chart, on what criteria and above all also will render a green / organic certificate. This uncertainty is the major tripping stone.

Certification problems

Organic produced cotton cannot be sold as "green" without certification. The present certification mode is very subjective, based on various inspections of the production material and methods of the registered farmers. Obviously, these standards vary from one agency to the other and need to be standardized. At present organic cotton cultivation proceeds according to the standards of International Federation of Organic agricultural Movement (IFOAM). In this context Govt of M.P. also involved in the promotion of organic cotton production and can also play a pivotal role in making the standards more applicable under local condition. They can also be vested with responsibilities to certify the produce. However, clear-cut standards have to be worked out for this purpose.

Suggestions for increasing organic cotton production

To tap the vast potential for organic cotton production, concerted research and development efforts are needed. The *G. arboreum* varieties under cultivation need to popularized among the farmers like Jawahar Tapti and JK-5 having high yield potential with inbuilt resistance mechanism against sucking pests. Still there need to identify ideal plant type suitable for organic agriculture couple with improvement in biotic tolerance and yield sustaining management practices. Though little input like fertilizer are applied, yet one should remain prepared for low yield under such system. Strong will is desired as today consumers may not be prepared to pay extra for eco-friendly products but in future all corporate decisions are bound to be based on environmental values, both producers and consumers are needed to be educated on the subject. State has good setup of agriculture department and they can expect to play a pivotal role in this endeavors. Clear-cut measures as to who will certify on what criteria's, how much would be the demand and of course, who would be the buyer at what premium are some of the bottlenecks and need to be developed. Once that is finalized, it will be possible to increase production of organic cotton in a well-organized manner.

An innovative effort to grow organic cotton is also under way in Madhya Pradesh. Since 1992, more than 1100 farmers from 77 villages (Anonymous 2001) in Khargone and Badwani districts of the state have been involved in a project called Maikaal Bio Re produce organic cotton. The area of cultivation extended to about 3265 hectares and the total production is estimated at about 3226 metric tons (Shastry and Koutu 2002). The project is in collaboration with Maikaal Fibres Limited (India) and Remai A.G., Switzerland. The state government of Madhya Pradesh also promotes the organic cotton cultivation by providing technical know how.

Quality control of organic manures

The awareness of the organic farming has increased with the consumer more concerned with the quality of the produce. Organic products also fetch a better price because of, this there is a great demand of organic manures. A wide variety of bio degradable wastes are used in the preparation of composts, resulting in wide variations in nutrient content and quality of the final product. There is at present no methodology to monitor the quality of compost and no standards have been developed in respect of quality control at different level. Thus a number of spurious or sub standards products are being marketed, which is a major limitation on the efficient use of compost in organic agriculture.

Maturity of the compost is an important component of quality. Immature compost have adverse effects on crop like cotton. The toxic elements derived from the raw material can be harmful to plants. This is especially true in the case of raw materials from municipal wastes. They can have high level of heavy metals like As, Cd, Pb etc. Phytotoxic compounds such as volatile acids are produced when wastes are stored under anaerobic condition.

Regulation for quality control

This is important in commercial production to regulate the quality produce. Government control over commercial production is required.

Monitoring of quality control

The guidelines for quality control have no meaning unless there is strict monitoring. For the monitoring to be effective, some Govt. body has to be involved in periodic sampling analysis from production sites and marketing point in huge quantity. Some accredited laboratories have also to be identified in the analysis of organic manures for identified parameters.

Commercial organic manure bags should also carry clear and informative labels. Good labeled bags enables the consumers to compare different brands in terms of nutrients and other quality parameters. This would eliminate substandard products from the market.

Yield loss in organic cotton

Yield loss in organic cotton is inevitable. No comparative statistics is available to evaluate the loss. However ICAC has given the yield loss estimate in organic cotton cultivation in few countries.

Technology for organic cotton cultivation

Considering the low productivity and the intrinsic risk and uncertainties in the cotton production certain low input sustainable production technologies was evolved to augment and stabilize the productivity of cotton in the region. The component of organic production system includes farmyard manure/ vermin compost, green manures, bio fertilizers, bio pesticides and bio-control agents for pest management.

Maintenance of soil fertility and nutrient management for organic cotton production

Improvement and maintenance of the organic matter in the soil is an essential precondition to sustain reasonable levels of organic cotton production. This would also increase water holding capacity, reduce erosion, improve soil stricture, besides enhancing the supply of nutrients, particularly, NP and S. Hence a good organic matter management programme is needed for fertility management under organic production system. Rain fed cotton crop in Central India removes around 5.8 kg N. 2.0 kg P and 6.6 kg K per 100 kg seed cotton produced (Pundarikakshudu 1985). With many such alternate uses of FYM, in such a huge quantities will required to meet the crops nutrient requirement which is generally not available. Hence, combination of sources with different biological properties must be resorted. The following components were standardized for integrated nutrient management under organic cotton production.

1. Farm Yard Manure: FYM @ 5 tones/ha with 0.6-0.8% N., 0.2-0.25% P with C:N ratio of 26-28% spread uniformly and harrowed 15 days prior to sowing.
2. Use of Dhaincha (Sesbania) as green manures: It is grown as green manure crop between two rows of cotton and turned down at 25 days after sowing. Its fast decomposing leaves provides N during the crucial early boll development period while the stalk acts as temporary mulch.
3. Vermicompost: It offers good scope for recycling of farm wastes. This is to be applied @ 1- 1.5 tones / ha . It contains several divers microflora and enzymes that add in good plant growth.
4. Use of Bio fertilizers: Continuous use of chemical fertilizers has resulted in soil degradation, multiple nutrient deficiencies and air and water pollution. To sustain soil health and high crop productivity, judicious use of different sources of nutrients such as chemical, organic and biofertilizers is essential. In this context bio-fertilizers assume importance as a component for organic cotton cultivation. Bio fertilizers are cheap and eco friendly and can supplement other sources of nutrients to an extent of 25-30 per cent.

Azospirillum

Azospirillum, a microaerophyllic bacterium, is known to enter into associative symbiosis with xylem vessels of plant roots. Besides its ability to fix elemental nitrogen. Azospirillum is also known to secrete growth promoting

Organic cotton production per hectare (1993)

S.No.	Country	Total organic production (tons)	Organic yield (kg/ha)	Conventional yield (kg/ha)	% Yield loss in organic production
1.	Argentina	1.8	290	451	-36
2.	India	124.6	181	280	-65
3.	Turkey	15.3	627	1009	-38
4.	USA				
	a. Arizona	1338.6	1076	1366	-21
	b. California	3363.5	1076	1509	-29
	c. Tennessee	150.7	538	504	+7
	d. Texas	655.2	538	544	-1
	e. Virginia	1.1	544	709	-23

substances like GA and IAA, which enhance root proliferation and growth of crop plants. They are known to add 25-40 Kg N/ ha /year.Azospirillum are effective both for seed treatment @ 50 kg/hectare and soil application @ 2 kg/ ac. Once the practice gets stabilized, farmers would be advised to increase its usages and reduce the usages of chemical fertilizers.

Phosphobacteria

Phosphobacteria, particularly those belonging to genera Bacillus and Pseudomonas, possess the ability to solubilise insoluble inorganic phosphorus and make it available to plants. It has been estimated that about 30 kg/ha/annum is solubilised by their application. Phosphobacteria is very much important component of organic cultivation in M.P. and state Govt. is providing it subsidized rate. Soil application @ 2 kg/ha along with Azospirillum (2kg/ha) is recommended

Use of drought and insect tolerant varieties

Use of drought and insect tolerant traditional varieties helps in sustainable cultivation of organic cotton production. Among the cultivated species of *cotton G. arboreum* varieties namely J. Tapti and Sarvottam are also found suitable for organic farming.

Insect management system

Unfortunately, in the last four decades, much emphasis has been given to chemical control at the expense of other alternative methods of control. The emergence of insect resistance to insecticides becomes a real problem which makes more people to appreciate the need for other alternatives. Perceiving the urgent need to give increased attention on this aspect it is essential to develop separate package of practices for eco friendly insect management.

a. Mono cropping of cotton is found to be conducive for pest development than mixed cropping i.e. cotton-soybean or cotton-groundnut. These systems also help to increase the abundance of predators and parasites in the cotton system.
b. The very late seeded crop harbored high population of leafhopper. Similarly higher dose of nitrogen and late sown crop invite the invasion of jassid attack.
c. Removal of weed plants that acts as alternate hosts for the pests is of almost importance.
d. Removal and destruction of crop residues, helps in reducing diapausing leaves of pink bollworms.
e. Botanical: Neem seed kernal extract and neem oil having an antifeedant action was found to be very affective against heliothis and whitefly.
f. Management of insects through natural enemies: In the eggs and larva parasites like Trichogramma sps helps in reducing spotted , pink and heliothis bollworms during early reproductive phase of the crop. Among the different predators, lady bird beetle and Coccinella sps were found to regulate the population of bollworms in the cotton ecosystem to varied degree.
g. Predatory birds also controlled the field population of heliothis bollworms up to 84%.
h. Application of NPV @ 250 LE/ha was found to be very effective in reducing the population of heliothis.
i. The application of *Bacillus thuringiensis* was also proved to be effective against heliothis.
j. g. Management of diseases through Biofungicides: Pathogens like Pythium, Fusarium, Rhizoctonia etc., cause diseases like damping off, root rot and wilt. Application of Trichoderma spp., a bio-control agent, has been found to be an effective alternative to manage root diseases. Seed treatment with trichoderma @ 50-100 g/kg of seed followed by soil application @ 2.5 kg with 100 kg of FYM, twice at 30 day interval has been recommended.
k. Traps: Pheromone and light traps were recommended. These traps helps in monitoring of adult population of pests like bollworm complex.
l. Topping: Topping is recommended not only to check the unnecessary vegetative growth but also to remove eggs laid by insects on top shoots thus aiding in their non-chemical control.
m. Removal of cotton stubble after harvest, without opting for a second (Ratoon) crop or prolong the crop growth, is recommended to break the cycle of pests.
n. Bird perches are recommended to attract birds which feed on caterpillars and helps in their natural control.

Experience about the production of organic cotton using variety Sarvottam

Progressive Cotton farmer Shri Om Prakesh Jain, Village Borasar Burhanpur MP was invited to share his experience of organic cotton cultivation during the Workshop on Eco-friendly Cotton, Organized by Indian Society for Cotton Improvement, Mumbai on 28th October (1998) . He narrated that in the month of May, 1995-96 a

programme was held in our village by the M.P. state Agriculture Dept. in which they expressed their own experience regarding organic cotton [Sarvottam] cultivation under the guidance of Agriculture Officers. After this I decided to grow this cotton. Four kg seed was received from Khandwa research center. SADO Burhanpur provided details regarding organic fertilizers, culture and neem oil.

Firstly I prepared my one acre land with the help of tractor deeply. Two trolleys of "Nadep Khad" were also used in this land which was prepared specially by me.

Before sowing, the seed was treated with Thiaram 3 g/acre and germination was 90-95 percent.

Square spacing of 45 x 45 cm was adopted. 15-20 days after germination, I used the method of inter cultivation in these plants. Besides this, 3 kg PSB + 1.5 kg Azotobacter culture was given according to the advice of agriculture Dept. Inter cultivation and other agricultural system which was useful for my crop was done by me.

Agriculture Dept. officials visited my field many times and gave me appropriate guidance. When crop was 60 to 90 days old, I used Neem oil 5 g/ l for spray. After rainy season in the end of the month of October, the plants were irrigated by me [two times]. In this way, I received 6.50 quintal cotton per acre on the basis of organic cultivation. The market value of cotton was 11250/- while expenditure was Rs. 3250/- and the profit was Rs. 8000/ - per acre.

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Identification of chilli varieties through total soluble seed proteins

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Abstract

Varietal development and its identification is one of the most important aspects of seed industry and seed trade. Due to continuous breeding programme by using elite lines, it has become difficult to identify and characterize these varieties on the basis of morphological characters alone. It has led to the exploration of new stable characters including genetic makeup to be used as markers for varietal identification. The present study includes the identification of different varieties (24) of chilli on the basis of their protein profile. Protein was extracted from sprouted seeds and subjected to Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Based on the resultant banding pattern and staining intensities all the varieties could be identified.

Keywords: Varietal identification, chilli, seed proteins, SDS-PAGE

Varietal development and its identification is one of the most important aspects of seed industry and seed trade. Several kinds of distinguishing traits are used to discriminate among families, genera, species, races, and individuals in populations. The usefulness of the classification procedure depends to a large extent on the number and kind of markers used. Until recently, earliness, distinctness, uniformity and stability (DUS) of any cultivar have relied on morphological methods, which are subjective and which may be influenced by environmental conditions (Goodrich et al. 1985). However the morphological makers were not quite enough to explore the genetic diversity between the morphologically overlapping cultivars and the morphologically identical accessions. Therefore, the need for new tool was disparate. The advent of electrophoresis as an analytical tool provide an indirect method for genome probing by exposing structural variations in enzymes or other proteins in the genome. Electrophoretic makers appear to be due to neutral genes, which are not linked to any loci that affect the cultivar and value. They are also independent of cultivar morphology and physiology, and offer significant advantages over morphological methods of variety and/or species identification in that they are rapid, relatively cheap, eliminate the need to grow plants to maturity and are largely unaffected by the growth environment.

Many workers have attempted to characterize crop plants by electrophoretic analysis of seed protein (Drzewiecki, 1990 in pea, Chakraborti et al. 1992, in tomato. Mudzana et al. 1995, in faba bean, Bonfitto et al. 1999, in melon, Mennella et al. 1999, in brinjal, Lucchese et al. 1999, in pepper, Wang et al. 2000, in tomato, Ahokas 2002, in barley, oat, wheat, peas and turnip, Yan-Min et al. 2003, in maize, capsicum and rice, Rahman et al. 2004, in *Brassica rapa*, Rani and Rathore 2006, in *Brassica juncea* L.) and have proposed the technique to be quick, reliable and relatively inexpensive laboratory method for varietal identification.

Most of the commercially grown chilli lack homogeneity in characteristics such as fruit shape, color and size. Further, the presence of large proportion of off-type individuals in seed lots results in negative effects on yield, uniformity and quality of the marketable produce. With the additional number of cultivars, due to continuous breeding programme and the corresponding increase in seed lots, there is a great probability of contamination and loss of identity of cultivar during seed multiplication, harvesting, processing, storage and distribution. Thus, the varietal characterization and identification would benefit the plant breeder, the seed producer, seed certification and testing agencies, seed merchants and farmers.

The aim of the present study was to evaluate the resolving power of total soluble seed proteins separated by SDS-PAGE for reliable identification and characterization of 24 cultivars of chilli.

Material and methods

Twenty-four public and private bred cultivars of chilli (Table 1) were used for characterization based on protein profiles.

Electrophoretic technique of total soluble seed proteins

SDS-PAGE of total soluble seed proteins was carried out by using 12 per cent gels according to the methods prescribed by Laemeli (1970) with slight modifications. Five seeds were grounded to fine powder to which 200µl Tris HCl extraction buffer (25 mM, pH 8.8) was added. The mixture was agitated thoroughly and kept for over night at 8°C for protein extraction. Then the mixture was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected. This protein extract was dissolved in an equal volume of working buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10 % glycerol, 0.025 % bromophenol blue) and incubated at 60-700 C for 10 minutes, cooled immediately for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. The supernatant was used for loading on to the gel. A current of 1.5 mA per well with a voltage of 80 V was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well and voltage up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. The gel then was stained using coomaasie brilliant blue solution overnight and destained using a mixture of 227 ml of methanol, 46 ml of acetic acid and 227 ml of distilled water until the bands were clearly visible. The electrophoretic run of 24 chilli cultivars was carried out in two groups (two gels) under same set of conditions. Group I consisted of cultivars 1 to 12 and Group II consisted of cultivars 13-24.

Results and Discussion

A wide variation was observed in the pattern of protein bands of studied cultivars. The cultivars differed in the number of bands, their relative mobility and intensity. The proteins separated on 12 per cent acrylamide gel could be distinguished and grouped based on the standard marker (97.4 KD). Entire protein banding profile was divided into seven regions (A to G) based on its decreasing molecular weight by comparing with standard protein marker. The seed protein weight of chilli ranged between 98 KD to 15 KD and relative mobility ranged between 0.030 to 0.813.

By using SDS-PAGE, the total soluble seed protein could be fractionated into 19 bands, which showed heterogeneity for number of bands among different cultivars. Maximum number of bands (19) was observed in cultivars TMR-23, X-235, Pusa Sadabahar, Shivani and Assam Local-1 whereas, paprika exhibited least number of bands (11). Although, not all the cultivars could be characterized based on number of bands alone, they could be differentiated clearly by their banding intensity and relative mobility. Each cultivar had its unique profile, which was different from other cultivars. Even though mobility was different between two groups (1-12 cultivars (group-1) and 13-24 cultivars (group-2), number of bands remained the same (19 bands in each group). Hence, profiles of all the cultivars were compared in the present study (Table 2, 3a & 3b and Fig. 1).

In case of 1-12 cultivars, nine bands were polymorphic (2, 7, 9, 11, 12,13,14,16 and 17) and ten bands were monomorphic (1, 3, 4, 5, 6, 8,10,15,18 and 19). While in case of 13-24 cultivars, eleven polymorphic (2, 4,5,6,7,10,11,14,15,16 and 17) and eight monomorphic (1,3,8,9,12,13,18 and 19) bands were obtained. In region A (> 97.4 KD; Phosphorylase b), five (band number 1, 2, 3, 4 &5) and six bands (band number 1, 2, 3, 4, 5 & 6) were observed in group-1 and group-2 varieties respectively. In group-1 cultivars, CO-2 and CO-3 can be identified by their conspicuous absence of second band (Rm: 0.050) and hence can be used for identification of these two cultivars while in Group-2 cultivars, Chikkaballapur was distinct from others by the absence of and second (Rm: 0.041) and fourth band (Rm: 0.069),

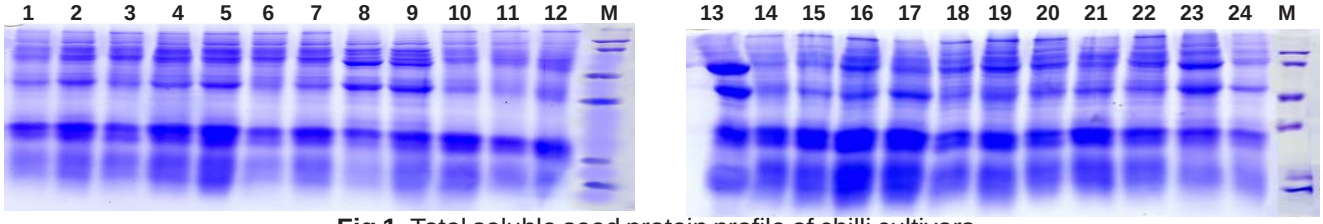

Fig 1. Total soluble seed protein profile of chilli cultivars

1. Arka Abhir 2. Arka Lohit 3. Arka Suphal 4. JCA 283 5. Khasi Anmol 6. LCA 206 7. CO-1 8. CO-2 9. CO-3 10. By. Dabbi 11. By. Kaddi 12. Dyavanur 13. Chikkaballapur 14. G 4 15. Phule Jyothi 16. Samrudhi 17. TMR 23 18. X 235 19. Pusa Sadabhar 20. Shivani 21. Assam local -1 22. Assam Local-2 23. Aparna 24. Paprika
M: Marker

while paprika was distinct by the absence of fifth (Rm:0.090) and sixth (Rm:0.117) bands.

In region B (66.0 to 97.4 KD; Bovine serum albumin), three bands (band no. 6, 7 and 8) were observed in group-1 cultivars, while two bands (band no. 7 and 8) were noticed in group-2 cultivars. In case of group-1 cultivars, fifth band was missing in cultivars CO-2 and CO-3 (Rm: 0.128), while in group -2 cultivars Paprika cultivar could be identified by the absence of seventh band (Rm: 0.134). For this region, Arka Lohit, Arka Suphal, JCA-283, Khasi Anmol, LCA-206 and CO-1 cultivars exhibited similar banding pattern and also similar banding pattern were observed with Bydagi Dabbi and Dyavanur cultivars.

In region C (43.0 to 66.0 KD; Ovalbumin), three bands (band no. 9, 10, 11, 12 and 13) were observed in

Table 1. List of cultivars used for the study

Cultivar	Source	Cultivar	Source
Arka Abhir	IIHR, Bangalore	KDC-1	DFU, Bangalore
Arka Lohit	IIHR, Bangalore	HMT-1	DFU, Bangalore
Arka Suphal	IIHR, Bangalore	PBC-613	DFU, Bangalore
JCA-283	IIHR, Bangalore	Phule Jyothi	DFU, Bangalore
Khasi Anmol	IIHR, Bangalore	Samrudhi	DFU, Bangalore
LCA-206	IIHR, Bangalore	Tejaswini	DFU, Bangalore
CO-1	IIHR, Bangalore	TMR-23	United Genetics, Bangalore
CO-2	IIHR, Bangalore	X-235	NSC, Bangalore
CO-3	IIHR, Bangalore	Pusa Sadabhar	NSC, Bangalore
AR-75	DFU, Bangalore	Shivani	East West Company, Bangalore
Byadagi Dabbi	DFU, Bangalore	Assam Local-1	Local variety, Assam
Byadagi Kaddi	DFU, Bangalore	Assam Local-2	Local variety, Assam
Chikkaballapur	DFU, Bangalore	Aparna	Lam station
Dyavanur	DFU, Bangalore	Paprika	Lam station
G-4	DFU, Bangalore		

DFU: Dry Farming Unit

Table 2. Number of total soluble seed protein bands observed in different chilli cultivars

Cultivars	Low intensity	Medium intensity	High intensity	Total
Arka Abhir	12	5	1	18
Arka Lohit	1	11	6	18
Arka Suphal	1	10	6	17
JCA-283	1	10	7	18
Khasi Anmol	0	10	8	18
LCA-206	4	9	4	17
CO-1	3	9	6	18
CO-2	3	8	5	16
CO-3	5	5	7	17
Byadagi Dabbi	5	6	3	14
Byadagi Kaddi	5	5	2	12
Dyavanur	5	6	3	14
Chikkaballapur	5	7	3	15
G-4	8	7	1	15
Phule Jyothi	6	6	2	17
Samrudhi	1	9	6	18
TMR-23	5	11	4	19
X-235	4	10	3	19
Pusa Sadabhar	1	12	6	19
Shivani	1	13	5	19
Assam Local -1	5	7	5	17
Assam Local -2	1	12	6	19
Aparna	2	11	5	18
Paprika	5	3	3	11

Table 3a. Intensity and relative mobility of total soluble seed proteins of 1-12 chilli cultivars

Region	Band No.	Rm value	1	2	3	4	5	6	7	8	9	10	11	12
A	1	0.034	+	++	++	++	+++	++	+++	+	+	++	++	++
	2	0.050	+	++	+++	+++	+++	+++	+++	-	-	+++	+++	+++
	3	0.072	+	+	++	++	++	+	+	+	+	+	+	+
	4	0.084	+	++	++	++	++	++	++	++	++	+	+	+
	5	0.094	+	++	++	+	++	++	++	++	++	+	+	+
B	6	0.113	++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
	7	0.128	+	++	++	++	++	++	++	-	-	++	+	++
	8	0.147	++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
C	9	0.169	-	-	-	-	-	-	-	+++	+++	-	-	-
	10	0.203	++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
	11	0.225	+	++	++	++	++	++	++	++	++	-	-	+
	12	0.250	+	++	++	++	++	++	++	-	+	+	-	-
	13	0.263	+	++	++	++	++	++	++	++	+	+	-	-
D	14	0.281	+	++	++	++	++	++	++	++	+	-	-	+
	15	0.309	++	+++	++	+++	+++	+	++	+++	+++	++	+	++
E	16	0.369	+	+	+	++	++	+	+	++	++	-	-	-
	17	0.488	+	-	-	++	++	-	+	++	++	-	-	-
	18	0.588	+++	+++	+++	+++	+++	++	+++	++	+++	+++	+++	+++
F	19	0.788	++	++	++	+++	+++	+	++	+	+++	+++	++	+++

Note: - Absent + Low intensity ++ Medium intensity +++ High intensity

1	Arka Abhir	4	JCA-283	7	CO-1	10	Byadagi Dabbi
2	Arka Lohit	5	Khasi Anmol	8	CO-2	11	Byadagi Kaddi
3	Arka Suphal	6	LCA-206	9	CO-3	12	Dyavanur

Table 3b. Intensity and relative mobility of total soluble seed proteins of 13-24 chilli cultivars

Region	Band No.	Rm value	13	14	15	16	17	18	19	20	21	22	23	24
A	1	0.034	+	++	++	++	++	++	++	++	+	++	++	+
	2	0.041	-	++	+++	+++	+	+++	+++	+++	+	+++	+	+
	3	0.055	+	+	+	++	+	+	++	++	+	++	++	+
	4	0.069	-	+	+	++	+	+	++	++	+	++	++	++
	5	0.090	+	+	+	++	+	++	++	++	++	++	++	-
	6	0.117	+	+	+	+++	++	++	+++	+++	-	+++	+++	-
B	7	0.134	++	++	++	++	++	++	++	++	++	++	++	-
	8	0.159	+	+	+	+++	++	++	+++	+++	+++	+++	+++	++
C	9	0.200	+++	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	10	0.234	-	+	+	++	++	++	++	++	++	++	++	-
	11	0.248	++	+	-	++	++	++	++	++	-	++	-	-
	12	0.262	++	++	++	++	++	++	++	++	++	++	++	+
D	13	0.303	+++	++	++	+++	+++	++	+++	++	+++	+++	+++	+++
	14	0.331	++	-	++	++	++	++	++	++	++	++	++	-
	15	0.345	++	-	++	++	++	++	++	++	++	++	++	-
	16	0.372	-	-	-	+	+	+	+	+	+	+	+	-
	17	0.400	++	-	++	++	++	+	++	++	++	++	++	+
	18	0.497	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
E	19	0.717	++	++	++	+++	+++	++	++	++	+++	++	++	++

Note: - Absent + Low intensity ++ Medium intensity +++ High intensity

13	Chikkaballapur	16	Samrudhi	19	Pusa Sadabhar	22	Assam Local -2
14	G-4	17	TMR-23	20	Shivani	23	Aparna
15	Phule Jyothi	18	X-235	21	Assam Local -1	24	Paprika

Table 3b. Intensity and relative mobility of total soluble seed proteins of 13-24 chilli cultivars

No.			Region					Band						
								Rm						
value	13	14	15	16	17	18	19	20	21	22	23	24		
A	1	0.034	+	++	++	++	++	++	++	++	+	++	++	+
	2	0.041	-	++	+++	+++	+	+++	+++	+++	+	+++	+	+
	3	0.055	+	+	+	++	+	+	++	++	+	++	++	+
	4	0.069	-	+	+	++	+	+	++	++	+	++	++	++
	5	0.090	+	+	+	++	+	++	++	++	++	++	++	-
	6	0.117	+	+	+	+++	++	++	+++	+++	-	+++	+++	-
B	7	0.134	++	++	++	++	++	++	++	++	++	++	++	-
	8	0.159	+	+	+	+++	++	++	+++	+++	+++	+++	+++	++
C	9	0.200	+++	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	10	0.234	-	+	+	++	++	++	++	++	++	++	++	-
	11	0.248	++	+	-	++	++	++	++	++	-	++	-	-
	12	0.262	++	++	++	++	++	++	++	++	++	++	++	+
D	13	0.303	+++	++	++	+++	+++	++	+++	++	+++	+++	+++	+++
	14	0.331	++	-	++	++	++	++	++	++	++	++	++	-
	15	0.345	++	-	++	++	++	++	++	++	++	++	++	-
	16	0.372	-	-	-	+	+	+	+	+	+	+	+	-
	17	0.400	++	-	++	++	++	+	++	++	++	++	++	+
	18	0.497	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
E	19	0.717	++	++	++	+++	+++	++	++	++	+++	++	++	++

group-1 varieties while four bands (band 9, 10, 11 and 12) were noticed in group-2 varieties. In case of group-1 varieties, band-9 (Rm: 0.169) was present only in varieties, CO-2 and CO-3, band-11 (Rm: 0.225) was absent in varieties, Byadgi Dabbi and Byadgi Kaddi, Band-13 (Rm: 0.260) was absent in varieties Byadgi Kaddi and Dyavanur. Hence, cultivars CO-2 and CO-3, Byadgi Dabbi and Byadgi Kaddi and Byadgi Kaddi and Dyavanur can be identified using this region. In group-2 cultivars for this region, Band-10 (Rm: 0.234) was absent in Chikaballpur and Paprika varieties and band-11 (Rm: 0.248) was absent in Phule Jyothi, Assam local -1, Aparna and Paprika. Hence, band 10 and band 11 could be used for the identification of these cultivars.

In region D (29.0 to 43.0 KD; Carbonic anhydrase), only two bands (band no. 14 and 15) were observed in group-1 varieties while five bands (band no.13, 14, 15, 16 and 17) were noticed in group-2 varieties. In group-1 cultivars, band-14 (Rm: 0.281) was absent in Byadgi Dabbi and Byadgi Kaddi cultivars and hence can be used for its identification. In group-2 cultivars, no band was observed for cultivar G-4 at 14 (Rm: 0.331), 15 (Rm: 0.345), 16 (Rm: 0.372) and 17th (Rm: 0.400) band, while band number 14, 15 and 16 was absent in Paprika cultivar. 16 th band was absent in cultivars Chikaballpur and Phule Jyothi.

In region E (20.0 to 29.0 KD; Soybean Trypsin Inhibitor), three bands (band no. 16, 17 and 18) were observed in group-1 varieties while only two bands (band no.18 and 19) was noticed in group-2 varieties. In case of group-1 cultivars, Band-16 (Rm: 0.369) and Band-17 (Rm: 0.488) was absent in Byadgi Dabbi, Byadgi Kaddi and Dyavanur, while Band-17 (Rm: 0.488) was absent in Arka lohit, Arka Suphal and LCA-206. In group-2 cultivars both the bands observed were monomorphic and hence are of little use for characterization purpose.

In region F (14.3 to 29.0 KD; Lysozyme), only one band was observed in both the group of cultivars which was monomorphic, while in region G (< 14.3 KD) no bands could be observed in both the groups of cultivars.

In conclusion, electrophoretic analysis of seed protein was able to characterize and identify all the twenty-four cultivars of chilli. Hence, this technique could be used as an effective means for analyzing seed proteins and subsequently identifying cultivars. As this technique is relatively easy to perform and do not require sophisticated laboratory facilities, it is attractive both for routine applications such as in seed testing and quality control and for research.

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Utilization of RAPD markers for identification of chilli cultivars

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Abstract

New chilli cultivars are continually being developed and released making cultivar identification and testing of this crop increasingly more difficult and complex. Recent studies have indicated that polymerase chain reaction technologies such as the generation of Random Amplified Polymorphic DNA (RAPD) markers have greater utility in cultivar identification at the seed level. After preliminary screening of 40 primers, this study demonstrated that ten primers generated highly reproducible polymorphic RAPD fragments differentiating 24 chilli cultivars. The total number of polymorphic bands was 62 out of 86 bands. Among the primers used in this study, OPAC-07 produced distinct band for most cultivars followed by OPAC-02 and OPAC-04. Therefore combination of these primers can be used to identify all the 24 chilli cultivars in the course of seed genetic purity testing. Hence, RAPD markers were found to be more useful than protein and morphological markers.

Chilli (*Capsicum annuum* L.) is one of the major commercial crops of the world. Most commercially grown Chilli cultivars are open pollinated and lack of homogeneity in characteristics such as fruit color, shape and size is often noticed. Further, the presence of large proportions of off type individuals in seed lots results in negative effects on yield, uniformity and quality of the marketable product.

Traditionally, varietals identification have been carried out by grow out test, which is based on the evaluation of morphological or physiological traits expressed by seed, seedlings or mature plants, which are often inaccurate because environmental stress conditions during seed or seedling/plant development can mask the expression of specific morphological or physiological traits. Besides, high cost, low polymorphism among the closely related varieties which are bred by using elite lines and prolonged time requirement have been the major limitations of morphological comparisons.

New techniques based on DNA profiling provide novel approaches to varietals identification and offers many advantages over traditional morphological comparisons. These advantages include potential resolving power, objective analysis of data, testing at all stages of development and cost effectiveness. Among these, random amplified polymorphic DNA (RAPD) markers have been successfully used for genetic finger printing in many vegetable crops including chilli (Votava et al. 2002) and this can be a powerful tool for analyzing the distinctness between closely related chilli varieties. The present investigation was carried out to characterize chilli cultivars using RAPD markers.

Material and methods

Freshly harvested seeds of 24 elite chilli genotypes of different origin, including released genotypes, were obtained from the AICRP on chilli, UAS, Bangalore. Thirty plants of each of chilli accession were grown in the greenhouse until 2-3 leaf stage for DNA isolation. Genomic DNA was isolated as per the modified CTAB (Cetyl Trimethyl Ammonium Bromide) method of Cao and Oard (1997). Finally, the extracted DNA pellet was dissolved in 50 µl of TE buffer and stored at -200C for further use.

PCR reaction

The RAPD reaction mixture consisted of 1.0 µl of template DNA, 2.0 µl primer, 2.0 µl dNTPs, 2.0 µl Taq, 2.0 µl of 1x PCR buffer (10 M Tris pH 8.0, 50 mM KCl, 1.8 mM MgCl2 and 0.01 mg/ml gelatin) and 12.6 µl of sterile water in a volume of 20µl. The lists of primers used in the study are given below (Table.1). PCR was carried out to screen 60 random primers of arbitrary sequence (Operon Technologies Inc.) to select primer that can amplify informative RAPD fragments. Of the 40 primers screened (OPAC series), 10 primers produced band with pooled DNA of all the varieties. These primers were used for PCR amplification and identification of cultivars.

Amplification was carried out on a MJ Research PTC 200 Thermal Cycler. The amplification profile was initial denaturizing temperature 94°C for 4 minutes, denaturizing 94°C for 1 minute, primer annealing 36°C for 1 minute, primer extension 72°C for 2 minutes; later three stages were repeated 35 times, complete primer extension 72°C for 5 minutes and soak temperature 4°C until PCR plate is removed.

Electrophoresis

The amplification products of 10 µl PCR reaction were separated on 1.5 per cent agarose gel together with DNA standard. PCR products were visualized under UV light and the DNA banding pattern was recorded directly using Polaroid camera. Polymorphisms at all loci were confirmed by two repeating tests at different times.

Results and Discussion

Analysis of RAPD profiles

The bands of DNA fragments were scored as present (1) or absent (0). The amplification profiles for all primers were compared with each other and unique bands and characteristic profiles were identified using 0, 1 matrices.

Table 1. Random decamer primers used for characterization of chilli cultivars

Primers	Nucleotide sequence
OPAC-01	5'TCCCAGCAGA3'
OPAC-02	5'GTCGTCGTCT3'
OPAC-04	5'ACGGGACCTG3'
OPAC-05	5'GTTAGTGCGG3'
OPAC-07	5'GTGGCCGATG3'
OPAC-09	5'AGAGCGTACC3'
OPAC-11	5'CCTGGGTCAG3'
OPAC-12	5'GGCGAGTGTG3'
OPAC-17	5'CCTGGAGCTT3'
OPAC-20	5'ACGGAAGTGG3'

RAPD polymorphism

The highest numbers of bands (13) were recorded with primer OPAC-01 while the least (05) was obtained with primer OPAC-11 and OPAC-17 (Table 3 and Fig. 1). The number of bands using the same primer is not always identical among the cultivars but a few primers shared the same behavior.

Reports have found that a change of one base pair in the target sequence of the genome might result in completely different RAPD profile (Williams et al. 1992). Since each 10 bp oligonucleotide primer only covers a very limited part of the genome, important differences located on non-amplified region could be missed. In the event of similar profiles obtained from two different cultivars using a particular primer could lead to a false conclusion that the two cultivars are same. Thus it is important use a series of primers for any sample to be tested.

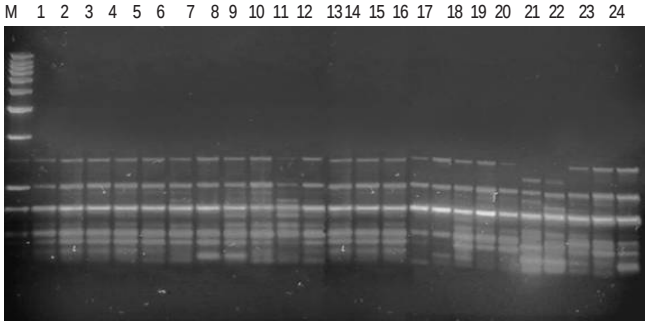

Fig. 1. DNA profiles of chilli cultivars amplified by primer OPAC 05

1. Arka Abhir
2. Arka Lohit
3. Arka Suphal
4. JCA -283
5. Khasi Anmol
6. LCA -206
7. CO-1
8. CO-2
9. CO-3
10. By. Dabbi
11. By. Kaddi
12. Dyavanur
13. Chikkaballapur
14. G -4
15. Phule Jyothi
16. Samrudhi
17. TMR -23
18. X -235
19. Pusa Sadabhar
20. Shivani
21. Assam local -1
22. Assam Local-2
23. Aparna
24. Paprika

M: Marker

When all the ten primers were considered together, complete identification was obtained for twenty four cultivars. Some of these primers were more successful in cultivar identification such as OPAC-02, OPAC-05 and OPAC-07 as they generated high number of RAPD markers with higher polymorphism and consequently more cultivars were identified.

OPAC-1 primer produced 13 bands of which 83.33 percent of the bands were polymorphic. Cultivar Assam Local-2 showed unique band (Band-12) which can be considered as novel sequence for this cultivar. Some

Table 2. Summary of RAPD analysis for different chilli cultivars

Parameters	Numbers
Total number of band levels	86
Total number of polymorphic bands	62
Total number of primers used	10
Maximum number of bands observed	13
Minimum number of bands observed	5
Average number of bands per primer	8.60
Average number of polymorphic bands per primer	6.20

bands were absent in some cultivars at different levels. Band 7 was absent in G-4 and Samrudhi, Band 9 was absent in cultivars G-4 and Assam Local-2, band 11 in Arka Abhir, Arka Suphal and G-4. OPAC-02 primer produced eight bands of which seven were polymorphic accounting for 87.50 per cent polymorphism. The primer failed to amplify at band level 5 for cultivars Phule Jyothi and at band level 7 for cultivars Shivani and Assam Local-1. Polymorphism in this primer was more due to the absence of bands in some cultivars than due to the presence of unique bands for the cultivars.

OPAC-04 generated 85.71 per cent polymorphic bands and at band levels one two and five the primer did not produce bands for the cultivars Byadagi Dabbi and Pusa Sadabhar. OPAC-05 primer was able to distinguish most of the cultivars with a polymorphism of 83.33 per cent for the 12 bands it generated. LCA-206, Byadagi Dabbi and Assam Local-1 were could be identified using this primer as it produced unique band for these cultivars. Arka suphal cultivar and Shivani and Assam Local-1 cultivars did not produce band at band levels 5 and 1 respectively. OPAC-07 primer generated highest number of polymorphic bands (90.90 %) among the different primers used in the study. Only Assam Local-1 cultivar had the presence of band at band level 1 and absence of band at band level 11 for this primer and could be utilized for its identification using this primer. OPAC-20 primer generated unique bands for Arka suphal, JCA-283 and TMR-23 at band level 3 and band 7 was present only in CO-1 and Dyavanur cultivars. OPAC-09 primer produced unique bands for the cultivars CO-2, TMR-23 and paprika at band level 5. OPAC- 11 primer did not produce any band for cultivars chikkaballpur and Phule Jyothi at band level one and at band level 4 no bands were to be seen for cultivars Arka Lohit, Byadagi Kaddi and G-4. Primer OPAC-09 produced only 37.50 per cent polymorphic bands and was found to be the least among the different primers used for the study. However it produced a unique band for Assam Local-2 cultivars at band level 1. OPAC-11 primer did not produce any band at band level 1 for the cultivars Chikkaballapur and Phule Jyothi, while primer OPAC-12 produced unique band for cultivars Shivani at band level 8 and at band level 2 for cultivars Assam Local-2 and Aparna. OPAC-17 primer generated only 5 bands of which most of the bands were monomorphic.

Table 3. Selected primers and their level of polymorphism in different chilli cultivars

Primer	Total No. of bands	Total No. of polymorphic bands	Per cent polymorphism
OPAC-01	13	10	76.92
OPAC-02	8	7	87.50
OPAC-04	7	6	85.71
OPAC-05	12	10	83.33
OPAC-07	11	10	90.90
OPAC-09	8	3	37.50
OPAC-11	5	4	80.00
OPAC-12	9	5	55.55
OPAC-17	5	3	60.00
OPAC-20	8	4	50.00
Total	86	62	-
Mean	8.60	6.20	72.10

Many primers producing unique bands which appeared in more than one cultivars but not more than five cultivars and these could also be used as novel sequences to identify these cultivars. Among the primers

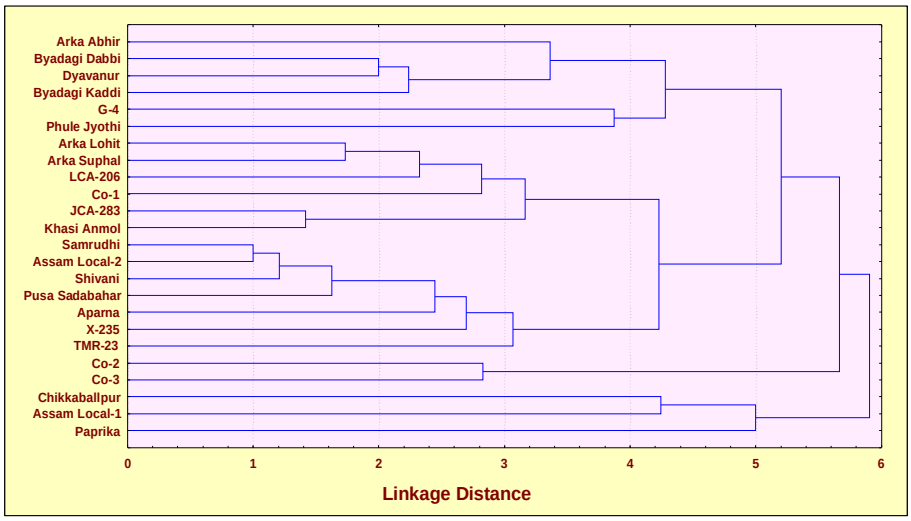

Fig. 2: Dendrogram showing genetic relationship among 24 cultivars of chilli for RAPD markers based on unweighted pair group mathematical average (UPGMA)

Table 4. Pairwise distance matrix of 24 chilli cultivars based on RAPD markers

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24
V1	0.00																							
V2	3.74	0.00																						
V3	3.46	3.16	0.00																					
V4	3.74	3.16	3.16	0.00																				
V5	4.24	3.46	3.16	3.46	0.00																			
V6	4.12	4.12	3.61	4.36	3.32	0.00																		
V7	3.74	4.69	4.00	4.47	4.24	3.87	0.00																	
V8	4.47	4.47	4.24	4.90	4.69	4.58	4.00	0.00																
V9	4.12	4.36	3.87	4.58	3.87	4.00	4.36	4.12	0.00															
V10	5.10	4.69	4.90	4.69	4.69	4.36	4.69	4.24	4.36	0.00														
V11	4.12	3.87	4.36	4.12	4.12	4.47	4.12	4.36	4.47	4.36	0.00													
V12	3.74	4.00	4.00	4.24	3.74	4.58	4.47	4.47	4.12	4.90	3.61	0.00												
V13	4.12	3.32	3.32	3.87	4.12	4.24	4.36	4.12	3.74	4.12	4.24	4.36	0.00											
V14	4.12	4.36	4.36	4.58	4.58	4.69	5.00	4.80	4.24	5.57	4.90	4.36	4.47	0.00										
V15	3.87	4.36	4.36	4.80	4.58	4.00	4.58	4.58	4.69	5.20	4.47	4.36	4.47	4.24	0.00									
V16	3.74	4.69	4.47	4.90	4.69	4.36	4.47	4.90	4.58	5.29	4.80	4.47	4.58	3.87	3.00	0.00								
V17	4.12	5.00	4.36	4.58	4.36	4.69	4.36	4.36	4.24	4.12	4.69	4.36	4.90	4.69	4.90	4.58	0.00							
V18	3.61	4.12	3.61	4.58	4.12	4.00	4.12	3.61	4.00	4.80	4.24	3.61	4.00	4.00	3.74	3.87	3.74	0.00						
V19	3.61	4.58	3.87	4.36	4.80	4.24	4.12	4.12	4.47	5.00	4.69	4.58	4.24	4.24	4.00	3.61	4.47	3.16	0.00					
V20	5.20	4.80	4.80	5.00	4.58	4.69	5.00	5.00	4.24	5.00	5.10	4.80	4.47	5.10	4.90	5.00	4.69	4.69	5.10	0.00				
V21	4.58	4.58	4.36	4.80	4.36	4.24	4.80	4.12	4.47	4.80	4.69	4.80	4.69	4.69	4.24	4.80	4.69	4.24	4.69	3.74	0.00			
V22	4.36	4.80	3.87	4.58	4.36	4.47	4.58	5.00	4.47	5.20	5.29	4.80	4.47	4.24	4.47	4.12	4.47	4.24	4.24	4.47	4.24	0.00		
V23	4.12	4.12	3.87	4.12	3.87	4.00	4.12	4.12	4.00	4.58	4.90	4.36	4.24	4.47	4.00	4.12	4.24	4.00	4.47	4.24	4.24	3.74	0.00	
V24	3.87	3.87	3.61	4.36	3.87	3.46	3.32	3.87	4.00	4.58	4.47	4.36	4.00	4.47	3.74	3.87	4.47	3.46	4.00	4.69	4.69	4.24	3.46	0.00

V1. Arka Abhir
V2. Arka Lohit
V3. Arka Suphal
V4. JCA-283
V5. Khasi Anmol
V6. LCA-206
V7. CO-1
V8. CO-2
V9. CO-3
V10. Byadagi Dabbi
V11. Byadagi Kaddi
V12.Chikkaballapur
V13. Dyavanur
V14. G-4
V15. Phule Jyothi
V16. Samrudhi
V17. TMR-23
V18. X-235
V19. Pusa Sadabhar
V20. Shivani
V21. Assam Local -1
V22. Assam Local-2
V23. Aparna
V24. Paprika

used in this study, OPAC-07 produced distinct band for most cultivars followed by OPAC-02 and OPAC-04. Therefore combination these primers can be used to identify all the 24 chilli cultivars in the course of seed genetic purity testing. However, further screening of primers is needed to look for the individual primer which can distinguish all the 24 cultivars.

The cluster analysis based on RAPD markers (Fig. 2 & Table 4) gave two major clusters, the first with 22 cultivars and the second with two cultivars, Shivani and Assam Local-1. The first major cluster was further divided into two subclusters. The first sub-cluster consisted of 20 cultivars while the second one had TMR-23 and Byadagi Dabbi clustered together. These two were also morphologically similar with respect to many of the plant and fruit characters (data recorded).

The first sub-cluster was again divided into two groups. The first group consisted of 11 cultivars while the second group consisted of 9 cultivars. The second group was further divided into two subgroups. The first subgroup consisted of seven cultivars, while the second subgroup consisted of two cultivars *viz*., Byadagi Kaddi and Dyavanur.

The first subgroup was divided into two sets, the first set consisting of cultivars Arka Lohit, arka suphal and JCA-283 being clustered together along with Chikkaballapur which was independent from these three cultivars. The second set consisted of khasi anmol and LCA-206 cultivars which were the only cultivars with neck at the base of their fruit. The first group that had 11 cultivars was further divided into two subgroups. The first subgroup had nine cultivars, while the second subgroup had two cultivars *viz*., Assam Local-2 and Aparna. The first subgroup was again divided into two sets. The first set with six cultivars which had Phule Jyothi and Samrudhi and Pusa Sadabhar and X-235 clustered together while Arka Abhir and G-4 were found to be independently clustered. The second set consisted of three cultivars *viz*., CO-1, Paprika and CO-2 which were also clustered together in morphological cluster also. All these three cultivars had bold fruits too (data recorded).

According to RAPD marker profile, the most closely related cultivars were Phule Jyothi and Samrudhi with a linkage distance of 3.00 followed by cultivars Arka Suphal and JCA-283, Arka Suphal and Khasi Anmol, X-235 and Pusa Sadabhar with linkage distances of 3.00. The most distant cultivars were Byadagi Dabbi and G-4 with a linkage distance of 5.57, followed by cultivars, Byadagi Dabbi and Samrudhi, Byadagi Kaddi and Assam Local-2 with linkage distance of 5.29.

In conclusion, all the chilli cultivars could be differentiated from each other based on RAPD markers, which makes these techniques useful for varietal finger printing. Further, these techniques can be applied for chilli varietal protection, pedigree analysis and seed purity detection.

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Association analysis among floral traits and yield in CIMMYT based CMS and its maintainers lines of wheat

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Abstract

Present experiment comprised of Ten CIMMYT based CMS (Cytoplasmic male sterile) and B (Maintainer) lines which were planted in 2:4 ratios in randomized completely block design with three replications to study the association analysis among floral traits and yield in wheat. Grain yield per plant showed positive significant correlation with number of tiller per plant in A line, seed setting percentage by stick pollination, stigma size in A line, anther size in B line, fertility in A lines, spike density and with days to heading in B lines. This indicates the importance of these characters in improving grain yield per plant in male sterile lines.

Key word: CIMMYT, Cytoplasmic male sterile, Maintainer lines, association analysis, seed setting percentage, stick pollination, stigma size, anther size

India achieved a record food production in the recent past. It may be considered a gradual transition from chromic shortage to self sufficiency in this important cereal crop. It has been possible through the better genetic understanding of this crop and consequent availability of right type of plant ideotype and sound production technology.

Wheat (*Tritium aestivum* L.) is an important staple food of millions. The area under wheat through out world and in India become nearly constant around 250 and 26 million ha respectively. A similar trend has been also observed in M.P. regarding area, production and productivity.

As per present population growth rate, population of India will be around 1.3 billion by 2025 AD assuming 25% more capita requirement of food grains due to better standard of living. Projected demand of 109 million tons by 2025 AD with productivity growth rate of 2.1% per year, India can able to harvest over 95 millions tones of wheat as per realized yield potential(Mishra et al 2007).There is no possibility in improvement of production and productivity through horizontal approach; it can be only possible through vertical approach. Thus, there is need to explore new innovative tools in order to break the yield barriers and make wheat cultivation more profitable as other cereals crops like rise and maize. In this constant, exploiting hybrid vigour at commercial level through development of hybrid wheat using cytoplasmic male sterility system will be a need to solve the yield barrier.

Association analysis between floral traits and yield is one of the important aspects which are to be studied for promoting cross pollination enhances the seed setting through out crossing. Therefore selection for floral traits viz. opening of flower, duration of flower, stigma size, stigma receptivity, pollen size, pollen viability, and synchronization of flowering, seed setting percentage by stick pollination, control pollination and seed vigour were the important tools in hybrid wheat development. Keeping in view the above facts the effort has been planned to study association analysis among floral traits and yield in CIMMYT based CMS and its maintainers lines.

Materials and methods

The present investigation was carried out during the rabi season of 2008-09 under Wheat Improvement Project, Department of Plant Breeding and Genetics, J. N.Krishi Vishwa Vidyalaya Jabalpur (M.P.).The experimental material consist of 10 A (Cytoplasmic male sterile) and B (Maintainer) lines. Both A and B lines were planted in 2:4 ratio in Randomized Completely Block Design with three replications. Each plot was accommodated in a four rows of 6 m length, with row to row distance of 23 cm. Every plot (2:4) treated as a block and there were 10 blocks in each replication .The distance between block to blocks was 1.5 m. The sowing was done on 25th November 2008 by dibbling of seeds in rows.The crop

Table 1. Experimental material

Block No.	CMS (A lines)	Maintainer (B lines)	Block No	CMS (A lines)	Maintainer (B lines)
1	JWH 1	JWB 1	6	JWH 16	JWB 16
2	JWH 5	JWB 5	7	JWH 17	JWB 17
3	JWH 8	JWB 8	8	JWH 20	JWB 20
4	JWH 10	JWB 10	9	JWH 23	JWB 23
5	JWH 14	JWB 14	10	JWH 4	JWB 4

JWH :(Jawahar Wheat Hybrid), JWB: (Jawahar Wheat Maintainer)

was raised under high fertility condition following recommended package of practices. Isolation provided to each block by fitting 2mt height of polythene around the block to protect the movement of undesirable pollen before anthesis. CMS lines were pollinated with respective B lines by control pollination (hand) as well as stick pollination by manual. After pollination with hand the spikes were covered with butter paper bags immediately to avoid the contamination. The pollination was made in between 9 to 11 am for better seed setting at the time of anthesis.

Observations were recorded on the basis of ten randomly selected plants of each CMS and maintainer lines in every replications for days to 50% flowering in A and B lines, days to maturity in A and B lines,Plant height in A and B lines (cm), nicking, flag leaf area (cm2),fertility in A lines (%), anther size in B lines (mm), stigma size in A lines (mm),spike length (cm), spiklets per spike, spike density, number of tillers per plant in A and B lines, seed setting percentage by control pollination, seed setting percentage by stick pollination and yield per plant in A lines (g).The data were subjected to find out the association analysis following method given by Miller et al *(*1958)

Result and Discussion

The analysis of variance for 21 different characters on floral biology, yield and its contributing traits showed that the mean square due to treatments were found significant for all the characters under study indicated the presence of sufficient variability in the material.

The values of phenotypic coefficients of variation are higher than those genotypic coefficients of variation for all the characters. The phenotypic and genotypic coefficient of variation where estimated to be high for grain yield per plant in A line, while it was moderate for nicking time, seed setting by stick pollination, fertility in A line and seed setting percentage by stick pollination. The remaining traits such as flag leaf area in A line, number of tillers per plant in B line, anther size in B line, number of tillers per plant in A line, stigma size in A line, seed setting percentage by control pollination, spike density, plant height in B line, plant height in A line , spike length in A line, days to heading in B line, days to heading in A line, spikelets per spike, days to maturity in B line and days to maturity in A line were exhibited lowest phenotypic and genotypic coefficient of variation. The estimates of phenotypic and genotypic coefficient of variation suggested that sufficient variation present in the material. Joppa et al. (1968) reported apparent genotypic differences for flowering among wheat cultivars as a result of deliberate selection by plant breeders. Phenotypic difference ; anther extrusion, anther size, stigma length and duration of floral opening where also observed by Chowdhary et al. (1994), Singh and Joshi (2003)and Singh et al. (2007). These findings are in agreement with present investigation.

Days to heading exhibited highly significant positive correlation with days to heading in A line, days to maturity in B line, nicking time, fertility percentage in A line and seed setting percentage by stick pollination. However, its associations were observed significant negative with anther size in B line, spike length and number of tiller per plant in B line. The above correlation with days to flowering in A and B line suggested that selection for above traits may have simultaneous improvement in the other traits and seed setting in male sterile lines.

Plant height exhibited significant positive correlation with plant height in B lines, flag leaf area, anther size in B lines and seed setting by stick pollination. However, it showed significant negative correlation with stigma size of A lines, and spike density. It indicated that association among plant height of male sterile line with pollen parent is of great importance in hybrid development programme for more seed setting resulted more grain yield. The close relation of seed set with extent of synchrony in heading of material and pollen parent was observed by Araki (1990) and the results are in agreement with the above finding.

The significant negative correlation of plant height in A and B line with stigma size and spike density indicates the recovery of desirable traits because selection for one trait could decrease value of the other and vice-versa and such correlation taken due consideration for further improvement in out crossing and seed setting.

Table 1. Phenotypic correlation for floral biology, yield and its contributing traits in A and B lines

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	X_{20}	X_{21}
X_1	**1.000**	0.7897**	0.2843	0.5702**	-0.0104	-0.1133	0.2180	-0.1258	0.4878**	0.3594	-0.0510	-0.0637	-0.1289	0.2789	-0.1465	0.3008	**0.0687**	0.3179	-0.0742	0.4738**	0.2401
X_2		**1.0000**	0.1433	0.6083**	-0.2195	-0.1812	0.4158*	-0.0638	0.5166**	-0.4018*	0.1564	-0.3625*	0.1146	0.1511	-0.3709*	0.3118	0.0193	0.3311	-0.0910	0.6017**	0.3979*
X_3			**1.0000**	0.7401**	0.0156	-0.2669	-0.1823	0.2488	0.0437	-0.0544	0.2780	0.1649	0.0242	0.2897	0.4924**	-0.0950	0.5748**	0.1551	0.4475*	0.0424	0.0102
X_4				**1.0000**	0.1574	-0.0397	0.1269	0.4461*	0.4246*	0.0771	0.2038	-0.0727	0.0528	0.2629	0.1709	0.2780	0.6207***	0.5711**	0.3823*	0.4409*	0.0025
X_5					**1.0000**	0.7911**	0.0373	0.5898**	-0.1565	0.7265**	-0.5217**	0.3248	-0.5469**	-0.0296	-0.0599	0.0143	0.2695	0.4491*	0.2660	-0.1217	-0.7069**
X_6						**1.0000**	-0.3330	0.5193**	-0.1647	0.8289**	-0.5194**	0.2153	-0.5574**	0.0957	-0.0217	0.1839	-0.0616	0.2499	-0.0735	-0.0455	-0.3857*
X_7							**1.0000**	-0.1512	0.3147	-0.4063*	0.1294	-0.3965*	0.3330	-0.2891	-0.6376**	-0.1474	0.1968	0.3982*	0.2813	0.2609	-0.1443
X_8								**1.0000**	0.0324	0.8209***	-0.2738	0.3908*	-0.3858*	0.1359	0.1133	0.2296	0.4284*	0.4926**	0.2757	0.1526	-0.3519
X_9									**1.0000**	-0.1231	0.3992*	-0.5457**	0.5635**	0.6510**	-0.1126	0.0858	0.5413**	0.7682**	0.4212*	0.9439**	0.5278**
X_{10}										**1.0000**	-0.3615*	0.3356	-0.4217*	0.1152	0.1834	0.1644	0.2509	0.3396	0.1642	-0.0470	0.4416*
X_{11}											**1.0000**	-0.7843**	0.8692**	0.2465	-0.1654	-0.5460**	0.4933**	0.1116	0.5787**	0.3368	0.5614**
X_{12}												**1.0000**	-0.8358**	-0.2082	0.4642**	0.4013*	-0.2988	-0.3031	-0.4235*	-0.5114**	-0.5120**
X_{13}													**1.0000**	0.2405	-0.1158	-0.3864*	0.4536*	0.2456	0.5158**	0.4474*	0.5175**
X_{14}														**1.0000**	0.2616	-0.1682	0.4380*	0.5031**	0.4109*	0.7341**	0.6350**
X_{15}															**1.0000**	0.3547	0.1323	-0.1214	-0.0812	-0.1828	-0.0814
X_{16}																**1.0000**	-0.2865	0.0528	-0.6200**	0.0581	-0.2003
X_{17}																	**1.0000**	0.7561**	0.9220**	0.4604*	-0.0263
X_{18}																		**1.0000**	0.6440**	0.7587**	-0.0066
X_{19}																			**1.0000**	0.3763*	0.0263
X_{20}																				**1.0000**	0.6194***

X_1= Days to 50%flowering in A line , X_2 = Days to 50%flowering in B line , X_3 = Days to maturity in A line , X_4 =Days to maturity in B line, X_5 = Plant height (cm) in A line, X_6 = Plant height (cm) in B line, X_7 = Nicking time, X_8 = Flag leaf area(cm), X_9 = Fertility in A line(%), X_{10} = Anther size in B line (mm), X_{11} = Stigma size in A line(mm), X_{12} = Spike length (cm) in A line, X_{13} = Spike density, X_{14} = Number of tillers/ plant in A line, X_{15} = Number of tillers/plant in B line, X_{16} = Number of spikelets/spike, X_{17} = Seed setting by control pollination, X_{18} = Seed setting by stick pollination, X_{19} = Seed setting by control pollination(%), X_{20} = Seed setting by stick pollination(%), X_{21} = Yield per plant in A line (gm)

*Significance at 5% level

**Significance at 1% level

Table 2. Genotypic correlation for floral biology, yield and its contributing traits in A and B lines

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	X_{20}	X_{21}
X_1	1.0000	0.7952	0.3004	0.5784	-0.0091	-0.1134	0.2397	-0.1288	0.4923	0.3727	-0.0452	0.0616	-0.1659	0.2772	0.01468	0.3079	0.0701	0.3410	-0.0750	0.4779	0.2439
X_2		1.0000	0.1498	0.6167	-0.2198	-0.1827	0.4756	-0.0652	0.5217	-0.4121	0.1555	-0.3691	0.1192	0.1508	-0.3803	0.3290	0.0189	0.3599	-0.0922	0.6076	0.4027
X_3			1.0000	0.7527	0.0153	-0.2694	-0.2032	0.2531	0.0441	-0.0554	0.2813	0.1798	0.0388	0.2973	0.4970	-0.0961	0.5785	0.1556	0.4518	0.0425	0.0096
X_4				1.0000	0.1572	-0.0402	0.1541	0.4488	0.4258	0.0841	0.2097	-0.0804	0.697	0.2615	0.1720	0.2814	0.6265	0.6070	0.3842	0.4431	0.0010
X_5					1.0000	0.7919	0.0395	0.5920	-0.1563	0.7406	-0.5372	0.3322	-0.6181	-0.0286	-0.0595	0.0153	0.2700	0.4820	0.2662	-0.1219	-0.7083
X_6						1.0000	-0.3592	0.5201	-0.1647	0.8451	-0.5384	0.2202	-0.6255	0.0965	-0.0224	0.1863	-0.0618	0.2679	-0.0735	-0.0456	-0.3863
X_7							1.0000	-0.1664	0.3399	-0.5012	0.1539	-0.4690	0.4447	-0.3045	-0.6958	-0.1776	0.2138	0.4721	0.3020	0.2795	-0.1556
X_8								1.0000	0.0323	0.8338	-0.2824	0.4077	-0.4334	0.1354	0.1141	0.2326	0.4297	0.5278	0.2758	0.1527	-0.3520
X_9									1.0000	0.1255	0.4108	-0.5681	0.6403	0.6522	-0.1141	0.0872	0.5425	0.8229	0.4214	0.9442	0.5278
X_{10}										1.0000	-0.3849	0.3473	-0.4560	0.1182	0.1808	0.1728	0.2560	0.3729	0.1646	-0.0483	-0.4500
X_{11}											1.0000	-0.8440	1.0306	0.2494	-0.1924	-0.5624	0.5120	0.1259	0.5963	0.3473	0.5767
X_{12}												1.0000	-0.9302	-0.2186	0.4861	0.4304	-0.3078	-0.3383	-0.4423	-0.5322	-0.5352
X_{13}													1.0000	0.2780	-0.1165	-0.4628	0.5123	0.2849	0.5815	0.5022	0.5908
X_{14}														1.0000	0.2618	-0.1699	0.4421	0.5388	0.4121	0.7367	0.6367
X_{15}															1.0000	0.3630	0.1334	-0.1306	-0.0823	-0.1840	-0.0834
X_{16}																1.0000	-0.2866	0.0691	-0.6265	0.0586	-0.2025
X_{17}																	1.0000	0.8070	0.9235	0.4611	-0.0259
X_{18}																		1.0000	0.6888	0.8123	-0.0072
X_{19}																			1.0000	0.3763	0.0263
X_{20}																				1.0000	0.6198

Nicking time exhibited significant positive correlation with days to heading in B lines, seed setting by stick pollination, where as, it was recorded significant and negative correlation with anther size of B lines, spike length and number of tillers per plant in B lines the above significant positive and negative correlation with nicking time suggesting the importance of this traits in adjustment of sowing date for better recovery of yield.

Anther size in B lines exhibited significant positive correlation with plant height in A and B lines and flag leaf area. However, it revealed significant and negative correlation with nicking time, stigma size of A lines and spike density. The association of anther size with stigma size is an establishment fact. It indicated that large stigmatic surface has maximum number of pollen grains accumulation promoted more out crossing resulted more seeds. Kherde et al (1967) reported positive association between anther size and pollen produce per anther which indirectly supported the above findings.

Stigma size in A lines exhibited significant positive correlation with fertility in A lines, spike density, seed setting by control pollination and seed setting percentage by stick pollination. However, it showed significant negative correlation with plant height in A lines, plant height in B lines, anther size of B lines and spike length.

Seed setting and seed setting percentage by control pollination exhibited similar trends and observed significant and positive correlation with days to maturity in B lines, flag leaf area, fertility in A lines, number of tillers per plant in A lines whereas days to maturity in A lines, stigma size and spike density had recorded different trends.

Seed setting and seed setting percentage by stick pollination exhibited common trends and noted significant and positive correlation with days to maturity in B lines, spike density, fertility in A lines, number of tillers per plant in A lines, seed setting and seed setting percentage by control pollination whereas stigma size recorded the different trend.

The above association of seed setting by control and stick pollination and its percentage indicated, importance of these traits in seed setting which also reflect the amount of out crossing. The male sterile lines having such characters are more useful to a crop breeders at the time of formulation of hybrid development programme because more setting definitely increased the grain yield which is prime objective. The present finding is in conformity with findings of Araki (1990), Singh *et al*.(2001) and Singh *et al*.(2008).

सीमिट आधरित गेहुँ की १० पुरूष बाँझ लाइनों एवं इनके बीज को नियत करने बाली "बी" लाइनों के उध्ययन हेतु बेतरतीब रचना में ३ प्रतिकृति में पुष्प गुणों तथा उपज के बीज सहसंवध हेतु लगाया गया । अध्ययन के तहतम उपज प्रति पौध का उच्चधनात्मक सहसंबध शाखा प्रति पौध, परागण छड़ी प्रतिशत एवं कलंक आधार का "ए" लाइनस में एवं पराग केशर आकार एवं कील धनत्व का "बी" लाइनस में पाया गया । यह अध्ययन गेहूँ संकर बीज उत्पादन के लिए पुष्प गुणों के सुधार हेतु तथा पुरूष बाँझ लाइनों के माध्यम से उपज प्रति पौध बढ़ने की महत्व को संकेतिक करता है ।

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Association analysis of morpho - phenological traits on yield in chickpea lines evaluated in normal and heat stress environments

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Abstract

Association and path coefficient analysis of various morpho-phenological characters with yield in thirty heat tolerant lines were worked under three environments during rabi 2010-2011.Very high magnitude of genotype x environment interaction with expression of these characters is evident from the change in the magnitude of association coefficient. Days to 50% flowering, days to pod initiation, days to maturity, total number of pods per plant, effective pods per plant, seeds per pod and biological yield showed significant positive relationship with most of the characters. Hence, these traits may be improved for developing varieties, suitable under normal sowing conditions (E-I). Likewise, under late sown (E-II) and very late sown(EIII) planting, characters like secondary branches, seeds per pod, plant height, days to 50% flowering, days to pod initiation, days to maturity, total number of pod per plant, effective pods per plant seeds per pod and biological yield showed significant positive correlation. Therefore, these traits should be given due importance while developing varieties for late and very late planting in chickpea. Days to maturity and biological yield per plant recorded positive direct effect in all the three environments except pooled analysis. Days to flower initiation exhibited negative direct effect in E-I and E-III, total number of pods per plant under E-II and E-III, whereas, days to 50% flowering in E-III, days to pod initiation under E-I, plant height under E-II, primary branches under E-III and secondary branches under E-II. It indicated that these characters have their specific contribution towards seed yield in E-I, E-II and E-III only. Therefore, selection for high biological yield and harvest index would lead to high seed yield and selection for pods per plant, primary branches per plant and plant height would facilitate for high biological yield.

Key words: Chickpea, correlation, path analysis, heat tolerant lines normal (E-I), late (E-II) and very late (E-III) planting

Chickpea is a cool season food legume and second most important pulse crop after dry bean as well as an important source of human food and feed. It also helps in improve soil fertility, particularly in dry lands. In India, chickpea is being grown in 7.10 mha area, with production 5.65 mt and productivity 795 kg/ha(www.newcrops.uq.edu). Madhya Pradesh, Uttar Pradesh, Maharashtra and Rajasthan are the major chickpea growing states in India, sharing 85% area. The maximum area (2.9 mha) and production (2.98 mt) of chickpea is recorded in Central Zone (http://wiki.icrisat.org). Madhya Pradesh covers 2.8 mha area with production 2.6 mt and productivity 931 kg/ha and contributing 37% of India's total chickpea production. Most of the characters of economic importance such as yield are complex in inheritance, which may involve several direct and indirect characters. This necessitates the study of their relationship with each other in order to achieve a balance improvement. The correlation is of practical importance since selection is usually concerned with changing two or more traits simultaneously. In such circumstances, correlation and path analysis provides an effective mean in finding out direct and indirect causes of associations. Thus, in the present study, an attempt was made to get the comprehensive information on these aspects in chickpea under different environments using heat tolerant lines.

Material and methods

Thirty chickpea genotypes were grown in a randomized completely block design with three replications under three different dates of sowing (normal planting on 19th November 2010 , late planting on 24th December 2010 and very late planting on 30th January 2011) . Each plot size was 4.0 m x 0.90m consisting of 2 rows of 4m length, the row to row distance was 45 cm and plant to plant spacing was 10 cm. The experiment was conducted with recommended agronomic practices. Five plants from each

Table 1. Correlation Coefficient analysis for yield and its contributing traits in heat tolerance lines in chickpea under various environments

Char.	ENV	FI	F 50%	PI	P 50%	Pl.ht (cm)	PB	SB	TNPP	EPPP	SPP	100 SW	BY(g)	HI (%)	SYPP
FI	E-I	1.0000	0.4839***	0.3694***	0.2521*	-0.0227	-0.1231	0.0066	0.1305	0.0803	0.1601	0.0080	0.1694	-0.1245	-0.0497
	E-II	1.0000	0.2094*	-0.0413	-0.0248	-0.0594	0.1804	0.2346*	0.2555*	0.3313**	-0.0438	-0.2510*	-0.0752	-0.2266*	-0.1994
	E-III	1.0000	0.4930***	0.3640***	0.0485	0.0241	0.1428	-0.2577*	0.3527***	0.0422	-0.0613	0.0616	-0.0305	0.0846	-0.4078***
	POOLED	1.0000	0.3431***	0.2038***	0.0745	-0.0345	0.0514	0.0269	0.1811**	0.1335*	0.0061	-0.0916	0.0415	-0.1271*	-0.2845**
F 50%	E-I		1.0000	0.3688***	0.3736***	0.0895	-0.0166	0.0996	0.1305	0.0803	0.1601	0.0080	0.1694	-0.1245	0.2485*
	E-II		1.0000	0.4793***	0.3143**	-0.1963	0.1503	0.2397*	0.3087**	0.1745	0.0221	-0.3182**	0.0192	-0.1074	-0.0135
	E-III		1.0000	0.1311	0.1931	0.1415	-0.0129	-0.2614*	0.1601	0.2924**	0.0244	-0.0589	-0.0648	0.4659***	-0.2880**
	POOLED		1.0000	0.3180***	0.3079***	-0.0020	0.0314	0.0166	0.2701***	0.2495***	0.0135	-0.2199***	0.0748	0.0929	0.1159
PI	E-I			1.0000	0.2280*	-0.0821	-0.1529	-0.0506	0.2676*	0.2177*	0.2098*	-0.3278**	0.0744	0.0100	0.0152
	E-II			1.0000	0.6061***	-0.1280	0.1726	0.2710**	0.0142	-0.0769	-0.0285	-0.2369*	0.0467	-0.0202	0.0955
	E-III			1.0000	0.1745	-0.0914	0.2546*	0.1688	0.1926	0.1437	0.0358	-0.4361***	-0.1356	-0.2135*	-0.2381*
	POOLED			1.0000	12.1861	0.3350***	0.0415	-0.1074	0.2144***	0.1619**	0.0827	-0.3398***	0.0182	-0.0749	0.0428
P50%	E-I				1.0000	0.2952**	0.1163	-0.2403*	0.2275*	0.1156	0.1106	-0.3994***	0.0512	-0.1982	0.0435
	E-II				1.0000	0.3458***	0.0289	-0.0674	0.0317	0.0326	0.0043	-0.3804***	0.2033	0.1643	0.2566*
	E-III				1.0000	0.3748***	-0.0874	-0.0209	0.2888**	0.3121**	0.0964	0.1550	0.1294	-0.0041	0.1951
	POOLED				1.0000	0.3350***	0.0415	-0.1074	0.1799**	0.1347*	0.0631	-0.1378*	0.1059	0.0114	0.0622
Pl. ht. (cm)	E-I					1.0000	0.0487	0.0651	0.4692***	0.3986***	-0.1916	-0.1711	0.3628***	-0.1761	0.2050
	E-II					1.0000	-0.3638***	-0.5311***	-0.0963	0.0139	-0.0826	0.0950	0.1589	0.4394***	0.3235**
	E-III					1.0000	-0.1090	-0.0714	0.1105	0.3821***	-0.1488	0.3148**	0.2790**	0.1781	0.2044
	POOLED					1.0000	-0.1374*	-0.1673**	0.2419***	0.2685***	-0.1302*	0.1027	0.2649***	0.1989**	0.4338***
PB	E-I						1.0000	0.0898	0.1247	0.0191	-0.0270	-0.0346	0.1320	0.1211	0.2397*
	E-II						1.0000	0.4465***	0.2866**	0.0180	-0.0188	-0.3480***	-0.1661	-0.1472	-0.0781
	E-III						1.0000	0.3377**	0.2803**	0.0376	-0.0705	-0.0508	0.3762***	-0.2099*	-0.0627
	POOLED						1.0000	0.2472***	0.1833**	0.0073	-0.0344	-0.1351*	0.0748	0.0577	0.0282
SB	E-I							1.0000	0.2572*	0.1644	-0.2717**	0.1603	0.2687*	0.1002	0.2844**
	E-II							1.0000	0.1930	0.0105	0.0421	-0.2264*	-0.1764	-0.2453*	-0.2809**
	E-III							1.0000	0.2990*	-0.1470	-0.0976	-0.0508	0.3762***	-0.2098*	0.2415*
	POOLED							1.0000	0.2218***	0.0305	-0.1034	-0.0283	0.1987**	0.1349*	-0.0044
TNPP	E-I								1.0000	0.8924***	-0.1838	-0.2821**	0.6094***	0.0963	0.5883***
	E-II								1.0000	0.7344***	-0.1726	-0.4441***	-0.1092	-0.1135	-0.2097*
	E-III								1.0000	0.0487	-0.0034	0.2011	0.6584***	-0.0876	0.1115
	POOLED								1.0000	0.7076***	-0.1139	-0.1014	0.5037***	-0.0137	0.4410
EPPP	E-I									1.0000	-0.1128	-0.3516***	0.5779***	0.0261	0.4926***
	E-II									1.0000	-0.2612*	-0.2858**	-0.2528	-0.0607	-0.1438
	E-III									1.0000	0.0306	-0.0015	0.0067	0.0301	0.0344
	POOLED									1.0000	-0.0927	-0.1678**	-0.3686***	0.0042	0.4410***
SPP	E-I										1.0000	-0.0530	-0.0542	-0.1022	-0.2019
	E-II										1.0000	0.1155	0.2447*	-0.3262**	-0.0396
	E-III										1.0000	0.0537	-0.0191	0.0108	0.1042
	POOLED										1.0000	-0.0104	0.0377	-0.1483	-0.1439
100 SW.	E-I											1.0000	-0.0030	-0.2693	0.0245
	E-II											1.0000	0.0341	-0.2332*	-0.0245
	E-III											1.0000	0.4670***	0.0330	0.0334
	POOLED											1.0000	0.1666**	0.1124	0.0334
BY(g)	E-I												1.0000	-0.0148	0.6842***
	E-II												1.0000	-0.2741**	0.6218***
	E-III												1.0000	0.0632	-0.0109
	POOLED												1.0000	-0.0630	0.2774**
HI (%)	E-I													1.0000	0.3730***
	E-II													1.0000	0.1274
	E-III													1.0000	0.4452***
	POOLED													1.0000	0.3684***
SYPP	E-I														1.0000
	E-II														1.0000
	E-III														1.0000
	POOLED														1.0000

E I, EII and EIII: Normal planting on 19th November 2010 , late planting on 24th December 2010 and very late planting on 30th January 2011

FI Days to flower initiation PB Primary branches BY Biological yield

F 50% Days to 50% flowering SB Secondary branches HI Harvest index

PI Days to pod initiation SPP Seeds per pod SYPP Seed yield per plant

P50% Days to maturity EPPP Effective pods plant TNPP Total numbers of pods per plant

Pl. ht. Plant height 100 SW Hunderd seed weight

Table 2. Path coefficient analysis for yield and its component characters in heat tolerance lines in chickpea under various environments

Characters		FI	F 50%	PI	P 50%	Pl.ht.	PB	SB	TNPPI	EPPP	SPP	100SW	BY(g)	HI (%)
FI	E-I	-0.1680	-0.0819	-0.0561	-0.0490	0.0031	0.0146	0.0011	-0.0307	-0.0169	-0.0121	0.0024	-0.0270	0.0130
	E-II	0.0907	0.0231	-0.0040	-0.0038	0.0058	0.0124	0.0218	0.0252	0.0297	0.0037	-0.0206	-0.0079	-0.0226
	E-III	-0.2412	0.1174	-0.0738	-0.0055	-0.0063	-0.0302	0.0587	-0.0803	-0.0116	0.0165	0.0222	0.0129	-0.0202
	POOLED	-0.3196	-0.0988	-0.0692	-0.0551	0.0202	-0.0344	-0.0194	-0.0777	-0.0585	0.0281	0.0002	-0.0300	0.0688
F 50%	E-I	0.0836	0.1714	0.0591	0.0691	0.0241	-0.0072	0.0141	0.0568	0.0510	-0.0113	-0.0757	0.0199	0.0044
	E-II	0.0192	0.0753	0.0347	0.0192	-0.0137	0.0120	0.0200	0.0197	0.0120	-0.0027	-0.0190	0.0031	-0.0101
	E-III	-0.0805	-0.1654	-0.0212	-0.0278	-0.0270	0.0021	0.0432	-0.0285	-0.0420	-0.0040	0.0149	0.0094	-0.0764
	POOLED	-0.0010	-0.0032	-0.0011	-0.0008	0.0000	-0.0005	0.0002	-0.0010	-0.0007	0.0006	0.0010	-0.0003	-0.0007
PI	E-I	-0.0347	-0.0358	-0.1039	-0.0253	0.0082	0.0202	0.0037	-0.0300	-0.0226	-0.0177	0.0344	-0.0087	0.003
	E-II	-0.0024	0.0252	0.0545	0.0340	-0.0071	0.0094	0.0150	0.0007	-0.0023	-0.0022	-0.0098	0.0017	-0.0001
	E-III	0.0015	0.0006	0.0048	0.0008	-0.0006	0.0011	0.0007	0.0008	0.0009	0.0002	-0.0020	-0.0008	-0.0011
	POOLED	0.0292	0.0470	0.1350	0.0416	0.0053	0.0149	-0.0205	0.0338	0.0219	0.0086	-0.0245	0.0143	-0.0258
P 50%	E-I	0.0381	0.0526	0.0318	0.1306	0.0405	0.0156	-0.0312	0.0304	0.0144	0.0132	-0.0510	0.0080	-0.0290
	E-II	-0.0027	0.0162	0.0398	0.0637	0.0229	0.0021	-0.0021	0.0036	0.0028	0.0015	-0.0215	0.0134	0.0141
	E-III	0.0030	0.0225	0.0219	0.1338	0.0480	-0.0119	-0.0014	0.0353	0.0385	0.0120	0.0184	0.0176	-0.0017
	POOLED	-0.0304	-0.0431	-0.0545	-0.1766	-0.0650	-0.0131	0.0145	-0.0442	-0.0301	0.0109	0.0165	-0.0276	-0.0236
Pl.ht. (cm)	E-I	-0.0002	0.0016	-0.0009	0.0034	0.0111	0.0006	0.0007	0.0050	0.0042	-0.0022	-0.0017	0.0040	-0.0019
	E-II	0.0098	0.0278	0.0199	-0.0551	-0.1532	0.0574	0.0782	0.0122	0.0005	0.0126	-0.0149	-0.0265	-0.0704
	E-III	0.0016	0.0100	-0.0074	0.0220	0.0614	-0.0093	-0.0057	0.0071	0.0216	-0.0116	0.0172	0.0152	0.0128
	POOLED	-0.0164	0.0036	0.0102	0.0954	0.2591	-0.0252	-0.0208	0.0629	0.0701	-0.0123	0.0069	0.0633	0.0525
PB	E-I	-0.0053	-0.0025	-0.0119	0.0073	0.0035	0.0610	0.0055	0.0070	0.0018	-0.0016	-0.0017	0.0078	0.0072
	E-II	0.0215	0.0251	0.0271	0.0051	-0.0589	0.1572	0.0672	0.0444	0.0102	-0.0010	-0.0533	-0.0258	-0.0236
	E-III	-0.0033	0.0003	-0.0062	0.0023	0.0040	-0.0264	-0.0089	-0.0070	0.0001	0.0019	0.0035	-0.0051	0.0045
	POOLED	0.0011	0.0016	0.0011	0.0008	-0.0010	0.0104	0.0016	0.0023	0.0005	-0.0032	-0.0002	0.0013	-0.0009
SB	E-I	-0.0006	0.0073	-0.0031	-0.0211	0.0053	0.0080	0.0886	0.0228	0.0150	-0.0241	0.0134	0.0232	0.0090
	E-II	-0.0346	-0.0384	-0.0398	0.0048	0.0737	-0.0618	-0.1444	-0.0225	-0.0026	-0.0123	0.0327	0.0228	0.0404
	E-III	-0.0236	-0.0253	0.0146	-0.0010	-0.0090	0.0327	0.0968	0.0239	-0.0107	-0.0095	-0.0020	0.0382	-0.0197
	POOLED	0.0019	-0.0015	-0.0048	-0.0026	-0.0025	0.0048	0.0314	0.0021	0.0029	0.0049	-0.0009	0.0027	-0.0016
TNPP	E-I	0.0595	0.1080	0.0939	0.0758	0.1485	0.0375	0.0837	0.3257	0.2894	-0.0566	-0.945	0.1939	0.0372
	E-II	-0.0110	-0.0104	-0.0005	-0.0022	0.0032	-0.0112	-0.0062	-0.0398	-0.0305	0.0061	0.0187	0.0039	0.0054
	E-III	-0.0243	-0.0125	-0.0116	-0.0192	-0.0084	-0.0193	-0.0180	-0.0729	-0.0057	0.0004	-0.0147	-0.0482	0.0052
	POOLED	0.0676	0.0825	0.0697	0.0695	0.0676	0.0630	0.0185	0.0782	0.2231	-0.0392	-0.0282	0.1358	-0.0324
EPPP	E-I	0.0094	0.0277	0.0202	0.0103	0.0355	0.0028	0.0157	0.0826	0.0930	-0.0115	-0.0341	0.0542	0.0034
	E-II	0.0066	0.0032	-0.0008	0.0009	-0.0001	0.0013	0.0004	0.0154	0.0201	-0.0053	-0.0075	-0.0014	-0.0011
	E-III	0.0011	0.0060	0.0045	0.0068	0.0083	-0.0001	-0.0026	0.0018	0.0235	0.0008	0.0004	0.0008	0.0005
	POOLED	0.0540	0.0679	0.0479	0.0502	0.0798	0.0156	0.0270	0.2365	0.2949	-0.0397	-0.0140	0.1303	-0.0247
SPP	E-I	-0.0001	0.0001	-0.0002	-0.0001	0.0002	0.0000	0.0003	0.0002	0.0001	-0.0011	0.0000	0.0001	0.0001
	E-II	-0.0050	-0.0044	-0.0049	0.0028	-0.0100	-0.0008	0.0103	-0.0188	-0.0323	0.1218	0.0167	0.0299	-0.0377
	E-III	-0.0082	0.0030	0.0047	0.0108	-0.0229	-0.0085	-0.0118	-0.0006	0.0044	0.1207	-0.0081	-0.0027	0.0016
	POOLED	0.0077	0.0150	-0.0055	0.0054	0.0041	0.0271	-0.0136	0.0123	0.0117	-0.0871	0.0064	-0.0044	0.0019
100SW	E-I	-0.0009	-0.0285	-0.0214	-0.0252	-0.0100	-0.0018	0.0097	-0.0187	-0.0236	-0.0029	0.0645	-0.0005	0.0175
	E-II	-0.0488	-0.0545	-0.0387	-0.0776	0.0210	-0.0731	-0.0489	-0.1015	-0.0806	0.0296	0.2156	0.0074	-0.0435
	E-III	-0.0245	-0.0239	-0.1099	0.0366	0.0747	0.0355	0.0056	0.0536	0.0042	-0.0178	0.2663	0.1272	0.0036
	POOLED	-0.0001	-0.0267	-0.0156	-0.0080	0.0023	-0.0019	-0.0026	-0.0087	-0.0120	-0.0063	0.0861	0.0109	0.0037
BY(g)	E-I	0.0177	0.0128	0.0092	0.0086	0.0398	0.0140	0.0289	0.0656	0.0642	-0.0078	-0.0009	0.1102	-0.0021
	E-II	-0.0289	0.0138	0.0104	0.0698	0.0573	-0.0544	-0.0522	-0.0322	-0.0235	0.0814	0.0114	0.3310	-0.0865
	E-III	-0.0149	-0.0158	-0.0440	0.0364	0.0687	0.0536	0.1093	0.1829	0.0098	-0.0061	0.1323	0.2769	0.0151
	POOLED	-0.0028	-0.0030	-0.0031	-0.0046	-0.0072	-0.0037	-0.0026	-0.0145	-0.0131	-0.0015	-0.0038	-0.0296	-0.0009
HI (%)	E-I	-0.0482	0.0160	-0.0016	-0.1390	-0.1047	0.0742	0.0637	0.0714	0.0226	-0.0662	0.1693	-0.0121	0.6251
	E-II	-0.2138	-0.1154	-0.0021	0.1900	0.3940	-0.1286	-0.2400	-0.1162	-0.0474	-0.2653	-0.1731	-0.2241	0.8576
	E-III	0.0055	0.0300	-0.0144	-0.0008	0.0135	-0.0111	0.0132	-0.0046	0.0015	0.0009	0.0009	0.0035	0.0650
	POOLED	-0.0758	0.0747	-0.0673	0.0471	0.0713	-0.0289	-0.0180	-0.0410	-0.0295	-0.0076	0.0150	0.0107	0.3522

E I, EII and EIII: Normal planting on 19th November 2010 , late planting on 24th December 2010 and very late planting on 30th January 2011

Env-1—(R^2=0.8052 Residual effect= 0.4414) Env3—(R^2=0.4560 Residual effect=0.7376)

Env-2—(R^2=0.5520 Residual effect= 0.6693) Pooled —(R^2 =0.5994 Residual effect= -0.6329)

replication were randomly selected and observation were made for the characters days to flower initiation, days to 50% flowering, days to pod initiation, days to maturity, plant height , number of primary and secondary branches, total number of pods per plant, effective pods per plant, seeds per pod, 100 seed weight(g), biological yield(g), harvest index (%) and seed yield per plant (g). The data were statistically analyzed to estimate correlation and path analysis by the methods given by Miller et al. (1958 and Dewey and Lu 1959) respectively.

Results and discussion

Secondary branches exhibited significant positive correlation with total number of pods per plant, biological yield and harvest index on pooled analysis basis. It had significant positive correlation with total number of pods per plant in E-I and E-II and with biological yield E-I and E-II. However, it revealed significant negative correlation with 100- seed weight, biological yield and harvest index in E-II and harvest index in E-III which is in accordance with the findings of Ali et al. (2010) in normal planting. Effective pods per plant recorded significant and negative correlation with 100- seed weight and biological yield, whereas,100- seed weight showed significant positive correlation with biological yield on pooled analysis basis. Number of seeds per pod recorded significant positive and negative correlation with biological yield and harvest index in E-II respectively. Hundred seed weight exhibited significant positive correlation with biological yield in E-III and pooled analysis environment, whereas, it was noted significant negative correlation in E-II which is also reported by Ali et al. (2010).

Days to 50% flowering exhibited positive association with days to pod initiation, days to maturity, total number of pods per plant and effective pods per plant on pooled analysis basis (Table 1). The results obtained by Paliwal et al. (1987) are the agreement with the present findings. It had also significant positive correlation with days to pod initiation in all the three environments and with days to maturity in E-I and E-II, number of secondary branches and total number of pods per plant in E-II and effective pods per plant and harvest index in E-III. Days to pod initiation recorded significant positive association (pooled) with plant height, total pods per plant, and effective pods per plant. However, it was noted significant negative association with 100- seed weight in all the three environments and for harvest index in E-III .It exhibited significant positive correlation with days to pod maturity, total number of pods per plant, effective pods per plant and number of seeds per pod in E-I whereas, it was significant positive with days to maturity and secondary branches in E-II.

From the present findings on correlation studies, it appeared that days to 50% flowering, days to pod initiation, days to maturity, total number of pods per plant, effective pods per plant, seeds per pod and biological yield showed significant positive relationship with most of the characters .The above findings is agreement with results of Thakur and Sirohi (2009). Hence, these traits may be improved for developing varieties, suitable under normal sowing conditions (E-I). Likewise, under late sown (E-II) and very late sown planting, characters like secondary branches, seeds per pod, plant height, days to 50% flowering, days to pod initiation, days to maturity, total number of pod per plant, effective pods per plant seeds per pod and biological yield showed significant positive correlation. Therefore, these traits should be given due importance while developing varieties for late and very late planting in chickpea.

The phenotypic path revealed that the harvest index showed the highest positive direct effect on seed yield per plant followed by 100 - seed weight and effective pods per plant in all the three environments including the pooled analysis. The similar results for 100- seed weight were recorded by Sontakey et al. (1991) and Derya et al. (2006). Thus, these traits were having maximum contribution towards seed yield per plant and direct selection may be applied to improve the yield. Highest positive direct effect on seed yield per plant was observed in harvest index followed by 100- seed weight and effective pods per plant in all the three environments including the pooled analysis (Table 2). The similar results for 100- seed weight were recorded by Sontakey *et al.* (1991). Thus, these traits were found having maximum contribution towards seed yield per plant and direct selection may be applied to improve the yield. However, days to maturity and biological yield recorded positive direct effect in all the three environments except pooled analysis; it means that these characters also showed more contribution towards yield per plant. Days to flower initiation exhibited negative direct effect in E-I and E-III, total number of pods per plant under E-I and E-III, whereas, days to 50% flowering in E-III, days to pod initiation under E-I, plant height under E-II, primary branches under E-III and secondary branches under E-II. It indicated that these characters have their specific contribution towards seed yield in individual environments.

Similarly, as direct effect, the indirect effects will also find it contribution via different traits towards seed yield per plant. Under all the environments secondary branches had positive indirect effect on seed yield per plant *via* days to flower initiation towards, secondary branches and biological yield through days to 50% flowering and primary branches, secondary branches and total number of pod per plant *via* days to pod initiation, days to 50% flowering, days to pod initiation, days to

maturity, plant height, total number of pods per plant, effective pods per plant, seeds per pod and biological yield *via* days to pod maturity, total number of pods per plant, days to 50% flowering and effective pods per plant *via* plant height, days to maturity and effective pods per plant through primary branches, days to 50% flowering, days to flower initiation and total number of pods per plant *via* effective pods per plant and days to maturity and plant height through biological yield. Thus, selection of these characters may enhance the yield potential widely in all the three environments under study.

Apart from performance of various characters as indirect effect in the entire environment there are many traits which exhibited their considerable improvement in two environments and selection in this direction may also improve the yielding ability. Under E-I and E-II, primary branches and plant height recorded positive indirect effect on seed yield per plant via flower initiation. Days to flower initiation, days to maturity, days to pod initiation, total number of pods per plant and effective pods per plant *via* days to 50% flowering, primary and secondary branches through plant height, secondary branches and total number of pods per plant *via* primary branches, plant height,100- seed weight and harvest index *via* secondary branches, plant height and biological yield *via* total number of pods per plant, primary and secondary branches through effective pods per plant and days to 50% flowering and days to pod initiation *via* biological yield.

Under E-I and E-III days to 50% flowering and, seeds per pod recorded positive indirect effect on seed yield per plant *via* days to flower initiation, primary branches *via* days to 50% flowering and days to maturity *via* days to pod initiation, days to flower initiation through plant height, days to 50% flowering *via* primary branches, harvest index *via* total number of pods per plant, days to maturity through seeds per pod, plant height and biological yield through 100- seed weight, 100- seed weight via biological yield and plant height through harvest index. Dasgupta et al. (1992) reported the similar results for the characters number of pods per plant, number of seeds per plant and number of seeds per pod. The indirect positive effect as obtained in present investigation is the contradictory with the results given by Bhambotia et al. (1994) The above finding suggested that these characters given due importance at the time of breeding programme for improvement of seed yield looking to their performance in E-II and E-III (late planting and very late planting).

एसोसिएशन और पथ विश्लेषण चना के तीस गर्मी सहिष्णु लाइनों में उपज के साथ विभिन्न बाह्य फलाद्गमिकी संबधी अक्षर के गुणांक विश्लेषण तीन वातावरण के तहत रबी 2010-11 के दौरान किया गया । इन वर्णो की अभिव्यक्ति के साथ जीनोटाइप x पर्यावरण बातचीत के बहुत उच्च परिमाण संघ गुणांक के परिमाण में परिवर्तन से स्पष्ट है । 50% फूल, फली दीक्षा दिनों, परिपक्वता तक दिनों, संयंत्र प्रति फली की कुल संख्या दिन, संयंत्र प्रति प्रभावी फली, बीज फली और जैविक उपज प्रति वर्णे की सबसे महत्वपूर्ण सकारात्मक संबंध दिखा । इसलिए, इन तत्वों विकासशील किस्मों, सामान्य बुवाई शर्तो (ई-I) के तहत उपयुक्त के लिए सुधार किया जा सकता है । इसी तरह, के तहत देर से (ई-II) बोया और बहुत देर से (ई-II) रोपण, माध्यमिक शाखाओं, फली, पौधो की ऊँचाई के अनुसार बीज, 50 प्रतिशत फूलदानों, फली दीक्षा दिनों, परिपक्वता तक दिनों, फली की कुल संख्या की तरह पात्रों के बोया संयंत्र के अनुसार, प्रति फली और जैविक उपज संयंत्र के बीज के प्रति प्रभावी फली महात्वपूर्ण सकारात्मक सह संबंध ा दिखाया । इसलिये, इन तत्वों के कारण महात्व दिया जाना चाहिए, जबकि चना में देर से बहुत देर रोपण के लिये किस्मों के विकास, परिपक्वता और संयंत्र प्रति जैविक उपज दिन जमा विश्लेषण को छोडकर तीनों वातावरण में साकारात्मक सीधा प्रभाव दर्ज किया गया । फूल दिक्षा दिन E-I और E-I और E-II और E-III के तहत् संयंत्र प्रति फली की कुल संख्या में नाकारात्मक सीधा प्रभाव का प्रदर्शन किया । इसलिये, उच्च जैविक उपज और फसल सूचकांक के लिये चयन उच्च बीज और संयंत्र प्रति फलि के लिये उपज चयन करने के लिये नेतृत्व करेंगे, संयंत्र और संयंत्र ऊँचाई प्रति प्राथमिक शाखाओं उच्च जैविक उपज के चयन के लिये महात्वपूर्ण होगा।

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Selection strategy for improving yield in desi chickpea genotypes evaluated under normal and heat stress environments in Kymore plateau zone of Madhya Pradesh

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Abstract

The present study was carried out considering forty eight promising desi chickpea genotypes sown on 22nd Nov 2009 (normal sown) and 2nd Feb 2010 (late sown). Different genetic parameters viz. genetic variability, heritability, genetic advance correlation and path analysis were carried out. Seed yield per plant was positively and significantly correlated with total and effective pods per plant, seeds per pod, biological yield per plant, 100 seed weight and harvest index in normal and late sown desi chickpea genotypes. Path coefficient analysis revealed that highest direct effect on yield was contributed by harvest index and biological yield per plant. Out of fourteen, six quantitative traits which were positively correlated with seed yield, being used to screen the heat tolerant genotypes. On comparing the mean values of the selected traits under both conditions, seven heat tolerant genotypes were identified i.e. Dilagi, ICC 9942, ICCV 10, ICCV 07115, JG 130 and JG11. The identified genotypes displaying fair yield potential and satisfying performance in both early and late sown conditions should test under different agro climatic conditions, with the aim of improving the traits directly affecting seed yield.

Keywords: Chickpea, Heat tolerance, Correlation coefficient, Path analysis

Chickpea (*Cicer arietinum* L.) family Leguminosae, is a self pollinated diploid (2n = 2x = 16) food legume originated in Turkey. It is the third most important food legume with 12.3 m ha area under cultivation and 9.8 m t production (FAO 2009). The expansion of irrigated agriculture in northern India has led to the replacement of chickpea with wheat. As a result, area under chickpea has reduced from 3.2 to 1.0 m ha in Punjab, Haryana and Uttar Pradesh, while it increased from 2.6 to 4.3 m ha in central and southern states (Madhya Pradesh, Maharashtra, Andhra Pradesh and Karnataka) (IIPR AICRP on Chickpea). Breeding efforts have contributed to improve yield potential regional adaptation through resistance to stresses, plant type and seed characteristics. Heat stress is major problem to chickpea production under warm environments. Changes in seasonal temperature affect the grain yield, mainly through phenological development processes. Winter crops like chickpea are highly vulnerable to high temperature during the reproductive stages. Chickpea growing season under rainfed conditions ranges from late-September to late-October, however there is a wide range in time of sowing under irrigated conditions that extends to the second fortnight of December. Irrigated chickpea in late sown conditions suffers heavy yield losses due to heat stress at the reproductive stage. Reproductive stages (flowering and podding) in chickpea are susceptible to changes in external environment and heat stress (Krishnamurthy et al. 2011). High temperature during the stage, results in reduction of seed yield (Summerfield et al. 1984). Frequent reductions in chickpea seed yields were observed when plants at flowering and pod development stages were exposed to high (>35 ^{0}C) temperatures (Summerfield et al. 1984, Wang et al. 2006).

Material and methods

The experimental material consisted of 48 high yielding desi chickpea genotypes. The genotypes were sown on 22nd Nov 2009 (normal sown) and 2nd Feb 2010 (late). Air temperature varied from 4.0°C being minimum in January and 42.5°C being maximum in May. Month of May is highly suitable for screening the heat tolerant genotypes under natural field conditions. The experiment was laid out in a randomized complete block design with two replications during Rabi 2009-10 under All India Coordinated Research Project on Chickpea, in the experimental field of Seed Breeding Farm, JNKVV Jabalpur (M.P). Size of each plot was kept 4.0 x 0.90 m,

with 2 rows of 4 m length. Row to row distance 45 cm and plant to plant spacing was 10 cm, fertilizer were applied 20:60:40 kg/ha. Observations were recorded on 14 quantitative traits including four phenological traits. The mean data of 10 plants were subjected to genotypic correlations which were computed by following the procedure of Miller et al. (1958) whereas path coefficient analysis was conducted according to Dewey & Lu (1959).

Results and discussion

Estimates of various parameters for assessment of genetic variability (Table 1) *viz,* mean, range of variability, heritability, genetic advance and coefficient of variation were analyzed for the traits which are directly affecting the seed yield. Heritability in broad sense involves total genetic variance which consists of additive, dominance and epistatic variances. As it is a reliable measure of genetic improvement under selection for polygenic traits. To this reason, estimates of heritability in broad sense and genetic advance have been interpreted together (Johnson et al. 1955) in the investigation. A relative study on comparison of magnitude of PCV and GCV for different traits interprets that seed yield per plant had maximum variability followed by total and effective pods per plant. High heritability with high genetic advance as percentage of mean was noted in days to flower initiation, seed yield, days to 50 % flowering, total and effective pods per plant in both normal and late sown desi chickpea genotypes (Table.1).

Table 1. Genetic parameters of different characters of normal and late sown desi chickpea genotypes

Characters		General mean	Range		Coefficient of variation (%)		Heritability	Genetic advance as % of mean
			Min	Max	PCV (%)	GCV (%)		
FI	NS	63.4	57	71	7.19	6.10	92.9	22.23
	LS	45.7	40.0	51.5	5.87	5.66	72.0	20.68
F 50%	NS	68.2	64	76	6.57	5.60	60.8	3.52
	LS	50.4	45	57.5	4.40	3.43	72.7	19.85
PI	NS	83.6	79	86	2.84	1.78	39.2	2.29
	LS	56.5	49.0	70.0	8.26	4.22	26.1	4.45
DM	NS	111.5	106.5	120.5	5.62	1.20	26.2	1.26
	LS	82.7	76.5	88.5	2.34	0.50	23.2	1.55
Ht. (cm)	NS	52.2	42.7	68.5	19.99	8.95	48.8	12.81
	LS	30.3	21.4	39.3	12.8	6.60	10.9	4.49
PB	NS	3.4	2.3	4.8	13.23	10.56	63.0	17.18
	LS	2.4	2.0	2.9	11.81	3.33	29.5	9.03
SB	NS	11.3	7.8	17.1	23.69	17.30	53.3	26.09
	LS	6.9	5.3	10.0	22.61	4.58	38.5	10.5
Total pods	NS	87.8	50.2	204.0	33.71	26.98	70.0	44.40
	LS	35.1	9.0	79.2	50.20	34.30	63.6	48.25
Effe. Pods	NS	71.1	39.8	172.7	32.75	22.43	64.1	48.54
	LS	28.8	7.8	60.8	58.81	38.87	50.7	45.93
Seeds/pods	NS	71.1	39.8	172.7	29.04	22.43	64.1	14.46
	LS	1.2	0.6	2.1	22.27	16.68	32.9	19.73
BY (g)	NS	41.6	28.5	67.5	28.46	19.78	48.3	28.32
	LS	20.4	9.5	35.0	40.94	21.78	28.3	23.86
100 SW (g)	NS	18.4	9.3	28.2	38.28	32.96	91.7	39.03
	LS	18.8	10.0	27.6	36.41	18.24	46.5	11.1
SY (g)	NS	16.6	5.7	41.3	42.65	43.41	70.4	43.76
	LS	5.49	1.4	19.4	65.70	35.40	53.6	39.05
HI (%)	NS	38.7	20.2	50.5	25.54	18.61	37.6	19.82
	LS	27.0	11.5	55.4	38.68	25.84	54.6	15.57

NS- Normal sown genotypes, LS- Late sown genotypes, FI-Days to Flower initiation, F 50%- Days to 50% flowering, PI- Days to Pod initiation, DM- Days to maturity,

Ht (cm)- Plant height, PB- Primary branches, SB- Secondary branches, Total pods, Effe. Pods-Effective pods, Seeds per pod, BY (g)- Biological yield,

100 SW- 100 seed weight, SY (g)- Seed yield, HI (%)- Harvest index

Table 2. Correlation analysis of yield and its component traits of normal sown (above diagonal) and late sown (below diagonal) desi chickpea genotypes

Characters	Fl	50%F	Pl	DM	PH	P Br	S Br	Pods/Pl	EP/Pl	Seeds/ Pods	BY/Pl	100SW	H I (%)	SY/Pl
Fl	1.0000	0.7835***	0.4122***	0.2835**	-0.0659	-0.1251	0.0778	-0.0037	0.0327	0.0587	0.2560*	-0.1409	-0.0236	-0.2136
50%F	0.9703***	1.0000	0.3167***	0.2371*	-0.1077	-0.0315	0.1162	0.0899	0.1231	-0.1132	-0.1877	-0.1888	-0.0681	-0.1724
Pl	0.5699***	0.6061***	1.0000	0.3444***	0.1323	0.0067	-0.0569	0.2022*	-0.1856	-0.1904	-0.1129	0.1131	-0.0507	-0.1211
DM	0.0206	0.0510	0.2618**	1.0000	0.2867**	0.0035	-0.0370	0.0950	0.0892	--0.0397	0.1666	-0.0014	0.0216	0.1034
PH	0.0733	0.0899	0.0988	-0.1804	1.0000	0.0310	0.2550*	-0.2436	-0.2376	0.0848	-0.1033	0.1027	0.0472	-0.0269
P Br	0.0454	0.0831	0.1094	-0.0892	0.1153	1.0000	0.1808	-0.1668	-0.1602	0.0487	0.2059*	0.1875	-0.1317	0.1083
S Br	-0.1221	-0.1008	-0.0326	-0.0079	0.1540	0.4850***	1.0000	0.0758	0.0525	0.1079	0.1996	-0.0075	-0.1606	0.1079
Pods/Pl	-0.0822	0.0968	0.0399	-0.0187	0.0044	0.3331***	0.4037***	1.0000	0.9639***	-0.0903	0.4483***	0.2812**	0.2236*	0.4397***
EP/Pl	-0.0457	-0.0551	0.0621	-0.0336	0.0272	0.3533***	0.3945***	0.9799***	1.0000	-0.1106	0.3648***	-0.3256	0.2833**	0.4240***
Seed/Pods	0.0479	0.0376	-0.0401	0.0637	-0.0058	0.2611*	0.1718	0.1654	0.1981	1.0000	0.2138*	-0.2078	0.1320	0.1869
BY/Pl	0.0785	0.0696	0.0785	0.2959*	0.2720*	0.2091*	0.1753	0.4038***	0.4329***	0.2315*	1.0000	0.3914***	0.1798	0.7837***
100SW	-0.0703	-0.0820	-0.0721	-0.1488	0.0681	0.0976	0.1183	0.1089	0.1455	0.3408***	0.0520	1.0000	0.3183***	0.5194***
H I (%)	-0.1900	-0.1849	-0.0679	-0.0320	-0.1210	-0.0693	0.0989	0.2923	0.3262***	0.1646	0.2226*	0.0635	1.0000	0.6586***
SY/PL	-0.0275	-0.0392	-0.0118	-0.1224	0.0034	0.1584	0.3453***	0.6067***	0.6242***	0.1868	0.4425*	0.1383	0.6149***	1.0000

Fl-Days to Flower initiation, F 50%- Days to 50% flowering, Pl- Days to Pod initiation, DM- Days to maturity, Ht (cm) - Plant height, PB- Primary branches, SB- Secondary branches, Total pods, Effe. Pods-Effective pods, Seeds per pod, BY (g)- Biological yield, 100 SW- 100 seed weight, SY (g)- Seed yield per plant, HI (%)- Harvest index

It indicates that most likely the heritability is due to additive gene effects contributing to these traits (Panse and Sukhatme 1967). It also reveals that selection would be ineffective for the characters which are highly influenced by environmental effects.

Correlation coefficient: Genotypic correlation coefficient of seed yield per plant was studied with different yield contributing characters. In case of normal sown desi chickpea genotypes (Table.2) seed yield per plant exhibited positive and highly significant correlation with biological yield (0.7837), harvest index (0.6586), 100 seed weight (0.5194), total pods per plant (0.4397), effective pods per plant (0.4240).

In case of late sown desi chickpea, (Table.2) seed yield per plant exhibited positive and highly significant correlation with effective pods per plant (0.6242), harvest index (0.6149), total pods per plant (0.6067), biological yield (0.4425), and secondary branches per plant (0.3453). (Arshad et al 2002) (Bakhsh et al 2006). Seed yield in normal and late sown desi chickpea genotypes were highly significant and positively correlated with total pods per plant (Farshadfar and Farshadfar 2008). Saleem et al. (2002) concluded that pod number and 100 grain weight were the most important traits in chickpea breeding programs. Positive and significant correlation of seed yield per plant with total and effective pods per plant and 100 seed weight can be considered in selection of heat tolerant genotypes (Saleem et al. 2002).

Path coefficient analysis: Genotypic path analysis of different traits contributing to seed yield showed (Table.3) that effective pods per plant (0.4264) and biological yield per plant (0.3873) had highest direct effect on seed yield in normal sown desi chickpea. While seeds per pod have highest indirect effect via harvest index (0.2218) (Arshad et al. 2004) In case of late sown desi chickpea (Table.3) harvest index (0.4466) has revealed highest direct effect. While effective pods per plant had highest indirect effect via total pods per plant (0.1555).

The present study conducted under high temperature conditions indicated that effective pods per plant, biological yield and harvest index had the maximum contribution in determining seed yield under heat stress in desi chickpea. (Yadav et al. 2001) Breeding strategies for improvement of yield potential in normal and late sown chickpea would aim on selection of plants having higher number of effective pods, high biological yield and harvest index along with earliness.

Considering, the mean values of six quantitative traits, *viz*. days to flower initiation, days to maturity, total and effective pods per plant, 100 seed weight and biological yield per plant which were significantly and positively

Table 3. Path coefficient analysis of normal and late sown desi chickpea genotypes

FI	NS	**-0.0980**	-0.0768	-0.0404	-0.0278	0.0065	0.0123	-0.0076	0.0004	-0.0032	0.0058	0.2251	0.0138	0.0023
	LS	0.1441	0.1398	0.0821	0.0030	0.0106	0.0065	-0.0176	-0.0118	-0.0066	0.0069	0.00113	-0.0101	-0.0274
F 50%	NS	0.2311	**0.2398**	0.2126	0.0054	-0.0043	-0.0013	0.0046	0.0036	0.0049	-0.0045	-0.0075	-0.0075	-0.0627
	LS	-0.0156	-0.0161	-0.0097	-0.0008	-0.0014	-0.0013	0.0016	0.0016	0.0009	-0.0006	-0.0011	0.0013	0.0030
PI	NS	0.2028	0.2021	**0.2067**	0.0023	0.0009	0.0001	-0.0004	0.0014	-0.0013	-0.0013	-0.0008	0.0008	-0.0003
	LS	-0.0203	-0.0216	-0.0356	-0.0058	-0.0035	-0.0039	0.0012	-0.0014	-0.0022	0.0014	-0.0028	0.0026	0.0024
DM	NS	0.0071	0.0035	0.2087	0.2252	0.1072	0.0001	-0.0009	0.0024	0.0022	-0.0010	0.0042	0.0001	0.0005
	LS	-0.0010	0.0025	-0.0080	-0.0495	0.0089	0.0044	0.0004	0.0009	0.0017	0.0032	0.0097	0.0074	0.0016
Ht. (cm)	NS	-0.0016	-0.0027	0.0033	0.0071	0.0247	0.0008	-0.0063	-0.0060	-0.0059	0.0021	-0.0026	0.0025	0.0012
	LS	-0.0014	-0.0017	-0.0018	0.0033	-0.0186	-0.0021	0.0029	-0.0001	-0.0005	-0.0001	-0.0032	-0.0013	0.0022
PB	NS	-0.0029	-0.0007	0.0002	0.0001	0.0007	0.2233	0.2042	-0.0039	-0.0037	0.0011	0.2048	0.0044	-0.0031
	LS	-0.0028	-0.0051	-0.0067	0.0055	-0.0071	-0.0614	0.0298	0.0205	-0.0217	-0.0160	-0.0128	-0.0060	0.0043
SB	NS	0.0051	0.0076	-0.0037	-0.0024	-0.0166	0.2118	0.2651	0.0049	0.0034	0.0070	0.2130	-0.0005	-0.0105
	LS	-0.0177	-0.0146	-0.0047	-0.0012	0.0224	0.0704	0.1451	0.0586	0.0573	0.0249	0.0254	0.0172	0.0144
Total pods	NS	0.0004	-0.0092	0.0207	-0.0097	0.0249	0.0171	--0.0078	-0.1022	-0.0986	0.0092	-0.0458	-0.2288	-0.0229
	LS	-0.0060	-0.0071	0.0029	-0.0014	0.0003	0.0244	0.0295	0.0731	0.0717	0.0121	0.0295	-0.0080	0.0214
Effe. Pods	NS	0.0140	0.0525	-0.0791	0.0380	-0.1013	-0.0683	0.0224	0.4110	0.4264	-0.0472	0.1555	-0.2388	0.1208
	LS	-0.0164	-0.0198	0.0223	-0.0121	0.0098	0.1269	0.1417	0.3521	0.3593	0.0705	0.1555	-0.0523	0.1172
Seeds/ pods	NS	-0.0097	-0.0187	-0.0314	-0.0066	0.0140	0.0080	0.0178	-0.0149	-0.0183	0.2652	0.2053	-0.0343	0.2218
	LS	-0.0040	0.0031	0.0033	0.0053	-0.0005	-0.0217	-0.0143	-0.0137	-0.0163	-0.0830	-0.0192	-0.0283	-0.0137
BY (g)	NS	-0.0991	-0.0727	-0.0437	0.0645	-0.0400	0.0798	0.0773	0.2736	0.1413	0.0828	0.3873	0.1516	0.0696
	LS	0.0111	0.0098	0.0111	-0.0277	0.0243	0.0296	0.0248	0.0571	0.0612	0.0327	0.1414	0.0074	0.0315
100 SW (g)	NS	-0.0541	-0.0726	0.0435	-0.0005	0.0395	0.0721	-0.0029	-0.1081	-0.1252	-0.0799	0.2505	**0.3844**	0.1223
	LS	-0.0127	-0.0148	-0.0130	-0.0268	0.0123	0.0176	0.0213	-0.0196	-0.0262	0.0614	0.0094	0.1801	0.0114
HI (%)	NS	-0.0085	-0.0245	-0.0182	0.0078	0.0169	-0.0473	-0.0577	0.0804	0.2018	0.0475	0.0646	0.2144	0.3595
	LS	-0.0848	-0.0826	-0.0303	-0.0143	-0.0541	-0.0310	0.0442	0.1305	0.1457	0.0735	0.0994	0.0283	0.4466

Table 4. Performance of heat tolerant genotypes grown under normal and high temperature stress condition

Chickpea lines	Days to flower initiation		Days to maturity		Total pods per plant		Effective pods per plant		100 seed weight(g)		Biological yield per plant (g)		Seed yield per plant (g)	
	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS
Dilagi	60	45	109	79	137	66	124	60	11.7	10.1	40.0	35.0	20.2	10.2
ICC 9942	66	46	111	88	80	39	62	34	10.2	10.0	28.5	18.0	10.3	8.5
ICCV 10	65	46	111	85	50	44	40	37	15.5	15.4	32.5	20.0	8.9	8.2
ICCV 07115	62	47	111	87	75	45	58	29	13.0	11.5	35.0	28.0	10.1	7.5
JG 11	61	43	112	85	55	53	39	41	23.3	21.0	45.0	26.0	8.6	8.9
JG 130	64	47	110	82	62	52	49	47	19.7	18.8	31.0	31.0	12.7	11.3

correlated with seed yield. On the basis of high seed yield in normal as well as late sown condition following genotypes identified as heat tolerant Dilagi, ICC 9942, ICCV 10, ICCV 07115, JG 130 and JG11. (Chaitanya and Chandrika 2006) and can be utilized in chickpea breeding programme.

अड़तालीस उन्नत देशी चना जीनोटाइप्स को अक्टूबर 2009 (सामान्य बोनी) और फरवरी 2010 (देर से) में बोया गया । विभिन्न अनुवांशिक मापदण्डों अर्थात अनुवांशिक परिवर्तनशीलता, अनुवांशिकता, अनुवांशिक अग्रिम और सह संबंध का विश्लेषण सामान्य और देर से बुवाई देशी चना में किया गया । सामान्य और देर से बुवाई देशी चना जीनोटाईप्स में, कुल फल्ली प्रति पौधा, बीज प्रति पौधा, जैविक उपज प्रति पौधा और 100 बीज वजन लक्षणों के बीज उपज प्रति पौधा शेष सकारात्मक सहबद्धता पाई गई । पथ गुणांक विश्लेषण से पता चला है कि उपज पर उच्चतम प्रत्यक्ष प्रभाव फसल सूचकांक और संयंत्र प्रति जैविक उपज के द्वारा किया गया चौदह में से छः मात्रात्मक लक्षणों को ।उच्च तापमान की स्थितियों का सामना करने वाली देशी चना जीनोटाईप्स को पहचानने के लिये स्तेमाल किया गया । दोनो ही स्थितियों की तुलना के आधार पर गर्मी सहिष्णु जीनोटाइप्स जो कि Dilagi, ICC 9942, ICCv 10, ICCV 07115, JG 130 और JG11 कि पहचान की गई । दोनो स्थितियों में उचित उपज क्षमता और संतोषजनक प्रदर्शन प्रर्दशित करने वाली इन सभी गर्मी सहिष्णु जीनोटाइप्स को विभिन्न कृषि जलवायु में परिक्षण करना चाहिये। जोकि उत्पादकता में सीधे बीज उपज प्रभावित करने वाले लक्षणों को सुधारने में मददगार हो सकती है।

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Characterization of chickpea cultivars grown under rice-fallow situation of Kymore platau zone of Madhya Pradesh, India

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Abstract

Chickpea is the most important rabi season food legume crop. Sixteen chickpea genotypes were evaluated in RBD with three replications (Second fortnight of December) grown under rice-fallow situation during rabi 2010-11 at JNKVV, Jabalpur. Large variation were observed in seed coat colour, Rajas reddish brown, JG11, GCP 105, Vaibhav and ICCV 07111 brown; DCP 92-3 yellow, Pusa 547 and PG 186 yellow brown, JG 14 and JG 16 dark brown ; JAKI 9218, ICCV 05106 and ICCV 07111 light brown. All genotypes had smooth seed surface and angular shape. Subhra, a kabuli genotype had irregular shape and beige seed coat colour. According to seed size Rajas, Pusa 372, Subhra, DCP 92-3, GCP 105, PG 186, JG 16, AIG 21, ICCV 07111 and JAKI 9218 are grouped under small seed size whereas, JG 11, Pusa 547, Vaibhav, ICCV 06107, ICCV 05106 and JG 14 are grouped under large seed size. Study on phenological and yield attributing traits Subhra, JG 14, JG 16, Vaibhav and Rajas were early, high yielding and disease resistance found suitable for rice-fallow situation.

Keywords: variety characterization, seed colour, seed shape, anthocyninin.

Chickpea (*Cicer arietinum* L.) is the most important food legume grown in Madhya Pradesh. Madhya Pradesh produces the major share of around 40% with 3085.5ha area, 3304.1 tonnes production and 1072 kg/ha productivity (http://wiki.icrisat.org). A substantial part of kharif rice area remains fallow during the rabi season. More than 15 million ha land in South Asia is left after rice is harvested at the end of Mansoon (Subbarao et al. 2001). Of this total area 11.65 million ha land is observed in India. Nearly 82% of the rice fallows are located in the Indian states Bihar, Madhya Pradesh, Chhattisgarh, Orissa and Assam. Rice-fallow land can be utilized to grow an additional crop to utilized soil moisture retained into the soil. Chickpea is most suitable crop that can be grown profitably on residual soil moisture in rice fallow with minimum irrigation. Minimum irrigation is a prerequisite to recharge rice fallow and utilize it to support the second crop. Chickpea is well adapted to growing on residual soil moisture in a post rainy (Rabi) season because of its deep and prolific root system (Saxena and Singh 1987). There is an ample scope of expansion of high yielding, disease resistant, short duration chickpea varieties. Early sowing, minimum tillage in rice fallow lands to make chickpea production more economical and sustainable (Pande et al. 2009).

Material and methods

Sixteen genotypes including 12 cultivars of chickpea, recommended for central zone and 4 promising lines from ICRISAT were evaluated in rice-fallow fields in randomized complete block design with three replications during Rabi 2010-11 under All India Coordinated Research Project on Chickpea, at Seed Breeding Farm, JNKVV, Jabalpur. Each genotype was accommodating in 12 rows plot of 4m length with spacing of 30x10cm. Recommended agronomic practices were followed. Visual observation and quantitative traits were reordered according to chickpea descriptors for the characterization of the genotypes (IBPGR ICRISAT; ICARDA 1993).

Results and Discussion

Visual traits are those morphological characters which can be assessed by seeing, touch and some traits can be measured. Seed traits are important for the consumer preference.

Presence of anthocynin

Pigmentation is important visual traits for identification of chickpea genotypes; it was noticed at vegetative stage

Table1. Presence of anthocynin in chickpea genotypes grown under rice-fallow situation

No anthocynin	Subhra, DCP 92-3, ICCV 07111
Low anthocynin	JG 11, GCP 105, PUSA 547, PG 186, JG 16, ICCV 05106, ICCV 06107, AIG 21, Rajas
High anthocynin	PUSA 372, Vaibhav, JG 14, JAKI 9218
Very high	-

of the plants, presence or absence of any other colour on the leaves and stem. It was characterized in four categories viz., no anthocynin, low anthocynin, high anthocynin and very high anthocynin were recorded on the plant. Kabuli types had large creamy seeds with no anthocyanin while desi chickpeas are small seeded with various colours, purplish flowers and have anthocyanin pigmentation (Ali et al. 2010 and Singh et al. 2006). No anthocynin was noticed in Subhra, DCP 92-3 and ICCV 07111 (Table 1). Low anthocynin was observed in Rajas, GCP 105, PUSA 547, PG186, JG16, ICCV 05106, ICCV06107 and AIG 21. High anthocynin was noticed in PUSA 372, Vaibhav, JG 14, and JAKI 9218. Bakhsh et al. (2005) noted presence of anthocynin pigment in Dasht, a chickpea variety.

Seed coat colour

Seed colour was observed by naked eye observations. Royal Horticultural Society (RHS) describes 21 colour codes for mature seed of chickpea. The ability to accurately select for seed coat color, an important export quality trait, would greatly benefit chickpea breeding programs (Hossain et al. 2011). Desi-type chickpea seed has a thicker, irregularly shaped seed coat ranging in color from light tan to black whereas the kabuli-type seed has a thin seed coat ranging in color from white to pale cream. Rajas and AIG 21 had the reddish brown seed coat colour. JG11, GCP105, Vaibhav and ICCV 06107 had the brown seed coat colour. DCP 92-3 showed yellow seed coat colour whereas, PUSA 547 and PG 186 showed yellow brown seed coat colour. JG 14 and JG 16 showed dark brown seed coat colour. JAKI 9218, ICCV 05106 and ICCV 07111 exhibited light brown seed coat colour (Table 2). A kabuli chickpea variety Subhra exhibited beige seed coat colour.

Table 2. Seed coat colour of chickpea genotypes grown under rice-fallow situation

Yellow	DCP 92-3
Yellow brown	PUSA 547, PG 186
Light brown	JAKI 9218, ICCV 07111
Brown	PUSA 372, JG 11, GCP 105, Vaibhav, ICCV 05106, ICCV 06107
Dark brown	JG 14, JG 16
Reddish brown	Rajas, AIG 21
Beige	Subhra

Table 3. Seed shape of chickpea genotypes grown under rice-fallow situation

Angular	Rajas, PUSA 372, JG 11, DCP 92-3, GCP 105, PUSA 547, PG 186, Vaibhav, JG 14, JG 16, JAKI 9218, ICCV 05106, ICCV 06107, ICCV 07111, AIG 21
Irregular	Subhra

Seed shape

Seed shape was grouped in three; all genotypes have angular seed shape (Table 3) except a kabuli genotype, subhra with irregular shape (owl's head shape). Knight et al. (2011) and Wood and Keir (2008) observed two distinct seed shape in desi chickpea lines viz., rounded and angular.

Seed surface

Seed surface categorized in rough, smooth and tuberculated. All genotypes were categorized under smooth seed surface (Table 4). Seed surface also noticed by Upadhyaya et al. (2002).

Table 4. Seed surface of chickpea genotypes grown under rice-fallow situation

Smooth	All sixteen genotypes
Rough	-
Seed size	

Seed size is market preference trait, categorized in two group large and small group on the basis of seed size calculated by seed weight of 100 seeds. 100 seed weight ranged from a minimum of 13g (DCP92-3) and maximum of 34.7g (AIG 21). Malhotra et al. (1997) and Sastry et al. (2007) observed seed size ranges from 10.42 to 58.42g/ 100 seed weight and 3.8 to 65.4g/ 100 seed weight respectively. Out of 16 genotypes nine genotypes viz., Rajas, PUSA 372, Subhra, DCP92-3, GCP 105, PG 186, JG 16, JAKI 9218 and AIG 21 grouped under small size group (<25g/100 seed weight) and seven genotypes viz., JG 11, PUSA 547, Vaibhav, JG 14, ICCV 05106, ICCV

06107 and ICCV 07111 were placed in the group of large size (>25g/ 100 seed weight). Upadhyaya et al. (2002) noticed that kabuli chickpea had greater seed size.

Table 5. Seed size of chickpea genotypes grown under rice-fallow situation

Large	JG 11, PUSA 547, Vaibhav, JG 14, ICCV 05106, ICCV 06107, AIG 21
Small	Rajas, PUSA 372, Subhra, DCP 92-3, GCP 105, PG 186, JG 16, JAKI 9218, ICCV 07111

Bakhsh et al. (2005) found that Dasht, a chickpea variety had large seed with yellow brown seed coat colour and round shape. Pundir et al. (1985), Gyandev and Kurdikeri (2009) also discussed different characters viz., seed shape, seed surface, seed colour and leaf pigmentation of chickpea.

Sixteen chickpea genotypes evaluated based upon naked-eye-observation method depicted that most of desi chickpea type were brown to dark brown seed coat colour and of angular shape. According to choice of consumer, it is important to take preference on seed coat colour. In this investigation 16 genotype of chickpea were grown in rice-fallow situation. Seed yield ranged from 1490 kg/ha to 3620 kg/ha, Subhra, JG 14, JG 16, Vaibhav and Rajas were identified as high yielding, early maturing and disease resistance genotypes under rice-fallow.

चना रबी मौसम की प्रमुख दलहनी फसल है । धान की कटाई के बाद भारत में रबी मौसम के दौरान 11.65 मिली. हेक्टेयर भूमि परती रहती है इस भूमि में उपस्थित नमी का उपयोग करने के लिए चना एक अतिरिक्त फसल के रुप में उगाया जा सकता है । चने की सोलह जीनप्रारुपों (दिसम्बर के द्वितीय पखवाड़े) को ज.ने.कृ.वि.वि., जबलपुर में रबी 2010-2011 के दौरान धान वाली परती भूमि में आर.बी.डी में तीन अनुकरण के द्वारा मूल्यांकन किया गया । सभी जीनप्रारुपों में से जे.जी. 11, जी.सी.पी. 105, वैभव और आई.सी.सी.व्ही. 0711 भूरे रंग के डी.सी.पी. 92-3 पीले , पूसा 547 और पी.जी. 186 पीले भूरे रंग के , जे.जी. 14 और जे.जी 16 गहरे भूरे रंग के, जाकी 9218, आई.सी.सी.व्ही. 05106 और आई.सी.सी.व्ही. 07111 हल्के भूरे रंग के , राजस लाल भूरे रंग के बीज पाये गये । सभी जीनप्रारुप चिकनी बीज सतह और कोणीय आकार के पाये गये । सुभ्रा, काबुली किस्म में अनियमित आकार और हल्के भूरे सफेद रंग के बीज पाये गये । राजस, पूसा 372, सुभ्रा , डी.सी.पी. 92-3, जी.सी.पी. 105, पी.जी. 186, जे.जी 16, ऐ.आई.जी. 21, आई. सी.सी.व्ही. 07111, जाकी 9218 छोटे बीज आकार के तहत समूहीकृत किये गये । अध्ययन में पाया गया कि सुभ्रा, जे.जी 14, जे.जी 16, वैभव और राजस शीघ्र पकने वाली अधिक उपज तथा रोग प्रतिरोधक क्षमता के रुप में धान वाली परती भूमि उगाई जाने वाली उपयुक्त किस्में हैं ।

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Identification of potential genotypes for drought tolerance in advance generation of bread wheat

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Abstract

Fifty four genotypes of advanced wheat lines were planted in a randomized complete block design with three replications at seed breeding farm of Plant Breeding & Genetics, JNKVV, Jabalpur in rabi 2007 under rainfed condition to asses the high temperature tolerant lines. Significant variation due to genotypes for all the characters revealed that the genotypes differ significantly for all the characters under study. In the present investigation, high heritability coupled with high genetic advance was exhibited for characters like plant high, tillers per plant, root biomass, biological yield per plant, grains per spike and spiklets spike density, biological yield per plant, spike length, harvest index, root biomass, 1000 grain weight were positively correlated with grain yield and also had positive direct effect and therefore these traits should be given due importance while practicing selection, aimed for improvement of grain yield. Comprehensive examination of result revealed that the advance generation wheat lines tested for suitability for drought tolerance viz. JWJ 13, JWJ 4, JWJ 27, JWJ 34 and JWJ 52 are found superior than the best check MP 3173. These lines have high grain yield, moderate root length and biomass and moderate to high stem thickness may be given due importance for selection of drought tolerance genotypes of wheat and can also be used as donor for development of drought resistant genotypes.

Keywords: Rainfed wheat, drought tolerance, thermo tolerance, correlation

Wheat (*Triticum aestivum* L. em Thall.) is the important staple food crop and contributes to the extent of about 27% of the total food grain production. In India wheat is being grown in 29.2 million ha area and produce 85 million tons. In M.P. the wheat is grown in 43 lakh ha with a production of 86 lakh tons. The productivity of wheat in M.P. is 1.9 tones. Which is quite low in comparison of productivity at national level (2.9tones/ha). One of the reasons of low productivity in M.P. is lack of high yielding varieties suitable for rainfed/partial-irrigated condition. High temperature during early vegetative phase and at post anthesis, low availability of water and unavailability of power are the major yield barriers. Damage caused by temperature extremes and drought could be minimized or eliminated by framing strategy to develop thermo tolerance genotypes during early growth period. Considering the above facts there is an urgent need to breed the high yielding wheat genotypes tolerant to high temperature and low moisture stress suitable under semi-irrigated condition in the state.

Material and methods

The experimental material comprised 54 genotypes of advance wheat lines including three checks viz., MP 3173, MP 3020 and HI 8627 under rainfed condition and were grown in a randomized completed block design with three replication. Each plot comprised of four rows of 2.5 m length spaced at 30 cm apart. Recommended package of practices were followed to raise a good crop. The observations of yield, yield contributing traits, root characteristics and stem thickness were recorded as per standard procedure. The data were subjected to statistical analysis to workout GCV, PCV, heritability, correlation and path coefficient analysis as per standard methods.

Results and Discussion

The mean, range, phenotypic and genotypic co-efficient of variation, heritability estimates and genetic advance percentage of mean are presented in Table 1. Significant variation due to genotypes for all the characters revealed that the genotypes differ significantly for all the characters under study. Considering the magnitude of phenotypic and genotypic coefficient of variation it was revealed that root biomass, biological yield per plant, number of tiller per plant, grain yield per plant, spikelets per spike, harvest index, spike density, plant height, spike length, grains per spike, root length per plant, thickness of stem and seed index had relatively larger amount of genetic variability. In the present investigation high heritability

Table 1. Parameters of genetic variability and its attributes in advance lines of wheat

Characters		Mean	Range		PCV(%)	GCV(%)	H^2(bs)	GA as %
			Min.	Max.			(%)	of mean
Days to 50% flowering	(X_1)	61.41	54.58	65.21	3.55	3.28	85.2	6.24
Days to maturity	(X_2)	133.96	126.67	139.83	2.48	2.32	87.3	4.46
Plant height	(X_3)	106.99	81.57	139.12	12.94	12.92	99.7	26.7
Number of tillers/plant	(X_4)	3.91	2.67	5.90	22.03	20.07	83.0	37.66
Spike length	(X_5)	9.30	7.21	11.70	13.10	11.74	80.3	21.66
No. of spikelets/spike	(X_6)	15.33	11.07	20.77	15.74	15.08	91.8	29.77
Spike density	(X_7)	1.66	1.18	2.24	15.73	13.77	76.4	24.75
No. of grains/spike	(X_8)	38.21	29.63	49.63	10.64	9.96	87.7	19.21
1000-grain weight (gm)	(X_9)	41.71	32.62	46.37	6.76	6.72	98.8	13.76
Biological yield/plant	(X_{10})	16.79	9.13	28.13	25.40	23.09	82.7	43.25
Harvest index	(X_{11})	41.81	22.29	58.33	16.90	14.23	70.8	24.81
Root length/plant	(X_{12})	11.38	9.07	14.59	11.71	9.27	62.6	15.11
Root biomass	(X_{13})	2.89	1.58	4.45	27.52	25.95	88.9	50.40
Thickness of stem	(X_{14})	4.36	3.63	5.22	7.88	7.64	93.9	15.25
Grain yield/plant	(X_{15})	6.86	4.37	9.66	21.51	18.33	72.7	32.32

coupled with high genetic advance was exhibited for characters viz., plant height, tiller per plant, root biomass, biological yield per plant, grains per spike and spike length. Such values indicated, predominantly the presence of additive gene action in the expression of these traits and consequently, greater chance of improving these traits through simple selection. High heritability coupled with low genetic advance was also observed for days to 50% flowering and days to maturity. Such values indicated non-additive gene action and influenced by the favorable environment rather than genotypes. These results are in agreement with result reported by Shukla et al. (2002), Kumar et al. (2002), Kumar et al.(2003) and Singh et al. (2003).

It was observed that grain yield per plant was positively associated with tiller per plant, biological yield per plant, root biomass, root length per plant, days to maturity, spike length, harvest index, spike density, thickness of stem and 1000 grain weight (Table 2). The path coefficient analysis (Table 3) revealed that spike density, biological yield per plant, spike length, harvest index, root biomass, 1000 grain weight had positive direct effect on grain yield per plant and therefore, these traits should be given due importance while practicing selection, aimed for improvement of grain yield in wheat. These results agree with the findings reported by (Mandal et al.1991; Singh et al. 2002; Shah and Deora 2002; Singh et al.2003; Nayeen and Baig 2003 and Kashif and Khaliq, 2004).

On the basis of findings, the advance generation wheat lines tested for suitability for drought tolerance viz., JWJ13, JWJ4, JWJ 27, JWJ 34 and JWJ 52 are superior than the best check MP 3173. These lines have high grain yield, moderate root length and biomass and moderate to high stem thickness. Thus these lines may be given due importance for selection of drought tolerance genotypes and can be used as donor for development of drought resistance genotypes in wheat.

पौध अवस्था एवं दाने भरने की अवस्था के दौरान अधिक तापक्रम, जल की कमी सिचाई के लिये विद्युत एवं अन्य साधन का आभाव तथा प्रमुख बीमारियॉं गेंहू की उत्पादकता को कम कर देती हैं । अधिक तापक्रम एवं तुषार से होने वाली हानि को गेंहूं में तापरोधी किस्मों के विकास से कम किया जा सकता हैं। अतः गेंहूं में तापरोधी किस्मों के विकास के लिये 54 जीन रूपों को रेंडमाइज ब्लाक डिजाइन के तीन रेप्लीकेशन में सन् 2007-08 में पौध प्रजनन एवं आनुवाशिकी विभाग के बीज प्रजनन प्रक्षेत्र पर असिंचित अवस्था में लगाया गया । समाविष्टों में उपज एवं उपज से संबंधित अन्य गुणों, जड़ संबंधित गुणों एवं तना की मोटाई का आंकलन मानक विधियों से किया गया । किस्मों के कारण सभी गुणों में बदलाव परिलक्षित हुये, इससे यह निश्कर्ष निकलता हैं कि सभी जीन रूप उपज एवं अन्य सभी गुणों को बदलाव के साथ प्रदर्षित करते हैं। अतः इनमें से उपयुक्त जातियों का चुनाव करना सम्भव हैं । परिणामों के विवेचना के बाद यह भी निश्कर्ष निकलता हैं कि गेंहूं कि लाइन क्रमशः जे.डब्लू जे.-13, जे.डब्लू जे.-4, जे.डब्लू जे.-27, जे.डब्लू जे.-34, जे.डब्लू जे.-52 गेंहूं की असिंचित अवस्था के लिये सर्वाधिक उपयुक्त किस्म एम.पी.3173 से अधिक उपज देने वाली एवं उपयुक्त हैं । अतः इन जीन रूपों को असिंचित अवस्था के लिये अनुमोदित किया जा सकता हैं । साथ ही साथ इन किस्मों को गेंहूं में तापरोधी एवं असिंचित अवस्था के लियें किस्मों के विकास में जनक के रूप में उपयोग किया जा सकता हैं ।

Table 2. Phenotypic correlation between yield and its attributes in advance lines of wheat

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
X_1	1.00	0.025	0.369**	0.045	0.752**	0.290**	0.116	-0.132	0.192*	0.165*	0.703**	0.200*	0.521**	0.651**	0.187*
X_2		1.000	-0.065	0.213**	0.115	0.160*	0.221	0.079	0.207**	0.289**	0.143	-0.151	0.153	0.072	0.016
X_3			1.000	0.227**	0.204**	0.016	-0.015	-0.041	-0.111	0.275**	0.393**	-0.122	0.337**	0.334**	0.160*
X_4				1.000	-0.041	0.056	0.356**	0.301**	0.106	0.334**	0.382**	-0.446**	0.251**	0.011	0.247**
X_5					1.000	0.300**	0.140	-0.117	0.263**	-0.030	0.709**	-0.091	0.491	0.751**	0.028
X_6						1.000	0.404**	-0.427	0.522**	0.212**	0.298**	-0.048	0.251**	0.308**	0.330**
X_7							1.000	0.645**	0.623**	0.292**	0.267**	-0.222**	0.359**	0.227**	0.479**
X_8								1.000	0.195*	0.112	0.001	-0.161*	0.143	-0.051	0.202**
X_9									1.000	0.036	0.238**	-0.094	0.270**	0.241**	0.360**
X_{10}										1.000	0.106	0.047	0.401**	0.064	0.391**
X_{11}											1.000	-0.527**	0.516**	0.664	0.214**
X_{12}												1.000	-0.112	-0.168*	-0.074
X_{13}													1.000	0.499	0.326**
X_{14}														1.000	0.328**
X_{15}															1.000

Table 3. Path coefficient based on phenotypic correlation coefficient (showing direct and indirect effect of characters contributing to grain yield per plant in wheat)

	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
X_2	-0.0214	-0.0015	-0.0043	0.0061	-0.0334	0.0455	-0.0184	0.0045	0.0082	0.146	-0.1118	0.0036	0.0028	0.0001
X_3	-0.0014	0.0227	-0.0046	0.0108	0.0032	-0.0033	0.0095	-0.0024	0.0078	0.3997	-0.0905	0.0080	0.0130	0.0008
X_4	0.0046	0.0052	-0.0202	-0.0022	-0.0117	0.0734	-0.0699	0.0023	0.0094	0.0389	-0.3304	0.0060	0.0004	0.0013
X_5	-0.0025	0.0046	0.0008	0.0531	-0.0627	0.029	0.0271	0.0057	-0.0008	0.0722	-0.0671	0.0117	0.0292	0.0001
X_6	-0.0034	0.0004	-0.0011	0.016	-0.2085	0.0834	0.099	0.0113	0.006	0.3028	-0.0357	0.0060	0.012	0.0017
X_7	-0.0047	-0.0004	-0.0072	0.0075	-0.0844	0.2063	-0.1495	0.0134	0.0082	0.2716	-0.1647	0.0086	0.0088	0.0025
X_8	-0.0017	-0.0009	-0.0061	-0.0062	0.089	0.133	-0.2319	0.0042	0.0032	0.0023	-0.1194	0.0034	-0.002	0.0011
X_9	-0.0044	-0.0025	-0.0021	0.014	-0.109	0.1285	-0.0456	0.0216	0.001	0.2418	-0.0699	0.0064	0.0094	0.0019
X_{10}	-0.0062	0.0063	-0.0067	-0.0016	-0.0443	0.0603	-0.026	0.0001	0.0028	0.1075	0.0346	0.0096	0.0025	0.0020
X_{11}	-0.0031	0.0089	-0.0077	0.0377	-0.062	0.055	-0.005	0.0051	0.003	1.0177	-0.3900	0.0123	0.0258	0.0011
X_{12}	-0.0032	-0.0028	0.009	-0.0048	0.0101	-0.0459	-0.0374	-0.002	0.0013	-0.5364	0.7400	-0.0027	-0.0065	-0.0004
X_{13}	0.0033	0.0077	-0.0051	0.0261	-0.0523	0.0741	-0.0332	0.0058	0.0113	0.5252	-0.0833	0.0238	0.0194	0.0017
X_{14}	-0.0015	0.0076	-0.0002	0.0399	-0.0643	0.0469	0.0119	0.0052	0.0018	0.6761	-0.124	0.0119	0.0389	0.0017
X_{15}	-0.0003	0.0036	-0.005	0.0015	-0.0688	0.0988	-0.047	0.0078	0.011	0.2182	-0.055	0.0078	0.0128	0.0052

X_1 Grain yield/plant
X_2 Days to flowering
X_3 Days to maturity
X_4 Plant height
X_5 Number of tillers/plant
X_6 Spike length
X_7 Number of spikelets/spike
X_8 Spike density
X_9 Number of grains/spike
X_{10} 1000-grain weight
X_{11} Biological yield/plant
X_{12} Harvest index
X_{13} Root length
X_{14} Root biomass
X_{15} Stem thickness

* Significant at 5% level; ** Significant at 1% level

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Breeding and evaluation wheat genotypes for terminal heat tolerance

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Abstract

The experimental material comprised of 18 uniform wheat genotypes (advance generation lines - F8) including 3 checks namely GW 366, DL 788-2 and HI 8498 grown under three replication in two date of sowing (irrigated late and very late sown condition) during rabi season 2007-08. Observation were recorded on five randomly selected plants from all the replications for the characters viz- days to 50 % flowering, plant height, grain filling rate –I, grain filling rate – II, number of tillers per plant , number of tillers per meter, spike length, number of spikelet per spike, kernel weight, grain yield per plant, gluten content and biological yield per plant. Analysis for both the date of sowing revealed significant differences for all the character. Large differences for genotypic and phenotypic variances were recorded for harvest index and biological yield per plant and grain yield per plant , number of tiller per plant, grain yield per plant , number of tiller per miter, grain filling rate –I, grain filling rate – II which indicated the substantial influence in the environment. Kernel weight and spike length had high heritability coupled with high genetic advance in both environments and grain filling rate – II in very late sown condition indicated importance of additive genetic variance in expression of these traits while, rest of the traits were seemed to be governed by non- additive variance. Advance lines of cross combination GW 173/UP 2494 followed by NGSN 3/IBWSN 84 and NGSN 5/ HTWYT 35 were found most promising and they are to be utilized in selection and hybridization for the development of variety under late sown and very late sown condition of Madhya Pradesh.

Keywords: Wheat, variability, coheritability, physiological attributes, economic attributes, terminal heat, late and very late sown.

Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and cultivated in different diverse agro climatic condition with an area of 27.0 mha with 80.7 tones production and one of the most important staple crops. Similarly the area, production and productivity in Madhya Pradesh. are 43.0 lakh hectare, 83.0 lakh tones and 1.6 tones respectively with the ratio of cultivated *aestivum* and *durum* wheat is 80:20.Due to high cropping intensity in Madhya Pradesh particularly in Kymore Plateau and Satpura hill zone the farmers used to cultivate wheat in the month of December and January after harvesting of rice, pea and potato.

Under the semi arid zone and under climate change late heat are increasingly frequent and limit grain yield since they coincide with the grain filling period of most cereals, including wheat. High temperature during post anthesis is one of the limiting factor for sustainable wheat production and have adverse effect on various growth stages viz- floral fertility, number of spikelets per plant, number of spikelets per spike, grain filling rate, partioning of photosynthetic, spike weight, grain number, grain weight per spike, biomass and yield. The most important parameter of the wheat for which genotypes are screened during the process of breeding is the grain yield. But determining grain yield is time-consuming as the wheat plants have to be bred until the maturity of the grains. Therefore the selection would be time and energy-saving if a standard test system were worked out based on the genetic variability and correlations of certain morphological, physiological parameters and the late heat tolerance and grain yield. In such condition they need early genotypes tolerance to terminal heat with average production. However many varieties are bred but very few of them are suitable for late sown condition.

In view of aforesaid problems present investigation is an attempt to identify genotypes tolerant to terminal heat, selection criteria and their contribution towards genetic improvement for heat tolerance in wheat during late and very late sown condition.

Materials and Methods

The experiment was carried out under Wheat Improvement Project, Department of Plant Breeding and Genetics at Seed Breeding Farm, College of Agriculture, nd Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur (M.P.) under

irrigated late and very late sown conditions. The experimental area occupied was quite uniform in respect of topography and fertility. Temperature vary from 1.4°C being minimum in December to 39.2 °C being maximum in April. The 77.2 mm precipitation received at grain filling stage in the last week of February and March help wheat crops and save one irrigation. The experimental material comprised 18 uniform wheat lines of advance generation (F7-F8) including three cheeks viz. GW 366, DL 788-2, HI 8498. These crosses were grown in a randomized block design with three replications. Each plot consists of four rows of 2.5 m length and 18 cm apart in both late sown and very late sown conditions. The sowing was done on 14/12/07 (Late sown condition) and 20/01/07 (very late sown condition) by dibbling of seeds in row. The recommended cultural packages of practices for late sown were followed to raise the healthy crop.

The observation were recorded on five randomly selected plants from each plot and from each replication for the character days to 50 % flowering, plant height, grain filling rate –I, grain filling rate – II, number of tillers per plant, number of tillers per meter, spike length, number of spikelet per spike, kernel weight, grain yield per plant, biological yield per plant and crude protein (%).

The data were subjected to analysis of variance. The genotypic and phenotypic coefficient of variances (PCV and GCV) was estimated as per (Burton and Devane, 1953). Broad sense heritability was estimated according to formula given by Hanson et al. (1956) and genetic advance as per formula suggested by Lush (1940) and Johnson et al. (1955). Coheritability was estimated following formula given by Bedard et al.(1971).

Results and Discussion

The analysis of variance for both the date of sowing revealed high significant differences for all the characters indicated the existence of sufficient genetic variability for the characters under studies. The wide ranges of variability is a better scope for selection and utilization of important physiological, yield component and quality attributes in wheat breeding. The larger differences in phenotypic and genotypic variance for harvest index, biological yield per plant, grain yield per plant, number of tillers per plant, number of tillers per meter, grain filling rate I and grain filling rate II indicated the substantial influence of environment. Sedimentation value, spike length per spike, protein content, dry gluten and number of spikelets per spike were recorded moderate genotypic and phenotypic variation where as days to maturity, wet gluten content, days to 50% flowering, plant height and 1000 grain weight were observed least phenotypic and genotypic variation (Table 1). Similar trends have been observed for high phenotypic and genotypic variance in second date of sowing as observed in first date of sowing. The character like kernel weight, protein, dry gluten content and spike length were exhibited moderate phenotypic and genotypic variance while sedimentation value and number of spikelet per spike recorded decreasing trend in second date of sowing. Days to maturity, days to 50% flowering, plant height were noted similar trends (low) whereas, kernel weight recorded decreasing trend in second date of sowing (Table 1).

Genotypic coefficient of variation is reliable measure of genetic variability for different characters as regard to genotypic coefficient of variation. Higher magnitude were observed for harvest index, biological yield, grain yield per plant, number of tillers per meter, grain filling rate-I, grain filling rate-II and number of tillers per plant for both date of sowing. Thus the above characters had sown considerable genetic variation and given due importance. However, low genotypic coefficient of variation was observed for days to 50% flowering, days to maturity and plant height in both date of sowing indicated the high influence of environment on the expression of these characters. These above results are agreement with the finding of Mandal and Sarkar(1996), Moghaddem et al. (1997),Sharma *et al*. (1998), Uddin et al. (1997), Shukla et al. (2000), who have reported high coefficient of variation for grain yield per plant, number of spikelets per spike, harvest index, number of tiller per plant and grain weight per spike.

High heritability coupled with high genetic advance as percentage of mean were observed for grain filling rate-II, spike length, protein content, dry gluten content, 1000 grain weight and number of tillers per plant for both the environment. This indicates substantial contribution of additive genetic variance for this character. On the other hand high value of heritability associated with low genetic advance as percentage of mean were recorded for days to 50% flowering, days to maturity, plant height , number of tillers per meter, number of spikelets per spike, biological yield , harvest index, grain yield per plant and wet gluten content in both date of sowing. This suggested the predominance of non additive variance in the expression of these characters.

In both the date of sowing, the coheritability estimate of grain yield per plant were observed high with number of tillers per plant, number of tillers per meter, number of spikelets per spike, harvest index, biological yield per plant in first date of sowing while, it was recorded high for grain filling rate-I, grain filling rate-II, number of tiller plant, 1000 grain weight, biological yield per plant and protein content in second date of sowing. However,

Table 1. Parameters of genetic variability for yield, its contributing traits and quality attributes of different crosses of wheat (First and second date of sowing)

Characters	Date of sowing	Mean	Range		C.V (%)	D^2P	D^2g	D^2e	PCV (%)	GCV (%)	H_2 (bs) (%)	GA	GA as % of mean
			Mini.	Maxi.									
Days to 50% flowering	I	69.71	62.33	83.50	7.42	27.63	27.11	0.53	7.54	7.47	98.10	13.65	19.58
	II	62.70	54.33	66.00	1.21	10.70	10.12	0.58	5.21	5.07	94.54	8.26	13.17
Days to maturity	I	110.74	104.50	118.50	4.45	25.00	24.71	0.29	4.51	4.49	98.88	13.85	12.59
	II	102.07	94.00	106.00	0.77	11.49	10.87	0.61	3.32	3.23	94.63	16.06	15.73
Grain filling rate-I (mg/day)	I	44.10	31.30	5.60	16.95	0.57	0.55	0.03	17.22	16.84	95.64	11.60	26.30
	II	36.38	24.00	48.00	11.34	0.46	0.29	0.17	18.73	14.90	63.29	9.09	25.05
Grain filling rate-II (mg/day)	I	29.80	22.00	4.00	15.43	0.22	0.20	0.10	15.67	15.13	93.28	15.26	51.20
	II	22.19	16.00	29.00	11.32	0.14	0.07	0.06	16.90	12.55	55.13	7.25	32.26
Plant height (cm)	I	87.85	79.00	105.00	7.64	46.22	40.75	5.47	7.73	7.27	88.16	15.63	17.79
	II	74.67	63.00	85.50	3.40	47.94	41.48	6.46	9.27	8.62	86.51	8.33	11.15
Number of tillers per plant	I	6.30	4.60	9.60	21.93	1.96	1.67	0.28	22.19	20.54	85.58	2.03	32.22
	II	4.10	2.80	5.30	17.42	0.92	0.41	0.51	23.48	15.75	44.97	1.84	44.87
Number of tillers per meter	I	101.59	64.50	133.50	21.12	475.39	410.45	64.94	21.46	19.94	86.34	6.53	6.46
	II	80.82	48.00	105.00	6.61	220.03	191.48	28.55	18.35	17.12	87.02	7.03	8.69
Spike length (cm)	I	10.10	6.80	12.10	13.35	1.85	1.55	0.30	13.47	12.33	83.75	5.43	54.30
	II	8.42	7.30	10.00	4.45	0.72	0.58	0.14	10.10	9.07	80.60	5.92	70.30
Number of spikelets per spike	I	17.98	15.40	21.80	11.21	4.09	3.59	0.50	11.26	10.55	87.75	3.92	21.80
	II	14.30	12.20	16.00	4.85	1.63	1.15	0.48	8.93	7.50	70.48	2.09	14.61
1000-grain weight (g)	I	42.76	34.00	46.20	7.05	9.39	9.2	0.18	7.16	7.09	98.07	14.45	33.79
	II	36.56	26.50	43.70	2.24	23.97	23.30	0.67	13.39	13.20	97.19	17.40	47.59
Biological yield per plant (g)	I	26.59	19.10	41.23	25.64	47.70	42.50	5.20	25.97	24.51	89.09	4.01	15.08
	II	15.11	9.90	23.16	8.58	11.47	9.78	1.68	22.40	20.69	85.31	6.53	10.12
Harvest index (%)	I	47.31	28.36	85.56	36.70	306.63	272.74	33.89	37.01	34.90	88.95	4.90	10.35
	II	47.83	31.26	62.20	13.72	96.54	53.42	43.12	20.54	15.27	55.33	7.80	16.30
Protein percentage	I	9.90	8.16	12.53	11.77	1.38	1.35	0.03	11.87	11.75	98.02	3.98	40.20
	II	10.08	7.80	12.60	2.12	2.05	2.00	0.04	14.21	14.06	97.99	3.36	33.33
Dry gluten (g)	I	31.35	27.60	38.83	10.20	10.56	10.54	0.01	10.36	10.36	99.86	13.08	41.72
	II	35.48	29.96	46.53	3.76	14.54	12.75	1.79	10.75	10.06	87.66	8.00	22.54
Wet gluten (g)	I	35.49	31.03	40.06	7.78	7.81	6.45	1.36	7.87	7.16	82.62	9.05	25.50
	II	39.61	32.86	51.66	3.17	18.09	16.50	1.59	10.74	10.25	91.20	5.92	14.94
Sedimentation value	I	33.71	20.50	45.50	18.30	39.35	39.18	0.17	18.60	18.57	99.56	10.18	30.19
	II	29.56	26.00	36.50	2.03	4.61	4.25	0.36	7.27	6.98	92.16	8.20	27.74
Grain yield/ plant (g)	I	11.38	6.90	19.63	25.46	8.64	8.09	0.54	25.80	24.99	93.72	2.12	18.62
	II	7.16	4.90	9.80	4.54	1.94	1.83	0.10	19.47	18.93	94.54	1.08	15.08

Table 2. Coheritability (%) values for different pairs of characters (First and second date of sowing)

Characters	Date of Sowing	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}
X_1	I	56.14	-19.66	-17.88	-19.16	-6.41	-31.11	30.64	4.16	-8.83	-3.46	1.12	-12.26	-3.68	-6.30	29.94	-10.90
	II	90.59	13.15	-20.93	-9.49	-5.22	-30.84	23.57	18.77	3.35	-1.08	-3.98	5.78	5.18	-4.83	11.57	0.94
X_2	I		-17.50	-33.61	-6.16	2.15	-22.64	-5.46	23.07	-11.42	8.44	-9.94	-6.89	3.78	21.48	26.59	-16.85
	II		16.25	-17.33	-0.18	-7.17	-22.40	-16.59	-13.88	0.99	-3.50	-1.36	5.93	0.81	-5.25	9.84	-5.56
X_3	I			59.41	32.05	5.25	-8.88	12.70	-11.80	24.14	46.36	-38.48	28.23	30.06	7.59	15.94	8.57
	II			46.73	-8.23	-17.06	-13.61	1.62	13.94	8.03	-8.39	37.96	19.72	1.35	8.74	-36.82	27.70
X_4	I				31.88	-0.06	-23.55	37.29	24.70	6.26	15.80	-16.26	43.96	29.88	10.12	-0.81	-5.96
	II				-13.50	-4.10	-27.75	5.27	37.40	27.82	4.14	34.79	-8.97	20.58	41.06	-20.59	34.65
X_5	I					14.89	-27.97	15.47	7.66	-7.37	31.48	-9.02	13.33	42.74	8.57	52.68	8.59
	II					-28.69	-1.73	20.87	33.13	-45.84	-29.20	8.99	-11.96	-15.67	-14.21	7.14	-28.93
X_6	I						12.20	6.82	-12.43	21.33	72.11	-24.18	15.35	22.93	23.52	-8.73	42.81
	II						11.26	-13.84	16.88	13.22	61.90	-24.44	66.38	19.46	23.69	-0.64	43.00
X_7	I							-33.99	-66.95	1.66	-31.94	54.57	-11.49	-36.49	-42.16	-34.61	42.94
	II							13.07	-20.36	-11.03	3.40	-0.69	23.08	-28.82	-25.83	-17.91	-2.72
X_8	I								47.77	24.45	19.93	4.41	33.07	-4.65	-6.65	29.86	12.67
	II								42.96	-32.74	-23.21	11.35	5.33	-36.14	-28.29	-13.78	-11.34
X_9	I									6.56	-3.08	-17.93	12.89	5.11	17.78	25.14	43.33
	II									-8.27	13.95	7.49	-4.98	11.00	18.43	-21.76	17.95
X_{10}	I										29.01	-18.76	6.75	-25.40	-34.84	6.51	-3.03
	II										28.22	20.53	-12.68	35.99	36.71	16.08	44.09
X_{11}	I											-51.63	26.97	31.75	26.77	16.44	26.49
	II											-30.22	18.28	35.45	36.16	-19.50	61.06
X_{12}	I												-1.45	-40.87	-40.98	3.16	50.76
	II												16.93	-14.62	-7.81	-5.71	24.59
X_{13}	I													-7.82	-13.48	16.49	17.78
	II													-32.43	-30.25	-54.16	30.79
X_{14}	I														70.28	29.80	-13.54
	II														76.00	10.78	22.75
X_{15}	I															-0.33	-14.86
	II															5.50	31.57
X_{16}	I																5.31
	II																-27.06

X1	Days to 50% flowering	X7	Number of tillers/meter	X13	Protein percentage
X2	Days to maturity	X8	Spike length (cm)	X14	Dry gluten (g)
X3	Grain filling rate-I (mg/day)	X9	Number of spikelets/spike	X15	Wet gluten (g)
X4	Grain filling rate-II (mg/day)	X10	1000-grain weight (g)	X16	Sedimentation value (ml)
X5	Plant height (cm)	X11	Biological yield/plant (g)	X17	Grain yield/ plant (g)
X6	Number of tillers/plant	X12	Harvest index (%)		

number of tiller per plant and biological yield per plant exhibited high coheritability with grain yield per plant in both date of sowing (Table 2).

Apart from grain yield per plant, days to 50% flowering with days to maturity and spike length, grain filling rate I with grain filling rate-II and protein content, grain filling rate-II with number of spikelets per spike dry gluten content, number of tillers per plant with biological yield per plant and grain yield per plant, biological yield per plant with dry gluten content and wet content, harvest index with grain yield per plant and dry gluten content with wet gluten content had high estimate of coheritability in both the environments. This suggests selection for either of these trends may result simultaneous selection for other co inherited characters. Gupta (1991) and Pateriya (1998) reported high coheritability between grain yield and 1000 grain weight and tillers per plant which is in agreement with the present finding.

Summing over the results, eventually it can be concluded that 1000 grain weight, biological yield and harvest index are the characters keeps immense value for the breeding of wheat suitable under late and very late sown condition. However, grain filling rate II (later stage of grain filling) has sown positive association with grain yield. On the basis of correlation and coheritability for second date of sowing draw attention of breeders should be needed during selection for high temperature condition. The findings which are common in both the environment may be exploited universally for the improvement of wheat in both the condition while results of specific to a particular environment may have greater significant to the particular condition only. These aspects must be considered while planning the breeding strategies and applying the selection pressure for the evaluation of future genotypes of wheat under very late sown condition.

On the basis of per se performance of different genotypes of advance generation crosses; cross GW 173 / UP 2494 followed by cross NGSN-3 / IBWSN-86 and NGSN-5 / HTWT-35 were account to be the best and ranked I, II, III, IV and V respectively. It indicates the stability for yield performance under high temperature at post anthesis in second date of sowing and genotype GW173 / UP 2494 was count to be least sensitive to high temperature as it has sown minimum reduction days for vegetative phase and reproductive phase under high temperature condition of second date of sowing. Crosses like NGSN-2 / CL1545, NGSN-1/ GW 337, PBW 343/ CDWR 9563, YCSN-1/ GW 2001-9 and NGSN-5 / HTWYT-35 also found quite favorable for high temperature condition for grain yield per plant under second date of sowing. The cross NGSN-5 / HTWYT-35, rank IV in second date of sowing and V for first date of sowing is common and least effected by changing the environment and suitable for irrigated late sown and very late sown condition of Madhya Pradesh.

गेहूँ की पीढ़ी 8 की 18 एक रूप लायनों एवं 3 नियंत्रणों (जी.डब्लू 366, डी.एल. 788-2 एवं एच.आई. 8498) को पश्चाताप अधययन हेतु दो विभिन्न बोनी की तिथियों में वर्ष 2007-08 में लगाया गया । पाँच बेतरतीब पौधों को जैसे 50% फूल का खिलना, पौध ऊँचाई, दाना भरण अवस्था 1 एवं 2, शाखा प्रति पौध, शाखा प्रति मीटर, बाली लम्बाई, दाना प्रति बाली, उपज प्रति पौध, ग्लूटेन मात्रा, जैविक उपज प्रति पौध को अवलोकन हेतु चुना गया । दोनों बुआई की तिथियों के गुणों में महात्वर्पुण अर्न्त पाया गया । जैबिक उपज हार्वेस्ट सूँची, दाना उपज प्रति, शाखा प्रति पौध, शाखा प्रति मीटर, दाना भरण अवस्था-1 एवं 2 गुणों का उच्च अन्तर जीनों टीपिक एवं फीनोटीपिक अध्ययन में पाया गया जो कि वातावरण का प्रभाव को इन गुणों पर दर्शाता है । 1000 दानों के वजन एवं बाली लम्बाई में उच्च आनुवांशिकता के साथ-साथ जनेटिक एडवांश दोनों बुवाई की तिथियों में दाना भरण अवस्था 2 में वातावरण के प्रभाव को दर्शाता है । इन गुणों की अभिव्यक्ति में एडीटीव जीन अपना प्रभाव दर्शाता है उपरोक्त अध्ययन में अग्रिम पीढ़ी की जी.डब्लयू 173/यू.पी. 2494, एन.जी.एस. एम. 3/आई.बी.डब्लयू.एस.एन. 84 एवं एन.जी.एस.एन. 5/एच.टी. वाइ.टी. 35 आशजनक पाई गई जिनका प्रयोग चयन एवं संकरण में उच्च पक्षचाताप अध्ययन हेतु देर से एवं बहुत देर से सिचिंत गेहूँ के अध्ययन में किया जा सकता है ।

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Crop contingent planning in *Kharif* season under scarcity condition of Northern Maharashtra

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Abstract

The experiment was conducted at National Agricultural Research Project, Dry land Sub-Centre, College of Agriculture, Dhule during *Kharif* 2009 regarding suitability of different contingent crops under delayed monsoon condition in scarcity area of Northern Maharashtra in respect of yield, economics, Growing Degree Days (GDD) and Helio Thermal Units (HTU). The result indicates that the first sowing date (7th July 2009) recorded significantly higher GMR (Rs. 37459/ ha.), NMR (Rs. 14809/ha) and B:C ratio (1.90) . The cost of cultivation was significantly higher with the second sowing date (22nd July 2009) followed by first sowing because it required one more harrowing than the first sowing date which resulted less weed infestation. The cost of cultivation of third sowing (6th August 2009) was significantly lowest as most of the crops failed and not harvested. Among the different contingent crops it is observed that groundnut recorded significantly higher NMR (Rs. 40612 /ha.) followed by pigeonpea at 1st sowing and at 2nd sowing. In second sowing pigeonpea registered significantly higher NMR (Rs.32865/ ha) but it was at par with fodder maize and groundnut. At third sowing date only pigeonpea registered significantly higher NMR (Rs. 22091/ha.) over the other crops. Similarly, significantly higher B: C ratio (3.18) was recorded by pigeonpea at 1st and 2nd sowing date (2.89) . Where as, it was at par with groundnut (2.53) and fodder maize (2.47). At 3rd sowing (6.8.2009) pigeonpea recorded significantly superior B:C ratio (2.23) over the rest of contingent crops. The highest values of GDD and HTU was recorded in crops sown on 1st sowing i.e. 7th July 2009. The highest GDD (3071) were required by the castor crop and lowest by the gaint bajra (fodder) crop. The same trend was also observed in case of HTU.

Keywords: Contingent planning, scarcity condition, growing degree days and helio thermal unit.

Occurrence of normal rain during South West Monsoon season (June – September) is very crucial for agricultural production as nearly 65% of Indian agriculture is rainfed. Even during a normal rainfall year some regions within the states may get less or scanty rainfall. Timely formulation and implementation of contingent agriculture plan helps to negate/ moderate the ill effects of low/scanty rainfall on production and productivity of crops in such regions. However, the aberration in temporal and spatial distribution makes the crop vulnerable to drought. Such adverse effects on crops can be combated by contingent measures, (Anonymous 2010 and Joel et al. 1996). A farmer may notice that droughts are becoming more persistent and may switch to more drought tolerant varieties of crops. Anticipatory adaptations are measures taken in advance of climate change. These are taken to minimize or offset the effects of climate change.

Material and methods

An agronomic investigation was carried out at National Agricultural Research Project, College of Agriculture, Dhule on medium deep black soil. A split plot design with three sowing dates and fifteen different crops replicated three times were tested. The gross and net plot size was 4.50 x 3.60 m^2. The crops were sown by dibbling method at spacing *viz.,* pigionpea- 45 x 22.5cm, cowpea, 30 x 10 cm, cowpea fodder-30 cm, sorghum fodder-30 cm, maize fodder – 30cm sunflower-45x30, Pearl millet-45x10, pearl millet fodder- 30 cm, soybean 30x10 cm,horse gram 30x15 cm, clusterbean-45x15 cm, castor-60x25 cm, Cotton-45x22.5 cm, groundnut-30x10 cm and sesame 30x10 cm. The recommended dose of fertilizer was applied as per the crops. The crops were sown at three sowing dates *viz.,* 7.7.2009, 22.7.2009 & 6.8.2009. The data on yield and monetary were worked out per hectare. The calculation of growing degree days and helio thermal units required by different sowing dates were worked out for different crops from weather data.

Table 1. Yield of different crops as influenced by sowing dates (2009- 10)

Crops/sowing dates	Grain yield (Qtls /ha)			
	S_1	S_2	S_3	Mean
Pigeonpea	15.28	14.00	11.31	13.53
Cow pea (P.Phandahri)	6.13	4.38	3.01	4.51
Sunflower	11.70	8.51	3.70	7.97
Pearlmillet	21.24	18.94	0.00	13.39
Soybean	19.23	11.37	0.00	10.20
Horsegram	4.77	4.89	6.28	5.31
Cluster bean	47.10	31.41	5.06	27.86
Castor	22.88	13.58	14.03	16.83
Cotton (Desi)	14.21	9.46	2.26	8.64
Groundnut	22.13	16.23	0.00	12.78
Sesame	7.89	3.68	0.00	3.85

Table 2. Green fodder yield of different crops as influenced by sowing dates

Crops/sowing dates	Green fodder yield (Qtls/ha)			
	S_1	S_2	S_3	Mean
Sorghum	448	429	218	365
Cowpea	314	316	168	266
Maize	504	574	176	118
Giant Bajra	687	565	273	508

Table 3. Straw yield of different crops as influenced by sowing dates (2009-10)

Crops/sowing dates	Straw yield (Qtls /ha)			
	S_1	S_2	S_3	Mean
Pigeon pea	10.49	22.22	17.90	16.87
Cow pea (P.Phandahri)	19.56	27.42	17.72	21.56
Sunflower	26.15	25.92	27.98	26.68
Pearlmillet	22.17	16.93	9.49	16.20
Soybean	35.40	31.20	18.10	28.23
Horsegram	21.60	19.75	0.00	13.78
Cluster bean	36.31	32.10	36.83	35.08
Castor	7.40	5.65	3.47	5.51
Cotton (Desi)	86.58	40.74	28.86	52.06
Groundnut	21.77	23.25	13.16	19.39
Sesame	33.12	29.35	14.60	25.69

Table 4. Economics as influenced by sowing dates and Kharif crops

Treatment	GMR (Rs/ha)	Cost of cultivation (Rs/ha)	NMR (Rs/ha)	B:C ratio
A) Sowing dates				
S_1-7.7.2009	37459	20009	14809	1.90
S_2-27.7.2009	28817	20173	8524	1.47
S_3-6.8.2009	11909	17118	-5431	0.67
SE±	1711	674	2206	0.08
CD at 5%	6720	2649	8664	0.34
B) *Kharif* crops				
C_1-Pigeonpea	48091	17341	30750	2.76
C_2-Sorghum(fodder)	18942	10972	4303	1.48
C_3-Cow pea	18542	19830	-1309	0.93
C_4-Cow pea(fodder)	21344	15592	5751	1.35
C_5-Sunflower	20265	16240	4025	1.22
C_6-Pearl millet	13403	13464	494	0.85
C_7-Soybean	22720	16896	5824	1.14
C_8-Horsegram	16664	15550	1082	1.06
C_9-Maize (fodder)	32590	16652	15940	1.91
C_{10}-Cluster bean	33542	28110	-17	1.13
C_{11}-Castor	34705	28536	2747	1.21
C_{12}-Giantbajra (fodder)	24401	14353	6156	1.69
C_{13}-Cotton	27237	34580	-6262	0.76
C_{14}-Groundnut	38880	25480	13399	1.45
C_{15}-Sesame	19550	12902	6628	1.20
SEm±	2454	00	2703	0.13
CD (P=0.05)	6908	00	7609	0.38
C) Interaction				
SEm± betn.levels of A	42.52	00	4683	0.23
CD (P=0.05)	11966	00	13179	0.66
SEm± betn.levels of B	4450	6746	5033	0.24
CD (P=0.05)	12794	26491	14577	0.66
CV%	28.26	00	136	30.21
Mean	26061	19100	5967	1.34

Market rates (Rs./Qtl)

1. Pigeonpea -3500/-
2. Cow pea -4000/-
3. Sunflower -2500/-
4. Pearl millet -1000/-
5. Soybean -2200/-
6. Horse gram-3000/-
7. Cluster bean-1200/-
8. Sesame-5000/-
9. Cow pea (Fodder)- 80/-
10. Maize (Fodder)-80/-
11 Sorghum (Fodder)-45/-
12. Gaint bajra-45/-
13. Cotton -3100/-
14. Groundnut-3000/-
15. Castor-2000/-

Table 5. Interaction effect of different sowing dates on the NMR (Rs/ha) of different crops

Crops Sowing dates	S_1	S_2	S_3
	7.7.2009	22.7.2009	6.8.2009
C_1 Pigeonpea	37294	32865	22091
C_2 Sorm (F)	9410	7856	-4356
C_3 Cow pea	5074	-2275	-6727
C_4 Cow pea (F)	9613	9246	-1605
C_5 Safflower	13157	4538	-5619
C_6 Pearl millet	5698	2780	-6994
C_7 Soybean	23025	5179	-10732
C_8 Horsegram	-29	-237	3505
C_9 Maize (F)	23364	25796	-1339
C_{10} Cluster bean	9906	8851	-18808
C_{11} Castor	9225	-561	-423
C_{12} Giant bajra (F)	5096	10775	2596
C_{13} Cotton	7593	-2736	-23644
C_{14} Groundnut	40612	22286	-22699
C_{15} Sesame	23104	3497	-6717
SEm±	4683		
CD (P=0.05)	13179		
Mean	5967		

Table 6. Growing degree days required by different sowing dates

Crops	Sowing Dates			
	S_1-7th July	S_2-22rd July	S_3-6th August	Mean
Pigeonpea	3039	2998	3092	3043
Sorghum(fodder)	1239	1230	1181	1216
Cow pea	1527	1235	-	1381
Cow pea(fodder)	1254	1237	1330	1273
Sunflower	1868	1800	1682	1783
Pearl millet	2074	1966	-	2020
Soybean	2047	1950	-	1998
Horsegram	2440	2298	2286	2341
Maize	1239	1230	1181	1216
Cluster bean	2486	2451	3039	2658
Caster	3306	3075	2834	3071
Giant bajra (fodder)	1164	1128	1330	1207
Cotton	2883	2653	2411	2649
Groundnut	2015	1723	2014	1917
Sesame	1452	1436	-	1444
Mean	1803	1894	2034	1947

Table 7. Helio thermal units required by different sowing dates

Crops	Sowing Dates			
	S_1-7th July	S_2-22rd July	S_3-6th August	Mean
Pigeonpea	16378	20699	19647	18908
Sorghum (fodder)	3856	5973	6659	5496
Cow pea	8318	7679	-	7998
Cow pea(fodder)	3584	5034	7377	5331
Sunflower	8355	9429	10279	9354
Pearl millet	9789	10897	-	10343
Soybean	9789	10759	-	6849
Horsegram	12840	12710	14071	13207
Maize	3856	6653	6820	5776
Cluster bean	12619	12124	12231	12324
Caster	19137	18733	18227	18699
Giant bajra(fodder)	5108	4236	7377	5573
Cotton	14365	14561	14558	14494
Groundnut	9515	8994	12062	10190
Sesame	6457	8043	-	14500
Mean	9597	10434	11754	10602

Results and discussion

Effect of sowing dates

The maximum grain and fodder yield of the different crops were reported by first sowing date where as in case of horsegram it was maximum at third sowing date. Similar results were also reported by Abdel Rahman et al. (2001) and Gujar et al. (2005).

The data regarding the monetary returns indicates that the first sowing at 7.7.2009 recorded significantly higher GMR (Rs. 37459/ha.), NMR (Rs. 14809/ha) and B:C ratio (1.90) .The cost of cultivation was significantly higher with the second sowing date followed by first sowing because it required one more harrowing than the first sowing date which resulted less weed infestation. The cost of cultivation of third sowing was significantly lowest as most of the crops failed and not harvested.

Interaction effect

The groundnut crop recorded significantly higher NMR (Rs. 40612/ha) followed by pigeonpea in 1st sowing (7.7.2009) and in 2nd sowing (22.7.2009). In second sowing pigeonpea registered significantly higher NMR (Rs.32865 / ha) but it was at par with maize fodder and groundnut. In

the third sowing date only Pigeonpea recorded significantly superior NMR (Rs. 22091/ha.) over the other crops (Dalvi et al. 2010).

The significantly higher B: C ratio (3.18) was recorded by pigeonpea in 1st sowing and second sowing date (2.89), and in 1st and 2nd sowing date It was at par with groundnut (2.53) and maize fodder (2.47). In 3rd sowing (6.8.2009) pigeonpea recorded significantly superior B:C ratio (2.23) over the rest of treatments.

Effect of growing degree days and helio thermal units

The growing degree days (GDD) and helio thermal units (HTU) influenced by different sowing dates and crops are presented in Table 6 and 7. It is observed that the highest value of GDD and HTU was recorded in crops sown on 1st sowing i.e. 7th July 2009. The highest GDD (3071) were required by the castor crop and lowest by the gaint bajra (fodder) crop. These results are conformity with Narwal *et al.* (2003) and Kichar and Ram Niwas (2003). The same trend was observed in case of HTU.

In contingent crop planning 15 days delayed sowing from the first sowing pigeonpea, maize fodder and groundnut were found statistically and economically beneficial . Also, it was observed that 30 days delayed sowing from the first sowing only pigeonpea crop was suitable under scarcity condition of Northern Maharashtra.

कृषि महाविद्यालय धुले (महाराष्ट्र) स्थित राश्ट्रीय कृषि अनुसन्धान परियोजना के अर्न्तगत शुष्क खेती के उपकेन्द्र में वर्ष 2009 के खरीफ मौसम के दौरान प्रयोग किया गया। जिसका उघेश्य महाराष्ट्र के उत्तरी भाग में त्रुटियुक्त वर्षा क्षेत्रों के लिये विभिन्न अनिश्चित फसलों को उगाने की उपयोगिता को फसलों की उपज, आर्थिक लाभ, जी.डी.डी., हिलीयों थर्मल यूनिट को ध्यान में रखते हुये ज्ञात करना था। प्रयोग के परिणामों से फसल की बोनी 7 जुलाई 2009 को करने से सार्थक रूपसे अधिक सकल आय (रूपये 37459/हेक्टेयर), शुध्द आर्थिक आय (रूपये 14809/हेक्टेयर) तथा लाभःव्यय अनुपात (1.90) होना पाया गया। काश्त हेतु अधिक सकल लागत व्यय 22 जुलाई 2009 को फसल की बोनी करने से हुआ हैं। प्रयोग में जॉची गयी फसलों में मूंगफली की फसल की बोनी दोनों तिथियों पर करने से अधिक शुध्द आय (रूपये 40612/हेक्टेयर) होना पाया गया। दूसरी तिथी (22 जुलाई 2009) पर बोनी करने पर अरहर की फसल से आर्थिक रूपसे अधिक शुध्द आय (रूपये 32865/हेक्टेयर) दर्ज की गई जो की चारेवाली मक्का एवं मूंगफल्ली की फसल से प्राप्त आय के समकक्ष थी। तिसरी बोनी की तिथि (6 अगस्त 2009) में अरहर की फसल व्दारा अधिक शुध्द आय (रूपये 22091/हेक्टेयर) अन्य फसलों की तुलना में दर्ज की गयी। इसी तरह सार्थक अन्तर रखते हुये अधिक्तम लाभःव्यय अनुपात (3.18) अरहर की बुआई प्रथम एवं व्दितीय तिथीयों पर करने से प्राप्त हुआ जो की अकेले मूंगफल्ली (2.53) तथा चारेवाली मक्का (2.47) की फसल को अकेले उगाने से प्राप्त होनेवाले अनुपात के बराबर था। अरहर फसल की बोनी 6 अगस्त 2009 को करने से प्राप्त लाभःव्यय अनुपात (2.23) अन्य फसलों से प्राप्त लाभःव्यय अनुपात की तुलना में सार्थक रूपसे अधिक था। अधिक्तम जी.डी.डी. तथा एच.टी.यू. मूल्य फसलों की बोनी 7 जुलाई 2009 कों करनें पर दर्ज किये गये। अधिक जी.डी.डी. (3071) अरंडी की फसल को तथा कम बाजरे की चारेवाली फसल को उगाने हेतू आवश्यक होना पाया गया। हिलीयों थर्मल यूनिट की आवश्यकता भी ठीक वही रही जो की जी.डी.डी. की थी।

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Evaluation of pigeonpea based intercropping systems under scarcity condition of Northern Maharashtra

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Abstract

The experiment was conducted at National Agricultural Research Project, Dry land Sub-Centre, College of Agriculture, Dhule during *Kharif* 2009 regarding suitability of Pigeonpea based intercropping systems under scarcity condition in respect of yield, economics and LER . The result revealed that the maximum pigeon-pea grain equivalent yield (25.12qha^{-1}**)** was recorded in pigeonpea + soybean (1: 3) intercropping system which was significantly higher than all other treatments except the treatment of pigeonpea + cowpea (1:3), pigeonpea + groundnut (1:3), sole cowpea and pigeonpea + sunflower (1:2) which were found at par with each other whereas, the lowest grain equivalent yield was recorded by sole pearlmillet. In case of economics the monetary returns and B:C ratio was found statistically superior due to different intercropping systems. The intercropping of pigeonpea + soybean (1:3) registered significantly higher gross monetory returns (Rs 87,917 ha^{-1}) and net monetary returns (Rs66930ha^{-1}) than other intercropping systems. The maximum cost of cultivation was required for sole groundnut followed by pigeonpea + groundnut (1:3) intercropping system. Among all intercropping systems the maximum B:C ratio (4.67) was reported by pigeonpea + sunflower (1:2) but it was at par with pigeonpea + soybean (1:3), sole cowpea and pigeonpea + cowpea (1:3) and minimum B:C ratio was registered by sole pearlmillet (1.75). In respect of different intercropping systems maximum LER (1.57) was recorded under pigeonpea + soybean (1:3) intercropping system followed by pigeonpea + groundnut (1:3), and pigeonpea + pearlmillet (1:2) while minimum LER (0.85) was observed in pigeonpea + rajmabean intercropping system.

Keywords: Pigeonpea, intercropping systems, scarcity

A medium-duration pigeonpea *(Cajanus cajan* L. Millsp) is usually grown as intercrop. A wide range of crop combination in pigeonpea-based intercropping systems is found in India and eastern Africa (Venkateswarlu and Subramanian 1990). The cropping intensity of dryland condition is also very low compared to other ecosystems. To improve the dryland productivity as well as soil fertility, it is worth to grow two or three crops together either in inter or mixed cropping which can protect crop from total failure due to natural hazards. Pigeonpea is a multipurpose legume crop which provides protein, fuel and organic nitrogen. It is a nutritious pulse yielding 20.40% protein (Razzaque et al. 1986).

Among the agricultural systems, an intercropping system has been proved as a boon to the Indian farmers. Its mean to stabilize the crop productivity in dry land areas and to increase its stability in rainfed area under existing inadequate land and rainfall situations. Under climatic condition of Northern Maharashtra (*Khandesh* region) intercropping of different crops are being considered for maximum yield and monetary returns.

The adoption of intercropping system to overcome the climatic situation is not practiced by the farmers at satisfactory level because of technical knowledge about which type of specific intercropping system should be followed under dryland condition. Therefore, the present investigation was undertaken to evaluate the pigeonpea based intercropping systems under scarcity condition of Northern Maharashtra.

Material and methods

An agronomic investigation was carried out at National Agricultural Research Project, College of Agriculture, Dhule (M.S.) on medium deep black soil. A randomized block design with thirteen intercropping system treatment combinations replicated three times were tested. The thirteen intercropping systems *viz.,* pigeonpea + soybean (1:3), pigeonpea + groundnut (1:3), pigeonpea + cowpea (1:3), pigeonpea + rajmabean (1:3), pigeonpea + sunflower (1:2), pigeonpea + pearlmillet (1:2), sole pigeonpea, sole soybean, sole cowpea, sole rajmabean, sole sunflower, sole pearlmillet and sole groundnut were included in the

investigation. The gross plot size was 4.50 x 3.60 m^2 and Net plot size was 4.10 x 3.00 m^2. The crops were sown by dibbling method except pearlmillet. The spacing for different crops were variable according to the crops *viz.,* pigeonpea -60 x 20 cm; soybean, groundnut, cowpea and rajmabean at 30 x 10 cm; sunflower -60 x 20 cm; pearl millet –45 x 10 cm. The recommended dose of fertilizer 20:40:0 (N:P:K) kg ha^{-1} for pulse crops, 50:75:0 (N:P:K) kg ha^{-1} for soybean and 60:30:0 (N:P:K) kg ha^{-1} for pearl millet and sunflower was applied as a basal dose. The data on preharvest studies *viz.,* mean plant height, number of branches were recorded. The data on post harvest studies *viz.,* grain yield, straw yield, land equivalent ratio and monetary returns were also recorded and worked out same were converted for per hectare.

Results and discussion

Yield

Maximum pigeonpea grain equivalent yield (25.12 qha^{-1}) was recorded in pigeonpea –ICPL-87 + soybean-DS-228 (1:3) intercropping system which was significantly higher than all other intercropping treatments except the treatment of pigeonpea + cowpea (1:3), pigeonpea + groundnut (1:3), sole cowpea and pigeonpea + sunflower (1:2) which were at par with each other (Table 2). The growth characters viz., height, number of functional leaves, leaf area, number of branches and number of pods were higher in same treatment which contributed for grain yield per unit area. Similar results were also reported by Holkar et al. (1991) and Kasbe et al. (2010). This may be because of initial growth of pigeonpea is slow which resulted in more space for soybean growth and development. The lowest grain equivalent yield (8.14 qha^{-1}) was recorded by sole pearlmillet. The second best intercropping system observed was pigeonpea + cowpea (1:3) which was found significantly superior over other treatments except the treatment of pigeonpea + groundnut (1:3), pigeonpea + sunflower (1:2) and sole cowpea.

Land Equivalent Ratio

The differences in land equivalent ratio presented in Table 2. Indicates that the maximum LER value (1.57) was found in pigeonpea + soybean (1: 3) intercropping system as compared to all other intercropping systems. The results are conformity with Katyama et al. (1995). This might be due to that the soil and climate of *Khandesh* region is quite suitable for growth, development and yield of pigeonpea and soybean. The adoption of intercropping system increases total crop yield and land equivalent ratio though the rainfall is uneven and erratic in northern Maharashtra.

Economics

The monetary returns and B:C ratio was found statistically superior due to adoption of different intercropping

Table 1. Mean plant height, number of branches/plant, grain and straw yield of pigeonpea based intercropping system as influenced by different treatments during (2009-10)

Treatment	Plant height (cm)	No. of branches/plant	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})
Pigeonpea + Soybean(1: 3)	143.5	6.5	15.38	34.31
	(44.6)	(5.2)	(15.06)	(12.17)
Pigeonpea + Groundnut (1: 3)	142.0	6.9	13.41	32.29
	(40.4)	(4.86)	(11.09)	(20.29)
Pigeonpea + Cowpea (1:3)	139.2	7.1	9.42	20.38
	(73.8)	(8.26)	(12.33)	(21.03)
Pigeonpea +Rajmabean (1:3)	140.5	7.3	15.39	34.4
	(25.76)	(3.53)	(0)	(0)
Pigeonpea + Sunflower (1:2)	139.3	6.6	12.64	28.25
	(159.2)	(16.62)	(11.59)	(31.07)
Pigeonpea +Pearlmillet (1:2)	135.3	5.9	10.18	22.78
	(170.1)	(5.66)	(22.45)	(42.19)
Sole Pigeonpea	152.8	5.3	18.07	40.38
Sole Soybean	52.15	5.06	20.53	14.20
Sole Cowpea	72.37	8.53	20.97	35.99
Sole Rajmabean	22.68	3.6	0	0
Sole Sunflower	150.22	20.86	15.58	29.56
Sole Pearlmillet	174.44	5.46	27.58	45.38
Sole Groundnut	39.54	5.06	20.99	25.97

Table 2. Mean grain equivalent yield, LER and economics of pigeonpea based intercropping system as influenced by different treatments during (2009-10)

Treatment	Pigeonpea GEY (q ha^{-1})	LER	GMR (Rs ha^{-1})	NMR (Rs ha^{-1})	Cost of cultivation (Rs ha^{-1})	B:C ratio
Pigeonpea + Soybean (1: 3)	25.12	1.57	87917	66930	20987	4.18
Pigeonpea + Groundnut (1: 3)	23.23	1.26	81345	55298	26047	3.11
Pigeonpea + Cowpea (1:3)	23.75	1.11	83148	62088	21060	3.94
Pigeonpea +Rajmabean (1:3)	15.58	0.85	54565	36505	18060	*3.01*
Pigeonpea + Sunflower (1:2)	21.23	1.43	74343	58463	15880	4.67
Pigeonpea +Pearlmillet (1:2)	16.97	1.26	59402	43472	15930	3..72
Sole Pigeonpea	18.31	1.00	64113	47303	16810	3.80
Sole Soybean	12.98	1.00	45457	25744	19713	2.30
Sole Cowpea	23.20	1.00	81201	61390	19810	4.09
Sole Rajmabean	000	000	000	-15810	15810	000
Sole Sunflower	11.29	1.00	39549	22996	16553	2.38
Sole Pearlmillet	8.14	1.00	28494	12241	16253	1.75
Sole Groundnut	18.13	1.00	63489	37029	26460	2.39
SEm +	1.36	NS	43500	4797	-	0.25
CD (P=0.05)	4.04	NS	128759	14199	-	0.75
CV %	13.86	NS	128.36	21.02	-	14.50

Market rates :- Pigeon pea - Rs 3500/qtl Soybean - Rs 2200/qtl, Pearl millet - Rs 1000/qtl Groundnut - Rs 3000/qtl, Cow pea, Sunflower, Rajma bean - Rs 4000/qtl and Straw - Rs 20/qtl

systems. Pigeonpea + soybean (1:3) intercropping system registered the significantly higher net monetary returns (Rs 66930 ha^{-1}) over the other intercropping systems but it was found at par with pigeonpea + groundnut (1:3), pigeonpea + cowpea (1:3), pigeonpea + sunflower (1:2) and sole cowpea (Katayama et al. 1995). The second best intercropping system observed in case of monetary returns was pigeonpea + cowpea (1:3) than other intercropping systems. The maximum B:C ratio (4.67) was noticed under the treatment of pigeonpea + sunflower (1:3) intercropping system which was also significantly superior over other intercropping systems except pigeonpea + soybean (1:3), pigeonpea + cowpea (1:3) and sole cowpea. Similarly, the pigeonpea + soybean (1:3) intercropping system also reported the maximum B:C ratio (4.18) than other intercropping systems.

From this study, it can be inferred that under scarcity condition of northern Maharashtra the sowing of pigeon pea based intercropping system as pigeonpea + soybean (1:3) was found most suitable to sustain under low rainfall, optimum moisture utilization, satisfactory growth and development of base and intercrops which overall affects on sustainable potential yield of the crops.

कृषि महाविधालय धुले (महाराष्ट्र) स्थित राष्ट्रीय कृषि अनुसन्धान परियोजना के अर्न्तगत शुष्क खेती के उपकेन्द्र में वर्ष 2009 के खरीफ मौसम के दौरान प्रयोग किया गया। अरहर आधारित अर्न्तवर्तीय फसल पद्धति का अभावग्रस्त स्थितियों में फसल की उपज, आर्थिक आय-व्यय तथा भूमि समतुल्य अनुपात के लिये उपयोगीता पता करना इस प्रयोग का उद्येश्य था। प्रयोगके परिणाम से अरहर + सोयबीन (1:3) अर्न्तवर्तीय फसल पद्धति से अधिक अरहर दानें की समतुल्य उपज (25.12 क्विन्टल/हेक्टेयर) दर्ज की गई जो की जॉचे गये अन्य सभी उपचारों सें सार्थक रूप से अधिक थी बजाय की अरहर + बरबटी (1:3), अरहर + मूँगफल्ली (1:3), शुध्द बरबटी तथा अरहर + सुर्यमुखी (1:2) फसल पध्दतियों के उपचारों के। इन सभी उपचारों के बीच दानों की समतुल्य उपज में सार्थक अन्तर नही होना पाया गया। शुध्द बाजरे की फसल को उगाने से सबसे कम अरहर समतुल्य उपज दर्ज की गयी। अरहर + सोयाबीन (1:3) अर्न्तवर्तीय फसल पध्दतिसे अधिक सकल आर्थिक आय (रूपये 87917/हेक्टेयर), शुद्ध आय (रूपये 66930/हेक्टेयर) एवं भूमि समतुल्य अनुपात (1.57) अन्य उपचारों की तुलना में दर्ज किया गया। यघपि शुद्ध मुंगफली की फसल उगाने में अधिक काश्त-व्यय हुआ एवं अरहर+सुर्यमुखी (1:2) अर्न्तवर्तीय फसल पध्दति अपनाने पर अधिक लाभ व्यय अनुपात (4.67) प्राप्त हुआ ।

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Micropropagation of Serpgandha (*Rauvolfia serpentina* Lin.)Benth

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Abstract

Rauvolfia serpentina,a medicinal shrub has been successfully micropropagated using shoot apex ,leaf and inflorescence explants cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of IAA, BAP and KIN in two different genotypes (RS-1 & Rajendra Serpgandh Local-1). The highest number of direct as well as callus mediated shoot proliferation was observed on MS medium supplemented with lower and equal concentrations of phytohormones (0.5mg?1-1.0mg?1) shoot apex and leaf explants respectively.The regenerated shoots were transferred to rooting medium containing IBA (0.5mg?1).After root proliferation, the *in vitro* derived plantlets were acclimatized and successfully transplanted into field condition.

Keyword: *Rauvolfia serpentina*, tissue culture, micropropagation

Rauvolfia serpentina(Linn) Benth, Serpgandha an important medicinal plant under shrub, belongs to the Apocynaceae family . The roots are tapering and bitter in taste .These are mentioned as a febrifuge, remedy for the bite of poisonous reptiles,cure for desentary and other painful afflictions of the intestine. The herbal plant is used as medicine for high blood pressure, insomnia, anxiety and other disorders of the central epilepsy (Ghani 1998).The plant is indigenous to India and found in Bihar, Orissa, Chhattisgarh, Madhya Pradesh, West Bengal, Andhra Pradesh, Tamil Nadu, and Kerala states of India. Its roots contain 50 indole alkaloids including the therapeutically important reserpine, deserpidine, rescinnamine, yohimbine, ajmalicine, ajmaline, isoajmaline, ajmalinine, chandrine, rauwolfinine, renoxidine,serpentine, serpentinine. In India *Rauvolfia serpentina* is becoming endangered due to its limited cultivation and indiscriminate collection from the wild. Recently the export of the crude drugs has been restricted by Government of India in order to conserve the natural growth and also to reduce its massive exploitation reported by Ghosh and Banerjee (2003). Seed, stem cutting and root cuttings can propagate the crop. Its seeds have poor viability and poor germination percentage, while propagation by root cuttings is also a limiting factor. This has resulted in the shortage of these alkaloids in the world market (Ghosh and Banerjee 2003, Chaturvedi et al. 2007) can be improved through tissue cultural approaches (Ilahi and Akram 1987, Anitha and Kumari 2006). *In vitro* propagation studies of different plant species has shown that this technique may be a solution for rapid propagation of such selected useful plant species and subsequent exploitation. *In vitro* regeneration of *Rauvolfia* has been reported by many authors (Kukreja et al. 1989, Roy et al. 1994, Baksha et al. 2007).The present paper deals with the plant regeneration from shoot apex, leaf from two different genotypes of *R.serpentina.*

Materials and methods

Two genotypes of *R. serpentina* namely; RS-1 (RS_1) & Rajendra Serpgandh Local-1 (RS_2) collected from J.N.K.V.V.(M.P.) and R.A.U,Pusa (Bihar) respectively were used for present *in vitro* studies. Shoot apex(2cm) and inflorescence (3-4cm) explants were collected from field grown plants but leaves (4-6mm) were collected from in vitro regenerated plants from two different genotypes.These explants were first washed with running water to remove the dirt particles. They were treated with 1% solution of teepol detergent for 10 minutes and then washed with distilled water thrice. The pretreated explants were surface sterilized with 0.1% $HgCl_2$ solution for 5 to 10 minutes under laminar flow chamber. After washing 3 times with sterile distilled water, explants were dissected and kept for inoculation.MS basal medium supplemented

with 3%(w/v) sucrose and 0.8%(w/v) agar was used in the present investigation. The shoot apex, leaf and inflorescence explants were excised and transferred to MS basal medium with different concentrations of phytohormones IAA, BAP and KIN for callogenesis and caulogenesis whereas the regenerated shoots gave the best response in rhizogenesis on IBA. The pH of all the media combinations was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving. Autoclaving was done at 1.06kg cm^{-2} at 121°C for 25 minutes. The cultures were incubated at 24± 2 °C under 2000 lux light intensity provided by white florescent lamp for 16 hours photoperiod. The *in vitro* regenerated plantlets were removed from the vessels, washed gently under running tap water and planted in the plastic pots containing a mixture of FYM(1:1:1).The plantlets were kept in greenhouse for acclimatization before subsequent transfer to the field.

Result and Discussion

The present study of *in vitro* propagation (direct and callus mediated) from two different genotypes of *Rauvolfia serpentina* includes callus formation, multiple shoot formation (Table-1), root formation and acclimatization of regenerated plantlets in the field.

Callogenesis

Callus induction observed from cultured shoot apex and leaf after 6-8 days.Poorest callus induction(17.8%) observed from genotype RS^2 of shoot apex explants only on media supplemented with IAA (1.0mg^{-1}), BAP (1.0mg^{-1}) and KIN (1.0mg^{-1}) contained white colour whereas the best callogenesis (72.9%) was observed from RS_2 on medium containing IAA(0.5mg^{-1}), BAP(0.5mg^{-1}) and KIN (0.5mg^{-1}) which gives white colour and compact friable in nature (Plate 1a).The specific concentration of phytohormones needed to induce callus varies from species to species and even depends on the source of explants(Shah et al.2003).The present study showed that the frequency of callus induction was improved on leaf explants in comparison to shoot apex. Callus induction depends on the plant genotype, the source or origin of the explant and the physiological state of tissue culture (Murashige 1974). Siddique et al.(2004) achieved best callogenesis on media having lower concentration of BAP in comparision to KIN in *Withania somnifera.*

Caulogenesis

Caulogenesis(direct and callus mediated) was developed from shoot apex and leaf explants in all the concentrations of the mentioned media but in case of inflorescence explant direct caulogenesis with average poor response observed after 7-10 days of culture only on medium supplemented with IAA(0.5mg^{-1}), BAP(0.5mg^{-1}) and KIN (0.5mg^{-1}) from both the genotypes (Plate 1b). The best callus mediated caulogenesis (63.2%) was observed after
medium containing IAA (0.5mg^{-1}),
KIN (0.5mg^{-1}) of leaf explants whereas
logenesis (82.7%) was found after 15-
ured shoot apex on the same medium
e RS_2 (Plate 1c,d).Direct organogenesis
her concentration of cytokinin ie; KIN,
ncentration of auxin ie; IAA but in reverse
ned on the leaf disc from *Withania*
ankar and Chinchanikar 1996),but
chieved from leaf disc of *W.somnifera*

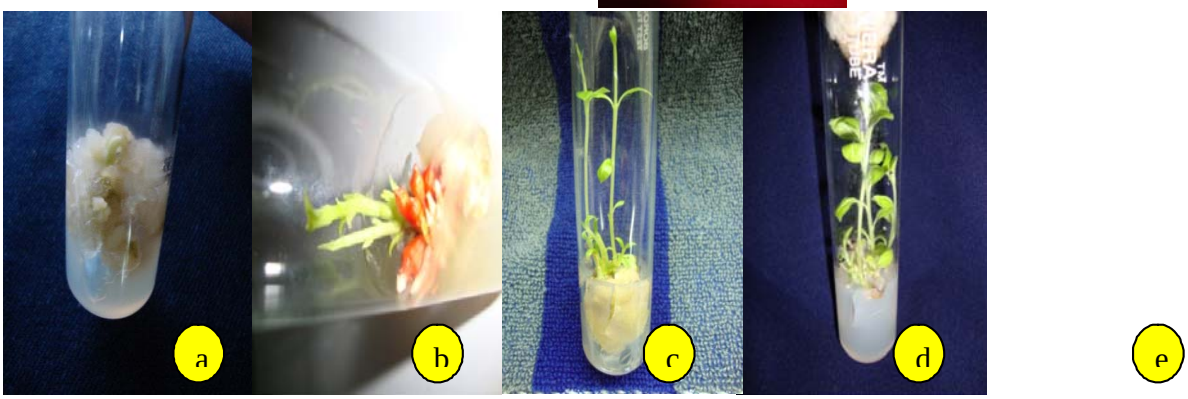

Plate 1: In vitro responses of *Rauvolfia serpentina* (a) Callus induction from leaf explants of *R. serpentina* (b)Initiation of multiple shoots from inflorescence of *R. serpentina* (c)Callus mediated shoot differentiation from leaf explants of *R. serpentina* (d) Direct shoot differentiation from shoot apex explants of *R. serpentina* (e)Rooting of regenerated shoots of *R. serpentina*

Table 1. *In vitro* response from different explants of *Rauvolfia serpentina* in different genotypes cultured on MS media in mg^{-1}

Explant	Response	IAA+BAP+KIN 0.5 + 1.0 +1.0		IAA+BAP+KIN 0.5 + 0.5+ 1.0		IAA+BAP+KIN 0.5 + 1.0 + 0.5		IAA+BAP+KIN 0.5 + 0.5 + 0.5		IAA+BAP+KIN 1.0 + 1.0 + 1.0		IAA+BAP+KIN 1.0 + 0.5 + 0.5	
		RS_1	RS_2	RS_1	RS_2	RS_1	RS_2	RS_1	RS_2	RS_1	RS_2	RS_1	RS_2
Shoot apex	1. Callus formation												
	a) % culture, callus formation	-	-	-	-	-	-	-	-	20.1	17.8	-	-
	b) Nature	-	-	-	-	-	-	-	-	CF	CF	-	-
	c) Colour	-	-	-	-	-	-			W	W		
	d) Callus growth	-	-	-	-	-	-	-	-	+	+	-	-
	2. Shoot differentiation												
	a) % culture, shoot differentiation	37.2	36.9	47.2	62.3	51.2	65.3	70.5	82.7	45.6	55.1	60.4	70.4
	b) No. of shoot/culture												
	Mean	1.70	1.43	1.68	2.11	1.75	3.85	3.11	5.38	1.76	2.45	2.78	3.00
	Range	1-9	1-7	1-10	1-8	1-9	2-11	2-7	2—15	2-6	2-5	1-10	2-8
	c) Shoot growth	++	++	++	+++	++	+++	+++	++++	++	++	+++	+++
Leaf													
	1. Callus formation												
	a) % culture, callus formation	-	-	50.3	42.3	58.5	50.4	70.6	72.9	-	23.5	21.7	-
	b) Nature	-	-	CF	CF	CF	CF	CF	CF	-	CF	CF	-
	c) Colour			WG	W	WG	W	W	W	-	W	GW	-
	d) Callus growth	-	-	++	++	+++	++	++++	+++	-	+	+	-
	2. Shoot differentiation												
	a) % culture, shoot differentiation	43.6	47.3	51.4	38.1	56.1	35.6	58.7	63.2	68.2	46.1	50.4	-
	b) No. of shoot/culture												
	Mean	1.98	1.69	3.12	1.78	2.98	2.31	2.76	3.87	2.11	1.79	1.66	-
	Range	1-5	1-5	2-6	1-7	1-7	1-6	2-5	2-7	1-10	1-9	1-6	-
	c) Shoot growth	++	++	++	++	+++	++	++	+++	+++	+	+	-
Inflore-scence													
	1. Shoot differentiation												
	a) % culture, shoot differentiation	-	-	-	-			34.7	18.7	-	-	-	-
	b) No. of shoot/culture												
	Mean	-	-	-				1.87	1.21	-	-	-	-
	Range	-	-	-				1-7	1-5	-	-	-	-
	c) Shoot growth	-	-	-				++	+	-	-	-	-

1.Callus growth: + Poor , ++ Moderate , +++ Good , ++++ Excellent . 2. Callus colour: W -White, WG -Whitish green,

3.Shoot growth: + Up to 1.0 cm , ++ 1.1 to 3.0 cm, , +++ 3.1 to 5.5 cm , ++++ More than 5.5 cm

cultured on MS medium supplemented with IAA,BAP,NAA,KIN at 0-14µM indivisually or in combination (Pawar et al.2001).The combination of IAA and BAP produced mostly shoot buds except the higher level of BAP where little amount of callus was formed from shoot tip of *Rauvolfia tetraphylla* (Ghosh and Banerjee 2003).In general, KIN has proved to be less effective in shoot bud formation but it was contradicted according to the report of Patil and Jayanthi(1997) who showed the KIN alone or in combination with IAA could induce adventitious shoot buds in axillary shoot cultures of *Rauvolfia tetraphylla*.These contrasting reports might be due to the difference in selecting the initial explants,while Patil and Jayanthi(1997) used the nodal segments Ghosh and Banerjee (2003),which supported the present work.

The present work has demonstrated that the combination of IAA, BAP and KIN in different concentrations is essential for induction of multiple shoots from cultured shoot apex and leaf on both genotypes. Genotype RS_2 was best for direct as well as callus mediated caulogenesis on medium containing IAA (0.5 mg^{-1}), BAP (0.5mg^{-1}) and KIN (0.5mg^{-1}).

Rhizogenesis

The presence of IBA in low concentration was found essential for rooting of R.tetraphylla in comparison to other growth regulators. Ghosh and Banerjee (2003); Baksha et al. (2007) suggested that lower concentration of NAA/ IBA showed best rhizogenesis in comparison to higher concentration in MS media in case of *R.serpentina*. So, for inducing roots, the regenerated shoot buds were separately transferred on MS medium supplemented with IBA containing 0.5mg^{-1} (Plate 1e) .The frequency of root formation was 90% with two weeks and rooted plantlets were successfully transferred to soil in green house.

The induction of direct multiple shoots through shoot apex and callus mediated multiple shoots through leaf from the same genotypes RS_2 has been now as a very powerful tool for micropropagation since the plantlets exhibit genetic stability and genetic variability, respectively.The present work demonstrates a simple procedure for rapid clonal propagation(direct and callus mediated) of *Rauvolfia serpentina.* Micropropagated plantlets can be acclimatized (67%) and subsequently established in the field.

रावलफीया सरपेन्टीना, एक औषधिय पौधे के दो विभिन्न जिनोटाइप्स (आर.एस. - 1 एवं राजेन्द्रा सर्पगंध - 1) के शिर्ष तना, पत्ते एंव पुष्प गुच्छ (एक्सप्लान्ट) के मुरेसिग एवं एस्कुग (एम.एस.) मिडियम जोकि ५।ए BAP एवं KIN के विभिन्न अनुपातो से बनाया गया, में कल्चर करके एक सफल सूक्ष्म प्रबंधन किया गया । प्रत्यक्ष एंव कैलस द्वारा अप्रत्यक्ष प्ररोह वृद्धि सबसे ज्यादा तना शिर्ष एंव पत्तियों (एक्सप्लांट) में क्रमानुसार देखी गयी, जिन्हें (एम.एस.) मिडियम के विभिन्न फाइटोहारमोन्स कम और बराबर मात्रा (0-5 एम.जी$^{-1}$ से 1.0 एम.जी.$^{-1}$) के अनुपात में उपलब्ध ा कराया गया। इन प्ररोहों को जड़युक्त बनानेवाली मिडियम, जिसमें IBA (0.5 एम. जी$^{-1}$) की मात्रा मौजूद है, में स्थानांतरित किया गया। जड़ वृद्धि के बाद इन विट्रो छोटे पौधों को वातावरणीय परिस्थितियों में नियंत्रण कर अंततः सफलतापूर्वक खेत में स्थानांतरित किया गया।

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Influence of plant growth regulators on callus induction and plant regeneration in Madagascar periwinkle (*Catharanthus roseus* L.)

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Abstract

Callus cultures of Madagascar periwinkle (*Catharanthus roseus* L.) were established. Growth regulator combinations were incorporated to Murashige and Skoog (MS) medium for callus induction. Present investigation suggested that, among the media attempted , maximum callusing efficiency was noticed on MS salts + 2mg/l 2,4-D + 2mg/l Kn +100ml/l coconut water followed by MS salts + 2mg/l 2,4-D +1mg/l Kn + 100ml/l coconut water and MS salts+1mg/l 2,4D. Shoot regeneration was maximum in MS salts + 1mg/l NAA +2mg/l BAP followed by MS salts+ 3.0mg/l NAA + 2mg/l Kn. For root regeneration, medium containing 1/2 strength MS salts + 2mg/l IBA was found the most suitable. Absence of light was favorable for root regeneration.

Keywords: Growth regulators, Shoot regeneration, IBA, 2, 4-D

Plant tissue culture technology has been extensively employed for crop improvement in several crops. A number of plant species have been used for generation and propagation of cell-suspension cultures, ranging from model systems like *Arabidopsis* to important monocotyledon or dicotyledonous crop plants like rice, Soyabean, alfalfa and tobacco. Madagascar periwinkle (*Catharanthus roseus* L.) is one of the most extensively investigated medicinal plant. The plant is known to posses more than hundred alkaloids in its various plant parts, many of which have remarkable pharmacological properties. These medicinally valuable alkaloids are present in all the parts of the plant viz., roots, stem, leaves and flowers (Verpoorte etal. 1997; Hughes and Shanks 2002; Samuelsson 1999). The root contains, the major alkaloids Ajmalicine and Serpentine, which are used in the treatment of circulatory diseases (Lemmens etal.1999). The importance of the plant is due to the presence of two bisindole antitumor alkaloids, vinblastine and vincristine. The vinblastine and vincristine tends to lower the number of white cells in blood. A high number of white cells in the blood indicate leukemia. Thus it acts as anti-cancer drug (Creasey1979).

Growth regulators 2, 4-D, NAA, BA and kinetin are frequently used to induce callus tissues in plant species (Misawa1994). They had been chosen for initiation of callus cultures of *C. roseus*. MS medium supplemented with NAA, and kinetin had been utilized by researchers for *in vitro* cultures of other species (Catapan et al. 2001; Catapan et al. 2002; Liung and Lai 2006; Kalidass and Mohan 2009).The seeds of periwinkle are very costly. Seeds collection due to indeterminate growth habit and shattering problem is difficult. The maintenance of seed purity due to its allogamous nature also poses difficulty. Therefore application of tissue culture techniques seems to have potential to overcome aforesaid problem. Hence, an attempt was made to get large population of periwinkle through fast multiplication using tissue culture techniques. The aim of this study is to develop conditions for callus cultures of *Catharathus roseus* L.

Materials and methods

The present experiments were carried out at Tissue Culture Laboratory, College of Agriculture Indore, a constituent campus of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, India.The seeds were obtained from AICRP-Aromatic and Medicinal Plant project, College of Agriculture, Indore. The seed of experimental material Madagascar periwinkle were grown and for *in vitro* culture three *viz.,* cotyledons, stem segment and leaf base were used.

Establishment of callus cultures

Leaf base, cotyledon and stem segments from mature plants of Madagascar periwinkle were used. The explants were collected, washed thoroughly under running tap water for 30 min to remove dirt or dead plant material. The explants were cut with sterilized razor and washed with liquid detergent (Teepol) for 10 min. Thereafter thoroughly washed with distilled water and transferred to 0.25% $HgCl_2$ solution for 5 min for surface sterilization. Finally, these were rinsed with sterile distilled water for four times prior to inoculation. Surface sterilized explants were inoculated onto devised culture medium with various combinations and a concentration of growth hormones. Inositol (100mg) was dissolved in 15ml autoclaved distilled water. A solution of growth hormones [Auxin – Napthalene acetic (NAA) and Indole butyric acid (IBA) and Cytokinin – Kinetin (Kn) and 6-Benzylaminopurine (BAP) of desired combinations and concentration was prepared. Auxins were dissolved in 90% ethyl alcohol and Cytokinins were initially dissolved in 3 drops of 1N HCl and 15ml-distilled water was added. Sucrose (20g) for MS and B5 media and 20g for White's medium was dissolved in100ml warm autoclaved distilled water. Agar (8g) was melted in 300ml-distilled water in two-litre volume conical flask. Then, 100ml of macronutrients, 1ml of micronutrients, 1ml of vitamins, 5ml of $FeNa_2EDTA$, 100mg of inositol and growth hormone solution (as per required composition) were dispensed in conical flasks containing melted agar and final volume of 1000ml was made by adding autoclaved distilled water. The mixture was stirred well and pH of 5.8-6.4 was adjusted. Prepared medium was then poured in test tubes (18ml/test tubes) and plugged with cotton. The medium was sterilized in autoclave at 121^0C for 20 min at15 lb. Inoculation was done under laminar airflow system. All cultures were incubated at 25 ± 2^0C for a photoperiod of 16 hr per day under fluorescent light (about 1200Lux). The experiment was repeated five times for all cultures. Periodic sub culturing was undertaken. Coconut milk was incorporated in the media to stimulate callus induction and shoot proliferation. In the present investigation, ripe coconuts (already dehusked) were selected and the milk created was collected by an air vent. The collected milk was filtered. Supernatant liquid was poured through filter paper to separate the precipitate. The filtered liquid was added to the medium used. About 100ml of coconut milk was used for 1 litre nutrient medium.

Regeneration of root and shoot from callus

The callus obtained from the explants were aseptically removed from the culture tubes and placed in sterilized Petri dishes on laminar airflow hood. The callus was then divided into 5mm pieces with the help of sterilized razor. Crumbs were then picked up by sterilized forceps and inoculated into the media. The culture tubes were then incubated at 25 ± 2'C and 16 hr (light) and 8 hr (dark) photoperiod per day. The shoot regeneration capacity of each medium was observed after 25 days. When shoots attained a height of 40-50 mm, these were removed from the shoot regeneration medium and placed on media in sterilized Petri dishes. Dry leaves and adhering calli were removed with the help of forceps and scalpel. Shoots were then placed onto different rooting media at the rate of one shoot per tube. The basal ends of shoots were slightly dipped into media and the cultures incubated at 25^0C under a 16h photoperiod. Observations were recorded on various explants *viz.*, cotyledon, stem segment and leaf base for induction of callus, and regeneration on different culture media.

Determination of average fresh callus weight

For obtaining average fresh callus weight, the average weight of non-cultured explants was calculated before its inoculation. For this the explants were first washed properly with mild detergent and rinsed several times, then placed on Whatman no1 filter paper. The blotted explants were then weighted on electronic balance and gross weight of ten explants was obtained, further the average weight of each explant was calculated. Then cultured explants in each replication exhibiting callus growth after 28 days of inoculation were transferred to a Petri dish containing filter paper, prior to placing them on electronic balance. The traces of agar medium was carefully removed prior to weighing and the average weight of each callus (culture explants) was calculated. Mean weight of non-cultured explants was subtracted from mean weight of callus samples to obtain average fresh callus weight (mg).

Determination of callusing percentage

Individual category of explants was inoculated into different media (100 test tubes with one medium), under nine replications (i.e. total of 900 test tubes per explants per medium). The callusing was calculated by the formula

$$\text{Callusing percentage (\%)} = \frac{\text{No. of test tubes responded}}{\text{No. of test tubes inoculated}} \times 100$$

The number of test tubes responded in each replication (i.e. the test tubes where the callusing was

observed from the fifteenth to twenty eight day after inoculation) was noted. Rest of the tubes which did not show any response, were considered as not responded or contaminated.

Determination of shoot regeneration capacity of callus

Shoot regeneration ability of all the nine devised culture media was tested. The callus obtained from the explants were aseptically removed from the culture tubes and placed onto sterilized petri dishes on laminar airflow. Callus was then divided into 1mm pieces with the help of sterilized sharp razor. Crumbs were then picked up by sterilized forceps and inoculated onto media. The culture tubes were then incubated at 25 $\pm 2^0$ C under cool white fluorescent tubes for 16 hr photoperiod.

Determination of root regeneration capacity

The root regeneration capacity of all the devised culture media, using their respective rooting medium was tested and reported in cm as the average root length of 25 shoots transferred to the rooting medium.

Statistical analysis

The present investigation was conducted in Completely Randomized Design (CRD) with five replications for each explant under each medium. The skeleton of analysis of variance for various media and explants is given, respectively.

1.Standard error of mean: SEm = $\sqrt{}$(EMS/r)

2. Critical difference: CD = SEm x $\sqrt{}$2x t (Edf at 5%)

Where,

D.F. = Degree of freedom; S.S. = Sum of squares; M.S.S. = Mean sum of squares; E df = error degree of freedom; t = Value of Fischer's table for error degree of freedom at 5% level of probability; r = Number of replications

Prior to carry out the analysis, the percentage data were transferred into angular values using angular transformation.

Result

Determination of best explant for callusing

The data of the callusing percentage and fresh weight using stem segment, leaf base and cotyledon as explants clearly indicated that medium M_4 was the most responding medium irrespective of the explants used (Table 1.2). The evaluation of various explants for their suitability to callusing was undertaken on the basis of Callusing percentage and Average fresh callus weight (Table 1.3). Therefore the best explant for callusing has been determined in M_4 only.

Callusing percentage of different explants on M_4 medium

The mean values of callusing percentage of different explants on the M_4 medium (Table1.4). Thirty eight percent leaf base responded to callusing in M_4 medium followed by stem segment 34.4% and cotyledon 27.8%.Thus, magnitudinal values suggested that the relative effectiveness of different explants for callusing percentage was leaf base > stem segment >cotyledon.

Average fresh weight of callus of different explants on M_4 medium

The average fresh weight of callus produced by different explants (Table1.5) on the M_4 medium revealed that leaf base gave the highest fresh weight (678 mg) followed by stem segment (634mg) and cotyledon (516mg).

It depicted that the leaf base was statistically superior to all the explants closely followed by stem segment. These two explants were though showing numerically and magnitudinal differences, but were found statistically at par. The cotyledon explant recorded the lowest average fresh weight of callus.

Determination of shoot regeneration capacity of callus

The callus with somatic embryos was inoculated onto different media for determining shoot regeneration capacity of callus (Table 1.6). Data recorded on various explants *viz.*, leaf base, stem segment and cotyledon for induction of callus, and regeneration on different culture media. Callusing percentage, weight of callus and regeneration capacity of callus into shoots and roots were the parameters of evaluation of the culture media.

Excellent shoot regeneration was observed on M_5 medium containing MS salts +1mg/l NAA+2mg/l BAP. The M6 medium also gave good results which had MS salt + 3.0mg/l NAA + 2mg/l Kn.M_2 and B_5 Media were unsatisfactory for regeneration. Other media also did not give any satisfactory results. Regarding shoot regeneration the leaf base was the best explant and it showed a very good response in M_5 medium.

Determination of root regeneration capacity of individual shoots on different media

Transferring the regenerated single shoots to rooting medium tested the root regeneration capacity of different media (Table 1.9). For rooting, the salt concentrations of all the media were reduced to half (since less salt concentration is desirable for root initiation) and then the media were compared for their ability to produce rooting on individual shoots. The M_1 was found the best medium for rooting that contained MS (1/2 strength salts) + 2.0mg/l IBA.

Discussion

Callus is an unorganized mass of plant cells and its formation is controlled by growth regulating substances present in the medium. The specific concentration of plant growth regulators needed to induce callus, varies from species to species and even depends upon the source of explant. The present study was conducted with the objectives to find out the best medium and explant for callusing and to study shoot and root regeneration capacities of callus in periwinkle commonly known as *sadabahar.* Nine media were tried under the present study. Of them, seven were of Murashige and Skoog's basal medium with various concentrations and combinations of growth hormones and the other two media were Gamborg B5 and White's media. The stem segment, leaf base and cotyledon were used as explants.

In the present study the mean values of callusing percentage of all three explant indicated that significant differences existed among different media in which M_4 was the best medium which contained MS salts + 2mg/l 2, 4-D + 2mg/l Kn +100 ml/l coconut water. This combination is the best for induction and growth of callusing for periwinkle .Similarly this medium has also been proved as best by many scientists *viz.,* Ahuja etal. (1982),Constabel et al. (1982), Junaid et al. (2006) and Miranda et al. (2003).In present investigation the decreasing order of effectiveness of different media tried was M_4> M_3> M_7> White's> M_2>M_5> B_5 > M_6> M_1.

The superior performance of medium M4 may be attributed to the presence of 2, 4- D and Kn in the medium. It is known that 2, 4-D is a hormonal stimulant for callus induction alone or in combination with Kn. M_3 medium contained 2mg/l 2, 4-D + 1mg/l Kn+100ml/l coconut water in MS salts. The media like M_7 and M_2, although contained fairly good amount of 2, 4-D as compared to kinetin yet showed adequate callusing. These findings were confirmed by Namdeo et al. (2006) who reported the effect of different concentrations of 2, 4-D and kinetin on the developement of callus initiation from leaves of *Catharanthus roseus*. They found that the leaves grown in MS medium supplemented with the 4.52aM 2, 4-D + 4.65 aM Kn +100 ml/l coconut water (MSD) showed the best callus induction (84%). Good friable callus, high biomass and maximum frequency of callus initiation (80%) in MS medium combinations containing 10% coconut water were observed. The effectiveness of different explants for callus induction was also considered and their responses were recorded. Regarding fresh weight of callus, the effectiveness of explants could be ranked as leaf base (38.6% response) > stem segment (34.4% response) > cotyledon (27.8% response). For shoot regeneration medium M5 with MS salts +1mg/l NAA+2mg/l BAP was found to be the best followed by M_6 Medium supplemented with salts + 3mg/l NAA + 2mg/l Kn. The overall superior performance of M_5 and M_6 media may be attributed to the presence of BAP and NAA in the media which are supposed to be highly effective in inducing multiple shoots from callus. Several workers have reported the efficiency of NAA and BAP in combination or alone in inducing morphogenesis of callus as Yuan et

Table 1. Different media used in experiment Media Protocol

Media	Combinations and Concentrations of Hormones (mg/l)
M_1	MS +2mg/l IBA
M_2	MS +1mg/l 2,4-D +1mg/l Kinetin
M_3	MS + 2mg/l 2,4-D +1 mg/l Kinetin +100ml/l Coconut water
M_4	MS+ 2mg/l 2,4- D +2mg/l Kinetin +100ml/l Coconut water
M_5	MS + 1mg/l NAA + 2 mg/l BAP
M_6	MS +3mg/l NAA+2mg/l kinetin
M_7	MS + 1 mg/l 2,4-D
B_5	B5 Salts +0.5mg/l NAA +2 mg/l BAP
White's	White's salts + 2mg/l NAA + 1 mg/l Kinetin +100 ml/l Coconut water

Table 2. Callusing percentage of different explants on different media

Media	Leaf base	Stem segment	Cotyledon
M_1	7.4	6.2	3.0
M_2	18.6	15.6	13.4
M_3	35.2	30.8	22.2
M_4	38 .6	34.4	27.8
M_5	16.0	13.2	11.8
M_6	8.0	6.6	5.4
M_7	26.6	24.2	19
B_5	13	11.8	9.6
White's	23	21	14.8

al. (1994) revealed that MS medium supplemented with 7.0 mg/l BAP and 1.0 mg/l NAA strongly stimulated the formation of shoots, in *Catharanthus roseus* similarly Frabetti et al. (2009) in *Teucrium fruticans* L. and Swanberg et al. (2008) in *Catharanthus roseus* also proved the same. Transferring the regenerated single shoots to rooting medium tested the root regeneration capacity of different media supplemented with IBA (2 mg/l), was proved to be the optimum for *in vitro* rooting. Various other scientists also favoured the results obtained during the present study e.g. Faisal et al. (2006) in *Rauvolfia tetraphylla* L.; Raha et al. (2001) in *Holarrhena antidysenterica* and Sharma and Batra (2006) in *Withania somnifera*.

Table 3. Fresh weight of callus (mg) of different explants on different media

Media	Leaf base	Stem segment	Cotyledon
M_1	111. 8	74. 8	54 .6
M_2	435.2	407	317
M_3	620.8	605. 6	451
M_4	678 .4	634 .8	516. 2
M_5	397	350.4	237. 8
M_6	211.8	162.8	130
M_7	572	547 6	422. 6
B_5	337	320	210
White's	479	452 .4	360. 4

Table 4. Callusing percentage of different explants on M_4 medium

Explants	Average callusing percentage
Leaf base	38 .6 (38.0)
Stem segment	34.4 (34.4)
Cotyledon	27.8(31.8)
SE (m)	1.65
CD (P=0.01)	7.12

Table 5. Average fresh callus weight (mg) of explants on M_4 medium

Explants	Average fresh weight (mg)
Leaf base	678.4
Stem segment	634.8
Cotyledon	516.2
SE (m)	12.93
CD (P=0.01)	55.76

Table 6. Shoot regeneration capacity

Media	Stem segment	Leaf base	Embryo
M_1	+ + +	+ + + +	+ +
M_2	+	+	+
M_3	+ +	+ + +	+
M_4	+ +	+ + +	+
M_5	+ + + +	+ + + + +	+ +
M_6	+ + +	+ + + +	+ +
M_7	+ + +	+ + +	+
B_5	+ +	-	-
White's	+ + +	+ +	+

- **- :** No response,
- **+ :** Poor less than 3 shoots
- **+ +:** Fair, between 4-9 shoots
- **+ + +:** Good, between 10-12 shoots,
- **+ + + +:** Very good, between 12-18 shoots,
- **+ + + + +:** Excellent, more than 18 shoots per 100 calli transferred to regeneration medium

Table 7. Root regeneration capacity of different media

Media	Average primary root length of 25 shoots (cms)	Remarks
M1	2.0	+ +
M2	0	-
M3	0	-
M4	0	-
M5	1.0	+
M6	0	-
M7	0	-
B5	0	-
White's	0.5	+

+ + : Very good root regeneration
+ : Slow growth
- : No response

Development of an efficient callus induction protocol for *Catharanthus roseus* is the first step towards the application of transgenic technology to improve *Catharanthus roseus. In vitro* regeneration system for *Catharanthus roseus* consequently will promote the application of plant tissue culture technology in the area of selection resistance, production of artificial seeds, and genetic transformation.

प्रस्तुत अन्वेशण में कैथेरेन्थस रोजियस या सदाबहार के कैलस तैयार किये गये । कैलस निर्माण के लिये मुराशिज एवं स्कूग (एम.एस.) के कल्चर माध्यम में विभिन्न वृद्धि नियंत्रकों का मिश्रण उपयोग किया गया । परिणाम प्रदर्शित करते हैं कि विभिन्न माध्यमों में अधिकतम कैलस निर्माण की क्षमता एम. एस. लवण + 2 मि. ग्रा./लीटर 2,4 - डी + 2 मि. ग्रा. प्रति लीटर कायनेटिन + 100 मि.ली./लीटर नारियल पानी तथा, जिसके बाद एम.एस. लवण + 2 मि.ग्रा./लीटर 2,4- डी + 1 मि. ग्रा./लीटर कायनेटिन + 100 मि.ली./लीटर नारियल पानी एवं एम.एस. लवण + १ मि.ग्रा./ लीटर २६ ४- डी आते थे । शाखा पुनः निर्माण में सर्वोत्तम एम. एस. लवण + १ मि.ग्रा./लीटर एन.ए.ए. + २ मि. ग्रा./लीटर बी. ए.पी. कल्चर माध्यम था, जिसके पश्चात् एम.एस. लवण + ३.० मि.ग्रा. /लीटर एन.ए.ए. + २ मि. ग्रा./लीटर कायनेटिन थे । जड़ पुनः निर्माण में १/२ एम.एस. लवण +२ मि.ग्रा./लीटर आई.बी.ए.सर्वोत्तम पाया गया । प्रकाश की अनुपस्थिति जड़ निर्माण के लिये अनुकूल है।

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Transient GUS expression and efficient transformation in tobacco (*Nicotiana tabacum*)

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Abstract

An easy, efficient, and reproducible protocol for *Agrobacterium*-mediated genetic transformation in tobacco has been established. Leaf disc explants were used to transform with *Agrobacterium tumefaciens* strain GV3101 containing pCambia 1305.1 vector. Explants cocultivated with bacterium for a cocultivation duration of 10 min resulted in to maximum transformation efficiency (>10%). The cocultivation medium consisting MS fortified with 2 mg.l^{-1} BAP and 1 mg.l^{-1} IBA resulted in to better shoot regeneration from leaf discs, whereas, MS media with 0.2 mg.l^{-1} BAP and 2 mg.l^{-1} NAA led to callus induction and rooting in regenerated shoots. Transformed plants were selected on hygromycin containing medium and verified through GUS expression assay and PCR-based molecular technique.

Keywords : Tobacco, Agrobacterium tumefaciens, PCR-Based molecular techniques, Antibiotic sensitivity

Genetic engineering, as a new area emerged in biology during the 1980s has provided an effective tool for solving fundamental problems in the field of plant genetics. Transgenic plants are suitable models for studying the mechanisms of interaction and regulation of plant genes (effects of *cis* and *trans* inactivation, gene silencing, cosuppression) and for studying the mechanisms of antivirus protection in plants. The simplicity of identification of marker and reporter genes permits assessing the function of transgenes at all possible levels: transcriptional, post-transcriptional and phenotypical (Permyakova et al. 2008).

Tobacco (*Nicotiana tabacum*) is widely used as a model plant system in transgenic research for several reasons: its molecular genetics is well understood, its genomic mapping is almost complete, tobacco plants survive well *in vitro* and under greenhouse conditions and tobacco produces large amounts of biomass. Tobacco plants can be used as living factories to produce proteins and enzymes, which can be extracted, purified and used for the manufacturing of pharmaceuticals and other valuable industrial compounds such as biopolymers. Transgenic tobacco plants are also ideal model organisms for the study of basic biological functions, such as plant-pathogen interactions, environmental responses, growth regulation and senescence.

Plant transformation mediated by *Agrobacterium tumefaciens*, a soil borne plant pathogenic bacterium, has become the most widely used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. There are several problems associated with genetic transformation in some agriculturally important crops like soybean, chickpea etc. In these crops transfer and integration of transgene and then confirmation is a big task for a researcher. During the present investigation, an effort has been made to develop an easy and reproducible protocol for transformation of tobacco as a model system with the aim to validate gene constructs made for other crops.

Materials and methods

Source of *Agrobacterium tumefaciens*

Agrobacterium tumefaciens strain GV3101 containing binary vector pCAMBIA1305.1 (11846 bp) having *GUS* gene with catalytic intron under the control of CaMV35S promoter and *nos* terminator; hygromycin phosphotransferase gene (*hpt*II) as plant selectable marker under the control of CaMV35S promoter and terminator (Plate1A) was obtained from NRCPB, IARI, Pusa, New Delhi. The catalytic intron specifies the expression of GUS only in eukaryotic cells.

Source of plant material

Tobacco seeds were procured from Anand, Gujrat and germinated *in vitro* on MS (Murashige and Skoog 1962) media to obtain sterile plant material. Fully expended leaves of four to five weeks old plants were used in experiments for *Agrobacterium*- mediated genetic transformation using leaf disc explants.

Maintenance of *A. tumefaciens* culture

A. *tumefaciens* was periodically cultured on Luria-Bertanni (LB) agar plates containing kanamycin (50 mg.l^{-1}) and Rifampicin (30 mg.l^{-1}) after every three weeks interval at 28°C in an incubator.

Preparation of explants for culture

Tobacco seeds were taken in 1.5 ml micro centrifuge tube and treated with Tween-20 and mercuric chloride (0.1% w/v) before inoculation into culture bottle containing MS media without any growth regulator and/or fortified with 0.2-0.5 mg.l^{-1} BAP. The bottles were incubated at 25°C and 12 hr photoperiod for about four to five weeks. Leaf discs of 1cm^2 were dissected for culture.

Antibiotic sensitivity of explants

The sensitivity of leaf discs to hygromycin was established prior to actual transformation experiments in order to determine the effective concentration for selection of transformants. The explants (leaf discs) were cultured on MS media with different concentrations of hygromycin (5 mg.l^{-1}, 10 mg.l^{-1}, 25 mg.l^{-1} and 50 mg.l^{-1}) with a control in each for the assessment of antibiotic sensitivity.

Cocultivation procedure

During cocultivation and regeneration experiments, MS media fortified with appropriate concentrations of plant growth regulator was used. The pH of media was adjusted to 5.8 ± 0.1 with addition of 1N HCl or 1N NaOH, and gelled with adding 8g agar per liter followed by steam sterilization at 121ºC under 15 psi pressure for 20 min. Warm culture medium, still in liquid state was poured either into pre-sterilized glass Petri dishes (30-35ml/ Petri dish) or culture bottles (40-45 ml/bottle) as per requirements under aseptic conditions of laminar air flow cabinet.

On first day the *A*. *tumefaciens* was cultured by inoculating a loop full of bacterial colonies in 20 ml LB broth with antibiotics rifampicin (30 mg.l^{-1}) and kanamycin (50 mg.l^{-1}) followed by incubation at 28°C with shaking at 190 rpm for 16 hrs in a incubator shaker. On second day, the bacterial culture was centrifuged at 10000 rpm for 5 min and the pellet was saved and supernatant was discarded. The pellet was suspended into 20 ml of liquid cocultivation medium (MS 0.2B2N or MS 2B1IBA). At the same time in a deep Petri dish, the leaves of tobacco were cut into small discs of size 1 cm^2. To prevent leaf discs from drying, the leaves were cut while immersed into liquid cocultivation medium. There after *Agrobacterium* suspension and leaf discs were cocultivated in a deep Petri dish for different time intervals (5, 10, 15 and 20 min) and swirled for proper contact of *Agrobacterium* and tobacco leaf discs. After cocultivation leaf discs were washed with liquid cocultivation medium, air dried on sterile blotting paper and then inoculated on solid cocultivation media. Inoculated plates were incubated in dark for three days at 25°C temperature.

Removal of *Agrobacterium* after cocultivation

After incubation in dark, cocultivated leaf discs were washed in liquid cocultivation medium containing cefotaxime for 10 to 15 min. and air dried on sterile filter paper. Antibiotic cefotaxime of concentration 250 mg.l^{-1} was used through out the study unless otherwise mentioned. The treated leaf discs were re-inoculated on MS media supplemented with BAP (2 mg.l^{-1}), IBA (1 mg. l^{-1}) and cefotaxime for direct shoot regeneration. For callus induction, MS media fortified with BAP (0.2 mg.l^{-1}) and IBA (2 mg.l^{-1}), containing cefotaxime was used. The plates were incubated at 25±2°C with a photoperiod regime of 12 hours at 1200 lux luminance provided by photo synthetically active radiation (PAR) lamps. The plates were subcultured within ten days on the same medium.

Selection of transformants

Two to three weeks after inoculation of cocultivated leaf discs, the regenerating shoots from leaf discs were transferred to MS media fortified with BAP (2 mg.l^{-1}), IBA (1 mg.l^{-1}), cefotaxime and hygromycin (50 mg.l^{-1}).

Acclimatization of putative transformants

Regenerated shoots were transferred to culture bottles containing MS media with cefotaxime for the development

of roots. The roots of regenerated plantlets were rinsed in luke warm sterile water to wash-off the adhered media and the plantlets were transplanted in plastic pots containing a 1:1:1 mixture of autoclaved sand, FYM and soil. Pots were kept in polyhouse under growth conditions of 30 ± 2oC and 60±5 % RH for 30 days. During this period half strength liquid MS salts were provided for nutrition.

Histochemical assay of GUS activity

The putative transformants regenerated after cocultivation of leaf discs with *Agrobacterium tumefaciens* GV3101 surviving hygromycin selection were analysed through histochemical assay for the presence of b-glucuronidase activity adopting the method described by Jefferson (1987).

DNA Isolation and quantification

Genomic DNA from putative transgenic plants was isolated adopting method described by Saghai-Maroof et al. (1984) with suitable minor modifications. Isolated DNA was quantified by measuring the absorbance at 260nm and 280nm in a UV-spectrophotometer and diluted to a concentration of 25 ng/ml.

Polymerase chain reaction (PCR) with transgene specific primers

PCR amplification was carried out in 20ml reaction volume containing 1 X PCR buffer, 1.5mM $MgCl_2$, 100µM dNTPs, 10pmol of each primer, 1unit Taq Polymerase and 25ng template DNA using Thermo Hybrid (*Px2*) programmable thermal cycler. CAMV 35S sequence specific primers (Forward: GCTCCTACAAATGCCATCA and Reverse: GATAGTGGGATTGTGCGTCA) and *hpt* gene specific primers (Forward: TCGTCCATCACAGTTTGCC and Reverse: AAAAGCCTGAACTCACCGC) were used for molecular confirmation of transformed plants. Reaction conditions for both the transgenes were initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 52 ºC for 1 min and primer extension at 72 ºC for 2 min, a final extension at 72 ºC for 10 min was given before completion of the reaction.

Gel electrophoresis of PCR products

PCR amplified products were mixed with gel loading dye and subjected to electrophoresis on 1.5 % agarose gel along with appropriate size DNA ladder (100bp or 1kb depending on expected size of products) and run under a constant voltage of 80 volt. The gels were stained in ethidium bromide solution and visualized under UV light in gel documentation system and photographed.

Results and Discussion

The present study was carried out in tobacco plant, *N. tabacum* because it is a plant that can grow quite easily in the sunny, hot and humid climates. The plant can be easily maintained in both *in vivo* and *in vitro* conditions, due to its growth conditions and various other factors (Pugalendhi et al. 2008).

The surface sterilized seeds were germinated on three different media combinations. The MS media without any growth regulator showed normal germination with roots and single shoot but with very low amount of leaf material. Since the experiment consisted of the use of leaf disc as explant, large amount of leaf material was the prime aim. So for, further experiments, seeds were germinated on MS media added with two concentrations of BAP i.e. 0.2 $mg.l^{-1}$ and 0.5 $mg.l^{-1}$. Addition of BAP resulted into multiple shoots from a single seed and produced large amount of leaf material for experiment (Plate 1). However addition of BAP influenced very slow rooting (>15 days) the leaves were thicker than normal. This was as consistent with the fact that a cytokinin e.g. BAP favours higher shoot proliferation. MS media without any growth regulators was used by Huang et al. (1997); Biemelt et al. (2003); Kang et al. (2003) and Tang et al. (2005) whereas, Soliman et al. (2009) used half strength MS medium for tobacco seed germination. Klaus et al., 2003 used B_5 medium to germinate the seeds of wild tobacco. The production of large number of shoots from a single seed on MS medium fortified with a small amount of BAP (0.2mg/l and 0.5mg/l) is a very useful way when there are limitations of seeds.

For the success of genetic transformation, establishment of *in vitro* plant regeneration system from explant culture is a prerequisite. Tobacco leaf discs of the size 1 cm^2 inoculated on MS 0.2B2N medium containing 0.2 $mg.l^{-1}$ BAP and 2 $mg.l^{-1}$ NAA showed callus induction from the periphery of the leaf discs after one week of the inoculation. Upon subculturing after 21 days on the same medium calli were developed profusely. However, some of the explants showed shooting and rooting on the same medium. Leaf discs derived calli after transfer to MS 2B1I medium containing 2 $mg.l^{-1}$ BAP and 1 $mg.l^{-1}$ IBA developed multiple shoots in two weeks of culture. This is probably due to the influence of cytokinin (BAP) which influenced shoot development. It was also

observed that leaf discs inoculated on this medium resulted direct shoot regeneration.

Regenerated multiple shoots were transferred to plain MS media (without any growth regulator). Rooting was observed within 1-2 weeks of culture. Completely developed plants were transferred for hardening.

In the absence of antibiotics, the leaf discs regenerated normally and produced multiple shoots on the periphery. The shoot regeneration capacity of leaf disc was inhibited even at 25 mg.l^{-1} concentration of hygromycin but they expanded to some extent before bleaching. However, the growth of leaf discs was considerably restricted and bleaching was complete at 50 mg.l^{-1} hygromycin within three weeks of culture. Hence, this concentration was used for the selection of transformed cells in cocultivated leaf discs. Higher concentrations of hygromycin were too toxic to leaf discs and caused immediate bleaching.

There was no significant effect of cocultivation medium on transformation frequency (Table 1), but it affected the morphogenesis from explants. The MS media fortified with 2 mg.l^{-1} BAP and 1 mg.l^{-1} IBA (MS2B1I) resulted direct shoot regeneration from leaf discs, whereas, MS media with 0.2 mg.l^{-1} BAP and 2 mg.l^{-1} NAA (MS0.2B2N) led to callus induction. The explants inoculated on MS 2B1I showed better regeneration of shoots as compared to MS 0.2B2N. Rooting from callus was observed within 10 to 15 days in MS 0.2B2N medium. Some non-morphogenic calli were also observed during experiments.

On the basis of the number of putative transformants obtained, cocultivation duration of 10 min was proved to be the best with higher transformation efficiency. The results obtained with different cocultivation periods are presented in Table 1. The transformation efficiency for different cocultivation durations was calculated as number of hygromycin resistant calli/shoots developed from total number of cocultivated explants during each exposure and expressed in percent.

Putative transformants were selected on hygromycin (50 mg.l^{-1}) containing medium which is lethal concentration for normal leaf discs. Nagl et al. (2005) used a lower concentration of hygromycin (20 mg.l^{-1}) whereas, higher concentrations 25 mg.l^{-1} (Singla-Pareek et al. 2003), 30 mg.l^{-1} (Islam et al. 2007) and 50 mg.l^{-1} (Bhatti and He 2009) of hygromycin were used for the selection of putative transformants of tobacco. However, lower concentrations of selection agent leads to more false positives. The variations in antibiotic concentrations may be associated with tobacco genotypes.

Table 1. Effect of cocultivation durations on transformation efficiency in tobacco

Co-cultivation duration	Cocultivation medium (MS with growth regulators) calli/shoots	Average number of Hygromycin resistant	Transformation frequency (%)
5 min	2B1I	11	8.01
	0.2B2N	9	8.04
10 min	2B1I	18	10.14
	0.2B2N	17	10.06
15 min	2B1I	9	8.22
	0.2B2N	12	8.04
20 min	2B1I	10	7.88
	0.2B2N	9	7.62

The cocultivated leaf discs were subjected to histochemical assay (Jefferson 1987) after three days of co-culture. Cocultivation duration had marked influence on the transient GUS expression. The GUS expression was higher (characterized with larger blue stained area) in leaf discs cocultivated for a time period of 10 min than that of 5 min cocultivation duration (Plate 1 E, F).

After selection on media containing hygromycin, the putative transformed calli and shoots were assayed for transient GUS expression. Blue stained areas could be clearly visualized on transformed calli and shoots (Plate 1 G, H) while untransformed calli and shoots were completely bleached and devoid of any stained spots. *GUS* positive reaction of these hygromycin resistant transgenic plants during histochemical assay indicates insertion of the whole *GUS* and hygromycin cassette and expression of these two foreign genes.

Since, complete sequence of the binary vector pCAMBIA 1305.1 is available at Genbank repository of NCBI, therefore *in silico* prediction of PCR amplified product was carried out using primer sequences used in present study through Primer-BLAST tool of NCBI. It showed a predicted product of size 195bp for CaMV35S primers and a product of size 499bp in case of hpt II primers.

Single colony of *Agrobacterium* strain was used as template to test the *in silico* predicted product size as well as for its further use as a positive control. In case of CaMV35S primers, a product of approximately 195bp was found while hptII primers led to a product size of 499bp as predicted *in silico*.

The genomic DNA from randomly selected transformed plants was analysed using gene specific primers through PCR with negative control (nontransformed plants) and the positive control (colony of *Agrobacterium*). A total of five samples selected as putative transformants on the basis of resistance for hygromycin in selection media after 15 days of co-culture were subjected to analysis. All the putative transformants gave positive results with a band size of approximately 499bp with gene specific primers for *hpt*II gene (Plate 1 K) and a product of 195bp size in case of CaMV35S specific primers. Same band was present in positive control, indicating successful transformation.

Histochemical and Molecular analysis employing polymerase chain reaction clearly indicated the integration of the transgene from the T-DNA region of *Agrobacterium* to the tobacco host genome. Although, the transformation efficiency was less i.e. approximately 10% but this could serve as a sound basis to prove the success of genetic transformation of tobacco in the present investigation.

तम्बाकु में आनुवंशिक रुपांतरण हेतु एग्रोबैक्टेरियम के माध्यम से एक सुगम, सक्षम एवम पुनः प्रस्तुत करने योग्य प्रोटोकॉल स्थापित किया गया है। पत्र चकत्ती (पत्ती के 1 से.मी × 1 से.मी टुकडे) को कर्तोत्तको के रुप मे इस्तेमाल कर पी कैम्बिया 1305-1 संवाहक वाले एग्रोबैक्टेरियम टुमेफेसिएन्स स्ट्रेन जी वी 3101 के साथ रुपांतरित किया गया। जीवाणुओ के साथ 10 मिनट की कोकल्टिवेसन अवधि तक कोकल्टिवेट किये गये कर्तोत्तको मे सबसे ज्यादा रुपांतरण क्षमता (>10% प्राप्त की गई। कोकल्टिवेसन माध्यमो मे से 2 मि.ग्रा./ली. BAP तथा 1 मि.ग्रा./ली. IBA से समृद्ध एम एस माध्यम पत्र चकत्ती से घड पुनर्जनन हेतु बेहतर माध्यम पाया गया, जबकि 0.2 मि.ग्रा./ली. BAP तथा 2 मि. ग्रा./ली. NAA से समृद्ध एम एस माध्यम से कैलस प्रेरण एवं पुनर्जनित धडो मे जड का विकास प्राप्त हुआ। रुपांतरित पोधो को हाईग्रोमाइसिन वाले माध्यम मे चयनित कर GUS अभिव्यक्ति परीक्षण तथा PCR आधारित आण्विक तकनीक द्वारा सत्यापित किया गया।

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Potential of hybrids in breaking yield barrier of pigeonpea in Madhya Pradesh

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Abstract

In India, Madhya Pradesh occupies an important place as far as pigeonpea (*Cajanus cajan* (L.) Millsp.) area and production are concerned. The productivity of the crop has not shown any significant positive change over a period of time. Therefore, to achieve a breakthrough in the yield potential of pigeonpea, the CMS-based hybrids developed at ICRISAT, were introduced and evaluated along with the best performing local check. In the on-station trials conducted at eight locations, the hybrids ICPH 2671 (51% superiority), 2740 (37% superiority), and 3762 (36% superiority) were found promising for seed yield. The on-farm trials, conducted at 7 locations spread over six agro-climatic zones in the eastern Madhya Pradesh during Kharif 2009-10, demonstrated 31.6% yield advantage of hybrid ICPH 2671 over check variety Asha. The data suggested that the pigeonpea hybrids have great potential for enhancing the crop productivity in Madhya Pradesh. The hybrid seed production programmes showed that quality hybrid seed can be produced successfully in Madhya Pradesh with the mean hybrid yield of 2242 kg ha^{-1}.

Keywords: hybrid seed production, CMS, agro-climatic zones

Pigeonpea is an important crop of Madhya Pradesh (M.P.) with annual area and production of 0.32 million ha (2008-09) and 0.26 million tons (2008-09), respectively. The stagnant productivity of pigeonpea 804 kg/ha (2008-09) had been a challenge to the plant breeders for the few decades. The absence of high yielding cultivars, losses due to biotic (insects and diseases) and abiotic (water-logging, drought) stresses, and problems in maintaining appropriate plant population in farmers' fields have always added to the difficulties encountered in realizing higher yields. To enhance the production and productivity of pigeonpea, ICRISAT developed a hybrid breeding technology using partial natural out-crossing (Saxena et al. 1990) and a male-sterility system (Saxena et al. 2005) of the crop. However, in spite of its high yield advantage, the first ever hybrid ICPH 8 developed in pigeonpea (Saxena et al. 1992) could not make any impact, and it was due to the genetic control of male-sterility. Consequently, its hybrid seed production became expensive and hence was not accepted by commercial hybrid seed producers. Soon after the development of cytoplasmic nuclear male-sterility (Saxena et al. 2005), a new series of hybrids with easy seed production technology and high yields were developed and hybrids ICPH 2671, ICPH 2740, and ICPH 3762 were found promising. Besides resistance to fusarial wilt and sterility mosaic diseases, these hybrids also posses considerable yield advantages over the standard pure line varieties (Saxena and Nadarajan 2010). The present investigations were carried to assess the adaptation and potential of pigeonpea hybrids in Madhya Pradesh by conducting on-farm trials in six agro-climatic zones.

Material and methods

The on-farm testing of pigeonpea hybrids was carried at locations, spread over six agro-climatic zones (Table 1) of Madhya Pradesh. Three hybrids ICPH 2671, ICPH 2740, and ICPH 3762 and a control variety Asha were included in non-replicated evaluation plots, measuring 21 X 20m, at Krishi Vigyan Kendras located in Betul, Chhindwara, Jabalpur, Mandla, Navgaon, Panna, Powarkheda, and Sagar, during *Kharif* 2009-10.

Pigeonpea hybrids ICPH 2740 and ICPH 2671 were evaluated in non-replicated plots measuring 0.2 ha each in the farmers' fields at Jabalpur, Narsinghpur, Rewa, Seoni, Raisen, Harda, and Panna districts. The large-scale seed production programme of the hybrid ICPH 2671 was organized on 11.5 ha with four locations in Kaymore Plateau & Satpura Hills, and at one location in Bundelkhand region. The planting was completed between 4 July and 3 September at 60cm row spacing. Major crop

Table 1. Characteristics of different agro-climatic zones involved in the testing of hybrids in Madhya Pradesh

Zone	Northern hills of Chattisgarh	Kaymore plateau and Satpura hills	Vindhyan plateau	Central Narmada valley	Bundelkhand	Satpura hills
Zone no.	III	IV	V	VI	VIII	IX
Locations	Mandla	Jabalpur, Panna, Rewa, Seoni	Raisen,Sagar	Harda, Narsinghpur, Narsinghpur Powarkheda	Chattarpur, Tikamgarh	Betul, Chhindwara
Latitude [N]	22°	24°-27°	23°-22°	21° -22°	24°	21°- 22°
Longitude [E]	80°	80°- 82°	82°- 88°	72°- 81°	80°	76°
Altitude [m]	457	559	400	500	330	500
Soil type	Red-yellow & brown-black	Mixed red & black	Deep medium black	Deep medium black	Mixed black & red	Shallow medium black
Climate	Humid & Cool	Summer hot winter cool	Summer hot winter cool	Summer hot winter cool	Summer hot winter cool	Summer hot winter cool
Rainfall [mm]	1400-1600	1000-1400	1200-1400	1200-1500	800-1400	1000-1200

Table 2. Seed yield (kg/ha) of pigeonpea hybrids at eight locations of the University farms recorded during 2009-2010

Location	ICPH 2671	ICPH 2740	ICPH 3762	Asha (C)
Betul	0850	1200	1012	0387
Chhindwara	1623	1194	1351	0840
Jabalpur	2621	2390	2330	1900
Mandla	1292	1667	1563	0958
Navgaon	0780	0877	0760	0429
Panna	1210	1165	1060	0671
Powarkheda	2678	2663	1537	2100
Sagar	3350	3250	2870	1750
Mean	1801cb	1801cb	1560b	1129a
Superiority (%) over control	37.3	37.3	27.6	

Mean not followed by same letters are significantly different at probability level of 0.5% according to Least Squares Mean Difference (Student's t-test)

Table 3. Seed yield (kg/ha) of two pigeonpea hybrids at farmers' fields during 2009-2010

Location	ICPH 2740		ICPH 2671		Asha (C)
	Seed yield	Superiority over control [%]	Seed yield	Superiority over control [%]	
Harda	0980	-07.1	1165	09.8	1050
Jabalpur	2150	21.4	2310	26.8	1690
Narsinghpur	1635	08.3	1950	23.1	1500
Panna	0936	46.6	1160	56.9	0500
Rewa	2080	20.2	2870	42.2	1660
Raisen	1842	24.0	2600	46.2	1400
Seoni	1860	20.1	1525	02.5	1487
Mean	1640b	12.4	1940c	21.5	1327a
Superiority (%) over control	019.1	-	031.6	-	-

Mean not followed by same letters are significantly different at probability level of 0.5% according to Least Squares Mean Difference (Student's t-test)

husbandry practices involved seed treatment with Thiometoxam (1g/kg) and Carbandazim (2 g/kg); Nitrogen (30 kg/ha), Phosphorus (80 kg/ha) and Potash (20kg/ha) supplemented as basal dose; irrigation before flowering; two hand weeding and two spray of insecticide at flowering and pod formation stage. Hybrid seed production programme involved row ratio of 5 female: 2 male and row to row to distance of 60 cm with meticulous rouging at the time of flowering stage.

Results and Discussion

Performance of pigeonpea hybrids at Krishi Vigyan Kendra

The hybrid evaluation trials were grown initially at 19 locations, however due to crop establishment problems caused by water-logging the trails were successful only at eight locations. In these trials all the three hybrids were significantly superior to the control, Asha (Table 2). The mean seed yield of the check was 1129 kg/ha with the maximum (2100 kg/ha) and minimum (387 kg/ha) productivity recorded at Powarkheda and Betul, respectively. Based on the mean, seed yield of the hybrids were significantly superior to the control variety Asha. However, the differences among the three hybrids were non-significant. The highest yield (3350 kg/ha) of hybrid ICPH 2671 was recorded in Sagar located in Vindhyan plateau and it was 91.5 % superior to the control. Similarly, the hybrids ICPH 2740 (3250 kg/ha) and ICPH 3762 (2870 kg/ha) produced higher seed yield in Sagar, respectively with 86% and 64% superiority over the check (1750 kg/ha).

Performance of pigeonpea hybrids in farmers' fields

In all the 7 locations performance of the hybrids ICPH 2671 and 2740 was better than the check Asha (Table 3). The mean performance of hybrid ICPH 2671 was significantly superior to hybrid ICPH 2740. The hybrid ICPH 2671 was found out-standing in Rewa (2870 kg/ha, 42.2% superiority) and Raisen (2660 kg/ha, 46.2% superiority). In one hectare the hybrid ICPH 2671 yielded 314 kg and ICPH 2740, 614 kg additional grain than the

Table 4. Overall performance of pigeonpea hybrids under different agro-climatic zones in the eastern Madhya Pradesh

Zone No.	Name	Locations	% Increase over Asha (C)	
			ICPH 2671	ICPH 2740
III	Northern hills of Chattisgarh	Mandla	35	75
IV	Kaymore plateau and Satpura hills	Jabalpur, Rewa, Seoni	43	29
V	Vindhyan plateau	Raisen, Sagar	89	62
VI	Central Narmada valley	Harda, Narsinghpur, Powarkheda	25	13
VIII	Buendelkhand	Chattarpur	28	40
IX	Satpura hills	Betul, Chhindwara	2	5
	Mean		4	7

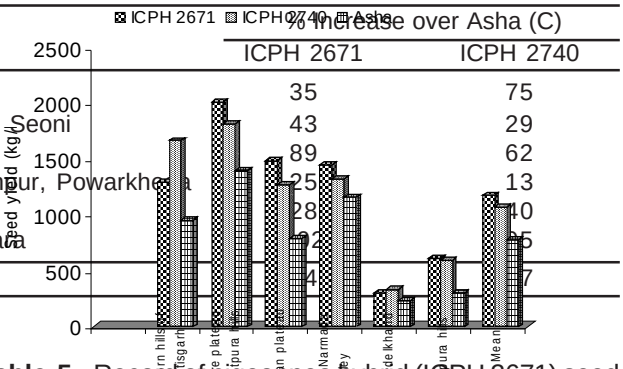

Fig 1. Performance of two pigeonpea hybrids under varied agro-climatic zones in the eastern parts of Madhya Pradesh

Table 5. Record of pigeonpea hybrid (ICPH 2671) seed production in Madhya Pradesh

Agro-climatic	Area(ha)	Production (kg)	Producibility (kg/ha)
Kaymore plateau and Satpura hills			
Jabalpur	1.5	2000	1333c
Katni	3.0	4350	1450 c
Rewa	1.0	1740	1740 c
Seoni	1.0	2500	2500 b
Bundelkahnd region			
Tikamgarh	5.0	15200	3040 a
Total/mean	11.5		2242 b

Mean not followed by same letters are significantly different at probability level of 0.5 % according to Least Squares Mean Difference (Student's t-test)

check (1326 kg/ha). The superior performances of the hybrids in farmers' fields at all the locations advocate the promotion of pigeonpea hybrids in state of Madhya Pradesh.

Overall performance of the hybrids

Two medium duration pigeonpea hybrids ICPH 2671 and ICPH 2740 were evaluated at 15 locations spread over six agro-climatic zones in the eastern Madhya Pradesh (Table 4). The most popular variety Asha was used as check to compare the performance. Maximum productivity of both the hybrids, and variety was recorded in Kaymore Plateau and Satpura hills agro-climatic zone and minimum in Bundelkhand zone (Fig 1). Over all 54 and 37% increase over the check variety Asha for seed yield/ha were recorded by the hybrid ICPH 2671 and ICPH 2740, respectively. High 102% (ICPH 2671) and 95% (ICPH 2740) increase over the check was recorded in Satpura hills followed by Vindhyan Plateau zone (Table 4). Whereas, low increases over the check were recorded in Central Narmada Valley agro-climatic zone in both the hybrids. These data support the promotion of medium duration pigeonpea hybrid in MP.

Large-scale hybrid seed production in Madhya Pradesh

Production of quality hybrid seed is another important factor in the promotion of hybrid technology. Apart from the calibrated seed production technology, identification of suitable locations for hybrid seed production is of the prime importance. To identify suitable locations the seed production programme of hybrid ICPH 2671 was taken in 11.5 ha at five locations in two agro-climatic zones (Table 5). In all, 25,790 kg hybrid seed was produced from 11.5 ha, with an average productivity of 2242 kg/ha. The hybrid seed productivity ranged between 1333 kg/ha and 3040 kg/ha. The maximum hybrid seed yield was recorded at Tikamgarh, located in Bundelkhand region and Seoni in Kaymore Plateau of Satpura hills. The seed production data indicate that the quality hybrid seed of pigeonpea can be produced successfully at all the location in the eastern parts of Madhya Pradesh.

भारत देश में, मध्य प्रदेश का अरहर कास्त के क्षेत्र व उत्पादन में विशेष स्थान है । अरहर की उत्पादकता में गत वर्षों में व्यंजक परिवर्तन नहीं हुआ है । इस कारण से, उपज-क्षमता में वृद्धि के लिए इक्रीसेट द्वारा बनाई गई "कोशिकाद्रव्य-जीन नर नंपुसकता" आधारित संकर किस्मों का तुलनात्मक परीक्षण वर्त्तमान में उगाई जाने वाली किस्मों के साथ किया गया । विश्व विद्यालय के विभिन्न केन्द्रों पर संकर आई.सी.पी.एच. 2671 (51% अधिक), आई.सी.पी.एच. 2740 (37% अधिक), व आई.सी.पी.एच. 3762 (36% अधिक) की पहचान की गई । म.प्र. के पूर्वी भाग स्थित छः कृषि जवालयु क्षेत्र के सात केन्द्रों पर संकर आई.सी.पी.एच. 2671 द्वारा आशा से 31.6% अधिक उपज प्रदान की । प्राप्त आँकड़े दर्शाते हैं की म.प्र. में अरहर की उत्पादन क्षमता बढ़ाने के लिए संकर किस्मों की विशेष क्षमता है । इसके संकर बीज का उत्पादन म.प्र. के पूर्वी क्षेत्रों मे औसत 2242 कि.लो./हे. उत्पादकता के साथ सफलतापूर्वक किया जा सकता है ।

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Efficacy of imazethapyr alone and in combination with other herbicides against weeds in soybean

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Abstract

Field experiment was conducted during *kharif* season of 2010 to test the efficiency of herbicide applied alone and in combination with other herbicides. The application of imazathapyr @ 200 and 400 g ha^{-1} provided 65.46 and 75.04% and 68.32 and 80.05% control over dominant broadleaf and grassy weeds at 40 DAS and harvest stage, respectively. While, combine application of imazethapyr and quizalofop-p-ethyl (100+50 g ha^{-1}) provide 76.60 and 90.30 % control of both type of weeds at 40 DAS and harvest stage, respectively. Two hand weeding (20 and 40 DAS) gave the excellent control of all weeds and recorded higher (2735 kg/ha) grain yield than the herbicidal treatments and control. However, combine application of imazethapyr + quizalofop-p-ethyl produced the grain yield of 2706 kg/ha which was more or less similar to weed-free treatment.

Keywords : imazethapyr, quizalofop-p-ethyl, soybean

Soybean suffers due to heavy weed competition, especially in early growth stages but also has good suppressing ability against weeds appearing late in the season. Being a rainy season crop, it has high yielding capacity but weed infestation is one of the major constraints in soybean cultivation (Bhan et al. 1974). Weed competition is one of the most important cause of yield loss in *kharif* soybean and is estimated to the tune of 30 to 80% (Yaduraju 2002). Soybean is one of the most important *kharif* oilseed, due to the profuse growth of weeds causing serious decline in yields. (Chhokar et al. 1995) revealed that weed free maintenance upto 45 DAS resulted in 96% increase in grain yield of soybean over weedy check. The effective and economical weed control in soybean on large scale is not possible through manual operations or use of mechanical means. Therefore, the application of post emergence herbicides like imazethapyr, quizalofop-p-etyl, fenoxaprop and chlorimuron ethyl have been recommended for weed control in soybean. Present investigation was carried out to study the efficacy of imazethapyr alone and incombination with other herbicides against weeds in soybean.

Material and methods

A field experiment was conducted during *kharif* 2010 at Research Farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). Jabalpur is situated at 23° 9' North latitude and 79°58' East longitudes with an altitude of 411.78 meters above the mean sea level. Jabalpur falls under "Kymore Plateau and Satpura Hills" agro climatic zone of Madhya Pradesh. The experimental soil was clayey in texture with pH 7.1, organic carbon 0.64%, medium in available nitrogen (372 kg N ha^{-1}), phosphorus (17.45 kg P_2O_5 ha^{-1}) and potassium (297 kg K_2O ha^{-1}). Nine weed control treatments (Table 2) were laid out in a randomized block design with three replications. Treatment consisted of T_1, T_2, T_3, imazethapyr at 100, 200, 400 g ha^{-1}, T_4 combine application of imazethapyr + quizalofop-p-ethyl (100+50 g ha^{-1}), T_5 imazethapyr + chlorimuron-ethyl (100+24 g ha^{-1}), T_6 hand Weeding (20 and 40 DAS), T_7 quizalofop-p-ethyl 50 g ha^{-1}, T_8 pendimethalin 1000 g ha^{-1} and T_9 weedy check. The soybean cv. JS 97-52 was sown on July 7th, 2010 at the seed rate of 80 kg ha^{-1} in rows 45 cm apart and crop was fertilized with 20 : 60 and 20 kg/ha as N, P_2O_5 and K_2O. Weed samples were collected by random placing of 50 x 50 cm quadrate in each plot at 40 DAS and at maturity stage. Weeds were cut down at ground levels and then identified, counted and the samples were kept in an oven at 70 ± 10 ^{0}C until they attained constant weight. Observations pertaining to the weed growth, dry matter production and crop growth and yield parameters were recorded. The data on weeds were collected through the square root transformation ?X + 0.5 for statistical analysis (Panse and Sukhatme, 1967).

Results and Discussion

Effect on weeds

The experimental field was mainly infested with *Echinochloa colona, Cyperus iria, Dinebra retroflexa, Commelina communis* and *Alternenthera philoxeroides* constituting, on an average 22.76 & 25.72%, 22.42 & 23.19%, 19.47 & 16.19%, 19.42 & 19.87% and 15.92 & 15.05% of total weed flora at 40 DAS and harvest stage, respectively in weedy check plot (Table 1). The infestation of monocot weeds was more than that of dicot weeds. Maximum weed infestation was observed in weedy check. Two hand weeding (20 and 40 DAS) recorded significantly lowest density of all prominent weeds (Table 2) than the other treatments.

Application of imazethapyr (100 g ha^{-1}) at lowest rate recorded the less weed control efficiency (56.20 and 67.20 %) against both monocot and dicot weeds but the efficiency was increased with the increase in doses to 200 and 400 g ha^{-1} (65.46 and 75.04 %) and 400 g ha^{-1} (68.32 and 80.05 %) at 40 DAS and harvest stage, respectively. The efficiency of imazethapyr was more pronounced (76.60 and 90.30 %) when it was applied in combination with quizalofop-p-ethyl (100+50 g ha^{-1}) and found at par with two hand weeding (20 and 40 DAS) and it was more effective against monocot as well as dicot weeds. Application of quizalofop-p-ethyl alone was more effective against grassy weeds only (Table 3).

Two hand weeding recorded significantly less (3.55 and 7.52 g m^{-2}) weed biomass than all other herbicidal treatments at 40 DAS and harvest stage, respectively. Among the herbicidal treatments, post-emergence

Table 1. Weed flora of the experimental field in weedy check plot at 40 DAS and harvest

Weed	Density (m^{-2})		Relative density (%)	
	40 DAS	Harvest	40 DAS	Harvest
Monocot weeds				
Echinochloa colona	70.63	65.36	22.76	25.72
Dinebra retroflexa	60.40	41.14	19.47	16.19
Cyperus iria	69.58	58.93	22.42	23.19
Commelina communis	60.27	50.49	19.42	19.87
Dicot weeds				
Alternanthera philoxeroides	49.41	38.24	15.92	15.05
Total	310.29	254.16	100.00	100.00

Table 2. Influence of imazethapyr on the density of weeds at 40 DAS and harvest stage in soybean

Treatments	Density (m^{-2})									
	Echinochloa colona		*Dinebra retroflexa*		*Cyperus iria*		*Commelinacommunis*		*Alternanthera philoxaroides*	
	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest
T_1 - Imazethapyr 100 g/ha	3.99 (15.44)	3.35 (10.78)	3.70 (13.20)	3.03 (8.69)	4.62 (20.90)	3.55 (12.36)	4.84 (23.35)	4.12 (16.53)	4.60 (21.07)	4.16 (16.85)
T_2 - Imazethapyr 200 g/ha	3.60 (12.53)	2.83 (7.53)	3.61 (9.48)	2.56 (6.14)	3.83 (14.19)	3.20 (9.81)	4.51 (19.81)	3.35 (10.77)	4.14 (16.70)	3.11 (9.23)
T_3 - Imazethapyr 400 g/ha	3.37 (10.87)	2.53 (7.05)	2.79 (7.33)	2.27 (4.84)	3.42 (11.36)	2.72 (6.96)	4.38 (18.75)	3.17 (9.55)	4.01 (15.78)	2.91 (8.09)
T_4 - Imazethapyr + Quizalofop-p-ethyl (100 + 50 g/ha)	2.99 (8.44)	0.71 (0.00)	1.94 (3.27)	1.60 (2.54)	2.58 (6.44)	2.05 (3.84)	4.09 (16.27)	2.03 (3.79)	3.59 (12.38)	1.53 (1.87)
T_5 - Imazethapyr + chlorimuron-ethyl (100 + 24 g/ha)	3.12 (9.26)	1.92 (3.32)	2.44 (5.69)	1.78 (2.68)	3.08 (9.02)	2.52 (5.98)	4.19 (17.12)	2.38 (5.20)	3.69 (13.19)	2.83 (7.52)
T_6 - Hand weeding (20 and 40 DAS)	0.71 (0.00)	0.72 (0.00)	0.69 (0.00)	1.48 (1.77)	0.75 (0.00)	1.64 (2.66)	0.81 (0.00)	1.59 (2.68)	0.71 (0.00)	1.24 (1.62)
T_7 - Pendimethalin 1000 g/ha	4.70 (21.81)	3.85 (14.53)	4.41 (19.08)	4.06 (16.01)	5.18 (26.37)	4.23 (17.43)	5.61 (30.94)	5.59 (30.72)	5.45 (29.32)	5.46 (29.30)
T_8 - Quizalofop-p-ethyl 50 g/ha	4.38 (18.98)	3.62 (12.69)	3.89 (14.84)	3.09 (9.11)	5.09 (25.39)	3.79 (14.03)	5.57 (30.64)	5.57 (30.64)	5.44 (29.36)	5.44 (29.36)
T_9 - Weedy check	8.43 (70.63)	8.11 (65.36)	7.80 (60.40)	6.45 (41.14)	8.37 (69.58)	7.71 (58.93)	7.79 (60.27)	7.14 (50.49)	7.06 (49.41)	6.25 (38.24)
SEm±	0.21	0.30	0.18	0.23	0.20	0.28	0.22	0.23	0.25	0.23
CD at 5%	0.65	0.91	0.54	0.70	0.60	0.85	0.65	0.70	0.74	0.70

( ) figure in parenthesis are the original value

Table 3. Influence of imazethapyr on the dry weight (gm^{-2}) of weeds at 40 DAS and harvest stage in soybean

Treatments	Dry weight (m^{-2})													
	Echinochloa colona		*Dinebra retroflexa*		*Cyperus iria*		*Commelina communis*		*Alternanthera philoxaroides*		Weed biomass (g m^{-2})		Weed control efficiency (%)	
	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest	40 DAS	At harvest
T1 - Imazethapyr 100 g/ha	5.32	4.11	4.24	3.29	4.47	3.85	5.42	4.59	5.35	4.20	24.80	20.04	56.20	67.20
	(27.85)	(16.49)	(17.45)	(10.31)	(19.44)	(14.42)	(28.94)	(20.59)	(28.23)	(19.06)	(121.91)	(80.87)		
T2 - Imazethapyr 200 g/ha	4.61	3.50	3.72	3.05	4.12	3.51	4.89	3.92	4.74	3.80	22.08	17.78	65.46	75.04
	(20.76)	(11.79)	(13.35)	(8.83)	(16.59)	(11.91)	(23.45)	(14.92)	(21.99)	(14.09)	(96.14)	(61.54)		
T3 - Imazethapyr 400 g/ha	4.32	3.12	3.64	2.41	3.96	3.23	4.78	3.80	4.46	3.31	21.16	15.87	68.32	80.05
	(18.26)	(9.28)	(12.79)	(5.45)	(15.28)	(9.99)	(22.45)	(14.02)	(19.41)	(10.45)	(88.19)	(49.19)		
T4 - Imazethapyr + Quizalofop -p-ethyl (100 + 50 g/ha)	3.93	0.71	2.83	1.46	3.60	2.64	3.99	3.06	3.83	2.55	18.18	10.42	76.60	90.30
	(14.98)	(0.00)	(7.70)	(2.03)	(12.80)	(6.77)	(15.48)	(8.99)	(14.18)	(6.12)	(65.14)	(23.91)		
T5 - Imazethapyr + chlorimuron -ethyl (100 + 24 g/ha)	4.19	2.34	3.22	2.20	3.85	3.03	4.37	3.68	3.97	3.16	19.60	14.41	72.95	83.29
	(17.09)	(5.15)	(9.88)	(4.59)	(14.37)	(8.70)	(18.65)	(13.14)	(15.28)	(9.63)	(75.27)	(41.21)		
T6 - Hand weeding (20 and 40 DAS)	0.69	0.71	0.71	1.47	0.71	1.77	0.71	2.14	0.71	1.43	3.55	7.52	100.00	95.08
	(0.00)	(0.00)	(0.00)	(1.67)	(0.00)	(2.72)	(0.00)	(5.11)	(0.00)	(2.62)	(0.00)	(12.12)		
T7 – Pendimethalin 1000 g/ha	6.13	5.26	4.70	4.07	5.61	4.23	5.76	5.16	5.88	5.25	28.08	23.97	43.74	53.26
	(37.04)	(27.42)	(21.56)	(16.59)	(30.99)	(17.61)	(32.73)	(26.34)	(34.28)	(27.30)	(156.6)	(115.26)		
T8 - Quizalofop-p-ethyl 50 g/ha	5.64	4.61	4.58	3.59	4.78	4.15	5.60	4.82	5.72	4.79	26.32	21.96	54.65	61.40
	(31.34)	(20.75)	(20.44)	(12.47)	(22.41)	(16.76)	(30.99)	(22.79)	(32.27)	(22.42)	(126.22)	(95.19)		
T9 - Weedy check	7.66	7.08	6.68	6.00	7.33	7.11	7.97	7.61	7.74	7.37	37.38	35.17	-	-
	(58.17)	(49.62)	(44.21)	(35.48)	(53.33)	(50.11)	(63.19)	(57.49)	(59.46)	(53.89)	(278.36)	(246.59)		
SEm±	**0.12**	**0.19**	**0.16**	**0.28**	**0.22**	**0.21**	**0.22**	**0.32**	**0.15**	**0.47**	**1.15**	**1.38**	**-**	**-**
CD at 5%	**0.35**	**0.57**	**0.48**	**0.83**	**0.65**	**0.62**	**0.66**	**0.98**	**0.44**	**1.41**	**3.44**	**4.15**		

( ) figure in parenthesis are the original value

Table 4. Effect of different weed control treatment on growth and yield attributing characters of the soybean crop

Treatments	Plant height (cm)				LAI		Pods/plant	Seeds/pod	Seed index	Seed yield	Stover yield	HI	B:C ratio
	30 DAS	60 DAS	90 DAS	harvest	30 DAS	60 DAS	harvest	harvest	harvest	(kg/ha)	(kg/ha)	(%)	-
T_1 - Imazethapyr 100 g/ha	27.02	72.12	74.38	73.86	2.39	7.35	81.23	2.38	9.64	2271.99	5235.57	30.26	2.13
T_2 - Imazethapyr 200 g/ha	27.88	72.37	75.19	74.44	2.43	7.57	87.06	2.44	9.73	2418.49	5315.84	31.27	2.09
T_3 - Imazethapyr 400 g/ha	27.92	72.81	75.61	75.05	2.50	7.72	90.88	2.50	9.85	2447.08	5365.32	31.33	1.85
T_4 - Imazethapyr + Quizalofop-p-ethyl (100 + 50 g/ha)	28.24	74.42	77.24	76.97	2.77	7.86	103.99	2.58	10.27	2706.58	5526.01	32.88	2.31
T_5 - Imazethapyr + chlorimuron-ethyl (100 + 24 g/ha)	28.08	73.06	76.43	75.71	2.65	7.75	97.85	2.55	9.99	2478.38	5384.76	31.51	2.29
T_6 - Hand weeding (20 and 40 DAS)	31.36	75.87	80.02	79.74	2.89	7.97	115.99	2.60	10.36	2735.69	5560.10	33.01	1.89
T_7 – Pendimethalin 1000 g/ha	25.96	70.44	73.95	73.49	2.30	7.16	75.47	2.27	9.02	1612.65	4525.15	26.27	1.52
T_8 - Quizalofop-p-ethyl 50 g/ha	26.97	70.91	74.22	73.63	2.34	7.29	78.30	2.33	9.48	2162.05	5135.28	29.63	2.01
T_9 - Weedy check	25.05	66.22	67.49	66.98	2.23	7.07	69.07	2.22	8.95	982.21	3625.93	21.24	1.06
SEm±	1.04	1.63	0.95	1.32	0.14	0.19	4.07	0.13	0.30	45.02	81.55	-	-
CD at 5%	3.12	4.92	2.85	3.99	0.41	0.58	12.27	NS	0.92	135.67	245.78	-	-

application of imazethapyr + quizalofop-p-ethyl (100+50 g ha^{-1}) lowered the total dry weight of weeds (18.18 and 10.42 g m^{-2}) than other herbicidal treatments (Table 3).

Effect on crop

The highest value of plant height and LAI of 31.36 & 75.87 cm and 2.89 & 7.97 cm at 30 and 60 DAS, respectively were recorded under weed free treatment. Weedy check recorded the lowest value of these parameters closely followed by the alone application of pendimethalin and quizalofop-p-ethyl. These variations in growth and yield attributes due to weed control treatments were evidenced by observation on weed count and weed dry weight (Table 4). Improvement in yield attributes due to effective weed management has also been reported by Patil et al. (2002). Yield attributing characters like pods per plant, seeds per pod and seed index were higher (104, 2.58 and 10.27) under the treatment (T_4) receiving combined application of imazethapyr + quizalofop (100+50 g ha^{-1}), while it was lowest under weedy check (69, 2.22 and 8.95). The grain yield and harvest index of soybean were also significantly higher (2706 kg ha^{-1} and 32.88) under T_4 and being at par with hand weeding twice (20 and 40 DAS) i.e. 2735 kg ha^{-1} and 33.01, respectively. Weedy check (982 kg ha^{-1} and 21.24) led to record the minimum grain yield and harvest index (Table 4). These results are in conformity to the finding of Vyas and Jain (2003), Kothawade et al. (2007) and Shete et al., (2008).

The economic viability of treatments is widely depended on benefit : cost ratio and the highest B:C ratio (2.31) was obtained with treatment T_4 i.e. combined application of imazethapyr + quizalofop-p-ethyl (100+50 g ha^{-1}) closely followed by T_5 imazethapyr + chlorimuron-ethyl (100+24 g ha^{-1}) i.e. (2.29). On the contrary, the lowest (1.06) B: C ratio was recorded under weedy check followed by alone application of pendimethalin 1000 g ha^{-1} (1.52).

वर्श 2010 के खरीफ में एक प्रक्षेत्र अनुसंधान खरपतवारनाशियों के अकेले एवं अन्य खरपतवारनाशियों के सोयाबीन फसल पर प्रभाव को देखने के लिये डाला गया । इमेजेथापाइर नामक दवा की 200 ग्रा. एवं 400 ग्रा. प्रति हेक्टेयर की दर से अलग-अलग डालने पर चौड़ी पत्ती वाले खरपतवारों का 65.40 प्रतिशत एवं 75.0 प्रतिशत नियंत्रण तथा घास कुल के खरपतवारों का 68 प्रतिशत एवं 80 प्रतिशत नियंत्रण हुआ, परन्तु इमेजेथापाइर 100 ग्रा./हे. एवं क्विाजालाफास इथाइल 50 ग्रा./हे. को मिलाकर डालने पर खरपतवारों पर 75-90 प्रतिशत तक नियंत्रण प्राप्त हुआ । इसके साथ ही अनुसंधान में यह भी देखा गया कि दो निंदाई (20 एवं 40 दिन बुवाई के बाद) करने से सोयाबीन की अधिकतम उपज (2735 कि.ग्रा./हे.) प्राप्त होती है, हालांकि इमेजेथापाइर एवं क्विाजालाफास इथाइल के सम्मिलित उपयोग से अधिकतम उपज 2706 कि.ग्रा./हे. प्राप्त हुई जो कि निंदाई से प्राप्त हुई उपज के लगभग बराबर है ।

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Long term effect of INM on biological properties, soil available zinc and sulphur and productivity of rice – wheat cropping system

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Abstract

An experiment has been conducted on integrated nutrient management in rice-wheat system at Jabalpur (MP) since *kharif* season 1987-88 to evaluate the productivity of rice-wheat cropping system on long term basis. The present paper deals with the studies during the year 2002-03 and 2003-04. Integrated use of 50% N through FYM/wheat straw/ green leaf manuring with sunnhemp + 50% NPK to rice followed by 100% NPK to wheat or 25% N through FYM/ wheat straw/green manuring with sunnhemp + 75% NPK to rice followed by 75% NPK to wheat were comparable to application of 100% NPK to both crops in terms of system productivity, soil biological properties and soil available zinc & sulphur nutrients in rice-wheat cropping system.

Keywords: System productivity, biological properties

Both rice and wheat crops grown in a sequence require high quantity of nutrients to harness their potential yields. However, it is unaffordable to poor and subsistence farmers of the country. The physical, chemical and biological properties of soils get to change due to continuous cropping and use of fertilizers alone or in combination with manures. Integrated nutrient management system is an important component of sustainable agricultural intensification. Organic manures generally add all the 16 essential elements in the soil, although the quantity is quite less, which are directly used by the crop plants. Organic manures contribute indirectly to the plant-nutrition by making the soil nutrients easily available and also provide food & nutrients for the soil micro organisms like PSB, actinomycetes, azotobacter etc. Therefore, a long-term experiment has been initiated on integrated nutrient management in rice-wheat system at Jabalpur (MP) since *kharif* season 1987-88 to maintain the productivity of rice-wheat cropping sequence without degradation of soil health under irrigated production system. The present paper deals with the studies during the year 2002-03 and 2003-04.

Material and methods

The soil of the experimental field was neutral in reaction (soil pH 7.7) and normal in EC (0.38 dS/m) with medium

Table 1. Treatments under different integrated nutrient management

T. No.	*Kharif* (Rice cv. Kranti)	*Rabi* (Wheat cv.Lok-1)
T_1	No fertilizers, no organic manures (Control)	No fertilizers, no organic manures (Control)
T_2	50% recommended NPK through fertilizers	50% recommended NPK through fertilizers
T_3	50% recommended NPK through fertilizers	100% recommended NPK through fertilizers
T_4	75% recommended NPK through fertilizers	75% recommended NPK through fertilizers
T_5	100% recommended NPK through fertilizers	100% recommended NPK through fertilizers
T_6	50% recommended NPK through fertilizers + 50% N through FYM	100% recommended NPK through fertilizers
T_7	75% recommended NPK through fertilizers + 25% N through FYM	75% recommended NPK through fertilizers
T_8	50% recommended NPK through fertilizer + 50% N through wheat straw	100% recommended NPK through fertilizers
T_9	75% recommended NPK through fertilizers+ 25% N through wheat straw	75% recommended NPK through fertilizers
T_{10}	50% recommended NPK through fertilizers + 50% N through green leaf manuring (Sunhemp)	100% recommended NPK through fertilizers
T_{11}	75% recommended NPK through fertilizers + 25% N through green leaf manuring (Sunhemp)	75% recommended NPK through fertilizers
T_{12}	Farmer's practice (40kg N + 20kg P_2O_5 + 3 tonnes FYM/ha)	Farmer's practice (40kg N + 20 kg P_2O_5/ha)

Recommended 100% NPK for both crops was 120 kg N + 60 kg P_2O_5 + 40 kg K_2O/ha through urea, single super phosphate and muriate of potash, respectively

organic carbon content (6.9 g/kg) and analysing medium in available N (260 kg/ha), P (16 kg/ha) and high in available K (448 kg/ha) contents. The rainfall was 1266 and 1756 mm during the two consecutive years i.e. 2002-03 and 2003-04. There were 12 treatments (Table 1). Rice cv. Kranti was grown under transplanting with the seed rate of 35 kg/ha at 20cm X 15cm planting geometry and the succeeding wheat cv. Lok 1 was grown by using 100 kg seeds/ha in rows 20 cm apart. The crops were grown under assured irrigation as per needs of crops. The system productivity of rice-wheat sequence was worked-out treatment-wise with the help of following formula as suggested by Tomar and Tiwari (1990)

$$\text{System productivity (kg/ha/day)} = \frac{\text{Wheat equivalent yield (kg/ha) of rice-wheat sequence}}{\text{Total duration (days) of rice and wheat crop sequence}}$$

Soil available Zn and S was determined by the Lindsay & Norwell (1978) method and Turbidimetric method (Chesnin & yien, 1951) respectively. Biological properties viz. count of actinomycetes, fungi, phosphorus solubilising bacteria (PSB), azotobacter and total bacteria of the soil of each plot was analyzed with the help of appropriate media after harvesting of wheat crop of each year. Different media and methodologies used for analysis of biological properties of soil as per the standard procedure. The data recorded on various treatments in both the years were tabulated and pooled when differences between the years were not significant. Afterthat data on various treatments were analyzed statistically in randomized block design as per the procedure suggested by Panse and Sukhatme (1967). The initial status of biological properties was not available, so they were not analyzed statistically.

Results and Discussion

Biological properties

The population of microbes viz. fungi, bacteria, azotobacter, phosphorus solubilising bacteria and actinomycetes etc. as affected by different treatments after completion of 17th crop cycles of rice-wheat system are given in Table 2. However, their deviation from the initial status of these microbes could not be assessed, because of unavailability of initial data. The population of these microbes obviously differed due to the effect of the treatments.

Fungal population

The fungi population increased under different rates of fertilizer application as 38.4, 40.7, 41.3 and 41.9x10^4/g under T_2, T_3, T_4 and T_5, respectively over control treatment. Plots treated with farmers' practice (T_{12}) had higher population of fungi (43.4x10^4/g) than T_5 – receiving 100%

Table 2. Effect of INM on biological properties, available S & Zn and system productivity of rice-wheat cropping system

Initial* Treatments	S (g/kg) 7.8*	Zn (g/kg) 0.35*	Fungi (10^4/g) NA	Bacteria (10^6/g) NA	AZB (10^6/g) NA	PSB (10^6/g) NA	ACT (10^7/g) NA	System Productivity
T_1	6.8	0.28	33.7	28.9	8.5	4.2	4.7	14.00
T_2	7.3	0.29	38.4	34.1	16.8	7.7	5.2	24.53
T_3	7.2	0.31	40.7	36.7	16.9	8.2	5.8	26.45
T_4	7.2	0.30	41.3	39.1	20.9	10.7	5.7	27.56
T_5	7.2	0.34	41.9	42.2	24.3	10.8	6.6	30.84
T_6	8.2	0.40	48.5	57.4	33.6	17.7	11.8	32.01
T_7	8.1	0.39	45.2	54.1	29.4	15.4	8.4	30.55
T_8	8.1	0.38	47.1	55.9	31.9	16.7	10.4	30.85
T_9	8.0	0.35	44.8	52.7	28.8	14.4	7.8	28.80
T_{10}	8.3	0.43	53.5	62.3	36.1	19.5	13.9	33.12
T_{11}	8.2	0.41	46.1	58.2	32.3	17.8	11.3	31.25
T_{12}	7.8	0.35	43.4	42.9	26.5	12.2	6.9	21.59
SEm±	0.2	0.01	–	–	–	–	–	0.36
CD at 5%	0.5	0.03	–	–	–	–	–	0.99

NA = Not Available, NS = Non Significant, AZB = Azotobacter, PSB = Phosphorus solubilising Bacteria, ACT = Actinomycetes, *Initial status during the year 1993-94.

recommended NPK through only fertilizers to both crops. All INM treatments had higher population of fungi (44.8 to 53.5x10^4/g) than T_5 and other treatments receiving plant nutrition only through fertilizers. Under high rate of integration of organic manures, green manuring had fungi population of 53.5x10^4/g followed by 48.5 and 47.1x10^4/g with FYM (T_6) and wheat straw (T_8) respectively. Similar, trend was observed with low rates of organic manure supplementation.

Bacterial population

Increasing rates of fertilizer application T_2, T_3, T_4 and T_5 showed corresponding increase in bacterial mass of soil as 34.1, 36.7, 39.1 and 42.2x10^6/g, respectively; and these densities were quite higher than T_1 (control). The farmers' practice of plant nutrition (T_{12}) had bacterial density of 42.9x10^6/g, which were slightly higher to T_5. Different treatments of INM recorded higher bacterial density than treatments in which, fertilizers are applied alone. Among the organic sources applied with different rates, green manuring (62.3 and 58.2x10^6/g under T_{10} and T_{11}, respectively) had higher bacterial density than FYM (57.4 and 54.1x10^6/g under T_6 and T_7 respectively) and wheat straw (55.9 and 52.7x10^6/g under T_8 and T_9 respectively) at the same levels of organic manures.

Azotobacter population

Absolute control plot (T_1) had minimum density of azotobacter (8.5x10^6/g), which became more than double to thrice when plant nutrition were applied only through fertilizers to both crops in rice-wheat system under T_2 (16.8X10^6/g), T_3 (16.9X10^6/g), T_4 (20.9X10^6/g) and T_5 (24.3X10^6/g) treatments. All INM treatments had nearly four times higher azotobacter population than control. The azotobacter population was maximum with T_{10} (36.1X10^6/g) followed by T_6 (33.6X10^6/g), T_{11} (32.3X10^6/g), T_8 (31.9X10^6/g), T_7 (29.4X10^6/g) and T_9 treatment (28.8X10^6/g) respectively. Obviously, population of azotobacter was nearly 1.2 to 1.5 times higher under different INM treated plots than T_5 (receiving full recommended dose of nutrients through fertilizers to both crops in rice-wheat system).

PSB population

Application of nutrients with different rates only through fertilizers under T_2, T_3, T_4 and T_5 had PSB populations as 7.7, 8.2, 10.7 and 10.8X10^6/g, respectively which were higher than T_1. Treatments with application of nutrients as per framers' practice (T_{12}) also had higher PSB population (12.2X10^6/g) than all above treatments. The PSB population markedly increased under all INM treatments ranging from 14.4 to 19.5X10^6/g over rest of the treatments described early. The INM with green manuring (T_{10} and T_{11}) had higher density of PSB than FYM (T_6 and T_7) and wheat straw (T_8 and T_9) at the same level of fertility status maintained.

Actinomycetes population

The density of microbes consisting with all actinomycetes differed among all the treatments after completion of 17th crop cycles under rice-wheat system. Their populations were minimum (4.7x10^7/g) under control (T_1), which increased under the treatments receiving only fertilizers at different rates. The treatment comprised with farmers' practice of plant nutrition (T_{12}) also had higher population of actinomycetes (6.9X10^7/g) than all the treatments comprises of fertilizer application alone. It is notable that its population was maximum (13.9X10^7/g) under T_{10} followed by T_6 (11.8X10^7/g), T_{11} (11.3X10^7/g), T_8 (10.4X10^7/g), T_7 (8.4X10^7/g) and T_9 (7.8X10^7/g) in descending order. Organic manures supply the food and nutrients to soil microorganisms.

Soil available sulphur

The initial status of S in soil of experiment was not available, but it was recorded first time from the soils of border area in the field during the year 1993-94 (Table 2). The residual soil S status showed declining trend over its initial status (7.8 g/kg) under control and treatments of fertilizers alone (T_2, T_3, T_4 and T_5) after completion of continuous 17th crop cycles in rice-wheat system. But soil S status was found stable under T_{12}. While it exhibited slight improvement over its initial status under different INM treatments (T_6, T_7, T_8, T_9, T_{10} and T_{11}) but soil S status was found stable (7.8 kg/ha) under the treatment of farmers' practice after the end of 17th crop cycles of rice-wheat system.

The soil S was minimum (6.8 g/kg) under control at the end of 17th crop-cycles, which increased due to application of fertilizers alone but differences among these treatments were not significant. Application of plant nutrition to crops under rice-wheat system as per farmers' practice (T_{12}) had significantly higher soil S (7.8 g/kg) than these above treatments. The residual soil S ranged from 8.0 to 8.3 g/kg under different INM treatments but differences were not significant. Application of 50% recommended NPK through fertilizers along with the 50% N through green leaf manuring of sunnhemp to rice followed by application of 100% recommended NPK

through fertilizers to wheat (T_{10} treatment) recorded significantly higher available S, than the treatments consisted of fertilizer application alone and T_{12} treatment also. Decomposition of organic manures, release considerable amount of various organic acids viz H_2CO_3, H_2SO_4, HPO_4, formic acid and butyric acids. These acids help in solubilising insoluable nutrient compounds.

Soil available zinc

After 17 years, the residual available Zn declined over its initial status 0.35 g/kg with the control treatment (T_1) and with T_2, T_3, T_4 and T_5 when doses of fertilizers alone were given to crops in rice-wheat cropping system (Table 2). Different combinations of fertilizer and organic manures increased the residual available Zn over its initial status, after the end of 17th crop cycles of rice-wheat system. Farmers' practice (T_{12}) and T_9 treatment maintained Zn status over its initial stage.

The residual soil Zn was minimum (0.28 g/kg) with control treatment closely followed by T_2, T_3 and T_4 (0.29 to 0.31 g/kg). The residual soil Zn significantly increased with T_5 (0.34 g/kg) receiving full recommended dose of fertilizers or T_{12} (0.35 g/kg) receiving nutrients as per farmers' practice or different INM treatments over control. The residual soil Zn under T_{10} treatment was maximum (0.43 g/kg) and significantly higher over all the treatments except T_{11} (0.41 g/kg). Bellakhi *et al.* (1998) also mentioned that available micronutrients increased significantly with organic sources of nutrients applied either alone or in combination with fertilizers over fertilizers alone.

System productivity

System productivity refers to be the total productivity per hectare per day under a particular treatment. Data pertaining to system productivity as affected by different treatments in both years of experimentation are given in Table 2. It is evident from the said data that system productivity was lowest (14.0 kg/ha/day) under control. It markedly increased with every increasing levels of fertilizers alone (T_2, T_3, T_4 and T_5). Among the different combinations of fertilizers and organic manures, system productivity was significantly maximum (33.12 kg/ha/day) under T_{10}. The next best treatment with this regard was T_6 (32.01 kg/ha/day), which was at par to T_{11} (31.25 kg/ha/day). The INM treatment T_8 led to record system productivity of 30.85 kg/ha/day, which was at par to T_5 (30.84 kg/ha/day) and T_7 (30.55 kg/ha/day). The INM treatment T_9 with system productivity of 28.80 kg/ha/day was significantly higher than remaining levels of fertilizer application (T_2, T_3 and T_4). Singh *et al.,* 2001 also recorded higher system productivity through INM in rice-wheat system.

धान एवं गेहुँ फसल प्रणाली की टिकाऊ उत्पादकता के मूल्यांकन हेतु, खरीफ 1987-88 से जबलपुर (म.प्र.) में समन्वित पोषक तत्व प्रबंधन पर एक प्रयोग चल रहा है । प्रस्तुत शोधपत्र, वर्ष 2002-03 एवं 2003-04 के परिणामों पर आधारित है । परिणामों की विवेचना से स्पष्ट होता है कि 50% नत्रजन गोबर खाद/गेहूँ भूसा/जूट की हरी खाद से + 50% नत्रजन, स्फुर एवं पोटाश समन्वित रूप से धान में देने पर तथा इसके बाद 100% नत्रजन, स्फुर एवं पोटाश गेहूँ में देने पर या 25% नत्रजन गोबर खाद/गेहूँ भूसा/जूट की हरी खाद से + 75% नत्रजन, स्फुर एवं पोटाश समन्वित रूप से धान में देने पर तथा इसके बाद गेहुँ में 75% नत्रजन, स्फुर एवं पोटाश देने पर धान-गेहूँ फसल प्रणाली की उत्पादक्ता, मिट्टी के जैविक गुणधर्म, मिट्टी में उपलब्ध जिकं एवं गंधक तत्वों की मात्रा, दोनों फसलों में 100% नत्रजन, स्फुर एवं पौटाश देने के समतुल्य पाये गये ।

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Authorship and ethical issues in agriculture publishing: an appraisal

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Abstract

As science is progressing and publishing is becoming easier the complexities in authorship are changing. Various authorship issues are merging up. Priorities of scientists researchers, academicians and students are shifting; almost a majority of scientific community wants quick success, gain world recognition, win awards, get promotion/career advancement and get financial grants. On contrary to the fact that they have soaring academic pressure to get their research published or else get perished. Interdisciplinary and collaborative works are increasing and so is the problem of author and authorship issues. Most of author, authorship and publishing issue arise up due to ignorance and lack of knowledge except a few which are done deliberately and intentionally to fulfill personal motives. The current paper highlights some such important facets to make the scientific community conscious, aware and alert about authorship and ethical issues in agricultural publishing.

Keywords: author, authorship, publication research, ethics

Agricultural advancement and development is always dependent upon experience and research findings of scientists apart from various biotic, abiotic, environmental and edaphic factors. The geography of our nation is such that agriculture varies across interstate boundaries and so is its development. Agricultural scientists work more on region specific problems. The future of our agricultural economy is based on agricultural research of the present. According to Neuberger (2011) science cannot progress unless the findings of research are published and accepted. Authorship is important for many reasons: for accountability, for credibility, and for fairness, so that those who contribute to the research are recognized. While reading the research paper the readers presumes that the authors are the original researchers, they believe in the author for their research, truthfulness, accuracy, accountability and responsibility. Authors publish their research findings due to academic reasons and for the want of recognition and excellence in their field.

The economy of our country is growing at a very fast pace, so is our agriculture. The share of agriculture in our nation's GDP (Gross Domestic Product) is 16.1% (http://business.mapsofindia.com/india-gdp/). Almost all forms of productive agriculture are input resource intensive. The demand for seed fertilizer and pesticide is growing and also the per capita price of these commodities is rising. Academic agricultural institutions play a major role in further development; role of agricultural universities is different from traditional universities, in the terms that one of the major responsibilities of such agricultural universities is extension apart from research and teaching. The prime aim of agricultural scientist is to bring their research to farmers so that they can adopt it. At present authorship of research papers is the most pertinent issue present in all agricultural publications. Agricultural researchers culminate their research in form of authored publication.

Publications should be based on ethics, morals, trust, faith, transparency, honesty and should be authentic, reliable, authoritative, genuine, convincing, original, legitimate, un-fabricated, indisputable, productive, correct, unadulterated, persuasive, incontrovertible, irrefutable and indubitable. For all this the author/authors have to be responsible, answerable, liable and accountable.

The trend for getting papers published is on the rise moreover many publications agencies have started publishing papers in the field of agriculture. Due to paucity of resources, funds and time peer review of submitted manuscripts is generally not done in many cases. Like a coin, authorship has two sides: credit and responsibility. One receives professional credit from his/her publications and takes responsibility for their contents (Biagioli et al. 1999).

According to Osborne and Holland (2009), authorship means playing a fundamental role in the creation of the product to be published. Hewitt (1957) stated that authorship cannot be conferred; it may be undertaken by one who will shoulder the responsibility that goes with it. Authorship credit is conceivably the most important and least understood area of professional life for members of the scientific community. Because promotion, prestige, and productivity are judged largely by publication activity, authorship credit has become the 'coin of the realm' in the scientific marketplace (Wilcox 1998). Progressive advancement in academic career is practically impossible without high impact factor good publications. The Internet has facilitated both the dissemination of anonymous texts as well as easy ''borrowing'' of ideas and words of others, this has raised a number of important questions regarding authorship (Stamatatos and Koppel 2011). On contrary prevention and unethical interpretation of research/publications require a comprehensive approach at different levels: individual, institutional and external controls as well as parties involved in the publication process; authors, editors, reviewers and readers (Regmi 2011).

Since the paper is about authorship it is essential to understand what authorship is about. Authorship is an important ethical facet of scientific research publication (Menezes et al. 2006), authorship is defined as any contribution that resulted in a name placement on the byline of the article (Dotson et al. 2011). Sheikh (2000) stated that authorship must bring with it responsibility as well as reward, considering the original Latin word *auctor* and the etymologic term authority. According to Frazzetto (2004) authorship on scientific publications has become the currency of modern science and a measure of a scientist's participation in the international scientific community. Unfortunately though the unethical and unjustified authorship practices have increased in recent years mainly due to the inadvertent pressure exerted by academia (Menezes et al. 2006).

The authorship is complicated, multidimensional and is of multiple types as mentioned below.

Gift Authorship : Inclusions, among the authors, of an individual who does not fulfill the requirement for authorship. It is common practice to oblige some seniors or colleagues. Gift authorship occurs between colleagues and collaborators. In this case a name of a colleague is unjustifiably added to the manuscript in the expectation that the favor will be returned. In this way both authors unethically increase the number of their publications (Gollogly and Momen 2006). Someone who has his or her name at the top of an article, but who has not had a major intellectual involvement this is not considered fair (Albert 2000). If the views of Kakkar (2004) are considered then he was of the opinion that strong practice of gift authorship is prevalent in India. Bhatia et al. (2007) relate gift authorship in India to '*guru-shishya parampara'*, a teacher–disciple relationship

Ghost Authorship : It is referred to denial of authorship to individuals who played an effective part in the work and were qualified for authorship. Ghost authors are individuals who have made contributions worthy of authorship but are not credited as authors (Flanagin et al 1998). This term is used to describe a professional writer who has done most of the work on a piece of writing and who may or may not be given any credit (Albert 2000). Also in ghost authorship no credit or acknowledgement is given to the original writer of the manuscript.

Honorary Authorship : Also known as courtesy authorship. It refers to those individuals receiving authorship credit without substantially contributing to a project (Rennie et al 1997). Honorary author have been used to describe individuals who, although listed on the byline as authors of an article, have not met authorship criteria for active participation in the research, in manuscript drafting, and in manuscript approval (Rennie et al 1997 and Rennie 1994).

Identity Theft Authorship: Identity theft authorship is a growing problem in academic community and has been used as a weapon for gainful activities and for fulfilling personal motives. With increase in learned individuals, academics and publishing, the problem is further mounting. Identity theft occurs when someone uses another individual's personal information to pose as that individual (Kent and Millett 2003). Uncountable numbers of papers are published every year and we see that in most of the cases full name and surname of the authors is not published and only initials are given and even gender is not mentioned likewise complete affiliation like position department, academic addresses are also not given. In most of the cases telephone and or email Id (identity) is

also not specified. All these shortcomings lead to identity theft authorship where person other than genuine author impersonates and uses the publications of other as his own for personal motives like promotion, getting grants, funds, recognition, improving annual confidential reports etc. The problems further aggravates since no legitimate mechanism is available for identity theft authorship detection and the problem remains undetected unless some personal level complain is done.

Coercive Authorship : Coercive authorship has been defined as authorship conferred to individuals in response to their exertion of seniority or supervisory status over subordinates and junior investigators of by use of intimidation tactics to gain authorship. Arguably it is a serious form of scientific misconduct (Kwok 2005 and Claxton 2005)

Incomplete Authorship : It is not acknowledging those who have contributed substantially to research leading to a paper (Huth 1986). Incomplete authorship is failure to include individuals who contributed substantially to a project; students are most frequently overlooked as authors (Irwin 2007).

Irresponsible Authorship : It includes unjustified authorship that is acknowledging as authors, individuals who did not have a substantial part in the research represented by a paper (Huth 1986). Here the problems are related to unjustified authorship, incomplete authorship, and/or inaccurate quotations and/or references (Irwin 2007). If the paper is having flaws then it is an indication of irresponsible authorship.

Corporate Authorship : The authorship of a firm or organization is known as corporate authorship. A corporate author is defined as, an organization or a group of persons that is identified by a particular name and that acts, or may act, as an entity (Wynar 1992). Authors generally work for a particular firm or an organization and the produce publication for them without taking or getting any credit for themselves. The copyright is owned by the corporate firm. The firm normally hires/employs people or authors to take up work for the firm.

Inappropriate Authorship: Inappropriate authorship signifies that the manuscript must not contain name of any author or coauthors who has not contributed toward the result presented in the manuscript. It also known as undeserved authorship. Inappropriate authorship just increases the number of authors without any gain.

Ascribe Authorship: The transfer of credit or discredit of any publication by the author, researchers, reviewer or editor should not be done either heedlessly, deliberately or evasively, all authors should bear the moral responsibility and accountability of the publication with clear distinction of each of the authors work.

Unearned Authorship : It is a form of science misconduct in which a person is listed as author, but did not 'earn' the right to be designated as such (Segen's Medical Dictionary 2011).

Multi-authorship : Any published article which is having two or more authors is considered to be a multi-authored paper. Whenever we go across any journal we see that the number of single authored papers are far less as compared to multi-authored paper, thus it can be presumed that the trend for multi-authorship is growing, it can also be attributed to the fact that since science is drifting more towards multidisciplinary facet. According to Monsen (1991) multi-authorship carries the risk of diminishing the contribution of respective authors. Multi-authorship trend reflects not only the increased complexity of modern research, but a growing entrepreneurial ethos and they see it as a problematic response to an increasingly competitive publication-based regime of credit and professional advancement (Angell 1986, Engler et al. 1987, Rennie and Flanagin 1994, Smith 1994 and Shapiro et al. 1994).

Credit Theft Authorship : This is a relatively new term and is most common in countries where the researchers are unaware, ignorant and unacquainted about their rights concerning publication issues. Such issue is of major concern especially in developing and under developed nations. Mostly for award of master's and doctorate degree the research component is done by the post-graduate student and after completion, most of the students leave academic field for the search of jobs and other purposes except a very few. The left over experimental research work in form of dissertation/theses is available with the supervisor/advisor. When this research work is published without giving credit to the original researcher or by removing the researchers name from first authorship place then such type of authorship is known as credit theft authorship or wangle authorship. Where the concerned author willfully, wishfully contrivance to extricate benefit out of others research work without giving requisite credit and authorship. We see many examples of it; in words of Hammersley (2001) sometimes advisors place themselves as first author inappropriately, while their students, who have done most of the work, are placed as

co-authors. It is an activity where the work is done by someone else and credit is taken by someone else. It is deceitful act and is different from gift, ghost or honorary authorship. In such cases the authorship is misrepresented and fudged.

Cyber-pseudepigraphy : This term is probably new for the readers of agricultural community and most of us do not know about it, Page (2004) has explained cyber-pseudepigraphy and could be defined as using the Internet to procure another person to write an academic essay or paper, pseudepigraphy, in a restricted sense, refers to the ancient Middle Eastern practice of writers ascribing a false name as the author of a particular work, usually to give a piece of writing greater authority or credibility in an extended sense, we can refer to pseudepigraphy as any attempt to assign a false name to a piece of writing.

Grafters : Rennie and Flanagin (1994) explained grafters as those who exact authorship in exchange for access to subjects, proprietary reagents or probes, funding or the like.

Guarantor : A guarantor is a person who acts to owe the complete of the published material. In most of the cases a guarantor is either the main author or one of the author/ corresponding author. In sole authorship normally the author is the guarantor. In terms of Rennie et al. (1997) guarantors are individuals who have contributed substantially to the manuscript and who have also made an extra effort to ensure the integrity of the paper as whole guarantors are prepared to be accountable for all parts of the completed manuscript, before and after publication.

Contributorship : Edwards (2009) advocates that, contributorship, applies in cases where clear attribution of content fails and authorship criteria are not met in full, contributorship credit can duly be given to someone for acquisition of funding, supervision of the research process or technical aspects of data collection.

Lazy Writing : Irwin (2007) has explained lazy writing as closely related to plagiarism except references are cited; paragraph after paragraph lifted out of one or more sources and presented as a paper.

Gatherer: A gatherer starts to prepare for writing by collecting. A gatherer collects all of the available books, articles, or other materials related to the subject, particularly those that are similar to the paper that is to be written the gatherer then reads all the material gathered and sifts through, analyses and possibly synthesis all the material from the sifting, analysis or synthesis, the gatherer organizes ideas in the material in what seems to be a logical sequence, and then begins to write (Dixon 2011) the paper. A gatherer acts as an individual who tries to generate information from preexisting published information.

Hunter Style : Dixon (2011) has explained this style, in hunter style, an author works through a systematic thought process and makes key decisions about the work the author wants to describe, before starting to write, the thought process includes defining what journal readers want to read about, answering key questions about the subject being written about and organizing the ideas into a logical structure, persons adopting this style are known as hunters and a hunter, first decides why the paper is needed, what will interest the readers and what is expected of the paper's writer.

Pretexting : Pretexting is the use of impersonation or fraud to trick another person into releasing personal information (http://epic.org/privacy/iei) or pretexting is a form of social engineering in which an individual lies to obtain privileged data, a pretext is a false motive (http://searchcio.techtarget.com). In other words we can say that pretexting is the practice of getting your personal information under false pretenses (http://www.ftc.gov). A pretexter is a person who is involved in the act and tries to impersonate as the original to obtain desired information. In academic world it is used to copy the ideas, future plans, projects, thoughts etc and take credit in ones name.

Falsification : it is a scientific misconduct, manipulating research materials, equipment, or processes, or changing or omitting data or results is known as falsification (http://www.ori.dhhs.gov). It is a type of scientific misconduct. Published data is always believed to be already validated hence falsification pose a threat to society and can lead to faults in other related experiments.

Fabrication : Making up data or results and recording or reporting them (http://www.ori.dhhs.gov). Fabrication is the invention of data arguably is the most blatant form of misconduct affecting truth claims. It ranges from the invention of all data reported to the invention of some of it (Holm 2007).

Collusion : The Durham University's definition of collusion

is as, working with one or more other students to produce work which is then presented as one's own in a situation in which this is inappropriate or not permitted and/or without acknowledging the collaboration (http://www.robots.ox.ac.uk/~gari/ethics. html). Barrett (2005) defined it as collusion is working together to produce assessed work in circumstances where this is forbidden. According to Curtin University, Australia, collusion means an agreement with another person to deceive others (http://www.academicintegrity.curtin.edu.au/global/staffbook.cfm). Whereas Jones et al (2005) delineate collusion as the collaboration without official approval between two or more students (or between student[s] and another person[s]) in the presentation of work which is submitted as the work of a single student; or where a student(s) allows or permits their work to be incorporated in, or represented as, the work of another student.

Paraphragiarism : Using the text of others with a few changes or mixing the others' texts without acknowledging the source(s). It is copying a portion of text from one or more sources, inserting and/or deleting some of the words, or substituting some words with synonyms, but never giving credit to its author nor enclosing the verbatim material in quotation marks (Howrd 1999; Levin and Marshall 1993).The word patch-writing (Howard 1999) is synonym to paraphragiarism (Levin and Marshall 1993).

Copyright Infringement : When an author publishes in a journal, the copyright of the author's work gets transferred to the publisher. The unauthorized duplication of work is copyright infringement (Adhikari 2010). Copyright infringement is against law and is considered as an illegal activity if it is done without obtaining proper permission from its copyright owners.

Plagiarism : This term has off late gained a lot of publicity, possibly due to the increase in number of educated, career conscious and shortcut adopting professionals, and now plagiarism is widely acknowledged to be a significant and increasing problem for higher education institutions (McCabe 2005; Judge 2008). Availability of computers, internet and unprincipled money oriented fast publishing has acted as a catalyst in promoting plagiarism. Cut, copy and paste options of word and image processing softwares are the best vehicle leading towards the path of plagiarism. According to Palmquist (2003), plagiarism involves intentionally or unintentionally using someone else's intellectual property without properly acknowledging the original source. According to the words of Bird (2002), plagiarism refers to claiming the words or ideas of another as one's own. In simpler words we can say that anything which is already published in some form should not be quoted in another publication as one's own publication. Whereas Siedlecki et al. (2008) argues that plagiarism means 'lifting an entire sentence or paragraphs and using it without quotation marks or correct citation. Thus to avoid plagiarism an author should always give proper citation and acknowledgement in the reference to the original source.

Self-plagiarsm : Self-plagiarsm is inappropriate recycling of text, it is nebulous, otherwise also known as auto-plagiarism (Jason 2010). One aspect, of self-plagiarism as noted by Roig (2005) is breach of copyright, Headache. (2009), have instituted policies that clearly note that self-plagiarism is a violation of ethical standards. Self-plagiarism is widespread and sometimes unintentional (WAME 2011). It is clear that unauthorized copying of self published matter and its repetition should be avoided in future publications if otherwise absolutely obligatory, if self-plagiarsm is done then copyright issues should be kept into consideration and proper citation be given.

Contract Cheating: This phenomenon is common among the college students. Contract cheating is defined as submission of work by students for academic credit, which the students have paid contractors to write for them (Clark and Lancaster 2006). Contract cheating is a form of academic dishonesty in which a student would pay someone (a contractor) to complete a given piece of coursework and then submit it as his or her own (Mahmood 2009). Contract cheating is widely adopted by the students for timely completion of projects and assignments given to them by their teachers; it also unethically aids the students to get good percentage/grades in the exam/evaluation process.

Salami Publication : Publication of each part of the results of one study in several papers, the practice of cutting up a body of data to yield several papers where one complete paper would be optimal (Laitman and Rikkers 2000). Making up of multiple publications in same or different journals out of results obtained from single study is known as salami publication or salami slicing. The term salami article refers to those authors who chop their research into many articles with common methodology to obtain a greater number of publications, without sufficient differences between them to justify this (Tramer et al. 1997).

Bologna, magnum opus, sibling publications and trivial publication are synonym to Salami. In other words we can say that it is a form of multiple publications generating from a single study/research. Thus if a reader wants to read a particular salami publication then he will have to read more than one paper of that particular author/authors to get complete information on that aspect. The main aim of salami publication is to increase the number of self publications to fulfill personal vested interests to meet out academic needs.

Duplicate Publication : It is also known as repetitive publication. Duplicate publication is a self explanatory term, it means republishing the paper once again without properly citing the first publication. Duplicate publication is an unethical practice. Duplicate or publication redundant is defined as a paper that overlaps substantially with one already published or submitted elsewhere (Mojon-Azzi et al. 2004). Its complexity increases if there is variation in authorship. It is also known as redundant publication, according to Roberts (2009), redundant papers may contain differences in how they are written but the data, outcomes, and conclusions are the same. Duplicate publication if prepared deliberately is done with a motive to increase ones publications.

Suspected Publication : Any published article or manuscript for which the journal editor receives some complain for ethical, legal and authorship issues or when such issues are detected at any instance of time after manuscript publication comes under the category of suspected publication. Any sort of allegation can bring manuscript under suspicion and scrutiny and if allegations are proved to be truthful the most quick action would be withdrawal of published paper from the journal apart from other actions.

Imalas Publication : Imalas publication is the sequential publishing of what are essentially the same results, but with a few new data included in the analysis each time (Holm 2007).

Data Augmentation : Data augmentation is a sort of malpractice and occurs when a researcher publishes a study, subsequently collects additional data, which typically end up strengthening the original effect, and publishes the combined results as a "new" study in a different journal. The reader is thereby misled into believing that two independent studies have been carried out (http://wwwfacpub.stjohns.edu/~roigm/ plagiarism/Salami%20slicing.html). Wahlstrom et al (2006) explained data augmentation as it is adding to the data in non-obvious ways, without altering their usefulness, reconstruction of original data can be prevented.

Fragmented Publication : In words of Armstrong (1982) a fragmented publication is whereby authors chop the study into small pieces to publish in various journals over a period of years. It is also known as piecemeal publication. It is expected from each author or a group of authors and coauthors to publish their findings as a single publication without other serving for the paper. Results from a single study/experiment should be presented in form of a sole report or research paper. Unwanted fragmentation of a research for personal enhancement is undesirable and should be avoided.

Data Dredging : it is defined as inappropriate fishing for information or data mining to find misleading relationships to form statistical bias, the relationship between data may be true within a small test set, but they would generally not have statistical accuracy to the wider population, the data dredging can be intentional or unintentional (http://www.businessdictionary.com/definition/data-dredging.html).

Data Torturing : Data torturing involves continued manipulation of the data till a desired result is obtained. Mills said that "if the data are tortured long enough they will give the researcher whatever he or she wants to hear" (Mills 1993).

Text Recycling : When a research is done at two places with similar methodology, using substantial chunks of the previous study including abstract, introduction and methodology is called text recycling (Adhikari 2010). In other words we can say that it is the possible recycling or reusing of parts and portions of text already published previously in some other form.

Verbatim Copying : The dictionary meaning of verbatim is, 'using exactly same words', or we can say that verbatim copying is using the text or any published materials of others without acknowledging the original source of the article or publication. The outcome of verbatim copying can be regarded as verbatim replica of original or verbatim repetition of text or original

Research Misconduct : Academic or publication misconduct is widespread among the scientific community (Council of Biology Editors 1990). The Clinton committee

has defined research misconduct as 'fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results (Valluri 2001). In the words of Goodman (1996) research misconduct is significant misbehavior that improperly appropriates the intellectual property or contributions of others, that intentionally impedes the progress of research, or that risks corrupting the scientific record or compromising the integrity of scientific practices. Research misconduct does not include honest error or differences of opinion (Anonymous 2011). The misconduct must be committed intentionally; the definition of misconduct can also extend to breaches of confidentiality and authorship/publication violations (http://www.apa.org/research/responsible/misconduct/index.aspx). Also assessing and managing research misconduct is undoubtedly complex (Scott-Lichter et al. 2009) phenomenon.

Scientific Fraud: In the words of Khalid (2010), scientific fraud is simply defined as counterfeiting information or statistical data to maintain certain results. Sometimes scientific fraud engulfs unfair evaluation of results and altering effecting data. In the context of scientific fraud comes the illegal ownership of information that should otherwise be accredited to others (http://www.mademan.com/mm/what-scientific-fraud.html).

Conclusion

We all belong to educational society and the time has come when there is an urgent need to maintain transparency in all our professional publication activities. It is essential to maintain scientific integrity in all publications. Authors are tempted towards publication of their work. The criteria developed by authorities towards career promotional policies, reward and funding grants is distracting some authors from following ethical, legal and moral values. It is extremely difficult to identify such issues. As far as possible human predicament should be prevented at all stages. The easiest approach to set right this problem is self-evaluation, self regulation, self-will control through effective motivation of possible authors. Keeping ourselves aware about the ethical and legal issues of publication is only an alternative.

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Leaf senescence and dry matter partitioning in relation to grain yield in early rice breeding lines under upland bunded environment

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Abstract

Pattern of Senescence appeared to differ in eight rice breeding lines under upland bunded environment during wet season of 2007 at College of Agriculture Farm, Rewa, JNKVV, M.P. Leaf area index, leaf dry matter, stem dry matter, total dry matter and panicle growth at flowering 10, 20 DAF and at maturity were determined. Breeding line AS-06016 had larger LAI (4.10), greater LDM 526 g^{-1} m^2 and SDM 361 g^{-1} m^2 at flowering and relatively gradual decrease in these parameters during grain filling phase exhibited superiority among other breeding line in pattern of dry matter partitioning. This leads to substantial increase in panicle growth (67.1 to 528 g^{-1} m^2), higher spikelets number 20.8 ($x10^3$)m^2, FGP 91.8%, HI 46.0% and grain yield 510 g-1 m2 in comparison to all others reflecting efficient translocation towards sink with delayed leaf senescence. Photosynthetic source component LAI had negative correlation to LDM and SDM at later stages of GFP, while, strong positive correlation with TDM and gain yield (r=0594*, 0.651*, 0.854** and 0.968**). LDM and SDM to TDM and grain yield at 20 DAF and maturity stage exhibited variation in degree of senescence and dry matter partitioning amongst breeding lines.

Keywords: Leaf area index, leaf dry matter, Stem dry matter, Grain filling period

Senescence is not just the running down of tissues or cell but a programmed part of development. During leaf senescence degradative processes are initiated in the chloroplasts and mitochondria as well as in the cell (Tanaka and Vergara 1967). This leads to great advantage to annual plants by withdrawal of carbohydrates from senescing cell to growing parts of the plants that is sink as well as essential to the functioning of xylem and phloem cell means translocation (Bidwell 1979). As the photosynthetic capacity of the whole plant is the result of various individual capacities of its leaves, the photosynthetic contribution of the leaf to the whole plant changes continuously with its age, i.e. the amount of photosynthate export from a leaf varied from time to time and delayed leaf senescence is desirable trait indicating more contribution of photosynthate for yield during grain filling phase (Murty and Venkateswarlu 1978).

The direction of photosynthate translocation also changes as the leaf ages with basic idea of phyllotaxic relationship that transport takes place via the most direct route to the nearest available sink (Wardlaw and Passioura, 1976) suggesting that the, contribution of flag leaf and penultimate leaves are more towards the sink.

Material and methods

A field experiment on early duration rice breeding lines under rainfed upland bunded conditions was carried out during 2007 at College of Agriculture, Rewa, JNKVV (MP), India, to assess the relationship between leaf senescence and dry matter partitioning during grain filling phase and yield realization in rainfed bunded conditions. Eight upland rice breeding lines including national two check varieties Annada and Tulasi were tested in a randomized block design with 4 replications.

The soil of the experimental field was silty clay loam in texture with normal pH (7.1), E.C. dSm^{-1}), low in organic carbon (0.58%) 1000 available P (8 kg ha^{-1}), medium in available N (237 kg ha^{-1}) and high in available K (517 kg ha^{-1}). The experiment was sown on 03.07.2007 by direct seeding method. The plot size was 5.0 x 2.0 m. The fertilizer was applied to the crop @ 60:40:40kg NPK ha. One third dose of the nitrogen and full dose of phosphorus and potash were applied at the time of sowing and remaining N was top dressed in two equal split doses at the time of maximum tillering and panicle initiation stages.

The observations were taken on leaf area index, leaf and stem dry matter at flowering of 10 DAF, 20 DAF and at maturity and sink size panicle growth at reproductive phase and their statistical correlation

(Federer 1955). Yield attributing parameters at harvest were recorded for the purpose.

Results and Discussion

Physiological parameters

Significant genotypic variations were recorded for LAI at flowering onwards (10 day intervals) till maturity. Breeding line AD-06016 and NDR-2705 had larger LAI (4.10) than other test genotypes at flowering. There is a declining trend in LAI with advancement of crop age towards maturity, (Khan et al. 1999) and it mean proportion was 6.6% at 10 DAF, 40.8% at 20 DAF and 81.9% at physiological maturity indicating that during GFP degradetive processes was initiated in the chloroplasts and mitochondria at 10 DAF (Tanaka and Vergara 1967) and become fast resulting faster senescence after 20 DAF. Delayed senescence in breeding line As-06016 (6.8% and NDR-2705 (5.8%) among the genotypes indicating efficient source utilization to long persistence of flag leaf and penultimate leaf may provide higher active photosynthetic surface area and contributed more carbohydrate to words sink during GFP (Murty and Venkateswarlu 1978).

Significant variation among the genotypes was recorded for LDM SDM and TDM (g^{-1} m^2) at flowering 10 DAF, 20 DAF and maturity. Breeding line AS- 06016 and NDR-2705 maintained significantly higher LDM during GFP while, VL-30424, AS-06016 and NDR-2705 had higher SDM during grain filling period which resulted into higher accumulation of total biomass. Mean decrease in LDM from flowering to 10 DAF, 20 DAF and at maturity was 14.5%, 25.4% and 32.6% respectively. The decrease in AS-06016 were 20.7%, 27.9% and 36.8% respectively during the same period which is 6.2%, 2.5% and 4.2% more than the mean decrease indicating an apparent positive relationship between the level of grain yield and magnitude of decrease in LDM during GFP.

Similarly the mean decrease in SDM from flowering to 10 DAF, 20 DAF, and maturity was 6.9%, 17.9% and 25.2% respectively during GFP with the highest value in AS-06016 exhibiting efficient translocation of stem reserves towards the sink. Mean dry matter decrease pattern in LDM and SDM also indicated that under upland condition mobilization of reserve toward sink is 7.4% faster in the leaves than in the stem (Sen et al 2000).

Significant variation were observed amongst breeding line for total dry matter the highest TDM was found in AS-06016 (954.1g^{-1} m^2) which maintained superiority till harvest while VL-30424 and NDR -2705 was found next to it for total biomass accumulation.

Mean panicle growth between flowering to 10 DAF varied from 49.5 to 2540.g^{-1} m^2, 10 DAF to 20 DAF from 133 to 396 g^{-1} m^2 and 20 DAF to maturity from 248 to 528g^{-1} m^2 and AS-06016 and greater panicle growth than other genotypes while, NDR- 2705 and VL-30424 were at per.

Phenology and yield parameter

The mean number of days taken to 50% flowering from sowing was 75 days Check CV Tulasi was very early (70DAS) among the genotypes while, HPR-2336 and VL-30249 took more days (78-79 DAS) for flowering indicating genotypic variability under rainfed bunded condition Significant genotypic variation were also recorded with regards to number of spikelets m^2 filed grain %, 1000 grain weight and harvest index (Table 1). Breeding line AS-06016 had higher value of these parameters due to more active photosynthetic surface area and higher accumulation of LDM, SDM and panicle growth at physiological maturity resulted into higher grain yield (510g^{-1} m^2).

Table 1. Penology, yield attributing characters and yield of rice breeding lines under rainfed condition

Breeding lines	Days to 50% flowering	Number of penicle (m)	Number of spikelets ($x10^3$)/m^2	1000 grain wt. (g)	Filled grain (%)	Harvest index (%)	Grain yield ($g^{-1}m^{-2}$)
IET-19836	78	301	15.1	23.7	86.6	44.0	430
AS-06016	73	366	20.8	24.6	91.8	46.0	510
VL-33424	72	321	16.8	23.5	90.5	42.7	450
VL-30249	79	294	16.4	22.8	84.5	46.3	401
HPR-2336	78	316	14.0	23.1	84.8	43.0	410
HDR-2705	72	330	19.6	24.0	90.7	47.8	495
Annada (C)	78	307	13.8	23.4	81.2	42.6	355
Tulasi (C)	70	315	12.7	21.5	80.8	40.9	305
Mean	76	319	16.1	23.3	86.4	44.1	419
CD (P=0.05)	1.2	4.62	1.63	1.35	2.50	4.16	39.9

Correlations

The photosynthetic source components LAI had negative correlation with LDM and SDM at maturity stage because of decreasing trends in dry matter while, strong positive correlation with TDM and grain yield (r-0.702**, 0.432* and 0.662*, 0.713*) respectively at 20 DAF and maturity stages (Table 2) indicating faster accumulation of reserve carbohydrate into the sink. Correlation of LDM to SDM, TDM and grain yield and SDM to TDM and grain yield were also found positive and significant during later part of grain filling phase exhibited variation in degree of senescence and dry matter partitioning among breeding lines.

Hence, breeding line AS-06016 having delayed leaf senescence by 6.8% higher active photosynthetic surface area in relation to mean LAI at physiological maturity contributed more photo assimilate towards the sink resulting into higher biomass accumulation and its partitioning and grain yield under upland bunded environment.

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Table 2. Correlation coefficient between LAI, LDM, SDM, TDM, and graind yield during grain filling period in rice under rainfed upland bunded environment

Parameters	Stages	LDM	SDM	TDM	GYL
LAI	Flow	0.110	0.179	0.324	0.103
	10 DAF	0.422	0.014	0.142	0.424
	20 DAF	0.353	-0.354	0.702**	0.662
	Mat.	-0.690	-0.469*	0.432*	0.713*
LDM	Flow		-0.222	-0.509*	-0.506
	10 DAF		0.368	0.072	0.164
	20 DAF		0.768**	0.092	0.462*
	Mat.		0.363	0.849**	0.436*
SDM	Flow			0.228	0.387
	10 DAF			0.378	0.98
	20 DAF			0.482*	0.526*
	Mat.			0.787**	0.493*
TDM	Flow				0.594*
	10 DAF				0.651*
	20 DAF				0.845**
	Mat.				0.968**

*, ** =significant at 0.05 and 0.01 probability level respectively

चावल की आठ प्रजनक पक्तियों में उचेंहन मेड़ वातावरण में पत्तियों के सूखने की अवधि एवं जैवभार उत्पादन तथा विभाजन का अध्ययन वर्ष 2007 में कृषि महाविद्यालय रीवा प्रक्षेत्र में किया गया। प्रजनक पक्ति ए. एस.-06016 में अधिकतम पर्ण क्षेत्रफल सूचकांक (4-10), उच्च पर्ण शुष्क भार (256 ग्रा./वर्ग मी.) एवं तना शुष्क भार (361 ग्रा./वर्ग मी) पुष्पावस्था में पाया गया, तत्पश्चात् इनमें निरंतर कमी दानों के भरने की अवस्था में देखी गई जो शुष्क जैवभार विभेदन की दृष्टि से अन्य प्रजनक पंक्तियों से इसे श्रेष्ठ बनाती है। इसके अतिरिक्त इस प्रजनक पंक्ति में उच्च शूकिका वृद्धि (64.1 से 528 ग्राम/वर्ग मी.), शूकिकाओं की संख्या (28.8 ग 103/वर्ग मी.), भरे दानों का प्रतिभात (91.8), कटाई सूचकांक (46 प्रतिशत) होने से अधिकतम उपज (510 ग्रा./वर्ग मी.) प्राप्त हुई। यह उपज अन्य प्रजनक पंक्तियों की तुलना में उत्तम प्रकाश संश्लेषण, भोज्य संवहन एवं देरी से पत्तियों के पीले पड़ने की वजह से हुई। विभिन्न गुणों का परस्पर सह संबंध अध्ययन दर्भाता है कि दाना भरने के बाद की अवस्था में पर्ण क्षेत्रफल सूचकांक का पर्ण शुष्क जैवभार, एवं तना शुष्क जैवभार से ऋणत्मक सह संवंध है, परन्तु मजबूत धनात्मक सह संबंध कुल जैवभार एवं उपज से है। पर्ण एवं तना शुष्क जैवभार का कुल जैवभार एवं उपज से धनात्मक सह संबंध पुष्पन के २० दिनों पश्चात एवं परिपक्वतावस्था में पाया गया जो कि पत्तियों के सूखने के क्रम एवं शुष्क जैवभार विभाजन, किस्मों में भिन्नताओं के परिणामस्वरूप पाया गया।

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Land use/Land cover mapping of Gusuru river watershed using Remote Sensing and GIS technique

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Abstract

Land use/land cover map of Gusuru river watershed in part of Panna and Satna district of Madhya Pradesh was prepared using satellite data. Six land use/land cover classes were identified in the study area. For satellite image classification unsupervised classification followed by visual interpretation technique was used. The study area has forest as the predominant land use/land cover class i.e. 88.03 per cent of total geographical area of the watershed.

Keywords: Remote sensing, GIS, watershed and Land use

Land is finite natural resources and there is no scope to increase the area under cultivation. The earlier efforts to cultivate crops in new areas where the forest area was converted to cultivated area has resulted in reduction of the forests by about 20 per cent of the total geographical area in India. The food production in India can only be increased by increasing the crop productivity (Sarkar 2005). The higher productivity can only be achieved with better information of land and its use. The knowledge of land use and land cover is important for many planning and management activities as it is considered an essential element for modeling and understanding the earth system. The term land use relates to the human activity or economic function associated with a specific piece of land, while the term land cover relates to the type of feature present on the surface of the earth.

A modern nation as a modern business must have adequate information on many complex interrelated aspects of its activities in order to make decisions. Land use is only one such aspect, but knowledge about land use/land cover has become increasingly important as the nation plans to overcome the problem of haphazard, uncontrolled development, deteriorating environmental quality, loss of prime agricultural lands, destruction of important wetlands and loss of fish and wildlife habitat. Land use data are needed in the analysis of environmental processes and problems that must be understood if the living conditions and standards are to be improved or maintained at current levels.

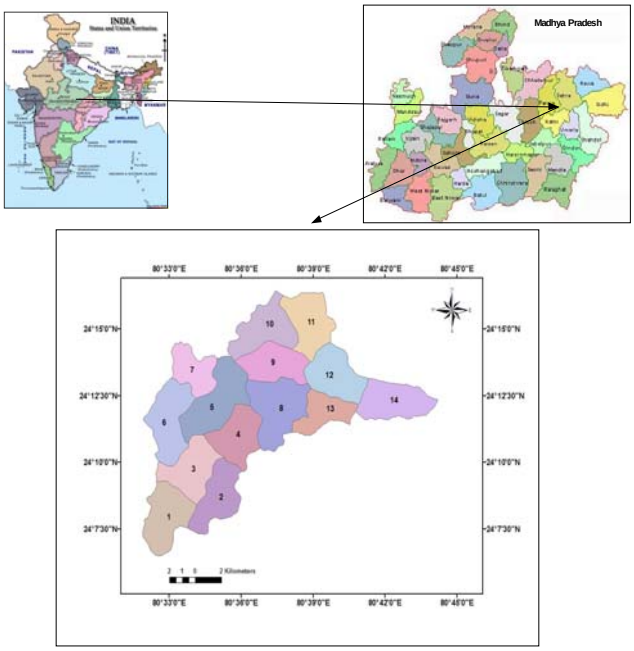

Fig. 1 Location map of study area

Remote sensing has emerged as a powerful tool in recent past for natural resources assessment with the ability of obtaining systematic, synoptic and repetitive coverage. The satellite remote sensing technology has found its acceptance world wide for rapid resources assessment and monitoring, particularly in the developing world. Geographical Information System (GIS) has made remote sensing a unique technology and widened the spectrum of sensing application in natural resources management. In this context, remote sensing technique plays an important role in the improvement of the present land use systems in the country (Vijay Kumar et al. 2004). Considering all these aspects an attempt has been made to prepare land use/land cover map using remote sensing and GIS technique of the study area.

Study area

The study area Gusuru river watershed is located in Part of Panna and Satna district of Madhya Pradesh and covers an area of 155 km^2 and is bounded between 80^0 32' 50.23" E and 80^0 37' 31.14" E longitude, 24^0 6' 32.75" and 24^0 16' 24.07" N latitude (Fig 1). The maximum and minimum elevation encountered in the watershed is 628 m and 339 m above mean sea level respectively. Temperature ranges between minimum of 40 ^{0}C during December or January months and the maximum of 45^0 C in May or June. Average annual rainfall is 1100 mm and is concentrated mostly between mid June to mid September with scattered rain during late December and January months.

Materials and methods

Base map of the study area was prepared using Survey of India (SOI) toposheets no. 63D/11, 63D/12 and 63D/16. Remote sensing data (toposheets basis) of IRS-P6 LISS-III for the study area acquired on 25-10-2007 were procured. The geocoded satellite data was procured from National Remote Sensing Agency (NRSA) Hyderabad. Digital image processing techniques were applied on the images making use of spatial and radiometric enhancement techniques in order to remove shadow and for proper tone and texture of the images. Then the watershed boundary of the study area was delineated using the SOI toposheets on 1:50000 scale and remote sensing data. The delineated watershed boundary was further subdivided into the micro-watersheds.

False Colour Composite (FCC) images of each micro-watershed were subjected to the process of classification. As a sample set a FCC of micro-watershed 1 is presented in Fig 2.

In the present study the land use/land cover categorization in the Gusuru river watershed is envisaged based on the classification scheme developed by NRSA

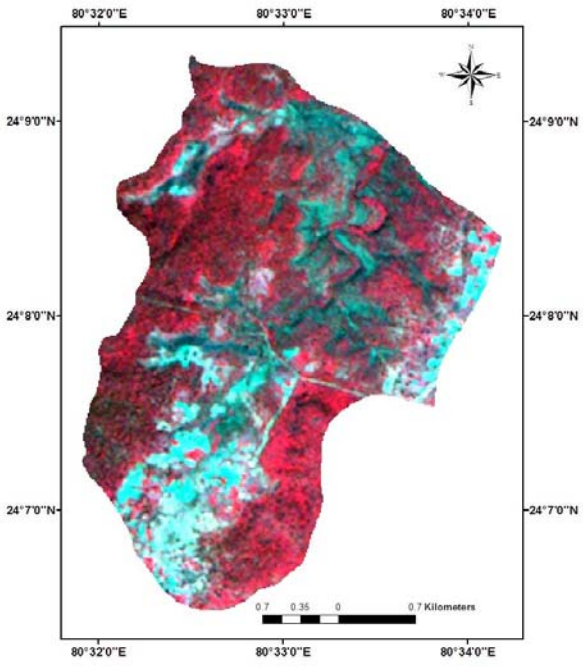

Fig 2 FCC of Micro-watershed 1

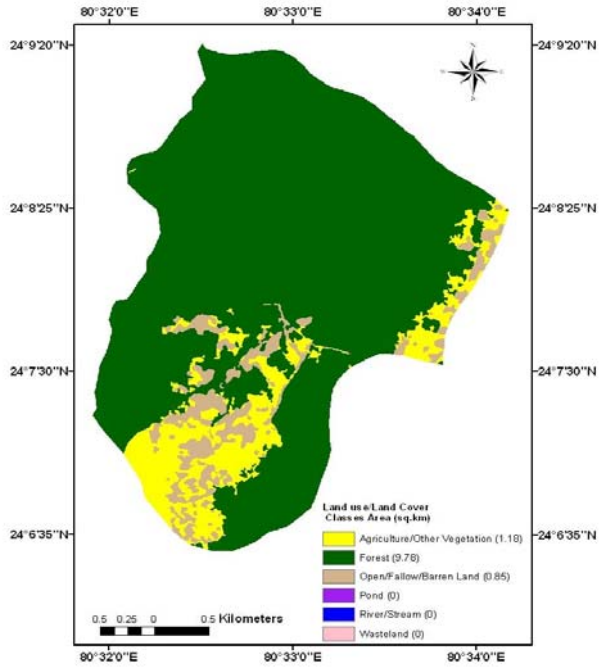

Fig 3. Land use/land cover map of Micro-watershed 1

Table 1. Interpretation key used in the On-screen Visual Interpretation Method for land use/land cover classification

Classes	Tone	Texture	Shape	Association	Pattern
Forest	Dark red/dark brown to red	Smooth	Regular	Everywhere	Contiguous
Open forest & Scrubs	Bright red	Smooth to course		Open forest, forest gaps, degraded forest, scrubs	Scattered
Agricultural/ Other vegetation	Red to Pink	Smooth	Mixed		Contiguous
Open/Barren land	Gray to Green	Smooth			Contiguous
Water body	Blue to Black	Smooth	Regular		Scattered
Wasteland	White to Whitish blue	Coarse	Uneven		Scattered

Table 2. Micro-watershed wise Land use/land cover distribution of study area

Micro watershed No.	Land use/land cover						
	River (km^2)	Pond (km^2)	Wasteland (km^2)	Agriculture/other vegetation (km^2)	Open/barren land (km^2)	Forest (km^2)	Total area (km^2)
1	0.000	0.000	0.000	1.180	0.850	9.783	11.813
2	0.020	0.000	0.000	0.790	0.370	9.526	10.706
3	0.030	0.002	0.000	0.150	0.410	10.540	11.132
4	0.020	0.000	0.000	0.005	0.002	10.477	10.505
5	0.030	0.000	0.000	0.013	0.027	14.400	14.470
6	0.030	0.000	0.000	0.510	0.450	11.584	12.574
7	0.000	0.000	0.000	0.110	0.140	7.645	7.895
8	0.030	0.000	0.000	0.630	0.320	13.743	14.723
9	0.008	0.000	0.000	0.420	0.200	9.556	10.184
10	0.008	0.000	0.000	0.000	0.820	10.750	11.578
11	0.020	0.000	0.000	0.230	0.190	10.636	11.076
12	0.020	0.000	0.000	0.840	0.980	9.647	11.487
13	0.020	0.000	0.000	0.520	1.060	6.185	7.785
14	0.030	0.030	2.190	1.710	3.150	2.139	9.249

(1995). Initially the unsupervised classification method was used for making the broad categories. Then the images were classified by onscreen visual interpretation technique, based on the available ancillary data, prior knowledge and sufficient ground truths using ERDAS Imagine software. The multispectral characteristics of the different class type i.e. variation in tone, texture, shape, association, pattern of various objects within the satellite data formed the basis for classification (Table 1).

Six categories were identified viz. forest, agriculture/ other vegetation, open/barren land, river, pond and wasteland. Forest and open forest & scrubs are classified as one class forest. Water body was classified into two categories river and pond. The areas with dark red to dark brown to red tone visible as smooth texture were considered as the forest (Fig 3).

Similarly, the entire crop lands, plantations and permanent pasture were put in one class naming as agriculture/other vegetation. Those regions with red tone to light to bright pink tones, visible as smooth texture, regular shape in image were considered as agriculture/ other vegetation (Fig 3). Likewise, regions with gray to green tone visible as smooth texture and contiguous in pattern were considered as open/barren land (Fig 3). Those regions with white to whitish blue tone, visible as coarse texture and scattered pattern were considered as wasteland. Similarly, regions with blue to black tone visible as smooth texture and linear in shape were considered as river and canals and put in one category i.e. river. Those regions with blue to black in tone visible as smooth texture and polygon shape were considered as pond. The doubtful areas or wrongfully interpreted areas were verified by ground truths.

Result and Discussion

The land use/land cover of Gusuru river watershed include forest areas, areas under agriculture and other vegetation, open/barren lands, water body and waste land (Table 2). Based on satellite data of 25th October 2007 forest occupies 88.03 per cent of total geographical area of watershed (155 Km^2). Open forest, degraded forest, dense forest and scrubs are clubbed as forest. Crop lands, plantations and pastures are included in agriculture/other

vegetation and they spread over 4.58 per cent of the area of watershed. Open/barren lands include the area which is not sown and fellow lands, in the study area it occupies about 5.77 percent of total geographical area of watershed. About 1.41 per cent of the total area of watershed is occupied by wastelands. Water bodies which has been grouped in river and pond is spread over 0.17 and 0.02 per cent of the area of watershed.

It is evident from the Table 2 obtained from land use / land cover analysis of the micro-watersheds of Gusuru river watershed, that except micro-watershed 14 remaining thirteen micro-watersheds have forest as the predominant land use/land cover out of six classes of land use/land cover in the study area. The forest cover for the remaining thirteen micro-watersheds ranges between 79.44 to 99.32 per cent indicating non significance of other land use / land cover classes. However, for the micro-watershed 14 which has forest cover of the order of only 23.126 per cent with barren land of 34.057 per cent and agriculture land of 18.488 per cent is distinct. This micro-watershed also has 23.678 per cent of wasteland while, the remaining thirteen micro-watersheds has no wasteland at all.

Conclusion

The digital image interpretation in conjunction with ground truth has greatly helped to assess the present land use/ land cover map of the study area. Unsupervised classification method followed by on screen visual interpretation resulted in six categories of land use/ land cover viz forest, agriculture/other vegetation, open/barren lands, river, pond and wasteland. Forest is predominant land use/land cover among all other classes identified in the study area. The remote sensing with its multispectral and synoptic view has the potential to provide accurate spatial information on land use/land cover of a region in a time and cost effective manner.

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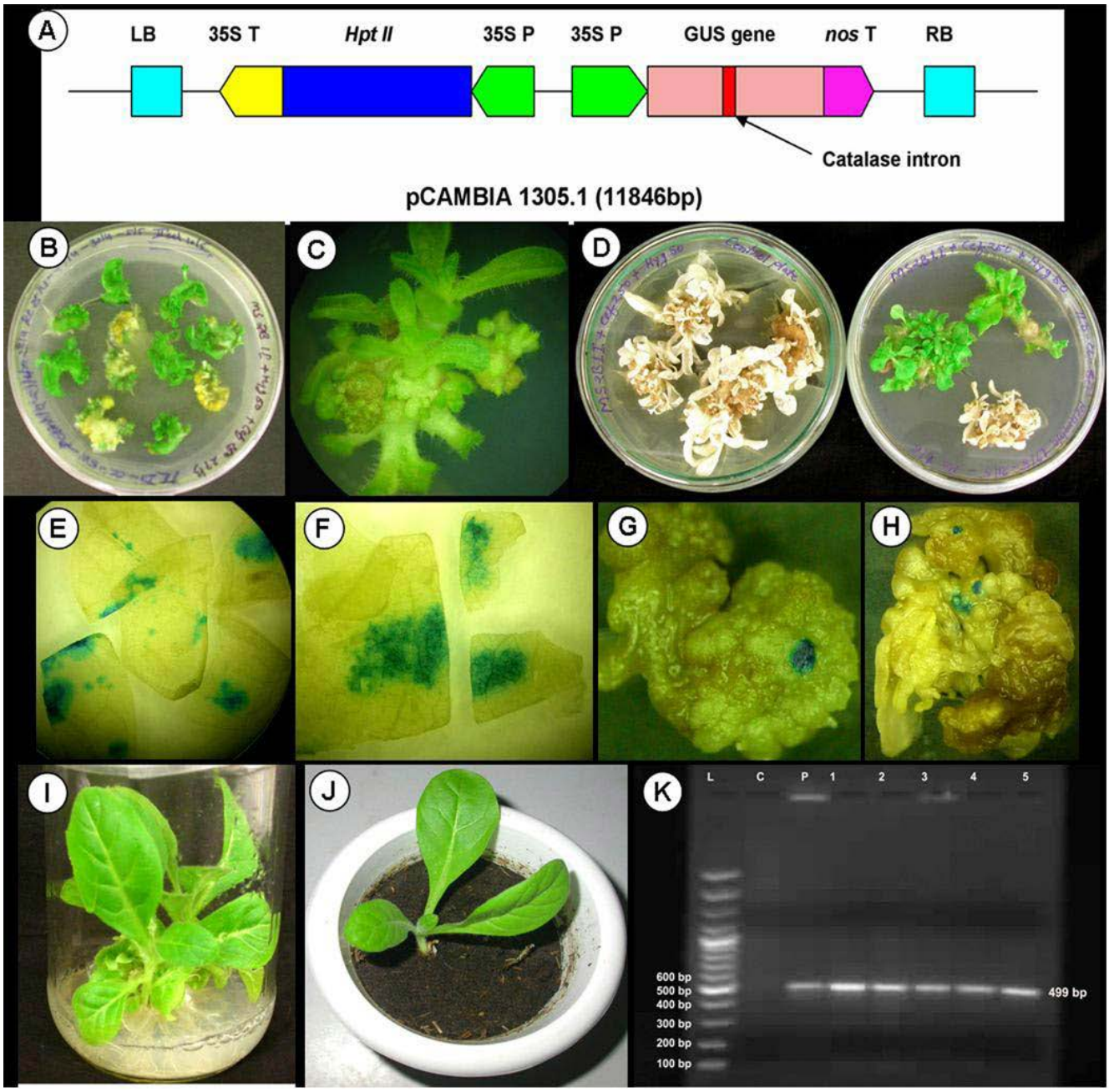

Plate 1. *Agrobacterium*-mediated transformation in Tobacco;
A: Vector construct, B & C: Shoot induction from cocultivated leaf discs,
D: Selection of transformed shoots in the presence of antibiotic Hygromycin,
GUS expression in transformed leaf discs (E & F), transformed callus (G & H),
I: Completely regenerated plant, J: Hardening of transgenic plant
K: PCR amplified 499 bp hpt II gene from transgenic plants (Lane 1 to 5),
Negative control (Lane C), Positive control (Lane P)