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SCREENING OF WHEAT GERMPLASM FOR STUDYING GRAIN LENGTH VARIATION DURING MATURITY AND GRAIN DEVELOPMENT STAGES

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops in the world. Grain traits are the important breeding objective in wheat, as phenotypically it is the most stable yield component. Large grain has been a key trait selected during wheat domestication and modern wheat breeding. Increasing grain size of wheat continues to be a major breeding target because of its effect on yield. Grain length (GL) has been found to be one of the important traits being neglected in improving grain weight of wheat as compared to rice. Wild germplasm of wheat found to be excellent source of variation in grain length to be exploited in wheat grain improvement. In the present study, wild progenitor of wheat including *Triticum dicoccoide s*(AABB), *Triticum monococcum* (A^mA^m), *Triticum boeoticum* (A^bA^b) and *Aegilopes speltoides* (SS) were understudied accessions as they are found to have great variations for grain length which could be exploited for increasing grain length thus grain yield in wheat. The mature seeds of each of these accessions were evaluated for GL and grain width (GW) for three consecutive years. GL varies from 6.5mm to 10.4mm while GW varies from 1.7mm to 3.6 mm in different accessions indicating wide variation of grain size. In long grain accessions maximum gain in GL is between 12 (Days Post Anthesis) DPA to 15DPA while in short grains accessions maximum gain of GL is between 9DPA to 12DPA. The richness in the genetic diversity enables to find the maximum grain size variation in the wild wheat germplasm whose exploitation could be helpful in cultivated ones. This study might enhance the potential for genetic manipulation of grain size in wheat along with understanding of the evolutionary dynamics of wheat which is likely to be useful for future wheat-breeding efforts.

***Keywords*:** Wheat, Grain length, Grain width, Days post anthesis, Genetic diversity

Introduction

Grain size in wheat is the most stable yield component and has a favorable effect on flour yield (Giura and Saulescu 1996). Wide variation among the grain morphological characters indicated wide genetic variation present among these accessions, which may be utilized for the selection of the parents for the plant breeding and production of new improved variety (Sinha and Mishra, 2015). Grain weight is largely defined by the size of individual grains and the morphometric components of grain area, length and width (Brinton *et al.*, 2017; Brinton and Uauy, 2019). The grain size is the main target as it mainly depends upon grain length, grain width. Archaeobotanical evidence from around the Fertile Crescent region indicates that the transition from the diploid wild einkorn (*Triticum monococcum* subsp. *aegilopoides*; AmAm) and tetraploid emmer wheat (*Triticum turgidum* subsp. *dicoccoides*; BBAA) to the domesticated forms (*T. monococcum* subsp. *monococcum* and *T. turgidum* subsp. *dicoccum*, respectively) was associated with a trend toward larger grains (Feldman, 2001; Fuller, 2007; Gegas *et al.*, 2010). Though, thousand grain weight measurement do not give an indication of variation within a sample. Detailed analysis of grain size measurement is cumbersome within sample and generally costly with low throughput. Contrastingly, grain size variation enables us to understand the diversification within a germplasm. Seed size was also a target of artificial selection during domestication, where large seeds are generally favored due to ease of harvesting and enhanced seedling vigor (Jahani *et al*., 2013). Grain size characteristics, which are immediately obvious to consumers, are major factors defining market value (Fitzgerald 2017). In rice, traits related to grain size and appearance have a large impact on market value and play a pivotal role in the adoption of new varieties (Juliano, 2020). Consumers mainly have a concept of quality on the appearance, size, and shape of the grain that boost the exporters as well. The maximum grain length variation presents in wild wheat germplasm to be exploited in the cultivated wheat for the grain improvement, ultimately grain yield. Though, thousand grain weight measurements do not give an indication of variation within a sample. Compared with rice, the grain length characteristics responsible for seed weight in other cereal crops, such as wheat (*Triticum aestivum*), maize, and sorghum (*Sorghum bicolor*), is lagging behind. The main attention of breeders and biotechnologists is to find out genetic variation which is an important aspect for better quality. Seed system utilize image analysis to give measures of size for individual seeds within a sample, allowing for a detailed understanding of variation, as well as an accurate estimation of the individual sample. Increasing grain size of wheat continues to be a major breeding target because of its effect on yield, offering new insights into the mechanisms controlling grain weight in cultivated wheat. Increasing grain size of wheat continues to be a major breeding target because of its effect on yield. It is also one of the main components that distinguish the domesticated species of diploid and tetraploid wheat from its wild ancestors. Due to the difficulty in quantifying, the phenotypic and genetic variation of wheat grain morphology is understudied. GLhas been found to be one of the important traits being neglected in improving grain weight of wheat as compared to rice. Wild germplasm of wheat found to be excellent source of variation in grain length to be exploited in wheat grain improvement. Consumers mainly have a concept of quality on the appearance, size, and shape of the grain that boost the exporters as well.

Materials and Methods

Plant Material

The present investigation include screening of four wild species including 144*Triticum diccocoides*, 165*T. boeticum, 58T. monococcum and 174Ae. speltoides* accessions during three consecutive years. Since long ago Punjab Agricultural University, Ludhiana collected wild germplasm from the different geographical locations from all over the world. All seeds were obtained from School of Agricultural Biotechnology, PAU Ludhiana.

Canon 5600 seed scanner

The grain length and grain width measurements were obtained through canon 5600 scanner using SEEDAN software. It has the capability to analyse the individual seed grain length and width. The data for some of the accessions were also calculated using vernier caliper to check the accuracy in the data. The seeds are placed on the scanner platform and covered with sheet. Afterwards, SEEDAN software measure grain length and width dimensions. The twenty seeds of each accession were randomly selected to calculate grain dimensions. The grain scanner enables robust and standardized study of grain size at very low cost with high throughput analysis.

Fig. 1 : Seedan system Wide grain length variation in wheat germplasm

Screening of seeds at developmental stages

The range of grain length and width of all the four wild species in three consecutive years were obtained. The selection of 20 long grain (9mm - 10.4mm) and 20 short grain (6mm - 7.0mm) accessions from all the four wild wheat species form the base for screening of grain length variation during developmental stages. Grain length and grain width were recorded in the developing grain starting DPA till the mature seed was obtained with the interval of 3 days. The flowering spikelet of the tiller were tagged during anthesis and only grains from florets were harvested with the same anthesis date were harvested. Three spikelets were selected from central part of spike and developing seeds were excised from the primary florets of wheat spikelet stored in 70 per cent ethanol for grain preservation. The seeds are placed on to the flatbed scanner in ascending order from 3 days to 30 days and grain dimensions were obtained in a standarised way.

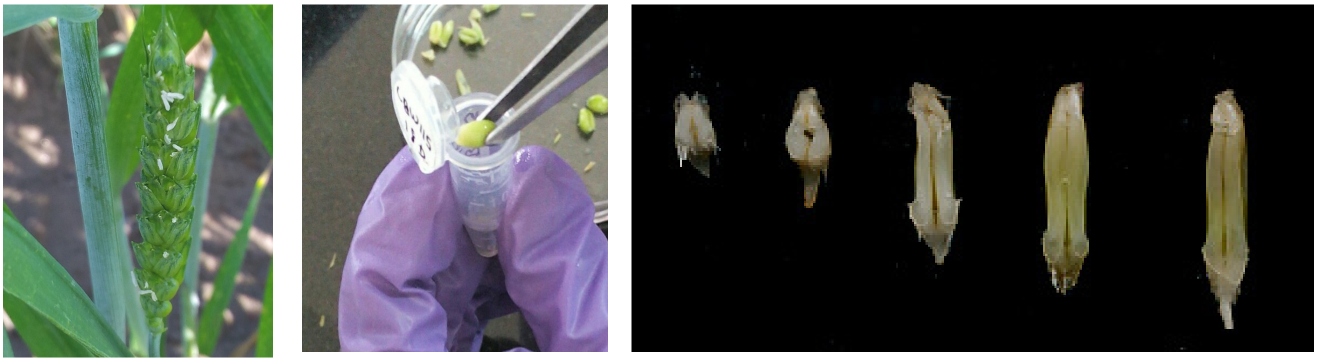


Fig. 2: (A) Wheat spikelet (B) Storage in 70% ethanol (C) Seed measurement at various developmental stages

Results and Discussion

Grain size measurement in wild species

The wild wheat progenitor species including *T. diccocoides, T.boeticum, T. monococcum* and *Ae. speltoides* were screened for grain length variation in three consecutive years. The twenty mature seeds from each accession were randomly selected to calculate grain dimensions i.e. grain length and width with canon 5600 scanner. The grain lengthand width (mm) of short and long grain accessions of *T diccocoides, T. boeticum, T. monococcum* and *Ae. Speltoides* were shown in Table-1. Overall, during three consecutive years, GL varies from 5.05-11.02, 5.95-9.71, 6.13-8.9 and 4.57-8.9 and GW varies from 1.67-3.6, 0.49-3.15, 0.4-3.4 and 1.28-2.78 in all the selected wild accessions *T diccocoides, T. boeticum, T. monococcum* and *Ae. speltoides* respectively. The grain length variation the wild wheat accessions provide the insight into the evolutionary features of grain size in wheat.

This illustrates the wide variation in grain length trait and the wide variation among the grain morphological characters may be utilized for the selection of the parents for the plant breeding and production of new improved variety (Sinha and Mishra 2015).

Table 1: Grain length and grain width of wild wheat accessions

Time		1st year		2nd year		3rd year	
Wild wheat species	No. of accessions	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)
T.diccocoides	144	6.5-10.4	1.7-3.6	5.53-10.48	1.67-3.53	5.05-11.02	1.67-3.53
T. boeticum	165	6.6-9.65	1.3-3.07	6.58-9.71	1.4-3.04	5.95-9.3	0.49-3.15
T. monococcum	58	6.54-8.17	2.1-3.3	6.13-7.96	1.6-3.11	6.3-8.9	0.4-3.4
Ae. speltoides	174	4.84-7.19	1.42-2.78	4.57-7.52	1.28-2.2	5.53-8.9	1.5-2.0

Lu *et al*. 2013 studied grain size variation in 127 rice varieties and found the GL ranged from 6.32 to 12.95 mm, GW ranged from 2.29 to 4.43 mm. A combination of different factors including GL, GW, LWR influence the grain size of rice which significantly correlate with grain weight (Tan *et al*., 2000). Therefore, wheat grain size and yield are closely related to one another and can be explained by a combination of grain size-related traits.

Grain size measurement at different developmental stages

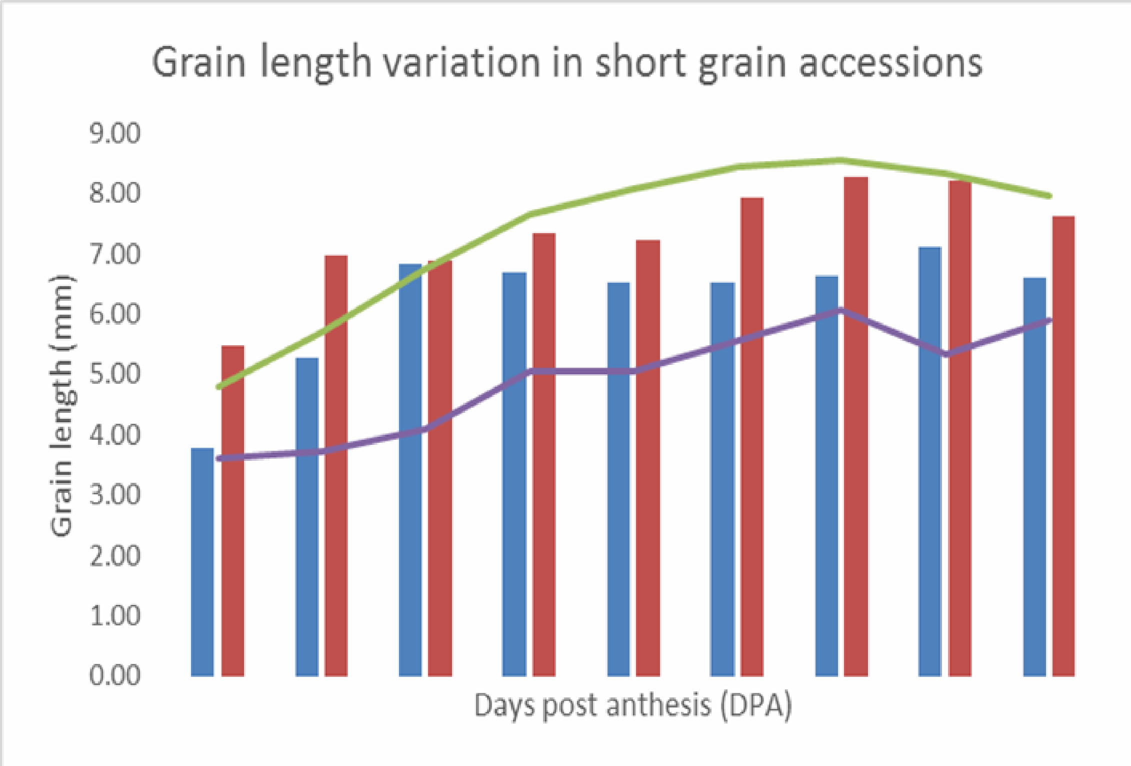
The screening of the variation in grain length during developmental stages after anthesis was done in 2017.The grain size mainly depends upon grain length, grain width and grain length to width ratio. In long grain accessions maximum gain in grain length is between 12 DPA to 15 DPA while in short grains accessions maximum gain of length is between 9 DPA to 12 DPA in all accessions. Interestingly, it has been observed that maximum increment in the length of long grains accessions observed in *T. boeticum, Ae. Speltoides* and *T. Diccocoides* during 12 DPA with GL (mm) increment of 0.83, 1.65 and 0.96 respectively. Contrastingly, The long grain accessions of *T. monococcum* shows the maximum GL enhancement of 1.5 mm at 15 DPA. Among short grain accessions of *T. monococcum, T. diccocoides,* shows the maximum GL increase at 12DPA while *T. boeticum and Ae. speltoides* shows the GL increment of 1.50 mm and 0.98 mm at 9DPA and 15 DPA respectively. Overall, the higher GL and GW increment during developmental stages was noticed in all the wild accessions from 9 DPA to 15DPA and 6 DPA to 15 DPA respectively. There is maximum increase in grain length upto12-18 DPA and thereafter decrease while changing to mature dry grain. The higher grain length and grain width was observed in *T. diccocoides* followed by *T. boeticum, T. monococcum* and *Ae. Speltoides* with *GL* of 10.02, 9.76, 7.90, 6.80. Among short grain accessions, the increasing order of grain length trend is same as long grain accessions.

Fig. 3: The variation of grain length and width (1) *T. monococcum* (2). *T. dicoccoides*at different developmental stages after anthesis; 3 DPA, 6DPA, 9DPA, 12DPA, 15DPA, 21DPA, 24DPA, 27DPA and 30DPA

Table 2 : Grain length of long and short grain accessions during developmental stages:

	Grain length (mm)							
	Long grain accessions				Short grain accessions			
	T. monococcu	*T.boeticum*	*T.dicoccoide*	*A.speltoides*	*T. monocc*	*T.boeticur*	*T.dicoccoi*	*A.speltoid*
6DPA	3.85	6.71	5.95	4.34	3.78	5.49	4.80	3.61
9DPA	5.26	7.46	6.60	5.54	5.28	6.98	5.69	3.72
12DPA	5.42	8.29	8.25	6.50	6.84	6.89	6.76	4.09
15DPA	6.58	8.75	9.03	5.66	6.69	7.34	7.64	5.07
18DPA	7.02	9.04	9.35	5.47	6.51	7.22	8.08	5.04
21DPA	7.60	8.86	10.22	5.36	6.52	7.94	8.46	5.56
24DPA	7.75	8.99	10.63	5.84	6.64	8.27	8.56	6.06
27DPA	7.60	9.56	10.11	6.70	7.12	8.23	8.33	5.34
30DPA	7.90	9.76	10.02	6.80	6.60	7.62	7.95	5.90

Fig. 4: Grain length variation in short grain accessions during developmental stages of all wheat wild accessions



Likewise, Grain width of all these four progenitor species were also calculated. It has been observed that among long grain accessions, maximum width is observed in *T. diccocoides* followed by *T. boeticum, T. monococcum and Ae. speltoides* with GW of 3.04, 2.69, 2.54 and 1.62. The range of increment of GW among long grain accessions of *T. diccocoides, T. boeticum, T. monococcum and Ae. speltoides*is 0.03-0.70, 0.02-0.26.0.04-0.39,0.11-0.25. The GW range of short grain accessions of all the four species namely *T. diccocoides, T. boeticum, T. monococcum and Ae. Speltoides* varies from 0.04-0.53, 0.01-0.18, 0.07-0.59 and 0.02-0.19 was lower than the long grain accessions. Among long and short grain accessions of selected four species, GW tends to increase in between 15 DPA and 24 DPA respectively. The long grain accessions of *T. monococcum* and *T. diccocoides* tends to increase from 24 DPA while *T. boeticum and Ae. Speltoides GW* tends to increase from 18 DPA and 15 DPA respectively. On the other hand a large variation of GW was observed in short grain accessions of, *T. monococcum T. boeticum, T. diccocoides and Ae. Speltoides tends to increase from 12 DPA, 24 DPA, 15 DPA and 18 DPA respectively.*

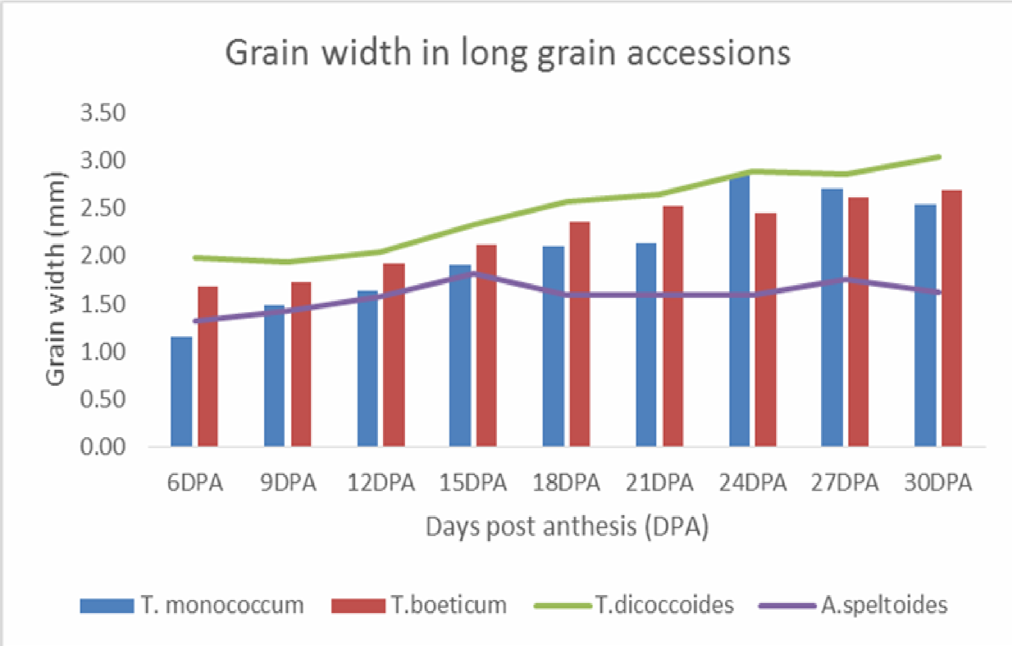


Fig. 5 : Grain width variation in long grain accessions during developmental stages of all wheat wild accessions

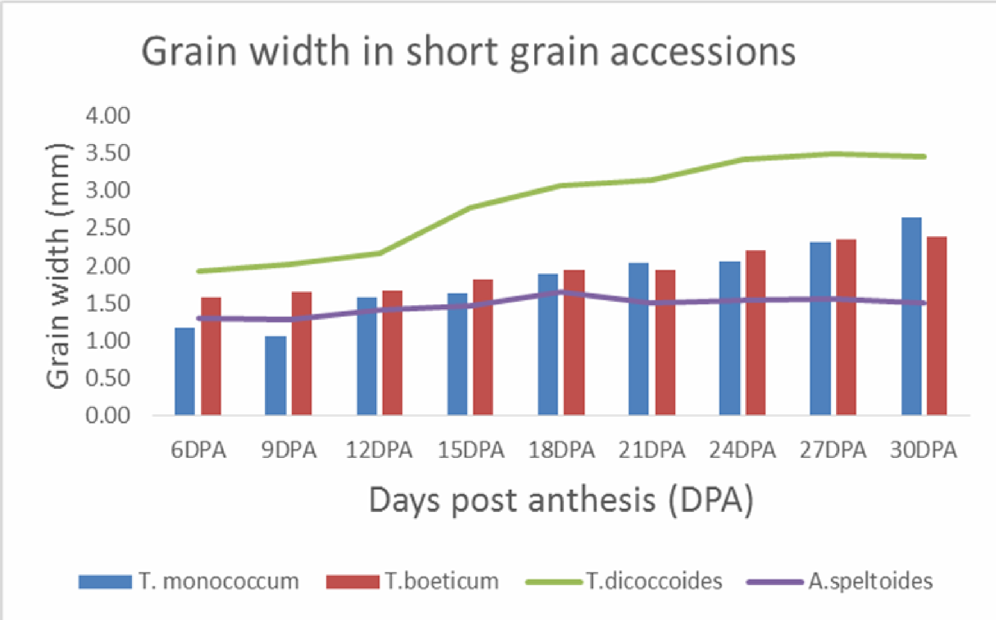
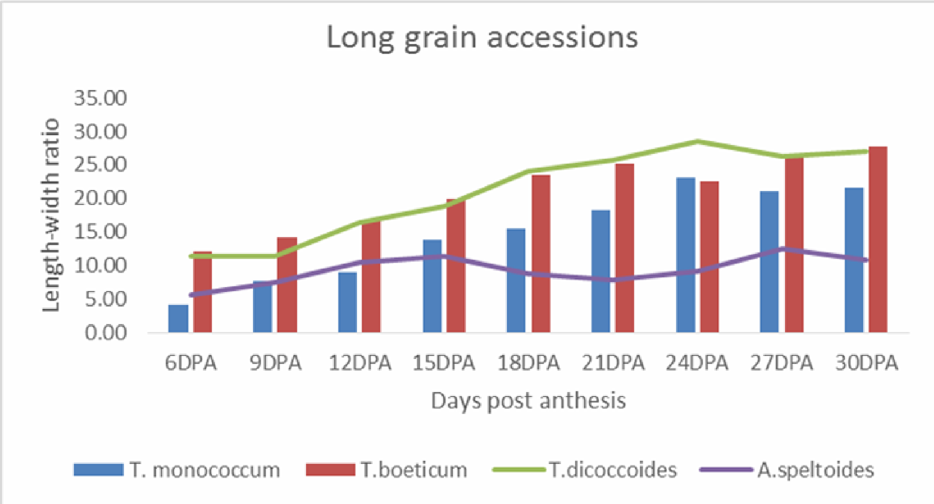


Fig. 6 : Grain width variation in short grain accessions during developmental stages of all wheat wild accessions

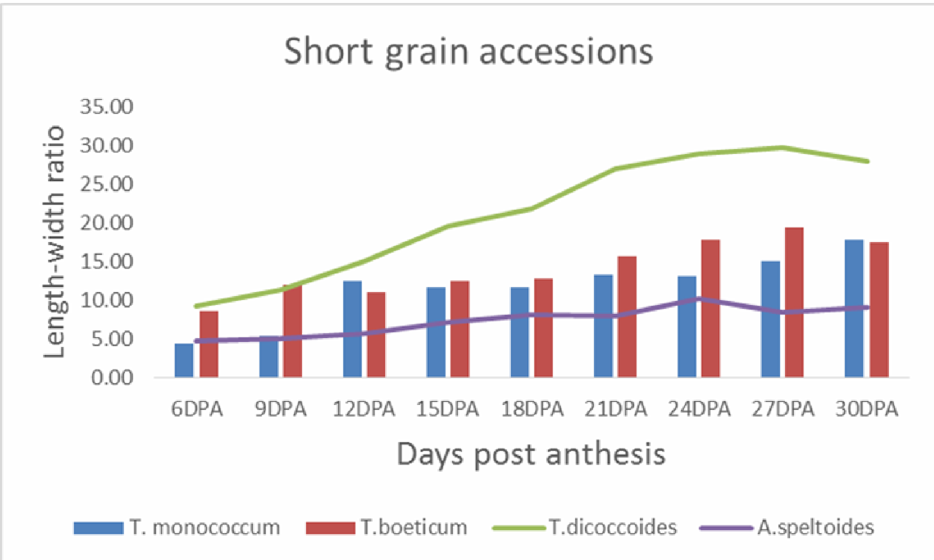
The length –width ratio gives a clear vision about the trend of increasing grain size among long and short wild wheat accessions. All the wild wheat species followed the same pattern of GL and GW increase from 6DPA to maturity. The range of long and short grains of *T. monococcum* range from 4.07-21.67 and 4.43-17.81 respectively. Likewise, the L x W ratio of long grains *T. boeticum*, T. dicoccoides, *Ae. speltoides* falls within the range of 12.16-27.86 , 11.45-27.0, 5.69-10.9 while short grains L xW ratio falls within range of 8.63-17.55, 9.23-28.02, 4.78-9.07 respectively during developmental stages. The highest L x W ratio observed in *T. dicoccoides* followed by *T. boeticum, T. monococcum* and *Ae speltoides*. Graham Acquah *et al*. 2018observed the L X w ratio of 50.8 in 45 rice varieties grown under irrigated lowland conditions. The correlation of length and length – width ratio of long grain accessions of *T. monococcum, T. boeticum, T. dicoccoides, and Ae. Speltoides* is 0.97, 0.95, 0.97, 0.86 while of short grain accessions correlation is 0.85, 0.93, 0.91, 0.48 respectively. Graham Acquah (2018) also observed the correlation of grain length and grain width ratio in the range of 0.50-0.70 in rice varieties.

Table 3 : Grain length width ratio of long and short grain accessions during developmental stages

	Length: Width Ratio							
	Long grain accessions				Short grain accessions			
	T. monococc	T.boeticum	T.dicoccoide	A.speltoides	T. monococc	T.boeticum	T.dicoccoide	A.speltoides
6DPA	4.07	12.16	11.45	5.69	4.43	8.63	9.23	4.78
9DPA	7.74	14.25	11.40	7.52	5.44	11.98	11.39	5.13
12DPA	8.90	16.56	16.41	10.43	12.54	10.98	15.03	5.81
15DPA	13.74	19.87	18.82	11.29	11.67	12.50	19.67	7.24
18DPA	15.40	23.49	24.09	8.73	11.64	12.90	21.89	8.14
21DPA	18.20	25.13	25.82	7.87	13.32	15.78	27.00	7.99
24DPA	23.18	22.52	28.59	9.08	13.12	17.89	28.89	10.20
27DPA	21.15	26.31	26.22	12.54	15.05	19.50	29.75	8.50
30DPA	21.67	27.86	27.00	10.90	17.81	17.55	28.02	9.07



A



B

Fig. 7 : Grain length and width ratio of (A) long and (B) short grain accessions during developmental stages

Conclusions

Agro-morphic characters of grains are an important pre-criterion for the selection of particular variety. Market value and the acceptability of crop solely depends upon the grain characteristics. It was observed that the grain length and grain width have compensatory effect as increase in grain length is compensated by decrease in grain width and vice versa (Giuraand Saulescu, 1996). The strongest effects on grain size, have been widely utilized in crop production (Lu *et al*. 2013). Cloning of genes from long and short grain length accessions is in progress to mine different alleles of grain length. It is also one of the main components that distinguish the domesticated species of diploid and tetraploid wheat from its wild ancestors. Due to the difficulty in quantifying, the phenotypic and

The genetic variation of wheat grain morphology is understudied. The grain weight is an important area of wheat genetic and breeding studies. The genetic diversity of wheat (*Triticum aestivum* L.) genotypes is useful for production of more efficient crops adapted to diverse conditions. Grain length is an important parameter responsible for increase in grain size and ultimately contributing towards increase in grain weight

Author's contribution

Preparation of manuscript, analysis of data and interpretation (Maninder Kaur), Designing of the research work (Satinder Kaur),

Declaration of Interest

The authors declare no conflict of interest.

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