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E: ISSN NO.: 2349-980X **Shrinkhla Ek Shodhparak Vaicharik Patrika**

Sodium Chloride and L-Phenylalanine Induced Modification in Biochemical Performance of *Mucuna Pruriens* Calli

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Abstract

Mucuna pruriens belongs to family Fabaceae and includes about 150 species of annual and perennial legume of tropical region especially Africa, India and West Indies. Mucuna formed almost same degree of friable callus from cotyledonary explants on MS medium supplemented with 2,4-D alone or with kinetin. Addition of 50 mM NaCl increased protein, phenolics and proline content. Further increase in NaCl concentration decreased proline, but increased phenolics and protein. Addition of 2 mM Phenylalanine to salt containing medium decreased phenolics and protein content, indicates survival of callus with vigour during salt stress.

Keywords: Mucuna pruriens, Phenylalanine, Sodium chloride

Introduction

The genus *Mucuna* belongs to family Fabaceae and includes about 150 species of annual and perennial legume of tropical region especially Africa, India and West Indies. Many species of the genus offer an excellent source as cover crop and green manure, in addition to their traditional use as feed and food (Janardhanan and Lakshmanan, 1985, Capo-Chichi *et al*., 2003). Almost all the species are reported to contain L 3,4 Dihydroxy Phenyl alanine (L-Dopa) a non protein amino acid that acts as precursor for the neurotransmitter dopamine, used in the treatment of Parkinson's disease (Manyam, 1995). *Mucuna* is traditionally used in various other applications like dye, treatment of pain and numbness of joints, irregular menstruation, anti-tumor (Gupta *et al*, 1997), antidiabetic (Rathi *et al*., 2002) and Antivenom (Guerranti *et al*., 2002). Hence, on the basis of these studies present work was taken up to evaluate the in vitro biochemical profile.

Tissue culture technique could play an important role in the production of active phytochemical substances. Plant cells grown in culture have potential to produce and accumulate chemicals similar to the parent plant from which they were derived. There are numerous reports describing the production of diverse metabolite through cell line selection or addition of precursor into the production medium (Khanna, 1985 and Mulabagal and Tsay, 2004).

Objective of the Study

1. To standardize high fidelity, rapid and reliable protocol for micropropagation of Mucuna pruriens.
2. To obtain maximum amount of bioactive principles through in vitro cultures from Mucuna pruriens.

Material and Methods

The pods of *Mucuna pruriens* were collected from the plants washed with Tween 20 thoroughly under running tap water. Further the pods were surface sterilized with 70% ethanol for about 3 to 4 minutes. Cotyledons were excised in Laminar air flow and inoculated on MS medium (Murashige and Skoog, 1962) supplemented with 3 % sucrose and varying concentration of phytohormones in aseptic condition. The cultures were grown at 25±2^{0}C with a photoperiod of 16 hours fluorescent light and subculture. The callus was harvested at the end of 4 weeks. For evaluation of the accumulation of active metabolite such as total phenolics, protein and proline activity in *Mucuna pruriens* was carried out from the 4 week old callus by using the following standard methods: for estimation of Protein (Lowry *et al.*, 1951), total phenolics were measured by the Folin-Ciocalteu

assay (Bray and Thorpe, 1954) and for the essay of proline content (Bates *et al*., 1973).

Results and Discussion

The different combination and concentration of different plant growth regulators showed varied results, 2, 4-D alone and in combination with varying concentration of Kn were effective for callus induction and growth in *Mucuna pruriens*. However, medium containing 2.0 mg/l 2, 4-D was found to be most effective for callus induction. Biochemical changes observed in the cotyledonary callus during the growth at 4 weeks old age callus in *Mucuna pruriens* revealed certain interesting results. Amount of phenolics was highest in 4 week old callus raised on MS medium supplemented with 2.0 mg/l 2, 4-D + 0.05 mg/l Kn + 100 mM NaCl (Fig-1). Phenolic content increased with addition of 50mM NaCl as compared to respective salt free control. Increased total phenolics also have been reported with moderate saline levels in red pepper by Navarro *et al.*, 2006. Increase in NaCl content upto 100mM NaCl led to extraordinary increase in phenolics and protein content indicating NaCl tolerance through enhancement of defense mechanism by callus (Jogaiah *et al*., 2004 in Grape cultivar). At higher salt concentration, increased phnolics oppose proline accumulation. The mechanism of salt tolerance by both the compounds is basically different. Whereas proline acts as nitrogenous osmoprotectant, the phenols act as carbonic antioxidants. Similar to phenolics, protein content also increased with increase in salt concentration (Fig-2). Jain and Padmaja (2004) have also reported accumulation of protein most of which appears to be stress tolerance proteins. Decline in proline levels could be accounted for increase in proline hydroxylase activity, which is generally a part of stress tolerating protein. Addition of 2.0mM phenylalanine along with varying concentration of salt, deceased salt (NaCl) induced protein accumulation, i.e helps in ameliorating the effect of salt upto a certain level. Phenylalanine supplementation leds to increase in proline accumulation in 100mM NaCl supplemented sets (Fig-3). Results indicate that addition of phenylalanine may possibly helps in suggesting the role of enzymes necessary for proline biosynthesis, during salt (100mM) stress.

Conclusion

From the present studies it can be inferred that callus raised on MS medium supplemented with 3% sucrose, 2.0 mg/l 2, 4-D with varying concentration of Kn with addition of salt can be a balanced source of protein and phenolics with a significant amount of callus growth indicating a possibility of further usage of the callus for secondary metabolite accumulation and isolation.

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Fig -1:% gain or loss in phenolic content of 4 week old Mucuna pruriens calli raised under MS+2.0 mg/l 2,4-D with varying concentration of Kn, NaCl and Phenylalanine compared to respective salt free control

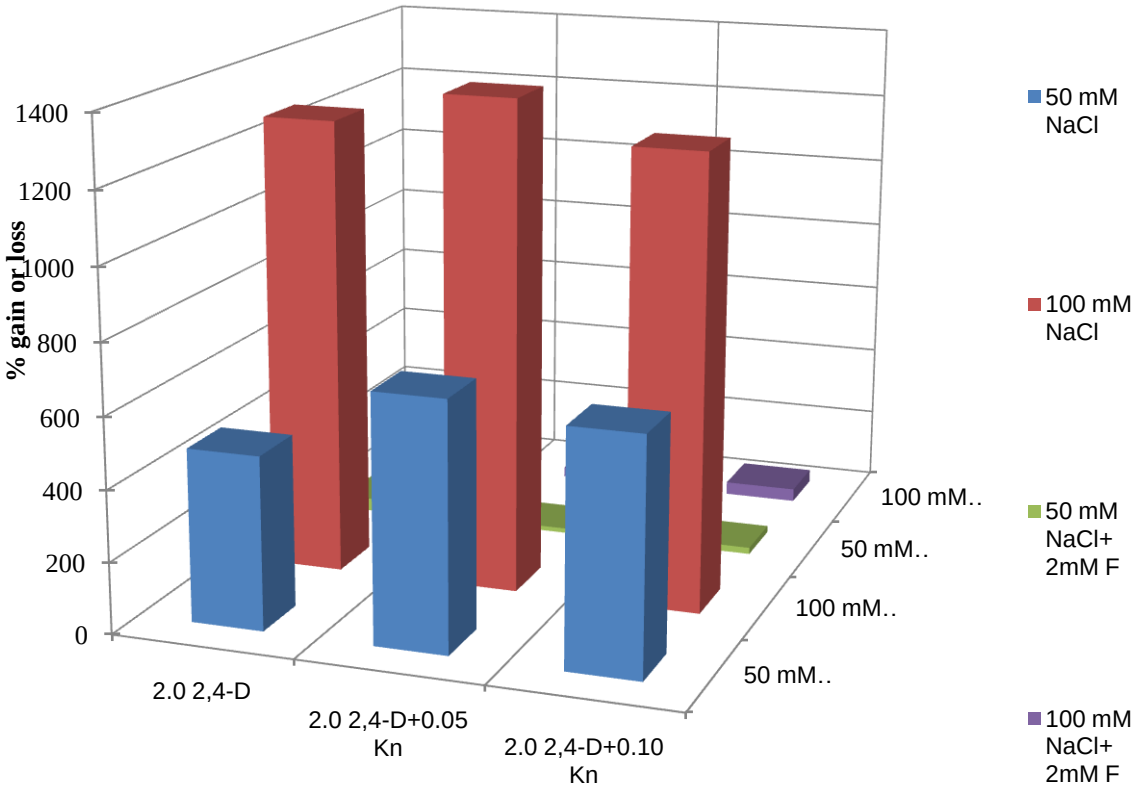

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Fig -2:% gain or loss in protein content of 4 week old Mucuna pruriens calli raised under MS+2.0 mg/l 2,4-D with varying concentration of Kn, NaCl and Phenylalanine compared to respective salt free control

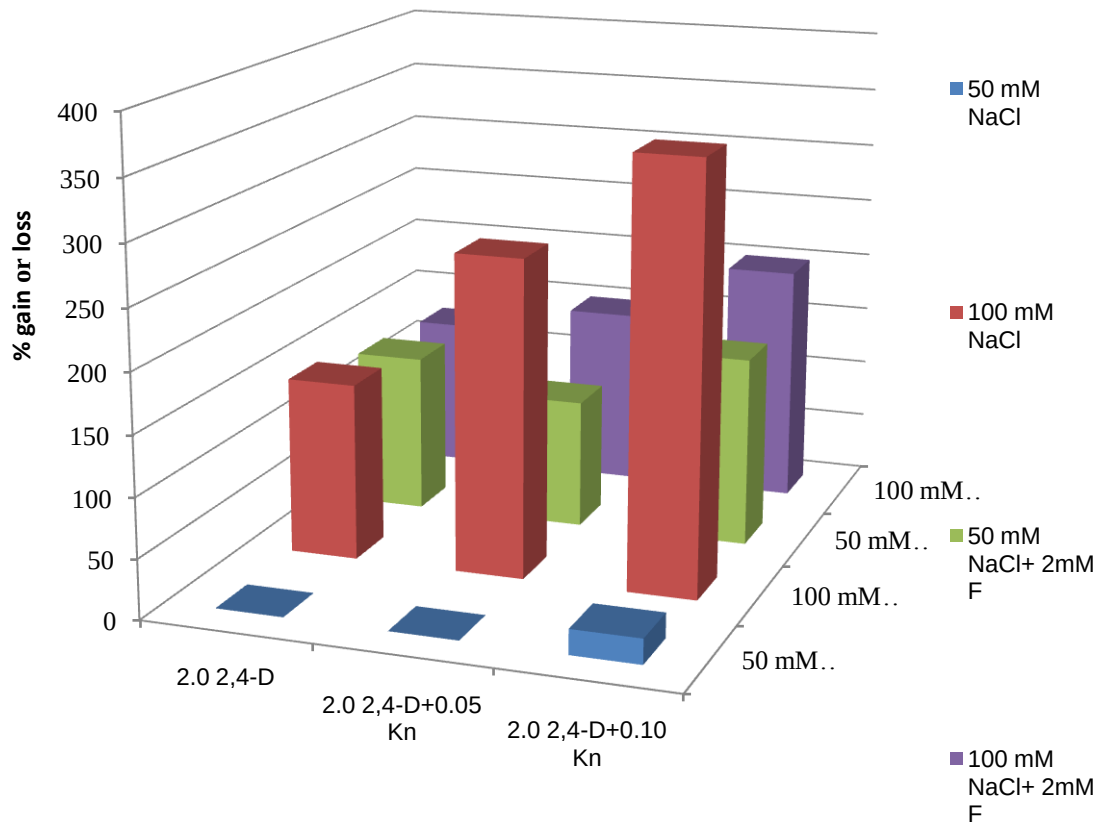

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Fig -3:% gain or loss in proline content of 4 week old Mucuna pruriens calli raised under MS+2.0 mg/l 2,4-D with varying concentration of Kn, NaCl and Phenylalanine compared to respective salt free control