Annals of Sri Lanka Department of Agriculture 2014. 16: 289-292

Short Communication

MICROPROPAGATION OF GRAPE VAR. THOMPSON SEEDLESS

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INTRODUCTION

Grape vine (*Vitis vinifera* L.) is one of the most widely grown nutritious fruit crops in the world (Mederos-Molina, 2007). There is a greater interest in the cultivation of the crop in Sri Lank and a better market has been created for farmers in Jaffna and Hambantota with the development of tourism. There were 52 ha of grapes in Jaffna in 2009 and the cultivated area increases gradually. However, scarcity of planting materials of suitable varieties is the major constraint faced by farmers. (www.agridept.lk). The propagation through tissue culture is the optimal method of propagation where no adequate amount of the mother plants for propagation by conventional methods are available (Abido *et al.*, 2013). Thompson seedless is an accepted sweet seedless variety which performs well under local conditions. Most of the conventional methods practiced under local conditions to produce planting materials of Thompson seedless had not shown satisfactory results. Thus, the objective of this study was to establish micro propagation protocol for grape variety Thompson seedless in Sri Lanka.

MATERIALS AND METHODS

Immature tender shoot tips were collected from a mature Thompson seedless vine grown at the Agriculture Research Station, Kalpitiya. Nodal shoot segments of 1.5-2 cm in length and shoot tips were used as explants. These were washed thoroughly for 1 hour under running tap water. For surface sterilization, explants were shaken with 10% (v/v) commercial chlorox with few drops of Tween 20 for 10 minutes. Explants were then dipped in 70% (v/v) ethanol for 1 minute and rinsed thoroughly with sterile distilled water.

Media preparation and Multiplication

The MS (Murashige and Skoog, 1962) media amended with range of BAP (Sigma-Aldrich) (0.3-1 mg/l) were tested in this experiment for shoot multiplication. *In vitro* shoots initiated from explants were transferred repeatedly (sub culturing) to the

same media for multiplication in 2 weeks interval. The culture medium also contained 3% commercial sugar solidified with 0.7% agar-agar (Himedia) and the pH was adjusted to 5.7. Culture vessels were kept at temperature 26±2 °C, light 3000 lux and 16 hours photoperiod.

Rooting

Multiplied shoots (about 2 cm) were excised, separated and transferred individually to full strength MS medium with 3% sucrose and two different IBA (1 – 2 mg/l) concentrations, and a MS medium free of hormones. The culture vessels were incubated under same conditions as multiplication phase. Plantlets were allowed to grow up to the culture tube height (8 cm).

Plant acclimatization

Culture vessels with well grown plantlets were kept 4 days under room temperature under diffused light. Then individual plants were transferred to poly bags containing potting mixture of sand, coir dust and topsoil (1:1:1). They were kept in a net house.

Statistical analysis

The results are presented as mean values. Experiments were repeated five times. The data on number of shoots per explants were subjected to analysis of variance (ANOVA) with the means separation ($p<0.05$) by least significant difference (LSD).

RESULTS AND DISCUSSION

Culture establishment

As it was a woody vine, the major task was the avoiding contaminations. The shoots collected during March to May were successfully established and observed the lowest contaminations (<30%) with the practice of surface sterilization procedure. Bud breaking occurred within 5 days after establishment in each treatment.

Shoot Multiplication

In this experiment we have used BAP concentrations less than 1 mg/l. At higher concentrations of BAP (>0.5 mg/l) callus formation occurred on the explants and at base

but produced shoot clumps which did not show elongation. About eight fold shoot multiplication rate could be achieved on this medium containing BAP 0.5-0.6 mg/l. According to the data analysis and observations made, BAP range of 0.5-0.6 mg/l was the best for the shoot multiplication of grape var. Thompson seedless (Table 1)

Table1. Effect of BAP concentration on shoot multiplication of grape var.Thompson seedless.

BAP concentration (mg/l)	Mean shoot multiplication ratio
0.3	2.35^{e}
0.4	2.91^{d}
0.5	8.46^{a}
0.6	7.56^{b}
0.7	4.9^{c}
0.8 *	0
0.9 *	0
1.0 *	0

Note: * Callus formed. CV (%)=4.91, LSD=0.468. Data presented as means with different letters indicating significant differences at $p<0.05$ according to Least Significant difference. Each mean represented five replications.

Root induction

Rooting performed equally well in all the treatments. During a month period about 8-10 cm long 2-3 major roots were observed. According to the observations made, adding of rooting hormones like IBA was not necessary. Therefore, MS medium with no growth regulators supplemented could be used as a rooting medium to reduce the cost of production as well.

Hardening of plants and Field Establishment

At the acclimatization phase 95% plantlets survived (Figure 1) and they showed vigorous growth at the field (Figures 2 and 3).

CONCLUSIONS

The study revealed the very efficient, reliable and reproducible micropropagation protocol for the propagation of planting materials of grape var.Thompson seedless. The BAP 0.5-0.6mg/l could be recommend as proper range of BAP for shoot multiplication on an average eight fold shoot multiplication rate could be achieved from an explants at this BAP concentration Rooting performed well in the MS media without growth

regulators. Further adjustments of BAP concentrations and combinations are recommended for the possible improvements of the propagation efficiency.

Figure1. Shoot multiplication

Figure 2. Hardened plants ready to field planting

Figure 3. Growing tissue cultured vine at the field

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