Temporal and Spatial Regulation of Cell Cycle Genes during Maize Sex Determination

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Maize (*Zea mays* L.) pistil cell death and stamen cell arrest are pivotal process on the sex determination, which diverges from bisexual state of floral meristem to unisexual state in staminate or pistillate floret. We investigated the temporal and spatial distribution of cell cycle gene expression during maize sex determination. The positive regulatory genes of cell cycle, *cyclin A*, *cyclin B*, *cyclin dependent kinase* (*CDK*) and *Mad2* were highly expressed in the developing pistil and stamen but the expression was disappeared in the dying pistil and arresting stamens. In contrast, the negative regulatory genes of cell cycle, *Wee1* and *CDK inhibitor* (*CKI*) were expressed in the arresting stamens in the wild-type ear and *tasselseed2* mutant tassel, however, these genes were not detected in dying pistil although the *cyclin B* gene expression was disappeared. These results suggest that both the pistil cell death and stamen cell arrest process in maize sex determination are involved in cell cycle regulation, but the different expression patterns of negative regulatory cell cycle genes in the arresting stamens and aborting pistils suggest that the two processes may have distinctive modes of action.

Key words – Maize, sex determination, cell cycle gene, pistil cell death, stamen arrest

Introduction

Most flowering plants are hermaphrodite, which produces both male and female reproductive organs within the same flower. In plant species, about 30% of them have evolved sex determination processes such as dicliny, dioecy, and monoecy that generate a unisexual state in the plant[3,7,15]. Maize is a monoecious plant that the male (tassel) and female (ear) florets are located in different space within one plant and evolves into increasing heterogeneity. The maize spikelet initial develops acropetally to two floral meristems, upper (primary) and lower (secondary) floret. Each floral meristem initiates a series of floral organs, a palea, two lodicules, three stamen initials and a central pistil. After floral organ initiation, unisexual stage is reached from bisexual stage by selective pistil cell death, pistil cell protection, and stamen cell arrest [2,8,14,27]. In the tassel spikelet, all pistils abort while stamens sexually mature. The *tasselseed* genes are involved in the pistil cell death process resulted in staminate floret. In the *tasselseed1* (*ts1*) and *2* (*ts2*) mutants, the sexual reverse occurred from staminate floret to pistillate floret[8,14,27]. The *TS2* gene encodes a member of the short- chain dehydrogenase/reductase (SDR) family of enzyme[9]. This gene is expressed in subepidermis of pistil cells at the time of their abortion in both tassel spikelets and the secondary ear spikelets[2]. In the ear spikelet, stamens fail to mature not because of cell death but because they are developmentally arrested. This process of stamen arrest requires the phytohormone gibberellin–biosynthetic or perception gibberellins that mutations (*dwarf* or *d1*, *d2*, *d3*, *d5*, *an1*, and *D8* mutants) prevent stamen arrest. In the dwarf mutants, all six stamens in the ear spikelets sexually mature, resulting in bisexual primary and staminate secondary florets[10,20]. In these mutations, exogenous treatment with biologically active gibberellins can reverse the mutant sexual phenotypes[19].

Plant cell cycle control is tightly linked to differentiation and development to accomplish normal plant growth[6], and plant cell divisions occur at specialized zones known as meristems, leaves and flowers are formed at the shoot and floral meristems, respectively[13]. The cell cycle composes of G1, S, G2, and M-phase, and many cell cycle genes have shown restrict oscillatory expression pattern, phase-specific expression. Generally, cyclin A play roles in G1/S transition, therefore, this genes are expressed highly through these phases. In contrast, *cyclin B* genes are expressed specifically at G2/M phase during the cell cycle. The phase specific cyclin-dependent kinases (CDKs) play a

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central role in mediating cell cycle progression[21,25]. The CDK activity is regulated by association with cyclin subunit, reversible phosphorylation and association with other regulatory factors. Both CDK A and B make a complex with cyclin A and cyclin B, respectively. The CDK/cyclin complexes are affected by a combination of different gene products and factors in which the progression of the cell cycle is initiated as an integral part of the growth and developmental program and in response to the environment. The mitotic arrest deficiency 2 (Mad2) spindle checkpoint protein inhibits anaphase-promoting complex or cyclosome (APC/C), a multi-subunit E3 ubiquitin ligase associated with proteosome-mediated proteolysis, through binding to its mitotic specific activator, Cdc20[23,28]. It has been reported that the maize Mad2 localization patterns in mitosis are basically conserved among eucaryote, and functions roles in the spindle checkpoint[29]. On the other hand, Wee1 and CDK inhibitor (CKI) were act as a negative regulator of CDK/cyclin complexes by phosphorylating to this CDK subunit[5,6]. In plant, maize *Wee1* gene expression is associated with endoreplication in highly polyploidy endosperm cells[26] and *Arabidopsis* Wee1 in fission yeast causes cell arrest[24]. However, it is not known well the relationship of cell cycle regulation with maize sex determination process in which the distinctive or specific regulation of cell cycle genes between pistil cell death and stamen cell arrest processes.

In this report, we examine the temporal and spatial expression of cell cycle genes in the two processing of maize sex determination, pistil cell death and stamen cell arrest. The positive regulator of cell cycle genes were expressed highly in the developing pistils and stamens but these gene expressions were declined in aborting pistils and arresting stamens. However, the negative regulator of cell cycle genes, *Wee1* and *CKI*, were solely expressed in the arresting stamens but not in the aborting pistil. Therefore, the two distinctive maize sex determination processes, pistil cell death and stamen arrest, may partially be caused by the specific cell cycle gene regulation.

Materials and Methods

Plant materials

The wild-type maize (*Zea mays* L.) inbred line W22 was used in this study. The *tasselseed2-reference* (*ts2*) was obtained from E. Irish and backcrossed into the inbred line W22[9].

RT-PCR analysis and cloning of cell cycle genes

Total RNA from dissected maize tissues was purified by the guanidine thiocyanate method[4]. First strand cDNA was synthesized using Superscript II (Gibco BRL) and an oligo-d (T) primer according to manufacturer's instructions. The *cyclin A*, *cyclin B*, *CDK A* (*cdc2*), *Mad2*, *Wee1*, and *CKI* cDNA clones were obtained by PCR amplification of first strand cDNA using primers as follows - *cyclin A* (P888: 5'TACATTGTGGGCAACA GACCAAGG3' and P889: 5' CCGACTCTGAGAACAGCCTAGCAAA3'); *cyclin B* (P893: 5'CCTGGACTCTGAGAACAGCCTACC3' and P889: 5'CCG ACTCTGAGAACAGCCTAGCAAA3'); *CDK A* (P905: 5'GG CATGGTAACAGCCT AACACATGG3' and P919: 5'TGGA CAGAGACCAAGGCGGTAATCT3'); *Mad2* (P958: 5'GAAG TACGGGCTTACCATGCTGCTC3' and P959: 5'AGCCAGA TCTTAAAGGCGCTGACTC3'); *Wee1* (P904: 5'TCACGCTA TATGCCT CCGGAAATG3' and P920: 5'CGCTGCCCAAA TGCAACTGATAAC3'); *CKI* (P975: 5'GGGTTACATTGGAG TTGAGGGACCA3' and P976: 5'CATG GGGAGGAATACC AGGGAGAC3'). Maize *actin* gene was used as a positive RT-PCR control (P213: 5'-CATGAGGCCACGTACAACTCC ATC3' and P214: 5'TCATACTCTCCCTTGGAGATCCAC 3'). PCR amplification was performed using the Expand Long Template PCR system (Roche) using the manufacturer's instructions under the following conditions: 94°C for 2 min, then ten cycles at 94°C for 10 sec, 65°C for 30 sec, and 68°C for 2 min, followed by an additional 20 cycles of PCR at 94°C for 10 sec, 65°C for 30 sec, 68°C for 2 min. with additions of 20 sec. at 68°C between each cycle. Amplification products used as *in situ* probes were cloned into the *pCRII* (Invitrogen), and sequenced to confirm their identity. The plasmid containing the *cyclin A*, *cyclin B*, *CDK A*, *Mad2*, *Wee1*, and *CKI* cDNAs are designated pYU1138, pYU1139, pYU1143, pYU1158, pYU1146, and pYU1174, respectively.

Preparation of *in situ* riboprobes

Plasmid DNA was prepared using Qiagen 100 columns (Qiagen Inc.) following the manufacturer's instructions. DNA templates for sense and antisense riboprobes were linearized at a restriction site flanking the cDNA inserts by digestion with an appropriate enzyme, which leaves a 5' overhang. *In vitro* transcription reactions using T7 or SP6 RNA polymerase and 11-digoxigenin-dUTP (Roche), riboprobe DNase treatment, and hydrolysis was according to the manufacturer's instructions. The riboprobe was subject to mild alkaline hydrolysis for varying times in 0.1 N NaOH to yield products in the 100-200 nucleotide range.

Cytological techniques and *in situ* hybridization

The formaldehyde tissue fixation, paraffin embedding and microtomy were essentially as described[16] except that Hemo-De (Fisher Scientific, Inc.) was substituted for Histoclear (National Diagnostics, USA), and the final concentration of riboprobe was adjusted to 10 ng/ml/kb and hybridization was performed at 55°C. After hybridization, slides were washed in 0.2X SSC (30 mM NaCl, 3 mM sodium citrate, pH 7.0) for 1.5 hrs (3 times for 30 min at 55°C, 60°C, 65°C, respectively), followed by a wash in 1X NTE (0.5 M NaCl, 10 mM Tris, pH 7.5) for 10 min at 37°C. Sections were treated with 20 μg/ml RNase A in 1X NTE for 30 min at 37°C, washed in 1X NTE for 10 min at 37°C, followed by a incubation in 0.2X SSC for 1hr at 55°C, and treatment in 0.5% blocking reagent (Roche) dissolved in 1X TBS (150 mM NaCl, 100 mM Tris pH 7.5) for 1 hr at room temperature. Blocking reagent was decanted and slides were incubated with an antibody solution containing a 1:1000 dilution of anti-digoxigenin-alkaline phosphatase-conjugated antibody (Roche) in 0.3% Triton 100, 1% BSA in 1X TBS for 5 hrs at room temperature. After incubation with antibody, slides were washed four times in 1X TBS for 10 min each, followed by a 10 min incubation in substrate buffer, containing 100 mM Tris pH 9.5, 100 mM NaCl and 50 mM $MgCl_2$. Approximately 300 μ l of substrate buffer containing 1 mM 4-nitroblue tetrazolium chloride and 0.4 mM 5-bromo-4-chloro-3-indolyl-phosphate (Roche) plus 1 mM levamisole (Sigma Co.) was pipetted onto each slide, a coverslip applied, and incubated for 3 days at room temperature in a dark, humid chamber. Slides were rinsed in 10 mM Tris pH 7.5, 1 mM EDTA, counterstained for 5 min at room temperature and mounted in 70% glycerol and 30% PBS.

Microscopy and image processing

All sections were examined using an Axioplan 2 microscope with digital image capturing on an HR and processing using AxioVision v4.4 (Carl Zeiss Inc.). Composites figures were generated and labeled using Adobe Photoshop 7.0 (Adobe systems Inc.).

Results and Discussion

Isolation of cell cycle genes from various maize tissues

To investigate the distribution of cell cycle genes during maize sex determination processes, we were trying to perform *in situ* hybridization using maize flowers, tassel and ear florets. To this end, we firstly examined RT-PCR analysis to verify which genes are expressed in dissected maize tissues and isolate cell cycle genes. Both tassel and ear samples were divided into two stages according to its size, early (<15 mm floret length) and late (15 - 20 mm floret length) stages. The early stage can be defined bisexual state in which the floral organ initiation growth and mature to early gynoecium or androecium development. In the late stage, the unisexual state was accomplished by pistil cell death or stamen arrest. Furthermore, other tissues such as root, stem, leaf, embryo and endosperm were collected. As shown in Fig. 1, the positive-regulated cell cycle genes such as *cyclin A*, *cyclin B* and *CDK A*, and a mitotic spindle checkpoint protein *Mad2*, were highly expressed in floral organs compared to other vegetative tissues. In the early and late stages of ear and tassel tissues, no big differences of these gene expression levels were observed. In case of negative-regulated cell cycle genes, *Wee1* gene was expressed highly in the late stage of floral tissues rather than the early stage. This result suggests that the Wee1 protein activates on the sex determining stages in which the pistil cell death and stamen arrest are occurred. On the other hand, the *CKI* gene, another cell cycle inhibitor, was also expressed in the tassel and ear

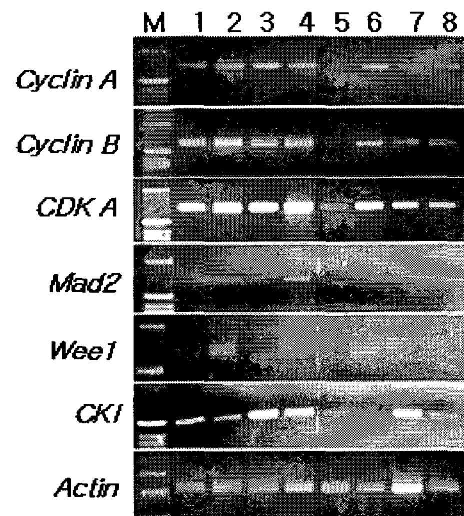

Fig. 1. RT-PCR analyses of cell cycle genes with various maize tissues. Total RNA was isolated from dissected maize tissues, and the first cDNA strand was synthesized using reverse transcriptase. RT-PCR was performed by its cell cycle gene specific primers. The *actin* gene was used as a positive control. M, size marker; 1, early ear; 2, late ear; 3, early tassel; 4, late tassel; 5, root; 6, shoot; 7, embryo & endosperm; 8, leaf.

through all stages but the expression levels were shown to reverse compared to *Wee1* gene expression pattern. Taken together, these results indicate that both the positive- and negative-regulated cell cycle genes used in this study are good indicators for cell cycle analysis on the sex determination process. Thus, all tested cell cycle genes were cloned and sequenced for *in situ* probes.

Temporal and spatial distribution of positive-regulated cell cycle genes in maize sex determination

To examine the temporal and spatial regulation of cell cycle genes in the dying pistil and arresting stamens, we conducted *in situ* hybridization using maize wild-type and *tasselseed2* mutant florets using digoxigenin-labelled antisense RNA probe. A spotty expression pattern was observed in all probes (Fig. 2). As shown in wild-type tassel (Fig. 2A-D), the positive-regulated cell cycle genes, *cyclin A*, *cyclin B*, *CDK A* and *Mad2* are highly expressed in the developing stamens but these expression was not detected in aborting pistil. In the wild-type ear (Fig. 2E, G and H), the developing pistils have shown to high these gene expression levels but these gene expression in the arresting stamens was not observed. The maize *tasselseed2* mutant tassel reverses sexual state resulted in pistillate[8,14,27]. As shown in the growing pistil of *tasselseed2* mutant tassel (Fig. 2F), the positive-regulated cell cycle gene (*cyclin B*) was expressed highly, but the arresting stamens were not expressed. The other positive cell cycle genes are working on the same way in the *tasselseed2* mutant (data not shown). Based on these results, we suggest that the spatial regulation of cell cycle genes is involved in the sex determination processes, pistil cell death and stamen cell arrest. To verify the temporal regulation of these genes, the *cyclin B* gene were chosen and performed *in situ* hybridization from very early to late stages of tassel florets. The *cyclin B* gene acts as a positive regulator in the G2/M phase of the cell cycle and its RNA is short-lived and present only in dividing cells in the G2/M transition[11]. As shown in Fig. 3A and B, the *cyclin B* gene was highly expressed in both pistil and stamen primodia at the very young bisexual

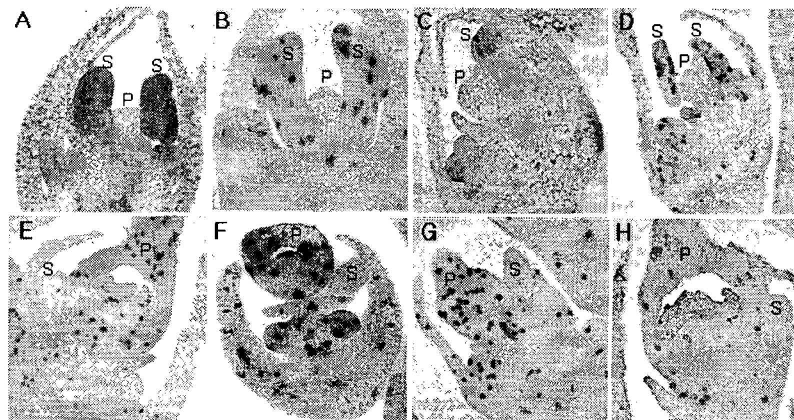

Fig. 2. *In situ* expression of positive regulated cell cycle genes with wild-type and *tasselseed2* mutant tassel florets. The wild-type tassel (A-D), wild-type ear (E, G and H), and the *tasselseed2* tassel (F) florets were used in this experiment. The antisense riboprobes, *cyclin A* (A and E), *cyclin B* (B and F), *CDK A* (C and G), and *Mad2* (D and H) were hybridized with longitudinal sections. P, pistil; S, stamen.

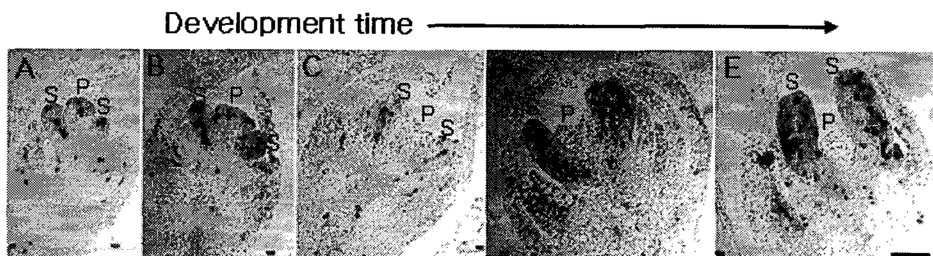

Fig. 3. *Cyclin B* gene expression in maize wild-type tassel according to developmental time. *In situ* hybridization was performed using *cyclin B* gene according to development time, from bisexual to unisexual state in the wild-type tassel. P, pistil S, stamen; scale bar = 100 μm.

stage. As tassel florets matured, the *cyclin B* transcripts became disappeared in the dying pistil and stopped the expression while staminate tassel mature (Fig. 3C-E). However, the growing stamens still have shown to high *cyclin B* gene expression (Fig. 3C-E). The same result occurred in the ear floret, the *cyclin B* gene expression was stopped in the arresting stamens (data not shown). These results implicate that the temporal regulation of cell cycle genes are important in the floral organ development. Taken together, these results suggest that cell cycle arrest or cell division cessation involved in the pistil cell death and stamen arrest process.

Arresting stamen specific expression of negative-regulated cell cycle genes

To exam whether the negative-regulated cell cycle genes are also expressed in the aborting pistil and arresting stamens, two negative regulator of mitosis genes such as *Wee1* and *CKI* were selected (Fig. 1). The Wee1 protein is a Thr/Tyr protein kinase that inhibits Cdc2 (CDK) activity and the progression of cells from G2/M transition in the cell cycle[18,22]. The maize *Wee1* gene expression is associated with endoreplication in highly polyploid endosperm cells[26]. Also the CKI has shown to be participated in the control of both the cell cycle and the endoreduplication cycle[1,5]. As shown in Fig. 4A and B, the arresting stamens in wild-type ear displayed specific expression of *Wee1* and *CKI* genes, respectively. The result coincided in the *tasselseed2* mutant tassel in which the arresting stamens have accumulated *Wee1* and *CKI* transcription messages (data not shown). Intriguingly, these two genes were not expressed in the aborting pistil in the wild-type tassel (Fig. 4C). These results refer a cell cycle block, possibly mediated by *Wee1* and/or *CKI*, negative regulators of cell cycle, in the process of arresting stamen cells but not in the pistil abortion process. The different expression patterns of those

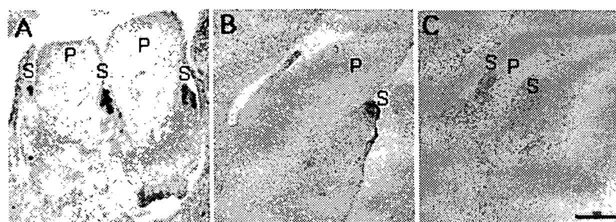

Fig. 4. *In situ* expression of negative regulated cell cycle genes with wild-type ear and tassel. The wild type ear (A and B) and tassel (C) samples were hybridized with *Wee1* (A and C), and *CKI* (B) antisense riboprobes. P, pistil; S, stamen; scale bar = 100 μm

genes between arresting stamen and aborting pistil tissues may refer thatthe pistil cell death and stamen cell arrest mechanisms are quite distinctive even though the disappearances of *cyclin B* gene expression was occurred in the both tissues(Fig. 2 and 3). In regarding to stamen arrest, it seems that the negative regulator of cell cycle genes function predominately. All together, we propose the extend mechanism of the maize sex determination that the temporal and spatial regulation of cell cycle genes play pivotal roles in pistil cell death and stamen arrest. Particularly, the stamen arrest event in the wild-type ear and *tasselseed2* mutant tassel may cause by cell cycle arrest with expressing negative regulated cell cycle genes such as *Wee1* and/or *CKI*.

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References

1. Bisbis, B., Delmas, F., Joubes, J., Sicard, A., Hernould, M., Inze, D., Mouras, A., and Chevalier, C. 2006. Cyclin-dependent kinase (CDK) inhibitors regulate the CDK-cyclin complex activities in endoreduplicating cells of developing tomato fruit. *J. Biol. Chem.* **281**, 7374-7383.
2. Calderon-Urrea, A., and Dellaporta, S. L. 1999. Cell death and cell protection genes determine the fate of pistils in maize. *Development* **126**, 435-441.
3. Charlesworth, D. 2002. Plant sex determination and sex chromosomes. *Heredity* **88**, 94-101.
4. Chirgwin, J. M., Przbyla, A. E., MacDonald, R. J., and Rutter, W. J. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294.
5. Coelho, C. M., Dante, R. A., Sabelli, P. A., Sun, Y., Dilkes, B. P., Gordon-Kamm, W. J., and Larkins, B. A. 2005. Cyclin-dependent kinase inhibitors in maize endosperm and their potential role in endoreduplication. *Plant Physiol.* **138**, 2323-2336.
6. de Jager, S. M., Maughan, S., Dewitte, W., Scofield, S., and Murray, J. A. 2005. The developmental context of cell-cycle control in plants. *Semin. Cell Dev. Biol.* **16**, 385-396.
7. Dellaporta, S. L., and Calderon-Urrea, A. 1993. Sex determination in flowering plants. *Plant Cell* **5**, 1241-1251.

8. Dellaporta,S. L., and Calderon-Urrea, A. 1995. The sex determination process in maize. *Science* **266**, 1501-1505.
9. DeLong, A., Calderon-Urrea, A., and Dellaporta, S. L. 1993. Sex determination gene *TASSELSEED2* of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. *Cell* **74**, 757-768.
10. Emerson, R. A., and Emerson, S. H. 1922. Genetic interrelations of two andromonoecious types of maize, *dwarf* and *anther ear*. *Genetics* **7**, 203-236.
11. Fobert, P. R., Coen, E. S., Murphy, G. J., and Doonan, J. H. 1994. Patterns of cell division revealed by transcriptional regulation of genes during the cell cycle in plants. *Embo J.* **13**, 616-624.
12. Gorbsky, G. J. 1997. Cell cycle checkpoints: arresting progress in mitosis. *Bioessays* **19**, 193-197.
13. Inze, D. 2005. Green light for the cell cycle. *Embo J.* **24**, 657-662.
14. Irish, E. E., Langdale, J. A., and Nelson, T. M. 1994. Interactions between *Tasselseeds* and other sex determining genes in Maize. *Developmental Genetics* **15**, 155-171.
15. Irish, E. E., and Nelson, T. 1989. Sex determination in monoecious and dioecious plants. *Plant Cell* **1**, 737-744.
16. Jackson, D. 1992. *In situ* hybridization in plants, pp. 163-174, In Gurr, S. J., M. J., and McPherson, Bowles, D. J. (eds.) *Plant Molecular Pathology: A Practical Approach*, Oxford University Press, Oxford.
17. Kimbara, J., Endo, T. R., and Nasuda, S. 2004. Characterization of the genes encoding for MAD2 homologues in wheat. *Chromosome Res.* **12**, 703-714.
18. Nurse, P., and Thuriaux, P. 1980. Regulatory genes controlling mitosis in the fission yeast Schizosaccharomyces pombe. *Genetics* **96**, 627-637.
19. Phinney, B. O. 1956. Growth response of single-gene dwarf mutants in maize to gibberellic acid. *Proc. Natl. Acad. Sci.* **42**, 185-189.
20. Phinney, B. O. 1961. Dwarfing genes in *Zea mays* and their relation to the Gibberellins, pp 489-501. In Klein, R. M., (ed.) *Plant growth regulation*, Iowa State University Press, Ames.
21. Potuschak, T., and Doerner, P. 2001. Cell cycle controls: genome-wide analysis in Arabidopsis. *Curr. Opin. Plant Biol.* **4**, 501-506.
22. Russell, P., and Nurse, P. 1986. cdc25+ functions as an inducer in the mitotic control of fission yeast. *Cell* **45**, 145-153.
23. Shah, J. V., and Cleveland, D. W. 2000. Waiting for anaphase: Mad2 and the spindle assembly checkpoint. *Cell* **103**, 997-1000.
24. Sorrell, D. A., Marchbank, A., McMahon, K., Dickinson, J. R., Rogers, H. J., and Francis, D. 2002. A WEE1 homologue from Arabidopsis thaliana. *Planta* **215**, 518-522.
25. Stals, H., Casteels, P., Van Montagu, M., and Inze, D. 2000. Regulation of cyclin-dependent kinases in Arabidopsis thaliana. *Plant Mol. Biol.* **43**, 583-593.
26. Sun, Y., Dilkes, B. P., Zhang, C., Dante, R. A., Carneiro, N. P., Lowe, K. S., Jung, R., Gordon-Kamm, W. J., and Larkins, B. A. 1999. Characterization of maize (Zea mays L.) Weel and its activity in developing endosperm. *Proc. Natl. Acad. Sci. U S A* **96**, 4180-4185.
27. Veit, B., Greene, B., Lowe, B., Mathern, J., Sinha, N., Vollbrecht, E., R., W., and Hake, S. 1991. Genetic approaches to inflorescence and leaf development in maize. *Development Supplement* **1**, 105-111.
28. Yu, H. 2006. Structural activation of Mad2 in the mitotic spindle checkpoint: the two-state Mad2 model versus the Mad2 template model. *J. Cell Biol.* **173**, 153-157.
29. Yu, H. G., Muszynski, M. G., and Kelly Dawe, R. 1999. The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. *J. Cell Biol.* **145**, 425-435.

초록 : 옥수수 성 결정에 있어서 세포주기 유전자들의 시간적, 공간적 조절

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옥수수 (*Zea mays* L.) 꽃은 암술 세포사멸과 수술 세포 성장정지 등을 통하여 양성상태에서 단성 상태로 성 결정 과정을 완성한다. 본 논문에서는 옥수수 성 결정 동안 세포주기 유전자들의 시간적, 공간적 발현조절을 조사하였다. 세포주기의 양성조절 인자 즉 *cyclin A*, *cyclin B*, *cyclin dependent kinase A* (*CDK A*), *Mad2* 유전자들은 성장하는 암술과 수술에서 높게 발현되는 반면 죽어가는 암술과 성장이 정지되는 수술에서는 이들의 발현이 사라졌다. 이와 반대로, *Weel*과 *CDK inhibitor* (*CKI*) 같은 세포주기 음성 조절유전자들은 야생형 암꽃과 *tasselseed2* 돌연변이 수꽃의 성장이 정지하고 있는 수술에서 발현이 증가되었지만, 흥미롭게도, 이들 유전자들은 죽어가는 암술세포에서는 발현되지 않았다. 이들 결과들을 통하여 옥수수 성 결정 과정 중에서 암술 세포사멸과 수술세포 성장정지는 세포주기조절과 밀접한 관계가 있으며, 특히 성장이 정지하는 수술과 죽어가는 암술에서의 음성 세포주기 조절 유전자들의 다른 발현양상은 이 둘의 성 결정 메커니즘이 구별 될 것이라고 사료된다.