Evaluation of reduced height dwarfing gene, Rht1 and Rhtt18 for coleoptile length in Durum wheat

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Abstract
A set of durum (*Triticum durum* Desf.) recombinant inbred lines with Rht18 semi dwarfing gene, their Rht1 semi dwarf parents, Rht18 parent Icaro and check varieties were evaluated for coleoptile length in a growth chamber. A screening system based on the "cigar roll" method was used for coleoptile length assessment. Eight seedlings per genotype with three replicates were placed in a darkened growth chamber at a constant temperature of 20°C and coleoptile length was recorded after seven days. The longest coleoptile amongst the Rht18 genotypes was longer than that amongst the Rht1 parents and check varieties. Also, the Rht18 genotypes as a group showed a longer coleoptile than the Rht1 checks and parent lines. However, there was significant variation for coleoptile length in both Rht18 and Rht1 backgrounds allowing selection for the trait. Rht18 appeared to be more conducive for the expression of coleoptile length than Rht1. Further research is needed to confirm the suitability of Rht18 to replace Rht1 in durum breeding.

Keywords
Coleoptile length, crop establishment, drought, semi dwarfing genes, yield.

Introduction
Durum wheat (*Triticum durum* Desf.*)* represents a small but important part of the global and Australian wheat production. To improve production, a reduced height gene (Rht-B1) has been in use since the 1960s but research has shown that this gene is associated with a reduction in coleoptile length. Rht1 gene results in dwarfing through insensitivity to gibberellic acid (GA). There are alternatives to this gene that are GA-responsive such as Rht18 (Konzak 1988). Increased coleoptile length is an important trait for the successful establishment of cereal crops under receding soil moisture and in situations where deep sowing is required.

Research in bread wheat (Rebetzke et al. 2012) has suggested that short coleoptiles are due to the Rht-B1 gene, and replacement of it with an alternative dwarfing gene, such as Rht18 is required for a longer coleoptile. However, some bread wheats have longer coleoptiles e.g. Harper, Frame, Yitpi and Magenta (Kunesch et al. 2019), which suggests that it is possible to develop longer coleoptile varieties in the Rht-B1 background. Bovill et al. (2019) have recently reported on the characterization of the Lcol-A1 gene with a major effect on coleoptile length in bread wheat. This gene is effective both in the presence and absence of dwarfing genes.

Most of the research information for Rht18 and its effect on coleoptile length has been obtained from bread wheat. There is no published research on this alternative dwarfing gene in durum and its effect on coleoptile length. Therefore, the objective of our study was to evaluate variation for coleoptile length in RIL populations derived from crosses between Australian Rht1 durum lines and Icaro

Methods
Plant materials
A set of 124 durum genotypes, in total with 115 random recombinant inbred lines (RILs) derived from crossing advanced durum lines from NSW DPI possessing Rht-B1(V980019, V230800, V260379, V230616, V230604, V260204) with Icaro, an Italian durum variety possessing Rht18 gene and six Australian Rht1 advanced lines, Icaro and two check varieties- Caparoi and Jandaroi, were used in this study. The genotype of these lines was confirmed by applying exogenous GA (100 ppm) to seedlings at 14 DAS in a glasshouse and measuring the final height response.

Coleoptile length determination
The coleoptile screening system was based on the "cigar roll" method (Amram et al. 2015). Eight uniformly sized seeds of each genotype were placed 2.5 cm apart and 4 cm above a base with the basal part of the seed lowermost on the wet germination paper (46 cm x 31 cm trimmed into 23 cm x 31cm, Futari Consulting P/L, Narrabri). The paper was then rolled, held tight with rubber bands at the base of each roll, wrapped with aluminium foil and placed in a 20 cm x 12 cm plastic tray with the water level monitored and maintained at a depth of 2 cm. Each line was replicated three times with each tray being a replicate. The trays were placed in a growth cabinet at a constant temperature of 20℃. The coleoptile length was measured after seven days from the base of the coleoptile to the tip, of each of the eight seedlings was recorded in millimetre.

Results
Analysis of variance showed a significant genotype effect ($P<0.001$) for coleoptile length (CL) among the 124 tested durum genotypes and varieties. For better clarity and legibility, every second Rht18 line on the continuum of CL was selected to clearly show that Rht1 genotypes occupied a similar continuum of CL as those of Rht18 (Figure 1).

Coleoptile length ranged from 19.58 to 36.13 mm, with the minimum CL measured on Rht1 and maximum on Rht18. The maximum expression of CL in GAR genotypes and varieties, was significantly longer than that of the GAI genotypes, at 36.13 mm (V191379-10) and 29.42 mm (V260204), respectively (Table 1). The shortest CL for Rht18 genotypes was recorded for V188180-7 (20.94 mm), in V230616 (19.58 mm) (Figure 1).

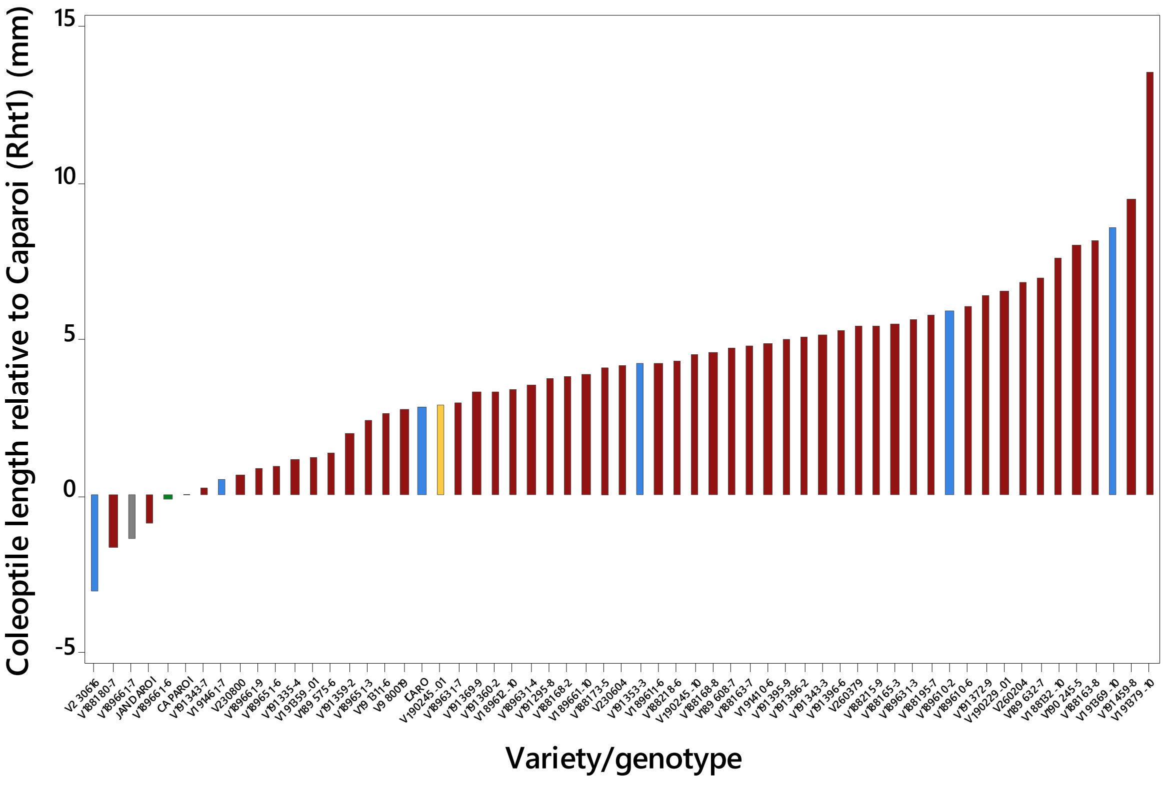

Figure 1. Coleoptile length (mm) relative to Caparoi (Rht1) after seven days of growth, in a temperature-controlled growth room at 20℃, for six Rht1 parents of RILs (blue bars) and 55 selected Rht18 genotypes (red bars), Jandaroi (Rht1, grey bar) and Icaro (Rht18, yellow bar). Values are means of three-block means, each determined from eight seeds per block per variety/genotype.

The mean CL also differed significantly between the Rht1 and Rht18 genotypes as groups. The mean CL for Rht18 genotypes, as a group, was 26.89 mm, while for Rht1 genotypes, it was slightly shorter at 24.64 mm. The difference in mean CL between Rht18 and Rht1 genotypes was small, but significant at P<0.001. The mean CL of the check variety, Caparoi (Rht1), and the Rht18 parent line, Icaro, were 22.65 and 25.45 mm, respectively.

Table 1. Mean coleoptile length (mm) measured after seven days of growth, in a temperature-controlled growth room at 20°C, for eight Rht1 genotypes, including Caparoi and Jandaroi, and 116 selected Rht18 genotypes, including the parent Icaro. Values are means of three-block means, each determined from eight seeds per block per variety/genotype. LSD values are for P<0.05*, with a level of significance for difference: P<0.001*.**

Gene	Rht1	Rht18
Mean	24.64 b	26.89 a
Min.	19.58	20.93
Max.	29.41	36.12
LSD (P<0.05)	0.48	
Sig.	***	

Relative to Caparoi, CL was 2 to 60% longer for 95% of Rht18 genotypes, whereas, for genotypes with Rht1 they were 2.6 to 30% longer for 75% of Rht1 genotypes. Coleoptile lengths shorter than Caparoi were observed for seven genotypes (five Rht18 and two Rht1) being up to 7.8% and 13.6%, respectively. Five Rht1 genotypes had longer coleoptiles than Caparoi (Figure 1). Relative to Icaro, three Rht1 genotypes had longer CL and 33 genotypes including Caparoi (28 Rht18 and 5 Rht1 genotypes) had shorter CL. In summary, highly significant phenotypic variation was observed among the 124 durum genotypes for coleoptile length with Rht18 genotypes producing a better expression of coleoptile length.

Discussion

Coleoptile length is an important factor in seedling emergence and crop establishment (Richards et al. 2002). Australian bread wheat cultivars containing GAI dwarfing genes reduced coleoptile length by as much as 40% (Rebetzke et al. 2012), also GAI wheat cultivars reduced yield by 37% under deep sowing conditions (Amram et al. 2015). Kirkegaard and Hunt (2010) showed that in Australia early sowing for higher yields requires deep sowing which in turn requires wheat varieties with longer coleoptiles.

In previous studies conducted in bread wheat, the GAI dwarfing genes, Rht1 and Rht2, were reported to possess shorter coleoptiles, which may reduce seedling vigour and crop yield stability under deep sowing conditions (Rebetzke et al. 2007; Amram et al. 2015). It has also been reported that these GAI dwarfing genes reduce coleoptile length by 30-40% when compared to standard-height cultivars (Mohan et al. 2013). In previous studies of the GAR dwarfing genes, a number of GA-sensitive Rht genes, such asRht4, Rht8, Rht12 and Rht13, were found to have little or no negative effects on seedling vigour or coleoptile length (Rebetzke et al. 2007),

In this study, the average coleoptile length of Rht18 genotypes was 9% higher than those with the Rht1 gene and was statistically significant (P<0.001) and it was consistent with results from Pandey et al. (2015). Whilst the observed magnitude of difference (2.2 mm) could appear very small they are useful for ranking the genotypes for coleoptile length and are not a full representation of the actual differences. We used a rapid protocol that did not allow the full development of coleoptiles but was successfully used by Amram et. al. (2015) in their study. The full development of coleoptiles could be considered in another experiment.

There was significant variation within both Rht-B1 and Rht18 genotypes suggesting that it is possible to select for longer coleoptiles in both genetic backgrounds. Whilst Rht18 did not appear to directly produce longer coleoptiles, it appeared more conducive to the expression of longer coleoptiles than Rht1. This observation is in agreement with the occurrence of longer coleoptile bread wheat varieties which most likely contain Rht1 or Rht2 (Kunesch et al. 2019).
For assured germination, wheat seed should not be planted deeper than its coleoptile length (Pumpa et al. 2013; Rebetzke et al. 2019; Mohan et al. 2013). To date, it appears improved coleoptile length has not been a breeding objective in Australian wheat breeding programs but under the climate change

scenarios it is likely that the frequency of droughts will increase and the increased temperature would have a negative effect on coleptile length (Rebetzke et. al. 2016) making it increasingly important to develop varieties with longer coleoptiles.

Conclusion

This study observed positive effects of, Rht18 dwarfing gene, on coleoptile length in durum wheat. The coleoptile length of Rht18 genotypes as a group was longer than that of the Rht1 group although a few Rht1 genotypes had longer coleoptiles than some Rht18 genotypes. Further studies are required to examine the prospects of Rht18, replacing the conventional Rht1 dwarfing gene in durum wheat in terms of its effects on yield and yield components and lodging tolerance.

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