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IN- VITRO PRPAGATION OF *Jatropha curcas* L. THROUGH THE EMBRYO CULTURE TECHNIQUE

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ABSTRACT

Jatropha. curcas is one of the highly sort after species. The species is said to have great medicinal properties. It has been used for the treatment of several ailments including cancer. It is a good source of biodiesel which is environment friendly, therefore the demand for it is expected to be high with time with increasing human population. It is observed that this species has scanty roots and delayed rooting of seedlings when propagated through vegetative cuttings thus paving way for the necessity of its micropropagation and mass-propagation. The explants used were embryos excised from the seeds. Three different media were used for this experiment with various treatments. These are Murashige and Skooge (MS), Preece and Woody Plant Media (WPM) media. After nine days, the embryos grew best on the MS medium treated with no hormones compared to those cultured on Preece and WPM media treated with no hormones. After twenty days, the MS medium still gave the best results.

Keywords: *J. curcas, in-vitro*, mass-propagation, embryo.

INTRODUCTION

Jatropha curcas, a multipurpose plant commonly known as physic nut belongs to the family *Euphorbiaceae*. It is widely grown for its seeds for biofuel production and medicinal purposes. In optimal conditions, about 5 tons/ha seeds of Jatropha is produced per year, oil content in the seed ranges from 30 to 50% by weight and the kernel contains 45 to 60% (Senthilkumar, *et. al.*, 2003 Liebig, *et. al.*, 2009), the oil can be combusted as fuel without being refined. The by product of jatropha seed after extraction which is the press cake, could also be considered for energy production.(Jongschaap, *et. al*., 2007) as biomass feed stock to power electricity plants used in ,fish or animal feed(if detoxified), or as high quality fertilizer. It can also be used as bio-pesticide (Achten, *et. al.*, 2008), and insecticide (Adio, *et. al.*, 2011).The latex of *J. curcas* contains an alkaloid

known as "jatrophine" which is believed to have medicinal properties (Kumar and Sharma, 2008). The sap of the plant is used for treatment of cancer, piles, snakebite, skin diseases, paralysis, dropsy, malarial fever, arthritis, gout, jaundice and resistance to various stresses (Heller, 1996; Openshaw, 2000).

The environmental friendliness of bio fuel and its synthesis from edible and non-edible oils makes it an attractive alternative to non-renewable crude oil. *J. curcas* is a promising substitute for diesel, kerosene and other fuels (Abdulla *et al*., 2011) However, despite the abundance and the uses of the species as oil and reclamation plant, none of the Jatropha species has been properly domesticated (Fairless, 2007) and as a result their productivity is variable (Igbinosa *et al.*, 2009), hence the need for the development of a sustainable and renewable source of energy such as *Jatropha curcas*. Scanty roots and delayed rooting of seedlings when propagated through vegetative cuttings informs the need for a more viable and sustainable means of mass propagation through which seedlings of the plant can be made available all the year round (Heller 1996; Openshaw 2000; Purkayastha *et al.*, 2010). Furthermore, it is necessary to develop a protocol for the production of plantlets of *J. curcas* in order to meet future demand for supply of quality planting material for plantations establishment in the absence of seeds (Mustafa, 2012). This will make the production of large quantities identical plants having desirable traits such as high quality seed, pest and disease free, to be possible on all year– round basis irrespective of the season and weather.

Several plant species have been propagated by tissue culture technology, mainly food crops and ornamental plants (Malik and Saxena, 1992). *In vitro* techniques such as micropropagation provide a fast and reliable method for production of large number of plantlets in short time. This technique will provide a higher multiplication rate than the conventional propagation methods and also minimize the risk of infections by microbes and insect pests, reduce genetic erosion, space requirements and expenses in labor costs.

Sujatha, *et. al*., 2005 indicated that *in vitro* plants of *J. curcas* produce a better yield and yield-related traits than seed-propagated plants. However, this system may be preferred above cuttings because of its flexibility to adjust to the market demand and the production of pathogen free material. Moreover, tissue culture technologies would help in producing the active compounds *in vitro* with better productivities without cutting down the natural resources (Wei *et al*., 2004). Genetic variation in seed morphology and oil content of *Jatropha* is of great potential in tree improvement programs, unfortunately no much work has been done on germplasm conservation (Misra and Misra, 2010). Tissue culture of *Jatropha curcas* will help in the application of molecular techniques to produce new plants and

improvement in terms of drought, cold tolerance and reduction in toxin productions (Varma, *et. al*., 2007).

The study was therefore undertaken to determine the culture conditions and efficient protocol for *in vitro* propagation of *Jatropha curcas* through embryo culture technique.

MATERIALS AND METHODS

The explants were collected from *Jatropha curcas* seeds. The material used was embryo of *Jatropha curcas* commonly called Barbados nut, obtained from Forest Research Institute of Nigeria (FRIN). Murashige and Skoog (MS), Preece medium and Woody Plant Media (WPM) were used. The pH of the media in each case was adjusted to 5.7 after which they were then dispensed in test tubes and sterilized using the autoclave at 121^{o}C for 20 minutes. Scalpels, forceps and Petri-dishes to be used for excision and inoculation were also sterilized. Hormones, (NAA and BAP), of varying concentrations ranging from 0.0 - 1.5mg/L BAP and 0.0-0.5mg/L NAA as shown in **Table 1.** The generated plantlets were subcultured into freshly prepared MS medium for mass propagation.

Table 1: Treatments

Medium	Treatment(s)	NAA (mg/L)	BAP (mg/L)
MS	**G_1**	0.0	0.0
	G_2	0.5	0.5
	G_3	0.5	1.0
	G_4	0.5	1.5
Preece	**H_1**	0.0	0.0
	H_2	0.5	0.5
	H_3	0.5	1.0
	H_4	0.5	1.5
WPM	**I_1**	0.0	0.0
	I_2	0.5	0.5
	I_3	0.5	1.0

	I_4	0.5	1.5

G_1, H_1, I_1 = Controls

The explants were surface sterilized using 70% alcohol for 5minutes and 10% sodium hypochlorite solution with 2 drops of Tween 20 for 15 minutes. The *Jatropha curcas* seeds were dissected to excise the embryos using the sterilized scalpel with sterilized blade and sterilized forceps under the lamina flow hood. Excised embryos were aseptically inoculated singly into each tube containing sterile medium. The cultured tubes were then sealed, labeled, and transferred into the growth room at 25^{o}C±2^{o}C with a 16 h light/8 h dark cycle. They were daily observed for growth response. The Completely Randomized Design was used for the analysis .

Shoot segments with lengths of about1.5cm to 2cm were excised from 20 day-old plantlets *in vitro* and used as initial material for the micropropagation in freshly prepared media with various hormone concentrations.

Table 2:

Treatment(s)	**NAA (mg/L)**	**BAP (mg/L)**
M_1	0.0	0.0
M_2	0.5	0.5
M_3	0.5	1.0
M_4	0.5	1.5

RESULTS

Table 2 shows the different media type used in the study and germination rates recorded after 9 days of inoculation. After 9 days of culture, the explants responded better to the MS and Preece media than WPM (**Table 3** and **Plates 1 - 3**). It was discovered that MS media supported the growth of *Jatropha curcas* with 80% germination; Preece medium has 70% germination while WPM has the lowest germination percentage of 30(**Table 3**). **Table 4** shows germination rate

after 20 days (Plates 4 – 6), explants cultured on MS medium still gave the best results with 90% germination. The was high significant difference between the results obtained from both MS and Preece compared with the WPM. **Table 5** shows the result of the sub cultured shoot cuttings from the generated plantlets of the embryo culture into MS medium 20 days. Treatment M_1 which is the controlled experiment has 30% germination, while M_2 formed calli. M_3 has 100% germination with M_4 also having 100% germination.

Table 3: Germination Rate in different media without growth hormone after 9 days

Treatment(s)	**No. of culture**	**No. of germinated plantlets**	**Average No. of shoots**	**Average No. of roots**	**% germination**
G_1	20	16	6	3	80
G_2	20	14	7	4	70
G_3	20	15	5	3	75
G_4	20	16	6	3	80
H_1	20	14	6	3	70
H_2	20	14	6	3	70
H_3	20	13	5	3	65
H_4	20	13	5	3	65
I_1	20	06	5	3	30
I_2	20	04	5	3	20
I_3	20	04	3	2	20
I_4	20	04	4	2	20

Table 3: Germination Rate after 20 days

Treatment(s)	No. of cultured tubes	No. of germinated plantlets	Average No. of shoots	Average No. of roots	% germination
G_1	20	20	13	6	100
G_2	20	18	14	5	90
G_3	20	19	10	6	95
G_4	20	17	11	5	85
H_1	20	17	12	5	85
H_2	20	18	12	5	90
H_3	20	17	9	5	85
H_4	20	16	9	4	80
I_1	20	11	9	4	55
I_2	20	10	9	4	50
I_3	20	7	6	4	35
I_4	20	4	7	3	20

Table 4: Growth response of sub cultured shoot cuttings to different hormone concentrations after 20 days

Treatment(s)	Av. No. of shoots			
	5 days	10 days	15days	20 days
M_1	-	1	3	3
M_2	-	-	1	-
M_3	-	3	8	10
M_4	-	5	9	10

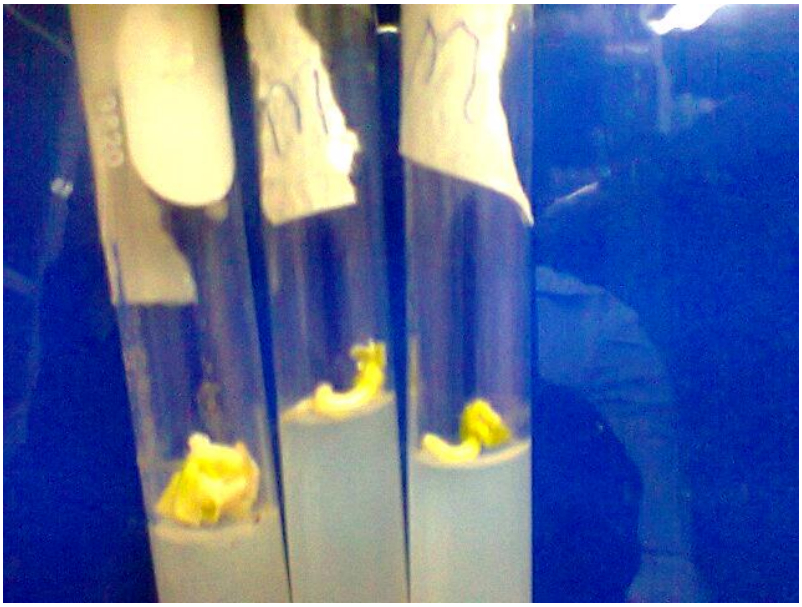

Plate 1: MS week one

Plate 2: PREECE week one

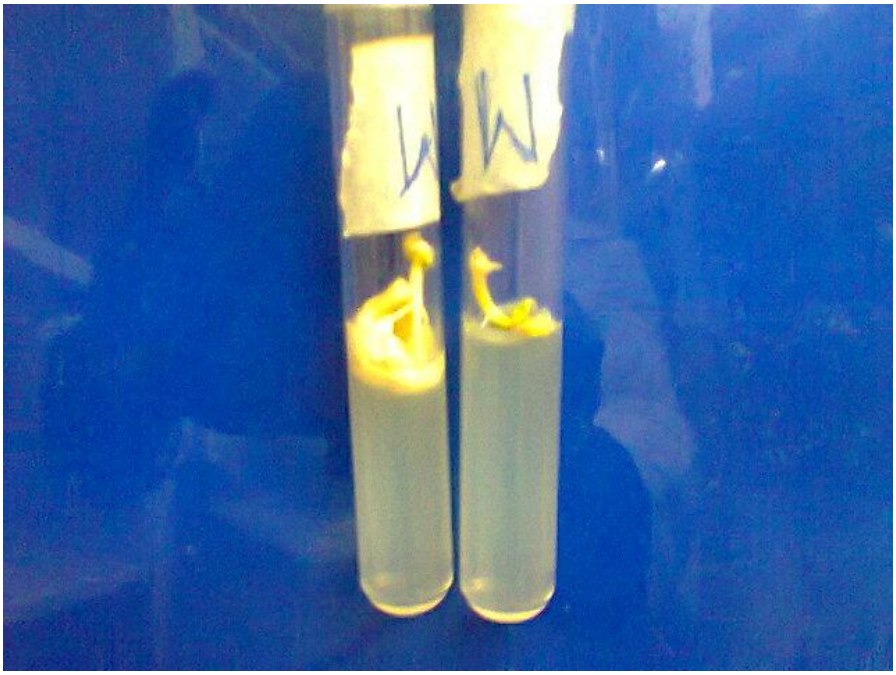

Plate 3: WPM week one

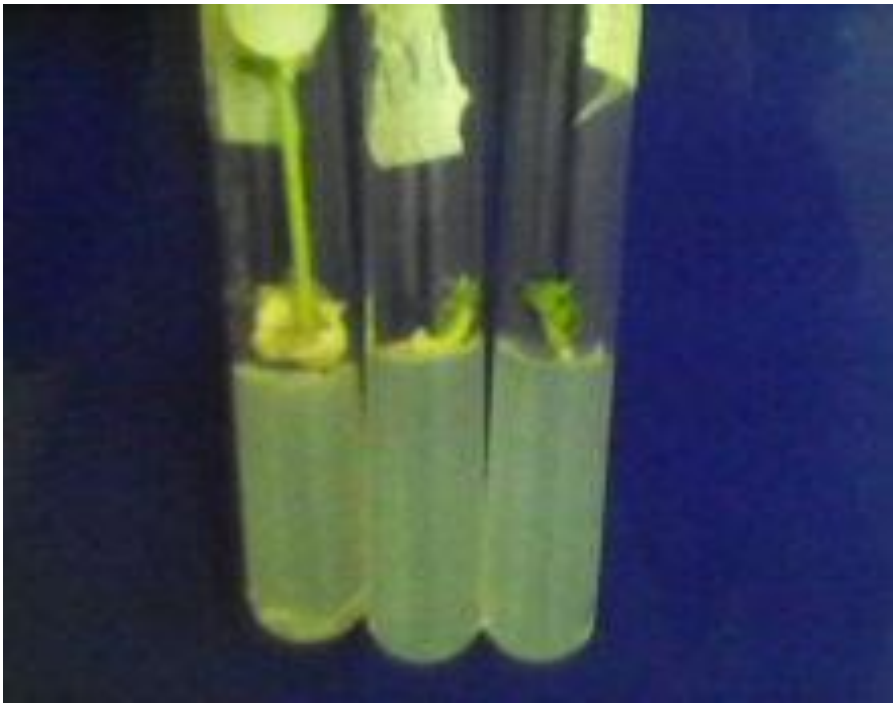

Plate 4: MS week three

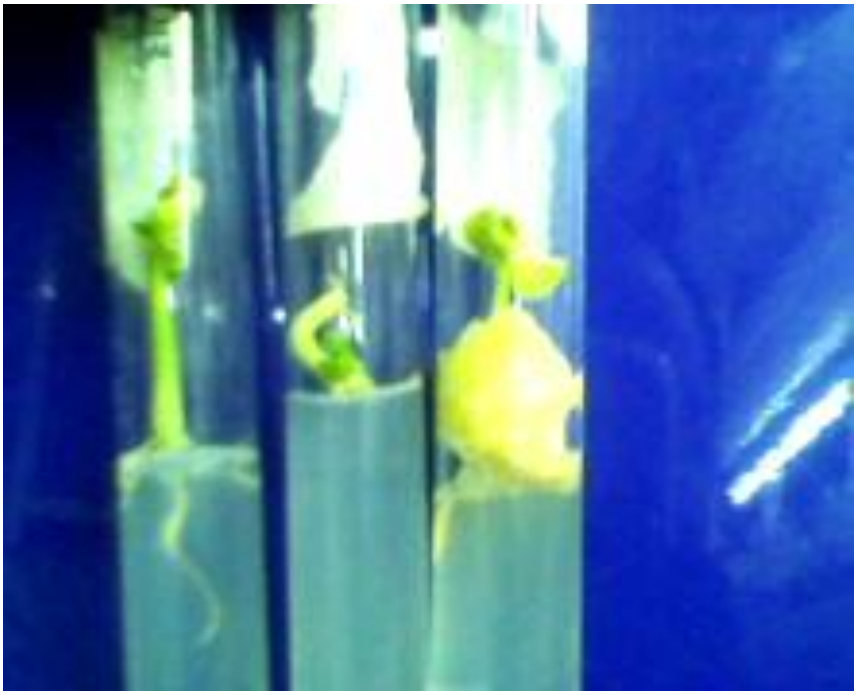

Plate 5: PREECE week three

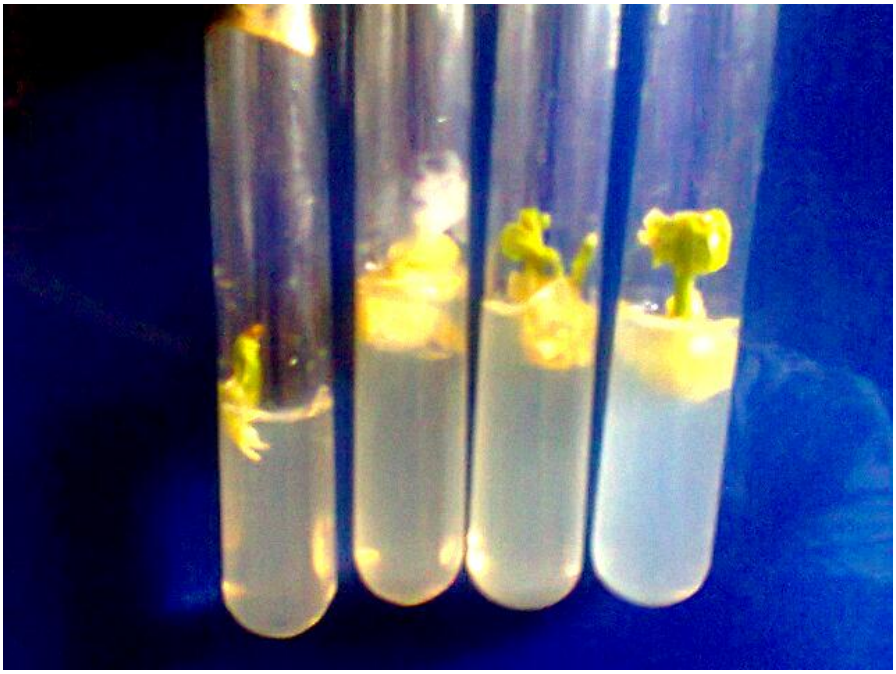

Plate 6: Growth response to different treatments (L-R) G1 – G4

DISCUSSION AND CONCLUSION

The results obtained in these experiments showed that embryo responded well in MS media without hormone compared to embryo cultured in Preece and WPM without hormones and the regeneration (sub-culturing) of the shoot cuttings responded well to various treatments in different ways, this is in line with report submitted by Lapitan (2009) that *J.curcas* propagated in vitro in different concentrations of growth hormones and formulated media.

The response of plantlets subcultured on MS media supplemented with NAA and BAP varied in line with Kumar, *et al*., (2008), who reported that subsequent growth of *J. curcas* promoted in MS medium supplemented with BAP and IAA. Sujatha, *et. al.*, (2005). He reported that the rate of shoot multiplication was significantly enhanced after transfer to MS basal medium supplemented with 2.3um (kinetin), 0.5um IBA and 27.8um adenine sulphate for 4weeks. Shoot buds developed into shoots when sub cultured on MS medium supplemented with 0.5mg/L BAP and 1.0mg/L IAA.

Based on the results obtained in this experiment, the treatments used played a major role in the growth response of the plantlets. In this study, treatment G4 which constitute MS, 0.5mg/L NAA and 1.5mg/L BAP showed the best result of growth and treatment G2 constituting MS, 0.5mg/L NAA and 0.5mg/L BAP showed a callus formation and according to Lapitan (2009), a balance of both auxin and cytokinin will often produce an unorganized growth of cells or callus because both cell division anSd cell expansion occur in actively dividing tissue, but the morphology of the outgrowth will depend on the plant species as well as the medium composition.

Kalimuttu, *et. al*., (2007), discussed that shooting was effectively achieved using nodal explants on MS supplemented with BAP (1.5mg/L), Kn (0.5mg/L) and NAA (0.1mg/L) and from this experiment, treatment G4 also support shooting generation. Therefore, production of high quality and uniform planting material that can be multiplied on a year- round basis under disease-free conditions anywhere irrespective of the season and weather can also be achieved with MS containing 1.5mg/L BAP and 0.5mg/L NAA supplement. Using plantlets, in vitro

propagation helps to reduce the space required for storage, and also facilitate germplasm transportation due to small size of plantlets while providing a rich source of biodiversity for breeders and farmers.

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