Effect of sodium and calcium chlorides, abscisic acid and proline on callus cultures of *Arachis hypogaea* L.

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Abstract

Callus cultures of *Arachis hypogaea* L. cv. JL-24 adapted to 200 mM NaCl (otherwise lethal to cells) were used for the study. Calli grew slowly when transferred to 250 mM NaCl, but the growth was enhanced when ABA was included in the medium. ABA induced increase in growth of callus was not accompanied by corresponding increase in internal free proline levels. 0.5 mM of $CaCl_2$ ameliorated the negative effect of NaCl indicating that cells require a specific Ca^{2+}/Na^+ ratio for their growth. Proline content also increased at this ratio thereby suggesting that increase in growth at 0.5 mM Ca^{2+} may be due to an increase in proline content. However, exogenous proline did not increase the growth of callus (adapted to 200 mM), and higher concentrations even inhibited the growth. This shows that proline is not required for growth or adaptation of cells to salt stress, but is produced as a consequence of stress.

Additional key words: adaptation, growth, salt stress.

Introduction

Accumulation of proline under stresss conditions is widely known in callus cultures (Pandey and Ganapathy 1985, Binzel *et al.* 1987, Chandler and Thorpe 1987, Lone *et al.* 1987, Hassan and Wilkins 1988, Kishore 1988) but it remains still controversial whether proline helps in adaptation of callus under stress conditions or it is just accumulated as a consequence of salt stress. In the present study we have tried to analyse the effects of ABA and $CaCl_2$ on growth of callus cultures under salt stress and to find relationship between growth and internal proline levels.

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Abbreviations: ABA - abscisic acid; BAP - 6-benzylaminopurine; EGTA - ethyleneglycol-bis-(β-amino ethyl ether)N,N,N',N'-tetraacetic acid; NAA - naphthalene acetic acid.

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Materials and methods

Callus cultures of *Arachis hypogaea* L. cv. JL-24 were initiated from hypocotyls in Murashige and Skoog (MS) medium containing 3 × 10^{-6} M BAP and 10^{-6} M NAA. Callus line adapted to 200 mM NaCl (otherwise lethal) was obtained by transferring the callus stepwise to higher concentrations of NaCl and tolerance was checked by growing in medium without salt and again transfer to medium containing NaCl. The growth and proline content in salt-tolerant line were similar to those in control callus cultures (Sarin *et al.* 1991). Adapted and unadapted callus cultures were further grown on medium with different concentrations of NaCl (0, 200, 250 mM), ABA (10^{-4}, 10^{-5}, 10^{-6} M), proline (1 - 70 mM), $CaCl_2$ and EGTA (0.1, 0.5, 1 and 5 mM) for 28 d.

Growth of callus was determined by recording fresh mass at the beginning and after 28 d. 10 replicates of each culture were taken. Proline content was measured according to Bates *et al.* (1973). Callus was homogenized in sulfosalicylic acid and supernatant reacted with ninhydrin and glacial acetic acid for 1 h at 100 °C. The reaction mixture was extracted with toluene and absorbance taken at 520 nm.

Results and discussion

Effect of ABA: ABA at concentrations 10^{-4} and 10^{-5} but not 10^{-6} M inhibited the growth of unadapted callus cultures. Growth of callus adapted to 200 mM NaCl was slower and enhanced at any concentration of ABA. However, when the callus cultures adapted to 200 mM salt were transferred to medium containing 250 mM NaCl, ABA partially ameliorated its negative effect on callus (Fig. 1*A*). The role of ABA in increasing the adaptation to higher concentrations of NaCl has also been

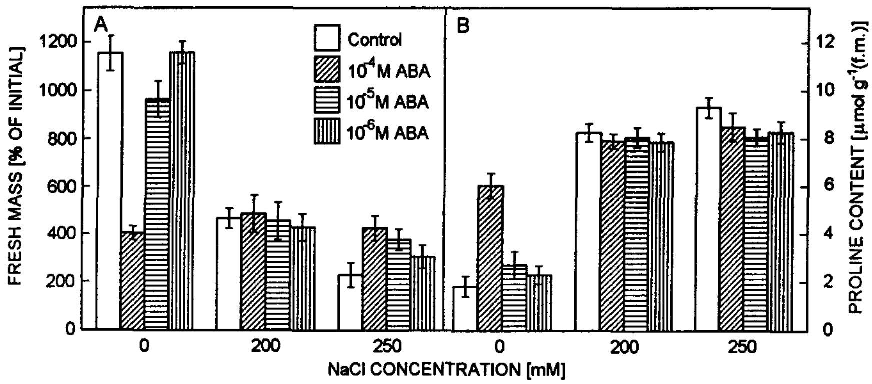

Fig. 1. Effect of various concentrations of ABA on growth (increase in fresh mass after 28 d treatment, initial fresh mass = 100 %) (*A*) and cytoplasmic free proline content (*B*) of unadapted calli, calli adapted to 200 mM NaCl and calli adapted to 200 mM NaCl and transferred to 250 mM NaCl (mean of 10 replicates ± S.D.).

reported by La Rosa *et al.* (1987). Proline levels of callus increased when it was transferred to 250 mM of NaCl (Fig. 1*B*), but less in the presence of ABA.

Effect of $CaCl_2$: $CaCl_2$ at a concentration of 0.5 mM had a significant ameliorating effect on the reduction of growth by salt stress (Fig. 2*A*). Lower or higher concentrations of $CaCl_2$ had no effect on growth of callus. The free proline content increased at higher concentration of NaCl and in the presence of 0.5 mM $CaCl_2$ further increase in proline content occurred (Fig. 2*B*).

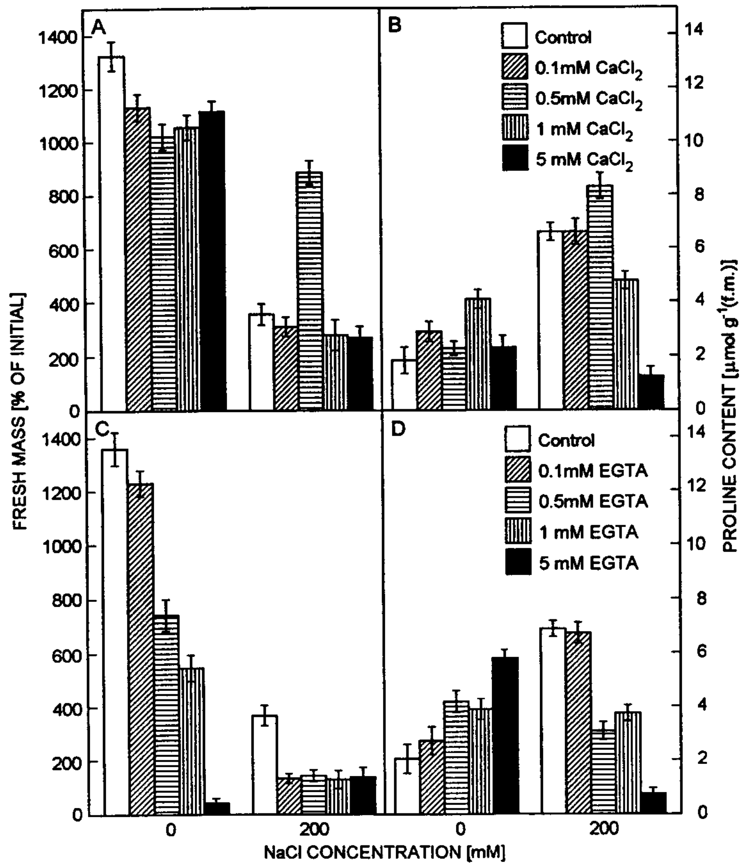

Fig. 2. Effects of different concentrations of $CaCl_2$ (*A*, *B*) and EGTA (*C*, *D*) on the growth (increase in fresh mass after 28 d treatment, initial fresh mass = 100 %) (*A*, *C*) and free cytoplasmic proline content (*B*, *D*) of unadapted calli and calli adapted to 200 mM NaCl (mean of 10 replicates ± S.D.).

The increase in proline content might suggest that Ca^{2+} is required for synthesis of proline and increase in growth of cells might be due to increase in proline content. However, after addition of EGTA to the medium, the growth of the cells decreased (Fig. 2*C*) while an increase in proline concentration was observed in the control cells

(Fig. 2*D*). In adapted cells, EGTA had additive effect on NaCl induced Ca^{2+} deficiency, thereby totally inhibiting the growth of cells.

Effect of exogenous proline: To check whether proline influences the adaptation of callus to NaCl, proline was included in the medium. Proline upto concentrations of 20 mM did not have any effect on the growth of cells and internal proline showed a slight increase (Fig. 3*A*). However, higher concentrations of proline inhibited the growth of callus cultures alongwith an increase in cellular free proline. The growth of cultures was totally inhibited at concentrations of 50 - 70 mM (Fig. 3*B*).

Since, ABA also does not increase growth by inducing the accumulation of proline, it suggests that proline is not the factor required for adaptation of cells at higher concentrations of NaCl (Duncan and Widholm 1991, Eberhardt and Wegmann 1989). This also shows that proline accumulation at higher concentrations than those normally found for a given NaCl level were toxic for the cells (Rodriguez and Heyser 1988).

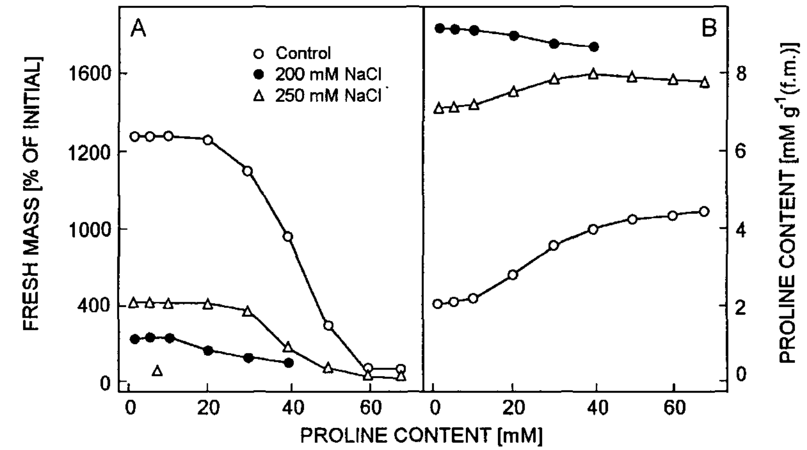

Fig. 3. Effect of exogenous proline on the growth (increase in fresh mass after 28 d treatment, initial fresh mass = 100 %) (*A*) and internal free proline content (*B*) of unadapted calli (*open circles*) and calli tolerant to 200 mM NaCl (*closed circles*) and tolerant calli moved to 250 mM of NaCl (*triangles*) (mean of 10 replicates ± S.D.).

In summary, both ABA and calcium help in decreasing salt stress and both are involved in determining the internal cytoplasmic content of proline. Further, proline does not have any adaptive role under salt stress.

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