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Efficient callus induction from various explants of *Jatropha curcas*

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ABSTRACT: *Jatropha curcas* L. is a tropical plant of the Euphorbiaceae family, characterized by its high oil content and ability to grow in various conditions, making it the most promising non-food biofuel crop in the world. The current study was directed to develop effective methods of tissue culture using this plant. The study was able to induce callus from cotyledons of sterile seedlings, as well as from true leaves taken from the field and from the embryo, using MS medium supplemented with interfering concentrations of Naphthalene acetic acid (NAA) with Benzyl adenine (BA), as the results indicated that The medium containing 0.5 mg L^{-1} for each of them gave the highest percentage of callus induction for the cotyledonous leaves, true leaves and embryonic peduncle, which reached 100%, 80% and 100%, respectively.
Keywords: *Jatropha curcas* L.; callus cultures; cotyledons; Benzyl adenine (BA); naphthalene acetic acid (NAA).

Indução eficiente de calos a partir de vários explantes de *Jatropha curcas*

RESUMO: *Jatropha curcas* L. é uma planta tropical da família Euphorbiaceae, caracterizada por seu alto teor de óleo e sua capacidade de crescer em uma variedade de condições, o que a tornou a cultura de biocombustível não alimentar mais promissora do mundo. O presente estudo foi direcionado para desenvolver métodos eficazes de cultura de tecidos para essa espécie. O estudo foi capaz de induzir calos de cotilédones de mudas estéreis, bem como de folhas verdadeiras retiradas do campo e do embrião, usando meio MS suplementado com concentrações interferentes de ácido naftaleno acético (NAA) com Benzil adenina (BA), como os resultados indicaram que o meio contendo 0,5 mg L^{-1} para cada um deles proporcionou a maior porcentagem de indução de calos para as folhas cotilédones, folhas verdadeiras e pedúnculo embrionário, que atingiram 100%, 80% e 100%, respectivamente.
Palavras-chave: *Jatropha curcas* L.; culturas de tecidos; cotilédones; Benzil adenina (BA); ácido naftaleno acético (NAA).

1. INTRODUCTION

Jatropha curcas L. is one of the genera of the Euphorbiaceae family. It is a woody shrub that is widespread in tropical and subtropical regions and is grown as an ornamental plant in home gardens in Asia and West Africa (OSENI; AKINDAHUNSI, 2011; POMPELLI et al., 2022).

Interest in this plant species has increased day by day because of its ability to thrive in degraded lands and soils of low natural fertility, its rapid growth and ease of reproduction, its drought tolerance, the preservation of photosynthetic pigments under prolonged drought, and its rapid recovery of its ability to photosynthesize when water is available. It possesses several industrial and medicinally important secondary metabolites (VERMA et al., 2021; SAPETA et al., 2023). It is also a rich latex source with a wide range of uses in the medical field. It is an excellent source of many bioactive compounds such as gallic acid, isoflavonoids, jatrophene, jatropham, phenolic acids, vanillic acid, free amino acids, organic acids, terpenoids, crocycline B, quercin, tannin, saponins and beeswax.

Scientific evidence has proven antifungal, antibacterial, anticancer, antiviral, antioxidant, and collagen stimulating activities from various studies (VIJAYALAKSHMI et al., 2022). It shows anti-hyperglycemic activity (HUTAPEA; FADILLAH, 2022). *J. curcas* are toxic in nature despite their medicinal properties. Previous studies in humans and animals have shown that they cause hepatotoxicity, cell death, diarrhea, depression, and gastroenteritis (SARABIA et al. al., 2022).

Plant tissue culture technology is a fast and effective way to regenerate various plants; a single piece is removed from the parent plant and transferred to an artificial growth medium. Several studies indicated the success of using plant tissue culture methods on different parts of *J. curcas*. One study formed vegetative branches directly from the callus of the cotyledon leaves of *J. curcas* (HEGAZI et al., 2020). A second study formed vegetative branches from cotyledon leaves, leaves and petioles (KHEMKLADNGOEN et al., 2011).

Another study was able to successfully apply an effective protocol for the induction of adventitious shoots and plant regeneration from young petioles and indicated that the use of high concentrations (5 to 120 mg L^{-1}) of thidiazuron (TDZ) solution for short periods (5 to 80 minutes) helped increase regeneration (LIU et al., 2015). Another study was able to form callus from leaves of three strains of *J. curcas*, using MS medium containing 4.44 µM BAP and 4.52 µM 2,4-D at 100% formed for all accessions, and the study succeeded in re-forming the plants from callus when using MS medium containing 2.27 µM TDZ with 0.49 µM IBA (FUFA; SHEKATA, 2020).

2. MATERIALS AND METHODS

2.1. Plant material and methods of sterilization

Jatropha curcas seeds were supplied from the Original Group for Exporting Agricultural Products - Egypt. Fifty seeds/experiment were used, removed their coat, then washed with running water and soaked for 24 hours with water only, then immersed in 70% ethanol for one minute, and then transferred to a 6.0% sodium hypochlorite solution (Commercial bleach, Babylon Comp. for Detergent, Baghdad) at a ratio of 2 volumes of it: 4 volumes of distilled water for 15 minutes (Comfort et al., 2018), washed them with sterile distilled water three times for two minutes/once. Sterilized seeds were planted on different media (cotton, MS, pet moss, sawdust, and MS with 2% activated charcoal).

True leaves excised from field-grown plants at 4 weeks were also used. They were washed with running water and immersed in 70% ethanol alcohol for one minute and immersed in a 6.0% sodium hypochlorite solution at a ratio of 2 volumes of it: 8 volumes of distilled water for 10 minutes (Khurana-kaul et al., 2010), washed them with sterilized distilled water three times for two minutes. Also, embryos were used after extracting them from the seeds soaked for 24 hours with water only and sterilizing them by immersing them in 70% ethanol for one minute, and transferred to a 6.0% sodium hypochlorite solution at a ratio of 1 volume of it: 9 volumes of distilled water for 10 minutes, washing them with sterile distilled water three times.

2.2. Callus induction

The cotyledons of sterile two-week-old seedlings of *J. curcas* were used and cut into pieces with an area of 1.0 cm^2. True leaves taken from the field at 4 weeks were also used and cut with an area of 1.0 cm^2. Also, embryos extracted from the embryos were used, and the parts were transferred separately into glass bottles of 100 ml at the rate of one piece/bottle containing MS medium (Murashige; Skoog, 1962) added to the interfering concentrations of NAA and BA (0.0, 0.1, 0.5, 1.0 mg L^{-1}) and the samples were kept in conditions of alternating light 16 hours light / 8 hours darkness and a light intensity of 2000 lux and a temperature of 25 ± 2 º °C to stimulate callus induction from them.

2.3. Callus subculture

Callus cultures were perpetuated periodically, once every 3-4 weeks, to avoid depletion of the components of the nutrient medium and its drying, depending on the appearance of brown pieces on it and cracking of the medium if the brown pieces are removed. It is cut into a piece weighing approximately 1.0 gm. Each piece is distributed in a glass bottle containing the same stimulation medium and re-kept in the same conditions mentioned above. The fresh weight of the callus species was estimated based on the differences in the weight of the callus directly when it was cultivated and after three weeks of cultivation on the medium.

3. RESULTS

3.1. Seeds germination

The results showed the difficulty and variation of the germination of *Jatropha* seeds in different development media. Despite this, the highest germination percentage was in the cotton medium (Table 1). The seedlings growing in the middle of the cotton were characterized as very good in their growth (Figure 1A). While the seedlings growing in the MS and peat moss medium were growing well (Figure 1B and 1C), when using sawdust, it did not significantly encourage the germination of seeds, except that they opened. The cotyledon leaves began to grow, then stopped and did not reach the emergence stage of the true leaves, and the seedlings did not continue to grow (Figure 1-D). In contrast, using activated charcoal with MS medium did not promote seed germination well.

Table 1. Germination of *Jatropha curcas* seeds in different growth media.
Tabela 1. Germinação de sementes de *Jatropha curcas* em diferentes meios de crescimento.

Media	The number of germinated seedlings	Sterilization efficiency (%)
Cotton	40	80
MS	10	20
Pet moss	30	60
Sawdust	10	20
MS with 2% activated charcoal	15	30

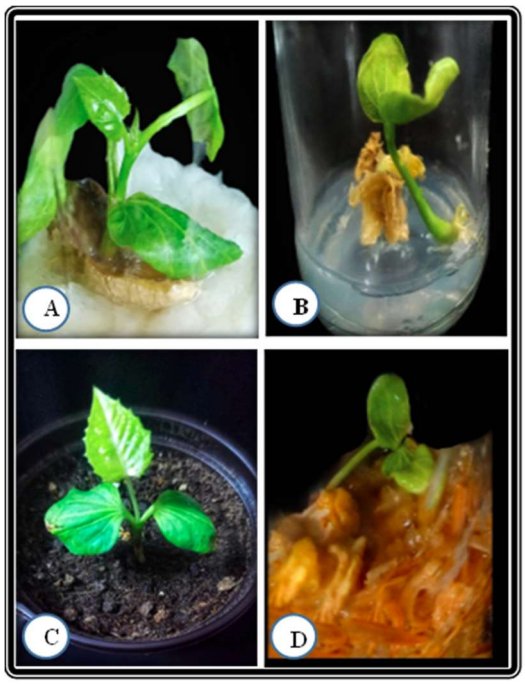

Figure 1. Production of *Jatropha curcas* seedlings from sterilized seeds growing in different media at the age of 5 weeks. *Seed germination in cotton (A), MS (B), Pet moss (C) and Sawdust (D) media.*
Figura 1. Produção de mudas de *Jatropha curcas* a partir de sementes esterilizadas crescendo em diferentes meios com 5 semanas de idade. *Germinação de sementes em meios de algodão (A), MS (B), Pet musgo (C) e serragem (D).*

3.2. Callus induction

Different explants showed clear and differentiated responses to callus induction in solid MS medium supplemented with different concentrations of NAA and BA. Cotyledons gave the highest response to stimulation of 100% when grown on a solid MS medium containing 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BA. While the response of true leaves reached 80%, and the embryo reached 100% response on the same medium (Table 2-4). The cotyledons required 5-6 days to induce callus, the true leaves took 7-8 days to induce callus, and the embryo took 2-3 days to induce callus.

The results showed that cotyledons stimulated the callus induction, starting from its edges (Figure 2A), after which it

continued with the callus formation during the second week (Figure 2B). The callus formation was completed in the third week of cultivation, and it was distinguished by its dark green color and compact texture (Figure 2C). As for the true leaf pieces, they also began to stimulate from their edges (Figure 2D) and continued with the formation of callus in the second week (Figure 2E).

The callus was formed in the fourth week, and it was distinguished by its yellow color and compact texture (Figure 2F). The results indicated that the embryos began to enlarge (Figure 2G), and then gradually turned into callus in the second week (Figure 2H). Its formation was completed in the third week, and its callus texture was compact and yellowish-green (Figure 2I).

Table 2. Callus induction from Cotyledon of *Jatropha curcas* on MS medium with a concentration of BA and NAA.
Tabela 2. Indução de calos do cotilédone de *Jatropha curcas* em meio MS com concentração de BA e NAA.

MS medium + BA/NAA	Callus induction (day)	Callus initiation (%)
0.0/0.0	0	0
0.0/0.1	0	0
0.0/0.5	0	0
0.0/1.0	0	0
0.1/0.0	7	10
0.5/0.0	7	20
1.0/0.0	7	20
0.1/0.1	7	40
0.5/0.5	6	100
1.0/1.0	5	70

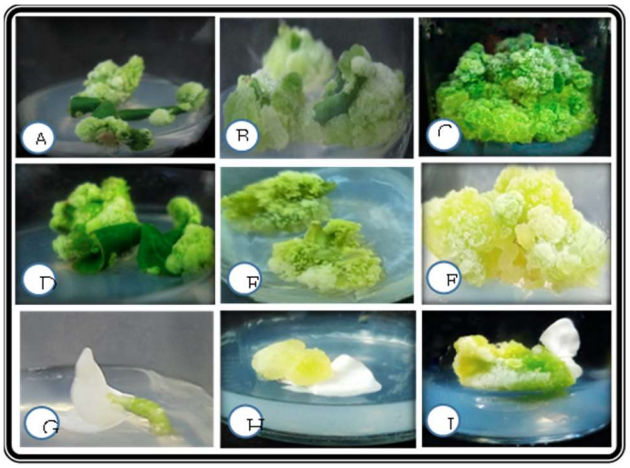

Figure 2. Induction of callus cultures from different explants of *Jatropha curcas* in MS medium containing 0.5 mg L^{-1} BA and 0.5 mg L^{-1} NAA. *(A) induction of callus from cotyledons, 6 days after cultivation; (B) callus produced from the cotyledons after 15 days of cultivation; (C) compact dark green callus, 21 days old; (D) induction of callus from true leaves, 8 days after cultivation; (E) callus produced from true leaves after 15 days of cultivation; (F) compact yellow callus, 30 days old; (G) induction of callus from embryo, 3 days after cultivation; (H) callus produced from embryo after 10 days of cultivation; (I) compact yellowish-green callus, 21 days old.*
Figura 2. Indução de culturas de calos de diferentes explantes de *Jatropha curcas* em meio MS contendo 0,5 mg L^{-1} de BA e 0,5 mg L^{-1} de ANA. *(A) indução de calo de cotilédones, 6 dias após cultivo; (B) calo produzido a partir dos cotilédones após 15 dias de cultivo; (C) calo compacto, verde escuro, com 21 dias; (D) indução de calo em folhas verdadeiras, 8 dias após cultivo; (E) calo produzido a partir de folhas verdadeiras após 15 dias de cultivo; (F) calo amarelo compacto, com 30 dias; (G) indução de calo do embrião, 3 dias após cultivo; (H) calo produzido a partir de embrião após 10 dias de cultivo; (I) calo compacto verde-amarelado, com 21 dias.*

The results also showed that there was a variation in the quantities of callus produced from the cotyledons, true leaves, and embryo, depending on the estimates of the fresh weight of callus when it was cultivated on the sustaining medium to determine the growth rate of callus induced from three types of explants (Table 5).

Table 3. Callus induction from True leaves of *Jatropha curcas* on MS medium with a concentration of BA and NAA.
Tabela 3. Indução de calos em folhas verdadeiras de *Jatropha curcas* em meio MS com concentração de BA e NAA.

MS medium + BA/NAA	Callus induction (day)	Callus initiation (%)
0.0/0.0	0	0
0.0/0.1	0	0
0.0/0.5	0	0
0.0/1.0	0	0
0.1/0.0	0	0
0.5/0.0	10	10
1.0/0.0	10	10
0.1/0.1	8	30
0.5/0.5	8	80
1.0/1.0	8	50

Table 4. Callus induction was performed from the embryo of *Jatropha curcas* on an MS medium with a concentration of BA and NAA.
Tabela 4. Indução de calos em embriões de *Jatropha curcas* em meio MS com concentração de BA e NAA.

MS medium + BA/NAA	Callus induction (day)	Callus initiation (%)
0.0/0.0	0	0
0.0/0.1	0	0
0.0/0.5	0	0
0.0/1.0	0	0
0.1/0.0	0	0
0.5/0.0	0	0
1.0/0.0	5	10
0.1/0.1	5	10
0.5/0.5	3	100
1.0/1.0	4	20

Table 5. Fresh weights of induced callus from three types of explants of *Jatropha curcas*.
Tabela 5. Peso fresco de calo induzido de três tipos de explante de *Jatropha curcas*.

Explant	Average of fresh weight (gr)
Cotyledon	0.728
True leave	0.656
Embryo	0.531

4. DISCUSSION

The poor germination of seeds by tissue culture may be attributed to their poor viability and low rates of germination and dormancy (Rizwan; Aftab, 2018) or due to the presence of toxic chemicals, protein, latex and endogenous bacteria in them (ISLAM; BARI, 2012). The formation of callus in most plant species, especially the herbaceous dicotyledons, has become easier and more routine than in woody plants. The reason may be attributed to woody plants' difficulty or slow behavior in forming these farms.

The formation of callus cultures from *J. curcas* in this study is an important and desirable matter because of the importance of this plant from an economic point of view. This system may help produce effective compounds and increase productivity compared to seed plants (EL-SAYED et al., 2020). The superiority of NAA and BA in callus induction may be attributed to its role in liberating protons

(H+) and increasing cell divisions, which results in a difference in the activity of some enzymes affecting metabolic activities and building proteins in callus cells (MOHAMMED; YAHYA, 2018).

Auxins and cytokinins in both the plant part or the protoplasts of plant cells cultured on the nutrient media have a major role in the induction of callus (AKITHA DEVI et al., 2018). The growth regulator NAA is considered an important auxin in stimulating cell division and increasing the rate of callus formation (Chen et al., 2019), and the change in the color of the callus of the three parts may be due to the role of growth regulators present in the nutrient media (ALWASH et al., 2020).

Cells on the absorption of water and nutrients from the medium and the interaction between the growth regulators added externally to the medium. Nutrition affects the fresh weight of the callus (ZAHARA et al., 2013) as the three parts differed in the time of their callus induction, which may be due to the part taken from the plant piece that forms the induced callus. In addition to the nature and variation of cellular tissues and their content of the number of cells that stimulate differentiation and division (MOHAMMED; YAHYA, 2018) in addition to genetic and non-genetic factors such as the age of the plant part and its size (AL-ABASI et al., 2020).

5. CONCLUSIONS

It can be said that plant tissue culture is an important method for obtaining and sustaining callus from different tissues of *Jatropha curcas* L., which is characterized by the difficulty of growing its seeds in different development media. We also conclude that establishing the path of callus formation from different explants is an important step in propagating this desirable plant in producing biofuel oil.

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