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Circadian oscillation of starch branching enzyme gene expression in the sorghum endosperm

Joel Mutisya,[1,2] Chuanxin Sun[1] and Christer Jansson[1,3,*]

[1]Department of Plant Biology & Forest Genetics; The Swedish University of Agricultural Sciences; Uppsala, Sweden; [2]Kenya Agricultural Research Institute (KARI); Nairobi, Kenya; [3]Lawrence Berkeley National Laboratory; Earth Science Division; Berkeley, CA USA

Expression of the three SBE genes, encoding starch branching enzymes, in the sorghum endosperm exhibited a diurnal rhythm during a 24-h cycle. Remarkably, the oscillation in SBE expression was maintained in cultured spikes after a 48-h dark treatment, also when fed a continuous solution of sucrose or abscisic acid. Our findings suggest that the rhythmicity in SBE expression in the endosperm is independent of cues from the photosynthetic source and that the oscillator resides within the endosperm itself.

Key words: barley, circadian rhythms, diurnal regulation, endosperm, oscillation, sbe, sorghum, starch

Abbreviations: ABA, abscisic acid; GBSSI, granule-bound starch synthase I; LD, light-dark cycle; DD, continuous dark; SBE, starch branching enzyme

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*Correspondence to:
Christer Jansson; Email: cgjansson@lbl.gov

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Starch, is the predominant storage carbohydrate in plants and the second most abundant biopolymer on earth, after cellulose. The path of starch synthesis rests on the conversion of sucrose to ADP-glucose (ADPG) and subsequent formation of the α-1,4 polyglucans amylose and amylopectin. Amylose is an essentially linear α-1,4 glucan with few α-1,6 glucosidic branches while amylopectin is highly branched. Granule-bound starch synthase (GBSS) catalyses the formation of new glucosidic linkages in amylose by transferring the glucose residue of ADPG to the non-reducing end of an existing α-1,4-linked glucan chain, thereby elongating it. Soluble starch synthase (SS) performs the same action for amylopectin. Branch points are introduced by starch branching enzyme (SBE), and debranching enzyme (DBE) may be involved in tailoring the branched glucans into a form capable of crystallization. All enzymes, including AGPase, exist in several distinct isoforms with specific roles in different organs and during different developmental stages. Other enzymes, such as glucan water dikinase (GWDK), starch phosphorylase (SP) and the D-enzyme, also participate in starch synthesis although their functions remain to be resolved. In the plant, starch is deposited as granules in chloroplasts of source organs such as leaves (transitory starch) or in amyloplasts of sink organs such as seeds, tubers and roots (storage starch). Typically, starch granules consist of 70% amylopectin and 30% amylose. Pakaging of amylopectin and amylose in starch granules is an intricate and complex process that is not fully understood. (reviewed in ref. 1).

Photocycles, thermocycles, and the circadian clock together phase cellular activities in plants to generate diurnal oscillations in gene expression, metabolism and physiology.[2,3] In Arabidopsis leaves, genes encoding enzymes involved in starch metabolism, particularly degradation, showed diurnal expression patterns.[4] Circadian regulation of the GBSSI gene in leaves has been reported for Arabidopsis,[5] sweet potato[6] and snapdragon.[7] Diurnal oscillations of starch synthesis gene expression has been observed also in sink organs; for the growth ring formation starch granules in potato tubers,[8] for the gene encoding the catalytic subunit of AGPase in potato tubers,[9] and the SBEI and SBEII genes in cassava storage roots.[10,11]

We have previously reported on the temporal and spatial expression profiles for the sorghum SBEI and SBEIIB genes.[12,13] We have also shown that SBE expression levels in sorghum endosperm showed a diurnal fluctuation with an induction in the light

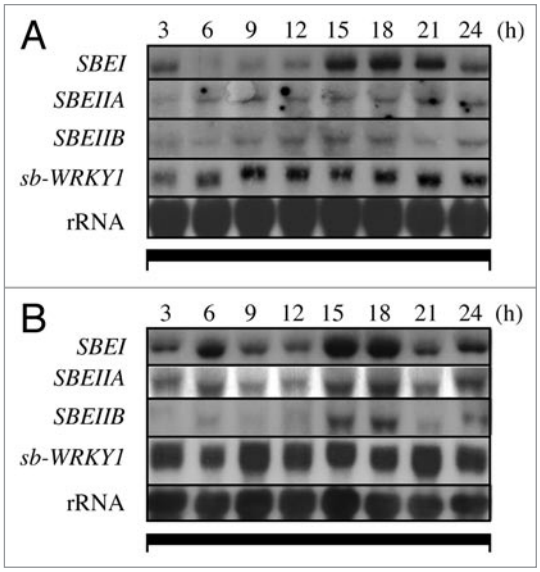

Figure 1. Oscillation of SBE expression in endosperms from cultured spikes detached from sorghum plants pre-conditioned to darkness for 24 h. Spikes were supplied with a continuous solution of 200 mM sucrose (A) or 10 μM ABA (B) and cultured for 24 h in darkness prior to sampling.

and decline in the dark.[14] To test whether the oscillations in endosperm transcript levels were acutely dependent on light or other cues from the source, we followed SBE expression in isolated seeds fed with a continuous supply of sucrose or ABA, two chemicals that have been shown to induce starch synthesis genes.[11,15-17] For these experiments, plants were first transferred to darkness for 24 h to remove endogenous sucrose form the sink tissues. Spikes from the pre-conditioned plants were detached and cultured in water, sucrose or ABA in darkness. We found that even in sucrose or ABA-fed seeds, SBE expression in the endosperm oscillated with a period of approximately 24 h (**Fig. 1**). The oscillatory pattern was observed also in the absence of exogenous inducers and in seeds isolated from plants grown under continuous dark (DD) conditions, although the rhythm in SBE expression for DD plants was less overt as compared to light-dark grown (LD) plants or seeds supplied with sucrose or ABA (data not shown). These results indicate that, (1) the oscillation in SBE expression in the endosperm is not dependent on information from the source, and, (2) the oscillator resides in the endosperm tissues. The nature of the oscillator is unknown.

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