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High-throughput analysis of rice genes by means of the heterologous full-length cDNA overexpressor (FOX)-hunting system

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ABSTRACT Mutant populations are indispensable tools for investigating plant gene functions. Gain-of-function technology is one of the approaches used for the systematic production of mutant resources and activation tagging is a well-established method to generate gain-of-function mutants in plants. As an alternative approach for the systematic generation of a gain-of-function mutant population, we developed the Full-length cDNA OvereXpressor (FOX)-hunting system in which full-length cDNAs (fl-cDNAs) are overexpressed in plants to quickly identify candidate genes. The FOX-hunting system was used for high-throughput analysis of rice (*Oryza sativa*) genes heterologously expressed in *Arabidopsis thaliana* (rice FOX *Arabidopsis* lines). A large screening to identify and characterize rice genes with rice FOX *Arabidopsis* lines revealed that one of the isolated genes, *BROAD-SPECTRUM RESISTANCE 1 (BSR1)* conferred multiple or broad-spectrum disease resistance in both a dicotyledonous and monocotyledonous plant. We found that expression of rice fl-cDNAs without a homolog in *Arabidopsis* affected morphological traits. In addition, overexpression of homologous genes of rice and *Arabidopsis* led to a similar phenotype. Thus, we conclude that the FOX-hunting system is an excellent heterologous system and offers a new tool with which to explore gene function in rice.

KEY WORDS: *full-length cDNA, rice, Arabidopsis, gain-of-function*

Introduction

Arabidopsis thaliana (L;) Heyhn. is the first higher plant of which the genome has been sequenced (Kaul *et al*., 2000). As the sequencing technology has progressed, the genomes of many other plant species have been determined (for a review, see Mochida and Shinozaki, 2010). Whereas *Arabidopsis* is very much the model dicotyledonous plant, rice (*Oryza sativa*) has been selected as the model monocotyledonous plant. In the meantime, the rice genome has been sequenced (Matsumoto *et al*., 2005) of which, to date, the quality is similar quality to that of *Arabidopsis*. Since the completion of the genome sequencing, researchers have focused on understanding the gene functions and the signaling networks involved in development and response to environmental changes. Functional genomics has become an important tool in the post-sequencing era to describe the biological function of every gene products. Research tools, such as transcriptome (Rensink and Buell, 2005), proteome (Agrawal and Rakwal, 2011) and computational annotation (Ouyang *et al*., 2007; Tanaka *et al*., 2008), have been created to understand gene functions in rice. These studies enabled us to analyze functionally rice genes on a large scale. In addition, biological resources have been developed for gene function identification. More than 28,000 full-length cDNAs (fl-cDNAs) of rice have been collected (Kikuchi *et al*., 2003). Mutant collections are other useful resources and high-throughput screens using these mutant populations should provide a means to analyze plant gene functions.

Some of the most important mutant resources are loss-of-function mutants, because their phenotypes are often the best clue to understand gene functions (Hirochika, 2001; Kuromori *et*

Abbreviations used in this paper: BSR1, BROAD-SPECTRUM RESISTANCE 1 gene; CaMV, Cauliflower mosaic virus; fl-cDNA, full-length cDNA; FOX, full-length cDNA overexpressor; TMV, tobacco mosaic virus.

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al., 2009). Large sets of loss-of-function mutant resources of rice are available, including natural variations and artificial mutants caused by chemically induced mutagenesis, *Tos17* transposon, and T-DNA insertional mutagenesis (for a review, see Hirochika *et al.*, 2004). Besides loss-of-function mutants, gain-of-function mutants are alternative resources (Kondou *et al.*, 2010). In rice, approximately 10,000 genes are grouped into families (Tanaka *et al.*, 2008). When functionally redundant paralogous genes of the gene of interest occur in the genome, its function is difficult or impossible to uncover with a knockout approach. Therefore, gain of function can complement a loss-of-function approach when redundant members of gene families are analyzed.

The classical approach for generating a gain-of-function mutant population in plants is the activation tagging system. This method has been developed by using transcriptional enhancers, such as the Cauliflower Mosaic Virus *35S* (*CaMV 35S*), to activate genes located proximally to the T-DNA or transposon insertion sites. We have developed an original gain-of-function mutagenesis method, designated Full-length cDNA OvereXpressing (FOX)-hunting system. In this system, fl-cDNAs are used for ectopic gene expression in plants. In this review, we describe two types of gain-of-function approaches and investigate a gene function in rice through analysis of these mutant resources.

Activation tagging in rice

Activation tagging, a well-established technology for generating gain-of-function mutants in plants, has been used for more than 10 years. A T-DNA-containing enhancer sequence is randomly introduced into the plant genome where it boosts the expression of adjacent genes (Weigel *et al.*, 2000). In rice, activation-tagging lines have been constructed since 2002 (Jeong *et al.*, 2002) and several groups have created more than 150,000 lines in total by using different activation-tagging vectors. From these lines, valuable genes have been identified (for a review, see Zhang *et al.*, 2009; Tsuchida-Mayama *et al.*, 2010; Lee *et al.*, 2011), but in most cases, multimeric *CaMV 35S* enhancers are used.

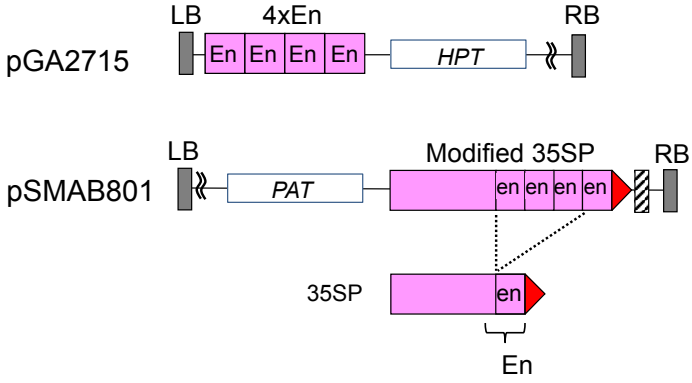

Fig. 1. Schematic representation of a traditional and a modified activation-tagging vector pGA2715 and pSMAB801, respectively. *Abbreviations: En,* CaMV *35S enhancer element (–417 to –86); en,* CaMV *35S enhancer element (–287 to –91);* HPT, *hygromycin phosphotransferase gene;* PAT, *phosphinothricin acetyltransferase gene; LB, left border sequence; RB, right border sequence; 35SP,* CaMV *35S promoter; pink box, 5′-upstream sequence of* CaMV *35S minimum promoter; red triangle,* CaMV *35S minimum promoter; hatched box, first intron of the* phaseolin *gene.*

Although pGA2715 is typical activation-tagging vector in rice (Jeong *et al.*, 2002) (Fig. 1), a modified activation-tagging vector, pSMAB801, has been developed that contains a *CaMV 35S* promoter with tetrameric enhancers (Mori *et al.*, 2007) (Fig. 1). Generally, in a vector, such as pGA2715, the -417 to -86 upstream region of the *CaMV 35S* promoter is used as the enhancer element and tetramerized (Weigel *et al.*, 2000; Jeong *et al.*, 2002), but, because the -287 to -91 region has a stronger enhancer effect in rice protoplasts, this shortened region is utilized as the enhancer in pSMAB801. In addition, a *CaMV 35S* minimum promoter and the *phaseolin* first intron have been placed downstream of the tetramerized enhancers to activate flanking genes more strongly (Fig. 1).

Approximately 13,000 activation-tagging rice lines were generated using pSMAB801 and many morphological mutants were obtained. *Spotted leaf 18* (*Spl18*) and *Short grain 1* (*Sg1*) were mutants isolated by visual screening (Mori *et al.*, 2007; Nakagawa *et al.*, 2012). In *Spl18,* overexpression of a new acyltranferase gene (*OsAT1*) induced a lesion-mimicking and disease resistance phenotype (Mori *et al.*, 2007). In *Sg1,* overexpression of the novel gene, *SG1*, induced a phenotype of short grains and dwarfing, reminiscent of brassinosteroid-deficient mutants. In contrast, knockdown rice plants that down-regulated both *SG1* and a related gene *SG1-LIKE PROTEIN1 (SGL1)* had longer grains (Nakagawa *et al.*, 2012). Both *SPL18* and *SG1* genes are specific genes for monocots and have homologs in rice. Therefore, the functions of these genes would not have been identified easily without using a gain-of-function approach.

FOX-hunting system

Although activation tagging is a very valuable tool for the generation of gain-of-function mutants, the overexpression effect depends on the insertion site of the T-DNA or transposable element. In addition, *CaMV 35S* enhancers can influence the activation of genes up to several kb from the insertion site (Hsing *et al.*, 2007), thereby, in some cases, complicating the identification of the genes responsible for the observed phenotypes.

To overcome these problems, a different approach has been developed to systematically generate gain-of-function mutants, the FOX-hunting system (Ichikawa *et al.*, 2006) (Fig. 2). As fl-cDNAs contain all the information for the production of functional RNAs and proteins, they were expressed to obtain gain-of-function mutations. With this system, approximately 10,000 fl-cDNAs obtained from the RIKEN *Arabidopsis* fl-cDNA collection (Seki *et al.*, 2002) were used to generate *Arabidopsis* transgenic plants. Each fl-cDNA was mixed at approximately the same molar ratio and cloned into an expression vector. To facilitate the translation initiation, Ω sequences of the Tobacco Mosaic Virus (TMV) were included into the expression vector. Expression constructs harboring the fl-cDNAs were transformed with *Agrobacterium tumefaciens* and, in turn, the *Agrobacterium* library to engineer *Arabidopsis* plants *in planta*. T_1 plants expressing individual fl-cDNAs were self-pollinated and the T_2 seeds were harvested. These transgenic plants are the *Arabidopsis* FOX lines and approximately 23,000 were obtained. The introduced fl-cDNA(s) can be identified quickly with vector-specific primers and, thus, the fl-cDNAs and their effects as transgenes can be analyzed easily.

On average, approximately 2.6 fl-cDNAs were introduced into each *Arabidopsis* FOX line and 1,487 morphological mutants were

TABLE 1

SOME OF THE RICE GENES FUNCTIONALLY CHARACTERIZED THROUGH THE RICE FOX *ARABIDOPSIS* LINES

Gene Name	Function/Description	Phenotype	Reference
OsHsfA2e	Heat stress transcription factor	Heat stress tolerant	Yokotani *et al.* (2008)
OsNAC063	NAC transcription factor	Salt stress tolerant	Yokotani *et al.* (2009b)
OsSMCP1	Small protein with a single C2 domain, a Ca^{2+}-dependent membrane-targeting domain.	Salt stress tolerant	Yokotani *et al.* (2009a)
OsLBD37	Lateral organ boundaries domain	Metabolite	Albinsky *et al.* (2010)
OsCEST	Unknown	Salt stress tolerant	Yokotani *et al.* (2011)
BSR1	Putative receptor-like cytoplasmic kinase	Resistance to rice blast	Dubouzet *et al.* (2011)
FNR1/FNR2	Ferredoxin $NADP^+$-oxidoreductase	Photosynthesis	Higuchi-Takeuchi *et al.* (2011)
Jamyb	R2R3-type MYB transcription factor	Salt stress tolerant	Yokotani *et al.* (2013)

found in 15,547 lines (Ichikawa *et al.*, 2006). Many different mutants were screened, including those in chloroplast development (Okazaki *et al.*, 2009), organ size (Breuer *et al.*, 2009), carbon and nitrogen responses (Sato *et al.*, 2009), trehalose resistance (Delatte *et al.*, 2011), and brassinosteroid response (Schneider *et al.*, 2012). Thus, the FOX-hunting system and the genetic lines are invaluable resources for high-throughput analysis of gene function in *Arabidopsis*.

FOX rice lines

To analyze rice genes, the FOX-hunting system was used by overexpressing rice fl-cDNAs (Nakamura *et al.*, 2007) and appoximately 12,000 lines were generated in which 13,980 independent fl-cDNAs were overexpressed. Various visible phenotypes were observed in the T_0 generation, involving changes in cell and tissue proliferation, organ morphology, plant height, growth habit, heading date, and seed fertility. Approximately 16.6% of these FOX rice lines showed altered growth or morphological characteristics. The observed phenotype could be reproduced by retransformation of the introduced fl-cDNA into rice and one dwarf mutant was characterized that was caused by overexpression of a novel gibberellin 2-oxidase gene (Nakamura *et al.*, 2007). By means of these FOX rice lines, green calli overexpressing the Golden2-like transcription factor (*OsGLK1*) were isolated and chloroplast development was demonstrated to be regulated by *OsGLK1* (Nakamura *et al.*, 2009). In addition, a mutant with increased plant height and seed size overexpressing the *TIFY* gene (*TIFY11b*) was identified and shown to increase its grain size by carbohydrate accumulation (Hakata *et al.*, 2012).

Rice FOX *Arabidopsis* lines

The advantage of heterologous gene expression is that it is possible to analyze gene function by using another plant species as a host. *Arabidopsis* is one of the best host plants, because a very efficient, fast, and high-throughput transformation system has been developed. Therefore, we utilized *Arabidopsis* as the host plant and rice as the heterologous fl-cDNA resource for functional analysis of rice genes. A rice fl-cDNA expression library was generated with approximately 13,000 independent rice fl-cDNAs. From this library, more than 33,000 independent *Arabidopsis* transgenic lines (rice FOX *Arabidopsis* lines) expressing rice fl-cDNAs were obtained (Kondou *et al.*, 2009). Several screening types were performed with these rice FOX *Arabidopsis* lines to isolate putative mutants with alterations in a range of phenotypes, including morphology, photosynthesis, metal element and pigment accumulation, hormone profiles, secondary metabolites, pathogen resistance, salt tolerance, UV signaling, high-light tolerance, and heat stress tolerance

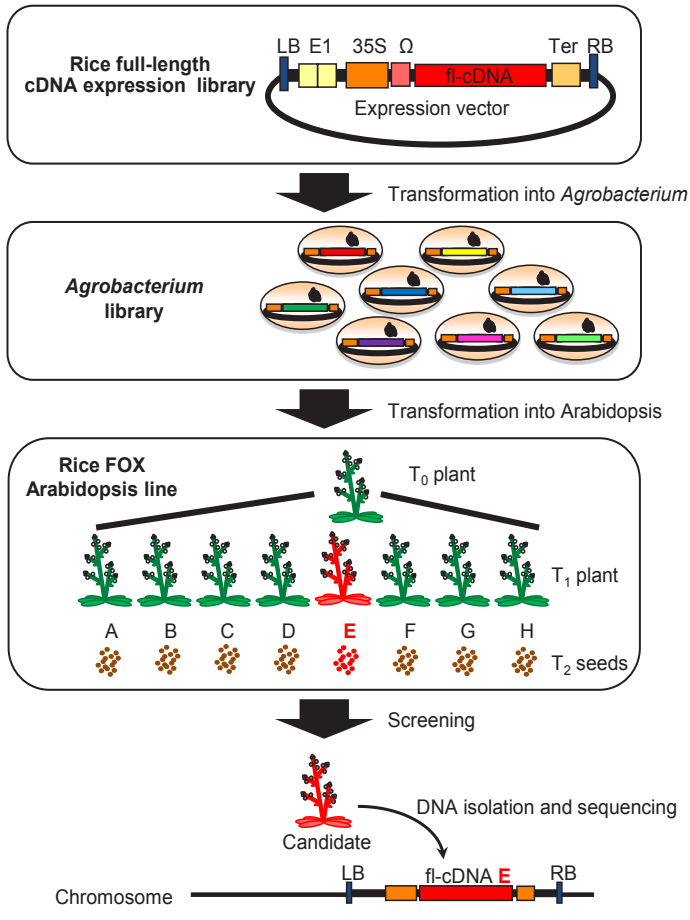

Fig. 2. Schematic representation of the FOX-hunting system. *The cDNA library is cloned into the expression vector and an* Agrobacterium *library carrying fl-cDNAs is generated. The* Agrobacterium *library is used to introduce the fl-cDNAs into* Arabidopsis *and generate T_1 seeds. T_1 plants are self-pollinated to generate T_2 seeds. The T_2 seeds can be used for several types of screening. The introduced cDNA is isolated from the candidate FOX line and the fl-cDNA responsible for the observed phenotype is determined. Abbreviations: E1, 5′-upstream sequence of the* CaMV *35S promoter (-419 to -90); 35S,* CaMV *35S promoter; Ω, translation enhancer of TMV; Ter, nopaline synthase terminator; LB, left border sequence; RB, right border sequence.*

(Kondou *et al.*, 2009). The screening results are available through the website (http://ricefox.psc.riken.jp; Sakurai *et al.*, 2011). The functions of several rice genes investigated with the rice FOX *Arabidopsis* lines are summarized in Table 1.

Flanking sequence tag analysis in transformed rice has been developed to discover the biological function of particular genes by means of a reverse genetics approach (for reviews, see An *et al.*, 2003; Hirochika *et al.*, 2004). To obtain information about the transferred fl-cDNAs of the rice FOX *Arabidopsis*, the fl-cDNAs of 18,547 rice FOX *Arabidopsis* lines were amplified by polymerase chain reaction and sequenced. The sequence analysis provided 6,501 fl-cDNAs from 13,018 (70.2%) of the tested lines, of which 3,309 fl-cDNAs were identified in more than two lines. The fl-cDNA information is available in the rice fl-cDNA overexpressed *Arabidopsis* mutant database (http://ricefox.psc.riken.jp; Sakurai *et al.*, 2011).

Abnormal morphological phenotypes were observed in the T_1 generation of 1,122 lines, of which 199 phenotypes reappeared in the T_2 generation, indicating that they were hereditary and not caused by environmental conditions. Of the 199 lines, we determined the fl-cDNAs in 172 lines, from which 182 fl-cDNAs were identified. Phenotypic analysis revealed that expression of 11 rice fl-cDNAs resulted in the same phenotype in more than two lines, suggesting that they were responsible for the mutant phenotypes. Of these, four proteins encoded by the fl-cDNAs (Os03g0142900, Os03g0184100, Os10g0546400, and Os03g0822400) were of unknown function. Interestingly, only eight fl-cDNAs have homologous genes in *Arabidopsis.*

Disease resistance gene isolated from rice FOX *Arabidopsis* lines

Broad-spectrum disease resistance against two or more types of pathogen species is desirable for crop improvement, but only a few genes have been identified until now. In rice, two of the most devastating pathogens are *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal bacterium of rice leaf blight, and *Magnaporthe oryzae,* the fungal pathogen of rice blast. To find rice genes conferring broad-spectrum disease resistance, rice FOX *Arabidopsis* lines were used because the small size and short lifespan of *Arabidopsis* enable high-speed and large-scale screening. Approximately 20,000 of the transgenic lines were screened for bacterial disease resistance against *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000). *Pst* DC3000 was selected not only because it is a widely used bacterium in *Arabidopsis* research, but also exhibits a type III secretion mechanism for infection, which is similar to that of *Xoo*. *Arabidopsis* is generally inoculated with *Pst* DC3000 by dipping or infiltration and the resistance level is evaluated by bacterial counts or lesion size in the infected plants. However, these conventional protocols are difficult to apply to 20,000 lines. Hence, the following high-throughput disease resistance screening system was developed. The rice FOX *Arabidopsis* lines were grown in 60-well plates for 3 weeks under aseptic conditions, dip inoculated with *Pst* DC3000, incubated, and evaluated for resistance in a binomial way (survive or die). After three rounds of independent screens, 72 lines that showed constant resistance were selected and the identities of the transgenes were determined. Most of the identified genes (including their orthologs in other species) had not been previously associated with resistance to any disease (Dubouzet *et al.*, 2011). Next, these 72 lines were screened for resistance to the fungal pathogen *Colletotrichum higginsianum* that had been chosen because it infects *Arabidopsis* by forming appressoria and penetration pegs, a process similar to that by which *M. oryzae* infects rice. Thirteen lines out of these 72 were also resistant to *C. higginsianum* and 11 causal genes were identified.

Several rice genes that conferred resistance to both *Pst* DC3000 and *C. higginsianum* in *Arabidopsis* were transformed into rice. The transformants were evaluated for their resistance to the rice bacterial pathogen, *Xoo.* One of the transgenic rice was highly resistant to *Xoo* and, interestingly, this line also showed high resistance to *M. oryzae* (Fig. 3). Therefore, the causal rice gene encoding a putative receptor-like cytoplasmic kinase was designated *BROAD-SPECTRUM RESISTANCE 1* (*BSR1*) (Dubouzet *et al.*, 2011). The resistance given by the *BSR1* gene is outstanding, because it confers multiple or broad-spectrum disease resistance to both bacterial (*P. syringae* and *X. oryza*) and fungal pathogens (*C. higginsianum* and *M. oryzae*) in dicotyledonous and monocotyledonous plants. To our knowledge, no other such disease-resistant monocotyledonous gene has been reported so far.

The BSR1 protein sequence is similar to that of the *Arabidopsis* BOTRYTIS-INDUCED KINASE1 (BIK1), it is not an orthologue. BIK1 is a receptor-like cytoplasmic kinase that has recently been shown to associate with pathogen-associated molecular pattern (PAMP) receptor complexes (such as the flagellin-sensitive2/brassinosteroid insensitive1 (BRI1)-associated receptor kinase1) mediate PAMP-triggered immunity signal transduction from multiple PAMP receptor complexes by phosphorylation. BSR1, like BIK1, is supposed to function by linking multiple PAMP receptor complexes to downstream intracellular signaling, in view of the broad-spectrum disease resistance of *BSR1* observed in both *Arabidopsis* and rice (Dubouzet *et al.*, 2011).

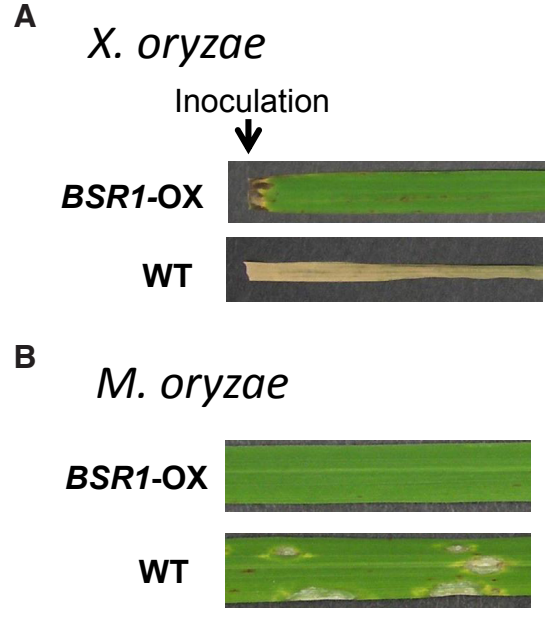

Fig. 3. Disease resistance of transgenic rice. *Transgenic rice overexpressing* BSR1 *shows resistance to the bacterial pathogen* Xanthomonas oryzae *pv.* oryzae **(A)** *and the fungal pathogen* Magnaporthe oryzae **(B)**. *An arrow indicates the point of inoculation. Abbreviations:* BSR1-*ox,* BSR1 *overexpressor, WT, wild type.*

Thus, the rice FOX *Arabidopsis* lines are very useful as a tool for the functional analysis of genes. Furthermore, the presence of common defense mechanisms between monocots and dicots has been proposed.

Phenotype of *Arabidopsis* plants expressing rice-specific full length cDNAs

We could determine 182 fl-cDNAs in the rice FOX *Arabidopsis* lines that showed morphological phenotypes in the T_2 generation as previously described. Detailed information for these 182 fl-cDNAs was obtained by using the KOME database (http://cdna01.dna.affrc.go.jp/cDNA/). The analysis indicated that 152 of the 182 fl-cDNAs had *Arabidopsis* homologs. Of the 30 remaining rice-specific fl-cDNAs, three provoked a similar morphological phenotype in two independent lines (Fig. 4A), indicating that they were candidates for inducing the mutant phenotypes. Interestingly, the functions of all three, *Os10g0546400*, *Os03g0822400* and *Os02g0180800*, are unknown. *Os10g0546400*, which is predicted to encode a 129-amino-acid protein of unknown function, was found in two rice FOX *Arabidopsis* lines, both of which showing notched rosette and cauline leaves. The predicted protein encoded by *Os10g0546400* is conserved in monocotyledonous plants only (purple false brome [*Brachypodium distachyon*], sorghum [*Sorghum bicolor*], maize [*Zea mays*], and barley [*Hordeum vulgare*]). *Os03g0822400*, encoding a conserved hypothetical protein of 90 amino acids was recovered from two rice FOX *Arabidopsis* lines that also showed notched rosette and cauline leaves and is conserved in monocots only. *Os02g0180800*, which is annotated as a nonprotein-coding transcript, was inserted into two independent lines that both had a greatly increased number of dark-green leaves. Part of its sequence is conserved in maize and barley. These three examples indicate that heterologous genes are expressed and can be functional in different plant species. Systematic heterologous expression can be useful introduce improvements into plants that cannot be achieved by conventional genetic methods.

Phenotypic comparison between rice FOX *Arabidopsis* lines and *Arabidopsis* FOX lines

We have reported that 1,487 morphological mutants were observed in *Arabidopsis* FOX lines overexpressing *Arabidopsis* fl-cDNAs (Ichikawa *et al.*, 2006). These lines have been sequenced for the transferred fl-cDNAs. We investigated whether overexpression of homologous genes in rice and *Arabidopsis* led to similar phenotypes. In the first example, two FOX lines with an altered leaf morphology (Fig. 4B) contained the fl-cDNAs *Os08g0566400* (rice) and *At4g28706* (*Arabidopsis*). The predicted amino acid sequences of these two genes showed 66% identity. The InterProScans of the predicted proteins of the two genes correlated with ribokinase, which participates in the first step of ribose metabolism, and is a member of the carbohydrate kinase superfamily. These results indicate that functional orthologous genes can be detected through database screening of two sequence libraries.

Another example involves the two isoforms of ferredoxin $NADP^+$-oxidoreductase (OsFNR1 and OsFNR2) (Higuchi-Takeuchi *et al.*, 2011) that catalyzes the reduction of $NADP^+$ by ferredoxin and provides the reducing power for CO_2 fixation in the Calvin cycle in chloroplasts. There is approximately 80% sequence identity at the amino acid level between the *Arabidopsis* and the rice FNR. Expression of rice fl-cDNAs of the FNR isoforms led to a similar phenotype of altered chlorophyll fluorescence and growth in both *Arabidopsis* and rice.

A third example is the ATP-dependent caseinolytic protease (Clp) family that is involved in chloroplast development (Olinares *et al.*, 2011). Expression of two rice fl-cDNAs of the Clp family (*Os12g0230100* and *Os03g0308100*) caused a pale-green phenotype, whereas in one *Arabidopsis* FOX line a pale-green phenotype was provoked by overexpression of the ClpR2 fl-cDNA (*At1g12410)* (Fig. 4C). These results suggest that FNR and Clp protease of rice and *Arabidopsis* can serve the same function in both species.

Comparison of the rice FOX *Arabidopsis* lines and *Arabidopsis* FOX lines resulted in the discovery of three homologous genes that showed common phenotypes, although 152 homologous gene pairs were identified in the rice FOX *Arabidopsis* lines and *Arabidopsis* FOX lines. Hence, in many cases, the overexpression of homologous and orthologous genes might induce different phenotypes and might indicate that a sequence ortholog is not necessarily a functional ortholog and that orthologous proteins cannot exert their function due to interaction specificity with other proteins.

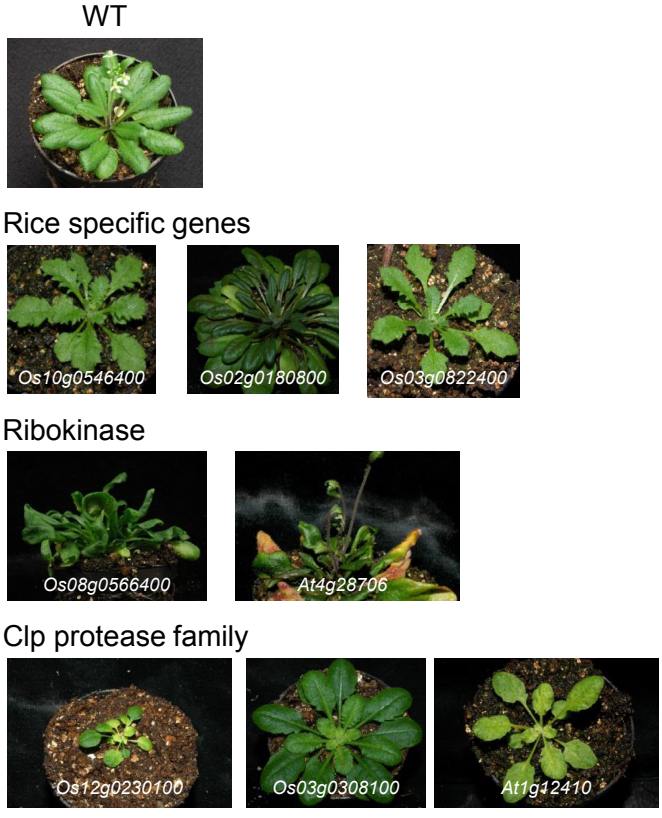

Fig. 4. Morphological mutants isolated from FOX lines. (A) *Rice-specific genes, from left to right,* Os10g0546400, Os02g0180800, *and* Os03g0822400, *expressed in* Arabidopsis *plants.* **(B)** *Introduction of ribokinase, from left to right, of* Os08g0566400 *and* AT4G28706. **(C)** *Introduction of the Clp protease family, from left to right, of the rice ATP-binding subunit clpA homolog (*Os12g0230100), *the rice peptidase S14, the ClpP family protein (*Os03g0308100*) and the* Arabidopsis *ClpP protease complex subunit ClpR2* (At1g12410).

Outlook

The FOX-hunting system can have many applications. To elucidate salt-stress responses, mini-scale FOX lines expressing 29 of the calcium-dependent protein kinase (CDPK) family were generated in rice (Asano *et al.*, 2010). They were screened for salt-stress resistance and salt-stress tolerant transformants overexpressing *OsCPK12* were identified. Of the many gene families in rice, the functions of most are still unknown. Undoubtedly, through the FOX-hunting approach, the function of a gene family can be clarified.

Heterologous approaches have been used in several plants with the *Agrobacterium* library. The *Arabidopsis* FOX *Agrobacterium* library was transformed into a supergrowing root culture of the legume *Lotus corniculatus* to identify genes involved in root growth (Himuro *et al.*, 2011). Some of these transformants showed useful traits, such as fast growth and thicker and longer roots. Thus, the FOX *Agrobacterium* library is a new biological resource for genetic analysis.

Recently, a chemical genomics approach has been developed to facilitate the understanding of gene function. By means of *Arabidopsis* FOX lines, overexpression of two enzymes involved in pectin modulation (pectin methylesterase and polygalacturonase) conferred resistance to cobtorin, an inhibitor of parallel alignment of cortical microtubules and cellulose microfibrils (Yoneda *et al.*, 2010).

A functional genomics approach is required to clarify the function of genes in many crop species other than rice. However, transgenic approaches for both forward and reverse genetics studies are not practical in plant species in which the transformation method is inefficient or not available. By using *Arabidopsis* or other transformable plants as a host plant, rapid gene function analysis of such plants will become possible after fl-cDNA preparation.

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