Combining Ability Analysis of Heat Tolerance in *Gossypium hirsutum* L.

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Abstract: In order to study the genetic control of heat tolerance, four varieties/lines of *Gossypium hirsutum* L., namely MNH-554, Cocker-304, Delcerro and Albacala (71) 1190, were crossed in all possible combinations. Heat tolerance of 12 hybrids and their parents was measured at reproductive phase using an electrolyte leakage technique. The genetic analysis of the data showed significant effects of specific combining ability and reciprocals, but the effect of general combining ability appeared to be non-significant. Comparison of the parents for heat tolerance revealed that Delcerro and MNH-554 were the best general combiners for the character. The crosses Cocker-304 × Delcerro and Albacala (71) 1190 × MNH-554 were determined as the best combinations for heat tolerance and they involved one good general combiner as a parent. Significant effects due to specific combining ability indicated the importance of non-additive gene effects controlling heat tolerance. This information suggests that development of heat tolerance in the present plant material may be difficult; therefore it is necessary to design a specific breeding programme for efficient transfer of the trait.

Keywords: combining ability; genetic basis; *Gossypium hirsutum*; heat tolerance; membrane thermal stability

Although the plant of *hirsutum* sp. is a "sun-loving" plant, an excessively higher temperature at reproductive phase (above 36°C) decreases its production significantly (Baloch *et al*. 2000). According to an estimate, the cotton (*Gossypium hirsutum* L.) plant sheds about 65–70% of its fruiting points due to heat-induced sterility, spotted bollworm attack and increased humidity during monsoon. The major proportion of this loss occurs due to high temperature which induces sterility in pollen grains (Taha *et al*. 1981; Kittock *et al*. 1988; Baloch *et al*. 2000). High temperature disrupts the movement of water, ion, and inorganic solutes across the plant membrane, which interferes with photosynthesis and respiration (Christiansen 1978). Clearly, an increase in high temperature at the reproductive phase is the major factor of low productivity of cotton varieties grown in the cotton belt of Pakistan (Anonymous 1994). Keeping in mind the importance of the problem in cotton production, it is necessary to introduce heat tolerance into modern cotton cultivars. A great deal of progress has already been made during the last two decades by developing short-timed and dwarf varieties like NIAB-78 and many others. However, the growing demand for edible oil and the expanding textile industry in the country make breeders improve the ability of the cotton plant to withstand under harsh climatic conditions.

The improvement of heat tolerance in cotton plant requires the knowledge of its genetic mechanisms. Thus before formulating a breeding programme aimed to develop cotton genotypes with increased heat tolerance, the scheme of a genetic experiment and analysis is essential. The phenomenon of heat tolerance was studied in *Triticum aestivum* L. (Saadalla *et al*. 1990a, b; Ibrahim & Quick 2001a, b), *Sorghum bicolor* (L.) Moench (Sullivan & Ross 1979), *Vigna unguiculata* (L.) Walp. (Marfo & Hall 1992) and *Brassica* species (Morrison & Stewart

2002); the results revealed their different genotypic responses to high temperature. In wheat, Ibrahim and Quick (2001a) measured heat tolerance using triphenyl tetrazolium chloride (TTC) and membrane thermal stability (MTS) techniques, with the values being intermediate to high (0.50–0.65) for TTC and relatively low (0.32–0.38) for MTS. In another study (Reynolds *et al*. 1994) MTS values were found to be positively associated with grain yield of wheat under heat stressed conditions. Ibrahim and Quick (2001b) performed genetic analysis of heat tolerance and found that mean squares of general combining ability (GCA) were four times higher than those of specific combining ability (SCA), suggesting the importance of additive gene effects for heat tolerance. Using diallel cross data, Moffatt *et al*. (1990) studied the genetic control of high temperature tolerance in wheat based on chlorophyll fluorescence and reported significant GCA and maternal effects and suggested that additive genetic effects may be exploited through breeding to induce tolerance to high temperature. Previous studies of heat tolerance in cotton showed that variation in this character existed in *hirsutum* sp., and researchers used different techniques to screen out heat tolerant genotypes (Rodriguez-Garay & Barrow 1988; Baloch *et al*. 2000; Sethar *et al*. 2001). The objective of the present study was to examine the genetic basis of variation in heat tolerance in cotton. For this reason four varieties were crossed and diallel data were analysed following the approach of Griffing (1956). The information reported herein could be helpful to cotton breeders for continuing improvement of heat tolerance in *G. hirsutum*.

MATERIALS AND METHODS

The cotton varieties/lines, MNH-554, Cocker-304, Delcerro and Albacala (71) 1190, were crossed in all possible combinations in a greenhouse to develop the experimental material for the study. The four parents belonging to *Gossypium hirsutum* L. were taken from the gene pool maintained in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The 12 hybrids and the parents were planted in the field in triplicate according to the randomised complete block design. Conventional agronomic practices and plant protection were adopted to obtain a healthy plant population.

Assessment of heat tolerance

Heat tolerance in the experimental material was assessed at the reproductive phase using an electrolyte leakage technique applied previously in cotton (Gorham *et al.* 1998) and wheat (Ibrahim & Quick 2001a, b). For this purpose three leaves of each plant of each entry were taken at the reproductive phase. The collection of samples continued for one week, at temperatures ranging between 27 and 38°C. Two discs of equal size were taken from each of the sampled leaves with a sharp steel pipe of 12.5 mm diameter and rinsed twice with deionised water. Six discs taken from each experimental plant were placed in 16 × 150 mm test tubes containing 10 ml deionised water. The test tubes were kept in a water bath at 49°C for 30 min, taking care the contents of the test tube were submerged in the water. After the treatment period the tubes were held overnight at room temperature and the leakage of electrolytes (conductance) was measured with electrical conductivity meter. The samples in the test tubes were autoclaved for 10 min at 120°C and conductance was measured again when the temperature of the tubes reached that of room. Membrane thermal stability (MTS) was calculated in percentage units using the following formula

$$MTS = (1 - T_1/T_2) \times 100$$

where: T_1, T_2 refers to conductivity readings after heat treatment and after autoclaving, respectively

Statistical procedures

The 4 × 4 diallel data were subjected to traditional analysis of variance to reveal the significant genotypic differences. Data were further analysed according to Griffing (1956) Method I, Model II.

RESULTS

Among the 12 hybrids, MTS values ranged from the high (40.72%) of the cross Delcerro × Cocker-304 to the low (17.21%) of the cross Albacala (71) 1190 × MNH-554 (Table 2). Genotypic differences between the 12 hybrids and their parents were highly significant, $P \leq 0.01$ (Table 1). The results of analysis for combining ability are presented in Table 3. Highly significant ($P \leq 0.01$) differences were observed for specific combining ability (SCA) and reciprocal effect, but the effects of general combing ability

Table 1. ANOVA for heat tolerance evaluation in F_1 hybrids and their parents of *G. hirsutum* L.

Source of variation	D.F.	Sum of squares	Mean squares
Replication	2	80.29633	40.1481
Genotypes	15	1598.942	106.596**
Error	30	170.6828	5.6894

**differences significant at $P \leq 0.01$

Table 2. Diallel analysis of heat tolerance in F_1 hybrids and their parents of *G. hirsutum* L.

Varieties/lines	MN554	Cocker-304	Delcerro	Albacala (71) 1190	Mean value	Standard error
MN554	37.78	27.04	23.42	29.95	29.55	3.05
Cocker-304	34.11	29.71	24.04	26.46	28.58	2.18
Delcerro	35.08	40.72	31.62	29.90	34.33	2.39
Albacala (71) 1190	17.21	27.05	30.74	36.25	27.81	4.01

Table 3. Combining ability analysis for heat tolerance of F_1 hybrids and their parents of *G. hirsutum* L.

Source of variation	D.F.	Sum of squares	Mean squares	Standard error
GCA	3	11.8785	3.960^{NS}	0.69
SCA	6	207.1506	34.525**	1.19
Reciprocal	6	313.9498	52.325**	1.37
Error	30	56.88	1.896	

**differences significant at $P \leq 0.001$; ^{NS}differences insignificant at $P > 0.05$

(GCA) appeared to be non-significant (P > 0.05). The values of general combining ability of different lines are given in Table 4. Although the effects of GCA were non-significant (P > 0.05), GCA of some parents was more pronounced than of the others. For example, the line Delcerro having the positive value 0.825 had better GCA than MNH-554 with the value 0.227. The other two parents, Cocker-304 and Albacala (71) 1190, having negative values –0.211 and –0.841, respectively, showed poor GCA for heat tolerance.

The values of specific combining ability of different parental combinations are shown in Table 4. The comparison reveals that three out of the six hybrids exhibited good SCA for heat tolerance MNH-554 × Cocker-304, Cocker-304 × Delcerro, Delcerro × Albacala (71) 1190, which attained 0.492, 1.701 and 0.269, respectively. The remaining three crosses, MNH-554 × Delcerro, MNH-554 × Albacala (71) 1190 and Cocker-304 × Albacala (71) 1190, had negative SCA values for heat tolerance.

The evaluation of parental line in reciprocal combinations showed that Albacala (71) 1190 × MNH-554 had the highest positive value (6.372) and proved to be the best combination, whilst other hybrids exhibited negative values.

DISCUSSION

After the emergence of cotton seedlings from the soil, various stresses, both biotic and abiotic ones, affect the growth and productivity of plants. The availability of information on the genetic mechanism of heat tolerance in cotton, i.e. additive, non-additive and epistasis, is essential in order to pursue a breeding programme effectively. In the present examination the sample of parents included in the crossing programme was small, yet the differences in MTS values were discernible. The lines MNH-554 and Albacala (71) 1190 with MTS values of 37.78 and 36.25, respectively, were found to be more stable under heat stress than

Table 4. General combining ability (GCA), specific combining ability (SCA) and reciprocal effects for heat tolerance in *G. hirsutum* L.

Parents	GCA	SCA	Reciprocal
MN554	0.227	–	–
Cocker-304	–0.211	–	–
Delcerro	0.825	–	–
Albacala (71) 1190	–0.841	–	–
$cd_1\ (g_i - g_j)$	1.349	–	–
Cross combinations			
MNH-554 × Coker-304	–	0.492	–3.538
MNH-554 × Delcerro	–	–1.871	–5.832
MNH-554 × Albacala (71) 1190	–	–5.875	6.372
Coker-304 × Delcerro	–	1.701	–8.342
Coker-304 × Albacala (71) 1190	–	–2.285	–0.293
Delcerro × Albacala (71) 1190	–	0.269	–0.423
$cd_1\ (s_{ij} - s_{ik})$	–	2.337	–
$cd_1\ (r_{ij} - r_{kl})$	–	–	2.699

Delcerro whilst Cocker-304 with low MTS values (29.71) was revealed to be heat susceptible (Table 2). The MTS values of 12 hybrids differed significantly from those of their parents (Table 1). Highly significant differences due to specific combining ability effects suggested that a major proportion of variation in heat tolerance was controlled by dominant properties of genes (Griffing 1956). Previous information on the genetics of heat tolerance in cotton is not available in the literature, however such studies in common bean (Shonnard & Gepts 1994) and wheat (Nayeem & Veer 1998) revealed that heat tolerance in these species was controlled by non-additive gene effects. It has been suggested that the magnitude of additive variance component and coefficient of heritability increase positively with the stress factor (Kacser & Burns 1981; Blum 1988), but in sorghum (Azhar & McNeilly 1988) and maize (Rao & McNeilly 1999), both studied for salinity tolerance, the additive variance was suppressed with the increasing concentration of NaCl and consequently with the estimates of narrow-sense heritability. The higher magnitude of non-additive variation revealed in the present plant material tested for heat stress suggests that the pattern of inheritance of heat tolerance in cotton is complex. Due to the same reason, the estimates of narrow-sense heritability for heat tolerance in cowpea (Marfo & Hall 1992) and green beans (Dickson & Petzoldt 1989) were low, 26 and 14%, respectively. Similarly for tomato (*Lycopersicon esculentum* L.), Shelby *et al.* (1978) reported that heat tolerance during the fruit set was conferred by a few partially dominant genes, but narrow-sense heritability was very low (8%). The presence of non-additive effects on the genetic control of heat tolerance suggests that selection for increased tolerance may not be made in the early breeding generations, and therefore recurrent selection with periodic inter-crossing appears to be the best method as followed in rice by Wu *et al.* (2000). Although there is sufficient evidence supporting the present studies, yet the information obtained cannot be generalised. Therefore it is suggested that another study involving a large number of varieties/lines differing in heat tolerance must be performed in order to clarify the present data.

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Souhrn

Azhar M. T., Khan A. A., Khan I. A. (2005): **Analýza kombinační schopnosti při studiu tolerance** *Gossypium hirsutum* L. **k vysokým teplotám**. Czech J. Genet. Plant Breed., **41**: 23–28.

U čtyř odrůd/linií *Gossypium hirsutum* L. (MNH-554, Cocker-304, Delcerro a Albacala (71) 1190) bylo provedeno dialelní křížení včetně reciprokých kombinací s cílem studia genetické kontroly tolerance k vysokým teplotám. Tolerance byla vyhodnocena u rodičů a jejich dvanácti hybridů v reprodukční fázi růstového cyklu měřením vodivosti na odstřižených listových discích. Analýza naměřených hodnot potvrdila statisticky významný podíl specifické kombinační schopnosti a vliv reciprokých křížení na rozdíl od obecné kombinační schopnosti, jejíž efekt nebyl statisticky významný. Při porovnání rodičů z hlediska jejich tolerance k vysokým teplotám Delcerro a MNH-554 měly nejlepší obecnou kombinační schopnost. Kombinace křížení Cocker-304 × Delcerro a Albacala (71) 1190 × MNH-554 byly potvrzeny jako nejlepší z hlediska tolerance k vysokým teplotám, přičemž v každém byla zastoupena odrůda/linie s nejlepší obecnou kombinační schopností. Na specifické kombinační schopnosti se významně podílely neaditivní účinky genů determinujících studovaný typ tolerance. Tento závěr naznačuje obtížnost šlechtění na zvýšenou toleranci k vysokým teplotám a předpokládá vypracování speciálního šlechtitelského postupu pro introdukci tohoto znaku.

Klíčová slova: kombinační schopnost; genetická analýza; *Gossypium hirsutum*; tolerance k vysokým teplotám; stabilita buněčných membrán

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