In vitro Propagation of Cassava (*Manihot esculenta* Crantz)

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Abstract

In vitro plant regeneration in cassava was obtained on MS medium supplemented with 2.0 mg/l BA + 0.1 mg/l NAA using nodal segment as explants. In this medium 90% explants induced shoots after 4 weeks. The average number of shoot per explant was 6.30 ± 1.20 and the average shoot length was 10.30 ± 0.70 cm. After 90 days average number of node per explant was 6.25 ± 0.45 in this same medium. Shoots rooted well when they were transferred into half strength MS media supplemented with 1.0 mg/l IBA. The average number of root per shoot was 8.50 ± 0.60 and the average root length was observed 9.90 ± 0.60 cm in this medium of culture after 30 days. No morphological variation was observed during the *In vitro* culture. After hardening about 90% plantlets were survived. In this experiment rapid multiplication of planting material in cassava through tissue culture techniques such as shoot initiation, micropropagation, rooting and hardening is developed.

Key words: *In vitro* propagation, cassava, nodal segments

1. Introduction

Cassava (*Manihot esculenta* Crantz.) is a perennial shrub and tropical root crop. It is cultivated for its starchy tuberous roots as a valuable source of calories. It belongs to the family Euphorbiaceae and requires at least 8 months of warm weather to produce a crop [1]. In the tropics cassava is the third most important source of calories with more than 600 million people in Africa, Asia and latin America depending on it [2-3]. The crop is valued in many areas for food security. In addition, the tuberous roots of cassava can be left in the ground for several years prior to harvest and providing security against famine [4]. It is also increasingly being used in processed food and fodder products and by the chemical, pharmaceutical, paper and textile industries [5]. The crop also can be used for bio-ethanol production. The total annual cassava root production worldwide is 184 million tones, out of which 50% production is in Africa, 30% in Asia and 20% in Latin America [6]. In addition, the average yield varies widely, e.g. 7 to 10 t ha^{-1} in Ghana, 26 t ha^{-1} in India and 37 t ha^{-1} in Thailand [6]. Cassava is slow growing vegetatively propagated crop and its multiplication is generally tedious [7]. Inadequate high yielding varieties and susceptibility to diseases were identified as the key challenge to the expansion of cultivation of the crop. These were compounded by the bulkiness and high distribution costs, low multiplication rates and poor storage quality of planting materials [8]. *In-vitro* techniques have been employed to produce high yielding varieties, disease-free and resistant planting materials, even from infected mother plants [9]. Production of a cultivar that will retain its distinguishing characteristics when bred with the same cultivar planting materials can also be achieved through *in-vitro* propagation. This propagation method can be carried out regardless of growing season, enable the year-round availability of planting materials and thus ensure the expansion of production. Tissue culture has been exploited for rapid clonal multiplication, transformation and conservation of cassava with higher yields [10] Cassava is mainly multiplied by stem cuttings, which is a slow process of propagation when compared with grain crops. It is mostly grown in small farms and the cuttings are usually planted at the start of the rainy season. In Bangladesh, cassava is a neglected crop grown primarily by farmers with the help of NGO's in marginal areas of greater Rangpur, Mymensingh, Tangail and Bogra. Cassava is a crop of great importance in the tropics because it is a readily available staple food, ease of cultivation and the ability to be transformed into different forms and stored as food for several years [11]. It is a valuable source of calories especially in countries where malnutrition [12]. In our country this crop can be an important alternative instant food crop for low fertile area and also for disaster periods like flood and draught. The large-scale cultivation is constrained by the lack of high quality planting materials and the low productivity and profit. Tissue culture method can be used to produce high quality vegetative planting material, which ensures productivity and free of diseases. In view of this, a high frequency cassava *In vitro* regeneration method is required. In this study, attempt was made to establish a protocol for availability of propagules round the year without any seasonal barrier through *In vitro* culture method and also to evaluate the response of various concentrations and combinations of plant growth regulators for *In vitro* plantlet production in cassava.

2. Materials and Methods

Nodal explants of field grown cassava were collected from Atomic Energy Research Establishment Campus, Savar, Dhaka. The explants were washed thoroughly using household detergent, Trix (commercial) for 20 minutes under running tap water. Subsequent sterilization was carried out in the laminar air flow cabinet. Nodal explants were sterilized with an aqueous solution of 0.1% mercuric chloride for 7 minutes. After that the explants were rings 4 times with sterile distilled water. Nodal explants of 2.0 cm

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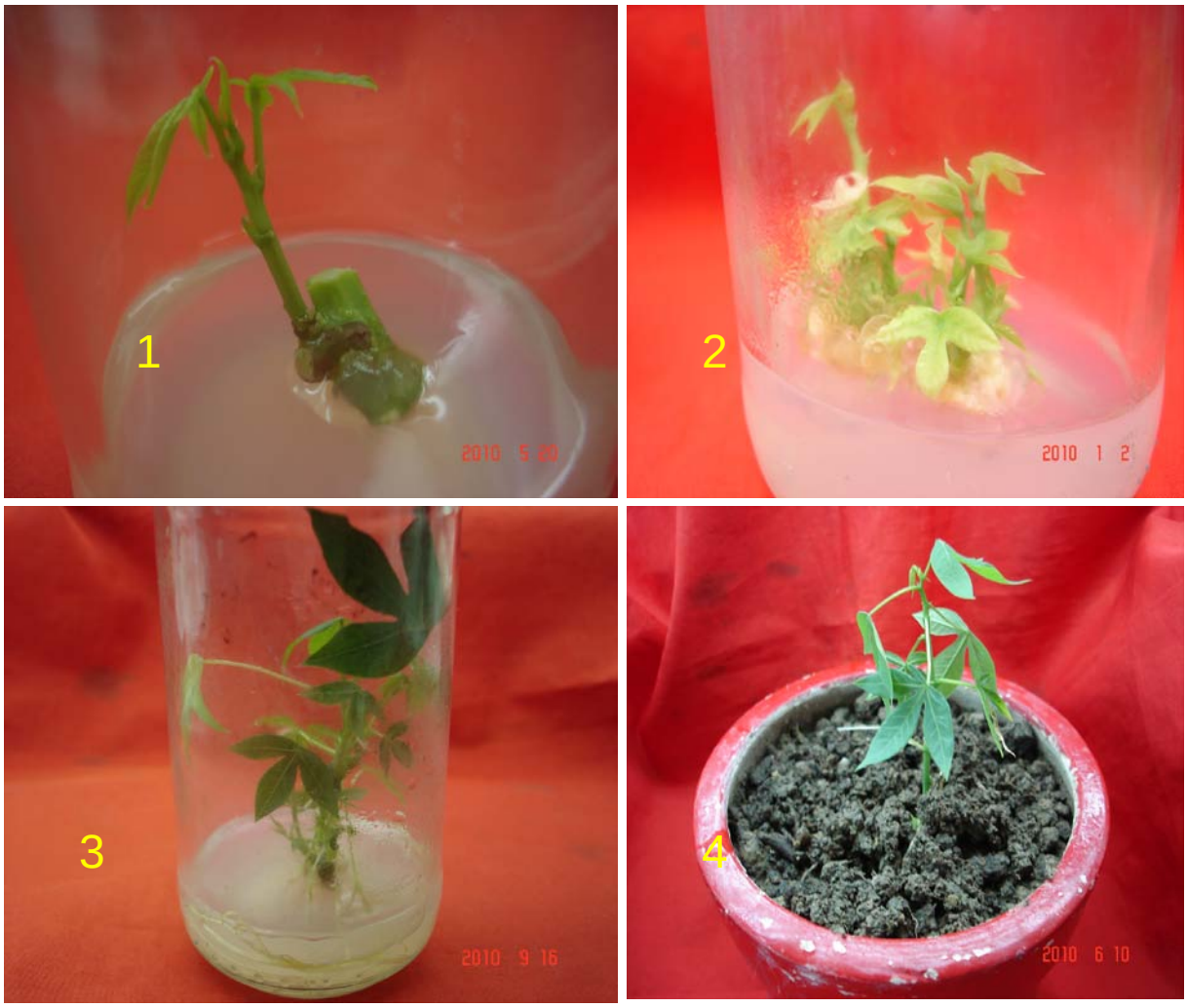
long were excised and inoculated on MS medium supplemented different concentrations of BA alone or BA in combination with GA3 and NAA for multiple shoot formation. Subculture was done 30 days interval on the same medium for promoting strong and healthy multiple shoots. Morphologically healthy shoots were transferred into half strength MS supplemented with different concentrations of IBA, IAA and NAA for root induction. The sucrose concentration was used 30 g/l and the pH of the media adjusted to 5.8 before autoclaving. Cultures were incubated at 26 ± 2ºC with a 16 hour illumination of 21.8 µmol/cm^2/s provided by fluorescent tubes. The experiment was conducted with 30 explants per media of 12 different concentration and combination of hormones in MS medium. Data were collected on different characters at day 90 for multiple shooting and at day 30 for rooting of shoots. Observations on culture were carried out daily.

3. Results and Discussion

Isolated *in vivo* nodal explants were cultured on MS media supplemented with different concentrations and combinations of plant growth regulators and responses of the explants are presented in Table 1. Successful responses of explants depend on the removal of exogenous and endogenous contamination. Therefore, an attempt was made for standardizing the exact duration of time for surface sterilization of explants. In this experiment 0.1% mercuric chloride solution was used at different period of time such as 5, 6, 7, 8, 9 and 10 minutes. Among the variants of time, 90% cultures were found to be contamination free when they were treated for 7 minutes (data not shown). Using the same concentration of mercuric chloride of contamination free cultures were observed in other plants [13]. Nodal explant grew up and produced new shoot buds and leaves



Figs. 1-4 *In vitro* plant regeneration of cassava through nodal segments 1) Shoot initiation on MS + 2.0 mg/l BA + 0.1 mg/l NAA. 2) Multiple shoot formation in the same medium at 3rd subculture. 3) Root induction on half strength MS + 1.0 mg/l IBA and 4) *In vitro* raised plant in earthen pot.

within 2 weeks of culture on medium containing MS + 2.0 mg/l BA + 0.1 mg/l NAA (Fig. 1). On the other hand, in the rest of the media explants were regenerated very few numbers of shoots except the media containing MS + 2.0

mg/l BA. Media containing MS + 2.0 mg/l BA showed shoot regeneration and multiplication like medium containing MS + 2.0 mg/l BA + 0.1 mg/l NAA. Among the media component used, MS + 2.0 mg/l BA + 0.1 mg/l NAA was found to be the best for multiple shoot formation at 3rd subculture, in which 90% explants produced multiple shoots (Fig. 2). The average number of shoot per explant was 6.30 ± 1.20, the average length of shoot was 10.30 ± 0.70 cm and the average number of node per explant was 6.25 ± 0.45 in the same medium. Many authors reported that BA is the best phytohormone for shoot initiation and shoot multiplication in cassava. [14-19]. The present study showed that BA in combination with NAA performed comparatively better in terms of multiple shoot initiation, which suggests that combination of growth regulators has good impact for *In vitro* shoot proliferation of cassava. Similar result observed by many authors by using different concentration and combination of BA + NAA in other plants including [20] sweet orange, [21] apple, [22] cabbage and [23] sugarcane. Shoot elongation inhabitation, browning of explants and callusing tendency at the base of explants were observed on comparatively higher concentrations of growth regulators used in the same media. This indicates that in case of cassava increased level of phytohormones did not enhanced stimulation of shoot bud proliferation, as almost all plant species have enough levels of endogenous hormones and require only optimum level of exogenous plant growth regulators for their regeneration and multiplication. This is supported by [24] his study on *In vitro* propagation of bulbs and corms, [25] also reported that synthetic cytokinins are inhibitory at high concentration, [1] used MS medium without plant growth regulators but fortified with NAA and GA_3 in case of micropropagation of cassava variety CM 6740-7 whilst [15] found better result by using MS medium without plant growth regulators in variety TMS 98/0379 and TMS 98/0581. These might be due to the effect of genotypes and explants source, which reinforced explants by culture media and environmental conditions. Many authors reported that table sugar (sucrose) was effective supplement for increasing the number of shoots or roots and or elongating shoots or roots for *In vitro* plant propagation [26-30]. The results obtained in this study also confirmed that sugar is suitable and cheap alternative source for carbohydrate (data not shown). The rooting responses differed among the auxins used (Table 2). IBA was found responsive for root induction and 1.0 mg/l IBA was found optimum, in which 90% shoot was rooted within 15 days of culture (Fig. 3). The average number of root per shoot was 8.50 ± 0.60 and the average root length of 9.90 ± 0.90 cm were observed in this medium within 30 days of culture. Poor root response was observed in NAA and IAA supplemented media.

Table 1. Effect of different concentrations and combinations of plant growth regulators on MS media for multiple shoot formation in cassava at 90 days

Different concentrations and combinations of plant growth regulators (mg/l)	% of shoot forming explants	Average number of shoot formed/explant (Mean ± SE)	Average length of shoot/explant (cm) (Mean ± SE)	Average number of node produced/explant (Mean ± SE)
MS	40	3.20±0.60	4.50±0.60	3.20±0.30
BA				
1.0	50	3.40 ± 0.20	6.20 ± 0.30	4.60 ± 0.45
2.0	60	4.70 ± 0.90	8.10 ± 1.10	4.10 ± 0.30
3.0	40	2.60 ± 0.10	4.60 ± 0.60	3.60 ± 0.20
4.0	20	2.30 ± 0.40	3.20 ± 0.40	3.20 ± 0.40
BA + NAA				
1.0 + 0.1	60	3.90 ± 0.40	7.60.0 ± 0.90	4.90 ± 0.40
2.0 + 0.1	90	6.30 ± 1.20	10.3 ± 0.70	6.25 ± 0.45
3.0 + 0.1	50	3.40 ± 0.30	6.40 ± 0.50	4.30 ± 0.60
2.0 + 0.1	30	2.60 ± 0.50	4.40 ± 0.20	3.20 ± 0.10
BA + NAA + GA_3				
1.0 + 0.1 + 0.1	50	2.70 ± 0.90	4.80 ± 0.40	4.20 ± 0.70
2.0 + 0.1 + 0.1	60	3.50 ± 0.20	6.20 ± 0.50	4.70 ± 0.60
3.0 + 0.1 + 0.1	30	2.60 ± 0.30	4.10 ± 0.70	3.20 ± 0.20
4.0 + 0.1 + 0.1	30	2.10 ± 0.20	4.20 ± 0.60	3.30 ± 0.40

Table 2. Effect of IBA, IAA and NAA on half strength MS media in root induction of *In vitro* raised shoots of cassava at 30 days

Different concentrations and combinations of plant growth regulators (mg/l)	% of root inducing shoots (Mean)	Average number of root induced/shoot (Mean ± SE)	Average root length/shoot (cm) (Mean ± SE)
IBA			
0.5	60	5.90 ± 0.40	5.20 ± 0.75
1.0	90	8.50 ± 0.60	9.90 ± 0.60
1.5	60	5.40 ± 0.30	6.90 ± 0.45
2.0	30	4.70 ± 0.45	5.40 ± 0.50
IAA			
0.5	10	3.20±0.30	3.20±0.30
1.0	30	3.80±0.60	5.20±0.70
1.5	30	3.40±0.50	4.20±0.60
2.0	20	2.30±0.40	2.10±0.30
NAA			
0.5	40	4.30±0.45	5.60 ± 0.45
1.0	60	5.40±0.90	6.20 ± 0.30
1.5	60	4.20±0.60	5.70 ± 0.40
2.0	40	3.65±0.40	3.60 ± 0.20

4. Conclusion

In this study, IBA was found to be the best for root induction. Similar observation was made by ref. [1] in cassava. The IBA was superior to IAA or NAA for rooting of many other *In vitro* cultured plants were described by refs [20, 22, 23, 31, 32].

The superiority of IBA for rooting over other auxins has also been reported by refs [33-36]. Morphologically strong and healthy rooted plantlets of about 5.0 cm tall were taken out from the culture vessels and washed gently under running tap water to get rid of agar. The *In vitro* raised plantlets were then transferred to a small earthen pot (Fig. 4) containing a mixture of soil and compost (2:1) and acclimated following the usual procedures of shade and misted twice a day. About 80% plantlets resumed new growth within 30 days of acclimation period. The use of plant growth regulators like BA + NAA has great potential and can offer meaningful and useful results in many crops for *In vitro* shoot multiplication like cassava.

In this study, BA + NAA also appear to be the most practical and effective for better *In vitro* cassava shoot multiplication. Thus, these findings would be useful for propagation of cassava round the year.

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