

**OPTIMIZATION OF MEDIA AND CONDITIONS FOR CALLUS INDUCTION FROM ANTHERS OF SESAME CULTIVAR MI 3**

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**Abstract :** Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), 3-indoleacetic acid (IAA) and 6-benzylaminepurine (BAP) were used to induce callus from anthers of sesame (*Sesamum indicum* L.) cultivar MI 3 as an initial step towards developing a protocol for the haploid production for use in the breeding. Anthers from flower buds 36-48 h before anthesis incubated at 8<sup>o</sup> C for 24 h in the dark gave the best results. Out of the media studied, MS<sub>5</sub> medium containing 10 mg/l of 2,4-D and 2 mg/l each of IAA and BAP gave the highest rate of callus induction (46%) within 2-3 weeks of plating. For sub-culturing, both MS<sub>5</sub> and MS<sub>4</sub> (5 mg/l of 3-indoleacetic acid and 3 mg/l of benzylaminepurine) media gave satisfactory results, with MS<sub>5</sub> recording 78% and MS<sub>4</sub> 60% callusing. Calli produced in these two media were soft and yellow-coloured. In MS<sub>5</sub> medium they exceeded 2 mm in diameter. These calli have been transferred to regeneration media for optimising conditions for haploid production.

**Key words:** Anther culture, Sesame, Gingelly, *Sesamum indicum* L., haploids, callus induction.

**INTRODUCTION**

Sesame (*Sesamum indicum* L.) is an important oilseed crop with a large number of land races and cultivars adapted to a wide range of climatic conditions.<sup>1-3</sup> It is economically the most important annual oilseed crop in Sri Lanka. For all practical purposes it is treated as a self-pollinated species although varying degrees of cross-pollination have been reported.<sup>1,3-7</sup> Doubled haploids (DH) offer an efficient and rapid method of improving self-pollinated crop species if suitable protocols for haploid production, diploidization and regeneration are available.<sup>8-10</sup> The DH method has already been used to improve a range of agronomically important characters in several crops such as rice,<sup>11,12</sup> barley,<sup>13,14</sup> wheat<sup>15,16</sup> and *Brassica* sp.<sup>17-19</sup> Modified Murashige and Skoog (MS) medium has been used successfully to induce callus from sesame anthers of cultivars of exotic origin.<sup>20,21</sup> However, under the same culture conditions significant differences among genotypes have been observed.<sup>10,15,22-25</sup> Therefore, efficient haploid production from locally adapted high yielding cultivars is a prerequisite for using this methodology in crop improvement.

Optimal stage of the explant and culture conditions for efficient callus induction and sub-culture of anthers of MI 3 variety, the only recommended white seeded sesame cultivar in Sri Lanka are reported here.

### MATERIALS AND METHODS

The cultivar MI 3 used in this study is the only recommended variety in Sri Lanka having white seeds. It has a non-branching stem, opposite leaf arrangement with three bicarpellate capsules per leaf axil. Flower buds in three stages of development were studied for callus formation from anthers. These were the small (48-60 h before anthesis), medium (36-48 h before anthesis) and large buds (24-36 h before anthesis). Flower buds were surface-sterilized with 70% ethanol for 5s and then rinsed in autoclaved distilled water prior to extracting anthers under aseptic conditions for plating. Callus formation from anthers treated at 8<sup>o</sup> C for 24 h and 48 h was compared with untreated ones which were directly plated without cold treatment.

Murashige and Skoog (MS) medium<sup>26</sup> has been the most effective one for callus induction in sesame.<sup>20,21</sup> Five MS based media with different levels of hormones were used in the present studies (Table 1). The pH value of media was adjusted to 5.8 ± 0.1 with 0.1 N NaOH or 0.1 N HCl. The effect of the presence or absence of light on the callus induction of plated anthers was also investigated. The effects of the total absence of light and a photoperiod of 12 h per day from fluorescent tubes at 2000 lux were compared. The plated anthers and subcultured calli were maintained at a temperature of 25 ± 1<sup>o</sup> C.

**Table 1: Hormone composition of MS media used in the experiments (mg/l)**

Hormones	Medium				
	MS <sub>1</sub>	MS <sub>2</sub>	MS <sub>3</sub>	MS <sub>4</sub>	MS <sub>5</sub>
2,4-D	10	15	20	-	10
IAA	-	-	-	5	2
BAP	1	1	-	3	2

### RESULTS

Flowers at the 'medium stage' having a length of 5-7 mm and 36-48 h prior to anthesis gave the highest rate of callus induction. The flower buds of this stage were therefore used in subsequent experiments. Out of a total of 1077 anthers which produced calli, 947 (88%) remained green until longitudinal splitting. Of the 6123 anthers which turned brown within a few days of plating, only 613 (10%) produced calli. Thus, 90% of the anthers which turned brown failed to produce callus.

In the preliminary studies with different temperatures of pretreatment of anthers, the highest number of calli (199 out of 1200 plated anthers) were produced when the anthers were incubated at 8<sup>0</sup> C for 24 h. Incubation at 8<sup>0</sup> C for 48 h resulted in the callus production from 154 out of 1200 anthers. Direct plating without a cold treatment resulted in only 71 anthers producing callus out of 1200 anthers. This experiment was conducted only with MS<sub>1</sub> medium and further experiments were carried out using anthers from flower buds of 'medium stage' of development incubated at 8<sup>0</sup> C for 24 h.

Among the media used MS<sub>1</sub>, MS<sub>2</sub>, MS<sub>4</sub> and MS<sub>5</sub> were capable of inducing callus from anthers of MI 3 variety (Table 2). All four media had one or two of auxins 2,4-dichlorophenoxyacetic acid (2,4-D) and 3-indoleacetic acid (IAA) and a cytokinin 6-benzylaminepurine (BAP), whereas MS<sub>3</sub> medium which failed to induce callus had only 2,4-D, but in greater concentration than the rest (Table 1). Callus induction in MS<sub>5</sub> medium occurred within 2 weeks of plating compared to 3-4 weeks in other media. A relatively greater proportion of anthers plated on this medium produced callus (Table 2). The first response of most anthers that gave callus was the longitudinal splitting within a week after plating. Continuous darkness was better than a photoperiod of 12 h per day on MS<sub>2</sub> and MS<sub>4</sub> media for callus formation (Table 2). Experiments on callus induction with MS<sub>5</sub> medium were therefore continued in the absence of light.

**Table 2: Callus induction capacity of media from anthers of medium size (5-7 mm) flower buds of sesame**

Medium	Treatment	Number of anthers producing callus*	Percentage of callus induction
MS <sub>1</sub>	Light	199	17
MS <sub>2</sub>	Light	80	7
	Dark	111	9
MS <sub>3</sub>	Light	0	0
	Dark	0	0
MS <sub>4</sub>	Light	60	5
	Dark	80	7
MS <sub>5</sub>	Dark	547	46

\* 1200 anthers were plated for each treatment.

Calli derived from anthers were cultured in four different MS based media viz. MS<sub>2</sub>, MS<sub>3</sub>, MS<sub>4</sub> and MS<sub>5</sub> (Table 1). Response to reculture was found to be different

in the four media (Table 3). The MS<sub>5</sub> medium containing three hormones proved to be the best among those tested. Calli formed on the MS<sub>2</sub> medium were relatively hard and brownish. They were globule like structures less than 1 mm in diameter. When these calli were transferred again to MS<sub>4</sub> or MS<sub>5</sub> media, they formed callus tissues similar to those usually grown in the latter two media, being yellowish and soft. However, calli subcultured from MS<sub>4</sub> and MS<sub>5</sub> media were more capable of callusing than those from MS<sub>2</sub>.

**Table 3: Callus formation on different media after reculture**

Medium	Response of plated callus		Characteristics
	Percentage of callus formation	Size of callus formed	
MS <sub>2</sub>	45.5 ± 0.7	+	brown, globular and hard calli
MS <sub>3</sub>	0	--	--
MS <sub>4</sub>	60.0 ± 1.7	+	yellowish soft calli
MS <sub>5</sub>	77.8 ± 1.8	+++	yellowish soft calli

+ - All calli less than 1 mm in diameter

++ - More than 80% of calli of 1-2 mm diameter

+++ - More than 80% of calli greater than 2 mm in diameter

## DISCUSSION

Shortening of the breeding cycle using DH technology can be achieved only if different stages take short periods.<sup>8,13,17,18</sup> All the media which were capable of producing callus were efficient in this context as they induced callus within 2-4 weeks. Nevertheless, MS<sub>5</sub> was the most successful medium as callus induction in it took place within 2 weeks compared to 3-4 weeks in other media. Moreover, a greater proportion of plated anthers produced callus in this medium.

The position of anthers on the medium at the time of plating also seems to have an influence on the callus induction. This aspect needs further investigations and should contribute to increasing the callus yield.

According to the only published results of anther culture in sesame, the Korean cultivars used have given 55.1% callus induction when 25 mg/l of 2,4-D and 1 mg/l of BAP were included in the MS medium.<sup>20</sup> Our experiments indicate that

concentration of 15 mg/l of 2,4-D with 1 mg/l of BAP (MS<sub>2</sub>) does not give a satisfactory rate of callus induction. The MS<sub>1</sub> medium containing 10 mg/l of 2,4-D with 1 mg/l of BAP recorded a higher rate of callus induction than MS<sub>2</sub> medium with 15 mg/l 2,4-D. These results are consistent with the findings that the different genotypes require different media for callus induction from anthers of the same species.<sup>10,15,22-25</sup>

Anthers from flower buds 36-48 h before anthesis, incubated for 24 h at 8<sup>0</sup> C when plated on MS<sub>5</sub> medium and kept in total darkness produced the highest rate of callus induction in the present experiment. With the objective of producing haploid plants, calli obtained from anthers of MI 3 variety have been transferred to regeneration media with different ratios of hormones. Besides, different media with natural sources are being tested for anther culture of several promising sesame genotypes including MI 3.

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