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PHYSIOLOGICAL RESPONSES OF *PISUM SATIVUM* PLANT TO EXOGENOUS ABA APPLICATION UNDER DROUGHT CONDITIONS

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Abstract

This study was carried out to investigate the possibility of using foliar ABA application with different concentrations (50,100 and 150 µg/L^{-1}) in order to decrease the adverse effect of water-deficit stress. The main parameters of interest were; growth parameters; endogenous phytohormones, proline; pigments contents; number and shape of stomata; minerals concentrations; isozymes; and SDS -PAGE protein profile in *Pisum sativum* plant at 36 days under drought and ABA treatments. The results showed that ABA reduces the reduction in both fresh and dry weights shoot and root of *Pisum* induced by drought stress. Proline content was increased in response to drought and ABA treatments. Water-stress caused significant reduction in Chl. a, b, whereas application of ABA significantly increased in total chlorohpyll. Water stress significantly decreased IAA, GA concentrations and increased ABA level in leaves than these of the control. ABA treatment partially overcame the decrease in IAA and GA contents. Water stress and ABA increased the activity of (POD) and (ACP) enzymes in *Pisum* plants. Proteins profile of *Pisum sativum* in shoot revealed qualitative and quantitative changes, also appearance or disappearance of some bands. After 54 days the yield harvested and total carbohydrates and SDS -PAGE protein profile in seed were investigated. The data showed that total carbohydrates contents were significantly decreased in plant under drought and ABA partially alleviate the adverse effect of water-deficit stress.

Introduction

Water is one of the most important ecological factors which mainly determined crop growth and development. Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle (Abdul Jaleel *et al.,* 2008). Water deficit influences plant growth at various levels from cell to community (Smith & Griffiths, 1993). It reduces plant growth through inhibition of various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones (Kramer, 1983).Also, water stress reduces the rate of photosynthesis (Chaves, 1991; Lawlor, 1995).

The production of reactive oxygen species is a common phenomenon in plants under drought stress. These reactive oxygen species (ROS) generations led to lipid peroxidation (Chen *et al.,* 2000) and protein degradation (Jiang & Zhang 2001). To alleviate adverse effects of reactive oxygen species, plants have evolved an antioxidant defense system that includes enzymes like superoxide dismutase, peroxidase and catalase etc. (Agarwal & Pandey 2004).

The alteration of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought tolerance. Both quantitative and qualitative changes of proteins were detected during water stress (Riccardi *et al*., 1998).

Moreover proline is one of osmolytes which increase faster than other amino acids in plants under water stress and help the plants to maintain the cell turgor (Valentovic *et al*., 2006). Therefore increasing proline concentration can be used as an evaluating parameter for irrigation scheduling and for screening drought resistant varieties (Gunes *et al*., 2008). Alexieva *et al.,* (2001) have found that proline content increased under drought stress in pea plants. The accumulation of proline in plant tissues is a clear marker for environmental stress, particularly in plants under drought stress.

Abscisic acid (ABA) is a plant hormone that plays a role in plant response to drought stress as cellular signaling in water movement from root to leaf (Alves & Setter, 2004), resulting in whole plant physiological and morphological adaptations (Yin *et al*., 2004). In addition, ABA is a stress signal generated in root tissues, carried up through xylem to shoot by transpiration stream and function as stomatal closure to reduce the water loss in transpiration (Seo & Koshiba, 2002).

Although, it has been shown that ABA can result in an oxidative stress, an enhancement in the capacity of oxidative stress tolerance may imply that plants need to mobilize the whole antioxidant defense systems including enzymatic and non-enzymatic constituents to resist oxidative damage in stressed plant tissues, rather than a few enzymes or metabolites. Different concentrations of ABA from 1 to 1,000 µM have been used to different plant tissues and have been shown to be able to induce gene expression and protein synthesis involved in antioxidative defenese (Guan *et al*., 2000). Drought stress significantly decreased IAA and GA concentration in leaves than that of control (Xie *et al.,* 2003). But under stress condition exogenous ABA caused an increase in the IAA and GA content compared to untreated plants (Farooq & Bano, 2006).

Pea (*Pisum sativum* L.), is a cool season food legume, has a wide variety of uses and is grown in Egypt and other countries of the Mediterranean region as a cheap source of protein.

Pea has high levels of the amino acids, lysine and tryptophan which are low in cereal grains and grain protein in pea can range from 19 to 27 percent but is most commonly 22 to 24 percent. Pea also contains high levels of carbohydrates and is low in fiber and contains 86 to 87 percent total digestible nutrients (Miller *et al.,* 2005).

The objective of this work is to investigate the effect of drought stress and ABA treatment on growth, protein profile, proline, stomotal growth and development, endogenous phytohormone level and activities of anti-oxidant enzymes in leaves after 36 days, and protein profile and total carbohydrates in seeds of pea plant.

Materials and Methods

The seeds of pea (*Pisum sativum*) were obtained from the Ministry of Agriculture, Agriculture Research Centre, Giza, Egypt. Seeds of pea were surface sterilized for 1 min in ethanol 70% (v/v), 20 min in 5% (v/v) sodium hypochlorite and rinsed five times with sterile double-distilled water. The seedlings were grown in a greenhouse and watered daily. When the second leaf was fully expanded, pots divided into 5 groups one set of pots was kept as control (well watered) and four other sets were used for drought (drought stress) and ABA (drought and ABA-treatment together) assessment, the concentrations of ABA were 50,100 and 150µg (ml-1). Drought was induced for a period of five days. While non-stressed plants continued to receive daily irrigation. After the second foliage leaf emersion, the treatments were given as foliar spray. After 36 days the effect of drought and ABA on photosynthetic pigments, activity of some antioxidant enzymes, electrophoresis SDS-PAGE, stomotal growth and development, endogenous phytohormone level, NPK and proline of pea plant were investigated in leaves. Yield was harvested after 54 days cultivation, total carbohydrate and electrophoresis SDS- PAGE of seeds were determined.

Plant growth: The plants with various treatments were up-rooted, washed with distilled water and then randomly selected at least seven seedlings and the fresh weights of shoots and roots and shoot length were determined. The samples were oven dried at 70°C for 72 h. and the dry weights of shoot and root were determined.

Biochemical analysis

Determination of photosynthetic pigment: Contents of photosynthetic pigments in treated and untreated of leaves of *Pisum* plant (100 mg) were extracted in 25 ml of chilled acetone solution in the dark. After centrifugation at 5000 rpm for 10 min, the absorbance of the supernatant was taken at 663, 644 and 452.5 nm against 80% acetone as blank. The chlorophylls content were estimated by the method of (Metzner *et al.,* 1965). The content of chlorophylls and carotenoid were expressed in mg g-1 f.w.

Determination of proline content: The proline content was determined using the method recorded by (Bates *et al*., 1973). Proline extracted from 100 mg of fresh leaves samples with 2 mL of 40% methanol. 1 mL of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3: 2, v/v) and 25 mg of ninhydrin mixed with 1 mL of the extract. After 1 h incubation at 100°C, the reaction terminated by putting the tubes in ice bath, 5 mL toluene added. The absorbance of the upper phase was spectrophotometrically determined at 520 nm.

Determination of phytohormone: Extraction, purification and quantitative determination of endogenous hormones, namely gibberellic acid (GA_3), indole acetic acid (IAA) and abscisic acid (ABA) were carried out according to the methods of Ünyayar *et al*., (1996) and Baydar & Ülger (1997). Analyses of GA_3, IAA and ABA were performed on a Model Varian 9050 HPLC equipped with UV detector and Model Varian 9010 pumps enabling the use of a concentration gradient of the mobile phase. Separations and determinations were performed on a nukleosil C18 column (4.6 mm x 150 mm). The Mobile phase yielded results of 30% methanol (adjusted to pH 3.0 with 0.1 M H_3PO_4) for GA_3, 55% methanol (in 0.1 M acetic acid) for ABA and 35% methanol (in %1 acetic acid) for IAA. The total run time for separations was approximately 15 min at a flow rate of 1 ml/min. GA_3, IAA and ABA were detected by their absorption at 280, 208 and 265 nm, respectively and the peak area of the samples were compared to the corresponding peak areas of standard solutions containing known concentrations of GA_3, IAA and ABA.

Measurement of minerals concentration: The concentration and total uptake of macronutrients (Nitrogen N, Phosphorus P, and Potassium K) in the Pea plants were determined using the standard method described by (Cottenie *et al*., 1982).

Extraction and assay of enzymatic activity: Isozymes were estimated in plants by using polyacrylamide gel electrophoresis (Native–PAGE). Two isozymes peroxidase (POD) (EC 1.11.1.7), and acid phosphatase (ACP, EC. 3.1.3.2) were estimated from the plant samples according to (Stegmann *et al*., 1985). After electrophoresis, the gels stained according to their enzyme system, incubated at 37°C in a dark room for complete staining, the staining gels were carried out according to Jonathan & Wendel (1990) and Graham *et al.,* (1964).

Polyacrylamide Gel Electrophoresis (SDS-PAGE): Protein extracts from the leaves of *Pisum* plant under treated and untreated were subjected to SDS - PAGE according to the method of Laemmli, (1970).

Examination of stomata samples: When plants harvested from the field, leaves of *Pisum* were preserved in formalin-acetic acid-ethanol (FAA; 1:1:9) for microscopic examination (Zwieniecki *et al*., 2004).The area stomatal opening were measured by light microscope (image analysis) at power 40x.

Determination total carbohydrates: Total carbohydrates were estimated by the method of Hedge & Hofreiter (1962) using anthrone reagent. Contents of total carbohydrates were expressed as mg g^{-1} d.w. using glucose as standard.

Statistical analysis: The obtained data was subjected to a statistical analysis using SAS Statistical Analysis System SAS. (2006). The statistical analysis model used was two ways analysis of variance with interaction at LS D. 5%.

Results

Growth parameters: The plant growth parameters of *Pisum* (shoot length, fresh and dry weights of shoots and roots) decreased significantly with drought stress as compared with control plants (Table 1). ABA treatment in combination with drought stress showed significant increase in all the growth parameters when compared with the drought.

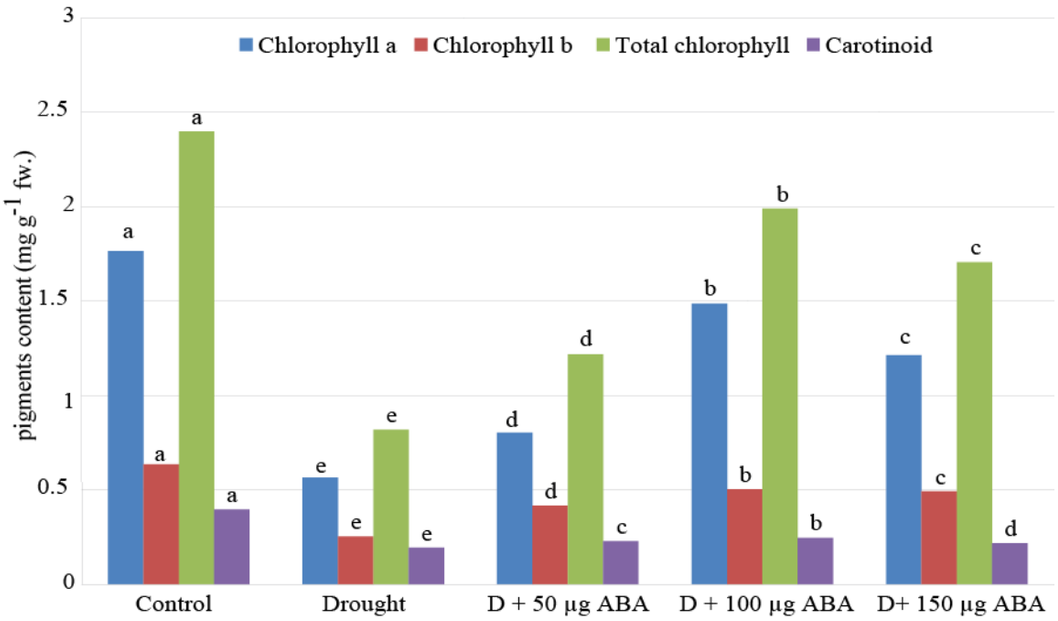
Table 1. Effect of drought and ABA on fresh weights, dry weights and shoots length in *Pisum sativum* plant.

Treatment	Shoot		Root		Shoot length (cm)
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	
Control	2.73[a]	0.97[a]	0.40[a]	0.09[a]	25[a]
Drought	0.91[e]	0.21[d]	0.22[c]	0.04[b]	15[d]
Drought + 50 µg ABA	1.22.[d]	0.58[c]	0.31[b]	0.05[b]	20[c]
Drought + 100 µg ABA	1.85[b]	0.70[b]	0.34a[b]	0.05[b]	22[b]
Drought + 150 µg ABA	1.77[c]	0.77[b]	0.29b[c]	0.04[b]	19[c]
LSD 5%	0.0182	0.083	0.083	0.0182	1. 8193

Data with different superscript letters were significantly different (p≤0.05)

Table 2. Effect of drought and ABA on GA_3, IAA and ABA contents in *Pisum sativum* plant.

Treatment	GA_3 mg/100g f.w.	IAA mg/100g f.w.	ABA µg /100 f.w.
Control	10.747	12.095	58.5
Drought	6.141	8.430	160
Drought+50µg ABA	7.258	9.542	213
Drought+100µg ABA	8.233	10.264	275
Drought+150µg ABA	8.018	9.447	312



Data with different superscript letters were significantly different (p≤0.05)
Fig. 1. Effect of drought and ABA on photosynthetic pigments content (mg g^{-1}fw.) in *Pisum sativum* plant.

Chlorophyll and Carotenoid Content: During the present investigation water stress significantly ($p<0.05$) reduced the contents of chlorophyll and carotenoid of leaf in *Pisum* on vegetative stage. But under stress condition exogenous ABA caused an increase in the contents of chlorophyll and carotenoid of leaf in *Pisum* plant (Fig. 1).

Responses of phytohormones to drought and ABA: Drought stress significantly decreased IAA, GA concentrations and increased in ABA concentration in leaves than that of control (Table 2).

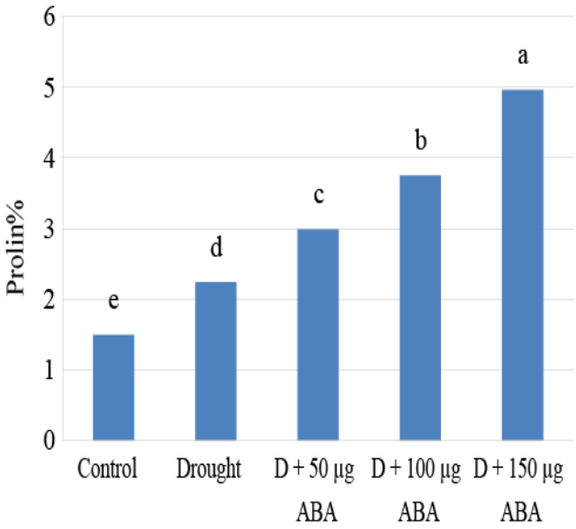
Responses of endogenous proline level to drought and ABA: Endogenous proline levels were significantly increased ($p<0.05$) affected by the application of ABA and drought stress when compared with untreated plants (Fig. 2).

Responses of minerals concentration to drought and ABA: This study finding that water stress led to reduction in the quantity of essential minerals potassium, phosphorous and nitrogen contents of pea plants (Table 3) which are very necessary for healthy growth and development, thus affecting efficacy and the pharmaceutical values of the leaves of the plants.

Polyacrylamide Gel Electrophoresis (SDS-PAGE): The SDS-PAGE results in shoot after 36 days showed that the presence and absence of protein bands (Table 4) revealed the differences among accessions as well as treatments. (Table 4, Fig. 3), showed that the number protein bands of *Pisum* were affected by the drought and ABA. New bands at M.W. 86, 50, 49, 48, 26 & 24 kDa appeared

respectively on drought and ABA. However at drought stress alone, the band at M.W. 142& 52 kDa disappeared. Also at drought stress and ABA, bands at M.W. 165,142 & 29 kDa disappeared.

The SDS-PAGE results in seed after 54 days showed presence and absence of protein bands (Table 5 Fig. 4), that new bands at M.W. 140.32, 77.23, 73.45, 61.37, 50.63, 46.71 & 27.3 kDa appeared respectively on drought and ABA. Although at drought and ABA stress, the bands at M.W. 163.18, 101.14, 87.44, 29.9, 25.8, 24.5 & 15.7 kDa disappeared respectively on drought and ABA.



Data with different superscript letters were significantly different ($p \leq 0.05$)

Fig. 2. Effect of drought and ABA on prolin percentage in *Pisum sativum* plant.

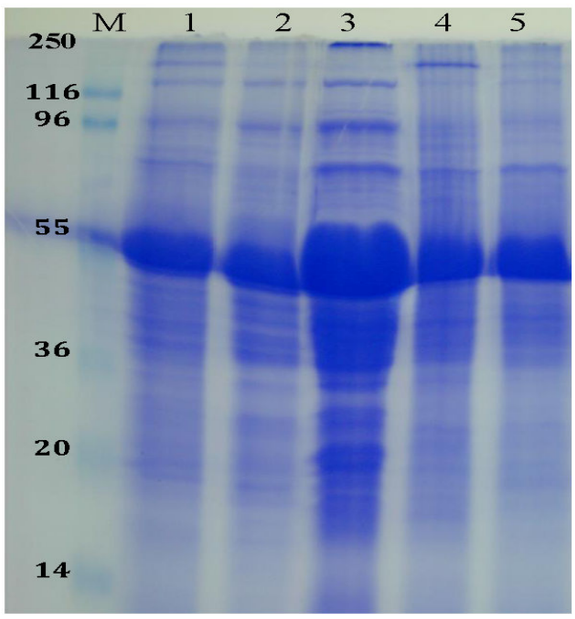


Fig. 3. Banding protein pattern of *Pisum sativum* shoot treated with different concentrations (50µM-100 µM -150 µM) of ABA. (Lane 1, Control; Lane2, Drought; Lane3, Drought + 50 µg ABA; Lane4, Drought + 100 µg ABA; Lane5, Drought + 150 µg ABA).

Enzyme activity: The activity of the antioxidants enzymes investigated in *Pisum* increased under water stress and ABA treatments. The induction of new isozymes and the change in the isoenzymes profiles play an important role in the cellular defense against oxidative stress, caused by water-deficit stress and ABA treatments (Table 6, Fig. 5).

Responses of stomata to drought and ABA: The result showed that stomatal aperture, size and form were quite different in the experimental plants than in the control plants. In drought and ABA treatments, the number of stomata per area was higher, the size was smaller, and the shape was more irregular and closed than in the control (Table 7, Fig. 6).

Total carbohydrates: Total carbohydrate contents decreased significantly with drought stress (Fig. 7) and treatment with ABA caused significant increase in the same contents in seed of pea plants. These results are in accordance with (Ahmed *et al.,* 1989).

Discussion

Drought stress reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq *et al*., 2008). Moreover such decline in shoot length in response to drought might be due to either decrease in cell elongation resulting from the inhibiting effect of water shortage on growth promoting hormones which, in turn, led to a decrease in each of cell turgor, cell volume and eventually cell growth (Banon *et al*., 2006). Creelman *et al*., (1990) showed that ABA is a regulator of shoot growth and development under water stress. Moreover, the role of ABA in closing stomata of drought-stressed plants has been widely reported. This effect is suggested to be vital for fast growth resumption and recovery of water content of plants (Zhang *et al.,* 2006).

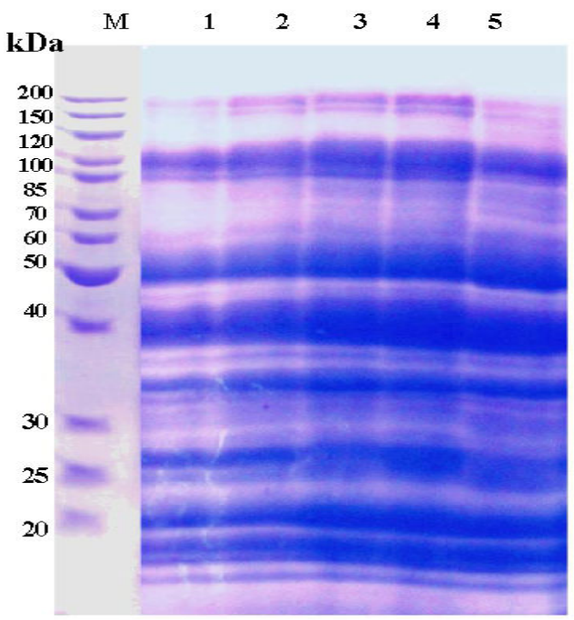


Fig. 4. Banding protein pattern of yield seeds of *Pisum sativum* plants treated with different concentrations (50µM-100 µM -150 µM) of ABA. (Lane 1, Control; Lane2, Drought; Lane3, Drought + 50 µg ABA; Lane4, Drought + 100 µg ABA; Lane5, Drought + 150 µg ABA).

Table 3. Effect of drought and ABA on minerals percentage in *Pisum sativum* plant.

Treatment	N%	P%	K%
Control	3.49[a]	0.849[a]	4.82[a]
Drought	2.14[b]	0.538[b]	3.73[b]
Drought + 50 µg ABA	1.99[c]	0.503[c]	2.33[c]
Drought + 100 µg ABA	1.95[d]	0.473[d]	1.59[d]
Drought + 150 µg ABA	1.26[e]	0.410[e]	1.45[e]
LSD 5%	0.0182	0.0083	0.0169

Data with different superscript letters were significantly different ($p \leq 0.05$)

Table 4. SDS-PAGE of protein patterns of *Pisum sativum* shoot treated with different concentrations (50µM -100 µM -150 µM) of ABA.

kDa.	1	2	3	4	5
165	+	+	+	-	+
142	+	-	-	+	+
120	+	+	+	+	+
86	-	-	+	-	-
66	+	+	+	+	+
58	+	+	+	+	+
52	+	-	+	+	+
50	-	+	+	+	-
49	-	+	+	+	-
48	-	+	+	+	-
45	+	+	+	+	+
41	+	+	+	+	+
39	+	+	+	+	+
38	+	+	+	+	+
35	+	+	+	+	+
29	+	+	+	+	-
26	-	+	+	+	+
24	-	+	+	+	+
23	+	+	+	+	+
21	+	+	+	+	+
Total	**14**	**17**	**19**	**18**	**15**

Lane-1, Control; Lane-2, Drought; Lane-3, Drought + 50 µg ABA; Lane-4, Drought + 100 µg ABA; Lane-5, Drought + 150 µg ABA

Table 5. SDS-PAGE of protein patterns of *Pisum sativum* seed treated with different concentrations (50µM -100 µM -150 µM) of ABA.

kDa.	1	2	3	4	5
192.76	+	+	+	+	+
163.18	+	+	+	+	-
140.32	-	-	-	-	+
101.04	+	+	+	+	-
87.44	+	+	+	-	+
77.23	-	-	+	+	-
73.45	-	-	-	+	+
61.37	-	+	+	+	+
50.63	-	+	+	+	+
46.71	-	-	+	+	+
39.19	+	+	+	+	+
36.55	+	+	+	+	+
33.85	+	+	+	+	+
31.75	+	+	+	+	+
29.9	+	-	-	-	+
27.3	-	+	+	+	-
25.8	+	-	+	-	+
24.5	+	+	-	-	+
19.5	+	+	+	+	+
15.7	+	+	-	-	+
15.2	+	+	+	+	+
13.1	+	+	+	+	+
Total	**15**	**16**	**17**	**16**	**18**

Lane-1, Control; Lane-2, Drought; Lane-3, Drought + 50 µg ABA; Lane-4, Drought + 100 µg ABA; Lane-5, Drought + 150 µg ABA

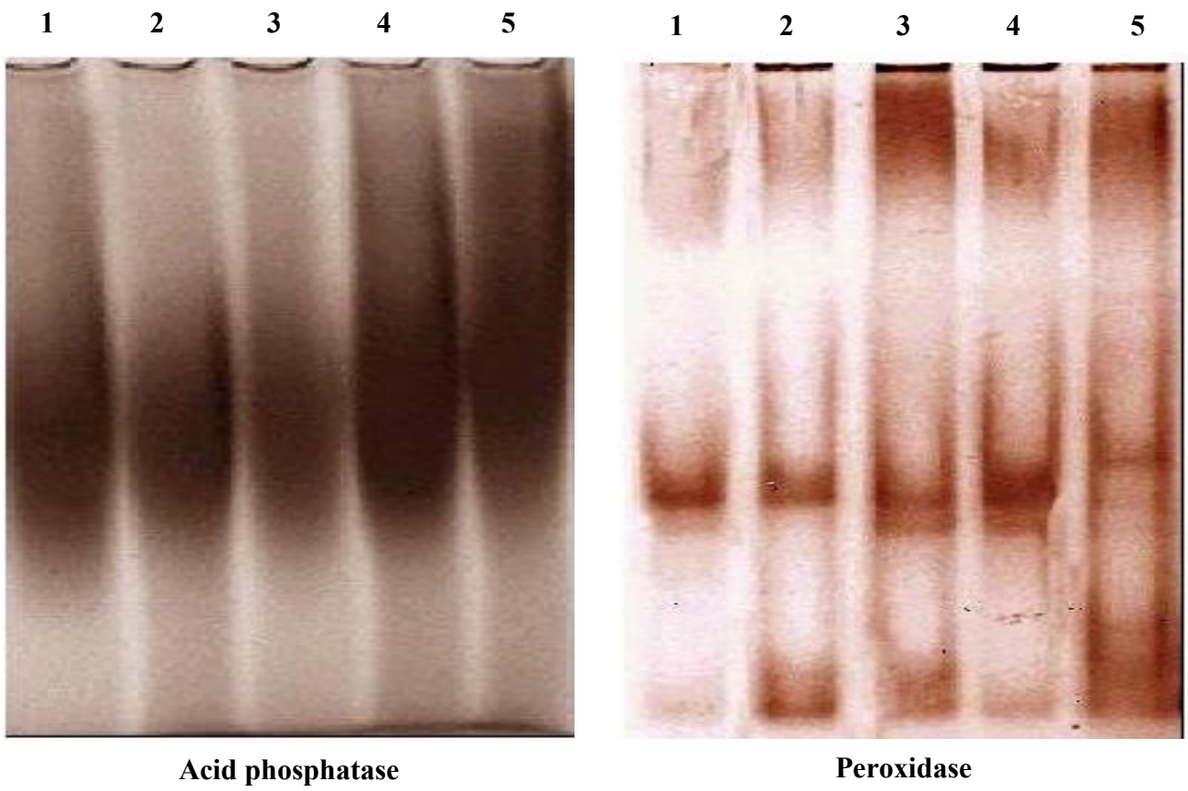


Fig. 5. Effect of drought and ABA on Zymogram of two enzymes, acid phosphatase and peroxidase on *Pisum sativum*. (Lane 1, Control; Lane2, Drought; Lane3, Drought + 50 µg ABA; Lane4, Drought + 100 µg ABA; Lane5, Drought + 150 µg ABA).

Table 6. The presence (+) and absence (-) of bands in two isozymes, peroxidase and acid phosphatase at the effect of drought and ABA on *Pisum sativum*

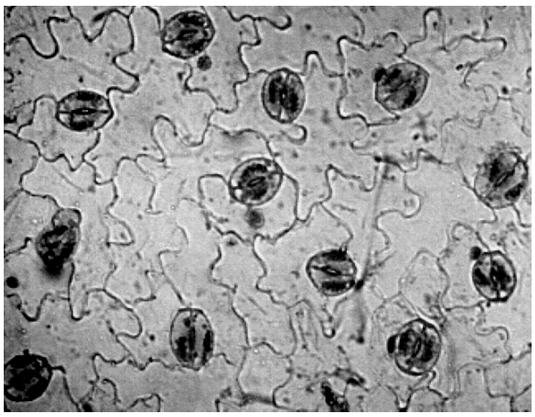
Peroxidase					
Rf.	**1**	**2**	**3**	**4**	**5**
0.14	+	+	+	+	+
0.66	-	-	-	-	+
0.71	+	+	+	+	+
0.75	+	+	+	+	+
0.90	+	+	+	+	+
Acid phosphatase					
Rf.	**1**	**2**	**3**	**4**	**5**
0.65	-	-	-	+	+
0.92	+	+	+	+	+

Lane-1, Control; Lane-2, Drought; Lane-3, Drought + 50 µg ABA; Lane-4, Drought + 100 µg ABA; Lane-5, Drought + 150 µg ABA

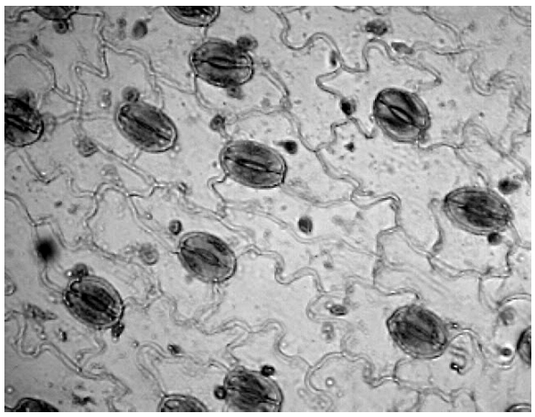
Table 7. Effect of drought and ABA on area of stomatal opening (µm)^2 in *Pisum sativum* plant.

Treatment	Control	Drought	Drought + 50 µg ABA	Drought + 100 µg ABA	Drought + 150 µg ABA
Area of stomatal opening (µm)^2	25.193^a	15.680^b	14.907^b	13.637^{bc}	8.411^c
L.S.D.5%			5.243		

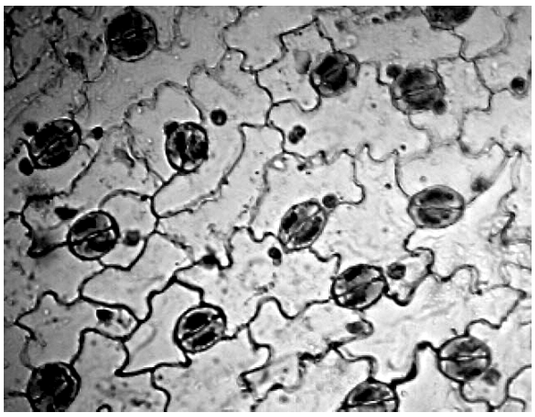
Data with different superscript letters were significantly different ($p \leq 0.05$)



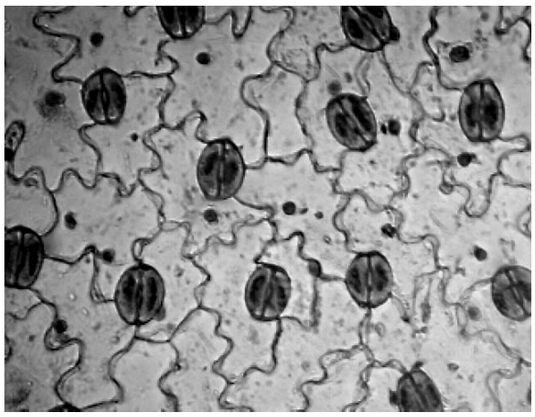
Drought



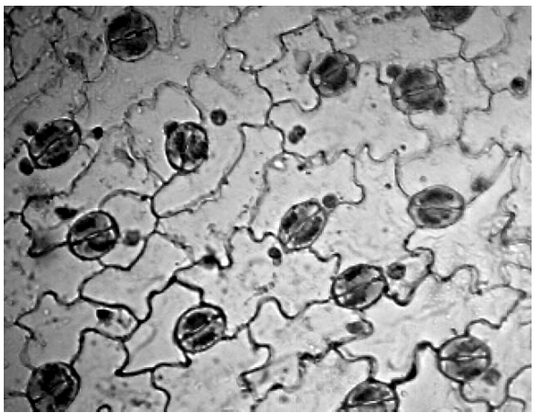
Control



Drought + 50 µg ABA



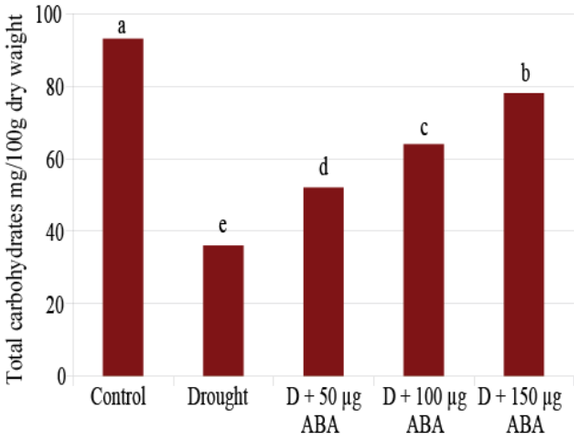
Drought + 100 µg ABA



Drought + 150 µg ABA

Fig. 6. Effect of exogenous ABA on stomata number and size in *Pisum sativum* plant under drought stress.

Chlorophyll content is often measured in plants to assess the impact of environmental stresses, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh, 1990). Similar to the results of Ashraf *et al*., (1994) the limitation of water supply induced faster chlorophyll degradation in present experiment. Carotenoids are also responsible for scavenging of singlet oxygen (Knox & Dodge, 1985) and the decrease in carotenoid under water stress might also have contributed to the increased ROS, which further oxidized the photosynthetic pigments. In the present experiment, the treatment with ABA partially ameliorated the adverse effect of water stress on chlorophyll content and carotenoid contents. .In the same trend, exogenous ABA exposure to wheat plants grown under field conditions of soil water restriction, resulted in the increases in chlorophyll and carotenoid contents in compared with treated plants, (Travaglia, *et al.,* 2007). ABA is supposed to maintain high chlorophyll and carotenoid contents the reason could be the increased stability of pigments by ABA (Gadallah, 1995) by increasing the activities of antioxidant and osmolytes as evident from the present investigation.



Data with different superscript letters were significantly different ($p \leq 0.05$)

Fig. 7. Effect of drought and ABA on total carbohydrates in *Pisum sativum* plant.

The decrease in IAA and GA was recorded previously by (Farooq & Bano, 2006). It may be due to decreased IAA and GA synthesis (Xie *et al*., 2004) or an increase in the destruction of IAA and GA by increasing the activity of oxidase. However treatment with ABA partially overcame the decrease in IAA and GA content observed under drought stress. Similar result was reported by (Maiti *et al*., 2000). Available evidence suggests that abscisic acid (ABA) synthesized in the roots under water stress and transported to the leaves may act as a root-to-shoot chemical signal of water stress conditions and, together with ABA synthesized in the leaves themselves, induce stomatal closure (Liang *et al.,* 1996). Moreover, plant hormones play a role in the transformation of stress-related signals into changes in gene expression needed for adaptation to suboptimal environmental conditions (Hare *et al*., 1997).

The result that proline content increased under drought stress in pea plants has been found by (Alexieva *et al*., 2001 and Hasson & Poljakoff-Mayber 1983). The induced proline may function in osmotic adjustment, ROS scavenging, and protein stabilization (Ramanjulus & Bartels, 2002), which are important for good adaptation to drought stress. The higher proline content could be due to enhance activity of ornithine aminotransferase (OAT) and pyrroline 5-carboxylate reductase (P5CR), the enzyme involved in proline biosynthesis as well as due to the inhibition of proline oxidase, proline catabolising enzymes (Debnath, 2008).

The effect of ABA on the concentration of mineral elements in plants was reported by Osuagwu *et al.,* (2010) who suggested that mineral elements in plants may be influenced by various agronomic and environmental factors such as water stress. This result was agree with (Yu *et al.,* 2007) who reported that water stress is also led to reduction in potassium content of plants. The reduction of potassium in the leaves of the plants might be due to the mobilization of potassium ion from leaves to the roots in response to water stress to increase the osmotic potential of the sap of the roots to assist the plants to withstand the effect of drought (Yu *et al.,* 2007). Also, some research reports have shown that water stress cause decrease in phosphorous and nitrogen content of plants, such as Ramoliya *et al*., (2004), who reported that water stress caused reduction in the phosphorous and nitrogen contents of the leaves of plants. Foliar exogenous ABA reduces potassium, phosphorous and nitrogen concentrations in *Pisum* plant under drought. It concluded that foliar exogenous ABA reduces transpiration by closing stomata and thus leads to reduce ion uptake in plant (Yeo *et al.,* 1985).

The effect of ABA on protein band was reported by Robinson *et al*., (1990) who suggested that the disappearance of polypeptides during stress were compensated by the increased synthesis of others. Ali & Basha (1998) showed that the total protein content of the leaves significantly increased when peanut plants were subjected to water stress for 5 to 20 as compared to irrigated controls. De- Britto *et al.,* (2011) reported that analysis of the leaf protein by SDS polyacrylamide gel electrophoresis showed higher levels polypeptides in stressed leaves. Changes in protein patterns induced due to drought play a pivotal role in the adaptive response of plants to the stress (Riccardi *et al*., 1998). Furthermore, recent identification and characterisation studies have demonstrated that most of the drought-responsive proteins are related to metabolism, energy, protein biosynthesis, cell defense, signal transduction, transport, and lignification (Rodríguez *et al*., 2006). Also, (Asghari & Ebrahimzadeh 2006) showed that the protein expression in leaves was increased under water stress and exogenous ABA treatments, so it is thought to be dependent on endogenous ABA. ABA foliar spray induced protein content in leaves under drought treatments. This may be due to the positive role of ABA on protein accumulation. Schmitz *et al.,* (2000) reported that protein synthesis in developing seeds is induced by ABA.

The amounts and activities of enzymes involved in scavenging active oxygen species are altered by environmental stresses such as water stress and salinity (Dalmia & Sawhney, 2004). In addition these stresses have been reported to increase acid phosphatase activity (Barret-Laennard *et al*., 1982). However, the application of exogenous ABA has also been reported to significantly increase the activities of POD in tomato species (Basak *et al.,* 2012). The increased activities of antioxidant enzymes act as a damage control system and thus provide protection from oxidative stress, which otherwise could cause lipid peroxidation resulting in damage to the cell membrane and organelles, protein and DNA structure and inhibit photosynthesis and other enzyme activities (Sairam & Saxena, 2000).

Our observations of increased stomatal number and smaller stomatal dimensions in ABA-treated plants are similar to those of Bradford *et al*., (1983) who grew tomato under artificially elevated ABA (leaves sprayed daily with 10 or 30 µMABA). Under drought-stressed conditions, stomata close in response to either a decline in leaf turgor and/or water potential, indicating that stomatal responses are closely linked to soil moisture content and leaf water status, (Wilkinson & Davies, 2002). The ability of plants to be able to regulate the size of the stomatal opening is a very important mechanism to control water loss and survive. To minimize the negative effects of water stress the plants respond by changing their growth pattern, producing stress proteins and chaperones, up-regulation of anti-oxidants, accumulation of compatible solutes, increasing the amount of transporters involved in water and ion uptake and transport and by closing the stomata.(Arve *et al.,* 2011). ABA can regulate stomatal aperture by promoting stomatal closure or inhibiting

stomatal opening, induced by changing the osmotic potential of guard cells, the mechanical properties of guard cells, or gene expression, (Hetherington 2001).

The reduction in total carbohydrate content induced by water stress treatments may be due to its inhibitory effect on photosynthetic activities, photosynthetic pigment concentrations or the activity of ribulose diphosphate carboxylase leading to decreases in all sugar fractions (Stibrova *et al*., 1986). Application of ABA induced increases ($p<0.05$) in the total carbohydrates in seed under drought. Similar result has been showed by (Bagniewska-Zadworna *et al.,* 2007 and Wattana, 2011). It suggested that ABA- induced sugar accumulation may partly increase osmotic adjustment in which helps the plant to better survive under drought condition (Wattana, 2011).

Conclusion

In summary exogenous ABA phytohormone reduced the deleterious effects of drought stress in the *Pisum* plant. Moreover, ABA induced a significant increase in proline and some antioxidant enzymes and endogenous ABA in plants exposed to water deficit stress, which played a major role in *Pisum* tolerance to drought stress.

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