NOVEL GENETIC DIVERSITY OF THE ALIEN D-GENOME SYNTHETIC HEXAPLOID WHEAT (2*n*=6*x*=42, AABBDD) GERMPLASM FOR VARIOUS PHENOLOGY TRAITS

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Abstract

The current study evaluates genetic penetrance and expressivity of an alien genome introgression in a set of 117 primary synthetic hexaploid wheat (SHW) accessions. These SHW's have originated from durum wheat /accessions with three sets of durum wheat cultivars ALTAR 84, D67.2 and CERCETA as the female and diverse *Ae. tauschii* accessions as the pollen parents. Diversity of the 12 important traits (Growth habit, pigmentation, chlorophyll content, leaf area index, crop digital ground cover, awn size, awn length, and several seed digital imaging parameters)revealed significant variation for the respective traits, leading to the conclusion that *Ae. tauschii* accessions have tremendous diversity than the durum controls. Further, the value deviations within each attribute had a range of being lower or higher than their durum wheat female parents and these observations allowed us to use the variations as selective sieves and narrow down the desirable SHW's that would be advantageous to exploit for wheat breeding and cultivar improvement programs. Selections were made and a group of 41SHWaccessions were identified that will after an intermediate DNA diversity evaluation form a crisper final set for user friendly utilization. The range of selections shows multiple trait advantages for exploitation in both irrigated and rain-fed conditions. This pivotal study sets the foundation to better define the D genome SHW's for efficient utilization in future research investigations. Our results have implications in widening the genetic base of hexaploid bread wheat and may facilitate the development of agronomically desirable wheat cultivars

Key words: Synthetic hexaploid wheats, D-genome, Genetic penetrance and expressivity, Phenology, Diversity.

Introduction

Interspecific hybrids between *Aegilops tauschii* Coss. (2n=2x=14; DD) accessions and *Triticum turgidum* L. (2n=4x=28; AABB) cultivars was an effort launched in 1987 at CIMMYT, Mexico (Mujeeb-Kazi, 2003a,b). As a consequence, hundreds of synthetic hexaploid wheat (SHW) lines have been developed over the last few decades (Ogbonnaya *et al.,* 2013), offering extensive diversity for addressing numerous biotic and abiotic stresses as well as wheat production constraints. Besides useful diversity and breeding potential, practical outputs of finished varietal products of the SHW in the form of the variety Chaunmai-42 have emerged, having a huge yield advantage over the existing cultivars grown in Sichuan, China. SHW's also confer resistance / tolerance to several biotic stresses (karnal bunt, powdery mildew, leaf rust, yellow rust, stem rust, spot blotch, leaf spot, barley yellow dwarf), abiotic stresses like drought, salinity, nutrient deficiencies, extreme temperatures, logging etc.and therefore, provide valuable resources for hexaploid bread wheat improvement (Mujeeb-Kazi *et al.,* 2008; Ogbonnaya *et al.,* 2013; Das *et al.,* 2015). This unexplored genetic variability for bread wheat makes SHW's ideal to address future food security issues of mankind under changing global climatic conditions (Mujeeb-Kazi, 2003a; Mujeeb-Kazi *et al.,* 2004; Trethowan & Mujeeb-Kazi, 2008; Mujeeb-Kazi *et al.,* 2008).

Ae. tauschii has been widely used in bread wheat improvement and cultivar development programs. To date, a number of useful genes in the form of chromosomal translocations have been introgressed from the D-genome into wheat backgrounds. Normal pairing (homologous with 7 bivalents) is the norm and thus maximum recombination takes place between the D-genome chromosomes of wheat and *Ae. tauschii* (Kihara,1944; McFadden & Sears, 1946; Miranda *et al.,* 2007). Two approaches have been used to incorporate alleles of the D-genome of *Ae. tauschii* into bread wheat either by the synthetic hexaploid route (Mujeeb-Kazi & Hettell, 1995) or via direct crossing between this diploid progenitor and suitable hexaploid wheats that require specific trait improvement alleles (Gill & Raupp, 1987). The contribution of the D-genome over the elite A and B genomes in SHW derived breeding lines acts as a conduit in the development of improved wheat cultivars (Ogbonnaya *et al.,* 2013; Khan *et al.,* 2016).

There are 1450 primary SHW's available that harbor a phenomenal genomic wealth. Here we have focused to comprehend the role of diverse *Ae. tauschii* accessions in some SHW's derived from same durum/different *Ae. tauschii* accessions. Further, we have thoroughly investigated to unravel as to how different *Ae. tauschii* accessions have modified the genetic and phenotypic attributes in uniform AABB durum genomes. This is an attempt to integrate well targeted SHW's in manageable numbers for wheat breeding, so that breeders may exploit these phenotypically and genotypically categorized genetic stocks swiftly to meet the time bound needs as they are reflected in the food security vision for 2050 as well as the 2030 projections of global "zero hunger" and sustainability development goals. The preliminary data presented here provide a baseline for future studies and will be pursued in depth on a much larger scale in future.

Material and Methods

Plant material: A total of 124 genotypes (Table 1) comprising of 117 D-genome SHWs developed from the crosses of three durum wheats (ALTAR 84, CERCETA (CETA), D67.2) and different *Ae. tauschii* accessions (Mujeeb-Kazi, 2003a). Three original durum parents and four local hexaploid wheats, Pasban-90, S-24, Shorawaki and PBW-343 were used as the control tolerant and susceptible checks. Phenological traits were recorded (summarized in Table 2) on different growth stages (Zadoks *et al.*, 1974).

Methodology

i. **Plant habit:** Plant habit was recorded at GS-31 as erect (1), semi-erect (2) and or prostrate (3).

ii. **Anthocyanin Pigment:** Anthocyanins play a protective role in plants under different stress conditions. Increased anthocyanin content is indicative of drought, cold, UV-B irradiation, toxic metals in soils or pathogen related stresses. It was recorded as either "present" or "absent" at Zadoks GS-26 stage for all the genotypes.

iii. **Leaf area index (LAI):** Leaf area index (LAI) is the area of green leaf lamina surface per unit of ground area. Measurement of leaf area index is related to light interception, photosynthetic performance, elucidates surfaces for transpiration and estimates crop biomass (Parry *et al.,* 2010). The photosynthetic capacity of the crop is related to the total leaf area and maintenance of the leaf area is essential for production of the carbohydrates used for grain filling. Leaf areas of 5 random leaves from each genotype were measured and LAI was calculated (Muller, 1991) at Zadoks GS-41 as follows:

Leaf area Index (cm2) = Leaf length (cm) × leaf width (cm) × 0.74

iv. **Chlorophyll concentration index (CCI):** Chlorophyll concentration index (CCI) was calculated with the SPAD-502 (Markwell et al. 1995) following manufacturer instructions (Minolta Camera Co., Osaka, Japan). SPAD readings have direct correlations with extracted chlorophyll from plants (Yadava, 1986). Five separate measurements were made on one leaf of all genotypes and averaged.

Higher the values of CCI directly related to the high photosynthetic rate.

v. **Crop digital ground cover:** Crop ground cover, or the percentage of soil surface enclosed by plant foliage, is an important observation of crop establishment and early vigor. Genotypes with higher ground coverage are able to better capture radiation thus reduces soil evaporation and thereby elevates water use efficiency. Early ground cover is also a valuable stress adaptive trait where, for example, it can reduce evaporative loss of soil moisture (Mullan & Reynolds, 2010). This can be measured using digital images that allow rapid and cost effective screening of large populations.

Photographs of ground cover for each genotype were taken at GS-32 with a digital camera and processed by using 'Adobe Photoshop CS3 Extended' software (Fig. 1).

The percentage ground cover (% GC) was calculated by the following formula:

$$\% \ GC = (Mean \ grey \ value \ / \ 255) \times 100$$

vi. Digital imaging (DI) of seed: Seed shape and size are among the most important agronomic traits due to their higher effect on yield and grain quality. DI technique was used to generate high-throughput photometric traits explaining various magnitudes of grain size and shape. DI has the ability to display the dimensions of grain morphology that are contributing to grain weight and size (Fig. 2). Twenty-five well developed seeds of each entry were photographed. Seeds were placed horizontally with equal distances on a black background to provide color contrast (Fig. 3). Area size (mm^2, perimeter length (mm), Length (mm), width (mm), Length to width ratio, Circularity, and distance between IS and CG were processed and computed with Smart Grain software version 1.1.

Smart Grain can analyse seed images captured on a scanner and in addition to seed area, it can also be used for measurement of perimeter length (PL), seed width (W), seed length (L), length to width ratio (LWR), circularity (CS), intersection of length and width (IS), centre of gravity (CG), and distance (DS) between IS and CG (Tanabata *et al.*, 2012).

Fig. 1. Crop ground cover images of the prostrate growth habit.

Table 1. Details of the germplasm used in the study.

S. No.	SH Entry	Pedigree
1.	433	ALTAR 84/*Ae. tauschii* (1012)*
2.	908	ALTAR 84/*Ae. tauschii* (1068)
3.	1010	ALTAR 84/ *Ae. tauschii* (1094)
4.	3	ALTAR 84/ *Ae. tauschii* (178)
5.	5	ALTAR 84/ *Ae. tauschii* (188)
6.	8	ALTAR 84/ *Ae. tauschii* (191)
7.	12	ALTAR 84/ *Ae. tauschii* (192)
8.	17	ALTAR 84/ *Ae. tauschii* (193)
9.	20	ALTAR 84/ *Ae. tauschii* (198)
10.	23	ALTAR 84/ *Ae. tauschii* (205)
11.	33	ALTAR 84/ *Ae. tauschii* (211)
12.	48	ALTAR 84/ *Ae. tauschii* (219)
13.	49	ALTAR 84/ *Ae. tauschii* (220)
14.	52	ALTAR 84/ *Ae. tauschii* (221)
15.	57	ALTAR 84/ *Ae. tauschii* (223)
16.	64	ALTAR 84/ *Ae. tauschii* (224)
17.	918	ALTAR 84/ *Ae. tauschii* (237)
18.	464	ALTAR 84/ *Ae. tauschii* (244)
19.	80	ALTAR 84/ *Ae. tauschii* (291)
20.	551	ALTAR 84/ *Ae. tauschii Ae. tauschii* (319)
21.	96	ALTAR 84/ *Ae. tauschii Ae. tauschii* (328)
22.	97	ALTAR 84/ *Ae. tauschii Ae. tauschii* (328)
23.	318	ALTAR 84/ *Ae. tauschii* (333)
24	923	ALTAR 84/ *Ae. tauschii* (380)
25.	419	ALTAR 84/ *Ae.tauschii* (502)
26.	572	ALTAR 84/ *Ae. tauschii* (539)
27.	993	ALTAR 84/ *Ae. tauschii* (793)
28.	187	ALTAR 84/ *Ae. tauschii* (JBANGOR)
29.	186	ALTAR 84/ *Ae. tauschii* (Y86-87 S401)
30.	607	D67.2/P66.270// *Ae. tauschii* (1009)
31.	608	D67.2/P66.270// *Ae. tauschii* (1015)
32.	610	D67.2/P66.270// *Ae. tauschii* (1017)
33.	906	D67.2/P66.270// *Ae. tauschii* (1032)
34.	785	D67.2/P66.270// *Ae. tauschii* (1054)
35.	771	D67.2/P66.270// *Ae. tauschii* (1057)
36.	892	D67.2/P66.270// *Ae. tauschii* (1068)
37.	894	D67.2/P66.270// *Ae. tauschii* (1074)
38.	896	D67.2/P66.270// *Ae. tauschii* (1085)
39.	899	D67.2/P66.270// *Ae. tauschii* (1090)
40.	909	D67.2/P66.270//*Ae. tauschii* (1093)
41.	584	D67.2/P66.270// *Ae. tauschii* (185)
42.	34	D67.2/P66.270// *Ae. tauschii* (211)
43.	37	D67.2/P66.270// *Ae. tauschii* (213)
44.	44	D67.2/P66.270// *Ae. tauschii* (217)
45.	47	D67.2/P66.270// *Ae. tauschii* (218)
46.	50	D67.2/P66.270// *Ae. tauschii* (220)
47.	53	D67.2/P66.270// *Ae. tauschii* (221)
48.	614	D67.2/P66.270// *Ae. tauschii* (1039)
49.	59	D67.2/P66.270// *Ae. tauschii* (223)
50.	590	D67.2/P66.270// *Ae. tauschii* (239)
51.	223	D67.2/P66.270// *Ae. tauschii* (257)
52.	593	D67.2/P66.270// *Ae. tauschii* (260)
53.	781	D67.2/P66.270// *Ae. tauschii* (288)
54.	594	D67.2/P66.270// *Ae. tauschii* (301)
55.	224	D67.2/P66.270// *Ae. tauschii* (308)
56.	595	D67.2/P66.270// *Ae. tauschii* (320)
57.	596	D67.2/P66.270// *Ae. tauschii* (368)
58.	782	D67.2/P66.270// *Ae. tauschii* (400)
59.	599	D67.2/P66.270// *Ae. tauschii Ae. tauschii* (416)
60.	603	D67.2/P66.270// *Ae. tauschii Ae. tauschii* (448)
61.	605	D67.2/P66.270// *Ae. tauschii Ae. tauschii* (497)
62.	847	D67.2/P66.270// *Ae. tauschii* (629)
63.	853	D67.2/P66.270// *Ae. tauschii* (633)
64.	854	D67.2/P66.270// *Ae. tauschii* (634)
65.	855	D67.2/P66.270// *Ae. tauschii* (635)
66.	260	D67.2/P66.270// *Ae. tauschii* (646)
67.	823	D67.2/P66.270// *Ae. tauschii* (657)
68.	861	D67.2/P66.270// *Ae. tauschii* (658)
69.	261	D67.2/P66.270// *Ae. tauschii* (659)
70.	865	D67.2/P66.270// *Ae. tauschii* (665)
71.	866	D67.2/P66.270// *Ae. tauschii* (666)
72.	867	D67.2/P66.270// *Ae. tauschii* (668)
73.	875	D67.2/P66.270// *Ae. tauschii* (709)
74.	803	D67.2/P66.270// *Ae. tauschii* (731)
75.	804	D67.2/P66.270// *Ae. tauschii* (741)
76.	884	D67.2/P66.270// *Ae. tauschii* (788)
77.	885	D67.2/P66.270// *Ae. tauschii* (791)
78.	887	D67.2/P66.270// *Ae. tauschii* (796)
79.	888	D67.2/P66.270// *Ae. tauschii* (797)
80.	889	D67.2/P66.270// *Ae. tauschii* (828)
81.	895	CETA/ *Ae. tauschii* (1085)
82.	440	CETA/ *Ae. tauschii* (1024)
83.	962	CETA/ *Ae. tauschii* (683)
84.	927	CETA/ *Ae. tauschii* (418)
85.	930	CETA/ *Ae. tauschii* (442)
86.	825	CETA/ *Ae. tauschii* (615)
87.	955	CETA/ *Ae. tauschii* (680)
88.	903	CETA/ *Ae. tauschii* (373)
89.	578	CETA/ *Ae. tauschii* (1055)
90.	449	CETA/ *Ae. tauschii* (166)
91.	448	CETA/ *Ae. tauschii* (1042)
92.	516	CETA/ *Ae. tauschii* (1043)
93.	517	CETA/ *Ae. tauschii* (1046)
94.	450	CETA/ *Ae. tauschii* (172)
95.	446	CETA/ *Ae. tauschii* (1030)
96.	477	CETA/ *Ae. tauschii* (371)
97.	454	CETA/ *Ae. tauschii* (200)
98.	483	CETA/ *Ae. tauschii* (445)
99.	513	CETA/ *Ae. tauschii* (1036)
100.	511	CETA/ *Ae. tauschii* (1031)
101.	515	CETA/ *Ae. tauschii* (1038)
102.	452	CETA/ *Ae. tauschii* (184)
103.	919	CETA/ *Ae. tauschii* (310)
104.	921	CETA/ *Ae. tauschii* (345)
105.	897	CETA/ *Ae. tauschii* (1090)
106.	600	CETA/ *Ae. tauschii* (416)
107.	429	CETA/ *Ae. tauschii* (540)
108.	460	CETA/ *Ae. tauschii* (235)
109.	1008	CETA/ *Ae. tauschii* (1093)
110.	786	CETA/ *Ae. tauschii* (356)
111.	573	CETA/ *Ae. tauschii* (541)
112.	640	CETA/ *Ae. tauschii* (299)
113.	655	CETA/ *Ae. tauschii* (408)
114.	485	CETA/ *Ae. tauschii* (450)
115.	622	CETA/ *Ae. tauschii* (199)
116.	479	CETA/ *Ae. tauschii* (391)
117.	673	CETA/ *Ae. tauschii* (519)
118.	-	Pasban-90
119.	-	Shorawaki
120.	-	PBW-343
121.	-	S-24
122.	-	ALTAR
123.	-	CETA
124.	-	D67.2

Table 2. Phenological outcomes of three durum wheats and their derived SHW.

Entry No.	Pedigrees	Values	CCI	LAI	GC%	AS	PL	L	W	LWR	CIR	DS
1-29	Altar/*Ae. tauschii+*	Low	33.97	17.02	17.84	17.56	22.6	9.12	3.03	2.29	0.4	0.64
		High	54.37	59.02	58.69	37.86	29.00	11.65	4.92	3.26	0.6	1.51
122	Control: Altar		48.63	32.56	32.92	35.52	28.42	10.28	5.18	1.98	0.6	0.86
30-80	D67.2/*Ae. tauschii+*	Low	31.93	17.61	15.08	24.49	25.24	9.22	3.64	1.91	0.4	0.72
		High	60.27	64.75	80.06	47.6	34.98	12.33	5.52	2.68	0.6	1.72
123	Control: D67.2		47.96	35.96	16.72	31.72	27.04	9.66	4.59	2.17	0.5	0.94
81-117	Cerceta/*Ae. tauschii+*	Low	31.30	17.76	34.46	30.32	27.28	9.78	4.29	1.85	0.50	0.77
		High	58.67	53.65	88.05	46.57	33.58	12.09	5.48	2.63	0.7	1.35
124	Control: Cerceta		36.97	24.93	44.14	31.35	25.42	9.51	4.63	2.08	0.6	0.90

Where, + = *Ae. tauschii* accession numbers in CIMMYT Wide Cross alien species working collection (Table 1), CCI= chlorophyll content index, LAI= leaf area index, GC%= growth cover, As= Area size, PL= perimeter length, L= length, W= width, LWR= leaf width ratio, CIR= Circularity, DS= Distance between IS and CG

Results and Discussion

Biotic and abiotic stresses will remain the major concerns of future food security and to address it properly, wheat breeders have to find appropriate germplasm with desirable traits. Therefore, utilization of the novel diversity from within the alien sources as well as SHW is of paramount significance (Das *et al.,* 2015; Khan *et al.,* 2016). Hexaploid bread wheat has enormous genomic plasticity, and several studies have shown that the effects of alien chromatin are less pronounced or buffered in the polyploid wheat compared to the diploids, being more sensitive to genetic imbalance (Dubcovsky & Dvorak, 2007; Schwarzacher *et al.,* 2011; Ali *et al.,* 2016). Further, both tetraploid and hexaploid wheat may completely mask the expression of alien parents involved in the hybrids formation (Kruse, 1973; Mujeeb *et al.,* 1978; Islam *et al.,* 1981). Although such hybrids possess the bi- or multi parental chromosomal complement (2n=4x=28, ABDH or 2n=3x=21, ABH) suggesting that there was full chromosomal penetration but no genetic expressivity was apparent. Thus the practical value of all wheat/barley hybrids could not be realized until the importance of co-dominant hybrid phenotype was understood as the initial observation for validating alien genetic expressivity (Mujeeb-Kazi *et al.,* 1987, 1989).

All these observations were related to intergeneric hybrids, however with the appearance of interspecifics like(SHW's) the alien phenotype was evident in all F1 and C-0 cross combination progenies and they all carried the tough glumes and the spring growth habit that controls the genetics of the spring/winter trait. However, Ma *et al.* (1995) reported that despite the evident phenotypic expression of co-dominance, often the alien donor trait of agronomic interest is suppressed as seen in some SHW derivatives where the genomic complement of the alien D genome donors existed but its high rust resistance response was masked by the recipient A and B genomes of the durum parent.

Various sub-sets are available within the ca. 1450 SHW's where the main categories include; a) Same durum female parent and varied *Ae. tauschii* accessions, b) same *Ae. tauschii* accession and varied durum female parents and c) durum parent/*Ae. tauschii* accessions and their reciprocal cross combinations to study the cytoplasmic effects. Here a sub-set of these primary SHW was assessed (Table 1) with an aim to validate the alien D genome accessional diversity across various traits. Within each similar durum/*Ae. tauschii* accessional group phenological variation was evident and it was depicted that the D genome was conferring the variable expression in a uniform A and B genome complement differentially or adding to the expressivity on its own (Figs. 4, 5). Although, we have selected a few major traits these expressivity findings shall be further investigated more intensively. The data gathered from the three SHW groups (Table 2) reflects evidence that the alien genetic penetrance of the *Ae. tauschii* genome complement depicts distinct expressivity as elucidated by majority of the traits compared to their respective durum control cultivars. In addition, using the varied expressivity values of traits we have been able to select SH parental stocks for applied agricultural usage efficiently. Although, selections could be made for individual traits but it is more advantageous to identify SHW's with multiple positive values across various traits as selections of a wider positivity would add more efficiency to breed these D genome primary SHW stocks with other elite germplasm and well adapted wheat cultivars.

All SHW's are facultative in growth patterns, and range between 105 to 130 cm in height, with or without anthocyanin pigmentation and also pubescence. The SHW's have tough lemma and palea that shows expressivity of the Q gene with minor modifier effects. Of these important genetic variability traits, the initial growth habit of SHW is characteristic that permits SHW's selection sieve for breeding targeted for drought or irrigated cultivation. Among the SHW's with prostrate growth habit e.g. with cv. ALTAR 84 the entries 2, 8, 9, 16, 17, 20, 29 with cv. D67.2 the entries 30, 33, 37, 45, 48, 61, 63, 67, 68, 69 and with CERCETA the entries 86,105 will be preferred for hybridization with wheat targeted for drought prone situations and for those countries where wheat is cultivated under irrigated and rain-fed conditions. All the three durum parents possessed the erect habit(1), indicative that the trait is an expression of the accessional diversity bestowed by the *Ae. tauschii* accession (Fig. 1). A similar variable trend was observed for the presence or absence of the anthocyanin pigmentation that none of the durum parents possessed, indicating its expression is regulated by the alien diploid accessions.
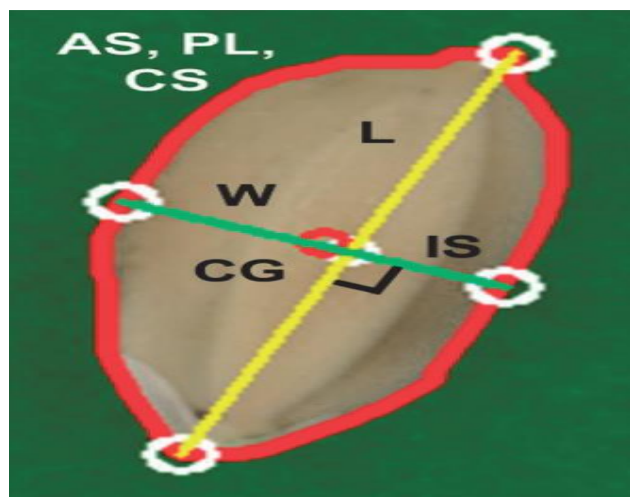
Fig. 2. Grain shape parameters: AS, within red line; PL, red line; CS, from red line; L, yellow line; W, green line; IS, white circle; CG, red circle.
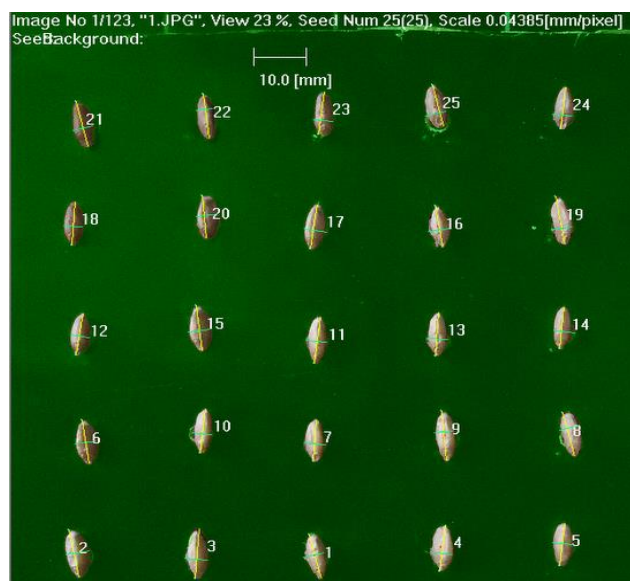

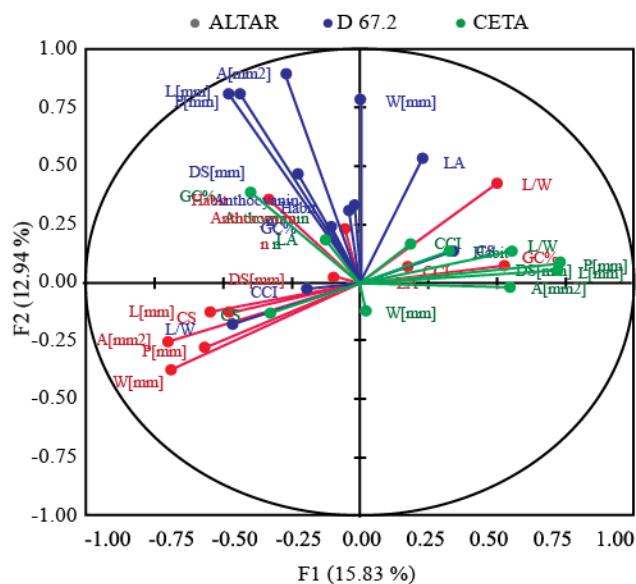Fig. 3. Horizontal and vertical seed image analysis by smart grain.

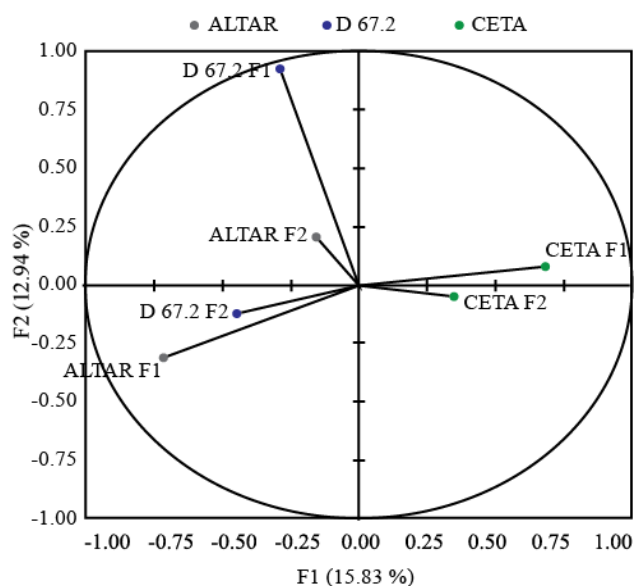Fig. 4. Multiple factor analysis of different variables.

Fig. 5. Partial axis analysis of durum parents.

For CCI the durum cultivars had values of 48.63 (ALTAR 84), 47.96 (D67.2) and 36.97 for CERCETA. The respective SHW's in each category possessed values that varied around the control cultivar values and as the higher readings of CCI in SHW's would represent a plus factor in selection several SHW fitted the criteria of having a better CCI than the durum controls. The SHW's with ALTAR (48.63) had entries that fell in the range between 33.97 up to 54.37, with D67.2 (47.96) the range was 31.93 up to 60.27 and for CERCETA (36.97) between 31, 30 and 58.67 (Table 2). Supporting this CCI variation were values of the leaf area index (LAI) that were 32.56 for ALTAR and its SHW's between 37.02 and 59.02; D67.2 (35.96) with its SHW's between 17.61 and 64.75; and CERCETA (24.93) with its SHW's ranging between 17.76 and 53.65. These ranges provided ample evidences that diverse accession of *Ae. tauschii* have expressed differentially in similar durum AB genomic

backgrounds and are an effective means to make narrow targeted selections around multiple aspects to make prudent SHW sets for breeding wheats.

Further data interpretation across other traits indicate that different *Ae. tauschii* accessions show a higher penetrance/ expressivity effect in D-genome synthetics. This was well pronounced for ground cover percentage (GC%) and digital imaging behavior. Data showing attribute details in Table 2 reflect the variation across the various SHW's and the influence of the *Ae. tauschii* accessions. The standard statistical values of the quantitative variables are in Table 3. The coefficient of variation (CV) represents the ratio of the standard deviation to the mean, and it is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from each other. The Pearson correlation coefficients are shown in Table 4. These data support the accessional trend of genetic expressivity of the various *Ae. tauschii* male parents used in the SH production. The negative values close to -1, shows negative relationship within the two variables e.g., -0.836 higher the width the lower will be LWR and vice versa. While positive values close to +1, shows the positive relationship within the two variables e.g., 0.946 explains greater the length of the seed higher will be its perimeter length and vice versa. Given the results it is possible to select SHW entries for further exploitation in breeding however, performance based information will be imperative for major traits that will benefit breeding targets that are set here for salinity tolerance complimented by positive values for other key attributes (Tables 2, 4).

Multiple factor analysis (MFA) is a statistical technique that determines the factors causing variation in a multivariate data on the basis of variables taken to study the population, the common factor is sorted out due to two or more correlated variables. In our study synthetics having the same durum inheritance grouped together and the three groups are named after their durum parent inheritance as ALTAR 84, D67.2 and CERCETA. MFA showed that the durum (ALTAR 84, D67.2 and CERCETA) with all the attributes had different patterns of variability as expressed through partial axes. Sub-Factors of CERCETA and ALTAR 84 are explained by Multiple Factor Partial Axis F1 and sub-factors of D67.2 have been explained by F2 (Figs. 4, 5).

CERCETA, ALTAR 84 and D67.2 as a factor were dissected into the respective parameters studied for each separately. Hence, these factors have the same number of variables studied. The magnitude of the studied variables graded the factors along the coordinates. It has specifically been observed that CERCETA with all its correlated attributes have been explained along X-axis at the positive side with respect to its variation in all observed individuals (Fig. 4). On the other hand the inheritance of D67.2 in SHs exhibited a different pattern compared to the CERCETA. With all those variables as of CERCETA variation was explained through Y-axis. Width (W), habit, anthocyanin and chlorophyll concentration index (CCI) found very near to Y-axis indicative of the same pattern of variability. Further, third factor of durum inheritance ALTAR 84 expressed in SHW's pertaining variation in the range of a factor score of -0.25 to -0.75 along X-axis and Y-axis in the negative coordinates (Fig. 4). Further, the MFA clearly dispersed the multivariate data into three groups undoubtedly classified by their different parental origin *i.e.,* CERCETA, ALTAR 84 and D67.2. This unbiased scattering of the data constituting three groups indicates that inheritance of CERCETA, ALTAR 84 and D67.2, though the common attributes. This grouping of SHW's assessed with characters (A: Area, W: Width, P: Perimeter, L/W: Length width ratio, DS: Distance, CCI: Chlorophyll concentration index, LA: Leaf Area) grouped the SHW's elucidating that ALTAR 84, CERCETA and D67.2 inheritances have different patterns of heredity highlighting the importance of MFA as a robust technique for assessing genetic penetrance and expressivity within populations.

Based upon the diverse phenological observations, we provide the baseline to initially select a potent sub-set of 41 SHW's (accession numbers 584, 594, 47, 593, 895, 224, 655, 930, 573, 867, 897, 825, 955, 448, 600, 785, 927, 516, 781, 449, 50, 44, 885, 460, 861, 962, 909, 866, 590, 261, 477, 896, 673, 572, 608, 614, 786,640, 454, 53 and 803) with outstanding breeding potential. It is our contention that we add the molecular inputs for refinement of the selected entries to be fingerprinted and to delineate the accessional dis-similarities and only to exploit those SHW's that demonstrate unique diversity in their DNA profiles.

Table 3. Descriptive statistics of quantitative variables.

	CCI	LA	GC%	AS	PL	L	W	LWR	CS	DS
Minimum	31.300	17.020	15.082	17.556	22.599	8.296	3.031	1.659	0.393	0.641
Maximum	60.270	64.750	88.049	47.602	34.982	12.327	5.651	3.264	0.673	1.719
Range	28.970	47.730	72.967	30.045	12.383	4.031	2.620	1.604	0.280	1.078
Mean	45.613	34.255	47.777	33.188	28.327	10.328	4.588	2.327	0.518	1.022
Variance	31.840	89.659	245.584	50.581	7.309	0.941	0.472	0.082	0.003	0.047
St. Dev	5.643	9.469	15.671	7.112	2.704	0.970	0.687	0.286	0.058	0.216
C.V	0.124	0.276	0.328	0.214	0.095	0.094	0.150	0.123	0.112	0.212
S.E	0.509	0.854	1.413	0.644	0.245	0.088	0.062	0.026	0.005	0.020

Table 4. Pearson correlation coefficient values of quantitative variables.

	CCI	LA	GC%	AS	PL	Length	Width	LWR	Circularity
LA	-0.069								
GC%	0.088	-0.046							
AS	-0.027	0.112	0.349						
PL	0.045	0.153	0.242	0.880					
Length	0.016	0.072	0.292	0.878	0.946				
Width	-0.014	0.128	0.323	0.913	0.725	0.679			
LWR	0.062	-0.100	-0.220	-0.589	-0.290	-0.189	-0.836		
Circularity	-0.130	-0.054	0.293	0.520	0.066	0.132	0.657	-0.791	
DS	0.002	0.055	0.185	0.458	0.530	0.483	0.269	-0.023	-0.006

Conclusion and Way forward

Synthetic hexaploid wheats possess enormous allelic diversity due to the contribution of the durum cultivars as well as *Ae. tauschii* accessions used in combinations with them. SHW's of positive resistance/tolerance after being screened are candidates for wheat breeding and ensuring resistance/tolerance expression that occurs from all three genomes (A, B and D). This study precisely defines that the contribution of the durum parent remains similar, and enormous variation observed is partitioned across the *Ae. tauschii* accessions. It also allows in case of SHW entries with major trait data similarity to infer that the accessions may not be diverse and allows duplicated accessions to be rejected, thus adding efficiency to SHW exploitation in breeding programs around uniqueness.

SHW's have been broadly associated with salinity tolerance and micro-nutrient level diversity *i.e.* iron, zinc, phytic acid (Gorham *et al.,* 1987; Jamil *et al*., 2016, Mujeeb-Kazi *et al.,* 1993, Mujeeb-Kazi 2003b, Trethowan & Mujeeb-Kazi 2008, Shah *et al.*, 1987). Our initial findings across some parameters of differential genetic expressivity are very encouraging and allow us to address both the above targets that are vital for wheat productivity and food security as projected for 2050 to support the nutrient rich food requirements of an estimated 9.2 billion people living on this planet (Ullah *et al.,* 2016). Pre-breeding programs using SHW's could supply the pertinent genetic expressivity facets as studied here to the wide array of these stocks that are globally available (Ogbonnaya *et al.,* 2013). Variability seen in this study across several parameters is encouraging and meaningful conclusions can be drawn and this shall renew optimism among wheat breeders for the targeted use of D genome derived SHW germplasm. Earlier data of Mujeeb-Kazi *et al.* (2000) has shown variation for the yield parameters particularly the 1000 kernel weight component and with these added trends we may select quality SHW's as donors for breeding and cultivar improvement programs. This has recently been seen for the karnal bunt (KB) tests done on the DArT mapping population has led to the conclusions that all entries tested within this population were immune to KB (Rizwan, 2014). A similar trend is quite possible for the salinity tests that are projected for the materials of this study but in order to make a well-defined user-friendly breeding set of "limited" entries, a multiple array of parameters information is more desirable. This was subsequently adopted for the KB tested germplasm (our unpublished data) where all SHW entries were immune to KB, a sub-set was made based upon other phenological traits having tremendous agricultural significance. The conclusions drawn above are analogous to the current wide usage of SHW's in breeding categorized under "bridge-crossing" and under exploited "direct-crossing" precision that stringently taps targeted *Ae. tauschii* accessions and offering better diversity definition.

Acknowledgement

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