

## Evaluation of Heat Shock and Salicylic Acid Treatments as Inducers of Drought Stress Tolerance in Hassawi Wheat

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**Abstract:** The present study was designed to evaluate the influence of salicylic acid (50 ppm) or/and heat shock on growth, some metabolic activities and protein patterns of Hassawi wheat (*Triticum aestivum* L. cv. Hassawi) plant. The experimental plant was subjected to three drought stress levels, 100, 60 and 30% field capacity. The results revealed that, there were no significant changes in dry weight and tissue water content in roots and shoots of Hassawi wheat at 60% field capacity, whereas they were significantly reduced at 30% field capacity. However, root/shoot dry weight ratio was increased by increasing of drought level. Drought stress especially at 30% field capacity induced a marked decrease in the content of photosynthetic pigments. The contents of soluble carbohydrate, protein and proline were significantly increased, while an opposite trend was obtained with respect to insoluble carbohydrates and proteins. The content of total free amino acids was significantly increased in roots, while it was decreased in shoots. Salicylic acid or/and heat shock treatments stimulated growth, photosynthetic pigments and accumulation of soluble and insoluble carbohydrates and proteins, but opposite effects were observed on the biosynthesis of free amino acids and proline. In addition to that, drought stress showed different *de novo* protein bands with different high molecular weights in plants treated or untreated with SA and heat shock either individually or in combination.

**Key words:** Amino acids, carbohydrates, photosynthetic pigments, proline, protein patterns

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### INTRODUCTION

Drought stress is characterized by water losses that exceed the absorption rate (Costa *et al.*, 2008). Water deficit can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle (Zhu, 2002). The lack of adequate moisture leading to water stress is a common occurrence in rainfed areas, brought about by infrequent rains and poor irrigation (Wang *et al.*, 2005). Saudi Arabia is predominately an arid country and the shortage of irrigation water, is one of the limiting

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factors for maximizing cropping intensities and obtaining higher crop yield. The problem of drought stress has presented a challenge to grow wheat in dry regions which represent the majority of the Saudi Arabia area.

Wheat occupies a unique position in the agriculture economy. Wheat (*Triticum aestivum* L. cv. Hassawi) is one of the most economic important crops and a major source of food in Saudi Arabia. Drought and heat stress are major limiting factors in crop production, because they affect almost all plant functions (Hern-Ndez *et al.*, 2001). The most common symptoms of water stress injury in plants is the inhibition of seedling growth, which is reflected in a reduction in the fresh and dry matter yields (Le Thiec and Manninen, 2003). It is well known that plant growth inhibition under water stress is associated with altered water relations (Dichio *et al.*, 2003). The detrimental effects of drought stress on plants are a consequence of osmotic strain on the cytoplasm. Water stress was reported to induce the accumulation of cytosolutes (soluble carbohydrates and proteins, free amino acids and proline) in various plants (Ain-Lhout *et al.*, 2001; Nath *et al.*, 2005; Sankar *et al.*, 2007) as well as variation on the amount of photosynthetic pigments, mainly chlorophylls and carotenoids (Chandrasekar *et al.*, 2000). The accumulation of these molecules may serve as means of osmotic adjustment, which improve plant's tolerance to oxidative stress (Ma and Turner, 2006; Mohsenzadeh *et al.*, 2006). The osmotic adjustment is considered an important mechanism developed by the plants to tolerate water stress, which promotes protection of plant cell structures (Martinez-Ballesta *et al.*, 2004). Nguyen *et al.* (2004) reported that drought resistance in rice depends mainly on the capacity of osmotic adjustment to allow plants to maintain turgor, protect meristems from desiccation and on the ability to control and reduce water loss.

The adaptation of plant to drought or heat stress is accompanied by alterations in the level of protein patterns. Under these conditions, synthesis of some proteins is repressed while the others start to be *de novo* synthesized. Some of these proteins were suggested to protect the cell against the harmful effect of drought or heat stresses. The alteration of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought tolerance (Chandler and Robertson, 1994; Ouvrard *et al.*, 1996). Changes in protein expression, accumulation and synthesis have been observed in many plant species as a result of plant exposure to drought or heat stress during growth (Saleh *et al.*, 2007; Mohammadkhani and Heidari, 2008). Proteins synthesized in response to drought stress are called dehydrins (dehydration induced). It has been proposed that those proteins have important functions in protecting cells from damage under drought stress and may also have a role similar to compatible solutes in osmotic adjustment (Bracale *et al.*, 1997).

The role of Salicylic Acid (SA) and Heat Shock (HS) under abiotic stress is critical in modulating physiological responses that will eventually lead to adaptation to an unfavorable environment. Exposure of wheat or rice seedlings to heat shock at 42°C resulted in a significant increase in tolerance to drought stress (Hamada and Khulaef, 1995; Sato and Yokoya, 2008). The SA is an endogenous growth regulator, which influence a range of diverse processes in plants. The SA treatment increased resistance to abiotic stresses of many crop plants such as maize, wheat and sunflower (Pál *et al.*, 2005; Wang *et al.*, 2006; Noreen and Ashraf, 2008), respectively. Exogenous application of SA decreased the inhibitory effect of drought stress in tomato, beans and wheat (Senaranta *et al.*, 2000; Sakhabutdinova *et al.*, 2003) and salt stress in broad bean (Azooz, 2009).

Many studies have focused on the impact of one single environmental stress event, such as drought stress or heat shock (Reddy *et al.*, 2004; Camejo *et al.*, 2005) whereas the combined effect of more than one type of stress on plant metabolism has received less

attention. The overlapping between more than one stress may explain the well-documented phenomena of cross tolerance in which a particular stress can induce in plant resistance to a subsequent stress that is different from the initial one (Bowler and Fluhr, 2000). Therefore, the objective of this study was to evaluate the effect of drought stress, SA and HS individually or in combination on growth, water status, some metabolic activities and protein patterns of Hassawi wheat. This study aims to investigate the application of SA or/and HS would protect Hassawi wheat plants from damaging effect of drought stress and determine whether application method would be more effective. This may be share to improve the resistance of Hassawi wheat plants to the action of drought stress and high temporarily temperature in Saudi Arabia.

## MATERIALS AND METHODS

### Growth Conditions

Seeds of Hassawi wheat; (a local variety from Al-Hassa, Saudi Arabia) were surface sterilized in an aqueous solution of 0.1% HgCl<sub>2</sub> for 1 min, with frequent shaking and then thoroughly washed three times with distilled water. The washed seeds were divided into 2 groups. The first group was soaked in distilled water and the second group was soaked in 50 ppm aqueous solution of SA for 8 h. They were germinated on filter paper moistened with distilled water in petri dishes until complete germination. Then, each group was divided into two sub-groups. The first sub-group of each was exposed to heat shock at 40°C for 3 h, while the others sub-groups were left without treatment. Thus, the experiment included four sub-groups; the first was untreated, the second and third groups were plants treated with SA and HS, respectively and the fourth sub-group was treated with both SA and HS. The germinated seeds of all these groups were sown in plastic pots containing 2 kg dry soil. Then they were placed in growth chamber maintained at 25/20°C light/dark (12 h) and 60% relative humidity and grown under fully conditions at 100% Field Capacity (FC) for 5 days. Thereafter, the pots of each sub-group were divided into three sets; the first set was maintained at 100% FC, the second and third at 60 and 30% FC, respectively for the entire experimental period. Six replicates from each treatment were prepared.

At the end of the experiment (21 days) the plants were uprooted and split up into root and shoot systems, washed with deionized water, blotted on paper towels and freshly weighed. To determine dry weight; roots and shoots were dried in an aerated oven at 80°C to a constant weight, then grinded into fine powder and stored for the various analytical experiments.

### Determination of Metabolic Activities

#### Photosynthetic Pigments

Chlorophylls (chl. a, b) and total carotenoid contents in Hassawi wheat leaves were estimated in 80% acetone extracts according to the method of Lichtenthaler and Wellburn (1983).

#### Determination of Carbohydrates

Carbohydrates of roots and shoots were extracted from plant tissues and determined by anthrone sulphuric acid method (Badour, 1959). One milliliter of the carbohydrate extract was mixed with 9 mL of anthrone sulphuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbency was read spectrophotometrically (Spectronic Genesys ZPC, Rochester, NY, USA) at 620 nm against blank containing only distilled water and anthrone reagent. The results were expressed as mg g<sup>-1</sup> dry weight.

### **Determination of Proteins**

The contents of soluble and insoluble proteins were determined using Bovine Serum Albumin (BSA) as a standard, according to the method of Bradford (1976). One milliliter of Bradford solution was added to 100  $\mu$ L protein extract and the absorbency was recorded at 595 nm. The protein concentration was calculated from a BSA standard curve and was expressed as mg g<sup>-1</sup> dry weight.

### **Determination of Total Free Amino Acids**

Total free amino acids content were extracted and determined according to the method of Lee and Takahashi (1966). Exactly 0.1 mL of the water extract containing free amino acids was mixed with 1.9 mL of ninhydrin-citrate-glycerol mixture in a test tube for 20 min at 100°C. The absorbency was recorded at 570 nm against blank (only distilled water and the same reagent).

### **Determination of Proline**

Proline content was determined according to Bates *et al.* (1973). Samples of roots and shoots (0.2 g) were homogenized in a mortar and pestle with 3 mL sulphosalicylic acid (3% w/v) and then centrifuged at 18,000 g for 15 min. Two milliliter of the supernatant were added to a test tube, to which 2 mL glacial acetic acid and 2 mL freshly prepared ninhydrin reagent were added. The test tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature. The reaction mixture was extracted with 4 mL toluene. The chromophore was aspirated from the aqueous phase and the absorbency was read at 520 nm in a spectrophotometer using toluene as a blank. The proline content was determined from a standard curve prepared with proline and the results were expressed as mg g<sup>-1</sup> dry weight.

### **Electrophoresis**

Shoot samples were homogenized and extracted in 50 mM sodium phosphate buffer (pH 7.5). Protein samples were prepared by mixing the extract with 2X SDS-PAGE treatment buffer and boiled for 4 min. The denatured protein samples were analyzed by vertical one dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to the procedure of Laemmli (1970). Protein bands in the gel were visualized by a Coomassie Brilliant Blue R-250 (CB) with Bismarck Brown R (BBR) according to Choi *et al.* (1996).

### **Statistical Analysis**

All data were analyzed statistically by one-way ANOVA based on SPSS (version 11.0) program. Values in the figures indicate the mean values $\pm$ SD based on six independent determinations (n = 6) and the Least Significant Difference (LSD) was used to test the difference between treatments; p = 0.05 was considered statistically significant (Steel and Torrie, 1980).

## **RESULTS**

### **Growth Parameters**

The results (Fig. 1a-e) concerning dry weight, water content and root/shoot dry weight ratio in roots and shoots of wheat (*Triticum aestivum* L.cv. Hassawi), subjected to drought stress either treated or non-treated with Salicylic Acid (SA) or Heat Shock (HS) alone or in

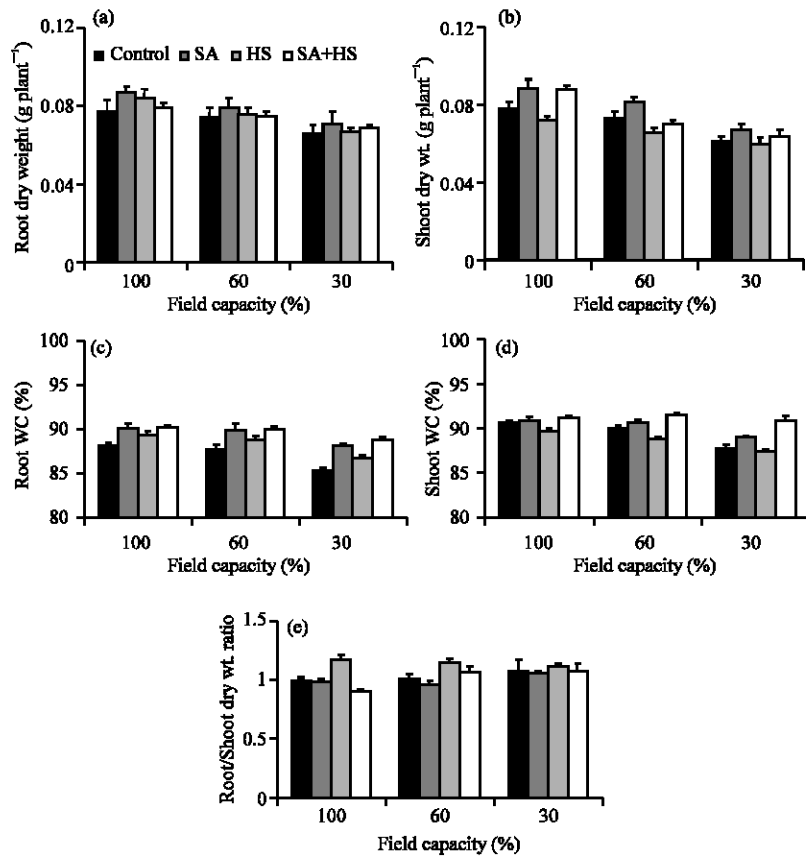


Fig. 1: Effect of drought stress, Salicylic Acid (SA) and Heat Shock (HS) alone or in combination on dry weight of (a) root, (b) shoot, (c) water content of root, (d) shoot and (e) root/shoot dry weight ratio of wheat (*Triticum aestivum* L. cv. Hassawi). Vertical bars represent  $\pm$ SD

combination showed that, drought stress at 60% FC exhibited insignificant changes of these parameters, while they were significantly decreased under drought stress at 30% FC. However, the values of dry weight of root/shoot ratio were increased (more than 1) with increasing drought stress. Application of SA or heat shock either alone or in combination induced a marked increase of these parameters particularly in plants subjected to the highest (30% FC) drought stress as compared with the untreated corresponding control plants.

### Photosynthetic Pigments

Total photosynthetic pigments (chl. a, b and carotenoids) did not decrease significantly (about 17%) in response to the mild (60% FC) drought stress, whereas they were significantly decreased (about 44%) at the highest (30% FC) drought stress in comparison with well-watered (100%) plants. Seeds pre-treatment with SA either with or without heat shock resulted in a marked increase in the pigment fractions in comparing to untreated corresponding control plants (Fig. 2a-d).

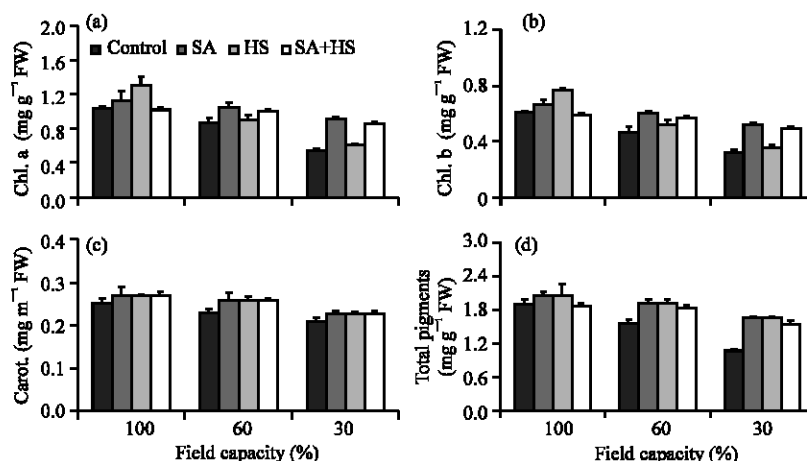


Fig. 2: Effect of drought stress, Salicylic Acid (SA) and Heat Shock (HS) alone or in combination on the contents ( $\text{mg g}^{-1}$  fresh weight) of (a) chl. a, (b) chl. b, (c) carotenoids and (d) total pigments of wheat (*Triticum aestivum* L.cv. Hassawi). Vertical bars represent  $\pm$ SD

### Carbohydrates

The effect of drought stress and seed treated with SA or heat shock individually or in combination on the contents of soluble and insoluble carbohydrates (Fig. 3 a-d), revealed that, drought stress stimulated soluble carbohydrates accumulation, whereas insoluble carbohydrates were markedly decreased in shoots and roots, as compared with well-watered (100% FC) plants. Presoaking of wheat seeds in SA with or without heat shock had a stimulation effect on soluble and insoluble carbohydrates as compared with those of corresponding untreated plants. This effect seems to be more obvious on highly stressed than well-watered plants.

### Proteins

Protein content (soluble and insoluble) showed variation as a result of drought stress (Fig. 4a-d). Soluble proteins were significantly increased in roots and shoots. Insoluble proteins were decreased in roots while they were increased in shoots with increasing of drought stress as compared with plants subjected to 100% FC. Heat shock or SA treatments either alone or in combination resulted in an increase of soluble and insoluble proteins as compared with those of corresponding untreated plants.

### Total Free Amino Acids and Proline

The content of total free amino acids (Fig. 5a, b) was varied among the two plant organs; while it was increased considerably in roots, the opposite trend was obtained in shoots. However, the content of proline (Fig. 5c, d) was significantly accumulated in both roots and shoots of the tested plants in comparing with well-watered control (100% FC) plants. The contents of free amino acids and proline were significantly decreased in both roots and shoots as a result of SA or/and heat shock treatments.

### Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Changes of protein patterns have been analyzed in shoots of Hassawi wheat (Fig. 6) to follow any possible alterations in gene expression in plants subjected to drought stress

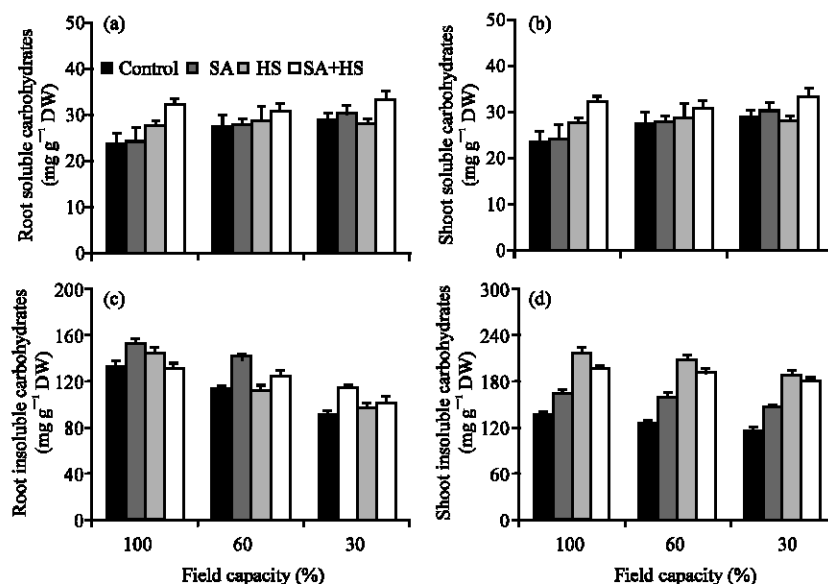


Fig. 3: Effect of drought stress, Salicylic Acid (SA) and Heat Shock (HS) alone or in combination on (a) soluble carbohydrate (mg g<sup>-1</sup> dry weight) of root, (b) shoot, (c) insoluble carbohydrate of root and (d) shoot of wheat (*Triticum aestivum* L.cv. Hassawi). Vertical bars represent  $\pm$ SD

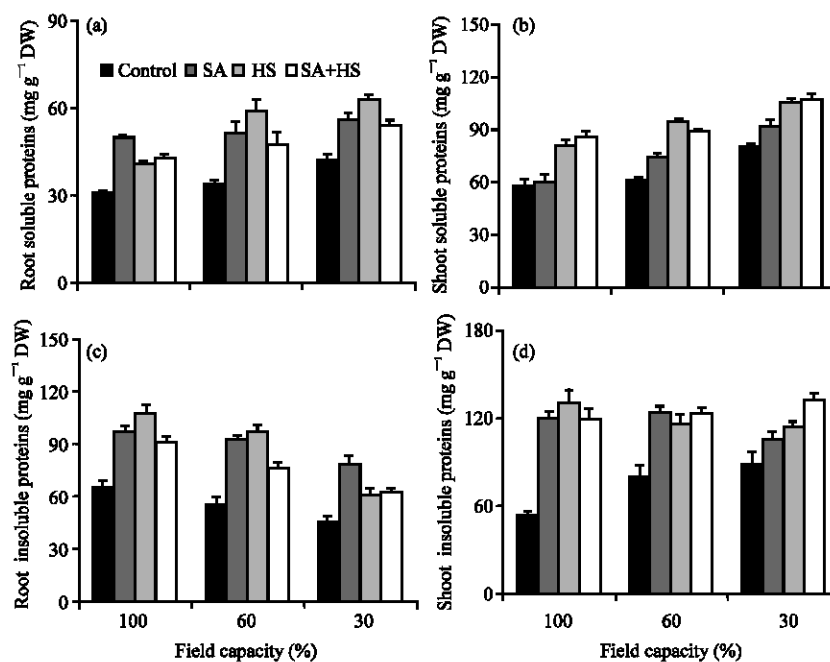


Fig. 4: Effect of drought stress, Salicylic Acid (SA) and Heat Shock (HS) alone or in combination on (a) soluble protein (mg g<sup>-1</sup> dry weight) of root, (b) shoot, (c) insoluble protein of root and (d) shoot of wheat (*Triticum aestivum* L.cv. Hassawi). Vertical bars represent  $\pm$ SD

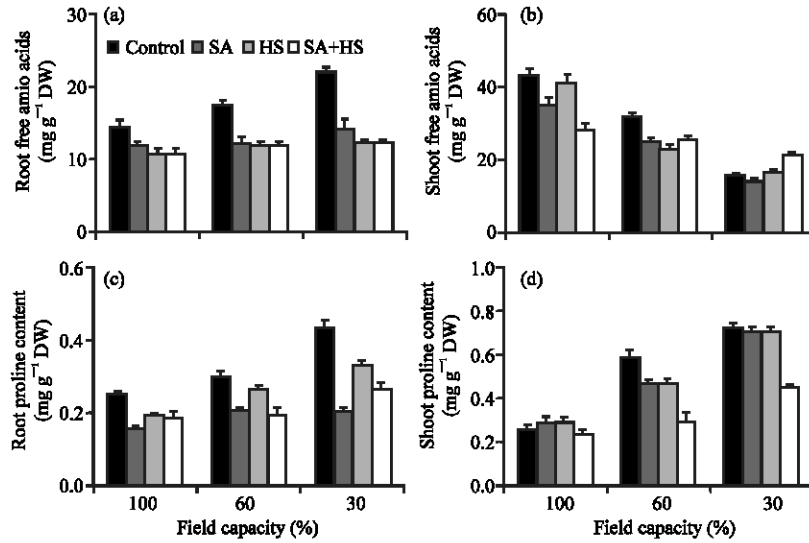


Fig. 5: Effect of drought stress, Salicylic Acid (SA) and Heat Shock (HS) alone or in combination on the contents ( $\text{mg g}^{-1}$  dry weight) of (a) total free amino acids in root, (b) shoot, (c) proline in root and (d) shoot of wheat (*Triticum aestivum* L.cv. Hassawi). Vertical bars represent  $\pm$ SD

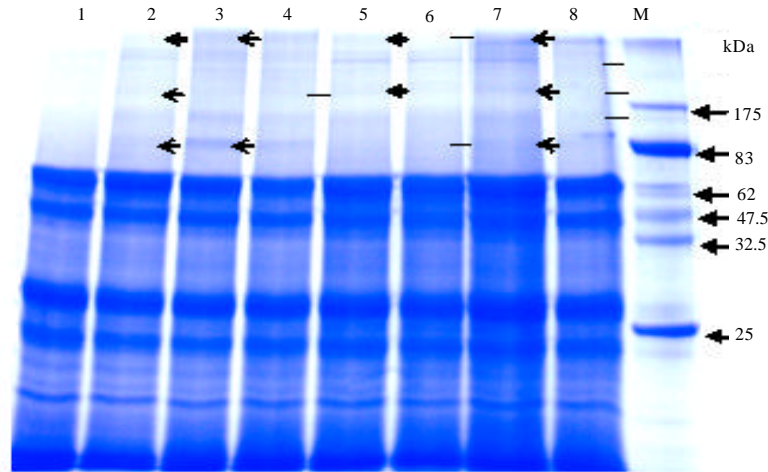


Fig. 6: Analysis of protein patterns by one-dimensional SDS-PAGE extracted from shoots of wheat (*Triticum aestivum* L.cv. Hassawi) plants treated with drought stress at 30% FC or in combination with Salicylic Acid (SA) or Heat Shock (HS) alone or together: M: Marker protein (25-175 kDa), Lane 1: Control (plants subjected to well-watered at 100% FC). Lane 2: Plants subjected to drought stress at 30% FC. Lane 3: Control plants treated with SA. Lane 4: Plants subjected to drought stress at 30% FC and treated with SA. Lane 5: Control plants treated with HS. Lane 6: Plants subjected to drought stress at 30% FC and treated with HS. Lane 7: Control plants treated with both HS and SA. Lane 8: Plants subjected to drought stress at 30% FC and treated with HS and SA. Newly appeared bands are indicated by (+) and the disappeared bands are indicated by (—)



at highest (30% FC) either treated or untreated with SA or/and HS in comparing with non-stressed control plants (plants subjected to well-watered at 100% FC). The treated plants induced variations in the appearance of new protein bands and in disappearance of others with different high molecular weights, whereas no changes in protein patterns with low molecular weights were observed. Drought stress (lane 2) induced the appearance of 3 new polypeptides (dehydrin proteins) with high molecular weights (630, 251 and 141 kDa), as compared with well-watered control plants. The SA or/and HS treatments indicated that: (1) in non-stressed plants; application of SA (lane 3) induced the appearance of two new polypeptides (630 and 141 kDa) and two heat shock proteins (HSPs) at 630 and 251 kDa in case of HS treatment (lane 5), while the combination effect of SA and HS (lane 7) resulted in appearance of three new polypeptides (630, 251 and 141 kDa). (2) In drought-stressed plants; it worthy of notice that, disappearance of one band (251 kDa) when these plants treated with SA (lane 4), two bands (630 and 141 kDa) in case of HS treatment (lane 6) and three bands (501, 251 and 158 kDa) as a result of the combined effect of SA and HS (lane 8).

## DISCUSSION

Plants avoid water stress by developing deep roots, minimizing water loss and accumulation of osmoprotective substances. The most striking feature in this study is that Hassawi wheat tolerated the drought stress up to the level of 60% FC and survived up to the level of 30% FC. This was judged by the elevated root/shoot dry weight ratio by drought stress (the root/shoot dry weight ratio on levels 100, 60 and 30% FC was 99, 101 and 108%, respectively) and the water content of roots and shoots which might used as a suitable selection criterion for the drought tolerance of Hassawi wheat. The high root/shoot dry weight ratio in the experimental plants was due to the inhibition of shoot growth compared with root growth. This ratio has been used previously as criterion for determination of varietal differences in the response to drought stress, which might used as an index of water or salt stress tolerance (Kramer, 1983; Azooz and Al-Fredan, 2009). The increase in root growth than shoot is a strategy used by the plants growing under arid conditions to increase the surface area for water absorption (Lobato *et al.*, 2008; Luvaha *et al.*, 2008). The reduction in shoot growth coupled with continued root growth will result in improving plant water statuses. Accordingly, Hassawi wheat plants maintained its tissue water content around the control value up to the level of 60% FC either under drought stress alone or in combination with heat shock. This was accompanied with the observable accumulation of cytosolutes (soluble carbohydrates, soluble proteins and proline) which of course could be share principally in osmoregulation as reported by Nguyen *et al.* (2004).

The reduction effect of drought stress on dry weight and water content of root and shoot are in agreement with those recorded by others (Kusaka *et al.*, 2005; Sankar *et al.*, 2007; Luvaha *et al.*, 2008). They suggested that, the quantity and quality of plant growth are depending on cell division, cell enlargement and cell differentiation which are affected by drought stress (Kusaka *et al.*, 2005; Sankar *et al.*, 2007). These processes are very sensitive to water deficit because of their dependence on cell turgor. The decrease in dry weight of roots and shoots, under the highest (30% FC) drought stress may be due to the considerable decrease in photosynthesis (Sankar *et al.*, 2007) and reduced cell turgor which affected cell division and expansion (Luvaha *et al.*, 2008).

Abiotic stress induces the transcription of genes that can be classified into two groups (Shinozaki and Yamaguchi-Shinozaki, 2000). The first group is involved in cellular protection including synthesis of compatible solutes, detoxification of harmful compounds, protein

recycling and membrane stabilization (Shinozaki and Yamaguchi-Shinozaki, 1997). The second group includes regulatory genes such as signaling molecules and transcription factors (Seki *et al.*, 2003). These mechanisms were recommended by our results when drought stressed plants treated with heat shock or/and 50 ppm SA, where the cytosolutes increased progressively, leading to the marked improvement of water status and plant growth. This was also associated with the marked enhancement in the biosynthesis of photosynthetically active pigments, which might be responsible for the regulation of carbohydrates and nitrogen components. The promotion effects observed when drought stressed plants treated with heat shock or/and 50 ppm SA indicated that these treatments alleviate to some extent, the suppressive effect of drought stress particularly at highest level. This means that SA or HS treatments may be increase the efficiency of water uptake and water status of drought stressed plants. Hamada and Al-Hakimi (2001) have shown that, treatment of wheat plants with 100 ppm SA through seed soaking was able to alleviate the inhibitory effect of drought and stimulate growth by enhancing photosynthetic rate. Sato and Yokoya (2008) reported that, exposure of rice seedlings to heat shock at 42°C resulted in a significant increase in tolerance to drought stress.

It is noted that the decrease in the content of photosynthetic pigment as affected by mild water stress was mainly due to a decrease in chl. a and to some extent in chl. b, while carotenoids content was unaffected in comparing with control (100% FC). The decrease in chlorophyll content in response to water stress has been found in various plants (Jaleel *et al.*, 2008; Farooq *et al.*, 2009). The reduction in photosynthetic pigments in response to drought stress has been reported to be due to the activation of chlorophyllase enzyme which catalyzes the first step in the catabolism of chlorophyll (Mujmda *et al.*, 1991). Carotenoids are responsible for quenching of singlet oxygen (Knox and Dodge, 1985) thus, the unchanged effect of drought stress on chlorophyll contents may be attributed to the higher level of carotene which may be protect plants against chlorophyll degradation, leading to their tolerance to some extent to the mild drought stress. Pretreatment of Hassawi wheat with SA or/and heat shock under drought stress, induced a significant stimulatory effect on the biosynthesis of photosynthetic pigments greater than estimated in untreated plants. This may be related to the inhibition of chlorophyllase activity and chloroplast membrane degradation (Quartacci and Navari-Izzo, 1992).

Drought stress stimulated the biosynthesis of soluble carbohydrates in roots and shoots of Hassawi wheat plants, while the insoluble carbohydrates were decreased. The increase in soluble carbohydrates was seemed to be at the expense of insoluble ones (Chaves Filho and Stacciarini-Seraphin, 2001; Lobato *et al.*, 2008). They reported that the increase in soluble carbohydrates in plants under drought stress due to the degradation of starch. The increase of soluble carbohydrates may be improve the tolerance of Hassawi wheat plants to drought stress as reported by Li and Li (2005) and Lobato *et al.* (2008). The applied SA or/and HS enhanced the biosynthesis of soluble and insoluble carbohydrates in roots and shoots of treated plants. Rizhsky *et al.* (2004) observed an accumulation of soluble sugars in *Arabidopsis* plants subjected to combination of drought and heat stress.

The results showed that two different situations in the relation between the accumulation of protein and amino acids among the two plant organs; while the insoluble protein content decreased in roots and increased in shoots by drought stress, amino acids increased in roots and decreased progressively in shoots. These opposite patterns in the relation between protein and amino acids and even proline might indicate the problematical behavior of nitrogen metabolism in glycophytes exposed to the environmental stresses. These complications might be linked directly and/or indirectly with the complications in gene

expression under stress conditions, which led to the synthesis of new proteins and disappearance of others with high molecular weights, in Hassawi wheat plants subjected to drought stress. This leads us to point out that, the drought tolerance in Hassawi wheat plants at moderate drought stress seemed to be linked with an equilibrium and interconversion between carbohydrates and nitrogen metabolism, whereas the water stress injury leads to the metabolic disturbances in both components. Most probably, the increase in the soluble protein content in shoots is the result of being incorporated of amino acids (which were found to be reduced by drought) into protein under drought stress while, the increase observed in the free amino acids in roots may be due to the high synthesis of amino acids from protein hydrolyses, in which the free amino acids are utilized by the plant to reduce the effects of the drought stress through organic solute accumulation and this way increased the water retention capacity (Sircelj *et al.*, 2005; Abraham *et al.*, 2008; Lobato *et al.*, 2008). In this context, Sankar *et al.* (2007) reported that, in inadequate conditions to the plant; pathway of proteins breakdown is active, because plants use proteins to synthesis nitrogen compounds as amino acids that might be auxiliary the plant osmotic adjustment. Present results revealed that, the increase in soluble protein in root may be due to the breakdown of insoluble protein which was found to be decreased by drought stress.

Presoaking of seeds in SA or exposure of germinated seeds to heat shock individually or in combination significantly increased the content of soluble carbohydrates and proteins. While free amino acids and proline were decreased in both roots and shoots as compared with untreated plants either under drought stress or non-stressed conditions. The stimulation effect of SA or HS on the biosynthesis of soluble carbohydrates and proteins, may indicate that the possibility of their involvement in osmotic adjustment and consequently drought tolerance which contribute in reducing the injurious effects of drought stress and accelerating the restoration processes during the period after action of stress, which might be a manifestation of the protective action of SA or HS on Hassawi wheat plant (Yadav *et al.*, 2005; Sato and Yokoya, 2008). The reduction of proline and free amino acids contents was associated with increasing of soluble proteins. This indicates that SA or/and HS treatments could stimulate the incorporation of free amino acids and proline into protein leading to increase Hassawi wheat growth.

The new bands of high molecular weights proteins in drought stressed or non-stressed plants treated with SA or/and HS may be due to *de novo* synthesis of these proteins or formation of peptides acting on higher molecular proteins (Gopala Roa *et al.*, 1987). These new proteins may have a specific function to protect Hassawi wheat plants from further dehydration damage and considered as a defense mechanism to drought stress as reported by Han and Kermode (1996) and Cellier *et al.* (1998). Drought induced polypeptides have been observed in many studies and are assumed to play a role in water stress tolerance (Arora *et al.*, 1998; Jiang and Huang, 2002). The disappearance of certain polypeptides in drought-stressed plants in response to SA or/and HS treatments may be related to increase RNAase activity (Bewley and Oliver, 1983). Present results suggested that the enhancement of drought tolerance by SA application, as manifested by higher cytosolutes during drought stress, was not related to the induction of dehydrins in Hassawi wheat. This explain that, the promotive effects of SA on the enhancement of physiological activities under drought stress was paralleled with the delayed induction of new proteins synthesis in Hassawi wheat.

## CONCLUSION

The results of this research suggested that, Hassawi wheat has the ability to tolerate drought stress up to the level of 60% FC and survive up to the level of 30% FC. Presoaking

of Hassawi wheat seeds in SA or exposure of germinated seeds to heat shock at 40°C, either individually or in combination, resulted in a significant increase in their tolerance to drought stress. This was associated with enhancement of dry weight root/shoot ratio, photosynthetic pigments and cytosolutes as well as appearance or disappearance of some protein bands with high molecular weights. The results support the hypothesis that SA or HS treatments might play an important role in modulating the physiological processes and gene expression which will eventually lead to adaptation of plants to an unfavorable abiotic stress. The overlapping between more than one stresses may be different than that caused by each of the different stresses applied individually. The presence of more than one stress can alter plant metabolism that may require a new type of response which would not have been induced by each of the individual stresses.

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#### REFERENCES

- Abraham, S.S., C.A. Jaleel, Z. Chang-Xing, R. Somasundaram, M.M. Azooz, P. Manivannan and R. Panneerselvam, 2008. Regulation of growth and metabolism by paclobutrazol and ABA in *Sesamum indicum* L. under drought condition. *Global J. Mol. Sci.*, 3: 57-66.
- Ain-Lhout, F., F.A. Zunzunegui, M.C.D. Barradas, R. Tirado, A. Clavijio and N.F. Garcia, 2001. Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. *Plant Soil*, 230: 175-183.
- Arora, R., D.S. Pitchay and B.C. Bearce, 1998. Water-stress-induced heat tolerance in geranium leaf tissues: A possible linkage through stress proteins? *Physiol. Plant.*, 103: 24-34.
- Azooz, M.M., 2009. Salt stress mitigation by seed priming with salicylic acid in two faba bean genotypes differing in salt tolerant. *Int. J. Agric. Biol.*, 11: 343-350.
- Azooz, M.M. and M.A. Al-Fredan, 2009. The inductive role of vitamin C and its mode of application on growth, water status, antioxidant enzyme activities and protein patterns of *Vicia faba* L. cv. Hassawi grown under seawater irrigation. *Am. J. Plant Physiol.*, 4: 38-51.
- Badour, S.S.A., 1959. Analytisch-chemische untersuchung des kaliummangels bei Chlorella im vergleich mit anderen mangelzuständen. Ph.D. Dissertation, Göttingen.
- Bates, L.S., R.P. Walderren and I.D. Teare, 1973. Rapid determination of free proline for water-studies. *Plant Soil*, 39: 205-207.
- Bewley, J.D. and M.J. Oliver, 1983. Responses to a changing environment at the molecular level: Does desiccation modulate protein synthesis at the transcriptional or translational level in a tolerant plant? *Curr. Trop. Plant Biochem. Biophys.*, 2: 145-146.
- Bowler, C. and R. Fluhr, 2000. The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends Plant Sci.*, 5: 241-246.
- Bracale, M., M. Levi, C. Savini, W. Dicorato and M.G. Galli, 1997. Water deficit in pea root tips: Effect on the cell cycle and on the production of dehydrin-like proteins. *Ann. Bot.*, 79: 593-600.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.*, 72: 248-254.

- Camejo, D., P. Rodríguez, M.A. Morales, J.M. Dell'Amico, A. Torrecillas and J.J. Alarcón, 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.*, 162: 281-289.
- Cellier, F., G. Conejero, J.C. Breidler and F. Casse, 1998. Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflowers: Accumulation of dehydrin transcripts correlates with tolerance. *Plant Physiol.*, 116: 319-328.
- Chandler, P.M. and M. Robertson, 1994. Gene expression regulated by abscisic acid and its relation to stress tolerance. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 45: 113-141.
- Chandrasekar, V., R.K. Sairam and G.C. Srivastava, 2000. Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. *J. Agron. Crop Sci.*, 185: 219-227.
- Chaves Filho, J.T. and E. Stacciarini-Seraphin, 2001. Changes in osmotic potential and soluble carbohydrates levels in *Solanum lycocarpum* St.-Hil. in response to water stress. *Rev. Bras. Bot.*, 24: 199-204.
- Choi, J., S. Yoon, H. Hong, D. Choi and G. Yoo, 1996. A modified Coomassie Blue staining of proteins in polyacrylamide gels with Bismarck Brown R. *Anal. Biochem.*, 236: 82-84.
- Costa, R.C.L., A.K.S. Lobato, C.F.O. Neto, P.S.P. Maia, G.A.R. Alves and H.D. Laughinghouse, 2008. Biochemical and physiological responses in two *Vigna unguiculata* (L.) walp cultivars under water stress. *J. Agron.*, 7: 98-101.
- Dichio, B., C. Xiloyannis, K. Angelopoulos, V. Nuzzo, S.A. Bufo and G. Celano, 2003. Drought-induced variations of water relations parameters in *Olea europaea*. *Plant Soil*, 257: 381-389.
- Farooq, M., A. Wahid, N. Kobashi, D. Fujita and S.M.A. Basra, 2009. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.*, 29: 185-212.
- Gopala Rao, P., C.D. Reddy and J.K. Ramaiah, 1987. Effect of B-vitamins on the protein component of clusterbeans *Cyamopsis tetragonoloba* L. *Taub. Ann. Bot.*, 59: 281-284.
- Hamada, A.M. and E.M. Khulaef, 1995. Effects of salinity and heat-shock on wheat seedling growth and content of carbohydrates, proteins and amino acids. *Biol. Plant*, 37: 399-404.
- Hamada, A.M. and A.M.A. Al-Hakimi, 2001. Salicylic acid versus salinity-drought-induced stress on wheat seedlings. *Rostlinná Viroba*, 47: 444-450.
- Han, B. and A.R. Kermode, 1996. Dehydrin-like proteins in castor bean seeds and seedlings are differentially produced in responses to ABA and water-deficit-related stresses. *J. Exp. Bot.*, 47: 933-939.
- Hern-Andez, A.J., A.M. Ferrer, A. Jimenez, R.A. Barcel and S. Francisca, 2001. Antioxidant system and O<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> production in the apoplast of pea leaves: Its relation with NaCl-induced necrotic lesions in minor veins. *Plant Physiol.*, 127: 817-831.
- Jaleel, C.A., P. Manivannan, G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam, 2008. Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. *Colloids Surfaces B Biointerfaces*, 61: 298-303.
- Jiang, Y. and B. Huang, 2002. Protein alterations in tall fescue in response to drought stress and abscisic acid. *Crop Sci.*, 42: 202-207.
- Knox, J.P. and A.O. Dodge, 1985. Singlet oxygen and plants. *Phytochemistry*, 24: 889-896.
- Kramer, P.J., 1983. *Plant Water Relations*. Academic Press, New York.
- Kusaka, M., A.G. Lalusin and T. Fujimura, 2005. The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum* (L.) Leeke) cultivars with different root structures and osmo-regulation under drought stress. *Plant Sci.*, 168: 1-14.

- Laemmli, U.K., 1970. Cleavage of structural protein during assembly of head of bacteriophage T<sub>4</sub>. *Nature*, 227: 680-685.
- Le Thiec, D. and S. Manninen, 2003. Ozone and water deficit reduced growth of Aleppo pine seedlings. *Plant Physiol. Biochem.*, 41: 55-63.
- Lee, Y.P. and T. Takahashi, 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.*, 14: 71-77.
- Li, T.H. and S.H. Li, 2005. Leaf responses of micropopagated apple plants to water stress: Nonstructural carbohydrate composition and regulatory role of metabolic enzymes. *Tree Physiol.*, 25: 495-504.
- Lichtenthaler, H.K. and R.R. Wellburn, 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.*, 11: 591-592.
- Lobato, A.K.S., C.F. Oliveira-Neto, B.G. Santos-Filho, R.C.L. Costa, F.J.R. Cruz, H.K.B. Neves and M.J.S. Lopes, 2008. Physiological and biochemical behavior in soybean (*Glycine max* cv. Sambaiba) plants under water deficit. *Aust. J. Crop Sci.*, 2: 25-32.
- Luvaha, E., G.W. Netondo and G. Ouma, 2008. Effect of water deficit on the physiological and morphological characteristics of mango (*Mangifera indica*) rootstock seedlings. *Am. J. Plant Physiol.*, 3: 1-15.
- Ma, Q. and D.W. Turner, 2006. Osmotic adjustment segregates with and is positively related to seed yield in F3 lines of crosses between *Brassica napus* and *B. juncea* subjected to water deficit. *Aust. J. Exp. Agric.*, 46: 1621-1627.
- Martinez-Ballesta, M.C., V. Martinez and M. Carvajal, 2004. Osmotic adjustment, water relations and gas exchanges in pepper plants grown under NaCl or KCl. *Environ. Exp. Bot.*, 52: 161-174.
- Mohammadkhani, N and R. Heidari, 2008. Effects of drought stress on soluble proteins in two maize varieties. *Turk. J. Biol.*, 32: 23-30.
- Mohsenzadeh, S., M.A. Maloobi, K. Razavi and S. Farrahi-Ashtiani, 2006. Physiological and molecular responses of *Aeluropus lagopoides* (Poaceae) to water deficit. *Environ. Exp. Bot.*, 56: 314-322.
- Mujmda, S., S.R. Ghosh, R. Bernard and E.B. Dumbroff, 1991. Activities of chlorophyllase, phosphoenol pyruvate carboxylase and ribulose, 5-bisphosphate carboxylase in the primary leaves of soybean during senescence and drought. *Physiol. Plant*, 81: 473-480.
- Nath, A.K., S. Kumari and D.R. Sharma, 2005. *In vitro* selection and characterization of water stress tolerant cultures of bell pepper. *Indian J. Plant Physiol.*, 10: 14-19.
- Nguyen, T.T.T., N. Klueva, V. Chamareck, Aarti and A.G. Magpantay *et al.*, 2004. Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Mol. Genet. Genomics*, 272: 35-46.
- Noreen, S. and M. Ashraf, 2008. Alleviation of adverse effects of salt stress on sunflower (*Helianthus Annus* L.) by exogenous application of salicylic acid: Growth and photosynthesis. *Pak. J. Bot.*, 40: 1657-1663.
- Ouvrard, O., F. Cellier, K. Ferrare, D. Tusch, T. Lamaze, J.M. Dupuis and F. Casse-Delbart, 1996. Identification and expression of water stress- and abscisic acid-regulated genes in a drought-tolerant sunflower genotype. *Plant Mol. Biol.*, 31: 819-829.
- Pál, M., E. Horváth, T. Janda, E. Páldi and G. Szalai, 2005. Cadmium stimulates accumulation of salicylic acid and its putative precursors in maize (*Zea mays* L.) plants. *Physiol. Plant.*, 125: 356-364.
- Quartacci, M.F. and F. Navari-Izzo, 1992. Water stress and free radical mediated changes in sunflower seedlings. *J. Plant Physiol.*, 139: 621-625.

- Reddy, R.A., K.V. Chaitanya, P.P. Jutur and K. Sumithera, 2004. Differential antioxidative responses to water stress among five mulberry (*Morus alba* L.) cultivars. *Environ. Exp. Bot.*, 52: 33-42.
- Rizhsky, L., H. Liang, J. Shuman, V. Shulaev, S. Davletova and R. Mittler, 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. *Plant Physiol.*, 134: 1683-1696.
- Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova, 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol.*, 1: 314-319.
- Saleh, A.A.H., D.Z. Abdel-Kader and A.M. El-Elish, 2007. Role of heat shock and salicylic acid in antioxidant homeostasis in Mungbean (*Vigna radiata* L.) plant subjected to heat stress. *Am. J. Plant Physiol.*, 2: 344-355.
- Sankar, B., C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram and R. panneerselvam, 2007. Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Botanica Croatica*, 66: 43-56.
- Sato, Y. and S. Yokoya, 2008. Enhanced tolerance to drought stress in transgenic rice plants overexpressing a small heat-shock protein, sHSP17.7. *Plant Cell Rep.*, 27: 329-334.
- Seki, M., Kamei A., K. Yamaguchi-Shinozaki and K. Shinozaki, 2003. Molecular responses to drought, salinity and frost: Common and different paths for plant protection. *Curr. Opin. Biotech.*, 14: 194-199.
- Senaranta, T., D. Touchell, E. Bunn and K. Dixon, 2000. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in plants. *Plant Growth Regul.*, 30: 157-161.
- Shinozaki, K. and K. Yamaguchi-Shinozaki, 1997. Gene expression and signaling transduction in water-stress response. *Plant Physiol.*, 115: 327-334.
- Shinozaki, K. and K. Yamaguchi-Shinozaki, 2000. Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.*, 3: 217-223.
- Sircelj, H., M. Tausz, D. Grill and F. Batic, 2005. Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. *J. Plant Physiol.*, 162: 1308-1318.
- Steel, R.G. and J.H. Torrie, 1980. *Principals and Procedures of Statistics*. 2nd Edn., McGraw-Hill Book Company Inc., New York, USA.
- Wang, F.Z., Q.B. Wang, S.Y. Kwon, S.S. Kwak and W.A. Su, 2005. Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. *J. Plant Physiol.*, 162: 465-472.
- Wang, L.J., S.J. Chen, W.F. Kong, S.H. Li and D.D. Archibold, 2006. Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. *Postharvest Biol. Technol.*, 41: 244-251.
- Yadav, S.K., N.J. Lakshmi, M. Maheswari, M. Vanaja and B. Venkateswarlu, 2005. Influence of water deficit at vegetative, anthesis and grain filling stages on water relation and grain yield in sorghum. *Indian J. Plant Physiol.*, 10: 20-24.
- Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Ann. J. Plant Biol.*, 53: 247-273.