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Water relation, solute accumulation and cell membrane injury in sesame (*Sesamum indicum* L.) cultivars subjected to water stress

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ABSTRACT

In this investigation, three cultivars of sesame (Sesamum indicum L.) were studied for their behaviors to water limited condition. The effect of water stress on relative water content, content of proline and total soluble sugar and cell membrane injury were determined. Five drought tolerance indices were calculated based on grain yield under drought (Ys) and normal (Yp) condition. The results indicated that water deficit reduced the relative water content of the leaves and increased proline in all of the studied cultivars. The studied cultivars don't showed a significant increase in their TSS when imposed in stress condition. Leaves of plants pre-stressed in field condition and then subjected to osmotic stress in laboratory exhibited about a % 40.5 lower membrane injury than other of not pre-stressed plant. The Oltan cultivar with having of the largest amount of STI and lower value of SSI and TOL recognized as a drought tolerance cultivar in compare with two other cultivars. Also, this cultivar had the highest increase in amount of proline content in water stress condition and showed not significant decrease in its leaf water content.

Key words: membrane injury, proline, RWC, *Sesamum indicum* , Water stress.

INTRODUCTION

One of the major environmental factors limiting the crop productivity in different parts of the world is on osmotic stress resulting from drought. Two major strategies of plant resistance to water deficit rely on water stress avoidance and/or water stress tolerance. Water stress avoidance consists of mechanisms enabling plant to maintain water uptake and high tissue water potential. Water stress tolerance refers to the ability of a plant to withstand dehydration [1]. In water stress condition, growth is inhabited and a variety of molecular, biochemical and physiological modification are produced [2]. Under severe water stress condition caused by high drought, plants stop growing completely and accumulate solutes in cell in order to maintain the cell volume and turgor against dehydration. This phenomenon is known as osmotic adjustment. Osmotic adjustment has been observed in root, stem, leaves and fruits [3].

Proline and quaternary ammonium compounds are key osmolytes, which help plant to maintain the cell turgor [4]. Moreover, there is additional evidence that these compatible solutes are activation of the enzymes or loss in membrane integrity due to a water deficiency [5]. Proline appeared to be mainly involved in protection against oxidative stress than osmotic adjustment during initial steps of water stress [6]. It has been indicated that proline lowers the generation of highly destructive free radicals species [1] [7]. Arish et al. [8] examined drought tolerance and drought sensitive genotypes of Cotton (*Gossypium hirsutum L.*) subjected to water stress. Water stress caused a significant increase in proline levels in leaves of both tolerant and sensitive genotypes. The leaf of tolerant genotype maintained higher RWC, photosynthetic activity and proline levels under water stress than that of drought sensitive genotype. Sucrose, as a member of the sugar family, is thought to function as a typical osmoprotectant, stabilizing cellular membranes and maintain turgor [9]. It has been suggested that under water stress soluble sugar can function in two ways which are difficult to separate: as osmotic agents and as osmoprotectors [10]. As osmoprotectors, sugars stabilize proteins and membranes, most likely substituting the water in the formation of hydrogen bonds with polypeptide polar residues [11].

Three cycles of drought generally induced lower leaf relative water contend (RWC) and cell membrane stability (CMS), and elevated concentration of soluble sugars was also observed following drought treatment [12]. Relative water content was found to be a major factor in a plant's capability to survive adverse water condition [13]. It is known that under stress condition the primary object of injury is the plasmalemma [1]. A consequence of the altered membrane integrity is increase of the cell permeability which is accompanied by electrolyte leakage from the cell [14]. The degree of cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance and can be used to screening drought- tolerant genotypes [15]. The research into drought stress has until now diverted toward whole plant with particular emphasis on the exploitation of high yield potential under normal and drought stress condition or selection the genotypes for morphological and physiological characters responsible for drought resistance [16]. If drought screening is based on grain yield, genotypes selected many have high potential yield or appropriate phenology, but not drought tolerance [17]. Among the stress tolerance indicators, a larger value of TOL and SSI represent relatively more sensitivity to stress, thus a smaller value of TOL and SSI are favored. Selection based on these two criteria favors genotypes with low yield potentional under non- stress conditions and high yield under stress conditions. On the other hand, selection based on STI and GMP will be resulted in genotypes with higher stress tolerance and yield potential will be selected [18]. The present investigation was conducted to evaluation of sesame cultivars under moisture stress and determination the role of proline and total soluble sugar accumulation in protecting cell membranes against injuries induced by water stress.

MATERIALS AND METHODS

Plant material and growing condition

The experiment was conducted at the experimental farm of Moghan agriculture and neutral resource research center, Iran (latitude 39° 39' N, longitude 47° 18' E and 78 m.s.l) from June to October 2008. Average rainfall is about 221 mm that most rainfall concentrated between winter and spring. The soil was silty loam with EC about 2 ds m^{-1} and pH about 8. The experiment was spilt plot in randomized complete block design with four replications. Two irrigation levels including irrigation after 90 and 120 mm evaporation from class A pan, as main factor and three sesame cultivars (Oltan, Hendi and Hendi 14) as subplot factor. Drought stress was imposed at irrigation after 120 mm, while the control plats were irrigated after 90 mm evaporation from class A pan (According to results of pervious experiments). Plot size was 4×3 m with 4 rows per plot, row spacing was 60 cm and distances between plants in the rows were 10 cm.

Stress tolerance indices

After separation of border effects from each plots, yield potential (Yp) and stress yield (Ys) were measured. Mean productivity (MP) and tolerance index (TOL) were calculated using the formula suggested by Rosielle and Hambelman. [19].

$$MP = (Y_P - Y_S)/2$$
$$TOL = (Y_P - Y_S)$$

For the calculated of stress susceptibility index (SSI) was used from the formula suggested by Fischer and maurer. [20]:

$$SSI = [1 - (Ysi / Ypi)] / SI$$

Where D is the stress intensity and calculated as:

$$SI = 1 - (\bar{Y}s / \bar{Y}p)$$

Also Stress Tolerance Index (STI) was calculated by the following formula: [18]

$$STI = (Ypi \times Ysi) / (\bar{Y}p)2$$

Where Y_P and Y_S are the yield of genotypes evaluated under stress and non-stress conditions and $\bar{Y}s$ and $\bar{Y}p$ are the mean yields over all genotypes evaluated under stress and non- stress conditions. Finally Y_P, Y_S, STI, SSI, TOL and MP were used to select drought tolerant lines.

Determination of relative water content (RWC)

To determine plant RWC, ten levees taken from the basal part of each plot, the samples of leaf were weighed (fresh weight, FW), then placed in a distilled water vial for 4h at 25°c and their turgid weights (TW) were measured. Dry weights (DW) were measured after oven-drying the sample for 24h at 75°c [21]. Relative water content was calculated by the following formula:

RWC= (fresh weight –dry weight) / (fresh weight of full turgor-dry weight) ×100 %.

Proline determination

The proline content was determined according to Bates et al. [22]. 0.5 g of leaf samples from each plot were extracted with 3 % (W/V) sulphosalycylic acid. After addition of 2 ml ninhydrin and 2 ml glacial acetic acid, mixture was heated at 100 c for 1 h in water bath and then was extracted with toluene (4 ml). Proline content was measured by a spectrophotometer and absorbance was read at 520 nm. Proline concentration was determined using calibration curve and expressed as m mol g^{-1} FW.

Measurement of soluble sugars

Sugar content was determined with the somewhat modified antron method [23].

Determination of membrane injury index

20 leaf discs (2 cm diameter) were taken from the basal part of stressed and unstressed plants and washed three times with 10 ml distilled water. Samples of each combination were divided into two groups. Leaf samples from one group of stressed and control plants subjected to osmotic stress evoked by immersion in 10 ml PEG solution (osmotic potential op -1.6 MPa) and kept for 24 h at 10 $^{\circ}$C. After stress treatment, leaf samples were washed with distilled water and then immersed in 20 ml of distilled water at room temperature. Leaf samples from another group (of stressed and control plant) also, were immersed in distilled water. After 24 h the electrical conductivity of the solutions was measured. Then the tissues were killed by autoclaving for 15 min, cooled to room temperature and the electrical conductivity of the solutions was measured once again. This technique is a modification of the method developed by Dexter et al. (1932). Membrane injury was estimated as the percentage injury according to the Sullivan [24] formula:

$$I= [1-(1-T_1/T_2)/ (1-C_1/C_2)] \times 100 \%$$

Where T_1 and T_2 represent conductivity values for water–stressed (exposed to an osmotic stress PEG-1.6 MPA) samples before and after autoclaving, respectively; c_1 and c_2 represent conductively valves of control samples before and after autoclaving, respectively.

Statistical analysis

Analysis of variance (ANOVA) for evaluated traits was performed using SAS program [25] and the mean values were compared with least significant difference (LSD) test.

RESULTS AND DISCUSSION

According to the decrease in yield of tested cultivars, the stress intensity estimated .119 in treatment with irrigation after 120 mm evaporation from class A pan. SI ranges between 0 and 1 and the lower value of SI, the mild is the stress intensity. Among the studied cultivars, the Oltan cultivar had the lowest SSI and TOL, also the largest STI and MP (Table.1). Generally, the cultivars with high value of STI and MP and low value of TOL have the high tolerant to stress condition [26]. The effect of water stress on Relative Water Content (RWC) in the leaves of the three tested cultivars is shown in (fig.1a). Water stress reduced the relative water content of the leaves. The three cultivars differed in their leaf water content under the applied stress. The Hendi 14 showed higher RWC compared to the other cultivars. It tend to loss more water when pass in to stress condition. Oltan had the lowest RWC, but had lost less water between the control and the stressed regime The Hendi 14 cultivar had the 21% reduction in RWC, followed by the Hendi (16%) but the Oltan cultivar don't showed a significantly decrees in RWC in drought condition (fig.1a). Relative water content was found to be a major factor in a plant's capability to survive adverse water condition [27]. Significant differences had been reported between cultivars. Resistant cultivars maintain high RWC than sensitive one[29]. The proline accumulation increased in leaves of the three cultivars when plant subjected to stress condition. Althout the initial amount of free proline in leaves of the three cultivars is similar in control, but the cultivars studied, showed a large and varying amount of proline accumulate in their leaves when imposed in stress condition. After imposition of mild stress the amount of proline content in the C. Oltan was twice the amount in Hendi 14 (Fig.1b). Proline, which increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selecting drought-resistant varieties [22]. Vendruscolo et al. [29] found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plant to avoid detrimental effects of water stress. The studied cultivars don't showed a significant increase in their TSS when imposed in stress condition (Table.3.).

The percent of membranes injury was estimated from the electrolyte leakage data of the stressed plants. Leaves of plant pre-stressed with mild water deficit and the subjected to sever osmotic stress exhibited about 45.5% lower membrane injury than those of not pre-stressed plants. It can be related to accumulate solutes in cell in order to maintain the cell volume during mild water stress and when plant subjected to sever osmotic stress (PEG -1.6 Mpa) can protection cellular structures during dehydration. Differences were noted in percentage of injuries between the cultivars. Dehydration tolerance was not enhanced equally in all examined cultivars. The largest difference in CMJ between the control and stressed plants was found in the Oltan (77/8 %) followed in decreasing order, by the Hendi 14 (35.7 %), and the Hendi (16.4 %). (Fig.1c).

In our experiments, generally the cultivar more tolerant to drought stress (Oltan) exhibited lower decrees in RWC when exposed in mild drought stress. While, Hendi 14 and Hendi cultivars which is characterized by the lower proline content in stressed leaves, exhibited a larger decrease in the leaf water content influence of mild water stress. Turkan et al. [30] found a higher proline accumulation in drought-tolerant *Phaseolus acutifolius* than in drought-sensitive *P.vulgaris*. They suggested that a directed consequence of higher proline concentration in tolerant species is the relatively higher water retaining capacity a reflected by RWC. Proline is one of the most water soluble amino acids and it is supposed to play a significant role in osmotic adjustment with regarded to reduction of osmotic potential due to a net accumulation of solutes [31]. Also Banduska. [21] reported that proline may take part in osmotic adjustment, which enables cell to maintain turgor during water stress.

Fig.1. Changes in:a) relative water content, b) proline content, c) injury index (%) in leaves of three sesame cultivars subjected to water stress.

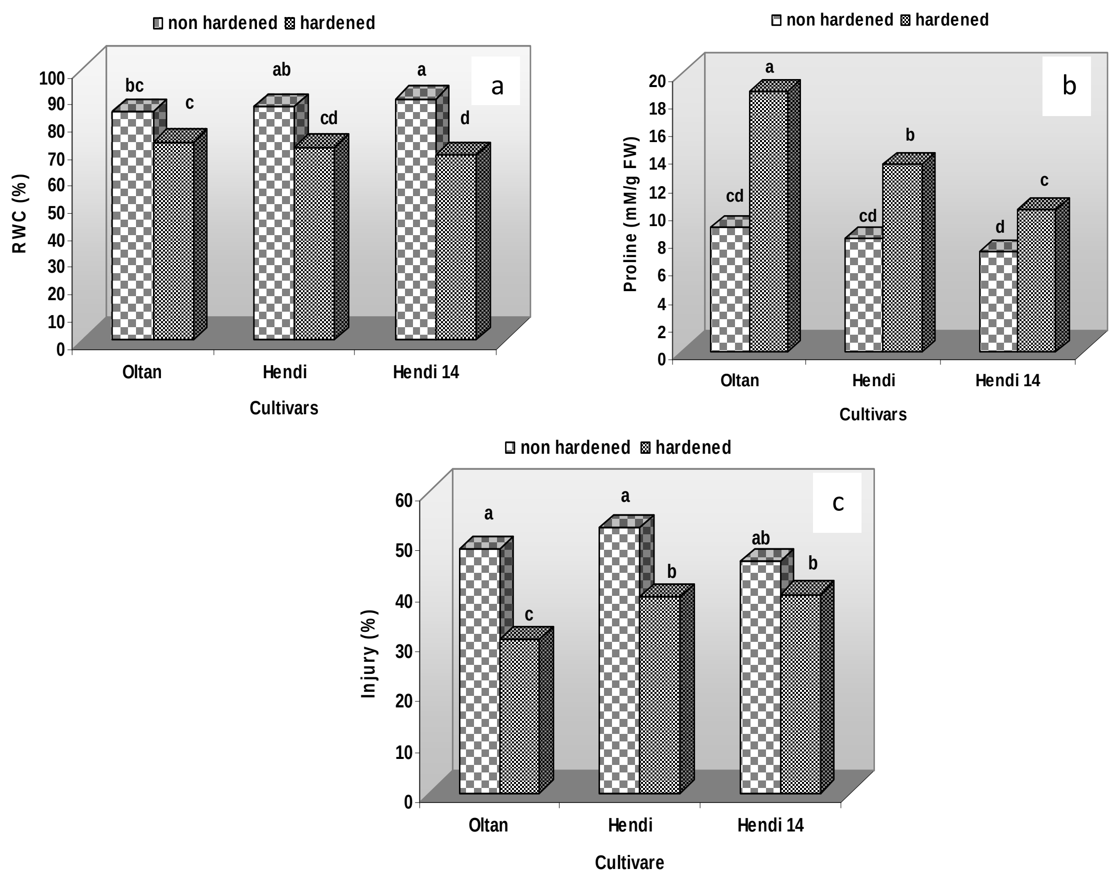

Table 1. Drought tolerance indices for studied cultivars

Genotype	Y_P	Y_S	SSI	STI	TOL	MP
Oltan	1433	1346	0.788	1.03	87	1385.5
Hendi	1299	1193	1.05	0.835	106	1246
Hendi 14	1354	1257	1.16	0.899	121	1293.5

Table 2. Simple correlation coefficient of the traits

	CMJ	Proline	TSS
CMJ	1		
Proline	-0.455*	1	
TSS	-0.152	0.266	1

**: significant at 5% level. CMJ: Cell Membrane Injury (%), TSS: Total Soluble Sugar*

Table 3: Mean squares from the analyses of variance

Source of variation	df	RWC	CMJ	Proline	TSS
r	3	9.819	21.069	0.603	6.409
irrigation	1	1584.37**	299.48*	214.32**	20.535
Error (a)	3	17.15	54.509	0.269	5.082
varity	2	2.041	29.101	52.65**	0.711
İ× V	2	71.375	149.781	25.58**	12.24
Error (b)	12	29.486	52.577	0.918	22.609

The Hendi 14 which is characterized by the lowest and similar accumulation of free proline in deferent of leaves after exposure to mild water stress, showed a perceptible decrease in the cell membrane injury in our leaves under condition of strong stress. Volair and Thomas. [32] reported that *dactylic glomerate* L. leaves exhibited a greater amount of membrane elasticity in drought resistant genotypes, there by having a greater RWC. For determinate the role of proline and soluble sugar accumulation in maintain of relative water content and cell membrane stability in leaves of plant under water stress condition simple correlation among the studied traits were calculated. There were a significant and negative correlation between proline accumulation and CMJ. (Table 3.) Synthesis of proline and proteins, which have been implicated to have a role in protecting cellular structures during dehydration and it, enables plant to survive cellular water deficits [33]. However, the accumulation patterns were reflected in a degree of alleviation of cell membrane injuries since almost similar effects were observed in the level of all genotypes regardless of accumulation proline level during accumulation. (fig 1a). Many authors reported positive correlation between the capacity for praline accumulation and dehydration tolerance ([34] and reference therein). The degration of phospholipids in response to drought stress has also been reported [24]. Jagtap and Bhargavas. [35] found that sorghum manipulated its antioxidant defense system and was able to lower free oxygen radicals in drought resistant genotypes. Membrane lipids would be protected by this action.

Volair and Thomas. (1995) reported that *dactylic glomerate* L. leaves exhibited a greater amount of membrane elasticity in drought resistant genotypes, there by having a greater RWC.

CONCLUSION

The effect of water stress on some physiological aspects of sesame (*Sesamum indicum* L.) cultivars studied in this work**.** Water deficit reduced the relative water content of the leaves and increased proline in all of the studied cultivars. But the cultivar more tolerant to drought stress (Oltan) exhibited lower decrees in RWC when exposed in mild drought stress which correlated positively with the higher proline content. Also significant negative correlation was found between cell membrane injury and proline content. This finding suggests the extent of the certain relationship between accumulation of proline and it resistance to drought or dehydration. Vasquez-Tello et all (1990) reported that when plant subjects to a mild water stress the proline accumulate in cell in order to maintain the cell turgor against dehydration and it can reduction of membrane injury index. An important strategy for the development of drought resistance in plants is the maintenance of cell membrane integrity after the imposition of water stress.

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