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Annals of Biological Research, 2012, 3 (8):3984-3989
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

The effects of seed priming with ascorbic acid on drought tolerance and some morphological and physiological characteristics of safflower (*Carthamus tinctorius* L.)

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ABSTRACT

The role of ascorbic acid as a key molecule antioxidant involved in biotic and abiotic stress has been well described. Thus, in order to study the effects of ascorbic acid on drought tolerance and some morphological and physiological characteristics of safflower (Carthamus tinctorius L.) under drought stress condition an experiment was conducted. The experimental design was factorial with two factors arranged in a completely randomized design (CRD), with three replications. The first factor was drought stress on 5 levels (control, -4, -6 , -8 and -12 bar) that was carried out by PEG 6000 and the second factor was ascorbic acid solution on 4 density (control, 55, 110 and 165 µm). The results showed that with increase in drought stress, germination percentage, shoot length, root length, seedling fresh weight, seedling dry weight and vigor index significantly decreased whereas catalase and peroxidase activity increased as compared to control with enhancement of drought stress. In general, priming with ascorbic acid significantly relived the harsh effects of drought stress on seedling growth, catalase and peroxidase activity of safflower and it seems that ascorbic acid was able to enhance the tolerant ability of the plant to drought stress.

Key words: Drought stress, Priming, Ascorbic acid, PEG

INTRODUCTION

Safflower as an oilseed crop is resistant to drought in arid and semi-arid regions of the world, but this plant germination and establishment phase is sensitive to drought stress. One of the major problems in safflower seedling emergence and establishment in arid regions is water shortage. Despite, water is one of abundant compounds on the earth and 2/3 of earth surface is covered by water, but water shortage is limitative to produce agriculture products in the world. Drought is one of the major physical parameters of environmental, which determines the success or failure of plants establishment [10]. Drought is the most important limiting factor for crop production and it is becoming an increasingly server problem in many regions of the world [23, 24]. The plants under dry condition change their metabolism to overcome the changed environmental condition. The complexity response of the plants to drought stress could be justified. Seed germination is one of the most important phases in the life cycle of plant and in highly responsive to existing environmental. Drought decrease germination percentage and seedling growth [10]. As well as under different condition particularly environmental stress, reactive oxygen species such as super oxide anion radicals, hydrogen peroxide and hydroxyl radicals, are generated , reactive oxygen species can damage essential membrane lipid as well as proteins and nucleic acids [20]. To counteract the damaging effects of ROS, plant cells possess an antioxidant system consisting of low-molecular weight antioxidants such as β-carotenes, ascorbic acid(AA), α-tocopherol (α-Toc), reduced glutathione(GSH)(non-enzymatic antioxidants), as well as antioxidants enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) [20]. Activity of antioxidant enzymes with detoxification and elimination of the harmful effects of reactive oxygen species reduces the severity of oxidative stress [17]. Increased cellular levels of ascorbic acid as an antioxidant can reduce oxidative stress by reducing reactive oxygen species. Ascorbic acid is an antioxidant molecule that acts as a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen [20].

Ascorbate has been shown to play multiple roles in plant growth, such as cell division, cell wall expansion and other developmental processes [25]. The present study was conducted to asses if the application of ascorbic acid could ameliorate the adverse effect of drought on *carthamus tinctorius* plants. For this purposes some morphological and physiological characteristics were measured.

MATERIALS AND METHODS

The experiment was carried out at the physiological laboratory of Faculty of Agriculture, Islamic Azad University, Saveh Branch. The cultivar of safflower was goldasht. The experiment was a factorial method with two factors arranged in a completely randomized design with three replications. The first factor was drought stress on5 levels (control, -4, -6, -8 and -12 bar) that was carried out by PEG 6000, the second was Ascorbic acid on 4 density (control, 55, 110 and 165µm). For ascorbic acid treatment, seeds of safflower were sterilized for 5 minute in sodium hypochlorite solution and in ethanol for 30 second and then rinsed by distilled water. Sterilized seeds were transferred in to sterile petri dishes contain filter papers and were added 10 ml ascorbic acid solution to each petri dish. Seeds of safflower were primed for 16 hours at 25 ^{0}C and dark conditions. Thereafter, the seeds treated with ascorbic acid solution rinsed with distilled water. Following this, the primed seeds were dried between two filter papers. Primed seeds were placed in petri dishes, on a layer of filter paper. Twenty five seeds were placed in each petri dish, the petri dishes were moistened with 5 ml of PEG 6000 solution at water potential of (0, -4, -6, -8 and -12 bar). The petri dishes were placed in germinator. The seeds were kept under aseptic condition for 7 days in 16h/8h light/dark cycle with a light intensity of 350 µmolm^{-2}s^{-1} and a relative humidity of 45% at 25$\pm$1^{0}C. Seed germination was recorded daily up 7 days after the start of the experiment. A seed was considered germinated when radical emerged by about 2mm in length. Moreover germination percentage was determined in the end of test. Germination percentage was calculated with the following formula:

$$GP = 100 (n / N)$$

N = Total seeds number n = Germinated seed number

To determine the radical and plumule length after 7 days, radicals and plumule produced in each petri dish were separated from the seeds, their length were measured with millimeter ruler. Seedling dry weight and seedling fresh weight were measured after the specified number of days. To deter mine the dry weight, seedlings were dried in aerated oven at 75^{0}C until constant weight. Vigor index as described by Abdul-baki and Anderson. [1] .

$$\text{Vigor Index} = \frac{\text{Germination percentage} * \text{Seedling lenght(cm)}}{100}$$

The remains of seedlings were frozen in liquid N_2 and stored under -80^{0}C until biochemical analysis.

Extract Preparation:
Seedling (0.02 gr) were homogenized in a mortar and pestle with 3ml of ice-cold extraction buffer (25 Mm sodium phosphate buffer, PH 7.8). The homogenate was centrifuged at 18,000*g for 30 minute at 4^{0}C and then supernatant filtered through Watman paper. The supernatant fraction was used as crude.

Catalase activity
Catalase activity was estimated by the method of Cakmak and Horst.[3]. The reaction mixture contained 100µl of crude enzymes extract, 500µl of 10 Mm H_2O_2 and1400µL of 25 Mm sodium phosphate buffer and the decrease in the absorbance was recorded at 240nm for 1 minute. Catalase activity of the extract was expressed as catalase units min^{-1}mg^{-1} protein.

Peroxidase activity
Peroxidase activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm [11]. The reaction mixture contained 100µl crude enzyme, 500µl H_2O_2 5Mm , 500µl guaiacol 28Mm and 1900µl phosphate buffer 60Mm (PH7.0).Peroxidase activity of the extract was expressed as peroxidase units min^{-1}mg^{1}protein.

Statistical analyze
All data were analyzed using SAS software SAS Institute Inc.[29]. Each treatment was analyzed in three replications. When analysis of variance (ANOVA) showed significant treatment effects, Duncan's Multiple Range Test was applied to compare the means at $p<0.05$.

RESULTS

Table1: Analysis of variance of the traits under study

S.O.V	Df	Germination percentage	Seedling Fresh weight	Seedling Dry Weight	Shoot length	Root length	Vigor index	CAT activity	POX activity
PEG	4	263.861**	18082.68**	325.838**	1026.72**	2070.79**	52.876**	288.92**	772.15**
AA	3	406.166**	3092.27**	162.055**	437.556**	368.37**	19.302**	333.81**	597.47**
PEG*AA	12	1.2171^{ns}	14.8130**	2.968^{ns}	5.119**	4.802*	0.073^{ns}	3.027**	21.846**
Error	40	1.030	2.482	2.5270	1.152	2.185	0.056	1.030	1.244
C.V		1.241	1.766	8.249	3.896	4.738	4.815	6.546	5.195

*ns=Non significant, * and** significant at 0.05 and 0.01 level of probability, respectively*

Analysis of variance (Table1) indicated that all of traits under study including germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length, vigor index, CAT and POX activity were significantly influenced by drought stress ($p<0.01$). The evaluated traits also were significantly influenced by priming with ascorbic acid ($p<0.01$). The interaction effects two-way (seed priming* drought stress) were significant for the studied traits except germination percentage, vigor index and seedling dry weight.

Table 2: The main effects of polyethylene glycol on the studied traits

PEG (bar)	**Germination percentage**	**Seedling Fresh weight** (mg)	**Seedling Dry weight** (mg)	**Shoot length** (mm)	**Root length** (mm)	**Vigor index**	**CAT activity** (units min^{-1} mg^{-1} protein)	**POX activity** (units min^{-1} mg^{-1} protein)
0	87.67^{a}	144.83^{a}	25.29^{a}	39.30^{a}	46.09^{a}	7.51^{a}	9.6^{e}	10.42^{e}
-4	84.95^{b}	112.78^{b}	24.30^{a}	34.01^{b}	43.54^{b}	6.62^{b}	12.53^{d}	17.08^{d}
-6	81.45^{c}	75.35^{b}	16.72^{b}	26.40^{c}	26.52^{c}	4.35^{c}	15.18^{c}	22.57^{c}
-8	78.67^{d}	60.98^{c}	16.63^{b}	21.74^{d}	24.38^{d}	3.67^{d}	17.84^{b}	26.15^{b}
-12	76^{o}	51.92^{d}	13.57^{c}	16.27^{e}	15.45^{e}	2.44^{e}	22.37^{a}	31.10^{a}

Difference between averages of each column which have common characters are not significant at probability level of 5%

All of the traits under study including germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length and vigor index decreased when drought stress level were increased from 0 to -12 bar whereas CAT and POX activity increased when drought stress level were increased from 0 to -12 bar (Table2).

Table3: The main effects of Ascorbic acid on the studied traits

AA (µm)	**Germination percentage**	**Seedling Fresh weight** (mg)	**Seedling Dry weight** (mg)	**Shoot length** (mm)	**Root length** (mm)	**Vigor index**	**CAT activity** (units min^{-1} mg^{-1} protein)	**POX activity** (units min^{-1} mg^{-1} protein)
0	75.68^{d}	71.34^{d}	15.48^{d}	21.79^{d}	25.43^{c}	3.66^{d}	20.63^{a}	29.80^{a}
55	79.24^{c}	84.31^{c}	17.63^{c}	24.43^{c}	28.72^{b}	4.29^{c}	17.92^{b}	22.58^{b}
110	85.24^{b}	97.24^{b}	21.36^{b}	30.32^{b}	34.96^{a}	5.63^{b}	13.48^{c}	18.19^{c}
165	86.84^{a}	103.62^{a}	22.59^{a}	33.63^{a}	35.68^{a}	6.09^{a}	9.98^{d}	15.29^{d}

Difference between averages of each column which have common characters are not significant at probability level of 5%

In seedling that were treated by ascorbic acid increased germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length and vigor index whereas decreased CAT and POX activity as compared to control. The best results were obtained from the seeds treated with 165µm ascorbic acid (Table3).

Difference between averages of each column which have common characters are not significant at probability level of 5%

Priming with ascorbic acid showed a significant effects on seedling fresh weight, shoot length, root length CAT and POX activity under drought condition (table4). The maximum seedling fresh weight was achieved when seedlings were primed with 165µm ascorbic acid under normal condition and maximum shoot length and root length were achieved when seedlings were primed with 110 and 165µm ascorbic acid under normal condition. The Minimum seedling fresh weight, shoot length and root length were observed in seeds untreated with ascorbic acid and -12 bar of PEG treatments. The Maximum CAT and POX activity were observed in seeds untreated with ascorbic acid and -

12 bar PEG treatment and minimum CAT and POX activity were achieved when seedlings were primed with 165µm ascorbic acid under normal condition.

Table4: Mean comparison of the drought stress level* seed priming interaction for the traits under study

PEG (bar)	AA (µm)	Seedling Fresh weight (mg)	Shoot Length (mm)	Root Length (mm)	CAT activity (units min-1 mg-1 protein)	POX activity (units min-1 mg-1 protein)
0	**0**	128.5d	33.45d	41.70c	14.53g	16.69k
0	**55**	140.2c	35.71c	43c	11.52hi	11.74l
0	**110**	151.3b	43.72a	48.85ab	7.55j	8.58m
0	**165**	159.2a	44.34a	50.79a	4.83k	4.65n
-4	**0**	94.24h	29.12e	38.42d	16.77ef	21.47gh
-4	**55**	110.2g	29.81e	40.64cd	16.16efg	18.71j
-4	**110**	121.3f	36.54c	47.22b	10.41i	16.42k
-4	**165**	125.3e	40.58b	47.87b	6.79j	11.72l
-6	**0**	58.3o	19.89h	20.79hi	20.49c	29.78d
-6	**55**	69.39l	23.07f	24.63g	17.82de	22.36g
-6	**110**	83.79j	29.81e	29.30ef	14.62g	19.54ij
-6	**165**	89.92i	32.83d	31.38e	7.79j	18.36j
-8	**0**	45.21r	15.67i	16.35j	23.63b	36.53b
-8	**55**	52.31p	20.77gh	22.77gh	19.53c	27.51e
-8	**110**	69.17l	21.9fg	30.61e	15.49fg	20.89ghi
-8	**165**	77.25k	28.62e	27.80f	12.71h	19.68hij
-12	**0**	30.46s	10.85k	9.87l	27.75a	44.52a
-12	**55**	49.38q	12.81j	12.56k	24.60b	32.57c
-12	**110**	61.47n	19.64h	18.82i	19.34cd	25.54f
-12	**165**	66.40m	21.78fg	20.55hi	17.79de	21.78g

DISCUSSION

Drought, salinity and temperature stresses are decreased germination percentage [30]. Demir and Van De Venter. [5] observed that drought may influence germination by decreasing that water uptake in watermelon. El-Midaoui et al. [8] reported that root and shoot growth significantly decreased by osmotic stress at -0.6 Mpa and above induced by PEG 6000. Murillo-Amador et al. [19] also found that seedling growth of cowpea inhibited by both NaCl and PEG, but higher inhibition occurred due of PEG. Also Murillo-Amador et al. [19] observed that seed germination and seedling growth in legumes decreased under drought condition. Okcu te al. [22] has found that -0.6 Mpa osmotic potential decreased pisum *sativum* seed vigor. This is in similar result with Murillo-Amador et al. [19] . Gamez. [10] in his investigation on pea, find that decrease germination percentage by increase of drought stress (to PEG), increase average time necessary for germination in day, decrease radical and plumule length, fresh and dry weight of radical and plumule. The decrease in seedling growth, under drought condition, maybe due to suppression of cell expansion and cell growth that is in response to low turgor pressure [13, 21]. the drought stress increases the production of reactive oxygen species (ROS) [18]. Plant can detoxify ROS by up-regulation antioxidant enzymes, such as SOD, CAT and POX as well as some non- enzymatic antioxidant compounds. It is evident that high levels of antioxidants are related to plant water deficit tolerance [28, 35]. The CAT and POX activity increased under drought stress when compared to control plants. Similar results reported under drought stress in wheat [31] and tomato plants [27]. CAT is tetrameric heme containing enzyme that is abundant in the glyoxysomes of lipid storing tissues[9]. The combined action of SOD and CAT converts the toxic O_2, H_2O_2 to water and molecular oxygen, averting the cellular damage under unfavorable condition such as drought stress [4, 26]. Like CAT and SOD the activity of POX increased under water stress. POX plays a key role in decreasing H_2O_2 content accumulation, eliminating MDA resulting cell peroxidation of membrane lipids and maintaining cell membrane integrity [14]. Ascorbic acid is one of the most extensively studied antioxidant and has been detected in majority of plant species, organelles and apoplast and is synthesized in the mitochondria and transported to the other cell components through a proton-electrochemical gradient or through facilitated diffusion [34] also it is one of the best indentified non-enzymatic compounds as antioxidant that plants bearing is increased to oxidative stresses [33]. It also plays a protective role against ROS that are formed during biotic and abiotic stress. Ascorbate is oxidized by oxygen free and dehydroascorbate is generated [20]. This leads to a decline in antioxidant activities. Ascorbic acid can also directly scavenge reactive oxygen radicals, thus providing membrane protection [36]. DolatAbadian and Modarres Sanavy. [6] reported that priming with ascorbic increased germination percentage, length of shoot and root, their dry weight and seedling total dry weight in sunflower and rapeseed and decreased CAT activity significantly than control treatment. One of the remarkable roles of ascorbic acid in seed germination and cell growth is anti-oxidant activity, rather than its possible utility as an organic substrate for respiratory energy metabolism. We have found that ascorbic acid increased length of root and shoot in treated seeds. In fact increase shoots and root length by ascorbic

acid might be due to the cell division and differentiation of meristem cells [16]. Ascorbic acid on plant survival is associated with the partial inhibition of a few interactions in reactive oxygen species production [32]. Hamad and Hamada. [12] observed on their experiment on wheat seeds, priming with ascorbic acid reduced harmful effects of drought stress on root and shoot fresh weight. Dolatabadian et al. [7] reported that salinity increased CAT and POX activity in leaves and roots of rapeseed while the application of ascorbic acid reduced the activity of these enzymes in salinity condition. Drought combined with ascorbic acid improves the biological status of rapeseed, Biological improvement is related to reduce production of harmful substances [2]. Ascorbic acid as an antioxidant, reduced catalase activity in pea under stress condition [15]. Ascorbic acid has antioxidant properties that can remove superoxide ion and prevents the production o hydrogen peroxide, thus CAT and POX activity is reduced because these enzymes play a key role in removing hydrogen peroxide. Generally, it is concluded that ascorbic acid as an antioxidant, can reduce the harmful effects of oxidative stress and improves plant growth in stress condition [7].

CONCLUSION

According to the results obtained, drought stress decreased germination percentage, seedling fresh weight, seedling dry weight, shoot length, root length and vigor index and increased catalase and peroxidase enzymes activities and priming with ascorbic acid significantly relived the harsh effects of drought stress on seedling growth, catalase and peroxidase activity of safflower.Totally, priming with ascorbic acid significantly relived the harsh effects of drought stress on seedling growth, catalase and peroxidase activity of safflower and it seems that ascorbic acid was able to enhance the tolerant ability of the plant to drought stress.

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