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Genetic improvement of durum wheat establishment under fluctuating environmental conditions

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Abstract. Early sowing is critical for achieving high grain yields in wheat, especially under semi-arid environmental conditions where terminal drought limit the season. Fluctuating and sporadic precipitation early in the season could delay sowing or even terminate seedling growth. By applying deep sowing technique farmers have a tool to ensure adequate seed-zone moisture before germination leading to increasing seed establishment. This is often not feasible because of the extensive growing of semi-dwarf wheat containing the gibberellin insensitive genes *Reduce height* (*Rht*)-*B1b*/*Rht-D1b* which have shorter coleoptiles and low vigor. On the other hand, several alternative dwarfing genes with longer coleoptile have been reported. Our working hypothesis is that introduction of these alternative dwarfing genes into modern durum cultivars can improve emergence and seedling establishment under fluctuating environmental conditions. Emergence tests of durum wheat landraces from across the Mediterranean region, alongside with elite Israeli durum wheat cultivars, showed significant advantage of these pre- "Green Revolution" cultivars to emerge from soil-depth. While the durum Israeli cultivars germinated poorly from 10cm depth, several durum landraces show improved emergence rate, establishment and early vigor. Our results demonstrate the potential of using alternative dwarfing genes to improve wheat seedling survival under fluctuating environmental conditions and enhance yields.

Keywords. Dwarfing genes – Durum landraces – Coleoptile – Emergence – *Rht* – Early vigor – Drought resistance.

L'amélioration génétique de l'ancrage au sol du blé dur dans des conditions environnementales fluctuantes

Résumé. *Le semis précoce est essentiel pour obtenir chez le blé des rendements en grains élevés, en particulier dans des conditions environnementales semi-arides où la sécheresse terminale limite la saison. Les précipitations fluctuantes et sporadiques au début de la saison pourraient retarder le semis ou même mettre fin à la croissance des plantules. En appliquant la technique du semis profond, les agriculteurs ont un outil pour assurer une humidité appropriée de la zone autour des semences avant la germination, conduisant à l'augmentation de l'ancrage au sol des semences. Ceci n'est souvent pas possible en raison de la culture extensive du blé semi-nain contenant les gènes insensibles aux gibbérellines, Rht -B1b / Rht-D1b, qui a des coléoptiles courts et peu vigoureux. D'autre part, plusieurs autres gènes de nanisme induisant des coléoptiles plus longs ont été rapportés. Notre hypothèse de travail est que l'introduction de ces gènes de nanisme alternatifs dans les cultivars de blé dur moderne peut améliorer la levée et l'ancrage au sol des semis sous des conditions environnementales fluctuantes. Des essais sur la levée des variétés locales de blé dur de toute la région méditerranéenne, en plus des cultivars de blé dur israéliens d'élite, ont montré que ces cultivars pré - «révolution verte» ont une plus grande capacité à émerger du sol profond. Alors que les cultivars de blé dur israéliens présentent une capacité de germination plus faible à 10cm de profondeur, plusieurs variétés locales de blé dur s'avèrent être plus performantes en termes de levée, ancrage au sol et vigueur précoce. Nos résultats démontrent le potentiel de l'utilisation de gènes de nanisme alternatifs pour améliorer la survie des semis de blé dans des conditions environnementales fluctuantes et augmenter les rendements.*

Mots-clés. *Gènes de nanisme – Variétés locales de blé dur – Coléoptile – Levée – Rht – Vigueur précoce – Résistance à la sécheresse.*

I – Introduction

Durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) MacKey.) is an important grain-crop, particularly in the Mediterranean basin. While in the past Israeli wheat fields were dominated by traditional durum wheat, nowadays only small portion of the fields is allocated for growing durum wheat and most fields replaced by bread wheat. The Mediterranean region is characterized by a long, hot, dry summer and a short, mild, wet winter (Loss and Siddique 1994). In recent years, global warming processes resulted in greater fluctuations in amounts and distribution of precipitation. In agreement with these environmental changes, farmers have shifted the sowing date from early November to mid-December. On the other hand, late sowing results in short growing season, as the terminal drought is common in early March. In cereals, yield is determined by five major components, including number of plants per unit area, number of spikes per plant, number of spikelets per ear, number of kernel-bearing florets per spikelet, and average grain weight. Drought has an effect on all the developmental stages of plant during the season and may cause a reduction in all yield components. Thus, more effective use of the entire growing season is essential for enhancing yield.

Early-season sowing into dry soil exposes the germinating seeds to risk of dehydration due to high precipitation fluctuation. Sporadic 30-50 mm of rainfall could suffice for emergence, but with no additional rainfall, drought stress will be imposed upon the young seedlings and in severe cases force re-sowing. This has become more important in the post- ‘Green Revolution’ cultivars containing the gibberellin-insensitive (GAI) dwarfing genes, ***R**educed **h**eigh**t*** (*Rht*)-*B1b* and *Rht-D1b*. These varieties have shorter coleoptiles and will not establish well if sown too deep (Allan, 1980). Several alternative dwarfing genes that are responsive to endogenous GA and exhibit no reduction in coleoptile length have been identified and characterized (Ellis *et al*., 2004). Lines containing these genes emerge more successfully when sown deep or when used in conservation farming systems (Rebetzke *et al*., 2012).

The objectives of the current study were to (*i*) characterize the field germination ability of modern Israeli cultivars sown in soil-depth (*ii*) test modern durum wheat cultivars and durum landraces for emergence ability, and (*iii*) develop the genetic infrastructure for future wheat breeding for improved tolerance to fluctuating environmental condition and enhance yields.

II – Material and methods

Field experiment: Two commercial modern Israeli wheat cultivars (Shaphir and Galil) were sown at two locations (Kfar Hanagid and Bet-Dagan, respectively) at two soil depths, 2 cm (control) and 10cm. Numbers of emerging seedlings were counted daily and after harvest, total biomass and grain yield were calculated.

Germination test: Eight modern wheat cultivars and 47 durum landrace wheat lines were tested in two soil depths (2 and 10 cm). Eight seeds were placed at the two soil depths in controlled conditions (dark, 15 ^{o}C). The numbers of emerging seedlings were recorded daily and coleoptile length was measured 10 days after sowing.

Field emergence assay: A subset of eight durum landrace wheat lines and one modern commercial durum cultivar (C-61, control) were sown in the field at 2 and 10cm soil depths. The numbers of emerging seedling, numbers of tillers, leaf size, plant height and grain yield were analyzed.

Gibberellin responsiveness assay: The response to GA was assessed by measuring coleoptile length of seedlings grown in hydroponic solution with GA_3 (10^{-5}M) or control.

III – Results and discussion

The 'Green Revolution' including the introduction of semi-dwarfing genes (*Rht-B1, Rht-D1*) led to impressive increases in wheat yields. The reduced culm length in these cultivars resulted from limited response to GA via DELLA proteins. As a consequence, most modern wheat cultivars have very short coleoptiles and show reduced emergence in deep sowing. Field experiments at two locations showed that deep sowing resulted in significant reduction in seedling stand (31.5 and 42% for Shaphir and Galil, respectively) as compared with the control treatment. As expected, this reduction in field stands was expressed later in lower grain yields (34 and 28.5%, respectively).

Controlled emergence tests from soil depth (2 and 10 cm) of 47 durum landraces, alongside with eight elite durum wheat cultivars showed significant differences: Most durum landraces (64%) emerge well from 10 cm soil-depth, with average of 6-9 days. On the contrary, the modern durum wheat cultivars were not able to emerge from soil-depth (with exception of cv. Afik which showed good emergence from 10cm soil depth). This higher emergence rate was associated with significantly longer coleoptile of the landraces lines as compared with the modern cultivars. For example, the landraces Abu Fashi and Gaza had a coleoptile length of 14.8 ± 0.6cm and 13.6 ± 0.7cm, respectively, while the modern cultivars Ayalon and C-9 were significantly shorter (8.3 ± 0.9cm and 9.4 ± 0.7cm, respectively). In agreement, responsiveness to exogenous GA test revealed positive significant effect in Abu Fashi seedlings while Ayalon and C-9 did not respond to the GA_3 treatment.

These greenhouse results were also supported by a field evaluation for emergence and development. Only slight or no significant reduction in emergence was observed for the local durum landraces were as commercial cultivar showed significant reduction in emergence rate when sown at 10cm. Furthermore, several landraces showed improved establishment and early-vigor which was manifested in minimal or no reduction in growth parameters (i.e. number of leaves, number of tillers and leaf width) when sown at soil-depth. Furthermore, most landraces presented enhanced early-vigor compare to the modern cultivars. For example, based on measurements of the last fully exposed leaf-width, the line Gaza showed significantly higher early-vigor compared to modern cultivars. In this line leaf width was not reduced in plots were seeds were sown at 10 cm. Interestingly other wheat lines which showed good emergence from soil-depth had relatively narrow leaf width (i.e., Abu Fashi). This might imply independent inheritance of the two traits.

In conclusion, our results demonstrate the breeding potential of replacing the GAI dwarfing genes with alternative dwarfing genes from pre "Green Revolution" germplasm, to improve wheat establishment under fluctuating water availability and enhance grain yields. Currently ongoing phenotypic and genotypic selection of crosses between modern Israeli cultivars and potential landraces lines with improved germination from soil-depth is underway.

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