Journal of Agricultural Biotechnology and Sustainable Development Vol. 2(2) pp. 030-034, February, 2010
Available online http://www.academicjournals.org/JABSD

Full Length Research Paper

Resynthesis of Ethiopian mustard (*Brassica carinata* L.) from related digenomic species: An unexplored possibility

F. A. Sheikh[1], S. Najeeb[2*], A. G. Rather[2] and Shashi Banga[3]

[1,2]Shere-e- Kashmir University of Agricultural Sciences and Technology Rice Research and Regional Station Khudwani, Anantnag (J and K)-192102, India.
[3]Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University Ludhiana -141001, India.

Accepted 6 October, 2009

The present study was undertaken to resynthesize *Brassica carinata* (BBCC, 2n = 34) from its related digenomic species viz. *Brassica napus* (AACC, 2n=38) and *Brassica juncea* (AABB, 2n = 36) for generating variability for oil and meal quality. Elite genotypes of *B. napus viz.* MHO-18-1-184 ("00") and *B. juncea viz.* NJHO-3-25('0' C22:1) were involved in interspecific hybridization followed by chromosome doubling, selfing and selection to extract *B. carinata* type plants without any backcrossing. Morphological and cytological assessment of the hybrids and derived *B. carinata* was carried out to check the breeding value and genomic stability of derived *B. carinata* types. The study was successful in developing two (NJ 2 and 3) derived *B. carinata* type plants, following hybridization between non parental amphiploids viz. *B. napus* and *B. juncea*. These derived plants showed zero erucic acid and high oleic acid content (49%), as in the two parental species. The derived *B. carinata* plants also exhibited sufficient genetic deviation from natural *B. carinata* and constitute an entirely new source of genetic variability.

Key words: *Brassica carinata,* interspecific hybridization, fatty acid.

INTRODUCTION

Ethiopian mustard (*Brassica carinata*) is currently being evaluated as an option to the traditional canola /mustard cultivation, especially for low rainfall areas of the world. In its area of adoption, the crop has been shown to possess acceptable yield levels as well as resistance to various biotic and abiotic stresses (Getinet et al., 1996). In spite of these strong positive attributes, the crop suffers from several agronomic limitations like low oil quality characterized by high level of erucic acid (Velasco et al., 1998) and unacceptable level of meal glucosinolates (Getinet et al., 1997). Restricted level of natural variability for these specific traits has greatly constrained the breeding programmes aimed at overcoming these limitations (Song et al., 1988). Resynthesis of *B. carinata* employing two diploid progenitor species viz. *Brassica oleracea* and *Brassica nigra* attempted in the past for variability enhancement; for oil and meal quality traits has not been not been effective as none of the diploid species had any history of human selection pressure for evolution as oil seed crop. Therefore, hitherto unexplored route of Resynthesis of *B. carinata* was attempted involving the elite lines of two related amphiploids viz. *Brassica napus* and *Brassica juncea*. Such approach may also help in capitalizing on gross structural evolutionary modifications that occurred in cohabiting genomes of *Brassica* digenomics during evolution (Song et al., 1995). The present work was a part of Ph. D research conducted at the research farm and molecular and tissue culture laboratory of Oilseed Section of Department of Plant Breeding, Punjab Agricultural University, Ludiana, Punjab (India).

MATERIALS AND METHODS

B. napus (AACC 2n = 38) cv. MHO-18-1-184("00") and *B. juncea* (AABB 2n = 36) cv. NJHO-3-25('0' C22:1) were used as parents for interspecific hybridization. The F_1 plants were confirmed at

*Corresponding author. E-mail: sfaroq4@rediffmail.com, sfaroq4@yahoomail.com.

Table 1. Meiotic studies in interspecific hybrid (F_1), amphiploid II (A_2), amphiploid III (A_3) generations of *B. napus x B. juncea*.

Generation	Number of plants sampled	PMCs	Meiotic profile		Mean frequency			
			Configuration	Proportion	II	III	IV	I
F1	10	197	10II + 1IV + 1III + 10I	0.05	12.48	0.75	0.17	9.08
			11II + 1IV + 11I	0.13				
			12II + 1III + 10 I	0.46				
			14II + 1III + 6 I	0.23				
			14 II + 9 I	0.13				
Amphiploid II (A_2)	50	119	11II + 2IV +1 III + 7 I	0.18	13.60	0.90	0.35	7.56
			12 II + 2III + 8I	0.23				
			15II + 8 I	0.33				
			15II + 1III + 7I	0.26				
Amphiploid III (A_3)	30	120	17II	0.73	16.70	_	_	1.08
			15II + 4I	0.27				

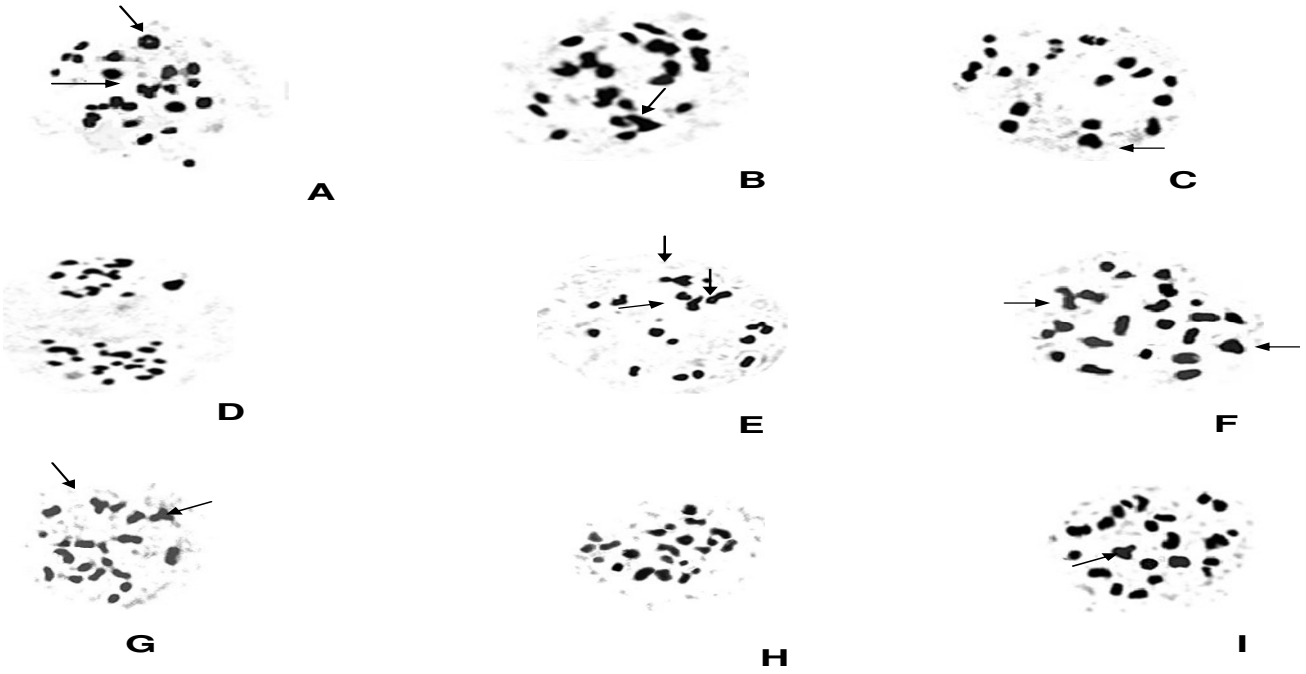

Figure 1. Meiotic configuration in PMCs of *B. napus* x *B. juncea* hybrid (A - D) and A2 amphiploid (E - I). A, 10II + 1IV + 1III + 10I; B, 12II + 1III + 10I; C, 14II + 1III + 6I; D, 17 - 20 distribution at anaphase; E, 11II + 2IV+1III + 7I; F, 12II + 2III + 8I; G, 12II + 2III + 8I; H, 15II + 8I; I, 15II + 1III + 7I.

morphological and cytological level (Figure 1 and Table 1). The flower buds were fixed around 6.00 to 8 a.m. in Carnoy's solution II (Ethanol: Chloroform: Acetic acid; 6:3:1), containing a few drops of ferric acetate (a filtered solution of saturated ferric acetate made by adding ferric chloride to glacial acetic acid). After 48 h of fixing, the young anthers were crushed in two percent acetocarmine on a slide and observed under inverted microscope to study the chromosome number and pairing behaviour of chromosomes. The photographs of the best cells were taken with digital camera and downloaded in the computer using the programme Camedia master.

The resultant F_1 plants were subjected to 0.2% colchicine treatment to induce sectorial polyploidy which on selfing were advanced to amphiploid II (A_2) and amphiploid III (A_3) generations based on morphological and cytological studies in each generation (Table1). The *B. carinata* plants in the advance A_3 generation having genomic stability akin to *B. carinata* (2n = 34) and high pollen fertility were tagged individually and subjected to fatty acid analysis as per the standard procedure of ethyl ester preparation (Appleqvist 1968) followed by gas liquid chromatography (GLC) and meal glucosinolate estimation using the method developed by Kumar et al. (2004).

RESULTS

The F1 hybrids of *B. napus* × *B. juncea* exhibited intermediate plant growth, early flowering and small pods

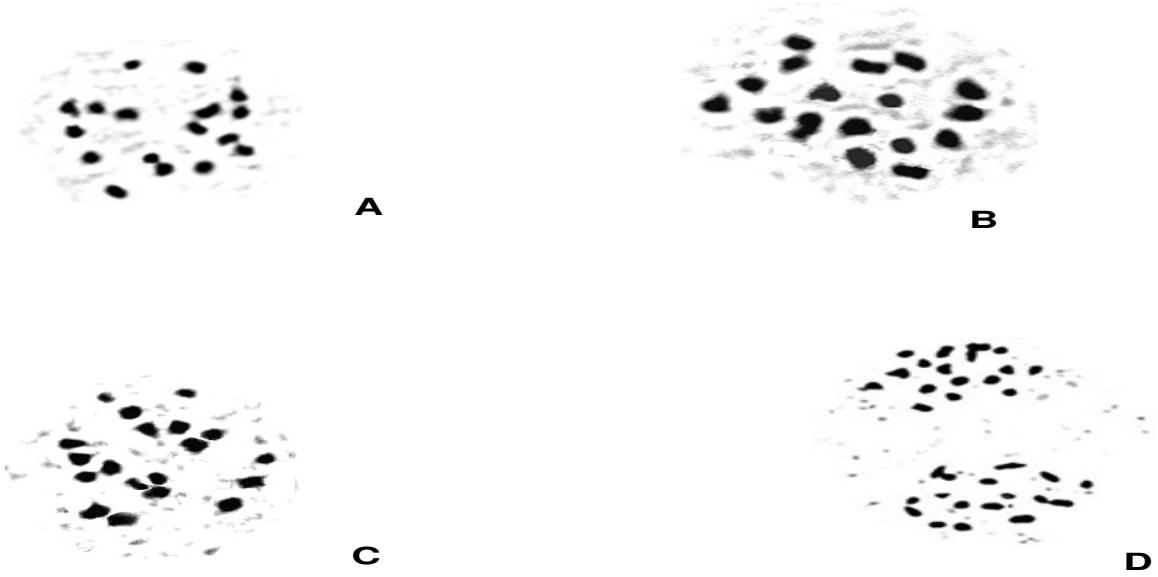

Figure 2. Meiotic configurations in PMCs of *B. carinata* type plants in A_3 generation of *B. napus* x *B. juncea* (A - D). A, 17II; B, 17II; C, 15II + 4I; D, 17 - 17 distribution at anaphase.

Figure 3. *B. napus* × *B. juncea* F1 showing amphiploid sectors.

with most of the pods empty. The cytological studies of pollen mother cells (PMCs) of the hybrids revealed a somatic chromosome number of 2n = 37 with presence of up to 14IIs and 11Is. Occurrence of quadrivalents and trivalents was observed in 87% of the cells. The mean bivalent frequency was found to be 12.48 with 12II + 1III + 10I being the predominant configuration occurring in 46% PMCs (Figure 1 and Table 1). The amphiploid shoots were identified by their deep green color, thick leaves, slower growth, bigger bud size, large flowers with well developed anthers carrying large pollen grains. The PMCs of these A_2 plants revealed the chromosome number to be 38 and 40 with mean bivalent frequency of 13.6 (Table 1 and Figure 1 and 2). A_3 cytological studies revealed a mean bivalent frequency of 16.7 (Figure 3. Three genomically stable derived *B. carinata* A_3 plants were tagged and the fatty acid profile as per expectation (Table 2) inherited the desired '0' erucic acid and fatty acid profile of the two parents (*B. napus* and *B. juncea*). Only exception being NJ-1 which had intermediate

Table 2. Fatty acid composition, oil content and glucosinolate content of derived *B. carinata* in comparison to natural digenomics.

Entries	Fatty acid composition (%)							Oil content	Glucosinolates (µ moles/g defatted meal)
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid		
NJ 1	4.1	0.0	26.6	23.8	12.3	10.1	23.0	*	106.8
NJ 2	5.1	1.6	42.5	24.4	12.9	13.0	0.3	*	80.5
NJ-3	5.3	1.8	49.0	21.0	10.8	11.5	0.3	*	81.0
NJH0 3 - 25	4.9	1.1	51.3	27.0	9.7	4.2	0.8	40.7	82.4
MHO 18-1-184	4.0	0.0	52.1	28.4	12.8	1.8	0.4	41.4	29.3
PC 5	3.5	0.6	11.2	18.1	11.1	9.6	45.9	34.3	107.5

*Oil content could not be estimated due to small quantity of seed.

erucic acid. Such a deviation was expected since NJ-1 was completely male sterile. Only open pollina-ted seed set, possibly through random pollina-tion with high erucic acid parent, was obtained and used for fatty acid analysis. Besides introducing '0' quality traits in these two plants, there was also a recorded increase in oleic acid content to approximately four fold than the existing *B. carinata* cv. PC5.

The mean glucosinolate level of two derived genotypes NJ 2 and NJ 3 are 80.5, 81.0 µ moles/g defatted meal,respectively, was closer to the *B. juncea* parent (82.4 µ moles/g defatted meal). This was in comparison to 107 µ moles in natural *B. carinata* cv. PC 5.

DISCUSSION

The interspecific hybrids of *B. napus* × *B. juncea* were partially (15-20%) male fertile due to abnormal chromosome behaviour (Qi et al., 1993; Rao et al., 1993). The higher number of bivalents against the normally expected 10IIs can be due to inherent homologies in combining diploid *Brassica* genomes (Roebbelen, 1960). Sandhu and Gupta (2000) noted up to 14IIs and 9Is at MI of meiosis in F_1 hybrids of *B. juncea* × *B. napus*. The high frequency of univalents led to unequal segregation and consequently steri-lity of these hybrids. Variable chromosome number and multivalent formation in early gene-rations of synthetic alloploids of *Brassica* is of common occurrence (Prakash et al., 1984). Presence of 9 - 17IIs and 0 - 4 quadrivalents has been reported in A_1 generation of *B. carinata* (Mizushima, 1950). Induction of ploidy in *Brassica* does not ensure only diploid chromosome behaviour as quadrivalents and trivalents have frequently been observed owing to close genomic affinities (Attia and Roebbelen, 1986).

The recorded decrease of erucic acid up to 0 level in the two derived plants indicate the inhe-rited alleles for low erucic acid located on B and C genomes from donor species of *B. juncea* and *B. napus*, respectively. The results are in accordance with the previous report that the erucic acid content of the seed oil in *B. carinata* is controlled by two genes; one each located on B and C genomes acting in additive fashion without any dominance (Fernandez-Escobar et al., 1988). This reduction of about 27 µ moles in derived *B. carinata* can be attributed to mutant alleles for low glucosinolate present on C genome, inherited intact from *B. napus* (*B. napus* donor in the present studies was '00' and *B. juncea* had high glucosinolates). Consequently, it can also be postulated that alleles located on B genome contribute more to total glucosinolate kitty in *Brassica* digenomics. The present set of derived genotypes of *B. carinata* will prove a useful material for transferring desirable quality traits to existing high yielding breeding material of *B. carinata*. In addition, this study will provide a greater insight into acquisition of variability by natural amphiploids after polyploidy.

REFERENCES

Appleqvist L A (1968). Rapid methods of lipid extraction and fatty acid ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. Ark Kenci 28: 351-70.

Attia T, Roebbelen G (1986). Cytogenetic relationship within cultivated *Brassica* analyzed in amphidiploids from the three diploid ancestors. Can . J. Genet. Cytol. 28: 323-29.

Fernandez-Escobar J, Dominguez J, Martin A, Fernandez-Martinez J M (1988). Genetics of the erucic acid content in interspecific hybrids of Ethiopian mustard (*B. carinata)* and rapeseed (*B. napus*). Plant Breed 100: 310-15.

Getinet A, Rakow G, Downey RK (1996). Agronomic performance and seed quality of Ethiopian mustard in saskatchewan. Can. J. Pl. Sci. 76: 387-92.

Getinet A, Rakow G, Raney J P and Downey RK (1997). Glucosinolate content in interspecific crosses of *Brassica carinata* with *B. Juncea* and *B. napus*. Plant Breed 116: 39-46.

Kumar S, Yadav SK, Chauhan JS, Singh AK, Khan NA, Kumar PR (2004) .Total glucosinolate estimation by complex formation between glucosinolates and tetrachloropalladate (II) using ELISA Reader. J. Food Sci. Technol. 41: 63-65.

Mizushima U (1950). On several artificial allopolyploids obtained in the tribe Brassiceae, Cruciferae. Tohoku J. Agr. Res. 1: 15-27.

Prakash S, Gupta S, Raut RN and Kalra A (1984). Synthetic *Brassica carinata Cruciferae* News lett. 9: 36.

Qi CK, Pu HM and Fu S Z (1993). Fertility and variation in characters in the progenies derived from F_1 of the interspecific cross *Brassica napus* x *B. carinata* . Oil Crops China, 2: 8-10.

Rao MVB, Rama Kumar PV, Murthy VVA (1993). Introgression of earliness in *Brassica napus*. II. An Interspecific *B. napus* and *B carinata* cross. Int. J. Trop. Agric. II: 20-26.

Robbelen G (1960). Beitrage Zur Analyse des Brassica–Genome. *Chromosome* II: 205-28.

Sandhu SK, Gupta VP (2000). Interspecific hybridization among digenomic species of *Brassica* Crop Improv 27: 195-97.

Song GK, Lu P, Tang K , Osborn TC (1995). Rapid genome Change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc. Natl. Acad. Sci. USA 92: 7719-23.

Song KM, Osborn TC, Williams PH (1988). Brassica taxonomy based on nuclear restriction fragment length polymorphism (RFLPs):1. Genome evolution of diploid and amphiploid species. Theor. Appl. Genet. 75: 784-92.

Velasco L, Fernandez-Martinez JM, Haro A-de, De-Haro A (1998). Increasing erucic acid content in Ethiopian mustard through mutation breeding. Plant Breed 117: 85-87.