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Interactive Effects of Salinity, Heavy Metal Stresses and Adaptive Compounds on Growth & Photosynthetic Pigments of Broad Bean Plants.

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ABSTRACT

The effects of NaCl, Cd and adaptive compounds namely salicylic acid and ascorbic acid, either alone or in combination, on growth and photosynthetic pigments of broad bean plants were investigated. Exposure of bean plants to these stressful factors induced variable decreases in the levels of growth parameters and chlorophyll content throughout the experimental period, as compared with control plants. The addition of 4 mM ascorbic acid and /or 0.09 mM salicylic acid to the stressful media induced, in general, significant protective changes in the detected parameters.

INTRODUCTION

Plant organisms subjected to different biotic and abiotic stresses show many changes in their common physiological processes. Salinity in soil or water is one of the major abiotic stresses in arid and semi-arid regions that substantially reduce the yield of major crops by more than 50% (Bray *et al.*, 2000; Khan and Panda, 2008). In these areas, salinity could be caused by low rainfall, high evaporation rate, proximity to the sea and poor water management. Salinity affects 7% of the world's land area for around 930 million ha (Munns, 2002).

Salinity becomes more toxic when plants are exposed to Cd ($CdNO_3$) stress by inducing physiological disturbs (Shafi *et al.*, 2009). Also it was known that salt stress can enhance Cd content in soil and promotes its uptake (Weggler *et al.*, 2000).

Cd is not an essential element; its content is estimated to be about 0.06–0.50 mg/kg in natural soils. Due to its large bioavailability in soil and high solubility in water, it impairs ecosystems by being readily absorbed by plant roots and transported through xylem to the vegetative and reproductive organs where it accumulates to high levels (Yang *et al.*, 1998). There, Cd affect many processes inside plant cells, resulting in decrease in the activity of photosynthesis process, chlorophyll content, plant growth and production and increase the activity of ROS (Florijn and Van Beusichem, 1993; Zhou and Huang, 2001; Yi and Ching, 2003; Zhou *et al.*, 2003). Furthermore, Cd stress is known to lead to protein degradation through amino acid metabolism resulting in decreased plant growth (Rai & Raizada, 1988).

Salicylic acid (SA) and ascorbic acid (AsA) are natural compounds involved in plant defense responses as well as the regulation of plant growth and development (Raskin 1992; Noctor and Foyer, 1998; Dempsey *et al.*, 1999).The application of these adaptive compounds has been repeatedly reported to ameliorate the damaging effects of both salinity and Cd stresses (Gunes *et al.*, 2007; Krantev *et al.*, 2008; Tayebi-Meigooni *et al.*, 2014; Moghadam, 2016).

MATERIALS AND METHODS

Plant material and growth conditions:

Homogeneous faba bean seeds (*Vicia faba* L. cv. Sakha 1) were selected and surface sterilized in 10^{-3} M $HgCl_2$ for 3 min. Seeds were then washed several times with distilled water. The sterilized seeds were soaked in distilled water at room temperature overnight with aeration to avoid anaerobiosis as a complicating factor. The seeds were then sown in plastic pots (25 cm in diameter; 5 seeds/ pot) each containing 4 kg of clay-sandy soil (2:1, v/v). The pots were irrigated with tap water and left to grow in a glass house under natural conditions. The irrigation of pots was repeated according to the soil moisture needs.

On the appearance of the first bi-foliate leaf, thinning and initial sampling was carried out and the left appropriate homogeneous plants were divided into 9 groups; each group being of 14 pots. The first group represents the control plants that were irrigated with 1/10 Hoagland nutrient solution only. The remaining groups were appropriately irrigated with 1/10 Hoagland nutrient solutions (Hershy, 1995); each being enriched with one of the appropriate treatments as follows:

- The 2nd group: 300 mM NaCl.
- The 3rd group: 1 mM Cd $(NO_3)_2.4H_2O$.
- The 4th group: 300 mM NaCl + 1 mM Cd $(NO_3)_2.4H_2O$.
- The 5th group: 4 mM AsA.
- The 6th group: 0.09 mM SA.
- The 7th group: 300 mM NaCl + 1 mM Cd $(NO_3)_2.4H_2O$ + 4 mM AsA.
- The 8th group: 300 mM NaCl + 1 mM Cd $(NO_3)_2.4H_2O$ + 0.09 mM SA.
- The 9th group: 300 mM NaCl + 1 mM Cd $(NO_3)_2.4H_2O$ + 4 mM AsA + 0.09 mM SA.

After 10 days from the beginning of treatment, samples were collected from 7 pots from each group, and the remaining 7 pots in each group were appropriately irrigated with the relevant solution according to the usual practice. After another 10 days, the second sampling was made and the experiment was terminated.

Sampling was carried out in a way so as to include all plants allotted for each treatment. The collected plants were used for assessment of the growth parameters (length of shoot and root, fresh weight of shoot and root, dry weight of shoot and root) and photosynthetic pigments in leaf (no.2).

Of interest in this connection, the net dry bioweight increase per unit dry weight per day is the relative

growth rate (RGR) that was herein calculated at the end of the experimental period as follows:

$$RGR = \frac{\ln (D_2) - \ln (D_1)}{t_2 - t_1}$$

Where, D_1 and D_2 is the plant dry weight at time t_1 and t_2, respectively.

The full data of the different stressed groups of broad bean plants were statistically analyzed using one way analysis of variance (ANOVA) and comparison among means was carried out by calculating the least significant difference test (L.S.D.) at 5% probability level. All the analyses were made using CoStat software (version 6.400).

Determination of photosynthetic pigments.

The plant photosynthetic pigments, chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids (cars) were estimated as described by Metzner *et al.,* (1965) and Horváth *et al*. (1972).

A known fresh weight of photosynthetic tissue was macerated in 85% acetone solution for 5 min. Small amount of $MgCO_3$ was added to reduce acidification. The filtration was carried out through filter paper. The filtrate was made up to a known volume with 85% aqueous acetone. The extract was measured spectrophotometrically against a blank of pure 85% aqueous acetone at three wavelengths of 480, 644 and 663 nm. The concentrations of the pigment fractions were calculated using the following equations:

Chlorophyll a = 10.3 A_{663} – 0.918 A_{644} µg / ml

Chlorophyll b = 19.7 A_{644} – 3.87 A_{663} µg / ml

Carotenoids = 5.02 A_{480} µg / ml

Then, the fractions were calculated as mg / g fresh weight.

RESULTS AND DISCUSSION

Changes in growth parameters

Treatment of broad bean plants with 1 mM Cd and 300 mM NaCl induced, in general, significant decreases in growth parameters except for root length as compared with control values. Combination of the two stresses (NaCl and Cd) led to a further significant reduction in these growth components as compared with those of controls. These observations appeared to coincide with the fact that RGR appeared to decrease from a control value of 0.68 g g^{-1} d^{-1} to lower values of 0.55 and 0.54 g g^{-1} d^{-1} when 1 mM Cd and 300 mM NaCl were administered either alone or in combination. The root length of the comparable treated plants appeared, in general, to be significantly increased on the 10th day after treatment; however the enhancement of root length being lower when plants were treated with combined NaCl and Cd stress. But, on the 20th day after treatment, root length appeared to decrease (Table 1).

In response to treatment with 4 mM AsA, an increase in all growth parameters was apparent on 10 and 20 days after treatment above the control values. In contrast, with 0.09 mM SA-treated broad bean plants, a decline set in for all growth attributes on 10 and 20 days after treatment. These results appeared to coincide with RGR values of 0.68, 0.86 and 0.53 g g^{-1} d^{-1} for controls, for the 4 mM AsA and for 0.09 mM SA-treated samples, respectively (Table 1).

Combination of NaCl and Cd with AsA and/or SA appeared to induce increases in all growth parameters compared to NaCl- and Cd-stressed plants on 10 and 20 days after treatment. The observed increments appeared to be most pronounced upon administration of AsA and SA followed by that induced by AsA when applied alone (Table 1).

Salinity and Cd stresses inhibited plant growth and this could be due to the negative effect of these stressful factors on the rate of photosynthesis and the decrease in the level of carbohydrates and growth hormones (Mazher *et al.*, 2007; Abdul Qados, 2011; Chen *et al.*, 2011; Liu *et al.*, 2014).

Table 1: Effects of NaCl and Cd either alone or in combination with AsA and/or SA on growth parameters (root length; cm plant^{-1}, shoot length; cm plant^{-1}, fresh weight; g plant^{-1}, dry weight; g plant^{-1}; water content; g plant^{-1} and RGR; g g^{-1} d^{-1}) of broad bean plants on 10 and 20 days after treatment.

Days after treatment	Treatments / Growth parameters	Shoot length	Root length	Fresh weight	Dry weight	Water content	RGR
	Initial	13.83	20.67	7.41	0.49	6.92	-
10 days	Control (1/10 Hoagland solution)	35.25	26.93	18.26	1.02	17.25	-
10 days	300 mM NaCl	29.50 *	41.77 *	15.71 *	1.09	14.63 *	-
10 days	1 mM Cd	31.67 *	40.33 *	14.84 *	0.93	13.92 *	-
10 days	300 mM NaCl + 1 mM Cd	28.50 *	29.23	13.54 *	1.05	12.49 *	-
10 days	4 mM AsA	36.00	30.50 *	21.22 *	1.35	19.87 *	-
10 days	0.09 mM SA	28.93 *	26.40	15.80 *	0.86	14.94 *	-
10 days	300 mM NaCl + 1 mM Cd + 4 mM AsA	33.00	34.87 *	15.83 *	0.94	14.88 *	-
10 days	300 mM NaCl + 1 mM Cd + 0.09 mM SA	30.50 *	34.00 *	14.73 *	0.96	13.77 *	-
10 days	300 mM NaCl + 1 mM Cd + 4 mM AsA + 0.09 mM SA	34.50	36.40 *	16.39	0.95	15.43	-
10 days	L.S.D.	3.564	3.465	2.155	0.610	2.208	-
20 days	Control (1/10 Hoagland solution)	51.50	45.33	26.30	1.98	23.42	0.68
20 days	300 mM NaCl	43.57 *	42.25 *	19.76 *	1.77	17.99 *	0.55
20 days	1 mM Cd	44.85 *	45.10	20.31 *	1.71	18.59 *	0.55
20 days	300 mM NaCl + 1 mM Cd	40.00 *	38.80 *	14.53 *	1.66	12.87 *	0.54
20 days	4 mM AsA	52.00	53.00 *	28.91 *	2.44	26.47 *	0.86
20 days	0.09 mM SA	43.65 *	39.17 *	21.48 *	1.68	19.80 *	0.53
20 days	300 mM NaCl + 1 mM Cd + 4 mM AsA	40.50 *	39.10 *	17.96 *	1.64	16.49 *	0.50
20 days	300 mM NaCl + 1 mM Cd + 0.09 mM SA	40.00 *	36.13 *	16.58 *	1.65	15.17 *	0.50
20 days	300 mM NaCl + 1 mM Cd + 4 mM AsA + 0.09 mM SA	44.5	42.50 *	24.04	1.99	22.05 *	0.69
20 days	L.S.D.	4.130	2.845	2.530	0.510	2.488	0.204

***The mean values are significantly different from the control at P ≤ 0.05.**

In parallel to our results, Raziuddin *et al.* (2011) found that Cd and NaCl either alone or in combination decreased growth by reducing root and shoot fresh and dry weights of *Brassica sp*. However, the combined effect of Cd and NaCl was more negative on these parameters than the sole effect of Cd and NaCl.

Shaddad *et al.* (1990) and Abd El-Aziz *et al.* (2006) reported an increase of growth parameter after application of AsA. The effect of AsA on plant growth may be due to the substantial role of AsA in many metabolic and physiological processes. According to several researchers application of SA inhibits seed germination (Guan and Scandalios, 1995; Rajjou et al., 2006; Xie et al., 2007). The effect of SA as a negative regulator of seed germination is probably due to SA-induced oxidative stress (Yusuf *et al.*, 2013).

According to many scientists, SA and AsA play an important role in plant defense against stressful factors. They alleviate the deleterious effects of salinity (Shakirova *et al.*, 2003; Hussein *et al.* 2007) and heavy metal stress (Zhou *et al.*, 2009; Moradkhani *et al.*, 2012).

Changes in photosynthetic pigments.

As shown in table 2, the various pigment fractions appeared to be slightly changed from the appropriate control values in response to NaCl and/or Cd stress on the 10th day after treatment. On the 20th day after treatment with NaCl, Cd and NaCl+Cd, an apparent significant decrease in all pigment fractions, except for cars and chl a/b, below control values, was observed.

Application of AsA alone induced slight changes in the different pigment fractions after 10 and 20 days in relation to the control levels. On the other hand, SA caused a significant increase in chl a, chl b, chl a+b and total pigments after 10 days from the treatment date, above the control values. However, SA-treated plants did not show any significant difference in the various pigment fractions after 20 days.

As regards the combination of the above mentioned adaptive compounds with NaCl and Cd, an increase in the determined pigment fractions was, in general, observed above those levels maintained in response to treatment with combined NaCl and Cd stress, throughout the experimental period. However, a statistically significant induction was only observed for chl a, chl b, chl a+b and total pigments when NaCl- and Cd-treated samples supplemented with SA after 10 days.

Biosynthesis of chlorophyll has been inhibited in plants due to metal and salt stress (Sinha *et al.*, 2003).The reduction in photosynthesis process under saline conditions is due to stomatal and non-stomatal factors. It also affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). According to Younis *et al.* (1993) and El-saht *et al.* (1994), broad bean, castor bean and maize plants exposed to saline conditions showed decrease in chlorophylls content in addition to a significant increase in carotenoids (Cars) contents throughout all stages of growth.

Cadmium is one of the most toxic heavy metals to both plants and animals. Once absorbed by plants, Cd has a negative effect on several cellular processes (DalCorso *et al.*, 2008), leading to inhibition in stunted growth and yield, leaf chlorosis, browning of root tips, increased leaf senescence and even death (Sanita di Toppi and Gabbrielli, 1999; Baryla *et al.*, 2001; Mallick and Mohn 2003; Metwally *et al.*, 2005). These visible symptoms reflect changes in plant biochemical components and metabolic pathways such as decreasing stomatal density and conductance to CO_2, thus reducing the photosynthetic rate, deficient nutrient uptake, disturbed water uptake and water relations (Sanita di Toppi and Gabbrielli, 1999; Baryla *et al.*, 2001; Schutzendubel *et al.*, 2001; Poschenrieder and Barcelo, 2004; Burzyński and Żurek, 2007).

Table 2: Effects of NaCl and Cd either alone or in combination with AsA and/or SA on the content of photosynthetic pigments of broad bean plants on 10 and 20 days after treatment. Values are expressed as mg g^{-1} fresh weight.

Days after treatment	Treatments	Parameters: Chl a	Chl b	Cars	Chl a+ b	Chl a/b	Total pigments
	Initial	0.49	0.18	0.13	0.67	2.79	0.80
10 days	Control (1/10 Hoagland solution)	0.72	0.34	0.20	1.06	2.12	1.27
	300 mM NaCl	0.70	0.31	0.18	1.01	2.32	1.2
	1 mM Cd	0.73	0.33	0.20	1.06	2.30	1.26
	300 mM NaCl + 1 mM Cd	0.63	0.31	0.18	0.94	2.05	1.12
	4 mM AsA	0.78	0.38	0.20	1.16	2.08	1.36
	0.09 mM SA	0.95 *	0.52 *	0.27	1.47 *	1.81	1.74 *
	300 mM NaCl + 1 mM Cd + 4 mM AsA	0.81	0.41	0.22	1.22	1.98	1.44
	300 mM NaCl + 1 mM Cd + 0.09 mM SA	0.92 *	0.54 *	0.28	1.46 *	1.71	1.74 *
	300 mM NaCl + 1 mM Cd + 4 mM AsA + 0.09 mM SA	0.73	0.36	0.20	1.09	2.04	1.29
	L.S.D.	0.143	0.117	0.116	0.251	0.444	0.339
20 days	Control (1/10 Hoagland solution)	0.83	0.45	0.26	1.28	1.84	1.53
	300 mM NaCl	0.59 *	0.29 *	0.16	0.88 *	2.12	1.05 *
	1 mM Cd	0.55 *	0.30 *	0.16	0.85 *	2.04	1.01 *
	300 mM NaCl + 1 mM Cd	0.59 *	0.31 *	0.15	0.90 *	1.96	1.05 *
	4 mM AsA	0.82	0.43	0.22	1.24	1.94	1.46
	0.09 mM SA	0.74	0.38	0.19	1.12	1.97	1.32
	300 mM NaCl + 1 mM Cd + 4 mM AsA	0.76	0.40	0.21	1.16	1.87	1.37
	300 mM NaCl + 1 mM Cd + 0.09 mM SA	0.70	0.36	0.19	1.06	1.92	1.25
	300 mM NaCl + 1 mM Cd + 4 mM AsA + 0.09 mM SA	0.72	0.38	0.19	1.10	1.89	1.29
	L.S.D.	0.144	0.130	0.140	0.244	0.712	0.328

***The mean values are significantly different from the control at P ≤ 0.05.**

Both Cd and salt stresses can interfere with plant growth and development by inducing physiological impairments. Increasing the concentration of Cd or salt was shown to affect nutrient uptake and carbohydrate metabolism (Moya *et al*., 1993), therefore, significantly decrease chlorophyll biosynthesis (Padmaja *et al*., 1990). Combination of salt and Cd stresses, increase the permeability of membranes and the activity of ROS (Mühling and Läuchli, 2003).

Many investigators studied the role of SA in improving plant response to several abiotic stresses. Guo *et al*. (2007) found that SA decreased the toxic effects of heavy metals in rice and He and Zhu (2008) recorded the same result under the effect of salt stress in tomato. The positive effect of SA and AsA on photosynthesis under Cd and salt stresses may be due to its role in protection of cells against oxidative stress (Krantev *et al.*, 2008; Tayebi-Meigooni *et al.*, 2014).

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التأثيرات التفاعلية لإجهاد الملوحة والعناصر الثقيلة والمواد الواقية على نمو وأصباغ التمثيل الضوئى لنباتات الفول.

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يهدف البحث الى دراسة تأثير العوامل المجهدة مثل كلوريد الصوديوم والكادميوم ومواد التكيف مثل حمض السلسليك والاسكوربيك ، إما منفردة او مجتمعة ، على نمو وأصباغ التمثيل الضوئى لنباتات الفول حيث أدى تعرض نباتات الفول لهذة العوامل المجهدة الى نقص فى النمو ومحتوى الكلورفيل خلال فترة التجربة مقارنة بالعينات الضابطة كما وجد أن اضافة حمض الاسكوربيك وحمض السلسيك الى البيئة المغذية المجهدة تستحث تغيرات معنوية فى نمو و أصباغ البناء الضوئى لنباتات الفول.