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GENETIC BASIS OF FLAG LEAF AREA IN BREAD WHEAT *(TRITICUMAESTIVUM* L.) UNDER DROUGHT CONDITION

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ABSTRACT

Using mixed inheritance analysis also called joint segregation analysis (JSA), six basic generations (P_1, F_1, P_2, BC_1, BC_2 and F_2) were developed and genetic effects in controlling flag leaf area were determined in four bread wheat crosses (1942 / Dera-98, SVP-74 / Zam-04, Pari-73 / Hashim-08, SVP-83 / Gomal-0) during 2012-2014. The results revealed that average population of F_1 exhibited almost intermediate flag leaf area to their parents in all the crosses except Pari-73 × Hashim-08 which exhibited increased leaf area than parent 1 and 2 indicating the accumulation of positive dominant genes in F_1 of the respective cross to control the trait. BC_1 and BC_2 showed little tendency towards their respective parents. F_2 population among all the crosses was almost equally distributed between the parents (P_1 and P_2). This continuous variation in F_2 and some BC_1 and BC_2 generations indicates that flag leaf area is under genetic control of mixed interaction of one to two major genes and several polygenes. Based on maximum log of likelihood estimates and AIC values, the best fit genetic model for cross 1942 / Dera-98 and SVP-74 / Zam-04 was E-1 which shows that the flag leaf area was controlled by the mixed action of two major additive dominant epistatic genes plus additive dominant polygene. The additive effect of 1st major gene (A) was positive and it was negative for 2nd major gene (B). The dominant effects (h_a, h_b) was recorded in the range of -0.2 - 6.9. The additive × additive effect (*i*) for cross 1942 / Dera-98 and SVP-74 / Zam-04 were -2.58 and -7.50, respectively. The non allelic dominant × dominant interaction (*l*) was -12.08 and 0.52 for cross 1942 / Dera-98 and SVP-74 / Zam-04 respectively. The additive × dominant effect (J_{ab}) of gene A over gene B and that of B over A for cross 1942 / Dera-98 was 5.34 and -4.87, respectively. Whereas for cross SVP-74 / Zam-04 was 8.53 and -3.09, respectively. The major gene variation was higher than that of polygene in both the crosses. Similarly, major gene heritability which is the most important second order genetic parameter was also higher in comparison to polygene heritability in both the crosses. Results revealed that best fit genetic model for cross Pari-73 × Hashim-08 and SVP-83 × Gomal-08 was D indicating that the trait was under the influence of mixed one major gene and additive dominance epistasis of polygene. The second order genetic parameters showed the phenotypic variation for BC_1, BC_2 and F_2 generation. The variation of single major gene in both the crosses was higher than corresponding polygenic variation. Similarly, the major genes heritability was also higher than polygene heritability in both the crosses. Results clearly revealed all genetic architecture about leaf area which recommended using this trait as a selection criteria for further breeding program.

Keywords: Bread wheat, flag leaf area, epistasis, major genes, polygenes inheritance

INTRODUCTION

Wheat (*Triticum aetsivum* L.) is a major cereal of Pakistan and has a key role in the agricultural policy and economy of the country. It is a staple food of about 40 percent among all over population of the world. Improved grain yield is the aim of cereal breeders and is based on the performance of yield components together with conducive environmental conditions [1]. Being the factories of photosynthesis, leaves have an obvious relationship with grain yield [2]. Compare to other leaves, the flag leaf contributes the most of photosynthetic assimilates to wheat kernel; therefore, it has the greatest importance in association with grain yield [3]. The dry mater contributed by the flag leaf to the grain in wheat accounts for 41 to 43% and is the major photosynthetic site during the grain filling stage [4, 5]. Leaf size accounts for as an important parameter for determining differences in biomass and grain yield in cereals and is related to photosynthetic area above the flag-leaf node; therefore, FLA was mentioned as one of the major objectives of plant breeding programs [6]. Based on conclusion of Sahin and Yildirim [1], larger flag leaf area is correlated with higher assimilates in wheat kernel due to efficient photosynthesis. All previous genetic studies relevant to the inheritance of flag leaf traits were either based on generation means analysis [7-9] or diallel analysis [1, 10] that measured the trait only as the polygenic system without measuring the effect of individual genes [11]. The statistical procedure used in the present studies has the power to determine individual effects up to two major genes as well as the collective epistatic effects of polygene [12]. Because of the high cost of the molecular techniques, population and sample size restriction, and the interference of errors, QTL technique has limited applications in breeding [13]. Based on the efforts of Wang [12], joint segregation analysis (JSA) is the method to identify the mixed inheritance model of QTLs and to estimate related genetic parameters; this utilizes the available sample size efficiently, for measuring plant quantitative traits [13]. In light of the superiority of the JSA over the previous statistical approaches, the present study was undertaken to find out (i) the genetic diversity for FLA among the genotypes to be used in cross combinations, (ii) genetic mechanism of FLA through hybridization between the parents of larger and smaller FLA and vice versa and (iii) the number and individual effects of major gene(s), and cumulative effect of the major as well as polygene, involved in controlling the FLA.

MATERIALS AND METHODS

Wheat germplasm consisting of 61 genetically variable genotypes (Table 1: please give the table) were planted in three replications in five meter long rows per entry with 50 seeds per row in randomized complete block design at experimental farm of the Department of Plant Breeding and Genetics, Faculty of Agriculture Gomal University, Dera Ismail Khan, Pakistan from Oct-May, 2010-2014. The site is situated at latitude 31.831482'N, longitude 70.911598'E and altitude 165m above mean sea level. Plot size per entry in each set was kept 1.2 m^2. For developing artificial drought conditions in the field, irrigation was ignored. All other cultural practices were performed normally. Data regarding plant height (cm), days to heading, days to maturity, flag leaf area (cm^2), number of ears per plant, number of spikelets per spike, number of grains per spike, 1000 grain weight (g), grain weight per spike (g) and grain yield per plant (g) were recorded on 10 randomly selected plants from each accession within each replication (Table 1).

Table 1: Cross-combinations attempted between parents during cropping season 2011-2012 (2^{nd} year) to produce F_1

1.	May-1942 × Dera-98
2.	SVP-74 × Zam-04
3.	Pari-73 ×Hashim-08
4.	SVP-83 × Gomal-08

Development of populations for Joint Segregation Analysis (JSA)

During growing season of the year, 2011 selected genotypes were sown on two different sowing dates i.e. October 24, 2011, November 05, 2011 in order to get floral synchrony among parents with different flowering times and to attempt maximum cross-combinations during flowering period. To develop six basic populations i.e. P_1, P_2, F_1, BC_1, BC_2 and F_2 for joint segregation analysis, crosses were attempted as presented in Table 1. F_1 seeds were separately collected from each cross combination on maturity. In next cropping season 2012-13 on two separate sowing dates (October 29, November 7) F_1 crosses were raised along with eight selected parents. On flowering F_1 was crossed with parent-1 (P_1) to have Back cross-1 (BC_1). Similarly, F_1 when crossed with parent-2 (P_2) resulted in the production of Back cross-2 (BC_2). It was separately exercised for each of the four crosses. During this year F_1 were used as pollen recipient parents and their parents (P_1 and P_2) were used as pollen donor parents, cross-combinations were attempted in order to produce first back crosses (BC_1) and second back crosses (BC_2) of all the F_1. It is elaborated as under

Table 2: Development of Back-crosses of experiment

First Back-crosses= BC_1 s = F_1 (pollen recipient) × P_1 (pollen donor)		
F_1	×	Parent-1
(May-1942 × Dera-98)	×	May-1942
(SVP-74 × Zam-04)	×	SVP-74
(Pari-73 × Hashim-08)	×	Pari-73
(SVP-83 × Gomal-08)	×	SVP-83
Second Back-crosses= BC_2 = F_1 (pollen recipient) × P_2 (pollen donor)		
F_1	×	Parent-2
(May-1942 × Dera-98)	×	Dera-98
(SVP-74 × Zam-04)	×	Zam-04
(Pari-73 × Hashim-08)	×	Hashim-08
(SVP-83 × Gomal-08)	×	Gomal-08

During same year some F_1 were bagged for self-pollination and obtained F_2 seeds of the attempted crosses. At maturity, seeds from F_1 F_2, BC_1 and BC_2 were harvested and stored for sowing in the field as an experimental trial during the next cropping season i.e., 2013-14.

Evaluation of six basic generations for Joint Segregation Analysis (JSA)

For field trials, seeds from the above mentioned crosses (Table 3) (F_1, F_2, BC_1 and BC_2) and those from their respective parents (P_1 and P_2 of the respective crosses) were sown during cropping season 2013-14 in the experimental area of Department of Plant Breeding and Genetics, Faculty of Agriculture Gomal University, Dera Ismail Khan, Pakistan, in artificially created water stress environment i.e. without applying irrigation water. Another set of the six populations (P_1, F_1, P_2, BC_1, BC_2 and F_2) was planted under normal conditions for recording data on yield and yield related traits as mentioned below. Both the experimental sets were planted in Randomized Complete Block Design (RCBD) in three replications. The row length was 5 meters and numbers of rows were two rows for all the four crosses and parents, F_1, BC_1, BC_2 and F_2 in each replication. Seeds were sown through dibbler with plant-to-plant distance of 10cm and row-row distance was kept 30 cm apart. For data recording, 50 randomly selected plants were taken from each parent and their respective F_1, 150 plants from each of BC_1 and BC_2 and 200 plants from each F_2 in each replication. On attaining maximum growth, flag leaves from main tillers were selected. Flag leaf area was measured in cm^2 using the formula [14] as, maximum width × length × 0.74. Average flag leaf area for each entry was calculated by dividing total area over number of observations.

Basics of genetic models for joint segregation analysis (JSA)

Morphological parameters were studied according to four kinds of genetic models as mentioned by Gai and Wang, [13], i.e. (1) one major-gene inheritance, (2) two major-gene inheritance, (3) polygene inheritance, and mixed one major-gene and (4) polygene inheritance. Data were recorded on randomly selected plants as mentioned earlier from six basic populations, i.e., parents (P_1 and P_2), first filial generation (F_1), backcrosses ($BC_1$and BC_2), and second filial generation (F_2). Aspects of the related materials are discussed in Table 3. The proposed assumptions of [13] are enumerated as below.

1. Diploid nuclear inheritance with no maternal or cytoplasmic effects,
2. No interaction or linkage between major genes and polygenes,
3. No selection and
4. The genetic effect of polygene and the effect of the environment in any segregating population followed a normal distribution, and variances

within the P_1, P_2 and F_1 populations were equal.

Based on these theories, five groups and 24 types of genetic models were developed as mentioned in Supplementary Tables S1 and S2 [13, 15]. If at one major locus if the two Parents differ in a particular quantitative trait, then only three major genotypes are possible. Let A-a represent the alleles of the locus, then accord to the Mendelian genetics/principles. The major genotypes for the two parents and the F_1 will be AA, aa and Aa, respectively. The genotypes for backcross BC_1 is a 1:1 mixture of AA and Aa, for BC_2 a 1: 1 mixture of Aa and aa, and for the F_2 a 1: 2: 1 mixture of AA, Aa and aa. Distribution forms of six basic generations are as under.

$$P_1 : X_{1i} \sim N(\mu_1, \sigma^2), \quad F_1 : X_{2i} \sim N(\mu_2, \sigma^2),$$

$$P_2 : X_{3i} \sim N(\mu_3, \sigma^2), \quad BC_1 : X_{4i} \sim (1/2)\, N(\mu_{41}, \sigma_4^2 + (1/2)\, N(\mu_{42}, \sigma_4^2).,$$

$$BC_2 : X_{4i} \sim (1/2)\, N(\mu_{51}, \sigma_5^2 + (1/2)\, N(\mu_{52}, \sigma_5^2) \text{ and}$$

$$F_2 : X_{6i} \sim (1/4)\, N(\mu_{61}, \sigma_6^2 + (1/2)\, N(\mu_{62}, \sigma_6^2 + (1/4)\, N(\mu_{63}, \sigma_6^2).$$

In six basic generations ten component distributions were supposed. When the genetic model was the mixed one major-gene and polygene, and all possible genetic effects existed, the ten components were different. Under some specific cases, some components remained unchanged. For example, when only the major gene existed without polygene (A-group model), the components were of the following relationships [13].

$$N(\mu_1, \sigma^2) = N(\mu_{41}, \sigma_4^2 = N(\mu_{61}, \sigma_6^2, \; N(\mu_2, \sigma^2) = N(\mu_{42}, \sigma_4^2 = N(\mu_{62}, \sigma_6^2$$

$$N(\mu_3, \sigma^2) = N(\mu_{52}, \sigma_5^2 = N(\mu_{63}, \sigma_6^2$$

When two major-genes were present without polygene in six basic generations, there were nine component distributions. In polygenic-inheritance model, each population of the six generations was calculated as a single normal distribution, and there were six different components in basic generations [13]. The genetic parameters presented in each model are given in Supplementary Tables 1 and Table 2.

Joint multiple-generation likelihood and an EM algorithm for Traits calculation

Based on EM algorithm [12, 16, 17] maximum-likelihood estimates were evaluated. Doctrine behind allthe models presented in Table 4 and 35 are nearly the same. In the E-step, the logarithm likelihood function of the complete data, classified by Bayesian rules is formulated as given in Table 3.3.

$$L_c(\Phi) = C + \Sigma \log f\,(X_{1j}; \mu_{1,}, \sigma^2) + \Sigma \log f\,(X_{2j}; \mu_2, \sigma^2)$$

$$+ \Sigma \log f(X_{3j};\mu_3,\sigma^2) + \Sigma[W_{4i1}\log f(X_{4i};\mu_{41},\sigma_4^2)+W_{4i2}\log f(X_{4i};\mu_{42},\sigma_4^2)]$$
$$+ \Sigma[W_{5i1}\log f(X_{5i};\mu_{51},\sigma_5^2)+W_{5i2}\log f(X_{5i};\mu_{52},\sigma_5^2)]$$
$$+ \Sigma[W_{6i1}\log f(X_{6i};\mu_{61},\sigma_6^2)+W_{6i2}\log f(X_{6i};\mu_{62},\sigma_6^2)] + W_{6i3}\log f(X_{6i};\mu_{63},\sigma_6^2)]$$

Where the range of summations is on individuals and $f(X_{1j};\mu_1,\sigma^2)$ shows the density function of the normal distribution $N(\mu_1,\sigma^2)$ and so on for the others. $W_{4i1}, W_{4i2}, W_{5i1}, W_{5i2}, W_{6i1}, W_{6i2}$ and W_{6i3} are rear probabilities of samples from BC_1, $BC_2$and F_2 populations under the initial parameter values. In the M-step, the maximum point of $L_c(\Phi)$ can be acquired for model D by calculating partial derivatives of $L_c(\Phi)$ for all traits and letting derivatives be zero. Where there were constraints in models D-1 through D-4, then the Lagrange-multiplicator (or λ – multiplicator method) was used in the maximization step for those models with constraints. As presented above, the technique to get maximum-likelihood estimates of traits was summed up as below:

(1) Initial values of component parameters were chosen according to the observations;

(2) Posterior probabilities $W_{4i1}, W_{4i2}, W_{5i1}, W_{5i2}, W_{6i1}, W_{6i2}$ and W_{6i3} were analyzed and therefore, obtained the logarithm likelihood $L_c(\Phi)$ (E-step) of the whole data;

(3) The maximum, or conditional maximum, of L_c was calculated and obtained the estimates of means and variances of the component distributions (M-step);

(4) Initial values with estimates from step (3) were replaced and then iterated steps (2) and (3) until a previously selected precision was achieved.

Table 3: The codes and parameters of P_1, F_1, P_2, BC_1, BC_2 and F_2

Generation	Code	Sample Size	Observation	Mean	Variance	Distribution
P_1	1	$n_1=60$	X_{1i}	μ_1	σ^2	$N(\mu_1,\sigma^2)$
F_1	2	$n_2=90$	X_{2i}	μ_2	σ^2	$N(\mu_2,\sigma^2)$
P_2	3	$n_3=60$	X_{3i}	μ_3	σ^2	$N(\mu_3,\sigma^2)$
BC_1	4	$n_4=150$	X_{4i}	μ_4	σ^2_{BC1}	Mixture of two or more normal curves
BC_2	5	$n_5=150$	X_{5i}	μ_5	σ^2_{BC2}	Mixture of two or more normal curves
F_2	6	$n_6=210$	X_{6i}	μ_6	σ^2_{F2}	Mixture of two or more normal curves

Model selection by *AIC* and test of fitness

In the joint segregation analysis of the mixed genetic model, Akaike's Information Criterion (*AIC*) [18] was employed to determine which of the following three statistics were most fitting.

$$1.\quad U_1^2 = 12[\sum F(X_{ii}) - n/2]^2, /n \sim \chi^2(1)$$

(to test whether the mean of Y_i is 1/2)

$$2.\quad U_2^2 = 45/4[\sum F(X_i)^2 - n/3]^2, /n \sim \chi^2(1)$$

(to test whether the 2nd momentum of Y_i is 1/3)

$$3.\quad U_3^2 = 180[\sum F(X_i) - 0.5)^2 - n/12]^2, /n \sim \chi^2(1)$$

(to test whether the variance of Y_i is 1/12)

Where;

$$AIC = (-2)\log(\mathit{Maximum\ likelyhood}) + 2(\mathit{Number\ of\ independent\ parameters})$$

Likelihood-ratio test (LRT) was used to choose the simplest type within the model group.

Where; $LRT = \lambda = 2\log(L_a) - 2\log(L_0)$

L_a and L_0 are the maximum likelihoods under H_a and H_0, respectively.

Two other important completely distribution free tests i.e. Smirnov's statics (nW^2) and Kolmogorove's statistics (D_n) where $D = \mathrm{Sup}\left|Fn(x) - F0(x)\right|$ were used as goodness-of-fit tests to determine whether the selected model sufficiently explains the data. If, for a particular genetic model, none of these five statistics were significant, then it was the indication that the data is adequately fit for the model.

Calculation of genetic parameters

Genetic parameters can be computed from the estimates of component parameters in the corresponding model. Taking model D as an example, the first-order genetic parameters can be calculated by least squares from the following equations [19].

$$\mu_1 = m + d + [d] + [i];\quad \mu_2 = m + h + [h] + [I];\quad \mu_3 = m - d - [d] + [i];$$
$$\mu_{41} = m + d + 1/2\,[d]\,1/2\,[h] + 1/4\,[i] + 1/4\,[j] + 1/4\,[l];$$
$$\mu_{42} = m + h + 1/2\,[d] + 1/2\,[d] + 1/2\,[h] + 1/4\,[i] + 1/4\,[j] + 1/4\,[l];$$
$$\mu_{51} = m + h - 1/2\,[d] + 1/2\,[h] + 1/4\,[i] - 1/4\,[j] + 1/4\,[l];$$
$$\mu_{52} = m - d - 1/2\,[d] + 1/2\,[h] + 1/4\,[i] - 1/4\,[j] + 1/4\,[l];\quad \mu_{61} = m + d + 1/2\,[h] + 1/4\,[l];$$
$$\mu_{62} = m + h + 1/2\,[h] + 1/4\,[l];\quad \mu_{63} = m - d + 1/2\,[h] + 1/4\,[l];$$

where m is the population mean, d and h are the additive and dominance effects of major genes, respectively and [d], [h], [i], [j] and [l] are additive, dominance, additive-additive, additive-dominance and dominance-dominance epistasis effects, respectively. The phenotypic variance (σ_p^2) of BC_1, BC_2 and F_2 was directly calculated from the observation data. σ^2 in the phenotypic variance of P_1, P_2 and F_1 was regarded as the environmental variance (σ_e^2). Since there is no genetic variation in each of the three populations; (σ_4^2) is the variance of component distribution in BC_1 which consists of polygenic variance (σ_{pg}^2) and environmental variance (σ_e^2). Thus $\sigma_p^2 = \sigma_{mg}^2 + \sigma_{pg}^2 + \sigma_e^2$ and $\sigma_4^2 = \sigma_{pg}^2 + \sigma_e^2$ for the

BC_1 population. Therefore, the major-gene variance σ^2_{mg} and the polygenic variance σ^2_{pg} in BC_1 were estimated, and the major-gene heritability (h^2_{mg}) and polygenic heritability (h^2_{pg}) were also estimated from $h^2_{mg} = \sigma^2_{mg} / \sigma^2_p$ and $h^2_{pg} = \sigma^2_{pg} / \sigma^2_p$. Same principle was used for calculating $\sigma^2_{mg}, \sigma^2_{pg}$, h^2_{mg} and h^2_{pg} in BC_2 and F_2 i.e $\sigma^2_{pg}(BC_2) = \sigma^2_p(BC_2) - \sigma^2_{mg}(BC_2) - \sigma^2_e$, $\sigma^2_{pg}(F_2) = \sigma^2_p(F_2) - \sigma^2_{mg}(F_2) - \sigma^2_e$,

$h^2_{mg}(BC_2) = \sigma^2_{mg}(BC_2) / \sigma^2_p(BC_2)$ and $h^2_{mg}(F_2) = \sigma^2_{mg}(F_2) / \sigma^2_p(F_2)$

Similarly, $h^2_{pg}(BC_2) = \sigma^2_{pg}(BC_2) / \sigma^2_p(BC_2)$ and $h^2_{pg}(F_2) = \sigma^2_{pg}(F_2) / \sigma^2_p(F_2)$

Posterior/rear genetic probabilities

Computation of posterior genetic probabilities was beyond the scope of the present study. As outlined by [13], the methodology and formulation for the computation of posterior genetic probabilities for general mixture with density function form $p(x;\phi) = \Sigma^k_{j=1} \pi_j f(x;\theta_j)$, the posterior probabilities, $W_t = (t = 1,....., g)$ of a sample having x is summarized as under:

$$W_t = \pi_t f(x;\theta_t), \sum_{t=1}^{g} W = 1$$

For model D, the posterior probabilities of individuals in BC_1, BC_2 and F_2 are calculated as:

$$BC_1: W_{4i1} = f(X_{4i};\mu_{41},\sigma_4^2)/[f(X_{4i};\mu_{41},\sigma_4^2) + f(X_{4i};\mu_{42},\sigma_4^2)]$$

$$W_{4i2} = f(X_{4i};\mu_{42},\sigma_4^2)/[f(X_{4i};\mu_{41},\sigma_4^2) + f(X_{4i};\mu_{42},\sigma_4^2)]$$

$$BC_2: W_{5i1} = f(X_{5i};\mu_{51},\sigma_5^2)/[f(X_{5i};\mu_{51},\sigma_5^2) + f(X_{5i};\mu_{52},\sigma_5^2)]$$

$$W_{5i2} = f(X_{5i};\mu_{52},\sigma_5^2)/[f(X_{5i};\mu_{51},\sigma_5^2) + f(X_{5i};\mu_{52},\sigma_5^2)]$$

$$F_2: W_{6i1} = f(X_{6i};\mu_{61},\sigma_5^2)/[f(X_{6i};\mu_{61},\sigma_6^2) + 2f(X_{6i};\mu_{62},\sigma_6^2) + f(X_{6i};\mu_{63},\sigma_6^2)]$$

$$W_{6i2} = 2f(X_{6i};\mu_{62},\sigma_6^2)/[f(X_{6i};\mu_{61},\sigma_6^2) + 2f(X_{6i};\mu_{62},\sigma_6^2) + f(X_{6i};\mu_{63},\sigma_6^2)]$$

$$W_{6i3} = f(X_{6i};\mu_{63},\sigma_6^2)/f(X_{6i};\mu_{61},\sigma_6^2) + 2f(X_{6i};\mu_{62},\sigma_6^2) + f(X_{6i};\mu_{63},\sigma_6^2)].$$

For determining the values of first order (μ_1 to μ_{69}) and second order genetic parameters ($\sigma^2_{41}, \sigma^2_{53}, \sigma^2_{61}, \sigma^2$) as well as $U_1^2, U_2^2, U_2^3, nW^2$ and D, the recorded data were analyzed by using statistical software Sin. Exe, the major gene-polygene mixed inheritance model to a joint analysis of multi-generations (Gai*et al.*, 2003) specially designed for six generations i.e. P_1, P_2, F_1, BC_1, BC_2, and F_2 generations. In case of best fit model the values of second order genetic parameters as well as σ^2_{mg} and σ^2_{pg} for BC_1, BC_2 and F_2 were worked out by using Microsoft excel program.

RESULTS

Frequency distribution of flag leaf area (cm^2) in different crosses

The data pertaining to frequency distribution of plant population in six basic generations regarding flag leaf area of four wheat crosses are presented in (Supplementary Table S3 and Supplementary Figures 1-8). Most of the plant population in the four crosses were recorded in the range of 25-31, 32-38, 39-45 cm^2 flag leaf area, followed by the 18-24, 46-52 and 53-59 cm^2 in all the crosses. On average F_1 exhibited almost equal flag leaf area to their parents in all the crosses except Pari-73 × Hashim-08 which exhibited increased leaf area than parent 1 and 2 indicating that dominant genes may be involved in controlling the trait. BC_1 and BC_2 showed little tendency towards their parents. F_2 segregating populations among all four crosses were almost equally distributed between the parents (P_1 and P_2). This continuous variation in F_2 and some BC_1 and BC_2 generations indicates that flag leaf area are genetically controlled by mixed interaction of one to two major genes plus polygene.

Genetic analysis of flag leaf area

The best fit model selected on the basis of maximum log of likelihood estimates and AIC values for cross 1942 × Dera-98 and SVP-74 × Zam-04 were found to be E-1 . Tests for goodness-of-fit regarding Flag leaf area (cm^2) of models E-1, D, D-2, E and B-1 for crosses has been shown in Supplementary Tables S5 and S6 while the most fit model for genetic behavior for flag leaf area has been shown in table S7. The model E-1 shows that the flag leaf area was controlled by the mixed action of two major additive dominant epistatic genes plus additive dominant minor genes (Table 4). The population means were 48.66 and 55.68 for cross 1942 × Dera-98 and SVP-74 × Zam-04 respectively. The additive effect of first major gene was positive in both the crosses and recorded as 7.01 and 13.65, respectively. Whereas that of second major gene in both the crosses were negative and recorded as -7.14 and -6.49, respectively. The dominant effects (h_a, h_b) was recorded as -4.09 and -0.28 for cross 1942 × Dera-98 and -2.13 and 6.99 for cross SVP-74 × Zam-04. The additive × additive (i) effect for cross 1942 × Dera-98 and SVP-74 × Zam-04 was -2.58 and -7.50, respectively. The dominant × dominant (l) non allelic interaction was -12.08 and 0.52 for cross 1942 × Dera-98 and SVP-74 × Zam-04 respectively. The additive × dominant effect of gene A over gene B (Jab) and that of B over A for cross 1942 × Dera-98 was 5.34 and -4.87 while for cross

SVP-74 × Zam-04 was 8.53 and -3.09, respectively. The second order genetic parameters for cross 1942 × Dera-98 and SVP-83 × Gomal-08 is also presented in table 4. The major gene variation was higher than that of polygene in both the crosses. Similarly, major gene heritability which is the most important second order genetic parameter was also higher in comparison to polygene heritability in both the crosses (Table 4). According to MLLE and AIC values (Supplementary Table 4) the best fit model for cross Pari-73 × Hashim-08 and SVP-83 × Gomal-08 was model D. the Model D indicates that the trait was under the influence of mixed one major gene and additive dominance epistasis of polygene. The additive effect of single major gene in cross Pari-73 × Hashim-08 was negative (-0.40) while the dominant effect (h) was positive (2.98). In cross SVP-83 × Gomal-08 the additive effect was positive (13.50) while dominant effect was negative. The second order genetic parameters shows the phenotypic variation for BC_1, BC_2 and F_2 generation which is further subdivided into genetic and environmental variation. The genetic variation is further divided into variation due to major and minor genes. The variation of single major gene in both the crosses was higher than corresponding minor gene variation. Similarly, the heritability of major genes was also higher than minor gene heritability in both the crosses. In four crosses of bread wheat gene action regarding flag leaf area was determined. Flag leaf area was under control of additive effects due to polygenes [d] in cross SVP-74 × Zam-04. Variations and heritability estimates were higher in case of major gene as compare to minor genes. It is due the epistatic effect of major genes plus polygenes but the main influence was of major genes. In both the crosses SVP-73 × Gomal-08 and SVP-83 × Hashim-08, one major gene and additive dominance polygenes were responsible for flag leaf area (model D). epistatic type of gene action was found among both major genes and polygenes due to additive [d] effect and dominant [h] effect of minor genes.

DISCUSSIONS

It has been found from different reports that the flag leaf area plays an important role in photosynthesis and accumulation of organic compounds [20, 21]. Simpson, [21] reported that there was a significant relationship between flag leaf area and photosynthetic rate. The selection of wheat genotypes on the basis of flag leaf area may be helpful to improve grain yield and production of wheat under biotic and abiotic stress conditions [22, 23]. In two crosses 1942 × Dera-98 and SVP-74 × Zam-04 model E-1 describing mixed two major additive-dominance epistatic genes and additive-dominant minor genes, which shows that flag leaf area was under control of it. These crosses shows that FLA is under the influence of epistasis along with over dominance type of gene action with additive effects due to polygenes [d] and favorable dominant effects due to major gene A and major gene B and additive effects. Similar results about over dominance type of gene action were also reported by Ajmal *et al*., [24]. Additive type of gene action along with partial dominance was reported by Bennett *et al*., [25] who says that selection in early generations is useful in that parameter. The environment was highly influenced on that parameter as shown by its variations. Various researchers have reported similar findings about flag leaf area role in improving grain yield and production of wheat. It have been studied that the selection on the basis of flag leaf area may be fruitful to wheat breeders to combat with changing environmental conditions and development of biotic and abiotic stress tolerance wheat varieties [1, 26-28]. Normal frequency distribution amongst F_{2S} denotes that Flag Leaf Area is quantitatively controlled traits which are clearly depicted from the values of major and minor genes [29]. Earlier research explains that significant additive gene effect is greatly involved in FLA i.e., generation means, diallel analysis and QTL analysis.

Table 4: Estimates of first and second order genetic parameters for Flag leaf area (cm^2)in four bread wheat crosses

Cross 1: 1942 × Dera-98 (Model E-1)

1st order parameter	Estimate	1st order parameter	Estimat	2nd order parameter	Estimates BC1	BC2	F2
m=	48.66	i =	-2.58	σ_p^2	258.34	216.75	259.96
d_a=	7.01	j_{ab} =	5.34	σ_{mg}^2	117.25	79.52	127.43
d_b=	-7.14	j_{ba}=	-4.87	σ_e^2	128.24	128.24	128.24
h_a=	-4.09	l =	-12.08	σ_{pg}^2	12.85	8.99	4.29
h_b=	-0.28	[d]	30.92	h_{mg}^2(%)	41.35	36.68	49.01
h_a/d_a	-0.58	[h]	12.44	h_{pg}^2(%)	4.97	4.14	1.63
h_b/d_b	0.04						

Cross 2: SVP-74 × Zam-04 (Model E-1)

1st order parameter	Estimate	1st order parameter	Estimate	2nd order parameter	Estimates BC1	BC2	F2
m=	55.68	*i* =	-7.50	σ_p^2	283.53	204.63	295.02
d_a=	13.65	j_{ab} =	8.53	σ_{mg}^2	166.91	110.13	198.21
d_b=	-6.49	j_{ba}=	-3.09	σ_e^2	86.87	86.87	86.87
h_a=	-2.13	l =	0.52	σ_{pg}^2	29.79	7.63	9.94
h_b=	6.99	[d]	30.73	h_{mg}^2(%)	58.86	53.81	67.18
h_a/d_a	-0.16	[h]	-19.55	h_{pg}^2(%)	10.50	3.72	3.36
h_b/d_b	-1.08						

Cross 3: Pari-73 × Hashim-08 (Model D)

1st order parameter	Estimate	1st order parameter	Estimate	2nd order Parameters	Estimates BC1	BC2	F2
M	42.64	D	-0.40	σ_p^2	326.98	313.22	294.63
m_1=	38.36	H	2.98	σ_{mg}^2	175.16	160.2	147.1
m_2=	87.91	h/d	-7.45	σ_e^2	143.93	143.93	143.93
m_3 =	48.69	[d]	1.55	σ_{pg}^2	7.89	9.09	3.6
m_4=	47.35	[h]	-3.56	h_{mg}^2(%)	53.56	51.14	49.92
m_5=	71.11	[i]	-7.00	h_{pg}^2(%)	2.41	2.90	1.22
m_6=	69.66	[j]	0.33				
		[l]	-10.64				

Cross 4: SVP-83 × Gomal-08 (Model D)

1st order parameter	Estimate	1st order parameter	Estimate	2nd order parameter	Estimates BC1	BC2	F2
M	160.54	D	13.50	σ_p^2	247.36	226.54	247.41
m_1=	28.62	H	-11.46	σ_{mg}^2	96.7	64.10	108.56
m_2=	56.65	h/d	-0.85	σ_e^2	135.14	135.14	135.14
m_3 =	55.46	[d]	1.55	σ_{pg}^2	15.95	27.30	3.71
m_4=	47.14	[h]	-3.56	h_{mg}^2(%)	39.09	28.29	43.87
m_5=	56.37	[i]	-7.00	h_{pg}^2(%)	6.42	12.05	1.49
h_b/d_b	289.37	[l]	-10.64				

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