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INCORPORATION OF SUPERIOR NUTRITIONAL QUALITY TRAITS IN INDIAN *B. juncea*

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The presence of high erucic acid in the seed oil and high gluconsinolate in the oil free meal limit the global utilization of Indian rapeseed mustard. In order to reduce the levels of erucic acid and glucosinolate in Indian mustard, *B. juncea* var. Varuna was crossed with low erucic acid *B. juncea* strains Zem-1 and TERI (OE)M21, and the low glucosinolate line BJ-1058 for transfer of low erucic/low glucosinolate characteristics. Simultaneously, a unique three way cross; *B. juncea* (var. Varuna × Zem-1) × BJ-1058 was made for transfer of double low characteristics. The fatty acids and glucosinolates were analyzed and monitored through improved methods of GC and HPLC respectively. The progenies of selected plants were advanced through pedigree method and recurrent selections. The paper reports the transfer of low erucic acid (0- 1.5%), low glucosinolate (9-25 µm/g oil free meal) and double low (0% erucic acid and 10-27 µm glucosinolate/g oil free meal) characteristics in *B. juncea* var. Varuna.

Key words : *B. juncea, B. napus,* erucic acid, glucosinolate, double low, rapeseed mustard

Brassica, commonly known as rapeseed mustard, is an important group of crop species being utilized as vegetables, condiments and edible oils. Among the oleiferous brassicas, approximately 80 per cent of the cultivated area is covered by *B. juncea*, the remaining being shared by *B. campestris* and *B. napus*. Altogether, they constitute a significant 25-30 per cent of the edible oilseeds produced in the country, thus contributing significantly to the national economy. The mustard oil is bestowed with lowest amounts of saturated fats, as compared to any other vegetable oil, and is a good source of the two essential fatty acids, linoleic and linolenic. The oil free meal is rich in proteins with well-balanced amino acids and minerals, and is a good source of animal feed. However, the rapeseed mustard varieties presently being grown in India have high amounts of erucic acid (40-50%) in the seed oil and glucosinolate (80-160µ/g) in the oil free meal. Experiments conducted on birds and animals suggested the adverse effects of diets rich in erucic acid (Gopalan *et al.*, 1974; Kumar and Tsunoda, 1980) and glucosinolate (Bille *et al.*, 1983; Bell, 1984). This led to the global research efforts towards lowering these two nutritionally harmful components. Consequently, the double low or 'OO' rapeseed varieties having less than 2 per cent erucic acid in the seed oil and less than 30 µm glucosinolate/g oil free meal were developed and commercialized in Canada and European countries ('Canola' quality; Downey 1990; Scarth 1995). These exotic canola quality rapeseed varieties were not found suitable to grow under Indian agroclimatic conditions (AICRP R&M, 1994), necessitating the research efforts for developing Canola quality rapeseed mustard varieties suitable to grow in India. The present work reports transfer of low

erucic acid, low glucosinolate and double low characteristics in *B. juncea* var. Varuna.

MATERIALS AND METHODS

B. juncea var. Varuna selected for transfer of low erucic acid/low glucosinolate/double low characteristics was used as a female parent. The exotic low erucic acid *B. juncea* var. Zem-1, the early maturing low erucic acid *B. juncea* strain TERI(OE)M21 (INGR No. 98001) and the low glucosinolate line BJ- 1058 were used as pollen donors. For the transfer of low glucosinolate, reciprocal crosses of *B. juncea* var. Varuna and BJ-1058 were attempted. For the transfer of double low characteristics, a three way cross (Varuna × Zem-1) × BJ-1058 was attempted.

The seeds of the female parent and pollen donors were sown in field/pots during the month of October following the recommended agricultural practices. Flower buds on selected inflorescences of the female parent. *B. juncea* var. Varuna were emasculted manually with the help of forceps and pollinated with pollen from freshly opened flowers of the respective male parent (as above). The F_1 seeds were harvested. Some of the F_1 plants of the cross *B. juncea* var. Varuna × BJ-1058 and Varuna × Zem 1 were backcrossed with Varuna to generate BC_1F_1 seeds. The F_1 plants of the cross Varuna × Zem-1 were also used for crossing with BJ-1058 to generate F_1 seeds of the three way cross. The F_1 and BC_1F_1 plants were grown and selfed to harvest F_2 and BC_1F_2 seeds. During next generation, the self and open pollinated (OP) F_3 and BC_1F_3 seeds were collected from a large number of individual plants.

The F_1 seeds of the cross *B. juncea* var. Varuna × Zem-1/M-21 were analyzed for their fatty acids content by an efficient GC method through the bulk method (Kaushik and Agnihotri 1997). The F_2 and BC_1F_2 seeds were analyzed by half seed technique (Downey and Harvey, 1963) to select plants having less than 2 per cent erucic acid. The seeds were germinated on a moist filter paper. The 24-36 hrs germinating seeds were hand pressed to remove the seed coat. The inner cotyledon with attached radicle was mounted in the numbered microtitre plates containing sterile *MS basal medium and kept in culture room at 25°C. The outer cotyledon was used to analyze* the fatty acids, and the low erucic acid plants were raised from the corresponding cotyledons in pots. The F_3 to F_5 plant progenies were raised following the pedigree method and recurrent selections. The plants were selected for early maturity and good agronomic attributes, selfed, and the self and OP seeds were collected in each generation. The harvested seeds were analyzed for their fatty acids content to confirm the quality characteristics. The F_6 plant population of the low erucic acid selections was grown in the field during Rabi 1997-98 for evaluation of yield potential and agronomical data was recorded.

The F_3 OP seeds of the crosses *B. juncea* var. Varuna × BJ-1058, BJ-1058 × Varuna, (Varuna × Zem-1) × BJ-1058 and the BC_1F_3 seeds of the cross (Varuna × BJ-1058) × Varuna were analyzed for their glucosinolate content by an improved HPLC method (Kaushik and Agnihotri, 1999). The selfed seeds of the plants, showing less than 30 µm glucosinolate/g oil free meal in the OP seeds, were utilized to grow single plant progenies during the next three subsequent generations following the pedigree method and recurrent selections. The plant progenies were grown and selfed, and the self and OP seeds were harvested from individual plants. The glucosinolate content was analyzed from OP seeds in each generation to select plants having glucosinolate less than 30 µm/g oil free meal. Simultaneously the plants were selected for early maturity in each generation.

The F_6 selfed seeds of the plants, from the cross *B. juncea* var. (Varuna × Zem-1) × BJ-1058 having less than 30 µm glucosinolate/g oil free meal, were analyzed for their fatty acids content. The double low plants having less than 2 per cent erucic acid in the seed oil and less than 30 µm glucosinolate/g oil free mean were identified for further progeny advancement. The F_6 single plant progenies of the double low *B. juncea* thus derived and the low glucosinolate selections derived from the cross *B. juncea* var. varuna × BJ-1058 were grown in the field during Rabi 1998-99 for collection of selfed and OP seeds. Selections were made for early maturity.

RESULTS AND DISCUSSION

Transfer of low erucic acid

The erucic acid content in the F_1 seeds of the crosses *B. juncea* var. Varuna × Zem-1/M-21 were intermediate (30- 35%) as compared to the high erucic acid parent Varuna (42%) and low erucic acid donor Zem-1/M-21 (0-2%). The erucic acid content in the BC_1F_2 seeds of the cross *B. juncea* var. (Varuna × Zem-1) × Varuna ranged from 23-52 per cent and none of the plants having low erucic acid could be realized. The erucic acid in the F_2 seeds ranged from 0-52 per cent in the cross Varuna × Zem-1 and 0-53 per cent in the cross Varuna × M-21. The erucic acid content in the seeds of single plant progenies of *B. juncea* var. Varuna derived from selected half seed having zero erucic acid, in the present study, remained 0-2 per cent in the subsequent generations (F_3 to F_6). In general the strains derived from the cross *B. juncea* var. Varuna × Zem-1 were late in maturity (140-145 days), tall and poor in agronomic attributes, as compared to those derived from *juncea* var. Varuna × M-21, which were comparatively early in maturity (135-140 days) and were further utilized for progeny advancement to derive the low erucic acid strains.

Table 1. The important agronomic and quality characteristics of the low erucic acid mustard (LEM), low glucosinolate mustard (LGM) and double low mustard (DLM) strains of *B. juncea* var. Varuna

Characteristics/ Strains	LEM	LGM	DLM	Varuna*
Plant height (cm)	195-205	170-197	198-242	190-200
Days to maturity	135-140	132-140	145-152	140
Seed yield (g/plant)	24-29	14-19	15-20	25-32
Oleic acid (%)	30-44	12-15	35-44	12.8
Linolenic acid (%)	28-31	17-24	43-48	16.9
Linolenic acid (%)	15-22	11-13	6-10	18.4
Erucic acid (%)	0-1.5	39-44	0.0	42.6
Glucosinolate (µm/g oil free meal)	127	9-25	10-27	163

*National check variety *B. juncea* var. Varuna

Transfer of low glucosinolate

The glucosinolate content in the F_3 seeds of the cross *B.juncea* var. Varuna × BJ-1058 ranged from 24-128 µm and in the reciprocal cross BJ-1058 × Varuna ranged from 25-130 µm/g oil free meal. The BC_1F_3 seeds of the cross (Varuna × BJ-1058) × Varuna had 81-176 µm glucosinolate/g oil free meal, and thus no plant having glucosinolate less than 30 µm/g oil free meal could be identified in the backcross progeny. From about 500 plants analyzed, only 1 plant from the *B. juncea* var. Varuna × BJ-1058 and only 2 plants from the reciprocal cross BJ- 1058 × Varuna were identified having less than 30 µm glucosinolate. The F_4 seeds from the single plant progenies of these low glucosinolate plants segregated to synthesize glucosinolate in the range of 40-70 µm in the forward cross (Varuna × BJ-1058) and again no plant having glucosinolates less than 30 µm/g oil free meal could be identified. The F_4 seeds from the single plant progenies of the low glucosinolate selections of the reciprocal cross BJ-1058 × Varuna synthesized glucosinolates

in the range of 10-84 µm and ten plants having less than 30 µm glucosinolate/g oil free meal were identified. In the single plant progenies of these low glucosinolate selections, some of the plants again segregated to synthesize more than 30 µm glucosinolate during F_5 and F_6 generations. Selections were made for the plant progenies where most of the plants synthesized < 30 µm glucosinolate, and the plants having low glucosinolate (i.e. 18-22 µm) were used for progeny advancement. Out of the 15 plant progenies forwarded from these low glucosinolate selections, only two showed stability where all plants synthesized < 30 µm glucosinolate/g oil free meal.

Transfer of double low characteristics

The glucosinolate content in the F_3 OP seeds of the three way cross, *B. juncea* var. (Varuna × Zem-1) × BJ-1058, made for transfer of double low characteristics, ranged from 16-134 µm/g oil free meal. Out of about 450 plants analyzed, only one plant was identified having low glucosinolate. The glucosinolate content in the F_4 seeds of the single plant progenies derived from the selected plant ranged from 19-55 µm. The self seeds of the plants synthesizing < 30 µm glucosinolate were analyzed for their fatty acid content, and only four plant progenies were identified having double low characteristics i.e. < 2 per cent erucic acid in the seed oil and < 30 µm glucosinolate/g oil free meal. Out of the twenty-five double low single plant progenies advanced from these selections, many of them segregated for their glucosinolate content, synthesizing glucosinolates upto 73 µm/g oil free meal. Only three plant pogenies remained stable for their quality characteristics in F_6 seeds, synthesizing 15-22 µm glucosinolate/g oil free meal and 0-2 per cent erucic acid.

The breeding for low erucic acid and low glucosinolate *B. juncea* becomes difficult due to the non-availability of desirable donor sources as well as the complicated genetic factors. The erucic acid content is under genetic control determined by the growing embryo (Harvey and Downey, 1964; Kondra and Stefansson, 1965). It is reported to be under the control of two genes with additive effects (Dorrell and Downey, 1964; Siebel and Pauls, 1989). Therefore, each seed from the same plant contains different genetic background resulting in seed to seed variation in the levels of erucic acid, and half seed technique, as applied in present study, is preferred for selection of zero erucic acid plants in the segregating population (Downey and Harvey, 1963). The high glucosinolate content is dominant over low glucosinolate types and are governed by at least three partially recessive genes (Kondra and Stefansson, 1970; Uzunova *et al.*, 1995). Both erucic acid and glucosinolates are inherited independently and a large number of segregating plant populations needs to be screened for several generations to select plants having desired quality parameters. Therefore, poor recovery of low glucosinolate plants in the present study is due to the involvement of several recessive genes resulting in segregation for high glucosinolate types even upto F_6 generation. Similar to our observations Malode *et al.* (1995) in their efforts to transfer double low characteristic in Indian *B. juncea* cv. Pusa Bold have also reported segregation for high erucic acid and high glucosinolate upto F_5 generations.

The international efforts for developing rapeseed mustard strains with single low/double low characteristics were initiated during early fiftees. Stefansson *et al.* (1961) developed the first zero erucic acid strains of *B. napus* in 1961. This was followed by the development of zero erucic acid strains of *B. campestris* (Downey, 1964). In similar studies, Kirk and Oram (1981) obtained low erucic acid strains of *B. juncea* by screening for low erucic acid in the F_2 progeny by half

seed technique. Alonso *et al.* (1991) have obtained the low erucic acid genotypes of Ethiopian mustard through continuous pedigree selection of plants with reduced erucic acid. Simultaneously, the efforts towards development of double low cultivars led to the release of world's first double low *B. napus* cv. Tower by Stefansson in 1974 and of world's first double low *B. campestris* cv. Candle by Downey in 1977 (Bell, 1982). During recent years, the low erucic acid/low glucosinolate/double low strains of many brassica species and its wide allies have also been reported from several countries (Scarth, 1995; Rakow, 1995; Potts *et al.*, 1999; Oram *et al.*, 1999).

Love *et al.* (1990) developed the low glucosinolate *B. juncea* through interspecific hybridization of *B. rapa* and *B. juncea*, and as a result produced the *B. juncea* low glucosinolate line BJ-1058 (used as pollen source in the present work). This first low glucosinolate line of *B. juncea* had poor fertility, high erucic acid and low oil content, and until the initiation of the present study (Rabi 1992-93) was the only known source of low glucosinolate *B. juncea* internationally. Using the low glucosinolate source Rakow *et al.* (1995) created improved lines by crossing with high yielding cultivars and further improved its fatty acids profile to bring it at par with canola quality standards (Potts *et al.* 1999). In Australia, a research programme was undertaken over a period of 25 years involving somaclonal variation, repeated mutagenesis and crossing between late maturing zero erucic acid cultivars with early maturing accessions from India and China. This lead to the development of early flowering, canola quality *B. juncea* germplasm (Oram *et al.*, 1999). Field trials are underway to test the yield potential of these canola quality *B. juncea* and efforts are being made for their acceptance for commercialization.

The Indian efforts towards this goal were initiated only about two decades ago and initially it remained confined to the testing of various exotic quality strains for their yielding capabilities at various locations. Most of them were late in maturity having poor seed set and were not found suitable to grow under Indian agroclimatic conditions (AICRP R&M, 1994). Recently, good progress has been made towards development of low erucic acid *B. juncea* (Agnihotri and Kaushik, 1998; Banga *et al.*, 1998) and the double low *B. napus* (Agnihotri and Kaushik, 1999). A double low *B.napus* hybrid (Hyola-401) developed by a private Australian seed company has been notified by ICAR (AICRP R&M, 1998). The low erucic acid strains of *B. juncea* and double low strains of *B.napus* developed at TERI have been registered by NBPGR-ICAR (Singh, 1998, 1999). Efforts are underway towards development of low glucosinolate and double low *B. juncea.*

The present work is an effort in this direction to introduce quality characteristics in *B. juncea.* The important agronomical and quality characteristics of the low erucic acid (LEM), low glucosinolate (LGM) and double low (DLM) mustard strains developed is given in Table 1. The erucic acid content in the LEM and DLM is reduced to the required international standard (< 2%) as compared to Varuna (44%), with corresponding increase in oleic acid content (30-44%) against only 12 per cent in Varuna. The LEM lines are being tested under AICRP multilocation trials. The glucosinolate content is reduced considerably (< 30 μ m) in LGM and DLM as compared to Varuna (163 μm) and is within the canola quality range. However, there is a need to select plants with lower levels of linolenic acid content from the present 6-22 per cent to < 5 per cent as desired for better oil stability (Scarth et al 1999). There is a scope to reduce the plant height, days to flowering/maturity and seed yield by crossing with high yielding cultivars with desired attributes. Further work is in progress in this direction.

The complete change over to canola quality cultivars in the major rapeseed mustard growing Asian countries, India and China, may take at least a decade (Downey and Chopra, 1996). Once achieved, the farmers and consumers will be able to derive the benefit through proper planning and initiatives for promotion of healthier oil for human consumption at a premium price and through high price of de-oiled cake. The work reported is a stepping stone in this direction providing valuable gene pool for low erucic acid, low glucosinolate and double low characteristics in Indian *B. juncea*. These can be utilized for developing high yielding cultivars with good agronomic attributes and superior nutritional quality.

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REFERENCES

Agnihotri, A and N. Kaushik. 1998. Transgressive segregation and selection of zero erucic acid strains from intergeneric crosses of Brassica. *Indian J. Pl. Genet. Resources* 11(2): 251-255.

Agnihotri, A and N. Kaushik, 1999: Transfer of double low characteristics in early maturing *B. napus*. *Journal of Oilseeds Research* (In Press)

AICROR&M. 1994. Annual Progress Report, All India Coordinated Research Project on Rapeseed and Mustard, ICAR, Govt. of India, New Delhi.

AICROR&M. 1998: Annual Progress Report, All India Coordinated Research Project on Rapeseed and Mustard, ICAR, Govt. of India, New Delhi.

Alonso, L.C., O. Fernandez-Serrano and J. Fernandez-Escobar, 1991. The outset of a new oilseed crop: *Brassica carinata* with low erucic acid. Proc. 8th Int. Rapeseed Conf., Saskatoon, Canada, 170-176.

Banga, S.S., S.K. Banga, M.L. Gupta and G.S. Sandha. 1998. Synthesis of genotypes having specialized fatty acid composition in Indian Mustard (*Brassica juncea* (L.) Coss). *Crop Improv.* 25(1): 21-25.

Bell, J.M. 1982. From rapeseed to canola. A brief history of research for superior meal and edible oil. *Poultry Sci.* 61: 613- 622.

Bell, J.M. 1984. Nutrients and toxicants in rapeseed meal a review. *Journal of Animal Sci.* 58: 996-1010.

Bille, N., B.O. Eggum, I. Jacobsen, O. Olseno and N. Sorensen. 1983. Antinutritional and toxic effects in rats of individual glucosinolates (+) myrosinases added to a standard diet. I. Effects on protein utilization and organ weights. Tierphysiol Tierer nahar Futtermittelkd 49: 195-210.

Dorrell, D.G. and R.K. Downey. 1964. The inheritance of erucic acid content in rapeseed (*Brassica campestris*). *Can. J. Plant Sci.* 44: 499-504.

Downey, R.K. 1964. A selection of *Brassica campestris* L. containing no erucic acid and in its seed oil. *Can. J. Plant Sci.* 44: 295.

Downey, R.K. 1990. *Brassica* oilseed breeding-achievements and opportunities. *Plant Breeding Abstracts* 60(10): 1165-1170.

Downey, R.K. and V.L. Chopra. 1996. Emerging trends in Oleiferous Brassica. In: Proceedings, 2nd International Crop Science Congress, Crop Productivity and Sustainability. Shaping the Future. p 167-179.

Downey, R.K. and B.L. Harvey. 1963. Methods of breeding for oil quality in rape. *Can. J. Plant Sci.* 43: 271-275.

Gopalan, C.D., D. Krishnamurthy, I.S. Shenolikar and K.A.V.R. Krishnamurthy. 1974. Myocardial changes in monkey fed on mustard oil. *Nutr. Metab* 16: 352-365.

Harvey B.L. and R.K. Downey. 1964. The inheritance of erucic acid contet in rapeseed (*Brassica napus*). *Can. J. Plant Sci.* 44: 104-111.

Kaushik, N. and A. Agnihotri. 1997. Evaluation of Improved method for determination of rapeseed-mustard FAMES

by GC. *Chromatographia* **44**: 97-99.

Kaushik, N. and A. Agnihotri. 1999. Separation and quantification of intact glucosinolates by HPLC to study the characteristics of glucosinolate in rapeseed-mustard. *Chromatographia*, **49** (5/6), p 281-284.

Kirk, J.T.O and R.N. Oram. 1981. Isolation of erucic acid-free lines of *Brassica juncea*. Indian Mustard now a potential oilseed crop in Australia. *J. Austral. Inst. Agr. Sci.* **47**: 51-52.

Kondra, Z.P. and B.R. Stefansson. 1965. Inheritance of erucic acid and eicosenoic acid content of rapeseed oil (*B. napus*). *Can. J. Genet. Cytol.* 7: 505-510.

Kondra, Z.P. and B.R. Stefansson. 1970. Inheritance of the major glucosinolates of rapeseed (*Brassica napus*) meal. *Can. J. Plant Sci.* **50**: 643-647.

Kumar, P.R. and S. Tsunoda. 1980. Variation in oil content and fatty acid composition among seeds from the *Cruciferae*. *In*: *Brassica crops and wild allies*: Biology and Breeding. Eds. S. Tsunoda, K. Hinata and C. Gomez-Campo. p. 235-252, Japan Scientific Societies Press, Tokyo.

Love, H.K., G. Rakow, J.P. Raney and R.K. Downey. 1990. Development of low glucosinolate mustard. *Can. J. Plant Sci.* **70**: 425-429.

Malode, S.N., R.V. Swamy and A.S. Khalatkar. 1995. Introgression of 'OO' quality characters in *Brassica juncea* cv. Pusa Bold. *In*: Proceedings, IX GCIRC International Rapeseed Congress, Rapeseed Today and Tomorrow, Vol. 2: 431-433.

Oram, R.N., Philip A Salisbury, John T O Kirk, and Wayne A Burton. 1999. Development of Early Flowering, Canola-Grade *Brassica juncea* Germplasm. *In*: Proceedings X GCIRC International Rapeseed Congress, Sept. 26-29, Canberra, Australia

Potts, D.A., Gerhard W. Rakow and Daryl R. Males. 1999. Canola- Quality *Brassica juncea*, A New Oilseed Crop for the Canadian Prairies. In: Proceedings, X GCIRC International Rapeseed Congress, Sept. 26-29, Canberra, Australia.

Rakow, G. 1995. Review: Developments in the breeding of edible oil in other *Brassica* species. *In*: Proceedings, IX GCIRC International Rapeseed Congress, Rapeseed Today and Tomorrow, Vol. 2: 401-406.

Rakow G., P. Raney and D. Males. 1995. Field performance of canola quality *Brassica juncea*. *In*: Proceedings, IX GCIRC International Rapeseed Congress, Rapeseed Today and Tomorrow. Vol. 2: 428-430.

Scarth, R. and B.E. Peter. 1999. Designer Oil Canola - A Review of New Food-Grade Brassica Oils with Focus on High Oleic, Low Linolenic Types: *In*: Proceedings, X GCIRC International Rapeseed Congress, Sept. 26-29, Canberra, Australia.

Scarth, R. 1995. Review. Developments in the breeding of edible oil in *Brassica napus* and *B. rapa*. *In*: Proceedings, IX GCIRC International Rapeseed Congress, Rapeseed Tody and Tomorrow, Vol. 2: 377-382.

Siebel, K. and K.P. Pauls. 1989. Inheritance patterns of erucic acid content in populations of *Brassica napus* microspore-derived spontaneous diploids. *Theor. Appl. Genet.* 77: 489-494.

Singh, B.B. 1998. Plant Germplasm Registration Notification. *Indian J.Pl. Genet. Resources* **11**(1): 121-122.

Singh B.B. 1999. Plant Germplasm Registration Notification. Indian J. Pl. Genet. Resources **11(2)** :

Stefansson, B.R., F.W. Hougen and R.K. Downey. 1961. Note on the isolation of rape plants with seed oil free from erucic acid. *Can. J. Plant Sci.* **41**: 218-219.

Uzunova, M., W. Ecke, K. Weibleder and G. Robbelen. 1995. Mapping the genome of rapeseed (*Brassica napus* L.). I Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. *Theor. Appl. Genet.* **90**: 194-204.