Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 3, p. 275-278, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Callus Induction on Sesame (*Sesamum indicum* L.) by 1-Naphthalene acetic acid and 2,4-Dichlorophenoxy acetic acid Hormones

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Article published on March 22, 2014

Key words: Sesame (*Sesamum indicum* L.), Callus, 2,4-D, NAA.

Abstract

Sesamum indicum L. belongs to family pedaliaceae that these family, including 60 species. Sesame is the most important oil-seed crop of semi-arid tropics and a source of high quality cooking oil and protein. In recent years, plant tissue culture techniques has become a powerful tool for the propagation of many plant species. In this research weight and diameter of callus were analyzed to determination of appropriate medium culture. This study were performed on fall of 2013 in complete randomized design with three replicated. The MS medium culture was contain NAA and 2,4-D hormones. ANOVA statistical analysis showed significant difference at 1% level. Highest callus diameter belong to medium contain 0.2 mg/l 2,4-D and 0.12 mg/l NAA, also maximum callus weight belong to 0.2 mg/l 2,4-D and 0.16 mg/l NAA. Therefore, hormones amount which used in this research can induced callus in sesame.

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Introduction

Sesamum indicum L. belongs to family pedaliaceae and is the most important oil-seed crop of semi-arid tropics and a source of high quality cooking oil and protein (Jeyamary and Jayabalan, 1997). It is grown in India, China, Korea, Russia, Turkey, Mexico, South America and several countries of Africa. It is cultivated on a total area of over 7.7 million hectares with total production of 3.3 million tons (FAOSTAT, 2008). The whole seeds are edible and are used in baking of breads and hamburger buns and for extraction of oil for cooking. The seed contains 50-60% oil, which has excellent stability due to the presence of natural antioxidants such as sesamolin, sesamin and sesamol and has medicinal and pharmaceutical value (Brar and Ahuja, 1979). The first report of tissue culture in sesame was reported by (Lee *et al*., 1985 and George *et al*., 1987). Since then number of reports have appeared and micro propagation using different explants (Gangopadhyay *et al*., 1998; Seo *et al*., 2007 and Chattopadhyaya *et al*., 2010). Among the explants used, cotyledon and /or hypocotyl explants gained utmost attention and have proved excellent source of explant for callus induction and subsequent regeneration (George *et al*., 1987; Jeyamary and Jayabalan, 1997; Rajender Rao et. al., 2002; and Seo et. al., 2007), however to date; only two reports have focused on somatic embryogenesis in S. *indicum* initiated from callus derived from hypocotyl and cotyledonary explant (Jeyamary and jeyabalan, 1997 and Xu *et al*., 1997). However, only one report published on successful conversation of somatic embryogenesis from seeding explants of Sesamum indicum. Somatic embryogenesis is a unique in vitro morphological appearance found only in plant system (Namasivayam, 2007) and somatic embryos have also been reported in many plant species (Ammirato, 1983 and Zimmerman, 1993). Progress in techniques for plant cell, tissue and organ culture makes it possible to introduce genetic variability and get desirable traits (Evans *et al*., 1983). *Sesamum indicum* is an important crop of semi-arid tropics and a source of high-quality cooking oil and protein. Regeneration of sesame from callus cultures has been reported by several authors (George *et al*., 1987; Dhingra and Batra, 1979; Weiss, 1971). However, introgression of useful genes from wild species into cultigens via conventional breeding has not been successful due to post-fertilization barriers. The only option left for improvement of *S. indicum* is to transfer genes from other sources through genetic transformation techniques. Is the recalcitrant nature of sesame to in vitro regeneration (Baskaran and jayabalan, 2006). Tissue culture, an important area of biotechnology can be use to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. Micro propagation is one of the important contribution of Plant Tissue Culture to commercial plant propagation and has vast significance. Tissue culture has helped to develop new strain of food crops, cereals, vegetables flowers, oil seeds and plantation crops such as spices, coffee, tea and rubber. in the present study, we have chosen callus mediated shoot organogenesis as an alternative method to achieve a higher rate of shoot multiplication for crop improvement.

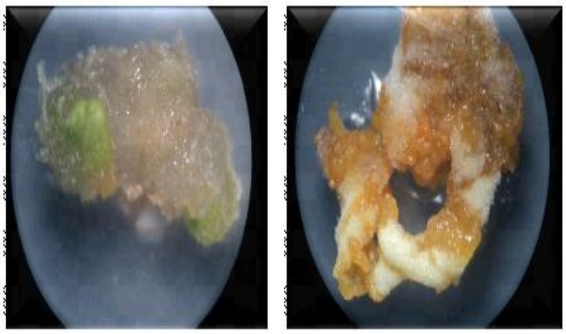
Material and Methods

Plant material and disinfection

The seeds were washed with running tap water for 20 minutes, followed by soaking in 3 % of teepol soap solution for 5 minutes then reported rinsing in distilled water. The seeds were disinfected with 70 % ethanol treatment for 45 seconds and washed with sterile distilled water for three times, followed by 0.1 % (w/v) aqueous mercuric chloride for 5 minutes and washed again three times with sterile distilled water. The disinfected seeds were germinated in 25 × 150 mm test tubes containing non-absorbent cotton. Initially the culture were maintained in dark condition for 48 h at 25 ± 2^{o}C and then under 16 h light and 8 h dark photoperiod condition with the light intensity of 3000 lux. Shoot tip and nodal segments were excised from seven-day-old aseptic seedlings and used as explants.

Culture Condition

MS basal medium (Murashige and Skoog, 1962) supplemented with 3 % (w/v) sucrose and 0.8 % (w/v) agar was used for subsequent experiments. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.8 with 1 N NaOH or 1 N HCL before galled with 0.8 % (w/v) agar (Himedia Ltd., Mumbia, india). The medium was dispensed into culture tubes (borosli, India) and autoclaved at 105 kPa and 121°C for 15 minutes. The shoot tipe and nodal explants were inoculated on the culture medium in test tubes 150 × 25 mm, containing 10 ml medium and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25 ± 2°C under 16/8 (light/dark) hours photoperiod of 30 µmol m^{-2} s^{1} of cool white fluorescent tubes (Philips, India). All subcultures were done at 20 or more day's intervals at appropriate stages.



1)2,4-D*NAA(0.4+0.12) 2) 2,4-D*NAA(0.2+0.012)

Fig. 1. callus induction of sesame by 2,4-D and NAA hormones.

Organogenic Callus Induction

shoot buds meristems segments from in vitro grown 12 days old seedlings were used as explants and placed on callus initiation medium which contained MS salts (Murashige and Skoog, 1962), B_5 vitamins (Gamborg *et al.*, 1968) supplemented with diverse concentration of 2,4-D (0.2 - 0.4 mg/l) and NAA (0.08 - 0.12 – 0.16 mg/l) alone or in combination 2.4-D and NAA for callus induction.

Results and Discussion

Callus culture induction

The shoot buds meristems were cut into small segments and used as explants. They were cultured on callus induction medium (MS) consisting of different auxins. Among the two auxins investigated 2,4-D with NAA were more effective than the other auxins with the highest percentage (60 %) of callus initiation (Table 1). The auxins in different concentration produced different types of callus. However, used 2,4-D with NAA at mid-level concentrations gave best percentage of organic callus induction (60 %, Table1) in the present study. Result of ANOVA statistical analysis according by Complete Random Design (CRD) with three replicates showed that the shoot buds meristems in MS medium with 2,4-D (0.2 mg/l) and NAA (0.12 mg/l) has produced high quality callus (Table 2). The results were obtained has many differences with Raja *et al* (2010) in amount of using 2,4-D hormone. Their result in compare with our research have significant in diameter and weight of callus. Therefore, we can introduce these hormone concentrations' for sesame callus induction.

Table 1. Data on effects of 2,4-D and NAA on callus induction and callus growth of shoot bud explants.

Plant Growth Regulators (mg/l)	Percentage of organogenic callus induction	Type and nature of callus
2,4 D		
0.2	0.48	Brown
0.4	0.60	Brown
NAA		
0.08	0	—
0.12	0	—
0.16	0	—
2,4 D+ NAA		
0.2+0.08	0.24	Light Brown
0.2+0.012	0.32	Brown
0.2+0.16	0.26	Brown and brittle
0.4+0.08	0.30	Brown
0.4+0.12	0.20	Dark Brown
0.4+0.16	0.18	Light Brown

Table 2. Sesame ANOVA statistical analysis.

Source	df	Diameter	Weight
2,4-D	2	874.80**	36.7366**
NAA	3	3.45	0.3719**
NAA×2,4-D	6	3.76	1.3407**
Error	24	4.08	0.1085

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