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Department of Horticulture, Faculty of Agriculture, Shiraz University, Shiraz, Iran

Effect of 24-epibrassinolide on salinity-induced changes in loquat (*Eriobotrya japonica* Lindl)

Fatemeh Sadeghi, Akhtar Shekafandeh*

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Summery

This study was carried out to investigate the role of 24-epibrassinolide (24-EBL) in inducing loquat plant salt tolerance. Plants were treated with five levels of salt, so that the electrical conductivity (EC) of 0.5 (control), 2, 4, 6 and 8 dS m^{-1} was established in pots. Then, the plants were sprayed with different concentrations of 24-EBL (0, 0.25, 0.5 and 0.75 mg l^{-1}). Under salt stress, the plant growth parameters and chlorophyll content decreased, while the total soluble sugars and proline contents considerably increased. However, the application of 24-EBL significantly ameliorated the plant growth by reducing the adverse effects of salinity on the examined parameters. The ion concentrations showed an increase in accumulation of Na^+ and Cl^- coupled with a decrease in K^+ with increasing salinity in medium. Exogenous application of 24-EBL had a significant effect on leaf Na^+, K^+, Cl^- contents. Out of different 24-EBL concentrations, the effects of 0.5 mg l^{-1} proved the best under stress conditions.

Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical evergreen fruit tree of Rosaceae family, (Lin et al., 2007; Zheng, 2007) which is grown commercially in subtropical to mild temperate climate. Loquat is commonly propagated by seed; as a result, the plants possess variable performance and different fruit characteristics owing to heterozygosis and cross-pollination. Therefore, in some parts of the world, the seedlings employ as a rootstock for cultivars with high fruit quality (Lin, 2007).

Salinity is one of the major abiotic stresses that affects plant production and growth in many arid and semi-arid areas throughout the world (Chelli-Chaabouni et al., 2010). Iran is now faced with salinity problems in about 34 % of its area in addition to harsh conditions of climate in about half of the country. This also signals that global climate change and consecutive years of drought is likely to increase salinity of the main agricultural lands (Rahimian et al. 2013). The deleterious effects of salinity on plant growth are associated with low osmotic potential (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these factors. All these factors cause adverse effects on plant growth and development at physiological and biochemical and molecular level (Ashraf and Harris, 2004).

Among the most common effects of salinity is growth inhibition by NaCl. In many plants, compatible osmoprotectant metabolites, such as proline, glycine-betaine, and soluble sugars are produced to protect the cells against the damaging effects from salt stress (Chelli-Chaabouni et al., 2010).

Although, soil salinity directly affects root system, hence breeding and selection of salt tolerant rootstock for sustainable fruit production is inevitable, but this is a time consuming. For reducing adverse effects of salinity different strategies have also been developed. One of these approaches is; employing different types of phytohormones (Houimli et al., 2008). Among them, Brassinosteroids, a recent class of plant hormone not only play prominent role in various physiological and biochemical processes in plants, like stem elongation, vascular differentiation, leaf bending and epinasty, induction of ethylene biosynthesis, regulation of gene expression, nucleic acid and protein synthesis and photosynthesis (Hayat et al., 2010; Krishna, 2003; Yu et al., 2004; Cao et al., 2005), but has also attracted increasing attention in studies addressing to adaptive response to environmental stresses, such as heavy metal (Ali et al., 2008; Hayat et al., 2007), salt (Ali et al., 2007), temperature (Wilen et al., 1995), drought (Zhang et al., 2008).

Loquat is considered as having a moderate tolerance to drought but there are some conflicting evidences on the loquat salt tolerance. Some sources have mentioned its moderate tolerance to NaCl (Gilman and Watson 1993) and others its salt-sensitive (http://aciar.gov.au/files/node/2275/mn050_part_5_pdf_19541.pdf). Knowing about salt tolerance limit of loquat helps us in extending its cultivation in area with certain levels of salinity which are not suitable for citrus. So, the present investigation was conducted to discover the salt tolerance of seedlings, effect of 24-EBL on its salt tolerance, and to determine the interactive effects of salinity and 24-EBL on plants morphophysiological characteristics.

Materials and methods

Plant material and treatments

The loquat uniform seeds were extracted from mature fruits and immediately washed with tap water and placed at 4 °C for 2 weeks. Germinated seedlings were allowed to grow in perforated polyethylene pots contained a mixture of peat-moss, sand and clay (1:1:1, v/v/v). Then, when the height of the seedlings in pots was about 30 to 40 cm, the same vigour seedlings were transferred to 7 litre plastic pots filled with 6 kg soil mixture as mentioned above. The field capacity of the soil used for potting was determined according to the protocol described by Richards (1949). Potted seedlings were irrigated for 8 months to field capacity level. In order to achieve optimum seedling vegetative growth, Fasemko complete fertilizer (pH 6.7) was applied to each pot with irrigation water each fortnight. The seedlings were grown in the greenhouse at day/night temperature: 30/25±4 °C and relative humidity of 40-45 % under natural sun light. After this period, when the seedlings were well established, a factorial experiment was conducted in completely randomized design with 4 replications. Treatments were 5 levels of salt (NaCl) × 4 levels of 24-epibrassinolide (24-EBL). The salts were applied to pots by irrigation water step-wise until an electrical conductivities (EC) of 0.5(control), 2, 4, 6 and 8 dS m^{-1} were attained. After that, plant were treated exogenously with 0.0, 0.25, 0.5 and 0.75 mg l^{-1} of 24-epibrassinolide (24-EBL) at run-off. This action was repeated 3 times with one week interval, and then six weeks after spraying, data were recorded.

Growth measurements

At the end of experiment, plants were harvested and divided into shoots and roots, after measuring of shoot and root fresh weight

* Corresponding author

(FW), they were dried in the oven at 75 °C for three days to determine their dry weights (DW). Leaf area was measured by Delta-Tdevices (England).

Chlorophyll content

Leaf chlorophyll content was measured with a chlorophyll-meter (SPAD-502, Minolta Co. Japan) using 3 full expanded leaves. Subsequently, 14 leaves with their chlorophyll content already measured by SPAD-502 were randomly detached from the plants to extract chlorophyll using the chemical method. Extraction was performed with 80 % ethanol and the yielded solution was centrifuged at 8000 rpm for 10 min. Chlorophyll content was colorimetrically determined using the following formula (A.O.A.C 1975):

Chlorophyll (mg g^{-1} fresh weight) = [20.2 (A 645) + 8.02 (A 663) x V] / (1000 W)

Where A is absorption value, V is ultimate volume of extract and W is leaf fresh weight. A regression line and equation were obtained using Excel software, considering the SPAD-502 readings for the mentioned 14 leaves and colorimetrically determined chlorophyll contents. This equation was later used to estimate other chlorophyll readings from SPAD-502.

Total Soluble Sugar

To measuring soluble sugars, 150 mg of dried leaf samples was extracted two times with ethanol. Sample were centrifuged at 3500 rpm for 10 min, the volume of upper phase was reached to 25 ml. Soluble sugars was measured according to method of DOBIOS et al. (1956), the absorption was recorded using spectrophotometer at 490 nm (Model UV-120-20.Japan).

Estimation of free proline content

Proline content was measured using the method of BATES et al. (1973). Free proline was extracted from 0.5 g of leaf samples with 5ml 3 % sulpho salicylic acid and centrifuged at 5000 rpm for 10 min. Supernatant was treated with acid-ninhydrin and acetic acid,

Tab. 1: Effect of 24-epibrassinolide on loquat plant growth parameters under salt stress conditions

24-EBL mg l^{-1}	NaCl EC dS m^{-1}	Shoot FW (g)	Mean	Shoot DW (g)	Mean	Root FW (g)	Mean	Root DW (g)	Mean
0	Cont(0.5)	297.4abc		105.1cd		80.2abc		30.8ab	
	2	298.7abc		129.26b		83.3ab		31.6ab	
	4	242.2f		92.2def		73.3bc		22.4de	
	6	161.8ghi		72.6ghi		58.1def		15.3fg	
	8	125.7j	224.1B	58.1i	91.5C	45fg	68B	13.1g	22.6B
0.25	Cont(0.5)	298abc		128.5b		83.6ab		31.2ab	
	2	290bcd		118.7bc		84.8ab		30.8ab	
	4	276.6cde		121bc		81.1abc		25cd	
	6	166.9gh		82.4efg		56.5efg		16.2fg	
	8	141.5ij	234.8AB	68.6ghi	103.8B	46.3g	70.5AB	15.3fg	23.7AB
0.5	Cont(0.5)	319.3a		148.2a		89.6a		32.2ab	
	2	304.9ab		155.2a		88.3a		35.1a	
	4	272.2de		104.1cd		89.6a		22.2de	
	6	163.5ghi		81.9efg		58.3def		20.5def	
	8	144.7hij	240.9A	71.2ghi	112.1A	51.2efg	75.4A	16.3fg	25.3A
0.75	Cont(0.5)	298.7abc		115.2bc		81.2abc		32.3ab	
	2	299.8abc		116.8bc		84.3ab		32.3ab	
	4	254.3ef		96.5de		70.3cd		20.5def	
	6	172.3g		76.7fgh		60.2de		17.9efg	
	8	129.4j	230.9AB	63.7hi	93.8C	55.1efg	70.2AB	17.3efg	23.3AB
Analysis of variance (F values)									
Salinity		333.03***		78.7***		50.73***		65.27***	
24-EBL		3.04*		11.3***		2.39ns		1.73ns	
Salinity × 24-EBL		1.0 ns		2.62**		0.88ns		0.80ns	

In each column, means with the same letters are not significantly different (P<0.05)
*, **, * * *; significant at 0.05, 0.01, and 0.001 levels, respectively. ns; non-significant

boiled for 1 h at 100 °C. The reaction was then terminated in an ice bath. Reaction mixture was extracted with 2 ml toluene. Absorbance of chromophore containing toluene was determined at 520 nm.

Mineral nutrient analysis

The mineral composition of each plant organ (roots and leaves) was determined at the end of the experiment. Leaves and roots were harvested and analysed for sodium, chloride and potassium. Roots were intensively washed with de-ionized water. One gram of leaf samples was ashed in a muffle a furnace at 550 °C for 5 h. Then the ash was dissolved in 10 ml 2 N HCl and reached volume to 100 ml with distilled water. Potassium and sodium were determined using flame photometer (Model PFP7, Jenway, England). The chloride content of leaves and root were determined following the method of Chapman and Pratt (1961).

Data analysis

Statistical analyses were performed by PROC GLM of SAS (SAS.9.1) and means were compared by LSD test at $P \leq 0.05$.

Result

Shoot fresh and dry weight

The results showed that salt stress caused a significant reduction in shoot fresh and dry weight. The main effect of exogenous application (foliar spray) of 24-EBL showed an increase in shoot fresh and dry weight and the highest values (240.9 and 112.1 g for fresh and dry weight respectively) were recorded in plants sprayed with 0.5 mg l^{-1} 24-EBL (Tab. 1). In the absence of 24-EBL, low concentration of salt (2 dS m^{-1}) increased significantly shoot dry weight in comparison with control.

There was a significant interaction between salinity and 24-EBL on shoot dry weight and the highest amount (155.2 g) was achieved in EC 2 dS m^{-1} when the plants were sprayed with 0.5 mg l^{-1} 24-EBL which was significantly higher than control and other salt and 24-EBL combinations. In EC 4 dS m^{-1} when plants were treated with 0.25 mg l^{-1} 24-EBL, shoot dry weight increased significantly by 28.8 % compared with non-treated plants in the same salt condition (Tab. 1).

Root fresh and dry weight

Root fresh and dry weight decreased significantly due to imposition of salt stress. Electro conductivity (EC) of 2 dS m^{-1} had not destructive effect on development (Tab. 1). The highest root fresh weight (89.6 g) achieved when plants were treated with 0.5 mg l^{-1} 24-EBL in EC of 4 dS m^{-1} which was significantly higher than the root fresh weight in the same condition without 24-EBL.

Leaf area

Salinity and 24-EBL, and their interaction significantly influenced average leaf area in loquat seedlings. Leaf area per plant decreased significantly in salt stress conditions (Fig. 1). In control (EC 0.5 dS m^{-1}), plant treated with 0.25-0.5 mg l^{-1} of 24-EBL led to maximum enhancement in leaf area (about 140.3 cm^2). In EC of 2 dS m^{-1}, 0.25 mg l^{-1} 24-EBL increased nearly 38 % in average leaf area per plant. In EC of 4 dS m^{-1} or higher, application of 24-EBL was not able to decreased harmful effect of salt.

Chlorophyll

Chlorophyll was also significantly influenced by salinity and 24-EBL application. With increasing salt concentrations in the medium, leaf chlorophyll content decreased. There was a high interaction between salinity and 24-EBL on leaf chlorophyll content (Fig. 2). In EC of 2 and 4 dS m^{-1}, the highest leaf chlorophyll content was observed when plants were sprayed with 0.5 mg l^{-1} 24-EBL which was the same as control.

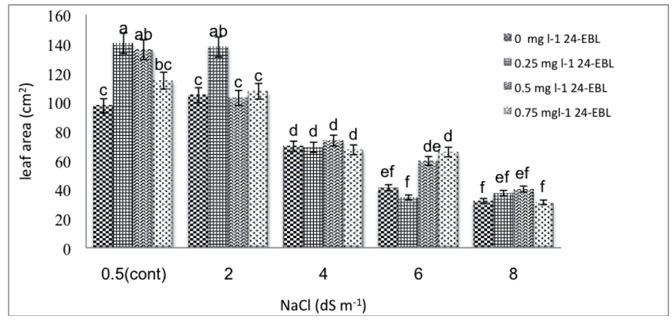

Fig. 1: Effect of 24-EBL on loquat leaf area under salt stress. Values are mean ± SE of 12 leaves. Different letters indicate significant differences between the treatments at 0.05 % level.

Total soluble sugars (TSS)

The results showed that with increasing salt concentrations, leaf total soluble sugar as an osmoprotectant increased (Fig. 3). In 6 dS m^{-1} NaCl, plants treated with 0.5-0.75 mg l^{-1} of 24-EBL accumulated significantly higher TSS than other combinations of salt and 24-EBL treatments.

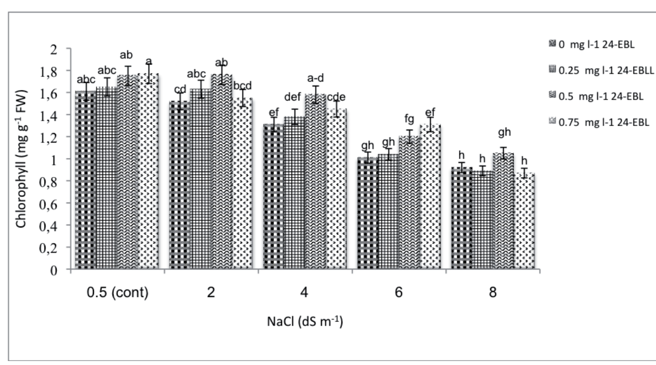

Fig. 2: Effects of 24-EBL on loquat leaf chlorophyll under salt stress. Values are mean ± SE of 12 leaves. Different letters indicate significant differences between the treatments at 0.05 % level.

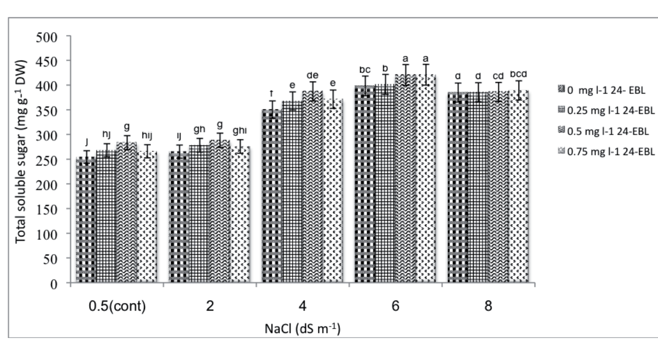

Fig. 3: Changes in total soluble sugars of loquat leaves under salt stress condition sprayed with different concentrations of 24-EBL. Values are mean ± SE of 6 plants. Different letters indicate significant differences between the treatments at 0.05 % level.

Proline

In the absence of 24-EBL, with increasing salinity in pots, leaf proline content increased. Application of 24-EBL enhanced the proline accumulation. For example, in EC 4 dS m^{-1}, 0.5-0.75 mg l^{-1} of 24-EBL increased significantly leaf proline content in comparison to non-treated plants in the same salt concentration (Fig. 4).

Mineral nutrients

Both sodium and chloride contents in the different plant organs (leaves and roots) increased with increasing salt concentration in the medium. The leaf sodium content was higher than that of root (Tab. 2, 3). Exogenous application of 24-EBL significantly changed the leaf Na^+ and Cl^- contents under saline conditions (Tab. 2). For example, in salinity of 6 dS m^{-1}, when plants were treated with 0.5 mg l^{-1} 24-EBL leaf Na^+ and Cl^- contents decreased by 8.7 and 35.5 % respectively with comparison to non-treated plant under the same salt concentration.

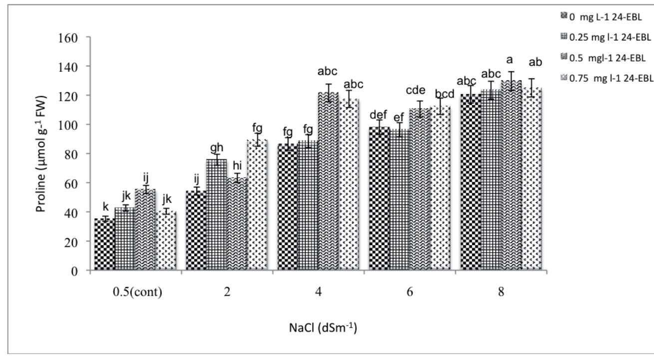

Fig. 4: Changes in proline of loquat leaves under salt stress condition sprayed with different concentrations of 24-EBL. Values are mean ± SE of 6 samples. Different letters indicate significant differences between the treatments at 0.05 % level.

Tab. 2: Effect of 24-epibrassinolide on leaf Na^+, K^+ and Cl^- ions of loquat plants under NaCl stress.

24-EBR (mg l^{-1})	NaCl EC dS m^{-1}	Leaf Na^+ (mg g^{-1} DW)	Mean	Leaf K^+ (mg g^{-1} DW)	Mean	Leaf Cl^- (mg g^{-1} DW)	Mean
0	Cont(0.5)	1.3g		13.7bc		1.3gh	
	2	1.5g		13.2cde		1.6f	
	4	8.5f		12.5fg		2.4de	
	6	14.9c		11.7hi		3.1c	
	8	16.3ab	8.5A	11.2i	12.5C	3.5ab	2.4A
0.25	Cont(0.5)	1.4g		13.6bc		1.2h	
	2	1.3g		13.4bcd		1.6fg	
	4	8.4f		12.5fg		2.3e	
	6	14de		13.7bc		2.2e	
	8	16.1b	8.2BC	11.7hi	13B	3.7a	2.2B
0.5	Cont(0.5)	1.3g		14ab		1.1h	
	2	1.1g		13.5bc		1.1h	
	4	8.1f		12.9def		1.5f	
	6	13.6e		13.3cde		2d	
	8	15.9b	8C	11.7hi	13.1AB	3c	1.9C
0.75	Cont(0.5)	1.2g		14.4a		1.1h	
	2	1.3g		13.3cde		1.1h	
	4	8.4f		12.7efg		1.6fg	
	6	14.2d		13.8abc		3.1c	
	8	14.2d	8.3AB	12.2gh	13.3A	3.3bc	2C
Analysis of variance (F value)							
Salinity		5093.7***		55.82***		226.3***	
24-EBL		6.38***		11.99***		13.46***	
Salinity × 24-EBL		1.81*		3.37***		4.85***	

In each column, means with the same letters are not significantly different ($P<0.05$)
*, **, * * *; significant at 0.05, 0.01, and 0.001 levels, respectively.

Tab. 3: Effect of 24-epibrassinolide on root Na^+, K^+ and Cl^- ions of loquat plants under NaCl stress.

24-EBR mg l^{-1}	NaCl EC dS m^{-1}	Root Na+ (mg g^{-1} DW)	Mean	Root K+ (mg g^{-1} DW)	Mean	Root Cl– (mg g^{-1} DW)	Mean
0	Cont(0.5)	0.9h		7.8b		3.7f	
	2	1.7g		7.8b		4.7e	
	4	2.3cde		6.6cde		7d	
	6	2.6b		5.8fg		8.7c	
	8	2.9a	2.1A	5.7g	6.7B	10a	6.8A
0.25	Cont(0.5)	0.9h		8.1ab		3.5f	
	2	1.6g		7.9b		4.6e	
	4	2.3cd		6.6cde		7.1d	
	6	1.9f		6.1fg		8.7c	
	8	2.3cd	1.7C	6fg	6.9AB	10a	6.7AB
0.5	Cont(0.5)	0.9h		8.2ab		3.5f	
	2	1.7g		8.2ab		4.5e	
	4	2ef		6.9cd		6.8d	
	6	2.2def		5.7fg		8.4c	
	8	2.5bc	1.8BC	6.24ef	7.1A	9.5ab	6.5B
0.75	Cont(0.5)	0.9h		7.7b		3.7f	
	2	1.5g		8.5a		4.2e	
	4	2.23cde		7c		7d	
	6	2.46bcd		5.9fg		8.8c	
	8	2.7ab	1.9AB	6.3def	7.1A	9.3b	6.6AB
Analysis of variance (F value)							
Salinity		189.08***		106.84***		934.01***	
24-EBL		10.31***		3.11*		2.70 ns	
Salinity × 24-EBL		2.44*		0.96ns		1.00 ns	

In each column, means with the same letters are not significantly different ($P<0.05$)
*, **, * * *; significant at 0.05, 0.01, and 0.001 levels, respectively. ns; non-significant

Although significant reduction in leaf and root K^+ occurred due to the addition of NaCl in the growth medium (Tab. 2, 3), exogenous application of 24-EBL altered the root and leaf K^+ of the salt stressed and non-stressed plants. The highest leaf K^+ (14.4 mg g^{-1} DW) and root K^+ (8.5 mg g^{-1} DW) contents were observed in control and EC 2 dS m^{-1} respectively, when plants were treated with 0.75 mg l^{-1} of 24-EBL. In EC 6 dS m^{-1}, exogenous application of 24-EBL (in all concentrations) increased significantly leaf K^+ content compared with non-treated plant in the same salt condition.

Discussion

After 10 days of salt treatment, leaves of plants grown in EC 8 dS m^{-1} showed the signs of toxicity. These symptoms were started from leaf margins and then extended on leaf surfaces. Injury symptoms may be related to accumulation of Cl^- and/or Na^+ in the leaves. It has been also reported that chloride injury develop as marginal chlorosis of leaves of broad leaved trees followed by extensive scorching of leaf blades (Kozolowski, 1997) which was in accordance with our observation on loquat plants. In EC 6- 8 dS m^{-1}, 24-EBL improved the growth of plants thus decreased the symptoms of toxicity (Fig. 5).

The increasing of salt concentration in growth media, shoot and root fresh and dry weight significantly decreased. The effect of salt stress on growth reduction has been reported on different plants by others (Ashraf and Harris, 2004; Anjum, 2008).

Application of brassinosteroids (BRS) considerably reduced the growth inhibitory effect of salt stress as reflected in the growth of the plants. The organ growth-promotive effects of BRs have been mainly related to cell expansion and division (Mayumi and Shibaoka, 1995) via enhancing microtubules and cellulose biosynthesis and thus changing mechanical characteristics of the cell wall (Fujioka and Sakurai, 1997). The role of homobrassinolide application in growth promotion under normal or stress conditions has also been reported in banana growth under stress conditions (Nassar, 2004). Houimli et al. (2008) also reported that 24-epibrassinolide increased salt tolerance in pepper plants which was in consistent with our result in loquat plants. It has been suggested that brassinosteroids could

Fig. 5: In high Salt condition (EC 8 dS m^{-1}), loquat plants were treated with different concentration of 24-EBR; 0.0, 0.25, 0.5 and 0.75 mg l^{-1} from left to right respectively. Application of 0.5 and 0.75 mg l^{-1} 24-EBL ameliorates loquat plant growth and decreased NaCl toxicity symptoms, two plants on the right.

have a positive role on root growth, if its concentration be greater than its threshold value and this is genotype dependent (MUSSIG et al., 2003).

In present study, salt stress reduced severely leaf surface area of loquat leaves. It is known that reduction in leaf area in salt-stressed plants can be explained by a decrease in leaf turgor, changes in cell wall properties and diminish in photosynthetic rate (RODRIGUEZ et al., 2005). BRs determine leaf size by controlling the cell proliferation gradient at the transition zone between cell division and expansion through positive regulation of either the rate or duration of the cell proliferation (GUDESBLAT and RUSSINOVA, 2011). EL-KHALLAL et al. (2009) indicated that increase in the leaf area induced by brassinolide was further translated into improved growth of the plants as reflected in the enhancement in fresh and dry weights of the shoot system.

Under saline conditions, reduction of leaf chlorophyll content may be attributed to the increase in the activity of chlorophyll degrading enzyme chlorophylase, (REDDY and VORA, 1986). It was observed that brassinosteroids reduced the inhibitory effects of salt stress on leaf pigment levels and this could be one of the reasons for induced growth stimulation by brassinosteroids under saline conditions (ANURADHA and RAO, 2003). CASTLE et al. (2003) also reported that epibrassinolide improved the tolerance of barley to salt stress, by reducing damage via protecting cell ultra-structure and chloroplast membrane system (CASTLE et al., 2003).

In present study, until certain level of salt stress (EC 6 dS m^{-1}) with increasing salinity total soluble sugar (TSS) increased and then decreased. It is known that sugars act as osmoprotectant in stress condition. The main functions of them are osmotic regulation, carbohydrate storage and also sugars can cause cell membrane and protein stability. These can be done through the formation of hydrogen bonds between the carboxyl groups of proteins and polar chain of sugar (KOSTER and LEOPOLD, 1988). Reduction of TSS in EC 8 dS m^{-1}, may be due to induction of high osmotic pressure which affects hydrocarbon enzyme synthesis and consequently sugar content declined (DORING and LUDDERS, 1986). The results showed that in EC 4-6 dS m^{-1}, exogenous application of 24-EBL enhanced leaf total soluble sugar content. Some investigations found increases in the activity of enzymes of carbohydrate metabolism such as sucrose synthase, sucrose phosphate synthase and acid invertase, in the presence of brassinosteroids, suggesting a regulatory role for this hormone in carbohydrate metabolism (YU et al., 2004; LISSO et al., 2006).

Leaf proline accumulation is a common metabolic response of higher plants to salinity stress. In addition to its role in osmotic adjustment as an osmolyte, it also plays an important role in a number of physiological and biochemical phenomena such as stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (SRINIVAS and BALASUBRAMANIAN, 1995; HARE and CRESS, 1997). SHAHID et al., (2011) reported that accumulation of proline in plant tissue due to EBL under stress may be associated with reduction in proline utilization due to the minimum protein formation, proline degradation and enhancement in proline formation due to the hydrolysis of proteins. The results of present study are also consistent with findings of WANG and ZHANG (1993) on rice and VARDHINI and RAO (2003) on sorghum.

Significant correlation was also observed between soluble sugar and proline ($r2 = 0.68$) (Fig. 6). STEWART et al. (1966) showed that proline accumulation was greater and most prolonged in leaves with higher sugar and starch contents. Based on these results, the interpretation of the role of sugar in proline accumulation is that the oxidation of sugars furnishes α-ketoglutarate and NAD(P)H for proline synthesis in leaves.

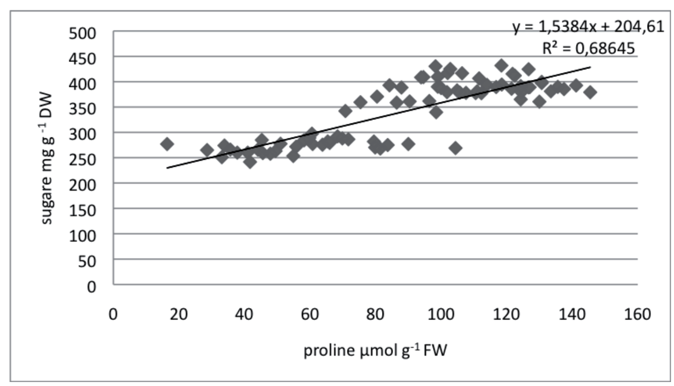

Fig. 6: Relationship between proline and total soluble sugars in leaves of loquat in response to salinity. Regression equation: $r2 = 0.68$; $P < 0.001$.

It has been observed that plants exposed to NaCl take up high amounts of Na^+, whereas the uptake of K^+ is significantly reduced (ASHARAF and AKHTAR, 2004). Similarly, the competition between K^+ and Na^+ in the roots of plants is well known (GARCIA-SANCHEZ et al., 2002; GARCIA-LEGAZ, 2008), suggesting that the uptake mechanism of the two ions (Na^+ and K^+) is similar (SCHROEDER et al., 1994).

Potassium ion is the major cation within plants which counterbalances the negative charge of anions. The K^+ ion stabilizes the pH, osmotic potential, and turgor pressure within cells. It also plays a crucial role in the activation of the enzymes involved in the metabolism and synthesis of proteins and carbohydrates (PAGE and CERA, 2006). Moreover, K^+ ions contribute to the osmotic adjustment of cells in salt stressed plants.

In present study, under salt conditions, the Na^+ content in the shoots was higher than it in the roots. The transport and accumulation of Na^+ in the leaves often characterize tolerant includer species that compartmentalize toxic ions in the vacuoles (ZID and GRIGNON, 1991; NIKNAM and MCCOMB, 2000; ASHRAF, 2004). However, if Na^+ sequestered in the vacuole of a cell, organic solutes should accumulate in the cytoplasm to balance the osmotic pressure of the ions in the vacuole, the compounds that accumulate most commonly are proline (MUNNS, 2000). As mentioned before, in our experiment the leaf proline contents increased.

Our results showed that in salt stress condition, the uptake of Na^+ reduced and that of K^+ increased by exogenous application of 24-EBL from root zone (Tab. 3). The beneficial effect of 24-EBL may be related to the improvement of the ion balance in salt treated cells due to its cationic nature as reported by PIROGOVSKYA et al. (1996). RONSCH et al. (1993) also suggested that BRs can be employed to plants for effective absorption of minerals from the soil.

It is worth noting that, where the amount of Na^+ decreased and the amount of K^+ increased, plant growth ameliorated via increasing in shoots and root fresh weight (Tab. 1).

The low concentration of Cl^- in the leaves indicates the ability of the different plant parts, especially the roots, to limit Cl^- transport to the leaves, where stronger damage could be produced. Leaf injury and defoliation are closely correlated with leaf Cl^- levels, and hence application of 24-EBL had an obvious influence on improving the damage. Also, the reduced accumulation of Cl^- in leaves of 24-EBL treated plants may be accentuated by the dilution effect of Cl^- because of the higher growth of 24-EBLtreated plants under saline conditions.

In conclusion, the detrimental effects of salt on growth and biochemical parameters were alleviated by 24-EBL application. Out of the four 24-EBL concentrations, the effects of 0.5 mg l^{-1} proved the best under stress conditions. The application of 24-EBL improved the performance of plants under low level of salt and also decreased salt harmful effect under medium-salt concentrations (EC of 4-6 dS/m) especially on leaf chlorophyll content and increased in leaf proline content as osmoprotectant. It seems that loquat is a moderate salt tolerance and application of this hormone may be useful for declining salt effect in area with EC less than 4 dS/m. However, field trail must be performed.

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Address of the author:
Dr. Akhtar Shekafandeh and Eng Fatemeh Sadeghi, Department of Horticulture, Faculty of Agriculture, Shiraz University, P.O. Box 7144465186, Shiraz, Iran
E-mail: shekafan@shirazu.ac.ir
E-mail: fateme.sadeghi2775@yahoo.com