Full Length Research Paper

Stress-induced changes in the free amino acid composition of two wheat cultivars with difference in drought resistance

Saeed Saeedipour[1]* and Foad Moradi[2]

[1]Department of Agronomy, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran.
[2]Agriculture Biotechnology Research Institute, Karaj, Iran.

Developmental changes in the levels of free amino acids of flag leaves in drought-tolerant (*Triticum aestivum* L. cv. Zagros) and drought-sensitive (cv. Marvdasht) wheat genotypes was investigated under controlled water deficit condition during grain filling. Cultivars were grown in pots and treated with either well-watered (control) or drought-stress (stress) from anthesis to maturity. Total soluble amino nitrogen in the leaves of two cultivars rose as relative water content (RWC) declined, the peak concentrations were attained early in the drought period (seven days after anthesis), when the Zagros and Marvdasht leaf RWC approached 80 and 70%, respectively. However, irrespective of water deficit, the total amino acids concentration declined with time. Interestingly, Zagros and Marvdasht did not accumulate the same amino acids. Zagros accumulated large amounts of proline and γ-aminobutyrate, whereas Marvdasht preferentially accumulated asparagine and arginine. It is concluded that; (i) modifications in the amino acid metabolism during dehydration stress were different depending on leaf development and (ii) proline and γ-aminobutyrate (Gaba) accumulation in Zagros leaves were essential for the acquisition of desiccation tolerance. On the contrary, the accumulation of large amounts of asparagine and arginine that serves as nitrogen and carbon reservoirs, in the Marvdasht during dehydration may be as a result of restriction of transport efficiency of storage material that led to low yield in drought sensitive cultivar.

Key words: Arginine, asparagine, γ-aminobutyrate, proline, water stress, *Triticum aestivum* L.

INTRODUCTION

Water shortage induces a deviation of plant metabolism from its normal pattern (Hsiao, 1973). When the water availability is limited, the accumulation of low molecular mass compounds, such as free amino acids, ensures the osmotic adjustment of the plants (Good and Zaplachinski, 1994; El Tayeb and Hassanein, 2000; Trotel-Aziz et al., 2003). Metabolic adjustments of plants to water deficit can be visualized as being of two distinct types: first, a breakdown of the steady-state induced by the action of the stress at susceptible points of metabolism; or, second, alterations in an adaptive sense, involving theregulation of metabolic processes in a way that plant survival is increased, either during or after the period of stress (Hanson and Hitz, 1982). Under water stress, a decrease in proteins could reflect either diminished synthesis or increased breakdown, leading to higher levels of free amino acids (Navari-Izzo et al., 1990). Since the effects of drought depend on species, tissue and age, as well as the nature, duration and degree of the stress, it is not surprising that marked differences

---

*Corresponding author. E-mail: saeeds79@gmail.com. Tel: +98-916-317-1978.

Abbreviations: ACC, Amino cyclopropan carboxylic acid; **Ala,** alanine; **Arg,** arginine; **Asn,** asparagines; **Asp,** aspartate; **DAA,** days after anthesis; **Gaba,** γ-aminobutyrate; **Gln,** glutamine; **Glu,** glutamate; **Gly,** glycine; **His,** histidine; **Ile,** isoleucine; **Leu,** leucine; Lys, lysine; **Met,** methionine; **Phe,** phenylalanine; **Pro,** proline; **RWC,** relative water content; **Ser,** serine; **Thr,** Threonine; **Tyr,** tyrosine; **Val,** valine.

have been found in the amino acid pattern for stress conditions (Hanson and Hitz, 1982). In the leaf, amino acid pools may be affected by many factors: (i) nitrogen assimilation into amino acids (through the glutamine synthetase/glutamate synthase cycle, GS/GOGAT cycle), (ii) amino acid interconversions (through aminotransferase reactions), (iii) active protein biosynthesis/ degradation, (iv) amino acid transport and (v) catabolism or utilization of amino acids for the biosynthesis of other metabolites (Noctor et al., 2002). Amino acid accumulation (especially proline) has been widely suggested to be directly involved as a protective mechanism in many water-stressed plants (Hare and Cress, 1997; Rhodes et al., 1999). The contribution of proline (Pro) to improved drought tolerance was shown in barley, where greater ability to accumulate Pro correlated with decreased membrane injury (Bandurska, 2000). In addition, a large increase in the Pro precursors, glutamate (Glu) and arginine (Arg) was observed in *Brassica napus* during drought (Good and Zaplachinski, 1994). Drought also induced more than five-fold increase in isoleucine (Ile), leucine (Leu) and aspartate (Asp) contents, as well as an increase, albeit small, in the levels of all other amino acids (Good and Zaplachinski, 1994). When water deficit was established, the level of free amino acids in sorghum plants increased from 32 to 39 mM under moderate stress and from 29 to 45 mM under severe stress (Jones et al., 1980). The amino acids which contributed most to this increase were aspartate, glutamate, proline, alanine and valine. Zagdanska (1984) reported an increase in aspartate, alanine and glutamate in stressed leaves of wheat, and suggested that this could help maintain energy fluxes of the chloroplast. Stewart and Larher (1980) found an accumulation of amino acids in the presence of water deficit, leading to a dynamic adjustment of N metabolism. Navari-Izzo et al. (1990) suggested that the increase in free amino acids could contribute to the tolerance of the plant to water deficit through an increase in osmotic potential, or as a reserve of N, principally for the synthesis of specific enzymes. The objective of the present investigation was therefore to determine the levels of free amino acids in response to water deficit, in two cultivars of wheat differing in drought tolerance during grain development.

MATERIALS AND METHODS

Experimental procedure, design and relative water content determination

Based on preliminary experiments (Saeidi et al., 2006), two contrasting winter wheat cultivars (*Triticum aestivum* L.) Marvdasht and Zagros (drought susceptible and tolerant during grain filling, respectively) were used in pot culture experiments during the growing season from 2009 to 2010 in the greenhouse of Agricultural Biotechnology Research Institute of Iran (48°20 N; 31°41 E; 20 m above sea level). Pots with a diameter of 23 cm and height of 25 cm were filled each with 8 kg pot^{-1} sieved yellow drab soil mixed with 20 g pot^{-1} manure fertilizer and 3.3 g pot^{-1} compound

fertilizer (N:P:K = 9:8:8). The soil contained organic matter of 1.48%, total N of 0.12%, available N of 82.3 µg g^{-1}, available P_2O_5 of 30.9 µg g^{-1} and available K_2O of 126.7 µg g^{-1}. Drought stress was imposed by withholding the amount of water applied in order to keep the soil moisture level at about 50% of the field capacity (FC). For non-stressed (control) treatments, the soil moisture was maintained in field capacity until the plants were harvested. 15 seeds per pot were initially sown and later thinned to five at the third-leaf stage. The pots were weighed daily and watered to restore the appropriate moisture by adding a calculated amount of water. The experiment was (2 × 2 × 4) (two cultivars and two water regimes) factorial design with four treatment. Each of the treatment had four replications with three sub-samples, in a complete randomized block design.

At each sampling point during dehydration stress, four leaf samples were utilized for the determination of leaf relative water content (RWC). The RWC was calculated according to the formula: RWC = (fresh weight – dry weight)/(full turgor weight – dry weight). Full turgor weight of the leaves was determined following a 24 h incubation in the dark and in flasks containing distilled water. Dry weight was determined after oven drying at 80°C for two days.

Amino acid analysis

Amino acids were determined after extraction of frozen ground leaf tissue (30 [mg] DW ml^{-1}) in a 2% (w/v) solution of 5-sulphosalicylic acid. The composition of individual amino acids was determined by ion-exchange chromatography using an Amino Tac JLC-500/V, JEOL, Croissy sur Seine, France (ninhydrin coloration) according to the instructions of the manufacturer. Total amino acid content was calculated by summing the 20 protein amino acids.

RESULTS

Plant water status

Withholding irrigation resulted in reduction in RWC in both drought- sensitive and tolerant genotypes; the reduction was more pronounced in the drought-sensitive cultivars, Marvdasht. At the beginning of water stress (seven days after anthesis (DAA)), RWC in Marvdasht was lower than that in Zagros (70% as compared to 80%), and decreased again thereafter, but with a sharp slop in Marvdasht (Figure 1). At the end of experiment, RWC dropped markedly at 31 DAA in both cultivars, however the loss was more marked in Marvdasht (10% as compared to 48%). The RWC of flag leaf in both cultivars remained high under well watered than water stressed condition.

Individual amino acid contents

Significant increases in total soluble amino nitrogen were detected in the leaves of both cultivars under water deficit (Table 1). Three main groups of amino acids could be roughly defined: (i) amino acids with similar levels in Zagros and Marvdasht and unchanged during dehydration: metionine, (ii) amino acids with similar levels in Zagros and Marvdasht and changed during dehydration: amino cyclopropan carboxylic acid and valine, and
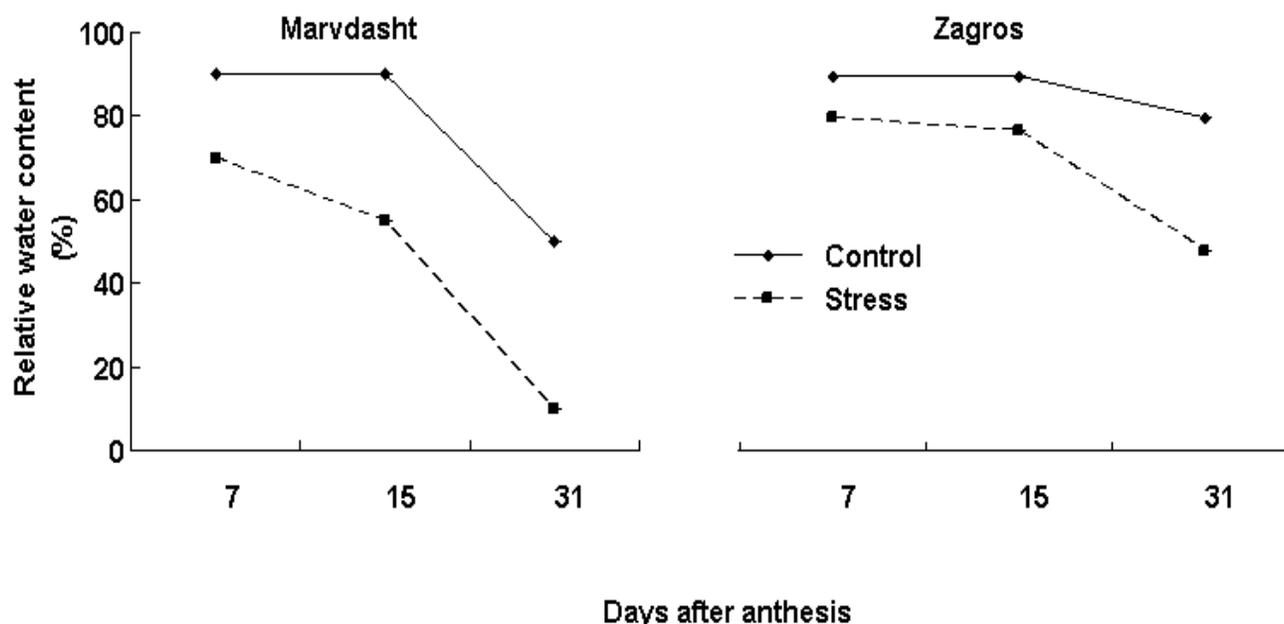
Figure 1. Changes in relative water content (RWC) under different water treatment: well watered (control) and withholding water (stress) from anthesis to maturity in flag leaves during grain filling in two wheat cultivars (*T. aestivum*). Data are means ± SE of four independent samples. SE bars are not shown where they are smaller than symbols.

Table 1. Free amino acid contents, in $\mu mol g^{-1}Fw$, of leaves of the wheat cv. Zagros and Marvdasht, subjected to different water status.

Amino acid	Zagros		Marvdasht		LSD(0.05)
	Control	Stress	Control	Stress	
Asp	3.07[b]	4.85[a]	2.45[c]	3.05[b]	0.074
Glu	1.25[c]	3.78[a]	1.11[d]	2.04[b]	0.037
Ser	4.51[d]	8.40[a]	4.70[c]	6.51[b]	0.182
Asn	1.55[d]	7.01[b]	4.79[c]	12.57[a]	0.304
Gly	1.19[a]	0.55[c]	0.67[b]	0.53[c]	0.045
Gln	1.18[d]	2.90[b]	2.25[c]	3.78[a]	0.126
His	0.01[c]	0.23[b]	0.23[b]	0.92[a]	0.026
Thr	1.18[b]	1.23[b]	0.96[c]	2.40[a]	0.064
Arg	0.23[c]	0.33[b]	0.33[b]	2.70[a]	0.045
Ala	1.82[d]	4.10[a]	2.22[c]	2.75[b]	0.078
Gaba	6.84[b]	6.64[c]	6.03[d]	8.99[a]	0.22
Acc	0.01[b]	0.05[a]	0.03[ab]	0.04[a]	0.026
Pro	0.54[d]	8.11[a]	0.78[c]	1.37[b]	0.052
Tyr	0.12[c]	0.16[b]	0.15[b]	0.58[a]	0.026
Val	0.50[c]	2.33[a]	0.52[c]	1.23[b]	0.045
Met	0.02[a]	0.04[a]	0.03[a]	0.04[a]	0.026
Ile	0.16[d]	0.48[b]	0.22[c]	0.51[a]	0.026
Lys	0.24[c]	0.37[b]	0.34[b]	1.50[a]	0.037
Leu	0.29[c]	0.29[c]	0.50[b]	1.26[a]	0.037
Phe	0.16[d]	0.23[c]	0.28[b]	0.89[a]	0.037
Total amino acids	24.87	52.08	28.59	53.66	

Ala, Alanine; Arg, arginine; Asn, asparagines; Asp, aspartate; Gaba, γ-aminobutyrate; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; RWC, relative water content; Ser, serine; Thr, Threonine; Tyr, tyrosine; Val, valine; ACC, amino cyclopropan carboxylic acid.
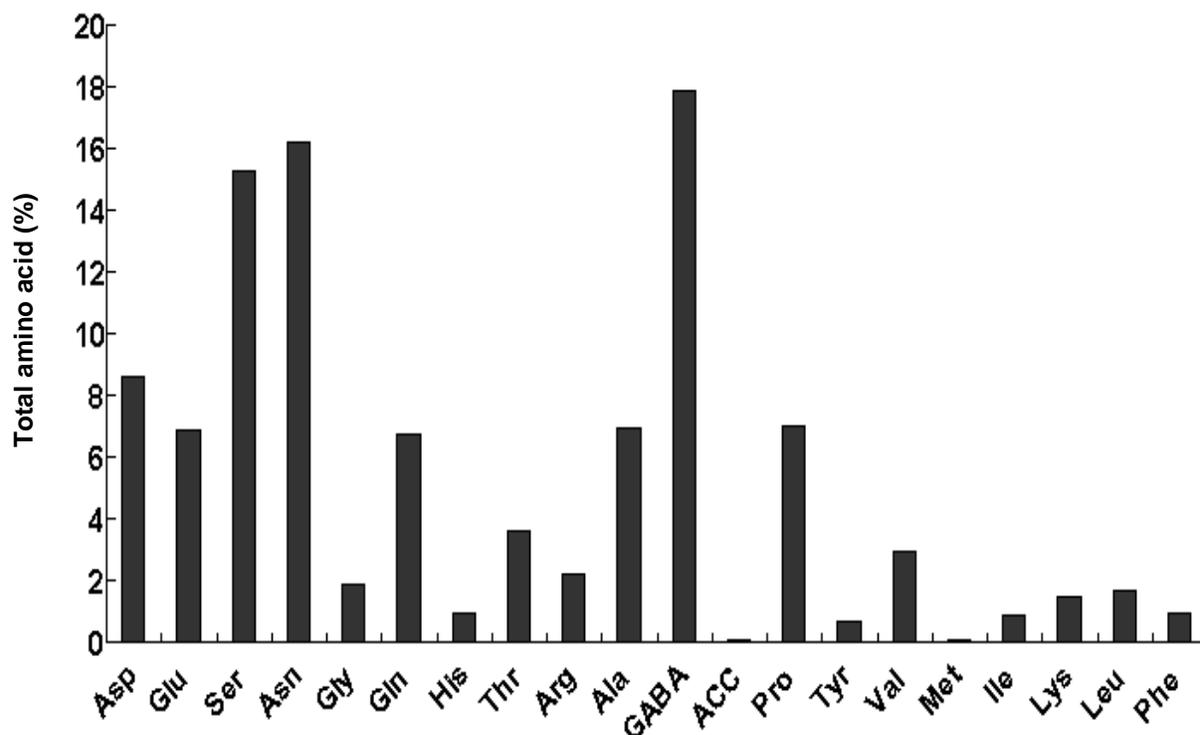
Figure 2. Average of individual amino acids concentration in fully expanded flag leaf of two wheat cultivars (Zagros and Marvdasht). Values are expressed as percentage of total leaf amino acids.

(iii) amino acids with dissimilar levels in Zagros and Marvdasht and changed during dehydration: all other amino acids. The only amino acid, which did not change significantly during dehydration in both Zagros and Marvdasht was Met. Met was low in stressed and unstressed Zagros and Marvdasht (ca. 0.02%).

Seven amino acids, namely γ-aminobutyrate (Gaba), glutamine (Gln), glutamate (Glu), aspartate (Asp), asparagine (Asn), alanine (Ala) and serine (Ser) make up the bulk of free amino acids in the leaf of both cultivars and together constituted c. 75% of the total free amino acid pool found in both unstressed Zagros and Marvdasht (Figure 2). These amino acids showed the highest changes during drought (Figure 3).

The amino acids that accumulated in a different extent in tolerant cultivar during dehydration were: aspartate, glutamate, proline, serine and γ-aminobutyrate (Figure 3). Pro content clearly rose in Zagros from the beginning of the dehydration stress (seven DAA). Pro content increased more rapidly in tolerant cultivar than in sensitive cultivar (during seven DAA) reaching 19% of the total amino acid content at 80% RWC (Table 1), whereas this difference was not evident until 31 DAA in Marvdasht (Figure 3L). One of the most striking results is that dehydrated Zagros and Marvdasht strongly and significantly differed in their Pro content.

Below 80% RWC, a drop in Pro content was observed in Zagros (15 DAA), and still constant till to the end of the experiment (Figure 3K). At the end of dehydration, Pro represented up to 11% of the total amino acid content in Zagros, whereas it was almost 5% of total amino acids in Marvdasht (Figures 3K and L). In contrast with Pro, Asn accumulated in Marvdasht at the end of dehydration stress (Figure 3H).

Asn represented 13 and 23% of the total amino acids contained in stressed Zagros and Marvdasht, at the end of experiment (31 DAA) (Table 1), respectively. During the seven to 15 of dehydration, Asn content dropped dramatically in both cultivars. However, Asn content in drought-sensitive was raised slightly during 15 to 31 DAA but remained at low levels in drought tolerant cv. Regardless of water stress, Asp content decreased slightly by time in both cultivars (Figures 3A and B) and during dehydration stress it represented c. 9.3 and 5.6% of total amino acids in Zagros and Marvdasht, respectively (31 DAA). Asp was weakly but significantly more abundant in dehydrated tolerant cultivar than in sensitive cultivar (Figures 3A and B).

At the beginning of water stress (during 7 DAA), Gln increased to a larger extent in Marvdasht than in Zagros and then decreased sharply in Marvdasht towards the end of the dehydration stress (31 DAA), and at 10 and 48% RWC, represented 4.3 and 5.4% of total amino acids in Marvdasht and Zagros, respectively (Figures 3M and N). Ala amounts did not significantly differ between Zagros and Marvdasht during 15 DAA, but increased to a
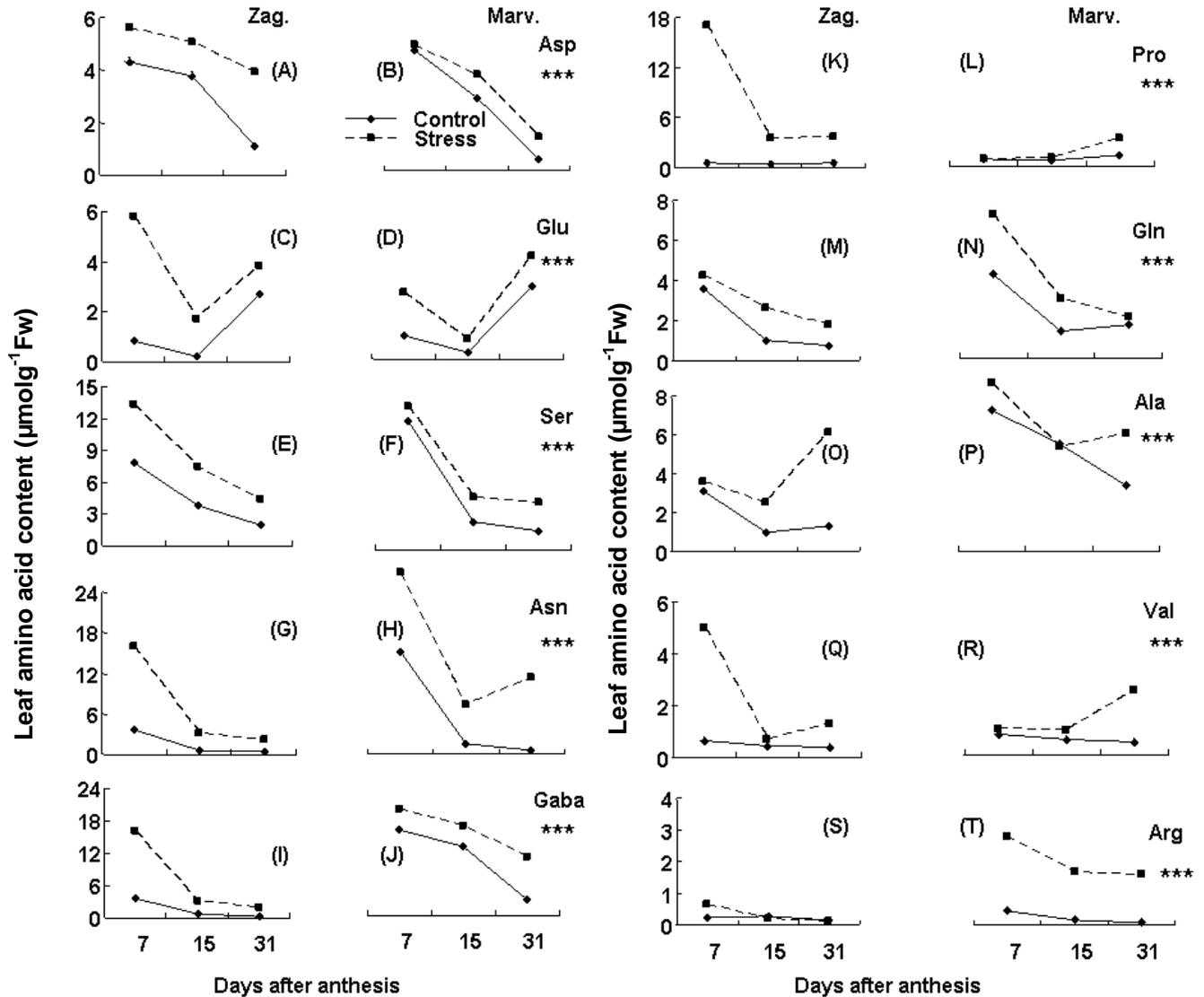
Figure 3. Leaf amino acid content in µmolg⁻¹Fw, of two wheat cultivars when plants were subjected to well watered (control) or water deficit (stress) condition from anthesis to maturity. Each point is the mean of four samples. Error bars are not shown when they smaller than symbols. Asterisks indicate significant differences between cultivars: *** P<0.001.

larger extent (nearly three times) in drought cultivar than sensitive cultivar under water stress condition (Figures 3O and P).

It must be noted that, together with Ser and Asn, Gaba is the most abundant amino acid found in wheat leaves. Like Pro, Gaba has been proposed to be important in the stress protection mechanism (Rhodes et al., 1999). This non-protein amino acid accumulated 28 and 21% of the total amino acids contained in unstressed Zagros and Marvdasht, respectively (Table 1). During the first step of dehydration (seven DAA), Gaba content rose dramatically in both cultivars. However, the concentration was more in tolerant, nearly three-fold with respective to control during seven days after anthesis as compared to sensitive cultivar. Irrespective of water stress, Gaba

levels decreased by time but remained at the higher level as compare to well watered treatment in both cultivars, although the level was more in Marvdasht than Zagros at the end of the experiment (31 DAA) (Figures 3I and J).

Another distinct feature between cultivars was found in leaf Ser concentration (Figures 3E and F), since water limitation cause substantial increment in Zagros (two fold) as compared to control treatment, while this differences was not detectable in Marvdasht during 7 DAA.

Water stress condition caused a substantial increment in Zagros leaf Val level during seven DAA, since the concentration was 7.7 times more than their respective control, whereas no significant differences was observed in Marvdasht leaf Val content until 31 DAA in water stress treatment (Figures 3Q and R). Conversely to Val levels,

the amount of Arg was much higher throughout the water deficit condition in drought-sensitive which is contrary to drought-tolerant cultivar (Figures 3S and T).

Concentration of leucine, lysine, phenylalanine, methionine, isoleucine, histidine, glycine, threonine and amino cyclopropan carboxylic acid (Acc) did not exhibit large or consistent changes during water stress, and absolute contents seldom exceeded 1 µmol g^{-1} on fresh weight basis during dehydration stress in both Zagros and Marvdasht, and did not show a clear trend (Table 1).

DISCUSSION

The identification, in wheat, of leaves that are drought-sensitive and leaves that are drought-tolerant made possible the physiological comparison of desiccation-tolerant and desiccation-sensitive leaf material for the discovery of specific physiological markers. The differential tolerance of wheat leaves depended on leaf age, and as is previously described (Saeidi et al. 2006), Zagros and Marvdasht proved to be drought-tolerant and drought-sensitive, respectively.

Since desiccation tolerance is related to leaf age, the question of the role of the developmental and physiological status of the leaves at the beginning of water stress was assessed. Interestingly, in hydrated tissues (90% RWC), the analyses to determine total amino acid contents showed similar results in Zagros and Marvdasht (Table 1). This finding suggests that the physiological differences that may explain the differential tolerance behaviour of Zagros and Marvdasht actually arose during desiccation stress.

At the beginning of water stress (seven DAA), total free amino acid content increased in both Zagros and Marvdasht, and decreased during seven to 15 DAA, thus suggesting that in the first half of dehydration, leaf nitrogen was translocated to other parts of the plant (roots).

This translocation hypothesis was supported by the high decrease in Asn between 80 and 60% RWC (Figures 3G and H). Previous reports proposed that Asn was utilized for nitrogen transfer to the roots, thus ensuring a metabolic safeguard in stress conditions (Chaffei et al., 2004; Olea et al., 2004). Although, the total amino acid concentration did not differ between Zagros and Marvdasht, individual amino acids differed and might have been individually important for the acquisition of desiccation tolerance. During drought studies with several genotypes of wheat, a concurrence between drought resistance and large increases in free amino nitrogen, or Pro, or both was observed. Pro has been widely proposed as an important amino acid involved in drought stress protection (Hare and Cress, 1997; Rhodes et al., 1999). In dehydrated leaves of wheat, Pro accumulation was only detected in the drought-tolerant Zagros and not in the drought-sensitive

Marvdasht (Figure 3K). Previously, a similar age-related trend in Pro accumulation was observed in wheat during salt stress (Colmer et al., 1995). Paleg et al. (1981) attributed a protective role to proline, as well as valine, against heat denaturation of several enzymes. The fully desiccation-tolerant Zagros accumulated Pro and Val until 80% RWC (seven DAA), had 19 and 5% of total amino acids, respectively, during seven DAA. This study clearly shows that Pro and Val were required as protectant in drought-tolerant cultivar.

Several reports emphasize the role of Gaba as a protectant against reactive oxygen species (ROS) in response to environmental stresses in plants (Rhodes et al., 1999). Similar changing pattern of Pro concentration, Gaba and Asn content in cv Zagros was markedly increased during the first step of leaves dehydration (seven DAA) leaves. Gaba increase in leaves of Zagros cv. suggested that the ROS level that was produced and increased under severe water stress can be controlled by Gaba shunt activation (Fait et al., 2005) in drought-tolerant cultivar. Finally, the accumulation of high concentration of Gaba has a function in the acquisition of desiccation tolerance in Zagros. Asn, being composed of two nitrogen and four carbon atoms, is an 'economical' way of storing nitrogen. Asn may also represent a suitable compound for the storage of nitrogen during the stress. Asn build-up has indeed been observed in several water-stressed plants (Stewart and Larher, 1980; Sieciechowicz et al., 1988) and has been proposed as a transient nitrogen storage metabolite in sugar-starved maize roots (Brouquisse et al., 1992) and seeds during dehydration and germination (Canas et al., 2006; Fait et al., 2006), and sink leaves during salinity stress (Gilbert et al., 1998). Under stressed water condition, Zagros leaf Asn levels increased 4.3 times with respect to control treatment, whereas in Marvdasht, the value reached up to 1.4 fold as compared to well watered condition. At the end of experiment, leaf Asn level in drought-sensitive was markedly higher than that in drought-tolerant, we speculated that the higher reserves deposited probably represent low transport efficiency of storage material, resulting in multiple factors like enzyme involved in carbohydrate utilization and storage (Wang et al., 1993).

The relative amount of Arg in Zagros leaf, opposite to Marvdasht exhibited little changes throughout water deficit condition (Figures 3S and T). Interestingly, considerable changes were observed in the proportion of the main Pro precursor, Glu, which may indicate that Pro is synthesized mainly using Glu as a precursor under stress conditions. In contrast, Marvdasht accumulated a wider pattern of amino acids. This is probably due to slower amino acid interconversions during the dehydration that finally resulted to higher levels of Leu, Lys, Tyr, Phe and His in dehydrated Marvdasht leaf.

According to principle component analysis (Figure 3) of the relative amounts of all amino acids, the greatest difference between the two genotypes in the amino acid

composition was observed at seven days after imposed water stress. The present report shows that Glu might have been channeled into two different pathways leading to Pro and Gaba biosynthesis in Zagros and Arg and Asn accumulation in Marvdasht. In addition, under stress, only the Arg pathway is used for polyamine synthesis, which could be a reason for the great decrease of relative Arg content in drought-tolerant cultivar (Galiba et al., 1993). The principal findings of this study include the large accumulation of Asn and Arg in the drought-sensitive Marvdasht and the accumulation of Pro and Gaba in the drought-tolerant Zagros.

ACKNOWLEDGEMENT

REFERENCES

Bandurska H (2000). Does Pro accumulated in leaves of water deficit stressed barley plants confine cell membrane injury? I. Free Pro accumulation and membrane injury index in drought and osmotically stressed plants. Acta Physiol. Plant. 22: 409-415.

Brouquisse R, James F, Pradet A, Raymond P (1992). Asparagine metabolism and nitrogen distribution during protein degradation in sugar-starved maize root tips. Planta, 188: 384-395.

Canas RA, De la Torre F, Canovas FM, Canton FR (2006). High levels of asparagine synthetase in hypocotyls of pine seedlings suggest a role of the enzyme in re-allocation of seed-stored nitrogen. Planta, 224: 83-95.

Chaffei C, Pageau K, Suzuki A, Gouia H, Ghorbel MH, Masclaux-Daubresse C (2004). Cadmium toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. Plant Cell. Physiol. 45: 1681-1693.

Colmer TD, Epstein E, Dvorak J (1995). Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and salt-tolerant wheat 3 *Lophopyrum elongatum* (Host) A. love amphiploid. Plant Physiol.108: 1715-1724.

El Tayeb MA, Hassanein AM (2000). Germination, seedling growth, some organic solutes and peroxidase expression of different *Vicia faba* lines as influenced by water stress. Acta. Agron. Hung. 48: 11-20.

Fait A, Yellin A, Fromm H (2005). GABA shunt deficiencies and accumulation of reactive oxygen intermediates: insight form Arabidopsis mutants. FEBS Lett. 579: 415-420.

Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, Fernie AR, Galili G (2006). Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. Plant Physiol. 142: 839–854.

Galiba G, Kocsy G, Kaur-Sawhney R, Sutka J, Galston AW (1993). Chromosomal localisation of osmotic and salt stressinduced differential alterations in polyamine content in wheat. Plant. Sci. 92: 203-211.

Gilbert GA, Gadush MV, Wilson C, Madore MA (1998). Amino acid accumulation in sink and source tissues of Coleus blumei Benth. during salinity stress. J. Exp. Bot. 49: 107-114.

Good AG, Zaplachinski ST (1994). The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. Physiol. Plant. 90: 9-14.

Hanson AD, Hitz WD (1982). Metabolic responses to water stress. Anal. Rev. Plant Physiol. 33: 163-303.

Hare PD, Cress WA (1997). Metabolic implications of stress induced proline accumulation in plants. Plant Growth Regul. 21: 79-102.

Hsiao TC (1973). Plant responses to water stress. Anal. Rev. Plant. Physiol. 24: 519-570.

Jones MM, Osmond CB, Turner NC (1980). Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. Aust. J. Plant Physiol. 7: 193-205.

Navari-izzo F, Quartaccr MF, Izzo R (1990). Water-stress induced changes in protein and free amino acids in field-grown maize and sunflower. Plant Physiol. Bioch. 28: 531-537.

Noctor G, Novitskaya L, Lea PJ, Foyer CH (2002). Co-ordination of leaf minor amino acids contents in crop species: significance and interpretation. J. Exp. Bot. 53: 939-945.

Olea F, Perez-Garcia A, Canton FR, Rivera ME, Canas R, Avila C, Cazorla FM, Canovas FM, De Vicente A (2004). Upregulation and localization of asparagine synthetase in tomato leaves infected by the bacterial pathogen Pseudomonas syringae. Plant Cell Physiol. 45: 770-780.

Paleg LG, Douglas TJ, Van Daal A, Keech DB (1981). Proline, betaine and other organic solutes protect enzymes against heat inactivation. Aust. J. Plant Physiol. 8: 107-112.

Rhodes D, Verslues PE, Sharp RE (1999). Role of amino acids in abiotic stress resistance. In: Singh BK, ed. Plant amino acids: biochemistry and biotechnology. New York: Marcel Dekker. pp. 319-356.

Saeidi M, Moradi F, Ahmadi A, Poostini K, Najafian G (2006). Effect of exogenous application of ABA and CK at different stages of grain development on some physiological aspects of source and sink relationship in two bread wheat cultivars. Iran. J. Crop Sci. 8: 268-282.

Sieciechowicz KA, Joy KW, Ireland RJ (1988). The metabolism of asparagine in plants. Photoch. 27: 663-671.

Stewart CR, Larher F (1980). Accumulation of amino acids and related compounds in relation to environmental stress. In: Miflin, B.J. ed. The Biochemistry of Plants: a comprehensive treatise, New York: Acad. Press. 5: 609-635.

Trotel-Aziz P, Niogret MF, Deleu C, Bouchereau A, Aziz A, Larher FR (2003). The control of Pro consumption by abscisic acid during osmotic stress recovery of canola leaf discs. Physiol. Plant. 117: 213-221.

Wang F, Sanz A, Brenner ML, Smith A (1993). Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol. 101: 321-327.

Zagdanska B (1984). Influence of water stress upon photosynthetic carbon metabolism in wheat. J. Plant Physiol. 116: 153-160.