Suppl. Table 1. List of sequences used in this study with GenBank or NtGI EST accession numbers.

Acc No/Protein/Corresponding gene	Organism	Predicted protein length in aa
p62/NBR1/Joka2		
NP_194200	*Arabidopsis thaliana*	704
XP_661032	*Aspergillus nidulans*	1524
NP_788814	*Bos taurus*	440
AAI49980	*Bos taurus*	986
NP_502280.2	*Caenorabditis elegans*	693
NP_501086	*Caenorabditis elegans*	498
AAI41800	*Danio rerio*	992
XP_001920616	*Danio rerio*	625
AAF53824	*Drosophila melanogaster*	599
XP_418128	*Gallus gallus*	956
XP_001233249	*Gallus gallus*	376
NP_003891	*Homo sapiens*	440
NP_005890	*Homo sapiens*	966
XP_001102347	*Macaca mulatta*	439
XP_001097043	*Macaca mulatta*	965
NP_035148	*Mus musculus*	442
NP_032702	*Mus musculus*	988
HQ025921	*Nicotiana tabacum*	843
NP_001047302	*Oryza sativa*	845
XP_518154	*Pan troglodytes*	440
XP_001155220	*Pan troglodytes*	895
XP_001765979	*Physcomytrella patents*	732
NP_001125548	*Pongo abelii*	440
NP_001127309	*Pongo abelii*	894
XP_002321635	*Populus trichocarpa*	673
NP_787037	*Rattus norvegicus*	439
NP_001019936	*Rattus norvegicus*	983
XP_002511320	*Ricinus communis*	754
XP_002454107	*Sorgum bicolor*	849
ABB18390	*Triticum aestivum*	870
XP_002277480	*Vitis vinifera*	910
ACN33320	*Zea mays*	842
ATG8/UBL family		
AAP21330.1/ATG8a/At4g21980	*Arabidopsis thaliana*	122
NP_849298/ATG8b/At4g04620	*Arabidopsis thaliana*	122
NP_176395/ATG8c/At1g62040	*Arabidopsis thaliana*	119
AAO23655.1/ATG8d/At2g05630	*Arabidopsis thaliana*	120
NP_850431/ATG8e/At2g45170	*Arabidopsis thaliana*	122
NP_849395.1/ATG8f/At4g16520	*Arabidopsis thaliana*	121
NP_191623/ATG8g/At3g60640	*Arabidopsis thaliana*	121
NP_566283/ATG8h/At3g06420	*Arabidopsis thaliana*	119
ABD38893/ATG8i/At3g15580	*Arabidopsis thaliana*	115
TC107227/NtATG8f	*Nicotiana tabacum*	122
TC99613	*Nicotiana tabacum*	120
TC105161	*Nicotiana tabacum*	125
TC115968	*Nicotiana tabacum*	117
TC89388	*Nicotiana tabacum*	122
TC93678	*Nicotiana tabacum*	118
TC92507	*Nicotiana tabacum*	126
TC96782	*Nicotiana tabacum*	122
TC96352	*Nicotiana tabacum*	122

TC95560	*Nicotiana tabacum*	126
TC115354	*Nicotiana tabacum*	125
AM841450	*Nicotiana tabacum*	117
FG635310	*Nicotiana tabacum*	118
TC92677	*Nicotiana tabacum*	127
TC95557	*Nicotiana tabacum*	119
UP9/LSU-like family		
DQ444223/UP9A	*Nicotiana tabacum*	117
AY547446/UP9C	*Nicotiana tabacum*	117
NP_190527/LSU1/At3g49580	*Arabidopsis thaliana*	94
NP_197854/LSU2/At5g24660	*Arabidopsis thaliana*	94
NP_190526LSU3/At3g49570	*Arabidopsis thaliana*	97
NP_568450/LSU4/At5g24655	*Arabidopsis thaliana*	92
Protein partners of UP9C described in this study		
DQ444220/Joka2	*Nicotiana plumbaginifolia*	467 (incomplete orf)
DQ444222/Joka8	*Nicotiana plumbaginifolia*	360 (incomplete orf)
DQ444221/Joka20	*Nicotiana plumbaginifolia*	161 (incomplete orf)

Suppl. Table 2. List of the plasmids used in this study.

Name	**Source/Reference/Description**
Vectors	
pDEST22	Invitrogen, PQ10001-01
pDEST32	Invitrogen, PQ10001-01
pK7CWG2	Karimi et al.[81]
pH7YWG2	Karimi et al.[81]
pH7WGC2	Karimi et al.[81]
pDONR221	Invitrogen, 12536017
pENTR-D TOPO	Invitrogen, K2400
pGEX4T-1	GE Healthcare Bio-Sciences AB,28-9545-49
pGBT9	GenBank Acc # U07646
pGAD424	GenBank Acc # U07647
pGAD10	GenBank Acc # U13188
pET28a	Novagen, 69864-3
pDEST15	Invitrogen, 11802-014
cDNA constructs made by traditional cloning	
pJoka2	Joka2 from *N. plumbaginifolia* (NpJoka2) in pGAD10
pJoka8	Joka8 from *N. plumbaginifolia* (NpJoka8)in pGAD10
pJoka20	Joka20 from *N. plumbaginifolia* (NpJoka20) in pGAD10
pJK1	UP9C from *N. tabacum* in pGBT9
pJK2	UP9C from *N. tabacum* in pGAD424
pLSU1	At3g49580 from *A. thaliana* in pGBT9
pLSU2	At5g24660 from *A. thaliana* in pGBT9
pLSU3	At3g49570 from *A. thaliana* in pGBT9
pLSU4	At5g24655 from *A. thaliana* in pGBT9
pJK11	NpJoka2 in pGBT9; ORF length as in pJoka2
pHis-UP9C	UP9C from *N. tabacum* in pET28a
pGST-PB1	0.7 kb EcoRI-EcoRI fragment from pJoka2 (1-684bp/1-228 aa) in pGEX4T-1
pGST-ZZ	0.74 kb EcoRI-EcoRI fragment from pJoka2 (685-1403bp/229-467 aa) in pGEX4T-1
pGST-Joka8	1.12 kb EcoRI-EcoRI fragment from pJoka8 in pGEX4T-1
pGST-Joka20	0.65 kb EcoRI-EcoRI fragment from pJoka20 in pGEX4T-1
pHis-ATG8f	ATG8f from *N. tabacum* in pET28a
cDNA constructs made by traditional cloning into entry vector	
pEntrPB1	PB1 domain (1-1266 bp/1-422 aa) from NtJoka2 in pENTR
pEntrPB1ZZ	PB1ZZ domain (1-2253 bp/1-751 aa) from NtJoka2 in pENTR
pEntrZZ	ZZ domain (316-2253 bp/106-751 aa) from NtJoka2 in pENTR
pEntrZZUBA	ZZUBA domain (316-2526 bp/106-842 aa) from NtJoka2 in pENTR
pEntrATG8f	NtATG8f cDNA in pENTR
cDNA constructs made by Gateway BP reaction	
pEntrJ	full -length NtJoka2 in pDONR221
pEntrB	UP9C in pDONR221
cDNA constructs made by Gateway LR reaction	
pJ4	full-length NtJoka2 from pEntrJ in pH7YWG2
pJ5	full-length NtJoka2 from pEntrJ in pK7CWG2
pATG8f	full-length NtATG8f from pEntrATG8f in pH7WGC2
pDEST22/PB1	PB1 domain (1-1266bp/1-422aa) of NtJoka2 from pEntrPB1 in pDEST22
pDEST32/PB1	PB1 domain (1-1266bp/1-422aa) of NtJoka2 from pEntrPB1 in pDEST32

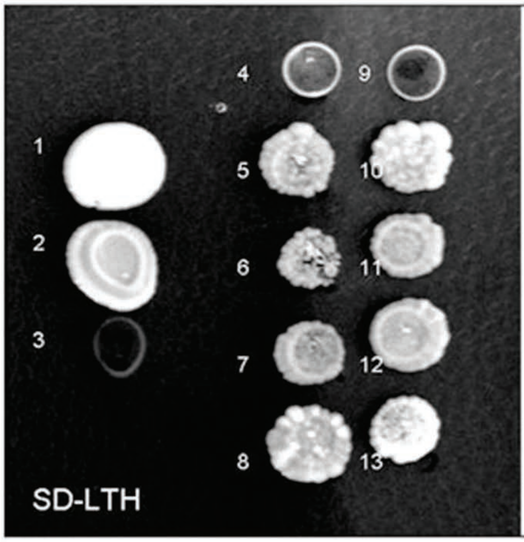
pDEST22/PB1ZZ	PB1-ZZ domains (1-2253bp/1-751aa) of NtJoka2 from pEntrPB1ZZ in pDEST22
pDEST32/PB1ZZ	PB1-ZZ domains (1-2253bp/1-751aa) of NtJoka2 from pEntrPB1ZZ in pDEST32
pDEST22/ZZ	ZZ domain (316-2253bp/106-751aa) of NtJoka2 from pEntrZZ in pDEST22
pDEST32/ZZ	ZZ domain (316-2253bp/106-751aa) of NtJoka2 from pEntrZZ in pDEST32
pDEST22/ZZUBA	ZZ-UBA domains (316-2526bp/106-842aa) of NtJoka2 from pEntrZZUBA in pDEST22
pDEST32/ZZUBA	ZZ-UBA domains (316-2526bp/106-842 aa) of NtJoka2 from pEntrZZUBA in pDEST32
pDEST22/ATG8f	full-length ATG8f from pEntrATG8f in pDEST22
pDEST32/ATG8f	full-length ATG8f from pEntrATG8f in pDEST32
pDEST22/UP9	full-length UP9C from pEntrUP9 in pDEST22
pDEST32/UP9	full-length UP9C from pEntrUP9 in pDEST32
pDEST15/Joka2	full -length NtJoka2 in pDEST15

Suppl. Table 3. List of the oligonucleotides used as forward (F) and reverse (R) primers. All primers are shown from 5’ to 3’.

Cloning by Gateway technology (start and stop codons are underlined)	
F: caccatggctatggagtctgct R: aatagtcccagtcccatcactg	used to amplify PB1 region of *NtJoka2*
F: caccatgtctactcccttacgatc R: ctggggtggtgcctgcg	used to amplify ZZ region of *NtJoka2*
F: caccatggctatggagtctgct R: ctggggtggtgcctgcg	used to amplifyPB1-ZZ region of *NtJoka2*
F: caccatgtctactcccttacgatc R: ctctccagcaataagatccatg	used to amplify ZZ-UBA region of *NtJoka2*
F: caccatggcaaagagttcattc R: ccatgaaagttacaccaagttg	used to amplify *NtATG8f*
F: ggggacaagtttgtacaaaaaagcaggctcaatggctatggagtctgctat R: ggggaccactttgtacaagaaagctgggtcctgctctccagcaataagatc	used to amplify *NtJoka2*
F: ggggacaagtttgtacaaaaaagcaggctcaatgttttcgacaattgctgt R: ggggaccactttgtacaagaaagctgggtcttgggaactgggaacggtaa	used to amplify *UP9C*
RT-PCR	
F: cctcccacatgctattctcc R: agagcctccaatccagacac	*Tac9* (GB# X69885)
F: cgggatccatgttttcgacaattgct R: gcaagcttggtacctcattgggaactgggaac	*UP9C/UP9A* (primers amplify both genes)
F: gcttcacgtctagtccaggt R: gggtgcctgcgtatctaca	*NtJoka2*
F: caccatggcaaagagttcattc R: ccatgaaagttacaccaagttg	*NtATG8f*
Other primers used in this work (restriction enzyme sites are underlined)	
R: agccgacaaccttgattggagac	used to sequence inserts in pDEST22 or pDEST32
R1: caggcatctagcgcatcatccaca R2: tcattcagggctgtgtggggctcgt	used for 5’RACE-PCR to amplify 5’-end of *NtJoka2*
F1: atgtggatgatgcgctagatgcctg F2: atgatgcagggagaacggctaaagct	used for 3’RACE-PCR to amplify 3’-end of *NtJoka2*
F: cggaattcttttcgacaattgctgtgc R: gcctgcagagcgtcaaactccattaccc	used for cloning *UP9C* into pJK1 and JK2
F: cggaattcgcgaaccgaggaggatgc R: cgctgcagctacgaggaagagacgacagaa	used for cloning *LSU1* into pGAD and pGBT
F: cgggatccatgttttcgacaattgct R: gcaagcttggtacctcattgggaactgggaac	used for cloning *UP9C* into pET28a
F: AGGATCCATGGCAAAGAGTTCATTC r: GGAATTCGAAAGTTACACCAAGTTG	used for cloning *ATG8f* into pET28a

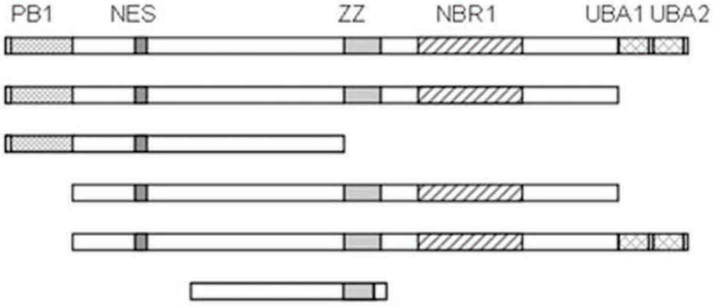
Supplementary Figure 1

A



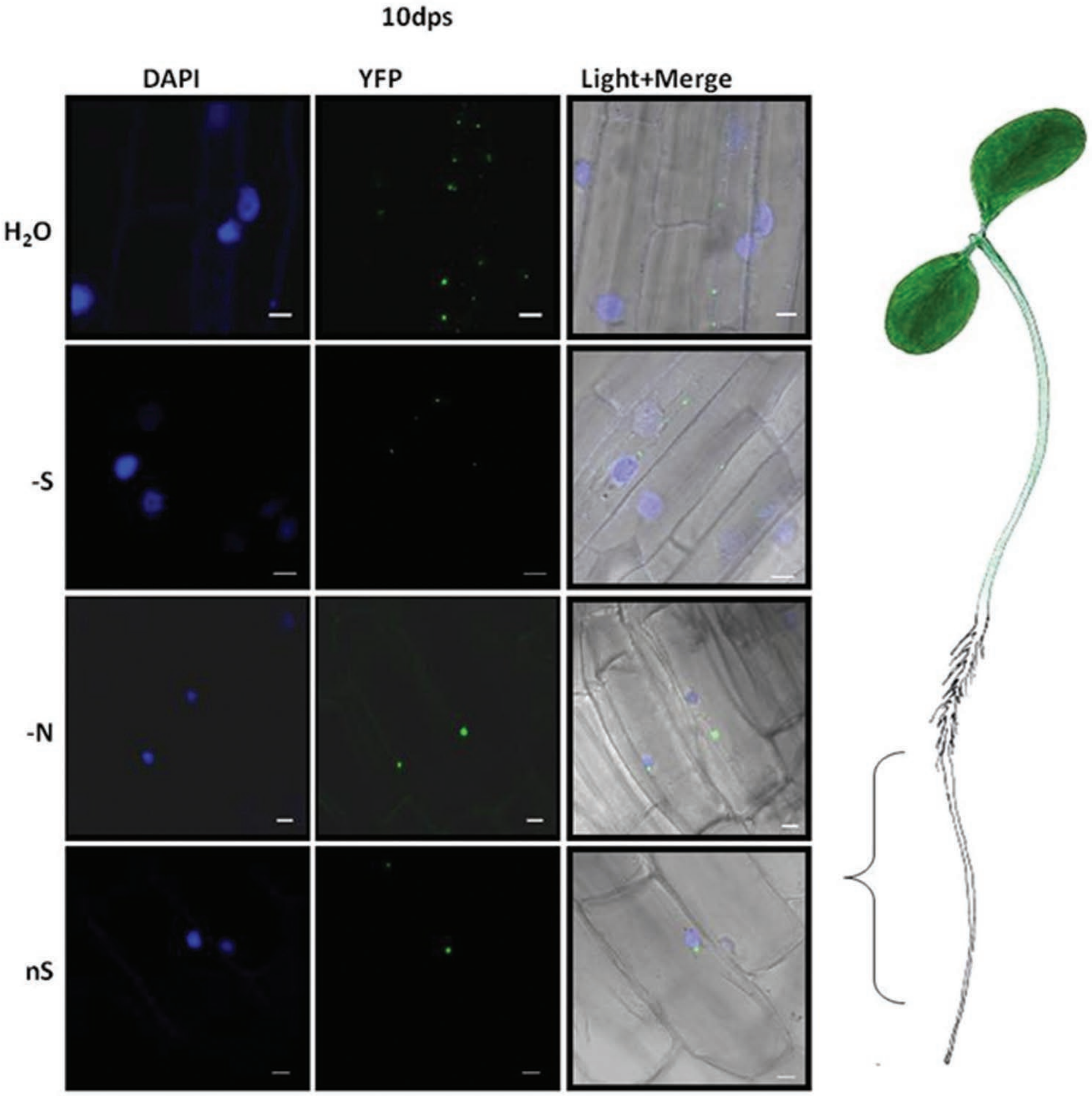
	AD	BD	growth
1.	Strong positive control		+
2.	Weak positive control		+
3.	Negative control		-
4.	PB1	UP9C	-
5.	PB1ZZ	UP9C	+
6.	ZZ	UP9C	+
7.	ZZUBA	UP9C	+
8.	Joka2	UP9C	+
9.	UP9C	PB1	-
10.	UP9C	PB1ZZ	+
11.	UP9C	ZZ	+
12.	UP9C	ZZUBA	+
13.	UP9C	Joka2	+

B

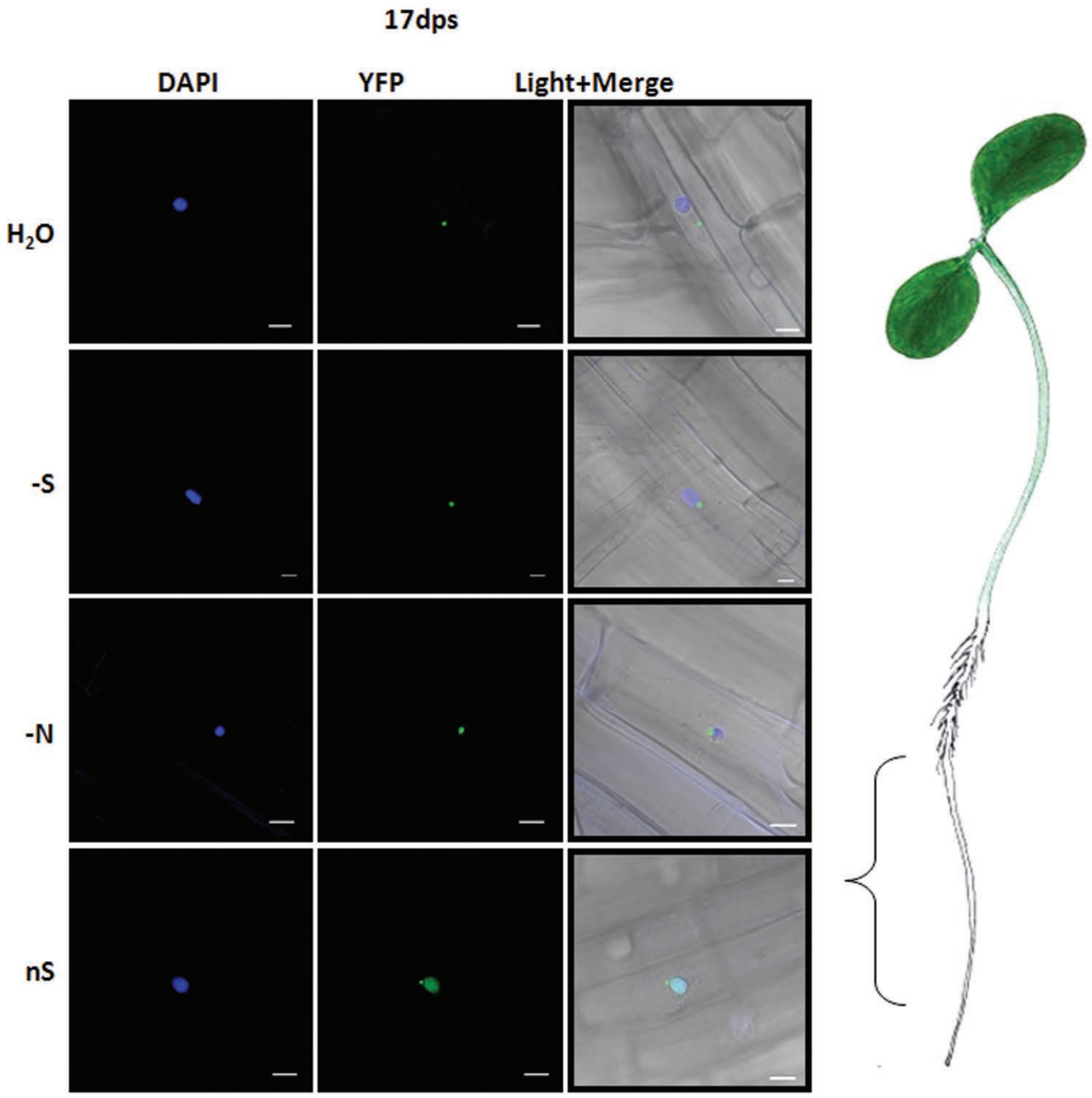


CONSTRUCT	INTERACTION
Joka2	+
PB1ZZ	+
PB1	-
ZZ	+
ZZUBA	+
2b	+

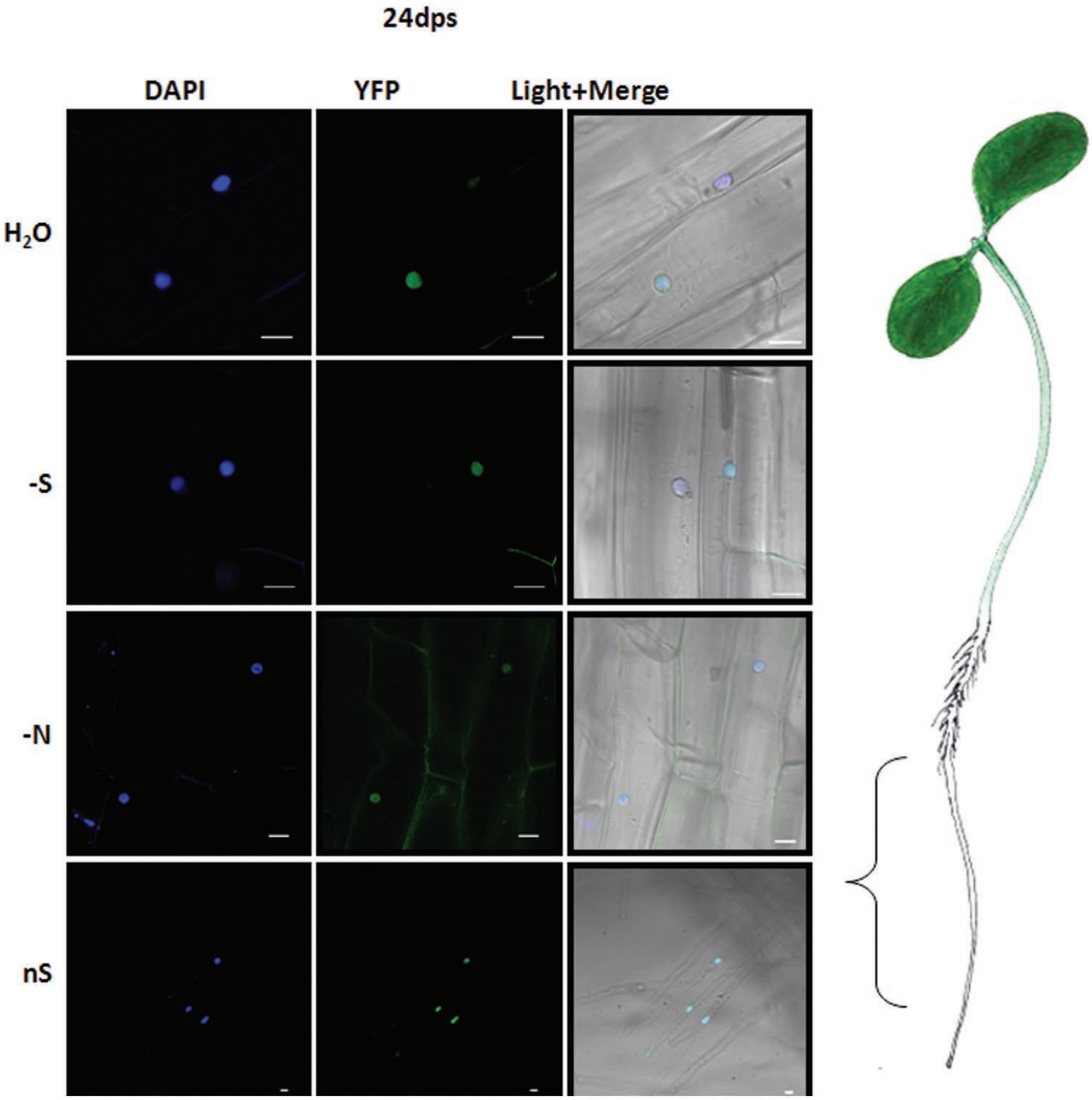
Supplementary Figure 2



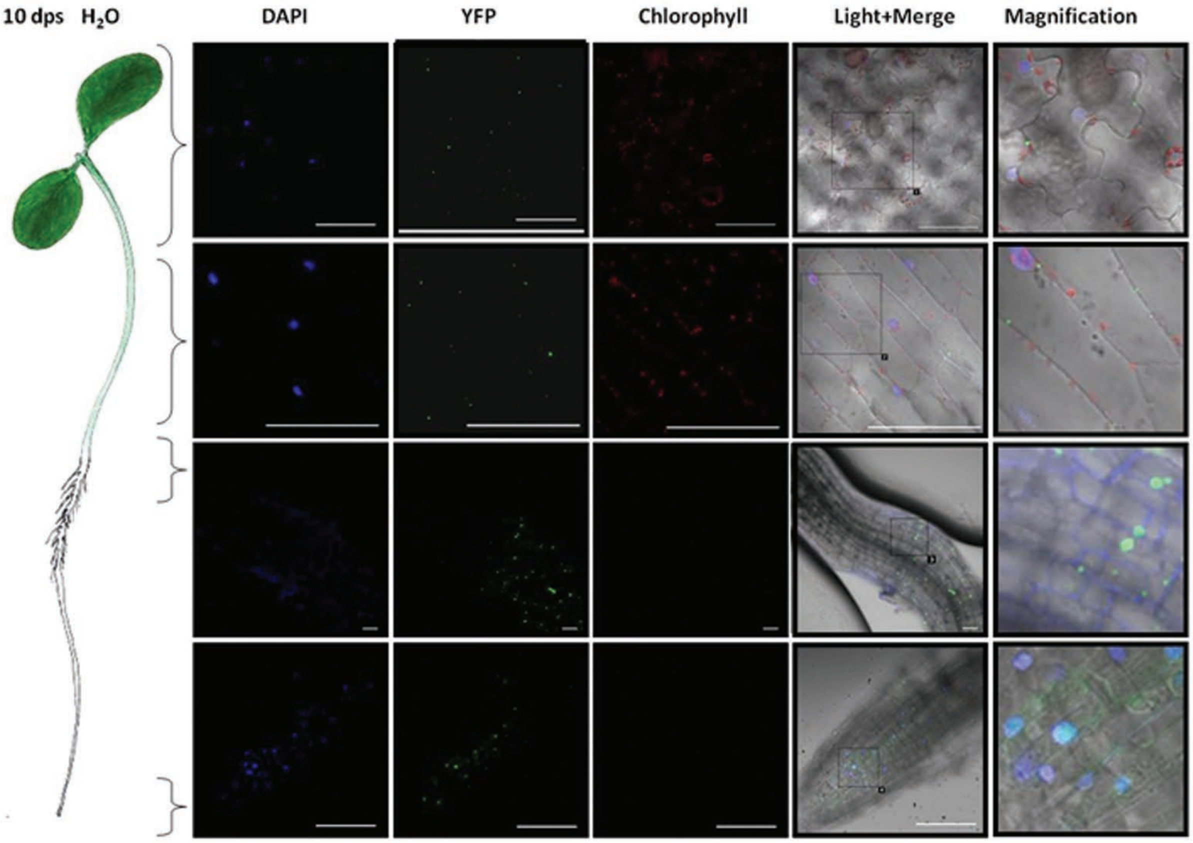
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 1. Mapping of the UP9C interaction to the ZZ domain of NtJoka2. The yeast colonies along with descriptions (A) and the schemes of the constructs used for the experiment (B) are shown. All constructs contain *NtJoka2* cDNA from *Nicotiana tabacum*, except 2b, which contains *NpJoka2* cDNA from *Nicotiana plumbaginifolia*. The proof for UP9C interaction with the 2b fragment comes from the pool down experiment (see Fig. 1B).

Supplementary Figure 2. Localization of Joka2-YFP in roots elongation zone of tobacco seedlings grown for 10 days in the indicated conditions (-S, sulfur starvation; -N, nitrogen starvation; nS, nutrient sufficient medium and water). The signal for Joka2-YFP, DNA staining with DAPI and overlays of both with the transparent view image are shown. The scale bars (10 μM) are indicated.

Supplementary Figure 3. Localization of Joka2-YFP in roots elongation zone of tobacco seedlings grown for 17 days in the indicated conditions (-S, sulfur starvation; -N, nitrogen starvation; nS, nutrient sufficient medium and water). The signal for Joka2-YFP, DNA staining with DAPI and overlays of both with the transparent view image are shown. The scale bars (10 μM) are indicated.

Supplementary Figure 4. Localization of Joka2-YFP in roots elongation zone of tobacco seedlings grown for 24 days in the indicated conditions (-S, sulfur starvation; -N, nitrogen starvation; nS, nutrient sufficient medium and water). The signal for Joka2-YFP, DNA staining with DAPI and overlays of both with the transparent view image are shown. The scale bars (10 μM) are indicated.

Supplementary Figure 5. Localization of Joka2-YFP in the indicated parts of tobacco seedlings grown for 10 days in water. The signal for Joka2-YFP, DNA staining with DAPI,chlorophyll (if present) and overlays of all with the transparent view image are shown. The right-hand panels are the magnification of the indicated parts. The scale bars (100 μM) are indicated.