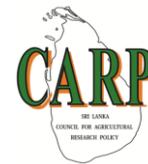


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## Research Paper

### Effect of BAP and IBA on Shoot Regeneration of Strawberry (*Fragaria x ananassa* Duch.) through Runner Tip Culture

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**Abstract:** Effect of three concentrations (0, 0.5 and 1.0 mg/L) of 6-benzylaminopurine (BAP) in combination with three concentrations (0, 0.5 and 1.0 mg/L) of Indole-3-butyric acid (IBA) on shoot regeneration of strawberry runner tips was investigated. Runner tips were rinsed with water containing Teepol™ for 5 min., kept under running tap water

for 30 min, and immersed in 0.5% Topsin™ for 30 min before sterilizing with 20% sodium hypochlorite solution (Clorox™) for 12 min. followed by 70% Ethyl alcohol for 1 min. After sterilization, explants were trimmed and tips of runners (1.0-1.5 cm long) were isolated and cultured on MS media supplemented with different concentrations of BAP and IBA. Among nine treatments, highest number of shoot buds were observed in 0.5 mg/L BAP alone medium followed by 0.5 mg/L BAP + 0.5 mg/L IBA supplemented medium. Highest number of leaves was also observed at the same concentrations. Among nine BAP and IBA concentrations, highest shoot and root length were observed at hormone free medium. Highest shoot girth was observed at 0.5 mg/L BAP + 0 mg/L IBA supplemented medium. It can be concluded that MS basal medium supplemented with 0.5 mg/L BAP could be used to regenerate shoots from runner tips for plant propagation of strawberry.

**Keywords:** BAP, IBA, *In vitro* culture, Runner tip, Strawberry



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## Introduction

Strawberry is an introduced horticultural crop in Sri Lanka and one of the best soft fruits in many parts of the world. The cultivated strawberry (*Fragaria x ananassa* Duch.) is one of the most important soft fruit worldwide. It is widely used due to its characteristic aroma, vibrant red colour, good juicy texture and sweetness. It is consumed in large quantities, either fresh or in prepared foods, such as preserved fruit juice, ice creams, jams, chocolates and milk shakes. Artificial strawberry

aroma is also widely used in many industrialized food products.

Strawberry has an increased potential of generating a higher income if it is cultivated in commercial scale. However, unavailability of suitable varieties, lack of support services, importing of plantlets with high price and under developed infrastructure facilities are major problems faced by strawberry cultivation.

Propagation of strawberry can be achieved by runners, division of plantlets, seeds or by *in vitro* micropropagation. Strawberry is difficult to propagate by seeds due to genetic variation and also rooting percentage of cuttings is relatively low (Mohan *et al.*, 2005). The division of plants and runners of strawberry are not always suitable because of their sensitivity to pathological agents (Nehra *et al.*, 1994). *In vitro* plants are more genetically uniform, produce higher number of runners, have better survival in the field and the fruit yield of strawberry increases in 24% than plants propagated by the traditional method (Kikas *et al.*, 2006). Meristem tips, generally obtained from tip of runners of virus-free plants, are commonly used to establish *in vitro* cultures for mass propagation or as a source of planting material for transformation experiments (Mercado *et al.*, 2007).

The concentration and combination of auxins and cytokinins in the culture medium is a key factor which determines the success of plant regeneration

## Materials and Methods

The experiment was conducted in 2018 at the Tissue Culture Laboratory at the Regional Agriculture Research and Development Center (RARDC), Bandarawela, Sri Lanka. Mother plants of strawberry (*Fragaria x ananassa* Duch.) cv. Sweet Charlie, grown in the field of Regional Agricultural Research and Development Centre, Bandarawela, were used as a source of explants during this experiment. Runner tips of strawberry were used for the establishment of *in vitro* culture.

### Sterilization of explants

Runner tips (cv 'Sweet Charlie') of 1.0-1.5 cm long were taken as explants. As soon as tips were separated from the mother plants, sterilization procedure was started. Otherwise runner tips became brown and it may lead to death of explants after *in vitro* culturing. In surface sterilization process, runner tips were firstly rinsed with water containing Teepol® for 5 min with shaking and then kept it under running tap water for 30 min. Then

of strawberry. The explants are placed on culture medium containing higher levels of cytokinins without or with low levels of auxins to induce axillary buds while preventing callus formation. The cytokinins are used to enhance the branching of lateral buds from the leaf axis. Additional shoots are produced through further axillary bud growth (Debnath, 2003). Micropropagation of strawberry from runners has been reported and efficiently produces a large number of disease free plants. In addition, the storage of tissue cultured propagules requires less space than traditional runner plant and the *in vitro* storage can be initiated at any time during the production cycle (Swartz *et al.*, 1981). *In vitro* strawberry plantlets are used for cultivation to prevent most of the plant and soil transmissible diseases. Hence, *in vitro* techniques are important for clonal multiplication and this study was focused on *in vitro* culture of strawberry cv. Sweet Charlie *via* runner tip culture for plant propagation with the ultimate objective of introducing it as new cultivars in Sri Lanka.

the runner tips were immersed in 0.5% Topsin® for 30 min and washed with sterilized distilled water for three times before sterilizing with 20% sodium hypochlorite solution (Clorox) containing two drops of Tween® 20 for 12 min. Thereafter, they were thoroughly washed with sterilized distilled water before dipping in 70% Ethyl alcohol for 1 min for further sterilization. Strawberry runner tips were finally washed with sterilized distilled water for three times.

### Inoculation of explants

The MS (Murashige and Skoog) basal medium supplemented with different concentrations of BAP and IBA were used as the culture media (Table 1) to inoculate the sterilized explants. After sterilization, explants were trimmed and tips of runners of 1.0-1.5 cm long were isolated as final explants. Those runner tips were cultured on the different culture media with good contact.

Table 1. MS basal media supplemented with different concentrations of BAP and IBA

Treatments	MS + Hormonal combinations	
	BAP (mg/L)	IBA (mg/L)
MS 1	0	0
MS 2	0.5	0
MS 3	1.0	0
MS 4	0.0	0.5
MS 5	0.5	0.5
MS 6	1.0	0.5
MS 7	0	1.0
MS 8	0.5	1.0
MS 9	1.0	1.0

MS – Murashige and Skoog basal medium

### Incubation of explants

After culturing (Figure 1), the culture vessels were sealed with lids and labelled with treatment number and date of culture, and kept in an

incubation room under white fluorescent light (16 h Light /8 h Dark, light intensity 1000-1500 Lux) and temperature at  $25 \pm 1$  °C as culture conditions.



Figure 1. The culturing of explant on culture medium

### Experimental design, data collections and statistical analysis

The experiments were laid down according to the Complete Randomized Design by using culture media with different concentrations of BAP and IBA as treatments. The *in vitro* strawberry plants grown for 8 weeks in different treatments are illustrated in Figure 2.

At 8 weeks, the number of shoots per plant, number of leaves per plant, length of shoots, number of roots, length of roots, girth of plant and fresh weight of plant were recorded. Data were analyzed using the SAS statistical software. Finally, the mean separation was done to identify the best medium with BAP and IBA concentration according to the Duncan's multiple range test at  $P=0.05$ .

## Results and Discussion

### Effect of BAP and IBA on number of shoot buds

The highest average number of shoots (auxiliary buds) was observed in the treatments with 0.5 mg/L BAP alone (MS2) followed by MS5 (0.5 mg/L BAP + 0.5 mg/L IBA) and MS8 (0.5 mg/L BAP + 1.0

mg/L IBA). A moderate numbers of shoots were obtained from treatments containing 1.0 mg/L of BAP, namely, MS3, MS6 and MS9. The lowest average number of shoots was observed at the BAP-free MS basal medium (Figure 3).



Figure 2. *In vitro* plants of strawberry grown in the culture media containing the different BAP and IBA concentrations after 8 weeks

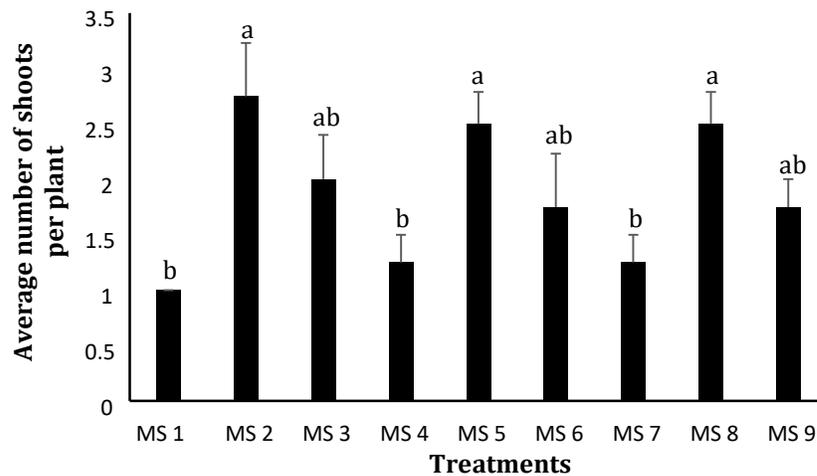


Figure 3. Response to BAP and IBA concentrations on average number of shoots (Vertical lines indicate the standard error of the means. The means with the same letter are not significantly different by the DMRT at P=0.05, n=4).

The results are consistent with the findings of Marcotrigiano *et al.* (1984) who studied the effect of BAP on *in vitro* multiplication of strawberry and observed that lower level of BAP (0.5 mg/L) was more effective for shoot proliferation as compared to 1.0 to 3.0 mg/L BAP. Hu and Wang (1983) reported that high concentration of cytokinin reduced the number of micropropagated shoots. This result has also been reported in *Fragaria indica* Andr. (Bhatt and Dhar, 2000). The results of the present study also proved that the high concentration of cytokinin reduced the number of shoots, and that 0.5 mg/L BAP is the optimum

concentration for shoot formation. On the contrary, Lal *et al.* (2003) found that the maximum number of shoots per explant was in the MS medium supplemented with BAP at 4.0 mg/L.

#### Effect of BAP and IBA on number of leaves

Figure 4 illustrates that the highest average number of leaves are in the treatment MS2. The culture media MS5, MS6, MS8 and MS 9 also showed the high number of leaves compared with other treatments except MS2. The lowest average number of leaves was observed in the BAP-free media (MS1, MS4 and MS 7).

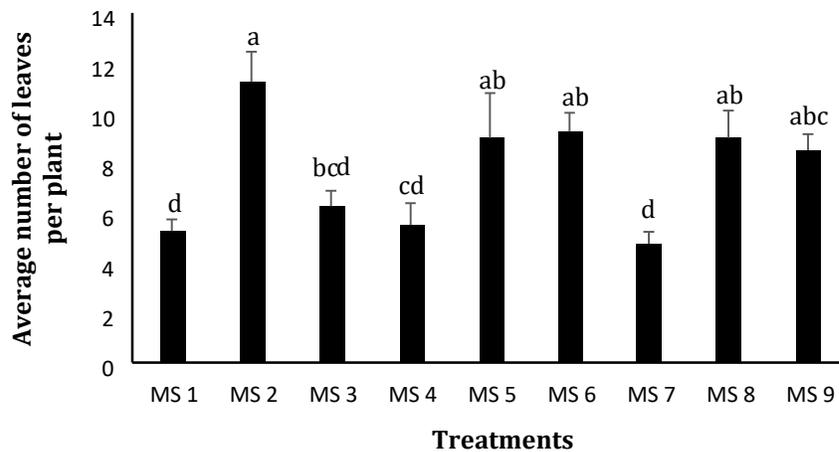


Figure 4. Response to different BAP and IBA concentrations on number of leaves (Vertical lines indicate the standard error of the means. The means with the same letter are not significantly different by the DMRT at  $P=0.05$ ,  $n=4$ ).

#### Effect of BAP and IBA on number of roots

The highest average number of roots per plant was observed at the hormone free medium (MS1; Figure 5). The MS medium supplemented with 0 mg/L BAP + 0.5 mg/L IBA hormone concentration also showed a significantly high number of roots than all other 7 treatments. There was no remarkable difference in number of roots between MS1 and MS4. Emarah (2008) revealed that MS medium supplemented with high IBA levels (0.5, 1.0, and 1.5 mg/L) showed the lowest roots response than lower concentrations of IBA. In the present study, 1.0 mg/L IBA treatment showed the lowest number of roots than 0 and 0.5 mg/L IBA. Moradi *et al.* (2011) reported that the maximum number of roots per explant was obtained in elongation medium in MS medium combined with 0.1 mg/L BAP with auxin 0.2 mg/L IBA.

#### Effect of BAP and IBA on shoot length

A significant difference ( $P<0.01$ ) was observed in shoot length among the treatments (Table 2). The shoot length (2.1 cm) was remarkably higher in hormone-free MS medium (MS1) and MS medium with 0 mg/L BAP + 1.0 mg/L IBA (MS7) than that in the other treatments, except those containing MS + 0.5 mg/L BAP + 0 mg/L IBA (MS2) and MS + 0 mg/L BAP + 0.5 mg/L IBA (MS4).

The shoot length in MS2 and MS4 were 1.9 cm and 1.8 cm, respectively. The lowest shoot length was observed in MS + 0.5 mg/L BAP + 0.5 mg/L IBA (MS5). No considerable variation in shoot lengths was observed in the *in vitro* plant cultured in concentrations of 0 and 0.5 mg/L BAP without IBA. However, there was significant difference in shoot bud formation (Figure 3).

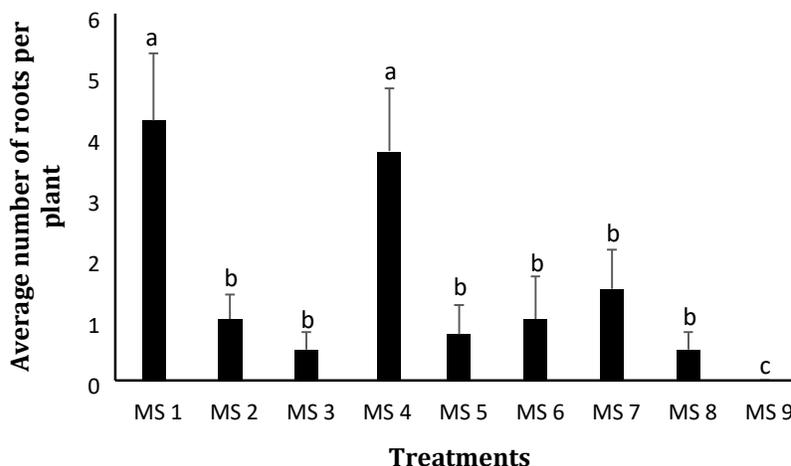


Figure 5. Response to different BAP and IBA concentrations on average number of roots per plant (Vertical lines indicate the standard error of the means. The means with the same letter are not significantly different by the DMRT at P=0.05, n=4).

Table 2. Response to different concentrations of BAP and IBA on shoot and root lengths of strawberry *in vitro* plant.

Treatments	BAP+IBA (mg/L)	Shoot length per plant (cm)	Root length per plant (cm)
MS 1	0 BAP + 0 IBA	2.1 <sup>a</sup>	2.6 <sup>a</sup>
MS 2	0.5 BAP + 0 IBA	1.9 <sup>a</sup>	0.2 <sup>c</sup>
MS 3	1.0 BAP + 0 IBA	1.2 <sup>c</sup>	0.07 <sup>c</sup>
MS 4	0 BAP + 0.5 IBA	1.8 <sup>ab</sup>	0.9 <sup>b</sup>
MS 5	0.5 BAP + 0.5 IBA	1.0 <sup>c</sup>	0.2 <sup>c</sup>
MS 6	1.0 BAP + 0.5 IBA	1.2 <sup>c</sup>	0.2 <sup>c</sup>
MS 7	0 BAP + 1.0 IBA	2.1 <sup>a</sup>	0.5 <sup>bc</sup>
MS 8	0.5 BAP + 1.0 IBA	1.3 <sup>bc</sup>	0.6 <sup>bc</sup>
MS 9	1.0 BAP + 1.0 IBA	1.2 <sup>bc</sup>	0.0 <sup>c</sup>
F test		P<0.01	P<0.01

Means followed by the same letter are not significantly different by the DMRT at P=0.05 (n=4).

Effect of BAP and IBA on root length

As shown in Table 2, the longest roots were obtained from the hormone free medium. It was showed significant difference (P<0.01) from all other treatments. The MS medium supplemented with 0 mg/L BAP + 0.5 mg/L IBA level showed the reasonable rooting. MS medium supplemented with 0.5 mg/L BAP + 0 mg/L IBA (MS 2), 1.0 BAP+0 mg/L IBA (MS3), 0.5 mg/L BAP + 0.5 mg/L (MS 5) and 0.5 mg/L + 1.0 mg/L IBA (MS 6) showed the lowest rooting compared with all other treatments except MS medium supplemented with 1.0 mg/L BAP + 1.0 mg/L IBA hormone concentrations (MS9) where no roots were observed. Sakila (2007) reported that out of different concentrations of IBA

(0.1-1.5 mg/L) and IAA (0.1–1.5 mg/L) tested, 1.0 mg/L IBA was the most suitable for root induction with 5.0 roots per explant and the average root length being 3.68 cm. Similar effects of IBA were also observed in *Calotropis gigantea*, *Capsicum annum* and *Prunus* sp. However, in the present study, the IBA-free media showed the best results on *in vitro* rooting. The MS medium with 0.5 mg/L IBA alone also showed higher rooting compared to having higher concentration of IBA.

Effect of BAP and IBA on shoot girth

According to the statistical analysis, there was significant difference (P<0.01) in shoot girth

among the treatments (Table 3). Shoot girth was remarkably higher in MS2 except MS5.

The treatment MS2 showed 1.5 cm average shoot girth and MS5 showed 1.3 cm shoot girth. It showed that 0.5 mg/L BAP was the best BAP concentration for shoot formation and shoot growth. Medium shoot girth was obtained from 1.0 mg/L BAP containing medium with different IBA concentrations. Lowest shoot girth was observed at the BAP free medium. According to the data collected, MS basal media containing 0 mg/L BAP

with 0 mg/L or 1.0 mg/L IBA showed lowest shoot girth compared with all other treatments.

#### Effect of BAP and IBA on fresh weight of *in vitro* plantlet

Fresh weight of the plantlet in MS8 (0.5 mg/L BAP + 1.0 mg/L IBA) showed a significant difference ( $P < 0.05$ ) from the other tested treatments. The average maximum weight of plantlet was 0.6 g. These plantlets have showed a maximum weight of plantlets than all other treatments.

Table 3. Effect of BAP and IBA on shoot girth and fresh weight of *in vitro* plantlet.

Treatments	Shoot girth per plant (cm)	Plantlet weight (g)
MS 1	0.5 <sup>d</sup>	0.1 <sup>b</sup>
MS 2	1.5 <sup>a</sup>	0.2 <sup>b</sup>
MS 3	1.0 <sup>c</sup>	0.1 <sup>b</sup>
MS 4	0.6 <sup>d</sup>	0.1 <sup>b</sup>
MS 5	1.3 <sup>ab</sup>	0.1 <sup>b</sup>
MS 6	1.2 <sup>bc</sup>	0.2 <sup>b</sup>
MS 7	0.5 <sup>d</sup>	0.1 <sup>b</sup>
MS 8	1.1 <sup>c</sup>	0.6 <sup>a</sup>
MS 9	1.0 <sup>c</sup>	0.1 <sup>b</sup>
F test	$P < 0.01$	$P < 0.05$

Means followed by the same letter are not significantly different by DMRT at  $P = 0.05$  ( $n = 4$ ).

## Conclusion

This study revealed a successful shoot regeneration method for strawberry cv. 'Sweet Charlie', where the runner tips of strawberry could be cultured on

MS basal medium containing 0.5 mg/L BAP without IBA.

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