

*Full Length Research Article*

## Combining Ability Studies for Stomata and Quantitative Indices in Wheat

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### Abstract

Wheat (*Triticum aestivum* L.) is the most precious staple food and is highly regarded in the world as king of cereals. The present experiment was conducted to determine the combining ability analysis involving six high yielding lines of wheat (*Triticum aestivum* L.) viz., 9481, 9486, 9490, 9862, 9863 and 9864 and three testers Pasban-90, Galaxy-13 and Millat-11 which were crossed in line  $\times$  tester mating design in the experimental area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The parental genotypes and their F<sub>1</sub> hybrids were examined in randomized complete block design (RCBD), with three replications under normal conditions in the season 2015-16. The data were collected for stomata and quantitative indices viz., plant height, flag leaf area, stomata size, stomata frequency, 1000-grain weight and grain yield per plant. The data were analyzed statistically and combining ability studies were done using line  $\times$  tester analysis, highly significant differences were shown among the lines and testers for most of the traits. For flag leaf area, stomata size, stomata frequency and grain yield per plant genotype 9481 proved to be good general combiner among the female parent. Among testers (males) Galaxy-13 exhibited positive general combining ability for the studied traits. Among the crosses, the hybrid 9481  $\times$  Galaxy-13 was the best specific combiner for most of the yield contributing traits. The superior genotypes and crosses can be combined to develop new promising and improved wheat varieties for obtaining high yield.

**Keywords:** Stomata, Quantitative, Wheat, Grain, yield

### Introduction

Agriculture is rightly considered to be the backbone for the economy of Pakistan as its share is 21% in gross domestic production (GDP). Agriculture is currently generating 45% employment opportunities for country's labor force and providing livelihood to 60% rural population GDP (Govt. of Pakistan, 2014-15). Most of the wheat is grown for human consumption and about 10 % is retained for seed purpose and industry. Wheat belongs to the family Poaceae and its genetics is more complicated than any other cereal and species because of its polyploidy level. It has three groups as diploid, tetraploid and hexaploid. Their chromosomes numbers are 14, 28 & 42 for diploid, tetraploid and hexaploid respectively. Saeed *et al.* (2005)

The population of Pakistan is increasing day by day and to nourish this burgeoning population, there is a clamant devoir to increase varieties having maximum yield potential (Awan *et al.* 2005). Wheat is planted for multiple uses as for consumption,

baking purpose and forages for livestock. Its gluten (a cohesive network of tough endospermic proteins) property is very important for chapatti making, more gluten produces more cohesiveness. In changing environment and increasing population, it has become necessary for breeders to develop such superior varieties to boost the wheat productivity (Khayatnejad *et al.*, 2010). To overcome this reduction in yield potential the genetic material of varieties and genotypes have to be reshuffled so that these varieties and genotypes may attain an ideal genetic makeup which would help to give better performance in a range of changing environment (Jain and Sastry (2012). Main objective of any wheat breeder is development of high yielding varieties all over the world. In an effective breeding program, planning and the choice of genotypes with desirable traits is important and contribution of selected traits to yield of a crop. Knowledge of the existing genetic variation is required for the development of high

yielding varieties. (Ahmed *et al.* 2015a). For the production of new cultivars the goal of the breeder is to find sufficient amount of variability, in which desired lines are to be selected in order to develop breeding lines. This could be accomplished by scrutinizing maximal genetic potential from the attainable germplasm.

Line  $\times$  tester analysis (Kempthorn, 1957) is most promising method in this respect which helps in the selection of parents and crosses for improvement in further wheat breeding program and provide information regarding genetic mechanisms controlling certain traits (Khan *et al.*, 2015). For the development of potential hybrids in several crop plants choice of suitable parents and understanding of SCA and GCA effects is of a great importance to plant breeders (Rashid *et al.*, 2007). The objective of this study was to use line  $\times$  tester analysis technique for evaluation of yield and yield components on the basis of combining ability effects for the selection of best combiners for a successful wheat hybridization program.

## Materials and Methods

The present study was carried out to estimate combining ability effects by using line  $\times$  tester analysis in the experimental area of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The experimental material comprised of six lines viz. 9481, 9486, 9490, 9862, 9863 and 9864 and three commercial varieties as testers' viz., Pasban-90 Galaxy-13, Millat-11. These wheat genotypes were crossed in line  $\times$  tester design during the month of February 2015. To accomplish crossing female spikes were hand emasculated and bagged to avoid contamination from foreign pollens. At harvesting the seeds of each cross were saved separately. In the second year of study the  $F_1$  seeds along with nine parents were planted in the field in randomized complete block design (RCBD) with three replications during 3<sup>rd</sup> week of November 2016. The varieties/ lines were assigned at random to experimental unit in each block. Each replication consisted of six varieties/lines and 18 crosses with one meter long single row for treatment. Plant to plant and row to row distance were 15 and 30 cm respectively. Two seeds per hole were sown with the help of dibbler and later thinned to one seedling per hole after germination. The experimental populations were kept under normal condition from sowing to maturity. Moreover efforts were done to implement proper production technology.

At maturity five guarded plants were randomly selected from treatment row of each

replication and data were recorded for quantitative traits like plant height (cm), 1000-grain weight (g) and grain yield per plant (g), flag leaf area (cm<sup>2</sup>) calculated by using the following method of Muller (1991), and stomata traits. The flag leaf strips used for studying stomatal frequency were also used for measuring size of stomata excluding guard cells using the Carnoy's solution. The stomata frequency (counts) per unit area were recorded on upper surface of flag leaf of the mother shoots using compound microscope at 10X magnification. The leaf strips were examined under higher power (40X) objective of microscope. Five stomata from each strip were measured at random for length and breadth with the help of an ocular micrometer and the average was calculated. The length and breadth were separately multiplied with standardized value (3.44). The stomata size ( $\mu\text{m}^2$ ) was calculated by multiplying the length and width (Ahmed *et al.*, 2017). The recorded data for all the characters were subjected to analysis of variance (ANOVA) technique as given by (Steel *et al.* 1997) and further partitioned by using line  $\times$  tester analysis (Kempthorne, 1957).

## Results and Discussion

Genetic makeup of any quantitative trait is highly correlated to its phenotype, so reliable selection can be made on the basis of phenotypic expression. However selection is difficult for quantitative traits because environmental factors can affect. Therefore breeders play important role in plant improvement and identification of greater genetic potential for yield and its components.

### Analysis of variance

The analysis of variance for all characters studied is given in Table 1. The genotypes showed highly significant differences for plant height while significant differences for flag leaf area, Stomata size, stomate frequency and grain yield per plant which indicates that sufficient variation was present among genotypes. These results are in line with the findings of Ahmed *et al.*, (2015a) and Ahmed *et al.* (2017). Among lines (female parents) highly significant differences were found for grain yield per plant. While significant results were obtained for flag leaf area, stomata size and stomata frequency. Significant differences were observed in tester (male parent) for flag leaf area, grain yield per plant, plant height and 1000-grain weight. On observing the parents significant differences were found for flag leaf area, Stomata size, stomata frequency, plant height, 1000-grain weight and grain yield/plant. Considering the crosses, significant differences were found for plant height, flag leaf area, grain

yield/plant, stomata size, and 1000-grain weight. Parent vs crosses showed significant differences for flag leaf area, stomata size, stomata frequency and grain yield per plant. Non-significant difference were found for plant height and 1000 grain weight. Line  $\times$  tester interaction was significant for plant height. Significant differences were observed for like, flag leaf area and 1000 grain weight. While remaining traits like plant height, stomata frequency, stomata size and grain yield per plant showed non-significant effects. Interpretation of line  $\times$  tester analysis revealed that genetic variability is present among the genotypes for yield and its related trait

#### **General Combining Ability (GCA) & Specific Combining Ability (SCA) effects:** **Plant height (cm)**

In case of plant height, negative GCA effects are more important since more emphasis placed upon selection for short stature progeny in segregating population because they ultimately turn out short stature line which would be more responsive to fertilizer and resistant to lodging. From this point of view, 9490 among female parents and Pasban-90 among male parents were potential parents and their values of general combining ability were -2.06 and -3.32, respectively. The SCA effects of crosses showed a wide variation from positive to negative values for plant height. Highest and positive SCA effects were observed for 9864  $\times$  Galaxy-13 (2.94) followed by the cross 9486  $\times$  Millat-11 (2.79). The highest negative SCA effects were observed for 9481  $\times$  Galaxy-13 cross combinations (Table 2). The best specific combiner for plant height was 9864  $\times$  Galaxy-13 among all crosses. Similar results were reported by Singh *et al.* (2013) and Ahmed *et al.* (2015b) for plant height.

#### **Flag leaf area (cm<sup>2</sup>)**

For flag leaf area, positive GCA effects are more important because flag leaf area has much contribution in the photosynthetic activity and ultimately the grain yield which is our main objective. So much emphasis is placed on the selection of genotypes with greater flag leaf area. Among the three male parents only Galaxy-13 showed the positive value of 1.29 for GCA effects of female parents 9862 (2.82). Out of six female parents, three parents exhibited negative GCA for this trait. Among crosses, 9481  $\times$  Galaxy-13 had highest and positive SCA effects (2.94). The highest negative SCA effects were observed for 9486  $\times$  Galaxy-13 cross combinations (Table 2). The 50% crosses had positive SCA effects and 50% had negative SCA effects. The best specific combiner for

spike length was 9490  $\times$  Millat-11 among other crosses. Similar results were reported by Srivastava *et al.* (2012), Ahmed *et al.* (2015b) and Ahmed *et al.* (2017). The findings of Khayatnejad *et al.* (2010) and Jain and Sastry (2012) were not similar.

#### **Stomata Frequency**

GCA effects calculated for these traits were of moderate magnitude. Among female parents, 9481 (2.98) exhibited higher and positive GCA effects followed by male parent Galaxy-13 (1.19). The cross combination 9864  $\times$  Galaxy-13 had highest and positive SCA effects (4.59), followed by 9481  $\times$  Pasban-90 (4.01). The maximum negative SCA effects were observed for 9863  $\times$  Galaxy-13 (-3.91). The 50% crosses had positive SCA effects and 50% had negative SCA effects (Table 2). Masood and Kronstad (2000), Jain and Sastry (2012) and Ahmed *et al.* (2017) also reported similar results in their experiments. The best specific combiner for stomata frequency was 9864  $\times$  Galaxy-13 among other crosses.

#### **Stomata size**

In this study three females parents viz., 9481 showed the positive GCA effects of 158.54 followed by 9864. Only three female parents showed negative GCA value, the remaining displayed positive GCA effects. Likewise, only one male parent showed positive GCA for this trait. So it can be concluded that above mentioned parents are desirable for use in future breeding programs. The cross 9862  $\times$  Millat-11 showed the higher value of SCA effects (87.13) for stomata size. While the crosses 9862  $\times$  Galaxy-13 (-68.76) showed negative SCA effects (Table 2). The negative SCA effects were observed in 12 crosses out of 18 crosses. Similar results were reported by Awan *et al.* (2005) and Ahmed *et al.* (2017). The findings of Saeed *et al.* (2005) were not similar.

#### **1000-grain weight (g)**

The female parent 9864 showed the maximum GCA effects with a value of 3.10 followed by 9481 with 2.57. Among the male parents, only Galaxy-13 showed positive effects of GCA with the value of 1.24. Three female parents out of six and two male parent out of three revealed negative GCA effects for 1000-grain weight. Among crosses, the cross 9481  $\times$  Galaxy-13 had highest and positive SCA effects followed by 9490  $\times$  Millat-11 (2.26). The best specific combiner for 1000-grain weight was 9864  $\times$  Galaxy-13 among other crosses (Table 2). Similar results were observed by Khayatnejad *et al.* (2010) and (Ahmed and Mustafa, 2017) for 1000-grain weight. The findings of Shabbir *et al.* (2012) and Ahmed *et al.* (2015a) were not similar.

#### **Grain yield per plant (g)**

Grain yield per plant is the ultimate objective which we want to improve. So, positive GCA effects contribute towards achieving our goal. For grain yield per plant, female parent 9481 showed the highest yield value 1.82 followed by genotype 9864 displayed positive GCA effects. Apart from the above parents, rest of other showed negative GCA effects. The highest and positive SCA effects were observed for the cross 9869 × Galaxy-13 (1.56). The negative SCA effects were observed for 9862 × Millat-11 (-1.61). The negative SCA effects were observed in 10 crosses out of 18 crosses (Table 2). The best specific combine for grain yield/plant was 9864 × Galaxy-13 among all other crosses. These results are in confirmation with Ahmed and Mustafa, 2017, while the results of Khan *et al.*, (2015) were not similar due to change in genotype and environment.

## Conclusions

It is concluded that significant variation exist among genotypes and cross combinations. According to the GCA and SCA effects the line 9481 showed potential general combiner and prove beneficial to generate desirable combinations. The crosses like 9481 × Galaxy-13 showed desirable SCA effects for yield and most of its components. This may yield transgressive segregants in the subsequent generations which can be selected and improved further. These results suggested that selection should be practiced in later generations to make it more effective to obtain desirable segregants in wheat breeding program for developing high yielding wheat varieties.

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**Table 1. Analysis of variance derived from line × tester for stomata and quantitative indices in wheat**

| Source of variation | df            | Plant height (cm)   | Flag leaf area (cm <sup>2</sup> ) | Stomata Frequency      | Stomata Size (μm <sup>2</sup> ) | 1000 grain weight (g) | Grain yield per plant (g) |
|---------------------|---------------|---------------------|-----------------------------------|------------------------|---------------------------------|-----------------------|---------------------------|
| Replication         | (R-1)=2       | 29.77 <sup>NS</sup> | 5.75 <sup>NS</sup>                | 7888.05 <sup>NS</sup>  | 20.01 <sup>NS</sup>             | 6.49 <sup>NS</sup>    | 5.42 <sup>NS</sup>        |
| Genotypes           | (G-1)=26      | 41.47**             | 35.15*                            | 49849.14*              | 193.85*                         | 33.38*                | 24.84*                    |
| Parents             | (P-1)=8       | 61.50*              | 39.38*                            | 29580.65*              | 160.56*                         | 25.07*                | 21.72*                    |
| Crosses             | (Cr.-1)=17    | 34.47*              | 30.57*                            | 56524.35*              | 61.69 <sup>NS</sup>             | 38.30*                | 9.52*                     |
| P. vs Crosses       | (2-1)=1       | 0.21 <sup>NS</sup>  | 79.15*                            | 98518.67*              | 2706.84*                        | 16.33 <sup>NS</sup>   | 310.17*                   |
| Lines               | (L-1)=5       | 25.76 <sup>NS</sup> | 55.67*                            | 138697.13*             | 116.70*                         | 64.07 <sup>NS</sup>   | 21.70**                   |
| Testers             | (T-1)=2       | 150.30**            | 23.71*                            | 68027.91*              | 23.89 <sup>NS</sup>             | 23.78*                | 3.00*                     |
| L x T               | (L-1)(T-1)=10 | 15.67 <sup>NS</sup> | 19.39*                            | 13137.24 <sup>NS</sup> | 41.74 <sup>NS</sup>             | 28.32*                | 4.74 <sup>NS</sup>        |
| Error               | (R-1)(G-1)=52 | 14.60               | 2.43                              | 7654.95                | 35.15                           | 4.76                  | 3.98                      |
| Total               | (GR-1)=80     | 23.71               | 13.15                             | 21373.89               | 86.35                           | 14.11                 | 10.80                     |

\* = Significant

\*\* = Highly significant

ns = Non Significant

**Table 2. Estimation of general combining ability (GCA) and specific combining Ability (SCA) effects for stomata and quantitative indices in wheat**

| Parents (GCA effects) | Plant height (cm) | Flag leaf area (cm <sup>2</sup> ) | Stomata Frequency | Stomata Size | 1000grain weight (g) | Grain yield per plant (g) |
|-----------------------|-------------------|-----------------------------------|-------------------|--------------|----------------------|---------------------------|
| <b>Lines (GCA)</b>    |                   |                                   |                   |              |                      |                           |
| 9481                  | 0.61              | 2.83                              | 2.99              | 158.54       | 2.57                 | 1.82                      |
| 9486                  | -0.76             | -2.69                             | -7.07             | -166.35      | -4.07                | -2.07                     |
| 9490                  | -2.06             | 0.22                              | 0.44              | -19.24       | 0.17                 | -0.81                     |
| 9862                  | -1.48             | -0.08                             | 0.29              | 5.76         | -0.11                | -0.71                     |
| 9863                  | 1.67              | -2.94                             | 1.6               | -98.02       | -1.65                | -0.04                     |
| 9864                  | 2.03              | 2.66                              | 1.76              | 119.31       | 3.1                  | 1.81                      |
| S.E                   | 1.27              | 0.52                              | 1.98              | 29.16        | 0.73                 | 0.67                      |
| <b>Testers (GCA)</b>  |                   |                                   |                   |              |                      |                           |
| Galaxy-13             | 1.96              | 1.29                              | 1.19              | 70.76        | 1.24                 | 0.11                      |
| Pasban-90             | -3.32             | -0.89                             | -1.11             | -40.3        | -0.2                 | 0.34                      |
| Millat-11             | 1.36              | -0.4                              | -0.08             | -30.46       | -1.04                | -0.45                     |
| S.E                   | 0.9               | 0.37                              | 1.4               | 20.62        | 0.51                 | 0.47                      |

| <b>Crosses (SCA)</b> |       |       |       |        |       |       |
|----------------------|-------|-------|-------|--------|-------|-------|
| 9481 × Galaxy-13     | -4.03 | 2.94  | 4.59  | 66.30  | 4.34  | 1.56  |
| 9481 × Pasban-90     | 0.42  | 0.68  | 2.33  | 55.85  | 0.55  | -0.10 |
| 9481 × Millat-11     | 0.59  | -1.99 | -4.70 | -27.31 | -0.34 | -0.30 |
| 9486 × Galaxy-13     | -1.01 | -3.81 | 1.42  | -27.31 | 3.56  | 0.03  |
| 9486 × Pasban-90     | 1.23  | 2.64  | -2.91 | -28.59 | 0.27  | -1.06 |
| 9486 × Millat-11     | 2.79  | 1.16  | 1.49  | 55.91  | -3.83 | 1.03  |
| 9490 × Galaxy-13     | 0.37  | -0.75 | -3.19 | -10.76 | -0.80 | -1.15 |
| 9490 × Pasban-90     | 1.71  | -2.18 | 4.01  | -28.54 | -3.54 | 1.53  |
| 9490 × Millat-11     | -2.08 | 1.31  | -0.82 | -55.54 | 2.26  | -0.38 |
| 9862 × Galaxy-13     | 1.53  | 1.55  | -1.27 | -68.76 | -0.91 | 0.71  |
| 9862 × Pasban-90     | -1.13 | -0.47 | -0.40 | -18.37 | 1.26  | 0.28  |
| 9862 × Millat-11     | -0.40 | -1.08 | 1.67  | 87.13  | -0.36 | -1.61 |
| 9863 × Galaxy-13     | 0.20  | -1.11 | -3.91 | 78.02  | -3.90 | -1.33 |
| 9863 × Pasban-90     | -0.80 | -0.33 | 0.75  | -46.26 | 1.76  | -0.23 |
| 9863 × Millat-11     | 0.60  | 1.45  | 3.16  | -31.76 | 2.14  | 0.40  |
| 9864 × Galaxy-13     | 2.94  | 2.81  | 2.36  | 57.35  | -0.22 | 1.34  |
| 9864 × Pasban-90     | -1.44 | -0.32 | -3.78 | -28.93 | -0.30 | -0.42 |
| 9864 × Millat-11     | -1.50 | -2.48 | -0.81 | -28.43 | -1.96 | -0.29 |
| <i>S.E</i>           | 2.21  | 0.90  | 3.42  | 50.51  | 1.26  | 1.15  |