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MICROPROPAGATION OF *JATROPHA CURCAS* L.

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ABSTRACT

A cost effective procedure was devised for efficient plant propagation of *Jatropha curcus* L. Direct and indirect *in vitro* regeneration was witnessed by addition of plant growth regulator (PGR) combinations. Callus cultures were initiated from CL, CNR, HC and RT explants on MS basal medium added with 2, 4-D, BAP and NAA in individual and combined applications. Excellent growth of callus was obtained in all explants with supplementation of BAP (2.0 mg/L) along with NAA (2.0 mg/L), 2, 4-D (0.5 mg/L) being operative for callus induction in only HC explants. These calli were tested for regeneration and shoot and root organogenesis was successfully induced respectively. Hypocotyl callus generated 4.0 shoots under the influence of 0.5 mg/L IBA and 1.5 mg/L BAP while CL explant generated 3.0 shoots per callus with slight change in concentration of BAP in same PGRs mixture (0.5 mg/L IBA and 1.0 mg/L BAP). Along callus mediated organogenic route of regeneration, direct organogenesis was achieved as well. In direct shoot regeneration ten shoots were formed on CNR with 1.5 mg/L BAP and 0.5 mg/L Kin. Incorporation of 0.25 mg/L IAA to the medium containing1.5 mg/L BAP + 0.5 mg/L Kin enhanced the regeneration percentage of shoots (11 per CNR explants). Root initiation in micro-shoots was attained on MS medium (half-strength) added with 0.1 mg/L IBA. The well-grown plantlets were shifted to peat moss substratum and maintained in field conditions after acclimatization. This regeneration protocol may also support genetic improvement efforts in *J. curcas*.
Abbreviations: CNR (Cotyledonary nodal region), CL (Cotyledonary leaf), HC (Hypocotyl)

Keywords: *Jatropha curcas,* BAP, CL, CNR, HC

INTRODUCTION

Biofuels from oil-yielding crops are an important source of renewable energy. This has led to an exceptional attention in production of biofuels and to make them low-priced than the fossil fuel oils (Mukherjee *et al*., 2011). Main sources of bio-diesel comprise Rapeseed, Soybean, Sunflower, Linseed, Cottonseed, Beef Tallow, Oil Palmand and *Jatropha* (Jayasingh, 2004). Among the likely candidate crops, *J. curcas* has received attention because it has ability to acclimatize in semi-arid marginal lands, its oil can be used as a biodiesel and it provides soil erosion control. Unlike other non-edible oils obtained from tree species having a lengthy developmental period, *J. curcas* produces fruits after two years of its plantation and annually per plant can produce 1 to 2 Kg of seeds when the age of plant is 2 to 3 years. The yield may increase with plant age. When cultivated as biofuel source, it can be the source of fuel on regular basis for 40 to 45 years. Sujatha *et al.* (2005) stated that *J. curcas* could bring massive profits to human society and land as an agro-forestry crop. Bio-diesel obtained from *J. curcas* is a non-toxic, bio degradable, free of chlorine and sulphur and have caloric content equivalent to mineral oil.

The major constraint in agriculture of *J. curcas* is that its yield of nuts is uncertain, making biofuel production unsustainable (Mukherjee *et al*., 2011). *Jatropha. curcas* being cross-pollinated plant bears seeds with unknown genetic prospective. Tree species proliferated using stem cuttings display a lesser life span and retain a lower disease and drought resistance than those propagated trough seeds. According to Sujatha *et al*. (2005), trees grown using stem pieces frequently yield pseudo-tap roots; the establishment of seed is also low in vegetatively propagated plants. Vast cultivation of *J. curcas* is still vital difficulty to determine the ultimate success. On the other hand practice of seed cultivation is seasonal, in seed cultivation heterozygosity cannot ensure quantity and quality of oil content and seed may transmit diseases to the seedlings. Considering *J. curcas* as the best candidate for biofuel, the development of suitable technology for rapid multiplication of this species is essential. The conventional method of propagation through seeds and cuttings will not meet the demand. *In vitro* regeneration of *J. curcas* offers a powerful method to overcome the problem (Kalimuthu *et al.*, 2007). The current research work was initiated to devise a protocol for micropropagation of *J. curcas.*

MATERIALS AND METHODS

Seed Collection

Fresh seeds of *J. curcas* taken from Botanic Garden GCU, Lahore were used as explant source for micropropagation studies.

Seed sterilization and seed germination

Soaked seeds were de-coated; surface sterilization was achieved by rinsing seeds in 0.1% (w/v) $HgCl_2$. The de-coated and sterilized seeds were aseptically germinated in jars provided with cotton pads moistened with distilled water.

Preparation and sterilization of the media

Murashige and Skoog, (1962) basal medium augmented with dissimilar PGRs was used as artificial nutrient medium in this study. Agar was used as gelling agent. The media was sterilized through autoclave at 121°C and 15 lbs inch^{-2} for 20 minutes and solidified at room temperature.

Explant preparation, culture and culture conditions

Different parts of (*in vitro* grown) five days old seedlings were excised such as CL, CNR, HC and RT and were cultured for callus initiation. The cultures were kept at 26 ± 1ºC under a 16/8 h light/dark period with 2500 Lux/m^2/s radiation.

Regeneration through callus

Data regarding observations on callus initiation duration, number of explants producing callus, type of callus induced (friable, compact, less compact), color of callus and callus induction percentage were recorded. Callus induction was followed under the influence of auxin and cytokinin alone and in combinations. All experiments were repeated thrice.

Calli appearing on the different media combinations were sub-cultured for regeneration under the influence of different levels of BAP, IBA, TDZ and IAA. Type of response (Callus growth, regeneration), callus initiation duration and number of regenerated shoots from callus were documented, after 6 weeks of inoculation.

Multiple shoot induction

Different explants were incubated on MS medium supplemented with dissimilar levels of BAP, Kin, IBA and IAA individually and in combinations for shoot induction. The shoots obtained in the above experiments were placed on shoot elongation medium containing changed levels of concentrations of GA_3 and BAP separately and in combinations.

Root induction and hardening

Well established shoots were sub-cultured to MS medium added with auxins like IBA, IAA and NAA to optimize protocol for root formation. Root initiation duration and percentage of root formation were recorded after 5 week of culture. Shoots with induced roots were shifted to pots containing peat moss for hardening/acclimatization.

Statistical analysis

All the experiments had parallel replicates. The data obtained for various parameters were analyzed statistically. Analysis of Variance (ANOVA) was followed on the data attained. Treatment means, standard errors were calculated in Microsoft Excel. Duncan's multiple range test (Steel and Torrie, 1960) was conducted as supplementary post-hoc test at probability level 0.05%.

RESULTS

Seed sterilization and seed germination

Surface sterilization of de-coated seeds was achieved with Mercuric Chloride (0.1% w/v). De-coated seeds treated for 7 min exhibited maximum sterilization (Table 1). After four hours soaking surface sterilized de-coated seeds were used for germination. Germination was witnessed after 4 days in glass jars containing moist cotton pads. The highest germination (100%) was recorded with 3 seeds per jar on cotton pad moistened with10 ml distilled water, whereas reduced germination percentage was witnessed in jars with 7 seeds (Table 1). Among variable soaking time durations such as 2, 4, 6, 12, 18 and 24 hours, soaking duration of 4 hours exhibited the uppermost germination limit i.e. 100% in jars placed at 26ºC in growth room (Table 1).

Table 1. Effect of amount of water on seed germination.

Moisture content (ml)	Seeds per culture jar	Germination initiation (days)	Germination (%)
5	3	5±00	75
10	3	5±00	100
15	3	7±00	50
5	5	8±0.5	20
10	5	6±0.1	50
15	5	7±2	25
5	7	0±00	0
10	7	8±3	20
15	7	0±00	0

*The separate trials indicated that temperature of 26°C and seed soaking for 4 h were optimal requirements for 100% seed germination.

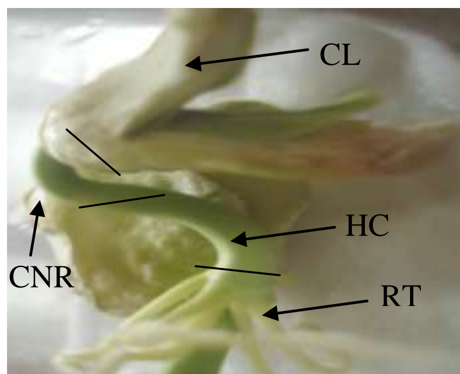

Fig. 1. Explants used for callus induction and multiple shoot induction (1X).

CL-cotyledonary leaf
CNR-cotyledonary nodal region
HC-hypocotyl
RT- root explants

Effect of various PGRs on callus induction in different explants

Plant growth regulators supplemented in MS medium described optimum medium for callus formation using different explants *viz.,* CL, CNR, HC and RT (Fig. 1). Under diverse concentrations of NAA, 2, 4-D and BAP applied individually and in combinations, in MS medium callus induction was witnessed readily in all types of tissues for all combinations. Callus initiation took place from cut surfaces of the every explant which turned into mass of callus by the 4th week. Hypocotyl explants initiated callus induction after one week of culture at cut edges. Amongst the different treatments applied, 0.5 mg/L 2, 4-D initiated callus earlier than others. The calli related to 2, 4-D concentrations were compact, globular and yellowish green to dark green in color (Fig. 2, Table 2). Callus induction in CL explants took 15 to 17 days. Among different treatments, 0.5 mg/L 2, 4-D initiated callus formation earlier (15 days) than others and the callus was mostly globular, compact and greenish (Fig. 2) but 2, 4-D for callus stimulation in CL explants was not very effective. Hypocotyl explants with BAP (2.0 mg/L) and NAA (1.0, 2.0 and 3.0 mg/L) initiated to produce callus after one week. Under the influence of BAP and NAA, the calli were globular, compact and yellowish green in color (Fig. 2). Explants such as CNR under combination of BAP (2.0 mg/L) and NAA (1.0, 2.0 and 3.0 mg/L) exhibited callus initiation after one week. The calli produced were green, compact and globular (Fig. 2). Cotyledon explants showed callus initiation after 7 days under BAP (2.0 mg/L) with NAA (1.0, 2.0 and 3.0 mg/L). Root explants produced callus with BAP + NAA after 10 days. The calli induced by combinations of NAA and BAP were globular and compact (Fig. 2). All treatments of BAP and NAA in combinations were observed to be suitable for callus induction in CL, HC, CNR and RT explants of *J. curcas*.

Response of callus types to regeneration

Calli obtained from CL, CNR, HC and RT were transferred to shoot induction medium (MS medium) added with different PGRs for regeneration. Response of different explant callus types to IBA and BAP for regeneration is presented in Table 3. Greenish, compact and globular calluses from HC and CL explants on culture media containing 2 mg/L BAP and 2 mg/L NAA were sub-cultured for shoot induction on said nutrient medium added with diverse levels of concentrations of IBA and BAP. Regeneration of shoots was achieved in HC callus with 1.5 mg/L BAP and 0.5 mg/L IBA after 6 weeks of sub-culture (Fig. 3), while CL callus exhibited shoot induction on 0.5 mg/L IBA and 1.0 mg/L BAP. Light green, compact and globular callus induced on medium containing 2 mg/L

BAP and 2 mg/L NAA was put to regeneration under the influence of diverse concentrations of TDZ and IAA (Table 3). After 25 days of sub-culture greenish calluses turned into quite green and friable but no shoot initiation was observed. At the beginning of 6th week of subculture browning and darkening of calluses consequently led towards necrosis (Fig. 3).

Table 2. Effect of 2, 4-D on callus induction in HC explants.

Type of explant	Type and conc. of PGRs (mgL^{-1})	Callus Initiation (days)	No. of explants producing callus	Callus Induction (%)	*Characteristics of callus
HC	2,4-D 0.1	7±0.5	3±00	50	Yellowish green, less compact
	0.5	6±00	6±00	100	Yellowish green, compact
	1.0	7±0.5	4±00	66.6	Yellowish green, less compact
CL	0.1	17±3	2±00	33	Dark green, compact
	0.5	15±2	3±00	50	Yellowish green, compact
	1.0	18±4	2±00	33	Dark green, compact
HC	NAA+ BAP 1+2	6±00	6±00	100	Yellowish green, Compact
	2+2	6±00	6±00	100	Yellowish green, compact
	3+2	7±00	6±00	100	Yellowish green, compact
CNR	1+2	7±00	6±00	100	Green, compact
	2+2	6±00	6±00	100	Green, compact
	3+2	7±00	6±00	100	Green, compact
CL	1+2	7±00	6±00	100	Light green, compact
	2+2	7±00	6±00	100	Light green, compact
	3+2	7±00	6±00	100	Light green, compact
RT	1+2	10±2	4±00	66.6	Green, friable
	1.5+2	11±1	6±00	100	Pale green, compact
	2+2	10±00	6±00	100	Light green, less friable
	2.5+2	10±00	6±00	100	Pale green, compact
	3+2	12±2	4±00	66.6	Pale green, compact

*All calli were globular.

Response of explant types to *in vitro* regeneration through multiple shoot induction

Different explants *viz.,* CL, CNR and HC (Fig. 4) from 5 days old *in vitro* seedlings were put to multiple shoot formation using BAP (1.0, 1.5 and 2.0 mg/L). Multiple shoot induction was witnessed only in CNR explants. Explants such as CNR exhibited shoot induction in 22 days and 25 days 1.0 mg/L and 1.5 mg/L BAP respectively (Table 4) and produced 5.0 and 3.0 shoots per explant under 1.0 mg/L and 1.5 mg/L BAP respectively (Fig. 4). Significant difference was observed among combinations and concentrations of PGRs tested for mean shoot number (Table 4). Shoot length of 1.25 cm was observed in CNR explants at 1.5 mg/L BAP, whereas 0.12 cm at 1.0 mg/L (Table 4, Fig.4). Difference was significant ($P \leq 0.05$) for mean shoot length. Kinetin, in individual application didn't show any influence on shoot proliferation in MS medium but, incorporation of BAP with Kin produced multiple shoots (Fig. 5). Culture medium, MS added with 0.5 mg/L Kin and1.0 mg/L BAP, responded earlier (21 d) to multiple shoot induction than other media with 1.5 mg/L BAP and 0.5 mg/L Kin and 2.0 mg/L BAP with 0.5 mg/L Kin (Table 4). Further, it was noted that CNR explants exhibited high number of shoots i.e.10 per explant at 1.5 mg/L BAP and 0.5 mg/L Kin as compared to other media like 1.0 mg/L BAP + 0.5 mg/L Kin (7 shoots per explant), and 2.0 mg/L BAP + 0.5 mg/L Kin (4.2 shoots). Among the levels of PGRs tested, highly significant differences ($P \leq 0.05$) were observed for mean shoot number. Maximum shoot length i.e. 1.55 cm was observed with medium containing 1.5 mg/L BAP + 0.5 mg/L Kin, whereas 1.33 cm with 1.0 mg/L BAP + 0.5 mg/L Kin, and 1.31cm with 2.0 mg/L BAP + 0.5 mg/L Kin. There were less significant differences ($P \leq 0.05$) between the levels of PGRs tested for mean shoot length (Table 4).

To determine the synergic influence of cytokinin and auxin, BAP and Kin were tested with IAA. CNR treated with media containing 1.5 mg/L BAP, 0.25 mg/L IAA and 0.5 mg/L Kin required long time (24 days) for shoot

induction initiation but with 1.0 mg/L BAP, 0.25 mg/L IAA and 0.5 mg/L Kin, shoot induction was observed in 22 days (Table 4, Fig. 6). Non-significant differences ($P \leq 0.05$) were found among the levels of PGRs tested for mean shoot formation. It was observed that CNR exhibited higher number of shoots i.e. 11 per explant at 1.5 mg/L BAP, 0.25 mg/L IAA and 0.5 mg/L Kin than 6.0 per explant at 1.0 mg/L BAP, 0.25 mg/L IAA and 0.5 mg/L Kin (Fig. 6). Significant difference ($P \leq 0.05$) was observed among PGRs tested for shoot number (Table 4). Shoot regeneration on medium containing BAP, or BAP with Kin or IAA infrequently resulted in stunted growth. Under the influence of GA_3 (0.25 – 0.75 mg/L) approximately three fold (3.6 cm) increase in shoot length was recorded in 4 weeks under 0.5 mg/L GA_3.

Table 3. Effect of BAP with IBA and TDZ with IAA on shoot induction from callus.

Primary Medium (MS + B5 vit.)		Explant used	Subculture Med. (MS + B5 vit.)		No. of shoots/ callus	Shoot length (cm)	shoot formation (%age)
PGR (mgL^{-1})	PGR (mgL^{-1})		PGR (mg L^{-1})	PGR (mg L^{-1})			
BAP 2.0	**NAA** 2.0	HC	**BAP** 1.5	**IBA** 0.5	4±0.3a	1.1±0.01a	45
2.0	2.0	CL	1.0	0.5	3±0.2a	1.0±0.02a	66
2.0	2.0		TDZ 1.25	IAA 0.5	0±00	0±00	0±00
2.0	2.0		1.5	0.5	0±00	0±00	0±00

Data was collected after 6 weeks of culture. Each value is a mean of three replicates with standard error (Mean ± S.E). Duncan' new multiple range test showed non-significant results ($P \leq 0.05$) between the concentration and combination of plant growth regulators tested for mean shoot length and %age of shoot formation.

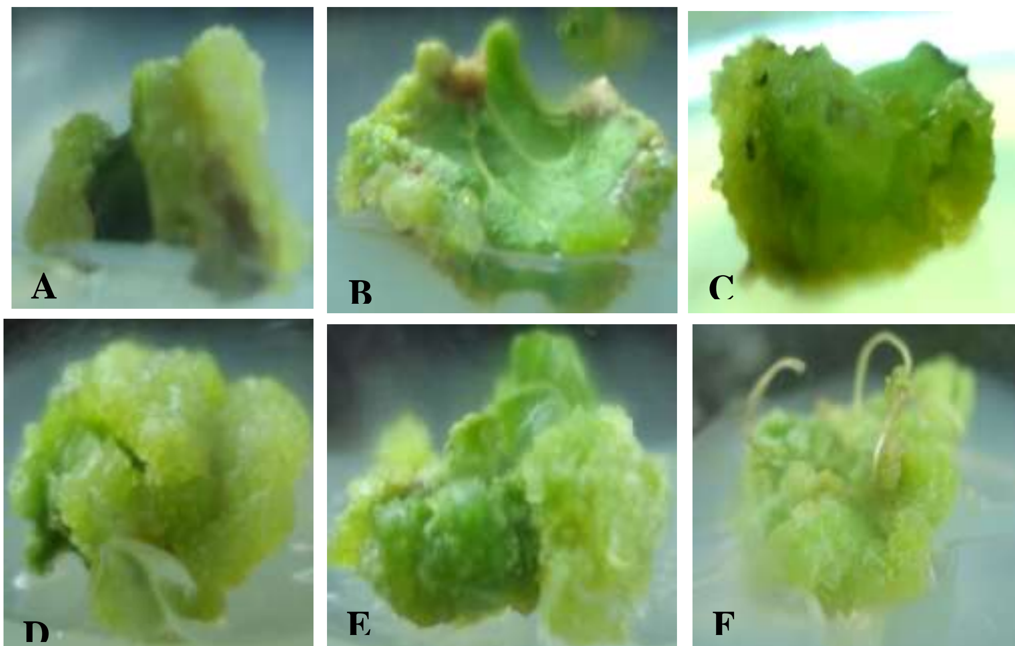

Fig. 2. Effect of PGRs on callus induction in different explants on MS medium after 7 days (1X). A) Callus induction on HC explant 0.5 mg L^{-1} 2, 4-D. B) Callus induction on CL explant 0.5 mg L^{-1} 2, 4-D. C) Callus induction on HC explants 2.0 mg L^{-1} BAP and 2.0 mg L^{-1} NAA. D) Callus induction on CNR explants 2.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA. E) Callus induction on CL explants with 2.0 mg L^{-1} BAP and 3.0 mg L^{-1} NAA. F) RT explant on MS medium supplemented with 2.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA.

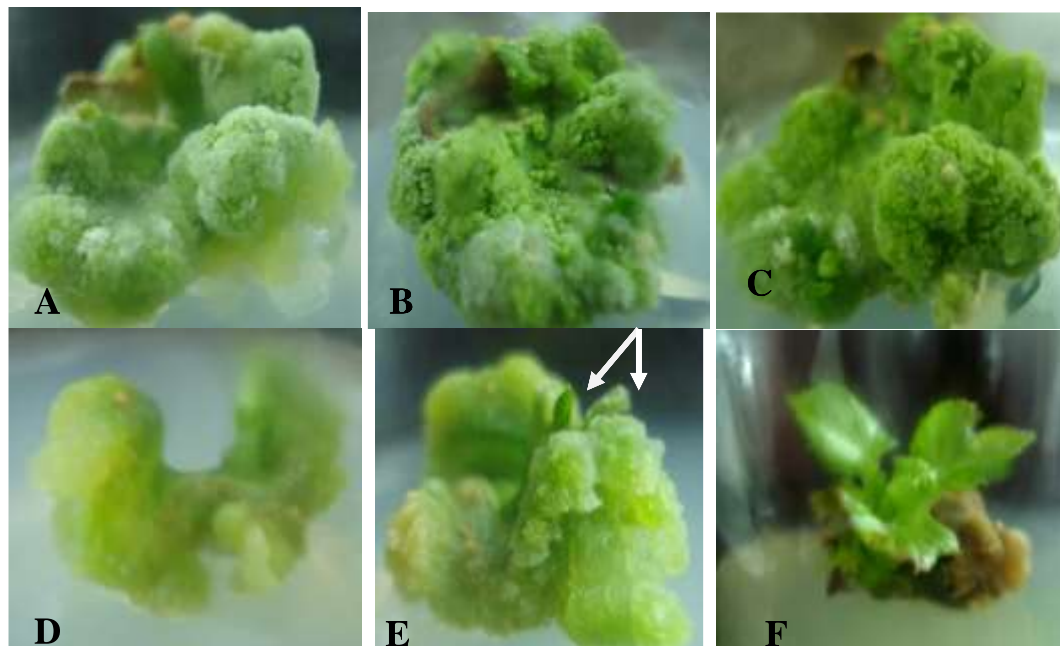

Fig. 3. Effect of TDZ with IAA and BAP with IBA on *in vitro* callus mediated regeneration from CL (A, B and C) and HC (D, E and F) explants respectively (1X). A) After 20 days of sub culture to regeneration medium (1.25 TDZ+ 0.5 IAA). B) After 30 days of sub culture to same regeneration medium. C) After 6 weeks of sub culture to same regeneration medium. D) After 2 weeks of sub culture to regeneration medium (1.5 BAP + 0.5 IBA. E) After 25 days of sub culture to same regeneration medium. F) After 6 weeks of sub culture to same regeneration medium.

Table 4. Effect of different concentrations of BAP and Kin on multiple shoot formation.

Explant type	Type and conc. of PGRs (mg L^{-1})	Shoot initiation Duration	*No. of shoot per explant	Shoot length (cm)	shoot formation (%age)
CNR	**BAP** 1.0	25±0.577a	3.0±0.145b	1.12±0.01b	45
	1.5	22±0.611a	5.0±0.166a	1.25±0.02a	66
CNR	**BAP + Kin** 1.0+0.5	21±0.585a	7.0±0.577b	1.33±0.03b	66
	1.5+0.5	23±0.577a	10±0.577a	1.55±0.028a	83
	2.0+0.5	23±0.577a	4.2±0.145c	1.31±0.044b	33
CNR	**BAP+IAA + Kin** 1.0+0.25+0.5	22±0.66a	6.0±0.44b	1.54±0.029a	69
	1.5+0.25+0.5	24±0.60a	11±0.88a	1.43±0.03b	80

* Data was collected after 6 weeks of culture. Each value is a mean of three replicates with standard error (Mean ± S.E). Mean with different letters (a,b,c) are significantly different from each other at 5% probability level by Duncan' new multiple range test. The results of ANOVA showed significant differences ($P \leq 0.05$) between the concentration and combination of plant growth regulators tested for mean shoot length.

Fig. 4. Multiple shoot induction on CNR explants using different concentrations of BAP after 6 weeks (1X). A) MS medium supplemented with BAP (1.0 mg L^{-1}). B) MS medium supplemented with BAP (1.5 mg L^{-1})

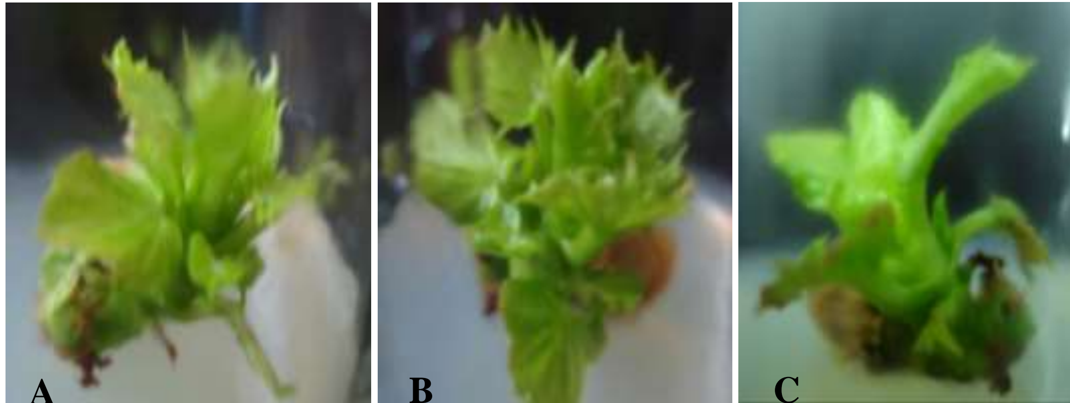

Fig. 5. Multiple shoot induction on CNR explants using different concentrations of BAP and Kin after 6 weeks (1X). A) MS medium supplemented with BAP (1.0 mg L^{-1}) and Kin (0.5 mg L^{-1}). B) MS medium supplemented with BAP (1.5 mg L^{-1}) and Kin (0.5 mg L^{-1}). C) MS medium supplemented with BAP (2.0 mg L^{-1}) and Kin (0.5 mg L^{-1})

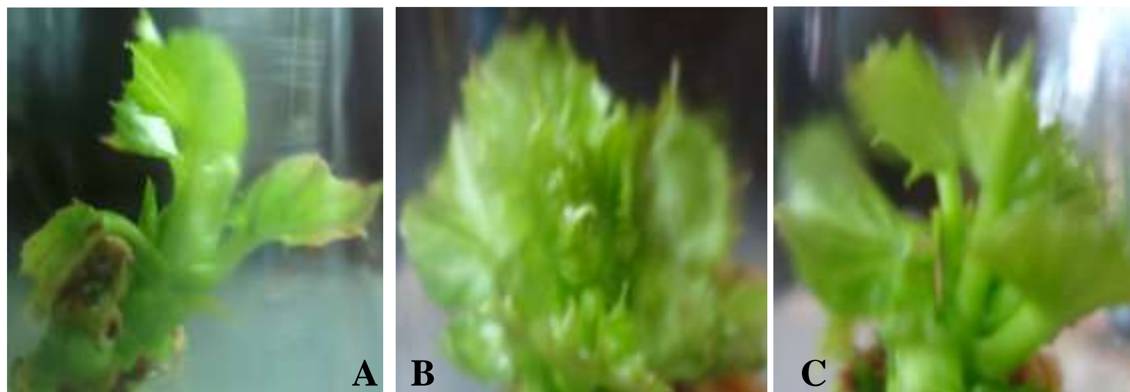

Fig. 6. Multiple shoot induction on CNR explants using different concentrations of BAP, IAA and Kin after 6 weeks (1X). A) MS medium supplemented with BAP (1.0 mg L^{-1}), IAA (0.25) and Kin (0.5 mg L^{-1}) **B)** MS medium supplemented with BAP (1.5 mg L^{-1}), IAA (0.25) and Kin (0.5 mg L^{-1}). C) Shoot elongation on MS medium supplemented with 0.5 mg L^{-1} GA_3, culture of 4 week after subculture (1X).

Table 5. Effect of IBA in ½ MS basal medium on rooting of *in vitro* shoots after 5 weeks of culture.

Conc. of IBA (mg L^{-1})	*Root initiation Duration	Root formation (%age)
0.1	16	70
0.25	NR	NR
0.5	NR	NR

NR= No Response

Fig. 7. Emergence of roots from regenerated shoot on root induction medium ½ MS + IBA 0.1 mgL^{-1} (1X).

Root induction in *in vitro* shoots and acclimatization of plantlets

The individual shoots from CNR explants from initial or subsequent cultures were subjected to root induction in MS media (full and half strength) with diverse concentrations of IBA. The root induction was inadequate in full strength MS medium containing variable concentrations of IBA. Shoots placed on MS medium (half strength) added with 0.1 mg/L IBA developed 70% roots taking 5 weeks (Table 5 and Fig. 7). MS medium lacking auxins didn't

support *in vitro* shoots to develop roots. The hardening of the *in vitro* plantlets was accomplished with survival percentage of 90%.

DISCUSSION

As the seeds of *J. curcas* exhibited poor response to germination, seeds were de-coated. Contamination reduced to minimum level when de-coated seeds were immersed in 0.1% (w/v) $HgCl_2$ solution as described by Kalimuthu *et al*. (2007). During surface sterilization of plant tissues requires disinfection of tissues with reduced cellular (Conger, 1987). The present study required use of $HgCl_2$ for seed sterilization to evade contaminants from *in vitro* cultures of *J. curcus*. The significance of removing the seed coats was early and efficient germination as described in *Embelia ribes* (Annapurna and Rathore, 2010). Seed soaking in the present study correlated to positive effects of soaking treatment in breaching dormancy and promoting germination (percentage) in different species by various studies (Brits *et al*., 1995; Thirunavoukkarasu and Saxena, 2002).

Formation of callus containing high morphogenetic potential is an initial step in tissue culture of any plant species for micropropagation. Among a number of PGR combinations tested for callus formation use of 0.5 mg/L 2, 4-D was more operative for callus formation in HC explants but was not effective in leaf explant. Callus was hard, compact and yellow green in HC explants. Kim *et al.* (2005) described off-white, yellowish and loose calluses in *Catharanthus roseus* with 2, 4-D. Though, it has been described that 2, 4-D is the frequently used auxin in tissue culture of cereals (Bhaskaran and Smith, 1990; Soomro *et al.,* 1993), other auxins at low concentrations with cytokinin were found to be more active for callus induction (Zouine and Hdrami, 2004; Datta and Conger, 1999; Pathirana and Eason 2006). Cultures required to be subcultured at an interval of two weeks at the beginning; otherwise the calli turned brown and dead. The browning of callus was perhaps due to phenolic compounds produced by explants (Monacilli *et al*., 1995). It was observed that a combination of BAP (2.0 mg/L) and NAA (1.0-3.0 mg/L) successfully induced callus on various explants of *J. curcas*. Present study established that NAA together with BAP was essential for induction of callus as described by Rajore and Batra (2007). As for as callus mediated regeneration of plantlets is concerned, only positive results were achieved via HC and CL calluses under the influence of BAP and IBA.

Production of plantlets with lower probability of genetic variations can be through direct plant regeneration. CNR explants derived from seeds were found to be ideal owing to their extensive proliferative ability. The effect of BAP to stimulate shoot multiplication was described for many plant species (Khalafalla and Hattori, 1999; Faisal *et al*., 2006). Ability of shoot bud to revive growth is directed by diverse internal factors (Ovecka *et al*., 2000). Kim *et al*. (2001) proposed that the competence of explants for shoot formation is associated to the levels of endogenous auxins and cytokinins and that the different riposte to different cytokinin may be attributed to the structural or chemical differences. In current study, MS medium with BAP and Kin was observed to be the most suitable treatment for multiple shoot induction and suggested that BAP combined with other cytokinin or auxin plays a significant role in revival of organized growth. It was observed that Kin individually was unable to initiate shoot at lower concentrations. The differential response of cytokinins, i.e. BAP and Kin, may be ascribed to levels of endogenous growth regulators, differences in uptake of PGRs and recognition by cells. Presence of Kin (0.5 mg/L) was found significant to increase shoot proliferation whereas increase in levels (2.5 and 3 mg/L) of BAP repressed shoot propagation as reported by Singh *et al*. (2010). In *J. curcas* BAP enhanced shoot formation from petiole and HC explants than Kin (Sujatha and Mukta, 1996). The positive effect of BAP on the morphogenic capability of the explants has also been described in *Euphorbia peplus* (Tideman and Hawker, 1982), *E. hirta* (Baburaj *et al*., 1987) and castor (Sarvesh *et al.,* 1992; Sujatha *et al*., 2008). The synergic effect of auxin and cytokinin was followed by applying the most responsive cytokinin combination i.e. BAP and Kin along with IAA. Addition of IAA (0.25 mg/L) with BAP (1.5 mg/L) and Kin (0.5 mg/L) improved shoot formation capability of CNR explant. The other concentrations of BAP, Kin and IAA i.e. 1.0, 0.5 and 0.25 mg/L, respectively were also found effective. The improvement of collective effect of Kin and IAA has also been described in CNR of Cashew (Philip, 1984). The medium, however supplemented with higher concentrations of IAA (0.5-1.0 mg/L) revealed no positive effect on shoot induction but callus induction occurred like already described results on many woody plant species (Xie and Hong, 2001; Nayak *et al*., 2007; Behera *et al*., 2008). It appears a common finding that cytokinin and auxin were found vital for shoot proliferation. The former studies reported that many types of explants of *J. curcas* retorted to these PGRs positively for callus induction, somatic embryos, shoots and roots (Sujatha and Mukta, 1996; Jyoti-Sardana *et al*., 2000). In the current study a lower level of Kin and IAA with an increasing concentration of BAP facilitated to produce better results for shoot induction. The increased concentrations of auxins normally hinder morphogenic responses and replacement with suitable auxin-cytokinin ratio is necessary to induce shoot and root primordia.

In present work, GA_3 and BAP resulted in increase in shoot length. The stimulated effect of GA_3 on shoot length has been reported (Sugla *et al*., 2007). GA_3 is assumed to rouse shoot length terminating the actions of auxins in meristematic regions (Taiz and Zeiger, 1998). Salts in nutrient medium have a vivid effect on root induction percentage and root number (Ohyama, 1970) in *in vitro* root induction. In the present investigation root induction response was meager in original strength of nutrient medium; however reducing the salt strength to half and addition of IBA improved root formation frequency. The advantage of IBA above other auxins in root induction to *in vitro* shoots was described in many tree plant species comprising *J. curcas* (Sujata and Mukta, 1996; Sujata *et al*., 2005; Thirunavoukkarasu *et al*., 2007). In current study higher levels of IBA caused failure in root induction. Differing to these results, Ndoye *et al*. (2003) described high root induction response in *Balnites aegyptiaca* at 49 µM IBA concentration. This can be described in perspective of basis of shoot initiation and influence of endogenous concentration of auxins at root induction time.

Conclusion

This study concluded that CNR explant is appropriate for clonal propagation of *J. curcas* thus it may be used for achievement of proficient shoot multiplication. This efficient and reliable direct plant propagation system can be subjugated for genetic transformation.

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