# Genetic enhancement in rapeseed- mustard for quality and disease resistance through *in vitro* techniques

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## INTRODUCTION

The yields of oilseed *Brassica* species, the economically important multi purpose oilseed crops and one of the major contributors to *Yellow Revolution* in India, are getting stagnated due to its susceptibility to a range of biotic and abiotic stress factors. The changing climatic conditions have compounded the adverse effect on oilseeds productivity due to unpredictable temperature fluctuations, and evolution of new biotypes/ pathotypes/ races of insects and disease. This has resulted in a huge gap in realized and potential yields and at present almost 50% of the edible oil demand in India is being met through imports. The presently cultivated Indian varieties have high erucic acid/low oleic acid against the international standards/ requirements of low erucic/high oleic acid genotypes desired for good health and shelf life. The defatted meal, containing about 40% protein with a well balanced amino gram, is an excellent source of proteins valued for animal nutrition. It is particularly rich in lysine and methionine, which are essential amino acids not found in cereal grains. However, the high glucosinolates in the oil meal make it less palatable for feed purposes. Moreover, the presently cultivated varieties of Indian mustard, *B.juncea*, are susceptible to fungal diseases resulting in huge economic losses, primarily as a result of narrow genetic base. The paper presents the application of tissue culture, molecular and bio-analytical techniques in breeding for improved nutritional quality and fungal disease resistance traits in Indian rapeseed- mustard.

## MATERIALS AND METHODS

### In vitro embryo rescue

To overcome the difficulties in crossing diverse varieties, an *in vitro* sequential embryo rescue technique- involving ovary, ovule and embryo culture- was established that allows for introgression of desired genes / traits across compatibility barrier (Agnihotri 1993). The plantlets were characterized through molecular markers by species specific probes. Such hybrids are realized in a very low frequency; thus the resultant hybrids were multiplied through axillary bud proliferation or secondary embryogenesis from the derived callus cultures (Agnihotri et al. 1990a, b). Three genotypes of *B. juncea*, RESBJ- 837, RESBJ-830 (F4 generation of a cross- involving selected S3 lines from cv. Kranti and cv. Krishna obtained from Dr Nashaat, Rothamsted Research, UK) and TERI (OE) M21-1 [near isogenic line for seed coat colour of TERI (OE) M21 (INGR: 98001) with low erucic acid] and *B. juncea* var. Varuna and Pusa Bold were used in the study. The exotic genotypes of *B. napus* Shiralee and Cyclone were used as double low donors.

The *B. juncea* genotypes Zem-1 and BJ- 1058 were the pollen donors for low erucic acid # the work was undertaken at Plant Biotechnology, TERI, Habitat Place, Lodhi Road, New Delhi and low glucosinolate content, respectively, and *B. carinata* var. Kiran (obtained from Dr Kolte, GBPUA.&T, Pantnagar) was used as the pollen donor for fungal disease resistance in *B.juncea*.

**Biochemical and molecular characterization**

The half seed technique was employed to select plants with specific fatty acids profile determined through GLC, followed by selection of plants with low glucosinolate in the oil free meal through HPLC/ Elisa (Kaushik and Agnihotri 1997, 1999). The desired genotypes of *B.napus* and *B.juncea* were developed through rigorous selections for agro-morphological traits under field conditions (Agnihotri et al.2004). A total of eight UBC ISSR primers (synthesized by Microsynth) were used for DNA amplification and observed on 15 g L−1 agarose gel by staining with ethidium bromide to characterize the hybrids (Gupta et al. 2004).

**Microspore mutagenesis and doubled haploids**

An efficient doubled haploid production protocol was established in *B.juncea* var. Varuna and Pusa Bold in order to minimize the breeding cycle (Prem et al. 2005, 2008). Haploid mutagenesis was exploited at three levels; isolated microspores, microspore derived embryos and donor plants. Based on the development and survival of target tissues/ cells, mutagenesis of microspore derived embryos was found to be most productive. The microspores derived embryos, after 1 hr treatment with 5.0 to 20.0 µM of chemical mutagens (ENU and EMS), were washed with sterile distilled water, air dried for 15 min in laminar flow and cultured on B5G medium containing 0.1 mg/l GA3, 2% sucrose (w/v) and 7 g/l agar. The Petri dishes containing mutagen treated microspore derived embryos were incubated in dark for 10 days at 4 ± $1^0$C in dark and then shifted to 25 $^0$C, 16 hr light/ 8 hr dark photoperiod. After 3 to 4 weeks of culture the roots of 3 to 5 leaf growth stage Mo plantlets were treated with 0.34% aqueous colchicine solution for 2h. The plantlets were hardened and transplanted; the doubled sectors of plants showing desirable agro morphological traits were bagged and seeds were harvested.

**Evaluation for fungal diseases and progeny advancement**

The mutant plants, as well as plants derived through embryo rescue aided hybridization, were screened under field conditions against natural occurring inoculums aided by artificial inoculation with field isolates of white rust and *Alternaria* blight, colleted from GBPUAT, situated at 250 Km from Delhi and a hot spot of fungal diseases. The response of plants to infection was assessed by calculating the disease index (DI) on a 0 (resistant) to 5 (susceptible) scales (Gupta et al. 2006). The progenies of selected plants were advanced through single seed descent/ backcrossing and selection followed by pedigree method.

## RESULTS AND DISCUSSION

**Nutritional Quality**

Several economically important traits were transferred *via* wide hybridization aided with embryo rescue; double low, high oil content, shattering tolerance in *B. napus*, and low erucic/high oleic acid, yellow seed coat color, double low, and resistance/ tolerance to fungal diseases, *Albugo candida* and *Alternaria brassicae* in *B.juncea.* Seven improved quality genotypes are registered at ICAR (Agnihotri et al. 2004; Table 1).

Table 1: The enhanced quality rapeseed-mustard strains registered at ICAR

| | |
|---|---|
| TERI GZ-05 - INGR 04078 [TERI-Uphaar] | High oleic and linoleic acid, yellow seeded, double low *B. juncea* |
| TERI (OO) R9903 - INGR 04077 [TERI-Uttam] | High oil content, canola quality, early maturing *B. napus* |
| TERI (OO) R986-INGR 99007 [TERI-Gaurav] | Early maturing, dwarf double low *B. napus* |
| TERI (OO) R985-INGR 99008 [TERI-Garima] | High oleic acid, double low *B. napus* |
| TERI (OE) R09-INGR 98005 [TERI-Shyamali] | Low erucic acid, high oleic *B. napus* |
| TERI (OE) R03-INGR 98002 [TERI-Phaguni] | Low erucic-acid, early maturing *B. napus* |
| TERI (OE) M21-INGR 98001 [TERI-Swarna] | Low erucic acid, yellow seeded, early maturing *B. juncea* |

*B.napus* (Gobhi-sarson), had earlier been restricted to only a few northern states in India. Therefore, efforts were made to develop low erucic / high oleic acid containing *B. napus* suitable for mustard growing belts. TERI-Unnat (INGR No. 98001) was identified for release by AICRPRM, ICAR (2001). The highlight is the notification of India's first double low *B. napus* var. TERI-Uttam- Jawahar [TERI (OO) R9903; INGR No.04077: National Identity no. IC 405232: Gazette of India July 2007], with >43% oil content. It has proven its suitability to grow in the mustard growing belts due to introgression of early maturity and shattering tolerance. During the on farm trials conducted in collaboration with JNKVV, Jabalpur, it gave higher wheat equivalent yield (WEY) as compared to safflower and wheat under limited irrigations, thus it can be an alternative crop in soybean based cropping systems (Agnihotri 2005). The meal from this variety, in the studies conducted at IVRI, has shown better digestibility as animal feed and is being explored as a new protein source for food and feed; a better quality meal for cattle and poultry at par with soybean meal (Ravichandran et al. 2008). Last but not the least, the oil from TERI- Uttam- Jawahar contain the negligible amounts of harmful trans- fatty acids, and conform to the codex standards for low erucic 'canola' oil as per the quality standards of Government accredited laboratory, thus has a good potential in the health oil sector. The successful incorporation of double low characteristics in Indian *B. juncea* cultivar Varuna has been achieved at TERI through a three way cross [(Varuna × Zem-1/ TERI(OE)M21) × BJ-1058] followed by backcrossing and selections by pedigree method. However, these strains need further improvement for productive agronomic traits (Agnihotri et al. 2004). Work is also in progress to transfer low glucosinolate in the background of agronomically superior Indian mustard genotypes through marker aided selection.

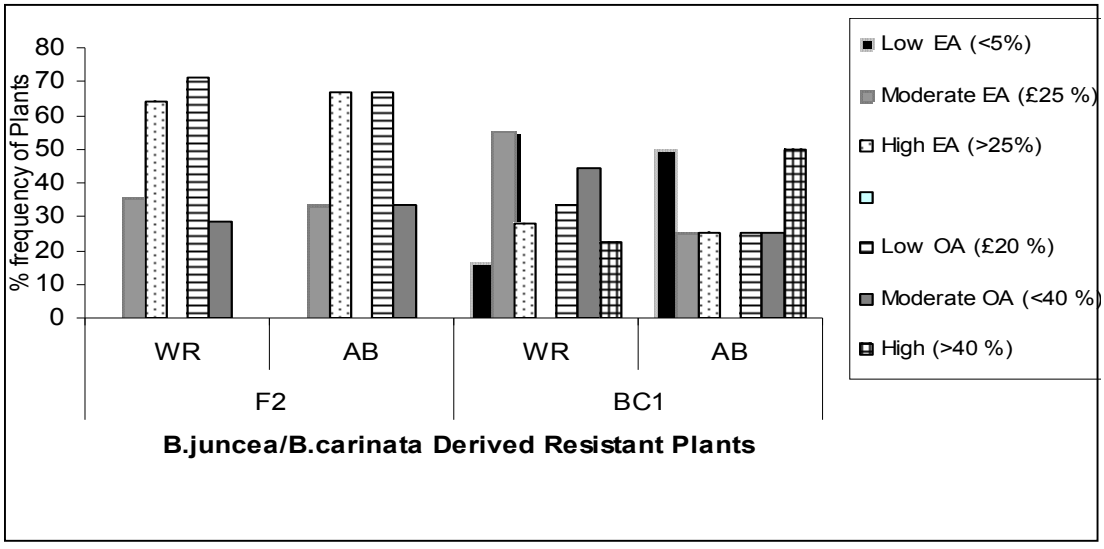
**Disease resistance through microspore mutagenesis**

The non–availability of several desirable genes or alleles within the *B. juncea* gene pool and limitations associated with the conventional breeding necessitates biotechnological interventions for induction of novel genetic variation. Among these the most noteworthy ones include the transgenic technology (Murphy 1999) and mutation breeding (Maluszynski et al. 2000). Several scientists have employed the use of tissue specific expression of transgenes to change endogenous biochemical profiles or to add novel biosynthetic pathways for qualitative traits; however, manipulating quantitative traits is as yet not feasible with this approach. During recent years, mutation research for genetic manipulation has been fairly successful in generation of novel genetic variation in *brassicas* (Potts et al. 2001). The use of seed mutagenesis is limited due to the chimeric nature of the mutant plants, adverse linkages and undesirable pleitropic effects, thus rendering this technique unpredictable and many a times unreliable (Maluszynski et al. 1995). These bottlenecks of the seed mutagenesis technique can be overcome by the use of doubled haploids (DH), which are produced from doubling the chromosome number of haploid plants (Kotts 1998). The doubled haploid plants, in the present study, were selected with good agro-morphological traits; high number of primary and secondary branches, high number of pods on the main shoots, and improved seeds/ siliqua and seed size. The seeds were evaluated for biochemical profile and a high variability was observed for different fatty acids with palmitic acid ranging from 3.22 – 16.0 %, oleic acid from 18.4 - 44.0 %, linoleic acid from 18.0 – 37.0 %, linolenic acid from 4.0 –16.0 % and erucic acid ranging from < 2.0 to 40.0 %. Mutant plants have been identified with low disease score; white rust disease

score ranged from 0.6 to 2.6 and for *Alternaria* blight DI ranged from 0.03 to 1.0 under field conditions and 1.3 to 3.3 *in vitro*, assessed by detached leaf method.

**Disease resistance through inter-specific hybridization via embryo rescue:**
The genetic base for the fungal diseases white rust and *Alternaria* blight are limited, and none of the cultivars of *B. juncea* are resistant or immune to fungal diseases (Kolte 2002; Yadav and Kumar 2004). Several *in vitro* techniques such as somaclonal variation, somatic hybridization and transgenics have been utilized to transfer resistance to *Alternaria* blight and white rust from secondary/ tertiary gene pools/ wide relatives. However, most of these techniques are either restricted to species other than *B. juncea* or the desired level of resistance has not been achieved. The most successful and widely used approach to realize incompatible hybrids is wide hybridization intervened with *in vitro* embryo rescue technique (ovary/ovule culture or sequential embryo culture) allowing transfer of desired genes from related species (Shivanna 1996). Resistance/ high tolerance to the most devastating fungal diseases, white rust (*Albugo candida*) and Alternaria blight (*Alternaria brassicae*), has been transferred from *B. carinata* to *B. juncea* through inter-specific hybridization aided by ovule culture. The hybrids were characterized through detailed morphological traits; leaf (shape, size, texture, colour, tip) and floral morphology (colour of petals, arrangement of flowers, anther shape and structure), they resembled more to the male parent in all morphological traits (Gupta et al, 2007). This was substantiated through molecular studies. The molecular markers ISSRs have been routinely utilized for genetic diversity, phylogenetic studies, and somaclonal variants in brassicas (Sarla et al. 2001). The ISSRs were used for the first time for hybrid characterization. The study elucidated that hybrids resembled more to the male donor (*B. carinata*) with a genetic similarity value of more than 60 % in comparison to the female parent thus indicating a strong influence of male donor (Gupta et al. 2004). The average distribution of early generation plant progenies for disease resistance and fatty acids profile is depicted in Figure 1. The elite genotypes, resembling *B. juncea* phenotype derived from advanced backcross progenies $BC_3F_2$ and $BC_2F_3$ of crosses from *B. juncea* genotype [RESJ 830/ RESJ 837/TERI(OE)M21-1] /*B. carinata* Var. Kiran have been selected for low erucic /high oleic acid and good tolerance to fungal diseases white rust and *Alternaria* blight (DI < 2). These genotypes can be used as valuable sources for developing Indian mustard with improved oil quality and reduced dependency on harmful chemical pesticides for oilseed sustainability in an environment friendly manner.

Fig 1.Percent frequency distribution of disease resistant plants in *B.juncea/ B.carinata* progenies.

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