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Investigation of 5-aminolevolinic acid (ALA) effects on seed germination and seedling growth of *Silybum marianum* under salinity stress

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Key words: 5-Aminolevolinic Acid (ALA), germination, milk thistle (*Silybum marianum*), salinity stress.

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Abstract

Salinity is a major environmental stress which adversely affects germination and seedling establishment in a wide variety of crops. However, studying of seeds with different plant growth regulators may helpful to alleviate the deleterious effects of salinity and enhance germination and seedling growth in salt stress environment. In present experiment we investigated the effects of 5-Aminolevolinic Acid (ALA) on germination and seedling growth parameters of milk thistle grown at different salinity levels. The experiment was arranged in factorial experiment in randomized complete block design with three replications. Results revealed that all parameters were significantly affected by salinity conditions and concentrations of ALA. In general, all parameters decreased with increasing NaCl concentrations, however the amount of these parameters increased with increasing ALA concentration. In addition, different between 0.25 and 0.50mM was not significant for germination percentage, shoot length, seedling fresh weight, seedling dry weight and leaf length. Interaction effects showed that highest value of root length and leaf length were produced from the 0.25 and 0.50mM ALA under the control treatment of salinity level. In contrast, the highest amount of seedling fresh weight were obtained from 0.25 and 0.50mM concentrations of ALA under control treatment of salinity. In general, our results suggest that ALA at low concentrations (0.25-1mM) has the potential to improve salt tolerance in milk thistle seedlings through concentrations treatment. We hope ALA may be useful in helping to solve serious problems occurring on a global scale, such as the desertification of green lands and salt damage to farm lands.

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Introduction

Milk thistle (*Silybum marianum* L.) is an annual plant belonging to the Asteraceae family, grows natively in the Mediterranean and is widespread in other regions in the world including Iran (Ghavami and Ramin, 2007). Since the 4^{th} century B.C. milk thistle extracts were used as medicine and became a favored remedy for *hepatobiliary* disease in the 16^{th} century (Burgess, 2003). Salinity is a major environmental stress affecting plant productivity and constitutes a problem concerning many areas, with an emphasis on region with hot and dry climates. More than 800 million hectares of land throughout the world are salt-affected. In many regions of the world and many areas of Iran, salinity stress may occur when crops are exposed to high levels of Na and Ca salts (Akhtar *et al.,* 2001). The ability of plants to cope with salinity stress is an important determinant of crop distribution and productivity in many areas, so it is important to understand the mechanisms that confer tolerance to salinity environment (Gilbert *et al.,* 1998).

Seed germination is a crucial stages in the life history of plants and salt tolerance during germination is critical for the establishment of plants that grow in saline soils. Selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seedling establishment. Numerous studied have demonstrated improvement in seed germination of different plant species under both normal and stress conditions in response to priming with plant growth hormones or other organic substances (Ashraf and Foolad, 2005). In recent studies, a number of plant growth regulators have been under trial to alleviate the oxidative damage caused by different abiotic stress. Gibberellic acid (GA3) is known to be concerned in the regulation of plant responses to the external environment (Chakrabarti and Mukherji, 2003), also, application of another plant growth bio-regulator has increased the saline tolerance of many crop plants (Haroun *et al.,* 1991). GA3 has also been shown to alleviate the effects of salt stress on water use efficiency (Aldesuquy and Ibrahim, 2001). Das Gupta *et al.* (1994) recorded that foliar application of plant growth regulators like IAA and GA helped the plant to restore retardation in water content in Mungbean plants subjected to water stress. In wheat, while seed germination decreased with increasing levels of salinity, the adverse effect of salinity was alleviated by soaking seed with IAA or NAA (Balki and Padole, 1982). On the other hand, 5-Aminolevulinic acid (ALA) is a key precursor of many tetrapyrrols including porphyrins for chlorophyll and haeme biosynthesis (Castelfranco *et al.,* 1974). It has been reported that ALA was used as a kind of herbicide at high concentration in agriculture (Duke and Rebeiz, 1994). However, several physiological effects of exogenous ALA at a low concentration have been found to regulate plant growth and increase the yields of some plants, including kidney bean, barley, potato and garlic by 10–60% (Hotta *et al.,* 1997). Recently, it was reported that a low concentration of ALA had a promotion effect on salt tolerance in cotton seedlings (Watanabe *et al.,* 2000), cold resistance in rice (Hotta *et al.,* 1998) and herbicide resistance in oilseed (Zhang *et al.,* 2008). ALA was also found to promote microtuber formation of potato under 0.5 % NaCl stress conditions and significantly enhanced photosynthetic gas exchange in date palm plants under salinity stress (Zhang *et al.,* 2006).

The role of plant growth regulators in overcoming the harmful effects of salinity on growth may be due to the change in the endogenous growth regulators which affects plant water balance. Thus the objective of this study was to investigate the response of milk thistle to different ALA and salinity levels during seed germination and seedling growth.

Material and method

Laboratory conditions and experimental design

This study was conducted in the laboratory of Biotechnology, University of Ilam at 2011. Seeds of milk thistle were surface sterilized by immersion for 1 min in sodium hypochlorite 10% solution, then repeatedly washed with deionized water. Twenty seeds in each treatment were allowed to germinate on a filter paper in 9 x 9 x 10 cm Petri dishes. The experiment was arranged in factorial experiment in randomized complete block design with three replications. The experimental treatments consisted of salinity levels (0 (distilled water) as a control, 50, 150 and 250mM NaCl) as the first factor, different concentrations of ALA (0 as a control, 0.25, 0.5 and 1mM) as the second factor were considered. The Petri dishes were placed in a growth chamber for 10 days at 25± 1 C° for germination.

Studied characters

Seed germination and seedling growth parameters

After complete germination ten seedlings were selected from each replicates and then seedlings were evaluated as follows:

1. Germination Percent (GP): according to the following equation described by (Ellis and Roberts, 1981):

GP = (Number of germinated seed / Total number of seed tested) x 100

2. Shoot length: the length of the ten seedlings from the seed to the tip of the plumule were recorded and expressed in millimeters (mm) as the shoot length.

3. Root length: the root length of ten seedlings from the seed to the tip of the root and recorded and expressed in millimeters (mm) as the root length

4. eedling fresh weight (FW): the weight of ten seedling was measure and expressed in milligram (mg) as the seedling fresh weight.

5. Seedling dry weight (DW): the weight of ten seedling were recorded and expressed in milligram (mg) after oven drying at 75 °c for 48 h.

6. Leaf length (mm)

7. Leaf width (mm)

Results

Analysis of variance the data showed (Table 1) that all parameters were significantly affected by salinity conditions and concentrations of ALA. Also, interaction effect between salinity conditions and concentrations of ALA on all parameters except of germination percentage and seedling dry weight was significant.

Table 1. Analysis of variance of the seed germination and seedling growth parameters affected by salinity and ALA treatments.

Source	d.f	MS							
		Germination percent	Germination rate	Root length (mm)	Shoot length (mm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Leaf length (mm)	Leaf width (mm)
Salinity (S)	3	4998.30**	3.86**	21128.07**	3470.78**	0.0749**	0.000016**	18.06**	15.41**
ALA (A)	3	404.55**	0.72**	290.99**	111.73**	0.0086**	0.000006*	9.03**	0.47**
S x A	9	10.46^{ns}	0.048**	103.77**	13.51**	0.039*	0.000002^{ns}	1.39**	0.29**
Error	45	38.72	0.016	14.87	3.47	0.00079	0.000077	0.13	0.03
CV%	-	9.02	5.32	8.42	6.59	20.65	11.52	3.95	3.36

ns, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Salinity and ALA concentration effects

Results in Table 2 revealed that salinity and ALA concentrations had a significant effect on averages of all parameters. Germination percentage decreased with increasing salinity levels concentrations. However, the difference between 0mM and 50mM was not significant. The highest and lowest germination percentage was observed in 0 and

250mM NaCl, respectively. On the other hand, this parameters increased with increasing concentration of ALA from control to 1mM, and the highest germination percentage was obtained in 0.5mM ALA. In the term of this parameter different between 0.25 and 0.5mM ALA was not significant. Root length and shoot length decreased with increasing NaCl concentration, however these parameters increased with increasing ALA concentration. In addition, different between 0.25 and 0.5mM ALA was not significant. Seedlings in the 0.5mM ALA showed higher root and shoot length. Increasing salinity levels from 50 to 250mM significantly decreased seedling fresh weight, however the highest seedling fresh and dry weight observed in 50mM NaCl than other levels of salinity. On the other hand, increasing ALA concentrations from 0 to 0.5mM significantly increased this parameter. The highest value of these parameters obtained under 0.25 and 0.50mM ALA, respectively. Also, Table 2 and 3 showed that increasing salinity levels from 50 to 250mM significantly decreased leaf length and leaf width, and in contrast increasing ALA concentrations from 0 to 0.5mM significantly increased leaf length and leaf width.

Table 2. Mean comparison of some of seed germination and seedling growth parameters under salinity and ALA concentrations in milk thistle.

Salinity concentration	Germination percentage	Root length (cm)	Shoot length (cm)	Seedling fresh weight (gr)	Seedling dry weight (gr)	Leaf length (cm)	Leaf width (cm)
0 mM	82.81^{a}	93.51^{a}	35.44^{b}	0.15^{b}	0.011^{a}	9.80^{b}	5.01^{c}
50 mM	82.18^{a}	52.55^{b}	44.77^{a}	$.21^{a}$	0.012^{a}	10.13^{a}	6.43^{a}
150 mM	65.62^{b}	27.32^{c}	21.00^{c}	$.12^{c}$	0.11^{a}	8.74^{c}	5.01^{c}
250 mM	45.31^{c}	9.77^{d}	11.71^{d}	$.05^{d}$	0.009^{b}	7.79^{d}	4.18^{d}
ALA concentration							
0 mM	64.06^{b}	40.12^{c}	25.97^{b}	0.120^{b}	0.010^{c}	8.01^{c}	5.14^{c}
0.25 mM	72.50^{a}	45.16^{b}	30.81^{a}	0.158^{a}	0.012^{a}	9.62^{a}	5.49^{a}
0.5 mM	74.06^{a}	50.01^{a}	30.20^{a}	0.154^{a}	0.011^{ab}	9.56^{a}	5.31^{b}
1 mM	65.31^{b}	47.84^{ab}	25.94^{b}	0.113^{b}	0.010^{bc}	9.27^{b}	5.51^{a}

Different letters at each column indicate significant differences at 5% probability level.

Table 3. Means of seed germination and seedling growth parameters of milk thistle under different salinity concentrations.

Salinity concentration	ALA concentration	Germination percentage	Root length (cm)	Shoot length (cm)	Seedling fresh weight (gr)	Seedling dry weight (gr)	Leaf length (cm)	Leaf width (cm)
	0 mM	78.75^{ab}	84.16^{b}	32.39^{f}	0.142^{bcd}	0.010^{a}	8.05^{e}	5.06^{ef}
0 mM	0.25 mM	85^{a}	86.28^{b}	36.40^{de}	0.167^{bc}	0.0124^{a}	11.08^{a}	4.83^{f}
	0.50 mM	87.50^{a}	101.97^{a}	38.05^{cd}	0.162^{bc}	0.0123^{a}	9.80^{c}	4.99^{ef}
	10mM	80^{a}	101.61^{a}	34.95^{df}	0.146^{bcd}	0.0107^{a}	10.26^{c}	5.15^{e}
	0 mM	78.75^{ab}	50.41^{c}	42.24^{b}	0.184^{b}	0.0118^{a}	8.72^{d}	5.72^{cd}
50 mM	0.25 mM	86.25^{a}	50.40^{c}	47.58^{a}	0.260^{a}	0.0132^{a}	10.85^{b}	6.65^{a}
	0.50 mM	86.25^{a}	54.17^{c}	49.11^{a}	0.263^{a}	0.0129^{a}	11.20^{b}	6.56^{a}
	10mM	77.50^{ab}	55.21^{c}	40.13^{bc}	0.161^{bc}	0.0112^{a}	9.76^{c}	6.80^{a}
	0 mM	60^{de}	20.63^{e}	18.70^{h}	0.098^{ef}	0.0117^{a}	8.02^{e}	5.74^{cd}
150 mM	0.25 mM	67.50^{cd}	32.16^{d}	25.78^{g}	0.146^{bcd}	0.0114^{a}	8.84^{d}	6.15^{b}
	0.50 mM	$72\ 50^{bc}$	33.63^{d}	21.07^{g}	0.135^{cde}	0.0110^{a}	9.04^{d}	5.53^{d}
	10mM	62.50^{d}	22.85e	18.45^{h}	0.105^{de}	0.0118^{a}	9.08^{d}	5.93^{bc}
	0 mM	38.75^{h}	5.29^{g}	10.54^{j}	$0.^{055fg}$	0.0082^{a}	7.26^{f}	4.07^{g}
250 mM	0.25 mM	51.25^{ef}	11.79^{f}	13.50^{i}	$0.^{060fg}$	0.0110^{a}	7.74^{ef}	4.34^{g}
	0.50 mM	50^{fg}	10.29^{fg}	12.58^{ij}	0.057^{fg}	0.0104^{a}	8.19^{e}	4.15^{g}
	10mM	41.25^{gh}	11.70^{f}	10.21^{j}	0.040^{g}	0.0100^{a}	7.98^{e}	4.16^{g}

Different letters at each column indicate significant differences at 5% probability level.

Interaction effects

Regarding to the interaction effects data illustrated in Table 3 clearly showed that root length, shoot length, seedling fresh weight, leaf length and leaf width were significantly affected by the interaction between salinity levels and ALA concentrations. Results clearly indicated that highest value of root length and leaf length were produced from the 0.25 and 0.50mM ALA under the control treatment of salinity level. While, the lowest amount of all parameters were obtained from the salinity 250mM concentration and 1mM concentration of ALA. Highest root length was produced under 0mM NaCl and 1mM ALA followed by 0mM NaCl and 0.5 mM ALA. In contrast, the highest shoot length and shoot length were produced under 50mM NaCl; 0 and 0.25mM ALA. In general, different between 0.25 and 0.5 mM ALA was not significant. Table 3 clearly showed that highest amount of seedling fresh weight were obtained from 0.25 and 0.5mM concentrations of ALA under 0mM NaCl. However, the lowest amount of this parameter was produced in the 1mM ALA under 250mM NaCl. The highest leaf width was observed in 50 mM NaCl with increasing concentration of ALA from 0.25 to 1mM.

Discussion

Milk thistle (*Silybum marianum* L.) grows natively in the Mediterranean and is widespread in other regions in the world including Iran (Ghavami and Ramin, 2007). This results indicate that different response of seed germination and seedling growth parameters under salinity levels and different concentrations of 5-Aminolevolinic Acid (ALA). Thus, it may be concluded that seed germination parameters and seedling growth characters could be used as selection criteria for salt stress tolerance at early growth stage. These results revealed that under salinity stress conditions germination percentage, root length, shoot length, seedling fresh weight, seedling dry weight, leaf length and leaf width decrease by high NaCl concentrations, and seedlings not be established well due to weak root growth. The decrease in the germination percentage, root and shoot length in response to increased salinity was also reported in wheat (Kandil *et al.,* 2012a), rice (Kandil *et al.,* 2012b) and milk thistle (Sedghi and Nemati, 2010). Some researchers believes that salinity stress has effects by osmotic pressure increase and water absorption reduction by seeds and in addition, by sodium and chlorine ions toxic effects, may affect seed germination (Wahid *et al.,* 2006; Gulzar and Ajmalkhan, 2001). On the other hand, there are various studies with plant growth regulators concerning that improvement of plant tolerance to salt, especially regarding the effects of salt on growth. For example, several studies using GA3 have attempted to reduce NaCl-induced inhibition of growth. Agakishiev (1964) found that spraying the leaf surface of cotton plants with GA3 solution caused a marked stimulation of growth despite a saline background (Aidid and Okkamoto, 1993). Our results showed that with increasing ALA concentration improved some of parameters such as germination percentage, root length, seedling fresh weight and leaf width under salinity conditions (Table 3). ALA is a key precursor in the biosynthesis of porphyrins such as chlorophyll, and it was recently discovered that at low concentrations ALA had a primitive effect on the growth and yield of several crops and vegetables (Hotta *et al.,* 1997a,b). Also, ALA was effective in counteracting stress damage such as a lack of cold resistance in rice seedlings (Hotta *et al.,* 1998). In this study we have shown that milk thistle seedlings treated with ALA can grow in salinity levels as high as 50-150mM NaCl. In general, our results suggest that ALA at low concentrations (0.25-1mM) has the potential to improve salt tolerance in milk thistle seedlings through concentrations treatment. We hope ALA may be useful in helping to solve serious problems occurring on a global scale, such as the desertification of green lands and salt damage to farm lands.

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