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# Phenotyping and Identification of Reduced Height (*Rht*) Alleles (*Rht-B1b* and *Rht-D1b*) in a Nepali Spring Wheat (*Triticum aestivum* L.) Diversity Panel to Enable Seedling Vigor Selection

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**Abstract:** Nepal is facing more intense early-season drought stress associated with climate change. The introgression of reduced height (*Rht*) alleles to enable stem dwarfism in bread wheat (*Triticum aestivum* L.) inadvertently reduced coleoptile length and growth plasticity in seedlings, making improved varieties less suitable for deep seeding; these alleles may have also reduced seedling root length. Therefore, with the long-term objective of breeding wheat for early-season drought stress, a Nepali spring wheat panel was evaluated to assess allelic variation at the most common dwarfing-associated loci (*Rht-B1*, *Rht-D1*) and their impact on coleoptile/seedling root traits, and to identify accessions with longer and/or more GA-responsive coleoptiles as parents for future breeding. Here, Kompetitive Allele Specific PCR (KASP) was used to genotype accessions. The panel was phenotyped using the cigar-roll method in the presence/absence of $GA_3$. Plant height was measured under field conditions. The results showed that Nepali landraces had a significantly higher frequency of the non-dwarfing allele *Rht-B1a*. The dwarfing alleles *Rht-B1b and Rht-D1b* had negative effects on coleoptile length but positive effects on the length of the longest seedling root. However, 40 potential semi-dwarf accessions (possessing *Rht-B1b* and/or *Rht-D1b* alleles) with long and/or more plastic coleoptiles suited for deep sowing were identified. This included 12 accessions that exhibited significant changes in coleoptile length in response to $GA_3$ treatment.

**Keywords:** *Rht*; coleoptile length; seedling root length; drought; deep seeding

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## 1. Introduction

The coleoptile is the structure that covers the first leaf before it emerges from the soil surface during germination. In cereal crops, including wheat (*Triticum aestivum* L.), the coleoptile is important for supporting and protecting the protruding leaf as it rises to the soil surface [1]. The length of the coleoptile determines seed sowing depth [2–4]. Longer coleoptiles allow deep sowing, which helps in the exploitation of soil moisture [5–7] and facilitates early establishment as well as weed suppression [8]. Therefore, wheat accessions having longer coleoptiles are better suited to areas with drought stress or drought at the early growth stage of the crop [4,5,9]. In this context, breeding wheat for longer coleoptiles is a strategy to cope with these challenges [4,9–11]. In addition to the coleoptile, different root architectural traits, including longer roots, contribute to improving drought stress tolerance in wheat [6,12,13]. Longer seedling roots contribute to plant establishment at the early stage [14]. Therefore, the phenotypic assessment of wheat root traits can be useful when selecting accessions for drought stress tolerance [15].

Genetic variation for wheat coleoptile length has been reported in many studies [4,5,16]. The trait is governed by many genes with a strong additive effect and high heritability [2,8]. Short coleoptiles in wheat are the result of pleiotropic effects associated with mutations in alleles at the reduced height genes, *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*), that encode DELLA proteins in the gibberellic acid ($GA_3$) signaling pathway [2,17]. Several *Rht* loci have been discovered [7,18–20], but among these, *Rht-B1b* and *Rht-D1b* are the most commonly used dwarfing alleles in modern wheat, derived from "Norin 10", a Japanese variety, at the start of the Green Revolution [21]. *Rht-B1b* and *Rht-D1b* have contributed to at least a 10% increase in wheat yield [22]. Today, most modern semi-dwarf wheat varieties have *Rht-B1b* and *Rht-D1b* alleles [8]. These varieties are insensitive to endogenous gibberellins, have shorter coleoptiles, and, if sown deep, have poor emergence [2,4,16].

Coleoptile response is also influenced by various environmental conditions, which may also be influenced by $GA_3$. The response of different wheat varieties was shown to vary based on the $GA_3$ concentration [23]. Exogenous application of $GA_3$ was shown to increase coleoptile length, and this response did not differ significantly between tall lines and lines with GA-responsive dwarfing alleles [24]. However, the increase in coleoptile length in response to $GA_3$ application was greatly reduced at higher temperatures [23] and also under a Mediterranean environment [1] in accessions with dwarfing alleles. These results suggest that the coleoptile response to $GA_3$ may be an indicator of environmental plasticity.

As already noted, seedlings with longer roots are more adapted to drought stress [12,13]. Studies on wheat seedling roots [25,26] have shown that *Rht-B1b* and *Rht-D1b* dwarfing alleles have a pleiotropic effect on root traits. A prior study [27] indicated only a slight effect of *Rht* loci on root length and density. Another study [22] showed that *Rht-B1b* and *Rht-D1b* negatively affect seedling root length. Similarly, it has been reported that *Rht-B1b* is associated with reduced coleoptile length and reduced seedling root length [20]. In contrast, findings have also shown that the selection of *Rht-B1b* decreased coleoptile length but increased root length [28]. Therefore, it is suggested that the relationship between dwarfing alleles and root traits should be cautiously reported [29]. In general, there are a limited number of studies on the effect of *Rht* genes on root length.

The above discussion suggests that breeding for longer coleoptiles and seedling roots is an important strategy to enhance seedling emergence, seedling vigor, and grain yield under early drought conditions. Recently, there has been discussion of the relative failure of grain yield of semi-dwarf lines containing *Rht-B1b* and *Rht-D1b* alleles compared to taller lines in drought environments [30]. Thus, there is a need to develop semi-dwarf wheat varieties with longer coleoptiles and longer seedling roots targeting wheat-growing regions that face early drought stress. One of these regions is Nepal. Wheat is the third most important cereal crop in Nepal, covering almost 22% of the total land acreage, and almost 75% of the wheat in the country is rain-fed [31]. The climate change predictions suggest that early-season drought stress will become more frequent and intense [32].

Findings from a recent study showed that despite the *Rht-B1b* allele pleiotropically affecting coleoptile length, concurrent selection strategies make it possible to develop semi-dwarf wheat varieties with longer coleoptiles [33]. With the long-term objective of breeding Nepali spring wheat with longer coleoptiles and longer seedling roots, here we analyzed a panel of 320 spring wheat accessions that was previously assembled from Nepal, termed as the Nepali Wheat Diversity Panel (NWDP) [34]. The panel was genotyped at the dwarfing loci *Rht-B1* and *Rht-D1* and simultaneously phenotyped for seedling vigor traits. Since these loci have historically been the targets of wheat breeding, it was also of interest to know to what extent the relevant Green Revolution varieties have affected the Nepali wheat germplasm population. For these reasons, the three objectives of this study were: (1) to measure allelic variation at the *Rht-B1* and *Rht-D1* loci in the NWDP; (2) to assess the NWDP for variation in the seedling vigor traits associated with early-season drought stress and the impact of the *Rht* alleles on these traits; and (3) to identify candidate accessions that possess the dwarfing alleles *Rht-B1b* and *Rht-D1b* yet have a longer and/or

more GA-responsive coleoptile (as a ratio of stem height at maturity) as potential parents for future breeding programs.

## 2. Materials and Methods

### *2.1. Plant Materials*

A diversity panel of 320 spring wheat genotypes termed the Nepali Wheat Diversity Panel (NWDP) was previously assembled (Supplementary Table S1). The panel included 167 Nepali landraces, 116 CIMMYT advanced breeding lines tested in Nepal for three seasons from 2011–2012 to 2013–2014 wheat-growing seasons, and 34 varieties released for commercial cultivation in Nepal. As the two major field experiments were conducted in Canada, three Canadian spring varieties, "Norwell", "Pasteur", and "AC Carberry", were also included in the panel [34,35]. The landraces in the diversity panel represented 29 districts of Nepal; the landraces were collected during different germplasm expeditions from the 1970s to the 1990s. The National Agriculture Genetic Resource Centre (NAGRC, Nepal) provided the landrace seeds; the Nepal Agriculture Research Council (NARC) and the National Wheat Research Program (NWRP, Nepal) of NARC provided the seeds of the released varieties. The seeds of the advanced breeding lines were acquired from CIMMYT, Mexico, and the seeds of the Canadian varieties were obtained from the Wheat Breeding Laboratory at the University of Guelph, Canada.

### *2.2. Phenotyping Coleoptile Length and Seedling Root Length*

The coleoptile length phenotyping experiment was conducted in the Crop Science Growth Facility at the University of Guelph using a two factorial randomized complete block design (RCBD) with three replications. The two factors were the genotype (see below) and treatment [application of gibberellic acid ($GA_3$) ($C_{19}H_{22}O_6$)].

The "cigar-roll" method was used to grow seedlings to assess coleoptile length and seedling root length [1,36–38]. Seeds were sterilized with 70% (*v*/*v*) ethanol for 30 s, rinsed with distilled water three times, and further sterilized for 10 min in a 4% sodium hypochlorite solution prepared from commercially available bleach and then rinsed with distilled water three times. Seven seeds from a single genotype were placed ~4 cm distance apart and ~4 cm from the top of a moistened sheet of germination paper (25 × 38 cm, Anchor Paper Company, St. Paul, MN, USA) and covered with a second germination paper. The papers were rolled to achieve a ~2 cm diameter and placed in a tray (32 × 24 × 14 cm), bending the lower part by ~5 cm to make it stable, where it was also supported in the vertical position by a ~3 cm by ~3 cm wire mesh in the tray. There were 40 rolls in each tray, with each cigar roll representing a different genotype. Of the 7 seedlings, the randomly selected 5 seedlings per cigar roll were counted as 1 replicate (averaged), and the three replications per +/− $GA_3$ treatment were placed in three separate randomized trays. All six trays relevant for each genotype were placed in the growth chamber at the same time, but to accommodate the large number of genotypes, they were staggered at different times.

The trays with the control treatment (0 mg/L $GA_3$) were supplied with two liters of deionized water, and the trays with the $GA_3$ treatment (50 mg/L $GA_3$) were filled with two liters of 50 ppm $GA_3$ solution (dissolved in deionized water) [39]. All trays were kept at 4 °C in a vernalization chamber for 48 h to achieve uniform germination. Finally, the trays were transferred to a Percival growth chamber for a period of seven days with a 16/8 h day-night using Sylvania Cool White F20T12/CW 20 watt fluorescent bulbs at 65–80 μmol $m^{-2}$ $s^{-1}$ (at the top of the trays), set at a constant temperature of 20 °C. The trays were moved around in the growth chamber every day. After seven days, the seedlings were removed from the growth chamber to measure coleoptile length. Out of the seven seedlings in each cigar roll, five were chosen randomly to measure the coleoptile length and seedling root length using a ruler. The seedling roots and shoots were dried at 60 °C for

three days, and the dry weight was recorded. The dry weight was recorded as the mean of five plants for each genotype per replicate.

### *2.3. Phenotyping for Plant Height*

Four field experiments were conducted in 2016 and 2017, both in Canada and Nepal, to evaluate the diversity panel for plant height (Supplementary Tables S2 and S3). The first field trial was conducted during the 2016 growing season, at the Elora Research Station of the University of Guelph, Canada (43°38′23.0″ N 80°24′11.0″ W). The seeds were planted on 11 May and harvested on 29 August. The second field trial was conducted during the 2017 growing season at the same research station (43°38′10.4″ N 80°24′07.6″ W). In 2017, planting was performed on 16 May, and the plots were harvested on 5 September. Both of these experiments were conducted in a rain-fed environment. The remaining two field trials were conducted in Nepal during the 2016–2017 wheat-growing seasons. One of these field trials was conducted at the Nepal Agriculture Research Council (NARC) Research Station located at Khumaltar, Lalitpur, Nepal (27°39′12.3″ N 85°19′33.7″ E) at an altitude of 1360 masl in collaboration with the Agricultural Botany Division (ABD) of NARC. The second field trial in Nepal was conducted at the National Wheat Research Program (NWRP), NARC, located at Bhairahawa, Rupandehi, Nepal (27°31′53.5″ N 83°27′32.2″ E) at an altitude of 107 masl in collaboration with NWRP. The planting and harvesting dates for the trial at the NARC station in Khumaltar were 23 November 2016 and 5 May 2017, respectively. The seed planting in NWRP, Bhairahawa, was performed on 30 November 2016, while the plots were harvested on 19 April 2017. All 4 trials in Canada and Nepal were conducted using an alpha lattice design [40] with 2 complete blocks and 20 incomplete blocks, including 32 accessions. Two accessions were excluded from the analysis as a high seed mixture was observed, i.e., only 318 accessions were used in the data analysis. The field experiment at the NWRP station was irrigated two times during the critical growth stages. At the Elora Research Station, each experimental plot consisted of a six-row plot (1 × 3 m) with 17.8 cm row spacing, and the plot-to-plot and range distances were 0.5 and 1 m, respectively. In the Nepal sites, due to limitations in the availability of seed, 2 m long, 2-row plots with 20 cm row-to-row spacing were used.

The diversity panel was evaluated for plant height (PH) at maturity. The PH was measured from the base of the plant to the tip of the spike (excluding the awns) on a handful of tillers from the middle of the plot. The mean PH at maturity was averaged across four field experiments conducted in 2016 and 2017: two at the Elora Research Station, University of Guelph, in 2016 and 2017, and two in Nepal during the 2016/2017 wheat-growing season. The average PH across the four experiments (Supplementary Table S2) was used to normalize seedling traits (coleoptile length and seedling root length) in this study.

### *2.4. DNA Extraction*

The spring wheat genotypes of the NWDP were also grown in the field in 2016 for DNA collection (Elora Research Station, Elora, ON). Leaf samples were collected from the field plots when the plants were at the 3–4 leaf stage. These samples were freeze-dried at −80 °C, and genomic DNA extraction was undertaken using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) using the manufacturer's protocol. The assessment of the quality of DNA was performed by determination of A260 nm and A280 nm using a NanoDrop (ND-1000) spectrophotometer (NanoDrop Technologies, Washington, DE, USA).

### *2.5. Genotyping PCR Amplification of Kompetitive Allele Specific PCR*

The diversity panel was genotyped by using Kompetitive Allele Specific PCR (KASP) markers (Table 1). The KASP assay was carried out in a 10 μL volume containing 5 μL of KASP Master Mix with low ROX (LGC group, Hoddesdon, U.K.), 0.14 μL of KASP assay mix, 1 μL of g DNA (concentration of 10–20 ng/μL) and 4 μL of water. PCR reactions and fluorescent readings were taken according to the LGC recommended protocol in 96-well plates using the QuantStudio™ 6 Flex Real-Time PCR machine with a fast reading block (Applied Biosystems, CA, USA).

**Table 1.** Kompetitive Allele Specific markers for alleles of the *Rht* loci with primer sequences and citations for the markers used in genotyping the panel of accessions.

| Marker Type | Genes | Primer | Primer Sequence (5′-3′) | Alleles | References |
|---|---|---|---|---|---|
| *KASP* | *Rht-B1* | FAM | CCCATGGCCATCTCCAGCTG | FAM (wt) | [41] [42] |
| | | HEX | CCCATGGCCATCTCCAGCTA | HEX (dwarf) | |
| | | Common reverse | TCGGGTACAAGGTGCGGGCG | | |
| | *Rht-D1* | FAM | CATGGCCATCTCGAGCTGCTC | FAM (wt) | [41] [42] |
| | | HEX | CATGGCCATCTCGAGCTGCTA | HEX (dwarf) | |
| | | Common reverse | CGGGTACAAGGTGCGCGCC | | |

### *2.6. Cluster Analysis of Genotypic Data*

Genotypic data (Supplementary Table S4) were imported into the software Graphical Genotype (GGT2.0) [43]. A matrix of the pairwise genetic distances was generated. This matrix was then saved as a MEGA file. The dissimilarity matrix was then exported to the MEGA X software [44], where an unweighted pair group method with arithmetic mean dendrogram (UPGMA) was calculated, and a dendrogram was generated.

### *2.7. Statistical Analysis and Data Visualization*

The coleoptile and seedling root length data, generated from the two factorial (genotype, $GA_3$ treatment) randomized complete block design (RCBD, with three replications), were analyzed using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC, USA). The model contained data from each plant (sample) nested in genotype-by-treatment due to the nature of the experiment. The Shapiro–Wilk test was conducted in PROC UNIVARIATE to test the normality of the residuals and to ensure that all the data points were independent and random; PROC SGPLOT was used to construct studentized residuals by predictor plots. The studentized residuals produced by the genotype-by-treatment combinations were considered outliers when they were >3.5 and <–3.5. These outliers were removed from the data set after confirming that they were true outliers. The least-square (LS) means were generated (Supplementary Table S5) for each genotype-by-treatment, where genotype was treated as the fixed effect. In addition, variance and correlation were analyzed using PROC ANOVA and PROC CORR. Tukey's test was used to compare phenotypic means. Principal component analysis was performed using PROC PRINCOMP and PRINQUAL in SAS to examine the effect of the relationship between observed traits as well as the effect of combined allelic variation on the coleoptile and root length traits.

The root to shoot biomass ratio was calculated as the ratio of mean root and shoot dry weight for each genotype. The change in coleoptile length as a result of $GA_3$ treatment was calculated using the following Equation:

$$\Delta\ change\ in\ CL = \left(\frac{CL_{GA3} - CL_C}{CL_C}\right) \times 100$$

where:
CL = Coleoptile length
$CL_{GA3}$ = Coleoptile length after $GA_3$ treatment
$CL_C$ = Coleoptile length under control conditions.

A similar equation was used to calculate the change in seedling root length as a result of $GA_3$ treatment.

For data visualization, graphs were generated in RStudio [45] using ggplot2, factoextra, and FactoMineR packages [46–48].

## 3. Results

### *3.1. Allelic Frequency at the Rht-B1 and Rht-D1 Loci*

Analysis of the NWDP with two gene-specific markers (Table 1) showed higher allelic variation within the population at the *Rht-B1* locus compared to the *Rht-D1* locus (Table 2). At each *Rht* locus, no heterozygotes were observed. Across the whole panel, at the *Rht-B1* locus, 173 accessions (54.40%) carried the homozygous wild-type allele, *Rht-B1a,* while 145 accessions (45.60%) carried the homozygous dwarfing allele, *Rht-B1b*. By contrast, at the *Rht-D1* locus, 283 accessions (88.99%) carried the homozygous wild-type allele, *Rht-D1a,* while only 35 accessions had the homozygous dwarfing allele, *Rht-D1b*. The majority of the landraces carried wild-type alleles at both *Rht-B1* (89.2%) and *Rht-D1* (91%) loci. However, a small proportion of landraces possessed dwarfing alleles at both *Rht* loci, which was a surprising result. Furthermore, a large proportion of the CIMMYT lines had dwarfing alleles at the *Rht-B1* locus (96.5%), but 93.9% of lines carried the wild-type allele at *Rht-D1* (Table 2).

**Table 2.** The allelic variation observed at the *Rht* loci in the Nepali Wheat Diversity Panel.

| Population/Group | Locus § | | | |
|---|---|---|---|---|
| | ***Rht-B1*** | | ***Rht-D1*** | |
| | **Wild Type (Allele *a*)** | **Dwarf (Allele *b*)** | **Wild Type (Allele *a*)** | **Dwarf (Allele *b*)** |
| Whole panel | 173 (54.40%) | 145 (45.60%) | 283 (88.99%) | 35 (11.01%) |
| Landraces | 148 (89.2%) | 18 (10.8%) | 151 (91%) | 15 (9%) |
| CIMMYT lines | 4 (3.5%) | 111 (96.5%) | 108 (93.9%) | 7 (6.1%) |
| Released varieties | 19 (55.9%) | 15 (44.1%) | 21 (61.8%) | 13 (38.2%) |

§ Each locus was homozygous for the alleles indicated.

### *3.2. Distinct Genetic Clusters Identified Based on Genotypic Data*

Four genetic clusters within the 318 accessions of the NWDP were identified using allele-specific markers based on the allele combinations present in each accession at the *Rht-B1* and *Rht-D1* loci (Figure 1). The largest cluster, consisting of primarily Nepali landraces (in red with 151 accessions), carried *Rht-B1a* and *Rht-D1a* (wild-type alleles), while the second-largest cluster of primarily CIMMYT lines (in cyan with 134 accessions) had the alleles *Rht-B1b* (dwarfing type) and *Rht-D1a* (wild-type). The third cluster in green had 27 accessions that carried *Rht-B1a* and *Rht-D1b* alleles. The fourth cluster included only six accessions, having both the dwarfing alleles (*Rht-B1b* and *Rht-D1b*). These results showed that a large majority of the introduced accessions into Nepal possessed dwarfing allele *Rht-B1b* while only a few accessions had *Rht-D1b*. Furthermore, surprisingly, some Nepali native accessions apparently possessed dwarfing alleles (Figure 1).

### *3.3. Effect of Rht-B1 and Rht-D1 Loci on Seedling Vigor Traits*

A significant difference ($p \leq 0.0001$) was observed among the genotypes in the NWDP for coleoptile length and seedling root length (Supplementary Table S6). Different alleles at the *Rht-B1* locus had significant effects on coleoptile length and seedling root length. Allelic variation at the *Rht-D1* locus had a significant effect on coleoptile length but not for seedling root length. The mean comparison showed that genotypes with either homozygous *Rht-B1b* or homozygous *Rht-D1b* had shorter coleoptiles, indicating that dwarfing alleles affected coleoptile length (Table 3). The interaction between the two *Rht* loci indicated that the presence of homozygous dwarfing allele *Rht-B1b* reduced coleoptile length

significantly compared to homozygous wild-type and homozygous *Rht-D1b* alleles. Although homozygous *Rht-B1b* exhibited a significant positive influence on the length of the longest seedling root, the interactions between the two loci did not show a significant effect on the length of the longest seedling root (Table 3).

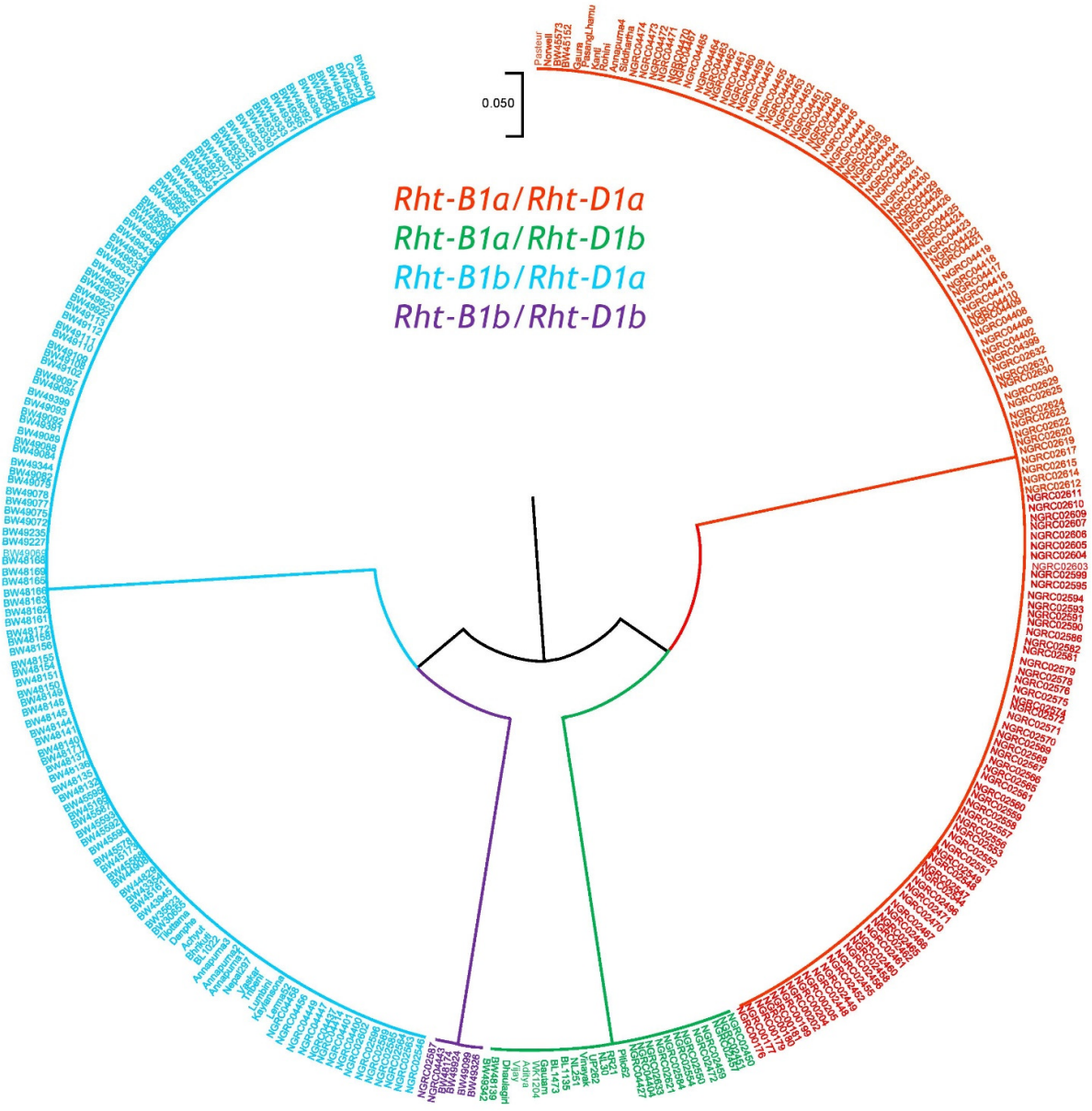


**Figure 1.** Dendrogram showing clusters based on accessions sharing the same allele combinations at the *Rht-B1* and *Rht-D1* loci.

**Table 3.** Least squares means for coleoptile and root length as a factor of *Rht-B1* and *Rht-D1* and their interaction without $GA_3$ treatment.

| Genes | § Alleles | Traits | | | |
|---|---|---|---|---|---|
| | | Coleoptile Length (mm) | | Seedling Root Length (mm) | |
| | | Mean * | SD | Mean * | SD |
| *Rht-B1* | *a* | 52.85 a | ±6.98 | 102.23 b | ±20.60 |
| | *b* | 39.56 b | ±4.36 | 107.59 a | ±17.82 |
| *Rht-D1* | *a* | 47.34 a | ±9.22 | 104.02 a | ±19.73 |
| | *b* | 43.59 b | ±4.11 | 109.62 a | ±17.59 |
| Gene Interactions | | | | | |
| *Rht-B1*/*Rht-D1* | *aa* | 54.52 a | ±6.23 | 100.49 a | ±20.75 |
| | *ab* | 44.13 a | ±3.10 | 111.45 a | ±17.41 |
| | *ba* | 39.59 b | ±4.23 | 107.89 a | ±17.85 |
| | *bb* | 40.73 b | ±7.56 | 99.79 a | ±16.89 |

§ Each locus was homozygous for the alleles indicated; a = wild-type allele, b = mutant allele. * Mean values within the same trait and genotype category having different letters are significantly different based on *t*-tests at the significance threshold of $p = 0.01$. Abbreviation: SD = Standard deviation.

Principal component analysis (PCA) was also performed to assess if the alleles at the two *Rht* loci differentiate the accessions with respect to the two seedling vigor traits, specifically the lengths of the coleoptile and longest seedling root. The biplots revealed distinct clusters based on the combined effects of alleles at the *Rht-B1* and *Rht-D1* loci (Figure 2). Accessions with the reduced height allele *Rht-B1b,* combined with the wild-type allele (*Rht-D1a*) (cyan dots in Figure 2), clustered distinctly from accessions possessing wild-type height alleles at both *Rht* loci (*Rht-B1a*, *Rht-D1a*) (red dots in Figure 2). However, despite the clustering, coleoptile length appeared to be mostly driven by the effect of the *Rht-B1* locus compared to that of the *Rht-D1* locus. In addition, the mean comparison of interactions revealed that the effect of the *Rht-B1* locus was stronger compared to the *Rht-D1* locus (Table 3). Similar to the results demonstrated in Table 3, the biplots also indicated that neither of the *Rht* loci had a significant influence on seedling root length. Interestingly, some accessions that carried dwarfing alleles clustered with accessions displaying a longer coleoptile (purple dots in Figure 2).

### *3.4. Effect of Breeding History on Coleoptile and Root Length*

The genotype analysis suggested that the majority of Nepali landraces did not have the dwarfing alleles *Rht-B1b* or *Rht-D1b* (red in Figures 1 and 2, Supplementary Table S4), opposite to the majority of CIMMYT lines (cyan in Figures 1 and 2). Consistent with this result, Tukey's pairwise comparison of the mean values (Table 4) revealed that CIMMYT lines and released varieties had significantly shorter coleoptiles than the landraces. Interestingly, the landraces had significantly shorter roots than the CIMMYT lines and released varieties. Therefore, the root to shoot biomass ratio was also calculated as an indicator of stress tolerance. The result showed that the CIMMYT lines and the released varieties had a significantly higher root to shoot biomass ratio compared to the landraces.

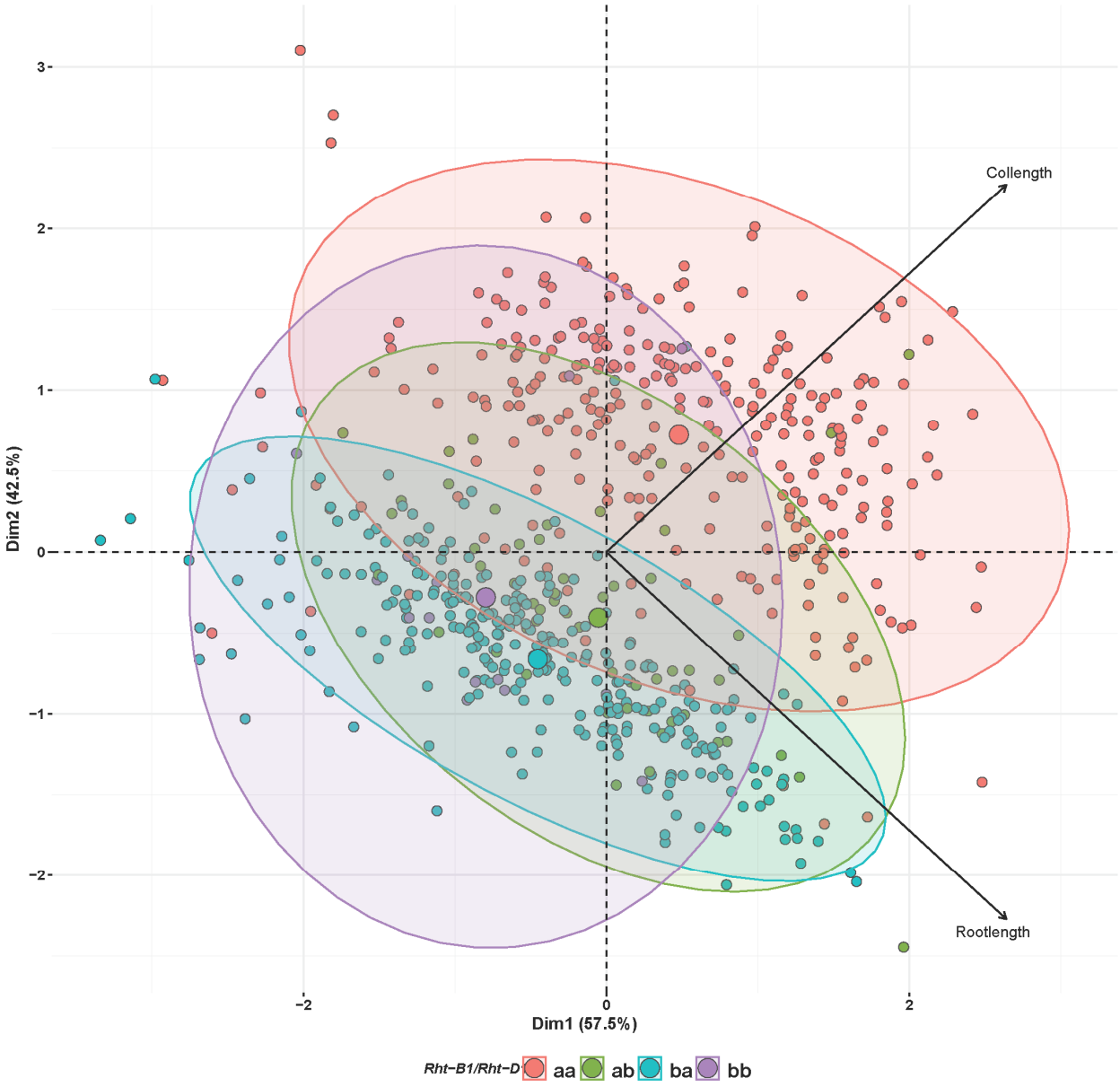


**Figure 2.** A biplot generated to distinguish the effect of allele combinations at multiple loci on NWDP accessions in the absence of a $GA_3$ treatment. The letters "a" and "b" indicate the allelic combinations for *Rht-B1*and *Rht-D1* loci where "a" and "b" represent the wild-type and mutant alleles, respectively.

**Table 4.** Tukey's pairwise mean comparison for genotypes segregated into three groups based on seed source.

| Seed Source Groups | Coleoptile Length (Control) (mm) § | Root Length (Control) (mm) § | Root to Shoot Biomass Ratio ^ |
|---|---|---|---|
| Landraces (166) | 52.7 a | 100.2 a | 0.59 a |
| CIMMYT lines (115) | 39.7 b | 107.9 b | 0.72 b |
| Released varieties (34) | 43.5 c | 115.5 b | 0.74 b |

§ Means that do not share the same letter within a column are significantly different. Significance level: $\alpha = 0.05$. ^ Based on dry weights of shoot and total root. Note: the three Canadian varieties were excluded from this analysis.

### *3.5. Identification of Likely Semi-Dwarf Accessions with Seedling Vigor Potential*

An objective of this study was to identify semi-dwarf accessions that nevertheless retained seedling vigor potential, given that *Rht* dwarfing alleles have previously been shown to reduce coleoptile length. The diversity panel was evaluated for seedling vigor traits. As breeders are interested in shorter plants at maturity but larger seedlings, only accessions with one or both *Rht* dwarfing alleles were analyzed, and the traits were normalized for stem length at maturity: the ratio of seedling coleoptile length to plant height at maturity (CL/PH), longest seedling root length to plant height at maturity (RL/PH), and seedling root dry biomass to plant height at maturity (RootB/PH). In addition, the seedling root biomass to seedling shoot dry biomass was calculated. The results are shown in Table 5, but only for those accessions having mean values greater than the mean of the entire NWDP population (318 accessions) for all four traits. In total, two landraces, six released varieties, and 21 CIMMYT lines were identified as candidates with high seedling vigor potential.

### *3.6. Effect of $GA_3$ Treatment by Genotype*

Apart from long coleoptiles, we were interested in determining if members of the NWDP have coleoptiles that are $GA_3$ responsive as this is a proxy for being environmentally responsive, i.e., emerging from deep seeding to escape early seedling drought. The effect of $GA_3$ treatment was significant for the entire NWDP ($p \leq 0.0001$) (Supplementary Table S6). Furthermore, a significant ($p \leq 0.0001$) genotype × treatment interaction was observed, which indicated an effect of the $GA_3$ application on coleoptile length as well as seedling root length. The interaction between *Rht-B1* and $GA_3$ treatment was significant for both study traits. However, the interaction between *Rht-D1* and $GA_3$ treatment was significant for only seedling root length, not for coleoptile length (Supplementary Table S6). Although multi-factor analysis may have provided a better explanation for this result, a possible reason could be that the majority of the accessions have *Rht-B1a* or *Rht-B1b* segregating in the background, which might have reduced the effect of altered alleles at the *Rht-D1* locus. A small but significant difference in mean coleoptile length in response to $GA_3$ was observed for the entire NWDP (Table 6), which suggested that at least a subset of accessions may be candidates for coleoptile plasticity. When the data were separated based on breeding history (Table 7), coleoptiles from the landrace group were found to be significantly more responsive to $GA_3$ treatment compared to CIMMYT lines and released varieties, but even these latter groups showed some $GA_3$ responsiveness.

**Table 5.** List of likely semi-dwarf accessions identified as having potential seedling vigor traits.

| | | ***Rht* Loci §** | | **Ratio** | | | |
|---|---|---|---|---|---|---|---|
| **Accession ID** | **Accession Group** | ***Rht-B1*** | ***Rht-D1*** | **CL/PH** | **RL/PH** | **RootB/PH** | **Root/Shoot Biomass** |
| NGRC02450 | Landrace | *Rht-B1a* | *Rht-D1b* | 0.054 | 0.142 | 0.000101 | 0.743 |
| NGRC04400 | Landrace | *Rht-B1b* | *Rht-D1a* | 0.051 | 0.159 | 0.000095 | 0.820 |
| Vaskar | Released variety | *Rht-B1b* | *Rht-D1a* | 0.053 | 0.155 | 0.000098 | 0.832 |
| Annapurna1 | Released variety | *Rht-B1b* | *Rht-D1a* | 0.052 | 0.156 | 0.000120 | 0.754 |
| BL1022 | Released variety | *Rht-B1b* | *Rht-D1a* | 0.068 | 0.180 | 0.000097 | 0.739 |
| BL1473 | Released variety | *Rht-B1a* | *Rht-D1b* | 0.055 | 0.204 | 0.000118 | 0.859 |
| WK1204 | Released variety | *Rht-B1a* | *Rht-D1b* | 0.054 | 0.153 | 0.000129 | 1.029 |
| Tilottama | Released variety | *Rht-B1b* | *Rht-D1a* | 0.058 | 0.158 | 0.000108 | 0.727 |
| BW43354 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.059 | 0.193 | 0.000093 | 0.723 |
| BW45593 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.054 | 0.155 | 0.000110 | 0.835 |
| BW45587 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.059 | 0.152 | 0.000106 | 0.869 |
| BW48133 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.055 | 0.161 | 0.000095 | 0.727 |
| BW48139 | CIMMYT line | *Rht-B1a* | *Rht-D1b* | 0.061 | 0.159 | 0.000112 | 0.793 |
| BW48162 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.058 | 0.188 | 0.000106 | 0.929 |
| BW49235 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.060 | 0.161 | 0.000122 | 0.763 |
| BW49079 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.053 | 0.177 | 0.000093 | 0.721 |
| BW49089 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.057 | 0.143 | 0.000112 | 0.766 |
| BW49112 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.052 | 0.168 | 0.000099 | 0.742 |
| BW49927 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.051 | 0.151 | 0.000121 | 0.805 |
| BW49931 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.058 | 0.162 | 0.000121 | 0.933 |
| BW49936 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.061 | 0.207 | 0.000112 | 0.802 |
| BW49943 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.056 | 0.155 | 0.000134 | 0.976 |
| BW49949 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.058 | 0.172 | 0.000112 | 0.940 |
| BW49954 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.051 | 0.146 | 0.000112 | 0.926 |
| BW49957 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.054 | 0.153 | 0.000114 | 0.860 |
| BW49958 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.055 | 0.174 | 0.000102 | 0.818 |
| BW49325 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.058 | 0.179 | 0.000097 | 0.802 |
| BW49392 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.056 | 0.173 | 0.000101 | 0.723 |
| BW49394 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 0.053 | 0.150 | 0.000102 | 0.736 |

Abbreviations: CL = coleoptile length, RL = root length, PH = plant height, RootB = root biomass. § Each locus was homozygous for the alleles indicated.

**Table 6.** Tukey's pairwise comparison of the mean response of NWDP seedling traits to $GA_3$ treatment at 95% confidence.

| **Treatment** | **Coleoptile Length (mm) §** | **Root Length (mm) §** |
|---|---|---|
| Control | 47.0 a | 104.8 a |
| $GA_3$ | 48.7 b | 98.2 b |

§ Means that do not share the same letter are significantly different. Significance level: $\alpha = 0.05$.

**Table 7.** Tukey's pairwise comparison of the mean response of seedling traits from different breeding history groups§ to $GA_3$ treatment at 95% confidence.

| | **Effect of $GA_3$ (% Change)** | |
|---|---|---|
| **Seed Source Groups** | **Δ Coleoptile Length §** | **−Δ Root Length §** |
| Landraces (166) | 5.25 a | 5.10 a |
| CIMMYT lines (115) | 0.32 b | 8.35 b |
| Released varieties (34) | 1.21 b | 5.77 ab |

§ Means that do not share the same letter within a column are significantly different. Significance level: $\alpha = 0.05$. Note: the three Canadian varieties were excluded from this analysis.

The landraces mostly contain the *Rht-B1a* allele, while the CIMMYT lines/released varieties contain the *Rht* dwarfing alleles (*Rht-B1b*) (Figure 1, Supplementary Table S4). When the NWDP accessions were separated based on the allele present at each *Rht* locus,

the effect of the $GA_3$ treatment on the coleoptile length was significantly larger in the wild-type height allele group (either *Rht-B1*a or *Rht-D1*a) than in the dwarfing allele (*Rht-B1b* or *Rht-D1b*) group (Figure 3).

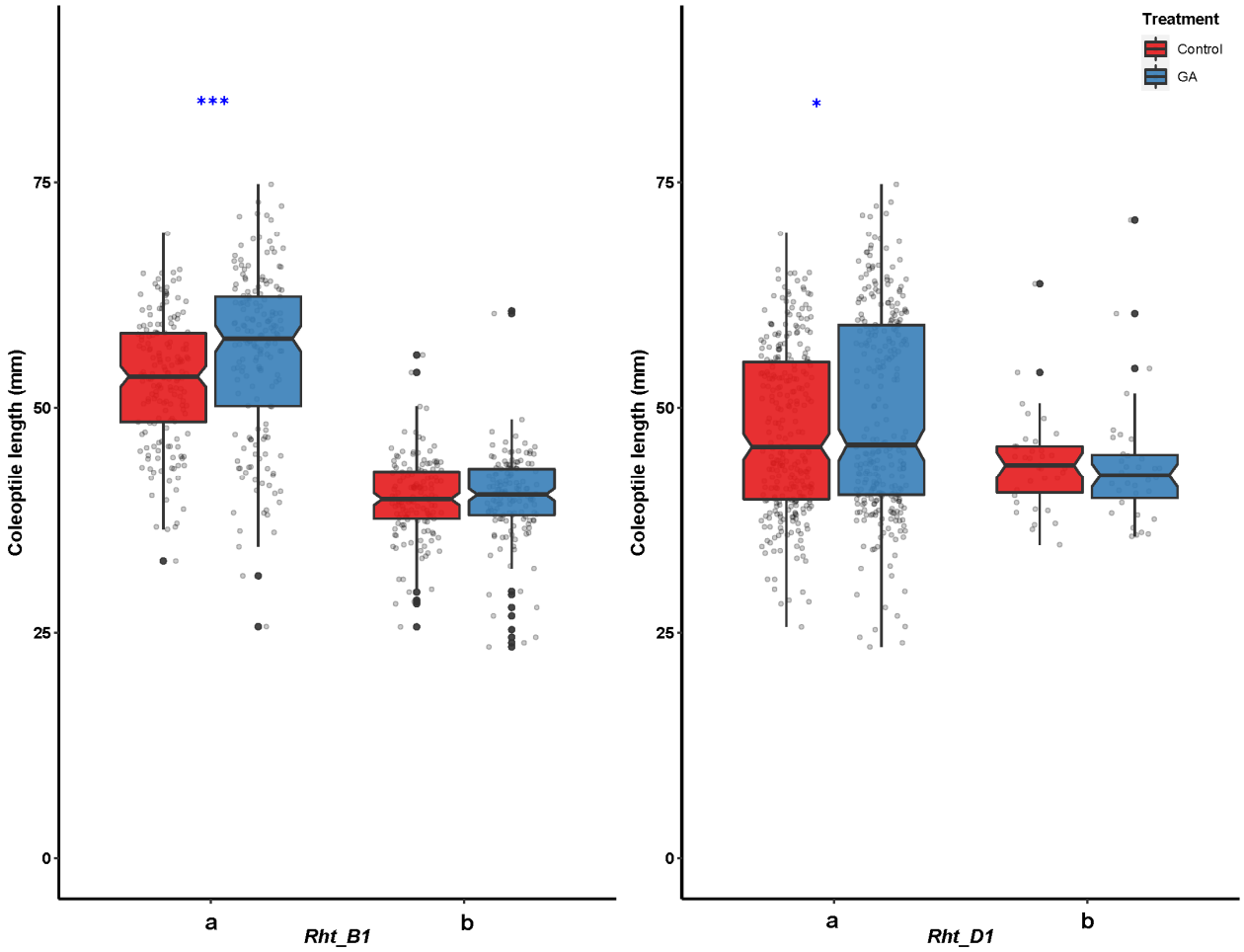


**Figure 3.** Effect of $GA_3$ treatment on coleoptile length as a factor of alleles at the *Rht-B1* and *Rht-D1* loci. Shown are GA-response box plots illustrating the frequency distribution of NWDP accessions for coleoptile length separated into allelic groups. The symbols * and *** indicate significant differences at $p = 0.05$ and $p = 0.001$, respectively. The solid dots are the outliers, and each box represents the interquartile range representing 50% of the values. Abbreviations: GA = gibberellic acid. The letters "a" and "b" indicate the allelic combinations for *Rht-B1*and *Rht-D1* loci where "a" and "b" represent the wild-type and mutant alleles, respectively.

However, among the accessions that contained either or both *Rht* dwarfing alleles, some accessions appeared to be more $GA_3$ responsive (boxed, Figure 4), suggesting a more detailed examination of these accessions should be pursued. In the case of the length of the longest seedling root, both *Rht-B1* allelic forms (wild type and dwarf) responded to $GA_3$ treatment, and they were significantly shorter compared to the control treatment, with similar trends at *Rht-D1* (albeit not significant for the *Rht-D1b* allele) (Supplementary Figure S1).

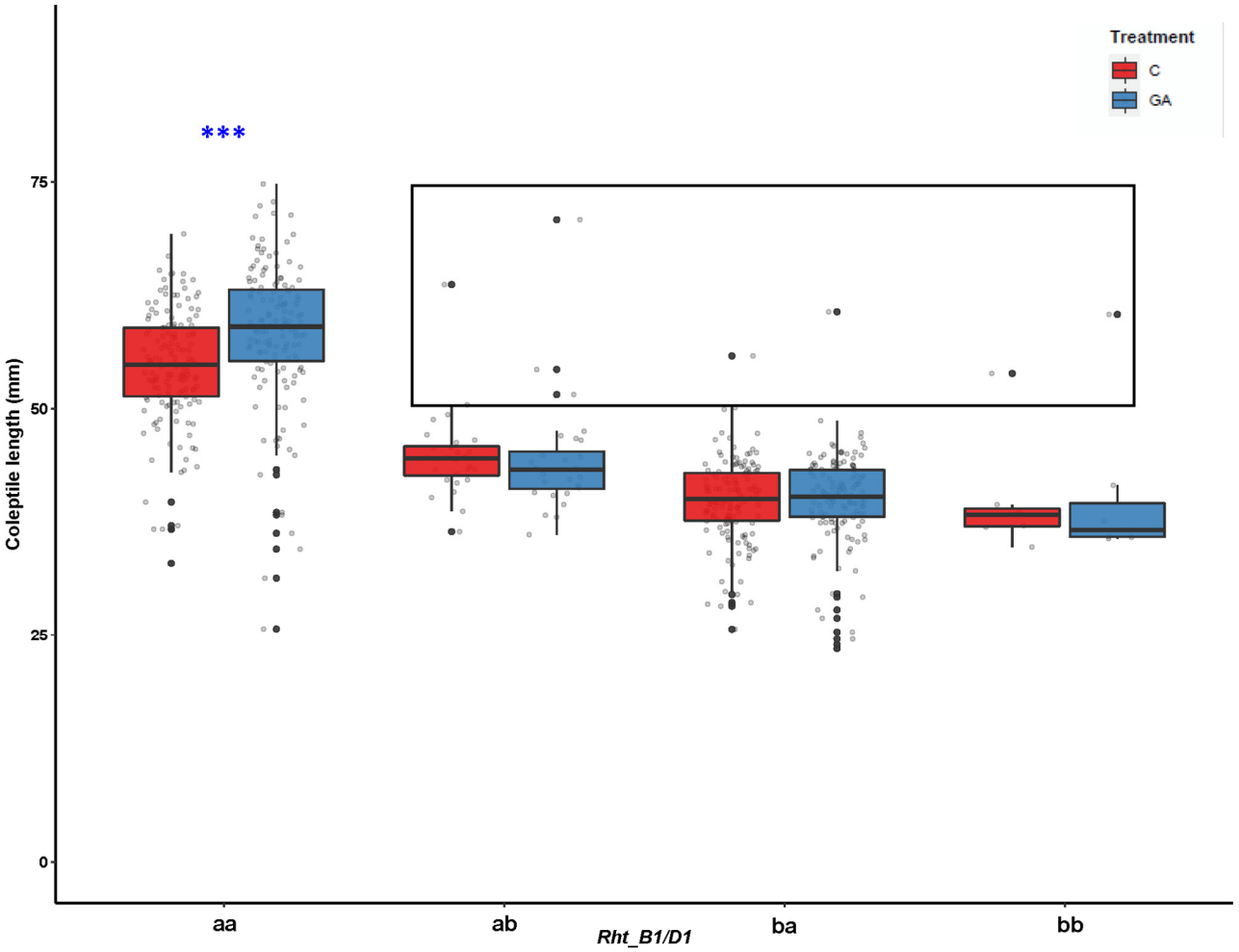


**Figure 4.** Effect of $GA_3$ treatment on coleoptile length as a factor of alleles at the *Rht-B1* and *Rht-D1* loci combined. Shown are GA-response box plots illustrating the frequency distribution of NWDP accessions for coleoptile length, separated into allelic groups. *** indicates a significant difference at 0.001. The solid dots are the outliers (contained in the large box), and each colored box represents the interquartile range representing 50% of the values. Abbreviations: C = Control, GA = gibberellic acid. The letters "a" and "b" indicate the allelic combinations for *Rht-B1*and *Rht-D1* loci where "a" and "b" represent the wild-type and mutant alleles, respectively.

### *3.7. Identification of Accessions with GA-Responsive Coleoptiles*

The above analysis showed that among accessions with one or two dwarfing alleles, a small subset appeared to be GA-responsive for coleoptile length. To identify the accessions with the most GA-responsive coleoptiles in the NWDP, and hence best candidates for deep seeding, the percentage change in coleoptile length and percentage change in the ratio of coleoptile length to plant height were calculated for each accession. As a filter, only those accessions with dwarfing alleles at the *Rht-B1* and/or *Rht-D1* loci were selected since only these genotypes would be the high-yielding semi-dwarfs. Furthermore, only accessions with values greater than a 10% threshold change for both traits were considered. These criteria resulted in 12 candidate accessions (Table 8). The findings showed that among the candidate landraces, NGRC04443, NGRC04466, and NGRC04427 were promising, while Nepal 297 appeared to be the best accession among the released varieties. Among the candidate CIMMYT lines, BW48136, BW48139, BW49333, BW49093, and BW48314 were observed to be the best GA-responsive accessions (Table 8). One accession was noteworthy, namely the landrace NGRC04443 because it had dwarfing alleles at both the *Rht-B1* and *Rht-D1* loci, whereas all the others had the wild-type allele at one of these loci.

**Table 8.** The accessions in the NWDP exhibiting the greatest change in coleoptile length in response to $GA_3$ application for those accessions with at least one dwarfing allele at the *Rht1-B1* or *Rht-D1* loci.

| Accession ID | Accession Group | *Rht-B1* Locus | *Rht-D1* Locus | % Change in Coleoptile Length:Plant Height Ratio with $GA_3$ Treatment | % Change in COLEOPTILE Length with $GA_3$ Treatment | Coleoptile Length (Control) (mm) | Plant Height (cm) |
|---|---|---|---|---|---|---|---|
| NGRC04427 | Landrace | *Rht-B1a* | *Rht-D1b* | 11.7 | 10.1 | 49.3 | 86.1 |
| NGRC04443 | Landrace | *Rht-B1b* | *Rht-D1b* | 12.5 | 12.0 | 53.9 | 96.1 |
| NGRC04466 | Landrace | *Rht-B1a* | *Rht-D1b* | 12.1 | 11.2 | 63.7 | 92.9 |
| Nepal297 | Released variety | *Rht-B1b* | *Rht-D1a* | 15.1 | 11.0 | 38.9 | 72.6 |
| BW48136 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 30.6 | 24.3 | 29.8 | 79.3 |
| BW48139 | CIMMYT line | *Rht-B1a* | *Rht-D1b* | 17.1 | 12.8 | 45.7 | 74.7 |
| BW48155 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 12.8 | 10.8 | 34.3 | 84.7 |
| BW48158 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 12.3 | 10.0 | 39.4 | 80.8 |
| BW48166 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 12.9 | 10.0 | 36.6 | 77.6 |
| BW49093 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 13.4 | 11.2 | 36.2 | 83.8 |
| BW48314 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 13.6 | 10.3 | 34.6 | 75.8 |
| BW49333 | CIMMYT line | *Rht-B1b* | *Rht-D1a* | 20.3 | 14.7 | 34.0 | 72.5 |

## 4. Discussion

### *4.1. Allelic Variation at Rht Loci in the NWDP Population*

The Nepali Wheat Diversity Panel (NWDP) was characterized by using diagnostic molecular markers for the alleles at *Rht-B1* and *Rht-D1* loci. The marker analysis revealed that ~46% of the population carried the dwarfing allele *Rht-B1b,* and only ~11% had the *Rht-D1b* allele. After the Green Revolution, dwarfing alleles at the *Rht-B1* and *Rht-D1* loci have been the most commonly used genetic variants in wheat improvement programs globally [1,49,50]. It is estimated that ~90% of wheat in the world is now semi-dwarf, carrying *Rht-B1b* or *Rht-D1b* alleles [51,52]. The *Rht-B1b* and *Rht-D1b* alleles were introduced from "Norin 10", a Japanese wheat variety, during the 1960s in the CIMMYT and USA wheat breeding programs to develop semi-dwarf commercial cultivars [41]. Yield improvement in semi-dwarf wheat was due to the positive pleiotropic effects of the dwarfing alleles, resulting in an increased number of grains per spike [53], partitioning of photosynthetic assimilated to grains, and also reduced lodging [54]. The low proportion of accessions in the NWDP having the reduced height alleles is likely due to the nature of the diversity panel since ~52% of the accessions in the panel were landraces [34]. The current study, however, showed that there were some landraces with reduced height alleles, suggesting that some of the accessions regarded as landraces may not be authentic landraces, consistent with our earlier analysis [34]. The introduction of foreign wheat genetic materials started in Nepal in the 1950s [55,56], and the semi-dwarf wheat varieties from CIMMYT (India) and USAID were introduced starting in the 1960s [55,57]. Currently, >95% of the cultivated area for wheat in Nepal contains semi-dwarf improved varieties [31,56,58]. As was detailed in our earlier study [34], perhaps the germplasm expeditions performed during the 1970s and 1980s collected some exotic germplasm as landraces since these were grown by Nepalese farmers for more than a decade. This is supported by the fact that some of the germplasm collections were directly from farmers' granaries, not the field, due to logistical challenges during these expeditions [34,59]. Low variation at the *Rht-D1* locus suggests that most of the CIMMYT genetic materials tested in Nepal were selected for the *Rht-B1b* allele.

*4.2. Effects of Dwarfing Alleles at Rht Loci on Coleoptile and Seedling Root Length*

The landrace accessions had significantly longer coleoptiles than modern germplasm (CIMMYT lines and released varieties). With the exception of a small number of accessions, all the landraces had the wild-type alleles (*Rht-B1a* and *Rht-D1a*) at the two *Rht* loci. The mean change in coleoptile length after $GA_3$ treatment was also significantly greater in the landrace group, indicating GA-responsiveness. On average, those accessions with the dwarfing alleles *Rht-B1b* and *Rht-D1b* were not responsive to $GA_3$ application, and this has been clearly shown in earlier studies [2,4]. Combined, these results show that alleles at *Rht-B1* and *Rht-D1* loci have highly influenced coleoptile length in the NWDP. These results support earlier studies that reported reduced coleoptile length associated with these dwarfing alleles [19,60]. Cell division in the intercalary meristem and cell elongation are affected by these dwarfing alleles, leading to reduced length of the epidermal cells and, as a result, a shorter coleoptile sheath [4,61–63]. The results from the interaction between the two *Rht* loci and $GA_3$ treatment (Supplementary Table S6), and the gene interaction between *Rht-B1* and *Rht-D1* loci (Table 3), also revealed that the *Rht-B1b* allele may have more influence on the reduction in coleoptile length compared to the *Rht-D1b* allele in the NWDP. However, the observation could be an artifact of the large number of accessions possessing the homozygous *Rht-B1b* allele compared to only 35 accessions possessing the homozygous *Rht-D1b* allele, and only 6 accessions harboring both *Rht-B1b* and *Rht-D1b* alleles in the population (Figure 1, Supplementary Table S4). Interestingly, some accessions with dwarfing alleles were observed to overlap in the cluster with the majority wild-type alleles in the PCA. These accessions could possibly possess GA-sensitive *Rht* genes such as *Rht8*, *Rht13,* and *Rht18* [64,65]. In the context that a longer coleoptile is one of the targets of breeding wheat for drought resistance [11], those few accessions that possessed dwarfing alleles but showed potential for longer coleoptiles are of high interest for future breeding efforts.

The current study showed that only the *Rht-B1* locus had a significant influence on the length of the longest seedling root (Table 3, Supplementary Table S6). The dwarfing allele caused roots to be more responsive (shorter) to $GA_3$ compared to the wild-type allele at the *Rht-B1* locus. Earlier studies on *Coleus blumei* [66] and *Eucalyptus* species [67] similarly reported reduced root growth with 50 mg/L $GA_3$ treatment. The root and coleoptile results are in agreement with an earlier study using a wheat RIL population that showed pleiotropic effects associated with the *Rht-B1* locus, specifically reduced coleoptile length but increased root length [24]. Seedling root length, such as plant height and coleoptile length, is also a GA-dependent process [36], and hence there may be a direct effect of *Rht-B1b* and *Rht-D1b* on seedling root growth, rather than a secondary partitioning effect [26]. However, the current results demand a further in-depth investigation to clarify the effect of *Rht* dwarfing genes on seedling root length.

*4.3. Identification of Candidate NWDP Accessions Possessing Rht-B1b and Rht-D1b Alleles for Longer Coleoptile Length and Other Seedling Vigor Traits*

It is has been established that longer coleoptiles are important for better plant establishment and better shoot development [5]. Shorter coleoptiles prevent deep sowing, which is important for wheat-growing regions with frequent drought stress [1,20,50]. Coleoptile length is reduced under warmer soil temperature conditions [61], which may have negative implications for semi-arid wheat-growing regions, including the Terai region of Nepal. Here, four traits were used to identify a total of 29 candidate accessions as promising for breeding improved seedling vigor, including longer coleoptiles (Table 5). First, since wheat breeders commonly target reduced height in breeding programs, only accessions with at least one dwarfing allele were considered for future selection. Semi-dwarf genotypes with *Rht-B1b* and *Rht-D1b* alleles have increased grain yield due to reduced lodging [68,69]. However, the reduction in plant height has been achieved at the expense

of shorter coleoptiles [2,19]. Therefore, the second trait used was a combination of the absolute coleoptile length (i.e., longer coleoptiles) and a high coleoptile length to plant height ratio. In addition, root length is an important trait for drought tolerance in wheat [6,11,70]; hence, root-related traits (i.e., high root length/biomass to plant height ratio) were the third criteria used here. The final trait selected was a high root to shoot biomass ratio, which has also been shown to be positively associated ($R^2 = 0.41$) with drought tolerance [70]. Based on these four criteria, among the 29 selected accessions, 21 were CIMMYT lines, six were released varieties, and the remaining two were landraces.

Complementary to the above approach, we also screened for accessions with the greatest GA-dependent percentage increase in coleoptile length and the greatest percentage increase in coleoptile length per cm of plant height. Here, only accessions having at least one of the dwarfing alleles (*Rht-B1b* and *Rht-D1b*) were included. In total, 12 potential candidates were identified that met these two criteria (Table 8). It was noteworthy that, of the 12 candidates identified, 3 of them were landraces. The landrace NGRC04443 requires further analysis, as unexpectedly, it was found to carry both the dwarfing alleles *Rht-B1b* and *Rht-D1b* and hence would have been predicted to have a coleoptile that was very unresponsive to $GA_3$. However, an earlier study reported that at least in some genotypes, plant height and coleoptile length can be under different genetic control in GA-sensitive wheat [71]. Furthermore, we are interested in the length of the coleoptile along with the plasticity of the coleoptiles on $GA_3$ treatment. With this said, the phenotypic plasticity could be due to the presence of GA-sensitive *Rht* genes such as *Rht8* and *Rht13* [64]. In this context, the three landraces (NPGR 04427, NPGR 04443, and NPGR 04466) and BW48139, which have longer coleoptile lengths among the 12 candidate accessions for plastic coleoptiles, may be of greater interest. The CIMMYT line BW48139 is especially noteworthy, as it appeared in both sets of assessments: for seedling vigor (Table 5) and GA-responsive coleoptiles (Table 8).

### *4.4. The Need to Genotype Other GA-Responsive Rht Genes*

While more than 20 dwarfing genes have been discovered [72], additional dwarfing genes may offer opportunities to breed for longer coleoptiles. Along with *Rht-B1b* and *Rht-D1b*, *Rht8* is the most abundantly used dwarfing gene among many *Rht* genes that have been identified [73]. *Rht8* is GA-responsive and has been shown to reduce plant height with a minimal effect on coleoptile length [2,51,74–76]. Approximately a 7% decrease in coleoptile length was observed in genotypes with *Rht8* compared to a ~22% reduction for genotypes with *Rht-D1b* in a study of Indian elite wheat cultivars [77]. Furthermore, in a recent study, 42% longer coleoptiles were observed in GA-responsive cultivars having *Rht8* and *Rht13* compared to GA-insensitive cultivars harboring *Rh-B1b* and *Rht-D1b* alleles [64]. In addition, the coleoptile length of *Rht18* lines was (coincidentally) 42% longer than lines with the *Rht-D1b* allele, and also no significant differences were observed in different yield attributing traits between these two sets of lines [65]. Additional options for exploration include *Rht-B1*e (*Rht11*) and *Rht-B1p* (*Rht17*), which restrict plant height but have lesser effects on coleoptile length [5,78]. Similarly, *Rht5* [19], *Rht12* [18], *Rht13* [19], *Rht14* [20], and *Rht24* [7,39,72] are the other GA-sensitive reduced height genes that may offer commercial potential in future wheat breeding efforts, though each may have its own limitations [18,27].

## 5. Conclusions and Future Perspectives

Nepal's wheat breeding programs have suffered from a lack of resources. Here, the accessions that comprise the Nepali Wheat Diversity Panel (NWDP) were characterized genotypically for the most common dwarfing alleles and phenotyped for seedling vigor traits to enable future breeding for early-season drought tolerance. The genotypic analysis showed that the dwarfing allele *Rht-B1b* was far more prevalent in the population than the *Rht-D1b* allele. Analysis of the seedling vigor traits showed significant variation among the genotypes and between the seed source groups, indicating the potential to

breed varieties with longer coleoptiles. Critically, 40 accessions with *Rht-B1b* and/or *Rht-D1b* dwarfing alleles possessed long and/or more plastic coleoptiles that may enable deep sowing to mitigate early-season drought. Some of these accessions may be used as parents in future breeding programs (e.g., BW48139). As the coleoptile and root length studies were conducted using the cigar roll method under controlled conditions, the promise of these accessions needs to be confirmed under field conditions. Furthermore, Nepali wheat germplasm should be genotyped at other dwarfing loci.

**Supplementary Materials:** The following are available online at www.mdpi.com/2073-4395/11/12/2412/s1, Figure S1: Effect of $GA_3$ treatment on root length as a factor of alleles at the *Rht-B1* and *Rht-D1* loci, Table S1: List of the 318 genotypes in the Nepali Wheat Diversity Panel included in this study, Table S2: Mean plant height data from four field experiments conducted at Elora Research Station, Canada; National Wheat Research Program, Nepal and Agricultural Botany Division, Nepal during 2016 and 2017, Table S3: Summary of weather data for the four field experiments conducted in Nepal and Canada during 2016 and 2017 wheat-growing seasons, Table S4: Alleles of *Rht-B1* and *Rht-D1* loci present in accessions in the NWDP, Table S5: Mean coleoptile length and longest seedling root recorded under controlled and $GA_3$-treated conditions, Table S6: Analysis of variance of 318 genotypes in the Nepali Wheat Diversity Panel for coleoptile length and seedling root length.

**Author Contributions:** K.K. and A.N. conceptualized the project. A.N. supervised the project. K.K. conducted the experiment, data collection, data analysis and wrote the manuscript. M.K. contributed to DNA isolation, molecular marker data collection, data analysis, and technical editing. M.N.R. assisted with the analysis and technical and language editing. All authors have read and agreed to the published version of the manuscript.

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