

Pathway-oriented gene discovery for abiotic-stress tolerant engineering of soybean plant



Tien-Dung LE (FPR 110001)
 Signaling Pathway Research Unit, RIKEN Plant Science Center
 Host researcher: Lam-Son Phan Tran

SUMMARY

Population of the earth is increasing rapidly, setting food security one of the world's major issues. In addition, climate change also puts a great burden on sustainable agriculture. Drought, flooding, unpredictable epidemics, soil erosion and environment pollutants are the factors threatening sustainable agriculture and, consequently, food security. Soybean provides an abundant source of oil and proteins for human consumption. Nevertheless, over the past few years, significant portions of the soybean producing areas have experienced severe drought, leading to significant yield reductions.

My study as an FPR fellow focuses on revealing the mechanisms and pathways of soybean plants in response to stress using comparative functional genomics and molecular biology approaches, thereby identifying target genes for genetic engineering. Specifically, I employed 4 approaches (1) *in silico* identification and analysis regulatory elements of the genes within pathways of interest, (2) high throughput transcription profiling by qRT-PCR and DNA microarray, (3) biochemical characterization, and (4) *in planta* verification of the candidate genes to target the following pathways and transcription factor family:

- The Two Component System (TCS) involved in cytokinin (CK) signal transduction
- The CK metabolic gene families, which control the levels of CK in the plant
- The NAC transcription factor family, many of which are stress-inducible
- The Methionine Sulfoxide Reductase enzyme family

OUTPUTS

Peer-reviewed papers:

1. Le D.T., et al.: Diversity of plant methionine sulfoxide reductases B and evolution of a form specific for free methionine sulfoxide. Submitted to *Biochemical Journal* (2013)
2. Ha C.V., Le D.T., et al., *BioMed Research International*, vol. 2013, Article ID 759657, 8 pages, (2013)
3. Liang X., Kaya A., Zhang Y., Le D.T., Hua D. and Gladyshev V.N., *BMC Biochemistry* 13:21 (2012)
4. Le D.T., et al., *PLOS ONE*, 7(11):e49522 (2012a)
5. Le D.T., et al., *PLOS ONE* 7(9):e46487 (2012b)
6. Le D.T., et al., *PLOS ONE* 7(8):e42411 (2012c)
7. Nishiyama R., Le D.T., et al., *PLOS ONE* 7(2): e32124 (2012)
8. Le D.T., et al., *DNA Res.* 18:263-276 (2011a)
9. Le D.T., et al., *DNA Res.* 18:17-29 (2011b)
10. Nishiyama R., Watanabe Y., Fujita Y., Le D.T., et al., *Plant Cell* 23: 2169-2183 (2011)

International Conference Posters:

1. Le D.T.: Characterization of the methionine sulfoxide reductase families from soybean. Gordon Research Conference: Thiol-Based Redox Regulation and Signaling, Bates College, Maine USA 2012, July 29 - August 03
2. Le D.T.: Physiological functions of Cytokinin metabolic genes in soybean plant as revealed by their transcriptome under normal and various drought models. American Society of Plant Biologists Minneapolis, Minnesota, USA 2011, Aug 06-12
3. Le D.T.: Molecular Detection of Nine Rice Viruses by RT-LAMP. 21st International Conference on Arabidopsis Research, PACIFICO Yokohama Japan 2010, June 06-10

Letter to Editor (not peer-reviewed):

1. Le D.T. and Le H.H.: Comments on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize". *Food, Chem. Toxicol.*

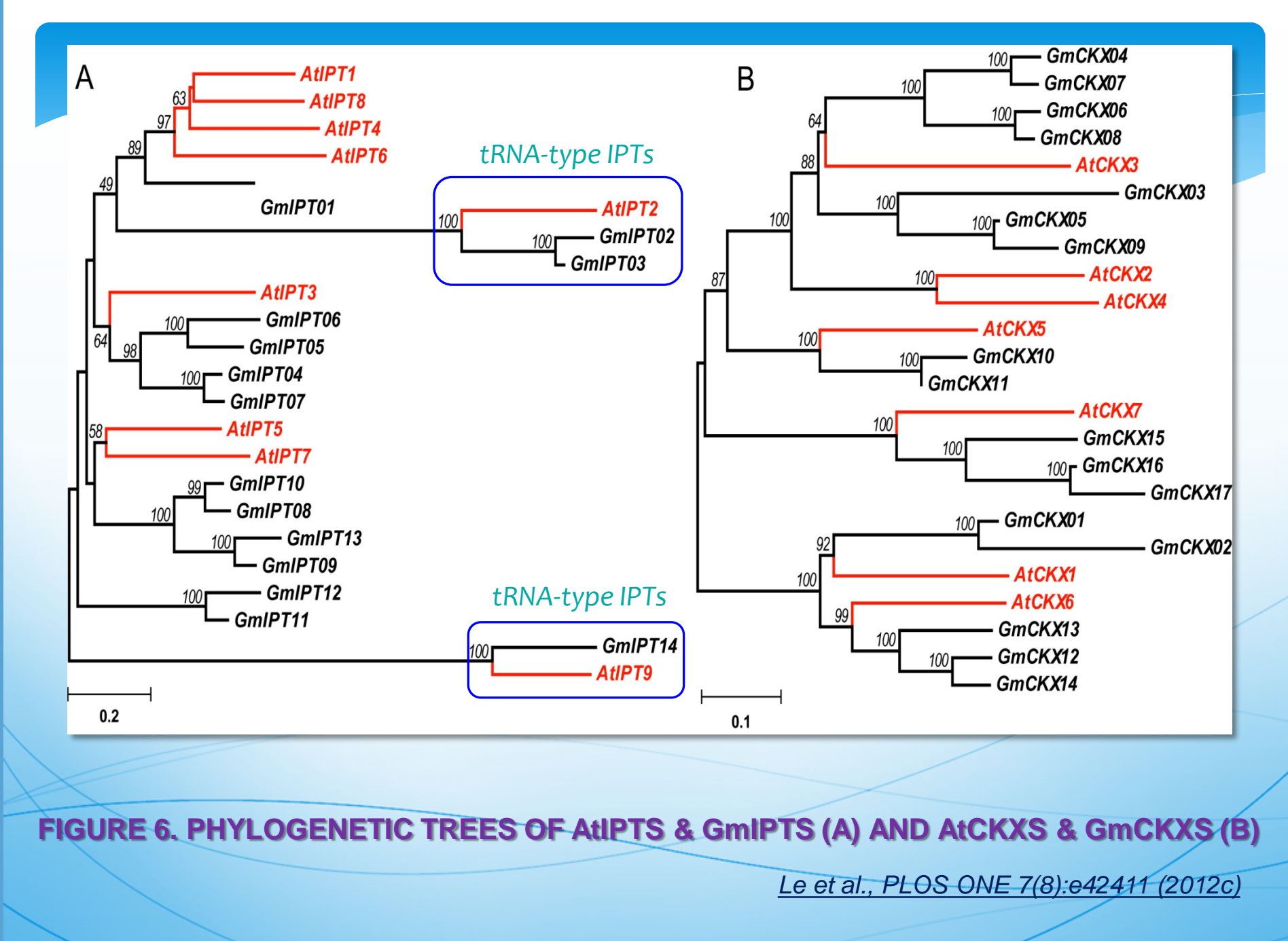
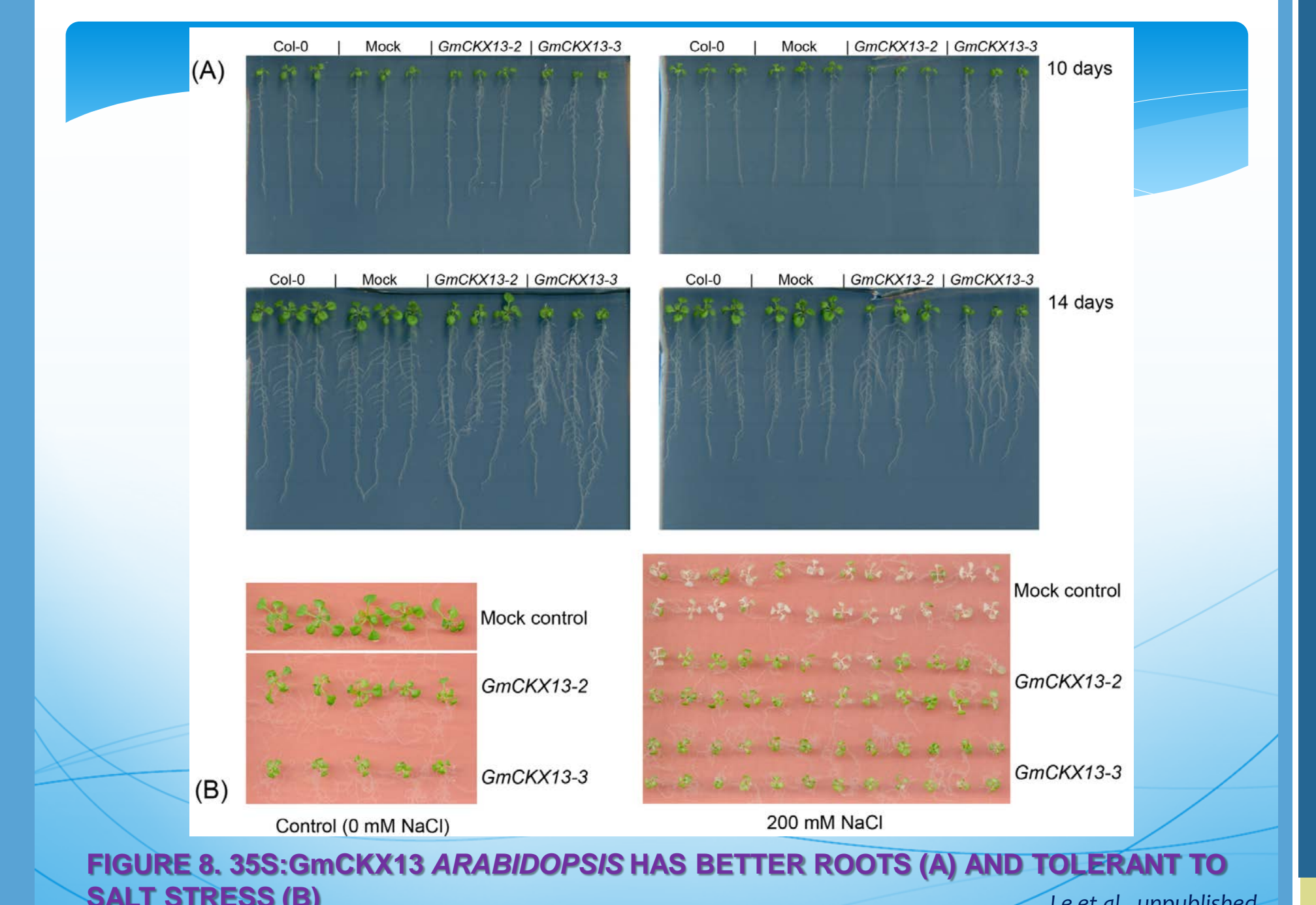
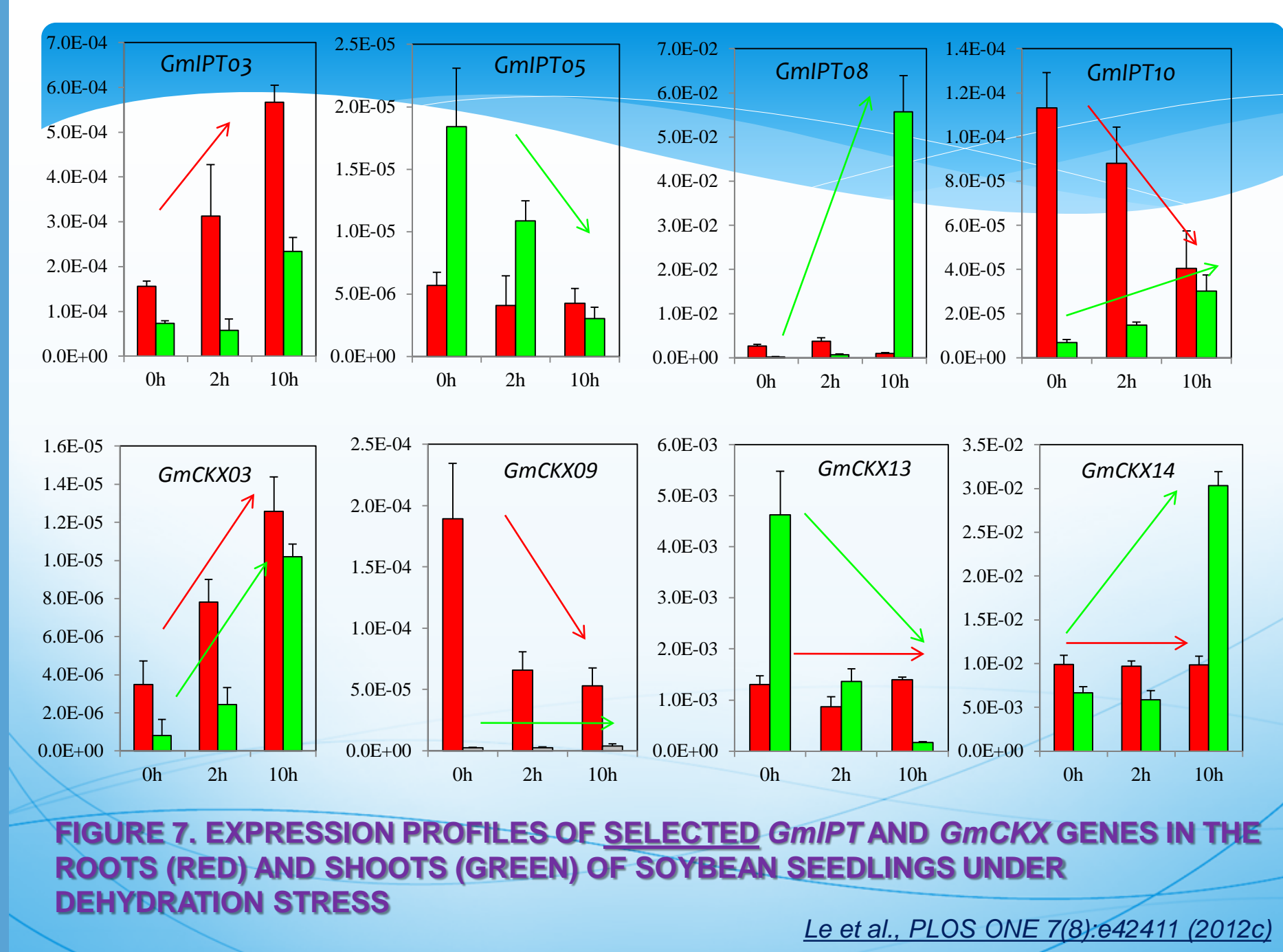
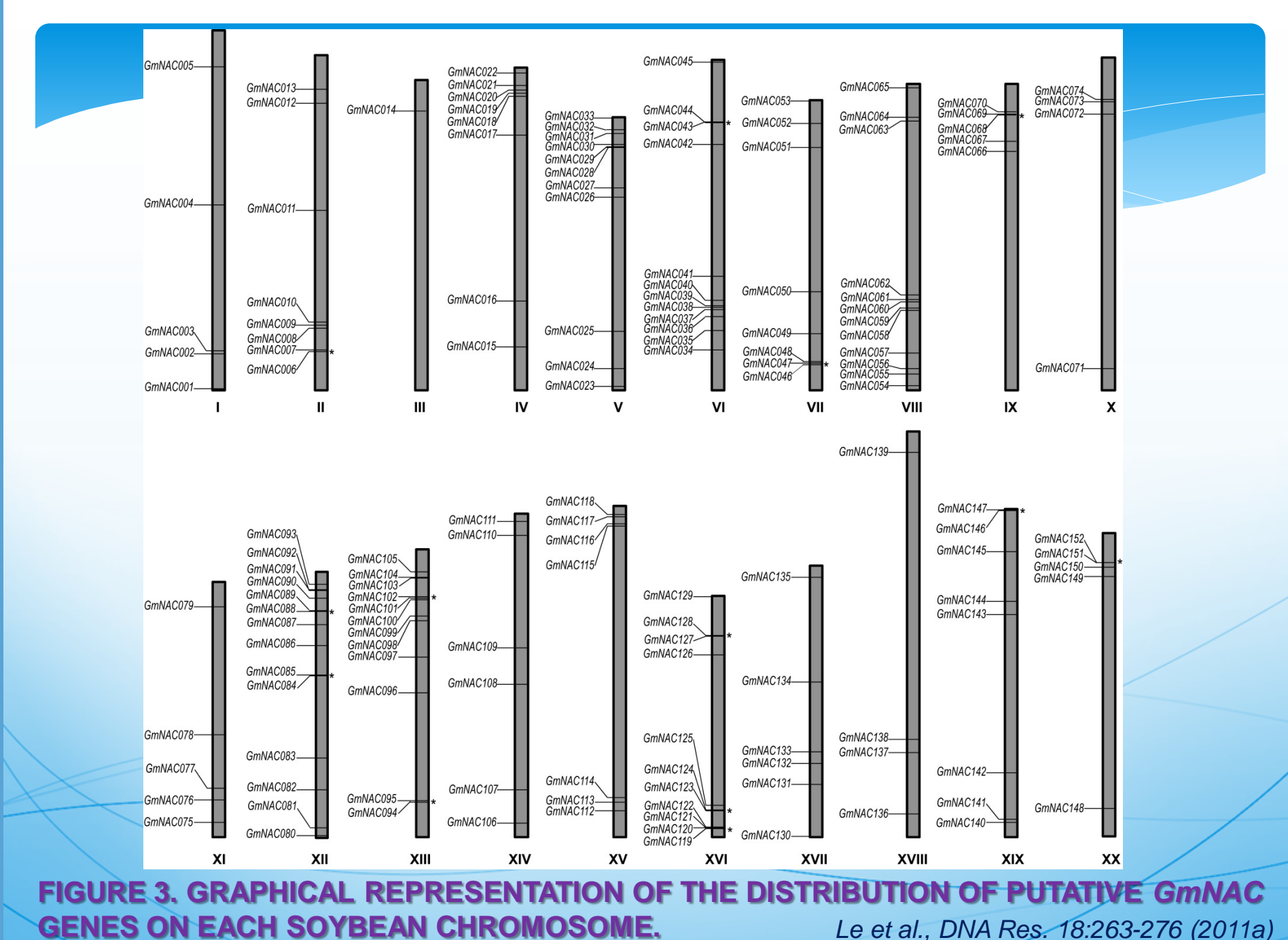
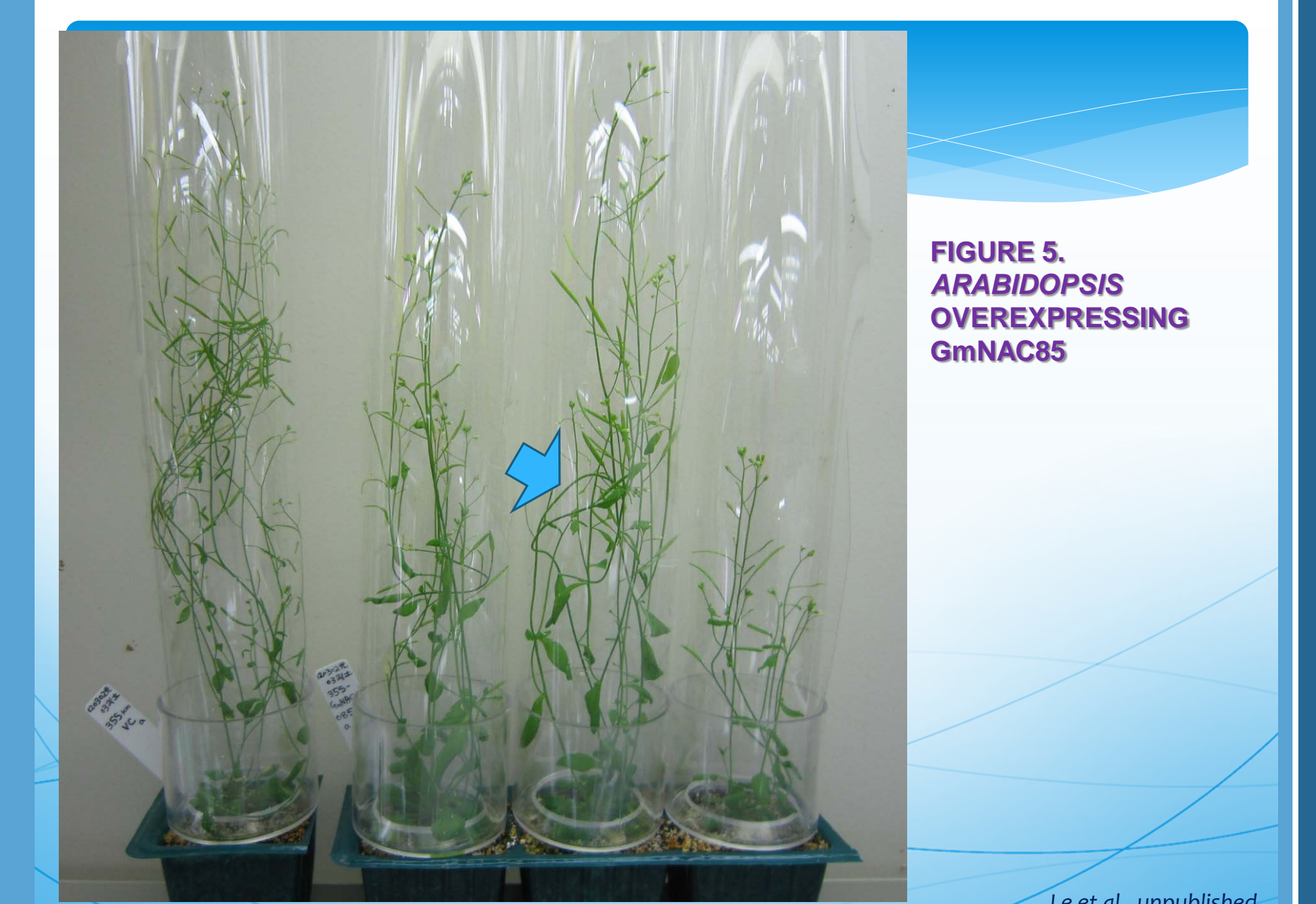
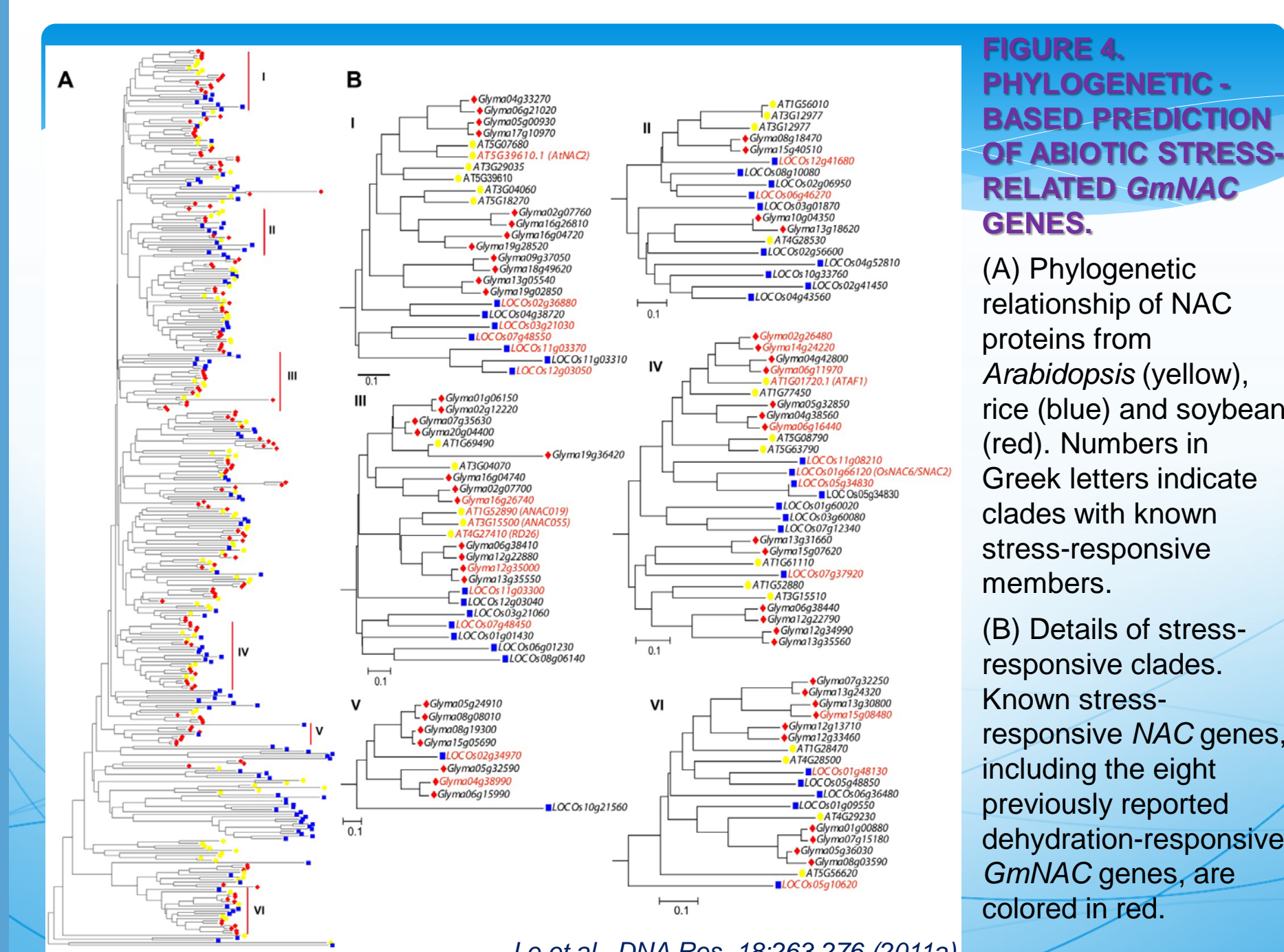
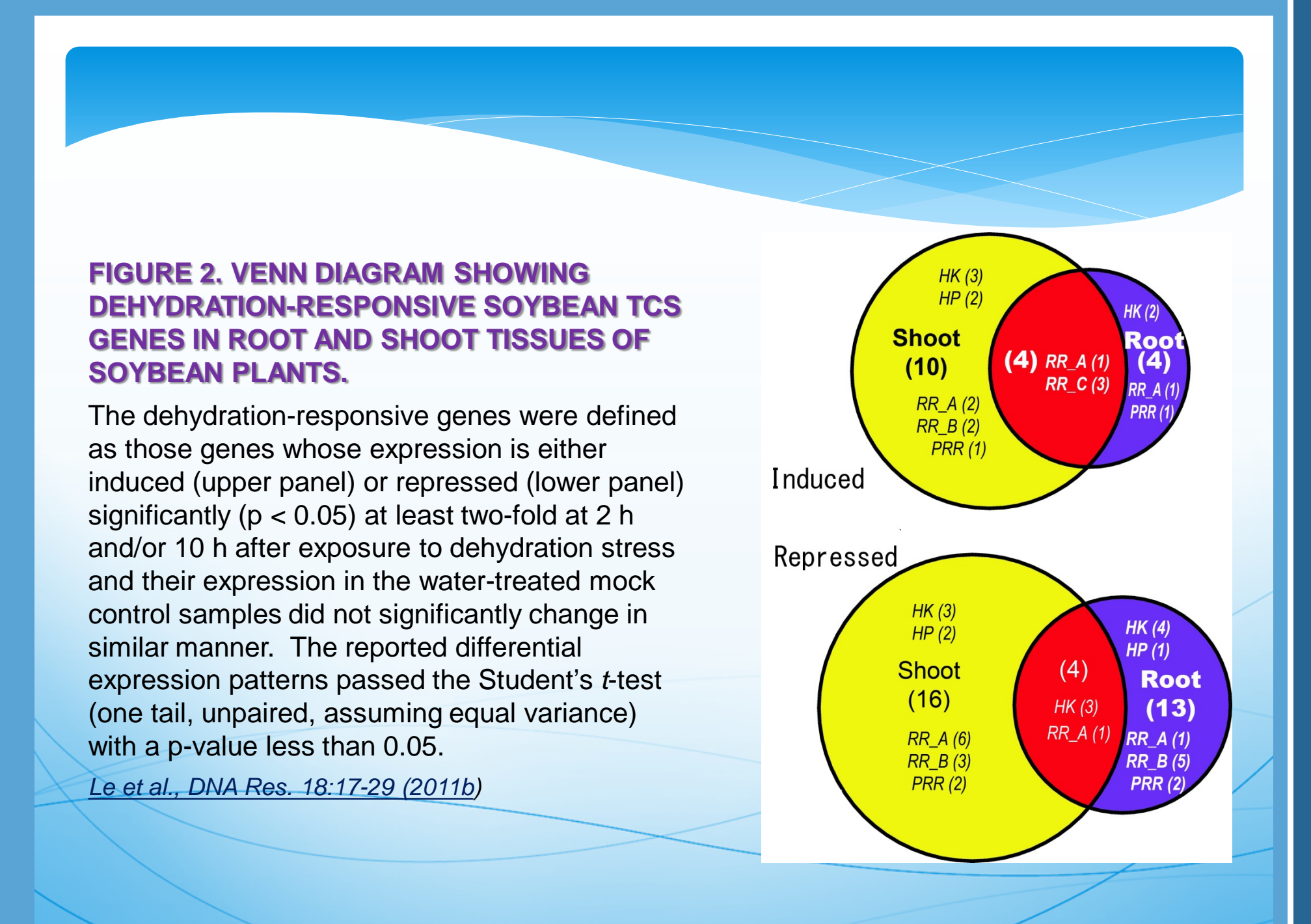
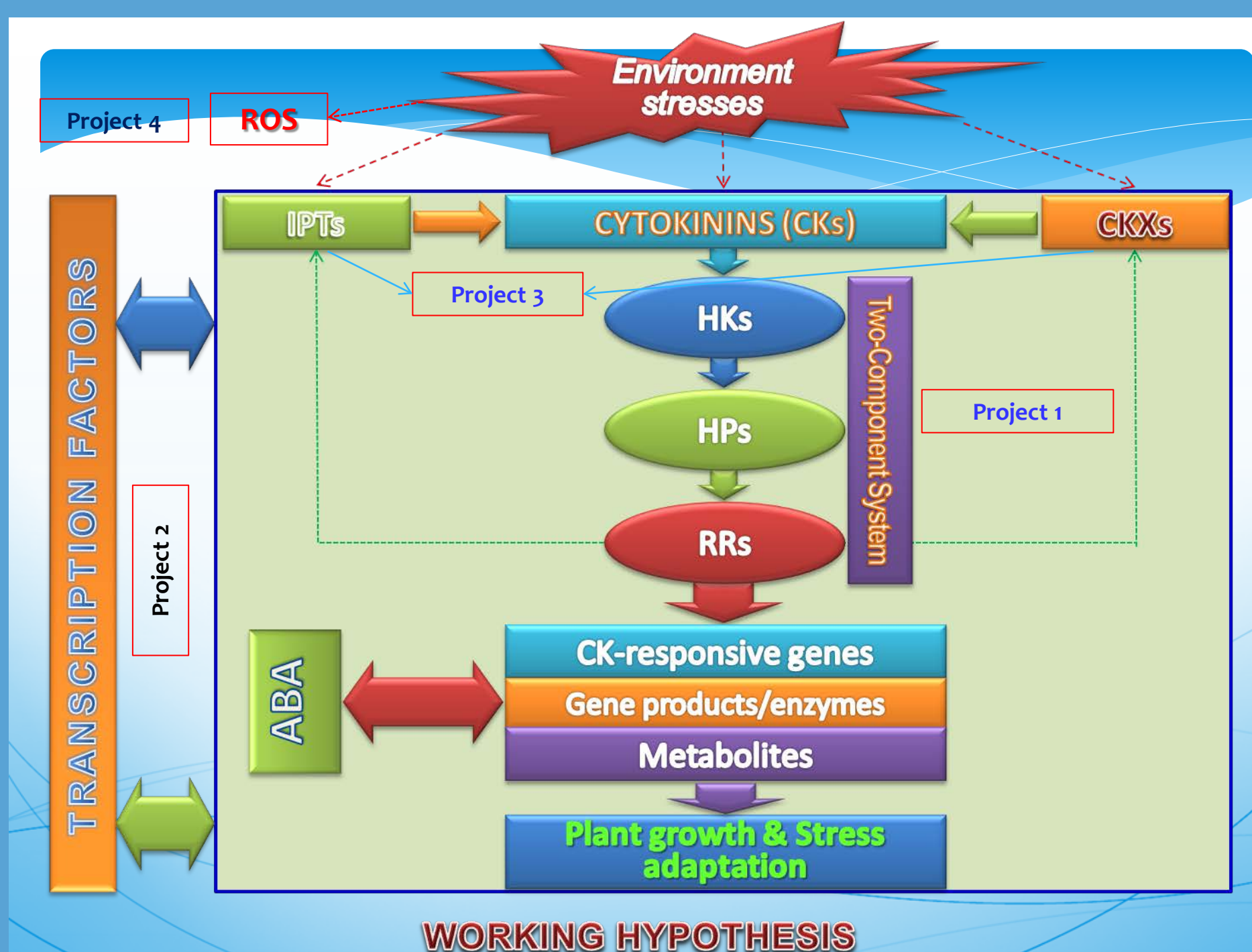
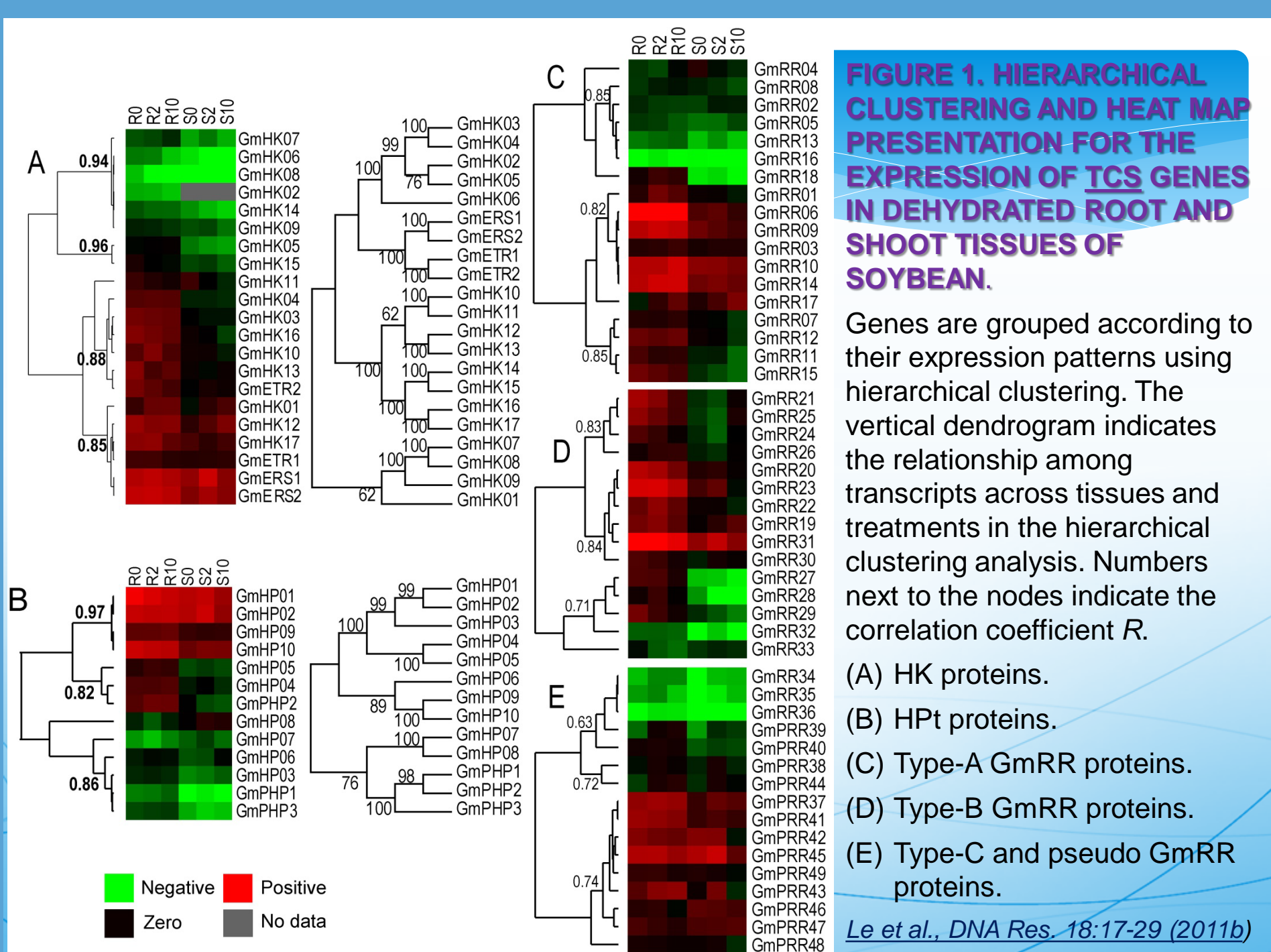


TABLE 1. CYCLE THRESHOLDS AND AVERAGE OF STANDARD DEVIATIONS OF DELTA CT OBTAINED FROM ISSUES UNDER ALL STRESS COMBINATIONS

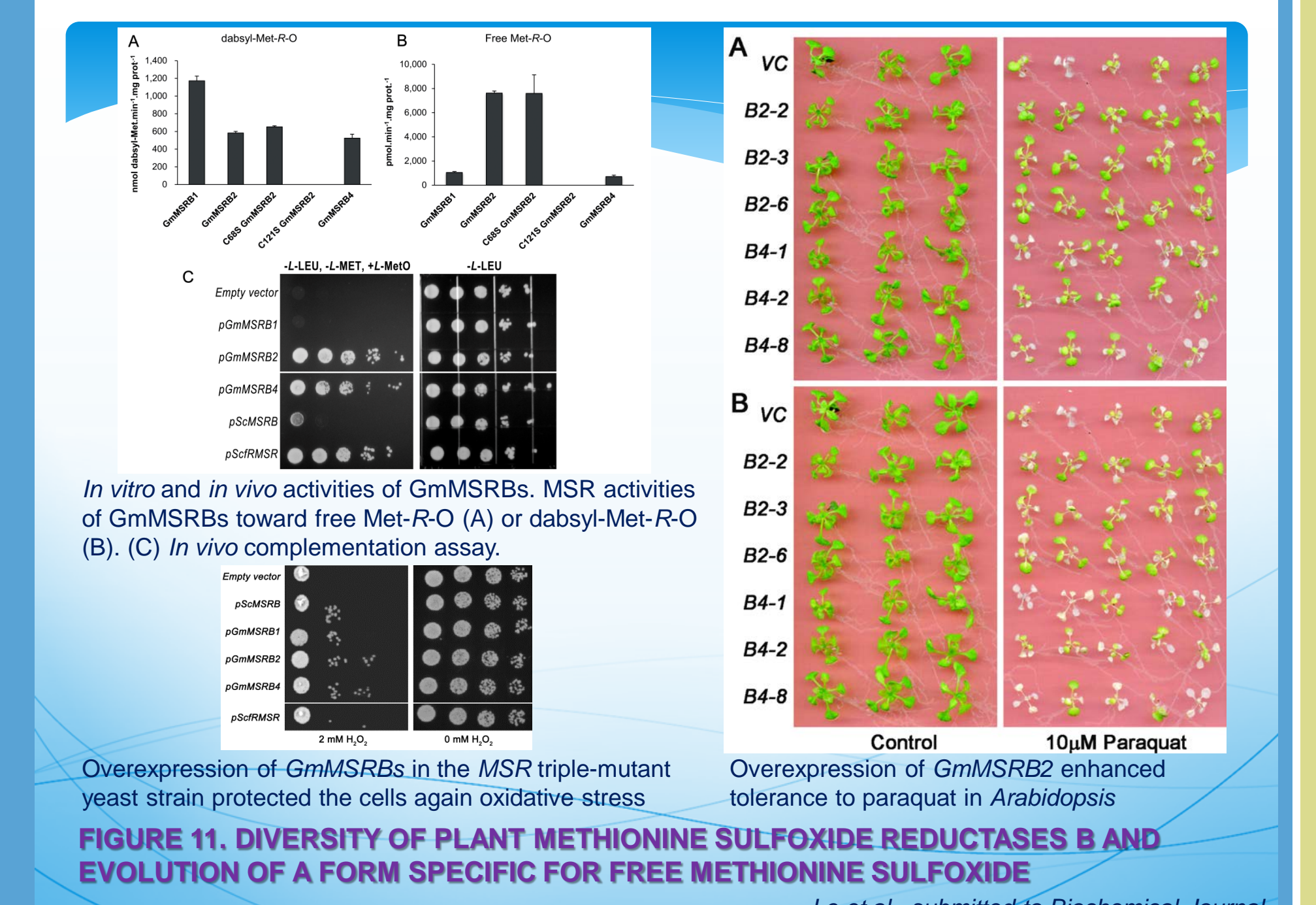
Gene	Mean CT		STDEV of CT		Average of STDEV of ACT	
	Roots	Shoots	Roots	Shoots	Roots & Shoots	Roots & Shoots
Act1	21.31	21.97	0.5507	0.8184	0.4274	0.5112
Act2	23.64	24.38	0.5522	0.6079	0.4580	0.5851
Act3	21.64	22.03	0.5793	1.0953	0.5325	0.5837
Act4	19.11	19.70	0.7287	0.7892	0.5772	0.5761
Act5	25.79	26.35	0.8712	0.8712	0.7810	0.8356
Act6	17.63	17.68	0.5359	0.5148	0.5897	0.5987
Act7	18.41	18.87	0.3853	0.7358	0.5210	0.5905
Act8	20.12	20.34	0.6676	1.0553	0.8089	1.1395
Act9	20.60	21.16	0.5545	0.7793	0.4704	0.5461
Act10	21.66	22.41	0.7632	0.6132	0.5284	0.5518
Act11	25.30	26.65	1.1050	0.8995	0.9455	1.1024
Act12	20.12	20.34	0.6676	1.0553	0.8089	1.1395
Act13	20.60	21.16	0.5545	0.7793	0.4704	0.5461
Act14	21.66	22.41	0.7632	0.6132	0.5284	0.5518

The top five genes are shown in red-colored letters, while the top two genes are in red-colored and bold letters.

FIGURE 10. EXPRESSION STABILITY OF THE CANDIDATE REFERENCE GENES IN ROOT AND SHOOT TISSUES OF SOYBEAN SEEDLINGS UNDER VARIOUS ABIOTIC STRESS AND HORMONAL TREATMENTS.

Soybean seedlings were subjected to dehydration, cold stress, salt stress and ABA treatments, and *gelnorm* was used to assess expression stability in (A) treated roots and shoots; (B) treated roots; (C) treated shoots.

Le et al., *PLOS ONE* 7(9):e46487 (2012b)



For additional information, please contact:
 Tien-Dung LE at letiendzung@psc.riken.jp or dunglt.agi@mard.gov.vn