



Genetic Inheritance Analysis of Wheat Spike-Related Traits in Wheat Segregating Population

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Abstract

An increase in food grain production is needed to feed the ever-increasing population, which is expected to reach 9 billion by 2050. Wheat (*Triticum aestivum* L.), one of the most important world food crops, is thought to play a major role in ensuring world food security. Wheat yield is a complex trait, and manipulating wheat spike attributes may lead towards improving yield plant-1 by increasing the spike length, selection for long awns and increasing the number of spikelets in the spike with supernumerary spikelets. This study was designed to investigate a wheat F₂ segregating population, developed from two parents with contrasting spike architecture, for genetic inheritance and variation in several spike-related traits such as awn status, supernumerary spikelet (SS) phenotype, awn length and spike length. Wheat F₂ population comprising 78 plants was developed by crossing landrace-91 bearing awnless but one terminal spike and non-SS spike with SS-spike bearing long awns. Chi-squared tests were used to determine the goodness of fit of observed data with expected segregation ratios for genetic inheritance analysis of spike-related traits. Statistics v.8.0 was used to calculate LSD values, while MS Excel was used for the graphical presentation of data. Genetic inheritance analysis of the F₂ population for awn status and SS-phenotype revealed that both awn development and SS-phenotype segregated in a 3:1 ratio, following simple single dominant gene inheritance. Considerable variations between parents and F₂ progenies for awn length and spike length were observed, with 107-2-F₂ having the maximum awn length of 11 cm and spike length of 22 cm. 107-2-F₂ line with maximum awn length, spike length and SS-phenotype was advanced to further generation for stable inheritance of the said traits and to further manipulate the line in enhancing yield/plant.

Keywords: Wheat spike, Awn, Supernumerary spikelet, Yield, Genetic inheritance

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INTRODUCTION

World food production is challenged due to loss of arable land, climate change, volatile prices, biofuel demand, high meat demand, and poor commodity distribution (Godfray *et al.*, 2010). An increase in food grain production is needed to feed the ever-increasing population, which is expected to reach 9 billion by 2050. Wheat (*Triticum aestivum* L.), one of the most important world food crops, will play a major role in ensuring world food security (Rajaram, 2002). However, in the last few decades, only limited breeding efforts were focused on enhancing the yield potential of this important crop (Reynolds *et al.*, 2011). Grain yield is a complex trait determined by multiple components, such as spikes per unit area, grain weight, and grains per unit area. In turn, these components are impacted by the inflorescence architecture, spike meristem growth, and spike fertility (Sreenivasulu and Schnurbusch, 2012). Considering that conventional wheat cultivars bear one spikelet per rachis node and produce between 20 and 50 kernels per spike, the implementation of exotic germplasm bearing more than one spikelet per rachis node has been suggested as a strategy to increase grain yield (Aliyeva and Aminov, 2011).

The wheat inflorescence classically consists of a spike with a main axis (the spike rachis) carrying lateral sessile spikelets that are directly attached to the rachis and also a terminal spikelet. The spikelet constitutes the basal unit of the spike inflorescence. Wheat yield in terms of grain production/plant mainly depends on the structural design of the inflorescence. Wheat scientists consider that managing spike architecture is one possible way to increase the per-plant yield. Dobrovolskaya *et al.* (2015) reported that genetic determinism of inflorescence development may permit new spike

architecture to be designed to improve grain production. New spike morphology may lead to an increase in the number of spikelets in the spike, i.e. making selections for wheat forms with supernumerary spikelets and branched spikes. These traits are natural variation in wheat inflorescence and hold great potential for boosting bread wheat yield by doubling the number of grains per spike (Martinek and Bednar, 2001).

The term supernumerary spikelet spike (SS) includes any additional sessile spikelets developed at a rachis node, such as multi-row spike (MRS), horizontal spikelets (HSs), and the ramified spike (RS) or branched spike. In tetraploid and hexaploid wheat species, the development of SS is a recessive trait (Dobrovolskaya *et al.*, 2009). A wheat spike normally bears one spikelet per rachis node, which arises directly on the main inflorescence axis; the formation of supernumerary spikelets (SS) is rare. The earliest studies in tetraploid wheat observed that SS is controlled by a single recessive gene (Sharman, 1967). In hexaploid wheat, however, three genes designated as Ramifera (*Rm*), Tetrastichon (*Ts*), and Normalizator (*Nr*) were reported to control SS (Koric, 1973). Fundamental genetic factors for supernumerary spikelet phenotype found to be assorted and exemplified for paired spikelet phenotype or multi-rowed spike (*mrs*) locus (Dobrovolskaya *et al.*, 2009). This study was designed to investigate spike-related traits in the wheat F₂ segregating population.

MATERIALS AND METHODS

Current research was conducted in an experimental area of Gomal University, Dera Ismail Khan, in the wheat growing season of November 2022-May 2023.

Research Design

Research design for this study was standardized as RCBD (randomized

complete block design) using three replications in the design. Experimental plot area was kept at 18 m × 5 m (90 m²) with 100 wheat germplasm replication⁻¹ having a size of each line being kept 5 m in length and a 30 cm distance between lines. Sowing was carried out in lines, and ten seeds per row were dibbled, keeping plant-to-plant distance of 15 cm and row to row-to-row distance standardized at 30 cm.

Practices

Land was prepared using 2-3 deep ploughings accompanied by rotavator operation. There was application of regular necessary agricultural practices such as fertilizer application of recommended doses of nitrogen and phosphorus, irrigation, hoeing, etc.

Material

Two wheat genotypes were used in this study, one landrace, namely landrace-91 (accession#11091) and second wheat variety, Zamindar-80. Both genotypes were acquired from National Agriculture Research Council (NARC), Islamabad.

Population development

For population development, two wheat genotypes with contrasting spike attributes were used in this study. Wheat landrace-91 was used as the recipient female parent and Zamindar-80 as male donor parent. F₁ progenies were developed by conventional hybridization technique.

Data collection and parameters

Data was recorded keeping selection intensity at 5% from every line. The protocol for data collection of parameters is as under.

Awn status

Awn status was scored as awnless (no awns), scurs (short distal scurs), short awns and long awns.

Awn length (cm)

Awn length was calculated by measuring the length from the base of the awn to the tip of awn.

Spike length (cm)

Spike length of the chosen plants was calculated after measuring the spike from base to the pinnacle of the spike, exclusive of awns, and further analyzed for their average.

SS Spike

SS spike includes any additional sessile/fertile spikelets developed at a rachis node. The SS spike phenotype was recorded as either SS-spike or non-SS spike.

Statistical Analysis

Chi-squared tests were used to determine the goodness of fit of observed data with expected segregation ratios for genetic inheritance analysis of spike-related traits. Statistix v.8.0 was used to calculate LSD values, means, while MS Excel was used for the graphical presentation of data.

Results And Discussion

Attributes of breeding parents and their F₁ progenies

Morphological assessment showed that landrace-91 possessed a bent-shaped long spike (13 cm), no awns (awnless), but one terminal scur, no supernumerary spikelets (non-SS), 18 spikelets/spike and non-waxy glumes. Zamindar-80 bears crooked-shaped long spikes (18 cm), long awns (6.5 cm), 4-5 supernumerary spikelets (SS)/spike, 22 spikelets/spike and medium waxy glumes. Spike attributes of their F₁ progenies showed crooked-shaped long spike (20 cm), terminal scur (1.9 cm), 4-6 supernumerary spikelets (SS)/spike, 21 spikelets/spike and strong waxy glumes (Fig.1). Spike architecture is a complex trait encompassing different characters such as spike length, shape, awn presence/absence, no. of spikelets/spike and no. of grains per spike. Awns, bristle-like structures extending from grass lemmas, protect against predators, contribute to photosynthesis and aid in grain dispersal. In wheat, selection of awns

with minimal extension, termed awnletted, has occurred during domestication by way of loci that dominantly inhibit awn development, such as Tipped1 (*B1*), Tipped2 (*B2*), and Hooded (*Hd*). Huang et al. (2020) showed that *B1* acts as a dominant repressor gene. Located on chromosome 5A, *B1* is a C2H2 zinc finger encoding gene with ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motifs. Constitutive over-expression of *B1* in awned wheat produced an awnletted phenotype with pleiotropic effects on plant height and fertility. *B1*, predominantly responsible for awn inhibition in wheat, is mainly responsible for awnletted wheats globally. The crossing of awnless landrace 91 with awned Zamindar-80 produced F_1 with awnless spike, showing that awn development was completely inhibited in F_1 (Fig. 1). This result shows that the genetic background of the recipient parent affects the expression of awn-related genes. On the contrary, the supernumerary spikelet (SS) phenotype was inherited successfully in F_1 (Fig. 1). Our findings are supported by Sun et al. (2009), who showed that supernumerary spikelet (SS) in the line 51885 was controlled by two dominant genes acting complementarily. Similarly, glume waxiness was also inherited from Zamindar-80 to F_1 (Fig. 1).

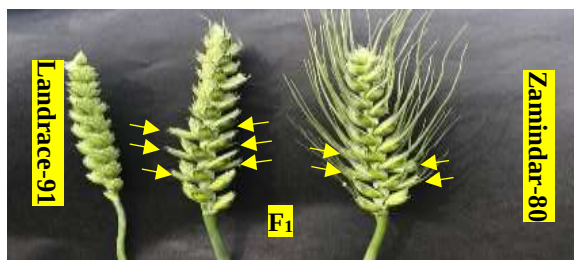


Fig.1 Spike attributes of breeding parents and their F_1 . Supernumerary spikelets (SS) are shown as yellow arrows.

Genetic inheritance analysis of the F_2 population

Awn development

An F_2 population comprising 78 plants was phenotypically evaluated for awn status using four attributes of awn: long (LA), short awn (SA), terminal scur (TS) and awnless (AL). Phenotypic evaluation showed that among 78 F_2 plants, 26 plants possessed long awns, 34 short awns, 12 terminal scurs, and 6 plants had awnless spikes. After clustering four attributes into two broad groups namely awned spike group (LA and SA) and awnless spike group (TS and AL), the results revealed the awned group possessed 60 plants and awnless group possessed 18 plants, perfectly fitting 3:1 ratio of a single gene controlling awn development dominantly (Table 1). Previous studies have had contradictory findings about the genetic inheritance of awn development. For instance, Huang et al. (2020) showed that awn development is controlled by a single dominant repressor gene *B1* located at chromosome 5A, while Yoshioka et al. (2017) showed that awn development in the common wheat gene pool is controlled by three dominant genes *Hd*, *B1* and *B2*. Similarly, Nishijima et al. (2017) identified a new awnless locus, *Anathera* (*Antr*), on the distal region of the short arm of chromosome 5D possessing a dominant awn-inhibiting gene related to differentiation of the variety *anathera* in the wild diploid wheat *Aegilops tauschii*. Further molecular analysis, cloning and functional validation of genes controlling awn development are needed to unlock the complex mechanism governing awn development and awn suppression in common wheat.

Table 1 Genetic inheritance analysis of F_2 population for awn status.

Population	Land race-91	Zamin dar-80	F ₂ -phenotype		Ratio	X ² _{3:1}	P-value
			LA TS	SA AL			
Landrace-91x Zamindar80	AL	LA	26 12	34 6	3:1	0.11	0.1
			60 18				

LA: long awns, **SA:** short awns, **TS:** terminal scurs, **AL:** awnless

Supernumerary spikelet (SS) spike

Phenotypic evaluation of F_2 plants showed that 55 plants reflected the SS phenotype similar to landrace-91, and 23 plants showed the non-SS phenotype similar to Zamindar-80. The segregation of F_2 plants into 55 SS and 23 non-SS fit a little distorted but near a 3:1 ratio of a single dominant gene controlling SS development in wheat spike (Table 2). A smaller deviation from a perfect 3:1 ratio indicates the possibility of the presence of minor effect genes or modification genes affecting the expression of the supernumerary spikelet. Moreover, a smaller population used in our study, only comprising 78 individuals, might have limited the perfect segregation of SS phenotype into a 3:1 ratio showing single dominant gene expression. This is also evident in the findings of Pennell and Halloran (1983) that the supernumerary spikelet in the wheat line 51885 was controlled by two dominant genes, which showed complementary function. Their findings also showed that transgressive segregation for increasing the number of spikelets was observed in the four crosses, suggesting the possibility of the presence of minor effect gene(s) or modification gene(s) affecting the expression of the supernumerary spikelet in addition to the two major dominant genes. The supernumerary spikelet character of bread wheat (*Triticum aestivum* L.) is an abnormal spike with an extra spikelet per spike. Relatively simple inheritance and stability of the SS-trait in Zamindar-80 can offer a valuable resource to be used in future breeding programs to increase yield/plant.

Table 2 Genetic inheritance analysis of the F_2 population for SS-phenotype.

Population	Landrace-91	Zamindar-80	F ₂ phenotype	Ratio	X ² _{3:1}	P-value
			SS Non-SS			
Landrace-91 x Zamindar-80	Non-SS	SS	55 23	3:1	0.17	0.1

Awn Length

Awns are the extension of lemma. Wheat awn plays a vital role in photosynthesis, grain production, drought tolerance, seed dispersal and protection against animals. Fig. 2 shows considerable variations between parents (landrace-91 and Zamindar-80) and F_2 progenies for awn length. Awn length ranged between 0.3-11cm. Maximum awn length was recorded for 107-2- F_2 (11cm), while the minimum awn length was recorded for 99-5- F_2 (0.3cm). The F_2 progenies will be advanced to F_3 to authenticate the stable inheritance of awn length to the next generation, and selection will be made for lines with maximum awn length. Previous studies have shown that awn length is highly associated with grain size. For instance, [Rebetzke et al. \(2017\)](#) showed that Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. Similarly, [Wang et al., \(2019\)](#) revealed that natural variations in the promoter of awn length inhibitor 1 (*ALI-1*) gene are associated with awn elongation and grain length in common wheat.

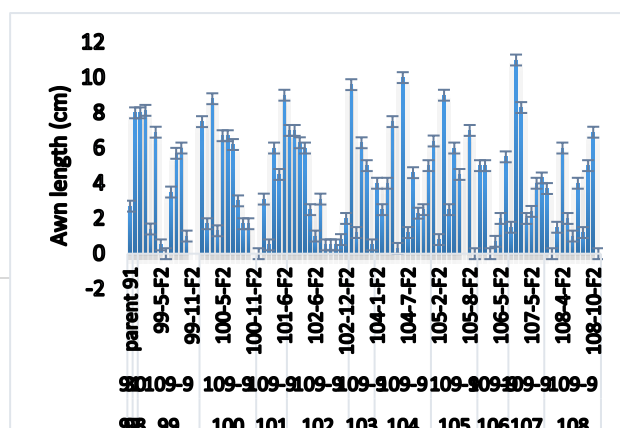


Fig. 2 Variations in awn length in the F₂ segregating population.

Spike length

Spike length is the most significant parameter of wheat yield. A lengthy spike is presumed to contribute to better yield. Since modern-day cultivars are bred for lengthy spikes bearing more grains. Considerable variations between parents and F₂ progenies were noticed for spike length. Mostly spike length ranges between 10-22 cm. Maximum spike length was recorded for 107-2-F₂ (22 cm), while the minimum spike length was recorded for 108-7-F₂ (10 cm) (Fig. 3). Progenies with maximum spike length were selected for further breeding for more grain yield.

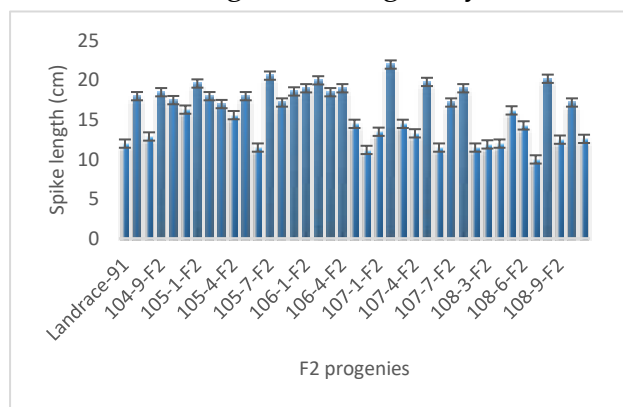


Fig. 3 Variations in spike length in the F₂ segregating population.

Conclusion

Spike architecture is one possible way to increase per-plant yield. However, spike architecture is a complex trait comprising several components such as awn presence/absence, awn length, spike length, spikelets/spike and supernumerary spikelets spike which individually or collectively contribute to

yield per plant. This study investigated the wheat F₂ segregating population, developed from two parents with contrasting spike architecture, for genetic inheritance and variation in several spike-related traits such as awn status, SS-phenotype, awn length and spike length. Genetic inheritance analysis revealed that both awn development and SS-phenotype segregated in a 3:1 ratio, following simple single dominant gene inheritance. Moreover, considerable variations were observed among F₂ progenies for awn length and spike length. F₂ line 107-2-F₂ with maximum awn length (11 cm), spike length (22 cm) and SS-phenotype was advanced to further generation for stable inheritance of the said traits and to further manipulate the line in enhancing yield/plant.

Authors Contribution

Nasr Ullah Khan conceived the idea, designed the study and drafted the manuscript. Saeed Ur Rehman and Maham Jamshed conducted the experiments and collected the data. Muhammad Anan Awais, Muhammad Yaseen and Fayaz Ullah helped in data collection and data analysis.

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