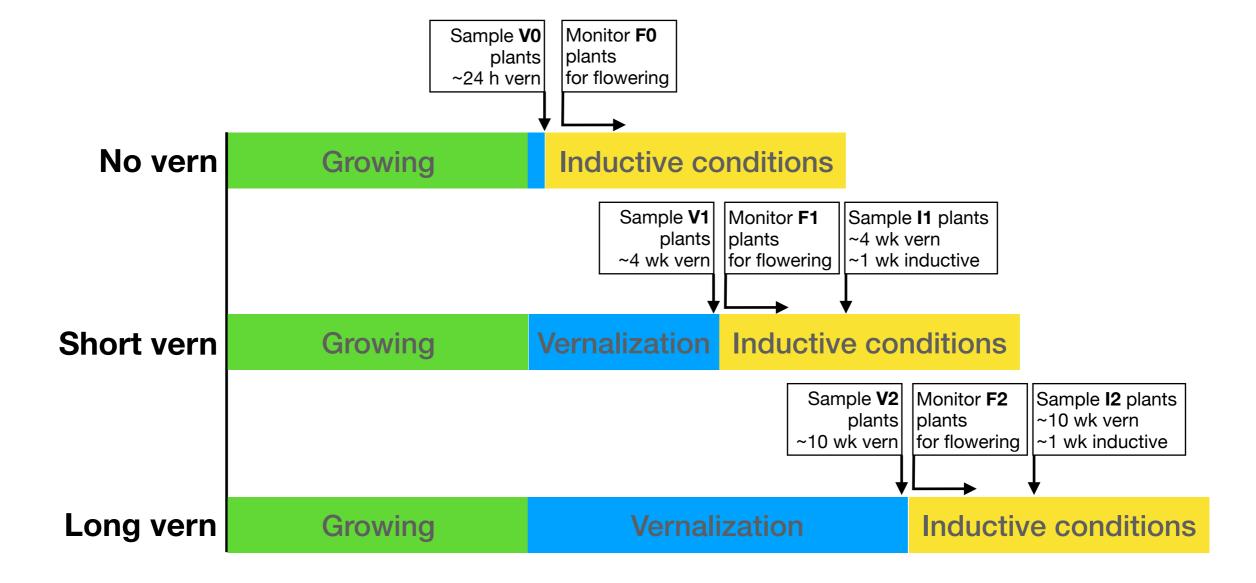
## Four latitudinal pairs of high and low elevation populations

Low elevation	High elevation	
TM2	LV3	
IHL	WL2	
BHR	YO1	
KC2	SQ3	

Question	Contrast	What will we learn?
1. How does gene expression change in response to vernalization in <i>S. tortuous</i> ?	Within each population: No vern (V0) vs. short vern (V1) No vern (V0) vs. long vern (V2) Short vern (V1) vs. long vern (V2)	What genes are differently expressed during vernalization? The typical suspects or a more diverse suite?
2. Do high and low elevation populations differ in gene expression during vernalization?	Within each latitudinal pairing (or with all low pooled and all high pooled): DEGs from Question 1 in high vs. low elevation populations	What genes differ in expression in high vs. low populations during vernalization? Contextualize with flowering responses. Can different responses be explained by differentially expressed genes?
3. Do all high elevation populations respond to vernalization with the same expression patterns/genes? Do all low elevation populations respond similarly?	Within elevation categories: DEGs from Question 1 in LV3 vs. WL2 vs DEGs from Question 1 in TM2 vs. IHL vs	Is adaptation to high elevation occurring through parallel mechanisms (or maybe a single origin that spread)? Assumes ancestral state is low elevation, we may be able to speculate about this if responses are more divergent in high vs. low or vice-versa.
4. How does expression differ between populations in inductive conditions after short vs. long vernalization?	As in Questions 1 and 2, but with samples from inductive conditions after short and long vern  11 vs. 12	How does inductive response differ with long vs. short vernalization?



## **Eight treatments/sampling points:**

No vernalization: expect no flowering

- FO phenotyped for flowering in inductive conditions after just 24 hours in vernalization chamber
- V0 apical meristem sampled after just 24 hours in vernalization chamber

Short vernalization: expect to meet vernalization requirements in low elevation populations\*, but not high elevation populations

- **F1** phenotyped for flowering in inductive conditions after short vernalization treatment
- V1 sampled in vernalization treatment after short vernalization
- I1 sampled in inductive conditions 7? days after coming out of short vernalization treatment

Long vernalization: expect to meet vernalization requirements in all populations

- **F2** phenotyped for flowering in inductive conditions after short vern vernalization treatment
- **V2** sampled in vern treatment after long vernalization
- 12 sampled 7? days after coming out of long vernalization treatment

<sup>\*</sup>but previous growout indicates there may be an age/size threshold for two of the low elevation populations