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Original article

Regulation of maize kernel weight and carbohydrate metabolism by abscisic acid applied at the early and middle post-pollination stages in vitro

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ABSTRACT

Abscisic acid (ABA) accumulates in plants under drought stress, but views on the role of ABA in kernel formation and abortion are not unified. The response of the developing maize kernel to exogenous ABA was investigated by excising kernels from cob sections at four days after pollination and culturing in vitro with different concentrations of ABA (0, 5, 10, 100 μM). When ABA was applied at the early post-pollination stage (EPPS), significant weight loss was observed at high ABA concentration (100 μM), which could be attributed to jointly affected sink capacity and activity. Endosperm cells and starch granules were decreased significantly with high concentration, and ABA inhibited the activities of soluble acid invertase and acid cell wall invertase, together with earlier attainment of peak values. When ABA was applied at the middle post-pollination stage (MPPS), kernel weight was observably reduced with high concentration and mildly increased with low concentration, which was regulated due to sink activity. The inhibitory effect of high concentration and the mild stimulatory effect of low concentration on sucrose synthase and starch synthase activities were noted, but a peak level of ADP-glucose pyrophosphorylase (AGPase) was stimulated in all ABA treatments. Interestingly, AGPase peak values were advanced by low concentration and postponed by high concentration. In addition, compared with the control, the weight of low ABA concentration treatments were not statistically significant at the two stages, whereas weight loss from high concentration applied at EPPS was considerably obvious compared with that of the MPPS, but neither led to kernel abortion. The temporal- and dose-dependent impacts of ABA reveal a complex process of maize kernel growth and development.

1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops; it directly or indirectly provides resources for food, feed, and bio-fuel throughout the world (Godfray et al., 2010). Although maize yield is principally determined by the kernel number per unit cultivation area (Edmeades et al., 1999), large deviations in crop yield calculations can occur due to differences in the weights of individual kernels (Borrás and Gambín, 2010). Clarification of the variation in kernel weight has important implications for yield stability.

Kernel weight formation is a product of endosperm proliferation and embryo growth. In maize, the endosperm cell multiplication stage occurs as polar nuclei divide and differentiate into endosperm cells after pollination, and this process lasts approximately 12–15 days (Kowles et al., 1990; Jones et al., 1996). In the endosperm cell expansion and enrichment period, which continues from 12 days after pollination (DAP) to maturity, starch, protein, and other stored substances accumulate in the endosperm cells (Ober and Setter, 1990; Ober et al., 1991). However, abiotic stresses, such as drought, adversely affect plant growth and the final yield performance of a crop (Edmeades et al., 1999; Ashraf and Foolad, 2007; Osakabe et al., 2014), especially maize (Otegui et al., 1995; Bruce et al., 2002; Kakumanu et al., 2012). Large amounts of abscisic acid (ABA) accumulate in plants under drought stress (Zhu, 2002; Fujita, 2005; Finkelstein, 2013; Muñoz-Espinoza et al., 2015; Sah et al., 2016) and are known to have a critical role in embryo maturation, seed development, seedling establishment, and transition from the vegetative to reproductive stage (Sreenivasulu et al., 2012; Daszkowska-Golec and Szarejko, 2013).

The detrimental effect of ABA on grains occurs in crops subjected to drought stress or exogenous ABA application after pollination, primarily via inhibition of the endosperm cells (Myers et al., 1990; Ober and Setter, 1992) and a decrease in seed weight (Schussler and Westgate, 1995; Zinselmeier et al., 1995). An even more serious effect leads to kernel abortion (Wang et al., 2002; Boyer, 2010), which is actually a

Abbreviations: ABA, abscisic acid; AGPase, ADP glucose pyrophosphorylase; CTK, cytokinin; CWI, acid cell wall invertase; DAP, days after pollination; EPPS, early post-pollination stage; MPPS, middle post-pollination stage; SAI, soluble acid invertase; SS, sucrose synthase; SSS, starch synthase; ZR, zeatin riboside

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noteworthy reduction of grain weight. Wang et al. (2002) indicated that the ABA content of maize kernels was remarkably enhanced by water deficit, and ABA enhancement increased sterility and abortion. This observation is in line with a study suggesting that high ABA concentration regulates maize kernel abortion under drought stress at the molecular level (Kakumanu et al., 2012). However, Asch et al. (2001) reported that ABA and kernel abortion are not associated with the maize ovary under prolonged water stress. Thus, consensus does not exist as to whether ABA leads directly to kernel abortion. Obviously, the role of ABA in regulating kernel formation and its mechanism require further study.

ABA is involved in the metabolic activity of key enzymes in sucrose decomposition and starch synthesis (Zhu et al., 2011; Travaglia et al., 2012) and regulates the process of kernel weight formation. Many enzymes are involved in sucrose-to-starch conversion in the developing crop endosperm, but five enzymes are considered to play key roles in this process (Kato et al., 2007). These enzymes are soluble acid invertase (SAI), acid cell wall invertase (CWI), sucrose synthase (SS), ADP-glucose pyrophosphorylase (AGPase), and starch synthase (SSS). Invertase and SS primarily catalyze sucrose decomposition and are considered the main controllers of sucrose conversion to starch and sink strength (Roitsch and Gonzalez, 2004). AGPase produces ADP-glucose, which is regarded as the rate-limiting enzyme in starch biosynthesis (Li et al., 2013; Tuncel and Okita, 2013; Huang et al., 2014). SSS activity is reported as being closely associated with the rate of starch synthesis and increases in the starch content of grains (Nakamura et al., 1989; Keeling et al., 1993). Nevertheless, the influence of ABA on enzymes in sucrose-to-starch conversion commonly induced under water stress has been investigated in wheat (Yang et al., 2004) and rice (Wang et al., 2015), and exogenous ABA application has been studied in rice (Zhu et al., 2011). However, little is known as to whether ABA directly impacts enzyme activities in the process of maize kernel development at the early post-pollination stage (EPPS) and middle post-pollination stage (MPPS).

Apart from the reports that ABA plays an important role in influencing grain growth in various plants, no other detailed or systematic studies exist on direct regulation of the same ABA concentrations and kernel weight and the involvement with enzyme activities in sucrose-to-starch conversion during maize growth at EPPS and MPPS. In this work, we used in vitro cultures to verify the maize kernel growth response at EPPS and MPPS to different concentrations of ABA (0, 5, 10, 100 μM). Our objectives were (a) to elucidate the relationship between ABA and kernel weight at different stages and whether it leads to abortion; and (b) to determine whether ABA affects the time course and induction extent of metabolic enzyme activities in sucrose-to-starch conversion during kernel growth.

2. Materials and methods

2.1. Plant materials

Maize (hybrid Zhengdan 958) plants were grown at the Wuqiao Experimental Station of China Agricultural University in Hebei Province, China. At the reproductive stage, uniform ears were bagged with paper bags before silking and hand-pollinated with fresh pollen from the same species at the silking stage. At 4 DAP, ears were detached, and a few layers of bract were removed and sterilized with 75% (v/v) ethyl alcohol. After sterilization, the ears were transferred to a clean bench and cut into nine-kernel cob sections. Each kernel/cob was selected at a proportion of 1/9 (i.e., the central kernel was retained, and the eight surrounding kernels were abandoned) and placed in culture medium according to Gengenbach (1977) with minor modifications. Prior to inoculation, culture medium was autoclaved at 121 °C for 15 min. Three cob sections were placed in one flask containing 50 mL of medium. The flasks were incubated at 25 ± 1 °C in the dark.

The response of the developing maize kernels to exogenous ABA was investigated by mixing ABA into the culture medium at the early- or middle post-pollination stages. At EPPS, kernels were cultured in medium initially containing 0 (A_0), 5 (E-A_5), 10 (E-A_{10}), or 100 μM (E-A_{100}) ABA. The kernels were sampled four times every four days (8, 12, 16, and 20 DAP), and reached maturity at 45 DAP. For the samples treated with ABA at MPPS, all kernels were continually cultured for 16 days in medium containing 0 μM ABA and transferred to new medium containing 0 (A_0), 5 (M-A_5), 10 (M-A_{10}), or 100 μM (M-A_{100}) ABA. The kernels were sampled every four days and four times after transfer (24, 28, 32, and 36 DAP), and reached maturity at 45 DAP.

The collected tissues were divided into two components. One component was instantly frozen in liquid nitrogen and stored at −80 °C prior to use, and the other was measured for weight and grain volume, dried to a constant weight, and preserved until analysis.

2.2. Determination of endosperm cell numbers and starch granules

Kernels were excised from the cob sections and immediately fixed with an ethanol: glacial acetic acid solution (3:1, v/v), vacuum infiltrated, and placed in storage for 12–24 h. The tissue was subsequently placed in 70% ethanol and stored at 4 °C until further processing. Stored tissue was dehydrated with ethanol, cleaned with xylene, and embedded in paraffin. Endosperm cells were stained with hematoxylin-eosin, and starch granules were stained with I_2-KI solution. One slice was successively cut into 10-μm thicknesses with a microtome blade, and the cross-sectional area of the kernel was observed under an Olympus microscope (CX41, Olympus Corporation, Japan).

2.3. Carbohydrate analysis

Amounts of 0.2 g dry matter were extracted with distilled water, and the extraction solution was prepared for soluble carbohydrate determination. Insoluble debris was extracted with HCl (3 M), and the extraction solution was used in starch analysis. Sucrose, glucose, and fructose were quantified via HPLC analysis, which was conducted on a Waters programmable liquid chromatograph with a 600 Controller and Waters 410 Differential Refractometer (Waters 2487). Starch and soluble sugars were measured with anthranone-H_2SO_4 using an UV–vis spectrophotometer (TU-1901, PERSEE, China) at a wavelength of 625 nm.

2.4. Enzyme activity analysis

Kernel tissues (300 mg fresh weight) were homogenized with 5 mL of 50 mM Hepes-NaOH buffer (pH 7.2) containing 10 mM $MgCl_2$, 15% (v/v) ethanediol, 1 mM EDTA, and 1 mM DTT on ice. The homogenate was centrifuged at 10,000*g* and 4 °C for 5 min. The supernatant was used in SAI, SS, AGPase, and SSS activity analysis. The sediment was washed three times with 5 mM Hepes-NaOH buffer (pH 7.2) to remove endogenous sugars, re-suspended in 5 mL of 50 mM Hepes-NaOH buffer (pH 7.2) containing 10 mM $MgCl_2$, 15% (v/v) ethanediol, 1 mM EDTA, 1 mM DTT, and 1 M NaCl for 4 h at 4 °C, and centrifuged at the same conditions described above. This supernatant was used in CWI activity analysis. Analysis methods according to Nakamura et al. (1989) and Zinselmeier et al. (1995) were followed with minor modifications.

2.5. Hormone extraction and quantification

The methods for ABA and zeatin riboside (ZR) determination according to Yang et al. (2004) and He (1993) were applied with minor modifications. Approximately 0.2 g of fresh sample was ground in a mortar on ice in 5 mL of 80% (v/v) methanol extraction medium containing 1 mM butylated hydroxytoluene as an antioxidant. The extract was incubated for 6 h and centrifuged at 4500*g* for 15 min at 4 °C. The supernatant was used in ABA and ZR determination. The