Characterizing *Arabidopsis thaliana* Mutants with Wheat Homolog Transfer Cell Genes Knocked-outs

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Abstract

The transfer cell is a style of adaptive differentiation to fulfill a local physiological need. The major characteristic of transfer cells is cell wall ingrowth. Cell wall ingrowth in wheat (*Triticum turgidum var.*) can be detected under light microscope in wheat grain at 25±3 days after anthesis. Transfer cells can help increasing yield and biomass production in wheat by increasing the surface area of the cell wall as much as 40 folds. More nutrients will be transported to phloem and put into biomass production. The objective of this study was to reveal the effect of T-DNA insertion during transfer cell development. The wheat homolog genes were studied in the model organism Arabidopsis (*Arabidopsis thaliana*) mutants with transfer cell genes knocked-out. T-DNA insertion were confirmed and amplified by PCR from eight mutants of Arabidopsis and wild type. Three primers were used in the PCR procedure. Photosynthetic capacity of eight mutants and wild type was measured under low light (105µE) and high light (488 µE) intensity. Light intensity treatment started at eight leaves stage after germination. First measurement started at seven days after light intensity treatment. High light intensity measurements started seven days after placed in the high light intensity environment. Length of light treatment was 14 hours per day. Environmental analysis was used for statistical purpose. Photosynthetic capacity results indicate that there is significant increase in photosynthetic capacity in wild type compare to knocked-out mutants after two weeks of high light intensity treatment ($p<0.0001$). Under low light intensity treatment and the first week of high light intensity treatment, there is no significant change in both wild type and mutants (LL, $p=0.866$; HL, $p=0.0828$). The results indicate that transfer cell development is important to plant biomass production. We also can upregulate these genes in wheat to increase the yield of wheat.

Introduction

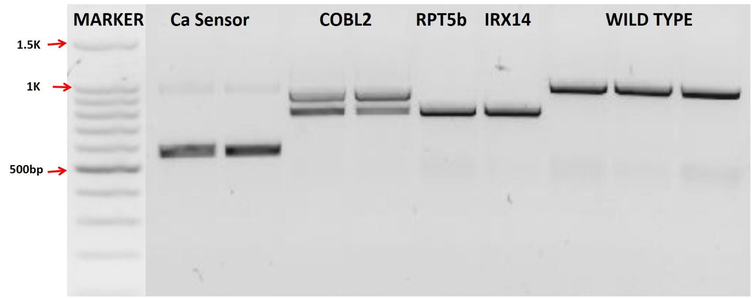
Transfer cells are highly specialized to accelerate facilitated membrane transport. The elaboration of transfer cells is one of the most impressive and powerful strategies that certain plants have developed to create a high capacity for membrane transport of assimilates and nutrients. Transfer cells are found in roots, leaves, and developing seeds of many economically important plants: Soybean, wheat, maize, rice, cotton, sunflower, barley, pea, faba bean, sugar beet, potato, tomato and the model plant *Arabidopsis.* Transfer cells in the nucellar projection of wheat grains at 25±3 days after anthesis have been examined using light and electron microscopy. Within the nucellar tissue, a sequential increase in non-polarized wall ingrowth differentiation and cytoplasmic density was evident. Cells located near the pigment strand were the least differentiated. (Wang, et. 1994) Wheat has a genome of 3000Mb, which is hard for genetic and physiological study. Arabidopsis' genome size is 125Mb total. It is one of typical plant research model organisms. Its genomes has been fully sequenced (http://www.arabidopsis.org/). Almost every arabidopsis gene has knocked out mutants and easy to study gene functions (http://signal.salk.edu/) . Arabidopsis develop phloem transfer cells in leaves when grew under high light intensity.

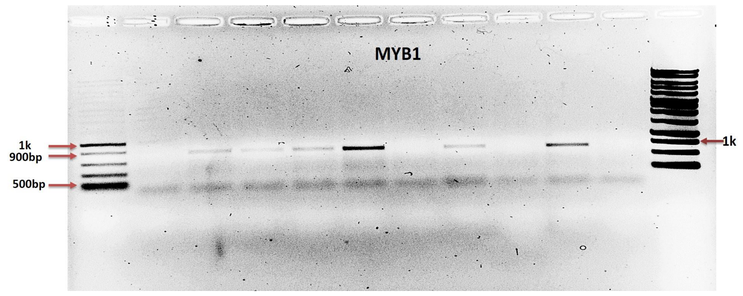
Methods and Materials

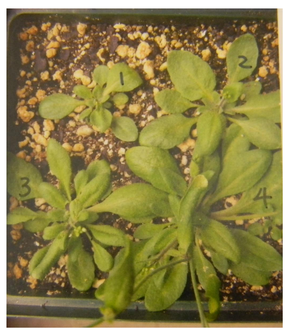
Salkline Name	Gene Position	Gene Name	Ecotype	HT Mutant
Salk_044883	At3g2981	COBL2	Col-0	YES
Salk_078827	At2g46600	Calcium sensor	Col-0	NO
Salk_032012	At4g36890	IRX14	Col-0	NO
Salk_069366	At1g09100	RPT-5b	Col-0	NO
Salk_004053	At3g11280	MYB1	Col-0	NO
Salk_047223	At1g76030	VHA-B1	Col-0	NO
		6HXK	Col-0	NO

Total eight mutants and one wild type of Arabidopsis were used in the study. T-DNA insertion were confirmed and amplified by PCR from eight mutants of Arabidopsis and wild type. Three primers were used in the PCR procedure. 400 – 900 bp PCR product was expected to produced in homozygote mutants; 1000 – 1200 bp PCR product was expected to produced in wild type; fragments with both size range were expected to produced in heterozygote mutants. Photosynthetic capacity of eight mutants and wild type was measured under low light (105µE) and high light (488 µE) intensity. Light intensity treatment started at eight leaves stage after germination. First measurement started at seven days after light intensity treatment. High light intensity measurements started seven days after placed in the high light intensity environment. Length of light treatment was 14 hours per day. Environmental analysis was used for statistical purpose.

Results





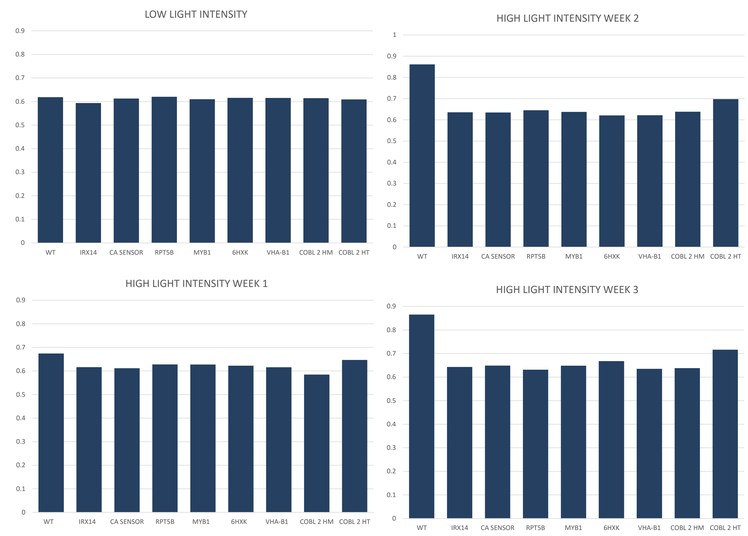


Plant 1: Homozygote mutants
Plant 2&3: Heterozygote mutants
Plant 4: Wild Type

Arabidopsis mutants Salk_069366(RPT5b) were planted in this pot

ENV	SOV	DF	Type III SS	MS	F Value	Pr > F
ENV 1	rep	1	0.00035187	0.000352	0.03	0.866
	Gene	8	0.00982592	0.001228	0.1	0.9992
ENV 2	rep	1	0.04333133	0.043331	3.02	0.0828
	Gene	8	0.15969145	0.019961	1.39	0.1974
ENV 3	rep	1	0.00273761	0.002738	0.19	0.6603
	Gene	8	2.19648911	0.274561	19.4	<.0001
ENV 4	rep	1	0.00242979	0.00243	0.18	0.668
	Gene	8	2.32279544	0.290349	22.01	<.0001

ENV 1: 7 days after treat under low light intensity (105µE)
ENV 2: 7 days after treat under high light intensity (488µE)
ENV 3: 14 days after treat under high light intensity (488µE)
ENV 4: 21 days after treat under high light intensity (488µE)



PHOTOSYNTHETIC CAPACITY UNDER FOUR ENVIRONMENTS

Discussion

Photosynthetic capacity results indicate that there is significant increase in photosynthetic capacity in wild type compare to knocked-out mutants after two weeks of high light intensity treatment ($p<0.0001$). Under low light intensity treatment and the first week of high light intensity treatment, there is no significant change in both wild type and mutants (LL, $p=0.866$; HL, $p=0.0828$).

Conclusion

The results indicate that transfer cell development is important to plant biomass production. We also can upregulate these genes in wheat to increase the yield of wheat.

Reference

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5. Heinz Walz, Mini-PAM user manual, 1999

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