BREEDING BREAD WHEAT FOR LOW PHYTIC ACID USING FULL DIALLEL CROSSES

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ABSTRACT

Phytic acid (Myo-inositol 1, 2, 3, 4, 5, 6 hexa-kisphophate) is a storage form of phosphorus and can accumulate to levels as high as 35% in the wheat kernel. Phytic acid acts as an anti-nutritional macromolecule (anti-nutrient) in the wheat kernel. Due to its inhibitory role, a high concentration of phytic acid is undesirable as it hinders the bioavailability of some essential nutrients such as Fe, Mg, Ca, Zn and Cu, etc. To see the inheritance of phytic acid in wheat, phytic acid concentration was initially determined in kernels of 10 wheat genotypes to identify two contrasting genetic groups for diallel analysis. Based on pre-screening results of 10 wheat genotypes, five wheat genotypes (3 with high and 2 with low phytic acid concentration) were crossed in all possible combinations during 2007-08 to generate a 5 × 5 full diallel set for studying the inheritance of phytic acid and other agronomic traits. All 20 F_1 hybrids and 5 parental genotypes were planted using a randomized complete block design with three replicates during 2008-09 at Khyber Pakhtunkhwa Agricultural University, Peshawar. Analysis of variance revealed significant differences for all traits, providing justification for diallel analysis. According to Hotelling's t^2 test and regression analysis, the additive-dominance model was adequate for phytic acid, plant height, flag leaf area, partially adequate for days to heading and grain filling duration. Values of D greater than H_1 and H_2 for flag leaf area and plant height indicated their additive nature, whereas values of D less than H_1 and H_2 for grain filling duration and phytic acid concentration accounted for non-additive control of these traits. The narrow and broad sense heritability estimates varied widely among traits for days to heading (0.07, 0.32), flag leaf area (0.31, 0.55), grain filling duration (0.24, 0.91), plant height (0.12, 0.28) and phytic acid concentration (0.01, 0.86). The values for phytic acid concentration ranged from 0.56 to 3.43% among F_1 hybrids and 1.06 to 3.67% for parental genotypes. The F_1 hybrids, Ps-2005 × Ghaznavi (0.56%), AUP-4006 × Ps-2004 (0.74%), Janbaz × Ps-2004 (0.89%) and Janbaz × Ps-2005 (1.01%), had the lowest concentration of phytic acid. This research confirms that F_1 hybrids with low phytic acid concentration could yield desirable segregants.

Key Words: **Wheat, Phytic Acid, Diallel Analysis, Inheritance, Heritability**

Citation: Ahmad, I., F. Muhammad and Aurangzeb. 2013. Breeding bread wheat for low phytic acid using full diallel crosses. Sarhad J. Agric. 29(1):33-42.

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to family Poaceae (Gramineae) of monocots and is one of the most important food crops covering two-thirds of the acreage of cereals in the world. It ranks first in terms of production and consumption in Pakistan and is one of the most abundant sources of carbohydrates. Although the total production of wheat in Pakistan has increased many fold over the past few decades and we have touched the level of self sufficiency in the recent past, yet we need to produce more wheat for export to earn foreign exchange. For export, we need to concentrate on nutritional quality of wheat grain in order to compete in the international market.

Bread wheat quality is degraded by phytic acid. Phytic acid (Myo-inositol1, 2, 3, 4, 5, 6, hexa-kisphosphate) is a constituent of cereal grain and is abundantly located in the bran. Human diets high in phytic acid can significantly decrease the absorption of Fe from the flour (Brune *et al* 1992). The interaction of phytic acid with protein, vitamins and minerals are important factors which limit the nutritive value of wheat. Phytic acid forms complexes with divalent (Ca^{+2}, Mg^{+2}) and trivalent (Fe^{+3}) metallic ions, which are not absorbed from the gastrointestinal tract and decrease the bioavailability of these elements leading to nutritional deficiency diseases (Walter *et al*., 2002). Higher temperature and longer fermentation period can decrease phytic acid contents tremendously (Bhatia and Khetarpaul, 2002).

Total phosphorus quantity in plants increases with increase in plant dry weight (Sanoka, 2006). The seed phosphorus content increases dramatically from anthesis, and the phosphorus distribution percentage in the seed reached 35% of the absorbed phosphorus at full maturity. The phytic acid concentration is negatively correlated with Ca, Mg, Zn, Mn and Fe concentrations in the seed, though the total phosphorus concentration in the seed is positively correlated. Rice bread is a potential alternative to wheat bread for gluten-sensitive individuals. Incorporation of rice bran into bread made from white rice flour adds flavor but also phytic acid, which can reduce the bioavailability of minerals (Kadan and Phillippy, 2007).

Estimation of heritability provides authentic information about the extent to which a particular genetic trait is transmitted to the successive generations and help in making desirable selections. Therefore, estimation of heritability is

essential for breeders to predict the genetic potential of breeding materials, identify effective and promising combinations in hybridization and to determine effective methods of selection. Higher the estimate of heritability the simpler and less time consuming is selection procedure and greater is the genetic improvement (Allard, 1960).

A diallel mating scheme was used for the mode of inheritance study. This scheme has been an important tool for genetic analyses and has been used all over the world by plant breeders. Researchers (Griffing 1956; Hayman 1954; and Mather and Jinks,1977) designed a procedures to analyze genotypes from all possible crosses.

Identification of wheat with relatively low phytic acid would be a step towards development of wheat cultivars with low phytic acid. Keeping in view the importance of phytic acid as a potent inhibitor for the bioavailability of micronutrients viz. Fe, Ca, Mn, Zn, Mg, Cu, etc., as well as macronutrients to gastrointestinal tract, a study was conducted. This study was initiated to cross two contrasting groups of wheat genotypes (2 with low and 3 with high phytic acid concentrations), selected on the basis of 10 bread wheat genotypes initial screening for phytic acid concentration.

Five wheat genotypes were crossed in 5 × 5 diallel with the objective of developing low phytic acid segregants of wheat and decrease the inhibitory effect of phytic acid and enhance bioavailability of micronutrients and macronutrients to humans. The specific objectives of the present project were to: (i) Determine the phytic acid profile and other agronomic traits of different bread wheat genotypes, (ii) Determine the mode of inheritance for phytic acid accumulation and other agronomic traits in bread wheat genotypes, and (iii) Estimate heritability for phytic acid accumulation and other agronomic traits in bread wheat genotypes.

MATERIALS AND METHODS

The experiment on the "Inheritance of phytic acid and other agronomic traits in bread wheat" was conducted in the Department of Plant Breeding and Genetics, Khyber Pakhtunkhwa Agricultural University, Peshawar- Pakistan during 2007-08 to 2008-09. Ten wheat genotypes, Uqab (2.20%), Tatara (2.40%), AUP-5006 (2.75%), Ghaznavi (1.06%), Saleem-2000 (2.43%), Pirsabak-2004 (1.77%), Fakhre Sarhad (1.64%), Pirsabak-2005 (2.89%), Janbaz (3.67%) and AUP-4006 (2.83%) were screened for phytic acid concentration in 2007 at the Nuclear Institute for Food and Agriculture (NIFA) Peshawar.

Based on the results of preliminary study two contrasting groups (one group with high phytic acid genotypes i.e. Pirsabak-2005, Janbaz and AUP-4006 and the other group with low phytic acid genotypes i.e. Pirsabak-2004 and Ghaznavi) were identified. These 5 genotypes were crossed in all possible combinations in 2007 to generate a 5 × 5 full diallel. Twenty F_1 hybrids (10 direct and 10 reciprocal) were generated with enough seed for planting experiment in 2008. All 20 F_1 hybrids along with 5 parental genotypes were planted with a plant to plant and row to row space of 25 cm with a row length of 3.75 m to maintain 160,000 plants per hectare using randomized complete block design with three replicates to investigate some of the important physiological, morphological and agronomic traits. Urea and DAP fertilizers were applied at the rate of 120 and 60 kg ha^{-1}, respectively, to crop for maintaining normal nutrients status of the soil. Half dose of urea and full dose of DAP were applied at the time of seed bed preparation while remaining half dose of urea was applied at the time of first irrigation. Standard practices including hoeing, weeding, irrigation etc were carried out for the experiment to reduce experimental error.

Observations

Data were recorded on 5 randomly selected plants for each population for the following parameters.

Days to Heading

Days to heading were recorded from the date of sowing to the date of heading based on the emergence of complete heads by an entry in each replication. The stage when spikes emerged fully after the unfolding of the flag leaf was regarded as heading stage.

Flag Leaf Area (cm^2)

For the calculation of flag leaf area (cm^2) in each entry, length and width of intact flag leaves on five randomly selected plants were measured by ruler and it was calculated using the formula proposed by Muller (1991) i.e. maximum width × length × 0.74.

Grains Filling Duration

Grain filling duration was determined by subtracting days to anthesis from days to maturity for each entry.

Plant Height (cm)

Plant height (cm) of five randomly selected plants was recorded from the ground level to the tip of the main spike (excluding awns) by a meter rod at the time of physiological maturity for each entry.

Phytic Acid

After threshing grain samples were drawn from each entry of experiment for phytic acid determination. The sensitive method of Haug and Lantzsch (1983) was adopted for the determination of phytic acid in the whole wheat flour samples.

Determination of Phytic Acid

The sample was extracted with 0.2 N HCl and heated with an acidic iron-III solution of known iron content. The decrease in the iron content was the measure of free phytic acid in supernatant.

Reagents

Phytic acid reference solution: Sodium salt of phytic acid (C_6 H_6 O_{24} P_6 Na_{12}) was used for reference. Stock solution was prepared by dissolving 0.15 g sodium phytate in 100 ml distilled water. The reference solution was prepared by diluting the stock solution with HCl in a range from 3 to 30 micro-grams (ug ml^{-1}) phytic acid phosphorus.

Ferric Solution

Ammonium Iron-III Sulphate.12 H_2O. Ferric solution was prepared by dissolving 0.2 g of ammonium iron-III sulphate.12H_2O in 100 ml of 2 N HCl and the volume was made upto 1000 ml with distilled water.

2, 2-Bipyridine Solution

Ten grams of 2, 2-bipyridine and 10 ml of Thioglycolic acid was dissolved in distilled water and the volume was made up to 1000 ml.

Protocol

Grain sample (10 g) of each genotype was finely grinded by grinder and a fine grade of flour was obtained (Haug and Lantzsch.1983). The defatted and finely ground wheat flour sample (0.06 g) was weighed and added in dry and clean screw cap test tube. Sample was extracted with 10 ml of 0.1 N HCl for 1 hour shaking in shaker. From this extract 0.5 ml in duplicate was taken into dry and clean screw cap test tubes. Ferric solution (1 ml) was added to these test tubes and closed by screw caps. These tubes were heated (105 ^{0}C) in boiling water bath for 30 minutes and were allowed to cool at room temperature. Reaction mixture was provided by 2 ml of 2, 2-biphyridine solution and mixed thoroughly by shaking. Reaction mixture was transferred to cuvet of spectrophotometer and optical density (OD 510 nm) was recorded. The absorbance was measured within 4 minutes. A standard curve was made in phytic acid and was determined by the following formula:

Phytic acid = Phosphorus phytic acid × 4.97 while

4.97 = 924/186 (Phosphorous phytic $acid^{-1}$)

Statistical Analysis

A. Analysis of Variance

Data were subjected to analysis of variance (Steel and Torrie 1980) using Stateview software version 5, developed by SAS Institute Inc. USA. The statistical model is as follows:

$$Y_{ijk} = \mu + T_{ij} + b_k + (bT)_{ij.k}$$

Where,

Y_{ijk} = jth observation on ith genotype in kth block

μ = the general mean

T_{ij} = the effect of i × jth genotype

b_k = the effect of kth block

$(bT)_{ij.k}$ = the error effect

B. Diallel Analysis

To accomplish a biometrical genetic analysis for genetic information and to find out the sufficiency of the genetic model, it is necessary to collect data from a number of consecutive generations or from unlike mating systems. When adequate number of inbred lines is available then according to Mather and Jinks, (1971, 1977 and 1982) an alternative approach is possible where inbred lines are crossed in all possible combinations including selfing and mutual crossing. Thus, n number of lines crossed in this manner will give up n^2 descendant families. This method, called complete diallel cross, allows a genetic analysis to be carried out after one generation and provides test of the competence of the model.

The variation in a diallel cross can be attributed to the differences among maternal parents, paternal parents or due to the interaction between them. These variations can be recognized by the analysis of a diallel table. A diallel table is the arrangement of data collected from n^2 progenies and consists of n number of rows and columns. Each row has a common female parent and each column has a common male parent, thus the parental combinations (self) are arranged in a leading diagonal. Diallel analysis was done by using a software Dial-98 (Yasuo Ukai 2007). The theory of diallel was developed by Hayman (1954a, b), Hayman (1957) and Jinks (1954) and was applied by Whitehouse *et al.* (1958) and Mather and Jinks (1971, 1977 and 1982). Since there is no limit on the number of parental inbred in an indeterminate diallel analysis (when the differences among the parental genotypes are undefined), five parents were employed to carry out suitable diallel analysis.

C. Genetic components of variation

The genetic components of variation were calculated by using the procedures given by Hayman (1954), and Mather and Jinks (1982) and as cited by Singh and Chaudhary (1985). The genetic parameters and their formulae are as follows:

Additive Variation (D)

$D = Vp-E$

Where, Vp = Variance of the parents

E = Environmental constituent of variation

Variation due to Dominant effect of Genes (H_1)

$$H_1 = Vp - \overline{4Wr} + \overline{4vr} - [(3n-2)/n]E$$

Where, $\overline{vr}$ = mean of the array variances, $\overline{Wr}$ = mean of the co-variances between parents and arrays, and n = number of parents.

Variations due to Dominant effect of Genes Correlated for Gene Distribution (H_2)

$$H_2 = \overline{4vr} - 4Vr - 2E$$

Where, Vr = variance of the mean of arrays.

Heritability (h^2)

$$h^2_{bs} = \frac{0.5D + 0.5H_1 - 0.25H_2 - 0.5F}{0.5D + 0.5H_1 - 0.25H_2 - 0.5F + E}$$

$$h^2_{ns} = \frac{0.5D + 0.5H_1 - 0.5H_2 - 0.5F}{0.5D + 0.5H_1 - 0.25H_2 - 0.5F + E}$$

RESULTS AND DISCUSSION

Mean Performance of F_1 and Parental Genotypes

A. Analysis of Variance

Analysis of variance for genotypes showed significant differences for all traits. Means of the genotypes (parents + F_1 crosses) for the traits studied are presented (Table 2).

Analysis of variance for days to heading revealed significant differences. Parental genotype Ps-2004 took maximum number of days to heading, followed by Janbaz and Ghaznavi while AUP-4006 appeared with less

number of days to heading. Among F_1 crosses, AUP-4006 × Ps-2005 scored maximum number of days to heading while Ghaznavi × Janbaz appeared with minimum value (Table 2). Rest of the F_1 crosses were at par with one another. These results are in agreement with those of Mohammad *et al*. (2001), Ahmad *et al*. (2003, 2006 and 2009), and Anwar *et al*. (2009) who also reported significant differences amid wheat genotypes for days to heading.

Table 2. ***Means of Parents and F_1s for days to heading (DH), flag leaf area (FLA), grain filling duration (GFD), plant height (PH) and phytic acid percentage (PA%) in 5 × 5 diallel cross of bread wheat***

S. No	Genotypes	DH	FLA (cm^2)	GFD	PH (cm)	PA%
1	AUP-4006	128.67	43.77	41.67	103.73	3.42
2	Janbaz	132.00	36.39	35.00	109.70	1.61
3	Ghaznavi	132.00	34.60	34.33	89.00	1.25
4	Ps-2004	132.33	41.20	34.67	102.80	1.66
5	Ps-2005	131.00	47.09	32.67	114.80	2.48
6	AUP-4006 × Ps-2004	131.33	35.69	37.33	101.26	2.38
7	Ps-2004 × AUP-4006	130.00	32.60	40.00	105.45	2.60
8	Ps-2004 × Ghaznavi	130.00	41.49	34.00	99.40	2.48
9	Ps-2004 × Ps-2005	132.67	41.70	35.33	111.47	1.46
10	AUP-4006 × Janbaz	131.00	39.30	38.00	105.67	2.83
11	AUP-4006 × Ghaznavi	132.00	37.94	39.00	96.00	2.81
12	AUP-4006 × Ps-2005	134.00	37.34	31.33	116.00	2.55
13	Janbaz × AUP-4006	130.00	41.60	37.00	106.80	2.65
14	Janbaz × Ghaznavi	130.00	37.99	33.67	102.30	1.63
15	Janbaz × Ps-2004	129.67	38.84	41.00	109.00	0.89
16	Janbaz × Ps-2005	129.67	42.81	38.67	100.20	1.01
17	Ghaznavi × AUP-4006	131.67	36.60	43.00	100.93	3.43
18	Ghaznavi × Janbaz	129.00	38.05	34.66	106.30	1.52
19	Ghaznavi × Ps-2005	131.00	45.81	34.33	103.80	2.81
20	Ghaznavi × Ps-2004	133.33	31.41	43.00	100.60	2.32
21	Ps-2004 × Janbaz	132.33	37.70	34.33	112.80	2.53
22	Ps-2005 × AUP-4006	132.00	35.84	34.33	110.30	2.83
23	Ps-2005 × Janbaz	132.00	38.71	40.00	99.73	2.77
24	Ps-2005 × Ghaznavi	130.00	40.70	35.67	99.73	0.56
25	Ps-2005 × Ps-2004	132.00	37.67	36.67	111.07	1.58
	Grand mean	131.86	38.91	36.78	104.75	2.16
	Parent mean	131.20	40.61	35.66	104.00	2.48
	Progeny mean	131.18	38.48	37.06	104.94	2.18
	LSD	**2.38**	**5.47**	**2.55**	**4.23**	**2.40**

Analysis of variance regarding flag leaf area (cm^2) revealed significant differences (Table 2). Parental genotype Ps-2005 appeared with larger flag leaf area while Ghaznavi with small flag leaf area (cm^2). Among F_1 hybrids, Ghaznavi × Ps-2005 showed large size flag leaves whereas Ghaznavi × Ps-2004 appeared with small size leaves. Flag leaf area is vegetative trait and highly affected by light and other abiotic environmental conditions. Maximum photosynthates to grains are supplied by flag leaf. A bigger flag leaf area; therefore, will increase the size of grains by storing more food and thus will increase grain yield. Flag leaf area has a direct relationship with yield and yield related traits (Riaz, 2003).

Statistical analysis for grain filling duration showed (Table 2) that parental genotype AUP-4006 took more time for grain filling, whereas short grain filling duration was recorded for Ps-2005. Among F_1 hybrids, Ghaznavi × AUP-4006 and Ghaznavi × Ps-2004 appeared with extended grain filling duration while cross Janbaz × Ps-2005 exhibited short grain filling duration. This trait is directly linked with temperature of the environment. Low temperature could extend grain filling duration. Data pertaining to plant height (cm) showed (Table 2) that parental Ps-2005 appeared with tall stature by gaining more height while Ghaznavi with short stature by scoring less height. F_1 crosses like Ps-2004 × Ps-2005 and AUP-4006 × Ps-2005 appeared with maximum plant height (cm) while cross AUP-4006 × Ghaznavi showed short stature by scoring less plant height value. Crosses like Janbaz × Ps-2005, Janbaz × Ghaznavi, Ghaznavi × AUP-4006 and Ghaznavi × Ps-2004 were at par with one another. Phytic acid concentration was determined by the sensitive method of Haug and Lantzsch (1983). Analysis of variance showed highly significant differences. Maximum phytic acid concentration was found in AUP-4006 while minimum in Ghaznavi among the parental genotype (Table 2). F_1 hybrids showed a wider range for phytic acid. Highest phytic acid concentration was observed in cross combination Ghaznavi × AUP-4006, followed by AUP-4006 × Janbaz whereas lower concentration was recorded for Ps-2005 × Ghaznavi and AUP-4006 × Ps-2004 among the F_1 hybrids. Many genotypes amid the F_1s were at par for phytic acid concentration.

B. Diallel Analysis

Data collected for phytic acid and agronomic traits were subjected to analysis of variance following Steel and Torrie (1980) before conducting diallel analysis by using Stateview software version 5, developed by SAS Institute Inc. USA. Significant genotypic differences were found for all the traits which provided justification for diallel analysis. Diallel analysis was carried out in two steps. In the first step formal 5 × 5 diallel analysis was carried out using software Dial-98. In the second step genetic parameters were calculated. Results of the traits studied are presented and discussed.

Days to Heading

Complete diallel analysis for days to heading was carried out using statistical package Dial-98 (Table 4), which revealed that item **a**, which was the measure of additive gene effect was significant and accounted for high proportion of total variation. Overall dominance component **b**, was highly significant, exhibiting the important role of dominance. The value of $\mathbf{b_1}$ was also highly significant which showed the existence of directional genes for days to heading. Highly significant value of item $\mathbf{b_2}$ accounted for the distribution of asymmetrical genes among parents whereas non-significant value of $\mathbf{b_3}$ was accounted for the absence of specific gene effect. The value of **c** (maternal effect) was non-significant and therefore there was no need for retesting of component **a**. Reciprocal effect (**d**) was non-significant and hence retesting of **b**, $\mathbf{b_1}$, $\mathbf{b_2}$ and $\mathbf{b_3}$ was not performed.

Table 3. ***Scaling tests of additive–dominance model for phytic acid and other agronomic traits in bread wheat for 5 × 5 diallel cross***

Parameters	t^2	Regression analysis (t value of b)		Conclusions
		b= 0	b= 1	
Phytic acid	-0.065^{ns}	0.98^{*}	1.64^{ns}	Model was adequate
Days to heading	0.236^{ns}	1.82^{ns}	0.21^{ns}	Model was partially adequate
Flag leaf area	0.042^{ns}	5.30^{*}	-034	Model was adequate
Grain filling duration	0.004^{ns}	0.46^{ns}	1.31^{ns}	Model was partially adequate
Plant height	0.032^{ns}	9.09^{*}	1.18^{ns}	Model was adequate

Additive dominance model was found partially adequate for days to heading after subjecting data to scaling tests (Table 3). For days to heading estimates of genetic components D, H_1 and H_2, were non-significant while environmental component was significant which played greater role in the manifestation of said trait (Table 5). However, D was greater than H_1, H_2 components and the trait was controlled by additive type of gene action. Narrow and broad sense heritability estimates were 0.07 and 0.32 respectively, (Table 5) for days to heading. Additive gene action for days to heading had also been reported by Al-Saheal (1985), Maloo (1987), Singh and Paroda (1988) and Chaudhry *et al.* (1994).

Table 4. ***Mean squares and degree of freedom for the analysis of variance of 5 × 5 diallel for days to heading***

Sources	Df	Ms	F
A	4	403.5	3.29^{*}
B	10	395.38	3.22^{**}
b_1	1	901.33	7.34^{**}
b_2	4	758.46	6.18^{**}
b_3	5	4.12	0.03^{NS}
C	4	0.47	0.00^{NS}
D	6	9.08	0.07^{NS}

P 0.05 **P 0.01

a = additive gene effect, b = dominance gene effect, b_1 = directional dominance deviation

b_2 = genes distribution among parents, b_3 = effect of specific gene c = maternal effect d = reciprocal effect

Flag Leaf Area

Diallel analysis for flag leaf area showed highly significant differences for item **a**, (measure of additive gene effect) which was responsible for a high proportion of the overall variation (Table 6). Overall dominance component **b**, was significant, indicating the important role of dominance for flag leaf area. The value of $\mathbf{b_1}$ appeared with non-significant differences which pointed out the lack of directional genes for flag leaf area. Asymmetry of genes allotment among the parental genotypes was clear by the significant value of item $\mathbf{b_2}$ while on the other hand non-significant value of $\mathbf{b_3}$ was held accountable for the absence of specific gene effect. The values of **c** (maternal effect) and **d** (reciprocal effect) were significant and due to this reason they were retested against items **a, b** and $\mathbf{b_1}$ which became non-significant.

Table 5. ***Estimates of genetic components of variation for days to heading***

Components	MS	SE
D	306.19^{NS}	±180.90
H_1	213.79^{NS}	±230.43
H_2	114.84^{NS}	±149.76
Heritability (ns)	0.07	
Heritability (bs)	0.32	

*= Value is significant when it exceeds 1.96 after dividing by its standard error.

Adequacy tests of additive-dominance model revealed adequate for flag leaf area (Table 3). The genetic components of variance for flag leaf area revealed that additive-dominance while H_1 and H_2 were non-significant (Table 7). Value of additive dominance showed that flag leaf was under the control of additive type of genes. Different values of H_1 and H_2 indicated distribution of positive and negative alleles in unequal frequencies. However the variation was small. Estimated values of narrow and broad sense heritability were being 0.31 and 0.55, respectively, for flag leaf area (Table 7).

Table 6. ***Mean squares and degree of freedom for the analysis of variance of 5 × 5 diallel for flag leaf area (cm^2)***

Sources	Df	Ms	F	Retesting against	
				c	d
A	4	92.17	10.44^{**}	1.71^{NS}	
B	10	39.78	2.04^{*}		0.83^{NS}
b_1	1	0.05	0.05^{NS}		
b_2	4	54.65	2.81^{**}		1.14^{NS}
b_3	5	35.82	1.84^{NS}		
C	4	53.67	2.76^{**}		
D	6	47.77	2.45^{**}		

*P 0.05 **P 0.01

a = additive gene effect, b = dominance gene effect, b_1 = directional dominance deviation
b_2 = genes distribution among parents, b_3 = effect of specific gene c = maternal effect d = reciprocal effect

Our results are in line with those of Masood *et al.* (2005) who also reported parallel findings for flag leaf area and other agronomic traits in bread wheat. Findings of our research are also supported by those of Hassan and Khaliq (2008).

Table 7. ***Estimates of genetic components of variation for flag leaf area (cm^2)***

Components	MS	SE
H_1	20.57^{NS}	±19.20
H_2	14.05^{NS}	±13.46
Heritability (ns)	0.31	
Heritability (bs)	0.55	

*= Value is significant when it exceeds 1.96 after dividing by its standard error.

Grain Filling Duration

Diallel analysis of variance for grain filling duration (Table 8) revealed significant differences for genetic components **a** and **b** which were the measure of additive gene effect and overall dominance, respectively. Genetic item $\mathbf{b_1}$ showed non-significant value which indicated the lack of directional genes for grain filling duration among parental genotypes. Highly significant value of genetic component $\mathbf{b_2}$ accounted for the preponderance distribution of asymmetrical genes among parental genotypes whereas $\mathbf{b_3}$ genetic component which was the measure of specific gene effect ($\mathbf{b_3}$) yielded highly significant value for grain filling duration. Maternal effect (**c**) indicated highly significant differences which allowed retesting of additive gene effect (**a**). After retesting highly significant value of **(a**) was remained significant which showed that maternal effect did not suppress the additive gene action. The values of genetic components **b**, $\mathbf{b_2}$ and $\mathbf{b_3}$ were retested against the reciprocal effect (**d**) which was significant and hence **b** and $\mathbf{b_3}$ became non-significant while $\mathbf{b_2}$ maintained its significant value.

Table 8. ***Mean squares and degree of freedom for the analysis of variance of 5 × 5 diallel for grain filling duration***

Sources	Df	Ms	F	Retesting against	
				c	d
A	4	37.3	15.47**	2.6*	
B	10	41.64	17.02**		0.63 NS
b_1	1	5.88	2.40NS		
b_2	4	83.52	34.14**		10.11*
b_3	5	15.28	6.25**		1.8NS
C	4	14.28	5.84**		
D	6	8.26	3.37**		

*P 0.05 **P 0.01

a = additive gene effect, b = dominance gene effect, b_1 = directional dominance deviation

b_2 = genes distribution among parents, b_3 = effect of specific gene, c = maternal effect, d = reciprocal effect

Additive dominance model was fully adequate for grain filling duration (Table 3). The estimates of genetic components of variance for D, H_1 and H_2, revealed significant differences (Table 9). Significant values of both D and H_1, H_2 genetic components showed that additive gene effect and dominance effect are involved in grain filling duration of wheat kernels. Long grain filling duration means more grain yield. Estimated values of narrow and broad sense heritability were being 0.24 and 0.91, respectively, for grain filling duration (Table 9).

Table 9. ***Estimates of genetic components of variation for grain filling duration***

Components	MS	SE
D	11.60*	±3.30
H_1	42.34*	±7.26
H_2	26.19*	±4.50
Heritability (ns)	0.24	
Heritability (bs)	0.91	

*= Value is significant when it exceeds 1.96 after dividing by its standard error.

Our results are in agreement with those of Masood *et al*. (2005) who also reported similar findings for grain filling duration and also stated that extended grain filling duration was directly related with more grain yield of the crop.

Table 10. ***Mean squares and degree of freedom for the analysis of variance of 5 × 5 diallel for plant height (cm)***

Sources	Df	Ms	F
A	4	394.83	3.61*
B	10	269.05	2.46*
b_1	1	1028.23	9.41**
b_2	4	340.41	3.12*
b_3	5	60.13	0.55NS
C	4	84.41	0.77 NS
D	6	85.67	0.78 NS

*P 0.05 **P 0.01

a = additive gene effect, b = dominance gene effect, b_1 = directional dominance deviation

b_2 = genes distribution among parents, b_3 = effect of specific gene, c = maternal effect, d = reciprocal effect

Plant Height

Analysis of variance for plant height revealed (Table 10) significant differences for item **a** and it was responsible as a principal contributing factor of the whole variation due to additive gene effect. Genetic component **b** (measure of the whole dominance) was significant, showing the fundamental role of dominance for plant height. The value of $\mathbf{b_1}$ showed significant difference which indicated the presence of directional genes for plant height. Asymmetrical genes distribution among the parents was obvious from the significant value of item $\mathbf{b_2}$. Absence of specific gene effect was directly linked with the non-significant value of $\mathbf{b_3}$. The values of **c** (maternal effect) and **d** (reciprocal effect) were non-significant and thus there was no need for retesting of item **a,** against **b**, $\mathbf{b_1}$, $\mathbf{b_2}$ and $\mathbf{b_3}$ respectively.

Table 11. ***Estimates of genetic components of variation for plant height (cm)***

Components	MS	SE
D	138.50NS	±119
H_1	82.19NS	±157.04
H_2	57.32NS	±111.14
Heritability (ns)	0.12	
Heritability (bs)	0.28	

*= Value is significant when it exceeds 1.96 after dividing by its standard error.

By subjecting the data to adequacy tests for additive dominance model it was found adequate for plant height (Table 3). Estimates of genetic components D, H_1 and H_2 for plant height in F_1 progenies were non-significant which played larger fraction in the appearance of the plant height. However, D was greater than H_1 and H_2 indicating that plant height was controlled by additive gene action. Greater value of H_1 than H_2 indicated that positive and negative genes were not equally dispersed. Estimates of narrow sense heritability 0.12 and broad sense heritability were 0.28 respectively (Table 11) for plant height.

Table 12. ***Mean squares and degree of freedom for the analysis of variance of 5 × 5 diallel for phytic acid***

Sources	Df	Ms	F
A	4	0.39^{NS}	1.76
B	10	2.92^{**}	13.16
b_1	1	3.85^{**}	17.31
b_2	4	3.39^{**}	15.26
b_3	5	2.36^{**}	10.64
C	4	4.29^{**}	19.30
D	6	1.65^{**}	7.42

*P 0.05 **P 0.01

a = additive gene effect, b = dominance gene effect, b_1 = directional dominance deviation

b_2 = genes distribution among parents, b3 = effect of specific gene c = maternal effect d = reciprocal effect

These results agree with those of Rasal *et al*. (1991), who reported additive gene effect for plant height and other agronomic traits. Iqbal *et al*. (1991), on the other hand reported partial dominance from their studies for plant height.

Table 13. ***Estimates of genetic components of variation for phytic acid***

Components	MS	SE
D	0.89^{*}	±0.26
H_1	2.43^{*}	±0.46
H_2	1.80^{*}	±0.33
Heritability (ns)	0.01	
Heritability (bs)	0.86	

*= Value is significant when it exceeds 1.96 after dividing by its standard error.

Phytic Acid

Two contrasting groups (one group with high phytic acid genotypes i.e. (Pirsabak-2005 (2.89%), Janbaz (3.67%) and AUP-4006 (2.83%) and other group with low phytic acid genotypes i.e. (Pirsabak-2004 (1.77%) and Ghaznavi (1.06%) were identified on the basis of preliminary study. Diallel analysis for 5 × 5 diallel cross was carried out by Dial-98 statistical package for phytic acid which revealed (Table 12) non-significant variation for genetic component **a** which was the of measure of additive gene effect. Genetic component **b**, for over all dominance was highly significant, indicating the importance of dominance. Genetic component $\mathbf{b_1}$ accounted for directional genes distribution among parent was also found significant for phytic acid. Distribution of asymmetrical genes ($\mathbf{b_2}$) among parents and existence of specific gene effect ($\mathbf{b_3}$) for phytic acid were also recorded with significant differences. Maternal effect (**c**) and reciprocal effect (**d**) score was also significant for phytic acid.

Additive dominance model was adequate for phytic acid due to non-significant values of t^2 test and regression analysis (Table 3). Estimation for genetic components of variations, D, H_1 and H_2 revealed significant differences (Table 13). Variance of additive gene effect was significant, but its value was less than both H_1 and H_2 indicating a lesser role of additive genes than dominant genes for phytic acid. Value of H_1 was a little bit greater than H_2 indicating more contribution of dominant genes. Narrow sense 0.01 and broad sense 0.86 heritability estimates were found for phytic acid (Table 13). Estimates of genetic components pleaded that phytic acid was under the control of dominant alleles. These results are in close agreement with the findings of Masud *et al.* (2007) who had reported variation in phytic acid concentrations levels in different cultivars of bread wheat and same cultivar of bread wheat at different locations.

CONCLUSION AND RECOMMENDATION

It was concluded from the present study that some of the F_1 hybrids like Ps-2005 × Ghaznavi (0.56%), AUP-4006 × Ps-2004 (0.74%), Janbaz × Ps-2004 (0.89%) and Janbaz × Ps-2005 (1.01%), had the lowest concentration of phytic acid. This research confirms that F_1 hybrids with low phytic acid concentration could yield desirable segregants.

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