Influence of NaCl Treatments on Micropropagation of Musa SPP. CV. Gaja Bantala

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Abstract

Salinity causes inhibitory effects on plant growth, morphology, and survival because of the toxicity of excessive Na^+ and Cl^- to the absorbance of water. In the present experiment micropropagated shoots of Musa paradisiaca cv. Gaja Bantala were cultured in rooting medium supplied with 1 mg/L IAA along with different concentration of NaCl. Various parameters including days of response, percentage of response,average numbers of roots etc. were taken after root formation from shoots inoculated with Murashige and Skoog (MS) medium. It was revealed that NaCl used in low concentration produced healthy and long roots whereas NaCl in medium at very high concentration (1 gm/L) affected the root formation in shoots more rapidly although the roots weregrown healthybut not too long. The percentage of response was declined to 50% and the average number of roots was 3.4. The leaves of few shoots turned yellow in color indicating decrease in chlorophyll content of shoots.

Keyword

Musa paradisiaca, Murashige and Skoog medium, Tissue Culture, NaCl, Gaja Bantala.

I. INTRODUCTION

Banana and Plantain represents the world's second largest fruit crop and fourth most important global foodcrop with an annual production over 100 million metric tons around the world. It is mostly propagated by vegetative means by using suckers which grow from lateral buds originating from corms and suckers are used for production of individual plants. This process is very slow as the rate of multiplication of suckers through conventional vegetative means has been found to express several negative impacts which include transmission of diseases, low production and poor preservation of original plant genetic material [9]. Tissue culture techniques have been applied to the plant species to produce new clones and cultivars with improved characteristics. The in vitro culture of banana includes many steps like shoot initiation, shoot multiplication and rooting. The cytokinins and auxins are of importance in in vitro culture as the later are concerned with root formation, the former is mainly required in the media for shoot formation and growth of buds [16]. Thus, numbers of researchers have suggested that cultured tissues and cells may prove useful both in selections of the salt-tolerant plants and in studies of the physiological basis for salinity tolerance [2, 19]. In vitro culture can also be used for studying the effects of different abiotic stress factors, like drought [21] and heavy metals [4]. Salinity is an important abiotic stress factor severely affecting productivity and survival of plant species. Scientists reported that salinity stress causes many adverse effects on the growth and development of millets [9]. Salinity causes inhibitory effects on plant growth, morphology, and survival because of the toxicity of excessive Na^+ and Cl^- to the absorbance of water. It is revealed that salt stress would cause an imbalance of the cellular ions resulting in ion toxicity and osmotic stress [12, 14].

The aim of the present study is to observe the different morphological abnormalities of banana shoots and roots grown on MS medium containing low NaCl concentration (100 mg/L, 200 mg/L) and high NaCl concentration (500 mg/L, 1000 mg/L).

II. MATERIAL AND METHODS

A. Plant material

Gaja Bantala variety of plantain belongs to the native places of Odisha and has a high demand by the farmers due to its fruit size and taste. For the initial culture healthy disease free 2-3 months old suckers were used as test material. The healthy shoots with 2-3 leafs were obtained from multiplication culture through the tissue culture of the Meristem inoculated withMurashige & Skoog medium containing growth regulator IAA. For the present study the shoots were collected from the multiplication phase of Gaja Bantala grown in vitro at Banana tissue culture laboratory of Regional Plant Resource Centre, Bhubaneswar Odisha, India.

B. Sucker sterilization

Suckers collected from the mother block contain many contaminations like bacteria and fungus that are present in soil. Before inoculation in media they were treated with different chemicals to make it sterilized. It was done by following steps. The trimmed suckers were kept under running tap water for 10 minutes. After processing suckers were washed in liquid detergent (Labolene) for 2-3minutes. Explants were then dipped in bavistin solution (1 %) for 30 minutes. After 30 minutes the suckers were washed with autoclaved double distilled water and transferred to mercuric chloride solution (0.5 %) for 45 minutes. The suckers were washed in 70 % alcohol solution for 1 minute. Finally the suckers were washed 3- 4 times with autoclaved double distilled water to remove excess chemicals from the sucker surface.

C. Culture medium

The most commonly used medium for banana tissue culture is Murashige & Skoog medium [15]. The phytohormone used for the root culture studies was Indole -3-acetic acid (IAA) at 1.0 mg/L.

D. Development of roots at different salt

concentration in rooting culture-To study the effects of salinity stress on the root induction of Musa paradisiaca cv. Bantala, sodium chlorite (NaCl) was used at different concentration following 100 mg/L, 200 mg/L, 500 mg/L, 1 gm/L. From the experiment it was found out that shoots cultured in rooting medium containing MS + 1 mg/L IAA showed excellent result.

The 1 mg/L IAA in MS medium was considered as the control for all the experiments. For each experiment the observation of 5 numbers of rooted plants were noted. The rate of contamination was in an average of 10% to 20%.

E. Inoculation of micropropagated shoots

The working area of the laminar airflow was first wiped with cotton moistened with 70 % ethanol and then irradiated with ultraviolet light for 30 minutes before inoculation. The shoots were taken out to a autoclaved petridish and shoots were isolated using sterilized scalpel and forceps. The shoots were inoculated in the culture vessel containing induction medium. The culture vessels containing the shoots were kept in culture rack maintained at 24°C to 26°C, 16 hr photo period of 35-50µEm-2s-1 intensity provided by cool white fluorescent tubes in the culture room of Banana Tissue Culture Lab.

F. *Analysis of Leaf Pigments*

Fresh leaf samples that were collected from the plantlets on 21st day after completion of rooting stage were homogenized and centrifuged. The supernatant was collected and the absorbency was noted. The O.D of all the samples was measured at 663 nm and 645 nm.

III. RESULTS AND DISCUSSION

A. Control for the studies

The control data taken for the present experiment was observed in miropropagated plantlets inoculated in MS medium containing 1 mg/L IAA without treated with NaCl. Auxins and other growth regulator such as gibberellins play important roles in the growth and differentiation of cultured cells and tissues [1]. Auxins such as Naphtalene acetic acid (NAA) have been reported to promote plant rooting in vitro [10]. During this experiment it was observed that shoots grown in 1 mg/L IAA had developed good numbers of roots in short duration. Generally higher auxin concentrations are required during the induction phase, whereas during the later stage it becomes inhibitory. This effect is also marked when micro cuttings are cultured continuously on media with auxins, studied by De Klerk et al. (1997) [5] in apple microcuttings previously exposed for 3 weeks to IAA and which rooted ex vitro more efficiently than those exposed to IBA or NAA.The data revealed from five cultured samples shown that the percentage response was 100 % with average roots number of 5. The root length varied in all the five samples with minimum root length of 1.81 cm to 5.78 cm.

B. Effect of NaCl on root proliferation

During this study effects of different concentration of NaCl on rooting culture of banana cv. Gaja Bantala has been marked phenotypically. The response of root induction gradually decreased from 100 % to 50 % as the concentration of NaCl increased. Similar results were also observed by Kumari and Vishnuvardhan, 2015 [13] while studing the effects of salinity on growth physiological activities and developmental processes of three Kodo Millet (Paspalum scrobiculatum).All the abiotic stresses such as water deficit stress (drought) or saline stress are probably involved in limiting plant growth significantly [8]. Both stresses (salinity and drought) are metabolically as well as physiologically related phenomena, as both induce osmotic stress on the affected plants [6, 20]. This increased damage is may be due to change in cell expansion process which is controlled by processes related to cellular water uptake and cell wall extension [3]. In many cases it was marked that as the number of roots increased the length of roots has been decreased. The root growth and development in higher concentration of NaCl was not as stout and healthy as comparison to control and medium having lower concentration of NaCl. Roots were thin with less

root hair in shoots cultured in high NaCl rooting medium.

No significant difference has been marked among the five rooted cultured plantlets in growth and development of shoots and leaves during the experiment. After applying stress (NaCl) the nitrogen, carbon and energy for recovery of stressed tissue [18].

B.2. Effect of sodium chlorite at 200 mg/L

M100. The root length varied in all the samples with minimum root length of 2.67 cm to 4.87 cm.

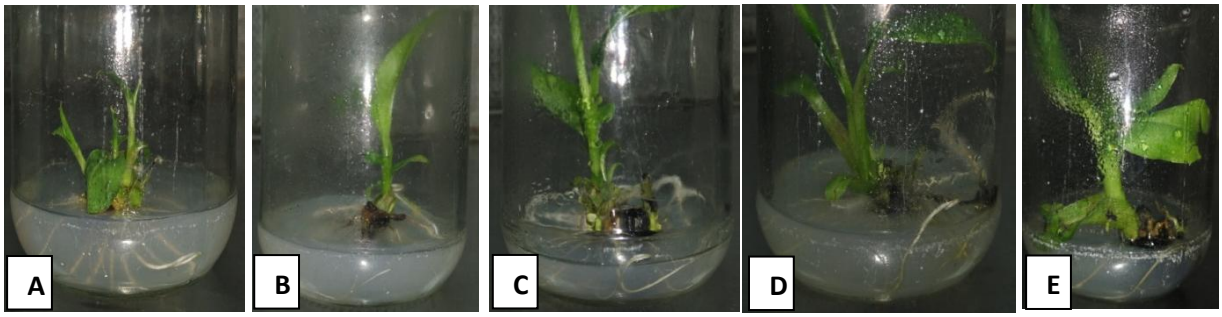

Figure 1. 3 weeks old micropropagated plantlets of banana (*Musa* spp.) cv. Gaja Bantala on different culture under *in vitro* conditions. A. Control (1 mg/L IAA, No NaCl); B. (100 mg/L NaCl); C. (200 mg/LNaCl); D. (500 mg/L NaCl); E. (1 gm/L NaCl).

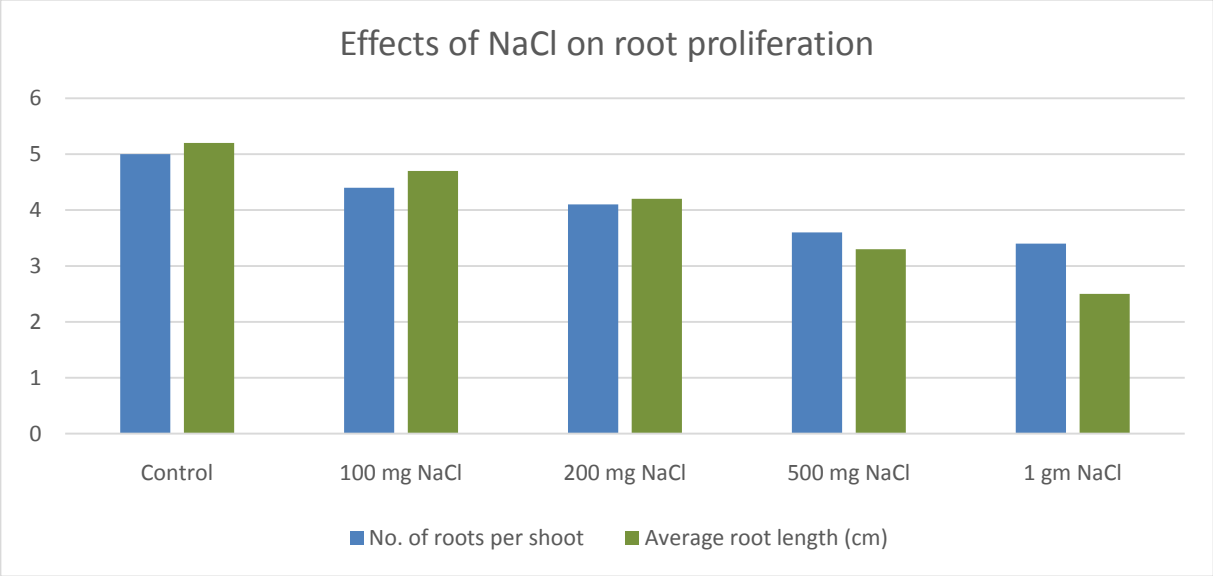

Figure 2. The above data revels the toxic effect of NaCl on root proliferation (Numbers of roots per shoot and Average root length in cm) of Gaja Bantala plantlets at different concentration (Control- 1 mg/L IAA, No NaCl)100 mg/L, 200 mg/L**,** 500 mg/L and 1 gm/L NaCl.

B.1.Effect of sodium chlorite at 100 mg/L

From the data it was observed that NaCl used in low concentration produced healthy and long roots. The percentage of response was 100% but the average number of roots reduced to 4.4 which was less in comparison to control (medium with no NaCl and only 1 mg/L IAA). The root length varied in all the sampleswith minimum root length of 1.73 cm to 5.00 cm.

B.3. Effect of sodium chlorite at 500 mg/

The percentage of response was 70% and the average number of roots was 3.6 in shoots cultured in 500 mg/L NaCl medium which is similar to shoots grown on medium containing 200 mg/L NaCl. Which mean with the increase in the salt concentration the root proliferation reduced but up to a certain salt concentration the effect was constant. The plantlets had shown certain resistant to salt stress.

B.4.Effect of sodium chlorite at 1 gm/L

From the data it was observed that salt at this concentration (1 gm/L) affect the root formation in shoots more rapidly although the roots grown were healthy but not too long. The percentage of response was decline to 50% and the average number of roots was 3.4.

was observed from the analysis of the leaf pigments of plants. Rooted plants grown on salt free medium has 2.88 mg/gm chlorophyll. Then chlorophyll content reduced rapidly to 2.30 mg/gm indicating the toxic effect on shoots cultured in rooting medium with 1 gm/L NaCl.

Figure 3. Rooted plants in rooting medium containing 1 mg/L IAA and NaCl at different concentration. A. (100 mg/L NaCl); B. (200 mg/LNaCl); C. (500 mg/L NaCl); D. (1 gm/L NaCl). The above figure shows the reduction in growth and proliferation of roots as well as shoots due to the NaCl toxic effects.

C.Analysis of Leaf Pigments-

Chlorophyll content is one of the most investigated physiological (but not specific) characteristics used for identification of physiological disturbance due to emission impact. It was established that some metals decreased chlorophyll content in many plant species [7]. With the increase in salt concentration the chlorophyll content in the plantlets decreases which

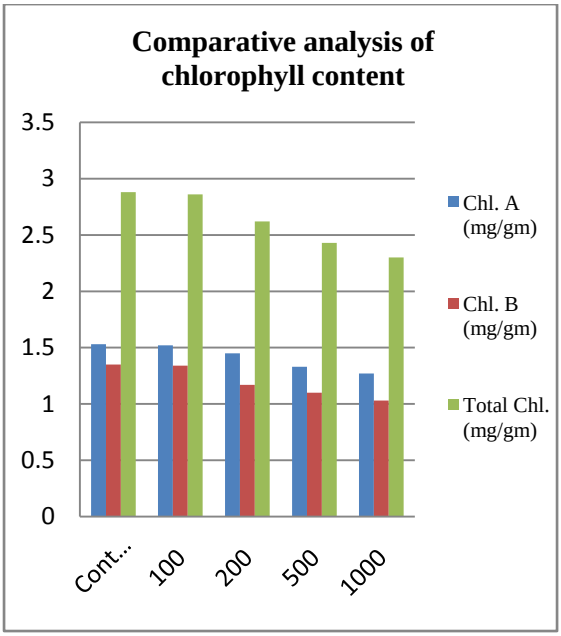

Figure 4. Comparative analysis of chlorophyll content in leave samples of Gaja Bantala plantlets cultured in rooting medium without NaCl (Control) and with NaCl (100,200,500 and 1000 mg/L).

Growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies [17]. Growth and development reduction was also noted with the increase of NaCl concentration in three pea species grown through in vitro culture [11]. As sodium chloride concentration increased the percentage of response of roots induction, root length and chlorophyll contentdecreased in this experiment. It was observed that roots formation at high NaCl concentration were unhealthy, weak and smaller in length in compared to the roots grown in low salt concentration (100 mg/L NaCl). Salinity causes to decrease in photosynthetic pigments (Chl a, b and total Chl) in banana plants (Figure 4). From this study it was depicted that Chl a is more sensitive than Chl b to salinity. Here in this experiment results are seems also similar with the findings of Haq et al., 2011 [22] studied in Sindhri Banana (Basrai) propagating plantlets through aseptic condition.

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