Early learnings from multi-site, multi-system assessment of new long-coleoptile genetics for deep sowing of wheat

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Take home messages

- Long coleoptile wheats provide successful establishment from deep sowing into subsoil moisture thus increasing the window when growers can sow into moisture. A longer window for sowing into moisture, reduces the need to sow dry with associated risk and uncertainty in some areas and seasons
- Yield was largely unaffected by deep sowing to 12 cm in long coleoptile Mace18 whereas yield penalties of up to 34% were observed with deep sowing of shorter coleoptile Mace
- Soil type influenced establishment of short coleoptile wheat when sown deep. On dry, sandier soils, leaf growth continued slowly upward to permit some seedling emergence. However, on heavier-textured, compacted and/or crusted soils, leaf growth was restricted and slow, commonly prevent seedling emergence.

Background

Timely and successful plant establishment is critical to crop productivity in rainfed farming systems. Early emergence combined with optimal phenology increases yield potential due to a longer duration for root, tiller and crop growth while ensuring conditions are suitable for growth and flowering, and during grain-filling. Well-established crops also provide ground cover to protect soils, reduce water loss through soil evaporation, and increase crop competition with weeds.

Changing weather patterns are associated with proportionally greater summer rainfall and increasingly later sowing breaks (Flohr et al. 2021; Scanlon and Doncon 2020). There is increasing interest in deep sowing at depths exceeding 10 cm to better utilise sowing opportunities after summer and early autumn rainfall and ensure earlier germination and establishment (Rich et al. 2021; Flohr et al. 2022). However, the shorter coleoptiles (65-95mm) associated with the green revolution *Rht1* and *Rht2* dwarfing genes in current wheat varieties limits sowing depths to less than

10 cm and commonly 3 to 5 cm. Coleoptile length is a key consideration with sowing depth as the coleoptile elongates from the seed through the soil protecting the elongating sub-crown internode and crown.

Alternative dwarfing genes have been identified with potential to reduce plant height and increase yields while increasing coleoptile length by 50-80% (e.g. Rebetzke et al. 2022). Some of these dwarfing genes (e.g. *Rht8* and *Rht18*) have been used commercially overseas but have not been assessed for use in Australia. Improved establishment and grain yield in a grower-led trial in 2020 highlighted the potential for long coleoptile *Rht18* wheats for earlier, deep sowing to make use of deep sowing opportunities arising from late summer and early autumn rainfall (Rebetzke et al. 2021). This paper reports on a series of subsequent experiments conducted across Australia examining deep sowing of long coleoptile wheats. A separate pot study investigated the influence of sowing depth on shoot and root growth in contrasting soil types.

Methods

Multi-location experiments were designed to investigate the potential for emergence with deep sowing of long coleoptile, *Rht18* breeding lines bred at CSIRO from an Italian durum wheat variety, 'Icaro', into the semidwarf variety 'MaceΦ'. Both MaceΦ and the *Rht18*-containing MaceΦ. 'Mace18', were grown together with the older, tall variety 'Halberd' (released in 1969) and two current semi-dwarf varieties, 'ScepterΦ' and 'CalibreΦ', at two depths (4 and 12 cm) at four sites in WA (Latham, Holt Rock, Hines Hill, Beacon). MaceΦ and Mace18 are closely related differing in the presence of the coleoptile-reducing *Rht2* and coleoptile-increasing *Rht18* dwarfing genes. Separate experiments containing many of the same entries were sown at Cootra (SA), Tabitta and Griffith (NSW). Plant number was recorded at 200ºCd (degree-days) and crops harvested at maturity for grain yield. Separate experiments were also conducted in southern and central Queensland but issues with seed quality reduced the performance of long coleoptile wheats.

A separate pot experiment was conducted in a temperature-controlled glasshouse to investigate the influence of soil type on emergence and plant growth with deep sowing. Both MaceΦ and Mace18 were sown at 4 and 12 cm depth in replicated deep pots (n = 8 reps) containing either a coarse-textured, sandy soil from Cootra (SA) or a heavy-textured, red-brown earth from Griffith (NSW). Plant growth measurements were undertaken at two times: an early sampling at 300ºCd post-sowing (1.5 leaves) and a later sampling at 600ºCd post-sowing (3.5 leaves). Seed used in all experiments were produced in the same environment and graded to the same size to minimise confounding maternal effects on seedling vigour.

Results and discussion

Sowing depth field experiments

Conditions were generally favourable at sowing and throughout the season across the different field sites in 2021. Establishment was excellent for shallow sowings with high emergence rates and final plant numbers at all sites (Fig. 1). Overall, plant number was reduced by an average 26% with deep sowing compared with shallow sowing. The largest reduction in plant number with deep sowing was at Beacon (WA) and Griffith (-32%), and the smallest reduction at Holt Rock (WA) (-17%) and Cootra (-20%). Across WA sites, percentage reduction in plant number with deep sowing was 54 and 3% for MaceΦ and Mace18, respectively, and 38 and 21% for ScepterΦ and CalibreΦ, respectively (Fig. 1). Plant number for Mace18 was not statistically different from Halberd while the ranking for plant number for the different wheat varieties was consistent across all four WA sites. Plant heights of MaceΦ and Mace18 were not different (data not shown) yet the coleoptile length of Mace18 (131mm) is significantly longer than MaceΦ (76mm) while Halberd and Mace18 have similar coleoptile lengths (Rebetzke et al. 2021). The moderately-longer coleoptile length of Calibre was associated with greater plant number with deep sowing compared with other shorter coleoptile *Rht2* varieties Mace and Scepter (Fig. 1).

Site mean grain yield ranged from 0.68 t/ha at Hines Hill (WA) (where crops were frosted) to 4.56 and 4.62 t/ha at Tabitta and Griffith in SNSW, respectively, where the latter sites received up to 550mm of rain in 2021. Shallow-sown Mace ranged in yield from 0.40 t/ha at Hines Hill to 5.78 t/ha at Griffith. In shallow sowings, Mace produced significantly ($P<0.05$) greater average yield than Mace18 (cf. 4.08 vs 3.81 t/ha). However, when sown deep, grain yields decreased to 3.11 t/ha (-20%) for Mace but was unchanged at 3.80 t/ha (-0.5%) for Mace18. The largest yield reduction with deep-sown Mace was at Griffith (-34%) with the smallest reduction at Cootra (-2%). These yield reductions appeared to reflect plant number with deep sowing at each of the sites assessed.

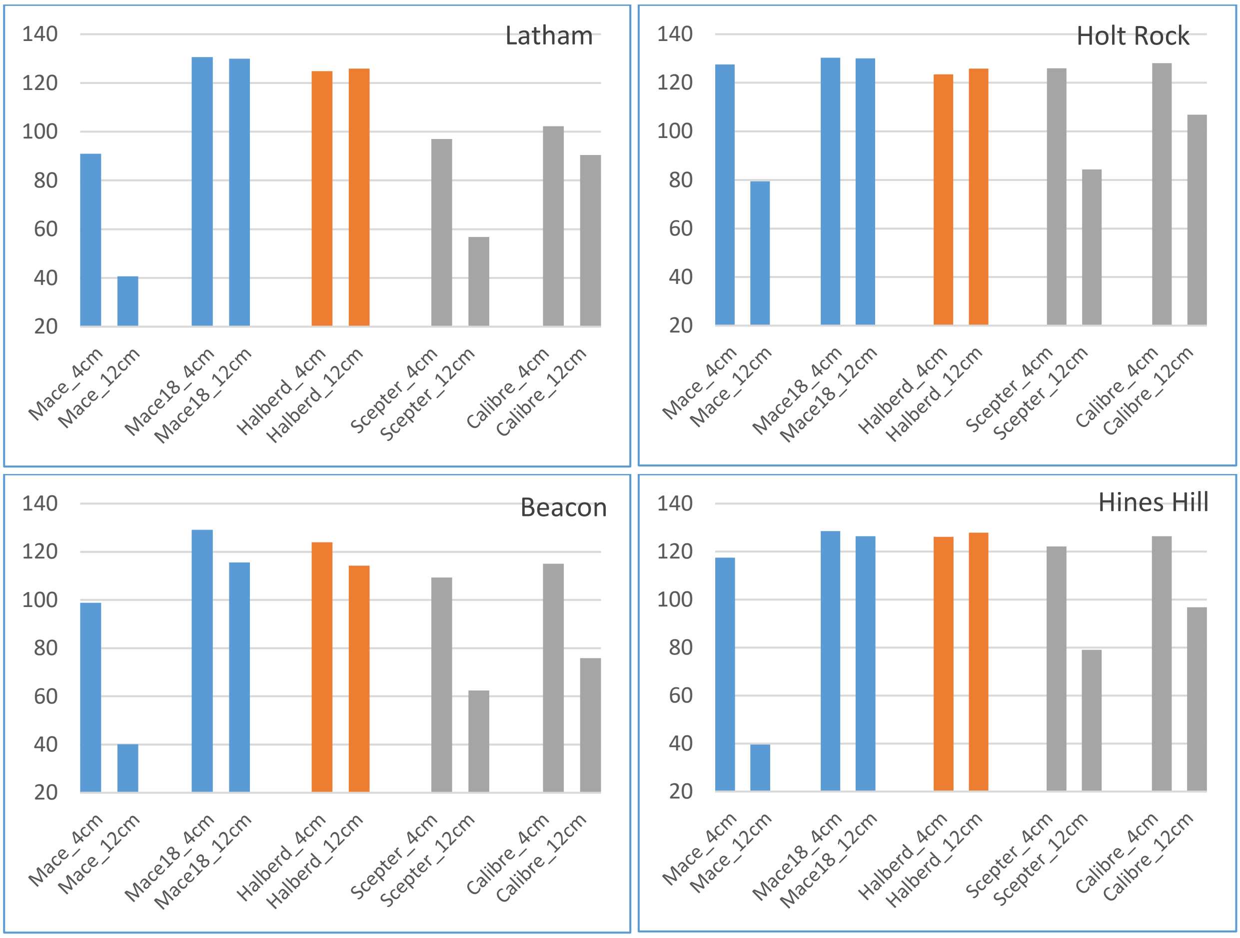

Figure 1. Mean numbers of plants per m² (at 200°Cd) at four WA sites for shallow-sown (4 cm) and deep-sown (12 cm) Mace *Rht2* and *Rht18* NILs, tall, long coleoptile variety Halberd ■, and commercial *Rht2* dwarfing gene varieties Scepter and Calibre ■. LSDs were 8, 16, 6 and 6 plants per m² for Latham, Holt Rock, Beacon and Hines Hill, respectively.

Sowing depth pot experiments

As reported, the Cootra and Griffith sites contrasted significantly ($P<0.05$) in plant establishment with deep sowing which was thought to be related to soil type. Pot experiments were designed to carefully examine seedling emergence and early seedling growth under controlled conditions in contrasting soils. In the early seedling assessment (at 300°Cd), coleoptile lengths were significantly ($P<0.05$) greater at 12 cm sowing depth, and were longer for Mace18 than Mace (Table 1). At 4 cm sowing depth, number of leaves per plant, and shoot and root length were similar for Mace and Mace18, and for both soil types. With deeper sowing to 12 cm depth, the sandy Cootra soil was associated with significantly (P<0.05) greater numbers of longer leaves, larger roots and fewer below-ground shoots than in the stronger Griffith soil (Table 1). Elongation of the first leaf to the soil surface is typically slow and restricted by soil type and factors including crusting and soil compaction. A soft, dry soil such as the Cootra soil allows for leaf elongation and emergence even with shorter coleoptile wheats sown deep (provided moisture at depth is adequate for germination). This contrasts with Mace in the Griffith soil where significant (P<0.05) shoot growth (as shoot length) was recorded below the soil surface (Table 1). There was a significant (P<0.05) variety × soil depth × soil type interaction with Mace18 producing a larger number of longer leaves, and greater root biomass than Mace particularly in the stronger Griffith soil. The reduced below-ground shoot growth for Mace18 reflected the long sub-crown internode and positioning of the Mace18 crown immediately below the soil surface (data not shown).

Table 1. Seedling growth characteristics at 300°Cd for the Mace and Mace18 near-isogenic lines (NIL) sown at 4 and 12 cm depths in a sandy Cootra and red-brown Griffith soil. All means are expressed on a single-plant or pot basis.

Seed depth	**NIL**	**Coleoptile length (mm)**		**Number of leaves (no)**		**Above-ground shoot length (mm)**		**Average root biomass (mg)**		**Below-ground shoot length (mm)**	
		Cootra	Griffith	Cootra	Griffith	Cootra	Griffith	Cootra	Griffith	Cootra	Griffith
4 cm	Mace	43	43	2.2	0.8	52	23	28	08	0	25
	Mace18	53	50	1.8	1.3	49	30	30	15	4	19
12cm	Mace	79	77	1.8	0.6	32	09	30	10	11	83
	Mace18	115*	121*	1.3	1.8*	38	43*	30	30*	34*	11*

*Mace and Mace18 means are statistically different at P = 0.05

Table 2. Seedling growth characteristics at 600°Cd for the Mace and Mace18 near-isogenic lines (NIL) sown at 4 and 12 cm depths in a sandy Cootra and red-brown Griffith soil. All means are expressed on a single-plant or pot basis.

Seed depth	**NIL**	**Number of leaves (n)**		**Shoot biomass (mg)**		**Root biomass (mg)**		**Number crown roots (no)**		**Number seminal roots (no)**	
		Cootra	Griffith	Cootra	Griffith	Cootra	Griffith	Cootra	Griffith	Cootra	Griffith
4 cm	Mace	3.8	2.6	389	206	301	95	2.6	1.1	6	5.7
	Mace18	4.1	3.8*	397	359*	202*	214	2.6	1.8	6	5.8
12cm	Mace	3.1	2.3	160	111	147	70	1.3	1.0	5.1	2.4
	Mace18	3.3	3.8*	185	216*	220*	163*	1.6	2.2*	5.9*	4.3*

*Mace and Mace18 means are statistically different at P = 0.05

Plants were predictably much larger with sampling at the later (600°Cd) seedling growth stage (Table 2). For example, average numbers of leaves more than doubled from 1.3 to 3.8 leaves from the earlier (300°Cd) seedling harvest (cf. Tables 1 and 2). Numbers of leaves, and both shoot and root biomass were reduced with deeper sowing with this reduction being greater for deep sowing in the Griffith soil. Numbers of crown and seminal roots were reduced at all depths in the Griffith soil (Table 2). Deep sowing was associated with fewer crown and seminal roots and particularly in the Griffith soil. Improved emergence and greater early seedling growth translated to increased shoot growth in Mace18 compared to Mace in the Griffith but not in the Cootra soil. In the Griffith soil, Mace18 produced significantly more leaves than Mace to increase shoot biomass. Root biomass was also significantly greater than for Mace reflecting larger numbers of crown and seminal roots (Table 2). Despite the similar shoot growth for Mace18 and Mace when sown deep in the Cootra soil, Mace18 produced greater root biomass and this largely reflected greater numbers of seminal roots when compared with Mace (Table 2).

The improved performance of Mace with deep sowing at Cootra appeared to reflect the observed ability of some short coleoptile wheats to continue growth of leaf 1 (and sometimes leaf 2) in soft, dry soils. Leaves continue to elongate upward until reaching the soil surface whereupon a crown is formed, and tillering commences. However, the reduction in seminal and crown root number, and reduced root biomass for the deep sown Mace (Table 2) does suggest that leaf growth through a soil might exhaust seed reserves to compromise early root development.

Figure 2. Long coleoptile Mace18 (L) and short coleoptile Mace (R) early and late seedling at 12cm seeding depth at Griffith

Field observations in the heavier, Griffith and Tabitta red-brown soils confirm the reduced above-ground shoot biomass and fewer crown and seminal roots in short compared with long coleoptile Mace near-isolines (Fig. 2). The slow movement of true leaves beyond the coleoptile can be supported with the promotion of new leaves and shoots initiated from nodal buds that would normally give rise to mainstem tillers. In some instances, as many as three nodes can initiate to support emergence with deep-sowing. However, early growth (leaf area and biomass) develops slowly with these commonly rare emerging seedlings.

Conclusions

Improved plant establishment with deep sowing at 12 cm confirmed the benefit of the long coleoptile trait first reported in separate on-farm experiments in 2018 and 2020. The 2021 studies highlighted the potential for increased grain yield with deep sowing for maximising water productivity. Improved performance in heavier soils suggests there may be potential for the long-coleoptile trait to aid in plant emergence and establishment in situations where furrow-fill occurs after sowing from wind or heavy rain, or with transient waterlogging at emergence (M. Lamond *pers. comm.*). The potential for coleoptile elongation should aid in ensuring emergence with variable depth control on large planters (B. Haskins *pers. comm.*), and with high soil temperatures when sowing early into warmer soils (Rebetzke et al. 2016).

Germplasm containing the *Rht18* dwarfing gene have been delivered along with selectable molecular markers for use in commercial breeding programs. Populations have been developed and are currently under assessment toward delivery of higher-yielding, long coleoptile wheat varieties for Australian growers.

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