Induced mutations in circadian clock regulator *Mat-a* facilitated short-season adaptation and range extension in cultivated barley

Shakhira Zakhrabekova[a], Simon P. Gough[a], Ilka Braumann[a], André H. Müller[a], Joakim Lundqvist[a], Katharina Ahmann[a], Christoph Dockter[a], Izabela Matyszczak[a], Marzena Kurowska[b], Arnis Druka[c], Robbie Waugh[c], Andreas Graner[d], Nils Stein[d], Burkhard Steuernagel[d], Udda Lundqvist[e], and Mats Hansson[a,1]

[a]Carlsberg Laboratory, 1799 Copenhagen V, Denmark; [b]Department of Genetics, Faculty of Biology and Environment Protection, University of Silesia, 40-032, Katowice, Poland; [c]James Hutton Institute, Invergowrie, Dundee DD2 5DA, United Kingdom; [d]Leibniz Institute of Plant Genetics and Crop Plant Research, 06466 Gatersleben, Germany; and [e]Nordic Genetic Resource Center, 230 53 Alnarp, Sweden

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Time to flowering has an important impact on yield and has been a key trait in the domestication of crop plants and the spread of agriculture. In 1961, the cultivar Mari (*mat-a.8*) was the very first induced early barley (*Hordeum vulgare* L.) mutant to be released into commercial production. Mari extended the range of two-row spring barley cultivation as a result of its photoperiod insensitivity. Since its release, Mari or its derivatives have been used extensively across the world to facilitate short-season adaptation and further geographic range extension. By exploiting an extended historical collection of early-flowering mutants of barley, we identified *Praematurum-a* (*Mat-a*), the gene responsible for this key adaptive phenotype, as a homolog of the *Arabidopsis thaliana* circadian clock regulator *Early Flowering 3* (*Elf3*). We characterized 87 induced *mat-a* mutant lines and identified >20 different *mat-a* alleles that had clear mutations leading to a defective putative ELF3 protein. Expression analysis of *HvElf3* and *Gigantea* in mutant and wild-type plants demonstrated that *mat-a* mutations disturb the flowering pathway, leading to the early phenotype. Alleles of *Mat-a* therefore have important and demonstrated breeding value in barley but probably also in many other day-length-sensitive crop plants, where they may tune adaptation to different geographic regions and climatic conditions, a critical issue in times of global warming.

earliness | food security | timing of flowering | molecular breeding | synteny

In all plant species, time to flowering is regulated by seasonal cues that include temperature and photoperiod, and it is tightly connected to the requirement for vernalization and the circadian clock (1–3). As early as the 1930s, Russian plant breeders identified the importance of early flowering as a breeding target (4), recognizing that, although earliness may limit high productivity in highly fertile areas because of inefficient use of the entire growing season, it allows marginal environments with a short growing season to enter into cultivation. These generally low-yielding areas constitute the majority of the land used for agriculture and therefore offer the greatest opportunity to substantially increase worldwide food production (5). Early cultivars are important for farmers, allowing them to manage the harvest by growing crops that mature at different times and establish an effective crop rotation.

Induced mutagenesis of barley (*Hordeum vulgare* L.) was reported in 1928 (6), only a year after Muller reported that ionizing irradiation could increase the mutation frequency in *Drosophila* (7). This finding promoted barley as a model plant and one of the first crops in which mutations were induced for applied purposes (8). In the 1940s, it was established that time to flowering in barley could easily be modified by physical or chemical mutagenesis (8). Although late maturity occurred more frequently in mutant populations, screening for early (or *praematurum*) mutants was much easier (9) and potentially more beneficial. Between 1941 and 1988, >1,200 early-flowering barley mutants were isolated at the Swedish Seed Association (later Svalöf AB), Sweden. Allelism tests using 195 mutants distinguished nine complementation groups (Table 1). The *praematurum-a* (*mat-a*) mutant group was the largest and covered 85 different alleles. It was also found that *mat-a* mutants were allelic to a mutant with erect growth, *erectoides-o.16* (*ert-o.16*) (10) and a series of *early maturity 8* (*eam8*) mutants from Japan (11, 12). Like the *mat-a* lines, *ert-o.16*, *eam8.q*, *eam8.r*, *eam8.s*, *eam8.u*, and *eam8.v* were induced mutants, whereas *eam8.k* occurred naturally in the cultivars Kinai 5 and Kagoshima Gold, and *eam8*.w occurred naturally in Early Russian (Fig. 1) (13).

Mutant line *mat-a.8*, under the name Mari, was the very first early barley mutant released as a commercial cultivar (in 1961) (14). It was derived as a one-step X-ray mutant 10 y earlier. In field trials in Sweden and under long-day conditions in phytotron experiments, it flowered 8–10 d earlier than the mother cultivar Bonus (14). In moderate day length, heading was found to be as much as 3 wk earlier (14), and field studies in Japan showed that heading of *mat-a.8* in a Bonus or a Tochigi Golden genetic background was at least 17 d earlier than for the parental cultivars (15). The remarkable earliness, combined with resistance to lodging, of Mari allowed two-row barley to break through a climate barrier, thereby extending barley cultivation into Northern Scandinavia and Iceland. However, due to its photoperiod insensitivity, Mari can also be grown near the Equator as a day-length-neutral plant. Its daughter cultivar Mona is high yielding even under tropical conditions at altitudes of ~350 m in Colombia (16). Consequently, breeders servicing a wide geographic range have frequently used Mari or its derivatives in their programs (Table S1).

Environmental stability is now prioritized by global organizations that seek to maintain crop yields under increasingly variable climatic conditions, reduced inputs, and expansion into marginal lands. Consequently, the *mat-a.8* mutant allele of Mari has once again emerged as a potentially valuable breeding trait. In this study, which was enabled by research conducted on flowering

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. JN180296 (Bonus *Mat-a*), JN180297 (Kinai 5; *eam8.k*), and AC244853.1 (BAC clone HVVMRXALLhA0624F14)].

[1]To whom correspondence should be addressed. E-mail: mats.hansson@carlsberglab.dk.

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Table 1. Early-maturity barley mutants and their distribution to nine assigned early-maturity (*Mat*) loci

Locus *Mat*	*-a*	*-b*	*-c*	*-d*	*-e*	*-f*	*-g*	*-h*	*-i*	l.n.d.*
Frequency of alleles	85	49	31	2	9	7	4	2	6	1,013
Days of heading earlier than wild type	9	7	7	3	6	3	3	6	6	

Shown are the number of early-maturity barley mutants isolated at the Swedish Seed Association (later Svalöf AB), Sweden, 1941–1988 and their distribution to nine assigned early-maturity (*Mat*) loci.
*l.n.d., locus not determined.

in *Arabidopsis thaliana* and by a collection of early-flowering barley mutants that were generated before the era of molecular biology, we identified and characterized the *Mat-a* gene and >20 *mat-a* mutant alleles at the DNA level.

Results

Fine Mapping of *Mat-a* and Identification of *Early Flowering 3* as a Candidate Gene. The natural early allele *eam8.k* of *Mat-a* has been mapped to the end of the long arm of barley chromosome 1H, 11.4 cM distal to the *third outer glume 1* (*trd1*) locus, 20.9 cM distal to the *Black lemma and pericarp 1* (*Blp1*) locus (12), and proximal to the RFLP marker ABG055 (GrainGenes; http://wheat.pw.usda.gov/GG2/). In wheat, a similar flowering phenotype, called *Earliness per se* (*Eps*), has been mapped as *Eps-A^{m}1* to a similar region between wheat chromosome 1A markers *Smp* and *VatpC* (Fig. 2*A*) (17). Sequence comparison of *VatpC* and ABG055 revealed 98% identity over a 66-bp region (*e* value = 10^{24}). To assess gene content at the *Mat-a* locus, we exploited the conserved synteny that exists between physical and genetic maps of sequenced and unsequenced grasses. In *Sorghum bicolor*, *VatpC* and *Smp* are the genes Sb09g030620 and Sb09g030810, respectively (18). The two genes flank a 267-kb region containing only 25 gene models (Fig. 2*A*). A *S. bicolor* homolog (Sb09g030700) of *A. thaliana Early Flowering 3* (*Elf3*; At2g25930), located 76 kb (12 gene models) proximal to Sb09g030810, was identified as a candidate for a *Mat-a* homolog. Mutant alleles of *A. thaliana Elf3* cause photoperiod-insensitive early flowering (19), similar to the phenotypes caused by mutations at the barley *Mat-a* and wheat *Eps-A^{m}1* loci. Of the 24 remaining genes, there were no other obvious candidates.

The likelihood of the barley ortholog of *A. thaliana Elf3* being a candidate gene for *Mat-a* was further strengthened by the location of an *Elf3*-like gene in a segment of *Brachypodium distachyon*

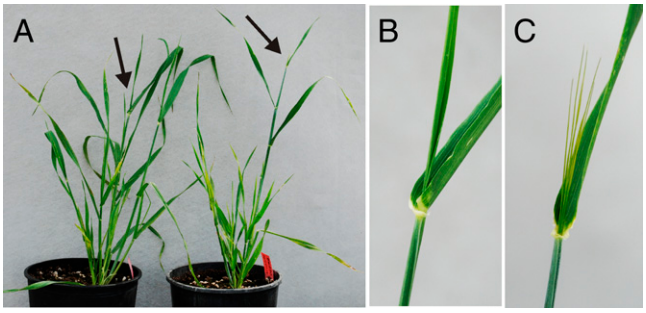

Fig. 1. Phenotyping of the heading date trait in barley based on appearance of the awns protruding from the flag leaf. (*A*) One-month-old barley plants carrying the wild-type *Mat-a* allele (*Left*) and the recessive *mat-a* allele (*Right*). Arrows indicate regions of the main stems around the flag leaf of these two genotypes, which are shown magnified in *B* and *C*. (*B*) The cultivar Bowman. (*C*) The Bowman backcross-derived line BW290 that carries the *eam8.w* allele on a 1.5-cM introgressed segment from Early Russian.

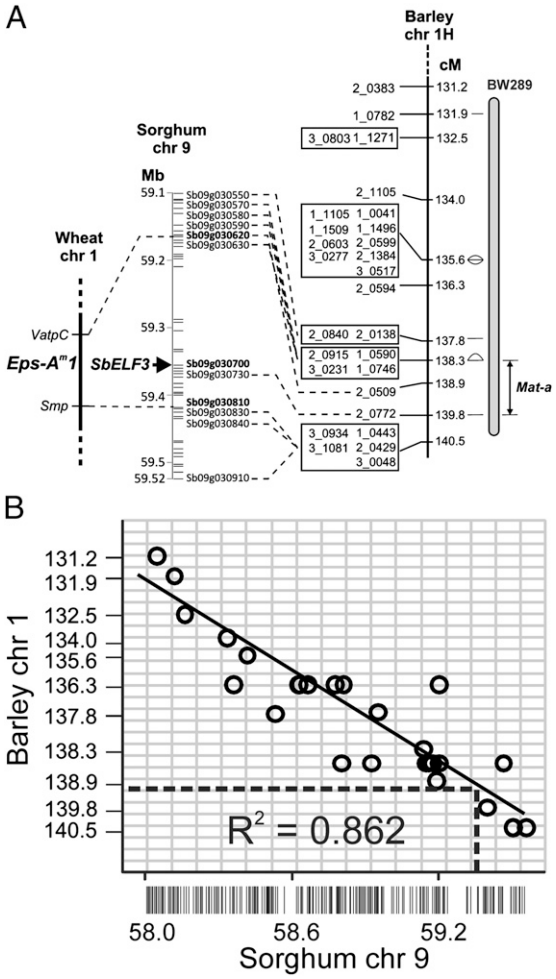

Fig. 2. Mapping and synteny at the *Mat-a* locus. (*A*) Inference of *HvElf3* as a candidate gene for *Mat-a* using genetic mapping data from wheat and barley and the *Sorghum* physical map. The wheat map shows a fragment of the *Eps-A^{m}1* locus. The *Sorghum* physical map shows only those gene model names that have barley homologs mapped on the telomeric region of barley chromosome 1H (shown as connecting lines to barley consensus map loci). Markers colocating on the barley consensus map (21) are boxed. The mapping of the backcross-derived near-isogenic line BW289, carrying the *Mat-a* allele *eam8.k*, is indicated. (*B*) Barley-*Sorghum* synteny model-based prediction of the genetic position of the *HvElf3* gene. Positions of barley genetic markers were regressed against physical map positions of homologous sequences in *Sorghum*. The scatter plot shows only those homologous pairs that map in the syntenic regions. R^2 is the coefficient of determination for the linear regression function. Physical distances (in kb) in *Sorghum* are shown on the *x* axis. Genetic map distances (21) on barley chromosome 1H are given on the *y* axis.

chromosome *Bd*02 (Bradi2g14290), which shows conserved synteny with the end of the long arm of barley chromosome 1H (20). In rice (*Oryza sativa*), *Elf3*-like genes are found on chromosomes *Os*01 (LOC_Os01g38530) and *Os*06 (LOC_Os06g05060), and not on chromosome *Os*05, which is the chromosome that shows the best conservation of synteny with the barley chromosome 1H (20). Using a barley–*Sorghum* synteny model (Fig. 2*B*), we predicted the position of the barley homolog of *Elf3* (*HvElf3*) to be between 138.9 and 139.8 cM on the barley consensus map (21). This genetic interval is in accordance with the recently reported mapping of the backcross-derived line BW289, carrying the *Mat-a* allele *eam8.k*, to the 130.4- to 140.5-cM region of barley chromosome 1H (Fig. 2*A*) (22).

Linkage of *mat-a* Phenotype and DNA Variation of the *HvElf3* Gene. Genetic linkage of the *mat-a* phenotype and *HvElf3* was further

supported by our finding of exact cosegregation of the earliness phenotype with a mutant allele of *HvElf3* in a population of F_2 recombinant lines. The near-isogenic line BW289, carrying the *eam8.k* mutation, was crossed with the cultivars Bowman and Barke. For each cross, 803 and 718 F_2 seeds were planted, and the day of heading was determined for each plant (Fig. S1*A*). All BW289 mutant plants headed at least 6 d earlier than the Bowman control plants and 17 d earlier than the Barke control plants (Fig. 3*A*). The *eam8.k* mutation is very distinctive (Fig. 4), allowing the design of primers for specific amplification of the mutant allele or the wild-type allele in the same PCR (Fig. S1 *B* and *C*). In both crosses, an early-heading group of recombinant lines was homozygous for the *eam8.k* mutation (Fig. 3 *B* and *C*), thus strengthening the hypothesis that *Mat-a* is *HvElf3*. The segregation in the cross to Bowman was 199, 394, and 210 for homozygous mutants, heterozygous plants, and wild-type plants, respectively (for 1:2:1 ratio, χ^2 test = 0.58, $P = 0.75$) (Fig. 3*B*). The mean heading dates of the mutant, heterozygous, and wild-type populations were April 25, May 4, and May 5, respectively. The mean heading date of the mutant population was significantly different from that of the heterozygous and wild-type populations (*t* test, $P < 0.001$). In the cross to Barke, the segregation was 185, 339, and 194 for homozygous mutants, heterozygous plants, and wild-type plants, respectively (for 1:2:1 ratio, χ^2 test = 2.5, $P = 0.29$) (Fig. 3*C*). The mean heading dates were April 25, May 5, and May 7, respectively. All three mean heading dates were significantly different from each other ($P < 0.001$). This result suggests that *eam8.k* exhibits partial dominance or epistatic interactions, which can be seen in a mapping population with a late-flowering cultivar like Barke.

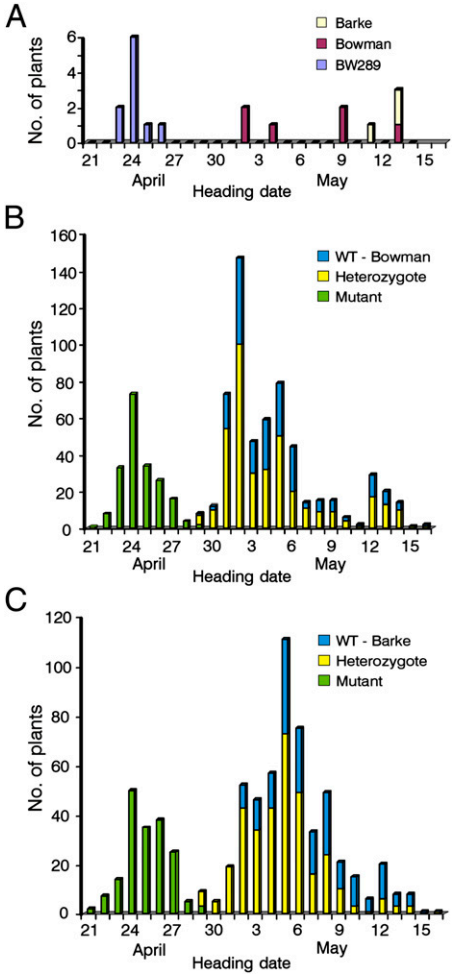

Fig. 3. Correlation between phenotype and genotype in control plants (*A*) and two F_2 mapping populations (*B* and *C*). (*A*) The day of heading for BW289 carrying the *eam8.k* mutation in comparison with the barley cultivars Bowman and Barke. (*B* and *C*) F_2 mapping population from the cross BW289 × Bowman (*B*) and BW289 × Barke (*C*).

Sequence Analysis of *mat-a* Mutants. The principal proof of identity of *HvElf3* as *Mat-a* came from comparative sequence analysis of *HvElf3* in 87 putatively independent *mat-a* mutant alleles and corresponding parental cultivars (Fig. 4, Fig. S2, and Table S2). The *mat-a.8* mutation associated with Mari is a 4-bp deletion, which causes a translational frame shift leading to a premature stop codon (Table S2). Truncated proteins were also the result of most other identified mutations due to deletions (48 cases in total), nonsense mutations (9 cases), or splice-site mutations (6 cases). Surprisingly, the Mari mutation was found in >40 mutant lines. At the moment, we cannot explain this finding, although we are aware that certain DNA structures can provide hotspots for mutations (23, 24). The original field books show that they were isolated, after various treatments, as independent lines between 1951 and 1983 (Table S2).

Nonsynonymous point mutations were only found in *mat-a.45* and *mat-a.1067*, leading to the amino acid substitutions N44I and R43H. Both N44 and R43 are fully conserved residues, located in the most conserved region of the ELF3 polypeptide (Fig. S3). The functional significance of conserved regions in ELF3 is yet to be determined. The *eam8.k* mutation was complex; an insertion of AGCTGCATGGCG at position 1,189 is followed by a deletion of 2,466 bp at position 1,189–3,656, directly followed by an inversion of bps 3,657–4,697, immediately followed by an insertion of CCGTCTCCTCCGCCTCCGCACCGTT and a deletion of 147 bp at position 4,698–4,845 (Fig. 4). In mutants *ert-o.16* and *mat-a.12*, no part of *HvElf3* could be amplified by PCR, whereas only the most 5′ and 3′ parts could be amplified in *mat-a.407*. These results suggest that the three mutations involve larger rearrangements, possibly deletions. Initially, no mutations were detected in 19 lines. Repeated phenotyping and genotyping showed that the majority of them (13 lines) were erroneous because they did not show an early-flowering phenotype (Table S2). The remaining six lines represented heterogeneous stocks consisting of mutant and wild-type alleles.

Thus, throughout this work, our DNA sequence analysis of *mat-a* lines has identified >20 different mutations in the barley *Elf3* gene that each lead to a clear nonfunctional putative translation product. This finding firmly establishes mutations in *Elf3* as causal for the *mat-a* phenotype.

Expression Analysis of *Gigantea* in a *mat-a* Genetic Background. The major components of the regulatory flowering pathways are conserved among distantly related plants. Among the major floral inducers in *A. thaliana* are CONSTANS, GIGANTEA (AtGI), and FLOWERING LOCUS T (1). In view of the present study, AtGI is of special interest because studies with yeast two-hybrid systems have demonstrated the physical interaction between AtGI, AtELF3, and an E3 ubiquitin–ligase, AtCOP1, leading to turnover of AtGI (19). We analyzed whether the expression of barley *Gigantea* is affected in a *mat-a* genetic background, which could help us to understand the early-flowering phenotype in these plants. The expression level of barley *Gigantea* was studied by real-time quantitative PCR using RNA samples from leaves of the near-isogenic *mat-a* mutant line BW289 (*eamk.8*) and Bowman. The plants were grown in 12/12-h light/dark cycles and were harvested every 3 h over a 24-h period at the third true-leaf stage. In Bowman, *Gigantea* had a pronounced expression during the latter half of the light period, whereas no expression was detected during the dark period (Fig. 5*A*). This expression profile was very

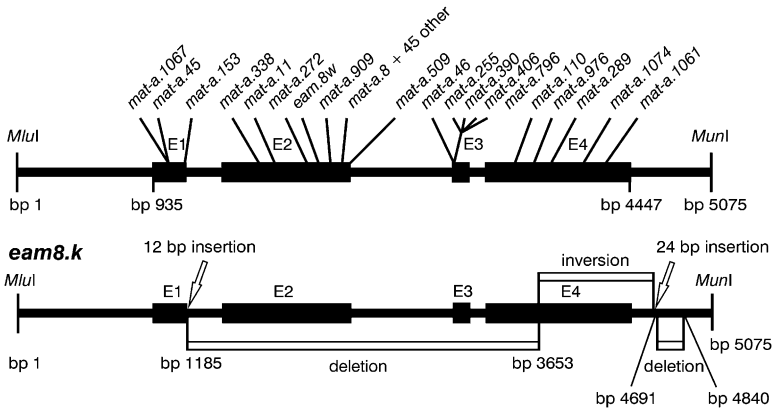

Fig. 4. Structure of the barley *Mat-a* gene and the positions of the detected mutations. A 5,075-bp MluI–MunI DNA fragment from BAC clone HVVMRXALLhA0624F14 (34) was used as template for resequencing 87 mutant alleles. E1–E4 designate exons. The complex *eam8.k* allele with two deletions, one inversion, and two small insertions is shown separately in *Lower*.

similar to those found earlier in barley and *A. thaliana* grown under 8/16- and 16/8-h light/dark cycles (19, 25). The level of *Gigantea* transcript in BW289 was similar to the peak value of Bowman at several time points and did not fluctuate to a similar extent. *Gigantea* expression was also pronounced during the dark period (Fig. 5*A*). Pronounced expression of flowering inducing genes like *Gigantea* might explain the early flowering of *mat-a* mutants.

HvElf3 in the Bowman wild type showed the lowest expression during the beginning of the light period and peaked at the end of the dark phase (Fig. 5*B*), similar to what has been found in *A. thaliana* (19). Interestingly, the peak expression value of *HvElf3* was 15 times higher in BW289 compared with Bowman (Fig. 5*B*). The abundance of *HvElf3* in the mutant is most likely due to lack of functional HvELF3 protein, because *AtElf3* has been shown to be autoregulated by its own gene product (26, 27).

Discussion

Global food production must increase substantially to meet the needs of the rapidly growing human population (28, 29). At the same time, warming of the global climate system threatens the productivity of existing agricultural land. However, it can also be predicted that other geographic areas will become more amenable to cultivation. The implications of these changes are that breeding of early cultivars will be essential to meet shorter growing seasons in many existing agricultural locations and that crop plants requiring adaptation to local light regimes and temperatures will be introduced into new geographic areas. The mutation characterizing the barley cultivar Mari drastically alters earliness, which is a useful trait when adapting cultivars to agricultural land with a short growing season. At the same time, the photoperiod insensitivity of Mari gives it the remarkable ability to give high yield close to the polar circle in Northern Scandinavia and also near the Equator—for example, under tropical conditions in Colombia (16). Over the years, many breeders in various geographic regions have used Mari as a parent to produce new cultivars (Table S1). Breeding populations containing Mari have been tested at several Mexican stations, on Cyprus, in Spain, Tunisia, Turkey, Jordan, Iraq, Iran, South Korea, and Peru. Apart from earliness, they maintained the ability to give high yield at all locations (9). The present identification of >20 different *mat-a* mutant alleles will significantly expedite the use of *mat-a* in plant breeding, especially because selection of earliness by phenotyping is labor-intensive and will be much more easily done directly using known *mat-a* mutations as genetic markers.

The molecular mechanism of the *Mat-a* gene product is yet to be determined, but it is likely to be identical in all plants. The early flowering and photoperiod insensitivity of *mat-a* mutants are also striking in *A. thaliana elf3* mutants under both long-day (16-h light/8-h dark) and short-day (8-h light/16-h dark) conditions. *A. thaliana elf3-8* plants bolted already when seven or eight rosette leaves had appeared. In contrast, the wild-type controls bolted after the appearance of 11.6 leaves under long-day conditions and 46.1 leaves under short-day conditions (19). A T-DNA insertion in one of the two *Elf3*-like genes of rice also resulted in photoperiod insensitivity but with a late phenotype, which should be seen in the light that rice is categorized as a short-day plant in contrast to barley and *A. thaliana*, which are long-day plants (30). Networks underlying early, photoperiod-insensitive flowering in *A. thaliana elf3* mutants include flowering time proteins such as

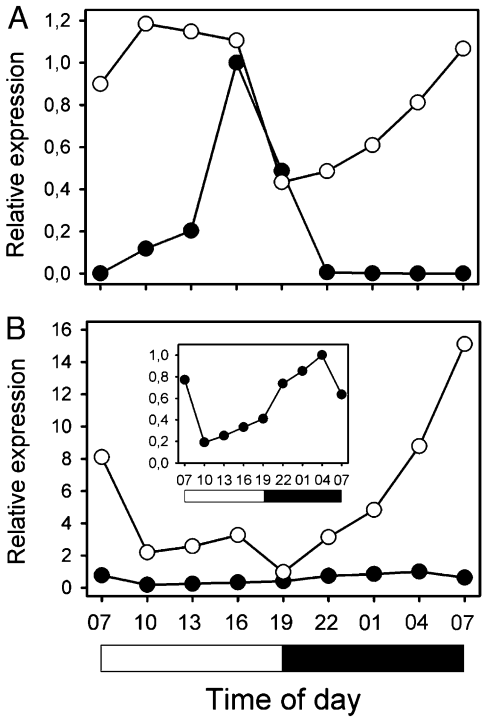

Fig. 5. Expression profiles of *Gigantea* (*A*) and *Elf3* (*B*) in the near-isogenic *mat-a* mutant line BW289 (open circles) and Bowman (filled circles). Plants were harvested at 3-h intervals at the three-leaf stage. The open and filled bars at the bottom indicate the light and dark periods, respectively. (*B Inset*) The *Elf3* expression in Bowman in more detail.

CONSTANS and GIGANTEA (2, 26, 31) and regulation by COP1 and miRNA172 (19, 32). COP1 is an E3 ubiquitin–ligase that mediates ubiquitination and targeted degradation of light-signal regulators (33). By allowing COP1 and GI interaction in a temporal COP1–ELF3–GI complex, ELF3 likely acts as a substrate adaptor for COP1 action on GI, which promotes ubiquitination and degradation of GI (19). Accordingly to this idea, *AtElf3* overexpression causes late flowering and nonfunctional mutations in *AtElf3* (19), and *HvElf3* show an early phenotype.

The work reported here is an example of how studies on a basic concept of plant physiology in *A. thaliana* can facilitate applied research in a crop plant. However, it also demonstrates the great potential of legacy collections of plant mutants and the importance of keeping them cataloged and alive, because multiple independent mutant alleles, when available, are the most effective approach to validating a candidate gene. Natural and induced mutations are also valuable assets for plant breeding, a topic of special importance in countries in which genetically modified plants are not publicly accepted. The *Mat-a* locus is an important breeding target in barley, but probably also in many other day-length-sensitive crop plants. Thus, alleles of *Mat-a* can be used by public and private plant breeders where it may be important in addressing problems of global food production and adapting crop plants to different geographic regions and climatic conditions, critical issues in times of global warming, and food security.

Materials and Methods

Plant Materials and Growth Conditions. Barley (*H. vulgare* L.) cultivars Bowman, Barke, Bonus, Foma, Kristina, and Maja; mutant lines Kinai 5 (carrying the *eam8.k* mutation), *mat-a* (84 different mutants), *ert-o.16*, BW289 (a Bowman near-isogenic line carrying the *eam8.k* mutation), and BW290 (a Bowman near-isogenic line carrying the *eam8.w* mutation); and F_2 mapping populations derived from crosses BW289 × Barke, and BW289 × Bowman were grown in a greenhouse at 18 °C under a cycle of 16-h light/8-h dark. Light intensity was set to a photon flux of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The F_2 mapping populations were planted on March 20, 2009. Phenotyping concerning date of heading was started April 19. Statistical analyses (*t* test) of differences in heading date were based on number of days between planting and heading for the different phenotypic groups. Mutant seeds are available from NordGen (Alnarp, Sweden; www.nordgen.org).

Cloning and DNA Sequence Analysis. By using the *A. thaliana* ELF3 polypeptide (At2g25930) as query, partial barley *Elf3* (*HvElf3*) DNA sequences were found in EST database (http://www.ncbi.nlm.nih.gov/nucest), and a consensus sequence of 1,643 bp could be assembled (72% of the coding gene). The 1,643-bp *HvElf3* DNA sequence was compared with randomly selected and sequenced BAC clones (http://webblast.ipk-gatersleben.de/barley/viroblast.php). *HvElf3* is present on BAC clone HVVMRXALLhA0624F14 (34). A 5,075-bp MluI–MunI DNA region was selected for sequence analysis from genomic DNA of barley mutants. DNA isolation and sequencing was performed by LGC Genomics. GrainGenes (http://wheat.pw.usda.gov/GG2/ggdb.shtml) and HarvEST (http://harvest.ucr.edu/) were used for map viewing and sequence searching. The Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/), Phytozome (http://www.phytozome.net/), and Gramene (http://www.gramene.org/) were used for plant comparative genomic analyses.

The barley Bonus *Mat-a* and the Kinai 5 (*eam8.k*) nucleotide sequences, as well as the draft assembly of BAC clone HVVMRXALLhA0624F14, have been deposited at the GenBank database under accession nos. JN180296, JN180297, and AC244853.1, respectively.

Genotyping of Mapping Population. Barley leaves (10 cm) were sampled and dried for a minimum of 3 d in plastic bags containing 50 mL of silica gel (catalog no. 1327-36-2; Azelis). Genomic DNA was isolated from dried leaves by using the REDExtract-*N*-Amp Plant PCR Kit (Sigma). Dried leaf pieces of 0.5 cm were transferred to 96-well plates with well volumes of 200 μL. Then, 40 μL of extraction solution was added to the plant samples and incubated at 95 °C for 10 min, which was followed by addition of 40 μL of dilution solution and vortexing. PCR amplifications were performed according to the manufacturer's protocol by using REDExtract-*N*-Amp PCR ReadyMix, which contains JumpStart Taq antibody for specific hot-start amplification. Primers specific for the wild-type allele or the mutant *eam8.k* allele were used (Fig. S1*B*). Primers F1 (GTCTGATTGGATTGGAAAACCTAG) and R1 (TGGGAAATTTTGCAGTTGG) allowed specific amplification of the wild-type allele, because primers F1 and R1 are constructed in a region deleted in *eam8.k*. Primers F2 (ACAAGCTGCATGGCGATAC) and R2 (TTTCGGTCGATCCAGATG) exploited the inversion of *eam8.k*, which made them specific to the mutant allele. All PCRs were mixed in 96-well plates by a Freedom EVO 200 robot (Tecan Group). Gel electrophoresis was performed on precast agarose gels (2% E-Gel 96 with SYBR Safe; Invitrogen). Statistical analysis of the segregation in the mapping populations was performed with a Pearson's χ^2 test.

Expression Analysis. BW289 and Bowman plants were grown under a constant temperature regime of 20 ± 2 °C. Light was given between 7:00 AM and 7:00 PM at an intensity of 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At the three-leaf stage, leaf material was harvested from five independent plants at the indicated time points. In the dark period, dim green light was used during the harvest procedure. Leaves for each time point were cut into 1-cm pieces and pooled. Portions of 100 mg were quick-frozen in liquid nitrogen and stored at −80 °C until further processing. Total RNA was isolated from pooled barley leaf material with the RNeasy Plant Mini Kit including the RNase-free DNase Set (Qiagen) according to the manufacturer's recommendations. In addition, RNA was treated afterward with DNaseI (Fermentas) according to the manufacturer's instructions. RNA integrity was checked on a 2% (wt/vol) agarose gel containing guanidinium thiocyanate and ethidium bromide. The concentration and purity of the extracted RNA was additionally measured spectrophotometrically (Nanodrop 1000; Thermo Scientific). The absence of residual genomic DNA was assured by a standard PCR using AmpliTaq Gold DNA Polymerase (Ultra Pure; Applied Biosystems), gene-specific primers for *HvElf3* (primer sequences as described below), and DNaseI-treated RNA as template. For real-time quantitative PCR, Applied Biosystems 7500 Real-Time PCR System was used in conjunction with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems) according to the manufacturer's instructions. Cycling conditions were 30 min at 48 °C, 10 min at 95 °C, 50 cycles of 15 s at 95 °C, and 1 min at 60 °C. This process was followed by a melting-curve program (60–95 °C). Fluorescence data were acquired at the 60 °C step and during the melting-curve program. Three replicate reactions were performed for each RNA–primer combination in the same run. As reference gene, *Gapdh* was used with published primers (35). Forward and reverse primer sequences were 5′GTGAGGCTGGTGCTGATTACG and 5′TGGTGCAGCTAGCATTTGAGAC for *HvGapdh*, 5′TTGTTCTGCAGGCTGAGAAG and 5′CAAGCATCCCATCTGTAGCA for *HvGigantea*, and 5′CCTACCGACAACAAGCAGAA and 5′CATGAATTCCCCAGCTGTAG for *HvElf3*, respectively. The binding sites of the F-Elf3 and R-Elf3 primers are indicated in Fig. S1*B*. Primer concentrations for forward and reverse primers in the reactions were 200/200 nM for *HvGapdh* and *HvGigantea* and 200/450 nM for *HvElf3*. For transcript-level comparison, RNA material from the 16 and 04 o'clock Bowman samples was included as a standard in the quantitative PCR of BW289. Analysis of relative transcription levels of the respective sequences was performed by using 7500 Software V2.0.5 (Applied Biosystems, Life Technologies).

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