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Research Article

Combining ability studies for heat tolerance traits in bread wheat [*Triticum aestivum* (L.) em. Thell]

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Abstract
A set of 28 hybrids were developed involving eight diverse parents (HD 2687, DBW 17, PBW 373 ,Raj 3765, Raj 3077, Raj 4037, Raj 4083and RKA 501) by using diallel mating system without reciprocals. The 28 F_1's and 8 parents were grown in Randomized Block Design with two replications during *rabi* 2008-09. Analysis of variance revealed that variance due to genotypes, parents, parents *vs.* crosses and crosses were highly significant and significant for all the traits studied. Significant mean squares due to general combining ability and specific combining ability in pooled analysis revealed significance of both additive and dominance gene effects in inheritance of all the heat tolerance traits in wheat. The low predictability ratio revealed that all the characters studied were predominantly controlled by non- additive genes action. The present study has identified desirable parents RKA 501, PBW 373, Raj 3077, Raj 3765 and HD 2687 for most of the heat tolerant parameters. The importance of additive but mainly non-additive gene action is highlighted. Improvement in heat injury and other heat tolerant parameters should be possible by resorting to biparental mating followed by recurrent selection or by diallel selective mating.

Key words
Combining ability, heat tolerant parameters, proline, Wheat

Introduction

Wheat is one of the most important cereals of India and known to be cultivated since pre-historic times (Tandon, 2000). Bread wheat belongs to the tribe triticeae and family poaceae. In wheat, plants can be injured at seedling emergence, reproductive development, stem elongation, heading and grain filling stages by high temperature. Wheat improvement for heat tolerance depends on intensive hybridization using heat tolerant donors and high yielding commercial cultivars. To evolve an effective hybridization programme combining ability analysis is used to test the performance of parents in different cross combinations and characterize the nature and magnitude of gene effects in the expression of various heat tolerant parameters. There are scanty references for genetic studies of heat tolerant parameters. Thus, the main objective of present studies was to identify the best combining parent and their crosses on the basis of their general and specific heat tolerant parameters *viz.*, proline content, stomatal frequency, heat injury, chlorophyll stability index and seedling vigour index under normal sown environment.

Materials and methods

A set of 28 hybrids were made by involving eight diverse parents (HD 2687 DBW 17 PBW 373 Raj 3765 Raj 3077 Raj 4037 Raj 4083 and RKA 501) in using diallel mating system without reciprocals during *rabi season* 2007-08. The 28 F_1's and eight parents were grown in Randomized Block Design with two replication during *rabi* 2008-09 at the Research Farm of Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur (Rajasthan). Udaipur is situated at an elevation of 579.50 meters above sea level on latitude of 24035' North and longitude of 37042' East.

Each treatment was sown in single row plot of 2 m length spaced 30 cm apart and plant to plant spacing of 10 cm. All the recommended agronomy practices were applied to the crop during the season, to raise the successful crop. Five plants in each line were randomly selected and observations were recorded for six heat tolerant traits *viz.*, proline content, stomatal frequency upper, stomatal frequency lower, heat injury (%), chlorophyll stability index, and seedling vigour index. The procedure followed for recording the observations on different characters are described below.

Proline content: At the time of anthesis the proline content was determined as per method given by Bates et al. (1973).

Stomatal frequency (upper and lower): The stomatal frequency of upper and lower surface of the fully expended flag leaf was calculated by xylene + thermocol print method.

Heat injury (%): Heat injury percent was measured as per Sulluvian's (1972) heat tolerance test which was further modified by Blum and Ebercon (1981) in wheat. The electrical conductance was recorded in normal and in high temperature incubated at 50^{o}c. Conductance was converted to conductivity by multiplying it with a cell constant. The heat injury was calculated by the formula given below

$$\text{per cent heat injury} = 1 - \frac{[1-(T_1.T_2)]}{[1-(C_1.C_2)]} \times 100$$

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Where, T_1 and T_2 initial and final reading of treated sample, and C_1 and C_2 initial and final reading of control sample. Heat injury was classified as tolerant (below 35 %) mid tolerant (35-50 %) and susceptible 50 % and above.

Chlorophyll stability index: Heat treatment of 65°C for one hr was given to the another parallel samples running same procedures as followed for estimation of the chlorophyll content and Chlorophyll stability index was calculated as follows (Murty and Majumber, 1962).

$$\text{Chlorophyll stability index} = \frac{\text{Chlorophyll in heated sample}}{\text{chlorophyll in normal sample}} \times 100$$

Seedling vigour index: The seedling vigour index was calculated as per formula given by Nayeem and Mahajan (1991). Seedling vigour index = Germination percentage x Shoot length. Combining ability analysis was performed by following Griffing (1956) Method I and Model II.

Results and discussion

The high temperature prevailing during winter season hinders the productivity of wheat. Various physico-chemical processes are responsible for heat tolerance in wheat genotypes. The genetic variation with regards to these parameters among certain genotypes and their crosses in wheat would be of great value in developing heat tolerant wheat genotypes. The negative *gca* and *sca* for heat injury and stomatal frequency showed their role in heat tolerant mechanism in wheat. The analysis of variance revealed that variance due to genotypes, parents, parents *vs.* crosses and crosses were highly significant and significant for all the traits studied. These results clearly showed that the genotypes including parent and crosses differed among themselves for all the traits. The highly significant variance due to parent *vs.* crosses for most of the traits showed that the existence of heterotic effect (Table 1). Significant mean squares (Table 2) due to general combining ability and specific combining ability in pooled analysis revealed significance of both additive and dominance gene effects in inheritance of all the heat tolerance traits in wheat. The low predictability ratio revealed that all the characters studied were predominantly controlled by non- additive genes action. Similar results were also reported by Nayeem and Veer (2000) for chlorophyll content, chlorophyll stability index, heat injury, proline content and stomatal frequency.

The estimates of *gca* effects showed difference between parents from trait to trait. The *gca* effects represent the additive nature of gene action. A high general combiner is characterized by its better breeding value when crossed with number of other parents. Knowledge of relative importance of additive and non-additive gene action is essential to a plant breeder for the development of an efficient hybridization programme (Ahmad *et al.*, 2014). In the present investigation, general combining ability effects of 8 lines were estimated to know their genetic worth for use in production of superior progenies. The results revealed that none of the parents was found excellent combiners for all the characters (Table 3). The parental lines HD2687 (1.02**) followed by PBW 373 (0.77**) and RAJ 4037 were having highly significant positive *gca* effects for proline content and were best general combiner for this trait. The negatively high significant *gca* effects for stomatal frequency on upper surface was observed for parent Raj 4083 (-1.57**) and on lower surface for the parent PBW 373 (-1.87**) and Deshpande and Nayeem (1999) reported good general combiners for heat tolerance, chlorophyll content and stomatal frequency. The parents PBW 373 and Raj 4037 had highly significant negative *gca* effects for heat injury. Parent HD 2687 showed highly significant *gca* effect for chlorophyll stability index. For seedling vigour index parents RKA 501 and HD2687 were having highly significant positive *gca* effects. These result indicated that these parental lines possess favorable genes and that improvement in heat tolerant traits may be attained if they used in hybridization programme.

The specific combining ability effects represent non-fixable components of gene action. Among all 28 crosses, 10 crosses exhibited significant positive specific combining ability effects for seedling vigour index. Out of 28 crosses, only five crosses *viz.*, HD2687 x RKA 501, DBW-17 x PBW 373, DBW-17 x RAJ 3765, RAJ 4037 x RAJ 4083 and RAJ 4037 x RKA 501 exhibited significantly positive *sca* value for proline content, chlorophyll stability index and seedling vigour index. In respect of chlorophyll stability index DBW-17 x PBW 373, Raj 4037 x Raj 4083, Raj 4037 x Raj 4083, Raj 4037 x RKA 501 and Raj 3765 x Raj 4083 were the best. Similar findings were reported by Nayeem and Veer (2000). On the other hand HD 2687 x RKA 501, DBW-17 x PBW 373, DBW-17 x Raj 3765 and Raj 4037 x RKA 501 were exhibited significantly negative *sca* effects for stomatal frequency (upper), stomatal frequency (lower) and heat injury. The combination of DBW-17 x Raj 3765, Raj 3077 x Raj 4037, Raj 4037 x RKA 501, HD 2687 x Raj 4083, HD 2687 x RKA 501 and DBW-17 x PBW 373 possessed high *sca* effects for heat injury. These crosses had combination of low x high, low x medium, low x low and medium x low *gca* parents with negative *sca* effects for heat injury indicating the importance of non-additive genetic variation which can be exploited by multiple crosses followed by intermating among desirable segregants (Nayeem and Veer, 2000). Two best specific combiners with *sca* values for heat tolerance traits are shown in (Table 4). The parental *gca* values were classified as high, medium and low, derived from the total range of the *gca* value for any characters which was divided into three parts where 'L' indicates the lower one third, 'M' indicates the middle one third and 'H' indicates the upper one third in the range. The desirable combinations involved high x high, high x medium, high x low, medium x medium, medium x low and low x low types of general combiners. The desirable performance of combination like high x low may be ascribed to the interaction between dominant allele from good combiners and recessive allele from poor combiners. Moreover, high x low crosses can result in strong transgressive segregants for desired characters due to segregation of genes with strong potentials and their specific buffers (Langham, 1961). Such combinations were observed in the hybrid DBW-17 x PBW 373 for chlorophyll stability index. These desirable cross combinations which involve high x low *gca* effects in this situation could be utilized in recombination breeding.

Crosses, Raj 4037 x Raj 4083, DBW-17 x PBW 373 for seedling vigour index, HD 2687 x Raj 4083 and DBW-17 x Raj 3765 for stomatal frequency (lower) involving low x low and low x high *gca* parents indicated importance of non-additive genetic variation which can be exploited by multiple crosses followed by intermating among desirable segregants (Nayeem and Veer, 2000). Crosses, DBW-17 x Raj 3765 for proline content ,Raj 4037 x Raj 4083 for chlorophyll stability index and Raj 4037 x Raj 4083 for stomatal frequency (upper) were involved medium x low and medium x high general combiners respectively and so may be used for obtaining superior recombinants in advance generation. The eight hybrids and their parents were ranked amongst themselves on the basis of seedling vigour index, chlorophyll stability index and their contributing traits (Table 5).

Ranking of best eight hybrids and their parents was done on the basis of *sca* for seedling vigour index and chlorophyll stability index as the base characters and the *per se* performance of their attributing characters. When the ranking of eight best hybrids and their parents was done, and furnished in order: HD 2687 x Raj 3765 ranked the first (3.67) followed by Raj 4083 x RKA 501 (3.83), Raj 4037 x Raj 4083 (4.00), HD 2687 x DBW 17 (4.33), DBW-17 x Raj 3765 (4.83), Raj 3077 x RKA 501 (4.83), DBW-17 x PBW 373 (5.00), HD 2687 x Raj 4083 (5.50), RKA 501 (3.83), PBW 373 (3.83), Raj 3077 (4.00), Raj 3765 (4.17), HD 2687 (4.17), DBW 17 (5.17), Raj 4037 (5.33) and Raj 4037 (5.50).

Conclusion

It can be summarized that analysis of variance revealed that variance due to genotypes, parents, parents *vs*. crosses and crosses were highly significant and significant for all the traits studied. Significant mean squares due to general combining ability and specific combining ability in pooled analysis revealed significance of both additive and dominance gene effects in inheritance of all the heat tolerance traits in wheat. The low predictability ratio revealed that all the characters studied were predominantly controlled by non-additive genes action. The present study has identified desirable parents, RKA 501, PBW 373, Raj 3077, Raj 3765 and HD 2687 for most of the heat tolerant parameters The importance of additive but mainly non-additive gene action is highlighted. Improvement in heat injury and other heat tolerant parameters should be possible by resorting to biparental mating followed by recurrent selection or by diallel selective mating.

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Table 1. Analysis of variance (mean sum of square) for six characters in wheat

Characters/ Source of variation	df	Proline content	Stomatal frequency (upper)	Stomatal frequency (lower)	Heat injury (%)	Chlorophyll stability index	Seedling vigour index
Replication	1	1.45	1.41	3.23	2.22	0.96	1.09
Genotypes	35	17.22**	36.67**	26.31**	80.15**	43.25**	8264.09**
Parents	7	4.93**	20.47**	7.65*	46.09**	25.28**	5131.75**
Crosses	27	20.83**	40.81**	31.26**	90.88**	48.32**	9238.11**
Parent *vs.* Crosses	1	5.64*	38.39*	23.22**	28.86*	31.88**	3891.88**
Error	**35**	**1.30**	**5.63**	**3.01**	**5.23**	**4.27**	**214.59**

*, ** Significant at 5 and 1 per cent level, respectively

Table 2. Combining ability analysis for heat tolerance in bread wheat

Characters/ Source of variation	df	Proline content	Stomatal frequency (upper)	Stomatal frequency (lower)	Heat injury (%)	Chlorophyll stability index	Seedling vigour index
Gca	7	7.21**	13.96**	17.49**	22.51**	4.90*	6145.01**
Sca	28	8.96**	19.43**	12.07**	44.46**	25.80**	3628.8**
$\sigma^2 g$		1.53	7.80	11.19	13.93	1.94	4226.40
$\sigma^2 s$		77.55	465.28	295.80	1171.77	662.78	98602.20
$\sigma^2 g/\sigma^2 s$		0.02	0.02	0.04	0.01	0.00	0.04
$(\sigma^2 A/\sigma^2 A+\sigma^2 D)$		0.04	0.03	0.07	0.02	0.01	0.08
Error	**35**	**0.65**	**2.82**	**1.51**	**2.62**	**2.13**	**107.29**

*, ** Significance at 5 and 1 per cent levels of probability, respectively

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Table 3. Estimate of general combining ability effects for different characters in bread wheat

Characters/ Parents	Proline content	Stomatal frequency (upper)	Stomatal frequency (lower)	Heat injury (%)	Chlorophyll stability index	Seedling vigour index
HD 2687	1.02**	-0.60	-0.60	-0.19	1.28**	27.65**
DBW 17	-0.66**	0.03	0.24	0.63	-1.02*	-4.71
PBW 373	0.77**	0.82	-1.87**	-1.97**	0.20	-19.65**
Raj 3765	0.16	-0.79	2.65**	2.95**	-0.57	0.68
Raj 3077	-1.49**	1.88**	0.79*	-0.64	-0.45	-37.43**
Raj 4037	0.73**	-0.91	-0.69	-1.39**	0.40	-11.89**
Raj 4083	-0.10	-1.57**	-0.46	-0.01	0.03	5.96
RKA 501	-0.45	1.13*	-0.05	0.61	0.13	39.38**
SE(gi)	0.24	0.50	0.36	0.48	0.43	3.06
SE(gi-gj)	0.36	0.75	0.55	0.72	0.65	4.63

*, ** Significance at 5 and 1 per cent levels of probability, respectively

Table 4. Superior F_1 hybrids based on the *sca* effect for heat tolerance traits in wheat

Characters	Superior F_1 hybrid	*sca* effect	*Per se* of crosses	*gca* effects of parent
Proline content	DBW-17 x Raj 3765	5.06**	17.65	M x L
	DBW-17 x PBW 373	4.50**	17.70	M x M
Stomatal frequency (upper)	Raj 4037 x Raj 4083	-7.46**	37.96	M x H
	DBW-17 x PBW 373	-7.30**	41.46	L x M
Stomatal frequency (lower)	HD 2687 x Raj 4083	-7.08**	24.05	L x L
	DBW-17 x Raj 3765	-5.63**	29.45	L x H
Heat injury (%)	DBW-17 x Raj 3765	-15.16**	36.58	L x H
	Raj 3077 x Raj 4037	-10.84**	35.28	L x M
Chlorophyll stability index	DBW-17 x PBW 373	9.04**	24.30	H x L
	Raj 4037 x Raj 4083	7.60**	24.10	M x L
Seedling vigour index	Raj 4037 x Raj 4083	93.81**	413.15	L x L
	DBW-17 x PBW 373	75.75**	376.65	L x L

*, ** Significance at 5 and 1 per cent levels of probability, respectively

Per se,; H - High GCA value, M - Medium GCA value and L - Low *gca* effect , derived from the total range of the parental *gca* effect for any character which was divided into three parts where L - indicates the lower one third , M - indicates the middle one third and H - indicates the upper one third in the range.

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Table 5. Ranking of eight best hybrids on the basis of *sca* and their parents on the basis of *gca* for heat tolerance traits in wheat

Hybrids /parent	*sca* (cross) and *gca* (parent) effects		Ranking according to *per se* performances for heat tolerance traits							
	Chlorophyll stability index	Seedling vigour index	Y1	Y2	Y3	Y4	Y5	Y6	Total rank	Mean rank
Raj 4037 x Raj 4083	93.81**	7.60**	3	8	5	5	2	1	24	4.00
DBW-17 x PBW 373	75.75**	9.04**	1	7	7	7	1	7	30	5.00
DBW-17 x Raj 3765	69.67**	7.17**	2	5	6	8	3	5	29	4.83
HD 2687 x Raj 4083	48.78**	-1.08	5	6	8	6	5	3	33	5.50
Raj 3077 x RKA 501	43.52**	3.09*	7	3	3	4	4	8	29	4.83
Raj 4083 x RKA 501	42.25**	-2.39	6	2	4	2	7	2	23	3.83
HD 2687 x DBW 17	37.85**	-5.08**	8	1	2	1	8	6	26	4.33
HD 2687 x Raj 3765	37.71**	-2.58	4	4	1	3	6	4	22	3.67
Parents										
HD 2687	27.65**	1.28**	2	8	5	6	1	3	25	4.17
DBW 17	-4.71	-1.02*	7	7	4	4	5	4	31	5.17
PBW 373	-19.65**	0.20	4	2	1	8	2	6	23	3.83
Raj 3765	0.68	-0.57	1	1	8	1	7	7	25	4.17
Raj 3077	-37.43**	-0.45	5	3	2	3	3	8	24	4.00
Raj 4037	-11.89**	0.40	6	4	6	7	8	2	33	5.50
Raj 4083	5.96	0.03	8	5	3	5	6	5	32	5.33
RKA 501	39.38**	0.13	3	6	7	2	4	1	23	3.83

Y1 - Proline content, Y2 - Stomatal frequency (upper), Y3 - Stomatal frequency (lower), Y4 - Heat injury, Y5 - Chlorophyll stability index and Y6 - Seedling vigour index