

Full Length Research Paper

Characteristics of micro-propagated banana (*Musa spp.*) cultures stressed with NaCl and polyethylene glycol

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The effect of NaCl and PEG was assessed on plant micro-propagation rate in banana (*Musa spp.*) cv., Basrai. Well micro-propagated plantlets were cultured on four different stresses of NaCl and PEG-4000 including control level: MS_{2b} (MS₀ + 3.0 mg l⁻¹ BAP), MS_{2c} (MS₀ + 100 mol m⁻³ NaCl), MS_{2d} (MS₀ + 5% PEG) and MS_{2e} (MS₀ + 100 mol m⁻³ NaCl + 5 % PEG) for 6-weeks. Efficiency of plant micro-propagation was reduced significantly among the stressed cultures. Similarly, photosynthetic pigments like chl *a* was decreased non-significantly but chl *b*, chl *ab* were decreased significantly. Total carotenoids were increased in the saline as well as PEG stressed cultures. Cell size of epidermis and aerenchyma was increased ($p < 0.05$), while parenchyma decreased. Proline and glycinebetain contents were increased ($p < 0.05$) in each stressed culture but were high in MS₂ than in MS₃ and MS₄ cultures. Meanwhile, proteins, sugars, phenolics and nitrates were observed to be in the reversed ($p < 0.05$) phenomena. In conclusion, NaCl treatment was observed to be most toxic than the PEG or PEG with NaCl on the banana micro-propagation.

Key words: *Musa spp.*, micro-propagation, NaCl (sodium chloride), PEG (polyethylene glycol), chlorophyll contents, proline, reducing sugars.

INTRODUCTION

Banana (*Musa spp.*) crop is a delicious fruit with 25% carbohydrates. Being an important staple food crop, it provides a valuable source of income for domestic's trade. Today, the rate of the world's banana production has been approximately 44 tons hectare⁻¹ (Frison et al., 1997; Roux et al., 2001; FAO, 2005). Commercial yield of banana is being lost by a number of variable environmental stresses.

Among the abiotic stresses, excessive saline conditions of the soil causes loss of both vegetative potential and reproductive plant growth in crops (Neumann, 1997). Inherent tolerance in crops and good management practices may enable partial amelioration of damage from soil salinity (Cherian and Reddy, 2003; Badiane et al., 2004; Flowers, 2004; Davenport et al., 2005). Similarly, drought requires proper understanding for better plant growth. It causes deficit water supply to the growing plants. Meanwhile, salinity causes inhibitory effects on plant growth because of the toxicity of excessive Na⁺ and Cl⁻ to the absorbance of water. It can be attributed to decrease in availability of the water required or imposed by developed osmotic stress (Kefu et al., 2003; Djibril et al., 2005; Munns et al., 2006).

However, both drought and salinity are involved in limiting plant growth. These are becoming widespread in

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Abbreviations: MS, Murashige and Skoog; NaCl, sodium chloride; PEG-4000, polyethylene glycol 4000; Chl, chlorophyll; IAA, indole acetic acid; BA, benzyleaminopurine; NaOCl, sodium hypochlorite; B5 vit, B5 vitamins.

the world and may convert more than 50% arable soil to saline soil in 2050 (Alam et al., 2002; Wang et al., 2003; FAO, 2005). With the passage of time, many crops have evolved certain mechanisms in metabolism to combat water deficit stressed conditions (Sadeghian and Yavari, 2004). Among the stressed plant cultures, one of such mechanism is ubiquitous, that is the accumulation of various organic metabolites especially during seed germination than in vegetative growth (Bewley and Black, 1994). Similarly, polyethylene glycol (PEG) induces water stress when supplied in plant growth cultures (Ruf et al., 1967; Kaufman and Eckard, 1971). It reduces the osmotic potential in nutrient culture medium. This inert osmoticum is not taken in the plant cell and phyto-toxicity (Lawlor, 1970).

Presently, aseptic plant cultures is gaining much importance for the development of abiotic stress resistant plants and also in elucidating physiological and biochemical operating mechanisms that involve under stressed conditions. These aspects are to keep plants survival under severe abiotic stresses at the cellular level against drought and salinity (Jain, 2001). Plant tissue culture provide a control and homogeneity stressed condition for the measurements of cell behaviors under stress conditions at the whole plant level; not for a single regulatory system (Lutts et al., 2004).

During this study, effects of both ionic and water deficit stresses under *in-vitro* condition through the application of NaCl and PEG or both were observed in micro-propagating plantlets of banana (*Musa* spp.). After 6-weeks of stress, a number of morpho-bio-components were studied. Differential phenomena were observed in the stressed cultures than in the control. This conducted work may be useful in the future for the improvement of salt or drought resistant plants or their selection.

MATERIALS AND METHODS

Apparently, healthy suckers of Basrai cultivar (*Musa* spp.) were collected from open-air banana fields. Inner portions of the sucker (meristem) were excised. They were sterilized with ethanol (90%) treatment for 1 min. They were washed with water and than stirred for 30 min in 30% Robin bleach (5.25% NaOCl). Organogenesis in the sterilized explants was induced by culturing them on MS₂ (MS (Murashige and Skoog, 1962) basal medium, B₅ vitamins (Gamborg et al., 1968) and 3% sucrose plant nutrient medium, supplemented with 10 μM IAA and 8 μM BA. After 3-weeks of culture, they were sub-cultured on MS_{2a} [MS, BA (15 μM), phytigel 1.0 g l⁻¹] medium for shoot induction for 2-weeks. This organogenesis culture was sub-cultured on MS_{2b} [MS, BA (15 μM), phytigel (2.0 g l⁻¹)] nutrient medium for multiplication of induced shoots (Haq and Dahot, 2007a).

Plantlets from (MS_{2b}) cultures were sub-cultured on differentially stressed micro-propagation cultures. They were represented as MS_{2b} (control), MS_{2c} (MS_{2b} + 100 mol m⁻³ NaCl), MS_{2d} (MS_{2b} + 5% PEG-4000) and MS_{2e} (MS_{2b} + 100 mol m⁻³ NaCl + 5% PEG-4000) cultures. Each established cultures were supplied with 3% sucrose (as carbon source) and 20 μM L-cystein. The 5.7 to 5.8 pH was adjusted before autoclaving (121 °C and 20 lbs for 15 min). Among the established cultures, each treatment was compromised on 7-replicates. These cultures were maintained under light conditions

with ~2000 lux light intensity for 18/6 h for 6 weeks at 25 ± 1 °C.

The micro-propagated plantlets from all the cultures excised and entangled medium was washed with tap-water. They were dried with filter-paper and data was collected. Numbers of plantlets propagated in the single explant were counted. Shoot height and its biomass were also measured. Photosynthetic pigments were determined as by Lichtenthaler (1987). Anatomy of leaf was also studied according to Gielwanowska et al. (2005) and Johansen (1940) methods. Both glycine-betain (Grieve and Gratter, 1983) and proline (Bates et al., 1973) contents were determined spectrophotometrically, by using fresh leaf tissues. Phenol contents were determined by using fresh leaves from each culture (Ozyigit et al., 2007).

Plant material was dried at 72 °C in electric oven for 2-days. It was used to study various bio-chemical characters. Protein contents (Bradford, 1976) Total carbohydrates (Dubois et al., 1956; Ciha and Brun, 1978), reducing sugars (Miller, 1959) and nitrate contents (Morris and Riley, 1963) were determined.

Data significance was calculated using COSTAT computer statistical software, including LSD at 5% level of difference among the cultures.

RESULTS AND DISCUSSION

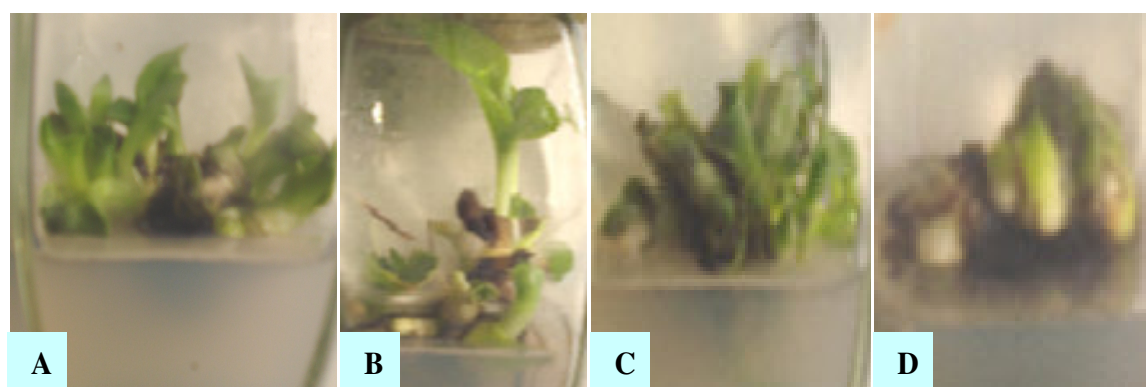
Versatile soil composition has been observed in nature. Some environmental factors are available in soils that are involved in plant growth inhibition. Similar experiment was conducted to assess the effect of NaCl and PEG separately as well as in combination on plant micro-propagation efficiency of banana (*Musa* spp.) cv., Basrai. In this experiment, 4-weeks old plantlets of the 3rd sub-culture with well propagation on MS_{2b} nutrient were sub-cultured on three differentially stressed cultures which were MS_{2c} (100 mol m⁻³ NaCl), MS_{2d} (5% PEG) and MS_{2e} (100 mol m⁻³ NaCl + 5% PEG) including MS_{2b} (best shoot multiplication culture) for 6 weeks. A number of variations among the bio-metrics of the multiplying plantlets were observed in comparison to the control culture (MS_{2b}).

Maximum shoot multiplication was observed on MS_{2b} medium supplemented with BA (Haq and Dahot, 2007b, c), while, multiplication rate was decreased significantly in all the stressed cultures in the order, MS_{2c} > MS_{2d} > MS_{2e}. These stresses not only reduced the multiplication shoots among the cultures, but also the shoot height, shoot fresh weight as well as the shoot dry weight were also decreased (p < 0.05). Including growth rate of the cultures, photosynthetic pigments were also affected (Haq et al., 2008; Cha-um et al., 2010). Chlorophyll *a* was decrease non-significantly, while chlorophyll *b* and total chlorophyll contents were also decreased but significantly. Total carotenoids were increased significantly in all the stressed cultures (Table 1; Figure 1). A significant increase in the epidermal cells and decrease in aerenchymatous cells (p < 0.05) was observed, while a non-significant change in the size of mesophyll cells occurred (Table 1; Figure 2).

Generally, all the abiotic stresses on plants cause injury in the chloroplasts that result to decrease in the amount of photosynthetic pigments under both salinity and/or

Table 1. Comparative growth-related characteristics under different NaCl and PEG stressed conditions in the micro-propagated banana (*Musa* spp.) cv., Basrai.

#s	Characters	MS _{2b} (0 mol m ⁻³ NaCl)	MS _{2c} (100 mol m ⁻³ NaCl)	MS _{2d} (5% PEG)	MS _{2e} (5% PEG+ 100 mol m ⁻³ NaCl)	Significance
A. Micro-propagation efficiency						
a.	# of plantlets / explant	^a 8.33±0.333	^b 5.33±0.333	^c 4.00±0.577	^d 2.67±0.333	***
b.	Plant height (cm)	^a 3.57±0.120	^b 2.57±0.120	^c 1.93±0.145	^c 1.63±0.088	***
c.	Fresh weight (g)	^a 6.391±0.170	^b 2.484±0.087	^c 1.164±0.065	^c 1.367±0.091	***
d.	Dry weight (g)	^a 0.502±0.042	^b 0.158±0.007	^{bc} 0.09±0.005	^c 0.075±0.002	***
B. Chlorophyll contents (mg g⁻¹ F.Wt)						
a.	Chlorophyll a	^a 0.978±0.036	^a 0.817±0.002	^a 0.669±0.012	^a 0.653±0.006	ns
b.	Chlorophyll b	^a 0.399±0.006	^a 0.428±0.009	^{ab} 0.436±0.01	^b 0.461±0.013	*
c.	Chlorophyll ab	^a 1.111±0.307	^b 1.254±0.005	^c 1.106±0.003	^c 1.124±0.021	***
d.	Total carotenoids	^a 258.62±8.88	^a 343.01±12.14	^a 329.83±7.57	^b 319.86±13.60	**
C. Anatomical features						
a.	Epidermal cells (μm) ²	^a 111.80±1.74	^{ab} 114.26±3.57	^{bc} 134.23±6.5	^c 147.44±10.57	*
b.	Parenchyma cells (μm) ²	^a 58.42±2.224	^a 63.77±0.387	^a 61.00±1.162	^a 57.88±2.505	ns
c.	Aerenchyma cells (μm) ²	^a 89.15±1.999	^b 91.68±2.696	^b 104.71±2.83	^b 86.18±3.534	**
D. Biochemical contents						
a.	Protein contents (mg g ⁻¹)	^a 2.517±0.021	^b 2.051±0.004	^c 2.039±0.004	^d 1.967±0.082	***
b.	Total sugars (mg g ⁻¹)	^a 0.643±0.249	^a 0.944±0.018	^b 0.837±0.019	^c 0.713±0.034	***
c.	Reducing sugars (mg g ⁻¹)	^d 0.794±0.007	^c 0.957±0.027	^b 1.281±0.012	^a 1.723±0.028	***
d.	Proline (mg g ⁻¹)	^b 0.833±0.006	^b 0.628±0.031	^b 0.637±0.035	^a 0.615±0.010	***
e.	Glycinebetaine (mg g ⁻¹)	^c 0.635±0.021	^b 1.007±0.019	^a 1.012±0.008	^b 1.451±0.060	***
f.	Phenol (mmol g ⁻¹)	^c 0.116±0.007	^b 0.180±0.013	^a 0.243±0.012	^a 0.262±0.010	***
g.	Nitrates (μ mol g ⁻¹)	^a 1.126±0.061	^a 1.124±0.006	^b 0.948±0.062	^b 0.797±0.047	**

**Figure 1.** Almost 6-weeks old micro-propagated plantlets of banana (*Musa* spp.) cv., Basrai on different stressed cultures under *in vitro* conditions. A, MS_{2b} (control); B, MS_{2c} (100 mol m⁻³ NaCl); C, MS_{2d} (5% PEG); D, MS_{2e} (100 mol m⁻³ NaCl + 5% PEG).

water deficit conditions concentrations of the free amino acids like as proline and glycinebetain. Within the mesophyll cells, both proline and photosynthetic pigments are synthesized from the same precursor. Under stressed conditions, this inter-connection is performed in an opposite manner; photosynthetic pigments decreases, while

proline contents increase under salinity (NaCl), water deficit (PEG) and salinity + water deficit conditions (Aspinal and Paleg, 1981).

Reducing sugar contents were also increased under the stressed conditions, while total proteins and carbohydrates decreased ($p < 0.05$). Phenolics increased

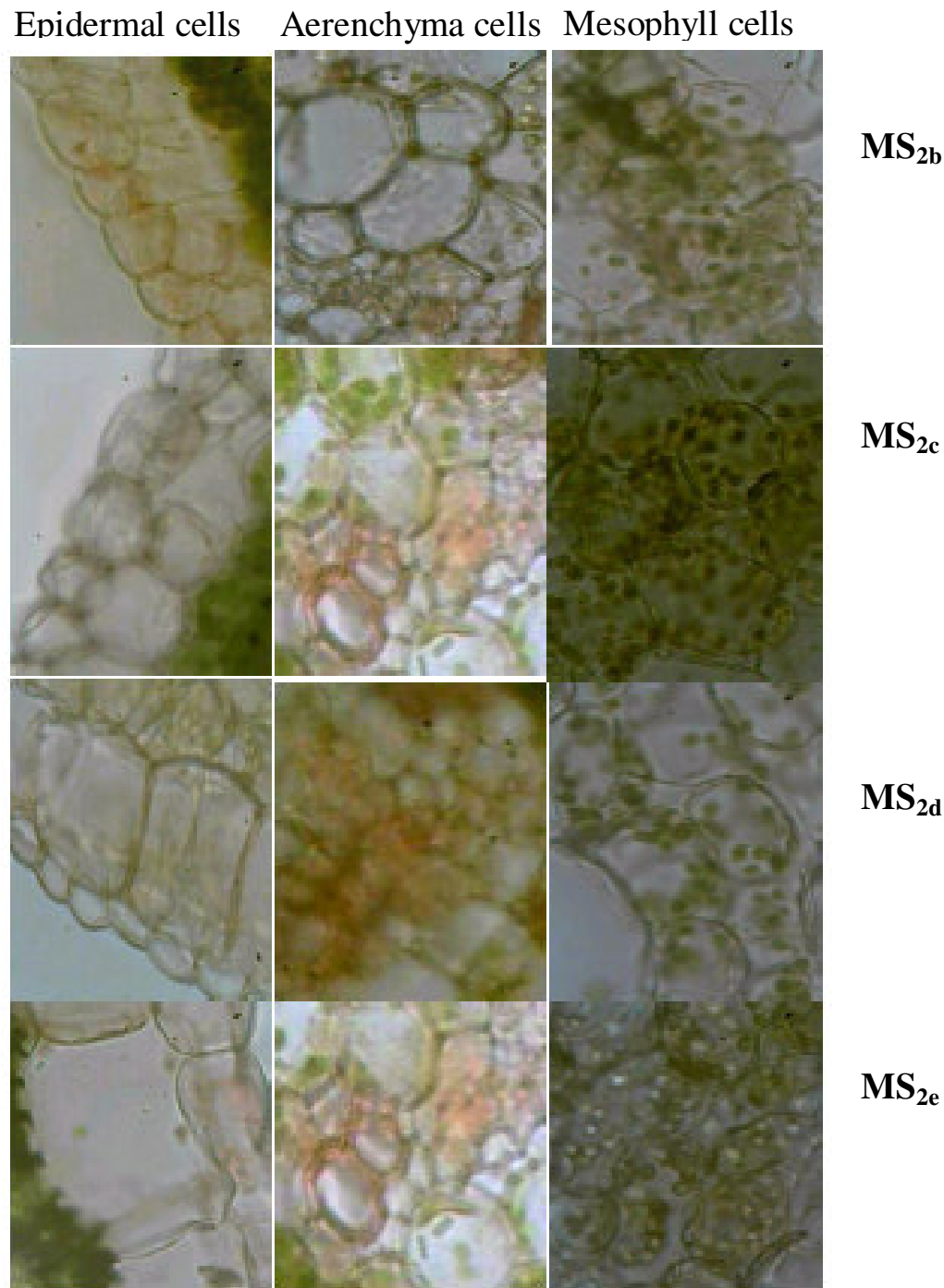


Figure 2. Anatomical aspects (cell sizes) of banana (*Musa* spp.) cv., Basrai cultured on different plant nutrient media supplemented with different salt and drought stressing agents. Epidermal, aerenchymatous and mesophyll cells in the plantlets cultured on MS_{2b} (control), MS_{2c} (100 mol m⁻³ NaCl), MS_{2d} (5% PEG) and MS_{2e}, (100 mol m⁻³ NaCl + 5% PEG).

($p < 0.05$), while nitrate contents were decreased significantly.

The accumulation of certain contents in the stress cultures may be for the safety of the cell at that moment or for the future. After the applied stress, the accumulated

bio-components indicated a source of carbon, nitrogen and energy for recovering stressed tissues, when stress-es were relaxed (Blum and Ebercon, 1976; Slama et al., 2007).

All the abiotic stresses such as water deficit stress (drought) or saline stress are probably involved in limiting

plant growth significantly (Hartmann et al., 2005). Both stresses (salinity and drought) are metabolically as well as physiologically related phenomena, as both induce osmotic stress on the affected plants (Djibril et al., 2005; Vanden and Zeng, 2006). In this experiment, almost all the parameters were considered to be affected significantly in the micro-propagating plantlets of banana (*Musa* spp.) cultivar Basrai. Both photosynthetic pigments and organic contents developed differential characteristics in the stressed cultures in comparison with the control.

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