Brassica Biotechnology

V. Dinesh Kuamr and SR Bhat

When Prof. Chopra was asked in 1985 to lead the establishemnet of a new discipline of Molecualr Biology and Biotechnology at IARI, he accepted the responsibility on the condition that he would continue to pursue the work he was engaged in at that time and integrate it into the new programme. Initially three major crops of national importance, namely, rice, chickpea and Brassica representing the three broad categories of food crops, cereal, pulse and oilseed were targeted for biotechnological improvement. As a Professor of Eminence, Prof. Chopra had initiated work on Brassica improvement and hence he chose to lead the Brassica programme. Incidentally, around this period (in 1986), Govt. of India launched 'Technology Mission on Oilseeds' with the aim of achieving self-sufficiency in edible oil production. Also, biotechnological techniques were being standardized in *Brassica napus*, a major oilseed crop of Europe, Canada and Australia. Thus the choice of Brassica, in particular the Indian mustard *B. juncea,* for biotechnological improvement was timely and well considered.

Prof. Chopra assembled a group comprising plant breeder, cytogeneticists, plant tissue culture specialists to apply the biotechnolgical tools to improve oilseed Brassica. Taking cue from the global trend, the group initiated work on plant tissue culture and set out to standardize tissue culture protocols for somaclonal breeding, anther/microspore culture and somatic hybridization and plant transformation. Once standardized, these protocols were to be applied for developing improved germplasm lines and varieties, novel cytoplasmic male sterility systems for heterosis breeding, introgression of genes for disease resistance, genetic transformation and so on. Down the line, work was initiated to clone genes and promoters and to develop molecular marker systems in Brassica. Below we present a summary of the Brassica research work conducted under the leadership of Prof. Chopra and highlight the salient contributions.

Optimisation of *in vitro* protocols

***In vitro* regeneration and its genetic control:** Initial work on plant regeneration showed that plants can be regenerated from various explants such as cotyledon petiole, hypocotyl, stem, and inflorescence of *B. juncea*. Further, strong genotypic effects on regenerability were reported (Jain *et al.* 1988; Narasimhulu & Chopra 1988, Narasimhulu *et al.* 1988). Prof. Chopra's group also studied the species- specific response to *in vitro* shoot regeneration by employing cotyledon explants of the naturally occurring diploid and amphidiploid species and observed that *B. rapa* (A genome) has the lowest *in vitro* regenerability while *B. nigra* and *B. oleracea* were most amenable to *de novo* shoot regeneration. *B. juncea* showed the lowest regeneration frequency among the amphidiploids. Subsequently, these studies were extended to synthetic amphidipoids and the earlier observations were confirmed based on naturally occurring genotypes. Where comparisons were based on responses of diploid progenitors and their synthetic amphidiploid products (Narasimhulu *et al.* 1988), the highest response was recorded in *B. carinata* and the lowest in *B. rapa.* The regeneration response in *B. juncea* was considerably lower than the progenitor species. These observations indicated an additive interaction between the constituent genomes on regeneration response in the corresponding amphidiploid. Influence of cytoplasm on regenerability was also evident. In reciprocal synthetic amphidiploids, *B. rapa* cytoplasm was found to negatively influence regeneration. However, in the synthetic *B. napus*, the regeneration response was intermediate between the low-responding *B. rapa* and the high responding *B.oleracea.* These studies indicated that the A genome has a negative effect on regeneration.

Influence of cytoplasm on *in vitro* shoot morphogenesis: Narasimhulu *et al.* (1989) observed the apparent contribution of cytoplasms to *in vitro* shoot regeneration, using *B. carinata* lines, which were synthesized from reciprocal crosses between *B. nigra* and *B. oleracea.* Cotyledon explants of the reciprocal allopolyploids were tested for *in vitro* shoot regenerability. *B. carinata* line synthesized from the cross *B. oleracea* x *B. nigra* and carrying C cytoplasm showed significantly higher *in vitro* regeneration capacity compared to the allopolyploids synthesized from *B. nigra* x *B. oleraccea* possessing the B cytoplasm. This study amply brought out the possibility of cytoplasm substitution as a means of improving the *in vitro* regenerability of recalcitrant species.

***In vitro* selection for useful variability:** *In vitro* culture technique offers the advantage of selecting for desirable attributes from natural or induced variability, since uniform conditions can be maintained in tissue cultures. Inclusion of the selection agent in the culture medium directly allows uniform selection for different inducible stresses and in a culture plate millions of cells can be screened. The probability of success could be further enhanced by a mutagenic treatment of cell cultures.

Jain *et al.* (1990) used an *in vitro* selection system to obtain salt-tolerant plants of *B. juncea* cv. Prakash by screening morphogenic cotyledon explant cultures on media containing sodium chloride. This resulted in the development of three salt-tolerant lines. Regeneration of plants from these cultures followed by screening of the selfed progenies for salt tolerance resulted in recovery of salt-tolerant lines with production of significantly higher amounts of proline. They were able to stabilize proline-over producing lines. By using somatic embryos derived from hypocotyl explants for *in vitro* selection, Kirti *et al.* (1991) were able to raise sodium chloride-tolerant plants of *B. juncea* which maintained their salt tolerance in the R_2 generation, indicating that the trait selected for resulted from a genetic change. The salt tolerance in these selected mustard lines was also correlated with proline overproduction.

Protoplast culture: A repeatable and efficient protoplast regeneration system is a pre-requisite for a variety of biotechnological applications including the production of somatic hybrids, cytoplasmic hybrids (or cybrids) and transgenic plants by direct plasmid delivery or through *Agrobacterium* based vectors.

Even though there were initial studies on protoplast regeneration in *B. juncea* (Sikdar *et al.* 1985), plating efficiency of protoplasts and frequency of shoot regeneration from calli were low. Prof. Chopra's group developed efficient regeneration protocols from hypocotyl protoplasts modifying the protocol developed for rapeseed hypocotyl protoplasts by Glimelius (1984) (Kirti and Chopra 1989, 1990). As in rapeseed, Kirti & Chopra (1989) found protoplasts isolated from dark-grown hypocotyls as ideal for further genetic manipulation. The protoplast-derived cell colonies regenerated shoots at about 35% frequency. Kirti & Chopra (1990) further modified the protocol to increase the shoot regeneration frequency to about 45% by resorting to liquid culture of protoplasts till they reached microcalli stage. This resulted in regeneration through direct and indirect somatic embryogenesis from protoplast cultures. Subsequently, protoplasts of hypocotyl origin were regenerated to plants in *B. nigra* (Gupta *et al.* 1990; Narasimhulu *et al.* 1993), *B. carinata* (Narasimhulu *et al.* 1992c), *B. oleracea* (Mukhopadhyay *et al.*1991) and vegetable brassicas (Kirti *et al.* 2001). Narasimhulu *et al.* (1993) observed that the regeneration frequency of protoplast-derived calluses of *B. nigra* was around 55% and those of *B. carinata* around 75%. Vegetable brassicas also showed high level of regeneration, in conformity with the observed genomic effects on regeneration (Narasimhulu and Chopra 1988a).

Thus, the establishment of efficient plant regeneration protocols from explants or through protoplasts laid the foundation for applying biotechnological tools for Brassica improvement and paved the way for somaclonal breeding, somatic hybridization mediated CMS line development and development of transgenics. In particular, identification of RLM 198 as the most responsive genotype to protoplast culture helped in generating a wide spectrum of somatic hybrids of Brassica coenospecies.

This period was marked by rare camaraderie and intense personal involvement with the work. Students, scientists, technical and supporting staff worked shoulder to shoulder and the lab was a hive of activity round the clock.

Somaclonal breeding and development of 'Pusa Jaikisan' mustard variety

During the 1980s somaclonal breeding was tested in several crops in Europe, USA and Australia with mixed results. Prof. Chopra's group (Anuradha *et al.* 1992) applied this method for the improvement of Indian mustard. The popular mustard cultivar 'Varuna' was subjected to *in vitro* culture and the plants regenerated from calli were evaluated in the field over several seed generations. A vast genetic variability was observed among regenerants for almost all important agronomic traits examined such as plant height, flowering time, branching pattern, seed yield, disease and pest tolerance. After rigorous screening on a massive scale over several generations, Dr. RK Katiyar selected a line Bio-902 which was processed and released for commercial cultivation in 1995 as 'Pusa Jaikisan'. This variety is characterized by bold, dark brown seeds, and early flowering, and yields around 25 q/ha. It is particularly suited to the major mustard belt in Rajastan, and remains one of the top three varieties even after two decades of release. *This is the only somaclonal variety of any field crop in the world that has attained such wide popularity.*

B. carinata is a species cultivated in Ethiopia and shows tolerance to Alternaria, high temperature and water stress. Efforts to introduce this species in India had failed. Exotic *B. carinata* accessions did not perform well under Indian conditions as they came to flowering very late. Based on the observation that early flowering was quite frequent in *B. juncea* somaclones, *B. carianta* was also subjected to somaclonal breeding. While a numebr of early flowering lines were obtained, they were not superior to the released varieites in yield.

It is pertinent to clarify here why despite this success, somaclonal breeding was not pursued in subsequent years. Our experience showed that somaclonal regenerants, like other mutagenesis approaches, yield variants that are generally inferior to the parent variety, and the variations generated fall within the range commonly found among natural accessions. Therefore, the probability of obtaining a novel and superior variant is far lower than that could be derived from recombination breeding involving selected parents. In fact, on hind sight, the development of Pusa Jaikisan is a testimony to the grit and determination of the team and the inspring leadership of Prof. Chopra.

Artifical synthesis of amphidiploids *B. napus, B. carinata* and *B. juncea*

B. napus (oil seed rape), a major oilseed crop in Europe and Canada, and *B. carinata* (Ethiopian mustard) possess several desirable agronomic attributes such as high productivity and resistance to white rust. Attempts at introducing exotic cultivars from Canada, Europe and Ethiopia to Indian agriculture proved unsuccessful because of their delayed flowering owing to photo- and thermo-sensitivity. Many cultivars remained in vegetative state and failed to bolt under Indian conditions. Artificial synthesis utilizing native accessions of the progenitor species was envisaged as an alternative to obtain agronomically suitable forms for

India. Likewise, artificail synthesis of *B. juncea* was imperative for enlarging germplasm pool of this species.

***Synthesis of B. napus and B. carinata* through sexual hybridization:** A wide spectrum of variability was generated in *B. napus* following hybridization between *B. rapa* ssp. *oleifera* var. brown sarson and *B. oleracea* var. *botrytis* (cauliflower) (Prakash 1980, Prakash & Raut 1983). However, the success rate of obtaining hybrids was very low (0.29%) and was possible only in one direction i.e. *B. rapa x B. oleracea.* Synthetic strains of *B. carinata* were derived from reciprocal crosses between *B. oleracea* vars. *italica* (broccoli), *botrytis* (cauliflower), *capitata* (cabbage) and *alboglabra* (Chinese kale), and early strains of *B. nigra* (Prakash *et al.* 1984). Synthesis of both the species resembled the natural forms in general morphology.

A synthetic B. napus line named ISN 706 developed at our Centre has been found to be a stable maintainer of 'polima' CMS of B. napus. Further, ISN 706 gave rise to the novel CMS line of B. juncea and formed the foundation of the DMH1 hybrid developed by Dr. Pental's group at Delhi University. Likewise, a synthetic B. carinata line has been derived which is dwarf (~90 cm) and early (100 days).

***B. carinata* and *B. juncea* synthesis through protoplast fusion:** Synthetic *B. carinata* lines were obtained through somatic hybridization by fusing *B. nigra* hypocotyl protoplasts with *B. oleracea* mesophyll protoplasts (Narasimhulu *et al.* 1992). Hybrids closely resembled natural accessions of *B. carinata* in general morphology. However, the inflorescence was very long as in *B. oleracea.* Variations were observed for plant height, flowering and maturity. Pollen fertility ranged from 36-87% while seed fertility was poor (~19%). Organelle genome constitution of four plants revealed that one plant had a unique combination of *B. nigra* mitochondria and *B. oleracea* chloroplasts while the other had the parental cytoplasms.

Likewise, synthetic *B. juncea* lines were derived following somatic hybridization (Bhat *et al.* 2005). Synthetic *B. juncea* were generally tall, broad leaved, late in flowering and produced high biomass like *B. nigra* parent. Despite forming 18 bivalents at metaphase I of meiosis, pollen fertility in A_1 generation was highly variable, ranging from total sterility to 90% viability and seed set was very low (0.1-0.3 seeds/silique). In generation A_2 and A_3, there was a marked improvement in pollen and seed fertility and most of the plant families in A_7 generation attained complete fertility. Molecular analysis revealed novel cytoplasmic organellar constituions in these synthetic lines. For instiance, three lines possessed *B. nigra* mitochondrial genomes while two had recombinant mt-genome. These synthetics subsequently produced not only novel morphological phenotypes but also plants with *B. nigra* cytoplasm which are not available in nature.

UTILIZATION OF WILD GERMPLASM

Heterosis breeding for improving productivity of mustard has been contemplated since long. However, non-availability of a suitable cytoplasmic male sterility and fertility restoration system prevented hybrid breeding. Dr. Shyam Prakash, a member of Prof. Chopra's team had a large collection wild allied species of Brassica and was engaged in wide hybridization. The newly gained tissue culture expertise for embryo rescue and somatic hybridization set the stage for utilizing the wild germplasm, and a programme to develop novel CMS systems in *B. juncea* and for introgressing genes for pest and pathogen tolerance was launched.

Development and refinement of CMS systems

***B. oxyrrhina* based CMS:** Both sexual and somatic hybridization approaches were used to transfer alien cytoplasm into *B. juncea* nuclear background. For sexual hybridization, *B. rapa* was used as the pollen parent with the wild species, and the resultant amphidploids were backcrossed with *B. juncea* to derive alloplasmic lines. The first CMS line (*B. oxyrrhina*) *B. juncea* obtained by sexual hybridization approach was found to be very stable with regard to male sterility (Prakash and Chopra 1988, 1990). However, leaves of the CMS line were chlorotic indicating incompatibility betweeen alien plasid genome and *B. juncea* nuclear genome. Further, the nuclear restorer gene for this CMS line was not found in *B. juncea* nor could it be introgressed from the wild species *B. oxyrrhina*. This experience indicated that somatic hybridization would be a better approach for generating CMS lines as it provides opportunity for selecting novel organellar genome combinations including organellar recombinations. Chlorosis corrected CMS (*B. oxyrrhina*) *B. juncea* lines were generated through somatic hybridization between (*B. oxyrrhina*) *B. juncea* and *B. juncea* (Kirti et al. 1993). This male sterility inducing cytoplasm has been subsequently transferred into *B. rapa* and *B. napus* nuclear backgrounds.

***Trachystoma ballii* based CMS system:** This CMS system is derived from sexual hybridization between *T. ballii* and *B. juncea*. The fertility restorer gene was introgressed from the wild species (Kirti et al., 1997). This CMS line was found to carry recombinant plastid genome (Baldev et al. 1998) but *T. ballii* mt-genome. In the CMS line anthers were converted to petals. Also, the female fertility was adversely affected. As a result, although genetically the system was stable and working, it was not suitable for practical breeding.

***Moricandia arvensis* based CMS system:** This system derived from the somatic hybrid *M. arvensis + B. juncea* carried mitochondria and chloroplast from *M. arvensis* (Prakash *et al.* 1998*)*. The male sterility phenotype was very good (flowers were almost normal except for non-dehiscing anthers) and female feritly was comparable to normal *B. juncea.* Fertility restoring gene for this CMS was introgressed from *Moricandia* (Prakash *et al.* 1998). However, CMS and fertility restorer lines exhibited severe chlorosis. Consequently, the system was unsuitable for practical deployment. A second round of protoplast fusion was carried out between chlorotic CMS line and normal *B. juncea* to obtain cybrids that carried mitochodrial genome from *M. arvensis* but chloroplast genome from *B. juncea* (Kirti *et al.* 1998). Such chlorosis corrected, green CMS lines showed normal developemnt and could be restored by the chlorotic restorer line making it a perfect CMS system for heterosis breeding. Bhat *et al.* (2005) worked out the mode of feritlity restoration and concluded that restoration is monogenic and gametophytic. This system has been provided to all public sector Brassica breeders in the country and also licensed to private seed companies. A commercial hybrid based on *moricandia* CMS has been released for commercial cultivation.

***Diplotaxis catholica* based CMS systems:** *D. catholica* was used in both somatic and sexual hybridization to develop CMS lines. The sexual CMS line was comaprable to *T. ballii* based CMS system in that CMS flowers showed petaloid anthers, crooked style poor nectaries and female fertility was severely affected (Pathania et al. 2003). On the contrary, CMS plants derived through somatic hybrids were green and were free from floral deformities. Flowers had small sized anthers and contained sterile pollen grain. Nectaries were excellent and the female fertility was normal (94%). Pods were also free of any deformities. Thus somatic hybrid derived CMS was superior. While feriltiy restorer for the sexually derived CMS line was available, somatic hydridization derived line lacked reliable fertility restorer. Hence this system also could not be used for heterosis breeding.

***Erucastrum canariense* based CMS system:** This system was derived from sexual hybridization (Prakash *et al.* 2001). The CMS phenotype was variable from petaloid anthers to nearly nromal anthers. In the initial years it showed stable CMS phenotype. However, in recent years, the system is showing sensitivity to temperature fluctuations. As temeprature rise in February, sterility breaks down (Chamola et al. 2012). Thus the system is not suitable to commercial deployment.

***D. erucoides* and *D. berthautii* based CMS lines:** These CMS lines were derived from sexual hybridiation and hence carry unaltered alien mitochondrial and plastid genomes (Bhat *et al.* 2005, 2006, 2008). Despite this, the plants are normal green and male sterile flowers show no abnormalities (have slender small sized indehiscent anthers and excellent nectaries). Female fertility is normal with more than 96% fertility. However, fertility restorer genes for these CMS lines could not be introgressed from the cytoplasm donor species.

Finding fertilty restorer gene from unexpected source

Conventional wisdom states that fertiltiy restorer (*Rf*) genes for alloplasmic CMS lines have to be derived from the respective cytoplasm donor species. Since we could not succeed in introgressing *Rf* genes for *B. oxyrrhina, D. erucoides* and *D. berthautii*, departing from the conventional approach, we tested whether *T. ballii, M. arvensis* or *E. canariense Rf* genes could rescue other CMS lines. To our pleasant surprise, *M. arvensis* resotrer line was found to restore *D. caholica, D. berthautii* and *D. erucoides* CMS lines (Bhat *et al.* 2003, 2005a, b). As a result, additional CMS systems became available for heterosis breeding. *This is a unique case where the morphologically different and highly divergent CMS systems are restored by a common restorer. Further, in D. catholica CMS it behaves as a sporophtic resotrer whereas in others it behaves as a gametophytic restorer.*

The Brassica research work initiated by Prof. VL Chopra continues to yield dividends. For instance, male sterility inducing cytoplasm has now been transferred into cauliflower (Chamola et al. 2013). The male sterility associated mitochondrial *orf*108 responsible for inducing male sterility in four CMS systems has been identified (Bhat et al., 2008, Kumar et al., 2012), Rf gene that restores fertility to *Moricandia* based CMS has been tagged (Ashutosh et al., 2007) and fine mapped (Bisht et al., 2015). The finding that different CMS share a common molecular mechanism is significant and was made possible by generating diverse CMS systems. Our group also showed that *D. erucudoides* is a good source of resistance to Alternaria (Sharma et al. 2003). In recent years we have developed introgression lines from backcrossing the amphidiploid *D. erucoides x B. rapa* hybrid with *B. juncea* and lines showing high degree of Alternaria resistance have been isolated following screening under both artificial and natural epiphytotic conditons (Bhat unpublished).

Although somatic hybridization was hailed as a great innovation in the 1980s, very few labs seriously pursued to develop lines of practical value. In this respect, the work carried out at NRCPB is noteworthy not only because it generated material of practical utility but also because it broadened our understaing of CMS.

Transgenic Brassicas

Work on developing transgenic brassicas started in the early nineties and was aimed at defining conditions for transformation using marker genes. *B. carinata* was found to be highly amenable to transformation and transgenics with *npt* and *hpt* genes showing Mendelian inheritance were produced (Narasimhulu *et al* 1992). *B.oleracea* despite being highly responsive to tissue culture regeneration was found to be recalcitrant to transformation

by *Agrobacterium.* After a detailed study of various parameters a repeatable protocol was developed for transformation of cabbage, cauliflower and broccoli (Chakrabarty *et al.* 2002).

In the second phase, transgenics for agronomic traits were targeted in *B.juncea*, *B. napus* and *B. oleracea*. Cabbage transgenics carrying *bet*A gene from *E.coli* and producing glycine betaine were developed, characterized and were shown to be tolerant to salt stress (Bhattacharya *et al*. 2003). Genetic engineering option was also explored to impart resistance to pests and pathogen. Kanrar *et al.* (2002) reported reduced incidence of black leaf spot disease caused by *Alternaria brassicae* in transgenic *B. juncea* lines expressing *Hevin* gene, a chitinase binding lectin. Likewise, transgenic mustard plants constitutively expressing another lectin, wheat germ agglutinin were found to adversely affect growth and fecundity of mustard aphid (*Lipaphis erysimi*) (Kanrar *et al*. 2002). The best promise of transgenic pest resistance appears to be offered by the *Bt* gene in vegetable brassicas. Synthetic *crylA(b)* gene introduced into cabbage (Bhattacharya *et al.* 2002), cauliflower (Chakrabarty *et al.* 2002) and broccoli (Viswakarma *et at*.2004) was found to impart high degree of tolerance to Diamond back moth larvae. Mortality of 40-60% second instar larvae was recorded after 48h of feeding in transgenics.

The work on transgenics for pest (aphid) resistance is being actively pursued by Prof. Chopra's student Dr. RC Bhattachrya, and some important leads have been registered to tackle this major pest.

Cloning of genes and promoters

Realising the importance of genes and promoters for transgenic improvement, Prof. Chopra initiated students to undertake such works. Initial efforts in this area were directed towards cloning of genes involved in fatty acid biosynthesis. The plastid localized stearoyl ACP desaturase gene responsible for conversion of stearic acid to oleic acid was cloned from *B. juncea* through PCR amplification (Vageeshbabu *et al.* 1996). Similarly, based on sequence information from *Arobidopsis, Fatty Acid Elongation 1 (FAE1)* gene involved in erucic acid biosynthesis was cloned (Venkateshwari et al. 1999). Different allelic forms of *FAE1* from *B. rapa* and *B. juncea* were also cloned and sequenced (Yadava et al.). Similarly, anther and seed specific promoter elements were PCR amplified using primers designed based on sequence information of tobacco and *B. napus* (Vageeshbabu & Chopra 1997). In the lab efforts were also made to develop transgenic mustard carrying *annexin* and *osmotin* genes to impart tolerance to both biotic and abiotic stresses. While these might appear inconsequential today, it propelled students to explore new areas and helped in building self confidence. Likewise, molecular marker work was initiated at an early stage which laid foundation of marker assisted breeding (see Dr. Mohapatra's account in this compilation).

In summary, the Brassica work illustrates how a well considered approach, team work and able leadership could make progress in multiple directions and provide solutions to practical problems.

Selected references

Anuradha G, Narasimhulu SB, Arunachalam V and Chopra VL. 1992. A comparative evaluation of somaclonal, gamma ray and EMS induced variation in *Brassica juncea*. J *Plant Biochemistry and Biotechnology*, 1:105-108

Ashutosh A, Kumar P, Sharma PC, Prakash S and Bhat SR. 2008. A novel *orf108* co-transcribed with atpA gene is associated with cytoplasm male sterility in *Brassica juncea* carrying *Moricandia arvensis* cytoplasm. *Plant Cell Physiol*, 49: 284–289

Ashutosh A, Sharma PC, Prakash S and Bhat SR. 2007. Identification of AFLP markers linked to the male sterility restorer gene of CMS (*Moricandia arvensis*) *Brassica juncea* and conversion to SCAR markers. *Theor Appl Genet*, 114:385–392

Baldev A, Gaikwad K, Kirti PB, Mohapatra T, Prakash S and Chopra VL. 1998. Recombination between chloroplast genomes of *Trachystoma ballii* and *Brassica juncea* following protoplast fusion. *Mol. Gen. Genet*, 260:357-361

Bhat SR and Chopra VL. 1998. Biosafety of transgenic crops – precautions for case-by-case assessment. *Current Science*, 91: 16-17

Bhat SR and Chopra VL. 2006. Chioce of technology for herbicide resistant transgenic crops in India: Examination of issues. *Current Science*, 91: 435-438

Bhat SR and Chopra VL.2005.Transgenic crops: priorities and strategies for India.*Current Science*, 88:886-889

Bhat SR, Haque A and Chopra VL. 2001. Induction of mutations for cytoplasmic male sterility and some rare phenotypes in Indian mustard (*Brassica juncea* L.). *The Indian Journal of Genetics and Plant Breeding*, 61 (4): 335-340

Bhat SR, Kumar P and Prakash S. 2008. An improved cytoplasmic male sterile (*Diplotaxis berthautii*) *Brassica juncea*: identification of restorer and molecular characterization. *Euphytica*, 159: 145–152

Bhat SR, Prakash S, Kirti PB, Dineshkumar V and Chopra VL. 2005. A unique introgression from *Moricandia arvensis* confers male fertility upon two different cytoplasmic male-sterile lines of *Brassica juncea*. *Plant Breed*, 124: 117–120

Bhat SR, Vijayan P, Dwivedi KK, and Prakash S. 2003. Diplotaxis erucoides-induced cytoplasmic male sterility in *Brassica juncea* is rescued by the *Moricandia arvensis* restorer: genetic and molecular analyses. *Plant Breed*, 125: 150-155

Bhattacharya RC, Maheswari M, Dineshkumar V, Kirti PB and Chopra VL .2004. Transformation of *Brassica oleracea* var.*capitata* with bacterial *bet*A gene enhances tolerance to salt stress. *Scientia Horticulturae*, 100: 215-227

Bhattacharya RC, Viswakarma N, Bhat SR, Kirti PB and Chopra VL. 2002. Development of insect-resistant transgenic cabbage plants expressing a synthetic *cryIA(b)* gene from *Bacillus thuringiensis. Current Science*, 83 (2). pp. 146-150

Bisht DS, Chamola R, Nath M and Bhat SR. 2015. Molecular mapping of fertility restorer gene of an alloplasmic CMS system in *Brassica juncea* containing *Moricandia arvensis* cytoplasm. *Mol Breeding*, 35:1-14

Chakrabarty R, Viswakarma N, Bhat SR, Kirti PB, Singh BD and Chopra VL .2002. *Agrobacterium*-mediated transformation of cauliflower: optimization of protocol and development of Bt-transgenic cauliflower. *J. Biosci*, 27: 495–502

Chamola R, Balyan HS and Bhat SR .2013b. Effect of alien cytoplasm and fertility restorer genes on agronomic and physiological traits of *Brassica juncea* (L.) Czern. *Plant Breed*, 132:681–687

Chamola R, Balyan HS and Bhat SR. 2013a. Transfer of cytoplasmic male sterility from alloplasmic *Brassica juncea* and *B. napus* to cauliflower (*B. oleracea* var. botrytis) through interspecific hybridization and embryo culture. *Ind J Genet*, 73:203–210

Chopra VL and Sharma RP. 1991.Biotechnology in crop improvement.*Current Science*, 60 (9-10): 543-547

Chopra VL and Vageeshbabu HS. 1996. Metabolic Engineering of Plant Lipids. *J Plant Biochemistry and Biotechnology*, 6: 53-62

Chopra VL, Narasimhulu SB, Kirti PB, Prakash S and Anuradha G. 1988. Studies of somaclonal variation in *Brassica juncea* and its relevance to improvement of stress tolerance and yield. *Review of Advances in Plant Biotechnology Ed.* , A. Mujeeb-Kazi, L. A. Sitch. pp228-238

Chopra VL, Prakash S and Kirti PB. 1996. Aceessing and exploiting genes of breeding value of distant relatives of crop Brassicas. *Genetica*, 97: 305 – 312

Jain A, Bhatia S, Banga SS, Prakash S and Lakshmikumaran M. 1994. Potential use of random amplified polymorphic DNA (RAPD) to study the genetic diversity in Indian mustard (*Brassica juncea* (L) Czern and Coss) and its relationship with heterosis. *Theor. Appl. Genet.* 88: 116–122

Jain RK, Jain S, Nainawatee HS and Choudhary JB. 1990. Salt tolerance in *Brassica juncea* L. I. in vitro selection, agronomic evaluation, and genetic stability. *Euphytica*, 45:141–152

Kanrar S, Venkateswari J, Dureja P, Kirti PB and Chopra VL. 2002. Transgenic expression of hevein, the rubber tree lectin, in Indian mustard confers protection against *Alternaria brassicae. Plant Science 162: 441-448*

Kanrar S, Venkateswari J, Dureja P, Kirti PB and Chopra VL. 2006. Modification of erucic acid content in Indian mustard (*Brassica juncea*) by up-regulation and down-regulation of the *Brassica juncea FAT TY ACID ELONGATION1 (BjFAE1)* gene. *Plant Cell Rep*, 25:148-155

Katiyar RK and Chopra VL. 1990. Somaclonally induced earliness in a *Brassica juncea* germplasm accession with field resistance to important diseases. *Plant Breed,* 104:262-264

Kirti PB and Chopra VL. 1989. A simple method of inducing somatic embryogenesis in *Brassica juncea (L)* Czern & Coss. *Plant Breed*, 102:73–78

Kirti PB and Chopra VL. 1989. Plant regeneration from hypocotyl derived protoplasts of (*Brassica juncea* (L) Czern and Coss. *Plant Cell Rep*, 7: 708-710

Kirti PB and Chopra VL. 1990. Rapid plant regeneration through organgenesis and somatic embryogenesis from cultured protoplasts of *Brassica juncea*. *Plant Cell Tiss Org Cult*, 20: 65-67

Kirti PB, Baldev A, Gaikwad K, Bhat SR, Dineshkumar V, Prakash S and Chopra VL. 1997. Introgression of a gene restoring fertility to CMS (*Trachystoma*) *Brassica juncea* and the genetics of restoration. *Plant Breed,* 116: 1179–1182

Kirti PB, Banga SS, Prakash S and Chopra VL. 1995. Transfer of Ogu cytoplasmic male sterility to *Brassica juncea* and improvement of the male sterile line through somatic cell fusion.*Theor Appl Genet.*, 91:517-521

Kirti PB, Banga SS, Prakash S and Chopra VL. 1995a. Transfer of Ogu cytoplasmic male sterility to *Brassica juncea* and improvement of male sterile through somatic cell fusion. *Theor. Appl.Genet,* 91: 517–521

Kirti PB, Hadi S, Kumar PA and Chopra VL. 1991. Production of sodium-chloride-tolerant *Brassica juncea* plants by in vitro selection at the somatic embryo level. *Theor.Appl. Genet,* 83: 233-237

Kirti PB, Mohapatra T, Baldev A, Prakash S, Chopra VL.1995. A stable cytoplasmic male sterile line of *Brassica juncea* carrying restructured organelle genomes from the somatic hybrid Trachystoma balli + B. juncea. *Plant Breed,* 114:434–438

Kirti PB, Mohapatra T, Khanna H, Prakash S and Chopra VL. 1995b. *Diplotaxis catholica* + *Brassica juncea* somatic hybrids: molecular and cytogenetic characterization. *Plant Cell Rep,*14:593-597

Kirti PB, Mohapatra T, Khanna H, Prakash S and Chopra VL. 1995c. *Diplotaxis catholica* + *Brassica juncea* somatic hybrids: molecular and cytogeneric characterization. *Plant Cell Rep.* 14: 593–597

Kirti PB, Narasimhulu SB, Mohapatra T, Prakash S and Chopra VL. 1993. Correction of chlorophyll deficiency in alloplasmic male sterile *Brassica juncea* through recombination between chloroplast genome. *Genet. Res. Camb,* 62: 11–14

Kirti PB, Narasimhulu SB, Prakash S and Chopra VL. 1992. Production and characterization of intergeneric somatic hybrids of *Trachystoma ballii* and *Brassica juncea.Plant Cell Rep*, 11:90-92

Kirti PB, Narasimhulu SB, Prakash S and Chopra VL. 1992. Somatic hybridization between *Brassica juncea* and *Moricandia arvensis* by protoplast fusion. *Plant Cell Rep*. 11: 318-321

Kirti PB, Prakash S and Chopra VL. 1991. Interspecific hybridization between *Brassica juncea* and *B. spinescens* through protoplast fusion. *Plant Cell Rep*, 9:639–642

Kirti PB, Prakash S, Gaikwad K, Bhat SR, Dineshkumar V and Chopra VL. 1998. Chloroplast substitution overcomes leaf chlorosis in a *Moricandia arvensis* -based cytoplasmic male sterile *Brassica juncea*. *Theor. Appl. Genet*, 97: 1179–1182

Kumar P, Vasupalli N, Srinivasan R and Bhat SR. 2012. An evolutionarily conserved mitochondrial *orf108* is associated with cytoplasmic male sterility in different alloplasmic lines of *Brassica juncea* and induces male sterility in transgenic *Arabidopsis thaliana*. *J. Exp. Bot,* 63: 2921–2932

Larkin PJ and Scowcroft WR. 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. Theor Appl Genet, 60: 197-214

Malik M, Vyas P, Rangaswamy NS and Shivanna KR. 1999. Development of two new cytoplasmic male sterile lines of *Brassica juncea* through wide hybridization. *Plant Breed,* 118: 75–78

Mohapatra T, Sharma RP and Chopra VL. 1992. Cloning and use of low copy sequence genomic DNA for RFLP analysis of somaclones in mustard *(Brassica juncea(L)* Czren and Coss*) Current Science*, 62 (6) : 482-484

Mukhopadhyay A, Topfer R, Pradhan A K, Sodhi Y S, Steinbi H-H, Schell J and Pental D. 1991. Efficient regeneration of *Brassica oleracea* hypocotyl protoplasts and high efficiency genetic transformation by direct DNA uptake. *Plant Cell Rep,* 10:375–379

Narasimhulu SB and Chopra. 1988. Species specific shoot regeneration response of cotyledonary explants of Brassicas. *Plant Cell Reports*, 7:104-106

Narasimhulu SB, Kirti PB, Bhatt SR, Prakash S and Chopra VL. 1994.Intergeneric protoplast fusion between *Brassica carinata* and *Camelina sativa*. *Plant Cell Rep,* 13:657-660

Narasimhulu SB, Kirti PB, Mohapatra T, Prakash S and Chopra VL. 1992b. Shoot regeneration in stem expiants and its amenability to *Agrobacterium tumefaciens* mediated gene transfer in *Brassica carinata*. *Plant Cell Rep*, 11:359-362

Narasimhulu SB, Kirti PB, Prakash S and Chopra VL. 1992. Somatic embryogenesis in *Brassica nigra* (Koch). *Journal of Experimental Botany*, 43: 1203-1207.

Narasimhulu SB, Kirti PB, Prakash S and Chopra VL. 1992a. Rapid and efficient plant regeneration from hypocotyl protoplasts of *Brassica carinata*. *Plant Cell Rep,* 11:159-162

Narasimhulu SB, Kirti PB, Prakash S and Chopra VL. 1992c. Resynthesis of *Brassica carinata* by protoplast fusion and recovery of a novel cytoplasmic hybrid. *Plant Cell Rep,* 11:428-432

Narasimhulu SB, Kirti PB, Prakash S and Chopra VL. 1993. Rapid and high frequency shoot regeneration form hypocotyl protoplasts of *Brassica nigra. Plant Tissue Org Cult. 32:35-39*

Narasimhulu SB, Prakash S and Chopra VL.1988. Comparative shoot regeneration responses of diploid brassicas and their synthetic amphidiploid products. *Plant Cell Rep,* 7:525-527

Ogura H .1968. Studies on the new male-sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. *Mem. Fac. Agric. Kagoshima Univ*, 6: 39–78

Pathania A, Bhat SR, Dineshkumar V, Ashutosh, Prakash S and Chopra VL. 2003. Cytoplasmic male sterility in alloplasmic *Brassica juncea* carrying *Diplotaxis catholica* cytoplasm: molecular characterization and genetics of fertility restoration. *Theor. Appl. Genet.* 107: 455–461

Pathania A, Kumar R, Dineshkumar V, Ashutosh, Dwivedi KK, Kirti PB, Prakash S, Chopra VL and Bhat SR. 2007. A duplication of *coxI* gene is associated with CMS (*Diplotaxis catholica*) *Brassica juncea* derived from somatic hybridization with *Diplotaxis catholica*. *J. Genet*, 86: 93–101

Prakash S and Chopra VL.1988. Introgression of Resistance to Shattering in *Brassica napus* from *Brassica juncea* through Non-Homologous Recombination. *Plant Breeding*, 101:167–168

Prakash S and Chopra VL .1988. Synthesis of alloplasmic *Brassica campestris* as a new source of cytoplasmic male sterility. *Plant Breed,* 101: 253

Prakash S and Chopra VL .1990. Male sterility caused by cytoplasm of *Brassica oxyrrhina* in *B. campestris* and *B. juncea*. *Theor. Appl. Genet*, 79, 285-287

Prakash S and Chopra VL. 1990. Reconstruction of allopolyploid Brassicas through non-homologous recombination: introgression of resistance to pod shatter in *Brassica napus* . *Genetical Research*, 56:1-2

Prakash S and Chopra VL. 1991. Taxonmy, distribution and cytogenetics. In: Oilseed Brassicas in Indian Agriculture (eds) Chopra VL and Prakash S, Vikas Publishing House, New Delhi pp 29-59

Prakash S and Chopra VL. 1993. Genome Manipulations. In: Breeding Oilseed Brassicas. Ed. Labana KS, Banga SS and Banga SK. *Monographs on Theoretical and Applied Genetics*, 19:108-133

Prakash S, Ahuja I, Upreti HC, Dineshkumar V, Bhat SR, Kirti PB and Chopra VL .2001. Expression of male sterility in alloplasmic *Brassica juncea* with *Erucastrum canariense* cytoplasm and the development of a fertility restoration system. *Plant Breed*, 120, 479-482

Prakash S, Bhat SR and Fu T .2009. Wild germplasm and male sterility. *In*: S. K. Gupta (ed.) Biology and breeding of crucifers, CRC Press, pp. 113–127

Prakash S, Gupta S, Raut RN and Kalra A. 1984. Synthetic Brassica carinata. *Cruciferae Newslett*, 9:36

Prakash S, Kirti PB, Bhat SR, Gaikwad K, DineshKumar V and Chopra VL.1998. *A Moricandia arvensis* based cytoplasmic male sterility and fertility restoration system in *Brassica juncea*. *Theor.Appl. Genet*, 97: 488–492

Sharma G, Dineshkumar V, Haque A, Bhat SR, Prakash S and Chopra VL. Brassica coenospecies: a rich reservoir for genetic resistance to leaf spot caused by *Alternaria brassicae*. *Euphytica* 125: 411-417

Srinivasan K, Malati VG, Kirti PB, Prakash S and Chopra VL. 1998. Generation and characterisation of monosomic chromosome addition lines of *Brassica campestris - B. oxyrrhina*. *Theor Appl Genet*, 97:976-981

Uprety DC, Prakash S and Tomar VK. 1990. Cytoplasm Influences the Photosynthetic Efficiency in Brassica carinata. Journal of Agronomy and Crop Science, *165: 207–210*

Vageeshbabu HS and Chopra VL. 1997. Genetic and Biotechnological Approaches for Reducing Glucosinolates from Rapeseed-Mustard Meal. *J Plant Biochemistry and Biotechnology*, 6: 53-62

Vageeshbabu HS, Venkateswari J, Kirti PB and Chopra VL. 1996. Molecular Cloning of the Gene Encoding Stearoyl-Acyl Carrier Protein Desaturase in *Brassica juncea*. *J Plant Biochemistry and Biotechnology*, 5: 51-53

Venkateswari J, Kanrar S, Kirti PB, Malathi VG and Chopra VL. Molecular cloning and characterization of *FATTY ACID ELONGATION1 (BjFAE1)* gene of *Brassica juncea*. *J Plant Biochemistry and Biotechnology*, 8: 53-55

Viswakarma N, Bhattacharya RC, Chakrabarty R,Dargan S, Bhat SR, Kirti PB, Fuchigami LH, Shastri NV and Chopra VL. 2004. Insect resistance of transgenic broccoli ('Pusa Broccoli KTS-1') expressing a synthetic cryIA(b) gene. *The Journal of Horticultural Science & Biotechnology* , 79:182-188

Yadav P, Bhat SR, Prakash S, Mishra LC and Chopra VL. 2009. Resynthesized Brassica juncea lines with novel organellar genome constitution obtained through protoplast fusion. *J Genet*. 88:109-12