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Original Article

ESTABLISHMENT OF CALLUS AND SUSPENSION CULTURE IN *SESAMUM INDICUM* L

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ABSTRACT

Objective: Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops in the world. We intended to evaluate the effects of different explants, carbon sources and growth regulators on callus induction and cell suspension culture of two Iranian sesame cultivars (Yekta and Karaj 1).

Methods: The explants (hypocotyl and cotyledon) were cultured on Murashige and Skoog (MS) basal medium supplemented with 30 g/l sucrose, 8 g/l agar, different concentrations and combinations of NAA, 2, 4-D, BAP and Kin hormones. To produce cell suspension culture, callus was excised and transferred to liquid MS medium supplemented with different concentrations of carbon source (glucose and sucrose) and growth regulators of auxins and cytokinins. The cultures were maintained under both dark and light conditions at *different rotation speeds* of the *gyratory shaker*.

Results: Callus produced from hypocotyl explants grew faster during 7 to 30 days of culture and after that stabilized at a lower growth rate. Maximum growth rate of suspension culture was achieved from friable green callus in MS liquid medium supplemented with 3 mg/l NAA and 0.6 mg/l BAP using hypocotyl callus.

Conclusion: The optimized cell suspension culture can be used to elicit the production of secondary metabolites, scale-up mass production in bioreactors and gene transformation.

Keywords: Sesame, Callus induction, Cell suspension culture, Hypocotyl, Plant growth regulators.

INTRODUCTION

Sesamum indicum L. is commonly known as sesame and queen of oil seeds belongs to the family of Pedaliaceae. This family consists of 40 species that 7 species have been cultivated in both tropical and temperate climates since ancient times [9].

There are numerous varieties and ecotypes of sesame adapted to various ecological conditions. However, the cultivation of modern varieties is limited due to insufficient genetic information and then many farmers continue to grow local sesame [14].

Beneficial health effects of sesame on human health have recently renewed the interest in this ancient crop [8]. Sesame has long been regarded in the orient as a health food for energy increasing and aging prevention. Sesame seeds contain rich nutrients such as oils, proteins, sugars, fiber, minerals and vitamins. Sesame oil, which occupies 50 to 60% of the seed mass, includes a large amount of unsaturated fatty acids [4]. In sesame seed, α-tocopherol and sesamol are known to be antioxidative compounds. Several lignin and phenolic compounds have potent antioxidative properties. It is considered that these antioxidative compounds play an important role in preventing oxidative damage in sesame seeds [15].

Among the explants, were used in sesame tissue culture, cotyledon and hypocotyl have proven to be an excellent source of explants for callus induction and subsequent regeneration [2, 9, 11]. Based on Were et al (2006) sesame shoots were only obtained from cotyledons whereas both cotyledons and hypocotyls produced roots. Modified MS medium with N6 macronutrients resulted in twice the shoot regeneration frequency obtained with ½MS macronutrients in the presence of thidiazuron (TDZ) [16]. Sesame hypocotyl was defined better than cotyledon for shoot formation in an Iranian sesame cultivar (Naztaksakhe). In addition, the best response to shoot induction was observed in medium supplemented with NAA and BAP hormones [6]. *Callus formation* of sesame was induced from hypocotyl and cotyledon explants in MS medium supplemented with NAA and BAP. High concentration of BAP in combination with casein hydrolysate increased multiple shoots formation while low concentration of BAP induced the formation of a single shoot [7]. Since there was no detailed protocol for obtaining sesame cell suspension culture, the aim of present study was to achieve and report high concentration of sesame suspension cells for the first time.

MATERIALS AND METHODS

Plant material and callus induction

Seeds of two Iranian sesame genotypes Yekta and Karaj1 were obtained from Seed and Plant Improvement Research Institute in Karaj, Iran. Yekta and Karaj1 are *high-yielding varieties* that cultivated more in Iran. Seeds were surface sterilized with 70% ethanol for 2 min followed by 3 times rinses by autoclaved distilled water. Then seeds disinfected with 2% sodium hypochlorite for 30 min and rinsed again three times with sterile distilled water. After surface sterilization, seeds were cultured on solid MS with 30 g/l sucrose, 8 g/l agar and incubated for 7 days in a growth chamber at 25±2 °C with a 16 h photoperiod. Explants were detached from 5-7 days plantlets and cultured on MS basal medium supplemented with 30 g/l sucrose, 8 g/l agar and different levels of plant growth regulators (2, 4-D, NAA, Kin and BAP). Hypocotyl explants placed vertically and horizontally in the agar. Cultures were maintained with sub-culturing every 2 weeks. This experiment was done in the factorial experiment based on completely randomized design in 3 replications. The statistical analysis of ANOVA was performed using the SPSS software and the *comparisons* among means were *calculated* using *Duncan's* multiple range test at 0.1 % probability.

Cell suspension, initiation and maintenance

The green friable callus was used for an establishment of homogenous suspension culture. Maximum growth rate of suspension culture was achieved in MS liquid medium supplemented with 3 mg/l NAA and 0.6 mg/l BAP using hypocotyl callus. One gram of friable green callus was excised in petri dish containing No.1 filter paper. Calli were carefully transferred with sterilized forceps to 250 ml narrow-necked Erlenmeyer flasks containing 100 ml liquid MS medium and different levels of growth regulators, sucrose and glucose.

The flasks were covered with aluminum foil and parafilm. The pH of medium was adjusted to 5.8 using 1N KOH before autoclaving the medium at 120 °C for 20 minutes. The flasks rotated at 110, 120 and 130 rpm on a gyratory shaker and incubated at 25 and 27 °C under light and dark conditions. For growth analysis, samples were taken

in the first day after sub-culturing at 48h intervals until 30th day of sub-culturing.

Cultures were maintained routinely by transferring 10 ml of culture into 250 ml narrow-necked Erlenmeyer flasks containing 100 ml fresh MS medium as mentioned above every 2 weeks.

Fresh and dry weight

For this purpose, contents of the flasks were aseptically passed through pre-weighed Whatman No.1 filter paper to collect the cells and measure fresh weight. Collected cells were dried for 6 h at 65 °C and their dry weight was recorded every two days during a month.

Fig. 1: Callus induction in hypocotyl (left) and cotyledon (right) explants

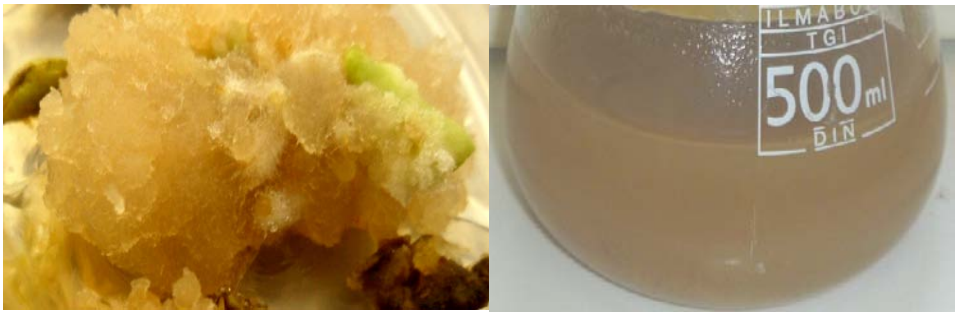

Fig. 2: Condensed cell suspension culture of Karaj1 (right) and hypocotyl callus was used (left)

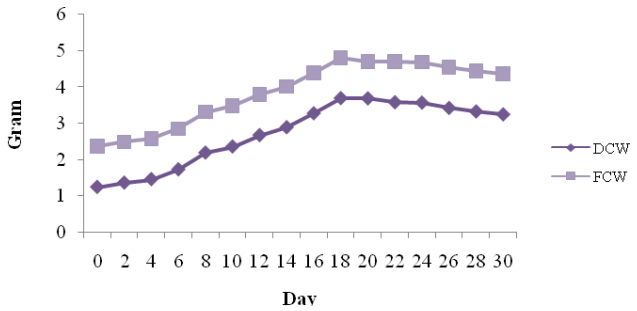

Fig. 3: Growth pattern of sesame cells and early stationary phase (D18). Cells were sub-cultured and cell growth monitored by determination of dry (DCW) and fresh (FCW) cell weight

Table 1: Variance analysis of Kin, NAA and explants effects on callus induction in *Sesamum indicum*

F	MS	Df	S. O. V
19.179**	7.841	1	Cultvar
0.568ns	0.232	3	Kin
86.482**	35.359	3	NAA
11.049**	4.518	2	Explant
24.412**	9.981	3	NAA × Cultivar
26.3**	10.573	3	Kin × Cultivar
8.992**	3.676	2	Explant × Cultivar
9.277**	3.793	9	Kin × NAA
12.694**	5.19	6	Explant × Kin
2.446*	1	6	Explant ×NAA
12.505**	5.113	9	NAA × Kin × Cultivar
5.281**	2.159	6	Explant × Kin × Cultivar
8.97**	3.6F68	18	Explant ×Kin × NAA
13.016**	5.322	6	Explant × NAA × Cultivar
7.752**	1.943	18	Explant × NAA × Kin × Cultivar
	0.409	192	Error
		288	Total

Table 2: Variance analysis of the effects of Kin, 2, 4-D and explants on callus induction in *Sesamum indicum*

F	MS	Df	S. O. V
79.261**	36.417	1	Cultivar
19.087**	8.77	3	Kin
93.173**	42.808	3	2, 4-D
257.144**	118.145	2	Explant
18.119**	8.325	3	Kin× Cultivar
14.446**	6.637	3	2, 4-D×Cultivar
1.682ns	0.773	2	Explant ×Cultivar
6.517**	2.994	9	Kin × 2, 4-D
2.592*	1.191	6	Kin ×Cultivar
29.915**	13.745	6	Explant ×2, 4-D
5.219**	2.398	9	2, 4-D×Kin× Cultivar
7.109**	3.266	6	Explant ×Kin×Cultivar
3.432**	1.577	18	Explant× Kin × 2, 4-D
1.604ns	0.737	6	Explant ×2, 4-D× Cultivar
4.079**.	1.874	18	Explant ×2, 4-D×Kin× Cultivar
		88.215	Error
		1300.632	Total

Table 3: The best concentrations of hormones for callus induction in *Sesamum indicum*±

Source		Concentration of hormones (mg/l)			
		BAP+NAA	Kin+NAA	Kin+2, 4-D	BAP+2, 4-D
Karaj1	hypocotyls laid horizontally	0.6+3.0	1.0+0.5	1.5+1.0	1.5+1.0
	hypocotyls laid vertically	1.0+3.0	0.0+3.0	1.5+1.0	1.5+2.0
	cotyledons	0.6+3.0	1.0+0.5	-	0.0+3.0
Yekta	hypocotyls laid horizontally	0.6+3.0	0.0+2.0	2.0+2.0	0.6+1.0
	hypocotyls laid vertically	1.0+2.0	2.0+0.5	2.0+1.0	1.5+2.0
	cotyledons	0.6+3.0	0.0+2.0	1.0+2.0	0.0+2.0

RESULTS AND DISCUSSION

Callus culture induction

Establishment of a reproducible tissue culture system is useful to obtain sufficient amounts of material for basic molecular analyses and for Agrobacterium-mediated *transformation* for foreign gene introduction into sesame. The efficiency in genetic transformation depends on the establishment of a robust and reproducible plant tissue culture system. Also cell suspension culture provides a useful material for further regulation of secondary *metabolites* biosynthesis and for enhanced production of valuable metabolites in sesame.

In the present study different explants were cultured on MS medium supplemented with various auxin and cytokinin concentrations and different combinations of them to assess the morphogenetic potential of the explants. Among Iranian sesame varieties Karaj1 and Yekta are the best due to seed yield and protein and *oil* contents [3]. Based on our data callus induction can be obtained from both cotyledon and hypocotyl explants because both explants showed an excellent response to callus induction media. However, hypocotyls were found to be superior over cotyledons (fig 1). Callus induction occurred within 15-20 days on MS medium containing different concentrations of auxins (2, 4-D and NAA) and cytokinins (Kin and BAP). Variance analysis results showed that callus induction was significantly affected by the type of explants, cultivars, different concentrations of NAA and reciprocal effects but different concentrations of Kin did not cause significant effect (table 1). Most of the induced calli were friable and suitable for the suspension culture but the best results of callogenesis (the highest fresh weight) were obtained using hypocotyls laid horizontally. The best media containing various *hormonal* treatments *tested* for *callus induction* in this study *was reported in* table 3.

Establishment of cell suspension culture

Cell suspension cultures were established from 4-week-old callus (hypocotyl and cotyledon derived) as inoculums. One gram of actively growing friable lush green color callus was excised and transferred to liquid MS medium supplemented with different concentrations of carbon source (glucose and sucrose) and growth regulators of auxins and cytokinins. The cultures were maintained under both dark and light conditions. In this study glucose and sucrose with concentrations of 20, 30 and 50 g/l were used. Our results showed that the liquid medium containing 30 g/l sucrose, dark condition and agitation speed of 130 rpm was more efficient than other conditions. In addition, NAA plus BAP combination yielded better results when compared to the other media including 2, 4-D and Kin, 2, 4-D and BAP, NAA and Kin or NAA and BAP. The good suspension culture was obtained by hepocotyl and cotyledon of both cultivars Yekta and Karaj1, but hypocotyl cleaved suspension cultures was grown faster and resulted in greater biomass accumulation in a medium containing 0.6 mg/l BAP and 3 mg/l NAA during 30 days (fig 2). In supplementation of *media* with *other hormones* much lower rates of cell division occurred.

After 3 weeks, a finely dispersed homogeneous and regenerable cell suspension culture obtained. The initial growth rate of the cells was slow during first 3 days (lag phase) but after around one week cells density increased significantly and large amount of the cells accumulated in a period of 18 days (log phase). The growth of sesame cell cultures followed standard growth kinetics in which the number of cells increased exponentially due to high rate of cell division followed by slow rate of cell increase during stationary phase. Cells number reached a peak at day 18 and then declined slowly (fig 3). Subculture was performed every 2 weeks. For maintenance of cell suspension culture it is necessary to subculture it regularly because the culture tends to form cell clusters in which cells aggregate and grow in clumps. As mentioned before *large amounts* of the cells were obtained in dark condition and agitation speed of 130 rpm. Interestingly, when the flasks rotated at 110 and 120 rpm *cells* showed *root formation.*

Contrast analysis indicated that there was a statistically significant increase in cell proliferation in treatments containing both auxins and cytokinins. These hormones are necessary for cell division at the G1-S and G2-M transitions in cultured plant cells and in planta as well. Auxin stimulates the acidification of the cell wall resulting in increasing extensibility, and also induces the transcription of specific mRNAs that code for proteins involved in the formation and operation of the miotic spindle. Cytokinins regulate cell division by induction of specific cell cycle genes like *CDK* and *CYCD* genes [10, 12, 13].

Hossain et al. (2007) reported that MS medium supplemented with 2 mg/L NAA, 0.05 mg/L BAP, and MS medium containing 2 mg/L 2, 4-D and 0.05 mg/L BAP showed the best growth of suspension culture of *Solanum melongera* [5]. Daud and Keng (2006) also found that MS medium containing 2 mg/L NAA was the most efficient medium in suspension culture of *Cyperus armaticus* [1].

Variance analysis results showed that callus induction was significantly affected by the type of explant, cultivar and different concentrations of Kin and 2, 4-D as well. Means comparison of interaction between cultivar, 2, 4-D and explant and interaction between cultivar and explant was not significant (table 2).

CONCLUSION

Suspension cultures, in which plant cells grow uniformly in liquid medium, provide an ideal tool for functional genomics approaches particularly for gaining the information of metabolite production pathway. In the present study, an establishment of fast growing callus and cell suspension culture of *Sesamum indicum* and their long term maintenance for a period of more than one year without apparent changes in the growth rate was achieved. MS medium supplemented with 3 mg/l NAA and 0.6 mg/l BAP showed the maximum cell growth and proliferation and cell culture at 18 days after sub-culture represented early stationary phase. Therefore, in order to elicit the production of secondary metabolites, scale-up mass production in bioreactors and gene transformation these results can be used.

CONFLICT OF INTERESTS

Declared None

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