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Generation Means Analysis for Five Physiological Traits of Bread Wheat under Rainfed Condition

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Abstract To understand the genetics of drought tolerance, two promising elite lines V-04189 and V-03138 were crossed to develop F_1, F_2 and back cross generations (BC_1 and BC_2). Six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) were raised with no recommended irrigations. Analysis of variance showed presence of sufficient genetic variability among six generations. Hybrid vigor was observed in most of the cases except flag leaf area. Generation means analysis revealed two parameter model [md] provided the best fit for relative water contents and excised leaf water loss which shows the presence of additive genetic effect is prominent with simple inheritance The remaining traits exhibited higher parameter models and indicating complex inheritance. Generation means analysis indicating the presence of additive genetic affects that can be fixed in early segregating generations.

Keywords Generation mean analysis, genetic variability

1. Introduction

Pakistan is an agricultural country. Agriculture is the backbone of Pakistan's economy and provides occupation to two third of the country's population. Water is the blood of crop which requires for certain metabolic activities and in the absence of water growth and development of plant ceases. Cultivated land in Pakistan is ranged from arid to semi-arid and deficient in water. In spite of having world's best canal irrigation, a large cultivated area (1.1 million ha) is rainfed with very low potential yield, Pakistan statistical year book, [1]. Developing wheat varieties with low moisture requirement and cope well with moisture stress is the only answer to overcome the oncoming perils. Evolution of wheat varieties with low moisture requirements is a long, hard and complex process, when the objective involved is the inclusion of high grain yield. Conventional and non-conventional means to create variability where selection is focus on grain yield and seeks out the character other than yield that may have an agronomic edge under water unavailability.

Photosynthesis is an essential event for plant growth that requires water as an essential ingredient. High leaf water potential and relative water contents increases the photosynthetic rate which were greatly reduced by water unavailability (Siddique *et al*., [2]: Bajji *et al*., [3]: Sairam and Srivastava [4] and Tas and Tas, [5]). High stomata frequency on flag leaf increased grain yield but under limited water it is undesirable (Yousufzai *et al*., [6]). Talebi *et al*., [7] reported that selection from moisture limited environment would improve yield for drought tolerance than non-stress. Khattab *et al.,* [8] revealed that epistatic gene effect create complexity and it cannot be overlooked in a new breeding program to improve wheat genotypes for economic traits. Khazaei *et al*., [9] reported significant variation among the wheat diploid, tetraploid and hexaploid species and found that stomatal frequency decreases from diploid to tetraploid and hexaploid which is reverse in case of stomatal length and width. Plant breeder has suggested to use morphological and physiological plant traits as a selection criteria for yield improvement (Farshadfar *et al*. [10] and Kumar and Sharma [11]). Sharma and Sharma [12] concluded that hybridization system, such as biparentals mating and/or diallel selective mating which exploited both additive and non-additive genetic effects.

Biometrical techniques always helping plant breeders to understand genetic components of crop plants. Most reliable technique to study gene action were described by Mather and Jinks [13] which implies at first degree statistics. For breeding drought tolerant wheat cultivars, availability of genetic variability and knowledge of gene action is needed otherwise it may not result in an appreciable improvement. The objective of present study is to evaluate six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of bread wheat to understand the gene action involved for better performance in rainfed environment.

2. Materials and Methods

Out of twenty six promising wheat lines from national uniform wheat yield trial (NUWYT) 2008-09, two elite lines V-04189 and V-03138 were selected on their higher performance in trial. Seed of the parents V-04189, V-03138 and hybrid (V-04189 × V-03138) grown at the crop research area of Deptt. Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan, during wheat crop season 2009-10 to develop F_2 and back crosses.

After booting, at anthesis selected spikes from parents and hybrid were crossed to develop, BC_1 (V-04189 × F_1), BC_2 (V-03138 × F_1) and some selected spikes from F_1 covered with butter paper bag to develop F_2 generations. All the precautionary measures adopted during crosses to avoid contamination. Seed of crosses and parents were harvested and threshed separately to avoid mechanical mixing and store under optimum conditions. The parents (V-04189, V-03138), F_1, F_2 and back cross (BC_1 and BC_2) generations were sown with pre-irrigation (no further irrigation) in triplicate by using randomized complete block design and subjected to complete their growth under rainfall during 2010-11. A single row for parents and F_1 generations, two rows for each back cross and five rows for F_2 generation were planted. The length of each row was five meters. Inter-row and inter-plant distance was kept 9 cm. Normal cultural practices except irrigation applied to the generations throughout their growing period.

Five plants were selected randomly for data recording from each row for each parent, F_1, twenty plants for back crosses and twenty five plants for F_2 generation in each replication. Data were recorded on the following traits when the plants were green and leaves were in fully expanded condition.

2.1. Flag Leaf Area (cm^2)

From the fully developed flag leaf of selected mother shoots, the maximum length and width was measured in centimeters. The data were recorded in the morning hours when leaf was fully turgid. Flag leaf area was calculated using the following function of Muller [14], Flag leaf area = Flag leaf length × Flag leaf Width × 0.74

2.2. Relative Water Contents (RWC)

The second leaf of each selected plants taken (in all the generations) to measure RWC. Leaf samples collected early in the morning were surface dried with the help of tissue paper and wrapped in polythene bag. Leaf samples were taken to the lab immediately and leaves were weighted to measure fresh weight (FW). These leaves were soaked in distal water overnight at room temperature to revive turgidity. The turgid leaves were blot to dry gently and weighted to measure turgid weight (TW). Turgid leaves were oven dried for 72 hours at 70^o C and dry weight (DW) was recorded with the help of electronic balance (COMPAX model no. CX-600). The RWC was calculated by the following formula as given by Rahman *et al*. [15]

RWC (%) = [(FW–DW) ÷ (TW–DW)] × 100

2.3. Excised Leaf Water Loss (ELWL)

Excised leaf water loss study carried out on third leaf (in all generations) and leaf samples were collected exactly the same way as for RWC above. Fresh weight of the excised leaf measured soon after collection in the field and leaf samples were spread on laboratory bench for six hours at room temperature. After six hours leafs were weighted again to obtain wilted weight than leaves were oven dried for 72 hours at 70^o C and dry weight was recorded with the help of electronic balance (COMPAX model no. CX-600). ELWL calculated using the following equation (Rahman *et al*., [15]).

ELWL = (Fresh weight – Wilted weight) ÷ Dry weight

2.4. Stomatal Frequency

Number of stomata per microscopic field at 10X magnification was counted for flag leaf of the mother shoot of each selected plant. Five strips were taken from the middle part of the flag leaf at the time when it was fully expanded. These strips were dipped into carnoy's solution to arrest the stomatal movement and removal of chlorophyll from the leaf tissue. After 48 hours, the strips were washed with acetone and stored in 70% ethyl alcohol for further examination.

2.5. Stomatal Size (µm)

Size of the stomata was measured in microns. An ocular micrometer (scaled at 10 mm), standardized using a 1.0 mm stage micrometer, was used. Each unit of ocular micrometer was found to be equal to 3.33 microns at 40X at the time of standardization. Length and width of three stomata per five strips was measured to calculate stomatal size and average stomatal size computed.

Data on individual plant basis on second and third leaf for RWC and ELWL, along with flag leaf for stomatal frequency and stomatal size under water deficient condition. Analysis of variance carried out to access significant differences between generation means according to Steel *et al*., [16]. Generation means analysis was performed following the procedure Mather and Jinks [13].

3. Results and Discussion

3.1. Flag leaf area (cm^2)

The six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of a cross V-03138 (P_1) × V-04189 (P_2) showed significant differences ($P \leq 0.05$) for flag leaf area (Table 1). Table 2 also depicted that the calculated flag leaf area of F_1 (34.96 cm^2) cross combination is between the two parents, which showed higher mean value over P_2 (34.33 cm^2) and lesser than P_1 (37.23 cm^2).

The generation means analysis revealed three parameters model [mdi] showed the best fit for flag leaf area from observed to expected generation means (Table 3). The additive genetic effects [d] significantly involved in the inheritance of flag leaf area and indicated that the selection in early segregating generations is useful. The positive and higher value of single epistatic effect (i) makes it possible to fix the additive × additive genetic effect to increase the flag leaf area. Importance of flag leaf area cannot be doubted for grain yield because it is the major site of photosynthesis and provides stored carbohydrates during grain filling. In this experiment it was observed that flag leaf area greatly reduced under rainfed condition (Nabipour *et al,* [17]). At post anthesis stage 98% reduction in the flag leaf area was observed by Kazmi *et al*, [18]. Flag leaf area considerably vary among different ploidy levels in wheat diploid (2n = 2X= 14) species have highest flag leaf area, that's why they have high photosynthetic ability (Maosong *et al*, [19]). The genotype showed high flag leaf area is more productive under moisture stress due to better grain filling. Breeding cultivars for low moisture requirement flag leaf area can be utilized as an essential morphological marker.

The results revealed that flag leaf area is predominantly under the control of additive type of gene action which has been previously reported by Chowdhry *et al*. [20], Awaad [21], Chowdhry *et al.* [22], Subhani and Chowdhry [23], Ambreen *et al*. [24], Mahmood *et al*. [25], Riaz and Chowdhry [26], Inamullah *et al.* [27], Slaeem *et al*. [28] and Munir *et al*. [29].

3.2. Relative water contents (RWC)

Relative water contents indicate the amount of moisture present in leaf. It is an important physiological trait for breeding cultivars under water scarce condition. High relative water contents is foremost need of plant to be successful under limited water condition. Parents V-04189 (P_1) V-3138 (P_2), first and second filial generations along with their back crosses showed significant differences ($P \leq 0.01$) for the relative water contents as displayed in Table 1.

The perusal of Table 2 indicated that hybrid (F_1) showed highest (83.22) relative water contents between the treatments. Malik and Aeright [30] found that drought resistant and drought susceptible genotypes greatly differ for their relative water contents and drought resistant genotypes showed higher relative water contents. Rahman *et al* [15] found non-significant differences between the generations for relative water contents. Siddique *et al* [2] reported that higher relative water contents increase the photosynthetic rate. Water unavailability creates significant reduction in relative water contents ultimately decreasing photosynthesis as agreed by Bajji *et al*. [3] and Sairam and Srivastava [4] but, Tas and Tas [5] claimed that hexaploid cultivars showed higher relative water contents under limited water environment.

The generation means study revealed two parameter model [md] showed fit best for relative water contents (Table 3). The additive genetic effects [d] significantly involved in the inheritance of relative water contents and indicated that the selection in early segregating generations will be fruitful. Absence of epistasis makes it possible to fix the additive genetic effect to increase the relative water contents to achieve the goal of breeding cultivars for stress environment. In the absence of non-allelic interaction the additive genetic effect found to be outstanding as reported before by Farshadfar *et al*. [10], Golparavar *et al*. [31] and Kumar and Sharma [11].

3.3. Excised leaf water loss (ELWL)

Two parents were crossed V-04189 (P_1) × V-3138 (P_2) to develop F_1, F_2 along with their back crosses and all the generations showed significant differences ($P \leq 0.01$) for the excised leaf water loss studied under rainfed condition (Table 1). On viewing Table 2 it is revealed that hybrid (F_1) showed higher value (4.61) of excised leaf water loss followed by P_1, F_2, BC_2, BC_1 and P_2 (4.59, 4.07, 3.87, 3.82 and 3.53 respectively).

The genetic analysis involved six generations in Table 3 revealed two parameter model [md] showed the best fit for excised leaf water loss. The additive genetic effects [d] significantly involved in the inheritance of excised leaf water loss and indicated that the selection in early segregating generations is useful. Absence of epistasis makes it possible to fix the additive genetic effect to control the excised leaf water loss to achieve the goal of breeding cultivars for better stress environment. Excised leaf water loss depicts the ability of leaf to maintain its turgidity when water is not available at field capacity. For breeding under rainfed condition low value of excised leaf water loss is needed because when moisture lost is plant will tend to survive stressful condition. This study reveals that excised leaf water loss is very sensitive to low moisture. In the absence of non-additive genetic effect breeder cannot go for hybrid because heterosis is insufficient to be utilized. In the absence of non-allelic interaction the additive genetic effect predominantly involved in the inheritance of excised leaf water loss as reported before by Farshadfar *et al*. [10] and Kumar and Sharma [11].

3.4. Stomatal frequency

Stomata are the site of gaseous exchange (intake of CO_2) between the leaf and air. Large number of stomata on leaf will increase the CO_2 intake ultimately increasing the photosynthesis. But, under stressful condition the higher stomata become a curse because 95% transpiration take place through stomata. Stomatal frequency showed significant differences ($P \leq 0.01$) among six treatments (P_1, P_2, F_1, F_2, BC_1 and BC_2) presented in Table 1. According to the Table 2 hybrid (F_1) showed higher number of stomata (125.44) as compared to the parents (P_1 = 80.66 and P_2 = 75.11). Nabipour *et al* [17] reported that under moisture stress stomatal frequency significantly increased. Maosong *et al* [19] and Khazaei *et al* [9] claimed that in wheat plant different ploidy levels showed significant diversity in stomatal frequency and diploid species showed highest number of stomata over tetraploid and hexaploid. That's why diploid species are more tolerant to water stress. Yousafzai *et al* [6] and Khazaei *et al* [9] concluded that higher stomatal frequency enhance the ability of wheat plant to withstand drought.

The perusal of Table 3 analysis of generation means indicated that three parameter model [mil] fitting best for stomatal frequency. It has been cleared that trait is under the control of non-additive gene action. The dominance × dominance [l] showed higher and positive value that determined the stomatal frequency had a breeding importance in later generations. The negative value of additive × additive [i] genetic effect makes the result more complex and indicated that selection is of no use in early segregating generations because there is no additive genetic effect to be fixed. Mahmood *et al*. [25] reported that additive genetic effects along with the partial dominance involved in the inheritance of stomatal frequency. The non-allelic interaction out of which additive × additive [i] and dominance × dominance [l] found to be outstanding Ambreen *et al.* [24].

3.5. Stomatal Size (μm)

According to the Table 1 parents F_1, F_2, BC_1 and BC_2 derived from a between cross V-04189 (P_1) × V-03138 (P_2) showed significant differences at significance level 1%. The perusal of Table 2 depicted that the stomatal size of hybrid F_1 (752.95) was higher than P_2 (748.88) and P_1 (148.88) parent. Maosong *et al.* [19] and Khazaei *et al.* [9] claimed that in wheat plant different ploidy levels showed significant diversity in stomatal size and hexaploid species showed higher stomatal size over tetraploid and diploid species.

The genetic analysis showed that three parameters model [mhi] showed the best fit for stomatal size from observed to expected generation means (Table 3). The additive genetic effect [d] is absent showed that stomata size is under the control of non-additive gene action and heterosis can be utilized in hybrid combinations. This may be due to over-dominance, unidirectional or dispersion of genes in the parents. The positive and higher value additive × additive [i] genetic effect favors the selection in early segregating generation and it is possible to fix the additive × additive [i] genetic effect to increase the stomata size. Hasan and Khaliq. [32] reported that stomatal size controlled by non-additive genetic effects and Ambreen *et al*. [24] also reported the presence of non-allelic interaction in the inheritance of stomatal size.

4. Conclusions

The data analysis revealed, all the traits studied have significant differences among the six generations. Generation means analysis concluded that most of the physiological traits controlled by additive type of gene action that's why, the selection in early generations could be helpful for improvement. In future, breeding cultivars against rainfed environment, these genotypes could be given a due importance.

Table 1. Analysis of variance for five physiological traits of six generations (P1, P2, F1, F2, BC1 and BC2) of wheat cross V-04189 (P1) × V-03138 (P2) evaluated under rainfed condition.

	MEAN SQUARES		
CHARACTERS	**REPLICATION (df=2)**	**GENOTYPES (df=5)**	**ERROR (df=10)**
Flag leaf area (cm^2)	0.62 $^{N.S}$	5.94**	0.55
Relative water contents (RWC)	0.082 $^{N.S}$	11.40**	0.85
Excised leaf water loss (ELWL)	0.24 $^{N.S}$	1.29**	0.18
Stomatal frequency	15.1*	969.8**	10.3
Stomatal size (µm)	178*	1206**	228

*P ≤ 0.05, ** P ≤ 0.01, N.S = Non-significant

Table 2. Generation means of five physiological traits of the wheat cross V-04189 (P1) × V-03138 (P2) evaluated under rainfed

Traits	**P_1**	**P_2**	**F_1**	**F_2**	**BC_1**	**BC_2**
Flag Leaf Area (cm^2)	37.23	34.33	34.96	32.90	34.80	35.22
Relative water contents (RWC)	82.65	80.73	83.22	82.71	79.21	79.53
Excised leaf water loss (ELWL)	4.59	3.53	4.61	4.07	3.82	3.87
Stomatal Frequency	80.66	75.11	125.44	98.44	94.44	94.66
Stomatal Size (µm)	751.10	748.88	752.95	703.00	725.57	724.09

Table 3. Estimations of genetic components of the best fit model on generation means for six physiological traits of the wheat cross V-04189 (P1) × V-03138 (P2) evaluated under rainfed condition

Traits	m ± S.E	[d] ± S.E	[h] ± S.E	[i] ± S.E	[j] ± S.E	[l] ± S.E	χ^2 (d.f)
Flag leaf area (cm^2)	66.41** ± 0.51	1.95** ± 0.52		1.96** ± 0.84			4.273(3)
Relative water contents (RWC)	146.07* ± 0.55	1.00* ± 0.83					7.621(4)
Excised leaf water loss (ELWL)	27.85** ± 0.14	1.25** ± 0.25					4.882(4)
Stomatal frequency	48.92* ± 1.77			-4.87* ± 2.22		12.28* ± 3.34	5.596(3)
Stomatal size (µm)	41.69** ± 15.63		4.51** ± 22.20	5.82** ±16.72			0.177(3)

m = Mean, [d] = Additive effects, [h] = Dominance effects, [i] = Additive × Additive effects, [j] = Additive × Dominance effects, [l] = Dominance × Dominance effects.

***P ≤ 0.05, ** P ≤ 0.01**

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