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山东农业大学

Studies on Plant VKOR, a homologue of human Vitamin K epoxide reductase

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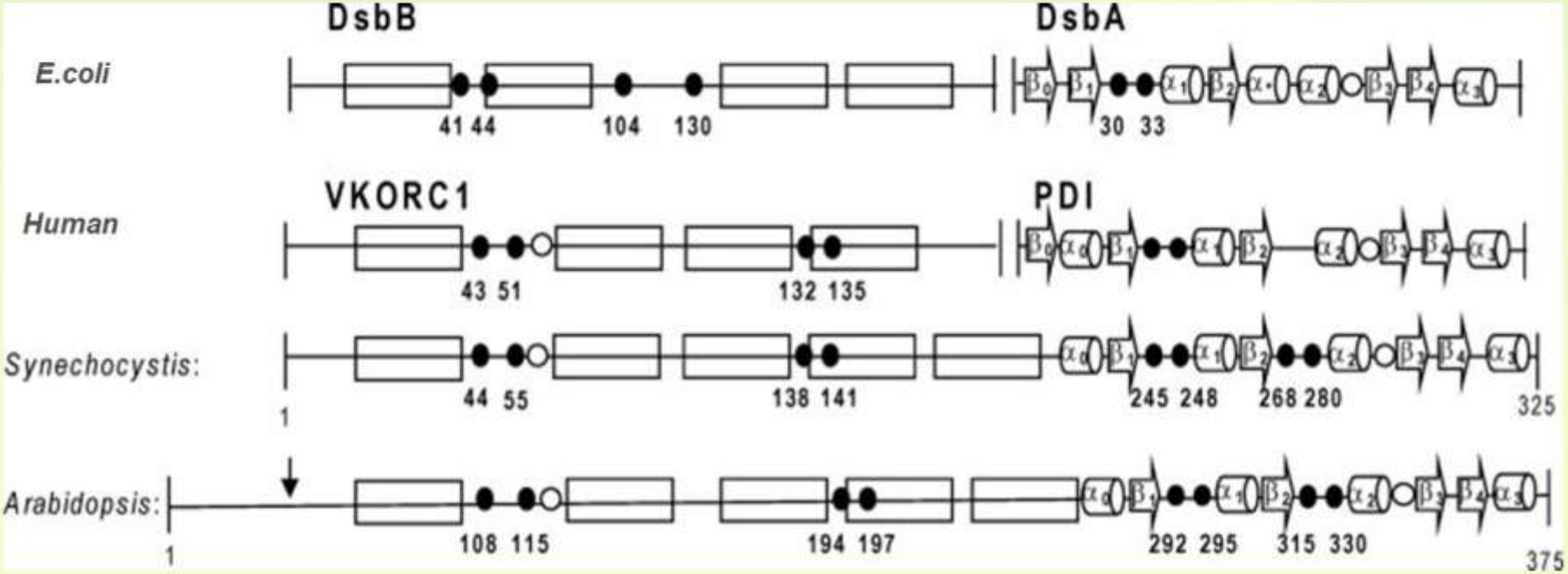
- A homologue of human Vitamin K epoxide reductase (VKOR) from *mycobacterium* was demonstrated to replace DsbB to catalyze the formation of disulfide bond.
- VKORs are present in bacteria, vertebrates, plants.
- In some plants, like *Arabidopsis*, the VKOR domain is fused with a domain homologous DsbA.

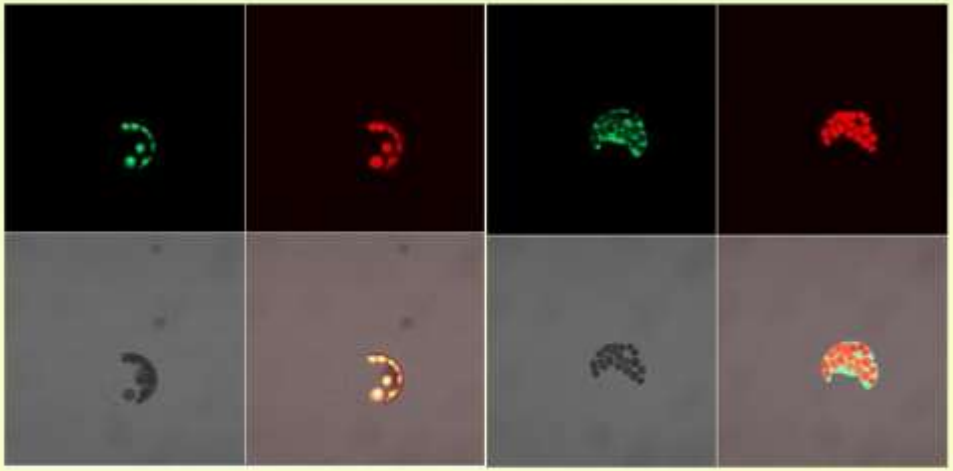


Multiple sequence alignment of diverse VKORs, homologues presented 70% consensus.

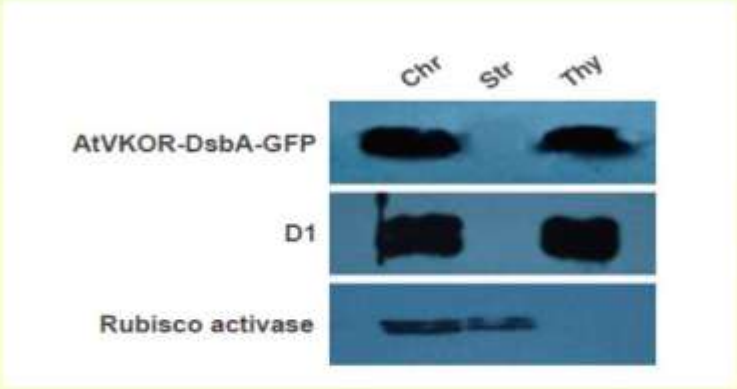
Questions

- What is localization of plant VKOR?
- Does it have function in formation of disulfide bond?
- What are the effects to plants if this gene is deleted?

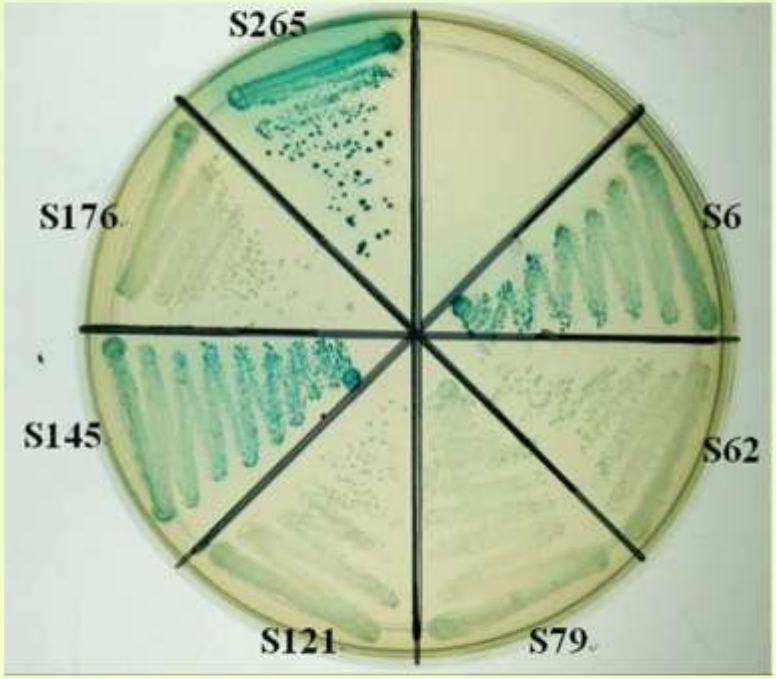
- In our study, different methods predicted *At*VKOR was localized in chloroplast and it might have a **transit peptide** (signal sequence) of 45 amino acids.
- Confocal analysis of GFP signals indicated the signal sequence targets to the chloroplast and Western blot anlysis proved *At*VKOR was localized at the thylakoid membrane.

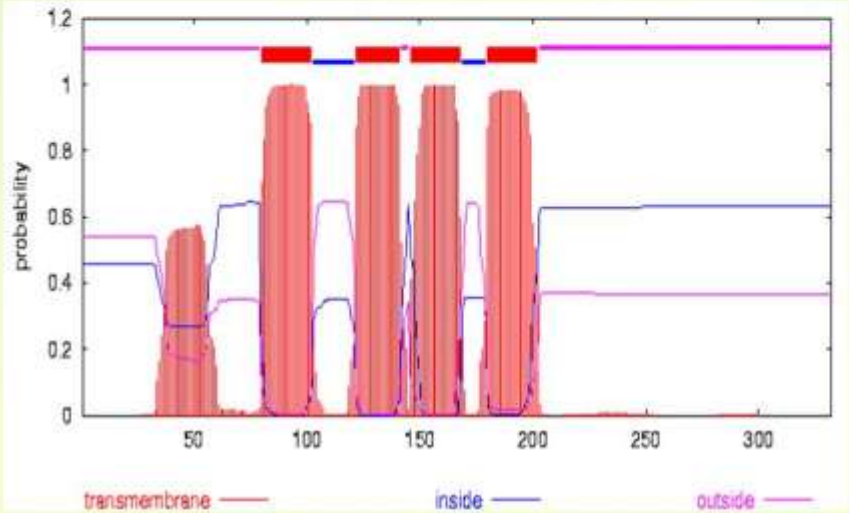


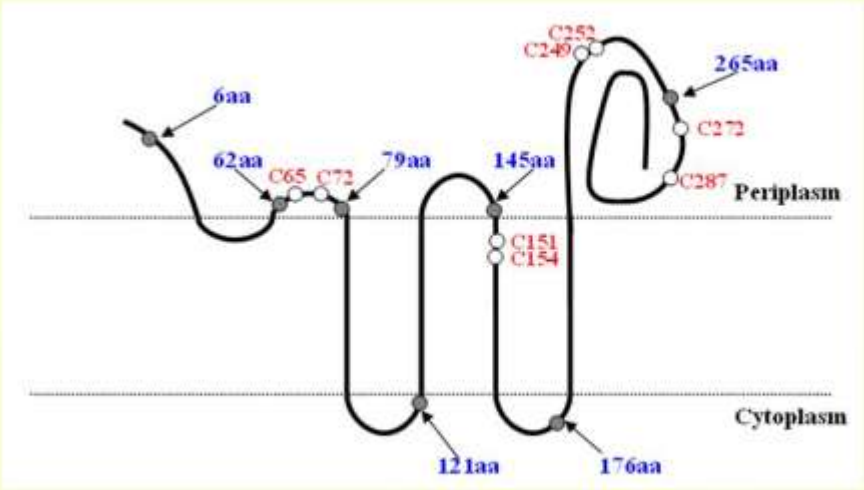
ss-VKOR-GFP Chlorophyll GFP Chlorophyll



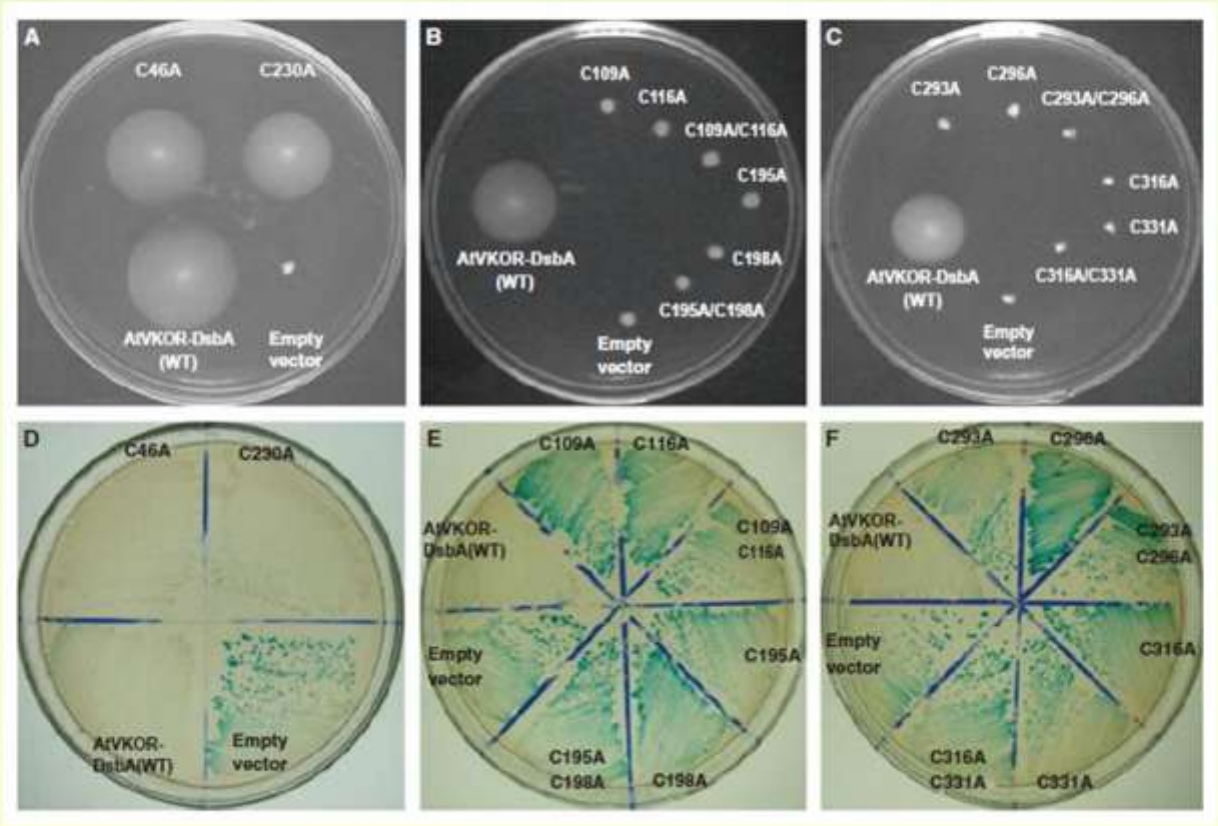
Topology of plant VKOR





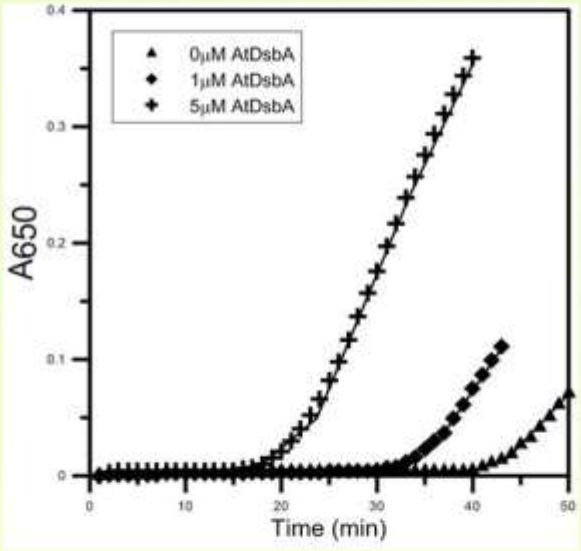


Plant VKOR has the function of disulfide bond formation in *E.coli* and the eight conserved cysteines are essential for the function.



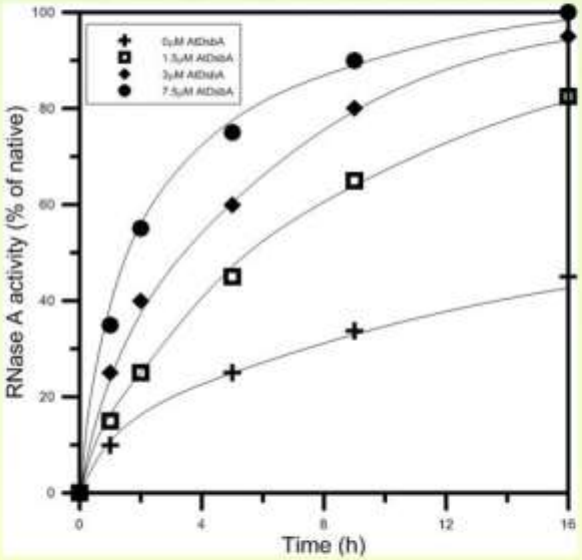
The function of disulfide bond formation and analysis of essential cysteines

The domain of *At*DsbA, like PDI, has the activities of reductase, oxidatase and isomerase.



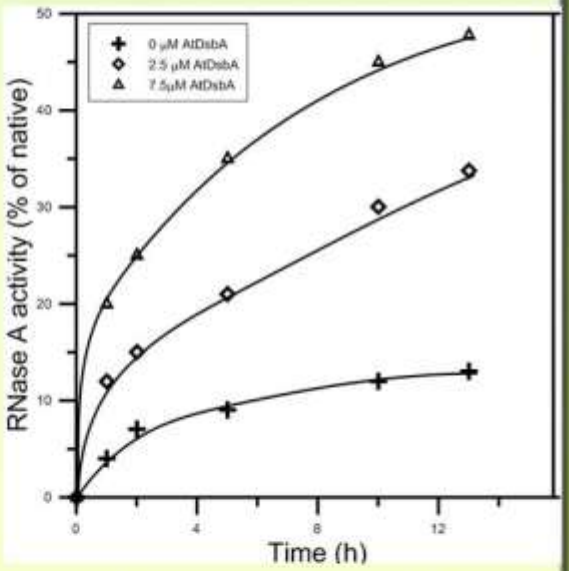
Reductase of *At*DsbA

(▲) 0 μM *At*DsbA; (◆) 1 μM WT *At*DsbA; (✚) 5 μM WT *At*DsbA.



Oxidatase of *At*DsbA

(✚) 0 μM *At*DsbA; (◘) 1.5 μM *At*DsbA; (◆) 3 μM *At*DsbA; (●) 7.5 μM *At*DsbA.

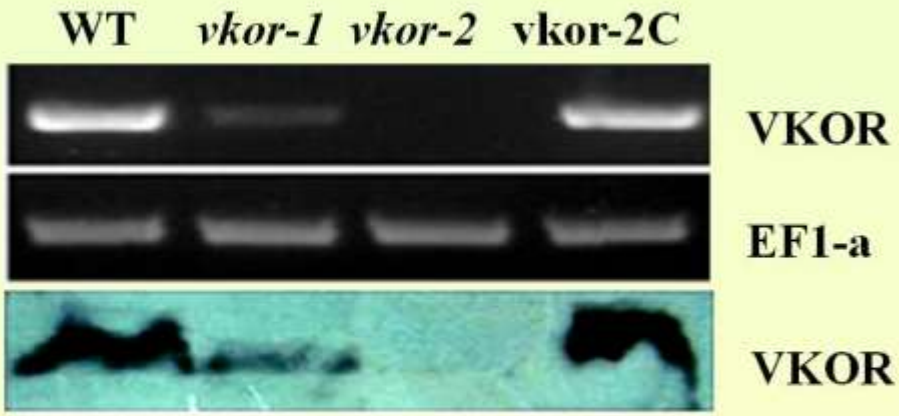


Isomerase of *At*DsbA

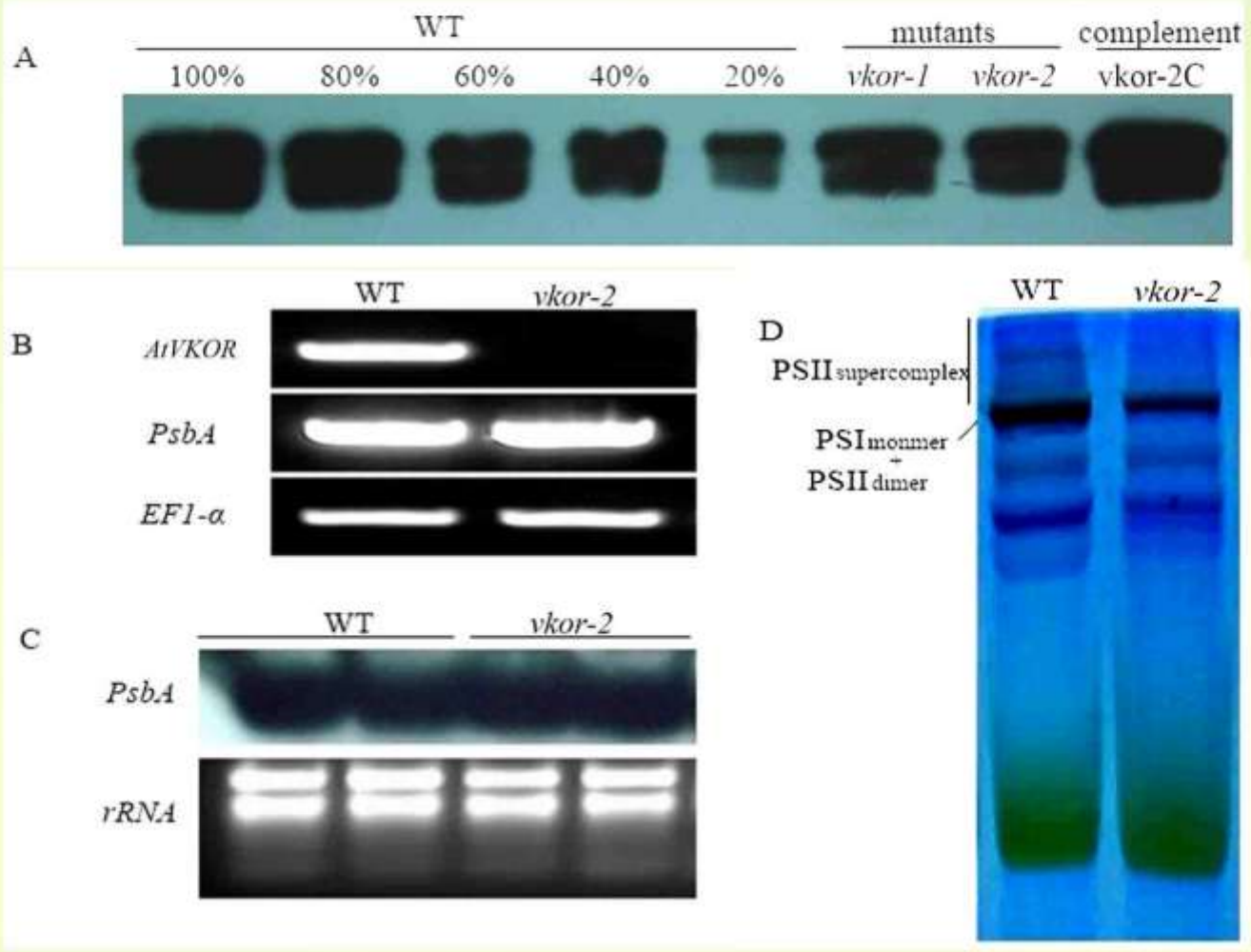
(✚) 0 μM *At*DsbA; (◘) 2.5 μM *At*DsbA; (Δ) 7.5 μM *At*DsbA

Loss of *At*VKOR caused the changes of phenotype of *Arabidopsis*

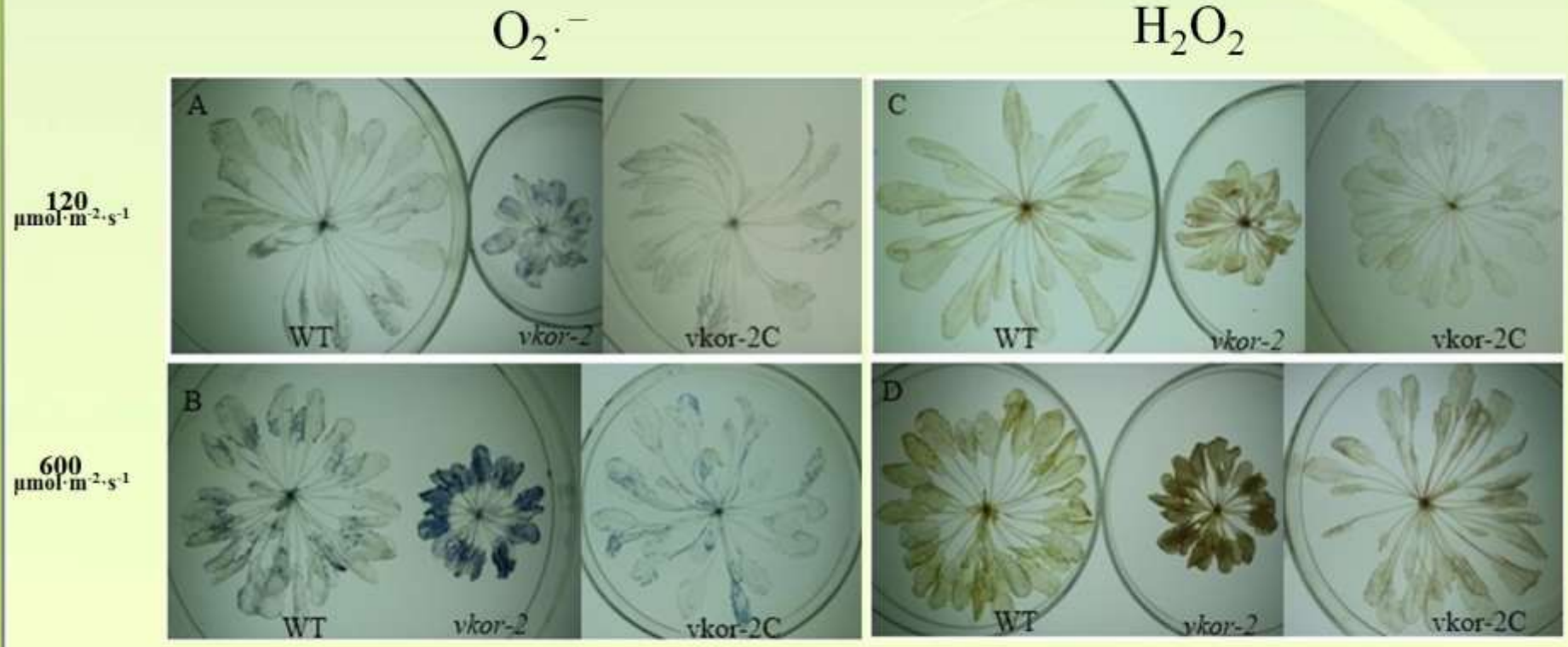
At4g35760



The quantity of core protein D1 in PSII in *vkor* mutant had a significant decrease. The deficiency of *At*VKOR enhanced the breakdown of D1.



The deficiency of *At*VKOR increased the accumulation of $O_2^{\cdot -}$ and H_2O_2 as the down-regulated activities of removal enzymes.



The accumulation of $O_2^{\cdot -}$ and H_2O_2 in plants of WT, *vkor-2* and vkor-2C

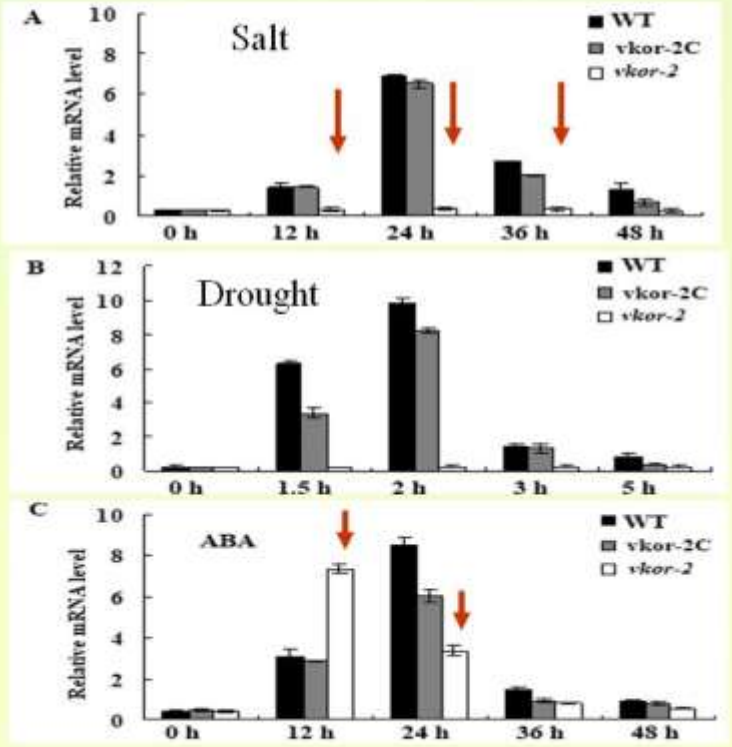
The deficiency of *At*VKOR may affect the activity of PSII

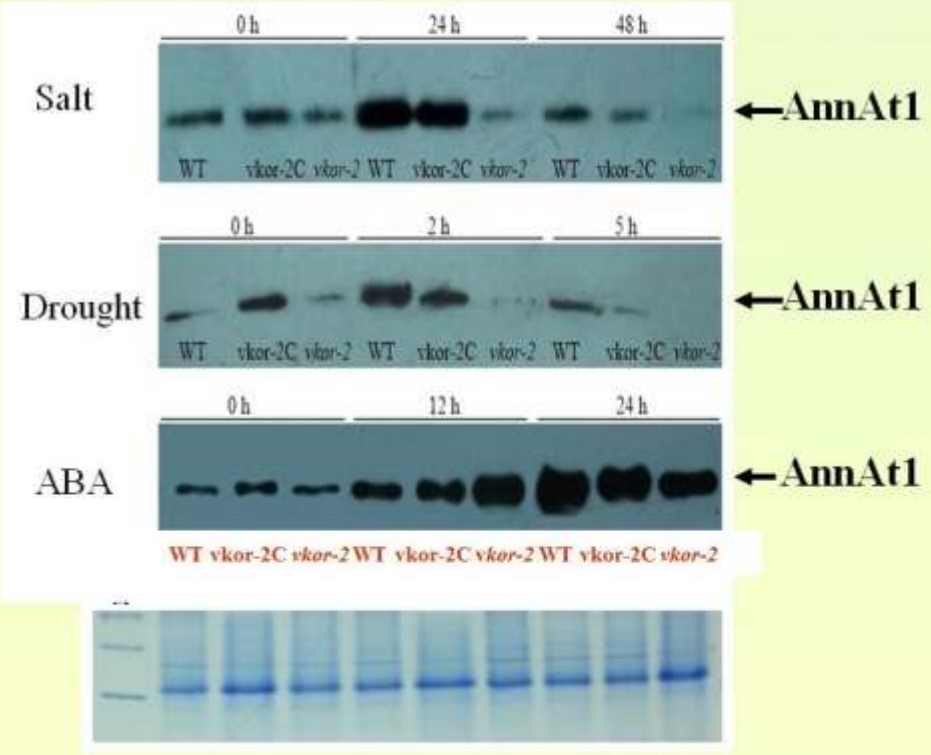
Parameters of chlorophyll fluorescence in 8-week-old wild-type and *vkor* mutants

Parameter	Growth Light			
	Wild-type	*vkor-1*	*vkor -2*	vkor-2C
Fv/Fm	0.84±0.01	0.58±0.01	0.53±0.01	0.81±0.01
φPSII	0.74±0.03	0.40±0.02	0.34±0.02	0.72±0.02
NPQ	0.20±0.03	0.14±0.03	0.10±0.01	0.18±0.03
1-qP	0.06±0.02	0.11±0.01	0.13±0.03	0.08±0.01

Growth Light intensity was120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fv/Fm, maximum quantum yield of PSII; φPSII, effective quantum yield of PSII; 1-qP, excitation pressure; NPQ, nonphotochemical quenching. Mean values ± standard deviation are provided. At least five leaves from different plants were measured.

_At_VKOR was involved in the response of ABA-mediated to osmotic stresses. The exogenous ABA treatment to _vkor_ mutant restore a WT-like pattern of gene expression.





Selected publications

- A protein oxidase catalysing disulfide bond formation is localized to the chloroplast thylakoids. ***FEBS J.*, 2011**, 278:3419-3430.
- A chloroplast membrane protein LTO1/*At*VKOR involving in redox regulation and ROS homeostasis. ***Plant Cell Reports*,** 2013, 32:1427-1440.
- The chloroplast protein LTO1/AtVKOR is involved in the xanthophyll cycle and the acceleration of D1 protein degradation. ***Journal of Photochemistry and Photobiology***, 2014, 130:68-75.
- Thylakoid membrane protein LTO1/*At*VKOR is involved in the response of ABA-mediated to osmotic stress in *Arabidopsis*. ***Physiologia Plantarum***, 2014. (accepted)
- Membrane topology and mutational analysis of *Mycobacterium tuberculosis* VKOR, a protein involved in disulfide bond formation and a homologue of human vitamin K epoxide reductase, ***Antioxid Redox Signal,*** 2011, *14*, 1413-1420.

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