CHAPTER -IV

RESULTS

4.1. Initiation of aseptic cultures

Shoot cultures of toxic and non-toxic cultivar (Plate 3I) were established within 6-8 weeks multiplied at the rate of 2-4 folds per month. Each micro-shoot attained a height of 3-4 cm within 6-8 weeks and formed the source of *in vitro* leaf and petiole explant (Plate 3N & O).

Both *in vivo* and *in vitro* seedlings (Plate 3J & K) were established within 2 weeks. These seedlings formed the source of in vivo and *in vitro* cotyledonary leaf and petiole explant (Plate 3L, M, P & Q).

PART A

4.2. REGENERATION

4.2.1. Regeneration of toxic cultivar of *Jatropha curcas*

4.2.1.1. Regeneration from *in vitro* and *in vivo* leaf explant

4.2.1.1. (a) Effect of plant growth regulators (PGRs) on shoot bud induction

The PGRs had a significant effect on shoot bud induction for all the three genotypes of toxic cultivar. The regeneration efficiency of all the genotypes were higher in TDZ containing medium as compared to BAP containing medium. When TDZ or BAP containing medium was supplemented with 1 mg/L IBA, regeneration efficiency decreased. The percent shoot bud induction and number of induced shoot buds per leaf explant was directly proportional to the concentration of PGRs. Of the different concentrations of TDZ tested alone and in combination with 1 mg/L IBA, highest percentage of shoot bud induction (91.22%) and highest number of shoot buds per leaf explant (15.11) was observed in the presence of 2.0 mg/L of TDZ (Tables 1 & 2) among all the three genotypes, however, further proliferation and elongation of shoot buds was inhibited due to compact shoot bud induction at this concentration. It was observed that 0.5 mg/L was found optimum for shoot bud induction and subsequent subculture. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 22.06-86.66% and number of induced shoot buds per leaf explant

varied from 3.88-10.10 among all the three genotypes (Tables 1 & 2). Of the different concentrations of BAP tested alone and in combination with 1 mg/L IBA, highest percentage of shoot bud induction (46.67%) and highest number of shoot buds per nodal leaf explant (6.12) was observed in the presence of 12.0 mg/L of BAP (Tables 3 & 4).

4.2.1.1. (b) Effect of explant sources on shoot bud induction

The sources of explant also influenced significantly the plant regeneration through shoot bud induction at all tested concentration of PGRs. *In vitro* leaf explant responded efficiently as compared to *in vivo* leaf explant in all the three genotype. The percentage of shoot bud induction varied from 6.56-91.22% in *in vitro* leaf explant (Plate 5A; Table 1) and 5.12-49.34% in *in vivo* leaf explant (Plate 5B; Table 2), and number of induced shoot buds per explant varied from 2.06-15.11 in *in vitro* leaf explant (Table 1) and 1.01-7.74 in *in vivo* leaf explant (Table 2) at tested concentration of TDZ alone and with 1 mg/L IBA among all the three genotypes whereas, on BAP alone and with 1 mg/L IBA containing medium the percentage of shoot bud induction varied from 9.11-46.67% in *in vitro* leaf explants (Table 3) and 8.01-41.23% in *in vivo* leaf explant (Table 4), and number of induced shoot buds per explant varied from 2.01-6.12 in *in vitro* leaf explant (Table 3) and 1.09-6.11 in *in vivo* leaf explant (Table 4) among all the three genotypes.

4.2.1.1. (c) Effect of genotype on shoot bud induction

Significant differences in percent shoot bud induction and number of induced shoot buds per explant was observed among the genotypes studied at tested concentration of PGRs. CSMCRI-JC-2 performed best at tested concentrations and combinations of PGRs both in terms of percentage of shoot buds induction and the number of shoot buds per explant followed by CSMCRI-JC-1. The percentage of shoot buds induction in CSMCRI-JC-2 and CSMCRI-JC-1 varied from 11.10-91.22% and 6.56-82.33% respectively and number of induced shoot buds per explant varied from 1.09-15.11 and 14.88 respectively at tested concentration and combinations of PGRs (Tables 1-4). The percentage of shoot bud induction in CSMCRI-JC-3 varied from 5.12-72.55%, and number of

Table 1. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* leaf explant of three genotypes of toxic cultivar of *J.curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	16.66 ± 1.51^{b}	40.33 ± 2.49^{b}	25.66 ± 1.52^{c}	4.53 ± 0.24^{ab}	6.06 ± 0.24^{a}	3.61 ± 0.21^{abc}
0.10	0	44.66 ± 2.51^{c}	59.66 ± 3.50^{d}	33.66 ± 1.48^{d}	5.63 ± 0.33^{ab}	8.30 ± 1.50^{ab}	3.06 ± 0.27^{ab}
0.20	0	51.33 ± 2.50^{d}	81.66 ± 3.49^{f}	39.33 ± 2.06^{e}	5.80 ± 0.67^{ab}	9.31 ± 1.55^{abc}	4.43 ± 0.39^{abc}
0.50	0	69.33 ± 2.31^{f}	86.66 ± 3.04^{fg}	60.66 ± 3.99^{g}	7.16 ± 1.15^{bc}	10.10 ± 1.71^{bcd}	5.13 ± 1.45^{bcd}
1.00	0	79.33 ± 4.71^{g}	90.33 ± 5.02^{g}	69.00 ± 3.60^{h}	10.44 ± 2.69^{d}	12.36 ± 1.98^{cde}	5.76 ± 1.65^{cd}
2.00	0	82.33 ± 5.76^{g}	91.22 ± 6.87^{g}	72.55 ± 4.98^{h}	14.88 ± 1.97^{d}	15.11 ±2.78^{e}	6.99 ± 1.94^{d}
0.05	1	6.56 ± 1.01^{a}	31.31 ± 2.50^{a}	15.56 ± 1.20^{a}	3.43 ± 1.21^{a}	6.01 ± 0.97^{a}	3.11 ± 0.9 0^{ab}
0.10	1	41.06 ± 1.50^{c}	49.61 ± 3.10^{c}	23.16 ± 1.31^{b}	4.12 ± 1.31^{ab}	7.99 ± 1.91^{ab}	2.06 ± 0.19^{a}
0.20	1	45.03 ± 2.10^{c}	71.56 ± 3.00^{e}	29.03 ±1.34^{cd}	4.71 ± 1.49^{ab}	9.00 ± 1.65^{abc}	3.13 ± 1.01^{ab}
0.50	1	59.31 ± 2.09^{e}	76.61 ± 2.81^{e}	50.16 ± 3.11^{f}	6.76 ± 1.95^{bc}	9.10 ± 1.76^{bc}	4.03 ± 1.35^{abc}
1.00	1	69.39 ± 3.99^{f}	80.31 ± 4.02^{ef}	59.10 ± 3.6^{g}	9.34 ± 1.91^{cd}	11.36 ± 1.28^{bcd}	4.06 ± 1.55^{abc}
2.00	1	72.13 ± 5.00^{f}	81.22 ± 6.0^{f}	62.45 ± 3.99^{g}	11.08 ± 2.67^{d}	13.11 ± 1.98 ^{de}	7.09 ± 1.84^{d}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 2. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* leaf explant of three genotypes of toxic cultivar of *J.curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	12.82 ± 2.19^{ab}	18.20 ± 2.71^{b}	7.32 ± 1.99^{ab}	2.21 ± 0.24^{ab}	2.89 ± 0.37^{ab}	1.98 ± 0.29^{a}
0.10	0	18.81 ± 3.47^{bc}	21.40 ± 3.40^{b}	9.41 ± 1.87^{abc}	2.58 ± 0.29^{ab}	3.26 ± 1.46^{ab}	2.26 ± 0.37^{abc}
0.20	0	21.80 ± 3.71^{cd}	22.81 ± 3.64^{b}	14.10 ± 2.44^{c}	2.51 ± 0.33^{ab}	3.82 ± 1.36^{ab}	2.98 ± 0.49^{bcd}
0.50	0	27.80 ± 4.25^{de}	34.20 ±4.18^{cd}	22.06 ±3.14^{de}	4.54 ± 1.08^{c}	5.40 ± 2.11^{bcd}	3.88 ± 0.91^{cde}
1.00	0	34.21 ± 4.31^{df}	39.33 ±4.56^{de}	26.61 ± 3.26^{e}	5.14 ± 1.09^{c}	5.54 ± 1.78^{bcd}	4.36 ± 1.09^{de}
2.00	0	41.24 ± 4.19^{g}	49.34 ± 4.85^{f}	36.11 ± 3.81^{g}	7.11 ± 2.11^{d}	7.74 ± 2.09^{d}	5.56 ± 1.21^{e}
0.05	1	8.82 ± 1.92^{a}	11.20 ± 2.63^{a}	5.12 ± 1.18^{a}	1.01 ± 0.11^{a}	2.09 ± 0.91^{a}	1.78 ± 0.60^{a}
0.10	1	10.81 ± 2.11^{a}	19.40 ± 3.66^{b}	8.41 ± 1.11^{ab}	2.08 ± 0.27^{ab}	3.16 ± 0.61^{ab}	2.16 ± 0.71^{ab}
0.20	1	18.80 ± 3.11^{bc}	20.81 ± 3.64^{b}	11.10 ±2.14^{bc}	1.91 ± 0.23^{a}	2.92 ± 0.61^{ab}	2.78 ± 0.33^{bcd}
0.50	1	23.80 ± 3.16^{cde}	30.20 ± 3.71^{c}	20.06 ±3.09^{de}	3.04 ± 1.57^{bc}	4.30 ± 1.51^{abc}	3.18 ± 0.71^{bcd}
1.00	1	29.21 ± 3.72^{ed}	32.33 ± 4.26^{c}	23.61 ±3.17^{de}	4.04 ± 1.90^{bc}	4.14 ± 0.88^{abc}	4.06 ± 1.79^{cde}
2.00	1	37.24 ± 4.41^{fg}	45.34 ±4.15^{ef}	31.11 ± 3.12^{f}	6.01 ± 1.69^{cd}	6.64 ± 1.99^{cd}	5.06 ± 1.61^{e}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 3. Effect of different concentrations of 6-benzylaminopurine (BAP) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

BAP (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
3.0	0	16.23 ± 2.0^{b}	18.31 ± 2.22^{b}	12.12 ±1.92^{ab}	2.21 ± 0.23^{ab}	3.87 ± 0.66^{a}	2.12 ± 0.56^{a}
6.0	0	22.24 ± 2.91^{c}	26.98 ± 2.89^{c}	19.31 ± 2.81^{c}	3.61 ± 0.67^{abc}	4.02 ± 0.74^{a}	3.13 ± 0.78^{ab}
9.0	0	38.34 ± 3.11^{ef}	42.65 ± 3.01^{ef}	33.31 ± 2.92^{e}	4.65 ± 1.01^{c}	4.12 ± 0.98^{a}	3.63 ± 0.97^{ab}
12.0	0	42.65 ± 4.01^{f}	46.67 ± 4.09^{f}	40.54 ±4.03^{f}	4.98 ± 1.11^{d}	6.12 ± 1.23^{b}	4.18 ± 1.01^{b}
3.0	1	11.13 ± 1.51^{a}	12.21 ± 2.01^{a}	9.11 ± 1.05^{a}	2.01 ± 0.13^{a}	2.77 ± 0.34^{a}	2.02 ± 0.46^{a}
6.0	1	17.14 ± 1.61^{b}	17.18 ±1.99^{ab}	14.41 ± 2.07^{b}	2.69 ± 0.57^{ab}	3.12 ± 0.54^{a}	3.03 ± 0.68^{ab}
9.0	1	30.32 ± 2.04^{d}	33.65 ± 3.81^{d}	28.14 ± 2.96^{d}	2.73 ± 0.87^{ab}	3.42 ± 0.87^{a}	4.05 ± 1.11^{b}
12.0	1	36.44 ± 3.12^{e}	37.87 ±3.09^{de}	32.55 ± 3.01^{e}	3.98 ± 1.91^{bc}	5.72 ± 1.01^{b}	4.05 ± 1.02^{b}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 4. Effect of different concentrations of 6-benzylaminopurine (BAP) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

BAP (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
3.0	0	11.13 ± 0.99^{a}	16.11 ±2.91^{ab}	8.08 ± 1.99^{a}	2.01 ± 0.26^{a}	3.27 ± 0.47^{b}	1.99 ± 0.59^{ab}
6.0	0	18.16 ± 2.31^{b}	22.01 ± 3.99^{b}	14.11 ± 2.92^{b}	3.21 ± 1.68^{ab}	3.92 ± 1.05^{b}	3.03 ± 0.78^{ab}
9.0	0	28.34 ± 2.91^{cd}	38.13 ±3.41^{de}	27.55 ± 2.72^{c}	4.05 ± 1.12^{b}	4.02 ± 0.99^{b}	3.13 ± 1.91^{ab}
12.0	0	32.61 ± 3.88^{de}	41.23 ± 4.89^{e}	30.11 ± 4.65^{c}	4.18 ± 1.11^{b}	6.11 ± 1.03^{c}	4.08 ± 1.42^{b}
3.0	1	10.03 ± 1.23^{a}	11.10 ± 1.91^{a}	8.01 ± 1.17^{a}	1.91 ± 0.27^{a}	1.09 ± 0.21^{a}	1.12 ± 0.57^{a}
6.0	1	16.24 ± 1.99^{b}	14.19 ± 1.51^{a}	12.11 ±1.03^{ab}	2.99 ± 0.89^{ab}	2.41 ± 0.51^{ab}	2.89 ± 0.69^{ab}
9.0	1	27.32 ± 2.63^{c}	29.61 ± 3.62^{c}	26.04 ± 2.91^{c}	3.79 ± 0.97^{ab}	2.62 ± 0.78^{b}	3.11 ± 1.82^{ab}
12.0	1	33.87 ± 3.76^{e}	34.14 ±3.91^{cd}	28.45 ± 2.91^{c}	3.11 ± 1.01^{ab}	3.17 ± 1.13^{b}	2.78 ± 1.89^{ab}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

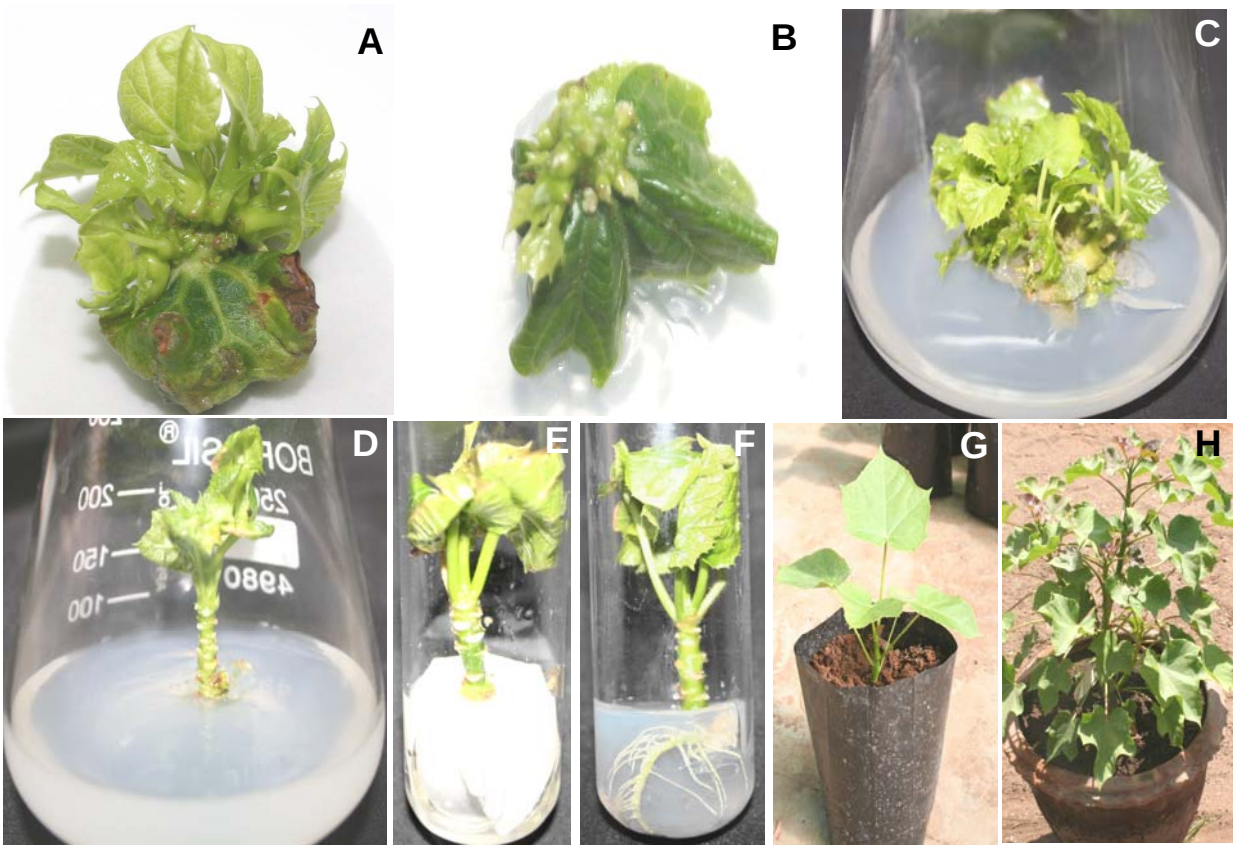

Plate 5. Direct shoot bud induction from leaf explant of toxic cultivar of *J. curcas*.

Direct shoot bud induction from (A) *in vitro* leaf explant and (B) *in vivo* leaf explant on MS medium with 0.5 mg/L TDZ. (C) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA . (D) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (E) Pulse treatment for root induction on half strength MS medium with 2% sucrose + 3 mg/L IBA + 1mg/L IAA + 1 mg/L NAA. (F) Root initiation and elongation on hormone free half strength MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (G) Regenerated plant in polybag. (H) Regenerated plant in pot after 6 month under natural condition.

induced shoot buds per explant varied from 1.12-6.99 at tested concentration and combinations of PGRs (Tables 1-4).

4.2.1.1. (d) Shoot proliferation and elongation from induced shoot buds

Approx 60-70%, 10-20% and 80-90% regenerated shoot buds on 0.05-0.50 mg/L TDZ with or without IBA, 1.0-2.0 mg/L TDZ with or without IBA and 3-12 mg/L BAP with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA in all the three genotypes (Plate 5C). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 5). Significant differences in elongation were observed at different concentrations and combinations of PGRs and genotypes. BAP and IAA combination was found best in all the three genotypes. The best elongation (3.11-3.99 cm) was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 5D). The elongation ranged from 1.98-3.99 cm on medium containing BAP and IAA combinations. The elongation inhibited on medium containing BAP and IBA. The elongation ranged from 2.01-2.54 cm on medium containing BAP and IBA combinations and 1.0 mg/L BAP and 1.5 mg/L IBA gave the best elongation (2.10-2.54 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 1.11-192 cm. The best elongation was observed in CSMCRI-JC-2 genotype (1.15-3.99 cm) followed by CSMCRI-JC-1 (1.11-3.61 cm). The least elongation was observed in CSMCRI-JC-3 genotype (1.11-3.11 cm) (Table 5).

4.2.1.2. Regeneration from *in vitro* and *in vivo* cotyledonary leaf explant

4.2.1.2. (a) Effect of PGRs on shoot bud induction

Shoot bud induction from cotyledonary leaf explant had also influenced by PGRs in all three toxic genotypes. Of the different concentrations of TDZ tested alone and with 1 mg/L IBA, highest percentage of shoot bud induction (93.42%) and highest number of shoot buds per leaf explant (26.21) was observed in the presence of 2.0 mg/L of TDZ (Tables 6 & 7) among all the three genotypes, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction. It was observed that 0.5 mg/L was found optimum

optimum for shoot bud induction and subsequent culture. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 80.06-87.16% and number of

Table 5. Effect of various plant growth regulators (PGRs) on elongation of proliferated shoot from leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

PGRs (mg/L)				Mean shoot length (cm)		
BAP	IAA	NAA	IBA	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.5	0.5	0	0	3.11 ± 0.43^{fg}	3.21 ± 0.30^{d}	2.99 ± 0.67^{e}
1.0	0.5	0	0	2.70 ± 0.28^{def}	2.83 ± 0.41^{cd}	1.98 ± 0.16^{bc}
0.5	1.5	0	0	3.61 ± 0.27^{g}	3.99 ± 0.43^{e}	3.11 ± 0.96^{e}
1.0	1.5	0	0	2.91 ± 043^{ef}	2.98 ± 0.74^{d}	2.86 ± 0.67^{de}
0.5	0	0.5	0	1.11 ± 0.11^{a}	1.15 ± 0.16^{a}	1.91 ± 0.10^{bc}
1.0	0	0.5	0	1.73 ± 0.22^{ab}	1.83 ± 0.27^{ab}	1.33 ± 0.17^{ab}
0.5	0	1.5	0	1.92 ± 0.31^{bc}	1.89 ± 0.32^{ab}	1.23 ± 0.22^{ab}
1.0	0	1.5	0	1.12 ± 0.17^{a}	1.17 ± 0.16^{a}	1.11 ± 0.19^{a}
0.5	0	0	0.5	2.11 ± 0.42^{bcd}	2.13 ± 0.51^{bc}	2.03 ± 0.23^{bc}
1.0	0	0	0.5	2.44 ± 0.41^{cde}	2.13 ± 0.51^{bc}	2.01 ± 0.13^{bc}
0.5	0	0	1.5	2.39 ± 0.39^{cde}	2.01 ± 0.42^{bc}	2.20 ± 0.25^{cd}
1.0	0	0	1.5	2.33 ± 0.55^{cde}	2.10 ± 0.52^{bc}	2.54 ± 0.41^{cde}

BAP, 6-benzylaminopurine; NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

induced shoot buds per cotyledonary leaf explant varied from 15.11-20.96 among all the three genotypes (Tables 6 & 7). Of the different concentrations of BAP tested alone and with 1mg/L IBA, highest percentage of shoot bud induction (56.18%) and highest number of shoot buds per cotyledonary leaf explant (6.99) was observed in the presence of 12.0 mg/L of BAP among all the three genotypes (Tables 8 & 9).

4.2.1.2. (b) Effect of explant sources on shoot bud induction

The sources of explant also influenced significantly the plant regeneration through shoot bud induction at tested concentration of PGRs. *In vitro* cotyledonary leaf explant responded efficiently as compared to *in vivo* cotyledonary leaf explant in all the three toxic genotypes.The percentage of shoot

Table 6. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* cotyledonary leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	27.42 ± 2.72^{a}	52.41± 5.51^{ab}	36.76± 3.52^{ab}	8.63 ± 1.65^{a}	12.01 ± 1.55^{a}	9.11 ± 1.21^{a}
0.10	0	57.26 ± 5.31^{bc}	70.66 ±7.51^{cd}	44.37 ± 4.52^{b}	11.43 ±1.78^{ab}	15.00 ± 1.50^{ab}	13.01 ± 1.85^{b}
0.20	0	62.45 ± 4.51^{bc}	74.56 ±7.51^{ef}	71.65 ± 4.08^{c}	17.65 ±2.06^{ab}	19.01 ± 1.55^{bc}	14.03 ± 1.83^{b}
0.50	0	85.46 ± 8.30^{d}	87.16 ±8.05^{fg}	81.36±8.51^{cde}	20.96 ±2.15^{ab}	20.11 ± 7.71^{bc}	15.11 ± 1.95^{bc}
1.00	0	91.76 ± 6.72^{d}	91.53 ± 6.03^{g}	90.02± 4.60^{de}	22.74 ±2.89^{ab}	22.16 ± 2.48^{cd}	18.66 ± 2.55^{d}
2.00	0	92.13 ± 5.77^{d}	93.42 ± 6.88^{g}	92.54 ± 6.09^{e}	24.81 ±2.96^{ab}	26.21 ± 3.76^{d}	23.09 ± 2.14^{e}
0.05	1	26.16 ± 2.02^{a}	42.11 ± 4.51^{a}	26.99 ± 2.02^{a}	8.83 ± 1.21^{a}	10.01 ± 1.55^{a}	9.10 ± 1.1 0^{a}
0.10	1	52.16 ± 6.51^{b}	60.51± 6.11^{bc}	44.65 ± 4.12^{b}	9.11 ± 1.31^{a}	12.31 ± 1.56^{a}	12.16 ± 1.15^{ab}
0.20	1	66.23 ± 6.11^{c}	71.36 ±7.01^{cd}	70.83 ± 6.43^{c}	14.61 ±1.39^{ab}	19.11 ± 1.95^{bc}	13.73 ± 1.21^{b}
0.50	1	81.21 ± 7.10^{d}	86.21 ±8.85^{fg}	80.36 ±7.51^{de}	18.76 ±1.85^{ab}	20.11 ± 2.79^{bc}	14.83 ± 1.35^{bc}
1.00	1	82.26 ± 8.01^{d}	90.11 ± 9.03^{g}	87.11± 8.61^{de}	20.14 ±2.51^{ab}	22.16 ± 2.49^{cd}	14.66 ± 2.55^{bc}
2.00	1	84.23 ± 8.01^{d}	92.32 ± 6.08^{g}	92.65 ± 5.92^{e}	21.01 ±2.67^{ab}	24.01 ± 2.98^{cd}	17.69 ± 2.04^{cd}

Valuesrepresent means ± SD of 25 explant per treatment in three repeated experiment
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 7. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* cotyledonary leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	23.61 ± 1.82^{a}	50.43 ±3.59^{ab}	35.38 ± 2.51^{b}	8.89 ± 2.25^{a}	12.45 ± 1.65^{a}	9.67 ± 2.20^{a}
0.10	0	52.66 ± 3.81^{bc}	69.16 ± 4.53^{c}	43.96 ± 2.53^{b}	11.13 ±2.30^{ab}	13.10 ± 1.59^{a}	13.76 ± 1.25^{b}
0.20	0	61.13 ± 4.47^{cd}	73.67 ± 5.61^{c}	69.13 ± 4.88^{c}	17.98±2.36^{cde}	19.11 ± 2.05^{b}	14.98 ± 1.30^{b}
0.50	0	79.63 ± 5.32^{e}	86.16 ± 7.15^{d}	80.06 ± 6.11^{d}	20.15±2.25^{def}	20.12 ± 2.71^{b}	15.33 ± 1.45^{b}
1.00	0	81.24 ± 5.92^{e}	90.93 ± 7.73^{d}	89.01± 7.10^{de}	22.65 ± 2.69^{f}	22.37 ± 2.48^{bc}	18.66 ± 1.65^{c}
2.00	0	83.01 ± 5.87^{e}	91.12 ± 7.18^{d}	91.65 ± 6.89^{e}	22.58 ± 2.77^{f}	25.01 ± 2.78^{c}	21.99 ± 2.04^{d}
0.05	1	22.41 ± 2.02^{a}	41.21 ± 4.11^{a}	25.16 ± 3.12^{a}	8.03 ± 2.21^{a}	10.05 ± 2.25^{a}	9.01 ± 2.1 0^{a}
0.10	1	51.46 ± 2.51^{b}	59.11 ± 4.51^{b}	43.56 ± 4.12^{b}	9.99 ± 2.31^{a}	12.56 ± 2.50^{a}	12.66 ± 1.15^{b}
0.20	1	65.93 ± 3.41^{d}	70.26 ± 6.11^{c}	64.03 ± 6.43^{c}	14.91 ±3.09^{bc}	19.41 ± 2.55^{b}	13.93 ± 1.21^{b}
0.50	1	79.11 ± 7.11^{e}	86.11 ± 6.85^{d}	79.66 ± 7.51^{d}	16.78 ±1.65^{cd}	20.11 ± 2.71^{b}	14.83 ± 1.35^{b}
1.00	1	81.00 ± 7.71^{e}	90.11 ± 7.03^{d}	88.76 ±8.61^{de}	19.54±1.51^{def}	22.66 ± 2.48^{bc}	14.86 ± 1.55^{b}
2.00	1	82.93 ± 6.91^{e}	91.42 ± 8.08^{d}	92.55 ± 9.92^{e}	21.71 ± 2.67^{f}	22.91 ± 2.78^{bc}	20.19 ± 2.04^{cd}

Values represent means ± SD of 25 explant per treatment in three repeated experiments
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 8. Effect of different concentrations of 6-benzylaminopurine (BAP) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* cotyledonary leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

BAP (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
3.0	0	26.33 ± 3.21^{b}	28.11 ± 3.11^{a}	22.12 ± 2.54^{a}	3.63 ± 0.98^{a}	4.77 ± 1.07^{ab}	3.02 ± 0.87^{a}
6.0	0	32.14 ± 2.81^{b}	36.18 ± 3.79^{b}	29.65 ± 3.9^{b}	3.99 ± 0.88^{a}	5.01 ± 1.05^{ab}	4.01 ± 0.99^{ab}
9.0	0	41.04 ± 3.81^{cd}	42.95 ± 3.71^{c}	43.11 ± 3.13^{c}	5.53 ± 1.08^{b}	5.11 ± 1.90^{ab}	5.15 ± 1.12^{ab}
12.0	0	45.15 ± 4.61^{d}	56.18 ± 4.69^{d}	50.01 ± 4.91^{d}	6.92 ± 1.83^{c}	6.99 ± 1.92^{b}	5.71 ± 1.62^{b}
3.0	1	20.11 ± 2.41^{a}	22.31 ± 2.91^{a}	19.11 ± 2.11^{a}	3.09 ± 0.74^{a}	3.67 ± 1.37^{a}	3.12 ± 0.87^{a}
6.0	1	26.04 ± 2.81^{b}	27.17 ± 1.79^{a}	24.91 ±3.19^{ab}	3.18 ± 0.98^{a}	4.02 ± 1.55^{a}	4.01 ± 1.49^{ab}
9.0	1	38.10 ± 2.88^{c}	43.55 ± 3.61^{c}	43.02 ± 4.23^{c}	3.83 ± 0.91^{a}	4.32 ± 1.88^{ab}	5.11 ± 1.02^{ab}
12.0	1	40.15 ± 3.92^{cd}	47.17 ± 3.93^{c}	46.14 ±4.21^{cd}	4.88 ± 1.90^{ab}	6.52 ± 1.03^{ab}	6.01 ± 1.05^{b}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 9. Effect of different concentrations of 6-benzylaminopurine (BAP) alone and in combination with 1 mg/L indole- 3-butyric acid (IBA) on shoot bud induction from *in vivo* cotyledonary leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

BAP (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary leaf explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
3.0	0	17.18 ± 2.61^{ab}	19.11 ± 2.01^{a}	14.10 ±1.41^{ab}	3.11 ± 0.54^{a}	3.98 ± 0.57^{ab}	2.89 ± 0.77^{a}
6.0	0	23.01 ± 2.99^{c}	29.18 ± 2.91^{b}	17.36 ± 1.89^{b}	4.71 ± 0.69^{ab}	4.52 ± 0.95^{ab}	3.63 ± 0.99^{a}
9.0	0	40.11 ± 3.71^{e}	44.03 ±4.11^{de}	36.01 ±3.93^{cd}	4.93 ± 0.99^{ab}	4.92 ± 1.09^{abc}	4.91 ± 1.12^{a}
12.0	0	42.51 ± 4.51^{e}	49.17 ± 4.19^{e}	44.66 ± 4.41^{e}	4.98 ± 1.02^{b}	6.52 ± 1.83^{c}	4.99 ± 1.82^{a}
3.0	1	12.01 ± 1.21^{a}	14.11 ± 1.41^{a}	11.01 ± 1.11^{a}	3.01 ± 0.74^{a}	2.79 ± 0.87^{a}	3.02 ± 0.77^{a}
6.0	1	19.45 ± 1.9^{bc}	19.18 ± 1.91^{a}	15.61 ±2.29^{ab}	3.09 ± 0.88^{ab}	3.82 ± 0.85^{ab}	3.73 ± 0.89^{a}
9.0	1	30.14 ± 3.81^{d}	35.75 ± 3.51^{c}	32.12 ± 3.03^{c}	3.13 ± 0.98^{ab}	3.99 ± 1.11^{ab}	4.90 ± 1.82^{a}
12.0	1	33.01 ± 3.31^{d}	39.27 ±3.90^{cd}	39.84 ±3.91^{de}	4.08 ± 1.72^{ab}	5.02 ± 1.13^{bc}	4.88 ± 1.09^{a}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

bud induction varied from 26.16-93.42% in *in vitro* cotyledonary leaf explant (Plate 6A; Table 6) and 22.41-91-65% in *in vivo* cotyledonary leaf explant (Plate 6B; Table 7), and number of induced shoot buds per cotyledonary leaf explant varied from 8.63-26.21% in *in vitro* cotyledonary leaf explant (Table 6) and 8.03-25.01 in *in vivo* cotyledonary leaf explant (Table 7) at tested concentration of TDZ alone and with 1 mg/L IBA among all the three genotypes. The percentage of shoot bud induction varied from 19.11-56.18% in *in vitro* cotyledonary leaf explant (Table 8) and 11.01-49.17% in *in vivo* explant (Table 9), and number of induced shoot buds per explant varied from 3.02-6.99 in *in vitro* cotyledonary leaf explant (Table 8) and 2.89-6.52 in *in vivo* cotyledonary leaf explant (Table 9) at tested concentration of BAP alone and with 1 mg/L IBA among all the three genotypes.

4.2.1.2. (c) Effect of genotype on shoot bud induction

Remarkable differences in percent shoot bud induction and number of induced shoot buds per explant was observed among the genotypes studied at tested concentration of PGRs. CSMCRI-JC-2 performed best at tested concentrations and combinations of PGRs both in terms of percentage of shoot buds induction and the number of shoot buds per explant followed by CSMCRI-JC-1. The percentage of shoot buds induction in CSMCRI-JC-2 and CSMCRI-JC-1 varied from 14.11-93.42% and 12.01-92.13% respectively and number of induced shoot buds per cotyledonary leaf explant varied from 2.79-26.21 and 3.01-24.81respectively at tested concentration and combinations of PGRs (Tables 6-9). The percentage of shoot bud induction in CSMCRI-JC-3 varied from 11.01-92.54%, and number of induced shoot buds per cotyledonary leaf explant varied from 2.89-23.09 at tested concentration and combinations of PGRs (Tables 6-9).

4.2.1.2. (d) Shoot proliferation and elongation from induced shoot buds

Approx 70-75%, 20-25% and 85-90% regenerated shoot buds on 0.05-0.50 mg/L TDZ with or without IBA, 1.0-2.0 mg/L TDZ with or without IBA and 3-12 mg/L BAP with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA in all the three genotypes (Plate 6C).Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated

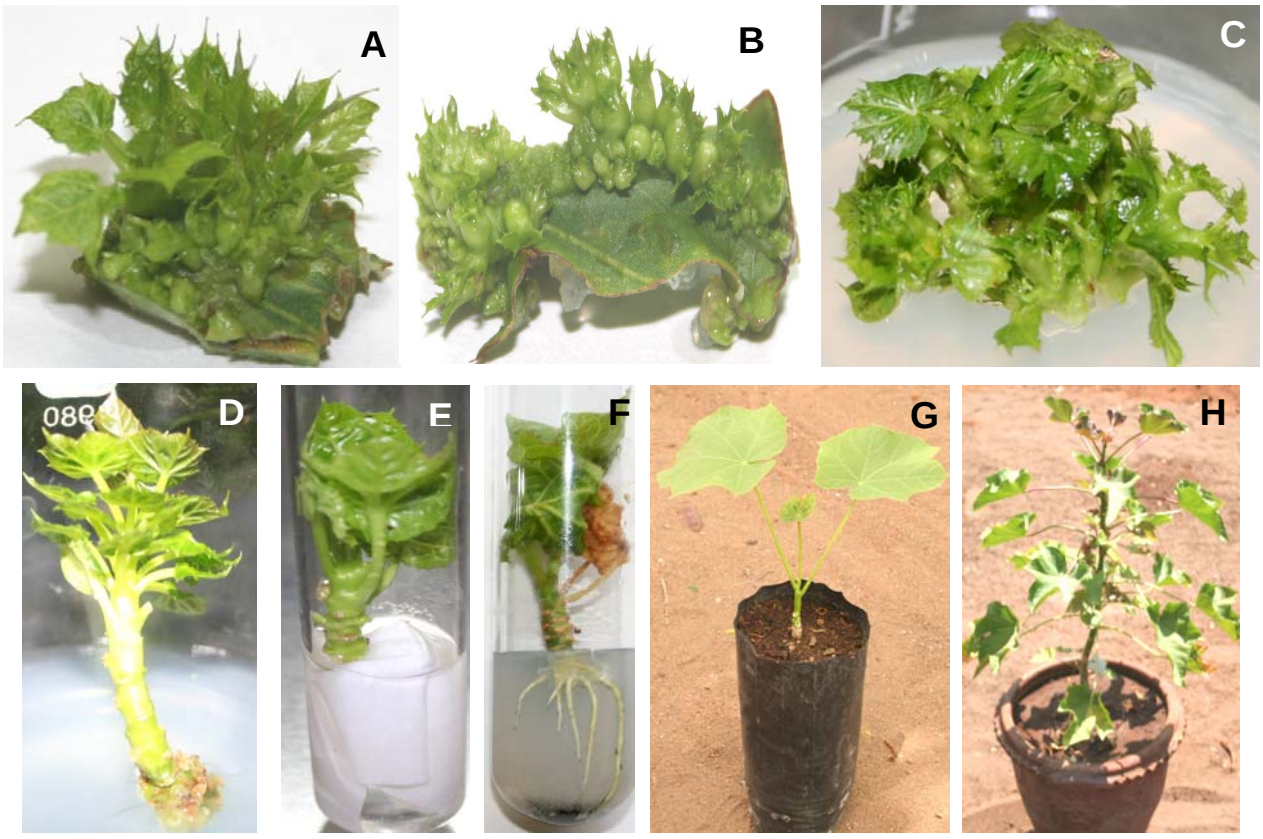

Plate 6. Direct shoot bud induction from cotyledonary leaf explant of toxic cultivar of *J.curcas*.

Direct shoot bud induction from (A) *in vitro* cotyledonary leaf explnt and (B) *in vivo* cotyledonary leaf explant on MS medium with 0.5 mg/L thidiazuron (TDZ). (C) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L kinetin (Kn) + 1 mg/L 6-benzyl aminopurine (BAP) + 1 mg/L α-naphthaleneacetic acid (NAA). (D) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP + 1.5 mg/L indole-3-acetic acid (IAA). (E) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1 mg/L IAA + 1 mg/L NAA. (F) Root initiation and elongation on half strength MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (G) Regenerated plant in polybag. (H) Regenerated plant in soil after 6 month under natural condition.

Table 10. Effect of various plant growth regulators (PGRs) on elongation of proliferated shoot from cotyledonary leaf explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

PGRs (mg/L)				Mean shoot length (cm)		
BAP	IAA	NAA	IBA	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.5	0.5	0	0	3.09 ± 0.42^{ab}	2.98 ± 0.48^{b}	2.96 ± 0.63^{a}
1.0	0.5	0	0	2.72 ± 0.29^{b}	2.23 ± 0.44^{c}	1.97 ± 0.16^{d}
0.5	1.5	0	0	3.63 ± 0.26^{a}	3.54 ± 0.75^{a}	3.13 ± 0.98^{a}
1.0	1.5	0	0	2.92 ± 044^{b}	2.80 ± 0.41^{bc}	2.87 ± 0.69^{a}
0.5	0	0.5	0	1.13 ± 0.11^{e}	1.55 ± 0.55^{f}	1.90 ± 0.11^{d}
1.0	0	0.5	0	1.71 ± 0.21^{d}	1.45 ± 0.26^{f}	1.35 ± 0.18^{de}
0.5	0	1.5	0	1.91 ± 0.33^{d}	1.57 ± 0.43^{ef}	1.21 ± 0.21^{e}
1.0	0	1.5	0	1.11 ± 0.07^{e}	1.09 ± 0.19^{f}	1.13 ± 0.18^{e}
0.5	0	0	0.5	2.13 ± 0.45^{cd}	2.22 ± 0.33^{cd}	2.13 ± 0.21^{bc}
1.0	0	0	0.5	2.41 ± 0.43^{bc}	2.10 ± 0.41^{de}	2.02 ± 0.13^{cd}
0.5	0	0	1.5	2.29 ± 0.37^{c}	2.41 ± 0.31^{cd}	2.22 ± 0.24^{b}
1.0	0	0	1.5	2.31 ± 0.54^{d}	2.41 ± 0.45^{d}	2.52 ± 0.41^{ab}

BAP, 6-benzylaminopurine; NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 10). Significant differences in elongation were observed at different concentrations and combinations of PGRs and genotypes. BAP and IAA combination was found best in all the three genotypes. The best elongation (3.13-3.63 cm) was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 5D). The elongation ranged from 1.97-3.63 cm on medium containing BAP and IAA combinations (Table 10).The elongation inhibited on medium containing BAP and IBA. The elongation ranged from 2.02-2.52 cm on medium containing BAP and IBA combinations and 1.0 mg/L BAP and 1.5 mg/L IBA gave the best elongation (2.31-2.52 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 1.09-191 cm. The best elongation was observed in CSMCRI-JC-1 genotype (1.11-3.63 cm) followed by CSMCRI-JC-2 (1.09-3.54

cm). The least elongation was observed in CSMCRI-JC-3 genotype (1.13-3.13 cm) (Table 10).

4.2.1.3. Regeneration from *in vitro* and *in vivo* petiole explant

4.2.1.3. (a) Effect of TDZ with or without IBA on shoot bud induction

The concentration of TDZ with or without IBA significantly influenced the response of direct shoot bud induction from petiole explant in all the three toxic genotypes. The percent shoot bud induction and number of induced shoot buds per petiole explant was directly proportional to the concentration of TDZ. Of the different concentrations of TDZ tested with or without 1 mg/L IBA, highest percentage of shoot bud induction (73.74%) and highest number of shoot buds per petiole explant (14.09) was observed in the presence of 2 mg/L TDZ among all the three genotypes, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction at this concentration. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 33.19-58.35 and number of induced shoot buds per petiole explant of nodal leaf varied from 4.18-10.10 among all the three genotypes (Tables 11-14).

4.2.1.3. (b) Effect of orientation of explant on shoot bud induction

The orientation of petiole explant significantly influenced the response of direct shoot bud induction at tested concentration of TDZ alone and with 1 mg/L IBA among all the three toxic genotypes. The percentage of shoot bud induction and number of shoot buds per explant was higher in horizontal position as compared to vertical position in all the genotypes. The percentage of shoot bud induction varied from 3.81-73.74% in horizontal position (Plate 7A & B; Tables 11 & 13) and 2.84-58.85% in vertical position (Plate 7C & D; Tables 12 & 14), and number of induced shoot buds per explant varied from 2.01-14.09 in horizontal position (Tables 11 & 13) and 1.01-13.08 in vertical position (Tables 12 & 14) at tested concentration of TDZ alone and with 1 mg/L IBA among all the three genotypes.

4.2.1.3. (c) Effect of explant sources on shoot bud induction

The sources of petiole explant also influenced significantly the plant regeneration through shoot bud induction TDZ alone and with 1 mg/L IBA among all the three genotypes. *In vitro* petiole explant responded efficiently as compared

to *in vivo* petiole explant in all the three genotypes. The percentage of shoot bud induction varied from 3.91-73.74% in *in vitro* petiole explant (Plate 7A & C; Tables 11 & 12) and 2.84-61.19% in *in vivo* petiole explant (Plate 7B & D; Tables 13 & 14), and number of induced shoot buds per explant varied from 2.02-14.09 in *in vitro* petiole explant (Tables 11 & 12) and 1.01-13.00 in *in vivo* petiole explant of nodal leaf (Tables 13 & 14) at tested concentration of TDZ alone and with 1 mg/L IBA among all the three genotypes.

4.2.1.3. (d) Effect of genotype on shoot bud induction

Differences in percentage of shoot bud induction and the number of shoot buds per petiole explant was observed among all the three toxic genotypes studied at tested concentration of TDZ with or without IBA used. CSMCRI-JC-2 performed best at tested concentrations of TDZ with or without 1 mg/L IBA both in terms of percentage of shoot buds induction and the number of shoot buds per petiole explant followed by CSMCRI-JC-1. The percent of shoot bud induction of CSMCRI-JC-2 and CSMCRI-JC-1 was 7.02-73.74% and 2.84-62.13% respectively and number of shoot bud per petiole expalnt of CSMCRI-JC-2 and CSMCRI-JC-1 was 3.07-14.09 and 1.01-11.21 respectively. The response of CSMCRI-JC-3 was poor, both *in vitro* and *in vivo* petiole explant (Tables 11-14).

4.2.1.3. (e) Shoot proliferation and elongation of induced shoot buds

Approx 60-70% and 10-20% regenerated shoot buds on 0.05-0.5 mg/L TDZ with or without IBA and 1.0-2.0 mg/L TDZ with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA in all the four genotypes (Plate 7E). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 15). Significant differences in elongation were observed at different concentrations and combinations of PGRs and genotypes. BAP and IAA combination was found best in all the three genotypes. The best elongation (3.01-3.92 cm) was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 7F). The elongation ranged from 1.88-3.91 cm on medium containing BAP and IAA combinations. The elongation inhibited on medium containing BAP

Table 11. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3- butyric acid (IBA) on shoot bud induction from *in vitro* petiole explant in horizontal position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	6.71 ± 1.17^{a}	10.67 ± 2.67^{a}	5.89 ± 0.90^{a}	3.19 ± 0.36^{ab}	4.12 ± 0.42^{ab}	3.54 ± 0.41^{ab}
0.10	0	14.48 ± 1.75^{ab}	21.21 ± 2.31^{b}	13.64 ±1.17^{ab}	4.23 ± 0.55^{a}	6.55 ± 0.70^{b}	3.55 ± 0.76^{ab}
0.20	0	21.73 ± 2.03^{c}	29.26 ± 2.99^{c}	19.03 ± 2.61^{b}	5.71 ± 0.79^{b}	9.11 ± 0.91^{cd}	4.63 ± 0.51^{b}
0.50	0	50.07 ± 3.44^{d}	58.35 ± 3.71^{d}	41.92 ± 2.04^{c}	7.36 ± 0.61^{c}	10.10 ± 1.01^{d}	5.66 ± 0.63^{d}
1.00	0	59.88 ± 4.35^{d}	65.32 ± 4.46^{e}	49.53 ± 3.92^{d}	9.14 ± 0.64^{d}	11.16 ± 1.01^{d}	5.81 ± 0.55^{d}
2.00	0	62.13 ± 4.91^{e}	73.74 ± 5.63^{f}	52.25 ± 3.21^{e}	11.21 ± 1.05^{e}	14.09 ± 1.05^{e}	6.70 ± 0.75^{a}
0.05	1	5.61 ± 0.91^{a}	8.51 ± 1.21^{a}	4.69 ± 0.62^{a}	2.79 ± 0.62^{a}	3.42 ± 1.25^{a}	2.64 ± 0.61^{a}
0.10	1	11.07 ± 1.21^{ab}	19.01 ± 1.95^{a}	11.74 ±1.69^{ab}	3.63 ± 0.72^{ab}	5.65 ± 1.41^{b}	2.95 ± 1.41^{a}
0.20	1	17.03 ± 1.79^{c}	22.17 ± 2.51^{b}	16.01 ±1.68^{ab}	4.91 ± 1.21^{b}	9.11 ± 1.51^{cd}	3.13 ± 1.07^{ab}
0.50	1	41.06 ± 4.08^{a}	56.25 ± 5.01^{d}	37.82 ± 3.07^{c}	6.96 ± 1.34^{c}	9.11± 1.88^{cd}	5.06 ± 1.36^{c}
1.00	1	49.08 ± 4.11^{d}	63.12 ± 6.37^{e}	44.83 ±4.16^{cd}	8.54 ± 2.67^{d}	19.06 ± 2.48^{f}	4.89 ± 1.30^{b}
2.00	1	51.11 ± 5.61^{d}	66.77 ± 6.04^{e}	48.15 ± 4.05^{d}	10.21 ± 1.51^{e}	13.19 ± 1.67^{e}	5.71 ± 1.33^{d}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 12. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* petiole explant in vertical position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	5.70 ± 0.50^{b}	9.17 ± 1.05^{a}	4.86 ± 0.82^{a}	2.20 ± 0.21^{a}	5.94 ± 0.95^{ab}	3.67 ± 0.30^{b}
0.10	0	13.81 ± 1.21^{d}	18.46 ± 1.99^{b}	11.69 ± 1.52^{b}	3.59 ± 0.39^{b}	7.95 ± 1.48^{c}	3.06 ± 0.27^{a}
0.20	0	19.47 ± 2.12^{f}	29.38 ± 2.47^{d}	19.80 ± 2.28^{c}	4.90 ± 1.15^{c}	8.21 ± 1.85^{d}	4.52 ± 1.26^{b}
0.50	0	39.76 ± 3.27^{h}	51.19 ± 2.54^{e}	38.66 ± 2.58^{d}	6.39 ± 1.51^{e}	9.65 ± 2.76^{e}	5.02 ± 1.48^{c}
1.00	0	48.60 ± 4.02^{i}	58.85 ± 4.94^{f}	46.54 ±3.85^{ef}	9.43 ± 1.99^{f}	10.39 ± 2.55^{fg}	5.40 ± 1.10^{d}
2.00	0	52.56 ± 4.05^{i}	61.22 ± 6.45^{f}	49.33 ± 4.44^{f}	10.85 ± 1.24^{g}	13.08 ± 2.69^{g}	5.65 ± 1.04^{a}
0.05	1	4.10 ± 1.22^{a}	8.06 ± 1.41^{a}	3.91 ± 0.97^{a}	2.02 ± 0.61^{a}	4.91 ± 0.97^{a}	2.97 ± 0.68^{a}
0.10	1	9.71 ± 1.70^{c}	15.16 ± 1.14^{b}	10.51 ± 1.76^{b}	3.01 ± 0.89^{b}	6.95 ± 1.57^{b}	3.00 ± 0.78^{a}
0.20	1	15.51 ± 1.36^{e}	23.37 ± 2.13^{c}	17.81 ± 1.91^{c}	4.01 ± 1.01^{c}	7.91 ± 1.88^{c}	4.02 ± 0.93^{b}
0.50	1	31.46 ± 3.79^{g}	49.10 ± 4.36^{e}	36.06 ± 3.08^{d}	5.71 ± 1.07^{de}	8.75 ± 2.09^{d}	4.11 ± 0.94^{b}
1.00	1	46.51 ± 4.12^{i}	51.71 ± 5.01^{e}	41.54 ± 4.09^{e}	8.48 ± 1.65^{f}	9.09 ± 2.19^{e}	5.01 ± 0.64^{c}
2.00	1	49.46 ± 4.13^{i}	53.21 ±5.11^{ef}	45.31 ±4.01^{ef}	9.86 ± 1.91^{f}	12.11 ± 2.11^{fg}	5.05 ± 1.01^{c}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 13. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* petiole explant in horizontal position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	4.87 ± 0.68^{ab}	8.92 ± 1.07^{a}	5.01 ± 1.51^{a}	3.07 ± 0.91^{a}	4.08 ± 0.52^{a}	3.39 ± 0.33^{b}
0.10	0	12.52 ± 1.86^{c}	19.67 ± 2.21^{b}	11.84 ± 1.71^{b}	4.62 ± 1.18^{b}	7.32 ± 0.91^{b}	3.56 ± 0.57^{b}
0.20	0	19.13 ± 2.16^{e}	24.29 ± 2.43^{b}	18.12 ± 2.20^{c}	5.09 ± 1.28^{d}	8.02 ± 1.88^{c}	4.09 ± 1.06^{c}
0.50	0	35.13 ± 3.16^{f}	46.19 ± 4.76^{c}	40.07 ± 4.46^{e}	7.09 ± 1.03^{e}	9.43 ± 1.16^{cd}	5.10 ± 1.02^{d}
1.00	0	44.08 ± 4.79^{g}	59.08 ±5.78^{de}	48.17 ± 4.51^{a}	8.49 ± 1.08^{fg}	12.27 ± 1.76^{d}	5.30 ± 0.95^{d}
2.00	0	51.22 ± 5.4^{h}	61.19 ± 6.59^{e}	48.25 ± 4.30^{a}	9.87 ± 1.67^{a}	13.00 ± 1.11^{a}	6.39 ± 0.61^{f}
0.05	1	3.81 ± 1.05^{a}	7.82 ± 0.91^{a}	4.01 ± 0.81^{a}	2.01 ± 0.19^{a}	3.07 ± 1.29^{a}	2.39 ± 0.22^{a}
0.10	1	9.49 ± 1.71^{b}	17.01 ± 2.11^{b}	10.64 ± 1.11^{b}	3.51 ± 0.67^{c}	6.12 ± 1.71^{b}	2.57 ± 0.29^{a}
0.20	1	17.12 ± 1.36^{de}	21.28 ± 2.53^{b}	17.11 ± 1.10^{c}	4.19 ± 0.67^{cd}	7.12 ± 1.99^{b}	3.09 ± 0.63^{ab}
0.50	1	31.03 ± 3.17^{f}	44.09 ± 4.56^{c}	37.06 ± 3.16^{d}	6.08 ± 0.93^{de}	8.33 ± 1.76^{c}	4.80 ± 0.73^{c}
1.00	1	41.18 ± 4.99^{g}	56.08 ±5.57^{de}	44.16 ± 4.71^{e}	7.39 ± 0.99^{f}	11.47 ± 1.98^{d}	4.99 ± 0.71^{d}
2.00	1	49.02 ± 4.01^{h}	58.17 ±5.24^{de}	47.16 ± 4.12^{e}	8.86 ± 0.91^{g}	11.01 ± 1.91^{d}	5.81 ± 0.97^{ef}

Values represent means ± SD of 25 explant per treatment in three repeated experiments
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 14. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* petiole explant in vertical position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	3.04 ± 0.53^{a}	8.01 ± 1.90^{a}	4.06 ± 1.08^{a}	3.04 ± 0.20^{ab}	4.03 ± 0.76^{b}	3.10 ± 0.30^{b}
0.10	0	11.53 ± 2.46^{b}	17.61 ± 2.31^{b}	13.15 ± 1.35^{b}	4.13 ± 0.91^{b}	4.25 ± 0.96^{bc}	3.03 ± 0.70^{b}
0.20	0	17.73 ± 2.11^{c}	23.80 ± 2.05^{c}	19.15 ± 2.18^{c}	4.15 ± 0.99^{a}	5.27 ± 1.31^{d}	4.08 ± 1.42^{c}
0.50	0	33.19 ± 2.65^{de}	43.55 ± 3.47^{d}	39.11 ± 2.30^{d}	6.10 ± 1.10^{d}	6.99 ± 1.71^{f}	4.18 ± 0.79^{c}
1.00	0	41.34 ± 3.03^{fg}	56.52 ± 4.11^{e}	41.08 ± 3.05^{e}	7.06 ± 1.16^{e}	9.33 ± 2.52^{h}	5.04 ± 1.04^{e}
2.00	0	47.76 ± 3.17^{h}	58.32 ± 4.14^{e}	46.14 ± 4.05^{f}	8.03 ± 2.03^{f}	11.30 ± 2.40^{a}	5.29 ± 2.04^{e}
0.05	1	2.84 ±0.87^{a}	7.02 ± 1.09^{a}	3.16 ± 0.62^{a}	1.01 ± 0.11^{a}	3.13 ± 0.41^{a}	2.83 ± 0.68^{a}
0.10	1	9.51 ±1.51^{b}	15.51 ± 1.51^{b}	11.05 ± 1.76^{b}	4.03 ± 1.02^{b}	4.15 ± 0.65^{b}	2.89 ± 0.39^{a}
0.20	1	15.63 ±1.61^{c}	21.61 ± 2.08^{c}	17.17 ± 2.05^{c}	4.05 ± 0.97^{b}	4.87 ± 0.76^{c}	3.78 ± 0.91^{bc}
0.50	1	29.09 ±2.73^{d}	42.45 ± 4.91^{d}	37.01 ± 3.12^{d}	5.19 ± 1.01^{c}	5.99 ± 1.01^{e}	3.68 ± 0.95^{bc}
1.00	1	37.64 ±3.85^{e}	56.41 ± 5.37^{e}	39.07 ± 3.61^{d}	6.16 ± 1.09^{d}	8.43 ± 1.67^{g}	4.74 ± 1.05^{d}
2.00	1	45.16 ±4.71^{gh}	56.12 ± 5.09^{e}	45.04 ± 4.13^{f}	7.19 ± 1.73^{e}	9.30 ±1.39^{h}	4.79 ± 1.11^{d}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

and IBA. The elongation ranged from 2.00-2.44 cm on medium containing BAP and IBA combinations and 1mg/L BAP and 1.5 mg/L IBA gave the best elongation (2.12-2.44 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 1.01-199 cm. The best elongation was observed in CSMCRI-JC-2 genotype (1.11-3.92 cm) followed by CSMCRI-JC-1 (1.01-3.52 cm). The least elongation was observed in CSMCRI-JC-3 genotype (1.22-2.89 cm) (Table 15).

Table 15. Effect of various plant growth regulators (PGRs) on elongation of proliferated shoots from petiole of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

PGRs (mg/L)				Mean shoot length (cm)		
BAP	IAA	NAA	IBA	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.5	0.5	0	0	3.01 ± 0.33^{ab}	3.11 ± 0.30^{b}	2.89 ± 0.67^{ab}
1.0	0.5	0	0	2.61 ± 0.29^{b}	2.73 ± 0.43^{c}	1.88 ± 0.15^{b}
0.5	1.5	0	0	3.52 ± 0.27^{a}	3.91 ± 0.33^{a}	3.01 ± 0.90^{b}
1.0	1.5	0	0	2.81 ± 033^{b}	2.94 ± 0.84^{bc}	2.80 ± 0.60^{b}
0.5	0	0.5	0	1.01 ± 0.13^{e}	1.11 ± 0.14^{f}	1.99 ± 0.11^{e}
1.0	0	0.5	0	1.71 ± 0.21^{d}	1.82 ± 0.28^{f}	1.32 ± 0.15^{d}
0.5	0	1.5	0	1.91 ± 0.30^{d}	1.88 ± 0.31^{ef}	1.22 ± 0.21^{d}
1.0	0	1.5	0	1.01 ± 0.19^{e}	1.10 ± 0.18^{f}	1.10 ± 0.20^{e}
0.5	0	0	0.5	2.37 ± 0.42^{cd}	2.17 ± 0.51^{cd}	2.02 ± 0.21^{cd}
1.0	0	0	0.5	2.41 ± 0.50^{bc}	2.11 ± 0.61^{de}	2.00 ± 0.23^{bc}
0.5	0	0	1.5	2.40 ± 0.40^{c}	2.17 ± 0.52^{cd}	2.30 ± 0.29^{c}
1.0	0	0	1.5	2.36 ± 0.65^{d}	2.12 ± 0.42^{d}	2.44 ± 0.41^{d}

BAP, 6-benzylaminopurine; NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

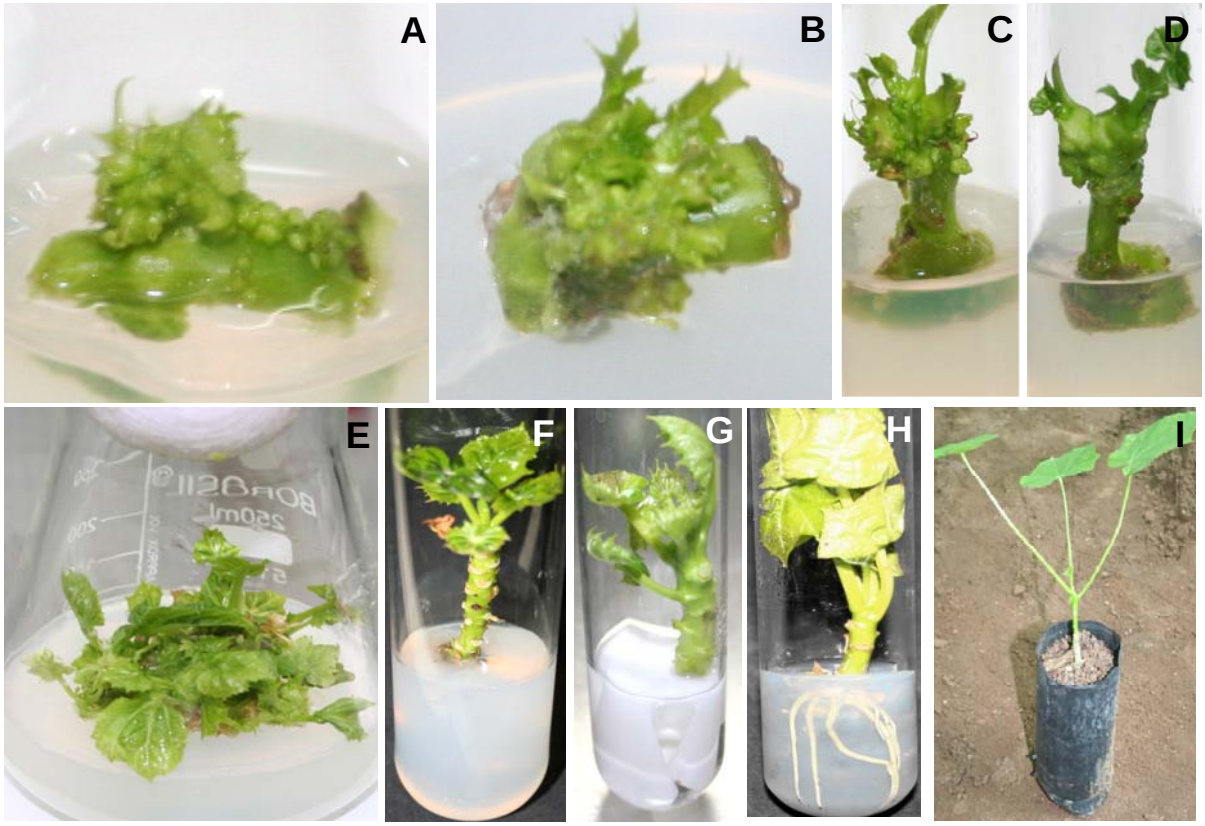

Plate 7. Direct shoot bud induction from petiole explant of toxic cultivar of *J.curcas.*

Direct shoot bud induction from (A) *in vitro* petiole in horizontal position (B) *in vivo* petiole in horizontal position (C) *in vitro* petiole in vertical position and (D) *in vivo* petiole in vertical position on MS medium with 0.5 mg/L TDZ. (E) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA. (F) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (G) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1mg/L IAA + 1 mg/L NAA. (H) Root initiation and elongation on hormone free half strength MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (I) Regenerated plant in polybag.

4.2.1.4. Regeneration from *in vitro* and *in vivo* cotyledonary petiole explant

4.2.1.4. (a) Effect of TDZ with or without IBA on shoot bud induction

The concentration of TDZ with or without IBA influenced the response of direct shoot bud induction from cotyledonary petiole explant in all the three toxic genotypes. The percent shoot bud induction and number of induced shoot buds per cotyledonary petiole explant was directly proportional to the concentration of TDZ. Of the different concentrations of TDZ tested with or without 1 mg/L IBA, highest percentage of shoot bud induction (66.97%) and highest number of shoot buds per cotyledonary petiole explant (13.76) was observed in the presence of 2 mg/L TDZ among all the three genotypes, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction at this concentration. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 39.69-51.19 and number of induced shoot buds per petiole explant varied from 4.75-9.75 among all the three toxic genotypes (Tables 16-19).

4.2.1.4. (b) Effect of orientation of explant on shoot bud induction

The orientation of cotyledonary petiole explant significantly influenced the response of direct shoot bud induction at tested concentration of TDZ alone and with 1 mg/L IBA among all the three genotypes. The percentage of shoot bud induction and number of shoot buds per cotyledonary petiole explant was higher in horizontal position as compared to vertical position in all the three toxic genotypes. The percentage of shoot bud induction varied from 4.01-66.97% in horizontal position (Plate 8A & B; Tables 16 & 18) and 3.01-62.97% in vertical position (Plate 8C & D; Tables 17 & 19), and number of induced shoot buds per cotyledonary petiole explant varied from 2.01-13.76 in horizontal position (Tables 16 & 18) and 1.91-10.18 in vertical position (Tables 17 & 19) at tested concentration of TDZ alone and with 1 mg/L IBA among all the three toxic genotypes.

4.2.1.4. (c) Effect of explant sources on shoot bud induction

The source of cotyledonary petiole explant also influenced significantly the plant regeneration through shoot bud induction TDZ alone and with 1 mg/L IBA among all the three toxic genotypes. *In vitro* cotyledonary petiole explant responded efficiently as compared to *in vivo* cotyledonary petiole explant in all

the three genotypes. The percentage of shoot bud induction varied from 3.01-66.97% in *in vitro* cotyledonary petiole explant (Plate 8A & C; Tables 16 & 17) and 6.01-63.02% in *in vivo* cotyledonary petiole explant (Plate 8B & D; Tables 18 & 19), and number of induced shoot buds per explant varied from 1.91-13.76 in *in vitro* cotyledonary petiole explant (Tables 16 & 17) and 2.01-13.28 in *in vivo* cotyledonary petiole explant (Tables 18 & 19) at tested concentration of TDZ alone and with 1 mg/L IBA among all the three genotypes.

4.2.1.4. (d) Effect of genotype on shoot bud induction

Significant differences in percentage of shoot bud induction and the number of shoot buds per explant was observed among all the three genotypes at tested concentration of TDZ with or without IBA used. CSMCRI-JC-3 performed best at tested concentrations of TDZ with or without 1 mg/L IBA both in terms of percentage of shoot buds induction per explant followed by CSMCRI-JC-1. The percent of shoot bud induction of CSMCRI-JC-2 was least in compare to other genotype. CSMCRI-JC-1 performed best at tested concentrations of TDZ with or without 1 mg/L IBA both in terms of number of shoot buds induction per explant followed by CSMCRI-JC-2. The percent of shoot bud induction of CSMCRI-JC-1, CSMCRI-JC-2 and CSMCRI-JC-3 were varied from 4.70-61.66%, 3.01-59.93% and 7.86-66.97% varied respectively and number of shoot buds per cotyledonary petiole expalnt of CSMCRI-JC-1, CSMCRI-JC-2 and CSMCRI-JC-3 were varied 1.91-10.75, 2.01-5.98 and 4.01-13.76 (Tables 16-19).

4.2.1.4. (e) Shoot proliferation and elongation from induced shoot buds

Approx 60-70% and 10-20% regenerated shoot buds on 0.05-0.5 mg/L TDZ with or without IBA and 1.0-2.0 mg/L TDZ with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA in all the three toxic genotypes (Plate 8E). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 20). Significant differences in elongation were observed at different concentrations and combinations of PGRs and genotypes. BAP and IAA combination was found best in all the three genotypes.The best elongation (3.01-3.61 cm) was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA.

Table 16. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* cotyledonary petiole explant in horizontal position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	6.97 ± 1.28^{a}	6.87 ± 0.67^{ab}	11.27 ±1.60^{ab}	2.21 ± 0.62^{a}	3.65 ± 0.37^{ab}	5.97 ± 0.96^{ab}
0.10	0	15.21 ± 1.70^{b}	14.09 ±1.88^{cd}	22.56 ± 1.25^{c}	3.39 ± 0.72^{abc}	3.98 ± 0.85^{abc}	7.24 ± 1.86^{abc}
0.20	0	22.12 ± 2.22^{c}	21.00 ± 2.31^{e}	31.08 ± 2.43^{d}	4.80 ± 0.96^{abc}	4.65 ± 0.93^{cde}	8.98 ± 1.92^{bcd}
0.50	0	49.11 ± 4.89^{d}	48.06 ± 4.58^{f}	51.19 ± 5.46^{e}	6.37 ± 0.97^{bcd}	5.85 ± 0.94^{de}	9.43 ± 2.70^{cde}
1.00	0	60.10 ± 6.32^{e}	56.04 ±5.22^{gh}	59.05 ±5.85^{fg}	9.83 ± 1.15^{de}	5.65 ± 1.34^{cde}	10.87 ± 2.09^{def}
2.00	0	61.66 ± 6.34^{e}	59.93 ± 5.56^{h}	66.97 ± 6.72^{g}	10.65 ± 1.71^{e}	5.98 ± 1.01^{e}	13.76 ± 2.08^{f}
0.05	1	5.90 ± 1.22^{a}	4.01 ± 0.67^{a}	9.86 ± 0.91^{a}	2.01 ± 0.21^{a}	2.87 ± 0.71^{a}	4.76 ± 0.81^{a}
0.10	1	12.11 ± 1.70^{ab}	12.01 ±1.76^{bc}	17.76 ±1.04^{bc}	3.07 ± 0.69^{ab}	3.76 ± 0.68^{ab}	6.87 ± 1.47^{abc}
0.20	1	18.41 ± 1.82^{bc}	19.11 ±1.91^{de}	29.97 ± 2.13^{d}	4.61 ± 0.91^{abc}	4.67 ± 0.93^{cde}	7.91± 1.41^{bcd}
0.50	1	43.44 ± 3.79^{d}	46.76 ± 4.08^{f}	53.20 ±5.36^{ef}	5.51 ± 1.07^{abc}	4.12 ± 1.04^{bcd}	8.98 ± 1.69^{bcd}
1.00	1	48.61 ± 4.12^{d}	51.84 ±5.09^{fg}	61.01 ± 6.01^{g}	8.49 ± 1.75^{de}	5.98 ± 1.34^{e}	9.65 ± 2.04^{cde}
2.00	1	59.46 ± 5.13^{e}	55.01 ± 5.01^{g}	63.21 ± 6.11^{g}	9.76 ± 1.91^{e}	5.13 ± 0.91^{cde}	12.76 ± 2.51^{f}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 17. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3 butyric acid (IBA) on shoot bud induction from *in vitro* cotyledonary petiole explant in vertical position of three toxic genotypes of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	5.37 ± 1.08 [a]	5.17 ± 0.49 [ab]	9.21 ± 1.10 [ab]	2.01 ± 0.42 [a]	3.05 ± 0.27 [ab]	5.17 ± 0.86 [ab]
0.10	0	13.11 ± 1.30 [b]	13.19 ± 1.68 [c]	21.16 ± 1.05 [c]	3.29 ± 0.52 [ab]	3.88 ± 0.75 [bc]	7.01 ± 1.67 [abc]
0.20	0	29.02 ± 2.02 [c]	20.00 ± 2.71 [d]	29.08 ± 2.03 [d]	4.10 ± 0.76 [b]	4.15 ± 0.63 [a]	8.67 ± 1.02 [cd]
0.50	0	47.91 ± 4.09 [d]	46.16 ± 4.18 [e]	49.19 ± 5.06 [e]	6.07 ± 0.57 [c]	4.75 ± 0.84 [c]	9.01 ± 2.11 [cd]
1.00	0	56.10 ± 5.02 [e]	54.04 ±5.12 [fg]	57.05 ± 5.05 [f]	8.13 ± 1.05 [de]	4.75 ± 1.04 [c]	10.06 ± 2.01 [cde]
2.00	0	59.16 ± 6.14 [e]	57.93 ± 5.16 [g]	62.97 ± 6.02 [f]	9.05 ± 1.71 [e]	5.08 ± 1.11 [c]	13.01 ± 2.11 [e]
0.05	1	4.70 ± 1.21 [a]	3.01 ± 0.37 [a]	7.86 ± 0.61 [a]	1.91 ± 0.11 [a]	2.07 ± 0.61 [a]	4.01 ± 0.71 [a]
0.10	1	11.19 ± 1.20 [b]	11.01 ±1.46 [bc]	15.74 ±1.14 [bc]	3.01 ± 0.59 [ab]	3.16 ± 0.38 [ab]	5.1 7 ± 1.41 [ab]
0.20	1	17.61 ± 1.02 [b]	17.11 ±1.11 [cd]	27.97 ± 2.03 [d]	4.01 ± 0.81 [b]	4.07 ± 0.63 [bc]	7.81± 1.31 [bc]
0.50	1	41.04 ± 3.09 [d]	44.76 ± 4.10 [e]	51.20 ± 5.06 [e]	4.71 ± 1.01 [bc]	4.04 ± 0.98 [bc]	8.18 ± 1.12 [bc]
1.00	1	46.61 ± 4.02 [d]	49.81 ±5.01 [ef]	54.01 ± 5.01 [e]	6.45 ± 1.65 [cd]	5.34 ± 1.04 [c]	9.01 ± 2.01 [a]
2.00	1	57.46 ± 5.03 [e]	51.01 ±5.21 [ef]	56.21 ±6.05 [ef]	8.16 ± 1.81 [de]	5.23 ± 0.92 [c]	11.71 ± 2.11 [de]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 18. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* cotyledonary petiole explant in horizontal position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	6.71 ± 1.68 [a]	5.88 ± 1.47 [a]	11.10 ± 1.60 [a]	2.21 ± 0.62 [a]	3.09 ± 0.37 [a]	5.84 ± 0.78 [ab]
0.10	0	18.91 ± 1.71 [b]	16.89 ±1.88 [bc]	17.96 ± 2.15 [a]	3.09 ± 0.92 [ab]	3.65 ± 0.75 [a]	7.45 ± 1.07 [bcd]
0.20	0	21.07 ± 2.22 [b]	21.81 ± 2.31 [c]	39.98 ±3.43 [bc]	4.40 ± 0.96 [bc]	4.74 ± 0.83 [cde]	8.01 ± 1.08 [bcd]
0.50	0	41.66 ± 4.89 [d]	41.69 ± 4.58 [d]	48.19 ±4.46 [de]	6.89 ± 1.07 [de]	5.62 ± 0.94 [de]	9.75 ± 1.09 [de]
1.00	0	48.99 ± 4.32 [c]	51.04 ± 5.22 [e]	56.15 ±5.85 [ef]	9.83 ± 1.65 [fg]	5.98 ± 0.64 [e]	10.59 ± 1.59 [ef]
2.00	0	51.00 ± 5.34 [c]	59.03 ± 5.56 [f]	63.02 ± 6.72 [f]	10.75 ± 1.71 [g]	5.54 ± 0.90 [cde]	13.28 ± 1.78 [g]
0.05	1	6.11 ± 1.22 [a]	6.91 ± 1.67 [a]	11.01 ± 1.51 [a]	2.82 ± 0.21 [ab]	2.97 ± 0.48 [a]	4.61 ± 0.76 [a]
0.10	1	10.01 ± 1.70 [a]	15.11 ± 1.76 [b]	16.06 ± 1.14 [a]	3.51 ± 1.09 [abc]	3.98 ± 0.98 [abc]	6.78 ± 1.07 [abc]
0.20	1	19.11 ± 2.32 [b]	19.21 ±2.01 [bc]	33.17 ± 3.13 [b]	4.51 ± 1.01 [bc]	4.32 ± 0.93 [bcd]	7.83 ± 1.38 [bcd]
0.50	1	33.06 ± 3.79 [c]	40.96 ± 4.08 [d]	43.21 ±4.36 [cd]	5.21 ± 0.99 [cd]	4.09 ± 0.94 [bcd]	8.35 ± 1.69 [cde]
1.00	1	46.91 ± 4.12 [de]	47.84 ± 4.09 [e]	51.91 ± 5.01 [e]	8.68 ± 1.15 [ef]	5.02 ± 1.04 [cde]	9.84 ± 1.09 [de]
2.00	1	49.06 ± 4.13 [c]	51.11 ± 5.01 [e]	53.01 ± 5.11 [e]	9.36 ± 0.92 [fg]	5.09 ± 1.01 [cde]	12.71 ± 1.71 [fg]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 19. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vivo* cotyledonary petiole explant in vertical position of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)			No. of buds / cotyledonary petiole explant		
		CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.05	0	6.11 ± 1.48 [a]	5.23 ± 1.17 [a]	9.11 ± 1.10 [a]	2.11 ± 0.51 [a]	3.01 ± 0.36 [ab]	5.01 ± 0.28 [ab]
0.10	0	18.01 ± 1.61 [b]	16.09 ±1.38 [bc]	16.98 ± 2.11 [b]	2.99 ± 0.82 [ab]	3.11 ± 0.71 [ab]	6.15 ± 0.97 [bcd]
0.20	0	20.07 ± 2.02 [b]	19.71 ± 2.11 [c]	37.90 ±3.41 [cd]	4.01 ± 0.91 [bcd]	4.01 ± 0.81 [bc]	7.01 ± 1.01 [cde]
0.50	0	40.36 ± 4.19 [d]	39.69 ± 4.08 [d]	47.17 ± 4.41 [e]	5.79 ± 1.17 [efg]	5.12 ± 0.91 [c]	8.15 ± 1.16 [def]
1.00	0	46.90 ± 4.12 [ef]	49.04 ±5.02 [ef]	54.15 ± 5.75 [e]	6.13 ± 1.45 [efg]	5.18 ± 0.74 [c]	9.19 ± 1.29 [ef]
2.00	0	49.10 ± 5.14 [f]	51.03 ± 4.87 [f]	61.02 ± 6.02 [f]	8.15 ± 1.11 [a]	5.19 ± 0.60 [c]	10.18 ± 1.88 [f]
0.05	1	6.01 ± 1.15 [a]	6.11 ± 1.07 [a]	9.01 ± 1.11 [a]	2.12 ± 0.11 [a]	2.01 ± 0.38 [a]	4.11 ± 0.66 [a]
0.10	1	9.11 ± 1.12 [a]	13.11 ± 1.06 [b]	14.06 ±1.01 [ab]	3.11 ± 1.01 [abc]	3.08 ± 0.88 [ab]	5.18 ± 1.17 [abc]
0.20	1	17.89 ± 2.78 [b]	17.21 ±2.11 [bc]	31.17 ± 3.89 [c]	4.01 ± 0.99 [bcd]	4.12 ± 0.83 [bc]	6.73 ± 1.28 [bcd]
0.50	1	31.06 ± 3.74 [c]	38.96 ± 4.18 [d]	40.11 ± 4.22 [d]	5.01 ± 0.92 [def]	4.08 ± 0.91 [bc]	7.31 ± 1.49 [cde]
1.00	1	44.71 ± 4.11 [def]	43.84 ±4.76 [de]	48.65 ± 5.11 [e]	6.18 ± 1.05 [efg]	5.01 ± 1.14 [c]	8.14 ± 1.29 [def]
2.00	1	43.06 ± 4.03 [de]	49.11 ±5.11 [ef]	51.01 ± 4.99 [e]	7.31 ± 1.02 [fg]	5.12 ± 1.01 [c]	9.70 ± 1.11 [f]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

Table 20. Effect of various plant growth regulators (PGRs) on elongation of proliferated shoots from cotyledonary petiole explant of three genotypes of toxic cultivar of *J. curcas* after 6 weeks.

PGRs (mg/L)				Mean shoot length (cm)		
BAP	IAA	NAA	IBA	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.5	0.5	0	0	2.99 ± 0.39 [e]	2.91 ± 0.41 [ef]	2.88 ± 0.53 [c]
1.0	0.5	0	0	2.66 ± 0.25 [de]	2.16 ± 0.41 [cd]	1.91 ± 0.14 [b]
0.5	1.5	0	0	3.61 ± 0.23 [f]	3.45 ± 0.71 [f]	3.01 ± 0.83 [c]
1.0	1.5	0	0	2.43 ± 044 [de]	2.51 ± 0.41 [de]	2.47 ± 0.61 [bc]
0.5	0	0.5	0	1.01 ± 0.11 [a]	1.15 ± 0.55 [a]	1.19 ± 0.11 [a]
1.0	0	0.5	0	1.41 ± 0.24 [ab]	1.41 ± 0.20 [ab]	1.25 ± 0.19 [a]
0.5	0	1.5	0	1.81 ± 0.31 [bc]	1.56 ± 0.41 [abc]	1.20 ± 0.22 [a]
1.0	0	1.5	0	1.01 ± 0.06 [a]	1.01 ± 0.16 [a]	1.11 ± 0.16 [a]
0.5	0	0	0.5	2.19 ± 0.43 [cd]	2.11 ± 0.23 [a]	2.01 ± 0.11 [b]
1.0	0	0	0.5	2.11 ± 0.33 [cd]	2.01 ± 0.31 [bcd]	2.01 ± 0.13 [b]
0.5	0	0	1.5	2.19 ± 0.27 [cd]	2.11 ± 0.11 [bcd]	2.01 ± 0.21 [b]
1.0	0	0	1.5	2.11 ± 0.51 [cd]	2.31 ± 0.41 [de]	2.12 ± 0.31 [b]

BAP, 6-benzylaminopurine; NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SE of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

The elongation ranged from 1.91-3.61 cm on medium containing BAP and IAA combinations (Plate 8F; Table 20). The elongation inhibited on medium containing BAP and IBA. The elongation ranged from 2.01-2.31 cm on medium containing BAP and IBA combinations and 1mg/L BAP and 1.5 mg/L IBA gave the best elongation (2.11-2.31 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 1.01-181 cm. The best elongation was observed in CSMCRI-JC-1 genotype (1.01-3.61 cm) followed by CSMCRI-JC-2 (1.01-3.45 cm). The least elongation was observed in CSMCRI-JC-3 genotype (1.11-3.01 cm) (Table 20).

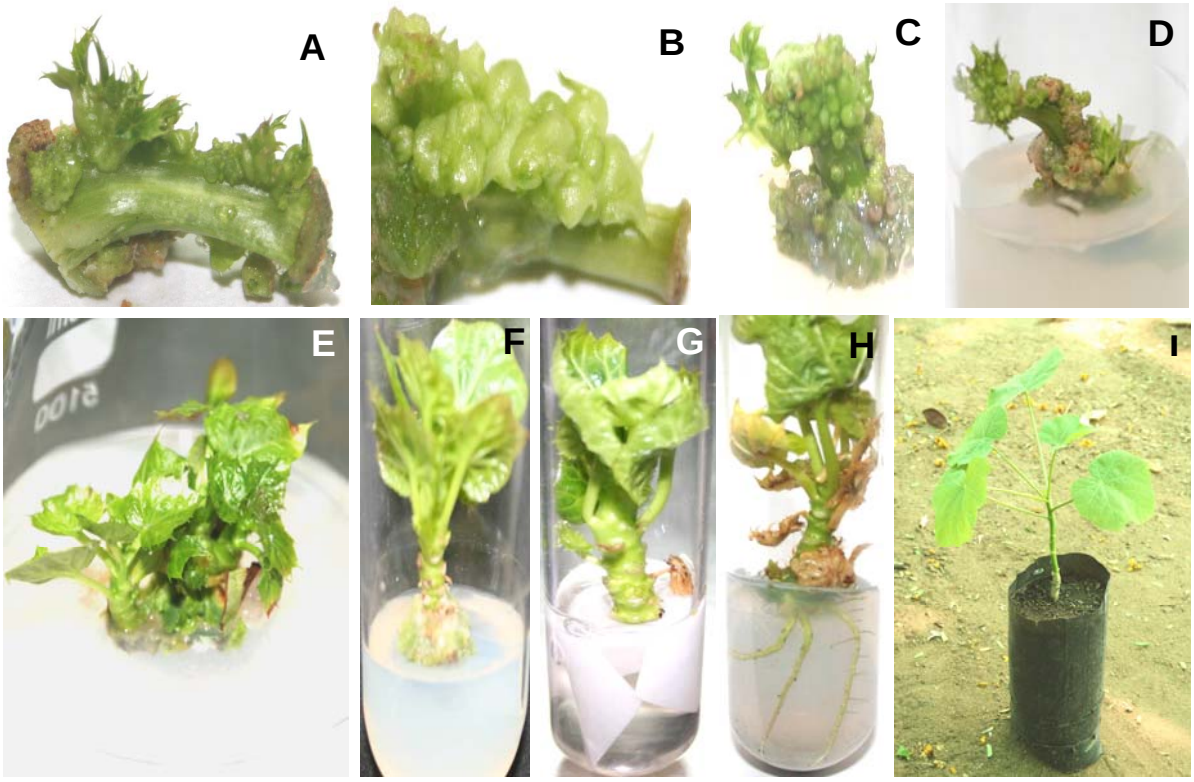

Plate 8. Direct shoot bud induction from cotyledonary petiole explant of toxic cultivar of *J.curcas.*

Direct shoot bud induction from (A) *in vitro* petiole in horizontal position, (B) *in vivo* petiole in horizontal position, (C) *in vitro* petiole in vertical position and (D) *in vivo* petiole in vertical position on MS medium with 0.5 mg/L TDZ. (E) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA. (F) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (G) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1 mg/L IAA + 1 mg/L NAA. (H) Root initiation and elongation on half strength of MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (I) Regenerated plant in polybag.

4.2.2. Regeneration in non-toxic cultivar *Jatropha curcas*

4.2.2.1. Regeneration from *in vitro* and *in vivo* leaf explant

4.2.2.1. (a) Effect of PGRs on shoot bud induction

The PGRs had effect significantly on shoot bud induction also in non-toxic *J.curcas* cultivar. The regeneration efficiency was higher in TDZ containing medium as compared to BAP containing medium. When 1 mg/L IBA supplemented either in TDZ or BAP containing medium, regeneration efficiency decreased. The percentage of shoot bud induction and number of shoot buds per leaf explant was directly proportional to the concentrations and combinations of PGRs used in the medium. Of the different concentrations of TDZ tested alone and with 1 mg/L IBA, highest percentage of shoot bud induction (68.36%) and highest number of shoot buds per leaf explant (5.71) was observed in the presence of 2.0 mg/L of TDZ, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction at this concentration. It was observed that 0.5 mg/L TDZ was found optimum for shoot bud induction and subsequent culture. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 31.22-63.68% and number of induced shoot buds per nodal leaf explant varied from 2.64-5.23 (Table 21). Of the different concentrations of BAP tested alone and with 1 mg/L IBA, highest percentage of shoot bud induction (49.67%) and highest number of shoot buds per leaf explant (3.99) was observed in the presence of 12.0 mg/L of BAP (Table 22).

4.2.2.1. (b) Effect of explant sources on shoot bud induction

The sources of leaf explant significantly influenced the plant regeneration through shoot bud induction. *In vitro* leaf explant responded efficiently as compared to *in vivo* leaf explant. The percentage of shoot bud induction varied from 19.31-68.36% in *in vitro* leaf explant (Plate 9A; Table 21) and 10.13-34.83% in *in vivo* leaf explant (Plate 9B; Table 21), and number of induced shoot buds per leaf explant varied from 3.01-5.71 in *in vitro* leaf explant and 0.75-3.14 in *in vivo* leaf explant (Table 21) at tested concentration of TDZ alone and with 1 mg/L IBA. The percentage of shoot bud induction varied from 13.92-49.67% in *in vitro* leaf explant and 7.02-25.11% in *in vivo* leaf explant and, number of induced shoot buds per explant varied from 2.05-3.99 in *in vitro* leaf explant and 0.63-

2.03 in *in vivo* leaf explant at tested concentration of BAP alone and with 1 mg/L IBA (Table 22).

Table 21. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* and *in vivo* leaf explant of non-toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / leaf explant	
		In vitro	*In vivo*	*In vitro*	*In vivo*
0.05	0	23.34 ± 2.32 [a]	11.14 ± 1.98 [ab]	3.11 ± 0.31 [a]	0.89 ± 0.15 [a]
0.10	0	34.61 ± 3.41 [bc]	15.80 ± 2.08 [bc]	4.31± 0.66 [ab]	1.64 ± 0.27 [abc]
0.20	0	44.19 ± 4.52 [d]	18.61 ± 2.60 [c]	4.73 ± 1.06 [ab]	1.84 ± 0.39 [bcd]
0.50	0	63.68 ± 5.14 [e]	31.22 ± 3.17 [de]	5.23 ± 1.41 [ab]	2.64 ± 0.69 [cde]
1.00	0	67.51 ± 6.16 [e]	33.82 ± 3.96 [de]	5.41 ± 1.43 [ab]	3.04 ± 0.95 [e]
2.00	0	68.36 ± 6.26 [e]	34.83 ± 3.76 [e]	5.71± 1.53 [b]	3.14 ± 0.85 [e]
0.05	1	19.31 ± 1.72 [a]	10.13 ± 1.01 [a]	3.01 ± 0.71 [a]	0.75 ± 0.09 [a]
0.10	1	31.67 ± 3.01 [b]	13.56 ± 2.07 [abc]	3.82 ± 0.81 [ab]	1.53 ± 0.21 [ab]
0.20	1	41.61 ± 4.41 [cd]	16.67 ± 2.05 [c]	4.03 ± 0.96 [ab]	1.51 ± 0.31 [ab]
0.50	1	59.67 ± 5.12 [e]	29.21 ± 3.56 [d]	5.03 ± 1.51 [ab]	2.43 ± 0.47 [cde]
1.00	1	63.65 ± 5.06 [e]	31.81 ± 3.45 [de]	5.12 ± 1.83 [ab]	2.94 ± 0.86 [de]
2.00	1	67.66 ± 6.01 [e]	31.81 ± 3.87 [de]	5.01 ± 1.63 [ab]	3.11 ± 0.91 [e]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.2.2.1 (c) Shoot proliferation and elongation from induced shoot buds

Approx 40-50%, 5-10% and 50-55% regenerated shoot buds on 0.05-0.5 mg/L TDZ with or without IBA and 1.0-2.0 mg/L TDZ with or without IBA and 3-12 mg/L BAP with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA (Plate 9C). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 23). Significant differences in elongation were observed at different concentrations and combinations of PGRs. BAP and IAA combination was found best. The best elongation 3.01 cm was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 9D).

Table 22. Effect of different concentrations of 6-benzylaminopurine (BAP) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* and *in vivo* leaf explant of non-toxic cultivar of *J. curcas* after 6 weeks.

BAP (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / leaf explant	
		In vitro	*In vivo*	*In vitro*	*In vivo*
3	0	14.11 ± 1.46 [a]	7.12 ± 1.61 [a]	2.09 ± 0.41 [a]	0.65 ± 0.21 [a]
6	0	26.01 ± 2.69 [b]	11.56 ± 1.91 [b]	3.11 ± 0.73 [b]	1.43 ± 0.69 [ab]
9	0	35.51 ± 3.51 [c]	13.66 ± 2.05 [b]	3.32 ± 0.41 [b]	1.49 ± 0.40 [ab]
12	0	49.67 ± 4.12 [d]	25.11 ± 2.17 [c]	3.99 ± 0.51 [b]	2.03 ± 0.57 [b]
3	1	13.92 ± 1.31 [a]	7.02 ± 1.51 [a]	2.05 ± 0.31 [a]	0.63 ± 0.15 [a]
6	1	26.00 ± 2.61 [b]	11.51 ± 1.31 [b]	3.01 ± 0.34 [ab]	1.41 ± 0.37 [ab]
9	1	34.91 ± 3.41 [c]	13.36 ± 1.49 [b]	3.21 ± 0.61 [b]	1.47 ± 0.51 [ab]
12	1	48.91 ± 4.82 [d]	25.01 ± 2.13 [c]	3.81 ± 0.81 [b]	2.01 ± 0.55 [b]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

The elongation ranged from 2.11-3.01 cm on medium containing BAP and IAA combinations (Table 23). The elongation inhibited on medium containing BAP and IBA. The elongation ranged from 1.94-2.11 cm on medium containing BAP and IBA combinations and 0.5 mg/L BAP and 0.5 mg/L IBA gave the best elongation (2.11 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 0.91- 1.71 cm (Table 23).

4.2.2.2. Regeneration from *in vitro* and *in vivo* cotyledonary leaf explant

4.2.2.2. (a) Effect of PGRs on shoot bud induction

The PGRs had a significant effect on shoot bud induction from cotyledonary leaf explant of non-toxic *J.curcas*. The regeneration efficiency was higher in TDZ containing medium as compared to BAP containing medium. When 1 mg/L IBA supplemented either in TDZ or BAP containing medium, regeneration efficiency was decreased. The percentage of shoot bud induction and number of shoot buds per cotyledonary leaf explant was directly proportional to the concentrations and combinations of PGRs. Of the different concentrations of TDZ tested alone and with 1 mg/L IBA, highest percentage of shoot bud

induction (81.07%) and highest number of shoot buds per cotyledonary leaf explant (20.17) was observed in the presence of 2.0 mg/L of TDZ, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction at this concentration (Table 24). It was observed that 0.5 mg/L TDZ was found optimum for shoot bud induction and subsequent culture. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 71.12-73.34% and number of induced shoot buds per cotyledonary leaf explants varied from 16.61-17.13 (Table 24). Of the different concentrations of BAP tested alone and with 1 mg/L IBA, highest percentage of shoot bud induction (51.07%) and highest number of shoot buds per cotyledonary leaf explant (3.95) was observed in the presence of 12.0 mg/L of BAP (Table 25).

Table 23. Effect of various plant growth regulators (PGRs) on elongation of proliferated shoots from leaf, petiole, cotyledonary leaf and cotyledonary petiole explant of non-toxic cultivar of *J. curcas* after 6 weeks.

PGRs (mg/L)				Mean shoot length (cm)			
BAP	IAA	NAA	IBA	Leaf	Petiole	Cotyledonary leaf	Cotyledonary petiole
0.5	0.5	0	0	2.11 ± 0.21 ^{bc}	2.31 ± 0.32 ^{b}	3.05 ± 0.48 ^{de}	2.33 ± 0.63 ^{b}
1.0	0.5	0	0	2.43 ± 0.21 ^{c}	2.11 ± 0.31 ^{b}	2.42 ± 0.44 ^{cd}	2.11 ± 0.17 ^{b}
0.5	1.5	0	0	3.01 ± 0.23 ^{d}	3.21 ± 0.71 ^{c}	3.26 ± 0.65 ^{e}	2.43 ± 0.61 ^{b}
1.0	1.5	0	0	2.11 ± 044 ^{bc}	2.19 ± 0.32 ^{b}	2.84 ± 0.63 ^{cde}	2.41 ± 0.21 ^{b}
0.5	0	0.5	0	0.91 ± 0.11 ^{a}	1.11 ± 0.25 ^{a}	1.23 ± 0.31 ^{a}	1.05 ± 0.11 ^{a}
1.0	0	0.5	0	1.21 ± 0.14 ^{a}	1.31 ± 0.10 ^{a}	1.16 ± 0.41 ^{a}	1.01 ± 0.12 ^{a}
0.5	0	1.5	0	1.71 ± 0.21 ^{b}	1.36 ± 0.31 ^{a}	1.39 ± 0.39 ^{ab}	1.01 ± 0.11 ^{a}
1.0	0	1.5	0	1.01 ± 0.12 ^{a}	1.13 ± 0.16 ^{a}	1.04 ± 0.14 ^{a}	0.71 ± 0.11 ^{a}
0.5	0	0	0.5	2.11 ± 0.41 ^{bc}	2.01 ± 0.19 ^{b}	2.16 ± 0.38 ^{bc}	2.09 ± 0.11 ^{b}
1.0	0	0	0.5	2.08 ± 0.31 ^{bc}	1.96 ± 0.31 ^{b}	2.13 ± 0.41 ^{bc}	1.99 ± 0.11 ^{b}
0.5	0	0	1.5	2.18 ± 0.26 ^{bc}	2.01 ± 0.13 ^{b}	2.17 ± 0.43 ^{bc}	1.91 ± 0.23 ^{b}
1.0	0	0	1.5	1.94 ± 0.41 ^{bc}	2.11 ± 0.41 ^{b}	2.11 ± 0.29 ^{bc}	1.89 ± 0.17 ^{b}

BAP, 6-benzylaminopurine; NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

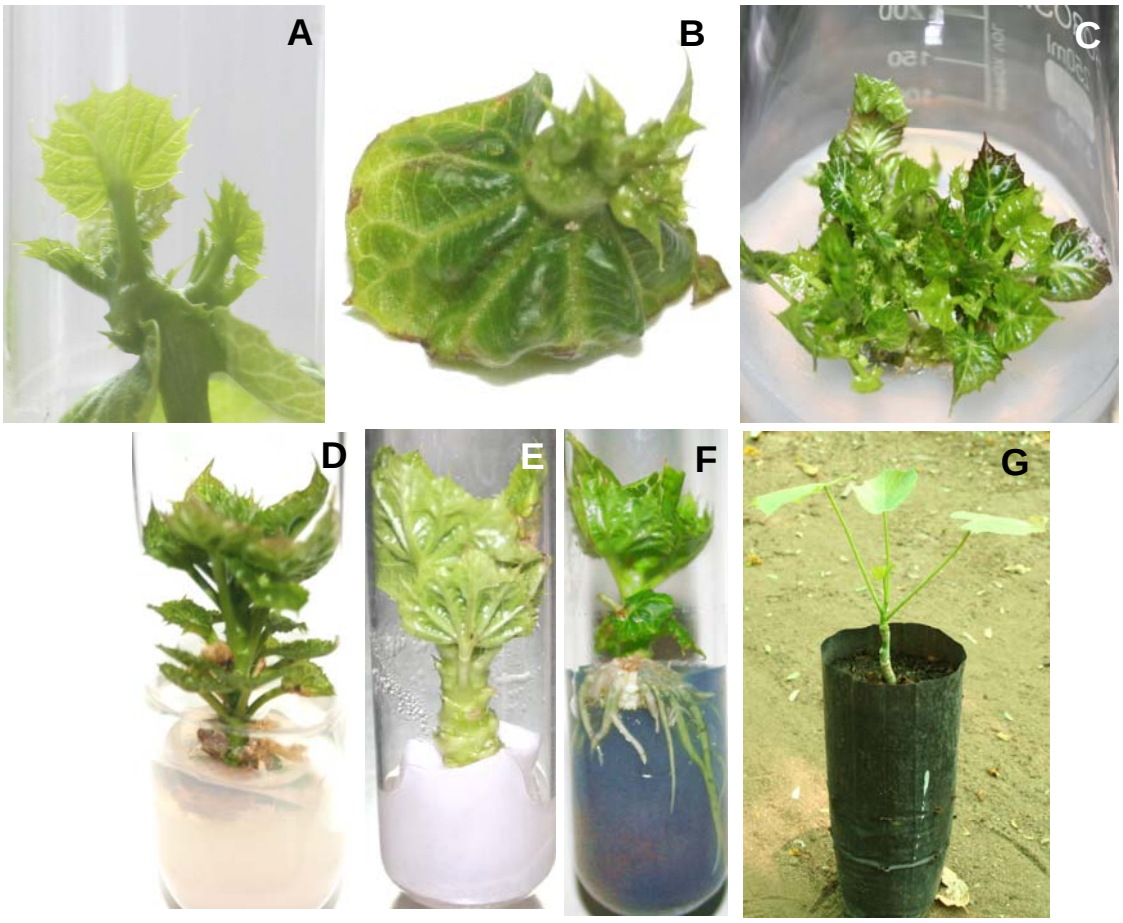

Plate 9. Direct shoot bud induction from leaf explant of non-toxic cultivar of *J. curcas.*

Direct shoot bud induction from (A) *in vitro* leaf explant and (B) *in vivo* leaf explant on MS medium with 0.5 mg/L TDZ. (C) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA. (D) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (E) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1 mg/L IAA +1 mg/L NAA. (F) Root initiation and elongation on half strength of MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (G) Regenerated plant in polybag.

Table 24. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from *in vitro* and *in vivo* cotyledonary leaf explant of non-toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / cotyledonary leaf explant	
		In vitro	*In vivo*	*In vitro*	*In vivo*
0.05	0	43.23 ± 4.52 ^{ab}	41.10 ± 4.98 ^{ab}	5.01 ± 1.21 ^{a}	5.00 ± 1.05 ^{a}
0.10	0	54.09 ± 5.51 ^{bcd}	51.98 ± 5.28 ^{cd}	9.13 ± 2.36 ^{b}	8.04 ± 1.20 ^{bc}
0.20	0	64.06 ± 6.51 ^{def}	61.11 ± 6.70 ^{def}	13.63 ± 2.56 ^{cd}	12.64 ± 1.33 ^{e}
0.50	0	73.34 ± 7.04 ^{fgh}	71.12 ± 7.27 ^{fgh}	17.13 ± 1.41 ^{de}	16.61 ± 1.38 ^{f}
1.00	0	77.90 ± 7.16 ^{gh}	75.72 ± 7.76 ^{fgh}	19.14 ± 1.43 ^{e}	18.74 ± 1.55 ^{f}
2.00	0	81.07 ± 8.26 ^{h}	79.11 ± 7.66 ^{h}	20.17 ± 2.53 ^{e}	19.24 ± 2.65 ^{f}
0.05	1	39.11 ± 3.42 ^{a}	37.87 ± 3.61 ^{a}	4.81 ± 1.21 ^{a}	4.75 ± 1.15 ^{a}
0.10	1	51.07 ± 5.01 ^{bc}	49.76 ± 4.67 ^{bc}	4.92 ± 1.31 ^{a}	4.53 ± 1.31 ^{a}
0.20	1	61.11 ± 6.41 ^{cde}	59.17 ± 5.65 ^{cde}	5.13 ± 1.66 ^{a}	5.51 ± 1.31 ^{ab}
0.50	1	69.04 ± 6.08 ^{efg}	65.11 ± 6.16 ^{efg}	11.53 ± 2.51 ^{bc}	9.93 ± 1.37 ^{cd}
1.00	1	71.75 ± 7.06 ^{fgh}	66.11 ± 6.05 ^{efg}	13.02 ± 2.83 ^{c}	12.04 ± 1.56 ^{de}
2.00	1	74.96 ± 7.01 ^{fgh}	66.11 ± 6.57 ^{efg}	14.11 ± 2.63 ^{cd}	13.65 ± 1.75 ^{e}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.2.2.2. (b) Effect of explant sources on shoot bud induction

The sources of cotyledonary leaf explant significantly influenced the plant regeneration through shoot bud induction. *In vitro* cotyledonary leaf explant responded more efficiently as compared to *in vivo* leaf explant of shoot culture. The percentage of shoot bud induction varied from 39.11-81.07% in *in vitro* cotyledonary leaf explant (Plate 10A; Table 24) and 37.87-79.11% in *in vivo* cotyledonary leaf explant (Plate 10B; Table 24), and number of induced shoot buds per cotyledonary leaf explant varied from 4.81-20.17 in *in vitro* cotyledonary leaf explant and 4.75-19.24 in *in vivo* cotyledonary leaf explant (Table 24) at tested concentration of TDZ alone and with 1 mg/L IBA. The percentage of shoot bud induction varied from 23.12-51.07% in *in vitro* cotyledonary leaf explant and 22.12-48.98% in *in vivo* cotyledonary leaf explant and, number of induced shoot buds per explant varied from 2.01-3.95 in *in vitro* cotyledonary leaf explant and

0.63-2.83 in *in vivo* cotyledonary leaf explant at tested concentration of BAP alone and with 1 mg/L IBA (Table 25).

Table 25. Effect of different concentrations of 6-benzylaminopurine (BAP) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot ud induction from *in vitro* and *in vivo* cotyledonary leaf explant of non-toxic cultivar of *J. curcas* after 6 weeks.

BAP (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / cotyledonary leaf explant	
		In vitro	*In vivo*	*In vitro*	*In vivo*
3	0	25.23 ± 2.31 [a]	23.11 ± 2.51 [a]	2.76 ± 0.45 [ab]	0.67 ± 0.10 [ab]
6	0	38.81 ± 2.99 [bc]	38.16 ± 3.41 [bc]	3.21 ± 0.63 [ab]	1.67 ± 0.39 [bc]
9	0	44.61 ± 4.01 [cd]	43.36 ± 4.45 [cde]	3.76 ± 0.71 [b]	1.89 ± 0.49 [cd]
12	0	51.07 ± 5.02 [d]	48.98 ± 4.11 [e]	3.95 ± 0.61 [b]	2.83 ± 0.77 [e]
3	1	23.12 ± 2.41 [a]	22.12 ± 2.50 [a]	2.01 ± 0.81 [a]	0.63 ± 0.09 [a]
6	1	36.87 ± 3.81 [b]	35.11 ± 3.31 [b]	3.11 ± 0.64 [ab]	1.61 ± 0.17 [abc]
9	1	41.11 ± 4.11 [bc]	40.26 ± 4.40 [bc]	3.67 ± 0.71 [b]	1.77 ± 0.41 [cd]
12	1	48.81 ± 4.12 [d]	46.11 ± 4.03 [de]	3.91 ± 0.81 [b]	2.71 ± 0.65 [de]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.2.2.2. (c) Shoot proliferation and elongation from induced shoot buds

Approx 50-60%, 5-10% and 60-70% regenerated shoot buds on 0.05-0.5 mg/L TDZ with or without IBA, 1.0-2.0 mg/L TDZ with or without IBA and 3-12 mg/L BAP respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA in all (Plate 10C). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 23). Significant differences in elongation were observed at different concentrations and combinations of PGRs. BAP and IAA combination was found best. The best elongation 3.26 cm was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 10D). The elongation ranged from 2.42-3.26 cm on medium containing BAP and IAA combinations. The elongation inhibited on medium containing BAP and IBA. The elongation ranged from 2.11-2.17 cm on medium containing BAP and IBA combinations and 0.5 mg/L BAP and 0.5 mg/L IBA gave the best elongation (2.17 cm). The BAP and

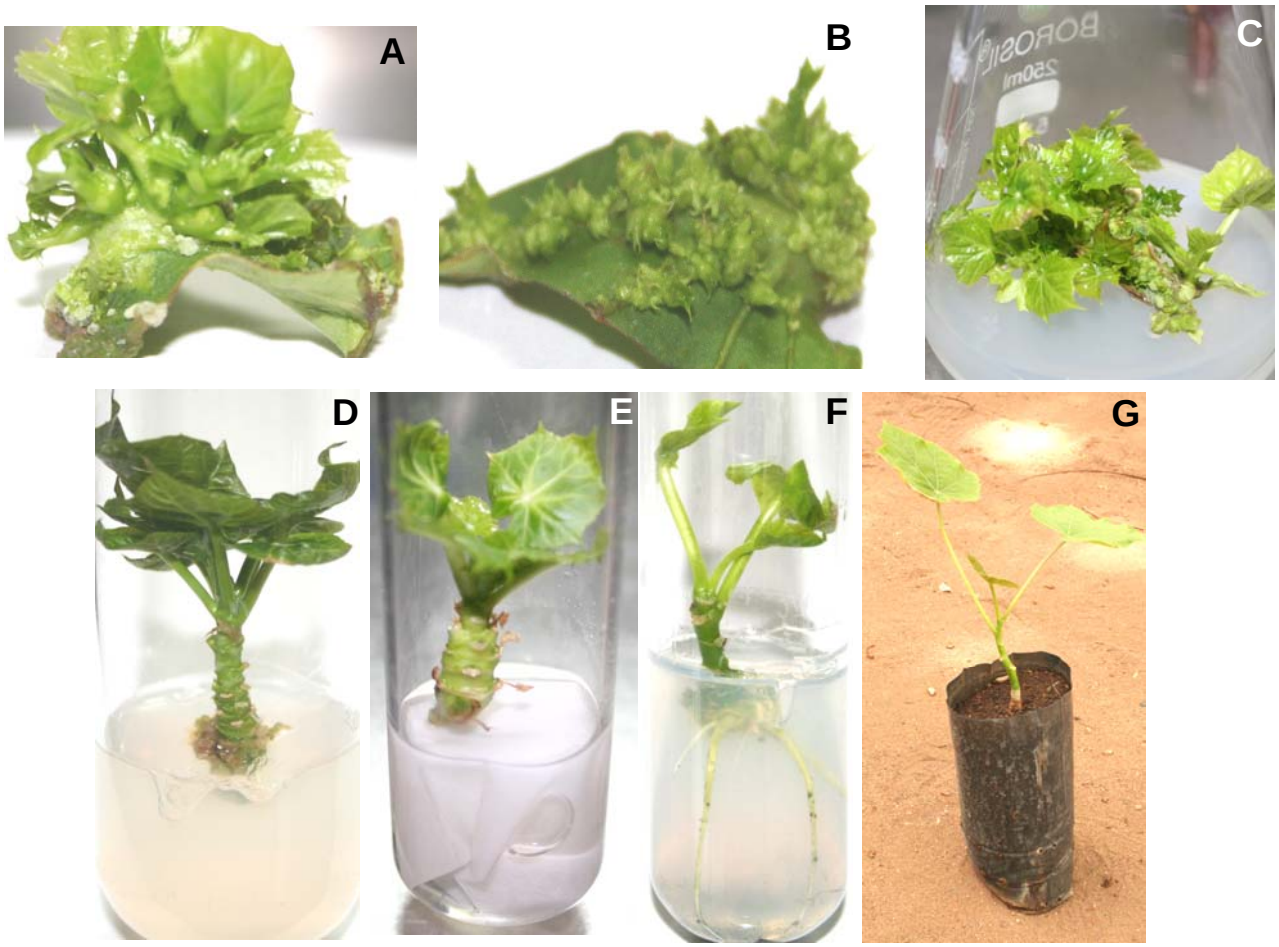

Plate 10. Direct shoot bud induction from cotyledonary leaf explant of non-toxic cultivar of *J. curcas.*

Direct shoot bud induction from (A) *in vitro* leaf explant and (B) *in vivo* leaf explant on MS medium with 0.5 mg/L TDZ. (C) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA. (D) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (E) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1mg/L IAA + 1 mg/L NAA. (F) Root initiation and elongation on hormone free half strength MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (G) Regenerated plant in polybag.

NAA combinations give the least elongation and the elongation ranged from 1.04-1.39 cm (Table 23).

Table 26. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from horizontally and vertically placed *in vitro* petiole explant of non-toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / petiole explant	
		Horizontal	Vertical	Horizontal	Vertical
0.05	0	17.21 ± 1.32 [a]	9.11 ± 1.51 [a]	2.99 ± 0.27 [a]	0.72 ± 0.13 [a]
0.10	0	29.37 ± 2.91 [b]	12.46 ± 2.57 [ab]	2.78 ± 0.29 [a]	1.48 ± 0.41 [ab]
0.20	0	39.52 ± 3.11 [c]	14.57 ± 2.51 [b]	3.98 ± 0.76 [ab]	1.49 ± 0.31 [ab]
0.50	0	57.61 ± 4.02 [de]	27.11 ± 3.06 [c]	4.98 ± 0.71 [a]	2.39 ± 0.46 [bc]
1.00	0	61.63 ± 4.01 [de]	29.18 ± 3.01 [c]	5.01 ± 0.81 [b]	2.83 ± 0.71 [c]
2.00	0	63.67 ± 4.11 [e]	30.71 ± 3.46 [c]	5.1 2 ± 0.91 [b]	3.01 ± 0.71 [c]
0.05	1	16.89 ± 1.29 [a]	9.08 ± 1.49 [a]	2.89 ± 0.37 [a]	0.71 ± 0.11 [a]
0.10	1	29.31 ± 2.89 [b]	12.05 ± 2.51 [ab]	2.71 ± 0.39 [a]	1.47 ± 0.42 [ab]
0.20	1	39.01 ± 3.01 [c]	14.08 ± 2.89 [ab]	3.91 ± 0.65 [ab]	1.46 ± 0.31 [ab]
0.50	1	56.01 ± 4.12 [d]	26.49 ± 3.11 [c]	4.78 ± 0.6 1 [b]	2.35 ± 0.42 [bc]
1.00	1	61.13 ± 4.11 [de]	28.18 ± 2.99 [c]	4.89 ± 0.81 [b]	2.81 ± 0.69 [c]
2.00	1	63.61 ± 4.21 [e]	30.01 ± 3.05 [c]	5.02 ± 1.11 [b]	2.99 ± 0.76 [c]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.2.2.3. Regeneration from *in vitro* and *in vivo* petiole explant

4.2.2.3. (a) Effect of TDZ with or without IBA on shoot bud induction

The concentration of TDZ with or without IBA also influenced significantly the response of direct shoot bud induction from petiole explant of non-toxic cultivar. The percent shoot bud induction and number of induced shoot buds per petiole explant of nodal leaf was directly proportional to the concentration of TDZ. Of the different concentrations of TDZ tested with or without 1 mg/L IBA, highest percentage of shoot bud induction (63.67%) and highest number of shoot buds per petiole explant (5.12) was observed in the presence of 2 mg/L TDZ, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction at this concentration. At 0.5 mg/L TDZ, the percentage of shoot

bud induction varied from 26.09-57.61% and number of induced shoot buds per petiole explant varied from 1.45-4.98 (Tables 26 & 27).

4.2.2.3. (b) Effect of orientation of explant on shoot bud induction

The orientation of petiole explant significantly influenced the response of direct shoot bud induction at tested concentration of TDZ alone and with 1 mg/L IBA. The percentage of shoot bud induction and number of shoot buds per explant was higher in horizontal position as compared to vertical position.

Table 27. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from horizontally and vertically placed in vivo petiole explant of non-toxic cultivar of J. curcas after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / petiole explant	
		Horizontal	Vertical	Horizontal	Vertical
0.05	0	16.08 ± 1.18 ^{a}	9.07 ± 1.48 ^{a}	2.71 ± 0.36 ^{abc}	0.70 ± 0.10 ^{a}
0.10	0	28.11 ± 2.71 ^{b}	11.01 ± 2.11 ^{a}	2.69 ± 0.37 ^{ab}	1.46 ± 0.41 ^{a}
0.20	0	37.01 ± 2.99 ^{c}	13.09 ± 2.99 ^{a}	3.82 ± 0.63 ^{bcd}	1.45 ± 0.30 ^{a}
0.50	0	55.11 ± 4.01 ^{d}	26.09 ± 3.01 ^{b}	4.76 ± 0.60 ^{cd}	2.41 ± 0.41 ^{b}
1.00	0	60.03 ± 4.01 ^{d}	27.28 ± 2.79 ^{b}	4.81 ± 0.71 ^{cd}	2.89 ± 0.69 ^{b}
2.00	0	61.11 ± 4.11 ^{d}	30.00 ± 3.04 ^{b}	5.01 ± 1.01 ^{d}	2.91 ± 0.75 ^{a}
0.05	1	15.02 ± 1.17 ^{a}	8.17 ± 1.48 ^{a}	2.69 ± 0.33 ^{ab}	0.69 ± 0.12 ^{a}
0.10	1	27.09 ± 2.62 ^{b}	10.09 ± 2.41 ^{a}	2.66 ± 0.35 ^{a}	1.45 ± 0.42 ^{a}
0.20	1	37.03 ± 2.79 ^{c}	12.99 ± 2.81 ^{a}	3.71 ± 0.62 ^{a}	1.44 ± 0.31 ^{a}
0.50	1	56.31 ± 4.1 1 ^{d}	25.99 ± 3.11 ^{b}	4.71 ± 0.59 ^{cd}	2.40 ± 0.40 ^{b}
1.00	1	59.02 ± 4.51 ^{d}	26.98 ± 2.41 ^{b}	4.80 ± 0.75 ^{cd}	2.87 ± 0.68 ^{b}
2.00	1	59.21 ± 4.91 ^{d}	29.56 ± 3.01 ^{b}	4.91 ± 1.02 ^{cd}	2.80 ± 0.74 ^{b}

Values represent means ± SE of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

The percentage of shoot bud induction varied from 15.02-63.67% in horizontal position (Plate 11A & B; Tables 26 & 27) and 8.17-30.71% in vertical position (Plate 11C & D; Tables 26 & 27), and number of induced shoot buds per explant varied from 2.66-5.12 in horizontal position (Tables 26 & 27) and 0.69-3.01 in

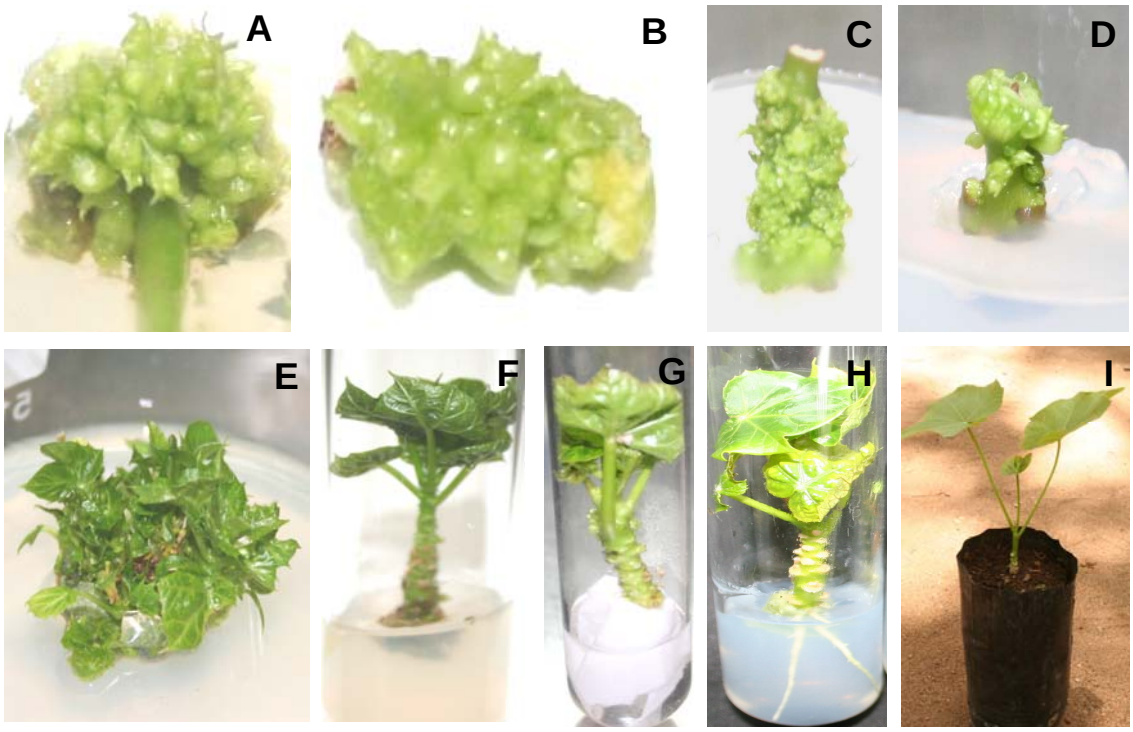

Plate 11. Direct shoot bud induction from petiole explant of non-toxic cultivar of *J. curcas*.

Direct shoot bud induction from (A) *in vitro* petiole in horizontal position, (B) *in vivo* petiole in horizontal position, (C) *in vitro* petiole in vertical position and (D) *in vivo* petiole in vertical position on MS medium with 0.5 mg/L TDZ. (E) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg /L BAP + 1 mg/L NAA. (F) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (G) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1 mg/L IAA +1 mg/L NAA. (H) Root initiation and elongation on half strength of MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (I) Regenerated plant in polybag.

vertical position (Tables 26 & 27) at tested concentration of TDZ alone and with 1 mg/L IBA.

4.2.2.3. (c) Effect of explant sources on shoot bud induction

The sources of petiole explant also influenced significantly the plant regeneration through shoot bud induction on TDZ alone and with 1 mg/L IBA containing medium. *In vitro* petiole explant responded efficiently as compared to *in vivo* petiole explant. The percentage of shoot bud induction varied from 9.08-63.67% in *in vitro* petiole explant (Plate 11A & C; Table 26) and 8.17-61.11% in *in vivo* petiole explant (Plate 11B & D; Table 27), and number of induced shoot buds per explant varied from 0.71-5.12 in *in vitro* petiole explant (Table 26) and 0.69-5.01 in *in vivo* petiole explant (Table 27) at tested concentration of TDZ alone and with 1 mg/L IBA.

4.2.2.3. (d) Shoot proliferation and elongation from induced shoot buds

Approx 30-49% and 5-10% regenerated shoot buds on 0.05-0.5 mg/L TDZ with or without IBA and 1.0-2.0 mg/L TDZ with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA (Plate 11E). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 23). Significant differences in elongation were observed at different concentrations and combinations of PGRs. BAP and IAA combination was found best. The best elongation (3.21 cm) was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 11F). The elongation ranged from 2.11-3.21 cm on medium containing BAP and IAA combinations. The elongation inhibited on medium containing BAP and IBA. The elongation ranged from 1.96-2.11 cm on medium containing BAP and IBA combinations and 1mg/L BAP and 1.5 mg/L IBA gave the best elongation (2.11cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 1.11-136 cm (Table 23).

4.2.2.4. Regeneration from *in vitro* and *in vivo* cotyledonary petiole explant

4.2.2.4. (a) Effect of TDZ with or without IBA on shoot bud induction

The concentrations of TDZ with or without IBA significantly influenced the response of direct shoot bud induction from cotyledonary petiole explant of non-

toxic cultivar. When 1 mg/L IBA supplemented in TDZ containing medium, regeneration efficiency become decreased. The percent shoot bud induction and number of induced shoot buds per cotyledonary petiole explant leaf was directly proportional to the concentration of TDZ. Of the different concentrations of TDZ tested with or without 1 mg/L IBA, highest percentage of shoot bud induction (67.97%) and highest number of shoot buds per cotyledonary petiole explant (5.92) was observed in the presence of 2 mg/L TDZ, however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction at this concentration. At 0.5 mg/L TDZ, the percentage of shoot bud induction varied from 41.23-59.68% and number of induced shoot buds per cotyledonary petiole explant varied from 2.49-5.01 (Tables 28 & 29).

4.2.2.4. (b) Effect of orientation of explant on shoot bud induction

The orientations of cotyledonary petiole explant of non-toxic significantly influenced the response of direct shoot bud induction at tested concentration of TDZ alone and with 1 mg/L IBA. The percentage of shoot bud induction and number of shoot buds per explant was higher in horizontal position as compared to vertical position. The percentage of shoot bud induction varied from 17.01-67.97% in horizontal position (Plate 12A & B; Tables 28 & 29) and 9.11-61.23% in vertical position (Plate 12C & D; Tables 28 & 29), and number of induced shoot buds per explant varied from 2.54-5.92 in horizontal position (Tables 28 & 29) and 1.61-5.67 in vertical position (Tables 28 & 29) at tested concentration of TDZ alone and with 1 mg/L IBA.

4.2.2.4. (c) Effect of explant sources on shoot bud induction

The sources of cotyledonary petiole explant also influenced significantly the plant regeneration through shoot bud induction on TDZ alone and with 1 mg/L IBA containing medium. *In vitro* cotyledonary petiole explant responded efficiently as compared to *in vivo* petiole explant. The percentage of shoot bud induction varied from 13.23-67.97% in *in vitro* cotyledonary petiole explant (Plate 12A & C; Table 28) and 9.11-61.11% in *in vivo* petiole explant (Plate 12B & D; Table 29), and number of induced shoot buds per explant varied from 2.12-5.92 in *in vitro* petiole explant (Table 28) and 1.61-5.61 in *in vivo* petiole explant of (Table 29) at tested concentration of TDZ alone and with 1 mg/L IBA.

Table 28. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from horizontally and vertically placed *in vitro* cotyledonary petiole explant of non-toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / cotyledonary leaf explant	
		Horizontal	Vertical	Horizontal	Vertical
0.05	0	21.11 ± 2.52 [a]	16.01 ± 2.43 [ab]	3.09 ± 0.97 [a]	2.91 ± 0.71 [ab]
0.10	0	31.47 ± 3.91 [b]	22.23 ± 3.41 [b]	2.98 ± 0.89 [a]	2.99 ± 0.79 [ab]
0.20	0	41.12 ± 4.11 [c]	36.47 ± 4.01 [c]	4.08 ± 0.86 [abc]	3.67 ± 0.89 [abc]
0.50	0	59.11 ± 5.02 [d]	54.21 ± 4.42 [de]	5.01 ± 0.91 [bcd]	4.16 ± 0.97 [bcd]
1.00	0	63.13 ± 6.01 [de]	56.57 ± 5.81 [de]	5.71 ± 0.88 [cd]	5.11 ± 1.08 [a]
2.00	0	67.97 ± 6.11 [e]	61.23 ± 5.71 [e]	5.92 ± 0.98 [cd]	5.67 ± 1.01 [cd]
0.05	1	19.99 ± 2.29 [a]	13.23 ± 2.19 [a]	2.99 ± 0.77 [a]	2.12 ± 0.59 [a]
0.10	1	29.81 ± 2.79 [b]	20.45 ± 2.27 [ab]	2.91 ± 0.79 [a]	2.63 ± 0.89 [ab]
0.20	1	39.71 ± 3.81 [c]	31.67 ± 3.94 [c]	3.99 ± 1.01 [ab]	3.01 ± 0.91 [ab]
0.50	1	57.81 ± 4.18 [d]	49.23 ± 5.98 [d]	4.98 ± 0.61 [bcd]	4.11 ± 1.23 [bcd]
1.00	1	61.44 ± 5.11 [e]	51.57 ± 6.61 [d]	4.99 ± 0.71 [bcd]	4.21 ± 1.37 [bcd]
2.00	1	65.01 ± 6.21 [de]	53.23 ± 7.01 [de]	5.62 ± 1.01 [bcd]	5.31 ± 1.36 [cd]

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.2.2.4. (d) Shoot proliferation and elongation from induced shoot buds

Approx 30-40% and 15-20% regenerated shoot buds on 0.05-0.5 mg/L TDZ with or without IBA and 1.0-2.0 mg/L TDZ with or without IBA respectively, proliferated on medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA (Plate 12E). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs like BAP, IAA, NAA and IBA (Table 23). Significant differences in elongation were observed at different concentrations and combinations of PGRs. BAP and IAA combination was found best. The best elongation (2.43 cm) was observed on medium containing 0.5 mg/L BAP and 1.5 mg/L IAA (Plate 12F). The elongation ranged from 2.11-2.43 cm on medium containing BAP and IAA combinations (Table 23). The elongation inhibited on

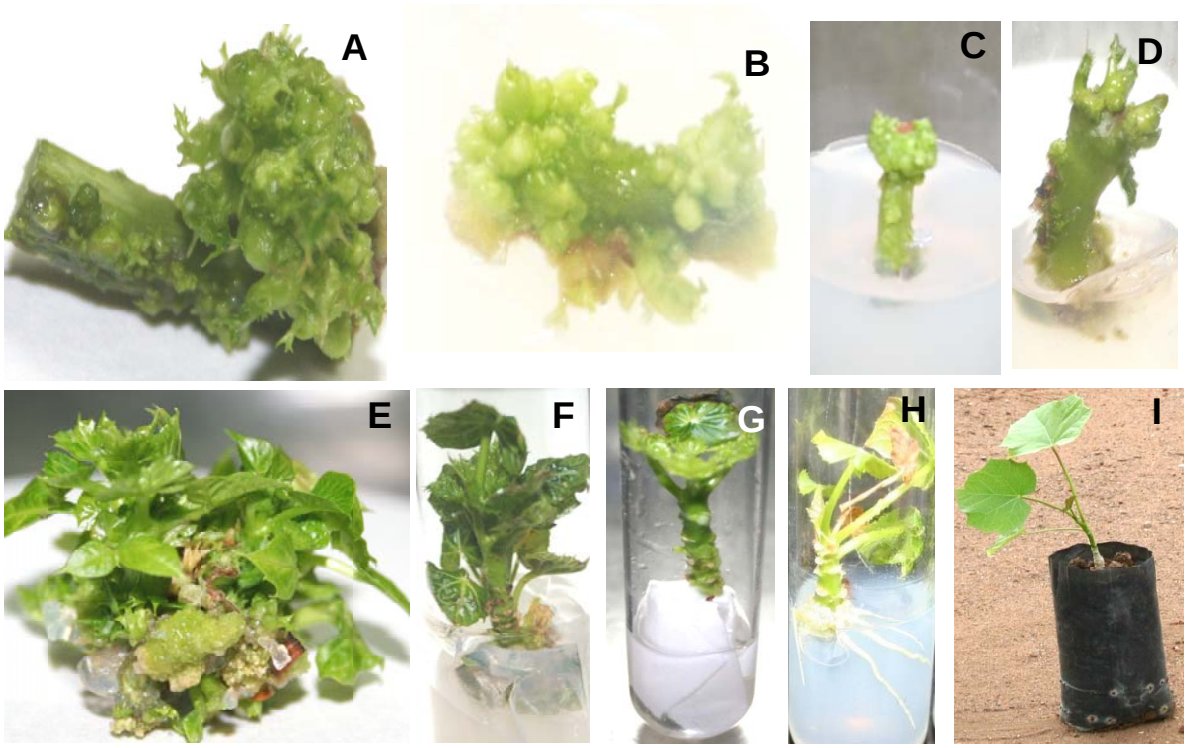

Plate 12. Direct shoot bud induction from cotyledonary petiole explant of non-toxic cultivar of *J.curcas.*

Direct shoot bud induction from (A) *in vitro* petiole in horizontal position, (B) *in vivo* petiole in horizontal position, (C) *in vitro* petiole in vertical position and (D) *in vivo* petiole in vertical position on MS medium with 0.5 mg/L TDZ. (E) Shoot proliferation of induced shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA. (F) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (G) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1 mg/L IAA + 1 mg/L NAA. (H) Root initiation and elongation on hormone free half strength MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (I) Regenerated plant in polybag.

Table 29. Effect of different concentrations of thidiazuron (TDZ) alone and in combination with 1 mg/L indole-3-butyric acid (IBA) on shoot bud induction from horizontally and vertically placed *in vivo* cotyledonary petiole explant of non-toxic cultivar of *J. curcas* after 6 weeks.

TDZ (mg/L)	IBA (mg/L)	Shoot bud induction (%)		No. of buds / cotyledonary petiole explant	
		Horizontal	Vertical	Horizontal	Vertical
0.05	0	19.87 ± 1.61 ^{ab}	11.01 ± 1.72 ^{a}	2.78 ± 0.69 ^{a}	1.67 ± 0.67 ^{a}
0.10	0	27.16 ± 2.77 ^{b}	19.27 ± 3.43 ^{ab}	2.56 ± 1.31 ^{a}	2.01 ± 0.87 ^{a}
0.20	0	37.47 ± 3.51 ^{c}	29.12 ± 3.91 ^{cd}	3.67 ± 1.36 ^{ab}	2.57 ± 1.06 ^{a}
0.50	0	51.01 ± 6.06 ^{d}	41.23 ± 5.87 ^{de}	4.59 ± 1.48 ^{ab}	2.49 ± 1.08 ^{a}
1.00	0	59.68 ± 5.01 ^{e}	49.06 ± 5.81 ^{e}	4.93 ± 1.81 ^{ab}	2.97 ± 0.96 ^{a}
2.00	0	61.11 ± 6.46 ^{a}	52.67 ± 6.87 ^{e}	5.61 ± 1.70 ^{b}	3.01 ± 0.89 ^{a}
0.05	1	17.01 ± 1.79 ^{a}	9.11 ± 2.07 ^{a}	2.72 ± 0.95 ^{a}	1.61 ± 0.51 ^{a}
0.10	1	27.15 ± 2.71 ^{b}	16.17 ± 2.89 ^{ab}	2.54 ± 0.63 ^{a}	1.99 ± 0.67 ^{a}
0.20	1	37.18 ± 3.89 ^{c}	21.52 ± 3.78 ^{abc}	3.63 ± 0.94 ^{ab}	2.23 ± 0.93 ^{a}
0.50	1	56.89 ± 5.11 ^{de}	37.47 ± 5.87 ^{cde}	4.31 ± 1.48 ^{ab}	2.29 ± 1.08 ^{a}
1.00	1	60.01 ± 6.99 ^{e}	44.23 ± 5.09 ^{de}	4.89 ± 1.68 ^{ab}	2.97± 1.01 ^{a}
2.00	1	61.91 ± 6.05 ^{e}	49.16 ± 5.87 ^{e}	5.01 ± 1.76 ^{ab}	3.01 ± 1.11 ^{a}

Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

medium containing BAP and IBA. The elongation ranged from 1.91-2.09 cm on medium containing BAP and IBA combinations and 0.5 mg/L BAP and 0.5 mg/L IBA gave the best elongation (2.09 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 0.71-1.05 cm (Table 23).

4.3. ROOTING

4.3.1. Effect of auxins

Significant differences in rooting percent were observed at different concentrations and combinations of auxins in toxic and non-toxic cultivar of *J.curcas.* However, the differences were not significant between toxic genotypes. Of the different concentrations and combinations of auxins tested, rooting percent varied from 5.11-51.96% and 2.01-21.71% in toxic and non-toxic cultivar respectively (Plate 5F, 6F, 7H, 8H, 9F, 10F, 11H and 12H; Tables 30-33). Highest percentage of root induction 51.96% and 21.71% were observed in toxic

and non-toxic cultivar respectively in the presence of 3 mg/L, 1 mg/L IAA and 1 mg/L NAA. Rooting percentage was higher when mixtures of auxins were used as compared to individuals in the medium.

4.3.2. Effect of strength of MS medium

Significant differences in rooting percentage were observed at different strengths of basal MS medium in toxic and non-toxic cultivar of *J.curcas* but not within toxic genotypes. Rooting percentage was more on half strength MS medium as compared to full strength MS medium. In toxic cultivar, rooting percent varied from 5.11-51.96% and 7.09-33.98% in half and full strength MS medium respectively whereas, in non-toxic cultivars rooting percent varied from 2.09-21.71% and 2.01-19.91% in half and full strength MS medium respectively (Tables 30-33).

Table 30. Effect of auxins with half strength MS medium on root induction in regenerated shoots from explant of toxic and non-toxic cultivar of *J.curcas.*

Auxins (mg/L)			Rooting percentage			
IBA	IAA	NAA	Toxic			Non-toxic
			CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	
1	0	0	8.21 ± 1.01 [a]	7.11 ± 1.23 [a]	5.11 ± 1.01 [a]	2.09 ± 0.17 [a]
2	0	0	12.13 ± 1.12 [a]	11.23 ± 1.01 [b]	9.09 ± 1.11 [b]	7.01 ± 0.21 [b]
3	0	0	17.10 ± 1.07 [b]	18.12 ± 2.01 [c]	15.13 ± 1.15 [c]	11.23 ± 1.21 [c]
1	1	1	20.21 ± 2.01 [bc]	21.12 ± 1.11 [cd]	19.11 ± 2.10 [d]	13.02 ±1.67 [cd]
2	1	1	22.18 ± 2.21 [c]	23.45 ± 2.32 [d]	25.05 ± 2.01 [e]	15.45 ±2.01 [de]
3	1	1	31.05 ± 2.06 [d]	27. 23 ± 2.23 [e]	21.03 ± 2.11 [d]	16.67 ± 1.23 [ef]
1	2	1	33.03 ± 3.91 [de]	31.01 ± 3.12 [ef]	27.07 ± 1.67 [e]	19.32 ± 2.01 [ef]
1	3	1	34.04 ± 3.11 [de]	23.12 ± 1.01 [d]	31.01 ± 3.91 [f]	8.09 ± 1.01 [b]
1	1	2	37.06 ± 2.91 [e]	31.92 ± 2.93 [ef]	36.06 ± 3.01 [g]	18.17 ± 2.88 [ef]
1	1	3	33.09 ± 3.12 [de]	29.11 ± 2.01 [ef]	31.98 ± 2.87 [f]	17.87 ± 2.55 [ef]

NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.3.3. Effect of culture type

Rooting percentage was higher when grownup shoots were pulse treated with different concentration and combinations of auxins and transferred on hormone free basal MS medium as compared to directly culturing on solid MS medium supplemented with auxins. In toxic cultivar, rooting percentage varied from 7.09-51.96% and 5.11-37.06% in pulse treated and directly cultured on auxins containing solid medium respectively, whereas, in non-toxic cultivar rooting percent varied from 8.71-21.71% and 2.01-19.34% in pulse treated and directly cultured on auxins containing solid medium respectively (Tables 30-33).

Table 31. Percent rooting on hormone free half strength solid MS medium after four days pulse treatment with various auxins with half strength liquid MS medium in grownup shoots from explant of toxic and non-toxic cultivar of *J.curcas.*

Auxins (mg/L)			Rooting percentage			
IBA	IAA	NAA	Toxic			Non-toxic
			CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	
1	0	0	10.45 ± 1.87 [a]	11.87 ± 1.09 [a]	10.54 ± 1.01 [a]	8.71 ± 1.01 [a]
2	0	0	12.78 ± 1.41 [a]	12.10 ± 1.54 [a]	13.01 ± 1.02 [a]	9.81 ± 1.02 [ab]
3	0	0	24.67 ± 1.65 [b]	23.89 ± 2.12 [b]	23.99 ± 2.98 [b]	12.78 ± 1.45 [c]
1	1	1	21.76 ± 1.87 [b]	22.89 ± 2.13 [b]	22.00 ± 2.09 [b]	11.61 ±1.72 [bc]
2	1	1	39.32 ± 3.01 [c]	40.54 ± 3.09 [c]	40.01 ± 3.05 [c]	12.76 ± 1.62 [c]
3	1	1	51.96 ± 3.76 [e]	49.87 ± 4.01 [e]	51.87 ± 4.01 [e]	21.71 ± 1.62 [e]
1	2	1	46.98 ±4.01 [de]	46.87 ±3.09 [de]	45.99 ± 2.98 [d]	11.82 ±1.41 [bc]
1	3	1	43.78 ±3.87 [cd]	42.98 ±3.01 [cd]	42.78 ± 3.09 [cd]	17.87 ± 1.44 [d]
1	1	2	41.81 ±3.98 [cd]	40.87 ± 2.87 [c]	40.99 ± 3.06 [cd]	19.32 ±1.41 [de]
1	1	3	41.76 ±3.01 [cd]	41.82 ±4.09 [cd]	42.89 ± 3.98 [cd]	11.12 ±1.52 [abc]

NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.3.4. Effect of cultivars

No significant differences were observed in rooting in toxic genotypes of *J.curcas* but significant diligences were observed between toxic and non-toxic cultivar. 5.11-51.96% and 2.01-21.71% rooting percent were observed in toxic and non-toxic cultivar respectively (Tables 30-33).

4.4. ACCLIMATIZATION

After 6-8 weeks, approximately 90% of plants survived. No morphological abnormalities were observed in regenerated plants (Plate 5G, 6G, 7I, 8I, 9G, 10G, 11I and 12I.)

Table 32. Percent rooting on hormone free full strength solid MS medium after four days pulse treatment with various auxins with full strength liquid MS medium in grownup shoots from explant of toxic and non-toxic cultivar of *J.curcas.*

Auxins (mg/L)			Rooting percentage			
IBA	IAA	NAA	Toxic			Non-toxic
			CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	
1	0	0	7.12 ± 1.02 [a]	7.09 ± 0.89 [a]	7.11 ± 1.01 [a]	8.98 ± 1.89 [a]
2	0	0	12.23 ± 1.23 [b]	11.89 ± 1.04 [b]	13.03 ± 1.23 [b]	9.34 ± 1.45 [a]
3	0	0	16.23 ± 1.67 [c]	15.45 ± 1.61 [bc]	14.99 ± 1.04 [b]	12.56 ± 1.98 [b]
1	1	1	18.29 ±1.81 [cd]	17.90 ± 2.90 [c]	19.11 ± 1.09 [c]	11.45 ± 1.34 [ab]
2	1	1	17.21 ± 1.03 [c]	18.12 ± 1.89 [c]	18.13 ± 1.04 [c]	12.62 ± 1.87 [b]
3	1	1	33.67 ± 3.01 [g]	32.31 ± 3.09 [e]	33.21 ± 3.87 [f]	17.34 ± 1.43 [cd]
1	2	1	23.01 ± 2.05 [e]	22.87 ± 1.98 [e]	24.87 ± 1.34 [d]	11.32 ± 1.01 [ab]
1	3	1	21.45 ±2.01 [de]	20.91 ± 1.98 [d]	22.54 ± 2.02 [d]	16.13 ± 1.87 [c]
1	1	2	29.78 ± 2.45 [f]	30.98 ± 2.98 [e]	29.98 ± 1.01 [e]	17.67 ± 1.98 [cd]
1	1	3	31.34 ±3.01 [fg]	32.98 ± 3.98 [e]	33.98 ± 1.03 [f]	19.91 ± 1.76 [d]

NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

PART B

4.5. GENETIC TRANSFORMATION

4.5.1. *Agrobacterium*-infection of leaf explant

All the concentrations i.e. 50, 100, 200 and 400μM of acetosyringone yielded 100% infection (Plate 13) in the *in vitro* leaf explant after dipping in *Agrobacterium* suspended liquid YMB medium for 30 min and co cultivation for 3 days.

Table 33. Effect of auxins with full strength solid MS medium on root induction in regenerated shoots from explant of toxic and non-toxic cultivar of *J.curcas.*

Auxins (mg/L)			Rooting percentage			
IBA	IAA	NAA	Toxic			Non-toxic
			CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	
1	0	0	9.12 ± 1.23 [a]	8.11 ± 1.91 [a]	7.65 ± 0.99 [a]	2.01 ± 0.09 [a]
2	0	0	12.32 ± 1.91 [ab]	11.23 ± 1.31 [ab]	10.11 ± 1.01 [a]	8.98 ± 1.09 [cd]
3	0	0	11.56 ± 2.01 [a]	12.32 ± 1.21 [b]	13.87 ± 1.12 [b]	7.05 ± 1.01 [bc]
1	1	1	17.12 ± 1.45 [bc]	18.98 ± 1.87 [c]	16.89 ± 1.23 [bc]	11.98 ± 2.03 [ef]
2	1	1	21.23 ± 2.09 [cd]	20.32 ± 2.03 [c]	19.89 ± 1.98 [cd]	6.06 ± 1.03 [b]
3	1	1	17.05 ± 2.98 [bc]	17.87 ± 2.03 [c]	18.98 ± 1.21 [cd]	7.87 ± 1.12 [bcd]
1	2	1	22.67 ± 2.87 [cd]	21.87 ± 2.95 [c]	20.12 ± 2.01 [d]	9.87 ± 1.88 [de]
1	3	1	31.67 ± 4.01 [e]	31.01 ± 3.09 [d]	30.98 ± 2.98 [e]	13.77 ± 1.99 [f]
1	1	2	34.45 ± 3.76 [e]	33.76 ± 3.03 [d]	31.23 ± 3.12 [e]	17.56 ± 1.77 [g]
1	1	3	19.56 ± 2.44 [cd]	19.09 ± 2.01 [c]	21.21 ± 2.04 [d]	9.09 ± 1.09 [cd]

NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; IAA, indole 3-acetic acid.
Values represent means ± SD of 25 explant per treatment in three repeated experiments.
Means in each column followed by same letters are not significantly different according to DMRT at α=0.05

4.5.2. Evaluation of the antibiotics effect on leaf explant and shoots

The effect of increasing concentrations of hygromycine was assessed separately on leaf explant and *in vitro* developed shoot separately. Hygromycine was highly effective even at low concentration (2.5 µg/ml). About 70–80% of the leaf explant/shoot was bleached by 30th days in culture medium containing 2.5 µg/ml hygromycine. The phytotoxic effect of hygromycine was noticeable at 5 µg/ml in the medium as leaf explant/shoot started to bleach between 10 days and 15 days following inoculation, resulting in a total loss of chlorophyll pigmentation (bleaching) by 30th day (Plate 14). All shoots became necrotic, and there was an absolute arrest of shoot-bud initiation from explant at this concentration (Plate 14). Hence, in subsequent experiments, we supplemented the shoot regeneration medium with 5 µg/ml hygromycine for the selection of putative transformed leaf explant/shoot. The three bactericidal antibiotics, namely

Plate 13. ***Agrobacterium*-infected leaf explant of *J.curcas***

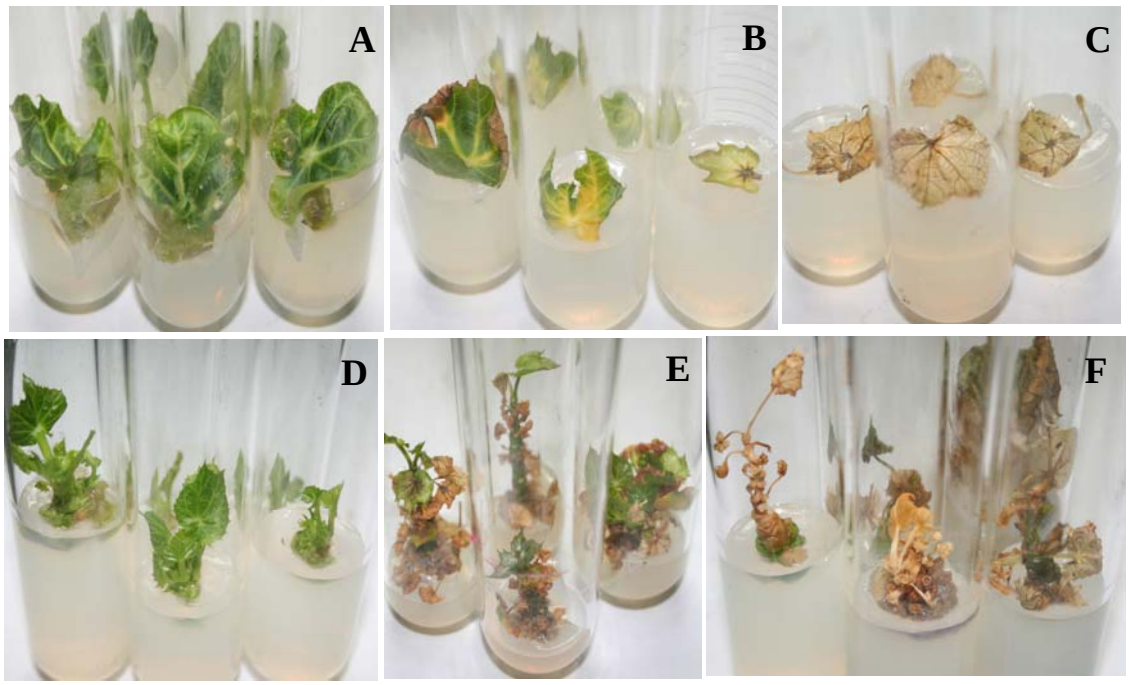

Plate 14. Determination of lethal dose (LD_{50}) of hygromycine in *J.curcas.*
Leaf explant: A=control, B=5 mg/L and C=10 mg/L of hygromycine,
Regenerated shoot: D=control, E=5 mg/L and F=10mg/L.

sporidex, carbenicillin and cefotaxime, were used at various concentrations (250, 500, 750 and 1,000 mg/L) to eliminate the *Agrobacterium* background. Antibiotics also had a detectable effect on shoot organogenesis. In the present study, 500 mg/L cefotaxime in the medium had a most suitable effect on bacterial elimination without adversely affecting the morphogenesis.

4.5.3. GUS expression in putative transformed shoots

The enzymatic activity of GUS was substantiated by the positive histochemical assay, as evidenced by the blue colour developed in leaf explants of transformed shoots (Plate 15). The explant which survived in the selection medium containing 5 µg/ml hygromycine was GUS-expressive.

4.5.4. Optimization of transformation parameters

4.5.4.1. Explant preculture

Prior to co-cultivation with *Agrobacterium*, leaf explant was cultured in the agar solidified shoot regeneration medium for a period varying from 1 day to 7 days. Leaf explant that was directly infected with *Agrobacterium* without pre-culture showed a lower transformation competence than the ones cultured in regeneration medium to bacterial treatment. Leaf explant pre-cultured for 4 days had the highest transformation competence (approx. 11.32%). Shorter durations of preculture (1-3 days) elicited a lower shoot organogenic response, while longer durations (5-7days) resulted in more non-transformed shoots. Transformation efficiency was measured by the surviving shoots in selective medium expressing the marker genes and tested positive by PCR (Plate 16).

4.5.4.2. Bacterial growth phase

To determine the right stage of *Agrobacterium* growth for high-efficiency transformation, we studied four different growth phases. At the late-log phase, corresponding to OD_{600}= 0.6, we obtained the maximum rate of transformation (13.55 %) as measured by the surviving shoots in selective medium expressing the marker genes and tested positive by PCR. An increase or decrease in the optical density of the *Agrobacterium* inoculum was not conducive to transformation, while extensive tissue damage occurred at optical density values greater than 1.0 because of bacterial overgrowth (Plate 17).

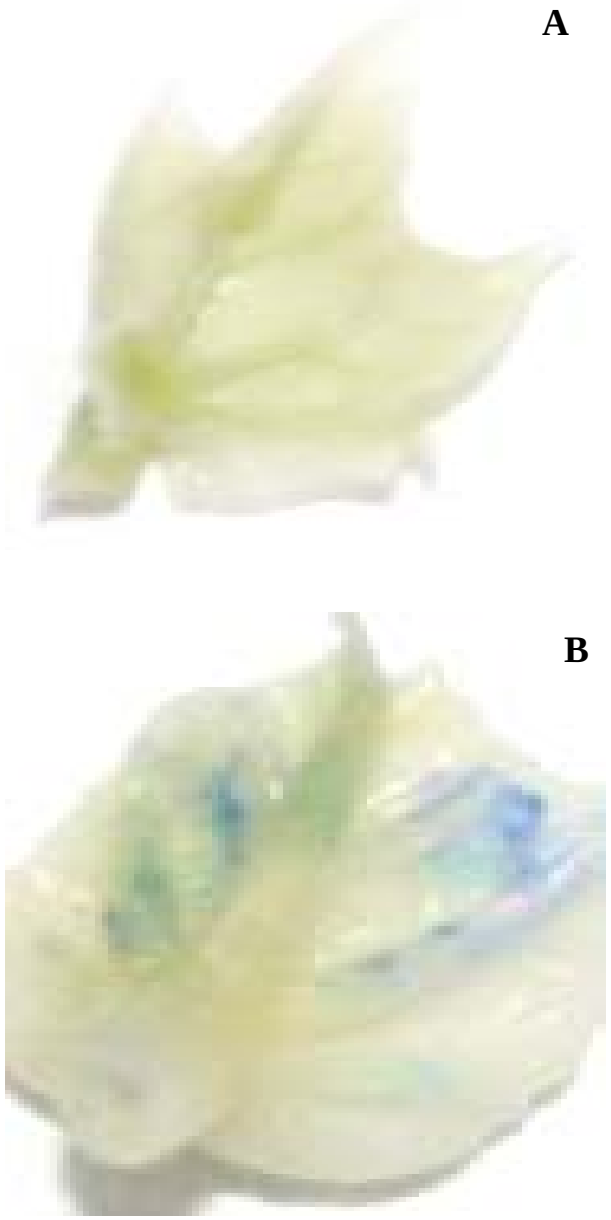

Plate 15. Gus expression as indicated by the blue spot on transformed leaf explant.

A. Non-transformed leaf explant

B. Transformed leaf explant

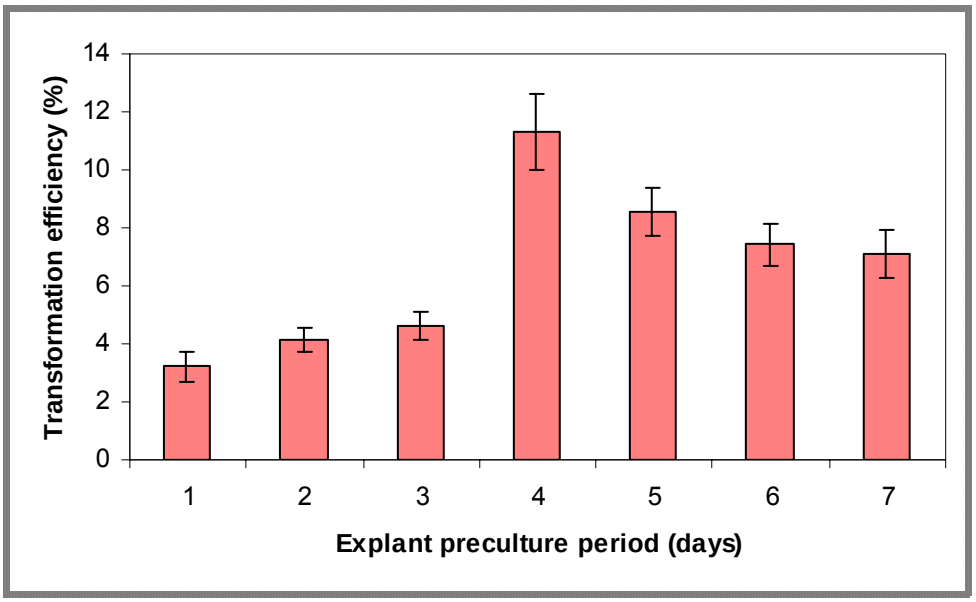

Plate 16. Effect of explant pre-culture period on transformation efficiency (%)

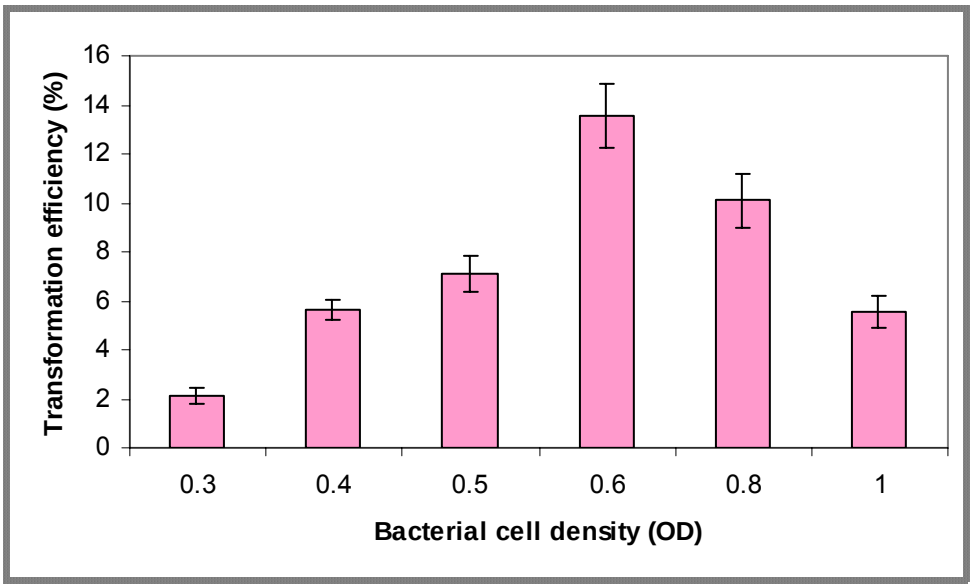

Plate 17. Relationship between growth stages of the *Agrobacterium* cultures (as measured by the optical density of the inoculum at 600 nm) and transformation efficiency (%)

4.5.4.3. Wounding effect

There is secretion of various phenolics compounds from the wound sites of leaf explant, which inhibits the transformation efficiency. The maximum transformation efficiency (8.99%) was scored using intact (unwounded) leaf explant. When leaf explant injured with either glass beads or hand pricking with hypodermic needle transformation efficiency decreased with a high rate of tissue browning. Thus, wounding was not only unnecessary for inducing transformation but also deleterious to regeneration (Plate 18). Transformation efficiency was measured by the surviving shoots in selective medium expressing the marker genes and tested positive by PCR.

4.5.4.4. Bacterial cell density and infection time

A range of bacterial cell densities (10^7-10^{10} cells/ml), adjusted by diluting the *Agrobacterium* suspension, was evaluated for explant infection. Optimum results were obtained with a density of 10^9 cells/ml. Of the range of increasing durations tested for explant infection with a diluted *Agrobacterium* culture, the maximum transformation efficiency (12.67%) was recorded with a 20 min long treatment at a bacterial cell density of 10^9 cells/ml (Table 34). Transformation efficiency was measured by the surviving shoots in selective medium expressing the marker genes and tested positive by PCR.

4.5.4.5. Co-cultivation period and medium pH

With a view to determining the suitable period for co-cultivation, leaf explant was cocultivated with above mentioned *Agrobacterium* strains for an increasing length of time (1-7 days). The maximum transformation efficiency (12.84%) was achieved after 4 days of co-cultivation. Co-cultivation periods longer than 4 days were unsuitable because of uncontrollable over- growth of bacteria. Explant co-cultivated for a shorter period (1-3 days) produced shoots, but only a few of these were resistant to hygromycine (3.43-7.65%) (Plate 19). A co-cultivation medium pH of 5.6 was found to be the best with respect to transformation frequency, with a decrease in transformation frequency occurring either below or above this pH threshold value (5.6) of the co-cultivation medium (Plate 20).

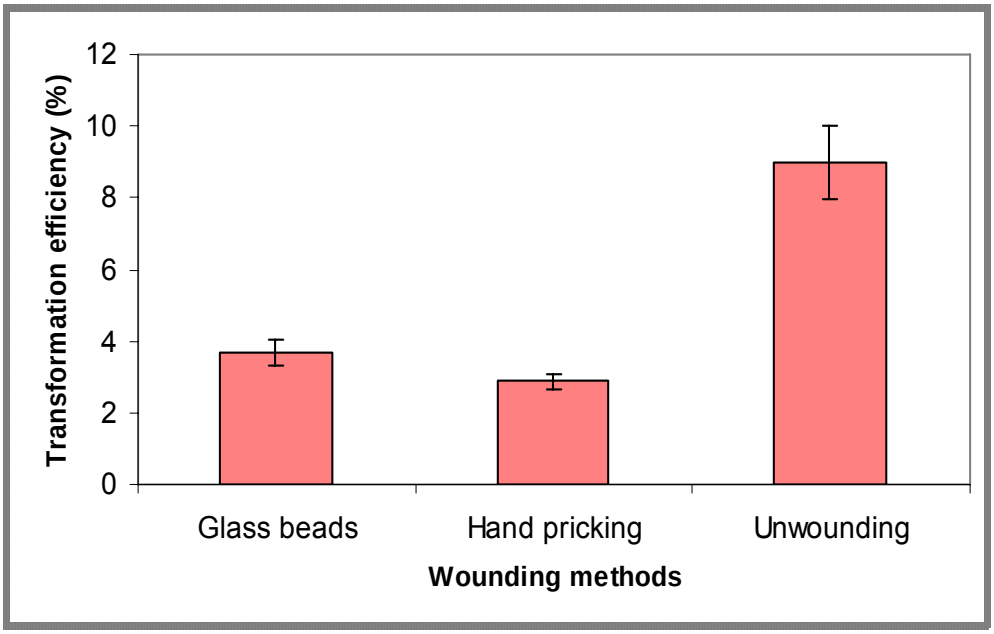

Plate 18. Effect of wounding method on transformation efficiency (%)

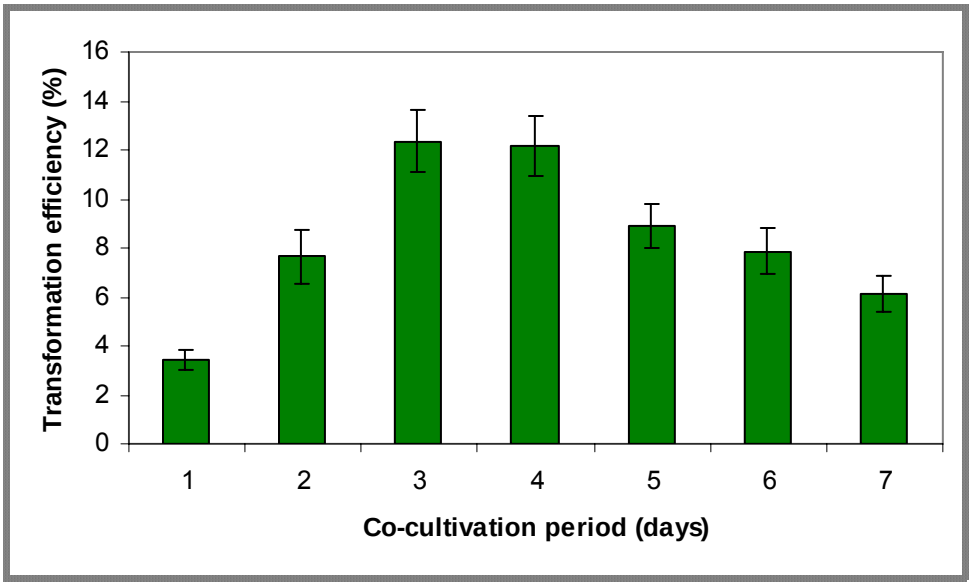

Plate 19. Effect of co-cultivation period on transformation efficiency (%)

Table 34. Effect of *Agrobacterium* cell density and duration of treatment on transformation efficiency (%)

Agrobacterium cell density	Transformation efficiency (%) Duration of treatment (min.)		
	10	20	30
10^7	5.11 ± 0.42	9.35 ± 0.99	9.55 ± 1.12
10^8	9.54 ± 0.62	10.45 ± 1.09	9.71 ± 1.56
10^9	11.01± 1.23	12.67 ± 1.94	11.98 ± 2.02
10^{10}	7.51 ± 0.98	7.76 ± 0.57	6.41± 0.63

4.5.4.6. Acetosyringone treatment

To determine the right concentrations of acetosyringone for high-efficiency transformation, we used different concentrations of acetosyringone (50, 100, 200 and 400 µM). At 100 µM we obtained the maximum rate of transformation efficiency (9.92 %) as measured by the surviving shoots in selection medium expressing the marker genes and tested positive by PCR. Acetosyringone (100 µM) was found to be the best with respect to transformation frequency, with a decrease in transformation frequency either below or above this concentration (Plate 21).

4.5.5. Molecular characterization of transformed shoots

4.5.5.1. Polymerase chain reaction analysis

DNA obtained from several independent hygromycine resistant, GUS-positive lines revealed the predicted amplification products of 400 bp and 866 bp with GUS (Plate 22) and DREB2A (Plate 23) gene-specific primers, respectively. This indicated the presence of both the linked marker transgenes, GUS and DREB2A as a single T-DNA in the transformed genome. No amplification product was detected in DNA from untransformed shoots when subjected to PCR amplification with either of the two primers.

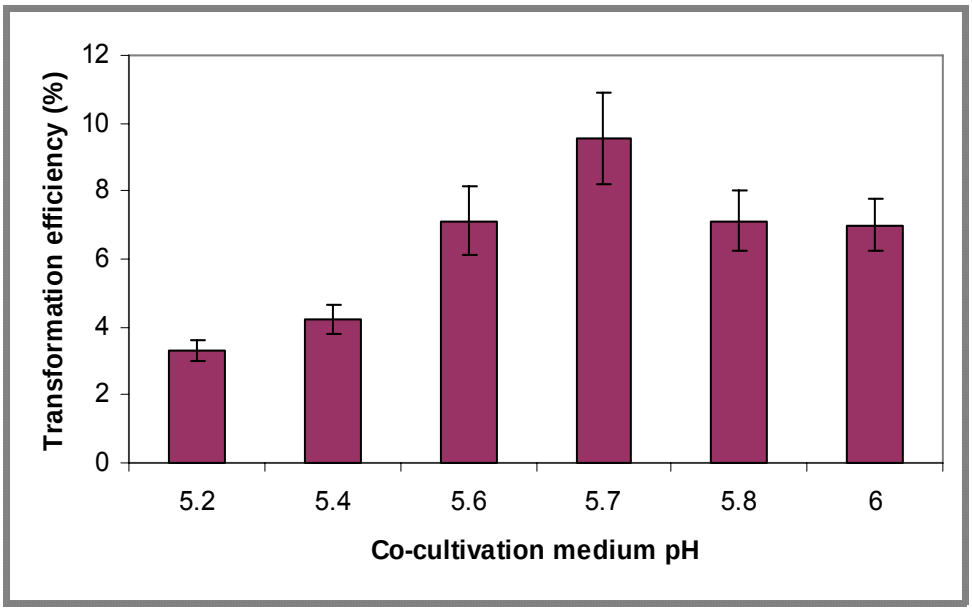

Plate 20. Effect of co-cultivation medium pH on transformation efficiency (%)

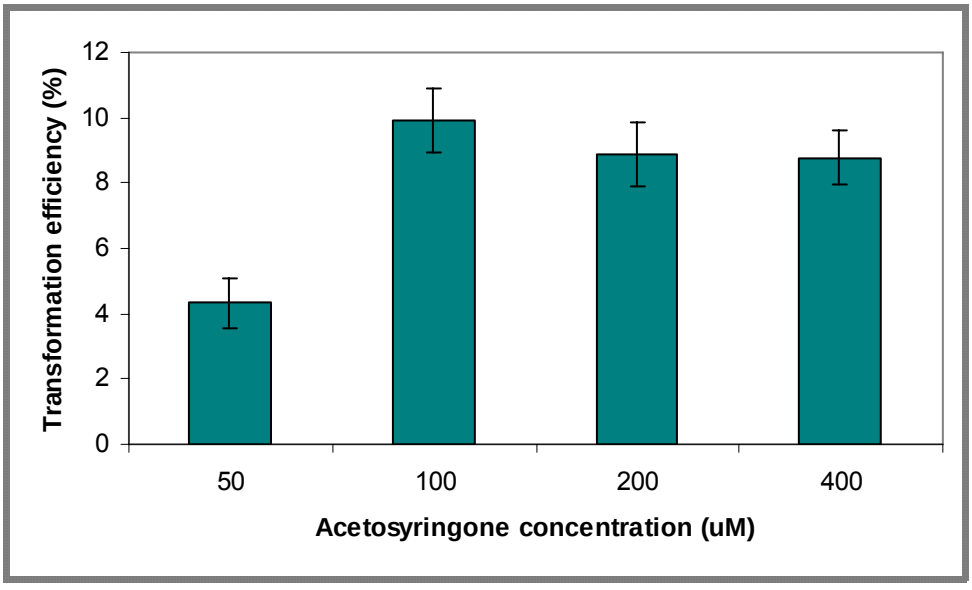

Plate 21. Effect of different concentrations of acetosyringone on transformation efficiency (%)

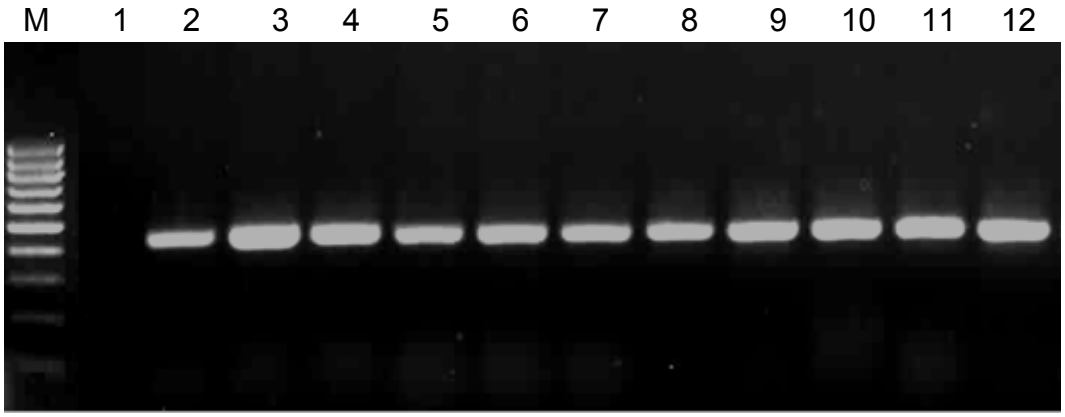

Plate 22. Molecular evaluation of putative transformed regenerated plants containing GUS gene through PCR

Lane 1: Negative control (Non-transformed)
Lane 2-11: Independent transformant lines containing GUS gene
Lane 12: Positive control (Plasmid DNA)
Lane M: 100 bp ladder

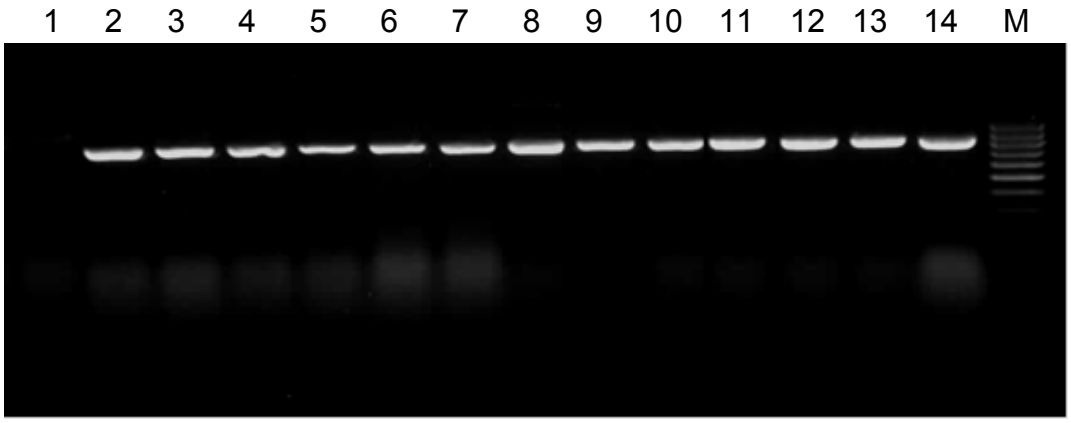

Plate 23. Molecular evaluation of putative transformed regenerated plants containing DREB 2A through PCR

Lane 1: Negative control (Non-transformed)
Lane 2-13: Independent transformant lines containing DREB 2A gene
Lane 14: Positive control (Plasmid DNA)
Lane M: 100 bp ladder

4.5.5.2. Dot blot analysis

When denatured DNA of different concentration were blotted on to positively charged nylon membrane and hybridized with Alkphos labeled DREB2A probe, positive signals were detected at all concentrations but deep signals were obtained at high concentration of DNA (45ng) in all transformed explant whereas, no signals were detected in non-transformed explant (Plate 24).

4.5.5.3. Southern hybridization analysis

Putative transformants analyzed with non-radioactive method of Southern hybridization to confirm the presence of DREB2A gene. Southern analysis confirmed the integration of DREB2A gene (lane 3-5) with 1-2 copy numbers. One transformed plant had a single copy of the DREB2A gene and other two transformed plants had two copies. No hybridization signal was observed in non-transformed plant leaf explant (Plate 25).

4.6. Multiplication and establishment of transgenic plants

Selected transformed regenerated shoot buds (on selection medium containing 5 mg/L hygromycine Plate 26A) were transferred to proliferation medium containing 2 mg/L Kn, 1 mg/L BAP and 1 mg/L NAA (Plate 26B). Individual (0.3-0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to elongation medium and approx 2.5 cm elongation was observed (Plate 26C). About 40% rooting was observed on half strength MS medium supplemented with 2% sucrose and 3 mg/L IBA, 1 mg/L IAA, 1 mg/L NAA and 0.25 mg/L activated charcoal for rooting (Plate 26D & E). After 6-8 weeks, 50-60% of plants survived (Plate 26F).

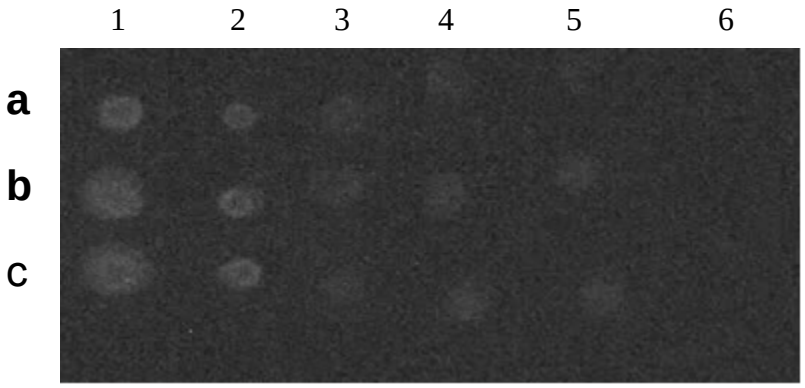

Plate 24. Dot blot of putatively transformed leaf explant at different dilutions of DNA (a) 15 ng (b) 30 ng and (c) 45 ng.

Lane 1: Positive control (Plasmid DNA)
Lane 2-5: Independent transformant lines containing DREB2A gene
Lane 6: Negative control (Non-transformed)

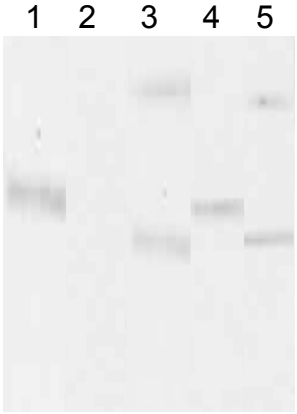

Plate 25. Southern hybridization of putatively transformed leaf explant.

Lane 1: Positive control (Plasmid DNA)
Lane 2: Negative control (Non-transformed)
Lane 3-5: Independent transformant lines containing DREB2A gene

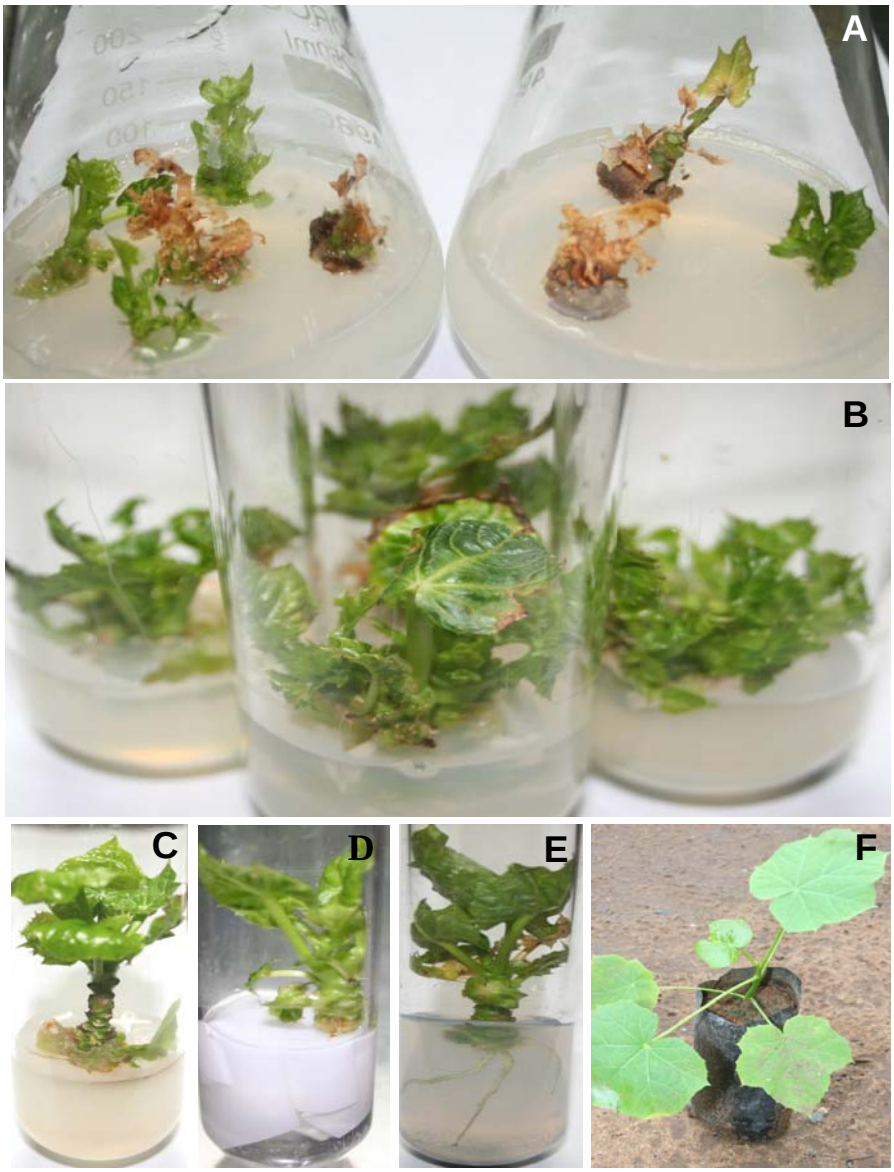

Plate 26. Selection, multiplication and establishment of transformed plant of toxic cultivar of *J. curcas.*

(A) Selection of transformed regenerated shoot buds on selection medium containing 5 mg/L hygromycine. (B) Shoot proliferation and multiplication of transformed shoot buds on MS medium with 2 mg/L Kinetin + 1 mg/L BAP + 1 mg/L NAA. (C) Elongation of proliferated shoot on MS medium with 0.5 mg/L BAP and 1.5 mg/L IAA. (D) Pulse treatment for root induction on half strength of MS medium with 2% sucrose + 3 mg/L IBA + 1mg/L IAA + 1 mg/L NAA. (E) Root initiation and elongation on hormone free half strength MS medium with 2% sucrose + 0.25 mg/L activated charcoal. (F) Regenerated transformed plant in polybag.