Breeding canola quality Indian mustard (*Brassica juncea* L.)

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ABSTRACT

Breeding canola *juncea* has been an important crop improvement activity in India for the last about two decades. However, despite earnest efforts it has not been possible to develop canola cultivars equalling the productivity and agronomic attributes of conventional mustard varieties. Introduced canola *juncea* genotypes have shown poor adaptation to Indian growing conditions. In order to achieve greater selection gains, a substantial germplasm assemblage of Australian and Canadian germplasm was involved in multiple crossings with locally bred canola *juncea*. Visual selection for yield attributes was carried out for 4 generations of selfing, after which 162 promising advanced generation progenies were evaluated for various yield contributing traits. Diversity analysis allowed grouping of these progenies into 13 clusters. Majority of the genotypes, however, assembled into five clusters .This indicated rather a narrow genetic base of breeding material despite involving large number of exotic accessions. Much of the diversity was ascribed to variation for plant height whereas contribution of branch number and pod length was negligible. Assessment of 22 selected progenies in large scale trial, however, suggested incremental yield gains with identification of putative genotypes matching productivity of conventional mustard cultivars. In order to expedite development of canola *juncea*, we are utilizing marker assisted backcross breeding to selectively refine productive cultivar RL 1359. Six primers namely, GER 1,GER 5, 5g101, 5gAJ30, 5g41 and 5gAJ67 are being used for the purpose.

INTRODUCTION

Brassica juncea is an important oilseed crop in India, China and in south-western areas of the former Soviet Union. Due to its relatively greater drought and heat tolerance it is considered as an alternate oilseed crop for dryer regions of Western Canada and Australia with considerable scope for expansion in United States. Besides greater abiotic stress tolerance. *juncea* possesses higher level of pod shattering resistance and has different genes for blackleg (*Leptosphaeria maculans*) resistance than *B. napus/ B. rapa* .Until recently most of the *B. juncea* types grown in the world were of conventional mustard quality as they contained high levels of glucosinolates in the meal and high levels of erucic acid in the oil fraction. During the past two to three decades , significant attempts have been made to introduce canola quality traits into *B. juncea* in an effort to change its seed quality while retaining its agronomic benefits .The first significant achievement in this direction was the development of low erucic acid *B. juncea* (Kirk and Oram, 1981). The next breakthrough occurred with the development of a low glucosinolate form of *B. juncea*(Love, et al.,1991) following interspecific hybridization between *B. rapa* and *B. juncea*. Both of these publicly available sources precipitated researches towards incorporating canola quality traits to *B. juncea.* Despite initial success to transfer low erucic acid genes ,fatty acid composition required more changes as low erucic acid forms had too low a level of oleic acid (C18:1) and too high of levels of linoleic acid (C18:2) and linolenic acid (C18:3) to be considered comparable to canola. This occurred because of the presence of desaturase promoter gene(s) on B genome which mediates increased desaturation of oleic acid. C genome of *B.napus* possesses desaturase promoter gene(s), resulting in low activity of desaturation step. Concerted efforts have allowed commercialization of canola *juncea*, albeit at ,a limited scale in Canada and Australia. In India similar attempts have not met with commercial success so far due to lower productivity levels of canola *juncea* as compared to conventional mustard cultivars. Greater access to Australian canola germplasm has helped in improving selection gains by enlarging available gene pool of canola germplasm for hybridization. This communication documents enhanced productivity levels and enlarged canola *juncea* gene pool at Punjab Agricultural University. Validation of molecular markers for glucosinolate content in Indian, Australian and Chinese germplasm accessions has indicated.

Key words: Diversity-multiple crosses-selection-MAS-glucosinolates-erucic acid

MATERIAL AND METHODS

The plant materials comprised 162 advanced generation progenies of *B.juncea* canola. These were evaluated in augmented design. The data for various morphological traits were subjected to Mahalanobis D^2 analysis using indostat software. In another set up of the experiment, 22 selected canola *juncea* progenies were evaluated in a large scale trial in RBD with three replications. Standard cultural practices were followed throughout the growing season to raise the crop. For developing marker assisted protocol , six molecular markers(GER 1,GER 5, 5g101, 5gAJ30, 5g41 and 5gAJ67) were validated for 9 Australian, 10 Chinese and 7 Indian Canola / non-canola genotypes to develop MAS.The protocol as described by Ramchiary et al (2007) was used . These markers are being used to screen backcross progenies in conversion of high yielding cv. RL 1359 to canola type.

RESULTS AND DISCUSSION

D^2 analysis allowed grouping of 162 genotypes into 13 clusters (Fig 1), of these four clusters were single genotype clusters. Majority of the genotypes fitted into five clusters .These were cluster 1,2,3,4 and 5 with 40,50,24,9 and 19 genotypes respectively. Intra-cluster grouping in these five clusters varied from 16.2 to 21.5, apparently indicating relatively homogeneous grouping. Of these maximum difference occurred between clusters one and five. Remaining multiple genotype clusters had 2-6 genotypes .Cluster thirteen was most diverged with intercluster distances of 292-371. Analysis of cluster means indicated wide divergence ,no single cluster had the optimum combination of all the characters. For plant height ,cluster one was the best. Other desirable clusters were cluster five for branch number ,cluster six for main shoot length and pods on main shoot ,cluster seven for seed yield and cluster nine for pod length .Maximum contribution to overall divergence was due to variation in plant height(55.4%),followed by main shoot length (22.7%) and pods on the main shoot(13.7).Contribution from yield was low . It was negligible for branch number and pod length. This suggested the necessity to incorporate variation for these critical yield contributing traits .This also reflected the necessity of using still wider germplasm base with better assemblage of productivity genes .The perceptible failure of multiple crossings to widen the genetic base can be attributed to the fact that the progenies used in these studies were developed using mostly the Indian canola and Australian canola genotypes .Initial Indian canola genotypes were developed using conventional Indian mustard and Canadian donors for quality attributes .The same is true for Australian genotypes ,most of them owe their origin to hybridization between Indian and Canadian germplasm. Closeness of Indian and Australian *B.juncea* was demonstrated earlier by our group based on DNA polymorphism generated by SSR markers . There is thus still a need to introduce more diverse germplasm ,especially for productivity traits. Chinese germplasm is an option as it is distinct and has high biomass productivity. However, it is not adapted to Indian conditions and it will require several generations of adaptive breeding before it can be utilized in conventional crop improvement programmes. Inspite of failure to substantially widen the germplasm base ,the specified strategy has helped in achieving incremental improvement in productivity of canola genotypes .The yield performance of selected genotypes is shown in Table 1. Out of 21 putative 00 genotypes, JC-91-4-66 (2522kg/ha), JC-1359-9-223 (2388kg/ha), JC-1359-23-527 (2432kg/ha) and JCR-30 (2278kg/ha) and JCR-91-4-61 (2218kg/ha) excelled the best check PBR-210 (2196kg/ha) by a margin of 14.8%,8.7%.10.7% 3.7 %and 1.0% . All the tested genotypes matured between 132 to135 days. Seed size ranged between 3.5 to 5.2 g/1000 seeds.Due to substantially higher productivity levels and superior agronomic features of Indian mustard genotypes, as an alternate strategy we have initiated marker assisted selection programme to selectively modify productive cultivars namely RL 1359 for canola characteristics .Due to its complex inheritance, breeding for low meal glucosinolate content is very difficult to breed and hence excellent candidate for marker assisted selection. Six primers have been developed by Ramchiary et al.(2007).These are GER 1,GER 5, 5g101, 5gAJ30, 5g41 and 5gAJ67.Of these primers GER 1 and GER 5 were designed using sequence information of two major candidate genes *GSL-ELONG R1* and *GSL-ELONG R4* which account for 80 % of aliphatic glucosinolates.Exepting one primer (5gAJ67) all primers are either co-dominant ,or dominant for low glucosinolates and are thus eminently suitable for marker assisted backcrossing programme. Recurrent

backcrossing can thus be carried out without the necessity of selfing after every backcross generation. These markers could be validated for almost all the test germplasm accessions Table 2.

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Fig 1: Grouping and Mahalanobis euclidean distances in canola *B.juncea*

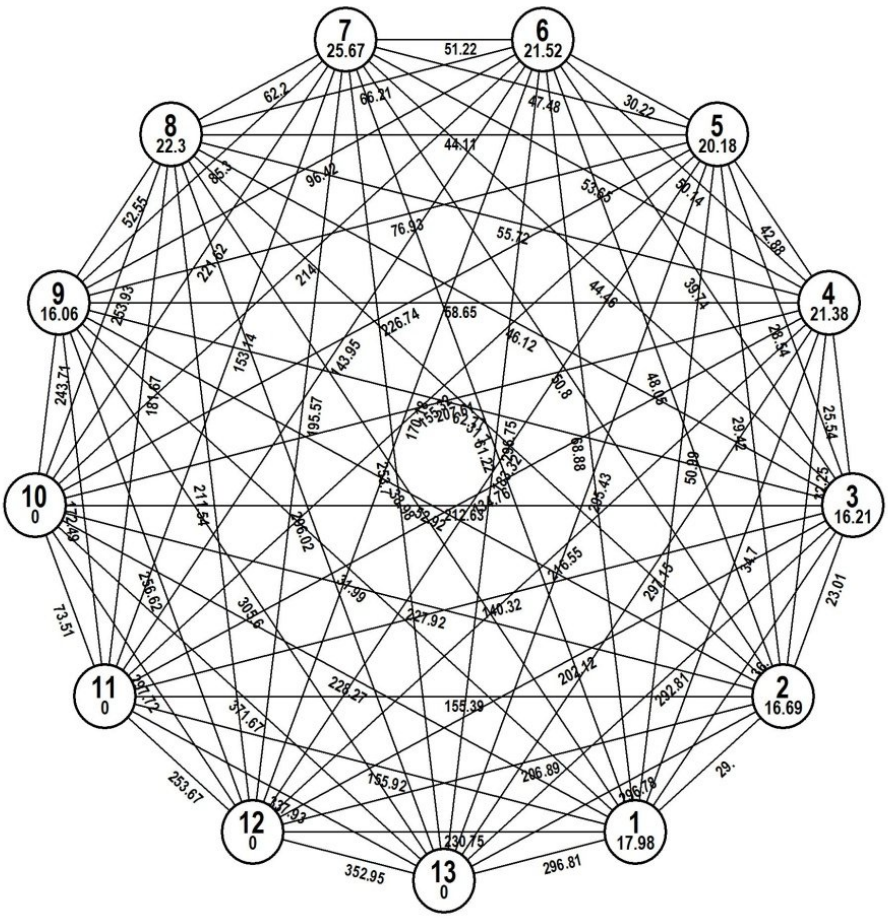

Table 1 : Performance of quality mustard genotypes in large scale trial

Genotype	Flowering (days)	Maturity (days)	Seed size (g)	Yield (kg/ha)
JCR-30	60	132	3.5	2278
JC-91-4-37	44	130	3.6	2125
JC-91-4-61	61	132	3.6	2218
JC-91-4-66	63	134	3.7	2522
JC-210-3-1-110	61	132	3.6	2085
JC-9-7-363	56	132	4.3	2199
JC-1359-23-5	48	130	3.8	2432
JC-1359-9-223	51	130	3.9	2388
JC-1359-28-494	46	130	4.6	2054
PBR-210	63	132	5.2	2196
CD (5%)	**--**	**--**	**--**	**325**
CV (%)	**--**	**--**	**--**	**9.8**

Table 2: Validation of molecular markers for diverse canola *juncea* genotypes

Entry*	Primer						Glucosinolates (µmol/g)
	GER1	GER5	5g101	5gAJ30	5g41	5gAJ67	
JN004(A)	I	I,h	I	I	h	I	<40
JO 006(A)	I,h	h	h	h	h	h	>70
JO 009(A)	I	I,h	I	I	h	h	<15
JN 010(A)	I	I	I	I	I	I	<30
JM 018(A)	I	I	I	I	h	h	<30
JN 028(A)	h	I	I	I	h	I	≈70
JN 031(A)	I	I	I	I	h	h	<30
JN 032(A)	I	I,h	I	I	I	I	40
JR 049(A)	I	I	I	I	h	I	<15
CBJ001(C)	h	h	h	h	h	I	<30
CBJ002(C)	I,h	I	I	I	h	h	<30
CBJ003(C)	h	h	h	h	h	h	105
CBJ004(C)	I	I	I	I	h	I	<30
TABP15(C)	h	I	I	I	h	h	<30
MPIR(C)	h	I	I	I	h	h	≈60
XINYOU(C)4	h	h	h	h	h	h	109
XINYOU5(C)	h	h	h	h	h	h	87
XINYOU8(C)	I,h	I	I	I	I	I	<30
XINYOU9(C)	I	I	I	I	I	I	<30
JC507(I)	h	I	I	I	I	I	≈50
JC527(I)	I	I	I	I	I	I	<30
JC664(I)	I	h	I	I	I,h	h	≈50
JC91-55(I)	I	I	h	I	I	I	<30
JC91-670(I)	I	I	I	I	I	I	<30
NUDH YJ-4(I)	I	I	I	I	I	I	<20
RL1359(I)	h	h	h	h	h	h	119

* Abbreviations in parentheses A = Australian, C = Chinese and I = Indian