JORDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

Role of GABA Shunt Pathway in Plant Abiotic Stress Tolerance

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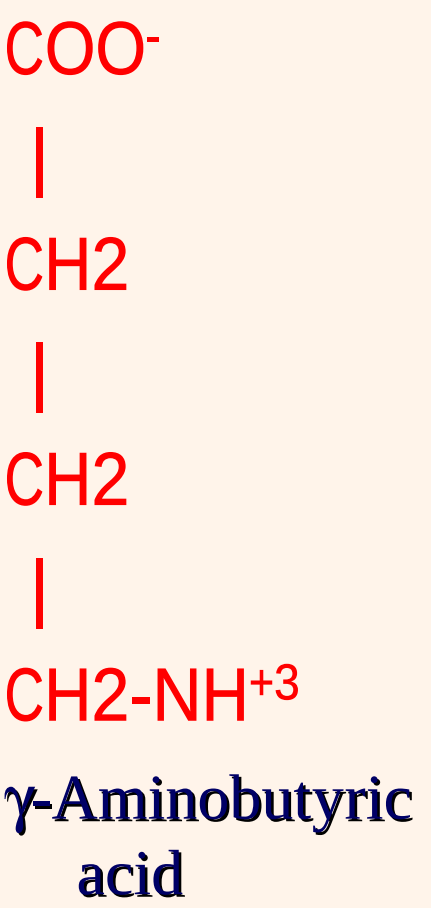
What is GABA?

- GABA : Gamma-aminobutyric Acid
- Four carbon non-protein amino acid
- It exists in non-bound form
- Highly soluble in water
- Has amino group on the γ-carbon rather than on the α-carbon
- GABA is a zwitterion (carry both + and – charges)
- Express in plant tissue under stress conditions.



γ-Aminobutyric acid

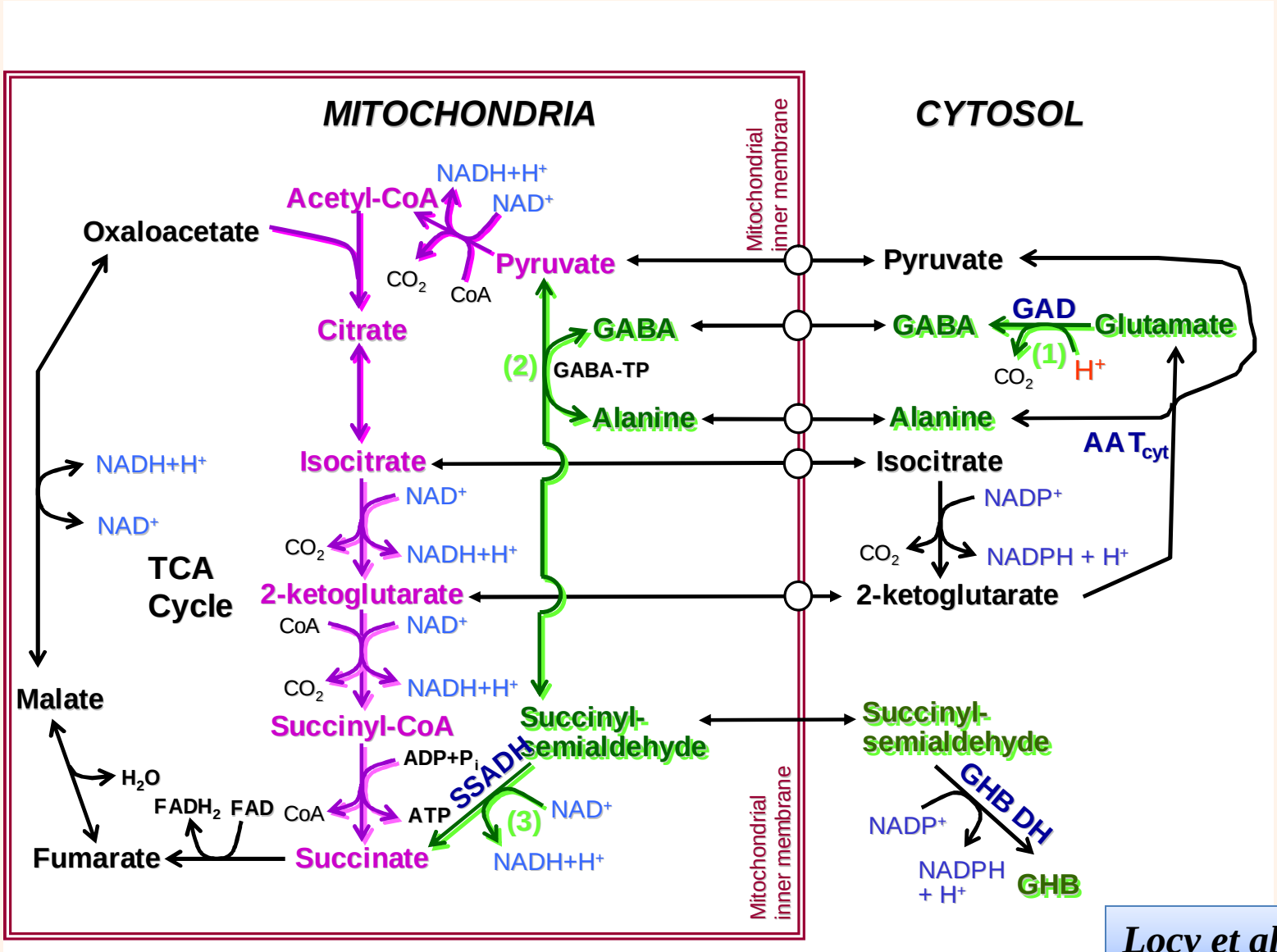
GABA Role in plants:

- GABA level in plant are low (0.03 to 2.0 µmol/g Fresh weight
- Increases in response to:
  - Heat shock
  - Cold stress
  - Mechanical stimulation
  - Drought stress
  - Salinity
  - Biotic stress

GABA shunt Enzymes in Plants

- Glutamate Decarboxylase (GAD)
- GABA Transaminase (GABA-TK) or (GABA-TP)
- Succinic Semialdehyde Dehydrogenase (SSADH)

GABA Shunt in Plants



Locy et al. 2000

Glutamate Decarboxylase (GAD):

- Cytosolic enzyme optimal PH ~5.5
- Calcium/Calmodulin binding protein
- In Arabidopsis two expressed isoforms:
  - GAD I : 58 KD (exclusively in roots)
  - GAD II: 56 KD (in all organs)
- Both under Calcium/ Calmodulin regulation
- 87% identical amino acid sequences

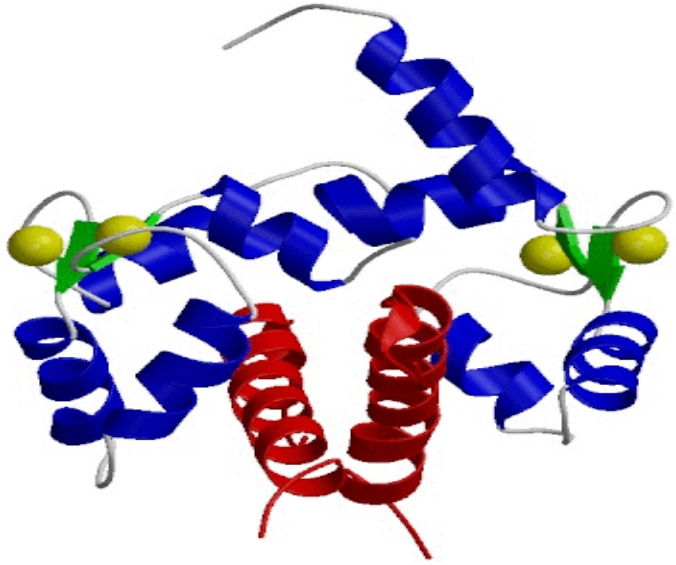
Ca^{+2}/CaM-GAD

Ca^{+2}/CaM

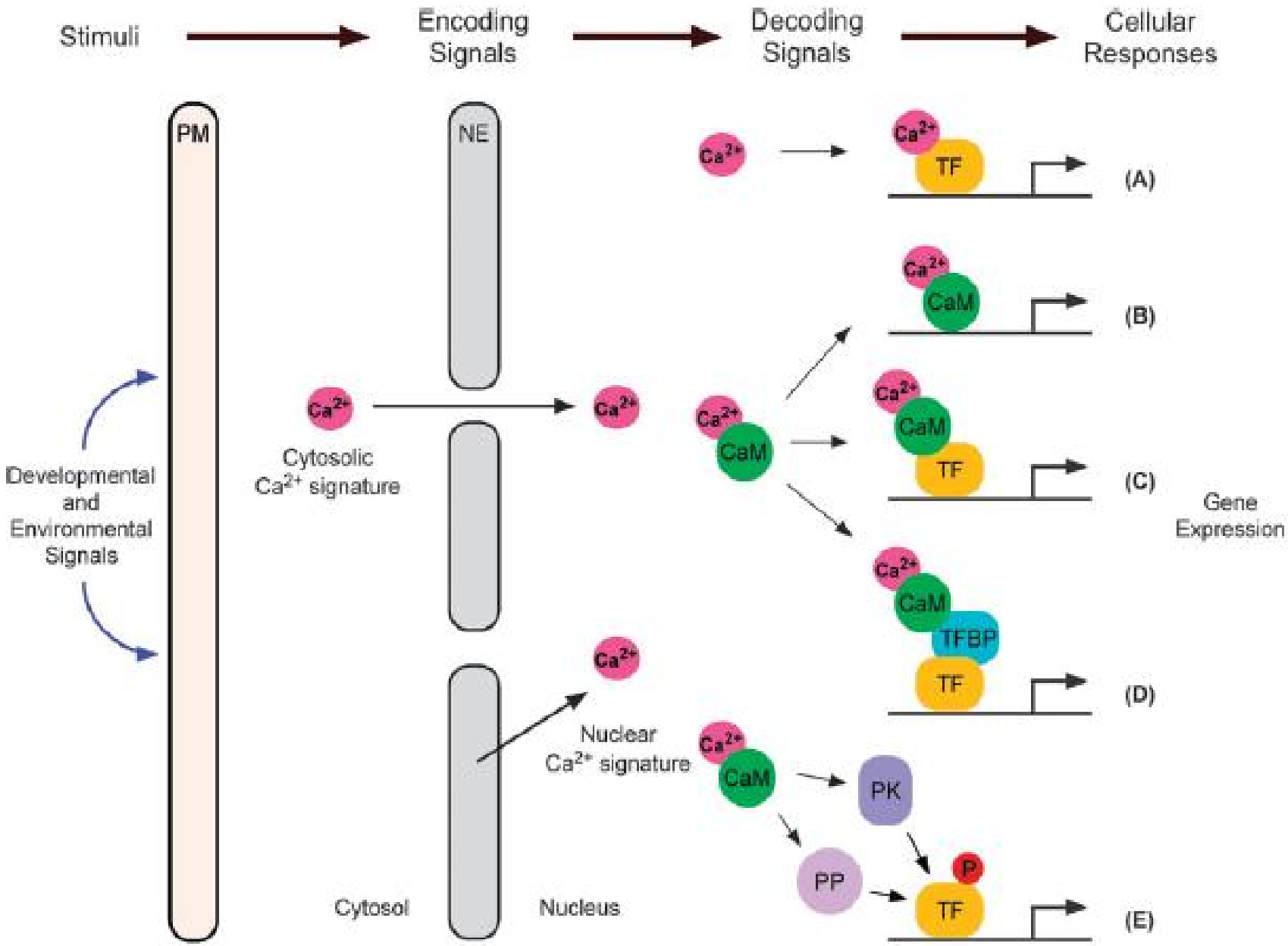
- Acidic protein (4 EF hand , 12 amino acids Asp and Glut)
- 4 Ca^{+2} binding sites
- CaM/Ca^{+2} Complex
- Modulation of various enzyme activities.

Abiotic stress:

- Ca^{+2} Elevation
-Ca^{+2}/CaM complex
-CaM/GAD Bound state
- GABA shunt
- CaM regulation of other proteins (CaM-binding proteins)

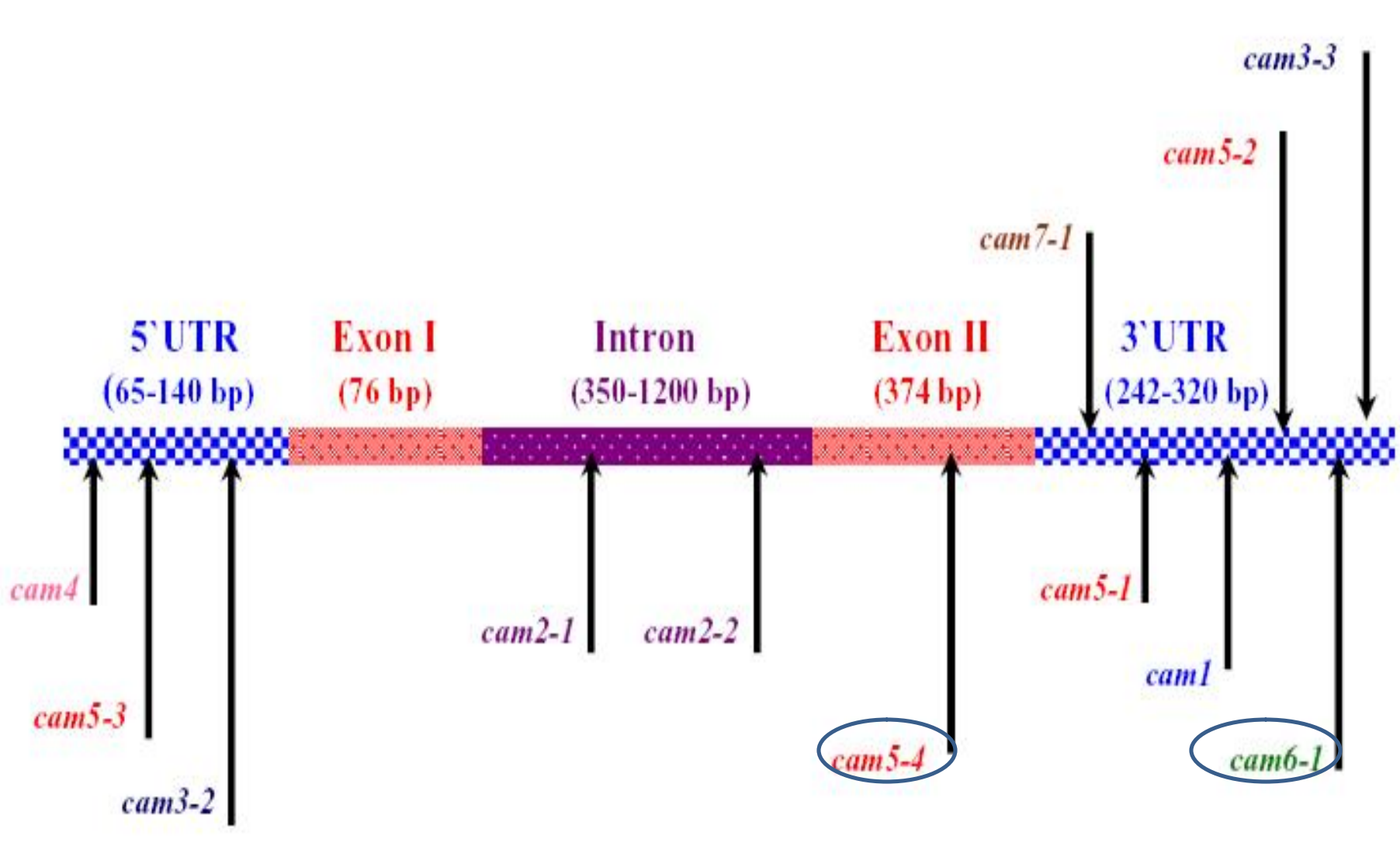


CaM-Ca /GAD



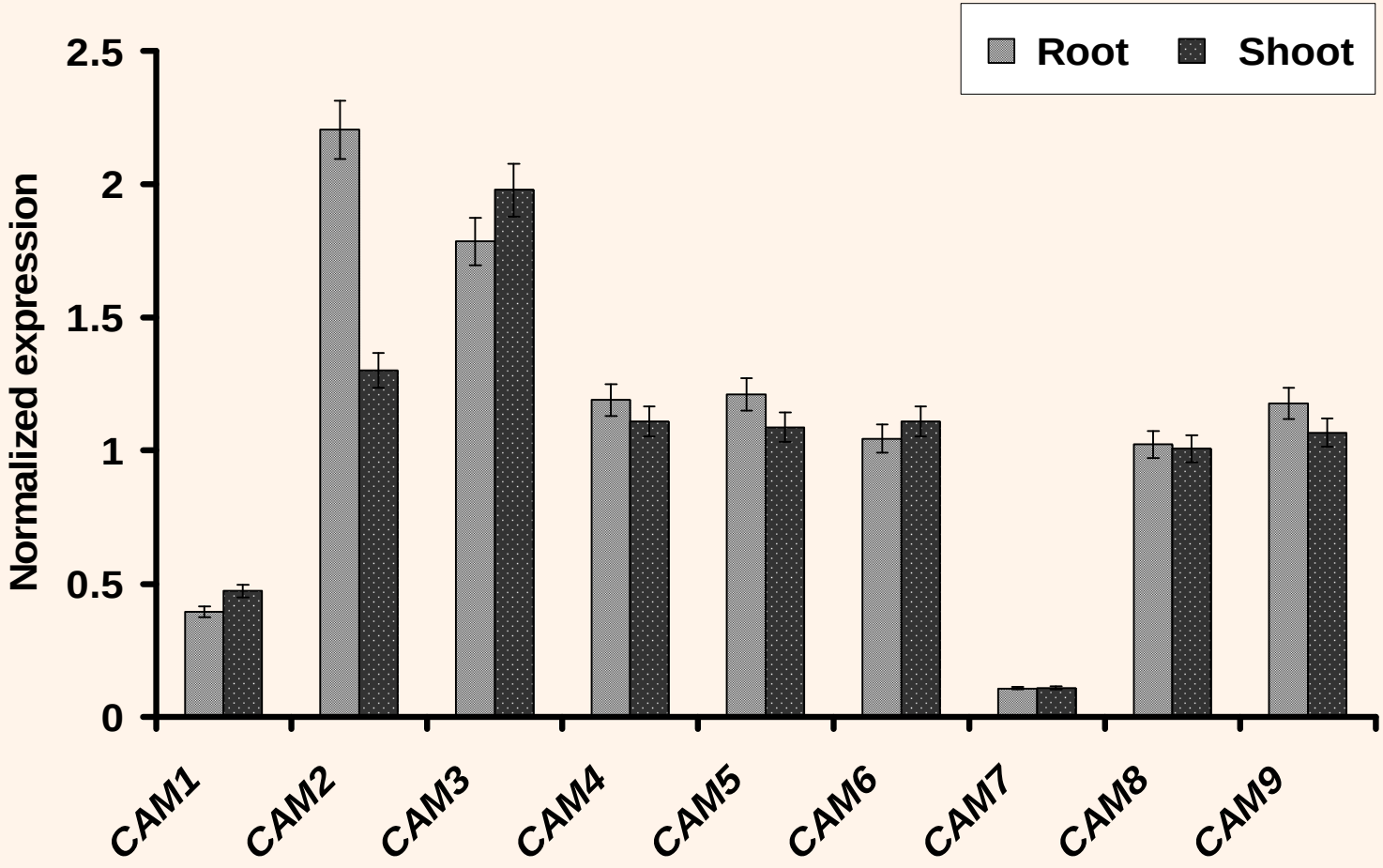
Molecular Plant • Volume 2 • Number 1 • Pages 13–21 • January 2009

Insertion sites in *CAM* genes



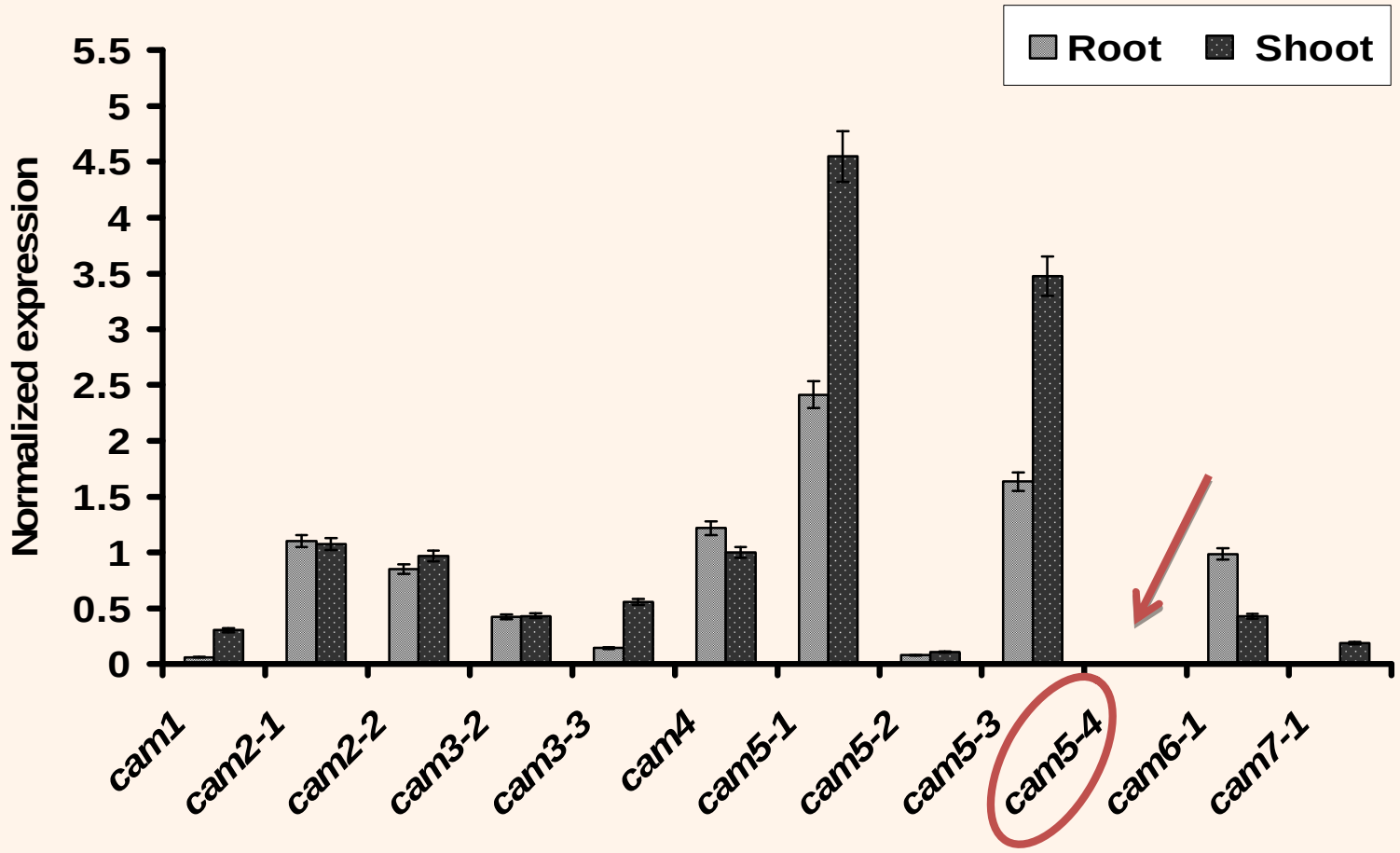
AL-Quraan et al. 2008

- **Normalized expression of nine *CAM* genes in root and shoot tissues of wild type *A. thaliana* grown at 25ºC.**

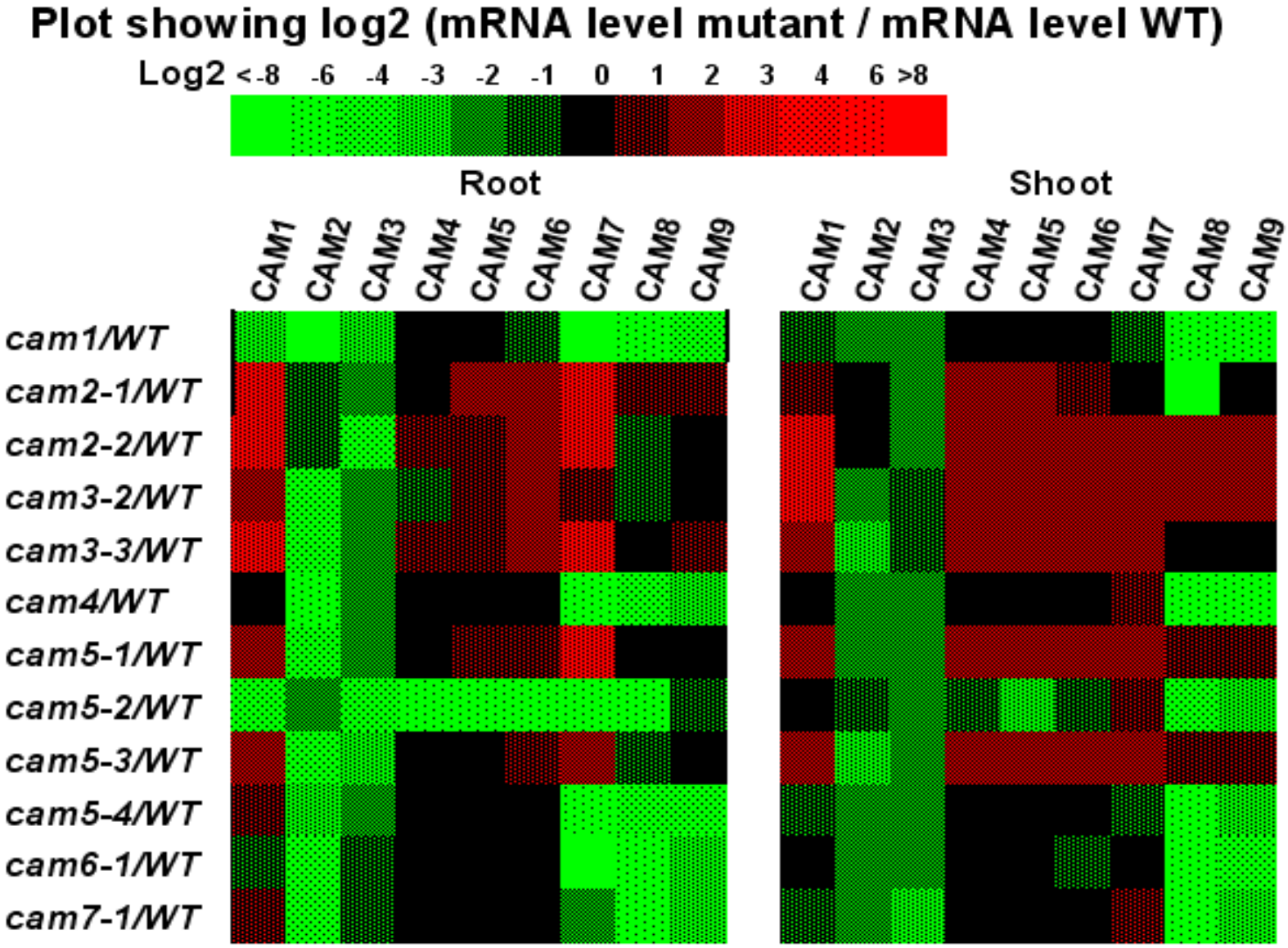


AL-Quraan et al. 2010

- **Normalized expression of *CAM* gene in root and shoot tissues of the corresponding *cam* mutants of *A. thaliana* at 25ºC.**

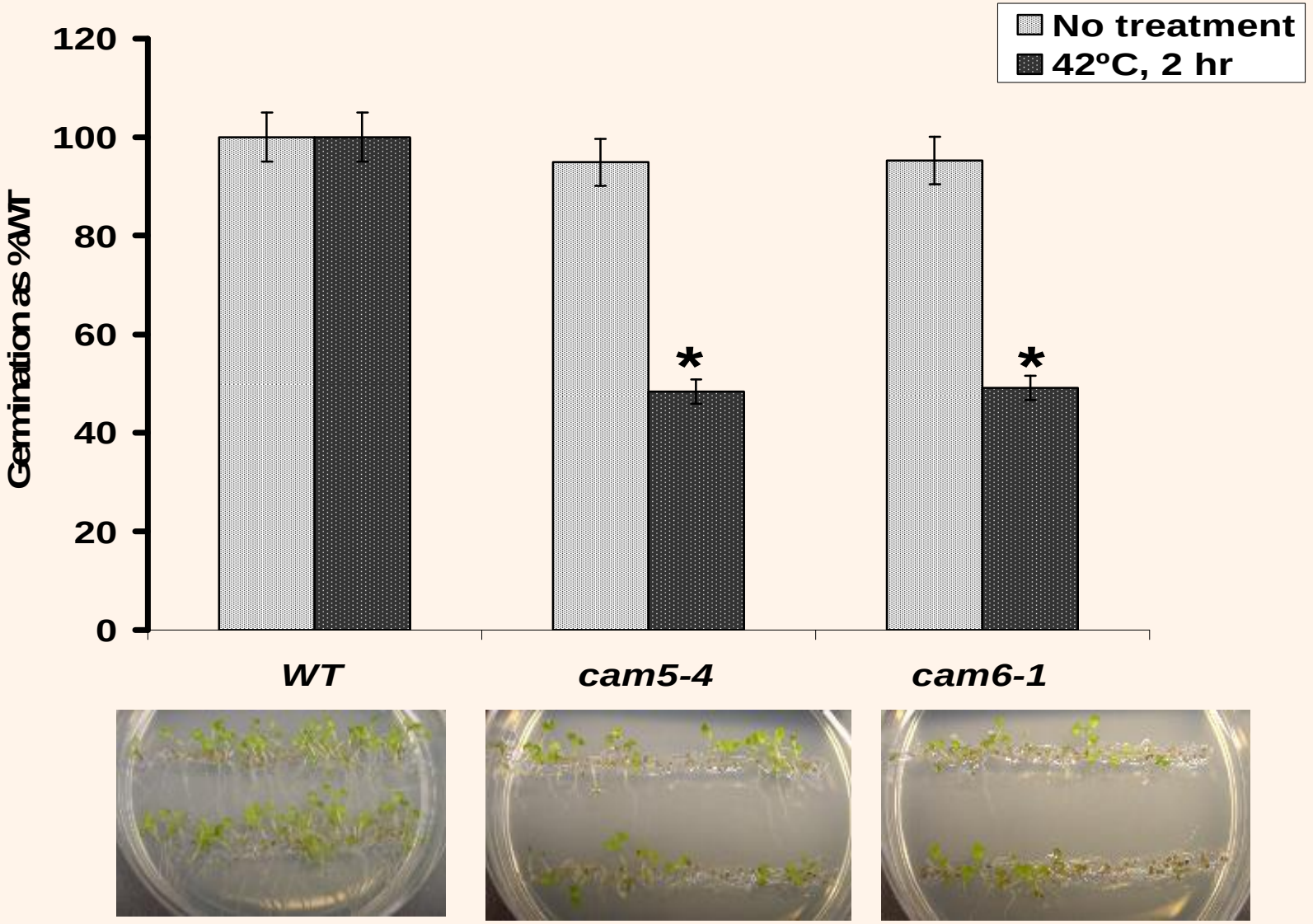


AL-Quraan et al. 2010



AL-Quraan et al. 2010

Seed germination: 42 °C



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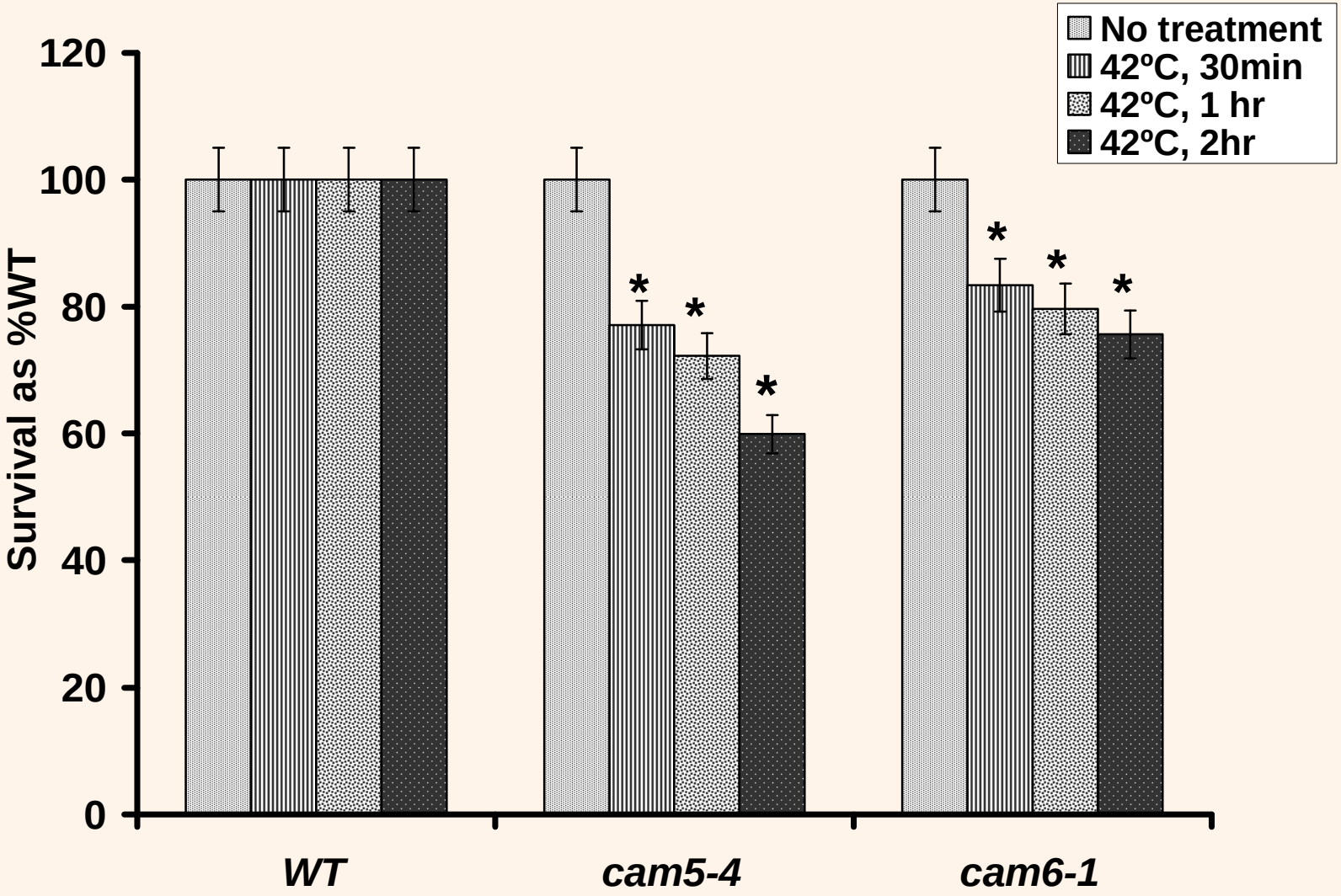
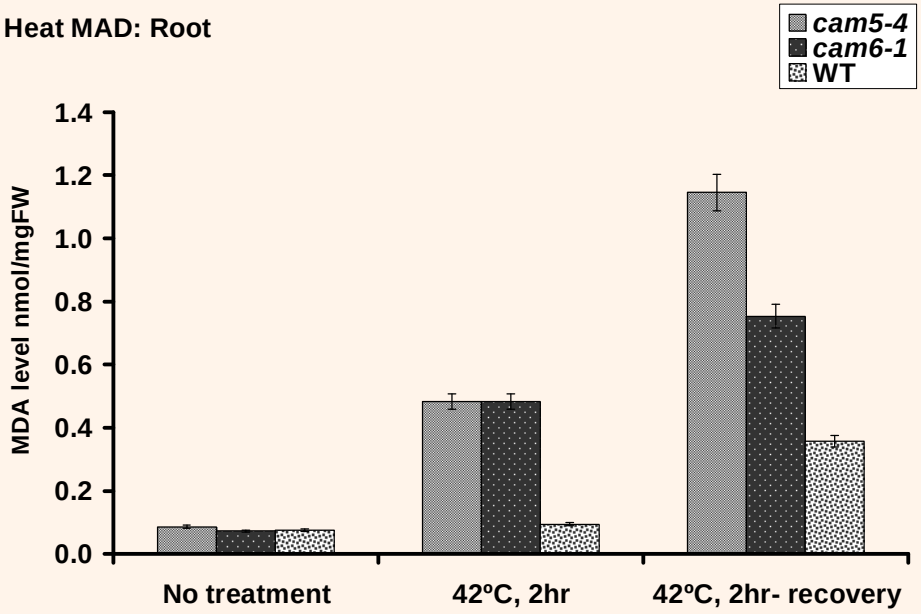
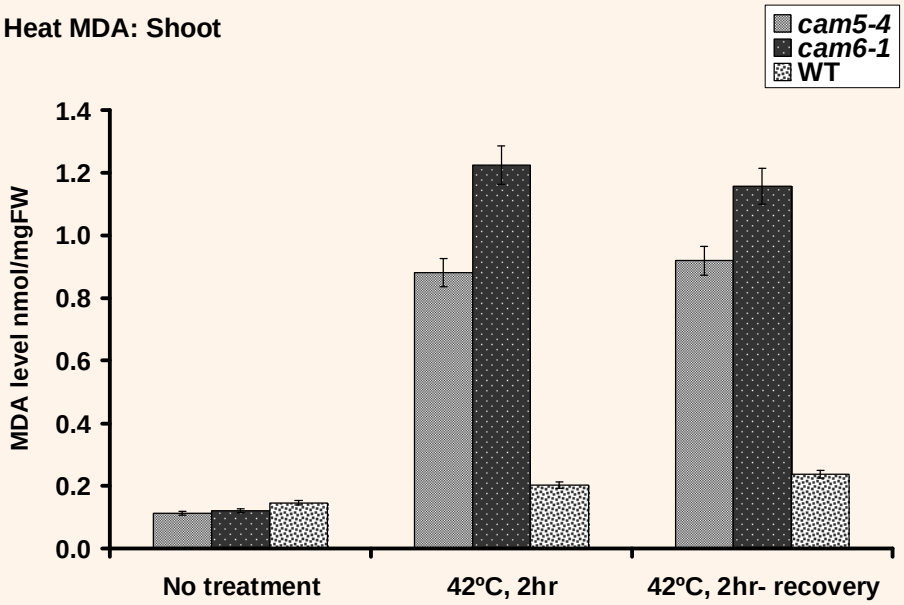
Seedling survival: 42°C



AL-Quraan et al. 2008

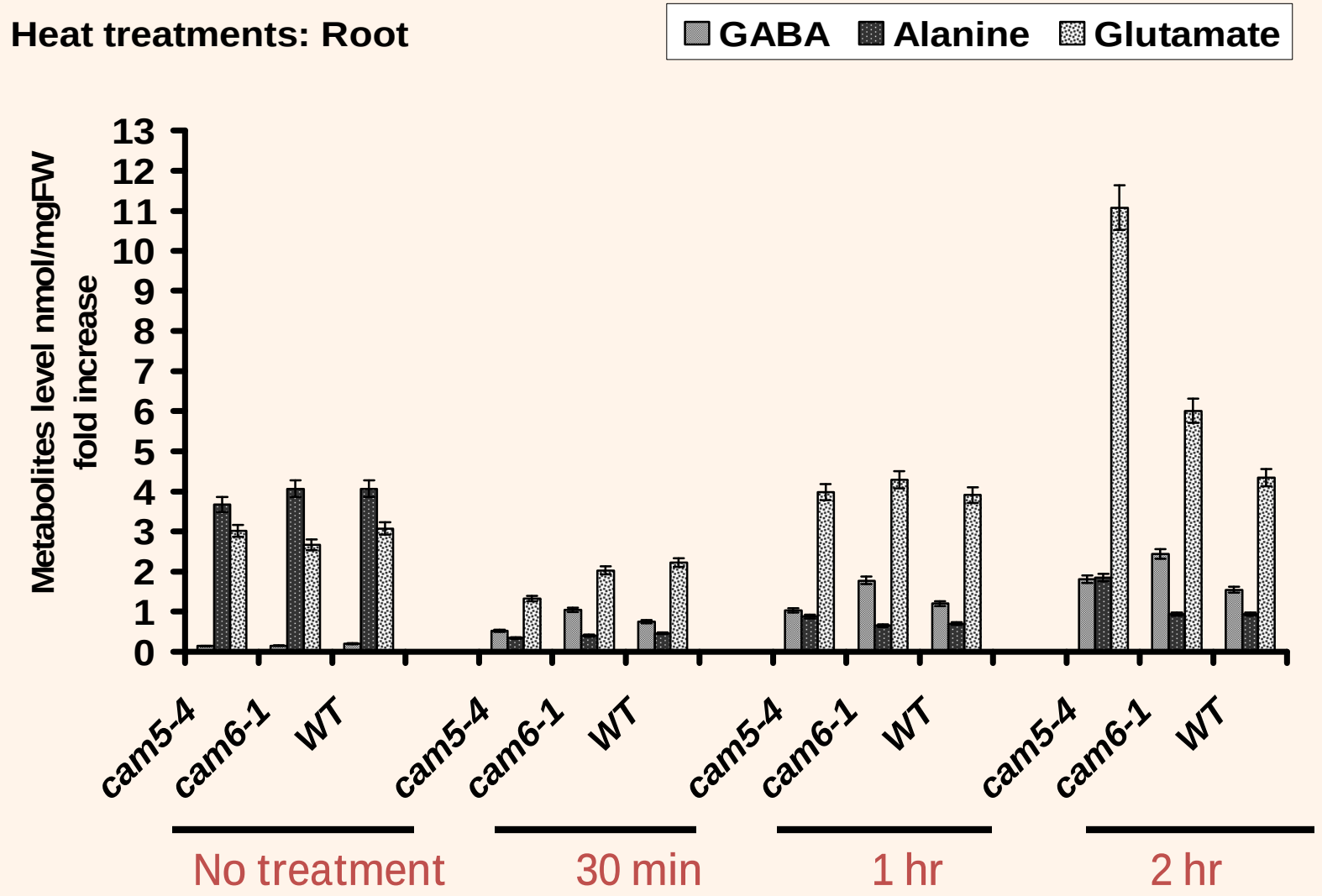
MDA level: 42 ºC





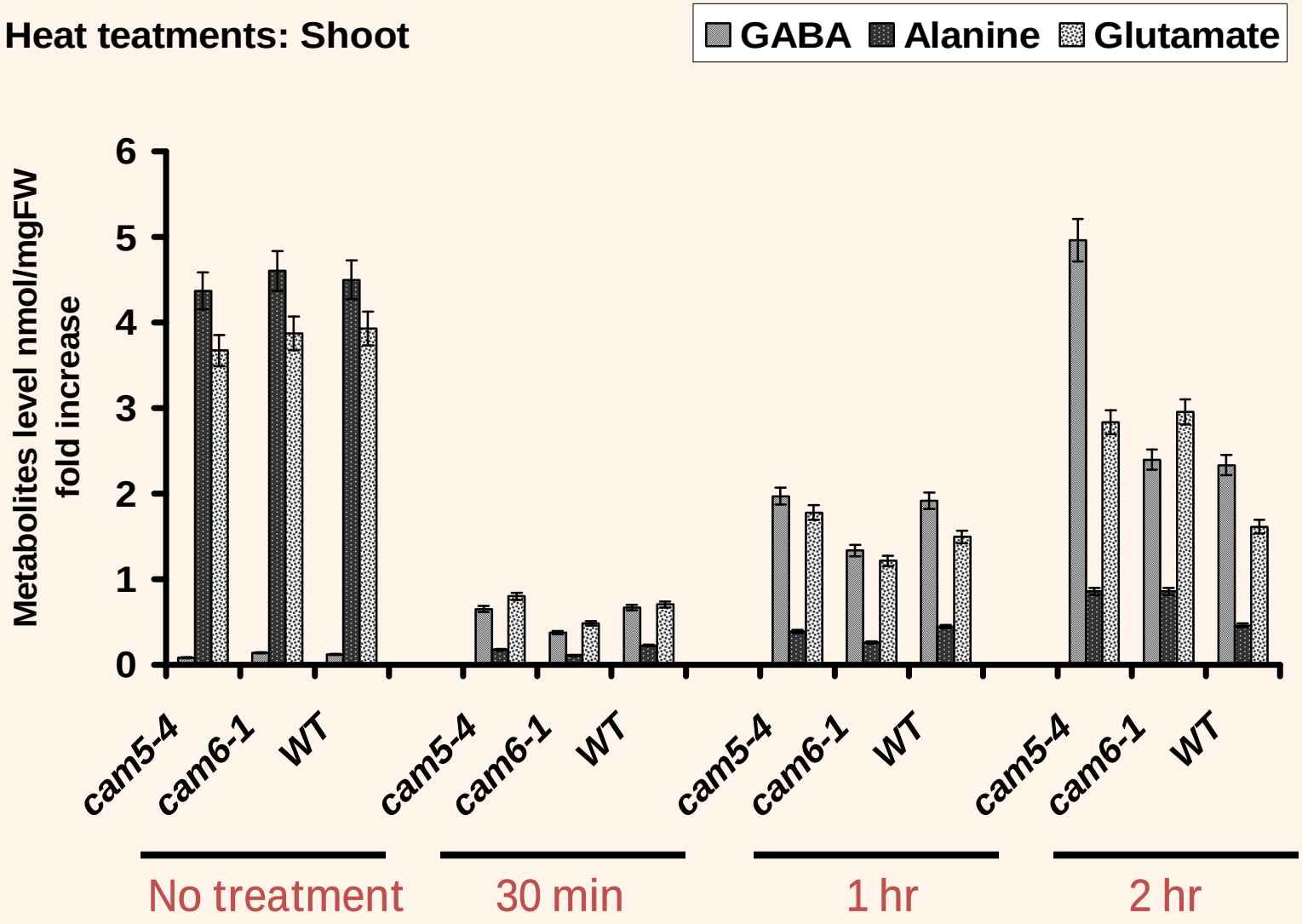
AL-Quraan et al. 2008

GABA shunt metabolites: 42 ºC (Root)

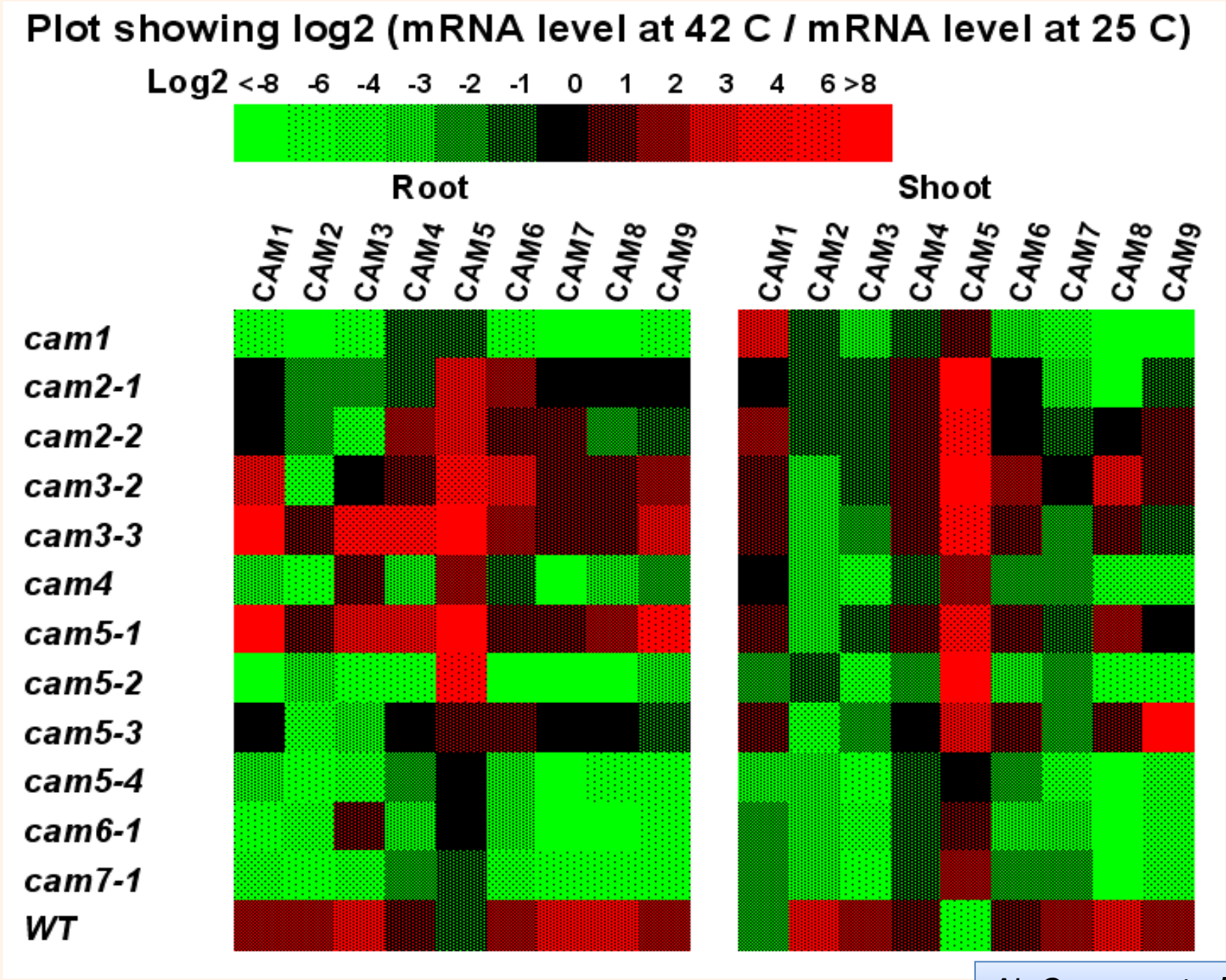


AL-Quraan et al. 2008

GABA shunt metabolites: 42 ºC (Shoot)

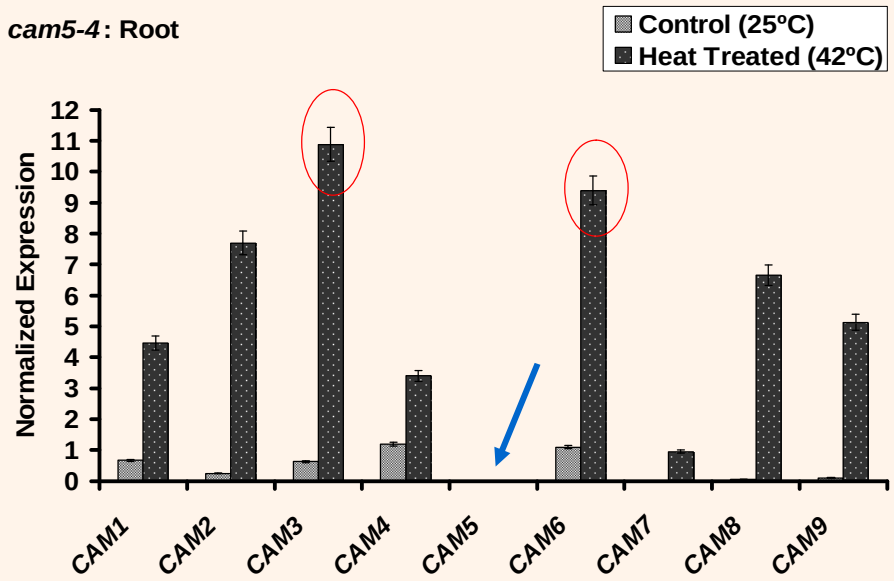


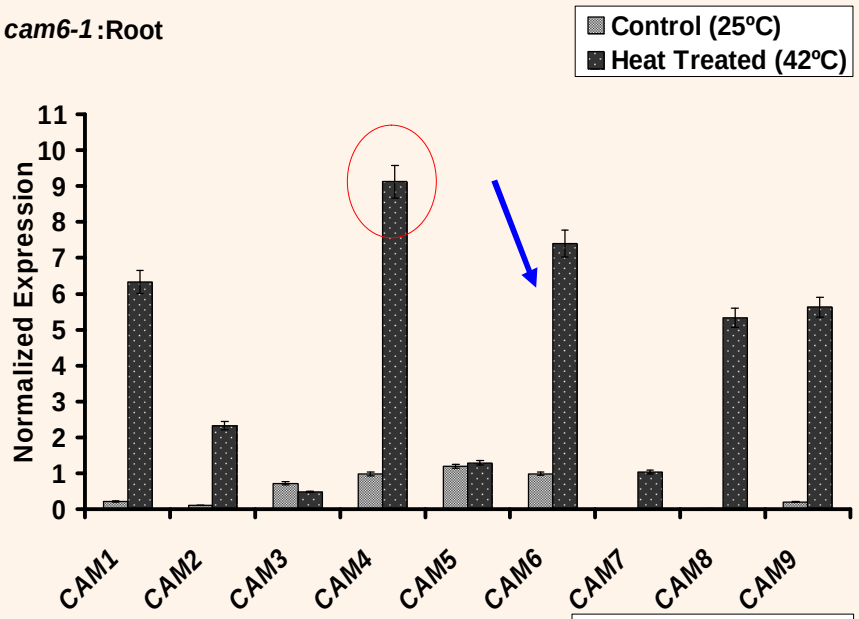
AL-Quraan et al. 2008

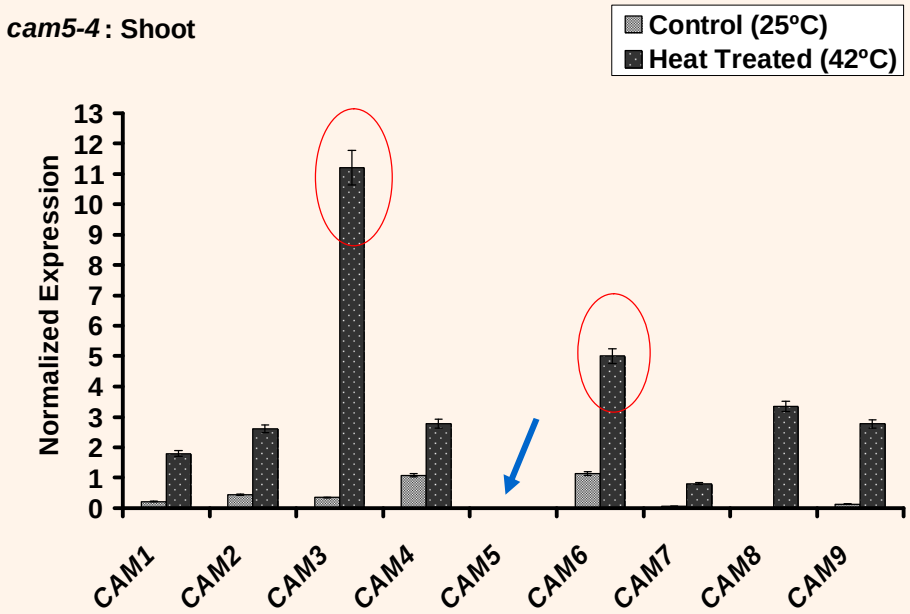


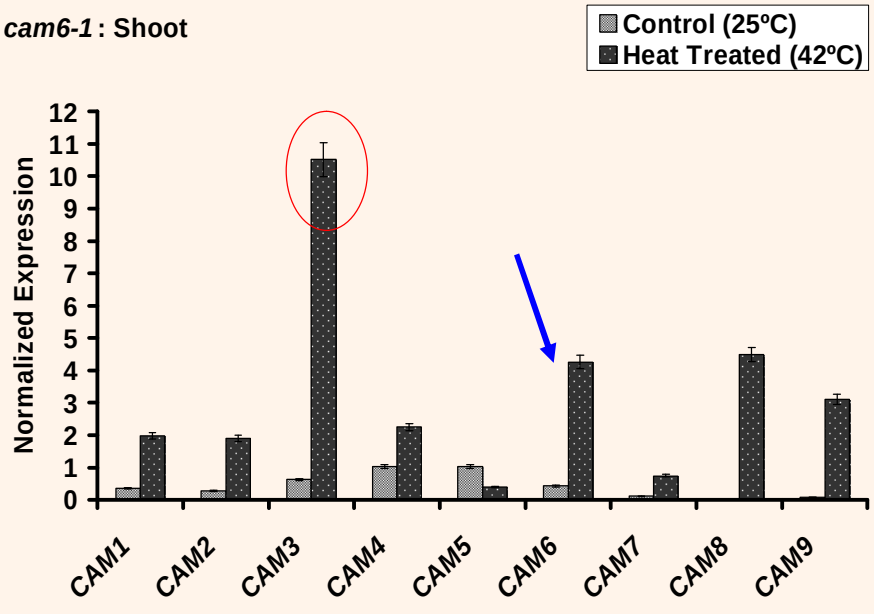
AL-Quraan et al. 2010

Expression of *CAM* genes in *cam5-4* and *cam6-1*









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Summary …….Conclusion

- ***CAM*** **genes showed differential expression and metabolites partition in root and shoot tissues after exposure to heat.**

- **Variations the expression of *CAM* genes in different *cam* mutants in control and in response to heat suggest interactions between calmodulin isoforms during expression of *CAM* genes in *Arabidopsis*.**

- ***cam*5-4 and *cam*6-1 mutants are sensitive to high temperature stress for seed germination, survival/ growth and oxidative damage.**

- **Sensitivity of cam5-4 presumably resulted from absence of CaM5 protein in *cam*5-4.**

- ***CAM*6 was expressed in *cam*6-1 mutant. Sensitivity of *cam*6-1 to stress may not be a direct consequence of this mutation.**
- **Differential tissue specific expression of *CAM* genes in root and shoot tissues**
- **Regulatory and compensatory interactions between calmodulin isoforms in expression of *CAM* genes in *Arabidopsis*.**
- **Important role for CaM5 protein in tolerance to abiotic stress in *cam*5-4.**

- **Sensitivity of *cam*6-1 to all environmental stress tested may be mediated indirectly through other alternative mechanism.**

- **Elevation in GABA shunt metabolites in response to heat stress in *cam* mutants suggests regulation of GABA level by CaM through GAD activation after the stress exposure.**

- **Later increase in metabolite level after long exposure may indicate involvement of reduction in cytoplasmic pH/or metabolite partitioning under stress.**

Major References

- **Locy, R.D., Wu, S-J, Bisnette, J., Barger, T.W., MacNabb, D., Zik, M., Fromm, H., Singh, N.K., Cherry, J.H.** (2000). The Regulation of GABA Accumulation by Heat Stress in *Arabidopsis*. Edited in J.H. Cherry et al: Plant Tolerance to Abiotic Stresses in Agriculture, Role of Genetic Engeneering, 39-52**,** Kluwer Academics. Netherland.

- **AL-Quraan, N.I.** Role of *Arabidopsis thaliana* Calmodulin Isoforms in Tolerance to Abiotic Stress. PhD Dissertation. Auburn University. Alabama.USA. August 2008.

- **AL-Quraan. N.I**., Robert D. Locy, and Narendra K. Singh. Expression of calmodulin genes in wild type and calmodulin mutants of *Arabidopsis thaliana* under heat stress. Plant Physiology and Biochemistry. 48: 697 – 702. 2010

Acknowledgement

- **Dr. Narendra K.Singh**
- **Dr. Robert D. Locy**

Auburn University , Alabama, USA

- **Dr. Hanan I. Malkawi**

Yarmouk University, JORDAN

- **Dr. Zuhair Amr**
- **Dr. Nizar Abu-Harfiel**

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