# Study of Deacclimation in Winter Canola (*Brassica napus* L.)

# Spring and Winter Canola

Spring Canola

- Planted early spring (March)
- Harvested around September
- Northern states

Winter Canola

- Planted in fall (September)
- Over winters and is harvested in June
- Yield 20 to 30% more than spring canola
- Warmers areas like the Southern Great Plains

*US Canola association*

# Cold acclimation

- Plants can increase tolerance to cold freezing temperature
- It starts when temperature drops below 10 C
- It takes up to 4 weeks
- Hardened winter canola can withstand temperatures below -15 C for up to 4 hours
- Freezing to -12 C following acclimation and deacclimation was best to highlight differences between genotypes

Winter canola (six leaf stage) → Cold chamber at 5C ( 4 weeks) → cold acclimated plants

# Deacclimation

- Acclimated plants upon exposure to warmer temperatures lose the freezing tolerance acquired during acclimation
- Leads to freezing damage if winter canola does not have a chance to reacclimate

- research on deacclimation is still lacking

# Objective 1

1) Assessing the effect of different climatic factors (temperature and time) impacting deacclimation in winter canola
    - We previously determined canola generally began to acclimate when temperatures dropped below 10C
    - The level of freezing tolerance was dependent on the acclimation temperature more than the time at a given temperature
    - We tried to find if a similar response would be observed during deacclimation
    - Preliminary studies indicated some differences in deacclimation rates among varieties, we wanted to verify that observation from this experiment

# Experimental approach

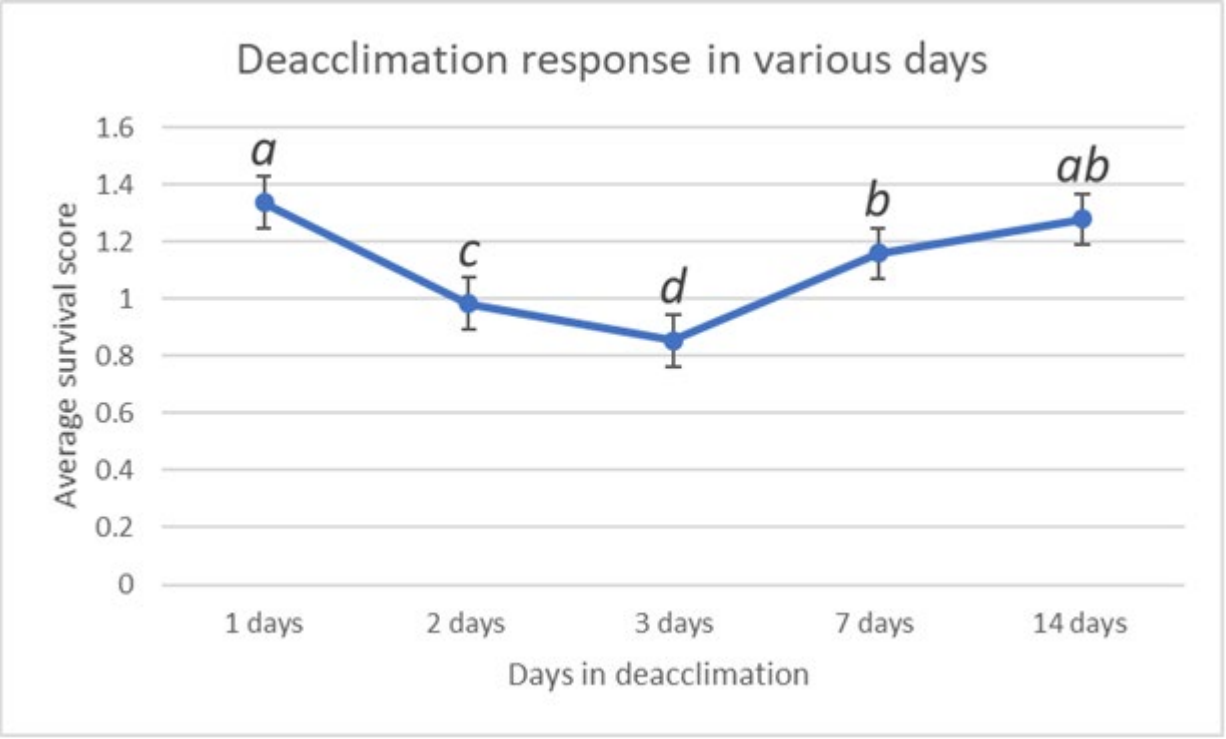
ars 189
ars 228
ars 269
ars 312
ars 346
ars 229
ars 261
ars 233
ars 246
ars 036
regina II
wester

→ Grown in greenhouse up to 6 leaf stage → Cold acclimation at 5C for 4 weeks → deacclimation at (5, 7, 10, 13, 15C) for 1, 2, 3, 7, 14 days → Freeze at -12C for 1 day

# Experimental approach

- Plants were scored after one week of recovery period based on visual damage on a 0-3 scale
- 0 being complete death
- 1 having  > 50% foliar damage but maintaining at least one living meristem
- 2 having between 50% and 10% foliar damage
- 3 having 0 -10% foliar damage

# Deacclimation results




- Deacclimation resistant at 1 and 14 days
- 3 days of deacclimation period affect the most on plant survival

# Deacclimation results

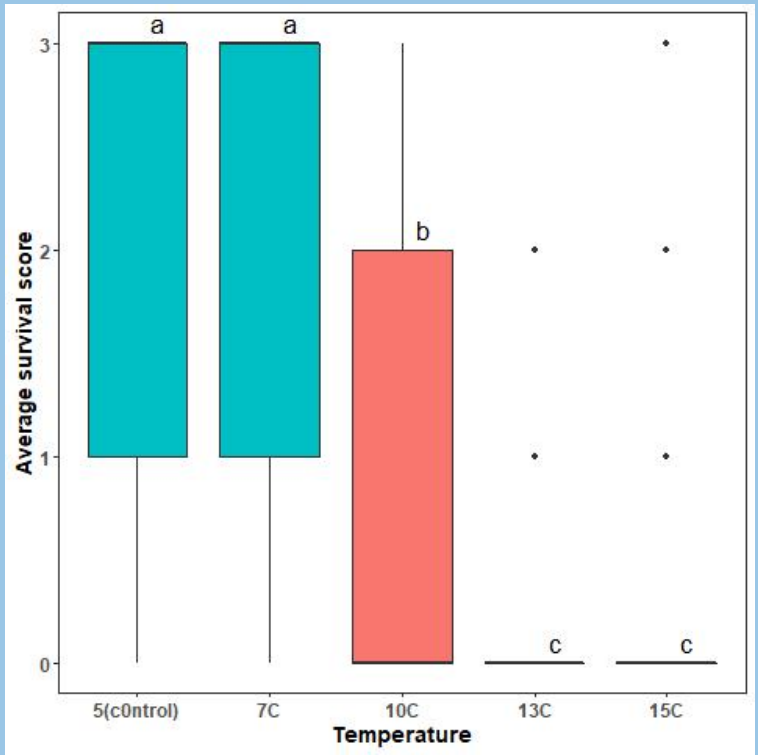



Fig: Overall deacclimation response of 5 different temperature

- No significant difference at 5(control) and 7C
- None of the plants have survived at 13 and 15C except in 1 days of deacclimation and in line ars229
- At 10C different lines showed different deacclimation response
- In 14 days deacclimation 7C response significantly different from 5C (Control)

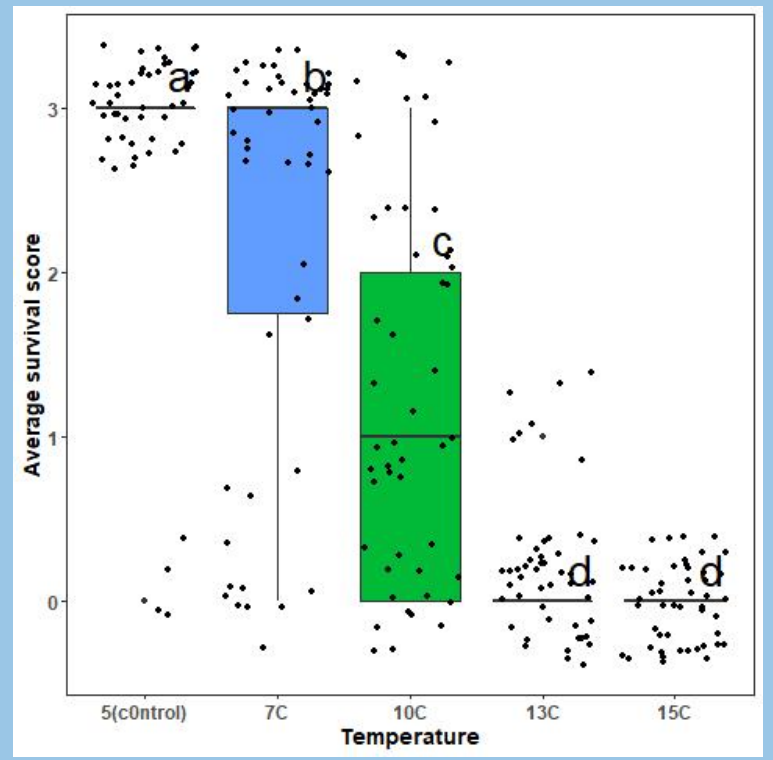
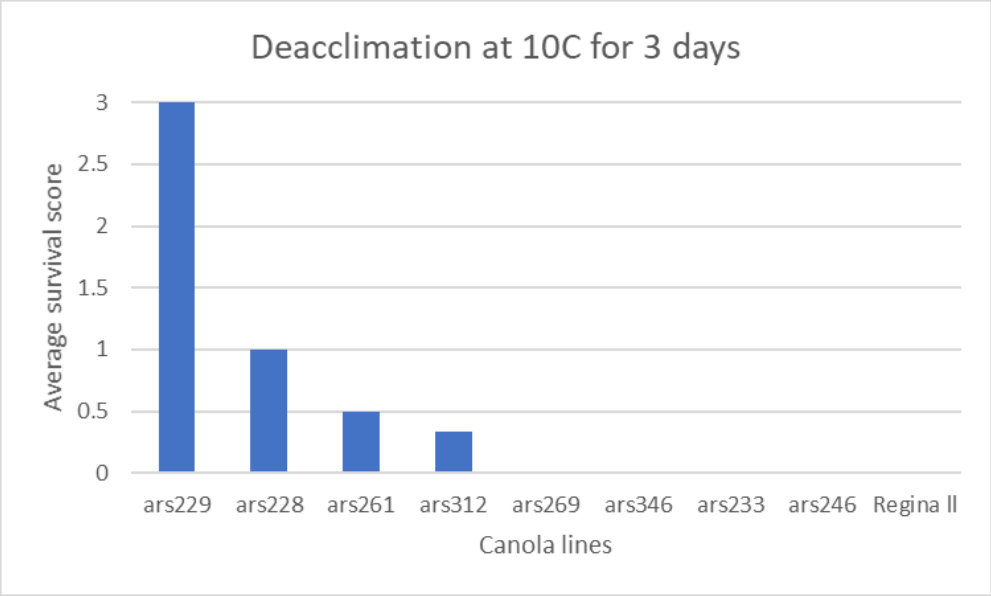



Fig: deacclimation response of 5 different temperature at 14 days

5C 7C 10C 13C 15C

Line 312 after 7 days deacclimation at the indicated temperatures.

# Deacclimation result




- ars229 is highly resistance to deacclimation among winter lines we studied

[1] "LSD results of canola lines at 14 days"

| MSerror | Df | Mean | CV | t.value | LSD |
|---|---|---|---|---|---|
| 1.045 | 228 | 1.279167 | 79.9155 | 1.970423 | 0.5200822 |

| | survival | groups |
|---|---|---|
| ars189 | 1.566667 | a |
| ars346 | 1.566667 | a |
| ars269 | 1.533333 | a |
| ars261 | 1.500000 | a |
| ars229 | 1.400000 | a |
| ars312 | 1.233333 | a |
| ars246 | 1.133333 | a |
| ars233 | 0.300000 | b |
| Regina II | 0.000000 | c |

- No significant difference among lines except ars233
- ars233 is highly freezing susceptible

# Bi-parental mapping

1. Examine genetics of acclimation and deacclimation by bi-parental mapping
2. significant differences for freezing tolerance and deacclimation resistance among canola lines

we made several crosses

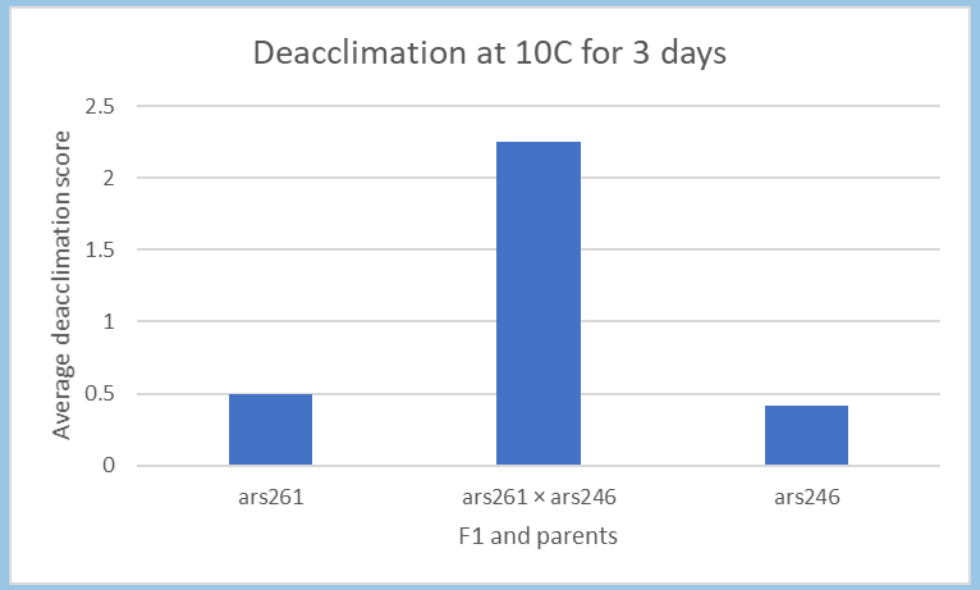
Freezing tolerant deacclimation resistant  Freezing tolerant deacclimation resistant

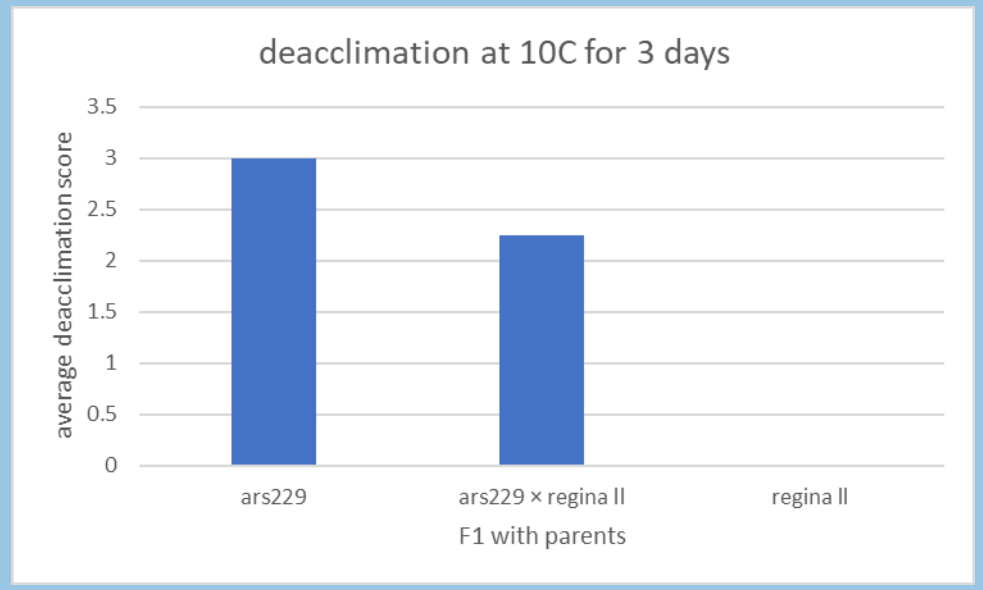
- (winter line 229) × (spring lines Regina II)

Freezing moderate deacclimation resistant

- (winter line 261) × (winter line 233) Freezing moderate deacclimation sensitive
- (winter line 261) × (winter line 246) Freezing tolerant deacclimation sensitive

# Results

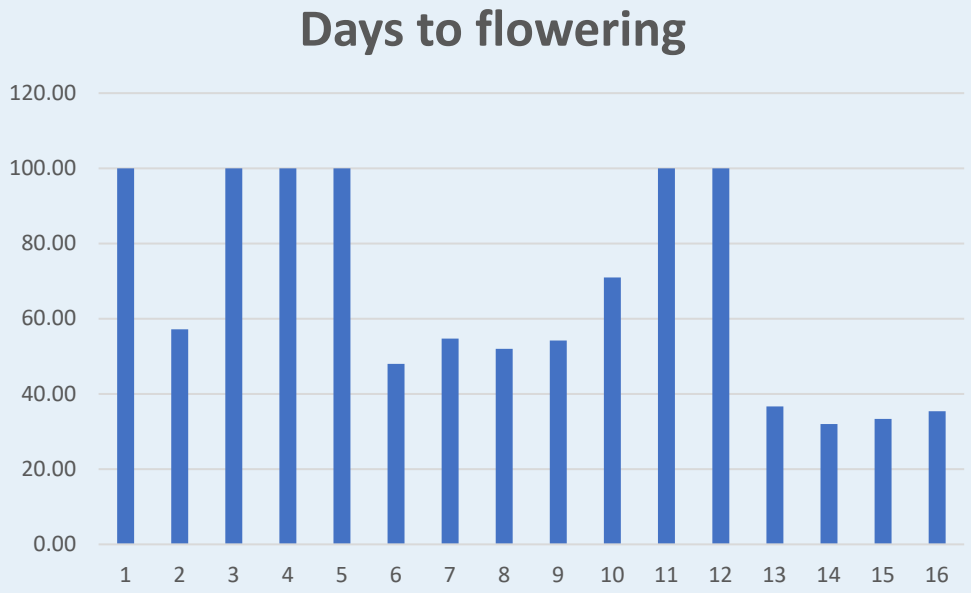


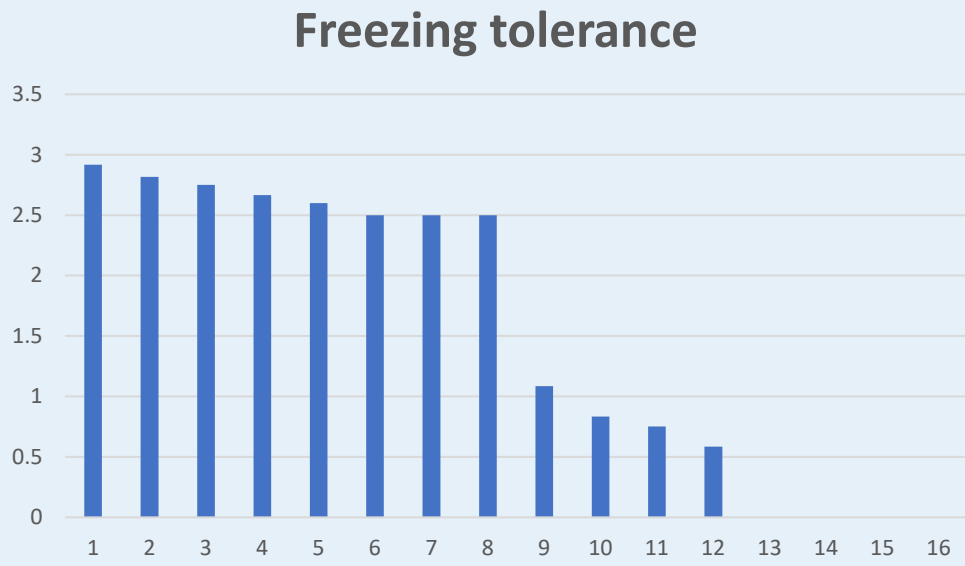




- Study F3 families from cross of ars229 × Regina II

# Conclusions

*Data from winter x spring camelina cross indicates we may be able to get spring lines with good freezing tolerance*




- Note F7 inbred lines 2, 6, 7 and 8 are spring biotypes but have very good freezing tolerance
- Lines 11 and 12 are winter types that now have poor freezing tolerance

**Thus, growth habit seems to be genetically independent from freezing tolerance in this related species**

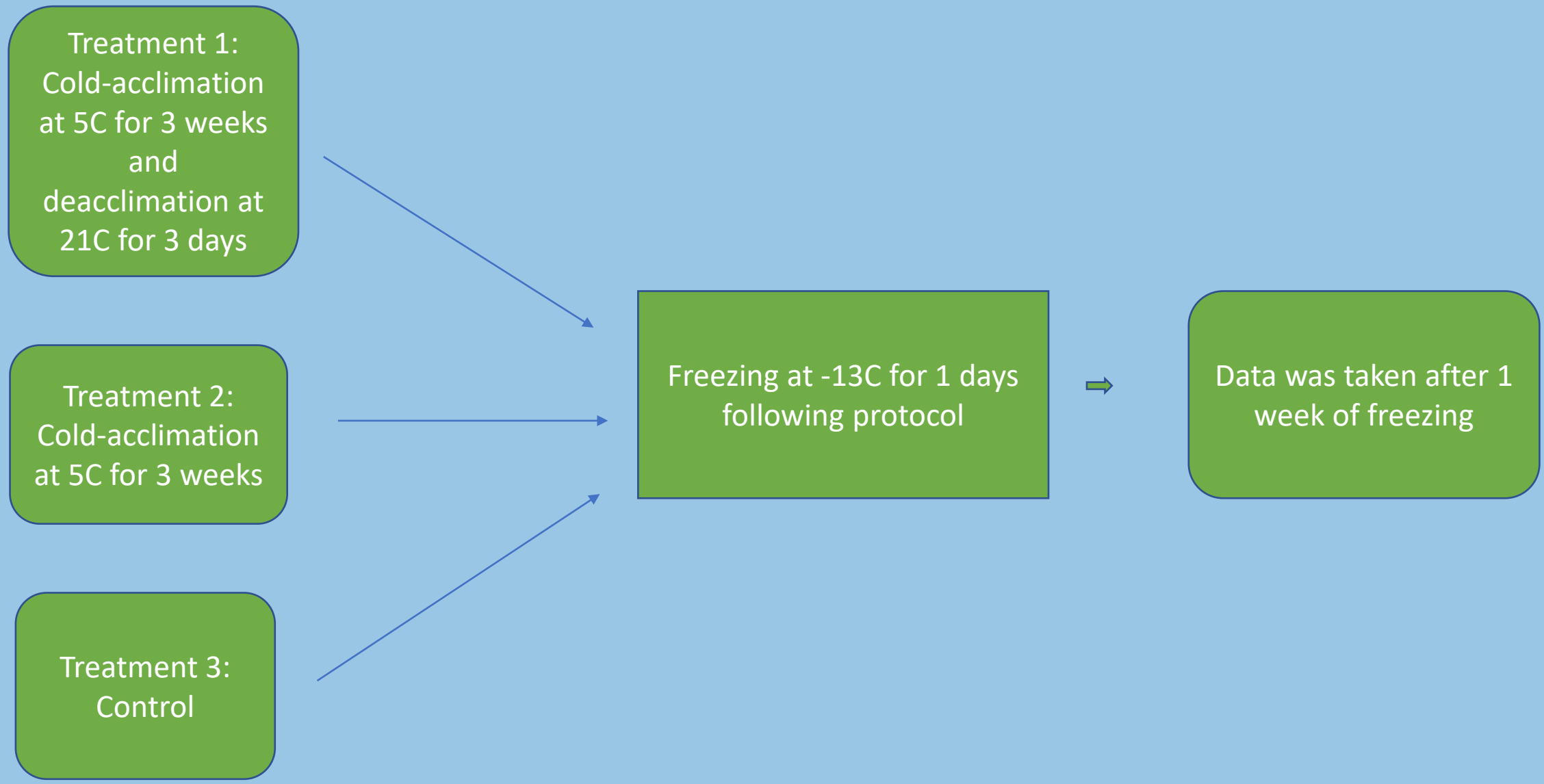
# Mutation analysis

1. Functionally test genes impacting deacclimation from GWAS studies

    1. Previous studies identified over 20 possible genes that might impact deacclimation in canola

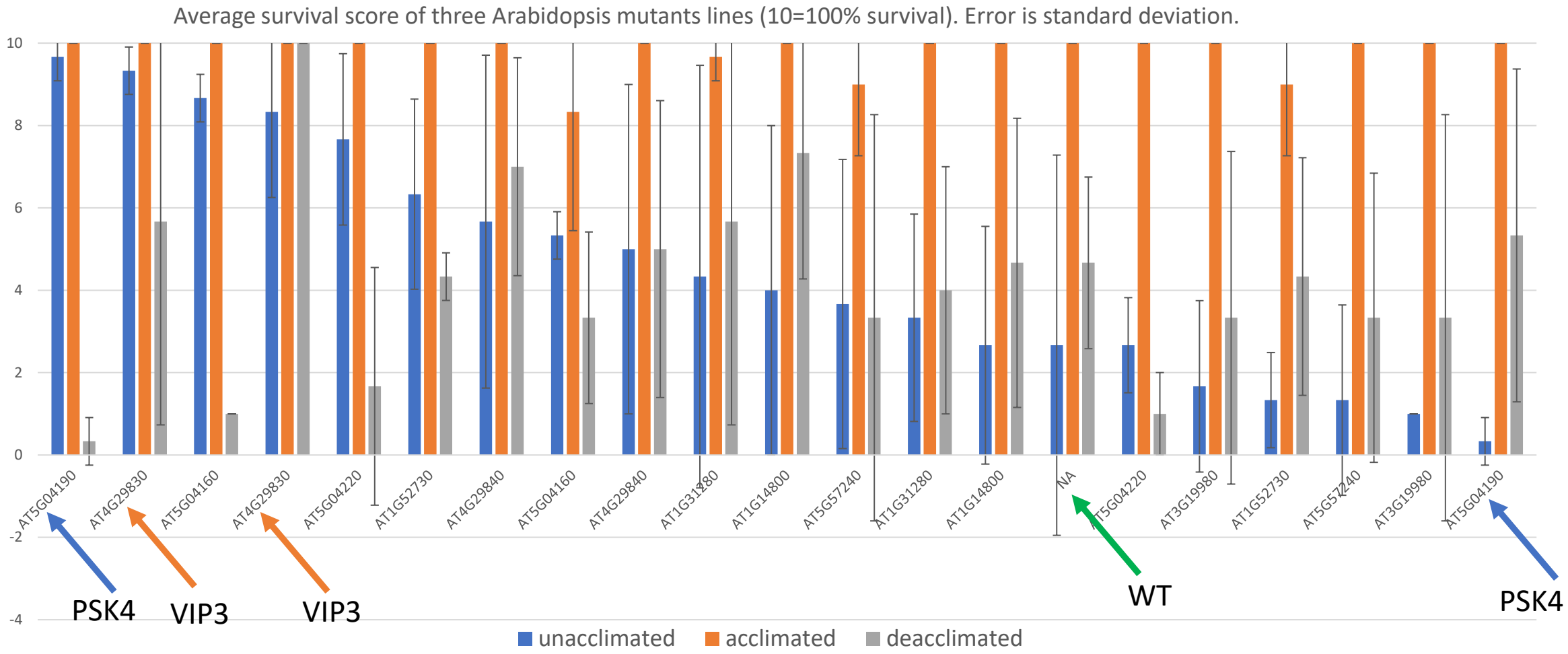
    2. Arabidopsis plants with mutations in those genes were obtained.

# Experimental Approach

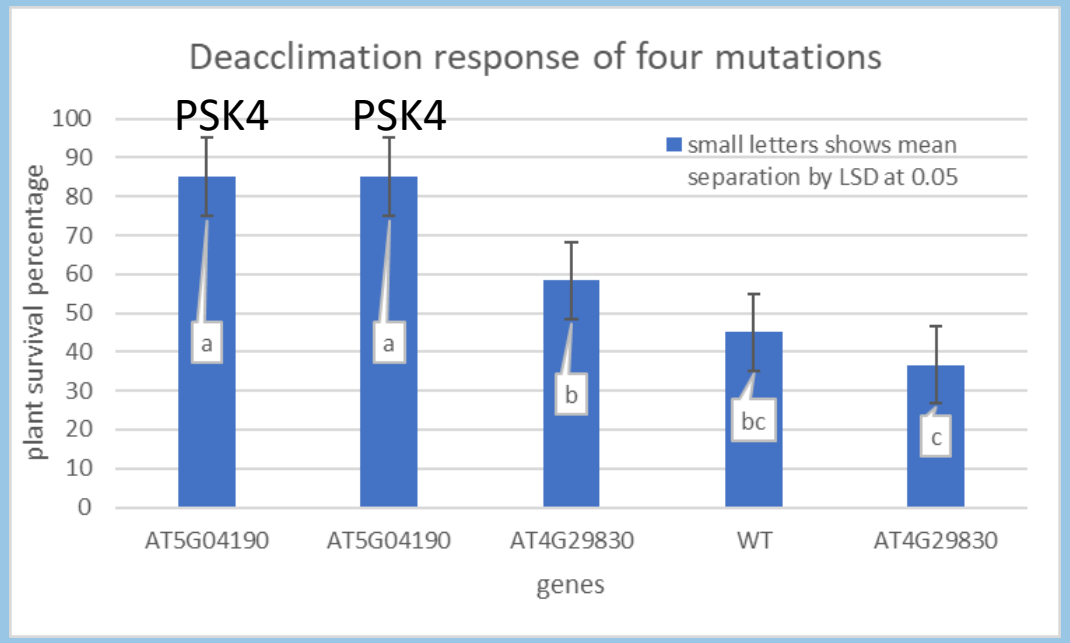


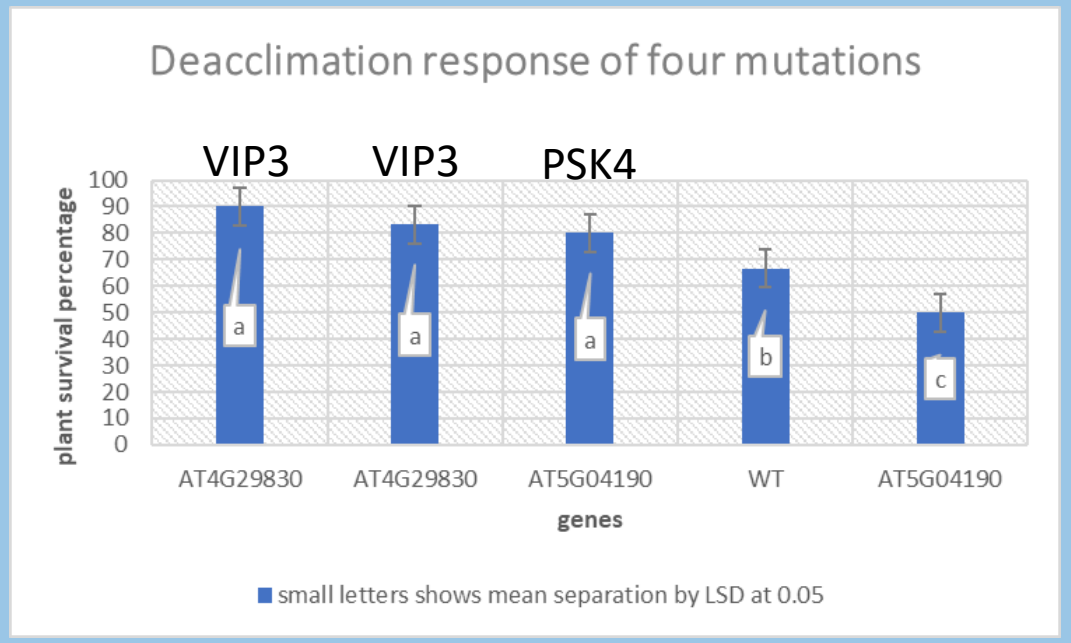
# Results

## Two genes (VIP3 and PKS4) showed promise in this screening



# Results on selected 4 mutations




1) At least one more experiment to confirm on these 4 mutations
2) Still 20 more mutations to analyze

# Thanking You