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RESEARCH ARTICLE

Transfer of GA-sensitive dwarfing gene *Rht18* by marker assisted breeding in HD3086 (*Triticum aestivum* L.) and multi-environment trials under varying seedling emergence regimes

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Abstract

The height reduction caused by the prevalent semi-dwarfing GA-insensitive gene *Rht-B1b* is associated with numerous negative pleiotropic effects, the major ones being reduced coleoptile lengths and lower early establishment rates, especially in moisture-stressed environments worldwide. Hence, breeding for wheat varieties with alternate GA-responsive (GAR) semi-dwarfing genes, such as *Rht18*, is an efficient approach for developing varieties for the moisture-stressed regimes globally. In this study, a structured marker-assisted backcross (MABC) breeding program was conducted to transfer the *Rht18* gene into the mega wheat variety, HD3086. A total of 46 *Rht18* positive BC_2F_5 lines were identified with an average background genome recovery of 88.34% using Wheat Breeder's 35K Axiom SNP Array. Multi-environment trials were carried out at three locations under two conditions, i.e., stubble retained (SR) and rainfed (RF). ANOVA revealed significant differences for most of the traits in these trials. Plant height (PH) reduction in *Rht18* introgressed lines ranged from 8.25–12.97 cm under SR and 5.25–10.15 cm under RF regimes. GGE biplots indicated specifically adapted and stable genotypes for yield in stubble-retained and rainfed regimes. These *Rht18* lines hold strong potential for varietal release with further multi-location and multi-season trials. Ultimately, they will contribute to broadening the genetic base of *Rht* genes, especially for improving wheat adaptation in moisture-stressed environments.

Keywords: Wheat, *Rht18*, marker-assisted backcross breeding, multi-environment trials, GGE biplots

Introduction

Wheat accounts for 21% of the world's food supply and is a vital source of carbohydrate and protein for the growing global population (Enghiad et al. 2017). However, its production faces threats from various biotic and abiotic factors, resulting in a huge gap between potential and actual yield (Golfam et al. 2021). A notable breakthrough in wheat yield improvement is the introduction of height-reducing genes (*Rht*) like *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*), which have transformed wheat into modern dwarf, daylight-insensitive wheat, which increased the production in countries such as Mexico, India, Pakistan and Turkey (Wilhelm et al. 2013). *Rht* genes (*Rht-B1b* and *Rht-D1b*) enhanced wheat yields by preventing lodging and resisting pre-harvest sprouting under high-input cultivation. This height reduction mechanism operates through mutations in DELLA proteins in the nucleus by inhibiting the GA signalling pathway (Van et al. 2021). The gibberellin insensitivity conferred by these genes not only affects other gibberellic acid (GA) dependent developmental processes such as α-amylase production in germinating seeds, root elongation, coleoptile length and

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leaf expansion but also has negative constraints on yield under abiotic stress conditions such as moisture stress, high temperatures and late sowing (Botwright et al. 2001; Alghabari et al. 2015). *Rht-B1b* and *Rht-D1b* reduced the coleoptile length of seedlings, thereby reducing the early establishment rates in the field (Botwright et al. 2001). In contrast, the alternate dwarfing genes lead to height reduction with an increase in grain yield by enhancing coleoptile length and improving early vigour (Wang et al. 2015). Hence, the alternate dwarfing genes have the potential to replace GA insensitive genes in regions with limited water availability. To date, 26 reduced height genes have been reported, including two major dwarfing genes, *Rht-B1b* and *Rht-D1b*, seven from tetraploid wheat and others from hexaploid wheat (Xu et al. 2023). One such alternate dwarfing gene mapped on chromosome 6A is *Rht18* from the durum wheat variety Icaro (Vikhe et al. 2017). These alternate dwarfing genes reduce the plant height without negatively affecting the coleoptile length and seedling vigour (Botwright et al. 2005; Ellis et al. 2004).

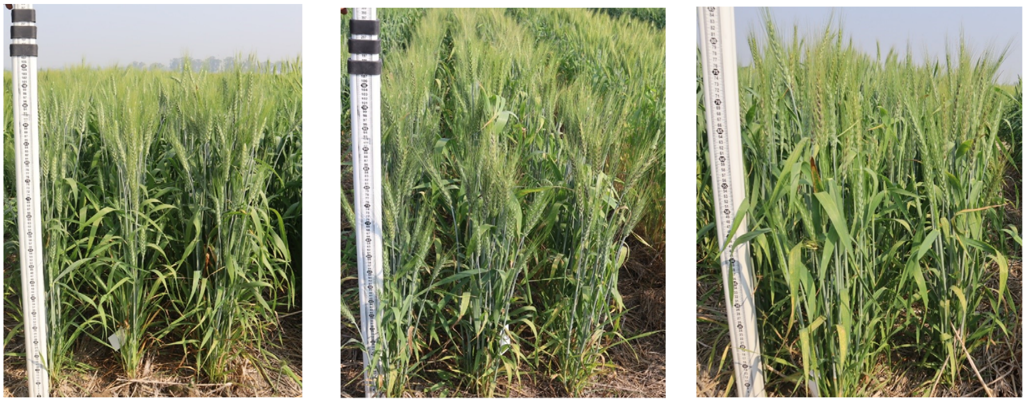
Breeding for moisture stress conditions and conservation agriculture is crucial for South Asian countries like India to combat declining wheat productivity, environmental degradation and climate change. The varieties should excel in emergence through deep residue loads, possess longer and stronger coleoptiles, demonstrate early vigour, have robust root systems and resist multiple diseases (Ranjan et al. 2021). Hence, it becomes imperative to develop and evaluate the varieties under moisture-stressed and residue-retained conditions. MABC is widely employed in wheat to improve economically valuable traits (Dharmaraj et al. 2024). MABC combined with multi-environment trials (MET) with different growth conditions will help breeders to identify genotypes suitable for a particular environment and also stable genotypes across all locations (Tekdal and Kendal 2018).

HD3086 (Pusa Gautami), which is a leading variety in the NWPZ and NEPZ, yields notably higher with timely sowing and occupies half of Northern India's wheat-growing area. Encompassing 11.59 Mha, the NWPZ is vital for India's wheat production and a key area with stubble burning activity (Yadav et al. 2021). HD3086 carries the major height reduction gene *Rht-B1b,* which has shown negative pleiotropic effects under stress conditions. Therefore, in the present study, we performed marker-assisted transfer of GA-sensitive alternate dwarfing gene (*Rht18*) in elite cultivar HD3086 and evaluated the semi-dwarf superior lines under residue retention and moisture stress conditions.

Materials and methods

Development of improved backcross lines through a planned marker-assisted backcross breeding program

The plant material for the study consisted of the hexaploid bread wheat cultivar HD3086 as the recurrent parent (RP) and the durum wheat cultivar Icaro (IC; PI503555) as the donor parent (DP). The initial cross was made between Icaro (DP) and NI5439, which is a tall hexaploid wheat lacking the *Rht-B1b* gene, aiming to introgress *Rht18* in the background of hexaploid wheat. Icaro was used as a female parent, and NI5439 was used as the male parent for the crossing program. Amongst the F$_1$ progenies generated, those displaying reduced height and carrying the *Rht18* gene were selected after confirming the hybridity with linked SSR markers. A backcross was performed using NI5439, and the resulting BC$_1$F$_1$ plants were crossed with HD3086 to generate the F$_1$ progenies. Two additional rounds of backcrossing were performed with HD3086, which resulted in the BC$_2$F$_1$ generation. Foreground selection was carried out in F$_1$, BC$_1$F$_1$ and BC$_2$F$_1$ generations. The plants solely positive for the gene(s) *Rht18* or *Rht18*+*Rht-B1b* combination were selected from the segregating population for the subsequent crosses. Strict phenotypic selection was carried out from the F$_1$ generation onwards to maximize the recovery of traits from HD3086. The selected BC$_2$F$_1$ plants underwent three rounds of selfing, leading to the development of the



HD3086 (*Rht-B1b*) **ATLA84 (*Rht18*)** **ATLA124 (*Rht18*)**

Fig. 1. Field image of the recurrent parent and introgressed *Rht18* positive genotypes

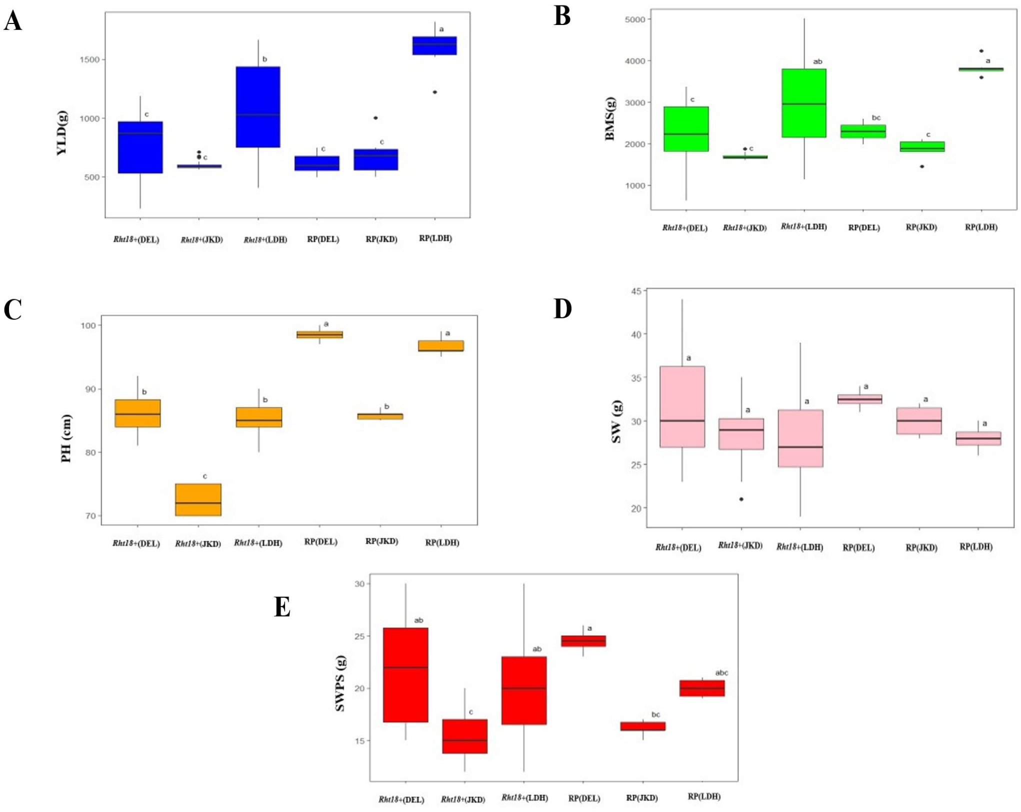


Fig. 2. Boxplots for the traits A. Grain Yield (g) B. Biomass (g) C. Plant height (cm) D. Spike weight (g) E. Seed weight per spike (g) under stubble retained conditions

BC_2F_5 generation. The number of plants forwarded in each generation is given in Table 1.

Foreground selection and allelic characterisation of Rht18 and Rht-B1

Foreground selection for *Rht18* was carried out using a linked SSR marker as described by Vikhe et al. (2017). The forward and reverse primer sequence of SSR marker, S470865SSR4, was 5′-CTAGGCACGAACAAATGGGC-3′ and 5′-GCACCATTCAATTCCCTCGG-3′. The *Rht-B1* gene was screened using a high-throughput fluorescence-based SNP genotyping technology using KASP markers (Ellis et al. 2002). The genomic DNA for genotyping was isolated from freshly collected leaf samples following the procedure detailed by Doyle and Doyle (1987). The PCR products were resolved using 3% agarose gel and visualized with a gel documentation system.

Recovery of recurrent parent genome (HD3086) through background selection

Background selection was performed using high-density SNP markers. *Rht18* positive BC_2F_5 lines, along with the recurrent parent HD3086 and donor parent Icaro, were genotyped using the Wheat Breeder's 35K Axiom Array SNP chips on the Affymetrix GeneTitan® system. The 35K Axiom Array consists of 35,143 evenly dispersed SNPs throughout the entire wheat genome, covering all chromosomes. A total of 14,957 polymorphic SNPs were selected for further analysis to determine the extent of background genome recovery.

Morphological evaluation of the selected lines for stubble retained (SR), rainfed (RF) and irrigated (IR) conditions

One hundred and nine lines were evaluated at three locations under the stubble retained (SR) condition. The study was conducted at three locations: IARI, New Delhi (DEL); BISA, Ludhiana (LDH) and IARI, Jharkhand (JKD). The genotypes were planted in an augmented design type II with six blocks and four checks (HD3086, HI1500, HD2967, HI1544). The fields were prepared by using rice stubble raised to a height of over 20 cm from ground level and covering them with dried rice straw. The crop was irrigated five times throughout the entire growth phase. Each plot consisted of four rows of plants of length 5.0 m. Data was recorded accordingly at the specific stages. The morphological traits evaluated included plant height (cm) (PH), spike weight (g) (SW), seed weight per spike (g) (SWPS), biomass (g) (BMS) and grain yield per plot (g) (YLD). PH was measured at the physiological maturity stage from the base

Table 1. Number of genotypes advanced in each generation for generating the lines positive for *Rht18* gene

Advanced generation	Total No. of plants	No. of plants advanced	Basis of advancement
BC_2F_3	300	215	FS+PS
BC_2F_4	215	109	PS
BC_2F_5	109	46	FS+PS
Final number of plants	46	21	FS+BS+PS

FS= Foreground selection, PS= Phenotypic selection, BS= Background selection

of the plant to the tip of the spike, excluding awns. SW was determined by weighing 10 random spikes using a standard electronic balance. SWPS was measured by dehusking the previously mentioned 10 random spikes and weighing the full, unbroken seeds. BMS was measured before harvest by cutting the crop at the base level, including grains and weighing the entire genotype planted per plot. YLD was measured by harvesting the plot and weighing the harvested grains. The details of the conditions for sowing are given in Supplementary Table S1.

The 109 genotypes were evaluated under rainfed (RF) conditions at three locations: IARI, New Delhi (DEL); IARI, Jharkhand (JKD), and ARI, Pune (PNE). The genotypes were planted in an augmented design type II with the above mentioned checks (Table S1). Each plot size was maintained at 1 m^2 with three rows of plants spaced 30 cm apart. The plants were given a single irrigation at 21 DAS to induce drought stress, and one pre-sowing irrigation. The morphological traits evaluated under rainfed conditions included PH, spike length (cm) (SL), and number of spikelets (NS). SL was measured using a metric scale from the base of the panicle to the tip, excluding awns. NS was counted manually from randomly collected five spikes and the average was calculated. Data were recorded for most of the traits as per Pask et al. (2014).

The genotypes were evaluated under irrigated conditions using an augmented block design II with the four specified checks. The crop received irrigation five times throughout its growth phase. The morphological traits evaluated under irrigated conditions included PH, SWPS, SW, NS, SL, YLD, BMS, harvest index (HI) and thousand grain weight (g) (TGW). TGW was measured by counting thousand grains and weighing them on an electronic weighing balance.

Statistical analysis and data visualisation

Analysis of variance (ANOVA) for each environment was conducted using RStudio version 4.3.2 with the 'augmented RCBD' package (Aravind et al. 2021). The boxplots were constructed using RStudio version 4.3.2 with the 'ggplot2' and 'plyr' packages. The evaluation of genotypes and their respective environments was carried out using genotype plus genotype by environment (GGE) analyses in the package "metan" (Olivoto and Lucio 2020).

Results

Development of the improved backcross lines following foreground and background analysis.

Foreground selection

SSR marker employed for foreground selection resulted in the identification of 215 *Rht18* positive plants. The genotypes were advanced to the BC_2F_4 generation, in which stringent phenotypic selection for disease resistance, absence of major dwarfing gene and superior agronomic traits resulted in the advancement of 109 plants to the BC_2F_5 generation. From these 109 lines, ultimately 46 *Rht18* positive lines were selected (Table 1). Throughout the generations, lines with both homozygous and heterozygous alleles for *Rht18* were retained for further development and evaluation.

Recovery of recurrent parent genome (HD3086)

Among the 46 selected lines harbouring *Rht18*, nine lines showing greater phenotypic similarity with the recurrent parent were genotyped using a 35K Axiom Array. The percent recovery on the carrier chromosome, i.e., 6A, is 64 to 84.8% (Table 2). Overall, the recovery per cent across all the chromosomes ranged between 72.78–94.36% in the improved backcross-derived lines. The average recurrent parent genome recovery was found to be 88.34%. Out of the nine genotyped lines, ATLA 131, ATLA 130, ATLA 200, ATLA 163, and ATLA 138 exhibited the maximum RPG recovery, which exceeded 90%. The maximum and minimum RPG recovery on chromosome 6A was observed in ATLA 131 and ATLA 114. The field image of HD3086 and the *Rht18* positive genotypes are depicted in Fig. 1.

Morphological evaluation of the selected lines for stubble retained (SR), rainfed (RF) and irrigated conditions

The analysis of variance (ANOVA) for various traits at each location for SR conditions is given in Table 3 ($p < 0.05$*, $p < 0.01$**). Significant differences were observed among all the selected lines for the studied traits across the three locations. On average, the PH and BMS were lowest in JKD, followed by DEL and LDH. Similarly, the average SW and SWPS were lowest in JKD, followed by LDH and DEL. The reduction in the following traits in JKD can be attributed to its distinct geographical location (Eastern India). The highest YLD was observed in LDH, while the lowest was recorded in JKD, indicating that the genotypes vary in their YLD performance across locations. The *Rht18* lines performed better than HD3086 in DEL, whereas HD3086 gave higher average yields than the *Rht18* lines in LDH and JKD (Fig. 2). For BMS and SW, HD3086 showed superior performance than the *Rht18* lines across the three locations. For SWPS, although HD3086 outperformed the *Rht18* lines in DEL and JKD, the performance was at par in LDH for both groups (Fig. 2). There were individual lines within the *Rht18* group that showed superior performance over HD3086. The PH was significantly reduced in the *Rht18* lines in comparison to HD3086 at all three locations. The PH was 12.40, 11.71 and 13.58 cm shorter than HD3086 in DEL, LDH and JKD, respectively. Three *Rht18* genotypes (ATLA132, ATLA142, ATLA163) showed higher SW, SWPS and BMS over HD3086, although it was not statistically significant over HD3086.

The results of ANOVA for the rainfed condition revealed significant differences for all the evaluated traits across locations except for SL in DEL (Table 4), indicating that the traits exhibit significant variations under rainfed conditions ($p < 0.05^*$, $p < 0.01^{**}$). The average PH was highest in DEL (84.26±1.76 cm) and showed a notable reduction in JKD (69.93±5.28 cm) and PNE (78.20±6.64 cm). NS exhibited the highest values in DEL, followed by JKD and PNE. The SL was highest in DEL, followed by a decreasing trend (DEL > JKD > PNE) (Fig.3). In DEL, three genotypes (ATLA1, ATLA68, ATLA166) showed superiority in NS and SL. YLD of *Rht*18 genotypes was comparable with HD3086 at DEL and JKD, while at PNE, *Rht*18 genotypes had higher YLD. However, ANOVA showed non-significant differences for YLD in JKD and PNE locations.

The selected BC_2F_5 lines were evaluated under irrigated conditions in DEL (Table 3). Amongst the seven traits evaluated at DEL, the four traits - PH, SWPS, BMS and YLD depicted highly significant variance. Ten *Rht18* genotypes had higher average YLD than HD3086. The maximum SW (45.60 g) and SL (14.00 g) were reported in *Rht18* genotypes, ATLA166 and ATLA142. SL showed an increase in the selected lines in DEL in rainfed and irrigated conditions.

Identification of stable lines by GGE interaction in stubble retained and moisture-stressed environment

In the stubble retained condition in DEL, six genotypes were specifically adapted, which included three double dwarf (*Rht18* +*Rht-B1b*) genotypes and three *Rht-B1b* genotypes (ATLA51, ATLA94, ATLA120). In LDH, the specifically adapted genotypes included one *Rht18* genotype (ATLA15), three double dwarfs, and one tall genotype. In JKD, the specifically adapted genotypes included four *Rht18* genotypes (ATLA57, ATLA130, ATLA175, ATLA176), three double dwarf and two *Rht-B1b* positive genotypes. The results of the GGE interactions indicate that the newly developed *Rht18* lines have good potential in the JKD location.

In rainfed conditions in DEL, two *Rht18* genotypes (ATLA1, ATLA166), three *Rht-B1b* genotypes, and one tall genotype were found to be specifically adapted. In PNE, four *Rht18* genotypes (ATLA21, ATLA59, ATLA84, ATLA107), one *Rht-B1b* genotype, four double dwarf genotypes, and one tall genotype were found to be specifically adapted. In JKD, three *Rht18* genotypes (ATLA99, ATLA114, ATLA155),

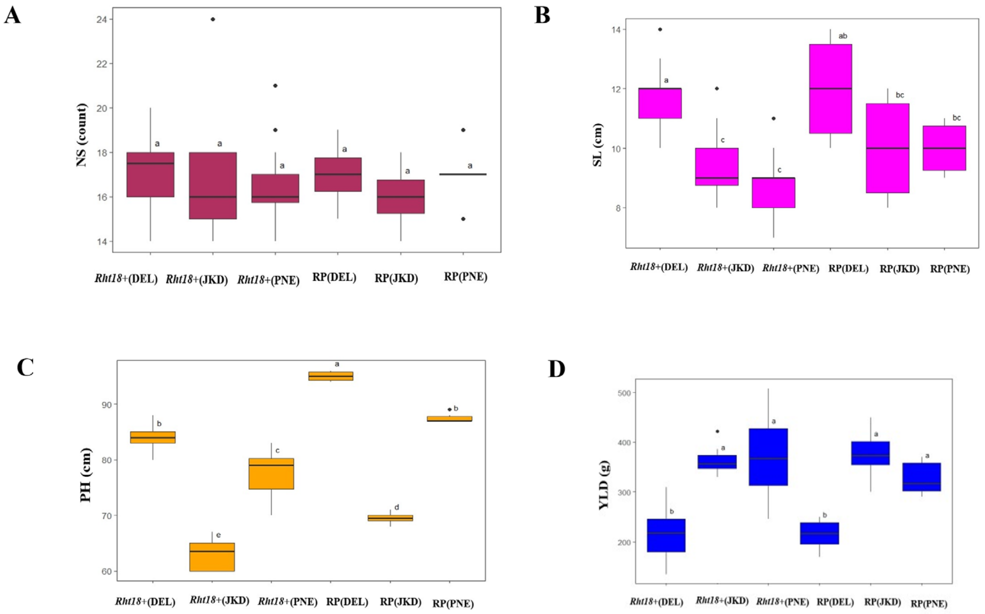


Fig. 3. Boxplots for the traits A. Grain Yield (g) B. Spike Length (cm) C. Number of spikelets (count) D. Plant height (cm) under rainfed conditions at DEL, JKD, PNE locations

three *Rht-B1b* genotypes, and four double dwarf genotypes were specifically adapted. JKD was the stable environment in both stubble retained and rainfed conditions (Fig. 4).

Discussion

The improved HYVs harbouring the major GA-insensitive semi-dwarfing genes (*Rht-B1b* and *Rht-D1b*) have brought significant yield gains through their synergistic effects with increased irrigation, mechanisation and higher fertiliser inputs (Vinod et al. 2022; Jobson et al. 2019); however, these genes exhibit negative pleiotropic effects under high temperature and moisture stress conditions. Therefore, deployment of GA responsive (GAR) semi-dwarf genes, which focus on the concept of Green Revolution 2.0, is imperative to address the above-mentioned pressing issues worldwide. A successful genetic transfer of the GA-sensitive semi-dwarfing gene *Rht18* was achieved in the background of HD3086 through a planned MABC programme. Five genotypes recorded a recurrent parent recovery per cent of >90% using high-throughput SNP genotyping (Table 2). High recovery per cent indicated that the improved lines have attained a high degree of phenotypic similarity to the RP (HD3086).

Rht18 introgressed lines evaluated in a multi-environment trial (MET) under rainfed conditions showed a reduction in PH by 2–10 cm (2.9–11.9%) compared to HD3086. These results corroborate earlier reports indicating that introgression of *Rht18* can reduce PH significantly from 12.4%-25% (Vikhe et al. 2019). An increase in YLD was seen in several *Rht18* lines under both stubble retained and

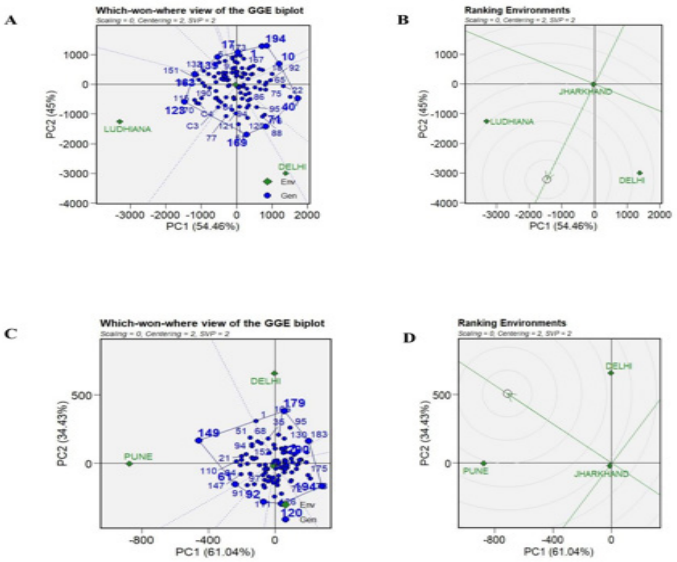


Fig. 4. Genotype and genotype plus environment interaction (GGE) Biplots for the 109 BC_2F_5 lines for grain yield per plot in A-B. Stubble retained environments C-D. Rainfed environment

Table 2. Background recovery percent of the whole genome of the recurrent parent (HD3086) and target chromosome (6A) used in the marker-assisted selection programme

Genotypes	Allele similar to RP (WG)	Allele similar to RP (6A)	Allele similar to DP (WG)	Allele similar to DP (6A)	Percent recovery (WG)	Percent recovery (%) (6A)
ATLA 114	9769	267	2953	118	72.78	64.05
ATLA 130	13154	371	1113	72	90.25	81.60
ATLA 131	13880	388	610	58	94.36	84.88
ATLA 138	12962	377	954	58	90.14	83.72
ATLA 142	12953	371	1190	72	89.32	81.60
ATLA 153	12932	371	1216	71	89.16	81.71
ATLA 163	13157	374	1119	71	90.24	83.08
ATLA 164	12890	371	1297	72	88.62	81.60
ATLA 200	13161	374	1120	71	90.25	82.02

WG= Whole genome, RP= Recurrent parent, DP= Donor parent

Table 3. ANOVA for the agronomic traits for the three locations evaluated in stubble retained and irrigated condition

	DEL (IR)			DEL(SR)			LDH(SR)			JKD(SR)		
	Treatment	Residuals	CV	Treatment	Residuals	CV	Treatment	Residuals	CV	Treatment	Residuals	CV
PH	322.66**	8.42	3.24	274.41**	6.52	2.91	276.37**	5.73	2.71	195.84**	15.98	5.24
SW	26.51^{ns}	27.49	19.77	25.29**	4.86	6.99	34.17**	5.22	7.73	14.42**	3.51	6.52
SWPS	13.13**	3.12	8.25	19.42**	3.56	8.51	22.94**	3.28	8.59	8.54*	3.03	10.7
BMS	112008.71**	12405.87	10.20	663550.72**	64786.14	12.21	420965.85**	122260.2	10.9	7637.90**	3416.11	3.43
YLD	17890.01**	4816.11	15.88	104551.16**	5962.22	11.64	150541.01**	30438.73	14.96	1751.02*	666.07	4.28
NS	2.77^{ns}	3.04	9.52	3.39*	1.31	6.30	-	-	-	-	-	-
SL	1.23^{ns}	0.75	7.31	1.34*	0.54	6.58	-	-	-	-	-	-

Table 4. ANOVA for the agronomic traits evaluated under rainfed conditions

	DEL			PNE			JKD		
	Treatment	Residuals	CV	Treatment	Residuals	CV	Treatment	Residuals	CV
PH	129.57**	11.96	3.72	111.97**	10.62	4.02	142.61**	11.40	5.13
SL	1.31^{ns}	0.85	8.02	0.53*	0.24	5.62	1.35**	0.35	6.31
NS	2.32**	0.60	4.35	2.45**	0.42	4.00	3.34**	0.16	2.38
YLD	45169.4**	469.65	9.21	9129.69**	1587.96	11.14	584.77*	71.32	2.31

rainfed conditions relative to HD3086. Additionally, certain yield contributing traits such as SL and NS increased in some *Rht18* lines under rainfed conditions. In PNE, these lines exhibited a higher average YLD than HD3086 under rainfed conditions. The results corroborate previous studies showing that GA-sensitive genes (*Rht*) increase grain yield (Khalid et al. 2023). The increase in YLD observed in the *Rht18* introgressed lines can be attributed to the reduction in PH, which likely reduces lodging and enhances partitioning of assimilates to the grain by reducing competition from the growing stem (Shah et al. 2017; Foulkes et al. 2011). Increased YLD may be influenced by other plant architecture-related factors, i.e., canopy structure (leaf angle, leaf size, LAI and branching), coleoptile length, seedling establishment, and tillering ability (Murchie et al. 2022). *Rht18* wheat lines are better able to withstand limited soil-moisture conditions owing to their improved emergence, seedling vigour, spike weight and more partitioning of assimilates to the grain compared to varieties carrying *Rht-B1b/Rht-D1b*, particularly when sown deep (Rebetzke et al. 2005; Tang et al. 2021).

In rice-wheat cropping systems, the leftover rice stubbles are often burnt, which contributes to atmospheric pollution. Under stubble-retained conditions, the improved lines showed a reduction in PH of 8–13 cm (10.2–13.2%). The introgressed lines showed improved coleoptile length and early seedling establishment, mitigating the negative pleiotropic effects of *Rht-B1b* under moisture stress (unpublished data). GA-sensitive dwarfing gene *Rht18* promotes better emergence of wheat seedlings through rice stubbles due to its longer coleoptiles, which may reduce the need for stubble-burning and thereby reduce atmospheric pollution. Our study showed that the BMS, SW and SWPS of several *Rht18* genotypes were higher than those of HD3086 under stubble retained conditions, which is in accordance with previous reports indicating that BMS is not compromised in gibberellic acid responsive (GAR) *Rht* genes. Genetically vigorous wheat genotypes are known to maintain higher biomass in the field (Rebetzke et al. 2014). The increased BMS observed in *Rht18* genotypes suggests greater vigour compared with *Rht-B1* under retained-residue conditions, thereby supporting conservation agriculture by reducing the likelihood of stubble burning. Additionally, an increase in TGW in *Rht18*-positive lines was observed, which may account for the overall increase in SW.

The genotype and genotype-by-environment interaction (GGE) analyses for YLD revealed that the *Rht18*, *Rht18*+*Rht-B1b*, and *Rht-B1b* genotypes specifically adapted to SR and RF conditions, respectively. In each location, double dwarf lines (*Rht-B1b*+*Rht*18) were also found stable, likely due to interaction among genes governing diverse traits that compensate for the negative effects of the *Rht-B1b* gene. Lines that performed consistently across all environments were also identified using the GGE biplots (Fig. 4). These stable *Rht18* genotypes can be considered suitable for release across, whereas the ones specifically adapted to the locations can be considered for release within those same locations.

In conclusion, the MABC programme and multi-environment trials (METs) targeting the alternate dwarfing gene *Rht18* successfully identified superior genotypes in the genetic background of a mega wheat variety (HD3086) with high background similarity to the RP. These improved lines have the potential to substitute the existing cultivars with the *Rht-B1b* semi-dwarf gene, especially for the Indo-Gangetic zone of India. It will aid in alleviating the challenges posed by moisture stress conditions and retained stubble by increasing early vigour and better establishment rates at the seedling stage.

Supplementary material

Supplementary Table S1 is provided, which can be accessed at www.isgpb.org

Authors' contribution

Conceptualisation of research (NJ, HK, RP, PKS, GPS); Designing of the experiments (NJ, HK, PKS, GPS); Contribution of experimental materials (HK, RP); Execution of field/lab experiments and data collection (AT, HK, NS, PKS, RP, PB, MK); Analysis of data and interpretation (AT, NS); Preparation of manuscript (AT, NJ, HK).

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Supplementary Table S1. Climatic conditions at the time of sowing and harvest in stubble retained and rainfed locations

Stubble retained			
	DEL	LDH	JKD
Date of Sowing	14-11-2022	7-11-2022	10-11-2022
Maximum Temperature	25°C	30°C	33°C
Minimum Temperature	17°C	13°C	18°C
Date of Harvest	13-4-2023	9-4-2023	11-4-2023
Maximum Temperature	26°C	31°C	33°C
Minimum Temperature	15°C	15°C	17°C
Rainfed			
	DEL	PNE	JKD
Date of Sowing	12-11-2022	2-11-2022	6-11-2022
Maximum Temperature	25°C	33°C	31°C
Minimum Temperature	15°C	21°C	16°C
Date of Harvest	13-4-2023	11-4-2023	8-4-2023
Maximum Temperature	26°C	33°C	30°C
Minimum Temperature	15°C	17°C	21°C

DEL- New Dehi, LDH- Ludhiana, JKD- Jharkhand, PNE- Pune