

Differences in Stem Soluble Carbohydrate Contents among Recombinant Chromosome Substitution Lines (RCSLs) of Barley under Drought in a Mediterranean-type Environment

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Abstract: Drought is one of the major abiotic stresses that dramatically threaten the global food supply and it is becoming an increasingly severe problem in many regions of the world, mainly in Mediterranean areas and/or climates. This study investigates the effect of drought on the stem soluble carbohydrate content and its role in grain filling in different barley genotypes – four recombinant chromosome substitution lines (RCSLs) and the recurrent parent cv. Harrington, which had been growing in two contrasting Mediterranean environments in central Chile. At anthesis, drought stress increased the stem glucose and fructose contents in lines 76 and 78 and fructans in all the genotypes. At maturity, in non-stressed plants the soluble carbohydrate content in the stem decreased, suggesting a mobilization of carbohydrates from the stem into the grain. Drought increased the stem content of fructose, sucrose and fructans in all genotypes. The accumulation of fructans was higher in RCSLs as compared to Harrington, providing evidence that the introgression of the wild ancestor (*Hordeum vulgare ssp. spontaneum*) into cv. Harrington increases the terminal drought tolerance of barley. Line 89 showed the maximal content of fructans and it could be considered as the most tolerant to terminal drought of all RCSLs. However, this genotype showed the lowest grain weight and yield, indicating that is the most susceptible line of those referred to as grain yield.

Key words: barley, carbohydrates, drought, fructans, grain, stem

INTRODUCTION

Drought is the major environmental factor limiting annual production of forages, cereals and other crops in temperate regions. The prospect of feeding an increasing global population in a world with increasingly erratic rain, temperature and drought patterns, as it is anticipated for the predicted changes in climate, presents a serious challenge to plant scientists and highlights the requirement to make crop improvement even more crucial for food production (Ji et al., 2010). Barley is one of the two major cereals cultivated in Mediterranean areas and the growth, development and yield of this crop is highly sensitive to a reduction in water availability in the soil.

Plants are able to limit water losses by closing their stomata. A decline in stomatal conductance, however, not only decreases water vapour loss, but also inhibits CO₂

influx leading to a reduction in carbon fixation and the inhibition of photosynthetic enzyme activity and regeneration (Flexas et al., 2004), which in turn alters carbohydrate metabolic equilibrium. Despite the reduced photosynthetic assimilation of CO₂ in drought-stressed leaves, plants accumulate a large content of water soluble carbohydrates such as glucose, fructose, sucrose, fructans, polyols, together with other compatible solutes such as amino acids, organic acids, quaternary ammonium and tertiary sulfonium compounds (Volaire et al., 1998; De Roover et al., 2000; Xue et al., 2008). These osmolytes are used for maintaining leaf cell turgor, protecting membrane integrity and preventing protein denaturation (Xue et al., 2008). Global gene expression studies have shown a reduction of the expression level of most genes encoding chloroplast enzymes involved in carbon fixation, while genes encoding cytoplasmic and vacuolar enzymes in the pathways leading to glucose, fructose and fructan production were up-regulated under drought stress (Xue et al., 2008), suggesting a coordination in the regulation of transcripts of key enzyme genes involved in carbon fixation and carbohydrate accumulation. Another conspicuous effect of water restriction on plants is growth reduction, mainly caused by an inhibition of leaf and stem elongation.

Carbohydrates act as nutrient and signalling molecules, modulating the expression of a large number of genes (Osuna et al., 2007) and they are also involved in the response to abiotic stresses. Fructans are widely believed to be involved in resistance to cold and drought (Vijn & Smeekens, 1999; Pérez et al., 2001). A positive correlation between the proportion of sugar in the form of polymeric fructan and drought resistance has been reported (Volaire et al., 1998). Fructans are sucrose-derived fructose polymers synthesized from sucrose by fructosyltransferases. Fructans from different sources exhibit different degrees of polymerization and different linkages between adjacent fructose residues. They can either be accumulated both as a short or long term reserve carbohydrate in heterotrophic organs such as roots, stems and grains or in autotrophic organs such as leaves (Pollock & Cairns, 1991). During vegetative and early reproductive growth of cereals, assimilated carbon is accumulated temporarily in the stem and leaf sheath as carbohydrate reserve. Much of this carbohydrate can be remobilized and transported to the developing grain, making a significant contribution to final grain yield (Schnyder, 1993). Thus, grain filling in cereals depends on two major sources of carbon, namely current photosynthesis in leaves and to some extent in spikes, and mobilization of stored water soluble carbohydrates from the stem into growing grains. In semiarid regions with terminal drought, grain filling in cereal crops may depend more on stem water soluble carbohydrate content than on current photosynthetic assimilates (Ehdaie et al., 2008). In turn, high water soluble carbohydrate content in the stem has been suggested as a selection criterion for use in breeding.

Changes induced by drought in the sugar content of the stem may be involved in a general response strategy to cope with this type of stress. The aim of the research reported in this paper was to investigate the effect of drought on the stem soluble carbohydrate content in different barley genotypes and their role on grain filling and further influence on grain weight and yield. In order to approach the natural environment of Mediterranean barley crops, this experiment was conducted in the field in two contrasting Mediterranean environments in central Chile, with and without

terminal drought stress from heading until maturity over a growing season. Four recombinant chromosome substitution lines and the recurrent parent cv. Harrington were used in order to test whether the introgression of the wild ancestor (*Hordeum vulgare ssp. spontaneum*) into cv. Harrington can increase the terminal drought tolerance of barley. The results could provide the basis for breeding approaches to improve reproductive stage drought tolerance in barley.

MATERIALS AND METHODS

Plant material and growth conditions

A subset of 4 (53, 76, 78, 89) out of 24 RCSLs barley lines described by Matus et al. (2003) and the recurrent parent, cv. Harrington, have been used in this study. The lines were obtained using two backcrosses with the recurrent parents and six generations of self-pollination (Matus et al., 2003). These RCSLs were selected for their agronomic characteristics and their contrasting yield response to soil water availability (Matus et al., 2003; Inostroza et al., 2009).

The field experiment was carried out in a Mediterranean environment at Cauquenes, Chile (35° 58' South; 72° 17' West; 177 m.a.s.l.; precipitation of 209 mm during the growing season) and Santa Rosa, Chile (36° 32' South 71° 55' West; 220 m.a.s.l.; precipitation of 182.7 mm plus 4 irrigations from heading to maturity) in 2007-08. Cauquenes was the site in which the crop remained under water stress during the grain filling period and Santa Rosa was the site with no water stress. The experiment was established in plots consisting of six rows 2 m long, each separated by 0.2 m. Seeding rate was 120 kg h⁻¹ and sowing dates were 01 June at Cauquenes, and 24 August at Santa Rosa, respectively. The experimental design in both sites was an α -lattice with four replications and five incomplete blocks per replication, each block containing all the genotypes. Plants were fertilized with the equivalent of 127 kg ha⁻¹ N, 120 kg ha⁻¹ P₂O₅, 44 kg ha⁻¹ K₂O, 36 kg ha⁻¹ MgO, 44 kg ha⁻¹ S plus B and Zn. Five main stems per plot were harvested at two growth stages – anthesis (27 September and 8 November) and maturity (12 November and 19 December) at Cauquenes and Santa Rosa, respectively. The plant material was stored at -80°C until analysis.

Carbohydrate extraction and determination

Frozen stem material was ground in liquid nitrogen to obtain a fine powder. For water soluble carbohydrate determination a subsample of the frozen material (~100 mg) was used for four consecutive extractions (with either 1 ml 80% ethanol (2 times), 40% ethanol or water, all of them, buffered with 10 mM Hepes-KOH pH=7.5 at 80°C for 30 min) and the extracts were pooled. Aliquots of 600 μ l of ethanol extracts were vacuum dried and resuspended in 1400 μ l water. Glucose, fructose, sucrose and fructans were analyzed in the extracts as described by Morcuende et al. (2004).

Statistical analyses

ANOVA were performed using PROC MIXED (SAS Inst., 2007). The threshold for significance was set at $P < 0.05$. The least square means of carbohydrates was calculated using the interaction between genotype, environment and growth stages.

RESULTS AND DISCUSSION

In the present study the sensitivity to soil water availability has been analyzed in four RCSLs barley lines and the cv. Harrington in two contrasting Mediterranean environments in central Chile, by studying different physiological and biochemical parameters related to the water content of the plant.

At anthesis the stomatal conductance of the four RCSLs and cv. Harrington was reduced in plants grown at Cauquenes as compared to those grown at Santa Rosa (54 and 217 $\text{mmol m}^{-2} \text{s}^{-1}$, respectively), suggesting that plants under drought conditions decrease water loss by closing their stomata. Such a parameter was also lower in the lines 53 (43 and 177 $\text{mmol m}^{-2} \text{s}^{-1}$) and 89 (39 and 197 $\text{mmol m}^{-2} \text{s}^{-1}$) as compared to cv. Harrington (55 and 230 $\text{mmol m}^{-2} \text{s}^{-1}$) at Cauquenes and Santa Rosa, respectively. There were no significant differences ($P < 0.05$) in stomatal conductance between lines 76 and 78 as compared to Harrington, in either environment. A decline in stomatal conductance has been frequently shown under drought stress (Xue et al., 2008), leading to a reduction in carbon fixation (Flexas et al., 2004).

At anthesis, in plants grown at Santa Rosa the glucose content in the stems of lines 76 and 78 was lower as compared to cv. Harrington, while the other lines, 53 and 89, showed a similar content of glucose as Harrington (Fig. 1A). However, drought stress significantly ($P < 0.05$) increased glucose content in lines 76 and 78 without surpassing the content found in the parental cultivar and the rest of RCSLs, in which drought tended to decrease the content of glucose. The fructose content showed the same pattern of changes described at this growth stage for glucose (Fig. 1B). Sucrose changed in exactly the opposite way from glucose and fructose (Fig. 1C) in both environmental conditions. Fructan content was similar in Harrington and lines 53 and 89 in the environment without restrictions of water, while it was slightly higher in RCSL 76 and lower in RCSL 78 as compared to Harrington (Fig. 1D). Drought led to a general increase of fructan content, line 78 showing the highest accumulation and 89 the lowest (Fig. 1D). These results are consistent with previous studies showing an accumulation of carbohydrates in the stem of cereals under drought stress (Volaire et al., 1998), which depends of the genotype and the environment (Ehdaie et al., 2008) and can play a role in grain filling. Fructans are believed to confer drought tolerance (Vijn & Smeekens, 1999; De Roover et al., 2000) and it has been suggested that, during drought, tolerant genotypes accumulate higher contents of fructans than more sensitive ones (Volaire & Lelievre, 1997). Transgenic fructan accumulating tobacco exhibited enhanced tolerance to drought stress (Pilon-Smits et al., 1995). Fructan accumulation has also been correlated with an induction of the gene encoding for sucrose: sucrose fructosyltransferase (1-SST), leading to a higher activity of the enzyme and further activation of fructan biosynthesis as compared to non-stressed plants (De Roover et al., 2000).

At maturity, in plants grown at Santa Rosa, the content of soluble carbohydrates decreased in all the genotypes as compared to the earlier growth stage (Figs. 1A, B, C, D), suggesting a mobilization of sugars from the stem into the developing grain (Schnyder, 1993). Drought stress tended to increase the content of glucose without reaching significance (Fig. 1A) and significantly ($P < 0.05$) increased the content of fructose (Fig. 1B), sucrose (Fig. 1C) and fructans (Fig. 1D) in all the genotypes. In the Cauquenes environment, line 89 accumulated enormous contents of fructose and

fructans (Fig. 1B, D) and the content of fructans in all the recombinant chromosome substitution lines was higher than in Harrington (Fig. 1D). In agreement with the role of fructans conferring drought tolerance, these results suggest that the introgression of the wild ancestor into cv. Harrington increases the terminal drought tolerance in barley.

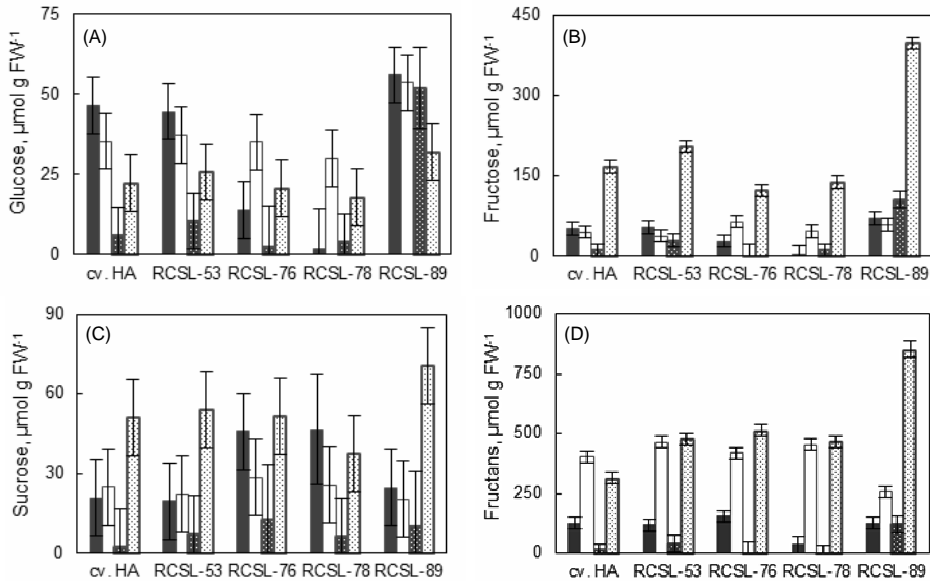


Figure 1. Effect of drought on the content of glucose (A), fructose (B), sucrose (C) and fructans (D). Plants were grown in the field in two Mediterranean environments in central Chile, without –Santa Rosa (black)– and with –Cauquenes (white)– terminal drought stress either at anthesis (without dots) or maturity (with dots) in five genotypes including cv. Harrington.

In our experiment, terminal drought stress tended to increase the number of grains in all the genotypes, while the thousand grain weight was decreased (data not shown), indicating that the interference of drought stress with assimilate accumulation affects not only grain size but also grain number (Ehdaie et al., 2008).

CONCLUSIONS

This study shows that line 89 accumulated the maximal content of fructans and other carbohydrates and it could be considered as the most tolerant to terminal drought of all RCSLs. However, this genotype showed the lowest grain weight and yield, indicating that is the most susceptible line of those referred to as grain yield.

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REFERENCES

- De Roover, J., Vandenbranden, K., Van Laere, A. & Van den Ende, W. 2000. Drought induces fructan synthesis and 1-SST (sucrose: sucrose fructosyltransferase) in roots and leaves of chicory seedlings (*Cichorium intybus* L.). *Planta* **210**(5), 808–814.
- Ehdaie, B., Alloush, G. A. & Waines, J. G. 2008. Genotypic variation in linear rate of grain growth and contribution of stem reserves to grain yield in wheat. *Field Crops Res.* **106**(1), 34–43.
- Flexas, J., Bota, J., Cifre, J., Escalona, J. M., Galmés, J., Gulías, J., Lefi, E-K., Martínez-Cañellas, S. F., Moreno, M. T., Ribas-Carbó, M., Riera, D., Sampol, B. & Medrano, H. 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. *Ann. Appl. Biol.* **144**(3), 273–283.
- Inostroza, L., del Pozo, A., Matus, I., Castillo, D., Hayes, P., Machado, S. & Corey, A. 2009. Association mapping of plant height, yield, and yield stability in recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp. *vulgare* background. *Mol. Breeding* **23**, 365–376.
- Ji, X., Shiran, B., Wan, J., Lewis, D. C., Jenkins, C. L. D., Condon, A. G., Richards, R. A. & Dolferus, R. 2010. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. *Plant Cell Environ.* **33**(6), 926–942.
- Matus, I., Corey, A., Filichkin, T., Hayes, P.M., Vales, M.I., Kling, J., Riera-Lizarazu, O., Sato, K., Powell, W. & Waugh, R. 2003. Development and characterization of recombinant chromosome substitutions lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp. *vulgare* background. *Genome* **46**, 1010–1023.
- Morcuende, R., Kostadinova, S., Pérez, P., Martín Del Molino, I.M. & Martínez-Carrasco, R. 2004. Nitrate is a negative signal for fructan synthesis, and the fructosyltransferase-inducing trehalose inhibits nitrogen and carbon assimilation in excised barley leaves. *New Phytol.* **161**(3), 749–759.
- Osuna, D., Usadel, B., Morcuende, R., Gibon, Y., Bläsing, O., Höhne, M., Günter, M., Kamlage, B., Trethewey, R., Scheible, W-R. & Stütt, M. 2007. Temporal responses of transcripts, enzyme activities and metabolites after adding sucrose to carbon-deprived *Arabidopsis* seedlings. *Plant J.* **49**(3), 463–491.
- Pérez, P., Morcuende, R., Martín del Molino, I., Sánchez de la Puente L. & Martínez-Carrasco, R. 2001. Contrasting responses of photosynthesis and carbon metabolism to low temperatures in tall fescue and clovers. *Physiol. Plantarum* **112**(4), 478–486.
- Pilon-Smits, E., Ebskamp, M. J. M., Paul, M. J., Jeuken, M. J. W., Weisbeek, P. J. & Smeekens, S. C. M. 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiol.* **107**(1), 125–130.
- Pollock, C. J. & Cairns, A. J. 1991. Fructan metabolism in grasses and cereals. *Annu. Rev. Plant Phys.* **42**(1), 77–101.
- Schnyder, H. 1993. The role of carbohydrate storage and redistribution in the source–sink relations of wheat and barley during grain filling – a review. *New Phytol.* **123**(2), 233–245.
- Vijn, I. & Smeekens, S. 1999. Fructan: More than a reserve carbohydrate? *Plant Physiol.* **120**(2), 351–360.
- Volaire, F. & Lelièvre F. 1997. Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought in the south of France. *Aust. J. Agr. Res.* **48**, 933–944.
- Volaire, F., Thomas, H., Bertagne, N., Bourgeois, E., Gautier, M-F. & Lelièvre, F. 1998. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: II. Water status, solute accumulation, abscisic acid concentration and accumulation of dehydrin transcripts in bases of immature leaves. *New Phytol.* **140**(3), 451–460.
- Xue, G., McIntyre, C. L., Jenkins, C. L. D., Glassop, D., van Herwaarden, A. F. & Shorter, R. 2008. Molecular dissection of variation in carbohydrate metabolism related to water-soluble carbohydrate accumulation in stems of wheat. *Plant Physiol.* **146**, 441–454.