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Rapid osmotic adjustment in leaf elongation zone during polyethylene glycol application: Evaluation of the imbalance between assimilation and utilization of carbohydrates

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ABSTRACT

This work aims to study rapid osmotic adjustment, in order to simplify and follow this mechanism with the changes of various sugars and potassium in parallel with growth and photosynthesis measurements. Four durum wheat varieties of *Triticum turgidum* subsp. *durum* L. designated as: "Inrat-var", "MBB-var", "OZ-var", and "Waha-var" were growing in continuously aerated-nutrient-solutions. The kinetic of the leaf elongation for leaf 3 were measured by the linear variable differential transducer (LVDT); osmotic potential (OP), carbohydrates, and potassium (K^+) concentrations. Growth recovered rapidly after a sudden full after stress. A considerable difference was noted in the recuperation (%) of the leaf elongation rate (LER) under the four varieties. The variation in the partial recuperation of LER was, therefore, associated with the aptitude of osmotic adjustment in the elongation zone (EZ) of the leaf growth. Osmotic adjustment is associated with soluble sugars and potassium accumulation. Compared to the accumulation of these solutes in the mature zone (MZ) and the photosynthesis process, several ideas related to this accumulation mechanism can be assumed among them, this accumulation process cannot be considered as a direct result of the reduction of the growth. Rather, it might be as the result of their mobilization from the MZ of growing leaf or other expanded leaves. The accumulation of these sugars probably is uncoupled with direct photosynthesis activity.

Keywords: Leaf elongation rate, Recovery, Rapid osmotic adjustment, linear variable differential transducer, Photosynthesis, *Triticum turgidum* subsp. *durum* L.

1. Introduction

The osmotic adjustment may supposedly be favorable to survive below extreme aridity and desiccation, although at the same time, they approved that it can endorse improved soil moisture capture leading to enhanced recovery below lack stress. In reality, plant turgor repairs due to the osmotic adjustment in roots or shoots and its improvement of root-growth and soil dampness extraction has been frequently described (Kusaka *et al.,* 2004; Velázquez-Márquez *et al.,* 2015). The purpose of the study of rapid osmotic adjustment (within hours) is to simplify and follow its mechanism with other physiological and biochemical variables. A fast osmotic adjustment or water stress compulsory on plants by sodium chloride (NaCl) or polyethylene glycol (PEG) in medium begets for certain vegetation a rapid osmotic adjustment (Fricke and Peters, 2002; Fricke, 2004; Mahdid *et al.,* 2014). The osmotic adjustment mechanism occurs after stress application is speedily within 1-2 h; however, the growth recovery is very rapid too. Studying osmotic adjustment by the side of this stage might limit and make simpler the presence or absence of the hydraulic factors implicated in the plant responses following the osmotic stress. The later behavior may possibly guide to an enhanced understanding of these responses. In contrast, in long term, more complicated properties such as anatomical and morphological proprieties may interfere and intervene with physiological responses. This may complicate any evaluation of the growth diminution and its relation to osmotic adjustment. Through previous reports, the relationship between osmotic adjustment and turgor recovery, in synchronization with the restoration of growth stay evident, despite that growth in the short term, did not regain its full rate before the intervention of the stress.

During of the rapid saline stress (short term) case, the inorganic salt solutes of medium contributed robustly to accelerate the osmotic adjustment of tissues (Fricke, 2004) and at the long term (Chen *et al.,* 2019)**.** For the rapid water stress, the accumulation of sugars was noted, and little accumulation of potassium (K^+) was found compared to sugars (Mahdid *et al.,* 2014). During the stress, the concentration of K^+ increases in some varieties for a short period. Thereafter, it remains minor except for the MBB-var (~29%) which we are interested in to follow the accumulation and the source of contribution of soluble sugars. So, many questions have been raised about the source of accumulated solutes in the EZ compared with the MZ. If it is as a result of deviation of these sugars from the

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growth process! Not using and not generating organic derivatives used for growth, or these accumulations as result of reduced growth after rapid stress؟

The current study tries to look into the varied involvement of sugars in a hasty osmotic adjustment of 4 varieties under osmotic stress. The greater part of previous works has not tried to scrutinize the relationship betwixt solutes accumulation and the decrease in growth following rapid stress, if this accumulation is a consequence of passive accumulation after growth inhibition. The work attempts to evaluate the balance between the assimilation of soluble carbohydrates; if the accumulation of carbohydrates for the first little hours of the water stress is compliant to the calculated reduction of growth. Consequently, it is imperative to control the relationship betwixt carbohydrates and photosynthetic yields and to reply to the question of whether an osmotic correction is an effect of accretion or translocation of photosynthates or an active accumulation method, e.g. a solutes mobilization or organic reserves utilize.

2. Materials and Methods

2.1. Growth conditions and rapid plant stress

Experiments were performed with 4 varieties of *Triticum turgidum* subsp. *durum* L.: Inrat-var, Mohamed Ben Bachir (MBB-var), Oued Zenati (OZ-var), and Waha-var, gained from the "Institut Technique des Grandes Cultures" (ITGC) in Algiers (Algeria) and chosen according to their growth analysis and degree of difference responses against water stress (Meziani *et al.*, 1992). Seeds were surface sterilized by NaCl (0.5%) for 15 min, washed three-folds with bidistilled water, and germinated on soaked-up filter paper in Petri dishes. After six-days, seedlings of like sizes were planted in ten-liter enclosed plastic basins including nutrient solutions. After that, plants were developed in a growth chamber, with a photoperiod of 14 h at photosynthesis photon flux density (PPFD) of 400 µmol/m^2/s and day/night temperature of 24/20°C as well as a vapor pressure deficit of 0.8-1 kPa as previously reported by the authors (Mahdid *et al.*, 2011).

The diluted nutrient solution, at pH 5.5, contained (in mM): calcium sulfate dihydrate: 0.5; potassium nitrate: 0.8; monopotassium phosphate: 0.3; magnesium sulfate heptahydrate: 0.2; ammonium nitrate: 0.4; Ferric-EDTA: 0.02; boric acid: 0.008; manganese sulfate monohydrate: 1×10^{-3}; sodium molybdate dihydrate: 0.1×10^{-3}; zinc sulfate heptahydrate: 0.2×10^{-3}; and copper(II) sulfate pentahydrate: 0.2×10^{-3}, was renewed periodically each four days. The measurement of the leaf elongation yield (LER) by means of LVDTs, in different conditions and PEG concentration was monitored as reported by the authors (Mahdid *et al.*, 2011).

2.2. Physiological ascertainment

The growth of the third leaf was released; the location of the EZ and the accurate distance of enlargement zone were established as reported elsewhere (Hu *et al.*, 2000). Then, it was confirmed by calculating the dislocation yields the length of the leaf axis with the stick in way (Schnyder *et al.*, 1987). The MZ is the rest of the leaf. The tissues were rapidly cut to little pieces set into microtubes having small plastic strainer, preserved, and speedily plunged into liquid nitrogen. The samples were defrosted and then spun in the centrifuge (10,000 rpm for 10 min). 20 µL of samples were collected and kept at -20 °C waiting for further experimental studies.

The potential of the osmotic adjustment was calculated and the relative water content (RWC) was ascertained as follows:

$$RWC = [(FW - DW) / (TW - DW)] \times 100 \quad \text{(Eq. 1)}$$

Where: FW, DW, and TW represent the fresh weight, the dry weight, and the turgid weight, respectively.

The osmotic potential or pressure (OP) at full plant turgor (π100) was calculated according to Wilson *et al.*, (1980) as follows:

$$OP = [\pi(RWC - 10)] / 90 \quad \text{(Eq. 2)}$$

The net photosynthetic rate (NPR) was calculated by a unique leaf chamber intended for durum wheat and linked to the Gas Analyzers from PP Systems (MA, USA) on four to six individual plants.

2.3. Biochemical determination

The total carbohydrates in a sample were estimated by the rapid colorimetric method of phenol-sulfuric-acid (Dubois *et al.*, 1956). The content of some carbohydrates e.g., glucose (Glu), fructose (Fru), and sucrose (Suc) were determined following enzymatic transformation to NADH (reducing agent), which is expected at the absorbance of 340 nm ($A_{340\ nm}$). The conversion of glucose was obtained via hexokinase (HK) to yield glucose-6-phosphate (G-6-P) which is changed with the G-6-P dehydrogenase (G6PDH) to yield NADH. The fructose conversion, however, requires the phospho-glucose-isomerase (PGI) which transforms the fructose-6-phosphate (F6P) to the G6P. The sucrose is the primary hydrolysis by means of β-fructosidase to yield Glu + Fru.

The oligo-fructans (DP1-DP7) were qualitatively estranged by thin-layer chromatography (TLC) as detailed elsewhere (Collins *et al.*, 1971). The obtained spots were visualized according to Wise *et al.*, (1956). The contents of carbohydrates in TLC-spots were determined to get an uneven approximation of the concentration of the fructan (Fig. 1). In the leaf sap, the concentration of potassium was calculated using a flame photometer. Every biochemical determination was made via the expressed sap extracted with liquid nitrogen (LN2).

2.4. Statistical analyses

The ANOVA unique factor betwixt groups was carried out under Microsoft Excel. The differences were deemed to be statistically significant at $p \leq 0.05$. The Student's t-tests were achieved by R software.

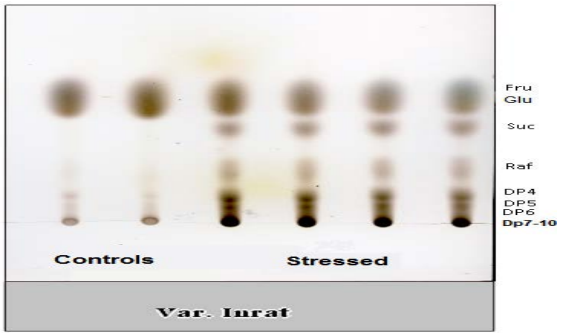

Figure 1. An image of obtained spots of the oligo-fructans from DP1 to DP10, as an example on experience; these spots were separated by TLC.

3. Results

3.1. Leaf growth recovery and rapid osmotic adjustment

Kinetics of short-term growth previous to and following the rapid stress is well-known (Mahdid *et al.*, 2014). Leaf elongation quickly finished within 2 minutes following the imposition of PEG 6000. Growth cessation continued for a few minutes in a steady rate for 2 h, growth stabilized to a new recovery rate below the pre-stress value (Fig. 2a). Data were used to estimate the rate of the leaf growth recuperation as denote steady LER in the previous phase of treatment connected to mean LER earlier than the stress. Significant difference was established betwixt varieties, the recovery achieved in MBB-var (72.72%) trail by Waha-var (62.51%). The lowly rate was reached in OZ-var (37.88 %) with a considerable difference (Fig. 2b).

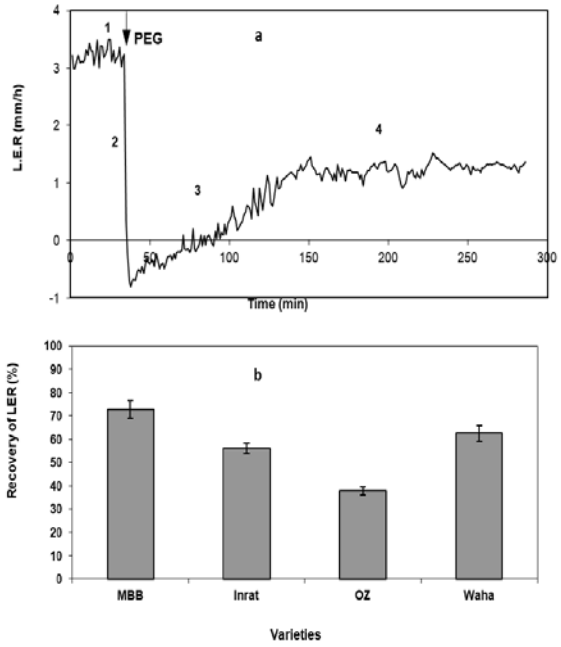

Figure 2. (a), LER kinetics (Four phases) calculated by LVDTs on the growing third *Triticum turgidum* subsp. *durum* L. as affected by water deficit imposed by adding PEG 6000 to the hydroponic's solution. (b), the percentage of leaf growth recovery represents the mean steady LER in the last phase (phase 4) of treatment related to the mean LER before stress (phase 1) in graph a.

The OP at full plant turgor (π100) at the same trend showed a dissimilar profile of reduces subsequent stress within four varieties. MBB-var showed constantly lower π100 from 1 h forward, attainment of 76 MPa (at 4 h) comparing to -1.24 MPa (control), Inrat-var, and Waha-var. By contrast, OZ-var showed no net decrease in π100 except a minor decrease 30 min after stress. Clearly, there are significant differences in the OP between the two zones (EZ + MZ), and the values of the osmotic adjustment at the finish of the stress period attained -0.52 (MBB-var) -37 (Inrat-var), and -0.33 (Waha-var). The OZ-var however, showed a small transient osmotic adjustment at 0.5-3 h (Fig. 3).

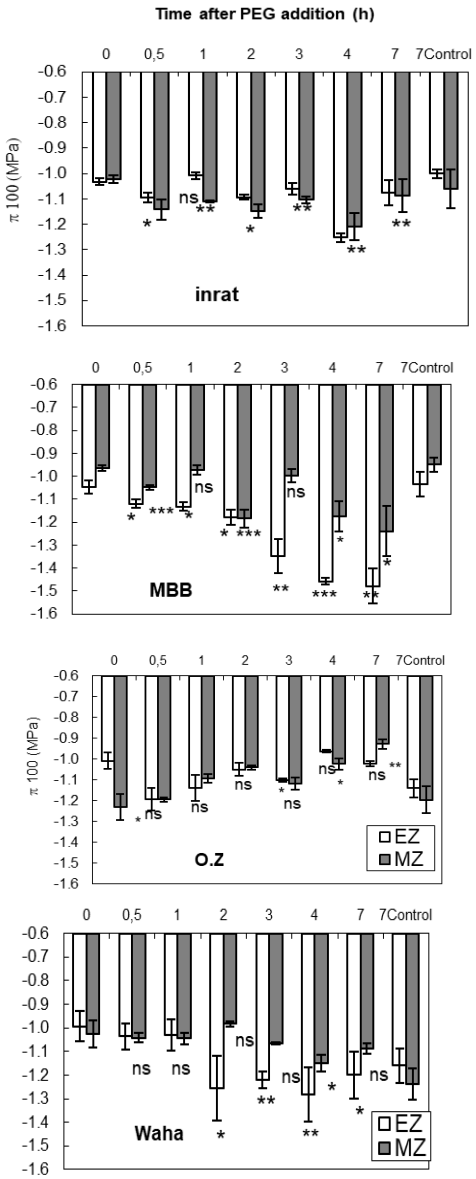

Figure 3. Modification in OP at full plant turgor (π100) of the bulk-tissue in elongation and both zones (EZ + MZ) of the third leaf of durum wheat previous before and after the supplementation of PEG 6000 to the root media. The data represents the average of at least 4 assays and ± SE are reported.

3.2. Rapid solute accumulation

3.2.1. Potassium

The increase in K^+ concentration occurred in all varieties through stress phase except OZ-var. The K^+ contribution to OP was important during and at the end of stress. This contribution to osmotic adjustment in other varieties was significantly towards the end of the stress period, between 29 and 56% except in Inrat-var, which occurred transiently. The results showed the predominance of this cation in the MZ compared to the EZ (Fig. 4).

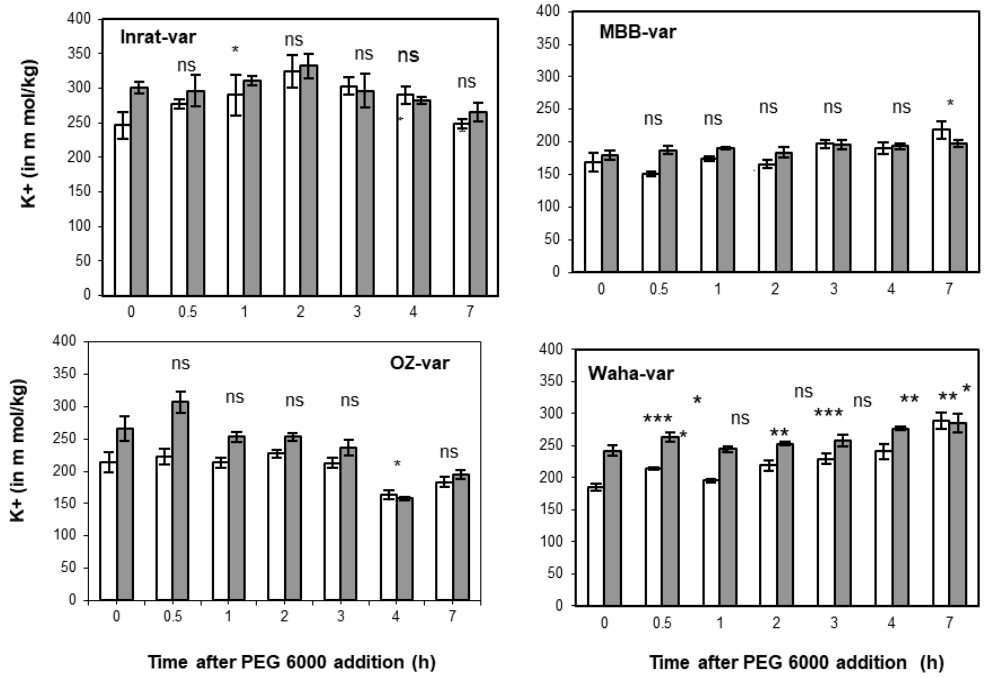

Figure 4. Modification in K^+ concentration at full plant turgor (π100) of bulk-tissue in the two zones (EZ + MZ) of third leaf of durum wheat previous to and next the supplementation of PEG 6000 to the root media. The data represents the average of at least 4 replicates ± SE.

3.2.2. Soluble sugars

The accretion of whole soluble carbohydrates was evidenced in the EZ and less in the mature one through the stress phase. The MBB-var demonstrated the highest accretion, followed by Inrat-var. However, the lowest accretion was achieved in OZ-var. The evaluation of individual carbohydrate concentrations by the enzymatic method exposed an augment in Glu, Fru, and Suc in both zones (EZ + MZ) of all varieties after the imposition of stress, and mainly in EZ (Table 1). In order to have an indication of the chemical (formula and range) of oligo-fructans as well as the comparison of sugars with the enzymatic method, data were obtained. The qualitative analyses of soluble carbohydrates allowed the determination of the degree of polymerization (Dp) from 3 to 7. The evaluation of total and individual sugar concentrations showed the presence of other sugars, especially oligo-fructans, with 22 and 56% of total sugars in EZ of the control and stressed, respectively, and to a smaller degree in MZ. The accretion of these carbohydrates was achieved in every variety excluding the OZ-var (Table 1).

The judgment of the reduction in the growth behind stress with the level of total carbohydrate accretion demonstrated no clear relationship betwixt the two variables, during the little-term stress until 7 h (Fig. 5). Similar deduction has been described between carbohydrate accumulation and photosynthetic rate during the stress (Fig. 6).

The photosynthesis rates graph indicated a slight decrease during stress and was not the same as the LER tendency during stress in all varieties (Fig. 7).

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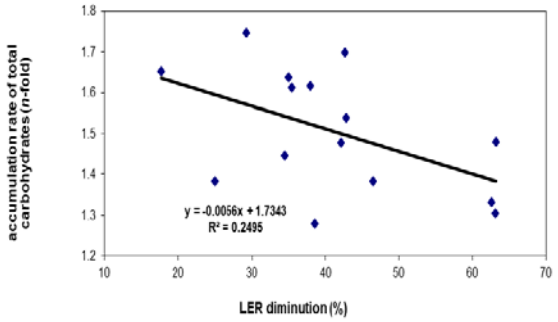

Figure 5. Correlation betwixt the accretion rate of the total sugars and the reduction of LER (%) at 7 h after stress in the four varieties of wheat. The data represents the average of at least 4 replicates.

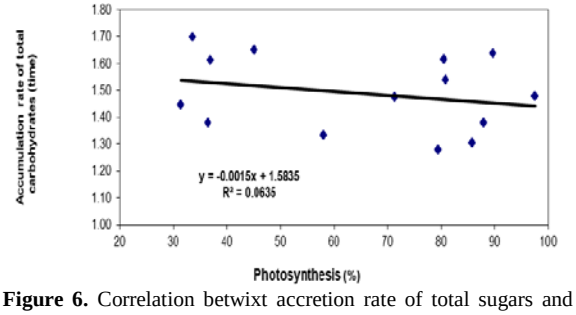

Figure 6. Correlation betwixt accretion rate of total sugars and photosynthesis decrease (%) at 7 h after stress in the four varieties of wheat. The data represents the average of at least 4 replicates

Table 1. Evaluation of total/other (g/kg) and individual (m.mol kg^{-1}) carbohydrate concentrations in EZ + MZ of MBB-var, Inrat-var, OZ-var, Waha-var of the last leaf of durum wheat calculated from the leaf sap tissue, behind the difference in concentrations betwixt two times (0 and 7 h). The data representing the average of at least 4 assays and ± SE are reported.

		Elongation zone (EZ)				Mature zone (MZ)			
		0h	7h	Difference		0h	7h	Difference	
MBB-var	Total Carbohydrates	53,05 ± 2,56	80,84 ± 5,38	27,79	***	37,76 ± 2,24	48,11 ± 1,29	10,3	**
	Glu	114,89 ± 2,62	158,36 ± 6,55	43,47	***	84,59 ± 7,05	98,87 ± 2,39	14,3	*
	Fru	33,19 ± 2,31	51,81 ± 4,16	18,62	**	19,71 ± 0,20	24,23 ± 2,02	4,52	*
	Suc	6,63 ±1,29	11,96 ± 1,32	5,33	**	7,97 ± 1,00	19,1 ± 0,23	11,1	***
	Other sugars	24,14 ± 2,18	38,92 ± 4,20	14,78	**	16,26 ± 1,14	19,41 ± 0,97	3,15	*
Inrat-var	Total Carbohydrates	31,89 ±2,43	45± 3,13	13,11	**	16,37 ± 1,19	29,13 ± 3,06	12,8	**
	Glu	61,94 ± 8,23	79,85 ± 5,25	17,91	*	30,55 ± 2,59	55,39 ± 7,70	24,8	**
	Fru	26,4 ± 3,26	38,8 ± 2,38	12,4	***	15,56 ± 1,84	20,55 ± 2,46	4,99	ns
	Suc	5,67 ± 1,59	4,8 ± 0,25	_	ns	4,85 ± 1,83	26,32 ± 4,36	21,5	***
	Other sugars	13,01 ± 3,11	25,88 ± 2,79	12,87	**	6,95 ± 1,73	13,91 ± 2,03	6,96	*
OZ-var	Total Carbohydrates	29,16 ± 1,08	38,92 ± 3,04	9,76	**	20,05 ± 0,52	21,13 ± 1,46	1,08	ns
	Glu	79,27 ± 2,22	106,34 ± 3,01	27,07	***	47,18 ± 1,17	56,32 ± 6,36	9,14	ns
	Fru	19,36 ± 1,60	29,15 ± 2,66	9,79	**	15,8 ± 1,10	24,21 ± 1,62	8,41	**
	Suc	2,65 ± 0,72	6,2 ± 1,68	3,55	*	4,53 ± 2,00	11,93 ± 4,11	7,4	ns
	Other sugars	10,50 ± 1,30	12,41 ± 1,92	1,91	ns	7,17 ± 0,45	2,55 ± 0,42	_	ns
Waha-var	Total Carbohydrates	25,71 ± 1,46	43,11 ± 2,89	17,4	***	9,76 ± 0,62	34,32 ± 1,81	24,6	***
	Glu	65,13 ± 4,32	96,22 ± 6,35	31,09	**	22,31 ± 2,33	46,41 ± 5,04	24,1	**
	Fru	23,15 ±1,59	35,61 ± 1,79	12,46	***	9,87 ± 0,41	23,04 ± 0,93	13,2	***
	Suc	3,07 ± 0,18	4,47 ± 0,27	1,4	**	5,82 ± 0,91	31,76 ± 5,30	25,9	***
	Other sugars	8,77 ± 0,51	17,85 ± 1,64	9,08	***	1,98 ± 0,41	10,95 ± 0,52	8,95	***

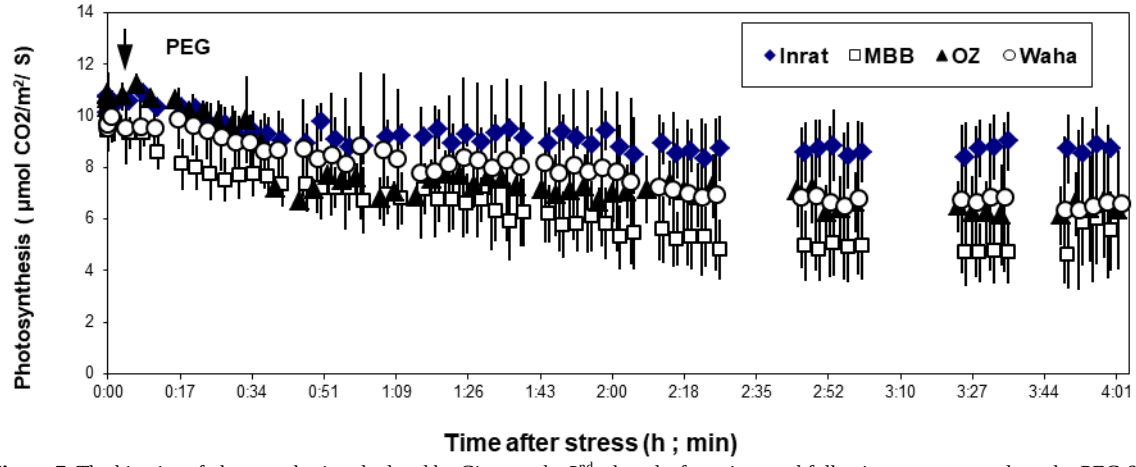

Figure 7. The kinetics of photosynthesis calculated by Ciras on the 2nd wheat leaf previous and following stress compulsory by PEG 6000 supplementation to the hydroponics solution.

4. Discussion

4.1. LER recuperation under prompt water insufficiency

Is very evident the reduction of the OP through the first rung of time hours in similar with the modifications of the LER. The source of the growth recuperation surely is linked to the water relations changes. The cells attuned osmotically inside 1 h, which designated that osmotic amendment is a speedy procedure produced by solute accretion. The continued reduction of OP behind 1 h resulted in a high recovery of the growth.

The osmotic adjustment in growing tissues gain water and thus turgor recovered immediately (Mahdid *et al.*, 2014), which the only explanation of LER changes due to turgor changes, where it turned out to be the reason for this is possibly owing to the vanishing of water possible gradient betwixt EZ cells and close to (mature) tissue and xylem due to the reduce in mature tissue and xylem water likely. Hence, this reduction shall ultimately result in minor water run and guide to the withering of the growing tissue (Mahdid *et al.*, 2011). The decrease of gradient water potential following the supplementation of PEG 6000 was pursued by a phase of rate of this gradient owing to the rapid reduce of the OP in the EZ.

The growth kinetics showed that the times' growth termination (little minutes) and recuperation (~1 h) may perhaps too rapid to generate the osmotic adjustment of lengthen tissue resulting to growth recuperation in the considered varieties. However, as in mainly earlier study, growth at time level hours did not recuperate to pre-stress levels, chiefly under conditions where elevated

concentrations of osmotic (PEG) or salt are current in the root medium, at least in the short-term (Munns *et al.*, 2000; Munns, 2002; Mahdid *et al.*, 2011). This perhaps owing to the modifications in biophysical characteristics of elongating cells, the hardening of the wall and the decrease of the extensibility provokes and leads to an additional turgor against wall hardness (Mahdid *et al.*, 2011). The osmotic adjustment collectively among cell wall extensibility regulates turgor in dehydration, where the previous is generally more efficient and widespread than the later (Bartlett *et al.*, 2012).

Considerable dissimilarity between the studied varieties was established in the amount of LER recuperation (%) at 2 h. Differences in the recuperation of LER (%) might not essentially signify the equal disparities in water stress acceptance between the varieties at the long-term. Other more complex responses, such as functional and structural responses may be implicated at a long-time scale.

The situation under sudden water stress is different, compared to saline stress. This situation may lead to variations in the metabolic procedures and fluctuations of endogenous solutes to rising zones transversely the plasma membrane. In this work, we attempt to match the differences in osmotic adjustment and growth recovery capacity between the four cultivars (Inrat-var, MBB-var, OZ-var, and Waha-var), that determine the velocity of osmotic adjustment in LER recovery at the short term under conditions of water stress. It also appears that the OP in the EZ is lower compared to the MZ. Dissimilarities in osmotic adjustment betwixt the varieties might be owing to the genetic variability. Thus, the degree of osmotic adjustment is correlated to the genetic potential of the plant. The osmotic mechanisms and possessions related with genotypic distinctions in osmotic adjustment have been elucidated in a lot of wheat research works (Morgan 1991; Blum, 2016; Morgan and Tan, 1996). This control behavior was maintained through another study. Indeed, the complex genetic control for osmotic adjustment has also been maintained through the quantitative trait locus (QTL) analysis in a population of sunflower plants in field environment, by the discovery of genomic regions related with water rank character and osmotic adjustment below water-stressed situations (Poormohammad Kiani *et al.*, 2007). We propose that a better considerate of this control at the physiological rank could help in propagation plants for arid and semi-arid conditions, and most important if we are looking at the impact of these mechanisms yield (Blum, 2016).

The magnitude of osmotic adjustment starting from 0.5 h may be due to the rapid accumulation of sugars and K^+, which indicates the accumulation so rapid of these solutes (especially sugars) in osmotic adjustment, particularly in the growing leaf tissue. Despite the small space occupied by vacuoles in growing cells, these rapid changes in K^+ concentration could be the result of increased mobilization and fluxes of endogenous solutes (including other ions) from MZ of grow athwart the plasma membrane, including potassium transporter channels (Osakabe *et al.*, 2013).

The phenol sulfuric way of full sugars does not permit the ascertainment of the DP of the accumulated sugars and consequently their involvements to osmotic adjustment. However, the enzymatic way applied for Glu, Fru, and Suc allowed the determination of the involvement of these regular sugars to osmotic adjustment.

The early accretion of whole sugars at 2 h gives considerable reduction in the OP (osmotic adjustment), attained betwixt 38 and 50% of osmotic adjustment at 7 h. The degree of whole sugars accretion illustrated a same outline in all varieties, e.g., MBB-var showed the highest accretion track by Inrat-var, Waha-var, and a lesser rate in OZ-var, which demonstrated the slightest rank of accretion. This outline was in accord with the modification in osmotic adjustment. Accumulation of sugars was primary announced in durum wheat but below long-term drought (Kameli and Lösel, 1995).

The outcome of this work designated a likely significant involvement of oligo-fructans to osmotic adjustment in every variety excluding the OZ-var. The function of oligo-fructan in lack resistance was further recommended by Hendry (1993), who declared that the emergence of fructan pro-suggest taxa matched with a climatological change on the way of cyclic drought and that the sharing of current fructan flora corresponds through areas of seasonal deficiency. Supplementary verification for a function of fructans in lack tolerance was given by the result in the genetically modified sugar beet, *Beta vulgaris*. Conversely, introduction of oligo-fructans in this non-fructan-producing species reconciles improved opposition to lack stress (Pilon-Smits *et al.*, 1999). Interestingly, the fructans enhance the growth in transgenic fructan-accumulating tobacco under water deficit (Pilon-Smits *et al.*, 1995). Was also considered as storage sugars of many species of grasses, and were given an important amount of cold tolerance (Livingston et al 2009).

More interestingly, many reports identify the opportunity that fructans might directly steady membranes below stress conditions (Hincha *et al.*, 2000).

The works examining the influence of fructan on liposomes showing that a straight relation betwixt membranes and fructan was promising. This novel field of investigation began to combine the fructan and its relationship by stress beyond simple association. This helps prevent leakage when water is isolated from the system either throughout drought (Livingston *et al.*, 2009).

The large accumulation of K^+, although its negligible concentration under the nutrient solution, it can indicate the physiological need of the plant to this cation. The involvement of K^+ to the osmotic adjustment has been exposed to increase with K^+ fertilization on the long-term in youthful durum wheat foliage below water stress (Damon *et al.*, 2011). As claimed by Fricke *et al.*, (2006); membrane potential is together a driving power and a probable monitor and is a significance of trans-membrane solute permeation and transport. This might propose that the rate in membrane possible agrees with growth recuperation and precedes solute accretion at osmotically considerable levels.

4.2. Balance betwixt carbohydrate accretion, photosynthesis, and LER throughout the stress

In terms of sugar stocks, we record here their levels in two periods of growth: The first, in normal conditions (before stress) followed by the second period of the stress response (osmotic adjustment). Data exposed no significant relationship betwixt the accretion of totality of carbohydrates and the reduction of LER throughout the stress, which might show that carbohydrate accretion, is not a direct result of growth decrease. This may be

associated with a complex development related to osmotic adjustment. These data maintain the active accumulation idea of solutes by the high permeability of sugars through the membrane or the hydrolysis of oligo-fructans or other sugars, as an osmotic reserve quite the passive accretion. It has been reported, that the water insufficiency usually augment the concentration of carbon in plant organs (Muller *et al.*, 2011), leading to an unbalance between expansion, the activity of organs, and photosynthesis. It seems, therefore, that metabolic processes and active accumulation of carbon depends on osmotic adjustment rather than on a passive accumulation of carbon as a result of the lack of growth during stress. In a second period, the growth recovery may be better associated with carbohydrate fluctuations introduced from the phloem and/or to carbohydrate gradients between the mature and elongating part of the growing leaf. The EZ has better osmotic adjustment and greater capacities to transport assimilate for growth, the same case for the fifth leaf of barley (Hein *et al.*, 2016).

In other studies, in reaction to the stress, abscisic acid (ABA) controls the activity of the β-amylase 1 (BAM1) and α-amylase 3 (AMY3) transcription via the ABA-dependent AREB/ABF-SnRK2 kinase-signaling pathway. A part of the released maltose from starch by the synergistic activity of amylases (BAM1 + AMY3) is export to the cytosol and metabolized into sucrose and free hexoses. The Suc is subsequently exported to the root or the buds to sustain osmotic adjustment. Hence, the creation of starch hydrolysis below abiotic stress shows to be an ordinary plant response as reported by Thalmann *et al.*, (2016) and Zanella *et al.*, (2016). Contrary to what was recorded in our previous results, starch levels were maintained or even elevated under long stress in barley leaves (Mahdid and Kameli, 1998). This led us to exclude the hypothesis of starch hydrolysis. We prefer here the mobilization of sugars to the EZ region across the membranes as an active transport.

. The insufficient recovery of growth despite the full recovery of turgor at short term stress may be signifying that additional mechanisms, probably concerning the cell wall rheology, growth, and cell function. As noted above (Bartlett *et al.*, 2012), and as detailed by Muller *et al.*, (2011), which suggested the possible implication of cell wall rheology or water flows to growing cells, override the role of C availability in sink organs such as mature leaves and take the lead on growth control.

The current research work does not specify also any relationship between the accretion of soluble carbohydrates and photosynthetic activity during stress which indicates that LER typically falls more quickly than photosynthetic revovery. Taking into consideration the transitive relationship between photosynthesis activity and LER diminution during stress, the accumulation of sugars by an osmotic adjustment is not a consequence of those intended for growth. The accretion of these carbohydrates is perhaps separated with direct photosynthesis activity. Muller *et al.*, (2011), mentioned this augment of the concentration of the carbon in plant organs may owe organ expansion (as a key carbon sink) being affected previously and more intensively than photosynthesis. The photosynthetic products (carbohydrates, etc.) can accrue to affect the osmotic adjustment when growth is initially condensed by water insufficiency whereas photosynthesis affected slightly. This reinforces the idea of the supply of these sugars from mature tissues, and this is evidenced by the increase in different soluble sugars in mature tissues

The inorganic solutes, e.g. potassium, are efficient in improving the osmotic adjustment. The maintenance of photosynthesis in water deficit is in line with other studies (Kaiser, 1987, Quick *et al.*, 1992; Bogeat-Triboulot *et al.*, 2007). As the data of Hasibeder *et al.*, (2015), who indicated that in drought conditions, the use of recent photosynthesis is shifted from metabolic activity to osmotic adjustment and storage compounds in grasses!

A partial stomatal aperture may allow the maintenance of photosynthesis through CO_2 supply, at least with low levels (Fig. 6). Hummel *et al.*, (2010), demonstrated that the rosette relative expansion yield of *Arabidopsis thaliana* is reduced further than photosynthesis in drought and that of the osmotic adjustment cost only a little percent of each day photosynthetic of the carbon fixation. This strengthens the suggestion of the recruitment of soluble carbohydrate or/and the employ of the carbon reserves for the osmotic adjustment of sink organs (old leaves or MZ).

As a conclusion, it is clear that the differences observed in the partial rate of LER are owing to the total plant turgor recovery (ΨP) caused by the fast osmotic adjustment. Nonetheless, the insufficient recovery of growth despite the magnitude of osmotic adjustment at short-term stress may propose that other mechanisms, may be relating to the cell wall rheology or wall extensibility changes. This fast osmotic adjustment occurs using chiefly entirety soluble sugars and potassium. We can consider the accumulation of total carbohydrates is uncoupled with direct photosynthesis or the reduction of the growth. Consequently, the increase of these carbohydrates possibly is undoing with direct activity of the photosynthesis. It is rather the mobilization of soluble carbohydrate or/and the exploit of the carbon reserves of nearby source organs in favor of EZ.

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Conflict of interest

The authors declare that they have no conflict of interest with this work and the preparation of the manuscript.

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