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RESEARCH NEWS

USE OF THERMOTHEREPY AND IN VITRO CULTURE TO ELIMINATE MEALY BUG WILT VIRUS IN PINEAPPLE (*Ananas comosus*)

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Pineapple (*Ananas comosus*) is considered as one of the important tropical fruits in the world. Mealy bug wilt of pineapple (PMWV) is a serious virus disease in pineapple and it is caused by two clostero viruses and one bacilliform virus. In Sri Lanka, the presence of clostero virus and pineapple bacilliform virus in mealy bug wilted pineapple were confirmed by Dassanayake *et.al* (2001). Reddening of leaves, twisting of their leaf tips and wilting of plants are the main symptoms of this disease, resulting in the reduction of fruit quality and yield. At present majority of pineapple fields are already infected with this virus. Although farmers grow pineapples it is difficult to find virus-free planting material. Therefore, production of virus-free planting material is important for new cultivations.

Techniques including thermotherapy or tissue culture and frequently a combination of both have been developed and successfully used to eradicate the virus from infected plant tissues (Walkey, 1980). At the high temperature virus synthesis stops, but degradation is continuous (Kassenis, 1957)

Pineapple slip suckers infected with pineapple wilt virus were collected. Suckers at desirable length (15 cm) were selected and indexed by indirect Enzyme Linked Immunosorbent Assay (ELISA) by using locally produced polyclonal antiserumm for pineapple mealy bug wilt virus (Dassanayake *et al.* 2001) and Polymerase Chain Reaction (PCR). After confirmation the disease status, suckers were cut into same size (8 cm). Different temperature treatments tested using a water batch were 45°C at 45 min, 50°C AT 30min and 60 min, 54°C at 20 min and 25 min, 55°C at 30 min, 57°C at 30 min, 58°C at 30 min, 59°C at 30min, 60°C at 30 min, 61°C at 30 min, and 62°C at 30 min, 20 min and 15min. Then the suckers were subjected to tissue culture process. For initiation Murasighe and Skoog (MS) (1962) medium containing 3 mg/l BAP was used. One month after *in vitro* condition, explants were indexed by ELISA and PCR. Six sub cultures after multiplication, plantlets were transferred to rooting medium (0.1 mg/lIAA) and acclimatization process was done. At different temperatures, the survival rate after heat treatment of suckers was recorded. It was not possible to obtain virus-free suckers from the heat treatments at 45°C - 59°C irrespective of the treatment duration. The survival after heat treatment was drastically reduced in this temperature range from 90 % to 50 %. Suckers subjected to heat treatment

within the temperature range of 60°C - 62°C at 15, 20, and 30 min showed that all the explants were free of virus diseases. Further, during this temperature range survival rate of suckers dropped from 40 % to 10 %. Therefore?, heat treatment at 60°C for 30 min duration in water bath was selected as the optimum temperature to eradicate viruses percent in pineapple. During the tissue culture process, high multiplication rate and good root formation were obtained. During a period of 2 years, about 20,000 virus-free plantlets were produced by using 60°C and 30 min heat treatment together with *in vitro* and *in vivo* cultures. Absence of viruses was confirmed by ELISA and PCR method at monthly intervals up to flower induction and fruiting under field condition. Eleven months after field planting, most of the plants produced flowers, without flower induction. Moreover, no growth and fruiting abnormalities were observed. Heat therapy coupled with- tissue culture can be used to obtain virus-free planting materials in pineapple.

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