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OSMOTIC ADJUSTMENT AND METABOLIC CHANGES UNDER DROUGHT STRESS CONDITIONS IN WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES

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Abstract

Osmotic adjustment (OA) of cells helps to conserve the water balance of the plant, and this adjustment is generally achieved through increased amounts of various common solutes. The core objective of this study is to determine whether the active accumulation of compatible solutes in salt tolerant accessions of bread wheat confers cross-tolerance to drought stress conditions. The work determines whether the salt tolerant cultivars W4909 and W4910 may also tolerate drought stress through osmotic adjustment and metabolic changes. The salt tolerant cultivars W4909 and W4910 had significantly higher amounts of total identified carbohydrates and amino acids in the evening and morning of the fifteenth day of drought stress as compared to Yecora Rojo. This suggests that W4909 and W4910 maintained efficient osmotic adjustment to conserve water, prevent the denaturation of soluble proteins and regulate ion transport. Under drought stress conditions, the salt tolerant cultivars responded by overproduction of different types of inorganic and organic solutes metabolites as osmolytes, showing that directed breeding for salt tolerance may confer cross-tolerance to drought in this major cereal crop.

Key words: Osmotic adjustment, Drought stress, Wheat, Osmolytes, Salt tolerant.

Introduction

Drought tolerance occurs when plants avoid injury through excess amounts of solutes at the cellular level, which enables plants to continue their growth and adjust to stress conditions. Under severe drought, plants are unable to prevent dehydration. Mechanisms therefore become essential for tolerating reduced water content, and to avoid cellular damage, maintain metabolic activities, and protect cell membranes and proteins. Numerous equivalent changes occur in the physiology of the plant during both salt and drought stress conditions; for example, active increases of compatible solutes to adjust osmotic pressure is a mechanism adopted by plants to tolerate both stresses (Colmer *et al*., 1995; De La Rosa-Ibarra & Maiti, 1995; Martin *et al*., 1993).

Plant responses to salt stress involve identical metabolic pathways to those affected by drought (Munns, 2002), implying that tolerance to one stress may confer cross-tolerance to the other. Kerepsi & Galiba (2000) suggest that soluble sugars might be central for conferring both salt and drought tolerance in crops. Changes in soluble sugars have great importance since they are directly related with a range of different physiological processes including photosynthesis, translocation and respiration. It has been proposed that there are three main responses which might be useful to tolerate stress: (a) homeostasis (including ion homeostasis, related to salt stress) and osmotic adjustment, (b) stress damage control and its repair (detoxification), and (c) growth control. Corresponding to these three responses, drought and salt stress signalling is distributed among three main categories (Zhu, 2002): (1) ionic and osmotic stress signalling, to re-establish the cellular equilibrium under stress conditions; (2) detoxification signalling, for controlling and repairing stress damage; and (3) signaling to co-ordinate cell division and cell expansion to certain intensities for the specific stress conditions.

The combination of salt and drought stresses causes major damage to our crops, drawing attention to the need for developing cultivars with tolerance towards abiotic stresses (Mittler *et al*., 2006). Tolerant plants can regulate their Ψ_s (solute potential) to compensate for the desiccating effects of water deficits on cells (Croteau *et al*., 2000), a process called osmotic adjustment. Osmotic adjustments occur during conditions of water deficit and, in this case, increasing solute concentrations within the cell directly creates a positive turgor pressure (Croteau *et al*., 2000). Within root tissues, osmotic adjustment will maintain root water potential (Ψ) lower than the soil water potential (Ψ), thereby allowing the continued movement of soil water to the crop. Osmotic adjustment has therefore played important roles in controlling plant tolerance of both drought and salinity stresses.

Osmolytes are organic compounds having osmotic activities and are soluble in the solution within the cell and its surrounding fluid. They are produced by plants, animals, and micro-organisms in response to abiotic stresses, and include specific amino acids, sugars, and methylamines. Their main role is to maintain cell volume, fluid balance and stabilize proteins, protect membranes against denaturation, and they can act as osmoprotectants (McNeil *et al*., 1999; Pradeep & Udgaonkar, 2004; Rose *et al*., 2006). Plants accumulate higher amount of osmoprotectants during exposure to various adverse environmental conditions, (McNeil *et al*., 1999). Under stress conditions, osmolyte accumulation will lower osmotic potential of the cells, thereby enhancing the intake of water and maintaining cell turgor. Osmoprotectants hence can be considered to play an important role in the alteration of cellular physiology to different abiotic stresses (Strange, 1993).

Under stress conditions, plants accumulate organic solutes having lower molecular weight, which are known

as compatible osmolytes (Naidu *et al*., 1992). These serve as osmo-protectants in counteracting the effect of osmotic stress, and have no interaction with biochemical reactions (Yoshiba *et al*., 1997). However, a high accumulation of osmolytes may upset the osmotic equilibrium of the vacuole and cytoplasm, and this disturbance in intercellular equilibrium may be restored by the synthesis (Chinnusamy *et al*., 2005; Flowers, 2004; Kuznetsov & Shevyakova, 1999). Many ions in the cell can harmfully upset metabolic activities at higher concentrations, probably by altering and binding the properties of co-factors, substrates, membranes and enzymes, or by going through the dehydration shells of a protein to enhance its denaturation. However, osmolytes lean to be neutrally charged at physiological pH or dipolar with spatially separated positive and negative charges, and are apart from the hydration shells of macromolecules.

Carbohydrates are the main product of photosynthesis and are synthesized by green plants when exposed to light. An alternative route is available via gluconeogenesis, a universal pathway for glucose/ carbohydrate synthesis, which utilizes many different small molecules. There are four major precursors for this process: glycerol, pyruvate, and 3-phosphoglycrate. During drought stress conditions, accumulation of carbohydrate forms an efficient stress tolerance mechanism adopted by plants (Galiba, 1994). The accumulation of carbohydrates has been reported in several parts of the plant in response to various abiotic stresses (Prado *et al*., 2000) and, among the soluble sugars, sucrose and fructans have potential roles in the adaptation to drought (Leshem & Kuiper, 1996). Changes in carbohydrates are particularly important as they have direct relationship with the physiological processes of photosynthesis, respiration, and translocation (Galiba, 1994; Kameli & Losel, 1993). In cereals, there are many published reports on the accumulation of soluble sugars during a variety of environmental stresses (Meier & Reid, 1982). Soluble carbohydrates have proved to be a reliable marker to select for drought tolerance in wheat as compared to proline (Al Hakimi *et al*., 1995).

Amino acids are the building blocks of proteins and are therefore crucial for life. However, under drought conditions, plants drop their osmotic potential by accumulating amino acids as organic solutes (Good and Zaplachinski, 1994). The higher accumulation of these solutes allows the tolerance of the drought by maintaining cell enlargement, plant growth and CO_2 assimilation under water stress conditions (Ibrahim *et al*., 2001; Navari-Izzo *et al*., 1990). It is therefore, important to understand how biochemical, metabolic and molecular responses are coordinated might help to improve tolerance to agriculture crops against abiotic stresses.

In this investigation, we evaluate the extent to which physiological and biochemical mechanisms of salt tolerance may also confer tolerance of drought conditions.

Materials and Methods

Plant materials: The seeds of three genotypes were obtained from the USDA (United States Department of Agriculture) and CIMMYT (International Maize and Wheat Improvement Centre Mexico). Two bread wheat cultivars W4909 and W4910 (salt tolerant) (*Triticum aestivum* L.) carrying registration numbers Gp 370 and Gp 371, PI 63114 and PI 63115 (Wang *et al*., 2003) were used along with cultivar Yecora Rojo (R.C. Wang-USDA-ARS- Forage and Range Research Laboratory, Utah State University, Logan, UT USA).

Measurement of soluble carbohydrates using Gas Chromatography (GC): Samples (approximately 0.2 g FW) of the fully expanded fourth leaf were weighed to ±0.001 g precision and then each separately extracted with 80 µl H_2O (UHP), 200 µl chloroform, and 470 µl methanol v/v/v (Overy *et al*., 2005). Supernatants were transferred into GC analysis vials (1 ml) then dried overnight at 40˚C in a vacuum drying oven (SAVANT, speed vacuum plus, SC2110A, with SAVANT Refrigerated Vapour Trap, RVT100, CFC Free, Thermo Fisher Scientific Incorporated, 81 Wyman Street, Waltham, MA 02454, UK) for one day or until the samples were completely dried. Dried samples were derivatised as described by Akinci (1997). The samples were dissolved in a mixture of 475 µl anhydrous pyridine and 25 µl N-trimethylsilyimidazol (TSIM added as the silylating reagent) then placed in a water bath at 70˚C for 30 minutes. During the reaction an active hydrogen is replaced by an alkylsilyl group (as described below), most often trimethylsilyl (TMS), which is volatile and less polar.

$$\text{H-C-OH} \longrightarrow \text{H-C-O-Si (CH}_3)_3$$

After the samples were cooled at room temperature, 10 µl of sample was injected onto the GC system (Varian 3500 GC with Varian 8035 auto-sampler (the Perkin Elemer Autosystem XL GC)). Separation was on a J and W DBS column (dimensions 30 m x 0.250 mm x 0.25 micron coil (VTW Scientific, California, USA) and film thickness of (0.25 µm), Varaian 2700, Mitchel Drive, Walnut Creek, California). Technical method: hydrogen was used as the carrier gas, 40cm/s. initial column temperature 120˚C, hold time 2 minutes. Final temperature 350˚C, hold time 10min. Rate 70˚C/min. Flame ionization detector 400˚C, injector A 50˚C and injector B 270˚C no hold time flow rate b= 1ml/min, split ratio b 50.

A mixture of sugar standards (glucose, fructose, sucrose, inositol, raffinose, mannose, maltose, galactose, trehalose, xylitol, erythritol, arabinose, ribose, xylose, sorbitol, lactose, lactitol, and melibiose) were prepared as follows: 3 mg of standard was dissolved in a 1.5ml Eppendorf tube and 10 µl of Phenyl α-D glucopyranoside (3 mg sigma mL^{-1}) added as a spike to each sample as an internal standard. The individual soluble carbohydrate was identified by its retention time and the amount of sugar by the area under the peak, calibrated using the known standards.

Measurements of insoluble sugar (starch): An enzymatic method was used to estimate starch amount in the samples. The residue of leaf sections after the methanol step was extracted in liquid nitrogen, suspended in 1ml of distilled water, then transferred to a screw cap 2 ml eppendorf tube, and autoclaved for 30 min at 121˚C. For starch digestion, a 50 µl aliquot from samples was mixed with 50 µl of MES buffer (pH 4.5). A 50 µl amount of each enzyme [Amyl glucosidase (20 units per assay) and α amylase (14 units per assay)] was added to the buffer mixture and then incubated for 5 hours at 37˚C or overnight at room temperature. The samples were vortexed and then centrifuged for 5 minutes. 10 µl aliquots of supernatants were placed into a 96 well plate and assayed as illustrated below (glucose reaction).

AMYLOGLYCOSIDASE/	Starch ⟶	AMYLASE Glucose
Glucose+ ATP	HEXOKINASE ⟶	Glucose + ATP
GLUCOSE 6-PHOSPHATE	DEHYDROGENASE ⟶	Glucose- 6 -phosphate
6-phosphogluconate	$+NAD^+$	+NADH

Quantification of amino acids using HPLC of OPA-derivatives: 200 µl of borate buffer [25 mM boric acid; pH 10 (NaOH)] was added to frozen samples collected using the method outlined in section 2.6.2.1. 100 µl of this solution was removed to a fresh 1.5ml microcentrifuge tube for derivitisation with 30 µl of OPA (ortho-phthaldialdehyde reagent solution, OPA, Sigma, reference P-0532) solution (prepared fresh on the day of use by mixing 300 µl of the OPA reagent (Sigma) with 2 µl mercapto ethanol). The sample was then shaken for 30 seconds, and a total of exactly 60 seconds was allowed for derivitisation before 10 µl was loaded onto the HPLC column. Reversed-phase HPLC separation was carried out on a Phenomenex LUNA C_8 column (250 x 4.6 mm; 5µm particle size; Macclesfield, UK) with a guard column (Phenomenex SecurityGuard; C_8; 4 x 3 mm; Macclesfield, UK), eluted using a gradient of aqueous phase [200 mM sodium acetate with 1.5% (v/v) tetrahydrofuran; pH 5.9 (acetic acid)] and methanol at 1.4 mL $minute^{-1}$. The elution gradient is shown in (Table 1) and was supplied by a Perkin Elmer Series 4 HPLC quaternary pump (Perkin Elmer, Massachusetts, USA). Derivatised amino acids were detected using an LS1 fluorescence detector (Perkin Elmer) with 340 nm excitation and detection at 455 nm. Peaks were quantified using a LC-100 laboratory computer integrator (Perkin Elmer).

Table 1. Gradient used for HPLC separation of OPA-derivitised amino acids.

Time (minutes)	Solvent delivery: Percentage aqueous phase
-5 – 0	Equilibrium at 75
0 – 2	Isocratic elution at 75
2 – 16	Linear reduction to 70
16 – 32	Linear reduction to 46
32 – 44	Linear reduction to 40
44 – 46	Linear reduction to 10
46 – 55	Isocratic elution at 10
55 – 60	Linear increase to 75

Peaks were identified by comparison with a mixed standard (Table 2) and the addition of individual amino acids to this standard was used to assign peak names. OPA derivitisation allows sensitive detection of a wide range of amines by UV fluorescence, but cestein conjugates are 40-fold less fluorescent (Lee & Drescher, 1979) and OPA does not conjugate with proline (Roth, 1971). Amounts of amino acid exuded were estimated by subtracting peak areas of a blank run (UHP water used in place of sample) from both standard and sample, followed by linear extrapolation using the mixed standard as a single point calibration. Single point extrapolation should be adequate because the range of the computer integrator was set within the experimentally defined linear range of detection using this system (personal communication, Professor W. P. Quick and Dr. D. Kinsman).

Table 2. Mixed amino acid standard, this standard solution was diluted 10-fold in borate buffer before derivitisation as described for samples. Analytical grade distilled water was used as the solvent. All amino acids were supplied by Sigma.

Amino acid	Concentration (µg ml^{-1})
Alanine	3.3
Arginine	3.0
Asparagine	3.4
Aspartic acid	3.3
GABA	3.2
Glutamic acid	3.1
Glutamine	3.2
Glycine	3.2
Histidine	3.2
Isoleucine	3.2
Leucine	3.8
Lysine	3.2
Methionine	3.8
Phenylalanine	3.3
Serine	3.8
Threonine	3.8
Tryptophan	3.3
Tyrosine	3.5
Valine	3.4

Quantification of proline: Quantification of proline was carried out as reported by Bates *et al*., (1973). Frozen fully expanded fourth leaves (200 mg FW) were ground and homogenized in 1 ml of 3% sulfo-calicyclic acid. After centrifugation for 5 minutes at 16000 g, a 200µl aliquot of the supernatant was placed in a glass tube with 1ml of glacial acetic acid and 1ml of ninhydrin acid (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved). The tubes were covered with glass caps then heated in a water bath at 100˚C for 1

hour. After fast cooling in an ice bath, 2 ml of toluene was added to each tube and the tubes were shaken vigorously. The toluene-phase-containing complex colour (pink) was measured at a wavelength of 520 nm using a spectrophotometer (Perkin-Elmer instruments, Lamibda-40UV/vis). The amount of proline was calculated from a standard curve using known amounts of proline (0-1.0 mg) and the data were expressed as means ± standard errors.

Statistical analysis

All statistical analyses were undertaken in Minitab 14.0 (Minitab Incorporated, USA) including general linear model analysis (statistically differences between day zero and the other days) and one-way ANOVA (statistical significant differences between genotypes one same day/data point) and drawing with SigmaPlot-10 (Systat Software Incorporated, Germany).

Results

Drought treatment

Total identified carbohydrates on the 15th day of the drought stress

Evening: Total identified carbohydrates were calculated for the evening of the fifteenth day of drought treatment and control in each determine the influence of each carbohydrate to the total pool. The salt tolerant cultivars W4909 and W4910 had significantly ($p<0.05$ and $p<0.01$) higher amount of total identified carbohydrates compared to the sensitive cultivar Yecora Rojo and control in the evening of the fifteenth day of the drought treatment. No effects were found in the total identified carbohydrates in the evening under control conditions in any of the cultivars (Fig. 1).

Morning: Total identified carbohydrates were calculated for the morning of the fifteenth day of drought treatment and control in each leaf, to investigate influence of each carbohydrate to the total pool. The salt tolerant cultivars W4909 and W4910 had significantly ($p<0.05$) higher amount of total identified carbohydrates compared to the sensitive cultivar Yecora Rojo and control on the morning of the fifteenth day of the drought treatment. Non-significant differences were observed in the amount of total identified carbohydrates in any of the genotypes under control conditions (Fig. 2).

Total Identified Amino Acids on the 15th day of the drought stress:

Evening: Total identified amino acids were calculated for the evening of the fifteenth day of drought treatment and control in each leaf, to investigate the relative contribution of each amino acid to the total pool. The salt tolerant cultivars W4909 and W4910 had significantly ($p<0.05$) greater amount of total identified amino acids than the drought sensitive cultivar Yecora Rojo and control in the evening on the fifteenth day of the drought treatment. Under control conditions, non-significant differences were recorded between genotypes in any of the three cultivars (Fig. 3).

Morning: Total identified amino acids were also calculated for the morning of the fifteenth day of the drought treatment and control in each leaf, to investigate the relative contribution of each amino acid to the total pool. The salt tolerant cultivars W4909 and W4910 had significantly ($p<0.01$ and $p<0.05$) greater amount of total identified amino acids than the drought sensitive cultivar Yecora Rojo and control in the morning of the fifteenth day of the drought treatment. Under control conditions, non-significant differences between genotypes were observed in any of the three cultivars (Fig. 4).

Discussion

This study shows that wheat cultivars bred for salt tolerance also have cross-tolerance for drought, which is mediated via the accumulation of organic solutes, particularly carbohydrates and amino acids. The work has identified the solutes concerned, highlighting particular roles for starch, sucrose and a range of amino acids. It has important implications for crop breeding for abiotic stress tolerance, showing that lines developed for one stress may be equally well adapted for others.

In order to maintain osmotic adjustment (OA) in equilibrium, plants must increase the accumulation of organic solutes in the cytosol and vacuole of the cell to maintain turgor pressure (Bartels and Sunkar, 2005; Taji *et al*., 2002). This is a part of the drought avoidance mechanism which is adopted under stress conditions (Cushman, 2001). OA develops slowly in response to water loss through stomata or tissue dehydration. As drought stress becomes severe, other responses will occur, such as changes in growth and photosynthesis. Plants accumulate higher amount of organic solutes including soluble sugars, amino acids, polyols and other solutes for OA as water stress becomes more severe (Ingram & Bartels, 1996; Rhodes & Hanson, 1993; Rodriguez *et al*., 1997; Zhang *et al*., 1999).

Carbohydrates tend to have specific significance under drought stress conditions, as are related with the physiological processes in plant s such as photosynthesis, translocation and respiration (Taji *et al*., 2002). In particular, sucrose and fructans have vital importance in plant adaptation under drought and salt stresses (Greger & Bertell, 1992; Hermans *et al*., 2005; Housley & Pollock, 1993; Williams *et al*., 1992). The salt tolerant cultivars W4909 and W4910 had significantly higher amount of fructose, galactose, glucose, mannitol, mannose, raffinose and sucrose in both the evening and morning compared to the Yecora Rojo under the drought treatment. This suggests that higher amount of sucrose and other organic solutes in these tolerant wheat cultivars could protect macromolecules and membranes from destabilization under drought stress. Previous work has also shown that tolerant wheat cultivars accumulated higher amount of fructose, galactose, glucose, mannitol, mannose, raffinose and sucrose compared to sensitive cultivars under drought stress conditions (Kameli & Losel, 1993; Leucci *et al*., 2008; Morsy *et al*., 2007; Wang *et al*., 1995). These metabolites can make significant contributions to osmotic adjustment and can act in water substitution to sustain membrane phosphor-lipids in the liquid crystalline phase, preventing the denaturation of soluble proteins (Kerepesi & Galiba, 2000; Nishizawa-Yokoi *et al*., 2008).

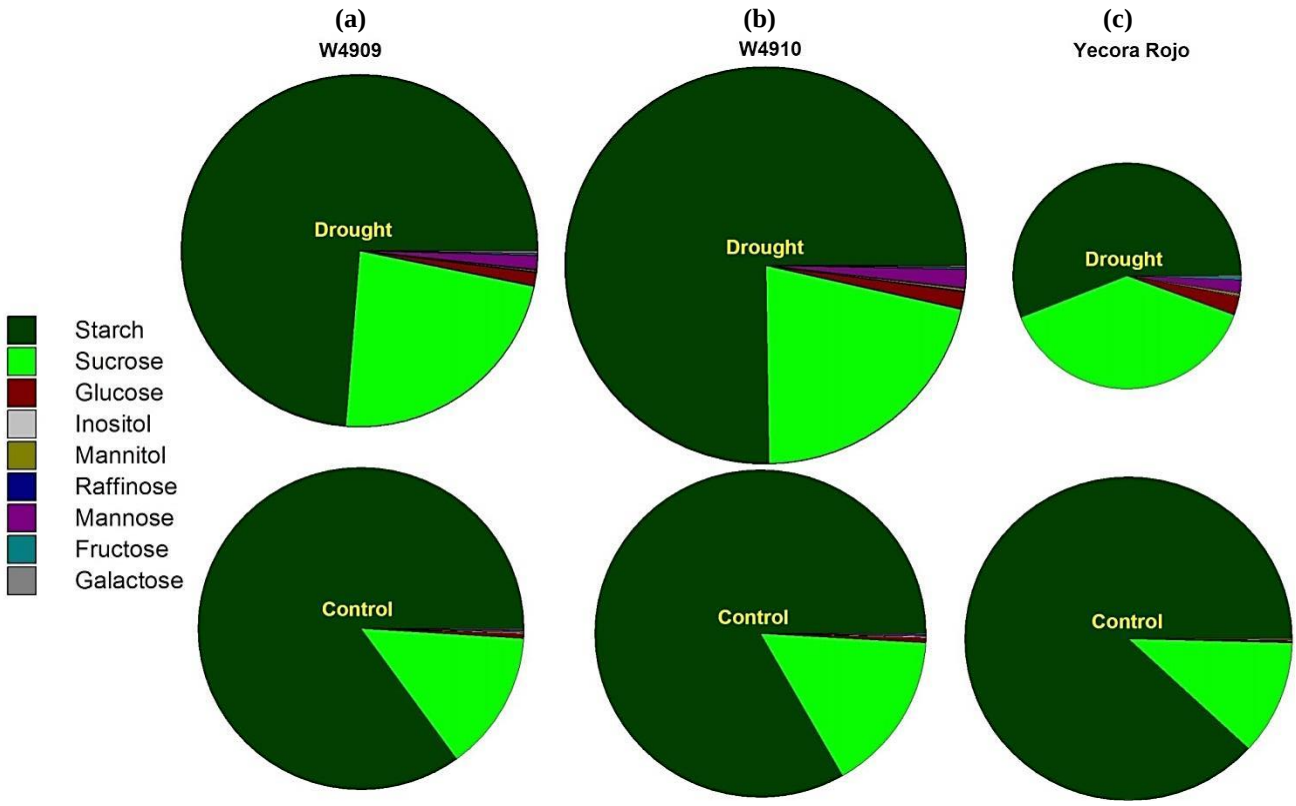

Fig. 1. Total identified carbohydrates in the evening on the fifteenth day of drought stress and control conditions for the three wheat genotypes; W4909, W4910 and Yecora Rojo. The different sizes of pie chart represent the different amount of total identified carbohydrates, and the different colours in each pie chart represent the different carbohydrates (see legend). (Starch=Glucose equivalents), letters show significant differences (p<0.05) among genotypes.

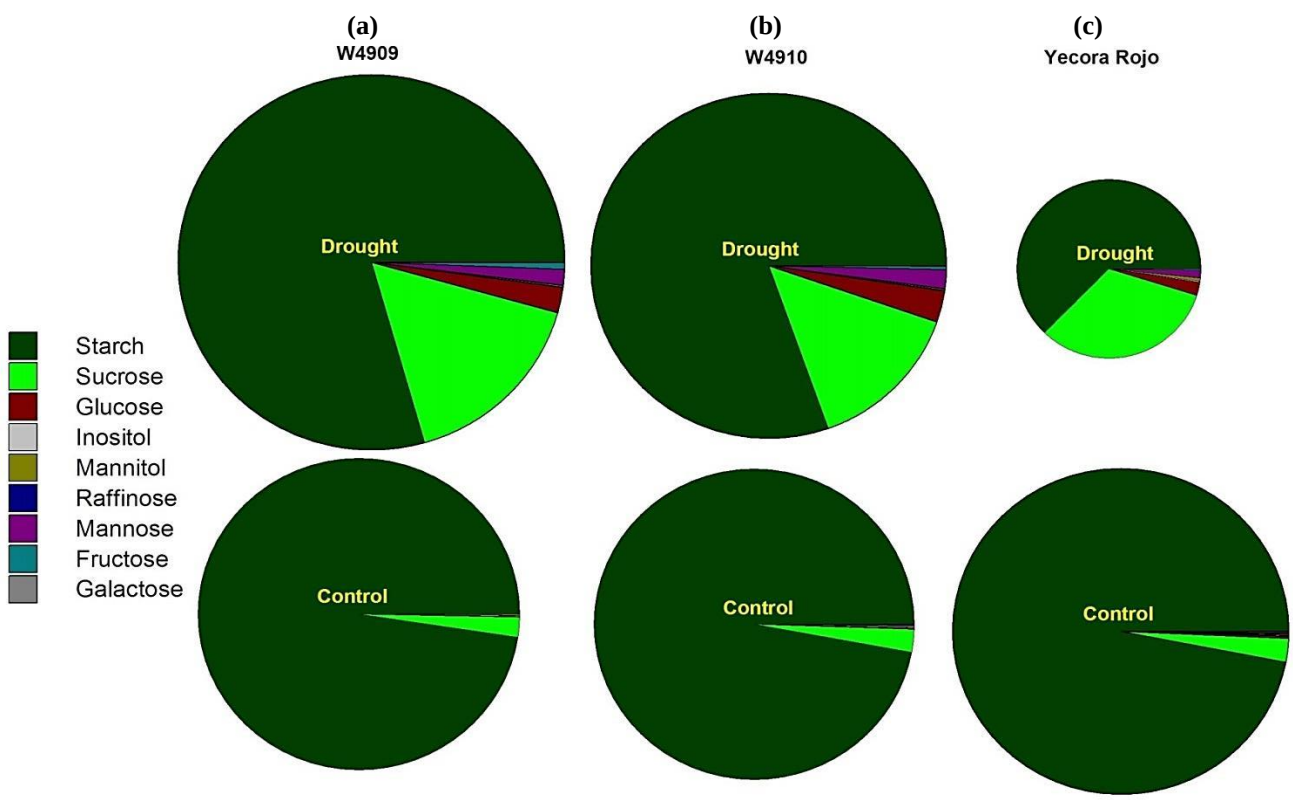

Fig. 2. Total identified carbohydrates in the morning on the fifteenth day of drought and control conditions for the three wheat genotypes; W4909, W4910 and Yecora Rojo. The different sizes of pie chart represent the different amount of total identified carbohydrates, and the different colours in each pie chart represent the different carbohydrates (see legend) (Starch=Glucose), letters show significant differences (p<0.05) among genotypes.

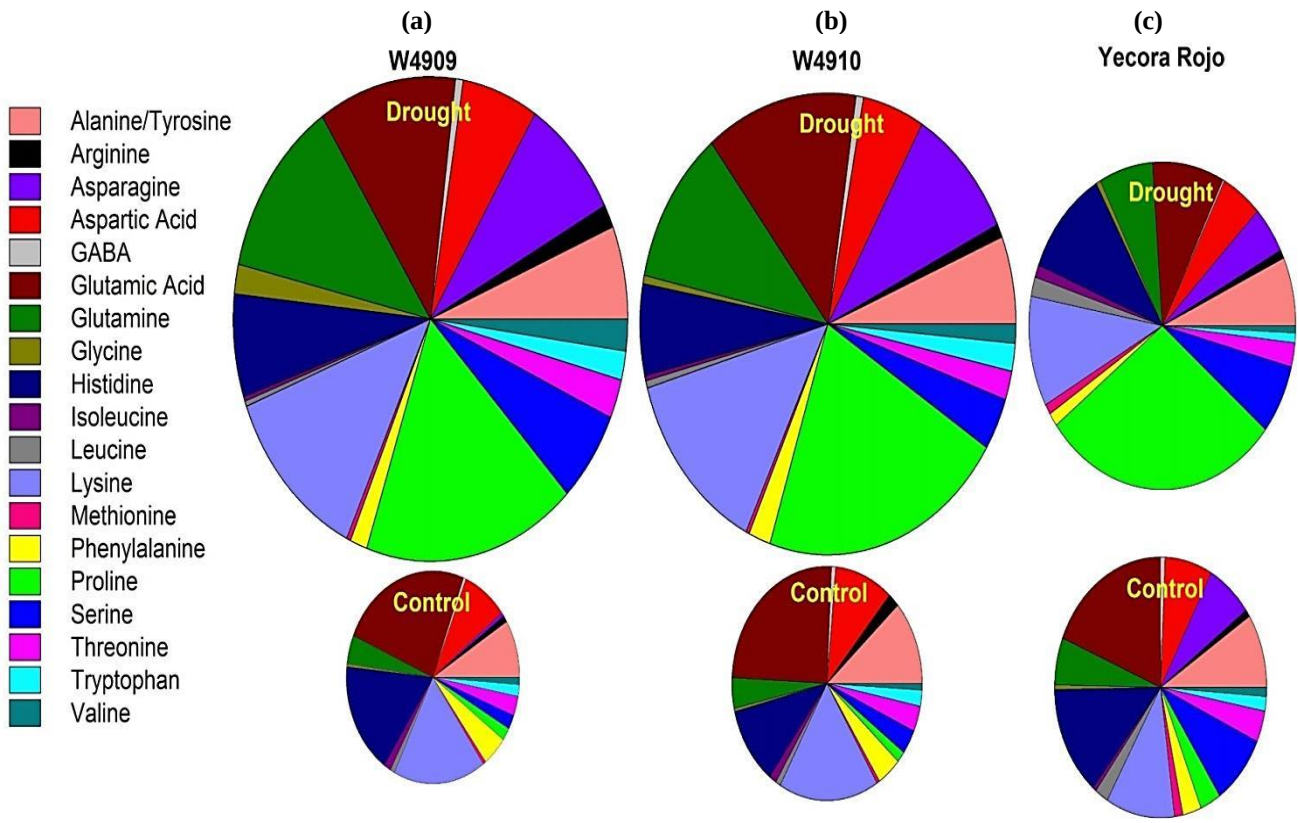

Fig. 3. Total identified amino acids in the evening of the fifteenth day under the drought and control conditions for three wheat genotypes; W4909, W4910 and Yecora Rojo. The different sizes of pie charts represent the total identified amino acids, and different colours in each represent the individual amino acids (see legend), letters show significant differences (p<0.05) among genotypes.

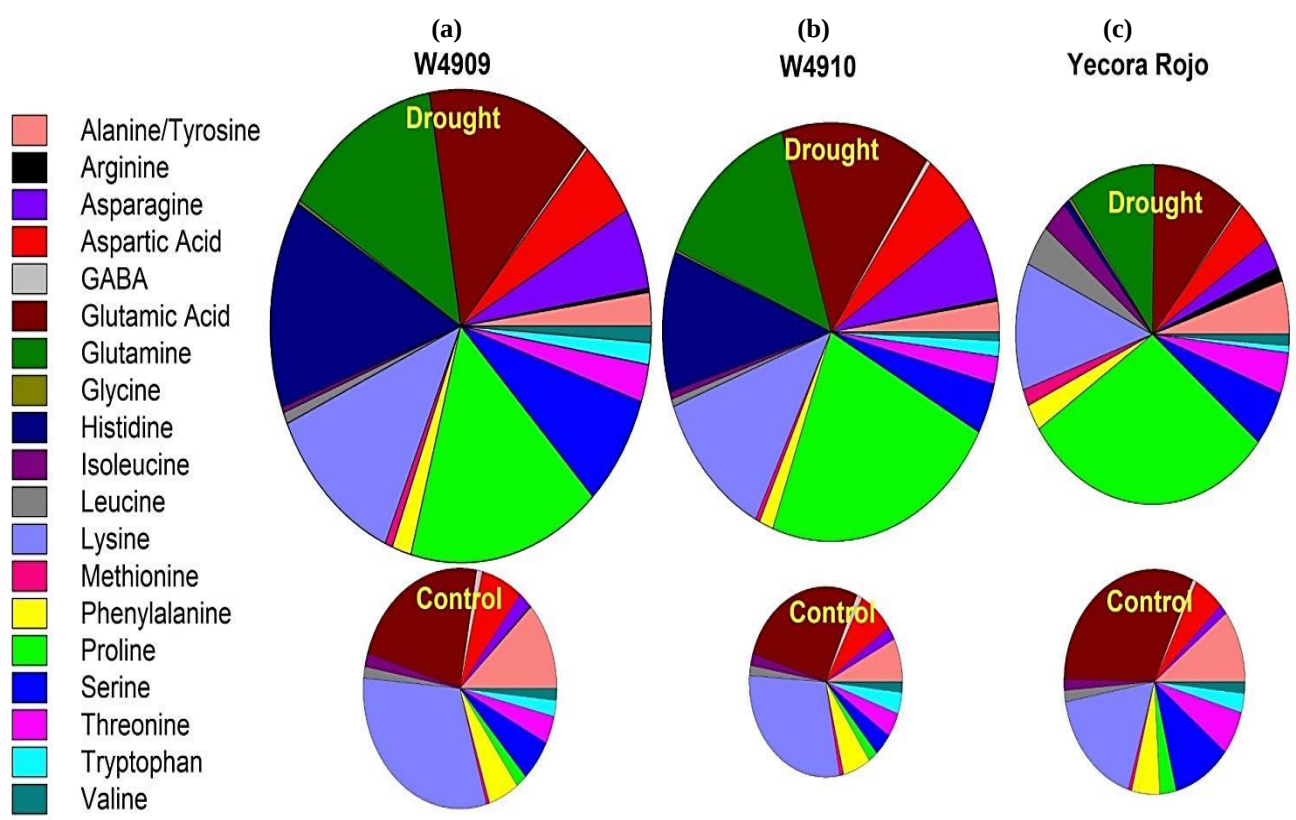

Fig. 4. Total identified amino acids in the morning of the fifteenth day under the drought and control conditions for three wheat genotypes; W4909, W4910 and Yecora Rojo. The different sizes of pie charts represent the total identified amino acids, and different colours in each represent the individual amino acids (see legend), letters show significant differences (p<0.05) among genotypes.

Sucrose is the most abundant sugar present in leaves of C_3 plants (Nishizawa-Yokoi *et al*., 2008), and it is also a major soluble sugar in wheat under the drought and control conditions in the present experiments. The salt tolerant cultivars W4909 and W4910 had significantly increased amount of sucrose under drought stress in the evening, suggesting that sucrose is one of the key organic compounds in the cytoplasm that can help plants to decrease water potential compared to the soil that can stabilize the water flow. These results are similar with other researchers, such as Sabry (1995), who observed significantly increased amount of sucrose in tolerant wheat cultivars subjected to drought stress. All the cultivars had significantly increased the amount of sucrose in the morning under drought stress, suggesting that tolerant cultivars might have sugar exported during the night. (Morsy *et al*., 2007), reported similar results and observed an increase in sucrose amount in a sensitive cultivar under water stress environment.

Drought stress alters the overall metabolism of carbohydrates for osmotic adjustment under water deficit condition (Hare *et al*., 1998; Kim *et al*., 2000; Martıinez *et al*., 2004; Pelleschi *et al*., 1997). The tolerant cultivars W4909 and W4910 had significantly higher amounts of total identified carbohydrates compared to the sensitive cultivar Yecora Rojo under drought stress in the evening and morning; signifying that the tolerant cultivars had efficient osmotic adjustment to lower the plant water potential compared with the soil. Results also suggest that the tolerant cultivars protected cell membranes by adapting to the drought stress conditions. The results are in agreement with the outcomes of Farshadfar *et al*., (2008) and Mohammadkhani & Heidari (2008), who found a massive increase in the amount of total carbohydrates under drought stress; as water potential decreased, the accumulation of sugars increased. Higher number of compatible solutes have been frequently observed in different crops under drought stress conditions (Chaves *et al*., 2003; Mack & Hoffmann, 2006; Rathinasabapathi, 2000). Plants also use insoluble carbohydrates for osmotic adjustment after conversion into soluble carbohydrates under drought stress conditions (Yoshiba *et al*., 1997; Zinselmeier *et al*., 1999). Plants respond to various abiotic stresses by overproduction of different types of organic solutes/metabolites as osmolytes to stabilize membranes and to achieve efficient osmotic adjustment (Rhodes & Hanson, 1993). These metabolites are often involved in osmotic adjustment with other organic solutes. Plant respond to drought by increasing a range of amino acids as osmolytes to control osmotic balance, to stabilize proteins, regulate ion transport, adjust stomatal opening and detoxify active oxygen species (Choluj *et al*., 2008; Erdei, 2002; Hong-Bo *et al*., 2006; Maathuis *et al*., 2003; Matysik *et al*., 2002; Mohanty & Matysik, 2001; Nezhadahmadi *et al*., 2013; Rai, 2002; Rhodes & Hanson, 1993).

Total amino acids were significantly higher in the salt tolerant cultivars W4909 and W4910 than in the drought sensitive cultivar Yecora Rojo in both the evening and the morning under chronic drought stress conditions. This result suggests that the total quantity of amino acids played a significant role in the drought tolerance, most likely as osmolytes (Ashraf & Harris, 2004). The results are in agreement with the finding of Sabry (1995), who reported a significant increase in total amino acids and genotypic differences in wheat under drought stress conditions. Increases in total amino acids have also observed in other crop species (Ashraf & Iram, 2005; Pustovoitova *et al*., 2001; Subramanian & Charest, 1995).

Conclusions

Total identified carbohydrates and amino acids were higher in the tolerant cultivars W4909 and W4910 compared to the sensitive cultivar Yecora Rojo in the evening and morning under drought stress conditions. Many studies have previously showed that soluble sugars and amino acids are better markers than only proline for selecting drought stress tolerance in wheat (Al Hakimi, *et al*., 1995). Drought stress altered the metabolism of plants, resulting in a higher accumulation of different soluble and insoluble carbohydrates and amino acids (Singh *et al*., 1993; Bussis & Heineke, 1998; Showler, 2002; Showler *et al*., 2007 and Showler, 2008) that was associated with drought severity. The salt tolerant cultivars W4909 and W4910 had higher amount of sucrose in the evening and morning under drought stress conditions suggesting an essential role in osmotic adjustment (Wang *et al*., 1995). Thus, these cultivars maintain their turgor pressure. It could be concluded that an increase in soluble carbohydrate content under drought stress could be responsible for lowering the water potential of the plant to maintain water channels and protect cell membranes, soluble proteins and phospholipids. This could be one of the core mechanisms adopted by the plants in order to tolerate drought stress.

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