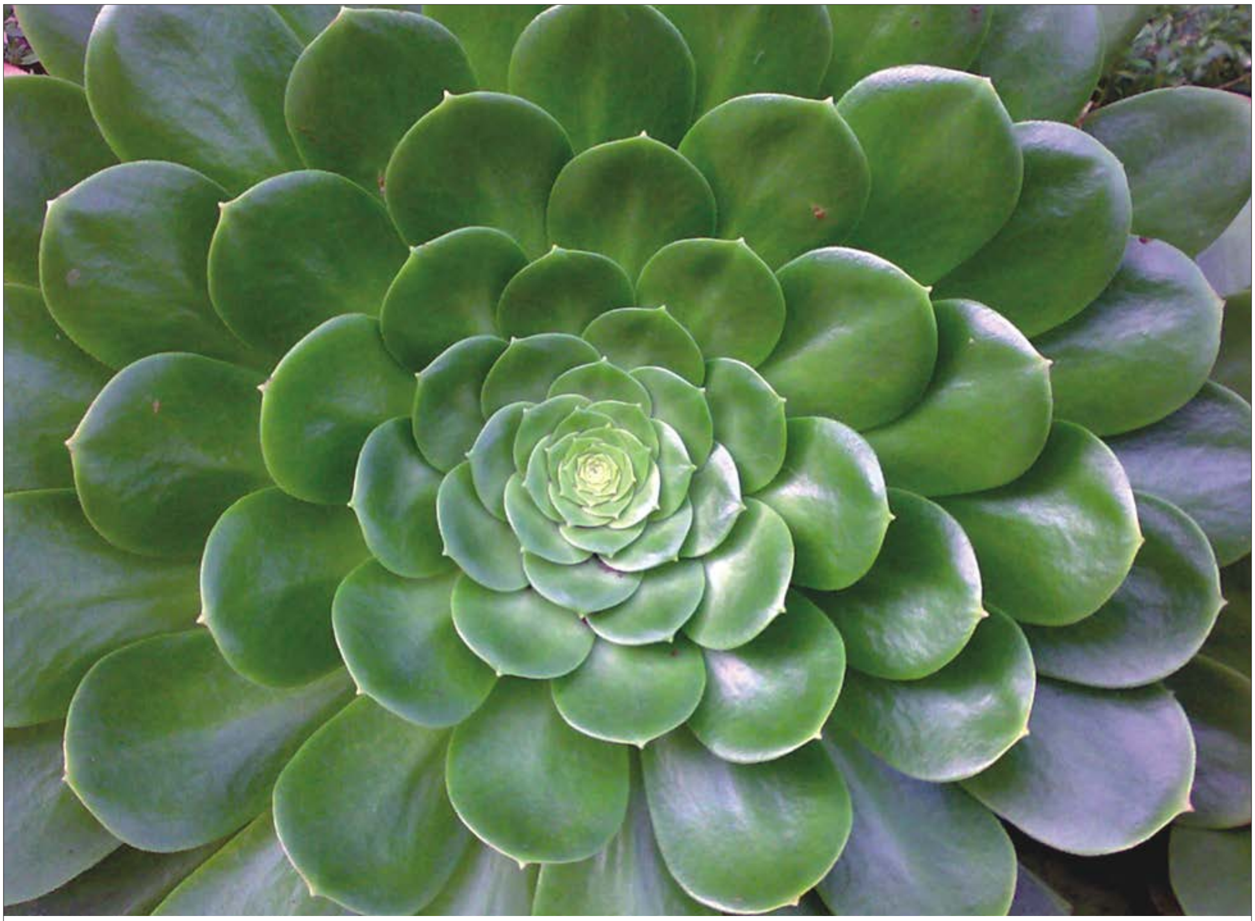

Journal of

Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 10 (4): 128-141, 2015
ISSN 1816-4951 / DOI: 10.3923/jps.2015.128.141

Induction of Drought Stress Resistance in Sesame (*Sesamum indicum* L.) Plant by Salicylic Acid and Kinetin

Yasser Hussein, Gehan Amin, Adel Azab and Hanan Gahin
Department of Botany, Faculty of Science, Zagazig University, Egypt

Corresponding Author: Yasser Hussein, Department of Botany, Faculty of Science, Zagazig University, Egypt

ABSTRACT

Sesame (*Sesamum indicum* L., family Pedaliaceae) is considered as one of the most important oil crops in Egypt due to its high seed oil content. This study was conducted to study the influence of exogenously applied phytohormones (Salicylic Acid (SA) and kinetin (Kin) on growth and physiological behavior in drought stressed sesame plants. The experiment was designed into 4 groups, control, drought stress, drought treated with SA (0.1, 0.5 and 1 mM) and drought treated with Kin (0.05, 0.1 and 0.5 mM). It was found that drought stress adversely affected all growth parameters of sesame including plant height, fresh weight, dry weight, number of leaves and leaf area. Drought stress caused significant decrease in endogenous phytohormones (SA and Kin), while on treatment of drought stressed sesame plants with SA and Kin increased level of endogenous SA and Kin. On the other hand, drought stress caused significant increase in total soluble protein, poly peptide bands, proline, moreover on treatments with SA and Kin improved all previous substances in drought stressed sesame plants. The SDS-PAGE detection of protein indicated some changes in amount and number of protein bands and also new protein bands appeared in both stressed and phytohormonal treated plants. Therefore, exogenous application of drought stressed sesame plants with salicylic acid or kinetin may induce resistance to drought stress by enhancement of growth and various physiological processes which are negatively affected by drought stress.

Key words: Drought stress, resistance, sesame (*Sesamum indicum* L.), salicylic acid, kinetin, growth, protein, proline, endogenous phytohormones

INTRODUCTION

Drought stress is a world-spread problem seriously influencing grain production and quality and with increasing population and global climate change making the situation more serious. Severe water stress may result in the arrest of photosynthesis and disturbance of metabolism (Jaleel *et al*., 2008). Drought stress leads to increase accumulation of Reactive Oxygen Species (ROS) in plants. Increased levels of ROS cause damage to various cellular mechanisms, such as enzyme inhibition, protein degradation, DNA and RNA damage and membrane lipid peroxidation, which ultimately culminate in cell death. In different crops, physiological responses to drought vary with the genotype (Anyia and Herzog, 2004; Bacelar *et al*., 2007; Centritto *et al*., 2009; Costa *et al*., 2012).

Sesame (*Sesamum indicum* L.) is an economically important oil seed crop which is widely cultivated in many parts of the world. Sesame is widely used in food, nutraceutical, pharmaceutical and industry in many countries because of its high oil, protein and antioxidant contents (Morris, 2002).

Increasing plant resistance to drought stress would be the most economical approach to improve agricultural productivity and reduce agricultural use of fresh water resources. It is generally accepted that treatments with phytohormones may lead to regulation of the plant metabolism and consequently plant performance (Itai *et al*., 1978). Similarity, the application of phytohormones to variously stressed plants may lead to counteraction of the adverse effects exerted by stress conditions (Heikal *et al*., 1982). In view of these findings, it was of interest to test whether the exogenous treatments of phytohormones such as Salicylic Acid (SA) and kinetin (Kin) can counteract the adverse effects of drought on sesame or not. Exogenous application of SA found to be effective in modeling plant responses to drought stress. Plant pre-treatment with SA resulted in higher tissue water content, increased antioxidant enzyme activities, decreased level of lipid peroxidation and membrane injury and it also protected nitrate reductase activity against inhibition under water deficit conditions (Sakhabutdinova *et al*., 2003; Umebese *et al*., 2009). Cytokinins (CKs) conduce to sustain plant tolerance to adverse abiotic conditions and suppress pathological phenotypes mainly due to their ability to promote cell division, keep meristematic cell identity and maintain high cellular redox potentials during drought (Werner and Schmulling, 2009).

Synthesis of stress proteins is a ubiquitous response to cope with prevailing stressful conditions including drought stress. Most of the stress proteins are soluble in water and therefore contribute towards the stress tolerance phenomena by hydration of cellular structures. Synthesis of a variety of transcription factors and stress proteins is exclusively implicated in drought tolerance (Wahid *et al*., 2007). The accumulation of some organic compatible compounds, such as proline, plays a significant role in plant protection and adaptation to a broad range of stress. Proline is one of the protective molecules that can unite oxygen and free radicals caused by stress. Therefore, one of the roles of proline in plants is probably reacting against drought stress (Ashraf and Foolad, 2007; Dias *et al*., 2014).

This study was conducted to study the influence of exogenously applied phytohormones (Salicylic Acid (SA) and kinetin (Kin)) on growth and physiological behavior in drought stressed sesame plants.

MATERIALS AND METHODS

The experimental plant used in this investigation was Sesame (*Sesamum indicum* L.) family Pedaliaceae Giza-32. The seeds were supplied by Crop Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Phytohormones (salicylic acid and kinetin) were supplied from SIGMA. Preliminary experiment was done to choose the concentrations of salicylic acid and kinetin. The concentrations used for kinetin were (0.05, 0.1 and 0.5 mM) and for salicylic acid were (0.1, 0.5 and 1 mM). The present work was carried out in the greenhouse of Department of Botany, Faculty of Science, Zagazig University during the summer season of 2012.

Growth conditions: Seeds of sesame (*Sesamum indicum* L.) were washed with tap water then were grown in plastic pots (16 cm in diameter and 19 cm in height) containing 3 kg soil, composed of clay and sand (2:1) in 21/4/2012. The mean temperature was about 30°C. The seeds were irrigated twice a week until germination and after 4 weeks of cultivation in 19/5/2012, uniform seedlings of 13 cm length and 6 leaves were selected and one seedling was left for each pot.

All pots were fertilized with NPK at ratio of 10, 20 and 25 mg kg^{-1} soil, respectively and watering according to the following groups:

Group A : "Control" plants were irrigated with 100% Water Holding Capacity (WHC) during plant life cycle
Group B : "Drought" plants were irrigated with 25% Water Holding Capacity (WHC)
Group C : "Drought+(kinetin)" plants were irrigated with 25% Water Holding Capacity (WHC) and treated with kinetin with different concentration (0.5, 0.1 and 0.05 mM)
Group D : "Drought+salicylic acid" plants were irrigated with 25% Water Holding Capacity (WHC) and treated with salicylic acid with different concentration (1, 0.5 and 0.1 mM)

Exogenously application of phytohormones: The plants of third and fourth groups were sprayed with serial concentration of selected phytohormones, respectively during different growth stages as follows.

Immediately after water-stressed treatment at early vegetative stage, after other three weeks at late vegetative stage and at flowering stage. Growth measurements and physiological analysis were carried out at two growth stages, vegetative and flowering.

Growth criteria measurements: Sesame plants were collected at vegetative and flowering stages for measurements of different growth criteria: (Height of the plant, root length, number of leaves/plant and leaf area).

Water Content (WC) was estimated according to the standard method developed by Weatherley (1950) as described by Bandurska (2000). Percentage of WC was estimated by measuring fresh weight and dry weight following oven drying of fresh samples at 60°C. Percentage of WC was calculated using the following equation:

$$\text{WC (\%)} = \frac{\text{Fresh sample} - \text{Dry sample}}{\text{Dry sample}} \times 100$$

Estimation of total soluble protein: The total soluble protein content was determined according to the method of Lowry *et al*. (1951). Fresh weight of plant material (0.5 g) was homogenized in 5 mL 1 N NaOH solution then left overnight. One milliliter of solubilized protein was mixed with 5 mL of the freshly prepared alkaline solution. After 10 min, 0.5 mL of diluted Folin reagent was added, left to stand 20 min, then the optical density was measured at 750 nm. The protein content was calculated using the standard curve of bovine serum albumin. The protein content was calculated using this standard curve and expressed as milligrams of protein per gram dry weight of sample.

Electrophoretic detection of protein by Sodium Dodecyl Sulphate, Polyacrylamide Gel Electrophoresis (SDS-PAGE): A known fresh weight of sesame leaves (0.3 g) was ground in liquid nitrogen in a mortar. Just as the liquid nitrogen evaporated, SDS sample buffer (Laemmli, 1970) was added and the tissues were further ground. After thawing, the homogenates were heated at 95°C for 5 min and then briefly centrifuged at 8.000 rpm ($205\times10^3\times$g) for 10 min. Supernatants containing water-soluble protein fraction were then kept under -20°C until used and subjected for further analysis by SDS-PAGE.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) as modified by Studier (1973). One-dimensional SDS-PAGE was run in a mini-gel electrophoresis apparatus (Cleaver Scientific Ltd, Omni PAGE

Gel Casting System). After running, the gel was immersed overnight on coomassie blue staining solution, with gently shaking at 50 rpm. After staining, the gel was washed by distilled water and immersed in de-staining solution till appearance of the protein bands. The gel was photographed by digital camera. The molecular biomass of the appeared protein bands was calculated from the inference of authentic protein marker, Broadway, pre-stained marker. Standard curve of protein marker was plotted based on the electrophoretic mobility (Rf) of proteins against their $\log_{10}$ molecular weights.

Estimation of proline: Free proline was estimated according to the method of Bates *et al*. (1973). Plant fresh weight (0.1 g) was homogenized in 10 mL of aqueous sulphosalicylic acid 3 (w/v). The homogenate was filtered using Whatman No. 1 filter paper. Two milliliter of glacial acetic acid and 2 mL of acid ninhydrin were added to 2 mL of the filtrate in glass test tube and heated in boiling water bath for 1 h. To stop the reaction, the tubes were placed in ice bath. Four milliliter toluene was added to the reaction mixture and stirred well for 20-30 sec. The toluene layer was taken and left at room temperature. The optical density of the produced color was measured at 520 nm on a spectrophotometer (WP 0803006). The amount of proline in the test samples was calculated from the standard curve of proline. The proline content on fresh-weight-basis was expressed as follows:

$$\text{Tissue } (\mu\text{mol g}^{-1}) = \frac{\mu\text{g proline / mL} \times \text{Toluene (mL)}}{115.5} \times \frac{5}{\text{Sample (g)}}$$

where, 115.5 is the molecular weight of proline.

Estimation of phytohormones content: Ten grams of fresh tissue were extracted and fractionated according to methods of Shindy and Smith (1975) and Chen (1990). The HPLC analysis was used to determine the phytohormones in the extract dissolved in 1 mL of analytical grade methanol. Analysis was performed on a model (HP 1050) HPLC equipped with UV detector in Agricultural Research Centre (Soils, Water and Environment Res. Inst. "SWERI"). Separations and determinations were performed on a C18 column (4.6×150 mm, 5 µm). Wavelength in the UV detector was 245 nm (Guinn and Brummett, 1990; Baydar and Ulger, 1998). Total run time for the separations was approximately 5 min at a flow rate of 1 mL min^{-1}. Peak identification was performed by comparing the retention times with pure standards (Sigma-Aldrich, Deisenhofer-Germany). Concentrations of phytohormones were calculated from corresponding standard curves.

Statistical analysis: The obtained data was analyzed statistically using one-way analysis of variance (ANOVA). Treatment means were separated by Least Significant Difference (LSD) multiple range tests at (Level 0.05).

RESULTS AND DISCUSSION

Plants are often exposed to environmental stresses through their life cycle. Among these stresses, in particular drought is the most serious problem that limits plant growth and crop productivity in agriculture. In the present study, drought stress reduced growth and yield of sesame plant. Treatment of drought stressed sesame plant by phytohormones (salicylic acid and kinetin) improved its growth and yield, which could enable sesame plant to resist drought stress.

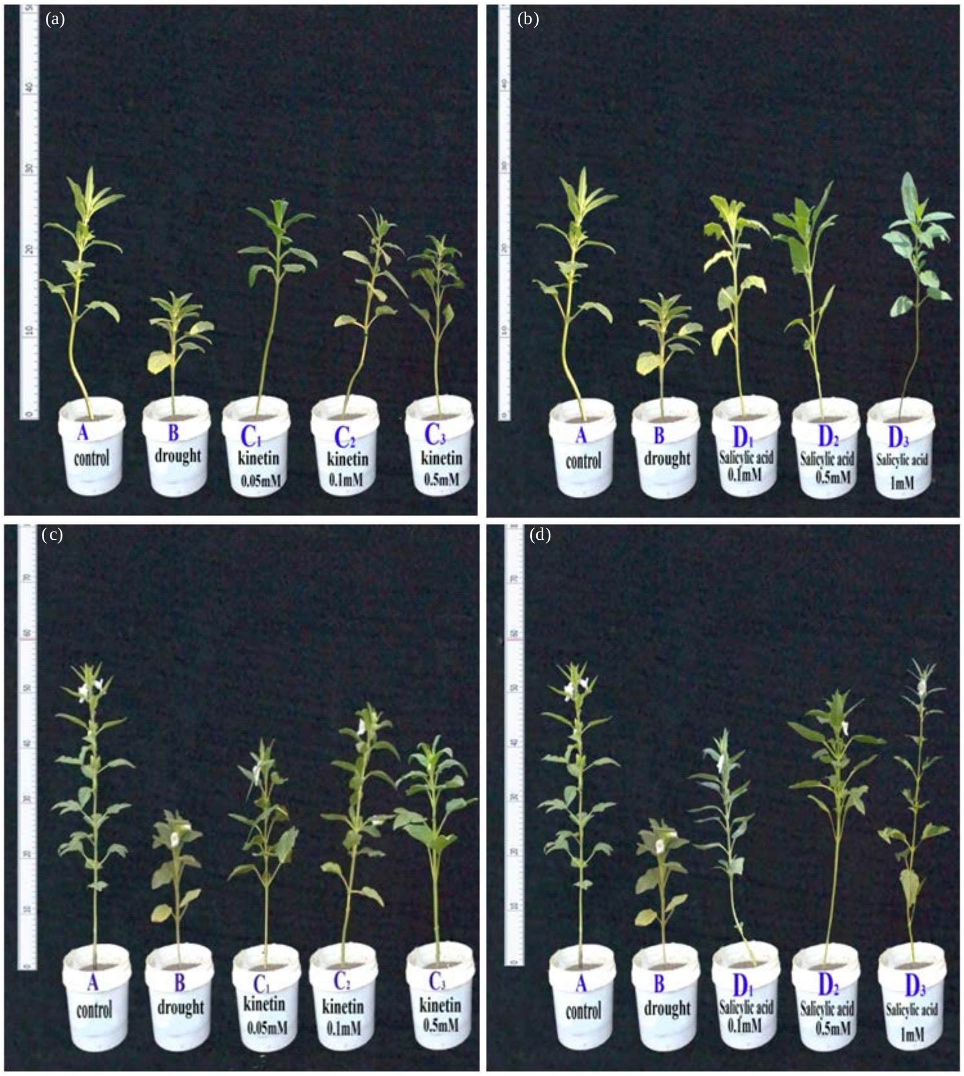

Fig. 1(a-d): Sesame plants treated with (a) Kinetin at vegetative stage, (b) Salicylic acid at vegetative stage, (c) Kinetin at flowering stage and (d) Salicylic acid at flowering stage

Growth criteria: Samples of sesame plants were collected at vegetative and flowering stages according to symptoms appearance. Results present in Table 1-2 and Fig. 1a-d show reduction in growth parameters including plant height, fresh weight, dry weight, number of leaves per plant and leaf area in drought stressed sesame plants as compared to the corresponding control while hormonal treatment with kinetin or salicylic acid with different concentrations enhanced all the previous growth parameters of sesame plants grown under drought stress at vegetative and flowering stages. These results were in agreement with those obtained by Fazeli *et al*. (2007), who found that drought stress decreased fresh and dry mass of sesame plants. Under drought stress, the rate of leaf elongation, leaf numbers, rate of expansion, final leaf size decreased. Drought stress influence total leaf area through its effect on initiation of new leaves, which is decreased under drought stress. Drought stress results in shorter internodes, possibly due to a reduction in leaf area and decrease in light interception, thereby leading to reduced dry mater production and reduced

Table 1: Effect of different concentrations of salicylic acid and kinetin on growth parameters of sesame plants grown under drought stress during vegetative and flowering stages

Treatments	Plant height (cm)		Root length (cm)		No. of leaves/plant		Leaf area (cm^2)	
	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
Control	31.6±0.0306[a]	55.0±0.0234[a]	7.5±0.0250[a]	11.5±0.0229[a]	18.6±0.0215[a]	56±0.0227[a]	45.6±0.0026[a]	101.66±0.0219[a]
Drought	16.8±0.0345[a]	29.0±0.0228[a]	4.5±0.0241[a]	10.3±0.0235[a]	11.8±0.0210[a]	32±0.0223[a]	22.77±0.0019[a]	43.85±0.0226[a]
Kinetin (mM)								
0.05	25.7±0.0309[a]	45.0±0.0224[a]	3.0±0.0234[a]	11.0±0.0234[a]	15.2±0.0202[a]	54±0.0229[a]	34.33±0.0013[a]	83.26±0.0233[a]
0.1	28.2±0.0231[a]	45.8±0.0238[b]	2.9±0.0256[a]	10.5±0.0341[a]	16.2±0.0229[a]	56±0.0228[a]	36.64±0.0025[a]	85.05±0.0204[a]
0.5	27.6±0.0249[a]	43.7±0.0229[a]	2.7±0.0260[a]	11.0±0.0235[a]	15.1±0.0318[a]	50±0.0225[a]	35.62±0.0319[c]	84.35±0.0258[a]
Salicylic acid (mM)								
0.1	28.0±0.0248[a]	51.0±0.0228[a]	3.5±0.0205[a]	9.9±0.0309[a]	15.6±0.0255[a]	50±0.0229[a]	40.9± 0.0198[a]	92.87±0.0190[a]
0.5	28.0±0.0245[a]	46.9±0.05852[a]	3.2±0.01924[a]	10.0±0.0183[a]	16.1±0.0222[a]	52±0.0227[a]	41.2 ± 0.0439[a]	93.31±0.0263[a]
1	31.2±0.0244[a]	54.0±0.0229[a]	3.6±0.0228[a]	10.2±0.0166[a]	15.3±0.0243[a]	50±0.0230[a]	44.0± 0.0023[a]	99.89±0.0276[a]
LSD	0.08	0.05	0.48	0.39	0.06	0.05	0.5	0.2

Data is the mean of 3 replicates ±SE, LSD: Least significant difference means for each category having different superscript letters are significant at $p<0.05$

Table 2: Effect of different concentrations of salicylic acid and kinetin on growth parameters of sesame plants grown under drought stress during vegetative and flowering stages

Treatments	Plant fresh weight (g)		Plant dry weight (g)		Water content (%)	
	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
Control	9.52±0.0194[a]	17.61±0.0296[a]	1.4±0.0019[b]	2.17±0.020[c]	85.2±0.0022[a]	87.6±0.0019[a]
Drought	5.06±0.0131[a]	9.48±0.0420[a]	0.57±0.0020[a]	1.39±0.0278[c]	88.7±0.0245[a]	85.3±0.0201[a]
Kinetin (mM)						
0.05	7.34±0.0114[a]	12.28±0.0249[a]	0.959±0.0015[a]	1.89±0.0263[c]	86.9±0.0178[a]	84.6±0.0131[a]
0.1	6.55±0.0135[a]	13.98±0.0259[a]	0.825±0.0015[b]	1.89±0.0262[c]	87.4±0.0015[a]	86.4±0.0153[a]
0.5	8.50±0.0291[a]	11.33±0.0350[a]	1.001±0.0012[b]	1.80±0.0282[c]	88.2±0.0138[a]	84.1±0.0211[a]
Salicylic acid (mM)						
0.1	8.02±0.0131[a]	14.70±0.0257[a]	1.02±0.0178[a]	1.84±0.02453[c]	87.2±0.00118[a]	87.4±0.0223[a]
0.5	7.08±0.0176[a]	14.83±0.0259[a]	0.990±0.0011[a]	1.86±0.0260[c]	86.01±0.0128[a]	87.4±0.0165[a]
1	8.99±0.0125[a]	15.15±0.0244[a]	1.2±0.0174[a]	2.05±0.0279[a]	86.6±0.0054[a]	86.4±0.0235[a]
LSD	0.08	0.16	0.04	0.15	0.06	0.11

Data is the mean of 3 replicates ±SE, LSD: Least significant difference means for each category having different superscript letters are significant at $p<0.05$

growth. The decrease in shoot length in response to drought may be either due to decrease in cell elongation resulting from water shortage which led to a decrease in each of cell turgor, cell volume and eventually cell growth and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through (Ajithkumar and Panneerselvam, 2013). The reduction of shoot length may protect the loss of water by mechanisms of mitigate drought stress.

Plant hormones are essential for the signaling involved in the induction of plant stress responses. It was observed that foliar application of SA or Kin increased all measured growth criteria in drought stressed sesame plants as shown in Table 1 and 2. These results were in agreement with those obtained by Hussain *et al.* (2008), who found that exogenous SA application was very effective in reducing adverse affects of drought stress in sun flower and improved the growth, enhanced photosynthetic capacity, water use efficiency, leaf turgor and relative water content of stressed sun flower. Salicylic acid and other salicylates are known to affect various physiological and biochemical activities of plants and may play a key role in regulating their growth and productivity. The SA and its close analogues enhanced the leaf area and dry mass production in corn and soybean (Hayat *et al.*, 2010). It has been implicated that kinetin is involved in the plant defense signal transduction pathway. There is a close correlation between the content of

Table 3: Effect of different concentrations of salicylic acid and kinetin on total soluble protein contents (mg g^{-1} dry weight) of sesame plants grown under drought stress during vegetative and flowering stages

	Total soluble protein (mg g^{-1} dry weight)	
Treatments	Vegetative stage	Flowering stage
Control	49.87±0.00852[b]	51.76±0.04547[b]
Drought	109.72±0.00708[c]	117.60±0.04382[a]
Kinetin (mM)		
0.05	85.57±0.04371[b]	116.44±0.0788[a]
0.1	62.89±0.04358[a]	76.86±0.04547[b]
0.5	81.16±0.0000[a]	91.66±0.04382[b]
Salicylic acid (mM)		
0.1	61.42±0.04642[a]	87.99±0.04382[c]
0.5	73.29±0.0741[a]	100.06±0.0000[a]
1	51.87±0.00718[a]	90.30±0.00841[a]
LSD	0.23	0.27

Data is the mean of 3 replicates ±SE, LSD: Least significant difference means for each category having different superscript letters are significant at $p<0.05$

endogenous kinetin and the degree of resistance in plants. Kinetin plays a regulative role in tolerance responses of plants to adverse environmental stresses (Chernyad'ev and Monakhova, 2003; Shao *et al*., 2010).

Total soluble protein contents: Results in Table 3 reveal that total soluble protein content show subsequent increase in drought stressed sesame plants as compared with control plant. These results were in agreement with those obtained by Anosheh *et al*. (2012), who stated that total soluble protein content increased in response to drought stress in wheat plants. Also, synthesis and accumulation of protein was investigated in two *Zea mays* species and remarkable changes were noticed in 78 out of 413 proteins of leaves. These evidences indicated that a kind of relationship existed between protein accumulation and plant physiological resistance against drought stress (Ghorbanli *et al*., 2013). The obtained results were in contrast with Fazeli *et al*. (2007), who said that water stress reduced protein synthesis in sesame leaf and suggested that water stress may cause generation of ROS. The ROS brings about inhibition of protein synthesis or causes protein denaturation.

The increase in total soluble protein level is a natural occurring under drought stress to increase plant adaptation to water deficit conditions. To avoid damage of endogenous components, antioxidant defense proteins and metabolites are essential to maintain cellular functions. The majority of new proteins belong to dehydrin-like proteins, which are abundantly induced during embryo maturation of many higher plants as well as in water stressed seedlings (Pelah *et al*., 1997).

Soluble-protein content in plants, an important indicator of reversible and irreversible changes in metabolism, is known to respond to a wide variety of phytohormones (Bajguz and Piotrowska-Niczyporuk, 2013). In the present study, exogenous application of SA or Kin resulted in an increase in the level of total soluble protein content in stressed sesame as compared to the control as shown in Table 3. The maximum contents are recorded (100.06 and 116.44 mg g^{-1}) in 0.5 mM of salicylic acid and 0.05 mM of kinetin, respectively. This is an indication of the positive effect of SA and kinetin in protein synthesis and improving plant adaptation to drought stress. These results were in agreement with those obtained by Anosheh *et al*. (2012), who stated that exogenous application of SA resulted in an increase in the level of total soluble protein by about 57%, compared to the control in wheat plants. In a previous study, exogenous application of SA was reported to enhance stress tolerance by affecting apoplastic proteins (Tasgin *et al*., 2003). Thus, SA-induced increases in protein content in the sesame plant treatments may be

Table 4: Molecular weights of the protein bands as detected by SDS-PAGE of the extracted protein of drought stressed sesame plants treated with salicylic acid and kinetin during flowering stages in addition to untreated control

				Kinetin (mM)			Salicylic acid (mM)		
				0.05	0.1	0.5	0.1	0.5	0.05
Bands	Marker M	Control lane 1	Drought lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
Molecular weights (kDa)									
Band 1	240	223	-	249	249	249	249	249	249
Band 2	140	-	-	-	140	140	223	223	223
Band 3	100	95	-	110	135	135	-	110	135
Band 4	70	-	-	-	-	-	-	-	-
Band 5	50	-	-	50	-	50	-	-	-
Band 6	35	-	47	47	47	-	47	47	47
Band 7	25	20	45	45	45	45	45	45	45
Band 8	20	19	42	19	19	42	19	19	19
Band 9	15	15	15	15	15	15	15	15	15
Band 10	7	-	-	-	-	-	-	-	-
No. of bands	10	5	4	7	7	7	6	7	7
Total molecular weights (kDa)	660	372	149	535	650	676	598	708	733

attributed to increased tolerance to drought stress. The addition of Kin slightly increased the content of proteins. Probably, the increase in protein level may be induced by elevated and potentially prolonged expression of target genes in response to the addition of the hormone (Zubo *et al.*, 2011). Kin may activate the synthesis of many proteins and involved in metabolism of vascular plants (Kudryakova *et al.*, 2013).

Electrophoretic detection of protein by Sodium Dodecyl Sulphate, Polyacrylamide Gel Electrophoresis (SDS-PAGE): Results in Table 4 show the electrophoretic patterns of protein extracted from drought stressed sesame plants treated with salicylic acid and kinetin as compared to control. There are a significant variations in the protein profile, number of polypeptide bands and total amount of protein in each band appeared in all sesame (*Sesamum indicum* L.) treatments as compared with non-treated control. The electrophoretic analysis (SDS-PAGE) of the protein provides information concerning structural genes and their regulatory system that control the biosynthetic pathway of that protein. Electrophoretic SDS protein profiles were successfully used to establish biochemical and genetic finger printing of many plants (Badr, 1995; Soliman and Ghoneam, 2004).

In the present study, drought significantly led to some modifications in cellular protein level in drought stressed sesame plant as compared to the control, which indicates synthesis and accumulation of several proteins in response to drought stress. Also, exogenous application of SA or Kin resulted in an increase in the cellular protein bands in sesame plants grown under drought stress as compared with control as shown in Table 4, which could be taken as an indicator of enhanced plant resistance to drought stress. According to the results of the present study, new protein bands appeared at molecular weights 42, 45, 47 kDa in drought plants, 42, 45, 47, 50, 110, 135, 140 and 249 kDa in kinetin application and 45, 47, 110, 135 and 249 kDa in salicylic acid application. Fazeli *et al*. (2007) reported that the strongest protein band with the molecular mass between 45-66 kDa was observed in drought stress sesame plants. Two new protein bands with the molecular mass higher than 66 kDa were detected. The number of polypeptide bands increase in drought stressed sesame plant treated with SA (7 bands) and Kin (7 bands) as compared to untreated control (5 bands) and drought stressed plants (4 bands). The total molecular weights (kDa) in each polypeptide bands increase in drought stressed sesame plants treated with SA (679 kDa) and Kin (620 kDa), while it decrease in drought stressed plants (149 kDa) as compared to control (372 kDa).

Table 5: Effect of different concentrations of salicylic acid and kinetin on proline content of sesame plants grown under drought stress during vegetative and flowering stages

	Proline (µg g^{-1} fresh weight)	
Treatments	Vegetative stage	Flowering stage
Control	1.08±0.00251^{b}	2.55±0.00291^{b}
Drought	1.71±0.00281^{a}	3.93±0.00266^{b}
Kinetin (mM)		
0.05	4.13±0.0061^{a}	4.63±0.0034^{c}
0.1	6.19±0.0037^{a}	7.57±0.0030^{a}
0.5	2.16±0.0030^{a}	3.22±0.00221^{b}
Salicylic acid (mM)		
0.1	5.23±0.0232^{a}	5.90±0.00271^{a}
0.5	3.56±0.00254^{a}	3.76±0.0026^{b}
1	3.35±0.0676^{a}	3.96±0.0031^{c}
LSD	0,044	0.058

Data is the mean of three replicates ±SE, LSD: Least significant difference means for each category having different superscript letters are significant at $p<0.05$

The SDS-PAGE detected a presence of number of new polypeptide bands in all hormonal treated samples forming a defense strategy to drought stress as compared with control. It might be due to almost all proteins are soluble in SDS and the resulting protein SDS-complexes are of high negative charges. The hydrogen bonds split up and by reducing the sulphur bonds the proteins may be dissolved into low and high molecular weight subunits and the electrophoretic separation of the protein subunits might be depends on only molecular weights. The main proteins those synthesized in response to drought stress are Late Embryogenesis Abundant (LEA), Heat-Shock Proteins (Hsps), desiccation stress protein, dehydrins, proteases, enzymes required for the biosynthesis of various osmoprotectants, the detoxification enzymes (SOD, CAT, APX, POD, GR). In addition, protein factors involved in the regulation of signal transduction and gene expression, such as protein kinases and transcription factors are also synthesized (Kavar *et al*., 2007).

The SA treatment may also lead to cyclophilins (drought stress protein) mRNA accumulation in bean leaves. The SA regulates many physiological processes and causes plants adaptation to environmental stress (Zhang *et al*., 2004). It was reported that SA would affect the defensive proteins, types of kinase protein and also it was proven that salicylic acid infuse the synthesis of the controlled proteins of plant or herb proteases (Raskin, 1992; Popova *et al*., 1997).

Proline contents: In the present study, drought significantly led to an increase in proline content in sesame plants as compared to the control plant in vegetative and flowering stages (Table 5), which could be due to the putative role of drought in increasing the production of proline precursor's. These results were in agreement with those obtained by Ghorbanli *et al*. (2013), who reported that the leaf proline increased significantly under mild and severe drought stress in comparison with control in tomato plants. Proline accumulation is the first response of plants exposed to drought stress in order to reduce injury to cells. Proline can act as an electron receptor preventing photosystems injuries in dealing with ROS function. Proline accumulation facilitates the permanent synthesis of soluble substances in closing stomata (Ghorbanli *et al*., 2013).

Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. Furthermore, it also contributes to stabilizing sub-cellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007; Dias *et al*., 2014).

Table 6: Effect of different concentrations of salicylic acid and kinetin on endogenous phytohormones content (SA and Kin) (mg g^{-1} fresh weight) of sesame plants grown under drought stress

Treatments	Phytohormones content (mg g^{-1} fresh weight) Salicylic acid	Kinetin
Control	3.95±0.0547[a]	0.85±0.00325[b]
Drought	4.167±0.04382[a]	0.91±0.02750[b]
Kinetin (mM)		
0.05	3.33±0.03451[a]	1.06±0.02214[b]
0.1	4.62±0.04336[a]	1.15±0.023254[b]
0.5	5.35±0.04155[a]	1.09±0.027894[b]
Salicylic acid (mM)		
0.1	4.41±0.05211[a]	1.03±0.002987[b]
0.5	4.36±0.04547[a]	0.86±0.019872[b]
1	4.91±0.04382[a]	0.96±0.019962[b]
LSD	0.3	0.12

All the data are the mean of three replicates ±SE, LSD: Least significant difference means for each category having different superscript letters are significant at $p<0.05$

Exogenous application of salicylic acid with all tested concentrations increase proline content in sesame plants grown under drought stress. The maximum content is 3.56 µg g^{-1} recorded in 0.5 mM of salicylic acid at vegetative stage and 5.90 µg g^{-1} recorded in 0.1 mM at flowering stage. In addition, kinetin application with all tested concentrations increase proline content in drought stressed sesame plants. The maximum content is 6.19 µg g^{-1} recorded in 0.1 mM of kinetin at vegetative stage and 7.57 µg g^{-1} recorded at flowering stage. These results were in agreement with those obtained by Anosheh *et al*. (2012), who reported that exogenous application of SA resulted in significant increases and changes in proline accumulation under drought stress. These changes are sometimes accounted for as adaptabilities which increase the tolerance or resistance of plants against the environmental factors (Metwally *et al*., 2003).

The SA seems to induce protective reactions to drought stress by increasing proline (Umebese *et al*., 2009). It was reported that increasing proline will cause the preservation of the cellular inflammation and reduction of the membrane damage in plants, so the osmosis regulation is as an adaptability which increases the plant tolerance or resistance to drought stress. In contrast with these results, Huq and Larher (1983) stated that after spraying with phytohormones, the accumulation of proline was considerably retarded, whatever the plant organ analyzed, the plant species tested and the level of stress or phytohormone used. This retardation may lead to the conclusion that the phytohormones could alleviate the adverse effects of drought stress. If proline accumulation is considered as an indication of stress injury, thus it can be said that, the exogenously applied growth hormones seem either to protect the plant against water stress injury and consequently the synthesis of proline is retarded or to play a specific role in proline transformations to other growth constituents.

Phytohormones (SA and Kin) content: Plant growth is known to be under the control of endogenous hormonal system and drought stress is known to change the balance of phytohormones content in plants. In the present study, drought showed a significant increase in endogenous SA and Kin contents of sesame leaves as compared to the control. Results in Table 6 show that exogenous application of salicylic acid with all tested concentrations increase endogenous phytohormones content (SA and Kin) in sesame plants under drought stress. Also, kinetin application with all tested concentrations increase phytohormones content (SA and Kin) in drought stressed sesame plants. The maximum content of SA is (5.35 mg g^{-1}) recorded in 0.5 mM of kinetin.

The maximum content of Kin is (1.15 mg g^{-1}) recorded in 0.1 mM of kinetin. These results were agreement with Kutlu *et al*. (2009) who reported that cytokinin levels increased in some plants under drought stress. There are some reports about increases in phytohormones during drought stress (Nilsen and Orcutt, 1996).

The obtained results indicate that these compounds play an important role in the response of sesame plants to drought stress. There are numerous examples with different plant species providing evidences for their ability to affect hormonal balance in plants (Yuldashev *et al*., 2012). Increasing the level of endogenous phytohormones for the improvement of drought tolerance in maize, or to alleviate the injuries produced by drought stress were reported by Wang *et al*. (2008). Our results were in contrast with those obtained by Farooq and Bano (2006), who reported that SA and Kin decreased under drought stress, which is possibly due to decreased SA and CKs synthesis or due to destruction of SA and CKs.

The SA and Kin influence the content of different phytohormones. The result indicated that the growth inhibition and cell damages induced by drought stress were rescued partly with the addition of SA or kinetin. The increase in the quantities of endogenous SA and Kin show that sesame plant becomes more resistance to injuries under drought stress.

CONCLUSION

Exogenous application of drought stressed sesame plants with salicylic acid or kinetin may induce resistance to drought stress by enhancement of growth and various physiological processes which are negatively affected by drought stress.

REFERENCES

Ajithkumar, I.P. and R. Panneerselvam, 2013. Osmolyte accumulation, photosynthetic pigment and growth of *Setaria italica* (L.) P. Beauv. under drought stress. Asian Pac. J. Reprod., 2: 220-224.

Anosheh, H.P., Y. Emam, M. Ashraf and M.R. Foolad, 2012. Exogenous application of salicylic acid and chlormequat chloride alleviates negative effects of drought stress in wheat. Adv. Stud. Biol., 4: 501-520.

Anyia, A.O. and H. Herzog, 2004. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur. J. Agron., 20: 327-339.

Ashraf, M. and M.R. Foolad, 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59: 206-216.

Bacelar, E.A., J.M. Moutinho-Pereira, B.C. Goncalves, H.F. Ferreira and C.M. Correia, 2007. Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environ. Exp. Bot., 60: 183-192.

Badr, A., 1995. Electrophoretic studies of seed proteins in relation to chromosomal criteria and the relationships of some taxa of *Trifolium*. Taxon, 44: 183-191.

Bajguz, A. and A. Piotrowska-Niczyporuk, 2013. Synergistic effect of auxins and brassinosteroids on the growth and regulation of metabolite content in the green alga *Chlorella vulgaris* (Trebouxiophyceae). Plant Physiol. Biochem., 71: 290-297.

Bandurska, H., 2000. Does proline accumulated in leaves of water deficit stressed barley plants confine cell membrane injury? I. Free proline accumulation and membrane injury index in drought and osmotically stressed plants. Acta Physiologiae Plantarum 22: 409-415.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Baydar, H. and S. Ulger, 1998. Correlations between changes in the amount of endogenous phytohormones and flowering in the safflower (*Carthamus tinctorius* L.). Turk. J. Biol., 22: 421-426.

Centritto, M., M. Lauteri, M.C. Monteverdi and R. Serraj, 2009. Leaf gas exchange, carbon isotope discrimination and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. J. Exp. Bot., 60: 2325-2339.

Chen, W.S., 1990. Endogenous growth substances in xylem and shoot tip diffusate of lychee in relation to flowering. Hortic. Sci., 25: 314-315.

Chernyad'ev, I.I. and O.F. Monakhova, 2003. Effects of cytokinin preparations on the pools of pigments and proteins of wheat cultivars differing in their tolerance to water stress. Applied Biochem. Microbiol., 39: 524-531.

Costa, J.M., M.F. Ortuno, C.M. Lopes and M.M. Chaves, 2012. Grapevine varieties exhibiting differences in stomatal response to water deficit. Funct. Plant Biol., 39: 179-189.

Dias, M.C., H. Oliveira, A. Costa and C. Santos, 2014. Improving elms performance under drought stress: The pretreatment with abscisic acid. Environ. Exp. Bot., 100: 64-73.

Farooq, U. and A. Bano, 2006. Effect of abscisic acid and chlorocholine chloride on nodulation and biochemical content of *Vigna radiata* L. under water stress. Pak. J. Bot., 38: 1511-1518.

Fazeli, F., M. Ghorbanli and V. Niknam, 2007. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biologia Plantarum, 51: 98-103.

Ghorbanli, M., M. Gafarabad, T. Amirkian and B.A. Mamaghani, 2013. Investigation of proline, total protein, chlorophyll, ascorbate and dehydroascorbate changes under drought stress in Akria and Mobil tomato cultivars. Iran. J. Plant Physiol., 3: 651-658.

Guinn, G. and D.L. Brummett, 1990. Solid-phase extraction of cytokinins from aqueous solutions with C_{18} cartridges and their use in a rapid purification procedure. Plant Growth Regul., 9: 305-314.

Hayat, Q., S. Hayat, M. Irfan and A. Ahmad, 2010. Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot., 68: 14-25.

Heikal, M.M., M.A. Shaddad and A.M. Ahmed, 1982. Effect of water stress and gibberellic acid on germination of flax, sesame and onion seeds. Biologia Plantarum, 24: 124-129.

Huq, S.M.I. and F. Larher, 1983. Effect of NaCl salinity on the growth and the nitrogen status of nodulated cowpea (*Vigna sinensis* L.) and mung bean (*Phaseolus aureus* L.). Zeitschrift Pflanzenphysiologie, 112: 79-87.

Hussain, M., M.A. Malik, M. Farooq, M.Y. Ashraf and M.A. Cheema, 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. J. Agron. Crop Sci., 194: 193-199.

Itai, C., H. Meidner, C. Willmer, J.D.B. Weyers and J.R. Hillman, 1978. Abscisic acid and guard cells of *Commelina communis* L. Nature, 271: 652-653.

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 Surf. B: Biointerfaces, 61: 298-303.

Kavar, T., M. Maras, M. Kidric, J. Sustar-Vozlic and V. Meglic, 2007. Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress. Mol. Breed., 21: 159-172.

Kudryakova, N.V., M.V. E mova, M.N. Danilova, N.K. Zubkova, V.A. Khripach, V.V. Kusnetsov and O.N. Kulaeva, 2013. Exogenous brassinosteroids activate cytokinin signalling pathway gene expression in transgenic *Arabidopsis thaliana*. Plant Growth Regul., 70: 61-69.

Kutlu, N., R. Terzi, C. Ekeli, G. Senel, P. Battal and A. Kadioglu, 2009. Changes in anatomical structure and levels of endogenous phytohormones during leaf rolling in *Ctenanthe setosa* under drought stress. Turk. J. Biol., 33: 115-122.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Metwally, A., I. Finkemeier, M. Georgi and K.J. Dietz, 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol., 132: 272-281.

Morris, J.B., 2002. Food, Industrial Nutraceutical and Pharmaceutical Uses of Sesame Genetic Resources. In: Trends in New Crops and New Uses, Janick, J. and A. Whipkey (Eds.). ASHS Press, Alexandria, VA., pp: 153-156.

Nilsen, E.T. and D.M. Orcutt, 1996. Phytohormones and Plant Responses to Stress. In: Physiology of Plant Under Stress, Nilsen, E.T. and D.M. Orcutt (Eds.). John Wiley and Sons, New York, USA., pp: 183-198.

Pelah, D., W.X. Wang, A. Altman, O. Shoseyov and D. Bartels, 1997. Differential accumulation of water stress-related proteins, sucrose synthase and soluble sugars in *Populus* species that differ in their water stress response. Physiologia Plantarum, 99: 153-159.

Popova, L., T. Pancheva and A. Uzunova, 1997. Salicylic acid: Properties, biosynthesis and physiological role. Bulgarian J. Plant Physiol., 23: 85-93.

Raskin, I., 1992. Role of salicylic acid in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol., 43: 439-463.

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. Bulgarian J. Plant Physiol., 29: 314-319.

Shao, R., K. Wang and Z. Shangguan, 2010. Cytokinin-induced photosynthetic adaptability of *Zea mays* L. to drought stress associated with nitric oxide signal: Probed by ESR spectroscopy and fast OJIP fluorescence rise. J. Plant Physiol., 167: 472-479.

Shindy, W.W. and O.E. Smith, 1975. Identification of plant hormones from cotton ovules. Plant Physiol., 55: 550-554.

Soliman, M.I. and G.T. Ghoneam, 2004. The mutagenic potentialities of some herbicides using *Vicia faba* as a biological system. Biotechnology, 3: 140-154.

Studier, F.W., 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol., 79: 237-248.

Tasgin, E., O. Atici and B. Nalbantoglu, 2003. Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves. Plant Growth Regul., 41: 231-236.

Umebese, C.E., T.O. Olatimilehin and T.A. Ogunsusi, 2009. Salicylic acid protects nitrate reductase activity, growth and proline in amaranth and tomato plants during water deficit. Am. J. Agric. Biol. Sci., 4: 224-229.

Wahid, A., S. Gelani, M. Ashraf and M.R. Foolad, 2007. Heat tolerance in plants: An overview. Env. Exp. Bot., 61: 199-223.

Wang, C., A. Yang, H. Yin and J. Zhang, 2008. Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. J. Integr. Plant Biol., 50: 427-434.

Weatherley, P.E., 1950. Studies in the water relations of the cotton plant: I. The field measurement of water deficits in leaves. New Phytol., 49: 81-97.

Werner, T. and T. Schmulling, 2009. Cytokinin action in plant development. Curr. Opin. Plant. Biol., 12: 527-538.

Yuldashev, R., A. Avalbaev, M. Bezrukova, L. Vysotskaya, V. Khripach and F. Shakirova, 2012. Cytokinin oxidase is involved in the regulation of cytokinin content by 24-epibrassinolide in wheat seedlings. Plant Physiol. Biochem., 55: 1-6.

Zhang, M., L. Duan, Z. Zhai, J. Li and X. Tian *et al*., 2004. Effects of plant growth regulators on water deficit-induced yield loss in soybean. Proceedings of the 4th International Crop Science Congress, September 26-October 1, 2004, Brisbane, Australia, pp: 122.

Zubo, Y.O., M.V. Yamburenko, V.V. Kusnetsov and T. Borner, 2011. Methyl jasmonate, gibberellic acid and auxin affect transcription and transcript accumulation of chloroplast genes in barley. J. Plant Physiol., 168: 1335-1344.