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Assessment of genetic variation among drought tolerant recombinant inbred lines (RILs) of Indian mustard (*Brassica juncea* L.)

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Abstract

The present study reveals the genetic variation among 225 Recombinant Inbred Lines (RILs) derived from the cross Rohini X PBR-97 which were evaluated under normal and water stress conditions. Significant genetic variation was observed for plant height, main shoot length, fruiting zone length, siliqua length, biological yield/plant, harvest index, seed yield/plant, fibre, phenol, days to flowering under normal conditions while plant height, main shoot length, fruiting zone length, siliqua length, seed/siliqua, harvest index, phenol, days to flowering were found to be significant under drought conditions. Heritability estimates were high (>70%) for main shoot length, siliqua length, seed yield/plant, and phenol content. Seed yield per plant was significantly and positively correlated with plant height (0.37**), main shoot length (0.19*), fruiting zone length (0.16*), siliqua length (0.19*), seeds/siliquae (0.16*), biological yield per plant (0.45**) and harvest index (0.61**) and negatively correlated with oil content (-0.13), fibre (-0.28**). Further, 25 promising RILs were identified based on seed yield/plant which can further be used in brassica improvement breeding programmes to develop drought tolerant brassica varieties.

Key words: *Brassica juncea, biochemical, correlation, drought stress, morphological, water deficit*

Introduction

Rapeseed-mustard group of crops is very important rabi oilseed and occupies a prime position due to its high oil content and ability to withstand fluctuating environmental conditions. Of these, Indian mustard [*Brassica juncea* (L.) Czern & Coss.], popularly known as 'raya' or 'laha' is a major rabi oilseed crop in India (Singh *et al*., 2016). Although a momentous success has been achieved in improving the production and productivity of Indian mustard during the last two decades, its domestic edible oil demand has not been met out fully. Thus, further improvement in the productivity of Indian mustard is an important breeding objective. It possesses enormous genetic variability for seed and drought tolerance characteristics which further leads to the genetic diversity. The genetic variability is the basic requirement for improving crops which further leads to progress in crop breeding. Thus, there are good chances to improve this crop by evaluating and utilizing genetic variation (Ullah *et al*., 2016). The information on the nature and magnitude of variability for different morphological and biochemical parameters is necessary to judge the potentiality of particular genotype.

In India, it is mostly grown on light textured soils as a rainfed crop and therefore later it results into drought stress during its reproductive stage (Kumar and Singh, 1998). Thus, development for drought resistance becomes the main objective for this crop (Singh and Choudhary, 2003). Drought is most serious problem for global agriculture, approximately affecting 40% of the world's land area (UN Environment Management Group 2011). The changing global climate scenario is definitely going to effect the crop growth and productivity by raising the temperature which will ultimately lead to severe drought stress causing alternations in metabolism and gene expression of crop plants (Mir *et al*., 2012). Thus, understanding plant responses to drought is the need of the hour so that stress tolerant crops can be developed to increase area and yield of the oilseed crops under such adverse conditions (Zhao *et al*., 2008).

Yield improvement in Indian mustard is primarily dependent on nature and magnitude of genetic variability for component characters. Loss of yield is the main concern of plant breeders and hence they emphasize on yield per-formance under stress conditions (Nouri *et al*., 2011; Meena *et al*., 2015). Thus, identification and development of genotypes possessing high yield and yield components is required to meet the increasing demands of growing population (Dilip *et al*., 2016). Thus, keeping all this in view, this study was designed to evaluate drought tolerant recombinant inbred lines (RILs) obtained from a cross between two diverse parents and determine 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 drought tolerant RILs based on yield and yield components which may further be used in mustard breeding programs.

Materials and Methods

Experimental design and genetic materials

Two hundred and twenty five RILs were developed by single-seed descent approach from commercially high yielding varieties Rohini (susceptible parent) and PBR97 (tolerant parent). These were evaluated along with checks under irrigated and water deficit conditions during two years in augmented block design, where each block had 10 RILs along with four checks (RB-50, RH-819, PBR-97 and Rohini). The crop was irrigated according to the standard practices under normal conditions while drought was stimulated by withholding water throughout the growing season. Moisture profiling of rainfed fields at sowing time was 7.5% (0-15 cm depth), 8.3 % (15-30 cm depth) and 7.8% (30-45 cm depth). After 45 days, moisture content reduced to 7.2 % (0-15 depth), 8.0% (15-30 cm depth) and 7.0% (30-45 cm depth) while after 120 days it was 7.0%, 7.7 % and 6.6 %, respectively. During irrigated conditions moisture content was 9.0% (0-15 cm depth), 10.5% (15-30 cm depth) and 8.4% (30-45 cm depth) at sowing time whereas it was 9.6 % (0-15 depth), 12.4% (15-30 cm depth) and 8.7% (30-45 cm depth) after 45 days and 9.1%, 11.5% and 8.0% after 120 days respectively. It is worthwhile to mention that crop received 574.4 mm rainfall during monsoon. Approx. 41.7 mm rain was recorded from October-February and 57.0 mm in March during 2014-2015.

Morphological and biochemical parameters

Five plants were randomly selected from each RIL and plant height (PH), main shoot length (MSL), fruiting zone length (FZL), siliqua length (SL), seeds/ siliqua, biological yield/plant (BY/plant), 1000-seed weight, harvest index (HI), seed yield/plant (SY/ plant), oil content (OC), glucosinolate (GSL), fiber and phenol contents were measured in both conditions. Harvest index (HI) was calculated by the formula: HI = grain yield/total above ground biomass × 100. Oil, glucosinolates, fiber and phenol contents were estimated using FT-NIR spectroscopy.

Statistical analysis

Analysis of variance (ANOVA) was performed as per the method suggested 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

Genetic variation

The RIL population was evaluated in augmented block design, where each block had 10 RILs along with two checks and parents. The results strongly suggested that selection may be expected for seed yield and related component traits in these RILs. A significant amount of variability was revealed among the RILs through the analysis of variance for seed yield and other traits indicating that RILs were differing for genes controlling yield and other traits (Golabdi *et al.*, 2006; Gholipouri *et al.*, 2009).

The blocks were observed to be nearly homogeneous as they exhibited non-significant variability for all the traits under both conditions. However, the check genotypes considered for the study differed significantly for siliqua length, seeds/ siliquae, 1000-seed weight and seed yield/plant under normal conditions while almost all characters were found to be significant except PH, MSL, FZL, SL

Table 1(a): Mean sum of squares for different morphological traits in RILs for normal condition.

Source	d.f.	Plant height (cm)	Main shoot length (cm)	Fruiting zone length (cm)	Siliquae length (cm)	Seeds/ siliquae	Biological Yield/ plant	1000 seed wt (g)	Harvest index	Seed yield/ plant (g)	Oil content (%)	Fiber	Phenol	DAF
Block	4	237.4	12.2	8..2	2.87	1.2	120.1*	0.1	30.3	18.4**	2.6	0.4	0.01	1.1
Treatment	228	238.6*	70.8**	69.9*	22.85**	1.5*	92.2*	0.0*	33.8*	14.3**	1.1	2.1**	0.04*	8.3*
Control	3	129.0	45.6	29.2	16.07**	11.4**	12.1	4.1**	21.7	14.6*	2.6	0.7	0.01	4.2
Progeny	224	230.3*	63.6**	59.3*	22.98**	1.3	92.1*	0.5	33.7*	13.5**	0.8	2.1**	0.04*	8.1*
Control v/sprogeny	1	2422.9**	1756.6**	2549.6**	13.72*	18.8**	199.2*	17.8**	98.0**	175.1**	64.1*	3.6*	0.31**	80.7**
Error	12	85.2	18.3	25.3	2.89	0.6	36.0	0.2	11.8	3.1	9.1	0.6	0.01	3.1

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

Table 1(b): Mean sum of squares for different morphological traits in RILs for drought condition

Source	d.f.	Plant height (cm)	Main shoot length (cm)	Fruiting zone length (cm)	Siliquae length (cm)	Seeds/ siliquae	Biological Yield/ plant	1000 seed wt (g)	Harvest index	Seed yield/ plant (g)	Oil content (%)	Fiber	Phenol	DAF
Block	4	63.8	20.3	17.9	4.5	0.4	311.1**	0.2	16.5	25.7*	0.7	0.1	0.01	8.8
Treatment	228	193.3**	30.5*	38.0**	20.8**	1.0**	107.5*	0.5	25.8*	13.4*	1.1	0.6	0.02**	10.9*
Control	3	93.6	6.3	14.3	7.6	3.4**	182.5*	2.7**	49.4**	21.5*	18.5*	1.7*	0.2**	10.8
Progeny	224	183.0**	30.8**	38.3**	20.7**	1.0**	104.1	0.4	25.5*	12.7	0.5	0.5	0.01*	9.8*
Control v/sprogeny	1	2790.2**	51.6*	43.4*	83.9**	0.01	644.2**	20.9**	32.1	145.1**	77.5**	26.6**	0.6**	271.5**
Error	12	62.6	10.9	7.9	2.6	0.2	47.6	0.4	10.0	5.8	4.8	0.4	0.01	4.3

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

and days to flowering under drought conditions (Table 1a,b). Similarly, treatments were also significant for all the characters except oil content in well watered conditions while 1000-seed weight, oil content and fibre were found to be non-significant under stress conditions (Table 1a,b). Under irrigated conditions RILs exhibited significant differences for PH, MSL, FZL, SL, BY/plant, HI, SY/plant, fibre, phenol, days to flowering while 1000-seed weight, seeds/siliqua and oil content were found to be non-significant. But, under drought conditions RILs showed non-significant differences for BY/plant, 1000-seed weight, SY/plant, oil and fibre content. The check versus RILs interactions showed highly significant differences for all the characters in both conditions except seed per siliquae and HI under water deficit conditions indicating that checks as a group differed significantly from RILs as a group (Table 1a, b).

A considerable reduction in most of the traits under study was observed as a result of water stress. It has been reported that the performance of most agro-morphological characteristics, such as PH, FZL, SL, BY/plant, 1000 seed weight etc. was reduced under drought conditions and these were the most important characters contributing to seed yield (Chauhan *et al.,* 2007). The main effect was on yield under stress conditions. Water deficit resulted in yield reduction in all the studied RILs compared with normal condition. The mean SY/plant was 17.6 g under normal conditions while it was 13.8g under drought conditions. Thus, on average, yield was reduced under water deficit by 21.7%. Seed yield/plant of RILs under stress condition varied from 7.3 g corresponding to RIL 213 to 20.9 g corresponding to RIL25 while under non-stress condition it ranged from 9.5g (correspond to RIL 60) to 21.3 g (correspond to RIL 25) (Table 2). A total of 25 best RILs were identified where seed yield/plant among the selected lines varied from 9.6 g (RIL126) to 20.9g (RIL25) under drought conditions whereas it was varying from11.3 g (RIL104) to 21.3 g (RIL25) under irrigated conditions and the reduction in yield for these RILs ranged from 2.2 to 6.3% (Table 3). For these RILs, other components were also recorded under both conditions where PH ranged from 145.7-195.9 cm

Table 2: Overall mean value of RILs, their range, genotypic and phenotypic coefficient of variation, heritability in broad sense and genetic advance

Characters	Mean		Range		GCV		PCV		h^2		GA	
	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal
Plant height (cm)	173.0	187.7	143.3-205.1	142.5-220.2	6.3	6.4	7.8	8.1	65.8	63.0	10.6	10.5
Main shoot length (cm)	56.6	66.6	43.0-77.6	42.2-90.2	7.9	10.1	9.8	12.0	64.4	71.2	13.0	17.6
Fruiting zone length (cm)	61.9	69.7	46.6-88.9	41.6-90.9	8.9	8.4	10.0	11.1	79.3	57.5	16.3	13.1
Siliquae length (cm)	42.9	44.1	33.4-61.7	32.7-73.9	9.9	10.2	10.6	10.9	87.4	87.4	19.1	19.6
Seeds / siliquae	11.9	11.7	9.0-15.6	8.4-15.4	7.3	6.7	8.4	9.6	76.1	48.8	13.1	9.6
Biological yield / plant (g)	52.6	61.1	31.4-84.8	30.6-92.8	14.3	12.3	19.4	15.8	54.3	61.1	21.7	19.8
1000 seed weight (g)	4.2	4.2	2.0-5.9	2.0-6.1	12.4	11.4	14.2	16.3	42.8	48.9	20.8	16.4
Harvest index (%)	26.3	29.1	14.6-38.7	15.4-38.8	14.9	16.1	19.2	19.9	60.6	65.0	23.9	26.7
Seed yield per plant (g)	13.8	17.6	7.3-20.9	9.5-21.3	19.0	18.3	25.8	20.9	54.0	77.2	28.7	33.2
Oil content (%)	39.2	39.6	33.2-41.3	32.8-41.7	*	*	*	*	*	*	*	*
Fiber	10.1	9.9	8.0-11.9	5.9-13.0	3.8	12.1	7.1	14.4	29.4	70.4	4.3	20.9
Phenol	1.4	1.5	1.0-1.9	1.0-2.4	3.8	11.5	6.9	13.3	30.0	75.0	4.3	20.6
Days to flowering	46.7	43.8	40.0-57.0	40.0-52.0	5.0	5.1	6.7	6.5	55.9	61.0	7.7	8.1

* Genetic parameters could not be calculated because of non-significant progeny mean squares

Table 3: Characterization of promising RILs for some important morphological and biochemical traits

RIL No.	Seed yield per plant (g)		Plant height (cm)		Main shoot length (cm)		Fruiting zone length (cm)		Siliquae length(cm)		1000 seed weight(g)		Oil content (%)	
	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal	Rainfed	Normal
25	20.9	21.3	194.6	180.6	53.2	72.0	59.3	75.3	4.3	4.4	5.7	5.6	39.7	40.2
43	20.8	21.3	188.3	178.5	53.5	76.3	59.0	79.6	3.4	4.3	6.0	4.0	37.9	38.6
186	20.4	21.1	194.6	192.3	56.8	48.8	62.6	54.3	4.5	4.5	4.3	2.6	39.1	39.8
193	20.3	21.1	195.9	178.0	55.5	61.3	60.5	61.5	4.4	4.4	3.3	3.2	40.2	40.3
75	20.6	21.0	180.0	171.9	55.2	65.7	59.9	68.8	4.3	4.6	5.1	3.5	38.4	38.2
84	16.7	17.4	165.0	196.4	61.2	65.1	68.0	77.0	4.4	4.4	5.0	5.0	38.0	40.8
26	18.9	19.7	185.3	178.2	55.6	70.1	60.7	74.5	3.4	4.4	5.0	4.6	39.9	40.0
188	19.3	20.1	195.7	188.0	59.6	57.1	53.3	62.5	4.5	4.5	3.9	4.5	39.8	39.8
27	18.8	19.7	180.6	167.1	57.2	50.6	62.2	54.1	4.2	4.4	4.5	4.2	40.3	39.9
2	11.1	11.6	147.3	179.6	56.5	57.6	61.9	64.4	4.4	4.5	3.3	3.7	39.5	40.3
35	18.7	19.6	172.2	175.7	54.2	72.2	59.0	72.8	4.3	3.2	5.2	5.4	40.7	40.4
89	18.6	19.5	181.3	214.2	63.0	66.6	63.7	68.0	4.5	4.4	4.2	4.4	39.1	39.8
30	11.0	11.5	145.7	163.3	46.5	56.1	51.3	60.0	3.6	4.3	2.8	4.0	38.8	40.4
202	18.2	19.2	171.8	181.5	57.9	57.6	66.0	59.1	4.4	3.6	4.1	4.9	39.0	40.2
11	11.0	11.5	153.4	193.1	58.8	70.5	64.4	73.1	3.6	4.3	4.5	4.9	38.4	39.0
214	18.1	19.1	166.1	199.4	48.3	66.5	53.0	69.2	4.3	4.4	3.9	6.1	39.4	40.2
218	18.1	19.1	163.0	183.0	57.0	66.8	65.9	68.1	4.5	3.5	4.0	4.7	39.2	40.3
79	18.7	19.8	180.5	194.3	56.9	68.7	62.1	75.8	4.4	4.6	3.9	5.0	40.2	40.1
32	15.8	16.8	149.8	175.1	49.1	78.2	54.3	82.9	4.3	4.3	4.9	3.9	38.8	39.6
34	15.8	16.7	154.0	200.5	49.0	70.3	54.8	69.5	3.4	4.9	3.3	5.9	38.7	39.9
9	12.9	13.7	183.0	186.5	52.3	63.1	54.7	65.5	3.3	4.5	4.4	2.3	39.7	40.1
126	9.6	10.2	187.4	155.6	51.7	52.5	56.5	53.0	4.0	4.4	3.6	3.9	40.2	39.3
12	12.9	13.7	169.6	181.3	57.3	69.1	61.4	71.7	4.1	4.2	4.7	4.6	37.5	39.7
74	12.7	13.6	183.0	191.9	61.4	62.2	67.0	66.9	4.3	4.5	4.8	4.0	37.1	39.5
104	10.6	11.3	162.3	187.9	53.0	69.9	59.0	70.2	4.4	4.60	4.2	3.4	40.2	40.3
CD (5%)	4.5	6.2	23.8	20.4	11.0	8.5	12.9	7.3	4.4	4.2	1.3	1.5	7.8	5.6
CV	10.0	17.5	4.9	4.6	6.4	5.8	7.2	4.5	3.9	3.8	11.7	14.0	7.6	5.6

and 155.6-214.2 cm;1000-seed weight varied from 2.8-5.9g and 2.3-6.1g; oil content from 37.08-40.67% and 38.2-4.8% under stress and non-stress conditions respectively (Table 3). Other important morphological, biochemical and physiological parameters were also recorded for these promising RILs as shown in Table 2. The estimates of mean for some other components such as PH, MSL, 1000 seed wt., HI and OC were 187.7 cm, 66.6 cm, 4.2 g, 29.1%, 39.6% and 173.0 cm, 56.6 cm, 4.2 g, 26.3%, 39.2% in normal and drought conditions respectively as indicated in Table 2. The ranges of all the components among all 225RILs were also estimated as shown in Table 2.

Character association

Seed yield is a complex trait that encompasses the interactions between different yield contributing traits. Therefore, selection should be based keeping in view the desirable traits and their correlation with seed yield (Grafius, 1964). The correlation coefficients for seed yield and morphological characters and their interrelationships were estimated in all possible combinations. In our study, significant and positive correlations of yield with these characters were found. The significant coefficient of correlation between seed yield and morphological characters ranged from 0.152* to 0.924**. It was concluded that seed yield per plant was significantly and positively correlated with plant height (0.366**), MSL (0.185*), FZL (0.161*), SL(0.188*), seeds/siliquae (0.179*), BY/plant (0.683**) and HI (0.607*) and significantly and negatively correlated with OC (-0.151*) and fibre (-0.283**) while negative and non-significant correlation of seed yield per plant was observed with days of flowering (-0.138), seed weight (-0.086) and phenol (-0.067). Similarly, BY/plant showed positive and significant correlations with PH (0.398**), MSL (0.291*), FZL (0.272**), seed/siliqua (0.400**) and HI (0.349**). PH was positively correlated with MSL (0.395**), FZL (0.576**, seed/siliquae (0.304**) and HI (0.212**). FZL was significantly positively associated with seed/siliqua (0.186**), BY/plant (0.272**) seed wt. (0.195*) and OC (0.176*) (Table 4). Similar, results have also been reported earlier by many workers (Singh *et al.*, 2013; Meena *et al.*, 2015; Dilip *et al.*, 2016). The associations between different yield attributing traits revealed the mutual relationship between two or more characters. Significant positive correlations of BY/plant with PH, MSL, FZL, HI were observed. Hence, selection for the higher values of these traits would be desirable for increasing the seed yield.

In the present study, we observed a narrow difference between the phenotypic and genotypic variances as expected in augmented designs in both conditions. Because of narrow difference between the variances, the values of heritability were generally higher leading to higher GA estimates (Table 2). Estimates of GCV and PCV for different characters considered for the study revealed that the phenotypic variances were significantly higher than the genotypic variances reflecting the role of environmental factors on character expression as reported earlier in oilseed Brassicas (Keer and Jakhar, 2012; Singh *et al.*, 2013; Bind *et al.*, 2014; Ullah *et al.*, 2016). For an Effective selection programme, variability present in a population and the extent to which it is heritable are the important factors. The genetic variability coupled with heritability helps in the identification of the characters that offer scope for improvement through selection and predict the expected gain (Meena *et al.*, 2015). In well watered conditions, PCV varied from 6.5 (Days to flowering) to 20.9 (SY/plant) whereas it was 6.7 (days to flowering) to 25.8 (SY/plant) for water deficit conditions. Similarly, the maximum GCV was observed for SY/plant (18.3%) followed by HI (16.1%) and BY/plant (12.3%) in non-stress conditions while it varied from 3.8% (Phenol) to 19.0% (SY/plant) in stress conditions (Table 2). Besides, genetic advance is another parameter which plays a crucial role in assessing the expected improvement in a character (Nadarajan and Gunasekaran, 2005). It is reported that high heritability assisted with high genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone (Bisne *et al.*, 2009; Verma *et al.*, 2016). In the present study, the heritability estimates were more than 50% for almost all the characters. High heritability values in conjunction with high genetic advance were observed for seed yield/plant (77.2%, 33.2%) in normal conditions whereas it was (79.3%, 16.3%)

for FZL in drought conditions (Table 2). Similar levels of heritability and genetic advance for different characters have already been reported earlier in Brassicas (Pandey and Singh, 2002; Mahla *et al.*, 2003; Ullah *et al*., 2016). This indicated that selection would be highly effective in the present material for these characters.

Conclusion

The plant performance is reduced significantly under water stress environment. Seed yield per plant under drought conditions can be improved by improving siliqua length, no. of siliqua per plant, 1000-seed weight etc. The 25 promising lines identified can be further considered for multi-location testing for varietal release or may be used as parents in the breeding programmes aiming at the development of drought tolerant brassica varieties. Thus, the information derived from the study will be helpful in breeding Brassicas for drought tolerance and early selection of genotypes with the desirable traits to be used in the breeding programmes which will ultimately further enhance breeding efficiency.

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