INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 6, p. 181-189, 2013

RESEARCH PAPER OPEN ACCESS

The effect of seed pretreatment by salicylhydroxamic acid on germination indices of safflower under salinity stress

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Key words: Catalase, Enzymes activity, Proxidase, NaCl.

doi: http://dx.doi.org/10.12692/ijb/3.6.181-189 Article published on June 22, 2013

Abstract

In order to study the effect of seed pretreatment by salicylhydroxamic acid on germination indices and enzymes activity of safflower (*Carthamus tinctorius* L. var. Esfahan 14) under salinity stress in Iran. The experiment was laid out in a factorial design with four salicylhydroxamic acid levels of 0 (control), 50, 75, 100 ppm and four levels of salt stress condition with NaCl including 0 (control), 75, 140, 210 mg/lit in three replications. The variety of the selected seeds is Esfahan 14. Pre-sowing seed treatments were applied for eight hours duration with salicylhydroxamic acid. The results of this investigation showed that salinity stress caused a significant reduction in germination percentage of the safflower seeds. Concentration of NaCl by 210 mg/ lit caused to the most reduction in germination as compared to the control treatment. Seed salicylhydroxamic acid-priming treatments improved seed germination and early seedling growth included germination percentage, coleoptiles and radical length and seedling dry matter accumulation of both control and salt stress conditions. Furthermore, the results of this experiment showed a significant reduction in enzyme activities in salicylhydroxamic acid seed priming. These results have practical implications in that the pre-sowing seed treatment with salicylhydroxamic acid could enhance the seed germination and early seedling growth characteristics of safflower plant in salinity condition.

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Introduction

In the nature, plants are exposed to the lots of the environmental variations like drought and salinity and these factors can limit plants growth (Bohnert *et al.*, 1995). The plants have some mechanisms to survey and adapt with these environmental variations. Some of these mechanisms are physiological and morphological mechanisms and molecular variations (Bohnert *et al.*, 1995). Salinity stress can be effective on the physiological processes from germination to development. Photosynthesis that is a main path in the plant physiology can be extremely influenced by salinity stress. The abcisic acid that produced under salinity stress caused the close of stomata and restricts entrance of carbon dioxide to the plant (Leungfar, 1994).

Gathering of different kinds of active oxygen in the cells and damaging to the membrane lipids, proteins and nucleic acids caused by salinity stress (Noctor and Foyer, 1998). The anti-oxidants that exist in the plants caused the neutralizing of these free radicals (Zhang and Kirkham, 1996). Also, Salicylic acids that are derived from SHAM have an important role in the resistance to the environmental stresses (Raskin, 1992). SHAM($C_7H_7NO_3$) is a phenolic compound which exists in lots of plants, these days this compound is identified as a hormone-like substance that plays an important role of salicylic acids in induction of flowering, growth, development, ethylene synthesis, stomatal opening and closing (Raskin, 1992).

For example, when Lily begins to flowering, it produces heat that is involved in the ion absorption by root and stomatal conductance (Raskin, 1992). The salicylic acids have protection role in crops which are under environmental stresses. Also, it causes the increase of resistance to salinity and water deficiency in wheat seedlings (Bezvukova *et al.*, 2001). This material causes the increase of resistance to the high and low temperatures in tomato and bean (Senarant *et al.*, 2000), and in rice causes the damage decrease of the heavy elements (Mishra and chudhari, 1999). Also, the protein production (Rkhanova, et al. 1999) and heat stock in the tobacco and lectin accumulation in the wheat (shakirova and Beerukova, 1977) are caused by salicylic acid. External application of the salicylic acid causes the resistance to the heat (Day *et al.*, 1998), chilling (Janda *et al.*, 1999) and salinity stress in dicotyledon (Borsani *et al.*, 2001). Also, salicylic acids cause some variations in the anti-oxidant enzymes activity in the maize at chilling time (Janda *et al*, 1999). Generally, salicylic acids have important effects in crops such as effect in nutrients absorption (Glass, 1975), membrane stability (Glass and Dunlop, 1971), water relations (Barhosky and Einhelling, 1993), stomatal function (Aldesuquy *et al.*, 1998), inhibition of ethylene synthesis (Srivastava and Dwivedi, 2000) and increase in growth (Rajasekaram and Blake, 1999).

Researches indicate that environmental stresses cause to produce oxygen free radicals in chloroplast and other cell organelles. These oxygen free radicals may turn to hydrogen proxide by super oxide dismutase and then turn to water in chloroplast by ascorbate proxidase and glutathione reductase. Catalase enzyme is involved in purification of active oxygen species that produced under salinity stress in rice (Shim *et al.*, 1999). Unusually, during stress times, the activity of some enzymes is stimulated like superoxide dismutase, ascorbateproxide and glothaionereductase (Mishra and Singhal, 1995).

Materials and methods

Method

The experiment was laid out in a completely randomized design (CRD) with three replications. The variety of seeds was Esfahan 14 prepared by seed and plant institute of Esfahan and the seeds were disinfected by sodium hypochloride solution by 5% for five minutes and then washed by distilled water for three times. The petri dishes were disinfected by oven before the experiment conduction. To treat the seeds by salicylhydroxamic acid, the seeds were placed in the darkness for six hours at 20 ^{o}C and put in the solutions that their concentration consisted of 0 (control), 50, 75, 100 ppm. The seeds were dried in the room temperature before germination test for 36

hours (for 0 mM of salicylhydroxamic acid used non-treated seeds). For germination test of the treated seeds, the seeds were placed in the petri dishes (30 seeds per petri dish) with Whatman filter paper. For germination, the seeds were placed in the petri dishes in growth chamber at 25±1 ºC for 14 days, irrigated daily with sodium chloride of 0 (control), 70, 140, 210 mg/lit to induce treatment of salinity stress. After that some traits were measured like germination percentage, length of radicle and coleoptiles, fresh and dry weight of seedling, activity of catalase and proxidase enzymes. The germinated seeds were counted with the intervals of less than 12 hours to calculate the percentage of germination. For counting, the seeds were known as germinated seeds that their radicles had a length of at least 2mm. The counting continued till three consecutive days. The number of the germinated seeds was constant in each sample. Germination percentage is determined by the following formula:

$$\text{Germination percentage} = \frac{\text{number of the germinated seeds till final day}}{\text{number of whole of the seeds}} \times 100.$$

The caliper was used to determine the length of radicle and coleoptiles. Also, the samples were measured by digital scales to determine the seedling weight, as well as to determine the dry weight of seedling; the samples were placed in the oven for two days at 70 ºC, and then measured by digital scales. To measure the enzymes activity, the seedlings were maintained in frozen liquid nitrogen in freezer until the bio chemical analysis was performed.

Measurement of the catalase enzyme activity

It was carried out by using Cakmak and Horst`s (1991) method. In brief, 0.2 g of new frozen tissue was attrited and chafed in liquid Nitrogen of 3 ml buffer with 25 mm sodium phosphate, pH=6.8 at 0-4 ºC. The obtained homogeneous was centrifuged in 15000 rpm for 15 minutes at 4 ºC and the obtained solution was used to determine the activity of catalase enzyme. Decomposition of hydrogen peroxide by reduce absorption was followed in 240 nm of wavelength and for per mg of protein was expressed in enzyme extract.

Measurement of the proxidase enzyme activity

It was done by Ghanati`s (2002) method. In brief, 0.2 g of new breezed tissue in liquid Nitrogen in 0.02 M of phosphate potassium buffer, pH=6.8 at 0-4 ºC was attrited and chafed. The obtained homogeneous was centrifuged in 12000 rpm at 0-4 ºC for 15 minutes and obtained solution was used to determine activity of peroxides enzyme. Enzymatic activity was read by adding the proper amount of enzyme extract, buffer, Grayacul with finally concentration of 28 Mm and hydrogen peroxide with finally concentration of 5 mm in 470 nm of wavelength by the spectrophotometer (Cintra 6 GBS) and enzyme activity was expressed for per absorption variation by mg of protein in per minute.

Results

The obtained results indicate that the salinity stress has affected on studied traits as significantly and salicylhydroxamic acid caused to make significantly difference on measured traits (Table 1). Salinity stress caused to reduce germination percentage of seeds that were under salinity stress and not treated by salicylhydroxamic acid. The seed germination percentage was reduced by increasing the salt concentration, while salicylhydroxamic acid causes to increase germination in salinity treatments. Most germination percentages were observed on 100 ppm of salicylhydroxamic acid and without salinity stress (Table 2). Pretreatment by salicylhydroxamic acid with 100 ppm concentration indicates good effect on germination and reduction of damaging effect of salinity stress. Seedling dry weight decreases by increasing the salt concentration (Table 2).

Most of the seedling dry weight was observed in 100 ppm of salicylhydroxamic acid and without salinity stress so there was a significant difference between the treatments mentioned above (Table 2). It is obvious that dry weight was caused by increase of the radicle and coleoptiles growth. The application of salicylhydroxamic acid was not under stress in radicle length of the treatments. Activity of anti-oxidant enzymes are extremely impressed and influenced by salinity stress (Table 2). Catalase activity increases

significantly in highest concentration of salt and without salicylhydroxamic acid pretreatment in comparison with control. Salicylhydroxamic acid in 100 ppm of concentration caused the decrease of catalase activity in 210 mg/lit of salt. The increase of salt concentration causes the increase of catalase activity (Table 2). Proxidase enzyme is influenced by the increase of salt concentration too and most of the amount of this enzyme is observed in treatment that has the maximum concentration and was not influenced by salicylhydroxamic acid.

Table 1. Analysis of variance of salinity stress and salicylhydroxamic acid on germination indices, Catalase and Peroxidase activity of safflower.

S.O.V	Df	Germination percentage	Fresh weight of seedlings	Dry weight Seedlings	Shoot length	Root length	Catalase activity	Peroxidase activity
Stress	3	2193.5**	35746.7**	821.83**	3585.54**	4136.53**	597.91*	2249.37 **
SHAM	3	246.8**	4592.8**	3.59**	2131.47**	251.64**	0.096 ns	247.73ns
Error	9	2.96	1.23	4.48	0.34	0.31	3.31	4.2

*, **, ns: significant at 5%, 1% level and not significant, respectively

Table 2. Means comparisons of germination indices, Catalase and Peroxidase activity of safflower under salinity stress and salicylhydroxamic acid.

Treatments	Stress	SHAM	Germination Percentage	Fresh weight of seedlings (mg)	Dry weight Seedlings (mg)	Shoot length (mm)	Root length (mm)	Catalase activity (1M H2)O2 min	Peroxidase activity (OD.g^{-1} FW.min^{-1})
*Stress SHAM	0	0	77.27h	103f	18.14d	35.20b	44.65d	17.74g	18.66g
	0	50	83.27e	114d	20.75c	36.47Ab	46.43c	14.39hi	13.66h
	0	75	89.27c	120.7b	23.32b	37.05a	50.89b	10.430j	10.180i
	0	100	92.60a	124.3a	25.01a	37.75a	53.63a	7.560k	6.940j
	70	0	71.90j	85.88h	13.65f	26.28c	39.31g	19.96de	23.46de
	70	50	77.89g	95.69g	16.43e	26.55c	41.7f	19.13ef	20.76f
	70	75	84.57d	108.5e	19.36d	26.83c	43.21e	13.32i	18.56g
	70	100	88.61b	116.7c	22.79b	27.13c	46.9c	9.410j	13.64h
	140	0	69.68k	74.15i	11.29hi	21.77d	31.08j	24.47b	31.76b
	140	50	72.49ij	75.29i	16.70e	22.24d	34.02i	20.54d	24.43d
	140	75	79.41g	86.61h	19.13d	22.47d	36.78h	17.54g	21.50f
	140	100	86.68f	96.68g	11.97gh	22.83d	40.12g	10.550j	20.66f
	210	0	59.36m	32.74m	6.670j	15.67e	24.57m	26.43a	38.55a
	210	50	64.05 l	46.55 l	10.10i	15.77e	26.15 l	22.67c	29.50c
	210	75	76.57i	61.67k	12.99Fg	16.23e	28.31k	18.19Fg	22.83e
	210	100	79.27g	68.72j	15.64e	16.67e	30.56j	15.48h	21.53f

Discussion

As it is indicated by the obtained results, the percentage of germination in safflower seed was decreased significantly by salinity stress, and pretreatment by salicylhydroxamic acid causes the increase of germination percentage. External application of salicylic acids is effective on some processes such as seed germination (Cut and klessing, 1992), absorption and transfer of ions (Harper and Balk, 1981), penetration to membrane (Barkosky and Einhelling, 1993). Also imagine that salicylhydroxamic acid regulates the ion absorption

by roots and stomatal conductance. Different hormones such as salicylic acids, absisic acid, jasmonic acid and ethylene have important role in plants response to stress. It seems that salicylhydroxamic acid causes the decrease of toxic and damaging effect of salinity stress and increases germination. Seedling's dry weight decreases extremely by increase of salt concentration (Ghoulam *et al*., 2001). Last researches show that salinity stress causes the growth decrease of different parts of plant, such as, stem and root. Also, it`s reported that the use of salicylic acid causes the increase of fresh and dry weight of radicles and coleoptiles in maize under salinity stress (Khodary, 2004). Those operations that lead to increase growth of roots and other parts of some plants by salicylic acids are not completely known. But it is probably due to salicylic acids via other materials such as Auxins that regulates cell division and cell enlargement (Shakirava and Sahabutdinova, 2003). The wheat that treated by salicylic acid has more cell divisions in apical merry stem of the early and primary roots that lead to increase the lengthy growth (Shakirava *et al*., 2003). Also the salicylic acid prevents Auxin oxidation (Fariduddin *et al*., 2003). It seems that the increase in seedling dry weight depends on increase in radicle and coleoptiles length by salicylic acid. Because salinity stress causes the decrease in cell division, the elements like cadmium and sodium cause the decrease of growth that is followed by reduction of cell division and cell enlargement by effect on protorpeumps and making problem for them (Liu *et al.,* 2003). Salicylic acids are involved in synthesis of special protein which is known as Kinase and these proteins have an important role in regulation and differentiation of cell division. Metabolic changes such as change of enzymes activity in response to osmotic stresses and unbalanced ions are made by effect of salinity stress (Bray, 1997). By environmental effects such as salinity stress, the oxidative stress is produced by production of oxygen free radicals and they are harmful for plants growth in this condition (Smirnoff, 1993). In this experiment, the salinity stress causes the increase of catalase activity while seed pretreatment by salicylhydroxamic acid was on inhibitor for this enzyme. Salicylic acids are a hormone-like phenolic compound that has an important role in defensive mechanism as a regulator against the bio and non-bio stresses (Szalia*et al*., 2000). The salicylic acids have been an inhibitor for catalase activity and this enzyme is a cleaner for hydrogen peroxide and finally causes the amount increase of this enzyme by reducing its activity (Janda *et al*., 2002). Although the hydrogen peroxide is toxic in high concentration and the cycle of anti-oxidant of ascorbateglothaione is destroyed by catalase enzyme and ascorbateproxidase, but in low concentration it can have message transporting role and activates the related genes to resist in the plant (Foyer *et al*., 1997). Some reports have been received about changes in activity pattern of anti-oxidant enzymes in heavy elements stress condition and other non-bio stresses, under salicylic acid treatment and without it (Matewally *et al*., 2003) that indicates that salicylic acid by making bond with catalase enzyme causes the reduce of its activity in tobacco (Chen *et al*., 1993) and some other crops (Sanchez-cases and klessing, 1994). Because the external application of salicylic acids increases the resistance to salinity and drought stresses (Tari *et al.* 2000), so salicylic acid by increasing the resistance of seedlings to salinity via increased enzyme activity, acts to stay in front of stress effects (Shirsu *et al*, 1997). Generally oxidative stress is the result of increase in free radicals level of oxygen in the cells. Proxidase are enzymes which have an important role in response to the non-bio stresses such as salinity stress. Proxidase are responsible to remove the extra hydrogen peroxide. Salicylhydroxamic acid activates the anti-oxidant enzymes directly and indirectly. Salicylhydroxamic acid can be used as a substrate to give electron to catalase and proxidase. In this research it has been observed that increased concentration of salicylhydroxamic acid by 100 ppm has increased enzyme activity than 50 ppm of its concentration. It seems that the increase of concentration acts as a stress in crop and causes the increase of enzymatic activity that was observed. Seed pretreatment by salicylic acid in maize causes the activity increase of anti-oxidant enzymes (Janda *et al*., 1999). So the

results show the application of salicylhydroxamic acid causes the partly elimination of some toxic effects of stress caused by chlorine and sodium in crop. Treating by salt and salinity stress cause the integrity reduction and being completing of cell wall and also causes the release of electrolytes and cell ingredient. These results are in accordance with the Bor`s (2003) results in which he showed that salinity stress causes the increase of lipid proxidation. In the leaves of sugar beet (*Beta Vulgaris*), he observed the seed which was not pretreated by increased Malone dialdehyde and increased Malone dialdehyde concentration was produced by increase of salt concentration (Bor *et al*., 2003). Reduction of cell membrane damaging in response to salicylhydroxamic acid treatment is obtained with the increase of seedling dry weight under stress. This can indicate the induction of defensive system of anti-oxidant by salicylhydroxamic acid by destroying free radicals directly or by anti-oxidant enzymes that can reduce damage to these activity species and finally lipid proxidation of membrane is increased. It seems that salicylhydroxamic acid prevents lots of oxidations and blocks the increase of Malone dialdehyde by cleaning the free radicals. Also these free radicals can damage the protein structure and reduce proteins content (Noctor and Foyer, 1998). Protein content is related to difference rate between its synthesis and decomposition. Several researches have reported the reduction of protein and increase of ammonium nitrate and free amino acids under saline condition (Yonis *et al*., 1993). Reduction of protein contents can be caused by reducing the activity of some enzymes such as nitrate reductase, glutamine synthesize, glutamine exaloglotarate, amino transfrase by effect of salinity stress. According to these results, this can be concluded that the pretreatment of safflower seed by salicylhydroxamic acid with 100 ppm, had a good and positive effect on germination indices by affecting on anti-oxidant defensive system of crop caused the increase of safflower seedling resistance in salinity stress and by this, can lead to more stability and increase of germinated seeds.

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