

ALLEVIATION OF ADVERSE EFFECTS OF DROUGHT STRESS ON COMMON BEAN (*PHASEOLUS VULGARIS* L.) BY EXOGENOUS APPLICATION OF HYDROGEN PEROXIDE

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Abstract

Effects of seed pretreatment by hydrogen peroxide (H_2O_2) on drought tolerance in common bean plants (*Phaseolus vulgaris* L.) were studied. Drought stress caused highly significant decrease in growth parameters, photosynthetic pigments, total carbohydrates and phytohormones. An increase drought stress caused highly significant increase in compatible solutes and polyamine contents as antioxidants and ABA contents in shoots of common bean plants. H_2O_2 -pretreatment of seeds enhanced all the above parameters than the water-pretreated seeds (control) under drought condition. Results suggested that H_2O_2 , a stress signal could trigger the activation of antioxidants in seeds which persists in the plants to alleviate the oxidative damage leading to improvements in physiological attributes for the plants growth under drought.

Introduction

Drought is a major abiotic stress that affects agricultural systems and food production and also induces several physiological, biochemical and molecular responses in several crop plants which give rise to excess concentrations of active oxygen species (AOS) resulting in oxidative damage at cellular level (Foyer and Noctor 2002). Drought inhibits the photosynthesis of plants causing changes of chlorophyll contents, damage the photosynthetic apparatus and decreases the activities of Calvin cycle enzymes (Monakhova and Chernyadev 2002).

Generally, the environmental stresses especially drought stress, give rise to accumulation of soluble carbohydrates, proline and free amino acids as well as antioxidants compounds. These solutes are low molecular weight, highly soluble compounds that are non toxic at high cellular concentration and protect cellular components from dehydration injury, thus are referred to as osmoprotectants and compatible solutes (Reddy *et al.* 2004, Shao *et al.* 2005).

Polyamines play an important role in maintaining membrane and nucleic acid integrity under most of the stress conditions (Erdei *et al.* 1996). However, both ionic deficiency and salinity, and osmotic stresses may influence polyamine metabolism in different manners and polyamines may have different and specific functions under these stress conditions (Zhou *et al.* 1995).

Water stress markedly reduced the amounts of auxins, gibberellins and cytokinin, while it reversibly raised the amounts of ABA (Abdalla and El-Khoshiban 2007).

Hydrogen peroxide is produced under various abiotic and biotic stresses. It is relatively stable and diffuses through membranes (Vranova *et al.* 2002), thus exogenous application of hydrogen peroxide at low concentrations stimulated and enhanced resistance to drought (He *et al.* 2009). H_2O_2 can serve as a second messenger in signal transduction pathways, leading to stress acclimation. Available information suggest that H_2O_2 directly regulates the expression of numerous genes involved in plant defense and the related pathways such as antioxidant enzymes, defense proteins and transcription factors (Hung *et al.* 2005).

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Common bean (*Phaseolus vulgaris* L., Fabaceae) is an important crop which is sensitive to water deficit when compared to other crops (Cruz *et al.* 1998). Therefore, it is important to elucidate drought tolerance mechanisms of this crop in order to improve its agronomic performances by exogenous application of hydrogen peroxide. Thus, this study aimed to explore the evaluation effects of hydrogen peroxide treatment for drought stress to better understanding of the physiological and biochemical mechanisms involved.

Materials and Methods

Seeds of common bean plants (*Phaseolus vulgaris* L.) were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Seeds were sterilized with 1% sodium hypochlorite for 5 min and rinsed with distilled water and divided into two groups. In the first group, the seeds were soaked in hydrogen peroxide (2%) for 4 hrs and then air dried. Ten seeds were sown in each pot (25 cm in diam) containing equal amount of homogeneous loamy clay soil. These pots were irrigated with 80% (serve as control), 60 and 40% of hold water capacity. In the second group, the seeds were soaked in distilled water for 4 hrs and then air dried. The seeds were sown in each pot and were irrigated with 80, 60 and 40% of hold water capacity. The experiment was conducted under natural conditions (day length 12 - 14 hrs, at 20 - 22° C and 70% humidity). After 60 days of sowing the plant samples were collected to determine certain morphological characters (shoot and root lengths and fresh and dry weights of shoots and roots) in addition to photosynthetic pigments, total carbohydrate, total soluble sugars, total free amino acids, proline, polyamine (putrescine, spermidine and spermine) and plant phytohormones (IAA, GA₃ and ABA).

Chlorophyll *a*, *b* and carotenoids were determined following Vernon and Seely (1966). Total carbohydrates were determined using the colorimetric method as described by Dubois *et al.* (1956). Total soluble sugars were estimated in ethanol extract of plant tissue by the phenol-sulphoric acid method as described by Dubois *et al.* (1956). Total free amino acids were determined using ninhydrin reagent (Moore and Stein 1954) using pure glycine as standard. Proline content was measured by using the method of Bates *et al.* (1973).

Putrescine, spermidine and spermine were extracted and determined using TLC according to Mietz and Karmas (1977) and Maijala and Eerola (1993) with some modifications. The method of extraction was essentially similar to that adopted by Shindy and Smith (1975). To estimate the amounts of acidic hormones IAA, GA₃ and ABA, the plant hormone fractions and standard ones were methylated according to Vogel (1975).

Data were statistically analyzed using F-test and LSD at 5 and 1% levels of probability according to SAS-Programme (1982).

Results and Discussion

The plant growth parameters of canola (shoot and root length, fresh and dry weights of shoots and roots) decreased significantly with increasing drought stress as compared with control plants (Table 1). H₂O₂ pretreatment alone or in combination with drought stress showed highly significant increase in all the growth parameters when compared with the control.

Drought stress resulted marked drop in plant growth parameters which may be attributed to damage of oxygen evolving complex of photosystem II and its reaction centers (Subrahmanyam *et al.* 2006). Hu *et al.* (2007) also found that shoot fresh weight of maize plants grown under drought stress was reduced as compared to control. Drought stress lowers the soil water potential resulting in reduction of plant growth (Munns 2002). Such decline in shoot and root 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), and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through (Lavisalo and Schuber 1998). Moreover, the decline in both fresh and dry weight of shoots and roots of common bean reveals the influence of water in stimulating and regulating the photosynthetic enzymes and growth promoting hormones, which thus influences dry matter production (Monti *et al.* 2006).

Table 1. Effects of exogenous application of H₂O₂ on growth parameters of *Phaseolus vulgaris* plants under drought stress.

Treatment	Shoot length (cm)	Root length (cm)	Fresh wt. of shoots (gm)	Dry wt. of shoots (gm)	Fresh wt. of roots (gm)	Dry wt. of roots (gm)
Control	18.7	21.4	5.6	0.65	1.8	0.11
H ₂ O ₂ 2%	21.3**	24.9**	6.5**	0.75**	2.5**	0.17**
Hold water capacity 60%	16.5***	19.6***	5.2***	0.55***	1.6***	0.09***
Hold water capacity 60% + H ₂ O ₂ 2%	23.7**	23.3**	7.1**	0.83**	2.4**	0.16**
Hold water capacity 40%	15.1***	17.6***	3.8***	0.42***	1.2***	0.06***
Hold water capacity 40% + H ₂ O ₂ 2%	20.9**	22.0**	6.0**	0.69**	2.0**	0.13**
LSD at 5%	0.514	0.415	0.183	0.023	0.079	0.006
LSD at 1%	0.739	0.597	0.263	0.034	0.113	0.010

Highly significant increase. *Highly significant decrease.

Ren *et al.* (2000) who observed that exogenous application of H₂O₂ to wheat enhanced the root growth and fresh weight under drought stress. H₂O₂ enhance cell division and promoted the secondary wall formation (Potikha *et al.* 1999). Anonymous (2002) also found that H₂O₂ can stimulate growth of wheat.

Photosynthetic pigments contents in leaves of common bean plants were decreased highly significantly with increasing level of drought stress (Table 2). H₂O₂ application alleviates the drought stress by improving pigment contents in leaves of common bean plants. Pretreatment with H₂O₂ alone caused highly significant increase in photosynthetic pigments as compared to control one (H₂O).

Table 2. Effects of exogenous application of H₂O₂ on photosynthetic pigment content (mg/g) of *Phaseolus vulgaris* plants under drought stress.

Treatment	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i> + <i>b</i>	Carotenoids	Total pigments
Control	12.21	3.32	15.53	1.32	16.85
H ₂ O ₂ 2%	13.22**	3.66**	16.88**	1.05***	17.93**
Hold water capacity 60%	10.01***	2.72***	12.73***	1.08***	13.81***
Hold water capacity 60% + H ₂ O ₂ 2%	14.09**	4.56**	18.65**	1.51**	20.16**
Hold water capacity 40%	7.92***	2.24***	10.16***	0.89***	11.05***
Hold water capacity 40% + H ₂ O ₂ 2%	13.60**	3.64**	17.24**	1.44**	18.67**
LSD at 5%	0.384	0.129	0.508	0.039	0.540
LSD at 1%	0.553	0.186	0.730	0.056	0.777

Highly significant increase. *Highly significant decrease.

Significant decrease in chlorophyll *a*, *b*, carotenoids and total pigments under drought stress amount due to water deficit and mainly because of the damage to chloroplasts by active oxygen species (Agastian *et al.* 2000). Similarly He *et al.* (2009) also found that H₂O₂ pretreatment enhanced the photosynthetic rate in wheat seedlings under PGE-induced drought condition.

Total carbohydrate contents decreased significantly with increasing level of drought stress (Table 3). Pretreatment of seeds with H₂O₂ caused highly significant increase in the same contents in shoots of common bean plants. These results are in accordance with Saleh (2007) who found that mung bean showed a significant decline in carbohydrate content when faced a chilling stress, but treating mung bean with hydrogen peroxide significantly increased carbohydrate content when compared to the positive control.

Table 3. Effects of exogenous application of H₂O₂ on total carbohydrate, total soluble sugars, total free amino acid and proline contents of *Phaseolus vulgaris* plants under drought stress.

Treatments	Carbohydrate (mg/100 g d.wt.)	Soluble sugars (mg/100 g d.wt.)	Free amino acids (µg/g f. wt.)	Proline (µg/g f.wt.)
Control	153.50	38.03	18.65	91.30
H ₂ O ₂ 2%	231.00**	48.58**	22.80**	97.44**
Hold water capacity 60%	141.50***	51.55**	23.58**	108.37**
Hold water capacity 60% + H ₂ O ₂ 2%	204.88**	67.73**	33.33**	116.30**
Hold water capacity 40%	118.00***	67.83**	46.08**	116.41**
Hold water capacity 40% + H ₂ O ₂ 2%	168.38**	81.98**	50.10**	126.35**
LSD at 5%	6.677	2.562	2.169	2.091
LSD at 1%	9.601	3.685	3.119	3.006

** Highly significant increase. *** Highly significant decrease.

Total soluble sugars content in shoots of common bean plants significantly increase under drought stress alone or in combination with H₂O₂ pretreatment. The increase in sugar concentration may be a result from the degradation of starch (Fischer and Höll 1991). Starch may play an important role in accumulation of soluble sugars in cells. Starch depletion in grapevine leaves was noted by Patakas and Noitsakis (2001) in response to drought stress.

The tolerance mechanism in water-deficit may be associated with accumulation of osmoprotectants such as soluble sugars. The accumulation of soluble sugars is strongly correlated to the acquisition of drought tolerance in plants (Hoekstra and Buitink 2001). The accumulation of soluble sugars compounds protects the cell under stress by balancing the osmotic strength of the cytosol with that of the vacuole and the external environment. The compound also interact with cellular macromolecules as enzymes and stabilize their structure (El-Tayeb 2006). Soluble sugars may also function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor.

Soluble sugars can function in two ways, which are difficult to separate: as osmotic agents and as osmoprotectors (Bohnert *et al.* 1995). As osmoprotectors, sugars stabilize proteins and membranes, most likely substituting the water in the formation of hydrogen bonds with polypeptide polar residues (Crowe *et al.* 1992) and phospholipid phosphate groups (Strauss and Hauser 1986).

The drought condition caused significant increase in the total free amino acids in shoot of common bean plants. Yadav *et al.* (1999) reported that amino acids content increased under drought stress apparently due to hydrolysis of proteins in chickpea. Ashraf and Iram (2005) stated that water deficit caused a significant increase in total free amino acids of all plant parts of

Phaseolus vulgaris and *Sesbania aculeata*. A maximum increase in free amino acids was observed in leaves and nodules of *P. vulgaris*.

Proline accumulation under drought stress may be that it contributes a protective role as scavenges of reactive oxygen species (ROS), resulted in improved adaptation ability and growth of plants under drought conditions (Turkan and Demiral 2009). Accumulation of proline is an important indicator of drought stress tolerance in higher plants (Ashraf and Iram 2005). Proline, has been suggested as one of the possible means for overcoming osmotic stress caused by the loss of water (Caballero *et al.* 2005). Proline is a non-protein amino acid that forms in most tissues subjected to water stress and together with sugar, it is readily metabolized upon recovery from drought (Singh *et al.* 2000). In addition to acting as an osmo-protectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger (Sharma and Dietz 2006), as a solute that protects macromolecules against denaturation and as a means of reducing acidity in the cell (Kishor *et al.* 2005). However, Vendruscolo *et al.* (2007) stated that proline might confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than as an osmotic adjustment.

Spermidine, putracine, spermine and total polyamine contents increased significantly in plants under drought stress (Table 4). In addition, pretreatment with H₂O₂ caused highly significantly increase in polyamine contents in shoots of common bean as compared to control plants except putracine which showed significant increase when seeds were pretreated with H₂O₂ alone.

Table 4. Effects of exogenous application of H₂O₂ on polyamine contents of *Phaseolus vulgaris* plants under drought stress.

Treatments	Spermidine (ppm)	Putracine (ppm)	Spermine (ppm)	Total polyamine (ppm)
Control	1.22	21.40	1.67	24.29
H ₂ O ₂ 2%	2.31**	28.6*	11.73**	42.64**
Hold water capacity 60%	2.75**	30.98**	14.2**	47.93**
Hold water capacity 60% + H ₂ O ₂ 2%	3.38**	54.96**	19.83**	78.17**
Hold water capacity 40%	5.39**	80.23**	24.55**	110.17**
Hold water capacity 40% + H ₂ O ₂ 2%	8.96**	128.04**	44.5**	181.5**
LSD at 5%	0.45	6.53	2.32	9.25
LSD at 1%	0.64	9.39	3.34	13.30

*Significant increase. **Highly significant increase.

Results show that spermidine, putracine and spermine significantly increased under drought stress alone or in combination with pretreatment of H₂O₂. These results confirm with results obtained by Kasukabe *et al.* (2004) working on transgenic *Arabidopsis thaliana* who found that plants exhibited a significant increase in spermidine synthase activity and spermidine content in leaves showing enhanced tolerance to various stresses including drought, salinity, freezing and hyperosmosis. The results strongly suggest an important role of spermidine as a signalling regulator in stress signalling pathways, leading to build-up of stress tolerance mechanisms in plants under stress conditions.

Polyamines (PAs) are ubiquitous in both eukaryotes and prokaryotes. The most common PAs in higher plants are putrescine (Put), spermidine (Spd) and spermine (Spm). In plants, PAs are commonly associated with responses to biotic and abiotic stresses and have been shown to function in drought and chilling tolerance in some situations (Kakkar *et al.* 2000).

Polyamines are involved in plant defense to environmental stresses (Bouchereau *et al.* 1999). In general, plant species and cultivars with high stress tolerance are endowed with a great capacity to enhance polyamine biosynthesis in response to environmental stresses including drought. The initiation of polyamine accumulation requires an osmotic signal (Imai *et al.* 2004) also suggest that an osmotic, rather than ionic effect is the main signal triggering the polyamine response under drought. It may act as a protective for the plasma membrane against stress damage by maintaining membrane integrity (Roy *et al.* 2005), preventing superoxide-generating NADPH oxidases activation (Shen *et al.* 2000) or inhibiting protease and RNase activity (Bais and Ravishanker 2002).

Data presented in Table 5 demonstrated that IAA and GA₃ levels were markedly reduced in common bean shoots with increasing levels of drought stress when compared with those of the control (80% hold water capacity). ABA content showed highly significant increase proportional to drought stress. The exogenous application of H₂O₂ alone or in combination with drought stress caused significant increase in both IAA and GA₃ contents. In contrast, ABA content showed highly significant decrease as compared with control plants.

Table 5. Effects of exogenous application of H₂O₂ on phytohormone contents (mg/100 g) of *Phaseolus vulgaris* plants under drought stress.

Treatment	IAA	GA ₃	ABA
Control	13.30	15.04	3.45
H ₂ O ₂ 2%	17.71**	31.30**	2.23***
Hold water capacity 60%	7.53***	12.90***	5.25**
Hold water capacity 60% + H ₂ O ₂ 2%	17.24**	25.32**	4.34**
Hold water capacity 40%	6.20***	10.04***	8.62**
Hold water capacity 40% + H ₂ O ₂ 2%	15.08**	21.43**	2.71***
LSD at 5%	0.78	1.27	0.35
LSD at 1%	1.13	1.83	0.50

Highly significant increase. *Highly significant decrease.

Phytohormones regulate the protective responses of plants against both biotic and abiotic stresses by means of synergistic or antagonistic actions referred to as signaling crosstalk. IAA and GA₃ levels were markedly reduced in drought stressed common bean plants as compared with those of the control while reversibly raised the abscisic acid (ABA). These results were similar to those of Zhang *et al.* (2006) and Abdalla and El-Khoshiban (2007) who found that water stress decreased the content of each of GA₃, GA₄, IAA and zeatin whereas it increased ABA values. Abdalla (2011) also found that drought stress in *Lupinus albus* gradually declined the contents of all growth promoting hormones (auxins, gibberellins and cytokinins) while reversibly raised the abscisic acid (ABA) amount below and above those of the untreated controls respectively.

Drought stress appeared to inhibit the biosynthesis of auxins and gibberellins and/or increase their degradation (Poljakoff-Mayber and Lerner 1993). Furthermore, Shi *et al.* (1994) working on maize seedlings proved that under water stress induced by polyethylene glycol, IAA and GA₃ content declined, while ABA content increased.

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