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Seedling elongation responses to gibberellin seed treatments in wheat

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Abstract

The wheat (*Triticum aestivum* L.) *Reduced height* (*Rht*) alleles are widely used to prevent lodging through semi-dwarfism. Seedling elongation and coleoptile length can be significantly decreased by some of these alleles, leading to reduced soil emergence after deep sowing in certain semi-arid environments. Application of the elongation-promoting hormone gibberellin A_3 (GA_3) as a seed treatment has been used as an alternative to improve seedling emergence. Seedling responses to GA_3 seed treatment were investigated under controlled conditions in a collection of varieties differing for *Rht* dwarfing alleles and the ability to emerge from deep planting. Data between treated and untreated seed were collected on overall coleoptile length and emergence from deep planting in simulated pot studies. Gibberellin-sensitive varieties, carrying either no dwarfing gene or the *Rht8* dwarfing gene, responded to the GA_3 seed treatment with increased coleoptile and subcrown internode elongation. Comparison of near-isogenic lines carrying no dwarfing allele or the GA-insensitive *Rht-B1b* and/or *Rht-D1b* semi-dwarfing alleles showed that GA insensitivity was associated with reduced seedling response to GA_3 seed treatment. However, there was variation in seedling elongation and GA_3 response in a collection of GA-insensitive varieties. Thus, it cannot be assumed that all *Rht-B1b* and *Rht-D1b* varieties will fail to respond to GA_3 seed treatment. Interestingly, some better emerging GA-insensitive varieties had longer coleoptiles after treatment, suggesting that selection for better emergence in semi-arid regions of the U.S. Pacific Northwest may have led to responses in GA_3 application independent of the dwarfing gene used.

1 | INTRODUCTION

The introgression of *Reduced height* (*Rht*) semi-dwarfing alleles into modern wheat (*Triticum aestivum* L.) breeding programs led to higher-yielding, short-statured wheat varieties (reviewed by Allan, 1986; Ellis et al., 2002; Gale et al., 1975; Peng et al., 1999; Schillinger et al., 1998). These *Rht* alleles control sensitivity to the plant hormone gibberellin-A (GA). The GA hormone promotes seed germination, cell elongation, the transition to flowering, and fertility (Sponsel, 2016). Decreased GA signaling due to loss of positive regulators or regulation results in insensitivity to GA hormone in

Abbreviations: GA, gibberellin-A; PNW, Pacific Northwest; *Rht*, reduced height.

dose-response experiments, whereas increased GA signaling due to loss of negative regulators of GA signaling results in increased sensitivity to GA hormone (Chandler & Robertson, 1999; reviewed by Nelson & Steber, 2016). Mutations resulting in reduced GA signaling or biosynthesis have been identified and used to breed shorter cereal crops to improve lodging resistance (Phillips, 2016). The current study examines the impact of GA_3 and *Rht* alleles on seedling elongation.

Most semi-dwarf wheat varieties in the U.S. carry either the GA-insensitive *Rht-B1b* or *Rht-D1b* alleles. These alleles represent mutations in the 17-amino acid DELLA (Asp-Glu-Leu-Leu-Ala) regulatory domain in protein homologues on chromosomes 4B and 4D of the allohexaploid wheat genome (Peng et al., 1999). The DELLA genes are highly conserved transcriptional regulators that repress GA responses in the plant and contain (a) an N-terminal DELLA regulatory domain needed for response to GA hormone, and (b) the C-terminal functional domain (reviewed in Hauvermale et al., 2012). Mutations in the two regions of the protein have opposite phenotypes. Mutations in the functional domain lead to loss of DELLA repression of GA responses like stem elongation, leading to a tall mutant like the barley *slender* (*sln*) mutants (Chandler & Robertson, 1999). Semi-dominant mutations in the regulatory domain interfere with the ability of GA hormone to turn off DELLA repression of stem elongation, leading to a repressor that is always on and a dwarf phenotype like the barley *Sln1d* mutant and wheat *Rht-B1b* and *Rht-D1b* (Chandler et al., 2002; Phillips, 2016). Gibberellin hormone stimulates stem elongation by targeting DELLA proteins for destruction by the 26S proteasome (Nelson & Steber, 2016). Gibberellin-binding to the GA receptor, GA-INSENSITIVE DWARF1 (GID1), allows GID1 to bind the N-terminal DELLA domain, thereby stimulating DELLA protein ubiquitination and destruction by the ubiquitin-proteasome pathway. The semi-dominant *Rht-B1b* or *Rht-D1b* alleles contain mutations within the N-terminal DELLA regulatory domain that stabilize the Rht-1 protein by preventing interaction with GA hormone and the GID1 receptor. This leads to GA-insensitive semi-dwarfism due to an inability to lift DELLA repression of stem elongation. The GA-insensitive *Rht-B1b* or *Rht-D1b* plants display semi-dwarf phenotypes in multiple vegetative tissues including reduced stem, leaf, coleoptile, and subcrown internode lengths (Allan, 1986, 1989; Ellis et al., 2004; Flintham et al., 1997; Youssefian et al., 1992). The reduced plant height phenotype prevents excessive stem elongation and lodging in response to nitrogen fertilizers. This, together with increased carbon partitioning to spikes vs. the stem, led to the yield increase referred to as the "Green Revolution." However, the reduced subcrown internode length and coleoptile length has led to problems with poor seedling emergence from deep planting (>15 cm) in semi-arid dryland wheat production systems (Schillinger et al., 1998).

Core Ideas

- GA_3 seed treatments cause increased seedling elongation.
- Most GA-insensitive *Rht-B1b* and *Rht-D1b* seedlings fail to elongate after GA_3 seed treatment.
- Seedlings of two good-emerging *Rht-B1b* varieties elongated after GA_3 seed treatment.
- Breeding for seedling emergence may select for suppression of *Rht-B1b* GA-insensitivity.

Successful emergence and good stand establishment of deeply planted wheat is one of the most important determinants of yield in semi-arid regions of the western United States (Schillinger & Papendick, 2008) where crops are planted deeply to reach available soil moisture. Farmers may use a planting depth of up to 20 cm to reach sufficient stored soil moisture for germination (Mohan et al., 1997). Poor emergence can result either from poor germination or from insufficient seedling elongation to reach the soil surface. Increased coleoptile elongation and better emergence of seedlings of semi-dwarf varieties has been improved through direct selection for emergence from deep planting (Sankaran et al., 2015; Schillinger et al., 1998). Many semi-dwarf varieties grown in the dryland Pacific Northwest (PNW), such as 'Eltan' (PI536994; Peterson et al., 1991), 'Otto' (PI667557; Carter et al., 2013), and 'Mela CL+' (PI675008; Gill et al., 2020), were selected for emergence from deep sowing through direct selection in crosses among semi-dwarf varieties. Other varieties like 'Farnum' (PI638535) and 'Sequoia' (PI678966; Carter et al., 2017) were selected from crosses among wild-type *Rht-B1a*/*Rht-D1a* parents without dwarfing alleles. Emergence has also been improved through the use of GA-sensitive alternative dwarfing alleles such as *Rht8*, a mutation associated with longer coleoptiles and better seedling vigor that causes reduced height associated with reduced sensitivity to the growth-promoting hormone brassinosteroid (Gasperini et al., 2012; Rebetzke et al., 2007b).

Concern over stand establishment has led growers and seed dealers to seek products that increase seedling emergence. Recently, GA_3 hormone seed treatments have been marketed to improve seedling emergence by stimulating germination (www.valent.com/products/release-lc). Unfortunately, in 2015, several western U.S. growers reported that GA_3 seed treatment of over 10,000 ha of the standard height *Rht-B1a/Rht-D1a* wildtype variety Farnum and the semi-dwarf (*Rht-B1b/Rht-D1a*) variety Mela CL+ resulted in excessive subcrown internode elongation and emergence of the crown root system from the soil after planting to a depth of 14 cm

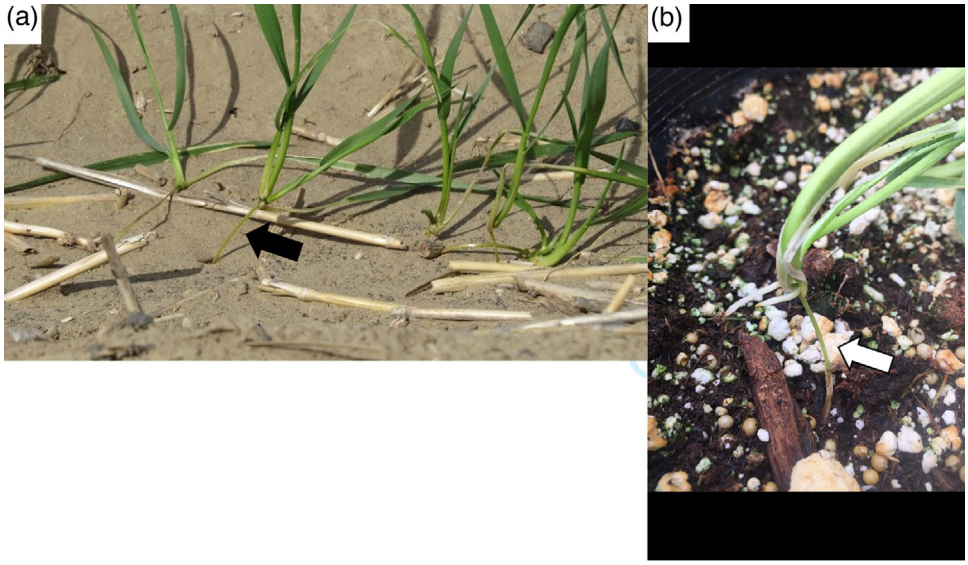

FIGURE 1 Winter wheat with exposed crowns after sub-crown internode elongation under both field (a) and greenhouse (b) conditions. The elongated sub-crown internode is indicated by an arrow

(Figure 1a). Because deeper root crown depth anchors the plant in the soil and is associated with winter survival (Allan, 1989), these hectares were replanted due to concern that the wheat seedlings might die of either cold exposure or desiccation of the crown roots. The current study was initiated in response to this problem in farmers' fields.

This phenomenon affecting both *Rht-B1b* and *Rht* wild-type varieties raised several interesting questions. Gibberellin stimulates the germination of dormant wheat grain (Tuttle et al., 2015). This subcrown elongation observed in 2015 suggested that the GA_3 seed treatment not only increased seed germination, but also increased elongation of seedling tissues including the subcrown internode. Additionally, the increased subcrown internode length was only seen in two of the many varieties that were treated that year, suggesting variation for GA_3 seedling response among varieties. The following hypotheses were tested: (a) seedlings of varieties carrying the GA-sensitive *Rht8b* dwarfing allele or wildtype height *Rht-B1a* and *Rht-D1a* alleles will show a stronger elongation response to GA_3 seed treatment than varieties carrying the GA-insensitive *Rht-B1b* or *Rht-D1b* dwarfing alleles, and (b) in a diverse panel of U.S. PNW wheat germplasm, GA_3 seed treatment will result in similar seedling elongation responses among varieties containing identical *Rht* alleles.

2 | MATERIALS AND METHODS

2.1 | Genetic material

A set of near-isogenic lines in the variety 'Brevor' (Cltr 12385; Heyne, 1959) carrying different alleles of *Rht-B1* and *Rht-D1* were used to evaluate the effect of dwarfing genes on seedling response to the GA_3 seed treatment (gift of R.E. Allan; Allan, 1980). The Brevor isolines included: (a) the wild-type homozygous for *Rht-B1a* and R*ht-D1a* alleles (hereafter called Brevor-wt), (b) Brevor homozygous for the semi-dwarf *Rht-B1b* allele (formerly *Rht1*) and wild-type *Rht-D1a* allele (hereafter called Brevor-*Rht-B1b*), (c) Brevor homozygous for the wild-type *Rht-B1a* allele and semi-dwarf *Rht-D1b* (formerly *Rht2*) allele (hereafter called Brevor-*Rht-D1b*), and (d) Brevor homozygous for both semi-dwarf alleles (hereafter called Brevor-*Rht-B1b/Rht-D1b*).

Additionally, a set of 11 varieties selected for differing emergence capacity and varying *Rht* alleles were examined for differences in seedling elongation response to GA_3 seed treatment. The varieties selected were Sequoia (*Rht-B1a/Rht-D1a/Rht8a*), Farnum (*Rht-B1a/Rht-D1a/Rht8a*), Eltan (*Rht-B1b/Rht-D1a/Rht8a*), Mela CL+ (*Rht-B1b/Rht-D1a/Rht8a*), 'Madsen' (PI511673; Allan et al., 1989; *Rht-B1b/Rht-D1a/Rht8a*), 'UI Bruneau' (PI 664304; *Rht-B1b/Rht-D1a/Rht8a*), 'LCS Azimut' (Limagrain Cereal Seeds; *Rht-B1a/Rht-D1b/Rht8a*), 'Norwest 553′ (PI655030; *Rht-B1b/Rht-D1a/Rht8a*), and Washington State University breeding varieties 'HRSW53-3T' (*Rht-B1a/Rht-D1a/Rht8a*), 'WA8212' (*Rht-B1a/Rht-D1b/Rht8a*), and 'FarEd176' (*Rht-B1a/Rht-D1a/Rht8b*). Hereafter, varieties will be referenced only by the allele they have conferring semi-dwarfism, or by wildtype (abbreviated wt), for those which have no semi-dwarfing allele. Alleles *Rht-B1* and *Rht-D1* were confirmed based on KASP marker assays as described by Grogan et al. (2016) and the SSR marker WMS261 (Korzun et al., 1998) was used for *Rht8*. Additionally, field emergence data was collected on varieties as part of the Washington

State University (WSU) Winter Wheat Breeding program trials. Emergence was collected from trials in 2020 at Lind, Kahlotus, and Ritzville, WA, and reported as the percentage of plants emerged from the ground after planting 15 cm deep, 5 wk after planting.

2.2 | Release LC seed treatments

Experiments used Release LC Plant Growth Regulator (Valent BioSciences Corporation), a commercial seed treatment product containing 97.6 mM gibberellin A_3 (GA_3) based on conversion of the manufacturer's label to metric units. The stock solution was aliquoted into 500-ml dark bottles and stored at 4 ˚C. The Release LC label recommended seed treatment with concentrations ranging from 2.4 to 38 mM GA_3. Serial dilutions of the seed treatment with sterile deionized water were prepared to obtain the following concentration of the active ingredient, GA_3, 0 mM (untreated control), 5, 10, and 20 mM. Please note that because experiments were performed using the commercial Release LC preparation, samples included decreasing concentrations of unknown inactive ingredients such as surfactants and solvents. A 10-g seed sample of each variety was placed into a 50-ml Falcon tube and treated with 152 microliters of the seed treatment. This liquid volume was chosen to be proportional to the liquid volumes recommended for commercial seed treatment with Release LC. To obtain an even coating, tubes were placed on a rotary mixer for 5 min at a rotation speed fast enough to avoid seeds sticking to the sides of the tubes (setting 40, ATR, Inc. RLVSD Rotamax Lab Mixer). The treated seeds were stored at room temperature in labeled paper envelopes for 24 h to allow the seeds to fully dry before subsequent coleoptile elongation experiments or planting in soil.

2.3 | Coleoptile elongation assay

Coleoptile elongation assays were performed essentially according to Murphy et al. (2008). Only non-dormant grain was used for these assays to avoid differences in seedling elongation due to differences in the timing of dark germination. Fifteen seeds of each variety–treatment combination were placed equidistant on separate sheets of germination paper (Nasco). The experiment was replicated twice for each variety–treatment combination. The germination paper was folded, moistened with water, and placed upright in a closed, opaque plastic container such that seedlings germinated and grew upright in the dark. The container was incubated in a Conviron growth chamber for 10 d in the dark with a 22 ˚C day and a 15 ˚C night temperature. Seedling coleoptile lengths were measured (mm). Ungerminated and mold-contaminated seedlings were not measured such that the actual sample size ranged from 12 to 15 per replication. Data were analyzed using a generalized linear model in SAS software v9.4 (SAS Institute) with the model $y = v + t + r + v \times t + e$; where y is the observed coleoptile length measured in millimeters, v is the variety effect, t is the GA_3 seed treatment concentration (0, 5, 10, or 20 mM), r is the replication (1 or 2), $v \times t$ is the interaction between varieties and treatment levels, and e is the error value. Differences among LSMEANS for each treatment were detected using pairwise comparisons via the LSMEANS statement in SAS. To determine the most effective concentration of GA_3 to elicit a measurable coleoptile response, seeds of the four Brevor isolines were treated with 0, 5, 10, or 20 mM GA_3. Based on the results of this experiment, subsequent experiments compared the effects of 0 and 20 mM GA_3 seed treatments on seedling growth.

2.4 | In-soil simulation assay

To evaluate seedling response to the seed treatment in the soil, seeds of each variety were treated with 0 mM (control) and 20 mM GA_3 before planting in 3-L plastic pots containing equal volumes of soil (Sunshine Mix #1/LC1 SunGro Horticulture), and one tablespoon of Osmocote fertilizer (Osmocote 14–14–14, Scotts Professional). Four pots were planted for each variety–treatment. Four seeds were planted equidistant from each other in each pot at a depth of 5 cm. Due to the variation that existed in the varieties to emerge from deep planting, we discovered a planting depth of 15 cm (typical of certain field conditions) provided too much variation in plant growth and emergence to evaluate treatment response. Plants were grown in a greenhouse with temperatures ranging from 21 to 24 ˚C during the day and 15 to 18 ˚C during the night with a 16-h photoperiod. Pots were watered on a regular basis. After 2 wk, the pots were emptied, and soil washed from the seedlings. Subcrown internode length was measured from the germinated seed to the crown root. The average response from all individuals per pot was counted as a single experimental unit, giving four replications per treatment by variety. If a seed did not germinate, it was reported as a missing data point. This entire experiment was replicated three times.

The data were recorded and analyzed as previously described, but with the addition of experiment and replication*experiment, and analyzed with the model $y = b + v + t + r(b) + v \times t + e$; where y is the response observed in mm, b is the experimental trial effect (1, 2, or 3), v is the variety effect (*Rht*-isoline), t is the treatment effect (0 or 20 mM), $r(b)$ is the replication within experiment, $v \times t$ is the variety × treatment interaction, and e is the experimental error value, which comprised a pooled error

TABLE 1 Field emergence data collected at three locations in Washington on 11 winter wheat varieties differing for their *Rht* alleles

Genotype	Emergence
Farnum (*Rht*-wt)	91%
Sequoia (*Rht*-wt)	87%
HRSW53-3T (*Rht*-wt)	76%
FarEd176 (*Rht8b*)	70%
Mela CL+ (*Rht-B1b*)	70%
Eltan (*Rht-B1b*)	69%
Madsen (*Rht-B1b*)	48%
UI Bruneau (*Rht-B1b*)	45%
Norwest 553 (*Rht-B1b*)	41%
Azimut (*Rht-D1b*)	37%
WA8212 (*Rht-D1b*)	ND

Note. ND, no data collected on this line; *Rht*, reduced height.

of the residual plus the insignificant lower order interaction terms. Means were separated using single degree of freedom contrasts.

2.5 | Analysis of untreated controls

The untreated individuals from the coleoptile elongation and field simulation assays were first analyzed to assess the varietal variation in coleoptile and subcrown internode length without GA_3 using the PROC GLM procedure in SAS v 9.4 (SAS Institute) as described for the dose–response assay, except without the treatment effects.

3 | RESULTS

3.1 | Genotypic variation in coleoptile elongation

In order to examine the effects of different *Rht* alleles on basal coleoptile lengths, dark coleoptile elongation was examined in individuals of differing *Rht* varieties without the Release LC seed treatment. Varieties were divided into two groups, the first consisting of the four Brevor *Rht*-isolines and the second group consisting of 11 varieties that differed for *Rht* alleles. The 11 varieties were also selected based on differences for emergence from deep planting, based on plant registration articles (cited in the methods) and field observations from the Washington State University Winter Wheat Breeding Program (Table 1).

The Brevor-wt displayed a longer coleoptile length than each of the mutant *Rht*-isolines under no treatment ($P < .0001$; Figure 2a). Brevor-*Rht-B1b* coleoptiles were not significantly longer than Brevor-*Rht-D1b* coleoptiles ($P = .591$). The Brevor*Rht-B1b/Rht-D1b* double mutant had a significantly shorter coleoptile than both the Brevor-*Rht-B1b* ($P = .002$) and Brevor-*Rht-D1b* ($P = .004$) single mutants.

In the group of 11 varieties varying for *Rht* alleles, the varieties containing the *Rht* wildtype and *Rht8b* alleles displayed longer coleoptiles than varieties with the GA-insensitive *Rht-B1b* and *Rht-D1b* alleles ($P < .0001$, Figure 2a). Significant differences in coleoptile length were observed between different varieties carrying the *Rht* wildtype allele. Variety HRSW53-3T had a significantly longer coleoptile than Sequoia, which had the shortest coleoptile of the *Rht* wildtype varieties. All three of these *Rht* wildtype varieties are considered excellent emerging under field conditions (Table 1). For varieties carrying the *Rht-B1b* allele, there was variation in coleoptile length from 50 to 39 mm. Mela CL+ had the longest coleoptile, which was not significantly different than Eltan, with these two varieties being two of the best emerging varieties containing *Rht-B1b*. Variety UI Bruneau had the shortest coleoptile length, which was not significantly different from either of the two *Rht-D1b* semi-dwarf varieties. These results demonstrate distinct differences in average coleoptile length in varieties based on the presence of differing *Rht* genes. It also demonstrates the variation that can exist among varieties with the same *Rht* alleles, indicating other genetic influences of this trait.

3.2 | Brevor isoline dose-response assay

To determine whether the Brevor-wt and the Brevor-*Rht* isolines show a dose-dependent seedling response to the Release LC seed treatment, coleoptile elongation in the dark was examined following seed treatments at GA_3 concentrations of 0, 5, 10, and 20 mM (Figure 3). The Brevor-wt isoline exhibited a significant increase in coleoptile length at 5 mM GA_3 ($P < .0001$) but did not show increasing elongation with increasing concentrations of GA_3. Thus, the GA_3 in Release LC appeared to cause an increase in coleoptile elongation at a threshold concentration of 5 mM GA_3. The Brevor-*Rht-B1b* and the Brevor-*Rht-D1b* isolines showed no significant response ($P > .05$) to increasing concentrations of GA_3. However, the Brevor-*Rht-B1b/Rht-D1b* isoline showed a significant response to a 5 mM ($P = .0460$), but no significant ($P > .05$) differences at the 10- or 20-mM concentration. This significant response resulted in a 3.6-mm decrease in coleoptile length at the 5-mM concentration. Based on the results of the dose response assay, all subsequent experiments were conducted using a Release LC seed treatment at 20 mM GA_3.

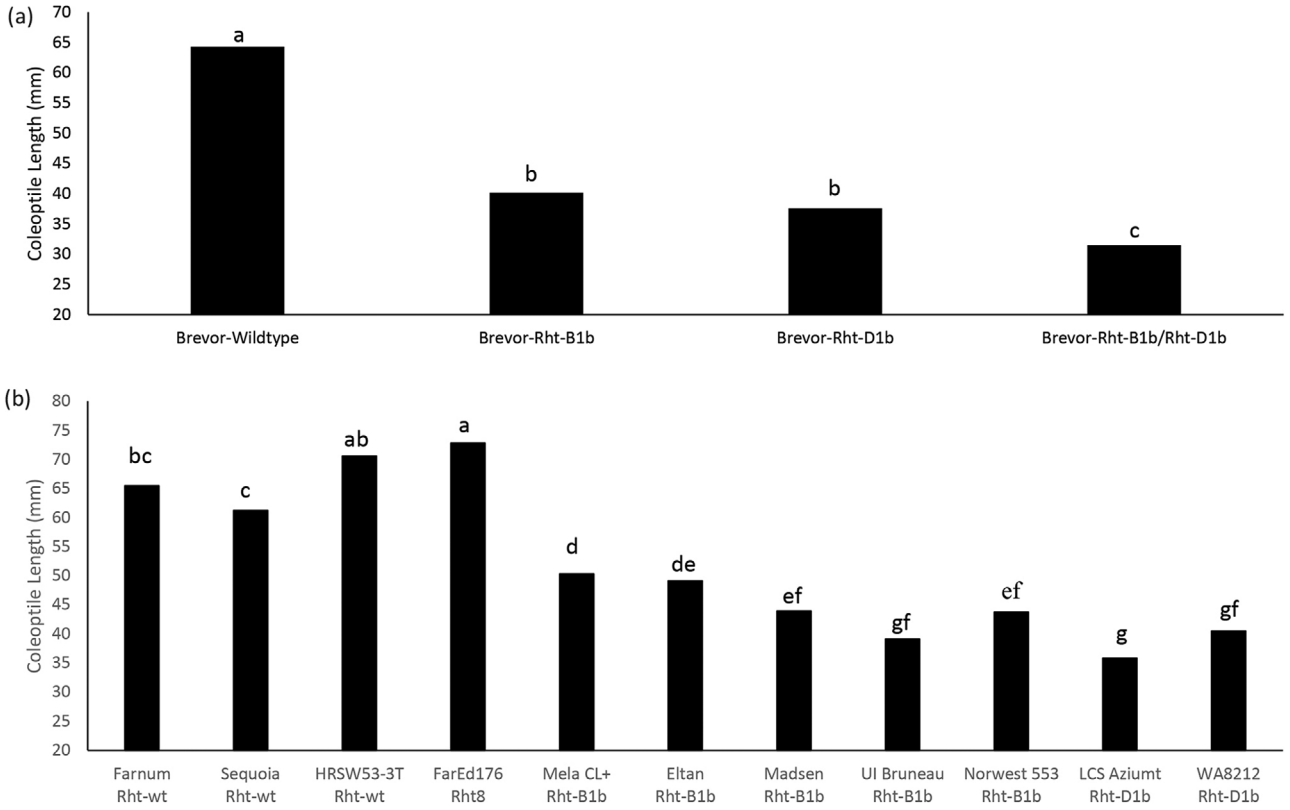

FIGURE 2 Comparison of coleoptile lengths of (a) Brevor near-isogenic lines of differing *Rht* genotypes and (b) winter wheat varieties with differing *Rht* genotypes. Letters above bars represent significant differences between lines

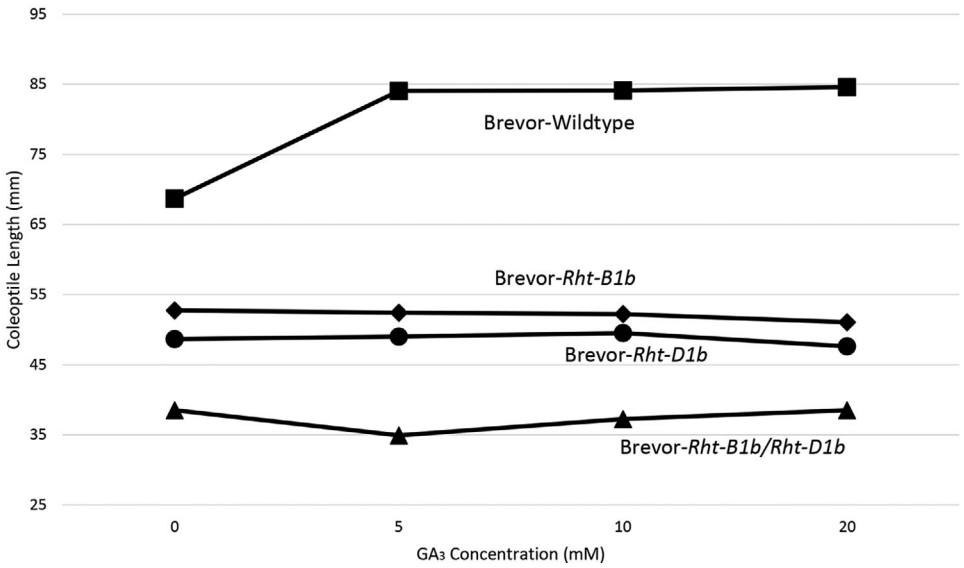

FIGURE 3 Coleoptile response of *Rht-B1b* and *Rht-D1b* Brevor isoline mutants and their respective average coleoptile length under a dose response assay using 0 mM (control), 5, 10, and 20 mM GA_3 seed treatments

3.3 | The effect of GA_3 seed treatment on coleoptile elongation

To examine the effect of the Release LC seed treatment on seedling elongation, the Brevor *Rht*-isolines and the set of 11 varieties were treated with a 0 mM (control) and Release LC treatment at 20 mM GA_3. The Brevor-wt isoline displayed a significant increase in coleoptile length of 11.5 mm following the GA_3 seed treatment, ($P < .0001$; Figure 4a). None of the *Rht* mutant isolines displayed a coleoptile-elongation response to the GA_3 seed treatment.

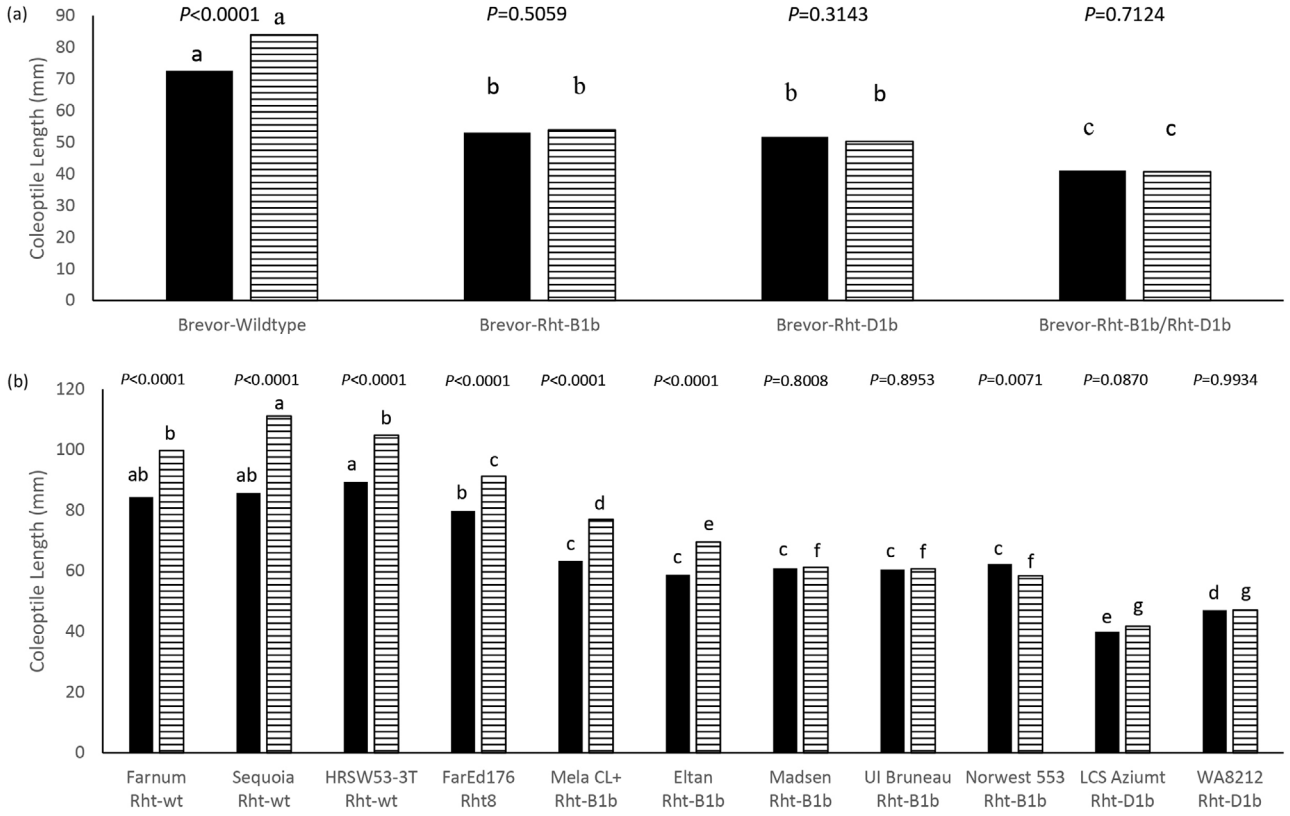

FIGURE 4 The effect of GA_3 seed treatment on dark coleoptile elongation of (a) the Brevor *Rht*-isolines and (b) a group of eleven winter wheat varieties carrying different *Rht* alleles. A pairwise comparison of coleoptiles treated with 0 mM (solid) or 20 mM (striped) GA_3 is presented with *P* values. Letters above bars represent significant differences across varieties within treatments

Similar to the Brevor-wt response, the GA_3 seed treatment resulted in a significant ($P < .0001$) increase in coleoptile elongation in the *Rht* wildtype varieties Farnum, Sequoia, and HRSW53-3T (Figure 4b). The FarEd176 variety with the GA-sensitive reduced height allele *Rht8b* also showed a significant ($P < .0001$) increase in coleoptile length in response to the GA_3 seed treatment. The five varieties containing *Rht-B1b* fell into two classes. In the first class, Eltan and Mela CL+ showed significant ($P < .0001$) increases in coleoptile length in response to the GA_3 seed treatment. Similar to the Brevor-*Rht-B1b* isoline, the remaining three varieties carrying the *Rht-B1b* allele failed to show significant coleoptile length increase in response to the seed treatment. Interestingly, Norwest 553 actually showed a significant ($P = .0071$) decrease in overall length when treated with GA_3. Neither of the two *Rht-D1b* varieties responded significantly to the GA_3 seed treatment (Figure 4b).

3.4 | Seedling elongation responses to GA_3 seed treatment in soil

Seeds were planted deeply in the soil following the GA_3 or control seed treatment, to determine if a greenhouse experiment could simulate the problem with excessive subcrown internode elongation observed after Release LC seed treatment in farmers' fields. The effect of the GA_3 seed treatment on seedling growth was measured based on subcrown internode length and the length of the first leaf.

To examine the contribution of varietal differences, subcrown internode length and first leaf length were first compared following the 0 and 20 mM GA_3 seed treatment. This was done in greenhouse experiments with seeds planted 5 cm deep. The Brevor-wt had a subcrown internode length similar to that of the Brevor-*Rht-B1b/Rht-D1b* isoline (Figure 5a). Both of these values were significantly ($P < .0001$) lower than either of the two *Rht-B1b* or *Rht-D1b* isolines. The Brevor-wt isoline was the only isoline to show a significant ($P < .0001$) response to 20 mM concentration of GA_3, almost doubling the average subcrown internode length (Figure 5a).

When comparing the 11 other varieties, the three wildtype varieties demonstrated a similar response as the Brevor-wt isoline. Farnum, Sequoia, and HRSW53-3T all showed significant ($P < .0001$) increases in average subcrown internode length after treatment of 20 mM of GA_3 seed treatment (Figure 5b). Similar to the Brevor-wt isoline,

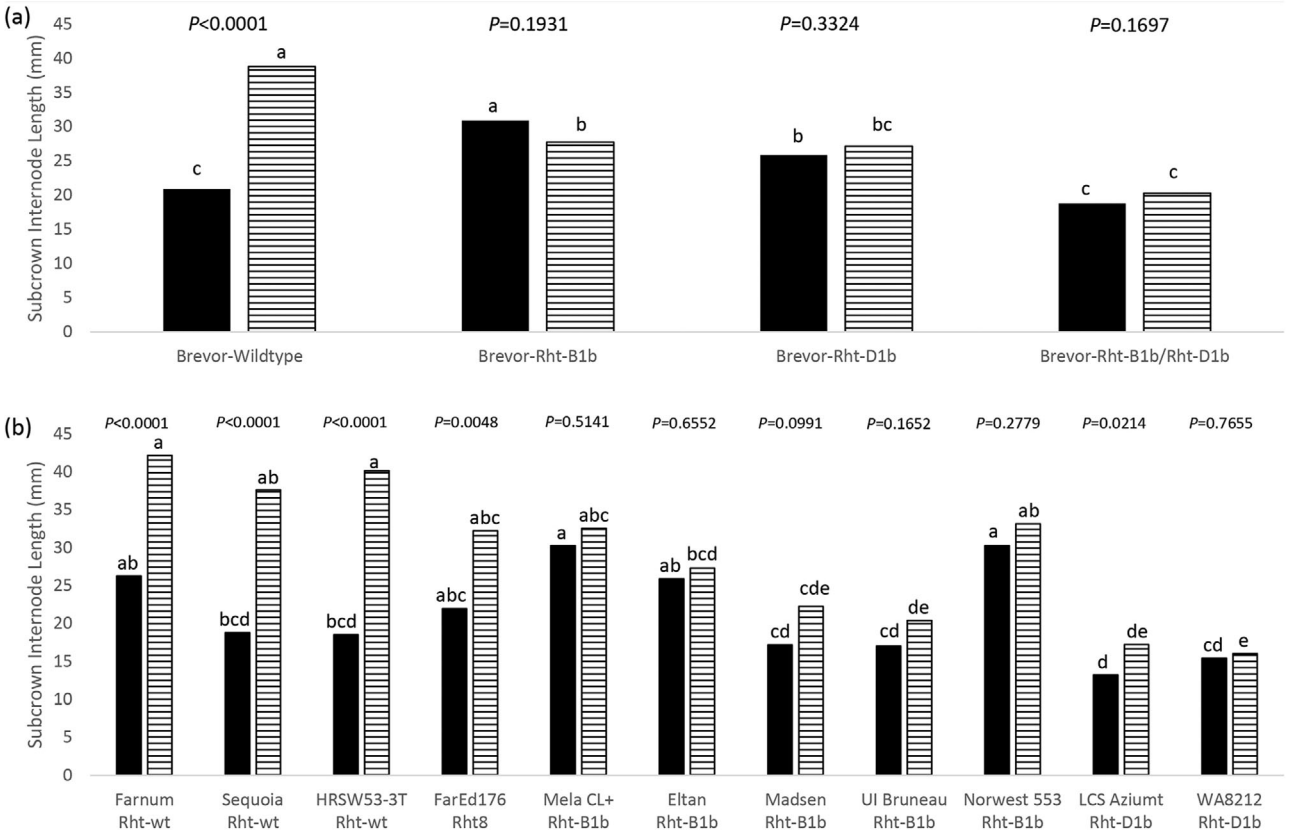

FIGURE 5 The effect of GA_3 seed treatment on subcrown internode elongation in (a) Brevor *Rht*-isolines and (b) the set of 11 winter wheat varieties carrying different *Rht* alleles. P-values indicate the significance of the response to the 20 mM GA_3 seed treatment (striped) compared to the 0 mM control treatment (solid). Letters above bars represent significant differences across varieties within treatments

Sequioa and HRSW53-3T showed significantly shorter subcrown internodes under no treatment as compared to some of the *Rht-B1b* varieties such as Mela CL+ and Norwest 553. The variety containing the *Rht8b* allele also showed a significant increase in subcrown internode length when treated with GA_3. None of the other *Rht*-insensitive allele containing varieties showed a significant increase in subcrown internode length when treated except for LCS Azimut. This variety has the shortest untreated subcrown internode length of all varieties, measuring an average of 13 mm. Although a significant increase ($P = .0214$), the increase was only to 17 mm, and was still one of the shortest of all subcrown internodes measured, regardless of treatment.

There were no significant responses in the length of the first leaf of any of the Brevor isolines (Figure 6a) or the 11 tested cultivars (Figure 6b), except for LCS Azimut. This line showed a significant ($P = .0457$) increase in average first leaf length, increasing from 125 to 132 mm when treated. The lines containing no dwarfing gene demonstrated significantly ($P < .0001$) longer first leaf length of all other varieties under either treatment, except for the varieties Mela CL+ and Eltan, where there was no significant difference (Figure 6b). The *Rht8b* allele containing variety had an intermediate first leaf length as did the variety Norwest 553. Madsen and UI Bruneau has the shortest first leaf lengths, as did the two *Rht-D1b* containing varieties.

In summary, all lines not containing dwarfing genes, as well as that containing the *Rht8b* allele, showed a significant increase in both coleoptile and subcrown internode length after treatment. Of the soft white winter wheat varieties containing *Rht-B1b*, Mela CL+ and Eltan both had a significant increase in coleoptile length after seed treatment, whereas Madsen and UI Bruneau did not. There was no significant response for subcrown internode. The hard red winter wheat variety Norwest 553, also containing *Rht-B1b*, had a significant response to treatment, only it was for a decrease in overall coleoptile length. Of the two varieties containing *Rht-D1b*, WA8212 showed no significant response. Azimut did show a response to treatment, with a small but significant increase in subcrown internode length.

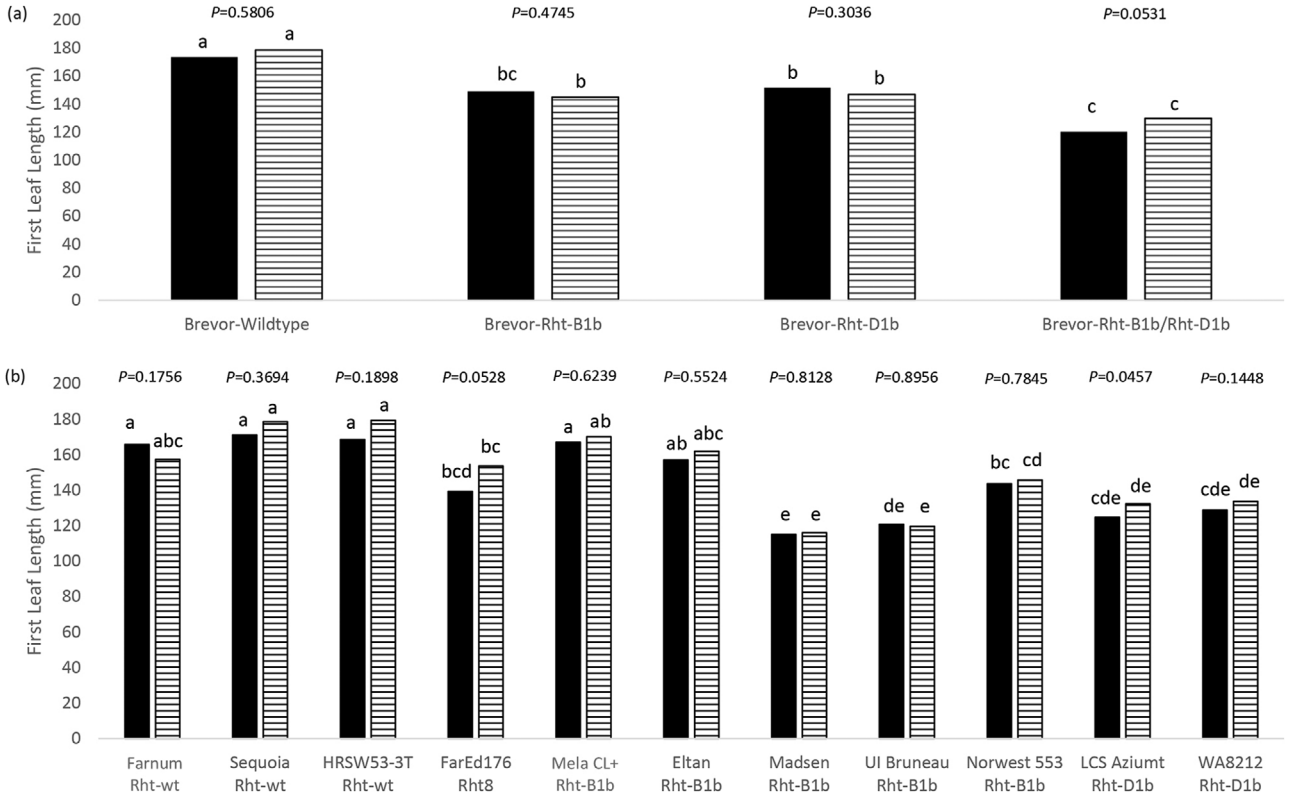

FIGURE 6 The effect of GA_3 seed treatment on first leaf elongation in (a) Brevor *Rht*-isolines and (b) the set of 11 winter wheat lines carrying different *Rht* alleles. *P* values indicate the significance of the response to the 20 mM GA_3 seed treatment compared to the 0 mM control treatment. Letters above bars represent significant differences across varieties within treatment

4 | DISCUSSION

4.1 | Identification of a potential Rht-independent GA response pathway

Decades of published research show that wheat's responsiveness to GA is regulated by the *Rht* genes (Allan, 1989; Ellis et al., 2004; Pearce et al., 2011; Peng et al., 1999; Pereira et al., 2002; Rebetzke et al., 2007b). The results presented here suggest the presence of other genetic contributions to GA response in addition to the well-studied *Rht* genes. The *Rht-B1b* varieties Mela CL+ and Eltan acted unlike any of the other *Rht-B1b* or *Rht-D1b* semi-dwarf varieties. In our experiments, Mela CL+ and Eltan exhibited significant coleoptile responses to GA_3 application. Mela CL+ and Eltan are both varieties that were developed for emergence from planting at depths of >15 cm (Gill et al., 2020; Peterson et al., 1991). Additionally, Mela CL+ is a backcross-derived variety of Eltan, so it is presumed that the same genetic mechanism for enhanced emergence from deep planting is in both varieties (Gill et al., 2020). These observations suggest that (a) Mela CL+ and Eltan undergo higher levels of cellular elongation throughout seedling development when compared to other *Rht-B1b* or *Rht-D1b* varieties, and/or (b) the coleoptiles of Mela CL+ and Eltan have the ability to respond to applied GA despite the presence of the *Rht-B1b* GA-insensitivity allele.

It appears that through decades of selection for improved seedling emergence in semi-dwarf varieties, breeders in the PNW have selected for coleoptile-specific suppressors of the seedling GA-insensitivity introduced by the *Rht-B1b* allele. This suppression pathway seems to restore elongation and GA-sensitivity during the early stages of seedling growth while preserving the semi-dwarf adult phenotype. Such suppressors could result either from mutations downstream of *Rht-1* in the wheat GA signaling pathway or from mutations in parallel pathways affecting seedling elongation. Previous studies have reported similar findings of significant loci associated with increased seedling elongation by suppression of the *Rht-B1b* or *Rht-D1b* dwarfing genes (Bovill et al., 2019; Chandler & Harding, 2013; Rebetzke et al., 2007b). For example, *Lcol-A1* is an *Rht*-independent locus that increases wheat coleoptile length. Further identification and utilization of suppressors of the *Rht-1* coleoptile elongation phenotype could be of great value in developing varieties that display semi-dwarfism at maturity, but do not lack GA-dependent

elongation early seedling development. Another approach to improving seedling emergence is to deploy alternative dwarfing genes that do not greatly decrease coleoptile length, like *Rht8* or *Rht18* (Ford et al., 2018; Gasperini et al., 2012; Grant et al., 2018; Mohan et al., 2013; Rebetzke et al., 2007b; Van De Velde et al., 2017). These could include genes in other hormone signaling pathways controlling plant height such as brassinosteroids or auxin.

4.2 | Varietal differences in seedling elongation

As expected based on previous work, the untreated wildtype Brevor had longer coleoptiles on average than either of the near-isogenic *Rht-B1b* or *Rht-D1b* lines (Allan, 1989). Allan (1989) reported that *Rht-B1b* displayed significantly longer coleoptile length than *Rht-D1b* in the Omar background but not in the Burt or Itana genetic backgrounds. The current study found no significant difference between coleoptiles in Brevor *Rht-B1b* and *Rht-D1b* lines (Figure 2a). When data for coleoptile length was examined over all the untreated selected genotypes, the wildtype and *Rht8* genotypes showed significantly longer coleoptiles than any of those with *Rht-B1b* or *Rht-D1b* (Figure 2b). Within the *Rht-B1b* genotypes, Mela CL+ had significantly longer coleoptiles than all other *Rht-B1b* and *Rht-D1b* varieties but for Eltan. This is consistent with the fact that northwest wheat breeding programs have been selecting for longer coleoptiles (without GA treatment) to improve emergence from deep planting. Keyes et al. (1989) showed that GA-sensitive varieties (those containing *Rht* wildtype alleles) tend to show higher rates of first leaf elongation when compared to their GA-insensitive counterparts. Interestingly, the strong emerging *Rht-B1b* lines Mela CL+ and Eltan had untreated first leaf lengths more like those of *Rht* wild-type lines than other *Rht-B1b* or *Rht-D1b* lines (Figure 6). Thus, suppression of dwarfism in Mela CL+ and Eltan appears to include first leaves, and these significantly longer first leaves may contribute to stronger emergence. Suppression of dwarfism in Mela CL+ and Eltan may also affect subcrown internode lengths. In the absence of hormones, the *Rht-B1b* varieties Mela CL+, Eltan, and Norwest 553 had significantly longer subcrown internode lengths than some of the *Rht* wildtype varieties (Figure 5b). In contrast, Allan (1989) found a small, sometimes statistically significant, decrease in subcrown internode lengths in *Rht-B1b* and *Rht-D1b* lines. This indicates that there is genetic variation for subcrown internode length within *Rht* subclasses and that such variation could be exploited to develop better-emerging varieties. This also suggests that the suppressor of *Rht-B1b* dwarfism in northwest wheat may increase subcrown internode lengths without GA seed treatment. Future work will need to investigate this notion using near-isogenic lines.

4.3 | GA seed treatment alters wheat seedling growth

In the past, seedling elongation responses of GA signaling mutants have been assessed by applying GA directly to the germinated or germinating seedling (Chandler & Harding, 2013; Ellis et al., 2004). In the current study, GA_3 treatment of seeds prior to germination resulted in significantly increased seedling elongation (Figures 4 and 5). This is interesting because this biological effect occurs well after the actual GA application. Results suggest either that the GA_3 persisted in the wheat embryo after germination, or that the resulting GA signaling had a long-term effect on the growth of embryo/seedling tissues present at the time of application. Another possibility is that the GA seed treatment altered responsiveness to hormone produced during seedling elongation. For example, the seedling elongation in these experiments may be a response to ethylene produced in response to etiolation since seedlings are elongating in darkness either due to incubation conditions or to deep planting in soil (Suge et al., 1997).

Several lines of evidence suggest that GA from the seed treatment does not persist indefinitely in the seedling. Although the pre-germination GA seed treatment resulted in a statistically significant stimulation of coleoptile growth in *Rht* wildtype and *Rht8* lines, this effect of the seed treatment was not clearly dose-dependent in Brevor-wt (Figures 3 and 4). This prolonged effect of GA seed treatment was more of a threshold than a dose-dependent effect, suggesting that a long-term effect on GA signaling may be more likely than the persistence of hormone in seedling tissues. The GA seed treatment appeared to have decreasing effects as the seedling grew since coleoptile and subcrown internode elongation but not first leaf elongation showed significant responses (Figures 4, 5, and 6). Some of the variability in seedling response to the GA seed treatment may be due to variation in either GA turnover rate or in the persistence of the GA signaling response. It is possible that both are influenced by variation in the environment.

4.4 | Seedling GA responses

The wildtype varieties and the *Rht8* varieties were sensitive to applied GA in the coleoptile and subcrown internode experiments, with coleoptile lengths increasing an average of 25%, and the subcrown internode almost doubling in length. This was expected as other reports have detailed the response of wheat varieties without dwarfing genes in response to GA application (Beharav et al., 1994; Ellis et al., 2004; Pinthus et al., 1989; Yang et al., 2017). Similar results were seen in the treatment of the Brevor-wt isoline. With the previously discussed exception of Mela CL+ and Eltan, none of the

remaining *Rht-B1b* and *Rht-D1b* lines responded to the GA seed treatment with increased coleoptile length. This was consistent with previous research (Allan, 1989). Interestingly, Norwest 553 demonstrated a decrease in coleoptile length when treated. Further research will need to examine whether Norwest 553 consistently shows this response and examine potential hormonal or genetic mechanisms. Of the *Rht-B1b* and *Rht-D1b* varieties, only the *Rht-D1b* variety LCS Azimut showed a significant increase in subcrown internode length in response to GA seed treatment. Curiously, there was no significant increase in Mela CL+ or Eltan subcrown internode length in response to GA (Figure 5). However, there was a problem with these two varieties showing crown emergence following GA seed treatment in farmers' fields in 2015. It is possible that limited sample sizes in the greenhouse experiment could not detect the effect that was apparent given the large number of seedlings in a farmer's field (Figure 1a).

Future work will need to examine the relative impact of coleoptile, first leaf, and subcrown internode elongation more closely to efficient seedling emergence from deep planting. It has been documented that under deep planting conditions, it is often the first true leaf that emerges and not the coleoptile. Many experiments have been conducted to try to determine the reason varieties emerge from deep planting such as coleoptile length (Amram et al., 2015; Mohan et al., 2013; Schillinger, 2011), and first leaf emergence force (Lutcher et al., 2019). Longer coleoptile length is not always associated with better field emergence (Mohan et al., 2013). However, the coleoptile is believed to protect the first leaf during elongation in the soil. Subcrown internode elongation is believed to be a response to ethylene in deeply sown wheat that may improve emergence (Suge et al., 1997). Increased GA or ethylene response in seedlings tissues within breeding programs may improve emergence.

The current study suggests that the seed industry should be cautious when using GA seed treatments to increase emergence. Such seed treatments likely improve emergence not only by increasing germination rates, but also by increasing seedling elongation. Crown emergence from the soil due to subcrown internode elongation may cause increased problems with winterkill (Loeppky et al., 1989). Thus, it would be wise to avoid such seed treatments in varieties that can respond to the GA seed treatment with increased coleoptile or subcrown internode lengths.

5 | CONCLUSION

Through observations of diverse PNW wheat germplasm, our data suggested that multiple genetic factors are involved in seedling elongation aside from the well-known *Rht-B1b* and *Rht-D1b* alleles. Variation in GA responsiveness was detected even among varieties containing identical *Rht* alleles. We believe that this variation is due to genetic suppressor(s) acting either in parallel to or downstream of the semi-dwarf *Rh-B1b* and *Rht-D1b* alleles to control seedling elongation phenotypes. Therefore, our research reached two main conclusions: (a) genetic factors exist that promote wildtype-like seedling elongation during emergence, yet do not inhibit the adult dwarfed phenotype in individuals carrying the *Rht-B1b* allele; and (b) due to the variation in seedling GA responsiveness among varieties with the same *Rht* allele, great caution must be taken, and thorough testing conducted, before administering large-scale GA_3 seed treatments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

A.M. Horgan: Formal analysis; Methodology; Writing-original draft. K.A. Garland Campbell: Conceptualization; Investigation; Resources; Writing-review & editing. A.H. Carter: Conceptualization; Funding acquisition; Investigation; Supervision; Writing-review & editing. C.M. Steber: Conceptualization; Investigation; Methodology; Resources; Supervision; Writing-review & editing. A.H. Carter and C.M. Steber contributed equally to this work.

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