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Chromosomal and cell size analysis of cold tolerant maize

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Summary. C-band number, guard cell length, and chloroplast number per guard cell were determined for eight maize populations. These populations consisted of maize selected for cold tolerance at the University of Nebraska as well as the original unselected populations. The genome size of these populations had previously been determined. C-band number fluctuated concertedly with the changes in genome size indicating that deletions and additions of constitutive heterochromatin occurred during selection, resulting in altered genome sizes. Guard cell size of all the cold tolerant populations was greater than the cell size of the respective nonselected populations. Chloroplast number per guard cell was also higher in all the cold tolerant populations than in their parental populations, but the increases were not statistically significant. The results indicate that changes in genome size that occurred during selection for cold tolerance are the result of changes in amounts of C-band heterochromatin and that the selection process results in an increase in cell size in the cold tolerant populations.

Key words: *Zea mays* – C-banding – Cell size – Chloroplast number – Cold tolerance

Introduction

The adaptation of plants to growth in cold environments is associated with several changes including adjustments in guard cell size and nuclear DNA amount. Limin and Fowler (1989) found that reductions in guard cell size accompanied adaptation to severe winter climates in the tribe Triticeae. Grime and Mowforth (1982) investigated the relationship of cell size and genome size to growth in different climates and found that plants which complete their life cycles during winter and early spring tend to have larger cells and genomes than those that mature in warmer months.

Bennett (1976) found a direct positive correlation between genome size and adaptation to growth at cooler northern latitudes of the United States for a number of grasses. An opposite phenomenon has been shown to occur in *Zea mays*, where DNA content decreases with increasing latitude. This decrease in genome size has been postulated to be an adaptation to growth in cooler, moister regions with shorter growing seasons (Rayburn et al. 1985). These observations led to the evaluation of genome size in populations of maize selected for cold tolerance (McMurphy and Rayburn 1991). While no unequivocal association between cold tolerance and genome size was observed, genome size appeared to be involved in adaptation to cold. Those cold-tolerant lines which exhibited a degree of freeze tolerance increased in genome size from the original populations, while the one population which was cold tolerant but extremely freeze sensitive had a decrease in genome size.

Maize chromosomes often contain heterochromatic knobs (McClintock 1929) that have been observed to be correlated with various environmental parameters. Maize populations from northern latitudes have fewer knobs than populations from southern latitudes (Longley 1938; Brown 1949; Rayburn et al. 1985). DNA content has been positively correlated with C-band (the mitotic equivalent of pachytene knobs) composition in maize (Rayburn et al. 1985). In some maize populations from the southwestern United States, however, variation in DNA content did not appear to be entirely accounted for by C-band positive DNA and supernumerary B-chro-

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mosomes (Rayburn and Auger 1990; Porter and Rayburn 1990).

The objective of the study presented here was to determine if cold tolerance in maize is associated with cell size and chloroplast number and whether the genome size variation of the cold-tolerant populations is a result of variation in amount of C-band (knob) heterochromatin.

Materials and methods

Plant material

The eight maize populations chosen for this study, NA, NA CT, NB, NB CT, NS, NS CT, NS CT-FT, and NS CT-HY, were obtained from Dr. Blaine Johnson, Department of Agronomy, University of Nebraska. The origins of populations NA, NB, and NS have been described by Gardner et al. (1987). NA CT, NB CT, and NS CT are the results of recurrent selection for cold tolerance out of NA, NB, and NS, respectively. NS CT-FT is a subpopulation out of NS CT selected for freeze tolerance. NS CT-HY is a selection for high yield of NS CT. All of the selected populations were developed at the University of Nebraska (Gardner et al. 1987). The populations used for this study were the same as those used by McMurphy and Rayburn (1991) for genome size determinations.

C-band observations

Seeds were germinated in a 1:1 mixture of perlite and vermiculite in an incubator set for an 8-h day at 32 °C and a 16-h night at 26 °C. After secondary roots appeared, seedlings were submerged in 0.05% 8-hydroxyquinoline for 2 h in the dark at 32 °C. The seedlings were then rinsed and fixed in 3:1 ethanol:glacial acetic acid for 24 h at 4 °C before being stored in 70% ethanol at 4 °C.

To prepare the chromosomes, fixed root tips were excised from the seedlings, rinsed in distilled water for 2–3 min, and hydrolyzed in 0.2 *N* HCl for 10 min at 37 °C. Root tips were then rinsed in cold water, placed in a depression well of an agglutination microslide, and treated with five drops of an enzyme solution (0.2 g cellulysin, 0.1 g macerase in 10 ml 10^{-3} *M* EDTA, pH 5.1–5.3) for 30 min. Slides were prepared as described by Rayburn and Gold (1982). Cell suspensions were dispensed onto slides using a 50-µl microcapillary tube and a Wiretrol plunger.

Chromosomes were C-banded following the method of Rayburn et al. (1985). Three to five plants per population were analyzed to determine C-band number. Bands proximal to the nucleolus organizing region of chromosome 6 were not counted. Photographs were taken using an Olympus Vanox AHBT microscope and Kodak Technical Pan film (2415).

Measurements of guard cell size

Plants were grown for 2 weeks on a plant cart with a 14-h daylength and irradiance of approximately 100 µmol photons $m^{-2} \cdot s^{-1}$. A small portion of abaxial epidermis was peeled from the most fully expanded leaf of the 2-week-old seedlings and mounted on a microscope slide in a drop of water. Guard cells were viewed using an Olympus Vanox AHBT photomicroscope with a fluorescence attachment model AH2-RF1 and excitor BP405. The excitation wavelengths were 395–415 nm, while the observed fluorescence wavelengths were above 455 nm. Photographs were taken using Kodak Tmax P3200 film.

The length of the guard cells was determined by photographing a stage micrometer at the same magnification as the guard cell photos. The micrometer photo was then compared to photographed guard cells to determine their length. Eight plants per population were examined, and at least ten stomates per plant were measured.

For statistical analyses, the populations were analyzed in pairs. Differences between each of the nonselected populations and their selected counterparts were tested for significance by *t*-tests. NS CT-HY and NS CT-FT were compared by *t*-tests to NS CT, the population from which they were selected.

Chloroplast number determinations

Chloroplast numbers per guard cell were determined from the same plants used for the measurements of guard cell size. Chloroplasts were counted using the technique of Ho et al. (1990). The same eight plants per population used to determine guard cell size were examined for the chloroplast study. Chloroplasts in at least 20 guard cells per plant were counted.

The populations were analyzed in pairs. *t*-tests were performed on the paired data as described above for the cell size experiment.

Results

C-band number

The mean C-band numbers of populations NA, NB, and NS were 10.4, 8.2, and 7.2, respectively. The number of C-bands as well as the range in C-band number of individual plants of each population examined are listed in Table 1. The data are designed to be examined in pairs. In one pair of the populations, NA and NA CT, the cold-tolerant population (NA CT) contained fewer heterochromatic C-bands than the unselected population (Fig. 1 A, B). The population pairs NB/NB CT and NS/NS CT exhibited an increase in heterochromatin of the cold-tolerant populations compared to their respective unselected populations. NS CT-FT and NS CT-HY both

Table 1. C-band number, guard cell size, chloroplast number per guard cell, and nuclear DNA content in the eight maize populations

Population	C-band number (range)	Cell size[a] (µm)	Number of chloroplasts per guard cell	DNA content per 2C nucleus[b] (pg)
NA	10.4 (10–11)	37.8	5.4	5.07
NA CT	8.8 (8–10)	42.9*	5.7	4.87
NB	8.2 (7–9)	42.9	5.7	4.98
NB CT	10.6 (10–11)	44.4	6.3	5.13
NS	7.2 (5–9)	41.7	5.9	5.05
NS CT	10.0 (10)	46.8*	6.0	5.18
NS CT	10.0 (10)	46.8	6.0	5.18
NS CT-FT	9.8 (8–11)	46.2	5.7	5.07
NS CT	10.0 (10)	46.8	6.0	5.18
NS CT-HY	8.0 (5–10)	45.0	5.8	5.03

[a, *] Significant increase at the 5% level, as compared to the corresponding non-selected parental population

[b] Cited from McMurphy and Rayburn (1991)

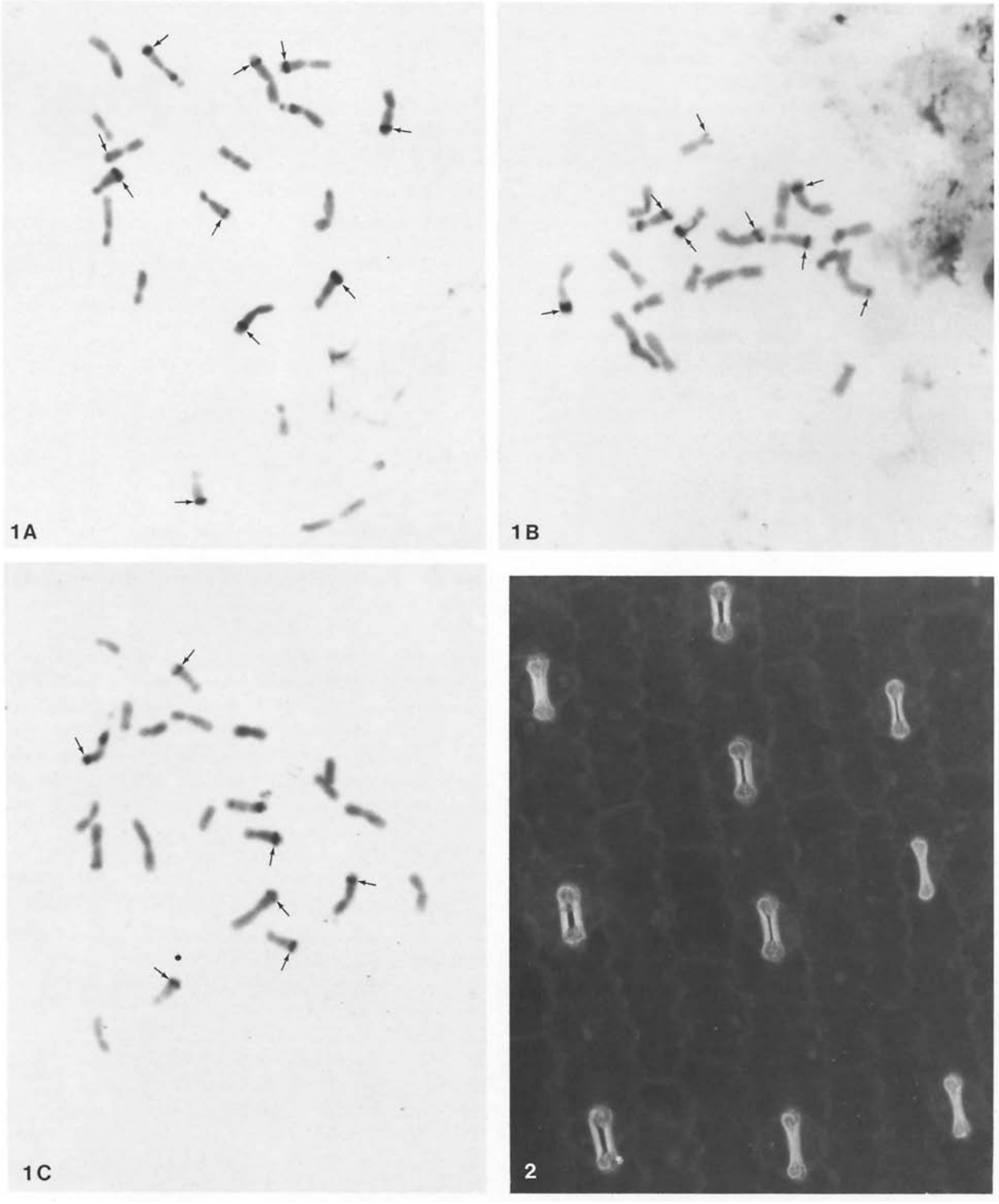

Fig. 1 A–C. C-banded karyotype of NA showing ten bands (**A**), NACT showing eight bands (**B**), and NB with 22 chromosomes showing seven bands (**C**). *Arrows* indicate C-bands

Fig. 2. Stomata of NA.

had fewer C bands than NS CT, the population from which they were selected.

One plant of population NB carried two B-chromosomes in addition to the basic set of 20 A-chromosomes (Fig. 1 C). All of the other plants examined had 20 chromosomes.

Guard cell size

The mean guard cell lengths of all of the populations examined are given in Table 1. A representative photograph of guard cells is shown in Fig. 2. In each of the population pairs NA/NA CT, NB/NB CT, and NS/NS CT, guard cell length increased in the cold-tolerant populations from that found in the unselected parent populations. The increase was significant in two of the three pairs compared. The other two paired analyses, NS CT/NS CT-FT and NS CT/NS CT-HY, revealed a nonsignificant decrease in cell size in the selected populations compared to their parental populations.

Chloroplast number per guard cell

Chloroplast number increased slightly in the cold-tolerant populations NA CT, NB CT, and NS CT compared to their unselected parental populations. Chloroplast number decreased slightly in NS CT-FT and NS CT-HY compared to NS CT (Table 1). Statistical analysis revealed no significant differences between the selected and the nonselected components of any population pairs.

Discussion

Selection for cold tolerance resulted in changes in C-band number and guard cell lengths in selected maize populations, and the former changed concertedly with reported changes in genome size (McMurphy and Rayburn 1991) of the selected populations. These facts indicate that all or most of the genome size changes were the result of changes in amount of repetitive heterochromatic DNA. These DNA sequences have no coding function, so the effect of deletion or addition of this genetic material is primarily nucleotypic (Bennett 1971).

The mean cell size of each cold-tolerant population was greater than that of its corresponding unselected population. This finding is in agreement with the hypothesis of Grime and Mowforth (1982) that plants which complete their life cycles during early spring and winter have larger cells than those that mature in the summer. Limin and Fowler (1989) would have predicted cells of the cold-tolerant populations to be smaller than those of the nonselected populations. A critical difference between the two studies is that Grime and Mowforth observed plants that were able to actively grow and reach maturity in cold environments, whereas Limin and Fowler studied the ability of plants to survive severe winter climates so that they could complete their growth when temperatures were more suitable. The selection pressures used in developing the cold-tolerant maize lines were similar to the early spring growth conditions that Grime and Mowforth associated with large cells. Also, Limin and Fowler focused on the tribe Triticeae. It has previously been determined that although a number of grasses, including some of the Triticeae, demonstrate an increase in genome size as latitude of cultivation progresses northward, maize follows a different trend, with genome size decreasing at northern latitudes. This may indicate totally different methods of adaptation to cold between Triticeae and maize.

It is interesting to note that in the case of NA/NA CT, changes in cell size did not reflect changes in genome size. Genome size decreased significantly in NA CT compared to NA, whereas cell size increased significantly in NA CT. Previous studies have indicated a correlation between genome size and cell size (Butterfass 1973; Limin and Fowler 1989). Our study suggests that cell size may be more greatly influenced by environmental adaptations than by genome size. DNA content may dictate a range of permissible cell sizes from which an ideal size is selected based on environmental pressures.

Although no statistical differences were found between chloroplast numbers of selected populations versus their nonselected counterparts, there was a trend toward higher chloroplast numbers in the cold-tolerant populations. This trend follows the changes seen in cell size. These findings, although statistically nonsignificant, support the hypothesis of Pyke and Leech (1987), which states that cell size is more important than genome size for determining chloroplast number per guard cell.

The differences in genome size among the populations examined are due to changes in heterochromatin amounts caused by adaptation to growth in cold environments. Populations which are both cold tolerant and freeze tolerant exhibit increased amounts of heterochromatin, whereas decreased heterochromatin amounts accompany cold tolerance alone. Cold tolerance with or without freeze tolerance is associated with increased guard cell size and increased chloroplast number per guard cell.

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