Drought Stress Modified Genetic Components and Combining Ability of Cotton Genotypes

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Research

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Abstract

**Background:** Gene action and combining ability of the genotypes are two important components in crop breeding and may be influenced by environmental stresses which sometimes mislead the selection process. Therefore, an experiment was conducted to assess the behavior of genetic parameters of cotton (*Gossypium hirsutum*) under drought stress during the year 2017-19. Initially, 50 genotypes of cotton were screened for drought stress tolerance potential by subjecting them to 4 week long drought stress (at 20% field capacity). Based on physiological parameters, 5 drought tolerant and 4 drought susceptible genotypes (lines and testers respectively) were selected and hybridized using Line × Tester technique to develop F$_1$ crosses. In the next year, 9 parents and their 20 F$_1$ hybrids were evaluated in field under normal irrigation and drought stress (at 50% field capacity).

**Results:** Stronger specific combining ability effects observed for seed cotton yield, number of bolls, boll weight and lint percentage (%) indicated active role of non-additive genes operating in the direction of lower parents with the exception for number of bolls where dominance worked towards superior parents. Best varietal combinations identified under water stress for number of bolls and boll weight were CIM-496 × NF-801-2-37, DPL-26 × NF-801-2-37, B-557 × NF-801-2-37, B-557 × MNH-129. Variety B-557 was found to be good general combiner followed by BOU-1724 for number of bolls, boll weight and lint % under drought.

**Conclusion:** Combining ability analysis indicated critical role of non-additive component in total variation suggesting weak heritability for all the traits. Direct selection in this material is therefore not advisable. Drought had suppressed variability and restricted the expression of yield characters, however, best identified varietal combinations for seed cotton yield, number of bolls and boll weight may be exploited for the development of hybrid to be grown in drought hit areas of Pakistan.

Introduction

Pakistan, geographically located in semi-arid region earns 90% of agricultural returns from irrigated lands, thus, canal irrigation is the main practice of this area. The country annually suffers 40-60% decrease in agricultural productivity due to extensive water loss owing to seepage and poor lining of water canals as reported by Cheema et al. (2014) and Lashari and Mahesar (2012). Water is the main input of agriculture sector in many regions of the world, but population explosion and unplanned urbanization had resulted in massive contraction of water reservoirs.

Cotton productivity markedly depends on regular supply of water obtained either through irrigation or rainfall (Iftikhar et al. 2012). Stable cotton yield is an important breeding objective of drought tolerance improvement programmes. Genetically controlled physiological responses in cotton plant for example, relative leaf water content, and Relative Cell Injury % help to sustain dry spells during its growing period and have been frequently used as indicator traits in identification of drought and heat tolerant plants (Amjid et al. 2015; Hejnak et al. 2015; Kader et al. 2015; Zahooor et al. 2017; Ahmad et al. 2020). Similar
information on genetic variability is required for yield related attributed which can be observed only at adult stage. Significant additive variation has been reported in yield associated characters for instance, average boll weight, number of bolls, boll diameter and lint % in drought stressed cotton plants (Alishah and Ahmadikhah 2009; Soomro et al. 2010; Panni et al. 2012).

This study was planned to provide information on water stress tolerance in cotton. Firstly, drought tolerant and susceptible cultivars were selected by subjecting 50 cotton cultivars to variable water conditions to determine variability for drought triggered physiological responses (relative water content; RWC, excised leaf water content; ELWL, relative cell injury %; RCI%) at seedling stage to accomplish selection of drought tolerant and susceptible cultivars. Line × Tester mating design developed by Kempthorne (1957) was used to make crosses using drought tolerant cultivars as lines and susceptible ones as testers to develop plant material for investigating genetic mechanisms controlling water stress tolerance in mature cotton. The advent of molecular techniques has restricted use of conventional breeding to certain extent. Like other environmental stresses, drought not only influences expression of genes but also modify the inheritance pattern, gene action and combining ability of the genotypes (Mahmood et al. 2020). Therefore, this study was planned to assess the impact of drought stress on inheritance pattern, gene action and combining ability of cotton genotypes in semi-arid regions. The information obtained from this study may facilitate plant breeders to make effective improvements in breeding program for development of drought tolerant cotton varieties.

**Materials And Methods**

This research was performed in research area of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad during May 2017-2019 in three phases (Seedling screening, development of crosses in Line × Tester technique and field evaluation of parents and their F1 hybrids) for yield traits.

**Physiological screening to select potential parents and hybridization**

During May-June (2017), presoaked seeds of 50 cultivars were sown at 2.5 cm depth in soil filled polythene bags arranged in a triplicated trial under normal (100% field capacity) and drought conditions (20% field capacity). During experiment, temperature was recorded to be 35ºC and humidity conditions 65-80% respectively. Stress was imposed at emergence of first pair of true leaves and continued till four weeks after which data were recorded on physiological traits, RWC, ELWL and RCI% using formulae developed by Clarke & Townley-Smith (1986) and Matin et al. (1989) respectively. RCI% was measured by protocol of Sullivan (1972). Third fully expanded leaf of each of the 50 cultivars was used to record data for RWC and ELWL whereas youngest leaf was used to assess RCI%. Data recorded were then analyzed using ANOVA (Steel et al. 1997). Results of physiological screening exhibited variable responses of cultivars to water stress, but some cultivars including DPL-26, CIM-496, B-557, 149F and BOU-1724 outperformed others by showing maximum water retention (approximately 70%), minimum water loss (38%) and RCI% (39%) under 20% field capacity and identified as drought tolerant whereas four cultivars, MNH-129, FH-1000, H-499 and NF-801-2-37 were shortlisted as drought susceptible for showing poor
water retention (below 38%) and maximum RCI% (more than 75%) under 20% field capacity. These nine cultivars were selected as parents and field planted during May to September (2018) to develop genetic material keeping tolerant ones as lines (female parents) and susceptible ones as testers (male parents). Seed cotton was harvested from selfed and crossed bolls and separated into lint and cotton seed using a single roller electric gin machine (approximately 20-25 seeds per cross were obtained).

**Field evaluation of parents and their hybrids at adult stage**

The performance of parents (five lines and four testers) and their 20 F₁ hybrids was evaluated during May 2019 by sowing seeds in triplicated field trial following completely randomized block design under two water levels; 100% (T₀) and 50% (T₁) field capacity. Presoaked seeds of 29 entries were sown 30 cm apart within rows and 75 cm between rows. Water stress was imposed at squaring stage (30 days after sowing) and continued till harvesting. Data were recorded on bolls per plant of each family, boll weight and lint % as described below.

**Boll number per plant**

At maturity, two pickings were made to collect bolls showing maximum opening from normal and water stressed cotton plants. Average boll number for each family was calculated by counting total bolls on each plant in each family to get total number of bolls of a family.

**Boll weight (g)**

Data regarding weight of boll were obtained by dividing total seed cotton yield of a plant by the respective number of bolls counted on that plant.

**Lint %**

For lint %, clean dried samples of seedcotton/family were weighed, ginned and lint % was calculated by following formula

\[
\text{Lint} \% = \left(\frac{\text{Weight of lint in a sample}}{\text{weight of seed cotton in a sample}}\right) \times 100
\]

**Results**

Mean data for average boll number, boll weight and lint % under two water levels were analyzed using ANOVA (Steel et al. 1996) for seeking significant differences among genetic material, water treatments and interaction between them (Table 1a, b). Genetic analysis of data was performed following Line × Tester technique (Kempthorne 1957) to estimate general and specific combining ability effects.
**Table 1a** Mean squares of number of bolls, boll weight and lint % under normal water supply

| Source of variation   | df | No. of bolls per plant | Boll weight | Lint %   |
|-----------------------|----|------------------------|-------------|----------|
| Replications          | 2  | 16.99<sup>ns</sup>     | 2.18<sup>*</sup> | 20.52<sup>ns</sup> |
| Cultivars             | 28 | 32.28<sup>**</sup>     | 0.45<sup>ns</sup> | 18.51<sup>**</sup> |
| Parents               | 8  | 50.28<sup>**</sup>     | 0.47<sup>ns</sup> | 33.08<sup>**</sup> |
| Crosses               | 19 | 24.07<sup>**</sup>     | 0.46<sup>ns</sup> | 11.04<sup>ns</sup> |
| Parents vs Crosses    | 1  | 44<sup>*</sup>         | 0.14<sup>ns</sup> | 43.75<sup>*</sup> |
| Lines                 | 4  | 48.29<sup>**</sup>     | 0.60<sup>ns</sup> | 12.47<sup>ns</sup> |
| Testers               | 3  | 42.65<sup>**</sup>     | 0.69<sup>ns</sup> | 25.43<sup>*</sup> |
| Line×Tester           | 12 | 11.36<sup>**</sup>     | 0.35<sup>ns</sup> | 6.97<sup>ns</sup> |
| Error                 | 56 | 10.75<sup>ns</sup>     | 0.46<sup>ns</sup> | 8.72<sup>ns</sup> |

ns: non significant, **: Highly significant (P ≤ 0.01), *: Significant (P ≤ 0.05)

**Table 1b** Mean squares of number of bolls, boll weight and lint % under water stress conditions

| Source of variation   | df | No. of bolls per plant | Boll weight | Lint %   |
|-----------------------|----|------------------------|-------------|----------|
| Replications          | 2  | 5.14<sup>ns</sup>     | 0.09<sup>ns</sup> | 52.34<sup>**</sup> |
| Cultivars             | 28 | 11.84<sup>**</sup>     | 0.28<sup>ns</sup> | 19.53<sup>**</sup> |
| Parents               | 8  | 14.02<sup>**</sup>     | 0.46<sup>*</sup> | 29.69<sup>**</sup> |
| Source               | Df | MS    | F    | P   |
|---------------------|----|-------|------|-----|
| Crosses             | 19 | 11.52 | 0.22 ns | 15.62 * |
| Parents vs Crosses  | 1  | 0.57 ns | 0.15 ns | 12.52 ns |
| Lines               | 4  | 26.46 ** | 0.50 * | 49.81 ** |
| Testers             | 3  | 5.84 ns | 0.08 ns | 20.36 ns |
| Line×Tester         | 12 | 7.96 ** | 0.16 ns | 3.04 ns |
| Error               | 56 | 3.21 | 0.22 ns | 9.98 |

ns: non significant, **: Highly significant (P ≤ 0.01), *: Significant (P ≤ 0.05)

The detailed result for screening based on three physiological traits (RWC, ELWL and RCI%) have been exempted from the text and only mean squares are given here which showed that 50 drought stressed cotton cultivars differed significantly (P ≤ 0.01) for RWC (MS = 218*) and ELWL (MS = 142.1*) but not for RCI% (MS = 0.49), therefore selection of potential parents was made on basis of RWC and ELWL. Although drought influenced water retention capacity of genotypes, yet some cultivars effectively respond, for example, BOU-1724, B-557, 149F, CIM-496 and DPL-26 maintained leaf water content above 70% and thus found to be tolerant to water stress. In contrast, MNH-129, FH-1000, H-499 and CIM-109 showed below 24% RWC indicating their vulnerability to water stress. When drought was applied, cultivars showing 38%-41% ELWL were characterized as tolerant cultivars (BOU-1724, 149F, B-557, CIM-496 and DPL-26) as compared to those which revealed >70% water loss. Similarly, minimum membrane leakage was observed in CIM-496, DPL-26 (43%), 149F (42%), B-557 (40%) and BOU-1724 (39%) indicating better adaptability to water stress. Cultivars H-499, S-12, NF-801-2-37, MNH-129 and FH-1000 suffered from severe membrane damage.

**Genetic analysis of total bolls, boll weight and lint %**

ANOVA using Line × Tester technique revealed significant variation (P ≤ 0.01) among 9 parents (5 lines and 4 testers) and their 20 F₁ hybrids for total bolls/plant, average boll weight and lint %. Under drought stress, 29 genotypes differed highly significantly for yield contributing traits (number of bolls and lint %) and non-significantly for boll weight. Significant effects of general combining ability (due to parents) were noted for number of bolls, lint % and boll weight. Number of bolls and lint % showed significant effects for specific combining ability (due to crosses). Mean squares due to 5 lines were highly significant (P ≤ 0.01) for number of bolls and lint %. Parental interaction (lines and testers) appeared to be strong only for number of bolls.
Genetic components of variation under normal supply and drought

Parental and hybrid genotypes grown under normal water supply showed dominant role of non additive genes for boll weight and lint % due to significant specific combining ability ($\sigma^2_{sca}$). Significant gca variance for number of bolls (0.20) showed strong contribution of additive genes. High specific combining ability ($\sigma^2_{sca}$) was noted in drought effected cultivars for number of bolls (1.58), boll weight and lint % suggesting active role of non additive genes. A slight contradiction in direction of dominance was observed where unlike boll number, environmental expression of boll weight and lint % was found to be more inclined towards lower parents. Variance ratio calculated for yield contributing traits was below unity and thus controlled by non additive genes. Estimated components of variation are presented in Table 2.

Table 2 Genetic components of variation for number of bolls, boll weight and lint % under normal supply and water stress conditions

| Traits | Normal water supply | Water stress conditions |
|--------|---------------------|------------------------|
|        | $\sigma^2_{gca}$  | $\sigma^2_{sca}$ | $\sigma^2_A$ | $\sigma^2_D$ | $\sigma^2_{gca}/\sigma^2_{sca}$ | $\sigma^2_{gca}$ | $\sigma^2_{sca}$ | $\sigma^2_A$ | $\sigma^2_D$ | $\sigma^2_{gca}/\sigma^2_{sca}$ |
| NBPP   | 0.37                | 0.20                  | 0.74           | 0.20          | 1.84                     | 0.10              | 1.58           | 0.21          | 1.58           |
| BW     | 0.003               | -0.04                 | 0.01           | -0.04         | -0.08                    | 0.002             | -0.02          | 0.004         | -0.02         | -0.08                  |
| LP     | 0.12                | -0.58                 | 0.24           | -0.58         | -0.20                    | 0.37              | -2.31          | 0.73          | -2.31         |

NBPP: number of bolls per plant, BW: boll weight, LP: lint %, $\sigma^2_{gca}$: estimate of GCA variance, $\sigma^2_{sca}$: estimate of SCA variance, $\sigma^2_A$: additive variance, $\sigma^2_D$: dominance variance, $\sigma^2_{gca}/\sigma^2_{sca}$: variance ratio

Cultivars 149F and B-557 were identified as good general combiners under drought stress for having maximum gca values for boll number and boll weight. BOU-1724 with numerical values of 0.55 and 1.81 showed strong general combining ability for number of bolls and lint %, respectively. Among testers, H-
499 was found to be better general combiner for boll weight and lint % than for number of bolls per plant. FH-1000 exhibited better general combining ability for lint % only while MNH-129 turned out to be poor general combiner for all traits (Table 3).

Table 3 General combining abilities of the cultivars under normal supply and water stress conditions

| Traits          | Normal water supply | Water stress conditions |
|-----------------|---------------------|------------------------|
|                 | NBPP    | BW   | LP   | NBPP    | BW   | LP   |
| Parents         |         |      |      |         |      |      |
| CIM-496         | -3.12   | -0.20| -0.46| -2.57   |
| -0.33           |         |      |      | -0.33   | -2.57|
| 149-F           | 0.52    | -0.17| 0.76 | 0.77    | 0.14 |
| -0.22           |         |      |      | -0.22   |      |
| DPL-26          | -0.46   | 0.30 | -1.55| 0.13    | -0.05|
| -1.00           |         |      |      | -1.00   |      |
| BOU-1724        | 0.76    | -0.11| 0.93 | 0.55    | 0.09 |
| 2.83            |         |      |      | 2.83    |      |
| B-557           | 2.29    | 0.17 | 0.31 | 1.13    | 0.16 |
| 0.97            |         |      |      | 0.97    |      |
| SE              | 0.95    | 0.19 | 0.85 | 0.51    | 0.13 |
| 0.91            |         |      |      | 0.91    |      |
| Testers         |         |      |      |         |      |      |
| FH-1000         | 1.78    | -0.04| 1.01 | -0.50   |
| 0.08            |         |      |      | 0.08    |      |
| NF801-2-37      | -1.94   | 0.26 | -1.73| -0.35   |
| -0.04           |         |      |      | -0.04   |      |
| Crosses          | NBPP  | BW   | LP   | NBPP  | BW   |
|------------------|-------|------|------|-------|------|
| CIM-496×FH-1000  | -0.89 | -0.09| -0.47| 0.05  | 0.35 |
| 0.16             |       |      |      |       |      |
| CIM-496×NF-801-2-37 | -0.25 | -0.13| 0.48 | 1.17* | -0.29|
| -1.48            |       |      |      |       |      |
| CIM-496×MNH-129  | -0.27 | -0.06| 1.08 | -1.02 | -0.33|
| 1.18             |       |      |      |       |      |
| CIM496×H-499     | 1.41  | 0.28 | -1.08| -0.20 | 0.27 |
| 0.14             |       |      |      |       |      |
| 149F×FH-1000     | -0.59 | 0.03 | -0.78| -0.25 | 0.09 |
| 1.05             |       |      |      |       |      |
| 149F×NF-801-2-37 | -1.51 | 0.07 | -0.08| -2.51 | 0.05 |
| -0.29            |       |      |      |       |      |

NBPP: number of bolls per plant, BW: boll weight, LP: lint %, SE: standard error

**Table 4** Specific combining abilities of the cultivars under normal supply and water stress conditions
| Combination                  | 0.52 | -0.32 | 0.63 | 0.89 | -0.09 |
|-----------------------------|------|-------|------|------|-------|
| 149F×MNH-129, -0.66         |      |       |      |      |       |
| 149F×H-499, -0.10           | 1.57 | 0.21  | 0.23 | 1.86*| -0.05 |
| DPL-26×FH-1000, -0.70       | 3.46*| -0.45 | 2.43*| 0.12 | -0.15 |
| DPL-26×NF-801-2-37, 1.08    | 0.24 | 0.60* | -1.07| 1.52*| 0.19  |
| DPL-26×MNH-129, 0.01        | -3.07| 0.19  | -2.03| -0.83| 0.03  |
| DPL-26×H-499, -0.39         | -0.63| -0.34 | 0.66 | -0.80| -0.08 |
| BOU-1724×FH-1000, -0.22     | -1.64| 0.17  | -0.88| 1.57*| -0.17 |
| BOU-1724×NF-801-2-37, -0.87 | 0.33 | -0.40 | -1.24| -1.85| -0.01 |
| BOU-1724×MNH-129, 0.55      | 1.08 | 0.10  | 1.93*| -0.32| 0.29* |
| BOU-1724×H-499, 0.54        | 0.23 | 0.13  | 0.19 | 0.60 | -0.10 |
| B-557×FH-1000, -0.34        | -0.34| 0.34  | -0.30| -1.50| -0.11 |
| B-557×NF-801-2-37, 1.55     | 1.19 | -0.15 | 1.91*| 1.67*| 0.06  |
| B-557×MNH-129, -1.08        | 1.74 | 0.09  | -1.61| 1.28*| 0.10  |
| B-557×H-499, -1.09          | -2.59| -0.28 | -0.01| -1.46| -0.05 |
| **Standard Error**           | 1.89 | 0.39  | 1.70 | 1.03 | 0.27  |
| **1.82**                     |      |       |      |      |       |
Comparison of sca estimates (Table 4) found that under fully irrigated conditions, H-499 and MNH-129 hybridized well with maximum parents, for instance, CIM-496, 149F and BOU-1724 for all three yield attributes. Evaluation of drought stressed hybrids for number of bolls revealed that CIM-496, DPL-26 and B-557 combined well with NF-801-2-37, whereas 149F and BOU-1724 made good combinations with H-499 and FH-1000 respectively. For boll weight, CIM-496 and BOU-1724 performed better with FH-1000 and MNH-129 respectively. B-557 and DPL-26 showed perfect hybridization with NF-801-2-37 for lint %.

Discussion

Drought tolerance, being a quantitative trait is considered as a challenging breeding objective in crop improvement tasks. In this study, examination of the data for 50 cultivars revealed variable responses to moisture stress. Data analysis showed adequate genetically controlled variation in the genetic material examined here, which can be exploited through hybridization. Genetic analysis dissected components of variation under moisture stress at maturity. Low general combining ability variance (\(\sigma^2_{gca}\)) found for yield contributing traits (boll number, boll weight, lint %), suggested non-additive genetic control under restricted water supply. Studies reported by Javaid et al. (2014; 2015), Khokhar et al. (2018) and Shahzad et al. (2019) also identified dominance variance in the inheritance of yield traits proposing use of such genetic material in hybrid breeding. The incidence of drought interferes with the functioning of regulatory genes by modifying their expression in order to achieve better adaptation to climate anomalies. Gene action under hostile environmental conditions could be easily altered depending on genetic diversity, linked loci, magnitude and direction of allelic polymorphism available in experimental material (Gioi et al. 2017). In the present studies, dissection of total variation into its components revealed the presence of greater sca variance for number of bolls, boll weight and lint % under water stress. Sprague and Tatum (1942) and Griffing (1956) stated that higher magnitude of sca signifies the value of non additive genes in determining expression of plant characters, and is an indication of low heritability (Falconer and Mackay, 1996). Direct selection of desirable plants is therefore not recommended in segregating populations. Previous studies reported by Magwanga et al. (2020) and Sarwar et al. (2012) for drought tolerance on cotton had documented dominant non additive genetic effects for boll number, boll weight and lint %, and thus are consistent with the present findings.

Comparison of general combining abilities of nine parents (five lines and four testers) revealed B-557 and BOU-1724 as good general combiners for number of bolls, boll weight and lint % under water stress treatment. Among testers, FH-1000 appeared to contribute positively as parent for boll weight and lint %. It is imperative to mention here that these inferences are confined to the present investigations. Six crosses revealed good manifestation for number of bolls, for instance, CIM-496 × NF-801-2-37, DPL-26 × NF-801-2-37, B-557 × NF-801-2-37, B-557 × MNH-129, BOU-1724 × FH-1000 and 149F × H-499, and parental cultivars involved in these six crosses had complementary gca, except for CIM-496 × NF-801-2-
37 which used both the parents with poor gca. Cultivar MNH-129 didn’t make sufficient contribution as parent for all the traits, however it combined well with B-557 and BOU-1724 (good general combiners) to exploit its potential for number of bolls and boll weight. Varietal differences observed in parents and hybrids could be justified on basis of genotype and environment interaction (Sezener et al. 2015; Rehman et al. 2020). Non-additive gene action has been considered as important feature for the development of hybrids, for which parents with high gca is not always obligatory, nonetheless, parents having different gca may also produce superior combinations as suggested by Fasahat et al. (2016).

Conclusions

Present investigations suggested postponement of selection for potential parents until fixation of desirable genes. The utility of this information can be extended by validating these results must be validated through other genetic study using large number of cultivars to draw logical inferences about selection of desirable plants showing wider adaptation potential across multiple environments. Crosses identified as superior for average boll number, boll weight and lint % may be utilized for development of hybrids to boost cotton production in water deficit areas. These findings may positively be used to harness water stress tolerance in commercial cotton cultivars grown in the cotton belt of Pakistan.

Declarations

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

All authors declare that they have no conflict of interest.

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Authors Contributions

Azhar FM designed and supervised the research, Javed A conducted experiment and wrote the manuscript. Shakeel A assisted in data analysis and interpretation. Khan Iftikhar Ahmad and Azhar MT proofread manuscript before final submission. All authors have read and approved the manuscript.

Consent for publication

All authors have agreed to submit the review article in Journal of Cotton Research.

Competing interests

The authors declare that they have no competing interests.

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