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Chromosome Variation in Callus Cells Derived from Different Cytogenetic Type Plants of *Scilla scilloides* Complex

Jae-Wook BANG*, Jae-Hyun PARK, and Eun-Young CHOI
Department of Biology, Chungnam National University, Taejon, 305-764. *Corresponding author.

세포유전적 유형이 다른 무릇 (*Scilla scilloides* Complex)에서 유도된 캘러스 세포의 염색체 변이

방재욱* · 박재현 · 최은영
충남대학교 자연과학대학 생물학과

Chromosome variation in callus cells initiated from different cytogenetic type plants of *Scilla scilloides* Complex was analysed. Considerable variation in both chromosome number and structure was found in type AA, while no autosomal variation was detected in type BB callus cells. In allotetraploid AABB, two hypoploid cells were found, while a hypoploid cell and three hyperploid cells were found in eutetraploid cells of BBBB. Autosomes in callus cells derived from the plant with B-chromosomes were more stable than those from the plant without B-chromosome. We doubt that B-chromosomes have a selective function for the autosomes in culture of *S. scilloides* Complex.

Key words: autosome, B-chromosome

Genetic variations which are occurred through the cell and tissue culture of plant species have been called somaclonal variation (Larkin and Scowcroft, 1981). Amongst somaclonal variations, chromosome changes are common phenomena and the frequency of chromosome variation in cultured cells is much higher than that in intact cells (Sacristan, 1971: Bayliss, 1973, 1980: Larkin and Scowcroft, 1981).

Numerous reports indicate that callus cells of a wide range of plant species show variation in chromosome number and structure (Yamane, 1975: Sunderland, 1977: Bayliss, 1980: Novak, 1974, 1981: Jha and Roy, 1982: Nair et al., 1993). Furthermore, chromosome instability of callus cells has been thought to be closely related to the loss of morphogenetic capacity (Murashige and Nakano, 1967: Karp et al., 1987).

Scilla scilloides Complex has various cytogenetic types which are composed of two well-differentiated genomes, A (x=8) and B(x=9) (Araki, 1971: Haga and Noda, 1976). Genotypic component may be important for chromosomal stability in cultures. The stability of A and B genomes in cultured cells may be different depending on the cytogenetic types. Chromosomal changes in callus cells were reported in diploid *Scilla indica* (Charkravarty and Sen, 1983), but no report has been shown on chromosomal stability in the callus cells derived from different cytogenetic type plants. The variation of B-chromosomes in callus cells was reported only in *Secale cereale* and the selective function of B-chromosome in cultures has been suggested (Asami et al., 1976).

In the present study cytological investigation was done in the callus cells derived from bulb segment cultures of *S. scilloides* Complex with different genome compositions. The behavior of B-chromosome and the relation between autosome and B-chromosome complements in callus cells were also discussed.

MATERIALS AND METHODS

Cytogenetic types were analysed from somatic cells of root-tip (Choi and Bang, 1990; Bang and Choi, 1991) and type AA(2n=16), BB(2n=18+2F+2f), AABB(2n=34) and BBBB(2n=36+1F+1f) plants were selected. The F is metacentric B-chromosome and f is telocentric B-chromosome (Choi and Bang, 1990).

For tissue culture, bulbs were washed with 70% ethanol for 30 sec, rinsed in sterilized distilled water (SDW) three times, sterilized in 10% sodium hypochlorite solution for 10 min and then, followed by washing with SDW. Scales of bulb were dissected into 5 mm×5 mm segments and placed on the MS (Murashige and Skoog, 1962) medium containing 2 ml/L of 2,4-D, 2 ml/L of NAA, 2 ml/L of BAP and 3% (w/v) of sucrose. The medium solidified with 0.8% (w/v) Bacto-agar and pH was adjusted to 5.8.

Cytologial examination of callus cells was done by Bang's method(1990). Actively growing calli were subcultured on the fresh medium at 20 days interval. For cytological analysis, small amount of subcultured callus was placed in the refrigerator (5° C) overnight, soaked in saturated 1-bromonaphthalene for 5 to 6 hours and then, fixed in acetic alcohol(1:3). Squash method was adopted in chromosome preparation.

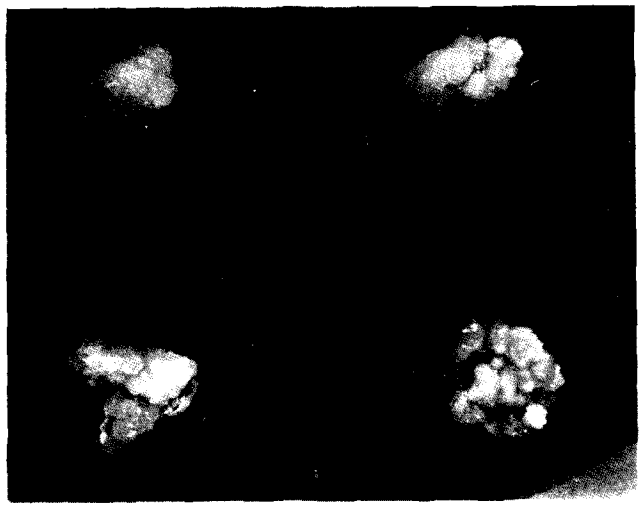

Figure 1. Calli produced from bulb segment culture of *Scilla scilloides* Complex. Upper: friable calli: lower: compact calli.

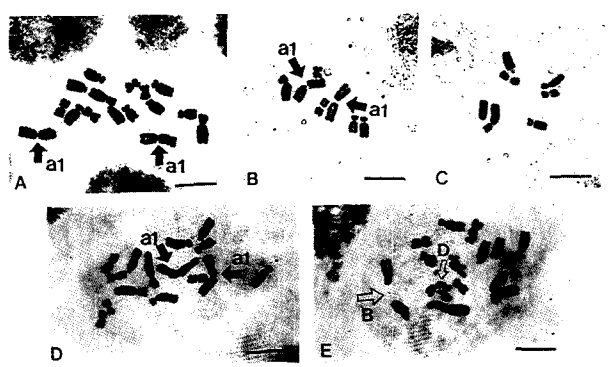

Figure 2. Chromosome complements in callus cells derived from AA genome plant of *S. scilloides* Complex (2n=16). A, Normal chromosome complement (2n=16): B, C and D, Chromosome complements showing loss of chromosomes. Arrows indicate deficient chromosomes: E, Chromosome breakage (B) and dicentric chromosome (D).

RESULTS AND DISCUSSION

Callus formed on the surface of the bulb segment after two weeks of culture. Two kinds of calli were found. One was yellow and compact shaped calli and the other was white and friable ones (Fig. 1). The former was subjected to chromosome analysis.

Considerable variation in both chromosome number and structure was observed in callus cells of type AA (Table 1). The chromosome complement of AA consisted of one pair of V-shaped large metacentric (a_1), five pairs of I-shaped subtelocentric (a_2 to a_6) and two pairs of v-shaped small metacentric (a_7 to a_8). The a_2 chromosome had secondary constriction (Araki, 1971: Choi and Bang, 1990). Elimination of chromosomes sporadically occurred in all the homologous pairs in type AA callus. Loss of whole homologous pair was found in chromosome a_1 (Fig. 2C), a_4 (Fig. 2B), and a_8 (Fig. 2C). Loss of one of homologous chromosomes was a common phenomenon. Structural changes such as dicentric due to centromeric shift and breakage of centromeric region were observed. Deficiency of chromosome arms was also found (Fig. 2C). The autosomal stability in callus cells of

Table 1. Chromosome variations in callus cells drived from different cytogenetic type plants of *Scills scilloides* Complex.[a]

Genome constitution of mother plant	Chromosome No. in callus cells	No. of cells analysed
AA (2n=16)	7	2
	12	2
	13	4
	14	7
	15	13
	16	245
	16+1S	1
	16+2S	2
	16+3S	1
total		277
BB (2n=18+2F+2f)	18	1
	18+1F+3f	1
	18+2F	8
	18+2F+1f	4
	18+2F+2f	57
	18+2F+3f	6
	18+3F+1f	1
total		78
AABB (2n=34)	19	2
	34	11
	34+1S	2
	34+1S	1
total		16
BBBB (2n=36+1F+1f)	29	1
	36	50
	36+1f	3
	36+1F+1f	8
	48	1
	68	1
	77	1
total		65

[a] F: metacentric B-chromosome, f: telocentric B-chromosome, S: chromosome segment.

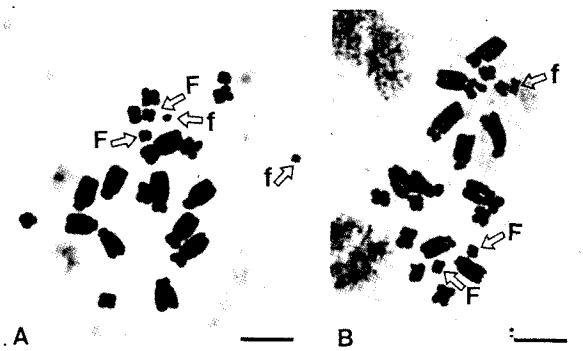

Figure 3. Chromosome complements in callus cells derived from BB genome plant of *S. scilloides* Complex (2n=18+2F+2f). A, Chromosome complement showing the same karyotype with mother plant. Arrows indicate B-chromosomes: B, Chromosome complement with to metacentric B-chromosomes (F) and one fused chromosome of telocentric B-chromosomes (f). Bars, 10 μm.

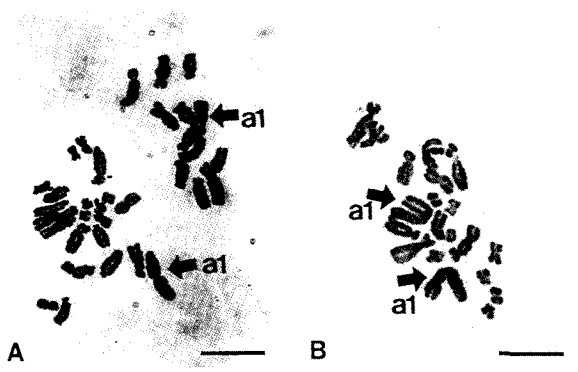

Figure 4. Chromosome complements in callus cells derived from AABB genome plant of *S. scilloides* Complex (2n=34). A, Normal chromosome complement. B, Hypoploid cell showing the elimination of fifteen chromosomes (2n=19). Arrows indicate chromosome a1. Bars, 10 μm.

AA was lower than that in any other types (Table 1).

Mother plant of type AA applied in cultures carried no B-chromosome. However, chromosome segments(S) were observed in four cells amongst 277 cells (Table 1). Occurrence of chromosome segments which were not found in the mother plants was reported in *Vicia faba* (Jha and Roy, 1982). Chromosome segment which newly appeared in cultured cells might supply a clue for the origin of B-chromosome.

No autosomal variation was detected in type BB, while numerical variations in B-chromosomes were found (Table 1). The chromosome complement of BB consisted of five pairs of subtelocentric (b_1 to b_5) and four pairs of metacentric (b_6 to b_9), and b_1 chromosome had secondary constriction (Araki, 1971: Bang and Choi, 1993). The frequency of B-chromosome variation was 26.9% and one cell out of 78 cells carried no B-chromosome. The fusion of small B-chromosomes was found in type BB (Fig. 3). In case of type BBBB, only eight cells amongst 65 cells carried the same number of B-chromosome of the mother plant. The frequency of cells with the same number of B-chromosomes

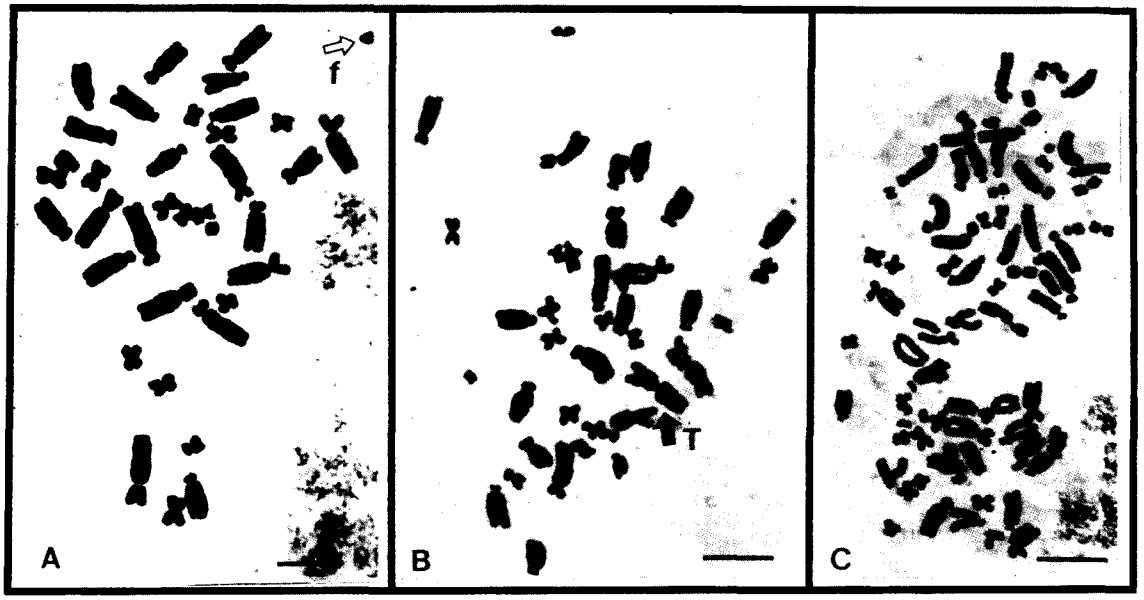

Figure 5. Chromosome complements in callus cells derived from BBBB genome plant of *S. scilloides* Complex (2n=36+1F+1f). A, Chromosome complement showing loss of one B-chromosome (2n=36+1f): B, Structural change of chromosome. T indicates translocated chromosome: C, Chromosome complement showing variation in chromosome number (2n=77). Bars, 10 μm.

of the mother plant in diploid BB was much higher than in eutetraploid BBBB (Table 1). It is interesting that the stability of B-chromosome was different depending on ploidy level. It suggests that the numerical variation of B-chromosomes originates from unequal distribution during cell division.

In allotetraploid AABB, hypoploid cells caused by elimination of chromosome were observed (Fig. 4), while a hypoploid cell and three hyperploid cells were found in eutetraploid cells of BBBB (Fig. 5). Chromosomal stability in suspension cells derived from diploid and tetraploid wild wheat was higher than that in hexaploid common wheat (Bang, 1990). However, in *S. scilloides* Complex autosomal stability in diploid, type AA was on a level of allotetraploid AABB, while autosomes in type BB with B-chromosome showed a high stability. The stability of autosome in type BB is due to the selective function of B-chromosome (Asami et al., 1976: Bang and Choi, 1990).

In eutetraploid BBBB, numerical and structural variations of autosome which was not detected in type BB were found. The multiplication of chromosome complement which was not found in other cytogenetic types was also found in type BBBB callus cells (Table 1). The elimination of B-chromosomes was the common phenomenon in eutetraploid BBBB.

Numerous factors such as ploidy level, genotype, regeneration procedure, source of tissue applied, hormonal composition in medium, endomitosis and nondisjuction affect the chromosome variation in cultured cells (Sunderland, 1977: Karp, 1988: Oh and Kim, 1988). We suggest that the present case of *S. scilloides* Complex, different cytogenetic type, ploidy level and B-chromosome may affect chromosome variation in the callus cells.

Callus cells of type BB and BBBB were derived from the plant with B-chromosomes. It is interesting that variation rate (87.7%) of B-chromosome in BBBB callus cells with 2F and 2f was much higher than that (26.9%) in diploid BB callus cells with 1F and 1f while autosomes in BB were more stable than in BBBB. That might be caused by ploidy level. B-chromosomes incidentally increase the frequency of crossing-over in autosomes (Carlson et al., 1993) and may carry a gene regulating expression of the structural gene (Ruiz Rejon et al., 1980). We doubt that B-chromosomes have effective role for stability of autosomes in culture of *S. scilloides* Complex.

적 요

세포유전적 유형이 다른 무릇 (*Scilla scilloides* Complex)

의 조직배양에서 유도된 캘러스에서 염색체 변이를 조사하였다. AA유형의 캘러스에서 심한 수적, 구조적 변이가 관찰된 반면, BB 유형의 캘러스 세포에서는 상염색체의 변이가 발견되지 않았다. 이질 4배체인 AABB유형에서는 두개의 hypoploid 세포만 관찰되었으나, B게놈의 진정4배체인 BBBB유형에서는 hypoploid 세포와 함께 hyperploid 세포가 관찰되었다. 캘러스 세포에서 상염색체의 안정성은 B염색체를 지닌 식물에서 유도된 캘러스에서 더 높게 나타났다. 이는 배양세포에서 B염색체가 상염색체의 안정성에 선택적으로 기능하기 때문인 것으로 여겨진다.

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