



Evaluation of recombinant inbred lines based on morphological and biochemical parameters in Indian mustard (*Brassica juncea* L.) derived from Rohini × PBR-97

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Abstract

Among the rapeseed-mustard (*Brassica* spp.) group of crops Indian mustard (*Brassica juncea* L.) is the principal crop which accounts for approximately 85% of the total acreage under rapeseed-mustard. Here in this study, a set of 225 recombinant inbred lines (RILs) developed through a cross between high yielding commercially released Indian mustard cultivars Rohini and PBR-97. were evaluated in $F_{6:7}$ generation during the *Rabi* season of 2012 -2013 and data were recorded for various morphological and biochemical parameters such as plant height, main shoot length, fruiting zone length, siliqua length, seeds/siliqua, biological yield/plant, 1000-seed weight, harvest index, seed yield/plant, oil content, and glucosinolates, fiber and phenol content. Significant genetic variation was observed among RILs for all the traits based on analysis of variance along with high genetic advance and high heritability for seed yield/plant, biological yield, 1000-seed weight, fibre and glucosinolates content. Seed yield/plant was found to be significantly and positively correlated with plant height, biological yield, number of seeds/siliqua and 1000-seed weight. Among these RILs, 25 RILs were identified as highly promising RILs on the basis of seed yield/plant which can be further used for multi-location testing for varietal release or used as parents in mustard breeding programs.

Keywords: *Brassica juncea*, Recombinant inbred line, morphological characterization, biochemical parameters

Introduction

Rapeseed-mustard (*Brassica* spp.) group of crops are the second most important *Rabi* oilseed crops in India. In 2013-14, these crops accounted for 24.2% of total oilseed production. Among various rapeseed-mustard species being grown in India, *Brassica juncea* L. commonly known as “Indian mustard” is the principal crop which accounts for nearly 85% of the total rapeseed-mustard acreage. Though, a significant achievement has been made in improving the production and productivity of Indian mustard during the last two decades, India still fall short of its domestic edible oil demand. Thus, further improvement in the productivity of Indian mustard is an important breeding objective. The country witnessed yellow revolution through a phenomenal increase in production and productivity from mere 2.68 mt and 650 kg/ha in 1985-86 to 8.0

mt and 1188 kg/ha in 2013-14 (AICRP-RM, 2015). However, the domestic production of oilseeds is still insufficient to meet the edible oil demand in the country and more than 50% of domestic edible oil demand is being met through costly imports. In 2013-14 alone, India imported about 11.62 mt of edible oil. Thus, increasing domestic oilseed production forms an important part of the strategy to enhance edible oil sustainability and to reduce the dependence on imports (www.seaofindia.com).

In rapeseed-mustard, as in other crops, seed yield is a complex trait that includes various components which finally decide yield structure and all this occur due to physiological and morphological processes during the phenological development (Sadat *et al.*, 2010). Thus, a more thorough study of individual yield components is required which may result into improvement of the genetic architecture of yield

(Marjanovic-Jeromela *et al.*, 2011). Therefore, breeders keep on creating new combinations of best traits from various genotypes and identify superior genotypes on the basis of highly heritable yield related components which further lead to improvement in seed yield. Hence, in the context of yield improvement, assessment of the nature and degree of variability existing within a breeding population is essential while selecting desirable genotypes. Moreover, it is also important to understand the interrelationship between each yield component and seed yield (Akram *et al.*, 2008). Thus, keeping this in view, current study was undertaken to develop a recombinant inbred line (RIL) population through cross between two high yielding commercially released cultivars Rohini and PBR-97 and to evaluate the extent of genetic variability, heritability, genetic advance and genotypic and phenotypic correlations for determining the degree to which various yield contributing characters are associated, and identify some of the superior lines for seed yield and related traits.

Materials and Methods

Plant material

Commercially released Indian mustard varieties Rohini and PBR-97 were used to develop RIL population. The seed samples of both the varieties were obtained from the Germplasm Unit, ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur.

Development and evaluation of RIL population

The two parental Indian mustard varieties Rohini and PBR-97 were crossed and 225 RILs were generated using a single-seed-descent approach. They were evaluated during *Rabi* 2012-13 under rainfed condition in augmented block design, where each block had 10 RILs along with four checks (RB-50, RH-819, PBR-97 and Rohini). Two rows of 5 m length spaced 30 cm apart with plant to plant spacing of 10 cm constituted one RIL. Five plants were randomly selected from each RIL to record data on plant height, fruiting zone length, main shoot length, seeds/silique, silique length, harvest index, seed yield/plant, biological yield/plant, 1000-seed weight. Harvest index (HI) was calculated by the formula: $HI = \text{grain yield} / \text{total above ground biomass} \times 100$.

Oil, glucosinolates, fiber and phenol contents were estimated using FT-NIR spectroscopy. RILs having higher mean value for a specific trait over respective check values were considered promising.

Statistical analysis

Analysis of variance (ANOVA) was performed as described by Federer (1956). The estimation of magnitude of variability (variance and coefficient of variation), heritability, genetic advance and correlation coefficients were done using the standard statistical procedures.

Results and Discussion

Recombinant inbred lines represent 'immortal' genetic populations. These populations represent defined genetic relationships within themselves and their highly homozygous nature limits phenotypic diversity to environmental variance components. In this sense, RIL populations are considered to be highly useful for studying quantitative traits such as seed yield. In the present study, a set of 225 RILs were evaluated to determine interrelationships between seed yield and its component traits, and to identify some promising lines with high seed yield. Based on ANOVA, a significant amount of genetic variability was reported among the RILs as indicated in Table 1 which reveals that selection may be expected for seed yield and related component traits in these RILs. The RIL population was evaluated in augmented block design, where each block had 10 RILs along with two checks and parents. The RILs were found to be significantly different for all the components except silique length, seed/silique, harvest index and phenol. The blocks were observed to be nearly homogeneous as they exhibited non significant variability for all the traits. However, the check genotypes considered for the study differed significantly for plant height, fruiting zone length, biological yield/plant, 1000-seed weight, harvest index, seed yield/plant, glucosinolate and phenol content. The check versus RILs interactions showed highly significant differences for all the characters except plant height and fruiting zone length indicating that checks as a group differed significantly from RILs as a group (Table 1).

Mean, range, genotypic and phenotypic coefficient

Table 1: Mean sum of squares for different morphological traits in RILs derived from the cross Rohini × PBR-97

Source	d.f.	Plant height (cm)	Main shoot length (cm)	Fruiting zone length (cm)	Siliqua length (cm)	Seeds/siliqua	Biological yield /plant (g)	1000 seed wt. (g)	Harvest index	Seed yield /plant (g)	Oil content (%)	Glucosinolates	Fiber	Phenol
Block	4	74.79	12.05	8.67	0.54	1.77	18.59	0.19	10.44	1.91	0.80	8.54	0.23	0.08
Treatment	233	67.32*	40.33*	33.10**	0.17	1.74	187.02**	0.67*	43.29*	25.08**	1.11*	395.55**	3.01**	0.06*
Control	3	100.98*	25.31	45.84*	0.29	1.53	75.23*	3.06**	75.30*	18.38*	0.17	179.64**	0.39	1.18**
Progeny	229	67.14*	39.67*	33.03**	0.16	1.69	188.47**	0.55*	41.24	24.12**	1.10*	399.63**	2.85**	0.04
Control	1	8.87	236.50**	10.67	2.18**	11.85**	191.45**	19.52**	415.32**	363.93**	5.06**	109.62**	45.85**	1.11**
v/sp progeny														
Error	12	24.74*	16.79	9.84	0.18	1.00	21.17	0.23	18.93	4.61	0.40	9.75	0.78	0.02

* and ** significance levels represents at 0.05 and 0.01 respectively.

of variation (GCV and PCV), heritability in broad sense and genetic advance were also calculated and it was observed that phenotypic variances were slightly higher than genotypic variances explaining the role of environmental factors on character expression. Similar observations were recorded in *B. juncea* by many researchers (Singh *et al.*, 2013; Adnan *et al.*, 2013; Bind *et al.*, 2014; Shekhawat *et al.* 2014; Singh *et al.*, 2016).

Moreover, such narrow differences between the phenotypic and genotypic variances are expected in augmented designs. Because of narrow difference between the variances, the values of heritability were generally higher leading to higher GA estimates. In the present study, the PCV ranged from 2.56 % (oil content) to 34.21% (glucosinolate) while GCV for these traits were 2.04% and 33.79%, respectively. These are very similar to the results obtained earlier in oilseed Brassicas (Singh *et al.*, 2013; Adnan *et al.*, 2013; Bind *et al.*, 2014; Shekhawat *et al.* 2014). In addition, harvest index ranged from 16.4-40.1% while seed yield/plant ranged from 10.1 to 32.9 g. On the other hand, oil content ranged from 38.6-43.2%.

For an effective selection programme, variability present in a population and the extent to which it is heritable are the important factors. Thus, genetic advance is another parameter which plays a crucial role in assessing the expected improvement in a character (Nadarajan and Gunasekaran, 2005). Here, we reported more than 50% heritability estimates for all the characters while genetic advance was observed in range of 3.1- 68.2. Such high genetic advance along with higher heritability is very essential and indicated that selection would be highly effective in the present material for these characters (Bisne *et al.*, 2009; Pandey and Singh, 2002; Mahla *et al.*, 2003).

Correlation coefficients for different parameters were also determined and fruiting zone length was showing highest and positive significant correlation with main shoot length (0.645**). Seed yield/plant was found to be significantly and positively correlated with plant height (0.207*), number of seeds/siliqua (0.169*), biological yield/plant (0.615**) and 1000-

Table 2: RILs selected on the basis of seed yield per plant and their different attributes

RIL No.	Seed yield /plant (g)	Plant height (cm)	Main shoot length (cm)	Fruiting zone length (cm)	Silique length (cm)	Seeds /silique	Biological yield (g)	1000 seed weight (g)	Harvest index (%)	Oil content (%)	Glucose-sinigrin-lactones	Fibre	Phenol
4	34.6	204.0	82.1	92.5	5.0	14.1	68.1	5.9	49.6	39.3	12.5	2.78	2.39
40	33.6	199.0	102.1	105.0	5.2	14.7	62.8	5.1	52.2	39.9	40.1	5.76	2.15
27	32.6	204.1	86.6	96.8	5.5	16.3	85.8	5.9	37.4	41.4	27.4	5.73	2.73
162	31.5	191.4	83.6	88.5	5.0	11.8	93.7	6.7	33.4	41.2	38.9	10.01	2.11
113	31.1	186.7	90.7	90.8	4.9	13.2	76.3	6.4	41.2	42.8	53.7	6.12	2.67
112	31.1	177.9	78.7	85.5	5.6	14.2	97.3	5.3	32.8	42.1	54.7	9.44	2.06
77	30.9	177.8	73.8	81.5	3.4	11.9	54.5	4.2	56.2	39.6	28.7	6.69	2.16
66	30.9	188.8	80.1	89.1	4.6	11.9	87.5	4.6	35.6	42.3	49.4	9.89	2.22
140	30.2	184.1	82.7	88.1	4.0	14.4	89.3	6.7	34.5	41.0	24.5	5.96	2.31
25	29.8	206.1	85.5	95.5	6.2	16.9	95.8	7.3	30.7	40.6	79.6	7.24	2.37
89	29.6	198.9	86.7	91.8	4.5	11.9	69.5	5.2	42.6	41.2	66.4	10.20	1.81
151	28.9	202.6	92.1	86.0	4.1	12.2	99.6	4.7	28.4	40.7	73.4	8.82	1.96
116	28.4	202.8	97.7	102.6	4.0	11.8	95.3	5.5	30.6	42.5	74.6	10.31	2.10
114	27.9	189.1	86.6	95.3	3.3	10.6	87.3	6.5	32.6	40.1	53.1	5.31	2.28
7	27.6	199.6	84.4	93.5	4.7	11.9	69.8	5.0	38.8	40.5	16.1	4.01	2.52
137	27.2	189.7	86.7	99.6	4.2	10.8	90.3	5.5	30.9	43.0	47.8	9.92	2.17
62	27.0	168.6	88.0	91.2	4.7	11.1	69.5	4.1	38.8	41.4	63.7	9.82	1.99
133	26.0	185.5	75.3	84.2	3.8	10.6	74.3	4.9	35.5	40.9	50.1	6.08	2.02
28	26.0	195.2	82.7	93.4	6.1	14.9	73.8	5.9	34.5	41.9	97.8	8.73	2.24
23	26.0	192.5	84.9	94.3	5.8	14.3	59.8	5.4	42.3	40.2	26.9	5.42	2.40
20	25.9	189.6	80.6	91.3	5.0	13.3	95.8	5.1	26.7	40.6	68.1	10.47	1.80
88	25.8	193.8	83.7	94.6	5.3	13.3	83.5	5.2	31.2	41.0	101.4	9.39	1.81
84	25.8	177.9	65.5	67.0	4.3	10.5	78.5	5.7	33.1	40.6	30.9	6.15	1.85
80	25.8	188.2	79.8	87.9	4.4	11.9	56.5	6.7	45.3	39.6	58.5	5.80	2.42
10	25.6	196.7	87.7	95.2	4.8	14.9	96.8	5.4	26.1	40.5	35.2	5.79	2.52
CD (P<0.05%)	5.5	12.8	10.6	8.1	1.1	2.6	11.9	1.3	11.2	1.6	8.1	2.28	0.42
CV (%)	11.5	2.6	4.9	3.5	9.3	7.9	7.2	9.1	14.7	1.6	5.3	9.97	7.90

seed weight (0.160*). Similar kinds of reports were also observed by many workers (Ali *et al.*, 2013; Islam *et al.*, 2016; Haider *et al.*, 2016).

The associations between different yield attributing traits revealed the mutual relationship between two or more characters. Main shoot length was found to be significantly associated with plant height (0.407**), fruiting zone length (0.645**) and biological yield (0.168*). Similarly, significant positive correlation of biological yield was observed with plant height (0.277**), main shoot length (0.168*), fruiting zone length (0.187*) and seeds/ siliqua (0.234**); number of seeds/siliqua with siliqua length (0.333**) and biological yield (0.234**); oil content with fibre content (0.302**). Thus, selection for the higher values of such parameters will be useful for increasing the seed yield.

In this study, 25 promising RILs were identified on the basis of seed yield/plant. The details are shown in Table 2. Seed yield/plant among the selected lines varied from 25.63 g to 34.63 g; plant height from 168.61-206.14 cm; 1000-seed weight from 4.11- 7.29 g; oil content from 39.32 to 42.99 % and harvest index from 26.13 % to 56.17 %. Further, these lines can be used as parents for breeding high yielding Indian mustard varieties and multi-location testing for varietal release. Since, the parental cultivars Rohini and PBR-97 used for developing RILs are known to vary considerably for drought tolerance in addition to morphological and physiological aspects, these RILs may also be used for identification of superior drought tolerant lines and also for the genetic dissection of drought related traits through mapping and analysis of major genes and/or QTLs.

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