Breeding for Seed Quality
(and some Advances in Seed Technology)

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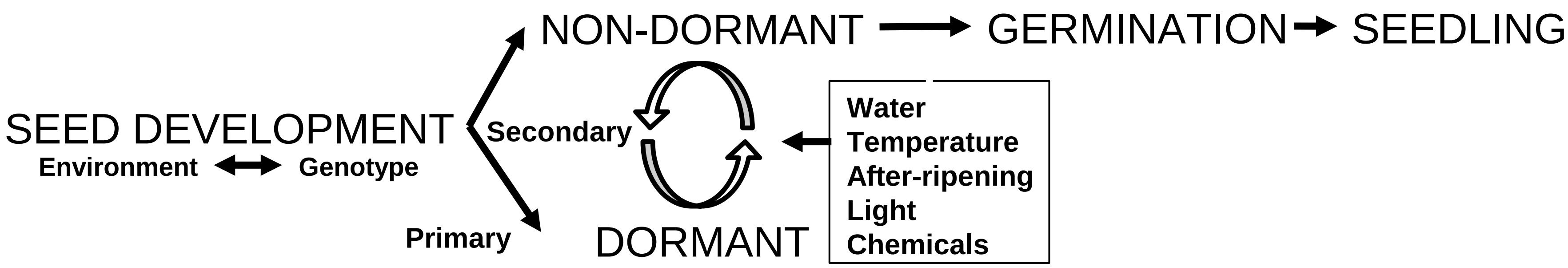
Seed Dormancy

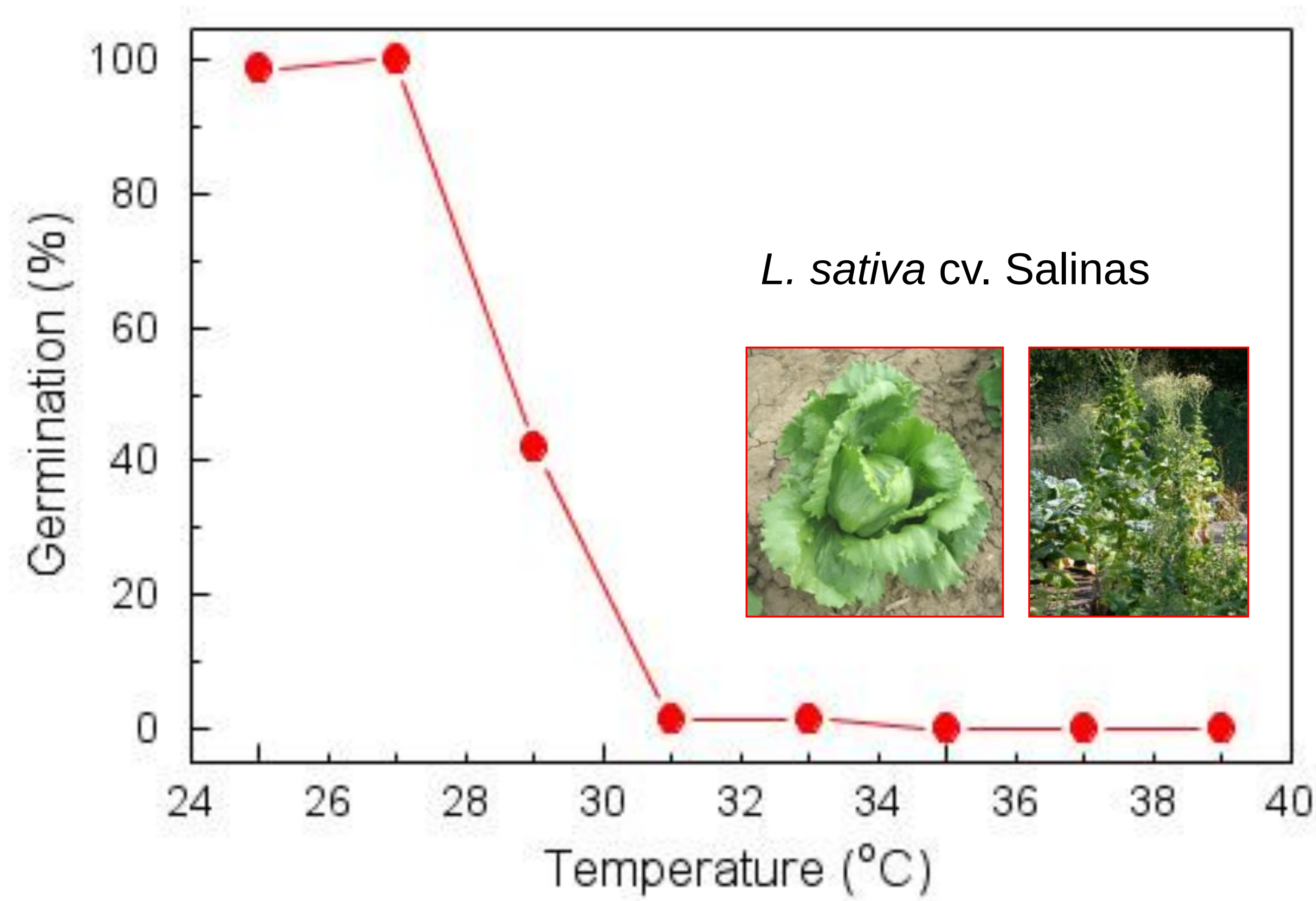
- Seed dormancy is the failure of a viable seed to germinate when provided with conditions normally conducive to germination (water, temperature, etc.).

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



Bewley et al. (2013) *Seeds: Physiology of Development, Germination and Dormancy*. Springer.

Thermoinhibition at High Temperatures



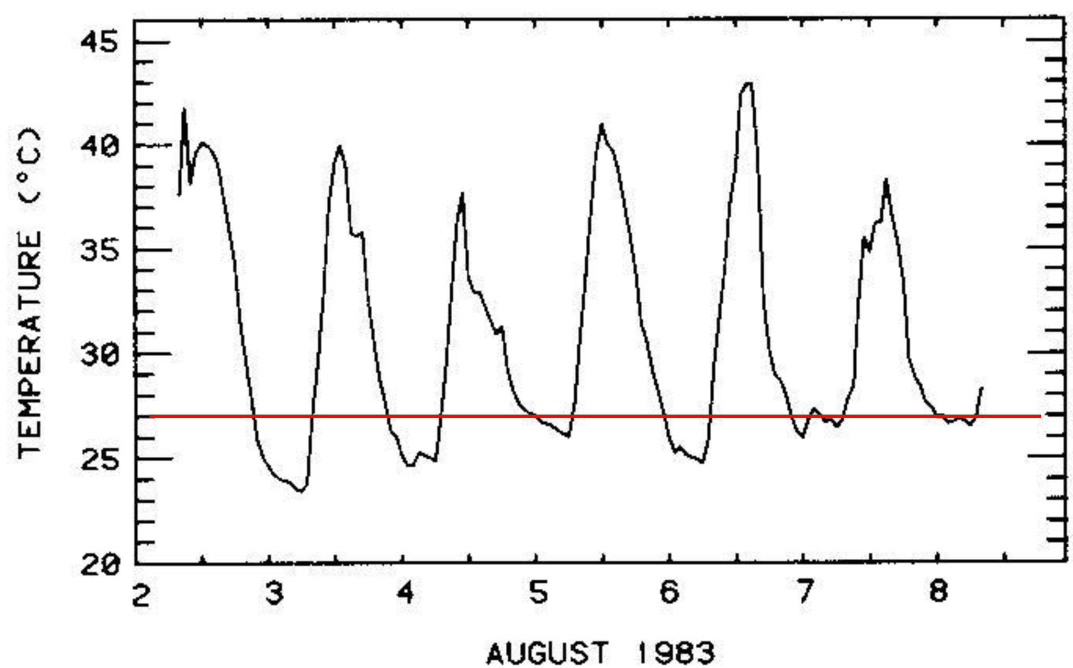
Argyris et al. (2005) Theor. Appl. Genet. 111: 1365-1376.

Soil Temperatures Often Exceed 30ºC in CA and AZ

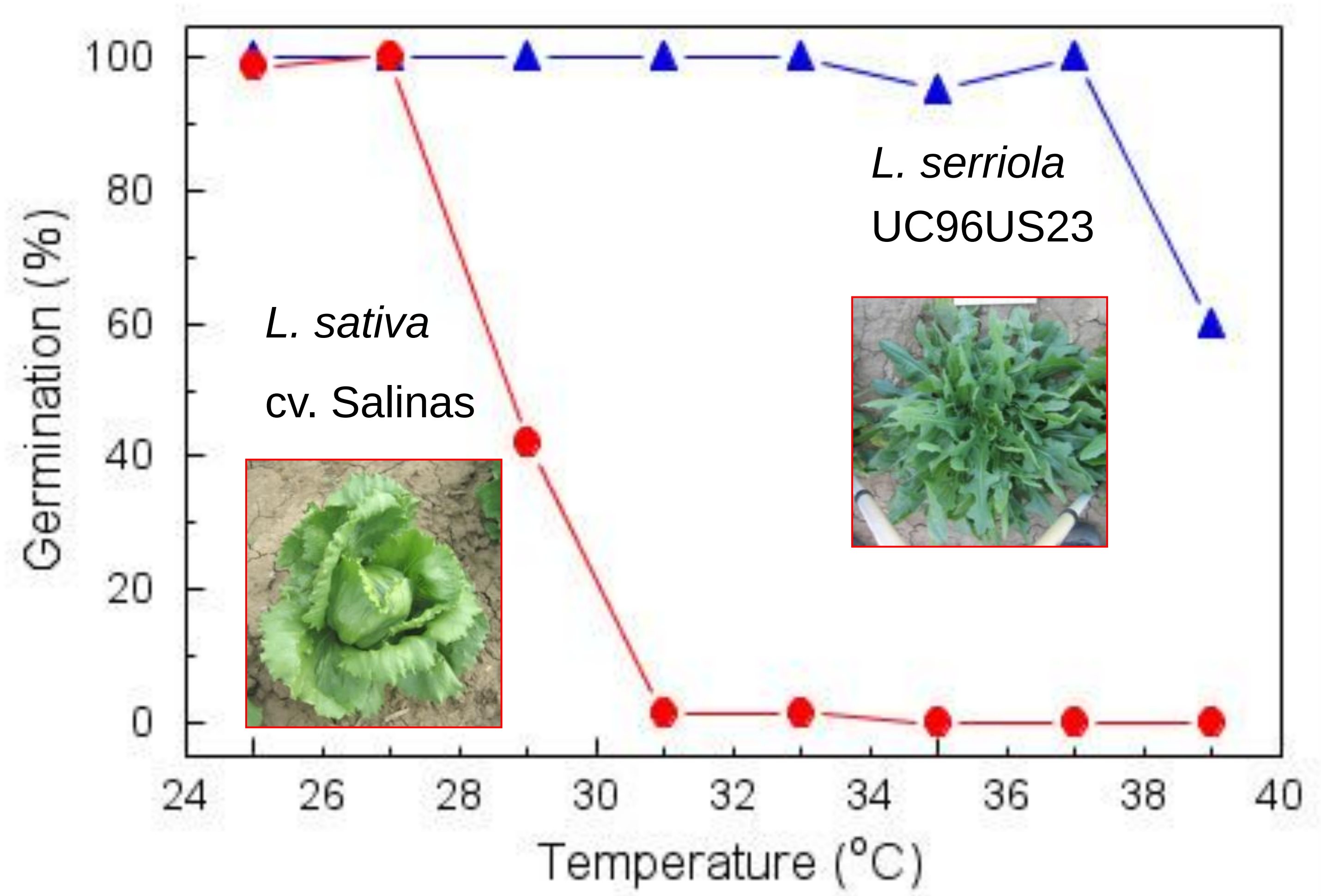
Over 95% of US lettuce production occurs in California and Arizona.

In November through March, production is focused in the desert regions of Imperial Valley, CA and Yuma, AZ.

Soil temperatures in August and September when these crops are planted generally exceed the upper temperature limit for lettuce seed germination.



Natural Variation for Thermoinhibition



Argyris et al. (2005) Theor. Appl. Genet. 111: 1365-1376.

Recombinant Inbred Line Population

L. sativa cv. Salinas X *L. serriola* UC96US23

↓

F1

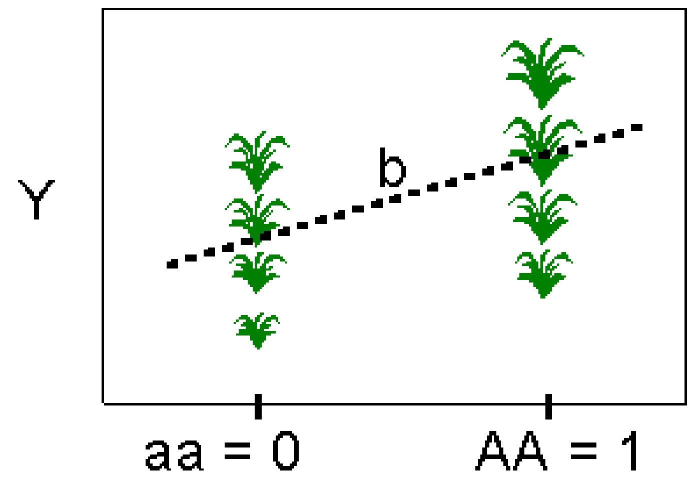
$F2_1$ $F2_2$ $F2_3$ . . . . $F2_{98}$ $F2_{99}$ $F2_{100}$

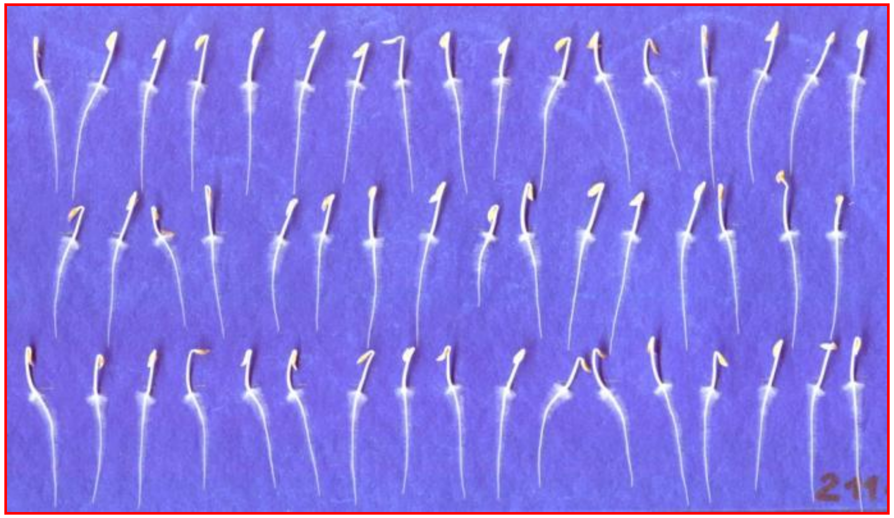
selfed families to F8 generation

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

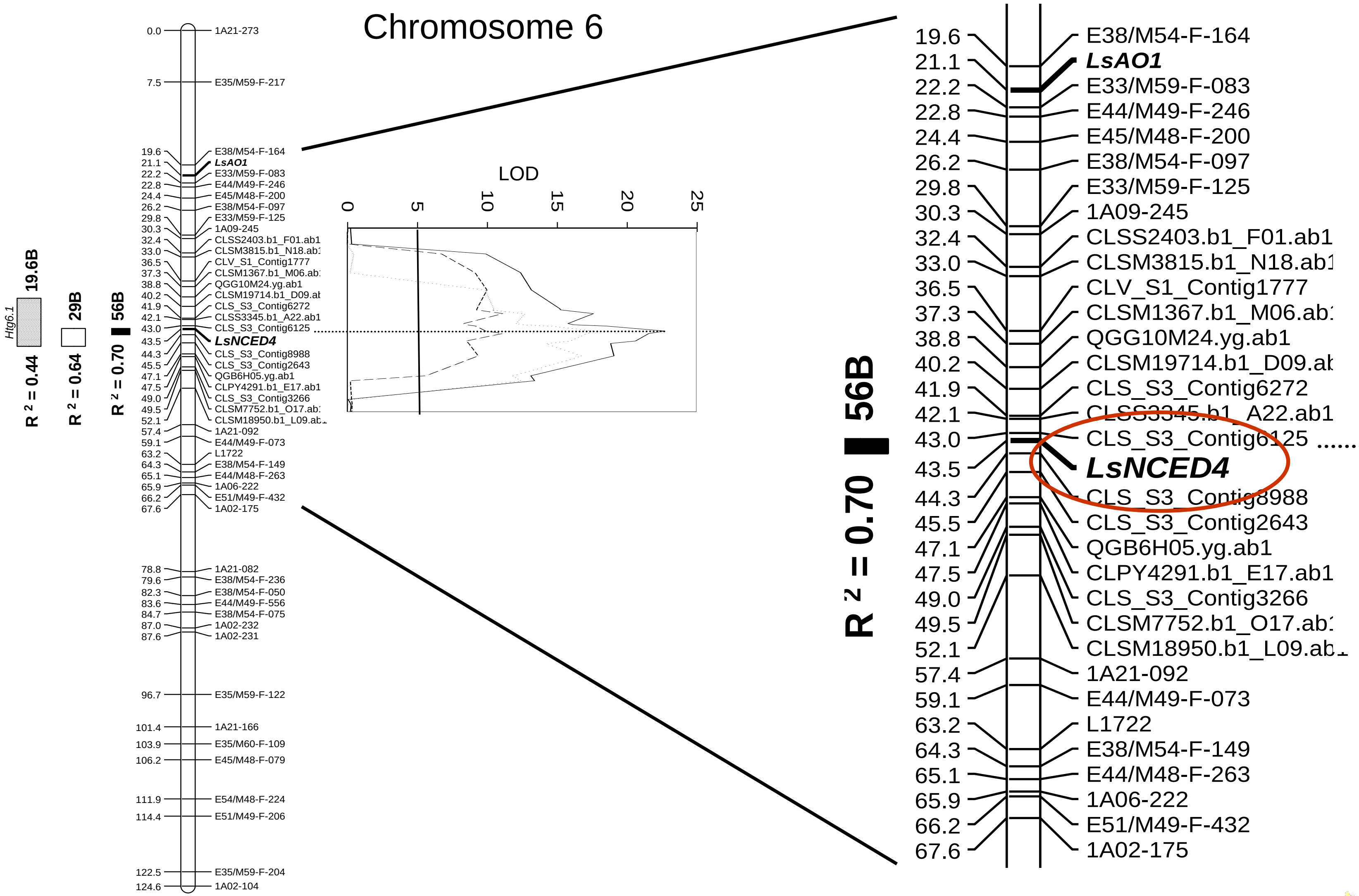
QTL Analysis – Phenotypic Data

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



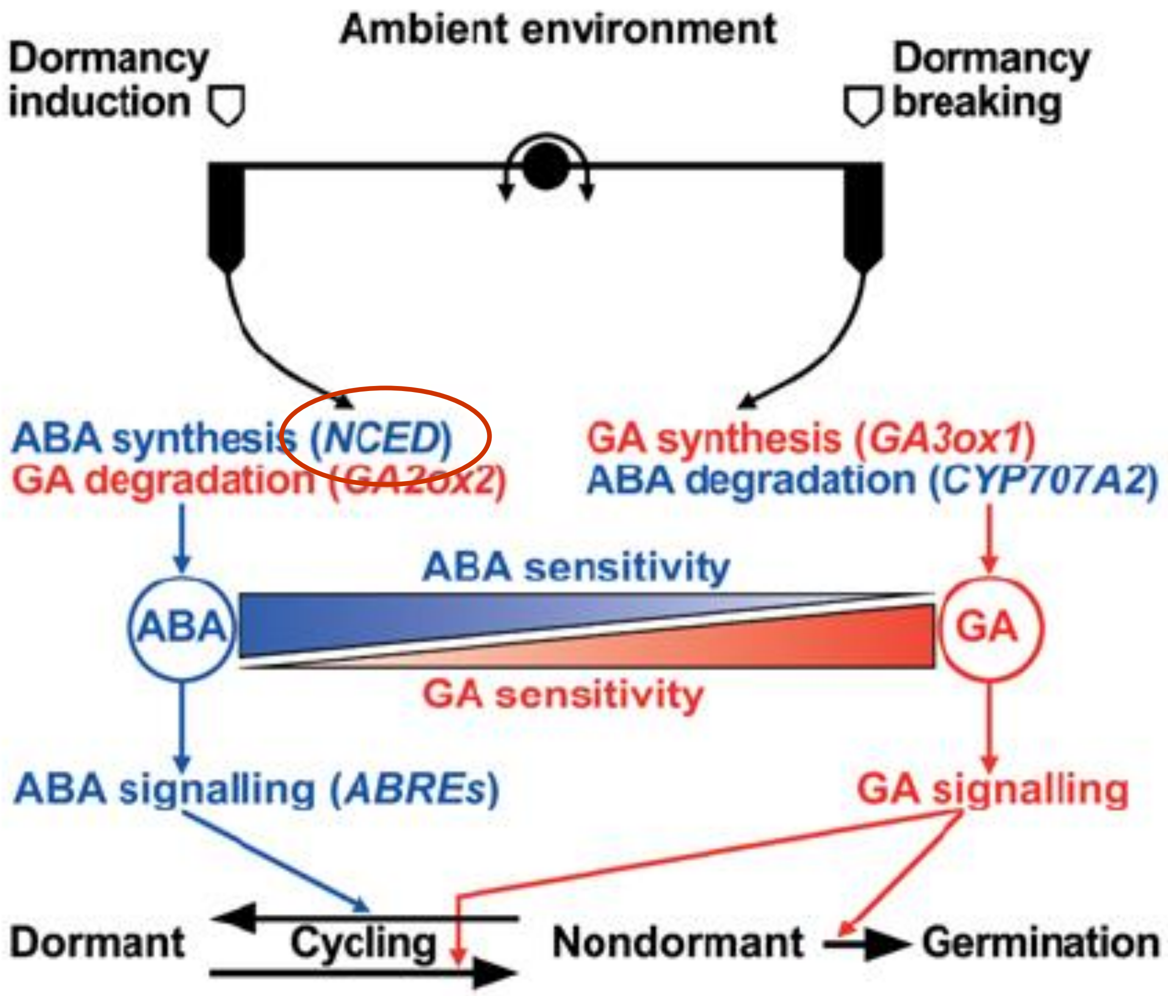


High Resolution Mapping of Htg6.1 in Lettuce



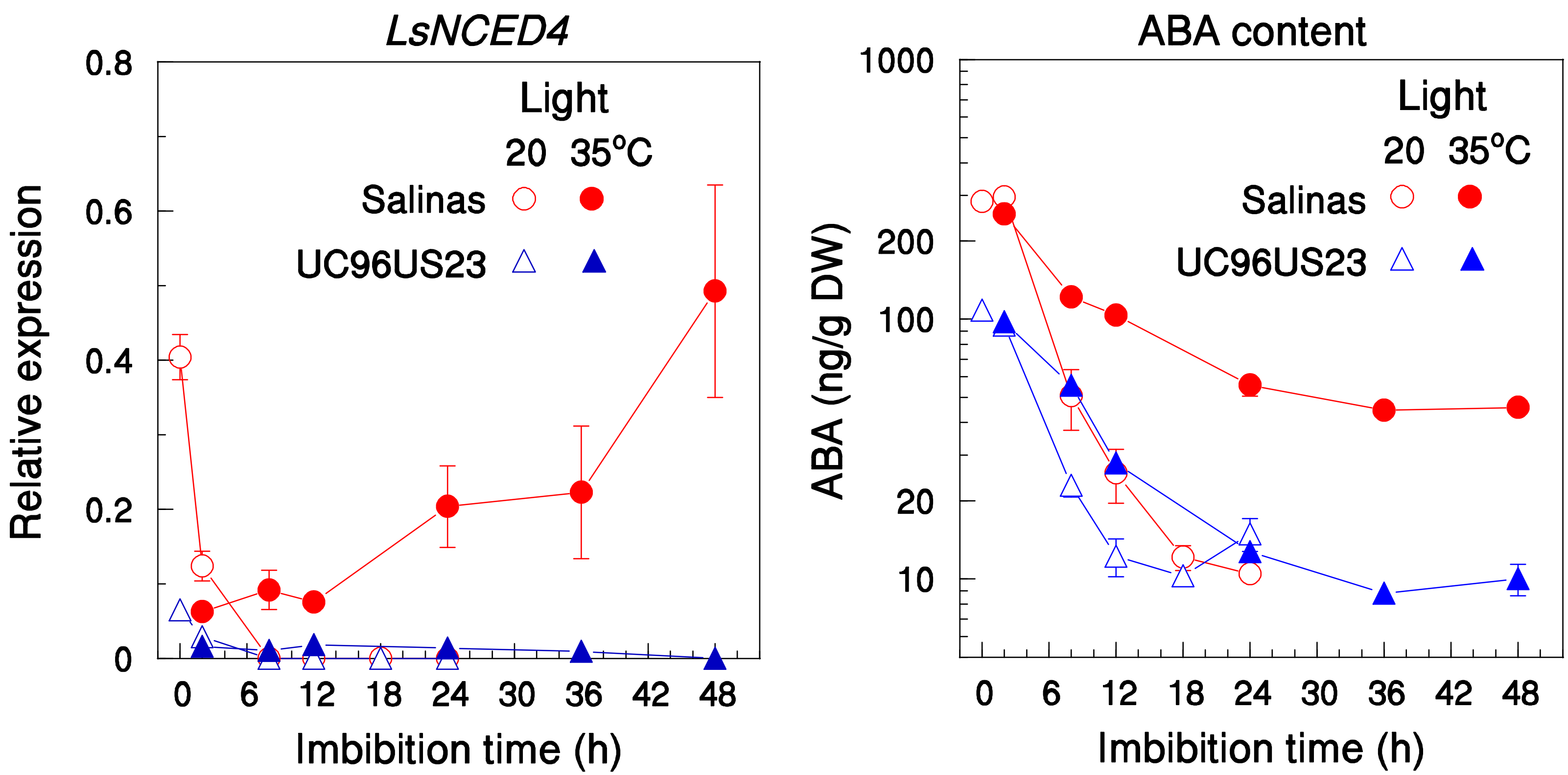
Argyris et al. (2011) Theor. Appl. Genet. 122: 95-108.

Hormone Balance Is Involved in Seed Dormancy



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

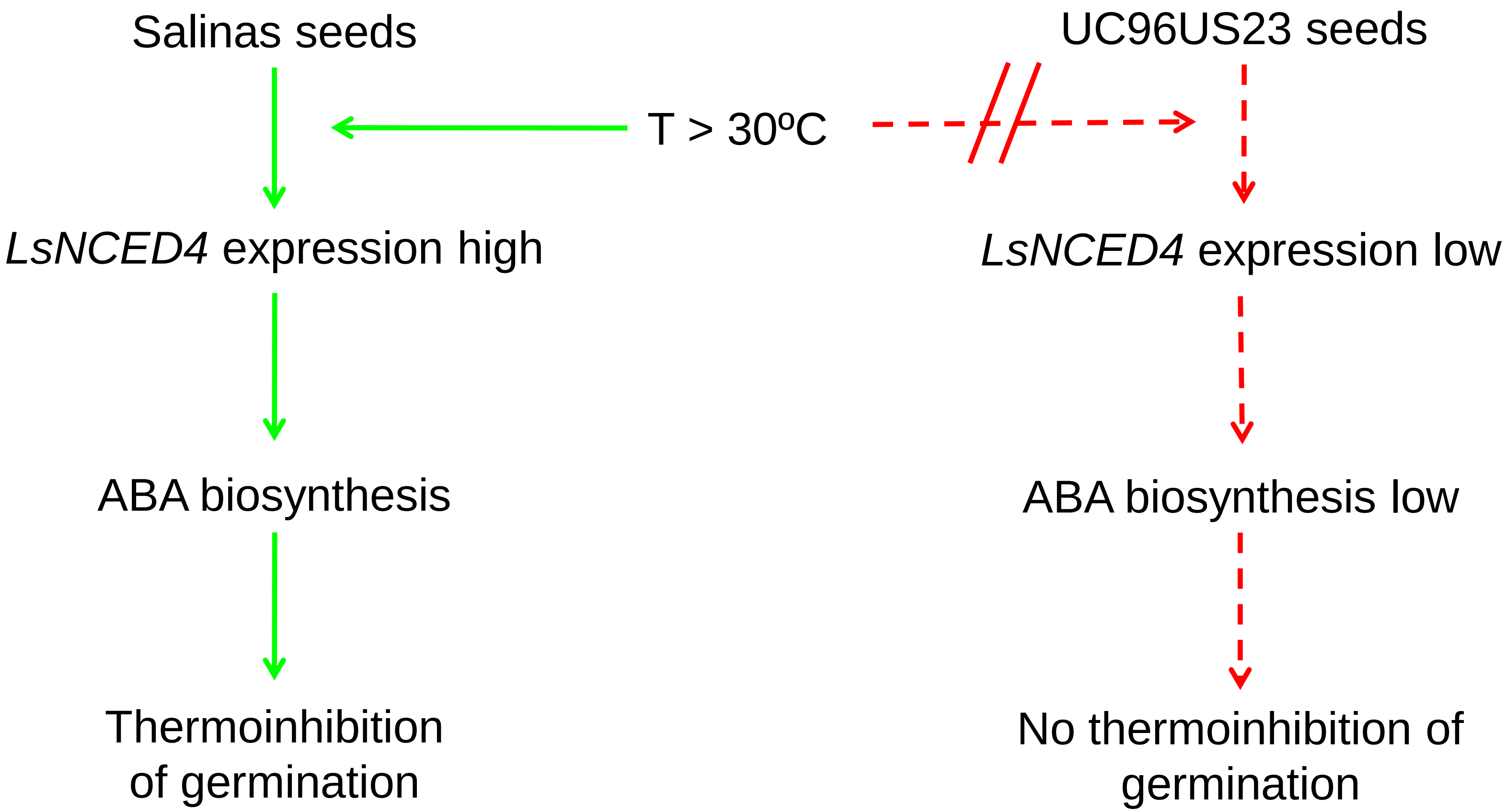
LsNCED4 Expression and ABA Content



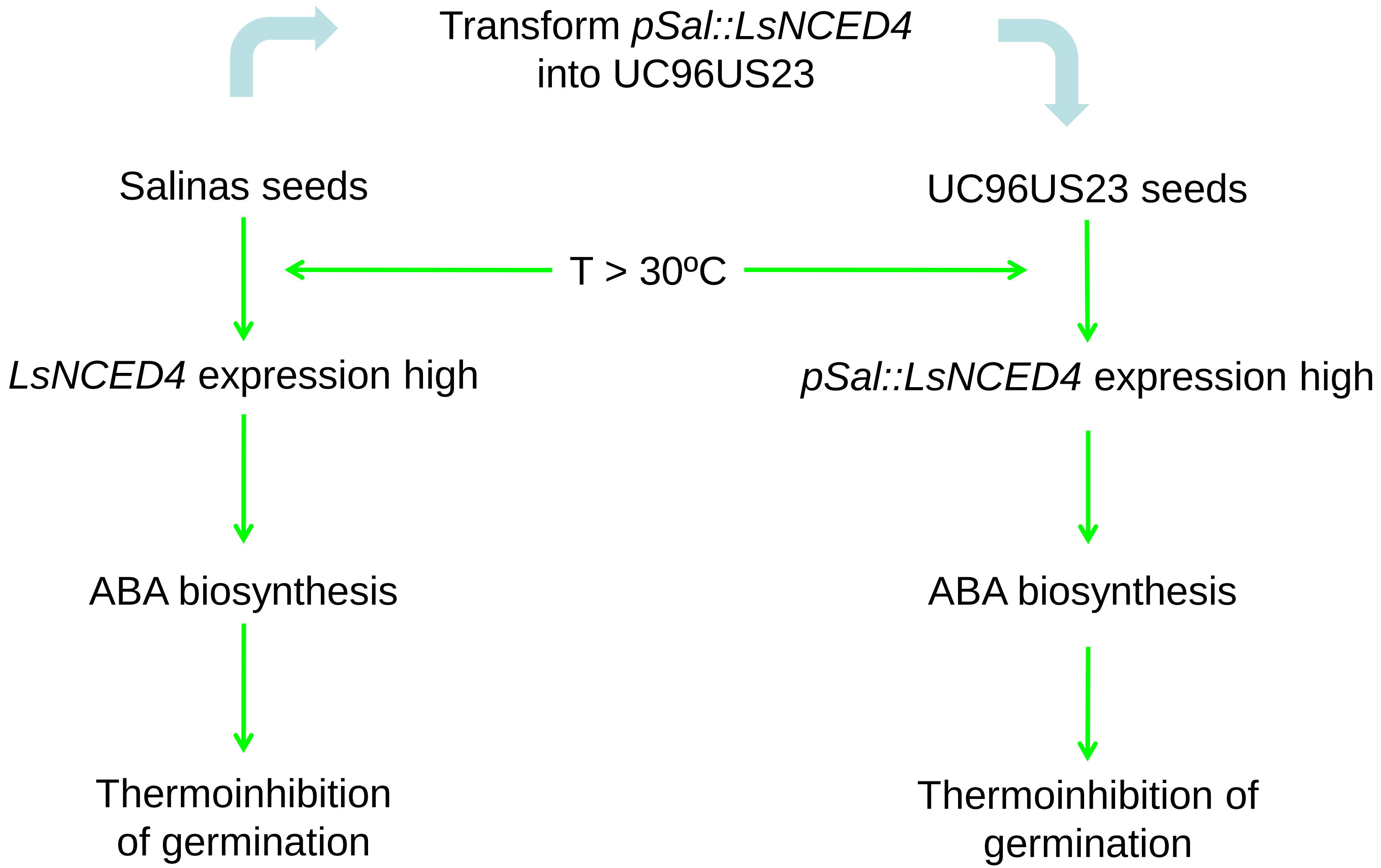
LsNCED4 expression and ABA content remain elevated only in seeds of the Salinas variety imbibed at 35°C, which exhibit thermoinhibition.

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

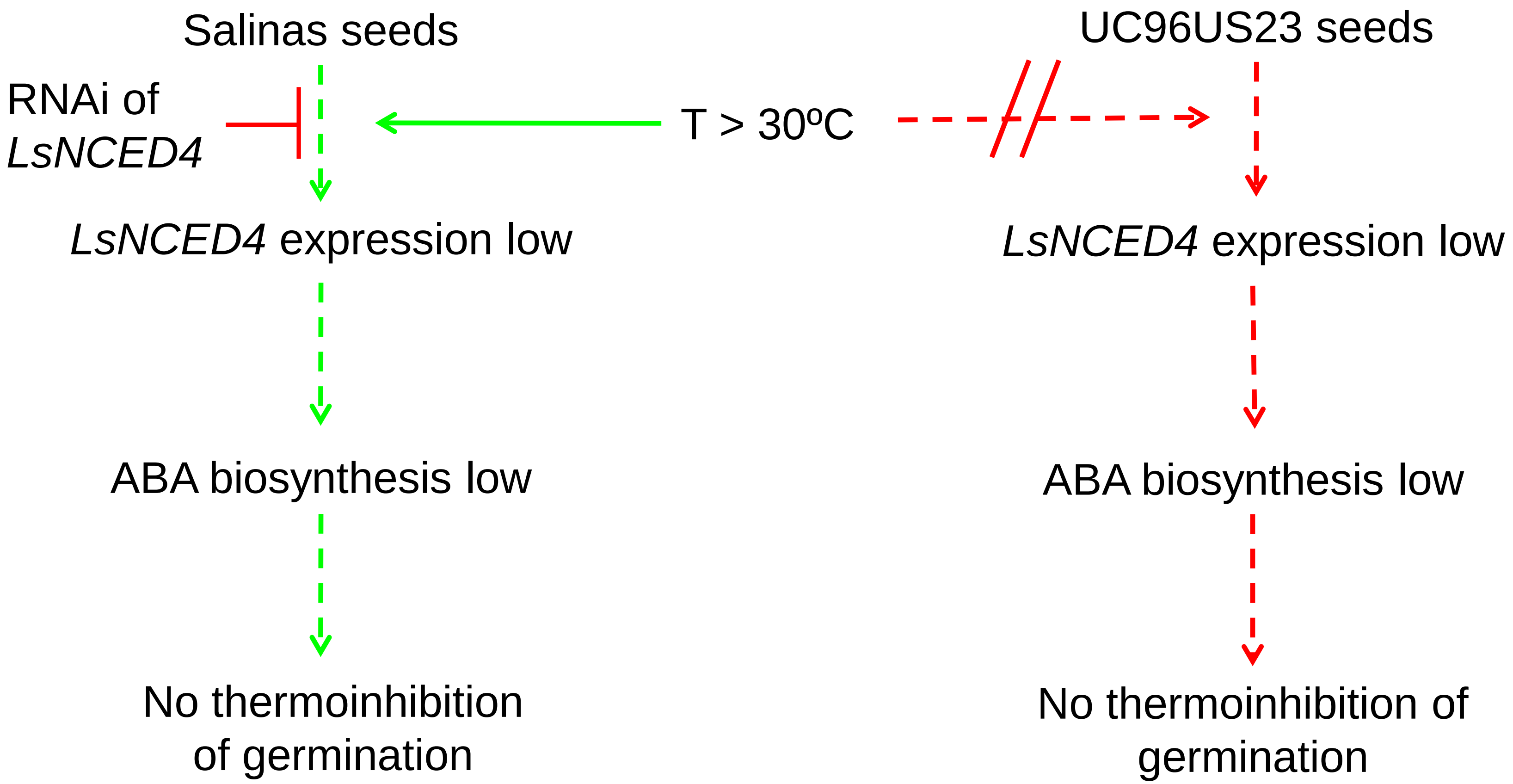
Functional Complementation of UC96US23



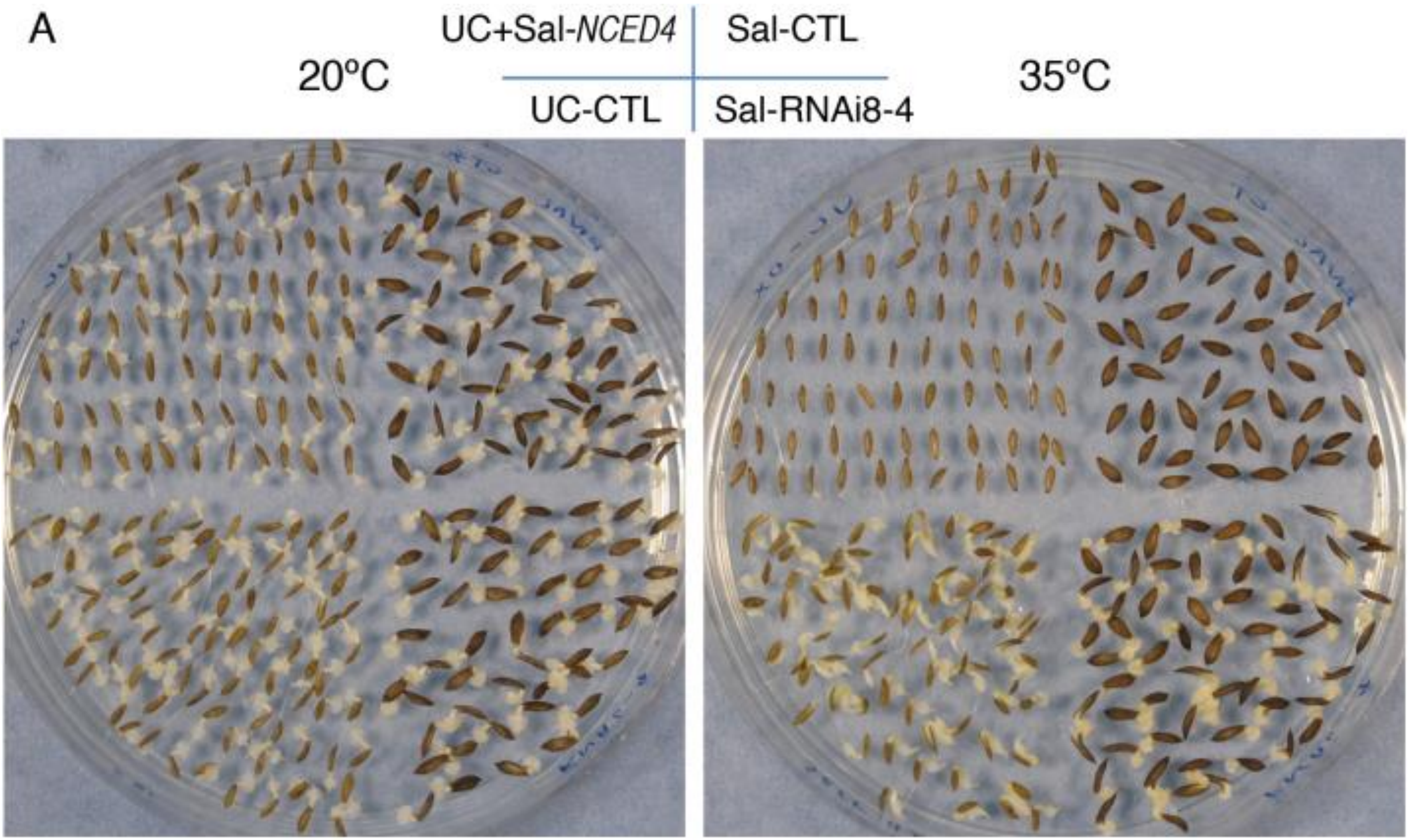
Functional Complementation of UC96US23



Silencing of Salinas LsNCED4



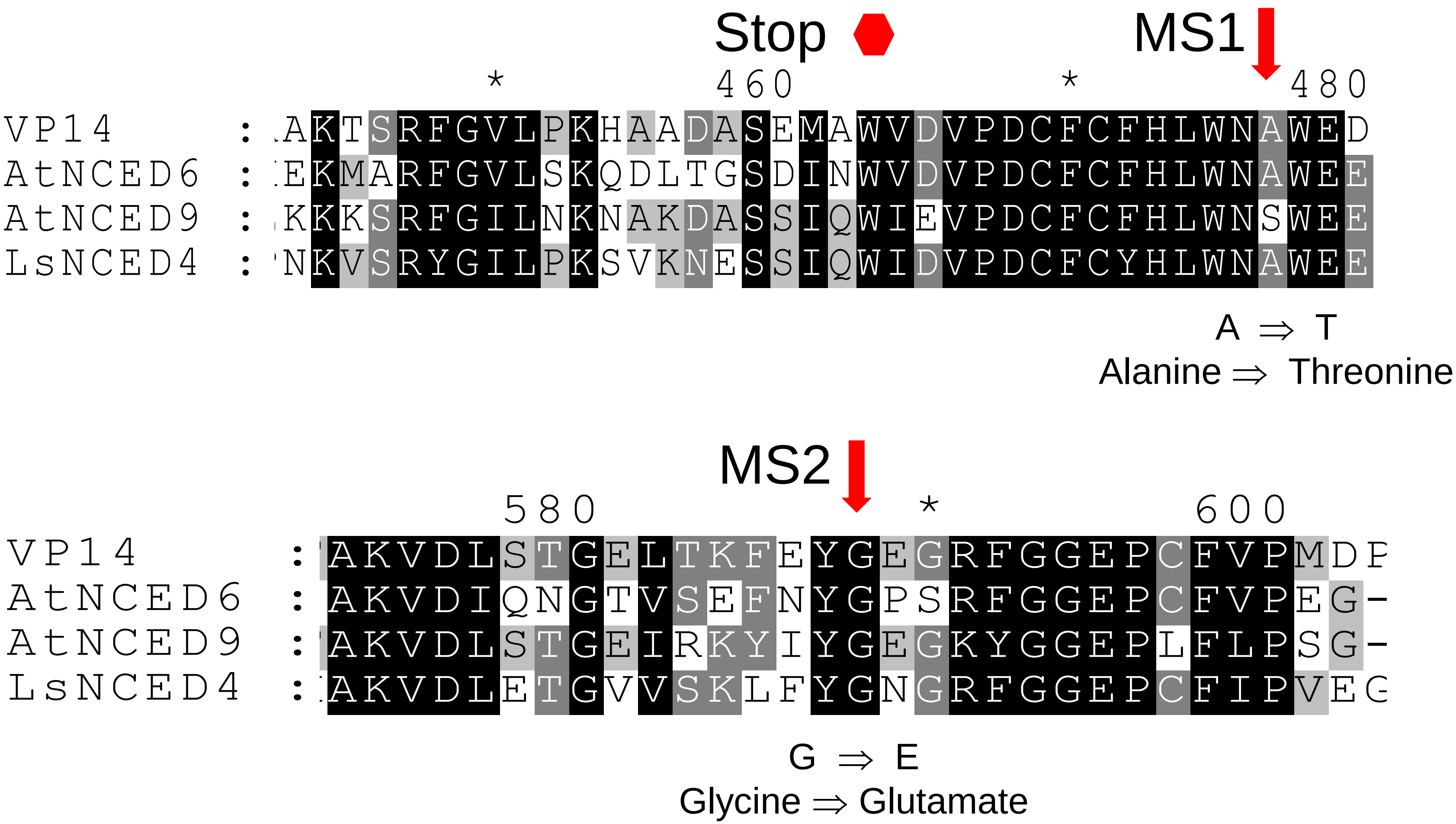
LsNCED4 Expression Is Required for Thermoinhibition



Transferring Sal-*NCED4* under its own promoter into UC makes it susceptible to thermoinhibition, while silencing *NCED4* in Sal makes it thermotolerant.

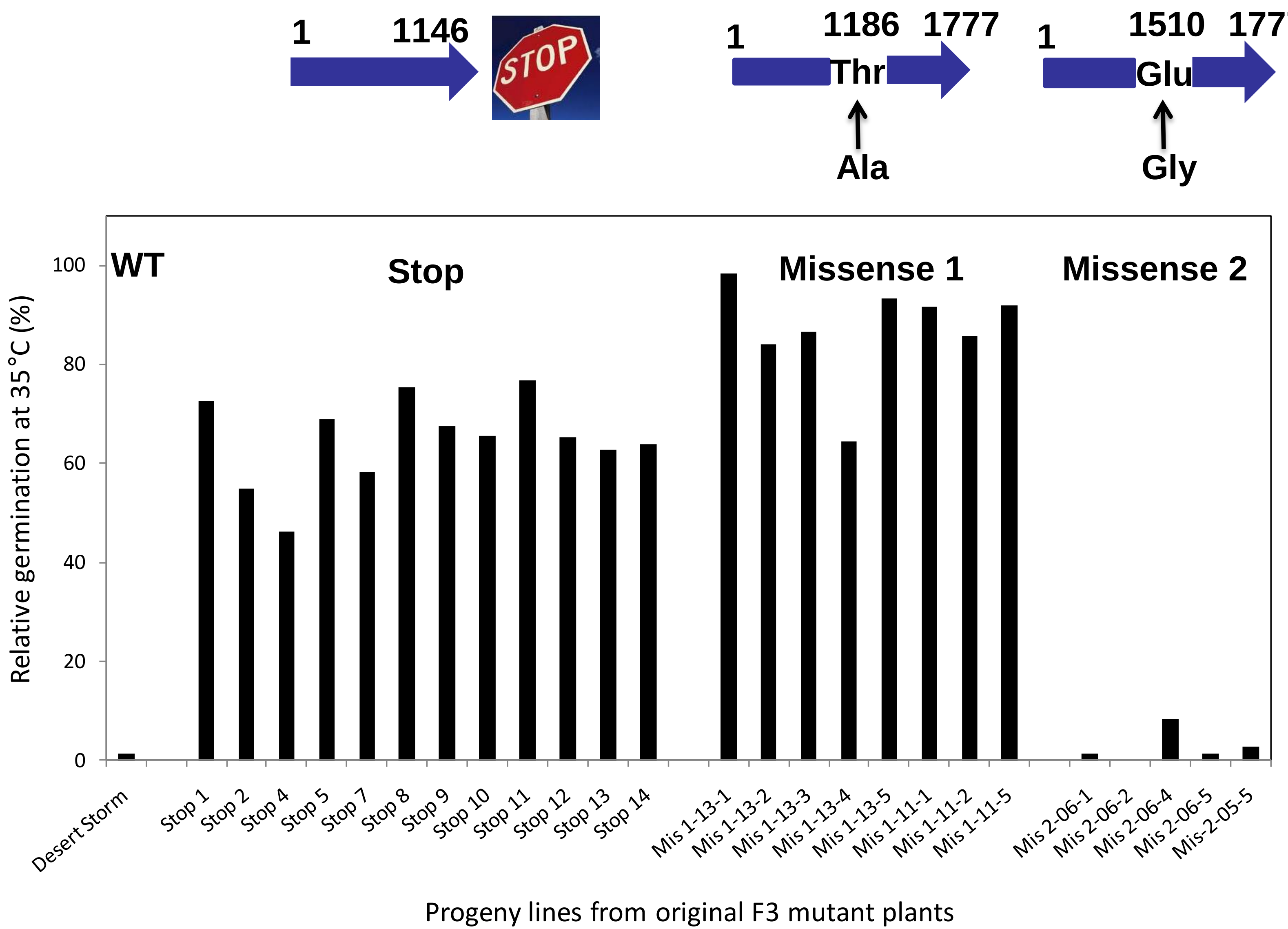
Huo et al. (2013) Plant Cell 25: 884-900.

TILLING Mutants of LsNCED4



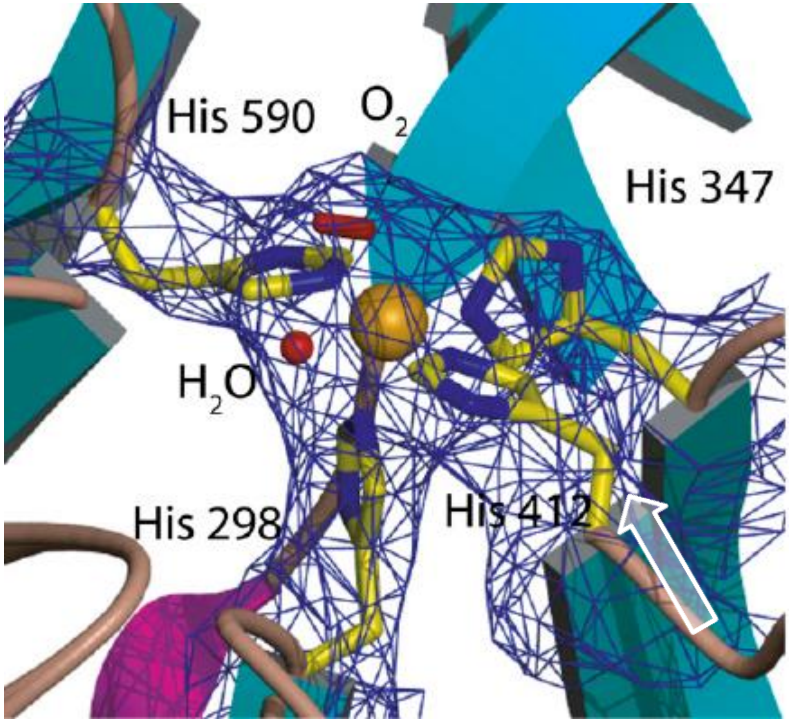
In cooperation with Arcadia Biosciences, an induced mutant population of lettuce cv. Desert Storm was TILLED to identify mutations in *LsNCED4*. Three were identified, one stop codon and two missense mutations.

TILLING Mutants of LsNCED4



Huo et al. (2013) Plant Cell 25: 884-900.

TILLING Mutants of LsNCED4



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.

Messing et al. (2010) Plant Cell 22: 2970-2980.
Huo et al. (2013) Plant Cell 25: 884-900.

USDA

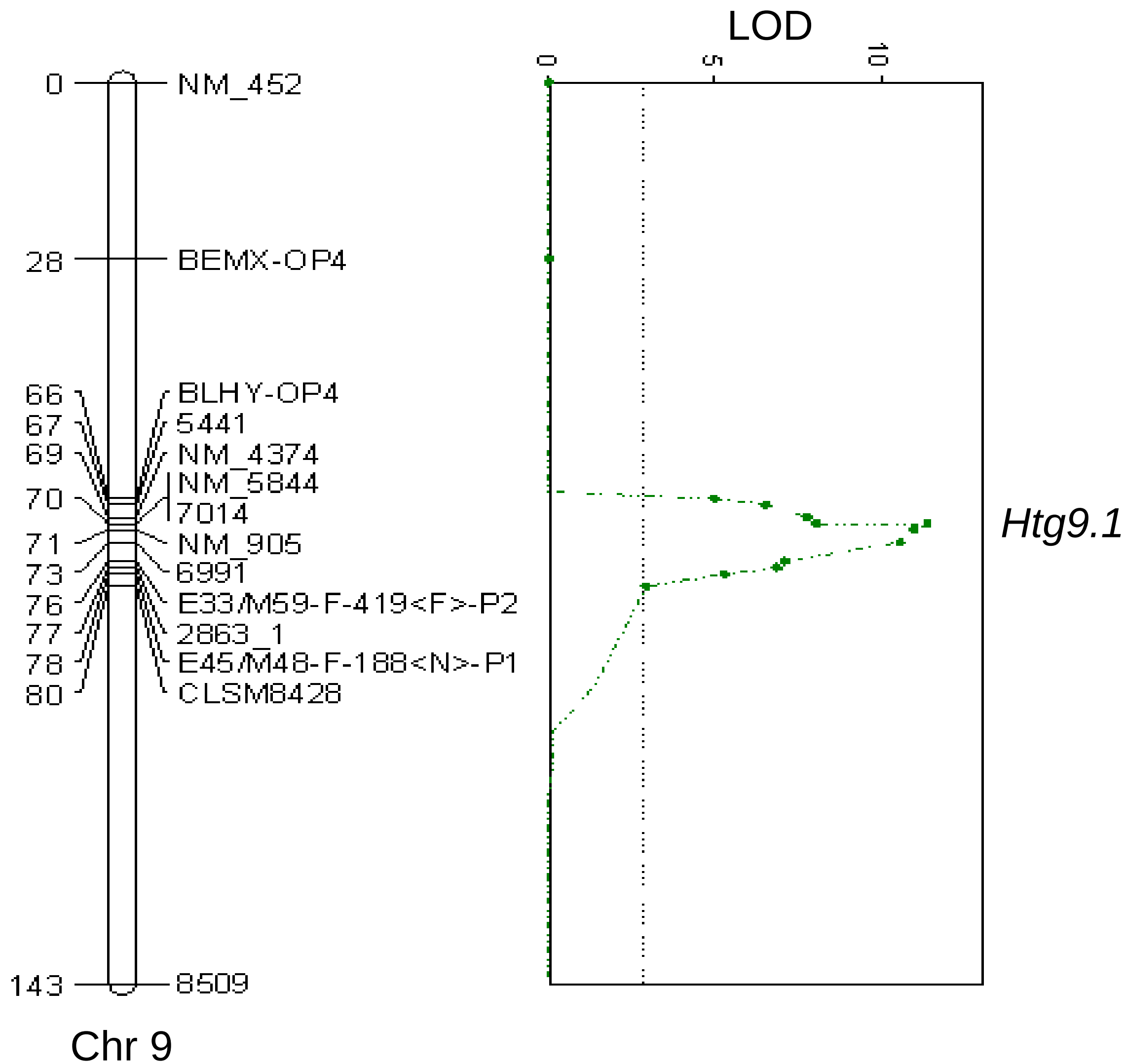
Germplasm for High Temperature Tolerance

- Parent line source UC96US23
- Native UC allele of *NCED4* introgressed into cultivated Salinas background
- Mutants in *NCED4*
- RNAi-*NCED4* silenced line

See poster session also

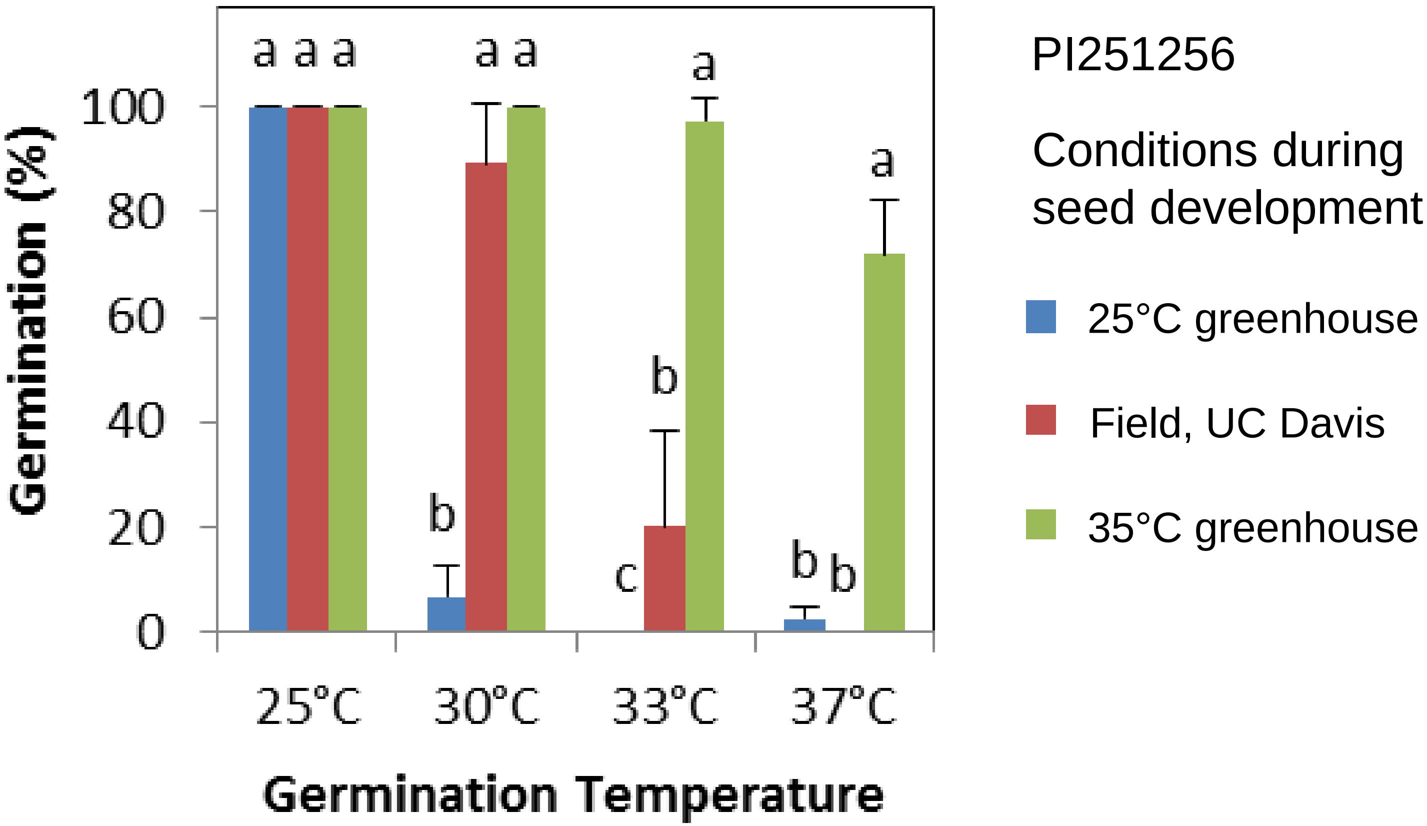
A Second HTG QTL

We are mapping another QTL involved in regulating high temperature germination in a lettuce population derived from a cross between PI251246 (primitive *L. sativa*) x cv. Salinas (population developed by USDA at Salinas). A major QTL has been identified on Chromosome 9, and fine mapping is in progress.



Yoong et al. unpublished.

Environmental Sensitivity of Seed Dormancy



The temperature during seed development can influence the subsequent thermosensitivity of the seeds during germination. We are pursuing this GxE interaction to identify loci and genes that control the seed dormancy response to maternal environment during seed development.

Mohan Niroula, unpublished results; see poster session

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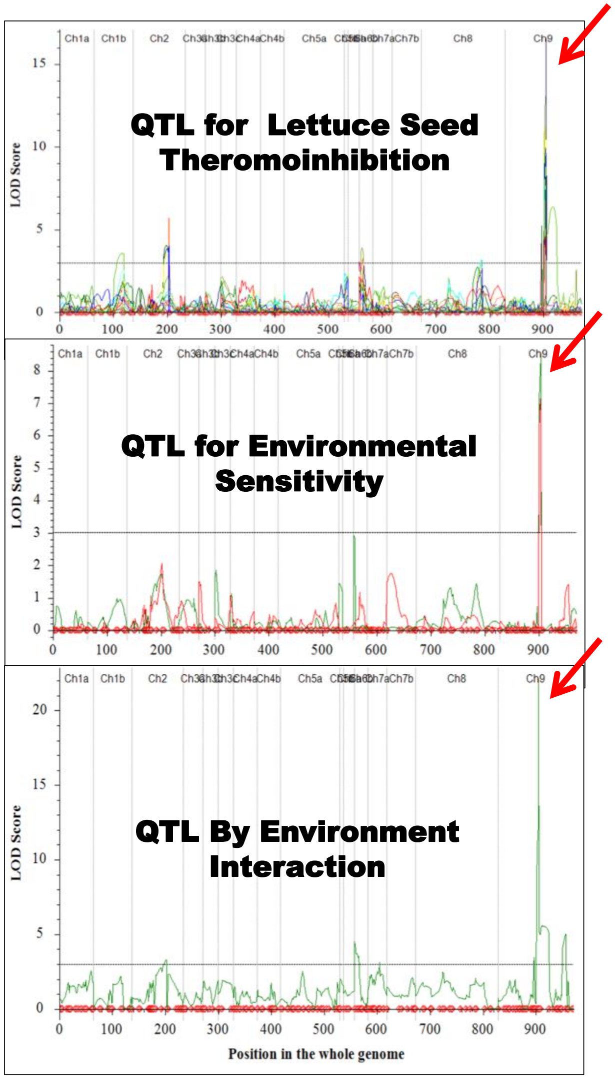
Environmental Sensitivity of Seed Dormancy



After growing the RIL population 4 environments and conducting seed germination tests at warm temperatures, we mapped both the germination capacity and the variation in the germination capacity for a given RIL across environments.

Using either the standard deviation across environments or a G x E analysis, a major QTL was found that collocated with the major trait for HTG in each environment.

Once a candidate gene is identified, its modification may improve both HTG and variability across environments.

Mohan Niroula, unpublished results; see poster session

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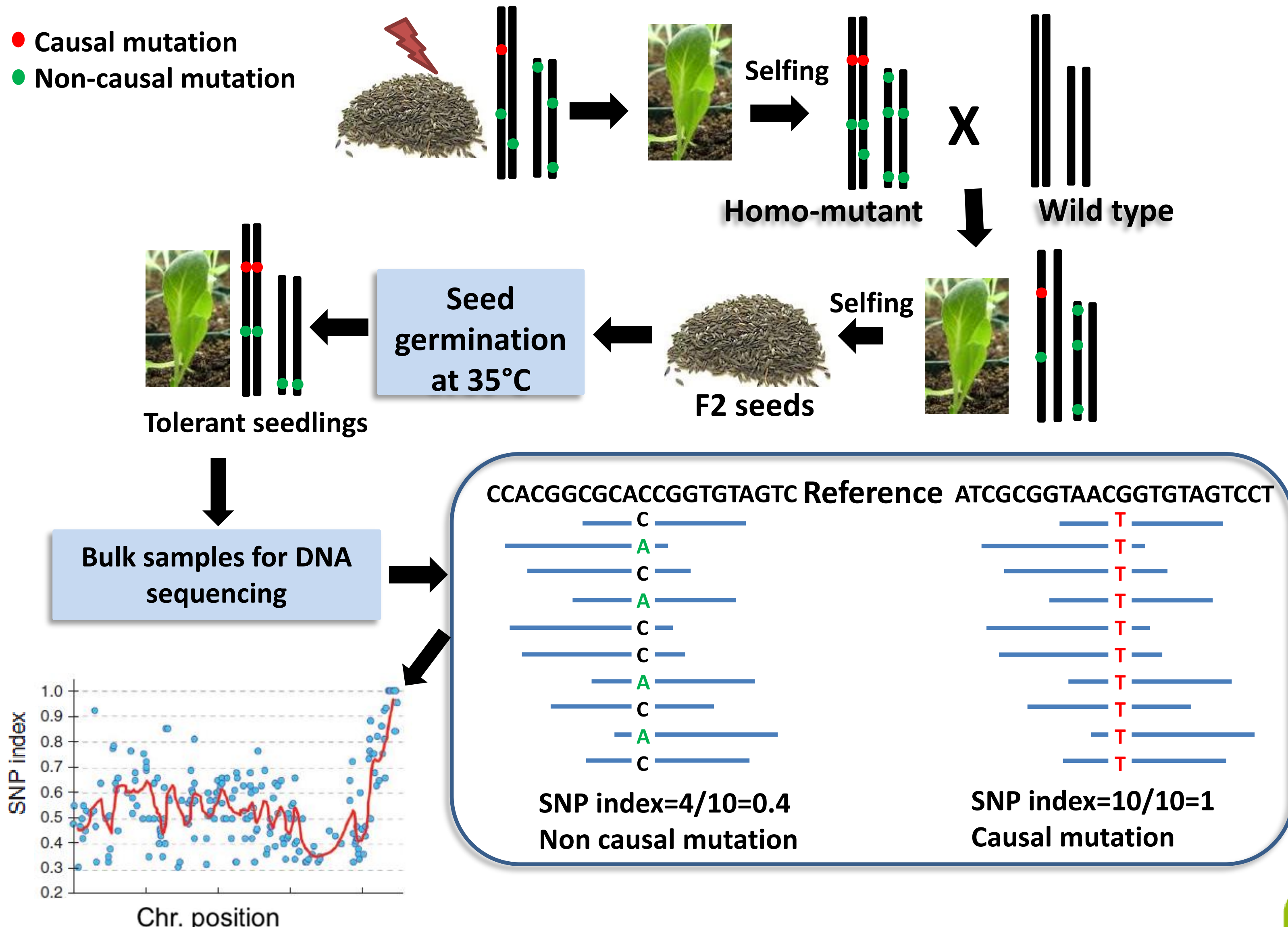
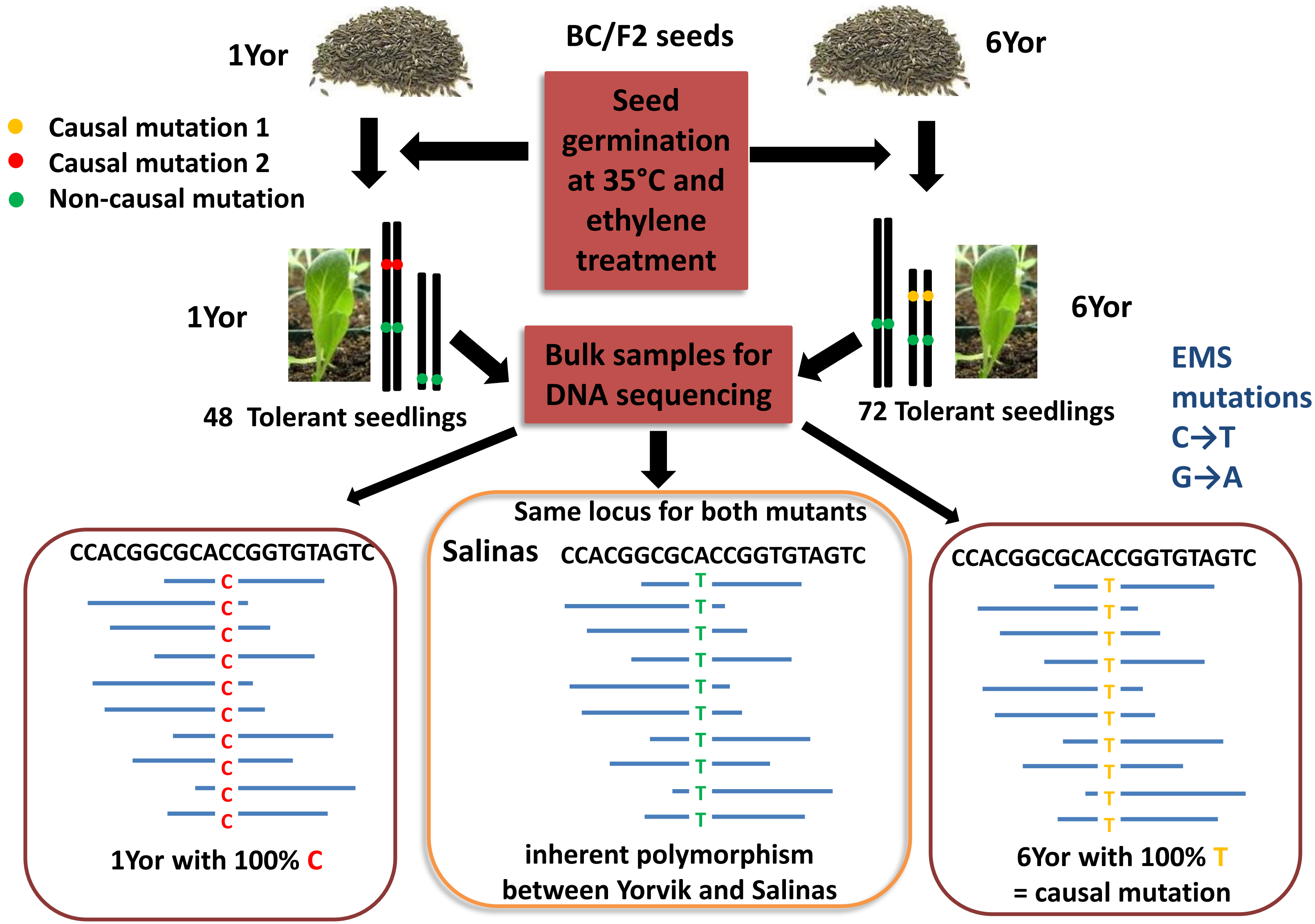
Mutation Identification by Bulked Segregant Analysis



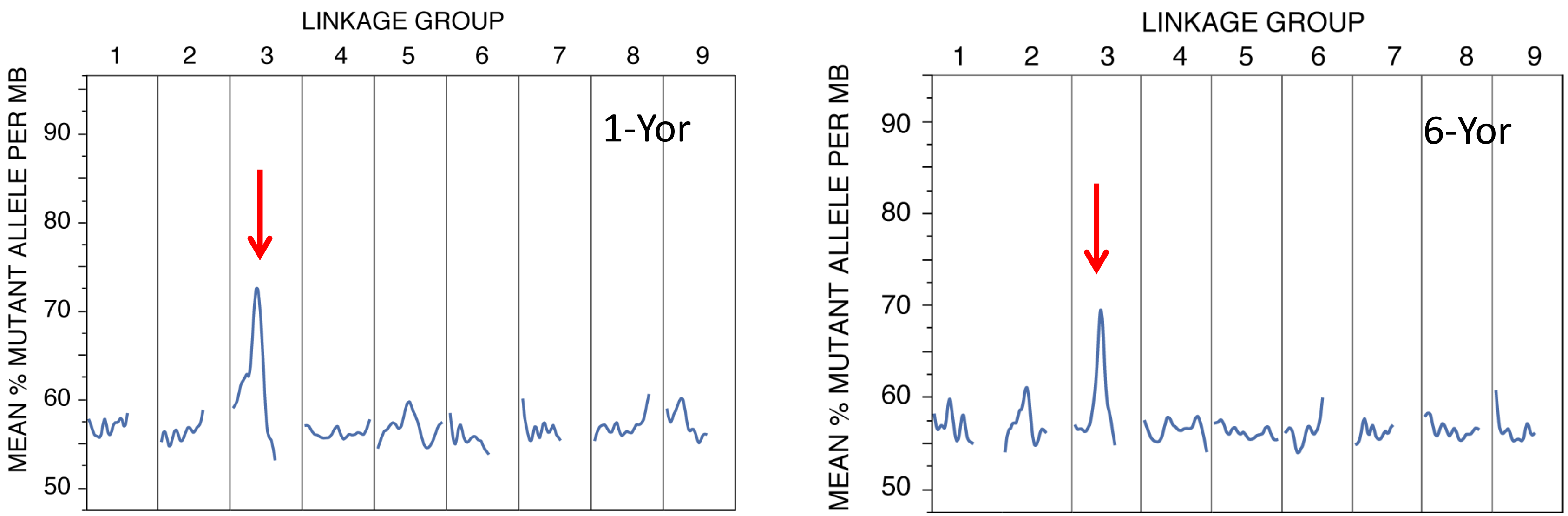
Luca Comai UCD   Eric Coppoolse RIJK ZWAAN

Mutation Identification by Bulked Segregant Analysis



Luca Comai, UCD Eric Coppoolse RIJK ZWAAN

Mutation Identification by Bulked Segregant Analysis



The percentage of mutant alleles per 1 MB bin was calculated across the genome. Peaks indicating a higher frequency of homozygous mutant alleles in the bulks would be candidates for the causal gene. Analysis of both mutant bulks identified a locus on chromosome 3.

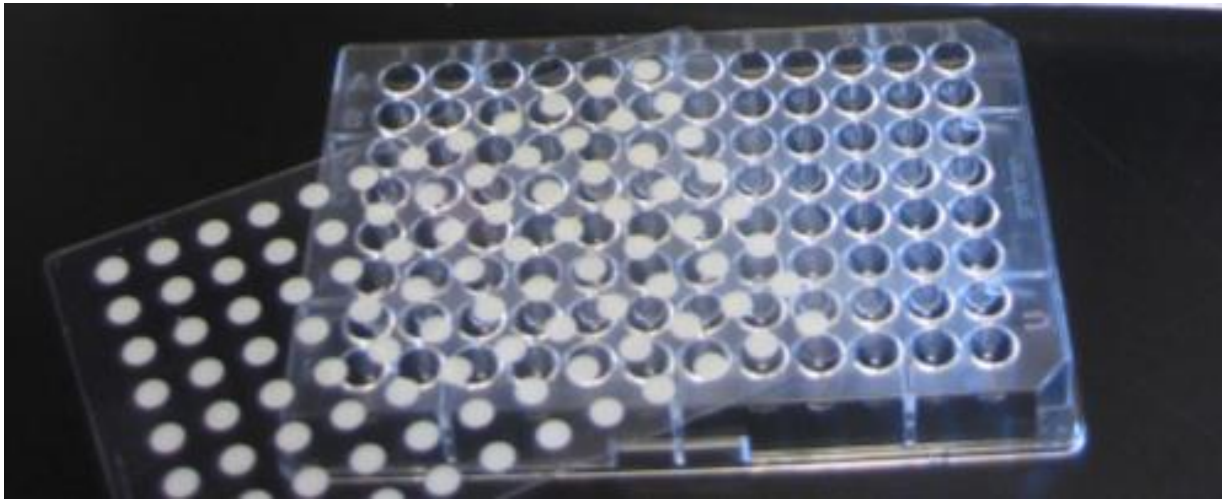
Mapping by Rijk Zwaan also independently located the causal mutations in this region of chromosome 3.

Luca Comai, UCD Eric Coppoolse RIJK ZWAAN

Q2 Seed Respiration Instrument

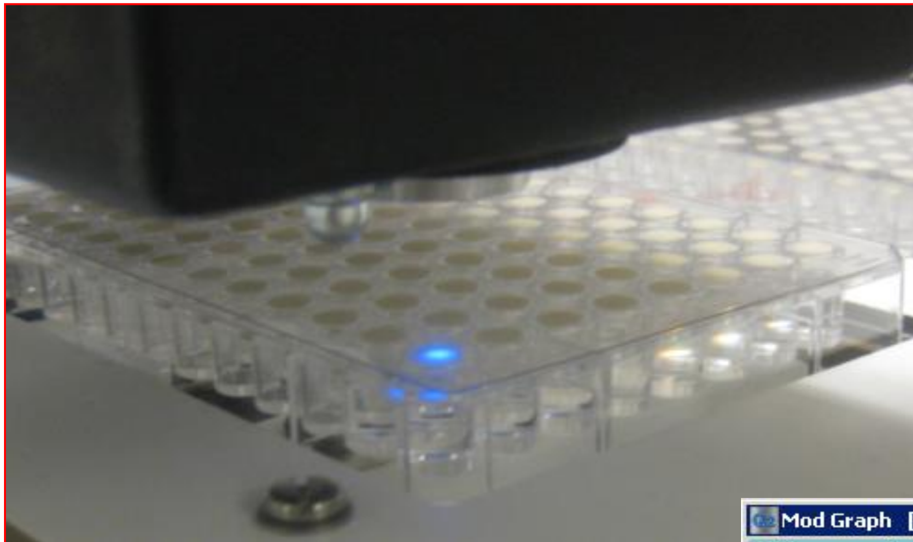
www.astecglobal.net

Q2 Seed Respiration Instrument



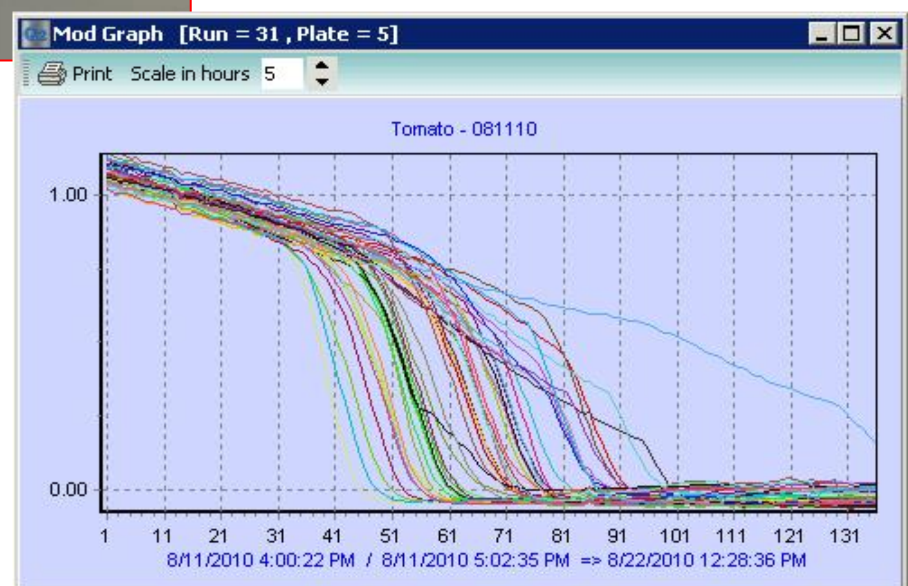
An oxygen-sensitive fluorescent dye is placed on a membrane (or vial cap) and sealed over a well containing an imbibed seed. The fluorescence of the dye is quenched by oxygen, so when illuminated by actinic light, the fluorescence increases as the seed consumes the oxygen in the well or vial. This provides a way to sample oxygen consumption over time of individual seeds.

Single-Seed Respiration Measurements

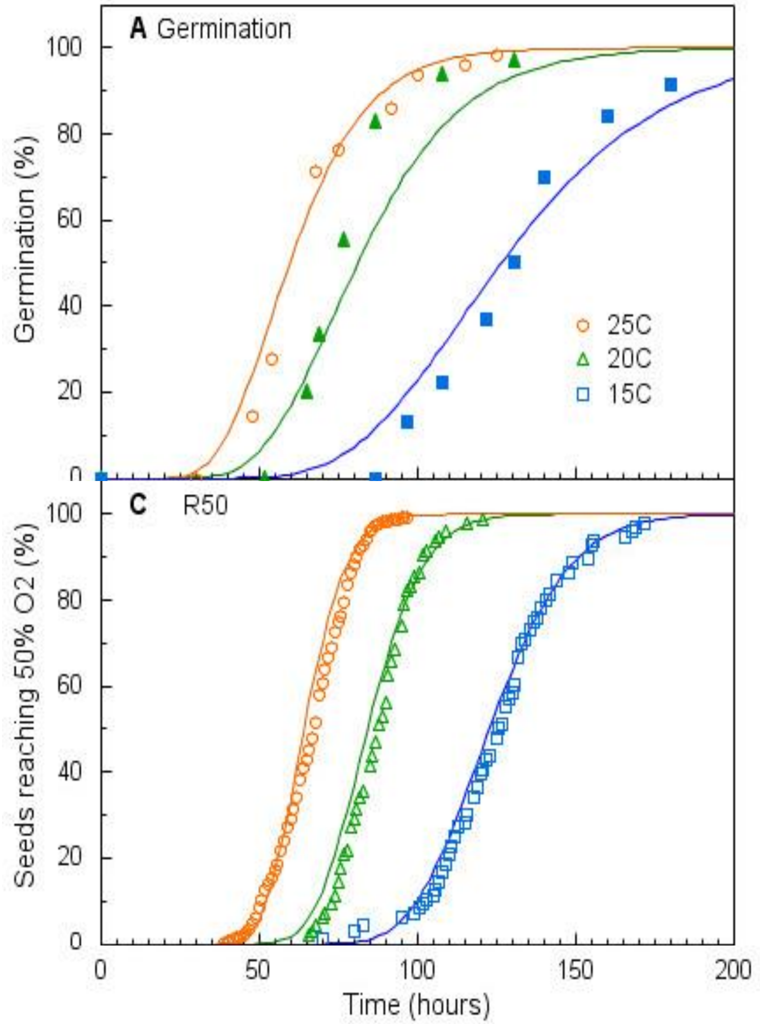


A light source/fluorescence sensor unit moves over the plates at specified intervals and records the oxygen depletion time courses for each well.

The instrument records the oxygen level in each well over time as it is depleted due to seed respiration.



Germination and Respiration Time Courses



Dahal and Bradford (1994) Seed Sci. Res. 4: 71-80.

Bello and Bradford, unpublished

Tomato seed respiration rates in response to temperature were described remarkably well by the population-based thermal time model (Dahal and Bradford, 1994).

The points are respiration data for each seed and solid lines indicate the predicted time courses based on the model.

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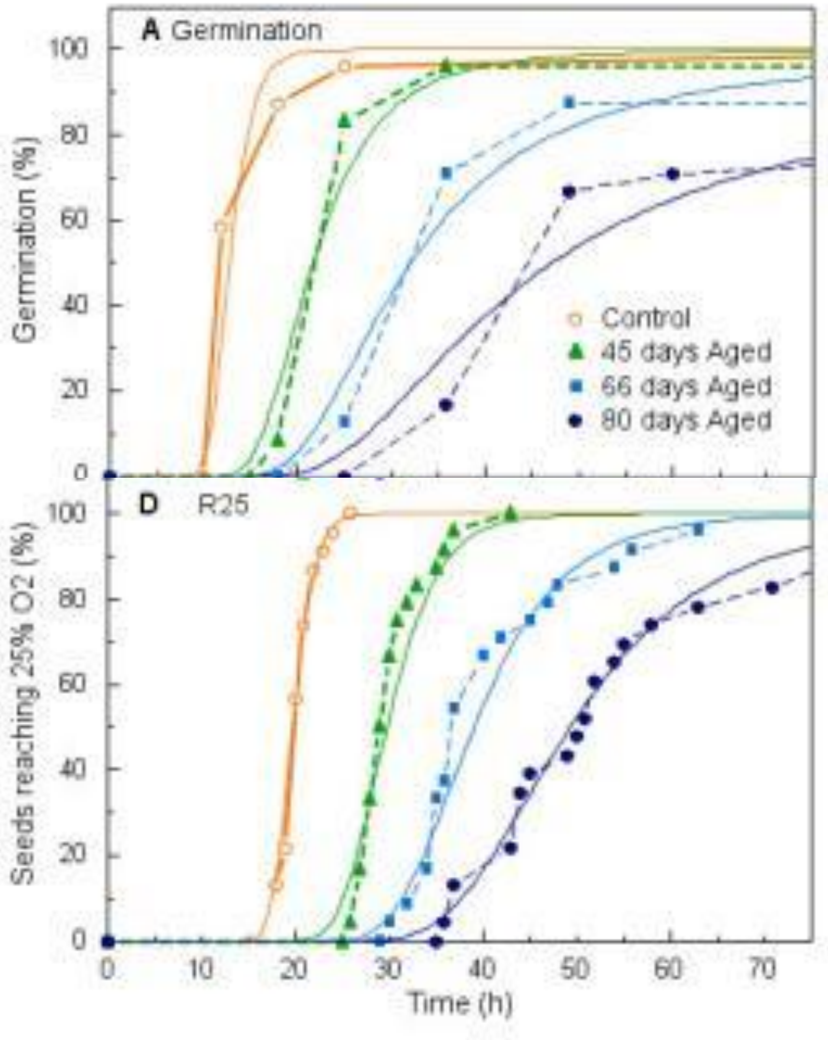
Aging Effects on Germination and Respiration



Germination and respiration time courses for radish seeds aged at 47% RH and 50°C for 0, 45, 66 or 80 days.

The respiration "time courses" matched well to the effects of aging on germination.

The aging-time model (Bradford et al., 1993) matched this data well (solid lines are model predictions).

Respiration time courses could be a sensitive and efficient way to detect and monitor early stages of seed deterioration during storage. It would enable the use of germination rates without labor-intensive repeated observations.

Bradford et al. (1993) J. Exp. Bot. 44: 1225-1234.

Bello and Bradford, unpublished

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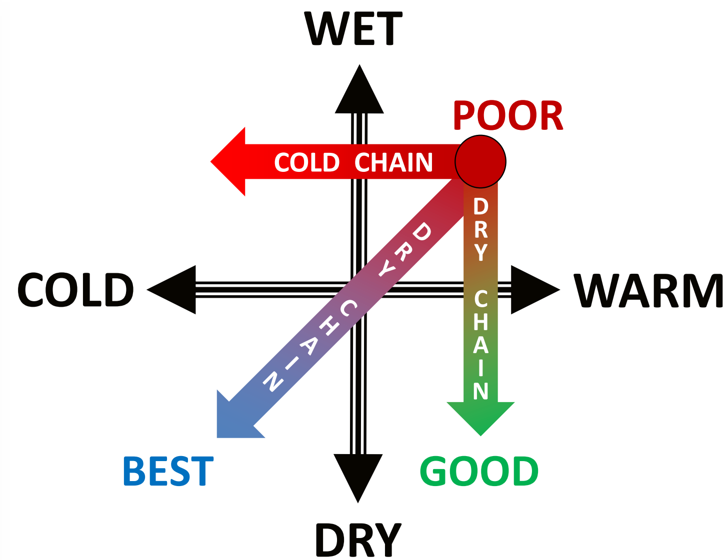
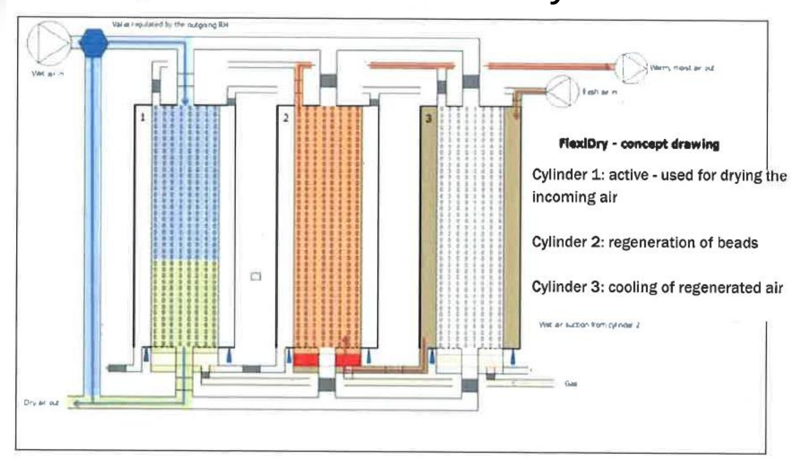
Drying Beads® for Seed Drying

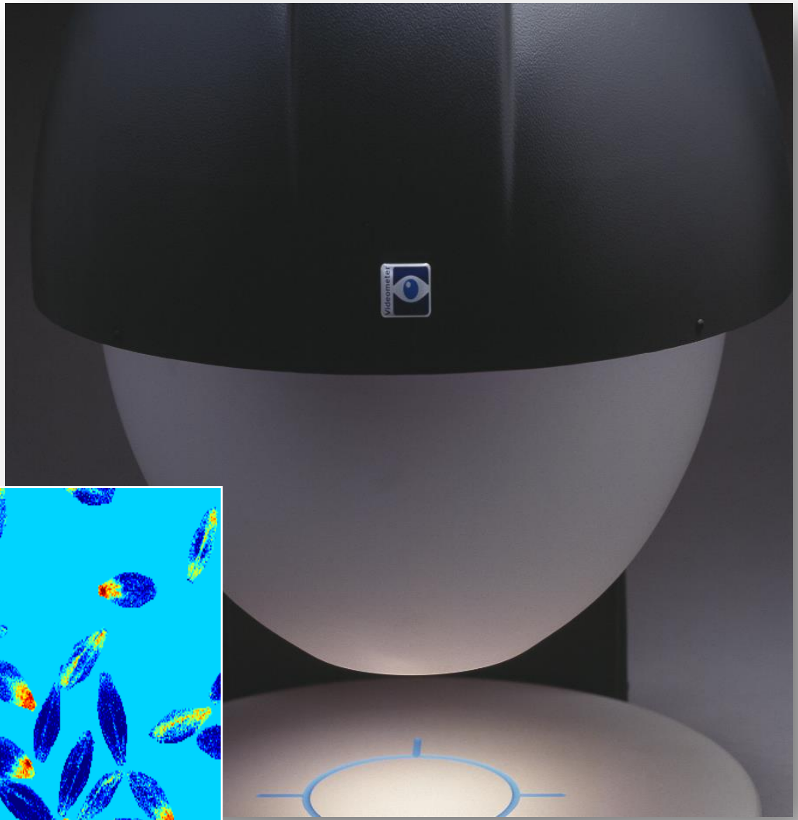


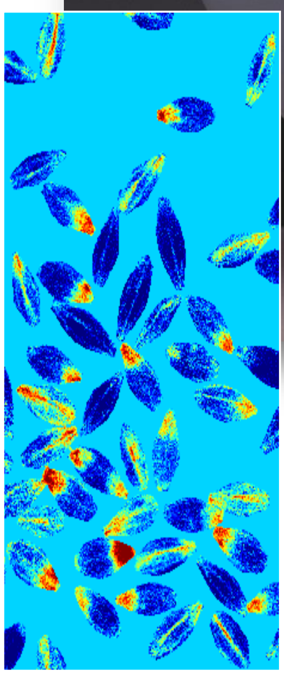
FlexiDry® Continuous dry air source



See also poster session www.dryingbeads.org

Videometer and CF Mobile





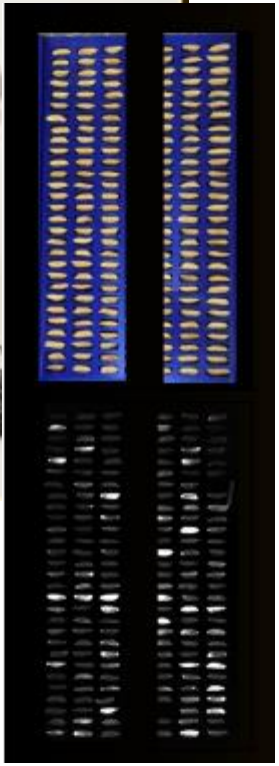


Image analysis applications for seed quality
See presentation in poster session

Acknowledgements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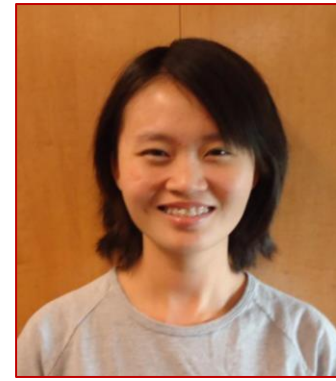
Fei-Yian Yoong


Jason Argyris


Richard Michelmore


Allen Van Deynze


David Still


Mohan Niroula

NSF

The Compositae Genome Project
http://cgpdb.ucdavis.edu/

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