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RECOVERY OF LEAF ELONGATION DURING SHORT TERM OSMOTIC STRESS CORRELATES WITH OSMOTIC ADJUSTMENT AND CELL TURGOR RESTORATION IN DIFFERENT DURUM WHEAT CULTIVARS

MOHAMED MAHDID[1,2*], ABDELKRIM KAMELI[1], CHRISTINA EHLERT[2] AND THIERRY SIMONNEAU[2]

[1]*Département des Sciences Naturelles, Ecole Normale Supérieure, Vieux Kouba, Alger, Algeria*
[2]*Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux,*
SupAgro-INRA, 2 Place viala, Montpellier, France.
[*]*Corresponding author' e-mail: mahdid_m@yahoo.fr*

Abstract

In order to investigate the responses of leaf elongation rate (LER), turgor and osmotic adjustment (OA) during a short-term stress (7 hours) imposed by PEG_{6000} and a recovery phase, three durum wheat (*Triticum durum* L.) varieties [Inrat; MBB; and OZ] were grown in aerated nutrient solutions. Leaf elongation kinetics of leaf 3 was estimated using LVDT. Turgor was estimated using a cell pressure probe; osmotic potential as well as total sugars and potassium (K^+) concentrations were estimated from expressed sap of elongation zone. Growth recovered rapidly and then stabilised at a lower value. A significant difference was found in % recovery of LER between the varieties. The cessation of growth after stress coincided with a decrease in turgor followed by a recovery period reaching control values in MBB and Inrat. A strong correlation ($R^2 = 0.83$) between the reduction in turgor (Δ turgor) and % recovery of LER was found at 7 hours after stress. The difference in the partial recovery of LER between varieties was thus related to the capacity of partial turgor recovery. Partial turgor recovery is associated with sugar or K^+ based OA which indicates its importance in maintaining high LER values under water deficit.

Key words: Leaf elongation rate; Recovery; Osmotic adjustment; Turgor; Durum wheat.

Introduction

A rapid osmotic or drought stress imposed to plants using salt or osmotica in medium solution influences plants in two sequential phases (Munns, 2002). A first phase that extent to up to several hours to about 1 day is followed by a second phase encompassing days to weeks. In the first phase, an osmotically induced water stress is imposed on plants by the lowered availability of water in the external solution. The growth reduction is very rapid and is followed by a recovery. In a second phase, longer term physiological adjustments take place.

Rapid and transient reductions in leaf expansion rates after a sudden increase in salinity have been recorded in many species including wheat and barley (Passioura & Munns, 2000; Munns, 2002). The same changes occur when KCl, mannitol or PEG are applied (Yeo *et al*., 1991; Chazen *et al*., 1995), showing that the responses are not salt-specific. Generally, inhibition of shoot growth is more pronounced than that of root growth (Rodriguez *et al*., 1997; Munns, 2002).

The control of leaf elongation in plants growing under water and salt stress remains a subject of extensive debate. Some authors have emphasized the role of cell turgor or leaf water status in determining leaf elongation rates (Frensch, 1997; Hsiao *et al*., 1998; Tang & Boyer, 2002), while others have emphasized the cell wall extensibility (Nonami & Boyer, 1990b; Cramer & Bowman, 1991; Neumann *et al*., 1994), or signals from roots such a ABA (Passioura, 1988; Gowing *et al*., 1990; Davies & Zhang, 1991). The debate has been sustained by a number of apparently contradictory findings on the extent to which leaf growth is controlled by leaf water status (Munns *et al*., 2000).

In order to determine the role of water status in expansion growth under environmental stress, Passioura & Munns (2000) found (using the pressurization technique) that the transient changes in leaf elongation rate (LER) disappeared in plants maintained at maximum water status (fully turgid) throughout sudden environmental changes, indicating that water relations were responsible for the sudden changes under saline and water stress conditions. These findings emphasized the role of cell turgor or tissue water status in determining leaf growth rates. However, it is not possible from those results, to confirm if pressurization has the same effect as cell turgor. It is also not clear if the lower rate of growth rate after recovery in a short-term stress is related to partial (insufficient) recovery of turgor or to other factors.

Two previous studies reported single-cell turgor measurements on NaCl-stressed cereal leaves of barley (Thiel *et al*., 1988) and wheat (Arif & Tomos, 1993). However, these studies focused on transient turgor changes in response to the addition of NaCl to the nutrient solution. We come across no reports studying the changes in turgor during recovery phase between different varieties.

In order to explain turgor recovery, measurements of osmotic potential and solute concentrations were made under water deficit, since most of the previous studies on the responses of LER on a short time scale, were made under NaCl in which Na^+ and Cl^- were available externally (Fricke, 2004).

Several studies carried out at tissue and cell level, concluded the importance of osmotic adjustment as a mechanism by which plants lower their osmotic potential allowing the maintenance of turgor at lower water status during drought on a long time-scale. However, the information available on the role of osmotic adjustment on a short time-scale (phase one) and in the recovery phase of turgor after saline stress and particularly, under water deficit is scarce. Fricke & Peters (2002) concluded that leaf cell elongation in NaCl-treated barley was probably limited by the rate at which solutes can be taken up to generate turgor, particularly at high NaCl levels where external osmolytes contribute strongly to osmotic

adjustment of tissues. This is not the case under water deficit. It is important to follow the changes of osmotic potential and accumulation in solutes under water deficit on a short term where no supply occurred from the external medium as it happens under salinity.

This work attempts to investigate the contribution of accumulated solutes (Sugars and K^+) in rapid osmotic adjustment and also attempts to clarify the relationship between shoot turgor and LER by following the variations in LER and turgor between varieties of wheat under short term water deficit (during the recovery of growth).

Materials and Methods

Plant material and growth conditions: The experiments were conducted using 3 durum wheat (*Triticum durum* L.) varieties: Inrat, Mohamed-Ben-Bachir (MBB), and Oued-Zenati (OZ), obtained from the ITGC institute (Algiers) and selected on the basis of growth analysis and differential responses to water stress. MBB is considered as a more tolerant variety compared to Inrat which is regarded as less tolerant on long term basis, while OZ is seen as intermediate in drought tolerance among the three varieties (Meziani *et al*., 1992). The seeds were surface sterilized with 0.5% NaOCl for 15 min, washed 3 times with distilled water then germinated on soaked filter paper in Petri dishes. After 6 days, seedlings of similar sizes were transferred to aerated nutrient solutions in 10 litres plastic containers under controlled environmental conditions. With a photoperiod of 14 h at 400 µmol PAR, 24/20c° day/night temperature, a RH air: 65%, and VPD: between 0.8-1. The hydroponics' solution was continuously aerated using air pump. Osmotic stress was induced using PEG 6000 to an osmotic potential of – 0.5 MPa.

The diluted nutrient solution contained: [$CaSO_4.2H_2O$: 0.5; KNO_3: 0.8; KH_2PO_4: 0.3; $MgSO_4.7H_2O$: 0.2; NH_4NO_3: 0.4; Fe-EDTA: 0.02; H_3BO_3: 0.008; $MnSO_4.H_2O$: 1 10^{-3}; $Na_2MoO_4.2H_2O$: 0.1 10^{-3}; $ZnSO_4.7H_2O$: 0.2 10^{-3}; $CuSO_4.5H_2O$: 0.2 10^{-3}]mM. The pH maintained between 5.5 - 5.8. The solution was renewed every 4 days.

LER was continuously measured on leaf 3 when it reached 6-8 cm length. Using linear variable differential transducers (LVDTs), (L100, Chauvin Arnoux, Paris, France) attached with a linen thread to the tip of the growing leaf. The LVDTs were connected to a data logger (CR10, Campbell Scientific Ltd), and data were stored every minute. All measurements and PEG addition were made in the morning approximately 4 hours into the photoperiod, after growth patterns had reached a steady state value.

Extraction and physiological measurements: The growing leaf three was disclosed, the location of the elongation zone (EZ) of the growing leaf and the exact distance of growth zone was found to be 3 cm long from leaf base (Hu *et al*., 2000) it was verified by measuring displacement rates along the leaf axis by the pricking method (Schnyder *et al*., 1987). Leaf tissue of EZ was quickly cut into small segments put into microtubes containing a small plastic sieve, sealed and quickly plunged into liquid nitrogen. Samples were thawed and then spun for 10 min. at 10.000 t/min. in the centrifuge. Samples (about 20 µL) were collected and stored in freezer (-20°C) until their analysis.

The osmotic potential was measured on the expressed sap using vapor pressure osmometer VAPRO 5520, after calibration with standards solution of NaCl of known osmotic potential, reading are expressed in m mol/kg. To convert to pressure units it was assumed that 40 mmol/kg water = - 0.1 MPa.

The relative water content (RWC) was determined according to the formula:

$$RWC = \frac{(FW-DW)}{(TW-DW)} \times 100$$

where: FW= fresh weight; DW= dry weight; TW = turgid weight

Osmotic pressure at full turgor (π 100) was calculated as (π (RWC-10))/90, with the assumption that tissue contains 10% bound water, and that it does not change with stress (Wilson *et al*., 1980). The application of this equation to obtain an idea of the component of changes in osmotic potential, that is due to solute accumulation rather than cell shrinkage.

Turgor was measured using Cell-pressure probe technique in epidermal cells of the leaf 3 elongating zone. The plant was kept in nutrient solution, placed vertically and attached by a plastic ribbon on plate plastic bar. To gain access to the growth zone of the third leaf, the coleoptiles, first and second leaves were separated. The newly exposed (uncovered) zone of third leaf was covered with thin film of Vaseline. Turgor was measured by cell impalement using a cell pressure probe made of borosilicate microcapillaris (Harvard Apparatus Ltd., Edenbridge, UK) which were sharpened and beveled to obtain a tip diameter of 6 µm and filled with silicon oil (type AS4, Wacker, Munich, Germany). The plant was placed in front of the pressure probe and a video microscope system (Leica, Buffalo, NY, USA) was used to adjust the microcapillary in front of the growth zone of leaf 3. Cell impalement resulted in the immediate appearance of oil/cell sap meniscus in the capillary. Adjusting the pressure in the microcapillary, the meniscus was brought back to its initial position and the required pressure value was read on a digital pressure indicator (DPI 260, Druck, Leicester, UK). Readings were repeated several times for one cell by moving the meniscus forwards and backwards.

Two controls were used in osmotic potential and turgor measurements, 0 hour control (before the stress), and 7 hours control at the end of the stress period. The 0 hour control was used to compare samples taken between 30 min and 4 hours, 7 hours control was used only to compare samples taken at 7 hours.

Biochemical measurements: Total carbohydrates were measured using the phenol sulfuric acid according to the method of Dubois *et al*., (1956).

Glucose, fructose and sucrose were estimated after enzymatic transformation to NADH, the latter is estimated at 340 nm on microplates. The transformation of glucose was obtained using Hexokinase (HK) to yield G-6-P which is then transformed using another enzyme glucose-6-phosphate dehydrogenase (G6PDH) to yield NADH. Fructose transformation requires a third enzyme Phospho Gluco Isomerase (PGI) which transforms Fructose-6-phosphate (F6P) to Glucose-6-phosphate (G6P). Sucrose is first hydrolysed using β-fructosidase to yield Glucose and fructose, which are then, estimated using the previously described methods.

The potassium concentration in leaf sap was measured by a flame photometer (SPECTRAA 220fs Varian) after a calibration with the standard solutions of KCl. Biochemical measurements were made using the expressed sap.

Statistical analysis: ANOVA single factor between groups was calculated by excel. The differences were considered significant at a probability level of $p \leq 0.05$.

T tests was performed using R software (allowing for differences in variance) in order to test for differences between any two groups.

Results

Leaf elongation rate: The kinetics of leaf elongation before and after stress indicated the presence of 4 growth phases (Fig. 1): before stress (1), rapid drop of LER down to zero (2), recovery phase (3) and steady state phase (4).

The parameters measured during the 4 phases are presented in table 1 for the 3 cultivars. LER was higher in OZ and Inrat compared to MBB before the addition of PEG. In each cultivar, Leaf elongation ceased within 1-2 min after the imposition of PEG. This period of no growth lasted for few minutes. LER recovered rapidly for a period between 105-130 min, with no significant differences between the varieties. The LER partly recovered, the % of recovery was dependant upon the varieties (significant difference). With MBB showing the highest recovery reached (72.7%) and OZ the lowest by 37.9% (Table 1).

In order to explain this cessation of growth after the osmotic shock and the growth recovery, the measurement of some parameters of water status such as osmotic potentials and turgor were made.

Osmotic potential and solute accumulation: Osmotic potential at full turgor π 100) showed different pattern of decrease following stress in the 3 varieties; OZ showed no net decrease in π 100 except a slight decrease 30 min after stress. Inrat showed minor decreases 2 and 7 h. By contrast, MBB showed consistently lower π 100 from 1 h onward, reaching –1.5 MPa as compared to –1.0 Mpa in the control and in the other varieties. (Fig. 2). Values of OA (difference between stressed and control plants) at the end of stress period reached -0.45 and -0.18 in MBB and Inrat respectively with no OA recorded in OZ.

An accumulation of total soluble sugars was recorded in EZ during the stress period; MBB showed the highest accumulation, followed by Inrat, the lowest accumulation was recorded in OZ (Fig. 3). The estimation of individual sugar concentrations using the enzymatic method revealed an increase in glucose and fructose in all varieties after the imposition of stress. Little accumulation of sucrose was recorded in Inrat & OZ (Table 2). Inrat showed a transient increase in K^+ during stress, whereas MBB showed a late accumulation at the end of stress period. In OZ however, the concentration of K^+ decreased during the stress period (Fig. 4).

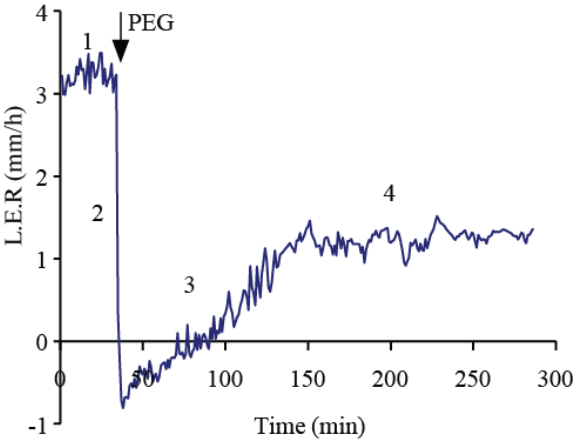

Fig. 1. Leaf Elongation Rate kinetics measured by LVDT on the growing third wheat leaf (*Triticum durum* L.) as affected by rapid water deficit imposed by adding PEG 6000 to the hydroponics solution, 1: LER before stress phase; 2: drop of LER down to zero; 3: recovery phase and 4: steady state phase. (This graph was obtained using the variety OZ).

Table 1. Leaf elongation rate parameters of the growing third leaf in 3 varieties of wheat before and following the addition of PEG (during 7 hours), values are means of six replicates ± SE.

Varieties	LER Parameters			
	LER before stress (mm/h)	LER after stress (mm/h)	Time of recovery phase (min)	% Recovery of LER at 7h
Inrat	3.37 ± 0.17 a	1.89 ± 0.09 a	131.88 ± 22.12 a	55.92 ± 2.24 a
MBB	2.26 ± 0.09 b	1.63 ± 0.08 b	132.64 ± 14.8 a	72.72 ± 3.79 b
OZ	3.07 ± 0.11 a	1.17 ± 0.10 c	105 ± 36.23 a	37.88 ± 1.8 c
ANOVA	***	***	ns	***

ns, Non-significant ; ***, Significant at p≤0,001. Values followed by letters are statistically different at p≤0.05 with R software test

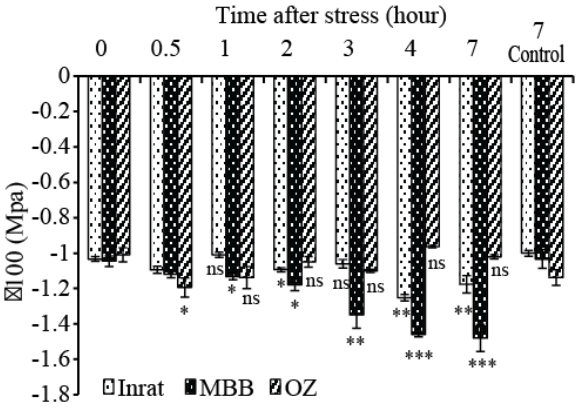

Fig. 2. Changes in osmotic potential at full turgor of bulk-tissue in EZ of growing leaf three of wheat in 3 varieties, before and following the addition of PEG to the root medium.
Values are the mean of 4 replicates ± SE (ns, non-significant; *, p≤ 0.05; **, p≤0.01; ***, p≤ 0.001, compared to time 0).

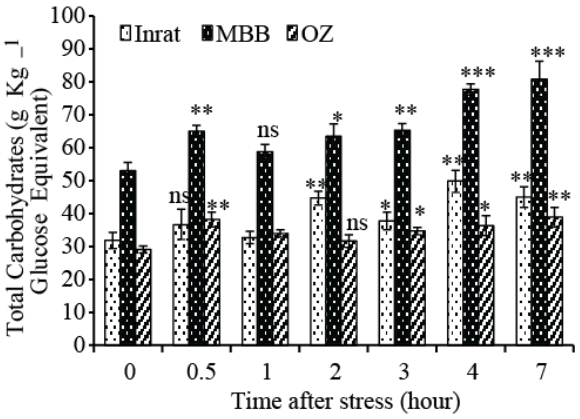

Fig. 3. Changes in total carbohydrates measured from the sap of bulk-tissue in EZ of growing leaf three of wheat in 3 varieties before and following the addition of PEG to the root medium.
Values are the mean of 4 replicates ± SE (ns, non-significant; *, p≤0.05; **, p≤ 0.01; ***, p≤ 0.001, compared to time 0).

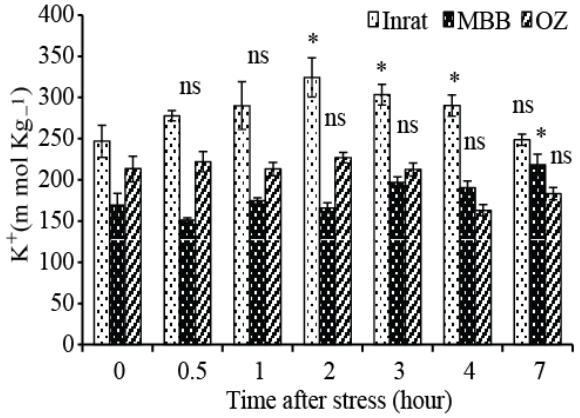

Fig. 4. Changes in K^+ measured from the sap of bulk-tissue in EZ of growing leaf three of wheat in 3 varieties, before and following the addition of PEG to the root medium.
Values are the mean of 4 replicates ± SE (ns, non-significant; *, p≤0.05; **, p≤0.01; ***, p≤ 0.001, compared to time 0).

Discussion

LER recovery under rapid water deficit: These results indicated that the times of zero growth (few minutes) and recovery (about 2 hours) may be sufficient for changes in the water status of elongating tissue leading to growth recovery in the varieties studied.

Growth did not recover to pre-stress levels, especially under conditions where high concentrations of salt or osmotica are present in the root medium.

Significant differences between the varieties were found in the extent of % LER recovery at 2 hours, in contrast to the differences in the time of recovery which were not significant. These differences in the % recovery of LER may not necessarily indicate differences in water stress tolerance between the varieties at long term.

The question of whether long term responses are different from short term responses is an important point to be raise. On the long time scale other and more complex responses such as morphological and anatomical may be involved.

These differences in tolerance are probably due to the genetic variability between these varieties. The results also indicate the possibility of using growth recovery on the short term (phase one) as a criterion for selecting varieties for drought tolerance. The genetic selection programs remain a subject of extensive research (Mirbahar *et al*., 2009; Kara & Zoghmar, 2011)**.**

The results of this study also indicated the presence of two types of genotypes differing in the extent of recovery LER to water stress; early and late physiological response genotypes.

Osmotic potential: The cells adjusted osmotically, which indicates the need of elongating cells to generate and maintain turgor. Under NaCl on the short term, Rodriguez *et al*., (1997), Fricke (2004) found a decrease in osmotic potential in roots and leaves of a number plant species.

The rapid decrease of osmotic potential 30 min after stress preceded the start of growth resumption which occurred at about 1 hour after stress. The continued decrease of osmotic potential after 1 hour resulted in higher magnitude of OA which could be the cause of high % growth recovery.

Under saline conditions, Fricke & Peters (2002) concluded that salt inorganic solutes from medium contribute strongly to OA of tissues. The situation is different under water stress, which suggests the possibility of mobilisation and fluxes of endogenous solutes to growing zones across the plasma membrane. Evidence for this has been found in growing tissues (Van Volkenburgh, 1999). In this study, the correlation between the differences in the capacity of OA and growth recovery found between the 3 varieties indicates the importance of OA in the recovery phase of LER even in the short term, under conditions of water deficit. These differences in OA between the varieties could be due to genetic variability. The osmotic components and properties associated with genotypic differences in OA, was reported in many studies for some time such as those of Morgan (1991), Morgan & Tan (1996) on wheat.

Recently, complex genetic control for OA has also been supported by QTL analysis in a population of sunflower plants in field conditions, by the identification of genomic regions associated with water status traits and OA under water-stressed conditions (Poormohammad Kiani *et al*., 2007). We suggest that a better understanding of this control at physiological level may help in breeding plants for arid and semi-arid conditions.

Table 2. Concentrations of glucose, fructose and sucrose (m mol kg^{-1}) in elongation zone measured from the sap of leaf tissue at 0 and 7h, followed by the difference in concentrations between the two times.

Varieties	Sugars	0h	7h		
	Glu	61.94 ± 8.23	79.85 ± 5.25	17.91	*
Inrat	Fruc	26.4 ± 3.26	38.8 ± 2.38	12.4	***
	Suc	5.67 ± 1.59	4.8 ± 0.25	–	ns
	Glu	114.89 ± 2.62	158.36 ± 6.55	43.47	***
MBB	Fruc	33.19 ± 2.31	51.81 ± 4.16	18.62	**
	Suc	6.63 ±1.29	11.96 ± 1.32	5.33	**
	Glu	79.27 ± 2.22	106.34 ± 3.01	27.07	***
OZ	Fruc	19.36 ± 1.60	29.15 ± 2.66	9.79	**
	Suc	2.65 ± 0.72	6.2 ± 1.68	3.55	*

Values are the means of 4 replicates ± SE (ns, non-significant; *, p≤0.05; **, p≤0.01; ***, p≤0.001)

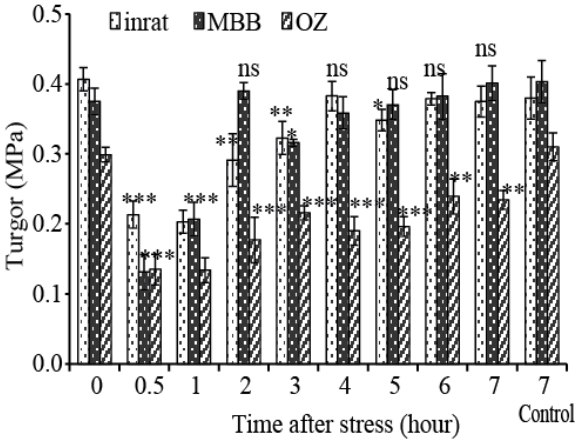

Fig. 5. Changes in turgor pressure measured by pressure probe in EZ of growing leaf three in 3 varieties of wheat, before and following the addition of PEG to the root medium.
Values are the mean of 6 replicates ± SE (ns, non-significant; *, p≤0.05; **, p≤0.01; ***, p≤0.001, compared to time 0).

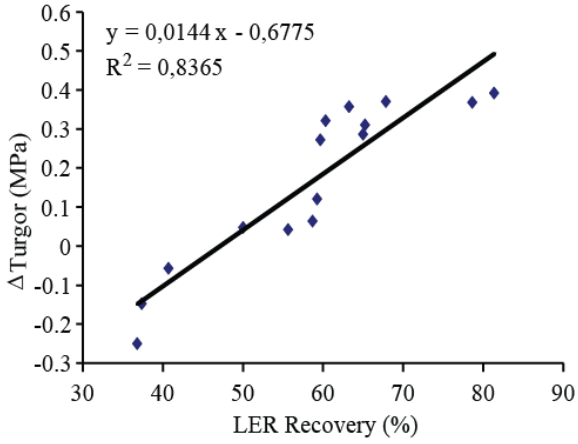

Fig. 6. The Correlation between turgor reduction (Δ turgor) and LER recovery (%) of growing leaf three at seventh hour after stress in 3 varieties of wheat. Each value is the mean of four replicates.

Turgor: Measurements of turgor on single cells in the EZ indicated a rapid decrease in turgor 30 min after stress, followed by a recovery period reaching pre stress values in MBB and Inrat. But in OZ, turgor values failed to return to controls at the end of the stress period (Fig. 5). The percentage recovery of LER was plotted against the reduction in turgor or Δ turgor at 7 hours of stress.

$$\Delta\text{Turgor} = \frac{\text{Final Turgor - Initial Turgor}}{\text{Initial Turgor}}$$

The results (Fig. 6) showed a good correlation between the two parameters during steady phase at the end of the stress period (R^2 = 83.65 %).

Turgor and % recovery of LER: The decrease in turgor noticed after the addition of PEG is probably due to the disappearance of water potential gradient between xylem and nearby tissue due to the decrease in xylem water potential. Therefore, this decrease will eventually results in lower water flow and lead to lower turgor, as also concluded by Tang & Boyer (2002).

The decrease of turgor after the addition of PEG was followed by a period of turgor recovery. Differences in the degree of turgor recovery were found between varieties. The importance of magnitude of turgor in growth recovery was noticed especially in MBB to a lesser extent in Inrat, while OZ showed less recovery of turgor which remained below the control values at the end of stress period. This recovery of turgor is probably due to OA of tissues described previously.

The results indicated an important correlation between the extent of turgor and LER recovery of these varieties. In another study (Bouchabké, 2003), LER was found to differ in the growing tissue of 5 lines of maize and turgor was not maintained under long term water deficit. These different turgor responses in these lines were mainly due to genetic variability. The main point which should be emphasized here is the similarity between the kinetics of both LER and turgor variations during cessation of elongation and during the recovery phase, with the exception of the steady state phase at end period of stress.

The role of cell turgor in controlling of leaf growth under water stress has been debated for a long time. Matsuda & Riazi (1981) observed that bulk-leaf turgor was largely unaffected in the basal leaf zone of osmotically stressed barley seedlings. Michelena & Boyer (1982) reached the same conclusion in maize. Termaat *et al*., (1985) concluded that turgor and therefore, the processes generating turgor, were not limiting shoot growth. Although turgor is necessary for growth, it could not entirely account for the decrease in leaf growth, which indicates that turgor is not the only factor regulating shoot growth.

It is not clear from the results why growth failed to return to pre stress values despite full turgor recovery. One explanation is the involvement of other factors such as cell wall extensibility. It is well known that cell turgor, hence favourable water status of plants is important to expansion growth. However, the role of turgor in the recovery phase is also an important question to be raised. Turgor was shown to recover in the growing leaf tissue a short time after applying stress due to OA.

Pressurization technique was used to cause step increases in (presumably) the turgor of growing cells, allowed to prevent the stop and decrease of LER several hours after sudden changes in the root medium, indicating that water relations are responsible for the sudden changes in LER, but failed to prevent an inhibition of leaf growth under salt stress over a time scales of 24 hours (Passioura & Munns, 2000). The question here, is whether the applied pressure exceeded the value of recovered turgor pressure indicated previously in our results? From the Lockhart (1965) equation, the excess pressure applied affected cell wall extensibility within hours which compensate for decreased extensibility found on the long term stress (more than 24 h). Cell wall extensibility decreases with increasing time exposure to stress. Direct measurements of cell-wall extensibility in growing tissues of intact plants have confirmed evidence of cell-wall hardening with the time in soybean and maize in response to water deficits or salinity (Nonami & Boyer, 1990a; Cramer & Bowman, 1991; Neumann *et al*., 1994).

Early changes in wall extensibility became large only about 5 to 10 h after transplanting the seedlings to vermiculite of low water content, and reached their lowest levels at about 40 h, the metabolic changes leading to altered cell walls and membranes required some time to be set in place (Nonami & Boyer, 1990a). Inhibition of cell expansion could then be associated with a hardening of the expanding cell walls rather than an inability to maintain turgor pressure.

Osmotic adjustment and solute accumulation: Solute were found to accumulate few hours after applying stress which indicates the importance of these solute (especially sugars) in OA in the growing leaf tissue.

The phenol sulfuric method of total carbohydrates does not allow the determination the DP of the accumulated carbohydrates and therefore their contributions to OA. The enzymatic method used for glucose, glucose and sucrose however, allowed the estimation of the contribution of these common carbohydrates to OA.

The early accumulation of total carbohydrates at 2 hours contributes significantly to the decrease in osmotic potential (OA), which reached between 38 and 50 % of OA at 7 hours.The extent of total carbohydrates accumulation showed similar pattern in all varieties i.e MBB showed the highest accumulation followed by Inrat and less in OZ which showed the least level of accumulation. This pattern was in agreement with the changes in OA. The same case was detected but on a longer term in growing leaves of wheat (Kameli & Lösel, 1995).

The comparison of growth reduction after stress with the level of total sugar accumulation reveal no clear correlation on a short term stress (hours), which may indicate that sugar accumulation is not a direct consequence of growth reduction and may be related to a complex process related to OA.

Slight accumulation of potassium (K^+) was found, despite its minor concentration in the nutrient solution. This may be related to the presence of small vacuoles occupying a limited volume of the cells in the growing tissue. This increase in K^+ concentration occurred transiently in Inrat and later in MBB during the stress period. The contribution of K^+ to osmotic potential was small at the end of stress except in MBB (about 29%). Previous studies on potassium during long term stress conditions showed a little accumulation in growing leaves of durum wheat (Kameli & Lösel, 1995). But on the other hand, a marked reduction was recorded in shoot bread wheat of tolerant genotypes under stress (Munns *et al*., 1979; Mujtaba *et al*., 2007). Recently, the contribution of K^+ to osmotic adjustment has been shown to be high with K^+- soil fertilisation on long term in young wheat leaves under water stress (Damon *et al*., 2011). According to Fricke *et al*. (2006); membrane potential is both a driving force and possible regulator, and is a consequence of trans-membrane solute transport and permeation. This may suggest that recovery in membrane potential coincides with growth recovery and precedes solute accumulation at osmotically significant levels.

It can be concluded from the results of this study that the differences observed in the % recovery of LER are at least partly due to plant water status. The results also suggested that OA was a rapid response and that this adjustment resulted in turgor recovery under a short term water deficit induced by PEG.

The OA occurred using mainly organic solutes 'sugars' in contrast to studies under salinity, where rapid OA was accounted for mainly by salt ions from the external medium. Finally, the results of this study indicated the role of turgor recovery in leaf elongation recovery; however, full turgor recovery alone does not necessarily result in full growth recovery which suggested that other factors such as cell wall extensibility changes may also be rapid and play a major role in expansion growth recovery under short term stress.

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