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Evaluation of synthetic hexaploid wheat (*Triticum aestivum*) derived RILs for kernel traits

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ABSTRACT

In bread wheat (*Triticum aestivum* L.), kernel parameters are significant grain quality characteristics that affect yield and milling quality as well as market price. Utilization of synthetic hexaploid donor to harness the diverse alleles for broadening the genetic base is a novel breeding strategy of the bread wheat breeding programs across the globe. An experiment was conducted during winter (*rabi*) season 2017–18 and 2018–19 at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi to evaluate 188 recombinant inbred lines (RILs) which were derived from HD 2932 (Indian bread wheat cultivar) and Synthetic 46 (Syn 46: Synthetic hexaploid donor) to identify the effect of the synthetic hexaploid donor on kernel parameters. Kernel parameters like kernel length (KL), kernel width (KW), kernel thickness (KT) and thousand kernel weight (TKW) were studied. Syn 46 had larger kernels and a high TKW with respect to HD 2932. Significant variation for all the traits was observed in the population with transgressive segregants. In this RILs population (188) KL ranged from 5.13–7.5 mm, KW ranged of 2.26–3.9 mm, KT ranged from 2.57–3.41 mm, and of TKW ranged from 25.2–53.17 g. Correlation analysis showed a significant positive correlation of TKW with KL and KT. Potential RILs identified with desirable allelic combinations may be used for future breeding programs, and RILs with contrasting kernel parameters may be used to create nested RILs for further generation of variability.

Keywords: Kernel size, Kernel length, Synthetic hexaploid wheat, TKW, Yield potential

Correlation of the kernel parameters with grain yield and milling parameters has been well documented in bread wheat (*Triticum aestivum* L.). Larger kernels positively affect seedling vigor, yield potential, milling quality and floor yield (Botwright *et al*. 2002, Sun *et al.* 2009, Cui *et al.* 2011, Williams *et al*. 2013). A positive correlation was observed between KL, weight, and area with milling score, i.e., a parameter derived from flour yield, endosperm separation index, and friability by Breseghello and Sorrells (2006). The association of kernel size with flour-water dough quality was reported by Morgan *et al*. (2000). However, genetic diversity within wheat cultivars facing a drastic bottleneck in the process of domestication and breeding (Kilian *et al*. 2010, Reif *et al*. 2011, Mondal *et al.* 2016). Synthetic hexaploid wheat (SHW) derived from tetraploid (*T. turgidum*) and diploid (*Ae. tauschii*) varieties represent a wider genetic basis because of the introduction of additional genetic resources. This artificially created hexaploid wheat can be an excellent choice to explore variability in the wheat genome (Yang *et al*. 2009, Li *et al*. 2014). The approach of resorting to wild relatives, landraces and cytogenetic stocks as donors in breeding program to broaden the genetic base has been adopted in the CIMMYT, Mexico, and across the globe. In particular, the use of synthetic wheat stocks in wheat breeding programs to avoid genetic erosion and broaden the genetic base is worth mentioning (Li *et al.* 2018, Rosyara *et al*. 2019).

The aim of the present study was to broaden the genetic base by utilizing Syn 46 as a donor parent, which is associated with larger kernels and high TKW. The study identified potential recombinant inbred lines (RILs) for kernel size traits, viz. KL, KW, KT, TKW, and length/width ratio (LWR), which can be used to increase the grain quality and yield potential of presently cultivated genotypes with no significant reproduction barrier.

MATERIALS AND METHODS

Seed material and field experiment condition: The 188 RILs and parent lines (Syn46 and HD2932) were timely

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sown at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi (28^{o} 38'N, 77^{o} 9'E, and 228.6 m AMSL) for season I ($F_{8:9}$); 2017–18; and season II ($F_{9:10}$); 2018–19 under irrigated conditions in a randomized complete block design (RCBD) in 2 replications with 3 rows (1 m length) per entry with row-to-row spacing of 25 cm. The parent lines were used as check after every 20 treatments and standard agronomic practices were followed to raise the crop. Spikes were harvested and threshed after physiological maturity. After harvesting, seeds were stored at room temperature and different morphological and agronomic parameters were recorded.

Phenotyping for kernel parameters: Randomly, 20 fully developed kernels were selected visually from both the field replication to record data for KL, KW, and KT; the traits were measured using Vernier calipers (Mititoyo vernier calipers 300 mm/12"). The mean of individual measurements of those kernels represented a data point and was used for statistical analysis. Similarly, 2 sets of 1,000 kernels were taken from each of the field replications to estimate the TKW, which was counted manually and weight was measured using electronic analytical balance.

Statistical analysis: Statistical analysis, including descriptive statistics and clustering analysis was done using the "pastecs" and "ape" packages respectively in R statistical software (v4.1.2; R core team 2021). Frequency distribution was done in XLSTAT statistical software (version XLSTA 14) and Pearson's correlation coefficient, was calculated using Minitab statistical software (version Minitab 21.1.0).

RESULTS AND DISCUSSION

Kernel parameters of the RILs: Results showed that Syn 46 showed higher TKW and larger kernel size than HD 2932 over the cropping seasons. In the RIL population, KL ranged from 5.13–7.5 and 5.6–7.26 mm, KW ranged from 2.79–3.68 and 2.26–3.98 mm, LWR ranged from 1.49–2.43 and 1.56–2.88, KT ranged from 2.6–3.41 and 2.57–3.39 mm, and TKW ranged from 25.2–51.4 g and 27.24–53.17 g for season I and II, respectively (Table 1). Significant variations were observed for all the traits in the population for both the years (Supplementary Table 1). These variations occurred within the population due to background recombination over the years. The continuous and normal distribution of the population indicates that these traits are polygenic and phenotypes are governed by multiple alleles. The results corroborate with the study of Ramya *et al.* (2010) who illustrated reasonable phenotypic variation among 185 recombinant inbred lines from the cross Rye Selection 111 × Chinese Spring grown in diverse environments for kernel parameters such as TKW, KL and KW.

Population distribution of kernel parameters: The frequency distribution for KL, KW, LWR, KT, and TKW for both seasons I and II indicated significant variation and normal distribution in the RIL population (Fig 1). In season I, KL population distribution had 9 class intervals, with the modal class (62 RILs) ranging between 6.5 and 6.75 mm. In season II, the population distribution of KL had 8 class intervals, with the modal class (45 RILs) ranging between 6.23–6.44 mm (Fig 1A, F).

In total, KW population distribution had 9 class intervals with the modal class (52 RILs) ranging between 3.34–3.45 mm and 10 class intervals with the modal class RILs (46) in the 3.16–3.34 mm range for season I and II, respectively (Fig 1B, G). The KT population was found to be distributed in 8 class intervals, with modal class (43 RILs) in the 2.82–2.93 mm range in season-I and 9 class intervals, with modal class in the 3.17–3.27 mm range (Fig 1C, H). Similarly, for TKW population was distributed in 10 and 9 class intervals, with the modal class (45) in the 36.8–39.7 g range and (48) in the 39.2–42.2 g range for season-I and II, respectively (Fig 1D, I).

Transgressive segregants identified for kernel parameters: Transgressive segregants for all the kernel parameters were identified during both the seasons (Table 2). Syn 46 had higher KL with respect to HD 2932 (Table 2). During the season I, for KL parameter, 9.04% RILs in the population surpassed recipient parent HD 2932 (Negative transgressive segregants), 13.30% surpassed recipient parent

Table 1 Phenotypic performance of recombinant inbred lines for two consecutive crop seasons I and II for kernel traits

Trait	Parental Genotype			Recombinant Inbred Lines (RILs)									
	Season	HD 2932	Syn 46	Min	Max	Mean	Range	Median	SE Mean	CI Mean	SD	CV (%)	W Value
KL	I	6.17	7.21	5.13	7.5	6.62	2.37	6.66	0.032	0.064	0.446	0.067	0.98
	II	6.07	7.02	5.6	7.26	6.52	1.66	6.51	0.03	0.05	0.35	0.05	0.97
KW	I	3.50	3.04	2.79	3.68	3.30	0.89	3.31	0.013	0.025	0.174	0.053	0.99
	II	3.31	2.97	2.26	3.98	3.26	1.72	3.26	0.02	0.04	0.3	0.09	0.98
LWR	I	1.76	2.37	1.490	2.430	2.011	0.940	1.990	0.012	0.024	0.164	0.082	0.99
	II	1.83	2.36	1.560	2.880	1.990	1.320	1.990	0.016	0.032	0.221	0.109	0.98
KT	I	2.68	3.26	2.60	3.41	2.99	0.81	2.99	0.013	0.025	0.177	0.059	0.98
	II	2.84	3.38	2.57	3.39	2.99	0.82	2.98	0.01	0.02	0.17	0.06	0.97
TKW	I	34.9	46.2	25.20	51.4	40.67	26.20	40.15	0.34	0.68	4.73	0.12	0.98
	II	35.4	46.3	27.24	53.17	40.48	25.93	40.28	0.36	0.7	4.9	0.12	0.99

Season I, 2017–18; season II, 2018–19.

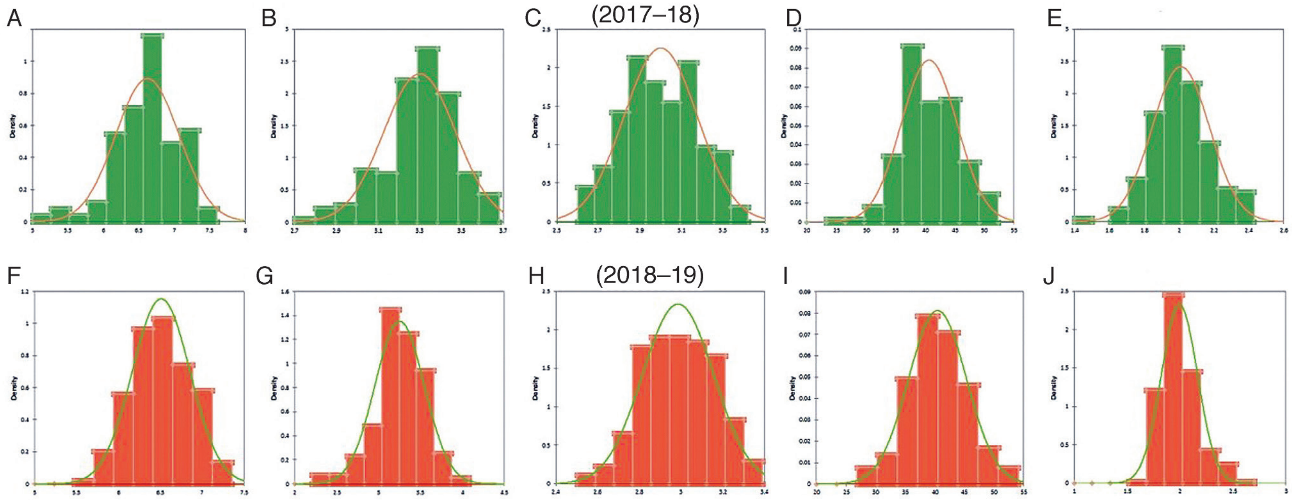

Fig 1 Frequency distribution of kernel size traits in the RIL population for seasons I (2017–18) and II (2018–19). KL (A, F); KW (B, F); KT (C, H); TKW (D, I); LWR (E, J).

Syn 46 (Positive transgressive segregants) and 77.66% RILs were observed to be recombinants. The parental lines were equivalent for KW (Table 2), however, 8.51% transgressive segregation was observed with respect to HD 2932, whereas 10.11% transgressive segregation was observed with respect to Syn 46 and 81.38% of the RILs were identified as recombinants. Donor parent Syn 46 had a higher KT with respect to recipient HD 2932 (Table 2). However, 7.98% transgressive segregation was observed with respect to HD 2932, whereas 3.19% transgressive segregation was observed with respect to Syn 46 and 88.83% of the RILs were identified as recombinants for KT parameter. Donor parent Syn 46 also had a higher TKW with respect to recipient HD 2932 (Table 2). For TKW parameter, 12.77% transgressive segregation was observed with respect to HD 2932, whereas 8.51% transgressive segregation was observed with respect to Syn 46 and 78.72% of the RILs were identified as recombinants.

During season II, the population showed 10.64% transgressive segregants with respect to recipient parents and 5.85% transgressive segregants with respect to donor parents, and 83.51% found recombinant. For KW, 11.7% and 43.08% of transgressive segregants were observed with respect to recipient and donor parents, respectively, and 45.21% were found to be recombinants. RILs population showed 21.27% and 0.053% transgressive segregants were observed with respect to recipient and donor parents, respectively, and 78.19% were found to be recombinants for KT. Similarly, 11.7% of transgressive segregants, were observed with respect to the recipient parent and 12.23%

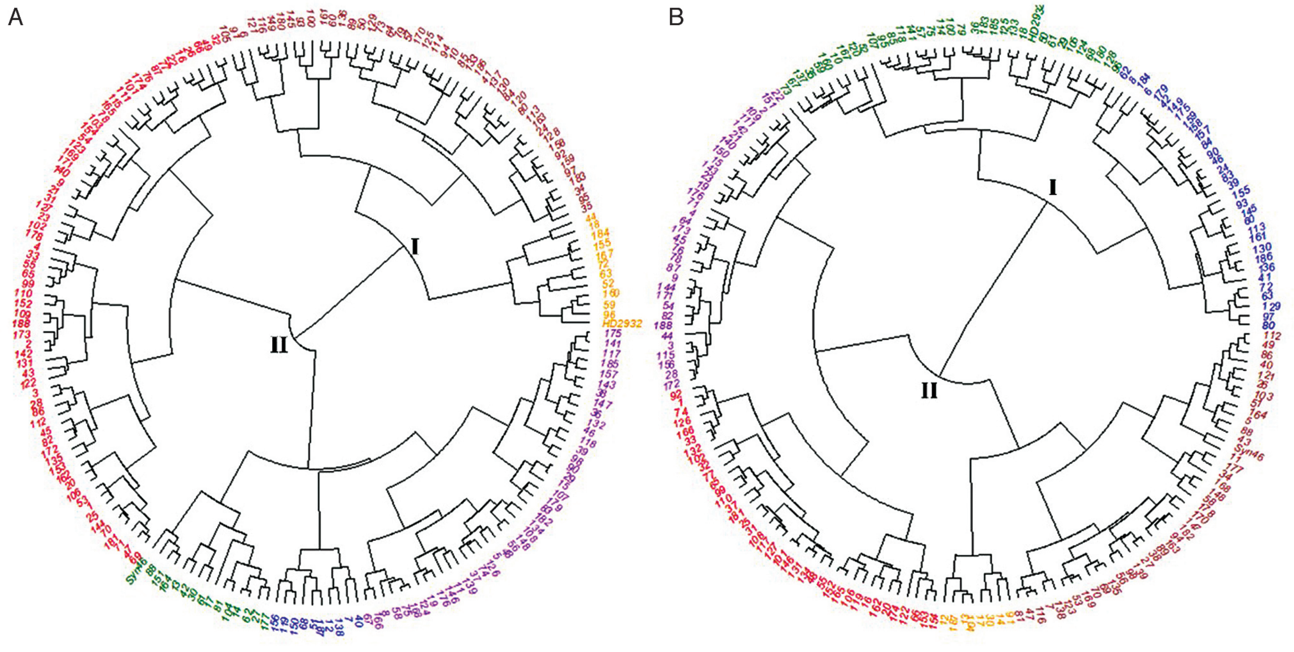

Fig 2 Neighbour-Joining cluster dendrogram of RILs based on performance for kernel traits: KL, KW, LWR, KT, and TKW during 2017–18 (A), and 2018–19 (B).

Table 2 Transgressive segregation analysis of kernel traits in the season I and II RILs population with parental lines

Season	KL	KW	KT	TKW
Season I (2017–18)				
HD 2932 transgressive segregation (%)	9.04	8.51	7.98	12.77
Syn 46 transgressive segregation (%)	13.3	10.11	3.19	8.51
Recombinant (%)	77.66	81.38	88.83	78.72
Season II (2018–19)				
HD 2932 transgressive segregation (%)	10.64	11.7	21.27	11.7
Syn 46 transgressive segregation (%)	5.85	43.08	0.53	12.23
Recombinant (%)	83.51	45.21	78.19	76.06

of transgressive segregants were found with respect to the donor parent, and 76.08% of recombinants were recovered for TKW (Table 2).

Segregation for all kernel parameters during both seasons and identification of transgressive segregants with respect to donor and recipient parents indicate the genetic component underlying the variation observed in the RIL population. The relative proportion of transgressive segregants and recombinants across the seasons varied only for KW and KT. This may be due to terminal heat stress or some other environmental stress factor faced by the crop.

Kernel parameter correlation: The Pearson's correlation co-efficient was determined with the pooled data of seasons I and II for pair-wise combinations of parameters of KL, KW, KT, and TKW (Supplementary Fig 1). Our results indicated that TKW had a significant positive correlation with KL (r=0.62, P<0.01), and KT (r=0.63, P<0.01). The correlation between KL (r=0.37, P<0.01) and KW (r=0.23, P<0.01) was moderate. A similar correlation was observed between KW and TKW (r=0.23, P<0.01). A highly negative correlation was observed between LWR and KW. Previous studies have reported significant positive correlations among kernel size traits for KL, KW, KT, and TKW in wheat (Dholakia *et al*. 2003, Cui *et al*. 2014). Strong correlation in kernel parameters in advanced breeding lines of RIL populations indicates there is a prospect of correlated multi-trait selection for recombinants with desirable allele combinations with a diverse genetic background.

Table 3 Contrasting RILs for kernel traits for season I (2017–18) and II (2018–19).

RIL	KL (mm)	RIL	KW (mm)	RIL	KT (mm)	RIL	TKW (g)
High (2017–18)							
RIL-104	7.50	RIL-55	3.68	RIL-156	3.41	RIL-22*	51.4
RIL-139	7.41	RIL-4	3.68	RIL-150	3.38	RIL-16	51.2
RIL-122*	7.39	RIL-65	3.61	RIL-111	3.35	RIL-49	51.2
RIL-81	7.34	RIL-122*	3.59	RIL-101	3.33	RIL-17	51.2
RIL-166	7.33	RIL-66*	3.59	RIL-151	3.32	RIL-32	50.2
Mean	7.39		3.63		3.36		51.03
Low (2017–18)							
RIL-52	5.13	RIL-100	2.79	RIL-93	2.60	RIL-6	25.2
RIL-155	5.20	RIL-61	2.85	RIL-184*	2.63	RIL-91	29.7
RIL-18*	5.34	RIL-30	2.86	RIL-137	2.64	RIL-149	31.0
RIL-184*	5.37	RIL-93	2.88	RIL-72	2.65	RIL-121	31.5
RIL-167	5.37	RIL-27	2.89	RIL-186*	2.65	RIL-180	32.8
Mean	5.28		2.85		2.64		30.0
High (2018–19)							
RIL-178	7.26	RIL-122*	3.98	RIL-122*	3.39	RIL-32*	53.17
RIL-148	7.26	RIL-71	3.94	RIL-106	3.34	RIL-66*	52.01
RIL-58	7.20	RIL-154	3.76	RIL-153	3.33	RIL-77	51.41
RIL-1	7.17	RIL-66*	3.74	RIL-11	3.30	RIL-122*	51.17
RIL-74	7.15	RIL-64	3.72	RIL-22*	3.27	RIL-22*	51.05
Mean	7.21		3.83		3.33		51.76
Low (2018–19)							
RIL-68	6.01	RIL-72*	2.94	RIL-72*	2.69	RIL-161	34.90
RIL-120*	5.92	RIL-90	2.94	RIL-8	2.68	RIL-8	34.65
RIL-145	5.88	RIL-84	2.92	RIL-120*	2.67	RIL-97	34.64
RIL-18*	5.88	RIL-63	2.89	RIL-130	2.65	RIL-175	34.47
RIL-113	5.87	RIL-155*	2.87	RIL-186*	2.63	RIL-61*	34.38
Mean	5.91		2.91		2.66		34.60

*represented the potential RILs with stable kernel parameters.

Clustering the RILs on the basis of kernel parameters:

Neighbor-joining clustering analysis was done using Euclidean distances (Fig 2), considering the kernel parameters separately for 2 cropping seasons (Fig 2 A, B). During both the seasons, the population was clustered into 2 primary groups, each containing the parents distinctly in each group. In season 2017–18, it was found that primary major group-I included recipient parent HD2932 and was comprised of 57 RILs, whereas primary major group-2, with donor parent Syn 46, was found to contain 131 RILs. However, in 2018–19, 67 RILs primarily co-clustered with recipient parent HD 2932 and 121 RILs co-clustered with donor parent Syn 46, respectively. It was also observed that the RILs with larger kernels were majorly found in group-II (Co-clustering with Syn 46) and the RILs with shorter grains were identified in group-I in both years. Studies indicated that major kernel trait expression was governed by the donor Syn 46, which showed the expression of alleles from wild diploid and tetraploid progenitors in RILs.

Stable desirable recombinants identified for kernel parameters: We investigated the stability of potential RILs for kernel traits (Table 3). Stable RILs with desirable kernel parameters were identified in both the seasons. RIL-122 had a high KL (7.39 mm) and KW (3.59 mm) during season I (2017–18), as well as a high KW (3.98 mm), KT (3.39 mm), and TKW (51.79 g) during season II (2018–19). In the season I, RIL-22 had a high grain weight, KT, and TKW. Similarly, RIL-66 was identified with high KW in both crop seasons and TKW in season II. RIL-155 had low KL and KW in seasons I and II. It was observed that contrasting RILs were found to be stably inherited for kernel size with different allelic combinations in both the cropping seasons. RILs with a small kernel size may be used to create nested RILs. Potential RILs with stable kernel size traits could be used as donors in wheat breeding programs.

Our study, established that synthetic wheat stock Syn 46 can be used as a donor to broaden the genetic base of a wheat breeding program and desirable recombinants for kernel parameters can be derived in advance of the generation of such crosses. The potential RILs identified with desirable kernel traits may be subjected to further yield trials for potential varietal development as well.

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