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Review

Flooding response in rice: Ethylene networks and sugar signaling

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Rice (*Oryza sativa*) is an aerobic organism, for which oxygen shortage poses a serious problem. Longtime complete submergence leads to damage or even death of the plant. Submergence-tolerant rice adapts to flood (complete submergence) in a long time. Ethylene regulation networks and sugar signaling are involved in the submergence response of rice. This is mainly through the regulation of many key genes such as *SUB1A* and *SK1/2*; therefore, the level of abscisic acid (ABA) and gibberellic acid (GA) was regulated. This paper reviews these two signaling pathways, understanding which can help us develop new varieties and improve existing cultivars.

Key words: Rice, ethylene regulation networks, sugar signaling, submergence.

INTRODUCTION

More than half of the world's population live on rice (*Oryza sativa*), especially in southeastern and eastern Asia, and it provides a large part of calories consumed every year (Hadiarto and Tran, 2011). Although, the world's total production of rice demonstrates annual increase, the intensively increasing world population has put a great burden on the production of rice. Flooding, submergence and water logging stress is one of the important abiotic stress factors which adversely affect the distribution and productivity of rice crop. The onset of flooding could lead to the condition of anaerobiosis or oxygen deprivation (partial or complete) as the diffusion of oxygen through water is 10^4-fold slower than in air. Furthermore, excessive water can also induce other changes in the soil that influence plants: hormone ethylene, products accumulation of anaerobic metabolism by soil micro-organisms, and the availability of shoots to carbon dioxide, light and oxygen typically diminished.

According to the ecological difference, rice cultivars can be divided into three groups: Upland rice, Lowland rice, and Deepwater or floating rice (Sauter, 2000). Deepwater rice is grown predominantly in low-lying areas of Southeast Asia which are flooded every year during the rainy season; it can avoid submergence stress by rapidly growing above the water surface and therefore restoring gas exchanges. In contrast, submergence-tolerant rice is well adapted to low oxygen supply in a complete submergence environment for 10 to 14 days, after the water subsides, the growth renew (Metraux and Kende, 1983; Sauter, 2000).

Stunt or elongated rice undergoes two opposite strategies to adapt to two kinds of flood respectively: deepwater flood, which occurs over a long period and involves deep water, and flash flood, which occurs over a short period and involves shallow water (Nagai et al., 2010). In both conditions, the ethylene which regulates many aspects of plant growth, development and senescence plays a very important role (Sauter et al., 2005). Ethylene has been identified as a key factor for initiating the internodes elongation which is the most important strategy taken by deepwater rice under the condition of deepwater flood. Many flood response genes are confirmed to be ethylene responsive (Van Der Straeten et al., 2001; Arumugam Pillai et al., 2002; Sauter et al., 2002; Zhou et al., 2002; Qi et al., 2004; Watanabe et al., 2004). Among those, several plant proteins containing ethylene responsive factor (ERF) domains (so called ERF factors) are indentified as the regulators of the abiotic and biotic stress responses (Gutterson and

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Reuber, 2004; McGrath et al., 2005). Furthermore, sugar signaling is also proved to be involved in flood recently (Chen et al., 2006). This article distinguishes itself by concentrating on ethylene reputation networks and sugar signaling involved in flood.

REGULATION OF ETHYLENE BIOSYNTHESIS UNDER THE PRESSURE OF FLOOD

The initial signal for flood response is the reduced partial pressure of oxygen in the submerged internodal tissues (Raskin and Kende, 1984). Both stunt and elongating strategies are the result of an interaction among three plant hormones: ethylene (ET), abscisic acid (ABA) and gibberellins (GAs). Ethylene causes a reduction in the level of abscisic acid, which is an antagonist of gibberellins action; and gibberellins are the ultimate hormone that promotes internodal growth (Hoffmann-Benning and Kende, 1992; Mekhedov and Kende, 1996; Olszewski et al., 2002). During the submergence period, the concentration of the ethylene increases more than 50 folds in the internodes of the deepwater rice. This is accomplished by not only the biosynthesis of ethylene from 1-aminocyckopopane-1-carboxylic acid (ACC) but also the trap of the ethylene in the internodes of the submergence part of the plant (Metraux and Kende, 1983; Zarembinski and Theologis, 1997). As summarized in Figure 1, biosynthesis of ethylene from ACC is regulated by ACC oxidase 1(AOC1) and ACC synthase 1/2/5 (OS-ACS1/2/5), besides, the high and continuous rates of ethylene synthesis also need the acireductone dioxygenase 1 (OsARD1) and the cross-talking with methioine cycle (Sauter et al., 2005).

OsARD1 is a metal-binding protein that belongs to the cupin superfamily. The mRNA levels showed a rapid, early and transient increase upon flood after treatment with ethylene-releasing compounds. As *OsARD1* transcripts accumulated in the presence of cycloheximide, an inhibitor of protein synthesis, *OsARD1* is probably a primary ethylene response gene. It is suggested that the early feedback activation methinoine cycle by low levels of ethylene is medicated by OsARD1 (Sauter et al., 2005), and ethylene controls *OsARD1* expression possibly through an EIN3-like transcription factor.

In rice, ACC oxidase contains six genes (*ACO1 to 5* and *7*) and one pseudogene (*ACO6*) (Iwai et al., 2006). The *ACO1* expression was localized in the basal parts of leaf sheaths immediately above nodes or the lower parts of elongating internodes as confirmed by in situ hybridization. At the heading stage, transcript of *ACO1* significantly accumulated in lower parts of elongating internodes. It is suggested that ACO1 effects on internode elongation at the heading stage in rice (Mekhedov and Kende, 1996; Iwamoto et al., 2010).

Partial submergence induces the expression of *OS-ACS1* and inhibits the expression of *OS-ACS2*. OS-ACS5 promotes rapid elongation growth of deepwater rice by contributing to the initial and long-term increase in ethylene levels (Van Der Straeten et al., 2001). Tissue localization of a submergence-induced *OS-ACS5* demonstrated that the expression of *OS-ACS5* in vascular bundles of young stems and leaf sheaths was strongly induced by complete submergence compared with air-grown rice seedlings (Zhou et al., 2002).

ETHYLENE REGULATION NETWORKS UNDER THE PRESSURE OF FLOOD

As summarized in Figure 1, the ethylene response factors SUB1A and SNORKEL1/2, which are in the downstream of ethylene network, have crucial function in response to flood in rice (Bailey-Serres and Voesenek, 2010). The understanding of their regulator/targeted genes will facilitate the understanding of the flood response.

Submergence-1 (Sub1) is a major quantitative trait locus affecting submergence tolerance in lowland rice. In submergence-intolerant japonica cultivar M202, this locus encodes two ERF genes, *Sub1B* and *Sub1C*. Relatively, in the tolerant near-isogenic line indica FR13A, M202 (Sub1), the locus additionally encodes the ERF gene *Sub1A*. Although, both of the *sub1a*/*sub1c* mRNA increases obviously after submergence, the *sub1c* mRNA seems to be more sensitive in intolerant cultivar. Hence, rapid induction of *Sub1A* may limit the expression of *Sub1C* (Fukao et al., 2006). It is suggested that Sub1A is one of the genetic determinants controlling submergence tolerance.

The *Sub1A* gene encodes a 281-amino-acid protein containing ERF domain and it belongs to B-2 subgroup of ERF. The submergence intolerant cultivar M202 introgressed with Sub1A shows more submergence tolerant compared with M202 (Perata and Voesenek, 2007). This is mainly due to the cross-talking between the gibberellin and ethylene. Ethylene causes internodal elongation in rice by increasing the activity of endogenous GAs (Raskin and Kende, 1984), and stimulates the expression of *Sub1A* in the condition of complete submergence. *Sub1A* increases the accumulation of the GA signaling repressors, Slender Rice-1 (SLR1) and SLR1 Like-1 (SLRL1), and concomitantly diminishes GA-inducible gene expression. This will block the function of GA on shoot elongation. Meanwhile, ethylene promotes GA responsiveness and shoots elongation in submergence-intolerant lines. Hence, Sub1A limits ethylene-promoted GA responsiveness during submergence by augmenting accumulation of the GA signaling repressors SLR1 and SLRL1 (Fukao and Bailey-Serres, 2008).

Besides the Sub 1A, Quantitative Trait Loci (QTL) analysis combined with positional cloning indicates that there are three major QTLs for deepwater response on chromosomes 1, 3 and 12 (Nemoto et al., 2004).

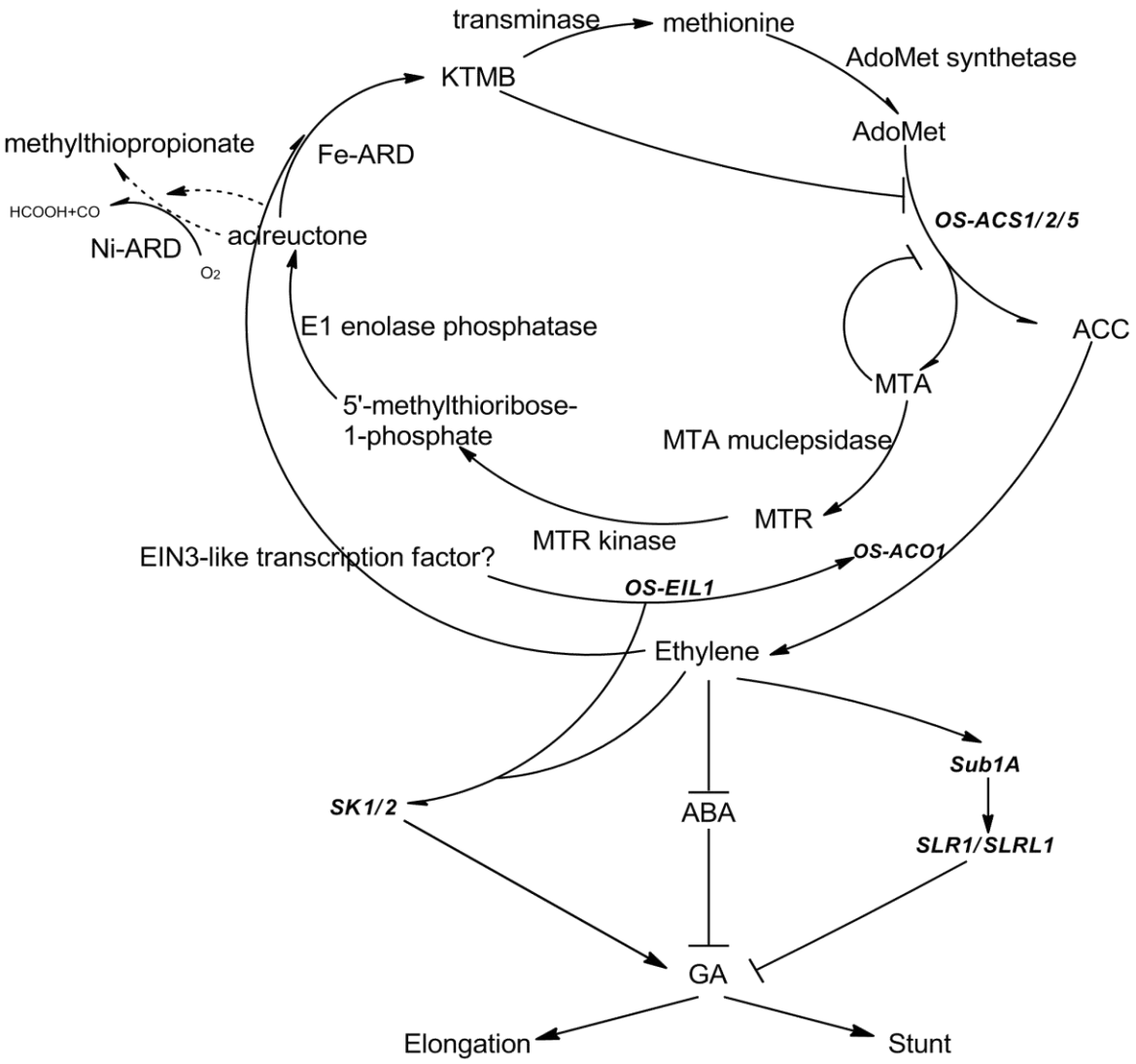

Figure 1. The overview of ethylene signaling pathway in response to submergerce.

SNORKEL1, SNORKEL2 (SK1, SK2) were first indentified in the QTL on chrome 12 by positional cloning and gain-of-function analysis. They possess a putative nuclear localization signal and localized to nuclei. All of them have a single APETALA2/ethylene response factor (AP2/ERF) domain. Except for the APETALA2/ethylene response factor domain, neither SK1 nor SK2 shows similarity to any other known genes (Fukao et al., 2006; Nakano et al., 2006; Xu et al., 2006). They are expressed in leaf blade, leaf sheath, and basal parts of the stem, including nodes and internodes, where the deepwater response occurs. Ethylene significantly up-regulated the expression of the *SK* genes which means SK1 and SK2 are responsive to ethylene (Hattori et al., 2009).

As ethylene accumulates in both deepwater and non-deepwater rice, the accumulated ethylene triggers the expression of the *SK* genes, leading to the induction of internode elongation in deepwater rice. And this induction is also medicated by GA, In the presence of the GA biosynthetic inhibitor uniconazole, internode elongation under deepwater conditions was repressed, while internode elongation was induced without uniconazole(Hattori et al., 2009).

SUGAR SIGNALING PATHWAY IN RESPONSE TO FLOOD

When flood occurs in the rice seeding period, sugar signaling also responds to the pressure of oxygen deficiency. In this condition, sugar in embryo is rapidly consumed, and as a result, α-amylase expression is up-regulated and the sugar is depleted in the scutellum between the embryo and endosperm (Chen et al., 2006). Meanwhile, the GAs is synthesized, which activates the synthesis and secretion of α-amylases and other

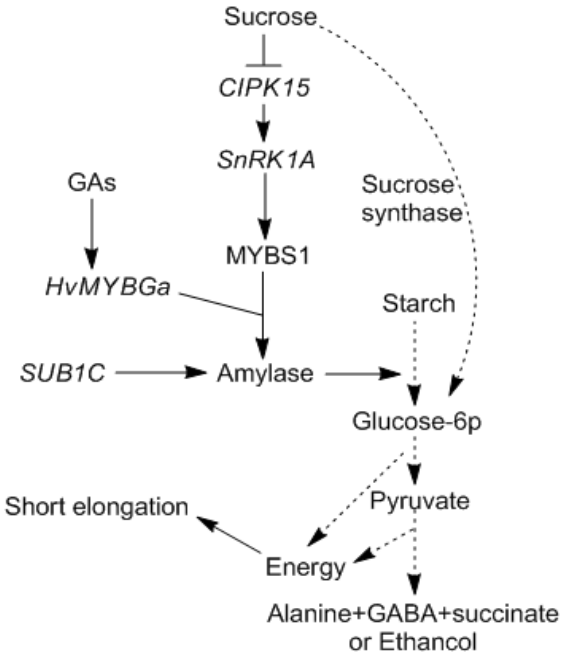

Figure 2. Overview of the sugar signaling pathway in response to submergerce.

hydrolases in aleurone cells surrounding the starchy endosperm. Under the promotion of those enzymes, energy produced in the digestion of starch and other nutrients stored in the endosperm to small molecules stored in the endosperm are taken up by scutellum and transported to the embryo to support seedling growth (Gubler, 1995). The mechanisms through which α-amylases are produced are widely studied. As demonstrated in Figure 2, CIPK15 plays a very important role in the sugar signaling pathway under the pressure of submergence.

Amylase is a key enzyme in sugar signaling, which regulates the abundance of eight amylase gene transcripts, some through the transcription rate, while the others through controlling the stability of their mRNAs (Sheu et al., 1996; Chan and Yu, 1998). The transcription factor MYBS1 is one of the three MYBs sharing conserved DNA binding domains (Sakura et al., 1989). MYBS1 binds to the TATCCA element which locates in-171 to-82 bp upstream of the α-amylase promoter and is essential for the high-level GA-activated transcription of α-amylase gene probably by forming a homodimer (Lu et al., 2002).

Genes encoding Snf1-related protein kinases (SnRK1s) are identified and characterized in several plant species, all of which play a very important role in sugar signaling. The SnRK1A expression in rice is regulated by sugars at the posttranscriptional level. In response to sugar starvation, the protein kinase SnRK1A regulates the interaction between MYBS1 and amylase sugar response complex (SRC). As a result, the expression of amylase is up-regulated in the condition of submergence. In this way, SnRK1A acts as a stress sensor (Lu et al., 2007).

The transmission of the signal from sucrose to SnRK1A is mediated by CIPK15. CIPK15 regulates the function of SnRK1A possibly though the direct interaction (Lee et al., 2009). CIPK15 belongs to the CBL-interacting protein kinases (CIPKs) group which containing a conserved 24–amino acid motif in the C-terminal nonkinase region that is sufficient and necessary for CIPK interaction with CBL, called the NAF (Asn-Ala-Phe) domain (Albrecht et al., 2001; Lee et al., 2009). This CBL-CIPK network were proved to participate in signaling pathways related to many stresses, including salt, drought, osmotic, cold, and ABA (abscisic acid) (Kolukisaoglu et al., 2004). CIPK15 controls sugar production required for seedling growth under submergence.

PERSPECTIVES

Rice is an important food crop and the only cereal which is well adapted to conditions of flooding. Moreover, rice is well suited for molecular and genetic analysis owing to the small genome size known gene sequence and the facile transformation mediated by *Agrobacterium*. Till this moment, many genes contribute to the flooding response but only a small number of genes were clarified clearly, and the small RNAs, which have functions in the stress response in plants, also have not been identified and verified. In the future, the crucial challenge is not only to identify more genes, small RNAs and clarify the mechanism during the flooding stress response, but also to illustrate the relationship and cross-talks among the ethylene, GA, ABA and sugar signaling pathway during response to the flooding stress in rice, as well as between the flooding stress process and the vegetal, developmental and other stresses responsive pathways in rice. Consequently, this research contributes to understanding the network and molecular mechanisms of the flooding stress responsive pathway in rice and may be useful in alleviating this agronomic problem in the breeding of rice.

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