

C-banding in maize

II. Identification of somatic chromosomes

Margarida L. R. de Aguiar-Perecin*
and Canio G. Vosa†

* Departamento de Genética, Escola Superior de
Agricultura "Luiz de Queiroz", Universidade de São
Paulo, Piracicaba, S.P., Brazil;

† Botany School, University of Oxford,
Oxford, England

A biometrical analysis of C-banded and Feulgen stained somatic metaphases of maize stocks with different knob constitutions has shown that large bands alter arm lengths of mitotic chromosomes. A representative diagram of the knobless maize somatic karyotype and of a highly knobbed stock are presented.

INTRODUCTION

The identification of maize chromosomes according to their relative length, centromere position, knobs and prominent chromomeres, determined from pachytene studies (Rhoades, 1950; Neuffer *et al.*, 1968; McClintock, 1978) is now well standardised and generally accepted.

Chen (1969) and Filion and Walden (1973), showed that maize somatic chromosomes can be identified according to their relative length and arm ratio in Feulgen stained metaphases.

C-banded somatic metaphases have revealed the presence of distal bands, that correspond with knobs (Vosa and Aguiar, 1972; Hadlaczky and Kálmán, 1975; Sachan and Tanaka, 1977; Ward, 1980; Aguiar-Perecin, in press), but no diagrammatic representation of the band positions on the chromosomes has ever been made.

In the present study, a biometrical analysis of C-banded karyotypes of maize stocks with different knob constitutions has shown that large bands alter arm lengths of mitotic chromosomes. A representative diagram of a highly knobbed stock and of a knobless one are presented.

MATERIALS AND METHODS

Inbred stocks of which C-banded karyotypes have been previously described (Aguiar-Perecin, in press) and derived from populations of the

Table 1 Designation and knob constitution of maize stocks

Stocks	Racial origin and knob constitution*
371A-4	<i>Ceremonial</i> , heterozygous for knobs at 2L (medium size) and 7L (small size).
12	<i>Ceremonial</i> , with medium size knobs at 2L and 5L and small ones at 6L ₂ , 6L ₃ , 7L and 8L.
18	<i>Zapalote Chico</i> , homozygous for large and medium sized knobs at 1S, 2S, 2L, 3S, 3L, 4S, 4L, 5S, 5L, 6L ₂ , 6L ₃ , 7S, 7L, 8L ₁ , 8L ₂ , 9S.

* Knobs 6L₂, 6L₃, 8L₁ and 8L₂ are telescoped together at mitotic metaphase and are represented as single bands in the ideograms of fig. 3.

Brazilian *Ceremonial* maize (Avati-Moroti racial group, according to Brieger *et al.*, 1958) and the Mexican race *Zapalote Chico* were used (table 1).

Measurements of the chromosome arm lengths and the relative positions of C-bands were made in micrographs taken from C-banded and Feulgen stained metaphases. Generally, the C-banding procedure previously described by Aguiar-Perecin (in press) was used. Feulgen staining was performed according to Chen (1969), with some modifications, as follows. Excised root tips from germinating seedlings were pretreated, in 8-hydroxyquinaline, for 2 hours. After fixation in alcohol-acetic acid (3:1), they were kept in 70 per cent alcohol at 5°C. Feulgen staining was made as follows: hydrolysis in 1N HCl at 60°C for 8 minutes, washing in distilled water, staining in leuco-basic fuchsin for 45 minutes, and washing in tap water for

45 minutes. The roots were then transferred to 45 per cent acetic acid for 5 minutes and squashed in 1 per cent acetocarmine. The cover-slips were removed in 45 per cent acetic acid, air dried and mounted in Canada balsam.

Suitably spread metaphases were photographed on Kodak High Contrast Copy film. Arm lengths were determined from photographic prints magnified to $\times 5000$, with a pair of callipers and read off to the nearest $\times 0.5$ mm on an amplified millimetre paper. Chromosome length estimates of each chromosome were derived by generating two chromosome indexes expressed as: (a) percentage of the haploid set length; (b) percentage of the length of chromosome 10. This latter ratio was included because chromosome 10 had no bands in the stocks analysed, so that its length represents a fixed parameter for comparing the lengths of each chromosome of the complement. The centromere position was expressed as arm ratio values (long/short arm). For each chromosome arm, band positions were determined by calculating the distance of the band centre from the centromere. This value was expressed as the percentage of the respective chromosome arm (see Paris Conference, 1971, and also Greilhuber and Speta (1976)).

For the diagrammatic representation of each chromosome of the complement, homologous pairs with and without bands were compared through a *t*-test, with suitable modifications. The *t*-test procedure was based on a previous comparison between sample variances (according to Steel and Torie, 1960). As Feulgen stained metaphases permitted more accurate measurements of chromosome arms, only these values have been used in the present statistical analysis.

RESULTS AND DISCUSSION

The analysis of the somatic karyotypes of the stocks used in the present study, has made possible the diagrammatic representation of maize karyotype without bands and of a karyotype showing bands corresponding to large and medium size knobs in almost all the positions known to occur in maize.

In the three stocks analysed, the chromosomes could be easily identified according to their relative length and arm ratio both in Feulgen and C-banded metaphases. Figs. 1 and 2 show the C-banded karyotypes of stock 18 of *Zapalote Chico* and of the hybrid 18 \times 371A-4 respectively. In the latter

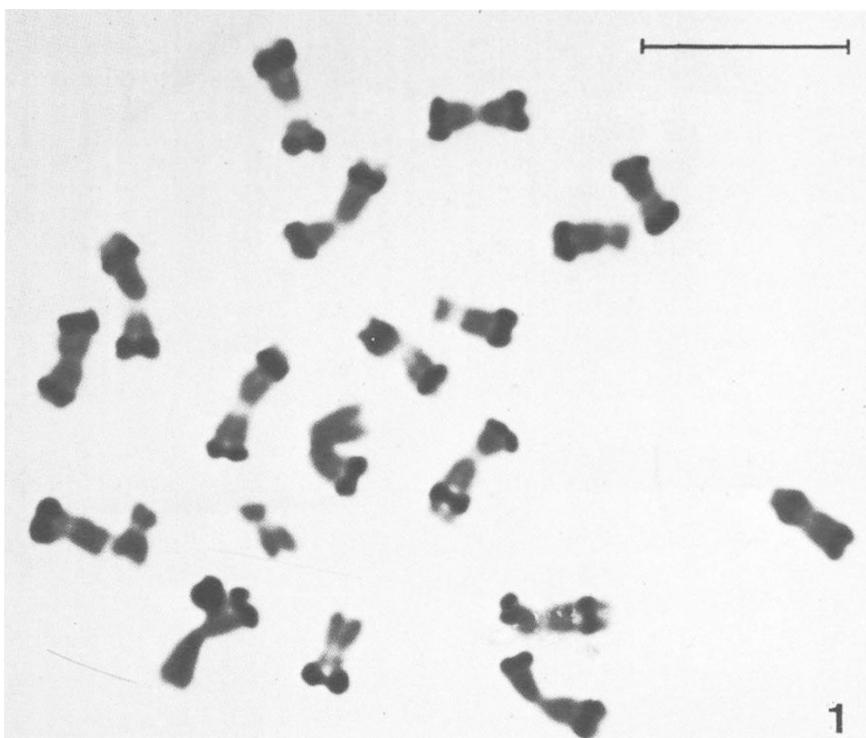


Figure 1 C-banded mitotic metaphase in stock 18 of the *Zapalote Chico* race. The dark stained bands correspond to knobs. Knobs at $6L_2$ and $6L_3$ and $8L_1$ and $8L_2$ in the pachytene stage appear as single bands in metaphase chromosomes.

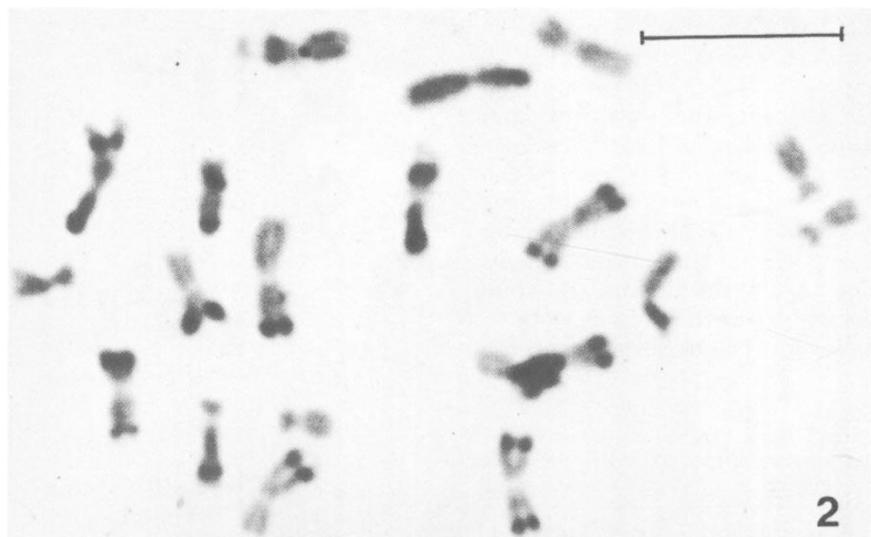


Figure 2 C-banded mitotic metaphase in the hybrid 18×371A-4.

karyotype it may be observed that bands corresponding to large and medium sized knobs alter the length of chromosome arms of the *Zapalote Chico* stock, as compared with the respective chromosomes from *Ceremonial* maize, which has bands only in the long arms of chromosomes 2 and 7.

No differences in the absolute length of chromosome 10 of both stocks have been found (table 2). Thus, this chromosome could be taken as "standard" for generating chromosome indices.

Table 2. Absolute length of chromosome 10 (expressed in arbitrary units) of chromosome 10 of *Zapalote Chico* and *Ceremonial*

Zapalote Chico (Stock 18) <i>n</i> = 22	Ceremonial (Stock 371A-4) <i>n</i> = 18	<i>t</i>
9.93 ± 1.58	9.66 ± 0.79	0.70 n.s.

n = Number of metaphases.

Table 3 shows the values of chromosome relative lengths, expressed as a percentage of the length of chromosome 10, calculated on 22 metaphases (from 5 plants of stock 18) and 18 metaphases (from 7 plants) of stock 371A-4 of *Ceremonial* maize. In stock 371A-4, length differences between the homologous chromosomes of pair no. 2, which is heterozygous for a knob in the long arm, could be detected, so that the two types of chromosomes are duly recorded in table 3. On the other hand,

no differences were found between the chromosomes of pair no. 7 which is heterozygous for a small knob at 7L.

The statistical comparison of these chromosome indices showed differences which are significant, at the 1 per cent level of probability between homologous chromosomes of *Zapalote Chico* and of *Ceremonial* maize. This finding

Table 3. Chromosome relative lengths expressed as percentages of chromosome of 10 of *Zapalote Chico* (stock 18) and *Ceremonial* (stock 371A-4)

Chromosome	Zapalote Chico (<i>n</i> = 22)	Ceremonial (<i>n</i> = 18)	<i>t</i>
1	226.63 ± 16.25	198.59 ± 11.60	6.15**
2	215.04 ± 16.50	K ⁺ 17.16 ± 14.61 K ⁻ 164.52 ± 11.21	7.60** 11.06**
3	204.09 ± 15.44	159.52 ± 10.42	10.44**
4	196.92 ± 15.69	151.39 ± 7.51	12.03**
5	182.88 ± 17.95	145.12 ± 8.88	8.66**
6	158.36 ± 11.58	131.14 ± 9.31	8.06**
7	164.46 ± 10.16	122.36 ± 6.80	15.03**
8	144.47 ± 11.92	120.31 ± 8.08	7.32**
9	145.14 ± 9.8	108.02 ± 5.04	15.44**
10	100.00	100.00	

** Significant at the 1% level.

n = Number of metaphases analysed.

K⁺ With knob.

K⁻ Without knob.

demonstrates that large and medium knobs of somatic chromosomes affect the lengths of somatic chromosome arms.

Estimates of maize somatic chromosome lengths have been expressed as a percentage of the total complement length (Chen, 1969; Filion and Walden, 1973). However, the statistical comparison of the total complement length expressed in arbitrary units of the two stocks, analysed in the present study, revealed differences at the level of 1 per cent of probability. This finding demonstrates that the calculation of chromosome relative lengths, as a percentage of the haploid set, would not be a system suitable for the present work for comparing homologous heteromorphic chromosomes (table 4).

Table 4 Relative chromosome lengths of *Zapalote Chico* (stock 18) and *Ceremonial* (stock 371A-4) expressed as percentage of haploid set

Chromosome	Zapalote Chico	Ceremonial
1	12.91 ± 0.62	14.13 ± 0.64
2	12.27 ± 0.47	12.14 ± 0.33*
3	11.72 ± 0.49	11.42 ± 0.48
4	11.3 ± 0.33	10.77 ± 0.30
5	10.54 ± 0.65	10.24 ± 0.37
6	9.32 ± 0.54	9.39 ± 0.47
7	9.51 ± 0.74	8.55 ± 0.33
8	8.44 ± 0.40	8.55 ± 0.46
9	8.19 ± 0.36	7.69 ± 0.23
10	5.82 ± 0.29	7.13 ± 0.33

* Without knobs.

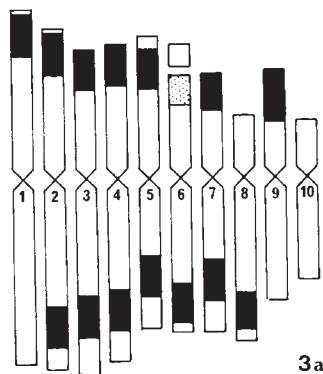
Table 5 Arm ratios of chromosomes of *Zapalote Chico* (stock 18) and *Ceremonial* (stock 371A-4)

Chromosome	Zapalote Chico (n = 22)	Ceremonial (n = 18)	t
1	1.13 ± 0.11	1.19 ± 0.07	2.0 ^{n.s.}
2	1.32 ± 0.10	K ⁺ 1.67 ± 0.16 K ⁻ 1.24 ± 0.13	8.07** 2.20*
3	1.58 ± 0.14	1.67 ± 0.18	1.78 ^{n.s.}
4	1.33 ± 0.17	1.37 ± 0.11	0.86 ^{n.s.}
5	1.08 ± 0.06	1.05 ± 0.04	1.82 ^{n.s.}
6	1.62 ± 0.18	1.60 ± 0.17	0.30 ^{n.s.}
7	1.46 ± 0.15	1.81 ± 0.20	6.32**
8	2.85 ± 0.29	2.17 ± 0.15	9.55**
9	1.08 ± 0.07	1.72 ± 0.14	17.67**
10	1.72 ± 0.07	1.76 ± 0.20	0.66 ^{n.s.}

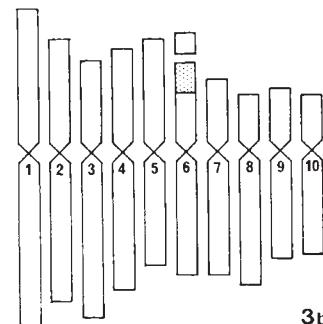
* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

The values of chromosome arm ratios are shown in table 5. Significant differences at the 1 per cent level were observed between homologous chromosomes of the pairs no. 2, 7, 8 and 9. This finding demonstrates that arm ratio values can be altered depending on the banding constitution of each chromosome.



3a



3b

Figure 3 Diagrammatic representation of the C-banded karyotype of (a) *Zapalote Chico* (stock 18) and (b) of the basic maize somatic karyotype without bands obtained from *Ceremonial* maize data.

Idiograms representative of the somatic maize karyotype without bands and of stock 18 of *Zapalote Chico* are shown in fig. 3. Band widths have been estimated from differences between heteromorphic homologous arms. As can be seen in this idiogram, bands corresponding to knobs observed at pachytene, most of them interstitial, appear to be subterminal or even terminal in somatic chromosomes due to their degree of contraction. In some cases and depending on the degree of condensation of metaphase chromosomes, the identification of some bands as terminal or subterminal may be rather difficult. This is the case of the short arm of chromosomes 1 and 2, in which the slightly subterminal appearance of the bands in their short arms can be better seen under the microscope in less condensed metaphases. Furthermore, the knob at 3S, which is clearly subterminal at pachytene, appears as terminal in somatic metaphases.

Further, it is also clear that there is a good correlation between band width and the degree of alteration of the length of a chromosome arm. The results presented in table 6 show significant differences between the homologous elements of pair

Table 6 Relative chromosome lengths of chromosomes 2, 6 and 8 with and without bands at their long arms (*Ceremonial* maize)

Chromosome	Band	Without Bands	t
2	176.0 ± 15.0 (n = 19)	164.0 ± 0.11 (n = 10)	2.89**
6	126.78 ± 11.13 (n = 12)	131.14 ± 9.31 (n = 18)	1.16 ^{n.s.}
8	114.34 ± 8.8 (n = 12)	120.31 ± 8.08 (n = 18)	1.91 ^{n.s.}

* Significant at the 1 per cent level.
n = Number of metaphases analysed.

no. 2 of stock 371A-4, which is heterozygous for a large knob at 2L. Conversely, no significant differences were found between chromosomes of pairs no. 6 and 8 whether with or without bands corresponding to stocks 12 and 371A-4 respectively. It should be emphasised that the knobs at 6L and 8L are small.

The present data are consistent with evidence from the literature that heterochromatic blocks stained by C-banding methods may represent additional material in the chromosomes (see Bennett *et al.*, 1977; see also Flavell, 1980).

A good example of chromosome polymorphism involving additional material is that provided by the human Y chromosome which has shown variability of its long arm among racial groups (Cohen *et al.*, 1966), which is due to differences in the length of the distal heterochromatic segment, as visualised through quinacrine fluorescence (Bobrow *et al.*, 1971) and Giemsa staining (Arrighi and Hsu, 1971).

In plants, many instances of polymorphism for telomeric and interstitial bands have been found in some genera. Correlations between the presence of large segments of heterochromatin and the increased length of chromosome arms, have been found in some investigations, as in species of the genus *Secale* (Bennett *et al.*, 1977).

Although a larger DNA content does not correspond to a larger amount of heterochromatin, Bennett *et al.* (1977) observed a positive relationship between the proportion of the complement represented by terminal heterochromatin and the 4C nuclear DNA content in some species of *Secale*. This provides the evidence in support of the notion that the increase in 4C nuclear DNA content in species of *Secale* has involved a large increase in the amount of telomeric heterochromatin. Controversially, a comparison of the total lengths of the haploid complement with the 4C DNA amount for nine *Secale* taxa, showed no significant relationship. However, this finding has been interpreted in the light of some evidence that metaphase chromosome volume can vary, according to the

metabolic activity of the cell or to various environmental factors.

In this context, it is interesting to emphasize the positive relationship that we have found between the values of the diploid complement length, measured in Feulgen stained metaphases and the frequency of knobs, in the maize stocks analysed in this study. Further, the use of a chromosome index expressed as a percentage of chromosome 10, has made possible the clear demonstration that the morphology of maize somatic chromosomes can be drastically modified according to the band patterns.

Very few references have been found in the literature, with respect to polymorphism of maize somatic chromosomes. Filion and Walden (1973) mentioned that variations of arm ratios could be observed in metaphase chromosomes of different maize stocks, but no evidence was shown of correlation between arm lengths and band widths.

The C-banded chromosome diagram presented in fig. 3(a), although representative only of the stock of *Zapalote Chico* used in this study, can be considered illustrative of band positions in maize somatic chromosomes and may be useful in the future standardization of maize somatic cytogenetics.

Acknowledgements One of the authors (M.L.R.A.P.) wishes to thank Dr Almíro Blumenschein of the Department of Genetics ESALQ, University of S. Paulo, Brazil for the valuable supply of seeds from which the stocks used in the above study were derived. She is also very grateful to Dr N. A. Vello of the same Department for help and advice in the statistical analysis.

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (P16 III/CNPq/Finep).

REFERENCES

- AGUIAR-PEREZIN, DE, M. R. L. 1984. C-banding in Maize: I. Banding patterns. *Caryologia* (in press).
- ARRIGHI, F. AND HSU, T. C. 1971. Localisation of heterochromatin in human chromosomes. *Cytogenetics*, 10, 81-86.

- BENNETT, M. D., GUSTAFSON, J. P. AND SMITH, J. B. 1977. Variation in nuclear DNA in the genus *Secale*. *Chromosoma*, *61*, 149-176.
- BOBROW, M., PEARSON, P. L., PIKE, M. C. AND EL ALFI, O. S. 1971. Length variation in the quinacrine-binding segment of the human Y chromosome of different sizes. *Cytogenetics*, *10*, 190.
- BRIEGER, F. G., GURGEL, J. T. A., PATERNIANI, E., BLUMENSCHEIN, A. AND ALLEONI, M. R. 1958. Races of maize in Brazil and other eastern South American countries. Nat. Acad. Sci. Nat. Res. Council, Washington, D.C. Publ. 593.
- CHEN, C. C. 1969. The somatic chromosomes of maize. *Can. J. Genet. Cytol.*, *11*, 752-754.
- COHEN, M. M., SHAW, M. W. AND MACCLUER, J. W. 1966. Racial differences in the length of the human Y chromosome. *Cytogenetics*, *5*, 34-52.
- FILION, W. G. AND WALDEN, D. B. 1973. Karyotype analysis. The detection of chromosomal alterations in the somatic karyotype of *Zea mays* L. *Chromosoma*, *41*, 183-194.
- FLAVELL, R. 1980. The molecular characterisation and organisation of plant chromosomal DNA sequences. *Ann. Rev. Plant Physiol.*, *31*, 569-596.
- GREILHUBER, J. AND SPETA, F. 1976. C-banded karyotypes in the *Scilla hohenackeri* group. *S. persica* and *Puschkinia* (Liliaceae). *Plant Syst. Evol.*, *126*, 149-188.
- HADLACZKY, GY. AND KÁLMÁN, L. 1975. Discrimination of homologous chromosomes of maize with Giemsa staining. *Heredity*, *35*, 371-374.
- MCCLINTOCK, B. 1978. Significance of chromosome constitution in tracing the origin and migration of races of maize in the Americas. In *Maize Breeding and Genetics*. Walder, D. B. (ed.) Wiley, New York, pp. 159-184.
- NEUFFER, M. G., JONES, L. AND ZUBER, M. S. 1968. The mutants of maize. Crop. Science Society of America, Madison, 74p.
- PARIS CONFERENCE. 1971. Standardization in Human Cytogenetics. Birth defects: Original articles series VIII: 7, 1972. The National Foundation, New York.
- SACHAN, J. K. S. AND TANAKA, R. 1977. Variation in pattern of C-banding in *Zea* chromosomes. *Nucleus*, *20*, 61-62.
- RHOADES, M. M. 1950. Meiosis in maize. *J. Hered.*, *41*, 58-67.
- STEEL, R. G. D. AND TORIE, S. M. 1960. Principles and procedures of statistics. McGraw-Hill, New York, 481p.
- VOSA, C. G. AND AGUIAR, M. L. R. DE. 1972. New techniques for knob detection in mitotic chromosomes in maize and teosinte. *Maize Genet. Coop. Newslett.*, *46*, 165-167.
- WARD, E. J. 1980. Banding patterns in maize mitotic chromosomes. *Can. J. Genet. Cytol.*, *22*, 61-67.