Pak. J. Bot., 40(6): 2355-2360, 2008.

AN OPTIMIZED AND IMPROVED METHOD FOR THE *IN VITRO* PROPAGATION OF KIWIFRUIT (*ACTINIDIA DELICIOSA*) USING COCONUT WATER

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Abstract

Kiwifruit (*Actinidia deliciosa*) is grown for its excellent food and nutritional value in many parts of the world. The main objective of this study was to optimize an efficient, reliable and economical protocol for *In vitro* micropropagation of Kiwifruit. The effect of coconut water along with BAP on shoot proliferation of Kiwifruit (*Actinidia deliciosa*) has been evaluated. It has been noticed that both the BAP and coconut water had a synergistic effect and none of them was found able to generate the maximum response when used separately. Maximum shoot length (7.2 ± 0.16), number of shoots (11.5 ± 1.5) and number of nodes (4.6 ± 0.22) were achieved on the MS medium containing 20% (v/v) coconut water with 2.0 mg/L of BAP (KW10). The use of coconut water also resulted in the longer sub-culturing time and the production of highly robust plants which were able to survive in the green house conditions. The proliferated shoots were subjected to root induction and half strength MS media with 0.2 mg/L IBA was found optimum for the root formation. The sufficiently rooted plantlets were transferred to green house for hardening. The potting mix comprised of 90% sand and 10% farm yard manure (v/v) and more than 95% of the plants, subjected to acclimatization, survived under green house conditions.

Introduction

The Kiwifruit (*Actinidia deliciosa*) is a large, woody, deciduous vine, native to the Yantze Valley of China. However, it is now widely cultivated in California, Italy, Japan, France, Greece, Spain, Australia, Chile and China. The fruit is borne on a vigorous, woody, twining vine or climbing shrub reaching 30 ft (9 m). The leaves are alternate, long-petioled, deciduous and shoots are coated with red hairs. Mature leaves are dark-green and hairless on the upper surface and have downy-white with prominent light colored veins beneath (Sale, 1990).

Kiwifruit is very well known for its great medicinal importance and has been considered for various treatments and onsets. The branches and leaves of the plant are boiled in water and the liquid has been used for treating mange in dogs (Anon., 2004). Fruit juice contains antioxidizing activity and the presence of glutathione may also account for the reduction of mutagenesis (Ferguson *et al*., 2004; Yao *et al*., 2004). Kiwifruit is also known to have laxative effect (Rush *et al*., 2002).

Micropropagation is not a new technology and application of innovative methods have served to overcome barriers to progress in the efficient multiplication of elite plant species and further improvements in the technique and methodology are anticipated. Growth and development *In vitro* are considerably influenced by several factors including genotype, the age of plant, the age of the tissue or organ (explant), the physiological state and many more. As a mean of securing pathogen free plants, culture of shoot apical meristem is ideal. Other

advantages that assure by this method are; micropropagation is faster and can be produced in large numbers in shorter periods, irrespective of the seasons, plants originate either from pre-existing meristem, without passing through a callus phase, produce uniform plants that are genetically stable traits, which are of great horticultural importance (George, 1993).

In the previous reports on micropropagation of Kiwifruit, 6-Benzyl amino purine (BAP) has been used for the multiplication of shoots (Moncalean *et al*., 2001). Whereas Takahashi *et al*., (2004) examined the effect of Zeatin on the formation of shoot buds from explants and callus tissues derived from stem segments of *Actinidia polygama*. As Zeatin is very expensive and cannot be used in commercial applications of micropropagation, so the objective of the study was to make use of less expensive and easily available coconut water and explore its effects on enhanced micropropagation of Kiwifruit for commercial multiplication in Pakistan.

Materials and Methods

Isolation of coconut water: The coconut water is simply drained from dehusked immature coconuts by drilling holes through two of the micropyles. Extract of water from each fruit separately was checked properly to ascertain that it is not fermented before addition to the bulk. Collected water from all the fruits was heated at 80-100 °C for 10 minutes with continuous stirring to precipitate out the proteins, fats and other materials. The precipitates were separated by filtration and the filtrate is stored at -20 °C for future use (George, 1993).

Harvesting, sterilization and germination of seeds: The seeds of Kiwifruit were collected from the pulp of the fruit separated with the help of muslin cloth and washed under tap water for 10 minutes. The seeds were sterilized according to Nasib *et al*., (2007) and Khan *et al*., (2007). The seeds of Kiwifruit were inoculated on simple MS medium and incubated at 25±2 °C with 16 hours photoperiod for 8 weeks.

Shoot multiplication: After 8 weeks of incubation, all the germinated seeds were transferred to the experimental media which comprised of full strength MS basal salts (Murashige & Skoog, 1962) supplemented with various levels of BAP (1.5, 2.0 and 2.5 mg/L) along with coconut water (15, 20 and 25 % v/v) as shown in Table 1. The pH of the media were adjusted to 5.77 prior to autoclaving by adding 1N NaOH and/or 1N HCl. The media were solidified with 2.5 gm/L phytagel added after adjusting the pH and before autoclaving. All the cultures were kept at 22±2°C with 16 hours photoperiod. Data regarding the length and number of shoots along with the number of nodes were recorded for 8 weeks and mean with standard deviation were calculated (Table 1).

Rooting and acclimatization: After eight weeks, the multiplied shoots were transferred on root initiation media comprised of half MS medium containing IBA 0.02 mg/L. Data were recorded after every week for four weeks (not presented here). The rooted plantlets were transferred to the green house for acclimatization. The potting mix used for acclimatization contained 90% sand and 10% farmyard manure (v/v).

Table 1. Effect of coconut water and BAP on shoot length of Kiwifruit.

Media Code	BAP level (mg/L)	Coconut water (% age)	Mean no. of Shoots*	Mean shoot length*(cm)	Mean no. of nodes
KW0	0.0	0	5.5 ± 0.9	2.2 ± 0.20	2.1 ± 0.19
KW1	0.0	15	6.7 ± 1.1	2.8 ± 0.22	2.2 ± 0.21
KW2	0.0	20	7.2 ± 1.4	3.0 ± 0.18	2.5 ± 0.20
KW3	0.0	25	7.4 ± 1.5	3.1 ± 0.21	2.5 ± 0.22
KW4	1.5	0	7.9 ± 1.2	3.4 ± 0.34	2.8 ± 0.21
KW5	1.5	15	8.1 ± 1.4	4.0 ± 0.22	3.0 ± 0.09
KW6	1.5	20	8.4 ± 1.1	3.8 ± 0.21	2.8 ± 0.29
KW7	1.5	25	7.9 ± 0.8	4.6 ± 0.10	3.2 ± 0.30
KW8	2.0	0	8.5 ± 1.8	4.0 ± 0.15	3.1 ± 0.25
KW9	2.0	15	9.4 ± 1.2	5.1 ± 0.19	3.6 ± 0.28
KW10	2.0	20	11.5 ± 1.1	7.2 ± 0.16	4.6 ± 0.22
KW11	2.0	25	10.5 ± 1.0	4.8 ± 0.07	3.3 ± 0.27
KW12	2.5	0	9.2 ± 1.2	5.1 ± 0.19	3.5 ± 0.21
KW13	2.5	15	9.4 ± 1.4	4.3 ± 0.12	3.3 ± 0.29
KW14	2.5	20	10.0 ± 0.9	4.5 ± 0.15	3.0 ± 0.26
KW15	2.5	25	9.9 ± 1.0	4.4 ± 0.45	3.2 ± 0.23

*Means are with ± Standard Deviation (SD)

Results and Discussion

The seeds of Kiwifruit started germination after 6 weeks and were fully germinated within 8 weeks. At the height of 1.5-2.0 cm, these germinated plantlets were transferred to the experimental media (Table 1). The number and length of the shoots along with the number of nodes were measured after every two weeks, upto eight weeks. The effect of different concentrations of BAP and coconut water on shoot multiplication is presented in Table 1. It was observed that both the BAP and coconut water had a synergistic effect and either of them was not able to produce the same result when used separately. It was noted that MS media containing 20% coconut water (v/v) with 2 mg/L of BAP (KW10) resulted in the maximum increase in number (11.5 ± 1.1) and length of shoots (7.2 ± 0.16) (Fig. 1A). The highest number of nodes (4.6 ± 0.22) was also recorded when the same KW10 medium was used (Table 1).

It was also noted that all the growth parameters were highly influenced by the addition of coconut water as the cultures grown on the media without coconut water (KW0, KW4, KW8, KW12) showed much lesser growth as compared to the cultures grown with the coconut water (Table 1). It was also observed that the cultures, grown without coconut water, turned brown after four weeks and need to be transferred to the fresh media whereas the addition of coconut water to the media had made cultures to survive for 8 weeks.

After 8 weeks of time, the multiplied shoots of good length (7.2 ± 0.16) were transferred on rooting media where the root initiation started within 10 days and prolonged enough in 4 weeks time. IBA (0.02 mg/L) induced sufficient rooting in Kiwifruit. The plants were then shifted to the green house and were well acclimatized using 90% soil and 10% farmyard manure as a potting mix. More than 95% of the plants, transferred to green house, survived under semi-controlled environment.

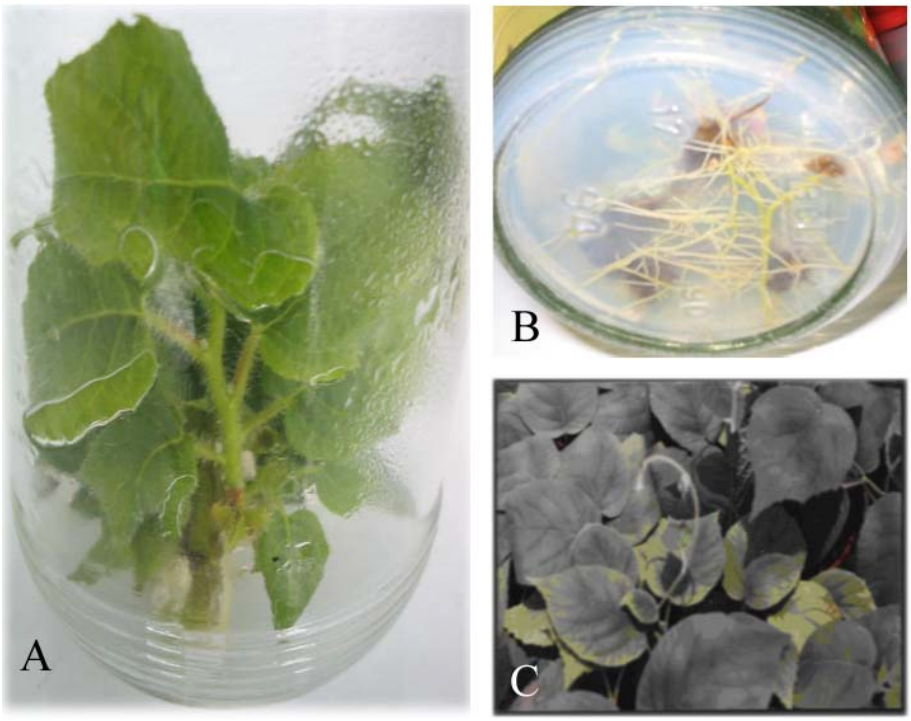

Fig. 1. (A) Axillary shooting after 6 weeks. (B) *In vitro* rooting of Kiwifruit. (C) Plants in Green house after acclimatization.

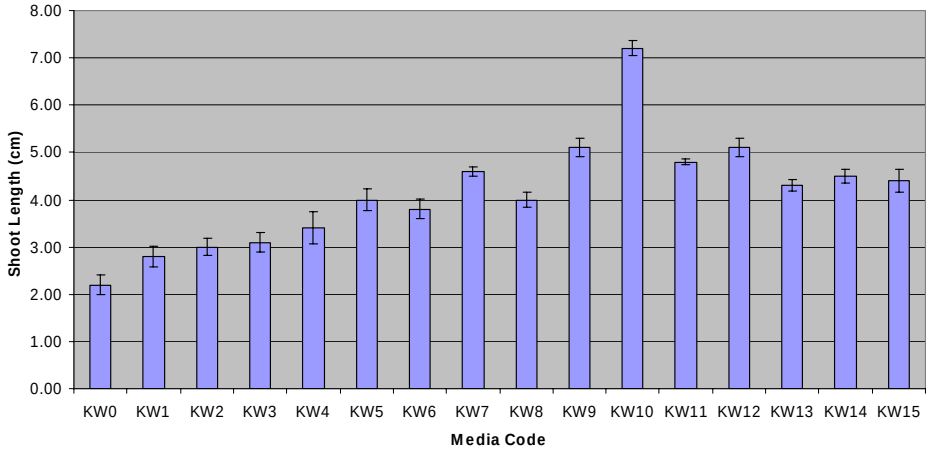

Fig. 2. Effect of coconut water on the mean shoot length of *Actinidia deliciosa* with ± Standard Deviation (SD).

Many undefined supplements were employed in early tissue culture media. Their use has slowly declined as the balance between inorganic salts has been improved and as the effect and role of growth substances have become better understood. Nevertheless several supplements of uncertain and variable composition are still in common use and coconut water is one of them especially in the tropical countries where this fruit is easily available at low price. Coconut water was first used in plant tissue cultures by Van Overbeek in (1941) for the development of embryos of *Datura stramonium* and then with the passage of time it was found that coconut water could be used to initiate and maintain tissue cultures of several plants. Coconut water is composed of many amino acids, nitrogenous compounds, inorganic elements, organic acids, sugars and their alcohols, vitamins, growth substances (cytokinins and auxins) and many other unknown components (George, 1993).

The effect of coconut water for the enhanced *In vitro* propagation of Kiwifruit was evaluated. During the course of study, apart from the enhanced shoot multiplication, two major effects of coconut water were observed. First the addition of coconut water to the media resulted in about 95% increment of overall phosphorus (P) content of the media (Mezetti *et al*., 1991). This effect of coconut water ultimately resulted in the doubling of sub-culturing time from four to 8 weeks. This elimination of transferring the plantlets to the fresh medium resulted in the reduction of overall cost of labor and chemicals as the number of plants produced was same. The second important effect of coconut water is that it proved as a very useful pre-conditioner to achieve bigger and more robust plants. The addition of coconut water to the culture media resulted in the plants with a greater nutritional and carbohydrates contents as coconut water itself contained 21.8 gm/L sugars in total (George, 1993). This high robustness and survival rate (>95%) of the *In vitro* grown plants might be due to their high carbohydrates contents which could be used to meet respiratory demands while surviving the physiological shocks of *ex vitro* procedures associated with the shifting of plant from controlled to semi controlled environment (Boase *et al*., 1993).

During this study, it was observed that the root induction was highly effected by the length of shoots and an appropriate length was pre-requisite for the efficient root formation. The use of coconut water also indirectly effected *In vitro* roots induction since during shoot multiplication; the addition of coconut water to the culture media resulted in maximum shoot length (7.2 ± 0.16) and hence facilitating the efficient root formation. This enhanced root formation ultimately resulted in the high survival rate (>95%) of the *In vitro* grown plants.

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(Received for publication 24 April 2007)