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# Coleoptile length comparison of three winter small grain cereals adapted to the Great Plains

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## Abstract

Successful crop stand establishment is critical to realize high yield potential, which is dependent on depth of seed placement to access soil moisture. The coleoptile determines sowing depth by its length and ability to emerge from depth. This study was conducted to assess coleoptile length among three sets of three Great Plains winter small grain cereals—wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and triticale (*X triticosecale* Wittm.)—and to evaluate the effect of the *Rht-B1b* dwarfing allele on coleoptile length in wheat and triticale. Fifty seeds of each genotype were sown in wet germination paper in two replications utilizing a randomized complete block design, which were placed in dark growth chambers at 25°C. Measurements were conducted after 7 d, and analysis of variance and comparison of least square means for coleoptile length among and within each set of genotypes were performed in Statistix 8.1 software using Fisher's protected least significance difference at the  $\alpha = 0.05$  significance level. Results revealed that triticale had the longest coleoptiles, which were significantly longer ( $P < 0.05$ ) than those measured in both barley and wheat. Additionally, significant variation in coleoptile length ( $P < 0.05$ ) was also found within each set of wheat (3.52–6.41 cm), barley (4.32–6.63 cm) and triticale (4.05–6.92 cm) genotypes, respectively. These findings confirm other reports that the presence of the *Rht-B1b* allele was pleiotropic for coleoptile length, but development of semi-dwarf wheats with longer coleoptiles is possible if breeders deploy concurrent selection strategies.

**Keywords** Coleoptile length · Seedling emergence · *Rht1* · Wheat · Triticale · Barley

## Introduction

Wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and triticale (*X triticosecale* Wittm.) are three grasses that belong to the monocotyledonous plant family *Poaceae*, which are cultivated worldwide for their nutritious small grains. Each of these cereal crops is critically important for human health and animal nutrition. For example, it has been estimated that wheat alone provides ~55% of carbohydrates worldwide (Gill et al. 2004); barley, with its multipurpose uses in the brewing sector and animal feed, is the fourth largest cereal in terms of global planting area and production (Shen et al. 2016) and triticale, a hexaploid hybridization of wheat and rye (*Secale cereale* L.), is valued for its ecological plasticity and ability to produce stable yields under tough environmental conditions (Chernook et al. 2019). With the global population recently estimated to reach ~9.7 billion people by the year 2050 and with drought severity and frequency predicted to increase due to global warming (Cai et al. 2020), identifying new ways for breeders to increase

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yields in each of these cereal crops will be of paramount importance to maintain global food security over the next 30 years.

The Green Revolution played a very important role in enhancing the grain yield potential in wheat and rice (*Oryza sativa* L.) and is one such example of breeder innovation that improved global food security by increasing cereal yields via the introduction of semi-dwarf genes for plant height (Hedden 2003). Reducing plant height was targeted as a breeding goal so that additional fertilizer could be used to increase grain yield without increasing lodging during the Green Revolution (Griffiths et al. 2012). Most of the dwarfing genes present in modern wheat cultivars are derived from ‘Norin 10’, the original line used by Dr. O. E. Vogel at Washington State University and shared with N. E. Borlaug in his Mexican Breeding Program and during the Green Revolution (Gale and Marshall 1976; Gale et al. 1981). Generally, dwarfing genes in wheat serve to decrease plant height by shortening the stem, thereby allowing for the redistribution of assimilates to the spike, resulting in more grain yield (Bandle and Knott 1986; Chapman et al. 2006; Rebetzke et al. 2012a, b; Liu et al. 2017; Brandle and Knott 1986; Chernook et al. 2019).

Additive genetic interactions between *Rht-B1b* and *RhtD1-b*, two homoeologous dwarfing alleles located corresponding to reduced height genes *Rht1* and *Rht2* on chromosomes 4BS and 4DS, respectively, produces a full dwarf phenotype (Gale et al. 1981). Breeders have also successfully developed semi-dwarf wheat cultivars that possess one or the other of *Rht-B1b* or *Rht-D1b* (Claeys et al. 2014). Both *Rht-B1b* and *Rht-D1b* alleles have been classified as being gibberellin (GA) insensitive and reduce plant height by the reduction or perception of GA, thereby reducing cell size (Keyes et al. 1990; Hoogendoorn et al. 1990; Mohan et al. 2013). Pleiotropic effects associated with *Rht-B1b* and *Rht-D1b* have also been reported for early growth and coleoptile length because of GA insensitivity (Ellis et al. 2004; Keyes et al. 1990; Hoogendoorn et al. 1990; Matsui et al. 2002; Rebetzke et al. 2007a). This result lies in contrast to *Rht8*, another characterized but perhaps less utilized reduced height gene on chromosome 2DS that is reported to reduce plant height without reducing coleoptile length because it is GA-responsive (Rebetzke and Richards 1999; Ellis et al. 2004). Many genetic mapping studies have consistently deduced that tight linkage between the *Rht* gene homologues and quantitative trait loci (QTL) identified for coleoptile length is tightly linked on chromosomes 4BS and 4DS; hence, inheritance of *Rht* alleles has served to pleiotropically reduce coleoptile length in wheat varieties classified as full dwarf and semi-dwarf (Spielmeyer et al. 2007; Yu and Bai 2010; Rebetzke et al. 2014; Singh et al. 2015). Therefore, most modern-day wheat dwarf and

semi-dwarf cultivars possessing *Rht-B1b* and *Rht-D1b* have short coleoptiles, although minor effect QTLs for coleoptile length have also been reported on wheat chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3BS, 3BL, 3D, 4A, 4BS (*Rht-B1b*), 4BL, 4DS (*Rht-D1b*), 4DL, 5A, 5B, 5D, 6A, 6B and 7B, indicating that coleoptile length can be increased in semi-dwarf varieties because it is quantitatively inherited (Rebetzke and Ellis 2007; Rebetzke et al. 2007b; Spielmeyer et al. 2007; Yu and Bai 2010; Rebetzke et al. 2014; Singh et al. 2015; Li et al. 2011; Li et al. 2017; Sidhu et al. 2020).

In monocotyledonous plants, the coleoptile is a protective sheath that serves to aid the emergence of the emerging first leaf shoot through the soil crust barrier (Sidhu et al. 2020). Allan et al. (1962) first reported the correlation between final stand establishment and coleoptile length in winter wheat. Generally, longer coleoptiles allow for deeper sowing depths and access to optimal soil moisture and temperature conditions to ensure seed germination, field emergence and proper stand establishment, the latter of which is an early predictor of high yield potential (Mahdi et al. 1998; Gan et al. 1992; Amram et al. 2015; Garnett et al. 2009; Rebetzke et al. 2005; Rebetzke et al. 2007b; Rebetzke et al. 2014). Deeper planting has the added benefits of further protecting the seed from being eaten by birds and rodents, as well as exposing seedlings from phytotoxic effects caused by pre-emergent herbicidal sprays (Brown et al. 2003; Flohr et al. 2018; Schillinger et al. 1998; O’Sullivan et al. 1985). Because more agricultural production environments are being afflicted by drought due to global warming, breeding programs have begun to re-focus on developing wheat cultivars with long coleoptiles, because shorter coleoptiles have been identified as a potential limiting factors in crop stand establishment and grain yield (Kirby 1993; Mohan et al. 2013; Whan 1976). On the Great Plains, for example, drought like conditions occurring during the fall and spring seasons has led to poor stand establishment in the past, thereby limiting hard winter wheat yields (Budak et al. 1995; Schillinger et al. 1998).

The objective of the present study was to compare the coleoptile lengths among and within three sets of wheat, triticale and barley genotypes that were developed for agricultural production in the Great Plains and to determine the effect of the dwarf *Rht-B1b* allele on coleoptile length among these genotypes.

**Table 1** Mean coleoptile length measurements and genotype calls at the *Rht1* locus for wild type *Rht-B1a* allele (CC) and dwarf *Rht-B1b* allele (TT) for three sets of wheat (*Triticum aestivum* L.) ( $n=122$ ), triticale (*X triticosecale* Wittm.) ( $n=29$ ) and barley (*Hordeum vulgare* L.) ( $n=39$ ) genotypes adapted for agricultural production in the Great Plains

Cereal	Genotypes	Coleoptile length (cm)	<i>Rht1</i> allele
Wheat	Scout66	6.41	C:T <sup>†</sup>
	WB4303 SC	6.37	T:T
	NE17576	6.35	C:C
	SCOUT 66 SC1	6.25	C:C
	CP7050CA SC	6.07	T:T
	NHH17450	6.04	T:T
	GOODSTREAK	5.89	C:C
	Monarch BBirr	5.80	T:T
	Goodstreak W	5.72	T:T
	WB-Grainfield WC	5.70	T:T
	CP7869 SC	5.69	T:T
	Guardin	5.65	T:T
	Turkey WC	5.64	C:C
	PRONGHORN	5.62	C:C
	Spur	5.60	T:T
	NE18466	5.59	T:T
	KS Western Star SC	5.58	T:T
	WB4401	5.57	C:C
	SY 517 CL2 WC	5.52	T:T
	NE18472	5.48	T:T
	SETTLER CL WC	5.46	T:T
	NE17589	5.45	T:T
	NW13570	5.45	T:T
	WB4462 WC	5.44	T:T
	CP7909 SC	5.43	T:T
	LCS CHROME SC	5.37	T:T
	SY AP 18AX WC	5.37	T:T
	CP7017CA SC	5.36	T:T
	NI17410	5.35	T:T
	SY Monument WC	5.35	T:T
	NE17441	5.33	T:T
	NE17544	5.33	T:T
	Zenda	5.32	C:C
	Whistler	5.32	T:T
	SY Rugged WC	5.31	T:T
	WB4418 WC	5.31	T:T
	NE17470	5.19	T:T
	Long Branch SC1	5.18	T:T
	CHEYENNE	5.16	C:T
	NE18573	5.14	T:T
SY Benefit SC	5.14	T:T	
Robidoux	5.13	T:T	
NI13706	5.11	T:T	
Crescent AX	5.11	T:T	
Tatanka WC	5.11	T:T	
NE18469	5.10	T:T	
KS Dallas Wc	5.10	T:T	
NE18509	5.09	T:T	
WB4792 WC	5.07	T:T	
ROBIDOUX W	5.03	T:T	

Table 1 (continued)

Cereal	Genotypes	Coleoptile length (cm)	<i>Rht1</i> allele
	LCS Link SC1	5.02	T:T
	NE10478-1	5.01	T:T
	LCS Valiant SC1	5.01	T:T
	SY Legend CL2 WC	5.01	T:T
	NE18644	5.00	T:T
	NHH144913-3 W	5.00	T:T
	NE18544	4.95	T:T
	PANHANDLE W	4.94	T:T
	NE18625	4.88	T:T
	WB4269 SC	4.88	T:T
	WB4595 WC	4.88	T:T
	PANHANDLE	4.87	T:T
	NE18412	4.84	T:T
	NI17411	4.84	T:T
	NE16562	4.83	T:T
	NE18583	4.81	T:T
	Langin	4.81	T:T
	RUTH SC1	4.81	T:T
	NE13434-1	4.79	T:T
	Breck BB Irr	4.79	T:T
	SIEGE(NE12561) SC1	4.74	T:T
	NE18562	4.71	T:T
	Wesley WC	4.71	T:T
	NE17443	4.68	T:T
	Ruth	4.68	T:T
	NE14494	4.67	T:T
	NE17572	4.65	T:T
	NHH17612	4.64	T:T
	AM Cartwright SC	4.64	C:C
	NE14691 W	4.64	T:T
	NE15410 W	4.64	T:T
	NE15420 W	4.64	T:T
	Sy Wolverine WC	4.62	T:T
	NE18517	4.61	T:T
	NE14691	4.60	T:T
	NW15443	4.60	T:T
	SY Sunrise WC	4.60	T:T
	NW15443 W	4.59	T:T
	CP7010 SC	4.57	T:T
	Canvas Wc	4.57	T:T
	NE16468	4.56	T:T
	NE15410	4.55	T:T
	NE18640	4.55	T:T
	NE18434	4.55	T:T
	NE18455	4.51	T:T
	NE14696	4.50	T:T
	NE15624	4.50	T:T
	NW13493	4.47	T:T
	FREEMAN	4.43	T:T
	NE18445	4.43	T:T

**Table 1** (continued)

Cereal	Genotypes	Coleoptile length (cm)	<i>Rht1</i> allele
	NW13493 W	4.43	T:T
	NE15624 W	4.43	T:T
	OVERLAND	4.42	T:T
	NE13425-1	4.41	T:T
	NE14696 W	4.40	T:T
	NE17433	4.38	T:T
	NE18630	4.33	T:T
	AM Eastwood BB Irr	4.28	T:T
	NE16424	4.27	T:T
	NE17626	4.26	T:T
	Freeman	4.21	T:T
	NE18435	4.19	T:T
	NE16562 W	4.10	T:T
	NE17534	4.06	T:T
	NE14434 SE	4.04	T:T
	NE14434	4.00	T:T
	NE12561	3.94	T:T
	WB4699	3.81	T:T
	PSB13NEDH-14	3.78	T:T
	NE17462	3.76	T:T
	NE17442	3.73	T:T
	NE16563V	3.52	T:T
Triticale	NT14433	6.92	C:C
	NT441	6.51	C:C
	a12-812-1T-4	6.39	C:C
	NT12404-1	6.38	C:T
	X13T1118-1B-1B-1T-9T	6.16	C:C
	X12T038-2B-1T-2	6.09	C:C
	NT12403	5.96	C:T
	NE03T416-1	5.93	C:T
	X13T1098-1B-1B-1T-8T	5.80	C:T
	X13T1074-1B-1B-1T-6T	5.74	C:C
	a12-812-1T-2	5.56	C:C
	X13T1064-2B-1B-1T-1T	5.55	C:T
	NT19406	5.43	C:T
	NT15406	5.41	C:T
	X12TO15-1B-1T-2	5.40	C:T
	NE03T416-3	5.29	C:T
	X13T1065-2B-1B-1T-10T	5.27	C:T
	NT07403	5.15	C:T
	X13T1016-1B-1B-1T-8T	5.12	C:T
	NT14407	5.09	C:T
	X13T1064-1B-1B-1T-1T	5.03	C:T
	X13T1049-1B-1B-1T-6T	4.97	C:T
	X10T008-7	4.94	C:T
	X13T1070-2B-1B-1T-2T	4.93	C:T
	X13T1074-1B-1B-1T-4T	4.61	C:T
	X13T1004-1B-1B-2T-4T	4.46	C:T
	NT19409	4.35	C:T
	X13T1124-2B-1B-1T-5T	4.30	C:T

**Table 1** (continued)

Cereal	Genotypes	Coleoptile length (cm)	<i>Rht1</i> allele
Barley	X13T1016-1B-1B-1T-2T	4.05	C:T
	NB14404	6.64	‡
	NB17411	6.57	
	NB17401	6.10	
	NB19422	5.59	
	NB15442	5.56	
	NB18416	5.51	
	NB17409	5.45	
	NB15415	5.42	
	NB19406	5.40	
	NB15417	5.39	
	NB17415	5.38	
	NB99845	5.35	
	NB19403	5.33	
	NB19420	5.33	
	NB19434	5.29	
	NB19417	5.27	
	NB18406	5.26	
	NB19408	5.24	
	NB11416	5.22	
	NB17431	5.14	
	NB14422	5.12	
	NB15420	5.11	
	NB18411	5.01	
	NB18435	5.00	
	NB19442	4.98	
	NB19433	4.97	
	NB19423	4.95	
	NB15441	4.94	
	NB18401	4.89	
	NB11414	4.87	
	NB19418	4.81	
NB18408	4.81		
NB18422	4.80		
NB16412	4.78		
NB12437	4.72		
NB18417	4.72		
NB19443	4.60		
NB18429	4.54		
NB18439	4.23		

†Heterozygote

‡Unable to genotype due to lack of B genome

## Materials and methods

### Plant materials

Three sets of wheat ( $n = 122$ ), barley ( $n = 39$ ) and triticale ( $n = 29$ ) genotypes were collected for analysis (Table 1). The

wheat genotypes were collected from each of the Nebraska State Variety Trial (SVT) nursery and the Nebraska Intra-state Nursery (NIN). The SVT is an elite trial of diverse lines being tested in Nebraska, while the NIN is an elite wheat nursery for the Nebraska small grains breeding program.



The barley and triticale nurseries each represent elite line trials for the Nebraska small grains breeding program.

### Coleoptile length measurement

For coleoptile length measurement, 50 uniform-sized seeds of each genotype were placed in the middle of a moist germination paper about 2 cm apart with germ end down in two replications. The germination paper was folded in half vertically and subsequently placed in a controlled environment growth chamber in the dark at 25°C. After seven days, the germination paper was unfolded, and 25 seedlings of each genotype were selected for coleoptile length measurements by measuring from the base of the seed to the coleoptile tip. The average measurements of 25 coleoptile lengths for each genotype were recorded in centimeters.

### Statistical analysis

The experiment was setup in a randomized complete block design with two replications. Analysis of variance and comparison of least square means for coleoptile length among and within each set of wheat, triticale and barley genotypes were performed using Statistix 8.1 software and Fisher's protected least significance difference at the  $\alpha=0.05$  significance level.

### DNA extractions and genotyping

Five seed for each of the wheat ( $n=122$ ) and triticale ( $n=39$ ) genotypes were grown in the University of Nebraska-Lincoln greenhouse. After three weeks, three to four young leaves from each genotype were bulked and harvested in 96-well plates. DNA extraction procedures followed the same protocol used by Belamkar et al. (2018) following manufacturer's protocol for BioSprint 96 DNA Plant Kits (QIAGEN, Inc.). Upon DNA extractions, the wheat and triticale breeding lines were subsequently genotyped using a Kompetitive allele-specific polymerase chain reaction (KASP) single nucleotide polymorphism (SNP) marker at the *Rht-B1* locus. KASP assays were performed in a 5  $\mu$ L reaction, which included 2.5  $\mu$ L 2X KASP master mix (insert manufacturer) containing a FAM<sup>TM</sup> and HEX<sup>TM</sup> specific FRET cassette, 0.14  $\mu$ L of primer mix (containing 0.7  $\mu$ L of each primer) and 2  $\mu$ L of genomic DNA (10–25 ng/ $\mu$ L). The PCR program for KASP was 94°C for 15 min, followed by 10 cycles of touchdown PCR from 65 to 57°C with a 0.8°C decrease per cycle, then followed by 26 cycles of 94°C for 20 s and 55°C for 1 min. Genotype calls for the tall, wild type *Rht-B1a* allele (CC), the dwarf *Rht-B1b* allele (TT) or heterozygotes (CT) were performed in a FLUOstar Omega SNP (BMG Labtech, Inc.) with Kluster Caller software and exported to an Excel file. The cultivars 'Goodstreak' and 'Pronghorn' (*Rht-B1a*) and 'Freeman' and

**Table 2** Analysis of variance with *F* values for mean coleoptile length for three sets of wheat (*Triticum aestivum* L.) ( $n=122$ ), triticale (*X triticosecale* Wittm.) ( $n=29$ ) and barley (*Hordeum vulgare* L.) ( $n=39$ ) genotypes adapted for agricultural production in the Great Plains

Cereal	Source	df	Coleoptile length	CV
Wheat	Genotype	121	3.21***	9.45
	Replications	1	24.99**	
Triticale	Genotype	29	2.68**	11.47
	Replications	1	1.94	
Barley	Genotype	38	2.01**	9.25
	Replications	1	3.46	

\*Indicates significance at the 0.05\*, 0.01\*\* and 0.001\*\*\* probability levels, respectively

**Table 3** Coleoptile length mean, range and mean standard error (SE) and SE for three sets of wheat (*Triticum aestivum* L.) ( $n=122$ ), triticale (*X triticosecale* Wittm.) ( $n=29$ ) and barley (*Hordeum vulgare* L.) ( $n=39$ ) genotypes adapted for agricultural production in the Great Plains

Cereal	Mean cm	Range	Mean SE	SE
Wheat	4.92	3.52–6.41	0.33	0.47
Triticale	5.38	4.05–6.92	0.44	0.62
Barley	5.18	4.32–6.63	0.34	0.48

'Ruth' (*Rht-B1b*) with known alleles at the *Rht1* locus were used as controls.

### Results

Analysis of variance identified significant differences ( $P<0.05$ ) for coleoptile length measurements among all wheat, triticale and barley genotypes evaluated in the present study. Similarly, significant differences in coleoptile length ( $P<0.05$ ) were also detected within each set of wheat, triticale and barley genotypes (Table 2). Replication effects were significant ( $P<0.05$ ) for analyses conducted on the entire set of genotypes and for the combined set of wheat genotypes, but they were non-significant ( $P>0.05$ ) for the wheat SVT and NIN nurseries run separately (data not shown) and within each set of triticale and barley genotypes (Table 2).

The mean coleoptile length for wheat (4.92 cm) was the shortest, followed by barley (5.18 cm) and triticale (5.38 cm), which had the longest mean coleoptile length (Table 3). Furthermore, the range of mean coleoptile length measurements was the greatest for wheat (2.89 cm), followed closely by triticale (2.87 cm) and barley (2.31 cm). The mean coleoptile lengths and the range observed within each set of genotypes are presented in Table 3. For wheat,

the longest coleoptile length was reported in Scout66 (6.41 cm), whereas the shortest was identified in NE16563V (3.52 cm) (Table 1). For triticale, the longest coleoptile was reported for NT14433 (6.92 cm), whereas the shortest was observed in X13T1016-1B-1B-1T-2T (4.05 cm), an experimental line. In barley, the longest coleoptile was measured for NB14404 (6.63 cm), whereas the shortest was measured for NB18439 (4.23 cm), also an experimental line (Table 1). For all genera, the coleoptile length measurements for most genotypes fell in the range of 4.60–5.50 cm (Table 1). Considering the distribution of coleoptile length among genera, wheat had the highest proportion of very short coleoptiles; for example, 36 genotypes had mean coleoptile length measurements in the 3.60–4.50 cm range. Conversely, triticale had the highest proportion of very long coleoptiles that were measured in the 5.60–6.50 cm range. (Table 1). This was not unexpected, because after the reduced height (*Rht*) genes were introduced by wheat breeders in the Green Revolution during the 1970's, wheat has mainly been bred for high grain yield and semi-dwarf stature, whereas triticale is more heterogenous and often bred for taller plants with increased biomass.

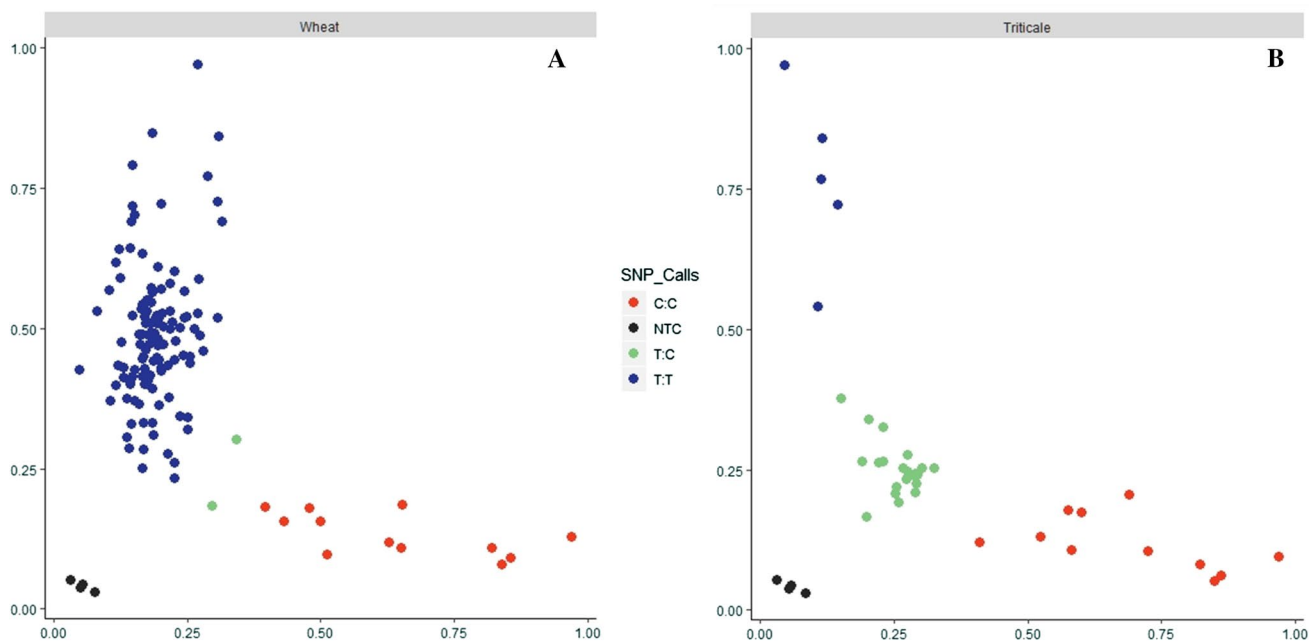
The genotyping results at the *Rht-B1* locus in wheat identified only eight genotypes with the *Rht-B1a* (tall) allele, depicted with blue dots: 'Am Cartwright' (4.64 cm), 'Zenda' (5.32 cm), 'WB4401' (5.57 cm), 'Pronghorn' (5.62 cm), 'Turkey' (5.64 cm), 'Goodstreak' (5.89 cm), 'NE 17,576'

(6.35 cm) and Scout66 (6.41 cm) (Fig. 1a; Table 1). The coleoptile length measurements for these eight genotypes averaged 5.68 cm, which was 0.82 cm longer than the mean (4.86 cm) for the wheat genotypes possessing *Rht-B1b*, indicating that the presence of dwarfing allele *Rht-B1b* was pleiotropic for coleoptile length.

Genotyping results for triticale identified seven genotypes that did not have the dwarf *Rht-B1b* allele and similarly to wheat three of these genotypes had the longest coleoptiles measured: 'NT14433' (6.92 cm), 'NT441' (6.51 cm) and 'a12-812-1 T-4' (6.39 cm) (Table 2). Of the 30 triticale breeding lines genotyped, twenty-two (~74%) were revealed to be heterozygotes (Fig. 1b) (Table 2) and no genotypes possessed the dwarfing *Rht-B1b* allele. This offers an explanation as to the greater overall mean coleoptile length in tetraploid triticale (5.38 cm) as compared to hexaploid wheat (4.92 cm). Interestingly, the mean coleoptile length for barley (5.18 cm) fell in the middle (Table 3).

## Discussion

Considerable variation in coleoptile length exists and was observed within and among the winter wheat, triticale and barley genotypes that were evaluated herein. Genotyping results revealed that the dwarfing *Rht-B1b* allele is a good screening tool for selection of semi-dwarf genotypes and that



**Fig. 1** Kompetitive allele specific polymerase chain reaction (KASP) single nucleotide polymorphism (SNP) genotyping results at the *Rht1* locus in wheat for a set of **a** wheat genotypes ( $n = 122$ ) and **b** triticale genotypes ( $n = 29$ ) adapted for agricultural production in the Great

Plains. Blue dots indicate presence of the dwarfing *Rht-B1b* allele (TT, HEX), whereas red dots indicate presence of the wild type *Rht-B1a* allele (CC, FAM). Heterozygotes are depicted by green dots

it would be possible to develop semi-dwarf wheats possessing longer coleoptiles if concurrent selection strategies were deployed. The selection of genotypes with longer coleoptiles is recommended and preferable for deep planting under drought stress. As expected, coleoptile length was reduced by the presence of the *Rht-B1b* allele in wheat and triticale (Rebetzke et al. 2014), because the semi-dwarfing alleles are strongly associated with the reduction of cell size and coleoptile length (Botwright et al. 2005; Yu and Bai 2010), which further confirms that the presence of dwarfing allele *Rht-B1b* was pleiotropic for coleoptile length among the wheat and triticale genotypes evaluated herein.

Interestingly, only two wheat genotypes were revealed to be heterozygous at the *Rht1* locus: NHH17450 and Cheyenne. Previous studies have reported that Cheyenne is known to carry non-dwarfing allele *Rht-B1a* (Guedira et al. 2010); hence, the seed source used for Cheyenne may have been contaminated with off-types that were mixed in the combine during harvest. A large amount of variation for coleoptile length was observed for the 112 genotypes that possessed the dwarfing *Rht-B1b* allele. For example, of the six genotypes with measured coleoptiles longer than 6.00 cm, each of ‘WB4303 SC’, ‘CP7050CA SC’ and ‘NHH17450’ possessed the dwarfing *Rht-B1b* allele, indicating that other genetic regions are contributing to coleoptile length as previously reported (Ellis et al. 2004; Keyes et al. 1990; Hoogendorn et al. 1990; Matsui et al. 2002; Rebetzke et al. 2007; Mohan et al. 2013). Therefore, it would be possible for breeders to develop semi-dwarf wheats with long coleoptiles if concurrent selection strategies are used. This research provides useful knowledge for farming communities in making selection decisions among small grains for coleoptile length and anticipated sowing depth in the Great Plains.

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**Author contribution** MA conducted the experiment, including the measurements, statistical analysis and helped write the manuscript. AE assisted in seed acquisition and reviewed the manuscript. FW assisted with DNA extractions, conducted genotyping and provided SNP analysis. JDB interpreted data, provided guidance and helped write and review the manuscript. PSB conceived the project, interpreted data, supervised and reviewed the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors have no conflicts of interest.

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