

New insights into the salt tolerance of alfalfa



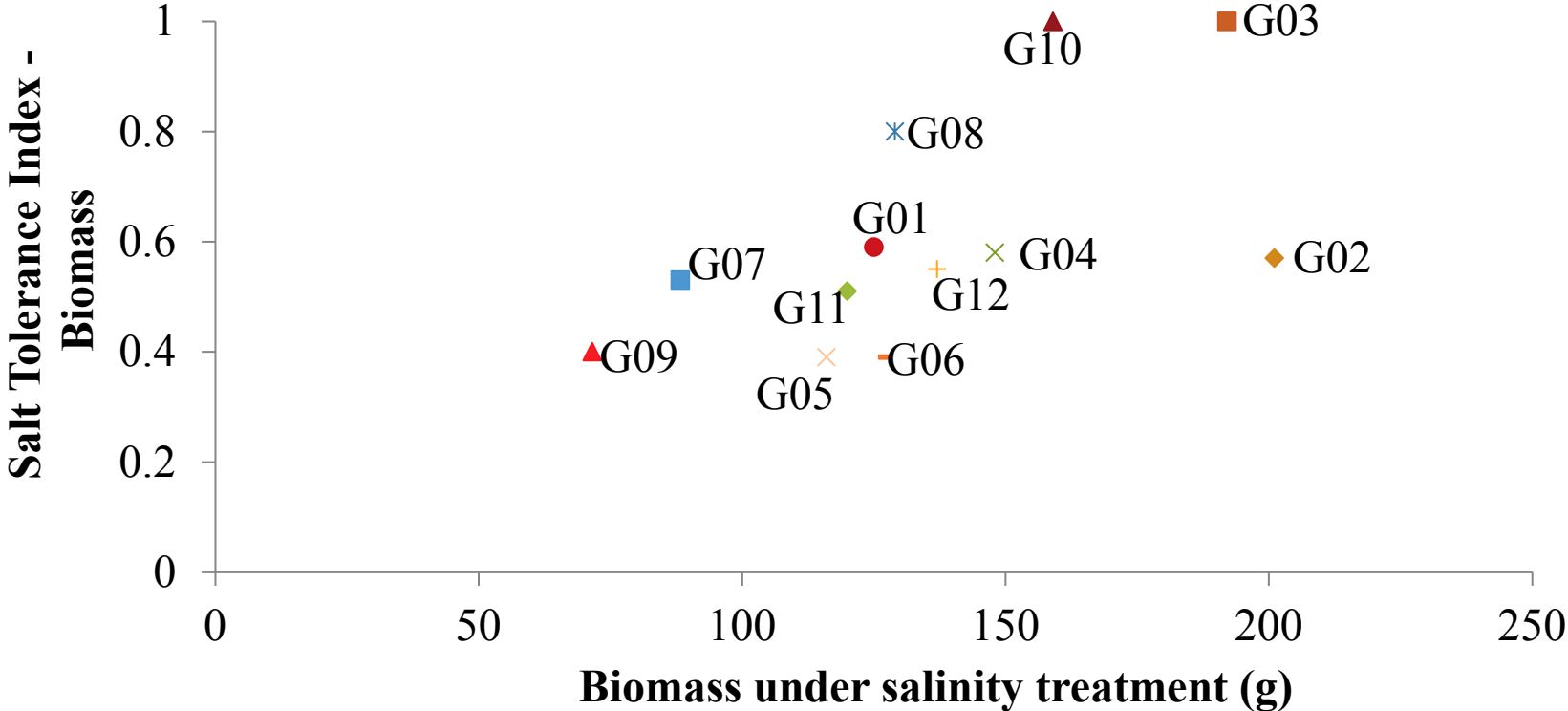
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Selection of Alfalfa genotypes based on Biomass production and Na, Cl, and K concentrations

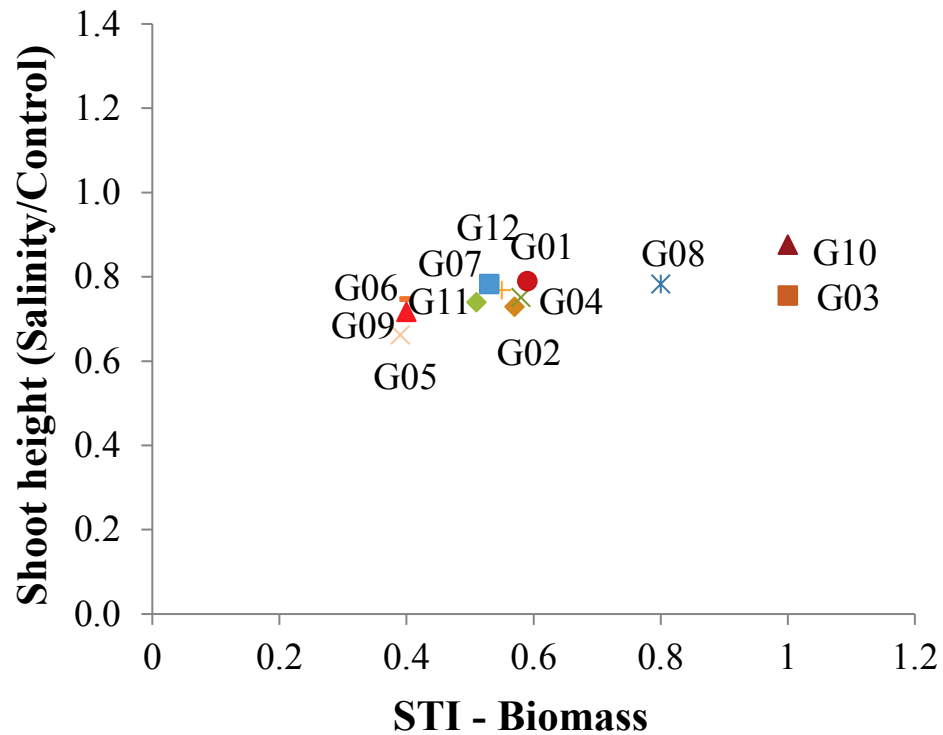
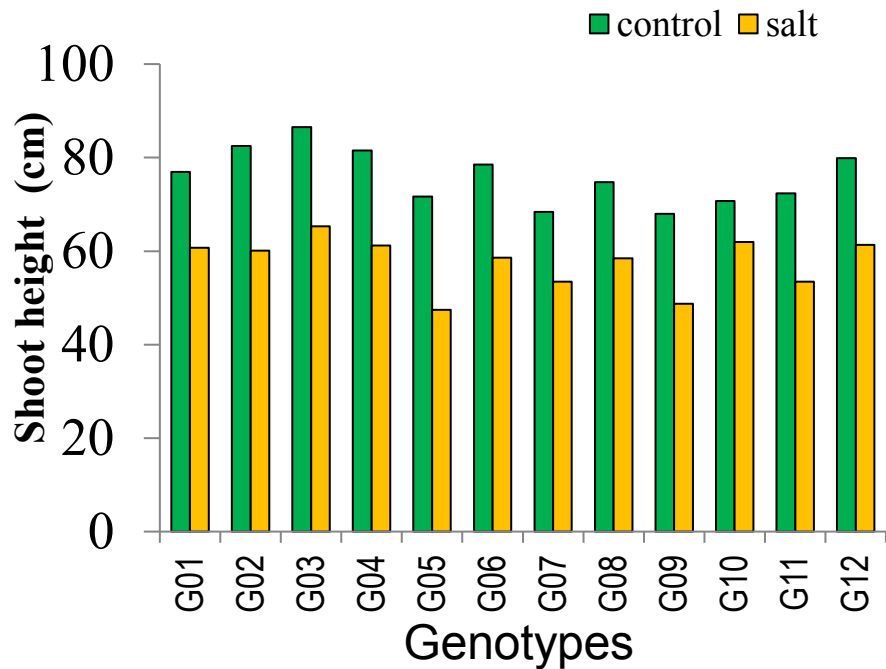
Genotype	#	Biomass per plant g (rank*)	Ion composition mmol kg ⁻¹ dw (rank)				Classification			
			Na	Cl	K	Total-S	Biomass	Na	Cl	K
		<i>Cl18.5 dSm⁻¹ (rank 1-60)</i>								
SISA 15	1	51.7 (1)	186 (56)	203 (56)	658 (40)	132	H	L	L	M
Cuf 101	2	51.5 (4)	539 (15)	393 (13)	533 (53)	135	H	H	H	L
SISA 14	3	43.5 (10)	186 (55)	176 (59)	685 (34)	123	H	L	L	M
Cuf 101	4	11.2 (39)	842 (1)	330 (23)	657 (41)	124	L	H	M	M
		<i>SO₄18.5 dS m⁻¹ (rank 1-60)</i>								
SISA 14	8	31.4 (1)	360 (40)	197 (44)	636 (39)	197	H	L	L	M
SISA 10	9	33.0 (25)	173 (60)	159 (56)	812 (9)	146	M	L	L	H
		<i>Cl24.5 dS m⁻¹ (rank 1-19)</i>								
SW9720	5	42.0 (1)	318 (7)	316 (10)	667 (13)	206	H	M	M	M
SISA 9	6	32.7 (6)	106 (19)	230 (17)	513 (19)	123	H	L	L	L
SISA 14	7	12.8 (11)	479 (2)	486 (2)	695 (9)	182	L	H	H	M
		<i>SO₄24.5 dS m⁻¹ (rank 1-21)</i>								
AZ-90 ST	10	51.7 (1)	331 (14)	161 (16)	692 (13)	161	H	L	L	M
SW9215	11	35.3 (2)	323 (15)	382 (5)	959 (2)	182	H	L	H	H
Salado	12	26.4 (8)	894 (4)	275 (11)	708 (11)	275	M	H	M	M

*Within treatments, rank 1 to higher ranks mean high to low biomass, and high to low K, Na and Cl contents.

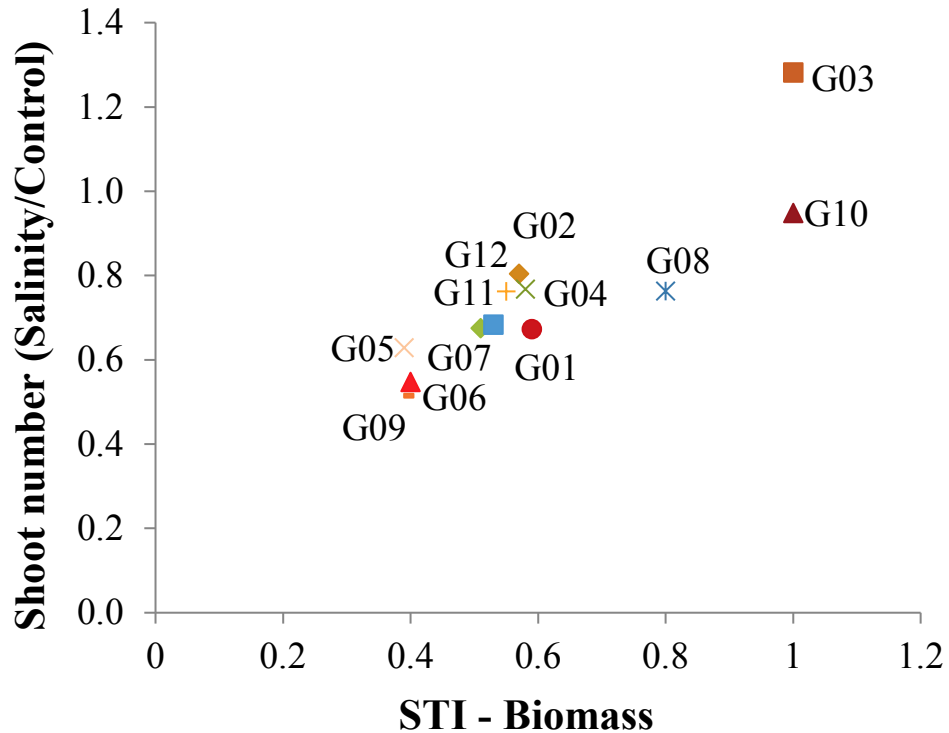
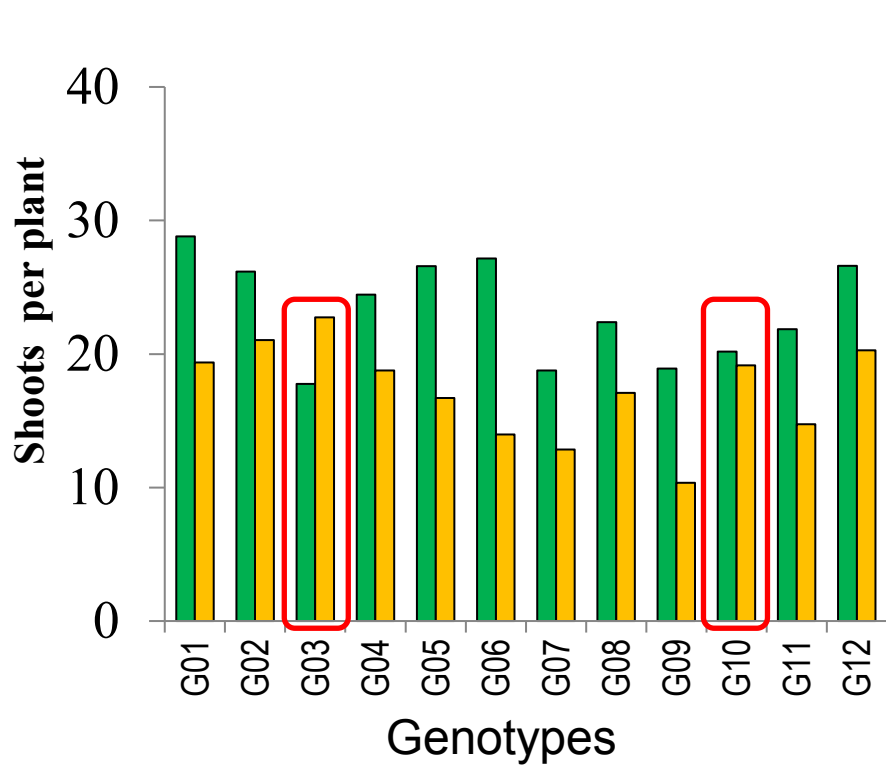
Salt Tolerance Index (STI) for biomass of 12 genotypes



Relationship shoot height with salt tolerance index (STI)

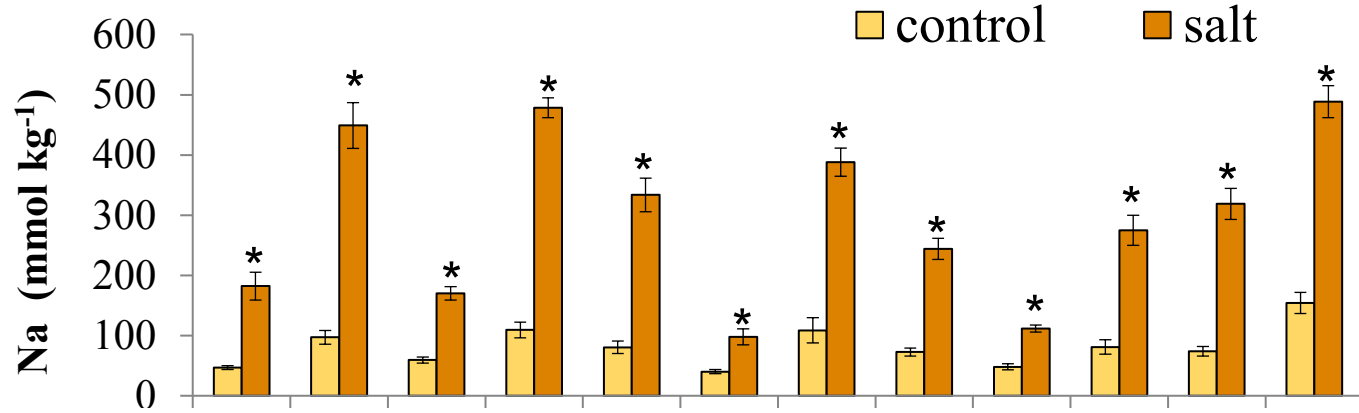


Relationship plant height and shoot number with salt tolerance index (STI)

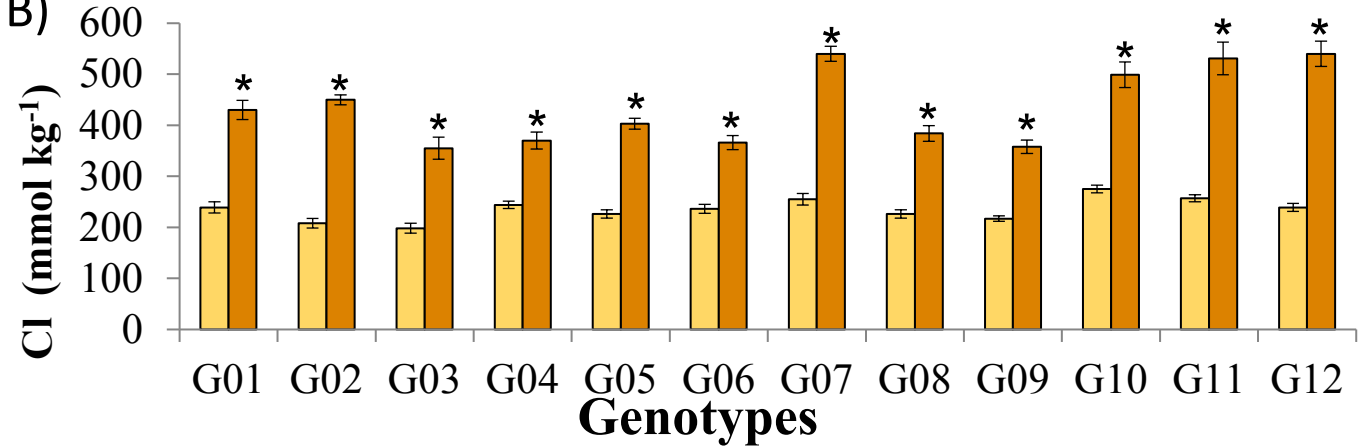


Shoot Na and Shoot Cl concentrations of 12 genotypes

A)



B)



Expression Analysis of 21 alfalfa genes

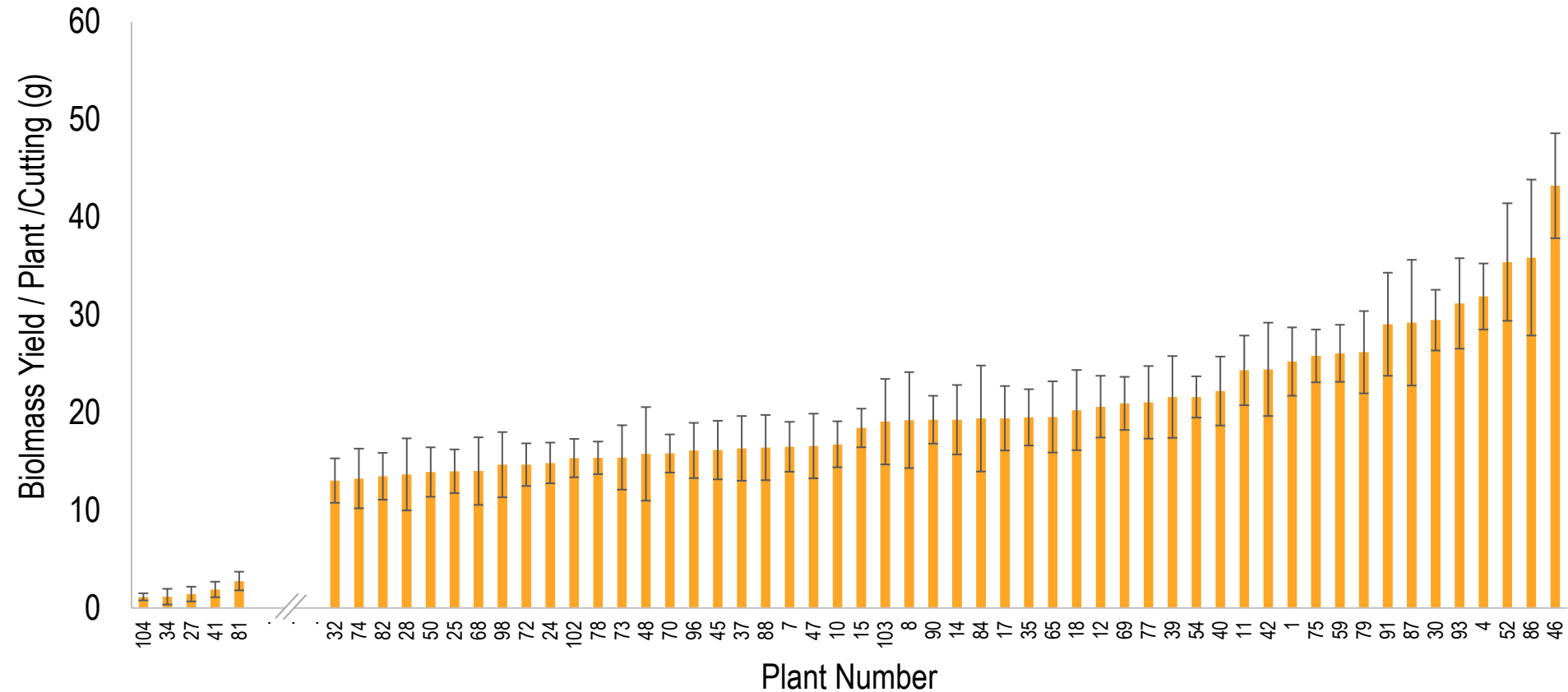


Crossing alfalfa genotypes based on the components of salt tolerance mechanisms and screening populations

Screening an F_2 population of a cross (G03 x G10) under salinity ($E_{c_{iw}}$ 18 dS m^{-1})



Biomass yield performance of a segregating population (G03 x G10) under salinity ($EC_{iw} 18 \text{ dS m}^{-1}$)

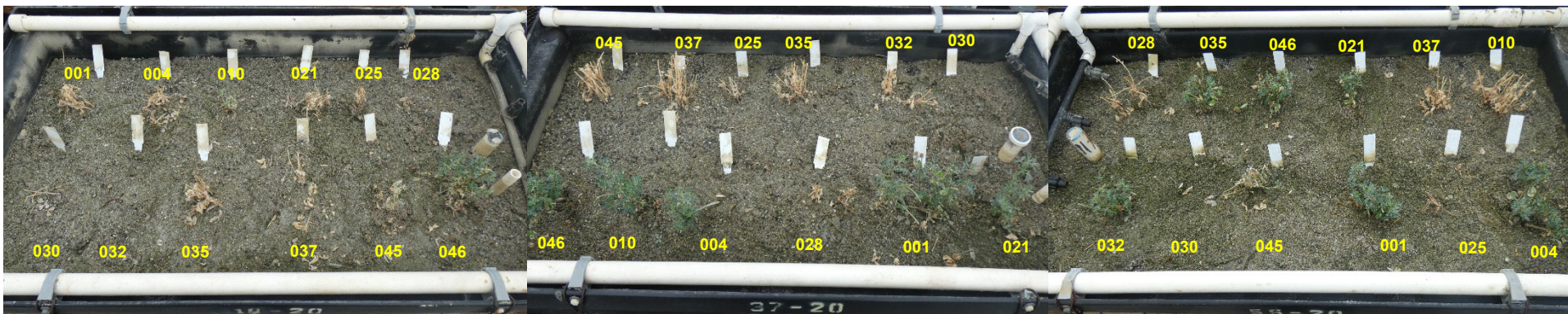


Screening selected genotypes for survival rate (EC_{iw} 27 dS m^{-1})

Control



Salinity



Screening selected genotypes for survival rate ($EC_{iw} 27 \text{ dS m}^{-1}$)

Plant # 46

Plant # 4

Plant # 37

Plant # 45



R1

R2

R3

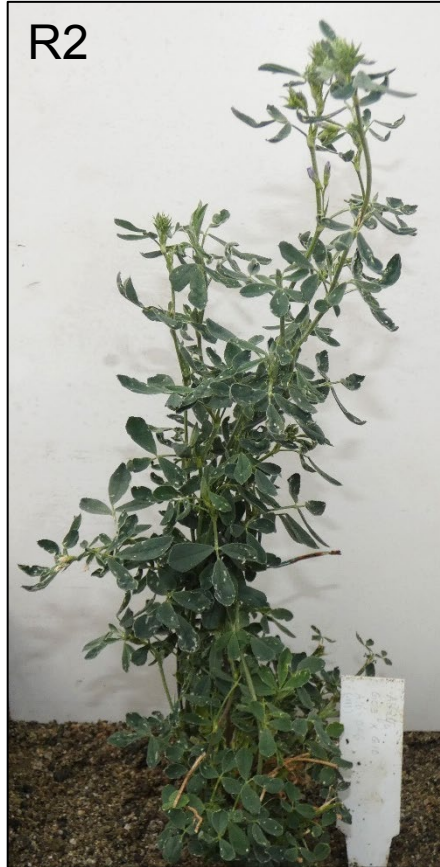
CONTROL SALT

CONTROL SALT

CONTROL SALT

CONTROL SALT

Plant # 46 after 3 months of salinity treatment ($EC_{iw} = 27 \text{ dS m}^{-1}$)



SUMMARY

- Selection based on total biomass and ion composition was highly efficient. Analysis on cloned plants was effective in clear discrimination between salt tolerant and salt sensitive genotypes
- Reduction in biomass under salinity was due to reduction in shoot number
- Gene expression analyses allowed us to classify genotypes based on their ability to regulate different components of the salt tolerance mechanism.
- Screening of a segregating population generated by crossing two salt-tolerant parents resulted in isolation of highly salt-tolerant genotypes that can tolerate salinity of $EC_{iw} = 27 \text{ dS m}^{-1}$.

ACKNOWLEDGMENTS

Funding: USDA-ARS

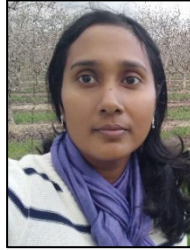
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**Dr. Don
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Undergraduate Students:



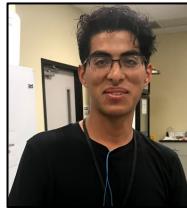
**Paul
Markley**



**Thomas
Forest**



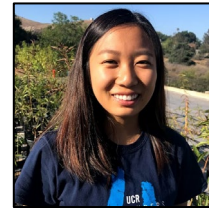
**Andrew
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**Marco
Duenas**



**Christina
Nguyen**



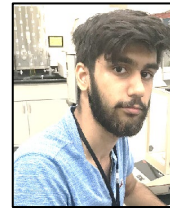
**Brianna
Song**



**Vanessa
Perez**



**Christian
Duenas**



**Ajashwar
Boparai**

Screening selected genotypes at EC 27 dS m⁻¹

A16-111-004



A16-111-046



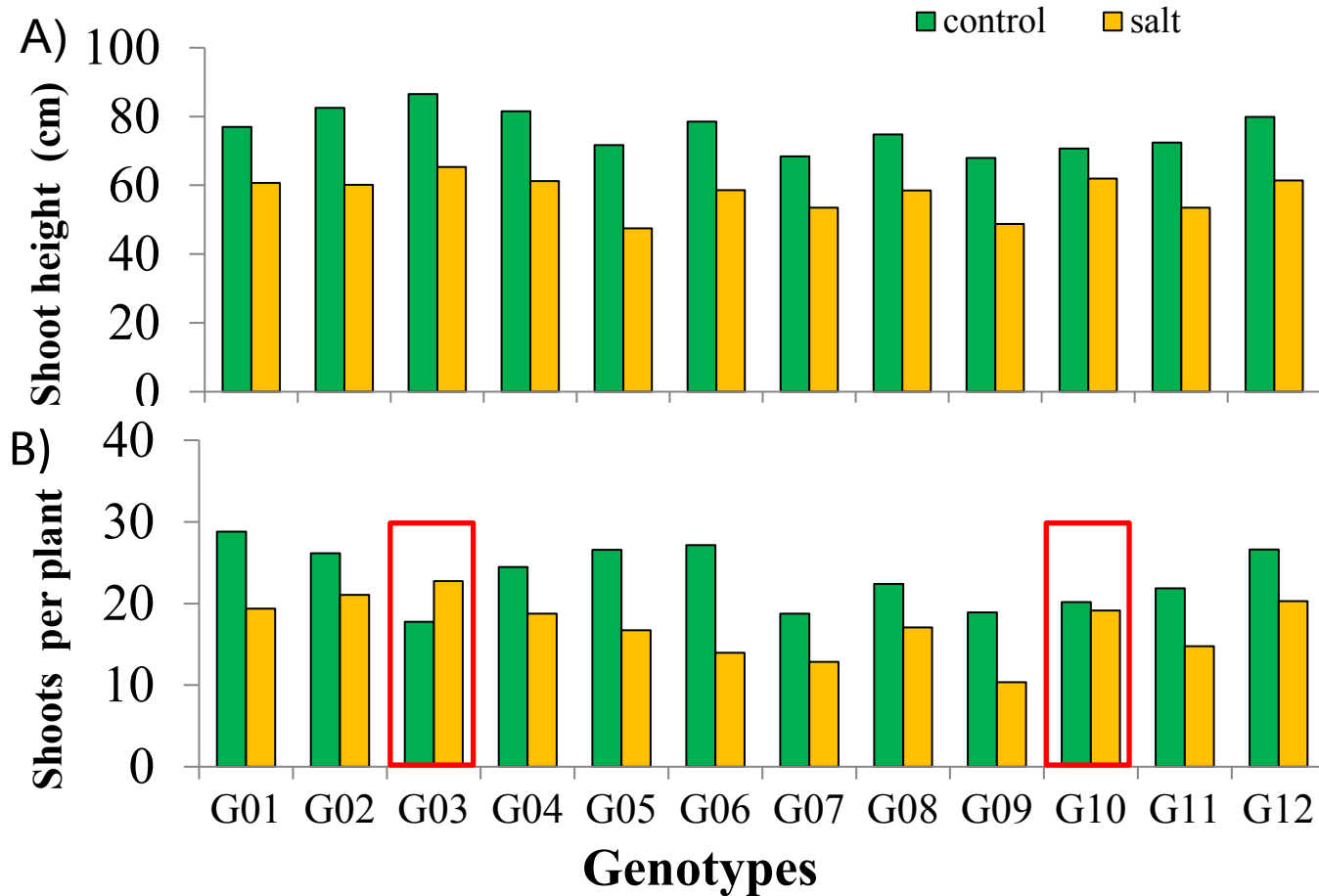
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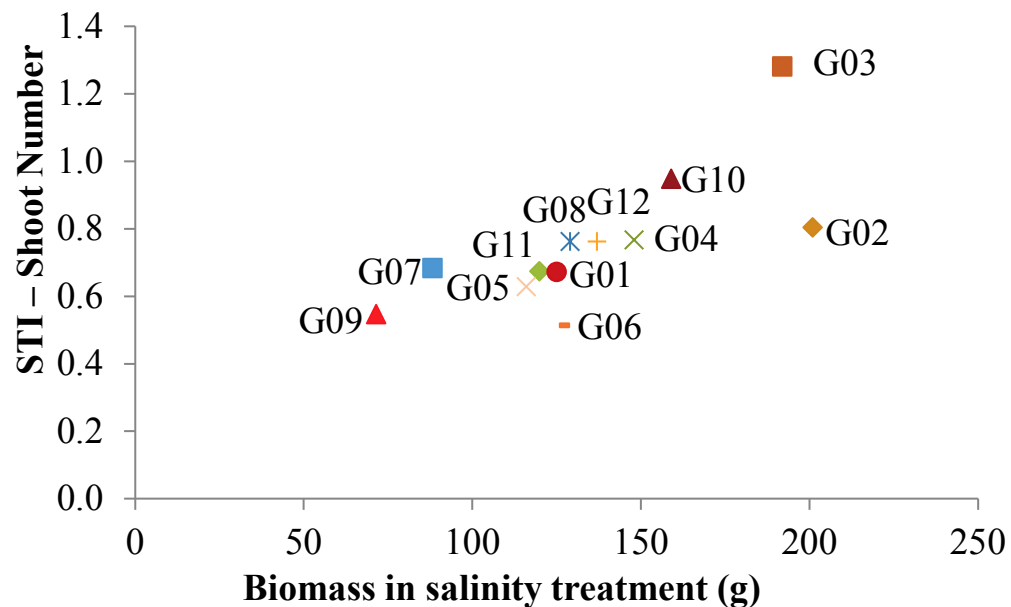
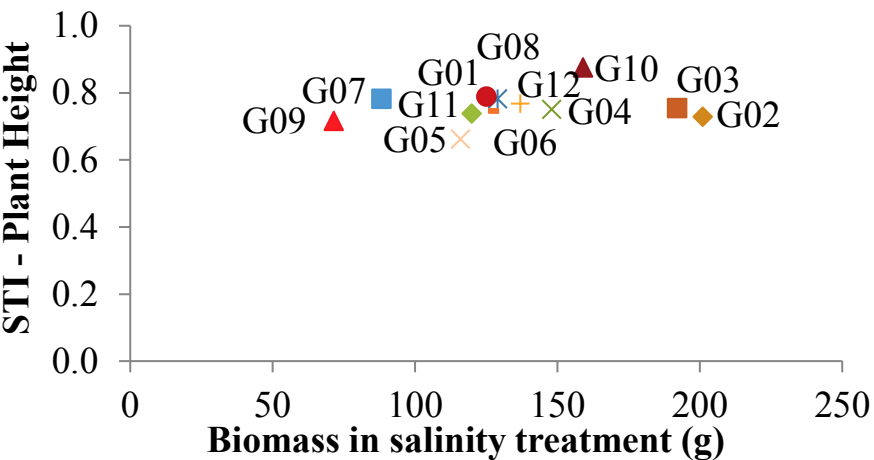
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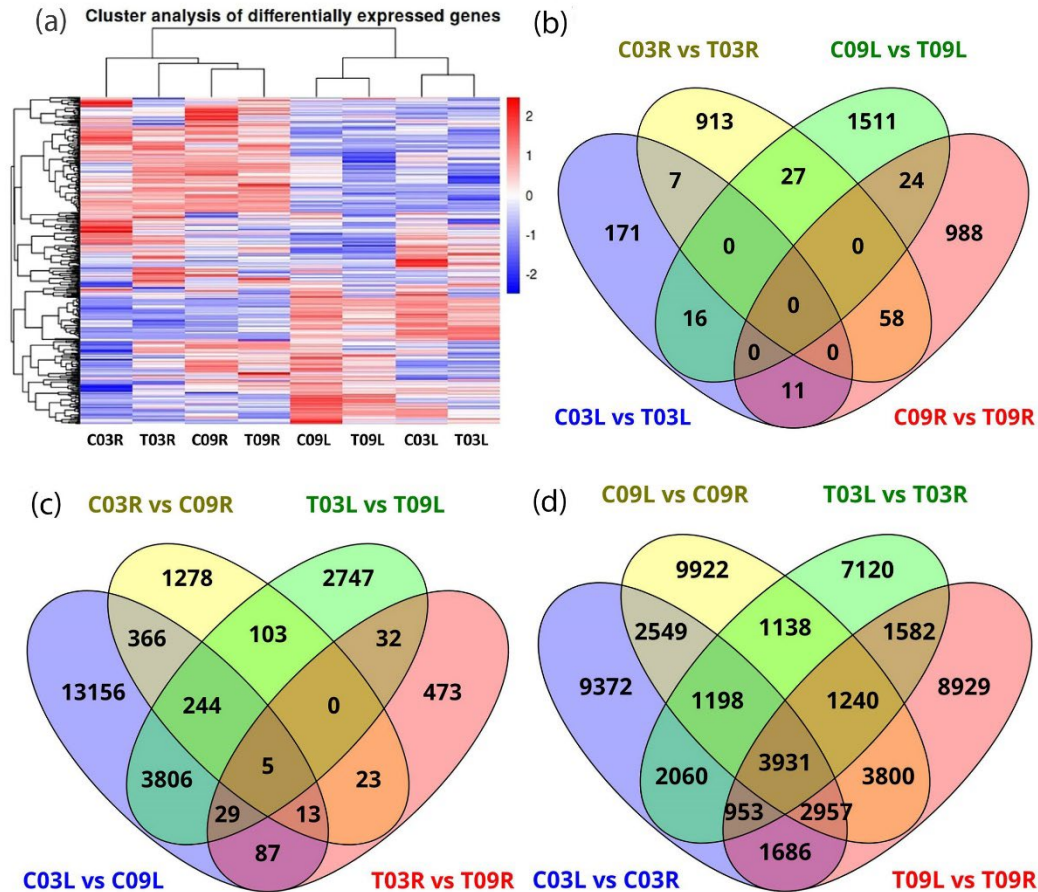
Shoot height and shoots per plant of 12 genotypes



Relationship between plant height and shoot number with biomass under salinity



Differentially expressed genes across eight samples



Important differentially expressed genes

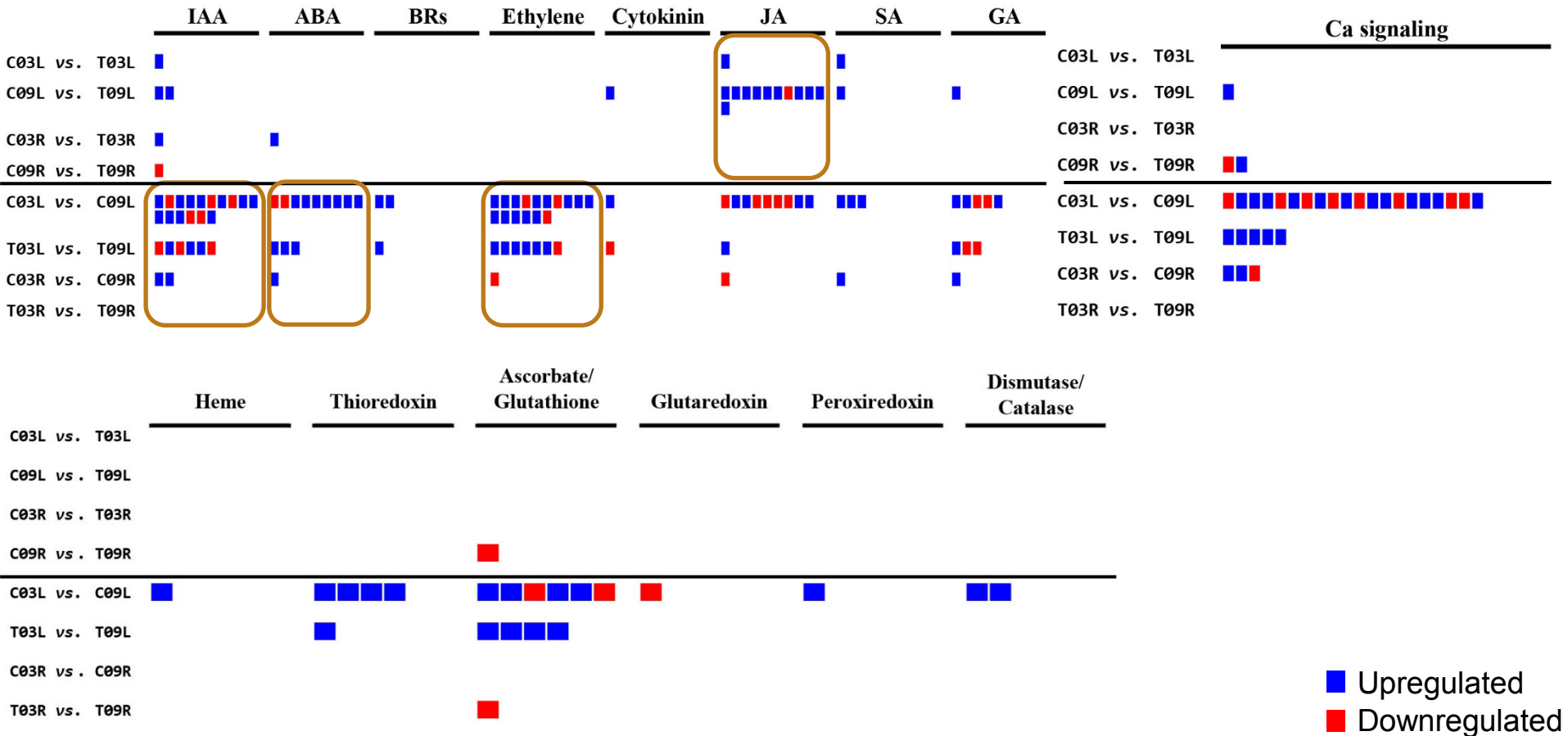
- Osmotic regulation

- Aquaporins – upregulated under treatment compared to control in G03 and downregulated in G09.

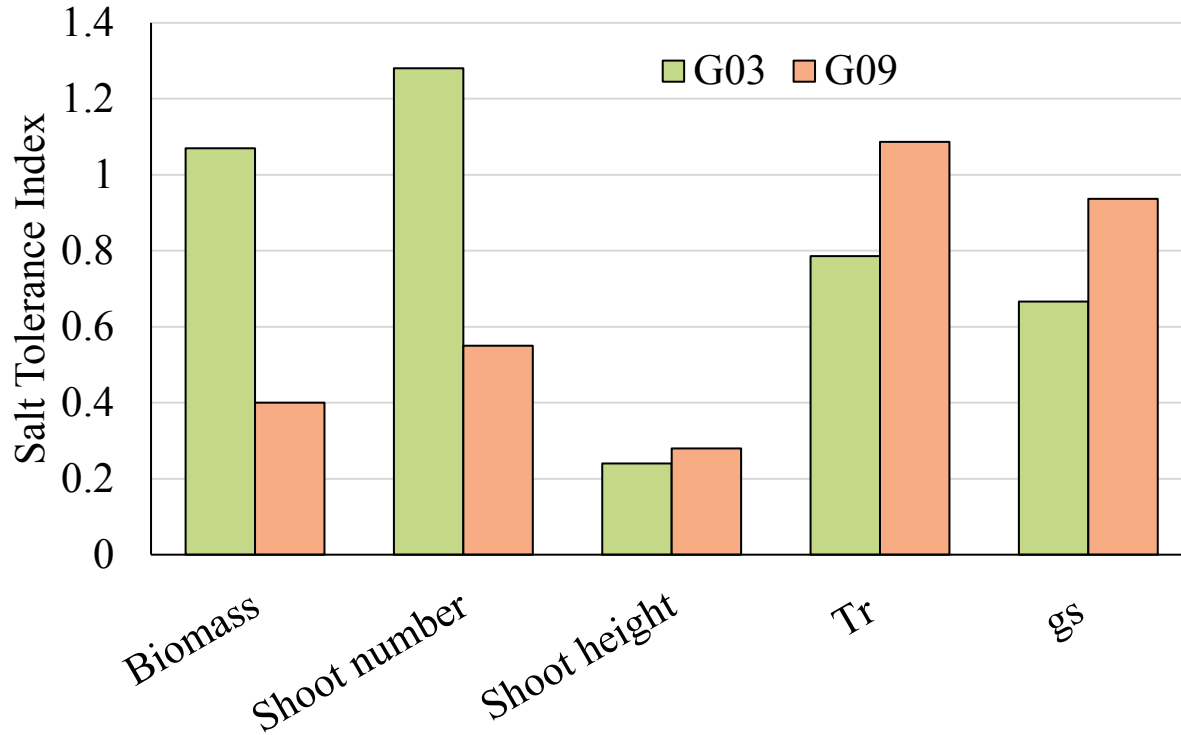
- Tillering

- GRAS family transcription factor genes – upregulated under treatment roots in both G03 and G09. Upregulated in G03 leaves compared to G09 leaves.
- Fructose-1,6-bisphosphate
- E3 ubiquitin ligase – negative regulator of tillering. Four genes encoding this enzyme were upregulated in G09 compared to G03.
- TCP transcription factor – negative regulator of Tillering

Genes associated with hormone-, Ca-, and redox-signaling pathways



Salt tolerance index for various traits between G03 (salt-tolerant) and G09 (salt-sensitive)



SUMMARY – Medicago Experiment

- Transcriptome analysis showed that the regulation of important genes involved in osmotic stress tolerance and tillering may be critical for performance difference between salt-tolerant and salt-sensitive genotypes.
- Differentially expressed genes involved in hormone-, calcium-, and redox-signaling showed treatment- and genotype-specific differences.
- Screening of a segregating population generated by crossing two salt-tolerant parents resulted in isolation of highly salt-tolerant genotypes that can tolerate salinity of $EC_{iw} = 27 \text{ dS m}^{-1}$.

Relationship plant height and shoot number with salt tolerance index (STI)

