DNA Endoreduplication in Maize Endosperm Cells is Reduced by High Temperature During the Mitotic Phase

G. Engelen-Eigles, R. J. Jones,* and R. L. Phillips

ABSTRACT

DNA endoreduplication is the replication of nuclear DNA without subsequent mitosis and cell division, and is believed to be important in maize (*Zea mays* L.) kernel development. DNA endoreduplication has been shown to be negatively affected by high temperature treatments (HTTs) imposed during early kernel development in maize; however, the specific period of endosperm development at which the process is most sensitive to HTT has not been determined. To address this issue, HTTs (4 and 6 d at 35°C) were applied to in vitro-grown maize kernels starting at 4, 6, 8, 10, or 12 d after pollination (DAP). Our approach was to isolate endosperms to determine the effect of elevated temperature on endosperm fresh weight (FW), number of endosperm cells, mitotic index, and DNA endoreduplication. The 4- and 6-d HTTs imposed during the mitotic phase of the endosperm cell cycle (4, 6, and 8 DAP) reduced nuclei number and kernel FW, delayed cell division, decreased average DNA content, and reduced the percentage of nuclei in the 24, 48, and 96 *C* classes (*C* is the DNA content of a haploid nucleus in maize). In contrast, delaying the imposition of the 4- or 6-d HTTs until 10 or 12 DAP (during the endoreduplication phase of the endosperm cell cycle) did not affect nuclei number, average DNA content, and DNA endoreduplication compared to the control. Thus, HTTs are most deleterious to DNA endoreduplication, endosperm FW, and nuclei number when applied during the mitotic phase of the endosperm cell cycle. These data further show that 4 to 10 DAP is the period during maize endosperm development that is most sensitive to high temperature, and that prolonged exposure restricts entry of mitotic cells into the endoreduplication phase of the cell cycle.

THE MAIZE ENDOSPERM makes up 70 to 90% of kernel mass; thus, factors that mediate endosperm development to a great extent also determine grain yield of maize. Previous studies have shown that the sink capacity of the endosperm is primarily determined by the number of endosperm cells and starch granules that are formed during the first 10 to 12 DAP (Capitanio et al., 1983; Reddy and Daynard, 1983; Jones et al., 1985). Recently, we also reported that the magnitude of DNA endoreduplication was highly correlated with endosperm FW, which implies an important role of DNA endoreduplication in the determination of endosperm mass (Engelen-Eigles et al., 2000).

Maize endosperm development consists of several different phases. The endosperm nucleus results from a fusion of one sperm with two polar nuclei. Mitotic divisions are observed within 3 to 5 h of fertilization (Kiesselbach, 1949). At 3 d after fertilization, cell walls are developed, and 2 d later the endosperm is completely celluarized (Kiesselbach, 1949). Most of the endosperm cells are produced between 4 and 12 DAP, with the mitotic index peaking between 6 and 8 DAP (Kowles and Phillips, 1988; Engelen-Eigles et al., 2000). DNA endoreduplication and starch synthesis start between 10 and 12 DAP (Jones et al., 1985; Kowles and Phillips, 1985).

DNA endoreduplication leads to enlarged nuclei with elevated DNA contents. DNA endoreduplication has been observed in the endosperm of *Z. mays* L. (Kowles and Phillips, 1985) and *Lycopersicon esculentum* Mill. (Bino et al., 1992), as well as in other tissues in plants, for example *Abies balsamea* (L.) Mill. (Mellerowicz and Riding, 1992) and *Arabidopsis thaliana* (L.) Heynh. (Galbraith et al., 1991). During maize endosperm development, the cell cycle takes two discrete forms: a mitotic cycle and an endoreduplication cycle. The mitotic cell cycle consists of G_1, S, G_2, and M; thus the nuclear DNA value of an endosperm mitotic cell is expected to be 3 or 6 *C*. However, during DNA endoreduplication, the cell cycle consists of G and S only; the cells do not divide, and no mitosis-like structural changes can be seen in the nucleus (Nagl, 1982, 1990). Recent research shows that endoreduplicating tobacco (*Nicotiana tabacum* L.) cells can revert back to cell division when supplied with auxin and cytokinin, resulting in a reduction of the DNA content per cell (Valente et al., 1998). The intricate details of the regulation of the endoreduplication cycle are beginning to be unraveled. Recent molecular studies suggest that the inactivation of $p34^{cdc2}$/cyclin B kinase, a mitotic kinase, and the activation of S-phase related protein kinases are required for DNA endoreduplication to occur (Grafi and Larkins, 1995; Nagl, 1995; Grafi, 1998).

Maize DNA endoreduplication starts at ≈10 DAP and peaks at 16 DAP, with the presence of at least four DNA content classes (12, 24, 48, 96 *C* or higher). By 18 DAP, the resolution of the individual *C*-classes becomes unclear, which is thought to be due to DNA degradation and/or starch granule interference, or it may be an artifact of the DNA preparation protocol (Kowles and Phillips, 1985). The onset of DNA endoreduplication and the number of *C*-classes depend on the maize genotype (Kowles et al., 1997) and environment (Meyers et al., 1992; Engelen-Eigles et al., 2000). The magnitude of maize endosperm DNA endoreduplication appears to be maternally inherited (Kowles et al., 1997), and the extra DNA synthesized by the endoreduplication process is likely important in maize kernel development (Kowles and Phillips, 1985; Kowles et al., 1992). It has been suggested that the increased DNA content during

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Abbreviations: *C*, the DNA content of a haploid nucleus in maize; DAP, d after pollination; FCM, flow cytometry; FW, fresh weight; HTT, high temperature treatment; LSD, least significant difference.

endoreduplication may provide for increased gene expression during endosperm development and kernel filling, since it coincides with increased enzyme activity and protein accumulation at this time (Kowles et al., 1992). DNA content and endosperm FW are highly correlated $r = 0.93$) between 4 to 18 DAP, indicating the importance of DNA endoreduplication in determining kernel mass (Engelen-Eigles et al., 2000).

Environmental perturbations such as water-deficit and exposure to high temperature affect the maize endosperm cell cycle and the magnitude of endoreduplication, and lead to reduced nuclei number and mature kernel mass (Meyers et al., 1992; Engelen-Eigles et al., 2000). In vitro studies have shown that kernel water potential decreases in response to decreased media water potential (Meyers et al., 1992). Media of −1.6 or −2.0 MPa resulted in a reduction of endosperm cell number compared with the nonstressed kernels (Meyers et al., 1992). Maize endosperm DNA endoreduplication was significantly reduced when water stress treatments were given from 1 to 10 DAP (Artlip et al., 1995). While the optimum temperature for maize kernel development is between 27 and 32°C (Keeling and Greaves, 1990), Jones et al. (1984) found that nuclei number, starch granule number, and kernel FW were reduced by exposure to 35°C for 6 to 8 d during the most sensitive phase of endosperm cell division (4 to 10 DAP). Night temperatures above 30°C during the early stages of maize endosperm development are especially detrimental to maize kernel development (Teixeira, 1995). Our recent studies show that the magnitude of maize endosperm DNA endoreduplication is reduced by 4- and 6-d HTTs imposed at 4 DAP. Since such treatment causes the endosperm cells to remain predominately mitotic, we concluded that HTT restricts the entry of mitotic cells into the endoreduplication phase of the cell cycle (Engelen-Eigles et al., 2000). However, the specific interval during the early stages of maize endosperm development during which the DNA endoreduplication process is most sensitive to HTTs remains unclear. Moreover, it is not known to what extent HTT affects DNA endoreduplication per se. Thus, the specific objective of this study was to determine when during endosperm development short-term (4 d) or long-term (6 d) exposure to HTT is most detrimental to endosperm DNA endoreduplication and kernel development in maize.

MATERIALS AND METHODS

Field-grown maize plants (cv. A619 × W64A) were self-pollinated and at 3 DAP the kernels were excised and cultured in vitro, as described by Gengenbach and Jones (1993). Five kernels were placed in a petri dish, and the control and each HTT consisted of three petri dishes. Kernels were placed in a 25°C incubator under complete darkness for 24 h to permit acclimation to the in vitro growing conditions. A portion of the cultured kernels were retained at 25°C and used as controls; the remainder were exposed to short-term HTTs (4 d at 35°C) starting at 4, 6, 8, 10, and 12 DAP. Other kernels were exposed to long-term HTTs (6 d at 35°C) starting at 4, 6, 8, 10, and 12 DAP. However, since the 6-d HTT results were comparable to the 4-d HTT, only selected components of the 6-d HTT will be discussed. At the end of each HTT, the kernel cultures were transferred back to 25°C for the remainder of the experimental period until the study was terminated at 18 DAP. Kernels were sampled on alternate days from 8 to 18 DAP and fixed in 3:1 ethanol (950 mL L^{-1}):propionic acid for 24 h. The samples were then transferred to 700 mL L^{-1} (v/v) ethanol and stored at −4°C (Kowles et al., 1994).

Endosperm nuclei (a combination of three endosperms per petri dish) were stained with mithramycin A (a GC-binding DNA fluorochrome; Sigma, St. Louis, MO) for analysis by flow cytometry (FCM) and were prepared as follows: endosperm cells were carefully dissected from the kernels and macerated with a flattened probe by forcing them through a 150-μm mesh screen (Bellco Glass Inc., Vineland, NJ) placed on top of a small funnel in a 1.5-mL microfuge tube. The screen was washed three times with 500 μL grinding buffer [100 m*M* glycine, 10.0 mL L^{-1} hexylene glycol (v/v), 1.0 mL L^{-1} Triton X-100 (v/v), and 2.0 g L^{-1} phenylmethylsulfonylfluoride (w/v)]. The sample was then centrifuged for 1 min at 180 g. The supernatant was decanted and the pellet resuspended in 400 μL mithramycin buffer [45 m*M* $MgCl_2$, 30 m*M* sodium citrate, 20 m*M* 3-(N-Morpholino) propanesulphoric acid, 1.0 mL L^{-1} Triton X-100 (v/v), pH adjusted to 7.0 with 1.0 *M* NaOH], and allowed to equilibrate for 30 min. The samples were again centrifuged for 1 min at 180 g, and the pellet was then resuspended in 200 μL mithramycin A stain solution (0.25 mg mithramycin A mL^{-1} mithramycin buffer). Endosperm nuclei were stained overnight in complete darkness (Kowles et al., 1994). The magnitude of DNA endoreduplication was determined with a Coulter Epics flow cytometer (Coulter Corp., Hialeah, FL). The water-cooled argon laser was aligned at 488 nm with DNA microspheres at a coefficient of variance <2.0. The laser was set to 455 nm for endosperm DNA content analysis. Up to 2000 nuclei were analyzed for each sample. The sample readings were gated to eliminate nuclear debris. Only samples up to 18 DAP were analyzed by FCM because later samples contained too much starch for accurate analysis.

Endosperm FW data were taken at the time of endosperm preparation for FCM; nine endosperms (three kernels per petri dish × three replicates) per HTT were weighed to determine endosperm FW. Nuclei number was determined by FCM by adding a known concentration of DNA microspheres (DNA Check, Coulter Corp., Hialeah, FL) to the endosperm nuclei preparation. The flow cytometer was programmed to run up to 2000 DNA microspheres while the nuclei were being counted. These data were used to calculate the number of nuclei per endosperm. The mitotic index was determined as the number of cells in prophase, anaphase, metaphase, and telophase divided by the total number of cells. For mitotic index determination, endosperm tissue was stained with acetocarmine and squashed with an iron needle. The preparation was briefly heated to clear the stain from the cytoplasm. Six endosperms (two kernels per petri dish × three replicates) were prepared, and 300 nuclei per endosperm were counted for mitotic index determination.

Statistical Design

Treatments were arranged in a complete randomized design. Each treatment consisted of 3 petri dishes with 5 kernels per petri dish. The experiment was performed in 1994 and repeated in 1995. Analysis of variance was calculated, and means were compared using the least significant difference (LSD) test at $P < 0.05$. There was no statistical difference

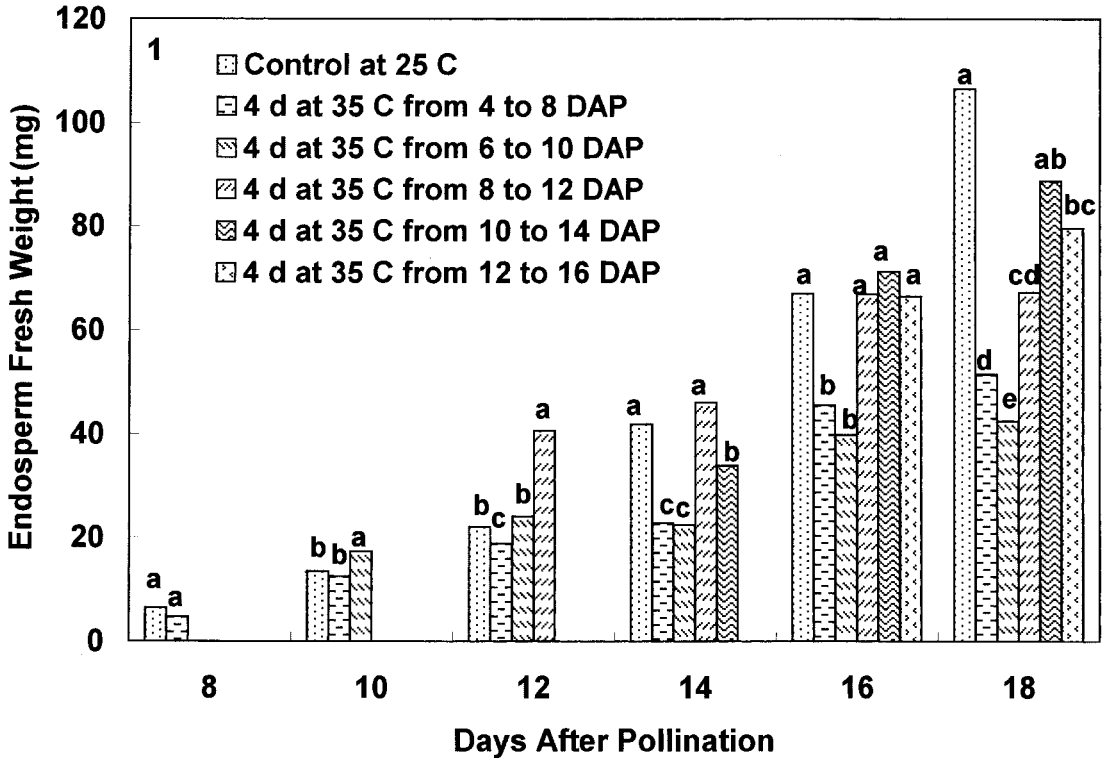

Fig. 1. The effect of sequential application of high temperature treatments (HTTs) (4 d at 35°C) on maize endosperm fresh weight (FW). Endosperms were weighed at the time of preparation for flow cytometry (FCM). Different letters indicate a significant difference of means, $n = 18$, LSD = 0.05.

between years, therefore the data were pooled. All data were analyzed with Statistix (Analytical Software, St. Paul, MN).

RESULTS

Endosperm Fresh Weight

The endosperm FW for the control kernels (constant 25°C) increased gradually from 8 to 12 DAP (Fig. 1). A rapid increase in control FW was observed between 14 and 18 DAP; this increase was likely due mainly to increased starch deposition during this period. The 4-d HTT reduced endosperm FW (Fig. 1); however, in most cases the detrimental effect of these treatments on endosperm FW was manifested after return to control temperature later in kernel development. The 4-d HTT applied at 4 or 6 DAP did not show a rapid increase in FW between 12 and 18 DAP, compared with the control endosperm (Fig. 1). The 4-d HTT imposed at 8, 10, or 12 DAP also resulted in reduced endosperm FW compared with the control endosperm, but the reduction was not as severe as when this treatment was imposed at 4 or 6 DAP. Similar to the control endosperm, the 4-d HTT starting at 8, 10, or 12 DAP showed a rapid increase in FW from 14 to 16 DAP (Fig. 1). The 6-d HTT results (data not shown) were similar to those observed for the 4-d HTT results. Therefore, collectively these data suggest that 4 to 10 DAP (the mitotic phase of the endosperm cell cycle) is the period during early kernel development in maize that is most sensitive to high temperature.

Endosperm Nuclei Number and Mitotic Index

The nuclei number of the control endosperm increased more than three-fold from 8 to 12 DAP (Fig. 2A), which corresponds to the period of maximum cell division (Fig. 2B). The control nuclei number continued to increase slowly at 14 DAP and peaked at 18 DAP, while the mitotic index decreased slowly to less than 1.0% at 16 DAP (Fig. 2B).

The time of initiation of the 4-d HTT affected endosperm nuclei number significantly, and delayed the occurrence of peak mitotic index. With the exception of the initial sampling date (8 DAP), the 4-d HTT initiated at 4 DAP resulted in significantly lower nuclei number compared with the control at all dates sampled (Fig. 2A). The 4-d HTT imposed at 6 DAP showed similar results, though the nuclei number for this treatment was not significantly different from the control at 14 and 16 DAP. The 4-d HTT beginning at 4 and 6 DAP resulted in a low mitotic index at the end of the treatment period (8 and 10 DAP). However, the percentage of the cells that were dividing increased significantly 2 d after the end of the HTT, and remained high at 12, 14, and 16 DAP compared with that observed for nuclei from control kernels (Fig. 2B). These data suggest that high temperature shifted the time of occurrence of maximum mitosis to later times in the endosperm.

The 4-d HTT initiated at 10 or 12 DAP did not result in lower nuclei numbers compared with the control at 16 and 18 DAP (Fig. 2A). The mitotic index was low for these treatments and not significantly different from

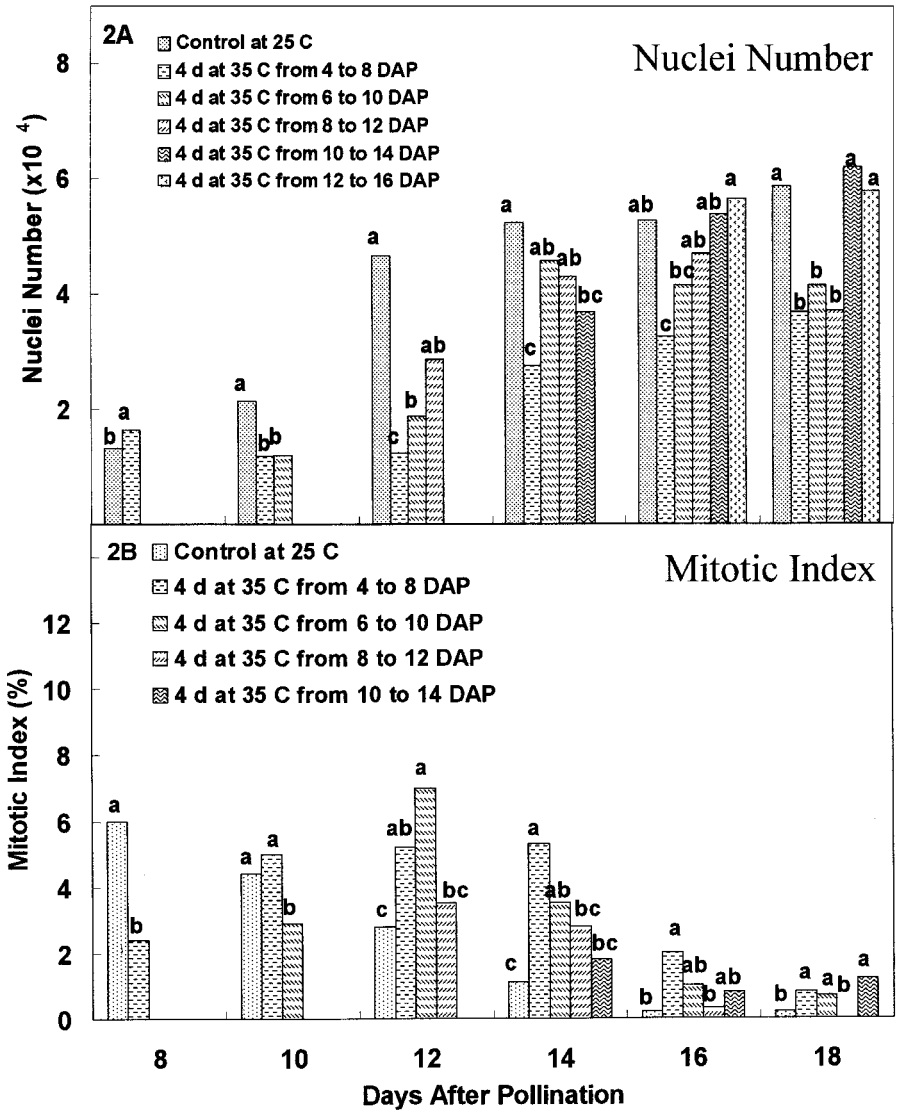

Fig. 2. The effect of sequential application of high temperature treatments (HTTs) (4 d at 35°C) on maize endosperm nuclei number (2A) and mitotic index (2B). Nuclei number was determined by flow cytometry (FCM). Different letters indicate a significant difference of means, n = 6, LSD = 0.05. Mitotic index was determined as the number of nuclei in prophase, metaphase, anaphase, and telophase, divided by the total number of nuclei viewed. Different letters indicate a significant difference of means, n = 6 endosperms, and 300 nuclei per endosperm were counted, LSD = 0.05.

the control (Fig. 2B). Comparable to the 4-d HTT, the 6-d HTT initiated at 4 and 6 DAP reduced the endosperm nuclei number more dramatically than when this treatment was started at 8, 10, or 12 DAP (data not shown). At 18 DAP, nuclei numbers in all 6-d HTTs were significantly lower than in the control endosperm. Analogous to the endosperm FW data, the endosperm nuclei number and mitotic index data also support the suggestion that 4 to 10 DAP is the most sensitive period to high temperature.

DNA Endoreduplication

The DNA content of maize endosperm cells are broadly divided into two levels: one level corresponds to cells with a mitotic DNA content of 3 or 6 *C*, and another level corresponds to cells with an endoreduplicating DNA content of 12 *C* or higher. It is important to note, however, that a cell with a 6 *C* content can divide and result in two 3 *C* cells, or it can continue into the endoreduplicating phase of the cell cycle. Unfortunately, the flow cytometric procedure employed in this study did not permit us to distinguish 6 *C* cells that are being committed to endoreduplication from those that remain mitotic. The mitotic DNA content (3 and 6 *C*) for the control nuclei declined over time from 84% at 10 DAP to 41% at 18 DAP (Fig. 3A). The mitotic DNA content for endosperms of kernels exposed to 4-d HTT imposed at 4 or 6 DAP also declined over the period sampled, but remained significantly higher compared with that of the control endosperm at 14, 16, and 18 DAP (Fig. 3A). With the exception of 16 DAP, the

mitotic DNA content for the 4-d HTT imposed at 10 DAP was not significantly different from that of the control. Likewise, the mitotic DNA content for the 4-d HTT imposed at 12 DAP was not significantly different from that of the control at all sampling dates (Fig. 3A).

At 10 DAP, DNA endoreduplication was already occurring in the control endosperm; 15% of the nuclei had an endoreduplicating DNA content (12 *C* or higher). All of these nuclei were in either the 12 or 24 *C* classes. The percentage of control nuclei with an endoreduplicating DNA content increased from 15% to 58% between 10 to 18 DAP (Fig. 3B). Clearly, over time, the majority of the control endosperm nuclei moved from a mitotic to an endoreduplicating DNA content. In addition, as development progressed to 16 and 18 DAP, a higher percentage of nuclei were in the 24, 48, and 96 *C* classes (data not shown).

To elucidate when DNA endoreduplication is most vulnerable to high temperature, both the 4- and 6-d HTTs were imposed at the time of high mitotic activity (4 to 10 DAP) and when DNA endoreduplication had been initiated (10 DAP). DNA endoreduplication was observed at 10 DAP for the 4-d HTT imposed at 4 and 6 DAP (Fig. 3B). At 10 DAP, the 4-d HTT imposed at 4 and 6 DAP showed 14% and 13% of the nuclei with an endoreduplicating DNA content (Fig. 3B). The endoreduplicating DNA content increased from 10 to 18 DAP for these two HTTs, but the increase in percentage of nuclei with an endoreduplicating DNA content was not as dramatic as that observed for the control endosperm and the HTT imposed after 8 DAP. For example, the 4-d HTT beginning at 4 DAP showed that only 23% of the nuclei left the 3- and 6-C pool for endoreduplication between 10 and 18 DAP (Fig. 3B). In contrast, the

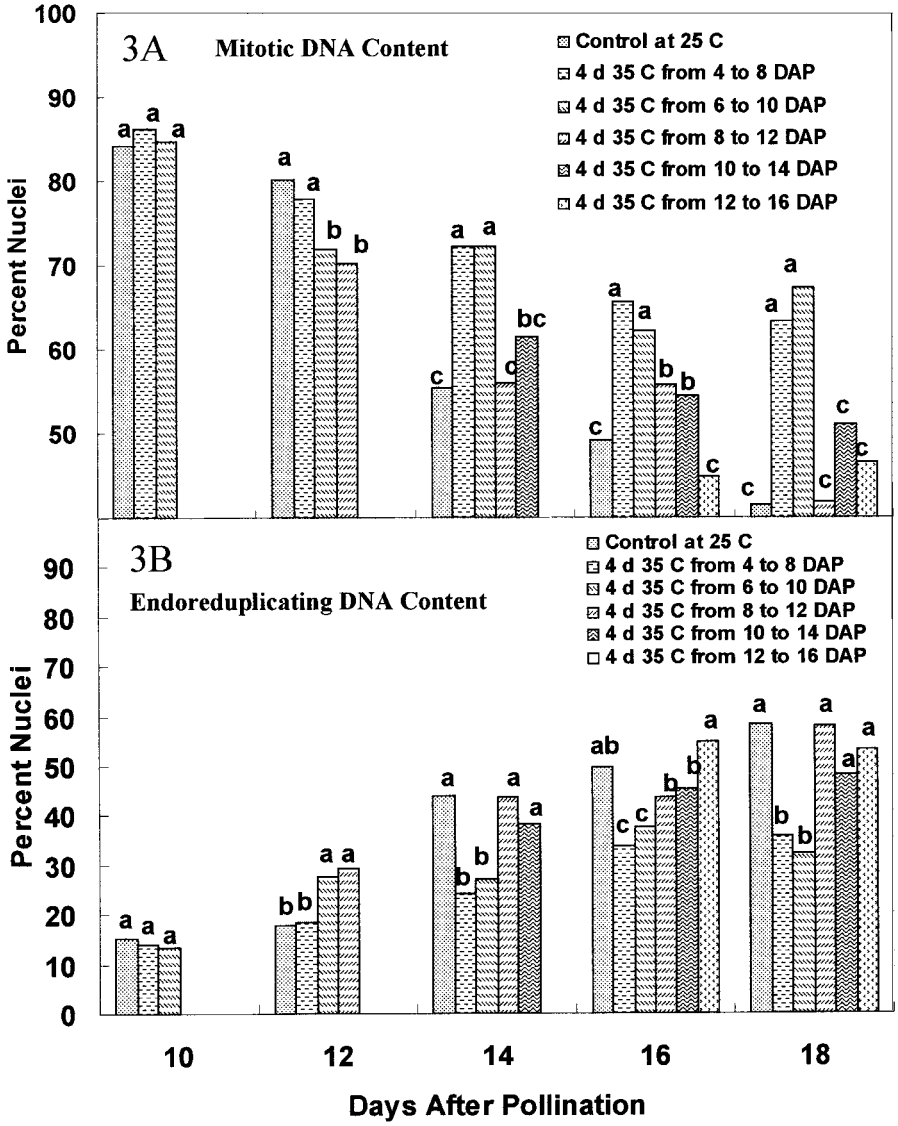

Fig. 3. The effect of sequential application of high temperature treatments (HTTs) (4 d at 35°C) on the percent nuclei with a mitotic DNA content (3A), and on the percent nuclei with an endoreduplicating DNA content (3B). The mitotic DNA class was comprised of the 3 and 6 *C* DNA content classes. The endoreduplication DNA classes were compiled by adding the percent nuclei in the 12, 24, 48, and 96 *C* classes. Different letters indicate a significant difference of means, $n = 6$, LSD = 0.05.

control endosperm showed 43% of the nuclei entering the DNA endoreduplication phase of the endosperm cell cycle during the same time interval.

The results from the 4-d HTT applied when DNA endoreduplication was in progress were considerably different from the results obtained when these HTTs were applied during the mitotic phase of endosperm development. The 4-d HTT imposed at 8, 10, or 12 DAP resulted in a percentage of nuclei with an endoreduplicating DNA content that was not significantly different from the control nuclei at 14, 16, and 18 DAP (Fig. 3B). The 6-d HTT yielded similar results to the 4-d HTT (data not shown). The results from the 4-d or 6-d HTT confirmed that DNA endoreduplication was most reduced when these treatments were applied during the mitotic phase of endosperm development (4–10 DAP). When these treatments were initiated at 10 or 12 DAP (i.e., during endoreduplication), the extent of DNA endoreduplication was much less affected. Therefore, the DNA endoreduplication process itself appeared to be quite resistant to either short- or long-term exposure to high temperature.

Average DNA Content

The average DNA content per endosperm was calculated by multiplying the percentage of nuclei in each *C* class by the *C* value of that class. Thus, a group of endosperm cells containing mostly endoreduplicating nuclei will yield a higher average DNA content than one with the majority of its nuclei as mitotic nuclei (3 and 6 *C*). The control endosperm showed a gradual increase in average DNA content to reach its maximum at 18 DAP (Fig. 4).

The 4-d HTT imposed during the mitotic cell cycle stage of the endosperm development (4 or 6 DAP) reduced the average DNA content significantly at 14, 16, and 18 DAP compared to that of control endosperm (Fig. 4). At 16 DAP, the average DNA content for the 4-d HTT beginning at 8 and 10 DAP was lower than that of the control, which was due to the higher mitotic DNA content at 16 DAP compared with that of the control (Fig. 3A). However, at 18 DAP, the average DNA content for the 4-d HTT starting at 8, 10, or 12 DAP did not differ from that of the control. Similarly, the average DNA content for the 6-d HTT beginning at 4 or 6 DAP was significantly lower than that of the control endosperm at 18 DAP (data not shown).

DISCUSSION

The objective of our study was to determine when during early maize kernel development DNA endoreduplication in the endosperm was most sensitive to high temperature. The approach was to impose 4- or 6-d HTTs sequentially at chronologically later stages during maize kernel development to determine their effect on DNA endoreduplication, endosperm FW, nuclei number, mitotic index, and average DNA content.

Maize endosperm FW was significantly reduced by the HTTs that were imposed during the mitotic phase (4–10 DAP) of the endosperm cell cycle. In contrast,

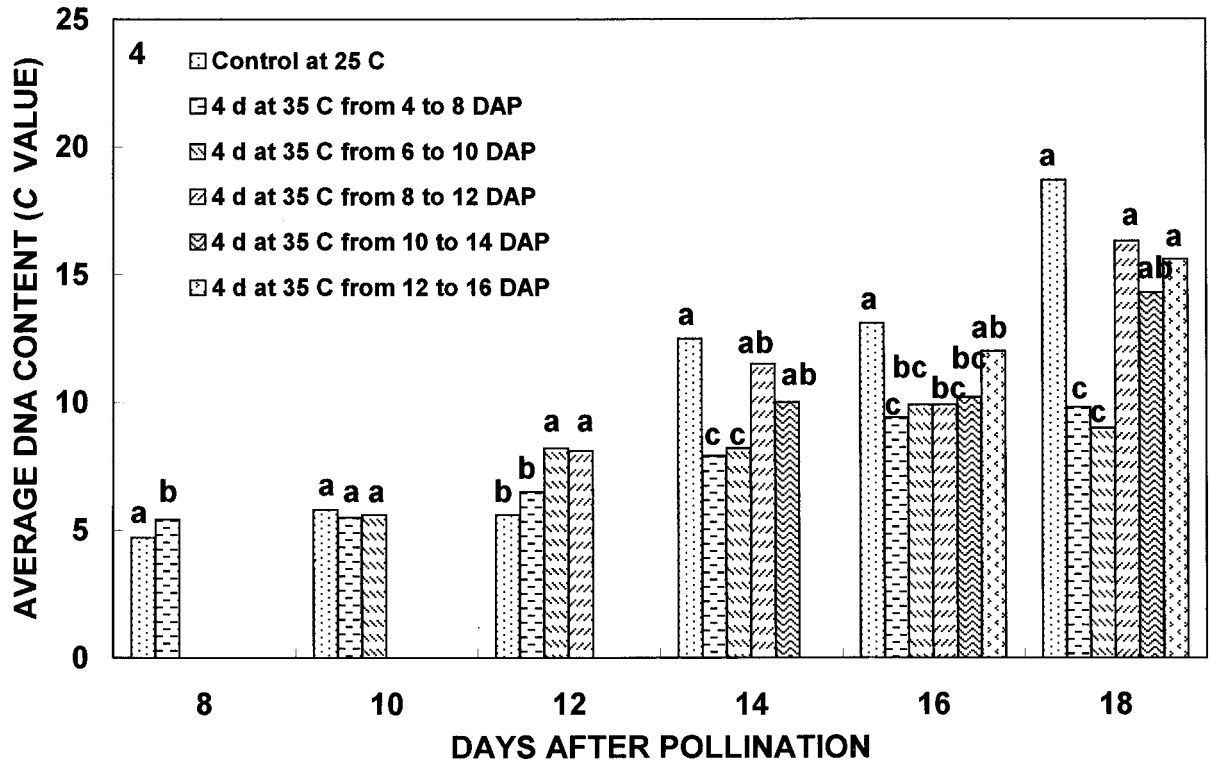

Fig. 4. The effect of sequential application of high temperature treatments (HTTs) (4 d at 35°C) on average DNA content in maize endosperm. Different letters indicate a significant difference of means, $n = 6$, LSD = 0.05.

the HTTs given during the endoreduplication phase of the endosperm cell cycle (10 to 16 DAP) resulted in a FW reduction that was less severe. The control endosperm FW increased steeply from 14 to 18 DAP, whereas the FW for the HTTs did not (Fig. 1). The reduction in FW by the HTT imposed during the mitotic phase of the cell cycle might have been the result of reduced cell proliferation and cell expansion growth. Indeed, a 4-d HTT imposed early in development resulted in ultrastructural changes that led to less endosperm cavity filling (Commuri and Jones, 1999). In addition, the FW data suggest that starch biosynthesis, which normally starts between 14 and 16 DAP, was optimal for the control endosperm and contributed significantly to the endosperm FW at 18 DAP. Starch biosynthesis may have been less optimal for the HTTs applied from 4 to 10 DAP, thus contributing less to the lower rate of endosperm growth. We have reported that starch granule number was reduced in maize endosperm that was exposed to 35°C during the early stages of kernel development (Jones et al., 1984 and 1985; Commuri and Jones, 1999). The 4- and 6-d HTTs applied early during kernel development (4 DAP) has been shown to reduce the activity of RNA transcript levels of the key starch synthesis enzymes such as ADPglucose pyrophosphorylase (ADPG-Ppase) and soluble and insoluble forms of starch synthase (Teixeira, 1995; Commuri, 1997). We surmise from the current data that such effects would be more severe when the HTT are imposed during the mitotic stage of endosperm development than during the endoreduplication stage.

The endosperm nuclei number (i.e., cell number) was also significantly reduced by the HTTs when applied early in endosperm development. These data are in agreement with earlier observations (Jones et al., 1984; Commuri and Jones, 1999; Engelen-Eigles et al., 2000). Coincident with the nuclei number data, the 4-d HTT imposed at 4 or 6 DAP delayed the peak mitotic index. Generally, the mitotic index for endosperm growing at 25°C peaks between 6 and 8 DAP (Fig. 2B) (Kowles and Phillips, 1988; Engelen-Eigles et al., 2000). The reduced nuclei number observed when the 4-d HTT was imposed at 4 or 6 DAP may be due to the disruption of mitosis as was shown by the delay in the mitotic index. Indeed, when the 4-d HTT was given after the mitotic phase of the endosperm cell cycle (e.g., at 10 or 12 DAP), nuclei number and mitotic index were not significantly different from those of the control endosperm. Therefore, the data show that the imposition of HTTs during the period of active cell division in the endosperm results in a significant decrease in kernel sink capacity via a reduction in the number of endosperm cells formed.

The magnitude of DNA endoreduplication is significantly affected when 4-d HTTs are imposed during the mitotic phase of kernel development; this may further contribute to the decrease in sink potential. These HTTs led to a reduced percentage of nuclei with an endoreduplicating DNA content (Fig. 3B) and to a significantly lower average DNA content (Fig. 4). The majority of nuclei remained mitotic (3 or 6 *C*) when the 4-d HTTs were initiated at 4 or 6 DAP (Fig. 3a), thus supporting the hypothesis that HTT seem to restrict the entry of mitotic cells into the endoreduplication cell cycle. Commuri and Jones (1999) have shown that kernels exposed to 4- and 6-d HTTs showed irregular-shaped nuclei and altered size of the nucleolus. Clearly, such structural changes may have resulted in copious numbers of cells with nuclei that had lost the competency to move to the endoreduplication phase of the endosperm cell cycle. In addition, the disruption of the endoreduplication cycle induced by the HTTs imposed at 4 and 6 DAP may have been due to the disruption of key molecular processes such as the inactivation of $p34^{cdc2}$/cyclin B kinase, believed to be part of the required molecular processes for DNA endoreduplication to occur (Grafi and Larkins, 1995; Grafi, 1998; Nagl, 1995). The prolonged cell divisions observed for these 4-d HTTs (Fig. 2A) suggest that the $p34^{cdc2}$/cyclin B kinase complex was not completely deactivated. These data suggest that further research should focus on the effect of HTTs on the molecular processes that control DNA endoreduplication.

From this study, it was clear that HTTs did not have a direct effect on DNA endoreduplication, since the 4-d HTT starting at 8, 10, or 12 DAP did not affect the magnitude of DNA endoreduplication and resulted in an average DNA content at 18 DAP that was similar to that of the control (Fig. 3 and 4). Thus, these data indicate that the DNA endoreduplication process itself is quite resilient to high temperature. It is the period before the cell cycle is committed to DNA endoreduplication (e.g., during the mitotic phase of the cell cycle) that is most sensitive to high temperature, which results in reduced DNA endoreduplication later in kernel development.

The present findings are in concert with the water deficit studies on maize endosperm DNA endoreduplication by Artlip et al. (1995). They concluded that DNA endoreduplication was significantly reduced when water stress treatments were given from 1 to 10 DAP. Water stress applied later in endosperm development was less deleterious to DNA endoreduplication than when given early in endosperm development. We conclude that the period from 4 to 10 DAP, that is, the time during which endosperm cells are in a mitotic cell cycle, is the most sensitive period to environmental perturbations. The magnitudes of endosperm FW, nuclei number, and endosperm DNA endoreduplication are clearly determined during this sensitive period in maize endosperm development.

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