

Potential Regeneration Capacity of *Jatropha Curcas* L Leaf Explants after Chemical Treatment for Aseptic In vitro Regeneration

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Abstract: Experiments were conducted to assess the potential tolerance of leaf explants of *Jatropha curcas* L to chemical components that are used during sterilization of explants for invitro regeneration resulting in generation of callus followed by shooting and rooting. Initially the seeds were sown in 96 well trays for the generation of plantlets in the green house using coco peat as the nutrient medium. Later leaf was selected as explant for invitro regeneration and was subjected to sterilization procedure. It was observed that, the leaf explants treated with 0.1% Mercuric chloride were devoid of contamination but resulted in chlorosis. However in almost all the explants, a part of the explant still responded on callus inducing media CI-1((MS+2.2µM BAP + 4.9µM IBA) resulting in generation of callus. But when these calli were transferred to shooting media SR-1((MS+8.9µM BAP + 4.4µM IBA) resulted in generation of embryoids. Further these embryoids were sub cultured on SR-1 to develop in to shoots. Later these shoots resulted in rooting when transferred to half strength MS medium along with 4.4µM IAA.

Keywords: *Jatropha curcas* L, Greenhouse, Embryoids, Chlorosis, Coco peat

1. Introduction

Jatropha curcas L (physic nut), a multipurpose tree of Latin American origin, belongs to the family Euphorbiaceae, and is widespread throughout the tropical regions of the World (Heller J 1996). It has been found to be a highly promising species, yielding oil seed as a source of energy in the form of biodiesel. Short gestation period, easy adaptation to different kinds of marginal and semi-marginal lands, makes this plant species attractive for cultivation (Heller J 1996; Gubitz GM et al 1999). Besides its importance as an energy crop, *Jatropha curcas* L has many medicinal uses, for instance in treatment of piles, scabies, eczema and ring worm (Rajore S et al 2005). The cultivation of *Jatropha curcas* L assumes utmost importance to meet the large-scale demand and ensure continuous supply of the elite material (Deore AC et al 2005). Tissue culture techniques offer rapid and continuous supply of the planting material. Although there are reports of plantlet regeneration in *Jatropha curcas* L by some workers, the number of shoots initiated in the explants is relatively low (Rajore S et al 2005; Sujatha M et al 1996; Sujatha M et al 2005). Also, the previous works done on the in vitro propagation of *Jatropha curcas* L suggest that the incorporation of two or more growth regulators in the culture medium results in its further proliferation and multiplication (Deore AC et al 2005). In the present study, we report an efficient and reproducible method for large-scale propagation of *Jatropha curcas* L using leaf explants from one month old plantlets grown in green house.

2. Methodology

2.1 Plant Materials

The seeds of *Jatropha curcas* L were procured from Suresh Kumar agro forestry seeds, Chikkabalar, Bangalore, Karnataka. The seeds were soaked in water for about three hours and inoculated in to 96 well trays for development of plantlet in green house using coco peat as the nutrient medium. The water sprayed for development of seeds was mixed with 1% Bavistin to prevent fungal contamination which may inhibit the growth of seeds. Then the leaf explants of one month old plantlets were selected for invitro regeneration studies. Leaves excised from one to two nodal tips were selected for culture (Shrawan Kumar et al 2010). The leaves were thoroughly washed under running tap water for about 10 minutes, then dipped in 1% Bavistin (BASF, India) for about 5 min and washed with autoclaved distilled water. Then the leaf explants were swirled with 3 to 4 drops of detergent Tween 20 for about 5 minutes, 0.1% HgCl₂ (w/v) solution for about 1 min and 70% alcohol for about 1 minute under LAF to maintain aseptic conditions. After each treatment the explants were washed with autoclaved distilled water for about 2-3 times, finally the explants were blotted on autoclaved Whatmanns paper. Subsequently, the leaves were cut into small pieces (5-7 mm) and placed with abaxial side on the MS medium.

3. Media and Culture Conditions

The medium used for callus development of leaf explants was CI-1 along with 30 g/L sucrose and 5.0 g/L agar. The

media was adjusted to pH 5.8 prior to adding agar and autoclaving. Explants of leaf were cultured in jam bottles containing 20mL of culture medium CI-1 for about 4 weeks for callus development. Then the callus generated in CI-1 were transferred to SR-1 which generated embryoids from callus and further developed to shoots within a period of 8 weeks through repeated subculture at regular intervals of time, approximately once in 15 days. Then the shoots were transferred to half strength MS medium along with 4.4 μ M IAA for rooting. Cultures were incubated at 25 \pm 2 $^{\circ}$ C with a 16-h photoperiod of 35- 40 μ mol .m⁻².s⁻¹ provided by cool white fluorescent lights for callus induction and shoot regeneration while for root development the tissues were provided with 8-h light and 16-h dark photoperiod.

4. Results and Discussion:

4.1 Effect of Mercuric chloride on leaf explants and generation of callus on CI-1

Leaf explants treated with 0.1% Mercuric chloride resulted in absence of contamination, and those not treated with Mercuric chloride resulted in contamination. However when treated with 0.1% Mercuric chloride most part of the explant resulted in chlorosis because of harsh treatment of Mercuric chloride. However CI-1 was effective in generation of callus at certain specific locations of explant where chlorosis was not observed. Lesser concentration of BAP in CI-1 was responsible for generation of callus in leaf explants.

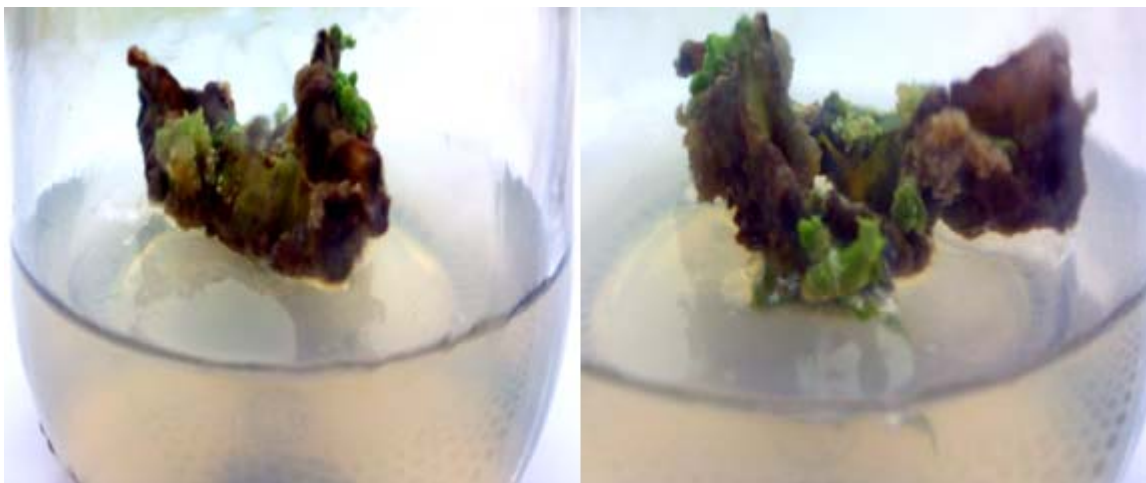


Figure 1: Development of callus from leaf explants of *Jatropha curcas* L on CI-1

4.2 Effect of SR-1 on callus for development of multiple embryoids and shoots.

Callus generated from leaf explant on CI-1 was transferred to SR-1 and cultured for about 8 weeks. The SR-1 was effective for generation of embryoids from callus. An average of 6 to 7 embryoids was generated out of each callus. Then the embryoids were sub cultured on to SR-1 for

development of individual shootlets and later transferred to half strength MS medium along with 4.4 μ M IAA for rooting. Higher concentration of BAP in SR-1 was responsible for regeneration of embryoids from callus which later developed in to shootlets. Leaf has higher proportion of meristematic tissue which was responsible for generation of more number of embryoids from each explant.



Figure 2: Development of embryoids from callus of leaf explants *Jatropha curcas* L on SR-1.

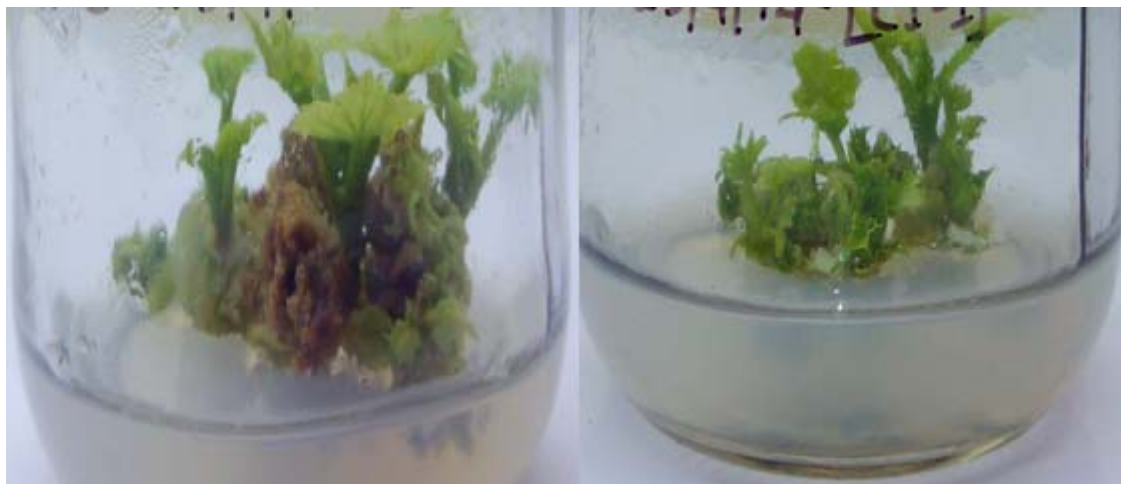


Figure 3: Development of shoot lets from embryoids of leaf explants *Jatropha curcas* L on SR-1

4.3 Effect of IAA on shootlets for rooting

The shootlets generated on SR-1 media from leaf explants were transferred to half strength MS medium along with $4.4\mu\text{M}$ IAA. The shootlets showed rooting after 5 weeks of culture.

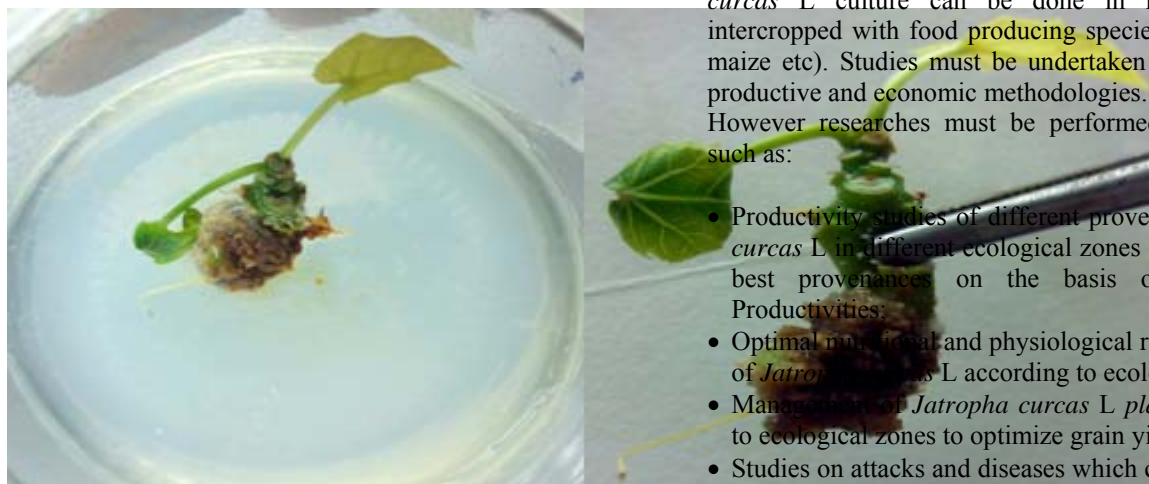


Figure 4: Rooting of *Jatropha curcas* L shoot cultured on SR-1 when transferred to half strength MS medium along with $4.4\mu\text{M}$ IAA.

The present study aims to characterize the explant that is most susceptible to chemical treatment and have maximum potential to generate shootlets for invitro regeneration of *Jatropha curcas* L plantlets. The development of appropriate techniques for *in vitro* culture and micropropagation of oil crops is necessary for germplasm collections, breeding program and mass propagation. The scope of the experiment to future is to develop a strategy for multiple embryoids production invitro from suitable explant of *Jatropha curcas* L for micropropagation. Since, *Jatropha curcas* L has gained much attention (Grimm 1996; Heller 1996; Henning 2000a; Pratt et al. 2002) for both non-oil producers and oil producers countries around the world.. The species has a high yield in oil, which can be used as fuel for diesel engines as well as for medical and insecticidal purposes (FACT Foundation, 2006).

5. Conclusion

Jatropha curcas L is becoming a new perennial cash crop for many regions of the imperative world. This makes it that special attention should be given to the way in which *Jatropha curcas* L plantations can be carried out. *Jatropha curcas* L culture can be done in marginal lands or intercropped with food producing species (millet, sorghum, maize etc). Studies must be undertaken on this feature for productive and economic methodologies. However researches must be performed in many aspects such as:

- Productivity studies of different provenances of *Jatropha curcas* L in different ecological zones and selection of the best provenances on the basis of seeds and oil Productivities;
- Optimal nutritional and physiological requirements studies of *Jatropha curcas* L according to ecological zones;
- Management of *Jatropha curcas* L plantations according to ecological zones to optimize grain yield per unit area;
- Studies on attacks and diseases which can affect seeds and oil content productivity of *Jatropha curcas* L plantations;
- *In vitro* propagation using various methods (from axillary buds, tissue culture,); necessary for germplasm collection, breeding programs and mass propagation.
- Constitution of *Jatropha curcas* L gene banks (cold room, laboratory, greenhouse, fields);
- Gene viability tests and renewal of stocks.
- Disinfection of basic plant material (Samba Arona Ndiaye Samba et al).

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Author Profile

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1. Molecular regulation of Santalol biosynthesis in *Santalum album* L published in Elsevier Science direct *Gene* 527 (2013) 642-648.
2. Invitro regeneration studies for multiple shoot induction through differential explants of *Jatropha curcas* L published in *IJPRBS journal* Volume 3 (4) : 606-612 August 2014.
3. Potential regeneration capacity of *Jatropha curcas* L leaf explants after chemical treatment for aseptic invitro regeneration accepted for publication in *IJSR journal* Volume 3, Issue 10 October 2014.