

Is HUB1 a hub for plant fitness?

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Abbreviations: HUB1, histone H2B monoubiquitination 1; FACT, facilitates chromatin transcription; OE-HUB1, overexpression of HUB1

Recently, we have identified circadian clock genes as targets of Histone Monoubiquitination1 (HUB1) in *Arabidopsis* from a transcriptome comparison between the *hub1-1* mutant and *HUB1* overexpression lines. HUB1 affected the amplitudes of the circadian clock gene expression profiles in the *hub1-1* mutant that coincided with reduced monoubiquitination of histone H2B at their coding regions. Here we showed that parameters for plant fitness are altered in HUB1 mutant and overexpression lines, suggesting that the histone H2B monoubiquitination status affects plant fitness.

The circadian clock is an important upstream control mechanism of the basic metabolism, and small deviations in its regulation are detrimental to plant fitness.^{1,2} The effects on plant fitness can be measured by parameters, such as photosynthetic capacity, growth and vigor, flowering time and seed set. Recently, we have shown that HUB1 positively regulates expression levels of circadian clock regulators and input and downstream effector genes through histone H2B monoubiquitination at their coding regions,³ and that it acts in concert with transcription regulators FACT and ELONGATOR.^{3,4} In the *hub1-1* mutant leaf growth was severely reduced by cell division and cell expansion defects,⁵ and an early flowering time phenotype of *hub1* alleles is well documented.^{6,7} The requirement of HUB1 for circadian clock genes to reach maximal transcript levels prompted us to analyze here the impact of HUB1 perturbations on plant fitness through chloroplasts, growth, vigor and seed yield parameters.

Germination vigor is a parameter that describes the synchronization of the germination rate and early growth performance that results in a competitive advantage in the field. We compared the germination vigor between vernalized low dormancy ecotype *Ler* and *hub1-1* and *OE-HUB1-7a*³ in the same background by following their germination time on MS media supplemented with 1% sucrose. The overexpression line was the first one to germinate on day 1 and 2, followed by wild-type *Ler* with the majority germinating on day 2, while for the *hub1-1* mutant the germination mainly occurred on day 2 and 3 (Fig. 1A).

To further investigate the role of HUB1 in growth, we analyzed how *HUB1* overexpression would enhance the juvenile leaf growth rate. *Arabidopsis* leaf growth occurs in three phases, namely proliferation, expansion and maturation, and these transitions coincide with 9, 12 and 18 d post germination, respectively.⁸

The leaf area, length and width of *OE-HUB1-7a*³ were analyzed every 24 or 48 h from day 7 to 25. The analysis of the leaf expansion rate showed an enhanced rate for overexpression lines (Fig. 1B) and the mature leaf areas were statistically significantly larger than those of the wild type ($p < 0.001$) (Table 1). To obtain similar insights into the underlying cellular responses in the overexpression lines as were shown for the mutant,⁵ the epidermal cell sizes were analyzed every 48 h between days 9 and 25 (Fig. 1C). Indeed, the calculated size of adaxial epidermal leaf cells in the *HUB1* overexpression line was significantly larger than that in the *Ler* control during all growth stages (Fig. 1C). Accordingly, in the *hub1-1* mutant, reduced cell areas were observed earlier despite increased endoreduplication levels that usually drive cell expansion.⁵

The leaf growth effects in *HUB1* misexpression lines could be influenced by developmental and cell cycle genes,⁵ as well as by reduced metabolic and photosynthetic activities as indicated by phenotypic and transcriptomic analysis.^{3,9} To verify the state of the photosynthetic apparatus in the lines, we analyzed the chloroplast ultrastructure in young leaves of wild type, and weak (rather normal leaves) and severe (small and pale-colored leaves) *hub1-1* mutants (Fig. 1D-F). Reduced thylakoid membrane and grana structures were observed in the *hub1-1* mutants in both short and long day conditions. In the most severe *hub1-1* mutant individuals, the thylakoid membranes were totally misoriented (Fig. 1F), while in the weak mutant individuals they remained poorly connected and poorly stacked (Fig. 1E). The number of plastoglobuli, which are spherical, lipid-rich bodies with a putative function in lipid and sugar metabolism and abiotic stress response, had increased in the stroma of the *hub1-1* mutants

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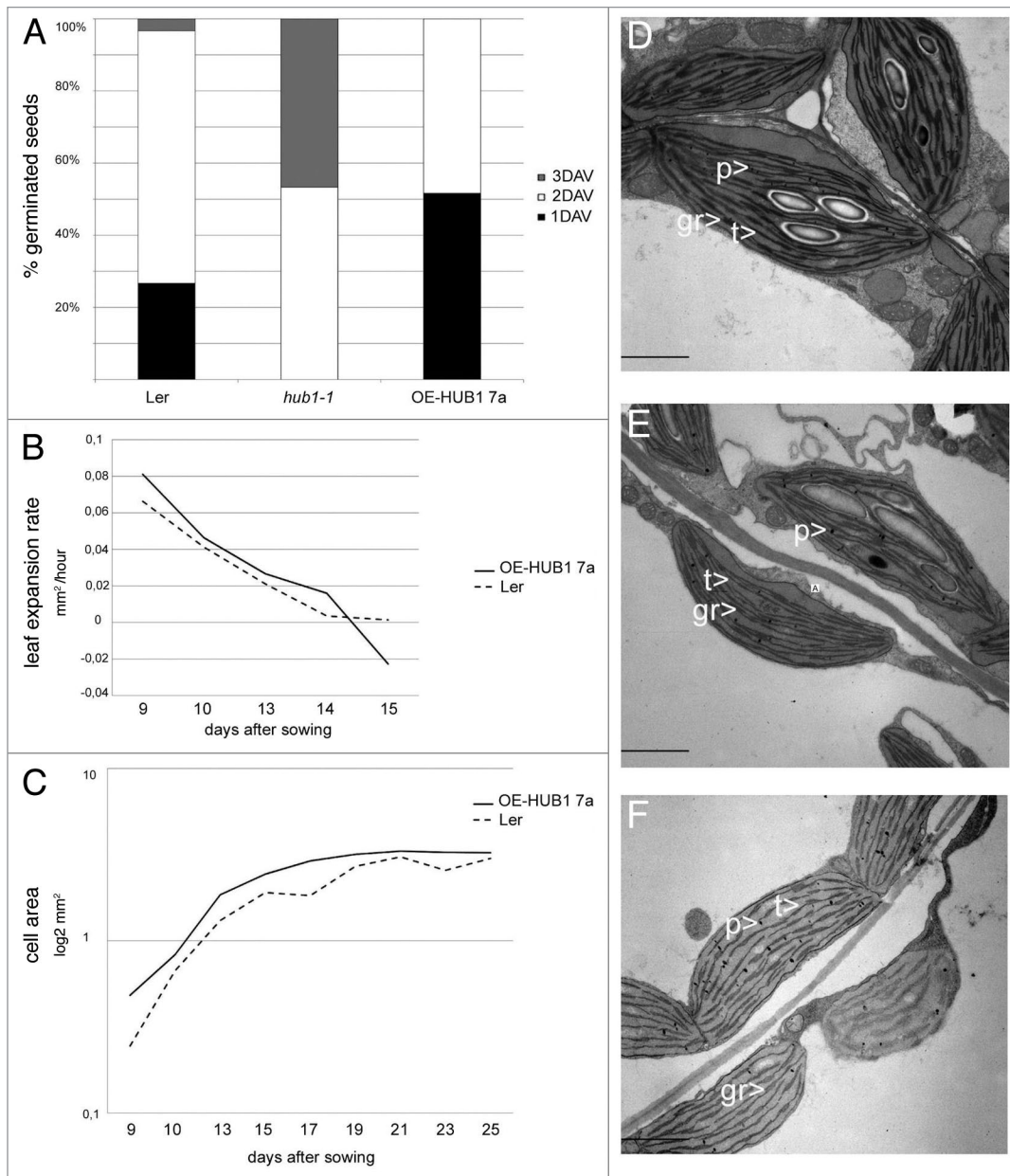


Figure 1. Fitness parameters in *HUB1* perturbed lines. (A) % of germinated seeds 1, 2 or 3 d after vernalization (DAV). (B) Leaf 1 and 2 expansion rate in *Ler* and *OE-HUB1-7a* line during 9 to 15 d after sowing. (C) Cell area increase in *Ler* and *OE-HUB1-7a* line between 9 to 25 d after sowing. (D) Chloroplast ultrastructure in *Ler* grown under short-day conditions, (E) *hub1-1* individual with weak phenotype and (F) *hub1-1* individual with severe phenotype. gr, grana; p, plastoglobuli; t, thylakoid membrane. Bar = 2 μ m (D, E and F).

(Fig. 1E and F). The overexpression line was visually comparable to the wild type (data not shown).

All *hub1* mutant alleles flower earlier than the wild type, predicting a reduced reproductive fitness. We analyzed the effects of the *HUB1* misexpression on silique and seed phenotypes such as silique number and shape, and size and number of seeds. The average number of siliques per plant was 115 siliques in the wild-type and overexpression lines, *OE-HUB1-7a*³ and *OE-HUB1-17*^{x3} and 55 siliques in the *hub1-1* mutant (Fig. 2A). The area and length of *hub1-1* siliques were severely reduced (Fig. 2B); whereas the silique thickness of the *HUB1*

overexpression lines had increased (Fig. 2C). In *hub1-1*, many seeds were missing due to 50% to 80% of unfertilized ovules (Fig. 2D). The total seed yield from dried siliques of the *hub1-1* plants was dramatically lower than that of the *Ler* wild type (Fig. 2E) and the seed size was reduced (Fig. 2F). While the silique and seed parameters were severely affected in the *hub1-1* mutant, no significant effects on the seed parameters were observed in the *HUB1* overexpression lines, probably because the *Arabidopsis* seed development does not depend on cell expansion. Taken together, important aspects of plant fitness were found to be affected by *HUB1* misexpression, namely

Table 1. Comparison of the mean area, length and width of juvenile leaf lamina (1st and 2nd) between *OE-HUB1-7a* and *Ler* (n = 15, mean ± SD)

Genotype	Area (cm)		Length (cm)		Width (cm)	
<i>OE-HUB1-7a</i>	27.333 ± 6.667	**	0.633 ± 0.097	*	0.547 ± 0.078	NS
<i>Ler</i>	20.033 ± 6.156		0.56 ± 0.097		0.505 ± 0.091	

* Significant (p < 0.05); **highly significant (p < 0.01); NS non-significant.

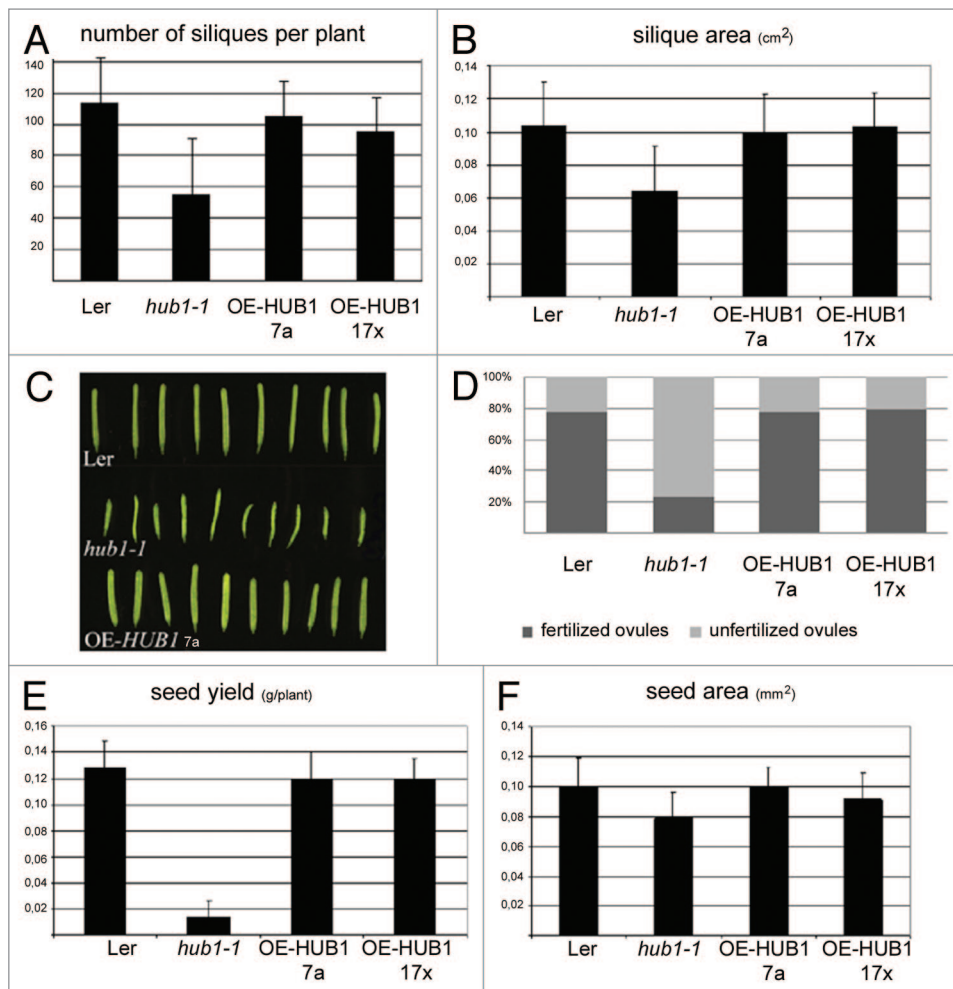


Figure 2. Mean silique parameters and seed yield ± SD of the *Ler*, *hub1-1* and *OE-HUB1-a* and *OE-HUB117* × lines with resp. 20- and 10-fold overexpression levels.³ (A) Number of siliques per plant. (B) Silique areas. (C) Siliques of *Ler*, *hub1-1* and *OE-HUB1 7a*. (D) Fertilized vs. unfertilized ovules in siliques. (E) Seed yield. (F) Seed areas.

HUB1 overexpression enhanced germination vigor and green biomass production, while the *hub1-1* mutation impaired seed yield.

The circadian clock has a well documented role in the temporal control of metabolic activities, and key metabolic processes are driven by genes that are expressed in a circadian manner.¹⁰ The phenotypes observed in *hub1-1*, such as reduced growth and seed yield, are characteristic for reduced fitness observed in mutants with defects in the circadian clock and/or photosynthetic capacity.^{1,2,11} On the other hand, the *HUB1* overexpression lines showed enhanced germination vigor as well as growth of leaves. *HUB1*-mediated activities in transcription elongation might account for reduced and enhanced germination vigor in the mutant and

overexpression lines because a new transcription activity during germination assists in the rapidity and uniformity of the process.¹² Taken together, our data indicate that *HUB1* might be a component of fitness and, through its role in the regulation of the transcription of several biological processes,^{3,5} could indeed be a hub for plant fitness.

Kinematic Leaf Growth Analysis

The kinematic leaf growth analysis was done as described.^{5,8} For statistical analysis, the data were compared pair wise and the leaf length, width and area and cell area measurements were subjected to t-tests.

Transmission Electron Microscopy of Chloroplasts

Leaves 1 and 2 from 3-week-old wild-type (*Ler*) and *hub1-1* seedlings, grown under short-day conditions, were cut into small pieces and immersed in a fixative solution of 2% paraformaldehyde and 2.5% glutaraldehyde, and postfixed in 1% OsO₄ with 1.5% K₃Fe(CN)₆ in 0.1 M Na-Cacodylate buffer, pH 7.2. Samples were dehydrated through a graded ethanol series, including a bulk staining with 2% uranyl acetate at the 50% ethanol step, followed by

embedding in Spurr's resin. Ultrathin sections were made with an ultramicrotome (Leica) EM UC6 and post-stained in EM AC20 (Leica) for 40 min in uranyl acetate at 20°C and for 10 min in lead stain at 20°C. Grids were viewed with a 1010 transmission electron microscope (JEOL; <http://www.jeol.com/>) operating at 80 kV.

Silique and Seed Analyses

The silique numbers and shapes were recorded by scanning and photography. For silique analysis, the green and mature siliques were harvested and the length and width parameters were

measured using the ImageJ software program. The number of unfertilized ovules was determined using microscopy. Seeds were harvested from the dried siliques and seed weight was determined by counting 200 seeds for weighing. The ImageJ software program was used to determine the area of the seeds. These yield parameters were subjected to t-tests.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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