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Modulating Rice Stress Tolerance by Transcription Factors

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Abstract

Plants are non-mobile organisms and have to adapt to environmental stresses mostly by modulating their growth and development in addition to physiological and biochemical changes. Transcription factors (TFs) regulate genome expression in response to environmental and physiological signals, and some of them switch on plant adaptive developmental and physiological pathways. One TF is encoded by a single gene but regulates the expression of several other genes leading to the activation of complex adaptive mechanisms and hence represents major molecular targets to genetically improve the tolerance of crop plants against different stresses. In this review an updated account of the discovery of TFs involved in biotic and abiotic stress tolerance in the model monocotyledonous plant, rice (*Oryza sativa* L.) is presented. We illustrate how the elucidation of the function of these TFs can be used to set up genetic engineering strategies and to rationalize molecular breeding using molecular assisted selection towards enhancement of rice tolerance to various stresses. Attempts have also been made to provide information on the molecular mechanisms involved in

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Abbreviations: ACC, 1-aminocyclo-propane-1-carboxylic acid; ABA, abscissic acid; BTH Benzothiadiazole; EtOH, Ethylene; MeJa, Methyl-Jasmonate; SA, Salicylic acid; TF, transcription factor; UV-B, Ultraviolet radiations B.

stress resistance or tolerance processes. We discuss how the comparison of the action of TFs isolated from the dicotyledonous model plant *Arabidopsis thaliana* in rice and vice versa can contribute to determine whether common or divergent mechanisms underlie stress tolerance in the two plant species. Lastly, we discuss the necessity to discover TFs controlling specifically the root adaptive development which constitutes a major way for the plant to escape to several stresses such as water deficit or mineral nutrient deficiency.

Introduction

Plants are fixed organisms that have no means to escape by mobility from the stochastic fluctuations of environmental conditions. They have to adapt to these fluctuating environmental conditions by producing protective molecules and by modulating their growth and development. This phenotypic plasticity is driven by the activation of specific genes encoding transcription factors (TFs). TFs are proteins able to specifically bind with short DNA sequences located in the promoter of genes and to interact with the preinitiation complex of transcription, conducting to activate or to inhibit the RNA polymerase II. Then TFs modulate the transcription rate of their target genes. One TF can modulate the transcription of several genes, including genes encoding TFs themselves, and reorients the cell and organism activity for the adaptation to a particular external condition. For this reason, TFs constitute key elements of the adaptation process of plants to their environment and are preferred targets for selection or engineering of complex agronomical traits of interest. For example, TFs have been used with success in the recent past to control and increase the production of valuable metabolites in plant cells (Gantet and Memelink, 2002). The evolution of many morphological traits during the domestication of plants has been associated with changes in TFs, as many biological processes in plants are regulated at the level of transcription. Any minor change in TF regulation, their sequences or into their target DNA sequences can greatly alter gene regulatory networks and plant physiology or morphology (Clark *et al*., 2006; Dias *et al*., 2003; Guo and Moose, 2003). Hence TFs are given adequate emphasis while creating varieties with a better tolerance to diverse stresses. The present review discusses recent advances in engineering rice (*Oryza sativa* L.) by TF. Rice is considered as a model for monocot plants because of its small genome size relative to other cereals (430 Mb), the ease of transformation, the amount of molecular and genetic resources available, and its economic importance. Rice is considered as the major source of carbohydrates for human consumption. Following recent annotation release, the rice genome contains 32,000 predicted genes compared to the 27,000 genes predicted in *Arabidopsis thaliana,* the model plant for dicotyledonous (Itoh *et al*., 2007). Eighty percent of the *A. thaliana* genes have a rice homolog whereas nearly 20% of the predicted rice genes have no homolog in *A. thaliana* (Itoh *et al*., 2007; Yu *et al*., 2002). On the other hand, rice homologs of 98% of the genes identified in cereals can be found in the rice genome, confirming the potential of this model cereal for deciphering the function of genes in other cereals (Delseny, 2003). Comparison among gene families encoding TFs in plant, animal and fungi genomes have revealed that this class of protein is more diverse in plants due to the existence of plant-specific families of TFs. Moreover, the number of genes inside TF families which are common to all organisms has been expanding more in plants than in animals compared to the evolution of the number of

genes encoding any other kind of proteins (Shiu *et al*., 2005). This shows that during evolution land plants have probably employed TFs to modulate genome expression in response to various environmental conditions in order to develop specific adaptive responses. This is true for rice, and this review throws light on how the knowledge on the function of regulatory genes encoding specific TFs can be used to improve the tolerance of this cereal to various biotic and abiotic stresses, which involve complex adaptation mechanisms, difficult to access through simple structural genes.

WRKY TFs are central elements for resistance of rice against pathogens

WRKY TFS CONSTITUTE A CLASS OF PLANT SPECIFIC TF MOSTLY INVOLVED IN THE REGULATION OF THE EXPRESSION OF DEFENSE GENES

Damage caused to rice by disease and insects represent annually a loss of around 25% of the total production and can induce locally dramatic loss of more than 80% (Khush, 2005). Plant defense against pathogens involves complex events of gene expression leading to the production of defense molecules such as toxic secondary metabolites (phytoalexins), pathogenesis related proteins (chitinases, glucanases) and cell wall reinforcement (Durrant and Dong, 2004). A class of TF, specific to plants, has been characterized to be more specifically involved in the regulation of various defense genes in response to pathogen aggression (Eulgem, 2006). These TFs are called WRKY because all of them possess a DNA binding domain consisting in 60 amino acids comprising a conserved WRKY tetrad. In *A. thaliana*, the expression of most of the 74 WRKY genes is induced in response to pathogen infection and other elicitation signals (Dong *et al*., 2003; Kalde *et al*., 2003). Basal defense and systemic acquired resistance are mediated by a plant hormone, Salicylic Acid (SA) (Pieterse and Van Loon, 2004). SA activates the translocation to nucleus of the transcriptional cofactor Nonexpresser of Pathogenesis Related genes1 (NPR1) where it interacts with a basic leucine zipper TF, TGA, and stimulates its binding to DNA target promoter sequences, resulting in the activation of the expression of a set of defense related genes (Despres *et al*., 2000; Thibaud-Nissen *et al*., 2006; Zhang *et al*., 1999; Zhou *et al*., 2000). This activation is relayed by the induction of expression of several WRKY genes comprising *AtWRKY18*, *AtWRKY53*, *AtWRKY54* and *AtWRKY70*. These WRKY TFs act downstream of NPR1 to regulate the expression of defense genes which results in the induction of resistance against different fungal or bacterial plant pathogens (Chen and Chen, 2002; Wang *et al*., 2006). In rice, the SA level is twofold higher than in dicots and does not increase after an infection by the bacterial pathogen *Pseudomonas syringae*, or the fungal pathogens *Magnaporthe grisea* or *Rhizoctonia solani* (Silverman *et al*., 1995). Nevertheless, analysis of SA concentrations in 28 rice cultivars suggested a correlation between SA levels and the resistance against *M. grisea* (Silverman *et al*., 1995). In addition, overexpression in rice of *NPR1* or its rice ortholog *NH1,* results in an increase of the resistance to *Xanthomonas oryzae*, the bacterial agent of bacterial blight, via the activation of a bZIP TF (Chern *et al*., 2005; Chern *et al*., 2001). This shows that a pathway involving SA defense signaling, mediated by NH1 and a bZIP TF is active in this species. It is now interesting to decrypt the gene regulatory network acting downstream to the NH1/bZIP TF. To accomplish this, the functions of rice WRKY TF have to be elucidated. In rice, 109 genes encoding WRKY TF have been identified (Zhang and Wang, 2005).

OSWRKY03 AND OSWRKY71 ACT UPSTREAM OF THE NPR1/NH1 REGULATORY PATHWAY

The transcriptome of rice seedlings treated by SA has been examined in order to identify rice WRKY TFs implicated in the induction of plant defense against pathogens. A total of 10 WRKY SA responsive TFs were characterized, and two of them, OsWRKY03 and OsWRKY71 further functionally studied (Liu *et al*., 2007; Liu *et al*., 2005b). OsWRKY03 has a sequence closely similar to that of AtWRKY29 which is involved in the response to bacterial and fungal pathogens in *A. thaliana* (Asai *et al*., 2002; Liu *et al*., 2005b). The expression of *OsWRKY03* is regulated by diverse stress-related signals such as SA, Benzothiadiazole (BTH), a functional analog of SA, Methyl-Jasmonate (MeJa), a stress and defense plant related hormone, 1-aminocyclo-propane-1-carboxylic acid (ACC), a precursor of ethylene (EtOH) another plant hormone, wounding and infection by *X. oryzae*. These regulations are all light-dependent. Expression of *NH1* and *PR1b*, a rice pathogenesis related protein, are also light-dependent (Agrawal *et al*., 2000; Chern *et al*., 2005). Constitutive overexpression of *OsWRKY03* leads to a dramatic dwarf phenotype and most overexpressing lines were mostly unable to survive when transferred into soil. The induction of plant defense mechanisms is very costly for plants and when they are constitutively activated, plants often fail to develop normally (Durrant and Dong, 2004; Zhang, 2003). In the *OsWRKY03* plants *NH1*, *PR1b*, *ZB8*, encoding the Phenylalanine ammonia-lyase, an enzyme involved in the synthesis of phenylpropanoid plant defense molecules (Zhu *et al*., 1995) and *POX22.3*, encoding a peroxydase (Chittoor *et al*., 1997) are constitutively induced. These data show that OsWRKY03 is acting upstream of the *NH1* defense regulatory gene and upstream of the other defense related genes (*Figure1*). This TF regulates the expression of NH1, which itself regulates the expression of defense genes, in response to pathogen attack and to plant stress signals. In this context, OsWRKY03 is also likely responsible of the light dependant expression of *NH1* and *PR1b*. It is interesting to note that in rice OsWRKY03 acts upstream of NH1 whereas in *A. thaliana* all WRKY TFs implicated in the regulation of defense genes act downstream of NPR1.This suggests that the gene regulatory networks involved in the activation of defense mechanisms in these two species have evolved with common elements but that these elements display a distinct organization between rice and *A. thaliana*. Similar information is available for *OsWRKY71* (*Figure1*), with the exception that the expression of this gene is not light dependent and that the effect of its overexpression on *ZB8*, *POX22.3* expression and on plant growth were not reported (Liu *et al*., 2007). OsWRKY71 is similar to the pathogen-inducible AtWRKY18 TF (Chen and Chen, 2002).

OSWRKY45 DEFINES A NEW PATHWAY FOR THE INDUCTION OF DEFENSE GENES, INDEPENDENT OF NPR1/NH1

OsWRKY45 was identified from a transcriptome analysis of leaves of BTH treated rice plants (Shimono *et al*., 2007). The induction of expression of *OsWRKY45* precedes the induction of defense related genes such as *PR1b*, *PBZ1*, a probenazole-inducible protein (Midoh and Iwata, 1996), *SA-GTase* and *RCI-1* a chloroplastic rice lipoxygenase (Schaffrath *et al*., 2000). BTH treatment or *OsWRKY45* overexpression enhances resistance of rice against the fungal pathogens *M. grisea* whereas the BTH-inducible resistance is compromised in *OsWRKY45* RNA interfered plants. The induction of the expression of *OsWRKY45* or *NH1* in response to BTH does not require

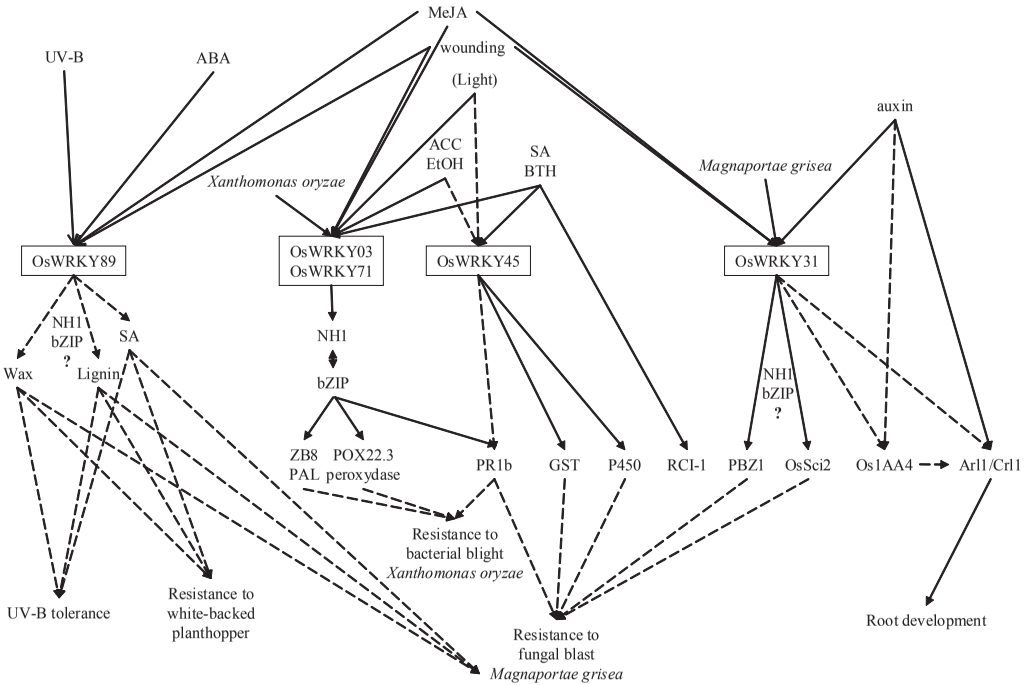

Figure 1. Networks involving WRKY transcription factors for plant resistance to biotic stresses in response to different environmental conditions, pathogen or hormonal signals. Unbroken arrows represent direct correlation between two elements of the network; broken arrows represent indirect relationship between two elements of the network. ? indicates that the involvement of the element X in the network has not been tested. ACC, 1-aminocyclo-propane-1-carboxylic acid, ABA, abscissic acid, Arl1/Crl1, adventicious root less1/ crown root less 1 BTH Benzothiadiazole, bZIP, basic leucine zipper, EtOH, Ethylene, GST, gene encoding glutathion-S-tranferase, MeJa, Methyl Jasmonate, NH1, rice ortholog of *A. thaliana* Nonexpresser of Pathogenesis Related genes1, OsSci2, gene encoding a subtilisin chymotrypsin inhibitor, PBZ1, gene encoding a probenazole-inducible protein, POX22.3, gene encoding peroxidase, PR1b, gene encoding pathogenesis related 1b protein, P450, gene encoding phytochrome P450, RCI-1, gene encoding a chloroplastic lipoxygenase, SA, Salicylic acid, UV-B, Ultraviolet radiations B, ZB8/PAL, gene encoding Phenylalanine ammonia-lyase.

NH1 or *OsWRKY45* respectively. This suggests that OsWRKY45 define a new defense transduction pathway acting independently of NH1 (*Figure1*). This is confirmed by the fact that two BTH induced genes (GST encoding a glutathione S-transferase, and P450 encoding a phytochrome P450) are under the control of *OsWRKY45* but do not require *NH1* whereas the expression of *PR1b* is mainly controlled by *NH1* but can be influenced to some extent by *OsWRKY45*. In addition, the *RCI-1* expression in response to BTH is independent of *OsWRKY45* and *NH1*. This revealed that the mechanisms of induction of defense genes by BTH and SA are more complex in rice than in *A. thaliana*.

OSWRKY31 LINKS DEFENSE ACTIVATION AND INHIBITION OF PLANT GROWTH

OsWRKY31 has been isolated from a rice cDNA library generated with elicitor-treated suspension cells (Zhang *et al*., 2008). The expression of *OsWRKY31* is induced by *M. grisea* inoculation, wounding and MeJa. Overexpression of *OsWRKY31* enhances resistance to *M. grisea* and the expression of *PBZ1* and *OsSci2*. *OsSci2* belongs to the subtilisin chymotrypsin inhibitor gene family. In this case it was not evaluated if the regulation of the expression by OsWRKY31 of the defense genes involves NH1. Interestingly, the plants overexpressing *OsWRKY31* exhibit a reduced number and a reduced growth of their lateral roots. *OsWRKY31* is also auxin inducible and its overexpression induces a resistance against TIBA an inhibitor of auxin transport. Auxin inducible and constitutive expression of *OsWRKY31* correlates with *OsIAA4*, a member of the early auxin response *Aux/IAA* gene family, and *ARL1/CRL1*, a gene involved in the control of adventitious and lateral root development (Inukai *et al*., 2005; Liu *et al*., 2005a). This suggests that this WRKY TF probably participates in the integration of the induction of defense and of a reduction of the growth. Studies of overexpressing or RNA interfered lines for *OsWRKY89*, a wound, MeJa, UV-B and ABA inducible gene, have revealed that this gene is involved in the regulation of the biosynthesis of wax, lignin and SA (Wang *et al*., 2007). Overexpression of OsWRKY89 in rice confers a reduced growth but an increased resistance to *M. grisea*, the white-backed planthopper insect pest and an enhanced tolerance to UV-B irradiation.

These data shows that WRKY TF play a central role in defense gene activation in plants, but that they act on different regulatory pathways in rice in comparison with what is known in *A. thaliana*.

Specific single TF of the AP2/ERF or NAC families are sufficient to confer rice tolerance to submergence, water deficit or salt stresses

SUB1-A1, A TF DISCOVERED IN LOWLAND RICE VARIETIES WHICH CONFERS RESISTANCE TO SUBMERGENCE

Abiotic stresses are diverse, often linked to a defect or excess of water, a defect in nutrients, an excess of salt or temperatures change. Because plants cannot move they have to adapt their physiology and development and change their metabolism to survive when they are submitted to these stresses. Some TF that integrate external signals and induce a complex adaptative response in plants have been characterized. This is the case of a particular Apetala 2 Ethylene Responsive Factor (AP2/ERF) TF, Sub1-A1 which confers submergence tolerance to rice. The root system of rice is adapted to

immersion and hypoxic conditions, by the development of a particular porous tissue, aerenchyma, which facilitates gas exchanges between shoot and the submerged roots. Nevertheless, most of the rice varieties are susceptible to a complete submergence and die in a few days. This is a major constraint for rice cultured in lowland, frequently sporadically inundated, in South and Southeast Asia. In these areas, some varieties have been selected from a long time, which can survive to a complete submergence during at least two weeks. Genetic analysis has revealed that this submergence tolerance correlate with a major Quantitative Trait Locus (QTL) located in the *Sub1* locus (Xu *et al*., 2000). The *Sub1* locus is characterized by the presence of 3 genes encoding AP2/ERF TF: Sub1-A, Sub1-B and Sub1-C (Fukao *et al*., 2006; Xu *et al*., 2006). All varieties tolerant to submergence possess the *Sub1-A1* allele whereas varieties susceptible to submergence either possess the *Sub1-A2* allele or in most cases the *Sub1-A* gene is absent. This suggests that *Sub1-A1* is responsible for submergence tolerance. This point was confirmed by the fact that transgenic overexpression or genetic introgression of *Sub1-A1* is sufficient to confer submergence tolerance. *Sub1-A1* and *Sub1-C* expressions are induced by submergence suggesting that these two genes of the *Sub1* locus are involved in the submergence response. Further functional studies were conducted using two near isogenic lines differing for the *Sub-A1* gene. When *Sub1-A* is absent the induction of *Sub1-C* by submergence is increased, suggesting that *Sub1-C* expression is negatively controlled by *Sub1-A1* and that *Sub1-C* participates to submergence susceptibility. Similar data were obtained in response to exogenous EtOH treatment. EtOH is an essential hormonal signal for acclimation to hypoxia conditions (Fukao and Bailey-Serres, 2004). In rice, after submergence, the EtOH level dramatically increases from one day of inundation and after 3 days the ethylene level is 1.6 lower in the *Sub-A1* line. *Sub1-A1* reduces the expression of expansin genes (*EXPA*), which are markers of cell elongation (Li *et al*., 2003). These data correlate with a reduction of plant growth under submergence observed in the *Sub1-A1* line. By contrast, in the lines where *Sub1-A1* is absent there is increase in growth in submergence conditions. During submergence, in the *Sub1-A1* line, the consumption of starch and soluble carbohydrate is slower as well as the induction of the expression of α-amylase genes (*RAmy3*) and sucrose synthase genes (*Sus*). Most *RAmy3* and *Sus* genes are EtOH inducible. By contrast genes encoding enzymes involved in fermentation such as *PDC* (pyruvate decarboxylase) and *ADH* (alcohol dehydrogenase) increase dramatically in the *Sub1-A1* line under submergence whereas their induction is limited in the line where *Sub1-A1* is absent. *PDC* and *ADH* genes are induced by EtOH. In addition, it was observed that chlorophyll breakdown induced by submergence was limited in the *sub1-A1* line. Taken together, these data show that Sub1-A1 TF regulates a set of adaptive mechanisms in condition of submergence such as chlorophyll protection, reduction of plant growth and carbohydrate catabolism as well as an increase in the ethanol fermentation pathway, resulting in plant surviving in submergence conditions (*Figure 2*). The *Sub1* locus, carrying *Sub1-A*, has been successfully introgressed by molecular assisted breeding in productive varieties, and protect these varieties against submergence.

NAC TF WHICH CONFER DROUGHT TOLERANCE IN RICE

SNAC1 (STRESS-RESPONSIVE NAC1) is another TF involved in stress resistance in rice (Hu *et al*., 2006). It corresponds to the *ONAC044* gene in rice (Ooka *et al*., 2003). NAC are TF specific of plants that are involved in stress response and in the

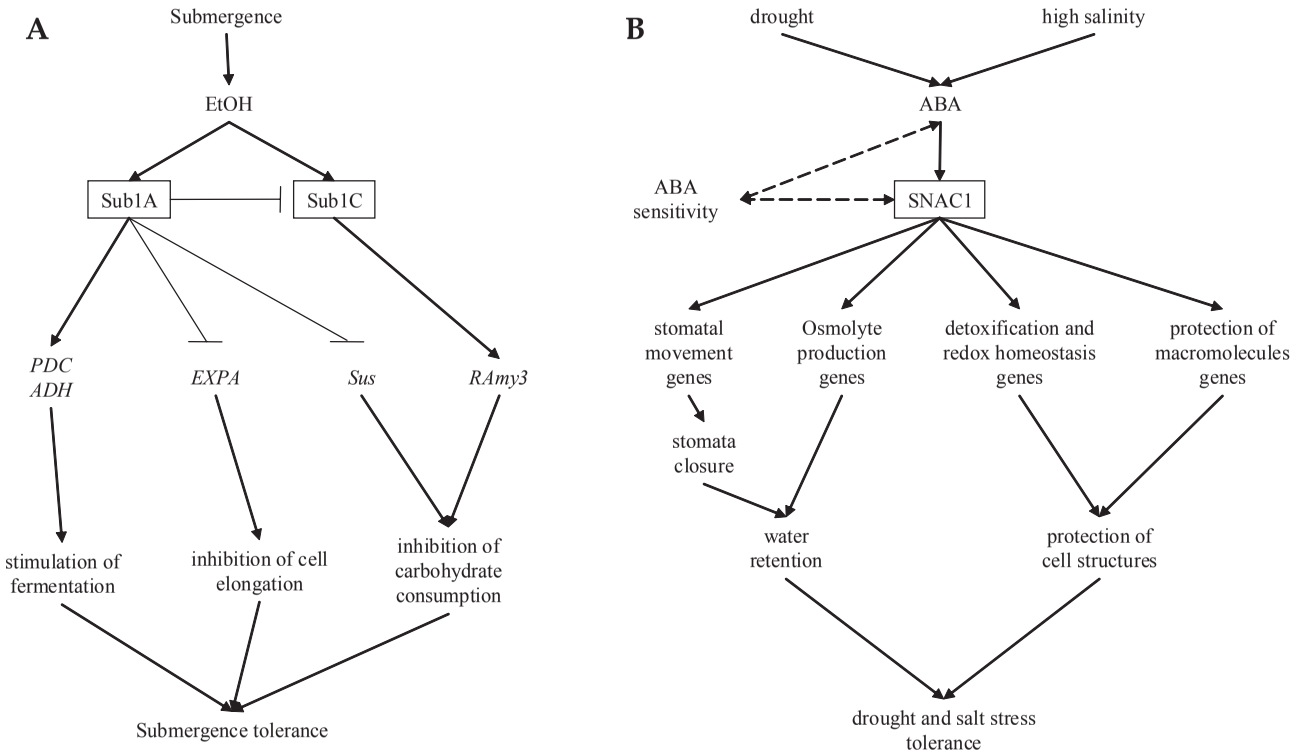

Figure 2. Networks involving AP2/ERF or NAC transcription factors for rice tolerance to water stresses. A: Network of rice tolerance to submergence. ADH, gene encoding alcohol dehydrogenase, EtOH, Ethylene, EXPA, genes encoding expansins A, PDC, gene encoding pyruvate decarboxylase, RAmy3, genes encoding α-amylases, Sub: genes encoding AP2/ERF transcription factors located in the sub locus corresponding to a major QTL for rice tolerance to submergence, sus, genes encoding sucrose synthases. B: Network of rice tolerance to water deficit. ABA, abscissic acid. Unbroken arrows represent positive direct causal relation between two elements of the network; broken arrows represent indirect causal consequences between two elements of the network. Lines ended by a perpendicular line indicate negative direct correlation between two elements of the network.

control of development (Ooka *et al*., 2003). A rice transcriptome analysis in response to drought stress revealed that *OsNAC1* is highly up regulated in response to this stress. Its expression is also induced by salt and cold stresses and by the stress hormone abscissic acid (ABA) involved in the induction of dehydration protective mechanisms in plants. Expression study of green fluorescent protein (GFP) placed under the control of *OsNAC1* promoter in plant, shows that this gene is normally expressed in ligulae, stamen and pistil and drop in leaves, predominantly in guard cells of stomata, when plants are subjected to drought stress. *OsNAC1* overexpression, has no visible effect in normal conditions but allows protection of plants against drought stress. All *SNAC1*-overexpressing plants produce more fertile spikelets under drought stress conditions than the WT plants. *SNAC1*-overexpression is associated to a better capacity of water retention in leaves linked to a higher rate of stomata closure without affecting the photosynthetic rate. This can be partially explained by the fact that *SNAC1*-overexpression seedlings have a greater sensitivity to ABA. *SNAC1*-overexpression allows also a better recovery of plants after rewatering. An enhanced tolerance of *SNAC1*-overexpressing plants against salt stress is also observed. A comparative transcriptome analysis between *SNAC1*-overexpressing plants and WT suggested that a set of 80 genes are upregulated in *SNAC1*-overexpressing plants. Forty of these genes are related to drought stress protection mechanisms such as specific signal transduction pathway, osmolyte production, detoxification and redox homeostasis, protection of macromolecules and stomatal closure. These data show that *SNAC1* control a complex gene regulatory network leading to drought and salt stress tolerance in rice (*Figure 2B*). Interestingly *SNAC1* overexpression does not affect plant growth and reproduction when cultured in well watered conditions. This is not the case for OsNAC6 TF. OsNAC6 is inducible by different abiotic stresses including cold, drought and hight salinity and by biotic stresses such as wounding and *M. grisea* (Nakashima *et al*., 2007). When overexpressed in plants it improves tolerance against dehydration, salt and *M. grisea*. Nevertheless *OsNAC6* overexpression also affects plant growth which is reduced in control culture condition. This disadvantage can be overcome if *OsNAC6* is expressed in plants under the control of its own promoter or with the stress inducible promoter of the *LIP9* gene (Rabbani *et al*., 2003) which encodes a low-temperature-induced protein. In these conditions plants present an increased tolerance to stresses but their growth is not affected in normal culture conditions (Nakashima *et al*., 2007). Trancriptome analysis has revealed that *OsNAC6* controls a set of 163 genes, among which 58 are inducible by drought, cold or salt stresses.

KNOWLEDGE OF *A. THALIANA* FACILITATES THE DISCOVERY AND THE ELUCIDATION OF THE FUNCTION OF NEW WATER-STRESS PROTECTIVE AP2/ERF TF ACTING IN RICE

Another important class of TFs involved in stress response and adaptation is the Dehydratation-Responsive Element-Binding protein (DREB1)/C-RepeaT (CRT)-Binding Factor (CBF). DREB1/CBF are members of the AP2/ERF family of TFs and bind Drought Responsive (DRE) and Cold Responsive CRE Elements and regulate plant response to stresses via a pathway independent of ABA. In *A. thaliana* several DREB/CBF TFs regulate the expression of genes related to cold, drought and salt resistance (Nakashima and Yamaguchi-Shinozaki, 2006). The activities of these TFs have yet to be studied in rice. Overexpression experiments in *A. thaliana* of OsDREB1A have

shown that this rice TF has a function similar of its *A. thaliana* homolog in the regulation of stress responsive gene expression and confers tolerance to drought, high-salt, and freezing stresses (Dubouzet *et al*., 2003). Similarly, two stress related *A. thaliana* TFs were overexpressed in rice: DREB1A/CBF3 and ABF3 (Oh *et al*., 2005). ABF3 is a bZIP TF which processes through a ABA dependent signal transduction pathway. It binds ABA-responsive elements to induce the expression of stress protection related genes. DREB1A/CBF3 enhanced tolerance to drought and to high salinity stresses in rice, whereas in *A. thaliana* this TF is mainly known to control a gene regulatory network involved in tolerance to freezing (Maruyama *et al*., 2004). ABF3 induce drought stress tolerance in rice and *A. thaliana* (Kang *et al*., 2002). These data have shown that the general function of stress related *A. thaliana* TFs is conserved in rice; however the function of DREB1A/CBF3 has differentially evolved in regard to the stress considered. As in these experiments the same TF has been overexpressed in A. thaliana or in rice, it is likely that the evolution has affected the target gene sequences of this TF leading to a regulation of gene regulatory networks related to the response to different stresses in each species. In addition, these data show that the ABA-dependent and ABA-independent stress signal transduction pathways are also conserved in rice and further that these transduction pathways have been used during the process of evolution to control the specific response and adaptation to different stress in these two species. Screening of an activation tagged mutant collection in *A. thaliana* has allowed the identification of *HARDY*, a gene encoding an AP2/ERF TF involved in drought and stress tolerance (Karaba *et al*., 2007b). When overexpressed in *A. thaliana* this gene promotes the mesophyll cell multiplication in leaves and root proliferation. This is associated with an increase of drought and salt stress tolerance. Similar phenotypes are observed in rice where *HARDY* overexpression increases the number of bundle sheath cells in leaves, root development in stress condition and enhances drasmatically the tolerance to drought stress. Physiologically these *HARDY* overexpressing lines are characterized by a better rate of photosynthesis carbon assimilation under stress condition, associated with higher efficiency of the photosystem II and lower levels of transpiration. In conclusion, the function of HARDY is mainly conserved in rice, and it is interesting to note that this TF seems to regulate drought and salt stresses tolerance in plants by acting both on tolerance (water use efficiency) and on escape (water uptake increase linked to an increased root development) strategies.

bHLH and other TFs control the adaptation to mineral nutrient deficiency

OSPTF1 AND OSPHR2 ENHANCE THE EFFICIENCY OF PHOSPHORUS UTILIZATION AND UPTAKE

Mineral nutrition of plants is one of the most limiting steps for their growth. Among the major mineral nutriment, phosphorus, absorbed by plants in the inorganic phosphate form (Pi), is required for growth, development and reproduction. In *A. thaliana*, a Myb transcription factor encoded by *PHR1* participates in the Pi starvation signaling system (Rubio *et al*., 2001) and Pi starvation induces complex transcriptional regulation responses that affect several genes encoding TF (Hammond *et al*., 2003; Wu *et al*., 2003). Nevertheless their functional roles in Pi starvation adaptation remain limited. In rice a cDNA encoding a bHLH TF, *OsPTF1* has been isolated from a cDNA subtracted

library obtained from roots of plant cultured in normal or Pi-deficient conditions (Yi *et al*., 2005). This TF possesses a DNA binding domain with residues typical of Gbox binding domains. G-box (CACGTG) is one type of E-box (CANNTG) recognized by bHLH TF. *OsPTF1* expression is constitutive in shoot but is induced in response to Pi starvation in all tissues of lateral roots and in the phloem cells of the elongation zone of the primary root. When *OsPTF1* is overexpressed, it does not change the phenotype of plants developing in high-Pi level condition. Whereas OsPTF1 overexpression stimulates, in comparison with control plants, tillering, biomass accumulation, panicle weight and Pi content when plants are cultured in low-Pi condition. Further studies indicate that *OsPTF1* acts on the stimulation of Pi uptake rate by stimulating root growth resulting in an increase of the root surface area. A transcriptome analysis has revealed that OsPTF1 overexpression has an impact on the expression rate of 158 genes most of them possessing an E-box element in their promoters and 20% of them a G-box, suggesting that at least these last ones could be direct target genes for OsPTF1. The genes regulated by OsPTF1 are known to play a role in the Pi starvation rescue (Bariola *et al*., 1994) such as RNS1 ribonuclease, H+-transporting ATPase and vacuolar H+-pyrophosphatase (Schachtman *et al*., 1998). But interestingly no high-affinity Pi transporter genes were found, suggesting that in this case these transporters are not essential for phosphate-starvation tolerance, but that this tolerance is mostly based on adaptive root development which constitutes the basis of the increase in Pi uptake rates, and of a better efficiency of phosphorus utilization (*Figure 3A*). *OsPHR2* was isolated and studied in rice on the basis of its similarity with AtPHR1 which plays a central role in Pi-starvation signaling in *A. thaliana* (Bari *et al*., 2006). In response to Pi-starvation, AtPHR1, a member of the MYB-CC TF family, controls the expression of a set of Phosphate Starvation Genes (PSI), including high affinity phosphate transporters and also the expression of miR399 which target the inhibition of PHO2. PHO2 is a ubiquitin conjugating enzyme which negatively regulates the expression of a subset of PSI genes. Interestingly, in this model, miR399 is the systemic signal for phosphate starvation (Bari *et al*., 2006). *OsPHR2* also encodes a MYB-CC TF (Zhou *et al*., 2008). Plants overexpressing OsPHR2 present an excessive Pi accumulation in shoots and modification of root morphogenesis characterized by an increased number and length of root hairs. In these plants several genes related to phosphate metabolism and uptake, including phosphate high affinity transporters, are also upregulated. An induction of the expression of rice *miR399* is also observed, but this augmentation is not correlated with a decrease in steady state level of *OsPHO2* mRNA. These data show that OSPHR2 is a central regulator of response to phosphate starvation in rice. Nevertheless, its action seems to be different in rice and in *A. thaliana* in regard to the regulation of the *OsPHO2* mRNA via *miR399*. This aspect has to be further studied and also the interactions between the phosphate starvation regulatory networks controlled by OsPTF1 and OsPHR2 (*Figure 3A*).

GRAMINACEOUS Fe ACQUISITION AND UTILIZATION INVOLVE SPECIFIC MECHANISMS CONTROLLED BY PARTICULAR TF DISCOVERED IN RICE, OSRO2 AND IDEF1

Iron is another essential mineral that plants take-up from the soil. Iron deficiency induces chlorosis, reducing crop yield and quality. At high pH, like in calcareous soils, iron is sparingly soluble and weakly available for plants. Two strategies have been developed by plants for Fe acquisition. The first one, used by non-graminaceous plants

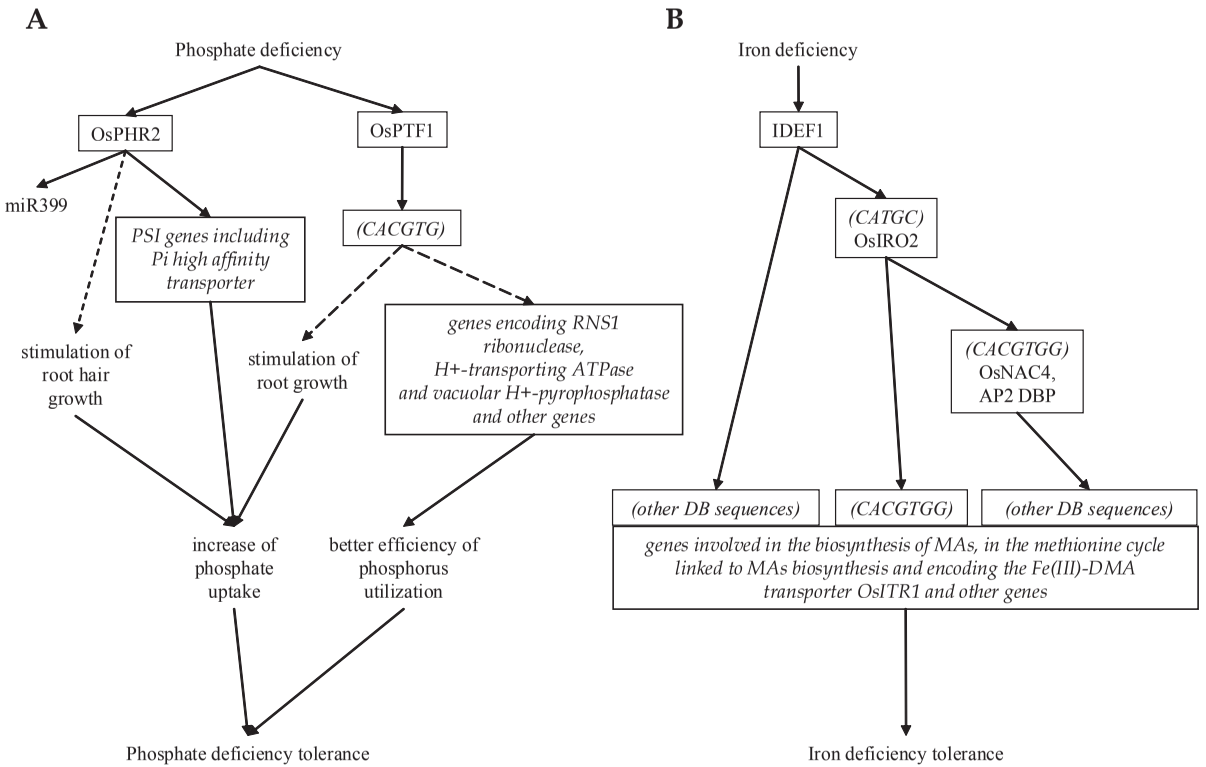

Figure 3. Networks involving bHLH transcription factors for tolerance to mineral nutrition. deficiencyA: Network of rice tolerance to phosphate deficiency. OsPHR2, gene encoding *O. sativa* PHR2 transcription factor of the MYC-CC family, Pi, phosphate, PSI, Phosphate Starvation Inducible, OsPTF1, gene encoding *O. sativa* Phosphate Transcription Factor of the bHLH family, (CATGC) or (CACGTGG) indicates the DNA binding sequence recognized by the transcription factor located just upstream in the network. B: Network for rice tolerance to iron. IDEF1: gene encoding the Iron Deficiency transcription Factor of the ABI3/VP1 family, OsIRO2, gene encoding the *O. sativa* IROn 2 bHLH transcription factor.

consists in protons excretion from roots to induce the reduction of Fe(III) in Fe(II) which is more soluble. This pathway is partially under the control of bHLH TF, FER in tomato and FIT1/TFU/AtbHLH29 in *A. thaliana* that regulates the expression of *FRO* encoding ferric chelate reductase and *IRT1* an Fe(II) transporter protein (Colangelo and Guerinot, 2004; Jakoby *et al*., 2004; Ling *et al*., 2002; Yuan *et al*., 2005). *FER* and *FIT1* are specifically expressed in roots whereas the most homologous gene in rice is not expressed in roots suggesting that TF involved in Fe deficiency response are different in rice (Li *et al*., 2006). The second strategy used for Fe acquisition by graminaceous plants, consists of the excretion of Fe(III) chelators such as mugineic acid family phytosiderophores (MAs). Genes encoding enzymes involved in the biosynthesis of MAs including *IDS2* encoding dioxygenase, are coordinately up-regulated by Fe deficiency (Kobayashi *et al*., 2005). In rice an Fe-deficiency-inducible bHLH TF, OsRO2 has been identified (Ogo *et al*., 2006; Ogo *et al*., 2007). This TF binds a G-box CACGTGG sequence. It is homologous to AtbHLH38 and AtbHLH39 which are also Fe-deficiency inducible in seedlings (Li *et al*., 2006; Vorwieger *et al*., 2007). *OsRO2* RNA interfered lines are more sensitive than WT or *OsRO2* overexpressing lines to Fe deficiency based on chlorophyll and Fe content measurements, and growth ability. A comparative transcriptome analysis between *OsRO2* RNA interfered lines and WT has revealed that *OSRO2* is involved in the regulation of all the genes involved in the biosynthesis of MAs, methionine cycle linked to MAs biosynthesis and the gene encoding the Fe(III)-DMA transporter OsITR1. Among these genes some of them possess the OSRO2 DNA binding sequences in their promoter and could be direct target of this TF. Two genes encoding TF, *OsNAC4* and the *AP2 domain-binding protein* gene, which possess also the OSRO2 DNA binding sequences in their promoters, were shown to be differentially regulated in transcriptome experiment. This suggests that these two genes are directly regulated by OSRO2 TF and their products might participate in the regulation of the other differentially expressed genes without the OsIRO2 DNA binding sequence. Interestingly the *OsIRO2* promoter contains a sequence similar to *IDE cis*-acting elements (Kobayashi *et al*., 2005; Ogo *et al*., 2006). *IDE1* and *IDE2* are two iron-deficiency-responsive *cis*-elements which were previously identified in the *IDS2* promoter in barley (Kobayashi *et al*., 2003). IDE1 includes a CATGC sequence found also in the *Sph RY* motif (TCCATGCAT) which interact with the B3 binding domain of the ABI3/VP1 TF family (Reidt *et al*., 2000; Suzuki *et al*., 1997). In rice the only member of the ABI3/VP1 TF family already characterized is OsVP1, the functional ortholog of the maize VIVIPAROUS 1 (Hattori *et al*., 1994). Further research for rice genes containing a B3 domain leads to the identification of four genes. Out of these, only *IDEF1* encodes TF able to bind the IDE1 sequence (Kobayashi *et al*., 2007). IDEF1 expression occurs in all vegetative organs of the plant but is not regulated by iron-deficiency. Overexpression of IDEF1 in rice confers tolerance of iron deficiency and correlate with an increase of the expression of OSRO2 TF and of its targets, suggesting that IDEF1 regulate the expression of *OSRO2* and by consequence of its downstream targets. This places IDEF1 TF upstream of a regulatory cascade leading to the iron deficiency tolerance in graminaceous species (*Figure 3B*). Because *IDEF1* expression is not regulated by iron deficiency, it is likely that the TF activity itself is directly regulated by post-translational mechanism.

The identification and functional characterization of OsTF1, OSRO2 and IDEF1 opens the way to the improvement of phosphate or iron deficiency tolerance by manipulating the whole gene regulatory networks controlled by these TFs.

Conclusions

RICE SUPPORTS THE DISCOVERY OF NOVEL TF AND NEW SPECIFIC REGULATORY MECHANISMS CONTROLLING THE TOLERANCE OF PLANT TO STRESSES

TFs allow controlling gene regulatory networks leading to the expression of complex phenotypes. In plants some TFs belonging to specific families have been employed during evolution to control defense against stresses. Some specific WRKY TFs play a crucial role in rice in the regulation of defense mechanism leading to resistance or tolerance against diverse bacteria, fungi or insect pests (Liu *et al*., 2007; Liu *et al*., 2005b; Shimono *et al*., 2007; Wang *et al*., 2007; Zhang *et al*., 2008) whereas some AP2/ARF or NAC TF are specifically involved in submergence (Fukao *et al*., 2006; Xu *et al*., 2006), drought, cold or salt stress tolerance (Dubouzet *et al*., 2003; Hu *et al*., 2006) and bHLH TF in mineral deficiency adaptation (Ogo *et al*., 2006; Ogo *et al*., 2007; Yi *et al*., 2005). When overexpressed in rice, a TF is generally sufficient alone to induce the expression of a set of genes encoding proteins necessary to induce a plant tolerance to the related stress. Interestingly these studies in rice have revealed that some TFs belonging to the same family are present in rice as well as in *A. thaliana* to control gene expression in response to a similar stress, although differences exist between the two species. This is well illustrated for the WRKY TF involved in the resistance against pathogens in rice and *A. thaliana*. They participate to a similar network including the regulatory protein NPR1 (in *A. thaliana*) or NH1 (in rice), but the difference is that in *A. thaliana* WRKY TF acts downstream of NPR1 to participate in the induction of defense genes whereas in rice WRKY TF acts upstream of NH1 or in NH1-independent pathway to regulate defense genes (Liu *et al*., 2007; Liu *et al*., 2005b; Shimono *et al*., 2007). Heterologous expression of AP2/ERF TF from rice to *A. thaliana* and vice versa suggests that TFs belonging to the same family have been selected for the defense against abiotic stresses. Whereas differences in function of these TFs in rice or in *A. thaliana* have been observed since they are not always involved in the response to the same stress (Dubouzet *et al*., 2003; Maruyama *et al*., 2004; Oh *et al*., 2005). This clearly shows that studying rice can lead to the discovery of new mechanisms for stress defense in plants in addition to that has been discovered in *A. thaliana*. Rice also opens access for deciphering new TF controlling mechanisms that are more specific to the biology of this species. This is substantiated by the discovery of the AP2/ERF Sub1-A1 TF involved in submergence tolerance mechanisms (Fukao *et al*., 2006; Xu *et al*., 2006) or by the bHLH OsRO2 and the ABI3/VP1 IDEF1 TF which control the graminaceous specific mechanisms for Fe uptake (Kobayashi *et al*., 2007; Ogo *et al*., 2006; Ogo *et al*., 2007). Further, it is also likely that what is discovered in rice will be useful to improve other graminaceous important crop species for Fe deficiency tolerance.

TF AND THE BALANCE BETWEEN RESISTANCE OR TOLERANCE MECHANISMS INDUCTION AND PLANT DEVELOPMENT

One of the limitations of overexpression of biotic or abiotic stress resistance TF is that as they act upstream of complex genetic regulatory cascade the positive effect that they confer in stress resistance improvement is counterbalanced by the defect in normal plant growth. (Liu *et al*., 2007; Wang *et al*., 2007; Zhang *et al*., 2008). Growth

repression is also an adaptive response to various stresses but from an agronomical point of view, where yield is a major trait of interest, this adaptive response should be avoided by placing stress related TFs under the control of stress inducible promoter in place of constitutive ones (Nakashima *et al*., 2007). By this mean the defense mechanisms controlled by the TF are induced only when the plant is submitted to a stress, but not in normal conditions where plant can develop normally. The mechanisms controlling the balance between the production of defense molecules and plant growth are not known, but the study of the WRKY TF OsWRKY31 can start to enlighten them. OsWRKY31 controls the expression of defense genes and of genes involved in auxin signaling and adventitious roots development (Zhang *et al*., 2008). How this TF links defense induction with the control of root development needs to be clearly understood. Sometimes modification of the development is part of the adaptive response of the plant to the stress. This is demonstrated by OsPTF1 TF that controls plant response to Pi deficiency by inducing genes involved in a better efficiency of phosphorus utilization concomitantly with an induction of root development and branching leading to an increase in root surface which allows an increase of Pi uptakes rates by the plant (Yi *et al*., 2005). This kind of adaptive response based on modification in root development is also involved in water deficit tolerance. At present, rice TFs involved in drought tolerance are TFs controlling genes related to the conservation of water and by promoting water use efficiency elevated rates (Karaba *et al*., 2007a). The other way for the plant is to escape water stress by an adaptive developmental response leading to an enhancement of its capacity to take water in soil by an increase in root development. Such a mechanism has been described in this review for Pi deficiency tolerance (Yi *et al*., 2005) and it is evident that adaptive root developmental responses might have a positive impact on the management by the plant to various soil related resources deficiency such as water and mineral nutrients.

DECIPHERING GENE REGULATORY NETWORKS INVOLVED IN ADAPTIVE ROOT DEVELOPMENT WILL GIVE NEW TOOLS TO IMPROVE WATER DEFICIENCY TOLERANCE

Water deficit is a major limiting step for rice culture. Rice uses 2 to 3 times more water than other food crop species. It is estimated that 30% of the freshwater used for crop worldwide is devoted to rice culture (Khush, 2005). Since root architecture is a major determinant of drought avoidance, a strategy to increase yield under limiting water availability is to develop new strategies to build optimal rice root architecture like producing a more branched and deeper root system, to improve soil exploration and water extraction. If breeding seems the most straightforward approach to select better adapted root architecture, selection for specific root ideotypes is hard and slow due to the low heritability of root morphology traits and also because of the high phenotypic plasticity of root development (Yue *et al*., 2006). A better knowledge of the key genes involved in root development will in the future help breeders to select improving rice cultivars using molecular assisted selection (MAS), a more direct and easier procedure when the phenotyping of the trait of interest is difficult. The elucidation of the function of rice genes encoding root specific TF possibly involved in root development will be a first step in this direction. Information regarding the gene regulatory network and related TF involved in root meristem maintenance, root radial patterning or root hair differentiation at present is available to a greater extent in *A.*

thaliana (Montiel *et al*., 2004). In rice, the *CRL1*/*ARL1* gene encodes an ASYMETRIC LEAVES2(AS2)/LATERAL ORGAN BOUNDARIES (LOB)-type TF which control the formation of adventitious crown-roots (Inukai *et al*., 2005; Liu *et al*., 2005a). Crown roots are adventitious roots which do not normally develop in *A. thaliana*. Interestingly, the expression of this gene is regulated by an Auxin Response factor (ARF) TF suggesting that auxin, ARF and other specific TF controls adventitious and lateral root organogenesis in rice. Further information on the function of CRL1/ARL1 TF and on its target genes have to be acquired in particular in regard to the control of adaptive adventitious root morphogenesis in response to stresses. In this regard it will be interesting to know by what mechanisms *CRL1/ARL1* is regulated by the stress responsive OsWRKY31 TF (Zhang *et al*., 2008). In *A. thaliana*, TF involved in the control of lateral root differentiation have been characterized. Lateral root initiation is under the control of auxin and two ARF, namely NPH4/ARF7 and ARF19. These two ARF TFs act redundantly for lateral root initiation via the activation of AS2/LOB TFs (Okushima *et al*., 2007). NAC1 is another TF that is implicated in lateral root formation downstream of auxin signal (Xie *et al*., 2000). *NAC1* transcripts were localized in the root tip and in the lateral root initiation region. Over-expression of *NAC1* increases the number of lateral root whereas *NAC1* antisense expression reduces lateral root initiation. After an initial auxin-triggered increase, NAC1 levels are reset by the ubiquitin dependant proteolysis pathway via SINAT5, a ubiquitin ligase and by RNA degradation via the micro RNA miR164, suggesting that the activity of this TF is regulated by different post-transcriptional or post-translational mechanisms (Guo *et al*., 2005; Xie *et al*., 2002). Lateral root development is also dependent on nutritional cues. Nitrate which is the major source of nitrogen plays an important role in lateral root growth. A MADS box TF (ANR1) has been shown to mediate nitrate-induced lateral root development (Zhang and Forde, 1998). *ANR1* is preferentially expressed in roots and is induced at the transcriptional level by nitrate. Antisense and co-suppressed transgenic lines for this gene present reduced lateral root elongation with increasing NO_3^- concentrations. *Agamous-Like 21 (AGL21)*, another gene encoding a MADS-box TF belonging to the *ANR1* monophyletic clade, has recently been isolated (Burgeff *et al*., 2002). It displays similar expression patterns as *ANR1* in the apex of the lateral root primordium but its function has yet to be determined. Other genes encoding MADS-box TFs such as *AGL12*, *AGL14*, *AGL17*, *AGL19*, *AGL52* are specifically expressed in roots (Burgeff *et al*., 2002), but since now their function remains to be discovered as loss of function for each of these genes does not rise to any visible root phenotype, suggesting the existence of genetic redundancy between them. Nevertheless, their root specific expression patterns suggest a possible role in root development. The functions of these TFs have now to be clarified in *A. thaliana* and rice. This will probably give rise to the discovery of new TFs involved in the adaptive root development allowing rice to escape water or mineral deficiency stresses and formulating novel genetics tools to improve rice, this worldwide important food crop specie.

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