Pak. J. Bot., 44(3): 1127-1130, 2012.

COMPARATIVE CHARACTERISTICS OF MICROPROPAGATED PLANTLETS OF BANANA FROM BBTV-INFECTED EXPLANTS TO ITS NORMAL AND SALINE STRESSED CULTURES

IKRAM-UL-HAQ[*], NAZIA PARVEEN [1], MUHAMMAD TAHIR RAJPUT[2] AND MUHAMMAD UMAR DAHOT

Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, 76080, *Pakistan*
[1]*Department of Statistics, University of Sindh, Jamshoro*, 76080, *Pakistan*
[2]*Institute of Plant Sciences, University of Sindh, Jamshoro*, 76080, *Pakistan*
[*]*E-mail:* rao.ikram@yahoo.com

Abstract

Effects of BBTV infection and NaCl stress were assessed in banana (*Musa* spp.) under aseptic conditions. Micropropagation efficiency in both BBTV infected and 100 mol m^{-3} NaCl stressed cultures was decreased significantly. Similarly plant height and its biomass were also remained low than control cultures ($p<0.05$). The stress related bio-contents like proline, reducing sugars and total carotenoids were increased, while total proteins and carbohydrates including chlorophyll contents decreased among the stressed cultures ($p<0.05$). The POX (peroxidase) activities of its soluble and ionic forms were significantly higher in both BBTV infected and salinity stressed cultures. Each developed parameters under vagrantly stressed cultures had been involved to direct differential bio-metrics among the micro-propagated plantlets.

Introduction

Banana (*Musa* spp.) is an important fruit crop. It is a source of cash as well as staple food for domestics. The demand of banana is high beacause of presence of various carbohydrates, minerals and vitamins in abundance that is equally beneficial nutrition for children as well as adults. At present, yield of banana has decreased due to a number of environmental stresses. Causing factors are gaining importance for purpose to obtain high yield of this crop (Kuo, 2003; Sahijram *et al*., 2003; Fsanz, 2006).

Among these stresses of banana, BBTV (*Banana bunchy top virus*) infection (biotic) and soil salinity (abiotic) are severely affecting vegetative growth and yield of this crop. Meanwhile, BBTV has been losing up to 100% yield of this important banana crop (Dale, 1987; Moffat, 2001). It is transmitted by vegetative planting materials or banana aphids (*Pentalonia nigronervosa*) (Magee, 1940; Wu & Su, 1990). Similarly, 20% of world's cultivated land is adversely affected by high salt concentration, which also inhibits both plant growth and yield (Tanji, 1990; Haq *et al*., 2008). Salinity is one of the major vegetative as well as reproductive growth limiting abiotic factors (Lauchli & Epstein, 1990). It decreases plant propagation efficiency under natural as well as artificial conditions.

Generally, micropropagation is implied to develop huge number of normal and pathogen free plantlets. This goal is achieveable through specification of concentrations and timing of supply of auxins and/or cytokinins at different stages of plant growth (Haq & Dahot, 2007a). Similarly propagation efficiency also depends on certain physical conditions like as light, temperature, pH and ratios of specific salts that are used in the plant growth medium (Alvard *et al*., 1993). Micropropagation technique is a useful tool for determination of abnormal features that are developed in the plantlets growing under biotic or abiotic stressed conditions. Applications of salts in plant propagation cultures, plant feel precise affect of that specific stress, like as when BBTV infected plants are cultured under aseptic conditions. Comparative decrease in growth efficiency is also observable (Adams *et al*., 1992; Lacerda *et al*., 2001; Grennan, 2006; Wang, 2006).

In present study, effects of BBTV infection and NaCl stress on micropropagation efficiency of banana (*Musa* spp.) cv., Sindhari banana (Basrai) was assessed. The severity of both stresses on plant multiplication causes differential biometrics in developed plantlets. This study can be helpful in making decision, whether plant materials (nursery) and soil or medium composition for propagation of this crop suitable or not.

Materials and Methods

a. Plant material and sterilization: Apparently BBTV infected and healthy young suckers of banana (*Musa* spp.) cv., Sindhari banana (Basrai) were collected from banana fields. BBTV infection was confirmed by PCR and ELISA based markers as reported by Haq *et al*., (2009). Meristematic tips were excised and sterilized for surface-growing pathogens by washing with ethanol (90%) for 1 min and then with 30% commercial bleach [5.25% sodium hypo-chlorite (NaOCl)] for 30 min separately. These were used as explants after washing with sterile distilled water.

b. Micro-propagation cultures: Sterilized explants of both healthy and BBTV infected plants were cultured on MS_2 [MS (Murashige & Skoog, 1962) basal medium with B_5 vitamins (Gamborg *et al*., 1968); 3% sucrose] medium, supplemented with benzyle aminopurine (8µM BAP) and indole acetic acid (10µM IAA) for 3-weeks separatly. After organogenesis, shoots were induced on MS_{2a} (MS; 15µM BAP; 1.0 g L^{-1} phytagel) medium in healthy explant and on MS_{2b} (in composition similar to MS_{2b}) in BBTV infected explants (Haq & Dahot, 2007a).

c. NaCl treatments and BBTV infected cultures: Almost 2-weeks old plantlets MS_{2b} cultures were excised and sub-cultured on MS_{2c} (MS_{2a} + 100 mol m^{-3} NaCl) medium. The cultures were maintained for 6-weeks.

d. Culture conditions: All cultures were supplied with 20µM L-cystein, 3% sucrose. The pH was adjusted to 5.7-5.8 before autoclaving (121°C and 20 lbs/in^2 for 15 min). Each culture was compromised on 7-replicates and maintained under 18/6 h photoperiod (light intensity ~2000 lux) at 25±2°C.

e. Data collection

a. Morphological parameters: After 6-weeks, plantlets on from all cultures, MS_{2a} (control healthy plantlets) MS_{2b} (BBTV infected plantlets) and MS_{2c} (NaCl stressed) were removed and washed with water. The number of plantlets per explant, plant height and plant biomass was measured.

b. Bio-chemical analysis: Chlorophyll contents and total carotenoids were determined in fresh leaf tissue (Arnon, 1949; Nagata & Yamashita, 1992). Plant material was dried for 2-days in electric oven at 72°C and then subjected to study different bio-chemical parameters.

Total carbohydrates were extracted according to Ciha & Brun, (1978) through homogenization in 10 ml extraction solution (glacial acetic acid: methanol: water, 1:4:5, v/v/v). Carbohydrates were measured by applying phenol-sulfuric acid assay (Dubois *et al*., 1956). Reducing sugars were analyzed by following Miller's method (1959), while total proteins were determined according to Bradford, (1976) by using BSA (*Bovine serum albumin*) as standard.

f. Statistical analysis

Data collected fom each culture was subjected for statistical analysis. Its significance was computed by using a *COSTAT* computer package (*CoHort Software*, Berkeley, USA) at 5%.

Results and Discussion

The growth expression potential during development of living organisms has been affected by a number of environmental stresses, either affected internally (viruses) or externally (medium composition or culture incubation conditions) results into growth limitations. During this experiment, 3 cultures were maintained for 6-weeks, viz., **a**, plant micropropagation culture (control) of healthy plantlets (MS_{2a}); **b**, micropropagation culture of BBTV infected plantlets (MS_{2b}) and **c**, Multiplication of healthy banana plants culture (as in **a**) under saline stressed (MS_{2c}) conditions. Significant differential bio-metrics in the micropropagated plantlets were observed among these stressed cultures (Fig. 1). The shoot multiplication medium (MS_{2b}) has favourable properties for banana micro-propagation (Haq & Dahot, 2007a, b). Any disorder casused through biotic or abiotic factors could be detected easily by culturing banana on this medium (Fig. 1).

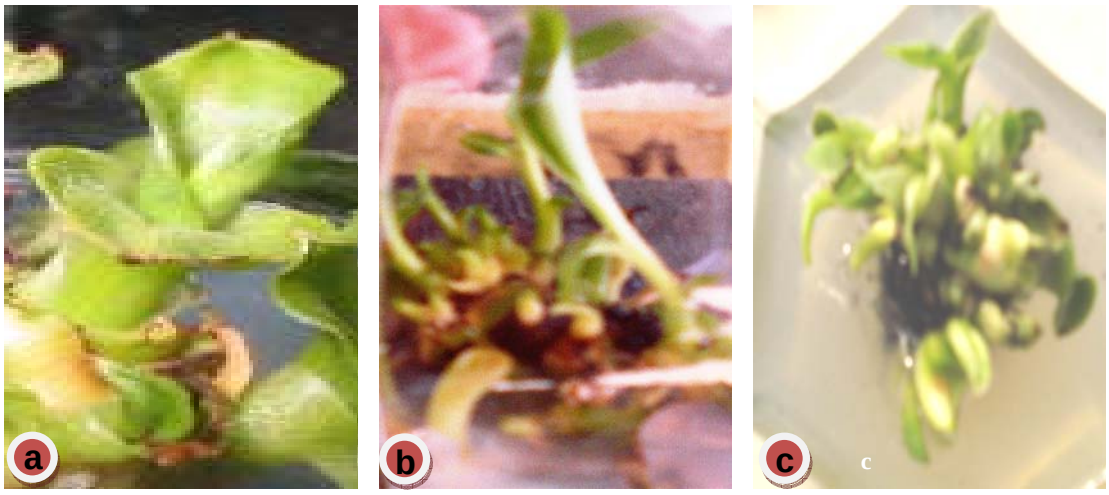

Fig. 1. Comparative presentation of 6-weeks old culture of NaCl stressed and BBTV infected banana (*Musa* spp.) cv., Basrai micropropagation under *in-vitro* conditions. a: Control banana micropropagation medium (MS_{2b}); b: Banana micropropagation culture on 100 mol m^{-3} NaCl stressed (MS_{2c}) medium; c: BBTV infected banana micropropagation culture on MS_{2b} medium (represented as MS_{2c}).

The cultures (3.25±0.23 plantlets per explant) that were stressed with salt (NaCl) or cultured plantlets infected with BBTV, in each case decrease in shoot multiplication rate (2.38±0.14 plantlets per explant) than control (6.01±0.13 plantlets per explant) was observed (Fig. 2). Among the cultures, certain bio-components like as carbohydrates and total proteins were decreased in comparison to control culture ($p<0.05$). Some of the growth related stress markers such as proline and reducing sugars were increased in saline stressed and BBTV infected cultures than the control plantlets (Ottow *et al*., 2005; Lopez *et al*., 2006).

With the decrease in micropropagation efficiency, plant height was also observed to be reduced. In both stressed cultures, decrease in chlorophyll contents was also observed. Chlorophyll a was decreased more in saline stressed plantlets while chlorophyll b in BBTV infected cultures ($p<0.05$). Sensitivity of chlorophyll type depends on the nature of stress applied on the multiplying plantlets. Meanwhile total carotenoids increased in both typed cultures (Fig. 2).

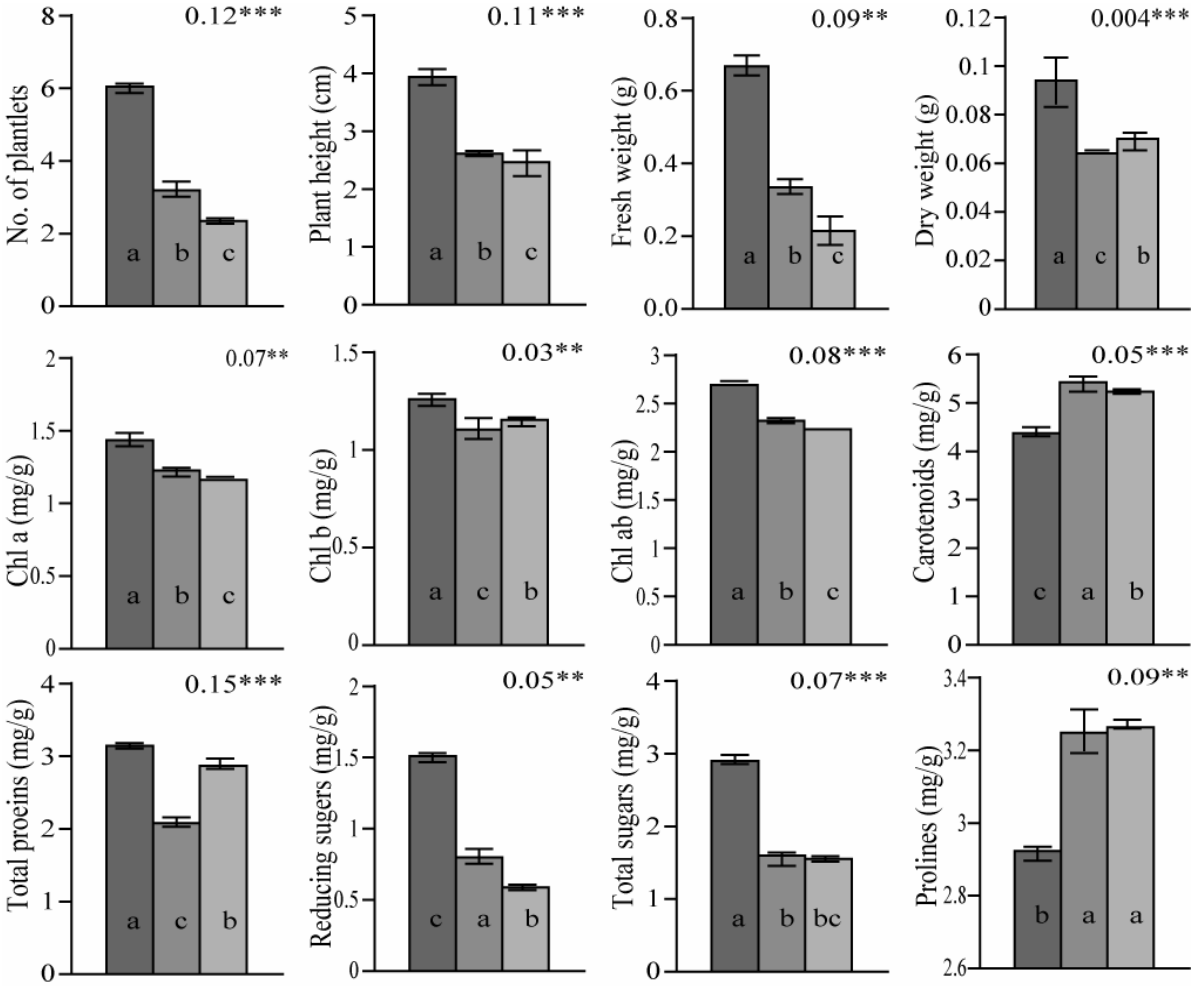

Fig. 2. Comparative bio-metrics of BBTV infected (▨) and saline stressed (▩) banana plantlets in comparison to control (□) cultures of Banana (*Musa* spp.) cv., Basrai micropropagated under *in-vitro* conditions (6-weeks culture).

The salinity and BBTV infection have been adversely affecting qualitative as well as quantitative characteristics of the multiplying banana plantlets. The growing plantlets are getting certain amendments in their internal constituents in according to the applied stresses (Haq *et al.*, 2011, 2012). The developed complex phenomena may be adopted by the plantlets in future. Early responses of the plantlets against applied stresses could usually be helpful to enhance their tolerance against the applied stresses. There accumulation of certain metabolites like as total carotenoids are enabling plantlets to remain functional under any applicable environmental stressed conditions. Carotenoids are acting as non enzymatic anti-oxidants involved in the prevention of lipid peroxidases disorders by losing H_2O_2 that develop when plant feeling somewhat applied stress against to their normal required growth conditions (Grassmann *et al*., 2002).

A large number of free amino acids are involved in regulation of different metabolic processes within the photo-assimilation region of the plants. All of theses including prolines are increased significantly in the tissues feeling any type of applied stresses (biotic or abiotic). Biosynthesis of proline involved in the prevention of stress injury among the tissues. It is an indicator for cell's injury as well as acts as osmoprotactent. Their over-productions can decrease the rate of injury due to saline stresses, enzyme inhibiting factors and pathogen toxins. They are developing stability for ongoing metabolism within the cells during different stages of the cell's cycles.

During this study, various plant characteristics were observed that altered because of both biotic (NaCl) and abiotic (BBTV-infection) stresses in multiplied banana plantlets. Each case is decreasing its multiplication efficiency. These stresses have been considered as main factors that are involved in banana growth and yield limiting factors. Complete elimination of these factors is being impossible but increase in tolerance among the banana cultivars could make us able to get high yields of this important fruit crop.

References

Adams, P., J.C. Thomas, D.M. Vernon, H.J. Bohnert and R.G. Jensen. 1992. Distinct cellular and organismic responses to salt stress. *Plant Cell Physiol*., 33: 1215-1223.

Alvard, D., F. Cote and C. Teisson. 1993. Comparison of methods of liquid medium culture for banana micro-propagation: Effects of temporary immersion of explants. *Plant Cell Tiss. Org. Cult*., 32: 55-60.

Arnon, D.T. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta Vulgaris*. *Plant Physiol.*, 24: 1-15.

Bradford, M.N. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*., 72: 248-254.

Ciha, A.J. and W.A. Brun. 1978. Effect of pod removal on nonstructural carbohydrate concentration in soybean tissue. *Crop Sci*., 18: 773-776.

Dale, J.L. 1987. Banana bunchy top: an economically important tropical plant virus disease. *Ad. Virus Res*., 33: 301-325.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 28: 350-356.

Fsanz. 2006. Nutrient Data for Australian Foods. Food Standards Australia New Zealand, available online at http://www.foodstandards.gov.au.

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res*., 50: 151-158.

Grassmann, J., S. Hippeli and E.F. Elstner. 2002. Plants defence and its benefits for animal and medicine: role of phenolics and terpenoids in avoiding oxygen stress**.** *Plant Physiol. Biochem*., 40: 471-478.

Grennan, A.K. 2006. Abiotic Stress in Rice. An ‘‘Omic’’ Approach. *Plant Physiol*., 140: 1139-1141.

Haq, I. and M.U. Dahot. 2007a. Effect of Permanent and Temporary Immersion Systems on Banana Micro-propagation. *Pak. J. Bot.*, 39: 1763-1772.

Haq, I. and M.U. Dahot. 2007b. Micro-propagation efficiency in banana (*Musa* spp) under different immersion systems. *Pak. J. Biol. Sci*., 10: 726-733.

Haq, I., F. Soomro, M.U. Dahot, Shahrrukh and U. Aiman. 2008. *In-vitro* multiplication of banana (*Musa* spp.) under different NaCl stresses. *Pak. J. Biotechnol*., 4: 25-30.

Haq, I., M.U. Dahot., S. Khan and N. Kousar. 2009. Screening of banana bunchy top diseased plants a way to control its spreading. *Plant Omics J*., 2: 175-180.

Haq, I., G.Yasin, M. Hussain and A.M. Dahri. 2012. Effect of abscisic acid on nacl stressed callus proliferation and plant regeneration in rice. *J. Life Sci*., 6(1): 48-54.

Haq, I., S. Memon, N.P. Gill and M.T. Rajput. 2011. Regeneration of plantlets under NaCl stress from NaN_3 treated sugarcane explants. *Afr. J. Biotechnol*., 10(72): 16152-16156.

Kuo, B.F. 2003. Fruit Finds: All about growing, eating and enjoying exotic fruit. The Fruit Gardener. California Rare Fruit Growers, Inc.

Lacerda, C.F., J. Cambraia, M.A. Oliva and H.A. Ruiz. 2001. Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. *Rev. Bras. Fisiol. Veg*., 13: 270-284.

Lauchli, A. and E. Epstein. 1990. Plant responses to saline and sodic conditions. In: (Ed.): K.K. Tanji. Agricultural Salinity Assessment and Management, pp. 113-137. ASCE, New York.

Lopez, M., J.A. Herrera-Cervera, C. Luch and N.A. Tejera. 2006. Trehalose metabolism in root nodules of the model legume Lotus japonicus in response to salt stress. *Physiol. Plant*, 128: 701-709.

Magee, C.J.P. 1940. Transmission studies on the banana bunchy top virus. *J. Inst. Agric. Sci*., 6: 109-110.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Anal. Chem*., 31: 426-429.

Moffat, A.S. 2001. Finding new ways to fight plant diseases. *Science*, 292: 2270-2273.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant*., 15: 473-497.

Nagata, M. and I. Yamashita. 1992. Simple method for simultanous determination of chlorophyll and caroteniods in tomato fruit. *J. Japan Soc. Food Sci. Technol*., 39: 925-928.

Ottow, E.A., M. Brinker, T. Teichmann, E. Fritz, W. Kaiser, M. Brosche, K. Kangasjarvi, X. Jiang and A. Polle. 2005. *Populus euphratica* Displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates and develops leaf succulence under salt stress. *Plant Physiol*., 139: 1762-1772.

Sahijram, L., J.R. Soneji and K.T. Bollama. 2003. Analyzing somaclonal variation in micropropagated bananas (*Musa* spp). *In-vitro cell Develop. Biol. Plant*, 39: 551-556.

Tanji, K.K. 1990. Nature and extent of agricultural salinity. In: Tanji KK (ed), Agricultural Salinity Assessment and Management, pp. 1-13. ASCE, New York.

Wang, Q., C.H. Wang, B. Zhao, Z.J. Ma, Y.Q. Luo, J.K. Chen and B. Li. 2006. Effects of growing conditions on the growth of and interactions between salt marsh plants: implications for invasibility of habitats. *Biol. Invas*., 8: 1547-1560.

Wu, R.Y. and H.J. Su. 1990. Purification and characterization of banana bunchy top virus. *J. Phytopathol.*, 128: 153-160.

(Received for publication 2 December 2010)