Development of an in vitro Callus Induction Protocol and Shoot Proliferation for Selected Jatropha (Jatropha curcas) Accessions

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Abstract

Jatropha (Jatropha curcas L.) is an oil bearing crop growing in tropical and subtropical parts of the world. The present study was undertaken to establish a protocol for in vitro callusing of three Jatropha accessions namely; Metema, Adami Tulu and Shewa Robit accession from leaf explants. The experiment was laid out in CRD with five replications in factorial arrangement. The medium supplemented with combination of 1.0 mg/L BAP and 1.0 mg/L 2,4-D resulted in maximum percentage of callus (100%) formed for all accessions. The maximum shoot regeneration (66.67%) from callus with 10.13 number of shoot was obtained from Shewa Robit accession in MS medium fortified with TDZ (0.5 mg/L) and IBA (0.1 mg/L). The presence of TDZ in the shoot regeneration medium has greater influence on the induction of adventitious shoot buds, whereas MS supplemented with BAP alone and combination with IBA did not induce shoot regeneration from callus culture. The results obtained in the present study would facilitate the high callus induction and regeneration responses in Jatropha for its improvement using biotechnological tools.

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1. INTRODUCTION

Jatropha (*Jatropha curcas* L.) is a succulent shrub or small tree, which belongs to the large Euphorbiaceae family. It is a multi-purpose plant which has been exploited for various purposes such as soil erosion control, firewood, hedges, green manure and traditional medicines (Carels, 2013). On the other hand, the seed oil of Jatropha is also used as soap manufacturing ingredient, paints and as a biodiesel to substitute kerosene (Kumar and Sharma, 2008). Among many other attributes and importance of Jatropha, in recent years it has gotten special attention for being a priority feed stock in production of biodiesel.

Biodiesel is an alternative diesel fuel made from different types of renewable sources such as plant oils and animal fats. It is environmentally friendly fuel with low emission profiles and also non-toxic and biodegradable (Abdulla *et al.,* 2011). Among the plant species producing raw materials for biofuels, Jatropha is one of the plant species that stimulates the highest interest in tropical and subtropical regions. It has been identified as most suitable oil seed bearing plant due to its various favorable attributes like high oil content, hardy nature, adaptability in a wide range agro-climatic conditions, need for less irrigation and less agricultural inputs, pest resistance, short gestation periods and suitable traits for easy harvesting (Heller, 1996; Edrisi *et al.* , 2015).

Because of the increased interest in the potential of Jatropha as an energy plant, more attention is given to methods that allow the mass production of elite material. However, several challenges remain before that plant biomass can be commercially exploited. Its supply on a large scale requires massive production of phenotypically uniform plant material of a very high quality within a short time-frame that is adapted to the growth conditions of the plantation areas (Medza Mve1*et al.* , 2013).

Traditionally Jatropha is propagated through seed and vegetative cutting. The most common method to obtain Jatropha plantlets is by seed germination, which can be severely limited by poor seed viability,

low germination percentage, inadequate rooting in growth plants in small pots and the delayed rooting of seedlings (Openshaw, 2000). Vegetative propagation of Jatropha through stem cuttings has been achieved however the established plants are not deep rooted and hence, they easily get uprooted when cultivated in lands with poor top soil (Openshaw, 2000). Therefore, to improve cultivation of this crop, the traditional inefficient mode of propagation should be changed and proper techniques need to be studied and put in place for mass production of the Jatropha plants.

The *in vitro* multiplication would be a useful alternative method for mass production of plant. It offers an opportunity for large scale production of uniform disease free planting material in a relatively short period of time and independent of the season (George, 2008). Callus induction, culture and regeneration optimization *in vitro*are important steps in the process of plant propagation and genetic transformation (Oliveira *et al.* , 2017). Therefore, efficient callus induction and *in vitro* regeneration system is highly required to enhance the use of modern techniques in genetic improvement (Pan *et al* ., 2010; Jose *et al.,* 2012). Callus also can be used for long term conservation of plant tissues by maintaining them under slow growth conditions, and as target tissue for genetic transformation (Ali *et al* ., 2007).

Among factors influencing the process of *in vitro* callus induction, the plant growth regulators (PGRs) can be considered as one of the most important ones. Two relevant classes of PGRs that are used to control organ and tissue development are the auxins and cytokinins (Ismail *et al* ., 2013). Both callus induction and plant regeneration from explants require the presence of appropriate concentrations and combinations of plant growth regulators in the culture media (Kalimuthu *et al.* , 2007). A sub-optimal culture medium may cause physiological disorders or death of tissue. Studies of auxins and cytokinins separately or their combinations to initiate*in vitro* callus induction and regeneration in Jatropha were reported (Sujatha *et al.,* 2005; Deore and Johnson, 2008; Oliveira*et al.* , 2017). In addition, genotypic differences in shoot organogenesis have been observed in a wide range of plant species including Jatropha. It has been reported that regeneration in Jatropha is highly genotype dependent (Kumar and Reddy, 2010; Kumar *et al* ., 2010; Mweu *et al* ., 2016). Keeping in view of the importance of the crop and its propagation methods, the present study was designed to optimize the concentrations and combinations of different growth regulators on callus induction and proliferation of shoots from three Jatropha accessions using leaf explants.

2. MATERIALS AND METHODS

2.1. Planting Material

The seed of three Jatropha accessions were collected from Amhara and Oromia region of Ethiopia and used for these tissue culture experiments (Table 1). The seeds were germinated on growth trays containing sterilized combination of soil, sand and manure in the ratio of 2:1:1, respectively and kept in the greenhouse condition of Holeta Agricultural Research center (HARC). They were watered thrice a week using a spraying can. After three weeks, the seed that germinated was transplanted into pot containing sterilized soil and kept as mother stock plant. After three months of growth (Fig.1); very young, health and vigorous part of the plant (nodal segment) was collected and used as a source of explants. The experiment was conducted at Plant Biotechnology Laboratory of Holeta Agricultural Research Center (HARC) 45 km West of Addis Ababa, Ethiopia.

2.2. Growth Regulators Stock Preparation

The Plant Growth regulators (PGRs) used for the study were the cytokinin, 6-benzyl aminopurine (BAP) and Thidiazuron (TDZ), and the auxins, 2, 4-dichlorophenoxyacetic acid (2,4-D) and indole-3- butyric acid(IBA). All PGRs stock solutions were prepared by weighing and dissolving the powder in distilled water at the ratio of 1 mg/ml. To begin the dissolving process, the powdered crystal of the PGRs was first weighed and dissolved in 3-4 drops of 1N NaOH and 1N HCl based on the type of PGR (NaOH for auxins and HCl for cytokinin). Then, the volume was adjusted by adding distilled water. Finally, growth regulators' stock solutions were stored in a refrigerator at a specific temperature for each growth regulator.

2.3. Culture Medium Preparation

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Culture medium was prepared by taking the proper amount of MS basal medium (Murashige and Skoog, 1962) stock solutions (mg/L) (Annex 1). Full-strength of MS with 30g/L of sucrose as carbohydrate source (w/v) for Callus induction and Shoot proliferation were used. The pH of the medium was adjusted to 5.8 (using 1N NaOH and 1N HCl) after addition of the growth regulators. Gerlite (2.5g/L) was added as a gelling agent after the volume and pH of the medium was adjusted. The media was sterilized by autoclaving at a temperature of 121°C with a pressure of 15 psi for 15 min and stored at room temperature.

2.4. Explants and surface disinfection

Leaf explant (2nd and 3rd node from the apex) was collected from healthy and vigorously growing mother stock plants. The excised explant materials were initially rinsed under tap water for 30 min to remove the dust particles from their surface. Then the explants were treated with commercial detergent (Largo, Ethiopia) for 5 minutes and were rinsed well with distilled water for three to five times. Under a clean Laminar flow hood, the explants were subjected to 70% (v/v) ethanol for one minute and rinsed with sterile distilled water three to four times. Further the explant materials were then surface sterilized by 2.5% of Local bleach (Berekina, Ethiopia) containing two drops of Tween 20 for 15min time of exposure. After that the explants were rinsed with sterilized double distilled water for three to four times to remove the residual effect of these sterilants. After sterilization process was completed, individual leaf explant was trimmed aseptically into approximately $1cm^2$ leaf disc segments and placed with the adaxial side in contact with the MS basal medium (Kumar *et al* ., 2010a).

2.5. Callus Induction

After sterilization, about $1cm^2$ leaf disc of the three Jatropha accessions (Metema, Adami Tulu and Shewa Robit accession) were transferred to callus induction media consisting of MS basal medium supplemented with various combinations of 2, 4-D (0, 0.5, 1.0, 1.5 mg/L) and BAP (0, 1.0, 1.5, 2.0 mg/L). Five leaf disc explants per jar were used and each treatment was replicated five times. All the cultures were incubated at 25 ± 2°C in darkness to promote callus formation and discourage greening of the callus. The percentage proportion of callus induction on leaf discs was evaluated at an interval of four weeks after inoculation of explant.

2.6. Shoot Proliferation from Callus

Well-established organogenic callus (4-week-old) grown on MS medium supplemented with combination of BAP (1.0 mg/L) and 2,4-D (1.0 mg/L) for all accessions were used. About 0.5gram of calli were transferred to shoot regeneration media, consisting of MS basal medium containing various combinations of cytokinins viz. BAP (0, 0.5, 1.0 mg/L) and TDZ (0, 0.25, 0.5, 1.0 mg/L) individually and in combination with different concentrations of IBA (0, 0.1, 0.2 mg/L). There were three callus clumps per jar and five replicates per treatment. Then the culture was maintained in a growth room at a temperature of 25 ± 2^0C and 16 h photoperiod provided by white florescent lamps. The cultures were subcultured once transferring into fresh media after three weeks for further initiation of adventitious shoots. During subculture removal of dead, dark brown cells was done. The percentage of shoot organogenesis and number of adventitious shoot initiated from callus was recorded after six weeks of transferring the callus on shoot regeneration media.

2.7. Growth conditions

All the cultures were kept under eight hour dark period and sixteen hour photoperiod in a growth room (except Callus induction). Artificial light was provided by parallel cool white fluorescent tubes installed above the cultures. The light intensity was regulated to 1500-2000 lux and the growth room temperature was adjusted at 25 ± 2 °C with relative humidity (RH) of 65-70%.

2.8. Experimental Design and Statistical Analysis

The experiment was laid out in Completely Randomized Design (CRD) for all the treatments. The experiment was comprised of different combination and concentrations of plant growth regulators combined with three accessions of Jatropha. Each treatment had five replicates of culture Jars and set as experimental unit.

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Data collected from each experiment was subjected to statistical analyses using the SAS statistical software (version 9.2) and ANOVA was constructed, followed by mean separation using Fisher's Least Significance Difference (LSD) at α=5%.

3. RESULT AND DISCUSSION

3.1. Effects of BAP and 2,4-D on Callus Induction

The analysis of variance (ANOVA) showed that there is highly significant ($P<0.01$) difference due to the main effect of PGRs combinations and concentrations on mean number of days taken to form calli formation and callus fresh weight. However, there were no significant ($P>0.05$) effects recorded due to difference in accession and the interaction effect of PGRs and accessions on days to callus formation and callus fresh weight. Besides to this, significant differences for percentage of callus induction were observed among the PGRs combinations and concentrations as well as interaction of PGRs with accessions. However, the percentage of callus induction was not significantly ($P>0.05$) different among the accessions.

The minimum number of days (13.6 days) to callus induction was recorded for explants cultured on MS media supplemented with combination of 1mg/L BAP and 1mg/L 2,4-D followed by (14 days) on MS media supplemented with 1.5mg/L BAP and 1mg/L 2,4-D. Whereas, the longest time (18.87 days) taken to form callus was observed when callus induction medium was supplemented with combination of 2mg/L BAP and 0.5mg/L of 2,4-D (Table 2). From these results, increasing BAP levels in media within constant rate of 2,4-D led to an increase in the mean days taken to form calli. The media which supplemented with BAP alone did not induce a callus. This indicated that presence of auxins in the media play a crucial role in the callus formation of Jatropha explants *in vitro* (Rajore and Batra, 2007; Kumar *et al* ., 2008; Kumar *et al.* , 2015). The result of this study also revealed that the number of days to callus emergence decreased with increased in concentration of 2,4-D (0.5-1mg/L) in the treatments. Kumar *et al.* (2015) also reported that leaf explants Jatropha started callusing within two weeks on the media containing 2, 4-D (10µM). However, further increasing 2,4-D levels in media led to an increase in the mean days taken to form calli. Radhakrishnan *et al* . (2001) reported that the cells used up 2,4-D as required and any excess began to actively show the herbicidal effects that therefore slowed down the callus induction process.

The best callus formation (100%) was observed when MS medium was supplemented with combination of BAP (1.0 mg/L) and 2, 4-D (1.0 mg/L) for all accessions. Whereas, the lowest percentage (47.8-48.6%) of callus formation was recorded for all the three accessions explants cultured on media supplemented with 2.0mg/L BAP and 1.5mg/L 2,4-D (Table 3). This indicated that callus induction frequency decreases with further increasing the concentration of both 2,4-D and BAP. Several authors reported that appropriate concentrations and combinations of cytokinins and auxins are important to produce Jatropha callus (Costa*et al.,* 2015; Kumar *et al* ., 2015). On the other hand, the MS media containing only 2,4-D, even at a low concentration resulted in a better callus formation (Fig. 2c). Similar observation was also made by Thao *et al* . (2003) and Soomro and Memon (2007) concluding that 2,4-D was pre-requisite for callus formation in many of plant species. Meanwhile, no callus formation was observed and the explants only showed leaf expansion on medium containing BAP alone (Fig.2b). These results are supported by Kaewpoo and Techato, (2009) who used different concentration of BAP (1mg/L, 2mg/L and 3mg/L) to induce callus from embryo cultures of Jatropha. The authors finding report showed that BAP alone has induced shoot rather than callus. It has long been suggested that strong auxins such as 2,4-D are mainly efficient in promoting cell clumping and further developing of the callus.

In case of Callus fresh weight the highest calli weight (2.23g) was recorded on MS media supplemented with combination of 1.0mg/L of BAP and 1.0mg/L 2,4-D followed by (2.06g) on media with 1.5mg/L of BAP and 1.0mg/L 2,4-D (Table 2). Whereas, the lowest overall mean calli weight (0.98g) were observed for explant induced on MS media containing high levels of BAP (2.0mg/L) and 2,4-D (1.5mg/L) were used. This results confirmed that despite 2,4-D being an effective auxin in producing callus in Jatropha, it was active for callus induction when used in small amounts (Soomro and Memon , 2007). Radhakrishnan *et al* . (2001) also reported that high 2,4-D concentration has been shown to have herbicidal effects on plants

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causing cell growth inhibition and at highest cell death.

3.2. Effect of cytokinins (TDZ &BAP) and Auxin (IBA) on Adventitious shoot proliferation from Callus

Analysis of variance showed that both of percentage of shoot regeneration and mean number of adventitious shoots per callus were significantly ($P<0.01$) affected by the type and concentration of PGRs, accessions and interaction effect of the two factors.

The highest percentage of shoot regeneration from callus (66.67%) was observed for Shewa Robit accession in MS medium supplemented with combination of 0.5mg/L TDZ and 0.1 mg/L IBA followed by 64% and 61.33% for Metema and Adami Tulu accessions, respectively, on the same PGRs concentrations as for Shewa Robit. Meanwhile, the lowest percentage (39.2-40.8%) of shoot regenerations were recorded for all accession when the callus was cultured on media supplemented with 0.25mg/L TDZ (Table 4a). However, MS supplemented with BAP alone and combination with IBA did not induce shoot regeneration from callus culture. Calli that were cultured on this medium (BAP supplemented) was proliferated large quantities of healthy, green callus, but failed to differentiate shoots on all accessions type (Fig. 3b). Even on subculturing to respective media it continued to form massive callus rather than formation of organogenesis. And also, on the control medium the shoot regeneration was not noticed. However, the addition of TDZ to the medium improved the shoot induction potential of the calli as many shoot primordia were observed after 45 days of culture (Table 4a; Fig. 3c). Similar results were verified by Khurana-Kaul *et al.* (2010) who also showed that the combination of TDZ and IBA was more effective than the combination of BAP and IBA in Jatropha shoot regeneration using leaf segments as explants. Aishwariya *et al* . (2015) also reported that Thidiazuron (TDZ) is among the most active cytokinin like substances and it induces greater *in vitro* shoot proliferation than many other cytokinins in many plant species. Variation in the activity of different cytokinins can be explained by their differential uptake rate in different genomes, translocation rates to meristematic regions and metabolic processes in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds also reported (Kaminek, 1992; Kumar *et al* ., 2011).

The highest adventitious shoot number (11shoot) was recorded for Shewa Robit callus cultured on MS media supplemented with 0.5mg/L TDZ and 0.1mg/L IBA followed by (10.4) and (9 shoot) shootlet per callus for Adami Tulu and Metema accessions, respectively. Whereas, the lowest shoot number (3.8-4) was recorded for all accessions, when the callus subcultured on MS media supplemented with 0.25mg/L TDZ (Table 4b). In this study, the number of shoots per callus was increased as the concentration of TDZ was increased from 0.25 to 1mg/L. These results suggest that TDZ plays a very important role in the formation of adventitious shoot buds of Jatropha, and these effects may be involved in stimulating *de novo* synthesis of auxins by increasing the levels of IAA and its precursor, tryptophan, as well as increase in contents of endogenous cytokinin (Murthy *et al* ., 1995; Murthy and Saxena, 1998). Besides, the ability of TDZ to induce high shoot regeneration efficiency in plant tissue has been reported for a number of species (Feyissa *et al* ., 2005; Landi and Mezzetti, 2006).

4. CONCLUSION

The present study concluded that medium supplemented with combination of 1mg/L BAP and 1mg/L 2,4-D had best callus responses from leaf disc explant for all Jatropha accessions. The maximum shoot regeneration (66.67%) from callus with 10.13 number of shoot was obtained from Shewa Robit accession in MS medium fortified with TDZ (0.5 mg/L) and IBA (0.1mg/L). However, shoot regeneration competence from the callus can differ depending on the type of accessions and plant growth regulators used. The callus induction rates obtained in this study were highest in comparison of earlier reports. Generally, the callus induction protocol thus developed can be used for the improvement of Jatropha through biotechnological tools.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

H.F., M.T. and T.M. designed the research, H.F. and J.D. performed the experiments.

H.F. and M.T. analyzed the data. H.F., M.T and J.D. wrote the paper.

REFERENCES

Abdulla R., Chan E. S. and Ravindra P. 2011. Biodiesel production from Jatropha curcas: a critical review. Critical Reviews in Biotechnology 31: 53-64.

Aishwariya V., Ravindra kale Ramrao, Kokila devi E., Arul L., Sudhakar D., Kumar K.K. and Balasubramanian P. 2015. Impact of TDZ (thidiazuron) pulse treatment in single and multiple shoot formation in calli of*Jatropha curcas* L. International Journal of Advanced Research 3(3): 879-884.

Ali A., Naz S., Iqbal J. 2007.Effect of different explants and media compositions for efficient somatic embryogenesis in sugarcane (*Saccaharum officinarum*). Pakistan Journal of Botany 39(6), 1961-1977.

Carels N. 2013.Towards the domestication of Jatropha: the integration of sciences, in Jatropha, challenges for a new energy crop. Springer, New York, pp. 263–299.

Costa J.L., Silva A.L., Bier M.J., Brondani G.E., Gollo A.L., Letti L.J., Erasmo E. L. and Soccol C.R. 2015. Callus growth kinetics of physic nut (*Jatropha curcas* L.) and content of fatty acids from crude oil obtained *in vitro* . Appl Biochem Biotechnol.176:892-902.

Deore A. C. and Johnson T. S. 2008. High-frequency plant regeneration from leaf-disc cultures of *Jatropha curcas* L.: an important biodiesel plant. Plant Biotechnology Reports2: 7–11.

Edrisi S.A., DubeyR.K., TripathiV., BakshiM., Srivastava, P. and Jamil, S. 2015. *Jatropha curcas* L.: a crucified plant waiting for resurgence. Renew Sustain Energy Rev. 41:855–62.

Feyissa T., Welander M. and NegashL. 2005.*In vitro* regeneration of *Hagenia abyssinica* (Bruce) J.F. Gmel. from leaf explants.Plant Cell Rep. 24:392-400.

George E.F., Hall M.A. and De Clerk G.J. 2008. Plant Propagation by Tissue Culture. 3rd Edition, Springer, Dordrecht.

Heller J. 1996. Physic Nut (*Jatropha curcas* L.) Promoting the Conservation and Use of Underutilized and Neglected Crops. International Plant Genetic Resource Institute, Rome.

Ismail I.B., Mustapha Y., Aliyu. 2013. Micropropagation as a tool for experimental breeding, crop production and crop improvement. Glo Adv Res J Agric Sci. 2:307-314.

Jose J., Nimisha K., Anu A., Nambisan P. 2012. Evaluation of somaclonal variation in callus cultures of *Jatropha curcas* maintained on different hormonal combination using RAPD makers. World J Agric Sci. 8:616-623.

Kaewpoo M and Te-chato S. 2009. Influence of explant types and plant growth regulators on multiple shoot formation from *Jatropha curcas* . Science Asia 35: 353–357.

Kalimuthu K., Paulsamy S., Senthilkumar R. and SathyaM. 2007. *In vitro* propagation of biodiesel plant of *Jatropha curcas* L. Plant Tissue cult.and Biotech. 17(2): 137-147.

Kaminek M. 1992.Progress in cytokinin research.Trends Biotech10:159–162.

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Khurana-Kaul V., Kachhwaha S. and Kothari S. 2010. Direct shoot regeneration from leaf explants of *Jatropha curcas* in response to thidiazuron and high copper contents in the medium. Biologia plantarum 54:369-372.

Kumar A. and Sharma S. 2008. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas*): A review. Industrial Crops and Products, 2:234–246.

Kumar N. 2008. Studies on regeneration and genetic transformation of*Jatropha curcas.* PhD Thesis, Bhavnagar University, Bhavnagar, India.

Kumar N. and Reddy M.P. 2010. Plant regeneration through the direct induction of shoot buds from petiole explants of *Jatropha curcas* : a biofuel plant. Ann. Appl. Biol.156:367– 375.

Kumar N. and Reddy M.P. 2010. Plant regeneration through the direct induction of shoot buds from petiole explants of *Jatropha curcas* : a biofuel plant. Ann. Appl. Biol. 156:367–375.

Kumar N., Vijayanand K.G. and Reddy M.P. 2010a. Shoot regeneration from cotyledonary leaf explants of *Jatropha curcas* : a biodiesel plant. Acta Physiol Plant 32:917-924.

Kumar N., Vijayanand K.G. and Reddy M.P. 2011. Plant regeneration in non-toxic *Jatropha curcas* - impacts of plant growth regulators, source and type of explants. J. Plant Biochem Biotechnol. 20:125-133.

Kumar S., Kumar V., Sharma M.K., Kumar N., Kumar A., Tomar K.S,, Sharma S.K., Singh M.K, Sengar R.S. and Jaiswal N. 2015. Effects of different plant growth regulators on *in vitro callus* induction in physic nut (*Jatropha curcas* L.). J. Appl and Nat Sci. 7:30-37.

Landi L. and Mezzetti B. 2005. TDZ, auxin and genotype effects on leaf organogenesis in *Fragaria* . Plant Cell Rep. 25:281-288.

Medza Mve1 D. S., Mergeai G., Druart P., Baudoin J. P. and Toussaint A. 2013. *In Vitro* Micropropagation of Jatropha curcas L. from Bud Aggregates. Journal of Technology Innovations in Renewable Energy 2013(2): 145-154.

Murashige T. and Skoog F. 1962.A revised medium for rapid growth and bioassays with tobacco cell cultures.PhysiologiaPlantarum15: 473-497.

Murthy B.S, and Saxena P.K. 1998. Somatic embryogenesis and plant regeneration of Neem (*Azadirachta indica* A. Juss). Plant Cell Rep. 17: 469-475.

Murthy B.S., Murch S.J. and Saxena P.K. 1995. TDZ-induced somatic embryogenesis in geranium cotyledonary cultures. Plant Cell Rep. 15: 423-426.

Mweu M. C., Nyende A. and Onguso J. 2016. Efficient somatic embryogenesis of Jatropha curcas L. from petiole and leaf discs. International Journal of Biotechnology and Molecular Biology Research 7(3): 29-35.

Oliveira A.B., Vendrame W.A., Londe, Cardoso Nogueira L. and Sanaey M. 2017. Induction of different types of callus in Jatropha curcas L. hybrid accession at in vitro condition. Australian Journal of Crop Sience 11(07): 874-879.

Openshaw K., 2000. A review of *Jatropha curcas* : an oil plant of unfulfilled promise. Biomass Bioenergy Vol. 19: 1-15.

Pan J., Fu Q., Zeng-Fu X.U. 2010. Agrobacterium tumefaciens-mediated transformation of biofuel plant *J. curcas* using kanamycin selection. Afr J of Biotechnol. 9:6477- 6481.

Radhakrishnan T.T., Murthy G. K., Chandran K. and Banyopadhyay A. 2001. Somatic embryogenesis in *Arachis hypogea* . Australian Journal of Botany 49: 753 – 759.

Rajore S. and Batra A. 2007. An alternative source for regenerable organogenic callus induction in *Jatropha curcas* L. Indian Journal of Biotechnology 6: 545-548.

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Soomro R. and Memon R.A. 2007. Establishment of callus and suspension culture in *Jatropha curcas* . Pakistan J. Bot. 39: 2431-2441.

Sujatha M., Makkar H. and Becker K. 2005. Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Growth Regulation 47:83–90.

Thao N.T., Ozaki Y. and Okubo H. 2003. Callus induction and plantlet regeneration in ornamental *Alocasia* . Plant Cell Tiss Org Cult. 73:285-289.

FIGURE LEGENDS

Figure 1. Mother stock plant of Jatropha accessions after three months. (a) Adami Tullu accession (b) Metema accession (c) Shewa Robit accession

Figure 2. Callus induction after four weeks of culture in different combination of BAP and 2,4-D for the three Jatropha accessions. (a) Explants cultured on hormone free MS medium; (b) Explants cultured on medium containing of BAP alone; (c) Callus induction by 2,4-D (0.5mg/L) only; (d) Leaf disc explants of Metema cultured on a medium containing 1mg/L BAP +1mg/L 2,4-D; (e) Leaf disc explants of Shewa Robit accession cultured on a medium containing 1mg/L BAP+1mg/L 2,4-D and (f) Leaf disc explants of Adami Tulu accession cultured on a medium containing 1mg/L BAP +1mg/L 2,4-D.

Figure 3. Multiple shoots regeneration from leaf derived callus of different Jatropha accession cultured at different concentration and combinations of either BAP or TDZ in combination with IBA after six weeks of culture. (a) Leaf derived callus cultured for shoot regeneration; (b) Leaf derived callus cultured on MS medium supplemented with 0.5m/l BAP + 0.1mg/L IBA; (c) Adventitious shoot formation of Shewa Robit accession from leaf derived callus cultured at 0.5mg/L TDZ; (d) Shoot regenerated from Shewa Robit leaf derived callus cultured on MS medium supplemented with 0.5mg/L TDZ and 0.1mg/L IBA; (e) Shoot regenerated from leaf derived callus of Metema accession cultured on MS medium supplemented with 0.5mg/L TDZ and 0.1mg/L IBA; (f) Shoot regenerated from leaf derived callus of Adami Tulu accession cultured on MS medium containing 0.5mg/L TDZ + 0.1mg/L IBA.

TABLES

Table 1 . Sources and growing altitudes of planting material of Jatropha accessions were used in this study

Table 2. Main effect of Plant growth regulators combination and concentrations on days to callus formation and callus fresh weight of leaf explants of Jatropha.

Table 3. Interaction effect of PGRs (BAP and 2,4-D) combination and concentration with different Jatropha accessions on callus formation percentage of explants excised from leaf after 30 days of culture growth.

Table 4. Effect of different concentrations of either BAP or TDZ in combination with IBA on percentage and number of shoot regenerated from leaf derived calli of Jatropha accessions after 45 days of culture growth. **(a)** Interaction effects of either BAP or TDZ in combination with IBA on percentage of shoot regeneration from callus derived from leaf of three Jatropha accessions. **(b)** . Interaction effects of either BAP or TDZ incombination with IBA on number of adventitious shoots regenerated from callus derived from leaf of three Jatropha accession.

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