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Effect of auxin type on growth *in vitro* of Kiwifruit axillary buds

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ABSTRACT

Kiwifruit Cultures were initiated from single-node explants on Murashige and Skoog medium containing 2 mg.l^{-1} BA. In shoot proliferation stage, 20 culture media containing MS supplemented with 3 concentrations of BA (0, 1, 2 mg.l^{-1}) individually and along with IAA (0, 0.1, 0.2 mg.l^{-1}) were compared. Maximum shoot proliferation from axillary buds was achived on media supplemented with 2 mg.l^{-1} BA alone. Formation of axillary shoots was influenced by concentration of cytokinin. In the rooting experiment, the shoots were cultured on MS medium containing BA (0, 1, 2, 3 mg.l^{-1}) with various concentrations of IBA or IAA (0, 1, 2, 3 mg.l^{-1}). Regenerated shoots produced prominent roots when transferred to MS medium supplemented with IBA (3.0 mg.l^{-1}). The effect of IAA on rooting was not significant. Approximately 80% of the rooted microcuttings were established successfully as container-grown plants.

Key words: *Actinidia deliciosa*, axillary bud, *in vitro,* proliferation, tissue culture.

Abbreviations: BA- 6-benzylaminopurine; IAA- Indole-3- acetic acid; IBA- Indole butyric acid; MS-Murashige & Skoog Medium.

INTRODUCTION

Kiwifruit is native eastern Asia. It is a fruiting vine wich, taxonomically, belong to the genus Actinidiaceae (Motohashi, 2002). Today, the kiwifruit is an important commercial crop grown in different parts of the world. The genus *Actinidia* contains about 60 species. All *Actinidia* species are perennial, climbing or scrambling plants, mostly deciduous although a few from warmer areas are evergreen (Ferguson, 1999).

Since ancient times people of that area have known of the very delicious flavor of kiwifruit. Today, the kiwifruit is an important commercial crop grown in different parts of the world (Ferguson, 1999). Until recently, the international success of kiwifruit could really be considered as the success of one fruiting cultivar 'Hayward' and associated male (pollenizer) cultivars. World trade has become restricted to this one cultivar (Ferguson, 1999). The kiwifruit is particularly attractive because of its high vitamin C content and large number of calcium, iron, phosphorus and protein as well as for its flavour (Monette, 1986). Kiwifruit juice inhibited growth of cancer cells (Motohashi, 2002).

Kiwifruit has been established in many countries principally USA, Australia, Belgium, Spain, France and Italy (Kumar and Sharma, 2002).

Kiwifruit propagation is normally carried out by cuttings or gtafting. The use of micropropagation techniques eliminates the seasonal limitations encountered with these methods and is favoured when a large number of plants is required in a short time (Wessels et al., 1984; Monette, 1986).

For commercialization of this fruit vine, however, planting material is required on large scale, which requires the development of an easier, quicker

and economically viable method of propagation. Several investigators have suggested that tissue and organ culture techniques could be the best alternative for kiwifruit propagation (Bonga, 1974; Abott, 1977, 1978).

In vitro culture techniques have been suggested as useful tools to test plant responses to variable compounds and to select for resistance/ tolerance to biotic and abiotic stresses as in vitro results were in some cases strictly related to those of in vitro trials, including fruit plants (Hammerschlag, 1992; Marino et al., 2000; Marino et al., 2004).

Although limited investigations have been carried out concerning the effects of various growth regulators on kiwifruit shoot proliferation, there is a scarcity of reported work regarding the effects of growth regulators on shoot proliferation and rooting in kiwifruit (Standardi and Catalano, 1985; Monette, 1986; Kumar et al., 1998; Kumar and Sharma, 2002).

The aim of this study was to investigate micropropagation of the kiwifruit (*Actinidia deliciosa* cv. Hayward) using different kind of growth regulators.

MATERIAL AND METHODS

Plant material and preparation of explant

Nodal shoots segments containing axillary buds were harvested from kiwifruit plants, that were cv.Hayward. The explants were harvested from newly developed fresh shoots during early in the morning and and proved to be the best time for explant collection.

Actively growing shoots were cut from female vine of *A. deliciosa* cv. Hayward. Initially expanded leaves were removed and axillary bud explants were surface sterilized by vigourous stirring for 30 second in ethanol (70% v/v) and 20 min in a 0.3% solution of commercial bleach containing 0.1 % Tween 20 that was used as a wetting agent (Jafari Najaf-Abadi and Hamidoghli, 2009). The explants were then rinsed three times in sterile distilled water, the first rinse lasting 2 min, the second 5 min and the final rinse lasted for 15 min. All plant tissue visibly damaged by the sterilant was removed. The plant material was cut into suitable sized explants and transferred onto the initiation medium containing major and minor salts as in Murashige and Skoog (MS, 1962) and 2 $mg.l^{-1}$ BA, 3% sucrose and %0.8 agar.

Effect of cytokinin concentration on explant proliferation

The shoots were transferred to proliferation medium one month later. The culture medium devised by MS medium supplemented with 3% sucrose and %0.8 agar, with different concentrations of BA (0, 1, 2 $mg.l^{-1}$) individually and along with IAA (0, 0.1, 0.2 $mg.l^{-1}$) was used for the proliferation of explants.

After six weeks of incubation in proliferation medium, shoots >10 mm long were harvested for rooting experiment.

Effect of auxin type and concentration on in vitro rooting

This experiment was adopted for standardizing most suitable auxin type and concentration for root induction. In this stage, shoots >10 mm were excised from explants of multiplication medium and inoculated on MS medium containing BA (0, 1, 2, 3 $mg.l^{-1}$) with various concentrations of IBA or IAA (0, 1, 2, 3 $mg.l^{-1}$).

The pH of all media adjusted at 5.8 prior to autoclaving at 121°C.and 142 kPa for 15 min. Cultures were routinely transferred every 2 weeks into fresh medium. All cultures, were incubated in a growth room with a culture environment of 24 ± 2°C with a 16 h light / 8 h dark cycle at an intensity of 57 $\mu mol.m^{-2}.s^{-1}$ provided by cool white fluorescent tubes.

Well-rooted shoots were removed from culture tubes after six weeks in rooting medium. Rooted shoots were washed thoroughly with tap water to remove the adhering medium and transferred to peat, perlite and compost (1:1:1). Transparent polyethylene bag or glass beaker was inverted over each plantlet to maintain high humidity.

Plantlets were monitored under the same environmental conditions as in vitro cultures for two

weeks and thereafter transferred to polyhouse for further growth. The transparent polyethylene bag or beaker was removed permanently upon new leaf appearance.

Statistical analysis

The data obtained were analysed by ANOVA. Three replicates were inoculated in each treatment of an experiment. Significant differences among mean values were separated using Tukey ($P<0.05$) test.

After six weeks of culture period, records were made on leaf number and shoot length per explant. For rooting experiment root number and root length were measured.

RESULTS AND DISCUSSION

Axillary bud Initiation and Shoot Proliferation

A prerequisite for successful culture is the establishment of an aseptic technique. For surface sterilization of explants, a solution containing 30% commercial bleach for 20 minutes gave the maximum aseptic cultures.

Almost all kiwifruit axillary buds placed on culture initiation medium were established and transferred to proliferation medium one month later (Fig. 1 and Fig. 2).

Only the effect of BA was significant on number of shoots and shoot height (Table 1).

The highest number of shoots and maximum shoot height were produced in medium containing 2 $mg.l^{-1}$ BA (Fig. 3).

In tissue cultures (as well as in intact plants and plant organs), cytokinins appear to be necessary for plant cell division. Cytokinins are very effective in promoting direct or indirect shoot initiation. To encourage the growth of axillary buds, and reduce apical dominance in shoot cultures, one

Table 1: Influence of BA on on kiwifruit axillary bud cultures

BA concentration ($mg.l^{-1}$)	No. of shoots±SE	Shoot length (cm) ±SE
0	$0.45±0.2^{b}$	$1.21±0.29^{b}$
1	$0.68±0.2^{ab}$	$1.23±0.29^{b}$
2	$1.2±0.2^{a}$	$1.33±0.29^{a}$

Means in a column with different small letters are significantly different at $p<0.05$ by Tukey.

Table 2: Influence of IBA on rooting of kiwifruit shoots produced *in vitro*

BA concentration ($mg.l^{-1}$)	No. of shoots±SE	Shoot length (cm) ±SE
0	0±0.11b	0±0.13b
1	0.13±0.11b	0.15±0.13ab
2	0.13±0.11b	0.13±0.13ab
3	0.68±0.11a	0.58±0.13a

Means in a column with different small letters are significantly different at $p<0.05$ by Tukey.

Fig. 1: Initial explant grown on MS+2mg.l^{-1}BA after 2 weeks

Fig. 2: Initial explant grown on MS+2mg.l^{-1}BA after one month

Fig. 3: Axillary shoots formed on MS+2mg.l^{-1}BA

Fig. 4: Adventitious roots formed on MS +3 mg.l^{-1}IBA mediuum

Fig. 5: *In vivo* acclimated plantlets of kiwifruit

Fig. 6:

or more cytokinins are usually incorporated into the medium at proliferation stage (George, et al.,2007). Our shoot proliferation are comparable with those reported by Wang et al. (2006), Wessels et al. (1984) and Monette (1986) and Standardi and Catalano (1985) for effective of cytokinins in kiwifruit proliferation. but this is contrary to the findings of Shen et al. (1990).

Wessels *et al.* (1984) cultured vegetative buds from actively growing shoots of *A. chinensis* on MS medium supplemented with various growth regulators. Shoot formation was obtained with all the growth regulators but was best with BA at 2 $mg.l^{-1}$. Shen et al. (1990) reported axillary shoot formation was influenced by the type and concentration of cytokinin used. Shoot multiplication from axillary buds was achieved on a medium supplemented with 1 $mg.l^{-1}$ BA together with 0.1 $mg.l^{-1}$ IAA.

Regeneration of roots and acclimatization

After elongation individual shoots measuring 10 mm were inoculated on MS medium supplemented with BA alone and in the combination with IBA or IAA for rooting.

Effect of IBA

The results indicated that root length and number of roots were significantly ($p<0.05$) affected by concentration of IBA (Table 2).

As expected, the auxins stimulated rhizogenesis. The regenerated shoots produced roots when transferred to MS medium supplemented with either IBA or IAA. Maximum root length and the highest number of roots were obtained with 3.0 $mg.l^{-1}$ and 2.0 $mg.l^{-1}$ IBA respectively, within six weeks in culture (Fig. 4).

Minimum root length and the least number of roots responses were observed in control medium without any auxins. Increasing the concentration of any auxin singly became effective to root induction.

In general, a high concentration of IBA seems to favour indirect rhizogenesis.

This agrees with data reported by Monette (1986), Marino and Bertazza (1990) for rooting of kiwifruit explants. According to Monette (1986), with increasing concentration of IBA from 5 to 10 $mg.l^{-1}$, rooting of explants was enhanced.

Effect of IAA

The effect of IAA was not significant on number of roots and root length. Maximum root length and the highest number of roots were produced in MS medium containing 3 $mg.l^{-1}$ IAA.

Although IAA induced rooting, the roots were thin and delicate and the plantlets did not survive the hardening in peat, perlite and compost (1:1:1). On the other hand, plantlets in which roots were induced as a result of their treatment with IBA had a survival rate of approximately 80% when hardened (Fig. 5).

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