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GENETIC MECHANISM OF LEAF VENATION AND STOMATAL TRAITS FOR BREEDING DROUGHT TOLERANT LINES IN WHEAT

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Abstract

Nature of gene action and combining ability for six physio-morphological traits were performed in a 6 × 6 combination of wheat genotypes. The mean squares for general combining ability (GCA) were highly significant for all traits except stomata frequency and flag leaf area which were non-significant. Highly significant mean squares for specific combining ability (SCA) were found only for flag leaf area, leaf venation and stomata frequency. Additive type of gene action was found to be of greater importance for all the characters except for leaf venation where non-additive gene action was more important. Variety Pasban-90 had high GCA effects for stomata size, epidermal cell size and hygrophilic collides. Lowest SCA effects were observed in cross the 2×3 for stomata size and stomata frequency while the cross 6 × 3 had high reciprocal effects for stomata size and epidermal cell size.

Introduction

Wheat (*Triticum aestivum* L.) is the major human consumable commodity in most of the areas of the world including Pakistan. It occupies 70% of Rabi (winter season) and 37% of total crop area in Pakistan (Ihsan *et al*. 2003). It is widely grown in the areas subjected to frequent drought stress and the need arises to evolve varieties having mechanism efficient enough to escape, avoid or better tolerate the drought conditions, so little success has been achieved to develop drought tolerant wheat varieties over the last decayed (Ejaz-Ul-Hassan and Khaliq 2008). A number of physiological traits like leaf venation, stomatal frequency and stomatal size that are associated with drought resistance have been identified in wheat and are relatively simple in inheritance (Ahmad *et al*. 2000). The major objective of the most wheat breeding programs is to increase grain yield on unit area basis. Moreover, selection and breeding for drought resistance in crops has been considered to be an economical and efficient means of overcoming drought problem (Ahmed *et al*. 2015a). Drought related plant traits including leaf venation, stomata size, stomatal frequency, epidermal cell size and hygrophilic colloids are important to select for developing drought resistant varieties/lines. More number of veins per unit leaf area will increase leaf area (Khan *et al*. 2015), so greater flag leaf area played an important role in yield increase under normal as well as water stress conditions (Ahmed *et al*. 2015b). Stomata play an important role in regulating drought condition, gaseous exchange and transpiration rate also depends upon the size of stomata aperture and its number per unit leaf area (Mackay *et al*. 2003, Jianwu *et al.* 2006). Less number and small sized of stomata can help the plant to use available water more efficiently. Similarly, stomata size and its frequency also help the plant to stand well even under shortage of water (Riaz and Chowdhry 2003). Denser leaf venation helps for survival of plant under shortage of water consume

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available moisture adequately, thus maintaining yield. Similarly, stomatal size and number of stomata also contribute for adequate water consumption thus these traits can be selected for developing normal and drought resistant wheat varieties (Ahmed 2004, Hassan and Khaliq 2008)**.** Combining ability studies are frequently used by the plant breeders to evaluate the nature of gene effects and classifying parental lines in terms of their hybrid performance. Therefore, estimation of available genetic variance in the early generation of crosses could be very helpful for a plant breeder. The present investigation was undertaken to derive information on the nature of combining ability operative in the inheritance of different physio-morphological traits for breeding drought tolerant lines in wheat.

Materials and Methods

Combining ability studies were carried out at Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Six wheat genotypes/lines *viz.*, Pasban-90, Ufaq-2002, millat-11, 9469, 9481 and 9486 available from the genetic stock from University of Agriculture Faisalabad, Pakistan and were crossed. The F_1 seeds along with their parents were planted in the field during the season 2015 using triplicated randomized complete block design. The inter-plant and inter-row space was 0.1 m and 0.3 m, respectively and one meter distance between replications. Single row of five meter length served as an experimental unit. Two seeds were sown per hole by dibbled method which were thinned to single per hole at seedling stage. All other treatments were kept constant for the entire experiment. Ten guarded plants were selected randomly from each row of each replication one month before maturity to record the data on flag leaf area, leaf venation, stomata size, stomatal frequency, epidermal cell size and hygrophilic collids on individual plant basis. Flag leaf area was measured in cm^2 using the following formulae of Muller (1991),

Flag leaf area = Flag leaf length × Flag leaf width × 0.74.

While the flag leaf weight was recorded in grams using electric balance after detaching it from the base excluding sheath and oven drying the sample at 70°C for 24 hrs. Hygrophilic colloids were estimated by the flag leaf. Dried leaves were ground to make fine powder using an electric grinder. About 1 g powder put within small crucibles of known weight and placed in a desiccator. After 24 hrs increase in weight was due to moisture gained by powder within crucibles. The absorption (%) was calculated as following formulae:

Absorption (%) = (Original weight of sample/weight gained by the sample after 24 hrs) × 100.

The number of stomata per unit area counted from the upper surface of the third nodal leaf of each randomly selected plant. The leaf strips, which were taken from the middle part of the leaf, were dipped in Carnoy’s solution (Sheehan and Hrapchak 1980) to arrest stomatal movement and removal of chlorophyll from leaf tissues. After 48 hrs the leaf strips were removed from solution, peeled off with razor and examined under 40× objective of microscope for stomatal frequency of selected tissues. The observations were taken from each foliar strip and the average was used for analysis. The same leaf strips were also used for measuring the size of the stomata under the 10× objective of microscope. Three stomata from each sample were measured at random for length and width of stomata with the help of ocular lens of microscope than multiplying length and width with standardized value of microscope which is 3.33 µ at 10× and average was calculated for analysis. Length and width of epidermal cells from the surface of same flag leaf strips was measure at 40× using micrometer. Three epidermal cells of each strip were measured to calculate the average size of epidermal cell. Leaf venation was recorded as number of longitudinal veins falling in low power (10×) microscope field. The mean of the 10 plants for each character were subjected to analysis of variance technique as suggested by Steel *et al*. (1997) with MSTAT-C software and the traits showing significant genotyping differences were further analyzed for combining ability following Griffing (1956) method I, model II.

Results and Discussion

Table 1 analysis of variance revealed that there were highly significant differences which showed a broad range of expression exhibited by all genotypes.

The analysis of variance for combining ability (Table 2) indicated that mean square due to general combining ability was non-significant (4.5) while the mean squares due to SCA (3.88) and reciprocal effects (4.14) were highly significant for flag leaf area. GCA mean squares were greater than SCA and GCA/SCA ratio with a value 1.2 indicating the presence of additive genetic effects for flag leaf area. Similar findings were also reported by Khan and Rizwan (2000) and Hmada *et al*. (2002) and Chowdhry *et al*. (2007). As the variances of SCA was higher than variance of GCA and reciprocal effects indicating the presence of non-additive gene action. Table 3 displayed that maximum positive GCA value was exhibited by 9486 (0.44) while the lowest GCA value was depicted by 9469 (-0.36). Direct 10 crosses out of 15 showed negative SCA effect. Maximum SCA effects were shown by cross combination 1 × 5 (1.40) while the lowest SCA value was recorded in the cross combination 1 × 2 (–1.20). The highest reciprocal effects was exhibited by 4 × 1 (1.82). Due to additive type of gene action selection would be possible in early generation for this trait.

For leaf venation in Table 2 it was found that SCA and reciprocal effects were highly significant for leaf venation while GCA mean squares were non-significant in this traits (Table 3). The presence of non-additive genetic effects was revealed due to greater SCA (3.09) mean square. Similar finding with Khan and Rizwan (2000). While GCA : SCA ratio indicating the presence of additive types of gene action involved in leaf venation. Iqbal (2004), Chaudhry *et al*. (2001) and Hassan and Khaliq (2008) also reported the same results. Estimates of GCA effects in Table 3 revealed that only genotype 9481 exhibited the highest positive GCA value which was 0.38 and the genotype Pasban exhibited the highest negative value –0.30. The cross 2 × 3 exhibited the highest positive SCA value which was 1.25 and the cross 1 × 3 exhibited the highest negative SCA value which was –0.76. In Table 4 highest positive reciprocal effects were shown by cross 5 × 4 with 0.77 value and highest negative value was exhibited by the cross named as 6 × 4 with –0.76. In view of non-additive gene action effective selection will be difficult for leaf venation in early generation.

Table 1. Analysis of variances mean square for physio-morphological traits in wheat.

Source of variation	df	Flag leaf area	Leaf venation	Stomata size	Stomata frequency	Epidermal cell size	Hygrophilic collides
Replications	2	1.504	1.640	9183.510	100.362	58145.371	0.008
Treatments	35**	4.074**	2.402**	17141.930**	346.717**	130901.545**	10.174**
Error	70	1.094	0.182	9108.643	47.341	54052.751	3.578

**Significant at 1%.

It is specious from Table 2 that estimates of mean squares were highly significant for GCA effects, and non-significant for SCA and reciprocal effects for stomata size. Mean squares due to GCA (43491) effects was greater than SCA (14693) effects, while the GCA:SCA ratio which was greater than one. These results indicate the involvement of additive genetic effects for the control of this trait. Similar conclusions have also been made by Iqbal (2004), Hassan and Khaliq (2008). Variety Pasban-90 had high positive GCA effects (58.20) for stomatal size where lowest effect was possessed by genotype Millat-11(–29.96) as displayed in Table 4. The top scorer for specific combining ability effect was the cross 3 × 6 with value 86.99, the lowest negative value (–82.23) was unveiled by the cross combination 2 × 3. With regard to indirect cross combination in Table 3, the cross 6 × 3 possessed the highest reciprocal effect (69.83), the lowest effect was recorded in 3 × 1 with the value –69.50. The selection for this trait in early generation would be informal due to no involvement of non-additive type of gene action.

Results from Table 3 showed that SCA and reciprocal effects mean squares were highly significant and non-significant for GCA effects for stomata frequency. Highly significant mean square due to reciprocal effects indicated that maternal effects involved for controlling the stomata frequency. Additive genetic effects was observed for this trait because mean squares due to GCA were greater than SCA, GCA : SCA ratio with a value (1.4) greater than 1 also designates additive gene action for this traits. These results are in agreement with those of Subhani *et al*. (2000); Hmada *et al*. (2002), Saeed (2010). A glance of Table 4 revealed that maximum GCA effects was shown by parent 9486 with value 3.51. Whereas, negative GCA effects were shown by varieties millat-11 with value –5.42. The highest positive SCA effects in Table 3 indicated by 3 × 4 with value of 8.62 followed by 1 × 6 with value of 7.80. The lowest SCA value was recorded by 2 × 3 with value of –10.35. In reciprocal crosses the highest reciprocal effects were depicted by 6 × 2 with value of 12.17, whereas the lowest value was exhibited by 3 × 1 with value of –16.25. Due to additive genetic effect, selection would be fruitful in early generation for stomata frequency.

Table 2. Combining ability analysis mean square for physio-morphological traits in wheat.

Mean square	df	Flag leaf area	Leaf venation	Stomata size	Stomata frequeny	Epiderml cell size	Hygrophilic collides
GCA	5	4.5	2	43491*	445	184828*	12.14
SCA	15	3.88*	3.09*	14693	323.191*	65729	7.59*
RE	15	4.14*	1.71*	10808	337.377*	178097*	12.1*
GCA/SCA		1.2	1	2.96	1.4	2.812	1.6

According to the analysis of variance for combining ability (Table 2) general and reciprocal effects mean squares were highly significant while mean square due to specific effects were non-significant for leaf venation. Maternal effects were present due to reciprocal effects were highly significant. The ratio of GCA variance to SCA variance was 2.812 and the mean square of GCA (184828) was greater than SCA (65729) as shown in Table 3 indicating the additive type of gene action. Chaudhry *et al*. (2001) and Ambreen *et al.* (2002) were also observed similar findings. The parent Pasban-90 proved to be a good general combiner as it exhibited the highest positive GCA value of 113.70 (Table 3) and highest negative GCA with –81.19 values shown by parent 9486. In case of SCA the cross 1 × 4 exhibited the highest positive SCA with 119.35 and cross 1 × 2 showed the highest negative value which was –213.84. Similarly in reciprocal effects the highest positive value was 2.467 observed in cross 6×3 with value 501.67 while highest negative reciprocal effects were observed in cross 2 × 1 which was –320.0 Additive genetic effects in the absence of epistasis suggest that in early generation selection will be effective for this trait.

Table 3. GCA, SCA and reciprocal effects for physio-morphological traits in wheat.

GCA	Flag leaf area	Leaf venation	Stomata size	Stomata frequency	Epidermal cell size	Hygrophilic collides
Pasban-90 (1)	–0.20	–0.30	58.20	–1.67	113.70	0.69
Ufaq-2002(2)	0.36	–0.25	–26.99	3.40	–13.27	0.33
Millat-11(3)	–0.34	0.13	–29.96	–5.42	–37.02	–0.08
9469(4)	–0.36	0.12	19.07	–1.63	56.04	–0.82
9481(5)	0.10	0.38	–25.27	1.80	–38.27	–0.50
9486(6)	0.44	–0.08	4.95	3.51	–81.18	0.38
SCA						
1 × 2	–1.20	–0.28	2.60	7.41	–213.84	1.19
1 × 3	–0.09	–0.76	6.07	2.97	78.24	–1.71
1 × 4	–0.49	–0.50	40.05	–2.06	119.35	0.38
1 × 5	1.40	–0.46	5.71	–2.59	–21.34	–1.41
1 × 6	0.58	0.35	–31.51	7.01	12.41	1.31
2 × 3	–0.72	1.25	–82.23	–10.35	–7.29	–0.06
2 × 4	0.49	–0.63	–0.26	5.27	22.99	0.08
2 × 5	–0.29	0.93	–36.26	1.02	–4.37	–0.12
2 × 6	–0.03	–0.32	64.35	–5.94	89.38	–1.06
3 × 4	0.08	–0.39	–56.12	8.46	–7.43	–0.08
3 × 5	–0.6	0.38	0.55	–5.24	–5.95	0.22
3 × 6	1.12	–0.53	86.99	–8.07	–180.43	–0.15
4 × 5	–0.17	0.37	63.02	–2.98	13.82	0.07
4 × 6	–0.06	0.29	–30.37	0.76	–6.59	0.02
5 × 6	–0.02	0.30	–10.37	4.33	111.05	1.32
Reciprocal						
2 × 1	–0.17	0.25	–32.00	0.01	–320.00	0.80
3 × 1	0.17	0.50	–69.50	–16.25	–111.67	–2.18
4 × 1	1.82	–0.74	24.50	10.50	50.84	–1.57
5 × 1	–1.17	0.48	13.17	7.70	–72.50	1.01
6 × 1	0.03	–0.20	–20.17	2.42	–161.67	–1.77
3 × 2	0.02	–0.31	–26.33	–2.50	–22.50	2.47
4 × 2	0.07	–0.51	60.00	1.87	139.17	0.11
5 × 2	–0.30	–0.68	57.00	3.00	77.50	0.03
6 × 2	–0.03	0.47	35.50	12.17	38.33	0.59
4 × 3	0.05	–0.61	–10.83	3.01	–61.67	0.06
5 × 3	0.83	0.43	–32.50	–8.00	–92.50	0.88
6 × 3	0.02	0.41	69.83	–6.88	501.67	–0.78
5 × 4	–0.43	0.77	–55.00	–5.05	–18.33	0.27
6 × 4	0.02	–0.76	–1.17	0.50	–25.00	0.03
6 × 5	–0.93	0.50	–12.50	–2.50	78.33	0.05

Table 2 showed that mean square due to GCA and reciprocal effects was highly significant while mean square due to SCA was non-significant for hygrophillic collides. The results clearly showed that this trait is controlled by additive gene action, because GCA effects were more than SCA effects as well as the ratio of GCA to SCA variance was 1.6 Similar results were also reported by Chaudhry *et al*. (2001), Ambreen *et al.* (2002). Table 3 indicated that highest positive GCA effect was exhibited by the parent pasban-90 (0.69) and parent 9469 (–0.82) showed the negative effects. Direct cross combination 5 × 6 with value 1.32 had positive effects while 1 × 3 displayed the lowest SCA effects –1.71, among reciprocal crosses 3 × 2 cross exhibited the high positive reciprocal effect and the lowest by 3 × 1. Due to the presence of additive gene action in early generation selection would be possible.

Combining ability analysis is mostly used to evaluate the nature of gene action and selecting parental lines on the basis of performance of their cross combinations. Drought affects every aspect of the plant growth and the ability to yield well under stress is conditioned by different physiomorphic traits. These traits are genetically complex and are not easy to manipulate. The magnitude of general combining ability was higher than specific combining ability thus additive (fixable) gene action was involved in all studied traits except leaf venation which controlled by non-additive gene action. The present study helps the plant breeder to evolve better drought tolerant varieties/lines and to grow efficiently under normal conditions and even in moisture stress conditions.

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