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A brassinosteroid analogue prevented the effect of salt stress on ethylene synthesis and polyamines in lettuce plants

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ABSTRACT

The present study was carried out to analyze the effect of treatment with DI-31, a brassinosteroid analogue (0.1 or 1 μM), on alleviating salt stress in lettuce plants. After application of DI-31, 100 mM NaCl treatments were administered. Samples that were separated into shoots and roots were taken 5 days after this application. DI-31 alleviated the reduction of weight due to NaCl with a greater effect at 1 than at 0.1 μM. The emission of ethylene increased with the application of NaCl, and the DI-31 treatment decreased this effect in shoots and roots. Similar results were obtained in ACC, since its concentration was increased by salinity, and DI-31 was effective in reducing it. The putrescine content decreased with salinity and DI-31 reversed the effect in the shoots, while it remained constant in the roots. The spermidine and spermine contents in roots increased with salinity. The DI-31 reversed the effect of NaCl in both shoots and roots in the two polyamines, except in shoots where the spermine content was maintained. Since the DI-31 brassinosteroid has partially reversed the negative effect of NaCl on lettuce plants, we conclude that the brassinosteroid has a protective effect against salinity.

1. Introduction

High salt concentrations in soil or irrigation water can have a devastating effect on plant metabolism, altering levels of growth regulators and uncoupling major physiological and biochemical processes (Hasanuzzaman et al., 2013). High salt concentrations are toxic to seed germination, seedling growth, vegetative growth, flowering and fruit set, and ultimately diminish economic yield and the quality of products (Munns and Tester, 2008). Lettuce is considered as a moderately salt tolerant vegetable (Ekinci et al., 2012). Salinity levels of more than 2.0 and 2.6 dS m^{-1} reduce lettuce fresh yield and plant growth, respectively (De Pascale and Barbieri, 1995). It has also been reported that NaCl concentrations above 50 mM produce decreases in weight and height of lettuce proportional to the concentration applied (Kim et al., 2008). Many growth hormone (or chemical) related studies have attempted to induce or increase salt tolerance in plants (Shahid et al., 2014). Among them, BRs have been widely used to confer resistance in plants against various abiotic and biotic stresses, including salinity (Fariduddin et al., 2014). 24-Epibrassinolide (EBL) has been used in most of the research to decrease the effects of salinity, although all investigations have been conducted in the laboratory (Karlidag et al., 2011; Ekinci et al., 2012). However, EBL is an expensive compound that cannot be used in field treatments, so we used a BR analogue in this research, DI-31 (also known as BB-16). This compound has already been successfully tested in field crops to increase lettuce production, resulting in a cheap and profitable alternative (Serna et al., 2012). DI-31 is characterized by the presence of a spiroketalic ring instead of the typical BR side chain, and these chemicals possess BR-like activity like BB6 and MH5 (Coll et al., 1995; Mazorra et al., 2004). DI-31 has been successfully used to counteract NaCl effects on rice seedlings (Núñez et al., 2003). On the other hand, a variety of stresses, such as salinity, are known to increase ethylene production, and increased ethylene levels aggravate the stress effect (Siddikee et al., 2012). The increase in ethylene levels inhibits plant growth and induces senescence, which leads to premature death (Mayak et al., 2004). Polyamines (PAs) are ubiquitous low-molecular-weight aliphatic amines that are involved in many physiological processes such as organogenesis, embryogenesis, floral initiation and development, leaf senescence, fruit development and ripening, and abiotic and biotic plant stress responses (Alcázar et al., 2010). Changes in plant polyamine metabolism occur in response to a variety of abiotic stresses such as salinity (Hasanuzzaman et al., 2013).

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On the other hand, salinity also increases ethylene production (Amjad et al., 2014). However, the results are controversial in relation to the effect of BR on ethylene synthesis. It has been found that frequent treatments with brassinosteroids lead to increases in the synthesis of ethylene by induction of ACC synthase gene expression (Korableva et al., 2002; Swarup et al., 2002; Joo et al., 2006; Hansen et al., 2009). In contrast, other authors have reported that brassinolide does not increase ethylene production in *Arabidopsis thaliana* (Arteca and Arteca, 2008). On the other hand, Wang et al. (2011) found that the reduction in ethylene evolution from imbibed seeds by salt stress was attenuated by EBL. However, there are no studies on the effect of treatments with BRs on ethylene production under NaCl stress in plants. Moreover, how brassinosteroid treatments affected the polyamines produced by Cr stress has been studied (Choudhary et al., 2011), but there are no studies on how BR treatments affect PA production under NaCl stress.

For these reasons, the aim of this study was to assess the effects of the application of DI-31, a BR spirostanic analogue, on the growth of lettuce plants that were treated with NaCl, in an attempt to prevent or reverse the adverse effects as a preliminary study for later potential use in the field, besides analyzing how these BR analogue treatments affect ethylene and polyamine contents in lettuce plants treated with NaCl.

2. Materials and methods

2.1. Plant material and experimental conditions

Experiments were carried out with lettuce (*Lactuca sativa*) var capitata cv Albanas provided by Rijk Zwaan Ibérica, S.A. (Almeria, Spain). Seeds were sterilized with a 5% (v/v) sodium hypochlorite solution for 5 min and washed thoroughly with distilled water. Seeds were germinated in trays containing moistened vermiculite covered with perforated aluminium paper and maintained in a germination chamber. Distilled water was added when necessary. The germination chamber was kept in the dark at a temperature of 20 °C and held for 2 days until the seeds germinated. Afterward, the seedlings were transferred to a growth chamber programmed at 22/18 °C. The light/dark cycle was 16/8 h and the relative humidity was 60/80%. The photon flux density was 400 μmol m^{-2} s^{-1}. After 3 days, the seedlings were transferred to 13 L pots containing aerated half strength Hoagland solution. The pH was adjusted daily to 5.5–6.0 and the volume of the solutions was maintained by the addition of deionized water. The nutrient solution was renewed every 7 days. Twenty-one pots were used, 3 for each treatment, with 20 seedlings per pot. Treatments were carried out 14 days after transferring the seedlings to the recipients, first with the DI-31 at 0.1 or 1 μM. The DI-31 applications were carried out by spraying the plants. Saline treatments, 1 mM NaCl for the control and 100 mM NaCl, were started the day after the treatment with DI-31. This NaCl concentration was chosen because previous studies have shown that 100 mM NaCl causes a significant decrease in the growth of lettuce, although this was not lethal (Zapata et al., 2007, 2008). To learn if the BR analogue could reverse the saline stress to a greater or lesser extent, the NaCl application was performed in two ways, in 1 day to create a greater osmotic effect on plants, and in 3 consecutive days so that the saline solution was progressively increased up to 33, 66, and 100 mM in order to avoid osmotic shock. It is important to know if the BR analogue can eliminate the adverse effect of salinity to be applied in 1 day since field irrigation water will be used this way. The different treatments were:

- 1 mM NaCl (control),
- 100 mM NaCl applied in 1 day without DI-31 (NaCl 1d + BR 0),
- 100 mM NaCl applied in 1 day + 0.1 μM DI-31 (NaCl 1d + BR 0.1),
- 100 mM NaCl in 1 day + 1 μM DI-31 (NaCl 1d + BR 1),
- 100 mM NaCl applied in 3 days without DI-31 (NaCl 3d + BR 0),
- 100 mM NaCl applied in 3 days + 0.1 μM DI-31 (NaCl 3d + BR 0.1),
- 100 mM NaCl applied in 3 days + 1 μM DI-31 (NaCl 3d + BR 1).

The BR analogue used in the present work, DI-31, was prepared by the Laboratorio de Productos Naturales at La Habana University, Cuba. The DI-31, a diosgenin derivative, with global formula $C_{27}O_5H_{42}$ (commercially known as Biobras-16 (BB16)) is the (25R), 3β, 5α, dihydroxy-spirostan-6-one (Jomarrón et al., 2000).

Five days after the last NaCl application, 12 plants from each pot were taken at random, and then divided into shoots and roots, and the following determinations were made: fresh weight, respiration rate, ethylene production rate, free and total ACC concentration, and polyamine concentration.

2.2. Biomass determination

Twelve plants were taken for each of the three replicates per treatment (36 plants per treatment) that were divided into two subsamples of six plants (6 subsamples per treatment). Fresh weight was recorded for shoots and roots from each 6 plants for each subsample and expressed as g per plant. Results are the mean ± SE ($n = 6$).

2.3. Ethylene and CO_2 emission

Immediately after fresh weight determination, the shoots or roots of each subsample were placed in glass jars of 500 and 120 mL, respectively, which were hermetically sealed for 1 h with a rubber stopper in order to quantify ethylene production and respiration rates at 20 °C and under light conditions similar to those of the growth chamber. The measurements of ethylene are best taken after the wound ethylene has emanated (2 h after cutting). In order to avoid stress by dehydration, which might affect ethylene and/or respiration rates, the shoots and roots were placed inside the glass jar on a sheet of Whatman No. 1 filter paper moistened with 5 ml of distilled water (Zapata et al., 2003). Ethylene concentrations were determined three times in each jar by extracting three samples of 1 ml of the head space atmosphere, in which the ethylene concentration was determined using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. Column temperature was 90 °C, and injector and detector temperature, 150 °C (Zapata et al., 2007). Ethylene production rate was expressed in nl g^{-1} h^{-1} and the results are the mean ± SE of the measurements per triplicate of each six subsamples per treatment ($n = 18$). Additionally, three samples of 1 ml of headspace atmosphere were taken to determine the respiration rate, monitoring the CO_2 concentration in a Shimadzu 14-A gas chromatograph with a thermal conductivity detector (TCD). Column temperature was 50 °C. Respiration rate was expressed as μg g^{-1} h^{-1} and the results are the mean ± SE of the measurements per triplicate of each six subsamples per treatment ($n = 18$). Subsequently, shoots or roots of each subsample were frozen and ground in liquid N_2, and used to determine free and total ACC and polyamines.

2.4. ACC determination

For extraction of free and total ACC, shoot or root tissue was macerated in a mortar with a pestle in 0.2 M trichloroacetic acid (1:3, w/v) and centrifuged at 10,000 × *g* for 15 min. The supernatant was used to determine free ACC concentration by chemical conversion of ACC to ethylene (Zapata et al., 2004). Conjugated ACC in the supernatant was hydrolyzed to free ACC with 2 N HCl, which