African Journal of Biotechnology Vol. 11(56), pp. 11967-11971, 12 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.339
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effect of exogenous gibberellic acid on germination, seedling growth and phosphatase activities in Lettuce under salt stress

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Accepted 22 June, 2012

The effect of gibberellic acid on germination and seedling growth of lettuce variety, Vista, under salinity conditions was studied. A reduction in germination percentage, roots and shoots length and fresh weight were observed under salt stress. At the same time, acid phosphatase and phytase activities in roots were reduced by NaCl. The exogenous application of gibberellin increased germination percentage and improved length and fresh weight of roots and shoots under salt treatment. It also increased both acid phosphatase and phytase activities in roots under this constraint. The application of gibberellic acid compensated for the negative effect of salinity.

Key words: Acid phosphatase, germination, gibberellic acid, lettuce, phytase, salinity, seedling growth.

INTRODUCTION

Salinity is a major problem that has negative effects on germination and seedling emergence by creating osmotic potential external to the seed and preventing water uptake through the toxic effect of Na^+ and Cl^- on germinating seeds (Khajeh-Hosseini et al., 2003). Salinity causes significant reductions in the rate and final percentage of germination (Almansouri et al., 2001). In many crops, the seed germination and early seedling growth are the most sensitive stages to environmental stresses, such as salinity (Sivritepe et al., 2003).

Abiotic stresses modify the level of plant growth hormones and decrease plant growth (Morgan, 1990). One of the most effective methods to overcome salinity problems is the use of plant growth regulators (Jamil and Rha, 2007). These hormones have been found to play a central role in integration of the plant responses expressed by plants to stress conditions (Amzallag et al., 1990). The inhibitory effect of salt stress on seed germination is alleviated by phytohormones, including gibberellic acid (GA) (Kaur et al., 1998; Khan et al., 2004; Kim et al., 2006; Atia et al., 2009), ethylene (Kepczynski and Kepczynska, 1997; Chang et al., 2010), cytokinin (Ismail, 2003) and messenger molecules such as nitric oxide (NO) (Zhao et al., 2007). Gibberellic acid has been reported to increase germination percentage and seedling growth and overcome the preventive effects of the salt stress on germination (Kabar and Baltepe, 1987). Kaur et al. (1998) reported that gibberellic acid increases percentage of germination and seedling growth under salt stress by enhancing the mobilization of starch reserves and increasing amylase activity in cotyledons, which ultimately leads to better seedlings growth. Several enzymes are secreted in response to gibberellin. Gibberellic acid (GA_3) is known to induce an increase in phytase and acid phosphatase activities in rye and barley during germination process (Centeno et al., 2001). Kaur et al. (1998) stated that the seeds whose germination was inhibited by high levels of salts may be grown in a medium supplied with gibberellic acid under salinity conditions.

The objective of the present study was to evaluate the effects of exogenous application of gibberellic acid on germination, seedling growth and acid phosphatase and

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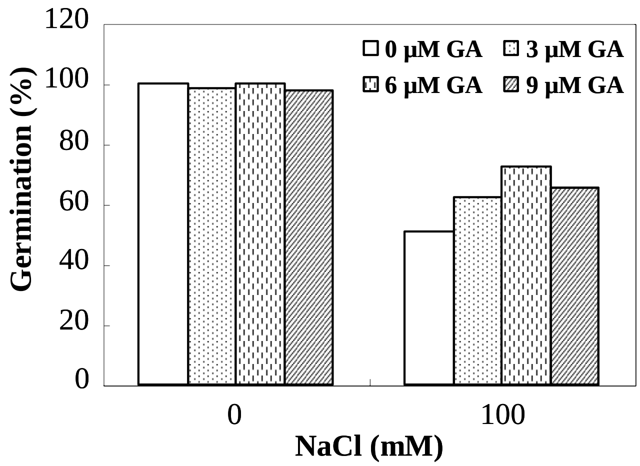

Figure 1. Effect of gibberellic acid (GA) on germination percentage of lettuce seed under normal (Control: 0 mM NaCl) or saline (S: NaCl 100 mM) conditions. Data are the mean of three samples of 25 seedlings, each one after 4 days.

phytase activities in lettuce seedlings variety Vista under salt stress conditions. Vista variety was selected in our previous study as a salt sensitive one during germination step (Nasri et al., 2011).

MATERIALS AND METHODS

Seed material and germination procedure

The seeds of lettuce (var. Vista) were provided by the Seed Laboratory of the Tunisian Ministry of Agriculture. For germination, seeds were soaked in distilled water or NaCl solution (100 mM) alone or provided with gibberellic acid applied at concentrations of 3, 6 and 9 µM at room temperature for 2 h. The seeds were then placed in Petri dishes with double-layer filter paper initially moistened with the same solutions and incubated for 4 days in the dark at room temperature (25 ± 2°C). Each treatment consisted of 25 seeds per Petri dish and was replicated 3 times. Seeds with emerged radicle were counted daily. Final germination percentage (FG%) was calculated as 100 x number of seeds germinated divided by the number of seeds sown. Lengths and fresh weights (FW) of roots and shoots under normal (Control: 0 mM NaCl) or saline (S: NaCl 100 mM) conditions were determined after 4 days.

Extraction and assay of acid phosphatase and phytase

Both acid phosphatase and phytase were extracted by grinding the tissues (root, shoot and cotyledons) after 48 and 96 h of germination at 4°C using 0.1 M sodium acetate buffer (pH 4.5) for the first enzyme and 0.1 M acetate buffer (pH 5.4) for the second one. The homogenate was centrifuged at 12000 g for 15 min and the supernatant was collected. Acid phosphatase activity was measured spectrophotometrically at 400 nm by monitoring the release of p-nitrophenol (pNP) from p-nitrophenyl phosphate. One unit of enzyme activity is defined as the amount of enzyme liberating 1 nmol of p-nitrophenol per minute (Saluja et al., 1989). Phytase activity was determined by measuring the release of phosphate from sodium phytate and was carried out as previously described (Nasri et al., 2011).

Statistical analysis

Data were presented as the mean of 4 seedlings for each treatment. Significant differences between treatments were analysed using analysis of variance (ANOVA) and mean comparison with Duncan test (Statistica®). Values were calculated at the $p \leq 0.05$ probability level.

RESULTS

Salinity caused a significant reduction in germination percentage as compared to control (no salt). However, exogenous application of gibberellic acid increased germination percentage of Vista variety treated with NaCl 100 mM. In all treatments, 6 µM of GA_3 was the most effective in increasing germination under salt stress (Figure 1). Further studies were carried out to observe the effect of salt stress on early seedling growth of germinating seeds. The results indicated that root growth was more affected by salt than shoot growth. Indeed, root length was restricted by 77% as compared to control (no salt), whereas shoot length was restricted only by 24% (Figure 2). On the other hand, root biomass was restricted by 48 and 40% for shoot biomass as compared to the control (Figure 3). However, GA_3 treated seeds showed less reduction in the size of root and shoot as compared to the control. Plant raised from seeds treated with 6 µM GA_3 had the maximum root and shoot growth (length and fresh weight) under both the salt and control treatments and shoots responded better than roots to GA_3 treatment (Figures 2 and 3).

Due to its positive effect on germination percentage and seedling growth of lettuce under salt stress, the concentration of 6 µM GA_3 was selected to investigate the effects of gibberellic acid on acid phosphatase and phytase activities in saline conditions. The influence of NaCl concentrations on acid phosphatase and phytase activities in root, shoot and cotyledons is shown in Table 1. Cotyledons of lettuce seedlings treated or not treated with NaCl contained higher levels of acid phosphatase and phytase activities than root and shoot. In the presence of salt, these enzymes activities were inhibited in the root and showed no decline in shoot and cotyledons after 2 and 4 days of germination.

The inclusion of GA_3 in germinated lettuce resulted in significant increase of acid phosphatase and phytase activities when compared with untreated seedlings at salt conditions. Moreover, a higher phosphatase activity was observed in shoot and cotyledons with GA_3 treatment, especially after 4 days of germination in normal and salt stress conditions (Table 1).

DISCUSSION

The plant growth hormones have been found to play a central role in the integration of plant responses under stress conditions (Amazallag et al., 1990). Gibberellic

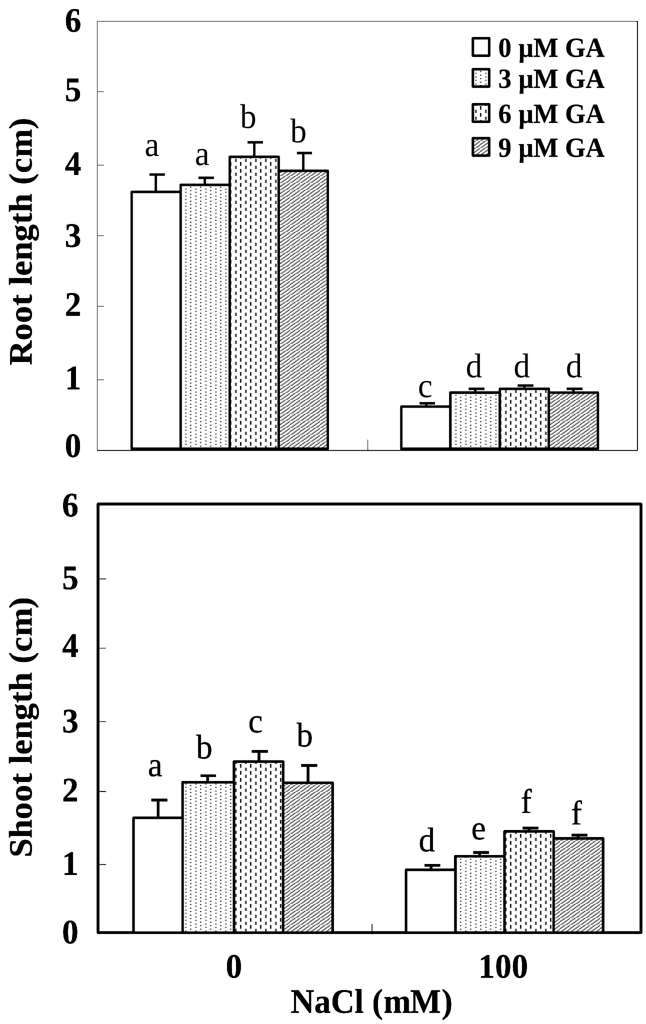

Figure 2. Effect of gibberellic acid (GA) on root and shoot length in lettuce seedlings under normal (Control: 0 mM NaCl) or saline (S: NaCl 100 mM) conditions. Values are means of four replicates ± SD. Means not sharing common letters are significantly different (*p≤ 0,05*) as assessed by Duncan's multiple range tests.

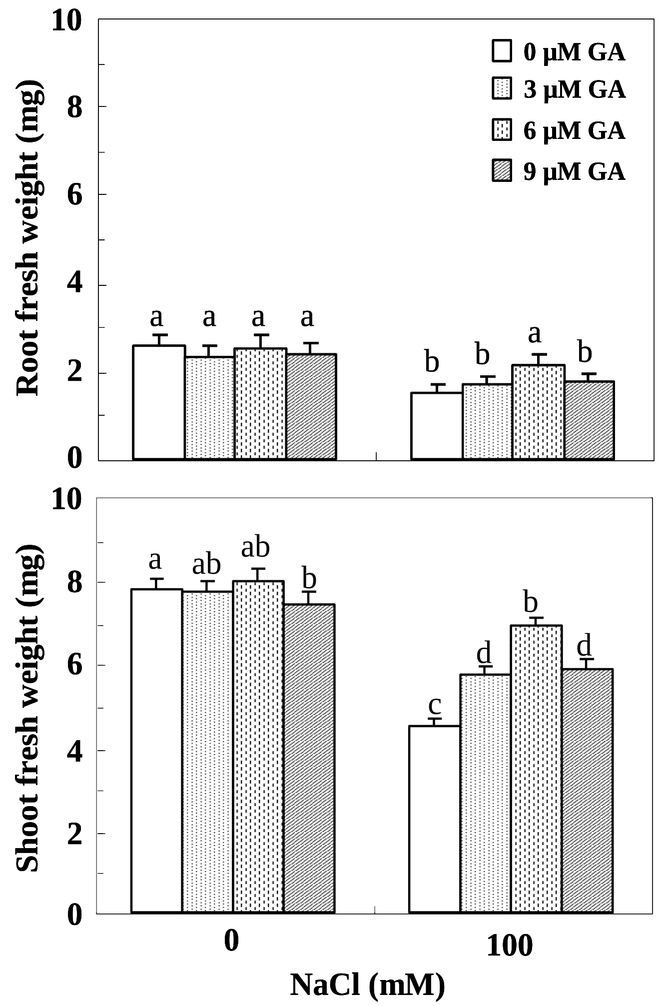

Figure 3. Effect of gibberellic acid (GA) on root and shoot fresh weight in lettuce seedlings under normal (Control: 0 mM NaCl) or saline (S: NaCl 100 mM) conditions. Values are means of four replicates ± SD. Means not sharing common letters are significantly different (p≤ 0,05) as assessed by Duncan's multiple range tests.

acid has been reported to increase percentage germination and seedling growth (Kaur et al., 1998). In this study, the exogenous application of GA increases the germination percentage and the length and fresh biomass of roots and shoots in lettuce under salinity conditions. These results agree with those of Mohammed (2007) in bean and Jamil and Rha (2007) in sugar beet. The stimulatory effect of gibberellic acid on germination percentage and seedling growth has been also reported in wheat (Akman, 2009). In fact, exogenous gibberellic acid causes an increase in germination and seedling growth by enhancing the availability of endogenous gibberellic acid (Kaur et al., 1998). This could be explained by the fact that GA_3 may reduce the abscisic acid level in seeds through the activation of their catabolism enzymes or by blocking the biosynthesis pathway (Toyomasu et al., 1994). The GA_3 are known to alleviate salinity effect in some halophytic seeds (Khan et al., 2004; Li et al. 2005; Gul and Khan, 2008; Atia et al., 2009). GA_3 is known to be a growth regulator and is used frequently in overcoming barriers to germination. Kabar and Baltepe (1990) stated that GA_3 was effective in removing heat and salinity stress in the germination of lettuce and barley seeds.

Gibberellic acid has been reported to reduce the inhibitory effect of NaCl on seedling growth in rice (Lin and Kao, 1995), chickpea (Kaur et al., 1998) and wheat (Akman, 2009). According to Lin and Kao (1995), in rice seedlings, GA counteracted the inhibitory effect of NaCl on shoot growth but not root growth, showing that under salt stress, the growth of shoots and roots respond

Table 1. Effect of gibberellic acid (GA) on acid phosphatase and phytase activities (nmol/min/organ) in root, shoot and cotyledons in lettuce seedlings under normal (Control: C: 0 mM NaCl) or saline (S: NaCl 100 mM) conditions 48 and 96 h after sowing. Values are means of four replicates ± SD. Means not sharing common letters are significantly different (p≤ 0,05) as assessed by Duncan's multiple range tests.

Parameter		Acid Phosphatase		Phytase	
		48 h	96 h	48 h	96 h
Root	C	5.38 ± 0.56^{a}	8.04 ± 0.61^{d}	0.59 ± 0.07^{a}	1.47 ± 0.29^{bc}
	C + GA	6.45 ± 0.91^{bd}	8.59 ± 0.67^{d}	1.19 ± 0.20^{bd}	1.81 ± 0.09^{c}
	S	3.65 ± 0.19^{c}	5.43 ± 0.29^{a}	0.43 ± 0.03^{a}	0.94 ± 0.16^{d}
	S + GA	6.00 ± 0.73^{ab}	8.19 ± 0.55^{d}	1.29 ± 0.14^{b}	1.32 ± 0.03^{b}
Shoot	C	28.66 ± 3.22^{ac}	21.58 ± 1.33^{b}	1.55 ± 0.05^{ad}	1.58 ± 0.13^{ad}
	C + GA	22.28 ± 3.53^{b}	25.90 ± 2.93^{cde}	1.61 ± 0.16^{ad}	1.76 ± 0.12^{bd}
	S	23.19 ± 3.61^{be}	24.58 ± 4.32^{ad}	1.72 ± 0.11^{ab}	1.30 ± 0.12^{c}
	S + GA	22.37 ± 5.43^{b}	28.84 ± 1.93^{be}	1.82 ± 0.04^{b}	1.54 ± 0.07^{a}
Cotyledons	C	106.28 ± 3.49^{a}	100.21 ± 1.40^{a}	2.71 ± 0.19^{a}	2.55 ± 0.28^{ad}
	C + GA	136.85 ± 6.60^{b}	176.91 ± 4.97^{d}	4.45 ± 0.16^{b}	3.52 ± 0.21^{c}
	S	102.28 ± 1.88^{a}	104.70 ± 11.39^{a}	2.23 ± 0.11^{d}	2.20 ± 0.21^{d}
	S + GA	122.16 ± 8.39^{c}	139.99 ± 3.91^{b}	3.50 ± 0.19^{c}	3.34 ± 0.25^{a}

differently to gibberellic acid.

The results of our analysis of acid phosphatase and phytase activities showed that gibberellic acid induces a significant increase in these enzyme activities under salinity conditions. Centeno et al. (2001) also found that the addition of GA_3 during the germination process induces an increase in acid phosphatase and phytase activities in rice and wheat seeds. Apparently, the presence of GA stimulates the activation and secretion, but not a new synthesis of acid phosphatase in wheat seeds (Bayley et al., 1976). Kaur et al. (1998) also refers to an increase in amylase activity in cotyledons and improvement in the transport of sucrose from cotyledons to the growing tissues of seedlings by gibberellic acid under salt stress. These authors found that gibberellic acid balance against the negative effects of salt stress by increasing mobilization of starch and amylase activity in cotyledons. Kim et al. (2006) suggested that reduction in root length and starch mobilization in rice seeds under salt stress will be recovered by exogenous application of gibberellic acid.

This study showed that exogenous application of gibberellic acid enhances germination and early seedling growth by stimulating phosphatase activity of lettuce seedlings, thus promoting more effective mobilization of the reserves of phosphorus in seeds subjected to salt stress.

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