Plant Biotechnology 30, 209–216 (2013)
DOI: 10.5511/plantbiotechnology.13.0318a

Review

Molecular mechanisms controlling plant organ abscission

Toshitsugu Nakano, Yasuhiro Ito*

National Food Research Institute, NARO, Tsukuba, Ibaraki 305-8642, Japan
*E-mail: yasuito@affrc.go.jp Tel: +81-29-838-8058 Fax: +81-29-838-7996

Received January 16, 2013; accepted March 18, 2013 (Edited by T. Ariizumi)

Abstract Plants shed organs by abscission, removing leaves, flowers or fruits when the organs are senescent, damaged, diseased or mature. Abscission also affects agriculture; for example, abscission of fruits or cereal grains can significantly reduce crop yield. Abscission of organs typically occurs in a predetermined tissue region, the abscission zone (AZ). Organ abscission can be disturbed in two ways, inhibition of AZ differentiation in the organ or suppression of abscission processes in AZ cells. Recent studies, mainly in Arabidopsis (*Arabidopsis thaliana*), rice (*Oryza sativa*), and tomato (*Solanum lycopersicum*), have identified many genes involved in regulation of AZ differentiation and activation of abscission of flowers or floral organs, seeds, and fruits. In this review, we discuss the functions of these genes, the developmental regulation of AZ tissues, and the signaling pathways that induce abscission. We also discuss the emerging concept that the regulation of abscission involves many of the same regulators that function in determination of shoot apical meristem cell fate.

Key words: Abscission zone, organ abscission, shoot apical meristem.

Leaves, flowers, fruits and other plant organs drop from the main body of the plant when they become senescent, mature or unneeded. This developmentally programmed abscission occurs within a specific tissue, the abscission zone (AZ), which forms at the junction of the organ and the main body of the plant. For wild plants, abscission of fruits or seeds from the main body is essential for dispersal of offspring. By contrast, for crop production, abscission of cereal grains or fruits is generally an undesirable trait, resulting in reduced yield as grain or fruit falls to the ground. Indeed, during domestication of cereal crops such as rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*), our ancestors selected plants with reduced abscission, which significantly improved yield (Doebley et al. 2006; Li et al. 2006; Lin et al. 2012). Abscission traits remain important in modern agriculture; for example, tomato (*Solanum lycopersicum*) mutants that do not have a pedicel AZ (Butler 1936; Rick 1956) have replaced abscission-competent tomato varieties for industrial processing of foods, such as tomato puree or juice. At harvesting, the tomatoes lacking an AZ detach without the stems and sepals, which remain on the plant; this results in reduced labor and time to remove the green tissues from the harvested fruits (Zahara and Scheuerman 1988). The tomato pedicel AZ-deficient mutations are called "jointless." Abscission is also important for other fruit crops. For example, fruit trees such as apple (*Malus*×*domestica*) abscise some of their young fruits in the so-called "June drop," because the trees usually bear too many fruits to support. However, too much fruit abscission causes lower yields, below the tree's yield potential; thus, balanced control of fruit-bearing is required for the highest yields. Moreover, strong winds in the harvest season can also reduce yields. For example, in Japan, typhoon season occurs at the same time as the apple harvest season. Strong typhoon winds often drop large numbers of apple fruits from the AZ, resulting in severe reduction of yield. Thus, resistance to dropping of fruits may decrease weather-related crop losses.

Abscission is an organized, regulated developmental process consisting of a few discernible steps: differentiation of AZ tissue, acquisition of competence to initiate abscission, and activation of abscission leading to cell separation. All of these steps are critical for the fate of organs that will be shed (Patterson 2001). The AZ tissue is composed of small, isodiametric cells with dense cytoplasm; these AZ cells bear some resemblance to undifferentiated or meristematic cells (Addicot 1982; Sexton and Roberts 1982). When physiological changes such as senescence or maturation occur in organs, abscission is induced through reduction of

Abbreviations: AZ, abscission zone; Cel, cellulase; PG, polygalacturonase; EXP, expansin; XTH, xyloglucan endotransglucosylase/hydrolase; LRR-RLKs, leucine-rich repeat receptor-like kinases; MAPK, mitogen-activated protein kinase; SAM, shoot apical meristem; AM, axillary meristem; TF, transcription factor; NPA, N-1-naphthylphthalamic acid; JA, jasmonic acid; ABA, abscisic acid.

This article can be found at http://www.jspcmb.jp/
Published online August 7, 2013

auxin level and induction of ethylene signaling (Meir et al. 2010; Taylor and Whitelaw 2001). Abscission is carried out by activation of cell wall degrading enzymes and remodeling proteins, including β-1,4-glucanase (cellulase; Cel), polygalacturonase (PG), xyloglucan endotransglucosylase/hydrolase (XTH), and expansin (EXP), resulting in breakdown of cell adhesion (Cai and Lashbrook 2008; Lashbrook and Cai 2008; Meir et al. 2010; Roberts et al. 2002; Tucker et al. 2007). These events are well conserved in different types of organs and in divergent plant species.

In this review, we summarize current knowledge on genes involved in regulation of AZ development and activation of abscission in various organs (Figure 1). We also examine the emerging idea that regulatory mechanisms for organ abscission may be shared with regulation of shoot apical meristems (SAMs).

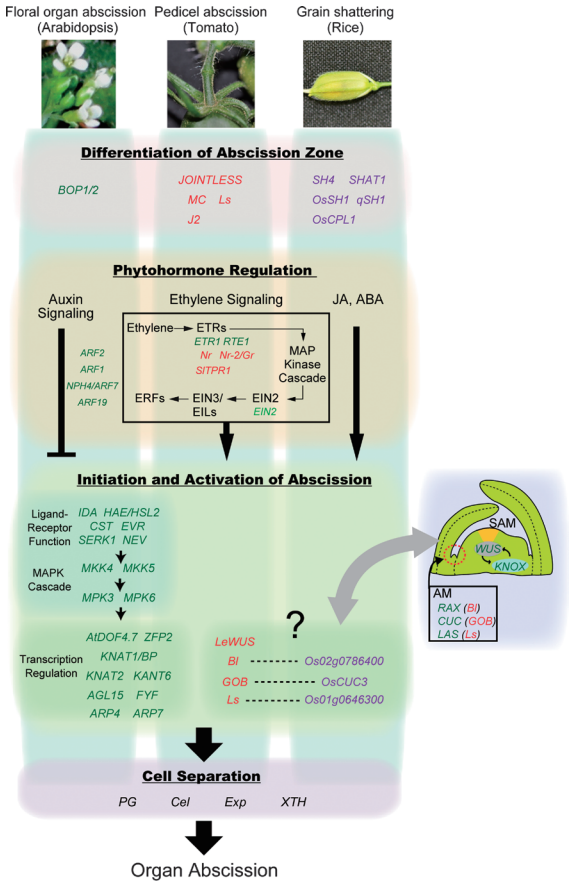

Figure 1. Genes regulating organ abscission. Genes for floral organ abscission in Arabidopsis, pedicel abscission in tomato, and grain shattering in rice are classified into four physiological steps: differentiation of the abscission zone, phytohormone regulation, initiation and activation of abscission, and cell separation. Several TFs that determine the fates of cells in SAMs and AMs may regulate the initiation and activation of abscission, but the functions of these genes in abscission remain unknown. Genes of Arabidopsis (green), tomato (red) and rice (purple) are shown. Genes that are commonly activated in the cell separation step in all organ abscission processes are shown in black letters.

Floral organ abscission

In flowers, AZs develop at the bases of floral organs such as petals or stamens; when the flower becomes senescent, abscission processes are activated in the AZs. In Arabidopsis (*Arabidopsis thaliana*), an AZ for floral organ abscission is composed of two to six layers of small cells (Cho et al. 2008). The double mutation in *BLADE-ON-PETIOLE1* (*BOP1*) and *BOP2* blocks the differentiation of AZs and thus the mutant never sheds the sepals, petals or stamens, even after senescence and wilting. *BOP1* and *BOP2* both encode BTB/POZ domain and four ankyrin repeat-containing proteins, and this double mutant phenotype indicates that they redundantly control the differentiation of floral organ AZs (Mckim et al. 2008). The tobacco (*Nicotiana tabacum*) homolog *NtBOP2* also regulates the differentiation of the AZ in corollas (petals) (Wu et al. 2012). The cells in the corolla AZ are shorter in length than the cells of neighboring tissues. Suppression of NtBOP2 activity by over-expression of the dominant-negative form of *NtBOP2* in tobacco BY-2 cells makes those cells elongated, suggesting that *NtBOP2* controls cell growth within the AZ tissues (Wu et al. 2012).

Once AZ tissues have formed, the tissues appear to remain in a quiescent or idling state. If abscission is triggered by an initiation signal, organ detachment is immediately carried out, but if there is no trigger, the tissues remain in the idling state and hold the organ on the plant indefinitely. Recent detailed investigations have identified a number of factors regulating the onset of floral organ abscission in Arabidopsis. Mutations inhibiting floral organ abscission have been identified in many genes, including those encoding the small secreted ligand peptide INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) and its probable receptors, leucine-rich repeat receptor like kinases (LRR-RLKs) HAESA (HAE) and HAESA-LIKE2 (HSL2) (Butenko et al. 2003; Cho et al. 2008; Jinn et al. 2000; Stenvik et al. 2008). Mutant analysis suggested that the IDA-HAE/HSL2 ligand-receptor interaction likely activates the mitogen-activated protein kinase (MAPK) cascade composed of MAPK kinase 4 (MKK4) and MKK5, and the subsequent MAP kinase 3 (MPK3) and MPK6 (Cho et al. 2008). The activation of MAPK cascade signaling is predicted to suppress the KNOX family transcription factor (TF) gene *KNOTTED-LIKE1* [*KNAT1*; also called *BREVIPEDICELLUS* (*BP*)], which is a negative regulator of the onset of abscission. KNAT1/BP restricts the expression of *KNAT2* and *KNAT6*, which positively regulate abscission processes (Shi et al. 2011). In addition, floral organ abscission requires *NEVERSHED* (*NEV*), which encodes an ADP-ribosylation factor GTPase-activating protein (ARF-GAP), which acts as a membrane trafficking regulator (Liljegren et al. 2009). The *nev* mutant blocks floral organ separation and

displays defects in Golgi apparatus structure, alteration of trans-Golgi network localization and accumulation of vesicles between the plasma membrane and the cell walls. Screening for mutations that suppress the *nev* mutant phenotype identified three genes, the receptor-like cytoplasmic kinase gene *CAST AWAY* (*CST*), and two LRR-RLK genes *EVERSHED* (*EVR*) and *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1* (*SERK1*) (Burr et al. 2011; Leslie et al. 2010; Lewis et al. 2010). The CST protein physically interacts with EVR and HAE at the plasma membrane (Burr et al. 2011). A current model suggests that CST, EVR and SERK1 block IDA-HAE/HSL2 activity by modulating the localization of HAE/HSL2 at the plasma membrane and controlling the movement of the receptor to the trans-Golgi network and early endosomes (Burr et al. 2011; Liljegren 2012). In addition, floral organ abscission is delayed by a loss of function mutation in the chromatin remodeling factor genes *ACTIN-RELATED PROTEIN 4* (*ARP4*) and *ARP7* (Kandasamy et al. 2005a; 2005b).

The initiation of floral organ abscission with the appropriate timing requires both positive and negative regulatory mechanisms. After initiation of abscission, transcripts of abscission-related genes, such as genes for cell wall hydrolysis enzymes and remodeling proteins (for example, *PG*, *Cel*, *XTH* and *EXP*), commonly show striking increases in different abscised organs (Belfield et al. 2005; Cai and Lashbrook 2008; Kalaitzis et al. 1997; Lashbrook and Cai 2008; Lashbrook et al. 1994; Meir et al. 2010; Tucker et al. 2007). Simultaneously, cell death is observed in tomato leaf and pedicel abscission and *LX ribonuclease* is up-regulated (Bar-Dror et al. 2011). Several TFs suppressing the activation of abscission have been identified. For example, overexpression or constitutive expression of Arabidopsis TF genes *FOREVER YOUNG FLOWER* (*FYF*), *AGAMOUS-LIKE 15* (*AGL15*), *Arabidopsis thaliana DOF4.7* (*AtDOF4.7*) or *ZINC FINGER PROTEIN 2* (*ZFP2*) caused retarded separation of floral organs (Cai and Lashbrook 2008; Chen et al. 2011; Fang and Fernandez 2002; Wei et al. 2010). AtDOF4.7 inactivates the expression of the PG gene *PG ABSCISSION ZONE ARABIDOPSIS THALIANA* (*PGAZAT*) by binding to its promoter. Protein interaction assays using yeast two-hybrid system and bimolecular fluorescence complementation (BiFC) indicated that AtDOF4.7 and ZFP2 can form a complex, suggesting that these TFs may be activated via complex formation (Wei et al. 2010). Overexpression of *FYF* inhibited floral organ abscission and also decreased the expression of genes encoding the abscission activators IDA, BOP2 and genes involved in the ethylene signaling pathway, clearly indicating the negative regulatory role of FYF in abscission (Chen et al. 2011). To maintain the pre-abscission quiescent or idling state, the negative regulators may suppress expression of abscission related genes. Following perception of an abscission initiating signal, the negative regulation appears to be cancelled, allowing the positive regulators to sharply up-regulate genes required for abscission.

Seed abscission

Seeds of specific plants such as soybean (*Glycine max*), pea (*Pisum sativum*), and Brassicaceae species, including Arabidopsis, are set within siliques or pods, a capsule-like fruit (Ostergaard et al. 2007). A stalk, the funiculus, supports seeds at the edge of the siliques and pods; AZs differentiate between seeds and stalks to allow the release of seeds (Leslie et al. 2007). An Arabidopsis seed AZ is composed of a few thin layers of small cells that differentiate after fertilization. Investigations of seed AZ-deficient mutants revealed that differentiation of the AZs requires the MADS-box TF gene *SEEDSTICK* (*STK*) and the bHLH family TF gene *HECATE3* (*HEC3*) (Ogawa et al. 2009; Pinyopich et al. 2003). Seeds of the plants with loss-of-function mutations in *STK* and *HEC3* lack AZs and thus remain attached to the pods even when the seeds are fully mature. This trait may be favorable for agricultural production because tight attachment of seeds to the fruit reduces yield loss during harvesting. In pea, the *development funiculus* (*def*) mutation inhibits the differentiation of the seed AZ, but the gene has not been identified yet (Ayeh et al. 2009).

Pedicel abscission in tomato

A pedicel is a stem or stalk that connects an individual flower or fruit to the inflorescence main stem. Solanaceous plants such as tomato and tobacco differentiate AZs within the pedicels to drop unfertilized flowers or mature fruits. The tomato pedicel region containing the AZ is morphologically distinct, displaying a knuckle or joint-like appearance. Tomato flower pedicels have long been used as a model system to investigate organ abscission (Jensen and Valdovin 1967; Meir et al. 2010; Roberts et al. 1984). Pedicel AZ defects have been found in tomato in the spontaneous mutants *jointless* (*j*) (Butler 1936) and *lateral suppressor* (*ls*) (Roberts et al. 2002). The *jointless* phenotype was also found in a related species, *S. cheesmaniae*, which was discovered in the Galapagos Islands. The *S. cheesmaniae* jointless trait has been introgressed into domesticated tomato cultivars by interspecific crossing; this locus is called *jointless-2* (*j2*) (Rick 1956). The *j* and *j2* mutations completely block the differentiation of the AZ and thus mutant plants develop AZ-less pedicels. The *ls* mutant was originally found as a lateral shoot-lacking plant, and was also found to develop incomplete pedicels (Nakano et al. 2012; Roberts et al. 2002). Molecular cloning revealed that *JOINTLESS* encodes a MADS-box TF and *Ls* encodes a GARS family TF (Mao et al. 2000; Schumacher et al. 1999). Meanwhile, the genomic

region around the putative *j2* locus has been sequenced but the gene has not been identified yet. Among the several genes identified in the sequenced region, a gene for C-terminal domain phosphatase-like1 (ToCPL1) is a candidate for the *j2* gene (Yang et al. 2005). In addition, a recent study using antisense suppression showed that *MACROCALYX* (*MC*), a tomato *APETALA1* family MADS-box gene, is also required for pedicel AZ formation (Nakano et al. 2012). The study also found that MC and JOINTLESS can form a heterodimer that binds to a DNA motif, the CArG-box, which is known as the target of MADS-box TFs, suggesting that MC regulates pedicel AZ differentiation via forming a MADS-box complex with JOINTLESS.

Pedicel abscission in rice grain shattering

During domestication of rice from its wild progenitor, reduced-shattering strains were preferentially selected for propagation because reduced shattering increased grain yield. So far, five rice genes that are required for the differentiation of the pedicel AZ have been identified: the Myb3 family TF gene *SHATTERING 4* (*SH4*) (Li et al. 2006), the BELL family TF gene *qSH1* (Konishi et al. 2006), the AP2 family TF gene *SHATTERING ABORTION 1* (*SHAT1*) (Hofmann 2012; Zhou et al. 2012), the YAB family TF gene, *OsSH1*, which is a homolog of the sorghum (*Sorghum bicolor*) *SHATTERING 1* (*SH1*) (Lin et al. 2012), and the *Oryza sativa carboxy-terminal domain phosphatase-like 1* (*OsCPL1*) (Ji et al. 2010). *SH4*, *qSH1*, *SHAT1* and *OsSH1* act as positive regulators to develop the pedicel AZs, and *OsCPL1* activity represses AZ differentiation. The *OsSH1* homologs in sorghum and maize are involved in the development of the pedicel AZ (Lin et al. 2012). Likewise, the wheat *Q* gene, a *SHAT1* homolog, affects seed shattering in wheat (Simons et al. 2006). These findings suggest that the conserved mechanisms regulating pedicel AZ differentiation in these cereal species evolved before these species diverged from a common progenitor.

The regulatory mechanisms acting in meristem cells may also be important for the function of pedicel AZ cells

A survey of the genes down-regulated by a defect in *MC* or *JOINTLESS* (Nakano et al. 2012) revealed another aspect of the regulation of organ abscission. Intriguingly, the set of identified genes included several TF genes whose homologs are involved in meristem cell fates, including *LeWUS*, *GOBLET* (*GOB*), *Ls*, and *Blind* (*Bl*), which are identical to the genes expressed in SAMs of tomato seedlings. These four genes are expressed in pedicel AZs, not in the surrounding pedicel regions (Figure 2; Nakano et al. 2012), suggesting their involvement in AZ function. *LeWUS* is a homolog

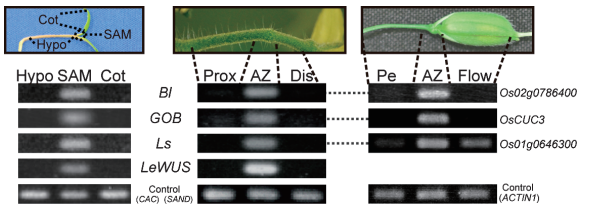

Figure 2. AZ specific gene expression in tomato and rice. Expression specificities of *LeWUS*, *Bl*, *GOB*, and *Ls* in tomato pedicels at anthesis and in young seedlings and expression of the rice homologs in anthesis flowers were analyzed by RT-PCR. *LeWUS*, *Bl*, *GOB*, and *Ls*, which are specifically expressed in SAMs in young seedlings, are also expressed specifically in AZs of anthesis flower pedicels. Expression analysis in an indica rice strain (Chinsurah Boro I) revealed that rice homologs of *Bl*, *GOB*, and *Ls* are transcibed in anthesis pedicel AZs at higher levels than in the neighboring tissues. The rice homolog of *Bl* is *Os02g0786400*, the *GOB* homolog is *OsCUC3*, and the *Ls* homolog is *Os01g0646300*. Hypo (hypocotyl), SAM (shoot apical meristem), Cot (cotyledon), Prox (proximal region of flower pedicel), AZ (abscission zone), Dis (distal region of flower pedicel), Pe (pedicel), and Flow (flower).

of Arabidopsis *WUSCHEL* (*WUS*), which encodes a homeodomain family TF and plays a critical role in the maintenance of SAMs (Mayer et al. 1998; Reinhardt et al. 2003). Meanwhile, *GOB*, *Ls* and *Bl* are homologs of Arabidopsis *CUP-SHAPED COTYLEDON* (*CUC*), *LATERAL SUPPRESSOR* (*LAS*) and *REGULATOR OF AXILLARY MERISTEMS* (*RAX*), respectively, and those Arabidopsis homologs act as regulators of axillary meristem (AM) development (Greb et al. 2003; Keller et al. 2006; Muller et al. 2006; Raman et al. 2008). Pedicel AZ cells remain small until the onset of abscission (Tabuchi et al. 2001). This cell property may be caused by the activities of *Bl* that regulates cell size in tomato (Busch et al. 2011). In addition, *GOB* may act to keep pedicel AZ cells small as the Arabidopsis homolog (*CUC2*) does (Peaucelle et al. 2007). Similar to SAM cells, tomato pedicel AZ cells also are competent to develop adventitious shoots (Nakano et al. 2012), suggesting that *LeWUS* activity may affect the fates of the AZ cells, acting in a similar fashion to the Arabidopsis homolog, which regulates stem cell activity in SAMs (Mayer et al. 1998). In addition, Arabidopsis *KNAT1*/*BP*, which acts in SAMs, determines the timing of floral abscission by restricting AZ cell size and number (Shi et al. 2011). These observations suggest that abscission and SAMs share specific regulatory mechanisms. Therefore, studies comparing AZ and SAM functions may provide insights into the molecular mechanisms governing both processes. Moreover, this conservation may extend beyond dicots; indeed, our recent experiments revealed that rice *Ls*, *GOB* and *Bl* homologs are also preferentially expressed in rice pedicel AZs (Figure 2).

Hormonal regulation of organ abscission

Accumulating data suggest that the timing of abscission is primarily determined by the interplay between

ethylene signaling and auxin responses (Meir et al. 2010; Roberts et al. 2002; Taylor and Whitelaw 2001); ethylene promotes abscission, but auxin inhibits abscission by rendering AZ cells insensitive to ethylene. It is also proposed that ethylene inhibits polar auxin transport or inactivates auxin action (Beyer and Morgan 1971; Taylor and Whitelaw 2001). Mutations in the ethylene receptor *ETHYLENE RESPONSE1* (*ETR1*) and the ethylene signaling gene *ETHYLENE-INSENSITIVE2* (*EIN2*) delay floral organ abscission in Arabidopsis (Bleecker and Patterson 1997; Patterson and Bleecker 2004). Similarly, a tomato fruit ripening mutation in the ethylene receptor gene *Never-ripe* (*Nr*) (also called *LeETR3*), which confers ethylene insensitivity, also causes delayed flower pedicel abscission (Lanahan et al. 1994). Tomato mutations of *Nr-2* and *Green-ripe* (*Gr*), which are allelic, inactivate a subset of ethylene responses, resulting in inhibition of fruit ripening and flower abscission (Barry and Giovannoni 2006; Barry et al. 2005). Studies of an Arabidopsis *Nr-2*/*Gr* homolog, *REVERSION-TO-ETHYLENE SENSITIVITY 1* (*RTE1*), revealed that RTE1 affects ethylene perception, possibly by modulating the conformation of the ETR1 ethylene receptor (Resnick et al. 2008; Resnick et al. 2006). SlTPR1, which is involved in floral organ abscission and pedicel AZ development, interacts physically with ethylene receptors Nr and LeETR1. The protein is proposed to play a role in crosstalk between ethylene signaling and auxin responses (Lin et al. 2008). Following binding of ethylene to the receptors, ethylene signals are relayed to the transmembrane protein EIN2, which activates accumulation of the EIN3/EIN3-LIKE (EILs) family TFs, which are readily degraded in the absence of ethylene signaling (Guo and Ecker 2003). EIN3 positively regulates transcription of *ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR1* (*ERF1*) by binding to the *cis*-regulatory elements (Solano et al. 1998). In tomato, simultaneous suppression of *EIN3-like* (*LeEIL*) genes depresses flower abscission and also inhibits fruit ripening (Tieman et al. 2001; Yokotani et al. 2009). Recently we identified an ERF family gene that plays a role in flower pedicel abscission in tomato (Nakano et al. in preparation). The suppression of *ERF* inhibits only pedicel abscission but not other ethylene responses such as fruit ripening, in contrast to many other ethylene signaling mutants, which result in pleiotropic effects on ethylene-mediated responses. This gene may be a promising target to improve fruit harvesting properties by controlling pedicel abscission.

The initiation of abscission can be inhibited by substantial amounts of auxin provided to AZs from source tissues such as leaf blades or flowers (Meir et al. 2006; 2010). Conversely, organ abscission can be activated by auxin-depressing treatments, such as removing source tissues or providing inhibitors of polar auxin transport [for example, N-1-naphthylphthalamic acid (NPA)] (Meir et al. 2006; 2010), indicating the importance of auxin in controlling abscission. AUXIN RESPONSIVE FACTORs (ARFs) are important regulators of auxin signaling, acting by binding to *cis*-acting DNA elements that called auxin-responsive elements (AuxREs). Generally, ARF activity is repressed by the ARF-binding proteins AUXIN/INDOLE-3-ACETIC ACIDs (Aux/IAAs); when auxin is supplied, Aux/IAAs are degraded, freeing the ARFs and restoring their activity (Guilfoyle and Hagen 2007). In Arabidopsis, a mutation in *ARF2* delays the timing of floral organ shedding, and the mutant phenotype is enhanced by a mutation in *ARF1* or mutations in *NONPHOTOTROPIC HYPOCOTYL 4* (*NPH4*, also called *ARF7*) and *ARF19* (Ellis et al. 2005; Okushima et al. 2005). Unlike typical ARF TFs, ARF2 may not participate in auxin signaling; the molecular mechanisms of ARF2 activity in organ abscission remain to be determined (Ellis et al. 2005; Okushima et al. 2005), but ARF2 may bind to AuxREs to prevent binding of other ARFs (Okushima et al. 2005).

Other than ethylene and auxin, the involvement in the regulation of abscission of other phytohormones, such as jasmonic acid (JA), abscisic acid (ABA), cytokinin and gibberellic acid, has been controversial (Addicot 1982; Taylor and Whitelaw 2001). However, a recent Arabidopsis study provided new evidence that JA and ABA act in the regulation of the floral organ abscission (Ogawa et al. 2009). That study found that the JA-deficient *allene oxide synthase* (*aos*) mutants and the ethylene insensitive *ein2* mutants showed delayed floral organ abscission; moreover, the delay increased additively in the *ein2 aos* double mutants. Furthermore, addition of the ABA-deficient mutation *aba deficient2* (*aba2*) to the *ein2 aos* double mutant combination enhanced the delay of abscission, demonstrating that these three phytohormones participate in regulation of the abscission activating processes.

Conclusion and perspectives

Recent advances in the understanding of the regulation of plant organ abscission have revealed many key genes involved in AZ development and activation of abscission. Abscission signaling cascades starting from the IDA-HAE/HAL2 interaction or ethylene stimulation have been extensively elucidated, although our understanding of the many identified genes remains fragmentary and incomplete. Moreover, it remains to be determined whether a general regulatory mechanism for abscission may exist among different organs and whether the mechanism may be conserved in different plant species. If a common regulatory mechanisms can be found, the applications in a wide variety of crop species could accelerate breeding to yield new crop cultivars with higher fruit harvesting efficiency, or ornamental

flowers with extended shelf life. The findings on AZ-specific expression of the genes regulating meristem cell fates may provide a clue to the general regulatory mechanism of abscission. Our advanced understanding of the regulatory mechanisms for SAM functions might lead to a better understanding of the roles of the genes expressed in AZs. The molecular mechanisms of ethylene and auxin signaling have been elucidated in various plant tissues and organs, and the accumulated knowledge could also provide insight into the molecular mechanisms regulating initiation of abscission by these phytohormones. Moreover, identification of abscission specific phytohormone regulation may enable development of methods to control abscission in practical agricultural applications, because the specific regulation may avoid undesirable side effects of phytohormones in non-target organs. For the long history of crop domestication, our ancestors have made great efforts to develop crops with reduced abscission. In keeping with this history, the elucidation of molecular mechanisms of abscission may expand potential applications for controlling abscission to a wide variety of agronomic traits in various crops.

Acknowledgements

We thank all the members of our laboratory. The work described here was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN) Japan.

References

Addicot FT (1982) Abscission. University of California Press, Berkeley, CA.

Ayeh KO, Lee Y, Ambrose MJ, Hvoslef-Eide AK (2009) Characterization and structural analysis of wild type and a non-abscission mutant at the development funiculus (Def) locus in Pisum sativum L. *BMC Plant Biol* 9: 76

Bar-Dror T, Dermastia M, Kladnik A, Znidaric MT, Novak MP, Meir S, Burd S, Philosoph-Hadas S, Ori N, Sonego L, et al. (2011) Programmed cell death occurs asymmetrically during abscission in tomato. *Plant Cell* 23: 4146–4163

Barry CS, Giovannoni JJ (2006) Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. *Proc Natl Acad Sci USA* 103: 7923–7928

Barry CS, McQuinn RP, Thompson AJ, Seymour GB, Grierson D, Giovannoni JJ (2005) Ethylene insensitivity conferred by the Green-ripe and Never-ripe 2 ripening mutants of tomato. *Plant Physiol* 138: 267–275

Belfield EJ, Ruperti B, Roberts JA, McQueen-Mason S (2005) Changes in expansin activity and gene expression during ethylene-promoted leaflet abscission in Sambucus nigra. *J Exp Bot* 56: 817–823

Beyer EM, Morgan PW (1971) Abscission: the role of ethylene modification of auxin transport. *Plant Physiol* 48: 208–212

Bleecker AB, Patterson SE (1997) Last exit: senescence, abscission, and meristem arrest in Arabidopsis. *Plant Cell* 9: 1169–1179

Burr CA, Leslie ME, Orlowski SK, Chen I, Wright CE, Daniels MJ, Liljegren SJ (2011) CAST AWAY, a membrane-associated receptor-like kinase, inhibits organ abscission in Arabidopsis. *Plant Physiol* 156: 1837–1850

Busch BL, Schmitz G, Rossmann S, Piron F, Ding J, Bendahmane A, Theres K (2011) Shoot branching and leaf dissection in tomato are regulated by homologous gene modules. *Plant Cell* 23: 3595–3609

Butenko MA, Patterson SE, Grini PE, Stenvik GE, Amundsen SS, Mandal A, Aalen RB (2003) Inflorescence deficient in abscission controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. *Plant Cell* 15: 2296–2307

Butler L (1936) Inherited characters in the tomato II—Joint pedicel. *J Hered* 27: 25–26

Cai SQ, Lashbrook CC (2008) Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: Enhanced retention of floral organs in Transgenic plants overexpressing Arabidopsis ZINC FINGER PROTEIN2. *Plant Physiol* 146: 1305–1321

Chen MK, Hsu WH, Lee PF, Thiruvengadam M, Chen HI, Yang CH (2011) The MADS box gene, FOREVER YOUNG FLOWER, acts as a repressor controlling floral organ senescence and abscission in Arabidopsis. *Plant J* 68: 168–185

Cho SK, Larue CT, Chevalier D, Wang H, Jinn TL, Zhang S, Walker JC (2008) Regulation of floral organ abscission in Arabidopsis thaliana. *Proc Natl Acad Sci USA* 105: 15629–15634

Doebley JF, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. *Cell* 127: 1309–1321

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005) AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in Arabidopsis thaliana. *Development* 132: 4563–4574

Fang SC, Fernandez DE (2002) Effect of regulated overexpression of the MADS domain factor AGL15 on flower senescence and fruit maturation. *Plant Physiol* 130: 78–89

Greb T, Clarenz O, Schafer E, Muller D, Herrero R, Schmitz G, Theres K (2003) Molecular analysis of the LATERAL SUPPRESSOR gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation. *Genes Dev* 17: 1175–1187

Guilfoyle TJ, Hagen G (2007) Auxin response factors. *Curr Opin Plant Biol* 10: 453–460

Guo HW, Ecker JR (2003) Plant responses to ethylene gas are mediated by SCF (EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. *Cell* 115: 667–677

Hofmann NR (2012) SHAT1, A new player in seed shattering of rice. *Plant Cell* 24: 839

Jensen TE, Valdovin JG (1967) Fine Structure of Abscission Zones. I. Abscission Zones of Pedicels of Tobacco and Tomato Flowers at Anthesis. *Planta* 77: 298

Ji H, Kim SR, Kim YH, Kim H, Eun MY, Jin ID, Cha YS, Yun DW, Ahn BO, Lee MC, et al. (2010) Inactivation of the CTD phosphatase-like gene OsCPL1 enhances the development of the abscission layer and seed shattering in rice. *Plant J* 61: 96–106

Jinn TL, Stone JM, Walker JC (2000) HAESA, an Arabidopsis leucine-rich repeat receptor kinase, controls floral organ abscission. *Genes Dev* 14: 108–117

Kalaitzis P, Solomos T, Tucker ML (1997) Three different polygalacturonases are expressed in tomato leaf and flower abscission, each with a different temporal expression pattern. *Plant Physiol* 113: 1303–1308

Kandasamy MK, Deal RB, McKinney EC, Meagher RB (2005a) Silencing the nuclear actin-related protein AtARP4 in Arabidopsis has multiple effects on plant development, including early flowering and delayed floral senescence. *Plant J* 41: 845–858

Kandasamy MK, McKinney EC, Deal RB, Meagher RB (2005b) Arabidopsis ARP7 is an essential actin-related protein required for normal embryogenesis, plant architecture, and floral organ abscission. *Plant Physiol* 138: 2019–2032

Keller T, Abbott J, Moritz T, Doerner P (2006) Arabidopsis REGULATOR OF AXILLARY MERISTEMS1 controls a leaf axil stem cell niche and modulates vegetative development. *Plant Cell* 18: 598–611

Konishi S, Izawa T, Lin SY, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. *Science* 312: 1392–1396

Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1994) The never ripe mutation blocks ethylene perception in tomato. *Plant Cell* 6: 521–530

Lashbrook CC, Cai S (2008) Cell wall remodeling in Arabidopsis stamen abscission zones: Temporal aspects of control inferred from transcriptional profiling. *Plant Signal Behav* 3: 733–736

Lashbrook CC, Gonzalez-Bosch C, Bennett AB (1994) Two divergent endo-beta-1,4-glucanase genes exhibit overlapping expression in ripening fruit and abscising flowers. *Plant Cell* 6: 1485–1493

Leslie ME, Lewis MW, Liljegren SJ (2007) Organ abscission. In: Roberts JA, Gonzalez-Carranza Z (eds) *Plant Cell Separation and Adhesion. Annual Plant Reviews Volume 25*. Blackwell Publishing, Oxford, pp 106-136

Leslie ME, Lewis MW, Youn JY, Daniels MJ, Liljegren SJ (2010) The EVERSHED receptor-like kinase modulates floral organ shedding in Arabidopsis. *Development* 137: 467–476

Lewis MW, Leslie ME, Fulcher EH, Darnielle L, Healy PN, Youn JY, Liljegren SJ (2010) The SERK1 receptor-like kinase regulates organ separation in Arabidopsis flowers. *Plant J* 62: 817–828

Li C, Zhou A, Sang T (2006) Rice domestication by reducing shattering. *Science* 311: 1936–1939

Liljegren SJ (2012) Organ abscission: exit strategies require signals and moving traffic. *Curr Opin Plant Biol* 15: 670–676

Liljegren SJ, Leslie ME, Darnielle L, Lewis MW, Taylor SM, Luo R, Geldner N, Chory J, Randazzo PA, Yanofsky MF, et al. (2009) Regulation of membrane trafficking and organ separation by the NEVERSHED ARF-GAP protein. *Development* 136: 1909–1918

Lin Z, Arciga-Reyes L, Zhong S, Alexander L, Hackett R, Wilson I, Grierson D (2008) SlTPR1, a tomato tetratricopeptide repeat protein, interacts with the ethylene receptors NR and LeETR1, modulating ethylene and auxin responses and development. *J Exp Bot* 59: 4271–4287

Lin Z, Li X, Shannon LM, Yeh CT, Wang ML, Bai G, Peng Z, Li J, Trick HN, Clemente TE, et al. (2012) Parallel domestication of the Shattering1 genes in cereals. *Nat Genet* 44: 720–724

Mao L, Begum D, Chuang HW, Budiman MA, Szymkowiak EJ, Irish EE, Wing RA (2000) JOINTLESS is a MADS-box gene controlling tomato flower abscission zone development. *Nature* 406: 910–913

Mayer KF, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T (1998) Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem. *Cell* 95: 805–815

Mckim SM, Stenvik GE, Butenko MA, Kristiansen W, Cho SK, Hepworth SR, Aalen RB, Haughn GW (2008) The BLADE-ON-PETIOLE genes are essential for abscission zone formation in Arabidopsis. *Development* 135: 1537–1546

Meir S, Hunter DA, Chen JC, Halaly V, Reid MS (2006) Molecular changes occurring during acquisition of abscission competence following auxin depletion in Mirabilis jalapa. *Plant Physiol* 141: 1604–1616

Meir S, Philosoph-Hadas S, Sundaresan S, Selvaraj KSV, Burd S, Ophir R, Kochanek B, Reid MS, Jiang CZ, Lers A (2010) Microarray Analysis of the Abscission-Related Transcriptome in the Tomato Flower Abscission Zone in Response to Auxin Depletion. *Plant Physiol* 154: 1929–1956

Muller D, Schmitz G, Theres K (2006) Blind homologous R2R3 Myb genes control the pattern of lateral meristem initiation in Arabidopsis. *Plant Cell* 18: 586–597

Nakano T, Kimbara J, Fujisawa M, Kitagawa M, Ihashi N, Maeda H, Kasumi T, Ito Y (2012) MACROCALYX and JOINTLESS interact in the transcriptional regulation of tomato fruit abscission zone development. *Plant Physiol* 158: 439–450

Ogawa M, Kay P, Wilson S, Swain SM (2009) ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are Polygalacturonases required for cell separation during reproductive development in Arabidopsis. *Plant Cell* 21: 216–233

Okushima Y, Mitina I, Quach HL, Theologis A (2005) AUXIN RESPONSE FACTOR 2 (ARF2): a pleiotropic developmental regulator. *Plant J* 43: 29–46

Ostergaard L, Borkhardt B, Ulvskov P (2007) Dehiscence. In: Roberts JA, Gonzalez-Carranza Z (eds) *Plant Cell Separation and Adhesion. Annual Plant Reviews Volume 25*. Blackwell Publishing, Oxford, pp 137-163

Patterson SE (2001) Cutting loose. Abscission and dehiscence in Arabidopsis. *Plant Physiol* 126: 494–500

Patterson SE, Bleecker AB (2004) Ethylene-dependent and -independent processes associated with floral organ abscission in Arabidopsis. *Plant Physiol* 134: 194–203

Peaucelle A, Morin H, Traas J, Laufs P (2007) Plants expressing a miR164-resistant CUC2 gene reveal the importance of post-meristematic maintenance of phyllotaxy in Arabidopsis. *Development* 134: 1045–1050

Pinyopich A, Ditta GS, Savidge B, Liljegren SJ, Baumann E, Wisman E, Yanofsky MF (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature* 424: 85–88

Raman S, Greb T, Peaucelle A, Blein T, Laufs P, Theres K (2008) Interplay of miR164, CUP-SHAPED COTYLEDON genes and LATERAL SUPPRESSOR controls axillary meristem formation in Arabidopsis thaliana. *Plant J* 55: 65–76

Reinhardt D, Frenz M, Mandel T, Kuhlemeier C (2003) Microsurgical and laser ablation analysis of interactions between the zones and layers of the tomato shoot apical meristem. *Development* 130: 4073–4083

Resnick JS, Rivarola M, Chang C (2008) Involvement of RTE1 in conformational changes promoting ETR1 ethylene receptor signaling in Arabidopsis. *Plant J* 56: 423–431

Resnick JS, Wen CK, Shockey JA, Chang C (2006) REVERSION-TO-ETHYLENE SENSITIVITY1, a conserved gene that regulates ethylene receptor function in Arabidopsis. *Proc Natl Acad Sci USA* 103: 7917–7922

Rick CM (1956) Genetic and Systematic Studies on Accessions of Lycospersicon from the Galapagos Islands. *Am J Bot* 43: 687–696

Roberts JA, Elliott KA, Gonzalez-Carranza ZH (2002) Abscission, dehiscence, and other cell separation processes. *Annu Rev Plant Biol* 53: 131–158

Roberts JA, Schindler CB, Tucker GA (1984) Ethylene-Promoted

Tomato Flower Abscission and the Possible Involvement of an Inhibitor. *Planta* 160: 159–163

Schumacher K, Schmitt T, Rossberg M, Schmitz C, Theres K (1999) The Lateral suppressor (Ls) gene of tomato encodes a new member of the VHIID protein family. *Proc Natl Acad Sci USA* 96: 290–295

Sexton R, Roberts JA (1982) Cell Biology of Abscission. *Annu Rev Plant Physiol Plant Mol Biol* 33: 133–162

Shi CL, Stenvik GE, Vie AK, Bones AM, Pautot V, Proveniers M, Aalen RB, Butenko MA (2011) Arabidopsis class I KNOTTED-like homeobox proteins act downstream in the IDA-HAE/HSL2 floral abscission signaling pathway. *Plant Cell* 23: 2553–2567

Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai YS, Gill BS, Faris JD (2006) Molecular characterization of the major wheat domestication gene Q. *Genetics* 172: 547–555

Solano R, Stepanova A, Chao Q, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev* 12: 3703–3714

Stenvik GE, Tandstad NM, Guo Y, Shi CL, Kristiansen W, Holmgren A, Clark SE, Aalen RB, Butenko MA (2008) The EPIP peptide of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to induce abscission in arabidopsis through the receptor-like kinases HAESA and HAESA-LIKE2. *Plant Cell* 20: 1805–1817

Tabuchi T, Ito S, Arai N (2001) Anatomical studies of the abscission process in the tomato pedicels at flowering stage. *J Jpn Soc Hortic Sci* 70: 63–65

Taylor JE, Whitelaw CA (2001) Signals in abscission. *New Phytol* 151: 323–339

Tieman DM, Ciardi JA, Taylor MG, Klee HJ (2001) Members of the tomato LeEIL (EIN3-like) gene family are functionally redundant and regulate ethylene responses throughout plant development. *Plant J* 26: 47–58

Tucker ML, Burke A, Murphy CA, Thai VK, Ehrenfried ML (2007) Gene expression profiles for cell wall-modifying proteins associated with soybean cyst nematode infection, petiole abscission, root tips, flowers, apical buds, and leaves. *J Exp Bot* 58: 3395–3406

Wei PC, Tan F, Gao XQ, Zhang XQ, Wang GQ, Xu H, Li LJ, Chen J, Wang XC (2010) Overexpression of AtDOF4.7, an Arabidopsis DOF family transcription factor, induces floral organ abscission deficiency in Arabidopsis. *Plant Physiol* 153: 1031–1045

Wu XM, Yu Y, Han LB, Li CL, Wang HY, Zhong NQ, Yao Y, Xia GX (2012) The tobacco BLADE-ON-PETIOLE2 gene mediates differentiation of the corolla abscission zone by controlling longitudinal cell expansion. *Plant Physiol* 159: 835–850

Yang TJ, Lee S, Chang SB, Yu Y, de Jong H, Wing RA (2005) In-depth sequence analysis of the tomato chromosome 12 centromeric region: identification of a large CAA block and characterization of pericentromere retrotranposons. *Chromosoma* 114: 103–117

Yokotani N, Nakano R, Imanishi S, Nagata M, Inaba A, Kubo Y (2009) Ripening-associated ethylene biosynthesis in tomato fruit is autocatalytically and developmentally regulated. *J Exp Bot* 60: 3433–3442

Zahara MB, Scheuerman RW (1988) Hand-harvesting jointless vs. jointed-stem tomatoes. *Calif Agric* 42: 14–14

Zhou Y, Lu D, Li C, Luo J, Zhu BF, Zhu J, Shangguan Y, Wang Z, Sang T, Zhou B, et al. (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor shattering abortion1. *Plant Cell* 24: 1034–1048