Salt and Cold Tolerance in Alfalfa

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Presentation Outline

Introduction

Alfalfa and Importance

Stress tolerance in alfalfa

A. Salt Stress

i. Growth and Biomass production

ii. Physiological Analysis

iii. Potential Mechanisms

B. Cold Stress

i. Screening

ii. Physiological Analysis

iii. Expression of cold responsive genes

Introduction: Alfalfa & Importance

- Alfalfa (*Medicago sativa* L.) is one of the most important forage legume crops in the world.

- Total value ~$27 billion/yr in the US after considering export and the benefit to ruminant livestock etc)

- 2011

South Dakota (Alfalfa/Alfalfa Mixtures Hay)
Area Harvested: **2350x1000 acres**
Production: **6345x1000 tones**
Rank: **Top 5 in the US**

USDA: Crop production 2011 summary, January 2012.

Effect of Cold & Salt Stress

Cold **Salinity**

Affects both yield and quality of alfalfa

50 to 75% of the agriculture yield is lost

Liu et.al., (2002), Eur J Agron 16:137-50. Bajaj et .al., (1999)

Objectives

- Understand how plants sense and respond to abiotic stress, such as salinity and cold
- Improve plant performance and production under stress conditions

Salt Stress

Characterization of physiological responses of two alfalfa half-sib families with improved salt tolerance

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

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Research article

Characterization of physiological responses of two alfalfa half-sib families with improved salt tolerance

CrossMark

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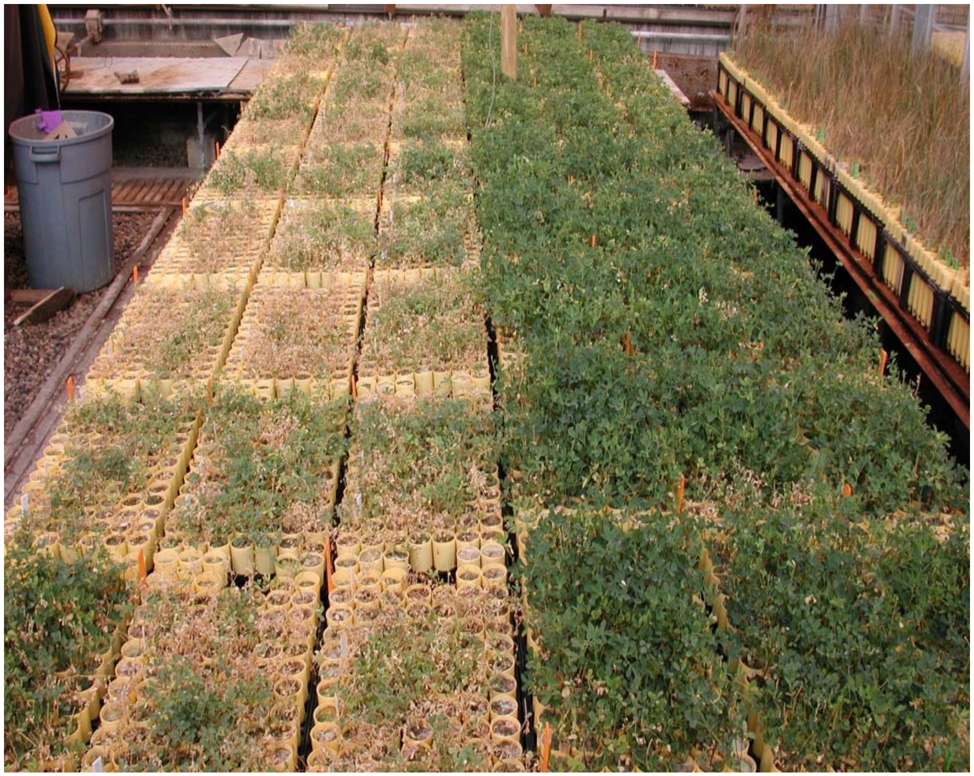
Plant Materials



Dramatic differences between a population of alfalfa (right) that has undergone three cycles of selection for ability to survive at 18.0 dS m^{-1} compared to unselected (left) alfalfa. Surviving plants were allowed to cross and subsequent generations were subjected to the selection protocol. (Drs. Mott & Peel)

❑Plant Materials

Melone
Mesasirsa
Saranac } P-A

CkSltn HS-A

BC 79 P-B

BC 11-1 HS-B

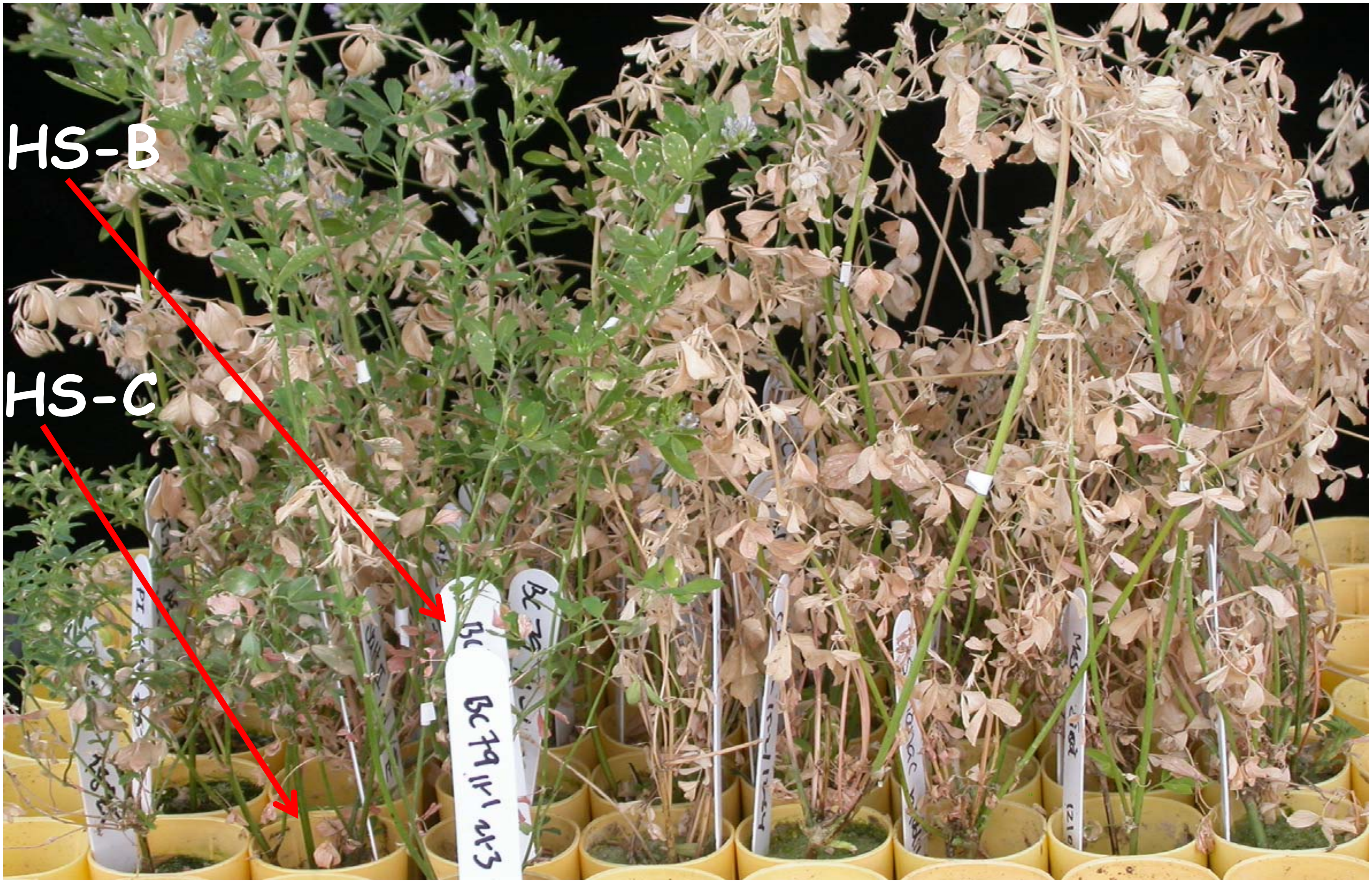
P53V08
Forage } P-C

Salt II HS-C

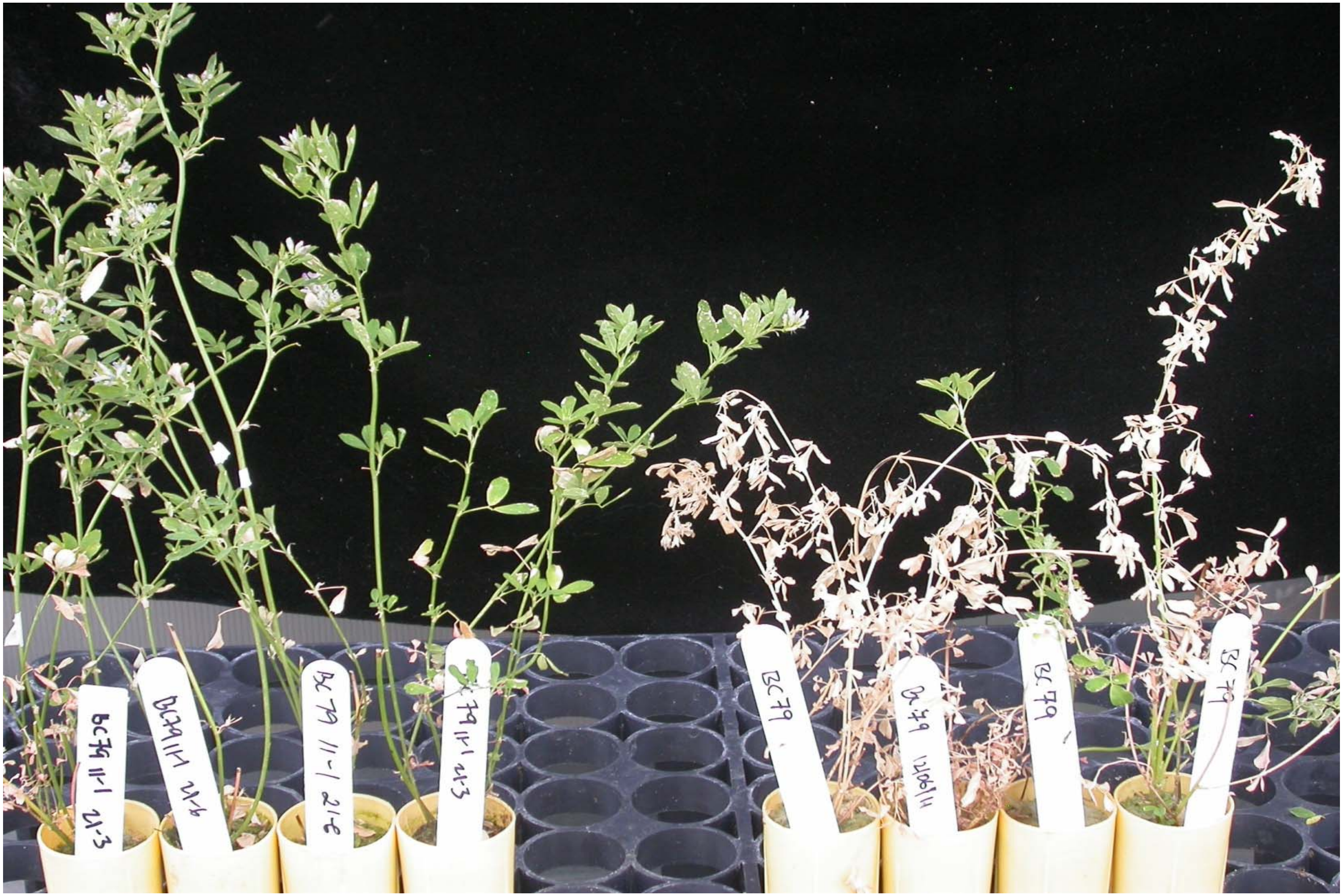
Salt Tolerant Selections

Salt-tolerant selections stayed mostly green while the original populations from which they were selected showed senescence one week after 12.0 dS m^{-1} treatment

7 Days After 12 DS (eq. 120 mM NaCl) Salt Treatment

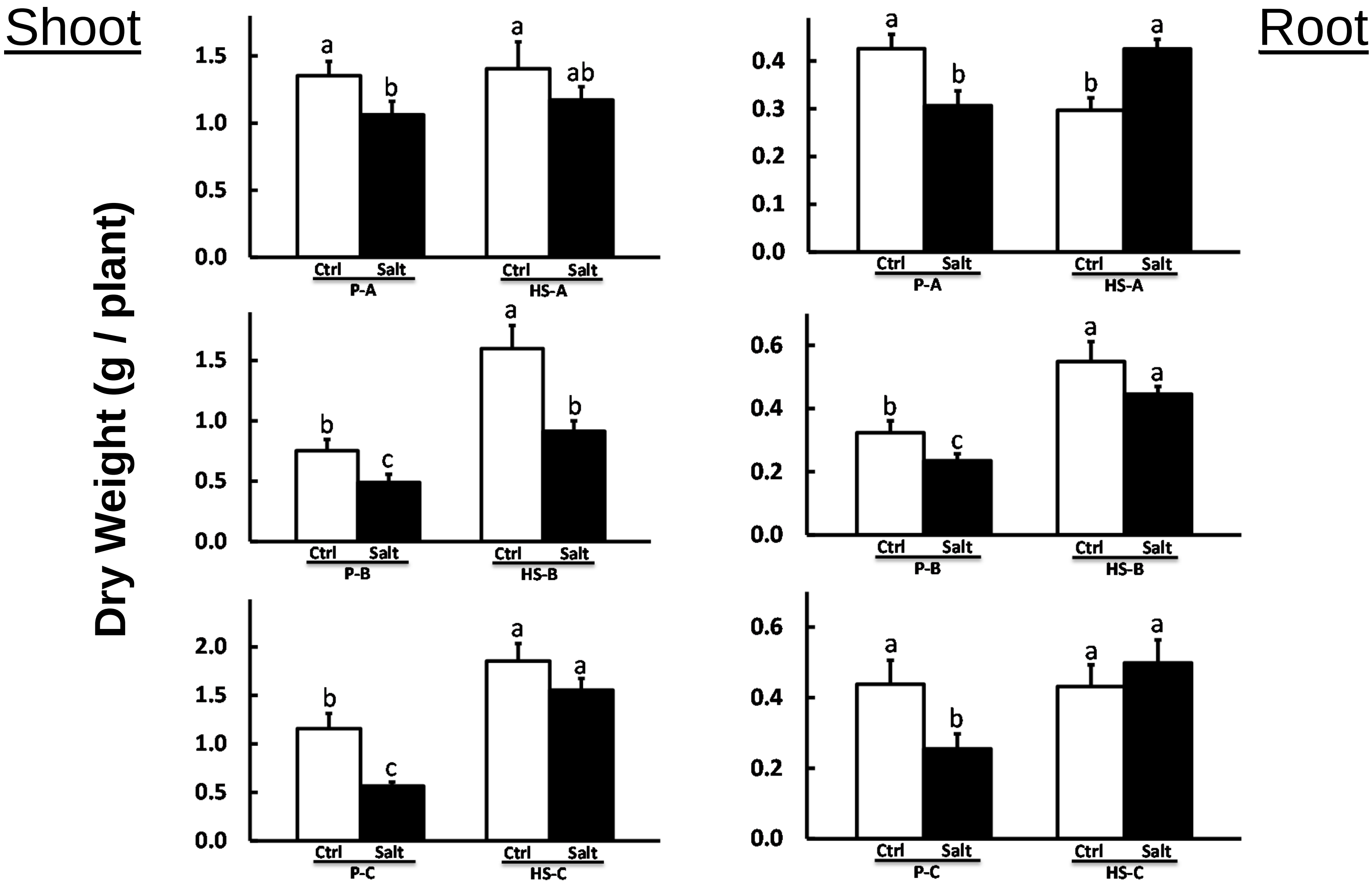


HS-B VS P-B After 12 DS Treatment

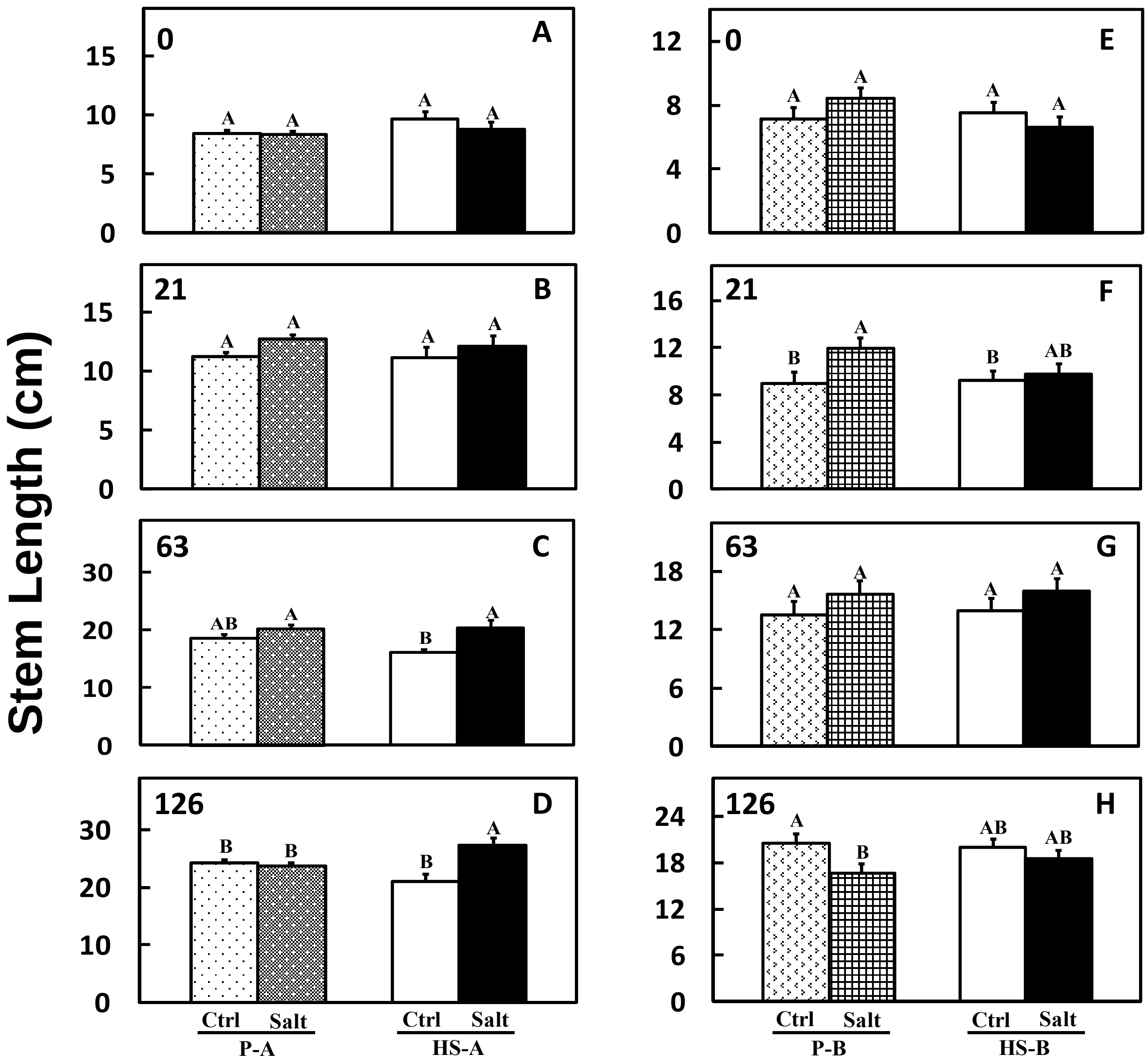


Selection: HS-B　　　　　　　　Parents: P-B

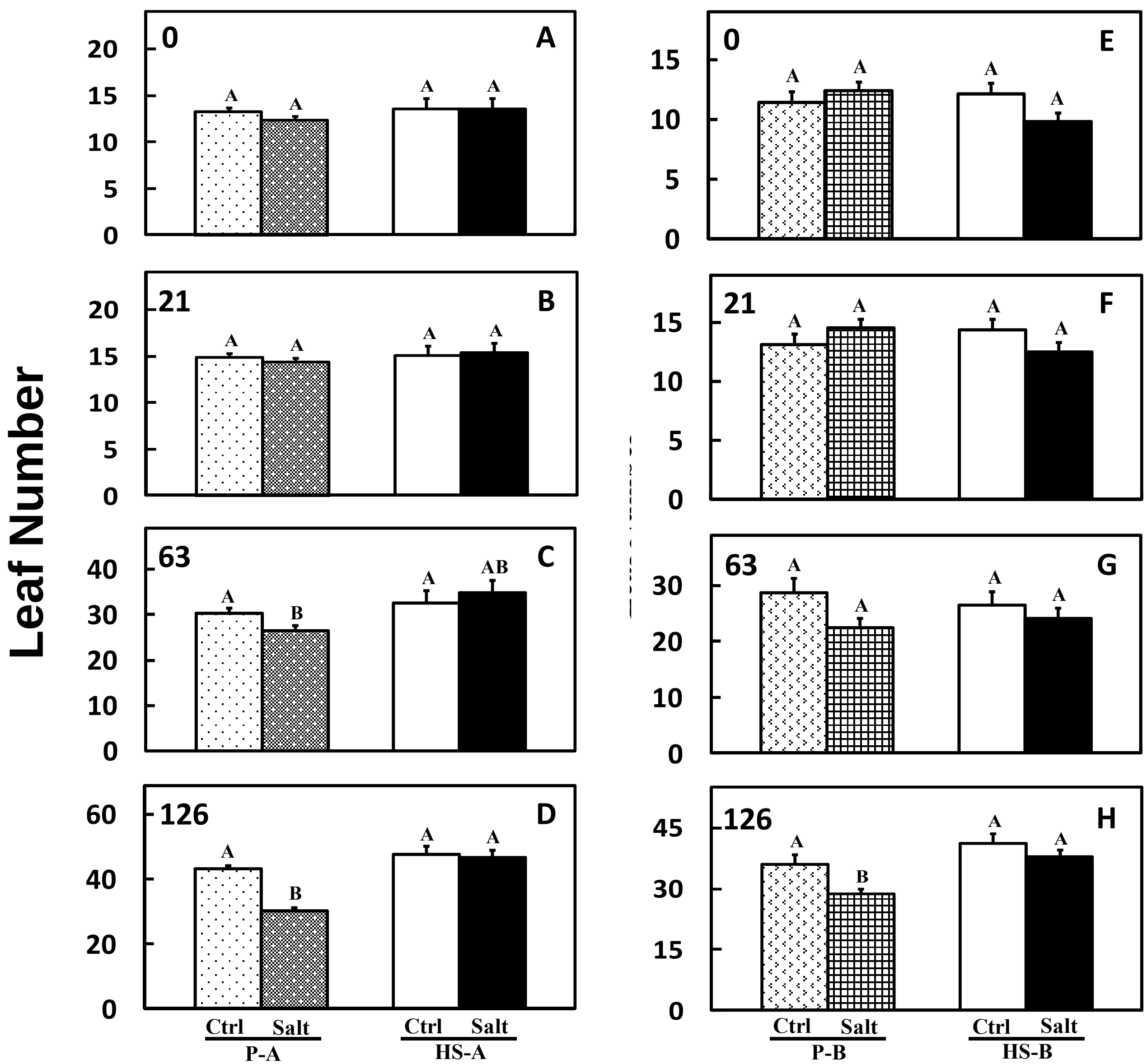
Improved Shoot & Root Biomass



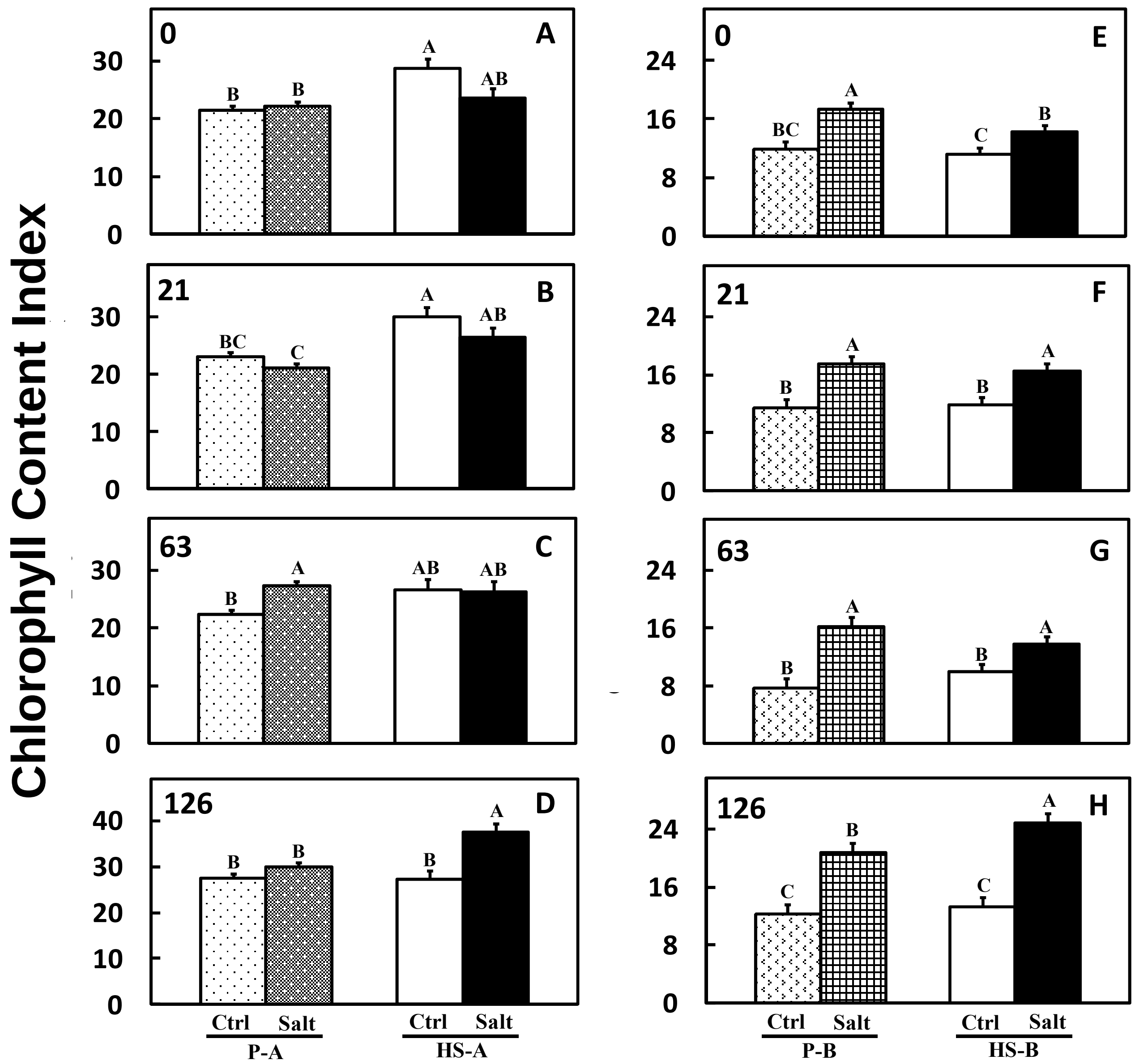
Maintained Stem Length in HS-B



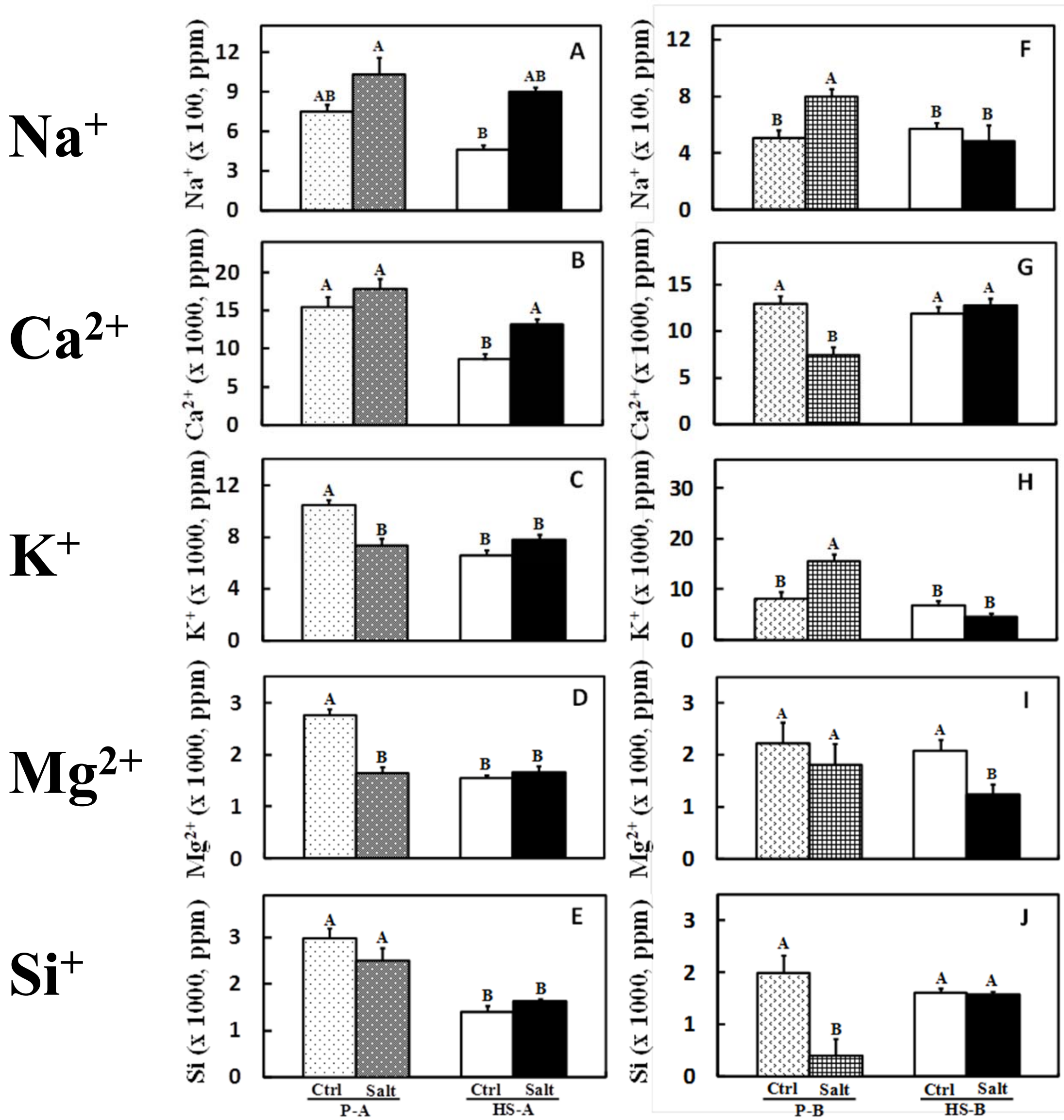
Maintained Leaf Number in Selections



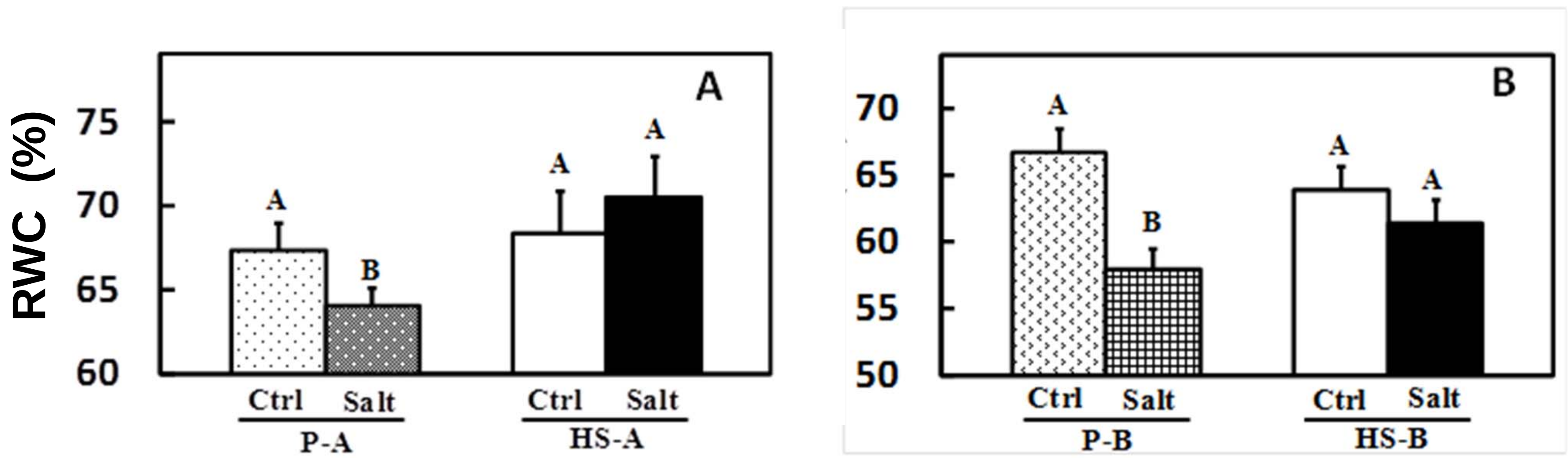
Higher Chlorophyll Content in Selections



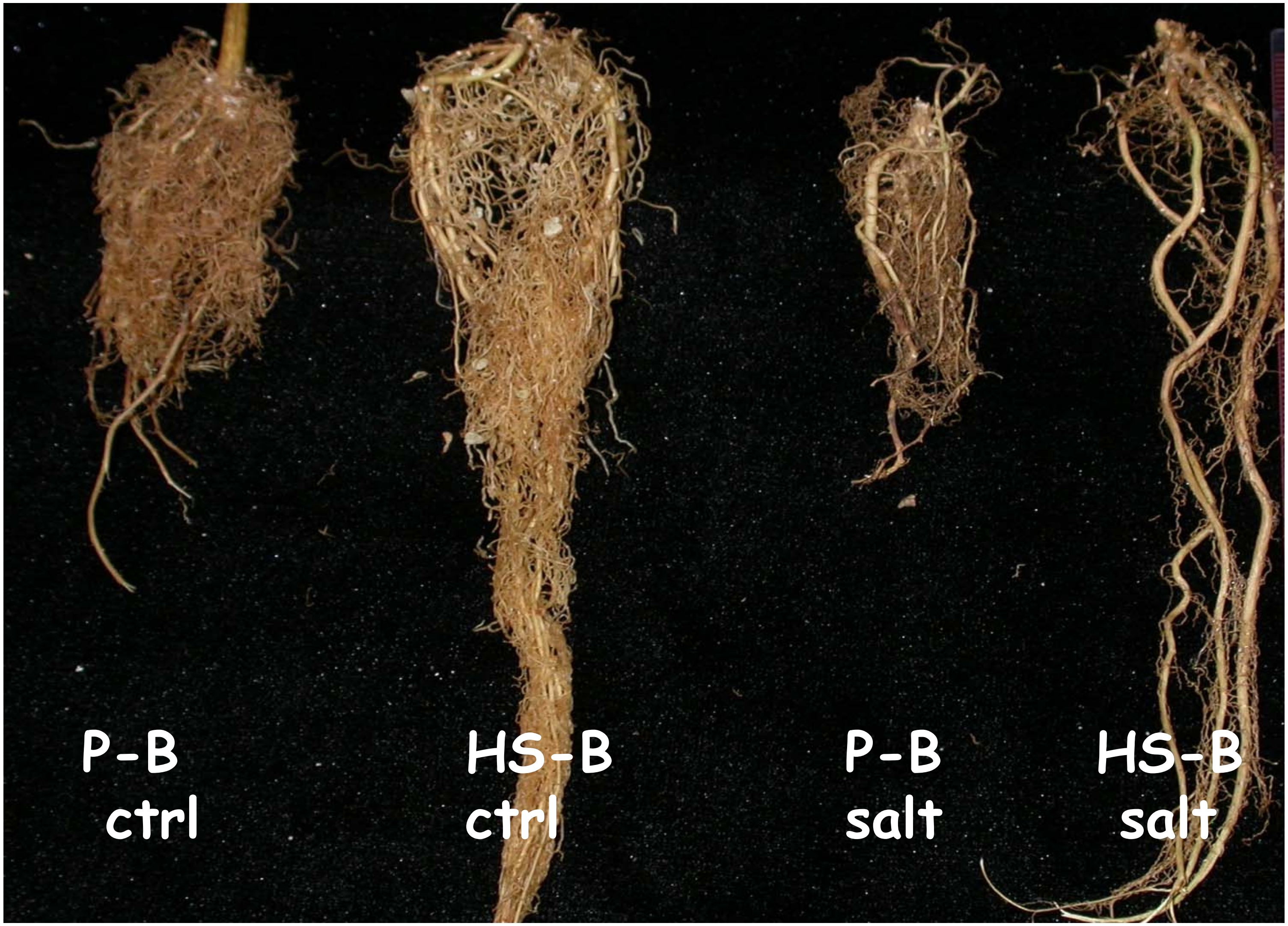
Inorganic Solutes Accumulation in Shoots



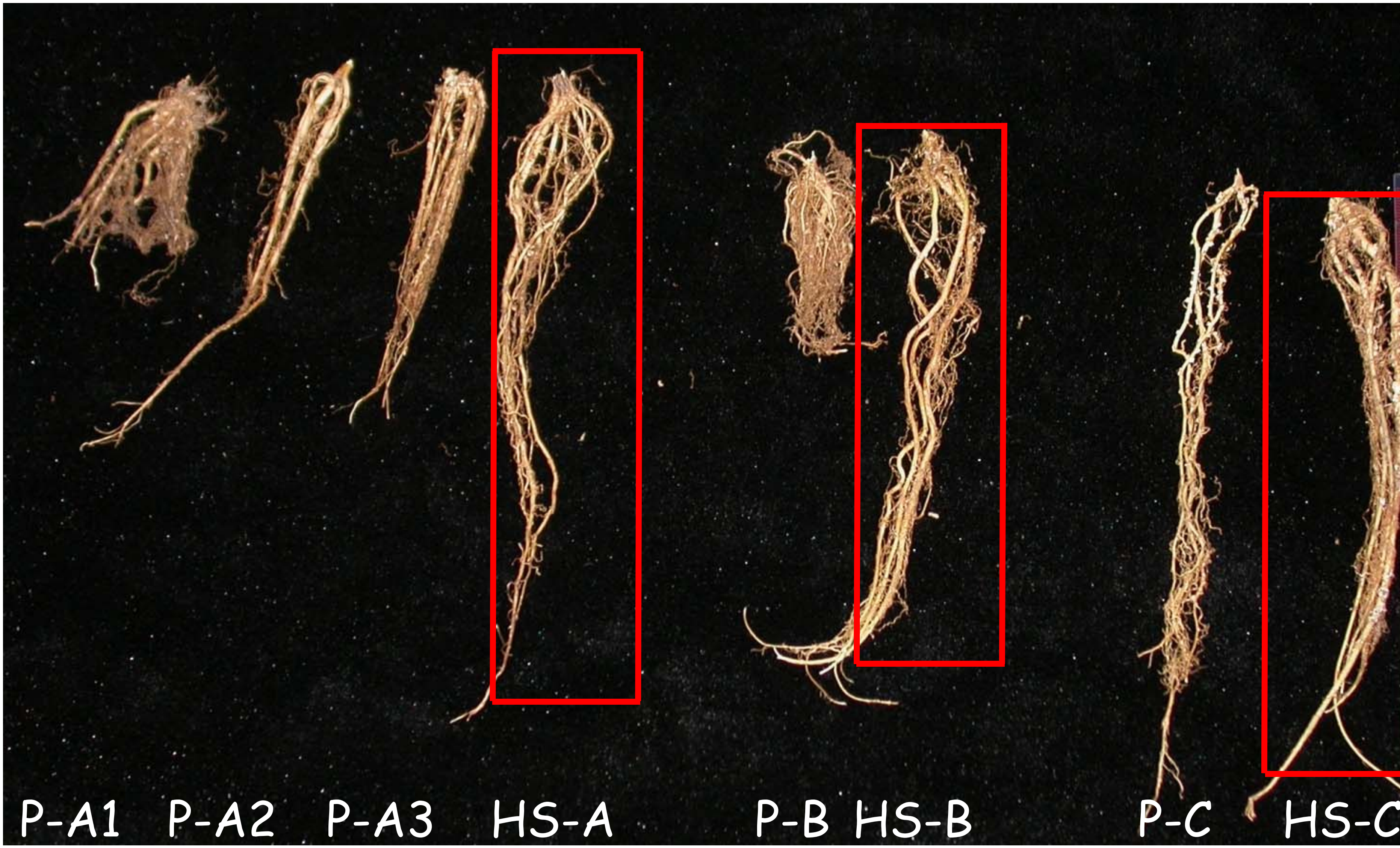
Maintained Relative Water Content



HS-B VS P-B Root After 7d at 12 DS



Root System After 7d at 12 DS



Summary: Salt Tolerance

Under salt stress, the selected lines HS-A & HS-B:

- Greater leaf number (72 & 84%)
- Better stem elongation (44%)
- Higher accumulation of chlorophyll (78 & 208%)
- Maintenance of RWC
- HS-B appeared to exclude Na^+
- Better root growth and biomass production in HS-A, HS-B and HS-C

Physiological Mechanisms in Salt Tolerance

Under salt stress, the selected lines HS-A & HS-B:

- Higher accumulation of chlorophyll – less reactive oxygen species (ROS) ?

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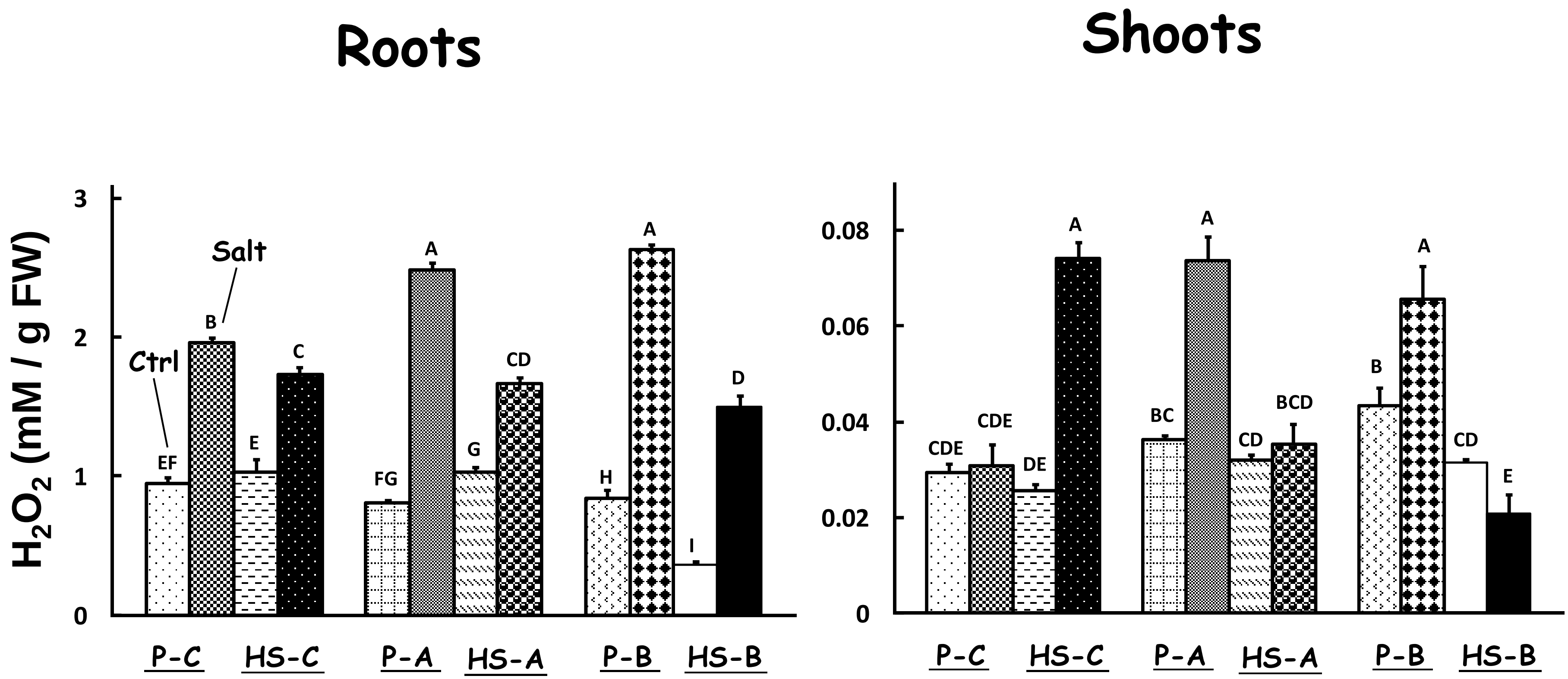
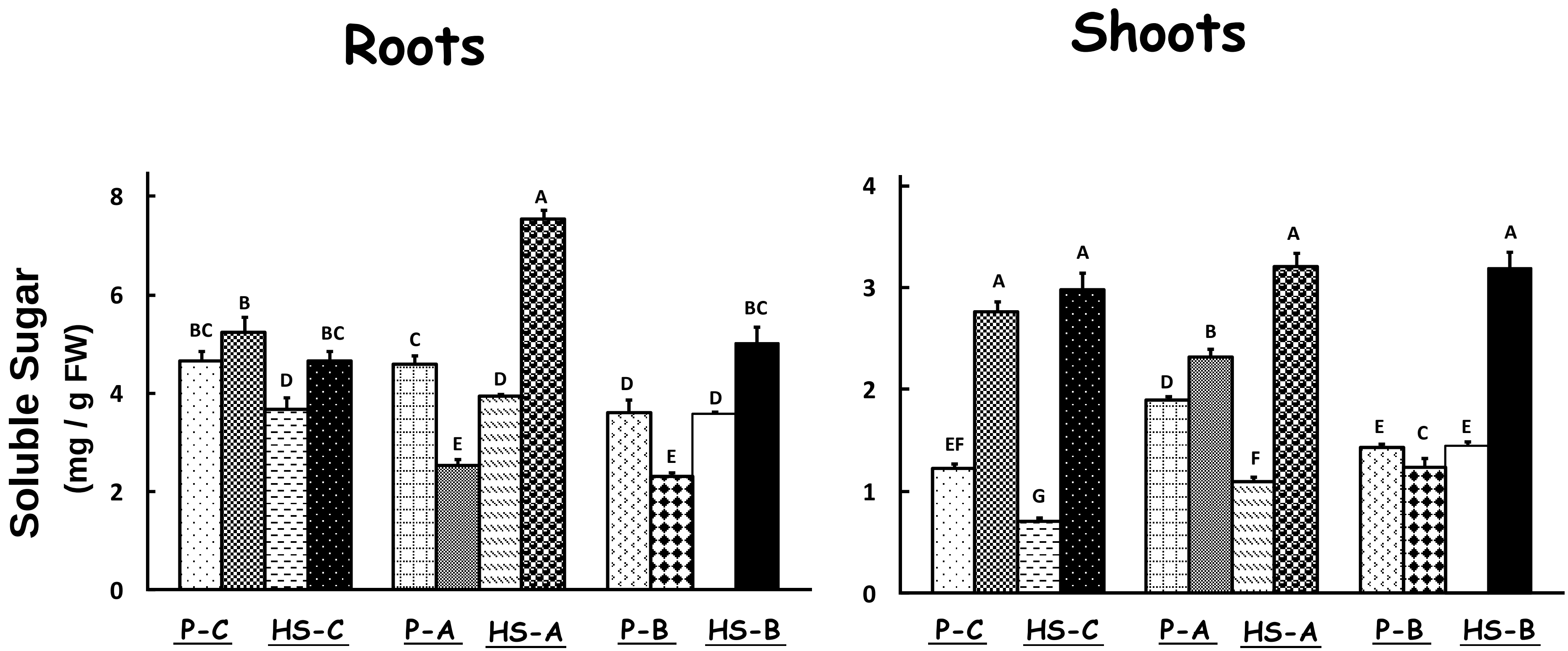
- Higher accumulation of chlorophyll – less reactive oxygen species (ROS) ?
- Maintenance of RWC – accumulation of osmotic solutes ?
- HS-B appeared to exclude Na^{+} - Na is located outside of the cell?

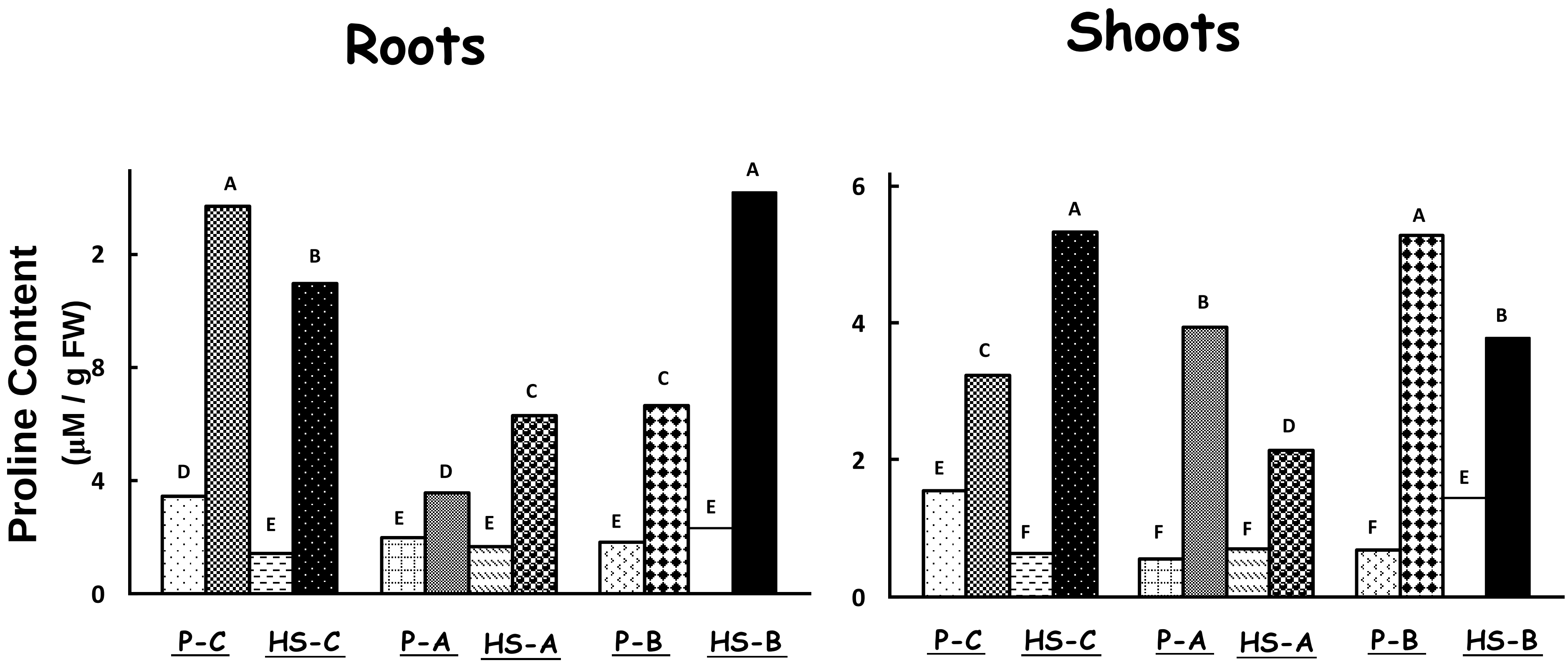
Less amount of ROS in selected genotypes



More soluble sugars in selected genotypes

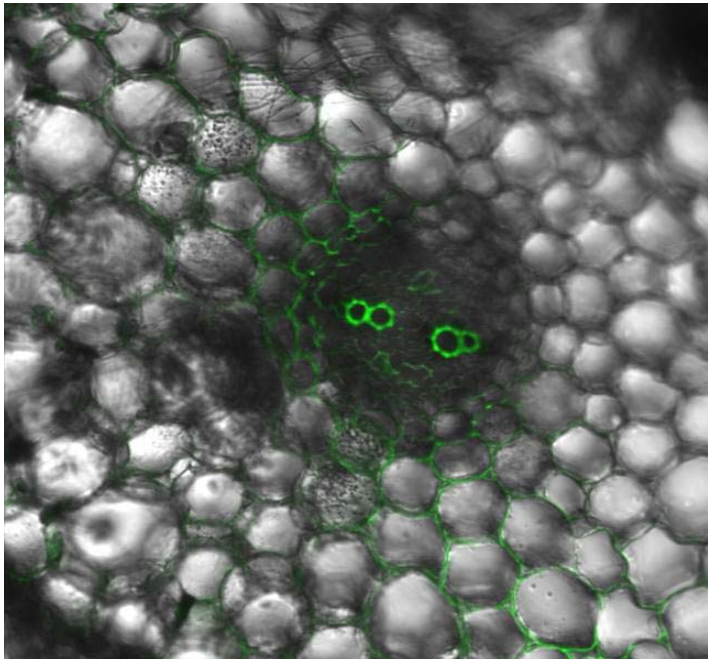


Proline Accumulation

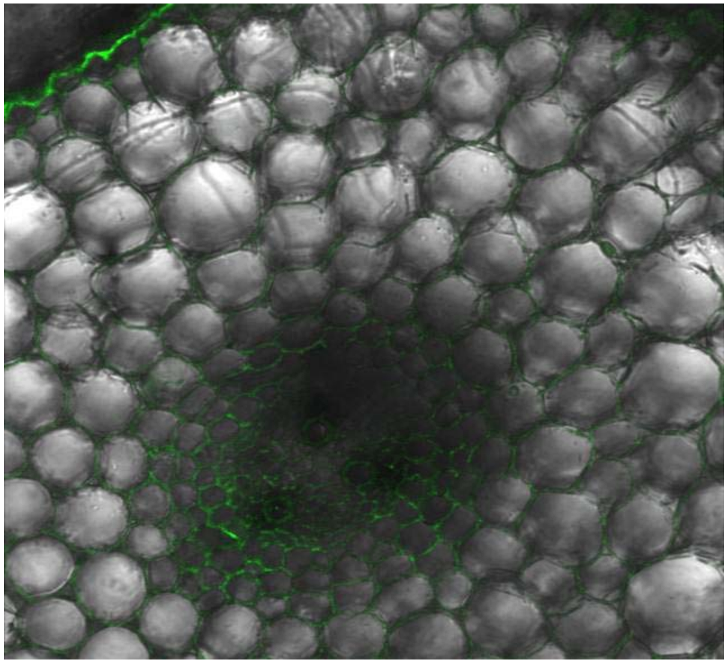


Na Localization using fluorescence dye

P-B



HS-B

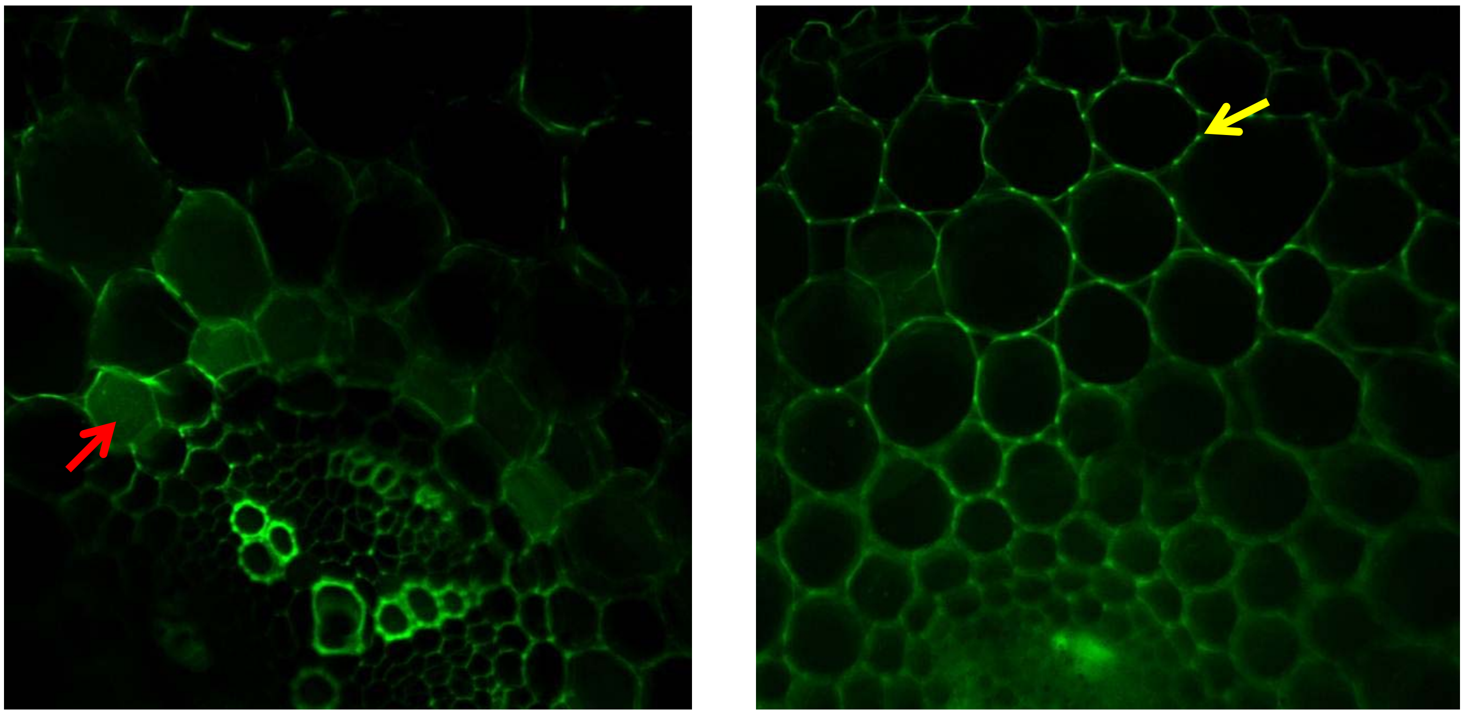


Root section W/O Salt treatment [FITC & UV, 10x] under confocal microscopy after 12 hrs staining with CoroNa-Green.(λ_{exc} = 543 nm, λ_{em} = 500-540 nm, XYZ scanning mode, image volume= 150 µm, thickness= 3µm)

o10 µM Cell-permeant CoroNa-Green Sodium Indicator (C-36676, Invitrogen)

Na Localization using fluorescence dye

P-B **HS-B**



Staining was done 12 hours after 9 dS/m (~90 mM NaCl) salt treatment [FITC & UV, 10x] In parental line (P-B) Na can enter root parenchyma cells (red arrow) and accumulated highly in xylem tissue. Selected plants (HS-B) however showed strong accumulation in cell walls (yellow arrow), especially high in the intercellular junction area of adjacent cells

Summary: Salt Tolerance

Under salt stress, the selected lines HS-A & HS-B:

- Less ROS amount in roots and shoots
- Greater accumulation of soluble sugar in roots and shoots
- Greater proline accumulation in roots
- Na^+ are seemingly localized in cell walls and intercellular space.

Cold Stress Tolerance

- Screening
- Physiological characteristics – electrolyte leakage assay
- Expression of cold responsive genes

Plant Materials

- Plant Materials

Alfagraze

Wind River

Don

SD-201

River Side

Bcbb-04

Chbb-04

Mt-0

A-1991

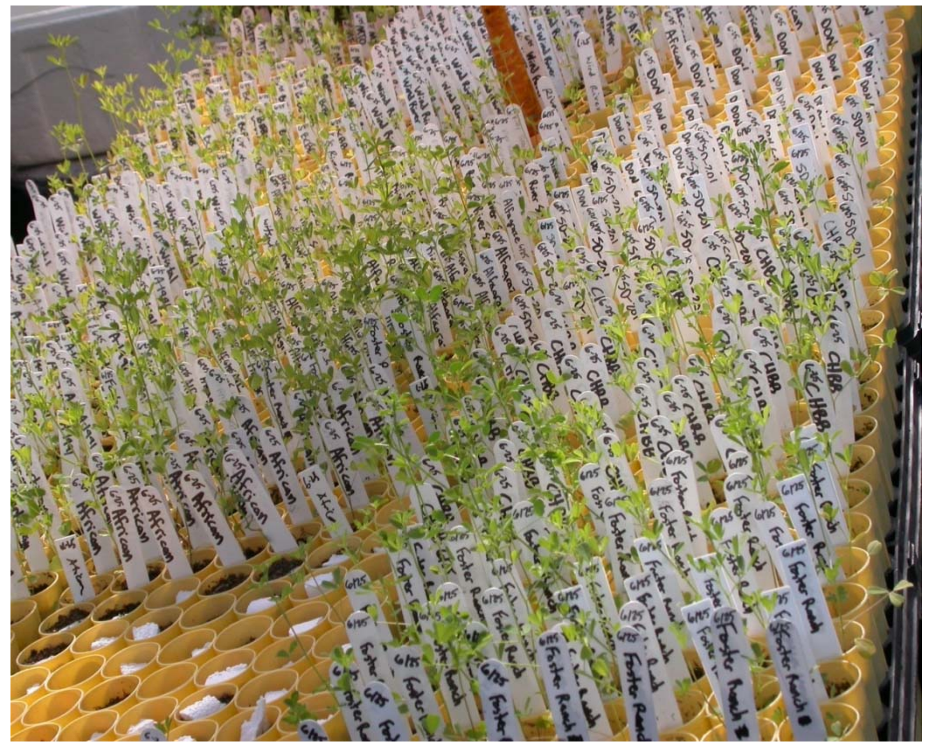
Foster Ranch

Apica

Caribou

Cuf-101

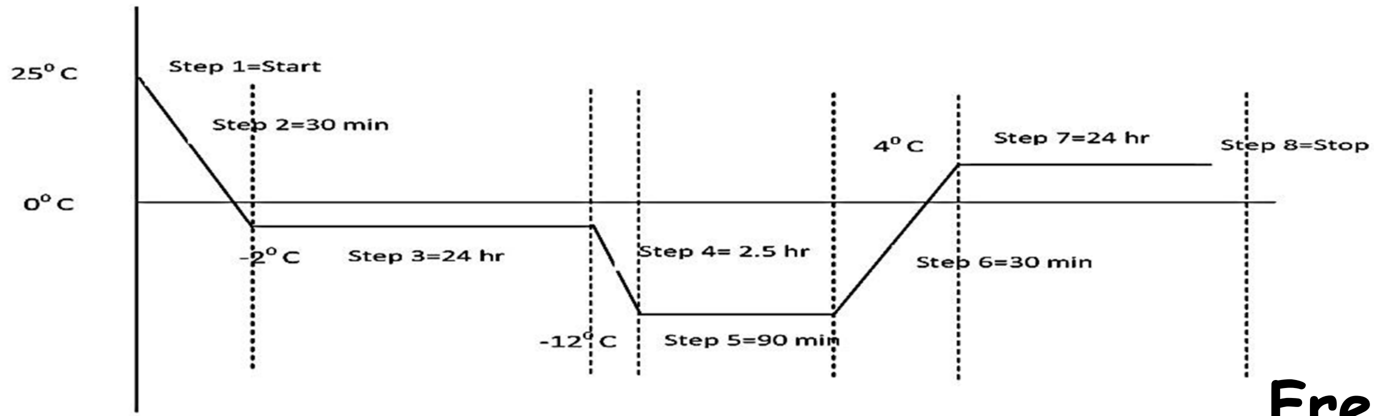
1. Screening of Cold Tolerance in Alfalfa



Freezing Test Program

May 23, 2010

Freezing Test Program: The freezing test program shown on sketch bellow.



Freeze Chamber

Step 1#: Setpoint 1=25°C, 1 second. Starts program at 25°C with 1 second as a starting point.

Step 2#: Setpoint 1=-2°C, 30 min. Decline the temperature down to -2°C in 30 min.

Step 3#: Setpoint 1=-2°C, 24 hrs. Holds at -2°C for 24 hrs.

Step 4#: Setpoint 1=-12°C, 2.5 hrs. Decline the temperature down to -12°C (at -2°C/30 min).

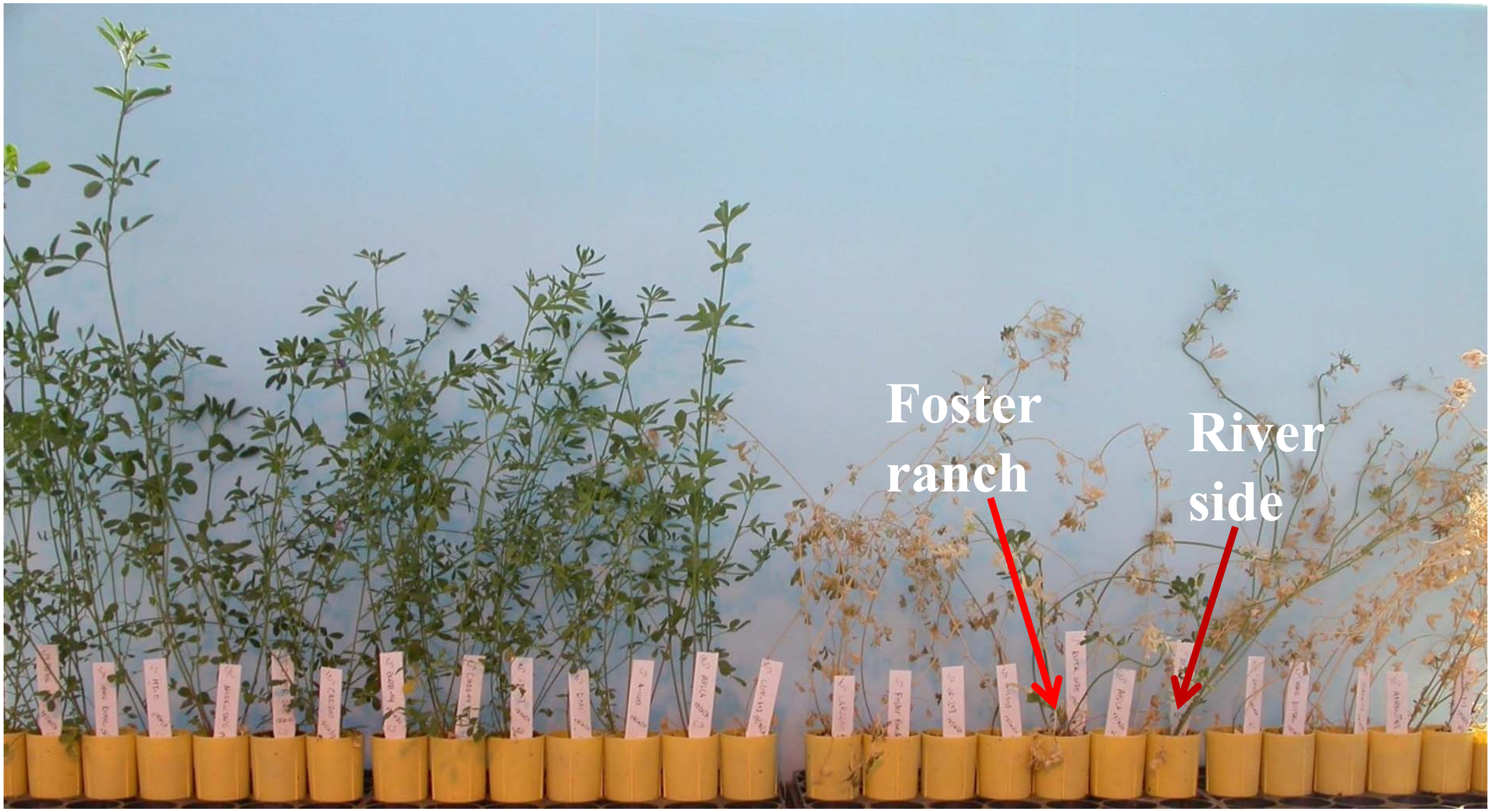
Step 5#: Setpoint 1=-12°C, 90 min. Holds at -12°C for 90 min.

Step 6#: Setpoint 1=4°C, 30 min. Ramps program up to 4°C in 30 min.

Step 7#: Setpoint 1=4°C, 24 hrs. Holds at 4°C for 24 hrs.

Step 8#: Stop program

Screening Results



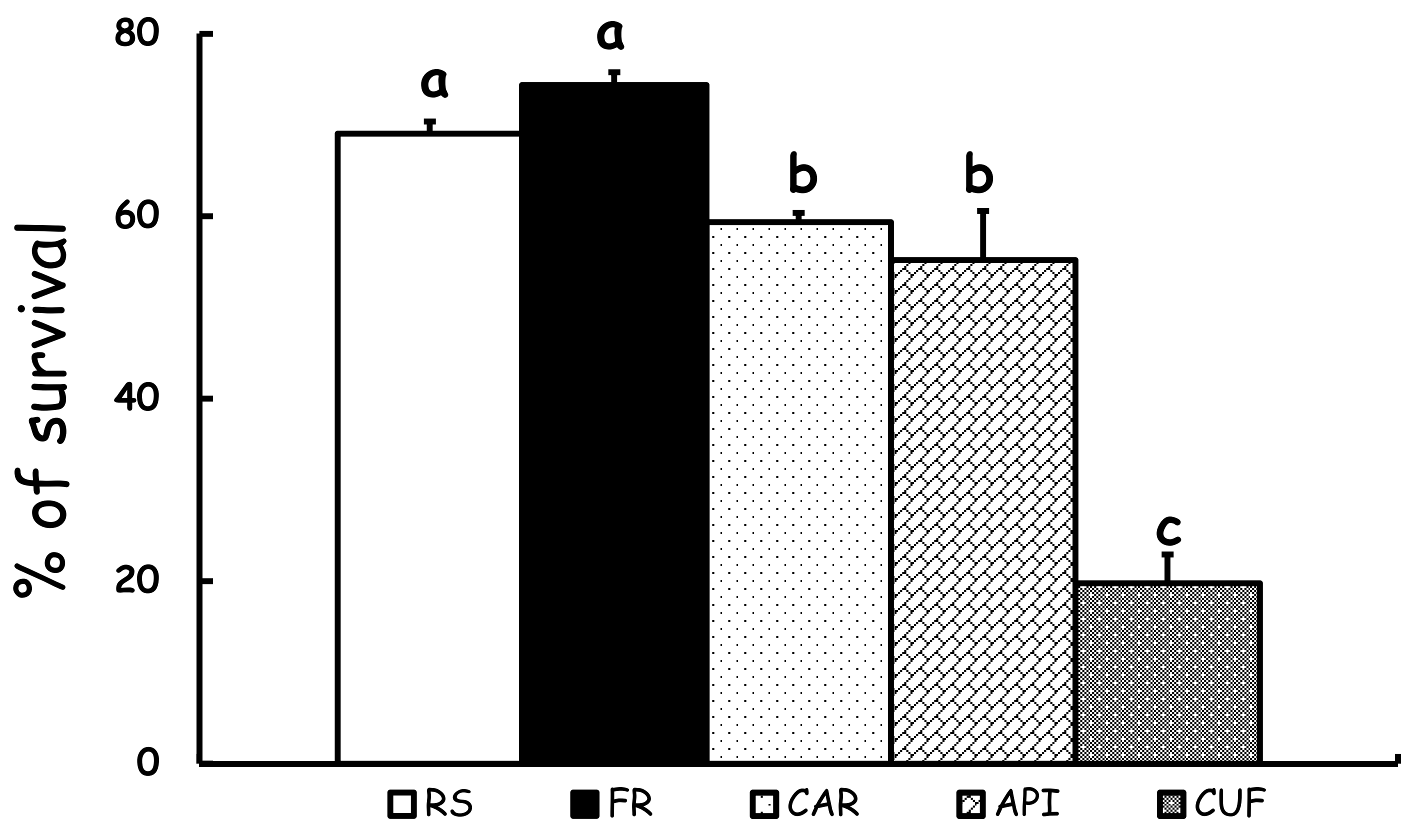
Control plants Cold treatments at -5^0 C

Freezing survival test of alfalfa seedlings. Temperatures were gradually dropped to $-5^{\circ}C$ and kept at the temperature for 1.5 h. Freezing treated plants were thawed at $4^{\circ}C$ for 24 h before returned to greenhouse. A few green ones (River side- "RS" and Foster ranch- "FR") on the right survived the freezing test.

Survival rate at different freezing temps

Cultivars	NA (-5°C)	CA (-5°C)	DA (-5 oC)	NA(-10 oC)	CA (-10 oC)	NA(-10 oC)
A-1991	44	100	44			
Alfagraze	56	100	67	89	78	33
BCBB-04	44	100	56			
CHBB-04	22	100	44			
Foster Ranch	56	100	67	78	100	61
Riverside	56	100	67	78	89	47
Wind River	44	100	44			
MT-0	44	100	56			
DON	11	78	22			
SD-201	11	89	22	0	78	33
Apica	44	100	44	78	67	67
Caribou	11	100	44	56	89	33
Ameristand	22	100	67			
CUF-101	11	67	22	11		33

Cold treatment at -10°C (Non-Acclimated)



Survival rate (%). Each Value represents the mean ± SE. The Different letter indicate significant differences ($p<0.05$) between treatments. The data combination of three freezing treatments (-5, -10 and -12 ^{0}C) of 24 tests ($p<0.05$).

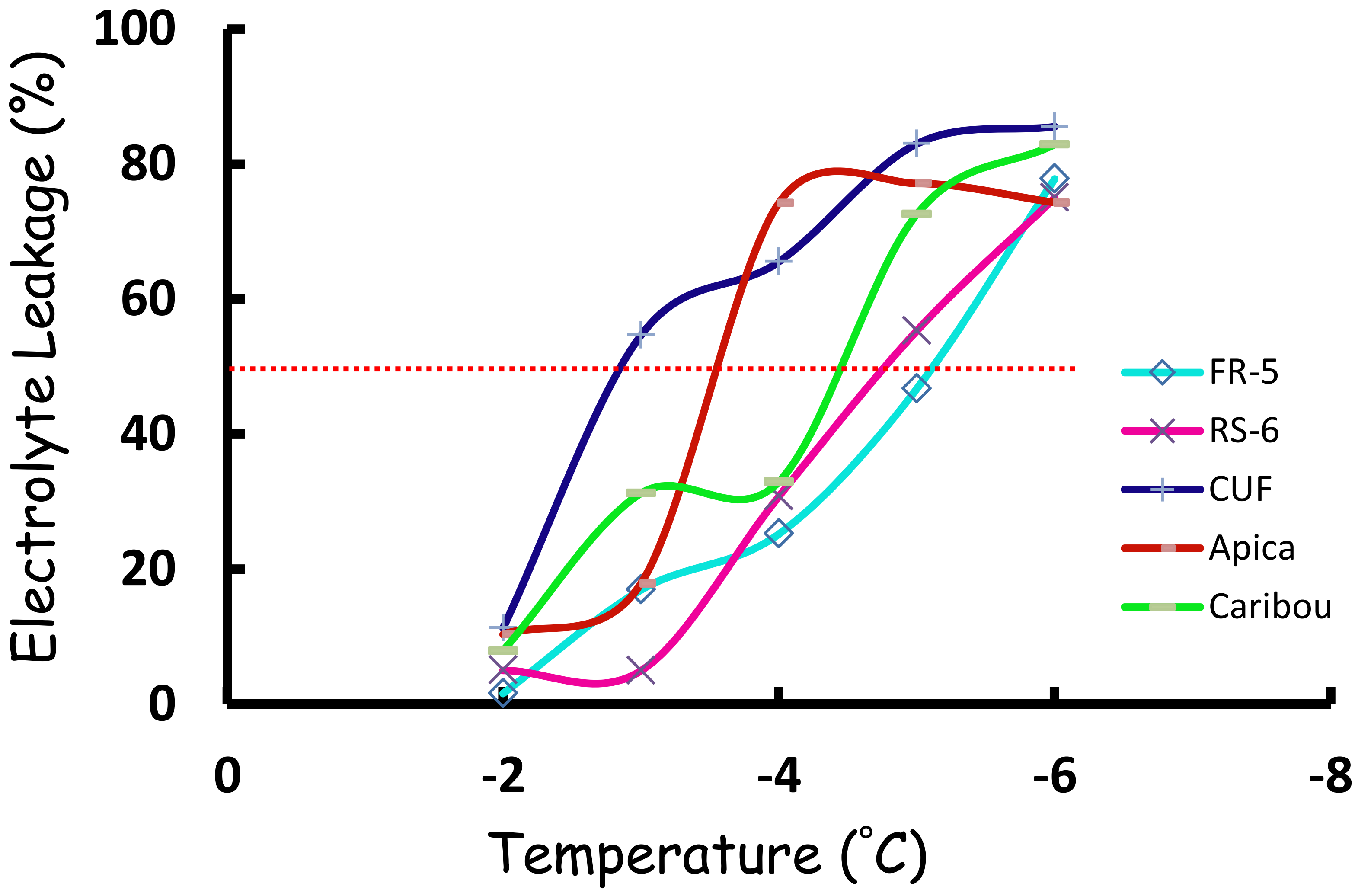
Selected VS Control 7d After -10°C Treatment

VS

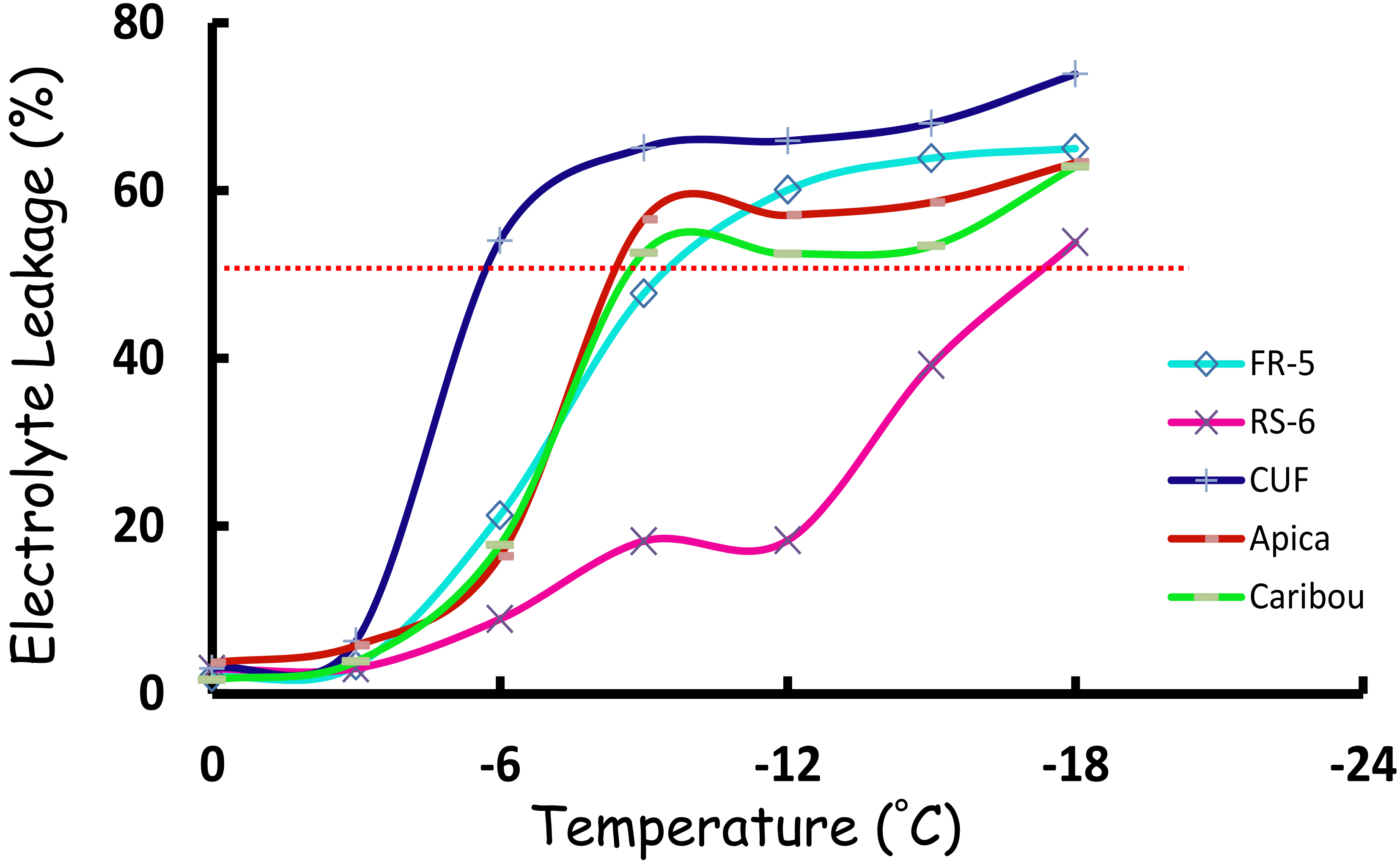
Selected Line

Control (PC, NC)

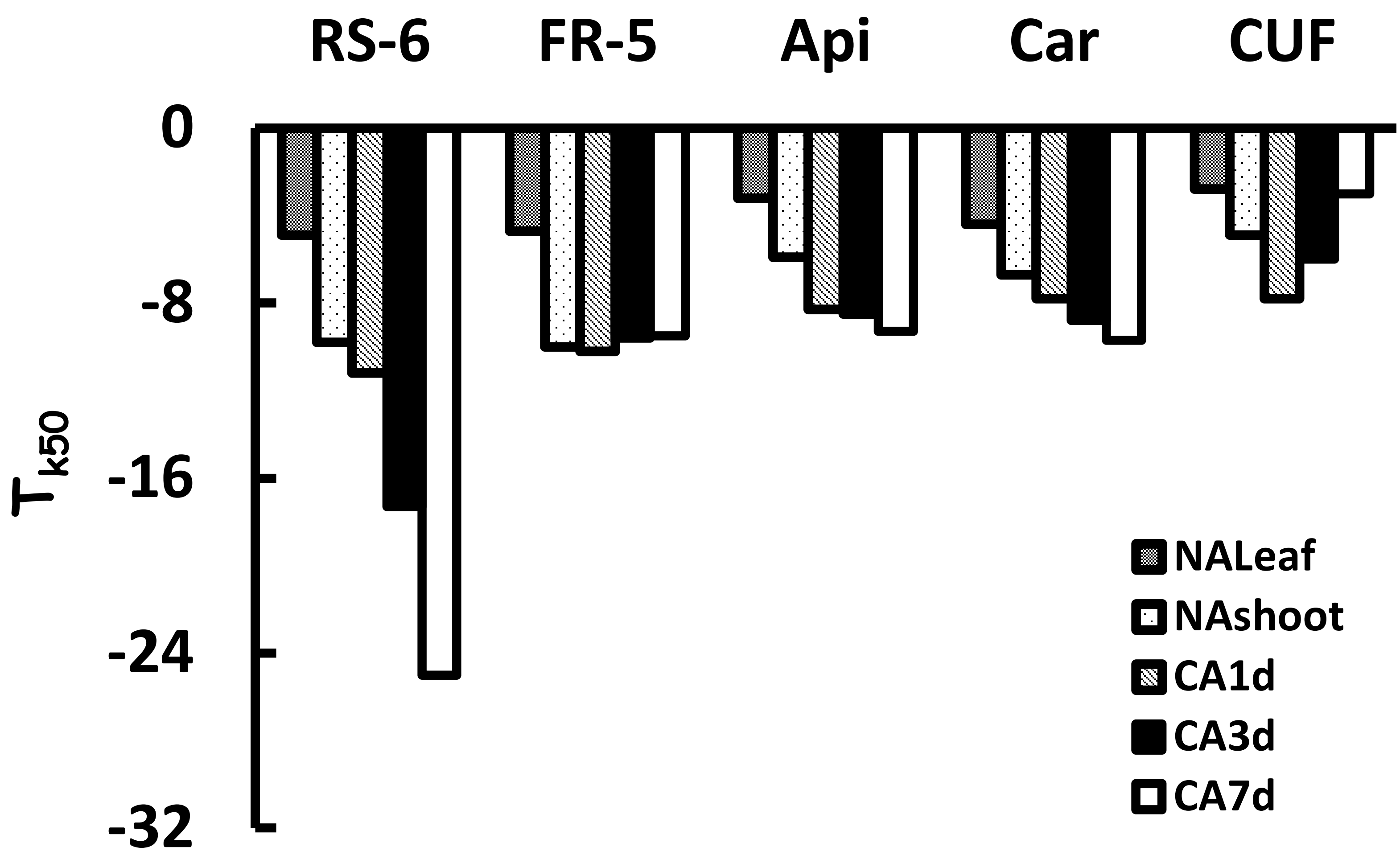
Leaf electrolyte leakage of non-cold acclimated plants



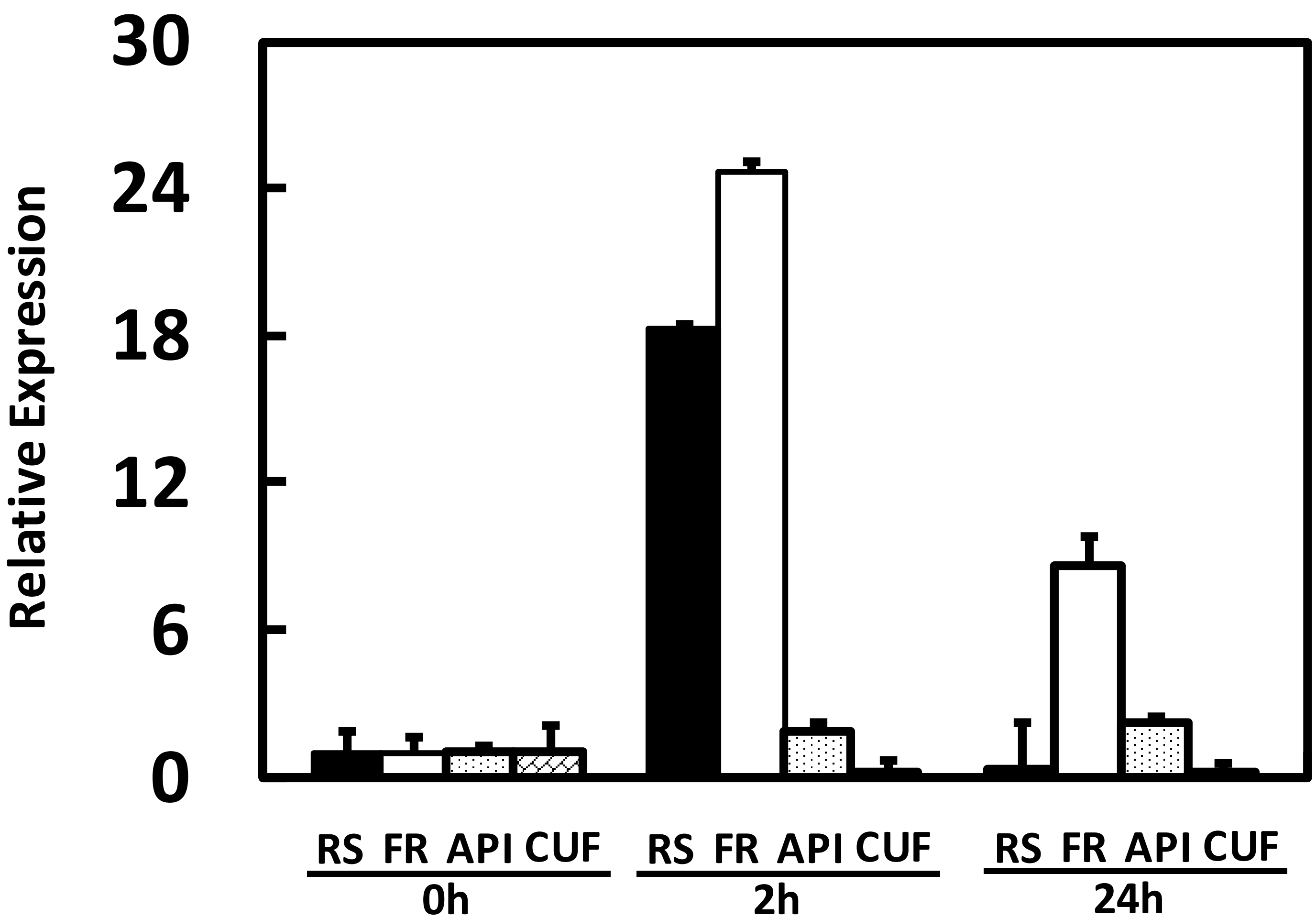
Leaf electrolyte leakage of cold acclimated plants



50% Killing Point (T_{k50} or LD_{50})

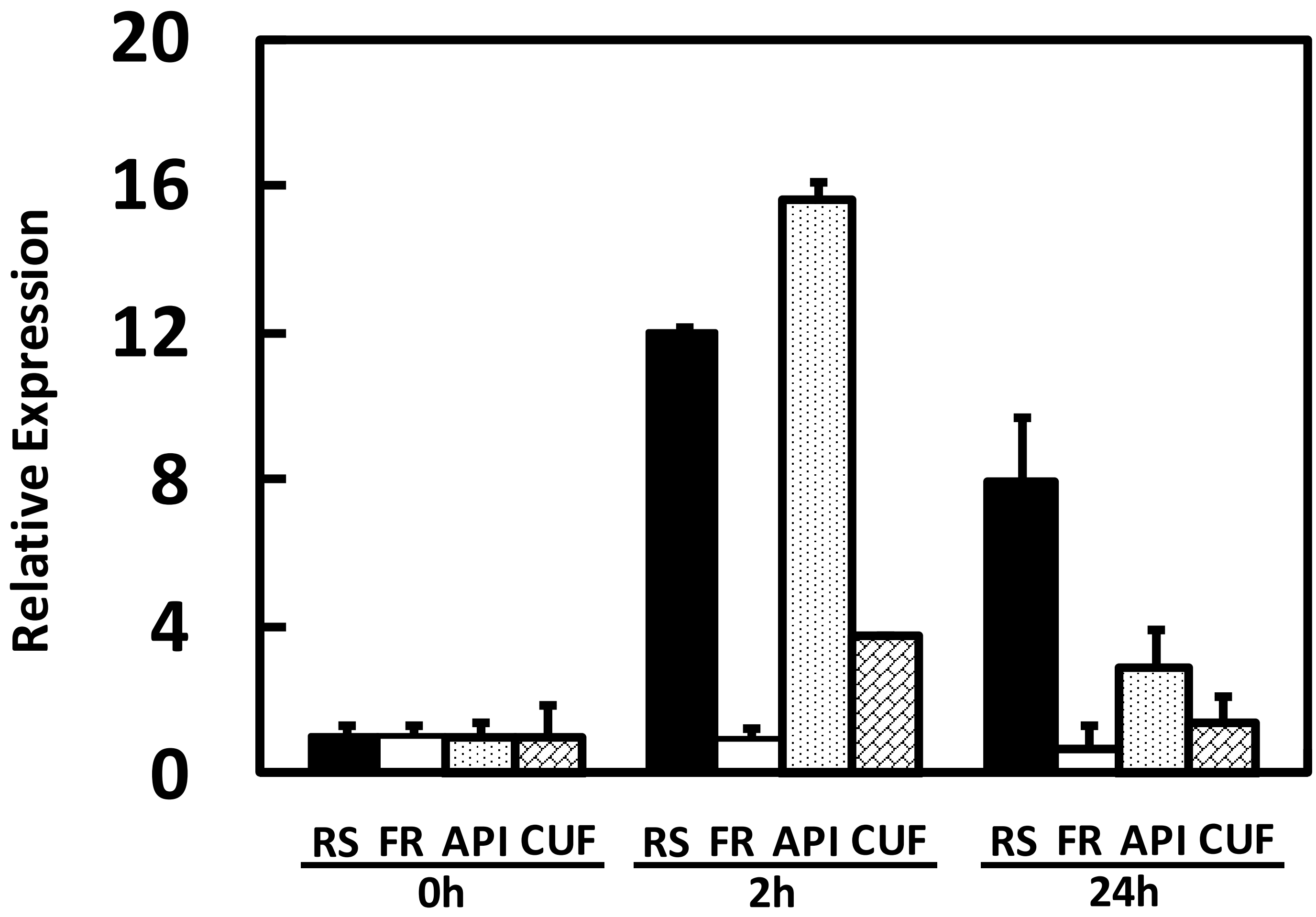


Gene Expression: *CBF1*



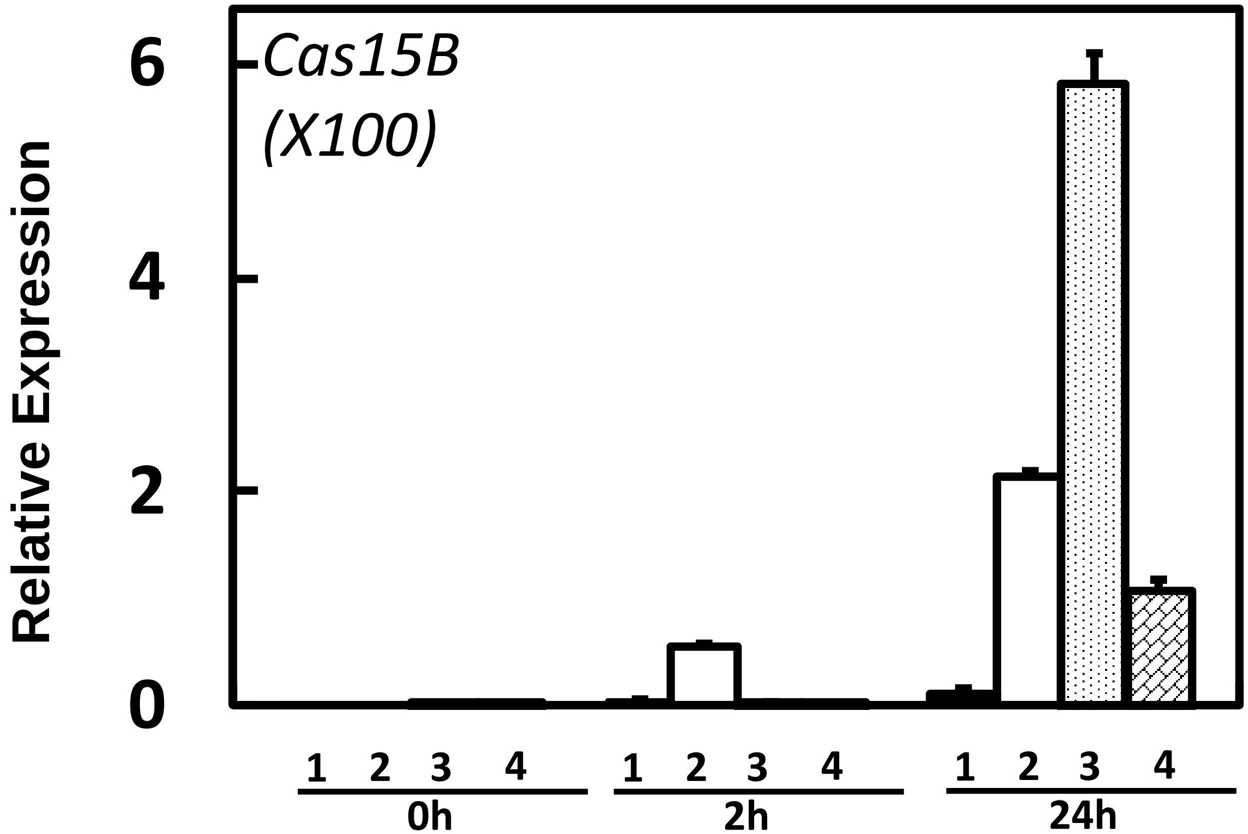
Change in CBF1 transcripts levels after cold treatment (2°C) in different genotypes.
RS: Riverside, FR: Foster Ranch, API: Apica, CUF: CUF101

Gene Expression: *CBF2*



Change in CBF2 transcripts levels after cold treatment (2°C) in different genotypes.
RS: Riverside, FR: Foster Ranch, API: Apica, CUF: CUF101

Gene Expression: *Cas15B*



Change in *cas15B* transcripts levels after cold treatment (2°C) in different genotypes.
RS: Riverside, FR: Foster Ranch, API: Apica, CUF: CUF101

Summary

- **Salt Stress**
  - ✓ Physiological analysis showed that the selected genotypes are more salt tolerant than their parental plants: better growth and biomass production, greener, and capable of maintaining RWC.
  - ✓ The salt tolerance is associated with lower ROS levels, greater accumulation of osmotic solutes, and limiting Na to enter the cells.

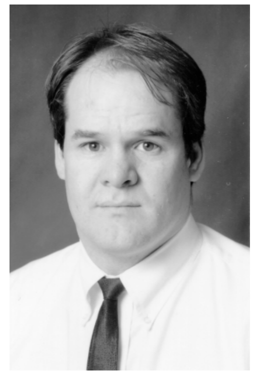
Summary

- **Cold Stress**
  - ✓ Our freezing tests suggested that two genotypes (River Side and Foster Ranch) have greater freezing tolerance as they have higher survival rate (%) , T_{k50}, lower EL (%) after freezing.
  - ✓ Gene expression analysis revealed that the selected genotypes showed more rapid and higher induction of known cold-responsive genes.

Summary

- Cold Stress
  - ✓ While CBF genes may play important role in freezing tolerance in the selected genotypes, specific genes involved and their regulation varied among genotypes.

Acknowledgements



Dr. Wu Dr. Mott Dr. Peel Dr. Fennell Dr. Boe

Thank You!