

Quantitative proteome analysis of alfalfa in drought stress under the influence of *miR156* (*Medicago sativa*)

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ABSTRACT

Medicago sativa is one of the most produced perennial forage crop and used as animal feed. Drought stress is a major form of abiotic stress, affecting the productivity and annual yield of alfalfa. A small non-coding RNA, *miR156* has a role in drought tolerance, while regulating downstream *SPL* genes. The objective of this research was to study the whole leaf proteome of *miR156*OE (A8) genotype of alfalfa under drought stress. In results, 3,000 proteins groups were identified using MaxQuant when searched against *Medicago truncatula* protein sequence database. Under control conditions, 68 proteins were upregulated in A8 and 84 proteins were downregulated, relative to EV (empty vector), whereas, under drought stress, 610 proteins were upregulated and only 52 proteins were downregulated. Functional analysis using DAVID online tool showed enriched proteins are involved in biological and molecular processes such as antioxidant response, response to stress, signal transduction and biosynthesis of secondary metabolites. Protein families related to signaling like MAPK, CDPK, PP2C, transcriptional regulators including bZIP and zinc finger proteins were found to be differentially regulated. The identified proteins families could be the potential targets of *miR156* in drought stress. This study provides a better understanding and an insight into the role of *miR156* in combating the drought stress in alfalfa at proteomic level.

Introduction

MiRNAs are 18-22 nucleotide long RNA molecules working as molecular scissors to alter the gene expression. *MiR156* targets members of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) gene family by mRNA cleavage and reduces their expression.¹ These *SPL* genes further regulate downstream genes and affect plant traits. *MiR156*OE genotype has shown increased biomass yield, increased root length and delayed flowering.¹ Also, overexpressed alfalfa has tolerance to drought and salinity stress.²

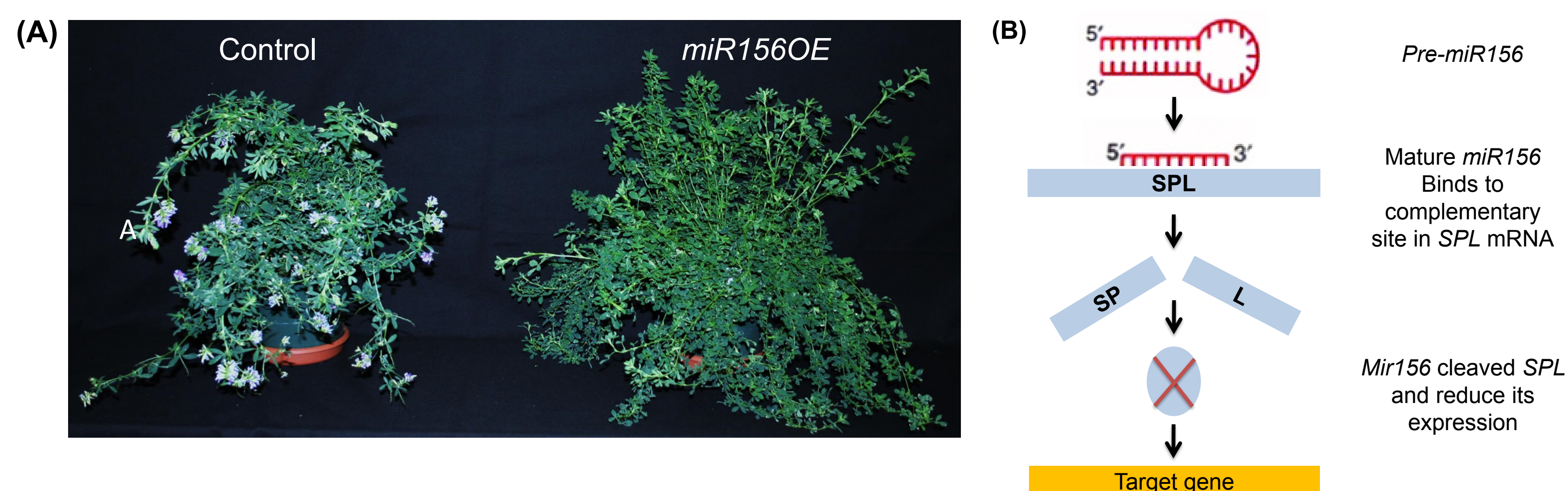


Figure 1: (A) Effect of *miR156* enhancement on shoot branching and delayed flowering in 6 month old alfalfa (B) Flow chart shows the *miR156*-*SPL* network.

Proteomics Work Flow

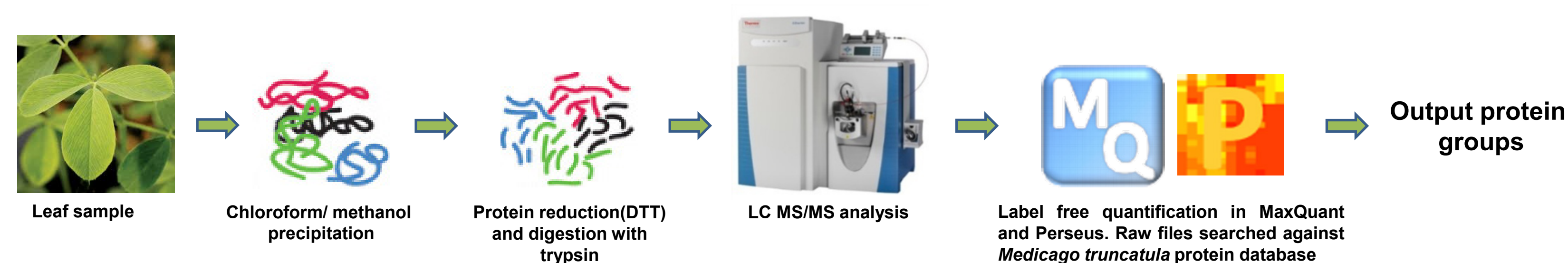


Figure 2: This is the bottom up LC MS/MS approach to study the whole proteome in plant tissue.

Results

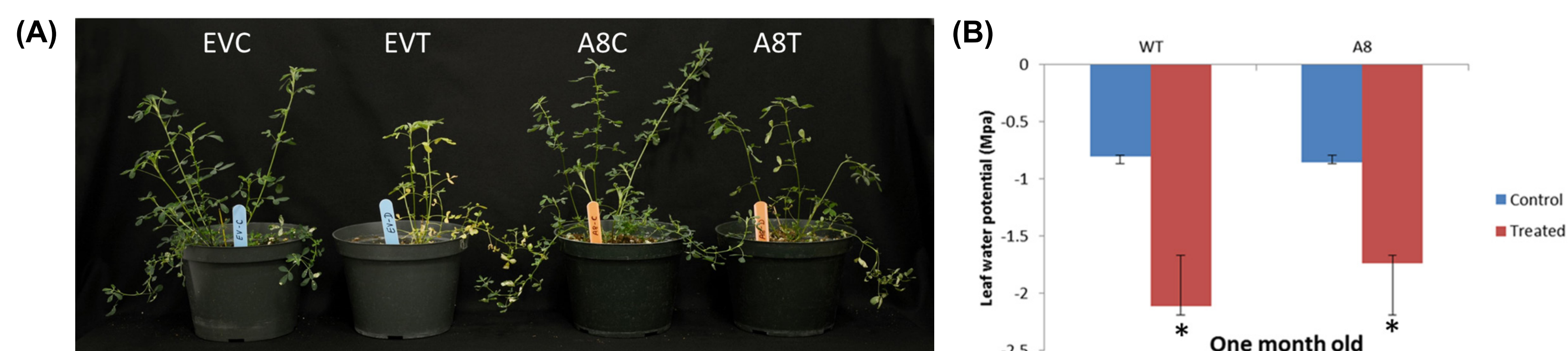


Figure 3: (A) One month old alfalfa plants under control and drought stress condition. *MiR156*OE genotype (A8) survives better in stress condition (B) Mid day leaf water potential shows that *miR156*OE genotype retains more water in drought condition than in empty vector (EV) control.

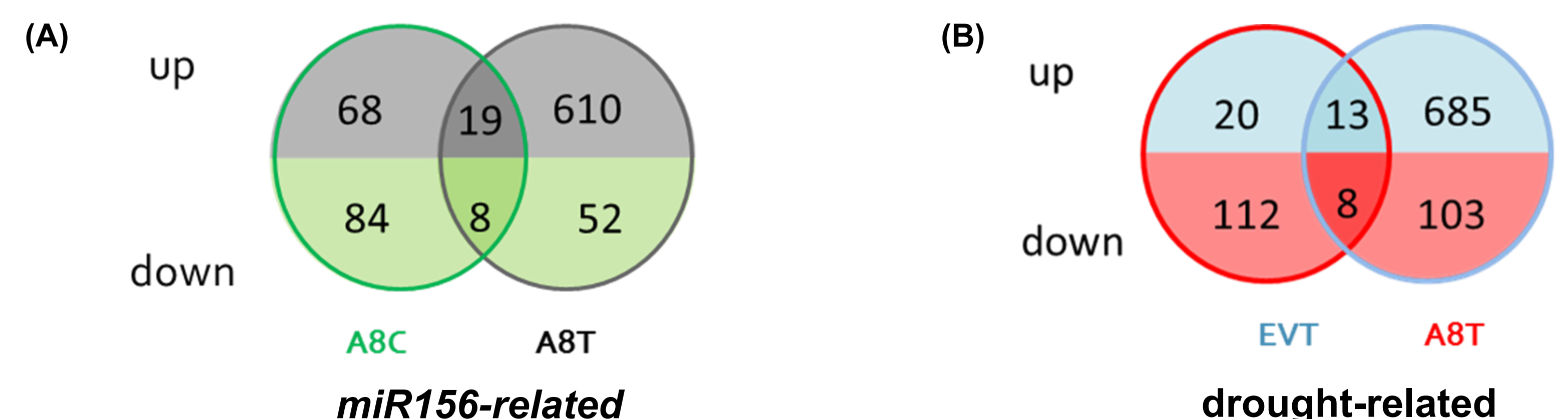


Figure 4: (A) Number of differentially regulated proteins in *miR156*OE genotype (A8) relative to EV control under stress and non stress conditions. (B) Differentially regulated proteins in EV control and A8 (*miR156*OE) relative to their respective controls.

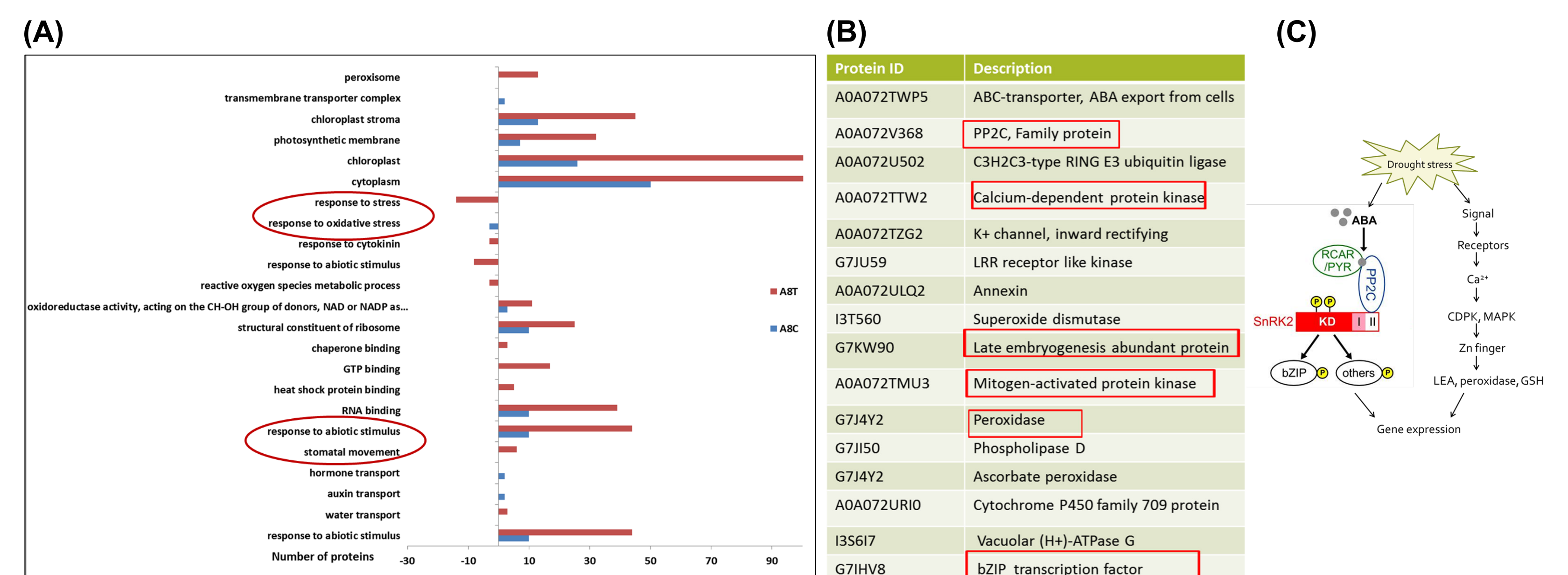


Figure 5: (A) Functional categories enriched in *miR156*OE genotype relative to EV control. (B) Represented protein families that are differentially regulated in *miR156*OE genotype under stress condition, when BLASTed against drought stress gene database³. (C) ABA dependent pathway and signaling cascade through Ca²⁺ as second messenger are well known pathways in drought stress. Perhaps, *miR156* helps in combating stress tolerance by activating these pathways.

Conclusion

MiR156 overexpression does effect the protein profiles. The protein families like PP2C, MAPK, CDPK and antioxidants could be direct or indirect targets of *miR156* to enhance tolerance in drought stress.

Future work

Study the whole leaf proteome of *SPL13* RNAi line in drought stress. *SPL13* is one of the targets of *miR156* that has role in enhancing drought tolerance.

References

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2. M. Arshad, B.A. Feyissa, L. Amyot, B. Aung, A. Hannoufa, MicroRNA156 improves drought stress tolerance in alfalfa (*Medicago sativa*) by silencing *SPL13*, Plant Sci 258 (2017) 122-136.
3. S. Alter, K.C. Bader, M. Spannagl, Y. Wang, E. Bauer, C.C. Schön, K. Mayer (2014).