In Vitro Callus Induction of *Jatropha curcas* from Mature Seed in Different Auxins Concentrations

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Abstract: The aim of this study to determine the effect of different concentrations of two types of auxins 2, 4-D (2, 4- dichlorophenoxy acetic acid) and NAA (Naphthalene acetic acid), on callus induction using leaves and hypocotyls explants of Jatropha curcas. 100% .&.90% of callus induction were achieved on Murashige and Skoog (MS) medium supplemented with different concentrations 2, 4 and 6.0mg/l, NAA &2,4-D hormones respectively using hypocotyls and leaves explants after three weeks. The maximum callus index observed on the MS medium containing 2.0 mg/l NAA was 4.0, when the hypocotyls segment was the explants. The callus was friable, nodular, granule and light green in colour .The percentage of callus initiation was increased by increasing of concentration of auxin 2, 4-D (2, 4 and 6mg/l), at the same time the percentage of callus formation was decreased by the increasing of concentration of auxin NAA (2, 4 and 6mg/l), using the explants leaves and hypocotyls. There is significant difference ($P \le 0.05$) between two hormones NAA and 2, 4-D on the percentage of callus, mean of callusing index and day of initiation. The auxin NAA in this study was found to be the suitable hormone to induce callus of Jatropha curcas for both types of explants used.

Keywords In vitro culture, Jatropha curcas, Growth regulators, Hypocotyls and leaves explant

1. Introduction

Ра

Jatropha curcas, belonging to *Euphorbiaceae* family, is a perennial, deciduous and oil-bearing shrub. Jatropha curcas is one of the most valuable crude drugs of primitive times and is still widely used in modern medicine. It has multipurpose plant valued not only for its medicinal properties and resistant to various stresses but also for its use as an oil seed crop [6]. In recent years this plant has received extensive attention of many scientists in view of its great economic importance, medicinal significant and for its seed oil as commercial source of fuel [4]. The superior quality oil can be extracted from the seeds. The oil is not edible due to the presence of toxic substance"Curcascine" [5]. It is also recommended as a drought resistant plant suitable for erosion control and is not palatable to grazing animals due to the toxicity [12].

Plant tissue culture is the method of culturing plant parts in artificial medium to regenerate into a new plant. This technique affords alternate solutions to problems arising due to current rate of extinction and decimation of flora and ecosystem. Plant cell cultures are generally more desirable than a solid medium because of higher growth rates resulting from a high medium to tissue contact. Plant cell and tissue cultures provide an alternative approach to the plants which are difficult to cultivate, or has a long cultivation period, or has a low yield, product yield by cell culture may be significantly produce a higher yield that obtained from the constituents of Jatropha curcas, such as curcin [3], 12-deoxy-10 hydroxyphobol [1], esterase and lipase have been isolated from seeds and characterized in succession, yet the full potential of Jatropha curcas is far from being realized [17]. To meet the large - scale demand and ensure easy supply of this elite material,

there is a need to establish mass multiplication technique. Macro propagation through stem cutting is possible but the seed yields are low and the established plants are not deep rooted and hence, are easily uprooted [6]. The propagation through seeds is dependent on good rainfall, moisture condition, sowing time and depth of sowing. This is major problem for establishment of plants propagated through stem cuttings and seeds on poor and marginal soils.

Tissue culture techniques offer rapid and continuous supply of material. Evaluation of tissue culture propagated plants of Jatropha curcas revealed that they were at par with seed propagated plants in terms of yield and yield related traits [18]. The improvement of micro propagation efficiency of Jatropha curcas is very important for its biodiesel production, also the plants regenerated are generally disease free. Despite the fact that nitrate and ammonium salts have been universally used as N source in tissue culture media, numerous reports specify that reduced nitrogen forms, particularly amides and amino acids, proline, and alanine, can improve cell proliferation as well as regeneration in specific genotypes [20]. Amino acids can adjust the nitrogen utilization of in vitro cultures by regulating primary nitrogen assimilation. Concurrently, many amino acids can be readily transformed into other amino acids and integrated into proteins in the cell culture [19].

Medza [9] studied the in *vitro* micropropagation of *Jatropha curcas L*. from bud aggregates, they reveal that the culture medium MS containg 6.65 μ M BA (benzyladenine) and 25 mg.l-1 L-glutamine gave the best results with an average of 64 buds per aggregate after three weeks for all accessions tested.

Rajore [14] found that, when leaf explants of *Jatropha curcas* was used, with high concentration of BA (5.0mg/l) with

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combination of NAA (1.0mg/l), the callus was induced within3-4weeks at the same time NAA (1.0-4.0mg/l) alone in half MS medium also induce callus.

Li [8] studied, explants epicotyl, hypocotyl, petiole and cotyledon of 8-day-old seedlings of J. curcas were utilized for callus induction on media supplied with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), naphthyl acetic acid (NAA) or indolebutyric acid (IBA) and 0.1 mg/L kinetin (Kin), and the results demonstrated that the combination of 1 mg/L NAA and 0.1 mg/L Kin was the best medium for callus induction.

2. Materials and Methods

Source of Plant Materials:

An identified seeds of *Jatropha curca* were collected from national research center, ministry of science & technology of Sudan, Khartoum.

Sterilization of Equipment and Glassware

All dissection instruments, glassware and other accessories were sterilized by autoclaving at $121 \circ c$ with 15 1b/in for15 min. Dissection instrument like scalpels and forceps after autoclaving were more sterilized by dipping in 90% ethanol for at least 15 minutes and flaming before used. The laminar-air flow cabinet was sprayed with 70% (v/v) ethanol. Irradiation of instruments with Ultraviolet light in laminar-air flow cabinet for 30 minutes prior to inoculation was used.

Seeds Treatment and Surface Sterilization

In order to germinate seeds of *Jatropha curcas*, they were treated by conc. H2So4 for 1min., and then rinsed by sterile distill water many times. Treated seeds were surface sterilized by soaking in 100% Clorox (0.5% free chlorine) with 2 drops of Tween-20 for 30min. and rinsed 3-4 times in sterile distilled water then soaked for imbibition in sterile distilled water for two days.

Seeds germination:

Soaked, surface sterilized seeds of Jatropha curcas were cultured on germination medium MS [11] basal medium.

Callus Induction:

Explants preparation and growth regulators:

Explants used for callus induction were leaves and hypocotyls, which were obtained from seedling of Jatropha curcas seven days old. Each of the sterilized explants was cut into 2-3 mm pieces using sterile scalpel. These explants were culture on MS medium supplemented with different auxins. Leaves and hypocotyls segments used for callus induction were inoculated in culture bottle (5*9cm) containing 25ml of MS media. Four explants per jar fortified separately with different concentrations of 2, 4-D and NAA (0.0, as control, 2,4and 6 mg/l) to assess their effects on callus induction for explants. To compare the effect of the presence of cytokinen on callus induction medium, two different concentrations of kinetin (0.05and 0.5mg/l) were used in combination with 4mg/l of 2, 4-D and 6mg/l NAA.

Five replicates were maintained for each experiment. Culture was marinated in a growth room at 25°C±2°C under dark photoperiod. Callus induction was observed regulatory.

Statistical Analysis:

Data were analysed by SPSS, considering each bottle with four explants as one replicate of each treatment. The percentage of callus induction and callus index (mean \pm SE) were evaluated [15].

3. Results and Discussion

Figure (1) represented the percentage of callus induction of Jatropha curcas after three weeks from hypocotyls and leaves explants, cultured on MS medium supplemented with auxin 2,4-D with different concentrations 2,4 and 6 mg/l .The hypocotyls explants induced by this auxin, were initiated callus after five days only, this finding was agreed with that obtained by [2], who found that the hypocotyls were suitable explants for callus initiation. On the other hand the leaves explants affected by the same auxins 2, 4-D concentration (2, 4 and6 mg/l) were initiated callus after 15 days. This result showed that the hypocotyls explants of Jatropha curcas initiated callus very rapidly after five days, when grown on MS medium supplemented with this hormone. This finding agreed with that obtained by Mona celli, [10], who found that callus produced with 0.5 mg /l 2, 4-D using hypocotyls explants of Jatropha curcas and showed fast rate during first 7 to 30 days of culture.



Figure 1: Effect of different concentration of hormone (2, 4-D) on callus induction of leaves and hypocotyls of Jatropha curcas in MS media

Results in figure (2) showed the effect of different concentrations 2.0, 4.0 and 6.0mg/l of auxin NAA on the percentage of callus induction of *Jatropha curcas* after three weeks, using hypocotyls and leaves explants. The hypocotyls explants induced by NAA auxin, were initiated callus after five days only and the percentage of callusing was 100% by auxin concentration of 2.0 mg/l



Figure 2: Effect of different concentrations of hormone (NAA) on callus induction of leaves and hypocotyls of *Jatropha curcas* in MS media

A previous studies have showed that auxins such as 2, 4-D and NAA are important for callus induction [8]; [16]; [13]; [21]. In this plant Li *et al* [8] have found that best result of callus induction was obtained when the medium supplied with 1mg/l NAA and 0.1mg/l kinten. The present study reveal that 2.0 mg/l NAA showed the best concentration of auxins for inducing callus from hypocotyls and leaves explants of *Jatropha curcas*, while the highest callus initiation percen-

tage(90%) from hypocotyls explants was obtained from 6.0mg/l 2, 4-D.

The difference between the percentage of callus initiation affected by auxins 2, 4-D & NAA with concentrations (2.0, 4.0 & 6.0mg/l) was significantly difference ($p \le 0.05$). The percentage of callus initiation was increased by increasing of concentration of auxin 2, 4-D (2, 4 and 6mg/l), at the same time the percentage of callus formation was decreased by the increasing of concentration of auxin NAA (2, 4 and 6mg/l), using the explants leaves and hypocotyls

Figure (3) showed the effect of combination of auxins 4mg/l 2, 4-D and kinetin with concentration 0.05 or 0.5 mg/l. The percentage of callus obtained were the same results obtained by 4mg/l 2, 4-D alone. 6mg/l of NAA with combination to 0.05 or 0.5 mg/l were gave the same results obtained by 6mg/l of NAA alone (fig.4). These findings agree with that obtained by Hoshino, *et al* [17]. These results contrary to that obtained by Li *et al.*[8], they have found that 1mg/l NAA and 0.1mg/l kinten was the most significant compared to 1mg/l and0.1mg/l kin of 2,4-D and IBA.



Figure 3: Effect of different combination of hormones (2,4-D+ Kinetin) on callus induction of leaves and hypocotyls of Jatropha *curcas* in MS media

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The texture of callus produced from leaves and hypocotyls was, friable, nodular and granule and light green in color. Fresh weight of callus was increased by the increasing of time till four weeks then decline, as shown in fig (5). These results agree with that obtained by Soomro and Memon, [17].



Figure 5: Fresh weight of callus of *Jatropha curcas* (hypocotyls explants)) for five weeks Induced by 2, 4-D&NAA

The fresh weight of callus induced by NAA and 2, 4-D after four weeks were 2.6 and 2.4 gm respectively.

Callus index produced from hypocotyls inducing by two auxins 2, 4-D (2,4and 6mg/l) and NAA (2,4and 6mg/l) Separately were increased by the increasing of time. The

maximum value of callus index obtained were 3.0 and 4.0

By the third week for the auxin 2, 4-D and NAA respectively as shown in (table 1& fig.6).

 Table 1: Callus Index of leaves and hypocotyls of Jatropha

 curcas during three week

Explant	hormones		Auxin concen-	Mean* + SF
part	NAA	2,4-D	tration (µM)	
leaves	-	-	2.0	2.5000ab ±3.00000
	-	-	4.0	4.5000a±2.5166 1
		2,4-D+kinti n	4+0.05	5.0000a±.00000
		2,4-D+kinti n	4+0.5	5.0000a±.00000
	-		6.0	4.0000ab±2.000 00
	NAA + kine- tin		6+0.05	3.0000ab±2.828 43
	NAA + kine- tin		6+0.5	3.0000ab±2.828 43
hypoco- tyls	-	-	2.0	1.5000ab±.5773 5
	-	-	4.0	3.0000a±00000
		2,4-D+kinti n	4+0.05	1.5000 a±.57735
		2,4-D+kinti n	4+0.5	1.5000ab±.5773 5

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		6.0	
	NAA + kine- tin	6.0+ 0.05	4.0000ab±00000
	NAA + kine- tin	6+0.5	4.0000ab ±00000
Mean* ±		2.7273±1.1037	6.5723ab±3.294
SD		1	6

*Means with different letters are significantly different at p = 0.05.



A - Callus induction from hypocotyls segments by NAA -concentration 2.0mg/l+-control) in MS media



B-Callus induction from hypocotyls segments by 2,4-D concentration 4.0 mg/l + control in MS media



C-Callus induction from leaves segments in by NAA concentration 2.0mg/l +control in MS media

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D-callus induction from leaves segments by2, 4-D (concentration 4.0 mg/l+ control)

Figure (6): Callus Induction from Hypocotyls Segments by 2mg/l of Hormones NAA &4mg/l 2.4D +control (A&B) and Leaves Segments by 2.0mg/l of NAA & 4mg/l 2, 4-D (C&D) in MS Media

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