SEED PHYSIOLOGY, PRODUCTION & TECHNOLOGY

Conjugated and Free Polyamine Levels in Normal and Aborting Maize Kernels

Yu-Ling Liang and Huu-Sheng Lur*

ABSTRACT

Kernel abortion in maize (*Zea mays* L.) can cause significant reductions in yield. Abortion usually commences early in development and frequently occurs because of stresses such as water deficit or low light. Polyamines have been implicated in development processes of fruits and seeds, but in maize the changes of polyamine levels during kernel growth are still unclear. The objectives of this study were to investigate the roles of polyamines in the early stages of kernel growth and kernel abortion. Two single-cross hybrids with different kernel abortion characteristics were grown in a greenhouse with shade treatment. During early development of normal, naturally aborting, and shade-induced aborting kernels, temporal changes in the concentrations of endogenous putrescine (PUT), spermidine (SPD) and spermine (SPM) and their free, perchloric acid (PCA)-soluble conjugated, and PCA-insoluble bound forms, were measured. Changes in DNA content and the number of endosperm nuclei were also measured. Total polyamine levels in normal kernels rapidly increased 4 to 6 d after pollination (DAP), and peaked during active endosperm cell division. The most abundant polyamines were, in order, PCA-soluble conjugated PUT, PCA-soluble conjugated SPD, and free PUT. Aborting kernels had significantly lower levels of polyamines than normal kernels after 4 DAP. The low polyamine levels were temporally associated with a low number of endosperm nuclei and low DNA content. The results suggest that polyamines may be involved in the regulation of early endosperm development in maize.

Kernel abortion in maize frequently occurs because of stresses such as water deficit or low light (Kiniry and Ritchie, 1985; Artlip et al., 1995). Abortions usually occur in the apical region of the ear and can significantly reduce yield. The physiological basis for kernel abortion is still unclear. Polyamines have been known to be involved in the regulation of fruit or seed development (Evans and Malmberg, 1989). The focus of the present study was to investigate the correlation between polyamines and kernel abortion in maize.

Abortion commences early in kernel development, before 12 d after pollination, at about the same period normal kernels undergo endosperm cell division, kernel enlargement begins, and DNA endoreduplicates (Kiniry and Ritchie, 1985; Artlip et al., 1995). During drought, kernel abortion may be caused by an inadequate supply of carbohydrates (Hanft and Jones, 1986). Kernel abortion was more closely associated with a reduction in leaf photosynthesis than to a shortage of carbohydrates within kernels (Reed and Singletary, 1989; Schussler and Westgate, 1991). However, factors other than an inadequate supply of assimilates may initiate the abortion process (Jones and Simmons, 1983).

Plant hormones, such as abscisic acid (ABA) and ethylene, may be involved in kernel abortion in maize. Aborted kernels had higher ABA and lower indoleacetic acid (IAA) concentrations than normal kernels (Reed and Singletary, 1989). A water deficit induced increase in ABA may inhibit endosperm cell division in kernels (Artlip et al., 1995). Whether stress induced ABA accumulation caused or initiated the abortion process is still unclear. Hanft et al. (1990) reported that application of 1-aminocyclopropane-1-carboxylic acid (ACC) inhibited kernel growth in vitro. In our previous study, the concentrations of ACC and ethylene in kernels increased significantly just 32 h after shade treatment began (Cheng and Lur, 1996). In addition, apical kernels were more sensitive to ACC than older, basal kernels. Application of ACC could inhibit endosperm cell division and the growth of very young kernels. The role of ethylene in kernel abortion still needs clarification.

Polyamines may regulate many development processes including DNA biosynthesis and cell division, especially in young, reproductive organs (Evans and Malmberg, 1989). High levels of free polyamines were detected during rice (*Oryza sativa* L.) and soybean [*Glycine max* (L.) Merr.] seed development (Sen et al., 1981; Lin et al., 1984). Significant amounts of conjugated polyamines (mostly hydroxycinnamic acid amides) were also detected in reproductive organs (Slocum and Galston, 1985; and reviewed in Martin-Tanguy, 1997). In maize, high concentrations of hydroxycinnamic acid amides were found in kernels with cytoplasmic male sterile genotypes (Martin-Tanguy et al., 1982). However, the function of free and conjugated polyamines in seed development is unknown and few studies have investigated the relationship between free and conjugated polyamines and kernel abortion. In addition, polyamines and ethylene share a biosynthetic precursor, *S*-adenosylmethionine (SAM), and metabolism of these two regulators can be mutually regulated (Apelbaum, 1990). The interaction between polyamines and ethylene in their biosynthesis may in turn regulate physiological processes.

In maize, little is known about the changes in polyamine levels and their roles during kernel growth, especially at the early developmental stage which is crucial to kernel abortion. In addition, our previous study revealed that ethylene may be involved in the regulation of kernel abortion (Cheng and Lur, 1996). Thus, to examine the possible involvement of polyamines during maize kernel abortion, we investigated fluctuations in free and conjugated polyamines in normally developing

Dep. of Agronomy, National Taiwan Univ., Taipei, Taiwan 106, Republic of China. This project was funded by the National Science Council, Republic of China. Received 18 Oct. 2000. *Corresponding author (lurhs@ms.cc.ntu.edu.tw).

Published in Crop Sci. 42:1217–1224 (2002).

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; DAP, days after pollination; IAA, indoleacetic acid; PCA, perchloric acid; PUT, putrescine; SPD, spermidine; SPM, spermine.

and aborting kernels. We also discuss the relationships between changes in polyamine concentrations, endosperm cell number, and DNA content for normal and aborting maize kernels.

MATERIALS AND METHODS

Plants

Two single cross maize hybrids, Tainung 351 (TN 351) and Tainung 1 (TN 1), were used in this study. Tainung 351 is an old cultivar and usually expresses abortion symptoms in the apical third of ears under field conditions in Taiwan. The abortion phenomenon has been greatly reduced in Tainung 1 by breeding, and the hybrid has become one of the most popular cultivars in Taiwan. Both hybrids were cultured in a greenhouse under ambient temperature and light condition (see Cheng and Lur, 1996) at the National Taiwan University Agriculture Experimental Station in Taipei, in 1998. The culture practice was the same as that commonly used in the field. Plants were grown in two rows per bed with 30 cm between rows and 20 cm between plants. Fertilizer rates were 100 kg N ha^{-1}, 50 kg P ha^{-1}, and 40 kg K ha^{-1}. Irrigation was supplied throughout the growing season as required. The ear shoots were bagged before silk emergence, and were hand-pollinated at about 4 d after silk emergence.

Shade Treatment

One or 2 d before pollination, a layer of commercial, black, polypropylene shade fabric was placed over the maize plants at about 3.5 m. The shade cloth excluded about 70% of the natural light. Light intensity and photosynthesis were monitored with a portable photosynthesis detector (LI-2100, LI-COR, Inc., Lincoln, NE, USA). One day after treatment, photosynthesis had decreased from 28 ± 2 to 6 ± 2 μmole CO_2 m^{-2} s^{-1}. Plants were shaded for 7 to 8 d. The light treatment is also described in Cheng and Lur (1996).

Kernel Sampling

The apical and basal regions of ears were defined as the apical 30% and basal 50% of the ear, respectively (Cheng and Lur, 1996). In general, aborted kernels appeared in the apical region of ears on plants in the shade treatment.

Kernels from the apical and basal regions were sampled at 0, 1, 2, 3, 4, 6, 9, and 12 d after pollination for polyamine and dry weight determinations. At each sampling date, kernels were cut from the apical and basal regions of two ears for each replication sample of treatments. Each replication consisted of 15 to 35 kernels, depending on the stage of development. Sample kernels were immersed in liquid N_2, freeze-dried and stored at −70°C. Four replications were performed for polyamine analysis. For endosperm nucleus number and DNA measurements, kernels were sampled at 4, 6, 9, and 12 d after pollination. Each sample consisted of 15 kernels dissected from an ear, and four sample replications were conducted for the endosperm nucleus number and DNA measurements.

The genotypes used were nonprolific types. At each sampling date, ears were chosen randomly from well-grown plants in control and shade treatments. In general, kernels from two ears (two plants) were sampled and pooled for each replication. Four replications were performed for the various analyses.

Endosperm Nucleus Number and DNA Content

Kernels were collected and then fixed in 950 g kg^{-1} ethanol. The endosperm was dissected from the kernel and the number of nuclei was counted using the method of Lur and Setter (1993). Four kernels of each sample were used for counting nuclei and the following DNA quantification. Endosperm DNA analysis was performed as described in Lur and Setter (1993), who modified the method of Giles and Myers (1965).

Polyamine Extraction

Polyamines were extracted by means of a modified version of the method described in Slocum and Galston (1985). Each freeze-dried sample (200 g) was homogenized in 5 mL 100 g kg^{-1} perchloric acid (PCA). After 60 min of incubation, samples were centrifuged for 10 min at 27 000 *g*. Polyamines in the supernatant are referred to as *free* polyamines. The supernatant was further hydrolyzed by combining 1 mL supernatant with 1 mL 12 *M* HCl in a glass ampule for 24 h at 110. The hydrolyzed solution was filtered through glass wool, dried at 80°C, and resuspended in 1 mL PCA. Polyamines released in the hydrolyzed fraction are called *PCA-soluble conjugated* polyamines. The pellet that was separated from the original supernatant was hydrolyzed by the same process. Polyamines released from this fraction are referred to as *PCA-insoluble conjugated* polyamines. At least four extractions were performed for each development stage and used for polyamine analysis.

Polyamine Analysis

For each extraction polyamines from the free, PCA-soluble conjugated, and PCA-insoluble conjugated fractions were benzoylated and quantified by the high-performance liquid chromatography procedure described by Lee et al. (1995), which was a modification of the method used by Flores and Galston (1982). The polyamines quantified in our experiments were PUT, SPD, and SPM. The polyamine levels were the average of four extractions for each development stage.

RESULTS

Dry Weight and Kernel Abortion

Normal kernels in the basal regions of ears from both cultivars commenced growing soon after pollination (Fig. 1). The dry weight of TN 1 kernels was usually greater than that of TN 351 kernels. In the control treatment, the dry weight of apical and basal TN 1 kernels increased normally (Fig. 1C and 1D). On shaded plants, the apical kernels showed little dry weight accumulation. The dry weight of the basal kernels increased continuously until 6 DAP. After 6 DAP, their weight increased more slowly than that of the basal kernels on plants in the control treatment. Shading caused significant abortion symptoms in the apical region of TN 1 ears.

In the apical region of TN 351 ears, kernel dry weight on ears of control plants increased only slightly and stopped increasing on ears of shaded plants (Fig. 1A). Abortion symptoms appeared in the apical regions of ears from plants in both the control and shade treatments. In this experiment, the dry weight and pattern of abortion of TN 1 and TN 351 kernels was consistent with that typically observed in the field. TN 1 usually has heavier kernels than TN 351, and kernel abortion frequently occurs in the apical region of TN 351 ears even under normal field conditions.

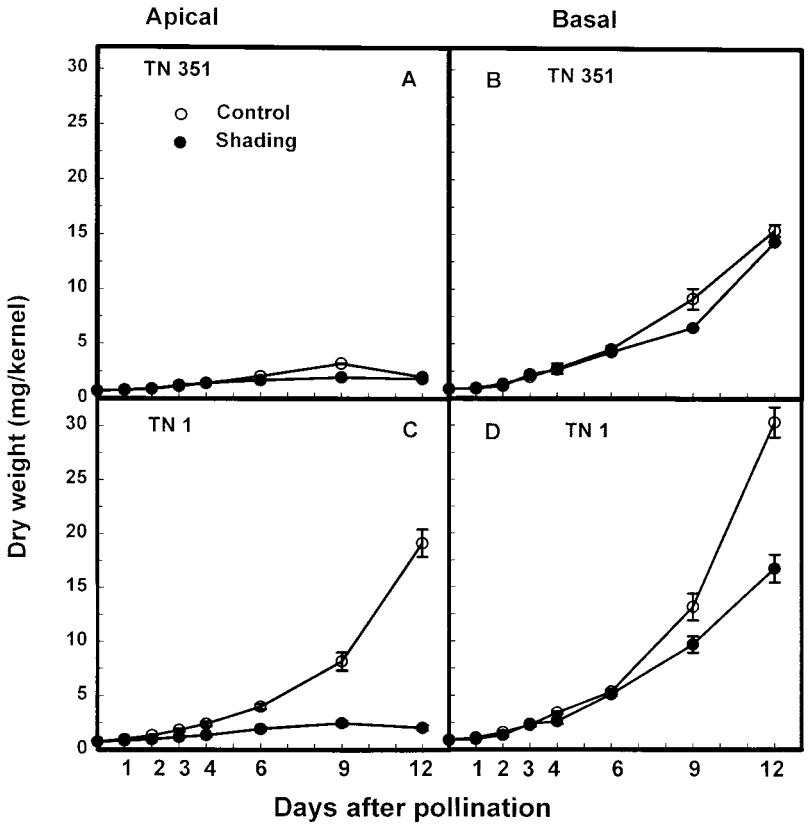

Fig. 1. Dry weight accumulation of kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four replicates.

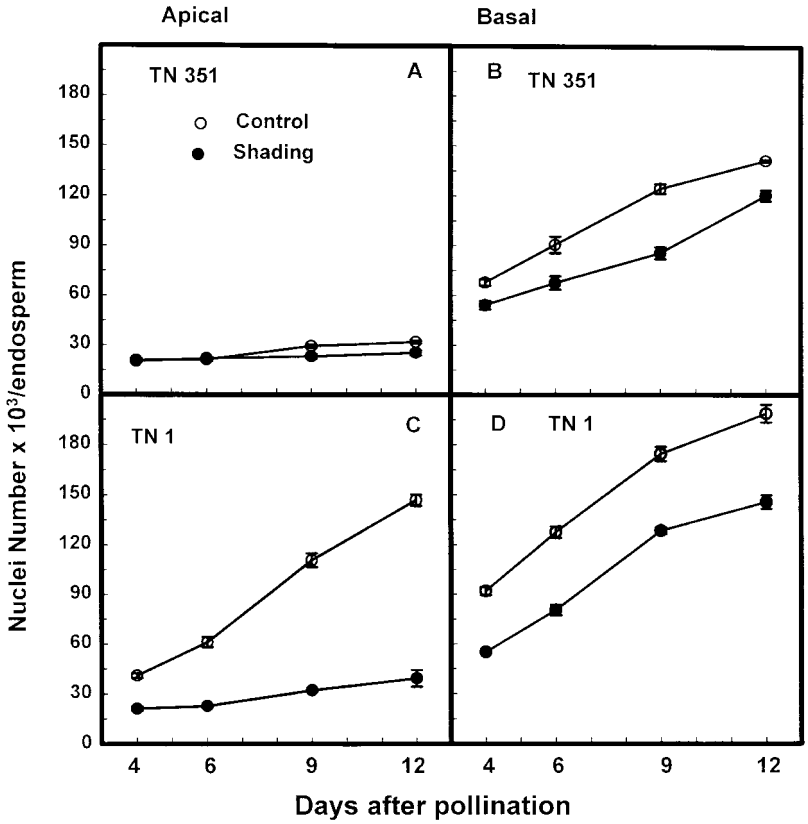

Fig. 2. Number of endosperm nuclei in kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four replicates.

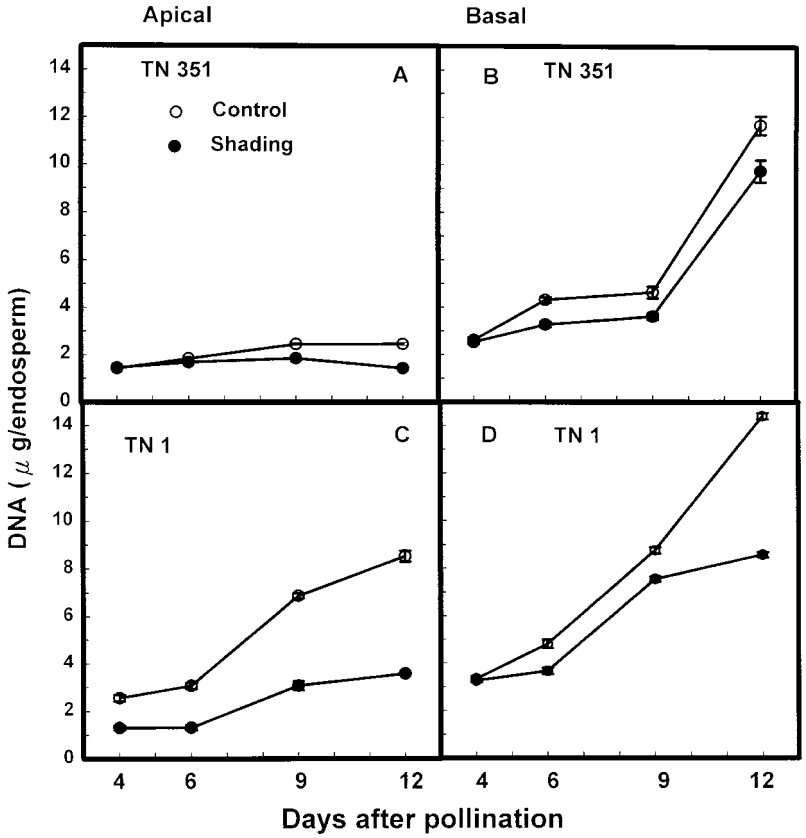

Fig. 3. Endosperm DNA levels in kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four replicates.

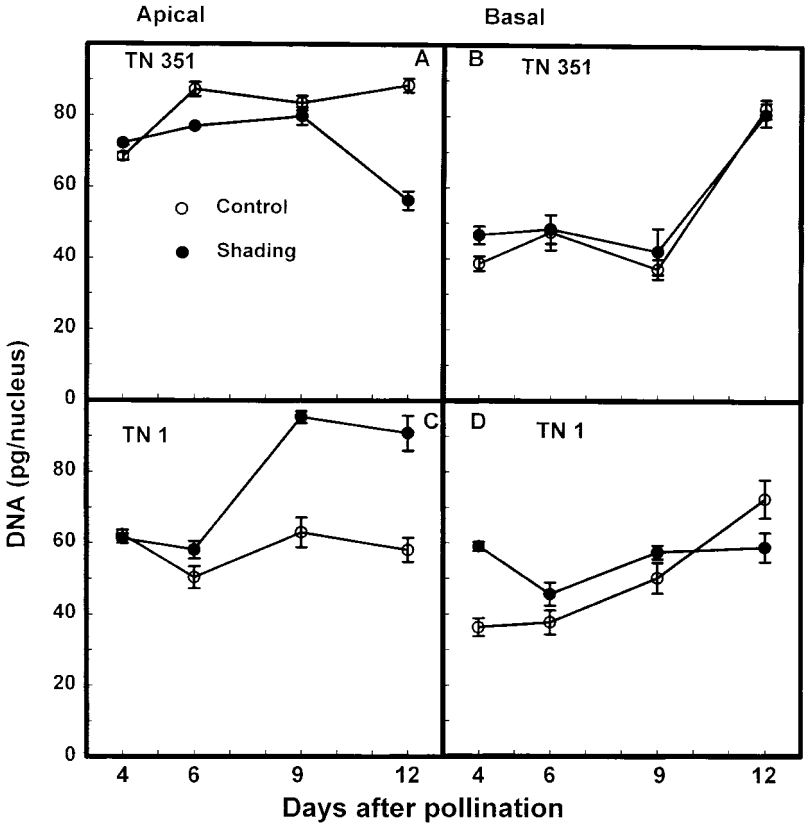

Fig. 4. Nuclei DNA contents for kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four replicates.

Changes in Nucleus Number

The number of endosperm nuclei in control kernels increased rapidly after pollination (Fig. 2). In general, TN 1 endosperm had a greater number of nuclei than TN 351 endosperm, and the number of nuclei in basal kernels was greater than that in apical kernels. Shade treatment reduced the increase in the number of nuclei for all plants of both cultivars. The effect of shading on nucleus number was observed as early as 4 DAP. The number of endosperm nuclei was essentially constant in the apical kernels of shaded plants (Fig. 2A and 2C). In TN 351, the number of nuclei in the apical kernels did not increase even in kernels on control plants (Fig. 2A).

DNA Content of Endosperm

The DNA content of the endosperm of normal, basal kernels increased after 6 DAP (Fig. 3). The increase in the DNA level per endosperm was faster in TN 1 than in TN 351. No DNA accumulation occurred in the apical kernels of TN 351 plants in the control and shade treatments. Shading also significantly reduced the DNA level per endosperm in the apical kernels of TN 1.

In the basal kernels of plants in the control treatment, the DNA content per nucleus continued to increase after 6 DAP, especially in TN 1. The DNA content per nucleus of TN 1 endosperm was even higher in plants of the shade treatment than in control plants (Fig. 4C). The apical kernels of TN 351 also had higher nuclear DNA content than the basal kernels (Fig. 4A, 4B).

Changes in Total Polyamine Levels

In normally developing kernels of TN 1, free and conjugated polyamines increased rapidly after 4 DAP and reached a maximum 6 to 9 DAP (Fig. 5C, 5D). Apical kernels contained lower levels of polyamines than basal kernels. During the first 3 d of treatment, there were no clear differences in the polyamine contents of kernels from shaded and control plants. However, after 4 DAP, the total polyamine levels in kernels of shaded plants were lower than in the kernels of control plants (Fig. 5A, 5C, and 5D). In TN 351, total polyamine levels were low in kernels from the apical region of ears on both control and shaded plants.

Putrescine Levels

In control plants, the putrescine content in the basal and apical kernels increased rapidly after 4 DAP (Fig. 6). The endosperm of apical kernels had less PUT than endosperm from basal kernels. The majority of putrescine in the endosperm of normally developing kernels consisted of PCA-soluble conjugated PUT. After 4 DAP, the concentration of PCA-soluble conjugated PUT was 3- to 4-fold greater than that of free PUT, and 10-fold greater than that of PCA-insoluble PUT. Levels of free PUT increased approximately 2 d earlier than the levels of the other types of PUT. For TN 351, there was no difference in the putrescine levels in the

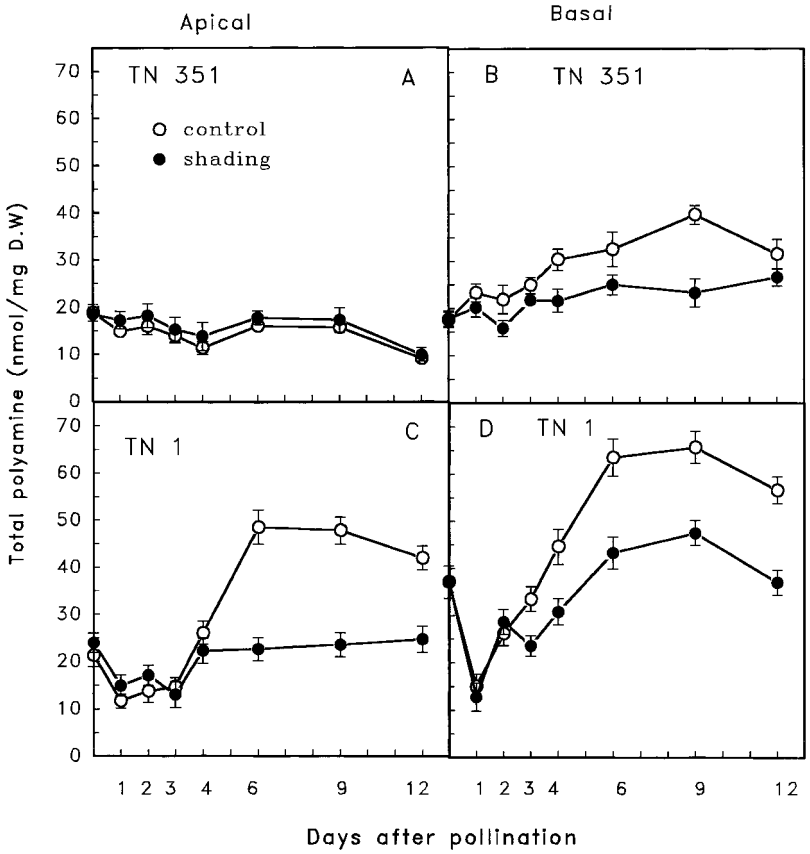

Fig. 5. Total polyamine levels in kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four separate extractions for each development stage.

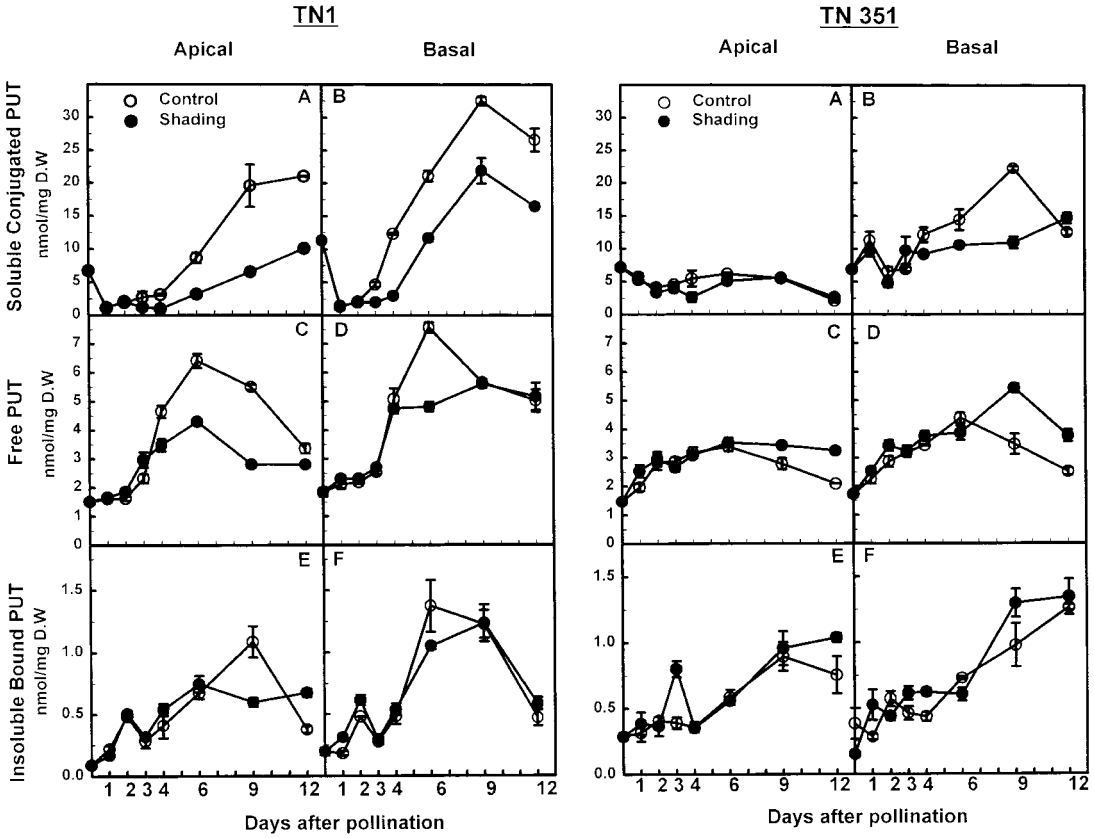

Fig. 6. Putrescine (PUT) levels in kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four separate extractions for each development stage.

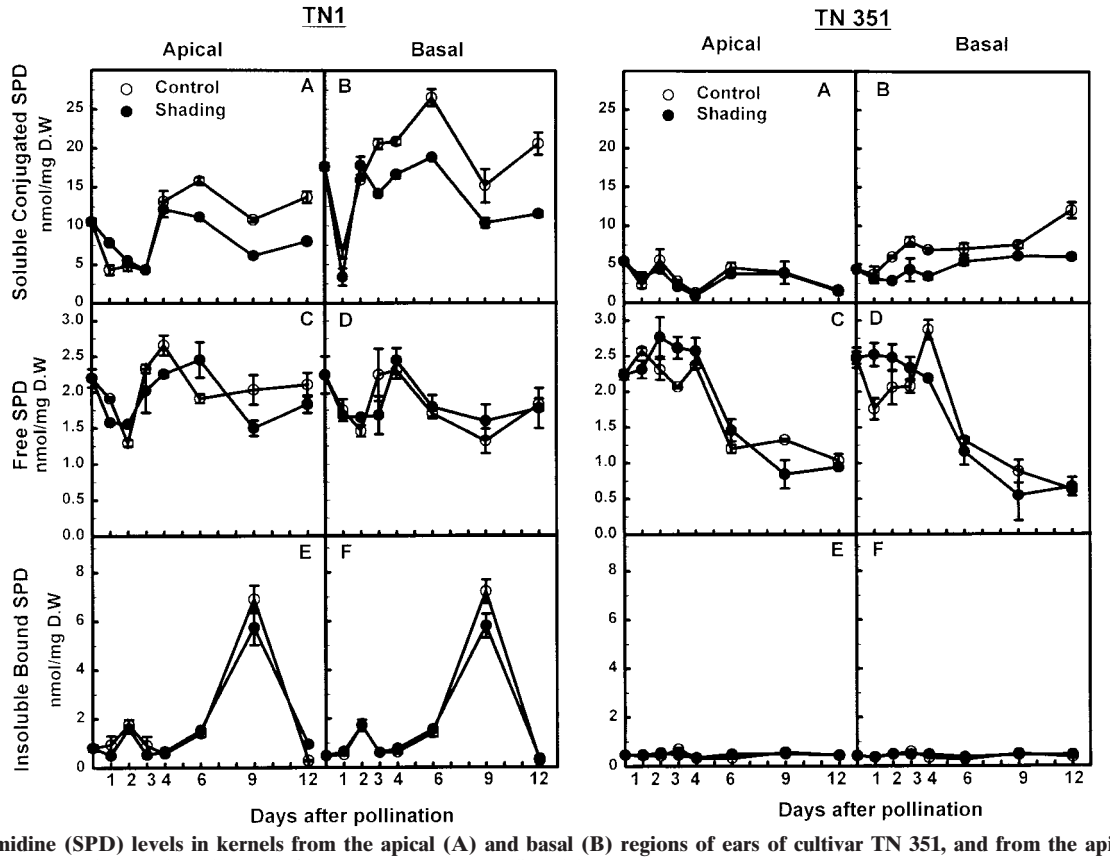

Fig. 7. Spermidine (SPD) levels in kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four separate extractions for each development stage.

apical endosperm of shaded and control plants. In the apical and basal kernels of shaded plants, the pattern of change in PUT concentration was associated with changes in dry matter accumulation.

Spermidine Levels

In TN 1, the pattern of change in levels of SPD was similar to that for PUT (Fig. 7). PCA-soluble conjugated SPD and free SPD content increased after 4 DAP, while the level of PCA-insoluble conjugated SPD rose after 6 DAP and reached a peak at 9 DAP. The levels of PCA-soluble conjugated SPD were 5- to 10-fold greater than that of free SPD and 3-fold greater than that of PCA-insoluble conjugated SPD. Endosperm from basal kernels contained more PCA-soluble conjugated SPD than endosperm from apical kernels. Kernels from shaded and control plants differed only in their PCA-soluble conjugated SPD content, which was lower in the endosperm of shaded plants.

Cultivar TN 351 had lower levels of PCA-soluble and PCA-insoluble conjugated SPD than TN 1. In the endosperm, free SPD content was high before 6 DAP and decreased thereafter. There was no difference in the SPD levels in endosperm from apical and basal kernels or in the endosperm from shaded and control plants.

Spermine Levels

In normally developing kernels SPM levels, like PUT levels, increased after 4 DAP (Fig. 8). PCA-soluble conjugated SPM was the most abundant type of SPM in the endosperm, and was nearly 3-fold and 10-fold more abundant than free SPM and PCA-insoluble SPM, respectively. The endosperm of control TN 1 contained slightly higher concentrations of PCA-soluble conjugated SPM than shaded TN 1. Levels of other types of SPM in the endosperm of shaded and control plants were not significantly different. PCA-soluble conjugated SPM was also the most abundant type of SPM in the endosperm of normal TN 1 kernels. The endosperm of shaded plants had a lower free SPM content than control plants. A similar trend of the changes in Spm was also found in TN 351, except that endosperms of shaded plants had a lower free Spm content than that of control plants.

DISCUSSION

We found that total polyamine content increased after 4 DAP, and is temporally associated with the accumulation of dry matter and an increase in endosperm nuclei in normally growing kernels. These results are similar to those for rice, soybean, and the ovary tissue of tobacco (Sen et al., 1981; Lin et al., 1984; Slocum and

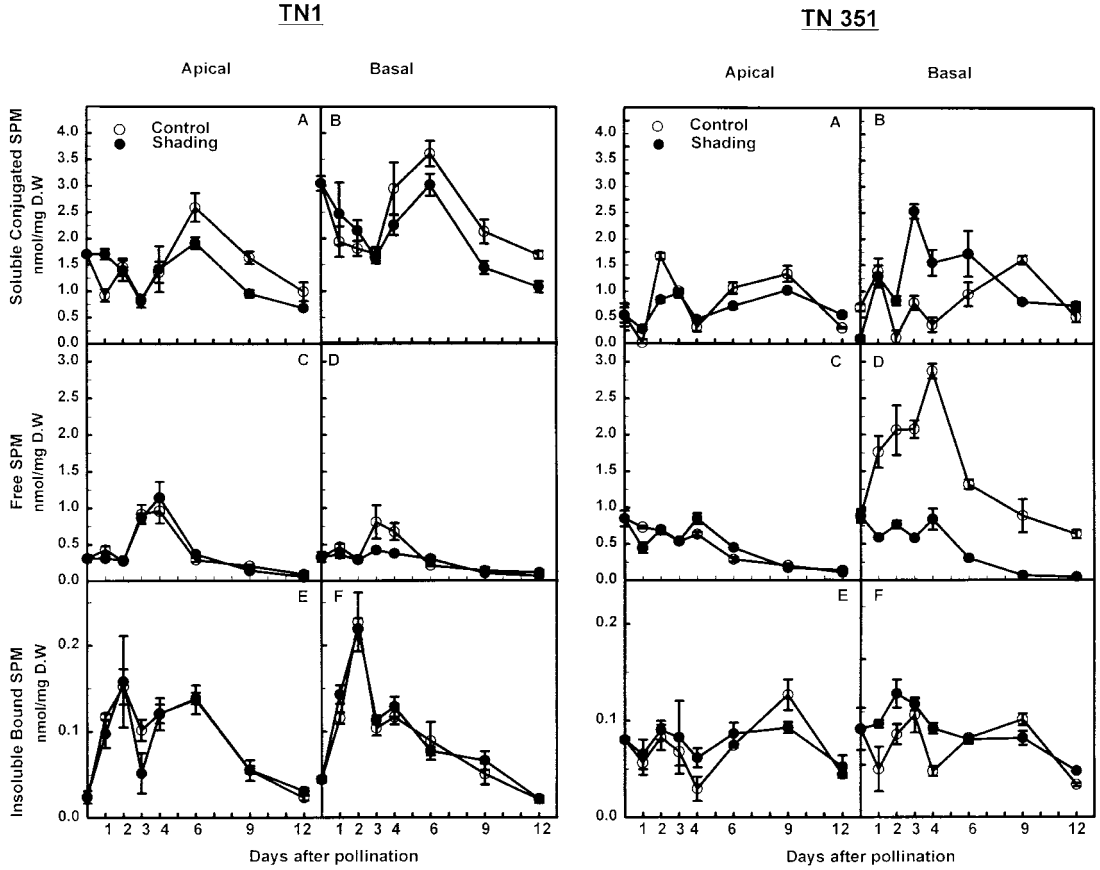

Fig. 8. Spermine (SPM) levels in kernels from the apical (A) and basal (B) regions of ears of cultivar TN 351, and from the apical (C) and basal (D) regions of ears of cultivar TN 1. Values are means ± SE of four separate extractions for each development stage.

Galston, 1985). Endosperm DNA levels increased continuously after 9 DAP, while total polyamine levels began to decrease at that time. Because nuclear DNA endoreduplication occurs mostly after 9 DAP, the increase in total polyamines is probably related to DNA synthesis during the division of endosperm nuclei. Endosperm DNA endoreduplication is a normal physiological process that occurs mostly after 6 to 8 DAP (Kowles et al., 1990). In addition, although the roles of DNA endoreduplication have not been elucidated, our results indicate that this process was not significantly influenced by the shade treatment. This finding is in accord with the suggestion that, in the endosperm, DNA endoreduplication may be less sensitive to stressful environments than cell division (Artlip et al., 1995).

In normal, growing maize kernels, PUT was the most abundant polyamine, followed by SPD and SPM. After 6 DAP, over 80% of the total polyamines consisted of PCA-soluble conjugated PUT and SPD. Similar results were obtained from developing tobacco (*Nicotiana tabacum* L.) ovary tissues (Slocum and Galston, 1985). Free PUT was the most abundant polyamine in developing rice seeds; however, conjugated polyamines were not quantified in the report (Sen et al., 1981). In contrast, SPD was the most abundant polyamine in soybean cotyledons during the rapid growth stage, and there were only limited amounts of conjugated polyamines (Lin et al., 1984).

Ethylene has been implicated in stress-induced kernel abortion in maize (Ober et al., 1991; Cheng and Lur, 1996). Although polyamines and ethylene share a biosynthetic precursor, the role of polyamines in the abortion process has not been investigated. We varied two factors, genotype (TN 1 and TN 351) and environmental stress (shade and normal light), to evaluate the relationship between polyamines and kernel abortion. In TN 351 low polyamine levels were temporally associated with the decrease of kernel growth, a low number of endosperm nuclei and low DNA content. In TN 1 the shade treatment induced abortion of the apical kernels, and the polyamine content of aborting kernels was lower than that of apical kernels from the control treatment. The low polyamine level was also temporally associated with a cessation in dry matter and DNA accumulation, and the lack of an increase in the number of nuclei. Thus, low polyamine levels and kernel abortion processes appear to be related, although causal relationships still need to be elucidated (Walden et al., 1997).

Although ethylene production began just 36 h after the start of the shade treatment (Cheng and Lur, 1996), the effect of shading on polyamine content was not apparent until 4 to 5 d after shading began (Fig. 5). Thus, shade stress seems to induce ethylene biosynthesis first. Increased ethylene production may consume the precursor for polyamine biosynthesis and it may inhibit the activity of the enzymes involved in polyamine biosynthesis (Apelbaum, 1990). Low polyamine content may in turn result in low DNA content and a low rate of nuclear division in the endosperm.

REFERENCES

Apelbaum, A. 1990. Interrelationship between polyamines and ethylene and it implication for plant growth and fruit ripening. p. 278–294. *In* H.E. Flores et al. (ed.) Polyamines and ethylene: Biochemistry, physiology, and interactions. American Society of Plant Physiologists, Baltimore, MD.

Artlip, T.S., J.T. Madison, and T.L. Setter. 1995. Water deficit in developing endosperm of maize: cell division and nuclear DNA endoreduplication. Plant Cell Environ. 18:1034–1049.

Cheng, C.Y., and H.S. Lur. 1996. Ethylene may be involved in abortion of the maize caryopsis. Physiol. Plant. 98:245–252.

Evans, P.T., and R.L. Malmberg. 1989. Do polyamines have roles in plant development. Annu. Rev. Plant Physiol. 40:235–269.

Flores, H.E., and A.W. Galston. 1982. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 69:701–706.

Giles, K.W., and A. Myers. 1965 An improved diphenylamine method for the estimation of deoxyribonucleic acid. Nature 3:93.

Hanft, J.M., and R.J. Jones. 1986. Kernel abortion in maize. Carbohydrate concentration patterns and acid invertase activity of maize kernels induced to abort *in vitro*. Plant Physiol. 81:503–510.

Hanft, J.M., A.J. Reed, R.J. Jones, and J.S. McLaren. 1990. Effect of 1-aminocyclopropane-1-carboxylic acid on maize kernel development *in vitro*. J. Plant Growth Regul. 9:89–94.

Jones, R.J., and S.R. Simmons. 1983. Effect of altered source-sink ratio on growth of maize kernels. Crop Sci. 23:129–134.

Kiniry, J.R., and J.T. Ritchie. 1985. Shade-sensitive interval of kernel number of maize. Agron. J. 77:711–715.

Kowles, R.V., F. Srienc, and R.L. Phillip. 1990. Endoreduplication of nuclear DNA in the developing maize endosperm. Develop. Genet. 11:125–132.

Lee, T.M., H.S. Lur, and C. Chu. 1995. Abscisic acid and putrescine accumulation in chilling-tolerant rice cultivars. Crop Sci. 35:502–508.

Lin, P.P.C., D.B. Egli, G.M. Li, and L. Meckel. 1984. Polyamine titer in the embryonic axis and cotyledons of *Glycine max* (L.) during seed growth and maturation. Plant Physiol. 76:366–371.

Lur, H.S., and T.L. Setter. 1993. Role of auxin in maize endosperm development. Timing of nuclear DNA endoreduplication, zein expression and cytokinin. Plant Physiol. 103:273–280.

Martin-Tanguy, J. 1997. Conjugated polyamines and reproductive development: Biochemical, molecular and physiological approaches. Physiol Plant. 100:675–688

Martin-Tanguy, J., E. Perdrizet, J. Prevost, and C. Martin. 1982. Hydroxycinnamic acid amides in fertile and cytoplasmic male sterile lines of maize. Phytochemistry 21:1939–1945

Ober, E.S., T.L. Setter, J.T. Madison, J.F. Thompson, and P.S. Shapiro. 1991. Influence of water deficit on maize endosperm development. Plant Physiol. 97: 154–164

Reed, A.J., and G.W. Singletary. 1989. Roles of carbohydrates supply and phytohormones in maize kernel abortion. Plant Physiol. 91: 986–992.

Schusser, J.R., and M.E. Westgate. 1991. Maize kernel set at low water potential: Sensitivity to reduced assimilates during early kernel growth. Crop Sci. 31:1189–1195

Sen, K., M.M. Choudhuri, and B. Ghosh. 1981. Changes in polyamine contents during development and germination of rice seeds. Phytochemistry 20: 631–633.

Slocum, R.D., and A.W. Galston. 1985. Changes in polyamine biosynthesis associated with postfertilization growth and development in tobacco ovary tissues. Plant Physiol. 79:336–343.

Walden, R., A. Cordeiro, and A.F. Tiburcio. 1997. Polyamines: Small molecules triggering pathways in plant growth and development. Plant Physiol. 113:1009–1013.