Salinity and hormone interactions in affecting growth, transpiration and ionic relations of *Phaseolus vulgaris**

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Abstract

Addition of either abscisic acid (ABA) or kinetin at 10^{-6} M to salinized media (20 - 120 mM NaCl) induced remarkable effects on growth of *Phaseolus vulgaris* plants. Whereas ABA inhibited the plant growth and the rate of transpiration, kinetin induced stimulation of both parameters. Moreover, ABA increased proline and phosphorus concentrations in the salinized plants whilst kinetin decreased them.

ABA induced stimulation of the transport of K, Ca and Cl from root to shoot, accumulation of K, Na and Cl in root cells and inhibits the transport of Na and accumulation of Ca. Kinetin appeared to inhibit the transport and accumulation of Na and Cl, transport of K, and stimulates the accumulation of K and Ca as well as the transport of Ca. The highest influence of both ABA and kinetin was mostly observed when these hormones were used in combination with the highest concentration of NaCl (120 mM) in the medium.

Introduction

The presence of both ABA and cytokinins in the salinized medium have the ability to affect the uptake and transport of ions through the root without inhibiting active transport into cortical cells (Cram and Pitman 1972). The dramatic increase in ABA content in stressed plants and its accumulation in water-stressed tissue (Rikin *et al.* 1976, Zeevaart 1983, Creelman and Zeevaart 1985, Neill and Horgan 1985, Abo-Hamed *et al.* 1990) stimulated research into possible roles or functions of ABA in stressed plants.

It has been shown that kinins are produced in the plant root and transported to the shoot and their transport to shoot to be affected markedly by water stress of the root (Van Steveninck 1976). Conversely, Most (1974) showed that ABA is accumulated in leaf as a consequence of stress conditions. In addition, Hocking *et al.* (1972) have shown that there is some transport of ABA from leaf to root. In this context, Tal and

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Imber (1971) reported that cytokinins decreased stomatal resistance by opening stomata and increased the resistance of root for water absorption. On the other hand, they found that ABA increased stomatal resistance but increased water permeability of root.

It is now well known that phytohormones interact with ion transport across membranes of plant cells and across plant roots (Van Steveninck 1976). Also, plant hormones appear to play a role in regulation of ion uptake and distribution within the entire plant (Van Steveninck 1972, Pitman *et al.* 1974, Karmoker and Van Steveninck 1979).

The influence of salinity on the internal solute concentrations in *Phaseolus vulgaris* was discussed in previous publication (Abbas *et al.* 1991). Thus, in the present paper, special emphasis will be imposed upon the influence of added phytohormones, ABA and kinetin, to the salinized medium on growth and internal solute concentrations in *Phaseolus vulgaris*.

Materials and methods

Seeds of *Phaseolus vulgaris* L. cv. Giza were germinated in the dark on moist tissue papers for 4 d at 25 °C. Uniform seedlings were selected and set up to grow for 2 d on 0.5 mM $CaSO_4$ solution in 1-litre black painted beakers at 25 °C. The 6-d-old seedlings were selected again for uniformity and transplanted, 4 seedlings per beaker, on full strength Long Ashton nutrient solution (Hewitt 1952), containing 0.1 mM Fe-EDTA and micronutrients according to Arnon (1938). At this plant age, nutrient solutions were suplemented with 20, 40, 80 or 120 mM NaCl and well aerated with humid air. Renewal of nutrient solutions was carried out at 48 h intervals. At 16-d ABA (10^{-6} M) was added to one set of NaCl concentrations, kinetin (10^{-6} M) to another set while a third set was maintained as control. The plants were allowed to grow on the hormone containing solutions for 4 d during which the rate of transpiration was measured. At 20-d-old, plants were harvested, their roots rinsed well in distilled water and then cut into shoots and roots. Fresh mass were measured for all samples before being dried at 80 °C to constant mass.

Dried samples were digested in concentrated nitric acid and made up to volume with distilled water. K and Na concentration were measured by flame-emission spectrophotometry and that of Ca was measured by atomic-absorption spectrophotometry.

Chloride was estimated after extraction in water at 45 °C for 1 h by conventional titration against $AgNO_3$.

The relative growth rate (RGR) was calculated according to the formula

$$RGR = (\log_e W_2 - \log_e W_1)/(T_2 - T_1)$$

where W_1 and W_2 are the dry masses at harvest times T_1 and T_2 *i.e.* 16 and 20 d, respectively, supposing that growth was exponential during this growth period.

The rate of transpiration was estimated gravimetrically from the decrease in the fresh mass of whole plant and culture solution on the basis of root fresh mass.

Proline was estimated colorimetrically according to Troll and Lindsley (1955).

Inorganic phosphorus was measured using the method described by Humphries (1956). Total phosphorus was measured in the same way after extraction with trichloroacetic acid and digestion in 10 N H_2SO_4.

Each experiment was repeated twice and each value represents the mean of 8 samples ± standard error.

Results and discussion

ABA appeared to inhibit, and kinetin to stimulate the growth of *Phaseolus vulgaris* plants (Fig. 1a). The rate of the increase induced by kinetin was slightly greater than the rate of decrease induced by ABA. The highest influence of both hormones on RGR was observed in combination with the highest NaCl concentration (120 mM). In kinetin treated medium, the RGR was about 160 % that of control whereas in ABA treated medium, it was reduced to 70 % only of the control at 120 mM NaCl.

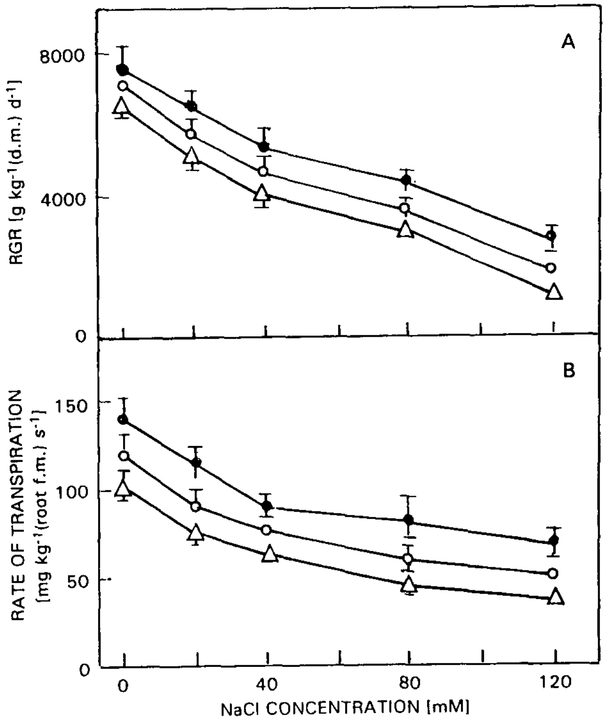

Fig. 1. Effect of 10^{-6} M ABA (*triangles*) and kinetin (*closed circles*) in comparison with control (*open circles*) in salinized medium on the RGR (A) and transpiration (B) of 16 - 20 d-old *Phaseolus vulgaris* L. plants.

These results are in agreement with observations of Skoog and Armstrong (1970) and Bialeck *et al.* (1973). ABA, as a growth inhibitor, is known to inhibit the plant growth whereas kinetin exerts influences on cell division, mobilization and retention of metabolites which would result in stimulation of plant growth (Van Steveninck 1976).

The rate of transpiration decreased by 20 % in the ABA-treated plants whereas approximately 40 % increase was recorded in those plants treated with kinetin compared to control plants at 120 mM NaCl (Fig. 1b). This observation is in consistence with the well-known influence of both hormones on transpiration (Van Steveninck 1976).

In general, K and Ca concentrations decreased whereas those of Na and Cl increased in both shoots and roots with increasing NaCl concentration in the culture medium (Fig. 2). Also, whereas K and Ca concentrations in shoots were greater than those in roots, Na and Cl concentrations in shoots were much lower than those in roots. It can be observed that both of the phytohormones increased the concentration of K in roots compared to that of control, though the increase induced by kinetin was greater in magnitude than that induced by ABA (Fig. 2A). In shoots, the influence of both hormones was different. Whereas ABA induced an increase in the concentration of K, kinetin induced a decrease in its concentration compared to that of control plants. On the other hand, both hormones decreased the concentration of Na in shoots but their influence was opposite in roots. Whereas Na concentration was increased in roots of ABA-treated plants, it was decreased in roots of kinetin-treated plants (Fig. 2C).

Except for roots of ABA treated plants, the changes induced by ABA and kinetin in the concentrations of K and Na were accompanied by an increase in the K/Na ratio in shoots and in the roots of kinetin treated plants. The total (K + Na) concentration was slightly decreased in shoots of ABA-treated plants due to the decrease in the concentrations of both K and Na, where it was almost unaffected in roots (Fig. 2E).

In kinetin treated plants, (K + Na) concentration was almost similar to that of control shoots, this was due to the decrease in Na and increase in K. On the other hand, (K + Na) in roots of kinetin treated plants was increased due to the induced increase in K and Na concentrations. Ca concentration was almost unaffected in the shoots and roots of ABA treated plants, but it was increased in both shoots and roots of plants treated with kinetin (Fig. 2B). Moreover, ABA caused a slight increase in Cl concentration in both shoots and roots whilst kinetin decreased this element concentration in both shoots and roots below the control levels. The highest influence of both ABA and kinetin on ion concentrations was observed in association with the highest concentration of NaCl in the culture media.

It is expected that the plant growth influences the demand for solutes which are necessary to sustain metabolic activities and to maintain the osmoregulation in the plant as its volume increases (Karmoker and Van Steveninck 1979). Hence, it is unlikely to separate the influence on ion transport and ion distribution within the whole plant. However, the fact that ABA stimulates K and Cl transport and accumulation despite of its inhibitory effect on the plant growth and transpiration

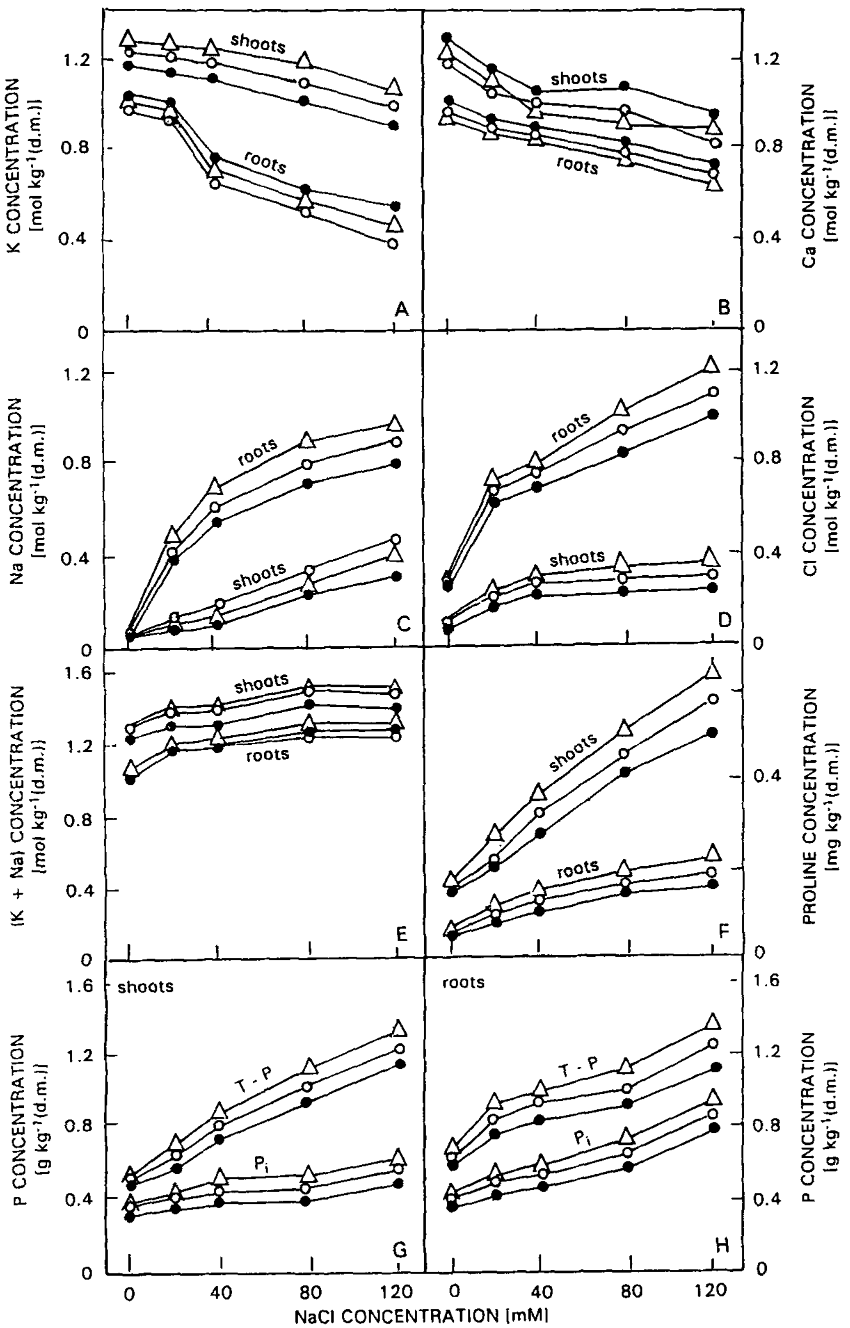

Fig. 2. Effect of 10^{-6} M ABA (*open triangles*) and kinetin (*closed circles*) in comparison with control (open circles) in the salinized medium on the concentrations of K (A), Ca (B), Na (C), Cl (D), K+Na (E), proline (F) inorganic and total P (G, H) in both shoots and roots of 16 - 20 d-old *Phaseolus vulgaris* L. plants.

whereas kinetin, on the contrary, inhibits ion transport and accumulation of solutes despite of its stimulatory effect on growth and transpiration, indicate predominance of the influences of both phytohormones on ion transport and solute accumulation over their influences on plant growth and water flux.

In accord with the effect of ABA on transport of ions, Van Steveninck (1974) found that ABA induced an enhancement of K, Na and Cl uptake in beet root discs, whereas in intact *Phaseolus vulgaris*, Karmoker and Van Steveninck (1979) found that ABA stimulated transport of Na and Cl but inhibited K transport. The inhibition of K transport in the intact plants was attributed to the decrease in transpiration rate induced by addition of ABA. Also, in consistence with the effect of kinetin, Hong and Sucoff (1976) found that kinetin inhibited Rb transport in roots of honey locust and attributed this inhibition to (1) an action of kinetin on the cell membranes and (2) its action as a competitive inhibitor for some enzymes controlling K uptake. Pitman *et al.* (1974) suggested that the stimulation of transport is a direct effect of ABA whereas inhibition may be produced as an indirect effect due to changes in the levels of endogenous growth substances and this may be an action of a cytokinin.

ABA, caused an increase in proline concentrations, whilst kinetin decreased it (Fig. 2F). The highest influence of the two plant hormones was observed in association with the highest NaCl concentration in the medium.

The concentrations of inorganic phosphorus (P_i) were, in general, higher in roots than in shoots (Fig. 2G and H). An opposite situation was observed for organic phosphorus ($T\text{-}P_i$) the values of which were simply obtained by subtracting values of P_i from the total phosphorus. ABA slightly increased the determined phosphorus fractions which were, on the other hand, decreased below the control levels in response to treatment with kinetin.

In general, the role of ABA in salinized plants appears to increase the internal solute concentrations including proline and inorganic phosphorus, whereas a reverse situation appeared due to the action of kinetin. One of the main effects of kinetin in salinized plants is the inhibition of uptake of Na and Cl, this would result in ameliorating the deleterious effect of salinity on the plant growth. In conformity with the present results, Adriana *et al.* (1978) found that kinetin applied simultaneously with NaCl to the leaf discs of *Nicotiana rustica* altered the stress responses. ABA, on the other hand, appeared to manifest the stress conditions. In conclusion, it seems that kinetin plays a key role in overcoming the effect of salinity in *Phaseolus vulgaris* plants, but the argument now arises about the role of ABA in stressed tissues, particularly if the dramatic increase in endogenous ABA levels in stressed tissues (Abo-Hamed *et al.* 1990) is taken into consideration.

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