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Research Article
Protocol of *in vitro* Jojoba (*Simmondsia chinensis* (Link) Schneider) Callus Induction

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Abstract

Background and Objectives: Presently, determination of optimum protocol for callus induction of any plant is an important issue in tissue culture technology. Therefore, the main objective of this study was to find out an optimum protocol for callus induction from *in vitro* cultured jojoba by determining the optimum explant and the best growth regulators mixture for callus induction. **Materials and Methods:** The study used three variant explants namely the leaf disks, seeds and nodal segments for callus formation. Different culture media containing basic Murashige and Skoog (MS) medium components supplemented with various concentrations of 2,4-dichlorophenoxy acetic acid as an auxin (2,4-D) and Kinetin (Kin) as a cytokinin with various concentrations ranging from 0.0, 0.5, 1.0 and 2.0 mg L^{-1} were used. The total number of treatments were 16. The callus was induced from all explants on MS medium containing the lowest concentration of 2,4-D 0.5 mg L^{-1} with any concentration of Kin. **Results:** The results showed that nodal segments were the best for callus formation followed by the leaf disks (leaves) and seeds, respectively. While, the best concentration of proliferation and development of the used explant was 2.00 followed in descending order by 1.00, 0.5 and 0.0 mg L^{-1}, respectively. **Conclusion:** The study find out that the best concentration of 2,4-dichlorophenoxy acetic acid as an auxin (2,4-D) and Kinetin (Kin) as a cytokinin was 2.00 followed in descending order by 1.00, 0.5 and 0.0 mg L^{-1}, respectively for callus induction.

Key words: 2,4-dichlorophenoxy acetic acid, callus induction, kinetin, leaf disks, nodal segments, seeds (immature zygotic embryos)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jojoba is a desert, wild, evergreen and a perennial plant for about 100-200 years old according to Verbanic[1]. Jojoba being a dicotyledonous and dioecious plant can grow under various plant growth conditions with air temperature variation between 5-54°C as stated by Al-Ani *et al.*[2]. Besides its ability to fight diseases and pests, the water requirement of Jojoba is low like that of date palm[3]. Therefore, it is important to grow jojoba in desert regions of Arabic peninsula to take the advantage of oil production to transfer it into permeate cheap fuel source for solving the energy shortage problem on cost effective basis in some Arabian countries due to persistent decline in carbonic fuel in the world[3]. It is also well known for its various medicinally important active constituents such as Simmondsia thus giving its scientific name as jojoba [*Simmondsia chinensis* (Link) Schneider] according to the findings of Gentry[4].

Tissue culture is an important and latest modern technology to overcome many problems and obstacles faced by the farmers who are following the old traditional methods[5]. Thus it encourages the scientists to carry further research studies on callus cells, tissue and explants *in vitro* which are difficult on the whole plant, especially for the perennial dioecious plant such as jojoba and date palm[6]. According to many researchers, the minimum maturity age of jojoba is three years[7,8].

Callus formation is one of the most important *in vitro* development and growth stages during which its undifferentiated parenchymal cells can be directed to form organs, roots, somatic embryos or to form many natural active constituents and secondary compounds of high medicinal value in a short period of time rather waiting for 3 years to achieve its maternity stage, because Jojoba produces the highest yield of seeds after 8 years of traditional culture[6,9].

Selection of proper explant for tissue culture is one of the most important factors for callus formation especially in perennial plants like Jojoba. There are many types of explants for use in tissue culture experiment based on the plant type (species) and the study objective. These explants are generally characterized with having meristem cells or being able to transform into meristem cells having maximum genetic stability that helps the cultured explant to achieve the best expected results[10].

Previous studies reported that the most commonly used explants in tissue culture of perennial plants and oil crops were seeds, fresh leaves and nodal segments as suggested by Jacoboni and Standardi[11]. On the other hand, this study counted on both the 2,4-D and Kin with different concentrations as representatives of auxins and cytokinins to determine an optimum mixture of interactive growth regulators for the growth and development of explant to produce callus *in vitro*.

MATERIALS AND METHODS

This experiment was conducted at Agricultural Biotechnology Department, Agricultural and Food Sciences College, King Faisal University, Al-Hasa, Kingdom of Saudi Arabia during March and May, 2015.

Plant material: Plant material was collected from semi-hardwood stems of field-grown female jojoba adult shrub. Single node cuttings (2 cm) of explants were excised from a 5-years-old shrub between March and May, 2015.

Sterilization *in vitro*: Explants were collected to initiate the cultures. The cuttings were thoroughly washed with 1% solution of savlon for 20 min and rinsed three times in Sterile Distilled Water (SDW). All the subsequent operations were carried inside a laminar air-flow cabinet. The clean cuttings were quickly rinsed for about 1 min in 70 % ethanol, followed by three washings in SDW. Then, these cuttings were surface-sterilized in 1.5 g L^{-1} mercuric chloride ($HgCl_2$) solution for 15 min and rinsed three times with SDW by following the procedures described by Jacoboni and Standardi[11] and Mohasseb *et al.*[8].

Establishment stage and callus production: The establishment stage and callus production was carried using the methodology of Murashige and Skoog[12]. The cuttings were slightly trimmed from both ends to expose the fresh tissue before planting to culture MS medium as described by Murashige and Skoog[12]. The culture medium solution (MS) consisted of inorganic salts, supplemented with (in mg L^{-1}): 100 myoinositol; 30000 sucrose; 7000 agar and different concentration of auxin 2,4-Dichlorophenoxy acetic acid (2,4-D) and Kinetin (Kin) (Sigma Chem. Co.). The pH of medium was adjusted at 5.7±0.1 before adding agar and autoclaving the medium at 1.02 kg cm^{-2} equivalents to 121°C for 15 min. The nutrient media was dispensed into autoclavable culture jars containing 25 mL of media. The details of nutrient media composition is presented in Table 1.

Table 1: Nutrient media composition for *in vitro* callus production of jojoba protocol and its sequence

	Kin (mg L^{-1})			
2,4-D (mg L^{-1})	0.0	0.5	1.0	2.0
0.0	A*	B	C	D
0.5	E	F	G	H
1.0	I	J	K	L
2.0	M	N	O	P

*Media code

All the cultures were maintained in diffuse light of 2000 lux for 16 h photoperiod at 25±2°C. The explants of each treatment were repeatedly sub-cultured to three subcultures after 4 weeks interval. For parameters of callus production stage, data were recorded for every treatment at the end of 3 months from the initial culture. Each treatment was replicated five times. Each replicate consisted of one jar containing three explants. The data were presented as an average per callus production as described below:

1-(Pro) = Proliferation (No.)
2-(CP) = Callus production (No.)
3-(FW) = Fresh weight (g)
4-(DW) = Dry weight (g)

Statistical analysis: Data was statistically analyzed by two way analysis of variance (ANOVA) for the completely randomized design. The treatment means were compared using Least Significant Difference (LSD) at 5 % level of probability. All the computations and statistical analysis was performed using a MSTAT-C computer program v.4 of Duncan[13].

RESULTS AND DISCUSSION

Specific effect of Kin on callus production: There was a significant effect of different concentration of Kin (0.0, 0.5, 1.0 and 2.0) on the response and proliferation of variant Jojoba explants (Table 2). The results in Table 2 showed only the effect of different concentration of Kin hormone without addition of the other hormone (2,4-D auxin) on callus production. Data in Table 2 showed the effect of Kin as cytokinin on different growth parameter of callus such as fresh and dry weight. The results showed direct relationship between the concentration and the response of cultured explant for proliferation and growth which increased by increasing the concentration of Kin. While, there was no clear response of all the explants cultured on hormone free medium except for nodal segments showing only little response. It was found that Kin did not play any effective role on callus formation directly. Whereas the interaction showed an indirect effect on callus formation in the presence of 2,4-D auxin. These results are in agreement with those reported by Hamama *et al.*[7] and Mohasseb *et al.*[8].

Specific effect of 2,4-D auxin on callus production: The results in Table 3 showed significant effect of 2,4-D auxin on the proliferation, development and growth of different cultured explants for callus formation due to their relation between the rate of callus formation and 2,4-D concentration. The results in Table 3 showed only the effect of different concentration of 2,4-D auxin hormone without addition of the other hormone (kin) on callus production. Data in Table 3 showed the effect of 2,4-D auxin on different growth parameters of callus such as fresh and dry weight of callus obtained from different explants of jojoba. The resultant callus formation increased by increasing the concentration of 2,4-D auxin which agreed with the findings of Gaber *et al.*[14]. Since, the callus formation was initiated from the immature embryos on medium supplemented with 2,4-D without kin (cytokinins). Therefore, the researchers observed that the addition of Kin is not necessary to obtain high frequency of callus production. Similar results were reported by Green and Philips[15] for maize and by Lazer *et al.*[16] for wheat crop.

Table 2: Specific effect of Kin on callus production and fresh and dry weight callus production from different explants of jojoba

		Leaf disks		Seeds		Nodal segments		Means	
Code	Kin (mg L^{-1})	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)
Callus production									
A	0.0	0.00^{b}	0.00	0.00^{b}	0.00	0.91^{a}	0.00	0.30^{c}	0.00
B	0.5	0.00^{c}	0.00	0.00^{b}	0.00	0.89^{a}	0.00	0.30^{c}	0.00
C	1.0	0.67^{b}	0.00	0.33^{a}	0.00	0.92^{a}	0.00	0.64^{b}	0.00
D	2.0	1.00^{a}	0.00	0.33^{a}	0.00	0.89^{a}	0.00	0.74^{a}	0.00
Means		0.42^{b}	0.00	0.17^{c}	0.00	0.90^{a}	0.00		
		Leaf disks		Seeds		Nodal segments		Means	
Code	Kin (mg L^{-1})	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
Fresh and dry weight callus production									
A	0.0	1.6250^{c}	0.4807^{b}	0.3120^{c}	0.1422^{b}	3.2500^{c}	0.9615^{b}	1.7290^{c}	0.5281^{b}
B	0.5	0.9480^{d}	0.4558^{b}	0.5980^{c}	0.1674^{b}	1.8960^{d}	0.9116^{b}	1.1473^{d}	0.5116^{b}
C	1.0	2.0963^{b}	0.3377^{c}	1.1890^{b}	0.5620^{a}	4.1927^{b}	0.6754^{c}	2.4927^{b}	0.5250^{b}
D	2.0	2.1600^{a}	0.5833^{a}	2.3600^{a}	0.7499^{a}	4.3200^{a}	1.1666^{a}	2.9467^{a}	0.8333^{a}
Means		1.7073^{b}	0.4644^{b}	1.1148^{c}	0.4054^{c}	3.4147^{a}	0.9288^{a}		

Pro: Proliferation, CP: Callus production, FW: Fresh weight, DW: Dry weight, different letters are significant at probability of 5%

Table 3: Specific effect of 2,4-D on callus production and fresh and dry weight callus production from different explants of jojoba

		Leaf disks		Seeds		Nodal segments		Means	
Code	2,4-D (mg L^{-1})	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)
Callus production									
A	0.0	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.91^{a}	0.00^{c}	0.30^{c}	0.00^{c}
E	0.5	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.93^{a}	0.00^{c}	0.31^{c}	0.00^{c}
I	1.0	1.00^{a}	0.30^{a}	0.00^{b}	0.00^{b}	0.89^{a}	1.00^{b}	0.63^{b}	0.77^{b}
M	2.0	1.00^{a}	0.30^{a}	0.67^{a}	1.00^{a}	1.00^{a}	3.00^{a}	0.89^{a}	2.10^{a}
Means		0.50^{b}	0.15^{b}	0.17^{c}	0.25^{b}	0.93^{a}	1.00^{a}		
		Leaves (leaf disks)		Seeds		Nodal segments		Means	
Code	2,4-D (mg L^{-1})	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
Fresh and dry weight callus production									
A	0.0	1.6250^{c}	0.4807^{c}	0.3120^{c}	0.1422^{c}	3.2500^{c}	0.9615^{c}	1.7290^{c}	0.5281^{c}
E	0.5	1.3507^{d}	0.5606^{b}	1.276^{b}	0.1026^{c}	2.7013^{d}	1.1212^{b}	1.7760^{c}	0.5948^{c}
I	1.0	2.1380^{b}	1.0009^{ab}	1.4580^{ab}	0.2238^{b}	4.2760^{b}	2.0017^{a}	2.6240^{b}	1.0755^{b}
M	2.0	2.6490^{a}	1.0746^{a}	1.5780^{a}	0.6818^{a}	5.2980^{a}	2.1492^{a}	3.1750^{a}	1.3019^{a}
Means		1.9407^{b}	0.7792^{b}	1.1560^{c}	0.6818^{c}	3.8813^{a}	1.5584^{a}		

Pro: Proliferation, CP: Callus production, FW: Fresh weight, DW: Dry weight, different letters are significant at probability of 5%

Table 4: Interaction effect of 2,4-D and Kin concentrations on callus production and fresh and dry weight of callus production from different explants of jojoba

	Treatments (mg L^{-1})		Leaves (leaf disks)		Seeds		Nodal segments		Means	
Code	2,4-D	Kin	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)	Pro (No.)	CP (No.)
Callus production										
A	0.0	0.0	0.00^{e}	0.00^{e}	0.00^{c}	0.00^{b}	0.91^{a}	0.00^{d}	0.30^{d}	0.00^{d}
F		0.5	0.00^{e}	0.00^{e}	0.00^{c}	0.00^{b}	0.67^{b}	0.67^{c}	0.22^{d}	0.22^{d}
G	0.5	1.0	0.67^{c}	0.20^{d}	0.00^{c}	0.00^{b}	0.67^{b}	2.00^{b}	0.45^{c}	0.73^{c}
H		2.0	0.33^{d}	0.20^{d}	0.50^{b}	1.00^{a}	0.93^{a}	2.00^{b}	0.59^{b}	1.07^{b}
J		0.5	0.33^{d}	0.20^{d}	0.00^{c}	0.00^{b}	0.76^{b}	1.00^{c}	0.36^{c}	0.40^{d}
K	1.0	1.0	0.67^{c}	0.40^{c}	0.50^{b}	1.00^{a}	0.90^{a}	1.00^{c}	0.69^{b}	0.80^{c}
L		2.0	0.89^{b}	0.75^{b}	0.50^{b}	1.00^{a}	0.67^{b}	2.00^{b}	0.69^{b}	1.25^{b}
N	2.0	0.5	0.67^{c}	0.40^{c}	0.00^{c}	0.00^{b}	0.92^{a}	2.00^{b}	0.53^{b}	0.80^{c}
O		1.0	1.00^{a}	0.80^{b}	0.89^{a}	1.00^{a}	0.90^{a}	2.00^{b}	0.93^{a}	1.27^{b}
P		2.0	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	3.00^{a}	1.00^{a}	1.67^{a}
Means			0.56^{b}	0.40^{b}	0.34^{b}	0.50^{b}	0.83^{a}	1.57^{a}		
	Treatments (mg L^{-1})		Leaves (leaf disks)		Seeds		Nodal segments		Means	
Code	2,4-D	Kin	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
Fresh and dry weight of callus production										
A	0.0	0.0	1.6250^{b}	0.4807^{c}	0.3120^{e}	0.1422^{e}	3.2500^{c}	0.9615^{c}	1.7290^{c}	0.5281^{c}
F		0.5	0.9480^{e}	0.4558^{c}	0.5980^{e}	0.3674^{c}	1.8960^{f}	0.9116^{c}	1.1473^{d}	0.5783^{c}
G	0.5	1.0	0.6790^{f}	0.3602^{d}	0.7590^{d}	0.0805^{f}	1.3580^{g}	0.7203^{d}	0.9320^{d}	0.3870^{d}
H		2.0	0.9517^{e}	0.5175^{c}	1.7050^{b}	0.5525^{b}	1.9033^{f}	1.0350^{b}	1.5200^{c}	0.7017^{b}
J		0.5	1.5813^{bc}	0.6059^{b}	0.2290^{e}	0.0818^{f}	3.1627^{c}	1.2117^{b}	1.6577^{c}	0.6331^{c}
K	1.0	1.0	1.4537^{c}	0.6013^{b}	0.7230^{d}	0.3038^{d}	2.9073^{d}	1.2025^{b}	1.6947^{c}	0.7025^{b}
L		2.0	0.9160^{e}	0.4548^{c}	1.8260^{b}	0.3643^{c}	1.8320^{f}	0.9095^{c}	1.5247^{c}	0.5762^{c}
N		0.5	1.6533^{b}	0.4838^{c}	1.1250^{c}	0.4515^{c}	2.1953^{e}	0.9677^{c}	1.7570^{c}	0.6343^{c}
O	2.0	1.0	1.0977^{d}	0.5479^{b}	1.9780^{b}	0.6438^{b}	3.3067^{b}	1.0959^{b}	2.0283^{b}	0.7625^{b}
P		2.0	1.9287^{a}	1.0607^{a}	2.5790^{a}	0.8182^{a}	3.8573^{a}	2.1213^{a}	2.7883^{a}	1.3334^{a}
Means			1.2834^{b}	0.5569^{b}	1.1834^{b}	0.3806^{b}	2.5669^{a}	1.1137^{a}		

Pro: Proliferation, CP: Callus production, FW: Fresh weight, DW: Dry weight, different letters are significant at probability of 5%

The results also indicated that nodal segments were the best for callus formation followed by leaf disks (leaves) and the seeds, respectively. On the other hand, the best 2,4-D concentration for proliferation and development of explants was 2.00, 1.00, 0.5 and 0.0 mg L^{-1}, respectively.

Interactive effect of 2,4-D, Kin and their concentrations on callus production: The results in Table 4 and Fig. 1 showed significant effect of various combinations of Kin as cytokinin and 2,4-D auxin concentrations on callus production and its growth parameters (fresh and dry weight) obtained from different explants. Because, the highest

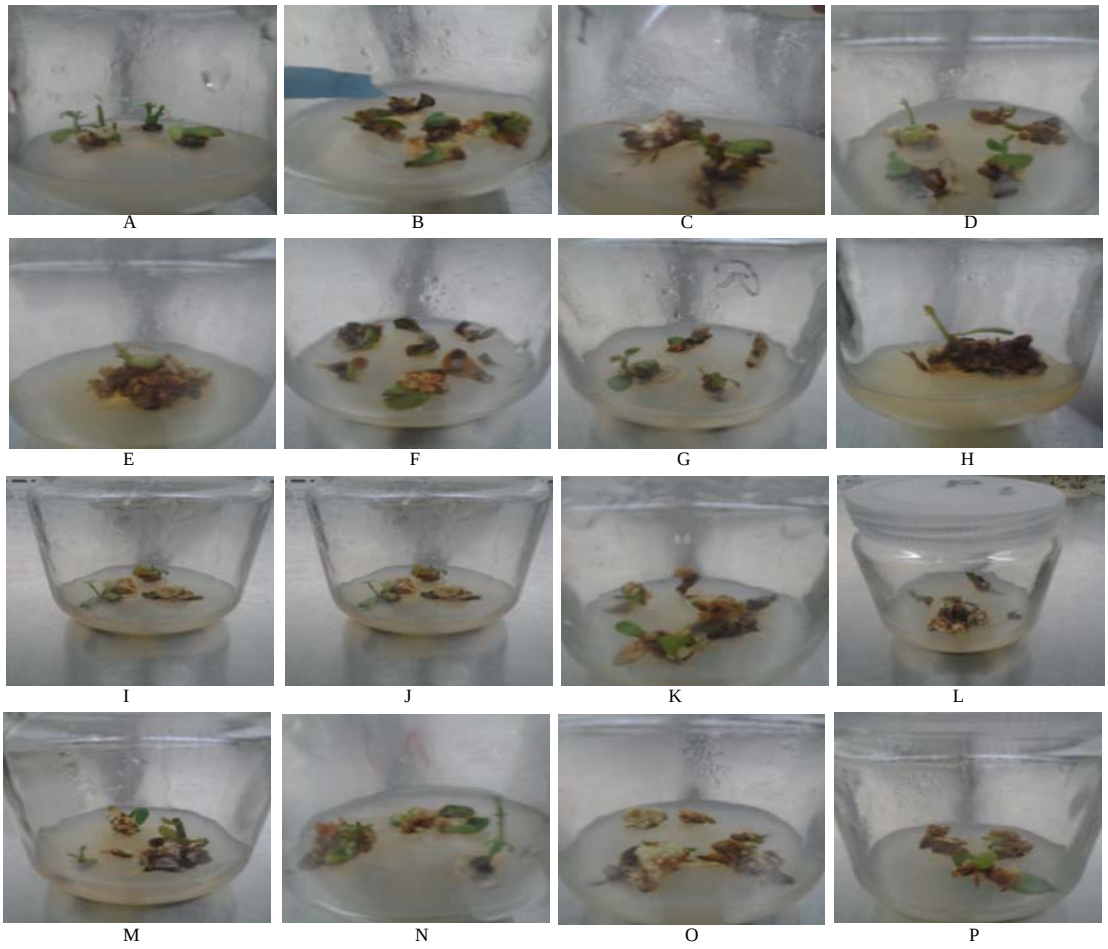

Fig. 1: Effect of 2,4-D, Kin and their concentrations on callus production from nodal segments of jojoba

response for callus formation was achieved at the concentration of 2.00 mg L^{-1} for both the 2,4-D and Kin. The results also indicated that 2,4-D played an important role on proliferation and development of explants for callus formation. Whereas, Kin played a contributory role on callus formation by increasing its concentration. However, the magnitude of explant response for proliferation and callus formation increased in combination with any concentration of 2,4-D. The cultured nodal segments achieved the best results on the development and proliferation of callus formation compared to other explants (seeds and leaf disks, respectively). The present results agreed with the findings of Al-Ani *et al.*[17].

The results showed the importance of addition of both the auxins represented by 2,4-D and cytokinins represented by Kin to obtain callus formation for different types of explants. Furthermore, the response of auxins started at the concentration of 0.5 followed by 0.1 and 2.0 mg L^{-1}, respectively.

The highest response of explant for callus formation was achieved by nodal segments followed by the leaves and seeds, respectively as indicated by the results in Table 4. This may be attributed to that activity and vitality of nodal segments, cells and tissues exceed those of leaves and seeds. The results also showed high rate of division, growth and development of cells and tissue of nodal segments than all the other cultured explants[8,17].

The lowest *in vitro* rate of callus formation was recorded using seeds. This lowest rate may be due to the loss of seed vitality and their low germination rate compared to fresh jojoba seeds which maintained their vitality and activity for a period of 5 years if provided with right and safe storage. This is generally preferred to use fresh seeds in tissue culture to achieve best germination and callus formation rates *in vitro* as reported by Roussos *et al.*[5].

The study results also indicated that nodal segments responded at the concentration of 0.5 mg L^{-1} 2,4-D, whereas, the response of leaves and seeds was observed at the

concentration of 1.0 followed by 2.0 mg L^{-1}, 2,4-D, respectively. Can Conning to cytokinins, the best results for all explants were achieved at concentration of 2.0 mg L^{-1} Kin.

Generally, the explant should have high ability to mitosis. Timing of cutting is very important factor for successful tissue culture[17]. In addition to the sterilization process, factors such as the physiological condition of the explant, its age, its characterization and development extent greatly affect the morphogenesis of the explants. This is demonstrated by the better results achieved by fresh leaves than the old ones or by stored explants like seeds which limit callus formation tendency[18].

Fresh leaves and nodal segments were obtained from *in vitro* grown plantlets have better ability for morphogenesis and regeneration than that taken from traditionally grown plants in the field or in the green houses[8].

Whereas in this study, the seeds were obtained from mature trees that are generally preferred to be cultured on a nutrient medium containing high level of cytokinins to increase their cell division rate according to the findings of George *et al*.[19].

It is generally well known that the germination rate of jojoba seeds is about 95%, because the seeds are characterized by their ability of keeping their bioactivity for about 10 years under proper storage conditions without loosing their vitality or their characteristic oil content. However, it is advisable to use fresh seeds than to use stored ones[5].

Normally, the yield of seeds from trees is about 250 g/tree after the 3rd year grown in the field which gradually increases till achieving the maximum productivity of 750 g/tree after the 8th year. This results in low production of oils and the natural active constituents of numerous uses and benefits thus leading to raising their prices and making it difficult to be obtained[20].

Plant growth regulators (auxins and cytokinins) are non-nutritious organic chemical compounds, naturally present in small quantities in plant tissues, which either promote, inhibit or modify physiological processes of the plant. They transfer from formation sites to sites of action within the plant[21]. The 2,4-D is one of the most commonly used auxins for callus development and embryos formation. Occasionally, it can be used as a replacement for plant auxins and cytokinins as it perfectly carry out their functions[22].

While, Kin is a synthetic physiologically active compound like cytokinins so it is a growth regulator rather than a phytohormone. Subsequently, 2,4-D and Kin promate elongation and cell divisions of the explant while the auxins enhance the growth of tissues and accelerate their cell division rates. While, cytokinins increase number of cells and their length during mitosis, thus it is very important to keep mutual balance between different growth regulators used in the experiment to achieve the maximum callus formation rate from *in vitro* cultured jojoba explants recorded at the optimum concentration of 2,4-D and Kin (2.00 mg L^{-1}). The findings of this study agree with the findings of Kumar *et al*.[23] who reported that higher percentage of callus proliferation (97.3%) was obtained from leaf explants, taken from field grown mature plant, when cultured on MS medium supplemented with 2,4-D (2.0 mg L^{-1})+BAP (0.5 mg L^{-1})+CH (100 mg L^{-1}) within 20-22 d of inoculation. The only difference with the present study is that different hormone such as cytokinin was used except 2, 4-D. However, the effect of 2,4-D, Kin and their concentrations on callus production from nodal segments of jojoba is presented in Fig. 1 showing various stages of *in vitro* callus development..

CONCLUSION

This study investigated the optimum protocol for callus induction from variant jojoba explants cultured on different concentrations of two types of hormones (2, 4-D and Kin). Thus, the resultant callus can be used for future research studies that seemed difficult for commercial cultivation of jojoba in the field such as the biochemical and physiological studies, in additions to that, for active constituents and secondary products formation. The use of hormones showed lot of potential rather than waiting for traditionally cultivated jojoba to reach maturity and fruiting stage in the field.

SIGNIFICANCE STATEMENT

This study discovered that the use of two types of hormones (2, 4-D and Kin) is the best option for callus induction from different jojoba explants cultured on different concentrations and combinations of the hormones. The best concentration of 2,4-dichlorophenoxy acetic acid as an auxin (2,4-D) and Kinetin (Kin) as a cytokinin was 2.00 for callus induction. The major contribution of this study is that 2,4-dichlorophenoxy acetic acid as an auxin (2,4-D) was used in combination with Kinetin (Kin) as a cytokinin in different concentrations using nodal segments for callus formation than leaf disks. While in other similar studies, the 2,4-D (auxin) was used with other different hormones. Therefore, this study introduced a new hormone in the form of kinetin for cal[us induction in different crop plants of importance. It is, therefore, recommended that in order to achieve viable callus formation, the use of the above mentioned hormones

should be encouraged among the progressive farmers for jojoba cultivation to obtain active constituents and for secondary products formation.

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