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In vitro Regeneration of Exotic Kiwi Fruit (*Actinidia deliciosa*) in Bangladesh

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Abstract

Exotic fruits play a crucial role in the agricultural landscape of Bangladesh owing to their significant market value and nutritional attributes. Efforts are being made to cultivate these fruits in various regions of the country. Hence, the establishment of a regeneration system for these fruits is imperative. The utilization of tissue culture systems is the optimal approach for the cultivation of exotic fruits on a large scale. This study focuses on the establishment of an *in vitro* regeneration protocol for exotic fruit, kiwi (*Actinidia deliciosa*). Various combinations of growth hormones have been employed in conjunction with MS medium. The optimal medium for shoot regeneration was MS supplemented with 2 mg/l BAP and 0.2 mg/l IBA. Additionally, the most favorable rooting outcome was observed when mature regenerated shoots were placed onto a half-strength MS basal medium supplemented with 0.2 mg/l IBA.

Introduction

Kiwi fruit (*Actinidia deliciosa*) belongs to the family Actinidiaceae and genus *Actinidia*. This fruit is approximately 3 inches long, with a brown hairy peel, green flesh and white pulp in the centre, containing minute black edible seeds. Kiwi fruit is enriched with vitamins, minerals and phytochemicals. *A. deliciosa* also helps against diseases associated with the cardiovascular system, diabetes, kidney problems, cancer, digestive disorders, bone and eye problems. Numerous pharmacological properties of kiwi, including anti-diabetes, anti-tumour, anti-inflammatory, anti-ulcer, antioxidant activity, hypoglycaemic, hypolipidemic are available in the kiwi fruit. Traditionally, kiwi has been used in the treatment of edema, hepatitis, kidney problems, rheumatoid arthritis, arthralgia and microbial infections (Satpal et al. 2021). The first commercial cultivation of kiwi fruit began in New Zealand during 1970s. In India, kiwi fruit was introduced in 1960s in parts of the Himalayan region from New Zealand and USA (Deb and Gangmei 2019).

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Grafting and occasionally cutting are the primary methods used to propagate kiwifruit; nevertheless, these techniques were not very successful because of dioecious nature, long juvenile period and rooting difficulties (Deb and Gangmei 2019). Plant tissue culture has emerged as a widely employed technique for the extensive regeneration of plants on a huge scale. The first micropropagation protocol for *A. deliciosa* was proposed by Harada (1975) and has been subsequently improved, such as shoot regeneration (Akbas 2007, Bourrain 2018, Zhong et al. 2021), organogenesis (Hanh et al. 2022, Purohit et al. 2020, Wu et al. 2011), somatic embryogenesis (Hanh et al. 2022, Oliveira 1999), etc. *In vitro* propagation of *Actinidia* spp. with many plants for the industry has been applied in many countries such as New Zealand, Italy, Franch, etc. (Testolin 2014).

Kiwi is not cultivated in our country; as a result, it is imported to Bangladesh. The three topmost exporters of kiwi to Bangladesh are India, Thailand and Malaysia. There is an increasing demand for kiwi in Bangladesh. In 2022, Bangladesh imported approximately 31 tons of kiwi fruits, showing a 21% increase compared to the previous year (https://www.indexbox.io/search/kiwi-fruit-price-bangladesh/). Due to its high price and status as a foreign fruit, kiwi is not widely available in Bangladesh. Every day, on an average around 1.68 million kilograms of exotic fruit are consumed in Bangladesh (Jahan 2023). To discourage the consumption of foreign fruits, Bangladesh government imposed duty and tax on fruit imports. People in Bangladesh are becoming more interested in growing foreign fruits these days. After several years of experimenting with soil treatment, several foreign fruits, such as dragon, strawberry, Saudi palm, apple, malta, grape, avocado, rambutan, almond, mangosteen, persimmon, Vietnamese coconut, Thai papaya, lime, durian etc. have been produced in our country (https://businesspostbd.com/editorial/exotic-fruits-grown-in-bangladesh-2022-10-24). To decrease the import of foreign fruits like kiwi in Bangladesh, tissue culture technology can play a vital role in producing foreign fruits in Bangladesh. To promote and increase the production of kiwi fruit, the current study aims to design an efficient, reproducible *in vitro* regeneration protocol for kiwifruit, a new fruit species for Bangladesh.

Materials and Methods

The current experiments were performed using *in vitro* raised leaf explants of kiwifruits. The premium quality kiwifruits were bought from the Unimart supermarket in Dhaka. The surface sterilization method of seeds was rigorously adhered to in accordance with the established protocol (Khan et al. 2016). Seeds were germinated on a sterilized cotton socked bed. After 8-10 days of germination, leaves were ready as explants. The pieces of leaf explants were introduced and cultured on a medium referred to as MS, which comprised a combination of plant growth regulators including BAP, NAA, IAA, Kn, 2,4-D) and IBA.

These plant growth regulators were used individually or in combinations to facilitate the process of *in vitro* shoot regeneration. The shoots that were regenerated *in vitro* were

consistently transferred to new culture media every 21-28 days to facilitate their further multiplication. The elongated shoots were isolated and subsequently transferred onto a specialized growth medium specifically formulated to facilitate the initiation and subsequent growth of root structures. Shoots measuring around 1-3 cm in length were isolated and subsequently cultivated on a rooting medium consisting of MS supplemented with different doses of IBA and NAA. The *in vitro* cultures were kept under illumination for 16 hrs per day, with a constant temperature of 25 ± 2°C. Subsequently, the plantlets possessing adequately developed root systems were transferred to an environment characterized by ambient temperature settings, where they were allowed to remain for 2-3 days. After this time frame, the specimens were transferred into plastic containers containing a blend of garden soil and compost, with a proportion of 2 parts soil to 1 part compost. Efforts were made to ensure that the plantlets were adequately supplied with moisture to promote effective hardening (Yeasmin et al. 2022, Banu et al. 2020).

Results and Discussion

A technique was devised to facilitate the *in vitro* regeneration of kiwi fruit characterized by its simplicity, efficacy and reproducibility. In this study, leaf explants were subjected to culture on MS medium with various growth regulators, such as BAP, Kn, 2,4-D, NAA, and IBA, either alone or in combination to observe their effects on proper regeneration. Careful consideration and selection of the medium type are pivotal in determining the success or failure of plant tissue culture (Bhattacharyya et al. 2016).

Table 1. Shoot regeneration of *A. deliciosa* from leaf explants on MS medium supplemented with various hormonal supplements.

Hormonal combinations (mg/l)	% of responsive explants	Days required for callus initiation	Mean no. of shoots/ explant (after 75 days)
MS + 0.5 BAP	Only swelling of explant	-	-
MS + 1.0 BAP	Only swelling of explant	-	-
MS + 0.5 Kn	Only swelling of explant	-	-
MS + 1.0 Kn	Only swelling of explant	-	-
MS + 1.0 BAP + 0.5 Kn	77.5	12-21	8.85 ± 2.06
MS + 1.0 BAP + 1.0 Kn	75.0	12-24	7.42 ± 2.35
MS + 1.0 BAP + 0.2 NAA	90.0	15-14	10.42 ± 1.48
MS + 2.0 BAP + 0.2 NAA	90.0	18-25	12.14 ± 1.48
MS + 1.0 BAP + 2.0 2,4-D	80.0	7-14	Only callus
MS + 2.0 BAP + 2.0 2,4 D	85.0	8-12	Only callus
MS + 2.0 BAP + 0.2 IBA	92.5	12-18	13.14 ± 1.35
MS + 2.0 BAP + 0.4 IBA	90.0	12-18	12.71 ± 1.03
MS + 1.0 BAP + 0.5 Kn + 0.2 NAA	70.0	11-18	9.42 ± 1.84

This experiment aimed to evaluate the effects of these growth regulators on shoot regeneration in the kiwi fruit plant. The leaf of kiwi exhibited varying responses to shoot regeneration, as indicated in Table 1. No morphogenic response was visualized when hormones were applied singly (Table 1). In contrast, Akbaş et al. (2007) obtained the best results for shoot proliferation on MS with 0.5 mg/l BAP from seed- germinated shoot tip explants of kiwi. Similar results were reported by Dev and Gangemi (2019), where they found maximum shoot bud formation from nodal explants on MS with 3 µM BAP supplemented media. This result contradicts the present experiments as in the present experiment no shoot formation was found when the explants were cultured on different BAP supplemented MS medium. This may happen due to the selection of different explants. As both selected node as well as shoot tip explants, respectively.

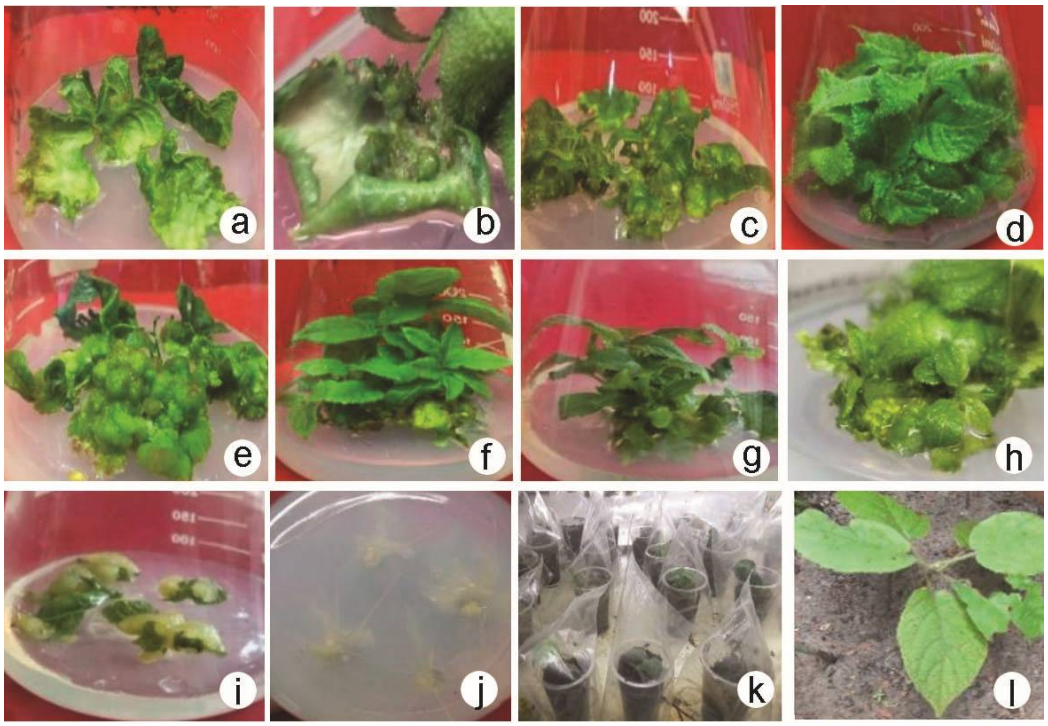

Fig. 1 (a-j). Different stages of *in vitro* regeneration from leaf segment explants of *A. deliciosa* in: (a) Initiation of callus from leaf explants on MS with 2.0 mg/l BAP and 0.2 mg/l IBA, (b) Shoot initiation from callus in the same combination as mentioned in Fig. a, (c-d) Multiple shoots as well as elongated shoots on same combination as mentioned in Fig. a respectively, (e) Callus formation and shoot initiation on MS with 2.0 mg/l BAP and 0.2 mg/l NAA, (f) Elongated multiple shoots on same media mentioned in Fig. e, (g) Multiple shoot formation on MS with 1.0 mg/l BAP and 0.5 mg/l Kn, (h) Formation of multiple shoot on MS with 1.0 mg/l BAP, 0.5 mg/l Kn and 0.2 mg/l NAA, (i) Formation of only callus on BAP and 2,4-D supplemented medium, (j) Root induction from regenerated shoots on MS with 0.2 mg/l IBA, (k-l) Hardening of rooted kiwi plants in plastic and clay pot respectively.

The leaf exhibited the greatest level of response (92.5%) in terms of shoot initiation when they were cultured on MS medium with 2.0 mg/l BAP and 0.2 mg/l IBA. In this combination, of BAP and IBA callus was initiated from lower parts of explants (Fig. 1a) and many shoots were produced from the callus (Fig. 1b and c). In this combination, occasionally direct shoots were produced from the cut end of the explants. Highest mean number of shoots/explants was recorded at 13.14 ± 1.35 (Fig. 1d) in this combination of BAP and IBA. Zhong et al. (2021) reported that MS with 1.0 mg/l BA and 0.2 mg/l IBA was the best adventitious bud induction medium from lamina of kiwi plants. For proliferation, they used MS + 1.0 mg/l BA + 0.1 mg/l IBA. Similar results were reported by Yu et al. (2014) and Long et al. (2010) for the shoot proliferation of kiwi fruit plants from stem and leaf as well as bud explants, respectively. The leaf segment culture experiment also yielded the significant favourable outcome when employing a two-way approach, specifically by supplementing the medium with 2.0 mg/l BAP and 0.4 mg/l IBA, as well as 2.0 mg/l BAP and 0.2 mg/l NAA. The emergence of callus initiation was undertaken between 12-18 and 18-25 days after inoculation respectively (Fig. 1e). The mean number of shoots/explants was very similar in both above mentioned combinations which was 12.71 and 12.14, respectively (Fig. 1f). Hanh et al. (2022) reported that MS medium supplemented with 0.02 mg/l NAA, 0.5 mg/l TDZ reflects the highest somatic embryogenesis (98.67%) and the best somatic embryoes (10.66 embryos) from leaf main vein explants of kiwi. Banu et al. (2017) reported that combinations of BAP and NAA show better results from node explants in *Gynura procumbens.* Goswami et al. 2018 also found a good response using BAP and NAA in *Brassica juncea*.

The response of leaf explants for regeneration was determined when utilizing MS medium with different concentrations of BAP and Kn as well as BAP, Kn, and NAA. Both MS with 1.0 mg/l BAP and 0.5 mg/l Kn, as well as the same concentration of BAP, Kn with 0.2 mg/l NAA showed a significant regeneration response (Table 1, Fig. 1g and 1h). It was noticed that for callus development BAP, Kn and NAA supplemented MS medium took less time compared to BAP and Kn fortified MS medium (Table 1). The inclusion of NAA in the TDZ-containing medium enhanced multiple shoot induction in *Mertensia maritima* (Park et al. 2019). Chamail et al. (1999) reported that the best callus induction happened on MS with 1.5 mg/l BA and 1.0 mg/l NAA from *in vitro* raised leaf segments of *A*. deliciosa, though they also used different concentrations of 2,4-D, 2iP, include and kinetin with BA in MS medium. Kinetin and 2iP failed to initiate calluses from the leaf segments. In the present experiment it was observed that when BAP and 2,4-D were added to MS medium, only callus was formed from the leaf explants. No sign of regeneration was observed from that callus after subculture (Fig. 1i). Shen et al. (1990) induced callus from the endosperm of Chinese gooseberry on basic MS medium with 3.0 mg/l zeatin, 0.5 mg/l 2,4-D and 400 mg/l cycloheximide and then transferred to MS with 1.0 mg/l zeatin and 400 mg/l cycloheximide for the development embryoids appeared and developed into plantlets.

Table 2. Root induction from the *in vitro* regenerated shoots of *A. deliciosa*.

Hormonal combinations (mg/l)	% of responsive shoots towards root induction	Days required for root initiation	Type of roots
MS + 0.2 IBA	85	10-15	Long and thin
½MS + 0.2 IBA	95	10- 14	Long and thin
MS + 0.2 NAA	65	10-15	Callus with root
½MS + 0.2 NAA	70	10-16	Callus with root
MS + 0.2 IAA	75	11-18	Callus with root
½MS + 0.2 IAA	75	12-18	Callus with root

The current investigation observed that leaf explants predominantly exhibited shoot induction followed by subsequent root initiation. The regenerated shoots, measuring 2-3 cm in length, were isolated and subsequently utilized to induce root formation. Full- and half-strength MS medium supplemented with 0.3 mg/l IBA, NAA and IAA were employed to induce roots from the shoots (Table 2). The media combination that yielded the highest rooting frequency (95%) was half-strength MS supplemented with 0.2 mg/l IBA (Fig. 1j). The optimal duration for root formation in these combinations was shown to be between 10 and 14 days, where long and thin roots were produced. Full- and half-strength MS supplemented with 0.2 mg/l IAA resulted in 75% root formation from regenerated shoots. The lowest frequency of root induction was observed on NAA-supplemented media. It was noticed that in NAA and IAA supplemented rooting media, callus was produced along with the roots. Zhong et al. (2021) reported that the highest (85.18%) roots were induced from kiwi microshoots on half- strength MS with 0.3 mg/l IBA. According to Akbas et al. (2007), the most effective root induction medium for kiwi was MS with 1.0 mg/l NAA. Deb and Gangmei (2019) got maximum (56.3%) rooting response from regenerated shoots of kiwi when they cultured the shoots on MS with 9 µM IBA supplemented media. Purohit et al. (2020) reported that rooting was achieved through half-strength MS medium supplemented with 1.5 µM IBA and 0.6 µM BAP for *in vitro* shoots of kiwi. Goswami et al. (2022) also reported root formation using MS with IBA in *Withania sp.* After the satisfactory growth of the plant roots, the plantlets were subsequently transferred to small plastic pots containing soil. These pots were then placed in a controlled culture room (Fig. 1k). Sixty percent of the plantlets survived in plastic pots (data not shown). The survived plantlets were relocated to large pot to facilitate their growth and establishment (Fig. 1l).

Based on the findings of this study, it can be inferred that the plant regeneration protocol described here exhibited notable features such as swift proliferation of shoots utilizing leaf explants, facile rooting of micro-shoots and successful acclimatization of plantlets to the surrounding environment. The established technique lays the groundwork for next studies on kiwi genetic transformation and large-scale multiplication, reducing dependency on imports and encouraging domestic production of this fruit.

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