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Diallel analysis for the inheritance study of phytic acid along with morpho-yield traits in bread wheat

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In the kernels of wheat, a macro molecule called phytic acid acts as an inhibitor of nutrients. Phytic acid in high concentration is undesirable as it hinders the absoption of other molecules. Regression analysis, the model of additive-dominance and Hotelling's t^2 test were adequate for biological yield, grains spike^{-1} and phytic acid, while partially adequate results were found for grain yield and 1000-grain weight. Greater values of H_1 and H_2 than D for biological yield, grain yield, 1000-grain weight, grains spike^{-1}, and phytic acid concentration indicated that these traits were under the control of non-additive gene action. Same results were also confirmed by average degree of dominance. Estimates of heritability for broad and narrow sense varied greatly for the traits of biological yield (0.89, 0.10), grain yield (0.98, 0.13), 1000-grain weight (0.68, 0.25) grains spike^{-1} (0.680, 05), and phytic acid concentration (0.86, 0.01). Phytic acid concentration ranged from 0.56 to 3.43% among F_1 crosses while for parental genotypes the range was 1.06 to 3.67%. 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%), indicated the lowest concentration of phytic acid. This research confirms that F_1 hybrids with low phytic acid concentration could yield desirable segregants.

Key words: Bread wheat, phytic acid, biological yield, grain yield, diallel analysis, inheritance, heritability.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a member of Poaceae (Graminae) belonging to monocoyledonae class of angiosperms which covers 2/3 area of cereals in the world and proves its most importance as a food. In Pakistan, bread wheat occupies first rank of cereal crop both in consumption and production and it is the richest source of carbohydrates. Wheat plays dual role on important food crop and a source of stabilizing indicator for the economy of Pakistan. Self sufficiency level has been reached by Pakistan due to increase in total production of wheat in the past few decades but yet we need to produce more wheat in order to earn earn foreign exchange by exporting wheat grain. In order to compete in the international market, we must focus and concentrate on the

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nutritional quality of wheat grain before export (Ijaz et al., 2013). In cereal grains, phytic acid is one of the constituents that is greatly present in the bran. Absorption of $Fe^{2+/3+}$ from the flour can vastly decline if humans consume food with high amount of phytate (Brune et al., 1992). Proteins, minerals and vitamins that make interaction are important factors which limit the nutritive value of wheat. Divalent (Mg^{2+}, Ca^{2+}) and trivalent (Fe^{3+}) metallic ions forms complexes with phytic acid in gastrointestinal tract which cannot be absorbed and these elements are no more available to cells, and are ultimately leading to nutritional disorders (Walter et al., 2002). Zn deficiency is direcly proportional with high phytic acid in diet (Linnerdal, 2000).

Five wheat genotypes were crossed in 5 × 5 full diallel on the basis of pre-screening results of 10 wheat genotypes, with the objective to develop low phytic acid segregants of wheat. The specific objectives of the present study were to determine phytic acid profile along with morpho-yield traits of different bread wheat genotypes and to estimate their heritabilities.

MATERIALS AND METHODS

In order to investigate "Inheritance of phytic acid and other morpho-yield traits in bread wheat", laboratory and field experiments were carried out jointly in the Department of Plant Breeding and Genetics, at The University of Agriculture, Peshawar and Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Khyber Pakhtunkhwa-Pakistan during 2007-08 to 2008-09. Primarily, 10 bread wheat genotypes (AUP-4006, Janbaz, Saleem-2000, AUP-5006, Tatara, Uqab, Ghaznavi, Fakhre Sarhad, Pirsabak-2004 and Pirsabak-2005) were screened for phytic acid concentration in 2007 at the Nuclear Institute for Food and Agriculture (NIFA) Peshawar (Masud et al., 2007). On the basis of preliminary data of 10 bread wheat genotypes for phytic acid, two contrasting groups (Group 1 with low phytic acid concentration, that is, Pirsabak-2004 and Ghaznavi) (Group 2 with high phytic acid oncentrations, that is, Pirsabak-2005, Janbaz and AUP-4006) were identified. Both groups (5 genotypes) were crossed to generate a full diallel set by using 5 × 5 full diallel in 2007.

At least 15 spikes of each variety were manually emasculated and bagged in order to prevent contamination by foreign pollens during flowering season of 2007. Receptive ovaries of the female spikes were pollinated by applying fresh pollens from the desirable male spike within two or three days after emasculation. Each variety was used as male and female and generated 20 F_1 hybrids, with enough seed for planting experiment in 2008. All F_1 hybrids (20) along with parental genotypes (05) were planted with a plant to plant and row to row space of 25 cm to maintain 160,000 plants/ha for investigating phytic acid and other morpho-yield traits of bread wheat. An experiment was planted in randomized complete block design with triplicate. Each entry comprised of one row having a length of 3.75 m.

Urea and DAP fertilizers at the rate of 120 and 60 kg ha^{-1} were added to experimental field for maintaining standard nutrients status of soil. Full dose of DAP and half dose of urea fertilizer were added to soil during prepration of seed bed whereas the remaining half dose of urea was applied along with first irrigation. Standard agricultural practices like weeding, irrigation and hoeing were carried out for decreasing experimental inaccuracy.

Observations

Data were recorded on five randomly selected plants of each population for the following traits:

1) Grains $spike^{-1}$
2) 1000-grain weight (g)
3) Biological yield (kg ha^{-1})
4) Grain yield (kg ha^{-1})

For each of the above mentioned trait, already adopted standard procedure was used.

5) Phytic acid: An adequate amount of sample of wheat kernels was drawn for phytic acid determination from each entry after manual harvesting and threshing. Kernels were grinded by blender and 0.06 g of flour was collected for phytic acid determination by adopting the sensitive method of Haug and Lantzsch (1983).

Determination of phytic acid

A very minute fraction of sample (0.06 g) was taken by weighing with the help of electronic balance and digested with 0.2 N HCl in test tube. Sample in the test tube was 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 ($C_6H_6O_{24}P_6Na_{12}$) was used for the preparation of reference solution. 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 $12H_2O$. 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 up to 1000 ml with distilled water.

2, 2-Bipyridine solution

This solution was prepared by dissolving 10 g of 2, 2-bipyridine and 10 ml of thioglycolic acid in distilled water and the final volume was raised up to 1000 ml.

Protocol

From each entry of the experiment, a representative grain sample (10 g) was weighed by an electronic balance and grinded by blinder for getting fine grade of flour. A minute quantity (0.06 g) was weighed and added in dry and clean screw cap test tube with a volume of 15 ml from each entry. Sample within test tube was digested by 0.1 N HCl (10 ml) by shaking for 1 h in shaker. From this extract, 0.5 ml in duplicate was taken into dry and clean screw cap test tubes. A quantity of 1 ml ferric solution (concentration = 23 ug ml^{-1} or 23 ppm solution) was added to these test tubes and closed by screw caps. These tubes were heated (105°C) in boiling water bath for 30 min and allowed to cool at room temperature. Reaction mixture was provided by 2 ml of 2, 2-biphyridine solution (concentration = 1% 2, 2 bipyridine solution) and mixed thoroughly by shaking. Reaction mixture was transferred to cuvet of spectrophotometer (UV-1800, Japan made) and optical density (OD 510 nm) was recorded. The absorbance was measured within 4 min. A standard curve was made in phytic acid and was determined by the following formula

Phytic acid = Phosphorus phytic acid × 4.97

Table 1. Means of parents and F_1s for biological yield (BY) (kg ha^{-1}), grain yield (GY) (kg ha^{-1}) grains $spike^{-1}$, 1000-grain weight (TGW in g), and phytic acid percentage (PA%) in 5 × 5 diallel cross of bread wheat.

S/N	Genotype	BY	GY	GSP^{-1}	TGW	PA%
1	AUP-4006	6958.90	1833.00	88.67	32.33	3.42
2	Janbaz	6668.03	2381.68	72.00	32.00	1.61
3	Ghaznavi	7278.08	2476.74	77.66	37.00	1.25
4	Ps-2004	6194.52	2138.61	94.67	31.00	1.66
5	Ps-2005	6593.21	1919.25	69.00	41.00	2.48
6	AUP-4006 × Ps-2004	8877.16	1838.31	69.00	36.00	0.74
7	Ps-2004 × AUP-4006	8485.38	2146.77	57.67	34.00	2.60
8	Ps-2004 × Ghaznavi	8296.81	1994.63	77.00	38.00	2.48
9	Ps-2004 × Ps-2005	7428.31	1892.37	64.00	35.00	1.46
10	AUP-4006 × Janbaz	6513.24	2443.89	84.00	35.00	2.83
11	AUP-4006 × Ghaznavi	7527.39	2220.89	80.00	39.00	2.81
12	AUP-4006 × Ps-2005	7575.34	2149.51	75.33	35.00	2.55
13	Janbaz × AUP-4006	8478.99	1926.66	75.33	40.00	2.65
14	Janbaz × Ghaznavi	8278.53	1956.16	73.00	36.70	1.63
15	Janbaz × Ps-2004	8098.32	2737.12	78.00	36.00	0.89
16	Janbaz × Ps-2005	7836.53	2330.82	74.00	31.00	1.01
17	Ghaznavi × AUP-4006	8173.97	2056.21	92.33	40.70	3.43
18	Ghaznavi × Janbaz	7962.92	2166.66	66.00	39.00	1.52
19	Ghaznavi × Ps-2005	8377.16	1818.32	95.00	36.33	2.81
20	Ghaznavi × Ps-2004	7834.74	2435.75	85.33	38.70	2.32
21	Ps-2004 × Janbaz	8391.32	1882.05	71.00	31.00	2.53
22	Ps-2005 × AUP-4006	8668.69	2530.95	70.33	38.33	2.83
23	Ps-2005 × Janbaz	7191.32	2058.31	78.66	35.00	2.77
24	Ps-2005 × Ghaznavi	7734.10	2068.21	79.66	40.70	0.56
25	Ps-2005 × Ps-2004	7616.43	2023.61	69.00	40.70	1.58

Statistical analysis

Analysis of variance

Analysis of variance (Steel et al., 1997) was performed for data of all traits.

Diallel analysis

Diallel-98 software was used for the analysis of 5 × 5 diallel cross, for calculating Griffing, ANOVA and estimates of genetic components of all traits.

RESULTS AND DISCUSSION

Data of phytic acid and other morpho yield traits for all genotypes (20 F_{1s} + 05) were subjected to diallel analysis for getting genetic information about various aspects.

Analysis of variance for F_{1s} and parental genotypes

All traits revealed significant differences after computing ANOVA. Means for the traits under study are presented (Table 1).

Data concerning biological yield revealed highly significant variations. Maximum biological yield was recorded for Ghaznavi and minimum Ps-2004 (Table 1). Among the F_1 progenies, maximum biological yield was recorded for AUP-4006 × Ps-2004 followed by Ps-2005 × AUP-4006, Ps-2004 × AUP-4006 and Janbaz × AUP-4006. Analysis of variance for grain yield was highly significant. Among the parents, highest grain yield was recorded for Ghaznavi followed by Janbaz while lowest was for AUP-4006. Amongst the F_1 hybrids, highest grain yield was recorded for Janbaz × Ps-2004 and Ps-2005 × AUP-4006.

Analysis of variance regarding grains $spike^{-1}$ was found significant. Among the parental genotypes, maximum grains $spike^{-1}$ was recorded for Ps-2004 and minimum grains $spike^{-1}$ for Ps-2005. Among the F_{1s} crosses, Ghaznavi × Ps-2005 produced more grains $spike^{-1}$ followed by Ghaznavi × AUP-4006 while cross combination, Ps-2004 × AUP-4006 yielded less grains $spike^{-1}$. Analysis of variance revealed significant differences for 1000-grain weight. Among the parents, Ps-2005 was found with maximum score for 1000-grain weight, followed by Ghaznavi. Janbaz and AUP-4006

Table 2. Additive–dominance model for phytic acid and other morho-yield traits in bread wheat for 5 × 5 diallel cross.

Parameter	t^2	Regression analysis (t value of b)		Conclusion
		b= 0	b= 1	
Biological yield	0.35^{ns}	4.89*	2.32^{ns}	Model was adequate
Grain yield	3.55^{ns}	1.17^{ns}	4.19^{ns}	Model was partially adequate
Grains spike^{-1}	0.07^{ns}	5.08*	0.46^{ns}	Model was adequate
1000-grain weight	0.28^{ns}	0.02^{ns}	0.46^{ns}	Model was partially adequate
Phytic acid	-0.065^{ns}	0.98*	1.64^{ns}	Model was adequate

were at par for the mentioned trait whereas Ps-2004 with lowest score was observed for the said trait among the parents. Maximum value of 1000-grain weight was observed for Ps-2005 × Ghaznavi, Ps-2005 × Ps-2004 and Ghaznavi × AUP-4006 and was at par among the crosses (Table 1).

Phytic acid indicated highly significant differences after ANOVA. High concentration of phytic acid was found in AUP-4006 while low concentration was observed in Ghaznavi among the parental genotypes. Among the hybrids (F_{1s}), highest phytic acid concentration was observed in cross combination Ghaznavi × AUP-4006, followed by AUP-4006 × Janbaz whereas lowest concentration was recorded for Ps-2005 × Ghaznavi and AUP-4006 × Ps-2004 and Janbaz × Ps-2004 among the F_1 hybrids (Table 1).

Diallel analysis

Data collected for phytic acid and other morpho yield traits was subjected to analysis of variance. Significant genotypic differences for all traits provided a full justification for diallel analysis. Diallel analyses (5 × 5) were performed by using Dial-98 software.

Biological yield

Biological yield indicated highly significant differences for an item after diallel analysis. Item **b** was also highly significant, displaying the contribution of overall dominance. Presence of directional genes ($\mathbf{b_1}$) for biological yield was justified by its significant value. Value of genetic item $\mathbf{b_2}$ with highly significant differences was the justification for the distribution of asymmetrical genes among parents whereas $\mathbf{b_3}$ was held responsible for the existence of specific gene effect. Maternal effect (**c)** was highly significant and it was a prerequisite for retesting of component **a**. Significant value of item (**a)** after retesting supported additive gene effect for maternal effect (Table 3).

Biological yield was adequate for diallel analysis due to non-significant values of adequacy tests (t^2 test and Regression analysis (Table 2). The estimates of genetic components **D, H_1, H_2, F, h^2** and **E** showed significant differences for biological yield. Less value of **D** than the values of both $\mathbf{H_1}$ and $\mathbf{H_2}$ suggested more contribution of dominance for biological yield. Average degree of dominance $(H_1/D)^{1/2}$ was 3.37 which is greater than unity, and is a clear indication of over dominance for the control of biological yield. Estimated value of **F** was positive with significant variation pleading for the existence of more dominant genes for the said trait. Both narrow sense and broad sense 0.10, 0.89 heritability estimates for biological yield are presented (Table 4).

Grain yield

Genetic component **a** which is responsible for the measurement of additive gene action and generally considered as a chief portion of total variation was found significant after conducting diallel analysis for 5 × 5 diallel cross for grain yield (Table 3). Item **b** which is a measure of overall dominance revealed highly significant differences which indicate the vital function of dominance for grain yield. Existence of directional genes for grain yield was mentioned by the significant value of $\mathbf{b_1}$. Distribution of asymmetrical genes among the parents was indicated by the significant value of genetic component $\mathbf{b_2}$ while there was non-significant value of item $\mathbf{b_3}$ which accounted for presence of specific gene effect. Maternal effect (**c**) was significant which is an important factor for retesting of genetic component **a**. Retesting of gentic component **a** decreased its value to non-significant level, thus showing that maternal effect suppressed the additive gene effect. Significant value of reciprocal effect (**d**) is mandatory for the retesting of **b**, $\mathbf{b_1}$, $\mathbf{b_2}$ and $\mathbf{b_3}$ which made them non-significant apart from **b** which kept its original value after retesting by **d**.

Regression analysis and t^2 test showed non-significant values (Table 2) for grain yield, justifying the adequacy of Additive-Dominance model. Significant differences were found for **D, H_1, H_2, F, h^2** and **E** genetic components (Table 3) for grain yield. Greater value $\mathbf{H_1}$ and $\mathbf{H_2}$ than additive gene effect specified crucial function of dominance for the abovementioned trait. Additive genes with dominance type nature are confirmed by the highly significant differences of **D** and $\mathbf{H_1}$ and $\mathbf{H_2}$ which showed

Table 3. Mean squares and degree of freedom for the analysis of variance of 5 × 5 diallel for gains spike^{-1}, 1000-grain weight, biological yield and phytic acid.

SOV	Grains spike^{-1}		1000-grain weight		Biological yield		Grain yield		Phytic acid	
	df	Ms	df	Ms	Df	Ms	df	MS	df	Ms
a	4	153.83^{NS}	4	19.72**	4	9235520**	4	74796**	4	0.39^{NS}
b	10	361**	10	13.65**	10	2452982**	10	195519.90**	10	2.92**
b_1	1	254.47^{NS}	1	20.80*	1	17649870**	1	112410**	1	3.85**
b_2	4	541.26**	4	13.91*	4	705571**	4	72530**	4	3.39**
b_3	5	237.52*	5	12.01*	5	811531**	5	108194**	5	2.36**
c	4	118.28^{NS}	4	10.25*	4	1124303**	4	163342*	4	4.29**
d	6	282.28**	6	16.86**	6	155731.50**	6	75210**	6	1.65**

*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.

Table 4. Estimates of genetic components of variation for phytic acid, grains spike^{-1}, 1000-grain weight, biological yield and grain yield.

Component	Phytic acid		Grains spike^{-1}		1000-grain weight		Biological yield		Grain yield	
	MS	SE	MS	SE	MS	SE	MS	SE	MS	SE
D	0.89*	±0.26	95.32^{NS}	±60.82	7.91*	±3.69	144228^{NS}	±103889	2968.52*	±149.10
H_1	2.43*	±0.46	285.26*	±111.6	8.47^{NS}	±4.57	1641443*	±300437	144838.60*	±977.32
H_2	1.80*	±0.33	193.70*	±75.20	6.58*	±3.34	1535619*	±269391	130338.10*	±854.71
F	1.50*	±0.39	178.13*	±92.26	7.50^{NS}	±4.57	151804*	±146298	7501.76*	±380.3
h^2	0.777	±0.40	41.22^{NS}	±65.71	3.68^{NS}	±4.06	3735413*	±697825	239805*	±21103.
E	0.07*	±0.01	24.52*	±5.00	1.31*	±0.27	51910*	±10516	7.33*	±1.48
$(H_1/D)^{1/2}$	1.65		1.72		1.05		3.37		6.98	
Heritability (ns)	0.01		0.05		0.25		0.10		0.13	
Heritability (bs)	0.86		0.68		0.66		0.89		0.98	

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

the aforesaid trait. Occurence of dominant genes for the trait of grain yield can be confirmed by F genetic item which was positive and significant. According to estimation of genetic components, the value of 6.98 for the average degree of dominance $(H_1/D)^{1/2}$ was responsible for over dominance type of gene action with additive effect. Values estimated for narrow and broad sense 0.13 and 0.98 heritability respectively were found for grain yield (Table 4).

Grains spike^{-1}

Diallel analysis (5 × 5) for item **a** was non-significant (Table 3) for the trait of grains spike^{-1}. The value **b**, with highly significant variation indicates the major role of dominance for mentioned trait. Dirctional genes are missing due to non-significant value of $\mathbf{b_1}$ for grains spike^{-1}. Existence of asymmetrical genes among parents for grains spike^{-1} is prominent from the significant value of $\mathbf{b_2}$. Specific gene effect was present due to significant value of $\mathbf{b_3}$. The value of **c** appeared with non-significant score. Reciprocal effect was significant and hence retesting of **b**, $\mathbf{b_2}$ and $\mathbf{b_3}$ was performed which rendered them non-significant except $\mathbf{b_2}$ which became significant.

Adequacy tests for additive dominance model showed partial adequacy of the data for grains spike^{-1} (Table 2). Estimated values of **D** and $\mathbf{h^2}$ were non-significant for grains spike^{-1} indicating lack of additive genes for this trait while $\mathbf{H_1}$, $\mathbf{H_2}$, **F** and **E** with significant variation demonstrated dominance gene effect for the said trait. Controlling of grains spike^{-1} is also evident from the significant and positive value of **F** genetic component. Over dominance type of gene action for grains spike^{-1} was also supported by average degree of dominance $(H_1/D)^{1/2}$ which was greater (1.72) than 1. Estimates of narrow sense heritability were 0.05 and broad sense heritability 0.68 for grains spike^{-1} (Table 4).

1000-grain weight

Diallel analysis for 1000-grain weight indicated major role of additive gene effect due to the significant value of **a**.

Genetic components b, b_1, b_2, b_3 were also highly significant for 1000-grain weight which pleaded for dominance type of gene action. Genetic component **d** was highly significant and hence retesting of **b**, $\mathbf{b_1}$, $\mathbf{b_2}$ and $\mathbf{b_3}$ was conducted which made them non-significant (Table 3).

Scalling tests indicated partial adequacy for 1000-grain weight (Table 2). Estimate of genetic component **D** was significant which suggested that 1000-grain weight is controlled by additive genes. More dominant genes for for 1000-grain weight are also supported by the significant value of **F**. Average degree of dominance $(H_1/D)^{1/2}$ was greater than unity, indicating over dominance type of gene action of additive nature for 1000-grain weight. Narrow and broad sense heritability estimates were 0.25 and 0.66, respectively, for 1000-grain weight (Table 4).

Phytic acid

Diallel analysis (5 × 5) for phytic acid revealed (Table 3) non-significant variation for additive gene effect (**a**). Overall dominance (**b)** was highly significant, indicating the importance of dominance for phytic acid. Directional gene distribution ($\mathbf{b_1}$**)** among parents was also found significant for the said trait. 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 2). Estimation for genetic components of variations, D, H_1, H_2, F, h^2 and E revealed significant differences. Additive gene action was less important as it was less than both H_1 and H_2 indicating a more role of dominance for phytic acid. Postive and significant value of F also pleaded for dominant type of genes for phytic acid. Mean degree of dominance was more (1.65) than unity, a clear cut proof of dominant type of gene action for phytic acid. Narrow sense 0.01 and broad sense 0.86 heritability estimates were found for phytic acid (Table 4).

Conclusion

From the present research, it can be concluded that phytate can also be decreased by breeding bread wheat genotypes up to the greater extent and even up to to the desirable level. By developing genotype/cultivar or line by breeding with low phytate, it would be a great step towards the improvement of bread wheat quality because in the international market now, the competition is on quality not on quantity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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