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Evaluation of Drought Stress and Foliar Chitosan on Biochemical Characterices of Castor Bean (*Ricinus communis* L.)

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Abstract: Drought is the most significant factor restricting plant production on majority of agricultural fields of the world. In order to study the effects of drought stress and chitosan foliar on antioxidant enzymes and proline content of three castor bean cultivars under field conditions. The results showed that with an increase in the intensity of drought stress on castor bean cultivars there was a increased CAT, POD enzymes activity. That highest CAT activity related to drought stress levels (D2, D3) and the most POD activity from of D3: Drought stress in beginning seedling stage. Between cultivars no had significant but factorial effect (Drought stress*cultivar) had a significant($p<0.05$) highest CAT activity related to D2: Drought in beginning flowering stage, V2: Ahvaz cultivar. Also drought stress caused increase significant ($p<0.05$) in proline content. There is chitosan foliar effect a significant ($p<0.01$) on CAT enzyme and POD mean comparison of CAT activity shows that highest CAT activity related to D2: Drought stress, M2: Chitosan foliar and maximum POD activity from of D3: Drought stress in beginning seedling and M2: Chitosan foliar. From this study, it is suggested that the severity of castor bean plant damaged from drought was reduced by chitosan application.

Key words: *Ricinus communis*, chitosan, water stress, Catalase (CAT), Peroxidase (POD), Proline (Pro)

INTRODUCTION

Water availability one of the most limiting environmental factors affecting crop productivity stress imposed during these periods drastically affects crop growth ultimately leading to a massive loss in yield and quality (Pirzad *et al*., 2011). Water stress usually induces the accumulation of Reactive Oxygen Species (ROS) (Sorial *et al*., 2010). Reactive oxygen species such as O_2 and H_2O_2 are produced during photosynthesis photorespiration, respiration, flowering and other reactions of cellular metabolism, plants posses a protective system composed of antioxidant such as peroxidase and catalase. Catalase is primary H_2O_2 scavenger in the peroxisomes and the mithochondria. An increase in peroxidase activity has been reported as an early response to different stresses and may provide cells with resistance against formation of H_2O_2 which is formed when plants are exposed to stress factor (Zolfaghari *et al*., 2010). Osmotic regulation will help to cell development and plant growth in water stress so plants reduce their osmotic potential for water absorption by congestion of proline (Keyvan, 2010). Proline plays a role in stabilising membranes and proteins. It can also act as antioxidant and regulates the cytosolic acidity (Akhkha *et al*., 2011). Castor (*Ricinus cummunis* L.) belong to the (Euphorbiaceace) family and one of the major commercial non-edible oilseed crop which grown semi-arid and arid regions of India, China, Russia and is reported to be drought tolerant castor bean is almost entirely grown under dry land conditions India first in castor bean production in the world (Vanaja *et al*., 2008; Babita *et al*., 2010). Castor oil normally contains a high concetration of ricinoleic acid >85% to which confers distinctive industrial properties to the oil. Such properties are of great value for a number application in many industrial fields such as paints and varnishes nylon-type synthetic polymers hydraulic fluids and lubricants and cosmetics (Babita *et al*., 2010).

The chitosan belongs to the carbohydrate family which contains unramified chains formula originally formulated from the glucose circle, however it contains a group of free amino carbon atom No. 2 (called glucose amino) which is similar to cellulose (Sheikha and Al-Malki, 2011). Chitosan can be extracted from the marine crustacean like shrimps, cramp and pinfish or from the exoskeletons of most insects and cell walls of fungi and some alage (Boonlertnirun *et al*., 2010; Sheikha and Al-Malki, 2011). It induces defence mechanisms in several plant species and increases the activity of Phenylalanine

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Ammonia-Lyase (PAL) and Tyrosine Ammonia-Lyase (TAL) key enzymes of the phenylpropanoid pathways associated with synthesis of secondary plant metabolites under unfavourable conditions (Boonlertnirun *et al*., 2010).

MATERIALS AND METHODS

Growth conditions and treatments: Field studies were conducted during the Spring of 2010, Damghan Branch, Iran. The experiment was performed in a split-factorial based on randomized complete block design with three replication. Water treatment including three levels control, cut of irrigation in beginning flowering and seedling stage, caltivars used (Commerical, Ahvaz local, Mashhad local) chitosan foliar application in 1 level (control, foliar in 5 g L^{-1} concentration) were allocated as main and sub-plots, respectively.

Enzyme assay: For preparation of crude enzyme extracts a 0.05 g sample of fresh leaves was ground in 2 mL of 0.1 M cool phosphate buffer (pH 6.8) on ice bath (Kar and Mishra, 1976). Crude extract was centrifuged at 15,000×g for 15 min at 4°C. The supernatant was used for catalase, peroxidase activity assay.

Free proline: Proline was determined following Bates *et al*. (1973). Fresh plant material (1-0.5 g) was homogenized in 10 mL of 3% sulfosalicylic acid and the homogenate filtered. The filtrate (2 mL) was treated with 2 mL acid ninhydrin and 2 mL of glacial acetic acid then with 4 mL of toluene, absorbance of the colored solutions was read at 520 nm.

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA) and means were compared using Duncan's range test at p = 0.05. All calculations were performed with the help of the SAS version 9.1 (2009) software for Windows program and Excel software was used for drawing diagrams.

RESULTS AND DISCUSSION

Catalase activity: The result from the ANOVA statistical analysis indicated that drought stress caused effect significant ($p<0.01$) in CAT activity. In effect, Fig. 1 shows that highest CAT activity related to drought stress levels (D2, D3) and lowest CAT activity related to control (D1 without stress). Between cultivars no had significant but factorial effect (Drought stress*cultivar) had a significant ($p<0.05$) (Table 1). The mean comparison shows that between cultivar in without stress levels no had significant but highest CAT activity related to D2 drought

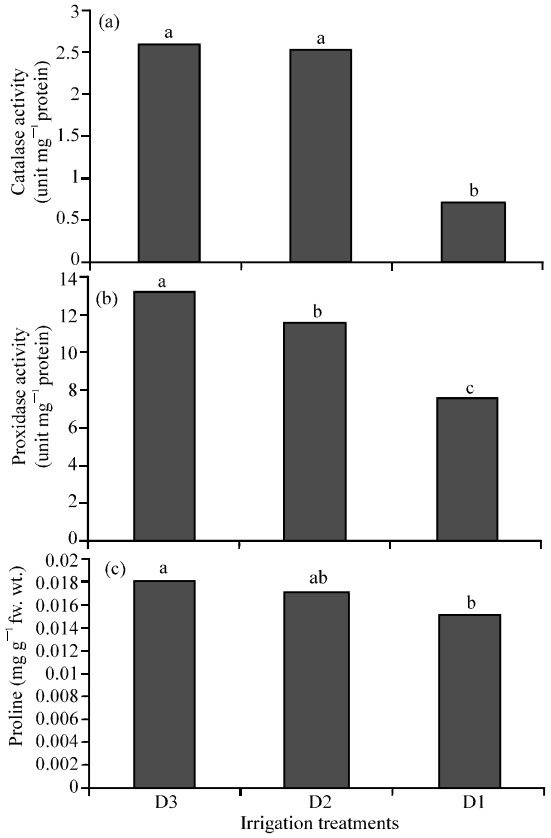

Fig. 1: Effect water stress on CAT activity: a) POD activity; b) Pro content and c) Letters on bars indicate results of Duncan's multiple range test different letters on the histograms indicate that the means differ significantly ($p<0.05$), D1: Without stress, D2: Drought stress in beginning seedling stage, D3: Drought stress in beginning flowering stage

Table 1: Analysis of variance (Mean square) water stress, cultivars and chitosan foliar on studied traits

Sources of variation	df	CAT activity	POD activity	Pro content
Replication	2	0.10^{NS}	2.40^{NS}	0.000007^{NS}
Drought stress	2	21.30**	153.20**	0.00005*
Main error	4	0.11	1.10	0.000007
Cultivar	2	0.15^{NS}	1.60^{NS}	0.0000008^{NS}
Solution	1	13.60**	99.90**	0.000001^{NS}
Cultivar*drought	4	0.49*	1.60^{NS}	0.000004^{NS}
Drought*solution	2	2.40**	3.00*	0.000001^{NS}
Cultivar*solution	2	0.01^{NS}	0.20^{NS}	0.000002^{NS}
Cultivar* drought*solution	4	0.01^{NS}	0.30^{NS}	0.000002^{NS}
Error	30	0.16	1.05	0.0000065
Coefficient of variation (%)		20.60	9.50	15.0

NS: Non Significant; * and **significant at $p<0.05$ and $p<0.01$, respectively; df: Degree of freedom

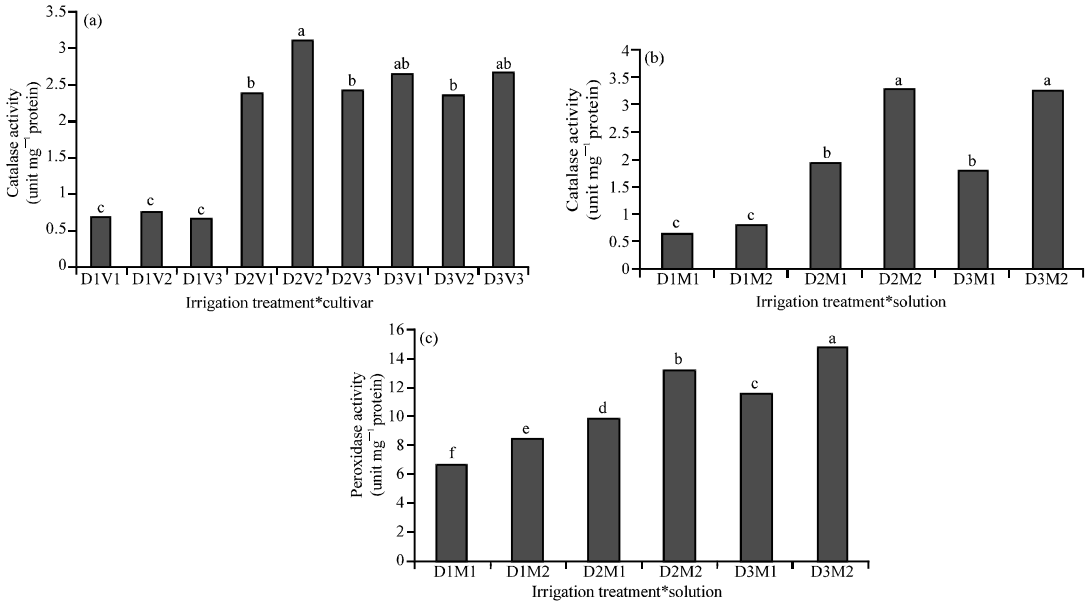

Fig. 2: Effect factorial: a) Drought stress*Cultivar on CAT activity; b) Chitosan foliar*drought stress on CAT activity; c) POD activity; V1: Commercial cultivar; V2: Ahvaz local; V3: Mashhad local

Table 2: Effect of various treatments of water stress, cultivars and foliar chitosan on studied traits

Treatments	CAT activity (unit mg^{-1} protein)	POD activity (unit mg^{-1} protein)	Pro (mg g^{-1} fw.wt)
Control	0.72^{b}	7.50^{c}	0.015^{b}
Drought in beginning flowering	2.64^{a}	11.56^{b}	0.017^{ab}
Drought in beginning seedling	2.56^{a}	13.16^{a}	0.018^{a}
Cultivarsss			
Commerical	1.91^{a}	10.98^{a}	0.017^{a}
Local Ahvaz	2.08^{a}	10.40^{a}	0.016^{a}
Local Mashhad	1.93^{a}	10.84^{a}	0.017^{a}
Solution			
Without foliar	1.47^{a}	9.38^{b}	0.016^{a}
Chitosan foliar	2.48^{b}	12.10^{a}	0.017^{a}

Data represent the mean values of three replicates. Within a column, mean values followed by different letters are statistically different based on Duncan's test at p = 0.05 range

in beginning flowering stage, V2: Ahvaz local cultivar and the lowest of CAT activity related to D1: Without stress, V1: Commercial cultivar (Fig. 2). The result from the ANOVA statistical analysis indicated that chitosan foliar caused effect significant in CAT activity ($p<0.01$) and factorial effect (Drought stress*solution) had a significant ($p<0.01$) (Table 1). The mean comparison of CAT activity shows that highest CAT activity related to D2: Drought stress, M2: Chitosan foliar and the lowest CAT activity related to D1: Without stress, M1: Without chitosan foliar (Fig. 2) result. Table 2 shows that no had a significant between drought stress levels and highest activity related to D2: Drought in beginning flowering, D3: Drought in beginning seedling stage but between control and drought stress treatment had a significant and minimum CAT activity from of without stress level. CAT is likely responsible for excess H_2O_2 removal under severe water stress conditions (Chang *et al*., 2012). Role of CAT in averting the cellular damage under unfavorable conditions like water stress has been suggested (Chugh *et al*., 2011). The increase in CAT activity might be useful in sdismuting H_2O_2 that is the key product in reducing senescence under moisture stress. In peroxisome the CAT have an essential role in the removal of toxic H_2O_2 which is continuously formed during photorespiration by the dismutation of the superoxide radicals goverated in the NADH dependent electron transport system of the peroxisomeal membrance (Pandey *et al*., 2010). An increase in CAT activity under drought stress of different crop has been reported (Huseynova *et al*., 2010; Vasconcelos *et al*., 2009; Bhardwaj and Yadav, 2012).

Peroxidase activity: The results from the ANOVA statistical analyises POD activity. Chitosan foliar caused effect significant ($p<0.01$) also factorial effect (drought stress*solution) had a significant ($p<0.05$) (Table 1). The results mean comparison show had a between treatment different significant. The most POD activity from of D3: Drought stress in beginning seedling stage and minimum POD activity related to D1: Without stress. Also between chitosan treatment had a significant and pox activity in chitosan foliar high rate of control (Table 2). Figure 1

shows that drought stress caused increase in POD activity also the result factorial effect shows maximum POD activity from of D3: Drought stress in beginning seedling stage and M2: chitosan foliar therefor minimum POD activity related to D1: Without stress, M1: Without chitosan (Fig. 2). POD enzyme can efficiently remove H_2O_2 both in the cytosol and chloroplast so increasing the activity of this enzyme in drought stress perhaps shows the accumulation of H_2O_2 in the condition (Sharifi *et al*., 2012) also (Klar *et al*., 2006) reported an increase of POD in tissues of wheat plants experiencing water stress, PO are often the first enzymes the activities of which are altered under stress condition. An increase in POD activity in drought tolerant as well as sensitive maize at seedling under osmotic stress has been reported (Chugh *et al*., 2011). It has been proposed that POD could act as efficient H_2O_2 scavening system in plant vacuoles in the presence of phenolices and reduced ascorbates phenolices are oxidized to phenoxyl radicals which can be reduced by ascorbate (Zivkovic *et al*., 2010).

Effect chitosan foliar on CAT, POD activity: The result shown chitosan played a key role in increasing cell membrane stability during drought stress POD and CAT are important antioxidant enzymes which synergistically protect cells against the detrimental effect of oxidative stress. The POD and CAT activities in castor leaf tissues were increase by treatment chitosan in drought stress (Fig. 2). The scavenging mechanism of chitosan may be related its structure which feature large numbers of hydroxyl and amino groups available to react with ROS (Yang *et al*., 2009). Chitosan may have acted as a signal for cellulose response of the chitinase enzyme in the plants to initiate their defense mechanism against phytopathogenic infections. Once the defense mechanism of the plants was initiated by the chitosan and chitinase enzyme activities also chitosan inducing the defense mechanism of the plants also provides protection against environmental stress such as drought and maintains stability of the plant (Burrows *et al*., 2007). It induces defence mechanisms in several plant species and increases the activity of Phenylalanine Ammonia-Lyase (PAL) and Ttyrosine Ammonia-Lyase (TAL) key enzymes of the phenylpropanoid pathways associated with synthesis of secondary plant metabolites under unfavourable conditions (Boonlertnirun *et al*., 2007, 2010). Ortega-Ortiz *et al*. (2007) reported the catalase activity increase in tomato fruit when chitosan were applied during the fruit growing. Previous study also showed how disease resistance and ROS metabolism in harvested navel oranges are affected by chitosan suggesting that chitosan treatment could induce navel orange fruit disease resistance by regulating the H_2O_2 levels antioxidant enzyme and ascorbate-glutathione cycle (Bae and Moon-Moo, 2010). In experimental the effect chitosan foliar on proline content no had significant.

Proline content: The results from the ANOVA statistical analysis indicated drought stress caused effect significant ($p<0.05$) and no had significant other treatments (Table 1). The mean comparsion indicated between treatment different irrigation had a significant. maximum proline content related to D3: Drought stress in beginning seedling stage and minimum proline content related to D1: Without stress (Table 2, Fig. 1).

This increase in proline concentration under water stress has been observed in other crops like marize, wheat and cowpeas. Although, proline is role in plant osmo tolerance remains controversial, it is however thought to contribute to osmotic adjustment detoxification of ROS and protection of membrance integrity during water stress (Vurayai *et al*., 2011). Proline is primarily accumulating in the cytoplasm. Beside, proline might play a more complex role in conferring drought resistance than simply contributing to osmotic adjustment (Shahraji *et al*., 2010). Also, proline is involved in the regulation of cellular redox potential, reducing stress induced cellular acidification and priming oxidative respiration, associated with increased maintenance respiration during stress to provide needed for recovery (Ditmarova *et al*., 2010). It has been shown that proline also plays a key role in stabilizating cellular proteins of osmoticun higher proline content in wheat plants after water stress has also been reported (Khalil *et al*., 2010).

CONCLUSION

Reactive Oxygen Species (ROS) play an important role in drought stress. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. An increase was observed in POD and CAT activity of three cultivars under stress conditions throughout the experiment. CAT activity increased in drought resistance cultivars under drought stress, however POD acts as the major antioxidant enzyme in castor bean leaves. Changes in the enzyme activity and content of substrates during dehydration may play an important role in the adaptation of water deficit and increase the overall plant resistance to stress conditions. Drought stress causes significant increase proline content in three cultivar castor bean. The results presented in this study demonstrate that foliar application of chitosan significantly increased cell membrane stability and antioxidant enzyme activities in

castor bean under drought conditions. These effects may be attributable to the active hydroxyl and amino groups in the chitosan structure which detoxify ROS by generating nontoxic macromolecular radicals. These results provide indirect evidence that chitosan can enhance the antioxidant capabilities of castor bean to drought stress. Therefore from this study, it is suggested that the severity of *Ricinus communis* damaged from drought was reduced by chitosan application. In addition, Damghan regions ingredient arid and semi arid in Iran also castor bean herb is drought tolerant the experimental but result shows factorial effect on CAT activity (Drought*cultivar) that Ahvaz local cultivar the most amount of CAT activity therefore suggested that Ahvaz local cultivar in drought stress condition rate of other cultivar toleranter therefore researchers can be with attention Damghan climate condition there cultivate Ahvaz local cultivar.

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