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Studies on combining ability and gene action for heat stress tolerance traits in Indian mustard (*Brassica juncea* L.)

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Abstract

In order to identify parents for suitable use in a breeding programme for development of high yielding varieties of Indian mustard with heat stress tolerant traits, the combining ability and gene action for certain physiological traits were investigated in half-diallel crossings among eight parental lines. The cultivars investigated were NRCHB 101, GM-2, NRCDR-601, BPR-543-2, BPR-549-9, JN-032, Urvashi and BPR-541-2 possess different tolerance levels to heat stress. Heat stress conditions were achieved through early sowing under conserve moisture condition. On the date of seeding (September, 28) of the year 2013-14, the maximum soil temperature at 0- 10 cm depth was 39.0^{o}C. Data were subjected to analysis of variance and combining abilities factor analysis. Analysis of variance for general combining ability (GCA) and specific combining ability (SCA) displayed significant general and specific combining effects for the seven seed yield and physiological traits *i.e.* population survival (%) 10 DAS, population survival (%) 25 DAS, membrane stability index (%), excised- leaf water loss (%), relative water content (%), water retention capacity of leaves (%) 24hrs and seed yield per plant (g). For all the traits the GCA effects were relatively more important than the SCA effects, indicating that additive genetic effects were predominant. Crosses displaying high SCA effects for relative water content (%), membrane stability index (%) and seed yield per plant (g) were observed to be derived from parents having various types of GCA effects (high x high, high x low, low x low and medium x low).The single seed descent method can be applied to exploit additive gene effects whereas dominance gene effects could be valuable in hybrid mustard breeding programmes. Among the parents, genotypes BPR-549-9, BPR-543-2 and Urvashi were found to be superior general combiners for seed yield and heat stress tolerance traits. Likewise, crosses involving diverse parents showed significant SCA effects for seed yield and other heat stress tolerance traits.

Key words: *Brassica juncea, combining ability, GCA, heat stress tolerance traits, SCA*

Introduction

Gaseous emissions due to human activities are substantially adding to atmospheric concentrations of greenhouse gases, particularly CO_2, methane, chlorofluorocarbons and nitrous oxides. In the atmosphere these gases trap heat radiated from the earth and thus increase global mean temperature. This rise in temperature may lead to altered geographical distribution and growing season of agricultural crops by altering the threshold temperature for the start of the season and crop maturity (Porter, 2005). High temperature is a major abiotic stress that severely restricts crop production (Hall, 1992). Impaired fertility and yield loss due to heat stress are widely reported for various crops, including wheat (Saini *et al.,* 1983, rice (Hall, 1992), corn (Schoper *et al.,* 1987) and cotton (Kittock *et al.,* 1988).

Oilseed brassicas are the second most important edible oilseed crop of India after soybean in terms of the acreage and production. More than 90% of the area under oilseed Brassicas in India is occupied by the Indian mustard (*Brassica juncea*) because of its relative tolerance to abiotic stresses in comparison with other oilseed Brassica species. An optimum average temperature of 26^{0} C is required for the proper germination and establishment of seedlings (Lallu and Dixit, 2008). Due to the changing climate, the temperature during the last 15 years, except 2010, was above this limit in the major rapeseed-mustard growing areas of the country. In addition, the cultivation of Indian mustard in Rajasthan is largely carried out under rainfed farming systems where sowing commences after south-west monsoon rains. Early rains may cause the farmers to sow the crop early in the season to take advantage of conserved moisture in the soil (Venkateswarlu and Prasad, 2012). But high temperature prevailing at the time of sowing reduces seed germination and causes seedling mortality, resulting in poor crop stand and reduced seed yield (Azharudheen *et al.*, 2013). Wide variations in diurnal soil temperatures ranging from 28 °C to 56 °C at the surface and from 33 °C to 37 °C at 300 mm depth were observed in Rajasthan (Gupta, 1986).

In breeding of high yielding varieties of crop plants, the breeder often faces with the problem of selecting parents and crosses. Combining ability analysis is one of the powerful tools available which estimates combining ability effects and aids in selecting desirable parents and crosses for further exploitation. Additive and non-additive gene action estimated through combining ability analysis in the parents may be useful for exploitation of heterosis and isolation of pure lines among the progenies of heterotic F_1s. Further, the diallel mating design provides an opportunity to mate the given set of parents in all possible combinations (Griffing 1956) and it provides information on combining ability and thus helps in the selection of desirable parents for utilization in the hybridization programme, as well as in the choice of appropriate breeding procedure for the genetic improvement of various heat stress tolerance traits in Indian mustard. Very few reports are made available on the use of this technique in Indian mustard. In view of this fact, present study was undertaken to estimate the general and specific combining ability effects under heat stress conditions.

Materials and Methods

Eight genetically diverse parents of Indian mustard with varying degree of heat tolerance namely; NRCHB-101, GM-2, NRCDR-601, BPR-543-2, BPR-549-9, JN-032, Urvashi and BPR-541-2, selected on the basis of high temperature tolerance, sensitive, high yielding, low yielding were crossed in diallel fashion (excluding reciprocals) during *Rabi* 2012-2013. The twenty eight crosses along with parents were evaluated at the experimental farm of the ICAR-DRMR, Bharatpur (77.27°E longitude; 27.12°N latitude and 178.37 m above mean sea level) in a randomized block design with three replications during *Rabi* 2013-2014. The soil of the experimental site was sandy loam with EC 1.5 dSm^{-1}, organic carbon (0.25 - 0.30%), available N (125-135 kg/ha), P (20-22 kg/ha), K of 240-260 kg/ha, and pH of 8.1. The crop was raised strictly under conserved moisture conditions. On the date of seeding (September, 28) of the year 2013-14, the maximum soil temperature at 0- 10 cm depth was 39.0°C. All parents and crosses were grown in two rows of five meter length; with row to row and plant to plant spacing of 30 cm and 10 cm, respectively. The recommended package of practices was followed to raise a good crop. Growth and physiological characters, including, population survival (%) at 10 and 25 days after sowing (DAS), membrane stability index (%), excised-leaf water loss (%), relative water content (%), water retention capacity of leaves (%), and seed yield per plant (g) were recorded on five randomly selected plants of each genotype.

Determination of growth and physiological parameters

The estimation procedures of physiological parameters *i.e.* membrane stability index (MSI), excised- leaf water loss (ELWL), relative water content (RWC), and water retention capacity of leaves (WRCL) were same as described in Ram *et al.* (2015).

Statistical analysis

The mean values were used for the analysis of variance. Data were first subjected to the usual analysis followed for a randomized block design for individual environment as suggested by Panse and Sukhatme (1967). The combining ability for 8 × 8 diallel analysis (excluding reciprocals) was carried out by Method II and Model I of Griffing (1956).

Results and Discussion

Temperature is an important factor which affects growth and development of plants. All plants require a certain amount of heat units during growth periods and the duration to achieve heat units depends upon the climatic conditions. For each set of experiments, high temperature stress was created by sowing in the last week of September under conserve moisture conditions. An understanding of the genetic control of the characters is the basic requirement for the purposeful management of the available genetic variability. The choice of the most suitable breeding method would depend mainly on the combining ability behaviour vis-a-vis nature of gene action involved in the control of the traits of interest to the breeder. Results indicate that analysis of variance showed a significant difference in studied traits (Table 1). Phenotypic values of population survival (10 DAS and 25 DAS), cell membrane stability index (MSI), excised-leaf water loss (ELWL), relative water content (RWC), water retention capacity of leaves (WRCL) and seed yield per plant, differed significantly among the eight parental lines and 28 F_1 crosses ($P< 0.01$). Both GCA and SCA were highly significant for population survival (10 and 25 DAS), membrane stability index, excised-leaf water loss, relative water content, water retention capacity of leaves and seed yield per plant ($P< 0.01$) (Table 1).

The concept of combining ability is important in designing plant breeding programmes; in particular, it is useful in testing procedures for the study and comparison of the performance of lines in cross combinations. Nature and magnitude of combining ability effects help in identifying superior parents and their utilization in further breeding programme. The magnitude and direction of combining ability effects are known to be useful in selecting parent plants in crop improvement programmes (Mather and Jinks, 1971).

The combining ability analysis showed that both general and specific combining ability effects played an important role in the control of the population survival of the genotypes studied, with the general effects being greater than the specific effects. This suggests a prominent role for additive genetic effects, although the significance of specific combining ability effects indicates that dominance and epistasis were also involved in the expression of the traits studied. Moreover, mean square values were higher for GCA than for SCA of population survival (10DAS), population survival (25DAS), membrane stability index and excised-leaf water loss; however, for relative water content, water retention capacity of leaves and seed yield per plant, the mean square of SCA was higher than the mean square of GCA, indicating the importance of both additive and non-additive gene effects. Similar results have also been reported in name of crop by other workers (Akbar *et al.*, 2008; Niranjana *et al.*, 2014; Singh *et al.*, 2017 and Singh B *et al.*, 2017).

Dhanda and Sethi (1998) reported that additive gene action, in general, played a major role in determining the inheritance of excised-leaf water loss and relative water content in wheat. General combining ability (GCA) was the main source of genetic variation among crosses, while specific combining ability (SCA) was negligible.

Heat shock increases cell membrane permeability, thereby inhibiting cellular function, as a result of the denaturation of proteins and increments of unsaturated fatty acids that disrupt water, ion, and organic solute movement across cell membranes. Thylakoid membranes typically show swelling, increased leakiness, physical separation of the chlorophyll light harvesting complex II from the PSII core complex, and disruption of PSII mediated electron transfer (Ristic *et al*., 2008). Cell membranes are main loci affected under heat stress conditions. In this investigation, cell membrane stability index (MSI) increased under heat stress in most genotypes

The cell membranes are thought to be the primary site of direct high temperature injury (Levitt, 1980; Blum, 1988).

Table 1.Mean squares for heat stress traits in diallel analysis in Indian mustard

Source of variation	df	Population survival (%) 10 DAS	Population survival (%) 25 DAS	Membrane stability index (%)	Excised Leaf Water Loss (%)	Relative Water content (%)	Water retention capacity of leaves (%) 24hrs	Seed yield per plant (g)
Replication	2	0.31	0.17	1.39	0.79	0.70	0.67	0.65
Treatments	35	227.9**	109.5**	216.1**	114.3**	160.1**	136.2**	142.2**
Parents	7	320.3**	99.5**	234.3**	121.0**	107.7**	101.4**	94.2**
Crosses	27	190.0**	104.3**	214.5**	109.3**	175.7**	152.1**	156.2**
Parents vs Crosses	1	602.9**	320.2**	134.8**	202.8**	105.8**	107.1**	102.3**
GCA	7	116.5**	58.9**	160.4**	58.3**	42.2**	39.5**	41.0**
SCA	28	65.8**	30.9*	50.0**	33.0*	56.2**	54.3**	61.2**
Error	70	0.25	0.18	1.32	1.42	2.30	3.65	3.75
Total	107	74.7	35.9	71.6	38.3	53.9	50.0	61.2

*, ** Significant at 5% and % level of probability, respectively.

Table 2.Estimates of general combining ability (GCA) effects for F_1 offspring of 8 Indian mustard parents and their mean performance (MP) for seed yield per plant and heat stress traits.

Parents	Population survival (%) 10 DAS		Population survival (%) 25 DAS		Membrane stability index (%)		Excised Leaf Water Loss (%)		Relative Water content (%)		Water retention capacity of leaves (%) 24hrs		Seed yield per plant (g)	
	GCA effects	MP	GCA effects	MP	GCA effects	MP	GCA effects	MP	GCA effects	MP	GCA effects	MP	GCA effects	MP
	F1		F1		F1		F1		F1		F1		F1	
NRCHB 101	-4.83**	66.0	-0.07	60.0	-3.0**	2.9	-1.4**	37.3	-0.8**	67.40	-0.75**	25.70	-0.86**	22.7
GM-2	-2.99**	68.7	0.7**	61.0	4.9**	6.4	2.3**	38.6	-2.7**	68.67	-1.90**	38.60	-2.12**	19.7
NRCDR-601	-0.55**	63.0	1.77**	53.0	1.8**	8.7	-2.7**	36.2	0.5	70.08	0.60	26.07	0.40	21.6
JN-032	-1.99**	72.0	3.05**	65.0	-6.0**	20.6	-3.1**	38.4	-2.3**	62.8	-2.06**	25.2	-1.86**	22.3
BPR-541-2	-0.09	74.0	1.78**	69.0	-3.8**	21.2	-1.3**	34.8	-0.6*	72.8	-0.56*	27.1	-0.67*	29.8
Urvashi	1.61**	76.0	-0.98**	70.0	-0.7**	27.5	0.95**	25.9	0.3	83.2	0.40	41.3	0.38	21.2
BPR-543-2	3.14**	86.0	-1.59**	79.0	2.6**	33.3	2.58**	24.5	2.6**	84.8	2.41**	42.7	1.52**	21.6
BPR-549-9	5.69**	88.0	-4.64**	77.0	4.3**	36.8	2.68**	24.2	2.96**	90.0	1.91**	43.4	1.98**	19.0
Xp		74.2		66.8		19.7		32.5		74.96		33.8		22.2
SE (gi) ±	0.08			0.07	0.11		0.20		0.25		0.21		0.23	
SE (gi-gj)	0.13			0.11	0.29		0.30		0.39		0.37		0.31	

**Significant at p = 0.01 by the F test.

Key: Xp = grand mean of Parents and F1; SE = Standard error; gi = General combining ability effects for line i; gj = General combining ability effects for line j.

Table 3.Estimates of specific combining ability (SCA) effects for F_1 offspring of 8 Indian mustard parents and their mean performance (MP) for seed yield per plant and heat stress traits.

Parents	Population survival (%) 10 DAS		Population survival (%) 25 DAS		Membrane stability index (%)		Excised Leaf Water Loss (%)		Relative Water content (%)		Water retention hrs capacity of leaves (%) 24		Seed yield per plant (g)	
	SCA effects	Mean	SCA effects	Mean	SCA effects	Mean	SCA effects	Mean	SCA effects	Mean	SCA effects	Mean	SCA effects	Mean
	F1		F1		F1		F1		F1		F1		F1	
(NRCHB 101x GM-2)	-8.9**	68.0	4.9**	62.0	-10.7**	11.4	3.3**	37.6	-2.0**	86.4	-1.51**	32.7	-3.0**	23.3
(NRCHB 101x NRCDR-601)	-9.1**	82.0	1.4**	75.0	-6.2**	32.8	-4.2**	34.3	-10.9**	89.3	-7.3**	36.3	6.8**	22.0
(NRCHB 101x JN-032)	4.2**	72.0	2.3**	65.0	-5.7**	26.5	-4.7**	32.2	0.9*	75.1	0.8*	30.3	10.0*	21.5
(NRCHB 101x BPR-541-2)	2.9**	68.0	0.0	62.0	2.1**	17.4	-4.6**	35.9	1.2**	79.7	1.1*	36.2	2.2**	17.5
(NRCHB 101x Urvashi)	7.5**	70.0	-6.2**	63.0	9.4**	22.9	7.4**	28.1	2.3**	82.1	1.1*	40.4	3.3**	25.2
(NRCHB 101x BPR-543-2)	11.8**	60.0	-6.9**	55.0	7.8**	16.6	7.1**	35.7	10.0**	88.9	4.2**	42.4	6.9**	29.5
(NRCHB 101x BPR-549-9)	12.8**	84.0	-4.1**	76.0	11.3**	33.4	7.7**	25.0	11.6**	89.5	8. 5**	43.1	7.6**	34.2
(GM-2x NRCDR-601)	13.1**	75.0	-1.3*	69.0	5.2**	19.4	2.3**	43.1	10.0**	75.6	8.1**	23.2	4.1**	30.2
(GM-2x JN-032)	8.3**	66.0	-4.7**	60.0	-1.2**	14.7	-3.3**	43.3	2.9**	70.4	1.2*	27.0	3.1**	32.0
(GM-2x BPR-541-2)	-2.7**	72.0	0.3	67.0	1.1**	21.2	0.9	32.9	-2.9**	76.3	-2.2**	32.2	-3.8**	24.2
(GM-2x Urvashi)	1.1*	81.0	-4.7**	74.0	0.5	28.9	2.8**	33.3	-1.8**	87.3	3.0**	38.3	2.3**	32.3
(GM-2 x BPR-543-2)	-6.7**	75.0	3.5**	70.0	4.0**	26.2	3.1**	25.9	-14.1**	84.3	-9.1**	35.5	-7.0**	34.2
(GM-2x BPR-549-9)	7.5**	83.7	-4.2**	80.0	2.9**	32.0	3.7**	23.8	9.5**	86.6	5.1**	40.8	5.5**	30.4
(NRCDR-601x JN-032)	-6.0**	78.0	5.3**	72.0	-2.8**	15.6	-1.6**	41.9	-6.4**	65.4	-5.0**	29.7	4.4**	33.5
(NRCDR-601x BPR-541-2)	-1.3**	65.0	-3.8**	59.0	0.7	18.5	1.9**	43.2	-2.1**	61.9	2.3**	24.7	-1.0*	22.9
(NRCDR-601x Urvashi)	4.6**	72.0	-0.6	66.0	8.7**	29.1	5.6**	26.1	6.0**	83.7	4.1**	39.9	2.1**	20.2
(NRCDR-601x BPR-543-2)	0.4	69.0	-7.5**	62.0	2.4	18.7	1.2*	34.2	-2.3**	72.7	-1.3**	30.7	-3.3**	21.0
(NRCDR-601x BPR-549-9)	3.6**	73.0	-6.5**	66.0	3.0**	29.7	6.4**	27.5	6.0**	79.5	4.1**	31.3	5.0**	20.7
(JN-032 x BPR-541-2)	-2.6**	67.3	5.3**	62.0	-5.8**	26.3	4.7**	39.3	-6.3**	69.9	4.2**	28.5	-2.2**	31.5
(JN-032 x Urvashi)	6.2**	87.7	-9.0**	82.0	13.0**	36.4	7.7**	30.5	-0.2	79.1	-0.3	35.1	10.0*	20.0
(JN-032 x BPR-543-2)	-5.7**	82.3	-0.4	76.0	-1.3**	16.4	-3.2**	36.9	-5.6**	69.8	-5.1**	34.4	6.2**	18.9
(JN-032 x BPR-549-9)	2.8**	77.7	-4.0**	71.0	3.8**	24.1	-2.7**	23.1	-1.9**	80.3	2.1**	39.5	1.0*	23.3
(BPR-541-2 x Urvashi)	11.7**	71.0	-3.4**	62.0	6.1**	14.0	1.1*	35.5	13.8**	69.5	11.0**	36.3	14.8**	18.7
(BPR-541-2 x BPR-543-2)	-9.9**	73.0	3.6**	67.0	-6.5**	22.8	-1.2**	38.4	6.1**	64.2	4.5**	24.8	-2.1**	21.6
(BPR-541-2 x BPR-549-9)	-4.7**	69.0	-7.2**	64.0	2.3**	29.4	3.7**	34.8	1.1*	71.7	1.1*	26.6	2.1**	22.3
(Urvashi x BPR-543-2)	-5.1**	83.0	7.8**	78.0	-15.1**	36.8	-13.1**	26.4	-4.1**	88.5	-3.1**	42.6	5.1**	29.5
(Urvashi x BPR-549-9)	-1.1*	82.0	7.3**	76.0	-9.3**	32.5	-11.4**	33.8	-8.5**	81.3	-6.4**	39.7	4.4**	32.5
(BPR-543-2x BPR-549-9)]	0.5	70.0	6.9**	65.0	-3.0**	23.8	0.04	29.7	2.2**	80.4	2.0**	36.0	4.2**	36.5
Xp	74.2		67.8		23.2		33.1		77.5		34.4		25.2	
SE Sij	0.26		0.22		0.60		0.6		0.8		0.7		0.7	
SE (Sij-Sik)	0.39		0.33		0.89		0.9		1.2		1.1		1.0	

** Significant at p = 0.01 by the F test.

Key: Xp = grand mean of Parents and F1; SE = Standard error; Sij = Specific combining ability effects between ith and jth lines; Sik = Specific combining ability effects between ith and kth lines.

Leakage of solutes through the membrane after heat stress has been measured by electrical conductivity (EC) and used in many other crops (eg: wheat, soybean, vegetables) as an index of membrane stability to identify heat tolerant genotypes (Sullivan and Ross 1979; Martineau *et al.,* 1979; Saadalla *et al.,* 1990; Shanahan *et al.,* 1990).

In this study, the variation exhibited by the seven characteristics under consideration indicated that selection for some of these heat-related characteristics; however, the selection efficiency is related to heritability. For the purpose of crop production, yield improvement and yield stability under heat-water stress conditions, the development of heat-drought tolerant varieties is the best approach (Siddique *et al.,* 2000). Therefore, physiological approaches are of great importance for deeper understanding of the complex responses of plants to heat stress, and the rapid development of new varieties.

The estimates of general combining ability of eight parents for seven characters are presented in Table 2. Among the parents the highest values for different variables were observed in the following parents: BPR-549-9 for population survival (10 DAS), membrane stability index (MSI), relative water content (RWC) and seed yield per plant; BPR-543-2 for population survival (10DAS), relative water content (RWC), membrane stability index (MSI) and seed yield per plant; JN032 for population survival (25DAS); BPR-541-2 for population survival (25DAS); GM-2 for membrane stability index (MSI) and NRCDR601 for membrane stability index (MSI) and population survival (25DAS). Thus, for general combining ability these lines can be considered as the most thermo-tolerant efficient cultivars based on their performance and also for their specific combining ability. It was observed that the parental lines which were high performing were also good general combiners for the respective characters. It can be inferred that the potential parents for utilization in breeding programmes to improve yield and its other heat stress related traits in Indian mustard may be judged on the basis of their *per se* performance.

Our present findings are in agreement with the earlier studies on wheat (Dhanda and Sethi,1998; Singh *et al,*. 2007; Rad *et al.,* 2013) and Indian mustard (Gautam and Chauhan, 2016; Singh *et al.,* 2017) with a different set of material. These outcomes reveal that there is a scope for improving combining ability of parents for heat stress tolerance traits, since good combiners for seed yield traits were not good for number of other heat stress tolerance traits. Therefore, efforts need to be made to improve the combining ability of heat stress tolerance traits which would, in turn, improve the gca of seed yield indirectly under heat stress conditions. In addition, crosses NRCHB101 x BPR-549-9, NRCHB101 x BPR-543-2, JN032 x Urvashi, NRCDR601 x Urvashi and BPR541-2 x Urvashi had high values for population survival (10 DAS), membrane stability index (MSI), relative water content (RWC), water retention capacity of leaves and seed yield per plant; however, the crosses Urvashi x BPR-549-9 and JN032 x BPR-543-2 has a negative value for excised leaf water loss under heat stress condition (Table 3).

In this study, GCA and SCA were highly significant; the heat tolerant genotype Urvashi, BPR-543-2, BPR-549-9 and GM2 possessed significantly high gca for seed yield as well as some of its heat stress tolerance traits under heat stress conditions. These genotypes shall be included in the breeding programme for accumulation of favourable alleles. Based on heat stress indices (PPS, MSI, RWC, WRCL, and ELWL), the line Urvashi x BPR-543-2, Urvashi x BPR-549-9 and JN032 x BPR-543-2 proved to be a heat tolerant genotypes. According to factors analysis and the relationship between factors scores and heat stress indices, MSI, RWC, and ELWL may be good criteria to identify heat tolerant genotypes with higher seed yield.

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