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Evaluation and utility of synthetic hexaploids in the improvement of wheat grain quality

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Abstract

A set of 75 synthetic hexaploid lines, derived from the hybridization of tetraploid (*T. turgidum* spp. Durum) and diploid (*Ae. tauschii*) wheats, was evaluated for grain quality traits. Since synthetic hexaploids have been developed using wild progenitors, the utility of synthetics in widening genetic base and improving wheat quality has been explored. Wide ranges of quality traits were exhibited in the synthetic hexaploids studied. There was higher protein content (>14%) in 14 lines along with higher grain weight (>50 mg). This is important for the improvement of protein content without decreasing the grain weight. Super soft grained lines were identified with having low hardness index (<20.0), such low values are rarely available in hexaploid wheats. Some of the very soft grained hexaploids had very low sedimentation value (<5.0 ml; 1 g test) also. This trait has utility in improvement of biscuit making quality of wheat. Similarly, synthetic lines with stronger gluten and hard texture were also identified. These can be used for improving bread and chapati quality. Since grain hardness and SDS sedimentation volumes are highly heritable, the criteria can be used for selecting desirable segregants among early generation progeny. Overall data demonstrated the availability of useful traits in synthetic hexaploids and their utility in the improvement of quality of wheats and widening their genetic base.

Key words: Synthetic hexaploids, wheat quality, grain hardness, gluten strength, sedimentation value.

Introduction

Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is an allo- hexaploid which evolved from the hybridization of a tetraploid wheat (AABB) with *Aegilops tauschii* (2n = 2x = 14, DD), syn. *T. tauschii*; *Ae. squarrosa* [1], This hybridization event is thought to have occurred more than once, but the exact number is not clear [2]. The small number of hybridization events and the restricted geographic origin of these events have resulted in a narrow genetic diversity for hexaploid wheat [2]. The D genome of wheat has remained largely unchanged from that of its wild relative *Ae. tauschii*, so much so that *Ae. tauschii* can be considered as an extension of the wheat gene pool [3, 4]. Therefore, *Ae. squarrosa* can be used as a source of genes for various traits still unexplored. Massa *et al.* [5] reported four alleles of puroindoline a and four alleles of puroindoline b determining grain softness among 50 *Ae. tauschii* accessions. The incorporation of *Ae. tauschii* genes into synthetic hexaploids facilitates the analysis of puroindoline sequence polymorphism and other genetic effects on kernel texture, using testing methods designed for hexaploid wheat, such as the Perten Single Kernel Characterization System (SKCS) or NIR. Analysis of synthetic hexaploids can also facilitate the analysis of the effect of the A and B genomes, contributed by the durum parent on kernel texture as well as gluten strength. Evaluation of the physical and chemical properties, rheological characteristics and bread baking tests has also identified potentially useful variation in the D genome of synthetic hexaploids [3, 6]. *Ae. tauschii* was found to carry novel glutenin proteins with positive effects for improving the bread making quality of wheat [3, 6]. Transgressive segregation has also been observed for kernel texture, indicating the presence of additional genes associated with the trait.

Two methods can be employed to incorporate *Ae. tauschii* genes into a hexaploid wheat breeding population. The direct cross method, which involves direct hybridization between *Ae. tauschii* and hexaploid wheat [7, 8], or an indirect method that relies on the intermediate step of producing synthetic hexaploids [9]. Synthetic hexaploid production mimics *T. aestivum* hexaploid wheat evolution by crossing tetraploid wheat *T. turgidum* ssp. durum and *Ae. tauschii*. Genes from *Ae. tauschii* are then available via direct crossing of synthetic hexaploids to *T. aestivum* [9]. CIMMYT (The International Maize and Wheat Improvement Center, Mexico) has produced numerous synthetic hexaploids facilitating the exploitation of genetic variability in *Ae. tauschii*. However, there are no such reports of utilizing synthetic hexaploids in breeding programme for improving quality of various end-use products. However, generally synthetic hexaploids have hard threshing and late maturity. Therefore, first desirable traits should be identified in synthetics and then should be used for transferring desirable traits into hexaploid wheats using back cross method and microlevel tests [10]. In the investigation efforts have been made to evaluate the potential of synthetic hexaploids for important quality traits in bread wheat.

Materials and methods

Large numbers of synthetic hexaploid lines were produced at CIMMYT, Mexico through the hybridization of 31 accessions of *T. turgidum* spp. durum (AABB) and many accessions of *Ae. tauschii*, (DD). The tetraploids included durum lines 'Doy 1', 'Croc 1', 'Altar 84', 'WARD', 'Laru', 'Rok/KML', D67.2/P66.270, YUK, Dverd 2, ACO89, 68.111, PG/USA2111 and 'Arlin 1' and *Ae. squarrosa* accessions. Ninety nine synthetic hexaploid lines with genome constitution AABBDD were procured from CIMMYT and maintained at DWR, Karnal. Entire set was grown at DWR, Karnal under normal dose of fertilizer and other agronomic package of practices. To maintain the purity of seeds, individual plants were harvested and threshed. Sufficient grains were obtained from 75 lines for quality analysis.

Grain hardness was measured using Single Kernel Characterization System (SKCS) Perten SKCS (AACC Method 55-31) on clean, unbroken wheat kernels. Fifty grains from each sample are analyzed for hardness index, grain weight, grain size and moisture content. Hardness index was calculated taking into consideration the moisture content, grain size and crushing force. Grain protein and moisture contents were estimated through standard NIR machine (Near infrared spectrophotometer) using AACC method 44-16. Grain protein content is expressed at 14% moisture basis.

SDS micro-sedimentation was measured by the modified method of Carter [11]. One gram whole meal flour of each cultivar was placed in graduated glass tubes (150 mm × 14 mm). Added 4.0 ml of distilled water to each sample. The samples were mixed, allowed to hydrate for 5 min, mixed again and then allowed to hydrate for another 5 min. SDS-lactic acid solution (12 ml) was added to each sample and the contents were mixed by inverting the tubes. The contents were allowed to settle for 10 min and the height (ml) of the sediment was recorded.

Single kernel characterization system (SKCS) hardness, kernel weight, kernel diameter and protein data were analyzed using simple correlation to investigate any association between these variables.

Results and discussion

Grain weight and grain hardness: A set of 75 accessions exhibited a high level of vigor and fertility and produced enough seed needed for the study. However, all the synthetics showed hard threshing and late flowering. Kernels of all the synthetic hexaploid accessions were large and plump, as indicated by their average weight and diameter, 32.16-56.21 mg and 2.97-3.40 mm, respectively (Fig. 1). Though, there was some reduction

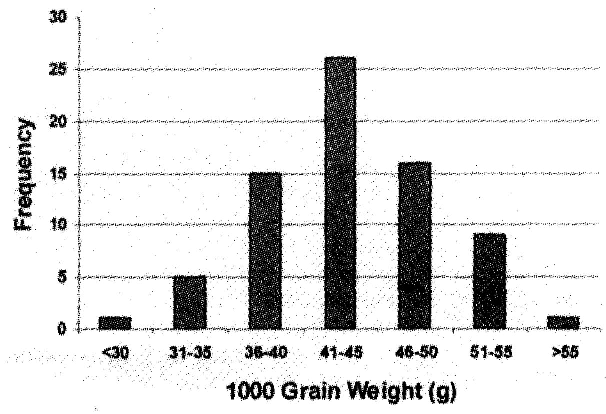

Fig. 1. Frequency distribution of 1000 grain weight (g) in a population of synthetic hexaploids

in the weight of grains because of climatic fluctuations during 2005-2006 crop season in comparison to earlier years. The strong positive correlation was observed between grain weight and diameter (r = 0.91) as expected. In contrast to earlier studies [4], there was significant (though moderate) positive correlation of grain weight and diameter with protein content (r = 0.37 and 0.34, respectively) in this set of synthetic hexaploids (Table 1). This indicates that grain size and weight can be impr‹·ved along with protein content utilizing synthetic hexaploids.

Table 1. Correlation coefficients among SKCS hardness index (HI), grain diameter (D), grain weight (W), protein content (P%) and sedimentation volume (ml) of 75 synthetic hexaploid accessions.

Character	HI	W (mg)	D (mm)	P (%)
W (mg)	–0.17ns			
D (mm)	0.03 ns	0.91***		
P (%)	0.16 ns	0.36**	0.34**	
Sed (ml)	0.11 ns	–0.34**	–0.32**	0.03 ns

ns = Not significant, **p = 0.01; ***p = 0.001

Significant variation in the average grain hardness among synthetic hexaploid accessions was observed (Fig. 2). Hardness index, as measured by SKCS, ranged from 12.6 to 94.8 with the average value of 48.1 indicating large variability in the trait obtained using different crosses. This enhances the utility of synthetics in making crosses for enhancing the quality and yield potential of bread wheat. Earlier reports [4] indicated lower range of HI (2.6 to 40.9) in a set of synthetic hexaploids. Standard deviation values ranged from 9.9 to 23.1 indicating homogeneous nature of samples. Based on kernel texture wheats are classified into hard

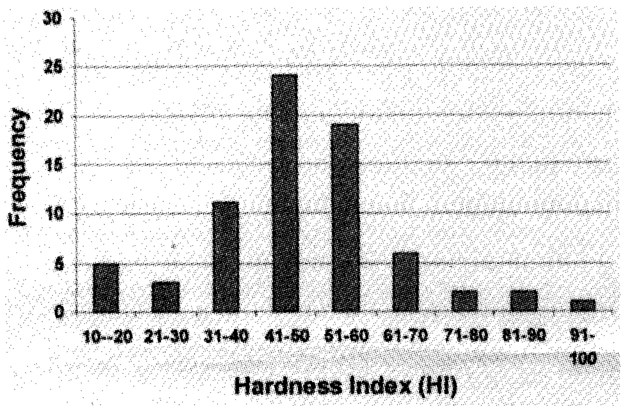

Fig. 2. Frequency distribution of SKCS Hardness Index (HI) in a population of synthetic hexaploids

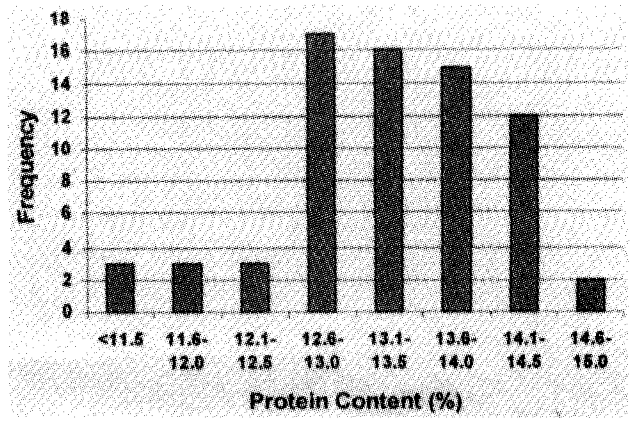

Fig. 3. Frequency distribution of protein content (% on 14% moisture basis) in a population of synthetic hexaploids

and soft category, occurrence of both hard and soft hexaploids in this population of synthetics is useful in improving wheat for diverse end-uses. Soft wheat with low protein content and weak gluten is primarily required for biscuit, cakes and noodles and hard wheat with strong gluten and high protein for bread making [12]. Therefore, availability of very soft synthetic hexaploids (HK20.0) in this population (such soft wheats are generally not available in bread wheat) has utility in improving biscuit making quality of wheat cultivars (Table 1). The softer grains in some of the accessions may be because of specific mutation in puroindolines, softness proteins associated with grain texture [4, 13]. Therefore, further characterization of softer alleles of puroindolines will help in producing super soft wheats with great potential for improving biscuit making quality. Efforts are on in this direction in our laboratory.

The correlation coefficients between SKCS hardness and the other kernel traits indicated that the variation in kernel texture of the synthetic hexaploid accessions was not primarily related to their diameter, weight or protein content (Table 1). Results of earlier studies indicate that though kernel texture vary due to environment, that genotype-by-environment interaction is relatively small and genotype rankings remain relatively constant. No correlation was observed between grain hardness and protein content and sedimentation volume, indicating that selection can be made for gluten strength in each class of wheat separately. This further improves greatly the utility of synthetics in wheat improvement.

Protein content and gluten strength: The kernel protein content of synthetic hexaploid lines ranged from 11.5 to 15.0% with the average value of 13.3% (Fig. 3). In this population protein content showed positive correlation with grain size and weight indicating that protein content can be improved along with grain weight. Since there was no correlation of grain hardness with protein content, it can be manipulated in each class of hard and soft wheat separately. Gluten strength is determined by the glutenin subunits which have important property of swelling in various non-reducing solvents (dilute acetic acid, lactic acid and SDS) and the swelling volume appears to be directly related to quantity and quality of glutenin [14]. Therefore, small-scale tests used to predict bread-making quality are based on glutenin swelling capacity or directly on insoluble glutenin content. The SDS sedimentation test is a modification of the Zeleny sedimentation test that requires only a small sample of whole meal flour, is simple to perform and is highly reproducible [15, 16]. Initially, 6 g of whole meal or 5 g of flour were used in the SDS sedimentation test. The amount of flour used for the test was later decreased to 1.0 g and the name of the test was changed to the SDS micro-sedimentation test [16].

Though SDS sedimentation test assesses protein quality, however, results also are highly influenced by protein concentration [15, 16]. Generally protein content is correlated to gluten strength positively. However, in this investigation no correlation was observed between protein content and sedimentation volume. No correlation between sedimentation and protein content may be because of diverse lines developed using diverse genotypes of tetraploids as well as *Ae. squarrosa*. Large variation was observed in sedimentation volume of all the synthetics studied and varied from minimum of 2.2 ml to 12.4 ml with an average value of 7.5 ml (Fig. 4). This shows that synthetics with very low gluten strength along with very soft grain characteristics can be used as a source for the improvement of biscuit making quality and synthetics with stronger gluten and hard lines for bread and chapatti making. Since grain hardness and SDS sedimentation volumes are highly heritable, the criteria can be used for selecting desirable segregants among early generation progeny (17). Our earlier studies also demonstrated very high heritability

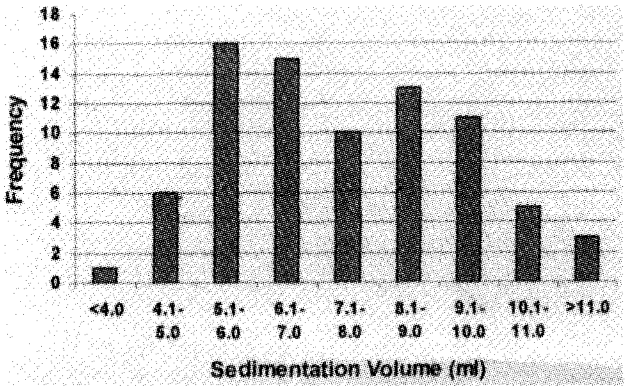

Fig. 4. Frequency distribution of sedimentation volume (ml) obtained using 1 g test in a population of synthetic hexaploids

for grain hardness index (H = 0.91) and high heritability for gluten strength (H = 0.71).

In conclusion, synthetic hexaploids with desirable traits were identified having their utility .in wheat improvement (Table 2). There was higher protein content (>14%) in 14 lines along with higher grain weight. Super soft grained lines were identified with very low hardness index (<20.0) not generally available in hexaploid wheats. Synthetics with very low gluten strength and very soft grain characteristics can be used as a source for the improvement of biscuit making quality and synthetics with stronger gluten and hard lines for bread and chapati making. Since grain hardness and SDS sedimentation volumes are highly heritable, the criteria can be used for selecting desirable segregants among early generation progeny. Overall results demonstrated the availability of useful traits in synthetic hexaploids generated by crossing different tetraploids and *Ae. squarrosa* lines, and thus can be used in widening the genetic base of hexaploids for improving wheat yield and quality *per se*.

Table 2. Quality traits of Promising synthetic hexaploid lines and PBW 343

Line	HI	Line	GW	Line	SED	Line	SED	Line	P (%)
SYN-99	12.6	SYN-50	51.90	SYN-70	2.2	SYN-41	10.4	SYN-30	14.1
SYN-18	15.2	SYN-51	52.10	SYN22	4.4	SYN-42	10.4	SYN-92	14.1
SYN-98	16.4	SYN-71	52.99	SYN 23	4.5	SYN-53	10.4	SYN-34	14.2
SYN-96	18.9	SYN-9	53.06	SYN-19	4.5	SYN-30	10.6	SYN-50	14.2
SYN-8	19.0	SYN-83	53.54	SYN-76	4.5	SYN-32	10.8	SYN-82	14.2
PBW 343	80.1	SYN-80	53.99	SYN 21	5.0	SYN-28	11.5	SYN-80	14.3
		SYN-79	55.86	SYN-88	5.0	SYN-77	11.8	SYN-98	14.3
		SYN-70	56.21	SYN-61	5.1	SYN-55	12.4	SYN-43	14.4
		PBW 343	38.12	PBW 343	7.2	PBW 343	7.2	SYN-60	14.4
								SYN-61	14.4
								SYN-87	14.4
								SYN-44	14.5
								SYN-59	14.8
								SYN-86	15.0
								PBW 343	11.8

HI = Hardness Index in lower range suitable for better biscuit making quality, GW = grain weight (mg), SED = sedimentation volume (ml) in lower range (for soft wheat products) and higher range (for hard wheat products) and P (%) = protein content in % at 14% moisture basis. Details of pedigree can be obtained from authors. PBW 343 is used as a control.

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