Survey of Rice Proteins Interacting With OsFCA and OsFY Proteins Which Are Homologous to the Arabidopsis Flowering Time Proteins, FCA and FY

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The FCA protein is involved in controlling flowering time and plays more general roles in RNA-mediated chromatin silencing in Arabidopsis. It contains two RNA-binding domains and a WW domain. The FCA protein interacts with FY, a polyadenylation factor, via its WW domain. We previously characterized a rice gene, OsFCA, which was homologous to FCA. Here, we found that the OsFCA protein could interact through its WW domain with the following proteins: OsFY, a protein containing a CID domain present in RNA-processing factors such as Pcf11 and Nrd1; a protein similar to splicing factor SF1; a protein similar to FUSE splicing factor; and OsMADS8. The FY protein is associated with the 3′ end processing machinery in Arabidopsis. Thus, we examined interactions between OsFY and the rice homologs (OsCstF-50, -64 and -77) of the AtCstF-50, -64 and -77 proteins. We found that OsFY could bind OsCstF50, whereas the OsCstF77 protein could bridge the interaction between OsCstF50 and OsCstF64. Taken together, our data suggest that OsFCA could interact with several proteins other than OsFY through its WW domain and may play several roles in rice.

**Keywords:** Flowering time • OsFCA • OsFY • Polyadenylation • Protein interaction • Rice.

**Abbreviations:** BiFC, bimolecular fluorescence complementation; CFIm and CFIm, cleavage factor I and II; CPSF, cleavage and polyadenylation specificity factor; CstF, cleavage stimulatory factor; GFP, green fluorescent protein; GST, glutathione S-transferase; ORF, open reading frame; PAP, poly(A) polymerase; RT–PCR, reverse transcription–PCR; YFP, yellow fluorescent protein.

**Introduction**

Several studies have demonstrated that proteins which play key roles in RNA metabolism affect flowering time in plants (Quesada et al. 2005). For example, ABA hypersensitive 1 (ABH1), which encodes nuclear mRNA cap-binding protein, regulates the level of cis-natural antisense transcripts (cis-NATs) to modulate CONSTANS expression (Kuhn et al. 2007). Moreover, ABH1 is associated with mRNA processing events of FLOWERING LOCUS C (FLC) (Michaels and Amasino 1999, Sheldon et al. 1999) and FLOWERING LOCUS M (FLM) (Sortecci et al. 2001). In addition, altered expression of a homolog of mammalian U2 small nuclear ribonucleoprotein auxiliary factor small subunit (U2AF35) affects the flowering time and splicing pattern of FCA pre-mRNA (Wang and Brendel 2006).

In eukaryotes, the formation of an mRNA 3′ end is one of several regulatory mechanisms by which cells can change the amount and type of mRNA derived from a distinct transcriptional unit (Zhao et al. 1999). A large number of studies in yeast and animals have demonstrated that cleavage and polyadenylation specificity factor (CPSF), cleavage stimulatory factor (CstF), cleavage factor I and II (CFIm and CFIm), and poly(A) polymerase (PAP) form the core polyadenylation machinery (Zhao et al. 1999). It has also been reported that there is a physical interaction between some of the
components of the splicing and the 3′ end processing machinery (Lutz et al. 1996, Kyburz et al. 2006). Recent studies in Arabidopsis have demonstrated that homologs of the yeast and mammalian polyadenylation machinery are involved in regulating mRNA 3′ end formation (Hunt et al. 2008). Of them, the best known example is FCA autoregulation by the FY protein (Quesada et al. 2003, Simpson et al. 2003, Henderson et al. 2005). FY is a part of the CPSF complex; thus, this protein complex regulates the 3′ end processing of FCA (Herr et al. 2006, Manzano et al. 2009). The FCA–FY interaction regulates the level of functional FCA–γ protein through a negative feedback mechanism by prematurely polyadenylating the FCA pre-mRNA within intron 3. The FCA–FY interaction is also required for FCA-mediated chromatin silencing (Bäurle et al. 2007, Liu et al. 2007, Manzano et al. 2009). In other plants, such as rice and ryegrass, the in yeast or in vitro interaction between FCA and FY homologs was also reported, suggesting that the role of the FCA–FY complex in controlling the amount of functional FCA protein is conserved (Winichayakul et al. 2005, Lu et al. 2006, Jang et al. 2008). Recently, the Arabidopsis Pcf11 homolog (PCFS4) was found to be in a complex with FY and thereby influences the flowering time (Xing et al. 2008a). A polyadenylation factor CLP1 (CLPS3) found in the same complex was associated with the CPSF complex, and thus it may bridge CPSF to the PCSF4 complex (Xing et al. 2008b).

We have previously shown that the OsFCA–γ protein, a homolog of FCA, could partially rescue the phenotype of the Arabidopsis fca-1 mutant, in terms of flowering time, through the up-regulation of SUPPRESSOR OF OVEREXPRESsion OF CONSTANS 1 (SOC1) mRNA (Lee et al. 2000, Samach et al. 2000) without concomitant down-regulation of FLC mRNA, and could not replace the FCA–γ protein in terms of negative feedback regulation of the FCA–γ mRNA level (Lee et al. 2005). The partial discrepancies in function between OsFCA and FCA raise the possibility that OsFCA may play another role in rice. Here, we demonstrate that the OsFCA protein interacts with a host of proteins including components involved in RNA processing using yeast two-hybrid, glutathione S-transferase (GST) pull-down and bimolecular fluorescence complementation (BiFC) analyses.

Results

Searching for proteins interacting with OsFCA

Analysis of the whole rice genome sequence revealed that no apparent FLC and FRIGIDA (FRI) homologs existed, although the autonomous pathway genes such as FCA and FVE homologs were present (Goff et al. 2002). We have previously shown that the OsFCA–γ protein, a homolog of FCA, could partially rescue the phenotype of the Arabidopsis fca-1 mutant in terms of flowering time but could not replace the FCA–γ protein in terms of negative feedback regulation of the FCA–γ mRNA level (Lee et al. 2005). These findings suggest that some functional differences exist between OsFCA and FCA. In order to gain insight into the function of OsFCA, we screened a yeast two-hybrid library of rice with the OsFCA protein. Three baits were used in this study: a full-length OsFCA protein (FL-OsFCA), a truncated OsFCA protein lacking the glycine-rich region (ΔGly-OsFCA) and the C-terminal half of the OsFCA protein containing only the WW domain (OsWW) (Fig. 1a). We used the protein lacking the glycine-rich domain as bait, since it may be made from the alternatively spliced OsFCA transcripts (OsFCA-3 and -4) (Du et al. 2006, Jang et al. 2009) and may interact with partners other than the full-length OsFCA protein. Based on the screens conducted with the three bait types, various kinds of proteins were found (Supplementary Table S2). Different sets of proteins interacted with each bait protein, i.e. only a few proteins showed up with more than two baits. Thus, the full-length open reading frames (ORFs) of all clones screened with each bait were examined in regards to their ability to bind all three bait proteins. Some of the results from the yeast two-hybrid experiments are shown in Supplementary Fig. S1. We then assessed whether the positive clones identified in the yeast two-hybrid assay could interact in vitro.

OsFCA interacts in vitro with several rice proteins

GST pull-down assays were performed in order to identify proteins that interact with OsFCA in vitro (Fig. 1b–g). From these assays the following proteins were identified: a protein (OsFy, AK111493) homologous to Arabidopsis FY, a protein (OsSF1, AK121107) homologous to splicing factor SF1 (Lorkovic and Barta 2002), a protein (OsFik, AK060799) similar to FUSE-splicing factor (Lorkovic and Barta 2002), a protein (OsPrr1B, AK101731) containing a CTD-interacting domain (CID) (Meinhart and Cramer 2004), a MADS transcription factor (OsMads8, AK072867) and a protein (OsDag-like, AK106007) similar to a chloroplast DAG protein (Chatterjee et al. 1996). In addition, truncated OsFCA proteins lacking the glycine-rich region (ΔGly-OsFCA) or containing only the WW domain (OsWW) interacted with the OsFCA-interacting proteins, indicating that the WW domain of OsFCA was important for binding with these OsFCA-interacting partners (Fig. 1b–g). All these proteins, except OsFik and OsMads8, have XPXPP motifs (where X is any amino acid) (Kato et al. 2004) (Fig. 2). Their Arabidopsis homologs also contain these motifs. This motif is known to be a ligand for group II/III WW domains such as that present in FCA and OsFCA (Sudol and Hunter 2000, Kato et al. 2004).

The interaction between OsFCA and its interactors was examined in more detail. In Arabidopsis, the second signature tryptophan residue of the WW domain of FCA protein...
is critical for the interaction between FCA and FY to occur (Simpson et al. 2003, Henderson et al. 2005). We examined if this was also true for the interaction between OsFCA and its interactors (Fig. 3). Although Arabidopsis FY could interact specifically with both FCA and OsFCA, rice OsFY appeared to bind rice OsFCA more specifically than FCA, because OsFY could still bind to the Arabidopsis WF mutant form to some extent (Fig. 3a, b). Other OsFCA-interacting proteins also did not bind the OsFCA WF mutant form, indicating that the WW domain is essential for their interaction (Fig. 3c–g).

Fig. 1 In vitro interactions between OsFCA and several rice proteins. (a) Illustration of the domain structures of the three OsFCA bait constructs. The AtSWI3B domain and the glycine-rich region (Gly) are shown in addition to the RRM and WW domains. These constructs were used as GST fusion proteins in the in vitro GST pull-down assays. The amount and quality of the GST fusion proteins used in the assays are shown in Supplementary Fig. S2. (b–g) In vitro analysis of the protein-protein interactions between OsFCA and OsFCA-interacting proteins. Beads carrying GST or GST fusion proteins were incubated with His-tagged (His) recombinant proteins, His-OsFY (b), His-OsFK (d) and His-OsMADS8 (f). The bands show the eluted His-tagged proteins visualized by immunoblotting with anti-His antibody. Ten percent of the His-tagged proteins are shown in the input lanes. In addition, beads carrying GST or GST fusion proteins were incubated with in vitro translated (IVT) proteins (35S-labeled), OsSF1 (c), OsRPRD1B (e) and OsDAG-like proteins (g). The bands indicate the eluted IVT proteins visualized by autoradiography. The input lanes show 10% of the 35S-labeled proteins. The band marked with an arrow in (b) corresponds to the partial form of the His-OsFY proteins.

OsFCA-interacting proteins

The expression pattern, subcellular localization and in planta interaction of OsFCA and its interactors

In order to determine whether OsFCA is spatially and temporally expressed with OsFCA-interacting genes, we evaluated the expression patterns of the genes that were found to interact with OsFCA in yeast and in vitro experiments (Fig. 4). OsFCA expression was abundant in panicles, calli, stems and, to a lesser degree, in roots and leaves. This ubiquitous expression pattern was also observed for the OsFY, OsSF1, OsFIK and OsRPRD1B genes. In contrast, the expression of OsMADS8 was much higher in only the late stages of panicle development. Meanwhile, the expression level of the OsDAG-like gene was lower to various extents in all tissues examined, except for the roots. These results suggest that these OsFCA-interacting proteins, except the MADS box proteins, are expressed in a spatial and temporal manner that is similar to OsFCA.

Interaction of OsFY with the CstF complex of the mRNA 3′ end processing machinery

Since OsFY was found to interact with the OsFCA protein, and because FY was part of the CPSF complex in Arabidopsis and a yeast FY homolog mediates interactions between the yeast versions of CPSF and CstF complexes (Ohnacker et al. 2000, Herr et al. 2006), we further examined the interaction network of OsFY with the CstF subunits of the rice 3′ end processing machinery (Fig. 7). The rice proteins homologous to Arabidopsis CstF-50 (OsCstF50, AK101437), -64 (OsCstF64, AK067921) and -77 (OsCstF77, AK102914) proteins were examined for interaction in vitro (Fig. 7a–c). We found that OsCstF77 bridged the interaction between OsCstF50 and OsCstF64, which has also been observed for the human Cst complex (Takagaki and Manley 2000). OsFY could interact with the OsCstF50 protein (Fig. 7d), but not with the OsCstF64 or OsCstF77 proteins (Fig. 7e). Since OsFIK and OsRPRD1B proteins contain domains that may be involved in RNA processing, we also examined if OsFY could interact with OsFIK and OsRPRD1B. OsFY interacted with OsFIK, but not with OsRPRD1B (Fig. 7f, g). The OsRPRD1B protein did not interact with either OsCstF64 or OsCstF77 (Fig. 7h).
In order to investigate in planta interactions, we first examined the subcellular localization of the OsCstF proteins and then performed the BiFC assay. Their GFP fusion proteins were found in the nucleus as expected (Fig. 8). The BiFC data were consistent with the in vitro pull-down assay data shown in Fig. 7 (Fig. 9). Other negative BiFC data except for two cases (N-YFP-OsCstF64/C-YFP-OsCstF50 and N-YFP-OsFY/C-YFP-OsCstF77) are not shown (Fig. 9c, d). These results suggest that OsFY may also interact with OsCstF50, thereby contributing to the connection between the CPSF complex and the CstF complex in rice. Taking all the results together, we summarized the interaction network between OsFCA and its binding partners as well as the CstF complex of the rice mRNA 3′ end processing complex (Fig. 10).

**Discussion**

**Interaction between OsFCA protein and its partners in vitro and in planta**

In this study, OsFCA was found to interact with various proteins including OsFY, a FY homolog. All of these proteins specifically interacted with OsFCA through the WW domain.

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**Fig. 3** FY and OsFY interact in vitro with their corresponding partners (FCA and OsFCA) as well as their counterparts through their WW domains. The second signature tryptophan residue of the WW domain was mutated to a phenylalanine residue in the rice OsWF or Arabidopsis WF construct. Beads carrying GST or GST fusion proteins were incubated with in vitro translated (IVT) recombinant OsFY (a) or FY (b) proteins (35S-labeled). The bands indicate the eluted OsFY or FY proteins visualized by autoradiography. The input lanes show 10% of the 35S-labeled proteins. The amount and quality of the GST fusion proteins used in the assays are shown in **Supplementary Fig. S2**. When GST fusion proteins and IVT proteins migrated to similar positions, the IVT protein appears to be at a lower position than that expected (a).
The WW domains of FCA and OsFCA are categorized as members of Group II/III, which are known to bind a ligand motif, XPXPP (where X is any amino acid) (Kato et al. 2004). Interestingly, all OsFCA-interacting proteins tested in this study, except OsFIK and OsMADS8, contain this motif. Meanwhile, OsFIK contains the PPXY motif of the Group I WW domain (Sudol and Hunter 2000) (Fig. 2). The FY protein contains two PPLPP motifs, both of which must be removed to disrupt interactions with the WW domain of the FCA protein in vitro (Henderson et al. 2005). However, the OsFY protein has two XPPXP motifs in addition to two PPLPP motifs. This may be the reason why OsFY could interact with the Arabidopsis WF form to some extent (Fig. 3a).

The WW domains could interact with components of the multiprotein complexes involved in transcription, RNA processing, chromatin remodeling and actin polymerization (Ingham et al. 2005). For example, the p68 protein of the CFIm 3′ end formation complex contains multiple proline-rich sequences that can bind different kinds of WW domains. It was also found that a WW domain could bind a group of proteins that had different functions and no apparent motifs (Ingham et al. 2005). This may explain why OsFCA could interact through its WW domain with various proteins that had different functional domains. A comparison between microarray data obtained from the wild type and fca mutants showed that there were additional target genes mis-regulated in fca mutants (Marquardt et al. 2006). FCA was also required for RNA-mediated chromatin silencing of single-copy and low-copy genes (Bäurle et al. 2007, Liu et al. 2007). This indicates that FCA may play other roles besides flowering control. This also suggests that OsFCA may play several roles in rice. Although the OsFCA protein could interact with a group of proteins, our data indicated that most of them except OsMADS8 appeared to be involved in aspects of RNA processing such as 3′ end processing and splicing. However, even though OsFCA interacted with these proteins in transiently expressed tobacco leaves, it remains to be determined whether these interactions are functionally significant in vivo. Transgenic rice lines expressing these proteins would provide further solid evidence for in vivo interactions.

Interaction network of the OsFY and CstF complex

The interaction of Arabidopsis homologs with some mammalian CPSFs, CstFs, CFIs and PAPs has been reported (Yao et al. 2002, Delaney et al. 2006, Forbes et al. 2006, Xu et al. 2006, Addepalli and Hunt 2007, Xing et al. 2008b). Mutations in some of these Arabidopsis homologs revealed that
they were essential for plant development. In addition, some allelic series of these mutations were also found to be involved in controlling flowering time (Henderson et al. 2005, Herr et al. 2006, Xing et al. 2008a). In this study, we demonstrated that OsFY interacted with OsCstF50, and OsCstF77 bridged interactions between OsCstF50 and OsCstF64 (Figs. 7, 9). A gene (AtCstF50, At5g60940) that is homologous to mammalian CstF50 exists in the Arabidopsis genome. However, unlike their mammalian counterparts, the AtCstF50 protein did not interact with the Arabidopsis CstF77 homolog (AtCstF77, At1g17760) in vitro and in yeast (Yao et al. 2002, Hunt et al. 2008). FY also failed to interact with AtCstF77 in yeast and in vitro (Hunt et al. 2008, Manzano et al. 2009). In yeast, the FY homolog Pfs2p interacts with the AtCstF77 homolog RNA14p (Ohnacker et al. 2000). This discrepancy between Arabidopsis and rice may be due to different experimental approaches taken or a difference in the characteristics of their CstF complexes. In human, CstF50 interacts with CstF77 through its WD40 repeats and also self-associates (Takagaki and Manley 2000). Recently, it has been shown that the human FY homolog WDR33 (WDC146) is a component of the CPSF complex in a purified functional 3’ processing complex, indicating that the human mRNA 3’ processing complex contains two WD proteins, one in CPSF and another in CstF (Shi et al. 2009). Interaction between CstF50 and WDR33 is not known; however, interactions among WD40 repeat proteins had been reported (Chen et al. 2004). As in FY and AtCstF50, OsFY and OsCstF50 both have the WD40 repeats but their amino acid sequence similarity is very low. Furthermore, only OsCstF50 could interact with OsCstF77 (Figs. 7a, e, and 9a, d). Further study will be needed to clarify the discrepancy in the interaction we observed.

Herr and colleagues found that FY was a component of the Arabidopsis CPSF (AtCPSF) complex and it interacted with ESP4/Symplekin (At5g01400) and ESP5/CPSF100 (At5g23880) within the complex (Herr et al. 2006). However, FCA was not included in the AtCPSF complex they had isolated. FY was also found in a complex containing the Arabidopsis homolog (AtPCFS4) of yeast Pcf11p protein, but there was no direct interaction between them (Xing et al. 2008a). A polyadenylation factor CLP1 (CLPS3) found in the AtPCFS4 complex.
Fig. 6 BiFC analysis for the interaction between OsFCA and its binding partners in Agrobacterium-infiltrated tobacco (N. benthamiana) leaves. Epifluorescence (I) and bright field overlay images (II) of tobacco leaf epidermal cells infiltrated with a mixture of Agrobacterium suspensions harboring constructs encoding the indicated fusion proteins. Confocal microscopy images of the infiltrated tobacco leaf epidermal cells expressing: (a) positive control, N-YFP-bzip/C-YFP-bzip, (b) N-YFP-OsFCA/C-YFP-OsFY, (c) N-YFP-OsFCA/C-YFP-OsSF1 (d) N-YFP-OsFCA/C-YFP-OsFIK, (e) N-YFP-OsFCA/C-YFP-OsRPRD1B (f) N-YFP-OsFCA/C-YFP-OsMADS8 and (g) N-YFP-OsFCA/C-YFP-OsDAG-like protein. Reconstituted BiFC signals were localized in the nuclear compartments for all combinations except the OsFCA and OsDAG-like protein pair (g) which shows no BiFC signal. Scale bars, 50 µm.
complex was associated with CPSF100 and 160 (Xing et al. 2008b). FCA appeared not to be in the complex. Both FY-mediated tandem affinity purification and co-immunoprecipitation assays failed to detect the FCA–FY interaction, suggesting that the interaction may be transient or regulated in vivo (Manzano et al. 2009). It would be interesting to compare these interactions between rice and Arabidopsis. It had been speculated that FCA as a regulator of 3′ end formation may be recruited in the standard 3′ end processing machinery to regulate the 3′ end formation of a specific set of transcripts or stabilize weak poly(A) site interactions with FY and the other 3′ end processing component. However, this was found to be an oversimplified view of the function of the FCA–FY interaction (Bäurle et al. 2007, Liu et al. 2007, Manzano et al. 2009). The target genes of OsFCA and OsFY have not yet been identified. Future investigations to determine the target genes of OsFCA and OsFY will be helpful to understand the significance of the protein interaction observed in this study.

**Materials and Methods**

**Plant material and growth conditions**

For the expression analysis of OsFCA and OsFCA-interacting genes, a variety of tissues (leaves, roots, stems, calli and panicles) from rice plants were obtained, as described previously (Lee et al. 2005).
RNA isolation and RT–PCR

Total RNA was extracted from various parts of rice plants as described previously (Lee et al. 2005), and first-strand cDNA was generated using the SuperScript III kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer’s instructions. Reverse transcription–PCR (RT–PCR) was then performed to detect the transcripts of the following genes: OsFY (AK111493), OsSF1 (AK121107), OsFIK (AK060799), OsRPRD1B (AK101731), OsMADS8 (AK072867), OsDAG-like (AK106007), OsCstF64 (AY730687) and OsUBQ (D12629). All PCR conditions and primers used in this study are listed in Supplementary Table S1.

Yeast two-hybrid assay

The three baits used in this assay were generated by PCR amplification from a full-length OsFCA cDNA. The amplified fragments for the OsFCA1–739 (FL-OsFCA), OsFCA112–739 (AGly-OsFCA) and OsFCA367–716 (OsWW) constructs (the numbers indicate the positions of amino acid residues in the full-length OsFCA-γ protein) were digested with EcoRI and SalI, and cloned into the same site of the pBD-Gal4-CAM Y2H bait vector (Stratagene, La Jolla, CA, USA). Three bait constructs were then transformed into yeast strain Y190 to screen the yeast two-hybrid cDNA library made from rice spikelets (Eugentech, Deajeon, Korea). The yeast two-hybrid screening and β-galactosidase filter assay were performed as described previously (Ham et al. 2006). Positive clones were subsequently sequenced and analyzed with the BLAST program (Altschul et al. 1997).

GST pull-down assay

The inserts of the three bait constructs described above and the full ORF of OsFIK were subcloned into the GST-tagged pGEX5X-1 vector (Amersham Biosciences, Uppsala, Sweden). The OsFCA570–739 (OsWW’) sequence was amplified using BamHI- and SalI-linked primer sets and cloned into the GST-tagged pETGST vector (a gift from Dr. Hyun Kyu Song). To produce recombinant proteins for interaction partners in Escherichia coli or the TNT-coupled Transcription/Translation System (Promega, Madison, WI, USA), the genes used in this assay were cloned into the 6×His-tagged pET21a vector (Qiagen, Novagen, Madison, WI, USA). The full-length OsFY, OsSF1, OsMADS8, OsRPRD1B, OsCstF64 (AK067921), OsCstF77 (AK102914) and other cDNAs were generated via PCR amplification from a rice cDNA library (Eugentech), and others were purchased from the Rice Genome Resource Center (Kikuchi et al. 2003). For construction of the GST recombinant proteins, the full ORFs of OsCstF50 (AK101437), OsCstF64 and OsRPRD1B cDNAs were cloned into the pGEX4T-3 vector (Amersham Biosciences), and the full ORF of the OsCstF77 cDNA was cloned into the pGEX5X-1 vector. The OsWF mutant form was generated via site-directed mutagenesis using the megaprimer PCR method. The details of the PCR conditions and primers used in this study are described in Supplementary Table S1.

The GST pull-down assay was performed as described previously (Kim et al. 2006). GST fusion recombinant proteins were mixed with the E. coli lysates or TNT translation products containing prey proteins, and the mixtures were gently rotated for 2 h at 4°C. Subsequently, they were washed three times with the washing buffer, and eluted with 10 mM reduced glutathione in 100 mM NaCl and 20 mM Tris–HCl, pH 7.2. Finally, the eluted protein samples were analyzed by 12% SDS–PAGE and visualized by Western blot analysis or autoradiography.

BiFC analysis and nuclear localization

The genes investigated in this assay were amplified from plasmid DNA templates by PCR using the gene-specific primers listed in Supplementary Table S1. PCR products
Fig. 9 BiFC analysis for interaction between OsFY and OsCstF components in Agrobacterium-infiltrated tobacco (N. benthamiana) leaves. Epifluorescence (I) and bright field overlay images (II) of tobacco leaf epidermal cells infiltrated with a mixture of Agrobacterium suspensions harboring constructs encoding the indicated fusion proteins. Confocal microscopy images of the infiltrated tobacco leaf epidermal cells expressing: (a) N-YFP-OsCstF50/C-YFP-OsCstF77, (b) N-YFP-OsCstF64/C-YFP-OsCstF77, (c) N-YFP-OsCstF64/C-YFP-OsCstF50, (d) N-YFP-OsFY/C-YFP-OsCstF77, (e) N-YFP-OsFY/C-YFP-OsFIK and (f) N-YFP-OsFY/C-YFP-OsCstF50. Reconstituted BiFC signals were localized in nuclear compartments in the cases of (a), (b), (e) and (f), whereas no signal was detected in the cases of (c) and (d). (e, f) 4′,6-Diamidino-2-phenylindole (DAPI) staining was used to localize nuclei. Negative BiFC assay data for cells expressing the following constructs are not shown: N-YFP-OsFY/C-YFP-OsCstF64, N-YFP-OsFY/C-YFP-OsRPRD1B and N-YFP-OsCstF64/C-YFP-OsRPRD1B. These data were consistent with the in vitro pull-down assay data shown in Fig. 7. Scale bars, 50 μm.
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References


Supplementary data

Supplementary data are available at PCP online.

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OsFCA-interacting proteins


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