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IN-VITRO MULTIPLICATION OF BANANA (*MUSA* SPP.) UNDER DIFFERENT NaCl STRESSES

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ABSTRACT

Salinity is a major abiotic factor. Its effects were assessed on the micro-propagation efficiency in Pakistani banana (*Musa* spp.) variety Basrai. Micro-propagation rate was decreased with an increase in NaCl level. Due to it, numbers of plantlets per explant, pseudostem diameter was decreased. Bio-chemical contents were also affected due to salinity such as K^+ was decreased while Na^+ and Cl^- increased significantly. So salinity is reducing not only micro-propagation efficiency in banana as well as decreasing its yield.

INTRODUCTION

Banana is one of the most important table fruit. Its annual production is more than 102 million tons per year (FAO, 2006). Banana is rich with various carbohydrates and minerals. It is equally beneficial diet for both children and adults (Vuylsteke and Ortiz, 1996). However, banana production is going to be decreasing due to a number of biotic and a-biotic factors.

Salinity is a serious growth retarding abiotic factor for agricultural crops. About 20% of world's cultivated land is adversely affected by high salt concentration, which has been inhibiting both plant growth and yield (Tanji, 1990, Huckle et al., 2000). Now days, great efforts are under way to solve salt stress problem, to ensure agricultural sustainability and crop production.

Plant micro-propagation is an efficient and safe route to develop a huge numbers but should be normal and fertile plantlets (Haq and Dahot, 2007). However, the rate of progress in a specific mode of development depends on different physical conditions (light, temperature, pH, salinity and drought) of growth cultures (Alvard et al., 1993, Escalona et al., 1999). Salinity is one among them, which has a serious effect on micro-propagation rate. It is effective not only under *in-vivo* but also *in-vitro*.

In-vitro is a useful tool to observe salt tolerance at cell level, through which the effects of salinity on plant morphogenesis can be studied easily. (Wang, 2006). In case of banana micro-propagation, like other species, the presence of NaCl in the cultures causes to reduce (Lexer and Fay, 2005, Lacerda et al., 2001 & 2003) or complete inhibition of growth. (Tejera et al 2004, Lopez et al., 2006)

The aim of present experiment was to check the severity of salinity on *in-vitro* multiplication efficiency in banana. On the basis of which we can suggest the farmers, about the type of soil (salt concentration) suitable for the cultivation of banana crop.

MATERIALS AND METHODS

A. Plant material and sterilization: Young banana meristem tips of banana (*Musa* sp.) cv. Basrai were excised. They

were surface sterilized by washing with ethanol (90%) for 1 min, than stirred with 30% commercial bleach (5.25% NaOCl) for 30 min, afterwards washed with sterile distilled water for 3-times (3x5 min).

B. Micro-propagation culture: The micro-propagation culture was establish by culturing micro-cuttings of suckers for organogenesis on MS basal medium (Murashige and Skoog, 1962) supplemented with vitamins B5 (Gamborg et al., 1968), 3% sucrose, 10 µM IAA and 8 µM BA and solidified with 3.60 g/L phytagel for organogenesis (MS_{2a}) for 3-weeks. The shoot induction was carried out by culturing them on MS medium supplemented with 15 µM BA, solidified with 1.0 g/L phytagel (MS_{2b}). After 3-4 weeks, numbers of plantlets were regenerated (Haq and Dahot, 2007).

C. NaCl treatments: After three weeks, micro-propagating plantlets were sub-cultured under different saline stresses (Table 1; Figure 1). Three stresses of salinity (NaCl) were maintained in shoot multiplication media including one control i.e., MS_{2b} (0mM NaCl) MS_2c (50 mM NaCl) and MS_2d (100mM NaCl) were maintained for again four weeks.

D. Culture conditions: All cultures were supplemented with 20.0 µM L-cystein, 3.0% sucrose and pH was adjusted to 5.7-5.8 before autoclaving (121°C and 20-lbs for 15 min). Each culture was maintained at 25±2°C with 18/6 h photoperiod (light intensity ~2000 lux).

E. Data collections

a. Morphological parameters: After four weeks, micro-propagating plantlets were removed from each culture. They were washed with water than number of plantlets per explant, pseudostem diameter and plants height was measured.

b. Bio-chemical analysis: After taking morphological parameters, plant material was dried in electric oven at 72°C for 2-days. Dried plant material was subjected to different bio-chemical analysis as described below.

i. Chloride contents: Chloride contents were measured by Chloro-Counter, by following the instruction in the instrument-operating manual (Marius Instrumenten, Utrecht, and The Netherlands).

ii. Na^+ and K^+ contents: Dried plant material was digested by acidic digestion. The sample extract was subjected to cations analysis (Na^+ and K^+) as described by Malavolta et al., (1989).

iii. Statistical analysis: Statistical analysis of date for all parameters collected during this experiment was computed by using a COSTAT Computer Package (CoHort Software, Berkeley, USA) at 0.5% level of significance.

RESULTS AND DISCUSSION

In this experiment, shoot multiplication medium (MS_{2b}) was used as a non-saline control medium. It is an optimized medium with favorable properties for the micro-propagation of banana (Haq and Dahot, 2007). This protocol is capable for developing normal and fertile plantlets. Two saline cultures were maintained by using a single salt (NaCl) in shoot multiplication medium (Table 1). So shoot multiplication cultures were maintained under both saline and non-saline stresses. It was observed that numbers of plantlets were decreased significantly at 100mM level of NaCl, during 28-days of culture.

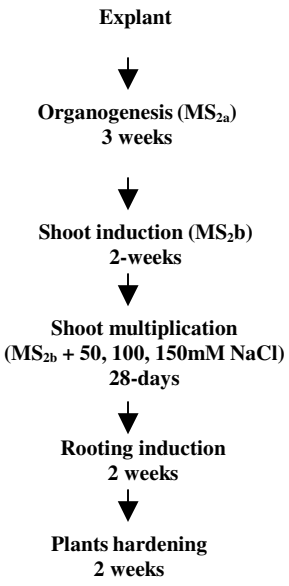

Figure 1. Schematic representation of the micro-propagating banana (*Musa* sp.) cv. Basrai with different NaCl stresses.

The plant height was also decreased but non-significantly under saline conditions during the whole growth period. Similar behavior for pseudostem diameter was also observed (Table 2, Figure 2). Inspite of vegetative growth parameters, other biochemical characters were also imbalanced with the increase in salinity levels (Ottow et al., 2005, Lopez et al., 2006).

With an increase in NaCl from control to 100 mM NaCl, both Na^+ and Cl^- were increased significantly, while a non-significant decrease in K^+ was observed. However, statistical analysis of data derived from the contents of Na^+ and K^+ in different cultures either stressed or unstressed are correlating with the data of culture growth (a visible indicator of tolerance). It leads to deduce that, accumulation of K^+ or Na^+ are the markers for either triggering or inhibiting the propagation rate among the cultures. Presence or retention of K^+ was a key factor for the indication of the non-saline stress. The positively correlation was observed with the growth of the plantlets. However, the correlation between growth and Na^+ was negative, when it increases, micro-propagation rate of cultured explants decreases significantly. While a positive correlation of K^+, seems to be a marker for efficient plant growth.

Both sodium and chloride ions were increased with the increase in salt levels but their specific amount in the plant tissue expected to be beneficial for plant growth due to their significance in the osmotic pool, while under higher salt stress, probably contributing to the higher reduction in the growth rate of the plantlets. So their abundance has negative effects on micro-propagation of banana.

Potassium ions are known to be a major component of osmotic adjustment during stress (Wu et al., 1996, Ottow et al., 2005), but the data of the present experiment for K^+ and Na^+ in stressed apparently was not consistent with the earlier finding of Dvorak and Gorham (1992) and Watad et al., (1991). However, plantlets on normal micro-propagation cultures were inherently rich in K^+ in comparison to that of saline cultures (Table 2), so K^+ was released in an environment rich in NaCl. The presence of a high concentration of K^+ in control one, which was supposed to act as the natural inorganic osmo-regulator (Chen et al., 2003, Munns, 2005) perhaps allowing Na^+ to enter in the tissue, which explains the situation of lower K^+ content after shock treatment as the physiological 'window' of optimum K^+ concentrations narrows in the presence of increasing amounts of Na^+ (Marschner, 1995, Maathius and Amtmann, 1999). A

higher level of endogenous K^+ content was also reported in Nona Bokra, which is a salt resistant cultivar (Lutts et al., 1999, Hartzendorf and Rolletschek, 2001).

It is concluded that salinity is really a major abiotic factor for plants including banana. During its micro-propagation, NaCl bears un-maintainable affects. These imbalanced morpho-biochemical aspects in multiplying banana either *in-vitro* or *in-vivo* reflects the ultimate loss/decrease its yield. The banana is a much sensitive crop for saline factor. It is the main reason for the lower regeneration and multiplication rate of banana in many saline areas in Pakistan.

Table-1: Different cultures used for assessing the effect of NaCl (28-days culture) on banana micro-propagation.

Media	Composition	Cultures
MS_0	MS salts	Rooting
MS_{2a}	MS salts + 10µM IAA and 8µM BA	Organogenesis
MS_{2b}	MS salts + 15µM BA	Shoot multiplication
MS_{2c}	MS salts + 15µM BA + 50mM NaCl	MS_{2b}+ NaCl
MS_{2d}	MS salts + 15µM BA + 100mM NaCl	"

Table-2: Effect of NaCl stress morpho-biochemical aspects of propagating banana (*Musa*) for 28-days culture.

Parameters	MS_{2b}	MS_{2c}	MS_{2d}	**ANOVA** (0.5%)
A. Morphology				
a. # of plantlets	5.85±0.152	2.32±0.12	1.25±0.69	*
b. Plant height(cm)	2.90±0.281	2.71±0.65	1.30±0.32	ns
c. Peudostem diameter (cm)	0.211±0.132	0.21±0.05	0.18±0.02	ns
B. Inorganics	3.50±1.00	4.25±0.90	7.20±1.20	***
a. Na^+	10.01±1.20	8.20±0.90	5.50±1.50	ns
b. K^+	4.40±1.50	6.22±1.28	8.50±1.20	*
c. Cl^-				

Figure-2: The morphological appearance of micro-propagating plantlets of banana cv Basrai on shoot multiplication medium supplemented with/ without different saline (NaCl) stresses. a: Shoot multiplication on MS_{2b} medium (with 0.0mMNaCl); b: MS_{2c} supplemented with 50mM NaCl and c: MS_{2d} supplemented with 100mM NaCl

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