RAPID PROPAGATION OF A BIODIESEL PLANT CASSAVA (*MANIHOT ESCULENTA* CRANTZ) THROUGH TISSUE CULTURE

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ABSTRACT

Cassava is an important tropical starchy root crop which is also used to acquire biodiesel. The aim of the present study was to develop an easy high frequency reproducible method for plant propagation and regeneration through plant tissue culture of cassava (*Manihot esculanta)*. Explant was initiated using Murashige and Skoog (Ms) with 3mg/L BAP and 1mg/L IBA. Adenine sulphate and glutamine were also added at the rate of 25 mg/L and 50 mg/L respectively, along with 0.6 g/L charcoal. Different concentrations (0.5-3 mg/L) of 6-Banzyl amino purine (BAP) and (0.025 and 0.5mg/L) of α-naphthaleneacetic acid (NAA) were applied for shoot elongation and multiplication. Most effective response of shoot length was obtained on medium having 1.5 mg/L BAP along with 0.025 mg/L NAA. Highly proficient root length was observed on MS in combination with 0.5 mg/L NAA.

Keywords: MS, Propagation, multiplication, biodiesel plant, Cassava.

INTRODUCTION

Manihot esculenta Crantz commonly known as cassava is a perennial shrub belongs to family Euphorbiaceae (Ian *et al.*, 2010). It is a carbohydrate rich root crop of comprehensive significance not only used for food but also in cosmetics, formation of glue, sugar, alcoholic drinks and acetone preparation. Its evaporated juice is helpful in preservation of meat and in certain table sauces. Wood of Cassava is utilized in chip and particle board (David, 2008; Christer *et al*., 2009; Deden and Herni, 2011).

Cassava is originated from South America and then it is distributed to subtropical and tropical regions of Asia and Africa (Ian *et al.*, 2010). In tropical areas the calorie resource after rice, maize, and sugarcane is the cassava shrub (Johanna, 1998). The demand of fuel is increasing day by day and to fulfill the requirement of fuel alternate sources are also considering, for this purpose biodiesel production is the alternate option (Villaluz and Corazon, 2008). Fuel ethanol is mostly prepared by corn starch, but Cassava is also considered useful for the production of biofuel The starch content of Cassava is reported to be 30%, which has gained attraction of various researchers recently. Another advantage of cassava growing is that it can be grown on infertile soil, drought and can withstand climatic variation, and it can stay on ground unharvested for longer period (Johanna, 1998).

Plant tissue culture is an inevitable technique used for growing isolated plant cells, tissues and organs under axenic conditions (*in vitro*) to regenerate and propagate entire plants. Tissue culture provides high fecundity of producing number of plants as compare to the conventional methods. Technique of plant tissue culture can be used for the production of large quantity of high quality plants instead of stem cutting multiplication which was traditionally used for Cassava plant as that process was very slow (Santana *et al*., 2009; Kwame *et al*., 2012). Here we report direct regeneration technique for cassava from nodal section. Cassava micropropagation is not done in Pakistan before and due to the fact of energy resource we are using this plant for tissue culture.

MATERIALS AND METHODS

The explants were taken from the healthy and profusely growing cassava plant at botanic garden, Center for Plant conservation, University of Karachi. The research was conducted at the tissue culture laboratory, Centre for plant conservation, University of Karachi. Sterilization was done by the thoroughly washing under running tap water with liquid detergent for 30 min. A quick dip of explants in 70% ethanol was also done in the sterilization process in order to remove surface adhered particles that may cause contamination. After that the explant was surface sterilized with 20% commercial bleach (Robin Bleach) [5.25 % Sodium hypochlorite (NaOCl) with 2-3 drops of Tween 20 for ten minutes followed by 3 times rinsing with autoclaved distilled water under laminar flow hood. After washing with sterilized distilled water the explant were initiated on MS (Toshio and Folke, 1962), 3 % sucrose solidified with 6% agar supplemented with 3mg/L BAP and 1mg/L IBA along with 25 mg/L adenine

sulphate and 50 mg/L glutamine. Charcoal was also added in the initiated medium at the concentration of 0.6 g/L. The pH of the medium was adjusted using 0.1M NaOH or 0.1M HCl, to give a final value of 5.75. The medium was sterilized by autoclaving at 15 psi for 20 minutes. For the multiplication MS basal medium with BAP (0.5, 1, 1.5, 2, 2.5, 3 mg.L^{-1}) together with NAA (0.025 mg. L^{-1}) were applied. The cultures were kept at 25 ± 1°C with 16 h light cycle of a light intensity of 2500 lux in every 24 h. Plantlets multiplied were then rooted in full strength and half strength Ms medium along with NAA.

RESULTS AND DISCUSSION

Cassava proves to be an auspicious biofuel crop and might emerge as a major alternative to diesel but like mostly vegetative crops it also has a slow rate of propagation. To produce high rate of plants in less time tissue culture method is adapted for the cultivation of important plants.

Kartha *et al*. (1974) described meristem culture in *Manihot esculenta* for the first In the present study cassava has been selected for micropropagation for its use as an alternative source of biofuel its edibility and starch . *Manihot esculenta* is described and propagated through tissue culture technique. Many scientists (Santana *et al*., 2009; Kwame *et al*., 2012) worked on cassava as low cost strategy for micropropagation, somatic embryogenesis (Jonathan *et al*., 2002; Tessy *et al*., 1999) .

Table 1. Effect of various concentrations of BAP, NAA and KIN in MS medium on *in vitro* shooting of *Manihot esculenta* Crantz.

BAP concentration mg/L	NAA concentration mg/L	KIN concentration mg/L	Shoot length (cm)
0	0	0	3.3 ± 1.68
0.5	0.25	-	8.5 ± 0.51
1	0.25	-	11.1 ± 0.73
1.5	0.25	-	13.2 ± 1.16
2	0.25	-	8.9 ± 0.99
2.5	0.25	-	9.7 ± 0.73
3	0.25	-	6.9 ± 0.85
1	-	0.5	2.1 ± 0.43
1	-	1	3.3 ± 0.26

Table 2. Effect of various Concentrations of NAA to MS medium on in vitro rooting of *Manihot esculenta* Crantz.

Medium (mg/L	Root Length (cm)
MS	3.8 ± 0.54
MS+0.25 NAA	4.5 ± 1.32
MS+0.5 NAA	7.2 ± 0.84
½MS	4.3 ± 0.68
½MS+0.25 NAA	5.2 ± 0.67
½MS+0.5 NAA	6.9 ± 0.49

Villaluz and Corazon (2008) also discussed about rapid propagation of cassava but in our study we described more comprehensive and rapid formulations without any complex addition in medium. In the Initiation medium MS with 3mg/L BAP and 1mg/L IBA along with 25mg/l adenine sulphate and 50mg/L glutamine and 0.6gm/L charcoal. Supplementation with activated charcoal had a positive effect on both differentiation and subsequent germination (Fig.1).The explants gave response in the medium along with charcoal within eight days. But Villaluz and Corazon (2008) ranges from 7.8 days to 13.5 days, after successful initiation plantlet were transferred to the multiplication MS basal medium with BAP (0.5, 1, 1.5, 2, 2.5,3 mg·L^{-1}) together with NAA (0.025 mg. L^{-1}) for the elongation and multiplication. It was noticed that MS medium having 1.5 mg/l BAP and 0.025 mg/l NAA gave the best result. (Table 1) (Fig. 2) After successful multiplication planlets were transferred to the rooting medium (Fig. 3, 4). Plantlets planted on the rooting medium gives proper well defined roots in MS medium in combination with 0.5 mg/L NAA within 15 days. (Table 2) After that plantlets were transferred to green house for acclimatization (Fig. 5). It is also noticed that plantlets survive more in sandy loam as compare to sandy loam combine with manure. But after 15-20 days plantlets growing in manure grow faster than that of without manure (Fig. 6).

Fig. 1. Initiation of Cassava.

Fig. 2. cassava at multiplication stage.

Fig. 3. cassava on rooting medium.

Fig. 4. cassava transplantation in green house.

Fig. 5. cassava in green house after 3 weeks in sandy loam soil.

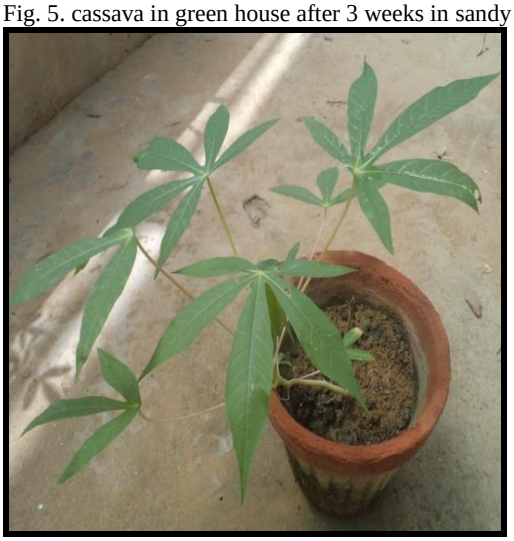

Fig. 6. Cassava after 3 weeks with sandy loam and manure.

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