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Research article

Changes in nutrient distribution are part of the mechanism that promotes seed development under severe nutrient restriction

Patricia Coello, Eleazar Martínez-Barajas*

Departamento de Bioquímica, Facultad de Química-UNAM, D.F. 04510, Mexico

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ABSTRACT

When bean fruits are detached from a plant at 20 days after anthesis (DAA), the material accumulating in the pod is relocalized to the seeds. This mobilization is more active during the first five days after the fruits are removed, which allows some seeds to continue their development. In freshly removed fruits, ^{14}C-sucrose was evenly distributed among seeds; however, in fruits that were removed three days before, the labeled sugar was concentrated in 1–2 seeds. In addition, in removed pods, embryos dissected from seeds that no longer continue development can assimilate and efficiently use sucrose for protein and starch synthesis. Our results support the hypothesis that most embryos in removed fruits are forced to stop developing by removal of the nutrient supply. We also observed that SnRK1 activity increased in embryos that were subjected to nutrient deprivation, supporting the role of SnRK1 in the metabolic adaptation to stress conditions.

1. Introduction

Carbon partitioning between source tissues and various competing sink tissues is a dynamic process (Lemoine, 2000). In legume seeds, nutrients (amino acids, sucrose and inorganic nutrients) are imported from the mother plant through the funiculus and symplastically transferred from the sieve elements into the seed-coat parenchymatous cells (Offler and Patrick, 1984). The unloading from the seed coat is a passive process (De Jong et al., 1996), and sucrose is removed from the space between the seed coat and cotyledons by the activity of an H^+/sucrose symporter in the cotyledonary cells (Lichtner and Spanswick, 1981). Early in development, most of the sucrose entering a legume seed is converted to hexose. During the synthesis of storage products, sucrose is the major sugar in the seed and in the embryo, and the synthesis of starch and protein becomes the primary fate of sucrose entering the seed (Weber et al., 1995, Borisjuk et al., 2003). Under most growing conditions, large numbers of ovules are initiated to maximize reproduction. However, at times of environmental stress, the nutritional demand of reproduction frequently exceeds the carbon and nitrogen resources that are allocated to the ovules, and some of the ovules are aborted (Sun et al., 2004). In general, the majority of reproductive abortion occurs early in development before most of the resources have been transferred to seeds and fruits (Stephenson, 1981). A stage called the "final stage in seed abortion" (FSSA) has been identified in pea seed development at which point seeds are approximately 10% of their final dry weight and the pod walls have reached their maximum weight. FSSA also marks the initiation of seed filling, the end of a period of active cell division in the embryo and the highest probability of seed abortion (Pegaire et al., 1986). Stressful environmental conditions can limit the nutrient availability at any time, and sugars (fructans, sucrose, glucose and fructose), which accumulate as reserves in different parts of the plant, are remobilized and transported to the developing grains to support grain yield under post-anthesis stress (Wardlaw and Willenbrink, 2000).

Plants have a regulatory mechanism to balance the supply of carbon with its use, allowing the growth to be sustained under a wide range of environmental conditions (Smith and Stitt, 2007). SnRK1 is involved in adapting metabolism to changing environmental conditions and coordinates the adjustment of physiological and metabolic demands with growth (Radchuk et al., 2010). *In vitro,* SnRK1 phosphorylates and inactivates 3-hydroxymethyl-3-methylglutaryl-CoA reductase (Dale et al., 1995), sucrose phosphate synthase, nitrate reductase (Sugden et al., 1999), trehalose phosphate synthase (Harthill et al., 2006), and non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase

Abbreviations: DAA, days after anthesis; FSSA, final stage in seed abortion.

* Corresponding author.
E-mail address: emtz@unam.mx (E. Martínez-Barajas).

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(Piattoni et al., 2011). SnRK1 also stimulates the redox activation of ADPGlu-PPase (Geigenberger, 2003) and modulates the transcription of a large number of genes (Baena-González et al., 2007), many of which could be important for the adaption of plants to hostile environments. In this paper, we describe how the remobilization and differential distribution of nutrients in the bean pod can support seed development when the nutrient supply is reduced and how SnRK1 activity increases in the embryo in response to nutrient deprivation.

2. Materials and methods

2.1. Plant material

Bean seeds (*Phaseolus vulgaris* cv V8025) were surface sterilized and germinated at 25 °C on wet filter paper. Seedlings were transplanted into 3-L pots that were filled with agrolite and grown in a greenhouse at 25 °C under a 16-h light/8-h dark regime. Plants were irrigated every day with half-strength Hoagland solution (Jones, 1982). The flowers were tagged at anthesis, and seed development was analyzed at 20, 21, 22, 23 and 25 days after anthesis (DAA). Some fruits were detached from the plant at 20 DAA and incubated at 25 °C in the dark, and seed development was analyzed at 1, 2, 3 and 5 days after the fruits were removed from the plant.

2.2. Sugar determination

Embryos were dissected and homogenized in 3 mL of 80% ethanol and extracted at 80 °C for 15 min. Ethanol extract was centrifuged at 12,000×*g* for 10 min, and the supernatant was used for the enzymatic determination of glucose, fructose and sucrose (Bernal et al., 2005).

2.3. Sucrose incorporation in fruits and embryos

The uptake and distribution of sucrose were analyzed in normally developed bean fruits at 23 DAA and in fruits that were removed from the plant at 20 DAA. The pedicel of the fruits was removed with a sharp blade, placed in 4 mL of water containing 20 μCi of sucrose (U−^{14}C, 565 mCi/mmol), and incubated in the dark at 25 °C for 48 h (Zhang et al., 2010). Then, the fruit was dissected. Individual seeds (coat and embryo were separated) and the pod segment corresponding to each seed were homogenized in 80% ethanol. A 100-μl aliquot was mixed with 3 mL of scintillation liquid (Ultima Gold XR, Perkin Elmer), and the radioactivity (cpm) was measured by liquid scintillation counting (Beckman LS 6500).

The embryos that developed in fruits at 23 and 25 DAA and those that developed from fruits that were removed from the plant at 20 DAA and incubated for 3 and 5 days at 25 °C in the dark were dissected and incubated in 10 mL of label solution (Melkus et al., 2009), supplemented with 200 mM sucrose (13 μCi/mmol) for 4 h at 25 °C and 250 rpm. Then, the embryos were rinsed three times in cold distilled water and homogenized in 80% ethanol. A 100-μl aliquot was mixed with 3 mL of scintillation liquid (Ultima Gold XR, Perkin Elmer), and total incorporation was measured by liquid scintillation counting. The ethanolic extract was centrifuged at 12,000 × *g* for 10 min, and the pellet was processed (Merlo et al., 1993) and used to measure the sucrose incorporation into starch and protein. The pellet was dissolved in 1.5 mL of 50 mM sodium acetate (pH 5.0) and heated for 3 h at 90 °C, after which 100 U/mL amyloglucosidase was added. The tubes were mixed and incubated at 37 °C for 12 h. After centrifugation (10 min at 12,000 × *g*), 100 μl of the supernatant was mixed with 3 mL of scintillation liquid (Ultima Gold XR, Perkin Elmer), and the incorporation into starch was determined by liquid scintillation counting. The pellet was resuspended in 1 mL of 50 mM Tris–HCl (pH 7.4) containing 30 U of pronase and incubated for 12 h at 30 °C. After centrifugation (10 min at 12,000 × *g*), the incorporation into proteins was determined using 100 μl of the supernatant mixed with 3 mL of scintillation liquid (Ultima Gold XR, Perkin Elmer).

2.4. SnRK1 activity

Embryo tissue was homogenized in extraction buffer (50 mM Tricine-NaOH (pH 8.0), 1 mM EDTA, 1 mM EGTA, 25 mM NaF, 12.5 mM β-glycerophosphate, 5 mM Na PPi, 1 mM Na orthovanadate, 10% glycerol, 0.02% (p/v) Brij 35, 1 mM DTT, 1 mM benzamidine, 0.1 mM PMSF, 1 × protease inhibitor cocktail (SIGMA P9599) and 1% polyvinylpolypyrrolidone). The extracts were centrifuged for 15 min at 15,000×*g* at 4 °C, and the supernatants were desalted using NAP10 (GE healthcare) columns that were equilibrated with the same buffer. The SnRK1 activity in desalted extracts (Zhang et al., 2009) was determined. Peptide phosphorylation was assayed in a final volume of 25 μl containing kinase buffer (40 mM HEPES-NaOH (pH 7.0), 5 mM $MgCl_2$, 20 mM NaCl, 2% (v/v) glycerol, 1 mM dithiothreitol, 50 mM sodium fluoride, 25 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA), 200 μM AMARA peptide (AMARAASAAALARRR) and 200 μM ATP (67 μCi [γ^{32}P] ATP/μMol ATP). After 6 min of incubation at 30 °C, 15-μl aliquots were removed and spotted onto 2 cm^2 phosphocellulose paper, which was placed in 500 mL of 1% (v/v) H_3PO_4. The squares were washed three times for 20 min each in 1% H_3PO_4 and once in acetone and then air-dried. Phosphocellulose squares were immersed in 3 mL of scintillation liquid (Ultima Gold XR, Perkin Elmer) and counted.

3. Results

From 20 to 40 DAA, the bean fruits showed a linear dry weight accumulation (Fig. 1A). At 20 DAA, the pods almost reached their maximum dry weight, and under normal conditions, their weight

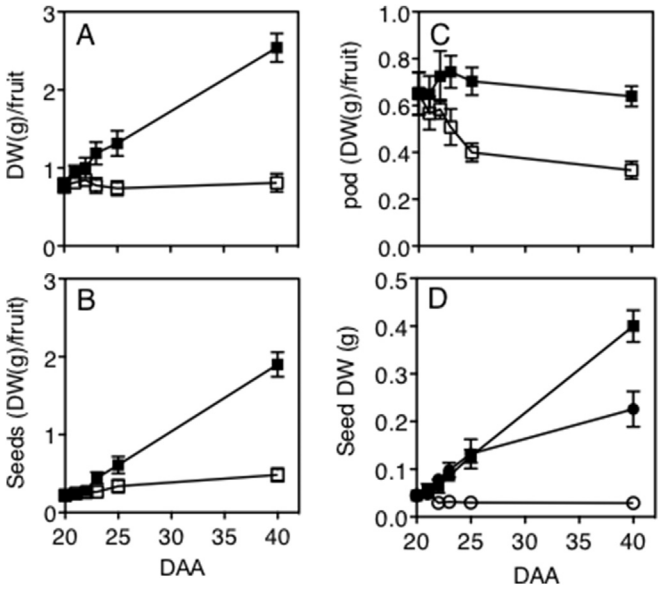

Fig. 1. Fruit (A), pod (B) and seed (C) dry weight in normally developed fruits (■) and in fruits that were detached from the plant at 20 DAA (□). Individual seed dry weight (D) developed under normal conditions (■), seeds that will survive (●) and seed that will die (○) in fruits that were removed from the plant at 20 DAA. Values are means ± SD (n = 50).