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Mini Review

Rice Plant Development: from Zygote to Spikelet

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Rice is becoming a model plant in monocotyledons and a model cereal crop. For better understanding of the rice plant, it is essential to elucidate the developmental programs of the life cycle. To date, several attempts have been made in rice to categorize the developmental processes of some organs into substages. These studies are based exclusively on the morphological and anatomical viewpoints. Recent advancement in genetics and molecular biology has given us new aspects of developmental processes. In this review, we first describe the phasic development of the rice plant, and then describe in detail the developmental courses of major organs, leaf, root and spikelet, and specific organs/tissues. Also, for the facility of future studies, we propose a staging system for each organ.

Keywords: Development — Rice — Staging.

Abbreviations: AC, archesporial cell; DAP, days after pollination; GMC, guard mother cell; LE, lateral element; PMC, pollen mother cell; PPC, primary parietal cell; PSC, primary sporogenous cells; SAM, shoot apical meristem; SC, synaptonemal complex; SEM, scanning electron microscope; SMC, subsidiary mother cell.

Introduction

Higher plants have many specialized organs, tissues and cells. All these components are derived from a single cell (fertilized egg, zygote) through a number of developmental events. In animals, developmental and differentiation processes are restricted mainly to a short period of embryogenesis. In contrast, development continues in plants until the end of the life cycle. Apical meristems repeatedly differentiate lateral organs. Meristematic tissues are generated in a variety of organs with proper timing to achieve successive development. Therefore, developmental studies of the entire plant life cycle are essential to elucidate the establishment of plant formation.

Importance of developmental study

Recent advancement of developmental studies has produced interesting results that show the way in which plant development is regulated genetically. One fruitful result is the establishment of the ABC model in flower development (Coen and Meyerowitz 1991), which facilitates our better understanding of flowers. Since the 1980s, numerous mutants have been identified and characterized to study plant morphogenesis and differentiation, and their causal genes have been cloned. Detailed developmental analyses of mutant phenotypes and spatiotemporal expression pattern of the causal genes contribute greatly to better understanding of plant architecture. In addition, developmental studies are expected to answer the identity of unknown organs, homology of similar organs and other important subjects.

For a description of the developmental process to be universal, for the description to be applicable to the comparison between wild type and mutant or between two species, categorization of the process with landmark events is essential. One categorization method is division into discrete stages. Stages should be characterized by landmark events. Although such landmark events are provided mainly by morphological and anatomical studies, recent genetic and molecular studies would make the staging system more available. For *Arabidopsis*, the developmental course of the wild-type flower has been divided into 12 stages using a series of events (Smyth et al. 1990). This staging is now used widely for describing mutant phenotypes and the site and timing of the cloned genes' expression.

Concomitant with the progress of molecular and genetic studies of plant development, a large amount of information, such as the expression pattern of genes and molecular functions of the proteins and their cellular localization, is being accumulated. These numerous data are being integrated in several website databases. To use information from various data sets more effectively, it is extremely useful to establish developmental staging based on internationally accepted terminology.

Rice is a model plant for developmental study

At present, *Arabidopsis* is a major source of information regarding plant development because it offers excellent advantages as a model plant for developmental study (Meyerowitz and Somerville 1994). *Arabidopsis* is a dicotyledonous species. Therefore, another model plant in monocotyledons is

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needed for a general understanding of angiosperms. Rice plant (Fig. 1) offers various advantages as an experimental plant compared with other monocot species, such as small genome size, a known genome sequence (Sasaki and Burr 2000) and self-fertilization. Moreover, it is an annual plant that is nevertheless able to survive for several years. It has a facility for *Agrobacterium*-mediated transformation, easy cultivation and accumulation of information on varieties and mutants. For those reasons, rice is considered a model plant of monocots. Rice belongs to the grass family (Poaceae) together with barley, wheat, maize and sorghum, which are important cereal crops that support the global food supply. In addition, these grasses are inferred to have a monophyletic origin (Clark et al. 1995), indicating that information obtained from rice is helpful for studying other cereal crops. In fact, several reports have elucidated genome synteny between rice and other grasses (Bennetzen and Ma 2003). Accordingly, rice is not merely a model plant for developmental study, it is a model crop for evolutionary and agronomical studies of cereals.

In this review, we describe rice development from zygote to spikelet, and attempt to categorize each developmental process into several stages. We hope that the staging systems proposed here are useful for describing mutant phenotypes and spatiotemporal expression patterns of development-related genes, and for comparative study with other species.

Categorization of Rice Plant Development

Plant development starts from egg cell fertilization with a sperm nucleus to form a zygote (fertilized egg). From the first zygotic division, plant development proceeds toward maturity through a number of stages that are discernible according to landmark events. Development finally culminates in the formation of male and female gametes that fertilize to form a zygote. The plant developmental course is roughly divisible into three phases: embryogenesis, vegetative and reproductive. Seed dormancy and germination, and the onset of inflorescence development typically delimit these three phases. In each phase, numerous events occur sequentially. Consequently, each phase can be divided into discrete stages. To date, developmental studies on rice have been carried out from the standpoint of morphological changes, and too detailed staging systems for several processes have been proposed (Matsushima and Manaka 1956, Kawata and Harada 1975). However, recent advancement in molecular biology and developmental genetics using useful mutants has revealed stage-specific gene expression and stage-specific defects of mutants. It has lent new insights into rice development. It would be useful to review rice development from a viewpoint that incorporates recent results of molecular and genetic studies.

Embryogenesis in most plant species is a period in which the plant body plan (body axis) is established. That is, the zygote undergoes cell division without morphogenetic events to form a globular embryo. It then differentiates the shoot apical meristem (SAM) and radicle in fixed positions that specify the body axis. For that reason, embryogenesis is an extremely important period for understanding the developmental nature of plants.

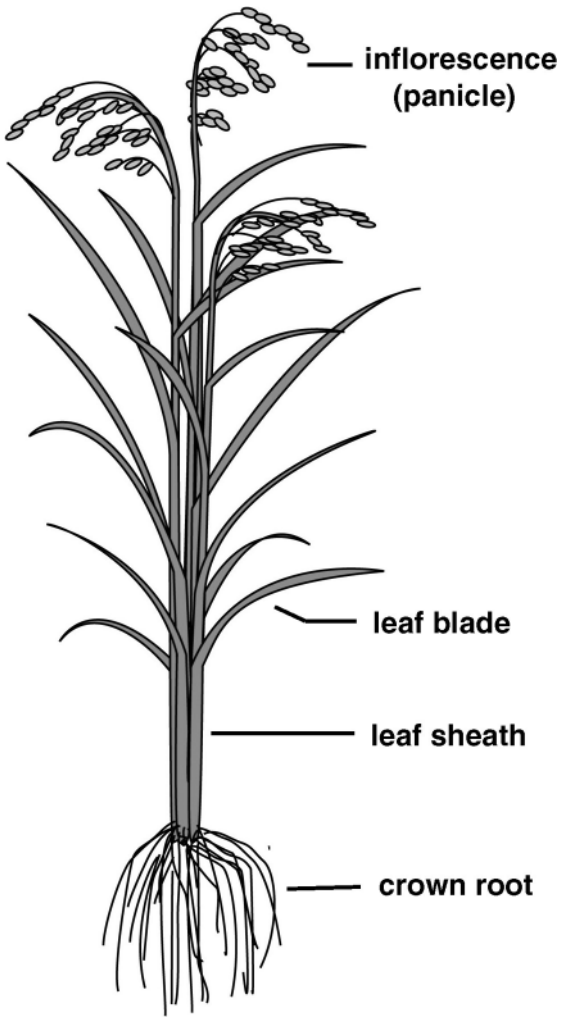

Fig. 1 Schematic representation of mature rice plant.

After seed germination, the plant undergoes vegetative development, usually the longest period, repeatedly forming a number of leaves and branches as lateral organs. During this phase, the stem also grows by the supply of cells from the rib zone of the SAM. Recent studies have indicated that the vegetative phase can be divided into two phases—juvenile and adult phases—which differ in many traits (Hackett 1985, Poethig 1990, Telfer et al. 1997, Asai et al. 2002).

The reproductive phase has been studied exhaustively because anyone can recognize the remarkable event in this phase: flowering. This phase marks a change of meristem identity from inflorescence meristem to floral meristem. In addition, flower formation is a complicated process because the floral meristem changes the identities of lateral organs sequentially: the sepal, petal, stamen and carpel (pistil). Therefore, the

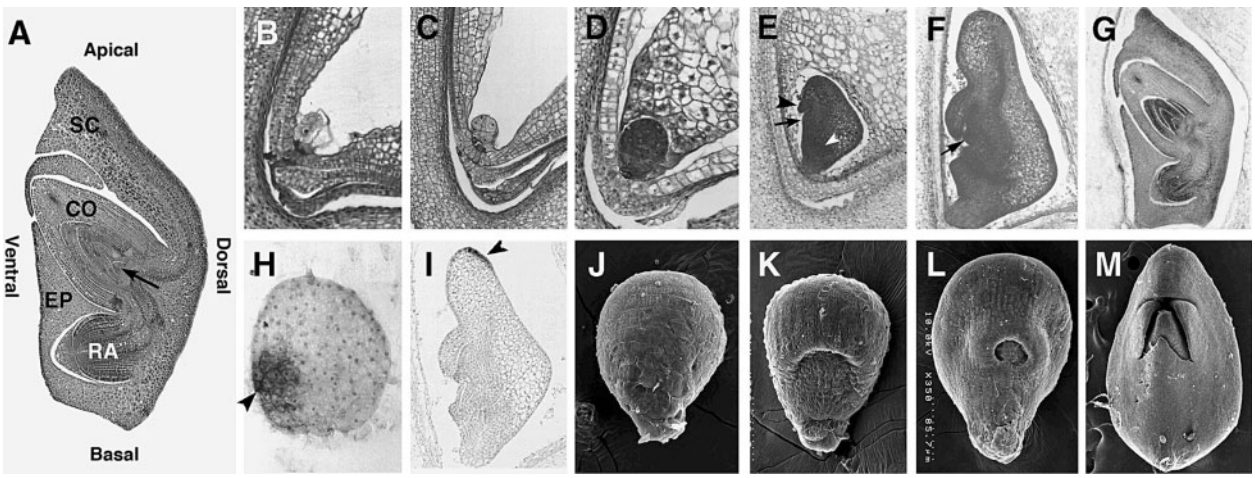

Fig. 2 Embryo development in rice. (A) Longitudinal section of mature embryo. Arrow indicates the SAM. SC, scutellum; CO, coleoptile; EP, epiblast; RA, radicle. (B) 1 DAP embryo. (C) 2 DAP embryo. (D) 3 DAP embryo. (E) 4 DAP embryo differentiating coleoptile primordium (black arrowhead), SAM (arrow) and radicle primordium (white arrowhead). (F) 5 DAP embryo differentiating the first leaf primordium (arrow). (G) Morphologically completed 10 DAP embryo. (H) *OSH1* expression (arrowhead) in 3 DAP embryo. (I) *RAmy1A* expression (arrowhead) in scutellar epithelium of 5 DAP embryo. (J–M) SEM images of 3, 4, 5 and 10 DAP embryos, respectively.

reproductive phase includes a large number of ingeniously regulated processes.

The following sections explain phasic development of rice plants with particular attention to the temporal landmark events that characterize each phase, and we propose staging systems that are useful for future study.

Embryo

DESCRIPTION OF DEVELOPMENTAL COURSE

First, it should be mentioned that several foliage leaves are present in mature grass embryos, including rice embryos (Fig. 2A). This fact contrasts with the situation in other monocot and dicot embryos, in which foliage leaves are not formed before dormancy and germination. This phenotype means that in grasses, early vegetative stages are incorporated into embryos before dormancy takes place. The temporal reversion of dormancy and early vegetative phase suggests that grasses are heterochronic mutants (Freeling et al. 1992, Asai et al. 2002). In this report, we regard rice embryogenesis as a period before germination.

In addition to a heterochronic nature, grasses are unique in that they have complex embryos (Fig. 2A). Several embryo-specific organs are present aside from the shoot (plumule) and radicle. The scutellum is the largest organ in the embryo corresponding to the cotyledon, and differentiates independently of the SAM, as indicated by Satoh et al. (1999). The epidermal layer of the scutellum (scutellar epithelium) comprises palisade-shaped cells. It is the site at which many maturation-related genes are expressed (Sugimoto et al. 1998, Miyoshi et al. 1999, Miyoshi et al. 2002). The coleoptile is the first recognizable organ post-globular stage, which protrudes from the ventral region just above the SAM. Although the identity of the coleoptile has been discussed, it may be an appendage of the scutellum. An epiblast, whose function is not clear, is formed in the basal region on the ventral side. The epiblast is not always observed in grass embryos; maize has no epiblast. Most of the dorsal region is occupied by the scutellum. The radicle is formed endogenously in the basal region. Thus, it is frequently considered as a kind of adventitious root.

Pollination occurs in rice at the same time as flower opening. It lasts for only about 15 min at around noon in summer. Fertilization takes place several hours after pollination. Due to the synchronous pollination (fertilization), it is possible to mutagenize a large number of zygotes, engendering efficient non-chimeric mutant production (Satoh and Omura 1979). The zygote reiterates cell divisions to form a globular embryo with no apparent morphological differentiation for 3 days after pollination (3 DAP) (Fig. 2B–D, J). In contrast to the *Arabidopsis* embryo, cell divisions are not regular, even at the very early stage. Although the first cell division usually occurs horizontally to form apical and basal cells, the directions of the second and following cell divisions are not fixed. At 4 DAP, the first morphogenetic event occurs: formation of SAM, coleoptile primordium and radicle primordium (Fig. 2E, K). Because the SAM, radicle and coleoptile initiate at fixed positions, embryonic axis and regionalization are inferred to be established in the globular embryo. The SAM at this stage is flat, not dome-like. The first leaf primordium is recognized at 5 DAP (Fig. 2F, L). Three foliage leaves are formed in the rice embryo. Soon after the formation of the third leaf primordium, the rice embryo is morphologically completed (Fig. 2G, M). Subsequently, maturation and dormancy processes take place.

For categorizing embryo development, it is reasonable to discriminate stages by specific morphogenetic events such as

Table 1 Staging of embryogenesis in rice

Stage		DAP [a]	No. of cells	Events	Expressed gene [b]	Related mutant
Symbol	Name					
Em1	Zygote	0	1	Fertilization		
Em2	Early globular stage	1	1–ca.25	First division of fertilized egg ca.25-cell stage		
				Rapid cell division		
Em3	Middle globular stage	2	ca.25–ca.150	ca.25–ca.150-cell stage		*gle1* [f], *gle2* [f], *gle3* [f]
				Globular-shaped		
				Relatively slow growth		
Em4	Late globular stage	3	ca.150–ca.800	Oblong-shaped	*OSH1* [c], *OsVP1* [d], *OsBZ8* [e]	*cle1* [f], *shl1* [g], *shl2* [g], *shl4* [g], *sho1* [h], *sho2* [h]
				Onset of exponential growth, dual rhythmicity of growth and cell increase		
				Gradient of cell size along dorsoventral direction		
				Pattern formation, regionalization		
Em5	SAM and radicle formation	4	ca.800–	Onset of coleoptile, SAM and radicle differentiation		*shl3* [g]
Em6	First leaf formation	5–6		Protrusion of 1st leaf primordium	*RAmy1A*	
				Enlargement of scutellum		
				Expression of *RAmy1A* in scutellar epithelium		
				Onset of juvenile vegetative stage)		
Em7	Second and third leaf formation	7–8		Protrusion of 2nd and 3rd leaf primordia in alternate phyllotaxis		
				Protrusion of epiblast		
Em8	Enlargement of organs	9–10		Enlargement of organs and morphological completion	*OsVP1* [d], *OSEM* [d], *RAB16A* [e], *REG2* [e]	*riv1* [i], *riv2* [i]
				Expression of maturation-related genes such as *OsEM*, *Rab16A*, *REG2*, etc.		
Em9	Maturation	11–20		Expression of maturation-related genes such as *OsEM*, *RAB16A*, etc.		
Em10	Dormancy	21–		Dormancy		

[a] Days after pollination, [b] the stage at which respective gene is first expressed is shown; [c] Sato et al. (1996), [d] Miyoshi et al. (2002), [e] Miyoshi et al. (1999), [f] Hong et al. (1995), [g] Satoh et al. (1999), [h] Itoh et al. (2000), [i] Miyoshi et al. (2000).

the initiation of new organs. However, during the globular stage, no apparent morphogenetic events occur. Marker genes that are expressed at a specific stage of globular embryo are not known, except *OSH1* (Sato et al. 1996). Considering the importance of the globular stage in embryo development, it is indispensable to reveal the events that take place over the 3 d that the globular embryo lasts. The number of cells in the embryo increases rapidly at 1 DAP, but after about the 25-cell stage, the rate of cell increase falls (Nagato 1978). That rate reduction suggests that actively dividing cells are restricted in the embryo. Detailed growth analysis reveals that another landmark event occurs at around the 150-cell stage. From this stage, the embryo undergoes dual rhythmic growth and cell division (Nagato 1976), and exponential growth (Nagato

1978). Furthermore, at about this stage, *OSH1* expression starts in a specific region where the SAM is later formed (Fig. 2H) (Sato et al. 1996).

Based on these events, we can imagine a developmental program in the globular embryo as follows. Initially, most zygote-derived cells divide several times. Then, actively dividing cells are restricted to a portion of the embryo (alternatively, some embryo cells stop dividing), resulting in some gradient of cell activity. The progress of this tendency may cause apical–basal or dorsal–ventral axis formation of the embryo. However, the embryonic axis does not immediately induce SAM and radicle formation. In this respect, the 150-cell stage seems to be a turning point for SAM and radicle differentiation and other morphogenetic events because SAM and radicle differentiation apparently start at this stage (dual rhythmicity of growth and recruitment of indeterminate cells, as deduced from *OSH1* expression). Correlation between *OSH1* expression and SAM formation is confirmed by analyses of *shl* mutants (Satoh et al. 1999, Satoh et al. 2003), strong *shl* alleles lacking the SAM express *OSH1* only in a small number of cells, and weak *shl* alleles, forming incomplete SAMs, show an intermediate number of *OSH1*-expressing cells between strong alleles and the wild-type allele.

Morphological differentiation of the embryo takes place at 4 DAP (Fig. 2E). The coleoptile, SAM and radicle are recognized almost simultaneously at 4 DAP, and the first leaf primordium at 5 DAP. Second and third leaf primordia differentiate within 3 or 4 d. Most morphogenetic events are completed by 10 DAP.

It is difficult to categorize late embryogenesis because morphogenetic events do not occur after 10 DAP: only a slight enlargement of organs occurs. In that case, maturation- related and dormancy-related genes would be useful. One ABA-regulated gene, *Rab16A*, is expressed after 10 DAP. Expression of another ABA-regulated gene, *REG2*, is down-regulated from the shoot and radicle after 10 DAP (Miyoshi et al. 1999). Accordingly, embryo maturation is considered to progress rapidly after 10 DAP. Although viviparous mutants have been reported (Miyoshi et al. 2000), late embryogenesis of rice has not been understood well.

Staging of embryo development—Based on the landmark events described above, we propose a staging system of embryo development in rice (Table 1).

Stage Em1: Zygote. Most of the egg cells are fertilized with self-pollen at around flower opening. On rainy or cool days, pollination frequently occurs in closed flowers. This stage lasts for several hours.

Stage Em2: Early globular stage. From first cell division to about the 25-cell stage at 1 DAP (Fig. 2B). This stage is characterized by rapid multiplication of cells. The cell division pattern is not conserved. The first division is horizontal in most cases, but oblique cell division is also observed. The second and subsequent cell divisions are variably oriented.

Stage Em3: Middle globular stage. From the approximately 25-cell stage to the approximately 150-cell stage at 2 DAP (Fig. 2C). During this stage, we have not observed any remarkable events. Cells on the dorsal side (endosperm side) seem to be somewhat larger than those on the ventral side. Interestingly, the maturation-related gene *OSVP1* is expressed at this stage (Miyoshi et al. 2002).

Stage Em4: Late globular stage. From about the 150-cell stage to the onset of organ formation at 3 DAP (Fig. 2D, J). During this stage, several events take place for differentiating embryonic organs such as the SAM and radicle. The embryo starts exponential growth that shows dual rhythmicity. The cell size gradient becomes apparent along the dorsoventral axis: ventral cells are smaller than dorsal cells. A rice *kn1*-type homeobox gene *OSH1* is first expressed in a small region on the ventral side (Fig. 2H) (Sato et al. 1996), indicating that indeterminate cells for constructing the SAM are recruited at this stage.

Stage Em5: Onset of coleoptile, SAM and radicle differentiation. When the embryo comprises 800–900 cells and becomes approximately 110 μm long at 4 DAP, the first morphogenetic event is visible as a protrusion of the coleoptile on the ventral side (Fig. 2E, K). Just below the coleoptile, the SAM is found. Its shape is flat, not dome-like. In addition, the scutellum is apparent in the apical region of the coleoptile. In the internal basal region, the radicle primordium is recognized as a population of densely stained small cells. *OSH1* is expressed in the SAM and the surrounding tissues.

Stage Em6: Formation of first leaf primordium. At 5 DAP, the first leaf primordium is visible on the opposite side of the coleoptile (Fig. 2F, L). Enlargement of embryonic organs, especially the scutellum, is remarkable. From this stage, the embryonic phase and juvenile vegetative phase coexist. A major α-amylase gene, *RAmy1A*, is first expressed in the apical region of scutellar epithelium (Fig. 2I), then its expression extends toward the basal scutellar epithelium. One maturation-related gene, *OSEM*, is expressed from this stage.

Stage Em7: Formation of second and third leaf promordia. Second and third leaves are formed at 7 and 8 DAP, respectively. By this stage, the SAM becomes dome-shaped. At this stage, organs are enlarging. The epiblast becomes enlarged; it is not present in maize embryos.

Stage Em8: Enlargement of organs. From 9 to 10 DAP, no more morphological change is observed, but enlargement of organs is marked (Fig. 2G, M). A maturation-related gene, *Rab16A*, is expressed from this stage. Expression of another maturation-related gene, *REG2*, is down-regulated from the shoot, radicle and vascular bundle.

Stage Em9: Maturation of embryo: 11–20 DAP. Enlargement of embryonic organs continues, but to a small extent. Maturation-related genes such as *OSEM*, *Rab16A* and *REG2* are expressed strongly, but the expression becomes weak at

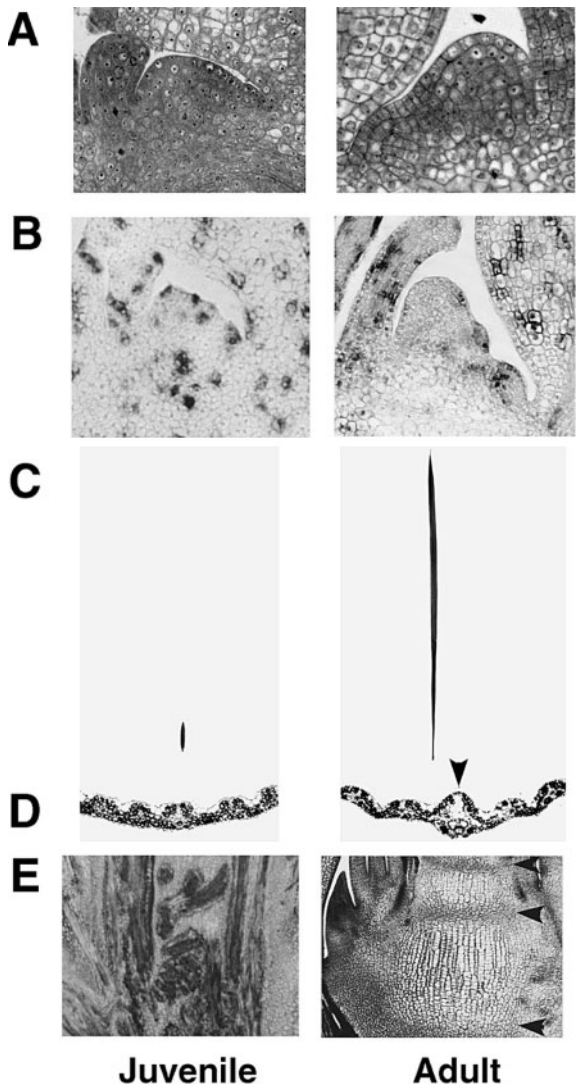

Fig. 3 Comparison of juvenile and adult traits in rice. (A) SAM at second (left) and 10th (right) leaf stages. (B) Histone *H4* expression in SAM at second (left) and 10th (right) leaf stages. (C) Second (left) and sixth (right) leaf blade. (D) Cross-sections of midrib region of second (left) and 10th (right) leaf blades. Arrowhead indicates midrib (E) Longitudinal sections of basal stem region where second and third leaves are inserted (left) and apical stem region where seventh and eighth leaves are inserted (right). Arrowheads indicate nodes.

around 20 DAP. Expression of these genes is also detected in the aleurone layers of the endosperm.

Stage Em10: Dormancy. After 20 DAP, the rice embryo would be dormant.

Vegetative phase

Rice plants produce 10 or more foliage leaves before entering the reproductive phase. Because leaf length differs with its position, the longest leaf being three or four leaves below the last one (flag leaf), the vegetative stage can be characterized by each leaf position. However, such a categorization would be complicated and not useful because the total number of leaves differs largely among cultivars and almost no difference is detected between two adjacent leaves except for the first and second leaves. Accordingly, in this review, we divide the vegetative phase into two phases: juvenile and adult. As in other species, many traits differ in juvenile and adult phases (Hackett 1985, Poethig 1990, Telfer et al. 1997). It is marked that the differences between juvenile and adult phases are not discrete, but continuous. For that reason, it is difficult to specify the boundary of the two phases explicitly. We only recognize the difference when two remote stages (e.g. 2nd leaf and 10th leaf) are compared. An intermediate period exists between juvenile and adult phases; the juvenile phase is converted gradually into the adult phase. For rice, Asai et al. (2002) first reported the differences between juvenile and adult phases in rice in analysis of a heterochronic mutant *mori1*. Those differences are observed in all the traits they examined. *mori1* is a heterochronic mutant that reiterates the second-leaf stage and suppresses the induction of adult phase. Their data suggest that the juvenile phase is the first and second leaf stages; third to fifth leaf stages are intermediate. The later stage is the adult phase. The following section describes how juvenile and adult phases differ in many traits, as summarized in Fig. 3 and Table 2.

The SAM becomes enlarged gradually with development. For example, the SAM at the 10th leaf stage is nearly two-fold larger than that at the second leaf stage (Fig. 3A). Expression of histone *H4* gene indicates that the cell division frequency in the SAM is higher at the second-leaf-primordium stage than at the stages after the fourth leaf primordium stage (Fig. 3B).

Various leaf traits vary largely during development. Plastochrons (the rate of leaf production) of the second and third leaves are approximately 1.6 d; after the fifth leaf, it is approximately 4 d. Leaf size and shape also vary with development. The first leaf is quite small and lacks a blade. The second leaf has both a blade and sheath, but it is very small (Fig. 3C). Subsequently, leaf size increases gradually, achieving its maximal size in the three or four leaves below the last leaf (flag leaf). The leaf blade shape is rather rounded in earlier leaves. Anatomical differences are also observed. Adult leaves are strengthened mechanically by the presence of a large midrib, whereas the midrib is not prominent in the second leaf; it is small in the third leaf (Fig. 3D). Regarding physiological traits, photosynthesis has been examined. The apparent photosynthetic rate per unit area is very low in the second leaf blade. It increases gradually in subsequently developed leaf blades. Low photosynthesis at the early stages engenders heterotrophism.

Stem structure also differs between juvenile and adult phases. In the adult stem, node and internode are differentiated clearly, but in the stem where first through third leaves are inserted, node and internode are not clearly distinguished (Fig. 3E). Vascular bundles are regularly oriented in the adult stem, but they are rather randomly oriented in the basal stem where the first through third leaves are inserted (Fig. 3E). Many

Table 2 Comparison of juvenile and adult characters in rice

Organ	Character	Juvenile	Adult
SAM	Size	Small	Large
	Cell division	Active	Inactive
Leaf	Size	Small	Large
	Shape	Round	Slender
	Midrib	Absent	Present
	Plastochron	Short	Long
	Photosynthetic rate	Low	High
Stem	Node	Absent	Present
	Axillary bud	Growing	Dormant
	Orientation of VB	Irregular	Regular
	Adventitious root	Many	Few or none
Whole plant	Trophism	Heterotrophic	Autotrophic
	Disease resistance	Weak	Strong

crown roots (adventitious roots) are formed from basal stem nodes, but almost no crown roots emerge from the adult stem nodes.

The traits described above indicate that juvenile and adult phases show not only quantitative differences, but also qualitative changes that are regulated by a developmental switch. The phase change is a simultaneous alteration in the expressionof many genes. Although *MORI1* is the only gene reported so far to be involved in the phase change of the whole plant, it is inferred that specific genes are responsible for the phase change of each organ and tissue.

Reproductive Phase: Inflorescence

DESCRIPTION OF DEVELOPMENTAL COURSE

When environmental conditions and internal factors become favorable for floral induction, a SAM that has regularly formed foliage leaves and tillers (branches) is converted into an inflorescence meristem. The inflorescence meristem is discernible from the SAM by the identity of lateral organs. The inflorescence meristem forms bracts (small degenerate leaves) and inflorescence branches that bear flowers (spikelets) later. The following sections describe inflorescence development and its staging only briefly because Ikeda et al. (2004) have already proposed a detailed staging system.

A mature rice inflorescence is shown in Fig. 4A. The rice inflorescence is categorized as a raceme together with those of *Sorghum*, *Panicum* and *Avena*, in which spikelets are not attached directly to the main axis, but are formed on the lateral branches. The rice inflorescence is also called a panicle because of its conical shape. In grasses, the main axis of the inflorescence is called the rachis (Bell 1991). Lateral branches that are attached directly to the rachis are called primary (rachis) branches. Those on the primary branch are termed secondary (rachis) branches. The mature inflorescence of rice has 10 or more primary branches that bear approximately 150 spikelets. Two types of inflorescence meristem are recognized: rachis and branch meristems. The rachis meristem forms bracts and branches as lateral organs, and finally aborts. In contrast, the branch meristem differentiates spikelets and branches; it is ultimately converted into a terminal spikelet. Moreover, the phyllotaxy of lateral organs differs for rachis and primary branch meristems. Primary branches are produced in spiral phyllotaxy, but spikelets on the primary branches are arranged in a biased distichous phyllotaxy. The length of primary inflorescence branches is reduced acropetally.

When the rachis meristem forms a bract 1 primordium, it is larger than the final SAM (Fig. 4B). The rachis meristem forms bracts and primary branches sequentially in 2/5 spiral phyllotaxy (Fig. 4C, E). The first primary branch primordium is visible at the axile of bract 1 when bract 2 primordium is formed. The rachis meristem increases its size, reaching its maximum size at the bract 3 primordium stage. Subsequently, it shrinks gradually. The growth of bracts is severely suppressed: the distal cells of the third and subsequent bracts become hairs (Fig. 4D, F). When all branch primordia are formed, the rachis meristem loses its activity and aborts. The first branch primordium does not elongate first: all branch primordia elongate simultaneously (Fig. 4F). When primary branches elongate to some extent, secondary branches are formed in the basal regions (Fig. 4G). Then the branch meristem is converted into a spikelet meristem to form glumes. Then it converts into a flower (floret) meristem to form floral organs (Fig. 4H). The inflorescence remains short (<4 cm) at this stage. It commences rapid elongation of rachis and branches after floral organ primordia are differentiated. Maturation of anthers and ovules takes place during rapid branch elongation.

Staging of inflorescence development—Development of rice inflorescence is categorized into nine stages (Table 3), according to Ikeda et al. (2004).

Stage In1: Establishment of rachis meristem. This stage is the period from the conversion of the SAM to rachis meristem until bract 1 primordium formation (Fig. 4B). The rachis meris-

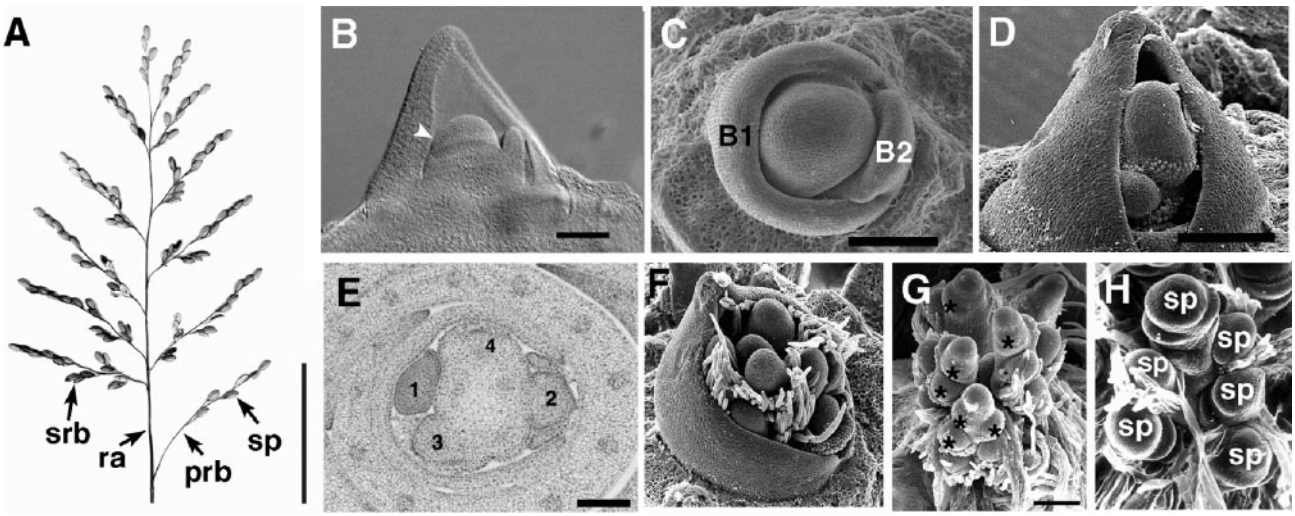

Fig. 4 Inflorescence development in rice. (A) Mature rice inflorescence. (B) Formation of first bract (arrowhead). (C) Second bract formation. (D) Early stage of primary branch formation. (E) Cross-section of rachis apex differentiating four primary branches in spiral phyllotaxy. Numerals indicate first, second, third and fourth branches, respectively. (F) Onset of primary rachis branch elongation. (G) Formation of secondary branches (*). (H) Formation of spikelets. ra, rachis; prb, primary rachis branches; srb, secondary rachis branches; sp, spikelet; B1, first bract; B2, second bract.

tem is larger than the last vegetative meristem. The –3 and –4 stem internodes have already started their elongation and the –2 internode starts elongation at this stage. Herein, the second, third and fourth stem internodes counted from the uppermost one are designated as –1, –2 and –3 internodes, respectively.

Stage In2: Formation of primary rachis branches I. During this stage, primordia of bract 2, bract 3 and the first primary branch are formed (Fig. 4C). At the end of this stage, the rachis meristem attains its maximum size. Most bract positions are determined at this stage, as inferred from the bract-specific expression of the *PLA1* gene (Miyoshi et al. 2004).

Stage In3: Formation of primary rachis branches II. This stage corresponds to the period of primary branch formation after the In2 stage (Fig. 4D). Primary branches are produced in spiral phyllotaxy (Fig. 4E). The number of primary branches depends on the time when the rachis meristem loses its activity (loss of *OSH1* expression). Stages In1—In3 are completed within 3 or 4 d. Elongation of the –1 stem internode starts at this stage.

The *lax* and *pap1* mutants would be related to this stage (Komatsu et al. 2001, Takahashi et al. 1998).

Stage In4: Elongation of primary branches. Although basal primary branch primordia are formed earlier than the apical ones, all primordia commence elongation simultaneously (Fig. 4F). Therefore, In4 is the stage from the onset of primary branch elongation to the formation of lateral organs. The epidermal cells of bracts elongate like hairs.

Stage In5: Differentiation of higher-order branches. Soon after the onset of elongation, primary branches (especially basal ones) produce secondary branches as lateral organs. For that reason, the stage In5 is the period when the primary branch meristem produces secondary branches, but not spikelets (Fig. 4G). Basal secondary branches frequently produce tertiary branches. Seven to nine days elapse from the conversion of the meristem until the end of this stage. The uppermost stem internode starts elongation at this stage. By this time, –3 and –4 internode elongation has been completed.

Stage In6: Differentiation of glumes. Primary and secondary branch meristems are converted into terminal spikelet meristems and form rudimentary glumes (Fig. 4H). Subsequently, lateral meristems become lateral spikelet meristems. Lateral organs of primary branches are arranged in a biased distichous phyllotaxy with a divergence angle of about 110° (Fig. 4H). Two empty glumes, lemma and palea, are formed after two rudimentary glumes.

Stage In7: Differentiation of floral organs (inflorescence length 1.5–40 mm). After glume formation, floral organs (two lodicules, six stamens and one carpel) are formed. At this stage, rachis elongation is not yet remarkable. Even when carpel primordia are observed in all flowers, the inflorescence is only 10 mm long. The –2 stem internode elongates rapidly.

Stage In8: Rapid elongation of rachis and maturation of reproductive organs. When the inflorescence becomes approximately 40 mm long, it starts rapid elongation. Each organ increases in size. Maturation of reproductive organs, the stamen and pistil, takes place to form pollen grains and the embryo sac. At this stage, the uppermost and –1 stem internodes elongate rapidly.

Stage In9: Heading and flowering. A few days after completion of flowers, the inflorescence emerges from the sheath of the flag leaf; flowering occurs at around noon.

Table 3 Staging of inflorescence development in rice

Stage		Meristem identity/lateral organ identity	Inflorescence length (mm) [a]	Events	Related mutant
Symbol	Name				
In1	Establishment of rachis meristem	Rachis meristem/primary branch	0.05–0.1	Conversion of vegetative meristem to rachis meristem. Enlargement of rachis meristem. Formation of bract 1 primordium. Onset of –2 stem internode elongation	
In2	Formation of primary branches I	Rachis meristem Branch meristem/primary branch	0.1–0.2	Rachis meristem reaches maximum size. Formation of first two primary branch primordia and bract 2 and 3 primordia	*pla1* [b], *lax*[c], *sp* [d] *Dn1* [e], *Dn2* [f], *Dn3* [g]
In3	Formation of primary branches III	Rachis meristem Branch meristem/primary branch	0.2–0.4	Formation of primary branch primordia in spiral arrangement. Abortion of rachis meristem at the end of this period. Onset of –1 stem internode elongation	*lax* [c], *pap1* [h]
In4	Elongation of primary branches	Branch meristem	0.4–0.6	Simultaneous elongation of primary branches	*sp* [e]
In5	Formation of higher-order branches	Branch meristem/ secondary and tertiary branches	0.6–0.9	Formation of secondary and tertiary branches. Onset of uppermost stem elongation	*lax* [c], *Dn1* [f], *Dn2* [g], *Dn3* [h]
In6	Differentiation of glumes	Spikelet meristem Floret meristem/glumes	0.9–1.5	Differentiation of two rudimentary glumes, two empty glumes, lemma and palea in 1/2 alternate arrangement	(see Table 6)
In7	Differentiation of floral organs	Floral meristem/floral organs	1.5–40	Differentiation of floral organs, two lodicules, six stamens and a carpel in whorl	(see Table 6)
In8	Rapid elongation of rachis and branches	Loss of meristem	40–220	Rapid elongation of rachis and branches. Completion of anther and ovule development	*ri* [e] (see Table 6)
In9	Heading and flowering		ca.220	Inflorescence emergence out of the sheath of flag leaf. Flowering at around noon	

[a] Values are for cv. Taichung 65.
[b] Itoh et al. (1998), [c] Komatsu et al. (2001), [d] Iwata and Omura (1971b) [e] Nagao and Takahashi (1963), [f] Jones (1952), [g] Futsuhara et al. (1979), [h] Takahashi et al. (1998).

Development of Major Organs

Leaf

Description of developmental course

Leaves are produced repetitively as lateral organs of the SAM. Analyses of a large number of mutants in several model plants (for reviews, see Scanlon 2000, Byrne et al. 2001, Bowman et al. 2002) suggest that leaf development is a complex process comprising cell division and expansion, axis determination, tissue differentiation and specification.

Grass leaves have a different architecture from those of dicots. For maize, developmental analyses of leaves have been made (Freeling 1992, Sylvester et al. 1996). However, for rice, only a few studies have been attempted from histological and morphological viewpoints (Kaufman 1959a, Hoshikawa 1989). This section presents a description of rice leaf development that incorporates recent genetic and molecular results.

The mature rice leaf is strap-like; it is usually divided into three distinct regions along the proximal–distal axis. The leaf blade (lamina) is the distal region of a leaf and a major site of photosynthesis (Fig. 5A). The leaf sheath is the proximal region and surrounds the shoot apex and younger leaves to protect them from physical damage. The boundary of the blade and sheath consists of three distinct parts: the lamina joint (collar), the ligule and the auricle (Fig. 5A). The lamina joint is a whitish region in the base of the blade; it functions in bending the leaf blade toward the abaxial side (Fig. 5A). The ligule is membranous and acuminate; it usually splits into two segments in adult leaves. The auricles are a pair of small appendages with long hairs that are positioned at the leaf margins (Fig. 5A).

The rice leaf is also polarized along the adaxial–abaxial axis (Fig. 5B, E). Numerous papilla and two kinds of trichomes are found over the entire leaf surface, except for the

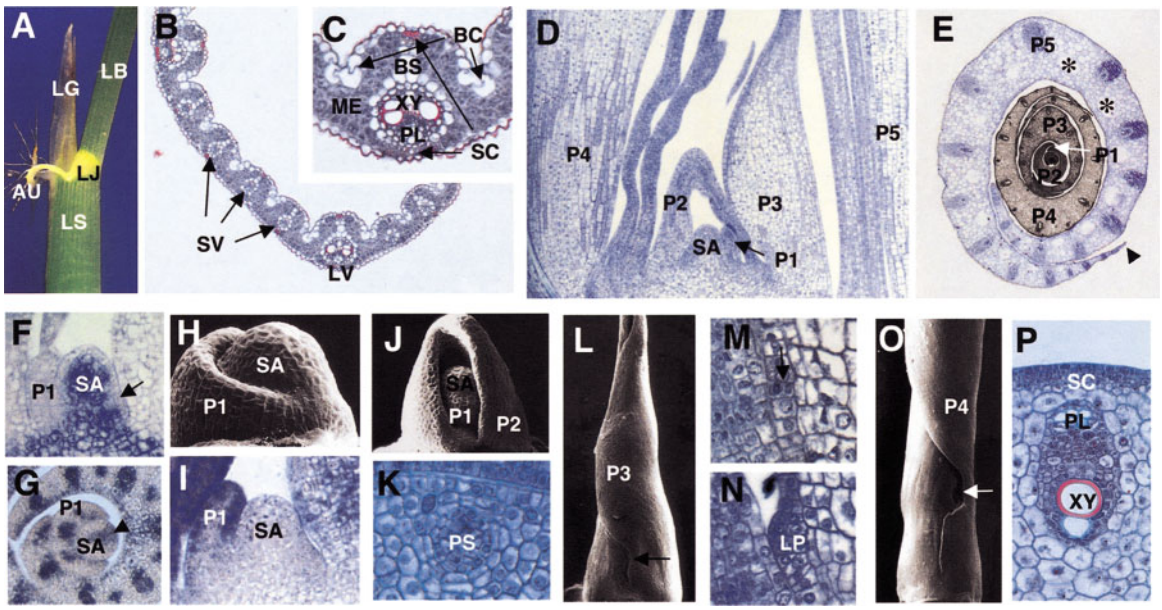

Fig. 5 Leaf development in rice. (A) Mature leaf. (B) Cross-section of mature leaf blade. Upper side is adaxial. (C) Close-up of the midvein in (B). (D) Longitudinal section of shoot apex. (E) Cross-section around shoot apex. Lacuna formation is observed in the leaf sheath of P5 (asterisk). Margin of the leaf sheath is pointed (arrowhead). (F) Expression of *OSH1* in the shoot apex. Down-regulation of *OSH1* is observed in the P0 (arrow). (G) Expression of *OsPNH1* in the shoot apex. The arrowhead indicates the expression in the central region of the P0. (H) SEM image of SAM and late P1 primordium. (I) Expression of *OsSCR* around shoot apex. *OsSCR* expression is observed in the P1 but not in the SAM. (J) SEM image of early P1 and P2 primordium. (K) Cross-section of the center of the P2 differentiating procambial strand. (L) SEM image of the P3. The arrow indicates the blade–sheath boundary. (M, N) Early development of ligule. Periclinal division is shown (arrow). (O) SEM image of the P4. Elongation of the sheath (below the arrow) does not yet start. (P) Cross-section of large vascular bundle of P4 leaf sheath. LB, leaf blade; LG, ligule; AU, auricle; LJ, lamina joint; LS, leaf sheath; LV, large vascular bundle; SV, small vascular bundle; SC, sclerenchymatous cell; PL, phloem; XY, xylem; BS, bundle sheath cell; BC, bulliform cell; SA, shoot apical meristem; PS, procambial strand; LP, ligule primordium.

adaxial surface of the sheath. In the adaxial epidermis of the blade, bulliform cells are arranged in vertical rows between vascular bundles (Fig. 5C). Two types of vascular bundles—large and small ones—are observed (Fig. 5B). The xylem is located on the adaxial side of the vascular bundles, and the phloem on the abaxial side (Fig. 5C). Bundle sheath cells (Fig. 5C) enclose the vascular bundles. Sclerenchymatous fiber cells are observed on the adaxial and abaxial sides of the vascular bundles of leaf blade (Fig. 5C) and on the abaxial side of the sheath. The shapes of the blade and sheath margins are different. The margin of the sheath is pointed and membranous (Fig. 5E).

Leaf primordium is first recognized as a small bulge on the flank of the SAM (Fig. 5J). After the protrusion, the bulge grows toward the apex and toward the opposite side of the SAM, forming a crescent-shaped primordium (Fig. 5H). The primordium becomes hood-shaped by rapid cell division and elongation in the apical and marginal regions (Fig. 5J). At this stage, initiation of the procambial strand is visible in the leaf center (Fig. 5K). After the two margins of the leaf primordium overlap and enclose the SAM, the shape of the leaf primordium becomes cone-like, and the blade (lamina)–sheath boundary is established (Fig. 5L). A protrusion of ligule primordium appears at the blade–sheath boundary of the adaxial surface. That protrusion originates from periclinal divisions of epidermal cells (Fig. 5M, N). Furthermore, the onset of various internal tissues occurs at this stage. The large vascular bundles cover the total width of the leaf, and xylem and phloem are recognized in the mid-vein. In addition, small vascular bundles are found between the large vascular bundles, and macrohairs differentiate on the leaf-tip epidermis. Stomata formation in the blade proceeds basipetally from the distal region, but the proximal region of the epidermis remains immature.

After differentiation of the ligule primordium, the leaf blade elongates rapidly. Although the leaf blade reaches its maximum length around this stage, leaf sheath elongation remains suppressed (Fig. 5O). Following the completion of leaf blade elongation, the leaf sheath commences rapid elongation. Epidermis-specific cell types such as bulliform cells, silica cells and stomata cells become apparent from the apex. Vascular bundles become mature, and sclerenchymatous cells differentiate just outside the vascular bundles (Fig. 5P). When the tip of the leaf blade emerges out of the sheath of the preceding leaf, the internal and epidermal structures of the leaf are almost complete except those in the most proximal region. Lacuna, which are air spaces interrupted by septa, are formed basipetally in the inner tissue of the midrib and the leaf sheath (Fig. 5E). The leaf blade bends at the lamina joint as a result of une-

Table 4 Staging of leaf development in rice

Stage		Events	Gene expression	Related mutant
Symbol	Name			
P0	Formation of leaf founder cells	Recruitment of leaf founder cells	Down-regulation of *OSH1* *OsPNH1* [a]	*sho1* [d], *sho2* [d], *lsy* [e]
P1	Formation of leaf primordium	Protrusion of primordium. Elongation of leaf margin around SAM	*OsPNH1* [a] *OsSCR* [b] *DL* [c]	
P2	Hood-shaped primordium	Hood-like shape. Overlapping of two margins. Differentiation of vascular bundle	*OsPNH1* [a] *OsSCR* [b] *DL* [c]	
P3	Formation of ligule primordium	Formation of ligule primordia. Formation of leaf blade–sheath boundary. Differentiation of sclerenchymatous cells. Initiation of epidermal specific cells	*OsPNH1* [a] *OsSCR* [b] *DL* [c]	*lg* [f]
P4	Rapid elongation of leaf blade	Differentiation of epidermal specific cells (bulliform cells, silica cells, cork cells and stomata). Elongation of leaf blade	Down-regulation of *OsPNH1*, *OsSCR* and *DL*	
P5	Rapid elongation of leaf sheath	Elongation of leaf sheath. Emergence of leaf blade from the sheath of preceding leaf. Formation of lacunae. Maturation of leaf epidermal cells		
P6	Maturation	Bending of leaf blade at the lamina joint		

[a] Nishimura et al. (2002), [b] Kamiya et al. (2003a), [c] Yamaguchi et al. (2004), [d] Itoh et al. (2000), [e] Obara et al. (2004), [f] Maekawa (1988).

qual elongation that occurs between the adaxial and abaxial cells (Maeda 1961).

Staging of leaf development—Although the word plastochron is originally defined as a time interval between the initiation of two successive leaves, plastochron number (Pi) is usually used to classify stages of leaf development (Sharman 1942, Hill and Load 1990). P1 represents the youngest leaf primordium just after the protrusion on the flank of the SAM. P2 is the next youngest primordium, and P3 represents the third youngest primordium (Fig. 5D, E). In addition, the term P0 is frequently used (Jackson et al. 1992, Sylvester et al. 1996). P0 represents a state of cells determined as a new leaf primordium in the SAM. Similarly, P–1 can be defined as a stage of cells that will become P0 cells in the next plastochron. It is reasonable to use the plastochron number to represent leaf stages because two successive stages (Pi and Pi+1) are easily distinguished. Moreover, it can be used ubiquitously in other model plants such as *Arabidopsis* and maize (Jackson et al. 1992, Lynn et al. 1999).

The developmental course of a rice leaf sometimes differs with position: the first and second leaves are quite different from adult leaves. However, adult leaves follow a similar developmental course. Here, we propose a staging system for adult leaves (Table 4).

Stage P0: Formation of leaf founder cells. Cells at the P0 stage, called leaf founder cells, are those whose fates are determined to be leaf primordium but are not distinguishable morphologically from other cells in the SAM. They are detected by down-regulation of *OSH1* expression (Fig. 5F). The leaf founder cells are distributed in a half-ring domain in the circumference of the SAM, opposite the youngest leaf primordium. *OsPNH1*, which is preferentially expressed in the developing vascular bundle, is first expressed in the central region of P0, where the midvein will develop later (Fig. 5G) (Nishimura et al. 2002).

Stage P1: Formation of leaf primordium. The P1 leaf primordium is a small protrusion on the flank of the SAM or a crescent-shaped primordium (Fig. 5H). Cell division activity in P1 is much higher than that in the SAM, as confirmed by histone *H4* expression (Itoh et al. 2000). Several molecular markers distinguish P1 from P0. The *OsSCR* expression starts in the P1 epidermal layer (Fig. 5I); it is then restricted gradually to the specific cell files of epidermis (Kamiya et al. 2003a). The *DROOPING LEAF* (*DL*) gene, a regulator of midrib formation and carpel specification, is first expressed in the central region of the P1 primordium (Yamaguchi et al. 2004).

Stage P2: Hood-shaped primordium. The P2 leaf primordium is hood-shaped (Fig. 5J). Molecular markers distinguishing P1 and P2 primordia have not been reported so far.

Stage P3: Formation of ligule primordium. The margins of the P3 leaf overlap and completely enclose the SAM. The P3 leaf is long and conical (Fig. 5L). The main morphological change at this stage is the establishment of the blade–sheath boundary. The ligule primordium is observed (Fig. 5M, N). In the leaf tip, epidermal cells are morphologically distinguished from the internal cells, indicating the cessation of apical meristematic activity (Kaufman 1959a). Epidermal differentiation and small vascular bundle formation proceed basipetally. Cell-file-specific expression of *OsSCR* in the epidermis is observed at the P3 stage (Kamiya et al. 2003a).

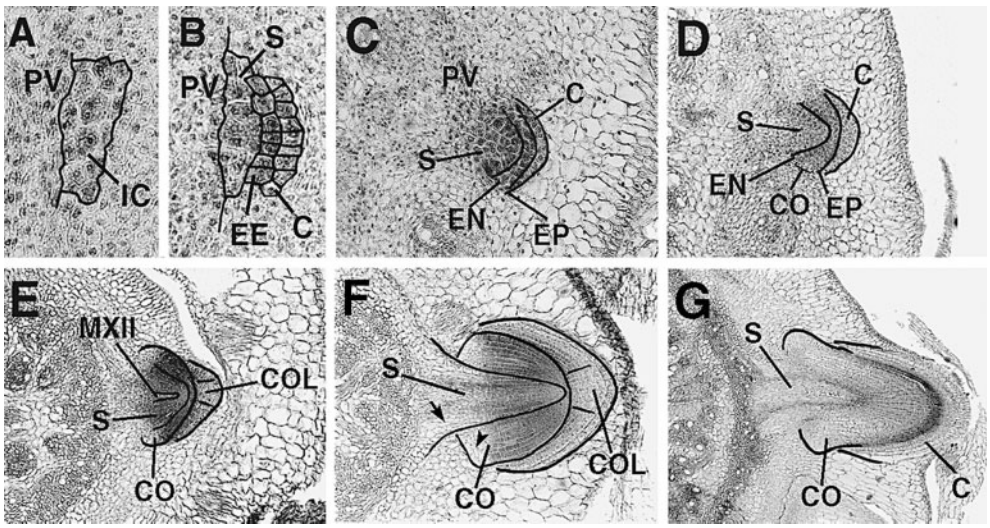

Fig. 6 Crown root development in rice. (A) Establishment of initial cells. (B) Establishment of epidermis–endodermis and root cap initials. (C) Differentiation of epidermis–endodermis initial into epidermis and endodermis. (D) Cortex differentiation. (E) Establishment of fundamental organization of root primordium. (F) Onset of cell vacuolation (arrowhead) in cortex and elongation (arrow) in stele. (G) Crown root emergence. IC, initial cells; PV, peripheral cylinder of vascular bundle; C, root cap or its initial; EE, epidermis–endodermis initials; S, stele (central cylinder); EP, epidermis; EN, endodermis; CO, cortex; COL, columella; MXII, late meta-xylem vessel.

Stage P4: Rapid elongation of leaf blade. A salient feature of the P4 leaf is its rapid leaf blade elongation (Fig. 5O). This elongation is believed to be attributable to the activity of the intercalary meristem located in the basal region of the leaf blade (Kaufman 1959a). Expression of genes that are associated with tissue differentiation, such as *OsPNH1*, *OsSCR* and *DL*, becomes down-regulated (Nishimura et al. 2002, Kamiya et al. 2003a, Yamaguchi et al. 2004).

Stage P5: Rapid elongation of leaf sheath. Following the completion of leaf blade elongation, rapid elongation of the leaf sheath occurs. The leaf blade tip emerges from the sheath of the P6 leaf. The lacuna are formed basipetally in the midrib and the sheath (Fig. 5E).

Stage P6: Mature leaf. The leaf becomes mature and growth is completed. Leaf blade bending occurs at the lamina joint.

Root (Crown Root)

DESCRIPTION OF DEVELOPMENTAL COURSE

The rice root system consists of seminal, crown and lateral roots, which correspond to pole-borne, stem-borne and root-borne roots, respectively (Barlow 1994). Generally, the root system of most dicot plants develops from the radicle formed in the embryo, whereas monocot plants have a so-called fibrous root system that is characterized by numerous crown roots (Klepper 1992). For example, a field-grown rice plant usually has several hundreds (sometimes over 1,000) crown roots (Kawata et al. 1978, Kawashima 1988). To date, organization and cell differentiation processes in lateral root development have been well characterized in a model dicot, *Arabidopsis* (Malamy and Benfey 1997). In contrast, only a few studies have explored root development in monocots. Recently, several molecular markers expressed in specific tissues of the rice root have been reported. The *QHB* gene is expressed specifically in the central cells of the quiescent center in the root apical meristem, and the *OsSCR* gene in the endodermis (Kamiya et al. 2003a, Kamiya et al. 2003b).

Based on morphological observations, Kaufman (1959b) and Kawata and Harada (1975) described root development in rice. They also proposed a staging system of crown root development. Here, we describe rice root development and propose a staging system with a small modification to that by Kawata and Harada (1975). The proposed system incorporates recent results of genetics and molecular biology.

The crown root primordium of rice originates from the innermost ground meristem cells, which are adjacent to the peripheral cylinder of vascular bundles in the stem (Fig. 6A). Those initial cells gradually differentiate various tissues in succession, such as the epidermis, endodermis, cortex, stele (central cylinder) and root cap, to form the complete organization of the apical meristem of the crown root. At the next stage, each tissue increases the number of cells through a regulated division pattern in the following manner. Initial cells of crown root primordium are formed in a few layers by one or two periclinal divisions of the innermost ground meristem cells (Fig. 6A). These initial cells and the nuclei of these cells become enlarged and their protoplasm density gradually increases. Subsequently, cells in the inner layers of the initial divide anticlinally and periclinally to form an epidermis–endodermis initial and a central cylinder initial. Then, the cells in the outer layer of the initial begin to divide mainly anticlinally and form the root cap initial (Fig. 6B). The epidermis–endodermis initial differentiates into epidermis and endodermis by periclinal divisions in a specific cell layer (Fig. 6C). The root cap initial cells and central cylinder initial cells undergo anticlinal and periclinal divisions and consequently increase their own size (Fig. 6C).

The endodermal cells undergo a number of periclinal, but asymmetrical divisions to produce several cortical cell layers (Fig. 6D) (Kawata and Lai 1965). Then root cap initial cells

Table 5 Staging of crown root development in rice

Stage		Events	Expressed gene	Related mutant
Symbol	Name			
Cr1	Establishment of initial cells	Establishment of initial cells of crown root primordium by periclinal divisions of ground meristem		*cr/1* [b]
Cr2	Establishment of epidermis–endodermis, central cylinder and root cap initials	Formation of epidermal–endodermal and central cylinder initials from the inner layer of the initial. Formation of root cap initial from the outer layer of the initial		
Cr3	Differentiation of epidermis and endodermis	Differentiation of epidermis and endodermis from epidermal–endodermal initial	*QHB* [a]	
Cr4	Differentiation of cortex	Formation of cortex by periclinal divisions of endodermal cells	*OsSCR* [a]	
Cr5	Establishment of fundamental organization	Establishment of fundamental organization of root. Root cap reaches the stem epidermis		
Cr6	Onset of cell elongation and vacuolation	Cells in the most basal region of stele start cell elongation		*crl2* [b]
Cr7	Emergence of crown root	Elongation of basal cells of all tissues. Emergence of crown root out of stem		

[a] Kamiya et al. (2003b), [b] Inukai et al. (2001).

form columella through periclinal divisions. The cells in the central cylinder initial (stelar initial) continue anticlinal and periclinal divisions to become dome-shaped (Fig. 6E). At the same stage, cells that constitute various tissues of the vascular bundle are gradually differentiated (Fig. 6E). Then, in the basal region of the primordium, cells of all tissues become vacuolated and elongated concurrently with the emergence of the crown root from the stem (Fig. 6F, G). It is noted that the stage when the vascular bundle system connects the stem and crown root coincides with the time of root emergence (Kawata and Harada 1975).

Staging of crown root development—Crown root development is divided into seven stages (Table 5).

Stage Cr1: Establishment of initial cells. Initial cells of crown root primordium are formed in a few layers by one or two periclinal divisions of the innermost ground meristem cells (Fig. 6A). The *QHB* and *OsSCR* genes are first expressed in the outer layer cells derived from the first periclinal division. Thereafter they maintain their specific expression: *QHB* in the quiescent center and *OsSCR* in endodermis (Kamiya et al. 2003a, Kamiya et al. 2003b). The first periclinal division is suppressed in the *crl1* mutant (Inukai et al. 2001).

Stage Cr2: Establishment of epidermis–endodermis, central cylinder and root cap initials. The initial cells of crown root primordium begin to divide anticlinally and periclinally to form epidermis–endodermis initial, central cylinder initial and root cap initial (Fig. 6B).

Stage Cr3: Differentiation of epidermis and endodermis. The epidermis–endodermis initial differentiates into epidermis and endodermis by periclinal divisions in a specific cell layer (Fig. 6C). The root cap initial cells and central cylinder initial cells undergo anticlinal and periclinal divisions (Fig. 6C).

Stage Cr4: Differentiation of cortex. Endodermal cells begin to form cortical cells by peliclinal divisions (Fig. 6D). When endodermal cells divide periclinally to produce cortex, the expression of *OsSCR* is down-regulated in the daughter cortex cells (Kamiya et al. 2003a).

Stage Cr5: Establishment of fundamental organization. Root cap initial cells form columella by periclinal divisions (Fig. 6E). In the central region of the stele, a large meta-xylem vessel is observed (Fig. 6E). At this stage, fundamental organization of the root is established.

Stage Cr6: Onset of cell elongation and vacuolation. At this stage, cells in the basal region of the stele show commencement of cell elongation and vacuolation, and those of the cortex show vacuolation (Fig. 6F). In the *crl2* mutant, the growth of these cells is suppressed (Inukai et al. 2001). Around this stage, the root apex reaches the stem epidermis.

Stage Cr7: Emergence of crown root. In the basal region of the root primordium, cells of all tissues elongate concurrently with the emergence of the crown roots (Fig. 6G).

Spikelet

Description of developmental course

Spikelet development and its staging have already been published in detail (Ikeda et al. 2004). Thus in this paper, spikelet development is reviewed only briefly.

The rice spikelet consists of a single floret because the spikelet meristem is converted into a floret meristem after producing two pairs of sterile glumes (rudimentary glumes and empty glumes) (Fig. 7A). Rice florets comprise lemma, palea and three kinds of organs: two lodicules (petals), six stamens and one pistil constituted by a single carpel (Fig. 7B)

After producing lateral branches, branch meristems (inflorescence meristems) are converted into spikelet meristems (Fig. 7C). The spikelet meristem first differentiates a pair of sterile glumes in a 1/2 alternate arrangement. They are rudimentary in their shape and bear no axillary buds (Fig. 7D). They are called

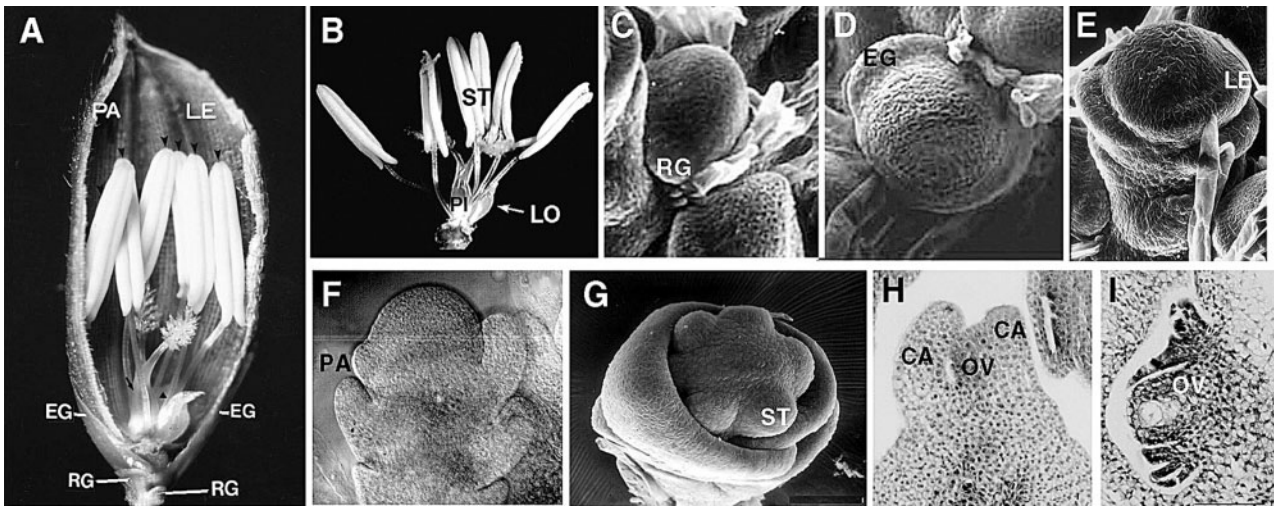

Fig. 7 Spikelet development in rice. (A) Mature spikelet. (B) Mature flower. (C) Early spikelet meristem producing rudimentary primordium. (D) Formation of empty glume primordia. (E) Formation of lemma primordium. (F) Formation of palea primordium. (G) Formation of stamen primordia. (H) Formation of carpel and ovule primordia. (I) Formation of embryo sac. PA, palea; LE, lemma; EG, empty glume; RG, rudimentary glume; ST, stamen; PI, pistil; LO, lodicule; CA, carpel; OV, ovule.

rudimentary glumes in rice or merely 'glumes' in other grasses. Grass species such as barley and wheat produce several florets in one spikelet after the pair of (rudimentary) glumes. In rice, however, one more pair of sterile glumes, called empty glumes, is formed (Fig. 7E); then the spikelet meristem is transformed to a floret meristem to produce two kinds of glumes (one lemma and one palea) and floral organs (Fig. 7F, G). The empty glumes are much larger than rudimentary glumes, but much smaller than lemma. The six glumes: two rudimentary glumes, two empty glumes, lemma and palea, are arranged in 1/2 alternate phyllotaxy. After the palea, floral organs are formed. The meristem at this stage is called a floral meristem. Rice has only three kinds of floral organs: two lodicules, six stamens and one pistil. The lodicules that are positioned on the lemma side are small and whitish. They correspond to petals. Sepals have been lost during the evolution of grasses. Six stamens are formed in a whorl. Finally, one carpel primordium is differentiated from the lemma side of floral meristem, and then encloses the floral meristem. The floral meristem is transformed to an ovule primordium; then an embryo sac is formed (Fig. 7H, I).

The rice (grass) flower structure differs from that of dicots and other monocots in that organs that correspond to sepals are lacking. In addition, only two lodicules (petal) exist and are biased to the lemma side, although the basic number of floral organs in monocots is three. Thereby, one lodicule to be formed on the palea side would have been lost in the past.

Recent studies have revealed that the ABC model is partially modified in rice (Nagasawa et al. 2003) even if we do not consider the loss of organs corresponding to sepals. Rice homologs of class A, B and C genes are expressed as expected (Kyozuka et al. 2000, Nagasawa et al. 2003). However, in contrast to *Arabidopsis*, carpel identity is predominantly regulated by the *DL* gene (Nagasawa et al. 2003).

Staging of spikelet development—Spikelet development is divided into eight stages (Table 6) according to Ikeda et al. (2004).

Stage Sp1: Formation of a pair of rudimentary glume primordia. The spikelet meristem first produces a pair of rudimentary glumes in a 1/2 alternate arrangement (Fig. 7C). The outer one is positioned on the adaxial side of the spikelet meristem. Two rudimentary glumes are vestigial.

Stage Sp2: Formation of a pair of empty glume primordia. Following two rudimentary glumes, a pair of empty glumes is formed in a 1/2 alternate arrangement (Fig. 7D). The empty glumes grow to some extent, but they are sterile. The *FZP* gene is expressed at this stage and is considered to be necessary for floret meristem formation (Komatsu et al. 2003).

Stage Sp3: Formation of lemma primordium. The lemma primordium is formed at a position that is 180° apart from the second empty glume (Fig. 7E). The two empty glumes are growing at this stage. The *LHS* gene regulates the identity of the lemma and palea (Jeon et al. 2000); *RAP1* is expressed in lemma (Kyozuka et al. 2000).

Stage Sp4: Formation of palea primordium. The palea primordium is formed at a position that is 180° apart from the lemma (Fig. 7F). The two empty glumes and lemma are growing. *RAP1* is expressed in the palea (Kyozuka et al. 2000). Many mutants are known to affect lemma and palea development (Iwata and Omura 1971a, Iwata and Omura 1971b).

Stage Sp5: Formation of lodicule primordia. Two lodicule primordia are formed on the lemma sides. The two empty glumes, lemma and palea are growing. The *RAP1*, *OsMADS45*, *OsMADS2* and *SPW1* genes are expressed in lodicules (Kyo-

Table 6 Staging of spikelet development in rice

Stage		Events	Expressed gene	Related mutant
Symbol	Name			
Sp1	Formation of a pair of rudimentary glume primordia	Formation of two rudimentary glumes in 1/2 alternate phyllotaxy	*FZP* [a]	*fzp* [a]
Sp2	Formation of a pair of empty glume primordia	Formation of two empty glumes in 1/2 alternate phyllotaxy		*ur2* [b]
Sp3	Formation of lemma primordium	Formation of lemma in 1/2 alternate phyllotaxy. Elongation of empty glumes	*LHS* [c], *RAP1* [d]	*lhs* [c], *bd1* [e], *bd2* [e], *An1* [f], *An1* [f], *An2* [f]
Sp4	Formation of palea primordium	Formation of palea in 1/2 alternate phyllotaxy. Elongation of empty glumes and lemma	*LHS* [c], *RAP1* [d]	*dp1* [g], *dp2* [h]
Sp5	Formation of lodicule primordia	Formation of two lodicules on the lemma side. Elongation of empty glumes lemma and palea	*RAP1* [d], *SPW1* [i], *OsMADS2* [d], *OsMADS45* [j]	*spw1* [i]
Sp6	Formation of stamen primordia	Formation of six stamen primordia in whorl	*SPW1* [i], *OsMADS2* [d], *RAG* [d], *OsMADS45* [j]	*spw1* [i]
Sp7	Formation of carpel primordium	Formation of carpel. Differentiation of stamen into filament and anther	*RAG* [d], *OsMADS45* [j], *DL* [k]	*dl* [i]
Sp8	Formation of ovule and pollen	Differentiation of integuments and embryo sac. Pollen differentiation	*MSP1* [ll], *OsMADS13* [m], *OsMADS45* [j]	*msp1* [l]

[a] Komatsu et al. (2003), [b] Iwata et al. (1983), [c] Jeon et al. (2000), [d] Kyozuka et al. (2000), [e] Misro (1981), [f] Nagao and Takahashi (1963), [g] Iwata and Omura (1971a), [h] Iwata and Omura (1971b), [i] Nagasawa et al. (2003), [j] Greco et al. (1997), [k] Yamaguchi et al. (2004), [l] Nonomura et al. (2003), [m] Lopez-Dee et al. (1999).

zuka et al. 2000, Nagasawa et al. 2003). In *spw1*, lodicules are transformed to glumes (Nagasawa et al. 2003). The number of lodicules is increased in *fon1* and *fon2* mutants (Nagasawa et al. 1996).

Stage Sp6: Formation of stamen primordia. Six stamen primordia are formed in the whorl (Fig. 7G). The *OsMADS45*, *SPW1* and *RAG* are expressed in stamens (Kyozuka et al. 2000, Nagasawa et al. 2003). The stamens are transformed to carpels in the *spw1* mutant (Nagasawa et al. 2003). The number of stamens is increased in *fon1* and *fon2* mutants (Nagasawa et al. 1996).

Stage Sp7: Formation of carpel primordium. A carpel primordium is formed on the lemma side of the floral meristem (Fig. 7H). At this stage, stamen primordia differentiate into an anther and filament.

OsMADS45, *RAG* and *DL* are expressed in the carpel primordium (Kyozuka et al. 2000, Yamaguchi et al. 2004). In the *dl* mutant, the carpel is transformed to stamens (Nagasawa et al. 2003). *fon1* and *fon2* increase the number of pistils (Nagasawa et al. 1996).

Stage Sp8: Formation of ovule and pollen. When the carpel primordium extends to the palea side, *OSH1* expression is down-regulated from the floral meristem. In addition, it is converted to ovule primordium (Fig. 7H, I). The carpel encloses the ovule at this early stage (Fig. 7H). The ovule undergoes female gamete formation. Simultaneously, pollen grains are formed in the anther. For detailed descriptions of ovule and pollen development, see below.

The *OsMADS45* and *OsMADS13* genes are expressed in the ovule (Lopez-Dee et al. 1999). The *MSP1* gene is expressed in the surrounding tissues of male and female sporocytes (Nonomura et al. 2003).

Development of Specific Organs/Tissues of Interest

Ovule

Description of developmental course

The ovule is a female reproductive organ where megasporogenesis, megagametogenesis, fertilization and embryogenesis take place. The ovule comprises the nucellus, chalaza and funiculus along the proximal–distal axis. In the radial direction, inner and outer integuments differentiate from the chalaza. To date, most studies on ovules have used dicot species. They have revealed a number of genes that are associated with placental formation, ovule primordium differentiation, ovule identity, differentiation of each ovule component and integument development (for review, see Gasser et al. 1998, Grossniklaus and Schneitz 1998, Skinner et al. 2004). Analysis of petunia MADS-box genes is a pioneer in the field of ovule identity establishment (Angenent et al. 1995, Colombo et al. 1995). By contrast, only a few studies have examined ovule development in monocots. In rice, the *OsMADS13* gene is considered to regulate ovule identity (Lopez-Dee et al. 1999). *OsMADS24* and *OsMADS45* are also considered to assist the function of *OsMADS13* (Favaro et al. 2002).

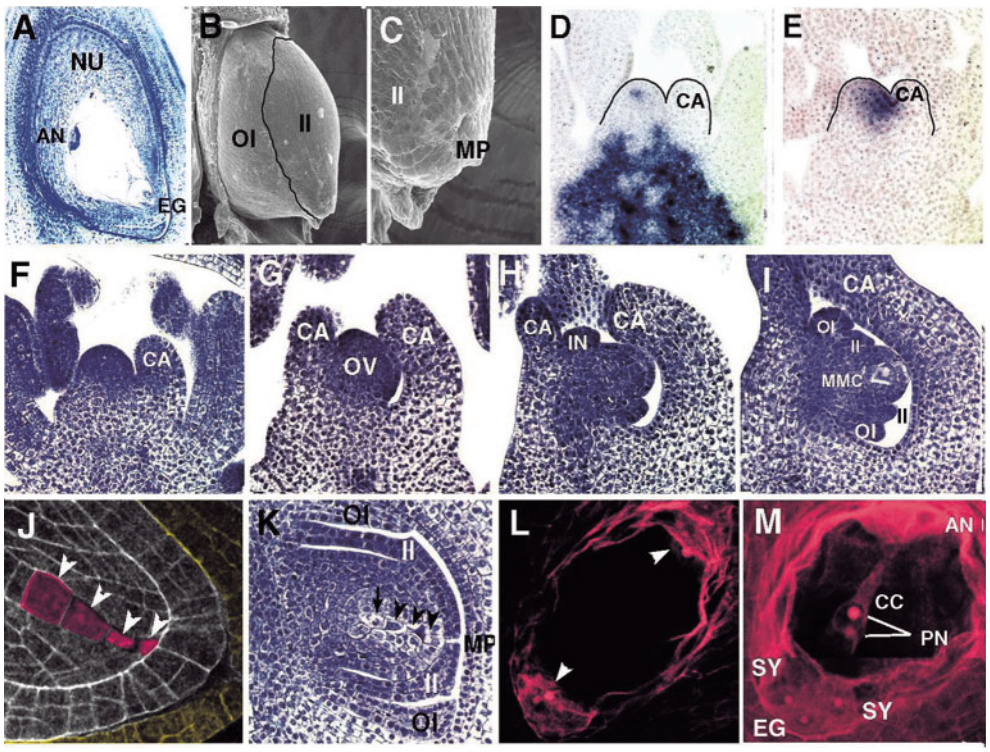

Fig. 8 Ovule development in rice. (A) Longitudinal section of mature semianatropous ovule. (B) SEM image of mature integument. The black line indicates the boundary between the inner and outer integuments. (C) Close-up image of micropyle region. (D) Down-regulation of *OSH1* expression from the floral meristem/ovule primordium when the carpel primordium is formed. (E) Expression of *OsMADS13* in the floral meristem/ovule primordium where *OSH1* expression is down-regulated. (F) The early stage of the ovule primordium. (G) Ovule primordium just before integument protrusion. (H) Formation of integument primordium. (I) Differentiation of integument primordium into inner and outer integuments. (J) Formation of linearly arranged four megaspores (arrowheads). (K) Degeneration of three megaspores and formation of micropyle. The arrow indicates a functional megaspore and the arrowheads three degenerating megaspores. (L) Formation of the eight-nucleate embryo sac. Four nuclei exist in each region indicated by an arrowhead. (M) Formation of cellularized embryo sac. NU, nucellus; AN, antipodals; EG, egg cell; II, inner integument; OI, outer integument; MP, micropyle; CA, carpel; OV, ovule primordium; IN, integuement primordium; MMC, megaspore mother cell; CC, central cell; PN, polar nucleus; SY, synergid.

Ovule development in rice was described correctly by Lopez-Dee et al. (1999). They also proposed a useful staging system for the ovule. Here, we describe rice ovule development and staging system with a small modification to that proposed by Lopez-Dee et al. (1999).

Rice has a hemianatropous ovule in the monocarpellary pistil. The ovule attaches its basal part to the ovary wall on the palea side; its apical part inclines toward the basal lemma side (Fig. 8A). The micropyle and egg apparatus are positioned in the receptacle end on the lemma side. In rice, the funiculus is vestigial; the chalaza seems to be attached directly to the ovary wall (Fig. 8A). The inner integument covers most of the nucellus leaving a small pit, a micropyle (Fig. 8B, C). The outer integument, however, covers only a quarter of the inner integument on the style side (Fig. 8B).

The floral meristem is converted into an ovule primordium when the carpel primordium formed on the lemma side of the floral meristem elongates to some extent (Fig. 8F). This conversion is confirmed by the down-regulation of *OSH1* expression and the complementary onset of *OsMADS13* expression (Fig. 8D, E), although the ovule primordium is first identified morphologically when the carpel primordium becomes ring-shaped (Fig. 8G). Just before the carpel primordium encloses the ovule primordium, a ring-shaped integument primordium is formed from the base (charaza) of the ovule (Fig. 8H). At this stage, the archesporial cell (AC) in the nucellus tip becomes enlarged. Soon after integument primordium differentiation, the carpel completely encloses the ovule primordium to form an ovary locule. Then, the single integument primordium divides into inner and outer integuments (Fig. 8I). The AC elongates and differentiates directly into the megaspore mother cell.

Although both inner and outer integuments elongate, elongation of the inner integument is remarkable. It envelops most of the nucellus except for the micropyle area near the megaspore mother cell (Fig. 8C). The outer integument elongates as long as the inner integument on the receptacle side, but that elongation is restricted to a quarter of the inner integument on the style side (Fig. 8B, K). The inner integument is stained more intensely than the outer integument by hematoxylin or

Table 7 Staging of ovule development in rice

Stage			Events	Inflorescence length (cm) [a]	Expressed genes
Symbol	Lopez-Dee et al. (1999)	Name			
Ov1	Ov 1	Ovule primordium differentiation		0.3–1	*OsMADS13* [a]
Ov2	Ov 2–Ov 3	Integument primordium differentiation	Integument primordium differentiation. Ovary locule formation. AC enlargement	1–3	Rice homolog of *ANT* [b]
Ov3	Ov 2–Ov 3	Division of integument primordium	Division of integument primordium into inner and outer integuments. MMC differentiation	3–5	*SPW1* [c] *MSP1* [d]
Ov4	Ov 4	Meiosis of MMC	Integument elongation. Micropyle formation. Meiosis of MMC	5–7	
Ov5	Ov 4	Degeneration of three micropylar spores	Integuments elongation. Degeneration of three micropylar spores	7–10	
Ov6	Ov 5	First mitotic nuclear division	First mitotic nuclear division of chalazal spore. Onset of ovule inclination	10–12	
Ov7	Ov 6	Second mitotic nuclear division	Second mitotic nuclear division. Vacuole formation in megagametophyte	12–14	
Ov8	Ov 7	Third mitotic nuclear division	Third mitotic nuclear division resulting in eight-nucleate megagametophyte	14–16	
Ov9	Ov 8	Polarization of nuclei	Polarization of nuclei. Completion of ovule inclination	16–18	
Ov10	Ov 9	Maturation	Maturation	18–25	

[a] Values are for Taichung 65.
[b] Lopez-Dee et al. (1999), [c] Yamaki, S., Ito, M. and Nagato, Y. (unpublished data), [d] Nagasawa et al. (2003), [e] Nonomura et al. (2003).

toluidine blue. Then, the ovule starts to incline toward the receptacle side.

Concomitant with integument elongation, the polygonum-type embryo sac is formed in the nucellus. The megaspore mother cell undergoes meiotic division and the four megaspores are arranged linearly in the micropyle–chalaza direction (Fig. 8J). Only the chalazal megaspore remains functional; the other three micropylar spores degenerate (Fig. 8K).

Following meiosis, the functional megaspore undergoes mitotic nuclear division to become a two-nucleate cell. The nucellar cells degenerate gradually and the megagametophyte becomes enlarged. In time, the megagametophyte forms a large vacuole and each of the two nuclei undergoes a second mitotic division. Successively, the megagametophyte becomes eight-nucleated through the third mitotic division.

After mitotic division, the polarization of egg cell, synergid cells, polar nuclei and antipodal cells occurs (Fig. 8L, M). The antipodal cells of rice continue to divide and form an antipodal cell cluster at the chalazal end, as in maize (Huang and Sheridan 1994). The ovule inclines to form a hemianatropous one. The pistil is enlarged along the style–receptacle axis.

Staging of ovule development—Based on the events described above, we propose a staging system of ovule development (Table 7). This system is a small modification of that by Lopez-Dee et al. (1999).

Stage Ov1: Ovule primordium formation. Soon after the carpel primordium is differentiated morphologically, the *OsMADS13* gene begins to be expressed in the cells where *OSH1* expression becomes down-regulated in the floral meristem (Fig. 8D–F). That expression is the first sign of ovule formation. The ovule primordium becomes obvious: the proximal end is attached to the palea-side carpel (Fig. 8G).

Stage Ov2: Integument primordium formation. The integument primordium protrudes from the base of the ovule primordium (Fig. 8H). The carpel encloses the ovule to form an ovary locule. The AC becomes enlarged. The rice homolog of the *AINTEGUMENTA* gene starts to be expressed in the integument primordium (Yamaki, S., Ito, M. and Nagato, Y. unpublished data).

Stage Ov3: Division of integument primordium. The single integument primordium divides into inner and outer integuments (Fig. 8I). The AC differentiates into a megaspore mother cell (Fig. 8I). *SUPERWOMAN1*/*OsMADS16* (Nagasawa et al. 2003) begins to be expressed in the abaxial side of the outer integument. *MULTIPLE SPOROCYTE1* (*MSP1*) is expressed

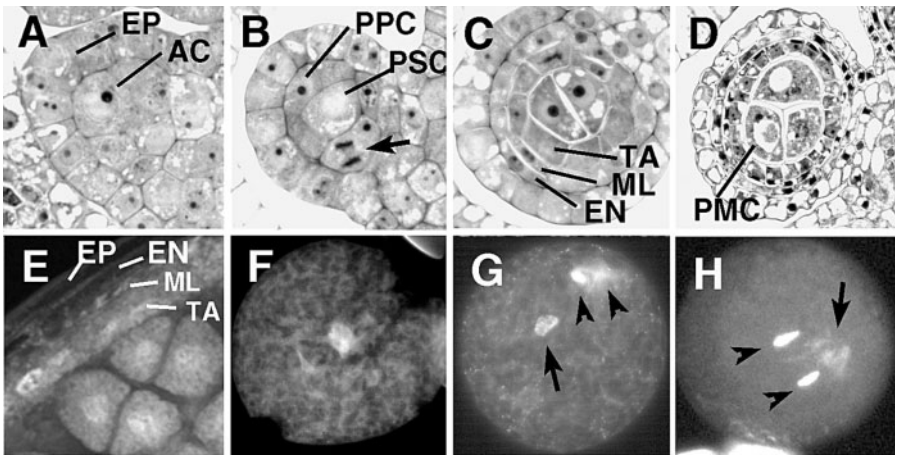

Fig. 9 Sporogenesis and gametogenesis in rice anther. (A) The ACs differentiate into the hypodermis of anthers. (B) The ACs continue periclinal division (arrow) and develop PSCs and PPCs. (C) Formation of four-layered anther wall through periclinal divisions of PSCs. (D) Formation of PMCs. PMCs enter into meiosis. (E) Formation of tetrad spores. (F) The uninucleated microgametophyte becomes spherical and enlarged. (G) A first pollen mitosis produces a vegetative nucleus (arrow) and generative nuclei (arrowheads). (H) Mature pollen grain contains two sperm nuclei (arrowheads), and an amorphous vegetative nucleus (arrow). EP, epidermis; EN, endocethium; Ml, middle layer; TA, tapetum.

in the whole carpel and ovule (Nonomura et al. 2003). The *msp1* mutant has a large number of female gametocytes.

Stage Ov4: Meiosis of megaspore mother cell. Inner and outer integuments elongate. Elongation of the outer integument on the style side is limited. The megaspore mother cell undergoes meiosis to form four linearly arranged megaspores (Fig. 8J).

Stage Ov5: Degeneration of three micropylar spores. The integuments complete their elongation and form a micropyle (Fig. 8K). At this stage, the ovule is anatropous. Three of the four megaspores degenerate: only the chalazal one develops to a functional megagametophyte (Fig. 8K).

Stage Ov6: First mitotic nuclear division. The nucellar cells gradually degrade. The enlarged megagametophyte undergoes the first nuclear division. The ovule starts inclining toward the receptacle side.

Stage Ov7: Second mitotic nuclear division. The two nuclei of the megagametophyte undergo a second nuclear division. Degeneration of nucellar cells continues. Ovule inclination continues.

Stage Ov8: Third mitotic nuclear division. A large central vacuole is formed in the megagametophyte and the third nuclear division occurs. Consequently, an eight-nucleated megagametophyte is formed (Fig. 8L). Ovule inclination continues.

Stage Ov9: Migration of nuclei and cellularization. The eight nuclei migrate to their respective positions, and cellularization occurs (Fig. 8M).

Stage Ov10: Maturation. This stage marks the completion of the hemianatropous ovule with embryo sac, comprising one egg cell, two synergids, one central cell (fusion of two polar nuclei) and antipodal cells (Fig. 8A).

Anther: Sporogenesis and Gametogenesis

Description of developmental course

In *Arabidopsis*, many mutants and genes have been reported as associated with anther development, sporogenesis and gametogenesis (Scott et al. 2004, McCormick 2004, Yadegari and Drews 2004). On the other hand, genetic regulation of these reproductive pathways remains unclear in rice, despite the fact that the staging and dissecting reproductive pathway of rice helps to surmount the reproductive barrier for introgression of agronomic traits into cultivars from distantly related species.

The stamen initiates in the third whorl of the rice flower. The anther primordium comprises three layers, designated L1, L2 and L3 (Satina et al. 1940, Goldberg et al. 1993). In rice the L1 layer gives rise to the epidermis and the stomium, which play important roles in anther dehiscence; the L2 layer develops into the archesporial cells, which are the primordial germ cells from which pollen and microsporangium differentiate; and the L3 layer gives rise to the connective cells, vascular bundles and circular cell cluster adjacent to the stomium (Raghavan 1988, Nonomura et al. 2003). The connective cells are positioned between microsporangia and vascular bundles, and degenerate during gametogenesis

In rice, a few cylindrical rows of hypodermal anther cells differentiate into ACs in the four corners of a transverse section (Fig. 9A). The ACs can be distinguished from other cells by a slightly enlarged nucleus and cytoplasm. After differentiation, they continue cell divisions to form primary sporogenous cells (PSCs) and primary parietal cells (PPCs) (Fig. 9B). The PSCs undergo several mitotic divisions and differentiate into pollen mother cells (PMCs). The PPCs repeat periclinal divisions and generate endothecium, a middle layer and a tapetum layer (Fig. 9C). Each of the four-walled layers expands through anticlinal divisions. The tapetal cells are binucleated and serve as a nurse tissue to provide nutrition and pollen wall materials for gametophytes (reviewed by Scott et al. 2004).

After completion of anther wall formation, the PMCs undergo pre-meiotic DNA synthesis (pre-meiotic S) and enter meiosis (Fig. 9D) (Nonomura et al. 2004b). Meiotic events are described in the following section of this review.

Spores develop into haploid gametophytes (Fig. 9E), which produce haploid gametes. Angiosperm sporophytes produce two types of spores—microspores and megaspores—

Table 8 Staging of anther development in rice

Stage		Events	AL [a] (mm)	FL [b] (mm)	Ovule stage	Expressed gene	Related mutant
Symbol	Name						
An1	Formation of hypodermal ACs	ACs differentiation at four corners of hypodermis of anthers	0.1–0.15	ND [c]			
An2	Formation of anther wall layer	Periclinal division of hypodermis to form layered structure of anther wall	0.15–0.3	0.7–2.0	Ov1–Ov2	*MSP1* [d]	*msp1* [d]
An3	Completion of anther wall layer	Establishment of PMCs and four-layered anther wall. Pre-meiotic DNA synthesis in PMCs	0.3–0.5	1.5–3.5	Ov3–Ov4		
An4	Meiosis	(see Table 9)	0.45–0.95	1.6–4.8	Ov5	(see Table 10)	(see Table 10)
An5	Formation of tetrad spores	Formation of four haploid spores. Onset of endothecium and middle layer degradation	0.8–1.1	4.0–6.0	Ov5		
An6	Formation of uninucleated gametophyte	Formation of spherical and enlarged microspore. Formation of exin, intine and a pore for pollen tube germination	1.1–2.2	5.0–7.0	Ov6–Ov8	*	**
An7	Formation of binucleated gametophyte	Formation of vegetative nucleus and a generative nucleus by pollen mitosis I	2.2	7.0	Ov9	*	**
An8	Formation of mature pollen	Formation of two sperm nuclei by pollen mitosis of generative nucleus	2.2	7.0	Ov9	*	**

[a] AL: anther length in cv. Nipponbare, [b] FL: floret length in cv. Nipponbare, [c] ND: not determined; [d] Nonomura et al. (2003), [e] Tsuchiya et al. (1994), [f] Zhu et al. (2004), [g] Lee et al. (2004), [h] Kazama and Toriyama (2003), [i] Komori et al. (2004).
* *Osc4* [e], *Osc6* [e], *AID1* [f], *OsCP1* [g], *Rf1* [h,i]. ** *aid1* [f] *oscp1* [g], *rf1* [h,i]

which give rise to pollen grain and embryo sac, respectively. After release from the tetrads, the free microspores become spherical and enlarged without mitotic cell division (Fig. 9F). In maize, intine and exine layers, which are components of the pollen wall, are formed at this stage (Chang and Neuffer 1989). In most flowers, this uninucleate status of male gametophytes is retained until the inflorescence heading. Although anther length is correlated roughly with development of microsporangia, most anthers reach their maximum length at the end of the uninucleate stage (Table 8). The first pollen mitosis produces a generative nucleus and a vegetative nucleus. The generative cell subsequently divides into two sperm cells (Fig. 9G). We have observed rice gametogenesis by the chromatin staining method. Therefore, we use the terms 'sperm nuclei' and 'binucleate stage' in this study in place of 'sperm cells' and 'bicellular stage', respectively.

Staging of anther development—Based on the developmental processes described above, we propose a staging system for anther development (Table 8).

Stage An1: Formation of hypodermal ACs. In transverse section, the anther initial is ovoidal; it subsequently becomes four-cornered in shape. At the four corners, the ACs initiate at the L2 layer (Fig. 9A).

Stage An2: Formation of anther wall layer. The ACs differentiate into PSCs and PPCs; the PPCs continue to divide periclinally (Fig. 9B). Transcripts of the *MSP1* gene, suppressing the AC derivatives entering into sporogenesis, start to accumulate in PPCs and are retained in the developed tapetum, but not in PSCs and PMCs (Nonomura et al. 2003).

Stage An3: Completion of anther wall layer. Formation of the four-layered anther wall is completed (Fig. 9C). PSCs mature into PMCs and undergo pre-meiotic S at this stage (Nonomura et al. 2004b) (Fig. 9C).

Stage An4: Meiosis. PMCs and a megaspore mother cell (MMC) enter into meiosis. The PMCs gradually become spherical as a result of callose accumulation in anther locules (Fig. 9D).

Stage An5: Formation of tetrad spores. The PMC produces four haploid spores (Fig. 9E). The tapetal cells are binucleated; the endothecium and middle layer begin to collapse (Fig. 9E). *MSP1* mRNAs in both anthers and ovules has disappeared by this stage (Nonomura et al. 2003).

Stage An6: Formation of uninucleate gametophyte. The microspores become spherical and enlarged (Fig. 9F), and a pore for pollen tube germination differentiates. In the 1.6 mm long anthers, tapetal cells have almost degenerated (Fig. 9F). At this stage, the expression of *Osc4* and *Osc6* mRNA is detected in tapetal cells, although the function of both genes remains unknown (Tsuchiya et al. 1994). Promoter activity of a cysteine protease gene *OsCP1*, whose T-DNA-tagged mutation affects pollen development, is also found (Lee et al. 2004). The *AID1* (Zhu et al. 2004) and *Rf1* (Kazama and Toriyama 2003, Komori et al. 2004) genes function at this stage, as inferred from their mutant phenotypes, although they have not yet been characterized histologically.

Stage An7: Formation of binucleate gametophyte. First pollen mitosis produces two nuclei, a vegetative nucleus and a generative nucleus (Fig. 9G).

Stage An8: Formation of mature pollen. The generative nucleus successively undergoes a second pollen mitosis. The mature pollen contains two smaller sperm nuclei, and a larger but amorphous vegetative nucleus (Fig. 9H).

Meiosis

Course of meiosis

Meiosis is a crucial event for the sexual reproduction of eukaryotes to form haploid spores and gametes. This event is characterized by a single round of pre-meiotic S followed by two rounds of chromosome segregation. The pre-meiotic S is known to affect meiotic events such as homologous chromosome pairing, reductional chromosome segregation and homologous recombination in wheat (Martinez-Perez et al. 1999), fission yeast (Watanabe et al. 2001) and *Arabidopsis* (Mercier et al. 2003).

Homologous pairing in early meiosis, which plays important roles in faithful cell division and homologous recombination, is divided into several substages based on the state of synapsis and condensation. Homologous chromosomes begin to condense at leptotene. They become partially synapsed at zygotene, become fully synapsed at pachytene, subsequently becoming desynapsed but held together by chiasmata at diplotene and diakinesis. The synapsis guarantees a strong and close connection of the homologous pairs, facilitated by a network of longitudinal and transversal protein fibers called the synaptonemal complex (SC) (Moses 1969, Westergaard and Wettstein 1972, Gillies 1975). The function of the SC in the early prophase remains to be elucidated.

Homologous recombination is also an important event for generating genetic diversity in offspring. In yeast, recombination initiation depends on DNA double-strand breaks by the SPO11 machinery (Bergerat et al. 1997, Keeney et al. 1997), and on the RAD51 and DMC1 complex, which catalyses homologous recombination (Masson and West 2001). Many genes and mutations such as *AtSPO11*, *AtRAD51* and *AtDMC1* have been reported in *Arabidopsis* (reviewed by Caryl et al. 2003). Two *DMC1* homologs, *OsDMC1A* and *OsDMC1B*, have been identified in rice, although their functions in meiosis remain unclear (Kathiresan et al. 2002).

A number of spontaneous and induced synapsis mutations have also been reported in rice (Kitada et al. 1983, Kitada and Omura 1983, Nonomura et al. 2004a, Nonomura et al. 2004b), but the genetic regulation of rice meiosis is poorly understood. The genus *Oryza* consists of more than 20 wild and two cultivated species. The interspecific F_1 hybrids between cultivars and wild species frequently show high sterility, which is partly attributable to the low ability of homologous pairing at meiosis (Katayama 1963, Brar and Khush 1997). Dissecting the meiotic process of *Oryza* will contribute to reducing reproductive barriers between different genome species.

Staging of meiosis—The staging of meiosis described here is based on events in anthers. Zhang and Zhu (1987) have estimated the absolute time course of male meiosis through statistical analysis in rice. In this review, anther length is used mainly as a parameter to represent the approximate time course in addition to the floret length because the longitudinal lengths of anthers and floret are correlated roughly with meiotic stages. Optical sectioning shows that the progression of female meiosis is synchronized loosely with that of male meiosis (Table 9).

Stage Mei1: Pre-meiotic S/G_2. Decondensed chromatins fill up the nucleoplasm (Fig. 10A). Pre-meiotic S occurs synchronously among the PMCs in anthers of 0.3–0.4 mm in length (Nonomura et al. 2004b). However, it is difficult to distinguish pre-meiotic S from G_2 in meiocytes. Localization of the PAIR2 protein into the PMC nuclei takes place subsequent to pre-meiotic S (Nakano et al. unpublished data). Results of RT–PCR and mutant analysis suggest that expression of *PAIR1* mRNA is linked to early meiosis or pre-meiosis (Nonomura et al. 2004a).

Stage Mei2: Leptotene. Meiosis-specific chromosome condensation starts (Fig. 10B). The lateral element (LE) of the SC is associated with chromosomal axes (Nonomura, K.I., Eiguchi, M., Nakano, M., Suzuki, T. and Kurata, N. unpublished data).

Stage Mei3: Zygotene. Synapsis of homologous chromosomes progresses to form central components of the SC between LEs of homologous chromosome axes (Fig. 10C). Chromosomes transiently become a compact sphere and adhere to the nucleolus at early zygotene to form a so-called synizetic knot (Nonomura et al. 2004b). The importance of the synizetic knot, however, is poorly understood.

Stage Mei4: Pachytene. Homologous chromosome synapsis is completed, and the paired chromosomes are thickened (Fig. 10D). Homologous recombination is inferred to occur at around this stage.

Stage Mei5: Diplotene. Because of the rapid removal of SCs from most chromosomal regions, homologous chromosomes are separated from each other, while pairing is maintained at the chiasmata (Fig. 10E).

Table 9 Staging of meiosis in rice anther

Stage		Events in PMC	AL [a] (mm)	FL [b] (mm)	Events in MMC	Expressed gene	Related mutant
Symbol	Name						
Mei1	Pre-meiotic S/G_2	Pre-meiosis specific DNA synthesis	0.02–0.45	0.9–1.6	ND [c]	*PAIR1* [d], *PAIR2* [d]	
Mei2	Leptotene	Initiation of meiotic chromosome condensation. Association of LEs to chromosome axes	0.40–0.55	1.5–2.1	ND		
Mei3	Zygotene	Establishment of central components between homologous chromosome axes	0.45–0.65	1.9–2.4	ND		*pair1* [d], *pair2* [e], *as* [f]
Mei4	Pachytene	Completion of SC formation. Homologous chromosome recombination	0.60–0.80	2.3–3.4	Zygotene to interkinesis		*ds1* [g], *ds2~d*, *s11* [h]
Mei5	Diplotene	Degradation of SC	0.75–0.85	2.8–3.4	ND		
Mei6	Diakinesis	Breakdown of nuclear envelope. Disappearance of a nucleolus	0.70–0.90	2.9–3.4	Pachytene to interkinesis		
Mei7	Metaphase I	Alignment of homologous chromosome pairs at equatorial plate	0.80–0.90	3.3–4.4	ND		
Mei8	Anaphase I/ telophase I	Reductional division of each homolog	0.80–0.90	4.0–4.8	ND		
Mei9	Interkinesis (prophase II)	Formation of daughter cells and nuclei	0.80–0.90	4.0–4.8	ND		
Mei10	Metaphase II	Alignment of sister-chromatid pairs at equatorial plates	0.80–0.90	4.0–4.8	ND		
Mei11	Anaphase II/ telophase II	Equational division of each sister chromatid	0.80–0.95	4.0–4.8	ND		
Mei12	Tetrad	Formation of four haploid spores	0.80–1.10	4.0–5.0	Interkinesis to tetrad		

[a] AL: anther length in cv. Nipponbare, [b] FL: floret length in cv. Nipponbare, [c] ND: not determined.
[d] Nonomura et al. (2004a), [e] Nonomura et al. (2004b), [f] Katayama (1963), [g] Chao and Hu (1960), [h] Kitada and Omura (1983).

Stage Mei6: Diakinesis. Chromosome pairs are highly condensed (Fig. 10F) and attached closely to the nuclear envelope. The nucleolus begins to disappear.

Stage Mei7: Metaphase I. The nuclear envelope is broken down. Bipolar kinetochores of homologous chromosome pairs are captured by microtubule bundles of bipolar spindles; the captured pairs align on the equatorial plate (Fig. 10G). The nucleolus disappears completely.

Stage Mei8: Anaphase I/telophase I. Homologous chromosomes separate and move to opposite poles (reductional division) (Fig. 10H). At the end of this stage, a phragmoplast, a cytoskeletal structure held by two arrays of microtubule bundles, determines the position of the cytokinetic plate (Nonomura et al. 2004a).

Stage Mei9: Interkinesis (prophase II). Two daughter cells and nuclei are reconstructed. Small nucleoli appear with condensed chromosomes (Fig. 10I).

Stage Mei10: Metaphase II. Chromosomes align on the equatorial plate of each daughter cell (Fig. 10J). The nucleoli disappear again.

Stage Mei11: Anaphase II/telophase II. Sister chromatids of chromosomes separate and move to opposite poles (equational division) (Fig. 10K).

Stage Mei12: Tetrad. Meiotic cell division is completed, producing four haploid spores (Fig. 10L).

Stomata

Description of developmental course

Stomata are cell complexes that are specialized for gas exchange between the leaf and its environment. Stomata in rice are distributed in vertical rows on the leaf surface (Fig. 11A), but those on the adaxial surface of the leaf sheath are rudimentary. Cell rows in which stomata are to be formed later are called stomatal cell rows. The distribution of stomatal cell rows in the epidermis is not random. In rice, the stomatal cell rows are located on the flanks of vascular bundles. The rows are usually separated, but are sometimes formed in two adjacent files. Stomata in rice consist of two guard cells that are narrow and have thickened walls and two subsidiary cells flanking the guard cells.

Development of stomata in rice has been well described by several authors (Kaufman 1959a, Hoshikawa 1989). Therefore, we briefly summarize the course while incorporating recent results. At the P3 stage of leaf development, the stomatal cell row is determined basipetally on the leaf epidermis (Fig. 11B). Symmetric transverse cell divisions in the stomatal cell

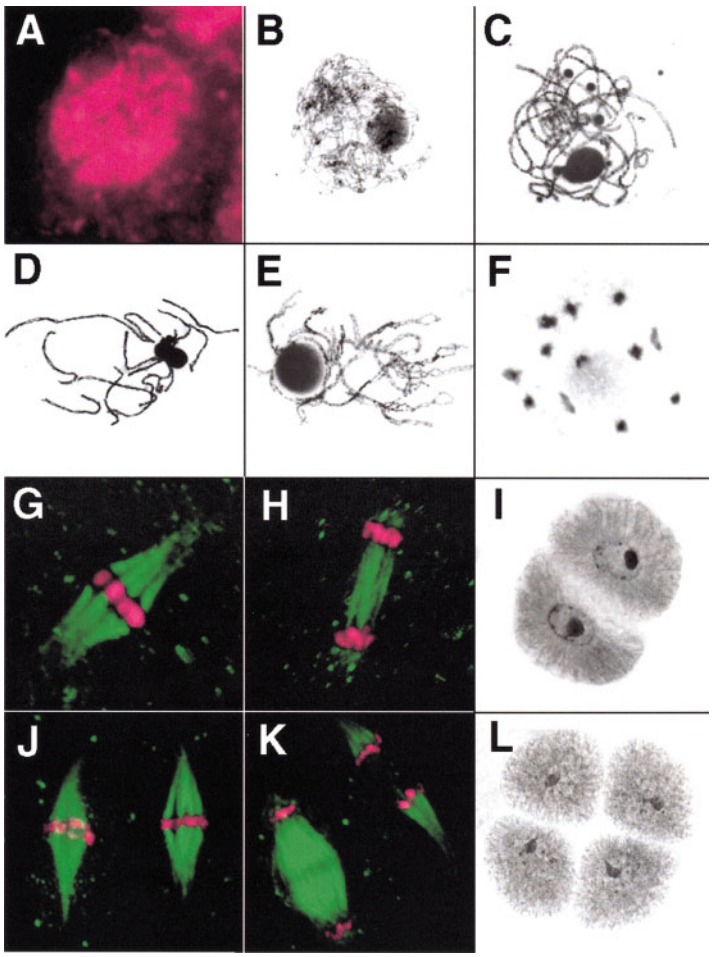

Fig. 10 Meiosis in rice anther. (A) Pre-meiotic stage. Chromosomes are stained with 4,6-diamidino-2-phenylindole (magenta). (B) Leptotene stage. (C) Zygotene stage. (D) Pachytene stage. (E) Diplotene stage. (F) Diakinesis stage. (G) Metaphase I. Spindle fibers (green) capture and align homologous chromosome pairs (magenta) at metaphase I plate. (H) Anaphase/telophase I. (I) Interkinesis. (J) Metaphase II. (K) Anaphase II/telophase II. (L) Tetrad stage.

row produce numerous cells smaller than those on either side of the row. The first asymmetric divisions produce guard mother cells (GMCs) and non-specialized epidermal cells (Fig. 11C). The GMC is a small cell that is stained strongly; the non-specialized epidermal cell is a large and weakly stained cell. The second asymmetric divisions occur in the two lateral epidermal cells (subsidiary mother cells, SMCs) adjacent to the GMC to form subsidiary cells, resulting in a three-cell complex (GMC + two subsidiary cells) (Fig. 11D). Finally, transverse symmetric divisions in GMC produce a guard cell pair (Fig. 11E). At maturity, the guard cells elongate and become dumb bell-shaped, while the subsidiary cells become ellipsoidal (Fig. 11F).

Staging of stomata development—Staging of stomata formation in rice was first proposed by Stebbins and Shah (1960). Kamiya et al. (2003a) subsequently described the staging in detail. According to Kamiya et al. (2003a), stomata formation is divided into six stages. Their staging system is based on the cell division pattern and the expression pattern of the *OsSCR* gene. The *SCR* gene was first reported in *Arabidopsis* as regulating asymmetric division of the cortex/endodermis in root. *SCR* expression is restricted to the quiescent center and the endodermal cell layer in root and some kinds of cell layers in above-ground tissue (Di Laurenzio et al. 1996, Wysocka-Diller et al. 2000). *OsSCR* is thought to be an orthologous gene of *SCR*. The expression pattern in the root is conserved (Kamiya et al. 2003a). Notwithstanding, *OsSCR* expression in rice leaves is different from that in *Arabidopsis*. The expression of *OsSCR* in leaves is correlated with stomatal development (Kamiya et al. 2003a). For that reason, the profile of *OsSCR* expression is a useful marker for stomata development and epidermal specification.

The staging suggested by Kamiya et al. (2003a) is reasonable in most respects, but their division of the subsidiary cell formation process into two stages (stage 2 and stage 3) is not explicit. Asymmetric division of the SMC and the formation of the three-cell complex are events in a single continuous process. Therefore, we unified their stage 2 and stage 3 as a single stage. In this review, we divide stomatal development into five stages (Table 10).

Stage Sto0: Determination of stomatal cell row. Cell rows (stomatal cell row) where stomata are to be formed later are determined (Fig. 11B), but stomata-specific cells are not yet

Table 10 Staging of stomatal development in rice

Stage		Events	*OsSCR* expression
Symbol	Name		
Sto0	Determination of stomatal cell row	Determination of stomatal cell rows	Uniform expression in the stomatal cell row
Sto1	Formation of GMC	Formation of GMC by asymmetric division in the stomatal cell row	Down-regulation in non-stomata-forming cells in the stomatal cell row
Sto2	Formation of three-cell complex	Asymmetric division of SMC. Formation of three-cell complex comprising GMC and two subsidiary cells	Localized expression in the SMC adjacent to GMC. Cell specific expression in GMC and subsidiary cell
Sto3	Formation of guard cell pair	Formation of a pair of guard cells by transverse symmetric divisions in GMC	Rapid down-regulation
Sto4	Completion of stomatal complex	Completion of stomatal complex formation	No expression

specified. *OsSCR* is specifically and uniformly expressed in the stomatal cell rows (Fig. 11G).

Stage Sto1: Formation of GMC. Asymmetric cell divisions in the potential stomatal cell row produce GMCs (Fig. 11C). *OsSCR* expression is maintained in the GMC but is down-regulated in other epidermal cells of the stomatal cell row (Fig. 11H). Polarized expression of *OsSCR* is observed on the GMC side of SMCs at the later stage of Sto1 (Fig. 11I).

Stage Sto2: Formation of three-cell complex. A pair of subsidiary cells is formed by asymmetric divisions of the two SMCs (Fig. 11D). As a result, a three-cell complex comprising a GMC and two subsidiary cells is formed. *OsSCR* is expressed in both the GMC and the two subsidiary cells (Fig. 11J).

Stage Sto3: Formation of guard cell pair. Transverse symmetric divisions of GMC produce a guard cell pair (Fig. 11E). At this stage, the level of *OsSCR* expression decreases rapidly (Fig. 11K).

Stage Sto4: Completion of stomatal complex. The formation of the stomatal complex, comprising two narrow guard cells and two large subsidiary cells is completed (Fig. 11F).

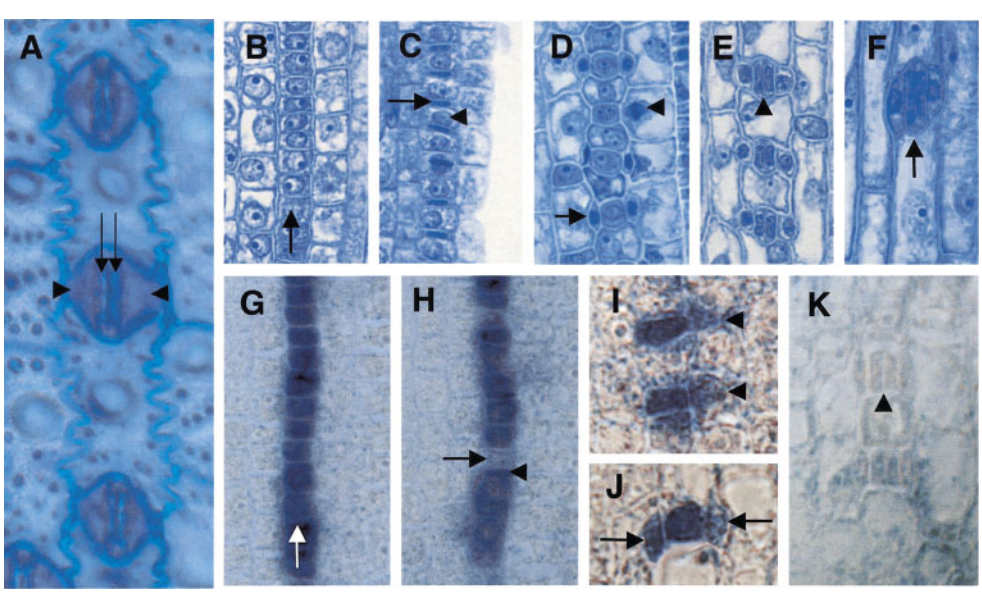

Fig. 11 Development of stomatal complex in rice. (A) Mature stomata in leaf blade. Arrows indicate guard cells and arrowheads subsidiary cells. (B–F) Longitudinal sections of developing stomatal complex stained with Toluidine Blue-O. (B) Stomatal cell row (arrow). (C) Production of GMC (arrow) and non-specialized cell (arrowhead) by asymmetric division. (D) Asymmetric division of the SMC (arrowheads) and the formation of a three-cell complex consisting of two subsidiary cells (arrow) and the GMC. (E) Formation of guard cell pair by transverse symmetric division (arrows). (F) Mature stomatal complex (arrow). (G–K) Expression pattern of *OsSCR*. (G) Uniform expression in the stomatal cell row (arrow). (H) Down-regulation in the non-specialized epidermal cell (arrow), with strong expression in the GMC (arrowhead). (I) Polarized expression on the GMC side of the SMC (arrowheads). (J) Expression in both the GMC and two subsidiary cells (arrows). (K) No expression in mature stomatal complex (arrowhead).

Concluding Remarks

This review has explicitly described a number of developmental processes that drive the life cycle of rice. The staging systems proposed here can be used in the description of mutant phenotypes and interpretation of gene expression and other biological aspects, as well as in establishing a conceptual framework of rice development. The rice plant is becoming an important model monocot plant and model cereal crop. For those reasons, elucidation of rice plant development contributes to the advancement of comparative biological studies regarding grasses (cereals) and between monocots and dicots. Many other processes that are not mentioned in this review also exist: endosperm development, vascular formation, stem development, air space formation in roots and leaf sheath (adaptation for anaerobic conditions) and others. These processes have not been examined sufficiently, but are expected to be understood soon. In addition, genes associated with rice development are being revealed rapidly. Thus, we expect that the staging systems described here will be refined in the future using new (molecular) markers.

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