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Something seedy going on: HEAT SHOCK PROTEIN90.6 links carbon and nitrogen metabolism in seed development

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Seed development is a highly coordinated complex process. The successive accumulation of nutrients and developmental signals in the seed requires orchestrated metabolism activities in the endosperm and the embryo. The endosperm plays a crucial role in seed development by passing maternally supplied resources (both nutrients and developmental signals) to the embryo (Lafon-Placette and Kohler, 2014). Major nutrients in seeds are accumulated and stored as carbohydrates, proteins, and lipids. Many studies have indicated that there is potential to modify the nutrient composition of important crop seeds such as maize (*Zea mays*) to enhance their usefulness as food sources (Huang et al., 2022); however, such manipulation is challenging. One challenge lies in the complex regulatory network of nutrient accumulation, which involves an orchestration of highly associated carbon (C) and nitrogen (N) metabolism (Zhang et al., 2015).

In the current issue of *Plant Physiology*, Xu et al. (2023) shed light on the association between C and N metabolism in seed development. The authors found a mutant producing shrunken and small seeds in an ethyl methanesulfonate (EMS)-mutant screen. A single-nucleotide mutation was identified in *HEAT SHOCK PROTEIN 90.6* (*HSP90.6*) in the EMS mutant via bulked segregant RNA sequencing (BSR-seq) technology. HSP90 genes encode chaperone proteins, and as a part of the superfamily of ATPases, HSP90s have diverse roles in cell signaling and stress responses. The EMS-generated mutation lies in the ATPase domain of *HSP90.6*.

The authors generated knock-out plants via CRISPR/Cas9 to validate the role of *HSP90.6* in seed development of maize. Seeds of knock-out mutants were highly distinguishable from the wild type (WT) as the mature *hsp90.6* seeds showed shrinkage, almost no filling, and the inability to germinate.

High expression levels of *HSP90.6* were detected in the early-stage embryo and endosperm via reverse transcription quantitative real-time PCR and *in situ* hybridization. Further, HSP90.6 localized to the nucleus and cytoplasm in a green fluorescence protein (GFP)-assisted subcellular localization assay. These findings provided clues that HSP90.6 might interact with other proteins in the nucleus and cytoplasm and affect seed development in an ATPase activity-dependent manner.

To test the hypothesis that the ATPase domain plays a role in the function of HSP90.6, Xu et al. (2023) expressed recombinant proteins of HSP90.6 originated from the WT and EMS mutant in *Escherichia coli*. The ATPase activity of the recombinant proteins revealed substantially decreased ATPase activity of the mutated protein. Thus, the interrupted ATPase activity of HSP90.6 may be responsible for defective seed filling of the mutant.

Further, RNA sequencing revealed disrupted expression of many genes associated with C and N metabolism in early-stage seeds of the *hsp90.6* mutant, suggesting a potential role of HSP90.6 in regulating C and N metabolism during seed development. The abundance of many amino acids and some sugar metabolites dramatically decreased in the early-stage seeds of the *hsp90.6* mutants, which supported the role of HSP90.6 in C and N metabolism during seed development.

To explore the molecular mechanisms underlying HSP90.6-regulated C and N metabolism, immunoprecipitation–mass spectrometry (IP-MS) was applied to identify proteins interacting with HSP90.6. A total of 52 specific proteins interacted with HSP90.6, three of which are involved in C and N metabolism: REGULATORY PARTICLE NON-ATPASE 6

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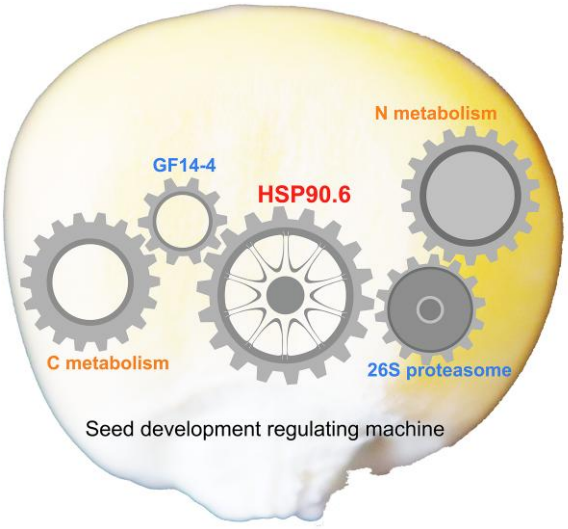

Figure 1 HSP90.6 regulates seed development of maize via manipulating carbon (C) and nitrogen (N) metabolism. Progressive and orchestrated processes of C and N metabolism are necessary for seed development. Xu et al. (2023) found that HSP90.6 plays a role in manipulating C and N metabolism via interacting with the 14-3-3 protein GF14-4 and the 26S proteasome subunits of RPN6 and PBD2.

(RPN6), PROTEASOME BETA SUBUNIT D2 (PBD2), and GENERAL REGULATORY FACTOR 14-4 (GF14-4). Subcellular localizations of RPN6, PBD2, and GF14-4 were predicted to overlap with HSP90.6, and colocalization of these candidates and HSP90.6 was verified in maize protoplasts via GFP-assisted subcellular localization assays as well as through yeast two-hybrid and luciferase complementation imaging (LCI) assays.

RPN6 and PBD2 are important components of the 26S proteasome with close interaction with each other (Tian and Trader, 2020). Considering that the 26S proteasome provides a principal machine for N cycling via degradation of ubiquitinated proteins in eukaryotic cells, Xu et al. (2023) speculated that there would be impaired activity of the 26S proteasome in the *hsp90.6* plants. Ubiquitylome analyses were performed to measure the hydrolytic activity of the 26S proteasome in WT and *hsp90.6*, and the activity was reduced by 74.9% in the mutant. Accordingly, western blotting showed that ubiquitinated proteins substantially accumulated in seeds of *hsp90.6*.

IP-MS was also applied to detect proteins interacting with GF14-4, which allowed identification of 25 candidates. Six of the candidate proteins were predicted to colocalize with HSP90.6 in the cytoplasm, three of which are involved in glucose metabolism: PHOSPHOGLYCERATE KINASE 3 (PGK3), PHOSPHOENOLPYRUVATE CARBOXYLASE 1 (PEP1), and GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE 1 (GPC1). LCI assays validated that GPC1 and PGK3 interact with GF14-4.

Taken together, the findings presented by Xu et al. (2023) provide useful clues for linkage between C and N metabolism via HSP90.6 during seed development (Figure 1). Specifically, HSP90.6 seems to affect C metabolism by interacting with GF14-4 and N recycling through the 26S proteasome degradation pathway. Although candidate proteins responsible for C and N metabolism have been identified to interact with HSP90.6, fundamental questions remain: Does HSP90.6 mediate a partition of energy and resources between C and N metabolism? Also, since seed development shows dramatic difference in dicots and monocots (Sreenivasulu and Wobus, 2013), will the conserved HSP90.6 allow us to illustrate a conserved network regulating coordination of C and N metabolism during seed development in angiosperms? Future study on HSP90.6 and associated pathways in other species would lead to clearer directions to manipulation of grain quality.

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