

IN VITRO PROPAGATION OF STRAWBERRY PLANTS (*FRAGARIA VESCA* CV. KENDALL)

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ABSTRACT

Initial experiments conducted with the strawberry cultivar Kendall showed that meristems with 2—4 leaf primordia grew into single shoots in Boxus medium. Transfer of these shoots to Murashige and Skoog (MS) medium supplemented with 2 mg/l Benzyl amino purine (BAP) gave highest shoot proliferation. In separate experiments carried out with multiple shoots of cultivars Kendall and Senga Sengara, addition of 5% coconut water (CW) to the MS medium gave similar shoot proliferation to 2 mg/l BAP. Increasing the CW concentration to 10% gave marginal increase in shoot proliferation over optimal concentrations of BAP.

KEY WORDS: Coconut water, Strawberry, Tissue-culture, Vegetative mass propagation

INTRODUCTION

Strawberry cultivation is gaining importance in Sri Lanka. One of the limiting factors for the increase in the area under cultivation of this crop is the non-availability of sufficient high quality uniform planting material within a stipulated time. The practice of using "stem separates" and runners for planting as is followed by most cultivators presents problems, in that they are not uniform in growth and productivity as they are aggregates from plants with diverse characters; they may not be free from disease and the quantity that could be obtained would also be limited due to the slow rate of vegetative multiplication of parent plants. Tissue culture provides a fast and dependable method of vegetative mass propagation whereby a large quantity of uniform plantlets could be produced in a short time throughout the year from parent plants with desirable characters.

The growth media used in *in vitro* propagation have been supplemented with coconut water (CW) in various percentages (Steward and Caplin, 1952: Pollard *et al.*, 1961). Since the procurement of some of the hormones is costly the use of a cheaper, easily available naturally occurring substitute will permit wider usage and also reduce the cost of production. This experi-

ment was therefore initiated to perfect the technique of mass propagation in strawberry and also to investigate the possibility of using CW as a substitute for the cytokinin, Benzyl amino purine (BAP) in the proliferation phase.

MATERIALS AND METHODS

Tips of runners of strawberry cultivar Kendall were collected from parent plants grown in the glasshouse at the Central Agricultural Research Institute, Gannoruwa. They were surface-sterilized by dipping in 70% alcohol for 30 seconds followed by immersing for 10 min. in 10% clorox (commercial bleach) to which "Tween 20" was added at the rate of 2 drops/100ml solution. The tips were then washed thrice in sterilized water and the meristem domes with 2—4 leaf primordia were dissected out under a dissecting microscope ($\times 16$ magnification). The explants were cultured in 25×150 mm culture tubes containing 25 ml of Boxus medium, to which activated charcoal was added at the rate of 2 g/l and solidified with 6 g/l agar.

After 8 weeks in culture the growing shoots were transferred to tubes containing Murashige and Skoog (MS) (1962) medium to which BAP was added at concentrations of 0, 1, 2, 4, 6 and 8 mg/l. Ten tubes were used for each concentration. The increase in shoot number was recorded and the concentration of BAP that showed the highest mean increase in shoot number was selected as the optimum level of BAP required for shoot proliferation. The MS medium with this level of BAP was used as the control treatment in the second experiment.

The material used in the second experiment was multiple shoots of cultivar Kendall and multiple shoots of cultivar Senga Sengara received as *in vitro* cultures from Italy. Five treatments consisting of MS basal medium supplemented with 5, 10, 15 and 20% CW and 2 g/l as control were used in this experiment.

Approximately 0.5g of shoots were transferred to jam jars of 165 ml capacity containing 25 ml of solid medium and subjected to a temperature of 25 ± 2 °C and 16 hr/day 1000 lux illumination for 3 weeks. Each treatment was replicated 10 times. At the end of 3 weeks the fresh weight of shoots in each jar was recorded and the increase in weight was considered as the degree of proliferation. The data were statistically analysed to determine the effect

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of treatments. The infected cultures in this experiment were discarded and their values were not included in the calculation of statistical parameters. The experiments with cultivars Kendall and Senga Sengara were carried out at two different time periods. At the end of the experiment multiple shoots were separated and rooted in MS medium with 0.5 mg/l Indole-3-butyric acid (IBA). The plantlets were transferred to a soil medium containing 1:2:1 garden soil: coir dust: cattle manure.

RESULTS

The effect of the different concentrations of BAP on the increase in shoots/culture of cultivar Kendall is shown in Fig. 1. The medium without BAP did not produce any shoots. Increase in concentration of BAP up to 2 mg/l showed increase in shoot number with a decreasing trend with further increase in concentration indicating that higher concentration of BAP was not beneficial. The medium consisting of MS nutrients with 2 mg/l BAP was therefore selected as the optimum medium for shoot proliferation and this medium was taken as the control for the second experiment.

Table 1 shows the effect of different concentrations of CW and the control treatment on the increase in weight of shoots. The weight increase with CW treatments did not show statistically significant differences over the control within both varieties tested. However the concentration 10 and 20% CW gave a higher increase in weight as compared to the control in both varieties. The trend in weight increase with increased CW concentration showed a quadratic trend with the highest value at 10% CW. A drastic drop in the value of 15% CW treatment compared to 10 and 20% CW in both varieties tested at two different times indicates an inhibitory effect on shoot proliferation at this concentration. The reason for this is not clear from this experiment. In the combined analysis of the data of both varieties the treatment effects mean over varieties show a similar trend as within individual varieties. Neither variety nor variety $\times$ treatment interaction was statistically significant.

The plantlets were successfully transferred to soil with more than 90% survival rate. The growth of tissue-cultured plants was more vigorous than the parent plant. Fig. 2 shows a tissue-cultured plant at fruit stage.

DISCUSSION

In the first experiment with single shoots BAP was very effective in shoot multiplication. Addition of 1 mg/l BAP induced shoot multiplication; better results were observed with 2 mg/l BAP. Eight mg/l BAP was not beneficial for shoot proliferation as it gave lower shoot number than even 1 mg/l though the results were not statistically significant. The concentration 2 mg/l appeared to be optimum for shoot proliferation.

In the second experiment the non-significant difference between the 2 mg/l BAP (control) and all the CW treatments showed that CW is a good substitute for BAP for micropropagation of strawberry plants. The evidence of 10% CW producing 18% increase in weight in the cultivar Kendall and 11% in the cultivar Senga sengara over 2 mg/l BAP suggests that 10% CW is marginally superior (as the lowest CW concentration 5% gives similar response as 2 mg/l BAP). Therefore, replacement of the costly BAP with readily available CW will be an advantage in mass propagation of strawberry plants. The similar trend in the response of both cultivars tested suggest that CW could be used in micropropagation of most strawberry cultivars.

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Table 1. Effect of coconut water (CW) on proliferation of strawberry cultivars

<i>Treatment</i>	<i>Increase in weight/culture (mean of 10 cultures)</i>		
	<i>Kendall</i>	<i>Senga Sengara</i>	<i>Mean</i>
MS+ 5% CW	3.25 ± 0.19	2.98 ± 0.14	3.13 ± 0.12
MS+10% CW	3.81 ± 0.31	3.30 ± 0.24	3.53 ± 0.19
MS+15% CW	2.97 ± 0.20	2.54 ± 0.17	2.76 ± 0.14
MS+20% CW	3.41 ± 0.31	3.04 ± 0.27	3.22 ± 0.20
MS+2 mg/l BAP	3.23 ± 0.31	2.97 ± 0.28	3.11 ± 0.21

Statistical Significance

Treatment	n.s.	n.s.	n.s.
Varieties	—	—	n.s.
Variety × Treatments	—	—	n.s.
CV (%)	23.2	23.6	20.2

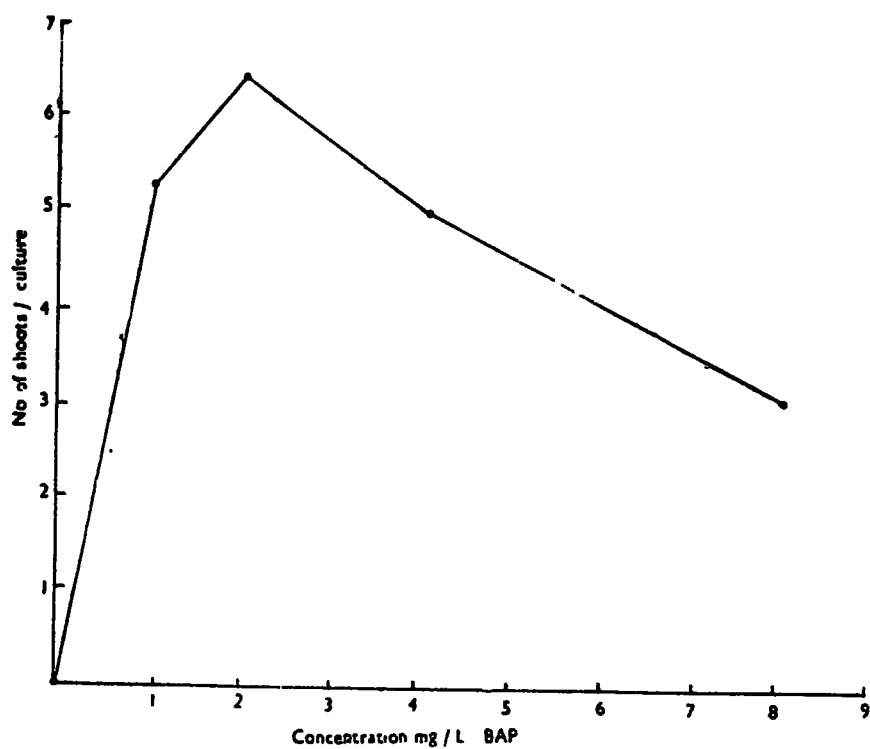


Fig. 1 Effect of BAP concentration on proliferation of strawberry.



Fig. 2 Tissue-cultured plants (Fruit Stage)