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Short Communication

Factors influencing the establishment of haploid embryogenic callus from anthers in *Jatropha curcas* (L.)

Vinod Saharan*, Deepti Shrimali[1], M. A. Shah[1], B. R. Ranwah[1] and Rahul Sharma

Department of Molecular Biology and Biotechnology, [1]Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur

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Jatropha curcas (L.) is a small perennial tree or large shrub widely cultivated throughout Central America, Africa and Asia. There are several reasons for developing *Jatropha curcas* as a new energy crop. As it does not compete with conventional food crops for land, water and manpower, it acquires a separate status [1]. Worldwide, introduction of Jatropha for biofuel met with limited success due to unreliable oil yields. Available planting material is indeterminate with variability in yield components and oil content. Currently crop improvement work in this species is limited [2]. The interspecific and intervarietal hybridization in Jatropha species can play a significant role in transferring useful traits such as high oil content, high number of seeds, and more femaleness for promotion of a highly heterozygous, long-generation Jatropha species. No reports are available for *in vitro* androgenesis in Jatropha. Thus, anther culture can be used with usual breeding practices to produce interspecific and intervarietal hybrids. Therefore, the present study was undertaken to study various factors for commencement of embryogenic callus line from anthers in Jatropha.

Flower buds of *Jatropha curcas* (L.) were procured from mature Jatropha plants (6-years old) growing in horticulture farm, Rajasthan College of Agriculture, Udaipur. The phenological correlation between size (length) of flower buds and required developmental stage of pollen was determined. The anthers from flower buds of different sizes (2.0-9.0 mm) were dissected, fixed in absolute alcohol: glacial acetic acid (3:1) and stored at 4^{o}C for 1 h. Fixed anther preparations squashed in a drop of (1%) acetocarmine solution with a brief warming up prior to their observation under the advance stage research microscope BX51 with image analyzer software (Olympus, Japan). For aseptic anther culture initiation, the flower buds of appropriate development stage were disinfected by treating with 0.4 % Bavistin (Carbendazim 50 % WP, BASF, India) coupled with freshly prepared 1.0 % Sodium hypochlorite (4-6 % active chlorine, Hi-media, India) solution for 15 min. with constant swirling at 110 rpm on shaker and rinsed 3-4 times with distilled sterilized water. Outer covering and connective tissues of surface sterilized buds *viz*., sepals, petals and filaments were removed watchfully with fine forceps and scalpel without damaging anther wall. Excised anthers were plated horizontally with the contact with nutrient media in Petri plates. In each Petri plates cultured with 20 anthers poured with 25 ml nutrient media. Effect of sucrose, maltose, amino acids were tried in N_6 media [3] (Table 1). The selected buds were subjected to cold pretreatment (in refrigerator at 4°C for 3 and 7 days) and heat pretreatment (in seed germinator at 35°C with 60 per cent humidity for 1 day). The pH of nutrient media was adjusted to 5.8 prior to sterilization at 121°C for 20 min at a pressure of 1.06 kg cm^{-2}. All the cultures were incubated at 24±1°C in dark. Statistical analysis for the data was performed with JMP software version 8 (SAS,

*Corresponding author's e-mail: vinodsaharan@gmail.com
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USA) using Tukey Kramer HSD test for determining significant differences among treatments at P = 0.05 level.

Male flower buds ranging from 2.0 to 9.0 mm lengths were used to determine uninucleate microspores stage (Fig. 1A). Image analyzer measured approximately 95-100 µm size of uninucleate microspores isolated from 3.0-4.0 mm size flower bud (Fig. 1B). Literature reviews in other crops illustrated that the uninucleate stage of microspore was ideal for androgenesis [4]. Isolated anthers of Jatropha were cultured on N_6 media supplemented with 1.0 mg/l 2,4-D + 0.5 mg/l kinetin with variable concentrations (0, 3, 6 and 9%) of sucrose (Fig. 1C). At 6% sucrose concentration, 70.3% of anthers showed callus covering the entire anther surface (Table 1). Severe browning and death of anther cultures was recorded on media combined with maltose and sucrose at 4% level. However, maltose alone or in combination with sucrose has been shown to be favourable for establishment of anther culture in wheat [5] and rice [6]. Media augmented with amino acids are effective for androgenic response in other crops [7]. Immaterial result for anther culture was obtained from glutamine, arginine, alanine and proline fortified media. Indeed, proline found toxic for anther culture, only 20% anther confirmed callus induction (Table 1). Cold pretreatment of 3-days was favorable for anther culture and provoke maximum percent of callusing (85%), similarly to rice anther culture [8]. Calli attained from cold pretreatment, were yellowish, compact, globular and easily disengage from mother tissue (Fig. 1D and E). Cytological studies confirmed emergences of callus from microspores (Fig. 1F). Calli raised from cold pretreatment of anther cultures depicted as haploid nature of embryogenic calli. In succeeding subculture, these callus lines sustained embryogenic nature. From the present investigation, it is evident that cold pretreatment of 3 days of flower buds were promising for induction of haploid embryogenic callus on N_6 media supplemented with 1.0 mg/l 2,4-D + 0.5 mg/l kinetin + 6% sucrose. Standardized protocol from present study for anther culture can be used in future for obtaining haploid plant of *Jatropha curcas*.

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Table 1. Effect of sucrose, maltose, amino acid and pretreatments (cold and heat) on callusing and browning in anther cultures grown on N_6 media supplemented with 1.0 mg/l 2,4-D + 0.5 mg/l kinetin + 6% sucrose (except sucrose and maltose variables)

Treatment	% Callusing[x]	% Browning[x]	Degree of callusing[y]
Sucrose (0%)	0 ± 0^{k}	100 ± 0^{a}	-
Sucrose (3%)	43.3 ± 1.7^{f}	56.6 ± 1.7^{f}	++
Sucrose (6%)	70.3 ± 4.4^{b}	21.6 ± 4.4^{j}	+++
Sucrose (9%)	25.0 ± 1.9^{i}	75.0 ± 2.3^{c}	+
Maltose (4%)	51.6 ± 4.4^{d}	48.3 ± 4.4^{h}	+
Maltose (4%) +Sucrose (4%)	20.0 ± 2.4^{j}	80.0 ± 1.2^{b}	+
Glutamine	45.0 ± 1.9^{e}	55.0 ± 1.9^{g}	++
Arginine	35.0 ± 2.5^{h}	65.0 ± 3.0^{d}	+
Alanine	40.0 ± 2.2^{g}	60.0 ± 1.1^{e}	+
Proline	20.0 ± 2.6^{j}	80.0 ± 2.0^{b}	+
Cold pretreatment (3 days)	85.0 ± 2.5^{a}	15.0 ± 2.0^{k}	++,+
Cold pretreatment (7 days)	55.0 ± 1.9^{c}	45.0 ± 1.1^{i}	++
Heat pretreatment (1 days)	51.6 ± 4.4^{d}	48.3 ± 4.4^{h}	+

[x]Each value represent mean ± SE of 3 replicates, each contained 20 anthers. In column mean ± SE followed by different letters are significantly different at P = 0.05 by TuKey-Kramer HSD test. [Y]The size of callus is directly related to the numbers of plus signs; +: microcallus, located in small spots along the anther surface; ++: covering the 70-75% of the anther surface and +++: covering the entire anther surface

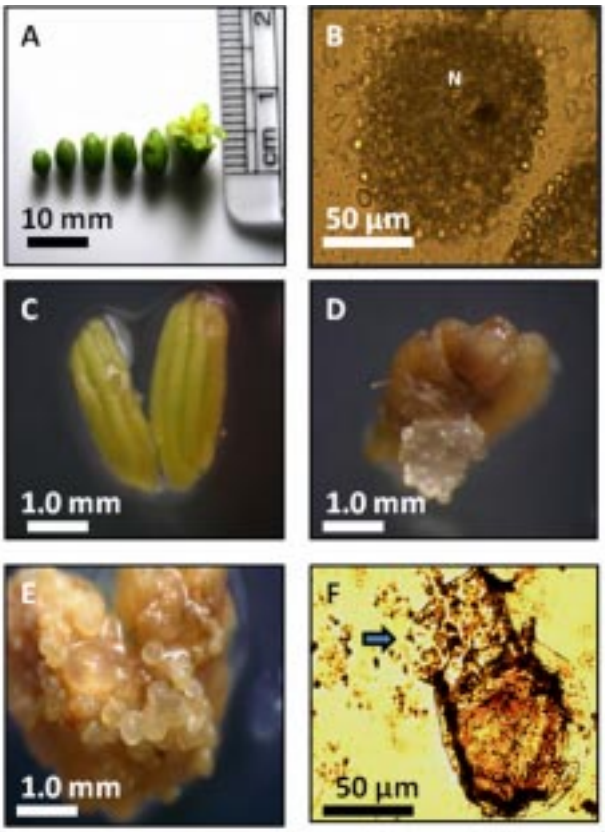

Fig. 1. Different stages of callus culture in Jatropha. (A) Different lengths of flower buds, (B) Uninucletae microspore, (C) Anther culture, (D) Embryogenic callus induction from anther culture, (E) Embryogenic callus culture, (F) Emergence of callus from microspore (arrow)

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