

7 Flooding Tolerance in Plants

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Abstract

Global warming is associated with an increase in flooding events, making many ecosystems worldwide vulnerable to submergence. Water submersion can severely affect crop production, since it drastically reduces oxygen availability necessary for plants' respiration, thus survival. Plants tolerant to flooding evolved morphological, physiological and biochemical adaptations to oxygen deficiency. Recently, considerable progress has been made in terms of understanding the molecular aspects governing these responses. However, the upstream stress-sensing mechanism of oxygen shortage has not yet been fully understood and many of the systems described in bacteria, fungi and animals have been excluded for plants. Many studies on oxygen deprivation stress have focused on rice (*Oryza sativa*), since it is one of the crops better adapted to a flooded environment. Beside being able to germinate under submergence, rice varieties display different mechanisms for a successful survival. Agronomically, the study of rice strategies to survive flooding in ecotypes adapted to extreme environments shows big potential in the context of climate change and the worldwide increasing need for food.

7.1 Introduction

Many ecosystems worldwide are vulnerable to flooding, either progressive or rapid, such as areas close to watercourses or exposed to monsoons. Man-made flooding can also occur, as in paddy fields where rice, which is well adapted to flooded environments, is cultivated. Global warming is associated with an increase in flooding events characterized by their unexpected occurrence, regimes and localization. In addition, the increase in the global demand for food requires a large increase in crop yields, especially in marginal agricultural areas. Unusual water submersion resulting in excessively wet or flooded soils can severely affect crop production coupled with a modification in plant distribution in natural ecosystems (Bailey-Serres and Voesenek, 2008).

Plants are aerobic organisms and need oxygen (O_2) to survive, and thus suffer severely from O_2 deprivation. Water submersion drastically reduces O_2 availability since it diffuses slowly in water, dropping to concentrations that can restrict aerobic respiration. Water is a remarkable barrier to general gas diffusion, leading to concomitant phenomena such as ethylene entrapment in submerged organs and, depending on the light conditions, increased CO_2 levels (Greenway *et al.*, 2006). A flooded environment can also suffer from low light, thus reducing photosynthesis, and from high concentrations of toxic soil compounds (Bailey-Serres and Voesenek, 2008). Consequently, an underwater scarcity of O_2 alone may not always be the major cause of injuries due to submergence, but a combination of different types of stress eventually leading to restrictions in ATP synthesis and carbohydrates.

Crop plants are usually very sensitive to submergence, thus the need for new genetic and agronomic strategies to increase production in flood-prone regions.

Studies on O₂ deprivation stress have often focused on flooding-tolerant species such as rice (*Oryza sativa*). The characterization of tolerance-related molecular traits could represent a major step towards breeding tolerant varieties (Bailey-Serres and Voesenek, 2010). However, rice ecotypes vary considerably in their responses to flooding, and only a limited number of varieties can withstand more than 2 weeks of complete submergence (Xu *et al.*, 2006). Adaptive responses to specific water regimes and unexploited resources of wild rice relatives seem to offer agriculturally important perspectives that have the potential to mitigate the environmental adversity aggravated by climate change (Brar and Khush, 1997; Voesenek *et al.*, 2004).

While initial efforts were aimed at describing morphological, physiological and biochemical aspects of plant adaptation to O₂ deficiency, considerable progress has recently been made in terms of understanding the molecular aspects governing these responses. Plant adaptation to submergence has been classified into two main strategies: the Low Oxygen Quiescence Syndrome (LOQS) and the Low Oxygen Escape Syndrome (LOES) (Colmer and Voesenek, 2009). A major feature of the LOQS syndrome is the reduction of underwater shoots growth, to conserve substrate availability until the water recedes. Instead, plants showing LOES are characterized by fast underwater shoot organs to reach the water surface and re-establish a gas exchange (Colmer and Voesenek, 2009). The genetic traits behind the LOQS and LOES have partially been understood in lowland rice (Fukao *et al.*, 2006; Xu *et al.*, 2006) and deepwater rice varieties (Hattori *et al.*, 2009), respectively.

This chapter focuses on the major metabolic problems caused by flooding stress, thus low O₂ in plants, and discusses the major findings related to the various mechanisms of adaptation. The different strategies

to survive O₂ deprivation in rice are then discussed in detail, in order to give an overview of the most recent and notable findings regarding the molecular aspects behind LOQS and LOES responses.

7.2 Plant Metabolism Under Low Oxygen

Oxygen is the final acceptor of electrons in the mitochondrial respiratory chain of plants. This is the last step in plant respiration that leads to ATP synthesis, which is necessary for all ATP-demanding processes, such as DNA synthesis and cell division (Gibbs and Greenway, 2003). With an O₂ deficit, a tight regulation of ATP production occurs through the shift of respiration from aerobic to anaerobic. This change relies primarily on glycolysis and fermentation to generate ATP and regenerate NAD⁺ to sustain glycolysis, respectively. However, because of a glycolysis inefficiency, an energy crisis ensues. Fermentation yields only 2–4 mol ATP per mol hexose compared to 30–36 mol ATP produced by aerobic respiration. Early reports described an increase in glycolytic ATP production under complete anoxia, named the ‘Pasteur effect’ (Neal and Girton, 1955; Vartapetian, 1982). However, under complete anoxia energy production can vary between 3 and 37.5% when compared to production in normoxic conditions (Licausi and Perata, 2009). This variation depends not only on the efficiency of the glycolytic flux, but also on the amount of polysaccharides (starch or sucrose) available for use as a substrate and/or the plant’s capacity to catabolise them (Gibbs and Greenway, 2003).

Under anaerobic conditions, a short initial lactic and a long-lasting ethanol fermentation occur after glycolysis (Perata and Alpi, 1993). Lactic fermentation is a one-step reaction from pyruvate to lactate, catalysed by lactate dehydrogenase (LDH) with the regeneration of NAD⁺. Ethanol fermentation is a two-step process regenerating NAD⁺ in which pyruvate is first decarboxylated to acetaldehyde by pyruvate

monitored in many plants under low O_2 , the results suggest that activation of ethanolic fermentation is one of the strategies for plants to survive under anoxia (Table 7.1).

An alternative hypothesis of the switch from lactic to ethanolic fermentation relies on the 'PDH/PDC stat'. This suggests a rise in pyruvate (subsequent to the block of aerobic respiration under O_2 deprivation), which becomes available for the PDC reaction, PDC having a higher K_m than pyruvate dehydrogenase (PDH) (Tadege *et al.*, 1999). Manipulation of the ethanol fermentation pathway suggested that PDC is the metabolic control point of the alcohol fermentation pathway. While *Arabidopsis* plants overexpressing *ADH1* did not show any increase in plant survival and a very small increase in ethanol concentration under low O_2 , *PDC1* and *PDC2* overexpressing plants improved in flooding tolerance and showed an approximate 50–150% increase in ethanol production (Ismond *et al.*, 2003). However, *ADH1* null mutants displayed a decreased tolerance, suggesting that a normal level of *ADH1* expression is critical to plant survival (Ismond *et al.*, 2003).

Other minor end-products of the anaerobic metabolism were also reported. Alanine is one of the amino acids that accumulates under anaerobic conditions (Ricard *et al.*, 1994). It is produced by a transamination from glutamate to pyruvate, via the action of alanine aminotransferase (AlaAT) to give 2-oxyglutarate (Streeter and Thompson, 1972; Reggiani *et al.*, 1988; Vanlerberghe *et al.*, 1990). Besides alanine, increases in gamma amino butyric acid (GABA) was also observed (Reggiani, 1999). Amino acid oxidation may help to minimize the decrease in cytosolic pH and reduce carbon loss via ethanol or lactate (Bailey-Serres and Voeselek, 2008). Since glutamate is the common precursor of both alanine and GABA, it is believed to play a central role in anaerobic amino acid metabolism. This compound was found to decrease after 2 h of anoxia in *Arabidopsis* (Branco-Price *et al.*, 2008). There are several possible sources of glutamate: the assimilation of ammonia by the glutamine

synthetase/glutamate synthase (GS/GOGAT) cycle (Reggiani *et al.*, 1988), and through glutamate dehydrogenase (GDH) (Fan *et al.*, 1997). The observation that the GS/GOGAT cycle needs 1 ATP and 1 NAD(P)⁺ while the GDH way regenerates 1 NAD(P)⁺, seems to favour the latter route of glutamate generation (Gibbs and Greenway, 2003).

7.3 The Morphological Adaptation of Plants to Oxygen Deprivation

Plant strategies to survive low O_2 are mainly represented by LOES and LOQS. While LOES has been described in many plants, LOQS has only been described in lowland tolerant rice varieties belonging to the *indica* subfamily (Xu *et al.*, 2006) and a few rice relatives (Niroula *et al.*, unpublished). The promotion of shoot elongation by submergence, which is part of the LOES, is known to occur in wetland and amphibious species over a wide taxonomic range, e.g. *Rumex palustris*, *Ranunculus sceleratus*, *Nymphoides peltata*, *Potamogeton pectinatus* and *P. distinctus* (Ishizawa *et al.*, 1999; Summers *et al.*, 2000; Sato *et al.*, 2002; Mommer *et al.*, 2005). Elongation is spectacular in the internodes of deepwater rice, which rapidly elongate under submergence (Hattori *et al.*, 2009).

Plants display several adaptive traits to ensure survival under low O_2 , often associated with LOES. These acclimative responses are genotype specific and include altered petiole/internode elongation rates, cell ultrastructure modifications, the development of lateral and adventitious roots and the formation of aerenchyma, together with the metabolic adaptations that were described in the previous section. These traits vary in importance depending on the water regimes and extent of submergence (Colmer and Voeselek, 2009).

Aerenchyma is hypothesized to be of fundamental importance under many flooding regimes (Colmer and Voeselek, 2009). It is characterized by intercellular

Table 7.1. Effect of anoxia on ethanol and acetaldehyde concentration in different plants under O₂ deprivation.

Experiment	Species		Ethanol	Acetaldehyde	Reference
24 h incubation in anaerobic conditions	Woody plant leaves	<i>Quercus alba</i>	175±76 ng ml ⁻¹ headspace	35±9 ng ml ⁻¹ headspace	Kimmerer and MacDonald (1987)
		<i>Liquidambar styraciflua</i>	241±97 ng ml ⁻¹ headspace	53±26 ng ml ⁻¹ headspace	
		<i>Fraxinus americana</i>	227±110 ng ml ⁻¹ headspace	26±8 ng ml ⁻¹ headspace	
		<i>Fraxinus pennsylvanica</i>	188±117 ng ml ⁻¹ headspace	32±25 ng ml ⁻¹ headspace	
		<i>Populus deltoides</i>	187±43 ng ml ⁻¹ headspace	130±63 ng ml ⁻¹ headspace	
4 h anoxia	Tomato roots (<i>Lycopersicon esculentum</i> M., var UC 82b)		226±13 ng ml ⁻¹ headspace	10±10 ng ml ⁻¹ headspace	Germain <i>et al.</i> (1997)
			0 nmol mg ⁻¹ FW +100 mM suc: ±0.2 nmol mg ⁻¹ FW +100 mM glu: ±13 nmol mg ⁻¹ FW		
anoxia and hypoxia	Rice coleoptiles (<i>O. sativa</i> L.)	cv. Calrose	3 days air + 2 days anoxia:		Gibbs <i>et al.</i> (2000)
			0.21±0.02 µl g ⁻¹ FW min ⁻¹		
		3 days hypoxia + 3 days anoxia:	0.20±0.01 µl g ⁻¹ FW min ⁻¹		
		5 days anoxia:	0.11±0.01 µl g ⁻¹ FW min ⁻¹		
cv. IR22	3 days air + 2 days anoxia:	0.09±0.01 µl g ⁻¹ FW min ⁻¹			
	3 days hypoxia + 3 days anoxia:	0.07±0.00 µl g ⁻¹ FW min ⁻¹			
14 h anaerobic conditions in the dark	Rice seedlings (<i>O. sativa</i> L.)	cv. FR13A, sub-tolerant	40±9 µl g ⁻¹ FW h ⁻¹	0.9±0.13 µl g ⁻¹ FW h ⁻¹	Boamfa <i>et al.</i> (2003, 2005)
		cv. CT6241, sub-intolerant	39±6 µl g ⁻¹ FW h ⁻¹	1.40±0.3 µl g ⁻¹ FW h ⁻¹	
12 h anaerobic conditions in the light	Rice seedlings (<i>O. sativa</i> L.)	cv. FR13A, sub-tolerant	8±0.9 µl g ⁻¹ FW h ⁻¹	0.05±0.013 µl g ⁻¹ FW h ⁻¹	
		cv. CT6241, sub-intolerant	11±1 µl g ⁻¹ FW h ⁻¹	0.10±0.02 µl g ⁻¹ FW h ⁻¹	

63 days of anoxic incubation	<i>Acorus calamus</i>	Roots	$\pm 80 \mu\text{mol g}^{-1} \text{FW}$	Schluter and Crawford (2001)
		Rhizome	$\pm 20 \mu\text{mol g}^{-1} \text{FW}$	
		Leaves	$\pm 10 \mu\text{mol g}^{-1} \text{FW}$	
	<i>Iris pseudachoris</i>	Roots	$\pm 60 \mu\text{mol g}^{-1} \text{FW}$	
		Rhizome	$\pm 20 \mu\text{mol g}^{-1} \text{FW}$	
		Leaves	$\pm 20 \mu\text{mol g}^{-1} \text{FW}$	
48 h anoxia	Rice coleoptiles (<i>O. sativa</i> L.)	cv. Leulikelash	$\pm 500 \text{ nmol coleoptile}^{-1}$	Kato-Noguchi and Morokuma (2007)
		cv. Asahimoki	$\pm 500 \text{ nmol coleoptile}^{-1}$	
		cv. Nipponbare	$\pm 1200 \text{ nmol coleoptile}^{-1}$	
		cv. Yukihikari	$\pm 1200 \text{ nmol coleoptile}^{-1}$	
4 h anaerobic conditions in the light	Rice seedlings (<i>Oryza sativa</i> L. cv. Cigalon)	root: $\pm 8 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 20 \mu\text{l g}^{-1} \text{FW h}^{-1}$	root: $\pm 0.25 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 0.30 \mu\text{l g}^{-1} \text{FW h}^{-1}$	Mustroph <i>et al.</i> (2006a)
	Wheat seedling (<i>Triticum aestivum</i> L. cv. Alcedo)	root: $\pm 1 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 0 \mu\text{l g}^{-1} \text{FW h}^{-1}$	root: $\pm 0.075 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 0.075 \mu\text{l g}^{-1} \text{FW h}^{-1}$	
4 h anaerobic conditions in the dark	Rice seedlings (<i>Oryza sativa</i> L. cv. Cigalon)	root: $\pm 0.15 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 2.5 \mu\text{l g}^{-1} \text{FW h}^{-1}$	root: $\pm 3 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 50 \mu\text{l g}^{-1} \text{FW h}^{-1}$	Mustroph <i>et al.</i> (2006b)
	Wheat seedling (<i>Triticum aestivum</i> L. cv. Alcedo)	root: $\pm 0.12 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $\pm 0.5 \mu\text{l g}^{-1} \text{FW h}^{-1}$	root: $\pm 2 \mu\text{l g}^{-1} \text{FW h}^{-1}$ shoot: $0 \mu\text{l g}^{-1} \text{FW h}^{-1}$	
After 4 h anoxic incubation	Wheat seedlings root (<i>Triticum aestivum</i> L. cv. Alcedo)		roots in anoxia, shoots in air: $149 \pm 7 \mu\text{g g}^{-1} \text{FW}$ plants in N atmosphere in light: $150 \pm 26 \mu\text{g g}^{-1} \text{FW}$ plants in N atmosphere in dark: $316 \pm 63 \mu\text{g g}^{-1} \text{FW}$	Mustroph and Albrecht (2007)
24 h anoxic stress	Coleoptiles	Barley (<i>Hordeum vulgare</i> L. cv. Ichibanboshi)	$\pm 9 \mu\text{mol g}^{-1} \text{FW}$	Kato-Noguchi <i>et al.</i> (2010)
		Rice (<i>Oryza sativa</i> L. cv. Nipponbare)	$\pm 9 \mu\text{mol g}^{-1} \text{FW}$	
		Oat (<i>Avena sativa</i> L. cv. Victory)	$\pm 29 \mu\text{mol g}^{-1} \text{FW}$	

FW, fresh weight

gas-filled spaces in plant roots and shoots, thus favouring the longitudinal transport of O_2 from air to submerged organs (Fig. 7.2) (Drew *et al.*, 1979; Kawase, 1981). Aerenchyma formation in plants occurs through two different processes named schizogeny and lysigeny (Sachs, 1882). Schizogenous aerenchyma involves cell wall reorganization and cell separation, and is characteristic of *Rumex* spp. (Laan *et al.*, 1989), *Epilobium parviflorum* (Seago *et al.*, 2005), *Acorus calamus* and *Epilobium irsutum* (Armstrong and Armstrong, 1994). Lysigenous aerenchyma is formed as a consequence of a programmed cell death (PCD) event (Campbell and Drew, 1983; Gunawardena *et al.*, 2001; Evans, 2003) and has been described in barley (Arikado and Adachi, 1955), wheat (Trought and Drew, 1980), rice (Justin and Armstrong, 1987) and maize (He *et al.*, 1996; Gunawardena *et al.*, 2001). Some species such as *Sagittaria lancifolia* can also form lysigeny and schizogeny together, although in different tissues (Schussler *et al.*, 1997).

Although aerenchyma develops further when the soil becomes waterlogged, in some cases it is already present in well drained conditions (Armstrong, 1971; Pradhan *et al.*, 1973; Das and Jat, 1977).

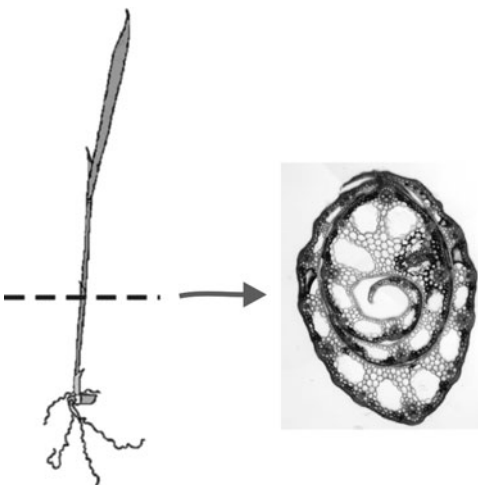


Fig. 7.2. Representative fresh cross-sections of rice leaf sheath showing aerenchyma formation.

The mechanisms responsible for aerenchyma have not yet been fully elucidated (Shiono *et al.*, 2008). However, it is known that it involves ethylene, which accumulates in submerged organs (Kawase, 1972, 1978; Könings and Jackson, 1979; Justin and Armstrong, 1991; He *et al.*, 1996; Zhou *et al.*, 2002; Lenochova *et al.*, 2009; Geisler-Lee *et al.*, 2010). In hypoxic roots of maize, exogenous ethylene applications induced aerenchyma formation while ethylene inhibitors repressed its development (Drew *et al.*, 1981; Könings, 1982; Jackson *et al.*, 1985). In addition, both 1-aminocyclopropane-1-carboxylate (ACC) synthase activity and ACC concentrations were found to be high in hypoxic maize roots (Atwell *et al.*, 1988; He *et al.*, 1994; Geisler-Lee *et al.*, 2010). Nevertheless, aerenchyma formation does not always require ethylene, as described for the roots of the wetland plant *Juncus effusus* (Visser and Bögemann, 2006). In rice stems, Steffens *et al.* (2010) demonstrated that constitutive aerenchyma forms in response to ethylene and H_2O_2 , in a dose-dependent manner. Moreover, the production of lysigenous aerenchyma in Arabidopsis under hypoxia was shown to require both ethylene and H_2O_2 signalling (Mühlenbock *et al.*, 2007) (Fig. 7.3). The identification of aerenchyma formation-associated genes expressed in maize roots under waterlogged conditions revealed the presence of mechanisms associated with the generation or scavenging of ROS, Ca^{2+} signalling, and cell wall loosening and degradation (Rajhi *et al.*, 2011). In submerged rice the couple ethylene/ H_2O_2 also plays a role in the aerenchyma formation of leaf sheaths (Parlanti *et al.*, 2011).

The volume of aerenchyma formed in submerged plant tissues depends on the species, as well as the cultivar/accession and environmental conditions (Colmer, 2003a, b). Wheat submergence-tolerant cultivars have significantly higher root porosities under submergence than sensitive cultivars (Huang *et al.*, 1994a, b, 1995a, b). Justin and Armstrong (1987) studied 91 plant species from wetland, intermediate and non-wetland habitats, and found that

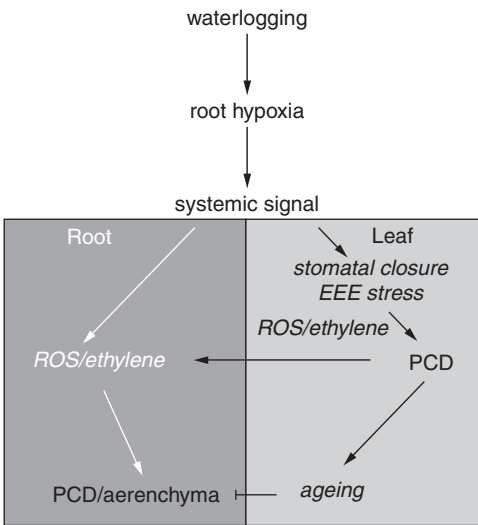


Fig. 7.3. Model proposed for aerenchyma formation in *Arabidopsis* roots under waterlogging (adapted from Mühlenbock *et al.*, 2007). Root hypoxia induces a systemic signal that leads to stomatal closure in leaves, with consequent excess excitation energy due to activation of photorespiration. ROS and ethylene induces programmed cell death (PCD) in both leaf and roots, leading to aerenchyma formation. Severe PCD during leaf ageing represses aerenchyma formation.

submergence tolerance is related to the extent of aerenchyma development.

The effectiveness of aerenchyma can be increased by the formation of a barrier to radial O₂ loss (ROL) in the epidermis and exodermis of roots, which inhibits O₂ diffusion, thus loss, from the roots to the surrounding anaerobic soil (Armstrong, 1979; Visser *et al.*, 2000; Colmer, 2003a, b). This barrier results from the deposition of suberin and lignin in the root exodermis cell wall, that form a physical resistance to O₂ diffusion (Shiono *et al.*, 2011). ROL barrier also enhances the O₂ movement toward the root apex, promoting a deeper rooting in waterlogged soils (Colmer and Voesenek, 2009). In some plants, ROL is constitutive (e.g. *Juncus effusus*) (Visser *et al.*, 2000), while in others it only increases under stagnant conditions (e.g. *Oryza sativa*, *Lolium multiflorum*, *Hordeum marinum*) (McDonald *et al.*, 2002; Colmer, 2003b; Garthwaite *et al.*, 2003). A further role

of ROL barrier is to limit the entry of toxic compounds, generated in highly reduced wet environments (Armstrong, 1979; Armstrong *et al.*, 1996; Armstrong and Armstrong, 2005). Root growth into waterlogged anoxic soils relies on internal diffusion of O₂ (Armstrong, 1979). ROL barrier, together with aerenchyma, likely contributes to the waterlogging tolerance of wetland species (Justin and Armstrong, 1987), including rice (Armstrong, 1971; Colmer *et al.*, 1998; Shiono *et al.*, 2011).

Many plants under water submergence show a hyponastic growth of leaves (e.g. *Rumex palustris*, *Ranunculus repens*, *Caltha palustris*, *Arabidopsis thaliana*) (Ridge, 1987; Cox *et al.*, 2003; Millenaar *et al.*, 2005). In *R. palustris*, leaf hyponasty results from the differential cell elongation of the petiole base and has been suggested to be a prerequisite for the subsequent petiole elongation (Cox *et al.*, 2003). Hyponastic growth is potentially important in order to reach the air surface above the water column, but reorientation itself can help in capturing light and in avoiding ground cover (Colmer and Voesenek, 2009). However, some sensitive plant species show epinastic leaf growth underwater (e.g. *Helianthus annuus*, *Nicotiana tabacum*, *Solanum lycopersicum*). This kind of growth has been suggested as reducing the dehydrating effects of the drop in water conductance that is observed under waterlogging (Jackson, 2002; Holbrook and Zwieniecki, 2003).

The formation of lenticels, plant openings that allow gas exchanges, at the stem bases and the development of adventitious roots and pneumatophores, which are specialized root structures that grow out of the surface of the water, can also increase the amount of O₂ that reaches the underwater organs (Kozlowski, 1984).

In addition to these morphological traits, the ability of the plants to form a leaf gas film, termed ‘plant plastron’ (analogous with the plastrons of aquatic insects), on submerged leaf surfaces can improve submergence tolerance (Raven, 2008). This gas film enables continuous gas exchange via stomata, bypassing cuticle resistance. Thus, it promotes gas exchanges, enhancing CO₂ uptake for photosynthesis under daylight,

and enhancing O₂ uptake for respiration during dark periods (Colmer and Pedersen, 2008; Pedersen *et al.*, 2009).

7.4 Low Oxygen Sensors and Sensing Mechanisms

Much progress has been made in terms of understanding plant signalling network(s) under O₂ deprivation, however the upstream stress-sensing mechanism has not yet been fully understood. Modifications in gene expression can be triggered by a direct sensor of O₂ concentration or by a change in cellular homeostasis. A plethora of indirect low O₂ sensing mechanisms has been suggested in plants, but so far a direct sensor has not been identified. One of the best O₂ sensor candidates, the plant O₂ binding protein haemoglobin, has been ruled out because of its low dissociation constant for O₂ (Dordas *et al.*, 2003). Nevertheless, non-symbiotic leg-haemoglobins are induced under low O₂ and have been suggested as playing a major role under hypoxia to scavenge NO and H₂O₂ (Perazzolli *et al.*, 2004).

In bacteria, fungi and animals, specific O₂ sensing regulatory systems and molecules have been found (for a review see Bailey-Serres and Chang, 2005). In mammals, the Hypoxia Inducible Factor (HIF) transcriptional complex plays a major role in low O₂ sensing. HIF is a heterodimer factor made up of the hypoxia-induced HIF1 α subunit and the constitutively expressed HIF1 β one. Under aeration, HIF is not active, since HIF1 α is hydroxylated at two prolyl residues by the prolyl-4-hydroxylase enzyme that requires O₂ as a co-substrate. The aerobically hydroxylated HIF1 α is degraded via the 26-proteasome (Acker *et al.*, 2006). Thus, reduced O₂ availability reduces the rate of HIF1 α degradation with the subsequent activation of the transcriptional complex, which is then translocated to the nucleus to activate downstream genes (Semenza, 2007). In plants, no HIF1 orthologues have been found so far.

Another possibility is that plants respond to variations in the concentration

of metabolic parameters that directly or indirectly depend on O₂ availability (Licausi, 2011). The most promising indicators are calcium flux, energy charge and reactive oxygen and nitrogen species (ROS and RNS). These parameters seem to be interrelated, thus suggesting the presence of downstream events that appear to cross each other.

Changes in Ca²⁺ have been reported in response to various stimuli, including hormonal changes, light, biotic and abiotic stresses (for a review see Lecourieux *et al.*, 2006). These changes are due to a transient change in plasma membrane permeability that seems to be a common occurrence in early plant defence signalling (Atkinson *et al.*, 1990). Calcium has also been proposed to trigger low O₂ signalling in plants, since an increase in cytosolic Ca²⁺ concentration has been observed in maize and Arabidopsis under hypoxia and anoxia (Sedbrook *et al.*, 1996; Subbaiah *et al.*, 2000).

In addition, maize cells seem to require Ca²⁺ for the expression of *ADH1* (Subbaiah *et al.*, 2000). Under anoxia, the Ca²⁺ response proved to be biphasic, composed of a slow Ca²⁺ spike, which takes a few minutes, and a subsequent sustained Ca²⁺ elevation, which takes hours (Sedbrook *et al.*, 1996; Subbaiah *et al.*, 2000). Transient Ca²⁺ changes are decoded by an array of proteins that fall into two main classes of Ca²⁺ sensor relays and sensor responders (Lecourieux *et al.*, 2006). They give rise to downstream events such as protein phosphorylation and gene expression. Of the Ca²⁺ sensor relays, the calcineurin B-like (CBL) proteins function through bi-molecular interaction (for a review see Luan *et al.*, 2002).

Rice tolerance to flooding has been shown to depend on a coordinated response to O₂ and sugar deficiency, regulated by the CBL Interacting Protein Kinase 15 (CIPK15) (Lee *et al.*, 2009). The specific CBL that interacts with CIPK15 under O₂ deprivation has not been determined. CIPK15 regulates the plant's global energy sensor Snf1 Related Kinase (SnRK1) to control sugar and energy production for growth underwater. CIPK15 has been shown to positively

regulate *ADH1* and *ADH2* in rice under submergence (Lee *et al.*, 2009). In addition, a partial overlap between energy deprivation and hypoxia has also been observed in *Arabidopsis*, where a subset of common genes appears to be regulated by the protein kinases AKIN10 and AKIN11 (Baena-González *et al.*, 2007). These proteins are members of the SnRK1 group.

Calcium fluxes and subsequent protein phosphorylation have also been reported to be required for the controlled generation of hydrogen peroxide (H_2O_2) (Neill *et al.*, 2002). However, Ca^{2+} independent H_2O_2 accumulation has also been observed (Chandra *et al.*, 1997; Mithöfer *et al.*, 2001). Emerging evidence suggests that a ROS-mediated plasma membrane Ca^{2+} channel activation is involved in general plant signal transduction and development (Lecourieux *et al.*, 2006). ROS and RNS have been indicated as key actors in plant responses to both biotic and abiotic stresses (for reviews, see Delledonne, 2005). While initially both ROS and RNS were thought to be only involved in degenerative processes, more recently they have emerged as signalling actors, participating in the sensing and signalling responses to different stresses. For a signalling molecule to be effective, it needs to be produced rapidly, to induce defined effects within the cell, and to be removed rapidly when no longer required (Pauly *et al.*, 2006). This is the case of ROS, whose production has been suggested as a necessary component of low O_2 signalling. A key manuscript by Baxter-Burrell *et al.* (2002) revealed that the activation of an RHO-like GTPase of plants (Rop) under low O_2 induces H_2O_2 accumulation. The ROP family modulates signalling cascades associated with different mechanisms in plants (Gu *et al.*, 2004). The Rop-dependent production of H_2O_2 via a NADPH-oxidase mechanism has been shown to be necessary for *ADH* expression, thus tolerance (Baxter-Burrell *et al.*, 2002). Tolerance to O_2 deprivation seems to require Rop to be activated but also to be negatively regulated through a feedback mechanism through a Rop GTPase Activating Protein (GAP), regulated by

H_2O_2 . The mechanism that drives the ROP rheostat activation under low O_2 is not currently known.

7.5 Transcriptional Regulations Under Low Oxygen

Many microarray datasets are currently available for *Arabidopsis* and rice plants under anoxia and hypoxia (Branco-Price *et al.*, 2005; Liu *et al.*, 2005; Loreti *et al.*, 2005; Lasanthi-Kudahettige *et al.*, 2007; van Dongen *et al.*, 2008; Banti *et al.*, 2010; Jung *et al.*, 2010; Licausi *et al.*, 2010; Mustroph *et al.*, 2010; Lee *et al.*, 2011). This considerable amount of information enables converging and diverging data to be gathered. These data identify common, core-response genes in addition to genes that are specifically regulated in different organs and after different low levels of O_2 (C. Pucciariello, unpublished).

One regulatory group of TF responding to a lack of O_2 regards members of the Ethylene Responsive Factors (ERF) belonging to family VII (Nakano *et al.*, 2006). In submerged rice, these factors respond to ethylene increased production/trapment under water. Genes belonging to this group have been found to be involved in both rice LOQS and LOES anti-theoretical responses (Fig. 7.4).

In some rice *indica* varieties, the ERF *Submergence 1A* (*SUB1A*) gene is thought to play a key role in the submergence tolerance of mature plants (Fukao *et al.*, 2006; Xu *et al.*, 2006). The resistance-related allele *Sub1A-1* is induced by ethylene under submergence and activates the *Slender-Rice 1* (*SLR1*) and *SLR1 Like 1* (*SLRL1*) genes. *SLR1* and *SLRL1* repress GA-mediated underwater plant elongation in order to save energy for the de-submergence phase (Bailey-Serres and Voosenek, 2008).

In deepwater rice the *Snorkel 1* (*SK1*) and *Snorkel 2* (*SK2*) genes, which also belong to the ERF family VII, mediate the opposite strategy of underwater elongation (LOES). LOES relies on fast elongation under submergence to re-establish contact with the air, thus favouring aerobic respiration (Hattori *et al.*, 2009).

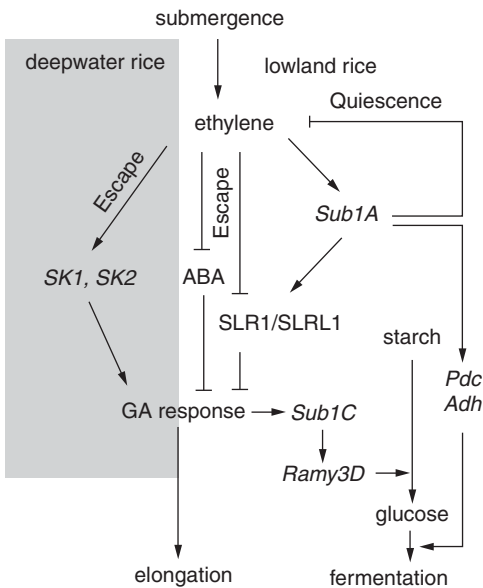


Fig. 7.4. The pathways in relation to Low Oxygen Quiescence Syndrome (LOQS) and the Low Oxygen Escape Syndrome (LOES) in lowland rice plants under submergence and the 'escape strategy' in deepwater rice (adapted from Hattori *et al.*, 2009 and Bailey-Serres *et al.*, 2010). In lowland rice, LOES promotes the GA-induced elongation with the subsequent starch and soluble sugar catabolism. Under the lowland rice LOQS, submergence stimulates production/entrapment of ethylene, which activates *Sub1A*. *Sub1A* positively regulates the two GA response suppressors SLR1/SLRL1. Consequently, GA signal is not transduced, thus explaining the lack of *Sub1C* induction. In deepwater rice, *SK1* and *SK2* are activated by the ethylene produced by submergence. *SK1* and *SK2* regulate internode elongation, and GA production/sensitivity seems to be involved in this response.

Genes which belong to the ERF family VII have also been found to be involved in Arabidopsis responses to low O_2 . The *Hypoxia Responsive ERF (HRE) 1* and *HRE2* transcription factors show a strong up-regulation under O_2 depletion, mediated by both ethylene-dependent and ethylene-independent signals (Licausi *et al.*, 2010; Yang *et al.*, 2011). Transgenic plants overexpressing either *HRE1* or *HRE2* showed an increased tolerance to anoxia, and double

knockout *hre1 hre2* plants displayed a decreased expression of several hypoxic genes.

In Arabidopsis, ethylene directly affects *RAP2.2* and *RAP2.3* genes, which are also members of the ERF family VII. Overexpression of *RAP2.2* in Arabidopsis plants increased the expression of a set of hypoxic genes and plant tolerance to O_2 limitation (Hinz *et al.*, 2010).

Group VII ERFs harbour a recognition site for the ATCTA motif, which is over-represented in the promoter of a set of hypoxic genes (Licausi *et al.*, 2011). This motif seems to be involved in the transactivation of *ADH* and *Hb1* promoters by HRE1 (Licausi *et al.*, 2011). However, the overexpression of these *ERF* genes did not increase the expression of hypoxic genes under normoxia (Licausi, 2011).

Interestingly, together with a common signalling mechanism, a side divergent pathway seems to be activated under anoxia and hypoxia (C. Pucciariello, unpublished). Heat Shock Transcription Factors (HSF) and Heat Shock Proteins (HSP) are highly induced under anoxia in a mechanism that overlaps with heat stress and identifies H_2O_2 as the common signalling element (Banti *et al.*, 2010). HSFs have been proposed to be specific H_2O_2 sensors in plants (Miller and Mittler, 2006). Arabidopsis seedlings that overexpress *HsfA2* are markedly more tolerant to anoxia as well as to submergence (Banti *et al.*, 2010). The induction of heat-related genes is likely restricted to anoxia treatment which, unlike hypoxia, could harbour a more complex mechanism of response/adaptation than the simple switch to fermentative metabolism, in agreement with Licausi (2011) (Fig. 7.5).

7.6 Rice Strategies to Survive Oxygen Deprivation

Rice is a semi-aquatic plant well adapted to surviving low O_2 environments, both when sowed in paddy fields, and as an adult plant as a consequence of natural and man-made flooding events. Rice feeds billions of people

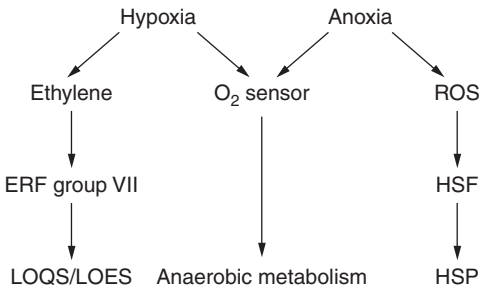


Fig. 7.5. Model of low O_2 signal transduction in plants (adapted from Licausi, 2011). Hypoxia and anoxia seem to be perceived by a common sensor, which is still unknown and which activates related anaerobic metabolism genes. Ethylene-related signalling is mainly activated under hypoxia. It regulates the ERF genes belonging to group VII, which stimulate or inhibit the signal transduction ways that lead to Low Oxygen Escape Syndrome (LOES) and Low Oxygen Quiescence Syndrome (LOQS). Anoxia activates a ROS-related pathway that includes the activation of Heat Shock Factors (HSF), which promote the transcription of Heat Shock Proteins (HSP).

and with increased global food demand, rapid increases in productivity are needed, especially in marginal lands. Ecologically, it can be classified into three different groups: upland rice, which grows in non-irrigated fields; lowland rice, which grows in rainfed or irrigated fields up to 50 cm deep; and deepwater rice, exposed to water exceeding 50 cm in depth (Sauter, 2000). Deepwater and lowland rice together account for 33% of global rice farmland, mainly located in India, Thailand and Bangladesh (Bailey-Serres *et al.*, 2010).

Different water cultivation regimes have favoured the selection of local rice landraces adapted to extreme environments, thus giving rise to the enormous ecological diversity of genotypes. Around 110,000 *Oryza* accessions, including wild species and related genera, are now present at the International Rice Research Institute (2011; <http://irri.org>) to preserve the germplasm.

In rice, two mutually exclusive major strategies to cope with submergence have been identified: the avoidance of anaerobiosis through rapid growth, called the ‘escape

strategy’, and tolerance of anaerobiosis through reduced growth, named the ‘quiescence strategy’. Each adaptation is useful under the appropriate environmental conditions in which it was developed. However, rice is an aerobic organism, thus it still suffers from O_2 deprivation: submergence stress has been identified as the third most important constraint to rice production in Indian lowland areas (Sauter, 2000).

7.6.1 Successful rice germination under oxygen deprivation

Rice successfully germinates under low O_2 (for a review see Magneschi and Perata, 2009). Only the coleoptile elongates, while the root fails to grow. In cereal grains, the starch stored in the seed endosperm is a major reserve. The starchy seeds are able to maintain a high energy metabolism under anaerobiosis through starch catabolism (Alpi and Beevers, 1983). Rice harbours a complete set of starch-degrading enzymes including α and β amylases, debranching enzymes and maltases. Indeed, α -amylases have a major role in degrading native starch granules in germinating rice (Murata *et al.*, 1968; Dunn, 1974; Sun and Henson, 1991).

α -Amylases are endo-amylolytic enzymes, which catalyse the hydrolysis of α -1, 4 linked glucose polymers of starch. Starch hydrolysis results in sugars that are translocated to the embryonic axis to be metabolized through the glycolysis, thus generating the energy and essential metabolites needed for growth. α -Amylases are not produced in anoxia-intolerant cereals such as wheat, barley, oat and rye, which under low O_2 consequently suffer from sugar starvation and eventually die (Perata *et al.*, 1992; Perata and Alpi, 1993; Guglielminetti *et al.*, 1995; Loreti *et al.*, 2003a).

In rice there are ten different isoforms of α -amylases, grouped into three sub-families: Amy1 (A–C), Amy2 (A) and Amy3 (A–F) (Rodriguez *et al.*, 1992). *Ramy1A* is hormonally modulated by gibberellins (GA) under aerobic conditions. Although *Ramy1A* has been detected in

anaerobic samples (Perata *et al.*, 1997), it is repressed by anoxia (Hwang *et al.*, 1999). The GA-independency of rice anaerobic germination has been demonstrated in the GA-deficient mutant *Tan-ginbozu* (Loreti *et al.*, 2003b). This mutant germinates under anoxia, although GA-dependent amylase gene induction is reduced (Loreti *et al.*, 2003b).

Ramy3D has been identified as the major actor in anoxic rice seedlings and is anoxia induced (Fig. 7.6) (Perata *et al.*, 1997; Hwang *et al.*, 1999; Lasanthi-Kudahettige *et al.*, 2007). *Ramy3D* is not induced by GA, since it does not possess the *cis*-acting element on the promoter region required for GA-responsiveness (Morita *et al.*, 1998; Loreti *et al.*, 2003a). Instead *Ramy3D* is regulated by sugar starvation, which suggests a link with the drop in soluble sugar content observed under anoxia (Gulgielminetti *et al.*, 1995; Perata *et al.*, 1996; Loreti *et al.*, 2003a). The expression of *Ramy3D* in rice embryos takes place 12 h after imbibitions, peaks 2 days later, and then starts to decline. After 5 days of imbibitions, *Ramy1A* also increases, thus suggesting that these two enzymes cooperate in the process of anoxic starch degradation (Loreti *et al.*, 2003a).

Cross-talk between sugar and O_2 -deficiency signalling has recently been demonstrated to be important for rice germination under low O_2 (Lee *et al.*, 2009). As indicated in the previous section, CIPK15 has been suggested as the key regulator of carbohydrate catabolism and fermentation in rice germination under flooding; *cipk15* mutants were found to barely germinate under water, also showing a reduced expression of *ADH* and the abolishment of *Ramy3D* mRNA accumulation (Lee *et al.*, 2009). The CIPK15 pathway is thought to work through the yeast-like general metabolic sensor SnRK1A, which plays a central role in the sugar signalling pathway, and the *MYBS1* transcription factor, in order to regulate *Ramy3D* expression (Lu *et al.*, 2007; Lee *et al.*, 2009). *MYBS1* binds specifically to the *Ramy3D* promoter TA box element, which belongs to the sugar-responsive element (SRE) and functions as a transcriptional activator of *Ramy3D* under sugar

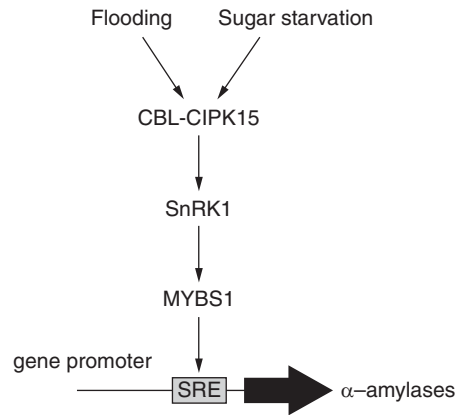


Fig. 7.6. The *Amy3* activation pathway that coordinates the rice seedling response to O_2 deprivation and sugar deficiency for tolerance to flooding (adapted from Lee *et al.*, 2009). CIPK15 acts as the main upstream positive regulator of SnRK1 at a post-transcriptional level in response to sugar starvation and flooding. SnRK1 is necessary for the activation of MYBS1, which then drives the *Ramy3D* expression by binding to the sugar responsive element (SRE) located on the promoter region of the gene.

depletion (Lu *et al.*, 1998; Toyofuku *et al.*, 1998; Chen *et al.*, 2002). The CIPK15 pathway has been proposed as being a principal actor in regulating starch catabolism under low O_2 , thus allowing rice to germinate (Lee *et al.*, 2009).

Another important trait related to rice germination under low O_2 is the capacity to elongate the coleoptile. Coleoptile elongation influences the establishment of rice in submerged fields, since it enables the seedlings to make contact with the atmosphere above the water column (Huang *et al.*, 2003). Considerable variations have been observed among different rice cultivars in their capacity to elongate the coleoptile (Setter *et al.*, 1994; Magneschi *et al.*, 2009a). A screening of 164 rice cultivars to assess their ability to elongate under anoxia revealed that anoxic coleoptile length is not correlated with glycolytic and fermentative related gene expression, or with carbohydrate content (Magneschi *et al.*, 2009a). Instead, an efficient ethanol fermentation appears to be critical to successful rice germination under

low O₂ (Setter *et al.*, 1994; Matsumura *et al.*, 1998; Magneschi *et al.*, 2009a).

Since coleoptile elongation under low O₂ is believed to be mostly due to cell expansion, cell wall loosening is thought to be involved. Of the enzymes involved in this process, it is likely that expansins play a role. (Cosgrove, 1999; Huang *et al.*, 2000). Expansins are encoded by a multigene family composed of two subfamilies: α and β expansins. In deepwater rice, rapid internode elongation mediated by GA induces the expression of *EXPB3*, *EXPB6*, *EXPB11* and *EXPB12* (Kende *et al.*, 1998). Increases in the mRNA levels of *EXPA2* and *EXPA4* were also observed in underwater internodes of deepwater rice (Cho and Kende, 1997). *EXPA7* and *EXPB12* have been proposed as possible regulators of anoxic elongation of rice coleoptiles (Lasanthi-Kudahettige *et al.*, 2007). In addition, the antisense expression of *EXPA4* resulted in a reduced coleoptile elongation under submergence (Choi *et al.*, 2003). However, the expression of 15 α and β expansin genes, in two rice cultivars showing long or short coleoptiles when germinated under anoxia, did not show any correlation with the ability to elongate (Magneschi *et al.*, 2009b).

7.6.2 Low oxygen escape syndrome in deepwater rice

Some rice varieties exposed to submergence try to escape low O₂ stress by improving the elongation of the stem and/or leaf, in order to reach the water surface and re-establish contact with the air. As previously indicated, lowland rice varieties risk starvation and death before they make contact with the air (Bailey-Serres and Voesenek, 2008). However, deepwater rice displays an extremely fast internode elongation in response to increasing water levels (for a review see Nagai *et al.*, 2010). Deepwater rice is cultivated in rainfed areas and tidal swamps due to its ability to elongate considerably under flooding (Catling, 1992). In these areas subjected to monsoons, paddy fields are seasonally covered with water

levels ranging from 1 to several m (Vergara and Mazaredo, 1975; Catling, 1992). Internodes of deepwater rice elongate with a daily increase of 20–25 cm and can even reach 7 m in height. Since inside the internode there is an empty hollow structure, air can circulate inside the plant thus reaching the underwater organs. Only a slight decrease in O₂ concentration was observed inside the culm of deepwater rice under submergence, with a concomitant rise in CO₂ (Stünzi and Kende, 1989). Oxygen concentration was in the range of 15 to 20%, being lower during the night than during the day. The CO₂ range was between 0.05% and 2%, with diurnal fluctuation inverse to that of O₂ (Stünzi and Kende, 1989).

Ethylene was initially suggested as having a role in internode elongation (Métraux and Kende, 1983), together with ABA and GA (Kende *et al.*, 1998). The deepwater response has now been partially clarified, through the analysis of major quantitative traits loci (QTL) detected on chromosome 12 (Hattori *et al.*, 2007, 2008, 2009). The two putative ERF transcription factors SK1 and SK2, which belong to this QTL and are induced by ethylene, have been shown to positively regulate internode elongation (Fig. 7.4). The overexpression of *SK1* and *SK2* in non-deepwater rice varieties promotes internode elongation, even in air (Hattori *et al.*, 2009). How *SK1* and *SK2* regulate internode elongation is still unclear. GA seems to be involved in this response, since treatments of deepwater rice with GA inhibitors show an arrest in elongation (Suge, 1987; Hattori *et al.*, 2009). However, the increase in GA content in deepwater rice under submergence is low, thus suggesting an increase in GA sensitivity, probably due to ethylene (Raskin and Kende, 1984; Hoffmann-Benning and Kende, 1992; Hattori *et al.*, 2009).

7.6.3 Quiescence strategy in tolerant lowland rice varieties

A limited number of lowland rice cultivars can tolerate more than 14 days of flooding

(Mackill *et al.*, 1996). Of these, FR13A has been used to study the molecular basis of tolerance related to the 'quiescence mechanism', thus growth arrest under flooding (for a review see Bailey-Serres *et al.*, 2010). As indicated above, rice plants that use a quiescence strategy to survive flooding tend to reduce growth and metabolic activity to minimum, thus storing energy for regrowth. QTL mapping has led to the identification of the *Sub1* locus, located near the centromere of the long arm of chromosome 9 (Xu *et al.*, 2006). *Sub1A* is indicated as the major source of flooding tolerance in rice and explains ~69% of the phenotypic variations in this trait. *Sub1* locus comprises, together with other genes, two to three closely related *ERF* genes named *Sub1A*, *Sub1B* and *Sub1C*, which are characterized by several allelic forms and function as transcriptional regulators (Fukao *et al.*, 2006; Xu *et al.*, 2006). *Sub1A-1* is positively regulated by ethylene, and rapidly increases its mRNA levels under submergence. (Xu *et al.*, 2006).

The presence of *Sub1A-1* allele seems to be crucial to mediate flooding tolerance and activate other *ERF* genes to promote survival (Jung *et al.*, 2010). At a molecular level, *Sub1A-1* activation reduces ethylene perception through a feedback mechanism, thus suppressing the ethylene-promoted GA-mediated induction of genes associated with carbohydrate catabolism and cell elongation (Fig. 7.4). Therefore, carbohydrate reserves are conserved for plant regrowth during re-oxygenation. In addition, the expression of *ADH* and *PDC* genes is also up-regulated in *Sub1A-1* harbouring plants under O₂ deprivation (Fukao *et al.*, 2006; Xu *et al.*, 2006). The direct influence of *Sub1A-1* has been evaluated, indeed the introgressed line M202(*Sub1*) that contains the *Sub1A-1* gene from the FR13A cultivar and has been found to be submergence tolerant when compared with the intolerant M202 cultivar (Fukao *et al.*, 2006).

The *Sub1C-1* allele has also been suggested as having a role in the tolerance mechanism. It seems to act downstream of the ethylene-promoted GA-dependent signalling response, thus enhancing the rapid shoot

elongation in intolerant rice genotypes (Fukao and Bailey-Serres, 2008). The transcript abundance of *Sub1C-1* is significantly down-regulated in rice accessions with the *Sub1A-1* allele (Fukao *et al.*, 2006; Xu *et al.*, 2006, Fig. 7.4). Tolerance via the quiescence mechanism is always associated with the presence of the *Sub1A-1/Sub1C-1* haplotype (Singh *et al.*, 2010).

Further studies revealed that *Sub1A-1* increases the accumulation of the DELLA protein SLENDER RICE 1 (SLR1) and the non-DELLA protein SLR-like 1 (SLRL1), both GA signalling repressors, thus limiting the shoot elongation (Fukao and Bailey-Serres, 2008). Moreover, Jung *et al.* (2010) showed that *Sub1A-1* regulates multiple pathways associated with tolerance such as anaerobic respiration, hormone responses and antioxidant systems.

In addition to its role under submergence tolerance, *Sub1A* has been shown to improve survival under dehydration, by enhancing the plant's ABA responsiveness and by activating stress-inducible genes (Fukao *et al.*, 2011). Submergence followed by drought and vice versa often occurs in rainfed lowlands, thus the improvement of a combined tolerance would substantially increase rice productivity in flood-prone areas.

7.7 Conclusions and Perspectives

Submergence stress is one of the major constraints to plant production worldwide. Metabolic changes due to low O₂ can be crucial to plant survival, due to the lower energy available for growth. Many morphological and metabolic adaptations have been described, and some traits related to tolerance have also been indicated. Molecular studies on *Arabidopsis* and rice plants have revealed the existence of specific genes activated under low O₂ and related to metabolic changes due directly to O₂ level fluctuation. In particular, ethylene production/entrapment seems to govern the expression of different *ERF* genes, which work through antithetical plant strategies

to survive flooding under different water regimes/durations in both *Arabidopsis* and rice. Nevertheless, anoxia seems to induce a side mechanism that resembles heat stress in the production of HSP. Although specific sensors of O₂ deprivation have not yet been found, indirect low O₂ sensing and signaling mechanisms have been proposed. Among them, Ca²⁺ flux, ROS production and energy charge variation are the most

promising and also seem to cross each other.

From an agronomic point of view, in the context of climate change and the increasing need for food, the study of rice strategies to survive flooding in ecotypes adapted to extreme environments shows great potential. Research has managed to shed light on the molecular mechanisms that govern the tolerance response to O₂ deprivation.

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