Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 317 (2004) 1–15
BBRC
www.elsevier.com/locate/ybbrc

Breakthroughs and Views

Rice octadecanoid pathway☆

Ganesh K. Agrawal,[a,b,*] Shigeru Tamogami,[c] Oksoo Han,[d] Hitoshi Iwahashi,[e] and Randeep Rakwal[a,e,*]

[a] *Research Laboratory for Agricultural Biotechnology and Biochemistry (RLABB), GPO Box 8207, Kathmandu, Nepal*
[b] *Plant Functional Genomics Laboratory (PFGL), National Institute of Agrobiological Sciences (NIAS), Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan*
[c] *Laboratory of Growth Regulation Chemistry, Department of Biological Production, Akita Prefectural University, Akita 010-0195, Japan*
[d] *Department of Applied Biotechnology, Agricultural Plant Stress Research Center and Biotechnology Research Institute, Chonnam National University, Kwangju 500-757, Republic of Korea*
[e] *National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*

Received 1 March 2004

Abstract

Plant jasmonic acid (JA) and structurally similar animal prostaglandins play pivotal roles in regulating cellular responses against environmental cues, including the innate immune response(s). In plants, JA and its immediate precursor 12-oxo-phytodienoic acid (OPDA) are synthesized by the octadecanoid pathway, which employs at least five enzymes (lipase, lipoxygenase, allene oxide synthase and cyclase, and OPDA reductase), in addition to the enzymes involved in the β-oxidation steps. Genetic, molecular, and biochemical analyses have led to the identification of almost all the genes of the octadecanoid pathway in *Arabidopsis*—a model dicotyledonous plant. In this regard, rice (*Oryza sativa* L.)—an important socio-economic monocotyledonous model research plant—remains poorly characterized. Until now, no gene has been specifically associated with this pathway. It is therefore of utmost importance to identify, characterize, and assign the pathway specific genes in rice. In this review, we have surveyed the rice genome, extracted a large number of putative genes of the octadecanoid pathway, and discussed their relationship with the known pathway genes from other plant species. Moreover, the achievements made so far on the rice octadecanoid pathway have also been summarized to reflect the contribution of rice towards extending our knowledge on this critical pathway in plants.

Keywords: Defense/stress response; Development; Genomic perspective; Jasmonic acid; 12-Oxo-phytodienoic acid

Plants have evolved remarkable defensive strategies during the millions of years of existence on this planet to protect themselves against a wide range of pests, pathogens, and other abiotic stresses. One of the most prominent early intra-cellular signaling events involved in triggering specific cell responses, including changes in genes, proteins, and metabolites, is the "octadecanoid pathway" ([1–7] and references therein). The octadecanoids have a central role in plant growth and development, and defense (Fig. 1), where most of our knowledge comes from extensive research in dicotyledonous (dicot) species [1–8]. The octadecanoid pathway in rice (*Oryza sativa* L.), a monocotyledonous (monocot) plant, is the least studied. Rice is a first reference model for cereal crops, whose genetic blueprint is now available [9–13]. The importance of rice lies in the fact that it is the main staple food crop for almost half of the world's population, where it plays an essential role in the existence (for around 9000 years) of the human society. Moreover, it has a compact genome (430 Mb) and is evolutionary related with other large genome cereals like sorghum (750 Mb), maize (2500 Mb), barley (5000 Mb), and wheat (15,000 Mb). Availability of rice genome sequence and very recently the full-length cDNA [12] has provided a unique opportunity to survey the genes of a particular pathway genome-wide, using the

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2004.03.020.

* Corresponding authors. Fax: +81-29-861-6066.
E-mail addresses: gkagrawal@onebox.com (G.K. Agrawal), rjunko@nifty.com (R. Rakwal).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.03.020

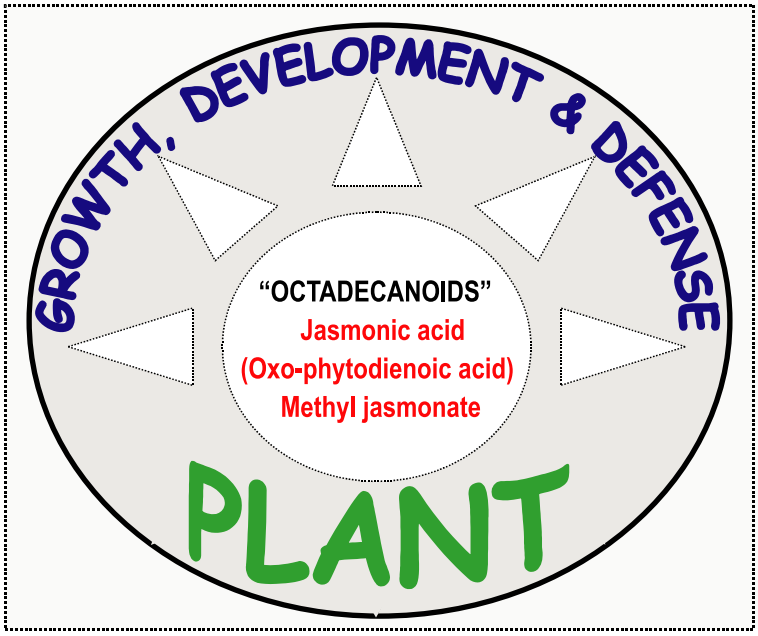

Fig. 1. Central role of the octadecanoids in plant growth, development, and defense.

characterized genes as a reference from other model plants, such as *Arabidopsis*, a dicot model plant. Keeping this in mind and the central role of the octadecanoid pathway in plants, and its functional similarity with the prostaglandins (PGEs) in animals, we have focused on the rice octadecanoid pathway mainly from genomic viewpoint in this review. Our database search of available full-length rice cDNAs (KOME website—http://cdna01.dna.affrc.go.jp/cDNA/ [12]) has revealed the presence of a relatively large number of putative genes (lipoxygenase, LOX; allene oxide synthase, AOS; allene oxide cyclase, AOC; 12-oxo-phytodienoic acid (OPDA) reductase, OPR, and jasmonic acid (JA) carboxyl methyltransferase, JMT), closely related to the assigned genes on the octadecanoid pathway in other model plants, primarily at the sequence level. Moreover, the recent progress seen on the rice octadecanoid pathway has also been discussed.

The octadecanoid pathway

The octadecanoid pathway leads to the production of the cyclopentanones JA and methyl jasmonate (MeJA), which are collectively referred to as jasmonates in plants. Intriguingly, this pathway is strikingly similar to the mammalian immune system, composed of the cyclopentanoic fatty acids such as PGEs and leukotriene A_4 ([2,14] inset, Fig. 2). In animals, the PGE biosynthesis is initiated via the conversion of arachidonic acid (20-carbon fatty acid) to cyclopentanoids by a cyclooxygenase. Arachidonic acid is also oxidized to the 5-hydroperoxide leading to the leukotrienes. A number of reports are there on the presence of PGEs in plants, of which only a few are backed by firm spectroscopic evidence ([2] and references therein). The PGEs, in particular, are involved in variety of physiological regulatory processes in a variety of animal organs, including the immune response [14]. Like PGEs in animals, the jasmonates also function as physiological regulators in a wide range of cellular processes, including development (tendril coiling, tuberization, germination, root growth, fertility, fruit ripening, and senescence) and defense (against pathogens and pests, wounding, abiotic stresses, and secondary metabolism). Commonality between PGEs and jasmonates in both development and immune responses further the importance of these compounds in the plant and animal kingdom.

In plants, JA is the key terminal product of the octadecanoid pathway (Fig. 2). It is synthesized via a series of steps starting with the release of α-linolenic acid (LA) via a lipase, its conversion to (13*S*)-hydroperoxylinolenic acid (13(*S*)-HPLA) by the action of a 13-lipoxygenase (13(*S*)-LOX), followed by sequential action of AOS and AOC giving rise to OPDA-JA precursor. The next step involves reduction of the cyclopentenone ring of OPDA by OPR, the last committed enzymatic step, followed by three cycles of β-oxidation to complete the synthesis of JA. Excellent review articles on JA biosynthesis are also available, and the reader is referred to these for additional details [2,4,15]. JA can be further catabolized by JMT to form its volatile counterpart MeJA [16], an important diffusible molecule involved in both intra- and inter-plant communication (Fig. 2). Apart from JA and MeJA biosynthesis, a variety of other compounds are synthesized from LA and

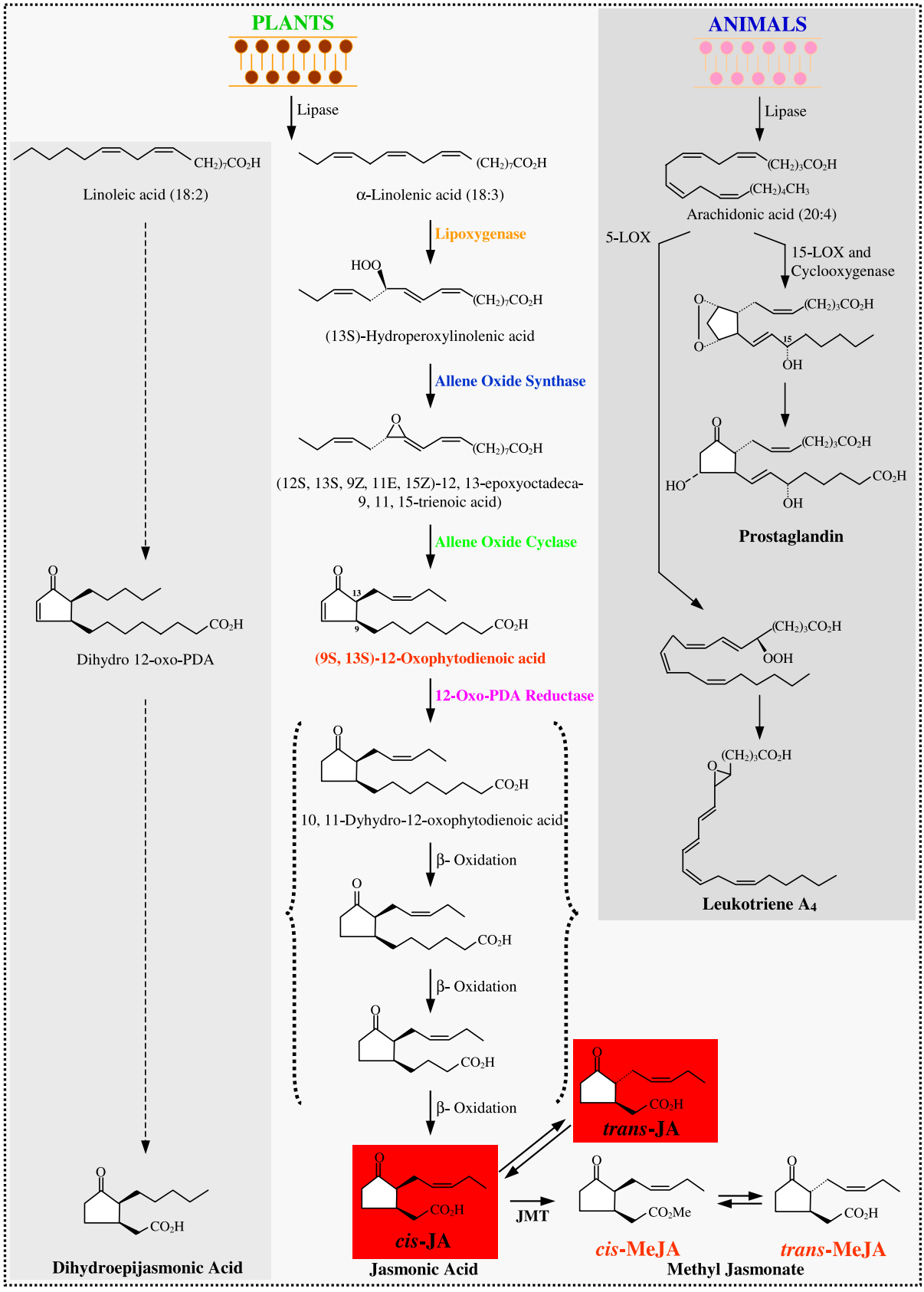

Fig. 2. The octadecanoid pathway leading to jasmonic acid (JA) via 12-oxo-phytodienoic acid (OPDA) biosynthesis in plants. In parallel, the analogous pathway in animals leading to the prostaglandins (PGEs) and leukotriene A_4 synthesis has been presented. Synthesis of dihydroepijasmonic acid from linoleic acid is also presented. Details have been described in the text.

13*S*-hydroperoxy-(9*Z*,11*E*,15)-octadecatrienoic acid (13(*S*)-HPOT) through different branches of the pathway [17]. In addition, dinor-OPDA has been identified in plants, and it is derived directly from plastid 16:3 fatty acid rather than by β-oxidation of 18-carbon OPDA [18]. All the synthesized metabolites from polyunsaturated fatty acids—including jasmonates and octadecanoids—are collectively named as "oxylipins." On the other hand, dihydroJA derived from linoleic acid (left inset, Fig. 2) was first isolated from *Botryodiplodia theobromae* [19]. DihydroJA was detected in *Vicia faba* at very low level [20], however no data on it are available in other plant species, including rice and *Arabidopsis*.

We still do not have much information on the methylation of JA and/or the relationship of endogenous MeJA and free acid (JA) in their roles as signals. The *cis*- and *trans*-isomers of both JA and MeJA are considered to be biologically active [2]. However, a firm conclusion—which isomer is more active—remains to be drawn. To address this question, an active investigation on it is needed to understand the structure–activity relationship, and ultimately the octadecanoid pathway. In rice, a racemic mixture of *cis*- and *trans*-isomers of JA has been mostly used to study phytoalexin production or endogenous analysis of JA in healthy and stressed plants [21,22]. We are currently looking on the activity and occurrence of *cis*- and *trans*-isomers of JA in rice plants.

In the following sections, we have discussed individually each enzyme involved in the octadecanoid pathway, starting from lipase to JMT, for simplicity and clarity. The sequence similarity among the genes has been given as supplementary data. Throughout the text, sequence similarity is mentioned at amino acid level, unless and otherwise stated.

Lipase

Phospholipases and LA are constitutively present in plant tissues. It has been previously suggested that increase in JA in wounded leaves could result from the activation of phospholipases that release LA from membranes [23]. Plant membranes, especially chloroplast membranes, are a rich source of LA esterified in glycerolipids and phospholipids. Early genetic study in *Arabidopsis* revealed that the *fad3-2 fad7-2 fad8* mutant has very low levels of LA, and is unable to accumulate JA in response to wounding [24,25], indicating that the level, distribution or availability of LA could determine the rate of JA biosynthesis. Two recent studies in *Arabidopsis* have at least provided evidence for the involvement of phospholipases (*AtPLDα1* and *AtPLA1*) in the octadecanoid pathway [26,27]. In the case of an *Arabidopsis AtPLDα1* anti-sense transgenic plant having suppressed level of AtPLDα1, the levels of JA and the expression of two JA responsive genes (*AtLOX2* and *AtVSP*) were reduced [26]. The *AtPLA1* gene (*defective in anther dehiscence1*, called *AtDAD1*) in the *Arabidopsis* mutant may function solely to provide JA needed for proper anther and pollen development, but not the wound-induced defense responses [27].

Considering the large number of lipases in plants, and as it is also true for rice, our particular focus on the characterized *Arabidopsis AtPLDα1* and *AtPLA1* genes has revealed the presence of at least three PLD and two DAD1 homologues in rice (Figs. 3A and B). Phylogram analysis reveals that the OsPLD1 is the most close (77% similarity) to AtPLDα1 and forms a group (B) with *Arabidopsis*, tomato, castor bean, and maize PLDs (Fig. 3A). On the other hand, OsPLD2 and OsPLD3 showing slightly lower similarity (67% and 59%, respectively) with AtPLDα1 form a separate group A. The percentage similarity among these lipases is presented in Supplementary Fig. 1. The OsPLD1 is predicted to be localized in the chloroplast stroma (CPS), whereas OsPLD2/3 are most likely to be localized in the microbody (MB). It should be noted that the AtPLDα1 and RcPLD are present in the cytosol (CS), and upon wounding they are translocated to the membranes. This is based on the finding that after wounding, membrane-associated PLDα activity is increased, which is accompanied by a decrease in soluble PLDα activity in leaves [26,28]. On the other hand, OsDAD1 and OsDAD2 have low similarity (34% and 24%, respectively) to AtDAD1 (group A), and do not belong to the same group as AtDAD1 and BrDAD1 (Fig. 3B). Moreover, compared to the chloroplastic (CP) localization of AtDAD1, OsDAD1, and OsDAD2 are predicted to have plasma membrane (PM) and CS localization, respectively. This analysis indicates that like *Arabidopsis*, rice possesses homologous phospholipase (*PLDs and PLA*) genes, and which are the most likely candidates for the octadecanoid pathway.

During the preparation of this review, McGee et al. [29] reported five phospholipase D isoforms from rice cultivar IRBB10 (carrying the *Xa10* gene for bacterial blight resistance), named RPLD1–5. RPLD1, 2, and 3 are almost similar to the OsPLD1, 2, and 3, showing 99.63%, 98.04%, and 99.39% homology, respectively. It should be noted that although both OsPLD1 and RPLD1 are present on chromosome 1, the OsPLD2 and 3 are located on chromosome 6 versus the tandemly arrayed RPLD2 and 3 on chromosome 5. With respect to OsPLD1, 2, and 3, the RPLD1, 2, and 3 show amino acid changes at two (P–S at 139 aa, C–S at 266 aa), twelve (G–D at 111 aa, S–C at 122 aa, G–R at 365 aa, A–G at 387 aa, D–Y at 391 aa, P–A at 395 aa, G–A at 480 aa, A–R at 521 aa, A–P at 551 aa, S–P at 691 aa, G–E at 695 aa, and R–G at 696 aa; possess an extra amino acid W at the 479 position), and five (N–K at 139 aa, G–R at 189 aa, G–E at 217 aa, I–L at 262 aa, and

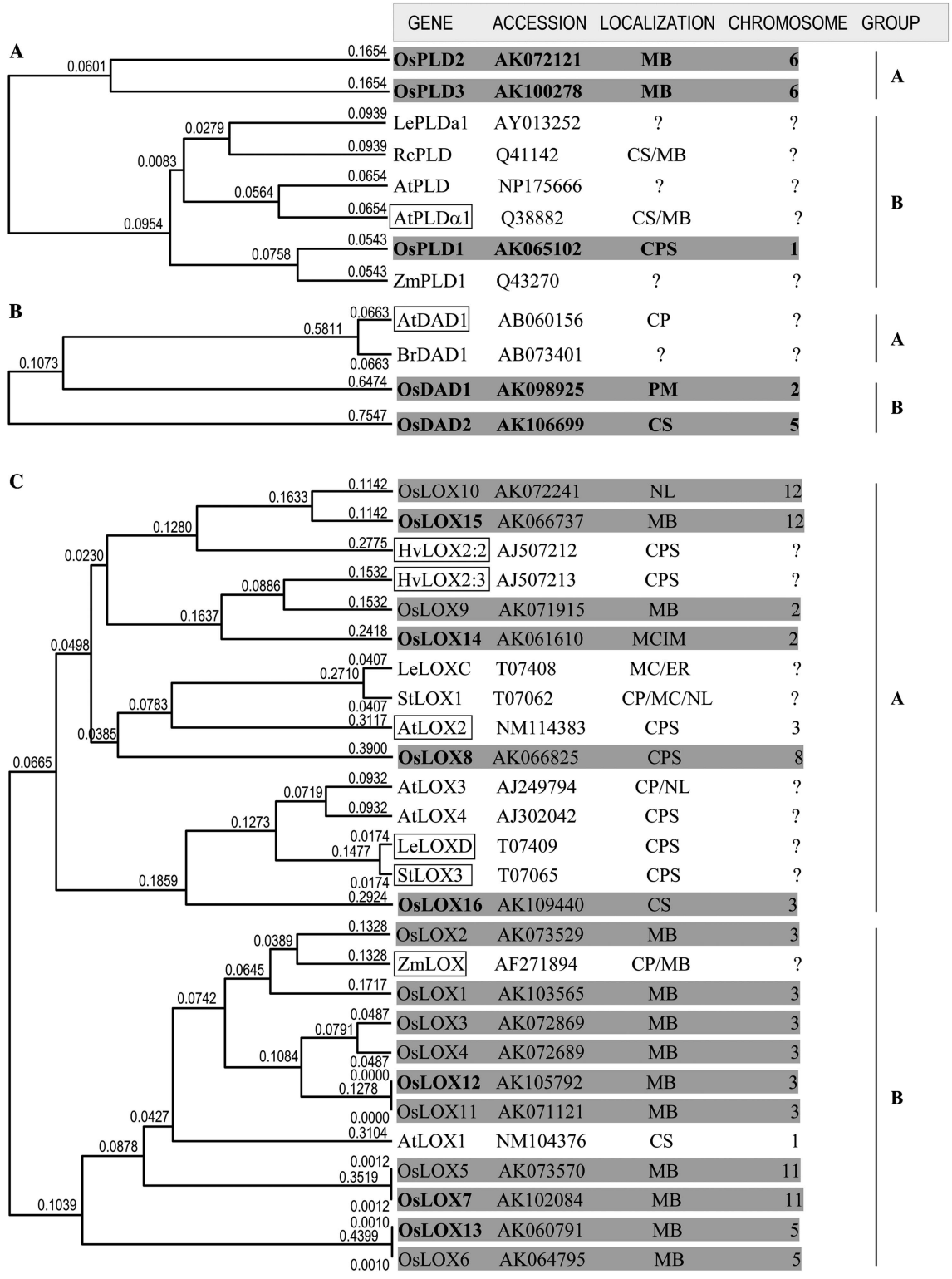

Fig. 3. Phylogenetic tree of rice phospholipases PLD (A) and DAD1 (B), and LOX (C) homologues and related proteins from other species. The phylogenetic tree was constructed by the UPGMA (unweighted pair group method arithmetic means) method, using the Genetyx program (SDC Software Development, Tokyo, Japan). Boxed gene(s) has been assigned on the octadecanoid pathway. Rice genes are shaded, and the candidate rice genes of the octadecanoid pathway have been highlighted in bold. The gene names, their accession numbers, their predicted localization sites (determined using Predotar, PSORT V6.4, and TargetP V1.0, at ExPASy www sever), chromosome position, and grouping are shown. Chromosome position and predicted localization sites of the rice genes are based on the KOME database, except for the previously characterized genes (*OsAOS1*, *OsAOC1*, and *OsOPR1*). *Abbreviations*: CP, chloroplast; CPS, chloroplast stroma; CS, cytosol; ER, endoplasmic reticulum; MB, microbody; MC, mitochondria; MCIM, mitochondrial inner membrane; NL, nucleus; and PM, plasma membrane.

N–S at 498 aa) places, respectively. Immunolocalization with peptide specific antibodies in leaves revealed the presence of RPLD1 in mesophyll cell wall, membranes, and chloroplasts, RPLD2 in the secondary walls of xylem vessels and guard cells, RPLD3 and 4 in the chloroplasts, and RPLD5 in the guard cells. Moreover, *RPLD1* and *2* were shown to be transcriptionally induced by wounding and pathogen (*Xanthomonas oryzae* pv. *oryzae*) infection. It was suggested that these RPLDs might have specific and distinct roles under stress [29].

Lipoxygenase

Most lipoxygenases (LOX, EC 1.13.11.12) are non-heme iron-containing dioxygenases catalyzing the hydroperoxidation of polyunsaturated fatty acids with a *cis*, *cis*-1,4-pentadiene structure [17,30–33]. 13(*S*)-LOX, which utilizes LA to synthesis 13(*S*)-HPLA, forms a major branch of the LOX pathway. Various isozymes of LOX are known in plants, but the physiological functions of specific LOX isozymes are still to be revealed. Accumulating evidence suggests that expression of the specific isoform of LOX may play important role in providing specific octadecanoid signaling molecules depending on the stress factors [17]. A number of LOX isozymes and their corresponding genes have been identified and characterized in plants, such as *Arabidopsis* [34–36], potato [37], tomato [38], barley [39,40], and maize [41]. From these studies, LOX responsiveness to wound- and/or JA/MeJA signals has emerged as one of the characteristic features of LOX genes.

In the *Arabidopsis* genome, 4 *AtLOX* genes have been identified to date, and only *AtLOX1* [34] and *AtLOX2* [35,36] have been characterized. Our analysis reveals the presence of at least 16 LOXs (termed OsLOX1–16) in rice. These genes are broadly classified into 2 groups, A and B (Fig. 3C). Further subclassification of these LOXs will be required, and that will depend on their functional properties. The percentage similarity among these LOXs ranged from 25% to 100% (Supplementary Fig. 2). Moreover, their putative localization sites are also varied, ranging from the MB, CPS, nucleus (NL), mitochondrial inner membrane (MCIM), and to the CS. Interestingly, among the 16 *LOX* genes in the rice genome, 7 LOXs (OsLOX7, 8, 12, 13, 14, 15, and 16; shown in bold letters) are predicted to be involved in fatty acid biosynthesis. OsLOX8 is the same as RLL, a LOX protein previously isolated from shoot and shown to be specific in its response to incompatible host-blast fungus pathogen infection [42]. These 7 LOXs, distributed in groups A and B, are present on 6 different chromosomes (2, 3, 5, 8, 11, and 12) and have a varied subcellular localization. These LOXs have 32–56% similarity to the AtLOX2, required for wound-induced synthesis of jasmonates in *Arabidopsis* leaves [36]. Of these, OsLOX8 is phylogenetically close (46% similarity) to AtLOX2, though OsLOX14, 15, and 16 show more similarity (52%, 54%, and 56%, respectively) with AtLOX2. Interestingly, the OsLOX14 and 15 are highly similar to the barley HvLOX2:3 (72%) and HvLOX2:2 (70%), proteins, whereas OsLOX16 is 68% and 69% similar to the tomato LeLOXD and potato StLOX3 proteins, respectively. All these characterized barley [39,40], tomato [38], and potato [37] LOXs are proposed to be important components of the octadecanoid pathway. Hence, these 4 LOXs (OsLOX8, 14, 15, and 16), belonging to the same group (A) as AtLOX2, LeLOXD, StLOX3, and HvLOX2:2/2:3, may be assigned as potential candidates for the octadecanoid pathway. The OsLOX7, 12, and 13 proteins, classified in group B, are close to the AtLOX1 (44%, 59%, and 48% similarities) and ZmLOX (52%, 71%, and 52% similarities) proteins. Interestingly, all OsLOXs of this group are localized to MB. In group B, ZmLOX is the best characterized. ZmLOX has recently been reported to be a non-traditional dual positional specific enzyme potentially involved in JA biosynthesis [43]. It was shown that the ZmLOX mRNA expression and accumulation of endogenous JA in wounded leaves occurred within 1 h. This analysis suggests that the OsLOX7, 12, and 13 might have dual positional specificity and may also be involved in the octadecanoid pathway. However, to come to any definitive conclusions, we need a complete biochemical and physiological characterization of at least these OsLOXs, as a first step. It is important to note that given the high number of LOXs in rice, it would not be surprising to presume that these LOXs might have a tissue specific expression with varying degree of responses to environmental stresses, and may control the JA level in different organs.

Allene oxide synthase

AOS is a cytochrome P450 enzyme of the CYP74A subfamily [44]. It is one of the key enzymes of the octadecanoid pathway, catalyzing the conversion of 13(*S*)-HPLA to produce 13(*S*)-HPOT. AOS cDNAs have been cloned and characterized in mainly dicot plants [44–46] and recently from two monocot species, barley [47] and rice [48,49]. In dicots, importance of AOS in JA biosynthesis comes from transgenic plants overexpressing the *AOS* gene [50–52]. Transgenic potato plants overexpressing the flax AOS showed increased JA level [50]. On the other hand, transgenic *Nicotiana tabacum* and *Arabidopsis* plants overexpressing AOS did not show increased JA level in control unwounded leaves, however these plants produced significantly more JA upon wounding [51]. These two studies suggested that either the amount of AOS protein or the production of LA or 13(*S*)-HPLA limits JA biosynthesis. Knock-out

mutants of AOS, exhibiting male-sterile phenotype, have been isolated from *Arabidopsis* (*aos*, [53]; *dde2-2*, [54]). The male-sterile phenotype was completely rescued by application of methyl jasmonate and by complementation with constitutive expression of the *AOS* gene. Wounding caused 100-fold increase in endogenous JA levels within 1 h in wild-type plants, but not in the *aos* mutant [53].

Our database search indicates that besides the characterized *OsAOS1* (*OsAOS* [49]), there are at least 4 additional *OsAOS* (*OsAOS2-5*) genes in the rice genome (Fig. 4A). Based on the phylogenetic tree, AOS proteins from different plant species could be categorized into 5 groups (A–E). Rice AOS proteins belong to groups A (OsAOS1, 2, and 3), B (OsAOS4), and E (OsAOS5). Most of the *OsAOS* genes are located on chromosome 3, except for the *OsAOS3* gene, which is on chromosome 2. The OsAOS proteins show putative localization sites ranging from mitochondria (MC), endoplasmic reticulum (ER), PM, MB, and to CS (OsAOS5). The AtAOS1 and LeAOS1 proteins along with HvAOS1, which interestingly does not contain a CP localization signal, were shown to localize to CP by immunocytochemical analysis. No experimental data on the localization of rice OsAOS proteins are available to date. Additional features on the rice OsAOS1 along with the other related AOS proteins are discussed elsewhere [49]. In group A, OsAOS1–3 along with the HvAOS1 and 2, and TaAOS form a separate subgroup consisting of the monocot AOS proteins. The only dicot AOS protein in group A is the tomato LeAOS3. OsAOS1–4 proteins are close to the monocot HvAOS1 and 2. OsAOS1 and OsAOS2 are 100% similar to each other, except for first 61 N-terminal amino acids in OsAOS2. OsAOS1 showed 47%, 51%, and 12% similarity to OsAOS3, 4, and 5 proteins, respectively (Supplementary Fig. 3). It should be noted that OsAOS5 shows the lowest similarity (11–14%) with the predicted AOS proteins, raising a doubt whether or not OsAOS5 is a member of the AOS gene family. Except OsAOS5, the percentage similarity between the rice AOS proteins with others ranges from 36% to 74%, respectively (for more details see Supplementary Fig. 3).

Allene oxide cyclase

AOC catalyzes the stereospecific cyclization of an unstable allene oxide to OPDA, the ultimate precursor of JA [4]. OPDA formed by this enzymes is the *cis*-(+) enantiomer having a 9*S*, 13*S* configuration. The first plant *AOC* gene cloned was the tomato *LeAOC* [55], and its specific occurrence in all vascular bundles and in flower tissues of tomato was seen [56]. Recently, the rice *OsAOC1* gene was also cloned and its expression was characterized against a variety of environmental cues [57]. In parallel, *Arabidopsis AOC* (*AtAOC1–4*) family genes were cloned and characterized with respect to various stresses, enzyme activity, and localization [58]. It was shown that the AOC genes in rice (*OsAOC1*, previously called *OsAOC* [57]) and in *Arabidopsis* (*AtAOC2*) are differentially regulated upon wounding, JA treatment, and other stresses. Furthermore, *AtAOC2* expression was detected locally and systemically in rosette leaves [58]. All the four AtAOCs (1–4) were enzymatically active, as they were able to form *cis*-(+)-OPDA, with AtAOC2 having the highest activity. However, it remains unknown, which one of these AtAOCs is actually involved in JA biosynthesis.

In rice, one more *AOC* gene, *OsAOC2*, located on chromosome 3 is identified (Fig. 4B). OsAOC1 is almost identical to OsAOC2, except for a single amino acid change, from L to I at position 90 from the first methionine. Phylogram reveals that rice OsAOCs form a separate group (C) among the broadly classified four groups (A–D). All *Arabidopsis* AtAOCs are clustered together in group D. All the four AtAOC proteins were found to be localized in the chloroplast as revealed by immunocytochemical analysis, using an anti-AOC2 antibody [58]. The tomato LeAOC protein also carries a chloroplast transit peptide and was shown to have chloroplastic localization by immunoblotting [55]. Given the presence of a chloroplast transit peptide signal in the N-terminal amino acid sequence of OsAOC1 and 2, and considerable similarity (50–64%, see Supplementary Fig. 4) with the AOC proteins from *Arabidopsis* and tomato, it is reasonable to suspect localization of OsAOCs to chloroplasts. Additional sequence features on AOC proteins are given elsewhere [57,58].

Oxo-phytodienoic acid reductase

OPR catalyzes the reduction of OPDA to 10,11-dihydro-12-oxophytodienoic acid (OPC 8:0), which is converted to the final product JA, by three rounds of β-oxidation. The β-oxidation pathway contains enzymes that degrade fatty acids by the sequential removal of two carbon units. In plants, β-oxidation is thought to occur almost entirely in glyoxysomes and peroxisomes. Among various plants species, the *Arabidopsis* and tomato are well characterized with respect to OPRs. In the *Arabidospis* genome, three *AtOPR* genes (*AtOPR1–3*) are known. *AtOPR3* is involved in JA biosynthesis because the encoded protein was demonstrated to efficiently reduce the natural isomer (9*S*, 13*S*) of OPDA to OPC 8:0 [59,60]. *AtOPR1* and *AtOPR2* encoded proteins had little ability to catalyze this step. Identification and characterization of AtOPR3 mutants further support the involvement of AtOPR3 in JA biosynthesis. AtOPR3 mutants (*dde1*, [61]; *opr3*, [62]) were found to be male-sterile. In leaves of the *opr3* mutant, OPDA

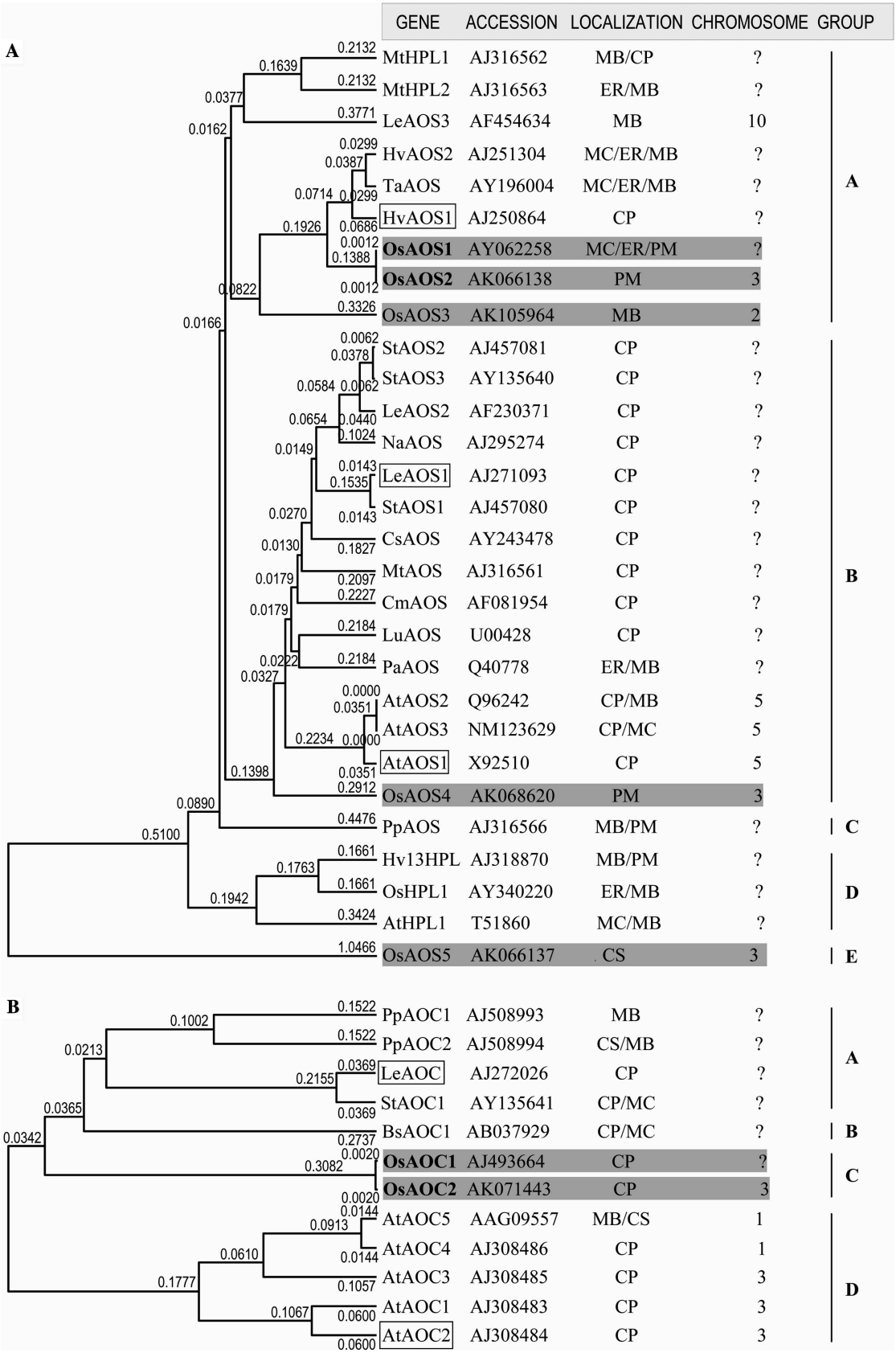

Fig. 4. Phylogenetic tree of AOS (A) and AOC (B) homologues and related proteins from other species. *Abbreviations*: CP, chloroplast; CPS, chloroplast stroma; CS, cytosol; ER, endoplasmic reticulum; MB, microbody; MC, mitochondria; and PM, plasma membrane. Rest is the same as mentioned in Fig. 3.

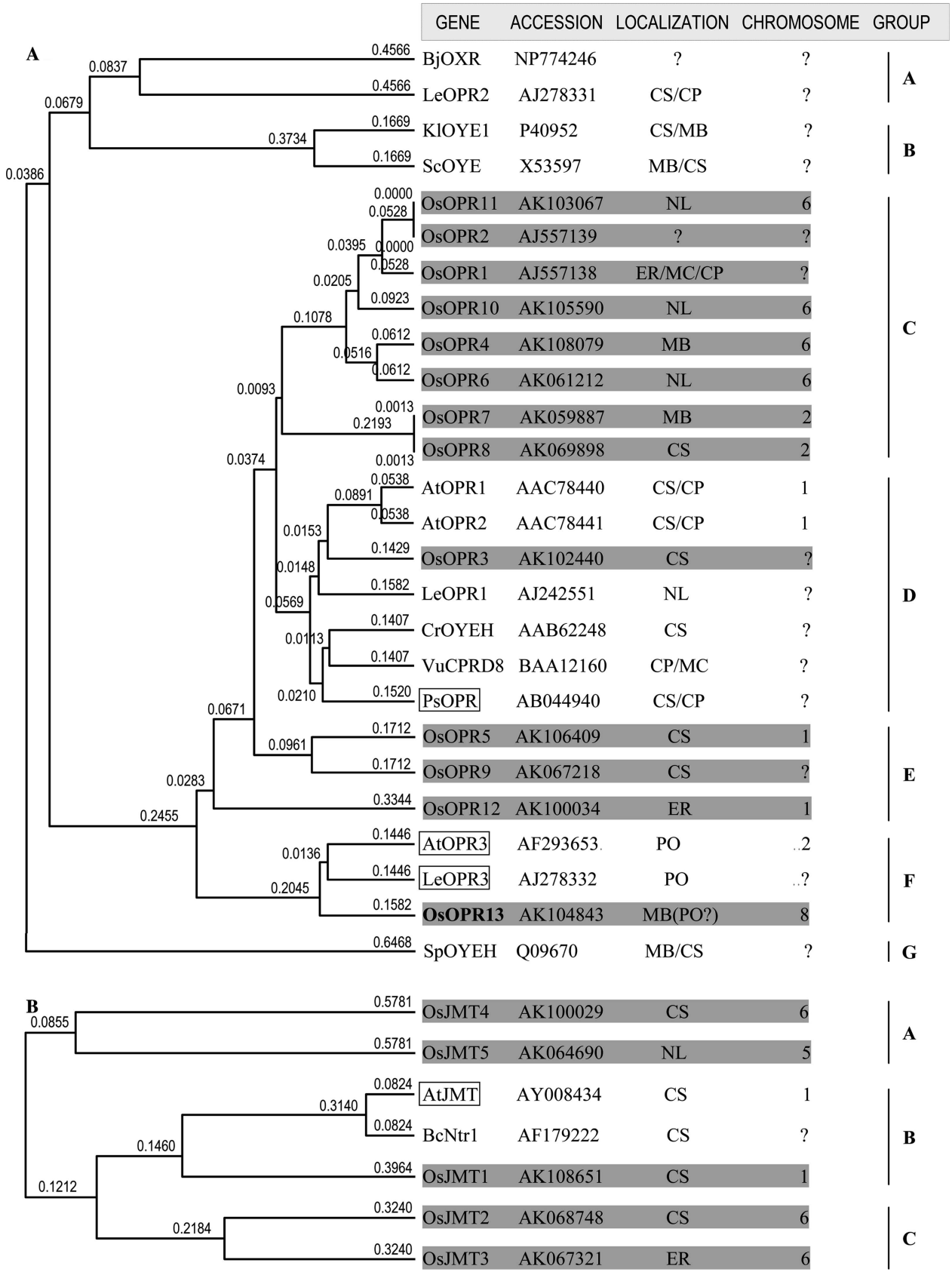

Fig. 5. Phylogenetic tree of OPR (A) and JMT (B) homologues and related proteins from other species. *Abbreviations*: CP, chloroplast; CPS, chloroplast stroma; CS, cytosol; ER, endoplasmic reticulum; MB, microbody; MC, mitochondria; NL, nucleus; PM, plasma membrane; and PO, peroxisome. Rest is the same as mentioned in Fig. 3.

accumulated upon wounding and plants were resistant to fungal and insect attack [63]. Tomato also has three *LeOPR* genes, *LeOPR1–3* [60]. The LeOPR3 has been placed on the octadecanoid pathway, based on the biochemical and cDNA microarray analyses [60]. LeOPR3 is closely related to AtOPR3, with respect to primary structure and substrate specificity, catalyzing the formation of 9*S*, 13*S*- and 9*R*, 13*R*-OPC 8:0 [60]. Using confocal laser microscopy of transiently expressed GFP fusion proteins, immunolocalization, and subcellular fractionation, the LeOPR3 and AtOPR3 proteins were shown to localize in the peroxisomes in tomato and *Arabidopsis*, respectively [60]. A pea OPR gene, *PsOPR* (originally called *PsOPDAR*) was cloned and characterized [64]. Upon examining the NADPH-dependent activity of the recombinant PsOPR protein, a higher affinity to OPDA than to 2-cyclohexen-1-one, a model substrate, suggesting the compatibility-specific activation of the octadecanoid pathway [64]. However, it should be noted that in presence of 2-cyclohexen-1-one as a substrate, all NADPH was consumed, however in presence of OPDA as a substrate, only half the NADPH was consumed. As the OPDA used in their system was the racemic mixture of the two *cis* stereoisomers, it is possible that PsOPR might have catalyzed only one stereoisomer of (9*S*, 13*S*)-OPDA or (9*R*, 13*R*)-OPDA. Moreover, as PsOPR has high similarity with AtOPR1 and 2, rather than AtOPR3, the possibility cannot be ruled out that PsOPR involves in the production of other JA-related compounds [64].

Surprisingly, rice has at least 13 OPRs (*OsOPR1–13*), a number that is dramatically higher than that seen in any plant species (Fig. 5A). The rice OsOPRs are tentatively classified into 4 groups (C–F). Groups C and E contain exclusively rice OsOPRs, whereas, OsOPR3 forms a separate group along with the other dicot OPRs. OsOPR13 belongs to group F along with the AtOPR3 and LeOPR3. Additional sequence features on the OPR proteins are published elsewhere [65]. Among 13 OsOPRs, OsOPR1 is the only gene characterized at transcriptional and enzymatic levels [65,66]. OsOPR1 catalyzes the reduction of (−)-*cis*-12-OPDA preferentially over (+)-*cis*-12-OPDA, a natural precursor of JA, indicating that OsOPR1 has OPR activity similar to those of AtOPR1/2 and LeOPR1. In this context, OsOPR1 may not be solely assigned on the octadecanoid pathway. Hence, which rice OsOPR is involved in JA biosynthesis remains unknown. OsOPR13 is the most potent candidate based on primary structure and phylogenetic relationship with AtOPR3 and LeOPR3. OsOPR13 has 70% and 71% similarity with the AtOPR3 and LeOPR3 proteins, respectively (Supplementary Fig. 5). LeOPR3 and AtOPR3 resemble each other and have 74% similarity. Other OsOPRs only show 40–54% similarities with these two functional OPRs. Rice OPR proteins have 43–100% similarity to each other.

JA carboxyl methyltransferase

The first *JMT* gene was isolated and characterized from *Arabidopsis* [16]. As JMT does not carry any transit signal peptides, it is presumably a cytoplasmic enzyme. Its expression was detected in almost all parts of mature plants, but not in young seedlings. Furthermore, JMT was shown to be induced by both wounding and MeJA. Its functional analysis was revealed in a transgenic experiment overexpressing the *AtJMT* gene. These transgenic plants were found to have elevated levels of genes responsive to jasmonate, even in the absence of wounding or jasmonate treatment. Moreover, transgenic plants had enhanced level of resistance against virulent fungus *Botrytis cinerea*. Induction of *AtLOX2* and *AtAOS* in *AtJMT* overexpressing plants suggests that AtJMT is part of feedback mechanism that could provide more substrate for MeJA synthesis and further induce jasmonate responsive genes [16]. Previously, a floral nectary-specific gene *BcNtr1* was isolated from *Brassica campestris*, and is a homologue of *AtJMT* [67]. However, it remains to be understood how MeJA biogenesis is regulated at the molecular level, and how it relates to jasmonate-responsive gene activation [7]. Nevertheless, characterization and transgenic research of the cellular component (JMT) that catalyzes the formation of MeJA has further increased our knowledge on the complexity of jasmonate-mediated plant responses.

In rice, we have identified 5 putative JMTs (named OsJMT1–5) having structural similarity with AtJMT (Fig. 5B). OsJMT1–5 possess the methyltransferase-7 domain—a *S*-adenosyl-L-methionine dependent carboxyl methyltransferase family signature. These putative rice JMTs show 27–39% and 28–35% similarities with the AtJMT and BcNtr1 proteins, respectively (Supplementary Fig. 6). Phylogram analysis reveals that OsJMT1 is the most close to AtJMT and BcNtr1 proteins, and having the same localization site (CS). On the other hand, OsJMT2/3 and OsJMT4/5 form a separate branch, and hence the JMTs can be broadly divided into 3 groups, A–C. It is interesting to note that OsJMT3 in group C, and OsJMT5 in group A, show putative ER and NL localizations, respectively. Considering the low similarity of OsJMTs with AtJMT/BcNtr1, it is necessary to know which OsJMT catalyzes methylation of JA.

Octadecanoid pathway transcripts and their significance

Detailed transcriptional profiling is one of the ways to probe the functional/physiological significance of a gene. The octadecanoid pathway transcript (OPTs) profiles (*OsAOS1*, *OsAOC1*, and *OsOPR1*), available to date in rice in development and against diverse biotic

and abiotic stresses [49,57,65], have been summarized and depicted in Fig. 6. The expression of the *OsAOC1* and *OsOPR1* genes, but not of *OsAOS1*, was observed during the vegetative and reproductive stages of young and mature plants (Fig. 6A, for further details see [57]). Moreover, the rhythmic expression of the *OsAOC1* gene was reported in the leaves of seedlings [57]. But it remains unknown whether a circadian clock controls the observed rhythmic expression of *OsAOC1*. To date, rhythmic expression of any other octadecanoid pathway genes is not known, and which will be worth investigating in other plants. Therefore, *OsAOC1* and *OsOPR1* appear to be part of a developmental program involved in rice growth and reproduction.

Among these genes, only *OsAOS1* was studied against blast pathogen (*Magnaporthe grisea*) attack (Fig. 6A).

A

GENE	Developmental Regulation			Rhythmic	Pathogen (M. grisea)	
	Leaf (Young/Mature)	Leaf Sheath (Young)	Flower	Young	Incompatible	Compatible
OsLOX	–	–	–	–	–	–
OsAOS1	NO/NO	NO	NO	NO	+++	+
OsAOC1	YES/YES	YES	YES	YES	–	–
OsOPR1	NO/YES	YES	YES	NO	–	–
OsJMT	–	–	–	–	–	–

B

TREATMENT		EARLY (within 120 min)					LATE (after 3 h)				
		OsLOX	OsAOS1	OsAOC1	OsOPR1	OsJMT	OsLOX	OsAOS1	OsAOC1	OsOPR1	OsJMT
	Wounding	–	△	▲	△	–	–	△	▲	–	–
Signaling Molecules	JA	–	▲	▲	▲	–	–	▲	△	–	–
	JA+SA	–	■	△	▲	–	–	–	–	–	–
	JA+ET	–	■	△	▲	–	–	–	–	–	–
	MeJA	–	–	–	–	–	–	–	–	–	–
	SA	–	△	▲	▲	–	–	▲	△	–	–
	SA+ET	–	■	△	▲	–	–	–	–	–	–
	ABA	–	△	▲	△	–	–	△	▼	–	–
	ET	–	△	▲	△	–	–	△	▼	–	–
	H_2O_2	–	△	▲	△	–	–	△	△	–	–
Protein Synthesis Inhibitors	CHX	–	▲	▲	▲	–	–	–	–	–	–
	JA+CHX	–	▲	▲	▲	–	–	–	–	–	–
	SA+CHX	–	▲	▲	▲	–	–	–	–	–	–
Protein Phosphatase Inhibitors	CN	–	▲	▲	▲	–	–	▲	△	–	–
	EN	–	▲	▲	▲	–	–	▲	△	–	–
	OA	–	▲	▲	▲	–	–	▲	△	–	–
Osmolytes	NaCl	–	–	–	▲	–	–	–	–	–	–
	Sucrose	–	–	–	▲	–	–	–	–	–	–
Environmental Stress Factors	Drought	–	–	–	▲	–	–	–	–	–	–
	42°C	–	–	–	–	–	–	–	–	–	–
	37°C	–	–	–	■	–	–	–	–	–	–
	12°C	–	–	–	■	–	–	–	–	–	–
	4°C	–	–	–	–	–	–	–	–	–	–
	O_3	–	–	–	▲	–	–	–	–	–	–
	SO_2	–	–	–	▲	–	–	–	–	–	–
	UV-C	–	–	–	▲	–	–	–	–	–	–
Heavy Metals	Copper	–	▲	▲	▲	–	–	▲	■	–	–
	Cadmium	–	▲	▲	▲	–	–	–	–	–	–
	Mercury	–	▲	▲	▲	–	–	–	–	–	–
Elicitors	CT	–	△	▲	▲	–	–	▲	▲	–	–
	BTH	–	–	–	–	–	–	–	–	–	–
	PBZ	–	–	–	–	–	–	–	–	–	–
	INA	–	–	–	–	–	–	–	–	–	–

Fig. 6. Response of rice octadecanoid pathway genes *OsAOS1*, *OsAOC1*, and *OsOPR1*. (A) Developmental, rhythmic, and pathogen response. (B) Effect of diverse environmental factors. Yes or no indicates presence of absence of the octadecanoid pathway transcripts. +++ indicates strong induction versus the weak induction (+). Upward and downward triangles indicate up- and down-regulation, respectively. Filled and open triangles indicate strong and weak expression levels, respectively. No change in expression is indicated by filled square. Gene that is not investigated is shown by dash. Details are mentioned in the text.

It was reported that the *OsAOS1* mRNA potently accumulated within 24 h in an incompatible versus compatible plant–pathogen interaction, suggesting a possible role for the octadecanoid pathway in rice plant response to pathogen [49]. It is quite apparent from Fig. 6B that all the genes (*OsAOS1*, *OsAOC1*, and *OsOPR1*) are dramatically and differentially regulated in leaves of two-week-old seedlings by various treatments. These data indicate that the octadecanoid pathway in rice is triggered by diverse environmental cues, a finding similar to that in dicot plants [6,58,60]. Furthermore, the expression profiles reveal several important features of the rice octadecanoid pathway genes. For example, cycloheximide (CHX, a protein synthesis inhibitor) and protein phosphatase (PP) inhibitor treatment of leaves potently enhanced the mRNA levels within 120 min (EARLY, Fig. 6B). CHX treatment alone or with signaling molecules (JA and SA) caused substantial increase in the OPTs with time upto 120 min, disrupting the transient behavior of the genes, indicating a possible role for negative *trans*-acting factor(s) in regulating these genes. Treatment with PP inhibitors cantharidin, endothall, and okadaic acid revealed different induction kinetics of the OPTs, suggesting that phosphorylation/dephosphorylation events of a kinase-signaling cascade(s) might be involved in early activation of the genes.

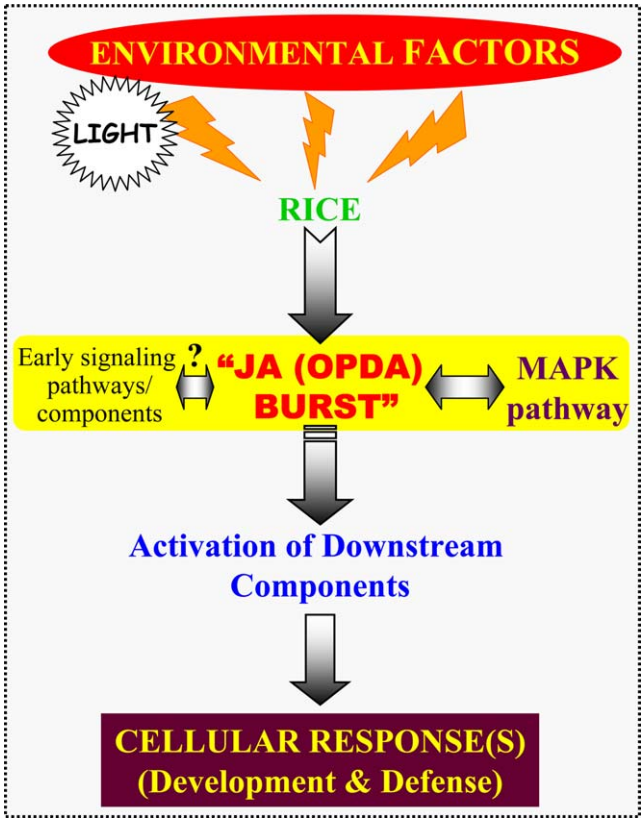

Fig. 7. The octadecanoid pathway plays a crucial role in rice plant response to environmental factors. Details are mentioned in the text.

JA (OPDA) burst

"JA (OPDA) burst" was recently reported in rice [22]. The burst was very rapid, detected within a few minutes in leaves of two-week-old seedlings upon wounding and fungal elicitor chitosan treatment. This finding implied that JA (OPDA) is an early signaling molecule in rice. Subsequent work revealed induction of an early signaling pathway—the mitogen-activated protein kinase (MAPK) cascade—by applied JA [68]. Mitogen-activated protein kinase (MAPK) cascade is one of the well-defined and characterized early signaling pathways that serve as a link in various ways between upstream receptors and downstream targets [68,69]. It is important to note that, to date, JA is not known to induce MAPKs in dicot plants. In Fig. 7, we have schematically presented how rice plants respond to a variety of environmental factors by triggering the rapid production of JA and OPDA followed by activation of downstream components to the cellular responses (Fig. 7). In this model, the MAPK pathway has been placed in parallel with the octadecanoid pathway based on our interpretation on wound (a common stress) signaling (for details see review [70]). How the octadecanoid and the MAPK pathways interact or coordinate each other against different environmental factors remains a question, and a subject of intense study in rice. Moreover, how the octadecanoid pathway interacts with other early signaling pathways/components is also largely unknown. Interestingly, OPT profiles have suggested a novel role for light signals in regulating the octadecanoid pathway components [49,57]. A strong support for this notion was recently revealed by the first octadecanoid pathway rice mutant *hebiba* [71].

Conclusions

The genomic overview presented on the rice octadecanoid pathway has helped us in identifying most, if not all, of the putative genes—a number significantly higher than that imagined, based on the *Arabidopsis* genome. Moreover, a comparison with the characterized pathway genes from other plant species, including *Arabidopsis* and tomato in particular, has resulted in predicting the rice genes that might encode functional enzymes of this pathway. For example, out of the 13 OsOPRs, OsOPR13 is the most likely candidate for conversion of OPDA to OPC 8:0, and so it can be selected for further biochemical and functional analysis. Based on genomic perspective, and the results summarized on the OPT profiles and the "JA (OPDA) burst," it is reasonable to consider the octadecanoid pathway as a "master switch" for a myriad of cellular responses in rice, and in plants, in general.

Future perspectives

Despite important progress made on the rice octadecanoid pathway, our knowledge on this crucial pathway is highly limited. We yet do not know the genes encoding enzymes involved in the octadecanoid pathway, leading to OPDA and JA biosynthesis. A systematic study on the candidate genes, as a first step, will undoubtedly accelerate and increase our understanding of the rice octadecanoid pathway, eventually leading to a greater insight into the plant immune response and growth and reproduction. To achieve the goal, future experiments should be directed towards: (1) identification of genes encoding functional proteins involved in JA biosynthesis, their localization, and their behavior against diverse environmental cues at transcript and protein levels, and (2) dissecting their function using either a transgenic approach (overexpression and RNA interference) or finding their mutants. It is also important to investigate those genes, not specifically involved in the octadecanoid pathway, to know their physiological function(s). For example, as predicted *OsOPR13* might act on the pathway, leaving open the question on the importance of the other 12 *OPRs* in rice. These studies will give us basic information on the octadecanoid pathway and resources, which will then help in addressing questions on the regulation and mode of action of the octadecanoid pathway. It is also important to investigate the role of the octadecanoid pathway in plant defense against pathogen attack. Whether JA and/or OPDA contribute to the establishment of compatibility/incompatibility is an important issue, and that needs to be addressed. Last but not the least, exploration of the octadecanoid pathway in different plant species is needed to establish a biological model that can explain more precisely the regulation and function of the octadecanoid pathway in distantly related plants.

Acknowledgments

R.R. and G.K.A. work at AIST and NIAS at the Tsukuba Science City, supported by the Japan Society for Promotion of Science (JSPS). We thank Drs. Vishwanath Prasad Agrawal (RLABB), Masami Yonekura (Ibaraki University, Japan), Akihiro Kubo (National Institute of Environmental Studies, Japan) and Nam-Soo Jwa (Sejong University, Korea), and Ms. Junko Shibato (AIST) for their encouragement and immense support.

References

[1] M. Hamberg, H.W. Gardner, Oxylipin pathway to jasmonates: biochemistry and biological significance, Biochim. Biophys. Acta 1165 (1992) 1–18.
[2] M.H. Beale, J.L. Ward, Jasmonates: key players in the plant defence, Nat. Prod. Rep. 15 (1998) 533–548.
[3] G.A. Howe, Cyclopentenone signals for plant defense: remodeling the jasmonic acid response, Proc. Natl. Acad. Sci. USA 98 (2001) 12317–12319.
[4] F. Schaller, Enzymes of the biosynthesis of octadecanoid-derived signaling molecules, J. Exp. Bot. 52 (2001) 11–23.
[5] H. Weber, Fatty acid-derived signals in plants, Trends Plant Sci. 7 (2002) 217–224.
[6] C. Wasternack, B. Hause, Jasmonates and octadecanoids: signals in plant stress responses and plant development, Prog. Nucleic Acid Res. 72 (2002) 165–221.
[7] J.-J. Cheong, Y.D. Choi, Methyl jasmonate as a vital substance in plants, Trends Genet. 19 (2003) 409–413.
[8] B.A. Vick, D.C. Zimmermann, The biosynthesis of jasmonic acid: a physiological role for plant lipoxygenase, Biochem. Biophys. Res. Commun. 111 (1983) 470–477.
[9] K. Shimamoto, J. Kyozuka, Rice as a model for comparative genomics of plants, Annu. Rev. Plant Biol. 53 (2002) 399–419.
[10] S.A. Goff, D. Ricke, T.-H. Lan, G. Presting, R. Wang, et al., A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*), Science 296 (2002) 92–100.
[11] J. Yu, S. Hu, J. Wang, G.K.-S. Wong, S. Li, et al., A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*), Science 296 (2002) 79–92.
[12] S. Kikuchi, K. Satoh, T. Nagata, N. Kawagashira, K. Doi, et al., Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice, Science 301 (2003) 376–379.
[13] The Rice Chromosome 10 Sequencing Consortium, In-depth view of structure, activity, and evolution of rice chromosome 10, Science 300 (2003) 1566–1569.
[14] M.J. Mueller, Radically novel prostaglandins in animals and plants: the isoprostanes, Chem. Biol. 5 (1998) R323–R333.
[15] M.J. Mueller, Enzymes involved in jasmonic acid biosynthesis, Physiol. Plant. 100 (1997) 653–663.
[16] H.S. Seo, J.T. Song, J.J. Cheong, Y.H. Lee, Y.W. Lee, I. Hwang, J.S. Lee, Y.D. Choi, Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses, Proc. Natl. Acad. Sci. USA 98 (2001) 4788–4793.
[17] I. Feussner, C. Wasternack, The lipoxygenase pathway, Annu. Rev. Plant Biol. Physiol. Plant Mol. Biol. 53 (2002) 275–279.
[18] H. Weber, B.A. Vick, E.E. Farmer, Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate family, Proc. Natl. Acad. Sci. USA 94 (1997) 10473–10478.
[19] O. Miersh, A. Preiss, G. Sembdner, K. Schreiber, (+)-7-Iso-jasmonic acid and related compounds from *Botryodiplodia theobromae*, Phytochemistry 26 (1987) 1037–1039.
[20] H. Gundlach, M.H. Zenk, Biological activity and biosynthesis of pentacyclic oxylipins: the linoleic acid pathway, Phytochemistry 47 (1998) 527–537.
[21] S. Tamogami, O. Kodama, Quantification of amino acid conjugates of jasmonic acid in rice leaves by high-performance liquid chromatography–turboionspray tandem mass spectrometry, J. Chromatogr. A 822 (1998) 310–315.
[22] R. Rakwal, S. Tamogami, G.K. Agrawal, H. Iwahashi, Octadecanoid signaling components "burst" in rice (*Oryza sativa* L.) seedling leaves upon wounding by cut and treatment with fungal elicitor chitosan, Biochem. Biophys. Res. Commun. 295 (2002) 1041–1045.
[23] E.E. Farmer, C.A. Ryan, Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors, Plant Cell 4 (1992) 129–1134.
[24] M. McConn, J. Browse, The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant, Plant Cell 8 (1996) 403–416.
[25] M. McConn, R.A. Creelman, E. Bell, J.E. Mullet, J. Browse, Jasmonate plays an essential role in plant insect defense, Proc. Natl. Acad. Sci. USA 94 (1997) 5473–5477.

[26] C. Wang, C. Zien, M. Afitlhile, R. Welti, D.F. Hildebrand, X. Wang, Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*, Plant Cell 12 (2000) 2237–2246.

[27] S. Ishiguro, A. Kawai-Oda, J. Ueda, I. Nishida, K. Kiyotaka Okada, The *defective in anther dehiscence1* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*, Plant Cell 13 (2001) 2191–2209.

[28] S.B. Ryu, X. Wang, Activation of phospholipase D and the possible mechanism of activation in wound-induced lipid hydrolysis in castor bean leaves, Biochim. Biophys. Acta 1303 (1996) 243–250.

[29] J.D. McGee, J.L. Roe, T.A. Sweat, X. Wang, J.A. Guikema, J.E. Leach, Rice phospholipase D isoforms show differential cellular location and gene induction, Plant Cell Physiol. 44 (2003) 1013–1026.

[30] J.N. Siedow, Plant lipoxygenases: structure and function, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 145–188.

[31] S. Roshal, Lipoxygenases in plants: their role in development and stress response, Z. Naturforsch. 51c (1996) 123–138.

[32] D.F. Hildebrand, T.R. Hamilton-Kemp, C.S. Legg, G. Bookjans, Plant lipoxygenase: occurrence, properties and possible functions, Plant Biochem. Physiol. 7 (1998) 201–219.

[33] A.R. Brash, Lipoxygenases: occurrence, function, catalysis, and acquisition of substrate, J. Biol. Chem. 274 (1999) 23679–23682.

[34] M.A. Melan, X. Dong, M.E. Endara, K.R. Davis, T.K. Peterman, F.M. Ausubel, An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate, Plant Physiol. 101 (1993) 441–450.

[35] E. Bell, J.E. Mullet, Characterization of an *Arabidopsis* lipoxygenase gene responsive to methyl jasmonate and wounding, Plant Physiol. 103 (1993) 1133–1137.

[36] E. Bell, R.A. Creelman, J.E. Mullet, A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*, Proc. Natl. Acad. Sci. USA 92 (1995) 8675–8679.

[37] J. Royo, G. Vancanneyt, A.G. Perez, C. Sanz, K. Stormann, S. Rosahl, J.J. Sanchez-Serrano, Characterization of three potato lipoxygenases with distinct enzymatic activities and different organ-specific and wound-regulated expression patterns, J. Biol. Chem. 271 (1996) 21012–21019.

[38] T. Heitz, D.R. Bergey, C.A. Ryan, A gene encoding a chloroplast targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate, Plant Physiol. 114 (1997) 1085–1093.

[39] K. Voros, I. Feussner, H. Kuhn, J. Lee, A. Graner, M. Lobler, Characterization of a methyl jasmonate-inducible lipoxygenase from barley (*Hordeum vulgare* cv. Salome) leaves, Eur. J. Biochem. 251 (1998) 36–44.

[40] A. Bachmann, B. Hause, H. Maucher, E. Garbe, K. Voros, H. Weichert, C. Wasternack, I. Feussner, Jasmonate-induced lipid peroxidation in barley leaves initiated by distinct 13-LOX forms of chloroplasts, Biol. Chem. 383 (2002) 1645–1657.

[41] A.B. Jensen, E. Poca, M. Rigaud, G. Freyssinet, M. Pages, Molecular characterization of L2 lipoxygenase from maize embryos, Plant Mol. Biol. 33 (1997) 605–614.

[42] Y.L. Peng, Y. Shirano, H. Ohta, T. Hibino, K. Tanaka, D. Shibata, A novel lipoxygenase from rice. Primary structure and specific expression upon incompatible infection with rice blast fungus, J. Biol. Chem. 269 (1994) 3755–3761.

[43] E.S. Kim, E. Choi, Y. Kim, K. Cho, A. Lee, J. Shim, R. Rakwal, G.K. Agrawal, O. Han, Dual positional specificity and expression of non-traditional lipoxygenase induced by wounding and methyl jasmonate in maize seedlings, Plant Mol. Biol. 52 (2003) 1203–1213.

[44] W.C. Song, C.D. Funk, A.R. Brash, Molecular cloning of an allene oxide synthase: a cytochrome P450 specialized for the metabolism of fatty acid hydroperoxides, Proc. Natl. Acad. Sci. USA 90 (1993) 8519–8523.

[45] D. Laudert, U. Pfannschmidt, F. Lottspeich, H. Holländer-Czytko, E.W. Weiler, Cloning, molecular and functional characterization of *Arabidopsis thaliana* allene oxide synthase (CYP 74), the first enzyme of the octadecanoid pathway to jasmonates, Plant Mol. Biol. 31 (1996) 323–335.

[46] G.A. Howe, G.I. Lee, A. Itoh, L. Li, A.E. DeRocher, Cytochrome P450-dependent metabolism of oxylipins in tomato. Cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase, Plant Physiol. 123 (2000) 711–724.

[47] H. Maucher, B. Hause, I. Feussner, J. Ziegler, C. Wasternack, Allene oxide synthases of barley (*Hordeum vulgare* cv. Salome): tissue specific regulation in seedling development, Plant J. 21 (2000) 199–213.

[48] S.B. Ha, B.C. Lee, D.E. Lee, Y.I. Kuk, A.Y. Lee, O. Han, K. Back, Molecular characterization of the gene encoding rice allene oxide synthase and its expression, Biosci. Biotechnol. Biochem. 66 (2002) 2719–2722.

[49] G.K. Agrawal, R. Rakwal, N.-S. Jwa, K.-S. Han, V.P. Agrawal, Molecular cloning and mRNA expression analysis of the first rice jasmonate biosynthetic pathway gene allene oxide synthase, Plant Physiol. Biochem. 40 (2002) 771–782.

[50] K. Harms, R. Atzorn, A. Brash, H. Kuhn, C. Wasternack, L. Willmitzer, H. Pena-Cortes, Expression of a flax allene oxide synthase cDNA leads to increased endogenous jasmonic acid (JA) levels in transgenic potato plants but not to a corresponding activation of JA-responding genes, Plant Cell 7 (1995) 1645–1654.

[51] D. Laudert, F. Schaller, E.W. Weiler, Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase, Planta 211 (2000) 163–165.

[52] D. Laudert, E.W. Weiler, Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling, Plant J. 15 (1998) 675–684.

[53] J.H. Park, R. Halitschke, H.B. Kim, I.T. Baldwin, K.A. Feldmann, R. Feyereisen, A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis, Plant J. 31 (2002) 1–12.

[54] B. von Malek, E. van der Graaff, K. Schneitz, B. Keller, The *Arabidopsis* male-sterile mutant *dde2-2* is defective in the *allene oxide synthase* gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway, Planta 216 (2002) 187–192.

[55] J. Ziegler, I. Stenzel, B. Hause, H. Maucher, O. Miersch, M. Hamberg, M. Grimm, M. Ganal, C. Wasternack, Molecular cloning of allene oxide cyclase: the enzyme establishing the stereochemistry of octadecanoids and jasmonates, J. Biol. Chem. 275 (2000) 19132–19138.

[56] B. Hause, I. Stenzel, O. Miersch, H. Maucher, R. Kramell, J. Ziegler, C. Wasternack, Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles, Plant J. 24 (2000) 113–126.

[57] G.K. Agrawal, N.S. Jwa, S.K. Agrawal, S. Tamogami, H. Iwahashi, R. Rakwal, Cloning of novel rice allene oxide cyclase (*OsAOC*): mRNA expression and comparative analysis with allene oxide synthase (*OsAOS*) gene provides insight into the transcriptional regulation of octadecanoid pathway biosynthetic genes in rice, Plant Sci. 164 (2003) 979–992.

[58] I. Stenzel, B. Hause, O. Miersch, T. Kurz, H. Maucher, H. Weichert, J. Ziegler, I. Feussner, C. Wasternack, Jasmonate biosynthesis and allene oxide cyclase family of *Arabidopsis thaliana*, Plant Mol. Biol. 51 (2003) 895–911.

[59] F. Schaller, C. Biesgen, C. Mussig, T. Altmann, E.W. Weiler, 12-Oxophytodienoic acid reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis, Planta 210 (2000) 979–984.

[60] J. Strassner, F. Schaller, U.B. Frick, G.A. Howe, E.W. Weiler, N. Amrhein, P. Macheroux, A. Schaller, Characterization and cDNA microarray expression analysis of 12-oxyphytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus systemic wound response, Plant J. 32 (2002) 585–601.
[61] P.M. Sanders, P.Y. Lee, C. Biesgen, J.D. Boone, T.P. Beals, E.W. Weiler, R.B. Goldberg, The *Arabidopsis delayed dehiscence1* gene encodes an enzyme in the jasmonic acid synthesis pathway, Plant Cell 12 (2000) 1041–1061.
[62] A. Stintzi, J.A. Browse, The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis, Proc. Natl. Acad. Sci. USA 97 (2000) 10625–10630.
[63] A. Stintzi, J. Weber, P. Reymond, J.A. Browse, E.E. Farmer, Plant defense in the absence of jasmonic acid: the role of cyclopentenones, Proc. Natl. Acad. Sci. USA 98 (2001) 12837–12842.
[64] Y. Ishiga, A. Funato, T. Tachiki, K. Toyoda, T. Shiraishi, T. Yamada, Y. Ichinose, Expression of the 12-oxophytodienoic acid 10,11-reductase gene in the compatible interaction between pea and fungal pathogen, Plant Cell Physiol. 43 (2002) 1210–1220.
[65] G.K. Agrawal, N.S. Jwa, J. Shibato, O. Han, H. Iwahashi, R. Rakwal, Diverse environmental cues transiently regulate *OsOPR1* of the "octadecanoid pathway" revealing its importance in rice defense/stress and development, Biochem. Biophys. Res. Commun. 310 (2003) 1073–1082.
[66] H. Sobajima, M. Takeda, M. Sugimori, N. Kobashi, K. Kiribuchi, et al., Cloning and characterization of a jasmonic acid-responsive gene encoding 12-oxophytodienoic acid reductase in suspension-cultured rice cells, Planta 216 (2003) 692–698.
[67] J.T. Song, H.S. Seo, S.I. Song, J.S. Lee, Y.D. Choi, NTR1 encodes a floral nectary-specific gene in *Brassica campestris* L. ssp. *pekinensis*, Plant Mol. Biol. 42 (2000) 647–655.
[68] G.K. Agrawal, H. Iwahashi, R. Rakwal, Rice MAPKs, Biochem. Biophys. Res. Commun. 302 (2003) 171–180.
[69] C. Jonak, L. Okresz, L. Bogre, H. Hirt, Complexity, cross talk and integration of plant MAP kinase signalling, Curr. Opin. Plant Biol. 5 (2002) 415–424.
[70] R. Rakwal, G.K. Agrawal, Wound signaling-coordination of the octadecanoid and MAPK pathways, Plant Physiol. Biochem. 41 (2003) 855–861.
[71] M. Riemann, A. Muller, A. Korte, M. Furuya, E.W. Weiler, P. Nick, Impaired induction of the jasmonate pathway in the rice mutant *hebiba*, Plant Physiol. 133 (2003) 1820–1830.