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EFFECT OF GROWTH REGULATORS AND TYPE OF EXPLANTS ON CALLUS INDUCTION IN CHICKOO (*Achras sapota* L.)

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Callus induction from leaf disc and cotyledon explants of Chickoo was investigated using different plant growth regulators in different concentrations. The germination of Chickoo seeds on various media composition was also studied. The highest percentage of seeds germination was 96% with 0.8% agar + 0.3% sugar. The best callus growth was observed with 2,4-D + Kinetin, while the highest value of shoots regeneration (25%) was recorded with BAP + GA_3. Callus was induced from leaf disc and cotyledon explants. The highest callus induction (75.55%) was recorded with 3 mg/l BAP, while 35% roots formation from the micro shoots was obtained with IBA (1 mg/l) + IAA (1.5 mg/l).
Keywords: *In vitro*, micropropagation, clonal propagation, PGR, callus induction, genetic improvement.

INTRODUCTION

Achras sapota (Sapotaceae), also known as "Sapodilla" is native to tropical America and widely growing tree in tropical countries, having a rich, sweet and delicious fruit taste due to high sugar content (19-24 °Brix). Sapota fruit, 100g in weight contains 98 kcal, 73.7g of water, 0.4-0.7g proteins and 28mg calcium. It is slowly growing long lived tree but may reach 20-30 m height after many years. It has high aesthetic value and is used in landscape due to its glossy leaves and beautiful, round canopy. The fruit of sapota, a berry varies from 5-10 cm in width having oblate shape (Peiris, 2007).

A. sapota is widely grown in tropical countries due to its delicious and sugar rich taste. Lack of suitable breeding methods and long breeding cycle make this plant very uneconomical. *In vitro* techniques besides budding and air layering are being increasingly applied for clonal propagation of selected tree species to supplement conventional methods, but the success rate is very low (Mascarenhas and Muralidharan, 1989). The genetic improvement through tissue culture is emerging as an important alternative in woody plants including fruit trees (Khurana and Khurana, 1999). The regeneration system to supplement the conventional methods and produce large number of genetically uniform propagules are required in *A. sapota*. Very few *in vitro* studies on proliferating shoot cultures, callus induction and early embryogenesis of *A. sapota* are reported (Bapat and Narayanaswamy, 1977). Therefore, callus induction from different explants was worked out *in vitro* using different culture media and growth regulators.

MATERIALS AND METHODS

Seeds were extracted from fresh fruits of Chickoo and washed with sterile distilled water containing few drops of Tween-80 with 1% NaOCl. The sterilized seeds were soaked in sterile water up to 12 hours. The hard seed coat was removed mechanically under aseptic conditions and inoculated on SH-medium with (agar 0.8% w/v) in culture tubes. Leaf disc 1.5-2.0 cm were taken from *in vitro* grown plants, and cultured in test tubes containing media supplemented with different PGR concentrations.

In vitro seed germination was tested on four media combinations (Simple SH-medium, ½ SH medium, 0.8% agar + 0.3% sugar and 0.4% agar). For callus induction the SH-medium with BAP (1, 2 and 3 mg/l), GA_3 (1, 2 and 3 mg/l), BAP + NAA (1+1, 1+2 and 3+3 mg/l, respectively), 2,4-D + Kinetin (1+1, 2+2 and 3+3 mg/l, respectively). For shoot regeneration from callus BAP + GA_3 (0.5+1 mg/l, respectively), BAP + Kinetin (1+1 mg/l, respectively), GA_3 + Kinetin (1.5+1.5 mg/l, respectively) and BAP + 2,4-D (2.5+3 mg/l, respectively) and for root regeneration NAA + IBA (1+1 mg/l, respectively), NAA + IAA (1+1 mg/l, respectively), IBA + IAA (1+1.5 mg/l, respectively), IBA + NAA + Kinetin (2+2.5+2.5 mg/l, respectively). Different stages of regeneration are shown in Fig. 1. The experiment was conducted in complete randomize design (CRD) with five replications. The means were compared through Duncan's new multiple range test (DMR-test).

Figure 1. Chickoo (*Achras sapota* L.) callus induction and shoot induction from cotyledon and leaf disc explants. A-B. Seed germination, C. Leaf disc culture, D. Callus induction, E. Callus growth, F. Callus growth after sub-culture, G. Root initiation, H. Shoot induction.

RESULTS AND DISCUSSION

***In vitro germination*:** Treatment comparisons showed that 0.8% agar + 0.3% sugar medium had 42% germination within 12 days and 96% germination with complete radical growth within 30 days which proved the best for achieving the highest germination percentage *in vitro*. It was followed by medium 0.4% agar with 34% germination after 12 days of culture and 92% germination after 30 days to culture. The ½SH medium yielded 24% and 73% germination at 12 and 30 days of culture, respectively (Table 1). The results are corresponding with Mascarenhas and Muralidharan (1989). In other *in vitro* studies, seeds of Chickoo took only 15-30 days for radical emergence, and 12-15 days for germination of seeds. Freshly harvested seeds cultured after proper sterilization showed high germination (Purohit *et al*., 1998).

Table 1. *In vitro* germination of Chickoo seeds on SH-nutrient media.

Treatments	Germination (%)		
	Week 2	Week 3	Week 4
Control	21.0e	23.2e	25.0e
Simple SH-medium	28.9bc	51.0cd	70.0cd
1/2 SH-medium	42.2a	62.3c	78.0bc
0.8% agar + 0.3% sugar	34.4b	76.2a	96.3a
0.4% agar	24cd	72.7ab	92.0ab

***Callus induction from leaf disc explants*:** The results showed that the BAP produced the highest callus induction (68%) followed by GA_3 (49%) after 60 days of culture; however, control treatment (SH-medium free of growth regulators) showed just 2% of callus induction at 60th day of culture (Table 2). When BAP applied with NAA, at 7th and 45th day to culture produced 18% and 34% callus, respectively. The level of callus induction was comparatively higher than BAP at 45th day (22%). In this study 13% cultures induced callus with 3 mg/l of BAP on 7th day and 25% with 2 mg/l on 45 days; however, 3 mg/l at 60th days (39%). The similar results are also reported in previous studies (Sachdeva and Mehra, 1986; Pumchaosuan and Wongroung, 2009; Hammerschlag *et al*., 1985). In another study, the combination of leaf disc and cotyledon explants showed the best callus induction (70%) and on maturity, callus developed embryoids (Table 2).

***Callus induction from cotyledon explants*:** SH medium supplemented with the treatment BAP + NAA gave the highest callus formation from cotyledon (40%) within 60 days of culture followed by 2,4-D + Kinetin (40%) as compared to control (2%). The callus produced was of friable nature and embryoids formation was less as compared to developed from leaf disc explants. In general, the study showed that the 2 mg/l each of BAP + NAA produced better results as compare to other treatments (Table 3). The present results are in line with previous findings which showed 80% callus induction from cotyledon explants on MS medium + 2,4-D (1 mg/l) or 2,4-D (1.5 mg/l). The similar results were also reported by Dong and Jia (1991).

***Shoots regeneration*:** It was observed that portion of leaf disc which was taken from close to midrib or with midrib facing towards front showed more shoot regeneration after shifting to shooting media (Table 2). BAP + Kinetin (2.5+2 mg/l,

Table 2. Callus induction (%) from leaf disc explants of Chickoo on different growth regulators.

Levels/ Treatments	Number of days								
	7 days			45 Days			60 Days		
	1 mg/l	2 mg/l	3 mg/l	1mg/l	2mg/l	3mg/l	1mg/l	2 mg/l	3 mg/l
Without PGR	0.42g	0.58g	0.43g	0.85h	0.71h	0.87h	1.32f	2.65f	1.38f
BAP	4.08f	23.23a	23.05a	6.93g	28.88c	28.78c	57.19e	69.22a	75.55abc
GA_3	4.15f	8.76de	11.35d	32.19bc	24.24d	29.02c	36.69ab	47.22bcd	57.90ab
BAP+NAA	10.66e	19.61b	22.96ab	34.21ab	37.54a	28.84c	37.06ab	38.95a	34.43abc
2,4-D+Kinetin	15.20c	2.59fg	7.86e	28.90c	11.66f	18.87c	31.07bcd	29.40cd	25.95d

Table 3. Callus induction (%) from cotyledon explants of Chickoo on different growth regulators.

Levels/ Treatments	Number of days								
	7 days			45 Days			60 Days		
	1 mg/l	2 mg/l	3 mg/l	1mg/l	2mg/l	3mg/l	1mg/l	2 mg/l	3 mg/l
Without PGR	0.65	1.69	0.96	1.34	1.07	2.06	2.32	1.99	2.28
BAP	9.58	12.22	7.84	10.89	11.32	13.67	12.32	23.43	35.56
GA3	1.29	5.47	4.76	12.76	23.24	19.42	10.54	20.77	33.85
BAP+NAA	11.56	24.67	17.76	17.56	34.07	24.78	37.06	37.79	45.67
2,4-D+Kinetin	12.78	17.56	18.78	9.98	21.76	34.67	21.65	43.88	52.97

respectively) showed maximum (28%) shoot regeneration and considered the best for shoots regeneration in Chickoo. Multiple shoots induction from callus with 4-5 shoots per test tube was observed with BAP + Kinetin. Subsequently, BAP+ GA_3 (1.5+1 mg/l, respectively) with 25% shoot regeneration was the second best combination, which produced 2-3 shoots per test tube. Ellis *et al*. (1991) found 57% shoot regeneration from callus when treated with BAP + Kinetin (2.5+2 mg/l, respectively) while 19% with TDZ and BAP (2+2 mg/l, respectively).

Position of explant (leaf disc) of *in vitro* grown seedlings affected shoot bud formation in Chickoo cultures. The Chickoo leaf disc segments from central portion of leaves showed more shoot regeneration as compared to sides of leaves. The similar results were also found in other studies (Chakravarty and Goswami, 1999; Sachdeva and Mehra, 1986; Grewal *et al*., 2000).

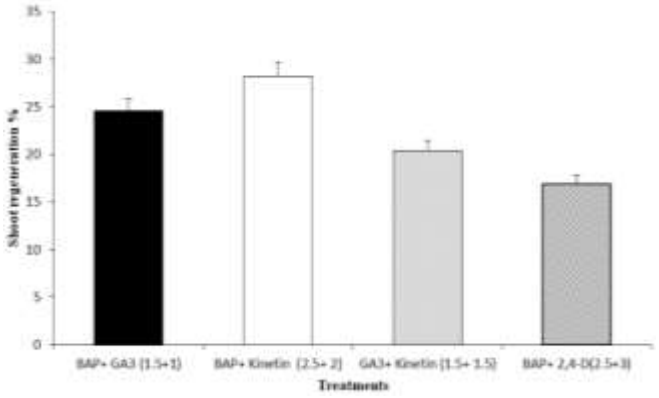

Figure 2. Effect of growth regulators on shoot regeneration from callus of Chickoo.

***Roots induction*:** First root was observed after 35 days of shifting micro-shoots (Fig. 3). IBA + IAA (1+1.5 mg/l, respectively) with 35% root induction was the best combination for root induction from micro-shoots. NAA + IBA (1+1 mg/l, respectively) with 24% root induction was the second for root induction from micro-shoots in Chickoo. In combination of NAA + IAA, just 16% root induction was recorded. Minocha (1987) reported that IBA alone or in combination with other growth regulators like NAA or IAA was the best for root induction from shoots in Chickoo. Mukhtar *et al*. (2005) observed the highest root induction with NAA and IBA (1+1.5 mg/l, respectively). In present study, 24% root induction was obtained from SH-medium + NAA + IBA (1+1 mg/l, respectively).

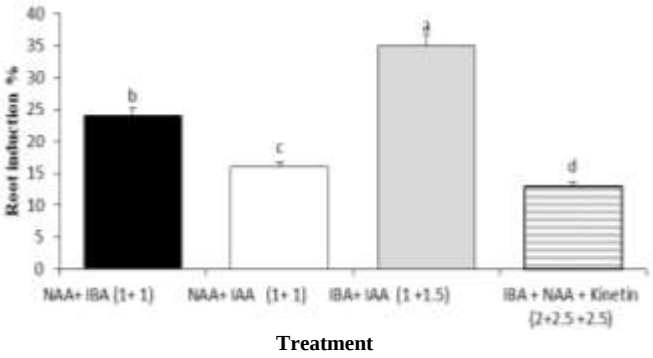

Figure 3. Root induction from microshoots of Chickoo on different growth regulators.

***Conclusion*:** The effect of plant growth regulators on callus induction of Chickoo plant was studied. Different concentrations of plant growth regulators were applied to check the germination rate, callus induction from leaf disc or cotyledons as well as shoot and root induction. The maximum callus induction from leaf disc was observed when 1 mg/l of BAP and 1 mg/l of NAA were added in MS media. The maximum callus induction from cotyledon was observed when 1 mg/l each of BAP and NAA was added in MS media. Shoot regeneration was the best observed when BAP and Kinetin were included in MS media at 2.5 and 2 mg/l, respectively, while root induction was maximum when IBA and IAA were included in MS media at 1 and 1.5 mg/L, respectively.

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