Journal of Stress Physiology & Biochemistry, Vol. 8 No. 3 2012, pp. 224-233 ISSN 1997-0838

ORIGINAL ARTICLE

24-epibrassinolide enhances flower and fruit production of pepper (*Capsicum annuum* L.) under salt stress

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Received June 9 2012

Among various environmental stresses, salt stress is extensively damaging to crops production in many areas of the world. The enhancement of plant tolerance is important for plant productivity. Brassinosteroids plays an important role in the plant response to adverse environmental conditions such as salt stress. Therefore, an experiment was conducted to explore the role of exogenously applied 24-epibrassinolide on flower and fruit production of pepper in presence or absence of NaCl-stress (70 mM). The plants were sprayed with 10^{-6}M of 24-epibrassinolide at vegetative, buds formation and early fruiting. 24-epibrassinolide improves flower number, fruit number and yield per plant, but was without effect on fruit mass and size. The detailed measurement of these parameters indicates that the effects of 24-epibrassinolide depend on the development stage of pepper plant and the application frequency of this hormone.

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Key words: 24-epibrassinolide / pepper / NaCl / flower/ fruit/ yield

Hot pepper (*Capsicum annuum* L.), is an important vegetable as well as spice crop of Tunisia. The pepper production is estimated to be 304,000 thousand tons from an area of 19,3 thousand hectares with an average yield of 15.75 tons per hectare (GIL, 2010). Despite the growth in acreage and production, pepper yields in Tunisia does not yet achieved the values recorded in other Mediterranean countries like Greece (23t/ha), Italy (28t/ha) and Spain (35t /ha) (Boughalleb and Mahjoub, 2005). These low yields are due to many constraints that affect yields and fruit quality. These constraints are related to differences in the environment of the plant, including temperature and salinity. Indeed, the production area is mainly located in the center region of Tunisia, where water resources are insufficient and threatened by salinization due to the arid climate and the overexploitation of groundwater (Hachicha and Braudeau, 1998).

Several solutions were advanced to attenuate the risks incurred by the agronomic production such as developing salt-resistant cultivars, leaching excess soluble salts from upper to lower soil layers, flushing soils that contain salt crusts at the surface, reducing salt by harvesting salt-accumulating aerial plant parts in areas with negligible irrigation water or rainfall for leaching, and ameliorating saline soils under cultivation (Qadir *et al.,* 2000). However, such solutions are expensive and difficult to implement. In recent years, a new approach has been developed to alleviate salt stress in plants, by using different types of phytohormones (Kaya *et al.,* 2009, Hamdia and Shaddad 2010, Javid *et al.,* 2011, Bayat *et al.,* 2012).

Brassinosteroids (BRs) are steroidal sixth group of phytohormones that are ubiquitously distributed in the plant kingdom (Clouse and Sasse 1998). They are implicated in a wide range of physiological and molecular responses in plants, such as stem elongation, pollen tube growth, leaf bending and epinasty, photosynthesis, ethylene biosynthesis, proton pump activation, vascular differentiation, gene expression, nucleic acid, and protein synthesis (Sasse 2003). BRs have been successfully demonstrated to improve seed germination, vegetative growth and elongation of shoot and root (Clouse and Sasse 1998). The beneficial effects of BRs have also been demonstrated for many crops such as vegetables, pulses, cereals, fruits and oil seeds (Hayat *et al.,* 2000; Hayat and Ahmed 2003; Janeczko *et al.,* 2010; Hayat *et al.,* 2012). In addition, BRs improves crop quality (Vardhini and Rao 2002; Ali *et al.,* 2006; Janeczko *et al.,* 2010).

For the last two decades, BRs has received much attention because of its involvement in the regulation of plant responses to biotic and abiotic stresses. These compounds have wide-range of biological activities that increase the crop yields by changing plant metabolism and protecting plants from environmental stresses (Krishna, 2003). BR regulated stress response because of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of protein synthesis, and the production of various chemical defence compounds (Bajguz and Hayat, 2009). The protective effect of BRs against abiotic stress factors such as toxic metals, heat stress, low temperature, and oxidative damage (Bajguz and Hayat, 2009) has been demonstrated. Furthermore, the role of BRs in inducing salt tolerance has been investigated in detail in many plant species (Rao *et al.,* 2002; Özdemir *et al.,* 2004; Hayat *et al.,* 2010; Hayat *et al.,* 2012; Ibn Maaouia-Houimli *et al.,* 2012). The aim of this work was to investigate the potential of BRs to ameliorate the productivity of pepper plants subjected to saline stress.

MATERIALS AND METHODS

The study was conducted in Chott Meriam (Tunisia), under glasshouse conditions. Pepper plants (*Capsicum annuum* L. cv. Beldi) were maintained under natural light conditions, approximate day/night temperatures of 30/20ºC and 70% relative humidity during the span of the experiment. Uniforms pepper seedlings were transplanted at a rate of one plant per 25cm plastic pot containing peat (pH= 5.8-6.8, EC 0.40 $dS.m^{-1}$, dry matter 35% and organic matter 25%). Fifteen day after planting (DAP) they are subjected to salt stress by addition of 70mM of sodium chloride to irrigation water. Watering plants is carried out 3 times a week. Plants were irrigated weekly with nutrient solution. The basal nutrient solution contained KNO_3 (16.83 $mg.l^{-1}$), Ca $(NO_3)_2.4H_2O$ (59 $mg.l^{-1}$), $NH_4H_2PO_4$ (115$mg.l^{-1}$), $MgSO_4.7H_2O$ (123 mg.l^{-1}), FeEDTA (5 mg.l^{-1}), $MnCl_2.4H_2O$ (1.8 mg.l^{-1}), H_3BO_3 (0.38 mg.l^{-1}), ZnSO4.7H2O (0.22 mg.l^{-1}), $CuSO_4.5H_2O$ (0.08 mg.l^{-1}), (NH4)6Mo7O_2.4H_2O (0.02 mg.l^{-1}).

The experiments were carried out in randomized block design with five replications. From each treatment, 10 plants were selected and observations were recorded. The plants were sprayed with 10^{-6}M of 24-epibrassinolide (EBL) at vegetative, buds formation and early fruiting (15, 45 and 75 days after planting DAP) following the schedule of treatments as mentioned below.

- C0 = Control + distilled water
- C1 = Control + EBL (at 15 DAP)
- C2 = Control + EBL (at 45 DAP)
- C3 =Control + EBL (at 15 and 45 DAP)
- C4 = Control + EBL (at 45 and 75 DAP)
- C5 = Control + EBL (at 15, 45 and 75 DAP)
- S0 = NaCl + distilled water
- S1 = NaCl + EBL (at 15 DAP)
- S2 = NaCl + EBL (at 45 DAP)
- S3 = NaCl + EBL (at 15 and 45 DAP)
- S4 = NaCl + EBL (at 45 and 75 DAP)
- S5 = NaCl + EBL (at 15, 45 and 75 DAP).

To measure generative development of the plants, the time span between transplantation and the formation of the first open flowers and first fruits harvested were recorded.

Total fruits yield and its components (early yield, total fruit number, diameter and length of fruits, fresh and dry matter of fruits). Number of seeds/fruit was counted and the total soluble solids (TSS), it was determined by abbey Refrectometer.

Statistical analyzes

Data were subjected to analysis of variance (ANOVA) to compare the effects of salt stress and EBL treatments. The differences between the means were compared using Duncan's test (at 5%).

RESULTS

First flower appearing

The first flower appeared 53 days after transplantation on control plants (C0) (Table 1). It appeared that NaCl delayed the formation of the first flower as it appeared only 62 days after transplantation on the plants grown under saline conditions (S0). a slight inhibitory effect provided by the application of EBL at vegetative stage for C1, C3, C5, S1, S3 and S5 plants compared to C0 and S0 plants. This effect is reflected particularly by a few days delay in the onset of the first flower. Spraying plants with EBL, at generative stage (buds formation), all stimulated flower formation, as the first flowers appeared at day 49, for C2 and C4 plants and at day 59, for S2 and S4 plants (Table 1).

First fruit ripening

Table 1shows that the effect of different treatments on the number of days required for the first fruit ripening per plant has the same trend as that of flowering. Control plants (C0) took 88 days before the first fruits were harvested. In contrast, it took 98 days before any fruits could be harvested from NaCl-stressed plants (S0). The application of EBL at vegetative stage, delayed fruit maturation by seven days for C1, C3 and C5 plants to nine days for S1, S2 and S3 plants compared to controls (C0) and stressed plants (S0) respectively. However, applying EBL at generative stage shortened the period of fruit maturation from 88 days (C0) to 84 days (C2 and T4), and from 98 days (S0) to 93 days (S2 and S4).

Number of flowers produced

The final average number of flowers per plant was increased in stressed and unstressed plants treated with EBL. In the absence of NaCl-stress, the application of 24-epibrassinolide seems to stimulate flowering (Table 1). The number of flowers produced is greater for C4 and C5 plants have a respective increase of 23% and 21% relative to controls (C0). Similarly, in the presence of NaCl-stress, stimulation of flowering is more important for S4 and S5 plants, it is 12% and 16% compared to S0 plants. The application of EBL at vegetative stage tends to delay the onset of the first flower. Subsequently, EBL- treated plants (C3, C5, S3 and S5) catch up and exceed C0 and S0 plants.

Total fruit number

In absence of NaCl-stress, the average number of fruit harvested from the plants that have received two (C4) or three (C5) applications of EBL are the best. Similar results are observed for the NaCl-stressed plants that have pulverized two (S4) or three (S5) times by EBL (Table 1). We also note that a single application of EBL at vegetative stage leads to a significant loss in fruits number by almost 20% for C1 plants compared to C0plants and 30% for S1plants compared to S0 plants.

Yield

In the absence of NaCl-stress, we obtained the best yields for plants that received the hormone and are on average, 446.4, 477.6 and 538.7 g / plant respectively for C3, C4 and C5 plants. The yield increases recorded in all three treatments were 21.9%, 36.5% and 54% relative to C0 plants. The difference was not significant between C3 and C4 and between C4 and C5. In contrast, it is highly significant between C3 and C5 and is manifested by an increase in yield of 112.2 g / plant.

In the presence of NaCl-stress, the results reveal an increase in yield based on the number of application of the hormone. This increase ranges from 18% for S2 plants to 49% for S5 plants. The yields averaged 284.2, 315.9 and 334.8 g / plant respectively for treatments S3, S4 and S5 (Table 1). However, the yields of plants that received only one spray by EBL at vegetative stage C1 and S1 are significantly lower by 14.6% and 11% compared with those given by untreated plants C0 and S0.

Fruit size

The results presented in Table 2 show that EBL-treatments have no significant effect on fruit length. We distinct two groups; the first consists of C4, C0, C1, C2, C3 and C5 plants, listed in descending order and have the most important lengths of fruits ranging from 13.3cm for C4 plants to 12.2cm for C5 plants. The second contains S1, S0, S2, S4 and S5 plants, cited in ascending order and has the lowest lengths of fruit that are between 8.8cm for S1plants to 9.8cm for S3 plants.

Statistical analysis revealed no significant differences between treatments for fruit diameter; it varied between 2.2 cm for C3 plants and 1.8 cm for S5 plants.

Fruit fresh and dry weights

The effects of EBL-treatments on the average fruit fresh weight are shown in Table 2. We distinct two groups; the first consists of C0, C1, C2, C3, C4 and C5 plants have fresh fruit weight between 15.7g for C0 plants and 14.5 g for C5 plants, form the best group that differs significantly from the group of S0, S1, S2, S3, S4 and S5 plants, whose fruit fresh weight range from 11.6 g for S2 plants to 10.8 g for S3 plants. It seems that only salinity affected the fresh biomass of the fruit.

Statistical analysis revealed no significant differences between treatments for fruit dry weight (Table 2). However, EBL produced an increase in fruit dry matter passing of 2.19 g for C0 plants to 2.45 g for C5 plants and 2.09g for S0 plants to 2.34g for S5 plants.

Seeds number

The analysis of Table 2, shows that in the absence of hormone treatment, salinity negatively affects seed formation. Indeed, the reduction rate of S0 plants is 22.4% compared to C0 plants. One spray of EBL, whether done with or without treatment with NaCl, does not induce any significant change in the number of seeds per fruit among C1, C2, S1 and S2 plants. However, two or three applications result in significant decreases in seeds number by 22, 26.2 and 31.3% for C3, C4 and C5 plants compared to C0 plants, and 12.3, 21 and 23% for S3, S4 and S5 plants compared to S0 plants.

Total dissolved soluble solids (TSS)

The % Brix index gives an indication on total dissolved soluble solids (TSS) within the fruit. The results of the fruit Brix index are illustrated in Table 2. We notice two distinct groups; C3, C4, C5, S3, S4 and S5 plants form the best group that differs significantly from the group of C0, C1, C2, S0, S1 and S2 plants.

Table 1. Effect of 24-epibrassinolide spray on days to first flower appearing, first fruit harveasting, flowers number, fruits number and yield of pepper plants.

Treatments	Days to first flower	Days to first fruit	Flowers number	Fruits number	Yield (g/plant)
C0	52,7 bc	87,8 c	40,4 ab	19 b	349,88 c
C1	57,3 b	94,2 b	37,4 b	15 bc	298,64 cd
C2	49,4 c	84,2 c	44,0 a	21 b	370,58 c
C3	56.0 b	95.1 b	41,8 ab	24 b	426,47 b
C4	49,7 c	84,3 c	47,7 a	30 a	477,58 b
C5	58,2 b	93,8 b	48,7 a	34 a	538,71 a
S0	62,1 ab	98,4 b	28,4 c	10 c	225,53 d
S1	67,8 a	106,5 a	25,4 c	7 c	201,13 d
S2	58,6 b	92,9 b	32,0 bc	13 c	266,22 cd
S3	68,7 a	107,8 a	32,2 bc	16 bc	284,21 cd
S4	59,4 b	93,5 b	35,7 b	19 b	315,94 c
S5	69,6 a	106,9 a	36,7 b	24 b	334,85 c

Means were separated by Duncan's test, different letters in a single column show statistically significant differences for $P<0.05$.

Table 2. Effect of 24-epibrassinolide spray on length, diameter, fresh mass, dry mass, seeds number and TSS of fruit pepper.

Treatments	Length (cm)	Diameter (cm)	Fresh mass (g)	Dry mass (g)	Seeds number	TSS (% Brix)
C0	12,08 a	2,22 a	15,35 a	2,19 a	42,8 a	4,84 b
C1	12,11 a	2,14 a	15,16 a	2,23 a	41,4 a	4,92 b
C2	12,40 a	2,16 a	15,18 a	2,21 a	29,4 b	4,94 b
C3	12,21 a	2,21 a	15,42 a	2,25 ab	33,4 b	5,40 a
C4	12,33 a	2,12 a	15,26 a	2,36 ab	27,6 c	5,56 a
C5	12,02 a	2,18 a	15,34 a	2,45 ab	24,4 c	5,84 a
S0	8,98 b	1,86 a	11,53 b	2,09 a	33,2 b	4,90 b
S1	8,78 b	1,87 a	11,44 b	2,12 a	34,0 b	4,92 b
S2	9,08 b	1,88 a	11,61 b	2,14 a	30,8 b	4,94 b
S3	9,14 b	1,87 a	11,15 b	2,19 a	32,8 b	5,24 a
S4	9,34 b	1,83 a	11,31 b	2,28 ab	26,2 c	5,66 a
S5	9,38 b	1,82 a	11,35 b	2,34 ab	25,6 c	5,90 a

Means were separated by Duncan's test, different letters in a single column show statistically significant differences for $P<0.05$.

DISCUSSION

This study show that 24-epibrassinolide treatments hastened flowering and harvesting time, when it pulverized at generative stage. Gabr *et al.,* (2011) revealed that BR has positive influences on advancing harvest dates of 'Canino' apricot fruits. Applied at vegetative stage, the effect of 24-epibrassinolide on flowering was less dramatic on the number of flowers produced in plants treated at generative stage.

The application of BRs at flowering generally leads to a considerable increase in the production of several crops, as has been shown in the fruits of pepper in this study. Sugiyama and Kuraishi (1989) reported that applications of BR, in navel orange, increased fruit set, as in japanese persimmon, grapevine and citrus (Watanabe *et al.,* 1997) which resulted in increased fruit number per tree and in turn yield per hectare in passion fruit (Gomes *et al.,* 2006). Ramraj *et al.,* (1997) detected an increase in the yield of wheat, rice, groundnut, mustard, potato and cotton grown in the field after application of BRs. The spray of BR solution on litchi leaves before flowering reduced the rate of bursting fruit and increased its commercial value (Peng *et al.,* 2004). The applications of BRs under salt stress have been known to increase growth and yield in many economically useful plant species. In cereals, BRs enhance the total grain yield and 100 grain weight of salt stressed plants (Ali *et al.,* 2008). In leguminous crops, the number of pods per plant and total seed yield increases after the application of exogenous BRs (Rao *et al.,* 2002).

Increasing the number of fruits per plant, in presence or absence of NaCl-stress, may be associated with improvement in photosynthetic rate of EBL-treated plants. (In a previous paper we showed that 24-epibrassinolide ameliorated the net assimilation in pepper plant (Ibn Maaouia-Houimli *et al.,* 2012). Similarly, in mustard, the application of BR led to an increase in photosynthesis is directly related to growth and seed production (Hayat et al, 2000). The application of 24-epibrassinolide in cucumber stimulated photosynthetic activity expressed by the acceleration in CO_2 fixation, increasing the biosynthesis of proteins and reducing sugar content (Yu *et al.,* 2004).

Kalinich et al (1985), Vardhini and Rao (1998) and Hayat *et al.,* (2000) reported that growth induced by BRs may be related to the increase in RNA and DNA and to improvement in the assimilation efficiency of photosynthetic carbon assimilation and protein biosynthesis.

In the present work, the 24-epibrassinolide has no effect on the length, size and mass of fresh and dry fruit. Thus, indicated that exogenous application of BRs to plant does not change its morphology (Khripach *et al.,* 1999; Gomes *et al.,* 2006). In contrast, this hormone reduces the number of seeds per fruit suggesting that the fruits of EBL-treated plants have a tendency to parthenocarpy. Fu et al., (2008) noted that 24-epibrassinolide induced parthenocarpic growth in cucumber accompanied by active cell division. It was suggested that the promoting effect on cucumber fruit growth is linked to the induction of cell cycle-related genes. Interestingly, application of 24-epibrassinolide to unpollinated cucumber flowers produced seedless fruits similar to those of the pollinated flowers. In horticulture, parthenocarpy can be exploited for increasing winter and early production (Pandolfini 2009); this means the possibility for the consumers to find fresh horticultural products in all seasons (Pandolfini 2009).

Total soluble solid concentration (TSS, measured as % Brix) of fruits is an important variable which is used to determine fruit quality because TSS is most commonly associated with sugar and organic acid concentrations (Young *et al.,* 1993). The % Brix index includes all soluble solids like sucrose, fructose, glucose, vitamins, amino acids, protein, organic acids, minerals and hormones. Each % of Brix is equal to 1 gram of soluble solids in 100 g of fresh mass. A high Brix % normally indicates high sugar content (Baxter *et al.,* 2005). Our data show that TSS of pepper fruits increase as the number of application of EBL increases. Similar result was found by Gomes *et al.,* (2006) in passion fruits. It can thus be postulated that EBL ameliorate the fruit quality, because TSS is most commonly associated with sugar and organic acid concentrations (Young *et al.,* 1993).

This study highlights the interest of 24-epibrassinolide application in agriculture under salt stress conditions. The effects of this hormone vary with the stage and frequency of application.

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