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Genetic variability for micro- and macro-nutrients in Indian mustard [*Brassica juncea* (L.)] under two environments

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Abstract

Twenty two Indian mustard [*Brassica juncea* (L.)] genotypes were evaluated in a randomized complete block design with three replications during *rabi* 2008-2009 under two dates of sowing, *viz.,* November 09 (E_1) and November 25 (E_2) to assess variability effects of environment on micro (Zn, Cu, Fe, Mn) and macro nutrients (Na, K). The results revealed that genotypes had substantial genetic variability for both micro- and macro-nutrients in seeds. However, the delayed sowing exposed the crop to heat stress especially during seed development appreciably reduced nutrients content. Both genotypic and phenotypic variations were high under delayed sowing (E_2) except for Cu and Fe content. Genotypes CS-3000-1-1-1-5, BPR-538-10 and BPR-560-6-B exhibited high Mn, Fe and Na content, respectively under both environments, E_1 and E_2 and would be potential sources for utilization in the breeding programme for enhancing nutritional value of the animal feed. Cu and Fe contents also showed consistently high heritability and genetic advance under both environments, implying that these characters could possibly be controlled by additive gene effects and could be improved by simple selection in segregating generations.

Key words: Genetic variability, micronutrients, Indian mustard, heritability, *Brassica juncea*

Introduction

Rapeseed-mustard, group of crops, in India is an important source of edible oil for human consumption and seed meal as animal feed. Genetic resources constitute an important reservoir of valuable genes and form the basis of breeding programme meeting the future challenges such as enhancing yield, nutritional quality and resistance to biotic and abiotic stresses. Hence, evaluation of available genetic resources and existence of adequate genetic variability for character(s) of interest is pre-requisite for the success of recombinant breeding. Vast information is available on extent of variability for grain yield and associated characters, biotic stresses, oil and seed meal quality [1]. However, information on nutritive quality of seed as determined by presence of micro- and macro-nutrients is scanty. Micronutrients such as Zn (zink), Cu (copper), Fe (iron) and Mn (manganese) do not form the part of the oil constitution but the presence in seed could help in enhancing the initial growth, vigor and establishment of the crop besides forming a part of seed meal which could serve as the nutritional supplement for animal feed. Amongst micronutrients, Zn plays an important role in growth, chlorophyll synthesis and photosynthesis [2, 3] and the quality of oil [4, 5]. Mustard is also grown in Rajasthan using saline water, therefore, Na (sodium) and K (potassium) content in seed is also important. Further, environmental conditions such as temperature, humidity and light intensity during crop growth also influence the expression of any character. The present study aims at evaluating selected promising Indian mustard genotypes for nutrient status in seed especially Zn, Cu, Fe, Mn, Na and K and also to analyze the effect under two environments.

Materials and methods

Twenty two genotypes of Indian mustard were grown in a randomized complete block design with three replications during *rabi* season of 2008-2009. The experiment was conducted at two dates of sowing, *viz.,* November 09, 2008 [(E_1: the maximum and minimum temperature at sowing time ranged from

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27.7-30.2^{0}C and mean of 29.2 ± 0.39^{0}C and 11.5-13.1^{o}C with a mean of 12.5±0.24^{o}C and at physiological maturity maximum and minimum temperature varied from 14.2-36.8^{o}C (mean 26.6±0.58^{o}C) and 2.2-18.7^{o}C (mean 8.9±0.37^{o}C)], respectively. In the November 25, 2008 sowing [E_2: the maximum and minimum temperature at sowing time ranged from 23.2-27.4^{o}C with a mean of 26.2±0.43^{o}C and 7.3-8.2^{o}C with a mean of 7.6±0.01^{o}C and at physiological maturity maximum and minimum temperature ranged from 19.8-36.8^{0}C (mean 29.3±0.50^{o}C) and 4.2-20.0^{o}C (mean 10.4± 0.45^{o}C)]. The crop under E_2 experienced heat stress during terminal stage. There were 5 rows of 5-m length for each genotype in a block. The row spacing was 30 cm and plant spacing within a row was maintained at 10 cm by thinning. A fertilizer dose of 40:40:20 kg/ha (N: P_2O_5: K_2O) was applied at the time of sowing and 40 kg/ha N was top dressed 3-4 days after first irrigation. The irrigations were given on December 19, 2008 and January 30, 2009 (E_1). In the E_2, the corresponding dates were January 4 and February 13, 2009. To protect the crop from the menace of aphid (*Lypaphis erysimi*) infestation, monochrotophos 36 % SL was sprayed @ 0.05% in 1000 litres water/ha on January 10, 21 and February 5, 2009 (E_1) and January 29 and February 11, 2009 (E_2). At the time of harvest, 10 random competitive plants from each replication were taken from the three central rows. The elemental analysis of the bulk seed samples of each genotype was carried out using wet digestion method [6]. Seed samples weighing 0.5 g were taken in conical flasks and digested in 9:3 ratio of nitric acid: perchloric acid digestion mixture and finally analyzed for Zn, Cu, Fe and Mn using Atomic Absorption Spectrophotometer (Thermo Electron Corporation, Model M 5). Na and K was analyzed using Flame photometer (Systronics model 128). The Na to K ratio was also worked out. The character means for each replication were subjected to analysis of variance for the factorial randomized complete block design [7]. Phenotypic (PCV) and genotypic (GCV) coefficients of variation were computed [8]. Heritability in broad-sense (h^2_b) was calculated [9] and expected genetic advance (GS) at 5% selection intensity was also estimated [10].

Results and discussion

Highly significant genotypic effects indicated that the genotypes had substantial genetic variability for the nutrients investigated. But the expression of the characters was highly influenced by environment as indicated by highly significant environmental effects (Table 1). Further, genotypes had differential response to both environments i.e., E_1 and E_2 as evident from highly significant genotype x environment (G x E) interaction effects. Therefore, the results for the two environments were discussed separately.

Range, mean, PCV and GCV

Environment 1 (E_1)

Phenotypic coefficient of variation (PCV) varied from 23.2% for Na content to 39.6% for Na/K ratio. The lowest and the highest genotypic coefficient of variation (GCV) were recorded for K (13.6%) and Fe (36.3%) content, respectively. Both the PCV and GCV with a value of >20 %, 15-20 % and < 15 % were classified as high, moderate and low, respectively. The PCV was high for all the nutrients, whereas, GCV

Table 1. Analysis of variance for Zn, Cu, Fe, Mn, Na, K content and Na/K ratio in seeds of Indian mustard under two environments

Source of variation	DF	Mean sum of squares						
		Zn	Cu	Fe	Mn	Na	K	Na/K ratio
Replication	2	32.38	11.20	1408.38	1.32	5364.61	3545999.55	0.003
Genotype	21	139.95**	58.12**	9102.16**	312.81**	121703.93**	1644687.92**	0.006**
Environment	1	286.39**	99.86**	32941.42**	774.89**	12822.74**	26217678.67**	0.023**
G x E	21	269.19**	45.02**	8478.70**	142.45**	82273.93**	2613712.87**	0.0078**
Error	86	36.55	3.89	382.06	33.49	9510.730	766855.76	0.002
General mean		35.13	12.13	156.65	35.01	687.66	4664.88	0.156
SEM (±)		0.84	0.39	5.03	0.87	17.22	104.46	0.005
CD (5%)								
Genotype		6.64	2.26	22.43	6.64	111.93	1005.07	0.045
Environment		2.09	0.68	6.76	2.00	33.75	303.047	0.013
G x E interaction		9.81	3.20	31.73	9.39	158.29	1421.38	0.063

was high only for Cu, Fe and Na/K ratio. Except K having low GCV the other nutrients showed moderate GCV (Table 2). The lowest and highest Zn content was observed for the genotype RGN-193 (20.6 ppm) and BPR-868-3 (55.3 ppm), respectively. Genotypes BPR-868-3, BPR-548-3 and BPR-141-B-205-43 had high Zn content ($\geq$43.2 ppm) with a mean value of 36.6 ±1.1 ppm. This character exhibited high PCV but moderate GCV. Copper content had a range of 4.00 ppm (RGN-193) to 19.7 ppm (BPR 868-3) having an overall mean of 13.0±0.6 ppm. This character had high PCV and GCV. The genotypes showing high Cu content $\geq$ 18.6 ppm were BPR-141-B-205-43, BPR-540-6 and RH-8814. Fe content had high PCV and GCV. Seed Fe content ranged from 70.4 ppm (BPR-868-3) to 294.8 ppm (RH-0216) with a mean value of 140.9±6.6 ppm. The genotypes having Fe content $\geq$ 203.4 ppm were RH-0216, CS-3000-1-1-1-5 and BPR- 538-10. Mn content varied from 23.8 ppm in RGN-193 to 51.2 ppm in Varuna. It had high PCV but moderate GCV. The genotypes having $\geq$ 50.2 ppm Mn concentration were Varuna, BPR-540-6 and CS-3000-1-1-1-5. The lowest and highest Na content was observed in the genotype RGN-193 (506.7 ppm) and BPR-560-6-B (923.3 ppm), respectively (Table 2). Genotypes BPR-560-6-B, RGN-73 and SKM-531 had $\geq$ 893.3 ppm Na content. This character exhibited high PCV but moderate GCV. The K content ranged from 3690.0 ppm (RH-0216) to 7546.7 ppm (RH-8814) with a mean concentration of 5110.6±156.7 ppm. The genotypes having more than 6073.33 ppm were RH-8814, BPR-868-3 and BPR-540-6. K had a high PCV and low GCV. The Na/K ratios ranged from 0.07 (RH-8814) to 0.24 (RH-0216) with a mean value of 0.14±0.017. Genotypes which had Na/K ratio $\geq$ 0.20 were RH-0216, RGN-73 and Bio-902. This character showed high PCV and GCV.

Environment 2 (E_2)

PCV varied from 22.4% for K content to 36.8% for Cu content. The lowest and the highest GCV were recorded as 15.7% and 32.2% for K and Cu content, respectively (Table 2). The PCV and GCV were high for all characters (Zn, Cu, Fe, Mn, Na and Na/K ratio). However, K content exhibited high PCV and moderate GCV. The lowest and the highest zinc content were observed in BPR-2 (15.1 ppm) and Urvashi (44.9 ppm) with an overall mean of 33.7±1.3 ppm. The genotypes having high Zn

Table 2. Range, mean, phenotypic (PCV) and genotypic coefficients of variation (GCV), heritability (h^2_b) and genetic advance as % of mean (GS) for micronutrients (Cu, Zn, Fe, Mn), macronutrient (Na, K) content (ppm) and Na/K ratio under two environments

Nutrient	E1						E2					
	Range	Mean ±SEM	PCV (%)	GCV (%)	h^2_b	GS	Range	Mean ±SEM	PCV (%)	GCV (%)	h^2_b	GS
Zn	20.62-55.33 (RGN-193- BPR-868-3)	36.61±1.09	24.6	17.3	49.4	25.0	15.06–44.94 (BPR-2-Urvashi)	33.66±1.25	30.2	25.3	69.9	43.5
Cu	3.98-19.73 (RGN-193-BPR-141-B-205-43)	13.00±0.58	36.1	33.4	85.5	63.6	6.6-17.31 (Bio-902 - BPR- 538-10)	11.26±0.50	36.8	32.23	76.7	58.1
Fe	70.39-294.83 (BPR-868-3-RH-0216)	140.86±6.55	38.3	36.3	89.5	70.7	96.97-289.33 (BPR-327-1- B-NRCDR 2)	172.45±7.17	34.0	31.8	87.3	61.2
Mn	23.79-51.15 (RGN-193-Varuna)	37.44±1.17	25.7	19.6	58.6	30.9	18.80-60.35 (BPR-548-3-CS-3000-1-1-1-5)	32.59±1.24	31.4	26.6	71.8	46.4
Na	506.67-923.33 (RGN-193-BPR-560-6-B)	677.80±19.15	23.2	17.4	56.4	26.9	212.00-1370.00 (BPR-2-CS-3000-1-1-1-5)	697.52±28.72	34.0	31.3	84.9	59.4
K	3690.00-7546.66 (RH-0216-RH-8814)	5110.55±156.67	24.2	13.6	31.6	15.7	2286.67-5031.00 (BPR-2- NPJ-112)	4219.21±115.45	22.4	15.7	48.8	22.6
Na/K ratio	0.072-0.240 (RH-8814-RH-0216)	0.142±0.007	39.6	24.2	37.5	30.6	0.093-0.317 (BPR-2-CS-3000-1-1-1-5)	0.169 ± 0.007	35.3	29.6	70.0	51.0

content ($\geq$ 43.9 ppm) were Urvashi, RH-8814 and RH-0216. Moreover, this character had high GCV and PCV. Copper content ranged from 6.6 ppm (Bio-902) to 17.3 ppm (BPR-538-10) and exhibited high GCV and PCV. The genotypes having high Cu content ($\geq$ 16.2 ppm) were BPR-538-10, RGN-193 and CS-3000-1-1-1-5. Genotypes showing lowest and highest Fe content were BPR-327-1-B (97.0 ppm) and NRCDR-02 (289.3 ppm), respectively and Fe content showed high PCV and GCV. Genotypes NRCDR-02, BPR-538-10 and RGN-193 had relatively high Fe content ($\geq$ 278.8) amongst the genotypes investigated with high PCV and GCV. The Mn content varied from 18.8 ppm in BPR-548-3 to 60.4 ppm in CS-3000-1-1-1-5 with high PCV and GCV. Genotypes CS-3000-1-1-1-5, NPJ-112 and RGN-193 had relatively high Mn content ($\geq$ 43.29 ppm). The Na content was the lowest in the genotype BPR-2 (212 ppm) and highest in CS-3000-1-1-1-5 (1370 ppm), with an overall mean of 697.5 ± 28.7 ppm. High PCV and GCV were observed for Na content with high concentration ($\geq$ 951.7 ppm) in CS-3000-1-1-1-5, BPR-560-6-B and BPR-545-2. The K content ranged from 2286.7 ppm in BPR-2 to 5031.0 in NPJ-112 with an overall mean of 4219.2 ± 115.5 ppm. K had a high PCV but moderate GCV. The genotypes having high K content (= 4976.7 ppm) were NPJ-112, RH-0216 and BPR-560-6-B. The Na/K ratio recorded in the genotypes was lowest in BPR-2 (0.09 ppm) and highest in CS-3000-1-1-1-5 (0.32 ppm). It also exhibited high PCV and GCV and relatively high Na/K ratio ($\geq$ 0.20 ppm) was recorded in the genotypes CS-3000-1-1-1-5, BPR-548-3 and BPR-560-6-B.

The results revealed that genotypes had substantial genetic variability for most of the characters investigated. However, the delayed sowing causing heat stress especially during seed development appreciably reduced the micro and macronutrients in seeds.

Heritability and genetic advance

Environment 1 (E_1)

The heritability estimates (h^2_b) ranged from 31.6 % for K content to 89.5% for Fe content and the genetic advance (GS) varied from 15.7 for K content to 70.7% for Fe content under E_1. The heritability values of >70%, 50-70% and < 50% were classified as high, moderate and low, respectively. The genetic advance was categorized as high (>30 %), moderate (20-30%) and low (<20%). Except Zn, K concentration and Na/K ratio, the rest of the characters had moderate to high heritability. Moderate heritability was exhibited by Mn (58.6%) and Na (56.4%) content but low for K (31.6%) and Na/K ratio (37.5%). The estimates for both heritability and genetic advance were high for Cu (85.5%, 63.6%) and Fe (89.5, 70.7%) content, respectively. However, moderate heritability (58.6%) and high GS (30.9%) for Mn content, low heritability (49.7%) and moderate GS (25%) for Zn content and low heritability (37.5%) and high GS (30.6%) for Na/K ratio were also exhibited. Heritability and GS were moderate for Na content and low for K content, respectively under E_1 (Table 2).

Environment 2 (E_2)

Except for Cu and Fe content both heritability and genetic advance (GS) were higher for Zn, Mn, Na, K and Na/K ratio under E_2 in comparison to E_1. The heritability ranged from 48.8% (K content) to 87.3% (Fe content), whereas, genetic advance ranged from 22.6% (K content) to 61.2% (Fe content). All the parameters (Cu, Fe, Mn, Na) except Zn content and Na/K ratio showed high heritability and genetic advance. However, in case of Zn content and Na/K ratio moderate genetic heritability was associated with high genetic advance (Table 2).

As per the requirement of the seed meal for the animal feed, presence of fairly higher concentration of micronutrients could act as biofortification for reducing malnutrition in the global scenario [11] and improve their productivity [12]. Addition of micronutrient has been the essential supplements like Zn (0.2-0.4%), Cu (500ppm-0.08%), Fe (2000ppm-0.55%) and Mn (0.085-0.1%) as suggested by BIS for animal feed [13]. Feed plans have also been suggested by Central Institute for Research on Buffalo [14]. However, the importance of micronutrient could only be evaluated when they are deficient in feed. Deficiency of copper could lead to disorder in the nervous system and sudden death due to heart failure, iron deficiency to anemia, poor growth, rough hairs and paleness of mucous membrane. Its lower availability could cause skeleton abnormalities and reduced reproductive performance [15]. Deficiency of Zn could reduce growth, fertility, thickening and scaling of skin cells, dermatitis, hair loss, increased susceptibility to foot infection and interference with the metabolism of Vitamin A in liver [14]. This problem could be overcome by breeding nutrient efficient genotypes of Indian mustard which may not only be adaptable to micronutrient deficient areas but also have the capacity of accumulating the micronutrients in the edible part of the plants [16].

Genotypes, CS-3000-1-1-1-5, BPR-538-10 and BPR-560-6-B exhibited high Mn, Fe and Na content, respectively under both environments, E_1 and E_2. Further, genotypes CS-3000-1-1-1-5 (Fe and Mn); BPR 868-3 (Zn and K); BPR- 540-6 (Cu, Mn and K) and BPR-141-B-205-43 (Zn and Cu) under E_1 exhibited high content for more than one nutrients. Similarly, under E_2 genotypes CS-3000-1-1-1-5 (Cu, Mn, Na, Na/K ratio); RH-0216 (Zn and K); RGN-193 (Cu, Fe and Mn); and BPR-540-6-B (Na, K and Na/K ratio) exhibited high level of more than one nutrients. These genotypes showing high content of various nutrients under both the environments and those having high content of more than one nutrients would be potential sources for utilization in the breeding programme for enhancing nutritional value of the animal feed.

The results revealed that Cu and Fe contents showed consistently high heritability and genetic advance under both environments, implying that these characters could possibly be controlled by additive gene effects and could be improved by simple selection in segregating generations. The rest of the characters had variable estimates for these parameters and thus their expression could be predominantly controlled by non-additive gene effects. The mean nutrient content of seed and Na/K ratio were largely influenced by the environment as evidenced by increased variability with lower mean. For improving these micro/macronutrients having moderate to low heritability and genetic advance, the appropriate breeding strategies would be bi-parental mating followed by selection.

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