Environmental and Hormonal Signaling in Seed Dormancy

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National Institute of Food and Agriculture

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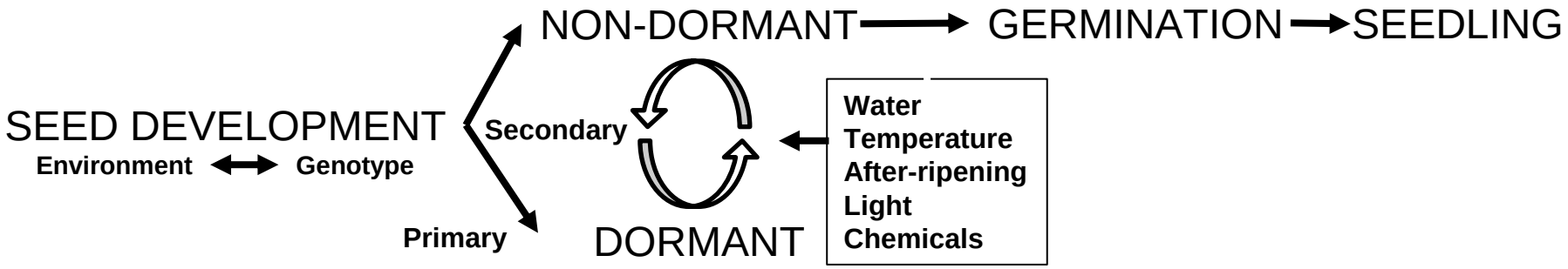
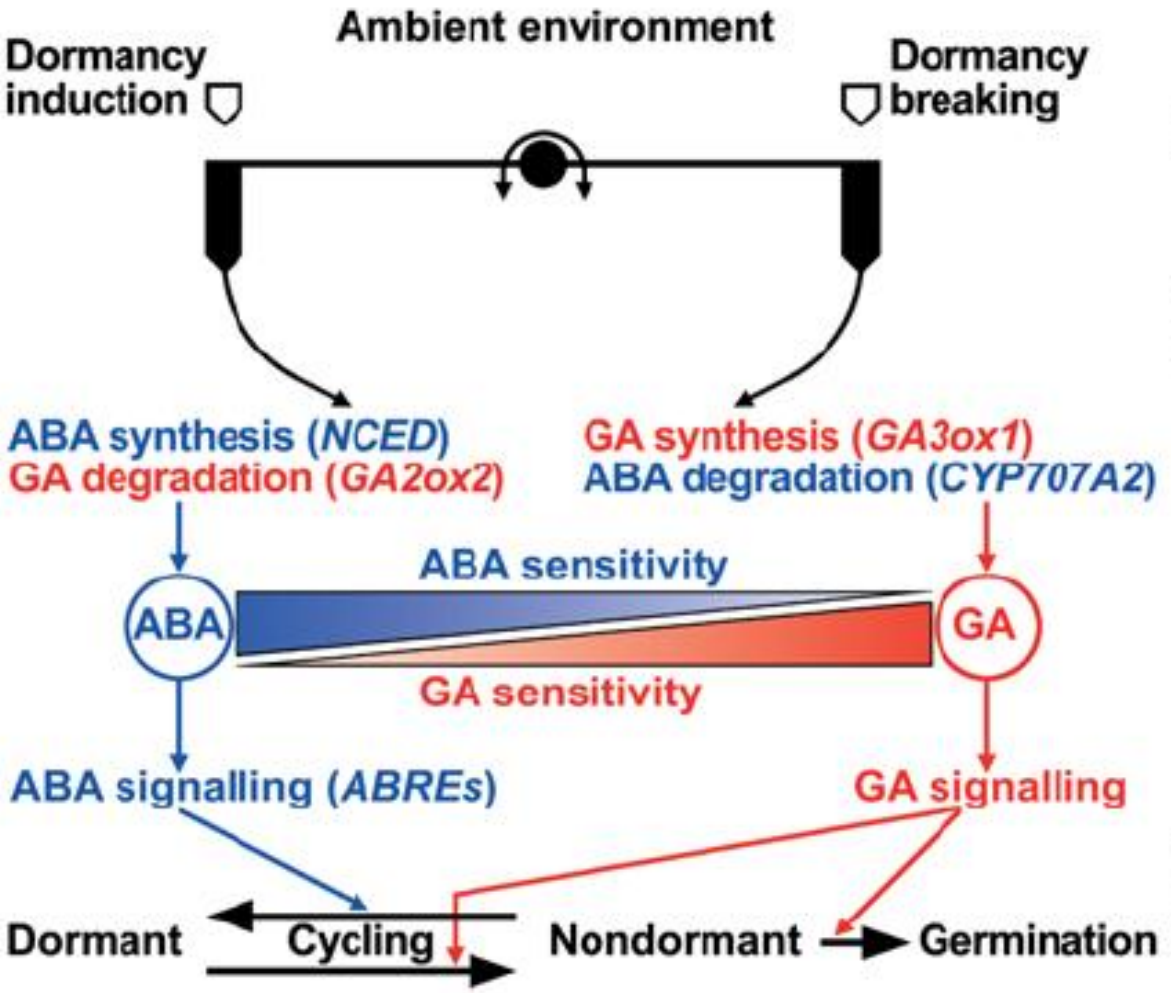
Seed Dormancy

- Seed dormancy is the failure of a viable seed to germinate when provided with conditions normally conducive to germination (water, temperature, etc.).

- Seed dormancy as an adaptive mechanism for successful propagation is generally sensitive to environmental conditions, particularly temperature, light and nutrients (i.e., nitrate).



Hormone Balance Is Involved in Seed Dormancy



Environment

Synthesis and degradation

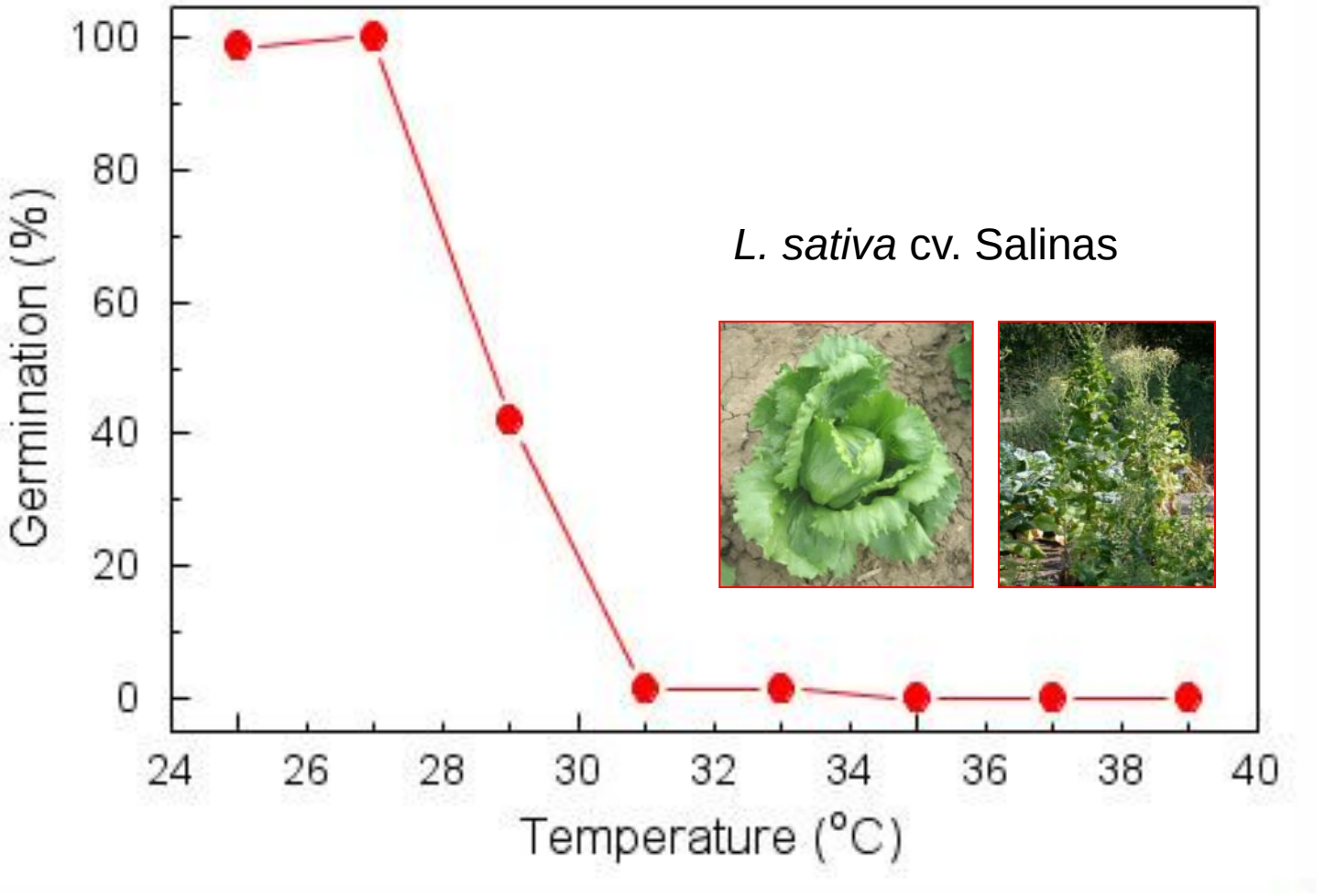
Sensitivity

Signaling

Response

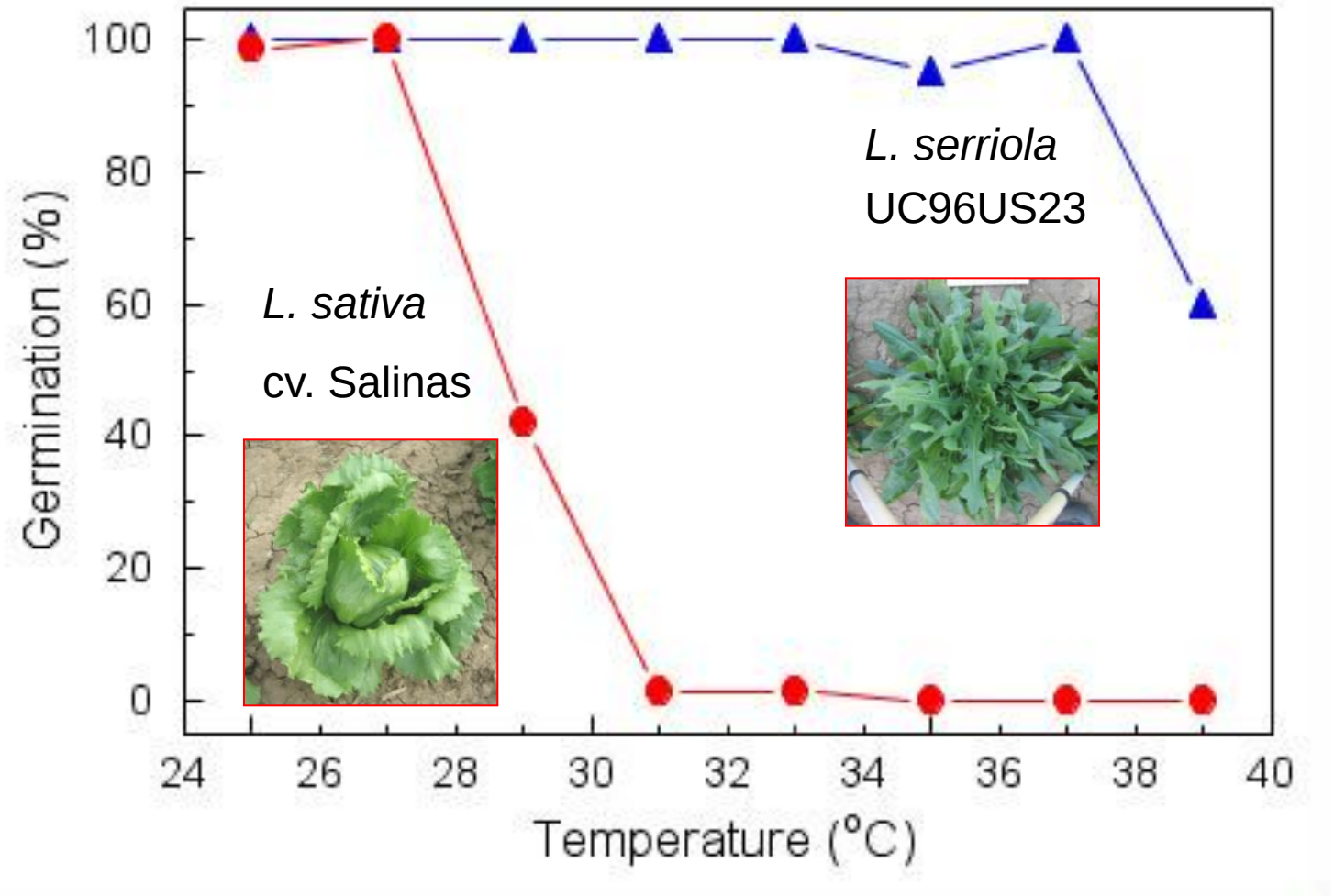
Finch-Savage and Leubner-Metzger (2006) New Phytologist 171:501–523.
Cadman et al. (2006) Plant Journal 46: 805-822.

Thermoinhibition at High Temperatures



Argyris et al. 2005. Theor.. Appl. Genet. 111: 1365.

Natural Variation for Thermoinhibition



Argyris et al. 2005. Theor. Appl. Genet. 111: 1365.

Recombinant Inbred Line Population

L. sativa
cv. Salinas

X

L. serriola
UC96US23

F1

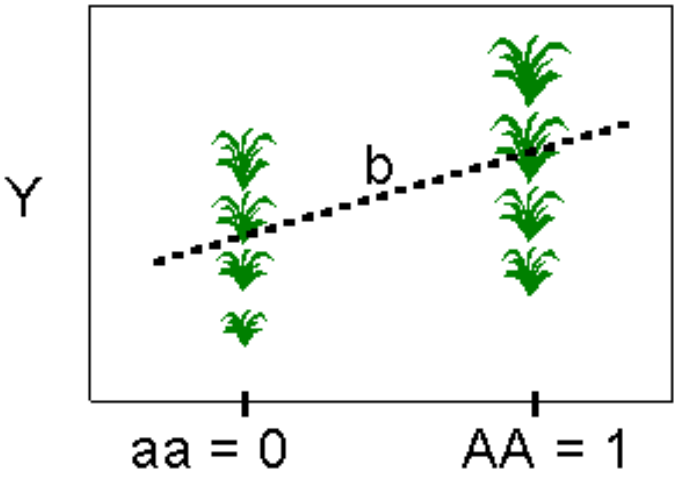
$F2_1$ $F2_2$ $F2_3$ . . . . . $F2_{98}$ $F2_{99}$ $F2_{100}$

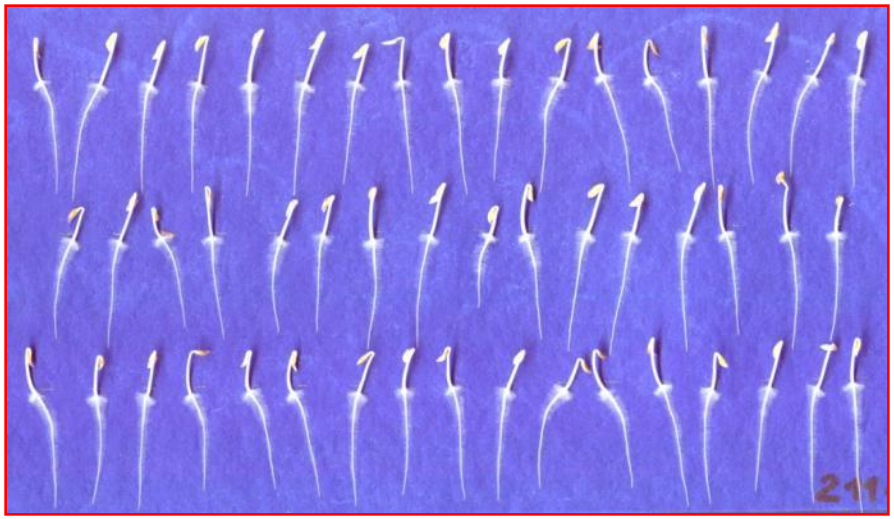
selfed families to
F8 generation

RIL_1 RIL_2 RIL_3 . . . . . RIL_{98} RIL_{99} RIL_{100}

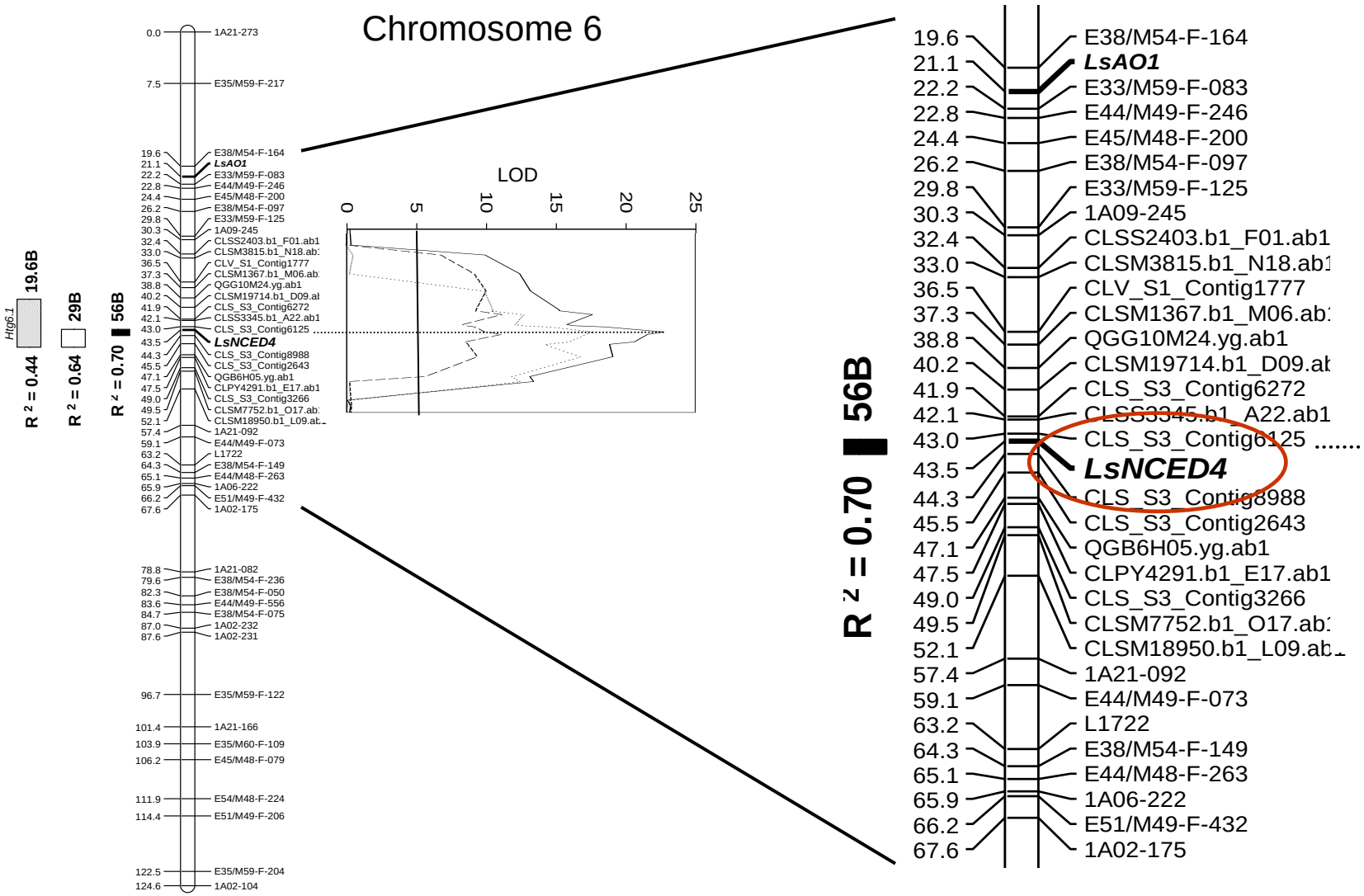
QTL Analysis – Phenotypic Data

- Phenotypes analyzed in RILs for QTL:
  - Germination/dormancy
    - Temperature response
    - Light and darkness
  - Seed traits (SWT, SOC)
  - Seedling characteristics
    - Root and shoot growth



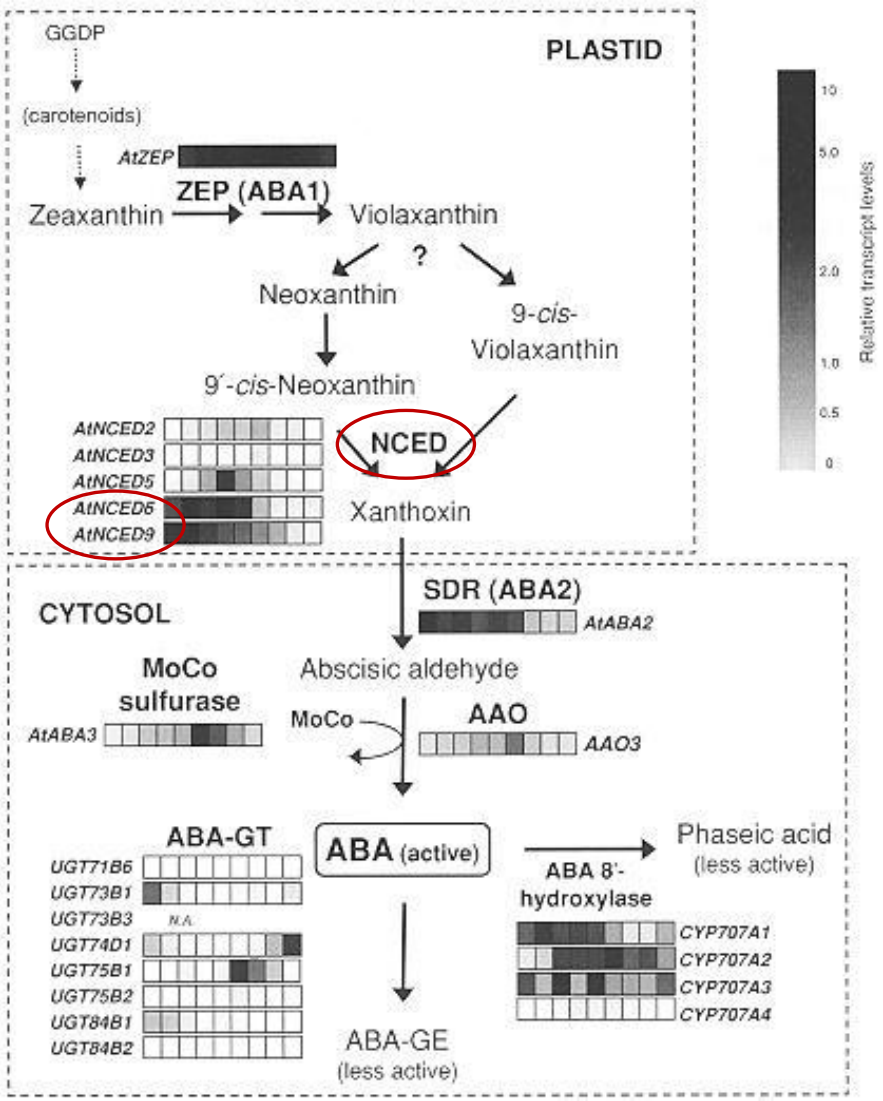


High Resolution Mapping of Htg6.1 in Lettuce



Argyris et al. 2011. Theor. Appl. Genet. 122: 95-108.

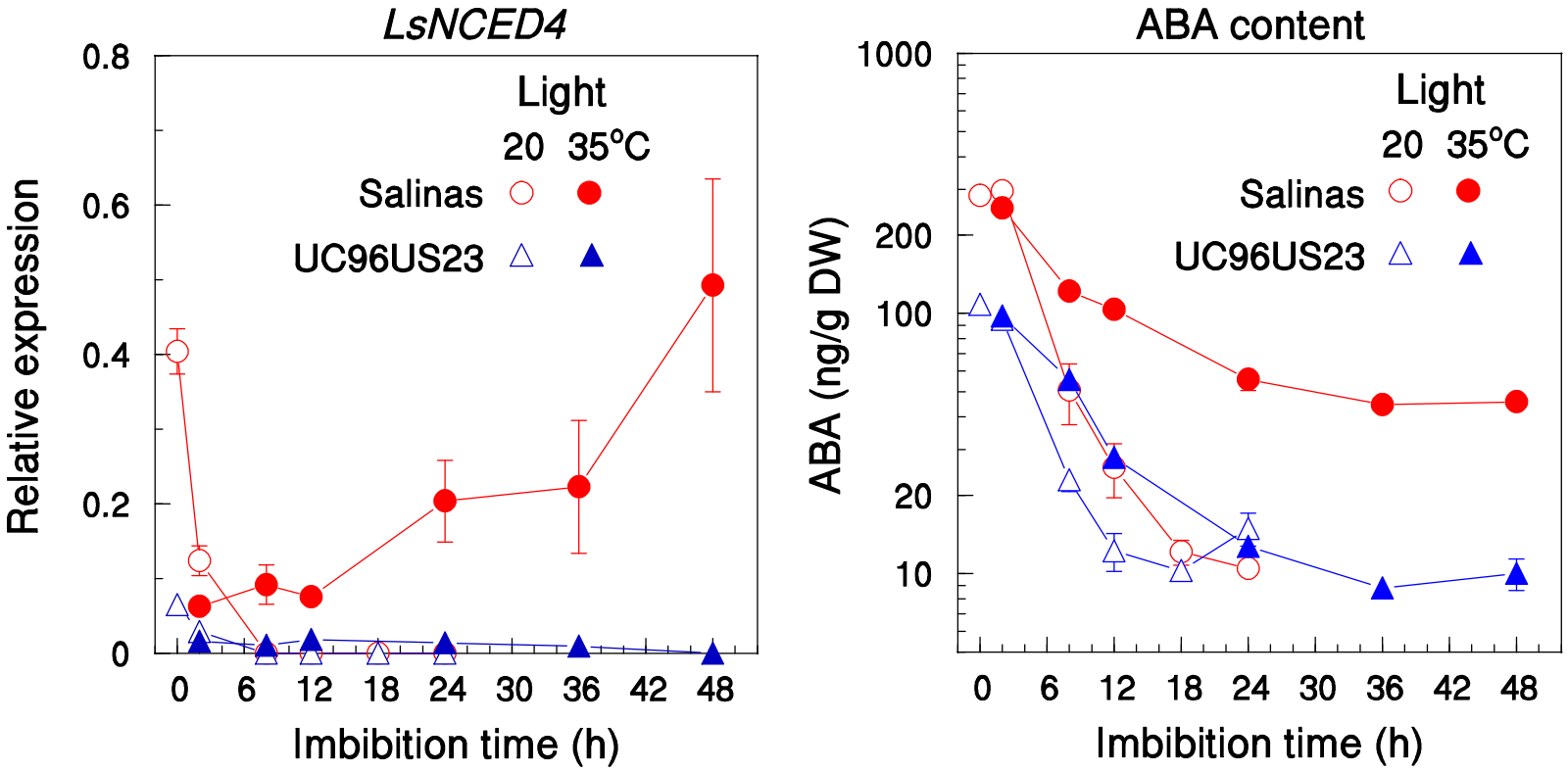
NCED Is in the ABA Biosynthetic Pathway



Yamaguchi et al. (2007) In Bradford and Nonogaki, *Seed Development, Dormancy and Germination*, Blackwell, pp. 224-247.

- *NCED* genes encode 9-*cis*-epoxycarotinoid dioxygenases that form xanthoxin in the ABA biosynthetic pathway.
- The closest homologs to *LsNCED4* in Arabidopsis are *AtNCED6* and *AtNCED9*, which are expressed during seed development.
- *AtNCED6 and AtNCED9* are involved in seed dormancy, while *AtNCED9* is involved specifically in thermoinhibition (Lefebvre et al. 2006; Toh et al. 2008).

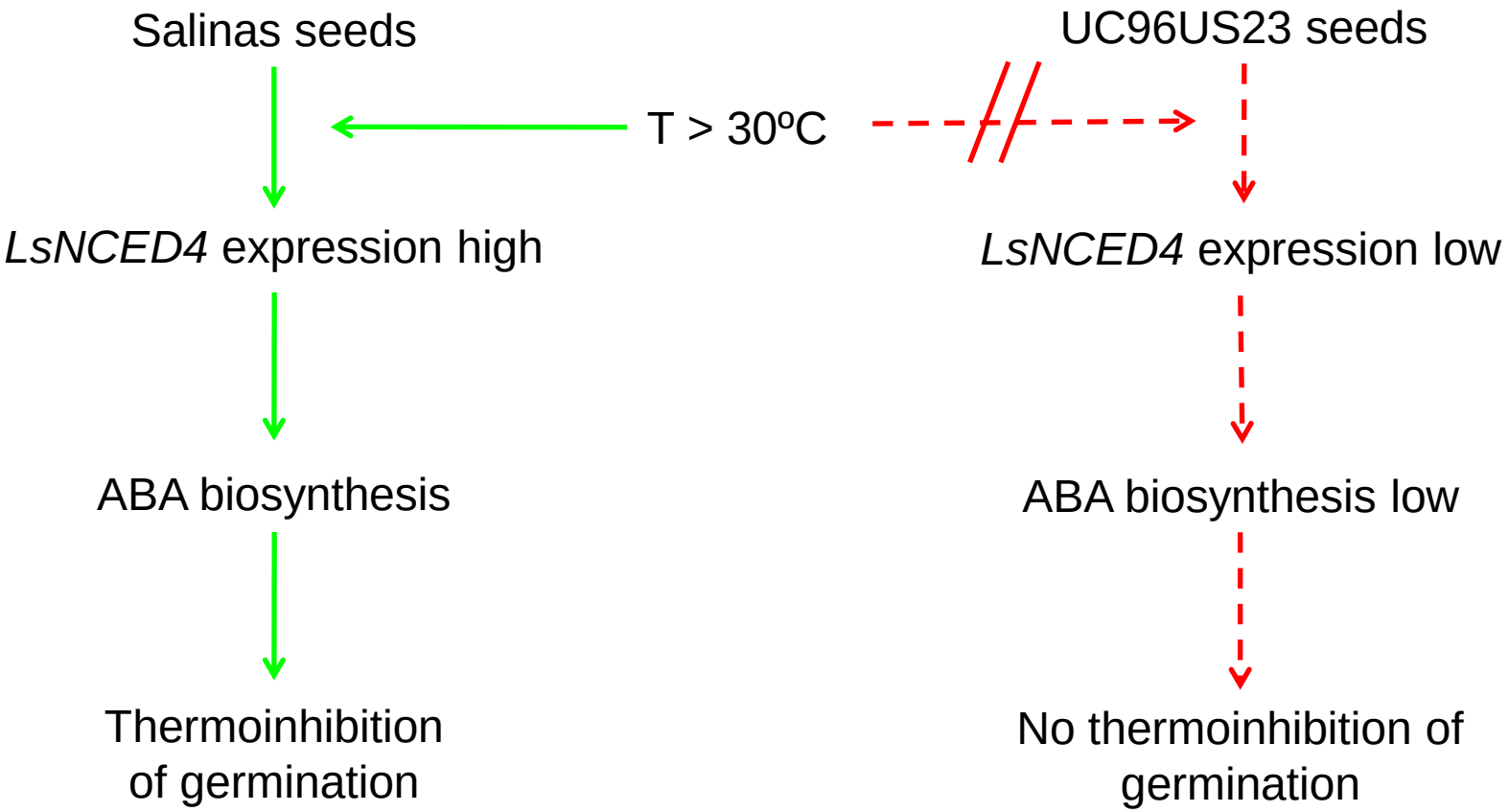
LsNCED4 Expression and ABA Content



LsNCED4 expression and ABA content remain elevated only in seeds of the Salinas variety imbibed at 35°C, which exhibit thermoinhibition.

Argyris et al. (2008) Plant Physiol. 148: 926-947.

Functional Complementation of UC96US23



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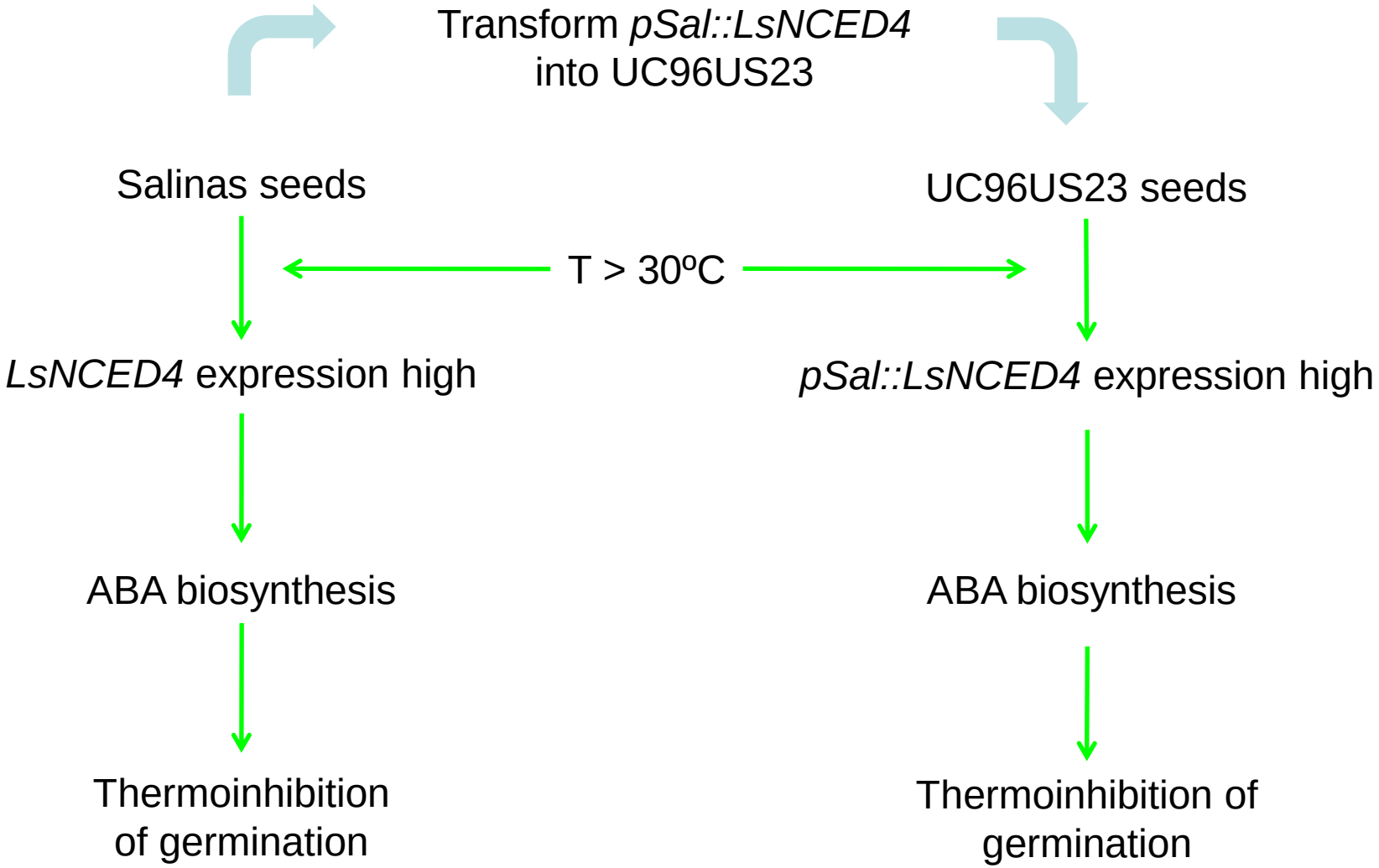
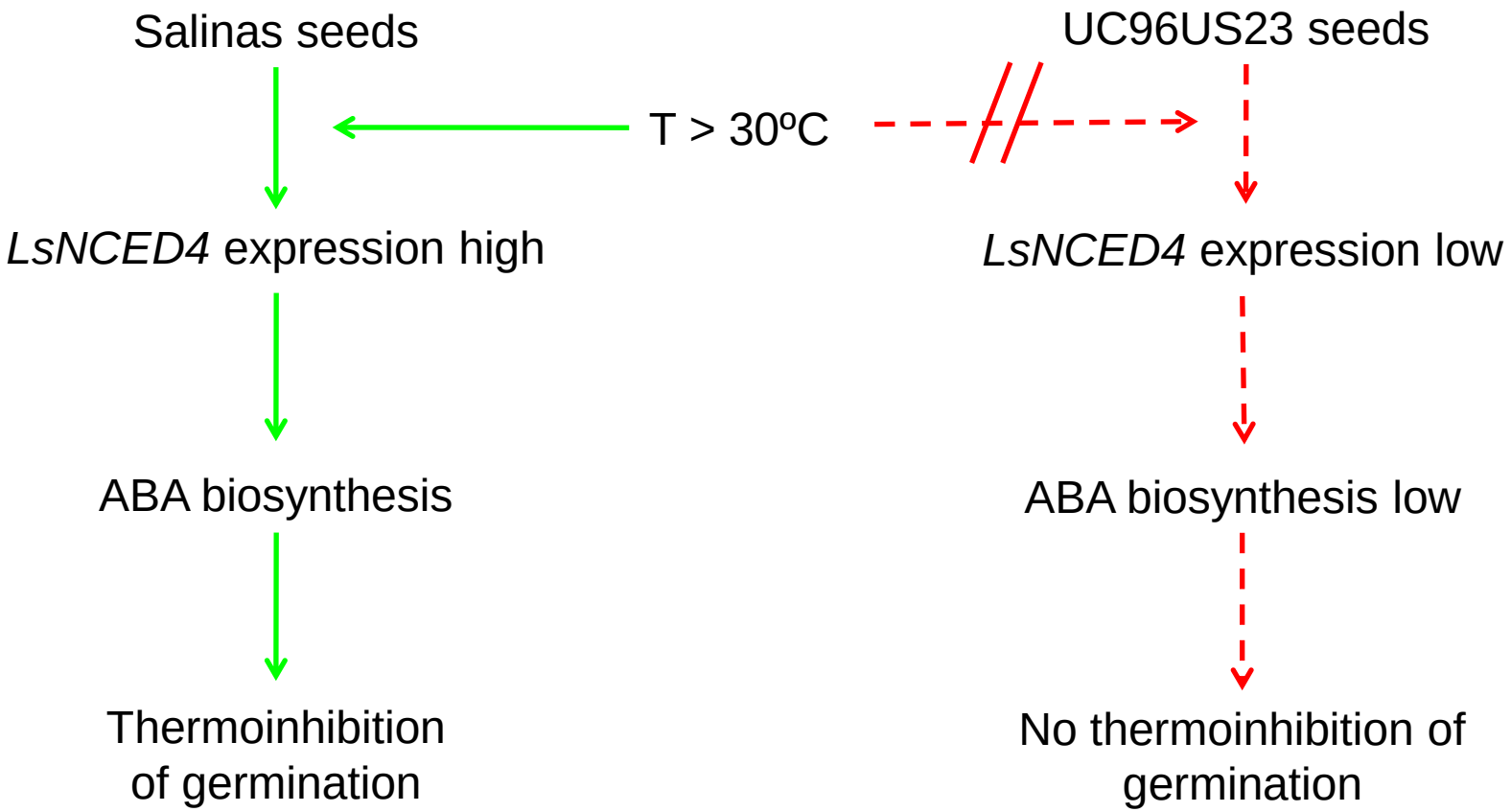
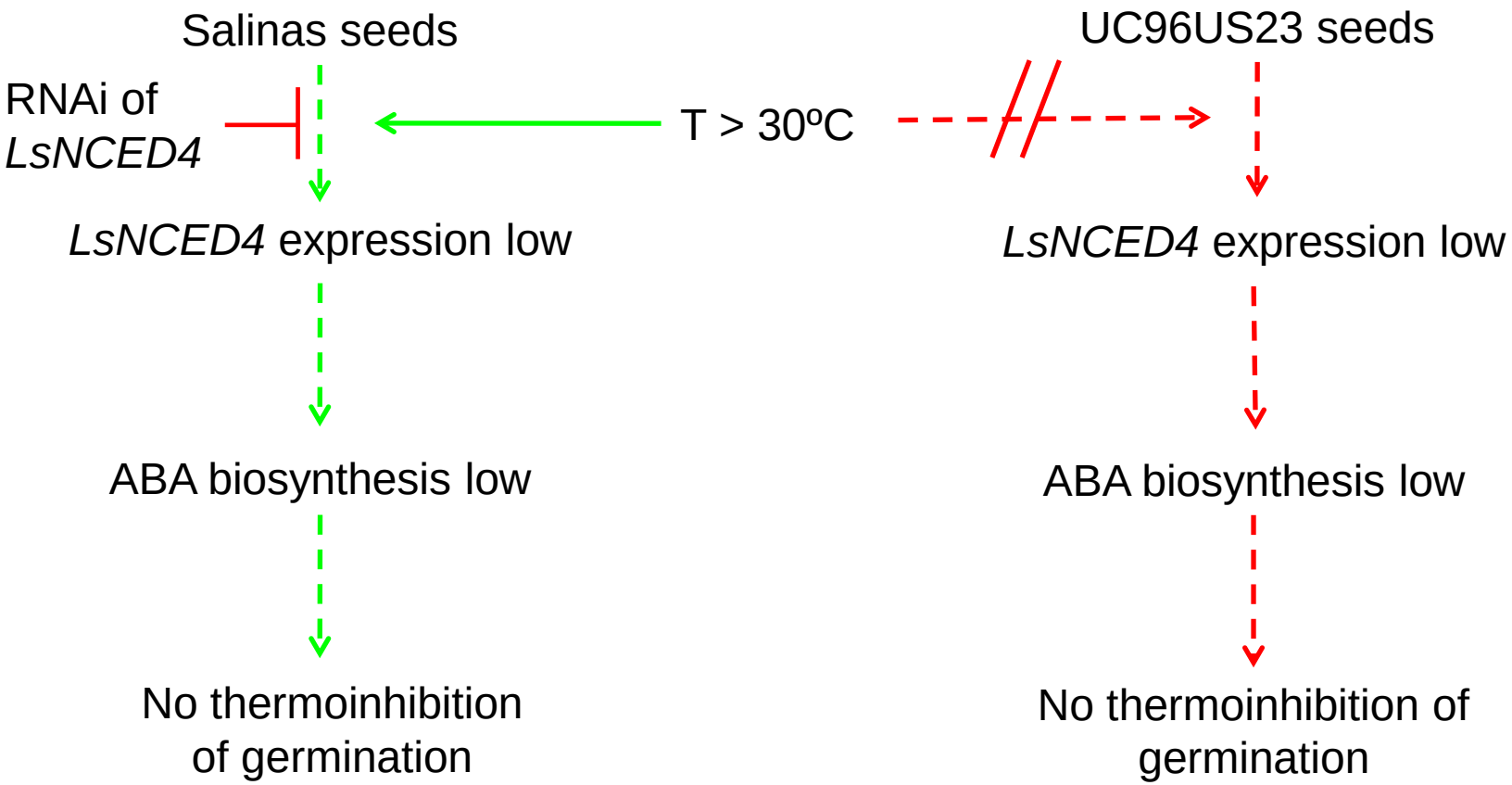


Silencing of Salinas LsNCED4



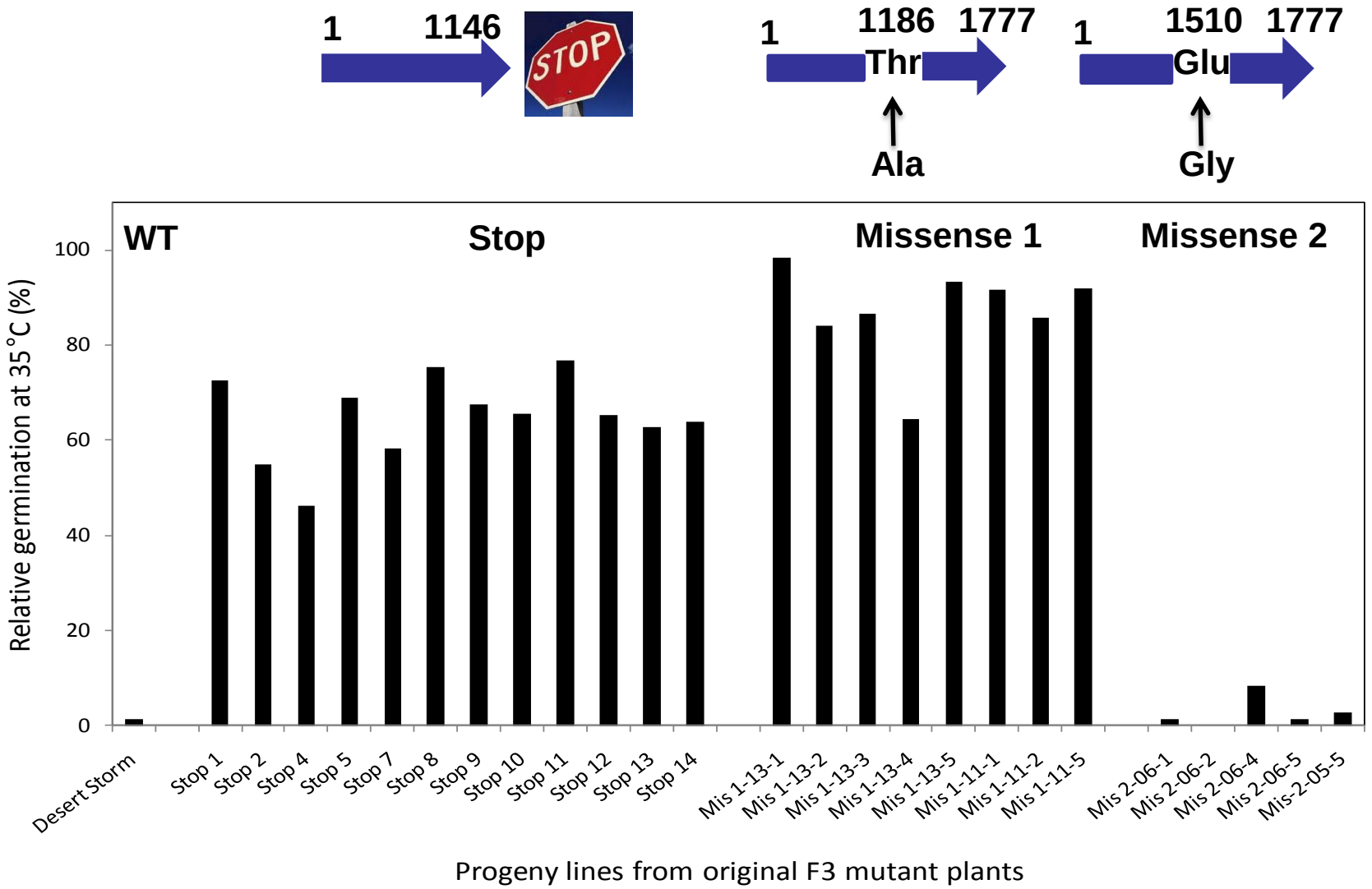
Silencing of Salinas LsNCED4



LsNCED4 Expression Is Required for Thermoinhibition

Both of these approaches worked as predicted, indicating that *LsNCED4* expression is required for the induction of thermoinhibition (unpublished data not shown).

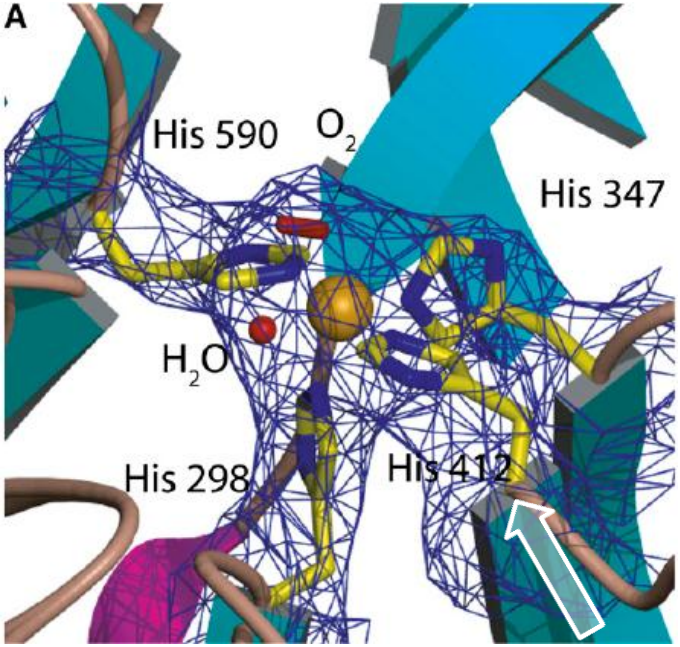
TILLING Mutants of LsNCED4



In cooperation with Arcadia Biosciences, an induced mutant population of lettuce cv. Desert Storm was TILLED to identify mutations in *LsNCED4*. Three were identified, one stop codon and two missense mutations.

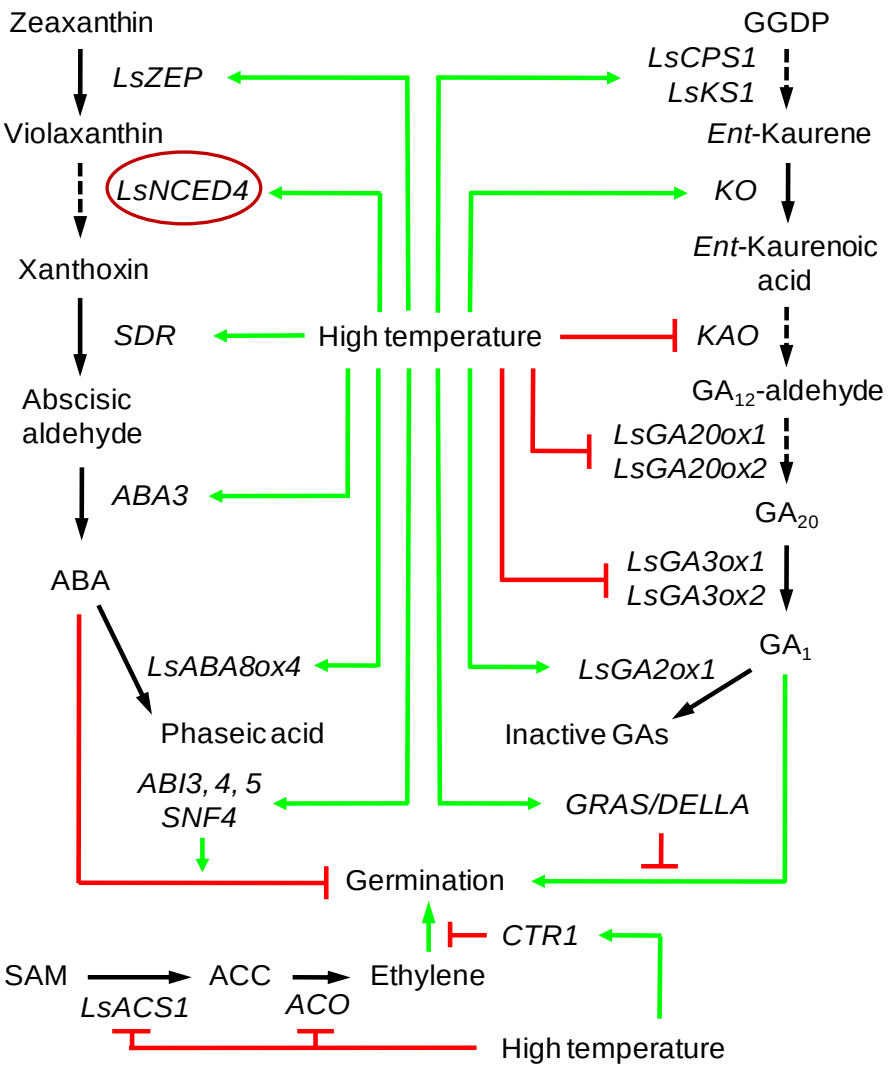
TILLING Mutants of LsNCED4

Messing et al. (2010) Plant Cell 22: 2970-2980.



By sequence homology with maize VP14, the Stop and MS1 mutations are near a histidine residue that is important for binding an iron atom that is involved in the active site of the enzyme. The MS2 mutation is not near the active site.

Hormone-Related Gene Expression



- In general, high temperature promotes expression of genes in the ABA biosynthetic and response pathways and inhibits expression of genes in the later stages of the GA biosynthetic pathway.
- Expression of ethylene biosynthetic genes is also inhibited.
- High temperature also promotes expression of germination repressors in the ABA , GA and ethylene action pathways.

Argyris et al. (2008) Plant Physiol. 148: 926-947.

Lettuce GeneChip® for Transcriptome Analysis

L. sativa	*L. serriola*	*L. saligna*	*L. perennis*	*L. virosa*
76,043 ESTs	**52,034** ESTs	**28,851** ESTs	**28,066** ESTs	**28,335** ESTs
↓	↓	↓	↓	↓
L. sativa **29,417** unigenes	*L. serriola* **22,327** unigenes	*L. saligna* **11,990** unigenes	*L. perennis* **12,661** unigenes	*L. virosa* **12,733** unigenes

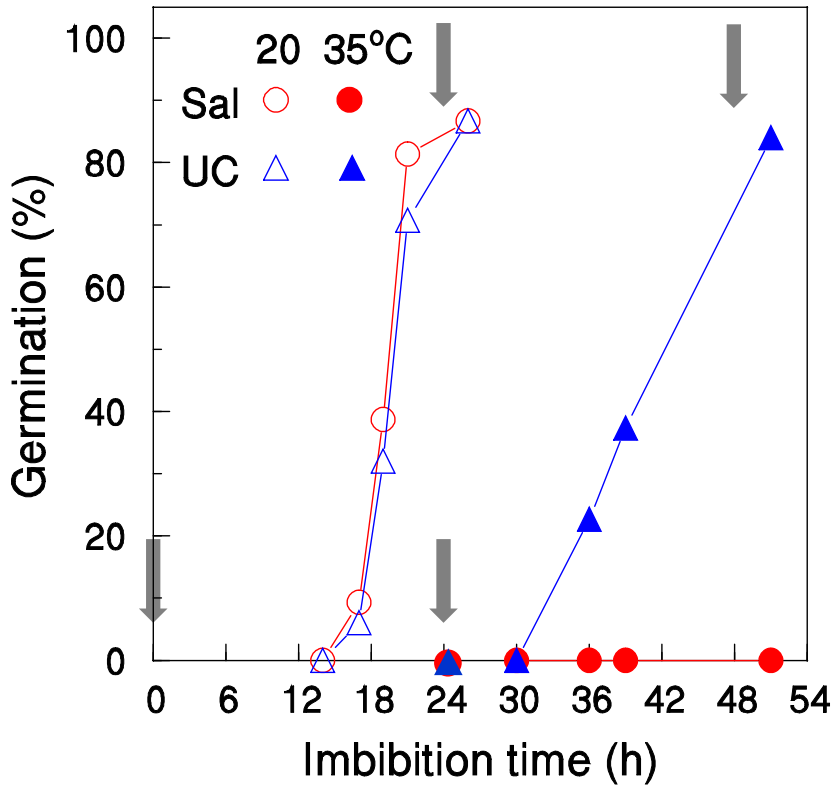
- Affymetrix platform: 6.6 million 5 µm features
- Average of 173 probes/unigene in 2 bp overlapping tiling path

35,747 unigenes
Represented on chip

- Identified and mapped over 15,000 new SNP markers in lettuce
- Used for high density mapping

Van Deynze, Michelmore, Van Leeuwen et al.

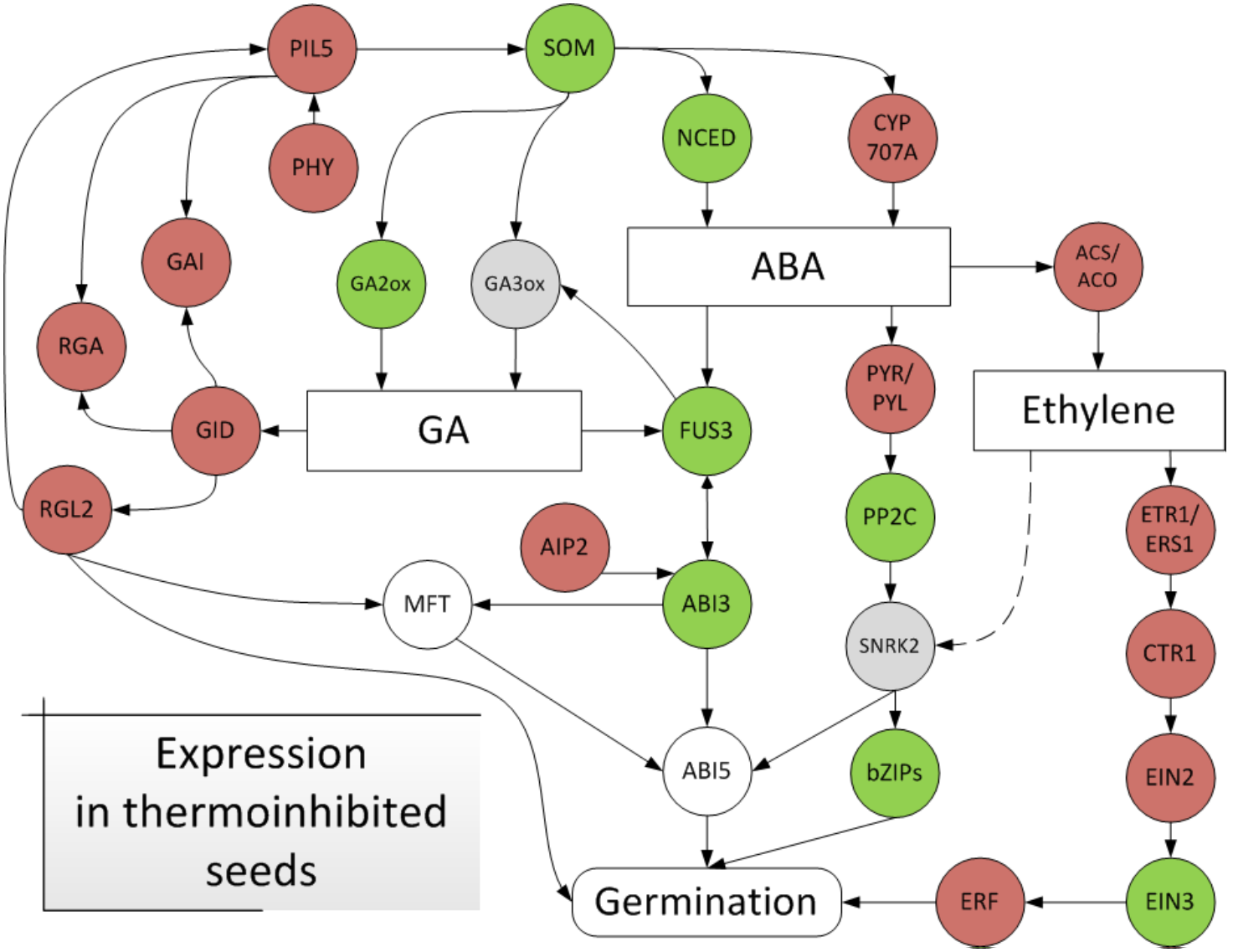
Germination Time Courses at 20 and 35ºC



- mRNA was extracted from dry Salinas and UC96US23 seeds and from seeds imbibed at 20 or 35ºC in the light for 24 or 48 h.
- Samples were hybridized to the lettuce microarray and ~13,000 genes differentially expressed above background levels were analyzed.

Argyris et al. (2008) Plant Physiol. 148: 926-947.

GA, ABA and Ethylene Pathways Respond to Temp



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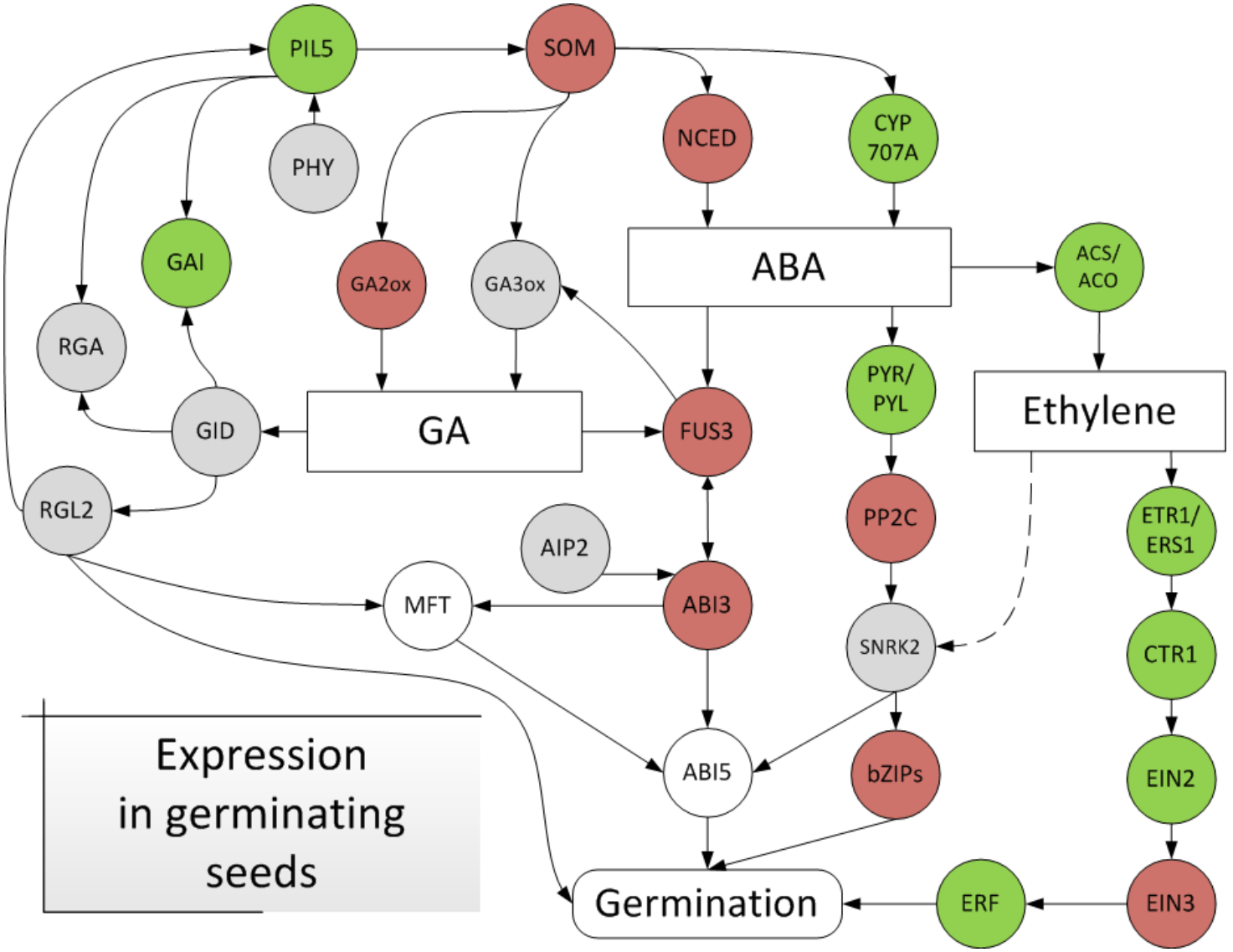
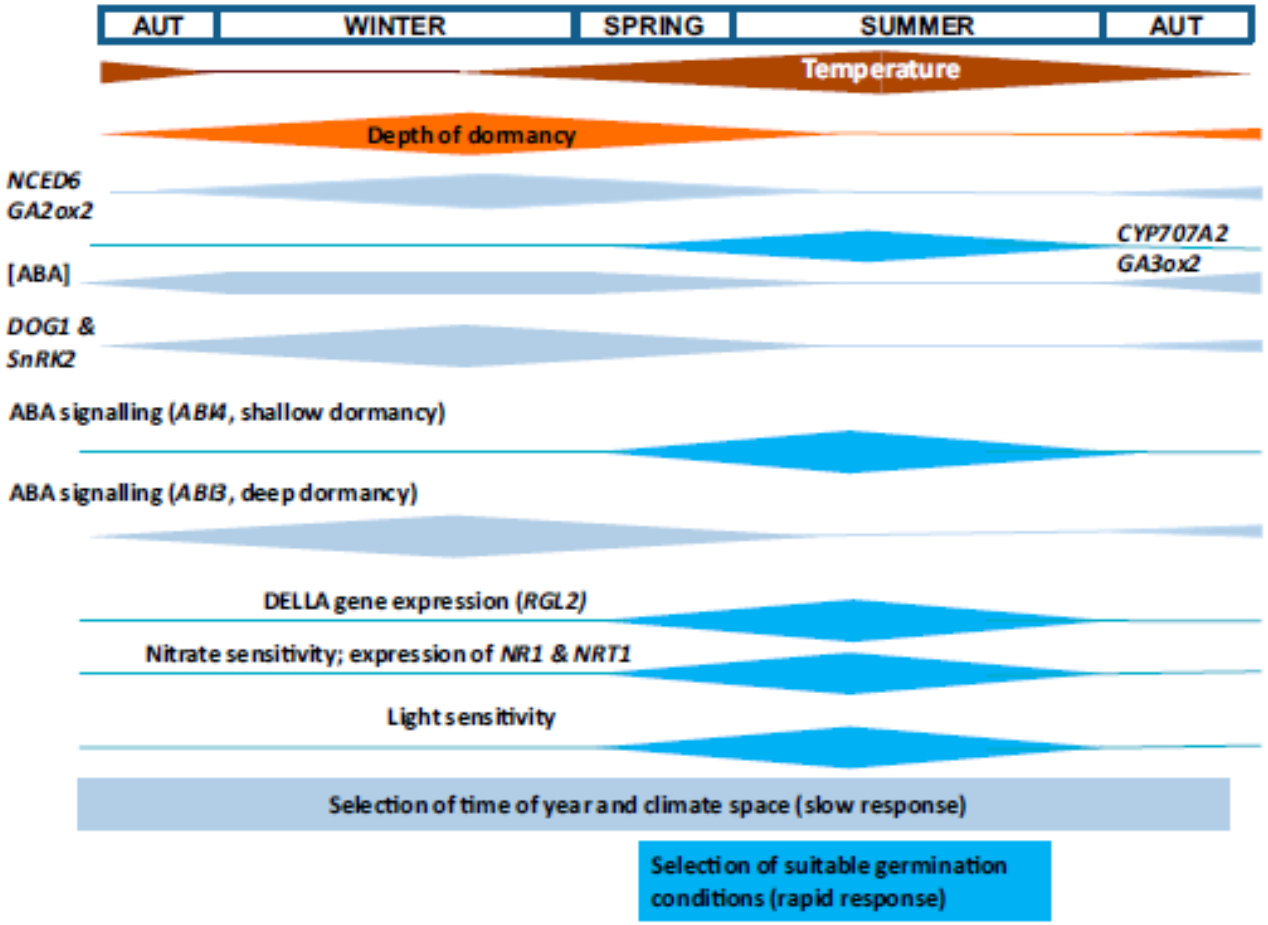


Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways

Steven Footitt, Isabel Douterelo-Soler[1], Heather Clay, and William E. Finch-Savage[2]

2011 PNAS 108: 20236-20241



Deep dormancy Shallow dormancy

Conclusions

- The *Htg6.1* QTL in lettuce is due to lack of induction of expression of *LsNCED4* in UC96US23 seeds in response to high imbibition temperature.
- Gene functional assays, RNAi silencing, mutations and gene transfers confirm that *LsNCED4* is necessary and sufficient for the induction of thermoinhibition in lettuce seeds.
- Markers, NILs and mutants are available for introgression of the alleles into lettuce cultivars to alleviate thermoinhibition in warm season plantings.
- A general picture is emerging of how environmental cues are transduced into hormonal signals regulating dormancy.

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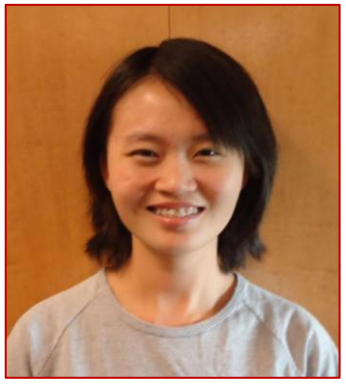

Fei-Yian Yoong

The Compositae Genome Project

http://cgpdb.ucdavis.edu/

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- Class IV of Davis PBA begins in Fall 2012
- Asian PBA to begin in Fall 2012 in Chang Mai, Thailand

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