

Mini-Review

Abscisic acid and the pre-harvest sprouting in cereals

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Pre-harvest sprouting (PHS) leads to loss of grain weight and a reduction in the end use quality of kernels in cereals, especially in wheat, and PHS in rice also becomes a more and more serious problem recent years. Many factors are involved in the controlling this complex trait. Only recently, we have reported the large scale screening and characterisation of the rice *phs* mutants, providing insight into the molecular mechanism of pre-harvest sprouting in rice. It has been shown that mutations of genes in synthesis of the carotenoid precursors of ABA resulted in the pre-harvest sprouting, which is consequence of ABA deficiency, and photobleaching is likewise due to the absence of photoprotective carotenoids. The further study of all different rice *phs* mutants will help us to elucidate the complex phenomena and finally capture the target for improving PHS in rice or other cereals.

Introduction

The phenomenon of germination of physiologically mature cereal grains in the ear or panicle, usually under wet conditions shortly before harvest, is termed as pre-harvest sprouting (PHS) or vivipary. PHS occurs in many cereal crops such as wheat, barley, maize, and rice in most region of the world. PHS not only causes reduction of grain yield, but also affects the quality of grains, resulting into significant economic losses.

During seed formation, embryo development can proceed through a maturation phase that allows the entry into a quiescent state, characterized by acquisition of desiccation tolerance, growth arrest and the entry into a dormancy period of variable length that is broken upon germination.¹ It is known that the plant hormone abscisic acid (ABA) is strongly involved in this process, but the mechanism is still not fully understood. It was found that ABA levels are low during embryogenesis, increase during the maturation phase, and then decrease when seed desiccation. So far a number of mutants with reduced capacity to synthesize ABA have been described, such as *f1c*, *not* and *sit* in tomato; *aba1* in *Nicotiana plumbaginifolia*; *aba1*, *aba2* and *aba3* in *Arabidopsis*; and viviparous mutants *Vp5*, *Vp7*, *Vp10/Vp13*, *Vp14*, *Vp15* in maize.²⁻⁴ Most viviparous mutants in

maize were blocked in biosynthesis of the carotenoid precursors for de novo ABA synthesis; these mutants produce albino or pale green, non-viable seedlings.⁵ However, some mutants in *Arabidopsis* involved in the carotenoid biosynthetic pathway did not lead to precocious germination.⁶⁻⁸ In rice, relatively few *phs* mutants have been reported so far, which only two genes related to vivipary were cloned, without further detail study.⁹ Recently, we have carried out an intensive screening of the rice mutant population and successfully isolated 27 *phs* mutants under the high humidity paddy fields.¹⁰ This review focuses on more recent studies of *phs* or viviparous mutants, and discusses the complex regulation of ABA synthesis and its physiological role in seed dormancy and germination.

Viviparous or Pre-Harvest Sprouting Mutants in Crops

Since pre-harvest sprouting in wheat is very intricate, most viviparous genes were identified through isolation of the mutants from other cereal crops, especially in maize. At least seven viviparous genes in maize including *Vp1*, *Vp5*, *Vp7*, *Vp8*, *Vp10/Vp13*, *Vp14*, *Vp15* were cloned.²⁻⁴ The *Vp5* and *Vp7* genes encode enzymes in the carotenoid biosynthetic pathway, and the mutants showed an albino phenotype with a reduced ABA level.^{11,12} The *Vp14* gene, on the other hand, is blocked in the first committed step in ABA biosynthesis, cleavage of epoxy-carotenoids to xanthoxin.^{13,14} The *Vp10/Vp13* and *Vp15* encode the enzymes in the molybdenum cofactor biosynthesis, the final step for ABA biosynthesis, oxidation of ABA-aldehyde to ABA by an aldehyde oxidase which requires molybdenum cofactor (MoCo). *Vp1* encodes B3 domain transcription factors, which controls multiple developmental responses associated with the maturation phase of seed formation.^{15,16} *Vp8*, encoding a putative altered meristem program1-like peptidase, regulates ABA accumulation and coordinates embryo and endosperm development.¹⁷ In addition, some other mutant loci in maize were identified in the carotenoid biosynthetic pathway, such as *Y1*, *Vp2*, *W3*, *Vp9* and *Y9*.^{11,18-20}

Carotenoids and *phs* Mutant in Rice

Recently we have identified a series of rice *phs* mutants simply categorized into three groups based on phenotypes besides vivipary, and the genes in four different loci were cloned in category I, that are all located in the carotenoid biosynthetic pathway.¹⁰ Unlike the viviparous mutants of maize, which accumulate carotenoids mainly in the endosperm, the rice counterparts accumulated carotenoids in seedling or embryo. It is thought that PSY, catalyzing the first

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committed step in carotenogenesis, is rate-limiting at least in many non-green tissues.²¹ There are three PSYs in rice, which OsPSY1 and OsPSY2 involved in carotenoid biosynthesis in photosynthetically active tissues, while OsPSY3 is devoted to abiotic stress-induced ABA formation. Interestingly, the transcripts for all three PSYs were not detected in rice endosperm.²² Therefore, the carotenoids do not accumulate in the endosperm of rice *phs* mutants. To this end, it is not surprising that we didn't identify any *psy* mutants in our large scale screening for *phs* mutants simply due to gene redundancy.

Carotenoids are integral and essential components of the photosynthetic membranes in all plants. In the chloroplast, they function in the protection against photo-oxidative damage and participate in the light harvesting process.²³ The rice *phs* mutants in the carotenoids biosynthetic pathway showed chloroplast damages, and *phs1*, *phs2* and *phs4* mutants are albino and lethal. Interestingly, the *phs3* mutant can survive and showed 'variegated' leaf at the tillering stage and completely leaf photobleaching during grain filling. The β -OsLCY RNAi plants also showed photobleaching leaf, and the levels of some PS II core proteins decreased in the plants.¹⁰ These results are consistent with the conclusion that carotenoids protect against oxidative damage.

ABA Biosynthesis and Pre-Harvest Sprouting

ABA is involved in several specific processes during seed development, such as the deposition of storage reserves, induction of primary dormancy. Evidence for the role of ABA in such processes has come from ABA-deficiency or -response mutants in Arabidopsis or maize.²⁴ Mutations in ABA biosynthesis fail to induce seed dormancy and exhibit a vegetative wilted phenotype, such as Arabidopsis *aba1* and tobacco *aba2* are known to be impaired in ZEP (zeaxanthin epoxidase), the first enzyme identified as an ABA biosynthetic enzyme;²⁵ The Arabidopsis ABA-deficient mutant *aba4* was recently identified in a screening for paclobutrazol-resistant germination and showed impairment in NSY (neoxanthin synthase).²⁶ The maize *viviparous14* (*vp14*) and tomato *notabilis* mutants are shown to be defective in NCED, 9-cis-epoxycarotenoid dioxygenase catalyzing the oxidative cleavage of xanthophylls, 9-cis-violaxanthin and/or 9'-cis neoxanthin to produce xanthoxin.^{14,27} Arabidopsis *aba2* and *aba3*, maize *vp10* and *vp15*, and tomato *flacca* and *sitiens* are typical mutants impaired in the later steps of ABA biosynthesis in the cytosol.²⁸⁻³¹ By comparison, ABA-deficiency or -response mutants in rice were scarcely identified. At present we have cloned other three genes from *phs* mutants besides *PHS1-PHS4* genes, and they are all involved in specific ABA biosynthetic pathway. Further detailed characterization with these mutants is underway.

ABA Response and Pre-Harvest Sprouting

Thus far, our knowledge on the signaling elements that mediate the regulation of seed dormancy and germination by ABA is primarily derived from genetic analysis. The maize *Vp1* locus was the first cloned gene in ABA response, and has been studied in detail.¹⁶ *Vp1* is a multidomain transcription factor that functions as both an activator and a repressor depending on the promoter

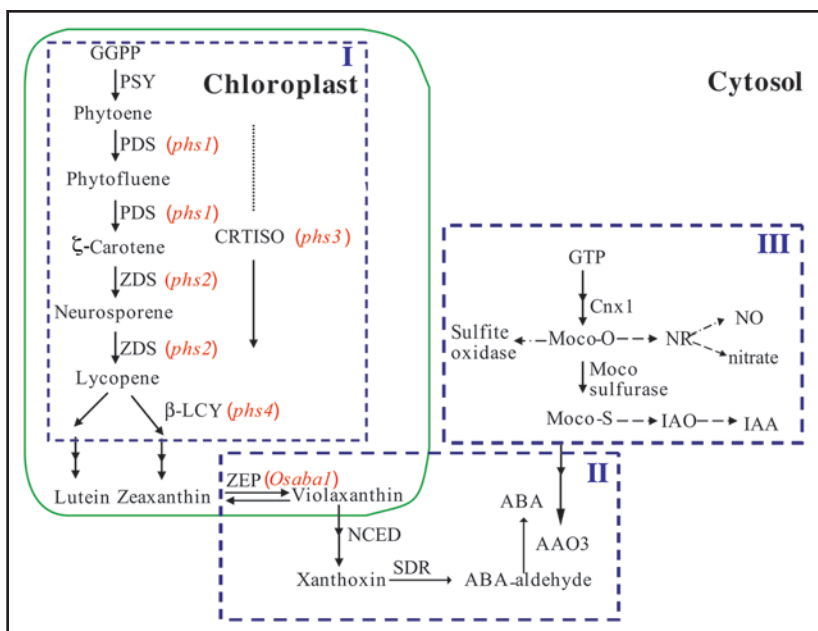


Figure 1. Pre-harvest sprouting mutants in rice localized in carotenoid and abscisic acid biosynthetic pathway. (I) Carotenoid precursor synthesis in the early steps of ABA biosynthesis. (II) Specific ABA biosynthetic pathway. (III) Molybdenum cofactor biosynthesis, molybdenum cofactor is a factor for active AAO3. GGPP, Geranylgeranyl pyrophosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ -carotene desaturase; β -LCY, lycopene β -cyclase; ZEP, zeaxanthin epoxidase; NCED, 9-cis-epoxycarotenoid dioxygenase; SDR, short-chain dehydrogenase/reductase; AAO3, Abscisic aldehyde oxidase 3; IAO, Indole-3-acetaldehyde oxidase; NR, Nitrate reductase; Cnx1, cofactor for nitrate reductase and xanthine dehydrogenase 1.

context.¹⁵ Interestingly, the missplicing of wheat *Vp1* genes and rice *Vp1* counterpart contributes to susceptibility to PHS in modern hexaploid wheat varieties and the sprouting susceptible rice varieties, respectively.^{32,33} *ABI3* is orthologous gene of *VP1* from Arabidopsis, *vp1* and *abi3* seeds share similar phenotypes including insensitivity to ABA, desiccation intolerance and premature activation of the shoot apical meristem.³⁴ Mutations in the *ABI4* and *ABI5* loci have similar *ABI3* qualitative effects on seed development and ABA sensitivity, but null mutations in *ABI3* are more severe than those in *ABI4* or *ABI5*.³⁵

In addition, several other loci in Arabidopsis have been identified that specifically affect seed maturation and germination but do not appear to be directly related to hormone synthesis or signalling. The leafy cotyledon (*LEC*) class genes, including *LEC1*, *LEC2* and *FUS3*,³⁶ play key regulatory roles in Arabidopsis affecting important traits of the maturation phase during seed development and the establishment and maintenance of dormancy.¹ However, very few information about the homologous genes in cereals was reported, which are worth for further studying.

Conclusions and Perspectives

Recent advances in ABA biosynthesis research have yielded substantial information on the pathways, genes and enzymes involved in the process.³⁷ We have learned that ABA is the major hormone involved in induction and maintenance of dormancy by pre-harvest sprouting or viviparous mutants in rice. Based on these results, the *phs* mutants in cereals are involved within three parts in the ABA biosynthesis pathway (Fig. 1). Part I represents carotenoids

biosynthesis, maize *vp5*, *vp7* and rice *pbs1-psb4* are localized in this part. Part II represents specific ABA biosynthesis, this part mutants include maize *vp14*, rice *Osaba1*. Part III is involved in molybdenum cofactor biosynthesis, maize *vp10/vp13* and *vp15* are in that part. We are going to identify the *pbs* mutants involved in all three parts. Identification of downstream targets of ABA and the genes that regulate ABA biosynthesis will help us to gain deep understanding on the dormancy mechanisms of cereals, and how ABA represses germination and prevent pre-harvest sprouting in crops.

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