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Full Length Research Paper

In vitro regeneration from different ages of petioles of physic nut (*Jatropha curcas* L.)

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***Jatropha curcas* L. is an important non-edible oil yielding plant growing in wasteland and dry lands. The experiment was conducted to study the plant regeneration of *J. curcas* from different ages of petioles. Petioles explant grown on Murashige and Skoog's (MS) medium supplemented with 1 mg/l α-naphthalene acetic acid and 0.2 mg/l kinetin showed the highest frequency of callus induction and the same medium was found to be best suited for callus growth in 1st petiole. Percent of somatic embryo formation was higher (35.16%) from the 1st petiole explant on 0.5 mg/l thidiazuron with 0.4 mg/l gibberellic acid. MS medium supplemented with 1 mg/l N^6-benzylaminopurine, 0.5 mg/l indole-3-acetic acid, 0.25 mg/l kinetin recorded the higher somatic embryo germination percentage (66.85%). MS basal medium supplemented with 0.5 mg/l thidiazuron, 0.1 mg/l N^6-benzylaminopurine, 0.4 mg/l gibberellic acid was found to be best medium for shoot elongation. Among the auxins tested, the higher frequency of root formation was observed in MS medium supplemented with 0.3 mg/l α-naphthalene acetic acid compared to indole-3-butyric acid and indole-3-acetic acid. The survival rate of *in vitro* rooted plantlets was dependent on the type of explant source and it varies from 47 to 60%.**

Key words: Euphorbiaceae, petiole, callus, indirect organogenesis, biodiesel, plant growth regulator.

INTRODUCTION

Jatropha curcas or physic nut is widely grown across the world as a biodiesel crop. It is a native of Central and South America and is now grown worldwide on waste-lands in the semi-arid tropics (Fairless, 2007; Achten et al., 2008). The most common *Jatropha* species are *J. curcas*, *J. integerrima*, *J. multifida*, *J. gossypifolia* and *J. podagrica* (Ye et al., 2009). *J. curcas* is a drought tolerant plant, growing in the marginal lands (Kheira and Atta, 2009) and as a hedge plant are not browsed by animals (Reinhard and Henning, 2004). The plant can survive more than 50 years (Takeda, 1982). The different parts of the plant like shoot, stem, leaves fruit, seed, bark and latex are used for the treatment of various diseases (Ginwal et al., 2005). Hence, *in vitro* direct shoot bud induction was tried in *J. curcas* from petiole (Singh et al., 2010; Kumar and Reddy, 2010), leaf (Sujatha et al., 2005; Kaul et al., 2010; Deore and Johnson, 2008; Misra et al., 2010) and nodal explants (Shrivastava and Banerjee, 2008; Datta et al., 2007; Rajore and Batra, 2005; Kalimuthu et al., 2007; Dubey et al., 2010).

Somatic embryogenesis studies have been carried out in *J. curcas* using different explants viz leaf (Jha et al., 2007; Deore and Johnson, 2008; Misra et al., 2010; Kaul et al., 2010; Sujatha et al., 2005; Rajore and Batra, 2007;

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Abbreviations: BA, N^6-Benzylaminopurine; **GA$_3$,** gibberellic acid; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid; **KN,** kinetin; **MS,** Murashige and Skoog (1962); **NAA,** α-naphthalene acetic acid; **TDZ,** thidiazuron.

Kumar et al., 2011), epicotyl (Wei et al., 2004), stem (Singh et al., 2010), axillary node (Sujatha et al., 2005 Shrivastava and Banerjee, 2008), embryo (Varshney and Johnson, 2010; Joshi et al., 2011), petiole (Kumar and Reddy, 2010; Rajore and Batra, 2007; Datta et al., 2007; Lu et al., 2003, Dubey et al., 2010), cotyledon disc (Khemkladngoen et al., 2011), *Agrobacterium* transformation (Purkayastha et al., 2010; Trivedi et al., 2010). Vegetative propagation studies were reported in *J. curcas* (Camellia et al., 2009; Kochhar et al., 2005, 2008; Kathiravan et al., 2009; Dhillion et al., 2009). Vegetative propagation of stem cuttings is not sufficient to meet the growing demand of *J. curcas* (Heller, 1996; Openshaw, 2000).

The oil content in the seed ranges from 30 to 50% by weight and the kernel contains 45 to 60% (Senthilkumar et al., 2003). The fruit ripening in *J. curcas* is not uniform in individual trees and in branches (Silip and Tambunam, 2008). The seed germination rate of *J. curcas* varies from 10 to 95% (Islam et al., 2009) and the seed dormancy is a major problem limiting crop productivity (Joker and Jepsen, 2003; Holmes et al., 1987; Verma and Gaur, 2009; Gutterman, 1980; Kobilke, 1989). There is a wide range of variation in seed and oil yield (Pant et al., 2006; Jha et al., 2007). The unreliable flowering, fruit and seed set in *J. curcas* are also important reasons for low productivity (Kochhar, 2005).

The review of literatures on tissue culture of *J. curcas* showed that limited attempts have been made using petiole explants for plant regeneration. The *in vitro* plants derived from petioles explants were found to be more resistant to genetic variation (Pierik, 1991). Therefore, the objective of the present study was to establish a viable protocol for *J. curcas* and to compare the regeneration potential in different ages of petiole. There are no reports available on the regeneration potential of different ages of petiole in *J. curcas* till date. Hence, the present study was undertaken to identify a suitable source of explants to maximize production of plantlets through tissue culture is important in the present scenario.

MATERIALS AND METHODS

Explant preparation and experiment al condition

The petioles starting from the tip 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th were collected from 150 days old seedling. The petiole was kept under running tap water for 20 min, followed by soaking in Tween-20 for 5 min and thoroughly washed 3 times using sterilized distilled water. Explants were surface sterilized with 4% sodium hypochlorite for 2 min and thoroughly washed 3 times with sterilize distilled water. Explants were rinsed with 70% ethanol for 5 min and thoroughly washed 3 times with sterilized distilled water. Further, the petioles were surface sterilized with 0.1% mercuric chloride for 5 min and thoroughly washed 5 times with sterilized distilled water. pH of the MS medium (Murashige and Skoog, 1962) was adjusted to 5.8 with 0.1 N NaOH and 0.1 N HCl before adding gelling agent viz., 0.25% phytagel (Sigma). The cultures were incubated at 25±1°C with a photoperiod of 16 h light at 3000 lux light intensity of cool white fluorescent light and 8 h dark condition.

Callus induction and growth

A total of 25 explants were used per treatment and these were replicated three times. The petioles were cultured in Murashige and Skoog's (MS) medium supplemented with α naphthalene acetic acid (NAA) and kinetin (KN) ranged from 0.2- 2.0 mg/l NAA and 0.2 mg/l KN in test tubes and incubated under dark condition for callus induction. 15 days old calli from the petiole were separated and sub-cultured in the same medium for callus growth at 20 days interval.

Somatic embryogenesis

Fifty-five (55) days old embryogenic callus were transferred to MS medium supplemented with 0.1-1.0 mg/l thidiazuron (TDZ) and 0.1-0.9 mg/l GA_3 for somatic embryos induction. The somatic embryos formed were transferred to MS medium containing various concentrations of 0.2-1.8 mg/l N^6-benzylaminopurine (BA), 0.1- 0.9 mg/l indole-3-acetic acid (IAA) and 0.1-0.45 mg/l KN for germination. The somatic embryos were cultured subsequently in MS basal medium supplemented with 0.1-1.0 mg/l TDZ, 0.02- 0.20 mg/l BA and 0.1-0.9 mg/l gibberellic acid (GA_3) for further growth and elongation of somatic embryos.

Rooting

The elongated shoots were transferred to MS medium with 0.1- 0.5 mg/l indole-3-butyric acid (IBA), 0.10- 0.40 mg/l NAA and 0.1-0.5 mg/l IAA for root induction.

Hardening

Rooted plantlets were washed gently under running tap water for few minutes. Then the rooted plantlets were transferred to sterile vermiculite pots. After 4 weeks, the rooted *in vitro* plantlets were acclimatized under controlled environment chamber (NK System LP-1PH) with 75% RH, 25°C and 13 h daylight conditions. The well acclimatized rooted plantlets were transferred to a mist chamber and subsequently to normal garden soil after five weeks.

Data analysis

The results were subjected to analysis of variance and significance test. The values of data are mean ± standard error of three replicates per culture. The experiments were repeated three times.

RESULTS

Callus formation

The frequency of callus formation varied depending upon the age of the petiole used and type of hormone combinations. Callus formation was observed from the wounded edges of the petiole. The frequency of callus formation was observed from 3.68 to 83.24%. The higher percenttage of callus formation was observed at 15 days in 1st (83.24%), 2nd (78.08%), 3rd (55.31%), 4th (42.62%), 5th (33.63%), 6th (18.21%) and 7th (7.12%) petioles (Figure 1A-G). There was no callus formation in 8th, 9th and 10th petiole (Figure 1H-J). Among the different concentration

Figure 1. A-Q, Plant regeneration from different ages of petiole of *Jatropha curcas* L.; A-J, callus formation from 1st to 10th petiole after two weeks of culture; K, callus growth on MS medium supplemented with 1 mg/l NAA in combination with 0.2 mg/l KN from 1st petiole after five weeks of culture (scale 10 mm); L and M, different stages of somatic embryogenesis (scale 10 mm); N, germination of somatic embryos resulting after 6 week culture (scale 10 mm); O, elongated shoots after three weeks of culture on MS with 0.5 mg/l TDZ, 0.1 mg/l BA and 0.4 mg/l GA_3 (scale 12 mm); P, *in vitro* rooted shoot on MS medium supplemented with 3 mg/l NAA (scale 12 mm); Q, *in vitro* rooted plants hardening at mist chamber (scale 15 mm).

Table 1. Effect of NAA and KN on callus formation in different ages of petiole of *J. curcas*.

Hormone (mg/l)		Mean callus formation in different ages of petiole explants ± S.E.[a]									
NAA	**KN**	**1st petiole**	**2nd petiole**	**3rd petiole**	**4th petiole**	**5th petiole**	**6th petiole**	**7th petiole**	**8th petiole**	**9th petiole**	**10th petiole**
0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	0.2	57.62±2.39	46.21±2.36	36.34±1.08	23.25±0.67	16.65±0.68	10.38±0.52	5.21±0.31	0.00±0.00	0.00±0.00	0.00±0.00
0.4	0.2	63.39±2.07	41.15±1.41	32.28±1.35	25.36±1.31	14.36±0.61	9.27±0.63	4.36±0.42	0.00±0.00	0.00±0.00	0.00±0.00
0.6	0.2	67.14±2.68	45.34±2.08	33.62±1.13	29.42±0.86	16.38±0.76	8.69±0.47	5.63±0.41	0.00±0.00	0.00±0.00	0.00±0.00
0.8	0.2	71.24±3.16	52.27±1.87	41.47±1.03	38.18±0.63	33.63±1.12	15.92±0.53	7.12±0.43	0.00±0.00	0.00±0.00	0.00±0.00
1.0	0.2	83.24±2.31	78.08±2.16	55.31±1.87	42.62±1.36	32.75±0.82	18.21±0.38	6.82±0.27	0.00±0.00	0.00±0.00	0.00±0.00
1.2	0.2	65.65±2.12	77.98±1.98	43.69±2.32	35.64±1.89	26.78±1.15	11.58±0.27	5.68±0.35	0.00±0.00	0.00±0.00	0.00±0.00
1.4	0.2	62.12±2.93	61.27±1.63	39.24±1.12	24.07±1.72	19.72±0.79	9.96±0.35	4.69±0.31	0.00±0.00	0.00±0.00	0.00±0.00
1.6	0.2	56.17±2.33	52.53±1.89	45.18±1.07	26.31±1.21	15.24±0.68	10.21±0.42	6.52±0.41	0.00±0.00	0.00±0.00	0.00±0.00
1.8	0.2	52.64±1.98	48.24±2.01	32.23±1.21	18.21±0.97	11.78±0.86	7.68±0.31	3.68±0.25	0.00±0.00	0.00±0.00	0.00±0.00
2.0	0.2	54.71±2.32	44.96±2.05	34.35±1.05	22.38±0.86	12.36±0.73	8.74±0.48	4.32±0.14	0.00±0.00	0.00±0.00	0.00±0.00

[a]Mean of three replicates.

of NAA and KN tested for callus formation, the highest percentage was observed at 1.0 mg/l NAA and 0.2 mg/l KN (Table 1). The 1st petiole (83.24%) recorded the highest percentage of callus formation followed by the 2nd petiole (78.08%). The concentration of NAA and KN increases, the percentage of callus formation also increases up to concentration of 1.0 mg/l NAA and 0.2 mg/l KN. The concentration beyond 1.0 mg/l NAA and 0.2 mg/l KN resulted in decrease of callus formation percentage.

Callus growth

The highest percentage of callus growth was observed at hormonal combination of 1.0 mg/l NAA and 0.2 mg/l KN (Figure 2). The 1st petiole (22.48 cm^2) recorded the highest percentage of callus growth followed by the 2nd petiole (21.25 cm^2), 3rd petiole (11.14 cm^2) and 4th (8.38 cm^2) (Figure 1K). The lowest percentage of callus growth was observed in the 4th petiole (4.28 cm^2).

Somatic embryo formation

Callus was transferred to somatic embryo induction medium and somatic embryo formation was observed after 3 weeks. TDZ at the concentration of 0.1 mg l^{-1} resulted in somatic embryo formation in 1st petiole (2.64%), 2nd petiole (1.85 %), 3rd petiole (0.97%) and 4th petiole (0.67%) (Table 2). The increased level of TDZ showed higher number of somatic embryo formation. The GA_3 supplementation with TDZ medium resulted in increased number of somatic embryo formation in 1st petiole (7.39%), 2nd petiole (5.69%), 3rd petiole (4.25%) and 4th petiole (2.18%) (Figure 1L and M). The hormone combination of 0.5 mg/l TDZ and 0.4 mg/l GA_3 recorded the highest percentage of somatic embryo formation. The highest somatic embryo formation was observed in the 1st petiole (35.16%), followed by the 2nd petiole (33.75%), 3rd (13.65%) and 4th (8.83%) (Table 2) (Figure 1M). Lowest somatic embryo formation was observed in 4th petiole (0.67%) at the hormone combination of 0.1 mg/l TDZ. The concentration beyond 0.5 mg/l TDZ and 0.4 mg/l GA_3 resulted decreased the number of somatic embryo formation.

Somatic embryo germination

The matured somatic embryos were transferred to MS medium supplemented with BA, IAA and KN. The hormone combination of 1.0 mg/l BA, 0.5 mg/l IAA and 0.25 mg/l KN recorded the maximum somatic embryo germination after 3 weeks (Figure 1N). The maximum percentage of somatic embryo germination was observed in 1st petiole (66.85%), 2nd petiole (62.17%) followed by 3rd petiole (21.12%) and 4th petiole (15.69%) (Table 3). The lowest percentage of somatic embryos germination was observed in the 4th petiole (%). The hormonal combination of 1 mg/l BA, 0.5 mg/l TDZ and 0.25 mg/l GA_3 resulted in higher percentage of somatic embryo germination. The

Table 2. Effect of TDZ and GA_3 in somatic embryo formation in different ages of petiole of *J. curcas*.

Hormone (mg/l)		Mean somatic embryo formation in different ages of petiole explants ± S.E.[a]			
TDZ	GA_3	1st Petiole	2nd Petiole	3rd Petiole	4th Petiole
0.0	0.0	0.00	0.00	0.00	0.00
0.1	0.0	2.64±0.16	1.85±0.13	0.97±0.08	0.67±0.07
0.2	0.1	7.39±0.19	5.69±0.18	4.25±0.13	2.18±0.11
0.3	0.2	7.94±0.17	6.71±0.16	5.38±0.24	3.89±0.16
0.4	0.3	22.51±1.23	24.68±1.21	11.23±0.42	7.36±0.21
0.5	0.4	35.16±1.11	33.75±1.32	10.54±0.31	8.83±0.20
0.6	0.5	26.63±0.85	25.35±0.96	13.65±0.36	7.36±0.12
0.7	0.6	18.92±1.21	16.56±0.48	8.64±0.14	7.61±0.14
0.8	0.7	17.34±0.79	11.31±0.35	6.48±0.12	4.25±0.11
0.9	0.8	9.84±0.34	6.54±0.23	3.96±0.11	2.37±0.09
1.0	0.9	8.46±0.41	4.67±0.17	5.41±0.09	3.28±0.06

[a]Mean of three replicates.

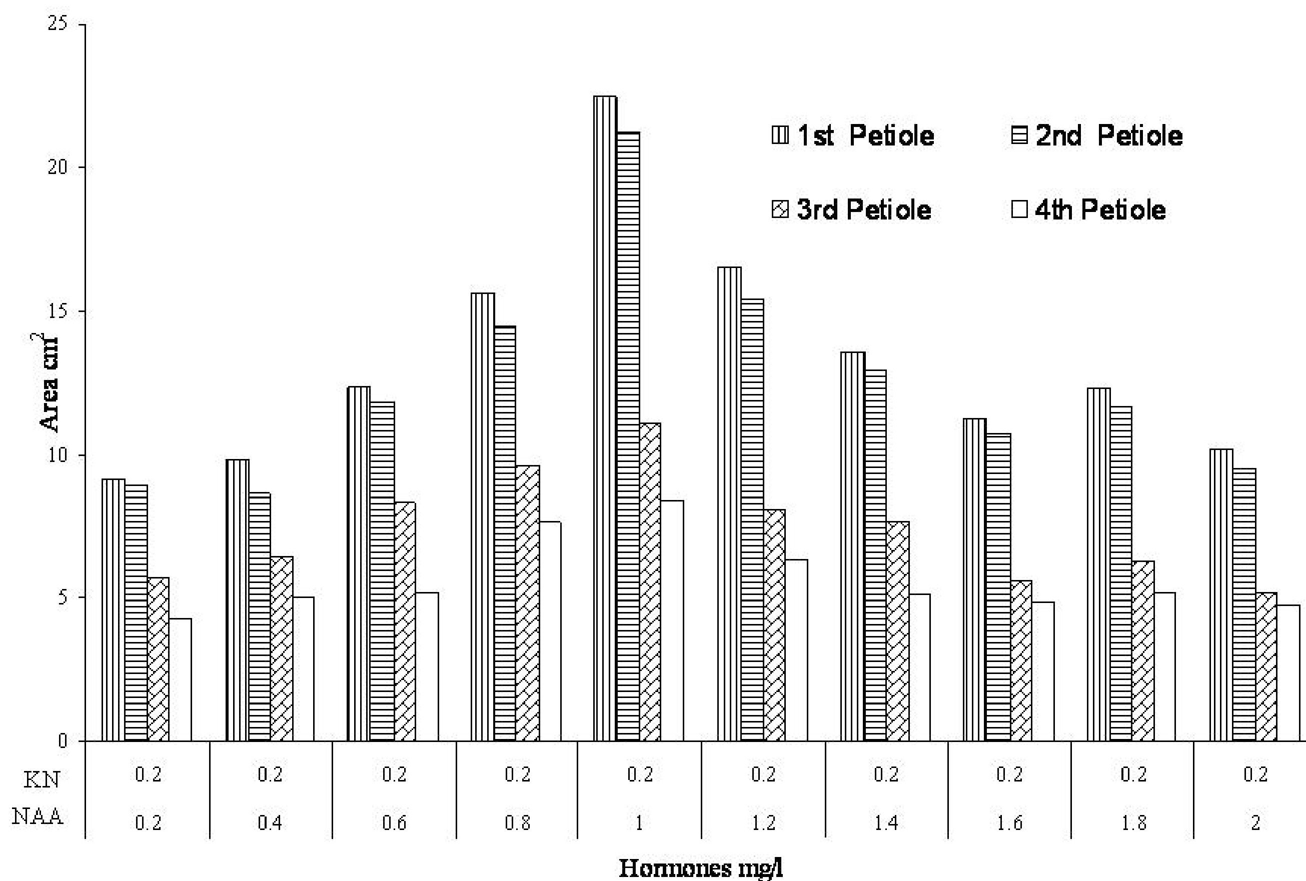

Figure 2. Callus growth from 1st to 4th petioles (area cm^2) of *Jatropha curcas*.

first petiole (32.56%) recorded the higher percentage of somatic embryo germination followed by 2nd petiole (31.58%), 3rd petiole (11.24%) and 4th petiole (7.95%) in BAP, TDZ and GA_3 combination.

Shoot elongation

The hormone combination of 0.5 mg/l TDZ, 0.1 mg/l BA and 0.4 mg/l GA_3 recorded the highest percentage of

Table 3. Effect of BA, KN and IAA on the somatic embryos germination in different ages of petiole of *J. curcas*.

Hormone (mg/l)					Mean somatic embryos germination in different ages of petiole explants ± S.E.[a]			
BA	IAA	KN	TDZ	GA_3	1st Petiole	2nd Petiole	3rd Petiole	4th Petiole
0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
0.2	0.1	0.0	0.0	0.0	8.62±0.13	7.58±0.21	4.69±0.24	3.78±0.14
0.4	0.2	0.1	0.0	0.0	22.36±0.34	12.75±0.63	6.78±0.37	8.45±0.18
0.6	0.3	0.15	0.0	0.0	39.84±1.25	25.28±0.14	10.11±0.46	13.37±0.32
0.8	0.4	0.2	0.0	0.0	43.96±1.35	39.43±0.86	12.85±0.53	10.53±0.52
1.0	0.5	0.25	0.0	0.0	66.85±1.85	62.17±1.21	19.46±0.24	12.34±0.13
1.2	0.6	0.3	0.0	0.0	24.67±0.75	29.68±0.69	21.12±0.31	14.36±0.24
1.4	0.7	0.35	0.0	0.0	17.39±0.62	22.96±0.35	17.35±0.41	15.69±0.16
1.6	0.8	0.4	0.0	0.0	12.78±0.24	18.62±0.42	12.14±0.32	12.47±0.41
1.8	0.9	0.45	0.0	0.0	7.57±0.19	11.36±0.38	8.78±0.24	10.15±0.73
0.1	0.0	0.0	0.1	0.0	3.42±0.14	2.77±0.16	3.15±0.13	1.25±0.08
0.1	0.0	0.0	0.2	0.1	8.34±0.36	4.96±0.22	4.22±0.14	1.34±0.06
0.1	0.0	0.0	0.3	0.15	14.58±0.74	6.24±0.13	5.38±0.21	2.45±0.12
0.1	0.0	0.0	0.4	0.2	24.25±0.36	22.37±0.46	7.63±0.23	4.85±0.14
0.1	0.0	0.0	0.5	0.25	32.56±0.92	31.58±0.75	9.65±0.42	7.95±0.21
0.1	0.0	0.0	0.6	0.3	21.35±0.64	17.46±0.52	11.24±0.56	5.36±0.12
0.1	0.0	0.0	0.7	0.35	13.63±0.43	19.37±0.34	8.54±0.41	4.27±0.06
0.1	0.0	0.0	0.8	0.4	9.58±0.31	8.58±0.52	7.38±0.23	5.43±0.07
0.1	0.0	0.0	0.9	0.45	10.11±0.63	12.22±0.22	5.96±0.17	5.52±0.11
0.1	0.0	0.0	1.0	0.5	9.14±0.37	10.4±0.19	5.79±0.16	4.91±0.13

[a]Mean of three replicates.

Table 4. Effect of TDZ, BA and GA_3 on mean shoots elongation in different ages of petiole of *J. curcas*.

S/N	Hormone (mg/l)			Mean number of shoots in different ages of petiole explants ± S.E.[a]			
	TDZ	BA	GA_3	1st Petiole	2nd Petiole	3rd Petiole	4th Petiole
1	0.0	0.0	0.0	0.00	0.00	0.00	0.00
2	0.1	0.02	0.0	5.27±0.35	4.82±0.26	3.34±0.08	3.87±0.08
3	0.2	0.04	0.1	9.34±0.17	5.94±0.18	4.59±0.09	3.86±0.06
4	0.3	0.06	0.2	12.52±0.23	8.76±0.18	4.92±0.11	5.91±0.04
5	0.4	0.08	0.3	18.74±0.38	11.58±0.21	5.37±0.13	6.67±0.13
6	0.5	0.1	0.4	32.68±0.69	24.93±0.23	7.61±0.14	5.39±0.11
7	0.6	0.12	0.5	25.92±0.44	16.61±0.24	8.38±0.16	4.21±0.08
8	0.7	0.14	0.6	21.14±0.37	13.57±0.17	4.46±0.08	7.34±0.11
9	0.8	0.16	0.7	19.27±0.42	11.68±0.15	5.63±0.11	2.18±0.07
10	0.9	0.18	0.8	16.34±0.36	9.53±0.16	6.68±0.13	6.17±0.06
11	1.0	0.2	0.9	11.47±0.27	7.49±0.17	5.91±0.07	5.28±0.04

[a] Mean of three replicates.

shoot elongation in the 1st petiole (32.68%) followed by 2nd petiole (24.93%) (Table 4) (Figure 1O). The TDZ and BA resulted in low percentage of shoot elongation in 1st petiole (5.27%), 2nd petiole (4.82%), 3rd petiole (3.34%) and 4th petiole (3.87%). The concentration of TDZ beyond 0.5 mg/l and 0.1 mg/l BA and 0.4 mg/l GA_3 decreased shoot elongation.

Rooting

4 weeks old elongated shoots were transferred to root induction medium with different auxins viz. IBA, NAA and IAA. The results of this study showed that the rooting percentage was higher in half MS medium supplemented with 0.30 mg/l NAA (Figure 3). First petiole obtained the

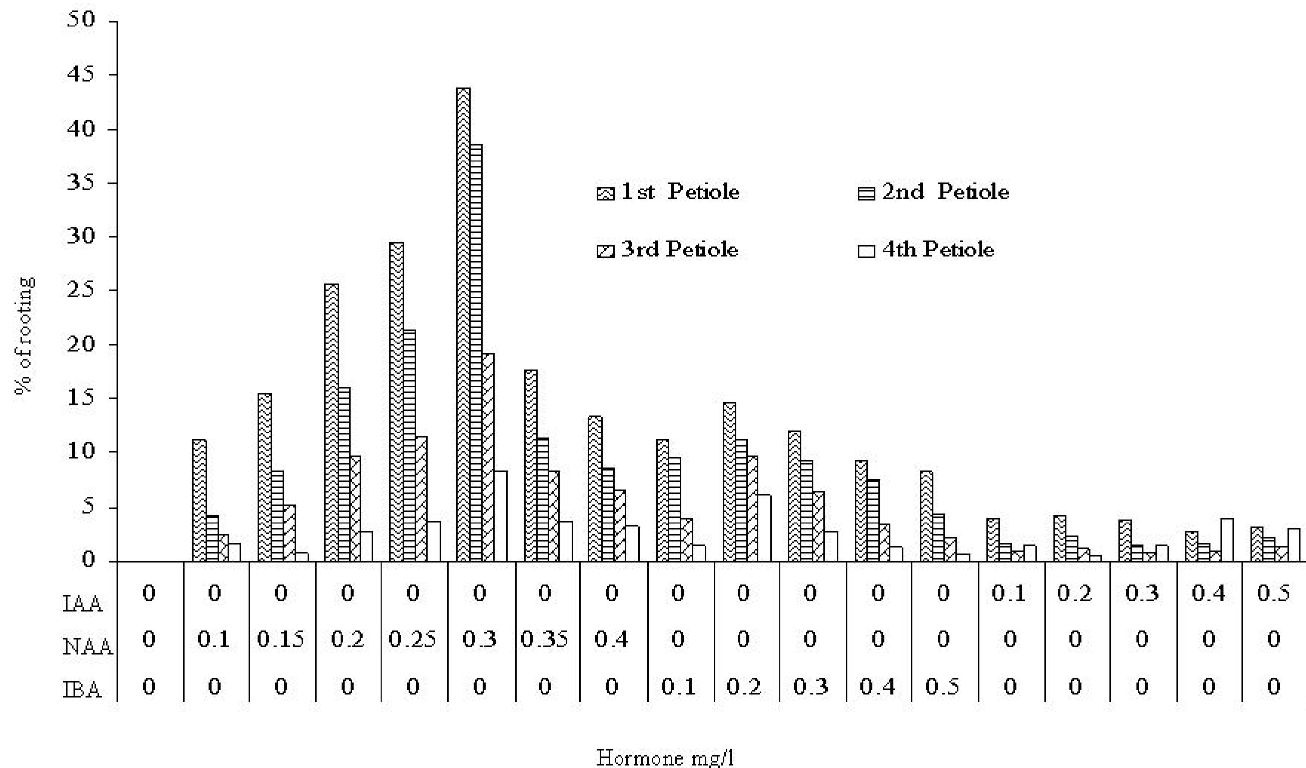

Figure 3. Effect of hormones and rooting percentage of *in vitro* shoots of *Jatropha curcas*.

maximum rooting of 43.77% followed by 2nd (38.61%), 3rd (19.24%) 4th (8.36%) petioles (Figure 1P).

Hardening

The survival percentage of plantlets varied according to the age of the petiole used as explant. The highest survival rate of plantlets was recorded in 1st petiole (60.32%) followed by 2nd petiole (56.14%), 3rd petiole (50.13%) and 4th petiole (47.28%) under hardening chamber. Further rooted plantlets were hardened at mist chamber (Figure 1Q).

DISCUSSION

An efficient regeneration protocol is a prerequisite for any successful genetic transformation in *J. curcas*. Therefore, an effort has been made to obtain regeneration using different ages of the petiole as explants. The callus induction differs according to the type of auxin, cytokinin, genotype and explants source (Varshney and Johnson, 2010) in *J. curcas* which is similar to our findings. From the present study, it was found that callus induction was achieved on MS medium containing NAA and KN from 1st petiole to the 7th petiole. The petiole older than 7th petiole has shown no callus formation which is similar to earlier reports in *Trifolium alexandrinum* (Abogadallah and Quick, 2010). Thepsamran et al. (2008) reported that the age of petiole determines the shoot regeneration of *J. curcas*. Prakash and Gurumurthi (2010) also observed in *Eucalyptus camaldulensis* that the age of explants played a major role in the determination of shoot and these results were similar to our findings. Misra et al. (2010) reported that the fourth leaf did not show response and died due to the maturity of the leaves and responded very late which is similar to our findings. In our experiments, the eight, nine and tenth petiole did not showed symptoms of callusing and completely dried.

The role of TDZ has been well documented for shoot proliferation in various plant species. TDZ stimulates higher axillary shoot proliferation in woody plants (Malik and Saxena, 1992; Huetteman and Preece, 1993). The role of TDZ has been well exploited in *J. curcas* (Kumar and Reddy, 2010; Misra et al., 2010; Deore and Johnson, 2008; Sujatha et al., 2005). In the present study, shoot buds cultured on TDZ medium did not elongate and resulted in rosette formation. The current finding is similar to those reported by Sujatha et al. (2005). The reason may be due to the presence of hormones in induction medium having a carry-over effect in differentiation medium and resulted in dwarfing effect in the shoots.

The induction of morphogenic responses with TDZ in *J. curcas* was found to be superior to BA (Kaul et al., 2010; Kumar and Reddy, 2010). Various studies have shown that BA has been found to be more effective than other cytokinin on multiple shoot induction in Euphorbiaceae (Tideman and Hawker, 1982) while BA in combination with

other cytokinin and auxins play an important role in regeneration and shoot elongation (Singh et al., 2010; Purkayastha et al., 2010; Kumar and Reddy, 2010; Deore and Johnson, 2008; Kaul et al., 2010) in *Jatropha curcas*.

Our results showed that BA, IAA and KN resulted in 66.85% shoot formation in the 1st petiole, followed by 2nd petiole (62.17 %), 3rd petiole (21.12%) and 4th petiole (15.69%) which is similar to the findings of Deore and Johnson (2008). Kinetin proved to be the best for axillary bud proliferation in *J. curcas* (Rajore and Batra, 2005; Kumar and Reddy, 2010). IAA enhanced callus formation and shoot organogenesis in *J. curcas* (Datta et al., 2007; Sujatha et al., 2005; Jha et al., 2007; Wei et al., 2004). GA_3 playing an important role in shoot elongation of *J. curcas* (Dubey et al., 2010; Deore and Johnson 2008; Kaul et al., 2010; Mazumdar et al., 2010; Li et al., 2008) and also in Castor (Sarvesh et al., 1992; Sujatha and Reddy, 1998; Kumari et al., 2008; Kumarijayashree and Thulaseedharan, 2001). In the present study, GA_3 at the concentration of 0.1 mg/l resulted in elongation of *J. curcas* shoots whereas higher concentration of GA_3 beyond 0.25 mg/l proves to be ineffective in elongation of *J. curcas*. Among the three hormones tested for root induction, half strength MS medium supplemented with NAA resulted in 43.77%. The percentage of rooting was very less in IBA and IAA. Kumar and Reddy (2010) reported that combination of IBA, IAA, NAA and activated charcoal was found to best for promoting rooting in *J. curcas*. Mazumdar et al. (2010) reported half MS basal medium supplemented with NAA resulted in 75% root induction and Datta et al. (2007) reported 52% root induction in MS basal medium supplemented with IBA in *J. curcas*.

Conclusion

The present study concludes that 32 shoots in 0.5 mg/l TDZ, 0.1 mg/l BA and 0.4 mg/l GA_3 in 1st petiole was higher than the previous protocol available from petiole explants of *J. curcas*. The current protocols can be used for the development of transgenic *J. curcas* plant for oil improvement programmes.

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