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Original Research Article

In vitro maintenance of bioactive Ca2+ and primary metabolite status in *Cissus quadrangularis* under salt stress

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Abstract: *Cissus quadrangularis* is a plant, which has been customarily used in the Indian subcontinent to hasten the process of healing in bone fracture. The production of callus tissue was achieved from mature nodal segments by culturing on MS medium supplemented with auxin and cytokinin. Addition of 50mM salt to the medium, decreased potassium and phosphorus content of callus, but increased nitrogen, sodium and calcium content. Further increase in NaCl concentration decreased phosphorus, but increased nitrogen, potassium, sodium and calcium content as compared to control (without salt). Accumulation of calcium ions at higher salt concentration may possibly help in faster remodeling of bones. Calcium ion also mitigates the adverse effects of salinity up to a certain level.

Keywords: Bioactive Ca^{2+}, *Cissus quadrangularis*, NaCl, Salt overly sensitive.

Introduction

Plants are not incompatible to salts, but most of the plants do not grow in saline soils. Globally, soil salinity is more common in arid and semi-arid regions than in humid regions. An understanding of responses of plants to salinity is of great practical significance. High concentrations of salts have detrimental effects on plant growth (1; 2). Many investigators have reported retardation of germination and growth of seedlings at high salinity (3; 1; 4). However, plant species differ in their sensitivity or tolerance to salts. There are many different types of salts and almost an equally diverse set of mechanisms of avoidance or tolerance. In addition, organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (5). *Cissus quadrangularis* L. (Vitaceae) commonly known as Hadjod is an important medicinal plant of great economic value (6). The species of Vitaceae found in India are edible, ornamental and have great medicinal properties including anthelmintic, alterative, dyspeptic, antiscorbic, stomachic, antioxidant and antimicrobial (7; 8; 9). It is a rambling shrub usually found in hotter parts of India, Sri Lanka, Malaysia, Java and West Asia. The stem is thick, fleshy, glabrous, and ridged and quadranglular at the nodes. It is referred as "Asthisamhari" in Sanskrit, "hadjod" in Hindi because of its ability to join bones and is commonly known as the "bone setter" in English. It is an edible plant; the stems are made into curries, chutneys and eaten in Southern parts of India.

The ash of the plant is being used as substitute for baking powder. The stem is useful in piles. The juice of the plant is used to control irregular menstruation, diseases of the ear and in nose- bleeding. A paste of the stem is given in asthma and may be useful for muscular pains, burns, and wounds. A decoction of the shoots with dry ginger and black pepper is given for body pain. The infusion of the plant is anthelmintic. The entire plant is being used in fractures, sprains, rheumatism and irregular growth of teeth, broken horn, anthrax, haematuria and elephantiasis, dislocation of hip, various wounds and cracked tail. The present investigation was carried out (i) to understand the adaptive features of *Cissus quadrangularis* which allow it to grow and survive in saline and arid regions, and (ii) to assess the pattern of macro- and micro-nutrient status within the tissues of this species in response to salt stress.

Material and Methods

Healthy plants of *Cissus. quadrangularis* were collected from the botanical garden, Department of Botany, C.C.S.U. Campus Meerut. The nodal segments were used as explants for callus initiation. These explants were cut 8-10 mm and washed under running tap water for half an hour, subsequently followed by 1% labolene (Qualigens, India) for 5 min and then washed with sterile distilled water. They were then surface sterilised with 0.1% $HgCl_2$ solution for 3-5 min followed by several rinses in sterile

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distilled water. The sterile explants were then transferred on MS medium (10). The medium was supplemented with various combinations of auxins and cytokinins for callus induction. The media were congealed with agar (0.8%) and sucrose (3%) was used as a source of carbohydrate. pH of the medium was adjusted to 5.8 using 0.1 N NaOH/0.1N HCl solutions before being autoclaved at 15 psi for 20 min. All the cultures were incubated at 30±2°C under 16 h photoperiod, illuminated by fluorescent light of about 2500-3000 lux intensity. Biochemical parameters like Nitrogen content (11), Phosphorus content (12), Mineral components (13) were analysed after six week of callus induction. All the experiments and measurements were made in the triplicate.

Results and Discussion

Callus growth was good to moderate in 2.5-3.0 mg/l NAA+0.5mg/l BA. Addition of 50mM NaCl led to increase in N (Fig. 1), Na^+, and Ca^{2+} but decline in growth (Table 1), P (Fig. 2) and K^+ content in the 6 week old calli. As the growth of callus is also reduced as compared to control, the primary metabolism may be considered to be under salt stress. The stress further enhanced by addition of 100mM NaCl to the basal MS medium. The energy metabolites (primary phosphorylating metabolite) reduced due to lower uptake of P in the presence of NaCl. Yet the interesting feature was increased uptake and accumulation of Ca^{2+} (Fig. 3) which in turn induces the SOS (Salt Overly Sensitive) pathway (14) for exclusion of Na^+. However, decline in K^+ (Fig. 4) at lower NaCl concentration under 2.5mg/l NAA+0.5mg/l BA excludes the possibility of using the PGR combination supplementation for growing salt stressed calli yielding high amount of bioactive principle. Using the 3.0mg/l NAA+0.5mg/l BA resulted in improvement in accumulation of P, K^+ and Ca^{2+} under 100mM NaCl along with moderately good growth of calli. This suggests that induction of SOS pathway by Ca^{2+} and also improvement of binding and uptake of NAA (15) under as low as 4.0mM Ca^{2+} which is provided in the standard nutrient composition of MS medium, helps in combating stress through increase in P uptake (though less than control) and K^+ accumulation along with maintenance of balance of Na^+ content equal to controls (Fig. 5). *In vitro* salt stress study in a plant like *Cissus quadrangularis* is suggestive of bioactive Ca^{2+} accumulation only at higher salt concentration given to at least 3.0mg/l NAA+0.5mg/l BA supplemented MS medium. It is important to note that *Cissus quadrangularis* does not exhibit good callus growth in various concentrations of IAA or 2, 4-D etc. (16; 17). NAA uptake is enhanced and materialized to result in good callus growth at low concentration of bioactive Ca^{2+}, which also helps in salt (Na^+) exclusion and maintenance of primary metabolism. *Cissus quadrangularis* is susceptible to salt stress (as low as 50mM NaCl), yet callus growth can be maintained if it is raised on 3.0mg/l NAA+0.5mg/l BA in the presence of low concentration of Ca^{2+} (as in MS Medium). This growth alongwith improvement in the amounts of P and K^+ are achieved as Ca^{2+} supposedly helps in binding and uptake of NAA (not of IAA or 2,4-D) alongwith activation of SOS pathway protecting the callus from injury. In this report we suggest the mechanism supporting bioactive Ca^{2+} uptake and maintenance of primary metabolism *In vitro* in *Cissus quadrangularis*.

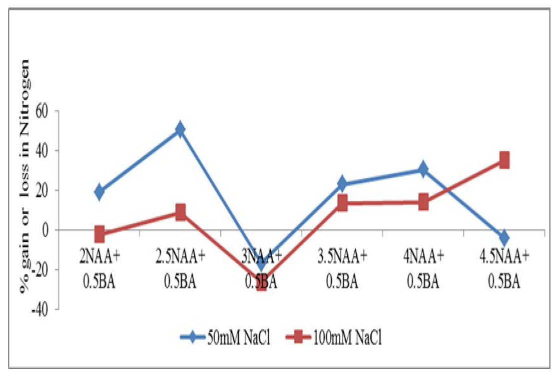

Fig.1: % gain or loss in Nitrogen (N) content in *Cissus quadrangularis* nodal calli on MS+PGRs+Salt supplemented media as compared to respective salt free control.

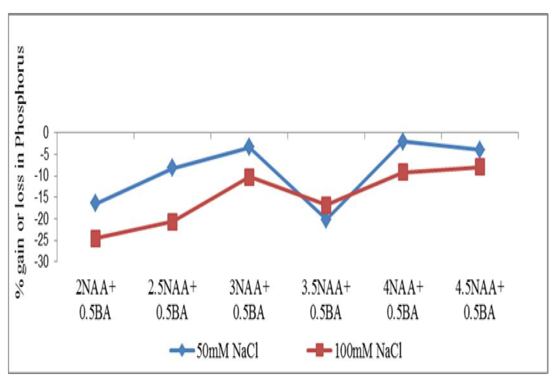

Fig. 2: % gain or loss in Phosphorus (P) content in *Cissus quadrangularis* nodal calli on MS+PGRs+Salt supplemented media as compared to respective salt free control.

Table.1: Effect of salt (NaCl) and Phytohormones (NAA & BA) on callus growth from nodal explants in *Cissus quadrangularis* calli.

S.No.	MS + Growth Regulators(mg/l)	Salt (mM NaCl)	Callus growth	Morphology of callus
1	2.0NAA+0.5BA	-	++	Loose, friable and brown
2	"	50mM	+	Loose, friable and brown
3	"	100mM	+	Loose, friable and brown
4	2.5NAA+0.5BA	-	++++	Loose, friable and brown
5	"	50mM	++	Loose, friable and brown
6	"	100mM	+	Loose, friable and brown
7	3.0NAA+0.5BA	-	+++	Loose, friable and brown
8	"	50mM	++	Loose, friable and brown
9	"	100mM	+	Loose, friable and brown
10	3.5NAA+0.5BA	-	++	Loose, friable and brown
11	"	50mM	+	Loose, friable and brown
12	"	100mM	+	Loose, friable and brown
13	4.0NAA+0.5BA	-	++	Loose, friable and brown
14	"	50mM	+	Loose, friable and brown
15	"	100mM	+	Loose, friable and brown
16	4.5NAA+0.5BA	-	++	Loose, friable and brown
17	"	50mM	+	Loose, friable and brown
18	"	100mM	+	Loose, friable and brown

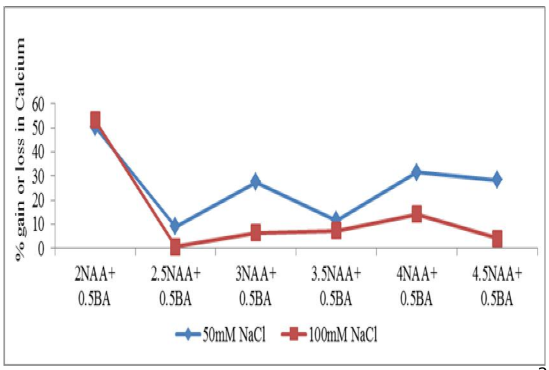

Fig.3: % gain or loss in Calcium (Ca^{2+}) content in *Cissus quadrangularis* nodal calli on MS+PGRs+Salt supplemented media as compared to respective salt free control

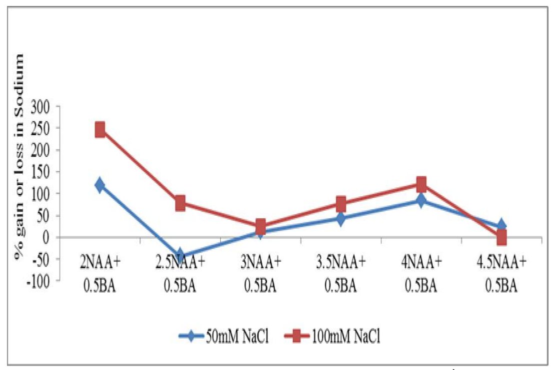

Fig.5: % gain or loss in Sodium (Na^{+}) content in *Cissus quadrangularis* nodal calli on MS+PGRs+Salt supplemented media as compared to respective salt free control

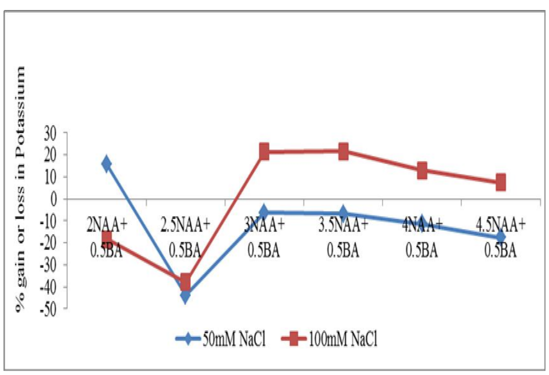

Fig.4: % gain or loss in Potassium (K^{+}) content in *Cissus quadrangularis* nodal calli on MS+PGRs+Salt supplemented media as compared to respective salt free control

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