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Shoot Proliferation Studies in Strawberry

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Abstract

A protocol for rapid multiplication of strawberry Fragaria x ananasa Duch cv. Chandler has been described. Meristem tips were cultured on MS and Knop medium containing benzylaminopurine (BAP) at 0.5, 1.0, 2.0 and 5.0 mg/l. Various clusters of micro-shoots (300 micro-shoots/culture) were obtained on MS medium with 1.0 mg/l BAP. Knops medium gave best response when enriched with 0.5 mg/l BAP. Complete plantlets were produced by transferring the shoot buds on hormone free MS or Knops medium containing 0.1 mg/l IBA.

Introduction

Strawberry (Fragaria x ananasa Duch) is a temperate herbaceous fruit mostly propagated by runners and rarely by seeds. Moreover, propagation by seeds is slow and produces variability in the progeny. Conventional propagation by runners is seasonal thus restricting the production of strawberry to the specific period of time. Micropropagation of strawberry has been successfully used to increase the speedy production of disease and virus free plant material (Boxus *et al.*, 1976). Furthermore, the tissue culture produced plants generate more runners as compared to conventional propagation methods (Swartz *et al.*, 1981). It has also been demonstrated that micro-propagated strawberry yield more as compared to field grown strawberry (Thuesen, 1984). Macrotinao *et al.*, 1984) reported the effect of growth regulator (BAP) on *in vitro* and subsequently field performance of tissue culture propagated strawberry plants. This study was conducted to establish an improved micro-propagation system for strawberry cv. Chandler.

Materials and Methods

Young runners of the cv. chandler were excised during its active vegetative growth and defoliated to expose the inner soft tissues. The apical meristimatic tip containing two or three leaf primordia were carefully dissected and dipped immediately in antioxidant solution containing 100 mg/l ascorbic acid and 100 mg/l citric acid for 24 hours.

Surface disinfection was conducted by dipping explants for 30 minutes in 50 per cent ethanol. After thorough rinsing, these explants were submerged for 10 minutes in a solution containing 1.5 per cent chlorox bleach and few drops of Tween 20. Three subsequent washing with sterilized distilled water were also done to remove sterilant.

Murashige and Skoog (1962) and Knops (1865) Medium were used in this study. These media were supplemented with sucrose (30 gm/l), thiamine HCl (0.4 mg/l), myoinositol (100 mg/l), nicotinic acid (0.5 mg/l) and pyridoxine HCl (0.05 mg/l). The pH of the media was adjusted to 5.8 prior to autoclaving. Agar was used at the rate of 5 mg/l to solidify media. Different concentrations of BAP ranging from 0.5-5.0 mg/l were used. Media were dispensed into 250 ml flasks which later on autoclaved for 15 minutes.

After inoculation, cultures were kept in 16 hours photoperiod under cool inflorescent light intensity of 2000 lux at a temperature of 24 ± 2 C.

Results and Discussion

Shoot proliferation in strawberry was studied using MS medium and Knops medium containing BAP at varying rates ranging from 0.0 to 5.0 (Table 1). On both media shoot tips turned green and initiated shoots after two weeks of culture. Number of shoots increased with the passage of time.

Maximum micro shoots (300) were observed on MS medium containing BAP at 1.0 mg/l while minimum shoots (5) when 5.0 mg/l of BAP was used. However, 1.0 mg/l of BAP seems to be optimum in this case. Also an increase in BAP (1.5 to 3.0 mg/l) produced compact but deformed shoots which could not be used for further subculturing. It was noted that at higher rates of BAP (4.0 to 5.0 mg/l) more micro-shoots with undifferentiated cells with a tendency to turn brown were produced. Present study showed that lower concentrations of BAP enhanced the shoot proliferation rate and shoots were vigorous and healthy in appearance. In the similar studies Macrotraigiano *et al.* (1984) observed the effect of BAP on the *in vitro* shoot initiation of strawberry and inferred that lower levels of BAP produced more vigorous shoots.

It was observed that explants when cultured on Knop medium supplemented with (0.0-5.0 mg/l of BAP), almost similar results were recorded i.e profuse tissue proliferation resulting in the formation of micro-shoots with healthy appearance (Table 1). These micro-shoots when sub cultured on the same medium, shoots with multidimensional runners were obtained. However, maximum shoots (15 were observed on 1.0 mg/l of BAP while minimum (32) o BAP at 3.0 mg/l. Here concentration of BAP at 1.0 mg also proved to be optimum for cv. chandler using Kno medium. In the parallel studies Sajjad *et al.* (199 optimized propagation system in Fragaria x ananasa Du using apical meristem on low auxins. Boxus (1976) a recommended the use of MS and Knops medium for clo multiplication of strawberry.

The cluster of micro-shoots thus obtained from both media were tested for rooting response using IBA ranging from 0.0-1.0 mg/l. Best root induction was noted on media containing 0.1 mg/l of IBA. Asahira and Kano (1977) examined root formation on the same media formulations. Maliaricikova and Mokra (1986) recorded root formation from in vitro raised shoots using similar concentration of the same auxin. Some contradictory findings have also been reported by few researchers which might be due to difference in the genotypes or growth conditions maintained for cultures.

A symmetrical trend was noticed in the two media (MS and Knops media) that with the increase in BAP levels shoot proliferation increased upto a certain limit and then start decreasing. In MS medium shoot multiplication get increased reaching upto 300 number of shoots and then start to decline upto 5 number of shoots. Similarly on Knop media the same fashion was repeated with a highest reach upto 158 and then gradual decline upto 32. The same concentration of BAP i.e 1.0 mg/l proved optimum for cv. Chandler in the both media giving rise to maximum shoot proliferation.

Table 1: Effect of BAP on shoot proliferation of strawberry cv. Chandler on MS and Knops media.

BAP (mg/l)	No. of micro-shoots at MS medium	No. of micro-shoots at Knops medium
0.1	155(++)	43(++)
0.5	162(++)	130(++)
1.0	300(++)	158(++)
1.5	271(++)	92(++)
2.0	155(+)	41(+)
3.0	45(+)	32(+)
4.0	21(+)	----
5.0	5(+)	----

++:Healthy and vigorous shoots; +: Poor and deformed shoots

MS medium confirmed better than Knops medium for the cultivar under study on the following grounds. 1. Number of shoots initiated on all concentrations of BAP were greater on MS medium as compared to Knops medium. 2. Higher levels of BAP (4.0 and 5.0 mg/l) on Knops medium did not respond while no such response in MS medium was recorded.

The micro-shoots raised through optimized media formulations from the two media showed a fairly high survival percentage in the glass house ranging from 83 to 97 per cent. The system of propagation could be conventional used for multiplication of strawberry in cv. chandler.

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