

Research Article

In vitro callus induction and plantlet regeneration of sesame (*Sesamum Indicum* L.)

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Citation

Muhammad Asad, Nadeem Ahmed, Amir Sohail, Jan Sher, Tanveer Burni, Fazal Hadi, Zarghoona Yousafzai and Salah ud Din. *In vitro* callus induction and plantlet regeneration of sesame (*Sesamum Indicum* L.). Pure and Applied Biology. Vol. 8, Issue 2, pp1307-1313. <http://dx.doi.org/10.19045/bspab.2019.80073>

Received: 18/01/2019

Revised: 08/04/2019

Accepted: 11/04/2019

Online First: 18/04/2019

Abstract

An *in vitro* propagation protocol was developed for Sesame (*Sesamum indicum* L. cv.SVPR - 1). In the present study the most important aspects of callus induction (CI) *in vitro* and regeneration of sesame seedlings (*Sesamum indicum* L.) were investigated. The explants were grown in basal medium Murashige and Skoog (MS) supplemented with different concentrations ratios of auxins and cytokines for the *in vitro* CI and the regeneration of plantlet. The callus commencement and induction was distinguished within 8 and 15 days after inoculation. Among the all combinations, 2, 4-dichlorophenoxyacetic acid (2, 4-D) and BAP with their respective concentrations of 2.0 and 0.5 ppm were more effective for IC frequencies in all explants. The maximum number and maximum length of shoots (11.8 ± 0.7 , 9.2 ± 0.6 cm), the maximum number and maximum length of roots (6.2 ± 0.7 , 6.6 ± 0.8 cm) were well demonstrated for MS with 2.0 ppm Kinetin (Kin) and 2 ppm indole-3-butyric acid in the explant of callus derived from the root. Seedlings with 3 to 5 shoots were transferred to pots of mixtures with equal amount of sand, soil and perlite for acclimation of field conditions and further multiplication.

Keywords: Callus induction; *In vitro*; Mass propagation; Sesame (*Sesamum indicum* L.)

Introduction

Sesamum indicum L. belongs to Pedaliaceae family and is known as an old oilseed crop and is also called the Queen of oilseeds. Sesame consists of 40 species which are grown in tropical and temperate zones [1]. They are geographically distributed in Madagascar, Tropical Australia, Tropical Africa, Pakistan, India and Afghanistan.

Almost an area of 8 million hectares is used for the growth of Sesame worldwide with a net yield of 3.3 million tons [2].

Sesame seeds are an important source of edible oil and are also used as spice. Sesame seeds by expression give a faxed oil consisting essentially of glycerides of oleic acid and linoleic acid with preparation of stearin, palmitin and myristin, solid fatty

acids are present at approximately 12-14% of 70% liquid fatty acids. It also contains potent antioxidants, lignins (sesamins), sesamol and phytosterol, which provide a high degree of resistance to oxidative rancidity [3]. Sesame oil is used in the Ayurvedic drug system [4].

The productivity of *Sesum indicum* L. is relatively low compared to other oil crops because the sesame crop is restricted by a poor soil [5, 6]. The present investigation is carried out to develop protocols for the induction of callus and the regeneration of seedlings from explants. Current techniques along with genetic engineering can be used for the improvement of this species. In the present experiment, explants bred in vitro were tested for so-called induction, their greatest growth and their efficiency for direct regeneration. The effects of different aid in the callus, the initiation of different explants in the MS medium and the reproducible protocol have also been given for the regeneration of plants from hypocotyl explants of *Sesamum indicum* L.

Materials and methods

Preparation of explants

Seeds of *Sesamum Indicum* L. cv. SVPR-1 for experimental work were obtained from the University of Agriculture in Peshawar. The sesame seeds were washed for 15 minutes with a tap under running water and were soaked in a 2% solution of Teepol soap for five minutes. The soaked seeds were then rinsed with distilled water repeatedly. The (seeds) of the explants were washed with 5% sodium hypochlorite solution for 5 minutes. It is then washed with 0.01% mercury chloride solution for 2 minutes. Wash 3 times with sterile distilled water. Explants of surface sterilization have finished. Subsequently, the explants were inoculated into test tubes (2.5 x 15 cm) containing nonabsorbent cotton. The cultures were allowed to retain in a dark state for duration of 48h at temperature $25\pm 2^{\circ}$ C and were then

exposed to a light and dark photoperiod for 16h and 8h respectively. The segments cut from the nodal, shoot tip and hypocotyl sections of the 8-day aseptic seedlings were used as explants.

Preparation of media

MS basal medium supplemented with 3% (w / v) sucrose and 0.8% (w / v) agar was used for subsequent experiments. The pH was adjusted to 5.6 by the addition of HCl or NaOH as required. Agar was added to the boiling medium at a rate of 8 g / l (0.8%) slowly and gradually with constant stirring to prevent clumping. After the addition of the agar medium it was placed in culture tubes. After preparation of the medium, it was sterilized in an autoclave at 121° C and under a pressure of 15 pounds for 15 minutes. The autoclave killed all microorganisms that were present from the beginning. Segments of hypocotyl, bud point and nodal explants were inoculated into the culture medium in 150 x 25 mm test tubes containing 10 ml of medium and covered tightly with non-absorbent cotton. Cultures containing explants were allowed to incubate at $25 \pm 2^{\circ}$ C for 16/8 light and dark photoperiod in $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ cold white fluorescent tubes (Pakistan). Sub-culturing was performed at intervals of 20 days or more at the appropriate stages.

In vitro callus induction

Leaf segments of 7-day-old seedlings cultured in vitro were used as explants and placed in a callus initiation medium containing MS salts [7], vitamins B5 [8], supplemented with different concentrations of 2,4-D, NAA and IAA (0.5-3.0 ppm) alone or in combination with BAP (0.2-2.0 ppm) for the induction of callus.

In vitro shoot bud proliferation from callus culture

In vitro callus obtained from the hypocotyl explant, they were transferred to the shoot bud induction medium. The medium for the proliferation of shoots containing the combined effect of a low amount of NAA

(0.02-0.10 ppm) and a high amount of cytokinins (Kin and BAP at 0.5-2.5 ppm) was tested for the proliferation of callus shoots.

Shoots multiplication and elongation

To obtain multiple recordings, three important steps were taken. In the first step, the individual treatment with different cytokinins (BAP and Kin) was evaluated. In the second step, at the appropriate concentration level of cytokinin for multiplication, various auxins (IBA, NAA and IAA) were evaluated at different concentrations. In the final phase, a new approach was designed for the high frequency recovery of multiple buds from leaf explants using different polyamines, amino acids, carbon sources and media supplements. The present experiment, the elongation of the induced shoots, was achieved in the same means of induction of multiple taps rather than using any other means of special extension.

Induction of root, hardening and acclimatization

The well-developed mature elongated shoots were transferred to the root induction in middle medium medium salt medium, Ganborge vitamins medium (B5) and various auxin concentrations (IAA, IBA and NAA at 0.01 - 0.05ppm). When the roots were regenerated, the plants were hardened in paper cups with a 1: 1: 1 mixture of sterile soil, sand and vermiculate. The plants were kept in an environmental growth chamber for good acclimatization and were transported successfully to field.

Statistical analysis

For statistical analysis the mean values with standard errors were used for a parametric moods median test [9]. Experimental data were analyzed for variance by Duncan's multiple range test (DMRT) using the SAS programmer (SAS Institute, Cary, N.C.).

Results and discussion

***In vitro* Callus induction**

Hypocotyl explants of the *in vitro* elevated seedlings were used for callogenesis. The small cut segments of Hypocotyl were used as explants. Different auxins were used as medium for callus induction to culture the explants segments. Among the all auxins 2,4-D with BAP was reported to be more effective which demonstrated the highest callus initiation of 85.8 % (Table 1, Figure 1J & N). Three types of auxins were used in different concentrations that produced different types of callus. Then callus was a subculture on medium with a large amount of auxin and a low amount of cytokinins. Among other types, green compact callus was best suited for regeneration than other types of callus. Auxin together with cytokinin improved the frequency of callusing. The combination of high concentrations of NAA and BAP induce green compact nature of callus, but not so in the other two combinations (Table 1). In present study 2, 4-D with BAP in the middle concentration range demonstrated the highest percentage of organic callus induction (85%, Table 1). Among other things, the type of auxin 2, 4-D resulted in high callus induction in (*Linum usitatissimum*) L. [10], in *Helictere sisora* L. [11] in sunflower [12] and in *Dolichos lablab* [13]. The high concentration of auxin (2,4-D) is used in combination with a low concentration of cytokinin (BAP) was used for the initiation of organogenic callus in *Holostemmaada-codin* [14] and *Malus domestica* [15].

Shoot bud induction from callus

For the shoot bud induction well-developed organogenic calluses were transferred on to shoot bud induction medium. Bud induction medium containing different concentrations of cytokinins (BAP, TDZ and Kin) along with lower concentrations of auxin NAA were used (Table 2). Adventitious buds regenerated from the organogenic calli occurred after 30 days and regeneration of multiple shoots from the adventitious buds

occurred in the next two subculture multi number of shoots was produced in organogenic callus derived from the hypocotyl explant (28.4 shoots/callus) (Table 2; Figure 1L). Regeneration from callus is reported from young leaves in *Linum* [10, 16] leaf segment in castor [17] and hypocotyl segments of in sesame [18].

Effect of cytokinin (BAP, TDZ and Kin) along with NAA

In the current study, a different concentration of BAP, TDZ and Kin with NAA was used for multiple shoot induction (Table 3). Maximum percentage of response (90.8%) occurred at BAP (2.0 ppm) with NAA (0.04 ppm). Multiple shoots exhibited the highest regeneration frequency in BAP with NAA combination followed by TDZ with NAA combination. Several cytokinins in combination with auxin are known to exhibit high levels of shoot formation in *Linum* [19, 20] and in sesame [21, 22].

Elongation, rooting and hardening

In vitro regenerated multiple shoots of hypocotyl-derived callus were transferred to MS medium enriched with GA3. The maximal percentage response was recorded in 0.6 ppm GA3 supplemented medium (Figure 1m & n). Prolonged shoots were transferred to root media enriched with various auxins (IBA, NAA and IAA), of the different concentration at which NAA (0.03ppm) produced the maximum number of roots (Table 3). Rooted plants were transferred to paper cups and kept in an environmental plant growth chamber for hardening. When the curing process was complete, the plants were transferred to earthen pots and then transferred to the field state. In vitro regenerated plants showed 77% survival rate under greenhouse effect. Therefore, the results obtained here could be useful for mass distribution of this plant. Such results were observed by [10, 23-25].

Table 1. Effect of different concentration of auxins along with cytokinin on Callus Induction from hypocotyl

Plant Growth Regulators (mg/l)	% of callus Induction	Nature and type of callus
NAA + BAP		
1.0 + 0.2	64.5 + 2.54	Yellowish Green Friable
2.0 + 0.4	70.4 + 3.12	Yellowish Friable
3.0 + 0.6	72.1 + 3.01	Green Friable
4.0 + 0.8	56.9 + 1.96	Green Compact
5.0 + 1.0	62.5 + 1.65	Green Compact;
IAA + BAP		
1.0 + 0.2	51.6 + 3.4	Yellowish Brown Compact
2.0 + 0.4	58.5 + 2.78	Green Friable
3.0 + 0.6	75.2 + 2.57	Green Friable
4.0 + 0.8	65.2 + 1.59	Yellowish Friable
5.0 + 1.0	50.5 + 1.85	Green Friable
2, 4 D + BAP		
1.0 + 0.2	56.6 + 1.65	Yellowish Friable
2.0 + 0.4	74.6 + 2.21	Green Compact
3.0 + 0.6	85.8 + 3.61	Green Friable
4.0 + 0.8	63.4 + 2.95	Green Friable
5.0 + 1.0	65.1 + 1.47	Yellowish Brown Compact

20-explants tested, values are means + SE of replication of 5 replication of 5 repeated experiments

Table 2. Effect of different concentration of cytokinin along with NAA on shoot bud Induction from callus

Plant Growth Regulators (mg/l)	% of shoot bud response	Mean number of shoots	Mean number of nodes / shoot
TDZ + NAA			
0.5 + 0.4	49.5 + 0.56	2.5 + 0.10	14.6 + 0.2
1.0 + 0.4	53.7 + 0.58	3.9 + 0.12	13.8 + 0.22
1.5 + 0.4	55.8 + 0.49	3.4 + 0.12	10.5 + 0.19
2.0 + 0.4	84.3 + 0.50	3.6 + 0.15	21.9 + 0.17
2.5 + 0.4	70.1 + 0.45	1.9 + 0.09	18.4 + 0.14
BAP + NAA			
0.5 + 0.4	60.3 + 0.59	3.5 + 0.11	20.0 + 0.25
1.0 + 0.4	75.8 + 0.65	3.9 + 0.14	22.4 + 0.22
1.5 + 0.4	88.4 + 0.67	4.1 + 0.13	21.9 + 0.19
2.0 + 0.4	90.8 + 0.83	4.5 + 0.16	24.8 + 0.29
2.5 + 0.4	80.7 + 0.72	4.0 + 0.15	19.5 + 0.18
Kin + NAA			
0.5 + 0.4	51.2 + 0.28	2.8 + 0.11	10.9 + 0.09
1.0 + 0.4	55.6 + 0.29	4.0 + 0.12	11.5 + 0.10
1.5 + 0.4	61.2 + 0.33	3.9 + 0.10	12.4 + 0.07
2.0 + 0.4	71.2 + 0.39	3.5 + 0.14	13.4 + 0.15
2.5 + 0.4	49.6 + 0.25	2.9 + 0.07	15.7 + 0.09

20-explants tested, values are means + SE of replication of 5 replication of 5 repeated experiments

Table 3. Effect of different concentration of auxins on root induction on elongated shoots

Con. of auxins (mg/l)	N0. of roots (Mean + SE)	% of roots Response (cm) (Mean + SE)	Length of roots (cm) (Mean + SE)	Callus formation
IAA				
0.01	4.0 + 0.15	62.2 + 3.5	09.1 + 0.54	-
0.02	4.8 + 0.14	82.9 + 4.0	10.0 + 0.51	++
0.03	5.6 + 0.19	91.8 + 4.7	12.4 + 0.65	-
0.04	5.2 + 0.10	75.2 + 4.0	09.8 + 0.48	-
0.05	3.8 + 0.12	60.4 + 3.9	08.5 + 0.43	+
NAA				
0.01	2.9 + 0.05	48.9 + 2.8	05.2 + 0.36	-
0.02	2.7 + 0.04	50.1 + 3.5	07.5 + 0.31	-
0.03	3.0 + 0.15	55.6 + 3.8	10.2 + 0.46	+
0.04	3.8 + 0.11	48.9 + 4.1	08.5 + 0.39	++
0.05	3.4 + 0.13	47.2 + 2.9	06.3 + 0.34	-
IBA				
0.01	2.7 + 0.16	45.8 + 2.5	05.3 + 0.21	-
0.02	3.0 + 0.12	50.9 + 2.7	06.2 + 0.28	-
0.03	2.5 + 0.11	49.7 + 2.4	09.8 + 0.30	-
0.04	1.9 + 0.10	46.5 + 2.1	04.3 + 0.25	++
0.05	1.7 + 0.07	34.1 + 1.9	05.4 + 0.20	+

20-explants tested, values are means + SE of replication of 5 replication of 5 repeated experiments

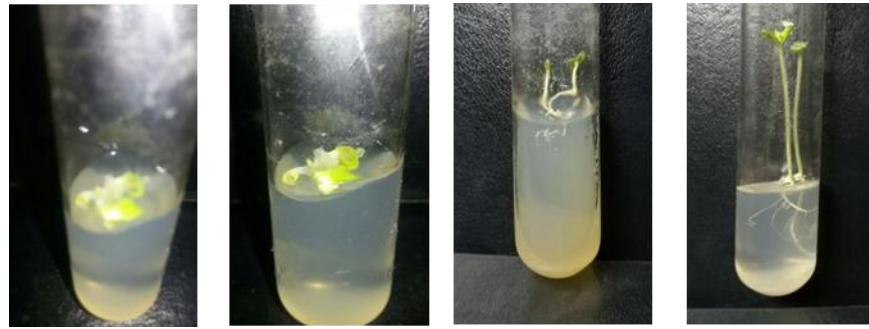


Figure 1. Different *In vitro* developmental stages of *Sesamum indicum* L

J- Callus induction; **L-** initiation of callus; **M** – initiation of shoot; **N-** elongation of shoot

Conclusion

In vitro culture of plants has gained importance during recent years because this technique can be used for the rapid multiplication and *ex-situ* conservation of some plants. The aim of the present work was to determine the culture conditions for micro propagation of this plant. The present study discovers nodal segment as effective explants which expressed maximum shoot induction in MS medium with different concentration of PGRs. Further this study showed that the high frequency organogenesis occurs by using BAP, IBA and IAA is possible. It could be used as a tool to protect the biodiversity/natural vegetation of *S.indicum* L.

Authors' contributions

Conceived and designed the experiments: N Ahmad, Performed the experiments: M Asad, Analyzed the data: A Sohail, R Ali & T Burni, Contributed reagents/ materials/ analysis tools: J Sher F Hadi, Z Yousafzai & S U Din, Wrote the paper: M Asad & A Sohail.

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