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Karyological Analysis of Somaclonal Variation in Callus Cells of *Allium victorialis* var. *platyphyllum*

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Calli obtained from basal disc explants of *Allium victorialis* var. *platyphyllum* were grown in three kinds of nutrient media (MS, BDS, and B_5), and the frequencies of mitotic index and the chromosomal aberrations were analysed. The mitotic index varied from 0.55% to 1.01% with respect to culture media and ages. The mitotic irregularities like micro-, bi- and multi-nuclei, chromosome bridge and laggards were noted in each types of calli. The chromosome number variations observed in metaphase stage were identified aneuploid and tetraploid. Structural variations such as dicentric chromosomes, centromere breakage and small chromosomes were observed. Relationship between basal medium and chromosomal variability was not observed in this study. But, in BDS medium, NAA and BA had a more effect on number variation than kinetin.

Keywords : *Allium victorialis* var. *platyphyllum*, mitotic abnormality, chromosomal aberration

Karyotypic patterns can be changed according to culture conditions. Larkin and Scowcroft (1981) argued its importance and proposed the term "somaclonal variation". Chromosomal changes are a clear indication of somaclonal variation (Lee and Phillips, 1988). Since D'Aamato (1952) first reported karyotypic alteration, many authors have been reported on the changes of chromosome number and structure in regenerants from tissue culture (Skirvin and Janick, 1976; D'Aamato, 1978). There are possible mechanism for the origin of somaclonal variation: genetic characteristics of explant, concentration of exogenous phytohormones or hormonal composition of culture medium and culture age (Karp and Bright, 1985; Lee and Phillips, 1988; Karp, 1989).

Callus phase is a common feature of regeneration and its genetic instability is well documented (Sacristan, 1971; Bayliss, 1973; Roy, 1980; Orton, 1983). The variation found in the callus cells originate either from perpetuating preexisting variation in the somatic cells of the explants or from new mutations generated during growth in culture. Tissue culture system which avoid callus phase is largely free of somaclonal variations and relatively low level of variations are observed in plants regenerated through somatic embryogenesis. This is due to the stringent selection in favor of cytologically normal cells during embryo differentiation (Morrish *et al.*, 1990; Eastmen *et al.*, 1991; Shenoy and Vasil, 1992; Vallés *et al.*, 1993).

The edible Alliums are of major economic and dietary importance in all over the world. For utilizing the potential value of wild relatives of Allium species, an efficient regeneration system of plants from in vitro cultures should be established (Novak, 1990; Viterbo *et al.*, 1992). The studies on tissue culture as materials of the genus *Allium* have been reported in *A. sativum* (Kim and Seo, 1991), *A. cepa* (Fridborg, 1971; Yamane, 1975), *A. wakegi* (Seo and Kim, 1988), and *A. senescens* var. *minor* (Nair and Seo, 1992, 1993).

The present paper reports chromosomal variation observed in callus cells which is induced in the dif-

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ferent kinds of media.

MATERIALS AND METHODS

Callus initiation

The plant materials, the bulbs of *Allium victorialis* var. *platyphyllum*, collected from natural populations of Ulreung island were surface sterilized immersing in 70% ethanol for 5 min and 7% sodium hypochlorite for 20 min and followed by washing three times in sterile distilled water. Callus was obtained from disc explants in MS, BDS and B_5 basal medium supplemented with various hormone concentrations and combination (Table 1). Callus were initiated under continuous illumination (white fluorescent lights, 3000 lux), 25± 1℃, 60± 5% relative humidity, and then transferred to dark condition for callus growth. First subculture was carried out in 210 days and next subculture was carried out after 30 days.

Mitotic index and chromosome analysis

To analysis the mitotic indices and the mitotic abnormalities, each of the first and the second subcultured callus were used. Callus was collected at random from ten flasks (50 mL, Erlenmeyer). Callus was fixed immediately in ethanol : glacial acetic acid (3 : 1) for 3 h, transferred to 70% ethanol, and hydrolysed in 1N HCl, 60℃ for 1 min and squash preparations were made in acetic-orcein. The mitotic index of the dividing cells was examined by counting about 5000 cells at random from each sample of callus. For chromosome analysis, callus was pretreated in 2 mM 8-hydroxyquinoline at 16℃ for 6 h before fixation. The total metaphase cell were observed about 50 cells for chromosomal variation analysis.

RESULTS

Callus was induced from explants in 40-60 days after culture and the growth rate of callus was very slow. After 210 days callus was sufficient for subculturing. The mitotic index of the callus was about 0.55-1.01% according to culture condition (Fig. 1). Among callus cells, various mitotic abnormalities including micro-nuclei, bi-nuclei, multi-nuclei, bridge, laggard, abnormal shaped chromatid and spindle abnormality were observed and their frequencies were 9.9-21.8% of the total mitotic cells (Table 1). Micro-nuclei cells were most abundant in all types,

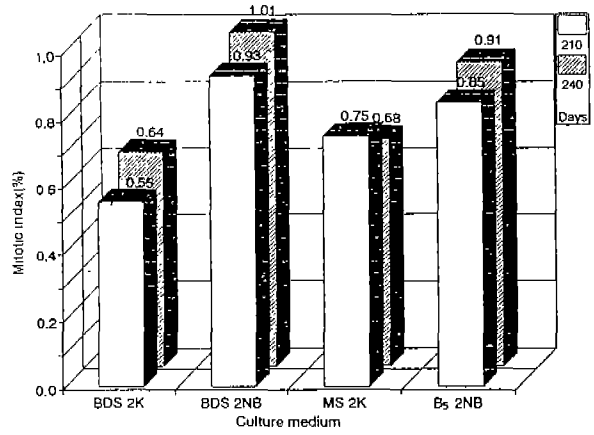

Fig. 1. Frequency of mitotic indices observed in callus cells.

Table 1. Mitotic abnormalities observed in four types of calli with respect to culture age

Basal media	Hormones		Culture age (days)	No. of Mitotic abnormalities(%)					Total cells counted
	Auxin	Cytokinin		micronuclei	binuclei	laggard	others[a]	total	
BDS	2,4-D[2]	KIN[1]	210	51(7.3)	7(1.7)	3(0.7)	21(4.8)	62(14.7)	422
			240	154(10.7)	8(1.6)	–	23(4.5)	85(16.8)	507
	2,4-D[1], NAA[1]	BA[1]	210	25(6.8)	4(1.1)	–	17(4.6)	46(12.4)	370
			240	71(11.5)	8(1.3)	2(0.3)	38(6.1)	119(19.2)	619
MS	2,4-D[2]	KIN[1]	210	22(5.3)	4(1.0)	–	15(3.6)	41(9.9)	413
			240	43(13.2)	1(0.3)	–	10(3.1)	54(16.6)	325
B_5	2,4-D[1], NAA[1]	BA[1]	210	77(17.1)	–	–	13(2.9)	90(20.0)	451
			240	28(13.3)	3(1.4)	3(1.4)	15(7.1)	46(21.8)	211

[1,2]Concentration of hormones showing 1 mg/L and 2 mg/L; Others[a] including multi-nuclei, chromatin bridge, chromatid connection and bouquet shaped chromatid.

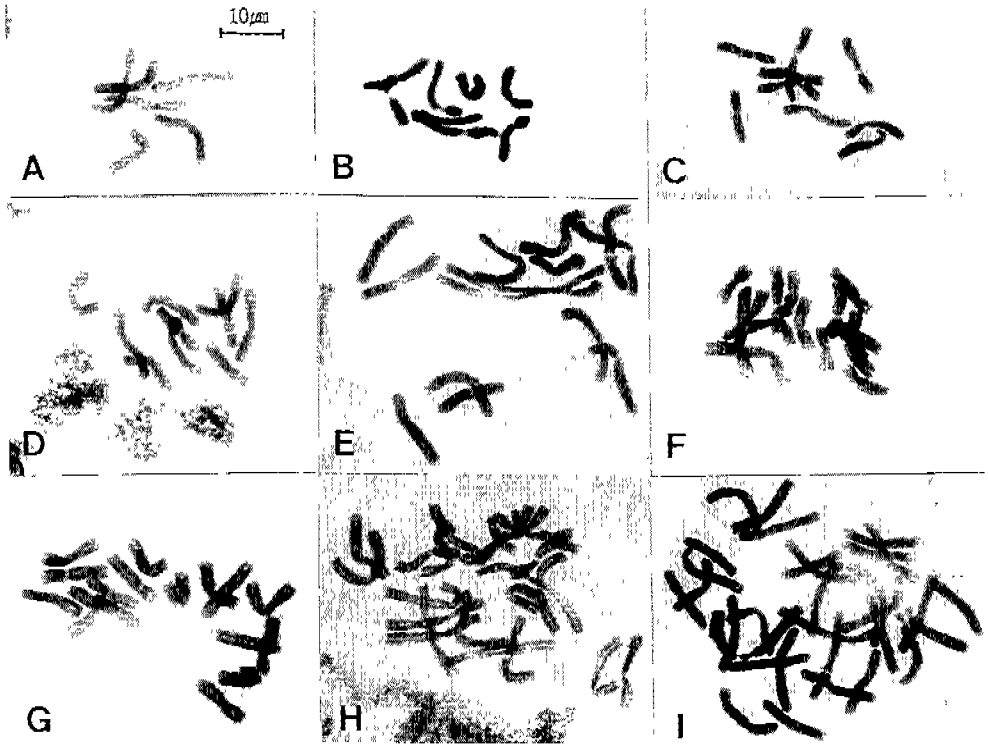

Fig. 2. The metaphase plates showing various chromosome numbers observed in the callus cells. A, 2n=6; B, 2n=8; C, 2n=9; D, 2n=10; E, 2n=14, F, 2n=15; G, 2n=16; H, 2n=21; I, 2n=32.

Table 2. Numerical variations of chromosome observed in the callus cell

Ploidy level	Plate No.[a]	Chromosome No.	Chromosomes							
			1	2	3	4	5	6	7	8
Haploid	A	6						−	−	
	B	8								
	C	9	−	+			+	−	+	
	D	10		+	−	−	+		+	+
Diploid	E	14		−				−		
	F	15					−			
	G	16								
	H	21	−	+,+	+		+		+	+
Tetraploid	I	32								

[a]The plate number of Fig. 2; +, chromosome addition; −, chromosome deletion.

and increased according to culture age except B_5 medium. A low frequencies of bi-nuclei or binucleate cell, and laggard were also observed among the callus cells throughout the culture period. Multinuclei, chromatin bridge, chromatid connection and bouquet shaped chromatid were common features. Non-polarization caused by spindle abnormality was observed in the callus cells.

Normal chromosome compositions of *A. victorialis* var. *platyphyllum* is 2n=16 which consist of a pair of subtelocentric with satellite, a pair of submetacentric, and six pairs of metacentric chromosomes (Kim *et al.*, 1990). As shown in Fig. 2 and Table 2, variable numerical variations were observed in callus cells, and their frequency with respect to culture condition and culture age were represented in Table 3. Even-though diploid population was prominent among the four callus lines, cells with various chromosome

Table 3. Frequecy of each ploidy level observed in four types of calli

Callus types	Culture age (days)	Ploidy level (%)					The rates of variated cell
		Hypodiploid	Diploid	Hyperdiploid	Tetraploid	Hypertetraploid	
BDS 2K[a]	210	6(9.7)	48(77.4)	5(8.1)	2(3.2)	1(1.6)	0.29
	240	6(13.3)	35(77.8)	4(8.9)	—	—	0.29
BDS 2NB[b]	210	9(19.1)	30(63.9)	7(14.9)	1(2.1)	—	0.57
	240	8(15.1)	36(67.9)	6(11.3)	2(3.8)	1(1.9)	0.47
MS 2K	210	8(13.8)	42(72.4)	4(6.9)	2(3.4)	2(3.4)	0.38
	240	6(13.0)	34(73.9)	3(6.5)	1(2.2)	2(4.3)	0.35
B_5 2NB	210	9(20.0)	25(55.6)	9(20.0)	1(2.2)	1(2.2)	0.80
	240	8(18.2)	24(54.5)	7(15.9)	3(6.8)	2(4.5)	0.63
Total		60(15.0)	274(68.5)	45(11.3)	12(3.0)	9(2.3)	0.46

2K[a], 2,4-D and kinetin; 2NB[b], 2,4-D, NAA and BA

number ranging from 2n=6 to 32 were observed in different frequency according to the culture age and culture medium. The rates of variated cell to normal diploid cells were 0.29-0.80 in 210 days old cells, and then more or less decreased in 240 days.

Structural variations like dicentric chromosome, acentric fragment or small chromosome and centromere breakage were also observed with not related to callus types (Figs. 3 and 4). Among them acentric chromosome or small chromosome observed in high frequencies. Fig. 4D was represented chromosome breakage in chromosome 2 and 6. Small chromosomes per abnormal cell ranges from 2 to 5, which might be effect of chromosome fragmentation. Due to small chromosome occurred by structural variation altered chromosome number, structural variations appeared with number variation.

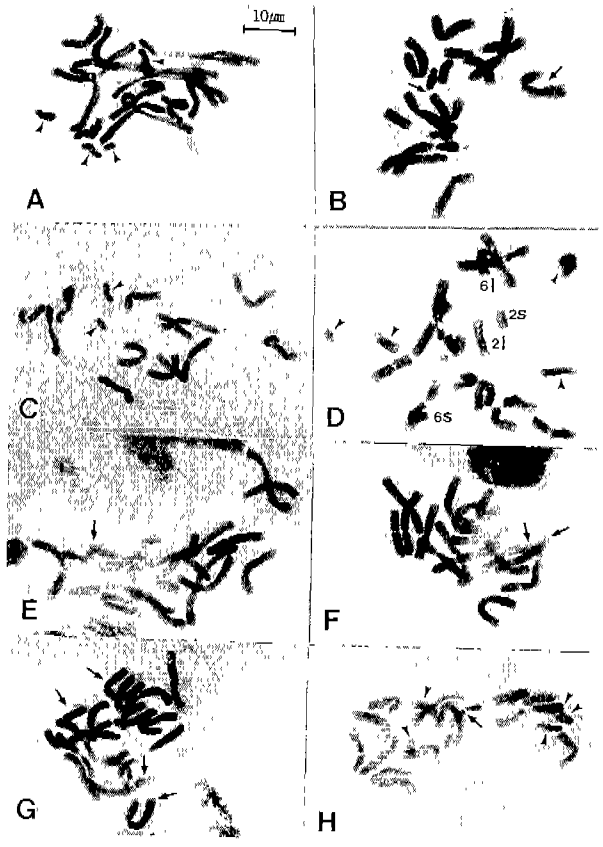

Fig. 3. The metaphase plates with structural variations observed in the callus cells. Arrows and arrow heads indicate dicentric chromosomes and small chromosomes, respectively. 2s and 2l indicate short arm and long arm of chromosome 2. 6s and 6l indicate short arm and long arm of chromosome 6. A, 2n=17; B, 2n=12; C, 2n=14; D, 2n=18; E, 2n=15; F, 2n=16; G, 2n=14; H, 2n=17.

DISCUSSION

Genotypic variation in *Allium* species during *in vitro* culture was mentioned in earlier reports (Selby and Collin, 1976; Phillips and Luteyn, 1983; Rauber and Grunewaldt, 1988; Seo and Kim, 1988; van der Valk *et al.*, 1992; Viterbo *et al.*, 1992; Nair *et al.*, 1993). Also effect of genotype and nutrient medium to produce organogenetic callus was reported in tomato (Ohki *et al.*, 1978), *Lillium longiflorum* (*Qu et al.*, 1988), potato (M'Ribu and Veilleux, 1990), alyceclover (Wofford *et al.*, 1992) and barley (Bregitzer, 1992). In *A. victorialis* var. *platyphyllum*, genotypic variation was not significant in callus initiation. Callus induction and subsequent growth in *Allium* species was strictly dependent on the presence of exogenous auxins (Havranek and Novak, 1973; Phillips and Lu-

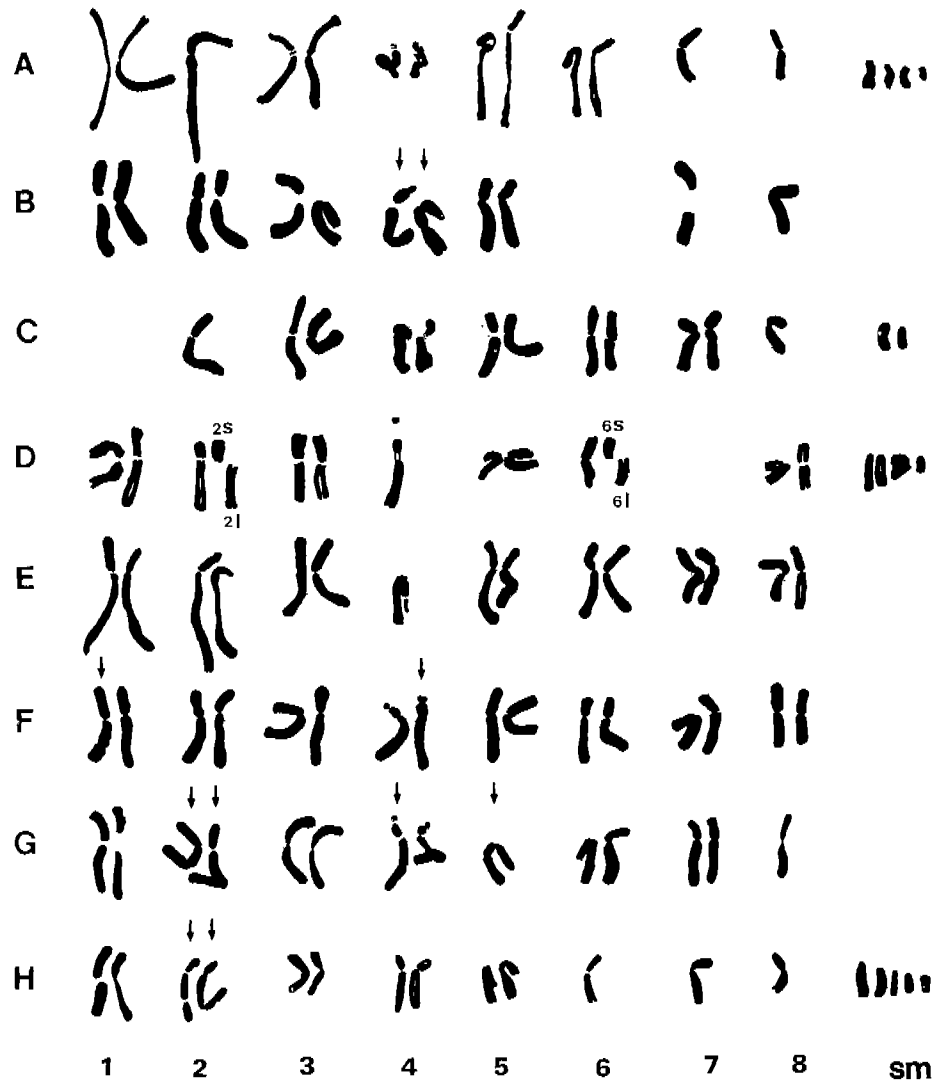

Fig. 4. The idiogram of chromosome complement in the callus cells observed in Fig. 3. Arrows indicate dicentric chromosomes. The sm represent small chromosomes.

Table 4. Structure variation of chromosomes observed in the callus cells

Ploidy level	Plate No.	Chromosome No.	Chromosomes								
			1	2	3	4	5	6	7	8	sm
Diploid	A	17		−					−	−	++++
	B	12				d,d		−,−	−	−	
	C	14	−,−	−						−	++
	D	17		c		−		c	−,−		++++
	E	15				−					
	F	16	d			d					
	G	14		d,d		d	d,−			−	
	H	17		d,d				−	−	−	+++++

+, chromosome addition; −, chromosome deletion; d, dicentric chromosome; c, centromere breakage; sm represented small chromosome.

teyn, 1983; Reichart and Novak, 1988). The presence of cytokinins was not usually necessary and callus formation obtained only when they are used in combination with auxins (Novak, 1990). Similarly this general assumption, in this study, callus formation obtained in media supplemented with combination of auxin and cytokinin but not with the cytokinin only (Data not shown).

Chromosomal behaviour in callus cells of *Allium* species has been reported by Roy (1980) and Novak (1981). In their reports, aneuploidy was increased in the callus cultures of *A. tuberosum* and polyploidy was increased in that of *A. cepa* when both species were grown in the same medium and culture conditions. Roy (1980) also reported that the difference in their chromosomal behavior was due to the genetic constitution of each species. In long term callus cultures of *A. sativum* an increase in polyploidy was reported (Novak, 1981). According to these reports the different ploidy level observed in the three types of calli maintained in different basal medium might be because of the variation of the nutrient levels in the basal culture medium. Chromosome variations were also observed due to the higher dose of nutrients like calcium chloride in potato and also an increase in chromosomal abnormalities observed according to the level of EDTA in *Haploppapus* cells (Sree Ramalu, 1984). Abraham *et al.* (1992) reported cytological abnormalities induced by magnesium sulphate in callus cultures of *Vicia faba*. In the present study, there are differences in chromosome number among different basal medium. Callus from B_5 medium supplemented with 2,4-D, NAA and BA showed maximum chromosome number variation (Table 3).

Bayliss (1973) reported that the chromosome numerical variation were induced by laggard and nonpolarization and Novak (1981) reported that the origin of aneploids directly has been related presence of micro-nuclei, chromosome bridge and laggard in callus cells. In *A. victorialis* var. *platyphyllum*, the chromosome number variations among the callus cells were correlated with the mitotic abnormalities (Tables 1 and 3).

Pavlica *et al.* (1992) reported that BA influenced on the mitotic cycle of the somatic cells in the root meristem as well as induced chromosome aberrations in *A. ascalonicum*. Ziauddin and Kasha (1990) reported that NAA induced chromosome structural variation in barley. Structural variations were not affected at different kinds of media in *A. victorialis* var. *platyphyllum*. The original chromosome number of small chromosome caused by structural variations could not be identified karyotypically therefore we consider that maybe, other method such as C- or G-banding should be applied for identification of that.

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산마늘의 캘러스細胞에서 體細胞群 變異의 核學的 分析

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적 요

MS, BDS 및 B_5에서 유도된 산마늘(*Allium victorialis* var. *platyphyllum*)의 캘러스 세포에 대한 세포 분열 지수와 염색체 변이를 분석하였다. 분열지수는 배지와 기간에 따라 0.55%에서 1.01%로 다소 낮은 편이었다. 소핵, 이핵, 다핵, 염색체교, 잔류 염색체 등의 분열 이상이 많이 관찰되었으며, 염색체의 수적 이상도 관찰되었다. 염색체의 구조적 이상도 수적 이상과 동시에 관찰되었다. 기본 배지와 염색체 변이와의 관계는 큰 차이가 없었으나 캘러스 생장은 관계가 있었다. BDS의 경우에서는 NAA와 BA가 kinetin보다 수적 변이에 보다 영향을 미치는 것으로 나타났다.

주요어 : 산마늘, 분열이상, 염색체 이상

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