[韓作誌(Korean J. Crop Sci.) 41(4) : 429~433(1996)]

C-banding Pattern of Mitotic Chromosome in Korean Indigenous Maize

In Sup Lee*, Bong Ho Choe** and J. P. Gustafson***

韓國 在來種 옥수수 體細胞 染色體의 C-分染패턴

이인섭* · 최봉호** · 제이. 피. 거스타프손***

ABSTRACT : A Giemsa C-banding method was used for the identification of somatic chromosomes of Korean indigenous maize(*Zea mays* L.). Two Korean indigenous maize stocks and an American hybrid maize for comparison were examined. Ten deeply stained heterochromatic knobs whose position and size were different between the genotypes, two satellites and interstitial bands were observed. The length of homologous chromosomes compared by the relative lengths of chromosomes presented as a percentage of the length of chromosome 10 were different between the genotypes. The Giemsa method proved to be useful for the identification of somatic chromosomes and for the characterization of different stocks of Korean indigenous maize.

Key words : Maize, Giemsa C-banding, Heterochromatic knob.

Zea mays is one of the most important cereals of the world and it is the cytogenetically best known organisms due largely to readily analyzable pachytene chromosomes. The identification of maize chromosomes according to their relative length, centromere position, knobs and prominent chromosomes, determined from pachytene studies(Rhoades, 1950 ; Neuffer *et al.*, 1968; McClintock, 1978; Aguiar-Perecin and Vosa, 1985) is now standardized and generally accepted. While meiotic chromosomes are well known, mitotic chromosomes have not been studied extensively because they are relatively small and the knob structures are invisible by conventional chromosome staining. Mitotic chromosomes of root tip cells can be identified in conventionally stained cells by arm ratio measurements(Chen, 1969; Horn and Walden, 1971; Filion and Walden, 1973), but knob constitution cannot be readily determined in most cells.

Now many techniques are available for preferential staining of heterochromatic region in mitotic chromosomes. In plants, most methods are C-banding procedures using bar-

*慶星大學校 (Dept. of Biology, Kyungsung University, Pusan 608-736, Korea)
**忠南大學校(Dept. of Agronomy, Chungnam National University, Taejon 302-764, Korea)
***미주리대학교(USDA-ARS, Dept. of Agronomy, University of Missouri, Columbia, MO. 65211, U.S.A.)
〈'96. 3. 28 接受〉

ium hydroxide treatment followed by incubation in saline citrate. Representative examples of these techniques for maize have been reported by Vosa and Aguiar(1972), Hadlaczky and Kálmán(1975), Sachan and Tanaka (1977), Ward(1980), and Aguiar-Perecin and Vosa(1985). They have revealed the presence of distal bands which corresponded with knobs on the C-banded mitotic chromosomes.

Korean indigenous maize lines have prominent characteristics(for example;multi-ear, multi-tillering and high protein content), and these can be utilized in maize breeding. In this study, karyotypes of C-banded mitotic chromosomes of Korean indigenous maize and C-banded mitotic chromosomes with different knob positions were examined.

MATERIALS AND METHODS

Inbred stocks of Korean indigenous maize, PI213749 and Waesungri, were used for this study. These stocks have characteristics of multi-tillering.

A modified Leishman C-banding employed by Bennet *et al*(1977) on rye (*Secale cereale* L.) was adopted as the basic treatment. The steps involved in the basic schedule for C-banding of maize were as follows.

1. Pretreat root tips in 0.04% 8-hydroxyquinoline for 3 hr. at room temperature.
2. Rinse root tips in distilled water and fix in 45% acetic acid overnight in the refrigerator.
3. Rinse root tips in distilled water and place them in 0.2N HCl for 50~60 min.
4. Rinse root tips in distilled water and place them in enzyme solution of 1.5% cellulase (Onozuka R-10; Yakult Honsha Co. LTD., Japan) for 20~25 min. at room temperature.
5. Squash root tips using 45% acetic acid, then remove cover slips by dry ice method.
6. Rinse slides in absolute EtOH for 1 hr., after air drying, stored slides in a desicator until use.
7. Place slides in saturated $Ba(OH)_2$ for 5 min. at room temperature.
8. Run distilled water into $Ba(OH)_2$ solution until $Ba(OH)_2$ is replaced, then place slides in a clean dish and rinse.
9. Transfer slides to two changes of 2XSSC solution.
10. Place slide dishes into preheated water bath and gradually raise the temperature to 60℃ and allow to incubate for a total of 1 hr.
11. Stain immediately in Leishman's stain (BDH Chemicals) diluted 1:20 in phosphate buffer (BDH Chemicals) at pH 6.8 for about 20 min. to 90 min. until desired density is obtained.
12. Dip slides once in distilled water and air dry.
13. Transfer slides to xylene for 24 hr. before mounting with permount.

Identification of chromosomes were referred to Brown(1967), Chen(1969), Horn and Walden(1971), and Margarida *et al.*(1985).

Chromosome length was determined from photographic prints magnified to ×5000, with a pair of calipers. Chromosome relative length was estimated by percentage of the length of chromosome 10.

Results and Discussion

Giemsa C-banded metaphase plates of PI 213749 and Waesungri are presented in Fig.

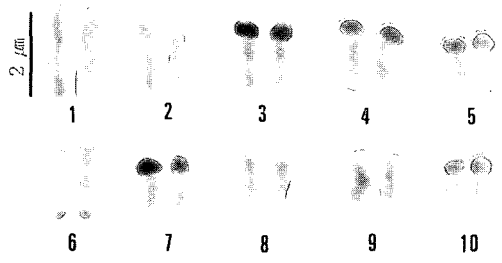

Fig. 1. The somatic karyotype of Korean indigenous maize.

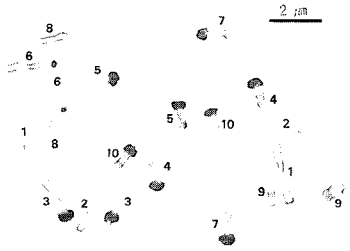

Fig. 2. Giemsa stained somatic metaphase of Waesungri.

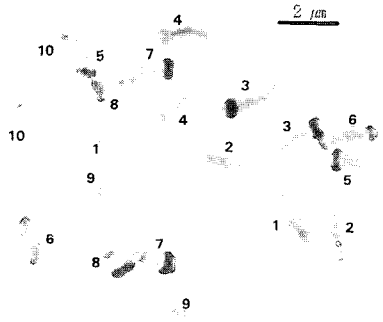

Fig. 3. Giemsa stained somatic metaphase of PI213749.

1, 2, and 3. Five pairs of the chromosome component show dark and heterochromatic bands and one pair has a satelite. According to the heterochromatic banding patterns as well as the arm ratios and relative length, the chromosomes can be identified as follows;

The chromosomes 3, 4, 5, 7 and 10 in Waesungri showed terminal dark knobs, whereas the chromosomes 3, 5, 6, 7 and 8 in PI213749 showed terminal dark knobs.

The knobs of the chromosome 4 and 10 in Waesungri and knobs of the chromosome 6 and 8 in PI213749 were unique to each stock.

Chromosome 1 and 2: These chromosomes appeared to have no heterochromatic knobs.

Chromosome 3: The long arm had a heterochromatic knob at the telomeric end and a bright portion at the telomeric end.

Chromosome 4: The chromosome had a heterochromatic knob at the end of short arm in Waesnugri, and the centromere had a small faint band at the short arm side in both stocks.

Chromosome 5: The chromosome had a heterochromatic knob at the end of long arm in both stocks. 2 spot-like bands were shown at centromeric protion in PI213749 stock.

Chromosome 6: The short arm had a intensive heterochromatic satelite. The long arm of this chromosome in PI213749 stock had a heterochromatic knob in the distal portion, and interstitial band was appeared in the centromere portion.

Chromosome 7: A deeply stained stained heterochromatic band was appeared near to the telomeric and of the long arm. A faint band was appeared at the short arm side of centromere in both stocks.

Chromosome 8: The long arm had a terminal knob and a faint band at the centromere portion in PI213749 stock. The short arm had a faint terminal band in Waesungri.

Chromosome 9: A faint band was appeared at the centromere portion in the Waesungri.

There was no band in PI213749.

Chromosome 10: A terminal knob was appeared at the end of the long arm in Waesungri. A faint centromeric band was appeard in PI213749.

Whereas, American hybrid maize(Mo17 × B73) showed 2 pairs of heterochromatic knob and a pair of satelite, and inbred maize (Pa405) showed 4 pair of heterochromatic knob and a pair of satelite.

Reeves(1944), Bianchi *et al.*(1963), and Smith and Goodman(1981) studied on number and position of chromosome knob of American maize and Italian maize, and reported that the number and the position of chromosome knob were varied to the genotypes and locations collected.

Korean indigenous maize showed 10 chromosome knobs and 2 satelites. This result showed that Korean indigenous maize have more chromosome knobs than American maize (4.21) and Italian maize (2.70). Korean indigenous maize also showed that the position and the size of chromosome knob were varied to the genotypes.

Table 1 shows the values of relative lengths of chromosomes presented as a percentage of the length of chromosome 10 and estimated on 10 metaphases of Waesungri, PI213749 and Mo17 × B73(American hybrid maize). As shown in Table 1, the relative lengths of chromosomes in Waesungri were generally varied more than those in PI 213749. Especially chromosome 1, 2, and 3 in Waesungri were relatively larger than those in PI213749.

Estimates of maize somatic chromosome lengths have been presented as a percentage of the total complement length (Chen, 1969; Filion and Walden, 1973; Aguiar-Perecin and Vosa, 1985). Aguiar-Perecin and Vosa reported that the statistical comparison of chromosome indices showed differences which were significant at 1% level of probability between homologous chromosomes of *Zapalote chico* and on *Ceremonial* maize. Above result revealed that the relative lengths of homologous chromosomes were different between the genotypes and showed the same tendency to the report of Aguiar-Perecin and Vosa.

Table 1. Relative chromosome lengths expressed as percentage of chromosome 10 in Waesungri, PI213749 and Mo17 × B73

Chromosome	Waesungri (n=10)	PI213749 (n=10)	(Mo17 × B73)*
1	223.22	192.03	244.50
2	193.23	161.34	188.99
3	178.16	157.73	174.94
4	161.41	152.74	161.51
5	147.23	140.58	145.48
6	144.56	139.69	134.16
7	124.73	137.06	131.75
8	123.84	119.67	124.69
9	114.71	110.20	111.57
10	100.00	100.00	100.00

n=Number of metaphases analyzed
* ; Ameriacan hybrid produced at the University of Missouri

The present study shows that the 10 pairs of chromosomes of maize can be distinguished from each other by the combination of their relative lenths, arm ratios and cytological markers (e.g., the satelite on chromosome 6). From the relation of the localization of heterochromatic bands to the distribution of heterochromatic knobs on metaphase chromosomes, it appears that the Giemsa C-banding procedure is relatively simple method to detect bands and knobs on somatic chromosomes of different stocks and hybrids of maize. The heterochromatic banding patterns and knobs on metaphase chr-

omosomes may be useful in maize cytogenetics such as chromosome mapping, detection of hybrid stocks and tracing out the ancestry of different stocks.

摘 要

Giemsa C-banding 방법에 의하여 한국 재래종 옥수수 핵형 및 염색체의 특성을 알아보고자 옥수수의 근단 생장점을 이용하여 조사 분석하였다. 이 방법은 한국 재래종 옥수수의 핵형 분석 및 염색체의 특성 연구에도 효과적으로 활용할 수 있는 방법임이 확인되었다. 조사된 재래종 옥수수에서는 각각 10개의 heterochromatic knob이 발견되었으며 이것들의 크기와 위치는 계통별로 다른 것으로 나타났고, 모두가 6번 염색체에 부수체를 가지고 있었다. 10번 염색체를 100으로 해서 비교해 본 각 염색체들의 상대적 길이는 조사된 계통별로 큰 차이가 있었다.

LITERRATURE CITED

1. Aguiar-Perecin, de, M. R. L. and C. G. Vosa. 1985. C-banding in maize; Ⅱ. Identification of somatic chromosomes. Heredity, 54 : 37-42.
2. Bennett, M. D., J. P. Gustafson and J. B. Smith. 1977. Variation in nuclear DNA in the genus Secale. Chromosoma, 61 : 149-176.
3. Bianch, A., M. V. Chatnekar and A. Ghidoni. 1963. Knobs in Italian maize. Chromosoma, 14 : 601-617.
4. Chen, C. C. 1969. The somatic chromosomes of maize. Can. J, Genet. 11 : 752-754.
5. Filion, W. G. and D. B. Walden. 1973. Karyotype anaysis: The detection of chromosomal alternations in the somatic karyotypes of *Zea mays* L. Chromosoma, 41 : 183-194.
6. Hadlaczky, G. Y. and Kálmán, L. 1975. Discrimination of homologous chromosomes of maize with Giemsa staining. Heredity, 35 : 371-374.
7. Horn, J. D. and D. B. Walden. 1971. Fluorescent staining of euchromatin and heterochromatin in maize(*Zea mays*). Can. J. Genet. Cytol. 13 : 811-815.
8. McClintock, B. 1978. Significance of chromosome constitution in tracing the origin and migration of races of maize in the Americans. In *Maize Breeding* and *Genetics*. Walden, D. B. (ed.) Wiley, NY. pp159-184.
9. Neuffer, M. G., L. Jones and M. S. Zuber. 1968. The mutants of maize. p74.
10. Reeves, R. G. 1944. Chromosome knobs in relation to the origin of maize. Genetics, 29 : 141-147.
11. Rhoades, M. M. 1950. Meiosis in maize. J. Hered. 41 : 58-67.
12. Sachan, J. K. S. and R. Tanaka. 1976. A banding methods for Zea chromosomes. Jpn. J. Genet. 51 : 139-141.
13. Smith, J. S. C. and M. M. Goodman. 1981. Comparison of chromosome knob frequencies between sympatric and allopatric populations of teosinte and maize. Amer. J. Bot. 68(7) : 947-954.
14. Vosa, C. G. and M. L. R. de. Aguiar. 1972. New techniques for knob detection in mitotic chromosomes in maize and teosinte. Maize Genet. Coop. Newsl. 46 : 165-167.
15. Ward, E. J. 1980. Banding patterns in maize mitotic chromosomes. Can J. Genet. Cytol. 22 : 61-67.