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Relationship between changes in endogenous polyamines and seed quality during development of sh_2 sweet corn (*Zea mays* L.) seed

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ABSTRACT

Polyamines putatively affect tolerance to abiotic stresses and are believed to be important in organogenesis. Present experiments investigate the relationship between polyamines (PAs) and seed quality. Therefore, during seed development, the changes in free putrescine (Put), spermidine (Spd) and spermine (Spm) and physiological and biochemical parameters in F_1 seeds of sh_2 sweet corn were compared. Concentrations of Put, Spd and Spm increased from 14 to 30 days after pollination (DAP). After 30 DAP Put concentration declined with an opposite trend to that of Spd and Spm. The regression analysis between PAs and seed quality described by physiological and biochemical parameters including germination percentage, germination energy, germination index, seed size, seed fresh and dry weight, total soluble sugar, total soluble protein, Malondialdehyde (MDA) concentration, electrolyte leakage, peroxidase (POD) and catalase (CAT) activity were conducted. Spd was observed to have a closer relation with the comprehensive physiological changes of seeds during their development than that of Put and Spm. Moreover, the Spm concentration might be more suitable to forecast seed germinability during seed maturation period than Spd and Put. It indicated that endogenous Spd and Spm in dissociated form had more effect than Put during seed development progress of sweet corn.

1. Introduction

Super sweet corn (with sh_2 gene) is planted all over the world. However, getting satisfactory stands is challenging because of poor seed quality (Fan et al., 1998). Seed quality is sum of those properties that determine the potential for rapid germination, uniform emergence, and development of normal seedlings. Thus, higher seed quality causes higher germination, satisfactory stands, and also has more interests in seed marketing of super sweet corn. Seed quality is affected during seed development (Cao et al., 2008b), and the most studies regarding sweet corn seed development were conducted to improve kernel quality and yield as they relate to food processing and suitable harvest timing (George et al., 2003; Wu and Chen, 1999; Ajayi et al., 2005).

Polyamines are small aliphatic polycationic nitrogenous compounds (PAs) and are ubiquitous in higher plants. PAs are important modulators of biological processes (Koetje et al., 1993), and are believed to play an important role in plant growth and development (Galston, 1983; Urano et al., 2004). Putrescine (Put), spermidine (Spd) and spermine (Spm) are the most common PAs in plants (Philip et al., 2000). These contents of various PAs are influenced by different developmental and physiological states (Paul et al., 1984). Moreover, it has been reported that PAs afford protection against environmental stresses, such as salinity, chilling, ozone exposure and potassium deficiency (Bouchereau et al., 1999; Gao et al., 2009). In sweet corn, the concentration of PAs in aborting seeds is lower than that in normal seeds during 8–12 days after pollination (Zhao and Qiu, 2003), and among Spm, Spd and Put, Put has the highest concentration during ripening of corn seeds (Zhang et al., 1999). However, little information is available on the relationship between changes in PAs during seed development and seed quality. In present study, the concentrations of free Spm, Spd and Put and seed quality parameters in supersweet corn seeds were assessed at different seed developmental stages to determine whether there are any relationships between polyamine levels and seed quality.

2. Materials and methods

2.1. Experiment material and planting

Supersweet corn seeds (F_1 hybrid with sh_2), cv. Supersweet 17, were used in these studies. The hybrid seeds were produced by synchronous hand cross-pollination of the parental inbreds in the

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field at the experimental farm of Zhejiang University using production practices common for the region.

2.2. Seed harvest

Normally, it takes 30–40 days for supersweet corn seeds to fully mature but seed germination is possible 14 days after pollination (Cao et al., 2008b). Supersweet corn ears were harvested at different developmental stages from 14 to 42 days (at an interval of 4 days) after pollination (DAP). At each harvest, 10 plants were randomly chosen and the first ear from the top of the plant was harvested by hand. Seeds from the middle part of supersweet corn ears were threshed by hand and bulked for further tests.

2.3. Germination test

Three replications of 50 seeds for each harvest were used, each 50 seeds were placed in a 12 cm × 18 cm germination box containing wet sand. Then, seeds were kept in a germination chamber at 25 °C with a diurnal cycle of 12 h light and 12 h darkness for 7 days. Seeds were considered germinated when a 1 mm length radicle protruded through the seed coat. The number of germinated seeds was counted daily. The energy of germination and germination percentage were calculated after 4 and 7 days, respectively. The germination index ($GI = \sum(Gt\ Tt^{-1})$) was calculated by the method of Zhang et al. (2007) where Gt is the number of the germinated seeds on day t and Tt is the time corresponding to Gt (in days). The energy of germination was the percentage of germinated seeds 4 days after planting relative to the number of seeds tested.

2.4. Measurements of physiological parameters

The seed length, width and thickness of 30 seeds were measured at each harvest manually with a ruler. The fresh and dry weights of 100 seeds were recorded at each harvest. Dry weights were recorded after 24 h in an oven at 80 °C (Cao et al., 2008a).

The evaluation of kernel nutritive quality was performed with 3 replications of 20 seeds for each harvest. Soluble protein was extracted from the seeds and determined by colorimetric assay with Coomassie Brilliant Blue G250 (Li, 2000). The concentration of total soluble sugar was determined by 3,5-dinitrosalicylic acid method (Jiang, 1999).

Malondialdehyde (MDA) concentration was determined using the thiobarbituric acid (TBA) reaction as described by Zhang et al. (2007). Briefly, 0.3 g fresh weight (FW) of corn seeds for each replication were collected at 14, 18, 22, 26, 30, 34, 38 and 42 days after pollination, and homogenized in 3 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 3600 × *g* for 10 min and 2 ml of 0.67% TBA was added to 2 ml of supernatant. The mixture was heated at 100 °C for 30 min, cooled to room temperature and then centrifuged at 1800 × *g* for 5 min. The optical density of the supernatant was measured at 450 nm, 532 nm and 600 nm. The MDA concentration was calculated according to the follow formula: $C\ (\mu mol\ l^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$, then the MDA concentration (nmol g^{-1} FW) was calculated.

Electrolyte leakage as a measurement for membrane permeability was determined according to the method of Liu et al. (2000). Fresh seeds were cut into segments of about 5 mm, respectively. Each tissue (0.2 g FW) was placed in the test tube with 10 ml deionized water and covered with a plug. After incubation at 25 °C for 6 h, the electrolyte leakage of the tissue was measured using a conductivity meter (DDS-11A, made in Shanghai, China), the value was named *E*1. Subsequently, the test tube was kept in water at 100 °C for 30 min, and then cooled to 25 °C, the second electrolyte leakage was measured as *E*2. The electrolyte leakage of deionized water was named *E*0. The relative electrolyte leakage (REL) was calculated as follows:

$$REL = \frac{E1 - E0}{E2 - E0} \times 100\%$$

Peroxidase (POD) and catalase (CAT) activities of from seeds of each harvest were measured. Seeds (0.5 g FW) were harvested and homogenized in 10 ml of 50 mM phosphate buffer (pH 7.8) containing 0.1 mM EDTA and 2 mM dithiothreitol. The homogenate was centrifuged at 10,000 × *g* for 15 min. The supernatant was used for enzyme assays and all of the extraction steps were carried out at 1–4 °C (Trevor and Fletcher, 1994).

POD activity was measured according to the method of Qiu et al. (2005). The assay mixture consisted of 3 ml of 50 mM phosphate buffer (pH 7.0), 1% guaiacol, 0.4% H_2O_2 and enzyme extract. The increase in absorbance due to oxidation of guaiacol was measured at 470 nm. Enzyme activity was calculated in terms of μmol of guaiacol oxidized $min^{-1}\ g^{-1}$ fresh weight at 37 °C, and was expressed as $U\ g^{-1}\ FW\ min^{-1}$. CAT activity was measured at 37 °C according to Trevor and Fletcher (1994). The enzyme assay contained 50 mM phosphate buffer (pH 7.0), 0.4% H_2O_2 and enzyme extract in a total volume of 4 ml. CAT activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm and was expressed as $U\ g^{-1}\ FW\ min^{-1}$. Both POD and CAT were determined for three replications.

2.5. Polyamine concentration assay

PAs concentrations were measured by HPLC according to the method of Flores and Galston (1982) with minor modifications. 0.3 g FW seeds of each harvest were homogenized with 3 ml 5% (w/v) chilling perchloric acid using a cooled mortar and pestle. The homogenates were kept in an ice bath for 1 h, and then centrifuged at 23,000 × *g* for 30 min at 4 °C; the supernatant was transferred to new centrifugal tube and were stored at −70 °C for quantification of PAs. Seed extracts were benzoylated. One ml 2 mol l^{-1} NaOH was mixed with 500 μl supernatant. After the addition of 10 μl benzoyl chloride, the samples were vortexed for 20 s then incubated for 20 min at 37 °C. Following the high temperature incubation, 2 ml saturated NaCl was added. Benzoyl-polyamines were extracted in 2 ml diethyl ether and vortexed for 10 s. After centrifugation at 1500 × *g* for 5 min at 4 °C, 1 ml of the ether phase was collected, evaporated to dryness under a stream of warm air, and redissolved in 100 μl methanol.

The benzoylated extracts were filtered through a 0.22 μm membrane filter, and then eluted at room temperature through a 6.0 mm × 150 mm, 5 μm particle size reverse-phase (C18) column (Shim-Pack CLC-ODS). PAs peaks were detected by an SPD-20A (Shimadzu) absorbance detector at 254 nm. The mobile phases consisted of methanol: water (64:36), at a flow rate of 1.0 ml min^{-1}. Three polyamine standards (Sigma Chemical Co.) of Put, Spd and Spm were prepared at different concentrations for the development of standard curves.

2.6. Statistical analysis

Statistical analysis was performed using the SAS software. Three replications were conducted for the parameter measurements. All obtained percentage values were arcsine-transformed prior to statistical analysis. Regression equations between polyamine concentrations and physiological parameters during seed development were calculated.