



Research Article

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HIGHER SALT TOLERANCE OF *PHYSALIS PERUVIANA* L. CALLUS WITH REDUCED PEROXIDASE ACTIVITY COMPARED TO ITS *IN VITRO* RAISED SEEDLINGS

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ABSTRACT

The present study was conducted to evaluate the effect of NaCl on the comparative growth and biochemical parameters of seedlings and callus of *Physalis peruviana*. The growth of seeds of *P. peruviana* was studied on MS (control), MS+50, 100 and 150 mM NaCl, respectively. The seeds germinated on all treatments but seedlings survived up to radicle emergence only, on MS supplemented with 100 mM and 150 mM NaCl. The callus induced on MS medium supplemented with BAP (0.2 mgL⁻¹) and NAA (1.0 mgL⁻¹) using stem explants from these seedlings (control) was sub-cultured on salt supplemented MS medium. The callus grew well up to 100 mM NaCl, whereas the seedlings failed to grow. Biochemical analysis indicated that *in vivo* the seedlings combat with the salt stress by accumulating more phenolics (especially in shoot) and undergoing higher peroxidase activity as compared to control. 100 mM NaCl raised callus (*in vitro*) has been found to be more tolerant to salt stress as compared to similarly treated seedlings, accumulating protein and proline rather than phenolics and peroxidases, indicating the mechanism of tolerance *in vitro* to be primarily through osmotic balance and generation of reductant (phenolics) diminishing the generation of free radicals vis-à-vis climacteric rise of peroxidase activity.

Keywords: *Physalis peruviana* L., NaCl, *in vitro*, *in vivo*, peroxidases.

INTRODUCTION

Salinity is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity¹. The saline area is three times larger than land used for agriculture². Total area under salinity is about 953 million ha covering about 8 % of the land surface. Excess salt in the soil solution may adversely affect plant growth either through osmotic inhibition of water uptake by roots or through specific ion effect. In many crop plants, seed germination and early seedling growth are the most sensitive, vulnerable stages to environmental stresses such as salinity due to less developed physiological mechanisms to combat stresses³. Rapid, uniform and complete germination is a prerequisite for successful transplant production and stand establishment in vegetable crops⁴. Solanaceae family is considered to be moderately sensitive to salinity. *Physalis peruviana* belongs to family Solanaceae and genus *Physalis*. High salt concentration in the soil has been found to disrupt several physiological processes leading to reduction in growth, small fruit size and low yield⁵. One of the most effective ways to overcome salinity problems is the introduction of salt tolerant crops. Therefore, this study was aimed to evaluate the effect of salinity on physiological and biochemical response of seedling and callus of *Physalis peruviana*, a common medicinal plant, to establish its potential for salt tolerance.

MATERIAL AND METHODS

Plant Material

Experiments on physiological and biochemical responses of seedlings and callus of *Physalis peruviana* to NaCl stress were carried out at CCS University Meerut during 2011-2012. Seeds were obtained from mature and ripe fruits. Seeds were sterilized by washing with Tween-20 thoroughly under running tap water. Further the seeds were surface sterilized with 0.01 % HgCl₂ for 5-7 minutes and finally rinsed five times with sterile double distilled water.

Seedling Culture

Seeds of *P. peruviana* were germinated on MS medium⁶ supplemented with or without different concentrations of NaCl (50-150 mM) and kept at 22 ± 2°C under 10 h PAR. After four weeks and eight weeks the seedlings were used for growth and biochemical analysis.

Callus Culture

Callus was initiated from the stem explants (1.0 cm²) of the seedlings germinated on MS medium supplemented with BAP (0.2 mgL⁻¹) and NAA (1.0 mgL⁻¹) without salt. After two weeks, the callus was transferred onto the same medium supplemented with 50, 100 and 150 mM NaCl with similar culture conditions as for the seedlings. At the end of four-week and eight -week period, the callus was taken for growth and biochemical analysis.

Biochemical Analysis

Evaluation of the active bio chemicals such as total phenolics, total protein content, proline content and activity of peroxidase enzyme (from fresh materials) in *P. peruviana* was carried out from leaf and stem explants, four and eight week-old callus using the following standard methods: total phenolics were measured by the Folin-Ciocalteu assay⁷ and for total proteins material was extracted using Tri-chloro acetic acid⁸. The proline content in the tissue was determined spectrophotometrically by recording absorbance at 520 nm against toluene blank⁹ and assay of peroxidase activity was carried out following Maehly and Chance method¹⁰.

RESULT AND DISCUSSION

Effect of NaCl on Relative Seed Germination and Growth of Callus

The effect of salt stress on seed germination was determined by adding 50, 100 and 150 mM NaCl to the MS medium (with 2 % sucrose) in different sets; radicle emergence occurred in all the tested concentrations, but, seedling growth took place only up to 50 mM NaCl (Table 1). Similar results were also observed at 90, 120 and 180 mM NaCl treatments in *Physalis* by Miranda *et al.*¹¹. The reduction in germination and relative growth under salt stress conditions has been reported in seedlings due to osmotic stress as well as salt injury under salinity stress conditions, which may constitute an adaptive strategy aimed at preventing germination in stressful environments¹²⁻¹⁴. The effect of salt stress on growth of callus was also determined by adding 50, 100 and 150 mM NaCl to the MS medium (supplemented with optimized concentrations of BAP and NAA, as mentioned in methodology). The growth of salt stressed callus was noted to be slightly reduced as compared to the control callus (Table 2). The reduction of relative growth under salt stress conditions has been generally reported in callus of several plants. But, in the present study, the callus was able to tolerate 100 mM NaCl as compared to seedlings (tolerating only up to 50 mM NaCl) and showed good growth up to 100 mM NaCl. Bahman *et al.*¹⁵ have inferred with salt untreated sets of wheat cultivar showing increasing callus volume with increase in salt concentration, that their relative growth rate and relative fresh weight accumulation decrease because of sodium influx in explants and cell membrane damages.

Effect of NaCl on Biochemical Parameters of Seedlings and Callus

Effect of NaCl on biochemical parameters of shoot and leaf of seedlings after four weeks

To MS+2 % Sucrose raised seedlings, addition of 50 mM NaCl led to increase in all the four parameters (protein content, proline content, phenolic content and peroxidases) in shoot. The phenolic content was considerably very high, indicating high level of antioxidant accumulation without equitable requirement of peroxide free radical scavenging activity. Stress-induced proline accumulation has often been argued to be

due to a disturbed nitrogen balance in the stressed tissue¹⁶. Recently, it was demonstrated that proline supplements enhanced salt tolerance in olive plants by improving photosynthetic activity and increasing the activity of enzymes involved in the antioxidant defense system¹⁷. In leaf also, all the four biochemical parameters increased (Figure 2a). However, phenolic content accumulated in higher amounts as compared to other tested bio chemicals in both stem and leaf in response to salinity (50 mM NaCl). Leaf phenolic contents are important protective components of plant cells. The potential of phenolics to act as antioxidant is mainly due to their properties to act as hydrogen donors, reducing agents and quenchers of singlet oxygen¹⁸. The synthesis of phenolics is generally affected in response to different biotic/ abiotic stresses including salinity¹⁹, as observed in the present study with seedling parts of *P. peruviana*.

Effect of NaCl on biochemical parameters of shoot and leaf of seedlings after eight weeks

To MS+2 % Sucrose raised seedlings, addition of 50 mM NaCl caused a considerable variation in biochemical parameters. In stem, 50 mM NaCl led to extraordinary increase in phenolics and peroxidase activity with an insignificant decline in proline content indicating stress combat with high antioxidant activity through reductant (phenolics) and free radical scavenger (peroxidase). In leaf, protein and proline content declined significantly whereas, phenolics and peroxidase activity increased compared to control (Figure 2b). Such differential response of plants in phenolic accumulation at different growth stages could be due to plant growth stage - dependent switching of accumulation of phenolics^{20,21}. Hichem *et al.*²² also reported that such variation in concentration of leaf phenolics within a plant under salt stress in relation to leaf age may be due to the reflection of different requirements for counteracting abiotic stresses at different growth stages. Gosset *et al.*²³ observed significant increase in peroxidase activity and greater decomposition of H₂O₂ in rice cultivars grown under salt treatment. It has been recognized that peroxidases are involved in H₂O₂ decomposition that initiates toxic oxygen production. These radicals are implicated in lipid peroxidation and in the denaturation of proteins and DNA as a result of the cellular de-compartmentation. Increased peroxidase activity under salt stress could result from the synthesis of isoperoxidases and/or the activation of existing peroxidases²⁴. Peroxidases are often used as molecular markers of biotic and abiotic stresses and could be used as tool for selection and as prediction model for greater yielding ability.

Effect of NaCl on biochemical parameters of callus after four weeks

To MS+2 % Sucrose + 0.2 mgL⁻¹ BAP + 1 mgL⁻¹ NAA raised calli, addition of 50 mM NaCl led to increase in all the four parameters except peroxidase, although proline content did not increase so much but there was more accumulation of phenolics. Interestingly, phenolics increased as opposed to proline accumulation in most of

the salt treatments. The mechanism of salt tolerance by both the compounds is basically different. Proline acts as nitrogenous osmoprotectant, the phenolics act as carbonic antioxidants. Increase in NaCl concentration to 100 mM led to extensive increase in proline, phenolics and protein content with a little increase in peroxidase activity indicating NaCl tolerance through the enhancement of defence mechanism (Figure 2c). Proline accumulation may be due to expression of genes encoding key enzymes of proline synthesis or low activity of the proline oxidizing enzymes²⁵. It is possible that, higher accumulation of proline may regulate multiple processes required for survival in salt stress conditions²⁶. Proline may either stimulate other responses or act as protective compound to minimize the salt induced damage. Datt *et al.*²⁷ have also reported accumulation of osmoprotectant (proline like) with stress. Proline accumulation in response to environmental stresses has been considered by a number of investigators as an adaptive trait concerned with stress tolerance. It may act as an enzyme protectant, stabilize membranes and cellular structures during hostile conditions, detoxify free radicals by forming long-lived adducts with them and affect solubility of various proteins by interacting with their hydrophobic residues²⁸.

Effect of NaCl on biochemical parameters of callus after eight weeks

To MS+2 % Sucrose + 0.2 mgL⁻¹ BAP + 1 mgL⁻¹ NAA raised calli, addition of 50 mM NaCl increased protein content with decrease in proline and phenolic content. Decline in proline could be accounted for increase in proline hydroxylase activity converting proline to hydroxyproline which is generally a part of stress tolerating protein. Increase in NaCl content up to 100 mM NaCl led to extraordinary increase in protein and proline, slight increase in phenolics with a decline in peroxidase activity indicating NaCl tolerance through enhancement of stronger defense mechanism by callus (Figure 2d). Thus, significant increase in protein and proline content occurs with increase in salt concentration providing sustenance to salt stress, but with no probable participation of peroxidase activity indicating no toxic principle to be formed as suggested by Rajeshwar *et al.*²⁹ in *Mucuna pruriens*. Our findings are in accordance with Jain and Padmaja³⁰ who have also reported accumulation of majorly stress tolerant proteins. Porgali and Yurekli³¹ reported lower amounts of proteins in salt treated, salt sensitive *Lycopersicon esculentum* plants as compared to control plants, whereas in salt tolerant *L. pennellii* plants, total protein amount was recorded to be more than the control plants.

Table 1: Effect of salt on seed and seedling germination of *P. peruviana* L.

Treatment	Radicle Emergence	Growth of Seedlings
MS (Control)	+	+
MS + 50 mM NaCl	+	+
MS + 100 mM NaCl	+	-
MS + 150 mM NaCl	+	-

Table 2: Effect of salt on callus growth raised on MS + 2 % sucrose + BAP (0.2 mgL⁻¹) + NAA (1.0 mgL⁻¹)

Treatment	Callus Growth	Morphology of Callus
Control	+++	Green and Compact
+ 50 mM NaCl	+++	Green and Compact
+ 100 mM NaCl	++	Yellowish Green and Compact
+ 150 mM NaCl	-	Brown

+++ Very Good Response ++ Good Response + Response - No Response

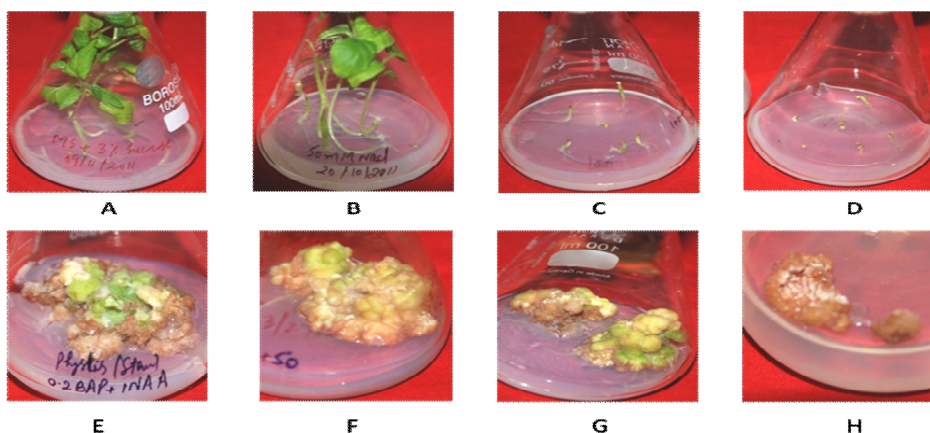


Figure 1: Effect of salt on seed germination, seedling growth (A-D) and callus growth after 4 weeks of induction (E-H) of *Physalis peruviana*
 A. Seed germination and seedling growth on MS (control). B. Seed germination and seedling growth on MS + 50 mM NaCl. C. Seed germination on MS + 100 mM NaCl. D. Seed germination on MS + 150 mM NaCl. E. Callus growth on MS + BAP (0.2 mgL⁻¹) + NAA (1.0 mgL⁻¹). F. Callus growth on MS + BAP (0.2 mgL⁻¹) + NAA (1.0 mgL⁻¹) + 50 mM NaCl. G. Callus growth on MS + BAP (0.2 mgL⁻¹) + NAA (1.0 mgL⁻¹) + 100 mM NaCl. H. Callus growth on MS + BAP (0.2 mgL⁻¹) + NAA (1.0 mgL⁻¹) + 150 mM NaCl

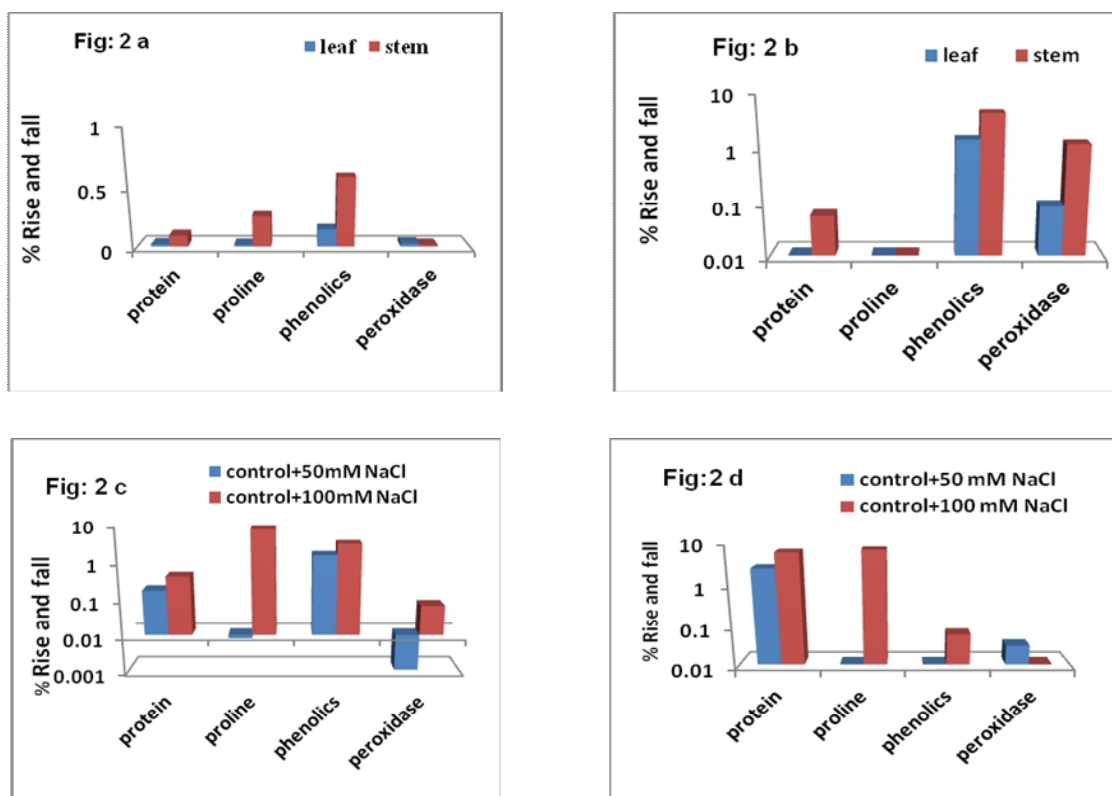


Figure 2: Biochemical parameters of 4 and 8 week old *P. peruviana* seedlings (a-b) under 50 mM NaCl treatment and of 4 and 8 week old callus(c-d) under 50 and 100 mM NaCl treatment

CONCLUSION

In conclusion, the callus has been found to be more tolerant to the salt stress compared to the explant (stem). The tolerance has been expressed as the metabolic response of tissue to salt in terms of accumulation of protein and proline contents, independent of peroxidase activity. Meaning thereby, *P. peruviana* callus undergoes salt stress due to osmotic imbalance rather than free radical formation, which is controlled by higher proline and protein synthesis. Accumulation of phenolics may reduce free radical accumulation and hence no increase in peroxidase activity has been noted in 8 week old 100 mM salt treated callus. This may be explored for higher extraction of medicinal principles in the light of available literature on allied plants.

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ABBREVIATIONS

MS - Murashige and Skoog medium
 BAP - 6-Benzylaminopurine
 NAA - 1-Naphthaleneacetic acid
 PAR - Photosynthetically active radiation

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