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Inheritance of nuclear DNA content in leaf epidermal cells of *Zea mays* L.

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Abstract The nuclear DNA content (ploidy level) of maize leaf-epidermal cells was investigated by Feulgen cytophotometry in two lines, Illinois High Protein (IHP) and Illinois Low Protein (ILP), their reciprocal hybrids, and their F_2s. Epidermal cells have a 2C, 4C or 8C nuclear DNA content. The mean DNA content per nucleus in IHP was significantly higher than in ILP; the mean DNA content per nucleus in hybrids was intermediate between the parental lines, and the same DNA content was measured in reciprocal crosses. In F_2s the same mean DNA content as in F_1s was observed but with larger variability than in the F_1, possibly indicating genetic segregation. It is inferred that the ploidy level in the leaf epidermis is inherited, and incomplete dominance occurs in hybrids. The same behaviour in the different genotypes was observed for epidermal cell-surface area, except that an increase of mean surface area occurred in the F_1, probably due to heterotic effects. The difference in the accumulation of 4C and 8C nuclei in leaf epidermis parallels that reported between two genotypes for the endosperm tissue: to the greater chromosome endoreduplication found in the endosperm there were correspondingly higher frequencies of 4C and 8C nuclei in the leaf epidermis, indicating a higher general tendency to chromosome endoreduplication in IHP than in ILP. It is suggested that the accumulation of 4C nuclei (G_2-block) in the leaf epidermis may be regarded as the initial step of chromosome endoreduplication, the two phenomena being related to the control of the sequence DNA synthesis-mitosis, possibly involving the same genes in both endosperm and leaf. However, the inheritance of DNA content per nucleus in epidermal tissue seems to be different from that observed in endosperm tissue of the same genotypes, suggesting that differences may occur in the regulation of the activity of these genes.

Key words Cell cycle · DNA content · Leaf epidermis · Maize · Ploidy level

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Introduction

In plants, differentiated cell nuclei generally maintain the same DNA content that they had at the end of the mitosis immediately preceding cell differentiation; i.e., the 2C DNA content typical of the DNA-presynthesis phase (G_1) of the diploid cell cycle (D'Amato 1977, 1985, 1989). However, in pteridophytes and the vast majority (about 90%) of angiosperms, some of the somatic cells undergo chromosome endoreduplication concomitant with differentiation (Evans and Van t'Hof 1975; D'Amato 1985; Nagl 1990). In these polysomatic species, the degree of endoreduplication may vary from tissue to tissue and is not generally uniform in a given tissue: a variable number of cells may remain at the 2C level, other cells have a 4C DNA content, and still others undergo chromosome endoreduplication (8C, 16C, 32C, etc.).

In some cases, the degree of endoreduplication in a given tissue is dependent on environmental conditions (Evans and Van t'Hof 1975; Van Oostveldt et al. 1976; Cavallini et al. 1995a). However, the occurrence of non-polysomatic species in certain genera and families indicates a genotypic control of nuclear stability at the diploid level (D'Amato 1985). Moreover, variations in the frequency of 2C, 4C and endoreduplicated nuclei have been observed even among genotypes of one and

the same species, cultivated under the same environmental conditions (Nagl and Capesius 1976; Herz and Brunori 1985; Cavallini and Natali 1994; Cavallini et al. 1995a). For example, in maize endosperm parenchyma, the mean ploidy level was found to vary among cultivars, lines or hybrids (Kowles and Phillips 1988; Kowles et al. 1992a; Cavallini et al. 1995b). In this tissue, the mean ploidy level was found to be under genetic control, with a maternal effect (Kowles et al. 1992b; Cavallini et al. 1995b). In particular, we studied two maize genotypes (Illinois High Protein, IHP, and Illinois Low Protein, ILP; Cavallini et al. 1995b) extensively: in both lines, chromosome endoreduplication occurs in endosperm cells within 24 days after pollination, attaining a maximum ploidy level of 384C (seven DNA replication rounds) in IHP and 192C (six replication rounds) in ILP. In the mature seeds, the endosperm of the two lines shows a different mean ploidy level. In reciprocal crosses between IHP and ILP, the F_1 endosperms have mean ploidy levels analogous to that of the maternal parent, demonstrating that the difference in ploidy level between the two genotypes is maintained. After selfing the F_1 plants, the difference in ploidy level between the two F_2 populations is reduced. In the F_2, the mean ploidy level is as variable as in the F_1, indicating the absence of genetic segregation.

From these data, it was apparent that both the genetic constitution (nuclear and cytoplasmic) of the maternal parent and the genotype of the individual endosperm affect the ploidy level. Since maternal effects are often found for endosperm characters (Reggiani et al. 1985), we analyzed nuclear DNA content (ploidy level) in a permanent somatic tissue (the epidermis of the adult leaf) of IHP and ILP plants and measured this parameter in the two genotypes, in the reciprocal hybrids, and in their F_2s. We also studied the effect of nuclear DNA content on the cellular dimensions of leaf epidermal cells. In this paper, we report the results of these analyses.

Materials and methods

Two strains of *Zea mays* L. were used: the High-Protein and the Low-Protein strains developed at the University of Illinois during at least 70 generations of selection for or against the seed protein content (Dudley 1974). Plants of the two strains, the reciprocal crosses and their F_2s were cultivated in the greenhouse until flowering.

The 5-cm-long central portion of the fourth leaf was used after fixation in ethanol-acetic acid 3:1 (v/v). Only this leaf portion was analyzed and compared in our experiments, because of the reported variability of nuclear DNA content among the leaves of a single plant (Baer and Schrader 1985b) and within one and the same leaf (Dolezelova et al. 1992). The adaxial epidermis was peeled off from leaves previously treated with a 5% solution of pectinase (Sigma) for 1 h at 37°C. The leaf peels were placed on gelatin-coated slides with their external face in contact with the gelatin, a procedure ensuring easy penetration of solutions into the epidermal cells. The slides were then simultaneously hydrolyzed in 1 N HCl at 60°C for 7 min, Feulgen-stained on 0.5% basic fuchsin for 1 h at room temperature, washed twice in SO_2 water for 15 min, dehydrated and mounted in D.P.X. balsam (BDH Chemicals). DNA content was estimated by a Barr and Stroud, GN5-type, integrating cytophotometer at a wavelength of 550 nm on nuclei belonging to cell rows containing neither hairs nor stomatal cells. Slides to be directly compared were concurrently stained; when simultaneous processing was not possible, due to the large number of preparations to be analyzed, squashes made with root tips of a single plantlet of *Vicia faba* (4C = 53.31 pg; Bennett and Smith 1976) were concurrently stained for each group of slides and used as standards to make all results comparable. Epidermal cell area was measured on the same slides after microphotographing; cell area was obtained by multiplying cell length by cell width.

The sample size was 300 cells per slide, one slide per individual, 20 individuals per genotype. The ploidy level of each genotype was calculated as the mean ($\pm$ SE) of the mean C-values of 20 individuals per genotype; the mean C-values were calculated on 300 nuclei per leaf.

Results

In the leaf epidermis of *Z. mays*, different cell rows may be distinguished: rows containing stomata, rows containing hair cells, and rows composed only of elongated cells (Fig. 1). Analyses on inter-hair and inter-stomatal cells showed that these cells usually maintain a 2C DNA content, whereas in elongated cell rows 2C, 4C and 8C nuclei occur.

Nuclear DNA content was determined in the leaf epidermis by Feulgen scanning cytophotometry. This technique may be subject to reservations when used to determine small variations in nuclear DNA content (Mello and Vidal 1980). On the other hand, only by this technique is it possible to measure nuclear DNA content after choosing cells to be analyzed. Anyway, our cytophotometric analyses always indicated the

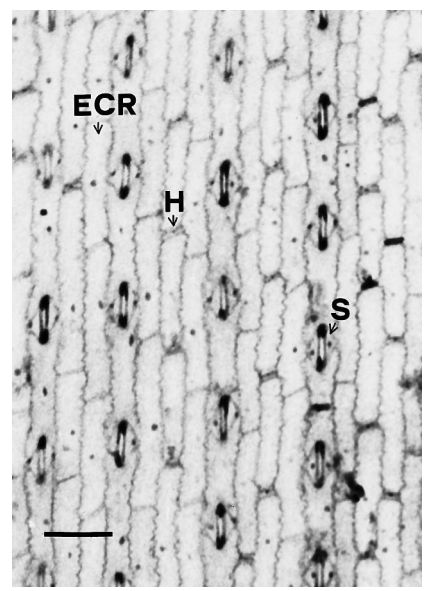

Fig. 1 Portion of an epidermal peel of the fourth leaf of *Z. mays*, line IHP, stained with Delafield's haematoxylin. *S* stoma; *H* hair; *ECR* elongated cell row. Bar = 500 μm

presence, in elongated cell rows, of distinct peaks for the 2C, 4C and 8C nuclei (Fig. 2).

In Fig. 3 are reported the frequency histograms of 2C, 4C, and 8C epidermal cell nuclei in the elongated cell rows of the fourth leaf of IHP, ILP, the reciprocal crosses and their F_2s. Statistically significant differences in the frequency of 2C and 4C nuclei are observed only between IHP and ILP, while no difference is found in reciprocal crosses and F_2s.

In Table 1 are reported the mean ploidy levels of the six genotypes tested; reciprocal crosses and their F_2s show a mean ploidy level intermediate between the two parental lines.

We calculated the mean ploidy level of elongated cell rows in leaves of single maize plants of IHP, ILP, the reciprocal crosses and their F_2s. The distributions of these values are reported in Fig. 4. Variability may be observed among individuals, in every generation. However, the interval distributions of IHP and ILP are clearly different, while the distributions of reciprocal crosses and those of their F_2s fall almost within the same interval. It may be noted that, in the F_2s, the distributions are much larger than in the other genotypes, possibly indicating the occurrence of genetic segregation among individuals.

On the same cells as were analyzed for their DNA content, cell area was measured on microphotographs. The mean leaf epidermal cell area of the six genotypes is reported in Table 1: again, a difference is observed between parental lines but not between reciprocal crosses or between their F_2s. It may be noted, however, that the mean cell area in the two reciprocal crosses is significantly larger than that of the parentals, indicating a possible heterotic effect of hybridization. The same results are suggested from the distributions of mean cell area in individual plants of the six genotypes (Fig. 5).

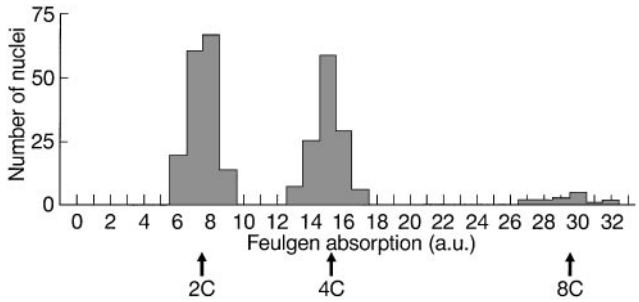

Fig. 2 Distribution of Feulgen/DNA absorption (arbitrary units) of nuclei of elongated cells rows in the mature fourth leaf of a IHP plant. Correspondence of peaks to DNA content (in C-values) is reported

Table 1 Mean DNA content per nucleus (C) and mean surface area of epidermal cells of the upper face of the fourth adult leaf of six maize genotypes; 1000 cells × 20 individuals per genotype were scored

Genotype	DNA content per nucleus ± SE	Mean surface area ± SE
IHP	2.98 C ± 0.06	69.913 ± 0.995
ILP	2.27 C ± 0.04	49.996 ± 0.901
IHP × ILP	2.68 C ± 0.06	79.475 ± 1.039
ILP × IHP	2.60 C ± 0.05	80.543 ± 1.053
IHP × ILP F_2	2.72 C ± 0.05	59.633 ± 2.012
ILP × IHP F_2	2.66 C ± 0.05	62.886 ± 2.310

Discussion

It has been reported in the literature that, in differentiated plant tissues, the arrest of cells in G_1 or G_2 may depend on environmental conditions. For example, in mature root tissues of *Pisum sativum*, the removal of cotyledons changed from 4C to 2C the arrest period

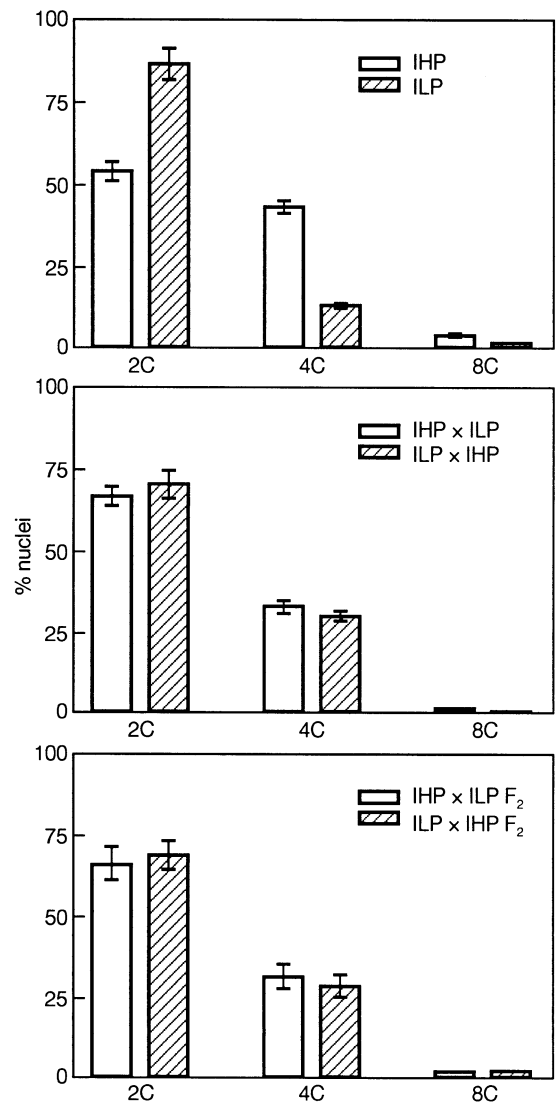

Fig. 3 Frequency histograms of 2C, 4C, and 8C epidermal cell nuclei in the fourth leaf of IHP, ILP, the reciprocal crosses and their F_2s. Fiduciary limits for $P \leqslant 0.01$

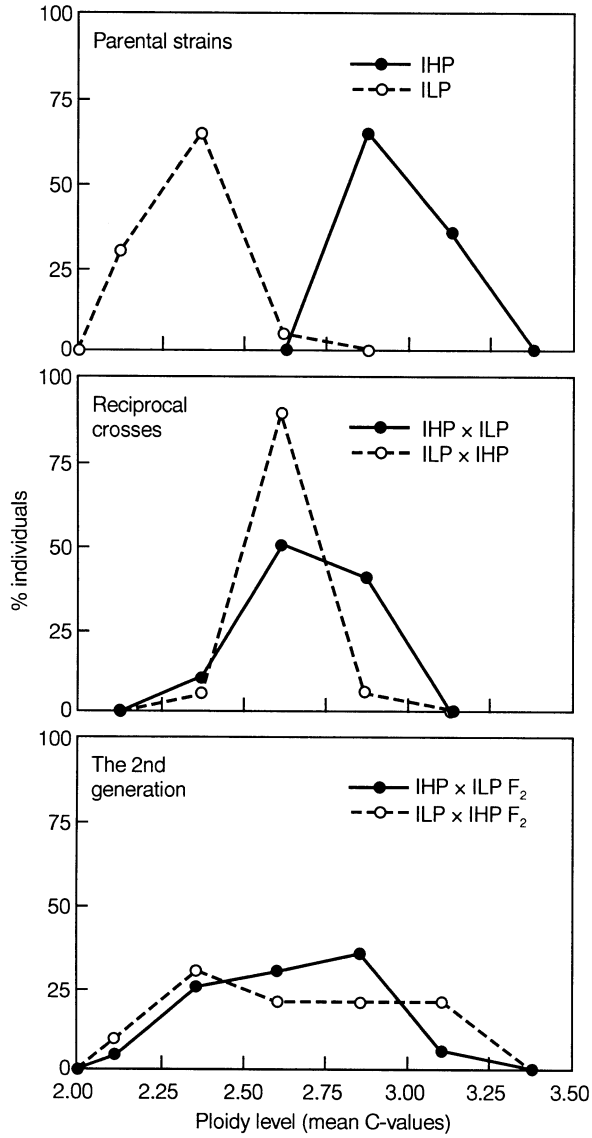

Fig. 4 Distributions of mean ploidy levels in leaves of individual plants of IHP, ILP, the reciprocal crosses and their F_2s

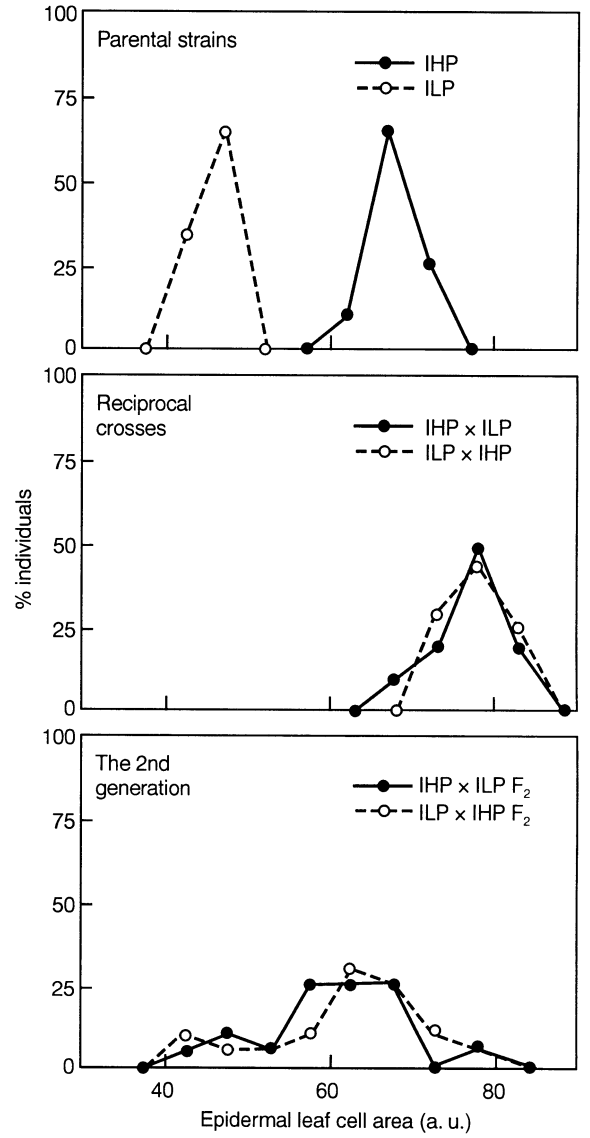

Fig. 5 Distributions of mean leaf cell areas in leaves of individual plants of IHP, ILP, the reciprocal crosses and their F_2s

(Evans and Van t'Hof 1974). At the same time, analyses on different species showed that the arrest phase, i.e. the frequency of 2C, 4C, or polyploid nuclei in differentiated tissues, is also under genetic control (Evans and Van t'Hof 1974; D'Amato 1985).

Maize leaf epidermis (Fig. 1) is excellent material in which to study nuclear DNA content in relation to cell differentiation, especially in those cell rows composed exclusively of elongated cells (Fig. 2), whereas cell rows containing hairs or stomata have mostly 2C nuclei, as already reported in another graminaceous species, *Triticum durum* (Cionini et al. 1983.

In the elongated cell rows, the frequencies of 2C, 4C and 8C nuclei, and hence the mean ploidy level, appear to be under genetic control. Statistically significant differences are found between IHP and ILP lines: more 4C and 8C cells are observed in IHP than in ILP (Table 1, Fig. 3). When considering mean ploidy levels in individual plants, the distributions of IHP and ILP individuals are almost completely separated (Fig. 4). The inheritance of mean ploidy level may be inferred from the results of reciprocal crosses and their F_2s. Reciprocal F_1s show the same frequencies of 2C, 4C, and 8C nuclei: they are intermediate between parental lines and are maintained in the F_2s (Table 1, Fig. 3). The distributions of mean ploidy levels of single F_1 individuals are similar; so are those of F_2s (Fig. 4), but larger distributions are observed in F_2s than in F_1s, indicating genetic segregation.

On the basis of the reported data we suggest that mean ploidy level in elongated cell rows of maize leaf epidermis is determined by one or more genes, with

incomplete dominance, although an environmental influence on mean ploidy levels can not be excluded, as indicated by the variability observed within parental or F_1 genotypes (Fig. 4). Similar conclusions may be drawn from flow-cytometric data (Baer and Schrader 1985a), though in these experiments analyses were made on whole leaves.

It is known that nuclear DNA content usually affects cell size, particularly in cell lineages undergoing chromosome endoreduplication (Nagl 1982). Our data on elongated leaf epidermis cells in IHP and ILP (Table 1, Fig. 5) confirm such a possible role of nuclear DNA content: mean cell surface area is larger in that line, IHP, where the frequency of 4C and 8C cells is higher. A similar relationship between cell size and nuclear DNA content has been reported in other species (Smith 1973; Cionini et al. 1983; Bryans and Smith 1985; Cavallini and Natali 1994). It is worth noting that reciprocal crosses between IHP and ILP show a similar mean cell area, but this is significantly larger than that of parental lines (Table 1, Fig. 5), possibly indicating a heterotic effect on the determination of this cell parameter. Heterosis for cell elongation has already been reported in hybrid maize embryos (Wang 1947). Our data indicate that the heterotic effect is largely limited to the first generation: in fact, mean elongated cell areas in the F_2 are nearly intermediate between those of parentals (Table 1, Fig. 5). The existence of heterosis for cell elongation, and the lack of heterosis for mean ploidy level, indicate that nuclear DNA content is not the only responsible factor for the final size of elongated cells, but that other genetic factors may also be involved in determining this character.

As for the genes involved in the inheritance of ploidy levels, some hypotheses may be suggested. Two important points are known to occur in the cell-cycle progression, the G_1/S and G_2/M transitions. In higher eukaryotes, these transitions are regulated by a class of specific proteins, the cyclins; in particular, cyclin E for the G_1/S and cyclin B for the G_2/M transition. Cyclins activate different forms of cyclin-dependent-kinases (CDKs), CDK 2 for the G_1/S transition and CDK 1 for the G_2/M transition. Cyclin B/CDK 1 is referred to as the mitosis-promoting factor (MPF), cyclin E/CDK 2 as the replication-promoting factor (RPF) (Murray 1994; Nagl 1995; Nasmyth 1995). It has been hypothesized that the switch from proliferation to polyploidization, in both animals and plants, is related to developmentally regulated repression and/or destruction of the MPF and the simultaneous activation of the RPF (Nagl 1995). This hypothesis has been supported by experimental evidence from maize endosperm (Grafi and Larkins 1995). It is plausible that, after repression of the G_2/M factor, different degrees of somatic polyploidy may be attained by plant nuclei according to the production rate of the active G_1/S factor.

As first discussed by Dewey and Howard (1963), and later specified by Cionini et al. (1983), G_2/4C is the stage when a cell takes a decision whether to remain in the cell cycle or to differentiate through chromosome endoreduplication. Differentiated 4C cells in the leaf epidermis of *Triticum durum* (Cionini et al. 1983) and in maize (this paper) represent the initial step of chromosome endoreduplication (cf. also Dolezelova et al. 1992). A difference in chromosome endoreduplication between IHP and ILP was reported in endosperm parenchyma cells by Cavallini et al. (1995b): the mean ploidy level was higher in IHP than in ILP, indicating a general tendency in IHP plants to switch more frequency from proliferation to polyploidization than in ILP plants.

It must be noted, however, that, while in reciprocal crosses between IHP and ILP the endosperm showed the same ploidy level as in the maternal plant, in elongated cell rows of leaf epidermis the ploidy levels of reciprocal crosses are the same and are intermediate between the two parentals, i.e. the inheritance of this character is different in the two tissues. This difference may be related to a different activity, and/or efficiency, of the genes involved in cell-cycle control in the two tissues, possibly mediated by auxin, which is known to affect cell-cycle progression in plants (Dudits et al. 1993; Nagl 1995) and is largely produced during the differentiation of maize endosperm (Lur and Setter 1993).

In conclusion, the present data show that the ploidy level in differentiated plant cells of maize is inherited. The availability of adequate DNA probes may now enable us to better understand these important aspects of cell differentiation in plants.

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