Biotechnology Applications for Improving Sweetpotato Production.

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Abstract

Sweetpotato, (Ipomoea batatas L.), is vegetatively propagated crop. Due to the following cycles of propagation, viruses are accumulated, which contributes to the decline of sweetpotato yield and quality. Stem cuttings of Abees cultivar were collected from several locations in Egypt and kept under controlled greenhouse conditions. Samples were tested for the presence of sweetpotato feathery mottle virus (SPFMV) infection using dot-ELISA. Infected plants were thermotherapy treated by incubation of plants at 42°C/ day and 39°C/ night for 3 weeks followed by meristem tip culturing and allowed to grow in *vitro*. RT-PCR was carried out to confirm the success of SPFMV elimination. Tissue culture formed plants were tested routinely for successive 2 years using dot-ELISA. 0% infection was reported in the *in vitro* propagated plants. Sweetpotato plants were assisted the private-sector to enhance the final yield in their bio-farming system.

Key words: Sweetpotato; virus-free; dot-ELISA; thermotherapy.

INTRODUCTION

Sweetpotato (Ipomoea batatas L.) is a dicotyledonous, perennial plant, belongs to the family convolvulacea. It ranks as the seventh important staple crop in the world and the fifth in developing countries after rice, wheat, maize and cassava (Loebenstein G., *et al*., 2003). Sweetpotato considered being the second important root crop after cassava in many tropical countries (FAOSTAT, 2006). Because of the enormous genetic diversity of sweetpotato and the accompanying diversity in phenotypic and morphological traits the crop has great potential for further development to accommodate specific uses.

Problem Definition

Nutritional studies in Egypt gave the evidence that vitamin A deficiency (VAD) is prevalent among pre-school children and their mothers (Nutrition Institute, 1995). VAD is a main cause of child blindness, and even in its less acute forms, it hinders normal growth and

development (WHO nutrition report, 2003). Orange fleshed sweetpotato varieties are rich in B-caroteen, the precursor of vitamin A. In Egypt, the sweetpotato variety Abees, a purple skin and orange flesh, has a high productivity per unit/area, good nutritional value and a big demand for exportation. However production is greatly constrained particularly by viral diseases (valverde, R.A., *et al*., 2007), since Sweetpotato is cultivated from shoot cuttings grown in the field from previous season. Owing to the great yield losses caused by virus diseases, their absence from propagation materials is essential for sustainable plant production. Shoot tip, meristem tip and heattherapy have been widely used to produce virus-free clones of crop plants which are propagated vegetatively. The aim of the present study is to improve the agronomic quality of deteriorated local cultivar, Abees using an efficient method for virus elimination by companying thermotherapy technique with meristem tip culture.

Methodology

Sweetpotato Abees cultivar apical cuttings were collected from several locations in Kafr El Shikh governorate, and kept under insect proof greenhouse. Cuttings were tested for the presence of SPFMV using dot-ELISA following the method described by (Ashoub, A. *et al* 2008). Infected plants were subjected to the thermotherapy treatment followed by meristem tip culture on MS salts medium. The cultures were grown at 28 ± 2 ºC under a 16-h photoperiod of 300 foot candle. Elongated shoots were tested for success of eradication of SPFMV using reverse transcriptase polymerase chain reaction, RT-PCR, Reverse transcription and PCR reactions were carried out in according to the described method (Ashoub, A. *et al*., 2008). SPFMV-free sweetpotato plants were massive *in vitro* propagated via nodal cuttings using the media and incubation conditions described above. To assure the absence of SPFMV from the micropropagated plant materials, leaf samples were collected every 2 months and tested by dot-ELISA.

RESULTS

Dot-ELISA carried out on sweetpotato plants collected from Egyptian fields showed 100% infection, Figure (1)

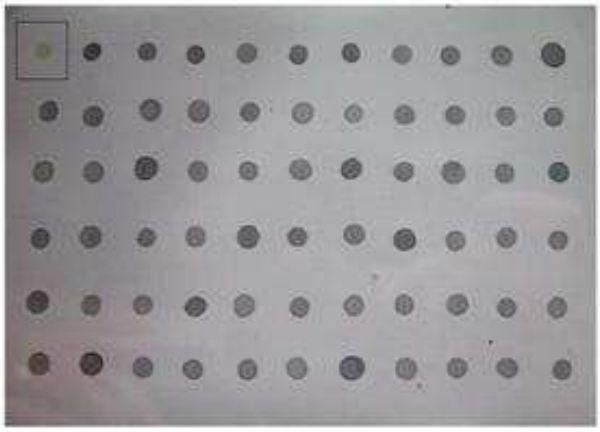

Figure 1
Dot-ELISA of sweetpotato samples collected form Egyptian fields. Healthy sample is indicated in a box at the top left handset.

Nearly, all (100%) of the heattherapy treated plants survived. The gradual regime used for raising the temperature allowed plants to survive. However the regrowth ability of the meristem tip decreased, only 96% of the meristem tips survived and formed shoots, After heattherapy and meristem tip culture, the *in vitro* rooted plantlets two months old, figure (2), subjected to RT-PCR analysis using specifically designed primers based

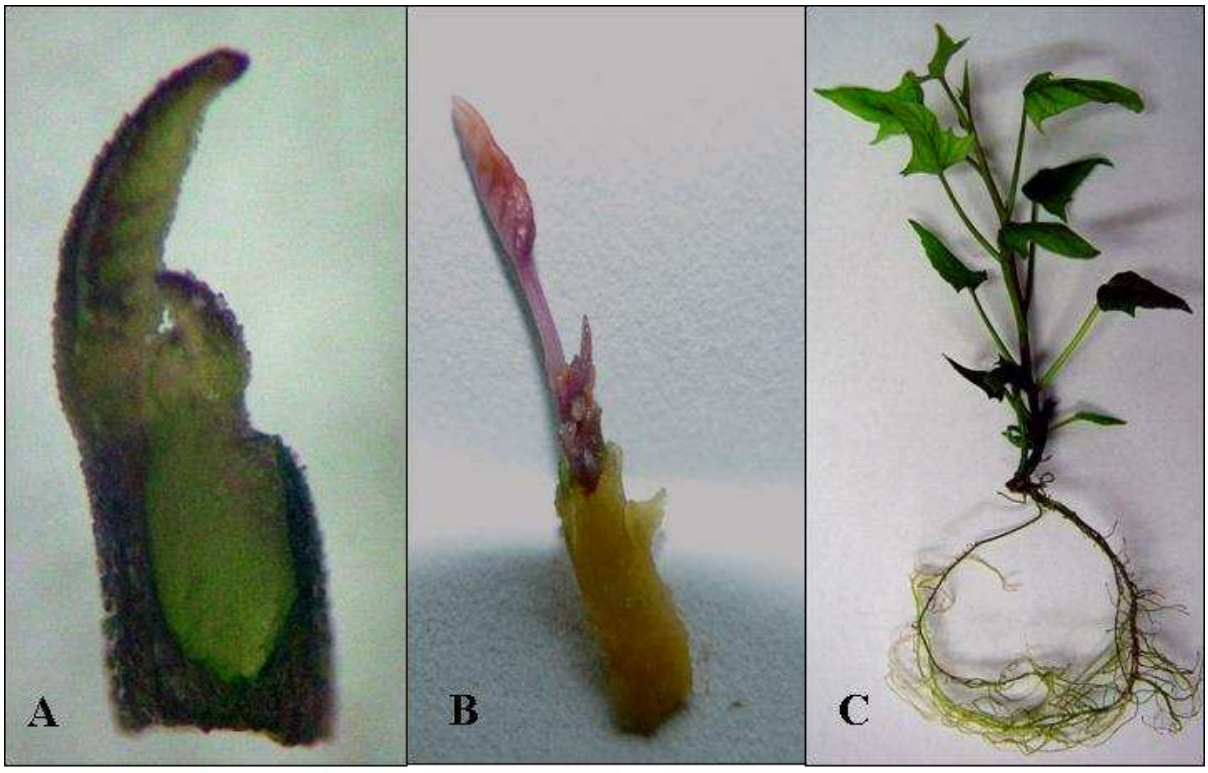

Figure 2
Production of viru-free sweetpotato plants using Heattherapy and meristem tip culture.
(A) A typical meristem tip (0.25 mm with two leaf primordia) used for virus eridecation.
(B) Regrowth of meristem tip after 4 weeks of culture on MS medium supplemented with 3% sucrose.
(C) An established healthy plant of sweetpotato Abees cultivar obtained from SPFMV-affected plant using heattherapy and meristem tip culture.

on the conserved regions present in all viral strains obtained from the NCBI database. All detected samples were found to be SPFMV-free, a single band corresponding to the expected size of the amplified product of ~300 nucleotides. Figure (3-A) represents an example of the

RT-PCR results obtained. Mein while, figure (3-B) showing an equal quality of RNA extracted from plants indicating that the absence of amplification in the treated samples is due to the absence of SPFMV and not to the quality of extracted RNA. Dot-ELISA was routinely used to evaluate tissue culture materials over 2 years to confirm that the absence of SPFMV from sweetpotato plants is due to the freeing of plants form infection and not that the virus is under detection level. In the present study, we report the successful elimination of SPFMV from sweetpotato the Egyptian Abees cultivar by heattherapy and meristem tip culture of infected plants.

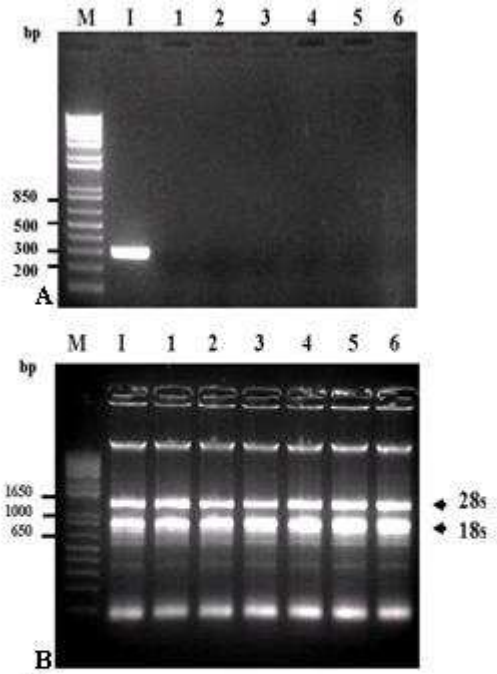

Figure 3
(A) RT-PCR of sweetpotato samples after thermotherapy and meristem tip culture. Positive control (I), one kb plus molecular weight marker, invitrogen, USA (M) and reformed sweetpotato plants (1-6).
(B) Aliquot of 2 µg total RNA extracted from positive control and sweetpotato samples. Arrows indicate the 28s and 18s ribosomal RNA.

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