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Induction of salt tolerance in pepper (*Capsicum annuum*) by 24-epibrassinolide

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Abstract
In order to study the effectiveness of brassinosteroids on the amelioration of the inhibitory effect of salinity on pepper plants, a short-term experiment was conducted in greenhouse to test different concentrations of 24-epibrassinolide (0.0, 0.01, 0.05, 0.1, and 0.5 mg L^{-1}) by foliar application on growth, relative water contents and chlorophyll fluorescence of pepper (*Capsicum annuum* cv. Beldi) plants irrigated with salt water (0.4 g L^{-1} NaCl). Salt decreases the different parameters of growth. However, its effects were more pronounced on the shoot than root growth. An exogenous supply of 24-epibrassinolide was found to be successful in alleviating of the inhibitory effects of salt stress on shoot growth parameters and the leaf relative water contents. However, non-significant effect of 24-epibrassinolide was observed on root growth and chlorophyll fluorescence. Out of the five concentrations, the effects of 0.5 mg L^{-1} proved the best under stress conditions.

Keywords: Chlorophyll fluorescence, 24-epibrassinolide, growth, relative water contents, salt stress.

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INTRODUCTION

Salinity is a major environmental stress that adversely affects plant growth and metabolism. Salts inhibit plant growth by osmotic stress, specific ion toxicity, ion imbalance and oxidative stress (Tester and Davenport 2003). Many crop plants including peppers are susceptible and cannot survive under conditions of high salinity or can survive but with decreased yields. To alleviate this problem, a number of studies were conducted with the aim of removing the inhibitory effect of salt stress on plant growth, using different types of phytohormones (Nandini et al. 2002, Chakrabarti et al. 2003, Hussein et al. 2007, Tuna et al. 2008). Among these, brassinosteriods (BRs) have been the main focus of many researches in the last 20-30 years (Rao et al. 2002, Anuradha and Rao 2003).

Brassinosteriods (BRs) are steroidal plant hormones, essential for plant growth and development (Sasse 2003). After the discovery and isolation of brassinolide from Brassica napus pollens, a large number of related steroids have been isolated and identified from various plant sources including angiosperms, gymnosperms and alga, and based on this Sasse (2003) suggested that these compounds are probably ubiquitous in the plant kingdom.

Research on brassinosteroids, has revealed that they elicit a wide spectrum of morphological and physiological responses in plants including stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis, proton pump activation, xylem differentiation and the regulation of gene expression (Li and

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Chory 1999, Mussig et al. 2002, Sasse 2003).

In addition to stimulating growth, they are effective in reducing abiotic stresses such as moisture, drought, low and high temperature, salinity, and heavy metal (Clouse and Sasse 1998, Rao et al. 2002, Anuradha and Rao 2003; Ozdemir et al. 2004, Ali et al. 2007, Hayat et al. 2007, Hasan et al. 2008) induced inhibitory effects (Rao et al. 2002). The objective of the present study was to observe the effect of the exogenous application of 24-epibrassinolide as a foliar spray on the growth and chlorophyll fluorescence of pepper plants under saline and non-saline conditions.

MATERIAL AND METHODS

A pot experiment was conducted to assess the interactive effect of the foliar application of 24-epibrassinolide (24-epbr) and root zone salinity on the morpho-physiological attributes of the pepper cultivar 'Beldi'. Seeds were obtained from Department of Agronomy, Institut Supérieur Agronomique de Chott Mariem, Sousse (Tunisie). Fifteen seeds were sown in each small plastic pot (10 pots) at 10 mm depth. Pots were filled with commercial peat. They were germinated in a greenhouse under natural light conditions, a daytime temperature of 24-30°C. After the second true leaf appeared, the combine effect of salinity and 24-epibrassinolide was investigated by applying two salinity levels (0 and 4 g L^{-1} NaCl) and five 24-epibrassinolide levels (0.0, 0.01, 0.05, 0.1, and 0.5 mg L^{-1}). 2 mg of 24-epibrassinolide (Sigma-E 1641) was dissolved in 2 mL of ethanol (stock solution). Different concentrations of 24-epibrassinolide were made by dilute a volume of stock solution with distilled water. The treatment was applied once with a manual sprayer, plants from each treatment were sprayed with 150 mL of each solution.

Growth measurement

After 12 days of treatment application, the data were recorded. Fifteen plants, representing each treatment, were up-rooted carefully and washed properly under tap water. Plant height and roots lengths were recorded. The plants were then separated into roots and shoots. Leaf area was measured with a LI-3100 leaf meter (LI-COR. Inc., Lincoln, NE, USA). Shoots and roots fresh weights (FW) were determined, and dry weights (DW) were obtained after oven drying the samples at 70°C for 72 h.

Leaf relative water contents

The relative water contents (RWC) in the leaf samples was estimated using the following equation Turner (1981):

$$RWC (\%) = [(FW - DW)/ (TW - DW)] 100$$

Where, FW is fresh weight, DW is dry weight and TW is turgid weight.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a portable fluorometer F.I.M, 1500, ADC (Fluorescence Induction Monitor 1550, Analytical Development Company Limited). The leaf was dark-adapted for 30 min, then dark fluorescence F0, maximal fluorescence Fm and photochemical yield Fv/Fm (where, Fv = Fm-F0) were recorded.

Statistical analysis

All analyses were done on a completely randomized. The results were subjected to one-way analysis of variance (ANOVA) and the mean differences were compared by the Duncan test at 5% significance level.

RESULTS

Growth

Salinity decreased the length, leaf area, fresh and dry mass of shoots (Fig. 1). Whereas the 24-epibrassinolide increased the values for both stressed and unstressed plants. Under the control conditions, all 24-epibrassinolide treatments had ameliorative effects on the shoot parameters growth. The 0.5 mg L^{-1} level was the most effective. However, under salt stress conditions, only the treatment with 0.5 mg L^{-1} of 24-epibrassinolide significantly increased the length, leaf area, fresh and dry mass (Fig. 1).

Exogenous application of different levels of 24-epibrassinolide to seedling pepper plants did not cause a significant increase or decrease effects on the root growth parameters including the length, fresh and dry mass of pepper plants under control or saline conditions, whereas, salinity had a significant

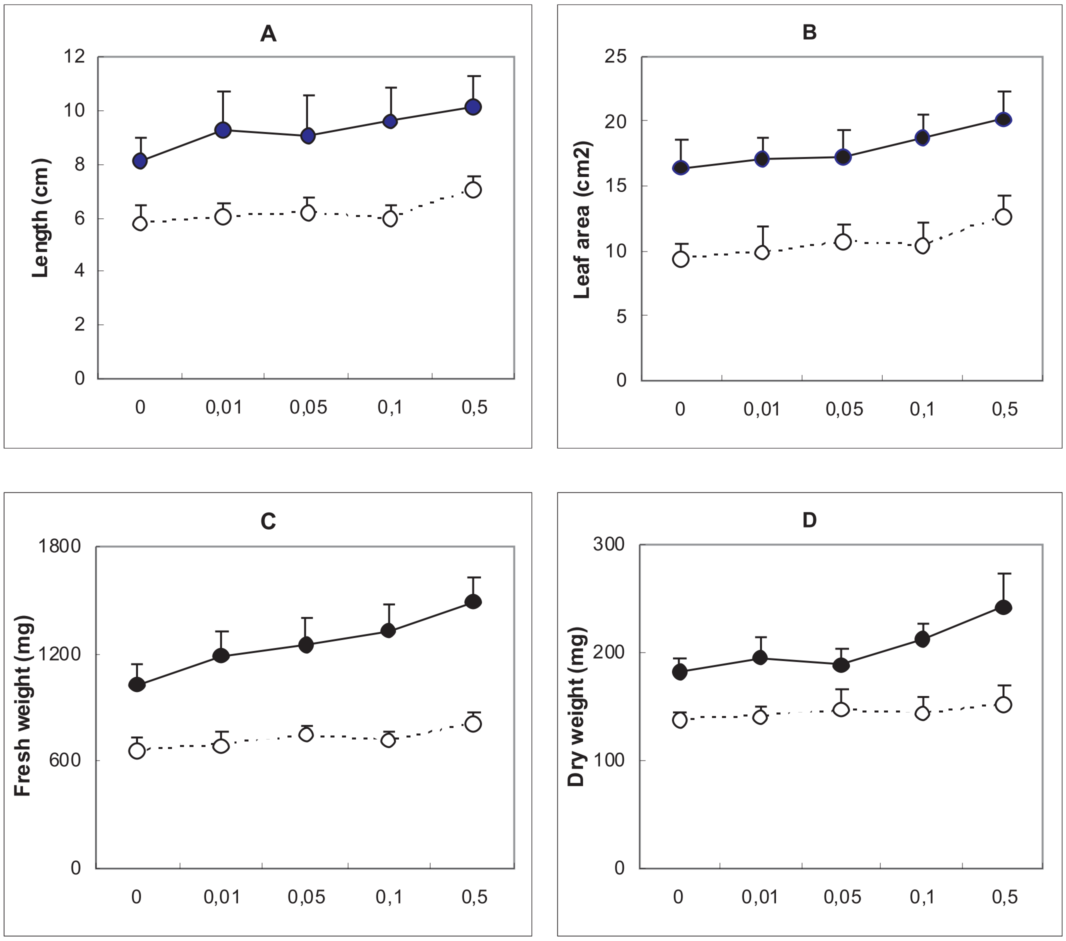

Fig 1. Effects of 24-epibrassinolide (EBR) at different concentrations on shoot growth parameters. (A) length (cm), (B) leaf area (cm2), (C) Fresh and (D) dry mass (mg) of pepper plants grown under control (closed circles) and saline conditions (open circles).

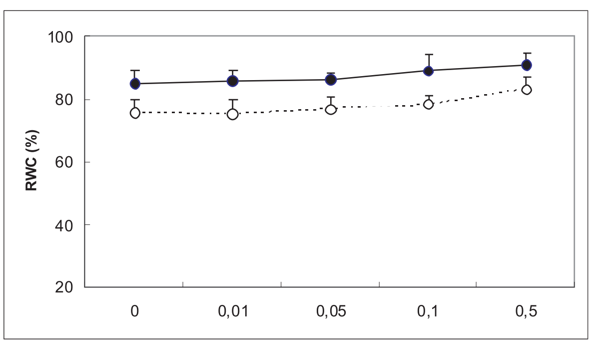

Fig 2. Effects of 24-epibrassinolide (EBR) at different concentrations on the relative water content of pepper plants grown under control (closed circles) and saline conditions (open circles).

($P \leq 0.05$) effect on reducing the root length, fresh and dry weights (Table 1).

Relative water contents

The leaf relative water contents (RWC) were decreased in the leaves of pepper plants grown at a high salinity compared to the unstressed control plants (Fig. 2). The 24-epibrassinolide ameliorated relative water contents partly and the values were still lower than that in the control plants (Fig. 2).

Chlorophyll fluorescence

The changes in PSII photochemistry were investigated in the dark-adapted leaves. Table 2 shows that neither salt nor the 24-epibrassinolide application affected the maximal efficiency of the PSII photochemistry (Fv/Fm) measured in the dark-adapted leaves.

DISCUSSION

Growth

Results from this study showed that NaCl treatment (4 g L^{-1}) caused reduction in growth

Table 1. Roots growth parameters: length (cm), Fresh and dry mass (mg) of pepper plants were subjected for different levels of foliar application of 24-epibrassinolide under control or saline conditions.

NaCl levels ($g\ L^{-1}$)	24–epbr levels ($mg\ L^{-1}$)	Root growth parameters		
		Length (cm)	Fresh mass (mg)	Dry mass (mg)
0	0	20.61 a	250 a	81 a
	0.01	19.96 a	239 a	77 a
	0.05	20.32 a	264 a	78 a
	0.1	21.14 a	259 a	83 a
	0.5	21.48 a	244 a	80 a
4	0	16.00 b	119 b	45 b
	0.01	14.56 b	106 b	42 b
	0.05	15.03 b	124 b	47 b
	0.1	15.49 b	129 b	50 b
	0.5	16.22 b	120 b	46 b

Data are reported as means (n = 15). Means were separated by Duncan's test, different letters in a single column show statistically significant differences for $P \leq 0.05$.

Table 2. Chlorophyll fluorescence parameters (F0, Fm and Fv/Fm) of pepper plants (var. Beldi) were subjected for different levels of foliar application of 24-epibrassinolide under control or saline condition

NaCl levels ($g\ L^{-1}$)	24–epbr levels ($mg\ L^{-1}$)	F_0	F_m	F_v/F_m
0	0	279 a	1496 a	0.813 a
	0.01	273 a	1478 a	0.815 a
	0.05	271 a	1459 a	0.814 a
	0.1	273 a	1470 a	0.814 a
	0.5	281 a	1532 a	0.816 a
4	0	276 a	1502 a	0.816 a
	0.01	275 a	1487 a	0.815 a
	0.05	280 a	1495 a	0.812 a
	0.1	276 a	1506 a	0.817 a
	0.5	278 a	1472 a	0.811 a

Data are reported as means (n = 6). Means were separated by Duncan's test, different letters in a single column show statistically significant differences for $P \leq 0.05$.

of pepper plants as compared to the control (Fig. 1). This is consistent with previous reports that pepper plants are relatively sensitive to salt stress (Chartzoulakis and Klapaki 2000, Lycoskoufis et al. 2005). Reduction in the plant growth has been attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of the ionic imbalance and decrease in many metabolic activities (Kumar et al. 2005). Brassinosteroids are thought by some investigators to be helpful in enhancing salt tolerance of rice (Anuradha and Rao 2003, Ozdemir et al. 2004), chickpea (Ali et al. 2007), barley (Kilic et al. 2007) and wheat (Shahbaz and Ashraf 2007).

Shoot growth was inhibited by salt stress (Fig. 1). According to Yasseen et al. (1987) NaCl inhibits growth by reducing both cell division and cell enlargement. The observed increased seedling growth of stressed pepper plants with 24-epibrassinolide treatment could be attributed to the positive effect of 24-epibrassinolide, which stimulate cell elongation and division.

Salinity reduced shoot elongation, whereas 24-epibrassinolide stimulated shoot elongation (Fig. 1). The physiological effects of these growth regulators on cell elongation was supported by Frank-Duchenne et al. (1998), he reported that epibrassinolide promoted stem elongation of two cultivars of sweet peppers. The same effect was also observed in the shoots of rice (Ozdemir et al. 2004) under salt stress.

Salinity reduced leaf area; this is compatible with reports by Chartzoulakis and Klapaki (2000). The decline in leaf growth is the earliest response of glycophytes exposed to salt stress. Reduction in the area available for transpiration and assimilate production could be a result of the osmotic effect of salts. Salt induced osmotic stress causes osmotic dehydration which leads rapidly to a decrease in the osmotic and water potential of cells and in cell volume (Levitt 1980).

Increase in the leaf area induced by 24-epibrassinolide was further translated into improved growth of the plants as reflected in the enhancement in fresh and dry weights of the shoot system. This is in accordance with literature reports that BRs influence cell division and consequently leaf size (Nakajima et al. 1996, Oh and Clouse 1998, Yu et al. 2004).

Foliar application of 24-epibrassinolide resulted in significant increases in growth parameters of plants grown at high NaCl but the values obtained in the highest 24-epibrassinolide treatment were all lower than for the control treatments. Similar results were observed in rice (Ozdemir et al. 2004) and wheat (Shahbaz and Ashraf 2007).

The imposition of salinity to the rooting medium had an inhibitory effect on the root growth of the pepper plants, but their growth rate is less affected by salts as compared to the shoots. Both promotive and inhibitory effects of brassinosteroids on root growth have been reported by Sathiyamoorthy and Nakamura (1990), Schumacher and Chory (2000), Mussig et al. (2003), and Ozdemir et al. (2004). However, application of 24-epibrassinolide did not ameliorate the adverse effects of salinity. This result obtained with 24-epibrassinolide was confirmed by Qayyum et al. (2007). According to Amzallag (2002) the effectiveness of 24-epibrassinolide on the growth of salt stressed plants depends on the type of plant species, growth stage, concentration of brassinosteroids, and application mode. In addition, Mussig et al. (2003) suggested that brassinosteroids had a positive role on the growth of roots if its concentration is greater than its threshold value and this concentration is genotype specific.

Relative water contents

RWC in leaves is considered as an alternative measure of plant water status, reflecting the metabolic activity in plant tissues (Flower and Ludlow 1986). Fig. 2 shows that salt stress significantly declined Leaf RWC compared to the control treatment. This decrease could be attributed to root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah 2000). The 0.5 mg 24-epibrassinolide treatment elevated RWC in salt-stressed plants to a level higher than the salt stressed plants but the other concentrations had little or no effect. Increased RWC by brassinosteroids application have been reported for Brassica juncea (Hayat et al. 2007) under cadmium stress and Vignia radiata (Ali et al. 2008) under aluminium stress.

Chlorophyll fluorescence

The reduction in growth observed for many plant species subjected to excess salinity is often associated with a reduction in photosynthetic capacity. The photosynthesis decreases could be due to stomatal or non-stomatal factors or both. On pepper plants, Bethke and Drew (1992) ascribed the observed reduction in photosynthesis to non-stomatal limitation. Measurements of chlorophyll fluorescence provide quantitative information about photosynthesis through non-invasive means and a variable to maximal chlorophyll fluorescence (Fv/Fm ratio) is an indicator of the efficiency of Photosystem II (Maxwell et al. 2000). The ratio for a normally functioning leaf varies between 0.75 and 0.85 and a decline in this ratio is indicative of photoinhibitory damage (DeEll et al. 1999).

In our study, no significant variation in the Fv/Fm ratio was registered, suggesting that the efficiency of the quantum yield of PSII was not affected by salinity. All Fv/Fm values indicated that leaves were photosynthetically active. The unaffected (stable) values of Fv/Fm mean that there is no loss in the yield of PSII photochemistry and confirms the resistance of the photosynthetic machinery to salt stress (Belkhodja et al. 1994, 1999). The 24-epibrassinolide concentrations have no effect on different variables of chlorophyll fluorescence. This results was in contrast to the results of Yu et al. (2004), who showed that a spray of 24-epibrassinolide at 0.1 mg L^{-1} on spinach plants led to an increase in the Fv/Fm ratio.

CONCLUSIONS

The study revealed that:

-The decline on plant growth was related mainly to the decrease in water availability, but not to injuries to the photosynthetic apparatus, since the PSII activity (estimated by the ratio Fv/Fm) of dark-adapted leaves was preserved under salt stress.

-The 24-epibrassinolide improved the tolerance of pepper plants to short-term salt stress by stimulating stem elongation, leaf area and shoot biomass production.

-The effect of 24-epibrassinolide on the growth of young pepper plant is related to concentration. The data presented in this study shows that 0.5 mg L^{-1} 24-epibrassinolide promotes growth, and the other concentrations cause no significant effect on pepper plants growth under salinity stress.

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24-epibrassinolit'le Biber (*Capsicum annuum*)'de Tuz Toleransinin Induklenmesi

Ozet

Biber bitkilerinde, tuzlulugun inhibitor etkilerini gidermede brassinosteroitlerin etkinligini arastirmak uzere, sera kosullarinda kisa donemli bir test gerceklestirildi. Tuzlu su ile sulanan biber bitkilerinde (*Capsicum annuum*) farkli 24-epibrassinolit konsantrasyonlarinin (0.0, 0.01, 0.05, 0.1, ve 0.5 mg L^{-1}) yapraklara uygulanmasinin buyume, nispi su icerigi ve klorofil floresansi uzerine etkileri incelendi. Tuz, cesitli buyume parametrelerini etkiler. Ancak, tuzun etkileri koklerden ziyade surgunlerde daha belirgindi. 24-epibrassinolit'in harici uygulanmasinin, surgun buyume parametreleri ve yaprak nispi su icerigi uzerindeki tuz stresinin inhibitor etkilerini gidermede etkili oldugu goruldu. Ancak, 24-epibrassinolit'in, kok buyumesi ve klorofil floresansi uzerinde onemli bir etkisi gozlenmedi. Bes konsantrasyon icerisinde, stres sartlari altinda 0.5 mg L^{-1}'nin en iyi sonucu verdigi goruldu.

Anahtar Kelimeler: 24-epibrassinolit, buyume, klorofil floresansi, nispi su icerigi, tuz stresi.

