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Responses of bean and pea to vitamin C under salinity stress

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Abstract: The relationship between compatible solutes and antioxidants are the strategies that plants have developed to tolerate salt stress. Proline and enzymes are the main metabolites that accumulate in vary species of higher plants in response to salt stress. In *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection pre-treatment with vitamin Cs (Vitamin C) led to enhanced seedlings tolerance to saline stress during germination , as greater growth of pretreated seedlings (vitamin C group) versus untreated ones (S group), evaluated through such parameters as length, water and chlorophyll content. During the germination period, a considerable increase was observed in proline levels in the seedlings subjected to saline stress (S), whereas in the vitamin C group, the proline increment was not significantly. Starting from the fourth day of germination, betaine levels in seedlings pretreated with vitamin c and then with water and in vitamin C showed a significant increase versus C and S seedlings, possibly because such a precursor promotes betaine biosynthesis. This could be responsible for the enhanced growth observed in vitamin C versus S seedlings, as well as for preventing the decrease in chlorophyll content in the vitamin C group. The accumulation of betaine seems to correlate with the greater tolerance of these seedlings against saline stress. This study concluded that a relationship between antioxidant glutathione and salt tolerance was observed in both plants, under the effect of vitamin c.

Keywords: Salinity, stress, vitamin c, beans, pea

INTRODUCTION

Since higher plants are immobile, they can't escape from environmental stresses. The ability of higher plants to scavenge the toxic effects of active oxygen seems to be very important determinant of their tolerance to these stresses. Antioxidants are the first line of defense against free radical damage. They are critical for maintaining optimum health of plant cells. There are several antioxidant enzymes, peptides and metabolites involved in the scavenging of active oxygen in plants, and their activation are known to increase upon exposure to oxidative stress[37].

Height salt concentration normally impair the cellular electron transport within the different subcellular compartments and lead to the generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals[7,11]. Excess of ROS triggers phytotoxic reactions such as lipid peroxidation, protein degradation and DNA mutation[37,36].

Antioxidants, is designing chemicals, when added in small quantities to a materials, react rapidly with the free radical intermediates of an autooxidation chain and stop it from progressing. An excellent example of this type of inhibitor is the synthetic hindered phenol 2,6-di-tert-butyl-4 methyl phenols often called BHT which react with mol- of peroxy radical and converts them to much less active products. It has been recognized for some time that naturally occurring substances including those found in higher plants, also have antioxidant activity. Recently, there has been increasing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders[21]. Accordingly attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them, and on the mechanisms of their action[41].

It has also been reported that plants with high levels of antioxidants, whether constitutive or induced have a greater resistance to such oxidative damage[23]. The primary components of this antioxidant system include carotenoids, ascorbate, glutathione, vitamin E (α-tocopherols) flavonoids, phenolic acids, other phenols, alkaloids, polyamines, chlorophyll derivatives, amino acids and amines and miscellaneous compounds. A number of studies indicated that the degree of oxidative cellular damage in plants exposed to a biotic stress is controlled by the capacity of antioxidative systems[21].

Vitamin C is a universal reductant and antioxidant of plants. It is found at concentration of 1-2 Mm in legume nodules[20] and is positively correlated with

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nodule effectiveness[6]. It is an essential metabolite for the operation of the ASC- GSH pathways, but it also has beneficial effects that do not require the presence of APX. ASC can directly scavenge ROS and reduce ferric Lb and Lb^{IV}[22]. It is also involved in hydroxylation of proline, regulation of the cell cycle and numerous fundamental processes of plant growth and development[26].

Some reports concerning the exogenous application of the vitamins to salinized plants and their role in stimulation of their growth are scarce[27]. These compounds were also scarcely tried to counteract some of the adverse effects of salinity stress[32]. Thus exogenous addition of such substances to the test organism could lead to growth stimulation through the activation of some enzymatic reactions[18].

The goal of the present work was to determine the influence of vitamin c pre-treatment on bean and pea seeds subjected to saline stress during germination through, growth criteria, compatible osmolytes and adaptive mechanisms of the antioxidant (Glutathione).

MATERIALS AND METHODS

Seeds Culture and Treatments: Surface sterilized seeds of broad been *Vicia faba* L. cv. Lara and pea *Pisium sativum* L. cv. Perfection were placed in petri dishes with distilled water in a controlled environmental chamber for 12 h in a glasshouse at the Faculty of Science, King Saud University, KSA. Soaked seeds were then distributed in plastic trays (15x15cm) with three layers of filter paper moistened with the solutions indicated to all treatments for 24 h, as follows:

- 100 mL of water (C).
- 100 mL of 150 mM NaCl (S).
- 00 mL of 200 mg/L vitamin C (V).
- 0 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).

Each treatment consisted of 10 plastic trays each containing 20 seeds. Samples were collected randomly at 2, 4, 6, and 8 days and the length of seedlings were measured. Relative water content was also measured and expressed as a percentage according to the following equation :

$$RWC\ (\%) = (FW - DW) / FW \times 100.$$

Recovery of Seedlings: At the end of the experiment described above (day 8), seedlings were transferred to 15 cm plastic pots containing vermiculite, maintained in the same environmental condition and watered daily with a half nutrient solution[13] for 15 days.

Analysis: Chlorophyll content: was determined, at 6 and 8 days after germination and in recovered plants .It was extracted by homogenizing 0.5 g fresh weight of green tissues (leaf plus stem) of the seedlings in 10 ml of 95% ethanol. After centrifugation for 10 min. at 5000 rpm, the chlorophyll content was analyzed spectrophotometrically on the ethanolic supernatant at 654 nm as described by Wintermans and De Mots[40].

Proline: 0.5 g FW were homogenized in 5 ml of 3% sulphosalicylic acid .After centrifugation for 10 min. at 5000 rpm, proline was estimated spectrophotometrically at 520 nm using the ninhydrin method[3]. Purified proline was used for standardization.

Betaine assay: 0.5 g FW were homogenized with 5ml of methanol and extracts were phase-separated with chloroform and water as described previously[29]. After evaporating the aqueous phase to dryness in an air stream of distilled water and 0.3 ml of a slurry of dowex -50 ion exchange resin in the H^+ form[16] were added .Betaine was determined according to the periodide method[39].

Glutathione determination: none-protein thiols were extracted by homogenizing 0.3 gm FW in 1.5 ml of 0.1N HCl .After centrifugation at 15000 rpm for 30 min at 4°C, the supernatants were used for analysis. Total glutathione was determined in the homogenates by spectrophotometry at 412 nm[31].

Index of halophytism (HIB) of broad bean and pea by vitamin C was obtained from the osmolytes (proline and betaine) and antioxidant (ghtathione) data according to the proposed equation as described by Abd El- Fattah[1].

$$HIB = \sqrt{\frac{bxg}{p}}$$

b = betaine content of the plant.
g = glutathione content of the plant.
p = praline content of the plant.

The HIB values classified in classes (I-V) named halophytism class.

Statistical Analysis: Figures in the text and tables indicate mean values±SEM .Differences between control and treated seeds were analyzed by one–way ANOVA, taking $P < 0.05$ a significant , according to Tukey s multiple range test .

RESULTS AND DISCUSSIONS

Results:

Effect of Pre-treatment with Vitamin C: To determine the growth of broad bean and pea seedlings starting from the second day of the experiment, their length and relative water content (RWC) were

Table 1: Effect of vitamin C treatment on relative water content (%) of broad bean and pea. Values are the means ±SEM of three replicated measurements.

Treatments	Time in days							
	2		4		6		8	
	P. vul.	*H. vul.*	*P. vul.*	*H. vul.*	*P. vul.*	*H. vul.*	*P. vul.*	*H. vul.*
C	48±3	30±2	56±3	39±2	72±4	47±2	89±4	66±3
S	41±2***	29±1	48±3**	35±2**	46±3***	40±2*	42±2***	58±2**
V	49±2	33±2*	58±2*	44±3**	80±3**	60±3***	93±3*	78±2***
SV	46±2	30±2	54±2	38±2	70±3	46±3	78±3	65±3

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Table 2: Effect of vitamin C treatment on the length (root plus shoot, cm.) during seedlings development of *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection. Values are the means ± SEM of three replicated measurements .

Treatments	Time in days							
	2		4		6		8	
	Bean	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*
C	1.8±.2	2.3±.2	6.3±.3	8.4±.4	15.5±.5	21.7±.6	20.1±.4	28.6±.6
S	.6±.1***	1.8±.1	2.6±.1****	6.1±.3	5.8±.2***	11.6±.4**	5.8±.2***	18.5±.4***
V	2.1±.1**	2.5±.2	6.8±.3	9.2±.4*	18.6±.6*	25.4±.5**	22.7±.6*	30.6±..4*
SV	1.4±.1**	2.1±.1	4.1±.2*	7.5±.4	11.2±.4**	18.5±.5*	14.6±.5**	25.4±.4*

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

measured. Relative water content was significantly lower in the S groups from the second day as compared with the C group. This decrease persisted up to the eight day, Table (1). The decrease was more in bean (44%) than in pea (15%) in S group compared with the C group at eight day.

The length of S seedlings was significantly lower than that of the control over the whole experiment in bean, reaching a length 4-fold less at the eight day of treatment (Table, 2). In pea the salinity was less effect over the whole experiment i.e the less at the eighth day of treatment reached to 36% (less than one fold). Stress induced root necrosis (in bean) but secondary roots failed to appear, and therefore root length was more affected than shoot length under salt stress conditions. The protective effect of vitamin C pre-treatment against saline stress was shown by the greater length and RWC of vitamin C versus S seedlings (Tables, 1, 2). On the eight day, mean vitamin C length was more than twice as much as S and was accompanied by the appearance of secondary roots in the former seedlings (bean). In pea vitamin C reached to nearest as C group. Pre-treatment with vitamin C increases the seedlings length and RWC than control, so it has activation the growth measurements in the two plants (Tables 1, 2).

Total chlorophyll was determined in green tissues in bean and pea. In S seedlings were lower than those of C seedlings of both plants, Table (3). On the eight day it was observed that the C seedlings had more than three fold that of S in bean, while about 1.5 fold that of pea. So bean more effective than pea. Seedlings pre-treatment with vitamin C prevented the decrease in chlorophyll caused by saline stress .Moreover seedlings pre-treatment with vitamin C have higher total chlorophyll contents than C seedlings in both plants.

Levels of Compatible Osmolytes: In the S groups, proline levels were significantly increased starting from the second day and peaking at the eighth day of the experiment, with an increase of roughly 340% in bean and about 278% in pea above the controls; while in vitamin C seedlings, significantly smaller increaments were observed (Table 4).

When betaine content was determined, it was observed that control seedlings reached the maximal betaine level (22.4 Um /g DW for bean and 8.2 Um /g DW for pea) on the fourth day after germination for

Table 3: Effect of vitamin C treatments on the chlorophyll content (µg/g DW) during seedlings development of *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection. Values are the means ±SEM of three replicated measurements.

Treatments	Time in days							
	2		4		6		8	
	Bean	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*
C	100±8	60±3	420±15	260±8	840±20	420±8	950±23	660±19
S	40±6***	46±4*	100±8***	120±8***	260±9***	320±5*	280±8***	480±15**
V	120±9*	80±6*	500±18*	290±13	1000±20	500±6*	1000±22	690±17
SV	75±7*	50±4	310±12*	200±10*	650±12*	400±7	920±10	600±13*

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Table 4: Effect of vitamin C treatment on the proline content (µg/g DW) during seedlings development of *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection. Values are the means±SEM of three replicated measurements.

Treatments	Time in days							
	2		4		6		8	
	Bean	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*
C	3.8±.2	1.8±.1	6.4±.3	3.5±.2	8.2±.3	4.2±.2	5.2±.2	4.2±.2
S	4.2±.2*	2.2±.1*	9.5±.4***	4.2±.2*	19.5±.4***	9.2±.3**	17.8±.4***	9.6±.2***
V	3.6±.1	2±.1	5.2±.2*	3.7±.2	9.5±.3	4.8±.3	4.4±.1	4.5±.1
SV	4±.2	2±.1	6.8±.3	3.6±.2	13.6±.4**	6.8±.3*	8.3±.3**	8±.2**

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Table 5: Effect of vitamin C treatment on the betaine levels (µg /g DW) during seedlings development of *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection. Values are the means ±SEM of three replicated measurements.

Treatments	Time in days							
	2		4		6		8	
	Bean	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*
C	5.2±.2	1.1±.1	14.6±.4	3.6±.2	22.4±1	8.3±.3	14.8±.6	5.4±.2
S	5±.2	1.6±.1*	12.5±.8*	5.4±.3**	20.2±1	9.3±..3*	13.4±.4	8.5±.5**
V	8.2±.4**	3.2±.1**	25.4±1**	9.4±.3***	43.6±2***	16.8±1***	33.5±1***	13.6±.4***
SV	3.4±.2*	6.3±.2***	18.7±1*	12.2±.5**8	38.5±1***	22.1±.5***	30±.6**	14.6±.5***

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

the two plants and thereafter, levels diminished .In seedlings subjected to saline stress there was little decrease in betaine content at bean seedlings, in contrast, there was an obvious increase at pea seedlings as compared to the control seedlings.

The seedlings pre-treated with vitamin C led to a significant increase in betaine of both plants. The peak betaine level (roughly 43.6 Um /g DW) in bean was reached on day sex.

Reduced glutathione content was constitutively lower in S group than in C group (Table 6). Glutathione levels were diminished in vitamin C and C groups in the experiment period as compared with shocked group. However, at vitamin C, the glutathione values showed about 100% increase compared with C group.

Table 6: Effect of vitamin C treatment on the glutathione content (µg/g DW) during seedlings development of *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection. Values are the means±SEM of three replicated measurements.

Treatments	Time in days							
	2		4		6		8	
	Bean	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*	*Bean*	*Pea*
C	10.6±.2	18.8±1	11.4±1	19.5±2	11.2±2	17.2±2	10.2±1	16.4±2
S	4.2±.2*	12.2±1*	9.5±.4***	14.2±.2*	8.5±.4***	9.2±.3**	6±.4***	9.6±.2***
V	10.9±1	19.2±2	12±.2*	20.7±2	12.5±3	18±3	10.4±1	17.5±2
SV	16.5±2***	27±3***	18±3***	36±.4***	23±4**	36±5****	28±5****	38±7***

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Table 7: Effect of vitamin C treatment on root length, shoot length and chlorophyll content after 8-days of seedlings development of bean and pea. Values are the means ±SEM of three replicated measurements.

Treatments	Bean				Pea			
	Rootlength	Shoot length	Chlorophyll a	Chlorophyll b	Rootlength	Shoot length	Chlorophyll a	Chlorophyll b
C	10.6±.2	18.8±1	0.114±1	0.195±2	11.2±2	17.2±2	0.102±1	0.164±2
S	4.2±.2*	12.2±1*	0.95±.4****	0.142±.2*	8.5±.4***	9.2±.3**	0.6±.4***	0.96±.2***
V	10.9±1	19.2±2	0.12±.2*	20.7±2	12.5±3	18±3	0.104±1	0.175±2
SV	16.5±2***	27±3***	0.18±3***	0.36±.4***	23±4**	36±5****	0.28±5****	0.38±7***

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).
*=Significant at P< 0.5 **=Significant at P< 0.1 ***=Significant at P< 0.05

Table 8: Effect of vitamin C treatment on halophytism index (HIB) and halophytism class (HCB) of *Vicia faba* L. cv. Lara and *Pisium sativum* L. cv. Perfection during seedlings development.

Treatments	Time in days															
	2				4				6				8			
	Bean		*Pea*		*Bean*		*Pea*		*Bean*		*Pea*		*Bean*		*Pea*	
	HIB	HIC	HIB	HIC	HIB	HIC	HIB	HIC	HIB	HIC	HIB	HIC	HIB	HIC	HIB	HIC
C	3.8	II	3.4	II	5.1	III	4.5	III	5.5	III	5.8	III	5.4	III	4.6	III
S	2.2	II	3	II	3.5	II	4.3	II	3	II	3.1	II	2.2	II	2.9	II
V	5	III	5.5	III	7.7	IV	7.1	IV	7.6	IV	7.9	IV	8.9	V	7.2	IV
SV	3.8	II	9.2	V	7	IV	10	V	8.1	V	10	V	10	V	8.3	V

100 mL of water (C).
100 mL of 150 mM NaCl (S).
100 mL of 200 mg/L vitamin C (V).
50 mL 150 mM NaCl + 50 mL 200 mg/L vitamin C (SV).

Plant Recovery: At the third day of recovery, at bean both vitamin C and control plants had two pair of leaves; while only on the fifth day did the second pair appear in the S seedlings. On the sixth day of recovery, it was observed that root length of vitamin C plants was 38% greater than that of S group (Fig. 1). Seedlings pre-treated with vitamin C and subjected to saline stress (vitamin C) had a greater recovery in chlorophyll levels (98% of the control) than those of the S group which reached 44% of the control value (Fig 1).

At pea the second leaf appear at second day in control as well as of vitamin C and vitamin C groups. At sixth day of recovery, it was observed that lateral roots length of vitamin C plants was 50% greater than that of S, while shoot length of vitamin C plants was 75% greater than that of S (Fig 1). Seedlings of H. vulgare pre-treated with vitamin C and subjected to

saline stress (vitamin C) had a greater recovery in chlorophyll levels (95% of control) than those of the S group, which reached 60% of control value.

Table (7) shows the effect of vitamin C on the halophytism index (HIB) under salt stress. The HCB was II in the two plants throughout experiment period under salt stress. In contras, HIB and HCB increased by vitamin C treatments in stress and unstress samples. The highest HIB and HCB (Table, 7) values were obtained at vitamin C group, Main while the lowest values were attained at S group.

The relationship between plant growth (shoot and root length) and HIB during seedlings development of bean and pea by vitamin C treatment were illustrated in Fig (2). It was cleared that a linear progressive relation between the growth of bean and HIB throughout the experiment period. While at pea the relation not progressive in beging of the treatments (Fig, 2), 2, 4 days. The linear relation starts at the day 6 of treatments.

Discussion: The large amount of literature on the effect of salinity on diverse plant tissues and cells, the function of compatible osmolytes in salt injury still remains controversial. To our knowledge, there are no previous reports on vitamin C pre-treatments during germination in bean plants subjected to salt stress in contrast that of pea.

Vitamin C has been proposed for a long time as a biological antioxidant. It exists in rather high concentrations in many cellular environments, such as the stroma of chloroplasts where its level is 2.3 x 10^{-3} M. Vitamin C has been demonstrated in many qualitative studies to possess significant antioxidant activity. For example $l0^{3}$ M vitamin C inhibited the photooxidation of a kampferol by illuminated spinach chloroplasts. Vitamin C reduces two equivalents of O^{-}_{2} produce H_2O_2 and triketo derivative dehydroascorbic acid. Vitamin C also reacts with $^{1}O_2$ at a relatively fast rate[22].

Pre-treatment of bean seeds with vitamin C led to enhanced seedling tolerance to condition of saline stress during germination , as evidenced by the greater growth of vitamin C versus S seedlings evaluated through such parameters as length, RWC and chlorophyll content (Tables, 1, 2, 3). Pea is less sensitive plant than bean, so its adverse saline effect is less. Also pre-treatment with vitamin C increase the tolerance of pea through the measure parameters.

During the germination period of the two plants, a considerable increase was observed in proline levels (up to 340%) in seedlings of bean and (up to 278%) in seedlings of pea subjected to sodium chloride treatment. However, such an increase in a protective osmolyte was insufficient to protect seedlings against damage caused by salinity conditions. In seedlings of both plants pre-treated with vitamin C and subjected to saline stress (vitamin C group), the proline increment was much smaller (about 60%). Hare *et al.,*[9] have contended that proline content increases when there is an injury to plant tissue. Possibly in seedlings pre-treated with vitamin C and subjected to saline stress, where growth is greater and plant status better, damage is less and therefore proline levels are hardly increased with respected to the control seedlings. On the other hand the inhibitory effect of betaine on proline accumulation would provide an alternative explanation for this finding, in agreement with the results obtained by Gibon *et al*.,[8] and Larher *et al.,*[15] with rape leaf discs .

Previous reports have shown that bean a member of the leguminosae family contains rich amount of betaine and accumulate less quantity under saline conditions. In contrast, pea a member of the graminae is salt tolerant contains less amount of betaine and is able to accumulate a greater quantity under conditions of saline stress.

Starting from the fourth day of germination, pre-treatment with vitamin C led to a significant increase in betaine levels in vitamin C and in vitamin C versus C and S seedlings in the two plants. Such an increase may be attributed to the fact that the addition of this precursor (vitamin C) promotes betaine formation by stimulating its biosynthesis[12].

The protective role of betaine against saline stress both in higher plants, in bacteria and animals is widely recognized[28]. The significant increase of this osmolyte in plant tissue from seeds pre-treated with vitamin C would help to explain the increase in tolerance to salinity. The accumulation recorded in seedlings starting from the fourth day could be responsible for the enhanced growth observed in vitamin C versus S seedlings, as well as for preventing the decrease in chlorophyll content in the vitamin C group.

Silvana *et al.*[34] reported that salt stress increased the accumulation in roots, stems and leaves of lipid peroxidation products produced by interactions with damaging active oxygen species. Additional ascorbic acid partially inhibited this response but did not significantly reduce sodium uptake or plasma membrane leakiness.

The mechanisms by which plants defend themselves against saline stress are indeed many fold; many are still unclear and they may vary according to the ontogenetic stage. Although bean is a betaine accumulating plant, it is sensitive to stress induced by sodium chloride. Meanwhile pea which is less sensitive to saline stress accumulates fewer amounts of a betaine as well as proline. Krishna[14], studies the effect of vitamin C on barley leaf cell ultrastructure and show that vitamin C had no effect on the leaf cell ultrastructure under normal conditions, but damage induced by salt stress on nuclei and chloroplasts was significantly reduced by vitamin C treatment. In

another study, rice seeds soaked in water or 150 mM NaCl in presence or absence of vitamin C were tested for germination and seedlings growth. When the salt solution was supplemented with vitamin C, the inhibitory effect of salt on germination was reduced considerably[14]. The promotion of growth by vitamin C under salt stress conditions was associated with enhanced levels of nucleic acids and soluble proteins[2].

The results obtained in this work strongly suggest that bean accumulate more betaine than pea thus was attributed to the less sensitive of pea to salt stress, so its low needs to osmolyte.

Pre-treatment with vitamin C showed an increase in osmolyte (proline and betaine) during germination, because its biosynthetic precursor, vitamin C, could activate BADH, thus enhancing the accumulation of proline and betaine. The accumulation of these osmolyte seems to correlate with greater tolerance against stress. In a more general context, it could be said that the formation of a compatible osmolytes such proline and betaine, capable of stabilizing membranes and proteins, is responsible for the increase in tolerance against saline stress.

Adaptation to high NaCl levels involves an increase in the antioxidant capacity of the cell to detoxify reactive oxygen species[4]. In concordance to Hernadez *et al*.,[10] the profile observed in glutathione (GSH) content could indicate that this antioxidant soluble compound was involved in the salt tolerance. The increase in glutathione content due to vitamin C treatment enhanced salt tolerance of both plants, may be totally or partly due to increased GSH synthesis and/or decrease rates of degradation[26].

The obtained result shows that the adaptation to high NaCl (Vitamin C) involves an increase in the antioxidant capacity (GSH) of the cell to detoxify reactive oxygen species through both enzymatic and non enzymatic reactions[34].

To sum up, the present work demonstrates clearly that adaptation to high NaCl (salt tolerance) involves ant increase in betaine and antioxidant (glutathione). In contrast salt stress produces increase in proline content[35]. Summing up, the adaptation to live in high salinity elucidate from the proposed index of halophytism.

The obtained results showed a clear link between the proposed HIB, HCB and the growth of the two plants under salt stress. In S group all HCB equal (II), meanwhile, it was III in control samples. In contrast, the addition of vitamin C to growth medium raises the HCB value with or without salt stress. In the vitamin C group the highest HCB values (V). So, it may be concluded that the addition of vitamin C to growth medium. The relationship between the halophytism index (HIB) and growth of the two plants were estimated. It may be concludes that, the interaction between osmolytes and antioxidant in HIB and HCB by vitamin C treatments was useful in crop improvement the tolerant to salt stress.

Once the mechanism of vitamin C action is better understood, new opportunities for agricultural biotechnology may become evident. Alongside unraveling the vitamin C mode of action, other aspects such as uptake, transport and stability of vitamin C as well as the development of vitamin C analogues with high activity, should continue to be explored. It is only with this combined knowledge that unique mechanisms of stress resistance can lead to implementations, with predictable effects of vitamin C application in the field, allowing for the full potential of vitamin C to be harnessed in the future.

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