

GER1, a GDSL Motif-Encoding Gene from Rice is a Novel Early Light- and Jasmonate-Induced Gene

M. Riemann^{1,2}, C. Gutjahr³, A. Korte³, M. Riemann³, B. Danger³, T. Muramatsu⁴, U. Bayer³, F. Waller⁵, M. Furuya⁴, and P. Nick¹

¹ Botanisches Institut 1, Technische Universität Karlsruhe, Kaiserstraße 2, 76128 Karlsruhe, Germany

² Department of Plant Physiology, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

³ Biologisches Institut II, Albert-Ludwigs-Universität Freiburg, Schänzlestraße 1, 79104 Freiburg, Germany

⁴ Hitachi Advanced Research Laboratory, Hatoyama, Saitama 350-0395, Japan

⁵ Institute of Phytopathology and Applied Zoology, Justus-Liebig-Universität Gießen, Heinrich-Buff-Ring 26–32, 35392 Gießen, Germany

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Abstract: The reaction of the rice mutant *hebiba* differs from that of wild-type rice in that the mutant responds inversely to red light and is defective in the light-triggered biosynthesis of jasmonic acid (JA). Using the wild type and the *hebiba* mutant of rice in a differential display screen, we attempted to identify genes that act in or near the convergence point of light and JA signalling. We isolated specifically regulated DNA fragments from approximately 10 000 displayed bands, and identified a new early light- and JA-induced gene. This gene encodes an enzyme containing a GDSL motif, showing 38% identity at the amino acid level to lipase Arab-1 in *Arabidopsis thaliana*. The GDSL CONTAINING ENZYME RICE 1 gene (*GER1*) is rapidly induced by both red (R) and far-red (FR) light and by JA. The results are discussed with respect to a possible role for *GER1* as a negative regulator of coleoptile elongation in the context of recent findings on the impact of JA on light signalling.

Key words: Jasmonate, GDSL motif, phytochrome, rice (*Oryza sativa* L. *japonica*).

Abbreviations:

JA: jasmonic acid
 OPDA: 12-oxo-phytodienoic acid (OPDA)
 R: red light (660 nm)
 FR: far-red light (730 nm)
 FDD: fluorescent differential display
 GFP: green fluorescent protein
 ER: endoplasmatic reticulum

Introduction

Plants respond rapidly to physical and chemical environmental changes. This is of special significance for the perception and transduction of the light signal, since the availability of light is crucial for plant life. Plants perceive environmental light information using different photoreceptor pigments such as phytochromes (Nagy and Schäfer, 2003), cryptochromes

(Lin and Shailitin, 2003), and phototropins (Briggs and Christie, 2002); in specific photoperiodic light signalling, blue light is presumed to be perceived through FKF1 (Imaizumi et al., 2003). The topic of light signal transduction in plant cells has therefore attracted considerable research (Quail, 2002). It is well known that phytohormones regulate the growth of plants, and therefore interactions between light and hormone signalling pathways occur frequently. As a consequence, the role of plant hormones in diverse light-mediated responses in plants has been analyzed in great detail, down to the molecular level (Halliday and Fankhauser, 2003). For the major growth-stimulating hormone, auxin, several auxin signalling genes, such as *Aux/IAA*, small auxin up-regulated RNAs (*SAUR*), and soybean *GH3* ([isolated by] *Gretchen Hagen 3*) have been shown to be influenced by light (Abel et al., 1995; Gil and Green, 1997; Guilfoyle, 1999; Tanaka et al., 2002).

A further possibility to explain cross talk between different hormones and light signalling pathways is protein degradation via the 26S proteasome (for review see Halliday and Fankhauser, 2003; Devoto and Turner, 2003; Devoto and Turner, 2005). Recently, Schwechheimer et al. (2002) demonstrated that the suppression of AtRBX1, an essential subunit of SCF-type E3 ubiquitin ligases, affects several signalling pathways, including the auxin signalling pathway, supposedly by inactivation of the SCF^{TIR1} complex, and the JA signalling pathway, probably by inactivation of the SCF^{COI1} complex. The light-dependent degradation of the transcription factors HY5 and HYH is also mediated by a proteasome-based process involving COP1, CIP8, and COP10 (see Schwechheimer et al., 2002 and references therein).

The rice coleoptile provides an excellent system to investigate the interaction between light, hormonal signalling, and photomorphogenetic responses. Exposure to light inhibits cell elongation of coleoptiles, rather than affecting their cell division rate. This is accompanied by an interruption of basipetal auxin transport from the perceptive site in the coleoptile tip to the major site of cell elongation in the basal region (Furuya et al., 1969). Previously, we have demonstrated that auxin (IAA) levels decreased upon irradiation with R light and the level of JA, a negative regulator of coleoptile growth, increased, whereas the concentration of abscisic acid (ABA) remained more or less constant during the first 2 h after irradiation (Riemann et al., 2003). The finding of Haga and Iino (2004) that the transcrip-

tion level of *OsAOS1*, a key enzyme of JA biosynthesis, is up-regulated by R light indicates a role for JA in phytochrome signalling of monocots. This is supported by recent work of He et al. (2005), who identified a novel light- and JA-regulated receptor-like kinase in maize, *Wound-Responsive and Phytochrome Regulated Kinase 1* (*WPK1*).

We attempted to identify genes that act at or close to the convergence point of JA and light signalling. We were especially interested in elucidating which light-regulated gene(s) directly affects coleoptile elongation. For this purpose, we chose the rice mutant *hebiba* that responds in a unique way to R light (Riemann et al., 2003) and provides an excellent model for this question: coleoptile growth is suppressed in darkness, and only elongates when it perceives a light signal. The light response of the *hebiba* mutant is thus an exact mirror image of behaviour in the wild type.

In the wild type, the JA biosynthesis pathway is induced after 30–60 min of R light irradiation, but this induction is blocked in the *hebiba* mutant, as is the de-etiolation response. Considering the relevant period for induction of JA biosynthesis, we isolated genes that are possibly involved in light-dependent activation of the JA pathway. This was done by comparing mRNA expression in coleoptiles of the mutant and the wild type, after irradiation with R and FR light, using a fluorescent differential display screen (Kuno et al., 2000a; Waller et al., 2002). These genes are expected to be relevant for the growth of coleoptiles, especially those regulated by both light and JA. We isolated such a gene containing a GDSL motif, which is classified as an enzyme. This is a new class of enzymes, predicted to possess lipolytic activity (Upton and Buckley, 1995; Brick et al., 1995; Akoh et al., 2004). In the present work, we demonstrated that this gene is an early light- and JA-induced gene, and that its response to R light is controlled by phytochrome A (Phy A) and B (PhyB).

Materials and Methods

Plant material

The *hebiba* mutant was obtained in a *japonica* background (*Oryza sativa* L. cv “Nihonmasari”), propagated in Japan (Hokuriku Experimental Station, Niigata) and subsequently in northern Italy (Almo Semi, Mortara). The mutant is male-sterile, such that it had to be maintained through heterozygotes. For each plant, small seed aliquots were checked separately to define the genotype of the population. An isogenic sister line homozygous for the wild-type allele was used as reference throughout the study.

Light sources and plant cultivation

The light sources for red light (R, 660 nm), far-red light (FR, 730 nm), and the green safelight (G, 550 nm) used in the photobiological studies are described in detail in Mohr et al. (1964). All light measurements were performed using a Tektronix-J16 photoradiometer (Tektronix, Beaverton, Oregon, USA). The seedlings were raised at 25 °C in photobiological darkness (using black boxes, black cloth, and isolated dark chambers) on floating meshes as described in Nick et al. (1994). Under these conditions, population germination was higher than 97% and seedling height varied by less than 5%.

Treatment of seedlings

In the time course experiment for red light irradiation 6-day-old seedlings were transferred into a R light ($3.4 \text{ mmol m}^{-2} \text{ s}^{-1}$) or FR ($15 \mu\text{mol m}^{-2} \text{ s}^{-1}$) light field at 25 °C. Seedlings used for the FDD screen were irradiated for 0, 15, and 30 min with R light and 15 min with FR light, those used for Northern blot analysis received 0, 30, 60, or 120 min.

For treatment of seedlings with methyl-JA (MeJA, Sigma-Aldrich, Neu-Ulm, Germany) or cycloheximide (Sigma-Aldrich, Neu-Ulm, Germany), 5 seeds were fixed in a row with B-400 Secure² adhesive (Factor II Inc., Lakeside, AZ, USA) on a glass slide. The glass slides were placed vertically in a conventional staining tray such that the row was horizontal, the tray was filled with water so that the seed row and the seeds were incubated under the same conditions as described above. After 6 days, the water was exchanged for different hormone solutions, cycloheximide, or water (as a negative control) and incubated for 1 h in darkness or in R light prior to harvest in green safelight (G, 550 nm). During incubation, the level of the respective liquids was kept slightly lower than the tip of the coleoptiles.

Phya, *phyb*, and *phyab* mutants (Takano et al., 2001, 2005) were raised in complete darkness for 5 days at 28 °C. Subsequently, they were either harvested immediately into liquid nitrogen, or transferred into a R light field ($15 \mu\text{mol m}^{-2} \text{ s}^{-1}$) for 2 min and incubated for 45 min in complete darkness prior to harvest.

Fluorescent differential display (FDD)

The FDD procedure was essentially the same as described in Kuno et al. (2000a) and Waller et al. (2002). First strand synthesis was performed with 2.5 μg of total RNA using a Texas Red-labelled 3'-anchored oligo(dT) primer (5'-Texas Red-T₁₄G-3; Yukigouseikagaku, Tokyo) and the Superscript Reverse Transcriptase Preamplification System (Gibco BRL). cDNAs were amplified by PCR using combinations of the Texas Red-labelled anchor primer and arbitrary 10-mer primers (Kit B, D, F, and X, Operon Technologies). Electrophoresis and detection of the PCR products were performed with an automated DNA sequencer (SQ5500, Hitachi).

Cloning of the cDNA of interest

The cDNA of interest was isolated by preparative gel electrophoresis and excised from the gel as described in Kuno et al. (2000a). The sequence of the cDNA 3'-end was obtained as described in Waller et al. (2002). To identify the complete cDNA sequence of the lipase, 5'-RACE PCR was conducted, but we were unable to amplify the 5'-end of this gene. However, a genomic clone in the rice genomic database of Monsanto (OSM143922), containing the sequence of the FDD fragment was identified. This clone, which is now publicly available on the gramene web page (<http://www.gramene.org/>), is located on chromosome 2 (Accession no. AP004018). Subsequently, we used an algorithm for eukaryotic gene prediction provided by Borodovsky and Lukashin on <http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi> to define the positions of exons and introns in the genomic sequence. According to this prediction, we designed different primer combinations in putative

Table 1 Identified cDNA fragments of the FDD screen. Four candidate genes were isolated from the FDD screen using different primer pairs. The D16 cDNA fragment showed an interesting and highly reproducible pattern of expression in the subsequent Northern blot experiments

Clone	Fragment size (bp)	PCR primers (5' to 3')	Best homology of the fragment (gene bank accession number)	Expression in <i>hebiba</i>
D16	750	GT ₁₅ G, AGG GCG TAA G	<i>Arabidopsis</i> lipase containing GDSL motif (S68410)	down
B10	480	GT ₁₅ G, CTG CTG GGA C	rice sucrose synthase (P30298)	constitutive
B6	600	GT ₁₅ G, TGC TCT GCC C	rice chloroplastic gene (AK104167)	up
F7	900	GT ₁₅ G, CCG ATA TCC C	soybean ethanolamine-phosphotransferase (T06384)	constitutive

5' and 3' regions. A specific primer combination (see below) yielded a product of the expected size, presumably representing the full length cDNA in a PCR using Eppendorf Triple Master System according to manufacturer's instructions. Subsequently, this product was cloned into Invitrogen TOPO vector according to manufacturer's instructions and sequenced.

- Forward primer: 5'-ATG GGC GCA GTT CCG GGG ATT TTG GT-3'.
- Reverse primer: 5'-AAG TGA CTT TCT CAT GAA GA-3'.

Isolation of RNA and Northern blot analysis

Coleoptiles were treated as described above, directly transferred to liquid nitrogen during harvest, and total RNA extracted with the RNeasy kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. 10 µg of total RNA from each sample were loaded on a 1.2% agarose gel. After electrophoresis, RNA was blotted onto a positively charged nylon membrane following standard protocols (Sambrook et al., 1989) and hybridized with a ³²P-labelled fragment of the *GER1* cDNA retrieved in the FDD screen. If not stated otherwise, the key results of Northern blot were reproduced 3–4 times using material from different experiments.

Intracellular localization by transient transformation

The *GER1* cDNA lacking the C terminal 19 nucleotides was cloned into a GFP vector (pMAV4, Kircher et al., 1999) under control of the 35S promoter using BamHI and PstI restriction sites. Rice coleoptiles were biolistically transformed as described by Holweg et al. (2004). The cells were viewed under a confocal laser scanning microscope (TCS SP, Leica, Bensheim, Germany) using a dual channel configuration with excitation from the 488-nm line of an argon-krypton laser and narrow bandwidth emission at 520–530 nm for GFP and 580–600 nm for YFP. Due to the rapid movement of peroxisomes, a fast scan option was used in combination with a time-lapse program, omitting frame averaging.

To test whether the observed pattern after transformation with *GER1*-GFP might result from a localization in the peroxisomes, we co-transformed rice coleoptiles with *GER1*-GFP and a YFP coupled marker for peroxisomes (designated POX-YFP in Fig. 5), as described in detail by Mathur et al. (2002).

Results

Fluorescent differential display (FDD) screen

Seedlings from wild type and *hebiba* were grown in complete darkness for 6 days, and either kept in darkness, or irradiated with R light for 15 min or 30 min, or with FR light for 15 min. After irradiation, coleoptiles were immediately excised, shock-frozen in liquid nitrogen, and used for isolation of total RNA. The eight different samples from the above treatments were compared by differential display for cDNA fragments that were differentially regulated between the wild type and the *hebiba* mutant.

After screening of about 10 000 cDNA bands and confirming the expression patterns with RNA isolated from three independent experiments, we isolated four candidate cDNA fragments that were differentially expressed between the wild type and *hebiba* (Table 1). The four cloned cDNAs showed homology to a sucrose synthase from rice, a chloroplastic gene from rice, a putative ethanolamine phosphotransferase from soybean, and an *Arabidopsis* lipase containing a GDSL motif. This putative lipase was termed *GER1* for *GDSL CONTAINING ENZYME RICE-1* and analyzed further, because the expression of *GER1* mRNA was constitutively much weaker in the *hebiba* mutant as compared to the wild type. The gene expression pattern of this cDNA observed in the FDD screen was confirmed by Northern blot analysis (Fig. 2), and the fragment was subsequently isolated and characterized.

Identification of the *GER1* gene

The complete cDNA sequence of the FDD fragment was identified through homology search in rice-genomic databases and application of gene prediction algorithms. The gene is located on rice chromosome 2. In order to verify the existence of the predicted gene, PCR reactions with different primer combinations using cDNA as a template were performed. With one of the primer combinations (see Materials and Methods), a gene of the appropriate size could be amplified and cloned.

The closest homologue found in the database is lipase Arab-1 (Brick et al., 1995) that shares 38% of identical amino acids with *GER1* (Fig. 1). The sequence of *GER1* cDNA is identical in both the wild type and mutant. We determined the rough position of the mutation in *hebiba* using a map-based cloning approach (unpublished data), and found that it does not map to chromosome 2. Therefore, we conclude that the mutant is effective in the regulation of *GER1* rather than in the function

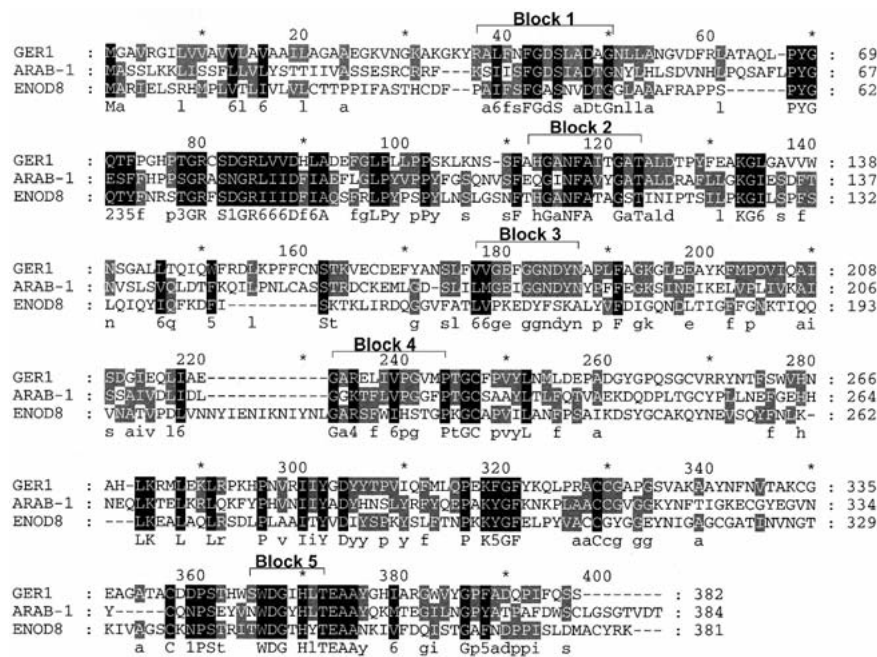


Fig. 1 Alignment of the GER1 protein sequence with homologous sequences from *Arabidopsis thaliana* (Arab Lipase-1) and *Medicago sativa* (ENOD8). 38% of amino acids of GER1 are identical to Arab Lipase-1 and 32% are identical to ENOD8. In the alignment, five blocks which have been described to be important for lipase function (Brick et al., 1995) are marked. Block 1 contains the GDSL motif.

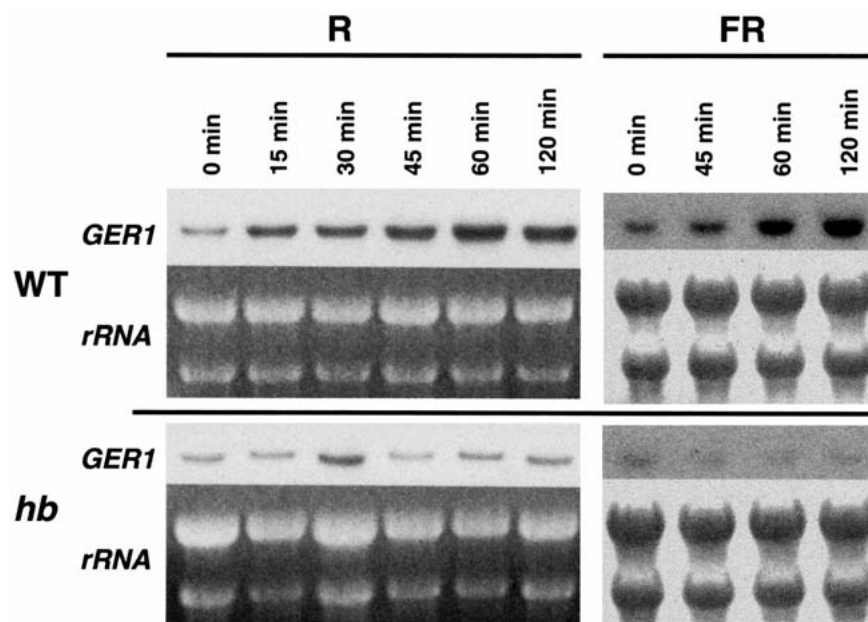


Fig. 2 Expression of *GER1* mRNA in etiolated rice coleoptiles of wild type (WT) and *hebiba* (*hb*) in response to continuous red light (R) and far-red light (FR) in a time-course experiment.

of the gene itself. Although the *Arabidopsis* gene is annotated as a lipase, we refer to the rice homologue as an enzyme containing a GDSL motif or *GDSL CONTAINING ENZYME RICE-1* (*GER1*) hereafter, according to the nomenclature of Beisson et al. (2003). The protein sequence of *GER1* also shows homology to proteins which contain a slightly altered GDSL motif, such as *ENOD8*, an early nodule-specific gene of alfalfa (Fig. 1; Dickstein et al., 1993).

There is one copy of *GER1* in the rice genome. It is located on chromosome 2 (bp 845152–8457973, see also “Materials and Methods”), and consists of 5 exons. Similar to its closest homologue in *Arabidopsis* (Brick et al., 1995), the transcripts

of this gene are also located in shoots. However, in contrast to *Arabidopsis*, the gene is only weakly expressed in etiolated shoots and is induced upon irradiation with R or FR light (Fig. 2).

The GDSL enzyme is an early light-induced gene under the control of PhyA and PhyB

To confirm the differential expression pattern in the FDD screen, we performed Northern blot analysis. *GER1* transcripts are up-regulated in the wild type by both R and FR light within 15 min, reaching maximal expression after 60 min (Fig. 2). The *hebiba* mutant shows a weak transient induction after 30 min

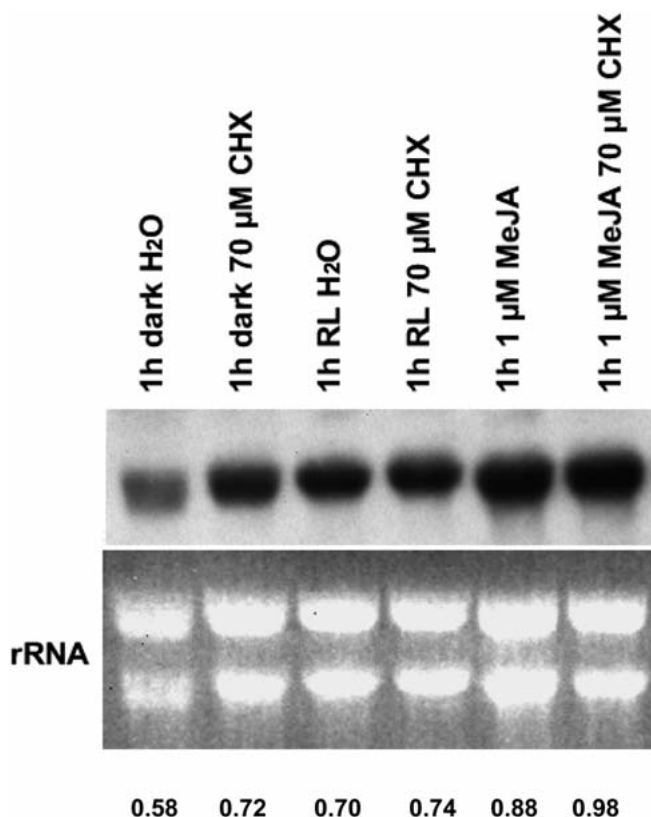


Fig. 3 *GER1* classifies as an early light- and early jasmonate-induced gene. Coleoptiles of wild-type seedlings were incubated in water or cycloheximide solution (CHX, 70 μ M), and either treated with red light or methyl-jasmonate solution (MeJA, 1 μ M). The numbers in the x-axis indicate the ratio of the signal intensity of *GER1* and the fluorescence of the upper rRNA band as determined with ImageJ software <http://rsb.info.nih.gov/ij/>.

in R, but during the subsequent time points and in FR light the expression level is as low as in the dark control.

Experiments in which we applied cycloheximide in order to suppress protein synthesis revealed that *GER1* can be classified as an early light-induced gene. The mRNA level of this enzyme is low in darkness in the absence of cycloheximide whereas it is induced after irradiation with R light both in the absence and presence of cycloheximide (Figs. 2, 3). Application of cycloheximide led to induction of the gene in darkness, indicating that in the absence of light the expression might be actively blocked by a suppressor that has to be continuously synthesized. Light inducibility is not affected by cycloheximide (Fig. 3), which leads to the classification of *GER1* as an early light-induced gene.

To address the question of which photoreceptor controls *GER1* expression in R light, we used rice *phyA*, *b*, and *ab* mutants (Takanokano et al., 2001, 2005), and compared *GER1* transcript levels in etiolated seedlings as well as 45 min after a R light pulse (Fig. 4). In *phyA* and *phyB* single mutants the expression could be induced by this treatment, while no induction was observed in *phyab* double mutant, indicating that *GER1* expression is redundantly controlled by *PhyA* and *PhyB* in R light.

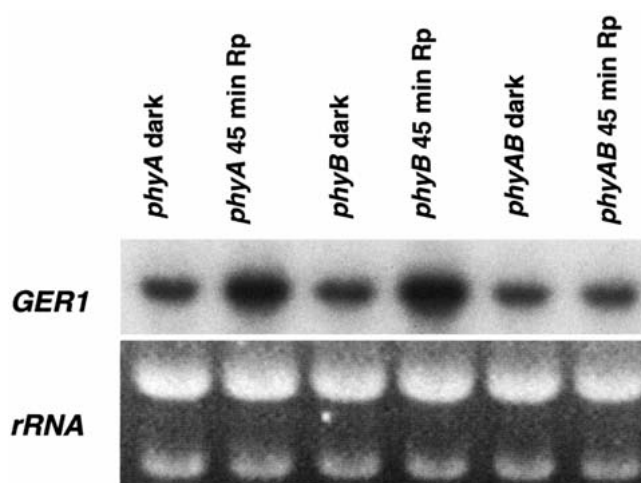


Fig. 4 Transcript levels of *GER1* in *phyA*, *phyB*, and *phyAB* mutants in darkness and after a red light pulse (Rp, 2 min of 15 μ mol $m^{-2} s^{-1}$ of red light). Irradiated seedlings were harvested 45 min after the end of the pulse treatment.

Exogenous jasmonate induces *GER1*

We have recently shown that, in rice coleoptiles, the JA pathway is triggered by irradiation with R light and that *hebiba* is impaired in this response (Riemann et al., 2003). We therefore tested *GER1* for a potential induction by exogenous methyl-JA (MeJA). Fig. 3 shows that 1 μ M of MeJA is sufficient to induce the enzyme and that simultaneous application of cycloheximide cannot suppress this induction, thus classifying *GER1* also as an early JA-induced gene.

Subcellular localization of *GER1*-GFP

In order to clarify the location of the *GER1* protein in the cell, we used transient transformation into the homologous system (rice coleoptiles). A partial *GER1* cDNA lacking the last 19 nucleotides at the 3'-end of the coding cDNA region was cloned into the GFP vector pMAV4 (Kircher et al., 1999). This vector was biolistically introduced into rice coleoptiles as described by Holweg et al. (2004). We had to use a shortened version of *GER1* for sterical reasons with respect to the available GFP vector. The missing nucleotides do not encode for any motif that is predicted to be critical for subcellular localization, which minimizes, but does not completely rule out, the possibility of different behaviour of the full-length protein.

After transformation with a control vector containing only GFP, the GFP signal was distributed in a diffuse pattern throughout the cytoplasm and the nucleus (Fig. 5A). In contrast, the *GER1*-GFP exhibited a characteristic pattern, where vesicular structures emerged from a cytoplasmic background (Fig. 5B), whereas the nuclear signal typical for GFP without fusion protein was absent (Fig. 5E). This pattern was consistent with localization in the endomembrane system, as predicted by different computer algorithms (see "Discussion"). We attempted to influence the protein localization by using external factors such as light, MeJA, BFA (brefeldin A), or auxin but could not find any difference from the respective control (data not shown).

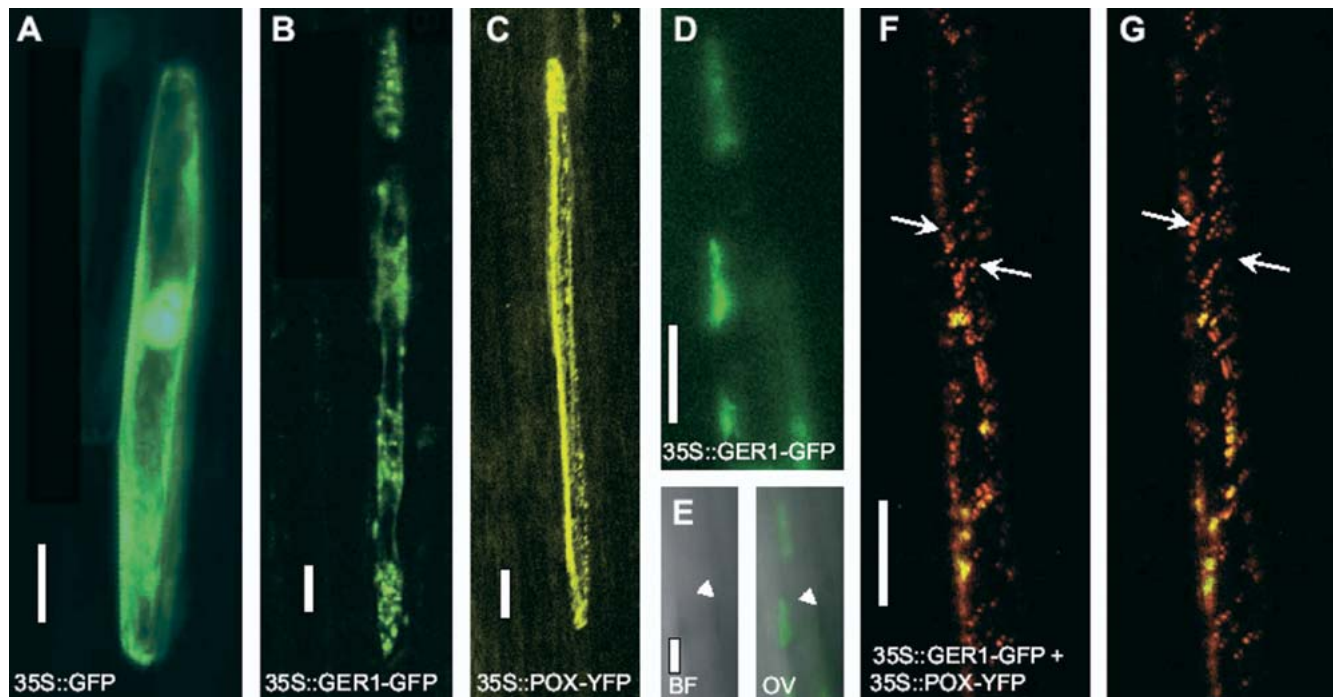


Fig. 5 Intracellular localization of rice GER1-GFP lacking the last 19 nucleotides of *GER1* cDNA at the C terminus upon transient transformation into rice coleoptiles. **(A)** Epidermal cell expressing GFP under control of the 35S promoter. **(B)** Epidermal cell expressing GER1-GFP. **(C)** Epidermal cell expressing a peroxisomal marker (POX) fused to YFP. **(D)** Detail of a cell that expresses GER1-GFP. **(E)** The same cell in a bright-field image (BF) and in an overlay of the bright-field image with the GFP signal (OV). The position of the nucleus is indicated by the

white arrowhead. **(F,G)** Two frames from a time series, where GER1-GFP and the peroxisome marker were followed within the same cell upon double transfection. The second frame was recorded 5 s after the first frame. Note that the smaller peroxisomes (red) move very rapidly along oblique tracks (white arrows), whereas the larger structures that are labelled by GER1-GFP (green) are less mobile. The yellow colour indicates the simultaneous presence of GER1-GFP and the peroxisome marker. Scale bar 10 μ m.

To test whether the GER1-GFP pattern was related to peroxisomes, we performed double transformation of rice coleoptiles with GER1-GFP and a peroxisome marker (Mathur et al., 2002) fused to YFP. This marker (POX-YFP in Fig. 5) was visible as highly mobile dots that were distributed throughout the cytoplasm, in addition to somewhat larger and mostly immobile vesicular structures that resembled the structures visualized by GER1-GFP (Fig. 5C). In cells that expressed both markers, the small mobile dots were exclusively labelled by the peroxisome marker, whereas the larger vesicles were characterized by the simultaneous presence of both markers (indicated by the yellow colour in Fig. 5F).

Discussion

Fluorescent differential display (FDD) was used to screen for new genes involved in JA-dependent phytochrome signal transduction. This method has already been used successfully to identify new genes involved in the auxin response of rice coleoptiles (Waller et al., 2002; Chaban et al., 2003). In the present study, we were interested to identify genes that are induced or repressed during de-etiolation and additionally are regulated by JA. Such genes are prime candidates for regulators of coleoptile elongation. For this purpose, we used the JA-deficient mutant *hebiba* as a tool because it reacts in the opposite way to the wild type upon irradiation with R light (Riemann et al., 2003). We compared gene expression after R light treatment in the mutant and the wild type.

GER1 is a member of a novel family of lipases

From the screening process we isolated the *GER1* cDNA, which encodes an enzyme containing a GDSL motif. This class of enzymes was previously described as representing a new class of lipases (Upton and Buckley, 1995), which is only present in bacteria and plants. The sequence of the *GER1* cDNA is identical in the wild type and the *hebiba* mutant, suggesting that *GER1* is functional in *hebiba*. In a map-based cloning approach, the putative position of the mutation in *hebiba* was determined to be located in a different genomic region. However, the product of the *HEBIBA* gene seems to be necessary to activate *GER1* during light signalling.

The predicted protein sequence harbours a putative transmembrane domain in the N terminus, as revealed by applying two different algorithms retrieved from the internet (<http://www.cbs.dtu.dk/services/TMHMM-2.0/> and <http://sosui.proteome.bio.tuat.ac.jp/sosui/frame0.html>). According to computer analysis with PSORT (Nakai and Kanehisa, 1991) and TARGETP 1.0 (Emanuelsson et al., 2000), the protein is predicted to be most likely located in the secretory pathway of the endoplasmic reticulum or, less likely, in the peroxisomes (Nakai and Kanehisa, 1991). The C terminal part of the protein, which is predicted to be located externally, should therefore be in the ER lumen or inside the peroxisomes. Our results on the co-localization of GER1-GFP and POX-YFP indicate that GER1 is localized in structures that are larger and mostly immobile

when compared to the bulk of peroxisomes. On the other hand, they seem to express the peroxisomal marker and therefore might be related to the genesis of peroxisomes. Alternatively, the localization pattern observed for the GER1-GFP fusion protein (Fig. 5) could be compatible with the so-called ER bodies, a novel ER-derived compartment (Hara-Nishimura and Matsushima, 2003). The formation of this compartment is induced upon wounding or treatment with JA in adult *Arabidopsis* plants and constitutively present in epidermal cells of cotyledons, but so far has not been reported for rice.

Although we obtained useful results from the experiments using GER1-GFP under control of the 35S promoter, we would like to extend this work by designing a vector containing the GER1 cDNA under control of its own native promoter. With such a tool, we hope to be able to examine the impact of different external signals such as light, plant hormones, or vesicle transport inhibitors on GER1 protein distribution (compare to "Results" section).

Recently, Lee and Cho (2003) identified a closely related GDSL motif-containing enzyme from Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). They also summarized the characteristics of the lipase II superfamily of plants. GER1 meets all criteria for a classification into this superfamily, especially in relation to the five conserved domains reported by Brick et al. (1995) which are preserved in GER1 (Fig. 1).

Light and JA regulate the expression of GER1

Northern blot analysis of GER1 expression revealed that it encodes a novel early light- and early JA-induced gene (Fig. 3). Data obtained with the *hebiba* mutant, which does not produce JA, suggest that GER1 is only weakly induced upon irradiation with R light (Fig. 2) and not induced by FR light. In summary, these findings show that GER1 is under control of both R light and the plant hormone JA. The same external stimuli also regulate the OPDA reductase of rice (OsOPR1), a JA-responsive gene in rice (Sobajima et al., 2003; Riemann et al., 2003). These findings are consistent with the abundance of light-, JA-, and pathogenesis-related motifs in the GER1 promoter (see supplemental data). GER1 might thus function in one of two JA-related processes: the GER1 gene product might participate in either the biosynthesis of JA itself or in JA signalling or response. Alternatively it could function in events that regulate these processes.

We found that GER1 is expressed after cycloheximide treatment even in absence of any other stimulus (e.g., light, MeJA). As mentioned in the "Results" section, it is possible that GER1 expression is actively inhibited in darkness by a repressor. To address question, it will be very interesting to study the presence of GER1 protein and, if possible, its activity. For this purpose, we will express GER1 in *E. coli* in order to obtain specific antibodies and to screen for a substrate for this enzyme.

Possible convergence points of light and JA signalling were recently described in *Arabidopsis*. A close homologue of the auxin-regulated soybean GH3 was identified as JAR1, a central molecule of JA signalling (Staswick et al., 2002). However, although this gene is allelic to FIN219, a component of phyA signalling which is regulated by auxin (Hsieh et al., 2000), the characterized JAR1 allele seems not to be influenced by light.

This apparent inconsistency is explained by the involvement of promoter methylation in FIN219 (Staswick et al., 2002). Yadav et al. (2005) found that AtMYC2, a transcription factor previously determined to be active in ABA and JA signalling, also functions as a repressor of blue light signalling in *Arabidopsis*. The authors speculate that this transcription factor acts very close to a putative convergence point of JA and blue light signalling.

Gutjahr et al. (2005) have shown that a general increase in jasmonic acid content in the lower as well as the upper coleoptile flank occurs during gravitropic stimulation. However, in the upper flank the level is higher than in the lower flank, which might promote the auxin-driven bending reaction of the coleoptile. Preliminary results on GER1 expression in upper and lower coleoptile flanks after gravitropic bending suggest that the expression pattern mirrors the level of JA (data not shown), which would further substantiate the correlation with GER1 induction under high JA levels.

Intriguingly, GER1 transcripts are induced by both R and FR light to the same extent (Fig. 3). Studies on *Arabidopsis* phyA (Whitelam et al., 1993) and phyB null mutants (Reed et al., 1993) in *Arabidopsis* had shown that PhyA is essential for the sensing of continuous FR light, whereas PhyB perceives continuous R light. However, there is evidence for a partial redundancy between PhyA and PhyB effects, for instance with respect to phytochrome-dependent gene expression (Kuno and Furuya, 2000b). Recently, by examining photobiological responses in rice phytochrome mutants (Takano et al., 2005), it was clearly demonstrated that PhyA, PhyB, and PhyC have overlapping functions in rice. Here, we have shown that PhyA and PhyB act redundantly to induce GER1 expression in R light. This is in line with the results of Takano et al. (2005), who have shown that PhyA and PhyB control Phy-dependent gene expression in R light, while PhyC function is restricted to FR light. In future it will be interesting to examine which Phys are controlling its expression in FR light, and whether blue light is an effective signal.

Is GER1 a functional phospholipase?

Considering the fact that Lee and Cho (2003) found about 90 putative proteins in the *Arabidopsis* genome belonging to the superfamily of enzymes containing a GDSL motif and roughly the same number in the rice genome, there is a high likelihood that different members of this family have specific physiological functions. These authors cloned a GDSL motif-containing enzyme from Chinese cabbage, called BRASSICA RAPA SALICYLATE-INDUCED LIPASE LIKE 1 (BR-SIL1). In contrast to GER1, BR-SIL1 is not induced by treatment with exogenous MeJA but is induced with salicylic acid (SA). Moreover, challenge with a non-host pathogen induced the expression of BR-SIL1. This result suggests that BR-SIL1 functions in defensive responses. While BR-SIL1 is related to SA-dependent defence processes, GER1 is regulated by JA-dependent processes, such as the response to light. In future, new regulatory patterns for other genes belonging to this superfamily of enzymes will emerge and the functions of these enzymes will be characterized in detail.

Ishiguro et al. (2001) published a phospholipase A called *DEFECTIVE IN ANther DEHISCENCE 1 (DAD1)*, which has been demonstrated to be the enzyme cleaving linolenic acid from a chloroplast membrane lipid and thus initiating the biosynthesis of JA. However, *DAD1* seems to function specifically during the development of pollen, not during pathogen-related processes. Additionally, *DAD1* does not seem to be the only lipase acting in the wound or pathogen-induced biosynthesis of JA (Turner et al., 2002). It is possible that *GER1* acts in an early step of JA synthesis in rice, in concert with other lipases. Since the activation of the JA pathway by light seems to be a peculiar feature of rice, it remains to be clarified which enzymes are active here. So far, there is no report on phospholipase A activity of an enzyme containing a GDSL motif in plants but, intriguingly, Flieger et al. (2002) characterized such an enzyme from the human pathogen *Legionella pneumophila*.

In addition to a possible direct function of *GER1* in JA synthesis, it is also possible that it acts in JA signalling or in a pathway connecting phytochrome signalling with the JA pathway. There are several signalling pathways in plant cells which depend on lipid-derived compounds, e.g., pathways involving phosphatidylinositol-3-kinases, those that involve phospholipase A activity, and also the pathway including diacylglycerol and inositol-3-phosphate as messengers (for review see Munnik et al., 1998). It will be a challenge for future research to identify such pathways as transmitters of a specific signal, such as JA, and put them into relation to known signalling components, such as the F-box protein *COI1* (Xie et al., 1998) or the recently described basic helix-loop-helix transcription factor *AtMYC2* which supposedly acts at the convergence point of blue light and JA signalling in *Arabidopsis* (Yadav et al., 2005).

To assess the function of *GER1* in more detail, we are planning to examine a rice mutant in which the *GER1* gene should be expressed constitutively by carrying an activation tag, as well as a *ger1* knockout mutant. These mutants will be helpful tools to elucidate the physiological function of this enzyme in future.

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M. Riemann

Botanisches Institut 1
Technische Universität Karlsruhe
Kaiserstraße 2
76128 Karlsruhe
Germany

E-mail: michael.riemann@bio.uni-karlsruhe.de

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