

## Mutations in leaf starch metabolism modulate the diurnal root growth profiles of *Arabidopsis thaliana*

Nima Yazdanbakhsh and Joachim Fisahn\*

Max Planck Institute of Molecular Plant Physiology; Potsdam, Germany

**R**oots of *Arabidopsis thaliana* exhibit stable diurnal growth profiles that are controlled by the circadian clock. Here we describe the effects of mutations in leaf starch metabolism on the diurnal root growth characteristics of *Arabidopsis thaliana*. High temporal and spatial resolution video imaging was performed to quantify the growth kinetics of *Arabidopsis* wild-type as well as *pgm*, *sex1*, *mex1*, *dpe1* and *dpe2* starch metabolism mutants grown in three different photoperiods. As a result, root growth patterns of all genotypes displayed characteristic modifications in their diurnal kinetics that were also affected by the photoperiod. To further investigate the role of starch derived substrate deficiency on root growth, the effect of 0.05% extracellular sucrose was studied in 12 h-12 h light-dark cycles.

Root growth of *Arabidopsis thaliana* is highly rhythmic with respect to the time of the day.<sup>1-3</sup> In general, root growth rates increase at night while most of the light period is characterized by declining elongation rates. Since a slow oscillation in root growth rate with a periodicity of approximately 24 h persists in free running conditions it was demonstrated that the circadian clock mediates these daily fluctuations.<sup>1</sup> Root growth at night is fueled by the degradation of starch within the leaves. Thus, a correspondence between the time taken to degrade starch reserves and the length of the night is important to optimize growth in C-limiting conditions. Gibon et al. observed a strong correlation between the rate of starch degradation and the relative growth rate when *Arabidopsis*

Col-0 was grown in a range of different photoperiods.<sup>4</sup> Therefore, to avoid periods of C starvation at the end of the night the circadian clock was postulated to function as a timer that adjusts degradation of starch to the prevailing length of the night.<sup>1,5</sup>

Root growth strongly depends on the supply of sucrose from the leaves. To investigate the effects of substrate depletion on root elongation at night, 12-day-old seedlings of Col-0, *pgm* and *sex1* growing in a 16 h photoperiod were previously investigated by digital time resolved video imaging.<sup>1</sup> As a result, the diel growth response was strongly modified in *pgm* and *sex1* as compared to the wild-type. Both mutants showed a pronounced inhibition of growth during the night and a gradual recovery of growth during the light period. To substantiate these findings, we here report on the root elongation patterns of additional mutants in starch metabolism, e.g., *mex1*, *dpe1* and *dpe2* detected at different photoperiods and elevated external sucrose supply.

### Diurnal Growth Profiles of Starch Metabolism Mutants in Short Days

We already reported on the diurnal growth profiles of *Arabidopsis* wild-type roots under short day photoperiods (8 h light/16 h dark).<sup>1</sup> In parallel, diurnal growth profiles of wild-type Col-0 seedlings displayed a strong increase in growth rate immediately after light on that was followed by a gradual decline during most parts of the light period (Fig. 1). A similar pattern in growth rate was detected in

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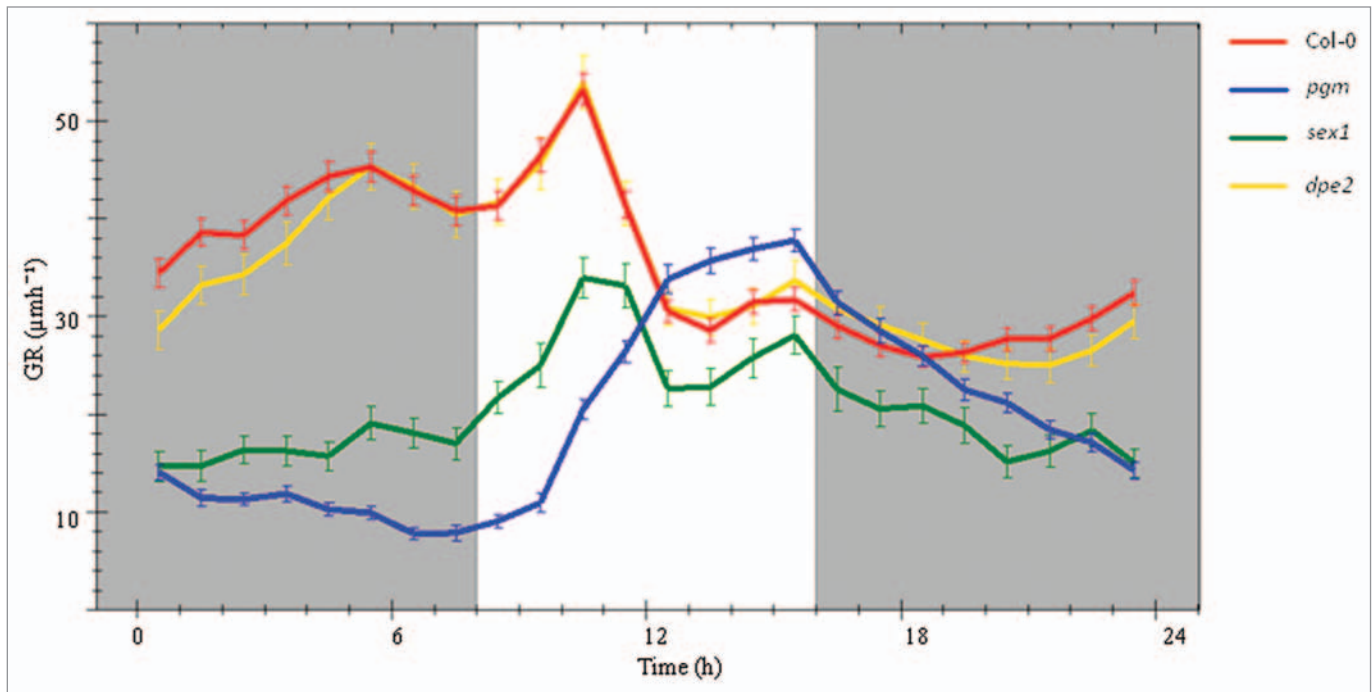
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\*Correspondence to: Joachim Fisahn;  
Email: Fisahn@mpimp-golm.mpg.de

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**Figure 1.** Root extension pattern of Col-0 (n = 24), *pgm* (n = 6), *sex1* (n = 8), *dpe2* (n = 13) seedlings in 8 h photoperiods. Seedlings were 21 days old and maintained at a day night temperature of 21°C. Measurements were performed on six consecutive days and averaged. GR: growth rate. Growth rates of Arabidopsis roots were recorded as described previously in reference 10–12.

the *dpe2* mutant (Fig. 1). However, due to the mutation in the disproportionating enzyme growth rates were reduced in this mutant between 20 p.m. and 4 a.m. in comparison to the wild-type (Fig. 1). This reduction in root elongation rate of the mutant indicates a deficiency in the supply of sucrose to the root at night. In accordance with measurements performed under long day conditions root growth rates of the *pgm* and *sex1* mutants when exposed to short day photoperiods declined strongly during the dark period (Fig. 1).<sup>1</sup> A time delay of 2 h was required to accelerate growth again in the roots of *pgm* after the induction of light (Fig. 1). Due to the absence of starch in the *pgm* mutant severe carbon starvation symptoms developed in the dark period that gave rise to delayed recovery of root growth upon illumination.

### Modulation of Root Elongation Kinetics by 0.05% Extracellular Sucrose in Equal Day/Night Length

To further investigate the impact of the photoperiod and the sucrose supply for

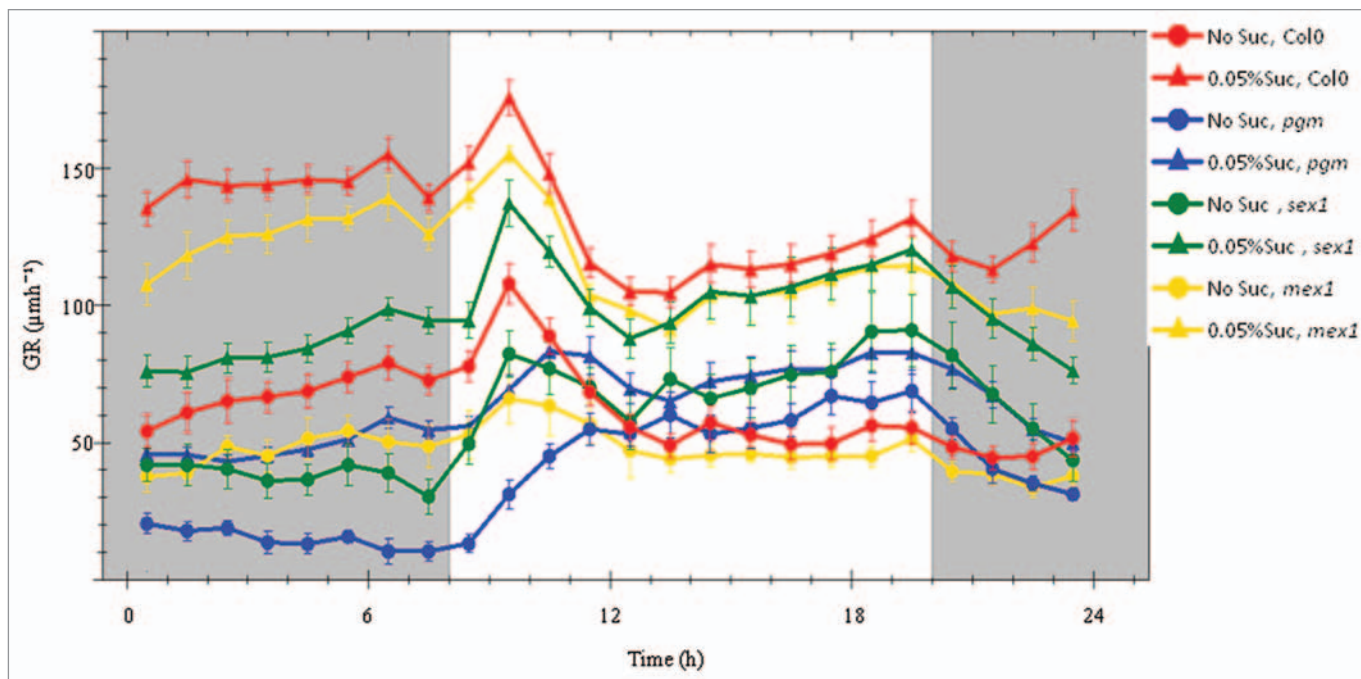
root expansion growth we exposed wild-type and mutant seedlings to 12 h/12 h photoperiods (equal nights; Fig. 2). Due to extension of the photoperiod absolute growth rates of Col-0 seedlings increased as described previously.<sup>1</sup> The delayed acceleration of root growth that emerged in the *pgm* mutants upon light on was almost abolished in equal photoperiods (Fig. 2). Increase in root elongation rates of *mex1* in the dark period occurred with a 1 h delay compared to the wild-type, probably indicating that sucrose supply to the roots is modified in the mutant.

Inclusion of 0.05% sucrose in the growth medium further increased the absolute root extension rates of wild-type Col-0 and the starch metabolism mutants *pgm*, *sex1*, *mex1* (Fig. 2). Col-0 seedlings supplied with extracellular sucrose exhibited a shorter growth inhibition period after transition to darkness which was followed by 4 h of strong increase in extension rate (Fig. 2). The growth rate stayed almost constant during the last part of the night. This phase resembled the growth behaviour of WS wild-type seedlings grown in the absence of sucrose.<sup>1</sup> Addition of 0.05% of sucrose to the growth

medium of *pgm* seedlings induced a major change in the diurnal response (Fig. 2). The strong and progressive inhibition of growth during the dark period was significantly repressed. The kinetics during the light period also became more similar to those of the wild type with a peak 3 h after illumination. In contrast to absence of sucrose the maximum elongation rate occurred between 10 and 11 am.

The *sex1* mutant also grew marginally different in the presence of 0.05% extracellular sucrose than in absence. While the strong inhibition of growth during the first 4 h after dusk was similar to the control seedlings, throughout the rest of the night we detected a slight recovery of growth. Upon light exposure, elongation rate decreased for an hour. In the second hour of illumination a strong increase in growth rate emerged which was followed by a 3 h decrease.

In general, root growth kinetics of *mex1* seedlings closely resemble that of the wild-type (Fig. 2). It had been reported that due to the mutation in the chloroplast envelope maltose transporter (*MEX*) gene, the predominant route for carbohydrate export from chloroplasts at night, the



**Figure 2.** Modulation in the diurnal root extension pattern of Col-0, *pgm*, *sex1* and *mex1* due to 0.05% sucrose. 24 h extension kinetics of Col-0 (n = 10), *pgm* (n = 5), *sex1* (n = 6) and *mex1* (n = 2) growing in no sucrose were compared with seedlings growing in 0.05% extracellular sucrose (n = 10, n = 8, n = 5 and n = 3, respectively) in 12 h photoperiods. Values represent averages of three consecutive days plus SE.

maltose levels in leaves of this mutant are at least 40 times as high as those of wild-type.<sup>6</sup> Starch levels are elevated and the rates of both synthesis and degradation are reduced. Levels of sucrose and hexoses are greatly elevated during the day but fall to levels comparable to those of wild-type leaves at night.<sup>6</sup> However, the existence of a glucose transporter in the chloroplast envelope potentially allows the export of glucose to the cytosol, thus providing substrate for root elongation at night.<sup>7</sup>

### Diurnal Growth Pattern of Starch Mutants under Long Day Conditions

Root growth kinetics of several *Arabidopsis* mutants compromised in starch metabolism as, *pgm*, *sex1*, *mex1*, *dpe1* and *dpe2* were compared to wild-type under long day conditions. Col-0 exhibited the typical pattern described before. The pattern of *pgm* and *sex1* resemble those described in Yazdanbakhsh et al.<sup>1</sup> Root growth kinetics obtained from *mex1* and *dpe1* seedlings resembled Col-0 wild-type (Fig. 3). However, small deviations were detected. *mex1* showed slightly decreased elongation rates after dusk for

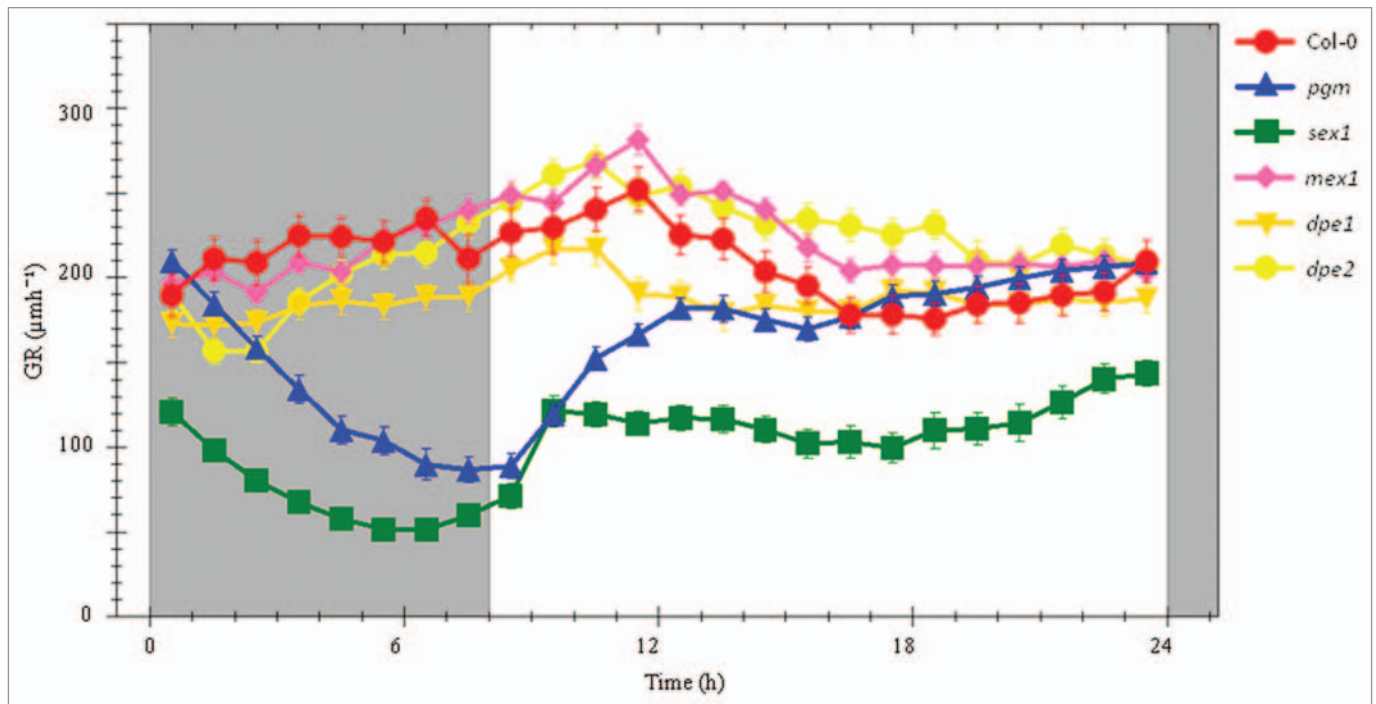
about 5 h and marginally increased rates in the light. Elongation of *dpe1* proceeded at reduced rates in comparison to wild-type during the night and the first part of the light period. *dpe2* displayed a more pronounced inhibition of elongation rate during the first 2–3 h of the dark period than the wild-type. Subsequently this decrease recovered towards the end of the night. At this time the growth kinetics of *dpe2* closely resembled that of the wild type.

The activities of most of the enzymes involved in starch metabolism had been reported not to be significantly different in *dpe1* compared to the wild-type. Exceptions are enzyme activities of  $\beta$ -amylase being twice that of wild-type and starch phosphorylase which is slightly increased.<sup>8</sup> Due to the mutation in the disproportionating enzyme, a plastidial  $\alpha$ -1,4-glucanotransferase, during the diurnal cycle the amount of leaf starch is higher in *dpe1* than in wild-type.<sup>8</sup> However, the amounts of starch synthesized and degraded are lower in *dpe1*. In contrast to the wild type at periods of starch degradation, a large accumulation of malto-oligosaccharides occurs in the mutant. Close similarity in the morphological phenotype

and the activities of enzymes involved in starch metabolism fit to the congruence in growth behavior of *dpe1* and the wild type.

### Final Remarks

Based on the deviation of the growth pattern of starch mutants in different day length from the wild type, the mutants can be sorted. *pgm* representing the most defected pattern would be followed by *sex1*, *dpe2*, *mex1* and finally *dpe1* which displayed kinetics most similar to the Col-0 wild type.<sup>9</sup> Interestingly, this order resembles the sequence of reactions in the starch metabolic pathway.<sup>9</sup> *pgm* seedlings are deficient in starch accumulation. *sex1* exhibits reduced rates of starch degradation suffering from a mutation in a downstream step in starch metabolism. Disproportionating enzyme (DPE1) has been shown to be required for conversion of maltotriose into larger maltooligosaccharides, which can be further attacked by amylolytic enzymes, and Glc, which is exported from the plastid.<sup>9</sup> *mex1* was shown to accumulate maltose and MEX1 encodes a novel transporter that is required for maltose export from the chloroplast.<sup>6</sup> DPE 2 has been shown to transfer



**Figure 3.** Diurnal extension profiles of Col-0 (n = 8), *pgm* (n = 9), *sex1* (n = 4), *mex1* (n = 6), *dpe1* (n = 11) and *dpe2* (n = 9). Root extension of 12-day-old seedlings grown in 16 h photoperiods, T = 21°C was monitored. Values represent averages of four consecutive days plus SE.

a glucosyl unit from maltose to glycogen in vitro, suggesting a novel pathway of carbohydrate metabolism in the cytosol of Arabidopsis leaves at night.<sup>8</sup> The mutation in this gene leads to accumulation of very high levels of maltose. However, the closer similarity in growth kinetics between *dpe1* and wild-type than *dpe2* might reflect the existence of two parallel routes for the export of starch degradation products from the chloroplast at night: the major flux of maltose by MEX1 and a minor flux of glucose derived from disproportionation of maltotriose by DPE1, by the glucose transporter.<sup>6,9</sup>

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