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Effects of salinity on growth and metabolism of *Phaseolus vulgaris*

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Abstract

Increasing salinity induced a marked reduction in the plant growth, though *Phaseolus* seedlings tolerated salinity up to 120 mM NaCl. A great reduction in sugar and protein contents occurred with increasing salinity, whereas soluble nitrogen compounds and the relative contents of the photosynthetic pigments were increased in the treated plants. Increasing Ca concentration in the salinized medium appeared to improve the plant growth and to increase the contents of saccharides and proteins in the NaCl-treated plants. This suggests that Ca could be added to salinized media to overcome the deleterious effects of salinity on the growth and productivity of leguminous crop plants.

Introduction

The general pattern of plant response to salinity is a growth suppression more or less in proportion to the solute concentration. Plants growing under such saline conditions accumulate various solutes as a result of alterations in their metabolic pathways.

Increasing salinity with NaCl in the medium was found to reduce photosynthesis (*e.g.* Mass and Hoffman 1976, Yeo and Flowers 1983, Yeo *et al.* 1985). These authors showed that the growth and photosynthesis are reduced by low, sublethal external salinity, although high tissue concentrations of salt are needed to cause reduction in chlorophyll content. Addition of Ca to the salinized medium has been found to significantly increase the salt tolerance of plants (*e.g.* Alit 1974, Wieneke and Läuchli 1980).

In present work, our aim was to study the influence of NaCl salinity on certain growth and metabolic aspects in *Phaseolus vulgaris* and to examine the role of Ca in overcoming the deleterious effects maintained by salinity.

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This paper to be considered as No XV of a series of papers on the general topic "Plant growth, metabolism and adaptation in relation to stress conditions".

Materials and methods

Seeds of *Phaseolus vulgaris* L. cv. Giza 3 were germinated and grown under conditions similar to those mentioned in a previous communication (Abbas *et al.* 1991). To the standard Long Ashton nutrient solution NaCl was added at concentrations 0 - 120 mM. In the experiments designed to study the influence of Ca, NaCl was added to the medium at a concentration of 100 mM, where Ca, as $Ca(NO_3)_2$, at concentrations 0 - 40 mM was added.

At the time of sampling, 12 seedlings were removed, 4 of them were used for determination of fresh and dry mass of roots and shoots.

The direct reducing value (reducing sugars) was determined following the procedure of Nelson (see Bell 1955). The total reducing value was assayed after hydrolysis of saccharose with an adequate amount invertase. The difference between total and direct reducing values is indicated as saccharose. Polysaccharides were estimated according to Younis *et al.* (1969).

Total nitrogen, total soluble nitrogen and ammonia nitrogen were estimated by the conventional micro-Kjeldahl method (Pirie 1955). Nitrate nitrogen was estimated by phenoldisulphonic acid method as outlined by Snell and Snell (1949) and amino-nitrogen was estimated as described by Muting and Kaiser (1963).

Chlorophyll *a*, chlorophyll *b* and carotenoids were determined in leaves following the spectrophotometric method as recommended by Metzner *et al.* (1965).

Each experiment was repeated twice and the values in figures are means of 8 samples ± standard error.

Results

With an increase in NaCl concentration in the growth medium from 0 to 120 mM both fresh and dry mass of shoots and roots was reduced (Fig. 1). The maximum

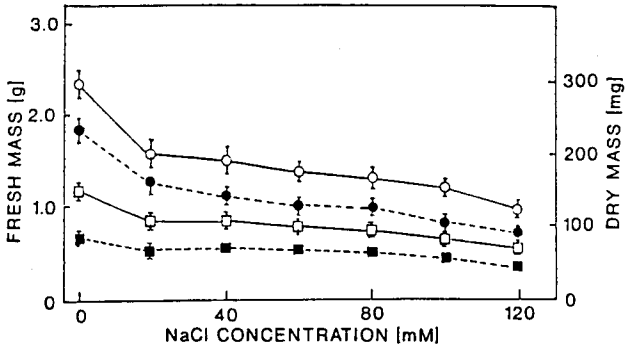

Fig. 1. Effect of increasing salinity in the growth medium on the fresh mass of shoots (*open circles*) and roots (*closed circles*) and on dry mass of shoots (*open squares*) and roots (*closed squares*) of 16-d-old *Phaseolus vulgaris* plants grown at 25 °C.

reduction in fresh mass reached to 58 % in shoots and 51 % in roots whereas the reduction in dry mass amounted to 65 % and 45 % in shoots and in roots, respectively.

Increasing NaCl concentration in the medium caused a progressive decrease in reducing sugars, saccharose, polysaccharides and consequently in the total saccharide content in both shoots and roots (Fig. 2). Compared to control, total saccharides decreased by 65 % on increasing NaCl concentration in the medium up to 120 mM.

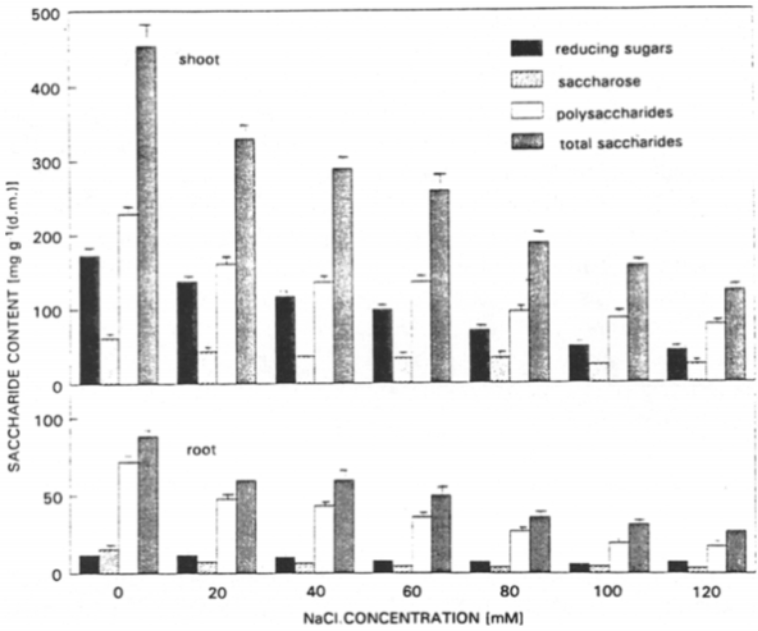

Fig. 2. Effect of increasing salinity in the growth medium on saccharide contents in both shoots and roots of 16-d-old *Phaseolus vulgaris* plants.

A progressive increase in the total soluble-N (two-fold increase at 120 mM NaCl) appeared (Fig. 3) concomitantly with a decrease in the protein content (only 25 % of the control value) at 120 mM. The decrease in the nitrate-N content was associated with an increase in ammonia and amino-N contents, though the magnitude of decrease in nitrate was greater than that of increase in ammonia. Amino-N appeared to increase from 2.1 in control shoots to 6.2 mg g^{-1}(dry mass) in shoots of plants grown at 120 mM NaCl whereas those comparable values in roots were 0.6 and 2.5 respectively (data are not given in the figure because amino-N contents are small enough to be presented graphically).

Surprisingly, the contents of chlorophyll *a*, chlorophyll *b* and carotenoids were increased in the leaves with increasing salinity in the medium (Fig. 4), the magnitude

of increase being most pronounced with chlorophyll *b* (63 % increase was maintained at 120 mM NaCl in relation to the control value).

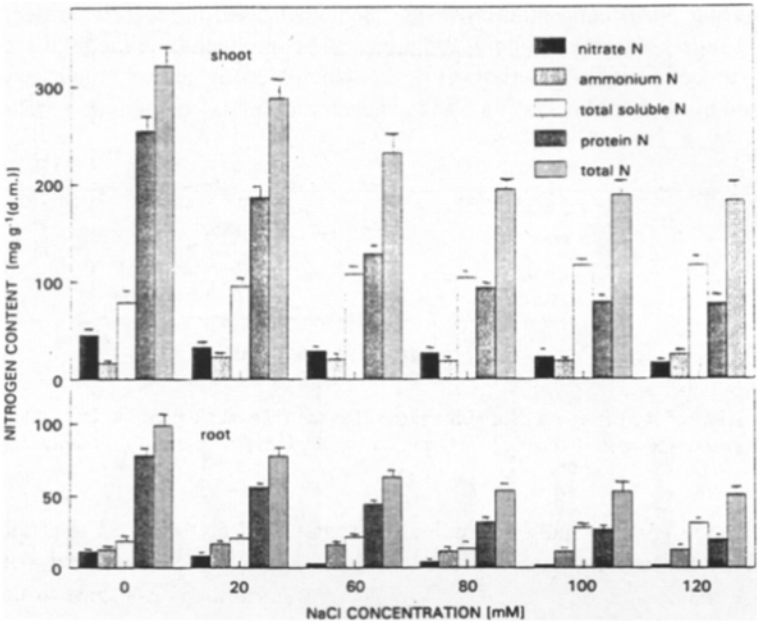

Fig. 3. Effect of increasing salinity in the growth medium on the nitrogenous compounds in both shoots and roots of 16-d-old *Phaseolus vulgaris* plants.

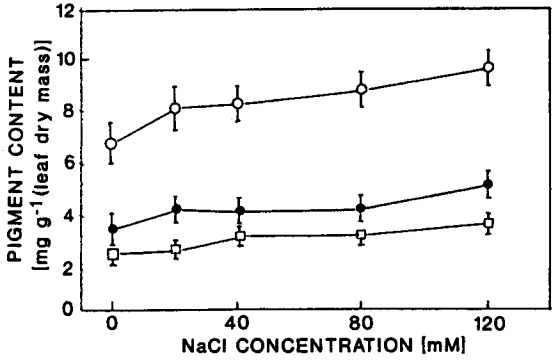

Fig. 4. Effect of increasing salinity in the growth medium on the contents of chlorophyll *a* (*open circles*), chlorophyll *b* (*closed circles*) and carotenoids (*squares*) in the leaves of 16-d-old *Phaseolus vulgaris* plants.

An addition of Ca (10 mM), as $Ca(NO_3)_2$, to the medium slightly improved the growth of salinized plants but with further increase in Ca concentration there was a progressive reduction in the fresh and dry mass of both shoots and roots (Fig. 5).

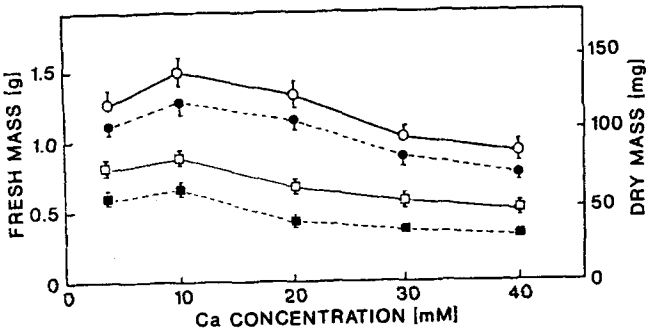

Fig. 5. Effect of increasing Ca^{2+} concentration in the salinized medium on the fresh mass of shoots (*open circles*) and roots (*closed circles*) and on the dry mass of shoots (*open squares*) and roots (*closed squares*) of 16-d-old *Phaseolus vulgaris* plants.

A progressive increase in reducing sugars, saccharose and polysaccharides contents was observed and consequently the total saccharides showed a maximum increase of 30 % upon increasing Ca concentration from 4 to 40 mM in the growth medium (Fig. 6).

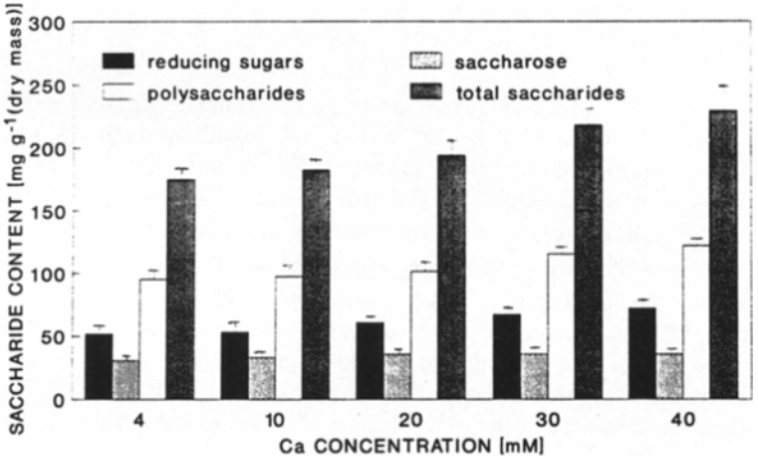

Fig. 6. Effect of increasing Ca^{2+} concentration in the salinized medium on the saccharide contents in 16-d-old *Phaseolus vulgaris* plants.

The total soluble-N was decreased by 25 %, whereas the protein content increased by 85 % with increasing Ca in the medium from 5 to 40 mM. Also, nitrate content was increased whereas ammonia and amino acid contents were decreased with increasing Ca. The total nitrogen content, like that of saccharides, was increased by about 30 % with increasing Ca concentration in the salinized medium from 4 to 40 mM (Fig. 7).

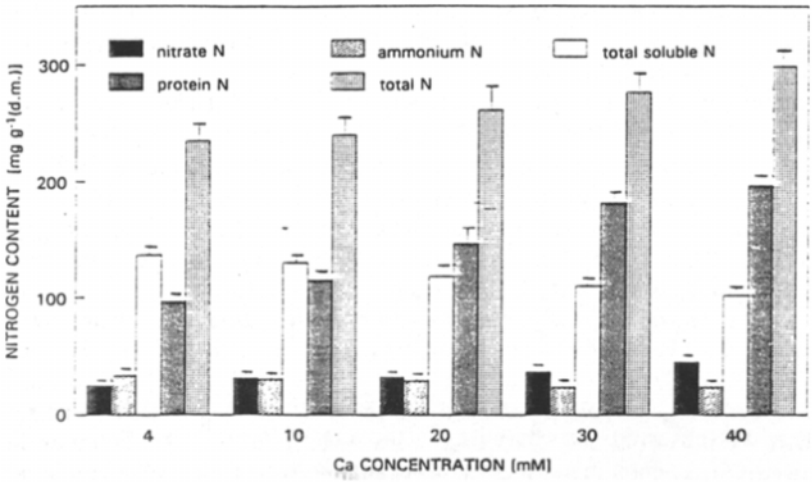

Fig. 7. Effect of increasing Ca^{2+} concentration in the salinized medium on nitrogenous compounds in 16-d-old *Phaseolus vulgaris* plants.

Discussion

The seedlings of *Phaseolus vulgaris* appeared to exhibit a significant ability to tolerate salinity with NaCl up to 120 mM in the growth medium. However, the growth of seedlings and dry mass production decreased with increasing NaCl concentration in the medium (Fig. 1). The poor growth of the plants at 120 mM NaCl was associated with decreases in organic matter which amounted to 65 % in shoots and 45 % in roots. This was mainly due to an inhibition in the rate of photosynthesis as it was found by Maas and Hoffman (1976), inhibition in the absorption of NO_3^- by roots from the medium due to increased Cl^- in the medium as it was suggested by Muraka *et al.* (1973), and inhibition in protein formation. Such effects of salinity on plant growth are in agreement with those observed by many other workers (*e.g.* Greenway 1962, Robinson *et al.* 1983, Imamul Huq and Larher 1983a, b, Younis *et al.* 1989).

Increasing NaCl concentration in the growth medium of *Phaseolus vulgaris* was accompanied by an increase of both Na^+ and Cl^- in both shoots and roots (Abbas *et al.* 1991). Ion excess in shoots seems to have inhibited the rate of photosynthesis as it is evident from the decrease in carbohydrate contents although the chlorophyll

concentration increased in leaves with increasing NaCl concentration. Concerning, chlorophyll contents in the NaCl-treated plants, similar observations have been found in *Lavatera arborea* (Okusanya 1980) and *Phaseolus aureus* (Imamul Huq and Larher 1982b).

NaCl caused a decrease in the nitrate content of the growing plants whereas ammonia, amino and total soluble nitrogen were increased. On the contrary, Imamul Huq and Larher (1983a) found that nitrate concentration was increased in roots of *Phaseolus aureus* with increased salinity and they suggested that nitrate uptake was maintained even in the presence of high NaCl concentrations in the medium in order to maintain osmoregulation in plants.

Protein content was markedly decreased with increasing NaCl, and in seedlings treated with 120 mM NaCl the protein content amounted to 30 % only of that in controls. It is suggested that, under salinity stress, plants accumulate organic solutes of which amino acids, particularly proline (Abbas *et al.* 1991) and other soluble nitrogenous compounds in order to maintain osmoregulation. This appeared to have occurred at the expense of protein formation. The present results are in consistence with those of Thomas *et al.* (1980).

It is well known that Ca^{2+} interacts with Na^+ and inhibits its absorption by the plant cells (Epstein 1976, Younis *et al.* 1986). The first action of Ca^{2+} is to decrease Na^+ concentration in shoots from 0.4 to 0.2 mmol g^{-1}(dry mass) and in roots from 0.95 to 0.35 mmol g^{-1}(dry mass) upon increasing Ca^{2+} concentration in the salinized medium from 4 to 40 mM. The progressive decrease in Na^+ concentration in plant tissue was accompanied by a progressive increase in sugars and total saccharide content (Fig. 6). Also, whereas the soluble nitrogenous constituents were decreased, those contents of nitrate and proteins were significantly increased with increasing Ca^{2+} in the salinized medium. Our results are in accordance with those of Wieneke and Läuchli (1980) and Imamul Huq and Larher (1983b), although Shere *et al.* (1974) found that addition of Ca^{2+} led to insignificant effects in increasing the salt tolerance in soybean. In conclusion, however, it seems evident that addition of Ca^{2+}, at certain concentrations, to the salinized media could overcome the salinity effects and at least improve the yield of crop leguminous plants as *Phaseolus vulgaris*.

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