식물학회지 제32권 제3호
Korean J. Botany 32(3) : 173-180(1989)

# Giemsa C-banded Karyotypes and Their Relationship of Four Diploid Taxa in *Allium*

**Seo, Bong Bo, Hyun Hee Kim and Ju Hwan Kim**
(Department of Biology, Kyungpook National University, Taegu)

# *Allium*屬 二倍體 4種의 C-分染 核型 및 類緣關係

徐 奉 甫·金 炫 希·金 周 煥
(경북대학교 생물학과)

## ABSTRACT

Giemsa C-banded karyotypes of *A. thunbergii*, *A. deltoide-fistulosum*, *A. cyaneum* and *A. cyaneum* var. *deltoides* were analyzed, and the interspecific relationships were investigated on the basis of the C-banding patterns. The banding pattern of each species was unique and made possible an easy and clear separation among them. *A. thunbergii* and *A. deltoide-fistulosum* revealed very close banding relationship but *A. cyaneum* and *A. cyaneum* var. *deltoides* showed significant difference in banding pattern in spite of their close plant systematic position.

## INTRODUCTION

The genus *Allium* is known to be distributed over the world and classified into about 500 species, and among them above ten species are grown in the wild population of Korea(El-Gadi and Elkington, 1975;Yu *et al.*, 1981). These species are in some difficulties to identify becausee they share very similar morphological characteristics and chromosomal complements.

From 1970's, chromosome identification and studies of the linear differentiation of chromosomes have greatly been improved by the analysis of preferential Giemsa staining of certain chromosomal regions resulting in specific banding pattern after incubation in solution of various reagents prior to the Giemsa stain. This technique for the identification of individual chromosome is used to distinguish each species and to resolve systematic relationships between related species(Schweizer, 1973). Giemsa C-banding karyotypes in genus *Allium* have been reported by many authors to resolve the interspecific relationships(Stack and Clarke, 1973;El-Gadi and Elkington, 1975;Vosa, 1976a, b; Al-Sheikh Hussain and Elkington, 1978;Kancko and

This is a part of "Giemsa C-banded karyotypes and phylogenic studies in *Allium* from Korea" supported by the Korean Science and Engineering Foundation(871-0406-014-2), 1987-1989

Tashiro, 1982;Kalkman, 1984;Cortes and Escalza, 1986;Nishitani and Yabuno, 1986;Cai and Chinnappa, 1987). But only a few seeies distributed in Korea were reported on the C-banding karyotypes(Seo and Kim, 1974; Seo, 1977).

In this paper we describe the Giemsa C-banding patterns and their relationships on the four diploid taxa which will be contribute to our aim to investigate the *Allium* speciation by means of C-banding karyotype.

## MATERIALS AND METHODS

The species studied are listed in Table 1. The identification of the species in each collection site was followed by the criteria of Yu *et al.*(1981).

Actively growing root tips excised from bulbs were pretreated in 0.002M 8-hydroxyquinoline for 6 hours at 18 C, fixed in a mixture of acetic acid-ethanol(1:3) for 2-3 hours at room temperature and stored in 95% ethanol at 4℃.

The conventional karyotypes were investigated using the 1% aceto-orcein stained squash preparation, and modified BSG technique(Schweizer, 1973) was applied to band the chromosomes. The root tips were macerated in 45% acetic acid for 2-3 hours at room temperature, hydrolyzed for 15 seconds in 1N-HCl at 60℃, and squashed in 45% acetic acid. After air dried overnight, the coverglasses were detached by immersion of the preparation in 10% acetic acid bottom side up and dried over one day. The dried slides were immersed in 8%(w/v) aqueous solution of barium hydroxide for 7 minutes at room temperature, washed in two changes of distilled water, incubated in 2XSSC at 60℃ for 30 minutes, and rinsed in two changes of distilled water. The slides were stained in a Giemsa solution which prepared freshly from the Giemsa stock solution by dilution to 3%(v/v) with M/15 Sörensen phosphate buffer, pH 6.8, for 15–20 minutes at room temperature, rinsed in distilled water, and mounted with Canada balsam through the processes using buthanol.

Well spreading and banding metaphases were selected and analyzed through microphotographs taken with Olympus microscopic camera set using double filters of green and orange colors which provided more apparent banding pictures in case of using the Fuji high contrast microfilm of ASA 32(Table 1).

**Table 1.** Plant materials used in this study

| species | somatic chromosome (2n) | collection site |
|---|---|---|
| *Allium thunbergii* | 16 | Prov. Jeonnam Hongdo Is. |
| *A. deltoide-fistulosum* | 16 | Prov. Jeonnam Segeol Mt. |
| *A. cyaneum* | 16 | Prov. Jeonbuk Jiri Mt. |
| *A. cyaneum* var. *deltoides* | 16 | Prov. Kyungnam Kaya Mt. |

## RESULTS

Four taxa investigated in this study are all diplod of 2n=16 which reveal the same morphology in conventional karyotypes(Fig. 1). The chromosome components are metacentrics to submetacentrics, excepting a pair of sat-chromosomes of subtelocentrics, and show a continuous transition in metaphase chromosomal length ranging from 5.9 to 12.4 $\mu$m. C-banding pattern of each species is unique but some common features are found among the banding patterns of the species. No centromeric band exists in these species.

***Allium thunbergii;*** In this species about 5.7% of the total chromosome length is banded. Banding patterns between homologues are more or less variable in chromosomes e, g and h. No band appears in chromosomes a and b, while others show the distinct bands. Sat-chromosomes have the bands only in the short arms, and chromosomes c only in the long arms whereas others in both arms(Figs. 2 and 3).

***Allium deltoide-fistulosum;*** The amount of band to the total chromosome length in this species is 5.9% which is distributed on six pairs of chromosomes. Chromosomes a and c show no band. Chromosomes b and d show the relativly thick bands in the intercalary parts of the short arms. Sat-chromosomes have characteristic bands in the short arms but a minor intercalary band appears in the long arm of one of the homologues. Three pairs of chromosomes(e, g and h)have the terminal and intercalary bands in both arms. Chromosomes f and g show somewhat differences in banding patterns within the homologues(Figs. 2 and 3).

***Allium cyaneum;*** In this species very small amount(about 1.5%) of the total chromosome length is banded. Bands distributed in telomeric parts of the seven pairs excepting no band of chromosomes b(Figs 2 and 3). Sat-chromosomes have relatively thick band in proximal part of the secondary constriction whereas other pairs have very thin telomeric bands in the only short arms or both arms. Chromosomes h show heterozygous in banding patterns on intercalary region of the short arm and telomeric region of the long arm.

***Allium cyaneum var. deltoides;*** The chromosome banding is distinctive, approximately 5.9% of the total chromosome length being banded. Six pairs of the complement chromosomes have bands, and each chromosome pair is distinguishable by the banding size and distribution. The terminal bands in the long arms of chromosomes e, g and h are heterozygous. The intercalary band in the long arms of sat-chromosomes is unpaired.

## DISCUSSION

Four taxa investigated in this study showed same diploid chromosome number of 2n=16 including a pair of sat-chromosomes and seven pairs of interspecifically undistinguishable chromosome complements by conventional staining method.

There were some intra-specific variations in banding formation, and so metaphases showing maximum banding response were selected as representative banding patterns of the species, and

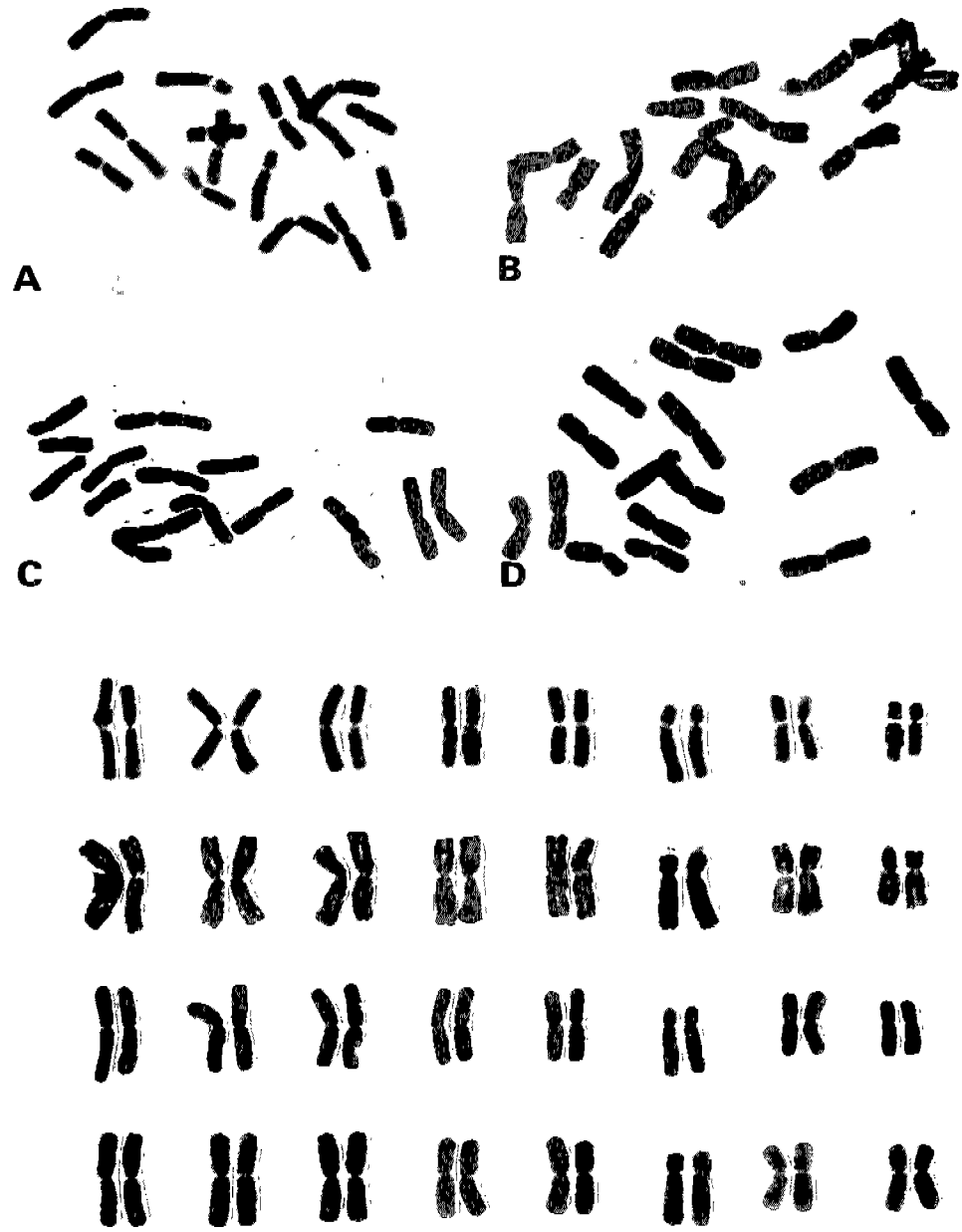


**Fig. 1.** Metaphase plates and idiograms of *A. thunbergii*(A & E), *A. deltoide-fistulosum*(B & F), *A. cyaneum*(C & G) and *A. cyaneum* var. *deltoides*(D & H). arrows:sat-chromosomes.

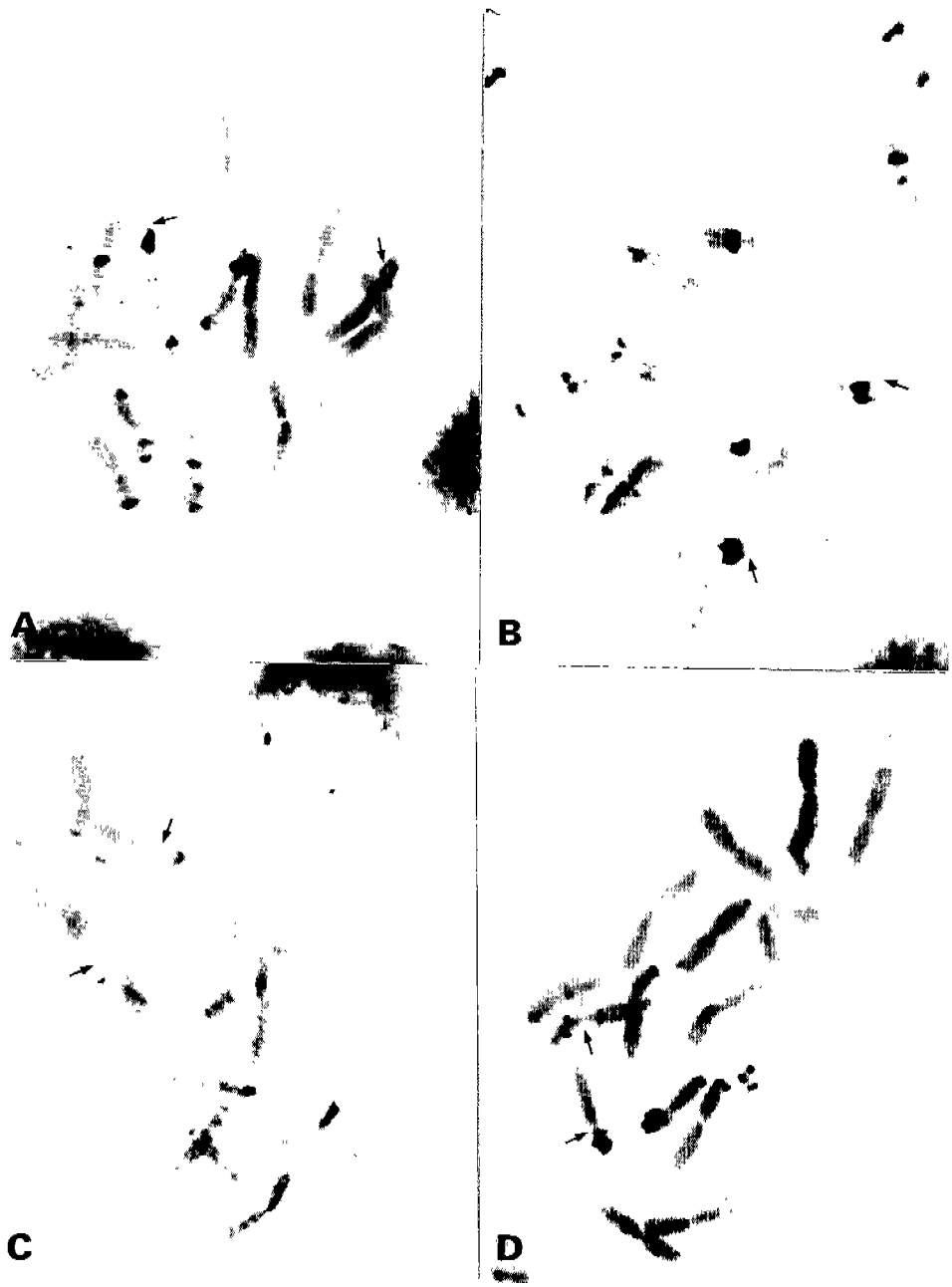


**Fig. 2.** C-banded metaphase plates of *A. thunbergii*(A), *A. deltoide-fistulosum*(B), *A. cyaneum*(C) and *A. cyaneum* var. *deltoides*(D). arrows:sat-chromosomes.

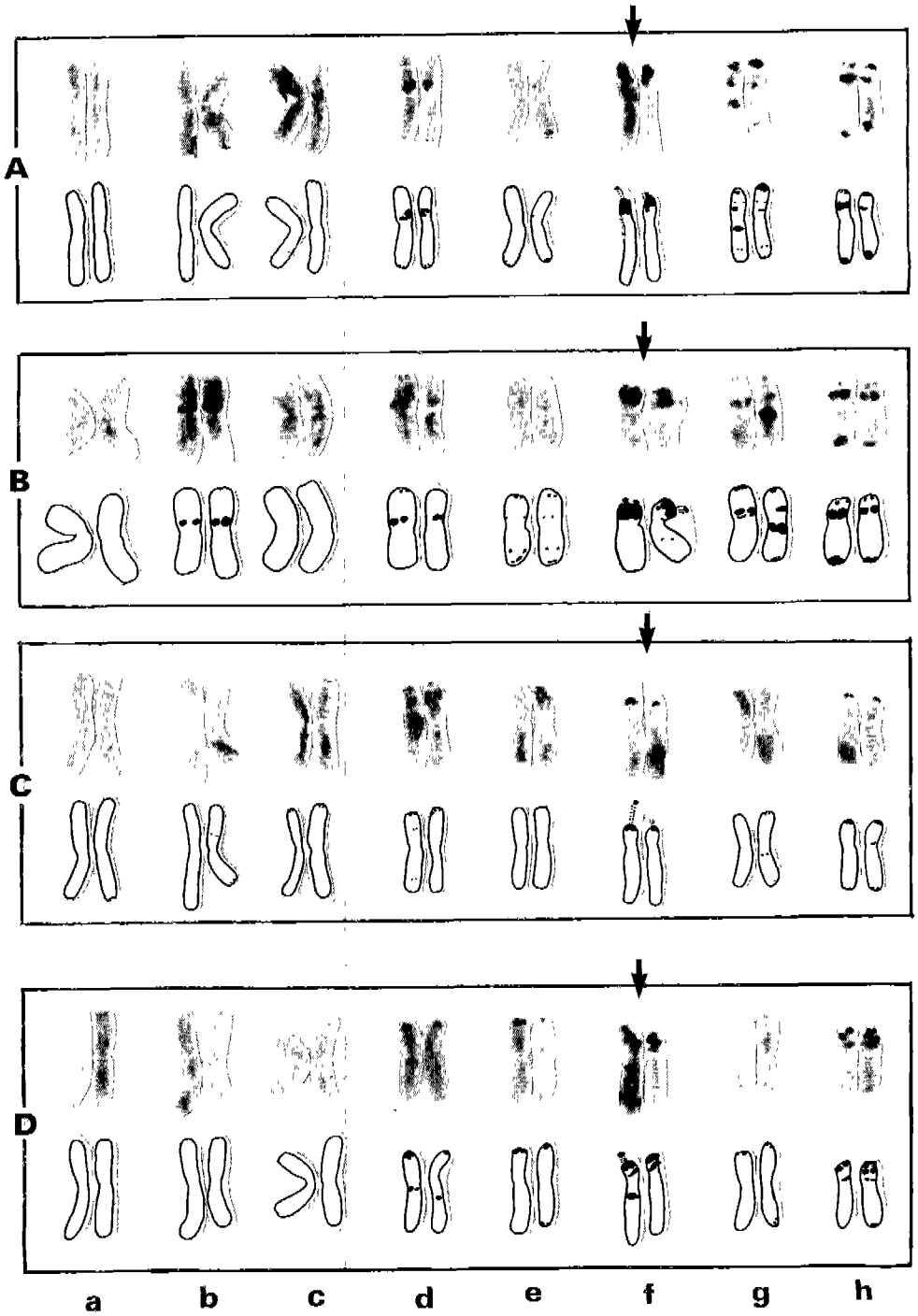


**Fig. 3.** C-banded idiograms and their diagrams of *A. thunbergii*(A), *A. deltoide-fistulosum*(B), *A. cyaneum*(C) and *A cyaneum* var. *deltoides*(D). arrows:sat-chromosomes.

analyzed as coupled with other banding metaphases showing variations. The banding variation was attributed to the technical differences for banding formation or to the genome itself. Schweizer and Ehrendorfer(1976) reported that there were several types of the constitutive heterochromatin detected by C-banding, and that the variation within and between individuals in banding patterns are considered largely due to the differences in amounts and types of constitutive heterochromatin linked with the degree of the chromosome contraction.

Interspecific differences in banding patterns were fully enough to separate the species in taxonomic situation. Vosa(1976) reported that the technique for the linear differentiation of the chromosomes provides a very useful tool for assessing taxonomic and phylogenic relationships. Although each species under this study showed the unique choromosome markers of banding patterrn, it is possible to compare the interspecific relationships on the basis of C-banding patterns.

*A. cyaneum* and *A. cyaneum* var. *deltoides* has been identified as a same species according to morphological taxonomy. Therefore these two taxa were supposed to reveal very closely related banding patterns considering their plant systematic position. But the banding types of these two species turned out to be considerably different except that the relatively thick bands appeared in the proximal parts of the secondary constrictions of the sat-chromosomes, and so the close relationship of the two taxa is questionable. In contrast to this, the C-banding patterns of *A. thunbergii* and *A. deltoide-fistulosum* were very similar in major bands, but the intercalary bands in the short arms of the two pairs(b and d) were retained in *A. deltoide-fistulosum while one pair(d) in A. thunbergii*. On the basis of the banding distribution and size, there were close relationship between *A. thunbergii* and *A. deltoide-fistulosum,* and limited relationship between *A. cyaneum* and *A. cyaneum* var. *deltoides*. Interspecific polymorphism of banding patterns in chromosomes d, f and g of these materials shall be explained by pericentric inversion or simple crossing over mechanism. The general interspecific relationship and phylogeny in genus *Allium* will be framed as a direction using the amounts of constitutive heterochromatin containing other wild species in further report.

## 적 요

산부추, 세모부추, 한라부추, 한라세모부추에 대한 Giemsa C-분염 핵형을 분석하고 분염 양상에 따른 종간 유연관계를 조사하였다. 분염은 각종에 따라 특징적인 양상을 보여 종 구분을 명확히 했다. 산부추와 세모부추는 분염양상에 상당한 유사성을 보였으나 한라부추와 한라세모부추는 분류학적 근연성에도 불구하고 상당한 차이를 보였으므로 분류학적 위치를 재고해야 할것으로 본다.

## REFERENCES

Al-Sheikh Hussain, L.A. and T.T. Elkington. 1978. Giemsa C-band karyotypes of diploid and triploid *Allium caeruleum* and their genomic relationship. *Cytologia* **43 :** 405–410

Cai, Q. and C.C. Chinnappa. 1987. Giemsa C-banded karyotypes of seven north American species of

*Allium*. *Amer. J. Bot.* **74** : 1087–1092

Cortes, F. and P. Escalza. 1986. Analysis of different banding patterns and late replicating regions in chromosomes of *Allium cepa, A. sativum* and *A. nigrum*. *Genetica* **71** : 39–46

El-Gadi, A. and T.T. Elkington. 1975. Comparison of the Giemsa C-band karyotypes and the relationships of *Allium cepa, A. fistulosum* and *A. galanthum*. *Chromosoma*(Berl.) **51** : 19–23

Kalkman, E.R. 1984. Analysis of the C-banded karyotype of *Allium cepa* L., Standard system of nomenclature and polymorphism. *Genetica* **65** : 141–148

Kaneko, K. and F. Tashiro. 1982. C-band karyotype of *Allium fistulosum*. *Bull. Fukuoka Univ. of Educ.* **32** : 55–58

Nishitani, S. and T. Yabuno. 1986. Cytological studies of *Allium togashii* Hara(Liliaceae). CIS **41** : 6–9

Schweizer, D. 1973. Differential staining of plant chromosomes with Giemsa. *Chromosoma* **40** : 307–320

Schweizer, D. and F. Ehrendorfer. 1976. Giemsa banded karyotypes, systematics, and evolution in *Anacyclus*(Asteraceae-Anthemideae). *Plant Syst. Evol.* **126** : 107–148

Seo, B.B. 1977. Cytogenetic studies of some tetraploids in *Allim*. *Korean J. Bot.* **20** : 71–76

Seo, B.B. and J. H. Kim. 1975. Karyotypic analyses based on the heterochromatin distribution in *Allium fistulosum* and *A. ascalonicum*. *Korean J. Bot.* **18** : 92–100

Stack, S.M. and C. R. Clarke. 1973. Differential Giemsa stain of the telomeres of *A. cepa*:observations related to chromosome pairing. *Can. J. Genet. Cytol.* **15** : 619–624

Vosa, C. G. 1976. Heterochromatic patterns in *Allium* I. The relationship between the species of the *cepa* group and its allies. *Heredity* **36** : 383–392

Vosa, C. G., 1976. Heterochromatic banding patterns in *Allium* II. Heterochromatin variation in species of the *paniculatum* group. *Chromosoma* **57** : 119–133

Yu S. O., S. T. Lee and W. T. Lee. 1981. A taxonomic study of the *Allium* species in Korea. *J. Korean Pl. Tax.***11** : 21–41

(1989. 6.20 接受)