Mustard breeding in India, some recent developments

D. Pental and A. K. Pradhan

Department of Genetics and Centre for Genetic Manipulation of Crop Plants
University of Delhi South Campus, Benito Juarez Road, New Delhi - 110021

Brassica juncea is a major oilseed crop of India and is grown in about six million hacteres of land, particularly in the rainfed areas of northern India during the winter growing season. Over the past few decades, breeding programmes, particularly in India, have followed pure line breeding methods for development of new varieties primarily through the exploitation of genetic variability that existed among the adapted pool of elite germplasm. It has resulted in only marginal improvement in productivity. To realize further gains in productivity, it is important to utilize new sources of variation which would lead to broadening the genetic base of the existing varieties. Productivity can also be substantially increased by heterosis breeding. It has been shown in the earlier work that there are two diverse gene pools for oilseed *B. juncea*, the east European gene pool and the Indian gene pool and hybrids between lines of the two divergent gene pools are heterotic for yield (Pradhan et al. 1993; Jain et al. 1994; Srivastava et al. 2000).

Apart from improvement in productivity, another important objective in *B. juncea* breeding is the improvement of oil and meal quality through the development of canola quality (low erucic acid and glucosinolates), high oleic, low PUFA and yellow seeded cultivars. Majority of these quality traits are available in the exotic east European germplasm. As all the cultivars grown in India are brown seeded and high in both erucic acid and glucosinolates, these quality improvements could be brought about only through the transfer of the desirable genes from the east European

germplasm. These exotic east European germplasm is ill-adapted to Indian agro-climatic conditions. Hence, the breeders in India have faced the problem of linkage drag while attempting to use these germplasm either through conventional pedigree breeding or backcross breeding. Moreever, there will be danger of dilution of heterotic gene pool if so many characters are to be mobilized from one heterotic pool to another through conventional approaches. These problems could be circumvented through the use of biotechnological tools such as use of DNA markers in precise transfer of genes from exotic line to elite varieties or through use of transgenic approaches.

Our lab at University of Delhi is actively involved in genetic improvement of oilseed mustard *B. juncea* through the use of conventional and biotechnological approaches. The lab is mainly concentrating on two major objectives: (1) Enhancement of productivity through development of hybrids and (2) improvement of oil and meal quality through development of canola quality mustard. The lab has developed both CMS-restorer system and transgenic barnase-barstar system which could effectively be used for large scale hybrid seed production in mustard. A high-density linkage map has been constructed using AFLP, RFLP and SSR markers. This map has been used to tag some of the agronomically important genes such as erucic acid, linoleic acid and seed coat colour and currently being used for the dissection of QTLs involved with yield and its components and glucosinolates. Antisense RNA technique has been deployed to develop high oleic and low linoleic and linolenic mustard lines. Microspore derived doubled haploids and marker assisted backcross breeding are being followed for precise transfer of low erucic acid and low glucosinolates from exotic east European lines to Indian cultivars.

Enhancement of productivity through development of hybrids

For large scale production of hybrid seeds in mustard, the lab has developed barnase-barstar male sterility and restorer system through transgenic approaches (Jagannath et al. 2001; Jagannath et al. 2002). The barnase and barstar transgenes were transferred to suitable heterotic combiners through recurrent backcrossing. Hybrid based on this male sterility system was tested in contained open field trials consecutively for two years during the mustard growing season in north India. Yield heterosis of 50 – 55% was recorded in transgenic hybrid over the national check variety. During the growing season of 2005-6, this hybrid will be tested in multi-location trials.

A novel CMS/restorer system has also been developed which could be used for large scale production of hybrid seed. The most unique and agronomically interesting fearure feature of this CMS is that any line of mustard can be used either to maintain the sterility (after certain number of backcrossing) or restore fertility (in the F1) and provides a wide choice of combiners and restorers for hybrid seed production (Patent filed and manuscript under preparation). The first hybrid based on this CMS system was tested in demonstration trials in the farmer's field in north India during 2004-05. Average yield heterosis of 31% (range 16-58%) was recorded in the hybrid over the local check varieties. During 2005-06 growing season, the hybrid will be tested in 500 farmer's field in one acre trials.

Improvement of oil and meal quality

In Indian *B. juncea*, low erucic acid is controlled by two recessive genes (Kirk and Hurlstone 1983) and low glucosinolate is controlled by 6-7 recessive genes (Sodhi et al 2002). These two traits are being transferred from a canola quality mustard line Heera to Indian cultivar through marker assisted backcross breeding through use of double haploids. Initially a high-density linkage map was developed in *B. juncea* using a F1 DH mapping population from the cross between Varuna (Indian cultivar) and Heera using 1029 AFLP and RFLP markers (Pradhan et al. 2003). The map has been further saturated with more AFLP, RFLP and SSR markers. The map was subsequently used to tag two loci of erucic acid genes by candidate gene approach (Gupta et al 2004) and two loci of seed coat colour by microsatellite markers (Padmaja et al 2005). These markers are being used in the marker assisted backcross transfer of low erucic acid and yellow seed coat color traits from east European lines to Indian variety. The map is now being used for tagging genes involved in the glucosinolate biosynthetic pathway and genes involved in the synthesis of linoleic and linolenic acid.

A high oleic and low linoleic mustard line has been developed by transgenic approach through the use of antisense RNA technique. A zero erucic acid mustard line has been modified for its fatty acid composition in the seed oil with antisense constructs using the sequence of *fad2* gene of *B. rapa*. The homozygous lines had 74% oleic acid and 8-9% each of linoleic and linolenic acid (Sivaraman et al. 2004). These lines are being made marker free through *cre-lox* technique and subsequently be transferred to zero erucic acid Indian mustard varieties.

Acknowledgements

This work was financially supported by the Dhara Vegetable Oil and Food Company Ltd. (DOFCO), a subsidiary of the National Dairy Development Board (NDDB), India.

References

Gupta, V., Mukhopadhyay, A., Arumugam, N., Sodhi, Y. S., Pental, D. and Pradhan, A.K. 2004. Molecular tagging of erucic acid trait in oilseed mustard (*Brassica juncea*) by QTL mapping and SNPs in FAE 1 gene. Theor. Appl. Genet. 108:743-749.

Jagannath, A., Bandyopadhyay, P., Arumugam, N., Gupta, V., Burma, P.K., Pental, D. 2001. The use of a Spacer DNA fragment insulates the tissue-specific expression of a cytotoxic gene (*barnase*) and allows high-frequency generation of transgenic male sterile lines in *Brassica juncea* L. Mol. Breeding 8: 11-23.

Jagannath A., Arumugam, N., Gupta, V., Pradhan, A., Burma, P. K., Pental, D. 2002. Development of transgenic *barstar* lines and identification of a male sterile (*barnase*) / restorer (*barstar*) combination for heterosis breeding in Indian oilseed mustard (*Brassica juncea*). Curr. Sci. 82: 46-52.

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

Kirk, J. T. O. Hurlstone, C. G. 1983. Variation and inheritance of erucic acid content in *Brassica juncea*. Z. Pflanzenzuchtg 90:331-338.

Padmaja, L. K., Arumugam, N., Gupta, V., Mukhopadhyay, A., Sodhi, Y. S., Pental, D. and Pradhan, A. K. 2005. Mapping and tagging of seed coat color and identification of microsatellite markers for marker assisted manipulation of the trait in *Brassica juncea*. Theor. Appl. Genet.. 111:8-14

Pradhan, A. K., Gupta, V., Mukhopadhyay, A., Arumugam, N., Sodhi, Y. S. and Pental, D. 2003. A high density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor. Appl. Genet. 106: 607-614.

Pradhan, A. K., Sodhi, Y. S., Mukhopadhyay, A., Pental, D. 1993. Heterosis breeding in Indian mustard (*Brassica juncea* L. Czern & Coss): analysis of component characters contributing to heterosis for yield. Euphytica 69:219-229.

Sivaraman, I., Arumugam, N., Sodhi, Y. S., Gupta, V., Mukhopadhyay, A., Pradhan, A. K., Burma, P. K. and Pental, D. 2004. Development of high oleic and low linoleic acid transgenics in a zero erucic acid *Brassica juncea* L. (Indian mustard) line by antisense suppression of the *fad2* gene. Mol. Breeding 13:365-375.

Srivastava, A., Gupta, V., Pental, D., Pradhan, A.K. 2001. AFLP-based genetic diversity assessment amongst agronomically important natural and some newly synthesised lines of *Brassica juncea*. Theor. Appl. Genet. 102: 193-199.

Sodhi, Y. S., Mukhopadhyay, A., Arumugam, N., Verma, J. K., Gupta, V., Pental, D., Pradhan A. K. 2002. Genetic analysis of total glucosinolate in crosses involving a high glucosinolate Indian variety and a low glucosinolate line of *Brassica juncea*. Plant Breeding 121:508-511.