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Characterizing reduced height wheat mutants for traits affecting abiotic stress and photosynthesis during seedling growth

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Abstract

Most high-yielding, semidwarf wheat (*Triticum aestivum* L.) grown around the world contains either *Rht1* or *Rht2* genes. The success of these high-yielding cultivars is greatest in the most productive farming environments but provide marginal benefits in less favorable growing conditions such as shallow soils and low-precipitation dryland farming. Further, growing evidence suggests semidwarf genes not only affect early seedling growth but limit grain yield, especially under abiotic stress conditions. There are 23 other reduced-height mutants reported in wheat, most of which have not been functionally characterized. We evaluated these mutants along with their parents for several traits affecting seedling emergence, early seedling growth, and photosynthetic efficiency. Two- to seven-fold differences in coleoptile length, first leaf length, root length, and root angle were observed among the genotypes. Most of the mutations had a positive effect on root length, while the root angle narrowed. Coleoptile and first leaf lengths were strongly correlated with emergence. A specialized deep planting experiment identified *Rht5*, *Rht6*, *Rht8*, and *Rht13* with significantly improved seedling emergence compared to the parent. Among the mutants, *Rht4*, *Rht19*, and *Rht12* ranked highest for photosynthetic traits while *Rht9*, *Rht16*, and *Rht15* performed best for early seedling growth parameters. Considering all traits collectively, *Rht15* showed the most promise for utilization in marginal environments followed by *Rht19* and *Rht16*. These wheat mutants may be useful for deciphering the underlying molecular mechanisms of understudied traits and breeding programs in arid and semiarid regions where deep planting is practiced.

1 | INTRODUCTION

The incorporation of height reducing genes in both rice (*Oryza sativa* L.) and wheat semidwarf high-yielding cultivars were instrumental to the "Green Revolution." These high-yielding, semidwarf wheat cultivars are resistant to lodging and have a high grain yield (due to increased productive tillers and biomass) in response to nitrogen fertilizer and water applications. Together, improved genetics and agronomic management greatly increased

grain yields during the 1960s, enabling autonomous wheat production in several developing countries including those in Latin America and Asia (Casebow et al., 2016; Gale & Youssefian, 1985). High-yielding, semidwarf cultivars contain the mutant form of reduced-height (Rht) gene *Rht1/Rht2* (*Rht-B1b/Rht-D1b*), incorporated from the Japanese dwarf wheat "Norin10." These genes were cloned and mapped to homoeologous group 4 (4BS/4DS) chromosomes and encodes a DELLA domain protein negatively regulating the gibberellin-based growth response in plants (Borojevic & Borojevic, 2005; Peng et al., 1999). Since their introduction into wheat, these genes have been extensively utilized in wheat breeding

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programs around the world. Currently, more than 95% of cultivated wheat contains at least one *Rht1* or *Rht2* allele (Hedden, 2003).

Similar to the gene transfer from the Japanese cv. Norin10, in the beginning of twentieth century, the reduced-height gene *Rht8* (mapped on 2DL) from "Akakomugi" was introduce into Italian wheat cultivars and later into wheat production regions of Argentina and south and central Europe (Borojevic & Borojevic, 2005). *Rht8* reduced plant height by ∼10% and did not have a negative effect on grain yield (Worland et al., 1998). The success of these genes (*Rht1*, *Rht2*, and *Rht8*) in reducing plant height is ancillary in comparison to increased lodging resistance, response to nitrogen fertilizer, and ultimately grain yield which allured many scientists to develop and identify other dwarfing genes in wheat.

Since the early twentieth century, the use of mutagens to generate induced mutations with their subsequent selection has been a popular method to create novel genotypes/phenotypes to develop improved and desirable cultivars (Ahloowalia et al., 2004). In wheat, after successful

utilization of the natural height mutants, many new height mutants were identified in the populations created by fast neutron radiation, γ-radiation, X-ray, Ethyl methanesulfonate, and N-Nitroso-N-methylurea (Konzak, 1987). So far, more than 25 reduced-height mutants have been identified in wheat (McIntosh et al., 2017). These reduced-height mutants are classified as Gibberellic acid (GA)-sensitive and GA-insensitive based on their response to the exogenous GA application (Ellis et al., 2004). Most of the mutants have a known chromosome location, however, chromosome locations of six dwarfing genes have not yet been determined (Table 1).

Even with the successful discovery of various reduced-height mutants in wheat, except for the natural mutants, *Rht1*, *Rht2*, and *Rht8*, others have not been utilized in wheat breeding due to lack of extensive agronomic and physiological studies. Although, some agronomic studies were performed on *Rht5*, *Rht12*, *Rht13*, and *Rht25* (Daoura et al., 2013; Ling et al., 2013; Mo et al., 2018; Wang et al., 2015; Watanabe, 2008). Further, *Rht8* classified as a GA-sensitive mutant, might be a brassinosteroids (BRs)

TABLE 1 List of reduced height mutants and parental genotypes

Rht gene	Acc. Name (NPGS-ID)	Parent (NPGS-ID)	Ploidy	Chromosomal location	Habit	Dominance	Mutation identified
Rht1	Isoline: Rht1rht2 (PI 518617)	Isoline: rht1rht2	AABBDD	4BS	S	SD	Breeding material
Rht2	Isoline: rht1Rht2 (PI 518621)	(PI 518625)	AABBDD	4DS	S	SD	Breeding material
Rht1Rht2	Isoline: Rht1Rht2 (PI 518613)		AABBDD	4BS, 4DS	S	SD	Breeding material
Rht3	Burt-Rht3(CI 17786)	Burt (CI 12696)	AABBDD	4BS	W	SD	Breeding material
Rht4	Burt Ert 937 (CI 15076)	Burt Mutant (CI 13728)	AABBDD	2BL	W	R	γ-radiation
Rht5	Marfed Ert 1 (CI 13988)	Marfed (CI 11919)	AABBDD	3BS	F	D	EMS
Rht6	Sel 14 (CI 13253)	Norin10 (PI 156641)	AABBDD	—	W	R	Breeding material
Rht7	Bersee (PI 168661)		AABBDD	2A	S	R	EMS
Rht8	Sava (PI 323638)	Redcoat (CI 13170)	AABBDD	2DS	W	R	Cultivar
Rht9	Lambro (PI 433757)	Capelli (PI 290505)	AABB	5AL	W	R	Cultivar
Rht10	Ai-bian No. 1 (PI 504466)		AABBDD	4DS		SD	Cultivar
Rht11	Karlik 1 (PI 504549)	Bezostaja 1 (PI 276704)	AABBDD	4BS	W	R	NMU
Rht12	Karcag 522 M7K (PI 503552)		AABBDD	5AL	S	D	γ-radiation
Rht13	Magnif 41 Ert 1 (CI 17689)	Magnif 41 (PI 344466)	AABBDD	7BS	S	SD	NMU
Rht14	Castelporziano (PI 347731)	Capelli (PI 290505)	AABB	6AS	S	SD	Thermal neutron
Rht15	Durox (PI 478306)		AABB	—	S	SD	EMS
Rht16	Edmore MUTSD1 (PI 499362)	Edmore (CI 17748)	AABB	6AS	S	SD	MNU
Rht17	MN 6616 M (CI 17241)	Chirs (CI 13751)	AABBDD	—	S	R	DES
Rht18	Icaro (PI 503555)	Anhinga (PI 428455)	AABB	6AS	S	SD	Fast Neutron
Rht19	Vic SD1 Line b (PI 503553)	Vic (CI 17789)	AABB	—	S	SD	EMS
Rht20	Burt M860 Line c (PI 503551)	Burt (CI 12696)	AABBDD	—	F	SD	γ-radiation
Rht21	XN0004	Qing 431/Xiaoyan 6	AABBDD	2AS	—	SD	
Rht22	Aiganfanmai		AABB	7AS	—	SD	
Rht23	NAUH164	Sumai 3	AABBDD	5DL	—	D	EMS
Rht24	Aikang 58/Jingdong 8		AABBDD	6AL	-	QTL	
Rht25	UC1110/PI610750		AABBDD	6AS	-	QTL	

Abbreviations: D, dominant; DES, diethyl sulfate; EMS, ethyl methanesulfonate; F, facultative; I, insensitive; NA, not available; NIL, near-isogenic lines; NMU, N-nitroso-N-methylurea; R, recessive; S, sensitive; S, spring wheat; SD, semidominant; W, winter wheat; *, *not used in this study*.

pathway mutant (Gasperini et al., 2012). Thus, testing mutants for their response to exogenous BR will help in further characterization.

The semidwarf alleles affect wheat growth and development throughout its entire growth cycle. The *Rht1/Rht2* mutations not only affect stem elongation but also negatively affect coleoptile length (Botwright et al., 2001), root architecture, grain weight (Casebow et al., 2016), root/aerial biomass (Subira et al., 2016), other root traits (Bai et al., 2013), seed size, and flag leaf photosynthesis (Jobson et al., 2019). Growing evidence further suggests that these mutants compromise the ability of plants to tolerate abiotic and biotic stresses. For example, presence of both *Rht1* and *Rht2* is associated with susceptibility to Fusarium head blight caused by *Fusarium graminearum* (James, 2011) and negatively affects yield under drought conditions (Jatayev et al., 2020). A significant amount of wheat is grown in dryland regions with <300 mm annual precipitation where drought is common and wheat is water stressed throughout most of its entire growth cycle (Donaldson, 1996). In such dry regions, wheat is commonly planted as deep as 20 cm below the soil surface to reach soil moisture adequate for germination and seedling emergence (Schillinger et al., 1998). In addition to planting depth, speed of emergence is also a critical factor because as little as 3 mm of rain occurring after planting but before emergence often creates a light surface-soil crust through which the elongating seedling cannot penetrate. Wheat cultivars with the semidwarf mutants *Rht1* and *Rht2* have poor emergence from such deep planting depths (Amram et al., 2015; Mohan et al., 2013; Schillinger et al., 1998).

The objective of our study was to characterize the known reduced-height mutants in wheat with special emphasis on their seedling emergence from deep planting depths and other traits important for tolerance to water stress conditions. All genotypes were evaluated for their photosynthesis parameters to identify the best photosynthetically efficient lines. Comparison of mutants with the parent provided an insight on mutation effects on a particular trait. Genotypes identified for specific traits could be utilized either as a donor in breeding programs or as a source to further characterize the less studied agronomically important traits like seedling emergence and photosynthesis in wheat.

2 | MATERIALS AND METHODS

2.1 | Germplasm

The known reduce-height mutants and their parental lines were collected from the Germplasm Resources Information Network (GRIN; https://www.ars-grin.gov/Pages/Collections#bkmk-1). Detailed information on the genotypes used in the present study is provided in Table 1.

2.2 | Early seedling growth measurements

2.2.1 | Coleoptile and first leaf length measurement

Seeds of each genotype were surface sterilized for 1–2 min with 70% ethanol followed by treatment with 1% sodium hypochlorite solution

for 5 min. Coleoptile and first leaf length measurement experiments were conducted as described in detail by Mohan et al. (2013). In brief, 15 seeds were placed in the germination paper (Heavy Germination paper #SD 7615L, Anchor Papers) about 2 cm apart with the germ side down. The germination paper was then folded in half vertically with the seeds in the crease. The folded half was again folded horizontally four times and placed in a plastic shelf bin (Uline; S-13396BLU) with holes at the base to drain any excess water. The plastic shelf bins were then placed side-by-side inside a storage bin, covered with a light-proof bag and placed inside a growth chamber at constant 22°C in complete darkness. After 10 days, coleoptile and first leaf length of 10 randomly selected seedlings were measured using a ruler scale to the nearest mm from base of the seed to the tip of coleoptile and first leaf. The experiment was replicated twice and mean lengths were reported.

2.2.2 | Biomass analysis

For fresh and dry weight analysis, the seedlings after coleoptile and leaf length analysis, were measured on an analytical scale to the nearest milligram. Subsequently the samples were dried at 55°C for 3 days before recording the dry weight to the nearest milligram and reported as mean percent dry matter.

2.2.3 | Root length and root angle measurement

Seeds were germinated on Murashige and Skoog agar petri plates under controlled growth conditions at 22°C. Root length was measured to the nearest mm from seed to the tip of the longest root on day 5 after germination. The petri plates were scanned using a HP Photosmart 7520 scanner before root length measurement. The scanned photos were imported into ImageJ 1.51h software (Schneider et al., 2012) for root angle measurements.

2.2.4 | Seedling emergence from deep planting

Seedling emergence was evaluated by specialized laboratory pot emergence test at the WSU Dryland Research Station, Lind, WA simulating deep-planting conditions for winter wheat following the detailed protocol described in Schillinger et al. (2017). The experiment was performed twice with four replicates each at a constant air temperature of 21°C. In brief, soil from the Lind Station (Shano silt loam) was collected from the surface 15 cm of a fallowed field in August and air dried to a water content of 1.5–2.0%. Soil was then screened through a 6 mm mesh to remove clods and straw. Next, the prescribed quantity of water was mixed with the soil to reach a target soil water potential. At the bottom of the 18 cm tall plastic pots, first 2.5 cm wet reserve layer (water 15% by volume) was placed and tamped with a specially designed hydraulic press followed by another 2.5 cm of compacted seed layer (water 12% by volume). Eight seeds of each line were placed on the compacted seed layer followed by

a 2.5 cm of loose, noncompacted seed layer of the same water content as layer two. Finally, 10 cm of dry soil was added to create a loose, dry soil layer. Thus, there was 12.5 cm of soil (2.5 cm moist plus 10 cm dry) covering the seeds. The entire protocol is illustrated in figures and photos in Schillinger et al. (2017). This method accurately simulates deep planting conditions encountered in the field. Seedling emergence was measured by counting individual emerged seedlings at 24-h intervals from days 6–14 after planting. On day 14, the soil was carefully removed to record coleoptile length and first leaf length of each seedling to the nearest mm (Figure S1).

2.3 | Hormone response assay

2.3.1 | GA response assay

A modified protocol of GA assay (Chandler & Robertson, 1999) and coleoptile measurement (Mohan et al., 2013) was followed. In brief, for each genotype, 15 seeds in the germination paper soaked in either phosphate buffer (1 mM potassium phosphate pH 5.5) or buffer solution containing 10 mM GA_3 (Sigma-Aldrich) was placed in a plastic shelf bin. Two plastic shelf bins were placed side-by-side in a storage box covered, with a light-proof bag and incubated in darkness at 22°C in growth chamber. Data on coleoptile and first leaf length measurements were recorded after 10 days to the nearest mm on two replicates of 10 seedlings each. The experiment was conducted twice and means were reported. The GA response of all genotypes was recorded compared to the buffer only control. The response of mutants was recorded compared to their respective tall parent.

2.3.2 | BR response assay

The BR response assay was modified from (Chono et al., 2003) using 24-epibrassinolide (epi-BL; Sigma). The sterilized seeds of each genotype were germinated on petri plates containing half strength Murashige and Skoog (MS) media with 3% phytagel in darkness at 4°C for 2 days. The petri plates were transferred to 16 h light and 8 h dark for 24 h before transferring to different concentration of 24-epi-brassinolide. Five uniform germinated seeds were transplanted on petri plates containing 0, 0.01, 0.1, and 1.0 μM of epi-BL under simulated 16 h daylight and 8 h darkness at 22°C for 5 days. The experiment was repeated twice with five replicates. Primary root length of the seedlings was measured on day 5 to the nearest mm using a ruler. Fresh weight of roots as well as of shoots was measured using an analytical scale. Root and shoot dry weights were measured separately after drying at 55°C for 3 days. The initial BR response of all the genotypes was recorded for the 0.01 μM epi-BL compared to the control (no epi-BL). The percent response for all the genotypes was calculated for the highest epi-BL (1.0 μM) concentration compared to their respective tall parent.

2.3.3 | CO_2 response measurements

Photosynthesis parameters were measured using the LI-6400XT portable photosynthesis system equipped with a 6400-02B LED red/blue light source (Li-Cor Inc.). On day 10 after planting, Z12/Z13 L2 was selected for each genotype (Zadoks et al., 1974). The plants were grown under glass house conditions at Washington State University Plant Growth Facility under simulated 16 h daylight and 8 h darkness and a constant daylight temperature of 22 and 18°C in darkness. Four hours after lights were activated and before starting the CO_2 response curve measurements, the middle of the leaf was adapted in the chamber head for 5 min to ensure that photosynthesis and stomatal conductance were stable. The first point selected for reference CO_2 concentration was the ambient level 400 ppm. A/C_i measurements were conducted at the reference CO_2 concentration of 400 ppm, followed by 50, 75, 100, 150, 200, 300, 500, 750, 1000, and 1500 ppm under saturated light conditions of 2000 μmol m^{-2} s^{-1}. While keeping the data collection method consistent across all data sets, data on three biological replicates was obtained for each genotype. A common vapor pressure deficit of 1.5–2.5 kPa was used for all gas exchange measurements.

2.4 | Statistical analysis

One and two-way analysis of variance (ANOVA) and t-test was conducted in R (R Core Team, 2020) and figures were produced using the packages ggplot2 (Wickham, 2016) and ggpmisc (Aphalo, 2020). The CO_2 curve fitting analysis was performed using the methods described in (Duursma, 2015) plantecophys package using 0.5 mol m^{-2} s^{-1} bar^{-1} for average mesophyll conductance for all wheat lines based on previous studies (0.5–1.05 mol m^{-2} s^{-1} bar^{-1}, Jahan et al., 2014; 0.19–0.42 mol m^{-2} s^{-1} bar^{-1}, Evans and Vellen 1996; and 0.43–0.68 mol m^{-2} s^{-1} bar^{-1}, Tazoe et al., 2011) and standardized leaf temperature to 25°C. Simple linear regressions were performed using Microsoft Excel. Mutants were ranked (1–20) for photosynthetic (A_n, V_{cmax}, J_{max}, WUE, and $_iWUE$), early seedling traits (coleoptile length, first leaf length, biomass, root length, and root angle) and for seedling emergence from best (1) to worst (20). The overall raking of mutants was assigned by the sum of the photosynthetic trait, early seedling trait, and percent emergence from deep planting to identify the overall best performing line.

3 | RESULTS

3.1 | Early seedling growth variation

3.1.1 | Coleoptile length

Coleoptile length showed a ∼3-fold variation among the parents and the mutants (Table S1). In general, the parents had a higher mean coleoptile length (86.1 mm) compared to mutants (70.6 mm). The

shortest coleoptile length was recorded for *Rht1* and *Rht3* (42.2 mm ± 6.98 and 42.9 mm ± 5.43) and the longest coleoptile was recorded for *Rht14* parent and mutant (127.8 mm ± 5.99 and 127.3 mm ± 4.04). Among the parents, the shortest coleoptile length was found in the tall *rht* line (51.9 mm ± 4.85). Mutations in *Rht3, 11, 17, 19,* and *20* had significantly shorter coleoptiles compared to their respective parents (Table 2; Figure 1). Mutations in *Rht2, 14,* and *18* showed no significant effect on coleoptile length. Mutation in *Rht5* increased the coleoptile length compared to the parent (Table 2; Figure 1).

3.1.2 | First leaf length

The first leaf length showed a ∼7-fold difference among the lines with parents having a higher mean first leaf length (125.5 mm) compared to mutants (107.6 mm). The shortest first leaf length was recorded for *Rht3* mutant (30.6 mm ± 10.31) and the longest for *Rht13* parent (206.1 mm ± 21.62) (Table S1). Among the mutants, *Rht9* (163.0 mm ± 13.43) attained the longest first leaf length, and among parents, tall *rht* isoline (69.0 mm ± 5.86) had the shortest first leaf. Similar to coleoptile length, the *Rht5* mutant had a longer first leaf compared to the parent while *Rht1, 8, 14, 16, 18,* and *20,* showed no

significant change compared to their respective parents (Table 2; Figure 1). On the other hand, the *Rht13* mutation negatively affected first leaf length.

Seedling percent dry matter of the genotypes varied from 5.6% in *Rht14* to 18.4% in *Rht19*. Among the parents, the lowest percent dry mater was recorded for *Rht14* parent (7.0%) and the highest for *Rht16* parent (13.2%). Compared to the parents, *Rht1, 4, 11, 16, 17,* and *19* positively affected early growth biomass while *Rht1Rht2, Rht2, 5, 8, 13,* and *18* had no significant effect. Mutation in *Rht6* and *14* decreased dry matter accumulation compared to their respective parent (Table 2; Figure 1).

3.1.3 | Root length

Root length directly affects the plant's ability to utilize water deep in the soil profile. Among the genotypes, root length varied from 55.5 to 108.3 mm with a mean root length slightly longer for mutants (80.1 mm) compared to parental genotypes (74.1 mm) (Table S1). In general, the mutants showed a wider range for root length (55.5 to 108.3 mm) compared to the parents (56.2 to 93.0 mm). Among the mutants, the shortest root length was recorded for *Rht7* and the longest for *Rht16*. While *Rht4* parent had the shortest root length, the

TABLE 2 Early seedling growth and emergence parameters of mutants compared to their respective parents

Genotype	Parameter					
	Coleoptile length	Leaf length	Biomass %	Root length	Root angle	Seedling emergence
Rht1	−	=	+	+	−	=
Rht2	=	+	=	+	−*	+
Rht1Rht2	−	−	=	+	−*	−*
Rht3	−*	−	−	−*	−	−*
Rht4	−	−	+	+*	−	=
Rht5	+	+	=	+	−	+*
Rht6	−	−	−	+	−	+
Rht7[a]	+	+	+	−	+	+*
Rht8	−	=	=	−	−	+*
Rht9	−	+	+	−	−	−
Rht11	−*	−	+	+	−	=
Rht12[a]	+	+	+	+*	−	+
Rht13	−	−*	=	x	x	+*
Rht14	=	=	−	−	−	=
Rht15[a]	+*	+	+	+	−	+
Rht16	−	=	+	+*	+	=
Rht17	−*	−	+	+	−	=
Rht18	=	=	=	+	−*	=
Rht19	−*	−	+	+	−	=
Rht20	−*	=	−	−	−	=

Abbreviations: −, decrease; +, increase; =, no effect, x, data not available.
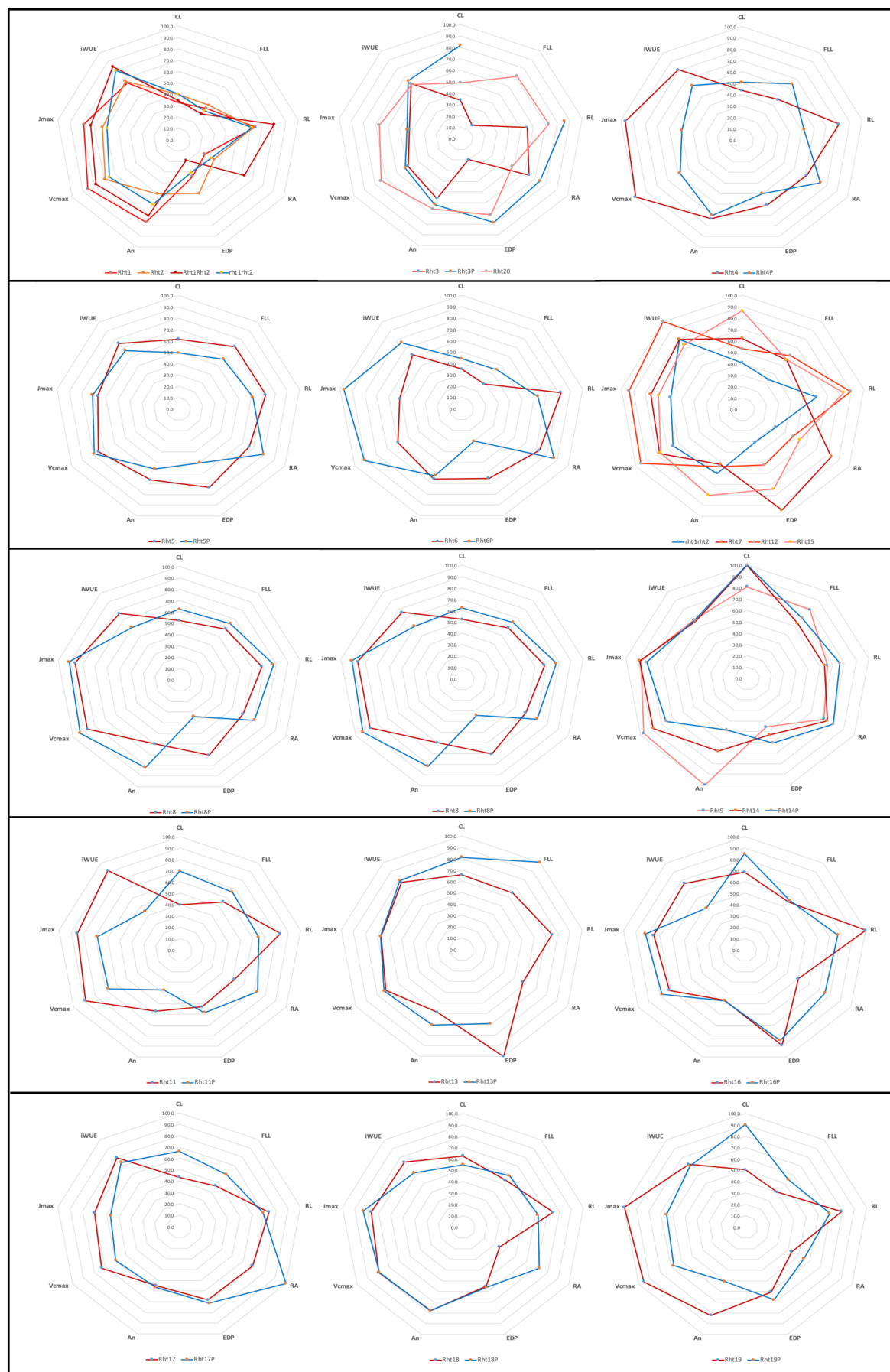[a]Compared with rht tall parent.
*Significance at 0.05.

FIGURE 1 Radar chart of various traits comparing mutants and parents. Red (shades of red) line represents mutants and blue represents parents

Rht3 parent had the longest. Compared to the respective parents, *Rht3*, *8*, *14*, and *20* mutants had a shorter root length while the remainder of the mutants had a longer root length than the parents. The difference from their respective parents was statistically significant for *Rht3*, *4*, and *16* (Table 2; Figure 1).

3.1.4 | Root angle

Like root length, root angle is an important trait controlling root architecture. We observed a large variation in root angle (Figure S2) among the genotypes. The narrowest angle was for *Rht1* (26.4° ± 2.42) and the widest angle for the *Rht17* parent (105.5° ± 3.64) (Table S1). Among the mutants, the widest root angle was observed for *Rht7* (88.9° ± 5.07). Although not significantly different, parents of mutant lines had a wider angle overall with the narrowest recorded for tall *rht* isoline (33.2° ± 2.31) and widest for *Rht17* parent (Table S1; Figure 1). Except for *Rht16*, all mutant lines had a narrower root angle compared to their respective parents. Significant reduction in root angle was recorded for *Rht2*, *Rht1Rht2* double mutant, and *Rht18* compared to their respective parents (Table 2).

3.1.5 | Emergence from deep planting depths

The ability for wheat seedlings to emerge from deep planting depths is a critically important trait for genotypes grown in many low-precipitation environments. Recorded as at least one seedling emerged (see Schillinger et al., 2017 for a detailed description of methods), the fastest emergence through the 12.5 cm layer of soil covering seeds was observed on day 7 after planting for *Rht13*, *Rht16* mutant, and parent, *Rht5* parent and *Rht12* (Figure 2). On day 8, more than 40% seedling emergence was recorded for *Rht7* (53.3%), *Rht13* (47.4%), *Rht8* (45.0%), *Rht16* parent (45.8%), and *Rht5* (44.1%). On day 14 after planting, *Rht1Rht2* double mutant had the lowest seedling emergence (14.8%) among all the genotypes, and highest was for *Rht13* (77%) (Figure 2). Among the parental genotypes, *Rht6* parents showed the least mean seedling emergence percentage (23.2%), while the *Rht16* parent had the greatest emergence (65.0%). Compared to their parents, *Rht1Rht2* and *Rht3* mutations negatively impacted emergence from deep planting depths, while *Rht5*, *8*, and *13* showed significant improvements (Figure 1). Relative to their corresponding parents, other mutations did not show significant effect on seedling emergence from deep planting. Selected mutants were also evaluated in a field deep planting emergence experiment (Mohan et al., 2013). Although, rain that occurred after planting caused some surface-soil crusting, we were still be able to identify *Rht7* and *Rht16* as the fastest emerging lines although *Rht16* was the more gradual in emergence. Overall, *Rht15* had 54% emergence followed by *Rht18* (33%) and *Rht7* (31%). *Rht15* started emerging late but attained the highest emergence percentage by day 14. *Rht1Rht2* double mutant followed by *Rht3* were the poorest emerging lines in the pot emergence test.

We also examined the coleoptile and first leaf length of the genotypes on day 14 by carefully removing the soil from pots and then the

soil from around the roots. We observed that the double mutant, *Rht1Rht2*, had the shortest coleoptile length (45.2 mm ± 2.46) and *Rht20* the longest (96.5 mm ± 4.18). Among the parental lines, the *Rht8* parent (53.4 mm ± 3.48) had the shortest and *Rht16* parent (93.4 mm ± 2.31) the longest coleoptile. Similarly, the first leaf elongation showed wide variation with the shortest for the *Rht3* mutant (87.9 mm ± 3.71) and longest for the *Rht16* parent (235.9 mm ± 11.46). In general, the parent lines had a longer first leaf length than their respective mutants, with the shortest recorded for the *Rht8* parent (158.4 mm ± 14.90). Among the mutants, the longest first leaf length was recorded for *Rht13* (225.3 mm ± 8.52).

To understand the relationship of coleoptile length, first leaf length, and emergence from deep planting depths, Pearson correlation was performed on rate of emergence on day 14 after planting (Figure 3A,B). Coleoptile length and mean seedling emergence was positively correlated ($r^2 = 0.36$; $p = 8.1e^{-21}$). Similarly, the first leaf length was also positively correlated ($r^2 = 0.44$; $p = 7.7e^{-27}$) with the mean emergence on day 14. Although both coleoptile and first leaf length were positively correlated, first leaf length seems to correlate better than coleoptile length for emergence capability.

3.1.6 | Photosynthesis trait variation

To identify the variation in germplasm and to understand effects of reduced height mutation on photosynthesis, measurements were performed and compared with their respective parents.

3.1.7 | Net photosynthesis (A_n; µmol CO_2 m^{-2} s^{-1})

Photosynthesis rates for each of the reduced-height genotypes were recorded for atmospheric gas concentrations and maximum light intensity (Table S2). The population showed ~2.6-fold variation with the *Rht11* parent having the lowest and *Rht9* the highest rate of net photosynthesis per unit leaf area. Overall, the mutant lines showed a wider range of photosynthesis rates than their parents. Most of the mutants compared to their respective parents did not show much difference in photosynthesis rate except for *Rht9*, *19*, and *11* that had higher photosynthetic rates than the parent genotypes. *Rht8* had a lower photosynthetic rate than its parent (Figure 1 and Table S3).

3.1.8 | Ambient intercellular CO_2 concentration (C_i; mmol m^{-2} s^{-1} pa^{-1})

The leaf internal CO_2 concentration at ambient conditions ranged from 21.9 to 29.2 mmol m^{-2} s^{-1} Pa^{-1} among all the genotypes. The mean C_i for parental genotypes (26.15 mmol m^{-2} s^{-1} Pa^{-1}) was not significantly different from the mutant mean (24.98 mmol m^{-2} s^{-1} Pa^{-1}) (Table S2). The lowest intercellular CO_2 concentration was present in *Rht12* and the highest in *Rht11* parent. *Rht20* had the highest C_i among the mutant
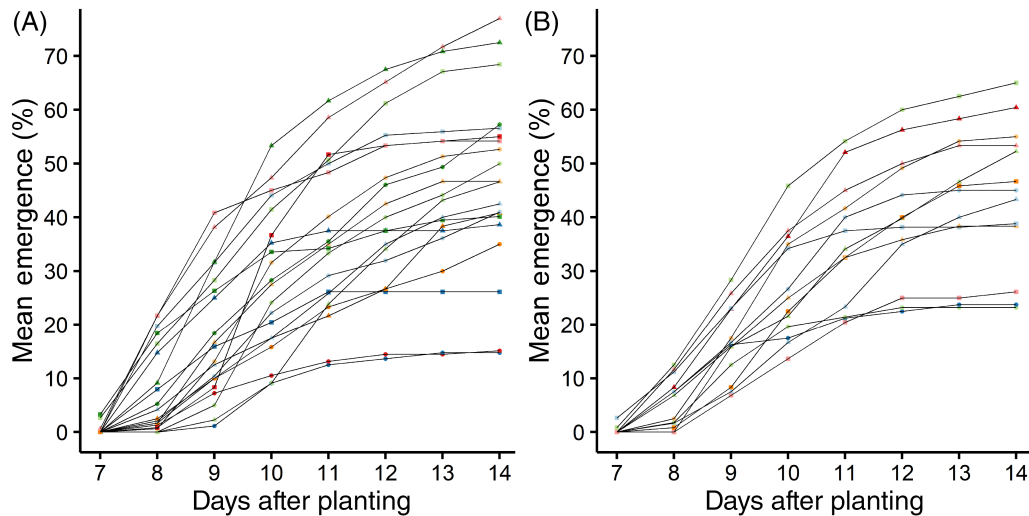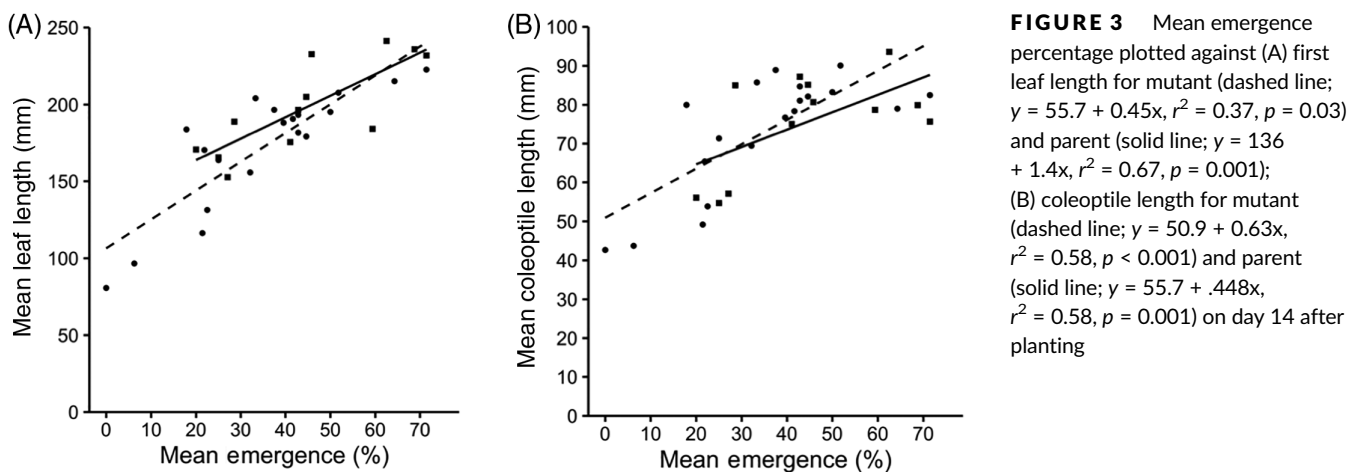
FIGURE 2 Mean percent seedling emergence from deep planting depths of (A) reduced height mutants and (B) standard height parent genotypes. Color and symbols represent respective mutant (a) and parent (b) in the figure are as follows: ■ Rht1, ▲ Rht14, ▲ Rht18, ■ Rht20, ● Rht6, ● Rht11, ● Rht15, ▲ Rht19, ● Rht3, ▲ Rht7, ■ Rht12, ■ Rht16, ● Rht1Rht2, ▲ Rht4, ■ Rht8, ▲ Rht13, ● Rht17, ▲ Rht2, ■ Rht5, and ● Rht9

FIGURE 3 Mean emergence percentage plotted against (A) first leaf length for mutant (dashed line; $y = 55.7 + 0.45x$, $r^2 = 0.37$, $p = 0.03$) and parent (solid line; $y = 136 + 1.4x$, $r^2 = 0.67$, $p = 0.001$); (B) coleoptile length for mutant (dashed line; $y = 50.9 + 0.63x$, $r^2 = 0.58$, $p < 0.001$) and parent (solid line; $y = 55.7 + .448x$, $r^2 = 0.58$, $p = 0.001$) on day 14 after planting

lines while the *Rht13* parent had the lowest internal CO_2 concentration among the parents. *Rht11* and *16* had lower C_i compared to their respective parental lines (Figure 1 and Table S3).

### 3.1.9 | Photosynthetic potential (CO_2 response curves)

Net photosynthetic rate versus internal CO_2 concentration curves were used to assess photosynthetic potential (Figure 4(A)) via the FvCB method of modeling. The parameter Rd was also fitted but was found to be poorly estimated by the model. The CO_2 response curves model provides a calculated estimate for in vivo apparent RUBISCO activity (V_{Cmax}) and the maximum rate of electron transport used in RuBP regeneration (J_{max}). In general, taken together, the photosynthetic potential of parents was higher than the mutants (Figure 4(B)). The *Rht4* recorded the highest value of V_{Cmax} while *Rht3* had the lowest value for both V_{Cmax} and J_{max}

compared to all the genotypes. Among the parents, the *Rht3* parent had the lowest rate while *Rht8* parent had the highest for V_{Cmax}. The V_{Cmax} for *Rht19* increased compared to its parent followed by *Rht1*, *11*, and *9* (Figure 1 and Table S2). *Rht6* showed the largest decrease in V_{Cmax} while *Rht18* remained relatively unchanged compared to the respective parents. Similarly, J_{max}, was highest in *Rht19* followed by *Rht4*. Compared to their respective parent, *Rht4* mutant had the largest increase, whereas *Rht6* had the largest decrease for J_{max}. *Rht13* changed the least between the mutant and the parent (Figure 1 and Table S3).

3.1.10 | Stomatal conductance (g_s; mmol m^{-2} s^{-1})

The stomatal conductance of the leaf is a measure of gas-exchange capacity and is influenced by the opening of stomatal pores and structural features in the mesophyll space. We observed ~2.5-fold variation in the g_s among the population while the range of g_s between the

FIGURE 4 Relationship between (A) net photosynthesis A_n and stomatal conductance g_s among mutants (dashed line) ($y = 4.45 + 45.6x$, $r^2 = 0.84$, $p < 0.001$) and parents (solid line) ($y = 6.81 + 33.1x$, $r^2 = 0.52$, $p < 0.001$); (B) the maximum rate of electron transport demand for RuBP regeneration (J_{max}) and maximum velocity of Rubisco for carboxylation (V_{cmax}) among mutants (dashed line) ($y = 50.2 + 2.74x$, $r^2 = 0.93$, $p < 0.001$) and parents (solid line) ($y = 82 + 3.1x$, $r^2 = 0.92$, $p < 0.001$)
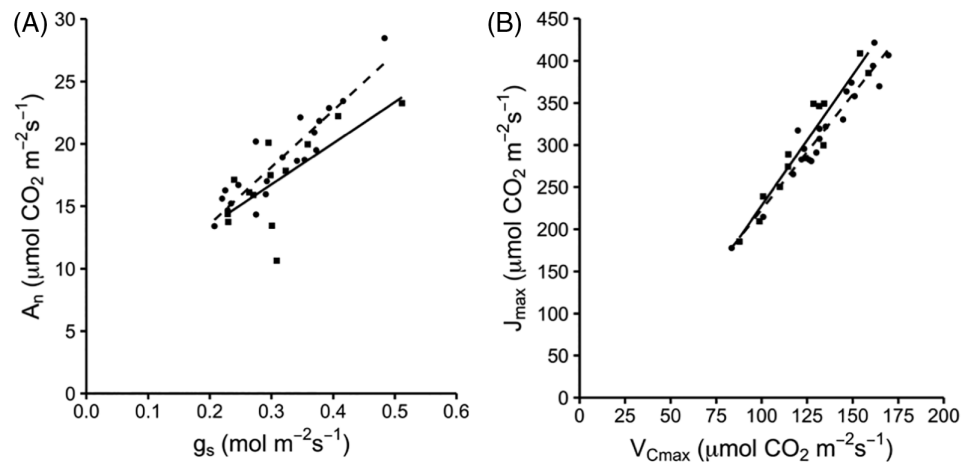
mutants and parents was similar. The maximum g_s recorded was for *Rht8* parent while *Rht16* has the lowest g_s (Table S2). *Rht9*, *19*, and *14* had a large increase in g_s compared to their respective parent while *Rht8* followed by *Rht16* had the largest decrease (Figure 1 and Table S3).

3.1.11 | Water use efficiency [WUE = A_n/E]

Water use efficiency (WUE) is an instantaneous measure of the ratio of net photosynthesis (A_n) to the rate of transpiration (E). It is physiologically and genetically complex and varies with crop development and environmental variables. A greater WUE can enhance crop productivity when water supply is limiting. Therefore, WUE is a useful measure for understanding genotype to phenotype relationships in the context of environmental interactions. Significant variation ($\sim$2.4-fold) in WUE was found (Table S2) among all the genotypes. *Rht11* parent had the lowest and *Rht12* had the highest WUE. Compared to their respective parent, the *Rht11* mutant had the highest improvement while *Rht6* followed by *Rht3* showed the largest decrease in WUE. While both *Rht1* and *Rht2* showed decrease in WUE compared to the tall *rht* isoline, the *Rht1Rht2* double mutant remained relatively unchanged in WUE (Figure 1 and Table S3).

3.1.12 | Intrinsic water use [$_i$WUE = A_n/g_s]

The $_i$WUE is a measure of the efficiency of the consumed water to the assimilated carbon at a given moment and is calculated from the ratio of net photosynthetic CO_2 assimilation rate to stomatal conductance (Tambussi et al. 2007, for review). *Rht11* parent was the lowest while *Rht12* had the highest $_i$WUE (Table S2). Compared to their respective parents, *Rht11* had the largest increase and *Rht6* closely followed by *Rht1*, and *Rht2* had a negative effect on $_i$WUE. *Rht9* changed the least compared to its parent (Table S3).

3.1.13 | Correlation analysis

Significant positive correlations were observed between A_n and g_s for both mutants and parents (Figure 4(A)). In general, A_n of mutants had

a higher correlation with g_s ($r^2 = 0.84$, $p < 0.001$) than parents ($r^2 = 0.52$, $p < 0.005$). A strong correlation between J_{max} and V_{cmax} was observed for both mutants ($r^2 = 0.93$, $p < 0.001$) and parents ($r^2 = 0.92$, $p < 0.001$) (Figure 4(B)).

3.2 | Exogenous response to hormones

3.2.1 | GA response

Genotypes showed a varied growth response to exogenous GA. The genotypes displayed a wide range of response in coleoptile length from suppression of growth (−36.8%; *Rht3*) to promoting growth (+53.7%; *Rht18*) by exogenous GA treatment compared with control (Figure S3). Similarly, for first leaf length the range of −60.7% (*Rht3*) to +65.8% (*Rht19* parent) was found (Figure S3). For both coleoptile and first leaf, the growth response in parents was narrower compared to controls −3.3% in *Rht11* to +50.2% in *Rht16* and −4.2% in *Rht11* to +65.8% in *Rht19*, respectively, for coleoptile and first leaf length (Figure S3a, b). Among the mutants, *Rht3* was the only mutant to have a significant negative response to exogenous GA treatment for coleoptile (−36.8%) and first leaf (−60.7%) growth compared to the control. *Rht4*, *8*, *9*, *13*, *15*, *18*, and *20* all had a positive growth response to exogenous GA treatment for coleoptile and first leaf length. *Rht5* and *19* had an increased growth response for coleoptile length and no response for first leaf length compared to the control. This trend was reversed for *Rht6*, *7*, and *16* with positive growth impact on leaf length and no response for coleoptile length in response to exogenous GA. The *Rht* lines *11*, *12*, *14*, *17*, and *Rht1Rht2* had no response to GA treatment in coleoptile and leaf growth compared to the control. The least responsive parental line was *Rht11* with 3.3% coleoptile and 4.2% leaf length response to GA treatment (Figure S3). For coleoptile length when mutants were compared to their respective parent, *Rht1*, *6*, *14*, *16*, *17*, and *19* response was negative; *Rht2*, *Rht1Rht2*, *4*, *9*, *8*, *11*, *13* had no response; and *Rht5* and *18* response was positive (Figure 5). In mutants this trend was different for first leaf length; *Rht1*, *2*, *Rht1Rht2*, *14*, *17*, and *19* had a negative growth response; *Rht4*, *5*, *6*, *9*, *11*, *13*, and *16* no response; and *Rht18* and *Rht8* a positive growth response compared to their respective parent (Figure 5).
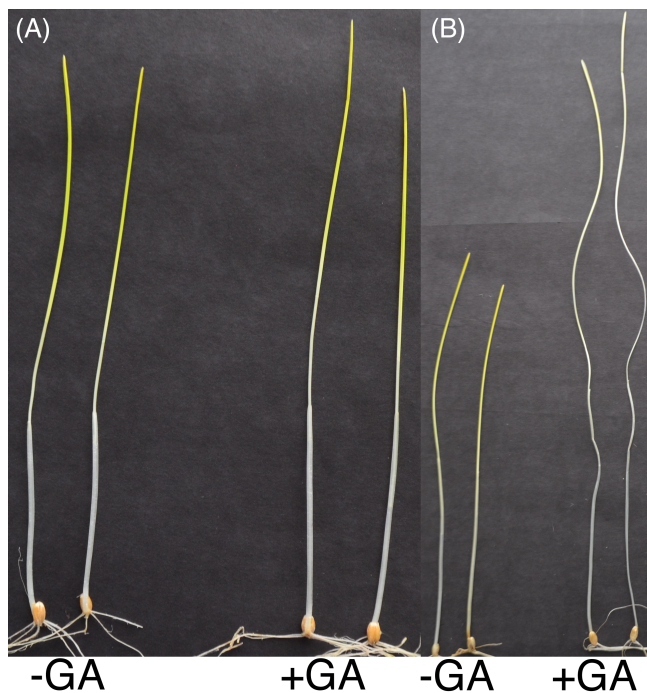
FIGURE 5 Representative genotype coleoptile growth response (A) no response (B) positive response to exogenous GA compared to control

3.2.2 | BR response

The genes involved in BR biosynthesis and signals are expressed in roots indicating its role in root regulation. Hence, the effect of exogenous application of epi-BL was quantified on root length and biomass. The genotypes responded differentially to varying concentration of epi-BL (Figure S4a). Based on their root lengths' response, genotypes were classified into three response groups: negative, no response, and positive. For the lowest concentration of 0.01 μM epi-BL, *Rht1Rht2*, *Rht4, 6, 8, 11, 12, 13, 15*, and *16* mutants and *Rht3, 5, 6, 16, 17*, and *18* parents responded negatively, while *Rht3, 5, 7, 17, 18*, and *20* mutants and *Rht4, 8* parents had no response to epi-BL treatment. Conversely, *Rht1, 2*, and *9* mutants and the tall *rht* isoline, *11, 14* parents responded positively to the exogenous application of 0.01 μM epi-BL. The percent response was recorded by calculating the change in root length in the highest concentration of epi-BL (1.0 μM) compared to the control. Overall, mutants decreased 36.7% in root length in response to exogenous application of epi-BL with the greatest effect on *Rht12* (−60.8%) and least in *Rht2* (−19.5%). For the parents, a 40.9% mean decrease in response to exogenous application was observed. Among all genotypes, the *Rht6* mutant showed the lowest response (0.8%) to exogenous application of epi-BL when compared to its parent. Compared to their parents, *Rht5* (18.4%) and *Rht8* (19.9%) had a positive response while *Rht4* (18.7%) showed a negative response. For the remaining mutants, response ranged from −2.5 to 7.6% when compared to the parents.

A differential response was observed in root dry matter to the exogenous application of epi-BL (Figure S4b). Among the genotypes,

Rht1, 3, 5, 8, 11, 12, 14, and *15* mutants and *Rht4, 6, 8, 11, 14, 16, 17, 18*, and *19* parents decreased in root dry weight. A positive response in root dry weight was found for *Rht1Rht2*, *Rht2, 3, 4, 7, 9, 17, 18, 19*, and *Rht5* parents. *Rht6, 13, 16* mutants, and the tall *rht* isoline displayed no response to exogenous application of epi-BL. Overall, mutants (−9.9%) had a negative mean response compared to their parents (20.7%). Among the mutants, *Rht11* (19.6%) had the greatest response while *Rht8* (−38.8%) responded most negatively. Among the parents, *Rht8* parent (−52.0%) had the greatest negative response while *Rht19* parent (5.3%) the largest positive response to exogenous application of epi-BL. *Rht11* (49.4%) had the greatest positive response while *Rht1Rht2* (−20.1%) the greatest negative response among all mutants compared to their respective parents.

Like roots, shoot dry matter also had a differential response to exogenous application of epi-BL (Figure S4b). Among the genotypes, *Rht5, 7, 8, 11, 12, 13, 14*, and *15* mutants and *Rht3, 4, 5, 6, 8, 14, 17*, and *19* parents decreased in shoot dry weight. *Rht3, 6, 16*, and *17* mutants and the tall *rht* isoline, *16, 18* parents had no significant response to exogenous application of epi-BL. Alternatively, *Rht1Rht2*, *Rht1, 2, 4, 9,18, 19*, and *20* mutants and *Rht11* parent had increased shoot weight when compared with control. Among all the mutants, *Rht5* (41.8%) showed the greatest positive response while *Rht14* (−37.7%) the largest negative response to exogenous application of epi-BL compared to the parent.

3.3 | Mutant ranking

3.3.1 | By traits

Based on all photosynthetic parameters, *Rht4* was the most photosynthetically efficient mutant followed by *Rht19* and *Rht12*. The *Rht3* was the least photosynthetically efficient line followed by *Rht6* and *Rht2*. *Rht16* followed by *Rht12* and *Rht15* performed better for all the early seedling growth parameters combined while *Rht3* followed by *Rht2* ranked lowest for early seedling traits. Of all the semidwarf mutants, under pot emergence from deep planting depths, *Rht13* followed by *Rht7* and *Rht16* attained maximum emergence while *Rht1Rht2* double mutant followed by *Rht3* had the fewest emerged seedlings. Based on all the traits combined, considering the context of dryland farming, *Rht15* followed by *Rht19* and *Rht16* mutants were ranked the best while *Rht3* followed by *Rht6 and Rht2* performed worst overall.

3.3.2 | By ploidy level

Reduce height mutants were grouped based on their ploidy level into two groups, hexaploid and tetraploid (Table 1). On average, tetraploids performed better for photosynthetic efficiency and early seedling growth than hexaploids while mean percent pot emergence from deep planting depth was not much different between the two classes (Figure S4a). Among the hexaploids, *Rht4* ranked highest for

photosynthetic traits followed by *Rht12*, while *Rht3* followed by *Rht6* ranked least efficient. Among the tetraploids, *Rht19* followed by *Rht9* ranked highest and *Rht16* followed by *Rht14* ranked lowest for photosynthetic traits. Among hexaploids, *Rht12* followed by *Rht11* performed best and *Rht3* followed by *Rht6* ranked lowest for early seedling traits. For the tetraploids, *Rht16* followed by *Rht15* ranked highest while *Rht9* followed by *Rht14* were among the lowest ranked mutants for early seedling traits. Among the hexaploids, *Rht13* followed by *Rht7* attained the most emerged seedlings on day 14 while *Rht1Rht2* double mutant followed by *Rht3* had the least emerged seedlings. *Rht16* followed by *Rht15* performed best while *Rht9* and *Rht14* had fewest emerged seedlings on day 14 among the tetraploids.

3.3.3 | By GA pathway

Plant height variation in wheat is achieved either by lowering the bioactive GA content or by inhibiting GA signaling. Based on these two pathways, mutants were classified either as GA biosynthesis (*Rht18, 16, 14*) or DELLA GA signaling (*Rht1, Rht2, Rht3, Rht11, Rht17*) mutants. The other mutants are not well characterized so were excluded from the grouping. We observed that the GA biosynthesis mutants had better early seedling growth, percent emergence, and photosynthetic traits compared to GA signaling mutants (Figure S4b). Among the GA biosynthesis mutants, *Rht18* performed best for photosynthetic traits and *Rht16* for early seedling emergence from deep planting. *Rht11* performed best for photosynthesis and early seedling traits and *Rht17* for emergence traits among the GA signaling mutants.

3.3.4 | By growth habit

The semidwarf mutants were classified into three groups based on their growth habits (Table 1). In general, winter wheat performed best for photosynthetic traits followed by spring and facultative mutants (Figure S4c). *Rht4*, *Rht12*, and *Rht5* were the most promising lines among winter, spring, and facultative mutants, respectively, for photosynthetic traits. Spring mutants had the most early seedling growth followed by facultative and winter. *Rht16*, *Rht20*, and *Rht11* had the highest seedling ranking among spring, facultative, and winter mutants, respectively. Maximum seedling emergence from pot experiment was observed for facultative (*Rht5*) followed by spring (*Rht13*) and winter (*Rht8*) mutants. Overall, *Rht3* and *Rht1Rht2* double mutants performed lowest for all the studied traits.

4 | DISCUSSION

Wheat yield has steadily increased since the introduction of semidwarf cultivars (FAO, 2020). The increase in grain yield of semidwarf wheat cultivars is largely attributed to their response to agricultural inputs like irrigation and nitrogen fertilizer. However, gains in grain

yield (∼40%) of semidwarf cultivars obtained in the Asian subcontinent have not translated to the African subcontinent, mostly due to lack of adequate agricultural production practices and infrastructure (Pingali, 2012). Further, semiarid Mediterranean regions of the world including the US Pacific Northwest, southern Australia, and other major Mediterranean wheat production areas have also failed to translate the same yield gain (∼10%) compared to as in Asia (Pingali & Kelley, 2007). Additionally, climate constrains and ever-increasing demand for food is pushing wheat scientists to find new ways to breed better wheat adapted to climate change while maintaining or increasing the productivity per unit land area. Of the various abiotic stresses wheat experiences during its life cycle, drought poses the greatest threat to wheat productivity (Kartseva et al., 2018). The success of *Rht1* and/or *Rht2* and *Rht8* semidwarf wheat cultivars allured scientists to find new ways to reduce wheat plant height. These efforts have so far resulted in identification of ∼25 reduced height genes/loci (Table 1). We have no known *Rht1* and *Rht2* mutation in our collection except for *Rht1*, *Rht2*, and double mutant. However, these developed lines were not used in breeding programs because they are not well characterized, and several mutants lack associated markers.

In this study, we observed that early growth in mutants was negatively affected (except for *Rht5* which was positively affected) compared to parents. In the field, *Rht5* was reported to decrease plant height and peduncle length while having no significant effect on biomass and harvest index (Daoura et al., 2013). Parents generally have a longer coleoptile and first leaf length compared to their mutant counterparts (Ellis et al., 2004). Roots play an important role in anchoring and absorption of nutrients and water from the soil. In dry areas, longer roots are desirable to reach any available moisture residing deep in the soil profile. In general, mutants in our experiment had longer roots on young seedlings compared to parents, except for *Rht3*, *8*, *9*, *14*, and *20*. There are contrasting reports about the mutation effect on root characteristics due to differences in genotype, background, and/or experimental variation (Wojciechowski et al., 2009). Most current genotypes carrying *Rht1* or *Rht2* genes are reported to have shorter root lengths than nonmutant types. This may be due to selection of lines over time in well-watered conditions. Root angle is highly heritable and plays an important role in root architecture in cereals, directly effecting their drought tolerance (El Hassouni et al., 2018; Richard et al., 2015; Uga et al., 2015). Narrowly angled and longer roots are more suitable for drought tolerance while wider-angle root structure is associated with phosphorous and nitrogen absorption (Lynch & Wojciechowski, 2015; Uga et al., 2015). We identified *Rht16* with a narrow root angle and longer root length that might be suitable for drought tolerance. Further, *Rht16* is among the fastest and best-emerging genotypes both in laboratory (this study) and deep planting field emergence experiments. We also observed *Rht7* to have a wide root angle and short root length showing fast emergence. Root angle and root length are certainly not the only factors affecting emergence from deep planting depth, but our study suggests they may influence the plant's ability to perform in water-stressed conditions during later stages of development.

Wheat seedling emergence is correlated with several early growth attributes as well as grain yield. Wheat seedling emergence from deep depths is of critical importance in dry and semiarid areas like the US Inland Pacific Northwest, southeastern and southwestern Australia, central Chile, and several countries surrounding the Mediterranean Sea (Schillinger et al., 2017). Rapid emergence in these geographic regions is positively correlated with adequate stand establishment, plant biomass, higher grain yield, and weed competitiveness (Coleman et al., 2001; Kirby, 1993; L'opez-Castaneda & Richards, 1994; Schillinger et al., 1998). We identified a few lines that emerge as early as day 7 after planting through 12.5 cm of soil covering the seed, including a line with over 50% emergence by day 8 after planting. Previously, we tested the mutant lines in a field experiment (Mohan et al., 2013) and found that *Rht16* and *Rht7* had rapid emergence from deep planting similar to our laboratory pot experiment data reported here. *Rht3* and *Rht1Rht2* mutants were among the worst for emergence in both the field and laboratory studies. *Rht3* (allelic *Rht-B1c* and also *Rht1Rht2*) severely negatively affects plant height, biomass, coleoptile length, and seedling establishment (Addisu et al., 2009; Ellis et al., 2004; Rebetzke et al., 2004). Coleoptile length and first leaf length are positively correlated with seedling emergence from deep planting depths (Amram et al., 2015; Mohan et al., 2013; Schillinger et al., 1998). The fast-emerging mutant, *Rht16*, had both a longer coleoptile and first leaf. GA responsive genotypes *Rht5* and *Rht13* are reported to significantly improve emergence from deep planting compared to the respective parents (Amram et al., 2015). We observed that first leaf length is slightly better correlated to seedling emergence than coleoptile length. This might explain the relatively poor emergence of the *Rht3* genotype as we measured both shorter coleoptile and asynchronous emergence of first leaf. As reported in Mohan et al. (2013), we also observed a similar trend of increased probability of emergence with increasing coleoptile and first leaf length. However, we also recorded good emergence in shorter coleoptile lines, indicating additional factors affecting emergence from deep planting (Figure 3(A, B)).

Plant hormones regulate plant growth and development and defects in hormone biosynthesis or signaling has altered plant morphology such as plant height. Other than GA, reduced height mutants in cereals are reported in auxin (Multani et al., 2003) and BR (Chono et al., 2003; Sakamoto et al., 2006). Growth response to exogenous hormones, therefore, to some extent can potentially determine the nature of mutation (Chandler & Robertson, 1999). Initially, wheat reduced-height mutants were characterized based on their exogenous GA response (Ellis et al., 2004). However, this interpretation is not precise as mutants initially classified in the GA pathway might be in the BR pathway (Gasperini et al., 2012). We have observed differential response of mutants to GA and BR. Although, most mutants are not well characterized, we quantified the response compared with their respective parents to shed some light on the effect of mutation. Although allelic to *Rht1*, *Rht11* mutation showed a different response to GA (no response) and BR (negative). The differences in exogenous hormonal response observed in the present study compared to earlier reports (Ellis et al., 2004; Gasperini et al., 2012; Konzak, 1988) might

be due to the differences in the genotypes used and experimental procedures. We observed that *Rht5* showed a similar trend as *Rht8* to BR and GA while maintaining the biomass, indicating that this mutation might be in the BR pathway. Further, our preliminary expression analysis with the *Brassinosteroid-Insensitive 1* (*BRI1*) gene also showed reduced expression in both *Rht8* and *Rht5* compared to their respective parent (data not shown). Further analysis will help characterize this mutation in *Rht5*.

Photosynthesis efficiency differs among crops with C4 pathway having an edge over C3 plants. In recent years, scientists have realized the potential of improving photosynthetic efficiencies to ultimately increase the yield potential of wheat (one of the goals of the International Wheat Yield Initiative). We observed >2-fold variation in the germplasm with regards to A_n, g_s, V_{Cmax}, J_{max} and other parameters (Table S4). Although there are contrasting reports for photosynthetic rate among the semidwarf mutants and the tall parent, we observed no significant differences in photosynthetic rates among *Rht1*, *Rht2*, and *Rht3* (Dobrikova et al., 2017; Nenova et al., 2014). However, in *Rht1* (*Rht-B1b*), under field conditions during early grain development, flag leaf photosynthetic rate per unit area, stomatal conductance (20.2%), photosynthesis ($\sim$17.8%), and evapotranspiration (13.7%) rate decreased significantly compared to the tall genotype (Jobson et al., 2019). Similar to our observation, Uprety et al., 2009 also observed that tetraploids combined together, ranked higher for photosynthetic traits. Higher A_n is well correlated with grain yield performance in warmer environments (Reynolds et al., 2000), hence *Rht9* might be used in breeding programs in warmer areas. We also observed reduced net photosynthetic rate and related parameters in *Rht8* compared to its parents. It is reported that BR positively affects photosynthetic rate by enhancing several photosynthetic processes and enzymes like efficiencies of PSII, carboxylation efficiency of RUBISCO, regeneration rate of RuBP, RUBISCO, and other Calvin cycle enzyme activity (see review by Siddiqui et al., 2018). Further, BR mutants showed reduced photosynthesis and stunted growth, while external application of BR improved photosynthesis efficiency and tolerance to abiotic stresses (Siddiqui et al., 2018). We observed *Rht8* reduced emergence from deep planting compared to *Rht1*, a GA mutant. WUE is particularly important in dry and drought prone areas of the world. In general, plants with higher WUE, or more specifically higher ᵢWUE, might have potential for breeding wheat suited for dry regions. In our study, *Rht12* had the highest ᵢWUE and second longest root length. Although we have identified a few potential lines having higher ᵢWUE, A_n, and root length, these lines should be evaluated under abiotic stress conditions to understand the genotype and environmental effect.

We reported significant variation for different traits in the present study. We found first leaf length strongly correlated with emergence from deep planting depths. Thus, while selecting for emergence from deep planting depths, considering both the coleoptile and first leaf length could be the best strategy. The identified germplasm could be utilized in breeding programs to improve traits not only for abiotic stress tolerance but also to improve photosynthetic capacity. Most of the traits we report here are not well characterized and the genes

controlling the traits are not identified. Hence, the contrasting lines could also be a valuable resource to dissect the molecular and physiological mechanism underlying the studied traits.

AUTHORS CONTRIBUTION

Amita Mohan and Kulvinder S. Gill conceived the original research. Amita Mohan and Nathan P. Grant performed the experiments and analyzed the data; William F. Schillinger coordinated the deep emergence laboratory pot experiment; Amita Mohan and Nathan P. Grant wrote the article; Kulvinder S. Gill and William F. Schillinger complemented the writing.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are available within the paper and within its material published online.

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SUPPORTING INFORMATION

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