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Efficient culture protocol for plant regeneration from petiole explants of physiologically mature trees of *Jatropha curcas* L.

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An efficient and reproducible protocol for induction of adventitious shoot buds and plant regeneration from petiole explant cultures of *Jatropha curcas*, an important biofuel crop, is described. Physiologically mature trees of three *J. curcas* genotypes were selected and explants were prepared from young petioles. Treating the explants with high concentrations (5 to 120 mg/L) of thidiazuron (TDZ) solution for short time periods (5 to 80 min) helped increase the regeneration frequency and improved the quality of the regenerated buds significantly. The age of the petioles and inoculation methods were found to influence the culture results. The best shoot buds induction (65.78%) and number of buds (6.77) per explant was seen in the second petiole explants of genotype M-1 treated with 20 mg/L TDZ solution for 20 min, followed by 35-day culture on hormone-free Murashige and Skoog medium. The regenerated buds could elongate to become shoots in a medium containing gibberellic acid. The elongated shoots initiated roots to become intact plantlets in rooting medium containing indole-3-butyric acid and L-glutamine (Gln), and supplementing 16 mg/L Gln into the rooting medium effectively stimulated the initiation and growth of roots, with the best rooting rate (51.72%). After acclimatization, these plantlets were transplanted to soil wherein normal growth was observed. Therefore, an intact plantlet could usually be obtained at 60 days of culture by using the culture protocol described in this study. This protocol can be used for mass production of true-to-type plants and the production of transgenic plants through *Agrobacterium* biolistic-mediated transformation.

**Keywords:** *Jatropha curcas*; petiole explants; regeneration; rooting; thidiazuron

**Introduction**

*Jatropha curcas* L., known by the common name physic nut, belongs to the Euphorbiaceae family.[1] *J. curcas* is a type of woody plant widely distributed in the tropical and sub-tropical areas.[2] The seeds and oil from *J. curcas* are unfit for human and animal consumption because of the presence of toxins such as curcin, phorbol esters and saponins.[3–6] The latex from *J. curcas* contains alkaloids such as jatrophine and jatropham, which are reported to have medicinal properties, and can be used for extracting pharmaceutical compounds and insecticides.[7–9] However, this tree is most famous for the high oil content in its seeds (up to 60%).[10] There has been a surge of interest in *J. curcas* cultivation around the world since studies showed that its methyl ester could yield biodiesel.[8,9,11,12]

The primary limitation in its large-scale cultivation as a biofuel crop is the low and inconstant seed yield due to the heterozygous nature of *J. curcas* plants.[13] In order to breed advanced *J. curcas* varieties for large-scale cultivation and produce more seeds, genetic transformation of the species might be a possible solution.[14–18]

In a genetic transformation system, regeneration of adventitious buds from the genetically transformed tissues (explants) is in most cases an essential step. Furthermore, most of the reported conventional regeneration methods for *J. curcas* are very time-consuming, and usually need 3–5 months for intact plant regeneration. Therefore, it is essential to develop highly efficient tissue culture methods, which facilitate large-scale production of quality planting material and enable species improvement through genetic engineering techniques. There have been some reports on the regeneration of plants in *J. curcas* tissue cultures using various organs as explant sources.[19–27] The petiole is a sporophytic tissue and plants propagated from petiole explants are considered less sensitive to genetic variation.[28] However, the regeneration efficiency is not satisfactory for the petiole explants of *J. curcas*. Here, we report an efficient protocol for the induction of adventitious bud formation and plant regeneration from petiole explants through thidiazuron treatment.
collected from physiologically mature trees, which can also be used for other explants of *J. curcas*.

**Materials and methods**

**Plant materials**

Three physiologically mature *J. curcas* trees of different genotypes coded M-1, M-3 and M-19 were the source of plant materials in this study. The M-1 and M-3 plants were collected from Indonesia. M-19 was collected from Yunnan Province of China and has been designated as superior clone 18.[29] All plants were grown at the farm of South China Agricultural University in Guangzhou, China. Among the three genotypes, M-1 was used for all the experiments and the others were used for comparison only.

**Preparation of explants**

Vigorously growing shoots with several young leaves were cut from the trees; and the petioles were isolated from the shoots. The petioles were surface-sterilized with 2% sodium hypochlorite (NaClO) for 15 min and rinsed 5 times in sterile distilled water. The sterilized petioles were cut into 4–6 mm long segments and used as explants for inoculation onto the medium. Petioles (about 2 cm in length) isolated from the second leaves counting from the top of the shoots were used for preparation of explants for all the experiments and the other petioles were used for comparison only.

**Preparation of thidiazuron (TDZ) treating solution**

TDZ (Sigma-Aldrich Co., St Louis, MO, USA) was dissolved in 1 mol/L NaOH solution. The solution was diluted with purified water to prepare the following concentrations: 0, 5, 10, 20, 30, 60 and 120 mg/L. These were adjusted with 1 mol/L HCl to a pH value range of 5.8–6.0, and filter-sterilized before treating the petiole explants.

**Treating the petiole explants with TDZ solution**

Petiole explants were soaked in glass bottles containing different concentrations (0, 5, 10, 20, 30, 60 and 120 mg/L) of TDZ solution for various time periods (0, 5, 20, 40 and 80 min). After treatment, the explants were briefly placed on sterile dry filter paper in Petri dishes to absorb excess moisture.

**Regeneration culture**

For inducing shoot-bud regeneration, petiole explants were inoculated horizontally on hormone-free Murashige and Skoog (MS) medium [30] after treatment with TDZ solutions for various time periods. The explants were inoculated vertically with their morphological up-side up only for comparison experiments. For comparison, petiole explants were also treated using conventional methods and inoculated horizontally on MS medium containing different concentrations of 6-benzylaminopurine (6-BA, 0, 0.2, 0.4 and 0.8 mg/L; Sigma-Aldrich Co., St Louis, MO, USA) or TDZ (0, 0.1, 0.3 and 0.6 mg/L) as reported previously.[22–24] The percentage of induction of shoot buds and the number of shoot buds per explant were scored after 35 days of culture.

**Shoot bud elongation culture**

For shoot bud elongation, the regenerated shoot buds were transferred along with the mother tissues (explants) to MS medium supplemented with 0.5 mg/L BA, 0.2 mg/L kinetin (KT; Sigma–Aldrich Co., St Louis, MO, USA), 0.25 mg/L indole-3-acetic acid (IAA; Sigma–Aldrich Co., St Louis, MO, USA) [21], and various concentrations (0, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/L) of gibberellic acid (GA3, Sigma–Aldrich Co., St Louis, MO, USA). The length of the elongated shoots was recorded after 15 days of culture.

**Rooting culture**

For rooting of the elongated shoots, shoots at least 1 cm in length were isolated from the mother tissues and inoculated onto fresh MS medium supplemented with 0.3 mg/L indole-3-butyric acid (IBA; Sigma–Aldrich Co., St Louis, MO, USA) and different concentrations (0, 4, 8, 16, 32, 64 and 128 mg/L) of L-glutamine (Gln; Sigma–Aldrich Co., St Louis, MO, USA) and the results were assessed on 10, 20, 30 and 40 days of culture. The Gln solution was filter-sterilized and added to the autoclaved culture medium as described previously.[31]

**Preparation of culture medium and culture maintenance**

Uniform culture conditions were applied in all experiments. Basal MS formula was used for all tissue culture experiments. All media used in our experiment contained 2.5% sucrose and were adjusted to pH 5.8–6.0 with 1 mol/L NaOH. Agar (0.5%) was added prior to autoclaving at 1.4 kg/cm² for 20 min. All culture treatments were kept at 25 °C ± 1 °C under a 12 h photoperiod of 60–80 μmol/(m²s) intensity (cool white fluorescent tubes).

**Acclimatization and transplantation of regenerated plantlets**

Regenerated plantlets were taken from culture bottles, washed thoroughly for removing residual medium, transplanted to pots with sterilized sand and soil in a 1:1 ratio and covered with a transparent plastic sheet for 2–3
weeks. The established plants were then transferred to a greenhouse (temperature 25°C ± 3°C and relative humidity 70–80%) for further growth.

**Evaluation of the results and data analysis**

All experiments were set up in a completely randomized factorial design and repeated three times with 30 replicates per treatment. ‘Regeneration percentage’ was calculated by dividing the number of explants that regenerated adventitious buds by the total number of explants. ‘Number of buds per explant’ was calculated by dividing the number of regenerated buds by the number of petiole explants that regenerated buds. ‘Length of shoot buds’ was calculated as the mean length of elongated shoot buds. ‘Rooting percentage’ was calculated by dividing the number of elongated shoots that regenerated adventitious roots by the total number of elongated shoots used for root induction. ‘Number of roots per shoot’ was calculated by dividing the number of regenerated adventitious roots by the number of elongated shoots that regenerated adventitious roots. ‘Average root length’ was calculated by dividing the total length of all regenerated roots by the number of regenerates roots.

Statistical analysis of the data was carried out using SPSS 17.0 software, and significance of differences among means was determined by Duncan’s multiple range tests at \( p \leq 0.05 \). The results were expressed as means ± SD (standard deviation) of three independent experiments.

**Results and discussion**

**Regeneration of adventitious buds from petiole explants with conventional culture methods**

Genetic transformation helps to modify one or a few traits of the species at a time. Physiologically mature plants possess all the traits for selection and are the best sources for potential genotypes for genetic modification of heterozygous plants. Since petiole explants are isolated from physiologically mature plants, they are suitable receptors for genetic transformation.[25,28,32]

Tissue culture methods for inducing plant regeneration from physiologically mature plants are very important for transformation. Conventional culture methods for inducing adventitious bud regeneration from *J. curcas* explants include direct inoculation of the explants on a medium containing cytokinins such as BA or TDZ at low concentrations (usually 0.05 to 2 mg/L), and the regeneration efficiency is usually proportional to the concentration of cytokinin used.[19,23,25,32] The best shoot buds induction (35.13%–55.11%) and number of shoot buds (4.76–9.43) per explant have been obtained when petiole explants are placed on MS medium supplemented with 0.5 mg/L TDZ after 6 weeks.[23,25] In our study, petiole explants from M-1, M-3 and M-19 plants without TDZ treatment were also inoculated onto MS medium containing different concentrations of BA and TDZ as reported previously.[9,23,25,32] The concentration of BA and TDZ in the medium influenced the response of shoot bud induction for all the genotypes tested (Figure S1 and Table S1 in the online Supplemental data). TDZ was much more effective for the induction of adventitious bud regeneration than BA, especially for M-19, which did not respond to any of the BA concentrations tested. Of the different concentrations of TDZ tested, the highest percentage of shoot bud induction (35.85%) and the highest number of induced shoot buds (3.89) per explant were observed when 0.6 mg/L TDZ was applied (Table S1 and Figure S1F in the online Supplemental data). Although the number of regenerated buds per explants was not small, most of the regenerated buds were very tiny and underdeveloped. Further elongation of shoot buds was slow and was inhibited at 0.6 mg/L of TDZ (data not shown). Therefore, increasing the TDZ concentration over 0.6 mg/L would not yield better results. The optimum TDZ concentration for the induction of shoot buds and subsequent sub-culture was 0.3 mg/L. The percentage of shoot bud induction was 27.19% and the number of induced shoot buds per explant was 3.21 at 0.3 mg/L TDZ (Table S1).

**Regeneration of adventitious buds from petiole explants treated with TDZ solution before culture**

In our study, the conventional methods showed low regeneration efficiency (below 40%), similar to previous reports.[19,23,25,32] In our previous study, high concentrations of BA treatment before inoculating hypocotyl explants on hormone-free MS medium increased the bud regeneration frequency in soybean.[33] To investigate whether this method could be used for *J. curcas*, petiole explants from M-1 were treated with various concentrations of TDZ solution for 20 min before being inoculated onto hormone-free MS medium. The concentrations of TDZ solution significantly influenced the response of adventitious buds induction (Figure 1 and Table 1). The results clearly showed that treatment with TDZ solution was more effective than conventional methods for induction of adventitious bud regeneration from petiole explants. The application of 20 mg/L TDZ resulted in the highest percentage of shoot bud induction (65.78%) and the highest number of induced shoot buds (6.77) per explant (Table 1 and Figure 1(D)). The percentage of induction of shoot buds and the number of induced shoot buds per explant were directly proportional to the concentration of TDZ when the concentration of TDZ was not higher than 20 mg/L. However, when TDZ was used at concentrations higher than 20 mg/L, the regeneration percentage of adventitious buds decreased significantly. Therefore, 20 mg/L TDZ solution was determined as the optimum concentration for the induction of shoot buds.
To study the effect of time duration of TDZ treatment on adventitious buds induction, petiole explants from M-1 were treated with 20 mg/L TDZ for various time periods before inoculation of explants on the hormone-free MS medium. The results showed that time duration of the treatment significantly influenced the response of shoot buds induction (Table 2). When the explants were treated with 20 mg/L TDZ solution for 5 min before culture, the percentage of shoot bud induction was 42.99%, and the number of regenerated buds per explant was 4.34. This was better than the best results obtained by using conventional culture methods (Table S1). Treatment with 20 mg/L TDZ solution for 20 min was most suitable and gave the highest regeneration percentage (65.78%) and the largest number of regenerated buds per explant (6.67) (Table 2 and Figure 1(D)). When the explants were treated with 20 mg/L TDZ for time periods longer than 20 min, i.e. 40 min and 80 min, the bud regeneration frequencies decreased significantly; the percentage of shoot bud induction varied from 39.03% to 54.24%, and the number of induced shoot buds per explant varied from 3.11 to 4.91 (Table 2).

In our study, the conventional methods showed low regeneration efficiency (Table S1). However, treatment of
petiole explants with a range of TDZ solutions at high concentrations (5 to 120 mg/L) for 20 min or treatment of petiole explants with 20 mg/L TDZ solution for different short periods of time (5 to 80 min) before inoculation on hormone-free MS medium increased the regeneration frequency and caused the formation of bigger buds (Table 1, Table 2 and Figure 1) as compared with the conventional methods. Further culture of the regenerated buds showed that the regenerated buds were easily elongated (Figure 2 (A)). In plant tissue culture, cytokinin is an essential factor for the induction of adventitious buds formation in most cases.[22,26,34–39] However, the mechanism underlying the induction of adventitious buds in our method is unclear. Our previous [33] and present studies suggest that our culture method might be applicable to a wide range of plant species, although different cytokinins should be tested for better results. It will be intriguing to see whether other cytokinins like BA or KT positively affect bud regeneration in J. curcas by using the protocol described in this study, especially because BA and KT are much less expensive than TDZ.

**Table 1.** Effect of treatment of petiole explants with various concentrations of TDZ solution on the regeneration of adventitious buds in J. curcas after 35 days of culture.

| TDZ concentration (mg/L)* | Regeneration percentage (%) | Number of buds per explant |
|--------------------------|-----------------------------|----------------------------|
| 0                        | 0**a**                      | 0**a**                     |
| 5                        | 28.89 ± 1.93d               | 3.84 ± 0.24d               |
| 10                       | 43.33 ± 3.33c               | 4.62 ± 0.05c               |
| 20                       | 65.78 ± 1.54a               | 6.77 ± 0.51a               |
| 30                       | 59.19 ± 1.40b               | 5.80 ± 0.31b               |
| 60                       | 55.18 ± 3.39b               | 5.33 ± 0.33b               |
| 120                      | 45.19 ± 2.79c               | 4.36 ± 0.69d               |

*Treatment time 20 min.
**Values represent means ± SD of 30 explants per treatment in three independent experiments. Data in the same column followed by different letters are significantly different at p ≤ 0.05 as determined by Duncan’s multiple range test.

**Table 2.** Effect of treatment of explants with 20 mg/L TDZ solution for various time durations on the regeneration of adventitious buds from petiole explants of J. curcas after 35 days of culture.

| Treatment duration (min) | Regeneration percentage (%) | Number of buds per explant |
|--------------------------|-----------------------------|----------------------------|
| 0                        | 0**a**                      | 0**a**                     |
| 5                        | 42.99 ± 1.39c               | 4.34 ± 0.15c               |
| 10                       | 54.76 ± 4.12b               | 5.35 ± 0.39b               |
| 20                       | 65.78 ± 1.54a               | 6.76 ± 0.51a               |
| 40                       | 54.24 ± 3.81b               | 4.91 ± 0.37b               |
| 80                       | 39.03 ± 4.09c               | 3.11 ± 0.03d               |

*Values represent means ± SD of 30 explants per treatment in three independent experiments. Data in the same column followed by different letters are significantly different at p ≤ 0.05 as determined by Duncan’s multiple range test.

**Effect of the age of petiole explants on regeneration**

It has been shown that the physiological age of the donor plant materials is one of the main factors influencing the regeneration and that mature explants usually have lower regeneration efficiency.[40–42] Attempts have been made to regenerate shoot buds from physiologically mature explants.[19,23,25,32] However, to the best of our knowledge, the effect of the physiological age of explants on bud regeneration has not been investigated before in J. curcas. The regeneration efficiencies were compared among petioles of different ages. In this study, petioles were isolated from the first leaf to the fifth leaf (counting from the top of M-1 shoots) and the length of the petioles were about 1, 2, 3, 4 and 5 cm, respectively, from the first petiole to the fifth one (Figure 1(I)). Petiole explants were treated with 20 mg/L TDZ solution for 20 min followed by inoculation onto hormone-free MS medium. Our results showed that the age of the petioles significantly influenced the regeneration ability of the explants; the percentage of induction of shoot buds varied from 12.31% to 65.78%, and the number of induced shoot buds per explant varied from 1.72 to 6.76 (Table 3). Among all petioles tested, the second petiole showed the best regeneration wherein the highest regeneration percentage of 65.78% and the largest number of regenerated buds per explant of 6.76 were observed (Table 1, Table 3 and Figure 1(D)). Our results showed that extremely young and extremely old petioles were not suitable for bud regeneration in J. curcas.

**Comparison of inoculation methods and genotypes on regeneration cultures**

To investigate the effect of inoculation orientation (horizontal or vertical) on adventitious bud induction, the petiole explants from M-1, M-3, M-19 were treated with 20 mg/L TDZ for 20 min and inoculated onto hormone-free MS medium horizontally or vertically. The orientation of explants significantly influenced the response of shoot bud induction. Our results showed that the method of inoculating the explants horizontally on the medium was more beneficial to the regeneration than inoculating the explants vertically, with no exception among all the three genotypes tested (Table S2 in the online Supplementary data). The percentage of induction of shoot buds varied from 55.40% to 65.78% in the horizontal position and from 15.87% to 32.09% in the vertical position among the genotypes, whilst the number of induced shoot buds per explant varied from 4.31 to 6.76 in the horizontal position and 1.25 to 2.49 in the vertical position among the
Genotypic effects on shoot regeneration have been observed in many species, although the precise mechanism remains unclear.[43–45] Differences in the percentage of induction of shoot buds and the number of induced shoot buds per explant were also observed among the genotypes studied. M-1 performed best both in terms of the percentage of induction of shoot buds (32.09%–65.78%) and the number of shoot buds per explant (2.49–6.76) (Table S2 in the online Supplemental data). These results showed the genotypic effects on adventitious buds regeneration using the new culture method, which are in agreement with previous findings.[22,23]

Effects of GA₃ on bud elongation

GA₃ has beneficial effects on the elongation of regenerated adventitious buds in a woody plant *Acacia mangium*.[46] This effect is consistent with the role of GA₃ in stem elongation.[47–49] Deore and Johnson showed that GA₃ aided the elongation of adventitious buds regenerated by using conventional tissue culture method in *J. curcas*.[21] To investigate the effect of GA₃ on the elongation of regenerated buds, mother tissues with regenerated shoot buds were transferred and inoculated onto fresh MS medium supplemented with 0.5 mg/L BA, 0.2 mg/L KT, 0.25 mg/L IAA and various concentrations of GA₃ (0, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/L). The results showed that GA₃ facilitated
Table 3. Effect of developmental age of the petioles on the regeneration of adventitious buds in J. curcas after 35 days of culture.∗

| Petiole position on the shoots** | Regeneration percentage (%) | Number of buds per explant |
|---------------------------------|-----------------------------|----------------------------|
| 1st                             | 20.82 ± 2.40****           | 2.53 ± 0.24****            |
| 2nd                             | 65.78 ± 1.54a              | 6.76 ± 0.51†              |
| 3rd                             | 53.33 ± 3.33b              | 4.63 ± 0.12b              |
| 4th                             | 30.07 ± 1.76c              | 3.26 ± 0.21c              |
| 5th                             | 12.31 ± 1.12d              | 1.72 ± 0.25d              |

∗TDZ concentration 20 mg/L; treatment time 20 min.
**Petioles were isolated from the first leaf to the fifth leaf counted from the top of the shoots of the M-1 genotype.
***Values represent means ± SD of 30 explants per treatment in three independent experiments. Data in the same column followed by different letters are significantly different at p ≤ 0.05 as determined by Duncan’s multiple range test.

Table 4. Effect of various concentrations of L-glutamine on the rooting of regenerated shoots from petiole explants of J. curcas.∗

| Culture time (days) | Gln concentration (mg/L) | Rooting percentage (%) | Average root length (cm) |
|---------------------|--------------------------|------------------------|--------------------------|
| 10                  | 0                        | 8.69 ± 2.73****        | 0.67 ± 0.19b             |
|                     | 4                        | 9.99 ± 1.46a           | 0.70 ± 0.14a             |
|                     | 8                        | 25.36 ± 1.76b          | 0.93 ± 0.26c             |
|                     | 16                       | 30.93 ± 3.40a          | 1.36 ± 0.18b             |
|                     | 32                       | 23.52 ± 1.65s          | 1.30 ± 0.15s             |
|                     | 64                       | 10.67 ± 1.60d          | 0.86 ± 0.09b             |
|                     | 128                      | 8.12 ± 2.51d           | 0.63 ± 0.12b             |
| 20                  | 0                        | 17.49 ± 2.22c          | 1.64 ± 0.19c             |
|                     | 4                        | 18.23 ± 3.40c          | 1.61 ± 0.09c             |
|                     | 8                        | 34.22 ± 2.94d          | 2.43 ± 0.24b             |
|                     | 16                       | 38.38 ± 2.10d          | 2.90 ± 0.34a             |
|                     | 32                       | 32.37 ± 1.67b          | 2.48 ± 0.17b             |
|                     | 64                       | 16.90 ± 2.03b          | 2.37 ± 0.21b             |
|                     | 128                      | 11.54 ± 3.08d          | 1.62 ± 0.23c             |
| 30                  | 0                        | 21.09 ± 1.11d          | 3.37 ± 0.32b             |
|                     | 4                        | 28.12 ± 3.30e          | 5.21 ± 0.45f             |
|                     | 8                        | 35.80 ± 3.47g          | 6.32 ± 0.20b             |
|                     | 16                       | 50.06 ± 2.09h          | 8.98 ± 0.42j             |
|                     | 32                       | 39.77 ± 4.36i          | 8.58 ± 0.29k             |
|                     | 64                       | 20.16 ± 3.58k          | 7.36 ± 0.41l             |
|                     | 128                      | 13.05 ± 2.29j          | 2.26 ± 0.31l             |
| 40                  | 0                        | 22.76 ± 2.03s          | 3.47 ± 0.46l             |
|                     | 4                        | 30.90 ± 2.84i          | 2.78 ± 0.39f             |
|                     | 8                        | 38.53 ± 1.31b          | 4.21 ± 0.33c             |
|                     | 16                       | 51.72 ± 3.52a          | 5.36 ± 0.38e             |
|                     | 32                       | 41.22 ± 1.90b          | 7.45 ± 0.31h             |
|                     | 64                       | 21.55 ± 2.00d          | 3.81 ± 0.49f             |
|                     | 128                      | 14.81 ± 1.09e          | 2.31 ± 0.29d             |

∗Total number of roots for a single shoot was calculated by adding both primary and secondary roots together. Values represent means ± SD (standard deviation) of 30 explants per treatment in three independent experiments.
**Number of roots per shoot = total number of roots/number of shoots that initiated at least one root.
### Effects of L-glutamine on shoot rooting cultures

Auxin-induced rooting is a common method for regeneration of viable plants from shoots.[50–52] IBA has been shown to be effective in the rooting of J. curcas.[6,13,21,53–55] As one of the main endogenous amino acids involved in plant metabolism, Gln provides nitrogen for the biosynthesis of amino acids, nucleic acids and other N-compounds, and is frequently applied in plant tissue culture medium as an organic nitrogen source.[56,57]

Studies have demonstrated that exogenous application of Gln increases the regeneration frequency and explant biomass in plant tissue culture.[58–63] Some reports also show that exogenous Gln affects rooting in regenerated shoots.[31,46,64,65] However, it is unclear whether exogenous Gln has a positive effect on the rooting of elongated shoots in J. curcas. To test whether Gln could facilitate elongated shoot-buds rooting in J. curcas, elongated shoots at least 1 cm in length were isolated from the mother tissues and cultured vertically in medium containing 0.3 mg/L IBA and various concentrations of Gln (0, 4, 8, 16, 32, 64 and 128 mg/L). The results were recorded at days 10, 20, 30 and 40 of culture (Table 4 and Figure 2).

Although the elongated shoot buds could be induced to regenerate adventitious roots in the MS medium containing 0.3 mg/L IBA and 0 mg/L Gln, the rooting efficiency was relatively low, and the highest rooting induction efficiency was 22.76% at 40 days of culture (Table 4).
Moreover, leaves of the elongated shoots easily turned yellow and even dropped from shoots early (Figure 2(B)), which was usually observed when regenerated shoots were transferred to rooting culture medium in J. curcas. [25,26,54] This phenomenon has been reported in Acacia catechu and was mitigated to a large extent when Gln (150 mg/L) was added to the medium.[64] Our results showed that supplementing Gln to the medium at certain concentrations effectively stimulated the initiation and growth of roots, with 16 mg/L having the best effect inducing the best rooting rate (51.72%) (Table 4). Furthermore, the growth of the regenerated shoots was greatly improved as a result of Gln application as indicated by the fact that the yellowing and/or early leaf shedding of elongated shoots could be effectively mitigated to a large extent when Gln (4-8 mg/L) was added (Figure 2(C) and 2(D)). Leaf shedding and yellowing could also be completely avoided by application of 16 mg/L or higher concentrations of supplemented Gln (Figure 2(E-H)). However, Gln concentrations higher than 16 mg/L inhibited the regeneration of adventitious roots significantly (Table 4). Therefore, the best and most effective results were obtained when 16 mg/L Gln was applied. Although root initiation could be observed even before day 10 of culture, longer time was needed for achieving better rooting results. A comparison of the data obtained between days 30 and 40 showed that culture periods longer than 30 days did not significantly enhance rooting. Therefore, a culture period of 30 days was considered adequate for rooting. Regenerated plantlets were acclimatized and transplanted successfully to the soil and the regenerated shoots showed normal growth (Figure 2(I)). To the best of our knowledge, this study for the first time showed that the application of exogenous Gln in a certain concentration range could improve both the rooting efficiency and the quality of rooting in plantlets in J. curcas.

Conclusions

An efficient in vitro culture protocol for obtaining regenerated plantlets for J. curcas was established in this study. With the improved shoot elongation and high efficiency of root induction, an intact plantlet could be obtained at 60-80 days of culture by using the described culture protocol, but not at 90-140 days of culture by conventional methods. This efficient and reproducible method would be useful for mass production of true-to-type plants and transgenic plants through Agrobacterium/biologist-mediated transformation.

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Disclosure statement

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Supplemental data

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