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# Genetic analysis of triple pistil wheat derived F<sub>2</sub> populations to enhance genetic yield potential

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## ABSTRACT

Triple pistil (TP) wheat is a novel genetic resource which holds the ability to produce three grains in a single floret. In this research, TP wheat derived two F<sub>2</sub> populations were studied to investigate genetic variability, heritability, genetic advance, genetic correlation, inheritance pattern and potential of TP trait for grain yield improvement. Phenological data were recorded for 12 morpho-agronomic traits. Most of the traits exhibited significant genetic variability ( $P \leq 0.05$ ) for further trait improvement. Strong positive genetic correlations ( $r \geq 0.99$  at  $P \leq 0.01$ ) were computed within yield contributing and yield limiting traits, indicating common genes controlling these traits and opportunity for simultaneous improvement. Inheritance analysis indicated double dominant (15:1) and double recessive (9:7) epistatic ratios in investigated populations, proposing that TP trait was controlled by two nuclear epistatic genes. In conclusion, TP wheat is a valuable germplasm resource for grain yield improvement and floral development studies.

**KEYWORDS:** Floral organs, grain yield, multi-ovary, *Triticum aestivum*, spike modification

## INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most productive and nutritionally important cereal crops in the 21<sup>st</sup> Century. Declining basic resources for crop production (water and nutrients), scarcity of fertile lands and alarming increase in population growth rate amounts to a perfect hunger storm. By 2050, global population is likely to surpass 9.1 billion mark, demanding almost 40% more increase in yield compared with current rate of annual genetic yield gain [1]. Genetic potential represents maximum possible yield that a crop can attain under ideal growth conditions. However, grain yield is the product of number of grains produced per unit of cropped area and average grain weight [2]. One way to improve wheat yield potential is to increase number of grains per spike without significant reduction in average grain weight [3]. Wide genetic variability present in inflorescence architecture of wheat can be manipulated to attain high grain numbers.

Four types of spike morphologies, namely, supernumerary spikelets [4,5], multi-row spikes [6], pistillody wheat [7] and triple pistil mutant [8] were ideal for increasing wheat yield because they can increase number of spikelets and grains per

spike. However, low kernel weight of supernumerary spikelet cultivars [4], delayed heading date and wrinkled grains of multi-row spikes [9] made many wheat breeders turn to explore other spike forms. Creation and research on new genetic variation to bolster grain numbers per spike [10,11,12] is still very interesting to breeders.

Among inflorescence modifications of wheat, triple pistil (TP) phenotype is unique, as this trait can set three grains per floret and total grain weight of tri-grain florets was significantly higher than single grain florets [13,14]. TP wheat was serendipitously identified in the experimental field of Chinese landrace “Ganmai 8” and entrusted to be unique due to formation of three close set grain in back-to-back fashion [15]. Florets of TP wheat consist of a lemma, a palea, two lodicules, three stamens and three pistils (carpel, gynocia or ovary). Although, one or two pistils die out in some florets before seed set due to competition for nutrients, pollen and space, but many florets exist which set three grains (Figure 1) [14]. TP wheat has normal inflorescence morphology and development process just like common hexaploid wheat, except the occurrence of two additional pistils. The additional pistils are completely fertile, holding ability to produce three normal grains and the trait is highly heritable to

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**Figure 1:** Spike of triple pistil wheat

the next generations [13]. Based on these reports, TP wheat suggested to be an interesting germplasm resource.

Previously, much work has been conducted on estimation of genetic parameters of common hexaploid wheat. However, studies focusing abnormal spike forms, especially triple pistil phenotype, are infancy. This research was carried out to envisage important genetic parameters of TP wheat derived two  $F_2$  populations to explore their genetic parameters and grain yield potential. Furthermore, inheritance analysis of TP trait was also carried out to determine approximate number of gene(s) associated with abnormal phenotype.

## MATERIAL AND METHODS

### Plant Material and Growth Conditions

This research was conducted at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan (located between longitude 73.8° East, latitude 31.43° North and 184 m above sea level). Two  $F_2$  segregating populations (1023 × BWL 812, 1916 × 1032; where 1023 & 1032 were TP lines) were sown during 2015/16 growing season under normal field conditions according to randomized complete block design (RCBD) with three replications. Sowing was done on December 11, 2015 and harvesting was done at maturity on April 26, 2016 after recording of field traits data. Seeds were sown in rows using hand dibbler keeping plant × plant and row × row distances 15 cm and 22.5 cm respectively. Fertilizers (NPK) were applied at a rate of 112:84:62 kg per hectare. Three irrigations were applied at crown root initiation, booting and grain milking stages. Standard agronomic and plant protection practices were followed.

### Phenotypic Measurements

At appropriate times, data of 12 morpho-agronomic traits was recorded from 88 plants of 1023 × BWL 812 and 191 plants of 1916 × 1032 populations. Moreover, morphometric data was also collected from 12 healthy and guarded plants of each parental line involved in these populations. Measurements of days to heading (DH), flag leaf area (FLA), number of productive tillers plant<sup>-1</sup> (PTPP), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), floret morphology, number of spikelets spike<sup>-1</sup> (SPS), number of grains spike<sup>-1</sup> (GPS), 100-grain weight (HGW), grain weight spike<sup>-1</sup> (GWPS) and grain yield plant<sup>-1</sup> (GYPP) were made from single plants. Flag leaf area was calculated before maturity, when leaves were green, fully developed and turgid in morning hours, by following the formula of Muller [16]. For

floret morphology, florets from four central spikelets of each mother shoot spike were visually examined before threshing. Those plants bearing at least one di-grain or tri-grain floret in each spikelet were considered as triple pistil plants, while those having single seed in each floret of all spikelets were considered as normal plants.

### Statistical Analyses

The recorded data of all traits, except floret morphology, was subjected to analysis of variance (ANOVA) technique [17] using Statistix 10.1 to calculate genetic variability among  $F_2$  plants and parental lines.

Broad sense heritability ( $h^2$ ), genotypic correlation, dendrogram for the distance matrix and biplot of principle component analysis (PCA) for studied traits were computed/constructed through CIMMYT META-R software v 6.0 [18]. Correlation figure was drawn using genesis software [19].

Genetic advance (GA) was calculated by following formulae:

$$GA = \sigma_p \times h^2 \times i$$

Where;

$\sigma_p$  = phenotypic standard deviation of  $F_2$  population

$h^2$  = heritability in fraction

$i$  = constant value for selection intensity.

The constant value used in this study at 10% selection intensity was 1.755.

Frequency distributions were computed by using Microsoft Excel 2016 Data Analysis Tool.

Inheritance pattern was calculated by following chi square goodness of fit test:

$$\chi^2 = \frac{(O - E)^2}{E} \times 100$$

Where;

$\chi^2$  = chi square calculated value, O = observed frequency, E = expected frequency.

## RESULTS AND DISCUSSION

### Presence of Variability, Heritability and Genetic Advance for Crop Improvement Through Early Generation Trait Selection

ANOVA results showed presence of significant amount of genetic variability for most of the traits studied, except for DH, DM and GWPS, which were non-significant under both populations. When TP line hybridized as female parent, highly significant variability ( $P \leq 0.01$ ) was recorded for HGW and GYPP. Similarly, significant genetic variability ( $P \leq 0.05$ ) was recorded for PTPP, PL, SL, SPS and GPS. In case of 1916 ×

1032, highly significant variability was recorded for FLA, PTPP, PH, PL, SL, SPS, GPS, HGW and GYPP, whereas DH, DM and GWPS were non-significant. Mean square and coefficient of variability (CV%) values of all traits are given in Table 1.

Low to high heritability and genetic advance estimates were recorded for all traits. Under both populations, heritability and genetic advance estimates varied from 0.00 to 0.99 and 0.00 to 33.23, respectively. Traits with significant or highly significant amount of genetic variability including FLA, PTPP, PH, PL, SL, SPS, GPS, HGW and GYPP showed high heritability estimates ( $\geq 0.65$ ). Whereas, moderate to low heritability estimates ( $\leq 0.60$ ) were calculated for non-significant traits such as DH, DM and GWPS. Genetic advance estimates varied greatly among all traits. High genetic advance estimates ( $> 5.65$ ) were calculated for PTPP, PH, GPS and GYPP. Moderate to low genetic advance estimates (3.00 – 5.64) were calculated for FLA and PL, whereas all other traits (DH, DM, SL, SPS, HGW, GWPS) showed low genetic advance estimates ( $< 3.00$ ). Heritability in broad sense and genetic advance estimates are given in Table 1.

Grain yield is a multi-factorial trait and is highly influenced by environmental fluctuations. Therefore, direct selection for grain yield as such could be misleading. For successful selection, occurrence of wide range of genetic variability and information on the association of morpho-agronomic traits with grain yield are necessary [20]. Occurrence of wide range of variability makes it expedient to select required traits quickly with greater precision [21]. Our results showed availability of sufficient genetic variability for most of the traits studied, except for DH, DM and GWPS (Table 1). Highest variability was recorded for yield contributing traits such as PTPP, GPS, HGW and GYPP indicating potential for further trait improvement. Previously, wide genetic variability has been reported in wheat germplasm for yield contributing traits [22,23,24,25,26,27].

Heritability and genetic advance estimates provide information about index of transmissibility of the quantitative characters and precise view of segregating generations for possible selection [23]. High heritability along with higher genetic advance estimates are helpful in genetic gain under selection. Combination of high estimates of these important genetic parameters indicate additive genetic effects and effectiveness of early generation trait selection [28]. Whereas, high heritability but lower genetic advance or vice versa suggest non-additive genetic effects and delayed selection for these traits. Yang and

Peng [29] studied nine agronomic traits of TP wheat line and observed high broad sense heritability estimates for all traits. Recently, Waqas *et al.*, [27] also reported high heritability and genetic advance estimates in eight  $F_2$  wheat populations for number of productive tillers plant<sup>-1</sup>, number of grains spike<sup>-1</sup>, grain weight spike<sup>-1</sup> and grain yield plant<sup>-1</sup>. In this research, higher broad sense heritability and genetic advance estimates were recorded for PTPP, PH, GPS and GYPP (Table 1). Selection in  $F_2$  population on basis of these traits would be helpful to select higher yielding plants. As described earlier, the availability of sufficient genetic variability for these traits, thus holding promise for crop improvement through early generation trait selection. All other traits showed higher heritability coupled with low genetic advance or vice versa, suggesting that selection for these should be delayed for efficient crop improvement.

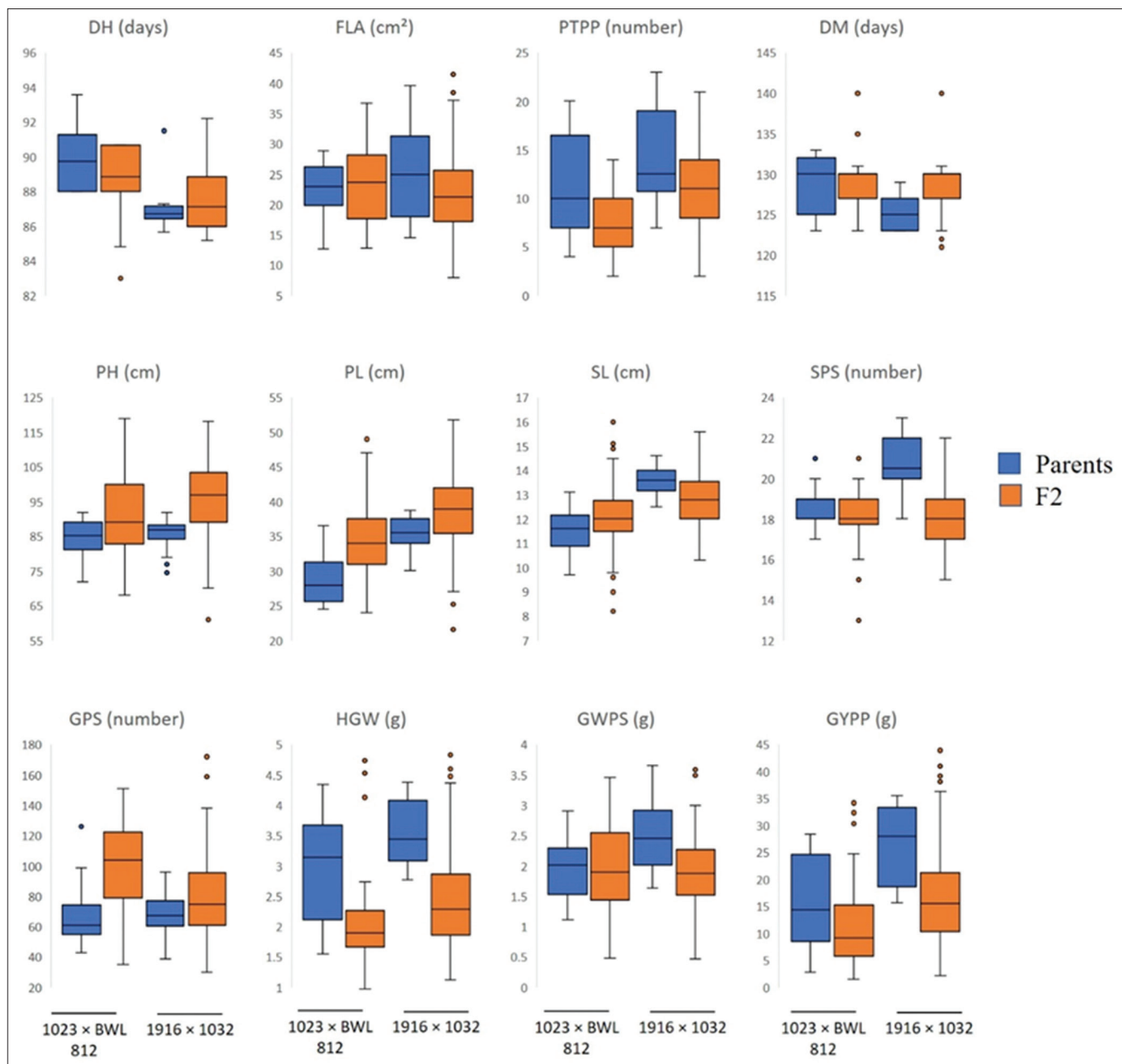
### Mean Performance Varied Among Studied Traits

All 12 traits showed a dispersive distribution (Figures 2 and 3) indicating the presence of complex underlying genetic mechanisms. We found that mean performance of  $F_2$  populations varied among traits. In case of 1023 × BWL 812, 72% plants had earlier heading date but 74% had delayed maturity date as compared with paternal parents. For yield limiting traits such as flag leaf area, plant height and peduncle length, most  $F_2$  plants (51% for FLA, 68% for PH, 86% for PL) showed performance better than that of their paternal parents. For grain yield related traits such as spike length, spikelets per spike and grains per spike, most  $F_2$  plants had better performance (72% for SL and 50% for SPS, 84% for GPS) but for productive tillers per plant, 100-grain weight, grain weight per spike and grain yield per plant, lesser number of plants had better performance (7% for PTPP, 3% for HGW, 45% for GWPS and 34% for GYPP) that that of parents. Similarly,  $F_2$  plants of 1916 × 1032 population showed dispersive distribution as 56% and 75% plants had delayed heading and maturity dates respectively. Plant height and peduncle length of most of the  $F_2$  plants increased (85% for PH and 83% for PL) but flag leaf area, spike length and spikelets per spike of few plants increased (25% for FLA & SL each and 4% for SPS). For grain yield related traits, same trend was observed as that of 1023 × BWL 812. GPS increased in most of the plants (62%) but plants with increased PTPP (26%), HGW (11%), GWPS (17%) and GYPP (9%) were lesser. This discrepancy in distribution between both populations might arise due to different population size and genetic backgrounds of both TP genotypes.

**Table 1: Mean squares, broad sense heritability and genetic advance estimates**

	DH	FLA	PTPP	DM	PH	PL	SL	SPS	GPS	HGW	GWPS	GYPP
<b>1023×BWL 812</b>												
Genotype	1.20 <sup>NS</sup>	29.52 <sup>NS</sup>	43.33*	0.27 <sup>NS</sup>	72.21 <sup>NS</sup>	41.49*	0.46*	1.69*	1376.9*	2.63**	0.11 <sup>NS</sup>	196.9**
C.V%	1.07	10.52	20.43	0.49	4.49	7.30	2.25	1.81	11.15	11.38	16.59	12.87
h <sup>2</sup>	0.10	0.56	0.79	0.00	0.55	0.70	0.65	0.83	0.83	0.92	0.03	0.96
GA	0.17	3.67	5.70	0.00	5.67	5.11	0.52	1.16	33.23	1.55	0.01	13.79
<b>1916×1032</b>												
Genotype	0.57 <sup>NS</sup>	113.6**	77.03**	4.10 <sup>NS</sup>	117.4**	12.12**	0.92**	6.50**	328.7**	1.45**	0.38 <sup>NS</sup>	283.8**
C.V%	0.83	8.43	7.02	0.84	1.31	1.98	1.53	2.18	5.88	5.75	11.79	4.18
h <sup>2</sup>	0.03	0.90	0.97	0.46	0.97	0.88	0.88	0.92	0.85	0.93	0.59	0.99
GA	0.04	10.08	8.69	1.18	10.72	3.24	0.89	2.44	16.50	1.17	0.43	16.94

<sup>NS</sup>Non-significant, \*Significant at  $P \leq 0.05$ , \*\*Highly significant at  $P \leq 0.01$ .



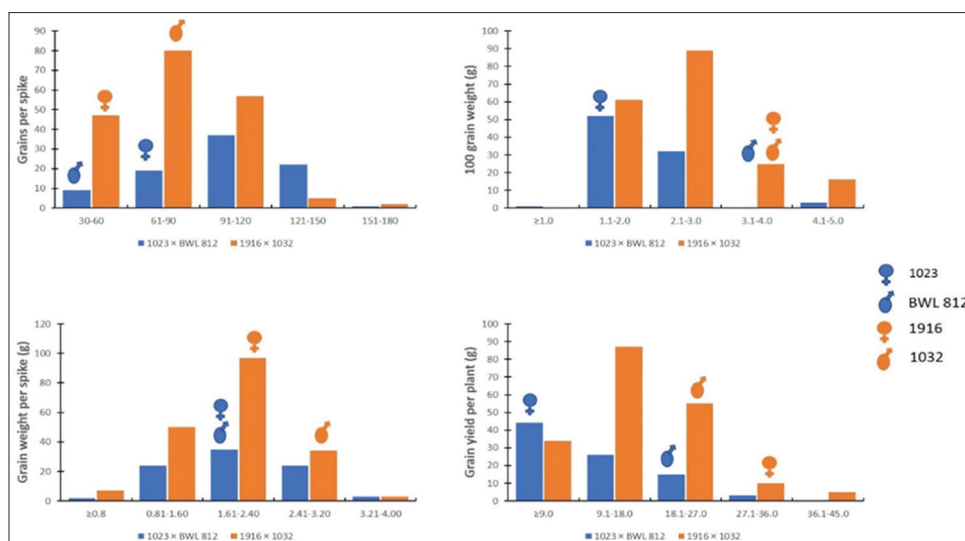
**Figure 2:** Mean performance of studied traits in both populations

### Genotypic Correlations Indicated Inherent Associations Among Yield Contributing and Yield Limiting Traits

Genotypic correlations were computed to determine inherent associations among different traits, cluster of traits controlled by common genes and their possibility for simultaneous improvement. In population 1023 × BWL 812, DH was positively and highly significantly correlated with FLA and GWPS but was negatively correlated with SL (Figure 4). FLA had strong non-significant positive correlation with GPS (0.81) but highly significant negative correlation with SPS, HGW, GWPS and GYPP. PTPP had highly significant positive correlation with HGW, GWPS and GYPP but was negatively and highly significantly correlated with PH, PL and GPS. PTPP also

had strong correlations with SL (-0.98) and SPS (0.93), however, both these were non-significant. Correlations of DM with other traits could not be computed due to null heritability and genetic advance estimates. PH had highly significant positive correlation with PL and GPS but also showed highly significant negative correlations with HGW, GWPS and GYPP. SL (0.99) and SPS (-0.94) had strong correlation with PH but were non-significant. PL had highly significant positive correlation with GPS but also showed highly significant negative correlation with HGW and GWPS. PL also showed strong correlations with SL (0.98), SPS (-0.90) and GYPP (-0.99) but all were non-significant. SL had highly significant positive correlation with GPS but was negatively and highly significantly correlated with GWPS. SL also exposed strong correlations with SPS (-0.64),



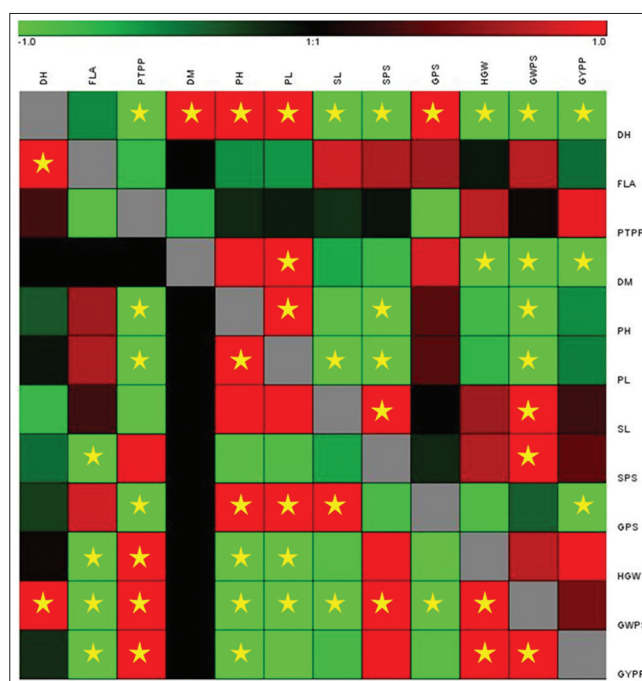


**Figure 3:** Frequency distribution for four major grain yield traits

HGW (-0.92) and GYPP (-0.84) but were non-significant. SPS had highly significant positive correlation with GWPS but also showed non-significant correlations with GPS (-0.87), HGW (0.98) and GYPP (0.98). GPS had highly significant negative correlation with GWPS but also showed strong non-significant correlations with HGW (-0.99) and GYPP (-0.95). HGW had highly significant positive correlation with GWPS and GYPP. Similarly, GWPS was positively and highly significantly correlated with GYPP.

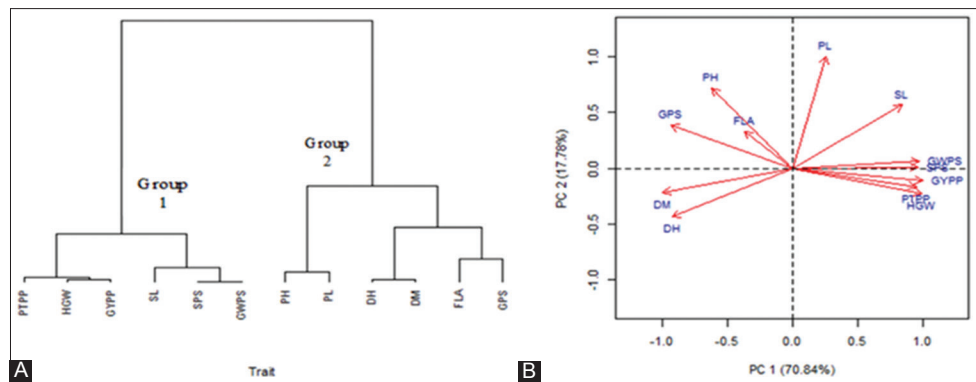
In case of 1916 × 1032, DH had highly significant positive correlation with DM, PH, PL and GPS but was negatively and highly significantly correlated with PTPP, SL, SPS, HGW, GWPS and GYPP (Figure 4). FLA and PTPP had non-significant correlation with all studied traits. DM was positively and highly significantly correlated with PL but had highly significant negative correlation with HGW, GWPS and GYPP. DM also showed strong correlation with PH (0.95), SL (-0.66), SPS (-0.80) and GPS (0.85) but all these were non-significant. PH had highly significant positive correlation with PL but was negatively correlated with SPS and GWPS. PH also had strong non-significant correlation with SL (-0.95) and HGW (-0.83). PL was negatively and highly significantly correlated with SL, SPS and GWPS. SL has highly significant positive correlation with SPS and GWPS. SPS had highly significant positive correlation with GWPS and GPS was highly significantly but negatively correlated with GYPP. HGW had strong non-significant positive correlation with GWPS (0.74) and GYPP (0.95), whereas GWPS had positive but weak correlation with GYPP. In conclusion, both populations exhibited highly significant positive correlations within yield contributing and yield limiting traits but highly significant negative correlations observed between these two groups of traits (Figure 5).

Selection of superior genotypes from segregating populations is very useful for cross breeding material and crop improvement. Genetic correlation provides better understanding about inherent association of different characters and possibility of



**Figure 4:** Genotypic correlation among traits. Bottom left panel for 1023 × BWL 812 and top right panel for 1916 × 1032. Yellow star indicates highly significant value at  $P \leq 0.01$ . Black boxes indicate that correlations were below threshold (0.05). Diagonal is indicated with grey colour boxes.

simultaneous improvement. Kashif and Khaliq [22] found that plant height, spike length, number of spikelets spike<sup>-1</sup>, number of grains spike<sup>-1</sup> and thousand grain weight were positively and significantly correlated with grain yield plant<sup>-1</sup>. Similarly, significant positive correlation was reported among number of productive tillers plant<sup>-1</sup>, spike length, number of spikelets<sup>-1</sup>, number of grains spike<sup>-1</sup> and grain yield plant<sup>-1</sup> [23]. In this research, both populations indicated clustering of all traits into two distinct groups. Group 1 (yield contributing) contained PTPP, SL, SPS, HGW, GWPS and GYPP, with exception of



**Figure 5:** (A) Dendrogram for the distance matrix and (B) biplot of the PCA analysis among studied traits. (A)Based upon genotypic correlation coefficients, all traits were clustered into two main groups: yield contributing (group 1) and yield limiting traits (group 2), with exception of GPS which was grouped with yield limiting traits. (B) Biplot of the PCA analysis showing direction and magnitude of variations explained by individual PC 1 and PC 2 out of whole system.

GPS (Figure 5A). Group 2 (yield limiting) contained DH, DM, FLA, PH, PL and GPS. Positive associations were present within both groups, whereas negative associations were present between groups. Those traits which were plotted on distal nodes far away from ancestral node, share common genes and can be improved simultaneously (Figure 5A). Furthermore, about 71% of the variation in whole system is described by group 1 (PC 1) only, whereas group 2 (PC 2) describes 18% of the total variation (Figure 5B). We found highly significant negative correlation between GPS and GYPP. The reason behind this antagonistic interaction is that when number of grains per spike are increased the average grain weight per spike tend to decrease which ultimately affect the final grain yield per plant [30]. When less number of grains per spike are produced, their 1000-grain weight is higher due to less competition for nutrient and space. In TP wheat, majority of the florets carry three grains and competition for nutrition and space was very high which lead to reduction in grain weight per spike and grain yield per plant. If TP trait is transferred into elite cultivars with more floral space, the negative correlation can be reversed.

**Oligogenic Control of TP Floret Trait**

A reciprocal cross involving genetically different TP and non-TP wheat lines was carried out to investigate inheritance pattern of TP trait. When TP wheat line hybridized as female parent (1023 × BWL 812), F<sub>2</sub> plants segregated as in double dominant epistasis (15:1) for the TP trait (79) to normal floret trait (9). On contemporary, hybridizing TP wheat line as male parent (1916 × 1032), double recessive epistasis (9:7) was observed for the TP trait (119) to normal floret trait (72) (Table 2). These results indicated that more than one nuclear epistatic genes are involved in governing the TP trait, either with or without cytoplasmic effects.

Previous studies reported that TP trait is controlled by single dominant gene, either with or without cytoplasmic effects [13,31,32,33,34] or by more than one dominant or recessive genes [35,36,37,38,39]. To validate previous findings, floret morphology of each F<sub>2</sub> plant was noted before threshing and chi-square goodness of fit test was applied to determine inheritance pattern. When TP wheat line was hybridized as

**Table 2: Segregation of TP and normal plants in both populations**

Cross Combination	Floret Type		$\chi^2$ value
	TP	Normal	
1023×BWL 812	79	9	2.24 <sup>NS</sup> (15:1)
1916×1032	119	72	2.88 <sup>NS</sup> (9:7)

<sup>NS</sup>Non-significant. Value for significant at P≤0.05 (df=1) is 3.84.

female parent (1023 × BWL 812), triple pistil to single pistil segregations were found to be following double dominant epistatic ratio (15:1). However, double recessive epistatic ratio (9:7) was observed when TP line was hybridized as male parent (1916 × 1032). Backcross of F<sub>1</sub> plants with respective parents could confirm either TP trait is affected by maternal cytoplasmic effects. But unfortunately, we were unable to perform backcross test due to non-availability of F<sub>1</sub> seeds. Our results suggest that TP trait is controlled by two nuclear epistatic genes, either with or without cytoplasmic effects. These results are consistent with previous reports [35,36,37] which suggested that TP trait is governed by two dominant or recessive non-complementary genes.

**Breeding for Higher Grain Yield Potential**

Alarming increase in human population demand at least 40% annual genetic gain in cereals production to ensure food security [1], which is a big task to achieve. TP wheat can produce three grains in all florets, more number of grains spike<sup>-1</sup>, higher grain weight spike<sup>-1</sup> and finally higher yielding potential. Recently, we have identified a F<sub>1</sub> hybrid with triple pistil and normal wheat cultivars background exhibiting up to 45% mid-parent heterosis for grain weight spike<sup>-1</sup> (Figure 6). However, this heterosis needs to be stabilized on small plots and at multi-locations.

Previously, Ma *et al.* [8] reported 3.41-42.83% heterosis in grain weight spike<sup>-1</sup> which is consistent with our results. Ma *et al.* [8] studied heterosis and its utilization in TP wheat. They produced 24 F<sub>1</sub> hybrids by crossing three multi-ovary wheat lines with eight common wheat lines. The heterosis was mainly observed for number of grains spike<sup>-1</sup>. High mid-parent heterosis for grain weight spike<sup>-1</sup> (32.71%) was also observed in 18 crosses. Later, Peng *et al.* [14]



**Figure 6:** Grain yield potential of TP wheat

reported that total grain weight of tri-grain florets was significantly higher than single grain florets. But the weight of 100-grains in tri-grain florets was lower than that of single grain florets. Based upon these results, an easy speculation can be made about enormous potential of TP wheat to raise genetic yield potential. It is evident that TP wheat line may not have same agricultural significance as solely TP trait holds. Introgression of TP trait into modern elite cultivars through marker assisted backcrossing or conventional backcrossing method will not only raise genetic yield potential but can also bring next wave of yield improvement. Further research efforts involving TP wheat would provide useful information to the wheat breeder to wisely utilize TP wheat lines as significant donor to potential grain yield related traits. Moreover, molecular aspects of floral development in economically important crops is also an interesting issue. Molecular characterization of TP wheat can be employed to identify ovule/ovary specifying gene(s) in wheat. This will not only advance research in *Triticaceae* but also in other important cereals. However, further detailed research efforts are required into this direction.

## CONCLUSION

In this research, genetic analysis of triple pistil wheat derived two  $F_2$  populations was carried out. Phenological data were recorded for 12 morpho-agronomic traits. Majority of the studied traits exhibited strong genetic variation for further trait improvement and higher heritability into succeeding generations. Strong genetic correlations were computed within yield contributing and yield limiting traits. Inheritance analysis indicated double dominant and double recessive epistatic ratios in studied populations. Introgression of TP trait into modern elite cultivars will not only raise genetic yield potential but can also bring next wave of yield improvement.

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