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ESTIMATION OF GENETIC VARIABILITY AMONG DROUGHT TOLERANT RIL POPULATION OF INDIAN MUSTARD (*BRASSICA JUNCEA* L.) DERIVED FROM ROHINI X RH819

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ABSTRACT

This investigation was aimed to study the patterns of genetic variation among 215 RILs derived from cultivars Rohini (drought susceptible) and RH819 (drought tolerant). The RILs were characterized and evaluated under stress as well as irrigated conditions along with parents and checks in augmented block design. Observations were recorded on 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 glucosinolates, fiber and phenol content. Analysis of variance indicated that significant differences were found among RILs for all traits except oil content under irrigated conditions while under stress conditions they were non-significant for seed/siliquae and BY/plant. The range of seed yield/plant, harvest index, 1000-seed weight was 6.49-20.52 g, 11.83-48.86%, 2.34-5.74g and 7.93-32.26g, 10.85-60.34%, 2.36-5.59g under stress and irrigated conditions respectively. Genetic components such as GCV, PCV and high heritability estimates (>50) were also calculated. Seed yield/plant was significantly and positively correlated with plant height (0.243**), fruiting zone length (0.203*), seeds/siliquae (0.343**), BY/per plant (0.444**) and harvest index (0.790**) and negative with oil content (-0.224**) and fibre (-0.213**). Out of these 215 RILs, 25 were identified as most promising lines which may further be used for multi-location trials and in various brassica improvement programs to develop drought tolerant varieties.

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INTRODUCTION

Brassicas are very important among the oilseed crops in our country and rank third after soybean and palm. Among Brassicas, Indian mustard (B. juncea) occupies approx. 80% of (Supriya et al., 2014). However, its growth and seed yield production have greatly decreased during last two decades and thus it has become a major challenge for maintaining world food supplies for the growing population. For the synthesis of genetically superior rapeseed, variety/hybrids possessing harmonious combination of desirable parameters and increased adaptability to a wide range of climatic conditions is prerequisite. The success of any crop improvement program depends upon the presence of substantial amount of genetic variability and heritability (Sidra et al., 2014; Laila et al., 2014; Shah et al., 2015). Thus, the measurement of genetic variation is important and understanding the extent and nature of this variation is essential for enhancing the breeding efforts

ICAR-Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur-321303, Rajasthan, India. such as selection and hybridization to improve breeding programs effectively. Brassicas possesses enormous genetic variability and several researches have worked on the extent and nature of genetic variation for Brassicass (Iqbal et al., 2014; Shafari et al., 2015; Verma et al., 2016). Heritability and genetic advance studies were also done by many researchers (Bind et al., 2014; Synrem et al., 2014; Ullah et al., 2016). Genetic variability is a measure of the tendency of individual genotypes in a population to vary from one another in a population. It is important for biodiversity as without variability it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction. Indian mustard is mainly grown as a rainfed crop and thus later it results into drought stress during its reproductive stage (Kumar and Singh, 1998). Thus, production and productivity of the crop is adversely affected. In the context of changing global climatic scenario, breeders need to develop drought tolerant varieties to mitigate climate change which may further be helpful to increase area and yield of the oilseed crops under such adverse conditions (Zhao et al., 2008). Morphological traits have been used to assess the genetic variation and relationships among populations of

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different oilseed species (Ullah et al., 2015; Singh et al., 2016). Seed yield as in other crops, is a complex character which is dependent on a number of other characters. Thus, to increase yield, the evaluation of direct and indirect effects of yield and its components provide the basis for successful breeding program (Gupta et al., 2015). In this sense, increase of seed yield can be more effectively achieved on the basis of performance and selection for closely associated traits. However, these traits should be highly heritable as the progress through selection depends on heritability, selection intensity and genetic advance of the trait (Meena et al., 2014). Hence, in the context of yield improvement, it is necessary to analyze the nature and extent of variation present within a set of breeding material and the interrelationships between yield and its components. Keeping all this in view, the present study was undertaken to (1) evaluate the RILs developed from a recombinant inbred line (RIL) population through cross between two high yielding commercial varieties Rohini and RH-819, and (2) to determine the extent of genetic variability, heritability, genetic advance and interrelationship of yield and yield components and identify superior drought tolerant lines.

MATERIALS AND METHODS

Plant material and assessment of RIL population

Commercially released Indian mustard cultivars Rohini and RH-819 were used to develop a RIL population of 215 individuals by single-seed-descent approach. These were evaluated along with four checks (RB-50, RH-819, PBR-97 and Rohini) during the rabi season of 2014-15 under rainfed and irrigated conditions in augmented block design. Drought was simulated by withholding water throughout the growing season, whereas, under normal conditions the crop was irrigated according to the standard practices. During irrigated conditions moisture content was 8.0% (0-15 cm depth), 9.5% (15-30 cm depth) and 7.7% (30-45 cm depth) at sowing time whereas it was 8.5 % (0-15 depth), 10.4% (15-30 cm depth) and 8.3% (30-45 cm depth) after 45 days and 8.2%, 9.9% and 8.0% after 120 days respectively. Moisture profiling of rainfed fields at sowing time was 6.8% (0-15 cm depth), 7.8% (15-30 cm depth) and 6.9% (30-45 cm depth). After 45 days, moisture content reduced to 6.4% (0-15 depth), 7.1% (15-30 cm depth) and 6.2% (30-45 cm depth) while after 120 days it was 6.1%, 6.8% and 6.0%, 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 Parameters

Plant height (PH), main shoot length (MSL), siliqua length (SL), fruiting zone length (FZL), seeds/siliqua, biological yield/plant (BY/plant), 1000-seed weight, harvest index (HI), seed yield/plant (SY/plant) were recorded for each RIL by selecting five plants randomly in both conditions. Harvest index (HI) was calculated by the formula: HI = grain yield/total above ground biomass \times 100. Oil, glucosinolates, fiber and phenol contents were estimated using FT-NIR spectroscopy.

Statistical analysis

Analysis of variance was performed as per the method suggested by Federer (1956). Genotypic and phenotypic

coefficient of variation, heritability, genetic advance and correlation coefficients were calculated using the standard statistical procedures.

RESULTS AND DISCUSSION

Morphological characterization

In the present study, RIL population was evaluated in augmented block design for yield and yield related components. The analysis of variance showed highly significant differences among RILs for these parameters indicating that RILs were differing for genes controlling yield and other traits (Golabdi *et al.*, 2006; Gholipouri *et al.*, 2009). Treatments were found to be significant for all the traits except days to flowering and seed/siliquae under irrigated and stress conditions, respectively. Similarly, RILs exhibited significant differences for PH, MSL, FZL, seed/siliqua, SL, BY/plant, HI, 1000-seed wt., SY/plant, fibre, phenol, RWC, days to flowering and SPAD while non-significant for oil content in well watered conditions (Table 1a, b).

On the other hand, RILs showed significant differences for all characters except seed/siliqua, and BY/plant under stress conditions. Under drought conditions, check versus RILs exhibited highly significant differences for PH, SL, 1000-seed wt., SY/plant, fibre, phenol, oil content, days to flowering and SPAD while they were significant for PH, SL, BY/plant, 1000seed weight, SY/plant, fibre, phenol, oil content, days to flowering in irrigated conditions indicating that checks as a group differed significantly from RILs as a group (Table 1a, b). Various agro-morphological characteristics such as PH, FZL, SL, BY/plant, 1000-seed weight are considered as the most important characters contributing to seed yield and it has already been reported that the performance of most of these characters was reduced under drought conditions (Chauhan et al., 2007; Tripathi et al., 2015). Similarly, in this study we observed reduction in most of the characters under drought conditions as indicated in Table 2 and the major effect was on vield under stress conditions. The mean SY/plant was 15.48 g under irrigated conditions whereas it was 10.38 g under stress conditions. Thus, on average, yield was reduced under water deficit by 32.94%. Seed yield/plant of RILs under non-stress ranged from 7.93 to 32.26g while it was from 6.49-20.52 g under stress conditions (Table 2).

The estimates of mean for some other components such as PH, FZL, 1000-seed wt., BY/plant and OC were 199.18 cm and 169.09 cm; 80.38 cm and 63.38cm; 4.21g and 4.35g; 60.01g and 48.68g; 39.41% and 39.33% under irrigated and drought conditions, respectively as indicated in Table 2. Similarly, mean and range for other important morphological and biochemical parameters were also recorded among these RILs as shown in Table 2. Among the 25 best selected RILs, seed yield/plant ranged from 7.25g (RIL180) to 19.22g (RIL110) under stress condition while it ranged from 7.93-20.59g under irrigated conditions and the yield reduction was 2.20 to 6.26% (Table 3). Various other yield components and biochemical parameters were also recorded for these RILs under both conditions where PH ranged from 153.82-194.16 cm and 154.00-214.16 cm; siliqua length from 3.4-5.1cm and 3.4-5.4 cm; oil content from 38.25-40.15% and 37.41-40.63% under drought and normal conditions respectively. The details are shown in Table 3.

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

Source	d.f.	Plant height	Main shoot	Fruiting zone	Siliquae	Seeds/	Biological	1000 seed	Harvest	Seed yield	Oil content	Fiber	Phenol	DAF
		(cm)	length (cm)	length (cm)	length (cm)	siliquae	Yield / plant	wt. (g)	index	/ plant (g)	(%)			
Block	4	34.65	20.72	105.45*	0.67	10.32**	183.06**	0.51**	11.71	3.01	0.11	0.59	0.01	2.17
Treatment	218	235.10*	95.35*	90.20**	28.48**	3.53**	189.12**	0.26**	63.42**	22.82**	1.40*	1.12**	0.04**	33.84
Control	3	189.38	19.27	24.11	19.55	9.10**	50.33	0.84**	22.63	11.10	3.04**	1.77**	0.005	0.40
Progeny	214	231.07*	96.64**	91.50**	24.85**	3.45**	182.68**	0.24*	64.23**	22.87**	0.87	0.88**	0.02**	33.15**
Control v/s progeny	1	1234.54**	54.95	10.43	832.01**	3.67	663.32**	2.66**	13.43	47.81*	110.28**	51.17**	3.28**	281.30**
Error	12	89.38	41.47	32.05	8.90	1.09	42.45	0.09	22.85	7.89	0.54	0.39	0.009	2.77

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

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

Source	d.f.	Plant height	Main shoot	Fruiting zone	Siliquae	Seeds/	Biological Vield / plant	1000 seed $ut_{(q)}$	Harvest	Seed yield	Oil content	Fiber	Phenol	DAF
		(CIII)	icingui (cini)	icingui (ciii)	icingtii (ciii)	sinquae	Tielu / plain	wt. (g)	mucx	/ plant (g)	(70)			
Block	4	60.30*	9.72	16.57**	4.62	0.57	92.80*	0.24	1.97	2.31	0.58	1.58**	0.008	3.17
Treatment	218	103.25**	18.79**	22.13**	14.97**	1.26	64.60*	0.35*	29.18**	8.14**	0.63**	0.67*	0.02**	22.25**
Control	3	114.04*	18.21*	20.20**	5.17	6.04*	64.20*	0.21	29.36**	20.76**	2.60**	0.44	0.04**	0.45
Progeny	214	103.12**	18.89**	22.25**	11.47**	1.18	64.50	0.34*	29.20**	7.89**	0.60**	0.63*	0.02**	22.07**
Control v/s progeny	1	97.49*	0.17	2.83	794.28**	4.02	89.12	2.21**	23.75	23.74**	1.63**	9.45**	0.24**	126.47**
Error	12	17.39	4.41	3.49	3.74	1.67	26.53	0.15	8.30	2.39	0.19	0.27	0.006	1.24

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

Table 2.	Overall mean value	of RILs. their ra	inge, g	enotypic and	phenotypic	coefficient of variati	on, heritabilit	v in broad sense and	genetic advance.
							- ,		B · · · · · · · · · · · · · · · · · · ·

Characters	Mean		Range		GCV		PCV		h ²	GA		
	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated
Plant height (cm)	169.09	199.18	146.62-199.34	144.72-236.92	5.47	5.97	6.00	7.63	83.14	61.32	10.28	9.63
Main shoot length (cm)	58.58	77.85	46.34-76.18	42.80-101.75	6.49	9.62	7.41	12.62	76.65	57.09	11.70	14.85
Fruiting zone length (cm)	63.38	80.38	47.82-80.48	46.44-102.08	6.83	9.59	7.43	11.9	84.31	64.97	12.91	15.92
Siliquae length (cm)	42.55	44.54	33.27-53.43	31.87-62.53	6.53	8.96	7.94	3.79	67.39	64.19	11.03	14.78
Seeds / siliquae	12.64	12.98	8.21-16.12	8.83-18.24	*	*	*	*	*	*	*	*
Biological yield / plant (g)	48.68	68.01	33.30-72.42	34.23-99.12	12.65	4.70	16.50	19.87	58.87	76.76	20.00	31.41
1000 seed weight (g)	4.35	4.21	2.34-5.74	2.36-5.59	9.89	9.19	13.33	11.63	55.88	62.50	15.35	14.68
Harvest index (%)	21.49	23.42	11.83-48.86	10.85-60.34	21.27	27.46	25.13	34.22	71.58	64.42	37.05	45.39
Seed yield per plant (g)	10.38	15.48	6.49-20.52	7.93-32.26	22.54	25.00	26.97	30.89	69.71	65.50	38.74	41.66
Oil content (%)	39.33	39.41	34.47-41.02	35.23-41.52	1.63	1.45	1.96	2.36	68.33	37.93	2.76	1.84
Fiber	8.60	9.57	4.46-10.99	6.58-12.52	6.98	7.31	9.19	9.8	57.14	55.68	10.81	11.15
Phenol	1.63	1.54	1.22-2.15	1.01-2.02	6.13	6.49	8.59	9.18	70.00	55.00	12.39	10.30
Days to flowering	41.45	45.38	36.01-57.21	40.64	11.00	12.14	11.31	12.68	94.38	91.64	22.00	23.92

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

S. No	b. RIL No. Seed yield per		ld per	Plant height (cm)		Main shoot length		Fruiting	zone	Siliqua	e length	1000 se	ed weight	Oil content (%)		Fibre (%)	
		plant (g)				(cm)		length (cm)	(c	em)	(g)	e				. /
		Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated	Stress	Irrigated
1	165	11.49	11.59	168.98	192.72	62.18	75.14	66.68	78.60	4.44	4.45	3.99	4.18	38.88	39.84	8.84	9.34
2	101	13.25	13.43	194.16	211.78	66.51	77.40	67.94	80.14	4.28	4.51	3.71	4.29	39.38	39.96	9.19	9.16
3	64	18.14	18.42	159.02	209.22	55.36	83.88	58.92	82.58	3.48	4.66	5.09	4.37	40.11	40.37	8.59	8.64
4	209	8.28	8.53	155.12	182.71	51.34	68.80	56.20	75.84	4.62	3.46	4.41	3.98	38.55	39.64	9.55	11.40
5	205	8.23	8.48	153.82	154.01	60.94	51.38	65.64	57.94	3.40	4.54	4.71	3.45	39.29	40.55	8.78	10.56
6	199	8.15	8.46	191.90	207.92	57.04	82.20	62.04	85.24	4.42	4.44	5.21	4.46	39.35	37.84	8.43	9.72
7	121	10.21	10.62	163.96	213.51	55.84	75.80	61.24	76.38	4.22	4.60	4.52	2.36	39.57	40.48	9.32	9.51
8	164	9.13	9.53	180.80	197.48	70.44	89.35	74.11	91.86	4.44	4.80	3.65	3.69	39.39	40.21	7.28	9.07
9	182	9.19	9.62	182.31	185.66	60.26	72.93	67.12	76.94	44	4.64	4.60	4.58	40.03	37.44	9.71	9.08
10	189	8.07	8.46	158.94	186.02	61.64	58.72	58.68	56.94	4.46	4.44	5.09	5.21	39.61	40.30	8.53	10.01
11	153	10.89	11.42	179.14	203.66	56.76	70.66	59.33	69.14	4.50	4.41	3.87	2.76	38.32	40.52	8.44	10.53
12	214	9.34	9.83	156.82	172.81	51.42	52.85	54.86	56.72	4.47	4.52	4.52	3.97	39.33	38.58	8.49	10.57
13	123	10.64	11.23	172.08	214.16	57.32	77.73	61.98	76.56	4.46	5.26	4.00	3.55	38.95	40.11	9.74	9.14
14	120	10.60	11.19	163.94	213.33	56.9	77.78	62.08	81.34	3.46	4.45	5.31	4.01	39.58	39.94	8.78	9.04
15	185	7.96	8.43	173.68	160.38	58.11	47.10	62.72	47.32	4.50	4.56	3.54	4.92	39.32	39.78	8.32	10.97
16	113	12.51	13.36	174.58	204.88	58.98	73.08	62.51	88.96	3.48	4.34	5.29	3.41	39.62	37.54	9.57	9.07
17	110	19.22	20.59	180.46	202.42	63.56	83.03	68.58	85.98	3.60	4.33	4.57	4.48	39.65	39.56	7.45	8.97
18	85	7.62	8.19	161.76	182.44	56.32	59.84	65.46	64.64	4.46	5.44	5.74	4.26	38.29	37.41	7.69	8.46
19	94	12.36	13.36	171.24	204.58	66.54	84.05	65.94	86.98	4.36	5.42	4.21	4.72	39.42	40.01	7.61	9.26
20	206	9.76	10.58	159.86	182.62	60.90	78.15	66.32	73.56	4.42	3.98	3.15	3.22	38.62	39.33	8.74	9.54
21	72	10.16	11.04	160.68	187.90	56.42	80.98	62.92	75.96	4.50	4.60	3.59	4.28	38.25	38.79	9.63	9.82
22	75	9.29	10.13	158.51	200.42	56.72	73.50	62.90	75.02	4.46	4.36	4.21	5.04	40.15	39.59	9.64	9.53
23	180	7.25	7.93	180.58	207.32	69.34	80.78	79.14	86.86	5.12	3.66	5.49	2.96	39.23	39.55	7.81	9.45
24	196	10.53	11.55	172.50	207.46	56.12	73.08	62.72	77.24	4.46	4.36	3.93	4.25	39.90	40.13	8.58	9.34
25	162	10.42	11.46	176.52	167.21	57.30	78.70	60.34	86.74	4.40	4.42	4.93	4.16	39.98	40.63	8.27	10.94
	CD (5%)	3.99	7.24	10.75	24.37	5.41	16.60	4.81	14.59	4.98	7.69	1.002	0.78	1.13	1.91	1.35	1.61
	CV	14.91	18.14	2.46	4.74	3.58	8.27	2.94	7.04	4.54	6.69	8.94	7.23	1.11	1.87	6.12	6.53

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

Genotypic and phenotypic coefficient of variation

Here in this study, the phenotypic variances were found to be significantly higher than the genotypic variances under both conditions thus reflecting the role of environmental factors on character expression. In addition, the differences between the phenotypic and genotypic variances were found to be very narrow as expected in augmented designs and because of these narrow difference between the variances, high heritability values were recorded which further gave higher GA estimates (Table 2). Similar observations were also reported earlier in oilseed Brassicas by various workers (Singh *et al.*, 2013, Bind *et al.*, 2014; Synrem *et al.*, 2014). Besides genetic advance, variability and heritability are the two important factors for an effective selection programme which plays a crucial role in assessing the expected improvement in a character (Nadarajan and Gunasekaran, 2005). It is reported that genetic variability, high heritability assisted with high genetic advance are normally more helpful to improve seed yield (Aytaç and Kinaci, 2009).

Under normal conditions, GCV varied from 1.45 (oil content) to 27.46 (HI) while it was 1.63 (oil content) to 22.54 (SY/plant) for water deficit conditions. The maximum PCV was estimated for SY/plant (26.97%) while it was lowest for oil content (1.96%) under drought conditions whereas it ranged from 2.36% (oil content) to 34.22% (HI) in irrigated conditions (Table 2). In this study, high heritability estimates (>50%) were recorded for almost all the characters. High heritability and high genetic advance combinations were reported for seed yield/plant (65.50%, 41.66%) and HI (64.42%, 45.39%), in irrigated conditions while it was (71.58%, 37.05%) for HI and (69.71%, 38.74%) for SY/plant under stress conditions indicating that selection would be highly effective in the present material for these characters (Table 2). Similar levels of heritability and genetic advance for different characters have already been reported earlier in Brassicas (Bind *et al.*, 2014; Iqbal *et al.*, 2014; Semahegn and Tesfaye, 2016).

Correlation coefficient

As seed yield is a complex trait, interactions and interrelationships between yield and its components are important for selection of germplasm (Grafius 1964). We reported significant and positive correlations between seed yield and other morphological characters which ranged from 0.150* to 0.886**. Seed yield per plant was significantly and positively correlated with plant height (0.243**), FZL (0.203*), BY/plant (0.444**), seed/siliquae (0.343**), MSL (0.232**) and HI (0.790^*) . Hence, selection for the higher values of these traits would be desirable for increasing the seed yield. SY/plant was significantly and negatively correlated with OC (-0.224**) and fibre (-0.213**). In addition, PH showed positive and significant correlations with MSL (0.463**), BY/plant (0.441**), FZL (0.556**), seed/ siliqua (0.463**), HI (0.195**) and Phenol (0.150*). MSL was found to be positively correlated with FZL (0.886^{**}) , seed/siliquae (0.431^{**}) and BY/plant (0.384^{**}) . HI was significantly positively associated with seed/siliquae (0.255**), BY/plant (0.389**) and seed yield/plant (0.790**). Thus, such correlations between different yield attributing traits revealed the mutual relationship between two or more characters. Such type of reports have ben also observed earlier by many workers (Singh et al., 2010; Yadav et al., 2011; Misra 2012).

Conclusion

The yield and yield components were reduced significantly due to water deficit. Seed yield per plant under stress could be improved by improving siliqua length, no. of siliqua per plant, 1000-seed weight etc. The 25 promising lines identified can be further used in the brassica breeding programmes to develop drought tolerant brassica varieties with desirable traits which will ultimately further enhance breeding efficiency. Moreover, the RIL population developed in this study may serve as a useful genetic resource for mapping genes/QTLs for seed yield or other related traits.

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