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ARTICLE

Marker-assisted introgression of genes into rye translocation leads to the improvement in bread making quality of wheat (*Triticum aestivum* L.)

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Introgression of genes from related species can be a powerful way to genetically improve crop yields, but selection for one trait can come at the cost to others. Wheat varieties with translocation of the short arm of chromosome 1 from the B genome of wheat (1BS) with the short arm of chromosome 1 from rye (1RS) are popular globally for their positive effect on yield and stress resistance. Unfortunately, this translocation (1BL.1RS) is also associated with poor bread making quality, mainly due to the presence of *Sec-1* on its proximal end, encoding secalin proteins, and the absence of *Glu-B3/Gli-B1*-linked loci on its distal end, encoding low molecular weight glutenin subunits (LMW-GS). The present study aims to replace these two important loci on the 1RS arm with the wheat 1BS loci, in two popular Indian wheat varieties, PBW550 and DBW17, to improve their bread-making quality. Two donor lines in the cultivar Pavon background with absence of the *Sec-1* locus and presence of the *Glu-B3/Gli-B1* locus, respectively, were crossed and backcrossed with these two selected wheat varieties. In the advancing generations, marker assisted foreground selection was done for *Sec-1*$^{-}$ and *Glu-B3/Gli-B1*$^{+}$ loci while recurrent parent recovery was done with the help of SSR markers. BC_2F_5 and BC_2F_6 near isogenic lines (NILs) with absence of *Sec-1* and presence of *Glu-B3/Gli-B1* loci were evaluated for two years in replicated yield trials. As a result of this selection, thirty promising lines were generated that demonstrated improved bread making quality but also balanced with improved yield-related traits compared to the parental strains. The study demonstrates the benefits of using marker-assisted selection to replace a few loci with negative effects within larger alien translocations for crop improvement.

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INTRODUCTION

Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD), the most important staple food globally, provides 50% of the total calories and 60% of the total proteins (Grote et al. 2021). It is sown on 23.9 million hectares (mha) of land with an estimated production of 137.5 million metric tons, feeding about 2.5 billion of the world's population (http://www.fas.usda.gov). With the growing wheat industry, consumer preferences have been changing and there is a shift in focus from "production" to "quality", leading to the development of diverse and improved baking and confectionery products (Kanojia et al. 2018).

Wheat is consumed in form of bread and bakery products and can be traced back to ancient times (10,000 BC) (Valavanidis 2018). These products are an important source of energy and a significant reservoir of protein, complex carbohydrates (mainly starch), dietary fiber, vitamins (especially B vitamins), and minerals (Kostyuchenko et al. 2021). More than 9 billion kilograms of bread products are produced annually, with an average consumption of 41–303 kg per year per capita (Dong and Karboune 2021). Bakery is currently one of the fastest-growing food industries, and wheat flour quality has a major role in sustainability of this industry (Longin et al. 2020).

The quality of wheat grain-based products is determined mainly by prolamins or storage proteins, that account for 45–80% of the total proteins in the grain of modern wheat cultivars. Prolamins includes high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (LMW-GS), and gliadins. The quality of dough is the cumulative effect of total protein content and the ratio of gliadins to glutenins (Meenakshi and Khatkar 2005; Suchy et al. 2003), with gliadins affecting the loaf volume potential and dough viscosity, and glutenins affecting dough development time and loaf volume (Kaur et al. 2020).

Glutenins and gliadins are encoded by group 1 wheat chromosomes (1A, 1B and 1D); the long arm glutenin loci *Glu-A1*, *Glu-B1*, and *Glu-D1* code for HMW-GS while the short arm loci *Glu-A3*, *Glu-B3*, and *Glu-D3* code for LMW-GS (Wang et al. 2020). *Gli-B1*, *Gli-B3*, *Gli-B5*, and *Gli-B6* loci also on the 1BS chromosome code for low molecular weight gliadins, with *Gli-B1* being tightly linked to the *Glu-B3* locus. In the gluten complex, HMW-GS and LMW-GS covalently interact with each other via intermolecular disulfide bonds, thus existing as glutenin macropolymers (GMPs). Glutamine-rich repetitive sequences that comprise the central part of these subunits are responsible for the elastic properties due to extensive

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arrays of interchain hydrogen bonds (Anjum et al. 2007). LMW-GS contains a long repetitive domain that facilitates the formation of more α-helices and β-strands and confers breadmaking quality (Wang et al. 2016). Gliadins exist mainly as monomers and interact non-covalently with GMPs. They act as plasticizers to modify the extensibility of gluten affecting the end-use traits (Wang et al. 2017). The balanced ratio of these monomeric and polymeric proteins provides the required amount of strength and viscosity for the dough (Wang et al. 2009). The loss of any of these loci will result in disproportion which will be reflected in the form of weakness and stickiness of the dough (Oak and Tamhankar 2017).

In the history of bread wheat breeding programmes, the rye 1RS chromosomal arm is the most widely used alien chromatin in the form of 1BL.1RS translocation, where the short arm of the wheat 1B chromosome has been replaced by the short arm of the rye 1R chromosome, (Lukaszewski 2014, Crespo-Herrera et al. 2017, Li et al. 2020). This is due to its remarkable yield potential and resistance toward disease and pest: loci for resistance against leaf rust (*Lr26*), stem rust (*Sr31*) (Mago et al. 2002), stripe rust (*Yr9*), and powdery mildew (*Pm8*) (Ren et al. 2009); higher yield potential with increased above-ground biomass, deep root system, canopy water status, and abiotic stress tolerance particularly, drought tolerance; and a wider range of adaptability (Ehdaie et al. 2003; Howell et al. 2014, Ren et al. 2017). But positive influence of this segment on yield and stress resistance, cannot serve the bread making industry as the addition of 1RS and removal of 1BS arm created imbalance in the ratio of monomeric to polymeric proteins and thus influenced bread making quality problems with dough stickiness ('sticky dough syndrome') and reduced dough strength. This was mainly due to addition of rye storage secalins with ω-and γ-secalins proteins encoded by the *Sec-1* locus on the proximal side of the 1RS arm, (Barak et al. 2013) and absence of LMW-GS and gliadins encoded by wheat *Glu-B3/ Gli-B1* locus on the distal end of this arm (Zhao et al. 2012, Wang et al. 2016, Sharma et al. 2020).

The deleterious effects of the 1RS chromosome on grain processing quality was rectified by replacement of distal and proximal segment to introduce the wheat *Glu-B3/Gli-B1* locus and to remove the rye *Sec-1* locus respectively on this arm. Howell et al. (2014) successfully generated two Near Isogenic Lines (NILs) in the background of cultivar Pavon, one with the absence of *Sec-1* locus (*Sec-1*$^{-}$/*Glu-B3*$^{-}$), and other with the presence of *Glu-B3* locus (*Sec-1*$^{+}$/*Glu-B3*$^{+}$) in the 1RS chromosomal arm. These two NILs were used as donors to improve the bread making quality of two popular Indian wheat varieties PBW550 and DBW17 with 1RS.1BL translocation (also having stripe rust resistance gene *Yr5*) by replacing these two loci on 1RS arm through marker-assisted backcross breeding. Performance of the selected improved lines was evaluated in relation to agro-morphological traits, resistance to stripe rust and bread-making qualities compared to the original lines.

MATERIALS AND METHODS

Plant material

Two NILs in the background of the cultivar Pavon having modified 1RS arm of chromosome 1RS.1BL translocation were used as donor parents. NIL Pavon 40:9, has the *Glu-B3/Gli-B1* wheat locus on the distal end of 1RS and designated as *Glu-B3*$^{+}$/*Sec-1*$^{+}$. In NIL Pavon 44:38, the *Sec-1* locus at the proximal end of 1RS has been replaced with corresponding wheat chromatin of 1BS and designated as *Glu-B3*$^{-}$/*Sec-1*$^{-}$ (Fig. S1) (Howell et al. 2014). The seeds of NILs were procured from Prof. Dubcovsky, UC Davis, California. Advanced versions of two wheat cultivars, PBW550 (WH594/ RAJ3856//W485) and DBW17 (CMH79A 95/3*CNO79// RAJ3777), both carrying the stripe rust resistance gene *Yr5*, were used as recurrent parents. PBW550 had been developed and released by Punjab Agricultural University, (PAU), Ludhiana for cultivation under timely sown irrigated (TSI) conditions of the Northwestern Plain Zone (NWPZ) of India (Sharma et al. 2021). DBW17 (CMH79A 95/3*CNO79// RAJ3777) had been developed and released by the Indian Institute of Wheat & Barley Research (IIWBR), Karnal for cultivation under TSI conditions in the NWPZ (Kaur et al. 2020).

Transfer of *Glu-B3*$^{+}$ and *Sec-1*$^{-}$ in PBW550 and DBW17

Pavon 40:9 and Pavon 44:38 donors were crossed as male with the advanced versions of cultivars PBW550 and DBW17 (Fig. 1). The four F_1s were backcrossed twice, and the generated BC_2F_1s were selfed up to BC_2F_6 generation (Table 1). To rapidly advance the generations, two generations were harvested in a year by shuttling the crop between PAU, Ludhiana (30.91°N, 75.85°E, grown between November and May, called main season —MS) and Regional Research Station of PAU at Keylong, Himachal Pradesh (32.71°N, 77.32°E, grown between May and October, called offseason—OS) with foreground and background marker-assisted selection (MAS) across the generations.

Marker-assisted foreground and background selection

Genomic DNA from parental lines and back cross progenies was extracted from the young leaf tissue using the CTAB method (Saghai-Maroof et al. 1984) and quantified on 1% agarose gels. The Polymerase chain reaction (PCR) was carried out in 20 µl reaction volume containing 35–50 ng of genomic DNA, 1-unit Taq polymerase (Homemade), 0.15 mM of each dNTPs, 0.38 µM of forward and reverse primers, and 1X PCR buffer (10 mM Tris–HCl pH 8.4). The PCR products were resolved using 2% agarose gels.

The details of the markers used for foreground selection are given in Table S1. Markers *omega p3, omega p4*, amplifying presence of the rye *Sec-1* locus (Froidmont 1998), and *wpt1911*, amplifying corresponding wheat chromatin (Howell 2014) were used to select the presence or absence of *Sec-1* loci. Presence of *Glu-B3/Gli-1* locus was amplified with marker *Psp3000* (Devos et al. 1993). The yellow rust resistance gene *Yr5* was selected with marker *STS-7/8* (Murphy et al. 2009). To ensure the integrity of chromosomal arm 1RS, (except two targeted loci), MAS was done for all genes present on this arm - as *Lr26/ Yr9/Sr31* with the *lag95* marker (Mago et al. 2002), *Pm8* with the *Sfr43* marker (Hurni et al. 2013). Selection was also done by amplifying the 1RS arm specific rye chromatin markers *rye F3/R3* (Katto et al. 2004) (Table S1). For recovery of the recurrent parent background, selection was done using 35 SSRs for the long arm of chromosome 1B and 20–30 SSR markers from each of the remaining wheat chromosomes.

SDS-PAGE

Seed storage proteins were sequentially extracted from grains of the developed NILs following the method of Smith and Payne (1984). After the removal of albumins and globulins from the flour, gliadin and glutenins were extracted using 1.5 M DMF (dimethylformamide) glutenin extraction buffer (50% Isopropanol, 50 mM Tris-HCl (pH 7.5), 1% dithiothreitol (DTT)). The presence/absence of *Glu-B3* encoded low molecular weight glutenin subunit (LMW-GS) proteins (42–50 kDa) and the *Sec-1* encoded secalin protein (42–55 kDa) were detected through SDS-PAGE on 10 and 15% SDS polyacrylamide gel, respectively (Walker 1996)

Evaluation for agro-morphological traits

BC_2F_5 and BC_2F_6 NILs (during 2018–19 MS—referred to as environment 1 (E1) and 2019–20 MS referred to as environment 2 (E2) from the four crosses along with the donor and the recurrent parental lines were evaluated in three replications in an alpha lattice design. Each trial entry was sown in four rows of 1.5 m length, with a row-to-row spacing of 25 cm, and plant to plant spacing of 10 cm. Data were recorded on plant height (PH in cm), spikelets per spike (SS), spike length (SL in cm), tillers per meter (TNpM), 1000 grain weight (TGW in g), yield per plot (YD in g), harvest index (HI in %).

Screening for stripe rust resistance

BC_2F_5 and BC_2F_6 NILs were also evaluated for resistance against stripe rust in E1 and E2. To create the artificial rust epidemic, the plant material was sprayed with a mixture of yellow rust races collected from farmer's fields. Since *Yr5* provides complete resistance, the material was scored as resistant (R) or susceptible (S).

Bread baking

Bread making is based on the principle of incorporating water into the flour, raising the volume of dough through the production of carbon dioxide by yeast enzymes, and stabilizing the structure at high temperatures. Whole wheat flour bread was made using the recipe of the Food technology Bakery, PAU, Ludhiana (Bhise and Kaur 2014). Briefly, the ingredients used for dough making (wheat flour, salt, sugar, water, fat) were mixed in a dough mixer (Sanco Instruments, New Delhi).

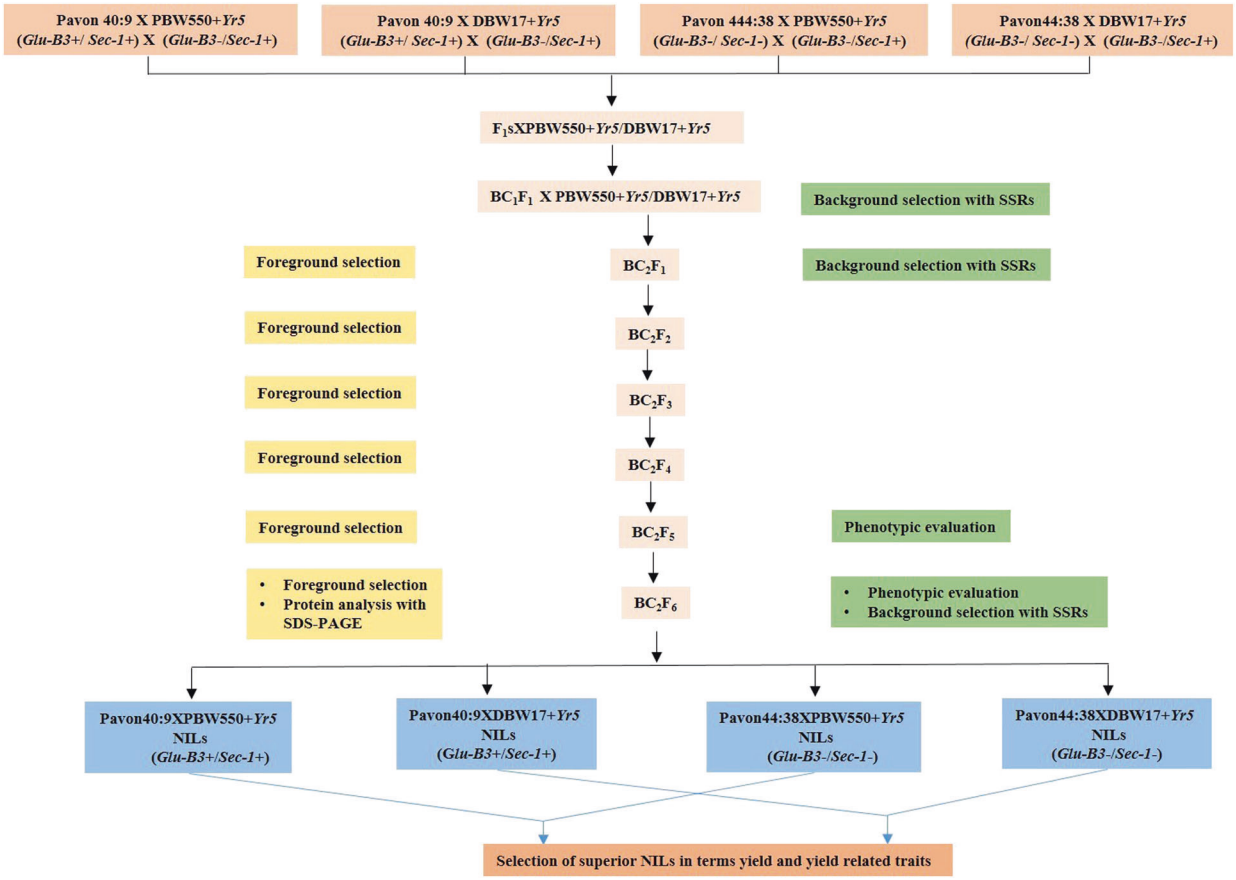

Fig. 1 Gene transfer through marker assisted selection. Schematic representation of the breeding scheme for marker assisted transfer of two loci *Sec-1*$^{-}$ and *GluB3/Gli-B1* in two wheat lines PBW550 and DBW17 carrying the stripe rust resistance gene *Yr5*.

Fermentation was done for 2.5 h at 30 °C 80% relative humidity (RH). Thereafter, the dough was remixed for 25 s to remove the CO_2 generated during fermentation. The dough was kept for 25 min to do the first proofing. The dough sheet was made with the help of a sheeter and moulder and rolled into a cylinder, followed by sealing of sides. The moulded dough was kept in the baking pan and the second proofing was done for 55 min. Baking was done at 230 °C for 25 min. After baking, the bread loaf was measured using the seed displacement method (Greene and Bovell-Benjamin 2004). The weight of bread was calculated 1 h after baking using a weighing balance. The crumb texture was examined by cutting the bread into slices, followed by tasting to test the flavor and chewiness.

Statistical analysis

Descriptive statistics were done using the Summary Tools v0.9.4 package in R-studio (Comtois 2020). The calculation of adjusted means or best linear unbiased predictions (BLUPs) was done using META-R version 6.0 (Alvarado et al. 2016) using a mixed linear model following the model:

$$Y_{ijk} = \mu + R_i + B_j(R_i) + G_k + \epsilon_{ijk}$$

where Y_{ijk} is the trait of interest, μ is the mean effect, R_i is the effect of the i^{th} replicate, $B_j(R_i)$ is the effect of the j^{th} block of i^{th} replicate, G_k is the effect of the k^{th} genotype, $\in_{ijk}$ is the error associated with the j^{th} block of i^{th} replicatefor the k^{th} genotype. $\in_{ijk}$ is assumed to be normally and independently distributed, with mean zero and homoscedastic variance σ^2. The genotypes, replicates, and blocks were considered as random effects to calculate adjusted means across the blocks and replicates.

Comparison of adjusted mean values (BLUPs: Best linear unbiased predictions) was made between genotypes to their respective recurrent parent independently for both the environments. The genotypic coefficient of variability (GCV) and the phenotypic coefficient of variability (PCV) were estimated according to Burton and Devane (1953), GCV was calculated using the equation

$$GCV = \frac{\sqrt{\sigma_g^2}}{\overline{x}} \times 100$$

where σ_g^2 is the genotypic variance, and $\overline{x}$ is the mean of all genotypes. The PCV was calculated using the equation

$$PCV = \frac{\sqrt{\sigma_p^2}}{\overline{x}} \times 100$$

where σ_p^2 is the phenotypic variance. The environmental/error coefficient of variability (ECV) was calculated using the equation

$$ECV = \frac{\sqrt{\sigma_e^2}}{\overline{x}} \times 100$$

where σ_e^2 is the error/environmental variance across the blocks and the replicates. The broad-sense heritability estimated the quality of the breeding program for the traits and the environments. The broad-sense heritability and genetic advance over mean were estimated using the formula by Allard (1960). Genetic advance is measured as low (for characters showing a GAM value of <10%), moderate (for characters showing a GAM value of 10–20%), and high (for characters showing a GAM value of >20%) (Johnson et al. 1955).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

$$GAM = \frac{h^2 \times K \times \sigma_p^2}{\overline{x}}$$

where K is selection differential at 5% intensity of selection (K = 2.06). The correlations were calculated as simple pairwise Pearson's correlations

Table 1. Summary of the number of plants selected in different generations for marker assisted transfer for the absence of *Sec-1*$^{-}$ loci and the presence of *GluB3/Gli-1* loci in two wheat cultivars PBW550 and DBW17 having *Yr5* gene.

Crosses	Year, generation and number of plants selected through foreground and background selection											
	2015 OS	2015 MS							2016 OS			
	2016 MS	2017 OS							2017 MS		2018 OS	
2018 MS	2019 MS	Background recovery										
Parents	F_1	BC_1F_1	BC_2F_2	BC_2F_3	BC_2F_4	BC_2F_5	BC_2F_6		Range		Average	
Pavon44:38 X PBW550+*Yr5*	Crosses done	Back-cross done	387/32	687/72	72/35	130	75	103	61	44.44–100		76.06
Pavon44:38 X DBW17+*Yr5*			319/29	723/63	63/23	165	52	69	49	44.44–100		73.05
Pavon40:9 X PBW550+*Yr5*			418/16	642/67	67/30	95	45	39	11	44.44–88.88		74.33
Pavon40:9 X DBW17+*Yr5*			429/28	578/79	79/32	100	54	15	5	66.66–88.88		77.77

OS-offseason wheat nursery at Keylong Himachal Pradesh, India; MS Main wheat season at PAU Ludhiana, India; BC –Backcross.
From BC_1F_1 to BC_2F_4 selection was done as a single plant while in BC_2F_5 and BC_2F_6 selection was done of progeny as whole.

among traits. It is defined as:

$$r = \frac{COV_{xy}}{\sigma_x^2 + \sigma_y^2}$$

where COV_{xy} is the covariance between trait x and trait y, σ_x^2 is the variance of trait x, and σ_y^2 is the variance of trait y. The coefficient of skewness (β_1) and kurtosis (β_2) is as:

$$\beta_1 = \frac{\mu_3^2}{\mu_2^2}$$

$$\beta_2 = \frac{\mu_4^2}{\mu_2^2}$$

where $\mu_2^2 = \frac{1}{n}\sum fi(Xi - \overline{X})^2$; $\mu_3^2 = \frac{1}{n}\sum fi(Xi - \overline{X})^3$; $\mu_4^2 = \frac{1}{n}\sum fi(Xi - \overline{X})^4$. And, fi is the frequency, Xi is the random variable and $\overline{X}$is the mean value.

The relationship among the traits was studied by Principal Component Analysis using FactoMineR v2.4 (Lê et al. 2008) and FactoExtra v1.0.7 (Kassambara and Mundt 2020) in R-studio v4.0.3. The principal components for both the years were plotted as biplots of eigen vectors. Structural equation modeling (SEM) was done using the package lavaan v 0.6-7 (Rosseel 2012) and visualized using package sem Plot v1.1.2 (Epskamp 2019) to identify the direct and indirect contributors of yield.

RESULTS

Transfer of *Glu-B3*$^{+}$ and *Sec-1*$^{-}$ locus into PBW550 and DBW17

The details of the number of plants selected in advanced backcross generations in each of four crosses are given in Table 1 and a schematic representation for the development of NILs with improved 1RS chromosomal arm is given in Fig. 1. In 2016 OS, a total of 1553 BC_1F_1 plants from the four crosses were planted and MAS was done for four genes in each of the crosses i.e., for two targeted genes, *Sec-1*$^{-}$ or *Glu-B3*$^{+}$ in addition to selection for three genes 1RS^{+}, *Pm8*$^{+}$ and *Lr26/Yr9/Sr31*$^{+}$cluster on chromosomal arm 1RS (Figs. S2, S3, S4, S5, S6, S7). The selected plants were backcrossed with respective recurrent parents and 2630 BC_2F_1 plants were sown in 2016–17 MS. The BC_2F_1 plants with two targeted genes in homozygous/heterozygous form and with 70% or more recurrent parent genome were selected. In 2017 OS, BC_2F_2 plants were sown as plant to row progenies and two plants per progeny, homozygous for targeted genes, were selected. In 2017–18 MS, BC_2F_3 plants were sown as plant to row progenies and the plants homozygous for targeted genes along with 80–85% background genome recovery were selected followed by harvesting of three single plants from selected progenies. A similar strategy of selection was employed for BC_2F_4 plants in 2018 OS. This was followed by sowing of BC_2F_5 and BC_2F_6 NILs in replicated yield trials in 2018–19 MS(E1) and 2019–20 MS(E2), respectively. The final selection of five plants/progeny was made after molecular characterization for four targeted genes along with background selection.

Phenotypic evaluation

The phenotypic evaluation of BC_2F_5 and BC_2F_6 NILs from four different crosses was done in the year 2018–19 (E1), and the year 2019–20(E2). In E1, 172 BC_2F_5 NILs in the background of PBW550 (103 NILs), DBW17 (69 NILs) with *Sec-1*$^{-}$ and 54 NILs with *Glu-B3*$^{+}$ gene in the background of PBW550 (39 NILs), DBW17 (15 NILs) were evaluated. The selected 126 BC_2F_6 NILs, comprising of 110 NILs with *Sec-1*$^{-}$ in the background of PBW550 (61 NILs), DBW17 (49 NILs) and 16 NILs with *Glu-B3*$^{+}$ gene in the background of PBW550 (11 NILs) and DBW17 (5 NILs), were evaluated in E2 (Table 1).

The adjusted means/BLUPs based on ANOVA of each year were compared with respective recurrent parents for the four crosses and selections were done for progenies equal to/outperforming their recurrent parents. Significant variation was observed across the panel of NILs, in both years (Fig. 2). The yield-related traits, i.e.,

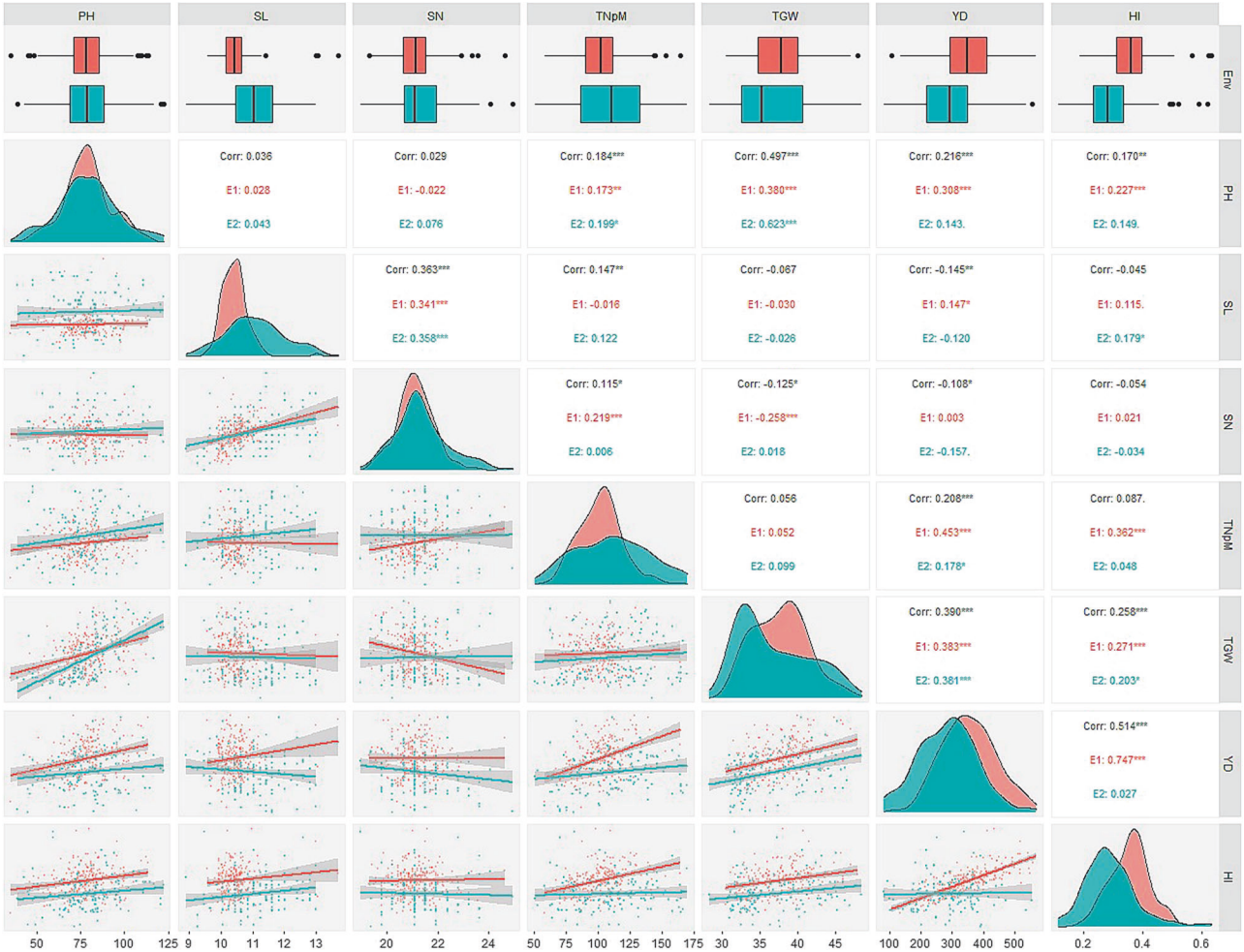

Fig. 2 Pairwise correlation analysis. Correlation coefficient of different yield-related traits in near isogenic lines (NILs) across two generations, BC_2F_5 and BC_2F_6. PH-Plant height, SL-Spike length, SN- Spikelet number/spike, TNpM-Tillers number per meter, TGW-Thousand grain weight, YD-Total Yield, HI-Harvest Index.

TGW, TNpM, HI, and yield, showed significant improvement over the recurrent parents, signifying the positive effect of selection across the 2 years (Fig. 2).

In the NILs selected for the presence of the glutenin (*Glu-B3*$^+$) gene, variations were observed for all the parameters scored. The PH of PBW550 NILs was relatively variable for both the years with a decrease in value observed in E2 (66.25 cm) compared to E1 (71.23 cm), while DBW17 NILs showed a similar trait value in both the seasons (73.08 in E1 and 71.23 cm in E2). While the trait value of PBW550 NILs was less for PH but the value of SL and SN was higher than DBW17.There was a significant decrease in the range of YD in E2 for both PBW550 (135.05–568.6 g in E1, 99.79–328.78 g in E2) and DBW17 (227.68–471.8 g in E1, 173.25–414.44 g in E2), with the average performance of DBW17 NILs superior across both environments. There was a decrease in value for YD-related traits in E2, both in the background of PBW550 and DBW17 for *Glu-B3*$^+$ NILs. Overall, DBW17 NILs outperformed PBW550 NILs in YD-related traits (Table S2). The final selection was made considering the performance of NILs relative to recurrent parents. *Sec-1*$^-$ NILs outperformed the *Glu-B3*$^+$ NILs in both backgrounds, as PH of *Sec-1*$^-$ NILs was optimum relative to*Glu-B3*$^+$ NILs. There was no significant difference in SL and SN in *Sec-1*$^-$ and *Glu-B3*+ NILs. The YD and YD-related traits performance were comparatively better in *Sec-1*$^-$ NILs compared to *Glu-B3*$^+$ NILs.

The overall average data and the range of the NILs for all the traits in E1 and E2 along with the recurrent parent values are given in Table 2. *Sec-1*$^-$ NILs in PBW550 background had higher mean values of PH, SL and SN as compared to *Sec-1*$^-$NILs in DBW17 in both environments. However, the average trait values for SL and SN was more in E2 than E1. For YD, NILs in both backgrounds had lower values in E2, ranging from 83.67 to 554.56 g compared to 108.38–568.60 g observed in E1. A similar trend was observed for both recurrent parents (PBW550- 409 g in E1, 402 g E1, and DBW17- 419 g in E1, 37.86 g in E2).The TGW of PBW550 NILs was almost similar in both environments, E1 (30.53–48.02 g) and E2 (30.97–48.51 g) whereas, for DBW17 NILs there was a decrease in value observed in E2 (28.38–45.27 g) compared to E1 (30.55–47.09 g), with the recurrent parents PBW550 (43 g in E1, 40 g E2), and DBW17 (39.77 g in E1, 37.86 g in E2) showing similar trends. HI was found to be constant across two environments, E1 and E2. However, based on the observed range of HI, PBW550 NILs (0.2–0.51) outperformed DBW17 NILs (0.19–0.63) in E1(Table S2). The final selection was done based on the better or comparable performance of NILs to the recurrent parents.

For both years, PH showed skewness of 0.03 and 0.15, with a leptokurtic distribution (0.29, 0.03), exhibiting a decrease in value for the 2019–20 year. SL showed positive skewness of 2.68 and 0.16 with platykurtic distribution (−0.4.2) in E2 compared to E1 (14.20). SN showed positive skewness of 0.57 and 0.65 with leptokurtic distribution for both years (1.91, 0.61). The TNpM exhibited positive skewness of 0.38 and 0.08 with leptokurtic distribution (0.56) in E1and platykurtic distribution in E2 (−0.79)

Table 2. Summary of the phenotypic variabilities of the NILs and parental lines across two environments E1 (BC_2F_5) and E2 (BC_2F_6) after foreground selection of *Sec-1*$^-$ and *Glu-B3*$^+$ loci and background selection of recurrent parents PBW550 and DBW17.

Trait	Env	PBW550	DBW17	Pavon40:9	Pavon44.38	Range	Mean	StdDev	Skewness	Kurtosis
PH	E1	84.18	77.36	66.25	79.91	35.37–113.66	78.71	13.69	0.03	0.29
	E2	86.92	80.99	72.09	81.98	39.13–122.18	78.97	17.13	0.15	0.03
SL	E1	10.24	9.64	10.15	10.00	9.57–13.68	10.45	0.48	2.68	14.20
	E2	11.24	11.83	11.63	12.80	8.91–12.99	11.07	0.90	0.16	−0.42
SN	E1	20.35	21.38	19.83	20.64	19.34–24.64	21.14	0.75	0.57	1.91
	E2	20.25	21.10	19.40	18.12	18.97–24.94	21.35	1.05	0.65	0.61
TNpM	E1	96.49	106.20	107.82	87.75	58.31–164.65	101.72	17.07	0.38	0.56
	E2	105.84	114.58	112.32	104.54	50.79–169.87	110.86	28.61	0.08	−0.79
TGW	E1	43.00	39.77	31.98	39.82	30.53–48.02	37.72	3.63	0.24	−0.41
	E2	40.16	37.86	37.53	38.04	28.38–48.51	36.84	4.78	0.56	−0.92
YD	E1	409.80	419.96	410.73	360.56	108.38–568.60	353.62	86.40	0.11	−0.30
	E2	402.25	347.45	421.60	441.05	83.67–554.56	283.91	92.27	0.11	−0.03
HI	E1	0.40	0.41	0.41	0.30	0.19–0.63	0.36	0.07	0.41	1.10
	E2	0.47	0.38	0.40	0.32	0.11–0.62	0.29	0.09	0.93	1.88

PH Plant height, *SL* Spike length, *SN* spikelet no. per spike, *TNpM* tiller number per meter, *TGW* thousand grain weight, *YD* yield per plot, *HI* harvest index.

Table 3. Summary of the genotypic variabilities of the NILs across two environments E1 (BC_2F_5) and E2 (BC_2F_6) generations after foreground and background selection.

Trait	Env	GCV	ECV	PCV	H2	GAM	LSD	CV
PH	E1	17.86	08.11	19.62	91.03	36.79	9.90	8.11
	E2	25.56	04.74	26.00	98.31	52.65	6.05	4.74
SL	E1	06.78	12.84	14.52	46.69	13.96	1.45	12.84
	E2	10.82	15.84	19.18	56.41	22.29	2.16	15.84
SN	E1	04.58	06.32	07.80	58.72	09.44	1.68	6.32
	E2	06.45	08.40	10.59	60.91	13.29	2.30	8.40
TNpM	E1	16.99	05.11	17.74	95.77	35.00	8.22	5.11
	E2	26.92	07.98	28.08	95.87	55.45	14.12	7.98
TGW	E1	10.01	04.89	11.14	89.86	20.63	2.85	4.89
	E2	13.22	05.90	14.48	91.30	27.23	3.39	5.90
YD	E1	24.65	05.07	25.16	97.97	50.78	28.55	5.07
	E2	32.68	07.99	33.64	97.15	67.33	36.62	7.99
HI	E1	23.28	22.90	32.65	71.30	47.96	0.11	22.90
	E2	47.35	29.71	55.90	84.70	97.53	0.17	35.77

h^2 Heritability broad sense, *GCV* Genotypic Coefficient of Variance, *PCV* Phenotypic Coefficient of Variance, *ECV* Residual/Environmental Coefficient of Variance, *CV* Coefficient of variation significant at $\alpha < 0.05$.

for the second year. The TGW showed positively skewed values 0.24 and 0.56 with platykurtic distribution for both years (−0.41, −0.92,) while the yield showed positively skewed values 0.11 and 0.11, with platykurtic distribution for both years (−0.30, −0.03).The HI showed positive skewness of 0.41 and 0.93, with leptokurtic distribution for both years (1.10, 1.88) (Table 2).

Genetic variability, heritability, and genetic advance

The estimates of genetic variability are given in Table 3. In the present study, moderate to high GCV and PCV were observed for all the traits except SL and SN. The high coefficient of variation suggests high variability among the NILs. The GCV value for all the traits was higher than the ECV value, conferring a lower effect of the environment.

The value of broad-sense heritability for various traits ranged from 46.69 to 98.31% across the 2 years. Moderate to high heritability was observed for SL and SN. High heritability was observed for PH, TNpM, TGW, YD, HI while SL and SN exhibited moderate heritability values. Also, higher GAM values were observed for PH, TNpM, TGW, YD, and HI. Although there is not much difference between the mean values of the traits for both years (Table 3), the GAM of the second year was significantly more than the first year, hence the superior selection of progenies.

Correlation analysis

Correlation studies were conducted to determine the relationship between various agro-morphological traits and yield (Fig. 2). For progenies, PH showed a positive correlation with yield for E1 (0.308) and E2 (0.143). SL showed a positive correlation with yield for E1 (0.147) and a negative correlation in the year E2 (−0.120). While SN had a positive correlation with yield for E1 (0.003), a negative correlation was observed in the year E2 (−0.157).

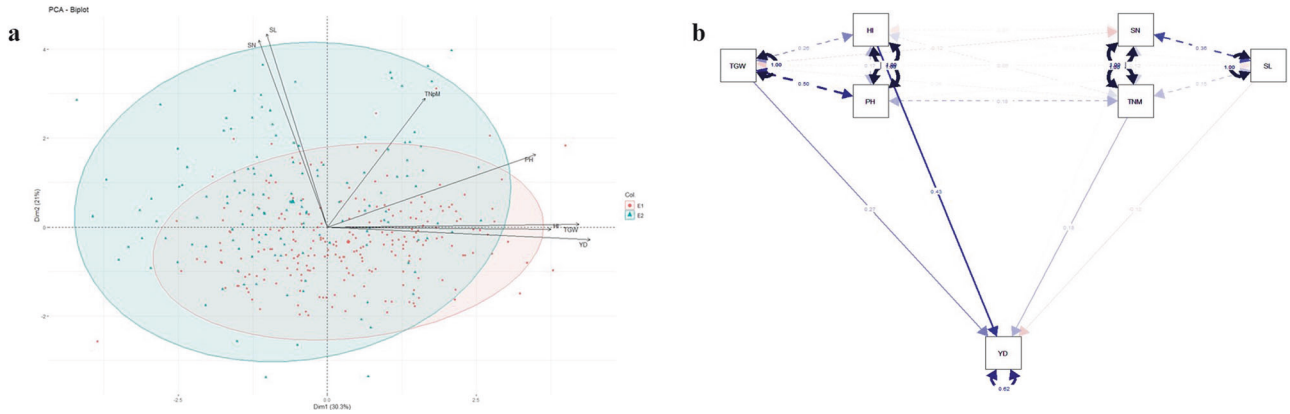

Fig. 3 Multivariate analysis. a Principal component analysis, and (**b**) Multivariate analysis by structural equation modeling among different yield-related traits in near isogenic lines (NILs) across two generations, BC_2F_5 andBC_2F_6. †Red and blue colour represents eigen vectors in season 2018–19, and 2019–20, respectively, for (**a**) ††Red, and blue colour represents negative, and positive contributions, respectively, for (**b**) †††PH-Plant height, SL-Spike length, SN- Spikelet number/spike, TNpM-Tillers number per meter, TGW-Thousand grain weight, YD-Total Yield, HI-Harvest Index.

However, both TNpM and TGW showed a highly significant positive correlation with yield. For the years E1 and E2, TNpM showed a positive correlation of 0.453 and 0.178, respectively. Likewise, TGW showed a positive correlation of 0.383 and 0.381 for the years E1 and E2. HI was also found to have a positive relationship with yield.

Principal component analysis

The principal component analysis, an exploratory tool for data analysis, offers details about traits by elucidating the population's maximum variability in the given environments (Fig. 3a). The eigen vectors in the first two principal components explained 51.3% of the total variability across the environments. The observed high GxE effect of the lines could be attributed to the selection of superior genotypes across years, differences in sowing dates, or differences in weather from year to year. Overall, PCA depicted YD to be dependent more on TGW, HI and least dependent on SL and SN. However, SN was more dependent on SL. Similarly, TGW was dependent on HI more than PH. YD was also dependent upon PH and TNpM, as discussed in correlation analysis.

Multivariate analysis by structural equation modeling

PCA revealed YD to be dependent on different characters to varying extents. Thus, Structural equation modelling (SEM) was done to investigate the direct and indirect variables that determined YD.The SEM showed that the TNpM, TGW, and HI were the main direct contributors of YD (Fig. 3b). However, PH contributed indirectly toward YD through TGW and TNpM.

Identification of superior NILs

After the transfer and selection of *Sec-1*$^{-}$ and *Glu-B3*$^{+}$ loci, 30 BC_2F_6 NILs were selected based on agronomic performance and absence/presence of protein encoded by these two loci (Table 4). Twenty-oneNILs with *Sec-1*$^{-}$weresuperior/comparable to respective recurrent parents, for yield-related traits. Of these, 8 NILs in PBW550 background had higher TGW of which NIL-102 also had higher YD than PBW550 (537.67 g). In the DBW17 background, 9 selected NILs had higher values for YD than the recurrent parent butfour of these had lower TGW than the recurrent parent DBW17. TNpM values of fiveof these NILs (besides NIL115, 128, and 108) was also better than DBW17.

In the background of PBW550 and DBW17, nine Glu-B3+ NILs with yield performance superior/comparable to the respective recurrent parent were chosen (Table 4). The YD value of five NILs (NILs-55, 6, 7, 53, 56, and 64) in the PBW550 background was fairly similar to the recurrent parent. For TGW, NIL-56 and 67 outperformed PBW550, whereas, for TNpM, NIL-56, 64, and 67 outperformed PBW550. NIL-58 and 61 had a higher YD value than the recurrent parent in the DBW17 background. NIL-61 and 57 had higher TGW than the recurrent parent, and all of the selected NILs had PH between 75 and 110 cm.

The presence of glutenins/gliadins encoded by the *Glu-B3*gene and the absence of secalin protein, as determined by SDS-PAGE, was a major determining factor in the selection of a final set of 30 best performing NILs (Figs. 4, 5). Of these, 21 selected NILs with *Sec-1*$^{-}$showed the absence of secalin proteins with sizes ranging from 42 to 55 kDa, while 9 NILs with *Glu-B3*$^{+}$showed the presence of LMW proteins with sizes ranging from 42 to 50 kDa. Thus, the yield-related components of the 30 selected NILs were comparable to PBW550/DBW17 and 1RS chromosomal arm substituted for loci *Sec-1−/Glu-B3+*.

Bread baking

The bread baking characteristics of four selected *Sec-1*$^{-}$NILs and four selected *Glu-B3*$^{+}$ NILs in PBW550/DBW17 background, along with donor and recurrent parents are given in Table S3. In comparison to sticky dough obtained from PBW550 and DBW17, *Sec-1*$^{-}$NILs had non-sticky dough and *Glu-B3*$^{+}$NILs had semi-sticky dough. All the selected NILs with *Sec-1*$^{-}$ and *Glu-B3*$^{+}$ had a lower bread weight than the recurrent parents PBW550 (144.21 g) and DBW17 (147.03 g). *Sec-1*$^{-}$ NILs had specific loaf volume ranging from 460 to 469cc, while *Glu-B3*$^{+}$NILs had loaf volume ranging from 455 to 465cc, with PBW550 and DBW17 having loaf volumes of 400 and 395cc, respectively. The crumb texture of PBW550 was semi-soft, as opposed to the hard texture of DBW17, and the NILs had a soft crumb texture.

DISCUSSION

In the present work two important wheat cultivars PBW550 and DBW17 were selected to replace two important loci on rye 1RS arm, (presence of *Sec-1* and absence of *Glu-B3/GliB1*) with corresponding 1BS wheat chromatin. As a result, lines with improved bread making quality balanced with yield-related traits, were generated using a combination of MAS and agro-morphological evaluation. The superiority of PBW550 and DBW17 varieties in terms of quality parameters makes them the most suited as recipients for alteration in two targeted loci. PBW550 is a medium short duration variety known for its bold grain and processing quality and has been cultivated in almost all wheat-growing regions of the country in the few years since its

Table 4. Summary of finally selected BC_2F_6 near isogenic lines (NILs) for different yield component traits with absence of *Sec-1*$^-$and presence of *Glu-B3*$^+$ loci on 1RS chromosomal arm.

Recurrent parent	PH	SL	SN	TNpM	TGW	YD	HI
PBW550	86.92	11.24	20.25	105.84	40.16	402.25	0.47
DBW17	80.99	11.83	21.10	114.58	37.86	347.45	0.38
Pavon44:38 × PBW550							
NIL-11	76.37	12.02	21.96	142.11	34.53	365.89	0.40
NIL-13	80.24	12.60	22.81	70.86	37.34	251.37	0.59
NIL-14	89.23	12.02	23.66	158.30	43.21	390.94	0.49
NIL-26	94.49	10.65	21.10	105.83	45.12	369.42	0.50
NIL-72	95.82	10.46	21.10	92.77	46.87	433.45	0.23
NIL-79	84.61	10.66	21.10	113.29	41.01	382.77	0.32
NIL-86	110.6	12.41	22.38	133.04	41.93	456.09	0.34
NIL-88	79.00	10.46	21.52	124.62	31.10	367.26	0.37
NIL-90	94.50	10.66	21.10	105.84	45.12	369.42	0.50
NIL-97	83.95	11.05	20.68	114.91	37.99	439.75	0.33
NIL-102	82.31	10.08	21.10	83.49	45.28	537.67	0.35
Pavon44:38 × DBW17							
NIL-41	83.62	11.44	21.96	147.29	39.47	360.18	0.32
NIL-107	82.42	11.63	21.10	133.69	34.67	384.93	0.32
NIL-108	83.04	10.08	20.25	100.23	33.85	360.58	0.30
NIL-110	97.79	9.69	20.68	144.05	39.16	448.66	0.36
NIL-115	74.73	11.05	20.68	108.75	38.91	409.98	0.35
NIL-119	107.9	11.24	21.53	138.55	36.58	366.66	0.34
NIL-122	94.17	10.27	20.68	126.57	45.10	424.76	0.30
NIL-123	83.95	10.69	19.40	134.01	42.67	381.05	0.36
NIL-128	74.73	11.05	19.83	109.40	36.47	395.20	0.32
NIL-131	82.86	11.63	21.53	125.92	34.81	360.24	0.37
Pavon40:9 × PBW550							
NIL-53	71.10	10.46	21.10	90.29	32.65	321.56	0.27
NIL-55	74.07	10.46	21.10	105.19	32.54	328.78	0.24
NIL-56	81.98	10.85	21.10	137.58	46.23	304.26	0.25
NIL-64	73.17	12.80	19.83	136.28	30.97	300.83	0.26
NIL-65	76.04	10.08	21.10	85.76	33.36	295.73	0.24
NIL-67	72.42	11.05	21.10	127.54	38.53	311.87	0.36
Pavon40:9 × DBW17							
NIL-57	74.73	11.05	21.10	94.50	38.34	264.33	0.35
NIL-58	72.76	10.91	20.25	73.78	32.19	367.64	0.37
NIL-61	88.90	9.88	21.53	107.46	45.27	414.44	0.27

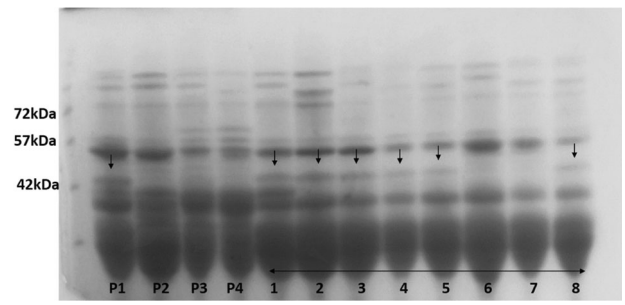

Fig. 4 Presence or absence of the *Glu-B3* locus encoded 42–50 kDa proteins in the parental lines and BC_2F_6 near isogenic lines (NILs), resolved on 10% polyacrylamide gel. P1-Pavon40:9, P2-Pavon44:38, P3-PBW550+*Yr5*, P4-DBW17+*Yr5* and 1 to 8- NILs derived from cross Pavon40:9XPBW550/DBW17; Arrows represent the presence of the *Glu-B3* locus encoded 42–50kDa proteins.

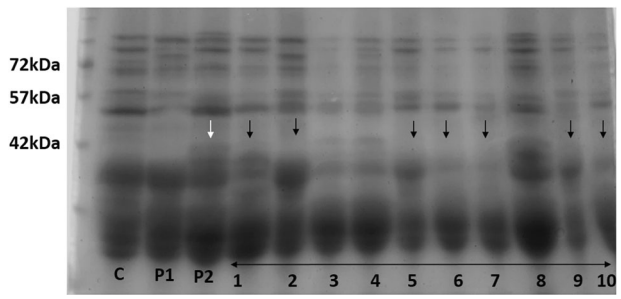

Fig. 5 Presence or absence of the *Sec-1* locus encoded 42–55 KDa proteins in the parental lines and BC_2F_6 near isogenic lines (NILs) resolved on 15% polyacrylamide gel. C- negative control (Chinese spring), P1-Pavon44:38, P2-PBW550+*Yr5*, and 1 to 10 - NILs derived from cross Pavon44:38XPBW550/DW17. White arrow represents the presence of the *Sec-1* locus encoded protein in P2 and black arrow represents absence of the *Sec-1* locus encoded proteins in NILs.

release in 2008 (Kaur et al. 2020). DBW17, another full duration cultivar with appropriate plant height and lodging tolerance, has a protein content ranging between 11 and 12%. The high extraction rate in DBW17 flour to the tune of 70.4% enhances its industrial suitability for more flour recovery (Yadav et al. 2010). Since stripe rust is a major disease of NWPZ, both the cultivars used for improving bread making quality were also pyramided with the stripe rust resistance gene *Yr5*.

Two backcrosses successively recovered the recurrent genotypes of PBW550 and DBW17 as evident from the marker data. For background recovery specifically for the 1RS.1BL chromosome, a comparatively higher number of markers were deployed from the long arm of chromosome 1 as the selection of more recombinants was expected for this chromosome while doing MAS for 1RS arm on this chromosome.

The NILs in PBW550 and DBW17 background were compared with each other to evaluate the effect of changing 1RS on individual genotype also as the positive effect of the translocation is also influenced by genetic background (Oak and Tamhankar 2017). NILs with superior performance for yield-related traits than their respective recurrent parents were selected based on phenotypic selections across the two-year trials (Table 4). Despite large GxE interaction of the lines across the years (Fig. 3a), many genotypes showed better performance than the recurrent parents in both years (Table 4). Large genetic variability among the NILs was detected within each of the two environments, which aided in the selection of better-performing lines (Singh et al. 2018; Dabi et al. 2019).

The exploitation of variability in the form of PCV, GCV, and heritability is essential for the proper selection of lines for any breeding programme (Neelima 2018). A higher magnitude of differences between the GCV and PCV reflects a large environmental effect on any trait (Osman et al. 2012). Moderate to high GCV and PCV for PH, TNpM, TGW, HI, and YD indicated that phenotypic selection was effective and the small differences between the PCV and GCV for most of the traits, i.e., PH, TNpM, TGW, YD, and HI, suggested only a small degree of environmental influence on the phenotypic expression of these characters in the respective years. The heterogeneity coefficients (GCV and PCV) values alone, however, are insufficient to determine the heritable portion of variance passed down from generation to generation, as expressed by broad-sense heritability, while also estimating the quality of the breeding programme for the traits and environments (Lush 1949). Moderate to high broad-sense heritability of the studied traits indicated a higher contribution of the genotypic component of variation for the traits than the environmental effect across the various blocks in each year trial (Table 3). The estimate of heritability is a predictive measure for scrutinizing the reliability of the phenotypic data, and genetic advancement as a percent of the mean is evidence of the expected result of the application of selection pressure on the pertinent population. Hence, heritability along with GAM offers a more consistent index for the selected value (Hanafi et al. 2020). The GAM of the second year was found to be higher than the first, which indicates the positive effect of the selection leading to the selection of superior lines.

Overall, the TNpM and TGW had a positive correlation with the YD. TGW is directly related to the grain yield and milling quality of the grain and impacts the seedling vigor and growth, indirectly affecting the yield (Botwright et al. 2002; Wu et al. 2018). Higher grain weights are positively associated with longer grain filling duration attributed to timely flowering and high grain filling volume (Zhang et al. 2010; Okami et al. 2016). As identified in the present study, this positive correlation has been reported in various studies (Kumar et al. 2014; Bhutto et al. 2016; Birhanu et al. 2017; Reddy et al. 2021). TNpM was found to have a positive correlation with yield across the years. Bhutto et al. (2016) published similar findings, demonstrating that an increase in the number of tillers leads to a proportionate increase in yield per plot. HI has shown a positive correlation with YD as previously documented in different studies (Yang and Zhang 2010; Duan et al. 2018; Porker et al. 2020). The positive association between yield and HI could be of major significance in encouraging breeders in their exploration for increased yield in wheat varieties (Foulkes et al. 2009; Aranjuelo et al. 2013; Duan et al. 2018).

The nature of gene action and the number of genes controlling the trait is usually measured by the critical analysis of distribution properties by third-order statistics such as skewness and kurtosis, which are more important than the first and second-order statistics that unravel only the interaction effects (Rani et al. 2016). Skewness indicates the cluster of deviations above and below the value of central tendency and defines the extent of deviations in the distribution of trait values, and thus could aid in the detection of varying effects like additive effects, dominance, and also epistasis. The positive skewness for all the traits in the present study for both years indicated the traits to be controlled by dominant and complementary gene action and the genetic gain obtained was through intense selection (Neelima 2018) (Table 2).

A Kurtosis indicates the level of peaks over the population with a leptokurtic distribution, which would mean that the trait in question is controlled by fewer genes, whereas a platykurtic distribution would mean that the trait is governed by many genes (Savitha and Kumari 2015). The decrease in the leptokurtic value of PH for the second year suggests that the selection leads to the removal of introgression lines with outlier trait values. The leptokurtic values for the second year could also be because of the decrease in the number of the lines that resulted in overall less distribution.

The principal component analysis is an exploratory tool for data analysis. It offers details about traits by elucidating the population's maximum variability in the given environments. A high GxE effect was observed across the years, depicted by lower variability (51.3%) explained by the principal component analysis (Fig. 3a). Similar results have previously been reported where lower variability has been associated with a large number of genic interactions among the traits due to the additive effect of the genes involved for each trait (Hailegiorgis et al. 2011; Degewione and Alamerew 2013; Nielsen et al. 2014; Mohibullah et al. 2017; Wani et al. 2018; Kiran et al. 2021). TGW was shown to be highly dependent on HI and TNpM and least dependent on SN and SL in the first two PCAs. Similarly, SN was heavily dependent on SL. On the other hand, YD was based on all of the characters analyzed, with HI, TGW, and PH being the most dependent variables (Tshikunde et al. 2019)., the SEM revealed that the main direct contributors to YD were the TNpM, HI, and TGW while PCA suggested that YD was affected by all the characters studied. (Fig. 3b). TGW also has an indirect effect on YD through HI. PH has an indirect effect on YD through HI and TNpM (Bhutta et al. 2006). SL has a direct negative effect on YD, while SN and SL directly affect TGW and indirect negative effect on YD, as explained in PCA (Iftikhar et al. 2012).

An agronomic evaluation of the NILs identified 30 lines that demonstrated substantial improvements for TGW, YD, and TNpM. In the absence of the secalin locus, 11 NILs in the PBW550 background had high TGW, TNpM, with some had high YD also (Sharma et al. 2018; Kaur et al. 2017) while 10 NILs in the DBW17 background had a high YD also. Similarly, for the *Glu-B3*$^{+}$ gene, nine NILs showed better performance for YD, TGW, and TNpM -two NILs in the DBW17 background had higher YD, while in the PBW550 background, two have high TGW and the other three have high TNpM and YD.

The high loaf volume of *Sec-1*$^{-}$ NILs represents the replacement of rye secalin proteins by wheat ω gliadins providing more elasticity to dough (Li et al. 2016). The better loaf volume of *Glu-B3*$^{+}$ NILs than recurrent parents is because of the addition of glutenins and gliadins encoded by *Glu-B3* loci, which provide sulfur residues required for disulfide linkage in intermolecular and intramolecular bonding (Sharma et al. 2020). The better loaf volume of *Sec-1*$^{-}$ NILs than *Glu-B3*$^{+}$ suggests that the effect of absence of secalin proteins is much more pronounced than the presence of glutenins and gliadins (Sharma et al. 2018). When compared for dough

consistency, though the performance of both *Sec-1*$^{-}$ and *Glu-B3*$^{+}$ NILs was better than the recurrent parent, *Sec-1*$^{-}$ NILs were better than *Glu-B3*$^{+}$ NILs. There is possibility of the negative dosage effect induced by duplication of sequences due to the addition of the *Glu-B3*$^{+}$ locus (Gabay et al. 2021).

Overall, the yield performance of the lines with substitution in the distal region of 1RS (*Sec-1*$^{-}$/*Glu-B3*$^{-}$), was better than those with proximal substitution (*Sec-1*$^{+}$/*Glu-B3*$^{+}$) indicating that removal of the proximal secalin locus was more rewarding for yield parameters also (Kaur et al. 2017; Howell et al. 2014; Gabay et al. 2021). Because of this, a comparatively higher number of NILs were finally selected with the *Sec-1*$^{-}$ locus as compared to the *Glu-B3*$^{+}$ locus. These new NILs with resurrected chromosomal arm 1RS have better bread-making quality along with resistance to stripe rust and yield traits comparable with recurrent parents. These selected lines with better performance for different quality, agronomic, and disease traits are potential candidates for the varietal release as well as the base material for future quality-oriented wheat breeding programs.

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AUTHOR CONTRIBUTIONS

SK*—conceptualization, funding acquisition, writing original draft, review and editing; RK, PT and DK—development of material, marker assisted selection in initial generations; RK and SK marker related work in advanced generations. GSD—field based studies, paper writing; GSD and AKaur—statistical analysis, data curation; AKumar—SDS-PAGE analysis; GSM, AS and PS—field evaluation; SKG—writing the paper; PC—conceptualization, development of material, finalizing the paper. All the authors have read the paper and approved it.

COMPETING INTERESTS

The authors declare no competing interests.

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