Drought effects on elongation kinetics and sugar deposition in the elongation zone of durum wheat (*Triticum durum* Desf.) leaves

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Abstract

The aim of this study was to analyze the effect of drought stress on the kinetics of leaf elongation in relationship to the variation of sugar concentrations and their net deposition rates along the elongation zone of leaf 4 of durum wheat plants. Plants were grown in soil in a naturally illuminated greenhouse, and water was withheld from seedlings for a period of 14 days. Leaf 4 of 26 day-old plants was used for growth measurements and tissue sampling. Relative elemental growth rates (REGR), cell displacement velocity (DV), and elongation zone length (EZL) were significantly reduced by drought treatment. Together, this resulted in a decrease in leaf elongation rate (LER) in drought-stressed plants. Epidermal cell length along the elongation zone was not significantly affected by drought stress, indicating that the decrease in elongation zone length was due mainly to a reduction in cell production rate. The concentration of total soluble sugars (TSS) and non-reducing sugars (NRS) was highest at the leaf base and decreased distally from 10 mm from the leaf base in plants grown under non-stressed (control) conditions. Drought stress caused a significant accumulation of TSS at the leaf base, mainly through an increase in non-reducing sugars. The continuity equation was used to calculate sugar net deposition rates. Drought stress increased the net deposition rates of non-reducing sugars in the first 10 mm from the leaf base. This increase was the principal source for the increase in non-reducing sugars concentrations at the leaf base in response to drought.

Keywords: Triticum durum; elongation zone; drought; leaf growth; sugar net deposition rates

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Introduction

Water stress is a major abiotic stress that affects plant growth and productivity world-wide. It limits crop productivity in part through reduction in leaf growth, which in turn limits whole plant photosynthetic capacity (Spollen and Nelson, 1994). Many studies have addressed the physiological and biophysical mechanisms through which growth is affected under drought. Some studies focused on cell wall properties (Hsiao and Xu, 2000) while others focused on tissue hydraulic properties (Nonami et al., 1997; Lu and Neumann, 1999) or the supply rates of

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solutes, including water soluble sugars, in growing tissue including roots (Sharp et al., 1990; Frensch, 1997; Hsiao et al., 1998).

Total soluble sugars, reducing sugars, and non-reducing sugars have been shown to accumulate significantly in wheat leaves under drought conditions (Munns and Weir, 1981; Kameli and Lösel, 1996). This increase in sugar levels was observed in both, mature and photosynthetically active leaf tissues (Kameli and Lösel, 1996; Biswal and Kohli, 2013) and in the basal elongation zone of leaves (Munns and Weir, 1981).

The elongation of the leaf blade in wheat and other grasses is restricted to the basal part of the leaf which is surrounded by the sheaths of older leaves (Kemp, 1980). In this region, called elongation zone, growth occurs through co-ordinated cell division and elongation (Schnyder and Nelson, 1988). The elongating zone is a strong sink for soluble sugars and other assimilates, which are closely associated with many metabolic and developmental processes in this region (Schnyder and Nelson, 1989; Hu et al., 2000b). Changes in the concentration of sugars and other assimilates in the elongation zone in response to drought or other environmental stresses may result from an alteration of several related mechanisms such as, relative rates of photosynthesis, translocation and utilization (polysaccharide biosynthesis and against dilution by growth-associated water uptake).

To understand the relationship between solute accumulation and growth, some physiologists have introduced the continuity equation, which is a statement of the law of mass conservation, to calculate the spatial distribution of net deposition rates of solutes in growing tissues of monocot plants (Schnyder and Nelson, 1988; Bernstein et al., 1995; Hu and Schmidhalter, 1998). This equation is used to couple growth velocity with patterns of element concentration in space and time. When the deposition rate is positive, the location is a sink for the material of interest (the material can be imported or produced by local metabolism); however, when the deposition rate is negative the location is losing material by catabolism or by export to other plant tissues (Silk and Bogeat-Triboulot, 2014).

The kinetics of growth in relationship to carbohydrate metabolism have been assessed under saline conditions for sorghum leaves (Bernstein et al., 1995) and spring wheat leaves (Hu and Schmidhalter, 1998; Hu et al., 2000a, b). To the best of our knowledge, no information is available about the effect of drought stress on the spatial distribution of sugars and their net deposition rates in relationship to growth kinetics in the elongation zone of durum wheat leaves. So, the main objective of this study was to analyse and quantify the effect of drought stress on the net deposition rate of carbohydrates and its implication on the elongation rate, in the elongation zone of leaf 4 of durum wheat (*Triticum durum* Desf.).

Materials and Methods

Growth conditions

Experiments were carried out using a durum wheat variety (*Triticum durum* L.*)* called Mohamed-Ben-Bachir (MBB), obtained from the ITGC institute (Algiers) Algeria. Seeds were sterilised with 0.5 % hypochlorite for 15 min, washed 3 times with distilled water, then soaked for 24 h and germinated in the dark on moist filter paper in Petri dishes for 48h at 25 °C. After germination, sets of 4-5 similar-sized seedlings were planted in 1l black plastic pots containing a mixture of (2:1:1) silt loam soil, sand, and compost.

The experiment was conducted in a naturally illuminated greenhouse. Average temperatures were 28 ± 2 °C day and 18 ± 2 °C night with a photoperiod of 14 h light and 10 h dark. The relative humidity (RH) was between 60% - 75%. After 12 days of growth with normal water supply, drought treatment was applied by withholding water from half of the pots, selected in a randomized manner. After 14 days from the start of drought treatment (three days after the leaf 4 emerged), elongating leaf 4 of 12-14cm length was used for growth measurements and tissue sampling. At this stage, relative water content (RWC) was 95% and 55% in control and stressed plants, respectively; leaf elongation was maximum and at the steady phase of elongation.

Growth measurements and analysis

Spatial distribution of growth was measured using the pinhole method (Schnyder and Nelson, 1988). The basal part of leaf sheaths of plants was pricked with a small device made with 15 small needles (0.2 mm diameter) spaced 3 mm apart and mounted in a piece of resin. Six to seven hours after pricking, leaves 1, 2, and 3 were removed and the pinholes displacement of the exposed fourth leaf was measured under a stereoscopic microscope. Relative Elemental Growth Rates (REGR), $mm^{-1}.mm^{-1}.h^{-1}$ or h^{-1} were calculated according to the following equation:

$REGR_i = (D_{i2} - D_{i1}) \times D_{i1}^{-1} \times (t_2 - t_1)^{-1}$

where D_{i1} represents the initial distance (mm) between neighbouring holes (i.e. 3 mm) for segment *i* at time (t_1) of making holes, and D_{i2} represents the distance between these same holes after a period ($t_2 - t_1$) of elongation.

The displacement velocity (DV, $mm.h^{-1}$) was calculated from REGR data according to (Schnyder and Nelson, 1987):

$DV = L \cdot (REGR_1 + REGR_2 + \ldots\ldots + REGR_{i-1}) + 0.5\ L \cdot REGR_i$

where L is the length of segment (i.e 3mm). The first term on the right side represents displacement due to elongation growth of all segments basal to the location of segment *i*. The second term describes displacement of the midpoint of segment *i* relative to its basal limit. DV was thus calculated for midpoints of segments.

Pricking resulted in a reduction of LER. The REGRs were corrected assuming that the reduction was uniform along the elongation zone (Schnyder et al., 1987; 1990).

Epidermal cell length determination

Leaf 4, at the steady phase of elongation, was freed from sheath of older enclosing leaves. The lowermost 30 mm of the elongating leaf 4 was placed on a thin layer of superglue spread on a microscope slide for 1-2 minutes and then carefully removed leaving epidermal cell imprints on the superglue layer. After drying, this was further divided into 2 mm long regions with a razor blade. The length of 10 epidermal cells was measured at each 2 mm from the leaf base to the end of the elongation zone (i.e., 30 mm from leaf base) using an eyepiece graticule fitted into a Zeiss microscope at a magnification of x400.

Sugar analysis

The elongation zones of leaf 4 was carefully freed from surrounding leaf sheaths, then cut with a razor blade, beginning at the leaf base which serves as the reference point and origin of the co-ordinate system, into six 5 mm-long segments. Segments of ten leaves per replicate were combined by position. After fresh weight measurements, extraction was made with 2 x 2 ml hot ethanol (80%) for 15 min.

Total soluble sugars (TSS) were determined according to the phenol – sulphuric acid method (Dubois et al., 1956), reducing sugars were measured using the alkaline ferricyanide method (Friedemann et al., 1962) and non-reducing sugars (NRS) were calculated as the difference between total sugars and reducing sugars.

Sugar deposition rates

Local net deposition rates of sugars (D, mmol Kg^{-1} H_2O h^{-1}) were calculated from data of elemental growth rates (REGR), displacement velocities (DV), and tissue substance concentrations using the one dimensional version of the continuity equation as described by (Silk, 1984; Hu and Schmidhalter, 1998).

$D = (\partial P/\partial t) + (DV \cdot (\partial P/\partial x) + (REGR \cdot P)$

where P is the substance concentration (mmol Kg^{-1} H_2O), t is the time (h) and x is the distance (mm) from the leaf base.

The first term ($\partial P/\partial t$) on the right side of the continuity equation, t, represents the local rate of change (temporal rate change in substance concentration at a fixed location from the leaf base). The second term, DV. ($\partial P/\partial x$), is the product of the DV and the spatial gradient in substance concentration and it is called the "convective rate of change". It represents the change due to the movement of cells away from the leaf base and was calculated as fellows (Schnyder and Nelson, 1987):

$(\partial P_i / \partial x_i) = 0.5 [(P_i - P_{i-1}) (x_i - x_{i-1})^{-1} + (P_{i+1} - P_i) (x_{i+1} - x_i)^{-1}]$

where P_i is the substance concentration in segment *i*, and x_i is the distance (mm) from the leaf base to the midpoint of segment *i.* For the first and the last segments, $\partial P/\partial x$ was calculated as $(P_{i+1} - P_i) . (X_{i+1} - X_i)^{-1}$ and $(P_i - P_{i-1}) . (x_i - x_{i-1})^{-1}$ respectively. The third term (REGR.P) is called "stretch rate" which represents the density to avoid dilution due to tissue expansion (Silk and Wagner, 1980).

Statistical Analysis

A randomized complete block design was adopted. Statistical significance of differences between means was determined with ANOVA two-factor (effect of drought and the location along the elongation zone) followed by Duncan's posthoc test at 0.1 and 5% significance level using the Statistica 6.1 Software package. The number of biological replicates is given in figure legends.

Results

Spatial distribution of growth and epidermal cell length

The spatial profile of REGR along the elongation zone of the developing leaf 4 of durum wheat is shown in (Fig. I.a). Leaf elongation occurred within a region extending from 0 to 27 and 0 to 21 mm from the leaf base in control and water-stressed plants, respectively. Thus, drought stress reduced the length of EZ by 6 mm. The distribution of relative elemental growth rates (REGRs) along the elongation zone was significantly (P≤0.01) affected by drought stress between 6 and 15 mm from the leaf base. The maximum REGR was reduced by 64% in water-stressed plants (0.054 h^{-1}) compared to control plants (0.15 h^{-1}). REGR was near zero at 27 and 21 mm in control and water-stressed plants, respectively,

The displacement velocity (DV) was near zero at the base and increased with distance to reach a constant value equal to LER at the distal end of the elongation zone (Fig. I.b). The DV of control plants was significantly (P≤0.01) higher than that of water-stressed plants, as was LER (control, 1.60 mm h^{-1}; drought treatment, 0.54 mm h^{-1}).

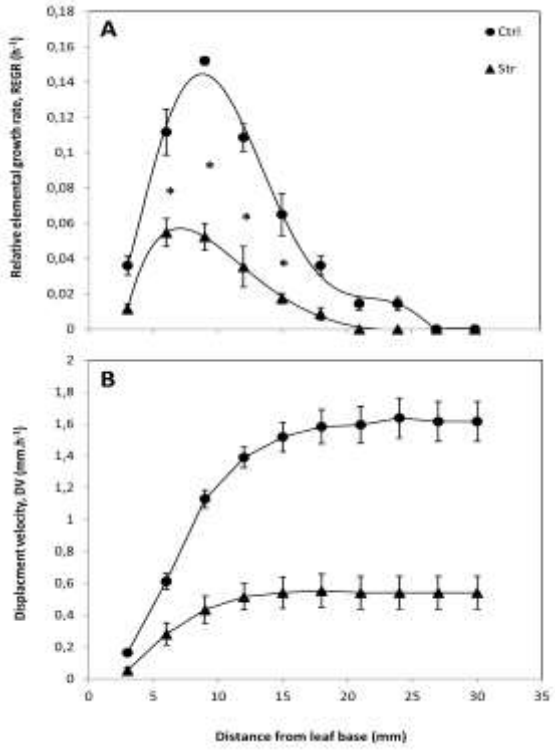

Fig. I. Drought effects on relative elemental growth rates, REGR (A), and displacement velocity DV (B) along the elongation zone of leaf 4 of durum wheat; Results are means ± SE of 10 plant analyses. Asterisks indicate significant differences between values (two-way ANOVA followed by Duncan's test at $p < 0.001$). Ctrl and Str indicate control and water-stressed plants respectively.

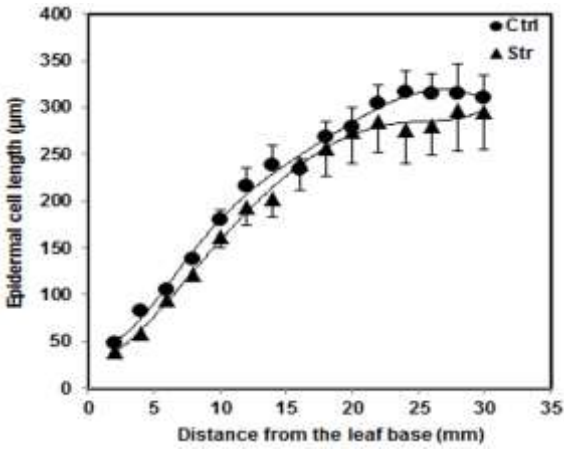

Fig. II. Spatial distribution of epidermal cell lengths within the elongation zone of leaf 4 of durum wheat in control (Ctrl) and water-stressed (Str) plants; Results are means ± SE of 10 cells from five different leaves.

The spatial distribution of epidermal cell lengths of leaf 4 during the steady phase of elongation was sigmoidal in both treatments (Fig. II). Epidermal cell lengths increased from the base to reach a maximum value of about 315 µm in

control plants and 295 µm in stressed plants. Drought stress did not significantly affect epidermal cell length along the elongation zone.

Spatial distribution of sugars

In control plants, TSS concentration was almost constant throughout the elongation zone (Fig. III.a); however, in water-stressed plants it was significantly ($p \leq 0.05$) higher at the leaf base (73 $mmol.kg^{-1}$ H_2O) and tended to decrease towards about 12 mm from the leaf base (the zone of most active elongation); distal to this location, TSS concentrations remained constant (~ 35 $mmol.kg^{-1}$ H_2O). The spatial distribution of TSS concentration was significantly ($p \leq 0.05$) affected by drought in the basal part (~ the first 10 mm from the leaf base) of the elongation zone.

In both treatments, NRS concentration was significantly ($p \leq 0.05$) higher at the leaf base compared with the remainder of elongation zone and decreased towards about 12 mm from the leaf base; beyond this location, NRS concentration remained almost constant. Drought treatment significantly ($p \leq 0.05$) increased NRS concentration in the basal portion of the elongation zone (Fig. III.b).

RS concentration was significantly ($p \leq 0.05$) lower at the leaf base compared with the remainder of elongation zone and increased with distance to reach a maximum at the end of the elongation zone in both control and water-stressed plants (Fig. III.c). RS concentration was not affected by drought conditions.

Net deposition rate of sugars

Net deposition rate of TSS ($mmol.kg^{-1}.H_2O$ h^{-1}) increased with distance from the leaf base to reach a maximum at about 7 mm and 12 mm in control and water-sressed plants, respectively, and then decreased distal to this location (Fig. IVa). No significant differences were observed between control and water-stressed plants in the basal portion (0-7 mm) of the elongation zone. Beyond this location, net deposition rates of TSS in control plants were significantly ($p \leq 0.05$) higher than in water-stressed plants. Negative deposition rates were noticed in water-stressed plants at about 19-26 mm from the leaf base.

Net deposition rates of NRS in water-

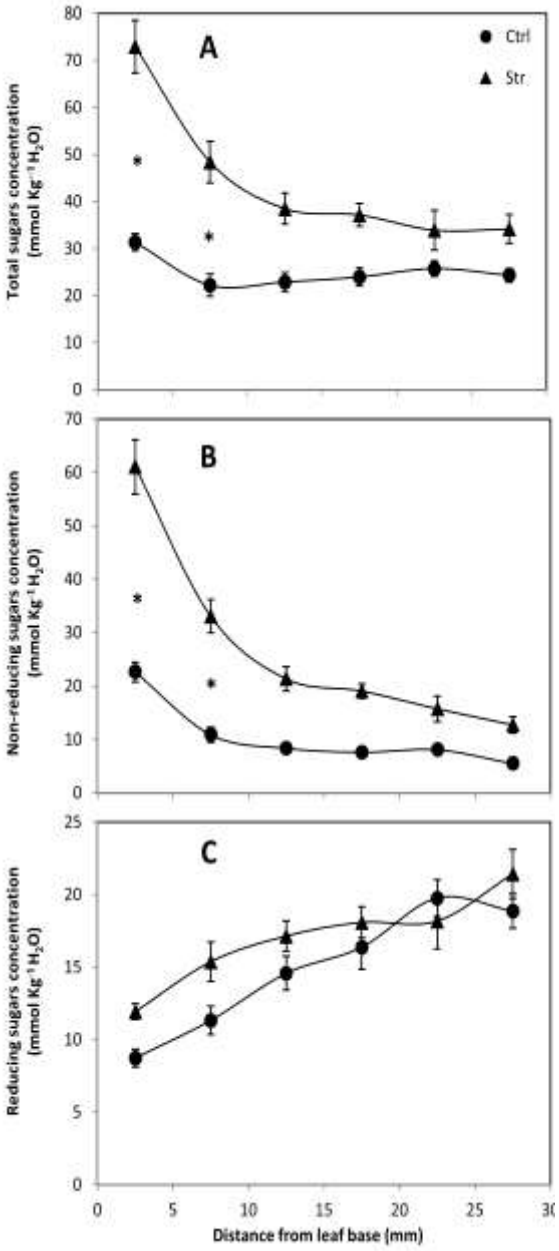

Fig. III. Distributions of total soluble sugars (A), Non-reducing sugars (B), and reducing sugars (C) concentrations along the elongation zone of leaf 4 of durum wheat; Results are means ± SE of 4 replicates of segments combined by position from 10 leaves. The statistical significance of differences between values is indicated by asterisks (two-way ANOVA followed by Duncan's test at $p \leq 0.05$). Ctrl. and Str. indicate control and water-stressed plants respectively.

stressed plants increased from the leaf base to 7.5 mm and then decreased sharply along the elongation zone (Fig. IV.b). In control plants, net deposition rates of NRS increased with distance to reach a maximum at 12.5 mm and then decreased gradually along the remaining portion of the

elongation zone. Net deposition rates of NRS between 0 and 10 mm from the leaf base were higher in water-stressed plants than in control plants; beyond this region, they were lower. These differences between treatments were not significant (p≤0.05). Negative deposition rates were noticed in water-stressed plants at about 17.5-30 mm from the leaf base.

Net deposition rates of RS increased from the leaf base to 7.5 mm and 12.5 mm in control and water-stressed plants, respectively, and then decreased throughout the elongation zone (Fig. IV.c). Net deposition rates of RS in control plants were significantly (p≤0.05) higher than those in water-stressed plants.

Discussion

Growth kinetics

The decrease in REGR and DV of water-stressed plants during the steady phase of leaf growth observed in this study (Fig. I) is consistent with those reported for wheat, maize, and Rhodes grass leaves under salt stress conditions (Hu et al., 2000a; Ortega and Taleisnik, 2003; Neves-Piestun and Bernstein, 2005; Ortega et al., 2006) and for tall fescue leaves under drought conditions (Durand et al., 1995). Leaf elongation rate (LER) is a function of the elongation zone length (EZL) and the relative elemental growth rates (REGR) (Hu et al., 2000a). Results of the present study showed a significant decrease in both REGR and EZL; therefore, the reduction of LER in response to drought stress during the steady phase of elongation was due to the decrease in both REGR and EZL. These results are in agreement with studies carried out with sorghum leaves (Bernstein et al., 1993a,b) and Rhodes grass leaves (Ortega et al., 2006) under salt stress conditions and with tall fescue leaves under drought conditions (Durand et al., 1995). However, they differ from others showing that salinity inhibited leaf elongation rates only by decreasing REGR of barley (Delane et al., 1982) and wheat leaves (Arif and Tomos, 1993; Hu et al., 2000a) during the steady phase of leaf growth.

In grasses, the length of the elongation zone depends on cell division and/or cell expansion. Thus, shortening of the elongation zone under stress conditions can result from the

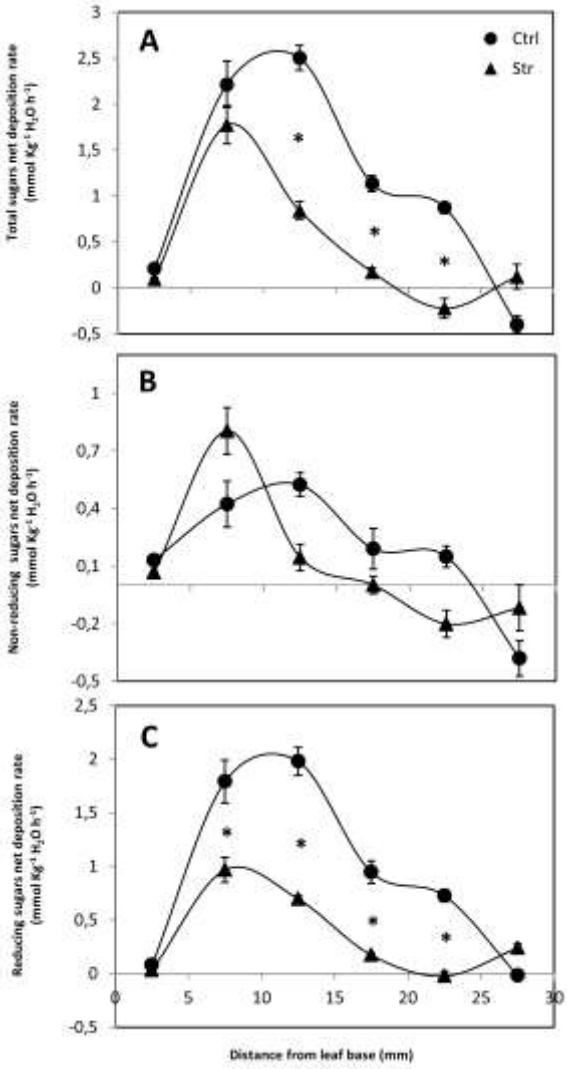

Fig. IV. Distributions of total soluble sugars (A), non-reducing sugars (B), and reducing sugars (C) net deposition rates within the elongation zone of leaf 4 of durum wheat; Results are means ± SE of 4 replicates. Statistical significance of differences between values is indicated by asterisks (two-way ANOVA followed by Duncan's test at p ≤0.05). Ctrl and Str indicate control and water-stressed plants, respectively.

reduced rates of cell production and/or final cell size (Durand et al., 1995). In the present study, the final cell size and cell size distribution along the elongation zone was not affected by water stress (Fig. II). Therefore, the decrease in elongation zone length have been caused by a reduced number of cells entering the elongation zone per unit time, due to a reduced cell production rate. Similar results were observed by Ortega and Taleisnik, (2003) and Ortega et al., (2006) in Rhodes grass leaves under salt stress conditions and Beemster and Masle (1996) in leaves of spring

wheat grown in soil with high resistance to root penetration.

The examination of displacement velocity profiles, which indicates the rate at which a given segment is pushed away relative to the leaf base due to elongation of subjacent tissue and partly to its own elongation, shows that tissue was being displaced more slowly throughout the elongation zone in water-stressed plants than in control plants. This suggests that metabolic and nutritional process had been significantly delayed by drought within the base of the leaf, as reported by Hilal et al., (1998) in soybean roots and Ortega and Taleisnik, (2003) in Rhodes grass leaves under salt stress conditions.

Spatial distribution of sugars along the elongation zone

In both treatments, our data showed that RS concentrations were low at the base of the leaf and increased along the elongation zone (Fig. III.c). Volenec and Nelson (1984) indicated that fructose and glucose were the principal components of monosaccharides in the elongation zone of tall fescue leaves which also contained myoinositol and small amounts of arabinose, mannose and galactose. Spollen and Nelson (1994) and Luscher and Nelson (1995) reported that the increase in monosaccharide concentrations from the base to the end of the elongation zone was due to an increase in the hydrolysis of non-reducing sugars (mainly sucrose and fructan). Drought conditions did not significantly affect reducing sugars along the elongation zone of durum wheat leaves in the present study. This is in agreement with results obtained for spring wheat leaves under salt stress (Hu et al., 2000b).

The spatial profiles of TSS and NRS along the elongation zone were comparable in the present study (Fig. III.a and b). This applied to control and drought-stressed plants and indicates that total soluble sugars were mostly in the form of non-reducing sugars (NRS). These results are consistent with those observed in the elongation zone of wheat leaves under salt stress (Hu and Schmidhalter, 1998; Hu et al., 2000b) and in tall fescue under drought stress (Spollen and Nelson, 1994).

Schnyder et al., (1987) reported that the fructan fraction with low molecular weight comprised most of the water soluble carbohydrates throughout the elongation zone of tall fescue leaves. However, Hu et al., (2000b) found that sucrose concentration was higher than fructan along the elongation zone of wheat leaves. Moreover, Spollen and Nelson (1994) reported that sucrose concentration increased while that of low-DP fructan decreased in the elongation zone of tall fescue leaves under water deficit which indicates that sucrose and fructan metabolism were tightly related.

Net deposition rates of sugars along the elongation zone

In the present study, drought stress caused a significant accumulation of total soluble sugars in the leaf base mainly in the form of non-reducing sugars (Fig. III). Water soluble sugars accumulation can result from two overall mechanisms, either a decrease in the rate of tissue expansion or an increase in their net rate deposition (including synthesis, catabolism, import, and utilization).

Many results suggested that the elongation zone of grass leaves is a strong sink for carbohydrates (Schnyder and Nelson, 1989: Berntein et al., 1995; Hu et al., 2000b). This is consistent with the results of the present work showing that net deposition rates of carbohydrates were high in the elongation zone in both treatments. Water soluble carbohydrates can be used either for structural component synthesis or osmotic function and growth-associated water uptake.

Net deposition rates of total sugars were not affected by water stress conditions in the base 7 mm along the elongation zone (Fig. IV.a), yet they decreased beyond 7 mm. Similar decreases were also noticed for reducing sugars along the elongation zone under drought conditions (Fig. IV.c). These decreases may reflect the effect of water stress conditions on the delay of carbohydrates metabolic processes in this region. Reducing sugars are involved in cellular polysaccharide synthesis used for new cell wall biogenesis during cell division. The shortening of the elongation zone in response to drought stress was attributed, in this work, to a decrease in cell

division in this region, and this may explain the decrease in net deposition rate of reducing sugars (i.e. mitotic activity inhibition caused by drought conditions may lead to a decrease in reducing sugars deposition rate).

Drought stress increased, though not significantly, the net deposition rate of non-reducing sugars in the basal 10 mm of the elongation zone. This increase, which can rise from an increase in fructan synthesis and sucrose import or a decrease in sucrose hydrolysis, was the principal source for the increase in non-reducing sugars at the leaf base in response to drought.

Negative net deposition rates were also observed in water-stressed plants in the regions extending from 19 to 26 mm for total soluble sugars and from 17.5 to 30 mm for non-reducing sugars. These negative deposition rates reflect an excess rate of sugar degradation over import (Hu et al., 2000b; Silk and Bogeat-Triboulot, 2014) and may be as much a consequence of lower REGR in this region as their cause, particularly in leaves of drought-stressed plants.

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