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GA-sensitive *Rht13* gene improves root architecture and osmotic stress tolerance in bread wheat

Muhammad Arslan Khalid[1], Zulfiqar Ali[2,3*], Latifa Al Husnain[4], Sajid Fiaz[5*], Muhammad Abu Bakar Saddique[1], Sabah Merrium[1], Kotb A. Attia[6], Sezai Ercisli[7] and Rashid Iqbal[8,9]

Abstract

The root architecture, more seminal roots, and Deeper roots help the plants to uptake the resources from the deeper soil layer to ensure better growth. The Gibberellic acid-sensitive (GA-sensitive) *Rht* genes are well known for increasing drought tolerance in wheat. Much work has been performed on the effect of these genes on the plant agronomic traits and little work has been done on the effect of *Rht* genes on seminal roots and root architecture. This study was designed to evaluate 200 wheat genotypes under normal and osmotic stress. The genotypes were sown in the solution culture and laid under CRD factorial arrangement with three replications and two factors i.e., genotypes and treatments viz. normal and osmotic stress (20% PEG-6000) applied one week after germination. The data was recorded for the root traits. Results demonstrated that out of 200 genotypes, the GA-sensitive *Rht13* gene was amplified in 21 genotypes with a fragment length of 1089 bp. In comparison, the GA-insensitive *Rht1* gene was amplified in 24 genotypes with a band size of 228 bp. From 200 wheat genotypes, 122 genotypes produced 5 seminal roots, 4 genotypes 4 seminal roots, and 74 genotypes 3 seminal roots. The genotypes G-3 (EBW11TALL#1/WESTONIA-*Rht5*//QUAIU#1), G-6 (EBW01TALL#1/SILVERSTAR-Rht13B//ROLF07) and G-8 (EBW01TALL#1/SILVERSTAR-Rht13B//NAVJ07) produced 5 seminal roots and have longer coleoptile (> 4.0 cm), root (> 11.0 cm) and shoot (> 17 cm) under normal and osmotic stress. Furthermore, Ujala 16, Galaxy-13, and Fareed-06 produced 3 seminal roots and have short coleoptile (< 3 cm), root (< 9.0 cm) and shoot (< 10.0 cm). These results showed that the genotypes showing the presence of GA-sensitive *Rht* genes produced a greater number of seminal roots, increased root/shoot growth, and osmotic stress tolerance compared to the genotypes having GA-insensitive *Rht* genes. Thus, the *Rht13* gene improved the root architecture which will help to uptake the nutrients from deeper soil layers. Utilization of *Rht13* in wheat breeding has the potential to improve osmotic stress tolerance in wheat.

Keywords Dwarfing genes, Breeding, Root architecture, Drought, Grain yield, Osmotic stress

*Correspondence:
Zulfiqar Ali
zulfiqarpbg@hotmail.com
Sajid Fiaz
sajidfiaz50@yahoo.com

Full list of author information is available at the end of the article

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop and is cultivated in all parts of the world, it is the main food source for about 30% of the world population [1]. In Pakistan, wheat was cultivated in an area of 9.60 million hectares with an overall production of 31.4 million tons [2], while in the world its production was recorded at 778.83 million tons [3]. Contrary to that the world population is daily increasing, at present the recorded world population is 7.90 billion [3]. Due to continuous increases in food consumption and increasing industrial uses, it is expected that the world wheat utilization will reach 741 million tons. In comparison, we have a wheat stock of 264 million tons only and the wheat stock likely decreased 3.3% at the end of 2019 [4]. So, to feed the increasing population which will double by 2050, there is a need to increase wheat production by 2% every year, especially in the underdeveloped countries of the world i.e., south-east Asia to fulfill the future demand of the increasing population. The assimilates portioning during the grain-filling period is supported when the above-ground biomass is more [5]. Furthermore, improving the root architecture reduces the requirement for organic fertilizers and improves abiotic stress tolerance [6].

The utilization of semi-dwarf wheat varieties extensively resulted in an increased wheat yield in the world during the 1960s and 1970s, which led to the Green Revolution [7]. The two main "green revolution" genes i.e., *Rht-B1b* and *Rht-D1b* encode DELLA protein-altered forms, which act as key repressors for the signaling pathway of gibberellin [8]. These dwarfing genes are well known to reduce cell size in coleoptiles, culms, and leaves of wheat. As compared to standard height genotypes (*Rht*-B1a and *Rht*-D1a) of wheat, gibberellin-insensitive wheat is poor in terms of leaf area development. Alternative height-reducing genes to *Rht*-B1b and *Rht*-D1b are accessible without compromising the adaptability traits [9]. The alternative height-reducing genes (GA-sensitive) *Rht8* and *Rht13* shorten plant height and enhance grain yield [9]. The *Rht13* gene reduces the plant height and peduncle length by 30 to 45% and 30 to 53%, respectively, while it increases the spike length, number of grains per spike, and grain yield per plant by 20 to 50%, 17 to 26%, and 29 to 50%, respectively [10]. The *Rht* genes play a significant role in improving drought stress tolerance and grain yield per plant in wheat.

In wheat, the root system contains two types of roots i.e., seminal roots (crown roots) and nodal roots (adventitious roots) [11, 12]. Both these root types play a vital role in plant growth and remain active during the whole plant life cycle. The number of seminal roots may be more crucial in specific conditions like drought stress, as these roots penetrate the deeper soil layers compared to nodal roots, making the accessibility of water to plants from deeper soil layers [13, 14]. The seminal roots also play a significant role in the establishment of crops, as these roots exist in the plant before the fourth leaf emergence. The genotypic variation in root number has been reported among genotypes of various crop plants like wheat [15–17], rice [18], cotton [19], and soybean [20]. These green revolutionary genes have a major role in the development of seminal roots, but little work has been reported on these plant roots [21, 22]. Studies showed that the increased number of seminal roots showed enhanced root length that allows to uptake of the soil moisture from the deeper soil layers resulting in increased osmotic stress tolerance [23]. The large root system is more efficient under drought stress even though it requires more share of plant assimilates [24]. Roots' inability to access water from soil is mainly due to lower root density and root length [24]. Previous studies at the seedling stage under osmotic stress using germination paper [25], hydroponic solution [26], small pots [27], wax layer screens [28], craft paper culture [29], and growth chambers filled with gel [30] reported that greater number of seminal roots associated with increased seedling growth and enhancing drought tolerance. The Rht genes were reported to significantly influence the number and length of seminal roots. The dwarfing genes i.e., *Rht8*, Rht12, and Rht18 produced more seminal roots (SR), longer SR, more root depth, and rate of root penetration [28]. Contrary to that the GA-insensitive genes (*Rht1* and *Rht2*) reduced the biomass of roots, while *rht-B1c* minimized the energy required for the roots development by increasing the length of root and cortical aerenchyma volume without affecting the ratio of root and shoot [31].

Significant studies were conducted on plant aerial parts, but little work was reported on the effect of GA-sensitive *Rht* genes on several seminal roots, coleoptile length, and root length under osmotic stress [32]. The present study aimed to evaluate the genotypes for seminal root diversity, root length, shoot length, and the role of GA-sensitive *Rht* genes on the number of seminal roots in modern wheat cultivars.

Materials and methods

Plant material and experimental layout

The experiment was performed at the Institute of Plant Breeding and Biotechnology, MNS University of Agriculture Multan, Pakistan. During 2020–2021 a set of 200 wheat genotypes collected from CIMMYT, MNS University of Agriculture Multan, and National Agricultural Research Institute (Annexure 1) were sown in solution culture as described by Hoagland and Arnon [33]. The recipe for the Hoagland solution is given in Table 1. The experiment was laid under a completely randomized

Table 1 The chemical composition of half-strength Hoagland solution. The chemicals from serial 1 to serial 4 are macronutrients. The first three macronutrients are mixed and kept in one bottle while 4th macronutrients are kept separate. The chemicals from serial 5 to 10 are micronutrients. All micronutrients except FeEDTA are mixed to get a master mix solution. Then this macronutrient master mix solution and micronutrient are dissolved in one-liter water and after that $(CaNO_3)_2$. H_2O and FeEDTA are mixed

Sr. No.	Chemical	Quantity/liter
1.	KH_2PO_4	0.5 ml
2.	KNO_3	2.5 ml
3.	$MgSO_4.7H_2O$	1 ml
4.	$(CaNO_3)_2.H_2O$	2.5 ml
5.	H_3BO_2	0.5 ml
6.	$MnCl_2.4H_2O$	0.5 ml
7.	$ZnSO_4.7H_2O$	0.5 ml
8.	$CuSO_4.5H_2O$	0.5 ml
9.	$H_2MoO_4.H_2O$	0.5 ml
10.	Fe EDTA	0.5 ml

design (CRD) with three replications in factorial arrangements with two factors i.e., genotypes and treatments viz. normal and osmotic stress (20% PEG-6000, prepared by adding 200 g of PEG-6000 and making the volume up to 1 L by adding Hoagland solution) applied one week after germination. The data were recorded two weeks after application of treatment from three plants (21 days age) of each genotype per replication per treatment for coleoptile length (cm) was measured from the length of the pointed protective sheath covering the emerging shoot, number of seminal roots was counted manually, root length (cm) was measured from the base of root to the tip of last root and shoot length (cm) was calculated from the joint of shoot with root to the tip of shoot. Collected data was analyzed to estimate correlation. Biplot analysis was also performed from mean best linear unbiased estimate (BLUE) values of 200 wheat genotypes [34].

Molecular analyses

Bioinformatics analysis

The protein sequences for different *Rht* genes were downloaded from Phytozome (https://phytozome-next.jgi.doe.gov/) by using the keyword search for a specific species. The domain of *Rht* genes was checked by the motif finder tool (https://www.genome.jp/tools/motif/). All protein sequences of *Rht* genes were utilized to perform the multi-sequence alignment using Clustal X (http://www.clustal.org/clustal2/) and phylogenetic analysis was performed using Mega X (https://www.megasoftware.net/dload_win_gui). For exons and introns prediction and organization in the gene structure display server was used (http//gsds.cbi.pku.edu.cn). The conserved motif was identified by the Conserved Domain Database (CDD) of NCBI (https://www.ncbi.nlm.nih.gov/) and TB tools (https://github.com/CJ-Chen/TBtools-II/releases) were used for the visualization of the conserved motif.

Table 2 List of primers

Locus	Gene	5′F	5′*R*	Fragment Length (bp)
Traes_7BS_54E859139.1 (primary)	*Rht1*	GAGAAGGT CCTGGGCA CCGT	AACAGCTGC CCCCCGATG AGA	228
Rht13	*Rht13*	ATGGCATAA TTTGGTGAA ATTG	TGTTTCAAG CCCAACTTC TATT	1089

DNA extraction

A nursery of 200 genotypes was raised and the sample was taken from young leaves at two leaf stages. The plant samples were ground using liquid nitrogen to break the cell wall, then CTAB was added to release DNA from cell and nuclear membranes, and after that chloroform was added to make the DNA sample protein-free. For further purification of the DNA sample, other reagents like RNase, and NaCl were also added and centrifuged for repeated times to finally obtain DNA in the form of a pellet. The 0.5 g of leaf samples were ground in pestle mortar with liquid nitrogen and this powder was transferred in a 2 ml tube. Then 0.75 ml CTAB extraction buffer was added to ground leaf samples and incubated at 65 °C for 45 min in a water bath. After incubation 0.75 ml chloroform isoamyl alcohol was added and centrifuged for 10 min at 9000 revolutions per minute (rpm) then the supernatant was transferred to another 2 ml tube. Then equal volume of chilled 2 propanol was added to precipitate the DNA and again centrifuged for 10 min at 9000 rpm at 9 °C after that supernatant was discarded and the pallet was washed with 70% ethanol and dried. The dried pallet was mixed in 30 µl of d_3H_2O. The extracted DNA quality and quantity were checked on the 1% agarose gel and the final concentration was recorded on a spectrophotometer.

PCR amplification

For designing the primers, coding DNA sequences were downloaded from Phytozome *Triticum aestivum* V2.2 (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Taestivum_er). The full-length primers were designed using Amplifx version 1.7.0 software. A list of primer sequences is mentioned in Table 2. During the designing of primers all rules for primer designing were strictly followed i.e., the length of primer ranged from 18 to 24 bases, no more than 4 to 60% GC contents, melting/annealing temperature ranged up to 50 to 60˚C, the forward and reverse primer temperature difference should not more than 5˚C.

Twenty µL final volume of PCR reactions containing 12.8 µL ddH2O, 1 µL genomic DNA (20 ng/µL), 0.4 µL of each primer (10 µM), 2 µLrTaq buffer (10X), 1.6 µL MgCl2 (25 mM), 1.6 µL dNTPs (10 mM), and 0.2 µLrTaq DNA polymerase (5 U/µL) (Takara Bio Inc; Shiga, Japan). PCR was performed in a thermo-cycler (T100 Thermal Cycler, Bio-Rad Laboratories, Inc.) set with initial denaturation temperature of 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 to 60 °C for 30 s and extension at 72 °C for 60 s/kb of amplicon followed by a final extension at 72 °C for 3 min/kb of amplicon. Resolve amplicons on 1% agarose gel [35, 36].

Statistical analysis

The data was collected in triplicate, and statistical analysis was undertaken via SPSS 2.0 and Microsoft Excel 2016.

Results

The phylogenetic analysis (Fig. 1) was constructed to find the evolution of these *Rht* genes. It was observed that primitive was the Unicellular organism then *Rht5* was evolved while *Rht1* and *Rht* genes in dicots have the minimum evolutionary era.

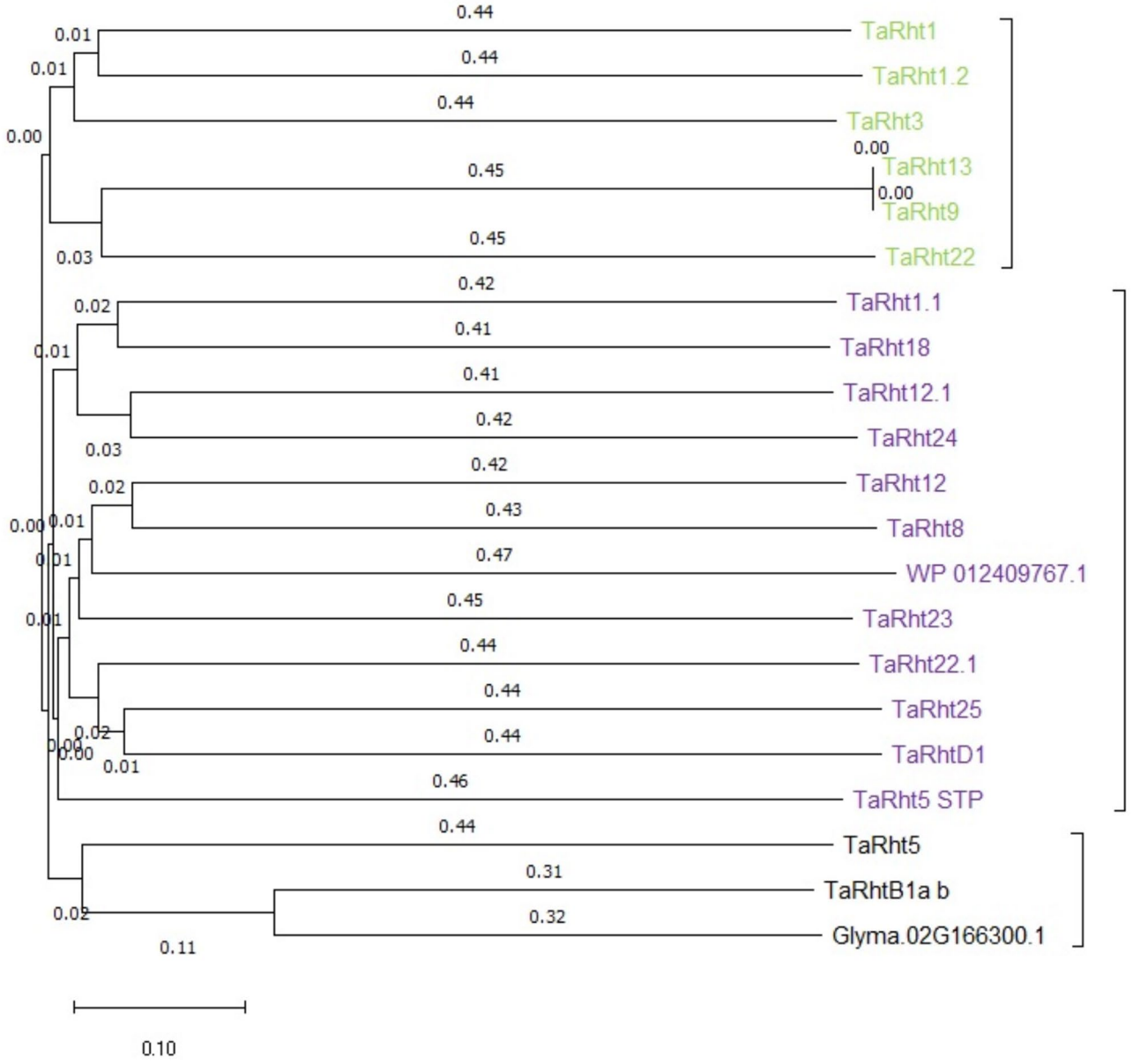

Fig. 1 Phylogenetic analysis of *Rht* genes in Wheat (Ta), Soybean (Glymax) and unicellular organism *Nostoc punctiforme* (WP) to find the evolutionary history of *Rht* genes

Structure analysis

To characterize the *Rht* gene's structural diversity, the analysis of exon-intron organization was performed. Most *Rht* genes showed two or three exons, while *Rht1*, *Rht2*, and *RhtD1* showed no intron. The *Rht12* genes showed three introns, *Rht12.1* showed seven introns, *Rht13* showed no introns, *Rht18* showed five introns, *Rht22* showed two introns, *Rht23* showed no introns, *Rht24* showed 11 introns, and *Rht25* gene showed six intros (Fig. 2). Most of the *Rht* genes showed both upstream and downstream regions while *RhtB1ab*, *Rht3*, *Rht9*, *Rht22*, *and Rht23* showed only upstream sequences with no downstream. *Rht24* showed no upstream or downstream sequences. It was observed that closely related *Rht* genes showed similar numbers of exons and lengths of introns, while distantly related genes have variable numbers and lengths of exons and introns, respectively.

Motif analysis

As Rht genes do not show high structural similarity, so motif analysis was performed. We have identified 20 conserved motifs. The genes *Rht 1.1*, *Rht1.2*, and *RhtB1ab* showed only one motif (Motif 17). The genes *Rht1*, *Rht3*, *Rht5*, and *RhtD1* showed no motif. It was also clear that the Motif 1 is conserved in *Rht2.1*, *Rht12*, and *Rht25*. A maximum of 10 motifs was observed in *Rht2.1*, *Rht9*, *Rht13*, and *Rht25*. The lowest number of motifs was observed in *Rht1.1*, *Rht1.2*, and *RhB1ab* which showed only one motif (Fig. 3). It was clear that structural motif composition varied among the different *Rht* genes, and it is similar among the *Rht* genes showing that these genes have conserved domains.

PCR amplification of *rht* genes

GA-sensitive and insensitive dwarfing gene primers were amplified on PCR displayed in Fig. 4. PCR results showed significant variation among wheat genotypes for the presence of *Rht* genes. The primer P1 (*Rht1*) was amplified at 228 bp (4a). The primer 2 (*Rht13*) gene showed a band size of more than 1 kb (4b). The genotypes having other different GA, BR, and dwarfing genes were also identified using the PCR amplification of selected primers. The wheat genotypes are identified as having GA-sensitive *Rht* genes alone or in combination with the GA-insensitive *Rht* genes.

Effect of GA-sensitive *rht* genes on the number of seminal roots

The mean sum of squares from the analysis of variance showed significant variability among genotypes and treatments for coleoptile length, seminal roots, root length, and shoot length. While coleoptile length and seminal roots showed non-significant differences for treatment (Table 3). Genotypes × Treatments interactions were not significantly different for seminal roots, coleoptile length, root length, and shoot length under both normal and osmotic stress.

Biplot analysis

The biplot analysis showed 35.3% explained variation for PC1 and 24.8% for PC2 (Fig. 5). Seminal roots had a positive correlation with coleoptile length, while they had a negative correlation with shoot length and root length both under normal (N) and osmotic stress (D). The genotypes were clustered according to the studied traits under normal and osmotic stress. Under normal stress,

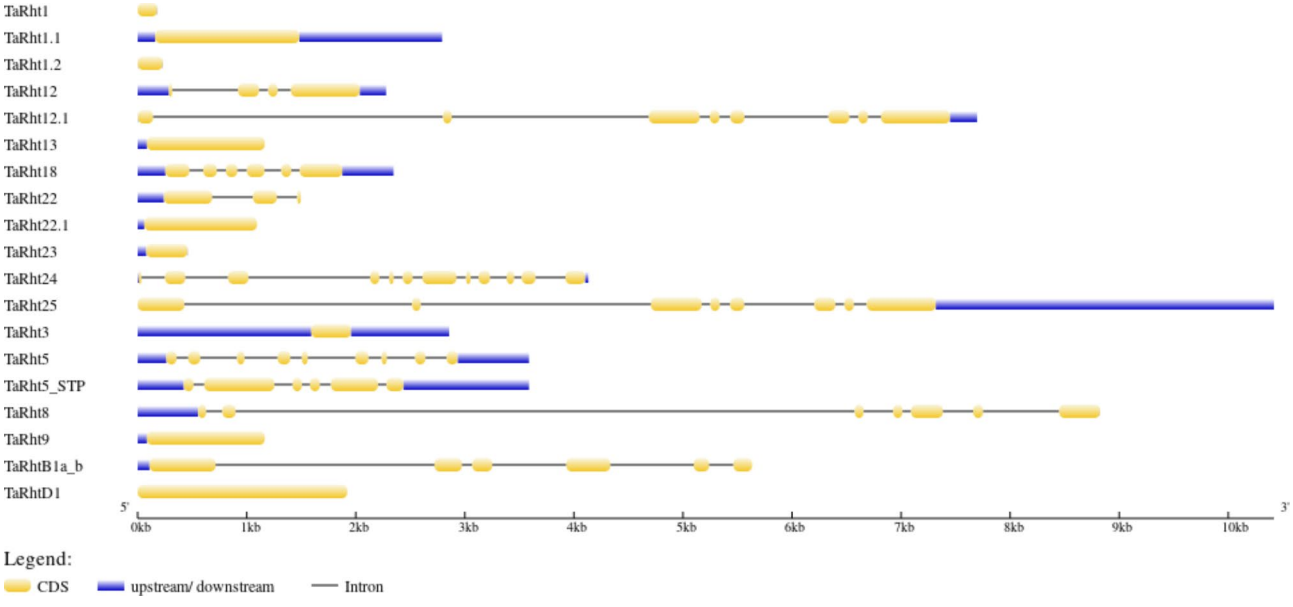

Fig. 2 Gene structure analysis of *Rht* gene(s) in wheat showing the arrangements of introns, CDS and upstream and downstream

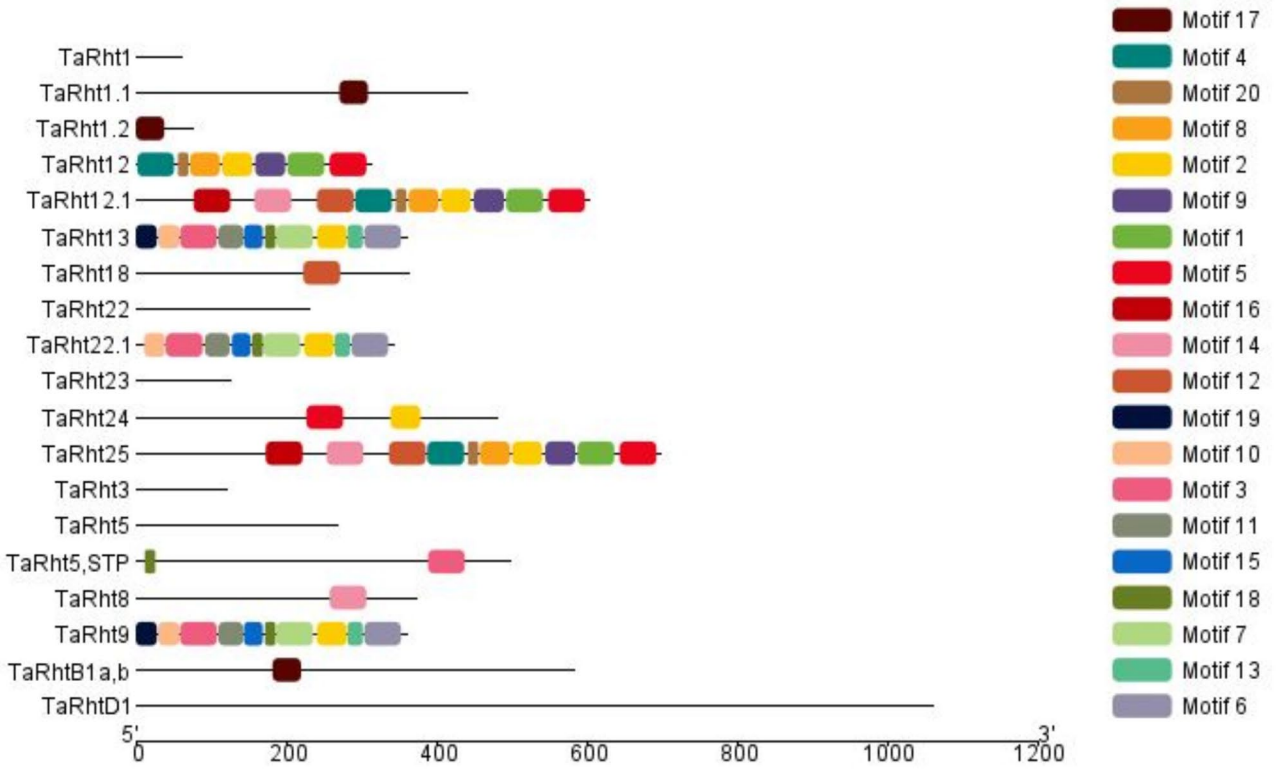

Fig. 3 Motif analysis of *Rht* gene(s) in wheat shows that the *Rht* genes are involved in various functions including Regulatory, ABA Responsiveness, Light Responsiveness, Cis-acting element in promotor and enhancer, Meristem expression, Anoxic specific inducibility, Low-temperature responsiveness, Drought inducibility, Auxin responsive element. The gene names are added on the vertical side and the different colors of the motif represent different functions involved

the genotype codes 163, 12, and 45 showed the highest scores for the seminal roots (SR) both under normal and osmotic stress. The genotypes coded with 3, 104, and 130 showed more mean value than the population means and appeared as the best genotype for coleoptile length (CL) both under normal and osmotic stress. Genotypes 38, 185, 40, and 19 showed more distance from the population mean towards the vector for Shoot length (SL) and root length (RL) both under normal and osmotic stress. Contrary to that the genotype codes 14, 103, and 95 fall opposite to the vector so their mean is less than the population mean and thus appeared as poor for the number of seminal roots (SR) both under normal and osmotic stress. The genotypes coded with 46,78 and 164, 155 showed less value compared to the population mean for coleoptile length and shoot and root length, respectively, both under normal and osmotic stress.

Mean performance of wheat genotypes

The mean performance of wheat genotypes under normal conditions (Annexure 1) showed that the genotype G-125 (ATTILA*2/PBW65*2//WHEAR) produced highest coleoptile length (4.1 cm), followed by the genotype G-3 (EBW11 TALL#1/WESTONIA-*Rht5*//QUAIU #1) having GA-sensitive *Rht13* produced maximum coleoptile length (4.0 cm), number of seminal roots (5), moderate root length (7.0 cm) and high shoot length (22.7 cm). The genotype G-40 (AAS-11) local check having GA-insensitive *Rht1* gene produced longer roots (14 cm), short coleoptile length (0.3 cm), intermediate shoot length (16 cm), and less number of seminal roots (3). The genotype G-46 (FSD-08) produced a shorter coleoptile length (0.1 cm), less number of seminal roots (3), shorter root length (3 cm), and shoot length (9 cm). Under osmotic stress (Annexure 2) the genotype G-3 (EBW11 TALL#1/WESTONIA-*Rht5*//QUAIU #1) having GA-sensitive *Rht* gene (*Rht13*) produced maximum coleoptile length (3.8 cm), more number of seminal roots (5), medium root length (8.9 cm) and more shoot length (24.22 cm). The greater number of seminal roots (5) and coleoptile length (3.6 cm) was recorded from genotype containing GA-sensitive *Rht* gene (*Rht13*) including G-3 (EBW11 TALL#1/WESTONIA-*Rht5*//QUAIU #1), G-6 (EBW01 TALL#1/SILVERSTAR-Rht13B//ROLF07) and G-8 (EBW01 TALL#1/SILVERSTAR-Rht13B//NAVJ07), while lower number of seminal roots (3) and root length (2.9 cm) was recorded from genotype Ujala-16. The genotype having 3 seminal roots and 5 seminal roots are shown in Fig. 6. Maximum root length (15.5 cm) and shoot length (26.9 cm) were recorded from the genotype

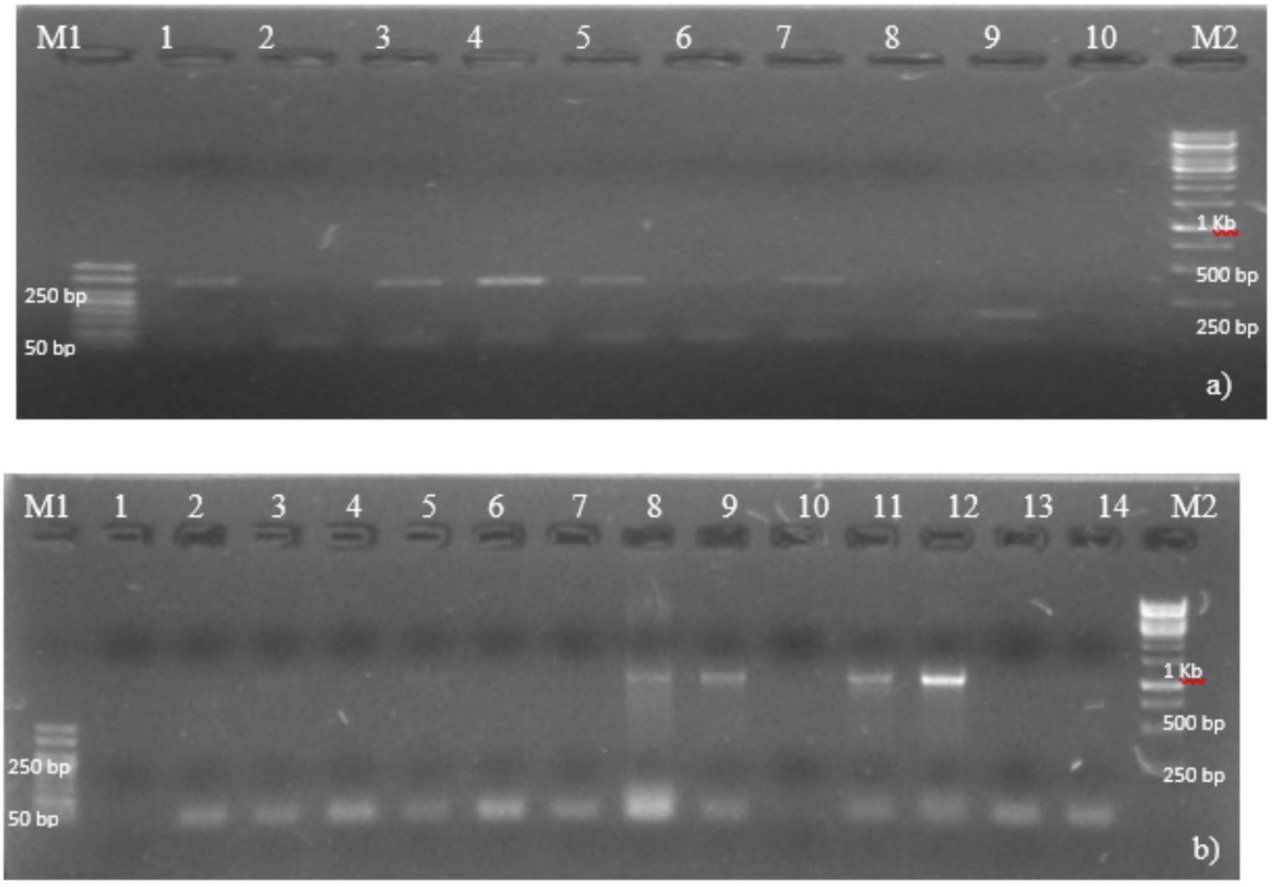

Fig. 4 PCR amplicons (a) P-1 = *Rht1* at 228 bp (b) P2 = *Rht13* at above 1 Kb. M1 and M2 show the ladder 50 bp and 1 Kb, respectively, while the number shows the PCR product of genotypes DNA. Figure that the genotypes 1, 3, 4, 5 and 7 *Rht13* gene above 250 bp. 8, 9, 11, and 12 showed the presence of *Rht13* gene at 1Kb

Table 3 Mean square values for the coleoptile length, seminal roots, root length, and shoot length of 200 wheat genotypes under normal and osmotic stress (20%, PEG-6000)

Source	DF	Coleoptile length	Seminal Roots	Root length	Shoot length
Genotypes	199	0.782**	2.414**	20.119**	40.322**
Treatments	1	0.819^{NS}	0.053^{NS}	129.955**	84.577**
Genotypes × Treatments	199	0.104^{NS}	0.193^{NS}	0.637^{NS}	3.096^{NS}
Error	800	0.309	13.391	0.446	22.412
Total	1199				

Where ** shows significant at $p \leq 0.01$, * = significant at $p \leq 0.05$ and NS = non-significant $p > 0.05$

G-3 (EBW11 TALL#1/WESTONIA-*Rht5*//QUAIU #1) and minimum root length (9.3 cm) and shoot length (10.2 cm) was recorded from genotype Galaxy 13 having GA-incentive *Rht* genes (*Rht1*). This shows that GA-sensitive *Rht* genes play a vital role in enhanced root and shoot growth which results in better seedling stand and crop growth.

Correlation estimates

The correlation estimates for studied traits showed that shoot length and root length were positively associated, while shoot length had a negative correlation with seminal roots and seminal roots, and coleoptile length was also negatively correlated under both normal and osmotic stress (Fig. 7a and b), respectively.

Discussion

The performance of plants is purely associated with plant growth and development. Many plant developmental features like plant architecture, leaf attribute, and architecture of vascular bundles are mean features that control the plant's overall performance. For example, the quality of light interception, plant source strength, and rate of photosynthesis is controlled by plant architecture and leaf features. These features aid in direct photosynthates mobilization from source to sink. These attributes are considered plant development part and showed that these enhance plant growth and yield, optimization of

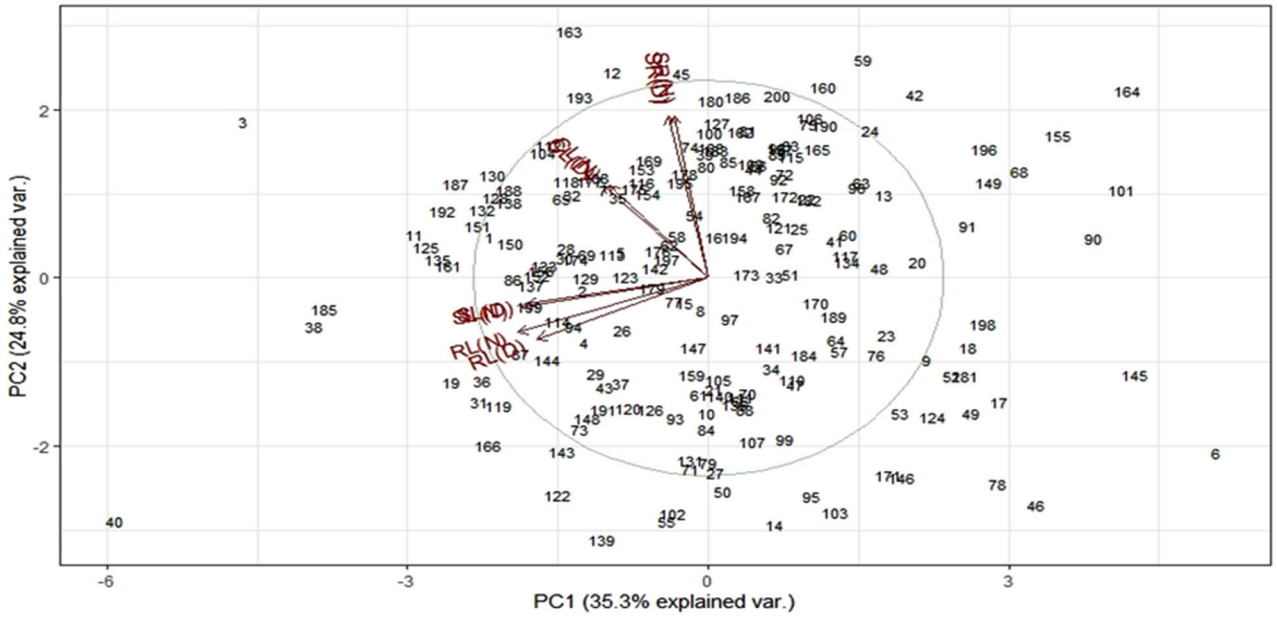

Fig. 5 Biplot display of seedling traits viz. coleoptile length (CL), seminal roots (SR), root length (RL) and shoot length (SL) of 200 wheat genotypes under normal (N) and osmotic stress (D) conditions. The Circle shows the theoretical highest extent of arrows, added by 68% of default confidence interval. Arrows depicts the correlation among these traits

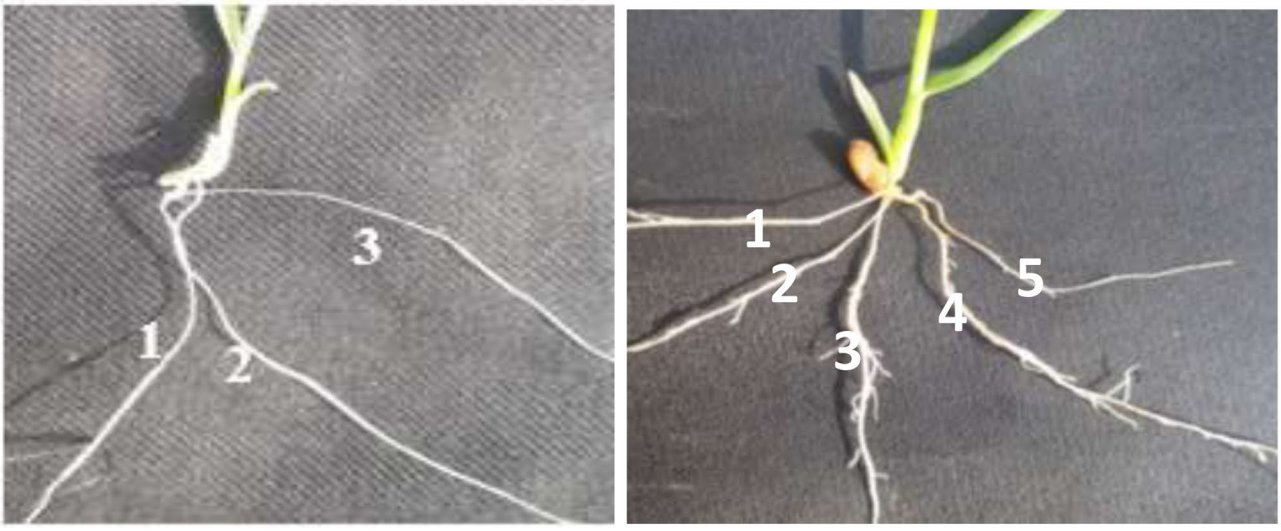

Fig. 6 The genotypes showing 3 seminal roots (Ujala-16) vs. 5 seminal roots (G-6 = EBW01 TALL#1/SILVERSTAR-Rht13B//ROLF07) grown in Hoagland nutrient medium

these crop development attributes is most crucial for efficient plant performance and yield [37].

The results demonstrated that *Rht* genes contain structural, functional, and regulatory domains showing that these genes are involved in many plants' growth functions. They play a significant role in increasing plant growth, reducing plant height (up to 50%) than taller plants of parental lines [38], or plant population varying for GA-sensitive and insensitive dwarfing alleles [39]. Plant molecular marker development [40] showed that dwarfing alleles showed a significant role in plant performance under various environments. These markers help to assess the effect of these alleles on specific traits important for selection and incorporation by crop breeding program. Furthermore, the use of these molecular markers helps in the targeted selection of desired traits in wheat breeding programs for adaptation to a variety of environments.

This study was planned to check the association of seminal roots with osmotic stress tolerance. The results

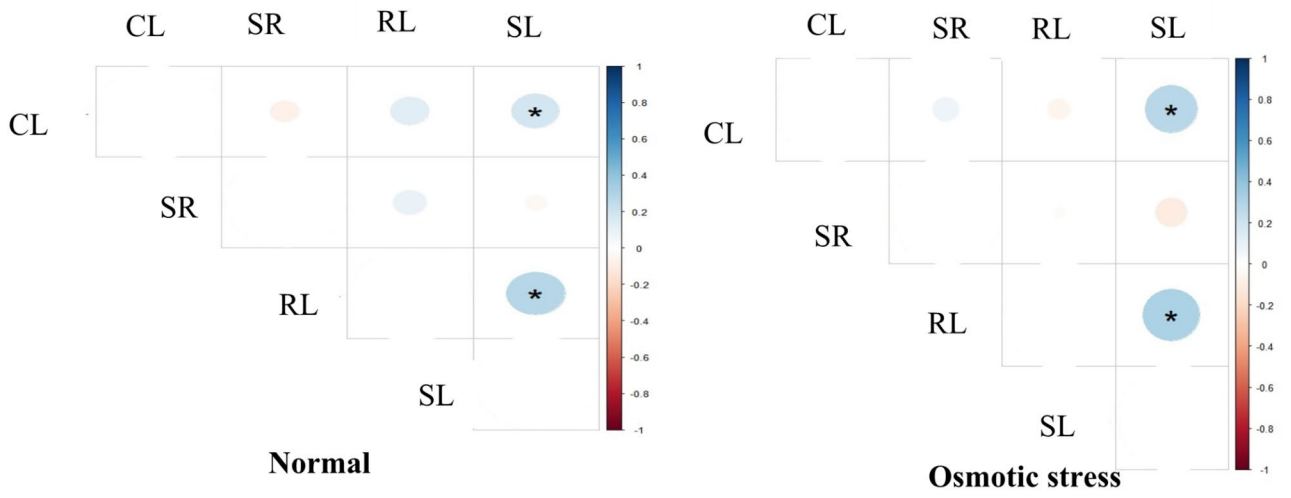

Fig. 7 Correlation estimates of seedling traits viz. coleoptile length (CL), seminal roots (SR), root length (RL) and shoot length (SL) of 200 wheat genotypes sown under the solution culture (Hoagland solution) in **a**) normal condition (no stress) and **b**) osmotic stress (20% PEG). Blue shade shows positive association, and the light pink shade depicts the negative correlation. The start on these shades shows the significance ($p \leq 0.05$) of correlation. The circle size showed the degree of association among the traits. More the size more will be the strong association

showed that the maximum number of seminal roots (5) and coleoptile length (3.6 cm) was recorded from genotype containing GA-sensitive *Rht* gene (*Rht13*) including G-3 (EBW11 TALL#1/WESTONIA-*Rht5*//QUAIU #1), G-6 (EBW01 TALL#1/SILVERSTAR-Rht13B//ROLF07) and G-8 (EBW01 TALL#1/SILVERSTAR-Rht13B//NAVJ07). It was also recorded that genotypes having a greater number of seminal roots showed increased root length, shoot length, and osmotic stress tolerance. It was observed that a greater number of seminal roots with narrow root angles showed increased seminal root length, which allows plants to absorb moisture from deeper soil layers thus enhancing drought tolerance in wheat [23]. It was reported that the number of seminar roots is associated with genetic and maternal control and the size, frequency, and number (two, three, four, five, and six) are associated with an increased in the size of the embryo. So the GA-sensitive *Rht13* gene plays a significant role in increasing seed number, seed volume, and embryo size compared to other GA-insensitive *Rht* genes [41, 42].

Roots' inability to access water from soil is mainly due to lower root density and root length (Gregory et al. 1978). Previous studies at the seedling stage under osmotic stress using germination paper [25], hydroponic solution [26], small pots [27], wax layer screens [28], craft paper culture [29], and growth chambers filled with gel [28] reported that greater number of seminal roots associated with increased seedling growth and enhancing drought tolerance. It was reported that the *Rht* genes significantly influence the number and length of seminar roots. The dwarfing genes i.e., *Rht8*, *Rht12*, and *Rht18* produced more seminal roots (SR), longer SR, more root depth, and rate of root penetration [28]. These results suggested that screening of genotypes for seminal roots is useful for selecting genotypes for drought tolerance at the early seedling stage.

The root morphology is the result of plant adaptations to a variety of environments, and the root morphology of plants is changed when plants are subjected to different abiotic and biotic stresses. Previous studies reported that drought and heat stress significantly affect root number, root length, and coleoptile length [43]. The morphology of roots in response to abiotic stress was regulated by plant hormones like gibberellins and ethylene production under extreme drought and heat stress [44]. Furthermore, an increase in hormone synthesis is also triggered by light intensity would lead to an increase the cell differentiation that promotes an increase in root number and root length in cereal crops [45]. The plant genotype (genetic background and dwarfing gene) has a positive impact on the plant root development and it was observed that the genotypes having *Rht13* and *Rht18* genes produced more root length, more root biomass, increased root/shoot ratio, and no effect on the plants assimilate portioning [46, 47].

Conclusion

This study has shown a significant role of the GA-sensitive *Rht13* gene on the number of seminal roots, coleoptile length, root length, and shoot length both under normal and osmotic stress compared to genotypes having the GA-insensitive (*Rht1*) gene. It was found that genotypes having a greater number of seminal roots with longer coleoptiles showed increased osmotic stress tolerance in wheat.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12863-024-01272-4.

Supplementary Material 1

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Author contributions
Muhammad Arslan Khalid and Zulfiqar Ali developed the idea of research and conducted experiments. Muhammad Arslan Khalid and Muhammad Abu Bakr Siddique collected data, data analysis, and collected a literature review for the article. Latifa AlHusnain, Sajid Fiaz, Rashid Iqbal, Sezai Ercisli and Kotb A. Attia helped in the revision, editing, and funding acquisition of the article. All authors revised the article and agreed to submit it for publication of the article.

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Data availability
Data will be made available upon reasonable request with the corresponding author.

Declarations

Ethics approval and consent to participate
The experiments did not involve endangered or protected species. No specific permits were required for these locations/activities moreover, all methods were carried out by relevant guidelines and regulations, under ethical approval and consent to participate.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan 66000, Pakistan
[2]Programs and Projects Department, Islamic Organization for Food Security, Astana 019900, Kazakhstan
[3]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan
[4]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
[5]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore 54590, Pakistan
[6]Center of Excellence in Biotechnology Research, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[7]Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey
[8]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[9]Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

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