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RESEARCH ARTICLE

IN VITRO PROPAGATION OF *Jatropha curcas* L. GROWING IN DRY LAND AREA

[1]Ravi Mahalakshmi, [*,1]Palanisami Eganathan and [2]Ajay Kumar Parida

[1]Plant Tissue Culture and Bioprospecting Laboratory, M. S. Swaminathan Research Foundation, 3rd Cross Road, Institutional Area, Taramani, Chennai- 600113, India
[2]Plant Molecular Biology Laboratory, M. S. Swaminathan Research Foundation, 3rd Cross Road, Institutional Area, Taramani, Chennai- 600113, India

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ABSTRACT

An *in vitro* propagation protocol was developed for large scale production of *Jatropha curcas*. Uninodal explants in Murashige and Skoog (MS) medium containing 1.33 µM Benzyl Aminopurine (BA) and 0.29 µM Gibberellic Acid (GA_3) was achieved shoot induction. *In vitro* shoots in MS medium combination of 7.36 µM Indole Butyric Acid (IBA) and 9.29 µM Kinetin (KN) was obtained multiple shoots. *In vitro* raised shoots were rooted in half strength MS medium supplemented with 1.34 µM Naphthalene Acetic Acid (NAA). Survival percentage of rooted plantlets was recorded in the growth chamber (92.2) and mist chamber (85.4).

INTRODUCTION

Jatropha curcas L. (physic nut) is a plant of Latin American origin, which is widespread throughout arid and semiarid tropical regions (Holm *et al*., 1979). *J. curcas* is a well known non-edible oil yield plant of Euphorbiaceae family and growing in wide range of agroclimatic conditions. *Jatropha curcas* is a suitable species for agroforestry programme, flood control, reducing nutrient leaching, controlling soil erosion and shifting of sand dune (Shrivastava and Banerjee, 2008). Extensive i*n vitro* propagation of *Jatropha curcas* was reported to develop plantlets using various explants (Pierik, 1991; Sujatha and Dhingra, 1993; Sujatha and Mukta, 1996; Sardana *et al*., 1998; Sardana *et al*., 2000; Rajore and Batra, 2005; Datta *et al*., 2007; Kalimuthu *et al*., 2007; Shrivastava and Banerjee, 2008; Dubey *et al*., 2010; Kumar *et al*., 2011; Biradar *et al*., 2012; Maharana *et al*., 2012; Shukla *et al*., 2013; Nahar *et al*., 2013). Although several tissue culture protocols are available, studies on *Jatropha curcas* growing in dry land areas have not yet attempted till date. The present study used uninodal explant from dry land accession of *Jatropha curcas* for rapid *in vitro* propagation for establishing of seed production orchards.

Corresponding author: Palanisami Eganathan
Plant Tissue Culture and Bioprospecting Laboratory, M. S. Swaminathan Research Foundation, 3rd Cross Road, Institutional Area, Taramani, Chennai- 600113, India

MATERIAL AND METHODS

Culture Initiation

Seeds of *Jatropha curcas* were collected from MSSRF-019 (IC567002) accession from *Jatropha* genetic garden, Kudankulam, Tiruvelveli District, Tamil Nadu, India, and soaked with DDH_2O (double distilled water) overnight at 25°C under dark condition. Later the seeds were treated with *Trichoderma viride*, sown in garden soil and regular irrigation provided. Nodal explants were collected from 3 months old seedling, and kept under running tap water for 20 minutes to remove exudates and subsequently rinsed with DDH_2O 5 times. A few drops of Tween-20 was added and rinsed for 5 minutes with DDH_2O and five times with DDH_2O.

Medium and culture

MS medium (Murashige and Skoog, 1962) supplemented with various concentrations of BA (0.44 - 4.44 µM) and GA_3 (0.29-0.58 µM) along with 30 g sucrose per liter, 100-300 mg/l Polyvinylpyrrolidone (PVP) and 0.8% agar (Qualigens Fine Chemicals, Mumbai, India) was used for experiments. The pH of the medium was adjusted to 5.8 and medium sterilized at 121ºC for 15 minutes uniformly distributed to test tubes. The uninodal explants were surface sterilized using 0.1% (w/v) $HgCl_2$ for 5 minutes, and subsequently washed with DDH_2O for 5 times under laminar air flow. The explants were trimmed

at both ends and inoculated vertically on medium. The cultures were incubated in a culture room at 27±2ºC and 80% relative humidity, and photoperiod of 16 h provided by cool white fluorescent tubes having a light intensity of 2000 lux. The newly developed shoots were transferred to fresh medium after 25 days for shoot multiplication with IBA and KN combination. Elongated *in vitro* shoots were transferred to ½MS medium, supplemented with various concentrations of NAA (0.27-13.43 µM) for root induction. Rooted plantlets were hardened in a growth chamber for 25 days at 26±1ºC, 80% relative humidity and irrigated with half strength MS salts. After 25 days, the plantlets were transferred to poly bags, mixed with organic manure and garden soil (1:3) and kept at mist chamber for two months hardening at 28±2ºC and 60% relative humidity. The experiments were repeated three times for ensuring consistency. Each combination was conducted three independent replicate and calculated mean value ± standard deviation.

RESULTS

Shoot initiation and multiplication

Initiation of shoots started from the eighth day of inoculation and maximum growth reached to 4.34 cm after 25 days (Table 1). Supplementation of plant growth regulators were BA (1.33µM), GA_3 (0.29µM) and 100 mg/l PVP in MS medium gave rise to better shoot induction and development (Figure 1A). *In vitro* shoots were sub cultured in MS medium containing 0.49 µM-9.8 µM IBA, 0.46-11.61 µM KN gave rise to rapid multiple shoots after 20 days (Figure 1B) and multiple shoot data presented in Table 2.

Table 1. Mean shoot induction and shoot length of *Jatropha curcas* using uninodal explants

BA µM	GA_3 µM	PVP mg/l	Shoot length (cm)*	Shoot induction (%)*
0.44	0.29	100	2.83±0.21	63.15±0.42
0.88	0.58	200	3.12±0.11	75.88±0.58
1.33	0.29	100	4.34±0.15	80.12±0.32
1.78	0.58	200	3.29±0.13	75.66±0.47
2.22	0.29	300	2.64±0.12	66.72±0.35
2.66	0.58	100	1.82±0.08	52.14±0.66
3.11	0.29	200	1.68±0.10	49.25±0.75
3.55	0.58	300	2.14±0.07	25.13±0.84
3.99	0.29	100	1.78±0.11	29.46±0.49
4.44	0.58	200	2.32±0.12	30.18±0.61

* Mean value of three independent replicates (mean ± SD)

Table 2. Mean number of shoot and shoot length of *Jatropha curcas* using *in vitro* shoots

Hormone concentration (µM)		Mean number of shoot and shoot length*	
IBA	KN	Number of shoots	Shoot length (cm)
0.49	0.46	3.23±0.12	1.35±0.08
0.99	0.93	2.37±0.20	1.28±0.10
1.47	1.39	5.33±0.13	1.67±0.14
2	1.86	5.62±0.18	1.56±0.09
2.46	2.32	4.14±0.10	1.36±0.05
2.95	2.79	2.26±0.09	1.74±0.11
3.44	3.25	5.34±0.12	1.53±0.10
3.94	3.72	4.23±0.08	1.86±0.12
4.43	4.18	5.17±0.13	2.12±0.07
4.9	4.65	5.54±0.17	3.14±0.13
7.36	9.29	9.36±0.20	3.68±0.24
9.8	11.61	4.27±0.18	1.42±0.11

* Mean value of three independent replicates (mean ± SD)

Rooting of *in vitro* shoots

The shoots inoculated in a plant growth regulators range (0.27µM -13.43µM NAA) responded efficiently between 25 - 30 days, among which the best response was observed on NAA (1.34µM) in terms of eight roots on an average with mean root length of 2.95±0.13 (cm) per shoot (Figure1C) and rooting of *in vitro* shoots presented in Table 3. The present result observed that survival percentage of rooted plantlets in the growth chamber (92.32±0.26%) and mist chamber (88.77±0.19%) (Figure 1D).

Table 3. Mean roots and survival percentage of *Jatropha curcas*

NAA µM	Number of roots*	Root length (cm)*	Percentage of rooting*	Survival percentage (Growth chamber)*	Survival percentage (Mist chamber)*
0.27	1.12±0.08	0.25±0.06	41.32±0.15	60.78±0.26	52.68±0.19
0.54	2.14±0.06	0.24±0.08	52.34±0.13	67.14±0.34	51.24±0.26
0.81	2.15±0.10	0.47±0.09	46.01±0.25	72.12±0.29	63.15±0.34
1.07	4.78±0.13	1.47±0.10	64.32±0.34	86.78±0.32	78.43±0.23
1.34	8.12±0.21	2.95±0.13	76.23±0.28	90.14±0.31	88.77±0.19
1.61	5.23±0.13	2.37±0.12	83.04±0.31	92.32±0.26	86.22±0.25
1.88	2.18±0.11	1.35±0.14	62.13±0.16	84.77±0.21	83.77±0.38
2.15	3.26±0.10	1.24±0.11	58.06±0.24	80.64±0.34	70.24±0.42
2.42	4.16±0.07	1.05±0.08	67.12±0.33	76.24±0.28	70.19±0.31
2.69	4.27±0.12	2.04±0.12	78.16±0.26	66.54±0.35	60.77±0.23
4.03	1.84±0.16	0.35±0.08	45.23±0.24	65.42±0.26	56.12±0.17
5.37	2.46±0.13	1.08±0.13	49.27±0.31	60.84±0.33	50.72±0.29
6.71	1.13±0.10	0.54±0.07	38.64±0.35	58.12±0.28	54.14±0.35
8.06	3.24±0.12	0.93±0.13	42.86±0.28	50.37±0.31	51.98±0.37
9.04	2.46±0.21	1.06±0.10	53.97±0.34	51.22±0.29	49.75±0.25
10.74	1.41±0.15	0.78±0.12	44.85±0.29	66.77±0.38	40.76±0.34
12.08	2.63±0.11	0.42±0.06	36.83±0.31	40.88±0.42	30.18±0.26
13.43	2.46±0.13	0.38±0.05	39.16±0.27	30.94±0.37	28.12±0.21

* Mean value of three independent replicates (mean ± SD)

Figure 1. Direct organogenesis of *J. curcas* from uninodal explants. A: Shoot initiation and development from uninodal explants, B: Multiple shoots from *in vitro* shoots, C Primary and secondary roots developed from shoots, D: Plants hardened in the growth chamber

DISCUSSION

Micropropagation protocol for *Jatropha curcas* described by Kumar and Reddy (2010) on MS medium using KN (10 µM), BA (4.5 µM) and NAA (5.5 µM) for shoot proliferation, reported that they achieved 10 shoots, rooting with NAA (5.5 µM). The role of BA in *Jatropha curcas* has been reported by Shamsiah *et al*. (2010) and Mve *et al*. (2013). Singh *et al*.

(2010) observed that BA and KN showed 10-15 bud breaks per explants. A maximum 24 shoots were obtained using BA, TDZ and KN combination (Sujatha *et al*., 2005). The present study obtained 4 shoots per *in vitro* explants with BA, GA_3 and PVP and all shoots are uniform of growth. Adding 100 to 300 mg/l of PVP to medium was found to better control browning of *J. curcas* and also enhance growth of shoots compared to control. This is similar to the findings of guava (Amin and Jaiswal, 1988), *Tectona grandis* (Gupta *et al*., 1980) and *Cleistanthus collinus* (Quraishi and Mishra, 1998). Out of the ranges of plant growth regulators tested, for root induction from *in vitro* shoots of *J. curcas*, MS medium supplemented with 1.34 µM NAA was found to yield the best results for rooting. Rooting of *in vitro* shoots achieved by Kumar and Reddy (2010) in MS medium with a combination of NAA (5.5 µM), 2% sucrose, 15 µM IAA showed higher percentage of survival. Sujatha *et al*. (2005) reported that 5.4 µM NAA induces rooting in *Jatropha curcas* and rooting response was least or almost negligible on NAA (Rajore and Batra, 2005). The present experiment showed that the survival percentage of rooted plants both in the growth chamber (92.32) and mist chamber (88.77) was high. Similarly, others reported *in vitro* survival rate of *Jatropha curcas* as 100% (Shrivastava and Banerjee, 2008), 98% (Mve *et al*., 2013; Purkayastha *et al*., 2010), 90% (Kumar and Reddy, 2010) and 87% (Datta *et al*., 2007).

Conclusion

In vitro propagation of *Jatropha curcas* growing under dry land condition can open up opportunities for rapid propagation and establishment of seed production orchards aiding in large scale cultivation of the species.

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