Organic Metabolite Accumulation in *Cissus Quadrangularis* Calli under Salt Stress

Abstract

Effect of NaCl on *in vitro* growth and content of sugars, proteins and phenolics in calli of stem explants of *Cissus quadrangularis* was investigated. The growth of calli decreased with addition of salt. Addition of salt (50mM) led to increase in all the studied parameters except reducing sugar. Protein content increased and phenolics, total sugars and non-reducing sugar content decreased at higher salt concentration (100mMNaCl) indicating a preference of nitrogenous metabolite accumulation to combat stress. Interestingly, reducing sugar content in the salt treated calli declined below control (salt free), indicating increased respiratory exhaustion or leaching of sugars.

Keywords: Please add keywords

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Introduction

Every plant require some abiotic factors, like light, temperature, air, water, inorganic and organic nutrient in optimum amount at a right time for its proper growth and maintenance. Any deviation from this optimal condition of any factor necessary for its growth will lead to aberrant changes in physiological processes and due to this plant body will experience tension referred as stress. Salinity stress is one serious threat to plants as it decreases crop yield and many products of economic value. During salt stress plants can survive by adaptive method such as ion transport, compartmentation, synthesis of secondary endodermis and accumulation of osmotically compatible solutes. The mechanism of plant salt tolerance is a topic of intense research in plant biology. The objectives are to understand the control of ion homeostasis and osmotic regulation, and to use the knowledge to engineer crop plants with enhanced salt tolerance (Zhu, 2007). *Cissus quadrangularis* L. (Vitaceae) commonly known as Hadjod (potential bone healer) is an important medicinal plant of great economic value (Panthong *et al.,* 2007). The species of Vitaceae found in India are edible, ornamental and have great medicinal properties including anthelmintic, alterative, dyspeptic, antiscorbic, stomachic, antioxidant and antimicrobial (kar and Borthakur, 2008, and Meher *et al*., 2010). The present report describes in vitro technique as an efficient method to study the effect of NaCl stress on organic solute accumulation as osmotic in callus tissue of Cissus quadrangularis in salt tolerance.

Aim of the Study

The present investigation was carried out to assess the in vitro bioactive metabolite status within the callus of this species in response to salt stress.

Review of Literature

Zhang in the year 2004 observed the accumulation of proline, glycine betaine and total sugars with increasing salt concentration in *Populus*. Niknam *et al,* 2006 also reported significant increase in protein content of *Nicotiana tobacum* plantlets over that of control at higher salt supplementation. SDS-PAGE analysis of total protein extracted from control and NaCl treated plantlets revealed that salt stress was associated with decrease or disappearance of some protein bands and induction of some new protein after exposure to 100 and 150mM NaCl concentration (Zhang and Wang, 2013). Bezirganoglu (2017), explained the accumulation of proline and sugars (upto 2 fold) in wheat callus as compared to salt free control.

Material and Methods

Healthy plants of *Cissus quadrangularis* were collected from the botanical garden, Department of Botany, C. C. S. University 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 forhalf an hour, subsequently followed by 1% labolene disinfection (Qualigens, India) for 5 minute and then washed with sterile distilled water. They were surface sterilized with 0.1% $HgCl_2$ solution for 3-5 minute followed by several rinse in sterile distilled water. The sterile explants were then transferred on MS medium. The medium was supplemented with various combinations of auxins and cytokinin for callus induction. The media was cogealed with agar (0.8%) and sucrose (3%) was used as a source of carbohydrate. pH of medium was adjusted to 5.8 by using 0.1 N NaOH/0.1 N HCl solution before being autoclaved at 15psi for 20 minutes. All the cultures were incubated at 30 ± 2^0C under 16 hour photoperiod, illuminated by fluorescent light of about 2500-3000 lux intensity. Biochemical parameters like, protein content (Bradford et al., 1976), phenolic content (Bray and Thorpe, 1954) and total sugar content (Nelson and Somagtyi, 1944) were analysed after six weeks of callus induction. All the experiments and measurements were made in triplicate.

Results and Discussion

In case of stem explants, calli accumulate maximum protein in MS medium supplemented with NAA (2.0mg/l) +BA (0.5mg/l) and NaCl (100mM), protein content increased gradually with addition of salt (Fig-1). Amount of protein in treated calli was higher in comparison to control probably due to synthesis of some defense proteins. In contrast, Handa *et al*., 1983 reported a decline in soluble protein levels in adapted cell lines. Accumulation of proteins showed abnormal pattern of rise and fall with increasing NAA (2-4.5mg/l) concentration. According to analysis of protein, NaCl induced quantitative and qualitative changes in proteins of calli. Proteins that accumulate in calli grown under saline conditions may provide a storage form of nitrogen that is reutilized when stress is over and may play a role in osmotic adjustment (Niknam *et al.*, 2004). Phenolic content also increased (Fig-2) generally with addition of salt (50mM), but a higher (100mM) salt concentration protein provided a better salt tolerance as compared to phenolics. Phenolic content also increased with augmentation of NAA upto 4.5 mg/l, followed by a decrease at higher concentration. Accumulation of phenolics may act as antioxidant by inactivating lipid free radicals during salt stress in case of *Cissus quadrangularis* calli.

Total sugar (Fig-3) and NRS content (Fig-5) increased with addition of salt (50mM) followed by a decrease at higher salt (100mM) concentration. The accumulation of sugars may play an important role in plant defensive mechanism of osmoregulation and energy preservation (Norwood *et al*., 2003 and Minorsky, 2003). Variation of NRS levels may affect the formation of RFOs (raffinose family oligosacharides) which may play a crucial role in maintaining membrane stability via interaction with phospholipid head groups and may scavenge reactive oxygen species when exposed to salt (Bohnert and Jensen, 1996, Bentsink *et al*., 2000). Reducing sugar content (Fig-4) in the salt treated calli declined (also reported by Pattanagul, 2008 in rice seedlings) below control (salt free), indicating increased respiratory exhaustion or leaching of sugars. Although researchers agree that salinity and water stress induce soluble sugar accumulation (Binzel *et al*., 1989, Wang and stutte., 1992 and Kameli and Losel, 1995) there are objections to the suggestion that metabolically labile primary metabolite, such as reducing sugars are compatible cytosolutes, since many of them have effects on cytoplasmic enzymes (Rozema *et al*., 1978). Thus we could assume that increase in NRS content, we observed in *C. quadrangularis* calli, was associated with an active osmotic adjustment. It is also possible that high NRS content was due to a low enzyme activity under salt stress. It could also be attributed to a general decrease in total metabolic activity caused by salt (NaCl) stress. Decrease in RS content was due to a more rapid hexose metabolism, supplying compound involved in water balance. This assertion is supported by studies with a variety of plants that demonstrate a salt or drought induced conversion of hexose and other carbohydrates into sugar alcohols and proline. Sugar is also considered as osmoprotectant and reported to accumulate during salinity (Kovacik *et al*., 2009).

Conclusion

Proteins that accumulate in callus grown under saline conditions may provide a storage form of nitrogen that is reutilized when stress is over and may play a role in osmotic adjustment. Soluble sugars, that are altered by water deficit and salinity may also act as signaling molecules under stress and also interact with hormones as part of the sugar sensing and signaling network in plants. Present investigation in *Cissus quadrangularis* is important as reports on understanding of its physiological status under presently increasing saline soil, is hardly available.

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Figure-1 % Gain Or Loss In Protein Content in *Cissus Quadrangularis* Nodal Calli on MS+PGR+Salt Supplemented Media as Compared to Respective Salt Free Control

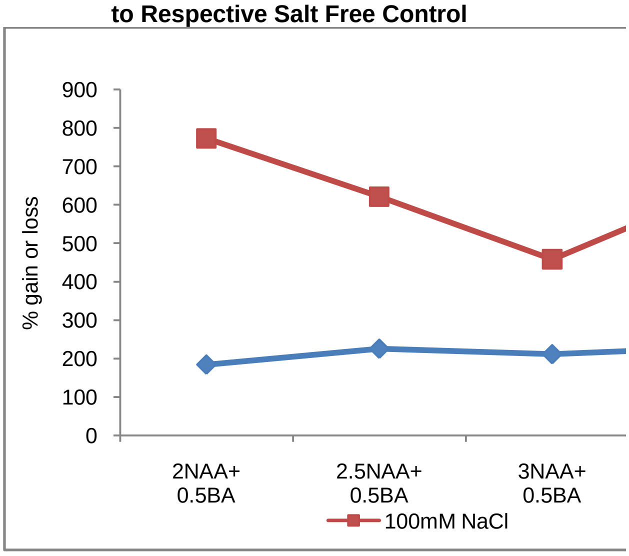

Figure-2 % Gain or Loss in Phenolic Content Of *Cissus Quadrangularis* Nodal Calli On MS+PGR+Salt Supplemented Medium as Compared to Respective Salt Free Control

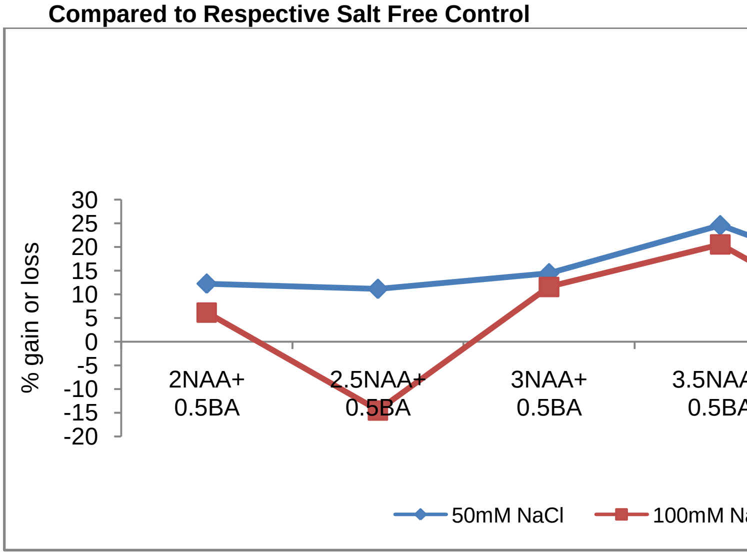

Figure-3 %Gain or Loss in Total Sugar Content of *Cissus Quadrangularis* Nodal Calli on MS+PGR Supplemented Medium as Compared to Respective Salt Free Control

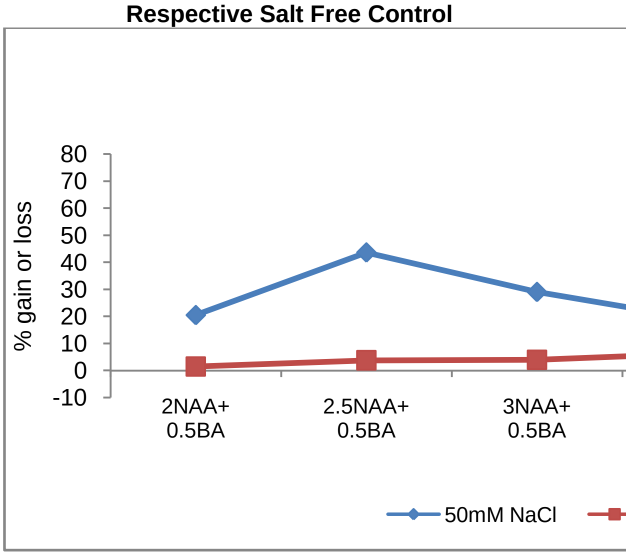

Figure-5 %Gain or Loss In Non Reducing Sugar Content of *Cissus Quadrangularis* Nodal Calli on MS+PGR Supplemented Medium as Compared to Respective Salt Free Control

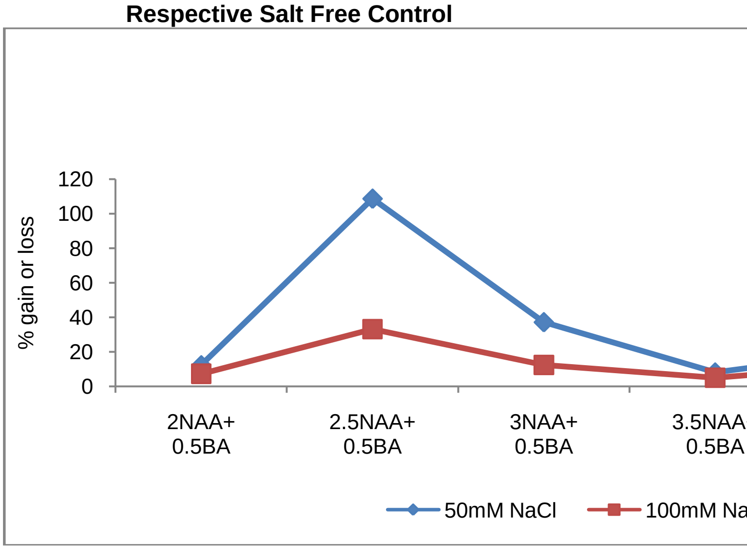

Figure-4 %Gain or Loss in Reducing Sugar Content of *Cissus Quadrangularis* Nodal Calli on MS+PGR Supplemented Medium as Compared to Respective Salt Free Control