Somatic Embryogenesis in Suspension Culture of Mature Seed of *Cucumis melo* L.

Kohji KAGEYAMA¹⁾, Kazunori YABE and Shigetoshi MIYAJIMA

Aichi-ken Agricultural Research Center, Aichi-gun, Aichi 480-11

The factors affecting somatic embryogenesis in suspension culture of melon seed and the process involved were described. The culture period in the initial culture medium supplemented with 1mg/l 2,4-D, 1mg/l NAA, 0.1mg/l BA and 30g/l sucrose, influenced the structural characteristic of the somatic embryo. When the suspension was cultured for 7 days in the initial medium, the somatic embryos which developed normal morphogenesis predominated. A larger number of normal somatic embryos were obtained when the 7-day-old cultures were washed with MS medium containing 0.5% activated charcoal. Somatic embryogenesis began in the filamentous embryo stage and proceeded from globular to torpedo stage.

KEY WORDS: *Cucumis melo*, somatic embryogenesis, suspension culture, activated charcoal, culture period.

Introduction

Somatic embryogenesis was first reported in carrots by STEWARD *et al.* (1958) and later have been derived from various tissues of more than 70 species (WILLIAMS and MAHESWARAN 1986). In melon, since ORIDATE and OOSAWA (1986) induced somatic embryo from mature seed, several attempts have been carried out to increase the efficiency of somatic embryogenesis and plant regeneration (HONMA *et al.* 1989, MOMONOKI *et al.* 1989). We reported the procedure using liquid culture instead of the conventional solid culture and determined its efficiency in bringing about somatic embryogenesis. We speculate that problems associated with somatic embryo development are attributed to structural abnormalities (KAGEYAMA *et al.* 1989).

The present paper discusses the process involved and the improvement in the efficiency of somatic embryogenesis using suspension culture of melon seed.

Materials and Methods

Mature seeds of *Cucumis melo* L. cv. Earl's Favourite Haru 1 were tested. After seed coats were removed, seeds were surface-sterilized by immersion in 10% hydrogen peroxide for 20 minutes and rinsed three times with sterilized distilled water. Sterilized seeds were plated on MURASHIGE and SKOOG (MS) medium (1962) with 0.8% agar. After 1 day in culture, seeds were cut horizontally approximately 1mm width from the side in which radicle develops, and three pieces per seed were used as explants.

Experiment 1: Six explants were cultured in a 100ml Erlenmeyer flask containing 50ml of MS liquid medium supplemented with 1mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 1mg/l 2-naphthaleneacetic acid (NAA), 0.1mg/l 6-benzylaminopurin and 30g/l sucrose, and placed on gyratory shaker at 115 rpm for 0~14 days. The cultures which had been harvested with

Received July 19, 1990.

1) Present address: Faculty of Agriculture, Gifu University, Gifu 501-11

75 μ m stainless steel screen were transferred to hormone-free MS liquid medium and cultured on the shaker for 2~4 weeks. Somatic embryos developing into more than 3mm were selected and their structural characteristics were determined.

Experiment 2: Six explants were cultured for 7 to 14 days with the same method as in Experiment 1. The cultures were harvested with 150 μ m stainless steel screen and then divided into 3 groups; not washed, washed with 100ml hormone-free MS medium per the culture in a flask, and washed with 50ml of hormone-free MS medium containing 0.5% activated charcoal and then with 50ml of hormone-free MS medium. The washed cultures were transferred to a 100ml Erlenmeyer flask containing 50ml of hormone-free MS medium and cultured on the shaker for 2 to 3 weeks. Somatic embryos developing into more than 3mm were selected and their structural characteristics were determined. Normal somatic embryos were transferred to hormone-free MS medium solidified by 0.2% Gelrite (Sanei Kagaku Kogyo Co., Osaka) and cultured to regenerate plants for 2 weeks.

All experiment cultures were incubated at 25°C under 16 hr photoperiod (5000 lux).

Results and Discussion

The culture period in the initial culture medium containing hormones influenced the structural characteristics of the somatic embryo (Table 1, Fig. 1). The somatic embryos were divided into two groups; those having two cotyledons and a long and thin hypocotyl as shown in common dicotyledon plants (normal), and those having more than three cotyledons and a short and thick hypocotyl (abnormal). As the culture period in the initial culture medium was longer, the percentage of normal somatic embryos decreased and the callus formation from the explants was promoted. The number of normal somatic embryos increased in the culture period of 5 to 7 days but decreased in that of 14 days, which suggests that 7 days seemed to be the optimum period in the initial medium for the development of somatic embryo with normal morphogenesis.

Activated charcoal enhances somatic embryogenesis and plant regeneration, and prevents abnormal morphogenesis in hypocotyl and leaf culture of cucumber (ZIV and GADASHI 1986,

Table 1. Effect of culture period in the initial culture medium on somatic embryo development

Culture period (days)	No. of somatic embryos ¹⁾			Percentage of normal somatic embryos (%)
	Normal	Abnormal	Total	
0	0	0	0	—
1	1.0	0	1.0	100
2	0.7	0	0.7	100
3	9.3	0.2	9.5	98
5	33.4	3.8	37.2	90
7	61.7	84.8	146.5	42
14	30.2	146.7	176.9	17

1) The number of somatic embryos indicated ones produced in a 100ml Erlenmeyer flask containing 6 explants and 50ml of culture medium.

Table 2. Effect of washing of the cultures after incubation in initial culture medium on somatic embryo development

Initial culture period (weeks)	Washing method ¹⁾	No. of somatic embryos ²⁾			Percentage normal somatic embryos (%)	Percentage of regenerated plantlets (%) ³⁾	
		Normal	Abnormal	Total		Normal	Abnormal embryo
1	①	73.0	39.5	112.5	65	54 (14/26)	55 (11/20)
	②	167.0	45.0	212.0	79	40 (32/80)	13 (5/40)
	no	67.5	76.0	143.5	47	80 (48/60)	63 (25/40)
2	①	15.0	108.5	123.5	12	70 (21/30)	35 (7/20)
	②	41.0	144.5	185.5	22	85 (34/40)	15 (6/40)
	no	11.5	111.5	123.0	9	90 (18/20)	15 (6/40)

1) ① Washing with 100 ml of hormone-free MS medium per the cultures in a flask.

② Washing with 50 ml of hormone-free MS medium with 0.5% activated charcoal and then with 50 ml of hormone-free MS medium without 0.5% activated charcoal.

2) The same as Table 1.

3) Somatic embryos were transferred to hormone-free MS medium solidified by 0.2% Gelrit and cultured to regenerate plants for 2 weeks.

Fig. 1. Somatic embryos with normal (left) and abnormal (right) morphogenesis derived from mature seed of melon.

CHEE and TRICOLI 1988) and in anther culture of *Anemone canadensis* (JOHANSSON *et al.* 1982). In order to increase the percentage of normal somatic embryos, the cultures obtained in the initial culture medium with hormones were washed and then transferred to hormone-free MS medium (Table 2). The cultures washed with the medium containing activated charcoal resulted into production of greater number of normal somatic embryos. The effect of activated charcoal was influenced by the culture period in the initial culture medium, that is,

the percentage of normal somatic embryos was higher in the 1-week-old cultures than in the 2-week-old ones.

ZIV and GADASHI (1986) suggested that the morphological quality of somatic embryo might be affected by a carryover of 2,4-D. It was considered that the shortened culture period in the medium with hormones and the washing of the cultures with the medium containing activated charcoal inhibited a carryover effect of 2,4-D and enhanced somatic embryo development with normal morphogenesis.

Somatic embryos from the cultures treated with activated charcoal less frequently developed into plantlets (Table 2), although normal morphogenesis was predominant. A number of regenerated plantlets had multiple shoots in the cultures treated without activated charcoal, which was seemed to be caused by the carryover effect of 2,4-D. Most of the plantlets regenerating both from the washed cultures and from those not washed had vitreous leaves,

Fig. 2. Stage of somatic embryo development. (Bar: 100 μ m) A,B: filamentous stage, C: globular stage, D: heart stage, E: torpedo stage.

and these plantlets can not be acclimated to natural environment. Subculture for maturing somatic embryo as shown in celery (ONISHI *et al.* 1988) and carnation (ZIV *et al.* 1982) may be needed to improve plant regeneration.

The process of somatic embryo development in melon was similar to that in carrot (HALPERIN and WETHERELL 1964). Filamentous stage, designated by HALPERIN and WETHERELL (1964), was observed in callus or seed tissue in the early stage (Fig. 2-A, B), which proceeded to globular and torpedo stage when the cultures were transferred into hormone-free medium (Fig. 2-C~E). The cells in callus or seed tissue in which somatic embryogenesis started were observed at radicle development side of torpedo stage embryo (Fig. 2-E). Therefore, it seemed that somatic embryo developed with the polarity of the root-shoot axis from the filamentous stage.

The liquid culture system in the present study may improve somatic embryo development with normal morphogenesis in melon. It is considered that the liquid culture system may be more efficient in cell selection for micropropagation and synchronization than the solid culture one. Further experiment is necessary to develop the procedure to mature somatic embryos and to prevent the vitrification of regenerated plantlets.

Acknowledgement

This work was partly supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.

Literature Cited

- CHEE, P.P. and D.M. TRICOLI 1988. Somatic embryogenesis and plant regeneration from cell suspension cultures of *Cucumis sativus* L. Plant Cell Rep. **7**:274~277.
- HALPERIN W. and D.F. WETHERELL 1964. Adventive embryony in tissue cultures of the wild carrot, *Daucus carota*. Amer. J. Bot. **51**:274~283.
- HOMMAN, Y., T. FURUMOTO and K. OOSAWA 1989. Refinement in production of melon somatic embryos on solid media. Japan. J. Breed. **39** (suppl. 1):12~13.
- JOHANSSON, L., B. ANDERSSON and T. ERIKSSON 1982. Improvement of anther culture technique: Activated charcoal bound in agar medium in combination with liquid medium and elevated CO₂ concentration. Physiol. Plant. **54**:24~30.
- KAGEYEMA, K., K. YABE and S. MIYAJIMA 1989. Somatic embryogenesis by suspension culture of mature seed in *Cucumis melo* L. Japan. J. Breed. **39** (suppl. 1):16~17.
- MOMONOKI, Y., Y. TABEL, S. NISHIMURA and T. KANNO 1989. Culture conditions of somatic embryogenesis in melon (*Cucumis melo*). Japan. J. Breed **39** (suppl. 1):20~21.
- MURASHIGE T. and F. SKOOG 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**:473~497.
- OONISHI, N., T. KAWANO, T. MASHIKO, S.C. SMITH and D. STUART 1988. Development of celery synthetic seed: Increase in germination rate of somatic embryos. Japan. J. Breed. **38** (suppl. 2):44~45.
- ORIDATE, T. and K. OOSAWA 1986. Somatic embryogenesis and plant regeneration from suspension callus culture in melon (*Cucumis melo* L.). Japan. J. Breed. **36**:424~428.
- STEWART, F.C., M.O. MAPES and K. MEARS 1958. Growth and organized development of cultured cells. II. Organization in cultures growth from freely suspended cells. Amer. J. Bot. **45**:705~708.
- WILLIAMS E.G. and G. MAHESWARAN 1986. Somatic embryogenesis: Factors influencing coordinated behaviour of cells as an embryogenic group. Ann. Bot. **57**:443~462.
- ZIV, M. and G. GADASI 1986. Enhanced embryogenesis and plant regeneration from cucumber (*Cucumis sativus* L.) callus by activated charcoal in solid/liquid double-layer cultures. Plant Sci. **47**:115~122.
- , G. MEIR and A.H. HALEVY 1983. Factors influencing the production of hardened glaucous carnation plantlets in vitro. Plant Cell Tissue Organ Culture **2**:55~65.

メロン種子の液体振とう培養による不定胚誘導

景山幸二¹⁾・矢部和則・宮島成寿(愛知県農業総合試験場, 愛知郡長久手町 〒480-11, 現¹⁾)

メロンの完熟種子からの不定胚誘導では、静置培養によるカルス形成、カルスのホルモンフリー培地での静置あるいは液体振とう培養が行われてきた。本実験では不定胚誘導の全ての培養を液体振とう培養で行い、効率的に不定胚を形成させる条件について検討した。

ホルモン添加培地での培養機関が不定胚形成に及ぼす影響を調べるため、完熟種子切片を 2, 4-D 1 mg/l, NAA 1 mg/l, BA 0.1 mg/l, ショ糖 30 g/l を含む MS 培地で 0~14 日振とう培養後、培養細胞塊をホルモンフリーの MS 培地に移植して振とう培養した。その結果、ホルモンを含む培地での培養期間は、形成される不定胚の形態および不定胚数に影響を及ぼした。不定胚は、細長い胚軸および 2 枚の子葉をもつもの（正常胚）と太短い胚軸および 3 枚以上の子葉をもつもの（異常胚）に分けられ、正常胚は培養期間 7 日で最も多く形成されたが、その占める割合は 5 日以前の培養期間のもので高かった (Table 1, Fig. 1)。

次に、正常不定胚の形成効率を高めるため、ホルモン添加培地からホルモンフリー培地への移植時の培養細胞塊の洗浄が不定胚形成に及ぼす影響を調べた。ホルモン添加培地で 1 または 2 週間培養して得られた細胞塊をホルモンを含まない培地に 0.5% で活性炭を添加した培地で洗浄したところ、活性炭添加培地での洗浄は不定胚数を増加させ、正常な不定胚形成の割合も高くする効果を示した (Table 2)。しかし、ホルモンを含む培地での培養期間が長い場合はその効果は少なかった。不定胚からの植物体再生率は、洗浄を行わない方が高い傾向を示したが、これらの植物体は多芽になるものが多かった。これらのことから、活性炭添加培地での洗浄は、形成される不定胚の形態や数のみならず不定胚から再生する植物体へも好影響を及ぼすことが明らかとなった。

不定胚は、カルスまたは外植体組織に細長い細胞塊が形成されることにより始まった。また、不定胚形成には初期段階から方向性があり、組織についている細胞側が幼根になるように生育が進んだ。生育過程は、ニンジン¹⁾の不定胚の場合と似ており球状、心臓型、魚雷型へと進んだ (Fig. 2)。

1) 現住所 岐阜大学農学部, 岐阜市柳戸 〒501-11