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Development of Salinity Resistant Somaclones of Justicia adhatoda- an Important Medicinal Plant with Bronchodilatory Effect

Linu Mathew*, Rashmi PA

School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, 686 560, India

ABSTRACT

Soil salinity is one of the major environmental abiotic stresses especially in arid and semi-arid regions and can severely limit plant growth and yield. Cell and tissue culture may serve as a useful tool for the assessment of salt tolerance competence in plants since it allows for relatively fast responses, short generation time and controlled environment. In the present investigation, experiments were conducted to study the effects of salinity on plantlet regeneration and secondary metabolite production in Justicia adhatoda L. callus culture. Sodium chloride tolerant calli of J. ahdatoda was obtained by exposing the callus to increasing concentration of Sodium chloride, 5.0mM- 100mM, in Murashige-Skoog medium for 30 days. The tolerant calli were grown better than the sensitive calli in 5.0mM-32mM concentration of Sodium chloride tested. Above 32mM Sodium chloride callus growth was inhibited. The growth behaviour, plantlet production and alkaloid production of the survived calli were characterised and compared with those of wild type plants.

Keywords: Soil salinity, salt tolerance, somaclones, Justicia adhatoda L.

Corresponding author

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INTRODUCTION

In order to meet the ever increasing demand of medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially, but the soil salinity and other forms of pollutions pose serious threats to plant production [1]. So it seems valuable, to test the important medicinal plants for their salt tolerance capacity.

Salinity is a global problem that largely limits crop production in irrigated areas of the world. Many techniques have so far been adapted to alleviate this problem. Of these, one is the selection of salt tolerant genotypes. This technique has successfully been used by many workers for the last many years. They reported that changes in salinized plants growth appear to be associated with accumulation of toxic elements and/or osmotic adjustment and turgor maintenance against these elements. In the last decade, this conventional technique was supplemented with in vitro techniques. Some workers reported that in vitro selection of plants cell lines that exposed to saline environment can be selected for enhancement of tolerance to salinity [2].

Moreover, studies at cellular level provide better knowledge to understand the mechanism of salt tolerance, since they require relatively little space and lower time for the selection, as well as controlled environment. Growth reductions and salt damage appear to be associated with ions toxicity, or disturbance of cellular and tissue water status [2]. In the present study this valuable technique is used for the assessment of sodium chloride (NaCl) tolerance in callus tissue of Justicia adhatoda L.

In Ayurvedic medicine, J. adhatoda has been used for a multitude of disorders including; bronchitis, tuberculosis and other lung and bronchiole disorders. The medicinal properties of J. adhatoda are well known in India and several other countries for many years. The plant is valued for containing bronchodilator alkaloids, mainly vasicine. All parts of the plant are used in herbal medicine and particularly the leaves [3].

MATERIALS AND METHOD

Callus establishment:

Callus was initiated from leaf, axillary bud and root tip explants on MS medium.

Salt tolerant Plantlet Regeneration

Three grams of thirty days old calli were inoculated on to Murashige-Skoog (MS) [4] medium supplemented with growth regulating factors and also containing increasing concentrations of NaCl, such as 5.0mM to 100mM(E. Merck (India) Limited).

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Each treatment per callus was replicated thrice. A control tube (0mM NaCl) also kept along with each concentration. Tubes were incubated for shoot induction for thirty days. Culture conditions were maintained as for callus induction.

Extraction of Secondary Metabolite

Secondary metabolite from 500 mg of plantlets and 500 mg of leaf were obtained by grinding the plant materials using mortar and pestle with 10ml volume of methanol(E. Merck (India) Limited).

Quantification of Vasicine

Quantification of total alkaloids was done by spectrophotometric method with tropaeolin 'OO' (Sigma Aldrich). Coloured complex developed was measured at 545 nm against blank. The amount of total alkaloids in the samples was calculated using standard curve of vasicine (SPIC India Ltd, Chennai).

RESULT AND DISCUSSION

In the present investigation, experiments were conducted to study the effect of salinity on callus growth, plantlet regeneration and secondary metabolite, vasicine, production of Justicia adhatoda under in vitro culture conditions. The plants were treated with different concentrations of NaCl, 5.0mM- 100mM. The salt tolerant calli showed better growth compared to sensitive calli. Calli produce plantlets on medium containing upto 32mM of NaCl but showed stunted growth (Plate3). But better proliferation was seen in media with 5.0mM-25mM of NaCl (Plate1). Healthy plantlets were obtained in those concentrations. Increased concentration of NaCl affected the survival of callus (Figure1). The callus survival and growth were completely inhibited resulting in tissue browning and subsequent death at NaCl above 34mM. (Plate2). When the cultures were subjected to higher levels of NaCl, symptoms of toxicity appeared as leaf damage, i.e. reduced size, marginal necrosis and ultimately explant necrosis. Only 8% of the calli were survived at 34mM of NaCl and showed stunted growth.

Plate. 1: Calli produce plantlet on medium containing upto 25mM NaCl

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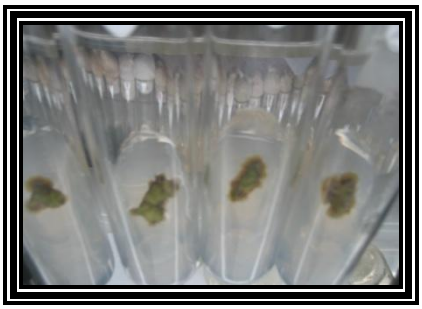


Plate. 2: Tissue shows browning and necrosis at NaCl above 33mM

Plate. 3: Plantlet shows stunted growth at NaCl above 25mMol

The secondary metabolite production by salt tolerant plantlets was at par with that of normal field grown plants (Table 1) [5].

Table.1: Quantitative Analysis of Vasicine Produced from Salt Tolerant Plants

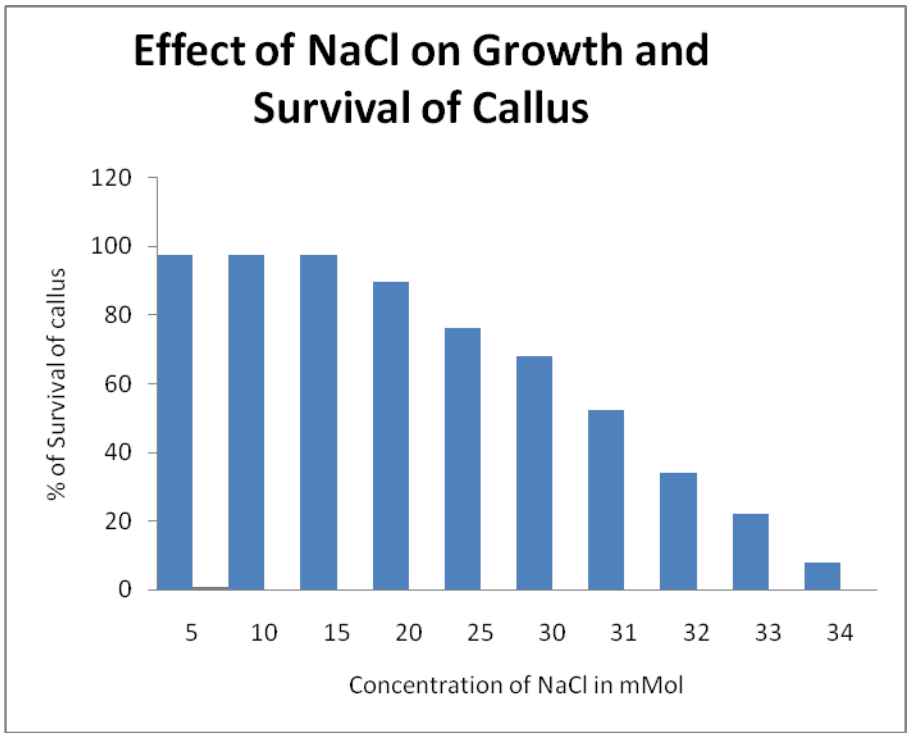
Sl No	Concentration of Vasicine (Mean) in mgL^{-1}	Average Production of Vasicine
Plant1	5.15	5.15±0.0433
Plant2	5.12	5.12±0.0260
Plant3	5.14	5.14±0.0141

Incorporation of salinity stress in the medium in the form of NaCl during callus induction and regeneration will pave the way for studying the effects of salt stress on the different stages of development. Increasing salinity induced a marked reduction in the plant growth, though the seedlings tolerated salinity upto 34mM NaCl.

Healthy and fast growing friable callus is the prerequisite of different biotechnological investigations. Callus consists of undifferentiated masses of cells developed on a semi-solid medium. The maintenance of such cultures depends on an adequate supply of nutrients, growth hormone and controlled sterile environment. The cells, although undifferentiated,

contain all the genetic information present in parent plant. By suitable manipulation of hormone and contents of the medium, it is possible to initiate the development of roots, shoots and complete plants from callus culture [6]. The nutritional requirements of plant cells and tissues vary from species to species.

Figure 1: Effect of NaCl on Growth and Survival of Callus



Salinity is one of the most serious environmental problems in influencing plant growth. Today the use of biofertilizers in agriculture is quite diffused, and good results have been obtained in terms of induction of resistance to biotic and abiotic stresses in plants [7]. Some medicinal plants need to be cultivated commercially in order to meet the ever-increasing demand for medicinal plants for the indigenous systems of medicine as well as for the pharmaceutical industry. In this regard, it seems significantly to test the important medicinal plant for their salt- tolerance capacity [8].

The finding of regeneration decrements in the present study were in agreement with many researchers who reported negative response of NaCl towards plant regeneration. Khorami and Safarnejad [9] mentioned that NaCl inhibited plant regeneration. Also, Priya et al. [10] reported that salinity strongly reduced the regeneration capacities of callus obtained from all cultivars and on all regeneration media tested, which is an agreement with the present study.

From the results of this investigation, it is clear that, the NaCl treatment caused alteration in growth of J. adhatoda but the secondary metabolite production was not affected

in the salt resistant plantlets. Thus the present study suggested a protocol for the cultivation of this plant under saline conditions.

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