

Bioinformatic study on the WRKY75 transcription factor regulates leaf senescence in Arabidopsis

Group 06

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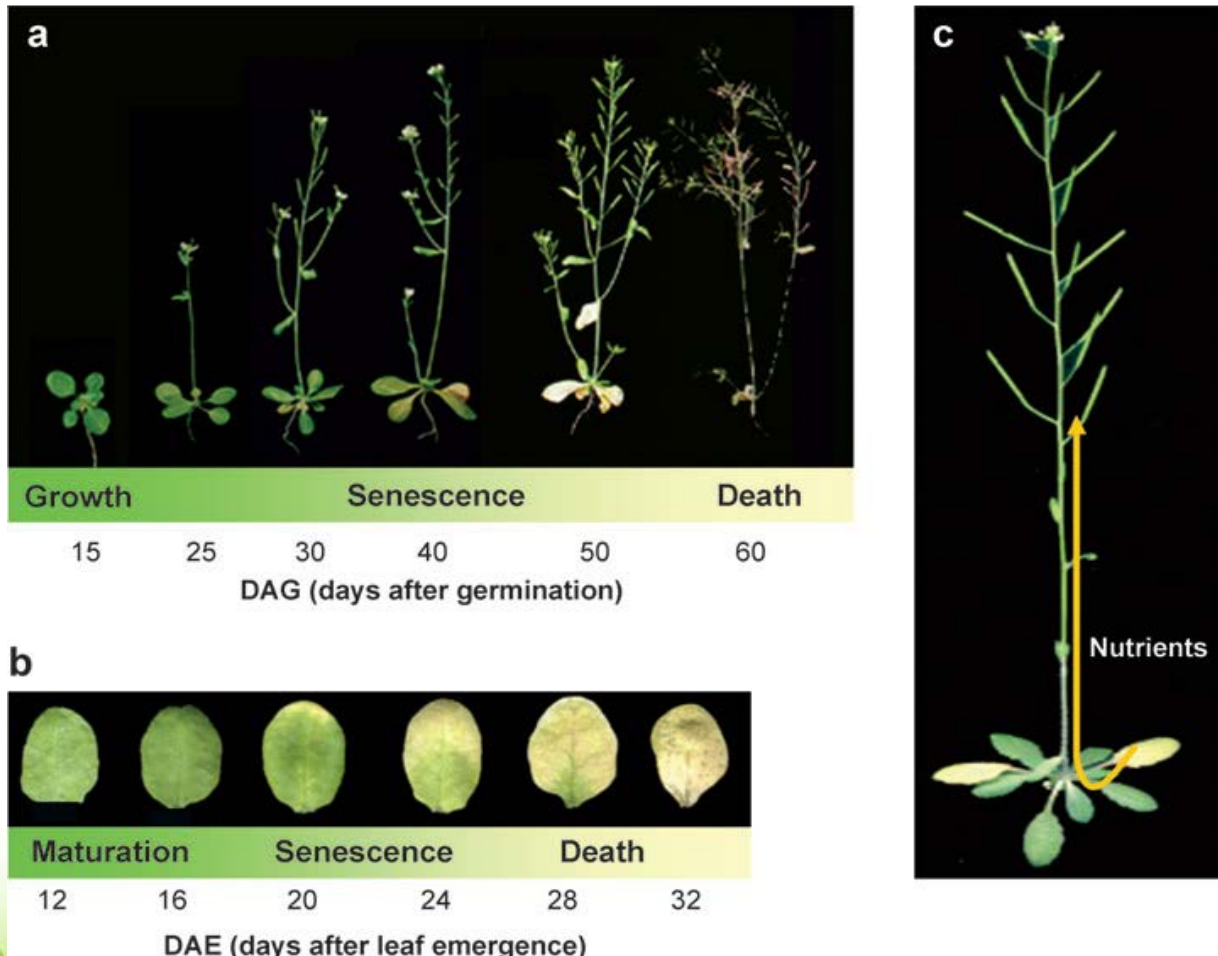
Outline

- Background
- Brief introduction of WRKY75 transcription factor
- Sequence analysis of WRKY75 transcription factor
- Interacting partners of WRKY75 transcription factor
- Protein characterisation analysis of WRKY75 transcription factor



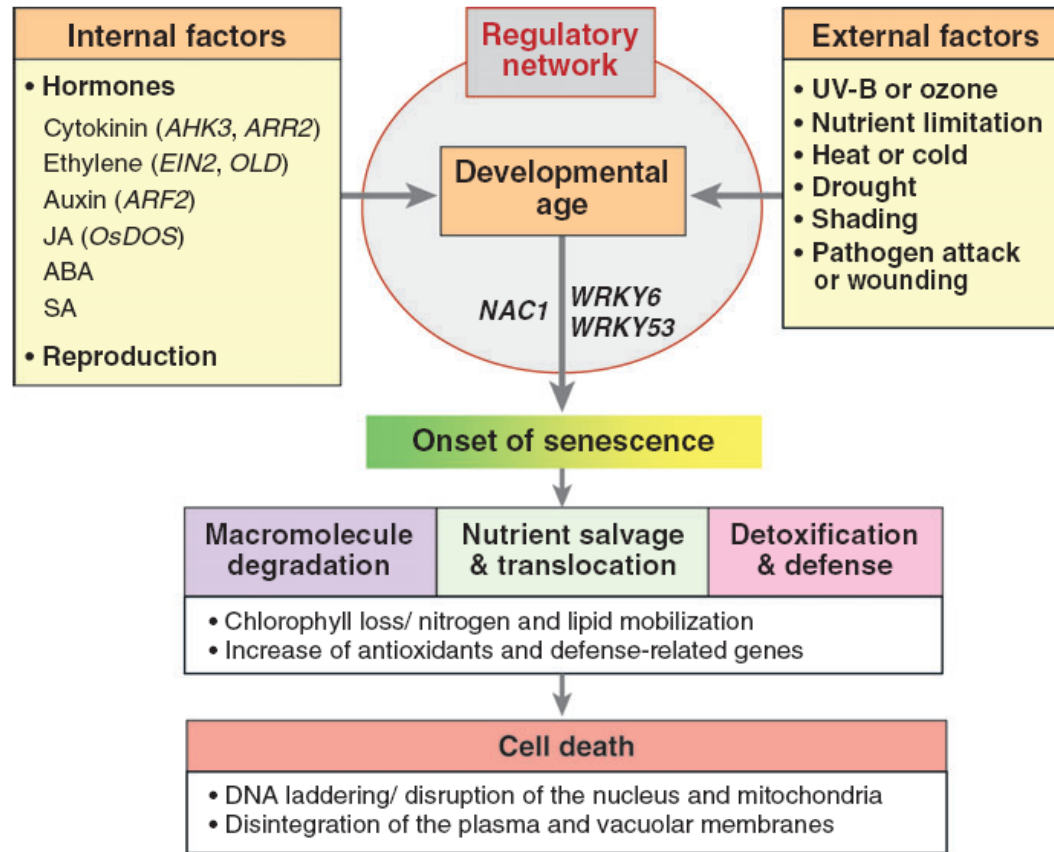
Background

The process of Age-dependent leaf senescence



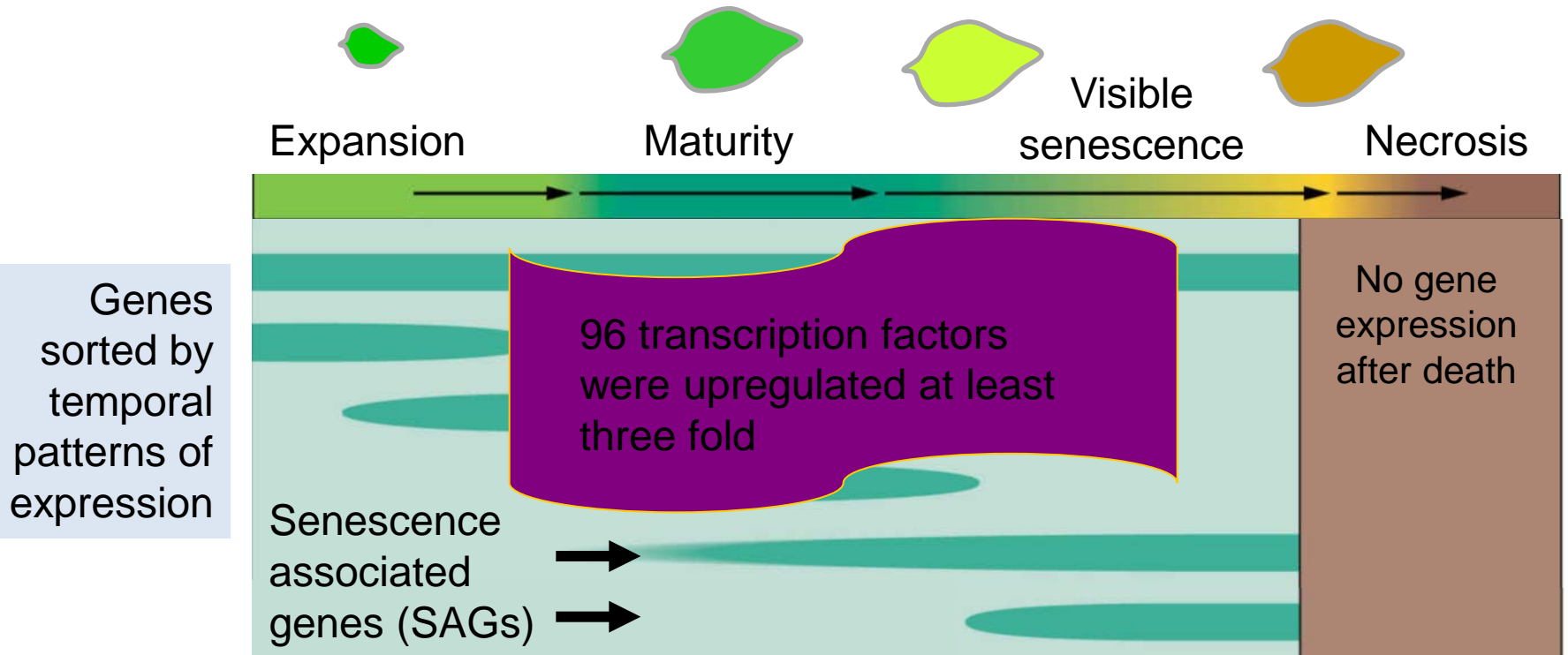
Background

Regulatory pathways in leaf senescence



Background

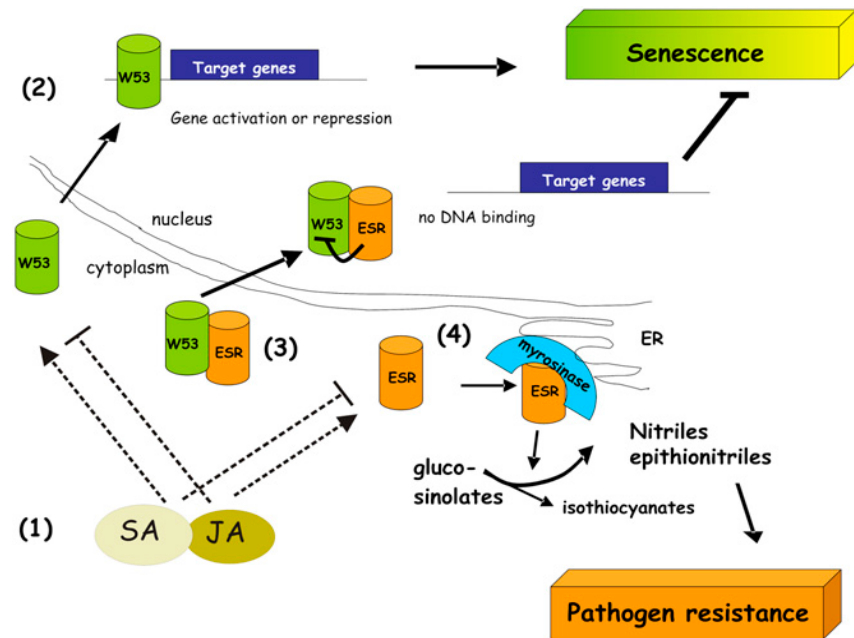
The onset of senescence brings about a change in gene expression



Background

The WRKY transcription factors regulate leaf senescence

WRKY53 is a positive regulator of age-dependent leaf senescence

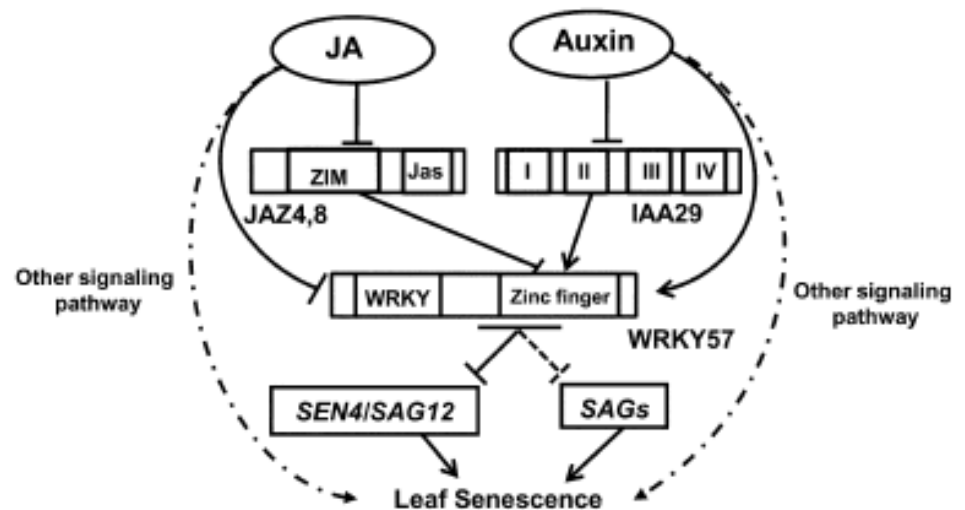
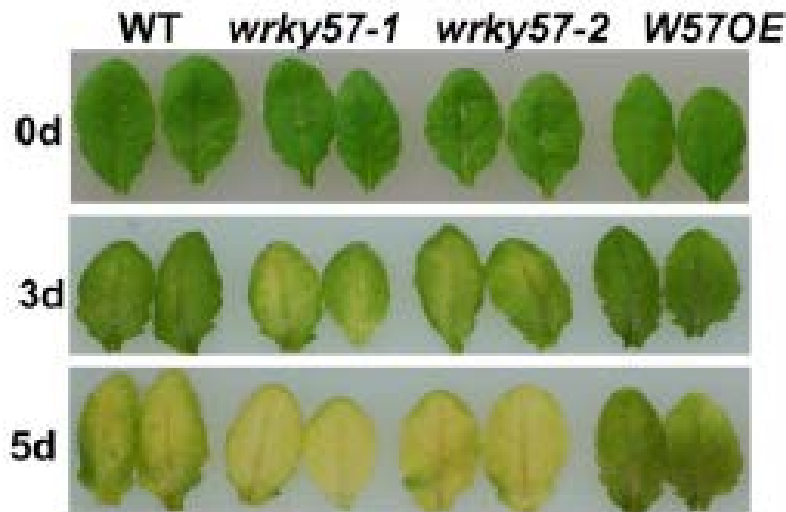


But *wrky53* mutant shows a weak delayed-senescence phenotype

Background

WRKY57 is a negative regulator of JA-promoted leaf senescence

wrky57 leaves showed a more severe JA-induced senescence phenotype than the wild type and overexpression line upon 100 μ M MeJA treatment.

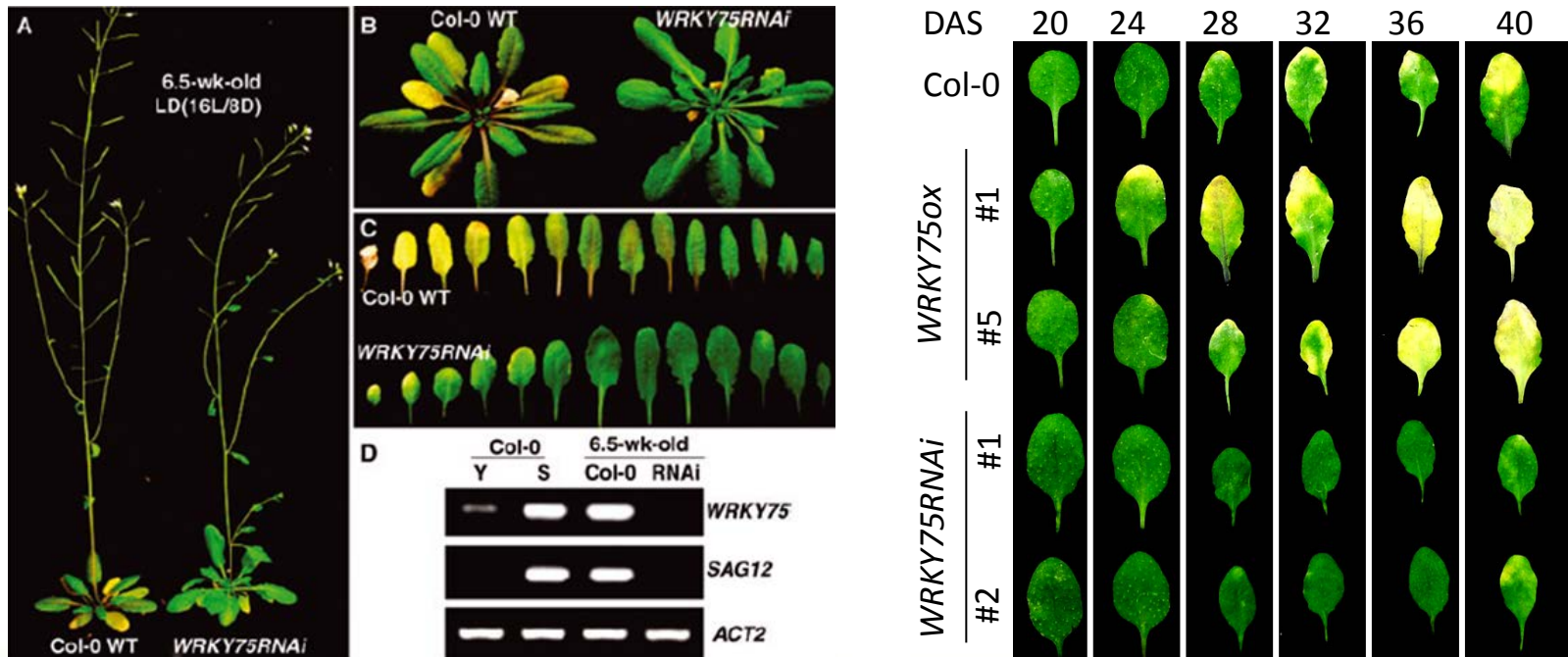


But the *wrky57* mutant shows no obvious senescence-related phenotype in the process of age-dependent senescence.

Background

WRKY75 is a positive regulator of age-dependent leaf senescence

The *wrky75RNAi* shows an obvious delayed senescence phenotype and *WRKY75ox* transgenic line shows an obvious accelerated senescence phenotype.



The molecular mechanism of WRKY75 regulates leaf senescence is unknown.

Our purpose:

Bioinformatic analyzing WRKY75 to help to investigate the molecular mechanism of WRKY75 regulates leaf senescence.



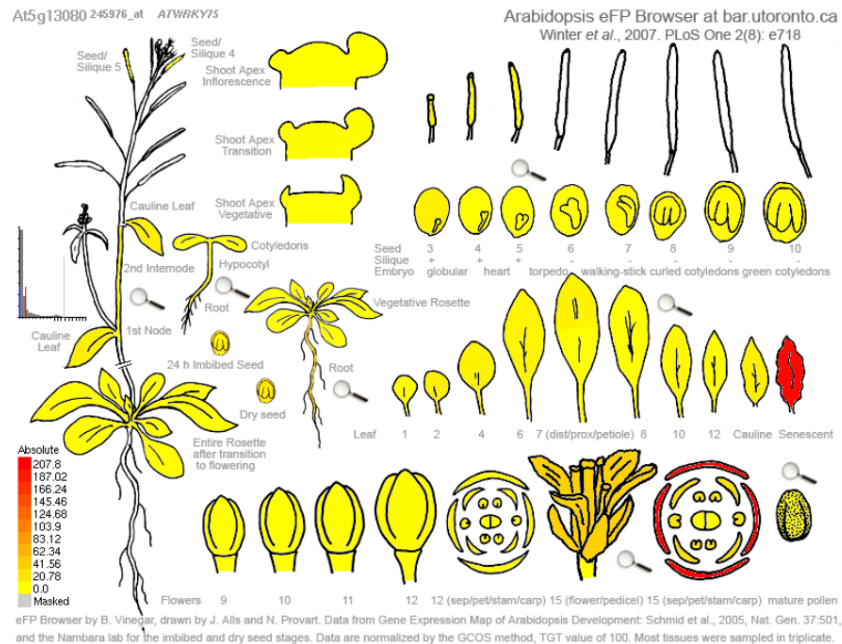
Brief introduction of WRKY75

WRKY75 transcription factor contains one WRKY DNA-binding domain

WRKY75 can specifically bind on the **W-box(TTGACC /T)** element of the promoter sequence.

Subcellular location: nuclear

Expression pattern:



Brief introduction of WRKY75

Binding sites in the promoter of WRKY75

W-box promoter motif	4151445	4151450	ttgact	WRKY
W-box promoter motif	4151975	4151980	ttgact	WRKY
W-box promoter motif	4152496	4152501	ttgacc	WRKY
W-box promoter motif	4153696	4153701	ttgact	WRKY
W-box promoter motif	4152979	4152984	ttgact	WRKY
W-box promoter motif	4152333	4152338	ttgacc	WRKY

RAV1-A binding site motif	4151588	4151592	caaca	ABI3VP1
RAV1-A binding site motif	4151819	4151823	caaca	ABI3VP1
RAV1-A binding site motif	4152072	4152076	caaca	ABI3VP1
RAV1-A binding site motif	4152355	4152359	caaca	ABI3VP1
RAV1-A binding site motif	4152898	4152902	caaca	ABI3VP1
RAV1-A binding site motif	4152642	4152646	caaca	ABI3VP1
RAV1-A binding site motif	4152059	4152063	caaca	ABI3VP1
RAV1-A binding site motif	4151908	4151912	caaca	ABI3VP1
RAV1-A binding site motif	4151902	4151906	caaca	ABI3VP1

Binding sites in the promoter of senescence-marker genes

SAG12

W-box promoter motif	18628572	18628577	ttgacc	WRKY
W-box promoter motif	18629158	18629163	ttgact	WRKY
W-box promoter motif	18628965	18628970	ttgacc	WRKY
W-box promoter motif	18627866	18627871	ttgact	WRKY

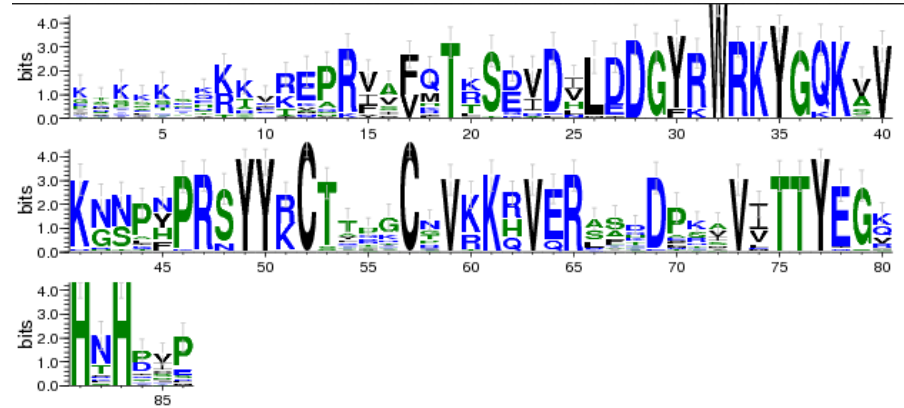
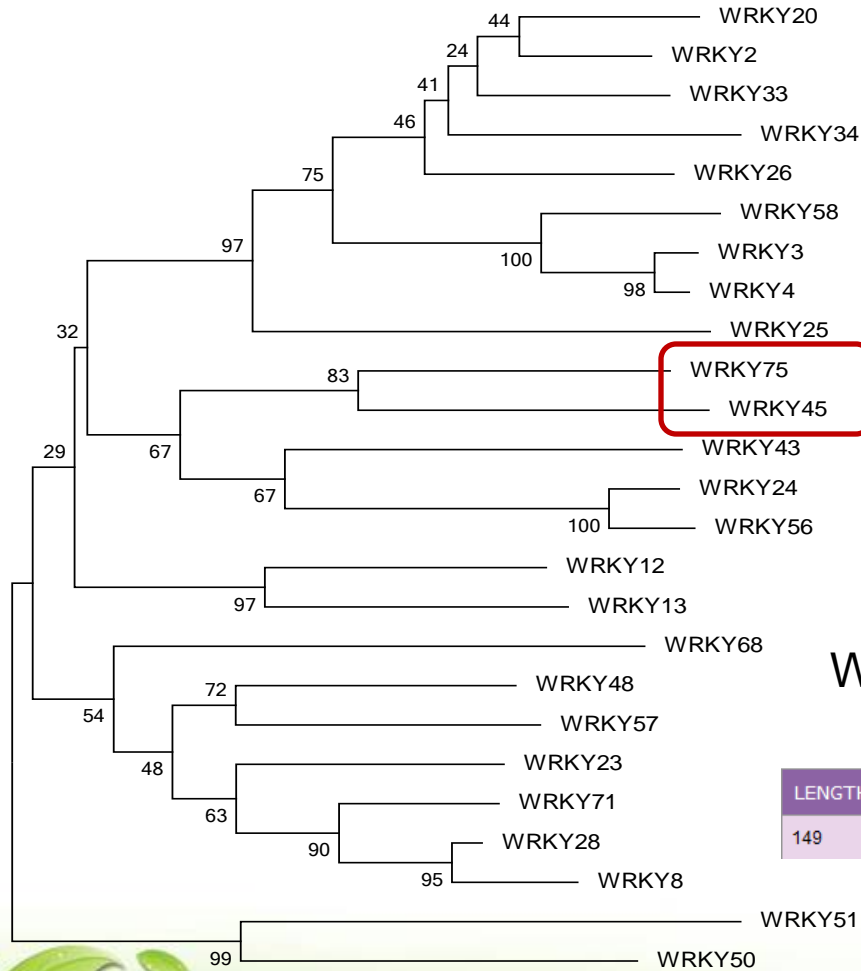
SEN4

W-box promoter motif	14822323	14822328	ttgact	WRKY
W-box promoter motif	14822361	14822366	ttgacc	WRKY
W-box promoter motif	14822624	14822629	ttgact	WRKY

CAB2

W-box promoter motif	10476545	10476550	ttgacc	WRKY
W-box promoter motif	10477027	10477032	ttgacc	WRKY

Sequence analysis of WRKY75

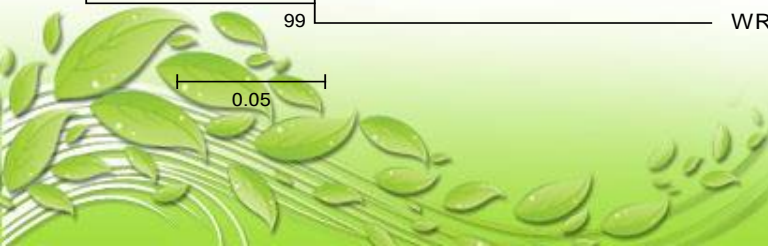


WebLogo 3.4

WRKY45 maybe the homologue of WRKY75

Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
149	398.0	82/149 (55.0%)	98/149 (65.8%)	6/149 (4.0%)

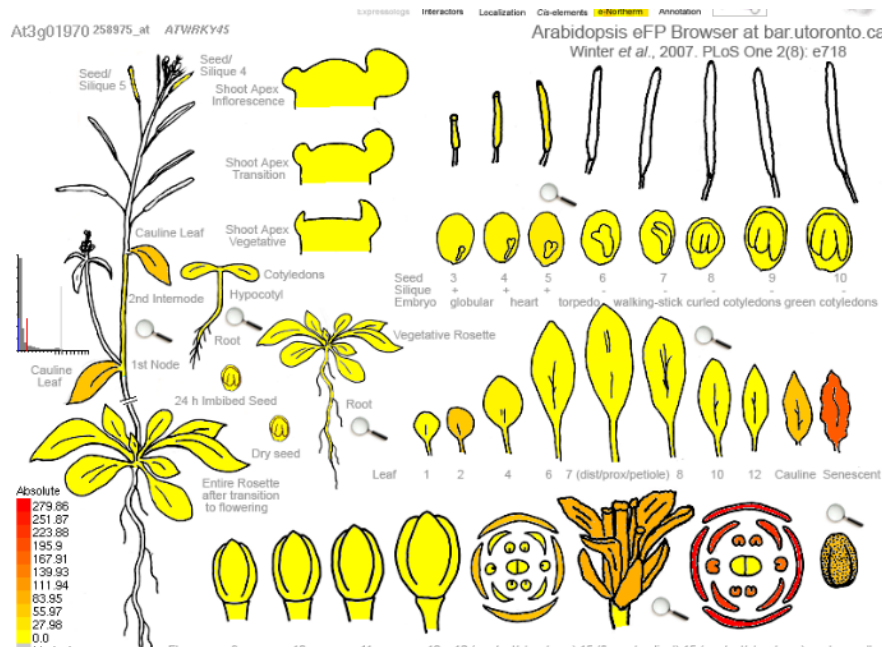


0.05

WRKY45 may involved in leaf senescence



Locus	Species	Function	Effect	Evidence
AT3G01970	<i>Arabidopsis thaliana</i>	Transcription regulation:WRKY	unclear	Genomic evidence:microarray data



senescence-phenotype analysis of *wrky45* single mutant and *wrky45 wrky75* double mutant



Sequence analysis of WRKY75

Mutiple alignment of WRKY75 WRKY53 and WRKY57

```

1 ----- 0 Q9FYA2 WRK75_ARATH
1 MNDPDNPDLSNDDSAWRELTLTAQDSDFDRDTSNILSDFGWNLHSSDHPHSLRFSDSL 60 Q9C983 WRK57_ARATH
1 -----MEGRDMLSWEQKTLTLLSELINGFDAAKK-----LQARLREAP--SPSSSFSPA 46 Q9SUP6 WRK53_ARATH

1 -----MEGYDNGSLYAP-----FLS--L-KSHSKPELHQGEE----ESSK- 33 Q9FYA2 WRK75_ARATH
61 TQ-----TTGVKPTTVTSSCSSAAVSVAVTSTNNNPSATSSSSSEDPAENSTA 108 Q9C983 WRK57_ARATH
47 TAVAETNEILVKQIVSSYERSLLLLNWSSSPSVQLIPTVTVVPVANPGSVP----ESPA 102 Q9SUP6 WRK53_ARATH
      . . : :. * .. .*

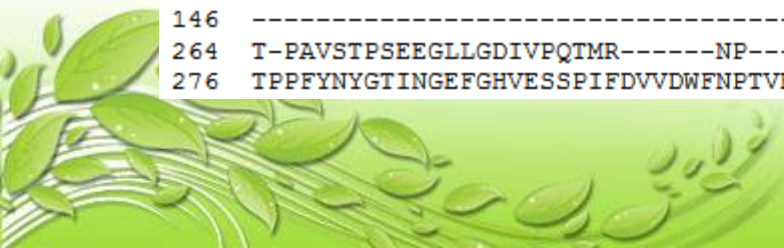
34 -----VRSEGCSKS-----VESSKKKGKKQRYAF-----QTRSQVDILDDGYR 71 Q9FYA2 WRK75_ARATH
109 SAEKTPPETPVKE-----KKKAQKRIRQPRFAF-----MTKSDVDNLEDGYR 151 Q9C983 WRK57_ARATH
103 SINGSPRSEEFADGGGSSESHHRQDYIFNSKKRKMPLPKWSEKVRISPERGLEGPQDDVFS 162 Q9SUP6 WRK53_ARATH
      * . :*: :. :. :* :

72 WRKYGQKAVKNNKFPRSYYRCTYG---GCNVKKQVQRLTVDQEVVVTTYEGVHSHPI--EK 127 Q9FYA2 WRK75_ARATH
152 WRKYGQKAVKNSPFPRSYYRCTNS---RCTVKKRVERSSDDPSIVITTYEGQHCHQT--IG 207 Q9C983 WRK57_ARATH
163 WRKYGQKDILGAKFPRSYYRCHTRSTQNCWATKQVQRSDGDATEVFEVTYRGTHTCSQAIT 222 Q9SUP6 WRK53_ARATH
***** : ***** * ..*:* * ..**.*

128 STENFEHILTQM--Q-----IYSSF----- 145 Q9FYA2 WRK75_ARATH
208 FPRG--GILTAHDPHSFTSHHLLPPPL--PNPY--YYQELLHQLHRDNNAPSRLPRPTTED 263 Q9C983 WRK57_ARATH
223 RTPPLASPEKRQDT-----RVKPAITQKPKDILESLSNLTVRTDGLDDGKDVFSFPD 275 Q9SUP6 WRK53_ARATH
      . :.

146 ----- 145 Q9FYA2 WRK75_ARATH
264 T-PAVSTPSEEGLLGDIVPQTMR-----NP----- 287 Q9C983 WRK57_ARATH
276 TPPFYNYGTINGEFGHVESSPIFDVVDWFNPTVEIDTTFPAFLHESIYY 324 Q9SUP6 WRK53_ARATH

```



WRKY75 interacting partners



the two genes are SAGs

Locus	Species	Function	Effect	Evidence
AT3G18360	<i>Arabidopsis thaliana</i>	Others	unclear	Genomic evidence:microarray data
Locus	Species	Function	Effect	Evidence
AT3G56880	<i>Arabidopsis thaliana</i>	Others	unclear	Genomic evidence:microarray data

VQ-motif containing proteins can interact with the WRKY transcription factor and act as transcription regulator(coactivator or supressor)of the WRKY.

Arabidopsis Sigma Factor Binding Proteins Are Activators of the WRKY33 Transcription Factor in Plant Defense ^W

Zhibing Lai,^a Ying Li,^{a,1} Fei Wang,^a Yuan Cheng,^{a,b} Baofang Fan,^a Jing-Quan Yu,^b and Zhixiang Chen^{a,b,2}

The Plant Journal (2013) 74, 730-745

doi: 10.1111/tpj.12159

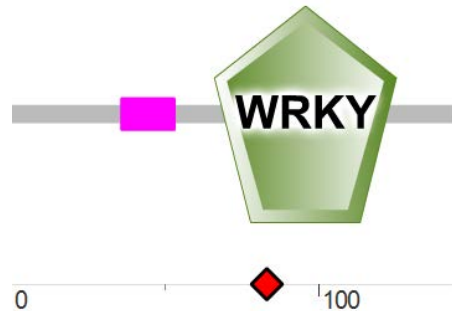
Arabidopsis transcription factor WRKY8 functions antagonistically with its interacting partner VQ9 to modulate salinity stress tolerance

(Zhibing Lai et al., *The Plant Cell*.2011)

(Yanru Hu et al.,*The Plant Journal* . 2013)

WRKY75 interacting partners

- 1 Senescence-phenotype analysis of the two VQ-motif containing mutant and over-expression transgenic line.
- 2 Confirm the interaction between the two VQ motif containing proteins and WRKY75 by yeast-two-hybrid
- 3 Narrow down the interacting region of WRKY75 between the two VQ motif containing proteins



- 4 Biological function analysis of the interaction(The VQ-motif containing protein may affect the DNA-binding ability of WRKY75)



Protein characterization analysis of WRKY75

Amino acid composition(percentages)

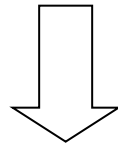
Amino Acid Composition (percentages)																			
A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
2.07	2.07	3.45	8.28	3.45	6.90	3.45	2.76	11.03	4.83	1.38	3.45	2.76	6.90	5.52	11.72	4.83	7.59	0.69	6.90

Biological process prediction

Biological Process Predictions			
GO term	Name	Prob	SVM Reliability
GO:0010468	regulation of gene expression	0.962	H
GO:0006355	regulation of transcription, DNA-dependent	0.908	H
GO:0006468	protein phosphorylation	0.867	H
GO:0006796	phosphate-containing compound metabolic process	0.747	H
GO:0006351	transcription, DNA-dependent	0.740	H
GO:0016310	phosphorylation	0.714	H
GO:0006810	transport	0.678	H
GO:0002682	regulation of immune system process	0.577	H
GO:0006811	ion transport	0.546	H
GO:0009887	organ morphogenesis	0.529	H
GO:0007166	cell surface receptor signaling pathway	0.521	H
GO:0006357	regulation of transcription from RNA polymerase II promoter	0.512	H
GO:0009888	tissue development	0.504	H

Protein characterization analysis of WRKY75

WRKY75 is a downstream component of MKK9-MPK3/
MPK6 in the regulation of Pi accumulation.



WRKY75 protein maybe phosphorylated by some
kinase (MPK3/MPK9)

(Lei et al., New Phytologist.2014.)

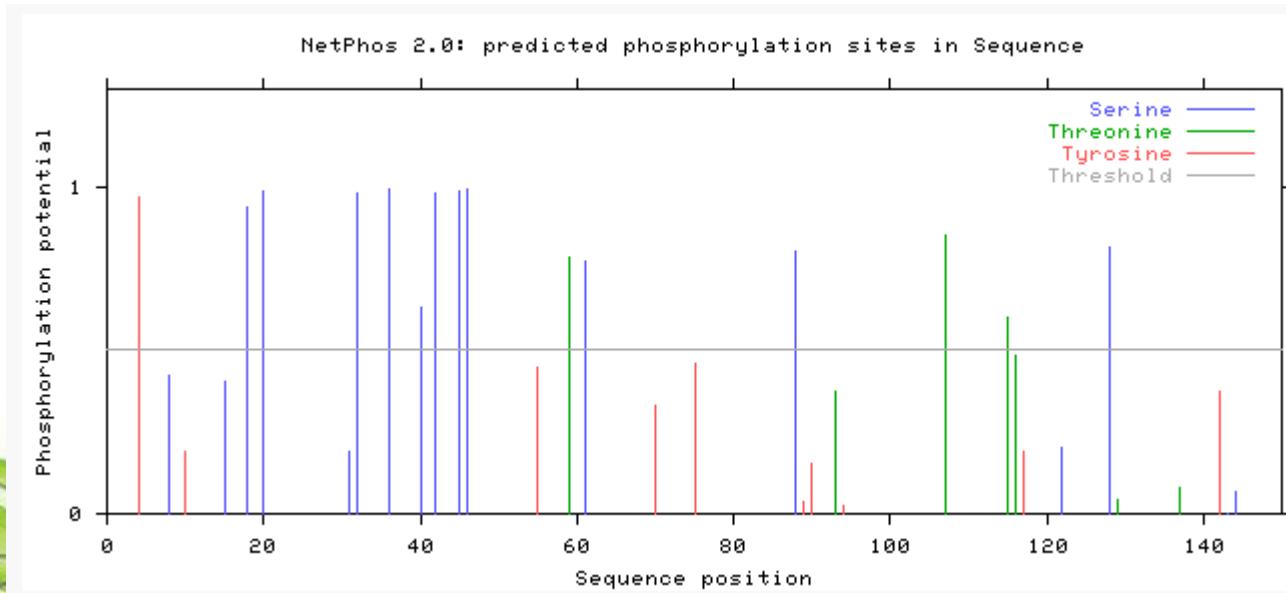


Protein characterization analysis of WRKY75

Prediction of phosphorylation sites

```
145 Sequence
MEGYDNGSLYAPFLSLKSHSKPELHQGEEESSKVRSEGCSKSVESKSKKGGKKQRYAFQTRSQVDILDDGYRWRKYGQKAV      80
KNNKFPRSYRCTYGGCNVKKQVQRLTVDQEVVVTTYEGVHSHPIEKSTENFEHILTQMQUIYSSF                      160
...Y.....S.S.....S...S...S.S..SS.....T.S.....
.....S.....T.....T.....S.....
```

Phosphorylation sites predicted: Ser: 11 Thr: 3 Tyr: 1



Protein characterization analysis of WRKY75

Amino acid composition(percentages)

Amino Acid Composition (percentages)

A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
2.07	2.07	3.45	8.28	3.45	6.90	3.45	2.76	11.03	4.83	1.38	3.45	2.76	6.90	5.52	11.72	4.83	7.59	0.69	6.90

Prediction of nuclear localization signals

Predicted NLSs in query sequence

```
MEGYDNGSLYAPFLSLKSHSKPELHQGEEESSKVRSEGCSKSVESSKKKG 50
KKQRYAFQTRSQVDILDDGYRWRKYGQKAVKNNKFRSYRCTYGGCNVK 100
KQVQRLTVDQEVVVTTYEGVHSHPIEKSTENFEHILTQMQUIYSSF 145
```

Protein characterization analysis of WRKY75

Prediction of ubiquitination sites

Peptide	Position	Score	Threshold
YAPFLSLKSHSKPEL	17	2.64	0.3
LSLKSHSKPELHQGE	21	0.80	0.3
QGEEESSKVRSEGCS	33	1.28	0.3
VRSEGCSKSVESKK	41	1.04	0.3
SKSVESKKKGKKQR	47	3.09	0.3
KSVESKKKGKKQRY	48	1.98	0.3
SVESKKKGKKQRYA	49	2.67	0.3
ESSKKKGKKQRYAFQ	51	3.10	0.3
SSKKKGKKQRYAFQT	52	2.65	0.3
RWRKYGQKAVKNNKF	78	2.27	0.3
KYGQKAVKNNKFPRS	81	2.57	0.3
QKAVKNNKFPRSYYR	84	0.31	0.3
TYGGCNVKKQVQRLT	100	1.36	0.3

Summary

- WRKY75 transcription factor may directly regulate some senescence-associated genes expression.
- WRKY45 maybe the homologue of WRKY75 and may involve in regulating leaf senescence.
- Two VQ-motif containing proteins may interact with WRKY75 and regulate the transcription ability of WRKY75
- WRKY75 maybe phosphorylated by some kinase in the process of rugulating gene expression during leaf senescence.



Thank you for your attention!

