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Thidiazuron (TDZ) induced plant regeneration from cotyledonary petiole explants of elite genotypes of *Jatropha curcas*: A candidate biodiesel plant

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ABSTRACT

Jatropha curcas, the energy plant has attained great attention in recent years because of its biodiesel production potential and medicinal value. This makes it imperative to search for techniques for its rapid propagation. In the present investigation an efficient and reproducible method for plant regeneration through direct shoot bud induction from cotyledonary petiole explants of elite genotypes (CSMCRI-JC-1, CSMCRI-JC-2 and CSMCRI-JC-3) of *J. curcas* was developed. The best shoot buds induction (51.19%) and number of shoot buds (9.75) per explant was observed when *in vitro* petiole explants were placed horizontally on MS medium supplemented with 2.27 μM TDZ after 6 weeks. The induced shoot buds were transferred to MS medium containing 10 μM kinetin (Kn), 4.5 μM 6-benzyl aminopurine (BAP) and 5.5 μM α-naphthaleneacetic acid (NAA) for shoot proliferation. The proliferated shoots could be elongated on MS medium supplemented with different concentrations and combinations of BAP, indole-3-acetic acid (IAA), NAA and indole-3-butyric acid (IBA). MS medium with 2.25 μM BAP and 8.5 μM IAA was found to be best combination for shoot elongation and 3.01–3.61 cm elongation was achieved after 6 weeks. However, significant differences in plant regeneration and shoot elongation were observed among the genotypes studied. Orientation (horizontal or vertical) and source (*in vitro* or *in vivo*) of explants also significantly influenced plant regeneration. The elongated shoots could be rooted on half-strength MS medium supplemented with 0.25 mg/L activated charcoal and different concentrations and combinations of IBA, IAA and NAA. Half strength of MS medium with 5 μM IBA, 5.7 μM IAA and 11 μM NAA was found to be best for rooting. The rooted plants could be established in soil with more than 90% survival rate.

1. Introduction

Tree-borne oilseeds are the best and potential alternative to mitigate the current and future energy crisis and also to transform the vast stretches of wasteland into green oil fields. The potential sources identified so far include *Jatropha curcas*, *Pongamia pinnata*, *Madhuca latifolia*, *Azadirachta indica*, *Calophyllum inophyllum* and *Simarouba glauca*. Among these *J. curcas* emerges as the most promising tree-borne oilseed on the basis of adaptability to wide range of edapho-climatic conditions, high oil content coupled with the suitability of Jatropha oil as a source of biodiesel. *J. curcas*, a multipurpose large shrub belongs to the family Euphorbiaceae, is native to South America and widely distributed in South and Central America, Africa and Asia. Biodiesel prepared from *J. curcas* has been sucessfully tested in both mobile and stationary engines without modification in any of the engine parts. Now there is a surge of interest in *J. curcas* as a biodiesel "miracle tree" to help alleviate the energy crisis and generate income in rural areas of developing countries (Francis et al., 2005).

Commercial plantations of this important crop are raised through seedlings and stem cuttings. The seed viability and rate of germination are low, and quality seed screening is another laborious task thus, propagation through seed may not provide quality planting material for sustainable use. It was also observed that huge quantities of seed is being used for raising of planting material, if any alternative propagation technique is developed the seed can be diverted for biodiesel preparation. Propagation can be also carried out by stem cutting but the limitation in generation of large scale planting material is (a) availability of sufficient quantity of material and (b) propagation is seasonal. Thus, conventional propagation through seeds is not reliable and vegetative cuttings are inadequate to meet the demand of large scale quality planting material. Therefore, *J. curcas* improvement programme by modern

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methods of agrobiotechnology are of interest worldwide. This has increased the importance of developing tissue culture methods to facilitate large scale production of quality planting material and for the improvement of the species using genetic engineering technique. Attempts have been made to regenerate *J. curcas* (Sujatha and Mukta, 1996; Wei et al., 2004; Sujatha et al., 2005; Rajore and Batra, 2007; Jha et al., 2007; Kumar, 2009; Kumar and Reddy, 2010; Kumar et al., 2010a,b,c, 2011a,b) either through callus mediated regeneration or direct shoot morphogenesis. In all the above reports, the frequency of regeneration has been low and unsatisfactory. It is also reported that regeneration in *J. curcas* is highly genotype dependent (da Camara Machado et al., 1997; Kumar et al., 2008; Kumar and Reddy, 2010; Kumar et al., 2010a, 2011b). Keeping the economical importance of *J. curcas in* mind and critical analysis of earlier reports, the objective of this study was to develop an *in vitro* regeneration method without formation of intervening callus in *J. curcas* for mass propagation and genetic improvement. This study also compared the regeneration efficiency of *in vitro* and *in vivo* cotyledonary petiole explants.

2. Materials and methods

2.1. Plant material and source of explant

The seeds of elite genotypes (CSMCRI-JC-1, CSMCRI-JC-2 and CSMCRI-JC-3) of *J. curcas* were obtained from Central Salt & Marine Chemicals Research Institute (CSMCRI) experimental plantation on wastelands at Chorvadla, India (21°75″N, 72°14″E) for the present study. Seedcoats were removed and then surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 15 min and rinsed five times in sterile distilled water. The sterilized decoated seeds were germinated on growth regulators free MS liquid medium (Murashige and Skoog, 1962) with the support of filter paper boats. After 2 weeks of germination, cotyledonary petiole explants were collected from cotyledonary leaves of germinated seedlings and used as *in vitro* explants. For *in vivo* explants, seedlings were raised in the nursery and cotyledonary petiole explants were collected from cotyledonary leaves of 2-week old seedlings, and sterilized by 0.1% mercuric chloride ($HgCl_2$) for 3 min and rinsed five times in sterile distilled water.

2.2. Shoot bud induction

The *in vitro* and *in vivo* cotyledonary petiole explants of all the three genotypes were cultured on MS medium supplemented with different concentrations of thidiazuron (TDZ) to find an optimum concentration of TDZ for shoot bud induction (Kumar and Reddy, 2010; Kumar et al., 2010a,b,c). Cotyledonary petiole explants were inoculated on medium surface in 200 mm × 38 mm culture tubes (Borosil, India) both in horizontal and vertical position. The percentage of induction of shoot bud and the number of shoot buds per explant were recorded after 6 weeks of culture.

2.3. Shoot proliferation and elongation from induced shoot buds

The induced shoot buds were transferred on MS medium supplemented with 10 μM kinetin (Kn), 4.5 μM BAP and 5.5 μM α-naphthaleneacetic acid (NAA) for 4 weeks for shoot proliferation (Reddy et al., 2008; Kumar and Reddy, 2010; Kumar et al., 2010a,b,c). Shoots were individually separated and further tested for their elongation on MS medium supplemented with different concentrations and combinations of BAP, indole-3-acetic acid (IAA), NAA and IBA. The length of elongated shoots was recorded after 6 weeks of culture.

2.4. Rooting and acclimatization

Green and healthy elongated shoots with three to four leaves were excised and cultured on half strength MS medium supplemented with different concentrations and combinations of auxins, *i.e.* IBA (5–15 μM), IAA (5.7–11.4 μM) and NAA (5.5–11 μM) rooting (Kumar and Reddy, 2010; Kumar et al., 2010a,b,c). The percentage of root induction per shoot was recorded after 4 weeks. Rooted shoots were carefully taken out of the medium and washed thoroughly in sterile distilled water to remove basal MS medium attached to the roots. The plants were transferred to plastic bags containing sterilized sand and soil in the ratio 1:1 and wetted with 0.02% (w/v) carbendazim and covered with transparent plastic bags to maintain humidity. After 3–4 weeks, the established plants were transferred to a greenhouse (temperature 25 ± 3 °C and relative humidity 70–80%) for further growth and the numbers of surviving plants were recorded after an additional 6–8 weeks.

2.5. Culture conditions and data analysis

Uniform culture conditions were applied in all experiments. The pH of the medium was adjusted to 5.7 using 1 N KOH or HCl, prior to autoclaving at 1.05 kg/cm^2 pressure at 121 °C for 20 min. The cultures were maintained at 25 ± 2° C under a 16 h photoperiod with light intensity of 35–40 μmol m^{-2} s^{-1} (cool white fluorescent tubes).

All the experiments were set up in a factorial completely randomized design (FCRD) and repeated three times with 25 replicates per treatment, with one explant per test tube. Data were subjected to analysis of variance (ANOVA), analyzed by four factor FCRD analysis for shoot bud induction and two factor CRD analysis for shoot elongation and rooting using a BASIC software package (Anand Agricultural University, Gujarat, India) at the 5% probability level. Statistical difference among the means was analyzed by Duncan's multiple range test using the SPSS (version 7.5). The results were expressed as mean ± standard error of three independent experiments.

3. Results

3.1. Effect of TDZ on shoot bud induction

The concentration of TDZ in the medium significantly influenced the response of shoot bud induction irrespective of genotype studied. The percentage of induction of shoot buds and the number of induced shoot buds per explant were directly proportional to the concentration of TDZ. Of the different concentrations of TDZ tested, the highest percentage of shoot bud induction (66.97%) and highest number of induced shoot buds (13.76) per explant were observed in the presence of 9.08 μM TDZ, among the genotypes studied. However, further proliferation and elongation of shoot buds were inhibited due to compact shoot bud induction at this concentration. It was observed that 2.27 μM TDZ was the optimum concentration for the induction of shoot buds and subsequent sub-culture. At 2.27 μM TDZ, the percentage of shoot bud induction varied from 39.69 to 51.19% and the number of induced shoot buds per explants varied from 4.75 to 9.75 among the genotypes (Tables 1 and 2).

3.2. Effect of orientation of explants on shoot bud induction

The orientation of explants significantly influenced the response of shoot bud induction at the tested concentrations of TDZ among the genotypes studied. The percentage of induction of shoot buds and the number of induced shoot buds per explant were higher in the horizontal position, as compared to the vertical position, irrespective of genotype. The percentage of induction of shoot buds