Integrative hormone and transcriptome analysis underline the role of abscisic acid in seed shattering of weedy rice

Hong Lang1 · Yuting He1 · Fengcheng Li1 · Dianrong Ma1 · Jian Sun1

Received: 1 February 2021 / Accepted: 17 April 2021 / Published online: 22 April 2021
© The Author(s) 2021

Abstract
Weedy rice is one of the most severe weeds in paddy fields, characterized by its high degree of seed shattering. Abscisic acid (ABA) serves as an abscission-accelerating signal and plays a critical role during abscission. However, mechanisms that link ABA and seed shattering remain elusive. In this study, WR04-6 (shattering) and SN9816 (non-shattering) were used to investigate the expression levels of genes involved in ABA biosynthesis and to determine the levels of ABA in tissues collected from the abscission zone (AZ) and the spikelet. ABA content in WR04-6, particularly in AZ, was significantly higher than in SN9816, significantly increasing prior to abscission. RNA-Sequencing and further expression analyses showed that the expression of OsNCED, the key gene involved in ABA biosynthesis, coincided with the increase of ABA content in the AZ and significantly increased during the seed shattering process. Additionally, the expression analysis of genes related to biosynthesis and metabolism of indole-3-acetic acid, gibberellin acid, and ethylene showed the greatest fold-change. Phytohormone levels associated with ABA co-expression-prediction revealed a potential signal transduction network among plant hormones involved in the regulation of seed abscission. Taken together, data presented in this study suggest that ABA contributes to seed shattering and transiently cooperates with other hormones, triggering a hormone imbalance that leads to the downstream activation of the AZ.

Keywords ABA · Hormone level · Seed shattering · Transcriptome analyses · Weedy rice

Abbreviations

| Abbreviation | Description                        |
|--------------|------------------------------------|
| ABA          | Abscisic acid                      |
| IAA          | Indole-3-acetic acid               |
| GA           | Gibberellin acid                   |
| ETH          | Ethylene                           |
| NCED         | 9-cis-epoxycarotenoid dioxygenase gene |
| ZEP          | Zeaxanthin epoxidase               |
| NDGA         | Nordihydroguaiaretic acid          |
| RNA-Seq      | RNA-Sequencing                     |
| RT-qPCR      | Quantitative real-time PCR         |
| PPI          | Protein–protein interaction        |

Introduction
Taxonomically classified as the same species as cultivated rice (Oryza sativa L.) (Cao et al. 2006; Reagon et al. 2010), weedy rice (Oryza sativa f. spontanea), is one of the most dominant and aggressive weeds found in paddy fields worldwide. It competes with cultivated rice for nutrients, water, sunlight, and other resources consequently affecting crop production (Chauhan and Johnson 2017). For example, in China, weedy rice can reduce rice crop yields by 10–50%, leading to serious economic losses (Zhao et al. 2017). Furthermore, it has many morphological and physiological traits related to weediness (black hull, long awn, and red pericarp) and is characterized by its high seed shattering rate (Sun et al. 2013, 2019). Weedy rice has evolved a system to control and adapt to the time when the seed population reaches final maturity based on the nutritional status, thus allowing seeds to separate from the parent plant facilitating seed
dispersal and long persistence in the field (Qiu et al. 2017). Excessive seed shedding in cereal crops is a major cause of yield loss, which consequently leads to a loss of interest for farmers (Hun et al. 2014). Moreover, controlling the degree of grain shattering is an important challenge for cereal crop breeding. Therefore, studying the mechanism of seed shattering in weedy rice is important for efficient regulation of crop productivity and effective management.

Abscission is the process of shedding vegetative or reproductive organs by a plant in response to developmental, hormonal, and environmental cues. This process occurs in a specialized group of cells collectively known as the abscission zone (AZ) (Glazinska et al. 2017). Previous studies on wild rice (Oryza rufipogon) and O. sativa ssp. indica identified several genes involved in seed shattering, necessary for AZ formation in the pedicel (Inoue et al. 2015). Shattering4 (Sh4) encodes a transcription factor, homologous to Myb3 and is necessary for the development of a functional abscission layer in the pedicel (Li et al. 2006). The underlying gene, qSH1, encodes a BEL1-type homeobox transcription factor that is often considered to be responsible for seed shattering in the indica subspecies (Konishi et al. 2006). Mutations in qSH1 or Sh4 show moderate shattering or even a non-shattering phenotype due to the impairment in the AZ development. Conversely, SH5 (another BEL1-type homeobox gene) is highly expressed in the AZ, suppresses AZ development via silencing and inhibits seed shattering (Yoon et al. 2014). The OsSHAT1 gene, which encodes an APETALA2 transcription factor, is also required for seed shattering by specifying AZ development in rice (Zhou et al. 2012). OsCPL1 was the first recessive shattering gene to be identified, encoding a carboxy-terminal domain phosphatase-like protein that acts as a repressor of AZ differentiation and thereby reduces seed shattering (Ji et al. 2010). Additionally, some related quantitative trait loci (QTL), such as qSH3 and qSH1, have been shown to determine whether rice grains shatter or persist on the spikelets (Zhu et al. 2012; Inoue et al. 2015; Jiang et al. 2019).

Phytohormones, such as abscisic acid (ABA), are considered to play vital roles in regulating organ abscission (Estornell et al. 2013). The role of ABA as a possible activator of the abscission process has been hypothesized as endogenous levels of ABA increase temporarily or constitutively during abscission. A previous study showed that the application of ABA-biosynthesis inhibitors, such as nordihydroguaiaretic acid (NDGA), reduced flower abortion in Lupinus luteus (Wilmowicz et al. 2016). Transcriptomic evidence indicated that the abscission-related ABA is biologically active and its increased biosynthesis is associated with the induction of the specific ABA-responsive 9-cis-epoxycarotenoid dioxygenase (NCED) gene (Giulia et al. 2013; Li et al. 2019a). These findings have led to the hypothesis that abscission is triggered by ABA activation. However, there is considerable controversy concerning the role of ABA in the promotion of abscission. Several researchers view ethylene (ETH) as the primary regulator of abscission, citing correlations of abscission with ETH production, and the inability of exogenous ABA to accelerate abscission in many cases (Marciniak et al. 2018). Moreover, the effect of ABA on abscission seems to depend on its interaction with auxin (such as indole-3-acetic acid, IAA) or ETH, rather than being directly involved on its own. Thus, ABA could have an intermediary role in organ abscission (Aurelio et al. 2000; Agustí et al. 2007). ABA is also believed to be the main regulator of ripening and plays a key role in the control seed maturation, in desiccation tolerance and dormancy (Sugimoto et al. 2010). Therefore, the determination of how ABA accumulation in seeds influences dormancy or seed abscission is critical. During seed development, ABA is known to control mid to late stages of embryo maturation and desiccation tolerance through the B3 domain transcription factor VIVIPAROUS (VP1) (Mccarty and Jones 1995). VP1 functions as a co-activator to regulate various seed maturation processes, including storage protein accumulation, oil deposition, and embryo degreening (Suzuki et al. 2003; Roschzttardtz et al. 2009; Delmas et al. 2013). However, the previous investigations of ABA functions in the abscission process of crop species (such as rice) are extremely rudimentary, and the processes underlying ABA cross-talk with other hormones to induce abscission in plant systems remain to be determined.

To provide a comprehensive understanding of ABA-associated seed abscission in weedy rice, endogenous ABA levels were measured in different tissues of the shattering weedy rice (WR04-6) and the non-shattering cultivated rice (SN9816). RNA-Seq was performed to identify differentially expressed genes (DEGs) putatively involved in ABA biosynthesis. The expression profile of genes involved in ABA biosynthesis during seed shattering was further examined by quantitative real-time PCR (RT-qPCR). Based on hormone levels and gene expression patterns associated with the ABA co-expression network, the function of ABA during seed shattering was comprehensively explored and discussed. These findings, therefore, lay a solid foundation for an in-depth understanding of the role of ABA in seed shattering of weedy rice.

Materials and methods

Plant materials and growth conditions

WR04-6 (Oryza. sativa f. spontanea), with seed shattering, red pericarp, and black hull phenotype, is a common weedy rice strain in the Liaoning Province. The seeds of WR04-6 were collected and preserved by Rice Research Institute of Shenyang Agricultural University. Temperate japonica


(\textit{Oryza sativa}) cultivar Shennong9816 (SN9816) is a non-seed shattering variety, which was bred by Rice Research Institute of Shenyang Agricultural University and used as a control in this study. The experimental materials were grown in the germplasm resources field at the Rice Research Institute of Shenyang Agricultural University, Liaoning Province, China. Seeds were sown on April 15, 2019, with seedlings transplanted to their final locations on May 26, 2019. Plants were spaced 30.0 x 13.4 cm apart. Fertilizer and water management followed the local standard management.

**Different developmental stage sampling**

Bootimg initiation and heading dates were recorded to ensure the precise timing of phenotypic evaluation and sampling due to variation in heading dates in the test populations. The emergence of the flag leaf was marked as the beginning of the booting stage. The booting stage was characterized as the period when anthers were fully developed and referred to as −5 DPA.

To detect the dynamic changes of ABA content during the growth and development of WR04-6 and SN9816, spikelets were randomly collected from nine plants of WR04-6 and SN9816 at −5, 5, 12, 17, and 35 DPA for hormone measurement. The samples were collected from nine individual plants (three biological replicates with three plants per time point). Then, the AZ tissues between rachilla and the palea and lemma at 15 DPA were collected by manually cutting at approximately 2 mm of the abscission fracture (three biological replicates with three plants per replicate) for further ABA content analysis.

The transcript patterns involved in ABA biosynthesis during seed shattering were determined by RT-qPCR analysis. AZ tissues were sampled at −5, 5, 10, and 15 DPA (three biological replicates with three plants per replicate) for RT-qPCR.

To determine whether ABA accumulation in spikelets of weedy rice acts on organ abscission or seed dormancy, spikelets at −5, 5, 10, 15, 20, 25, 30, and 35 DPA were selected for expression characteristic of \textit{OsVP1} and immunoblot analysis. All of the samples were flash-frozen using liquid nitrogen and stored at −80°C until further use.

**Seed shattering measurement**

Breaking tensile strength (BTS) was used as a quantitative standard to evaluate seed shattering at 0, 5, 12, and 17 DPA. BTS is inversely proportional to seed shattering and measures the maximum amount of weight (g) that a single flower or grain can hold before releasing (Thurber et al. 2011; Nunes et al. 2014). To evaluate seed shattering, five spikelets or grains were selected from the main panicles in five plants using a Digital Force Gauge (aiPLi, China). An individual grain was detached from the panicle by holding the seed with a clip and the peak measurements were recorded when the grain was removed.

**Morphological analysis of the AZ**

To observe the morphological differences in the AZs (the rachilla below the floret) between WR04-6 and SN9816, spikelet samples at −5 (booting stage), 0, and 7 DPA were collected for scanning electron microscopy (SEM) analysis and observed using a microscope (Hitachi TM3030, Japan). At least three inflorescences from WR04-6 and SN9816 were collected and dissected.

**ABA, IAA, GA3, and ACC extraction and analysis**

The levels of ABA, IAA, GA3, and ACC were determined by Zoonbio Biotechnology Co., Ltd (Nanjing, China). Approximately 0.5 g of fresh tissue was finely ground in liquid nitrogen and extracted with 5 mL of extraction buffer (isopropanol/hydrochloric acid) and 8 μL internal standard (1 μg/mL) in each sample tube. The mixture was incubated on a shaker for 30 min at 4°C. Then, 10 mL of dichloromethane (CH2Cl2) was added and the sample was again incubated on a shaker for 30 min at 4°C. The sample was then centrifuged for 5 min at 13,000 rpm at the same temperature and the lower, organic phase was extracted. The organic phase was dried under N2, dissolved in 400 μL of methanol (0.1% methane acid) and filtered through a 0.22-μm filter membrane. ACC determination was achieved by adding an external standard method (Hermann et al. 2007).

The purified product was then subjected to high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis and the methods were modified from those described by a previous study (You et al. 2016). Three independent replicates were performed for each measurement.

**Western blotting**

A primary antibody targeting OsVP1 was generated by GenScript Co., Ltd. (Nanjing, China) using a synthetic peptide (SKQPKPSPEKPKPKC) derived from OsVP1. The secondary antibodies, anti-Bip-2 (AS09481), was generated by Agrisera AB (Sweden). Total protein was extracted from spikelets collected from WR04-6 and SN9816 at 5, 15, 25, and 35 DPA according to the manufacturer’s instructions of the Minute™ Total Protein Extraction Kit for Plants Tissues (Invent Biotechnologies, Inc., Beijing, China). Protein concentration was determined by the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA). The extracted proteins were separated using 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).
Following electrophoresis, the separated proteins were transferred to a polyvinylidene fluoride (PVDF) membrane for immunoblot analysis. Quantification analysis of the protein band intensities from immunoblot was performed by Gel-Pro Analyzer 4 software (Switzerland). Each experiment was repeated at least three times and one representative result was shown.

**RNA-sequencing and comparative transcriptome analysis**

Samples collected from the AZ (≤ 2 mm in length) of WR04-6 and SN9816 at 15 DPA were used for total RNA isolation. Total RNA was isolated for cDNA library construction. The cDNA libraries were sequenced using Illumina HiSeq™ 2500 sequencing platform (Illumina Corp., San Diego, CA, USA) by Gene De novo Biotechnology Co., Ltd (Guangzhou, China). In total, six samples (two materials × three biological replicates) were sequenced. RNA-Seq reads were examined to remove low-quality (number of bases with Q-value ≤ 20) reads. The reference genome of *Oryza sativa* ssp. *japonica* (Os-Nipponbare-Reference-IRGSP-1.0) was obtained from The Rice Annotation Project Database (RAP-DB) (https://rapdb.dna.affrc.go.jp/index.html). Cleaned short reads were aligned to all exon sequences using HISAT2 (Kim et al. 2015) and the expression abundance was calculated by RSEM (Pertea et al. 2015) with default parameters. Genes with an expression Log2 fold-change of ≥ 1 and a false discovery rate < 0.05 were considered as significant DEGs. Then, DEGs were subjected to enrichment analysis of GO function and KEGG pathways, and the Protein–Protein Interaction (PPI) network of hormone-related DEGs was constructed using the Cytoscape software based on STRING Database (https://string-db.org/).

**cDNA synthesis and gene expression analysis by RT-qPCR**

Total RNA was isolated using MiniBEST Plant RNA Extraction Kit (TaKaRa, Dalian, China) based on the manufacturer’s instructions. PrimeScript™ RT II 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) and TB Green ® Premix Ex Taq™ II (Tli RNaseH Plus) (TaKaRa, Dalian, China) were used for synthesizing first-strand cDNA and RT-qPCR according to the manufacturer’s instructions, respectively. RT-qPCR was performed using an Applied Biosystem 7500 Real-Time PCR System (Thermo Fisher Scientific, USA) under the following conditions: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 34 s. The transcript levels were normalized to the reference gene *OsActin* following the 2−ΔΔCt method (Livak and Schmittgen 2001). Three biological replicates were quantified for RT-qPCR analysis. The gene-specific primers used in the RT-qPCR are listed in Supplementary Table S1.

**Statistical analysis**

Data were analyzed by analysis of variance and for significance (*p* < 0.05) of treatment differences using Duncan’s test on SPSS software version 19.0 (Student’s *t* test: **p** ≤ 0.0005; *p* ≤ 0.005; *p* ≤ 0.05). Results are presented as means ± standard error (SE) of three biological replicates.

**Results**

**Identification of the seed shattering phenotype**

A common weedy rice strain WR04-6 (*Oryza sativa* f. *spontanea*) and non-seed shattering *temperate japonica* (*Oryza sativa*) cultivar SN9816 are significantly different in seed shattering behavior. The evaluation of seed shattering was performed using BTS. The BTS value of SN9816 was similar to WR04-6 at anthesis (0 DPA) and then slowly decreased from 5 to 17 days post-anthesis (DPA) (Fig. 1a). However, the reduced BTS values were not sufficiently low for grain shattering. Furthermore, the BTS value of WR04-6 slightly increased from 0 to 5 DPA, subsequently, BTS value decreased more rapidly that resulted in lower BTS values at 12–17 DPA than that of SN9816. In WR04-6, a small number of seeds completed the transition from milk to the wax stage, the testa showed a high degree of lignification and the hull color changed from green to dark brown (Fig. 1b). The grain phenotype of WR04-6 was severely dispersal at 17 DPA, while SN9816 displayed a compact panicle.

**Anatomy of the abscission zone**

Different degrees of seed shattering may be accompanied by different anatomical structures. Here, SEM was used to analyze the differences in the AZ between WR04-6 and SN9816. AZ generally forms in rice pedicel tissue 16–20 days before heading (Ji et al. 2006; Yoon et al. 2017), and the value of BTS in WR04-6 sharply decreased from 5 to 12 DPA (Fig. 1a), which suggested that the AZ tissues in WR04-6 may have already generated a significant difference before this period. Therefore, tissues of AZ from −5 (booting stage), 0, and 7 DPA were used for further SEM analysis. Results showed that there were no obvious phenotypic differences between WR04-6 and SN9816 at the booting and anthesis stages (Fig. 1c). However, the distinct interspace between rachilla and the palea and lemma of WR04-6 was detected at 7 DPA, and only a small part of the tissue was connected. On the contrary, the connection of the rachilla in SN9816 remained almost unchanged from −5 to 7 DPA.
These anatomical observations corresponded with the trends observed in the BTS value.

Biological function of ABA during seed shattering

The dynamics of ABA levels during spikelet development of WR04-6 and SN9816 were examined (Fig. 1d). ABA concentration significantly increased from 5 (7.70 ng/g) to 12 DPA (19.17 ng/g) in WR04-6, which was consistent with the BTS values measured at this time point. Subsequently, ABA concentration continuously decreased during mid to late-phase embryogenesis, with the lowest concentration detected at 35 DPA (3.88 ng/g). The ABA level trends in SN9816 were similar to WR04-6. However, the overall concentration was much lower in SN9816 during the same period, except at 17 DPA. ABA concentration reached 17.72 ng/g in SN9816 at 17 DPA, which was 1.18-fold higher than in WR04-6 (14.96 ng/g). When the seeds reached physiological maturity, the ABA level was higher in SN9816 compared to WR04-6.

For convenience of sampling, the AZ at 15 DPA was therefore selected for further ABA content detection in WR04-6 and SN9816. As expected, the ABA level of AZ tissues in WR04-6 (30.97 ng/g) was more than three-fold higher than in SN9816 (9.15 ng/g) at 15 DPA (Fig. 1e).

OsVP1 is a ABA-responsive gene, which positively regulate the expression of seed dormancy gene Sdr4, and is a global regulator of seed dormancy. To distinguish whether ABA accumulation in spikelet of weedy rice acts on organ abscission or seed dormancy regulation, the gene and protein expression patterns of OsVP1 were detected. The expression level of OsVP1 in both WR04-6 and SN9816 increased from −5 to 35 DPA during spikelet development (Supplementary Fig. S1). Samples of spikelets from WR04-6 and SN9816 at representative time points (5, 15, 25, and 35 DPA) were selected for immunoblot analysis of OsVP1. There was no significant difference in the OsVP1 protein level at different
stages of seed development between WR04-6 and SN9816 (Fig. 1f, Supplementary Fig. S2), indicating that ABA accumulates abundantly in spikelets was responsible for seed abscission rather than seed dormancy.

Comparative transcriptome analysis in AZ tissues

To elucidate the molecular basis of the observed differences in seed abscission, the transcriptomic analysis was performed to investigate the genome-wide gene expression profile in AZ tissues from WR04-6 to SN9816 at 15 DPA. After filtering out genes with low expression, a total 7082 DEGs were identified, including 2926 up-regulated and 4156 down-regulated DEGs (fold change (FC) > 2, false discovery rate (FDR) < 0.05; Fig. 2a, Supplementary Table S2). These were classified into 128 biochemical pathways, including biosynthesis of secondary metabolites, metabolic pathways, phenylpropanoid biosynthesis, alpha-linolenic acid metabolism, starch and sucrose metabolism, and fatty acid metabolism (Fig. 2b).

Genes related to plant hormone biosynthesis and signaling pathways

Since hormones act as internal cues that initiate the abscission process, the hormone biosynthesis and signal transduction-related DEGs were thoroughly analyzed. Overall, 39 DEGs involved in ABA biosynthesis or signaling pathway were detected in the transcriptome analysis (Supplementary Table S3 and S4). OsNCED3 and OsNCED5 genes were up-regulated (Fig. 2c) and among these were six DEGs encoding a putative aldehyde oxidase (AAO) that catalyzes the conversion of abscisic aldehyde to ABA, five genes were down-regulated, and only one was up-regulated (Supplementary Table S4). OsPYL3 and OsPYL7 were down-regulated and encode the ABA receptor family (PYR/PYL). The expression of genes encoding proteins belonging to the PP2C complexes (OsABIL1, OsABIL3, and OsSIPP2C2) and SnRK2 families (OsSAPK1 and OsSAPK6), involved in ABA signal transduction, were significantly higher in ABA tissues in WR04-6 than in SN9816. Furthermore, OsHVA22E and OsGEM are the two types of ABA-responsive protein-encoding genes, five and three genes were identified as significantly expressed in the AZ tissues, respectively. Among the five OsHVA22E genes, only one gene was up-regulated and others were down-regulated. Among the three OsGEM genes, two genes were up-regulated, except that LOC_Os02g42430 was down-regulated (Supplementary Table S4).

Seventeen, twenty one, and nine genes were found to be related to biosynthesis or signaling transduction pathways of IAA, ETH, and GA, respectively (Supplementary Table S3 and S5). Among the 17 IAA-related DEGs, 16 genes were down-regulated that encode intermediates of IAA biosynthesis (OsFIB, OsYUCCA3, OsYUCCA5, OsYUCCA7, and OsYUC9), GH3 protein (OsGH3-2, OsGH3-1, and OsGH3-11) (Supplementary Table S5) and AUX/IAA proteins, while only one gene was up-regulated. In ETH-related genes, three ACO genes (OsACO1, OsACO5, and OsACO7) and three putative ACO were identified as significantly down-regulated and are the key enzymes in ETH biosynthesis (Chersicola et al. 2017). Moreover, OsACO2 and LOC_Os08g30100 were up-regulated. In this study, nine GA-related genes were found to be involved in seed shattering. GA 2-oxidase is responsible for deactivating bioactive GAs to reduce GA levels, which were encoded by OsGA2ox genes (Li et al. 2019b). Transcriptome data showed that OsGA2ox genes were up-regulated and the expression of OsKS, OsKOS, and OsGA2ox genes was down-regulated. There were five genes related to JA biosynthesis or signaling transduction and these genes were up-regulated. Contrarily, there were six genes related to CK signaling and all of them were down-regulated (Supplementary Table S5).

To provide a general view of the protein functions and signaling circuitry in the AZ tissues at the last stage of abscission, the PPI network for the phytohormone-related proteins was predicted. The gene expression data (log2FC > 1, FDR < 0.05) for DEGs were mapped to the PPI network. A total of 88 DEGs (30 up-regulated and 58 down-regulated genes) related to ABA/IAA/ETH/GA biosynthesis and signaling transduction pathways were selected for PPI construction (Fig. 2d). PPI network complex had two significant modules and centrality analysis showed that OsNCED3 had strong connectivity with genes belonging to GA (OsGA2ox5 and OsGA2ox9) and IAA (OsORR1 and OsSaur11) biosynthesis or signal transduction pathway. OsNCED1 was considered to work as an important regulation effect strongly associated with GA (OsKSI, OsKSI, and OsGA2ox1) and ETH (OsACO2) biosynthesis genes, while OsIAA1 and OsSaur8 were predicted to interact with OsNDE4.
Expression patterns of ABA biosynthesis-related genes

To monitor changes of the gene expression levels from the ABA biosynthesis pathway, AZ tissues and the spikelets during –5–15 DPA were sampled and significant differences were observed in RT-qPCR analysis (Fig. 3). Results showed that the expression of zeaxanthin epoxidase (OsZEP) and OsNCEDs remained at a basal level in the spikelet, while specifically up-regulated in the AZ tissues of WR04-6 and SN9816. In the AZ of WR04-6, the expression level of OsNCED2 and OsNCED4 were barely detectable, while the transcripts of OsNCED1, OsNCED3, and OsNCED5 exhibited similar trends, increasing throughout the abscission stage. The expression level of OsNCED3 decreased after 15 DPA, while the expression of OsNCED1 and OsNCED5 continued to increase. In SN9816, OsNCEDs expression was lower than in WR04-6 during the period investigated.

Measurement of IAA, GA₃, and ETH levels in spikelets

To further verify RNA-Seq analysis and investigate the role of other hormones in the abscission process, levels of IAA, GA₃, and ETH in spikelets at different developmental stages in WR04-6 and SN9816 were quantified. Results showed that endogenous IAA and GA₃ levels in WR04-6 were significant lower than those in SN9816 at different stages of spikelet development, which was consistent with the down-regulation of genes related to IAA and GA biosynthesis or signaling transduction pathway (Supplement Fig. S3). At the initial seed shattering of 17 DPA, IAA and GA₃ content in WR04-6 was 0.40 and 0.47-fold compared to SN9816, respectively. 1-aminocyclopropane-1-carboxylic acid (ACC) serves as a precursor of ETH synthesis and its content is closely linked to the ETH level produced by plants (Ruduš et al. 2012). The ACC concentrations both decreased in WR04-6 and SN9816 following spikelet development, while WR04-6 displayed a significantly lower level than in SN9816 (Supplement Fig. S3). In the process of seed shattering of WR04-6, ABA and ETH abundantly accumulate in the AZ tissue, while IAA, GA₃ decrease significantly, indicating that a complex crosstalk network of phytohormones was involved in the variations of seed shattering (Fig. 4).

Discussion

Seed shattering in weedy rice is an adaptive trait for seed dispersal which is dominated by natural selection. In *Oryza* plants, extensive research has been conducted on the abscission characteristics in recent years and several QTLs/genes...
that control seed shattering have been identified through genetic methods (Konishi et al. 2006; Subudhi et al. 2014; Yao et al. 2015). *Japonica* type weedy rice possessed the ancestral type *qsh1* allele and derived type *sh4* allele, which was the same as *indica* rice. However, the shattering of weedy rice is much stronger than that of *indica* rice. Based on this fact, crossing *japonica*-type weedy rice with *indica* rice to construct a genetic mapping population will help isolate the weedy-specific shattering genes. On the other hand, it is also a worthwhile research strategy for isolating shattering genes by using genome-wide association study (GWAS) in a mixed natural population of weedy rice and cultivated rice with significant phenotypic variation of shattering.

Phytohormones are considered to play vital roles in regulating organ abscission acting at different levels in the regulation of gene expression. Previous studies performed on various species have characterized the main hormone responsible for organ abscission, while the physiological and biochemical aspects underlying the abscission process in weedy rice remain poorly understood. Thus, understanding the mechanisms that regulate seed abscission is of great significance to weedy rice management. In this study, hormone levels and gene expression patterns were comprehensively analyzed between WR04-6 and SN9816 and results contribute to an in-depth understanding of the role of ABA in seed shattering of weedy rice.

**Weedy rice showed different seed shattering behavior from cultivar rice**

BTS value of weedy rice (WR04-6) was similar to that of cultivar rice (SN9816) during the anthesis stage. However, WR04-6 showed a remarkable decrease accompanied by an increased level of seed shattering at 12 DPA (Fig. 1a). The time of seed shattering observed in WR04-6 was consistent with several weedy rice strains and the BTS value was in a similar range to the previously published studies (Konishi et al. 2006; Thurber et al. 2011), suggesting that most weedy rice strains may have similar growth and developmental process. The most obvious change was found in AZ tissues of WR04-6 at 7 DPA as part of the seed had a narrow gap between the lemma and rachilla, while there was no change in the non-shattering cultivar SN9816 (Fig. 1c). Anatomical observations further confirmed the differences between WR04-6 and SN9816 in AZ tissues. Although previous studies showed that the formation of AZ tissue is a universal prerequisite for abscission, the narrow gap between the lemma and rachilla may be another phenotypic trait of weedy rice that has adapted to seed shattering (Htun et al. 2014).

**The roles of ABA in seed shattering acquisition**

The level of ABA in WR04-6 was significantly induced during seed shattering. In spikelets, ABA accumulation of WR04-6 and SN9816 showed a similar trend (Fig. 1d), while ABA levels in WR04-6 were higher than in SN9816, indicating that the ABA content variation, particularly in the AZ tissues, may be associated with abscission. This is similar to apple, where a statistically significant correlation was calculated between fruitlet abscission and ABA content (Giulia et al. 2013). Generally, ABA accumulation in seeds during early embryogenesis is low but increases during the transition of developing embryos into the maturation phase, usually peaking around mid-maturation. ABA levels usually
...sues of WR04-6 during seed abscission, but remained at a relatively low expression level in SN9816 (Figs. 2c and 3). Similar expression patterns have been previously observed in tomato (Nakano et al. 2013), citrus (Xie et al. 2018), Lupinus luteus (Glazinska et al. 2017), and sugarcane (Li et al. 2016) during organ abscission, showing up-regulation in NCEDs expression levels. These results suggest a positive correlation between ABA biosynthesis and seed shattering. ABA signaling transduction occurs via type 2C protein phosphatase (PP2C) and SNF1-related kinase (SnRK2), which act immediately downstream of the receptors to transmit signals from ABA (Pilati et al. 2017). In this study, three OsPP2C genes (OsABIL1, OsABIL2, and OsSIPP2C2) and two OsSnRK2 genes (OsSAPK1 and OsSAPK6) were up-regulated in the AZ tissues of WR04-6, consistent with increased ABA biosynthesis. Thus, ABA levels and transcriptomic profiling studies have reinforced the idea that seed shattering relies on the up-regulation of ABA levels and the results further confirmed that ABA participates in significant regulation during abscission in different species or organs (Giulia et al. 2013; Wilmowicz et al. 2016; Li et al. 2019a).

ABA plays a central role in acquiring embryonic dormancy during seed maturation (Tuan et al. 2018; Xing et al. 2020). As OsVP1 serves as a seed-specific transcription factor that primarily acts in the late embryo stage, functioning during desiccation tolerance and dormancy (Brady et al. 2003), its level was further investigated by western blot. Results showed that there was no significant difference between WR04-6 and SN9816 in the OsVP1 protein level at different development stages in spikelets (Fig. 1f), suggesting that the increase of ABA content in the spikelet may be responsible for seed abscission rather than seed dormancy. Together, the differences in the levels of ABA and OsVP1 protein expression between WR04-6 and SN9816 suggest that ABA was involved in the process of signal perception and transduction in the last stage of organ abscission.

The interplay of plant hormones has been extensively reported (Gao et al. 2019; Khan et al. 2020). Previous studies show that many genes participate in IAA biosynthesis, signaling, transportation or degradation during organ abscission, including AtAUXs, AtLAXs (Basu et al. 2013), CitAUX/IAA, CitGH3 (Xie et al. 2015), and SIPIN1 (Shi et al. 2017). In this study, some genes (such as OsYUCCAs, OsGH3s, and OsIAAs) were found to be down-regulated in the AZ tissues of WR04-6. Similarly, some genes related to GA biosynthesis were largely suppressed (Supplementary Table S3 and S5). ETH serves as a pivotal effector of plant organ abscission and application exogenous ETH or its promoters could accelerate organ abscission in apple (Cin et al. 2005), peach (Rasori et al. 2002), and mango (Ish-Shalom et al. 2011) species. Transcriptome data showed that one OsACS gene (OsACS2) and two OsACO genes (OsACO2 and LOC_ Os08g30100), encoding the key enzymes in ETH biosynthesis, were significantly induced and multiple ETH-response genes were up-regulated during seed shattering in WR04-6. These results were consistent with the study carried out in tomato (Chersicola et al. 2017), which demonstrated that ABA and ETH synergistically regulate seed shattering in weedy rice. Additionally, PPI prediction highlighted a potential signal transduction network of plant hormones involved in regulating seed abscission (Fig. 2d).

**Complex crosstalk between phytohormones during seed shattering**

The generally accepted model of abscission induction involves the decrease of IAA levels and the increase of GA and ETH levels (Meir et al. 2010; Nakano and Ito 2013; Marciniak et al. 2018). Endogenous levels of IAA, GA₃, and ETH were determined in the spikelets in WR04-6 and SN9816 (Supplement Fig. S3). As expected, seed shattering was accompanied by the decrease of IAA levels, while with a contrary result to the previous study (Marciniak et al. 2018) that was GA₃ significantly lower than in SN9816 during seed shattering process. The divergent results obtained in various species may be due to the existence of a unique regulatory mechanism of the abscission of different organs. ACC is the directed precursor in ETH biosynthesis and its content is closely correlated with ETH release (Larsen 2015). Results showed that the ACC level in WR04-6 was lower than in SN9816, indicating that WR04-6 released more ETH than SN9816. Based on these results, we hypothesized that increasing ABA levels could inhibit the expression of genes related to the IAA/GA pathway, thereby affecting plant hormone homeostasis in response to seed abscission (Fig. 4). In general, weedy rice showed different hormone levels from cultivar rice and thus the interplay of different plant hormones affecting seed shattering requires further study.

**Conclusions**

In the current study, we employed phytohormone measurements and transcriptomic analysis to characterize the role of ABA on seed shattering acquisition in weedy rice. Phytohormone levels and RT-qPCR data supported the model
for ABA function in seed shattering regulation of weedy rice. Changes in IAA, GA, and ETH levels accompanied by down-regulated gene expression patterns in the AZ tissues of WR04-6 highlighted a potential signal transduction network of plant hormones involved in regulating seed abscission. The results provide molecular information for future investigations and further understanding of the processes and the roles of phytohormones in seed shattering of weedy rice.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10725-021-00714-8.

Acknowledgements This work was supported by Liaoning Revitalization Talents Program (Grant No. XLYC1808003).

Author contributions DM and HL conceived the project. HL and YH conducted gene expression phenotypic measurement; JS, and FL analyzed and interpreted the data; HL drafted the manuscript; DM, JS, and FL critically revised the manuscript. All authors read and approved the final manuscript.

Data availability Raw sequence data were deposited in the NCBI Short Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA656624.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Agusti J, Zapater M, Iglesias DJ, Cercós M, Tadeo FR, Talón M (2007) Differential expression of putative 9-cis-epoxy-carotenoid dioxygenases and abscisic acid accumulation in water stressed vegetative and reproductive tissues of citrus. Plant Sci 172:85–94. https://doi.org/10.1016/j.plantsci.2006.07.013

Aurelio GC, Janel M, Tadeo FR, Primo-Millo E, Talón M (2000) Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta 210:636–643

Basu MM, Gonzalez-Carranza ZH, Azam-Ali S, Tang S, Shahid AA, Roberts JA (2013) The manipulation of auxin in the abscission zone cells of Arabidopsis flowers reveals that indoleacetic acid signaling is a prerequisite for organ shedding. Plant Physiol 162:96–106. https://doi.org/10.1104/pp.113.216234

Brady SM, Sarkar SF, Bonetta D, McCourt P (2003) The abscisic acid insensitive (ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in Arabidopsis. Plant J 34:67–75. https://doi.org/10.1046/j.1365-313x.2003.01707.x

Cao Q, Lu B, Xia H, Rong J (2006) Genetic diversity and origin of weedy rice (Oryza sativa f. spontanea) populations found in the north-eastern China revealed by simple sequence repeat (SSR) markers. Ann Bot 98:1241–1252. https://doi.org/10.1093/aob/mcl210

Chauhan BS, Johnson DE (2017) Weedy rice (Oryza sativa). I. Grain characteristics and growth response to competition of weedy rice variants from five Asian countries. Weed Sci 58:374–380. https://doi.org/10.1614/WS-16-09.10071.1

Chersiciola M, Kladnik A, Znidaric MT, Mrak T, Gruden K, Dermastia VD, Danesin M, Boschetti A, Dorigoni A, Ramina A (2005) Ethylene biosynthesis and perception in apple fruitlet abscission (Malus domestica L. Borkh). J Exp Bot 56:2995–3005. https://doi.org/10.1093/jxb/eri296

Delmas F, Sankaranarayanan S, Deb S, Widdup E, Bourronville C, Bollier N, Northey JGB, McCourt P, Samuel MA (2013) ABI3 controls embryo degreening through Mendel’s I locus. Proc Natl Acad Sci USA 110(40):E3888–E3894. https://doi.org/10.1073/pnas.1308114110

Estornell LH, Agusti J, Merelo P, Talon M, Tadeo FR (2013) Elucidating mechanisms underlying organ abscission. Plant Sci 199–200:48–60. https://doi.org/10.1016/j.plantsci.2012.10.000

Frey A, Godin B, Bonnet M, Sotta B, Marion-Poll A (2004) Maternal synthesis of abscisic acid controls seed development and yield in Nicotiana plumbaginifolia. Planta 218:958–964. https://doi.org/10.1007/s00425-003-1180-7

Gao Y, Liu Y, Liang Y, Lu J, Jiang C, Fei Z, Jiang C, Ma C, Gao J (2019) Rosa hybrida RfERF1 and RfERF4 mediate ethylene-and auxin-regulated petal abscission by influencing pectin degradation. Plant J 99:1139–1171. https://doi.org/10.1111/tpj.14412

Giulia E, Alessandro B, Mariano D, Andrea B, Benedetto R, Angelo R (2013) Early induction of apple fruitlet abscission is characterized by an increase of both isoprene emission and abscisic acid content. Plant Physiol 161:1952–1969. https://doi.org/10.1104/pp.112.208470

Glanzinska P, Wojciechowski W, Kulasek M, Glinkowski M, Marciniak K, Klajn N, Kesy J, Kopcewicz J (2017) De novo transcriptome profiling of flowers, flower pedicels and pods of Lupinus luteus (yellow lupine) reveals complex expression changes during organ abscission. Front Plant Sci 8:641. https://doi.org/10.3389/fpls.2017.00641

Hermann K, Meinhard J, Dobrev P, Linkes A, Pesek B, Hess B, Machackova I, Fischer U, Leubner-Metzger G (2007) 1-Ami- nocylopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (Beta vulgaris L.): a comparative study of fruits and seeds. J Exp Bot 58:3047–3060. https://doi.org/10.1093/jxb/erm162

HunTM, Inoue C, Chhourn O, Ishii T, Ishikawa R (2014) Effect of quantitative trait loci for seed shattering on abscission layer formation in Asian wild rice Oryza rufipogon. Breed Sci 64:199–205. https://doi.org/10.1270/jsbbs.64.199

Inoue C, Hun TM, Inoue K, Ikeda K, Ikeda T, Ishikawa R (2015) Inhibition of abscission layer formation by an interaction of two seed-shattering loci, sh4 and qSH3, in rice. Genes Genet Syst 90:1–9

Ish-Shalom M, Dahan Y, Maayan I, Irihimovitch V (2011) Cloning and molecular characterization of an ethylene receptor gene, MiERS1, expressed during mango fruitlet abscission and fruit ripening. Plant Physiol Biochem 49:931–936. https://doi.org/10.1016/j.plaphy.2011.05.010

© Springer
Ji HS, Chu SH, Jiang W, Cho YI, Hahn JH, Eun MY, McCouch SR, Koh HJ (2006) Characterization and mapping of a flowering mutant in rice that corresponds to a block of domestication genes. Genetics 173:995–1005. https://doi.org/10.1534/genetics.105.054031

Ji H, Kim SR, Kim YH, Kim H, Eun MY, Jin ID, Cha YS, Yun DW, Ahn BO, Lee MC, Lee GS, Yoon UH, Lee JS, Lee YH, Suh SC, Jiang W, Yang JI, Jin P, McCouch SR, An G, Koh HJ (2010) Inactivation of the CTD phosphatase-like gene OsCPL1 enhances the development of the abscission layer and seed shattering in rice. Plant J 61:96–101. https://doi.org/10.1111/j.1365-313X.2009.04039.x

Jiang L, Ma X, Zhao S, Tang Y, Liu F, Gu P, Yu Y, Zhu Z, Cai H, Sun C, Tan L (2019) The APETALA2-Like transcription factor super-numerary bract controls rice seed shattering and seed size. Plant Cell 31:17–36. https://doi.org/10.1105/tpc.18.00304

Khan N, Bano A, Ali S, Babar MA (2020) Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regul 90:189–203. https://doi.org/10.1007/s10725-020-00571-x

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357–360. https://doi.org/10.1038/nmeth.3317

Konishi S, Iakeshi T, Lin S, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. Science 312:1392–1396. https://doi.org/10.1126/science.1126410

Larsen PB (2015) Mechanisms of ethylene biosynthesis and response in plants. Essays Biochem 58:61–70. https://doi.org/10.1042/bse0580061

Li C, Zhou A, Sang T (2006) Rice domestication by reducing shattering. Science 311:1936–1939. https://doi.org/10.1126/science.1125604

Li M, Liang Z, Zeng Y, Jing Y, Wu K, Liang J, He S, Wang G, Mo Z, Tan F, Li S, Wang L (2016) De novo analysis of transcriptome reveals genes associated with leaf abscission in sugarcane (Saccharum officinarum L.). BMC Genomics 17:195. https://doi.org/10.1186/s12866-016-2552-2

Li C, Ma X, Huang X, Wan H, Wu H, Zhao M, Li J (2019a) Involvement of HD-ZIP I transcription factors LeHB2 and LeHB3 in fruitlet abscission by promoting transcription of genes related to the biosynthesis of ethylene and ABA in litchi. Tree Physiol 39:1600–1613. https://doi.org/10.1093/treephys/ppt071

Li C, Zheng L, Wang X, Hu Z, Zheng Y, Chen Q, Hao X, Xiao A, Wang X, Wang G, Zhang Y (2019b) Comprehensive expression analysis of Arabidopsis GA2-oxidase genes and their functional insights. Plant Sci 285:1–13. https://doi.org/10.1016/j.plantsci.2019.04.023

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. Methods 25:402–408. https://doi.org/10.1006/jmth.2001.1262

Marciniak K, Kucko A, Wilimowicz E, Swidzinski M, Przedniczek K, Kopcewicz J (2018) Gibberellic acid affects the functioning of the abscission layer and seed shattering in rice. Plant Biol (Stuttg) 16:888–906. https://doi.org/10.1111/plb.12133

Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol 33:290–295. https://doi.org/10.1038/nbt.3122

Pilati S, Bagagli G, Sonego P, Moretto M, Brazzale D, Castorina G, Simoni L, Tonelli C, Guella G, Engelen K, Galbiati M, Moser C (2017) Absciscic acid is a major regulator of grape berry ripening onset: new insights into ABA signaling network. Front Plant Sci 8:1093. https://doi.org/10.3389/fpls.2017.01093

Qiu J, Zhou Y, Mao L, Ye C, Wang W, Zhang J, Yu Y, Fu F, Wang Y, Qian F, Qi T, Wu S, Sultana MH, Cao YN, Wang Y, Timko MP, Ge S, Fan L, Lu Y (2017) Genomic variation associated with local adaptation of weedy rice during de-domestication. Nat Commun 8:15323. https://doi.org/10.1038/ncomms15323

Rasori A, Ruperti B, Bonghi C, Tonutti P, Ramina A (2002) Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. J Exp Bot 53:2333–2339. https://doi.org/10.1093/jxb/er097

Reagan M, Thuerber CS, Gross BL, Olsen KM, Jia Y, Caicedo AL (2010) Genomic patterns of nucleotide diversity in divergent populations of U.S. weedy rice. BMC Evol Biol 10:180. https://doi.org/10.1186/1471-2148-10-180

Roschitztardtz H, Fuentes I, Vásquez M, Corvalán C, León G, Gómez I, Araya A, Holuiugue L, Vicente-Carabajao J, Jordana X (2009) A nuclear gene encoding the iron-sulfur subunit of mitochondrial complex II is regulated by b3 domain transcription factors during seed development in Arabidopsis. Plant Physiol 150(1):84–95. https://doi.org/10.1104/pp.109.136531

Ruduš I, Sasiak M, Kępczyński J (2012) Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene. Acta Physiol Plant 35:295–307. https://doi.org/10.1007/s10457-011-9576-9

Shi Z, Jiang Y, Han X, Liu X, Cao R, Qi M, Xu T, Li T (2017) SLP/NJ regulates auxin efflux to affect flower abscission process. Sci Rep 7:14919. https://doi.org/10.1038/s41598-017-15072-7

Subudhi PK, Singh PK, DeLeón T, Parco A, Karan R, Biradar H, Cohn MA, Sasaki T (2014) Mapping of seed shattering loci provides insights into origin of weedy rice and rice domestication. J Hered 105:276–287. https://doi.org/10.1038/jhered/est0089

Sugimoto K, Takeuchi Y, Ebana K, Miyao A, Hirochika H, Hara N, Ishiyama K, Kobayashi M, Ban Y, Hattori T, Yano M (2010) Molecular cloning of Sdr4, a regulator involved in seed dormancy and domestication of rice. Proc Natl Acad Sci USA 107:5792–5797. https://doi.org/10.1073/pnas.091165107

Sun J, Qian Q, Ma D-R, Xu Z-J, Liu D, Du H, Chen W (2013) Introgression and selection shaping the genome and adaptive loci of weedy rice in northern China. New Phytol 197:290–299. https://doi.org/10.1111/nph.12012

Sun J, Ma D, Tang L, Zhao M, Zhang G, Wang W, Song J, Li X, Liu Z, Zhang W, Xu Q, Zhou Y, Wu J, Yamamoto T, Dai F, Lei Y, Li S, Zhou G, Zheng H, Xu Z, Chen W (2019) Population genomic analysis and de novo assembly reveal the origin of weedy rice as an evolutionary game. Mol Plant 12:632–647. https://doi.org/10.1016/j.molp.2019.01.019
Suzuki M, Ketterling MG, Li QB, McCarty DR (2003) *Viviparous1* alters global gene expression patterns through regulation of abscisic acid signaling. Plant physiol 132(3):1664–1677. https://doi.org/10.1104/pp.103.022475

Thurber CS, Hepler PK, Caicedo AL (2011) Timing is everything: early degradation of abscission layer is associated with increased seed shattering in U.S. weedy rice. BMC Plant Biol 11:14. https://doi.org/10.1186/1471-2229-11-14

Tuan PA, Kumar R, Rehal PK, Toora PK, Ayele BT (2018) Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. Front Plant Sci 9:668. https://doi.org/10.3389/fpls.2018.00668

Wilmowicz E, Frankowski K, Kucko A, Swidzinski M, de Dios AJ, Nowakowska A, Kopcewicz J (2016) The influence of abscisic acid on the ethylene biosynthesis pathway in the functioning of the flower abscission zone in *Lupinus luteus*. J Plant Physiol 206:49–58. https://doi.org/10.1016/j.jplph.2016.08.018

Xie R, Pang S, Ma Y, Deng L, He S, Yi S, Lv Q, Zheng Y (2015) The *ARF*, *AUX/IAA* and *GH3* gene families in citrus: genome-wide identification and expression analysis during fruitlet drop from abscission zone A. Mol Genet Genom 290:2089–2105. https://doi.org/10.1007/s00438-015-1063-1

Xie R, Ge T, Zhang J, Pan X, Ma Y, Yi S, Zheng Y (2018) The molecular events of IAA inhibiting citrus fruitlet abscission revealed by digital gene expression profiling. Plant Physiol Biochem 130:192–204. https://doi.org/10.1016/j.plaphy.2018.07.006

Xing W, Pi Z, Li X, Zou Y, Wang M, Liu D, Wang Q, Wu Z (2020) Comparative transcriptome analysis reveals an ABA-responsive regulation network associated with cell wall organization and oxidation reduction in sugar beet. Plant Growth Regul 91:127–141. https://doi.org/10.1007/s10725-020-00592-6

Yang B, Cheng J, Wang J, Cheng Y, He Y, Zhang H, Wang Z (2019) Physiological characteristics of cold stratification on seed dormancy release in rice. Plant Growth Regul 89:131–141. https://doi.org/10.1007/s10725-019-00516-z

Yao N, Wang L, Yan H, Liu Y, Lu B (2015) Mapping quantitative trait loci (QTL) determining seed shattering in weedy rice evolution of seed shattering in weedy rice through de-domestication. Euphytica 204:513–522. https://doi.org/10.1007/s10681-014-1331-x

Yoon J, Cho LH, Kim SL, Choi H, Koh HJ, An G (2014) The BEL1-type homeobox gene *SH5* induces seed shattering by enhancing abscission-zone development and inhibiting lignin biosynthesis. Plant J 79:717–728. https://doi.org/10.1111/tpj.12581

Yoon J, Cho LH, Antt HW, Koh HJ, An G (2017) KNOX protein OSH15 induces grain shattering by repressing lignin biosynthesis genes. Plant Physiol 174:312–325. https://doi.org/10.1104/pp.17.00298

You C, Zhu H, Xu B, Huang W, Wang S, Ding Y, Liu Z, Li G, Chen L, Ding C, Tang S (2016) Effect of removing superior spikelets on grain filling of inferior spikelets in rice. Front Plant Sci 7:1161. https://doi.org/10.3389/fpls.2016.01161

Zhao C, Xu W, Song X, Dai W, Dai L, Zhang Z, Qiang S (2017) Early flowering and rapid grain filling determine early maturity and escape from harvesting in weedy rice. Pest Manage Sci 74:465–476. https://doi.org/10.1002/ps.4730

Zhou Y, Lu D, Li C, Luo J, Zhu BF, Zhu J, Shangguan Y, Wang Z, Sang T, Zhou B, Han B (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor shattering abortion1. Plant Cell 24:1034–1048. https://doi.org/10.1105/tpc.111.094383

Zhu Y, Ellstrand NC, Lu BR (2012) Sequence polymorphisms in wild, weedy, and cultivated rice suggest seed-shattering locus *sh4* played a minor role in Asian rice domestication. Ecol Evol 2:2106–2113. https://doi.org/10.1002/ece3.128

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.