Plant Science 177 (2009) 499–510

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

Germins: A diverse protein family important for crop improvement

Rebecca M. Davidson [a], Patrick A. Reeves [b], Patricia M. Manosalva [c], Jan E. Leach [a,*]

[a] Department of Bioagricultural Sciences and Pest Management and Program in Plant Molecular Biology, Colorado State University, Fort Collins, CO 80523-1177, USA
[b] USDA-ARS, National Center for Genetic Resources Preservation, Fort Collins, CO, USA
[c] Boyce Thompson Institute for Plant Research, Ithaca, NY, USA

ARTICLE INFO

Article history:
Received 7 April 2009
Received in revised form 19 August 2009
Accepted 19 August 2009
Available online 25 August 2009

Keywords:
Germin-like protein
Oxalate oxidase
Superoxide dismutase
Biotic and abiotic stress
Quantitative disease resistance

ABSTRACT

The germin protein family is comprised of two main subgroups in plants, oxalate oxidases (OXOs) and germin-like proteins (GLPs). These proteins are implicated in a variety of plant processes including germination, development, pollen formation, and response to abiotic and biotic stress. Here, we examine the phylogenetic relationships and functional diversity of the germin gene family across diverse genera, and then focus on rice (*Oryza sativa*) as a model. In general, germin genes are expressed in all tissue types and are induced by biotic and/or abiotic stresses. In rice, many of the stress-induced germin genes physically co-localize with quantitative trait loci (QTL) for disease resistance, and emerging evidence suggests that small RNAs may regulate their transcript abundance. We analyze the extensive sequence, gene expression and functional data for germin family proteins and relate them to QTL map locations to predict additional candidate germin genes for future crop improvement.

© 2009 Elsevier Ireland Ltd. All rights reserved.

Contents

1. Introduction . . . 499
2. Developmental regulation of germin expression . . . 500
   2.1. Diversity of germin gene expression among tissue types . . . 500
   2.2. Can phylogenetics predict functional roles of germin family proteins? . . . 502
3. Germins and broad-spectrum disease resistance . . . 503
   3.1. Germins and stress responses in monocots and dicots . . . 503
   3.2. Potential for regulation of rice GLP expression by small RNA molecules . . . 503
   3.3. Germin associations with quantitative trait loci in rice and other cereal crops . . . 504
4. Summary and prospects . . . 507
   Acknowledgements . . . 508
   References . . . 508

1. Introduction

The germin family comprises a group of proteins belonging to the cupin superfamily. All germins contain the characteristic germin motif that gives rise to a jellyroll β-barrel structure involved in metal binding [1,2]. The term "germin" was coined after a family member was initially identified as a specific marker for germination in wheat embryos; that member was later identified as a homohexamer glycoprotein with oxalate oxidase activity [3–5]. Proteins with an average of 50% identity to this wheat germin, that also contained the germin motif, were found in several plants other than cereals and were designated germin-like proteins (GLPs) [1,6]. Most share biochemical attributes such as resistance to extreme heat and denaturing agents, homo-polymer formation, glycosylation and cell wall localization, though they differ in their tissue specificities and enzyme activities [3,7–10]. Germin family proteins have been widely studied in crop plants due to their diverse roles in important plant processes [1]. We focus this review on two major groups within the germin protein family, oxalate oxidases (OXOs) and GLPs.

OXOs, also referred to in the literature as "true germins", possess oxalate oxidase activity and are the products of a highly

Abbreviations: GLP, germin-like protein; OXO, oxalate oxidase; SOD, superoxide dismutase; chr, chromosome; QTL, quantitative trait loci.

* Corresponding author. Tel.: +1 970 491 2924; fax: +1 970 491 3862.
E-mail address: jan.leach@colostate.edu (J.E. Leach).

0168-9452/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2009.08.012

homogenous group of gene homologues present only in true cereals [3,11–13]. Proteins with oxalate oxidase activity have also been discovered in beet, spinach, banana and sorghum, however, limited evidence suggests they are distinct proteins from the germin-type OXOs [14–16]. Potential substrates for the OXO enzyme are endogenously produced oxalic acid (oxalate) and calcium oxalate, an insoluble crystal form stored in plant vacuoles and cell walls [17]. Oxalate is also a known pathogenicity factor produced by *Sclerotinia* fungi [18]. The enzymatic products include hydrogen peroxide (H_2O_2) and carbon dioxide [4,17,19]. *OXO* gene expression is detected in both epidermal and mesophyll tissues [20]. The OXO proteins contain N-terminal secretion signals and are extracted from soluble and cell wall plant protein fractions, indicating that these proteins are secreted into the apoplast [9,21,22]. Germins with OXO activity are found only in cereals, and may play roles in calcium regulation, oxalate metabolism, and response to pathogenesis.

*GLP*s are encoded by a heterogeneous group of genes present in many land plants including monocots, dicots, gymnosperms and moss. GLP is a term referring to all germin motif-containing proteins with unknown enzyme activity or those that do not possess oxalate oxidase activity [1]. The diversity of possible functions and enzymatic activities for GLPs is reflected in the many synonyms for names found in the literature, including oxalate oxidase-like proteins, nectarins, rhicadhesin-like receptors and ADP glucose pyrophosphatase/phosphodiesterase (AGPPase) [1,7,11,21,23,24]. Many GLPs, including those from moss (*Barbula unguiculata*; *Bu*), barley (*Hordeum vulgare*; *Hv*), wheat (*Triticum aestivum*, *Ta*; *Triticum monococcum*, *Tm*), tobacco (*Nicotiana* sp.; *Na* or *Np*), pea (*Pisum sativum*; *Ps*), azalea (*Rhododendron mucronatum*; *Rm*) and grape (*Vitis vinifera*; *Vv)* possess superoxide dismutase (SOD) activity [24–29]. SOD converts superoxide anions into H_2O_2. One GLP from barley does not possess OXO or SOD activity, but, instead, has ADP glucose pyrophosphatase/phosphodiesterase activity and catalyzes the hydrolytic breakdown of activated monosaccharides [7,20]. GLP transcripts are detected primarily in epidermal tissues [20] and native and recombinant GLP proteins are found primarily in cell wall fractions [20,27,29,30]. Like OXO proteins, GLPs also contain N-terminal secretion signals, and the targeting of these proteins to the cell wall and extracellular matrix [30] is consistent with their putative roles in cell wall expansion and response to environmental stress [8,10,22,29,31,32].

Enzyme activity and gene expression data suggest that both *OXO* and *GLP* genes and their encoded proteins play roles in plant defense responses. Barley and wheat *OXO* gene expression and enzyme activity were induced in response to powdery mildew infection [9,33]. A family of *OXO* genes from ryegrass is associated with wound-induced H_2O_2 accumulation [12]. In rice (*Oryza sativa*, *Os*), there is a strong genetic association between a cluster of four *OXO* genes and rice blast resistance [34]. Cultivar-specific induction of one *OsOXO* gene was observed after infection with *Magnaporthe oryzae*, the rice blast pathogen [11]. Rice *GLP* genes, including a tandemly duplicated cluster of seven members, are differentially induced in rice leaves after inoculation with *M. oryzae*, and contribute collectively to disease resistance against two very different fungal pathogens, *M. oryzae* and *Rhizoctonia solani* [35]. *GLP* induction after fungal infection has also been observed in barley and wheat leaf tissue [20,22,26,33], barley spikelets [36], and grape leaves and fruits [29]. Finally, oxalic acid produced by *Sclerotinia* fungi suppresses the oxidative burst in host plants and promotes pathogen ingress and disease [37]. Resistance to *Sclerotinia*-induced diseases was enhanced by over-expressing monocot *OXO* alleles in dicot crop plants such as peanut, sunflower, tobacco and rapeseed [38–41]. Because *OXO* and *GLP* genes play important roles in general defense against a wide variety of pathogens, they are desirable candidates for utilization in crop improvement programs.

OXOs and GLPs are encoded in large gene families in barley, wheat, rice, maize, ryegrass, grape and *Arabidopsis thaliana* [11–13,29,42,43]. Given the potential importance of these genes to disease resistance and crop improvement, this review aims to elucidate commonalities in functional attributes of rice germins and well-studied germins from other crop plant species. First, we describe similarities among 90 germin family proteins from 17 plant species with regards to developmental deployment, stress-response and catalytic activity. Next, focusing on rice, we use genome-wide expression data to explore germin gene expression profiles during diverse stress responses. By combining information on the physical location of germin genes relative to disease resistance QTL and differences in their stress-response profiles, we predict the utility of these genes for crop improvement. The predictive power was further improved by adding comparisons of functionally characterized germins across crop species, including cereals such as barley, wheat and maize.

2. Developmental regulation of germin expression

2.1. Diversity of germin gene expression among tissue types

Germins have been studied in many plant species in a multitude of contexts. A meta analysis of transcript data for all 41 rice genes plus an additional 48 genes from 17 other species from 19 published studies was performed to explore the diversity of germin gene expression in crop plants with respect to tissue specificity and stress responsiveness (Fig. 1; Tables 1 and 2). Two additional data sources were used for the rice germins, the Massively Parallel Signature Sequencing (MPSS) Rice Expression Atlas (http://mpss.udel.edu/rice/; University of Delaware [44] and the Rice Genome Annotation Digital Northern database (http://rice.plantbiology.msu.edu/dnav.shtml; Michigan State University [45]). The MPSS database allows genome-wide quantitative assessment of mRNA transcripts or small RNAs by identifying short sequence signatures that can be used to estimate relative gene expression. The MPSS signatures uniquely identify >95% of all rice genes in 80 normalized MPSS transcript libraries encompassing various tissue types and stress treatments [44]. Most of the transcript data were derived from the reference rice cultivar, Nipponbare. Among all MPSS libraries, expression levels were above five transcripts per million (TPM) for 27 of 41 rice germins. The Rice Genome Annotation Digital Northern database is an annotated, curated collection of rice ESTs from many cultivars [45]. Using their Rice Gene Expression Anatomy Viewer, EST abundances, by gene locus identifier (Table 2), were obtained for four tissue types for 26 of 41 rice germins. There was no evidence of transcription for six rice germins in either database.

Among the 89 germin family genes considered, transcripts were observed for genes under non-stress conditions in all major plant organs including seeds, flowers, leaves and roots (Fig. 1). Most germin genes (74%) are expressed in leaf tissue including cotyledons, shoots, young and mature leaves, and somewhat fewer are expressed in root tissue (61%) including young and mature roots. Relatively fewer germins are expressed in flowers (29%) and seeds (45%). Most germins are constitutively expressed in more than one tissue type. Four genes (*AtGer2*, *VvGLP5*, Os03g59010 and *OsOXO1*) were expressed exclusively in reproductive tissues, and two (*AtGLP5* and Os03g08150) were expressed only in leaf tissue.

The diversity of germin family gene expression among plant tissue types is consistent with their potential roles in tissue expansion and growth, from seed to flower. One third of rice germins are expressed in developing and/or germinating seeds, consistent with early discoveries and naming of germins as markers of seed germination [1]. Although initially discovered in

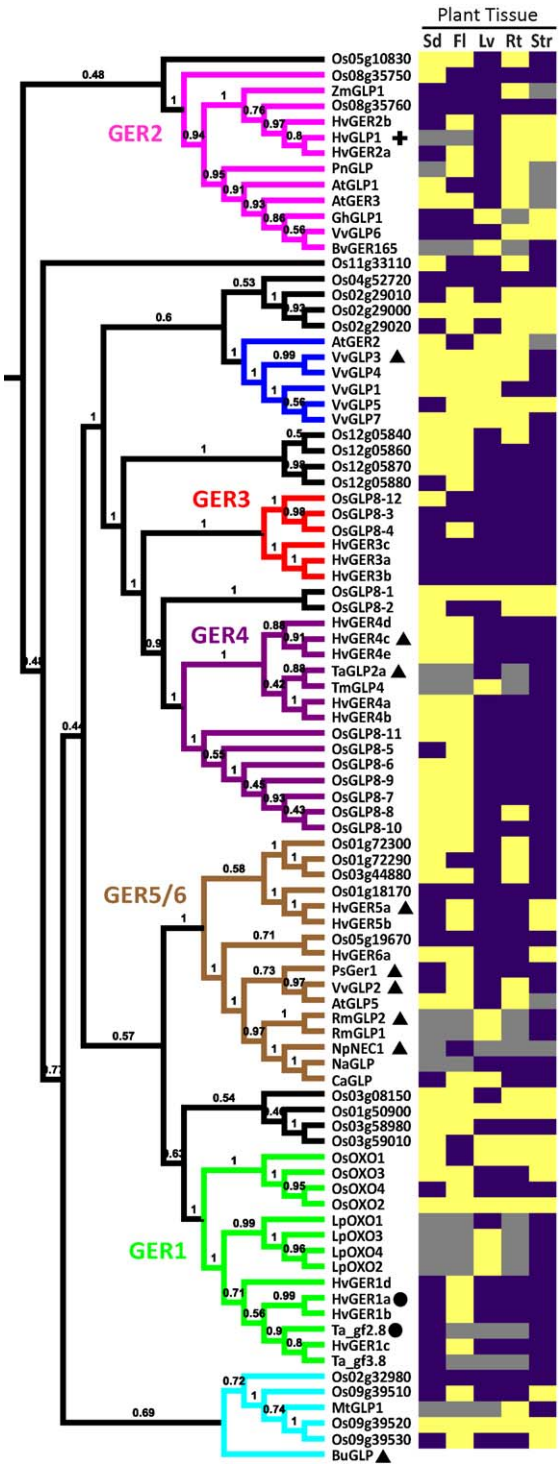

Fig. 1. Relationships of germin family genes in rice and 17 other plant species. The phylogenetic tree is based on amino acid sequences listed in Tables 1 and 2. Sequences were aligned using Dialign-TX [90] and the gene tree estimated using Bayesian maximum likelihood [91]. The root position was inferred using a relaxed molecular clock [92]. Clade support is shown as posterior probabilities at nodes. The symbols next to gene names indicate verified enzyme activity; oxalate oxidase (circle), superoxide dismutase (triangle) and ADP glucose pyrophosphatase/phosphodiesterase (cross). The heat map is based on expression data from published literature, and for the rice genes, from MPSS rice expression libraries [44] and the MSU rice digital northern database [45]. Gene expression is indicated as present (purple), absent (yellow) or no data (grey) in seeds (Sd), flowers (Fl), leaves (Lv), roots (Rt) or stress induced (biotic or abiotic; Str). Transcripts were considered present if bands were visible in published gel images. Gene expression in four tissue categories refers to non-inducing conditions, and stress-induced expression includes gene induction above basal expression levels.

Table 1
Accession numbers of germin family gene and protein[a] sequences used in phylogenetic analyses.

Gene name	Accession no.[b]	Plant species	Reference
AtGER1/AtGLP1	U75206	*Arabidopsis thaliana*	[32,82]
AtGER2	X91957	*Arabidopsis thaliana*	[32]
AtGER3/AtGLP3b	ATU75195	*Arabidopsis thaliana*	[32,82]
BuGLP	AB036797	*Barbula unguiculata*	[25]
BvGER165	AF310016	*Beta vulgaris*	[56]
CaGLP	AY391748	*Capsicum annuum*	[31]
GhGLP1	AF116537	*Gossypium hirsutum*	[46]
HvGER1a	DQ647619	*Hordeum vulgare*	[20]
HvGER1b	BI949639	*Hordeum vulgare*	[20][c]
HvGER1c	BY847725	*Hordeum vulgare*	[20][c]
HvGER1d	BG366014	*Hordeum vulgare*	[20][c]
HvGER2a	DQ647620	*Hordeum vulgare*	[20]
HvGER2b	BY837622	*Hordeum vulgare*	[20][c]
HvGER3a	DQ647621	*Hordeum vulgare*	[20]
HvGER3b	n/a	*Hordeum vulgare*	[20][c]
HvGER3c	DN178806	*Hordeum vulgare*	[20][c]
HvGER4a	CD056200	*Hordeum vulgare*	[20][c]
HvGER4b	n/a	*Hordeum vulgare*	[20][c]
HvGER4c	DQ647622	*Hordeum vulgare*	[20]
HvGER4d	DQ647623	*Hordeum vulgare*	[20]
HvGER4e	BQ762852	*Hordeum vulgare*	[20][c]
HvGER5a	DQ647624	*Hordeum vulgare*	[20]
HvGER5b	AV923347	*Hordeum vulgare*	[20][c]
HvGER6a	DQ647625	*Hordeum vulgare*	[20]
HvGLP1	Y15962	*Hordeum vulgare*	[8]
LpOXO1	AJ291825	*Lolium perenne*	[12]
LpOXO2	AJ492380	*Lolium perenne*	[12]
LpOXO3	AJ504848	*Lolium perenne*	[12]
LpOXO4	AJ492381	*Lolium perenne*	[12]
MtGLP1	AY184807	*Medicago truncatula*	[50]
NaGLP	AY436749	*Nicotiana attenuata*	[49]
NpNEC1	AF132671	*Nicotiana plumbaginifolia*	[24]
PnGLP	D45425	*Ipomoea nil*	[48]
PsGer1	AJ250832	*Pisum sativum*	[27]
RmGLP1	AB272079	*Rhododendron mucronatum*	[28]
RmGLP2	AB272080	*Rhododendron mucronatum*	[28]
Ta_gf2.8	M63223	*Triticum aestivum*	[13]
Ta_gf3.8	M63224	*Triticum aestivum*	[13]
TaGLP2a	AJ237942	*Triticum aestivum*	[9]
TmGLP4	AY650052	*Triticum monococcum*	[57]
VvGLP1	EF064171	*Vitis vinifera*	[29]
VvGLP2	DQ673106	*Vitis vinifera*	[29]
VvGLP3	AY298727	*Vitis vinifera*	[29]
VvGLP4	EF064172	*Vitis vinifera*	[29]
VvGLP5	EF064173	*Vitis vinifera*	[29]
VvGLP6	EF064174	*Vitis vinifera*	[29]
VvGLP7	EF064175	*Vitis vinifera*	[29]
ZmGLP1	AAQ95582	*Zea mays*	[47]

[a] Protein sequences were predicted from genomic DNA sequences using FGENESH HMM gene structure prediction program. http://linux1.softberry.com/berry.phtml.

[b] Gene sequences were acquired from the NCBI sequence database http://www.ncbi.nlm.nih.gov/. Germin sequences from crop plants with published gene and/or protein expression data were included in the analysis.

[c] Designated barley (*Hv*) sequences were extracted from the TGI database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gireport.pl?gudb=Barley) and analyzed by [20,35].

cereals, germins are expressed in developing siliques of *A. thaliana* [32], the skin and pulp of grapes [29] as well as expanding cotton (*Gossypium hirsutum*, *Gh*) fibers [46]. Germins are expressed in expanding shoots of developing seedlings, including young whorl leaves of maize [47], cotyledons of *Pharbitis nil* (*Pn* [48]), and young leaves of rice, barley and grape [20,29]. Germin genes are expressed in mature leaf tissue of most plants, and also in root tissue of rice, barley [20], grape [29], pepper (*Capsicum annum*, *Ca* [31]), *Nicotiana attenuata* (*Na* [49]), pea (*Ps* [27]) and alfalfa (*Medicago truncatula*, *Mt* [50]). Finally, only 13 rice germins are expressed in floral tissues such as mature pollen, ovary/mature stigma and immature panicles, and two are flower specific (Os03g59010, *OsOXO1*). Five of the 13 are related to nectarin 1, encoded by *NpNEC1*, from *Nicotiana* sp. Nectarin 1 produces H_2O_2

Table 2
Rice germin family genes, locus identifiers[a], and chromosomal coordinates.

Locus ID	Chromosome	5′ Coordinate	3′ Coordinate
LOC_Os01g18170	1	10166094	10164664
LOC_Os01g50900	1	29558747	29559638
LOC_Os01g72290	1	42241533	42242785
LOC_Os01g72300	1	42244715	42245955
LOC_Os02g29000	2	17160127	17159318
LOC_Os02g29010	2	17161547	17166870
LOC_Os02g29020	2	17169352	17170023
LOC_Os02g32980	2	19592747	19591621
LOC_Os03g08150	3	4136722	4137613
LOC_Os03g44880	3	25269898	25271593
LOC_Os03g48750	3	27729269	27728259
LOC_Os03g48760	3	27733754	27733065
LOC_Os03g48770	3	27738087	27737226
LOC_Os03g48780	3	27741221	27740193
LOC_Os03g58980	3	33525399	33526261
LOC_Os03g59010	3	33530129	33531009
LOC_Os04g52720	4	31176126	31173818
LOC_Os05g10830	5	5965397	5966166
LOC_Os05g19670	5	11446464	11447387
LOC_Os08g08920	8	5180531	5181354
LOC_Os08g08960	8	5202041	5203225
LOC_Os08g08970	8	5215873	5216960
LOC_Os08g08980	8	5222478	5223690
LOC_Os08g08990	8	5227424	5228454
LOC_Os08g09000	8	5232663	5233804
LOC_Os08g09010	8	5236151	5237313
LOC_Os08g09020	8	5242322	5243488
LOC_Os08g09040	8	5247942	5248886
LOC_Os08g09060	8	5253808	5254949
LOC_Os08g09080	8	5257901	5259045
LOC_Os08g13440	8	7989374	7988056
LOC_Os08g35750	8	22421190	22420215
LOC_Os08g35760	8	22426255	22425051
LOC_Os09g39510	9	22695664	22694639
LOC_Os09g39520	9	22697908	22697264
LOC_Os09g39530	9	22700478	22699440
LOC_Os11g33110	11	19084161	19084993
LOC_Os12g05840	12	2688828	2687775
LOC_Os12g05860	12	2692513	2691487
LOC_Os12g05870	12	2696216	2695409
LOC_Os12g05880	12	2699188	2698387

[a] Rice germin family protein sequences were acquired from the MSU Rice Genome Annotation Database http://rice.plantbiology.msu.edu/ [45].

in the nectar of tobacco flowers (Fig. 1, brown clade) [24]. The unique combinations of germins expressed among rice tissue types suggest that particular suites of genes contribute to distinct developmental processes.

2.2. Can phylogenetics predict functional roles of germin family proteins?

Phylogenetic relationships were analyzed for the germins discussed above to determine if functional roles are conserved among germin gene lineages (Fig. 1; Tables 1 and 2). The tree suggests a complex evolutionary history with recent gene duplications. Thus, it is difficult to identify orthologs of particular rice germins in other species. However, subfamilies of germin genes have been described for rice and barley [20,35]. Within subfamilies, which we expand to include germin sequences from additional taxa (Fig. 1), relationships among gene lineages are roughly congruent with established phylogenetic relationships among taxa. For example, relationships among gene lineages in the GER1 subfamily (Fig. 1, green clade) correspond closely with phylogenetic relationships among rice, ryegrass, barley, and wheat [51,52].

The phylogenetic tree suggests conservation of tissue specific expression pattern within a few subfamilies. Members of the GER3 subfamily (Fig. 1, red clade) are expressed in all tissue types in both rice and barley, with only a few exceptions [20,35]. Similarly, expression of GER4 subfamily genes from barley, rice and wheat (Fig. 1, purple clade) is largely restricted to vegetative tissue. However, expression patterns are highly variable within the majority of clades. This suggests that, over evolutionary time, germins with similar or identical catalytic properties were recruited for new uses via deployment to different tissues or altered time of expression. The four predicted rice oxalate oxidases of the GER1 subfamily (Fig. 1, green clade) are an excellent example. *OsOXO1* is only expressed in flowers, *OsOXO2* is not expressed in any of the four tissues assayed, *OsOXO3* is expressed in leaves and roots, and *OsOXO4* is expressed in leaves, roots and seeds. Only *OsOXO4* is induced by stress (Fig. 2 and [11]). Diversity in expression patterns may reflect divergence in regulatory sequences and cis-acting elements that direct expression to specific tissues [53].

GLPs from seven species have SOD activity [20,24–29]. These sequences occur in the GER4 and GER5/6 subfamilies as well as two additional major clades in the gene tree (Fig. 1). The observation of SOD activity in divergent germin lineages suggests an origin of SOD function near the base of the germin gene tree. Moreover, we can infer that a germin with SOD activity existed in the common ancestor of bryophytes and angiosperms because *BuGLP*, from the moss *B. unguiculata*, has SOD function [25]. Hence, SOD activity is likely an ancient gene function within the germins that has persisted at least since the early diversification of land plants. The precise ancestral function of the germin gene products, however, cannot be definitively concluded. A distinct clade of genes, tentatively named here as GER2, is another early germin lineage. The catalytic activity of only one GER2 gene (*HvGLP1*) is known, and it has AGPPase activity [7]. Therefore, the possibility that

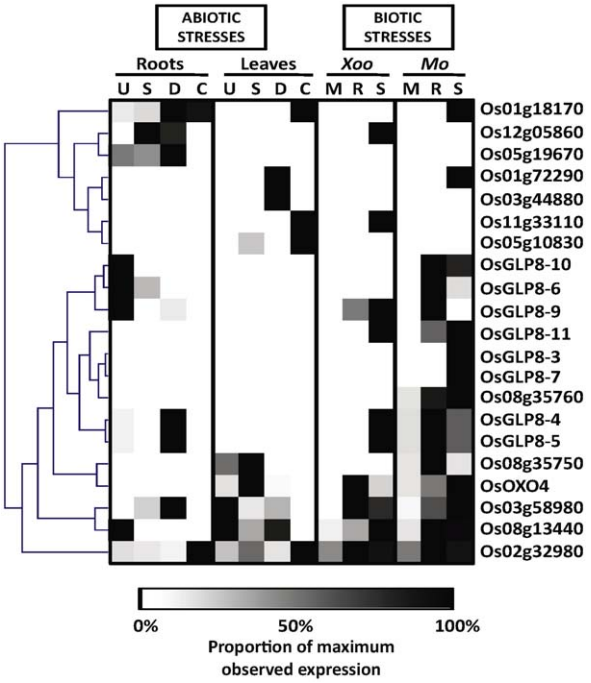

Fig. 2. Rice germin family gene induction after abiotic and biotic stresses. Genome-wide transcript data from 34 MPSS stress libraries [44] showed that 21 rice germin genes were induced by abiotic or biotic stresses or both. Abiotic stresses included salt (S), drought (D) and cold (C) treatments in roots or young leaves compared to untreated (U) controls. Biotic disease treatments included resistant (R) and susceptible (S) interactions with *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Magnaporthe oryzae* (*Mo*) compared to mock (M) inoculations. Proportions of maximum observed expression values, by gene, for each of four stress categories (black boxes; root/abiotic, leaves/abiotic, *Xoo*, *Mo*) are shown in the heat map. Clustering of genes was performed by hierarchal clustering based on induction patterns using MeV [93].

AGPPase is the ancestral catalytic activity within the germins cannot be excluded.

Two proteins in the GER1 subfamily (Fig. 1, green clade) have OXO activity, a catalytic property unique to this clade. OXO activity has only been experimentally demonstrated for the wheat and barley proteins HvGER1a and Ta_gf2.8 [12,54,55]. However, because patterns of relationships among gene lineages within the GER1 subfamily reflect phylogenetic relationships among the taxa from which they were derived, it is reasonable to predict that other genes within the GER1 lineage may also exhibit OXO activity. Based upon the gene tree, germin-type OXO activity appears to be a derived function that has evolved from ancestral genes with SOD activity, presumably following gene duplication.

The sugarbeet germin *BvGER165* is intriguing in that it is speculated to encode a protein with OXO activity [56]. Expression of *BvGER165* correlated with increased OXO activity and H_2O_2 production in stressed seedling tissue, though the protein was not purified and the specific enzyme activity was not confirmed [56]. *BvGER165* would represent the first germin with OXO activity from a dicot if ultimately shown to possess OXO activity. Curiously, the terminal branch length of *BvGER165* in the gene tree was much longer than neighboring branches suggesting the possibility of accelerated evolution in this sugarbeet gene, consistent with parallel acquisition of a novel oxalate oxidase catalytic function.

BvGER165 belongs to the GER2 subfamily of *GLP*s (Fig. 1, pink clade). The related proteins *GhGLP1* and *HvGER2a* tested negative for OXO and SOD activities [10,20]. Another protein in this subgroup, barley HvGLP1, has a distinct AGPPase enzyme activity [7]. This barley protein was first identified in cell wall fractions of seedling tissue and was noted for its insolubility after stress inducing treatments [8]. Transient silencing of the related gene (*HvGER2a*) did not result in loss of resistance to powdery mildew infection [20]. Although the evidence is limited, it suggests that the GER2 subfamily is distinct from other germin subfamilies in both enzymatic function and possibly its role in the defense response.

3. Germins and broad-spectrum disease resistance

3.1. Germins and stress responses in monocots and dicots

Germin gene expression is consistent with participation of the encoded enzymes in stress responses. Indeed, two-thirds of the germins in our analysis were induced by a variety of biotic (fungal, bacterial and viral infections, herbivore damage, mycorrhizal association) and abiotic (mechanical wounding, excess salt, drought, cold, iron and atmospheric nitrogen dioxide) stresses (Figs. 1 and 2).

OXO genes of the GER1 subfamily were responsive to stress induced by pathogens, wounding and elevated osmotic conditions (Fig. 1, green clade). Barley *(HvGER1)* and wheat (*gf2.8*) *OXOs* are important for resistance to *Blumeria graminis*, and both *OXOs* have been expressed in dicots such as peanut, sunflower and oilseed rape to enhance resistance against *Sclerotinia* pathogens [9,33,38,39,41]. One of a cluster of four rice *OsOXOs* was induced by the rice blast fungus, *M. oryzae* [11], and the same gene showed induction by the bacterial blight pathogen (*Xanthamonas oryzae* pv. *oryzae*, *Xoo)* and salt stress in MPSS expression libraries. Wounding and other abiotic stresses induced rice (*Os*) and ryegrass (*Lp*) *OXOs* [12] (Figs. 1 and 2).

GLP genes from all major clades are induced by biotic or abiotic stresses. Three subgroups contain genes associated with broad-spectrum fungal disease resistance in grape, barley and rice (Fig. 1, blue, red and purple clades). In grape, several *VvGLPs* were differentially induced by three pathogens, *Erysiphe necator*, *Plasmopara viticola* and *Botrytis cinerea* [29]. Both barley *HvGER3* and *HvGER4* genes were induced in leaf tissue by *B. graminis* f. sp. *hordei* [20], and *HvGER3* was upregulated in spikelets by *Fusarium graminaerum* [36]. Transient silencing of *HvGER4* genes resulted in loss of resistance to *B. graminis*, but silencing of *HvGER3* did not [20]. Silencing of closely related rice *OsGLP*s in the GER4 subfamilies conferred loss of resistance to *M. oryzae* and *R. solani* [35]. The GER4 subfamily members are likely involved in basal defense responses based on their non-specific pathogen induction and the broad-spectrum loss of resistance in silenced plants.

The other major groups of GLPs have different pathogen specificities and responses to abiotic stress (Fig. 1, light blue and brown clades). For example, pepper *CaGLP* was induced in leaf tissue by *tobacco mosaic virus* (*TMV*) and in resistant and susceptible interactions with *X. campestris* pv. *vesicatoria* [31]. Root specific *GLPs* in these subgroups include *MtGLP1*, a gene induced by mycorrhizal infection, *PsGLP*, a pea gene detected in root nodules, and *NaGLP,* a gene important for protection against the herbivore *Manduca sexta* [27,49,50]. As many as 13 rice *GLPs* are induced by abiotic stresses including drought, cold and salt. Rhododendron *GLPs*, *RmGLP1* and *RmGLP2*, were upregulated after treatment with the environmental pollutant nitrogen dioxide [28]. Finally, a gene from a wild species of wheat, *TmGLP*, in the GER4 subfamily, was induced by excess iron application; the authors speculated that the H_2O_2 produced by oxidative enzymes regulates cell wall iron homeostasis after pathogen attack [57].

Half (21 of 41) of all rice germins are induced by stress (Fig. 2). Most were induced by both biotic and abiotic factors, though seven genes were induced only after pathogen infection. Rice germins were induced in both resistant and susceptible interactions suggesting that they are involved in basal defense rather than resistance gene specific responses. The expression of some GER4 subfamily members contributing to broad-spectrum fungal disease resistance in leaf tissue [35] is suppressed in roots after abiotic stress (Fig. 2).

The literature is mixed on the contribution of germins to the oxidative burst that occurs shortly after elicitation of stress [17,58]. OXO proteins using oxalate as a substrate influence local pH and ion fluxes [17]. Extracellular GLPs with SOD activity may modulate the balance between superoxide radical anions and H_2O_2. SOD-type GLPs also can regulate apoplastic superoxide production by membrane associated NADPH oxidases which are activated after biotic infection [59]. Both OXO and SOD reactions produce the active oxygen species, H_2O_2. H_2O_2 in the extracellular matrix can serve as a signaling molecule and also can participate in several cell wall remodeling processes, including cell wall polymer cross-linking, peroxidase-dependent wall lignification, formation of wall appositions (papillae), and cell expansion and elongation [17,60]. Currently, only correlative evidence for OXO and/or GLP contribution to stress-induced H_2O_2 accumulation is available, and the evidence is contradictory. For example, in ryegrass, an increase in wound-dependent OXO enzyme activity was temporally correlated with H_2O_2 accumulation [12]. In barley, induction of *HvGLP4* (*HvGER4*) was correlated with papillae formation after *B. graminae* infection [22]. However, in another study, there was no change in H_2O_2 production in neighboring cells after transient silencing of *HvGLP4* and subsequent pathogen infection [26]. The interpretation of these studies is complicated by the known induction or presence of several other germins as well as other active oxygen generating enzymes.

3.2. Potential for regulation of rice GLP expression by small RNA molecules

Eukaryotic organisms produce endogenous small RNA molecules (approximately 21–24 nucleotides) of two general types, microRNA (miRNA) and short interfering RNA (siRNA) [61]. Small RNAs are non-coding regulatory RNA molecules that control gene expression by mediating RNA degradation, translational inhibition, or chromatin modification [61]. These small RNAs have important

Table 3
Number of small RNAs[a] that potentially target specific rice GLPs or multiple genes within germin (GER) subfamilies in units of transcripts per million.

Putative gene targets[b]	Unique siRNA sequences[c]	Germinating seedling control	Germinating seedling after *Mo*[d]	Control for ABA	ABA treated[e]
GER3	9	2	0	0	16
GER4	10	5	0	11	0
Os01g50900	1	22	0	0	0
Os02g29000	2	4	0	0	0
Os02g29010	7	7	0	13	56
Os02g29020[f]	1	0	0	0	0
Totals	30	40	0	24	72

[a] Small RNA libraries were from MPSS data at [44].
[b] Putative gene targets of small RNA based on sequence identity. Small RNAs putatively target individual genes or multiple genes within a germin (GER) subfamily.
[c] Number of unique small RNA sequences with identity to putative targets.
[d] RNA samples were obtained 24 h post-inoculation with the rice blast pathogen, *M. oryzae* (*Mo*).
[e] RNA samples were obtained from seedling tissue 24 h post-application with abscisic acid (ABA).
[f] This small RNA sequence was detected only in the stem small RNA library.

roles in many aspects of gene regulation in plants, controlling developmental processes, signal transduction, protein degradation, and response to abiotic and biotic stress [62]. Small RNAs can regulate the expression of tandemly arrayed, multi-gene family members [63]. For example, regulation of a cluster of highly related resistance genes in *Arabidopsis* by small RNAs was predicted to play an important role in optimizing plant defense response to pathogen attack [64,65]. Many rice germin family members are tandemly arrayed on the chromosomes [11], including members of GER3 and GER4, and several have complex expression patterns (Figs. 1 and 2). Therefore, we explore the possibility that small RNAs regulate rice germins.

A scan of six small RNA libraries at Rice MPSS (http://mpss.udel.edu/rice/; [44]) revealed 30 unique small RNA signatures that map to predicted coding sequences and 5′ untranslated regions (UTR) of rice germin genes (Table 3). One unique small RNA sequence putatively targets a GLP on chr 1, and 10 unique small RNAs target three tandemly arrayed, highly related GLP genes on chr 2. Nineteen small RNAs putatively target 10 genes in the GER3 and GER4 subfamilies and three related genes on chr 12; all of these are highly similar and tandemly arrayed.

An interesting example of possible small RNA regulation of germins is found in rice stem tissue (Fig. 3). Of the five germin subfamilies previously categorized [35], genes in three (GER2, GER5 and GER6) are expressed in non-stressed stem tissue while transcripts from genes in the GER3 and GER4 subfamilies were not detected. Interestingly, the corresponding stem small RNA library contains small RNAs with putative targets in the GER3 and GER4 subfamilies that are involved in plant defense responses after fungal infection [35]. These observations are consistent with a hypothesis that small RNAs downregulate the expression of this subset of germins in non-stressed stem tissues.

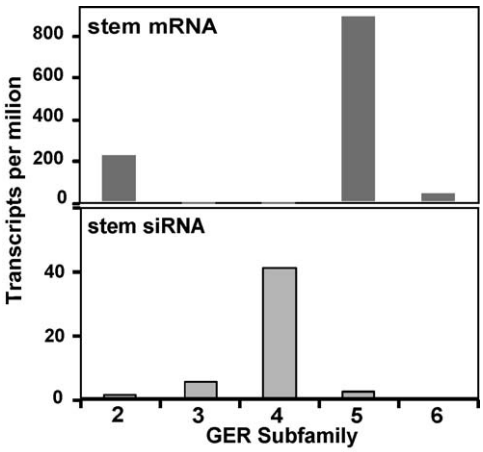

Fig. 3. Evidence for potential small RNA regulation of GLPs in rice stem tissue. MPSS mRNA and small RNA (siRNA) signature data corresponding to GLPs belonging to GER subfamilies described in Manosalva et al. [35] were extracted from rice MPSS [44] are shown in units of transcripts per million. The stem siRNA transcript population includes four unique small RNA signatures that putatively target GER3 subfamily members, and four others that target GER4 subfamily members.

The potential targeting of GER3 and GER4 subfamily members on chr 8 by small RNAs is of particular interest because of their roles in QTL-governed resistance [35]. The apparent downregulation of rice GER3 and GER4 genes by small RNAs in non-stressed rice tissue may reflect coordinated regulation of genes involved in defense (Fig. 3). Consistent with this are the presence of the small RNAs corresponding to GER3 and GER4 family members in the control seedlings and their absence in the seedlings after blast infection (Table 3). Such regulation may optimize defense responses by reducing the fitness costs to the plant. For example, inducible rather than constitutive responses may minimize energy requirements and enhance the capacity for response to pathogen attack.

Abscisic acid (ABA), an important plant hormone, is implicated in determining the outcome of interactions between many plants and their pathogens. Increased concentrations of ABA within *Arabidopsis* leaves correlate with susceptibility to an avirulent strain of a bacterial pathogen [66]. Conversely, the ABA signaling pathway is required for plant resistance to a necrotrophic fungus [67]. After ABA treatment of rice, subfamily specific changes occur in small RNA populations that putatively target genes in the GER3 and GER4 subfamilies (Table 3). Small RNAs targeting GER3 increase after ABA treatment, while those targeting GER4 disappear, suggesting that these gene family members are oppositely regulated by this hormone. Rice GER4 genes are predicted to play a general role during defense responses since they are effective against two fungal pathogens using different infection strategies (hemibiotrophic vs necrotrophic) [35]. The differences in regulation of these two subfamilies by ABA and pathogen treatment provide testable hypotheses regarding possible overlaps in regulation of plant defenses.

3.3. Germin associations with quantitative trait loci in rice and other cereal crops

Polymorphic markers from barley *HvOxOa (HvGER1*, germin with OXO activity*)* and *HvOXOLP* (*HvGER4*, GLP with SOD activity) were used as candidate genes in multiple rice QTL mapping experiments and were associated with reductions in disease [34,68,69]. The *HvGER1* probe co-localized with a cluster of four *OsOXO* genes on rice chr 3, and the *HvGER4* probe associated with a cluster of *OsGLP*s on rice chr 8. These rice

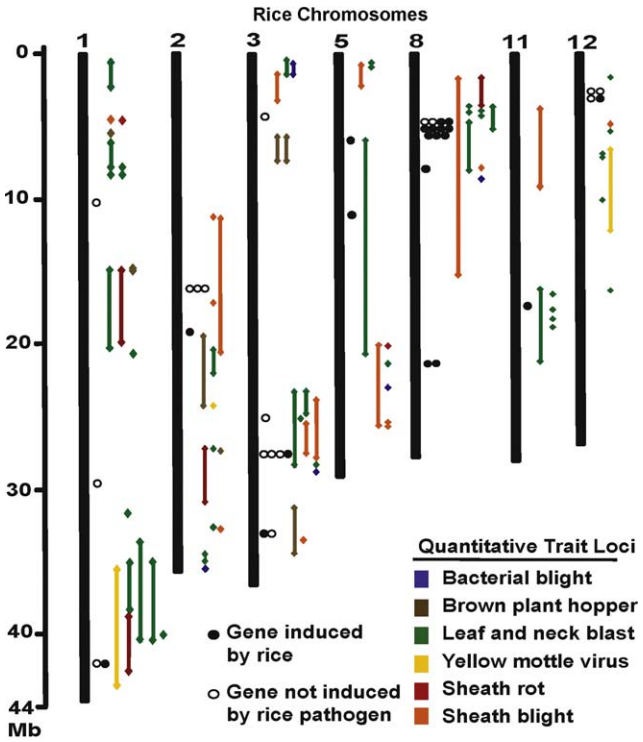

Fig. 4. Rice germin co-localization with quantitative trait loci. Both GLPs and OXOs physically co-localize with quantitative trait loci for resistance against biotic pathogens. Molecular marker data from 19 published QTL mapping studies were physically mapped on the rice genome (see Table 4). Individual points show single marker associations with disease phenotypes, and line segments show QTL intervals. Chromosomal positions of germin genes that are induced (filled circles) or not induced (open circles) by either *X. oryzae* pv. *oryzae* or *M. oryzae* in MPSS expression libraries are indicated next to the chromosomes. One GLP on chr 4 and a cluster of three GLPs on chr 9 are not shown because they are not associated with QTL in these mapping studies.

germin subfamilies were functionally characterized and found to contribute to broad-spectrum fungal disease resistance ([11,35], and R. Davidson, unpublished). *HvGER4* co-localized with barley QTL for fungal resistance [70], and the barley markers flanking this QTL were physically mapped to rice chr 8 [35]. The same barley marker, *HvOXOLP*, co-localized to a wheat QTL for resistance against *Pyrenophora tritici-repentis*, the causal agent of tan spot [71]. In maize, the OXO probe (*HvGER1*) mapped to five maize chromosomes, and the GLP probe (*HvGER4*) mapped to four chromosomes [43]. As in rice, barley and wheat, both germin markers co-localized to known maize QTL for broad-spectrum disease resistance, including resistance to *Cercospora* spp., the causal agent of gray leaf spot, and *Exserohilum turcicum*, the causal agent of northern corn leaf blight [43]. Given the above examples of germin genes co-localizing with disease resistance QTL and the functional validation of several in rice and barley disease resistance, we propose that combining gene location, expression profile, and QTL associations will facilitate the identification of additional candidate germin genes for disease resistance.

Molecular marker data from 19 published rice QTL mapping studies were mapped onto the rice genome along with the physical locations of all germin genes to predict additional rice germins that may contribute to complex, multi-gene resistance (Fig. 4; Tables 2 and 4). The cluster of *OsGLP*s on chr 8 co-localized to the originally targeted blast resistance QTL [69] and another rice blast QTL in a different mapping population [72]. For both studies, the chr 8 QTL regions contributed over 30% of the phenotypic variation in different mapping populations [69,72]. In addition, a sheath blight QTL [73] co-localized with the genes, consistent with the loss of resistance in the silenced mutants to both the blast and sheath blight pathogens [35]. Four clustered *OsOXO*s on chr 3 co-localized with multiple rice blast QTL [34,74], two sheath blight QTL [75,76] and a bacterial blight QTL [68]. QTL in this chr 3 region contributed 10–26% of phenotypic variation [34,73,75]. The addition of the expression data for the germins to the alignment adds another dimension to the analysis, and increases confidence in the predictions. For example, some family members that were confirmed to co-localize with chr 8 and 3 QTL were induced after pathogen challenge (Fig. 2).

Six other rice germins from the analysis may be good candidates for sources of resistance because they (1) co-localize with one or more disease resistance QTL; (2) are induced after pathogen or abiotic stress; and (3) are phylogenetically related to defense response germins. For example, a *GLP* on chr 1 (Os01g72990) is physically located under QTL for sheath rot and yellow mottle virus [77,78]. This GLP was induced in leaves by drought stress and *M. oryzae* infection in MPSS libraries, and is closely related to the barley *HvGER5* genes that were induced by infection with *B. graminis* [20]. A *GLP* on chr 3 (Os03g58980) is within a minor QTL for resistance to brown plant hopper [79], and is induced by infection with *M. oryzae* and *X. oryzae* pv. *oryzae* as well as drought stress. A chr 11 *GLP* (Os11g33110) co-localizes with several rice blast QTL [34,72,80], and is induced by *X. oryzae* pv. *oryzae* and cold stress. A *GLP* on chr 2 is located under a sheath blight QTL [75] and is induced by cold stress and infection with *M. oryzae* and *X. oryzae* pv. *oryzae*. Lastly, two *GLP*s on chr 5 (Os05g10830 and Os05g19670) that exhibit stress induction are within a large rice blast QTL [72]. We propose that polymorphic markers in these genes could be successful in locating QTL for disease resistance.

Table 4
Estimated physical coordinates of molecular markers and QTL intervals in disease resistance mapping studies.

QTL paper reference, parental cultivars, rice disease, causal organism[a]	Chr	Flanking markers[b]		Estimated coordinates[c]	
		5′	3′	5′	3′
Alam and Cohen [79], IR64 × Azucena, brown planthopper, *Nl*	1	AMY1B	RZ276	14442432	14603974
	2	RG157	RZ318	19865083	24565710
	3	RG191	RZ678	5709784	7327744
	4	RG143	RG620	33630028	34663799
	4	RZ590	RG163	32668236	*
	6	RZ144	RZ667	6718648	6928404
Albar et al. [78], IR64 × Azucena, rice yellow mottle virus	1	RZ730	C112	35265873	43281154
	2	RZ318	RG95	24565710	*
	4	RG190	RG788	8595185	*
	9	RZ228	RG667	18513777	20481607
	12	R617	Y6854R	7477327	13429316

Table 4 (*Continued*)

QTL paper reference, parental cultivars, rice disease, causal organism[a]	Chr	Flanking markers[b]		Estimated coordinates[c]	
		5′	3′	5′	3′
Chen et al. [70], Zhenshan 97 × Minghui 63, rice blast, *Mg*	1	C161	R753	196624	1887955
	1	RM259	RM243	7443429	7968524
	1	RG532	RM259	5759761	7443429
	1	G393	R2201	31373895	*
	1	RM212	C547	33381385	40391516
	1	C547	C2340	40391516	34798491
	1	C2340	C86	34798491	38041145
	2	RM213	RM208	34652316	35135783
	3	RZ403	R19	23044452	24551002
	7	RG528	RG128	1570817	2315990
	7	RG128	C1023	2315990	7231613
	7	RM234	R1789	25471987	26527687
	8	RG333	RM25	4100172	4372099
	9	RM201	C472	20174289	14594969
	9	RM257	RM242	17719660	18810067
	9	RG570	RG667	19946740	20481607
	9	RM215		21189110	
	9	R1952		2260721	
Fukouka and Okuno [83], Nipponbare × Owarihatamochi, rice blast, *Mg*	4	G271		20156485	
	9	G103		10799952	
	12	C625		11058522	
Han et al. [84], Zhenshan 97 × Minghui 63, sheath blight, *Rs*	5	C624	C246	21351430	27057523
	5	C246	RM26	27057523	27321395
	9	C472	R2638	14594969	17835012
	9	RM257	RM242	17719660	18810067
Huang et al. [85], B5 × Minghui 63, brown planthopper, *Nl*	3	R1925	R2443	35385979	31892032
	4	C820	R288	6882835	8198506
Li et al. [73], Lemont × Tequing, sheath blight, *Rs*	3	RG348	RG944	1430443	3219536
	4	RG143	RG214	33630028	31881866
	8	RG20	RG1034	2029021	16241105
	12	RZ397	RG214A	5758288	*
Li et al. [86], Lemont × Tequing, bacterial blight, *Xoo*	2	RG520	RZ476A	35662091	*
	3	C515	RG348	698124	1430443
	4	RG214	Ph	31881866	*
	4	RZ69	RG190	11217892	8595185
	8	G104	G1314A	8917705	*
	9	RZ404	RG451	22194746	*
	10	C16	RG1064F	20840254	*
Liu et al. [69], SHZ-2 × LTH, rice blast, *Mg*	2	RM262	RM5789	20790000	22380000
	7	RM3404	RM3826	20110000	20810000
	8	RM310	RM3215	5020000	8560000
	10	RM8207	RM3311	9550000	10360000
Liu et al. [75], Jasmine 85 × Lemont, sheath blight, *Rs*	1	RM1361	RM104	39407759	40494732
	2	RM424	RM5427	11389704	21519085
	2	RM112	RM250	32013785	*
	3	RM16	RM426	23082184	27544733
	3	RM5626	RM426	24820470	27544733
	3	RM514	RM85	35227302	*
	5	RM507	RM7349	80784	3225473
	6	RM435	RM190	537327	1764638
	9	RM409	RM257	14372062	17719660
	9	RM215	RM245	21189110	*
Pinson et al. [87], Lemont × Teqing, sheath blight, *Rs*	1	RG532		5759761	
	3	RG348		1430443	
	3	RZ474		25084678	
	4	RZ590		32668236	
	6	RZ508		30945227	
	8	G104		8917705	
	9	RZ404		22194746	
	10	RG561		21518696	
Ramalingam et al. [68], IR64 × Azucena, brown planthopper, *Nl*, bacterial blight, *Xoo*	2	RG181	XLRFRI2	27488270	*
	3	OXO	CDO337	28539529	*
	3	RG191	RZ678	5709784	7327744
	4	RZ675	RG163	24131148	*
	5	RZ70	XLRFRA1	24241592	*
	7	RG477	NLRIN121	6778514	*

Table 4 (*Continued*)

QTL paper reference, parental cultivars, rice disease, causal organism[a]	Chr	Flanking markers[b]		Estimated coordinates[c]	
		5′	3′	5′	3′
Sirithunya et al. [74], CT9993-5-M × Khao Dawk Mali 105, leaf and neck blast, *Pg*	3	RM16	RM168	23082184	28047759
	5	C597	RM122	238174	289101
	6	R2171	RG64	8062746	9549516
	6	C1478	R2171	6822190	8062746
	7	G20P	RM2	17525116	15969160
	7	RM10	OSR22	22135223	*
	7	R3089	G20P	18334208	17525116
	9	R1687	RG553	8346089	9216376
Srinivasachary et al. [77], C039 × Moroberekan, sheath rot, *So*	1	RG140	RG612	5093011	*
	1	RZ276	CDO920	14603974	19926001
	1	RG109	RG236	38525259	42296614
	2	RG102	RG73	27483091	31106412
	5	RG573	RG360	21488330	*
	6	RG172	RG192	24072058	*
	7	RZ272	RG511	4657304	5131861
	8	RG20	RG333	2029021	4100172
Tabien et al. [88], Lemont × Tequing, rice blast, *Mg*	1	RZ14		40015287	
	2	RG520		35662091	
	3	RZ474		25084678	
	5	CDSR49		22497768	
	12	RZ397		5758288	
Wang et al. [72], CO39 × Moroberekan, rice blast, *Mg*	1	RZ276	RZ744	14603974	20926224
	1	RG140	RG612	5093011	*
	2	RG102		27483091	
	3	RG104A	RG348	463840	1430443
	4	RG214		31881866	
	5	RG182	RG573	6112140	21488330
	6	RZ744	WAXY	20926224	1764623
	6	RG64	RG172	9549516	24072058
	7	RG351	CDO553	29413782	*
	8	RG333	RZ562	4100172	5415950
	11	RG103		20301909	
	11	RG16		18143751	
	12	RG574		1594835	
	12	RG869B		7731471	
	12	RG9		18050023	
Wu et al. [34], Moroberekan × Vandana, rice blast, *Mg*	2	RM250		32774365	
	3	RM168		28047759	
	9	RM215		21189110	
	11	RM21		18973973	
Zenbayashi et al. [80], Norin × Chubu, rice blast, *Mg*	11	RM209	G4001	17771755	22781184
Zou et al. [76], Lemont × Jasmine 85, sheath blight, *Rs*	2	G243	RM29	11750492	*
	2	RG171	RM29	17590791	*
	3	R250	C746	25071688	27084381
	7	RG30	RG477	12785785	6778514
	9	C397	G103	12289952	10799952
	9	RG570	C356	19946740	18525201
	11	G44	RG118	9934607	4413928

[a] Disease-causing organisms used in phenotypic assays of mapping populations: *Nilaparvata lugens* (*Nl*), *Magnaporthe oryzae* also called *M. grisea* (*Mg*) and *Pyricularia grisea* (*Pg*), *Rhizoctonia solani* (*Rs*), *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *Sarocladium oryzae* (*So*).

[b] Polymorphic molecular markers associated with reductions in measured disease symptoms. Marker data are reported as QTL intervals with 5′ and 3′ flanking markers, or as single markers that are significantly associated with phenotype.

[c] The estimated physical coordinates of molecular markers relative to the rice genome were acquired at Gramene ([89], http://www.gramene.org/) and MSU Rice Genome Annotation [45], http://rice.plantbiology.msu.edu/index.shtml) databases. Coordinates were also estimated by performing BLASTN of primers and other associated sequences against the reference japonica rice genome at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Asterisks indicate molecular markers that could not be physically mapped to the rice genome. Entire QTL intervals and single markers that could not be physically mapped are not shown.

4. Summary and prospects

Germins are a functionally diverse protein family, with at least three different enzyme activities. They are encoded by large gene families with distinct subgroups. Most germin genes are expressed in multiple tissue types including seed, flowers, leaves and roots. There is evidence of cell type specificity among germin transcripts; some *GLP*s are restricted to epidermal cells and *OXO*s are present in epidermal and mesophyll cells [9,20]. The conservation of germin-box containing proteins in multiple species from bryophytes to angiosperms suggests that, as a class, they are essential for basic plant function.

There are 41 predicted germin genes in rice distributed on nine chromosomes. Six of these may be non-functional, suggested by the absence of gene transcript evidence in multiple genome-wide expression databases. Over half of germin family genes in rice are induced by abiotic and biotic stresses. Phylogenetic reconstruction suggests multiple gene duplication events in rice. Gene expression data indicate that the majority have been leveraged to serve novel functions. The diversification of germins is likely attributable to

two processes. Following duplication, changes in coding sequences allowed evolution of diverse enzyme activities. Mutations in regulatory regions promoted changes in temporal and spatial expression patterns of closely related paralogs. The result is a genomic suite of gene family members with slightly modified functional attributes that have been effectively deployed to support a variety of basic plant functions, including basal resistance to pathogens.

OXO and GLP have long been speculated to function as components of general basal defense responses; general responses are predicted to be effective against diverse types of pathogens and pests [1,4,20,29]. Their predicted roles in basal defense are several. They may facilitate cell wall strengthening through lignification and papillae formation; these would impede penetration and reduce access by cell wall degrading enzymes. In addition, the H_2O_2 produced is an active oxygen species that is directly toxic to pathogens [58]. Finally, H_2O_2 is a known signaling molecule that is involved in multiple pathways of plant innate immunity. Using silenced or mutant plant lines, future work will allow dissection of how different gene family members individually and collectively function in basal defense responses.

The apparent cell type and tissue specificity of germin gene expression as well as predictions of small RNAs putatively targeting rice *GLP*s provide well-founded hypotheses regarding germin family gene regulation and acquisition of defense-related functions. For example, regulatory regions of epidermis-specific genes could be compared to genes expressed in the mesophyll for identification of novel cis-elements controlling cell type localization. Comparisons of 5′ regulatory regions of tandemly repeated *OsGLP* genes on chr 8 suggest that some members have accumulated putative defense-related cis-elements such as W-box motifs ([81], Davidson et al, unpublished). For these genes, an increase in the number of putative W-box motifs corresponds with increased induction by pathogen challenge (Davidson et al, unpublished) and improved broad-spectrum disease resistance [35]. Acquisition of epidermal specificity and the ability to be activated by pathogen-induced WRKY transcription factors (targeting W-box motifs) may explain the functions of particular germin genes in fungal disease resistance. The identification of small RNAs that putatively target defense-related *OsGLP*s suggests another possible layer of gene regulation that could be investigated.

In addition to studying diversification of germins within a plant genome, it is also important to identify natural variation in alleles among plant varieties. The identification of polymorphic loci in rice, wheat and maize implicated *OXO* and *GLP* genes in QTL-based disease resistance [68,70,71]. Limited evidence in rice suggests that insertions and/or deletions in 5′ regulatory regions and differential gene expression among cultivars may explain functional distinctions between 'resistant' and 'susceptible' alleles ([11], Davidson et al. unpublished). The genetic background within which to deploy these 'good' alleles is also critical to consider. Transient silencing of *HvGER4* (*HvGLP4*) in barley conferred increased susceptibility to *B. graminis* but was genotype dependent [26]. The effects of genetic background are also evident when comparing the same germin-containing QTL across diverse rice mapping populations. The level of partial resistance conveyed by a particular genomic region varies across populations and is likely dependent on the allelic states of other loci contributing to the complex phenotype.

The comprehensive data available for rice, including a well-annotated genome sequence, robust genome-wide expression data, and extensive published QTL data, provided the opportunity to identify additional candidate germin genes for disease resistance. Rice germins previously confirmed to contribute to disease resistance were shown in our analysis to co-localize with numerous additional QTL from different mapping populations (Fig. 4). Furthermore, based on co-localization with QTL, expression profiles and relationships to other defense-related germins, we predict six additional rice germins as promising candidates for being defense response genes. Rapid identification of additional candidate genes for disease resistance using bioinformatic approaches will facilitate future crop improvement efforts.

The importance of germins is not limited to rice. Our comparative approach predicted candidate germin gene lineages with possible relevance to disease resistance in other taxa. Based upon phylogenetic relationships in the reconstructed gene tree, and the conservation of disease resistance attributes of genes across taxa, we predict that the GER1 and GER4 lineages are the most likely to contain genes involved in broad-spectrum disease resistance. This hypothesis could be tested in QTL studies using markers developed for genes belonging to these lineages. More broadly, we propose that application of such a comprehensive, comparative approach will expedite identification of other types of candidate genes underlying QTL for various traits useful for crop improvement.

Acknowledgements

We thank Adam Heuberger for technical assistance. Rebecca Davidson and research described were supported by USDA-CSREES-NRI-Rice-CAP grants 2004-35317-14867 and 2006-35604-16628, a Ford Foundation Diversity Fellowship, the Generation Challenge Program, and the Colorado State Experiment Station. Patricia Manosalva was supported by a project from the USDA-CSREES-NRI #2003-01551.

References

[1] J.M. Dunwell, J.G. Gibbings, T. Mahmood, S.M.S. Naqvi, Germin and germin-like proteins: evolution, structure, and function, Crit. Rev. Plant Sci. 27 (2008) 342–375.
[2] L. Requena, S. Bornemann, Barley (*Hordeum vulgare*) oxalate oxidase is a manganese-containing enzyme, Biochem. J. 343 (1999) 185–190.
[3] E. Woo, J. Dunwell, P. Goodenough, A. Marvier, R. Pickersgill, Germin is a manganese containing homohexamer with oxalate oxidase and superoxide dismutase activities, Nat. Struct. Biol. 7 (2000) 1036–1040.
[4] B.G. Lane, Oxalate, germins, and higher-plant pathogens, IUBMB Life 53 (2002) 67–75.
[5] A.S. Jaikaran, T.D. Kennedy, E. Dratewka-Kos, B.G. Lane, Covalently bonded and adventitious glycans in germin, J. Biol. Chem. 265 (1990) 12503–12512.
[6] B. Dumas, A. Sailland, J.P. Cheviet, G. Freyssinet, K. Pallett, Identification of barley oxalate oxidase as a germin-like protein, Comptes rendus de l'Academie des sciences 316 (1993) 793–798.
[7] M. Rodriguez-Lopez, E. Baroja-Fernandez, A. Zandueta-Criado, B. Moreno-Bruna, F.J. Munoz, T. Akazawa, J. Pozueta-Romero, Two isoforms of a nucleotide-sugar pyrophosphatase/phosphodiesterase from barley leaves (*Hordeum vulgare* L.) are distinct oligomers of HvGLP1, a germin-like protein, FEBS Lett. 490 (2001) 44–48.
[8] L. Vallelian-Bindschedler, E. Mosinger, J.P. Metraux, P. Schweizer, Structure, expression and localization of a germin-like protein in barley (*Hordeum vulgare* L.) that is insolubilized in stressed leaves, Plant Mol. Biol. 37 (1998) 297–308.
[9] P. Schweizer, A. Christoffel, R. Dudler, Transient expression of members of the germin-like gene family in epidermal cells of wheat confers disease resistance, Plant J. 20 (1999) 540–552.
[10] H.J. Kim, T.C. Pesacreta, B.A. Triplett, Cotton-fiber germin-like protein. II: Immunolocalization, purification, and functional analysis, Planta 218 (2004) 525–535.
[11] M.G.C. Carrillo, P.H. Goodwin, J.E. Leach, H. Leung, C.M. Vera Cruz, Phylogenomic relationships of rice oxalate oxidases to the cupin superfamily and their association with disease resistance QTL, Rice 2 (2009) 67–79.
[12] E. Le Deunff, C. Davoine, C. Le Dantec, J.P. Billard, C. Huault, Oxidative burst and expression of germin/oxo genes during wounding of ryegrass leaf blades: comparison with senescence of leaf sheaths, Plant J. 38 (2004) 421–431.
[13] B.G. Lane, F. Bernier, E. Dratewkakos, R. Shafai, T.D. Kennedy, C. Pyne, J.R. Munro, T. Vaughan, D. Walters, F. Altomare, Homologies between members of the germin gene family in hexaploid wheat and similarities between these wheat germins and certain *Physarum* spherulins, J. Biol. Chem. 266 (1991) 10461–10469.
[14] S. Singh, S.N. Mishra, C.S. Pundir, Purification and properties of oxalate oxidase from NaCl stressed grain sorghum seedlings, J. Plant Biochem. Biotechnol. 15 (2006) 55–57.
[15] B.G. Lane, Oxalate oxidases and differentiating surface structure in wheat: germins, Biochem. J. 349 (2000) 309–321.
[16] P. Varalakshmi, K.E. Richardson, Studies on oxalate oxidase from beet stems upon immobilization on concanavalin-A, Biochem. Int. 26 (1992) 153–162.
[17] B.G. Lane, Oxalate, germin, and the extracellular matrix of higher plants, FASEB J. 8 (1994) 294–301.

[18] G. Godoy, J.R. Steadman, M.B. Dickman, R. Dam, Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*, Physiol. Mol. Plant Pathol. 37 (1990) 179–191.
[19] T.S. Gechev, F. Van Breusegem, J.M. Stone, I. Denev, C. Laloi, Reactive oxygen species as signals that modulate plant stress responses and programmed cell death, Bioessays 28 (2006) 1091–1101.
[20] G. Zimmermann, H. Baumlein, H. Mock, A. Himmelbach, P. Schweizer, The multigene family encoding germin-like proteins of barley. Regulation and function in basal host resistance, Plant Physiol. 142 (2006) 181–192.
[21] F. Bernier, A. Berna, Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? Plant Physiol. Biochem. 39 (2001) 545–554.
[22] Y. Wei, Z. Zhang, C. Andersen, E. Schmelzer, P. Gregersen, D. Collinge, V. Smedegaard-Petersen, H. Thordal-Christensen, An epidermis/papilla-specific oxalate oxidase-like protein in the defense response of barley attacked by the powdery mildew fungus, Plant Mol. Biol. 36 (1998) 101–112.
[23] M. Mathieu, M.A. Lelu-Walter, A.S. Blervacq, H. David, S. Hawkins, G. Neutelings, Germin-like genes are expressed during somatic embryogenesis and early development of conifers, Plant Mol. Biol. 61 (2006) 615–627.
[24] C. Carter, R.W. Thornburg, Tobacco nectarin I – purification and characterization as a germin-like, manganese superoxide dismutase implicated in the defense of floral reproductive tissues, J. Biol. Chem. 275 (2000) 36726–36733.
[25] T. Yamahara, T. Shiono, T. Suzuki, K. Tanaka, S. Takio, K. Sato, S. Yamazaki, T. Satoh, Isolation of a germin-like protein with manganese superoxide dismutase activity from cells of a moss, *Barbula unguiculata*, J. Biol. Chem. 274 (1999) 33274–33278.
[26] A. Christensen, H. Thordal-Christensen, G. Zimmermann, T. Gjetting, M. Lyngkjaer, R. Dudler, P. Schweizer, The germinlike protein GLP4 exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley, Mol. Plant-Microbe Interact. 17 (2004) 109–117.
[27] S. Guicciardo, J.P. Wisniewski, N.J. Brewin, S. Bornemann, A germin-like protein with superoxide dismutase activity in pea nodules with high protein sequence identity to a putative rhicadhesin receptor, J. Exp. Bot. 58 (2007) 1161–1171.
[28] K. Kondo, K. Yamada, A. Nakagawa, M. Takahashi, H. Morikawa, A. Sakamoto, Molecular characterization of atmospheric NO_2-responsive germin-like proteins in azalea leaves, Biochem. Biophys. Res. Commun. 377 (2008) 857–861.
[29] D. Godfrey, A. Able, I. Dry, Induction of a grapevine germin-like protein (*VvGLP3*) gene is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? Mol. Plant-Microbe Interact. 20 (2007) 1112–1125.
[30] B.G. Lane, A.C. Cuming, J. Fregeau, N.C. Carpita, W.J. Hurkman, F. Bernier, E. Dratewkakos, T.D. Kennedy, Germin isoforms are discrete temporal markers of wheat development, Eur. J. Biochem. 209 (1992) 961–969.
[31] C.J. Park, J.M. An, Y.C. Shin, K.J. Kim, B.J. Lee, K.H. Paek, Molecular characterization of pepper germin-like protein as the novel PR-16 family of pathogenesis-related proteins isolated during the resistance response to viral and bacterial infection, Planta 219 (2004) 797–806.
[32] N. Membre, F. Bernier, D. Staiger, A. Berna, *Arabidopsis thaliana* germin-like proteins: common and specific features point to a variety of functions, Planta 211 (2000) 345–354.
[33] F. Zhou, Z. Zhang, L. Gregersen, J. Mikkelsen, E. de Neergaard, D. Collinge, H. Thordal-Christensen, Molecular characterization of the oxalate oxidase involved in the response of barley to the powdery mildew fungus, Plant Physiol. 117 (1998) 33–41.
[34] J.L. Wu, P.K. Sinha, M. Variar, K.L. Zheng, J.E. Leach, B. Courtois, H. Leung, Association between molecular markers and blast resistance in an advanced backcross population of rice, Theor. Appl. Genet. 108 (2004) 1024–1032.
[35] P.M. Manosalva, R.M. Davidson, B. Liu, X. Zhu, S.H. Hulbert, H. Leung, J.E. Leach, A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice, Plant Physiol. 149 (2009) 286–296.
[36] M. Federico, F. Iniguez-Luy, R. Skadsen, H. Kaeppler, Spatial and temporal divergence of expression in duplicated barley germin-like protein-encoding genes, Genetics 174 (2006) 179–190.
[37] S.G. Cessna, V.E. Sears, M.B. Dickman, P.S. Low, Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant, Plant Cell 12 (2000) 2191–2199.
[38] D. Livingstone, J. Hampton, P. Phipps, E. Grabau, Enhancing resistance to Sclerotinia minor in peanut by expressing a barley oxalate oxidase gene, Plant Physiol. 137 (2005) 1354–1362.
[39] X. Hu, D.L. Bidney, N. Yalpani, J.P. Duvick, O. Crasta, O. Folkerts, G. Lu, Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower, Plant Physiol. 133 (2003) 170–181.
[40] A. Berna, F. Bernier, Regulated expression of a wheat germin gene in tobacco: oxalate oxidase activity and apoplastic localization of the heterologous protein, Plant Mol. Biol. 33 (1997) 417–429.
[41] J. Thompson, T. Gibson, F. Plewniak, F. Jeanmougin, D. Higgins, The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Res. 25 (1997) 4876–4882.
[42] A. Druka, D. Kudrna, C.G. Kannangara, D. von Wettstein, A. Kleinhofs, Physical and genetic mapping of barley (*Hordeum vulgare*) germin-like cDNAs, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 850–855.
[43] G.X. Wang, Y. Chen, J.R. Zhao, L. Li, S.S. Korban, F.G. Wang, J.S. Li, J.R. Dai, M.L. Xu, Mapping of defense response gene homologs and their association with resistance loci in maize, J. Integr. Plant Biol. 49 (2007) 1580–1598.
[44] K. Nobuta, R.C. Venu, C. Lu, A. Belo, K. Vemaraju, K. Kulkarni, W.Z. Wang, M. Pillay, P.J. Green, G.L. Wang, B.C. Meyers, An expression atlas of rice mRNAs and small RNAs, Nat. Biotechnol. 25 (2007) 473–477.
[45] S. Ouyang, W. Zhu, J. Hamilton, H. Lin, M. Campbell, K. Childs, F. Thibaud-Nissen, R.L. Malek, Y. Lee, L. Zheng, J. Orvis, B. Haas, J. Wortman, C.R. Buell, The TIGR rice genome annotation resource: improvements and new features, Nucleic Acids Res. 35 (2007) D883–D887.
[46] H.J. Kim, B.A. Triplett, Cotton fiber germin-like protein. I. Molecular cloning and gene expression, Planta 218 (2004) 516–524.
[47] Z.M. Fan, H.Y. Gu, X.W. Chen, H. Song, Q. Wang, M.H. Liu, L.J. Qu, Z.L. Chen, Cloning and expression analysis of Zmglp1, a new germin-like protein gene in maize, Biochem. Biophys. Res. Commun. 331 (2005) 1257–1263.
[48] M. Ono, K. SageOno, M. Inoue, H. Kamada, H. Harada, Transient increase in the level of mRNA for a germin-like protein in leaves of the short-day plant *Pharbitis nil* during the photoperiodic induction of flowering, Plant Cell Physiol. 37 (1996) 855–861.
[49] Y. Lou, I. Baldwin, Silencing of a germin-like gene in *Nicotiana attenuata* improves performance of native herbivores, Plant Physiol. 140 (2006) 1126–1136.
[50] J. Doll, B. Hause, K. Demchenko, K. Pawlowski, F. Krajinski, A member of the germin-like protein family is a highly conserved mycorrhiza-specific induced gene, Plant Cell Physiol. 44 (2003) 1208–1214.
[51] P. Catalan, E.A. Kellogg, R.G. Olmstead, Phylogeny of Poaceae subfamily Pooideae based on chloroplast ndhF gene sequences, Mol. Phylogenet. Evol. 8 (1997) 150–166.
[52] N.P. Barker, L.G. Clark, J.I. Davis, M.R. Duvall, G.F. Guala, C. Hsiao, E.A. Kellogg, H.P. Linder, R.J. Mason-Gamer, S.Y. Mathews, M.P. Simmons, R.J. Soreng, R.E. Spangler, Phylogeny and subfamilial classification of the grasses (*Poaceae*), Ann. Mo. Bot. Gard. 88 (2001) 373–457.
[53] D. Ober, Seeing double: gene duplication and diversification in plant secondary metabolism, Trends Plant Sci. 10 (2005) 444–449.
[54] B.G. Lane, J.M. Dunwell, J.A. Ray, M.R. Schmitt, A.C. Cuming, Germin, a protein marker of early plant development, is an oxalate oxidase, J. Biol. Chem. 268 (1993) 12239–12242.
[55] M. Sugiura, H. Yamamura, K. Hirano, M. Sasaki, M. Morikawa, M. Tsuboi, Studies on enzymes. 159. Purification and properties of oxalate oxidase form barley seedlings, Chem. Pharm. Bull. 27 (1979) 2003–2007.
[56] B.G. de los Reyes, J.M. McGrath, Cultivar-specific seedling vigor and expression of a putative oxalate oxidase germin-like protein in sugar beet (*Beta vulgaris* L.), Theor. Appl. Genet. 107 (2003) 54–61.
[57] G.S. Liu, D.L. Greenshields, R. Sammynaiken, R.N. Hirji, G. Selvaraj, Y.D. Wei, Targeted alterations in iron homeostasis underlie plant defense responses, J. Cell Sci. 120 (2007) 596–605.
[58] P. Wojtaszek, Oxidative burst: an early plant response to pathogen infection, Biochem. J. 322 (1997) 681–692.
[59] M. Sagi, R. Fluhr, Production of reactive oxygen species by plant NADPH oxidases, Plant Physiol. 141 (2006) 336–340.
[60] A.A. Rodriguez, K.A. Grunberg, E.L. Taleisnik, Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension, Plant Physiol. 129 (2002) 1627–1632.
[61] H. Jin, Endogenous small RNAs and antibacterial immunity in plants, FEBS Lett. 582 (2008) 2679–2684.
[62] M.W. Jones-Rhoades, D.P. Bartel, B. Bartel, MicroRNAS and their regulatory roles in plants, Annu. Rev. Plant Biol. 57 (2006) 19–53.
[63] Y.D. Lu, Q.H. Gan, X.Y. Chi, S. Qin, Roles of microRNA in plant defense and virus offense interaction, Plant Cell Rep. 27 (2008) 1571–1579.
[64] H. Yi, E.J. Richards, A cluster of disease resistance genes in Arabidopsis is coordinately regulated by transcriptional activation and RNA silencing, Plant Cell 19 (2007) 2929–2939.
[65] N.A. Eckardt, Positive and negative feedback coordinate regulation of disease resistance gene expression, Plant Cell 19 (2007) 2700–2702.
[66] P.G. Mohr, D.M. Cahill, Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in *Arabidopsis* infected with *Pseudomonas syringae* pv. *tomato*, Funct. Integr. Genomics 7 (2007) 181–191.
[67] B.A.T. Adie, J. Perez-Perez, M.M. Perez-Perez, M. Godoy, J.J. Sanchez-Serrano, E.A. Schmelz, R. Solano, ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis, Plant Cell 19 (2007) 1665–1681.
[68] J. Ramalingam, C.M. Vera Cruz, K. Kukreja, J.M. Chittoor, J.L. Wu, S.W. Lee, M. Baraoidan, M.L. George, M.B. Cohen, S.H. Hulbert, J.E. Leach, H. Leung, Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice, Mol. Plant-Microbe Interact. 16 (2003) 14–24.
[69] B. Liu, S. Zhang, X. Zhu, Q. Yang, S. Wu, M. Mei, R. Mauleon, J. Leach, T. Mew, H. Leung, Candidate defense genes as predictors of quantitative blast resistance in rice, Mol. Plant-Microbe Interact. 17 (2004) 1146–1152.
[70] H. Chen, S. Wang, Y. Xing, C. Xu, P. Hayes, Q. Zhang, Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 2544–2549.
[71] J.D. Faris, W.L. Li, D.J. Liu, P.D. Chen, B.S. Gill, Candidate gene analysis of quantitative disease resistance in wheat, Theor. Appl. Genet. 98 (1999) 219–225.
[72] G. Wang, D.J. Mackill, J.M. Bonman, S.R. McCouch, R.J. Nelson, RFLP mapping of genes conferring complete and partial resistance to blast resistance in a durably resistant rice cultivar, Genetics 136 (1994) 1421–1434.
[73] Z. Li, S. Pinson, M. Marchetti, J. Stansel, W. Park, Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*), Theor. Appl. Genet. 91 (1995) 382–388.

[74] P. Sirithunya, S. Tragoonrung, A. Vanavichit, N. Pa-In, C. Vongsaprom, T. Toojinda, Quantitative trait loci associated with leaf and neck blast resistance in recombinant inbred line population of rice (*Oryza sativa*), DNA Res. 9 (2002) 79–88.
[75] G. Liu, Y. Jia, F.J. Correa-Victoria, G.A. Prado, A. McClung, J.C. Correll, Mapping quantitative trait loci responsible for resistance to rice sheath blight disease using greenhouse methods, Phytopathology 99 (2009) 1078–1084.
[76] J.H. Zou, X.B. Pan, Z.X. Chen, J.Y. Xu, J.F. Lu, W.X. Zhai, L.H. Zhu, Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.), Theor. Appl. Genet. 101 (2000) 569–573.
[77] S. Srinjvaschary, K.G. Hittalmani, H.E. Kumar, M.G. Shashidhar, Vaishali, Identification of quantitative trait loci associated with sheath rot resistance (*Sarocladium oryzae*) and panicle exertion in rice (*Oryza sativa* L.), Curr. Sci. 82 (2002) 133–135.
[78] L. Albar, M. Lorieux, N. Ahmadi, I. Rimbault, A. Pinel, A.A. Sy, D. Fargette, A. Ghesquiere, Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology, Theor. Appl. Genet. 97 (1998) 1145–1154.
[79] S.N. Alam, M.B. Cohen, Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population, Theor. Appl. Genet. 97 (1998) 1370–1379.
[80] K. Zenbayashi, T. Ashizawa, T. Tani, S. Koizumi, Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32, Theor. Appl. Genet. 104 (2002) 547–552.
[81] T. Eulgem, P.J. Rushton, E. Schmelzer, K. Hahlbrock, I.E. Somssich, Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors, EMBO J. 18 (1999) 4689–4699.
[82] C. Carter, R.A. Graham, R.W. Thornburg, *Arabidopsis thaliana* contains a large family of germin-like proteins: characterization of cDNA and genomic sequences encoding 12 unique family members, Plant Mol. Biol. 38 (1998) 929–943.
[83] S. Fukuoka, K. Okuno, QTL analysis and mapping of pi21, a recessive gene for field resistance to rice blast in Japanese upland rice, Theor. Appl. Genet. 103 (2001) 185–190.
[84] Y.P. Han, Y.Z. Xing, Z.X. Chen, S.L. Gu, X.B. Pan, X.L. Chen, Q.F. Zhang, Mapping QTLs for horizontal resistance to sheath blight in an elite rice restorer line, Minghui 63, Acta Genetica Sinica 29 (2002) 622–626.
[85] N. Huang, A. Parco, T. Mew, G. Magpantay, S. McCouch, E. Guiderdoni, J.C. Xu, P. Subudhi, E.R. Angeles, G.S. Khush, RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a doubled haploid rice population, Mol. Breeding 3 (1997) 105–113.
[86] Z.K. Li, L.J. Luo, H.W. Mei, A.H. Paterson, X.Z. Zhao, D.B. Zhong, Y.P. Wang, X.Q. Yu, L. Zhu, R. Tabien, J.W. Stansel, C.S. Ying, A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*, Mol. Gen. Genet. 261 (1999) 58–63.
[87] S. Pinson, F. Capdevielle, J. Oard, Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines, Crop Sci. 45 (2005) 503–510.
[88] R.E. Tabien, Z. Li, A.H. Paterson, M.A. Marchetti, J.W. Stansel, S.R.M. Pinson, Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties, Theor. Appl. Genet. 105 (2002) 313–324.
[89] C.Z. Liang, P. Jaiswal, C. Hebbard, S. Avraham, E.S. Buckler, T. Casstevens, B. Hurwitz, S. McCouch, J.J. Ni, A. Pujar, D. Ravenscroft, L. Ren, W. Spooner, I. Tecle, J. Thomason, C.W. Tung, X.H. Wei, I. Yap, K. Youens-Clark, D. Ware, L. Stein, Gramene: a growing plant comparative genomics resource, Nucleic Acids Res. 36 (2008) D947–D953.
[90] A.R. Subramanian, M. Kaufmann, B. Morgenstern, DIALIGN-TX: greedy and progressive approaches for segment-based multiple sequence alignment, Algorithms Mol. Biol. 3 (2008) 6.
[91] F. Ronquist, J. Huelsenbeck, MrBayes 3: Bayesian phylogenetic inference under mixed models, Bioinformatics 19 (2003) 1572–1574.
[92] A.J. Drummond, S.Y. Ho, M.J. Phillips, A. Rambaut, Relaxed phylogenetics and dating with confidence, PLoS Biol. 4 (2006) e88.
[93] V.T. Chu, R. Gottardo, A.E. Raftery, R.E. Bumgarner, K.Y. Yeung, MeV+R: using MeV as a graphical user interface for Bioconductor applications in microarray analysis, Genome Biol. 9 (2008) R118.