Effects of ascorbic acid, charcoal, glucose, and salicylic acid in nutrient solutions on vegetative growth and the susceptibility of *Phaseolus vulgaris* to sodium chloride

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ABSTRACT
The effects of ascorbic acid, charcoal, glucose and salicylic acid, each added separately to normal nutrient solution, on the vegetative growth of *Phaseolus vulgaris* and also their ameliorative role when 75 mM NaCl was added were studied. Application of 20, 40 and 80 mg l^{-1} ascorbic acid and 20 mg l^{-1} salicylic acid generally induced larger leaf area and higher leaf, stem and root dry weights compared with normal nutrient solution. Glucose at 0.5 g l^{-1} increased root dry weight, but at higher concentrations glucose and charcoal inhibited growth. In all the treatments containing NaCl, leaf succulence and foliar injury developed and vegetative growth was drastically reduced. However, 20 mg l^{-1} ascorbic acid and to a lesser extent, 0.5 g l^{-1} glucose, partially ameliorated the NaCl stress by increasing leaf area, stem and total plant dry weights, relative to treatment with NaCl alone. All other treatments either had little ameliorative effect or increased the susceptibility of *P. vulgaris* to NaCl stress.

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RÉSUMÉ
BUXTON, G., GEYER, B. & HUYSKENS-KEIL, S.: *Effets d'acide ascarbique, charbon de bois, glucose et acide salicylique en solutions nutritives sur la croissance végétative et susceptible de* Phaseolus vulgaris *au chlorure de sodium.* Les effets d'acide ascorbique, charbon de bois, glucose et acide salicylique chacun ajouté séparément aux solutions nutritives normales, à la croissance végétative de *Phaseolus* et aussi leur rôle améliorati f lorsque 75 mm de NaCl était ajouté, étaient étudiés. L'application de 20, 40, et 80 mg l^{-1} d'acide ascorbique et 20 mg l^{-1} d'acide salicylic provoquait en général une surface foliaire plus large et des poids de feuille, de tige et de racine sèches plus élevées par comparaison avec une solution nutritive normale. Le glucose à 0.5 g l^{-1} augmentait le poids de racine sèche, mais aux concentrations plus élevées le glucose et le charbon de bois empêchaient la croissance. Dans tous les traitements contenant NaCl, la succulence de feuille et la lésion foliaire dévélopaient et la croissance végétative était considérablement réduite. Toutefois, 20 mg l^{-1} d' acide ascorbique et dans le plus faible mesure, 0.5 g l^{-1} de glucose améliorait partiellement la tension de NaCl par l'augmentation de surface foliaire, les poids de tige et de plante totale sèches, relative au traitement avec NaCl seul. Tout autre traitements avaient un petit effet amélioratif ou bien augmentaient la susceptibilité de *Phaseolus* à la tension de NaCl.

Introduction

The sensitivity of many glycophytic plants to salinity stress has been a problem affecting production agriculture in arid and semi arid regions (Liang *et al.*, 1996) and in areas where saline water is used in irrigation practices (Awada *et al.*, 1995; Khan, Ahmed & Blume, 1996). Salinity adversely affects the physiology, growth and yield of crops (Greenway & Munns, 1980); therefore, there is the need to improve plant performance under salinity stress.

Others have reported that certain solutes, when added to the root medium, alleviate the harmful effect of salinity and improve stress resistance. Nitrogen fertilization, for example, has improved plant growth, reproduction, and physiology under salinity stress (Bernstein, Francois & Clark, 1974; Feigin *et al.* 1987, El-Siddig & Lüdder, 1993). Similarly, addition of Ca (LaHaye & Epstein, 1971; Cramer, Epstein & Lauchli, 1988; Zekri & Parson, 1990; Akhavan-Khazian *et al.*, 1991; Lopez & Satti, 1996; Duvenport, Reid & Smith, 1997), Zn (Shukla & Makhi, 1980; Khan *et al.*, 1996), Si (Bradbury & Ahmed, 1990; Liang *et al.*, 1996), K (Lopez & Satti, 1996), and P (Awad, Edwards & Campbell, 1990; Zaiter & Saade; 1993; Aslam *et al.*, 1991) to saline growing media have mostly improved growth and development of plants. However, the ameliorative effects of these antistressors have not been shown in some reports (LaHaye & Epstein, 1971; Akhavan-Khazian *et al.*, 1991; Rengel, 1992).

Phaseolus vulgaris is sensitive to salinity and suffers growth reduction even at moderate salinity (LaHaye & Epstein, 1971; Smith & Mc-Comb, 1981; Akhavan-Khazian *et al.*, 1991). The addition of Ca to the root medium improves its performance under salinity (LaHaye & Epstein, 1971; Cachorro, Ortiz & Cerda 1993a; Awada *et al.*, 1995). Ascorbic acid (Golan-Goldhirsh, Mozafar & Oertli, 1995), salicylic acid (Kauss *et al.*, 1992), and charcoal (Ishii & Kadoya, 1994; Paul & Rajeevan, 1992) have been observed to promote growth of plants considerably under some other stressful conditions.

In this study, the effectiveness of these solutes and glucose in promoting vegetative growth of *P. vulgaris* under normal conditions, and also their possible ameliorative role when the plant is growing under salinity stress was evaluated.

Materials and methods

Seeds of *P. vulgaris* L. var. Manus Brilliant were germinated on moistened filter paper in the greenhouse of the Humboldt University, Institute for Horticultural Crop Science, Quality Dynamics/ Post Harvest Physiology, Berlin. When the seedlings were 3 days old, they were placed individually in holes in the lid of 3.2-*l* containers filled with aerated distilled water. After 5 days, the distilled water was replaced with aerated nutrient solution of the following composition: $Ca(NO_3)_2$, 510.8 g; KNO_3, 332.0 g; KH_2PO_4, 168.0 g; (30 % K_2O and 10 g MgO), 336.3 g; $Na_2B_4O_7$, 5.0 g; $MnSO_4$, 2.5 g; $CuSO_4$, 0.25 g; $ZnSO_4$, 0.25; $(NH_4)_6Mo_7O_{24}$, 0.05 g 1000 l^{-1} of distilled water.

The seedlings were grown in nutrient solution alone or nutrient solution to which 75 mM NaCl has been added. Twenty, 40 and 80 mg l^{-1} ascorbic acid, 10 g l^{-1} activated charcoal, 0.5, 1.0, and 2.0 g l^{-1} glucose, and 10 and 20 mg l^{-1} salicylic acid were each added to the nutrient solution with or without 75 mM NaCl. In all, there were 20 treatments and each treatment consisted of four replicates with three plants in a replicate.

The *p*H and electrical conductivity (EC) of the experimental solution were measured twice weekly. Table 1 summarizes the results. Mean day and night greenhouse temperatures were 24.8 ± 1.9 and 19.0 ± 0.2 °C, respectively, while mean relative humidites were 32.0 ± 3.9 and 46.5 ± 1 per cent for day and night, respectively. The greenhouse was lighted for 11 h per day using Osram HQI-TS 400 W light. Light intensity at plant tops was 4600 Lux m^{-2}. Daily visual observations on NaCl toxicity symptoms were made. Plants were harvested 21 days after the treatments had been applied. Leaf area (single sided) was measured with a leaf-area meter (LI-COR Type: LI-3100). Root, stem, and leaf dry weights were determined after the samples were dried at 80 °C for 72 h. Leaf succulence (increase in leaf thickness of treated plants relative to control) was calculated as fresh weight/leaf area.

The completely randomised block design was used for the experimental set up. The data were analysed statistically using the analysis of variance, and the least significant difference was used to test treatment means.

TABLE 1

pH and Electrical Conductivities (µS/cm⁻¹) of the Nutrient Solutions Used

Experimental solution	*Range of pH*	*Electrical conductivity (µS/cm⁻¹)*
Normal nutrient solution (NNS)	6.6-8.0	2.5 ± 0.2
NNS + 75 mM NaCl	6.7 - 7.3	10.1 ± 0.3
NNS + 20 mg l^{-1} ascorbic acid	6.6 - 7.8	2.4 ± 0.2
NNS + 20 mg l^{-1} ascorbic acid*	6.5 - 7.7	10.4 ± 0.4
NNS + 40 mg l^{-1} ascorbic acid	6.5 - 8.1	2.6 ± 0.3
NNS + 40 mg l^{-1} ascorbic acid*	6.8 - 7.6	10.3 ± 0.7
NNS + 80 mg l^{-1} ascorbic acid	6.9 - 8.3	2.5 ± 0.3
NNS + 80 mg l^{-1} ascorbic acid*	6.5 - 7.8	10.3 ± 0.3
NNS + 10 g l^{-1} charcoal	6.7 - 7.6	2.3 ± 0.2
NNS + 10 g l^{-1} charcoal*	6.8 - 7.7	10.3 ± 0.2
NNS + 0.5 g l^{-1} glucose	6.5 - 8.2	2.6 ± 0.3
NNS + 0.5 g l^{-1} glucose*	6.5 - 8.2	10.3 ± 0.2
NNS + 1.0 g l^{-1} glucose	6.7 - 8.7	2.4 ± 0.3
NNS + 1.0 g l^{-1} glucose*	6.4 - 8.8	10.3 ± 0.5
NNS + 2.0 g l^{-1} glucose	6.6 - 8.7	2.2 ± 0.2
NNS + 2.0 g l^{-1} glucose*	6.7 - 8.8	10.2 ± 0.7
NNS + salicylic acid 10 mg l^{-1}	6.7 - 8.0	2.3 ± 0.2
NNS + salicylic acid 10 mg l^{-1}*	6.6 - 8.0	10.1 ± 0.3
NNS + salicylic acid 20 mg l^{-1}	6.8 - 7.9	2.5 ± 0.3
NNS + salicylic acid 20 mg l^{-1}*	6.7 - 7.7	10.1 ± 0.3

* 75 mM NaCl added

Results

Foliar injury

The results of the study showed that by day 5 (d5), young leaves on plants in nutrient solution with added charcoal had become chlorotic (pale yellow). These leaves later developed reddish spots. Chlorosis (pale green) was observed in young leaves of treatments with 2.0 g l^{-1} (d5) or 1.0 g l^{-1} (d7) glucose (Table 2).

In NaCl-treated plants, mature leaves developed tip and marginal necrosis which spread later to the blade. Injury appeared first in plants treated with salicylic acid (d5), followed by those with 80 mg l^{-1} ascorbic acid (d7), then the treatments with 75 mM NaCl alone, 1.0 g l^{-1} glucose, 40 mg l^{-1} ascorbic acid, or 10 g l^{-1} charcoal (d9). Foliar injury was not observed in plants treated with 20 mg l^{-1} ascorbic acid, and 0.5 and 2.0 g l^{-1} glucose until day 16. However, the intensity was low. Under salinity also, charcoal induced chlorosis (pale yellow, d7) with red spots in newly developed leaves.

Effect on leaf growth

Fig. 1a shows the effect of the treatment without NaCl on leaf area of *Phaseolus*. Treatments with 20, 40, and 80 mg l^{-1} ascorbic acid and 20 mg l^{-1} salicylic acid increased leaf area to 113, 117, 106 and 115 per cent of control, respectively. However, the increases were not significant compared with the controls. On the other hand, leaf area was significantly reduced to 47, 70 and 20 per cent for 10 g l^{-1} charcoal, 1.0 and 2.0 g l^{-1} glucose, respectively, relative to the control. The effect of 0.5 g l^{-1} glucose and 10 mg l^{-1} salicylic acid on leaf area of *Phaseolus* was generally similar to that of the control. All the treatments to which 75 mM NaCl was added reduced leaf area significantly (Fig. 1b). In nutrient solution with NaCl, leaf area was reduced to 26 per cent of the control, but the corresponding reductions were 37 and 32 per cent of the control for NaCl treatment with 20 mg l^{-1} ascorbic acid and 0.5 g l^{-1} glucose, respectively. Thus, relative to treatment with NaCl alone, 20 mg l^{-1} ascorbic acid increased leaf area significantly by 46 per cent and 0.5 g l^{-1} glucose by 23 per cent, though the result was not significantly different. Sodium chloride treatments with 40 and 80 mg l^{-1} ascorbic acid, 10 g l^{-1} charcoal, 1.0 and 2.0 g l^{-1} glucose, and 10 and 20 mg l^{-1} salicylic acid further reduced leaf area relative to the result from treatment with NaCl alone; with 2.0 g l^{-1} glucose producing the poorest growth (16 % of NaCl value).

Fig. 2 shows the effect of the treatments on leaf dry weight. All the three levels of ascorbic acid (20, 40 and 80 mg l^{-1}) and 20 mg l^{-1} salicylic acid when added separately to nutrient solution

TABLE 2

Foliar Injury Symptoms Induced in P. vulgaris

Treatment	*Tip and marginal necrosis*	*Chlorosis*
	Days after treatment	
Normal nutrient solution (NNS)	-	-
NNS + 1.75 mM NaCl	9	-
NNS + 20 mg l^{-1} ascorbic acid	-	-
NNS + 20 mg l^{-1} ascorbic acid*	16	-
NNS + 40 mg l^{-1} ascorbic acid	-	-
NNS + 40 mg l^{-1} ascorbic acid*	9	-
NNS + 80 mg l^{-1} ascorbic acid	-	-
NNS + 80 mg l^{-1} ascorbic acid	7	-
NNS + 10 g l^{-1} charcoal	-	5
NNS + 10 g l^{-1} charcoal*	9	7
NNS + 0.5 g l^{-1} glucose	-	-
NNS + 0.5 g l^{-1} glucose*	16	-
NNS + 1.0 g l^{-1} glucose	-	7
NNS + 1.0 g l^{-1} glucose*	9	-
NNS + 2.0 g l^{-1} glucose	-	5
NNS + 2.0 g l^{-1} glucose*	16	-
NNS + 10 mg l^{-1} salicylic acid	-	-
NNS + 10 mg l^{-1} salicylic acid*	5	-
NNS + 20 mg l^{-1} salicylic acid	-	-
NNS + 20 mg l^{-1} salicylic acid*	5	-

* 75 mM NaCl added - No symptoms observed

increased leaf dry weight slightly to 112, 118, 109 and 110 per cent of the control values, respectively (Fig. 2a). Similar to the results for leaf area, ascorbic acid at 40 mg l^{-1} was most effective. Application of 0.5 g l^{-1} glucose and 10 mg l^{-1} salicylic acid did not generally affect leaf dry weight whilst 10 g l^{-1} charcoal and 1.0 and 2.0 g l^{-1} glucose significantly decreased leaf dry weight to 54, 84 and 25 per cent of the control, respectively. Leaf dry weight was drastically reduced in all the treatments containing NaCl. In nutrient solution with NaCl alone, leaf dry weight was 37 per cent of the control, but increased to 49 per cent in NaCl treatment with 20 mg l^{-1} ascorbic acid. This shows a significant improvement in leaf dry weight of 31 per cent over the result of the NaCl treatment alone. On the other hand, 0.5 g l^{-1} glucose in NaCl nutrient solution did not affect leaf dry weight appreciably, but all the other treatments further reduced leaf dry weight relative to the treatment with NaCl alone (Fig. 2b).

Effect on stem growth

Stem dry weight was increased by 20, 40 and 80 mg l^{-1} ascorbic acid and 20 g l^{-1} salicylic acid to 125, 120, 103 and 112 per cent, respectively, of the control values (Fig. 3) when they were added separately to normal nutrient solution. However, only the increases induced by ascorbic acid at 20 mg l^{-1} were significantly different from the controls. The effect of 0.5 g l^{-1} glucose or 10 mg l^{-1} salicylic acid on stem dry weight was generally similar to that of the control. On the other hand, 10 g l^{-1} charcoal and 1.0 and 2.0 g l^{-1} glucose each in nutrient solution significantly reduced stem dry weight (60, 76 and 38 per cent of control, respectively) with the latter treatment showing pronounced effect. Stem growth reduced significantly in all the treatments containing NaCl (Fig. 3b). In the treatment with NaCl alone, stem dry weight decreased to 42 per cent of the control value; but in NaCl medium with 20 mg l^{-1} ascorbic acid and 0.5 g l^{-1} glucose, stem dry weight was 57 and 50 per cent of the control, respectively, thus giving significant increases of 36 and 19 per cent, respectively, relative to the treatment with NaCl alone. In contrast, 40 mg l^{-1} ascorbic acid did not improve stem dry weight whilst all the other remaining treatments further reduced stem dry weight when added to medium containing NaCl.

Effect on root growth

Fig. 4a shows the effect of the treatment on root dry weight. Only 40 mg l^{-1} ascorbic acid and

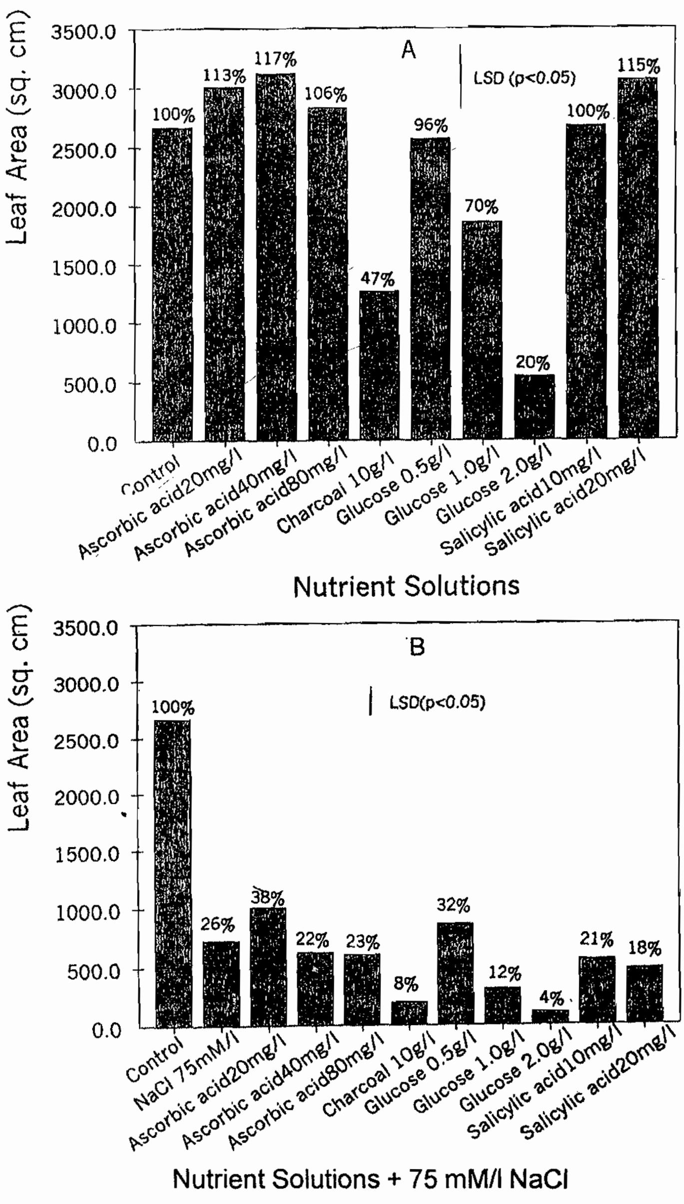


Fig. 1. Effect of ascorbic acid, charcoal, glucose and salicylic on leaf area of *Phaseolus* grown in nutrient solution (A) and in nutrient solution with 75 mM l^{-1} NaCl added (B) compared with control.

Values shown above bars are percent of control.

0.5 g l^{-1} glucose caused appreciable increase in root dry weight (though, not significant) to 118 and 110 per cent relative to the control, respectively. Application of 10 g l^{-1} charcoal and 1.0 and 2.0 g l^{-1} glucose each in nutrient solution significantly reduced root dry weight to 74, 87 and 47 per cent, respectively. In treatments with NaCl, root dry weight was significantly reduced (Fig. 4b). In treatments with 20 and 40 mg l^{-1} ascorbic acid, 0.5 g l^{-1} glucose and 10 mg l^{-1} salicylic acid, root dry weight was reduced to 57, 41, 56 and 42 per cent of the control, respectively. These were not significantly different from the value (51% of control) recorded for treatment with NaCl alone. In contrast, all the other treatments further reduced root dry weight compared with treatment with NaCl alone.

Effect on total plant growth

Fig. 5a shows the effect of the non-saline treatment as depicted by overall total plant dry weight. Ascorbic acid at 40 mg l^{-1} increased plant dry weight significantly to 118 per cent of the control. The application of 20 mg l^{-1} ascorbic acid (114 %), 20 mg l^{-1} salicylic acid (109 %), and 80 mg l^{-1} ascorbic acid (107 %) increased plant dry weight but not significantly, compared with the control. Ten mg l^{-1} salicylic acid and 0.5 g l^{-1} glucose had generally no effect (99 and 97 per cent of control, respectively), whilst 10 g l^{-1} charcoal and 1.0 and 2.0 g l^{-1} glucose significantly reduced plant dry weight to 60, 75 and 46 per cent of the control, respectively (Fig. 5a).

Salinity caused significant reductions in overall plant growth (Fig. 5b). In treatment where NaCl

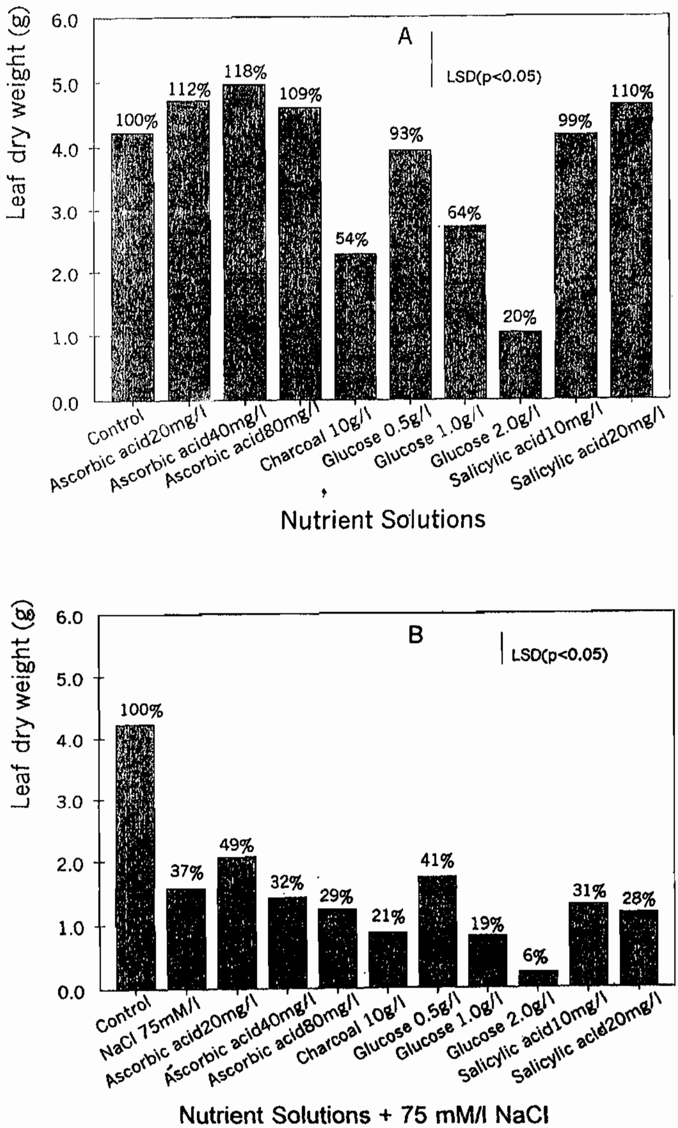


Fig. 2. Effect of ascorbic acid, charcoal, glucose and salicylic on leaf dry weight of *Phaseolus* grown in nutrient solution (A) and in nutrient solution with 75 mM l^{-1} NaCl added (B) compared with control.

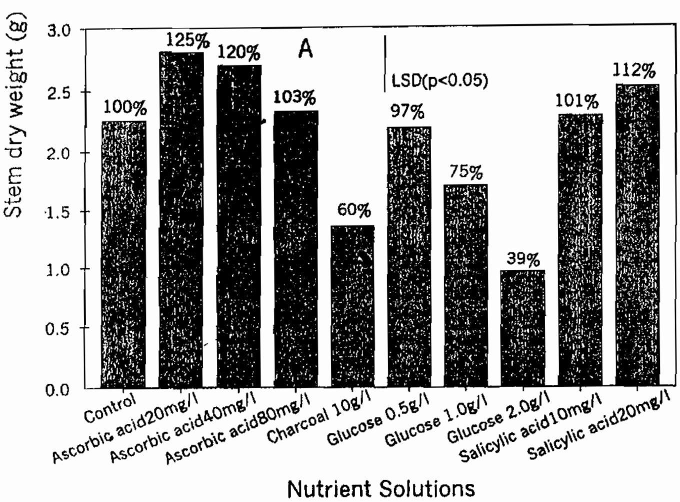
Values shown above bars are percent of control.

alone was added to the nutrient solution, total plant dry weight was reduced to 41 per cent of the control, but the corresponding reductions were 53 and 46 per cent of the control for NaCl treatment with 20 mg l^{-1} ascorbic acid and 0.5 g l^{-1} glucose, respectively. Thus, relative to treatment with NaCl alone, 20 mg l^{-1} ascorbic acid increased plant dry weight significantly by 29 per cent and 0.5 g l^{-1} glucose by 12 per cent, although this was not significant. In contrast, the other treatments did not improve the inhibitory effect of NaCl stress on the growth of the plant; rather, 10 g l^{-1} charcoal and 1.0 and 2.0 g l^{-1} glucose significantly reduced the overall plant dry weight further, compared with treatment with NaCl alone (Fig. 5b).

Effects on shoot root ratio

Table 3a shows the shoot root ratio of the various non-saline treatments. Treatments with 20 mg l^{-1} ascorbic acid and 20 mg l^{-1} salicylic acid increased shoot root ratio slightly to 111 and 109 per cent, respectively, of the control, but like treatments with 40 and 80 mg l^{-1} ascorbic acid, 10 mg l^{-1} salicylic acid and 0.5 g l^{-1} glucose, the results are not significantly different from the control. On the other hand, 10 g l^{-1}, charcoal and 1.0 and 2.0 g l^{-1} glucose, when added to normal nutrient solution, significantly reduced shoot root ratio to 75, 75 and 56 per cent, respectively, of the control.

Salinity significantly reduced shoot root ratio (Table 3b). In NaCl nutrient solution, shoot root ratio was reduced to 79 per cent of the control. The addition of 20 mg l^{-1} ascorbic acid to the NaCl containing media reduced shoot root ratio but only to 89 per cent of the control, resulting in a significant increase of



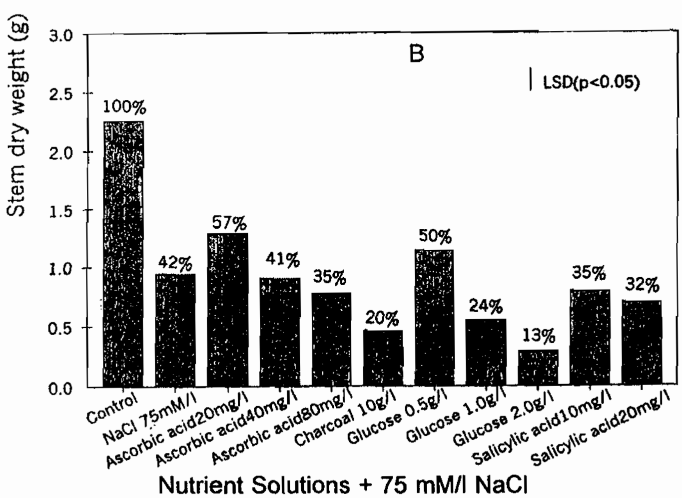


Fig. 3. Effect of ascorbic acid, charcoal, glucose and salicylic on stem dry weight of *Phaseolus* grown in nutrient solution (A) and in nutrient solution with 75 mM l^{-1} NaCl added (B) compared with control.

Values shown above bars are percent of control.

15 per cent relative to treatment with NaCl alone. Forty and 80 mg l^{-1} ascorbic acid, 0.5 g l^{-1} glucose, and 10 and 20 mg l^{-1} salicylic acid did not improve shoot root ratio, whilst all the other treatments further reduced shoot root ratio compared with treatment with NaCl alone.

Effect on leaf succulence .

Table 3a shows the results of leaf succulence for non-saline treatments. All levels of ascorbic acid and salicylic acid and also 0.5 and 1.0 g l^{-1} glucose did not significantly affect leaf succulence. In contrast, 10 g l^{-1} charcoal and 2.0 g l^{-1} glucose significantly induced succulence by increasing leaf thickness to 125 and 115 per cent, respectively, compared to the control.

With the exception of the treatment with 2.0 g l^{-1} glucose, all treatments containing NaCl significantly induced leaf succulence (Table 3b). The treatment containing NaCl alone produced the maximum succulence of 145 per cent relative to the control (no salt), with treatments containing 20 mg l^{-1} ascorbic acid (133 %), 0.5 g l^{-1} glucose (136 %), or 10 mg l^{-1} salicylic acid (131 %) producing significantly similar effect like the treatment with NaCl alone. However, leaf succulence induced when 40 and 80 mg l^{-1} ascorbic acid, 10 g l^{-1} charcoal, 1.0 and 2.0 g l^{-1} glucose, and 10 and 20 mg l^{-1} salicylic acid were added separately to the NaCl nutrient solution was less pronounced. The values ranged from 125 to 129 per cent of the control, and were significantly lower than the treatment with NaCl alone. In media containing NaCl, 2.0 g l^{-1} glucose did not induce leaf succulence significantly.

Discussion

The study shows that ascorbic acid and salicylic acid are generally beneficial to vegetative growth

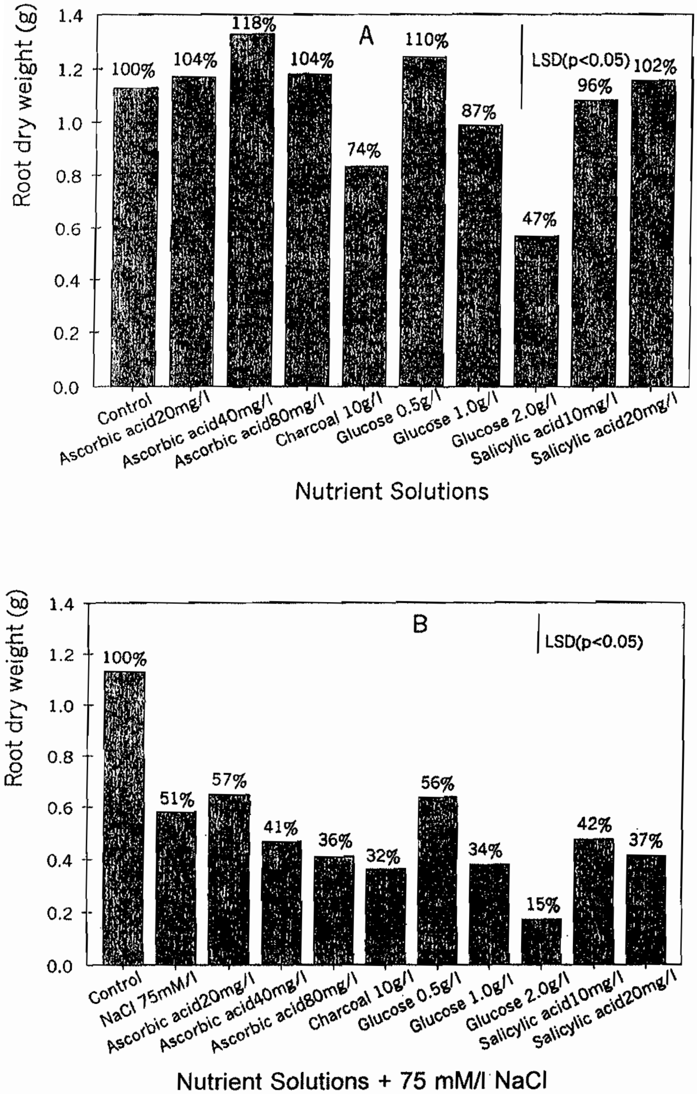


Fig. 4. Effect of ascorbic acid, charcoal, glucose and salicylic on root dry weight of *Phaseolus* grown in nutrient solution (A) and in nutrient solution with 75 mM l^{-1} NaCl added (B) compared with control.

Values shown above bars are percent of control.

of *Phaseolus* when added separately to normal nutrient solution. These two solutes each increased leaf area of *Phaseolus,* with ascorbic acid at 40 mg l^{-1} producing the largest leaf area. Ascorbic acid is known to accelerate physiology of synthesis in plants (Purves, Oriang & Heller, 1992), and it has also been associated with cell wall expansion (Smirnoff, 1996). These properties could partly have contributed to the increase in leaf area. Salicylic acid, on the other hand, is known to occur in a variety of plants, and is thought to be an important plant growth substance (Raskin, 1992 a & b). Its growth promoting effect on *Phaseolus* may possibly be by altering the balances in plant growth regulators in such a way as to favour leaf expansion. Through the production of larger leaf areas (compared to control), ascorbic acid and salicylic acid could directly have a positive effect on the photosynthetic machinery which directly determines growth and dry matter production. Thus, plant dry weight was increased by ascorbic acid or salicylic acid in a manner which could be generally associated with the observed increases in leaf area. Again, 40 mg l^{-1} ascorbic acid produced maximum overall plant dry weight and also leaf and root dry weights.

Plant growth stimulating effect of ascorbic acid and salicylic acid has been reported. Saraswathamma (1981) observed that presoaking seeds of *Trigonella faerumgraecum* L. var pusa in ascorbic acid promoted shoot and root growth of the subsequent seedlings developed. Raskin (1992b) reported that salicylic acid affects a variety of plant processes including the stimulation of plant height and flowering. Salicylic acid also stimulated

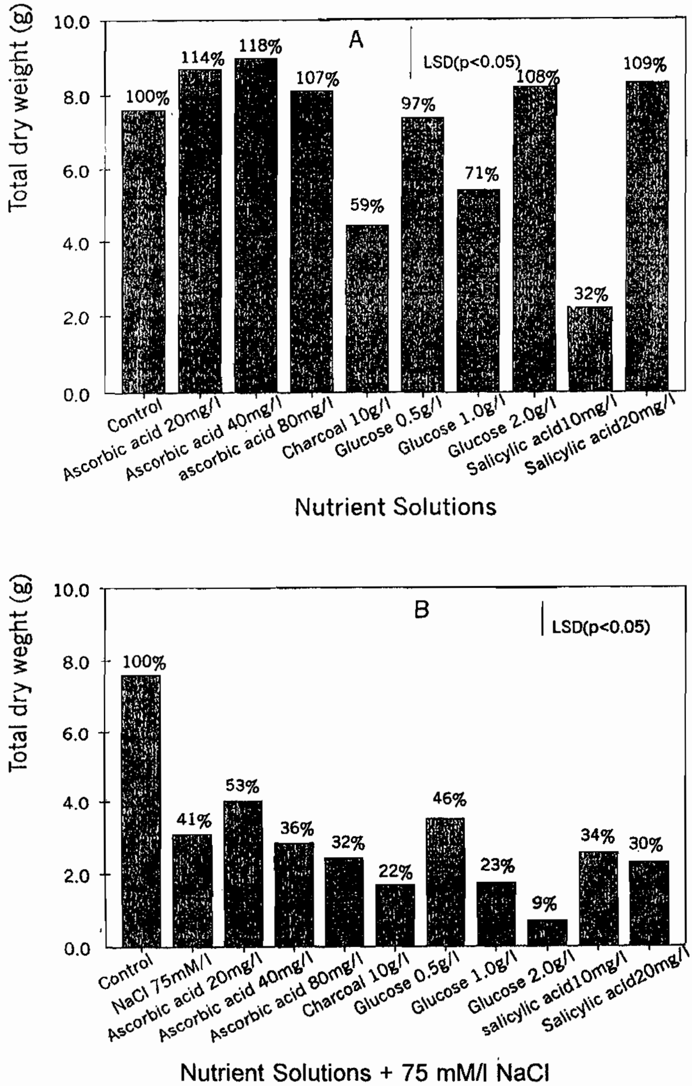


Fig. 5. Effect of ascorbic acid, charcoal, glucose and salicylic on total plant dry weight of *Phaseolus* grown in nutrient solution (A) and in nutrient solution with 75 mM l^{-1} NaCl added (B) compared with control. Values shown above bars are percent of control.

adventitious root development of cuttings of *Phaseolus* (Kling & Meyer, 1983). However, its beneficial effect on root growth was not shown in this study on whole plants of *Phaseolus*.

Unlike ascorbic acid and salicylic acid, charcoal and glucose (particularly at the higher concentrations, 1.0 and 2.0 g l^{-1}) were inhibitory to leaf, stem, and root growth of *Phaseolus*. Charcoal and glucose also induced leaf chlorosis, suggesting that they adversely affected chlorophyll synthesis and or caused chlorophyll destruction. This response, together with the observed small leaf areas they produced, could have reduced the C-assimilation capacity of the leaves and consequently poor growth. Furthermore, the observed significant decrease in shoot root ratio may suggest that the carbon partitioning processes in the plant were also altered by charcoal and glucose at high concentrations. Glucose of lower concentration (0.5 g l^{-1}) did not induce chlorosis, and like treatment with 40 mg l^{-1} ascorbic acid, it improved root growth. Increase in root growth may suggest that more water is absorbed by the roots for growth and physiological processes. This perhaps partly explains the better growth with lower glucose concentration than with higher concentrations. Unlike its growth inhibitory effect on *Phaseolus,* charcoal has been observed to increase vegetative growth in *Dendrobium* (Paul & Rageevan, 1992; Kumar, 1992), in tomato (Teo & Tan, 1993), and in *Citrus,* particularly the stimulation of root elongation (Ishii & Kadoya, 1994).

In all the treatments with NaCl, vegetative growth was severely inhibited but leaf succulence was increased. Such responses have been reported as the normal growth behaviour of

TABLE 3

Effect of Ascorbic Acid, Charcoal, Glucose and Salicylic Acid on Shoot/Root Dry Weight and Leaf Succulence of Phaseolus *Grown in Nutrient Solution (A) and in Nutrient Solution with 75 mM l^{-1} NaCl added (B) Compared with the Control*

(A)

Experimental solution	*Shoot root dry weight*	*Leaf succulence (leaf fresh weight/leaf area × 10^{-3})*
Normal nutrient solution (NNS)	5.7	1.66
NNS + 20 mg l^{-1} ascorbic acid	6.3 (111)	1.4 (99)
NNS + 40 mg l^{-1} ascorbic acid	5.7 (100)	1.66 (100)
NNS + 80 mg l^{-1} ascorbic acid	5.8 (101)	1.161 (97)
NNS + 10 g l^{-1} charcoal	4.3 (75)	2.08 (125)
NNS + 0.5 g l^{-1} glucose	5.1 (89)	1.4 (99)
NNS + 1.0 g l^{-1} glucose	4.3 (75)	1.71 (103)
NNS + 2.0 g l^{-1} glucose	3.1 (5)	1.90 (115)
NNS + 10 mg l^{-1} salicylic acid	5.9 (104)	1.68 (101)
NNS + 20 mg l^{-1} salicylic acid	6.2 (109)	1.66 (100)
LSD ($P<0.05$)	1.0	0.20

(B)

NNS	5.7	1.66
NNS + 75 mM l^{-1} NaCl	4.5 (79)	2.40 (145)
NNS + 20 mg l^{-1} ascorbic acid	5.1 (89)	2.20 (133)
NNS + 40 mg l^{-1} ascorbic acid	4.7 (83)	2.10 (127)
NNS + 80 g l^{-1} ascorbic acid	4.8 (85)	2.14 (129)
NNS + 10 g l^{-1} charcoal	3.6 (64)	2.09 (126)
NNS + 0.5 g l^{-1} glucose	4.4 (78)	2.25 (136)
NNS + 1.0 g l^{-1} glucose	3.5 (62)	2.14 (129)
NNS + 2.0 g l^{-1} glucose	3.1 (54)	1.74 (105)
NNS + 10 mg l^{-1} salicylic acid	4.5 (79)	2.17 (131)
NNS + 20 mg l^{-1} salicylic acid	4.6 (80)	2.08 (125)
LSD ($P<0.05$)	0.5	0.40

75 mM NaCl added. Figures in brackets are percent of NNS

Phaseolus under salinity stress (Lessani & Marschner, 1978; Cachorro, Ortiz & Cerda, 1993b; Awada *et al.*, 1995). According to Greenway & Munns (1980) and Wyn Jones (1981), salinity inhibits plant growth through a combination of osmotic stress causing plant water deficit, specific ion toxicity, and imbalances in essential nutrient ions. The observed foliar injury of *Phaseolus* is a clear indication of ion toxicity which is associated with imbalance in the plant tissue (Cheeseman, 1988; Awada *et al.*, 1995). Similar visible symptoms of salt toxicity associated with poor growth of *Phaseolus* have been reported by Zaiter & Mahfourz (1993) and Cachorro *et al.* (1993b). They explained that *Phaseolus* is unable to compartmentalise effectively, the excess salt it accumulates and ion toxicity therefore develops, disrupting the photosynthetic machinery. In this study, salinity induced leaf succulence in *Phaseolus,* but did not cause enough tissue dilution to offset the development of the toxicity symptoms (Jennings, 1976).

Ascorbic acid and salicylic acid improved vegetative growth of *Phaseolus* under normal conditions, and were therefore expected to be of some benefits to growth under salinity stress. However, only treatments containing 20 mg l^{-1} ascorbic acid and to a lesser extent, 0.5 g l^{-1} glucose, partially alleviated the adverse effect of salinity stress on *Phaseolus* by increasing plant growth. It appears that when salinity stress is over a short duration (about 2 weeks), low concentrations of ascorbic acid or glucose in the root medium suppress the development of ion toxicity in the plant and thus partially alleviated the adverse effect of NaCl stress on plant growth. This effect perhaps may involve some mechanisms which prevent or reduce salt uptake and transport to the leaves and or allow some degree of compartmentalization of the salt in the plant tissue. As to how ascorbic acid operates to effect these suggested mechanisms is not clear (Flowers & Yeo, 1986). On the other hand, it seemed that glucose, a respirable material, at low concentration was beneficial to the respiratory processes of *Phaseolus.* Thus, presumably, it contributed to the high energy demand for either salt exclusion from the root and or for compartmentalization of excess internal ions (Cheeseman, 1988).

Salicylic acid is reported to regulate some aspects of disease resistance in plants and thus, prevents the development of stressful conditions in plants (Malamy & Klessig 1992; Kauss *et al.,* 1992). Activated charcoal is also known to be a protectant against phytotoxicity induced by some stress (Mueller-Warrant, Mellbye & Aldrich-Markham, 1991). However, under NaCl stress, it seemed these two solutes enhanced NaCl phytotoxicity as was shown by the early development of severe leaf injury symptoms, and thus, they could not ameliorate the susceptibility of *Phaseolus* to NaCl stress.

In conclusion, supplementing normal nutrient medium with 20, 40 or 80 mg l^{-1} ascorbic acid or 20 mg l^{-1} salicylic acid is beneficial in improving vegetative growth of *Phaseolus*, while glucose or activated charcoal inhibits growth. Also, low concentrations of ascorbic acid, and to a lesser extent, low concentrations of glucose, partially alleviate growth inhibition by salinity stress. It is suggested that low levels of ascorbic acid and glucose may prove beneficial to growth during short-term exposures of *Phaseolus* to salinity stress.

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