PLANT MEDIATOR TO TACKLE CLIMATE CHANGE

Wathugala G.D. Lakmini[1], Heather Knight[2] and Marc R. Knight[2]

[1]Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka,
[2]School of Biological and Biomedical Sciences, Durham of University, South Road, Durham, United Kingdom. DH1 3LE

INTRODUCTION

The current rate of climate change predicts that plants will become subject to increasing extremes of environmental stress. Rapid population increases in developing countries also demand higher yield from crop production, often from sub-optimal agricultural areas. Genetic engineering can help meet these needs through the development of crops with greater stress tolerance. Mediator is transcriptional co-activators which convey DNA bound transcriptional regulators and enhancers to the general RNA polymerase II transcription machinery and mediator genes are recently identified in plants (Fig. 1). So far it has showed their great involvement in regulation of plant stress tolerance by controlling transcription of stress genes (Fig. 2). SFR6 (SENSITIVE TO FREEZING6) is one of plant mediator protein which has identified first with its involvement to tolerance against freezing in *Arabidopsis*. The freezing sensitivity of *sfr6* mutant is lack of expression of downstream genes in CBF cold response pathway (Fig. 3). Apart from that there was preliminary evidence that *sfr6* mutant is sensitive for other biotic and abiotic stresses. Therefore, the objective of this research was to screen the involvement of SFR6 to regulate other environmental stresses.

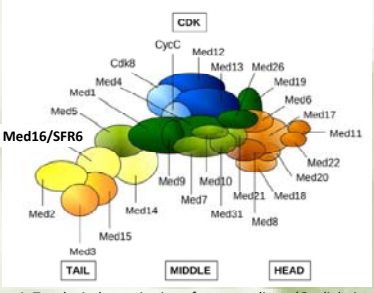
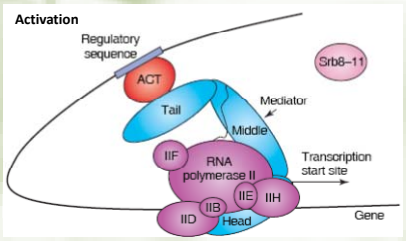


Figure 1: Topological organization of yeast mediator (Guglielmi *et al*., 2004)



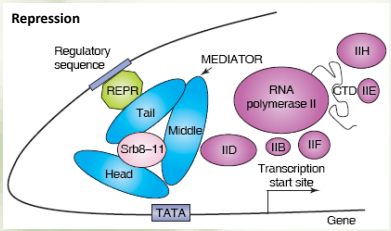


Figure 2: Function of mediator in transcriptional activation and repression (Bjorklund and Gustafsson, 2005)

Figure 3: Involvement of SFR6 upon low temperature induced gene expression

MATERIALS AND METHODS

To examine the role of Med 16/SFR6, *At4g04920* was over-expressed in wild type *Arabidopsis* and *sfr6-1* mutant. Then freezing sensitivity and *KIN2* expression were measured in transgenic plants. To examine the sensitivity of *sfr6* mutant to different environmental stresses, *sfr6* mutants were subjected to range of environmental stresses along with wild type *Arabidopsis*. Homologue was cloned from rice and its orthology was tested transferring *OsSFR6* to *sfr6-1* mutant.

RESULTS

Complementation of *sfr6-1* mutant by wild type *AtSFR6*

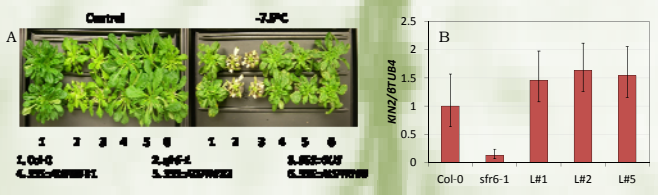


Figure 4: (A) Transformation of wild type *AtSFR6 in* to the *sfr6-1* mutant restores its freezing tolerance. Line 3, 4, 5 and 6 are in *sfr6-1* background. **(B)** Real time PCR of *KIN2* transcripts in 7 day old seedlings of *sfr6-1* overexpressing *35S::AtSFR6*, subjected to 4°C for 6 h. Error bars show ±RQ.

Introducing the wild type *SFR6* gene into the s*fr6-1* mutant should rescue the mutant phenotype. This assay shows that *At4g04920* in the *sfr6-1* mutant compliments mutant phenotypes. The transgene rescues seedling colour from yellow green to dark green, the plants regain freezing tolerance (Fig.4A), and express *KIN2* gene to wild type levels (Fig.4B).

AtSFR6 orthologues from other crop plants

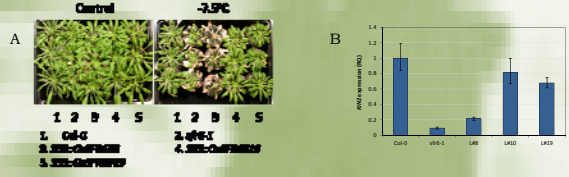


Figure 5: (A) Transformation of Os*SFR6 in* to the *sfr6-1* mutant restores its freezing tolerance. Line 3, 4 and 5 are in *sfr6-1* background, **(B)** Real time PCR of *KIN2* transcripts in 7 day old seedlings of *sfr6-1* overexpressing *35S::OsSFR6*, subjected to 4°C for 6 h. Error bars show ±RQ.

AtSFR6 homologue from rice was identified (OsSFR6), and its functional complementation was tested by transferring *OsSFR6* to *sfr6-1* mutant. Transgenic plants complemented all *sfr6-1* mutant phenotypes including freezing sensitivity (Fig.5A) and *KIN2* expression (Fig.5B).

Sensitivity of *sfr6* mutants to UV radiation and biotic stresses

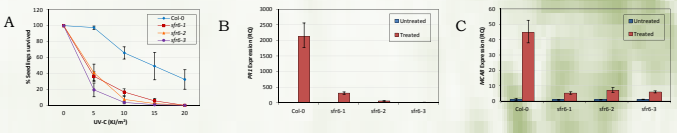


Figure 6: Sensitivity of *sfr6* mutants to UV Irradiance. **(A)** Number of seedlings survived 10 days after UV irradiance. **(B,C)** Real time PCR of *PR1* and *MC8* transcripts in 7 day old seedlings of *sfr6* alleles subjected to 5KJ/m² UV irradiance. Error bars show ±RQ.

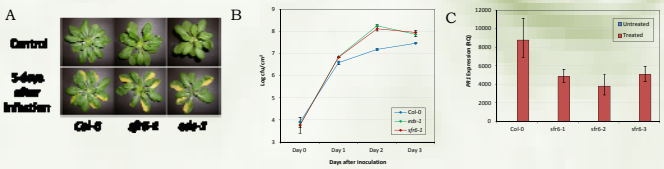


Figure 7: Sensitivity of *sfr6* mutants to virulent *P. syringae* infiltration. **(A)** Comparison of the spread of lesions caused by *P. syringae,* 5 days after infiltration. **(B)** The growth of strain by scoring colony forming units (cfu). **(C)** Real time PCR of *PR1* transcripts in 5 weeks old plants of *sfr6* alleles inoculated with *P. Syringae* virulent strain. Error bars show ±RQ.

Sensitivity of *sfr6* mutants to range of environmental stresses were tested and found, in addition to known roles of SFR6, SFR6 also has roles in protecting against UV irradiance and pathogen infection in *Arabidopsis*, by showing reduced level of UV (Fig. 6) and pathogen (Fig. 7) induced gene expression.

CONCLUSION

Results demonstrate the requirement of SFR6/MED16 for the activation of many but not all stress response gene expression, and indicated conserved *AtSFR6* function in rice. However, the mechanism of regulation of stress induced gene expression via SFR6/MED16 remains to be further investigated. The future research on specific roles of individual subunits and of the whole complex of plant mediator will widen our knowledge of the transcriptional regulation of gene expression in plant and will create new routes to improve crop tolerance to environmental stresses.

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