Enraizamiento a partir de callos de jatropha cuneata (wiggins & rollins) in vitro

Rooting from callus of Jatropha cuneata (Wiggins & Rollins) in vitro

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Resumen

El género *Jatropha* es importante por sus propiedades medicinales y se piensa que podría llegar a constituir una fuente de aceite con posibilidades de desplazar en un futuro a las fuentes de combustibles convencionales.

En el estado de Sonora existen varias especies de Jatropha, una de ellas es *Jatropha cuneata*, la cual necesita ser estudiada debido a que es una especie con gran potencial económico, ya que representa una posible alternativa para el desarrollo energético sostenible de biodiesel.

A través del cultivo *in vitro*, se realizan estudios con la planta *Jatropha cuneata*. Para ello se utilizan hojas de la planta, se desinfectan y siembran asépticamente en medio MS con el regulador de crecimiento ANA (0, 1, 1.5 y 2 mGL^{-1}) combinándolo con 0, 0.5 y 1 mGL^{-1} de cinetina, lo cual produjo mayor formación de callos con 1 mgL^{-1} de ambos reguladores de crecimiento. Los callos obtenidos se subcultivaron en medio de cultivo conteniendo las

combinaciones de ANA con cinetina: ANA en concentraciones de 0, 1, 1.5 y 2 mGL^{-1} y cinetina en las concentraciones de 0, 0.5, 1, 1.5 y 2 mGL^{-1} ; se obtuvo enraizamiento en los callos que estuvieron sometidos en medios de cultivo con las concentraciones de 1 y 1.5 mGL^{-1} de ANA. También se subcultivaron callos en 0, 1, 1.5, 2 y 2.5 mGL^{-1} de 2, 4-D y en 0, 1, 1.5, 2, y 2.5 mGL^{-1} del regulador de crecimiento de cinetina, no mostrando diferencias significativas con los callos sometidos en 1, 1.5 y 2 mGL^{-1} de 2, 4-D y cinetina.

Palabras clave: *Jatropha cuneata*, callos, ANA, cinetina, 2, 4-D.

Abstract

The Jatropha genus is important for its medicinal properties and it is thought that it could constitute a source of oil likely to displace conventional fuel sources in the future.

There are several species of Jatropha in Sonora State, one of them is Jatropha cuneata, which needs to be studied since it is a species with great economic potential, since it represents a possible alternative for sustainable energy development of biodiesel.

Through in vitro culture, are carried out studies with the plant Jatropha cuneata. Leaves of the plant are used for this, are disinfected and sown aseptically in MS medium with ANA growth regulator (0, 1, 1.5 and 2 mGL-1) combined with 0, 0.5 and 1 mGL-1 of kinetin, which produced more callus (mass of unorganized parenchyma cells derived from plant tissue (explants)) formation with 1 mgL-1 both of growth regulators. Obtained callus was subcultured in a culture containing combinations of ANA with kinetin: ANA at concentrations of 0, 1, 1.5 and 2 mGL-1 and kinetin at concentrations of 0, 0.5, 1, 1.5 and 2 mGL-1; He was rooting in the calluses that were subjected in culture media with concentrations of 1 and 1.5 mGL-1 of ANA. She is also subcultured callus in 0, 1, 1.5, 2 and 2.5 mGL-1 of 2, 4-D and in 0, 1, 1.5, 2 and 2.5 mGL-1 of kinetin growth regulator, showing no significant differences with the tripe in 1, 1.5 and 2 mGL-1 of 2, 4-D and kinetin.

Keywords: Jatropha cuneata, callus, ANA, kinetin, 2, 4-D.

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Introduction

The main types of vegetation recognized in Mexico include the Euphorbiaceae (Martinezgordillo et al., 2002). Jatropha L. is a genus in this family with 175 species distributed in America, Africa and India (Rodríguez-Acosta et al., 2009). In Mexico, there are 45 species of Jatropha. It grows in a tropical climate zones in the regions series and subtrophical and it can be grown in regions with scarce rainfall and poor soils (Renuga and Rajamanickam, 2014). Seven species, some in arid and semi-arid areas have been found in the State of Sonora (Gil-Montaño et al, 2010).

Crops that take advantage as oil for conversion to biodiesel producers are: rapeseed (Brassica napus) in United States, sunflower (Helianthus annus) in Italy and the South of France, soybean (Glycine max) in the United States and Brazil, and oil palm (Elaeis guineensis) in Malaysia (Sujatha et al., 2005), but they have the disadvantage that coming from arid climates, could not grow or achieve survive in places with aridity and drought, which are not a suitable alternative for marginal land, while Jatropha species are crops that do not compete with other crops since they survive and grow in poor agricultural areas, such as arid and semi-arid areas supporting dry climates; in addition, they have the advantage of not be eaten as food. The above makes it necessary to carry out studies with plants that develop and manage to survive in such conditions.

The demand for biodiesel has created a growing interest in finding new and potentially generating energy species (Ricci et al., 2012). Jatropha seeds are producing energy. Attention in vegetable oils increases day by day for its applications in different industries such as cosmetics, food and energy. Jatropha has proven to be of great importance because of its potential as a producer of agroenergetic and the oil from its seeds can be used in the production of biodiesel (Adriano-Anaya et al., 2014). Some parts of this plant have proven medicinal, as peel which contain tannins (Sharma and Kumar, 2008), latex, containing an

anticancer alkaloid jatrophina (Das-Gupta et al., 2011), and in general all parts of the plant have been used in traditional veterinary medicine, as seeds that have been used for arthritis and gout, diseases and dermatomucosas sap extracts parts of the plant to allergies, wounds and inflammations . Gossypiifolia parts of Jatropha has been used in both human and veterinary uses (Félix-Silva et al., 2014A), and can be used in treatments for cardiovascular disease because it has antioxidant and anticoagulant activities without showing toxic effects (Felix Silva et al., 2014b).

Studies have been conducted with different species of Jatropha, J.curcas being studied to contain the oil that meets the requirements to function as biofuel, and having the advantage of not being edible contain toxic substances (Falasca and Ulberich, 2008) . In addition, they have made studies with J. gossypifolia, because some of its parts are used in some countries in different ways: with the leaves make cures when people have dermatitis, fever, rashes and skin inflammations, ulcerations in tongues babies, stomach pains and venereal diseases (Balee, 1994). The water decoction of the leaves of this plant used to bathe people with skin infections, seeds are purgative and used in ailments such as headaches, body and as blood purifier (Oduola et al., 2005) .

Plant micropropagation is an alternative method for obtaining plants from different tissues, many commercial laboratories use tissue culture to rapid multiplication of plants, conservation of germplasm, elimination of pathogens, genetic multiplication, and for production of secondary metabolites (Alonso and Perez-Jimenez, 2011). Tissue culture is a good alternative for the propagation of plants of economic and medicinal interest by these biotechnological techniques is possible to obtain good plants seizing appropriate features provided by a single mother plant. The tissue culture plants, coupled with genetic engineering are tools widely used in plant biotechnology to carry out various studies with very promising both woody plants and ornamental plants for indoor and rapid spread results, however generally research related to the production of plant secondary metabolites through in vitro culture have focused on the use of undifferentiated tissue (callus cell suspensions) (Pérez-Alonso and Jiménez, 2011). Callus, cell suspensions, root hairs and multiplication of stems are used for micropropagation studies as well as studies of plant-pollutant interactions under aseptic conditions (Couselo et al., 2012).

In the state of Sonora there are some species of Jatropha, including Jatropha cuneata Wigg. et Rollin, whose common name is matacora (Velderrain-Algara, 2010) (Figure 1). Cuneata Jatropha seeds have low germination percentage, so the use of tissue culture techniques intended to conduct studies with the plant, power seat bases and achieve their production in vitro.

Materials and methods

Medium

culture medium was prepared using Murashige and Skoog (Murashige and Skoog, 1962), with deionized water, the pH adjusted to 5.7, 3% sucrose and 8.5 g agar, growth regulators (NAA, kinetin added and 2, 4-D) in different concentrations was heated with stirring and added 10 to 20 mL test tube 25 x 150 mm, then sterilized in an autoclave model STERILMATIC- for 15 minutes, 121 ° C and 15 pounds pressure. It is cold and the culture medium proceeded to disinfect samples of the plant and the aseptic seeding leaves in laminar flow chamber Edge Gard Hood.

Obtaining explants

We first tried to germinate seeds collected in Bahia Kino, Sonora plant, earning 3% germination percentage, because it is a very low percentage of germination was decided to use sheets of Jatropha cuneata, which were collected from plants growing wild in the 93 km road to Bahia de Kino, Sonora (figure 1). The leaves had an approximate size of 1.0 cm long and 1.0 cm wide; due to contamination otherwise it proceeded to have vegetative material for in vitro sowing, so stems of wild plants were cut and laboratory placed in a container with tap water; week there were new leaves (Figure 2), which were taken sample to be inoculated in the culture medium.

Figure 1. *Jatropha cuneata* in Bahía de Kino, Sonora.

Figure 2. Stalks *Jatropha cuneata* with leaves growing in the laboratory.

Disinfection of samples:

The plant leaves were disinfected with 70-75% ethyl alcohol for 1-3 minutes, containing 0.5% Tween 20, they were immersed in a solution of 10-15% commercial bleach for 8-12 minutes, rinsed in several times with sterile deionized water and then proceeded to planting under aseptic conditions being planted whole leaves and leaf cuttings in MS culture media (Murashige and Skoog, 1962) sterile. Planting material and disinfected aseptically seeded in culture media, the aseptic environment was provided by the laminar flow chamber model Edge Gard Hood, which is previously cleaned. And the seeding of the samples in the MS medium with growth regulators α-naphthalene acetic acid (NAA) and kinetin at different concentrations, we proceeded to incubation. Explants in nutrient media were placed in

incubation conditions: 25 ± 1 ° C in temperature, 75% relative humidity with 16 hours light.

To stimulate the induction of cell growth disorganized (callus), plant leaves were used, disinfected and sown on MS medium containing the growth regulator ANA at concentrations 0, 1, 1.5 and 2 mGL-1 kinetin in combination with concentrations of 0, 0.5 and 1 mGL^{-1} .

To stimulate organogenesis calluses obtained, MS medium was prepared by adding 0, 1, 1.5 and 2 of ANA mGL-1 with 0, 0.5, 1.0, 1.5 and 2 mGL-1 kinetin; calluses obtained were subcultured and placed under incubation conditions described above. Callus on MS medium were also transferred to growth regulator acid 2, 4-dichlorophenoxyacetic acid (2, 4-D): 0.1, 1.5, 2 and 2.5 mGL-1 and kinetin (0, 1, 1.5, 2 and 2.5 mGL-1).

Experimental design

A completely randomized design with three replications. To determine significant differences analysis of variance approach we were used. As there are significant differences LSD test of means comparison was carried out, with a significance level of 95%. The results were statistically analyzed by the program Statgraphic, Plus Windows version 1.5.

Results and discussion

The leaves of the plant inoculated in MS medium with growth regulators: ANA combined with kinetin showed early callus growth medium containing 1 mGL ANA-1 1 mGL-1 kinetin, at 8 days incubation continued to grow (Figure 3), and 30 days of incubation showed higher growth of calluses (undifferentiated cell growth) of good characteristics, proliferation and growth, which was observed on the leaves planted in the other combinations of regulators growth. The calli were subcultured on MS medium with ANA: 0, 1, 1.5 and 2 mGL-1 with 0, 0.5, 1.0, 1.5 and 2 mGL-1 kinetin, achieving the presence of roots on MS medium with 1 ANA mGL- 1 (Figure 4) at 15 days of incubation and 1.5 mGL-1 (Figure 5) at 20 days of incubation, from callus from plant leaves, not when presenting rooting kinetin was present in the culture medium.

Figure 3. Callos obtenidos en MS con 1mGL^{-1} de ANA con 1mGL^{-1} de cinetina

Figure 4. Enraizamiento en callos de hojas de J. cuneata en el medio MS con 1mGL^{-1} de ANA.

Figure 5. Raíces en callos con 1.5 mGL^{-1} de ANA.

The results obtained in this study showed that the presence of auxin is required for induction and growth of cell mass as Jatropha cuneata leaves planted in MS culture media with ANA showed callus growth at 30 days of incubation. When transferring the calluses obtained MS nutrient media with ANA and kinetin, root growth was observed in ANA: 1 and 1.5 mg L-1, Suarez and Salgado (2008) mention that the lack of organogenesis from callus often reported in certain species, especially those that are recalcitrant for propagation in vitro, however, this study was possible to observe the development of roots. For tissue culture propagation of Jatropha there are limitations, such as latex which contains, which makes this recalcitrant type of propagation, however, in vitro studies with Jatropha curcas have obtained good results (Datta et al., 2007) . Renuga and Rajamanickam, (2014) developed an efficient method for propagating Jatropha curcas in vitro from nodal segments system. Also with Jatropha curcas used the nutrient medium MS with different concentrations of sucrose, for stimulation of zygotic embryos from immature fruit, being that concentration of 60 GL-1 sucrose was the best (Ferreira et al., 2008) while in another job with the same level and with 22.2 uM BAP they managed the best results for the growth of stems in vitro (Sujatha et al., 2006).

In this study, to transfer plant callus in MS medium containing concentrations of 0, 1, 1.5, 2 and 2.5 mgL-1 growth regulator 2, 4-D, it was observed that the best growth responses these calli were obtained at concentrations of 1, 1.5 and 2 mg L-1 (Fig. 6), showing statistically significant differences with increasing concentration of the growth regulator (Table 1). These results agree with those obtained in J. curcas, in which the highest rate of callus formation was induced with 1.5 and 2 mg L-1 2,4-D (Solis et al., 2013). In studies with J. curcas fastest growing callus obtained with 2, 4-D (Coutiño-Cortés et al., 2013). In this work it was observed that increasing the concentration of the growth regulator, ie, with 2.5 mg L-1 of 2, 4-D, decreased cell proliferation. Increased production of callus after 30 days of incubation (Figure 7) was also observed. After this time, growth and callus production is decreased, which is not consistent with the results obtained with J. curcas, in which combinations of BA mGL-1 0.25 0.5 mGL AIB-1 presented to callus formation 21 days of incubation (Bermejo-Cruz, 2010).

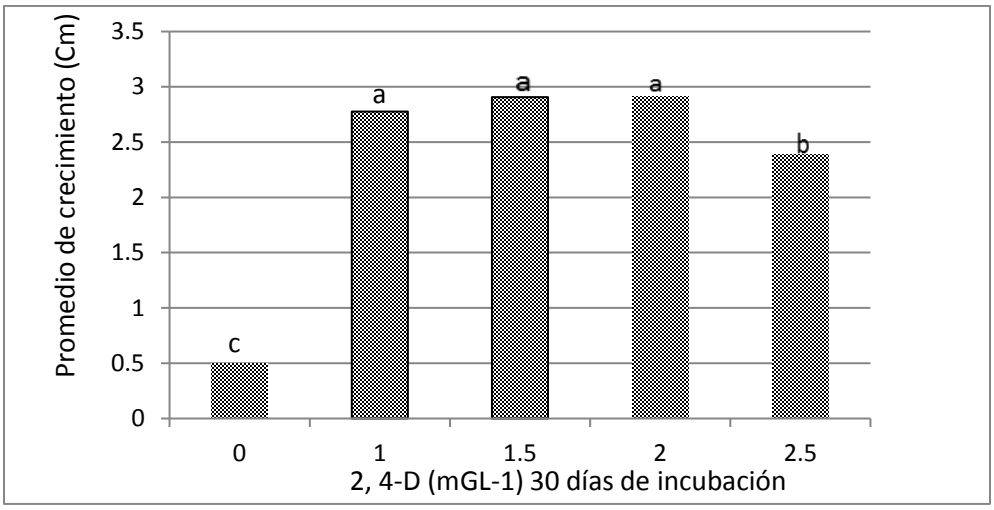

Figure 6. Promedio de crecimiento de callos en 0, 1, 1.5, 2.0 y 2.5 mGL^{-1} de 2,4-D.

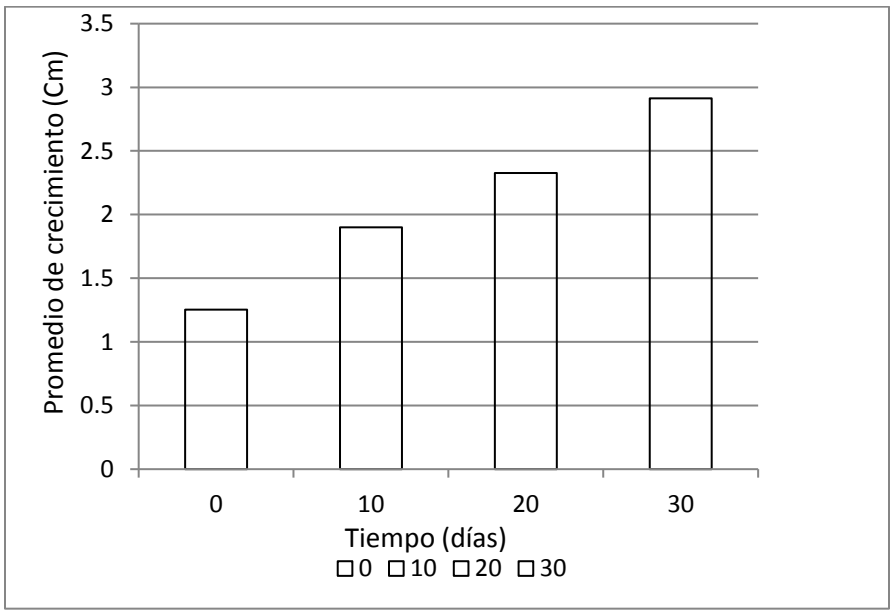

Figure 7. Promedio de crecimiento de callos de J. cuneata en 10, 20 y 30 días de incubación con el regulador de crecimiento 2,4-D (2 mGL^{-1}).

Table 1. Effects of growth regulator 2, 4-D for callus four weeks of incubation.

2, 4-D (mgL^{-1})	Crecimiento (cm)	Diferencia estadística
0	0.5	c
1	2.77	a
1.5	2.90	a
2	2.91	a
2.5	2.38	eb

Securities not associated with the same letter are significantly different ($P<0.05$).

Calluses obtained in MS medium with kinetin at concentrations of 0, 1, 1.5, 2 and 2.5 mg L-1, finding the best answers in concentrations of 1.5 mg L-1, when analyzing the data also subcultured statistically showed no difference significant with calluses undergoing concentrations of 1 and 2 mg L-1 (Table 2).

Table 2. Effects of kinetin growth regulator on callus J. cuneata at 30 days of incubation.

Cinetina (mGL^{-1})	Crecimiento (cm)	Diferencia estadística
0	0.5	c
1	2.54	ab
1.5	2.63	a
2	2.55	ab
2.5	2.26	b

Securities not associated with the same letter are significantly different ($P<0.05$).

Conclusions

Crops that have been used as sources of biodiesel, traditional crops have been used in human feeding. We need to increase research into plants that potentially could be used as crops for the production of biodiesel and that do not compete with crops that are used as a food source for humans.

The results of this study show that Jatropha cuneata is able to produce favorable results in conditions in vitro, so it is necessary to continue studies of this kind, because they are plants that survive in adverse weather conditions for other crops, may used on marginal land and with the advantage that they do not compete with plants that are harvested for food production. Cuneata Jatropha can be used by their potential to produce biodiesel.

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