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Heterochromatic Knob Number And Karyotype in Korean Indigenous Waxy Corn by Giemsa C-banding Pattern of Mitotic Chromosome

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A Giemsa C-banding method was used for the identification of somatic chromosomes and heterochromatic knob position in Korean indigenous waxy corn (*Zea mays* L.). 5 inbred stocks were examined and their heterochromatic knob numbers ranged from 6 to 12. In comparison of homologous chromosomes of two stocks of YS-1 and MY-1, knob numbers, knob positions, arm ratios and relative length of chromosomes were different between the genotypes. The length of homologous chromosomes in YS-1 were generally larger than those of MY-1. The Giemsa method was proved to be useful for the identification of somatic chromosome and a C-banded diagram showing knob positions, arm ratios and relative length of chromosome could be used as a good tool to compare the characteristics of chromosomes of Korean indigenous waxy corn stocks.

Key words – Giemsa C-banding, Heterochromatic knob, Indigenous waxy corn

Intoduction

The chromosomes of corn have a number of distinguishing features to identify a chromosome to the other. Among them dark staining spot known as heterochromatic knob is one of the important features. These knobs have been shown to appear at certain points on certain chromosomes and knob number is constant for any individual plant. Therefore, it can be used as one criterion in determining the relationship among various kinds of corn.

Heterochromatin is cytologically recognizable in interphase and early stages of mitosis and meiosis as highly condensed regions of chromatin. Heterochromatin can be visualized with Giemsa stain as knobs or bands in mitotic or meiotic metaphase preparations [2]. The preferential staining ability is probably due to a DNA-protein interaction involving protein that are specifically associated with the heterochromatin [7]. Heterochromatin is known to consist of highly repetitive DNA, and to be genetically silent [10]. The presence of heterochromatin blocks in the genome has been shown to influence both chiasma formation and genetic recombination in *Zea mays* L [15]. In corn, furthermore, chromosome knob has been used to suggest evolutionary relationship between different taxa of this genus [12,17].

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While C-banded somatic metaphases have revelled the presence of distal bands that correspond with knobs [8,11, 16,18,19], cytogenetic studies such as karyotypes, chromosome knob number and position, and chromosome arm ratio on Korean indigenous corn have been a few [12].

In the present study, to know the knob numbers of Korean indigenous waxy corn, 5 inbred stocks were investigated. Also arm ratio and relative length of chromosome of two representative stocks which had different knob positions on the chromosome were measured, and diagrams reveling the knob positions, arm ratio and relative length of chromosome were presented.

Materials and Methods

Five inbred stocks of Korean indigenous waxy corn were used for this study. The stoks and collection sites are shown in Table 1.

A modified Leishman C-banding employed by Bennet et al. [2] on rye (*Secale cereale* L.) was adopted as the basic treatment. The C-banding procedure previously described by Lee et al. [11] was used. Chromosome was investigated

Table 1. Stocks used for the study and their collection site

Stock	Collection site	Stock	Collection site
YS-1	Yangsan	KH-2	Kimehae
MY-1	Miryang	HA-2	Haman
SC-1	Sancheong		

with phase-contrast microscope (Olympus, B202). Identification of chromosomes was referred to Chen [5], Horn and Walden [9].

Chromosome length was determined from photographic prints magnified to ×5,000, with a pair of calipers. Arm ratio showing short arm versus long arm and chromosome relative length estimated by percentage of the length of chromosome 10 were calculated with 5 samples.

For the diagrammatic representation of each chromosome of the complement, homologous chromosome pairs were compared 5 times, respectively

Results and Discussion

Five inbred stocks of Korean indigenous waxy corn were stained by Giemsa C-banding method and heterochromatic knobs and chromosome size were investigated under phase-contrast microscope. The knob numbers observed are shown in Table 2.

In Table 2, knobs in these stocks ranged from 6 to 12, and average number was 9.2. The knob number on the chromosome vary from stock to stock, and the results show the same tendency to Central and South America corn, the United States corn and Italian corn.

Reeves [14] collected corn seeds from Central and South America, and investigated chromosome knob. The knob numbers ranged from 0 to 9 with the greatest number in Guatemala, the lowest in Ecuador.

Brown [4] reported that the knob numbers of the United States corn were from 0 to 12 with the greatest number in the Southern Dents, the lowest in the Northern Flints, and high numbers of knobs were positively correlated with the following external features of ear and plant ; high row numbers, denting, absence of husk leaves, many seminal roots, and irregular rows of kernels.

Bianch et al. [3] reported that the knob numbers Italian corns ranged from 0 to 8 with the greatest number in Northern Italy (average number : 3.3), the lowest number in Southern Italy (average number : 1.7). Lee and Lee [12] reported that the knob numbers of 10 inbred stocks of Korean indigenous corn ranged from 8 to 12 (average number : 9.4) and Korean indigenous corn lines had more knobs than those of the United States corn, Central and South American corn or Italian corn.

Table 2. Numbers of heterochromatic knob in 5 Korean indigenous waxy corn stock

Stock	Knob no.	Stock	Knob no.
YS-1	10	KH-2	10
MY-1	12	HA-2	8
SC-1	6		

In this study, Korean indigenous waxy corn also had more knobs than those of other countries.

Giemsa C-banding metaphase plates of YS-1 and MY-1 are presented in Figure 1.

Four pairs of the chromosome component show dark and heterochromatic knobs and one pair has a satellite in YS-1, and six pairs show the knobs and one pair has a satellite in MY-1.

The chromosome 6, 7, 8 and 9 in YS-1 show terminal heterochromatic knobs, espcially chromosome 8 show terminal heterochromatic knobs both sides, whereas the chromosome 1, 2, 3, 6, 7 and 9 in MY-1 show terminal heterochromatic knobs. Chromosome 9 show both of central and terminal heterochromatic knob, and chromosome 4, 5 and 10 have no heterochromatic knob in both stocks.

Table 3 shows the values of arm ratio relative chromosome length expressed as a percentage of the length of chromosome 10 in YS-1 and MY-1. The values of chromosome arm ratio vary from 1.10 (chromosome 5) to 2.37

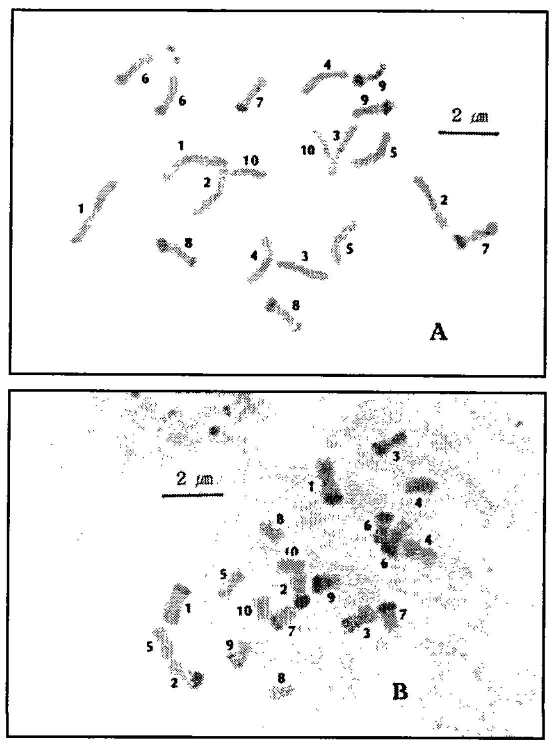

Fig. 1. Giemsa stained somatic metaphase of YS-1(A) and MY-1(B) of Korean indigenous waxy corn lines.

Table 3. Arm ratio and relative chromosome length expressed as percentage of chromosome 10 in YS-1 and MY-1

	YS-1		MY-1	
No.	Arm ratio	Length	Arm ratio	Length
1	1.11	217.81	1.25	216.67
2	1.13	183.56	1.31	186.90
3	1.24	178.08	1.21	184.52
4	1.14	155.48	1.22	177.38
5	1.10	151.37	1.29	169.05
6	1.62	127.40	1.65	156.08
7	2.37	124.66	1.56	152.38
8	1.25	123.29	1.31	123.81
9	1.61	114.38	1.19	120.23
10	1.21	100.00	1.33	100.00

(chromosome 7) in YS-1 and from 1.19 (chromosome 9) to 1.16 (chromosome 6) in MY-1.

The comparison of the values of arm ratios between homologous chromosomes in the two stocks show the positive correlation ($r=0.65^{*}$), but the variation of the values in YS-1 (1.27) is larger than those in MY-1 (0.46). In the values of relative length of chromosomes do not show the significant corelation ($r=-0.1^{ns}$).

Above result show that the arm ratios and the relative length of homologous chromosomes are different between the genotypes, and show the same tendency to the report of Aguiar-Perecin and Vosa [1].

Idiograms representing the somatic corn karyotype of YS-1 and MY-1 are show in Figure. 2.

These idiograms show arm ratios, relative length of chromosomes and knob positions. As can be seen in these idiograms, 4 terminal knobs (6L, 7L, 8L and 8S), 1 subterminal knob (9L), 1 heterochromatic band of centromere (9) and 1 satellite (6) are shown in YS-1 (Fig. 2A), whereas 6 terminal knobs (1L, 2L, 3L, 6L, 7L and 9L), 1 heterochromatic band of centromere (9) and 1 satellite (6) are shown in MY-1 (Fig. 2B). In the stock A 6 chromosomes have no knob but chromosome 8 has two terminal knobs. Whereas in the stock B only 4 chromosomes have no knob and no chromosome has two terminal knobs.

Lee and Lee [12] reported that the values of arm ratio, the relative length of homologous chromosomes, the knob numbers and positions were different between the genotypes in the Korean indigenous maize. Filion and Walden [6] mentioned that variations of arm ratios could be observed in metaphase chromosomes of different maize stocks, but no evidence was shown of corelation between arm length and band width. Aguiar-Perecin and Vosa [1] mentioned that the C-banded chromosome diagram could be considered illustrative of band positions in maize somatic chromosomes and might be useful in the future standardization of maize somatic cytogenetics.

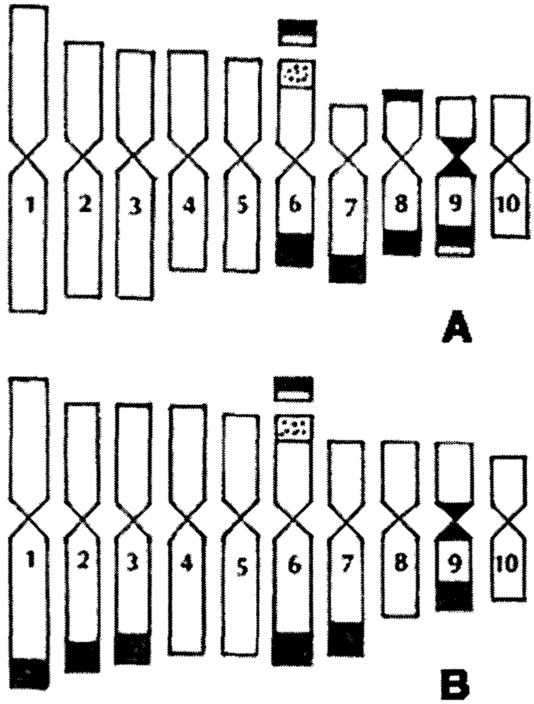

Fig. 2. Diagram of the C-banded corn somatic karyotype of YS-1(A) and MY-1(B).

Above result shows that knob position and heterochromatic band of centromere as well as arm ratio and relative length of chromosome are different between the genotypes. And the result shows that the C-banded chromosome diagram can be considered illustrative of band positions, arm ratios and relative chromosome length in somatic chromosomes of Korean indigenous waxy corn.

To know more information to the heterochromatic knob number of Korean local waxy corn by the characteristics of the corn and by the cultivation regions, more study is required.

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초록 : C-banding 패턴에 의한 한국 재래종 찰옥수수 염색체의 Heterochromatic knob 수와 핵형

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Giemsa C-banding 방법으로 한국 재래종 찰옥수수의 핵형 및 염색체 상에 존재하는 knob의 수와 위치 등을 확인하고 모식도를 통하여 염색체의 특성을 나타내고자 재래종 찰옥수수 5개의 자식 계통 (inbred stock)을 조사하였다. knob의 수는 6-12개이었고 평균 9.2개이었으며 계통별로 차이가 있었으며, knob을 가지고 있는 염색체도 계통별로 차이가 있었다. YS-1의 경우 6번, 7번, 8번 그리고 9번 염색체에서 knob을 가지고 있었는데, MY-1의 경우 1번, 2번, 3번, 6번, 7번 그리고 9번 염색체에서 knob을 가지고 있었다. 염색체의 장완과 단완의 비율, 염색체의 상대적 길이 등을 비교해 보기 위하여 YS-1과 MY-1 두 계통을 조사해 본 결과 두 계통 간에 차이가 있었고, 염색체의 상대적 길이는 YS-1이 큰 것으로 나타났다. Giemsa C-banding 법은 재래종 찰옥수수의 계통별 특성과 이를 이용한 계통 분류에도 효과적으로 이용할 수 있을 것으로 생각된다.