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Efficient Callus Induction and Plant Regeneration in *Sesamum* species

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Hypocotyl and cotyledon explants excised from two *in vitro*-grown sesame species, *Sesamum indicum* and *Sesamum orientale* were inoculated for callus induction on MS media supplemented with different concentrations and combinations of α-naphthaleneacetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D) and indole-3-acetic acid (IAA), and 6-benzylaminopurine (BAP). A combination of 1-2 mg$\cdot l^{-1}$ NAA and 0.2-0.6 mg$\cdot l^{-1}$ BAP was efficient for the formation of calli from hypocotyl and cotyledon explants. After three to four weeks of inoculation, embryo-like structures were formed in hypocotyl-derived calli on the medium supplemented with only 1 mg$\cdot l^{-1}$ 2, 4-D. Addition of casein hydrolysate (1-2 g$\cdot l^{-1}$) to the regeneration media containing 0.1 mg$\cdot l^{-1}$ NAA plus 1-4 mg$\cdot l^{-1}$ BAP effectively increased the rate of adventitious shoot formation from the hypocotyl-(22-42%) and cotyledon-derived calli (16-34%) in *S. orientale*. However, in *S. indicum*, only hypocotyl-derived calli showed an increase (14-44%) in adventitious shoot formation rate. Generally, high concentrations of BAP (3-4 mg$\cdot l^{-1}$) in combination with casein hydrolysate increased the formation of multiple shoots while low concentrations of BAP (1-2 mg$\cdot l^{-1}$) induced the formation of a single shoot. Even in case of low BAP, addition of a high concentration of casein hydrolysate tended to increase the multiple shoot formation. Regenerated shoots formed roots on 1/2 MS medium supplemented with 0.5 mg$\cdot l^{-1}$ NAA.

Introduction

Sesame (*Sesamum indicum* L.) is one of the most important oil crops, and has been extensively cultivated in both tropical and temperate areas from ancient times. Recently, as the cultivation area has been extended, new strains or mutants which enable it to be cultivated in a variety of environments[1] have been found. The seeds of sesame contain approximately 50% oil and 20-25% protein, and the defatted meal contains about 50% protein and may be eaten together with its oil. The essential amino acid composition, with the exception of lysine, of sesame seed protein is noted to be comparable to those of the proteins from beef and casein[2,3].

Generally, the productivity of sesame is relatively low as compared to that of other oil crops because the cultivation of sesame is restricted by poor soil[4] and short cultivation periods. Recently, cell culture techniques have been successfully utilized to obtain useful variants such as high lysine mutants[5,6], salt[7,8], aluminium[9,10] and herbicide[11,12] tolerant cell lines which may represent a new and useful source of genetic variations.

Several studies have been reported on the tissue culture of sesame ; anther culture[13,14], protoplast fusion between the same sesame species[15], single plant regeneration from shoot tip[16], and multiple adventitious shoot formation from the culture of shoot tip with an attached cotyledon[4]. In addition,

organogenesis from leaf-derived calli in *S. indicum* has been reported by Datta and Biswas[17] with a plant regeneration rate of 22.5%. In protoplast culture of sesame, protoplast isolated from cotyledon contains lipid, and hence fails to induce calli and further regenerate the plant. Thus, it was indicated that cotyledon was not a suitable material for protoplast culture[18]. However, plant regeneration from hypocotyl-derived calli in sesame has not yet been achieved.

This study was carried out to establish an efficient callus induction and plant regeneration system in *S. indicum*, a cultivated species in genus *Sesamum* with reference to a wild species, *S. orientale*.

Materials and Methods

Seeds of two sesame species, *S. indicum* cultivar Danbaek and Ansan (from Korea) and a wild type, *S. orientale* collected from Ghana, Africa, were surface-sterilized by treatment with 2% (v/v) sodium hypochlorite solution added with one drop of Tween-20 for 15 min., and then washed three times with sterile distilled water. These seeds were cultured on the hormone-free MS medium[19] containing 30 g$\cdot l^{-1}$ sucrose and solidified with 8 g$\cdot l^{-1}$ agar to obtain sterile seedlings.

From the 5 to 7-d-old sterile seedlings, hypocotyl (1 cm) and cotyledons were excised and cultured for callus induction on MS media containing 30 g$\cdot l^{-1}$ sucrose and 8 g$\cdot l^{-1}$ Bacto-agar (Difco) and supplemented with various combinations of hormones as shown in **Table 1**. The cultures were maintained under constant illumination at 1,500 lux and 27±1 °C. After one month, the compact calli were transferred onto MS media containing 30 g$\cdot l^{-1}$ sucrose and 8 g$\cdot l^{-1}$ Bacto-agar (Difco) and supplemented with various combinations of hormones as shown in **Table 2**, or casein hydrolysate (Difco) combined with 0.1 mg$\cdot l^{-1}$ NAA and 1-4 mg$\cdot l^{-1}$ BAP combinations for shoot regeneration (**Table 3**). These calli were transferred to fresh regeneration media at three week intervals. The cultures were maintained under constant illumination at 3,000 lux and 27±1 °C. All the media were adjusted to pH 5.8 before autoclaving for 20 min. at 121 °C. The regenerated shoots were transferred onto 1/2 MS medium supplemented with 0.5 mg$\cdot l^{-1}$ NAA under the same condition for root formation.

Results and Discussion

Callus formation rate from hypocotyl and cotyledon of two sesame species after one month of culture on callus induction media are shown in **Table 1**. NAA and 2, 4-D were generally more suitable for the callus formation in both explants of *S. indicum* cultivar Danbaeck and Ansan compared with IAA. However, no difference was observed in callus induction among the additions of NAA and 2, 4-D regardless of the concentration of BAP (0.2-0.6 mg$\cdot l^{-1}$). In contrast with two cultivars of *S. indicum*, high callus formation was observed in *S. orientale* on MS media supplemented with IAA in the combination with BAP. These results indicate that IAA significantly influences callus induction in tissue culture of *S. orientale*. Generally, synthetic auxins such as NAA, 2, 4-D and conjugated auxins may not be destroyed by oxidases such as IAA oxidases and/or peroxidases. On the contrary, IAA which occurs in plants may be degraded by IAA oxidases and become inactive[20]. Furthermore, it appears that the difference in the rate of callus formation between *S. indicum* and *S. orientale* may be attributed to the difference in response to IAA.

Calli produced from hypocotyl and cotyledon of two cultivars and a wild type which were cultured on the media supplemented with different concentrations of auxins showed variations in color and texture. Hypocotyl- and cotyledon-derived calli only from the media with 2, 4-D in combination with BAP were soft, friable and light brown. After three to four weeks of inoculation, embryo-like structures were formed (**Fig. 1-A**) in hypocotyl-derived calli on the MS medium supplemented with

Table 1. Effect of 2, 4-D, NAA, IAA and BAP on the callus formation from hypocotyl and cotyledon of two sesame species after four weeks of incubation*[1].

Cultivar	Auxin (mg·l^{-1})	Explant*[2]		BAP concentration (mg·l^{-1})			
				0.0	0.2	0.4	0.6
Danbaek (*S. indicum*)	NAA	1.0	H	96*[3]	92	100	98
			C	90	88	92	94
		2.0	H	98	100	100	100
			C	90	92	94	86
	IAA	1.0	H	54	52	58	46
			C	62	58	66	64
		2.0	H	62	68	52	64
			C	56	62	64	66
	2, 4-D	1.0	H	90	86	82	86
			C	88	90	86	92
		2.0	H	92	88	84	90
			C	84	90	98	86
Ansan (*S. indicum*)	NAA	1.0	H	88	90	86	84
			C	92	90	92	86
		2.0	H	92	94	88	96
			C	94	96	90	86
	IAA	1.0	H	58	52	56	52
			C	54	60	62	54
		2.0	H	60	64	56	48
			C	52	58	54	58
	2, 4-D	1.0	H	92	82	90	88
			C	90	92	88	90
		2.0	H	82	86	92	86
			C	82	92	94	84
Wild type (*S. orientale*)	NAA	1.0	H	92	98	98	94
			C	94	100	94	96
		2.0	H	100	100	96	98
			C	94	94	92	94
	IAA	1.0	H	92	90	92	92
			C	92	94	96	88
		2.0	H	94	100	94	96
			C	94	96	94	92
	2, 4-D	1.0	H	94	100	96	92
			C	90	94	98	96
		2.0	H	98	98	100	94
			C	94	92	94	98

*[1] Fifty explants per treatment.
*[2] H : Hypocotyl, C : Cotyledon.
*[3] Callus formation rate (%).

only 1 mg·l^{-1} 2, 4-D. Similarly, formations of embryo-like structures from hypocotyl-derived calli on MS liquid or solid medium supplemented with 1 mg·l^{-1} BAP and 0.1 mg·l^{-1} NAA were reported by George *et al.*[4] On the other hand, calli consisting of both compact and friable tissue were induced from both hypocotyl and cotyledon explants cultured on the media supplemented with either NAA or IAA in combination with BAP.

After six weeks of culture on regeneration media, the rate of green spot and adventitious shoot

Table 2. Adventitious shoot formation from hypocotyl- and cotyledon-derived calli in two sesame species after six weeks of incubation*1.

Cultivar	NAA (mg·l^{-1})	Callus*2	BAP concentration (mg·l^{-1})			
			1.0	2.0	3.0	4.0
Danbaek (*S. indicum*)	0.0	HC	0*3 (32)*4	0 (27)	8 (54)	6 (44)
		CC	0 (28)	2 (34)	0 (43)	4 (57)
	0.1	HC	4 (35)	6 (41)	10 (58)	8 (61)
		CC	0 (18)	4 (39)	4 (47)	6 (53)
	0.5	HC	0 (25)	0 (25)	6 (51)	4 (49)
		CC	2 (24)	4 (41)	0 (39)	2 (55)
Ansan (*S. indicum*)	0.0	HC	2 (28)	2 (30)	6 (43)	6 (54)
		CC	0 (34)	4 (26)	0 (38)	4 (58)
	0.1	HC	0 (22)	2 (37)	8 (52)	4 (48)
		CC	0 (36)	0 (40)	4 (41)	4 (53)
	0.5	HC	4 (24)	4 (33)	4 (54)	8 (45)
		CC	0 (27)	0 (47)	0 (44)	2 (51)
Wild type (*S. orientale*)	0.0	HC	2 (34)	6 (35)	2 (49)	2 (65)
		CC	0 (38)	4 (31)	6 (52)	4 (58)
	0.1	HC	4 (29)	6 (48)	2 (47)	8 (53)
		CC	0 (47)	0 (42)	0 (65)	0 (56)
	0.5	HC	6 (37)	6 (36)	8 (53)	6 (54)
		CC	4 (38)	4 (53)	6 (55)	4 (55)

*1 Fifty embryogenic compact calli with and without green spot per treatment.
*2 HC : Hypocotyl-derived callus, CC : Cotyledon-derived callus.
*3 The rate of adventitious shoot formation (%)
*4 Numerals in the parentheses indicate the rate of green spot formation (%).

Table 3. Effect of casein hydrolysate on the adventitious shoot formation from hypocotyl- and cotyledon-derived calli in two sesame species after six weeks on the MS medium supplemented with 0.1 mg.l^{-1} NAA and combinations with BAP*1.

Cultivar	CH*2 (g·l^{-1})	Callus*3	BAP concentrasion (mg·l^{-1})			
			1.0	2.0	3.0	4.0
Danbaek (*S. indicum*)	1.0	HC	4/5*4 (18)*5	5/7 (24)	6/11 (34)	5/12 (34)
		CC	2/1 (6)	3/3 (12)	3/1 (8)	3/2 (10)
	2.0	HC	4/3 (14)	9/13 (44)	4/16 (40)	6/12 (36)
		CC	2/2 (8)	3/0 (6)	2/3 (10)	2/4 (12)
Ansan (*S. indicum*)	1.0	HC	5/2 (14)	10/5 (30)	9/4 (26)	8/7 (30)
		CC	3/0 (6)	4/2 (12)	2/3 (10)	3/3 (12)
	2.0	HC	11/7 (36)	12/5 (34)	8/10 (36)	11/5 (32)
		CC	4/1 (10)	6/2 (16)	5/1 (12)	3/4 (14)
Wild type (*S. orientale*)	1.0	HC	6/5 (22)	9/5 (28)	6/10 (32)	8/13 (42)
		CC	5/3 (16)	9/3 (24)	9/5 (28)	11/5 (32)
	2.0	HC	9/3 (24)	8/10 (36)	5/11 (32)	7/13 (40)
		CC	7/2 (18)	8/9 (34)	7/3 (10)	9/6 (30)

*1 Fifty embryogenic compact calli with and without green spot per treatment.
*2 CH : Casein hydrolysate.
*3 HC : Hypocotyl-derived callus, CC : Cotyledon-derived callus.
*4 Number of regenerated shoots (single shoots/multiple shoots).
*5 Numerals in the parentheses indicate the rate of adventitious shoot formation (%).

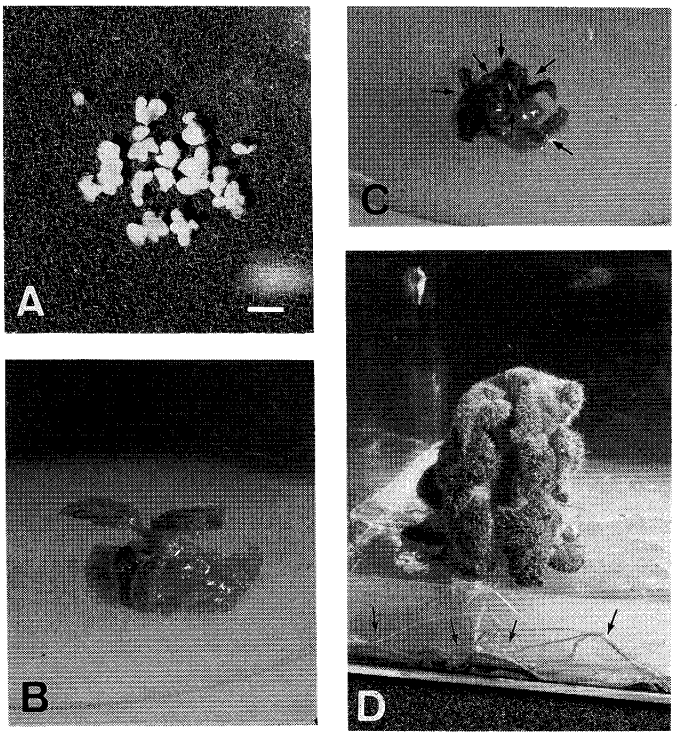

Fig. 1 Plant regeneration from hypocotyl-derived calli in *S. indicum*.
A : Embryo-like structures obtained in MS liquid medium supplemented with 1 mg•l^{-1} 2. 4-D four weeks after culture. Bar indicate 3 mm. B and C : Single (B) and multiple shoot (C) formation on MS medium supplemented with 0. 1 mg•l^{-1} NAA, 2 mg/l BAP and 2 g•l^{-1} casein hydrolysate after two to three weeks of culture (arrows indicate adventitious shoots). D : Regenerated plantlets on 1/2 MS rooting medium supplemented with 0. 5 mg•l^{-1} NAA (arrows indicate formed roots).

formation from hypocotyl- and cotyledon-derived calli in two sesame species was examined, which are shown in **Table 2**. Small green primordia appeared approximately 10 days after inoculation, and single (**Fig. 1-B**) or multiple shoots (**Fig. 1-C**) were formed on hypocotyl- and cotyledon-derived calli after two to three weeks of culture on the regeneration media. The rate of green spot formation from hypocotyl- and cotyledon-derived-calli in two species increased with the increase in BAP concentration regardless of NAA concentration. Furthermore, a relatively high rate of green spot formation from both calli of the two cultivars and a wild type occurred on MS media containing NAA (0. 0-0. 5 mg•l^{-1}) and BAP (3-4 mg•l^{-1}). Hewever, a lower rate (0-10%) of adventitious shoot formation from both call in *S. indicum* and *S. orientale* was recorded on regeneration media regardless of the concentration of NAA and BAP (**Table 2**).

The effect of casein hydrolysate on adventitious shoot formation from hypocotyl- and cotyledon-derived calli of two sesame species is shown in **Table 3**. The addition of casein hydrolysate (1-2 g•l^{-1}) on the regeneration media containing the combinations of 0. 1 mg•l^{-1} NAA and 1-4 mg•l^{-1} BAP effectively increased the rate of shoot regeneration from the hypocotyl-(22-42%) and cotyledon-derived calli (16-34%) in *S. orientale*. On the contrary, calli cultured without casein hydrolysate had a low adventitious shoot formation rate (0-10%) as shown in **Table 2**. Thus, shoot regeneration from hypocotyl-derived calli in sesame appeared to require other substances such as casein hydrolysate. It has been reported that the rate of plant regeneration and somatic embryogenesis increases with the addition of casein hydrolysate[21-23] and amino acids such as

glutamine[24,25], asparagine[26] and proline[27-29]. However, in the two cultivars of *S. indicum*, the rate of adventitious shoot formation increased in only hypocotyl-derived calli (14-44%). The low rate of adventitious shoot formation from cotyledon-derived calli in two cultivars of *S. indicum* even with the addition of casein hydrolysate to the regeneration media could be attributed to organ- and species-specificity in the response to hormone combinations. Therefore, it appears that the medium composition significantly influences adventitious shoot formation in tissue culture of *S. indicum* and *S. orientale*.

The numbers of single or multiple shoots were influenced by BAP and casein hydrolysate concentrations. Generally, high concentrations of BAP (3-4 mg$\cdot l^{-1}$) in combination with casein hydrolysate increased the formation of multiple shoots while low concentrations of BAP (1-2 mg$\cdot l^{-1}$) induced the formation of a single shoot from the culture of shoot tip as observed by George *et al.*[4] and Lee *et al.*[16]. Even in the case of low BAP, addition of a high concentration of casein hydrolysate tended to increase the formation of multiple shoots. The most efficient adventitious shoot formation (44%) was accomplished on the regeneration medium supplemented with 0.1 mg$\cdot l^{-1}$ NAA, 2 mg$\cdot l^{-1}$ BAP and 2 g$\cdot l^{-1}$ casein hydrolysate. In the regenerated shoots which were transferred onto 1/2 MS medium supplemented with 0.5 mg$\cdot l^{-1}$ NAA, the initiation of rhizogenesis was observed after two weeks (**Fig 1-D**).

The efficient plant regeneration from hypocotyl- and cotyledon-derived calli of *S. indicum* and *S. orientale* will contribute to further experiments of sesame such as cell and protoplast culture and cell selection. However, the difficulty of regenerating plantlets from cotyledon-derived calli in *S. indicum* cultivars must be investigated in further research.

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《和文要約》

ゴマにおける効率的カルス誘導と植物体再分化

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無菌的に発芽させた幼植物ゴマの胚軸および子葉からのカルス誘導にはMS基本培地にNAA 1～2 mg/lおよびBAP 0.2～0.6 mg/lの組合せの添加が有効であった．2.4-D 1 mg/l添加培地で胚軸由来カルスから胚様体が形成された．カゼイン分解物1～2 g/l，NAA 0.1 mg/lおよびBAP 1～4 mg/lの添加は，*S. orientale*の胚軸および子葉由来カルスからのシュート形成率を高めたが，*S. indicum*では胚軸由来カルスのみでシュート形成率が高まった．カゼイン分解物との組合せで高濃度（3～4 mg・l^{-1}）のBAP添加は，カルス当たりの分化シュート数を増加させた．しかし，低濃度（1～2 mg/l）のBAP添加ではカルス当たりシュート数は1本のみであった．ただし，この場合もカゼイン分解物高濃度添加は分化シュート数を増加させる傾向を示した．再分化したシュートは，NAA 0.5 mg/l添加した1/2MS培地に移植することによって容易に根を分化した．