

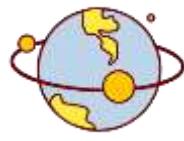
Tomato tolerance to salinity as mediated by rootstock origin

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הרצאה ביום עיון שרש דבר
במסגרת יום פתוח מו"פ ערבה
מרכז ויידור, תחנת יאיר 23/2/2022





The increasing effect of salinity



- > 50% of global arable land would be affected by soil salinity by 2050
- Food demand is to grow by 70% by 2050
- The progress in breeding for salinity tolerance requires *considerable time and cost*



Grafting: One potential approach to improve crop tolerance to salinity



(Robert Sharrock, 1672)

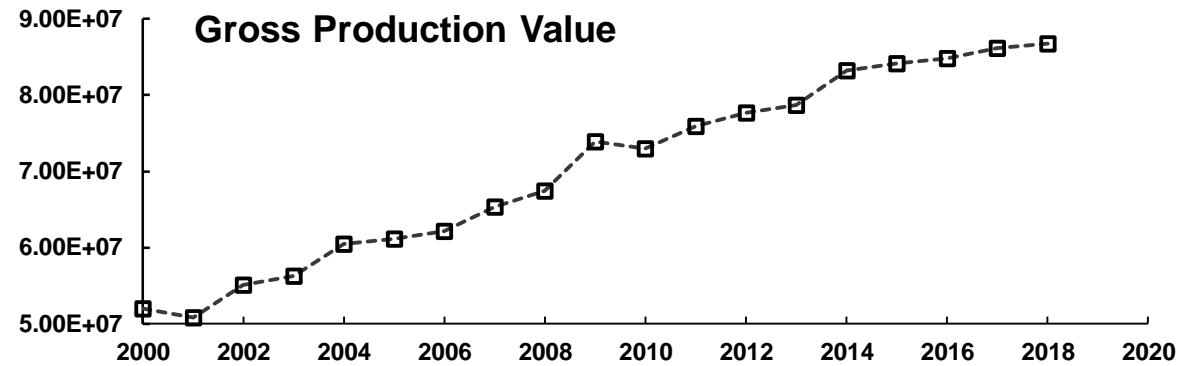
- ❖ Strengthen tolerance to abiotic stress
- ❖ Reduce biotic infection
- ❖ Increase nutrient and mineral uptake to the shoot
- ❖ Boost plant growth and development





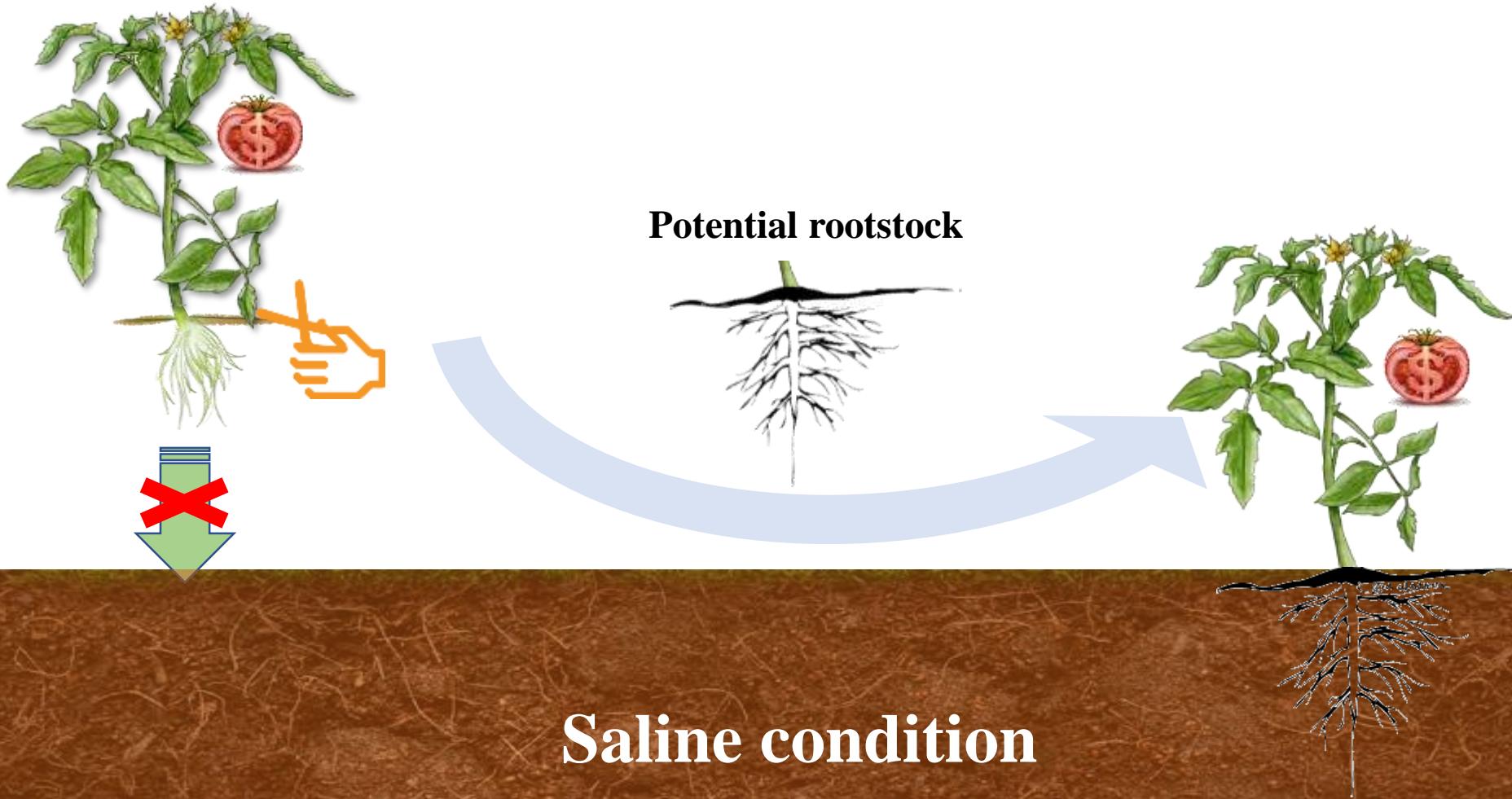
Tomato : an important economic crop

- ❖ Tomato grafting have been using for many years
- ❖ Diversity of tomato in response to abiotic stress





The main idea to increase tomato tolerance to salinity





Objectives



1. To screen the *graft population* for tolerance to saline condition by monitoring scion performance in respect to *commercial traits*
2. To elucidate *rootstock effect on morphological traits* of the scion
3. To explore the molecular basis of the rootstock mediated enhanced tolerance to salinity

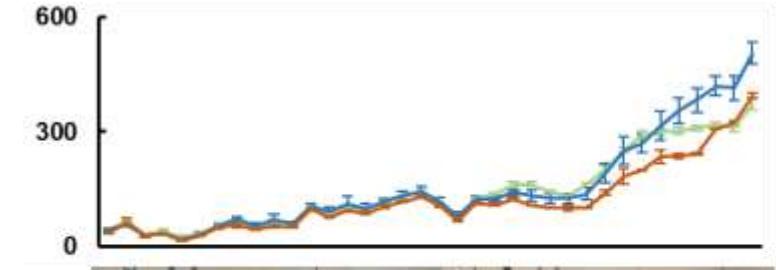
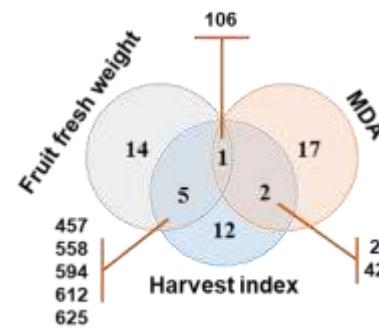
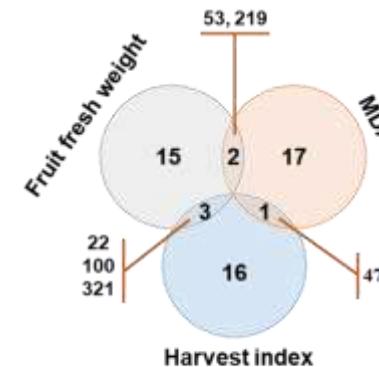
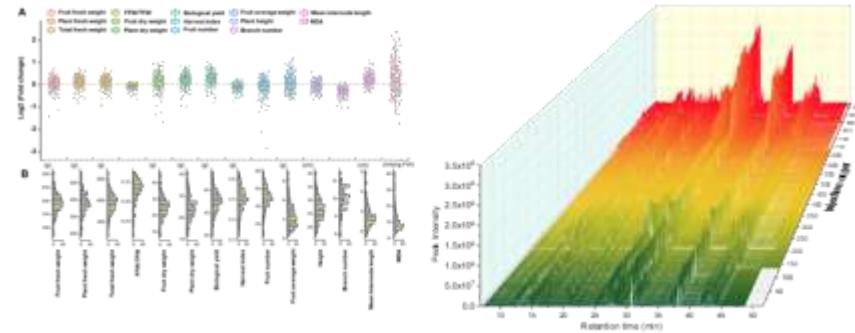


Research outline

254 tomato grafts

Phenotype-targeted selection

Physiological response



Preliminary experiment for selecting salt concentration (Non-grafted M82)



Control



75 mM



150 mM



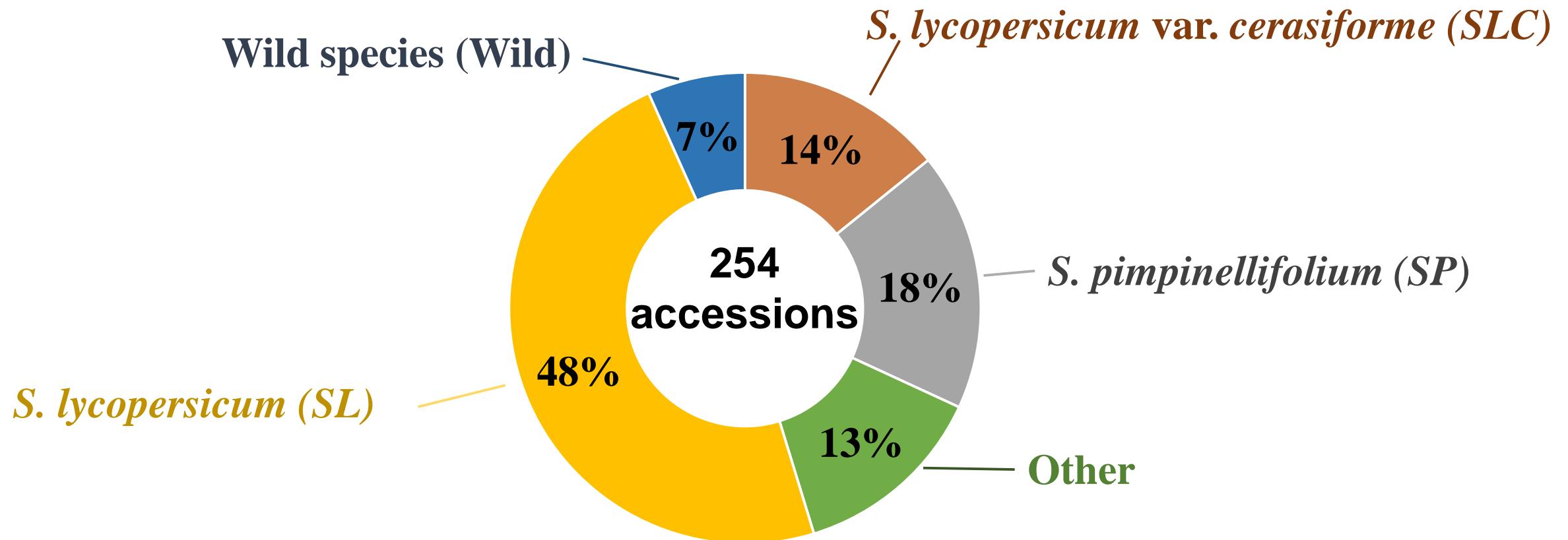
250 mM

➤ 200mM NaCl was chosen to be applied in the filed experiment



Experimental setup

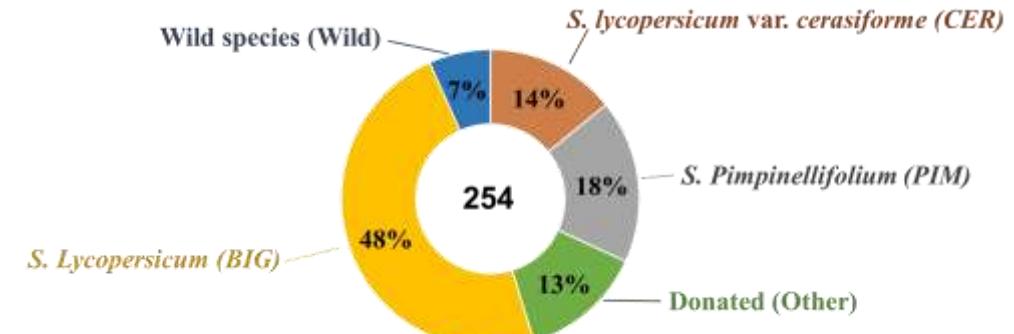
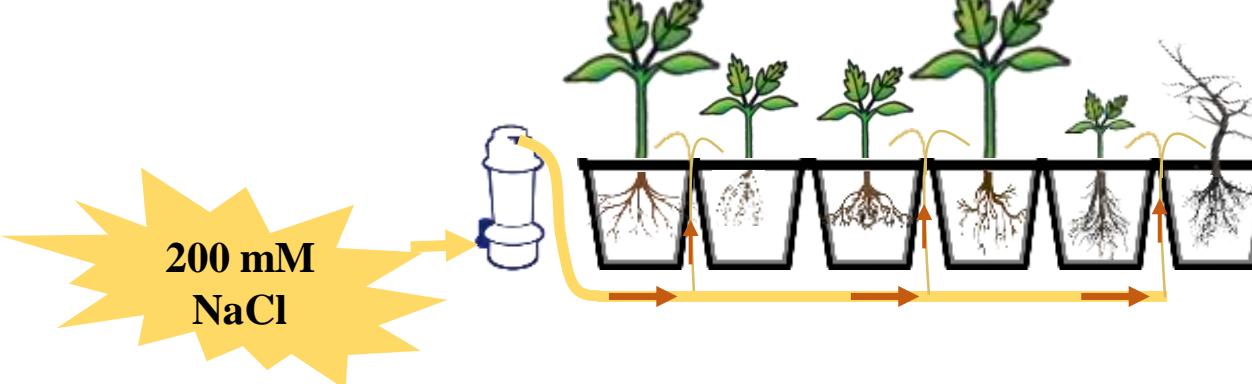
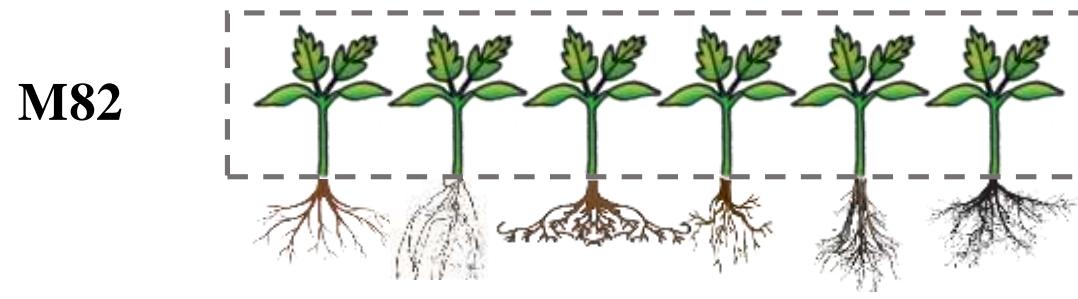
- 254 tomato accessions of different origins were used as rootstocks





Experimental setup

➤ Same scion was grafted onto different rootstocks





Experimental setup

254 grafts
(3-4 replicates)



Naturally salt-affected field:

$$0.75 \text{ dS m}^{-1} < \text{EC} < 1.8 \text{ dS m}^{-1}$$



Saline soil:

$$\text{EC} > 4 \text{ dS m}^{-1} \text{ (equivalent to 40 mM NaCl)}$$

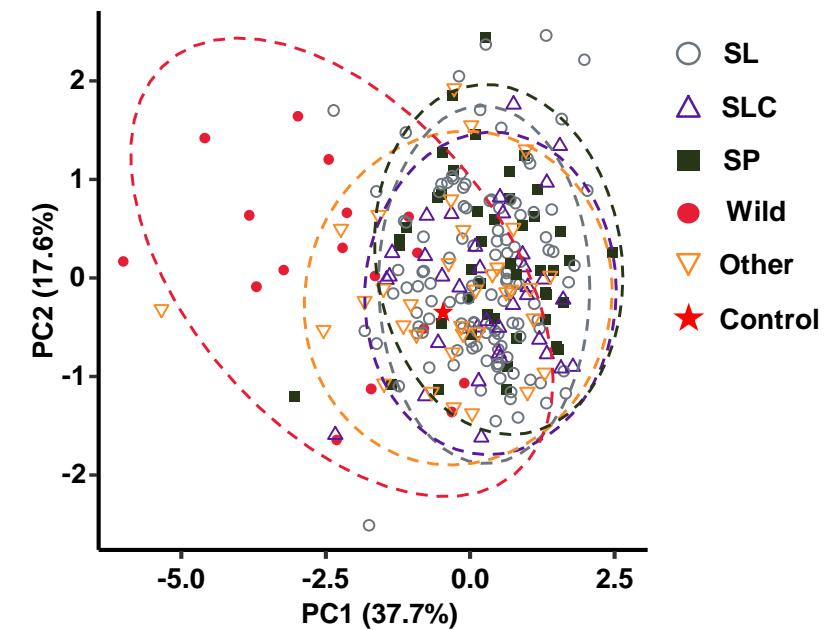
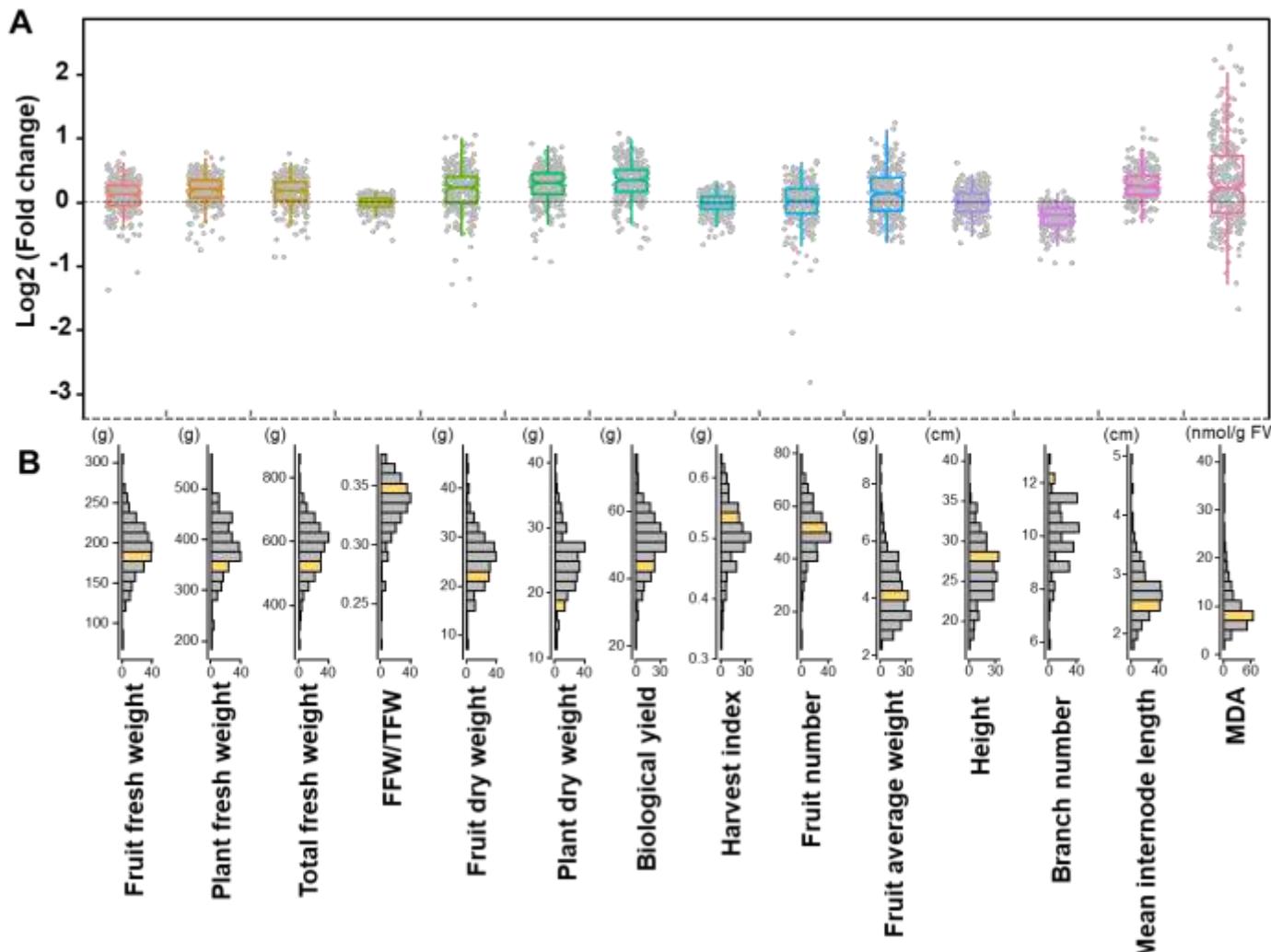
(Munns, Day et al. 2020)



In our experiment:

$$\text{EC} = 20 \text{ dS m}^{-1}$$

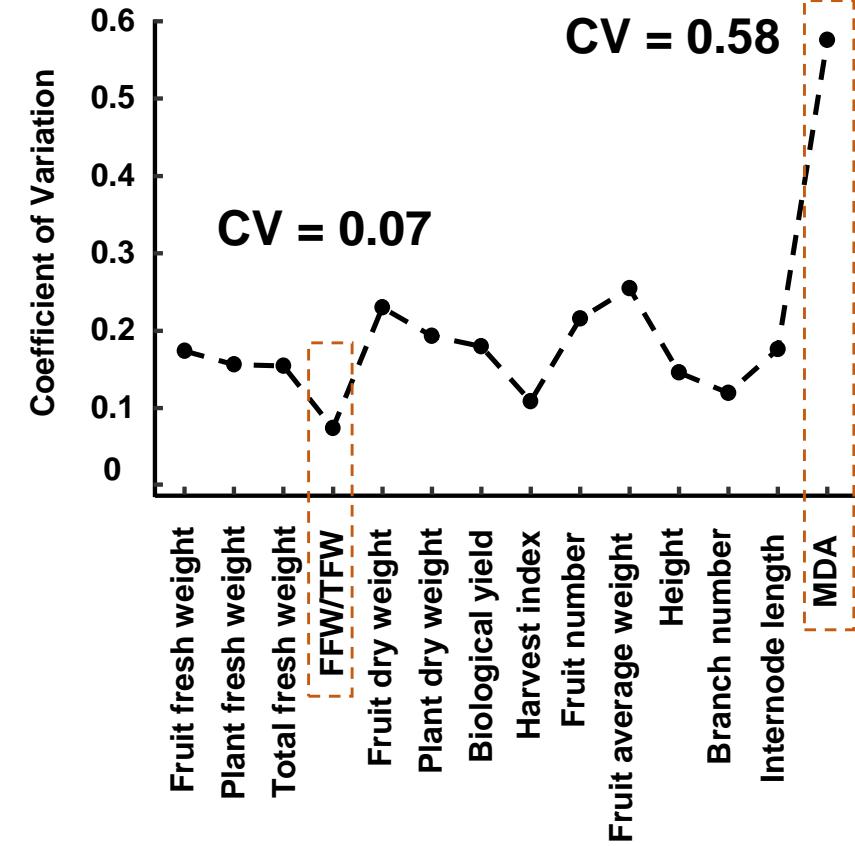
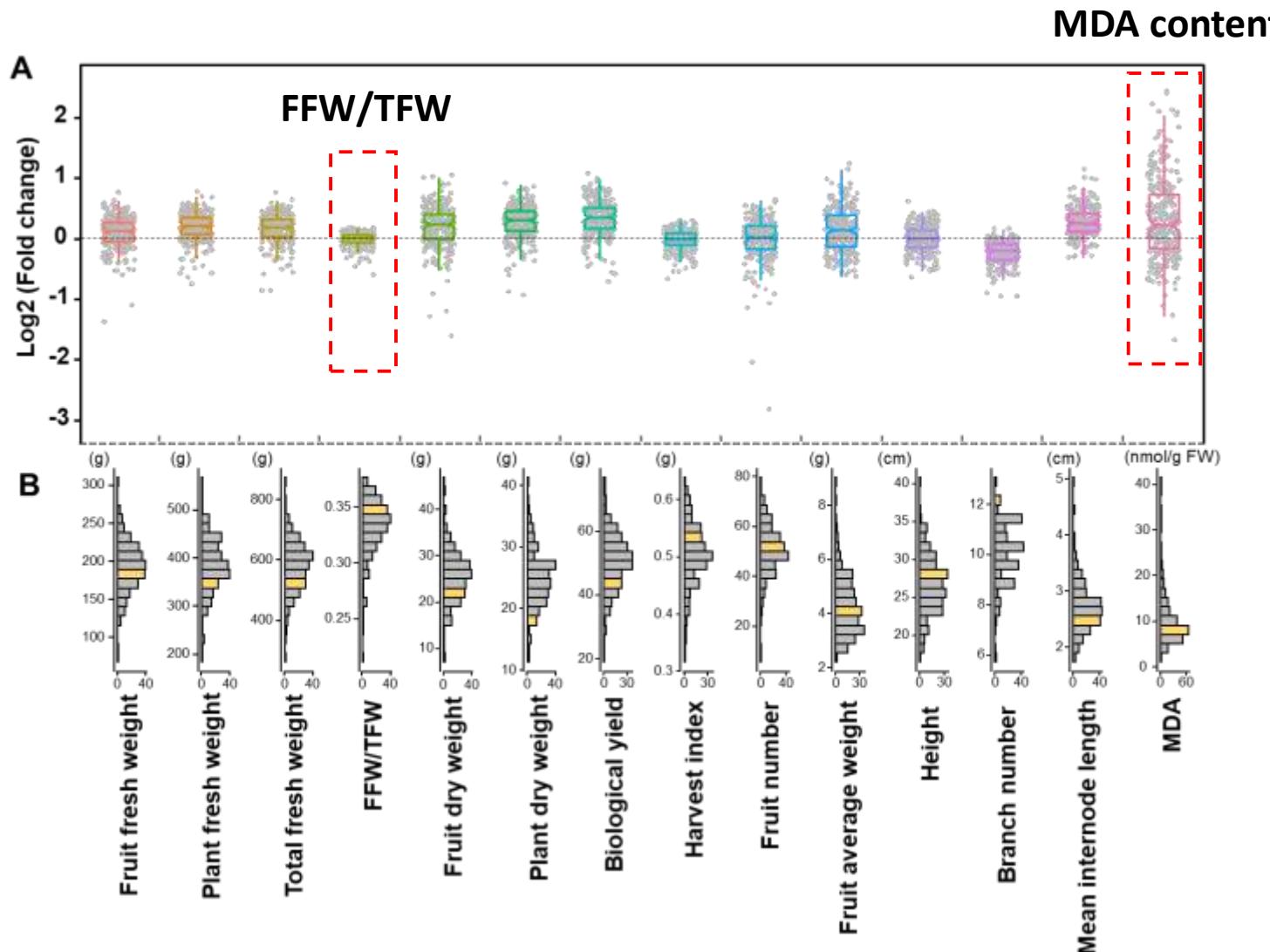
Grafting M82 onto different rootstocks resulted in phenotypic diversification



□ The PCA result revealed that the *origins of rootstocks* had a *minor role* in phenotypic diversification

* Fold change = Mean of replicates to self-grafted M82

FFW/TFW is regarded as an intrinsic trait for M82

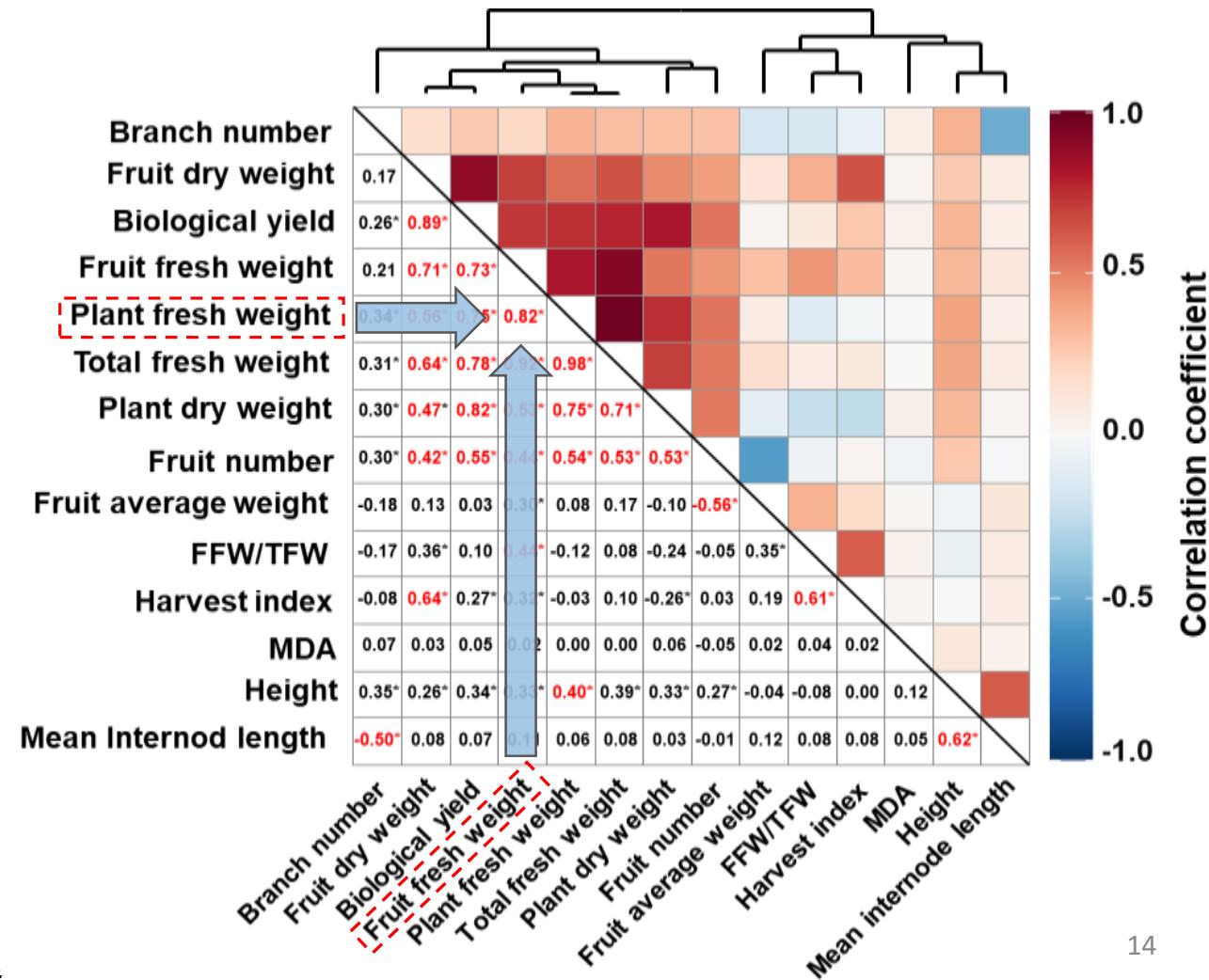


FFW, fruit fresh weight; PFW, plant fresh weight; TFW, total fresh weight

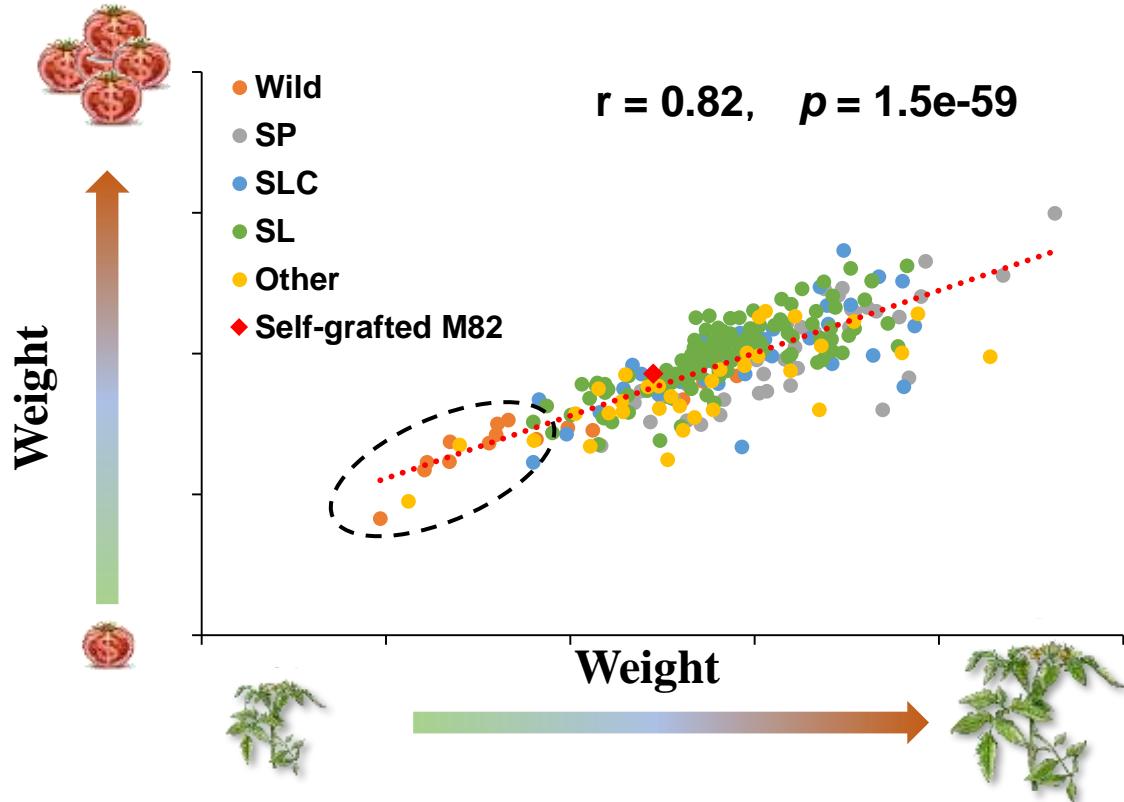
* Coefficient of variation = Standard deviation / Mean

Correlation analysis revealed the strong relationship between FFW and PFW

$$\begin{aligned} \text{FFW/TFW} &= \frac{\text{FFW}}{\text{FFW} + \text{PFW}} \\ &= \frac{1}{1 + \frac{\text{PFW}}{\text{FFW}}} \end{aligned}$$



Will the more tolerant rootstocks give us higher FFW?



The higher the PFW, the higher the FFW

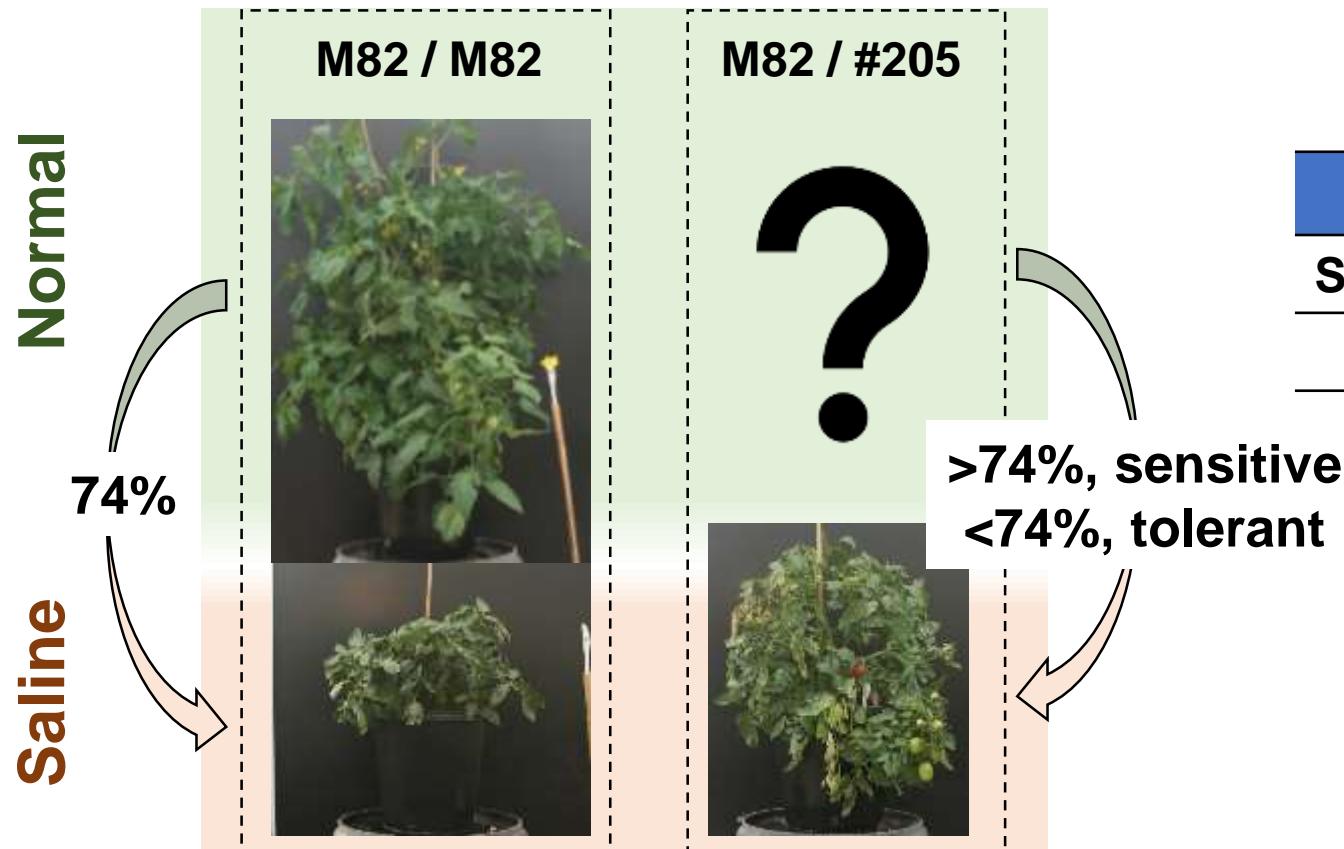
(the PFW refers to the *final* vegetative weight of plant)

Question

The more tolerant the grafted plant
to salt stress, the higher the PFW??

Will the more tolerant rootstock give us higher FFW?

- Salt tolerance: a relative concept

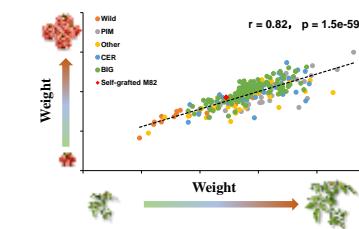
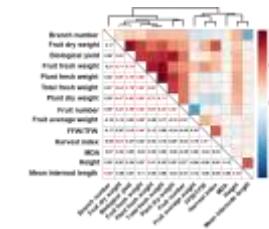
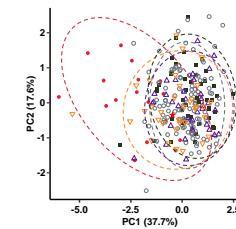
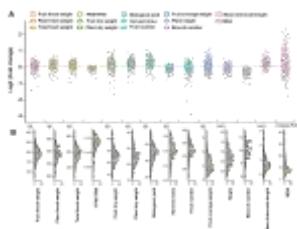


- The higher the PFW, the higher the FFW

Combination	PFW (g)	FFW (g)
Self-grafted M82	283	128
# 205	541	288

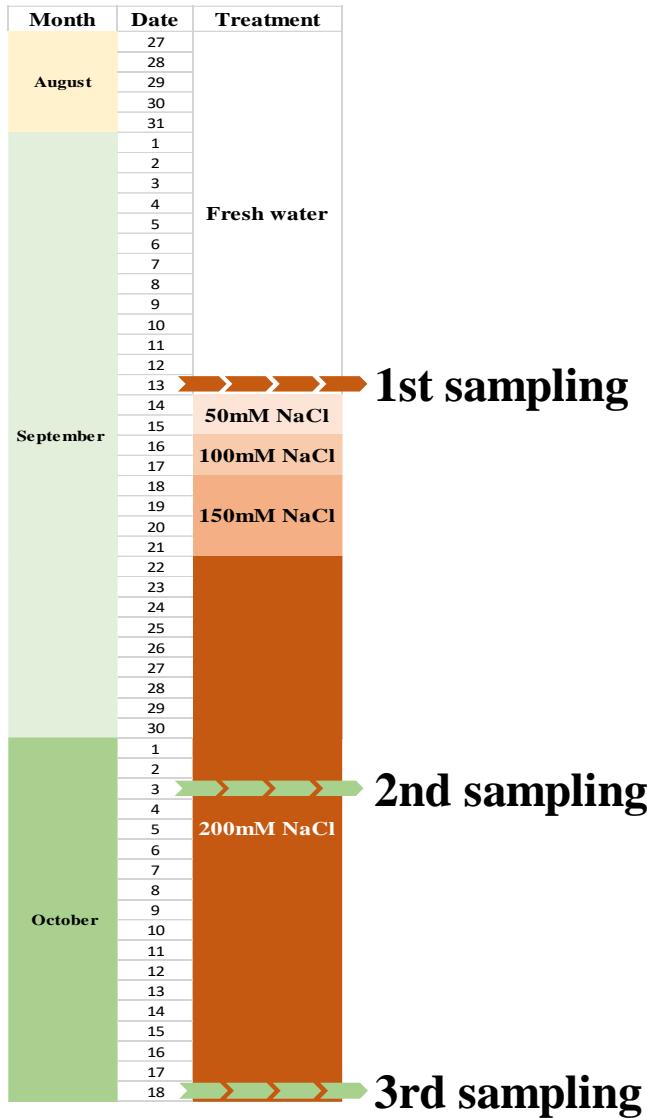
Short summary

- Grafting M82 onto different rootstock resulted in **phenotypic diversification**; Rootstocks' **origins** had a **minor role** in the plant growth
- The lowest CV of **FFW/TFW** suggested that it is an **intrinsic trait for M82**, irrespective of the “**R × S**” interaction
- **The higher the final PFW, the higher the FFW**

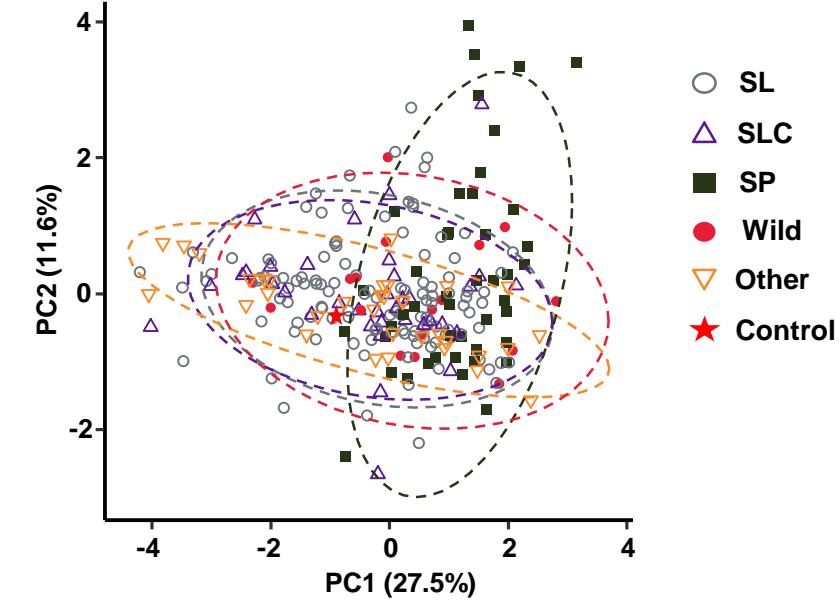
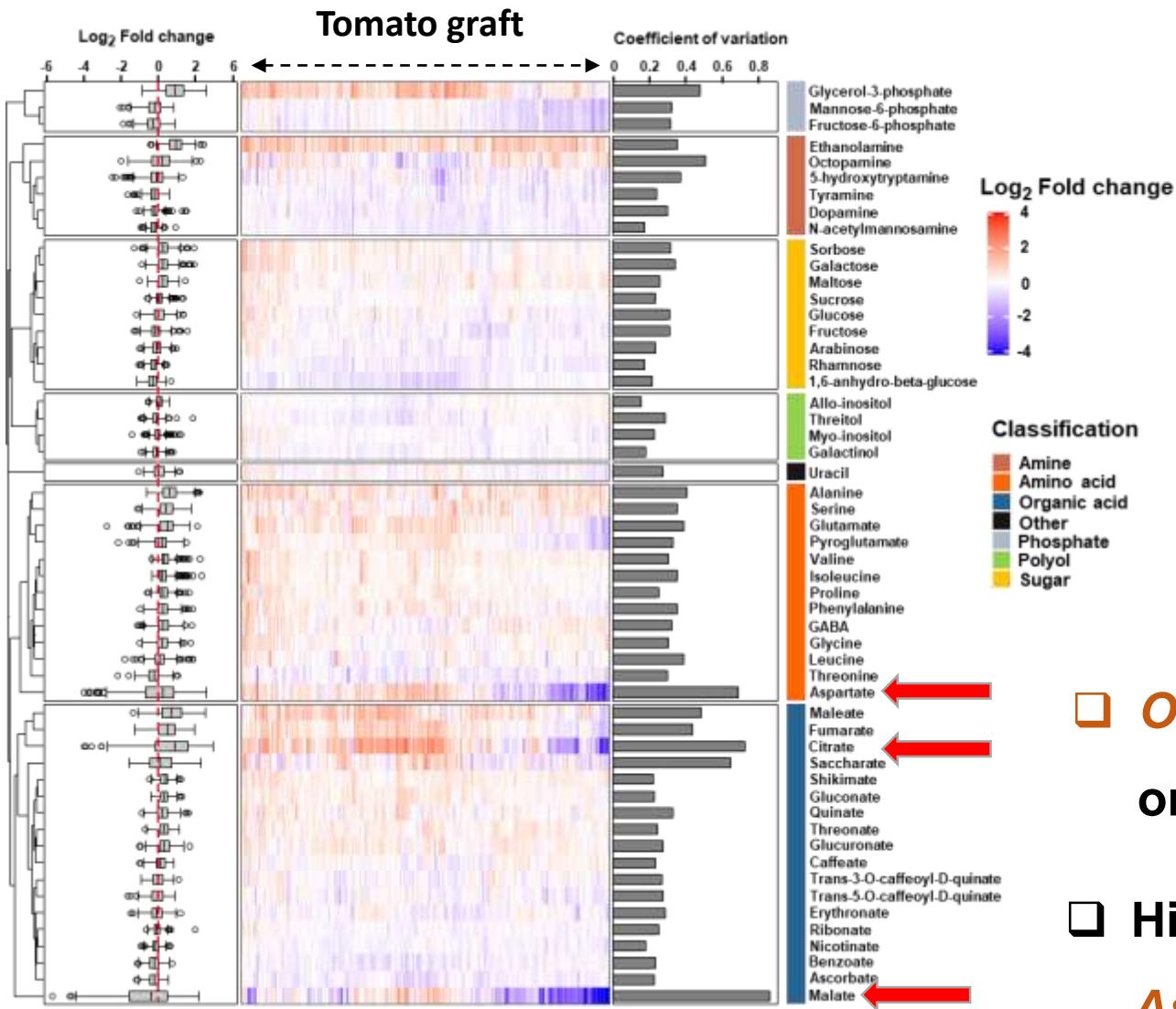


Metabolite profiling for 1000 grafted plants

254 lines
(3-4 replicates)



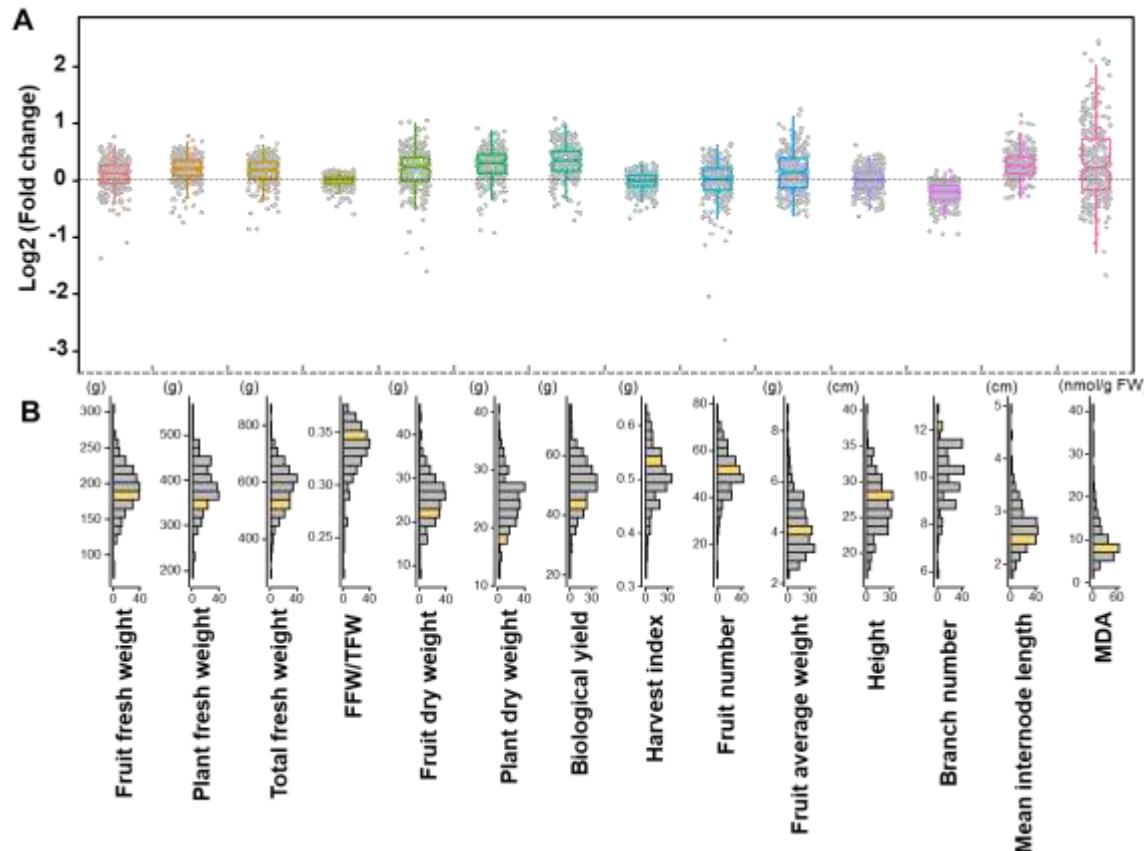
Grafting M82 onto different rootstocks resulted in metabolic variations under saline condition



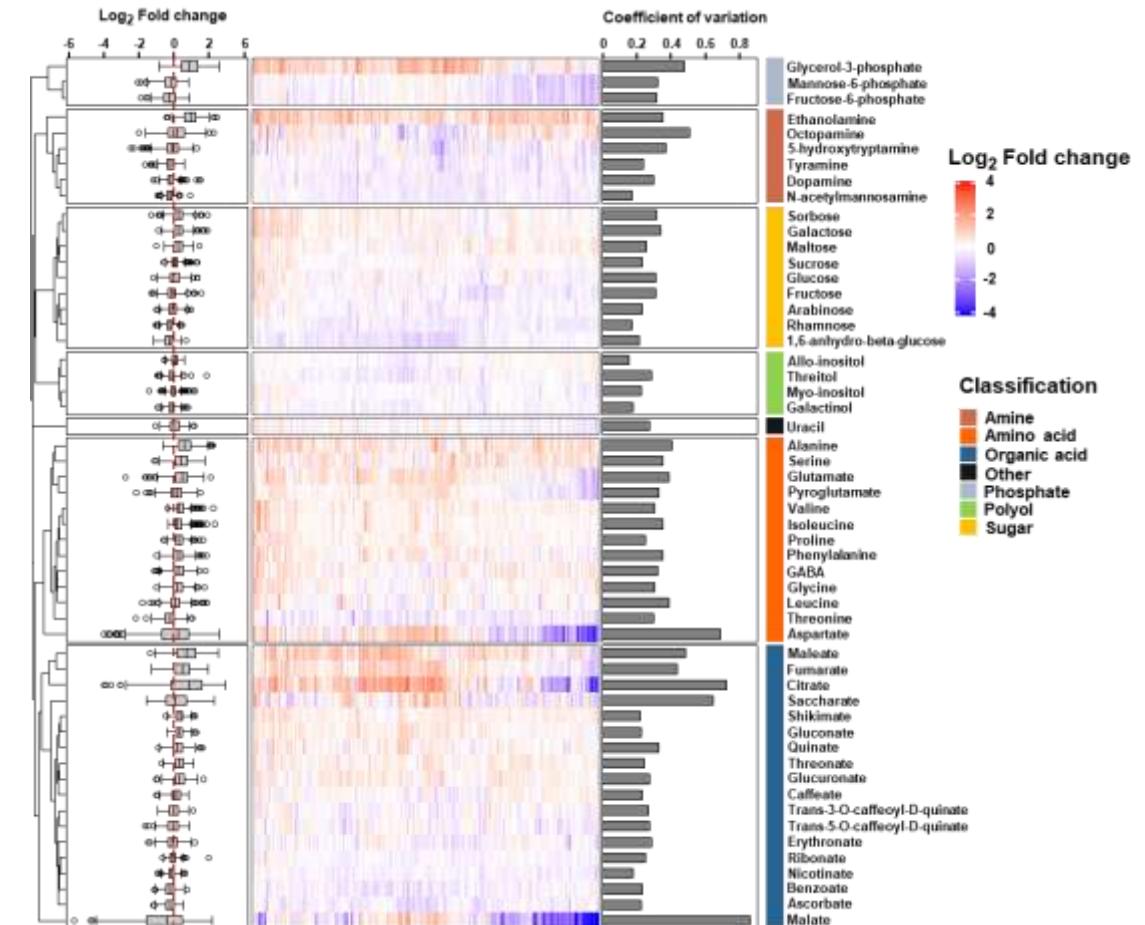
- Origins of rootstocks showed a *minor effect* on the metabolic variations
- Highly responsive metabolites: *Citrate*, *Malate*, *Aspartate*

Integrating morphological and metabolic data

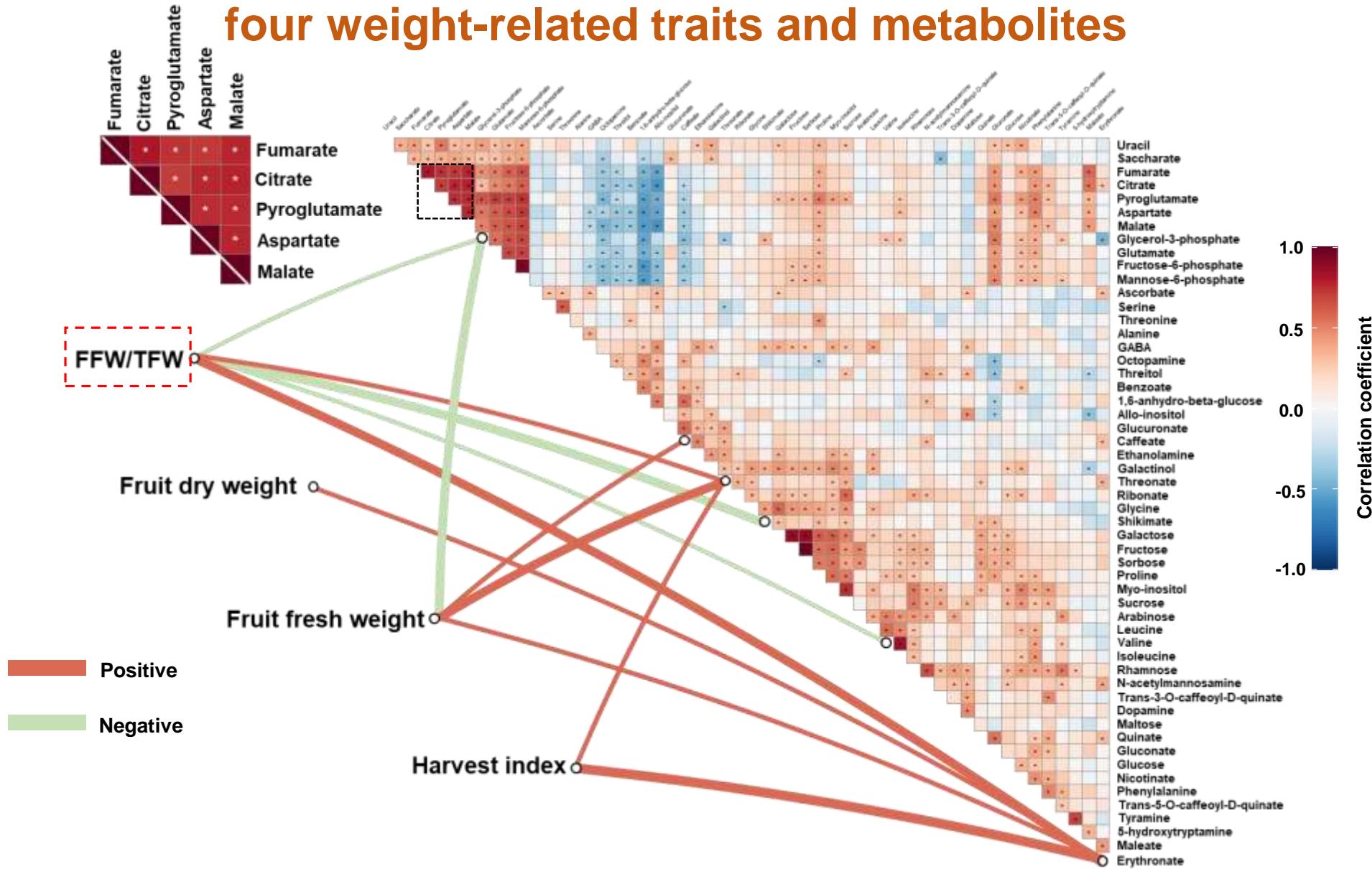
□ Phenotypic diversification



□ Metabolic variations

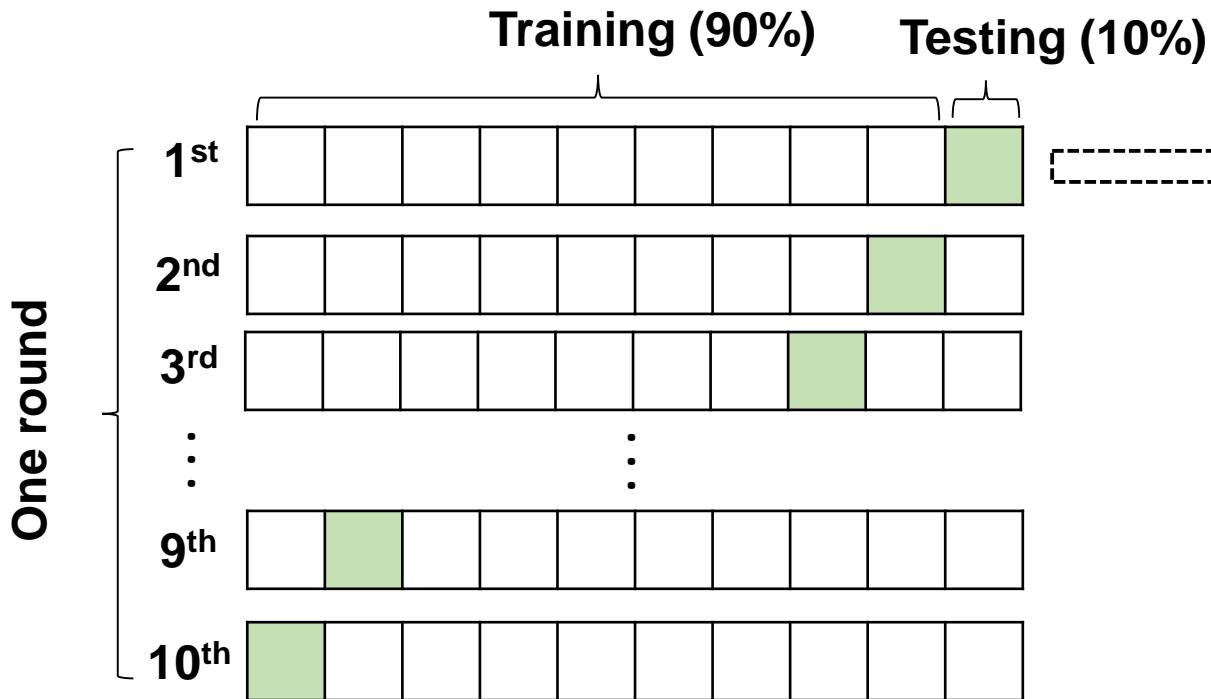


Correlation analysis highlighted linear relationship between four weight-related traits and metabolites

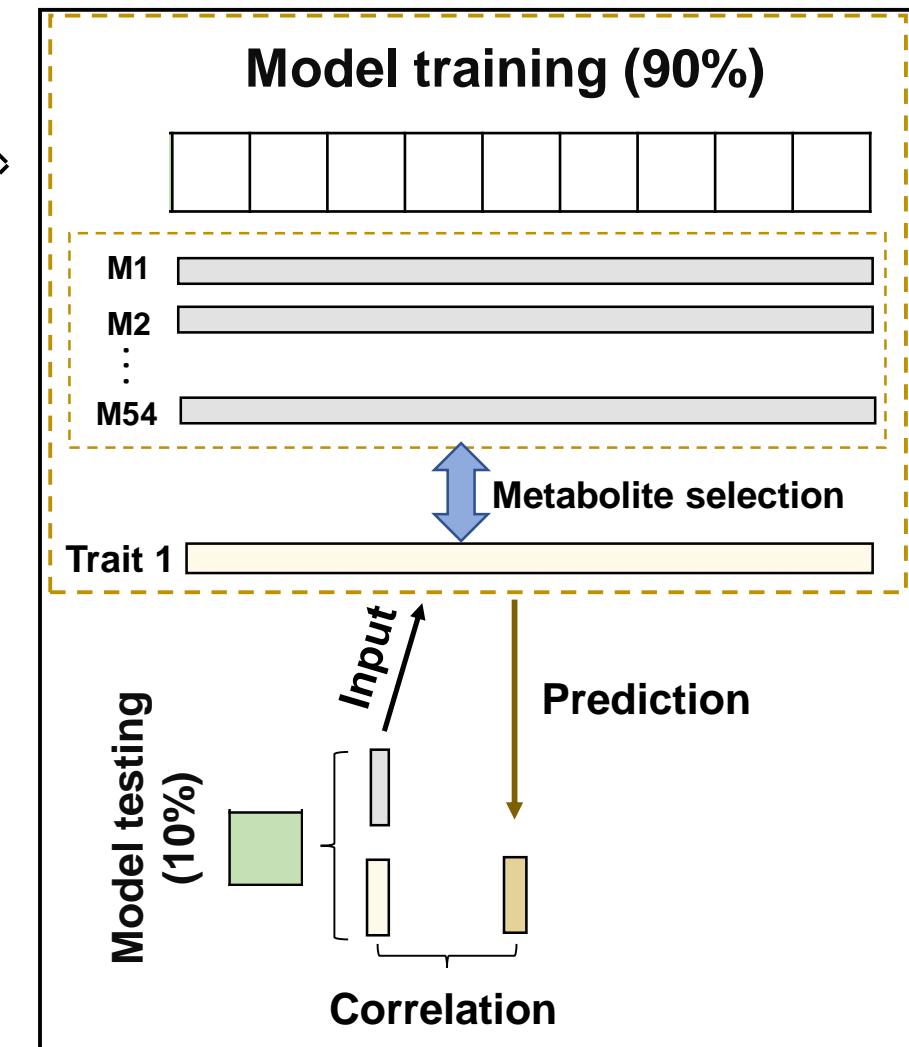


Metabolic prediction of four weight-related traits using LASSO

(Least Absolute Shrinkage and Selection Operator)

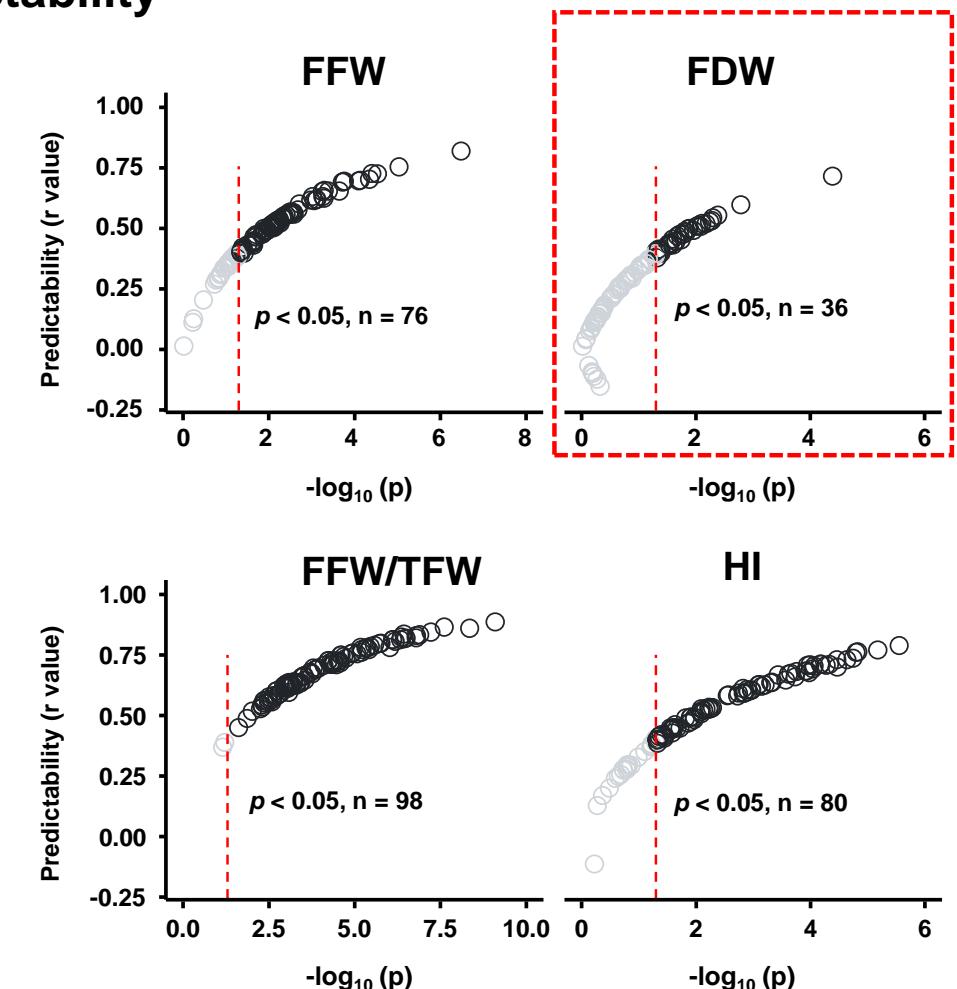
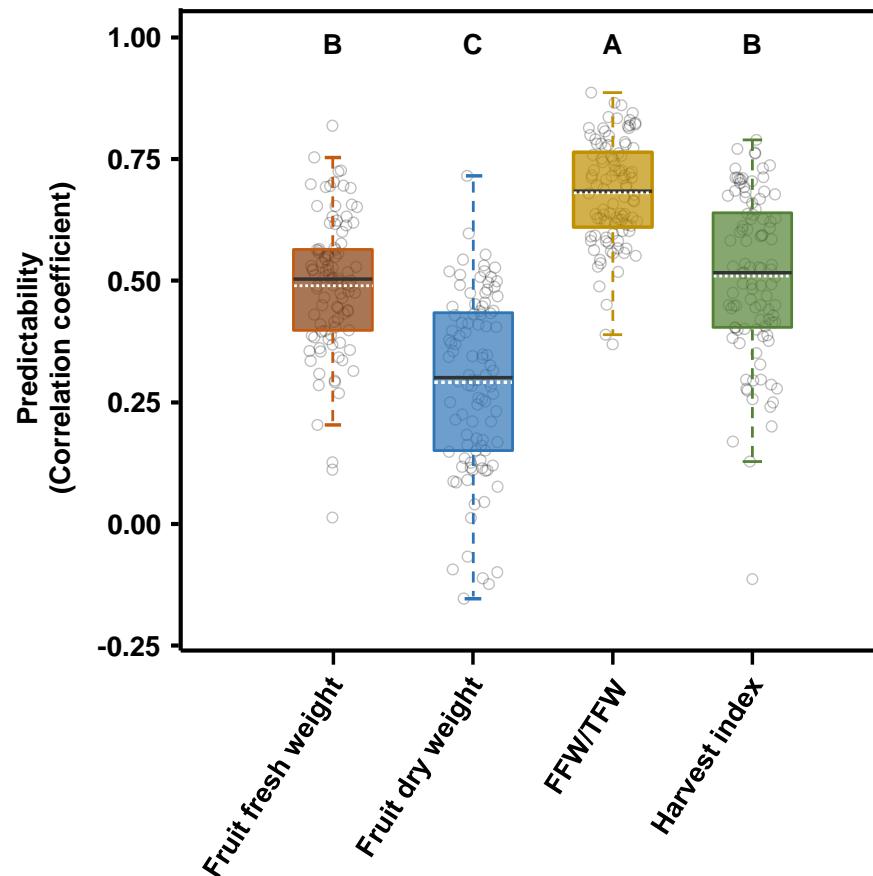


- **Predictability** is defined as the **correlation** between **predicted** and **observed** values



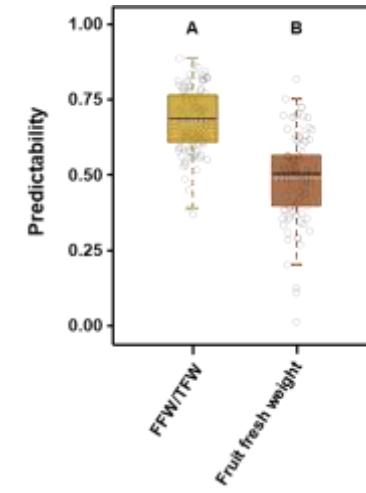
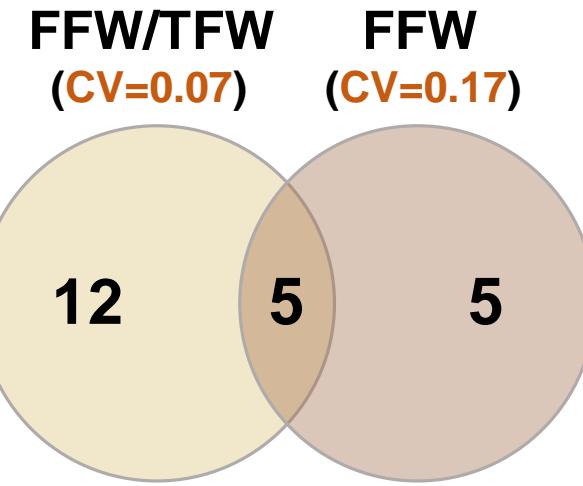
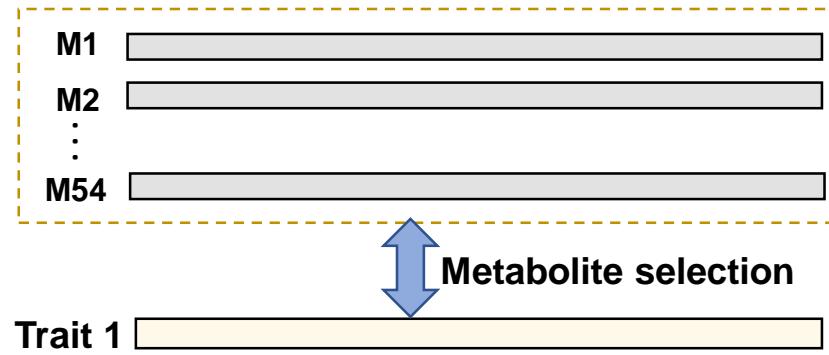
Metabolic prediction indicated that FFW, FFW/TFW, and HI are significantly predictable

- The metabolic prediction yielded three levels of predictability



Predictability is defined as the correlation between predicted and observed value

Prediction model revealed the vital metabolites contributing to the maintenance of plant growth



□ The *more frequently selected* the metabolite, the *more important* the metabolite to the prediction of *targeted trait*.

□ *More extensive metabolites* of importance are associated to maintain the *intrinsic trait* FFW/TFW *against* the impact of the "*R* × *S*" *interaction*.

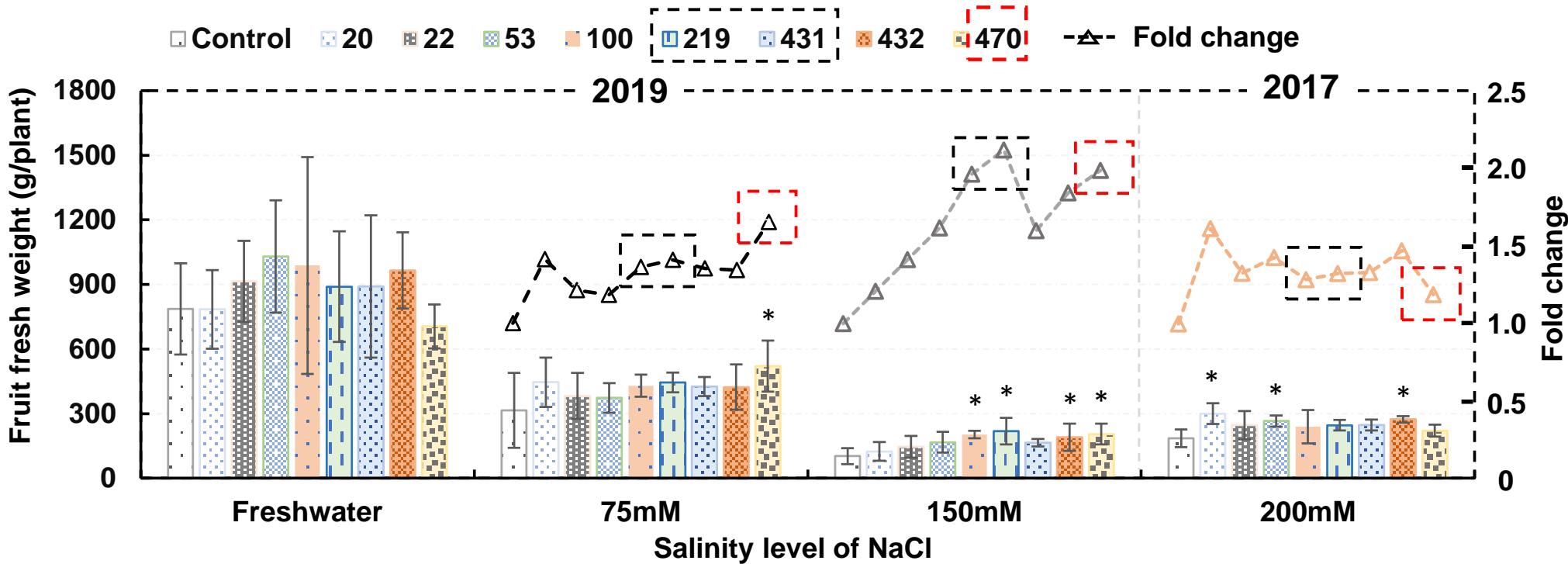
Phenotype-targeted selection based on FFW, HI, and MDA

- 8 “best” tomato grafts were selected as compared to self-grafted M82

Type	Rootstock (ID)	Rootstock origin	Fruit Fresh Weight			Harvest Index			MDA	
			Mean	SD	p-value	Mean	SD	p-value	FC	SD
Control	M82	<i>S. lycopersicum</i>	185.7	40.4	-	0.5	0.1	-	-	-
Best	20	<i>S. pimpinellifolium</i>	299.8	48.4	0.001	0.51	0.04	1.000	1.32	0.40
	22	<i>S. pimpinellifolium</i>	246.7	65.7	0.167	0.61	0.10	0.611	0.88	0.09
	53	<i>S. pimpinellifolium</i>	265.5	26.8	0.031	0.55	0.04	1.000	0.62	0.08
	100	<i>S. lycopersicum</i>	238.9	77.5	0.297	0.62	0.07	0.477	1.16	0.78
	219	<i>S. lycopersicum</i>	246.0	24.2	0.177	0.54	0.03	1.000	0.60	0.24
	431	<i>S. lycopersicum</i> var <i>cerasiforme</i>	247.4	25.1	0.158	0.59	0.08	0.894	0.98	0.38
	432	<i>S. lycopersicum</i> var <i>cerasiforme</i>	273.6	15.2	0.014	0.55	0.02	1.000	0.700	0.14
	470	<i>S. lycopersicum</i>	220.7	27.5	0.776	0.57	0.08	0.988	0.59	0.16

FC: fold change

The “best” tomato grafts showed different trends in the relative changes of FFW across saline conditions

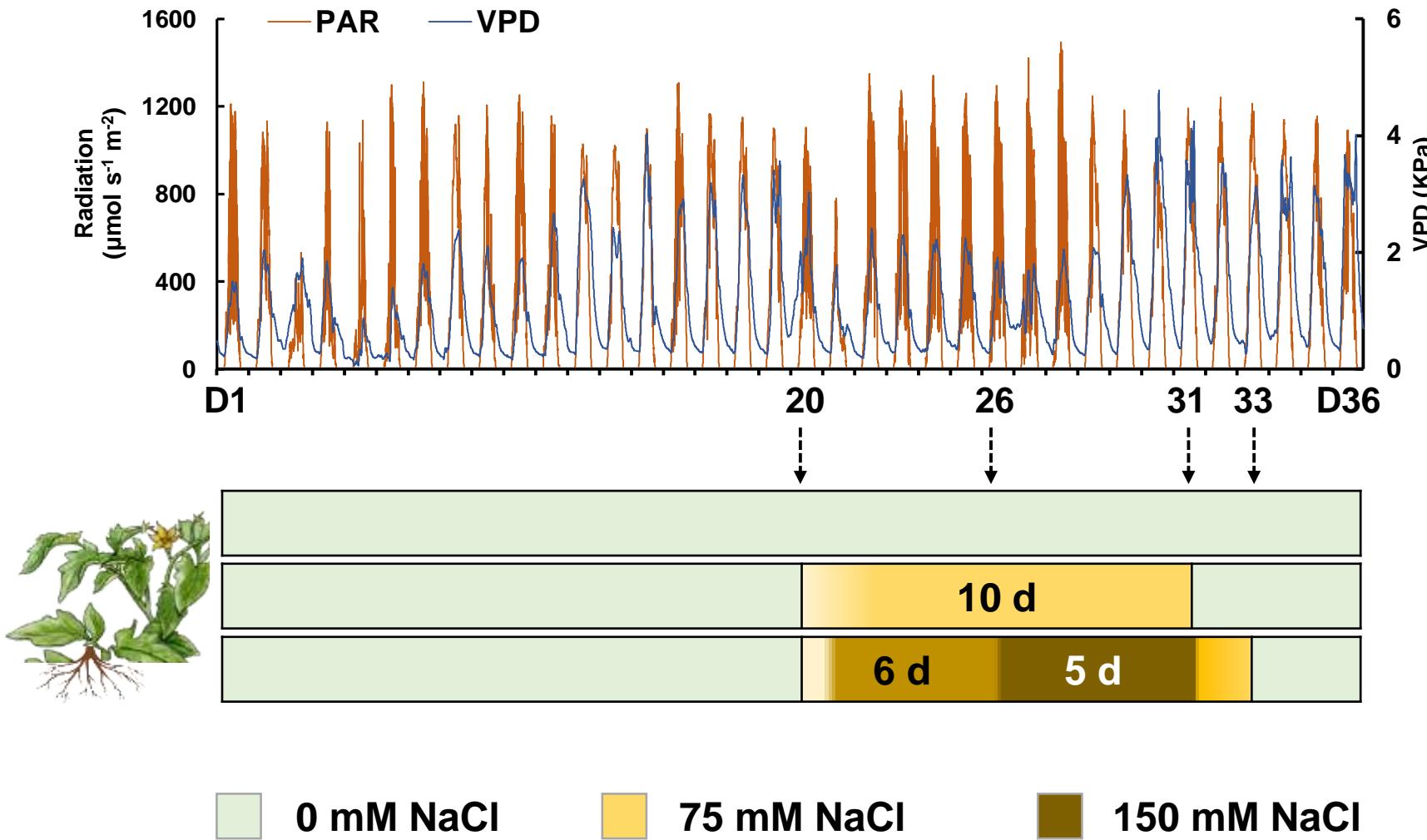


* indicates a significant difference between each tomato graft and self-grafted M82

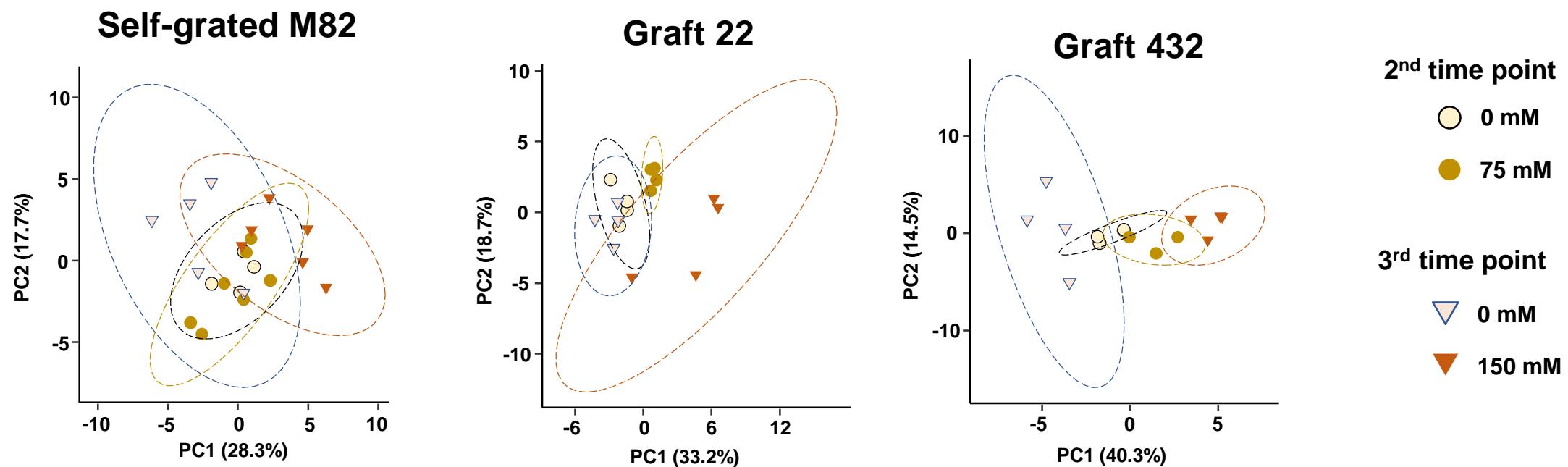
Conclusion

- Rootstocks' *origins* showed a *minor role* in the plant growth and metabolism.
- The *prediction model* highlighted the vital role of metabolites in *morphological performance* of tomato grafts in response to salt stress.
- Taken together our results emphasize the importance of rootstock collections in breeding efforts towards improved combinations.

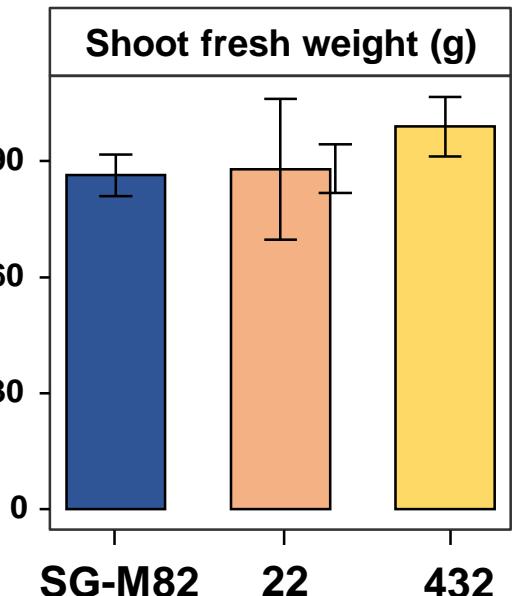
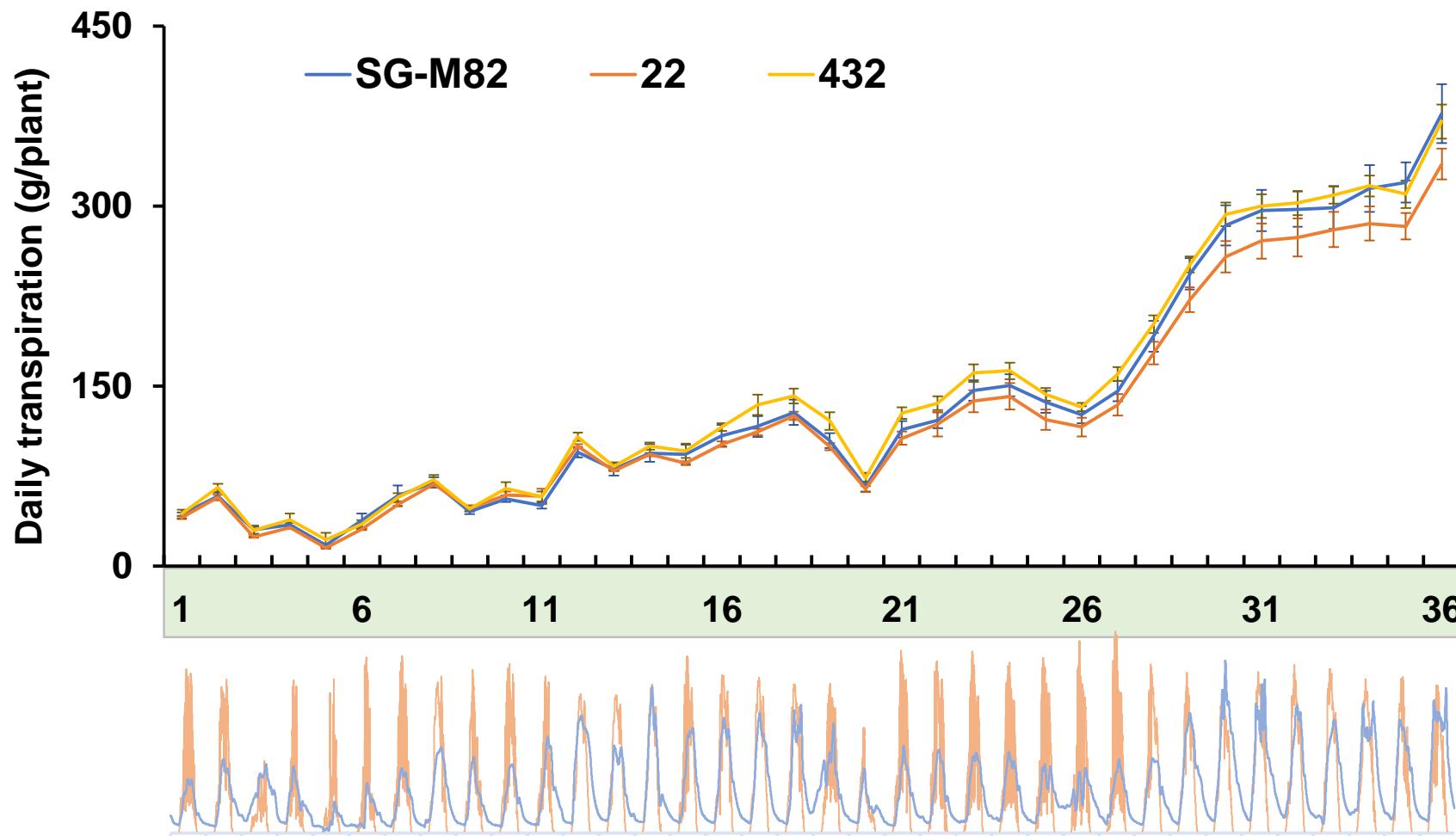
The response of selected tomato grafts to different saline conditions and recovery of freshwater



More severe salt stress resulted in greater alterations in metabolite profiles.

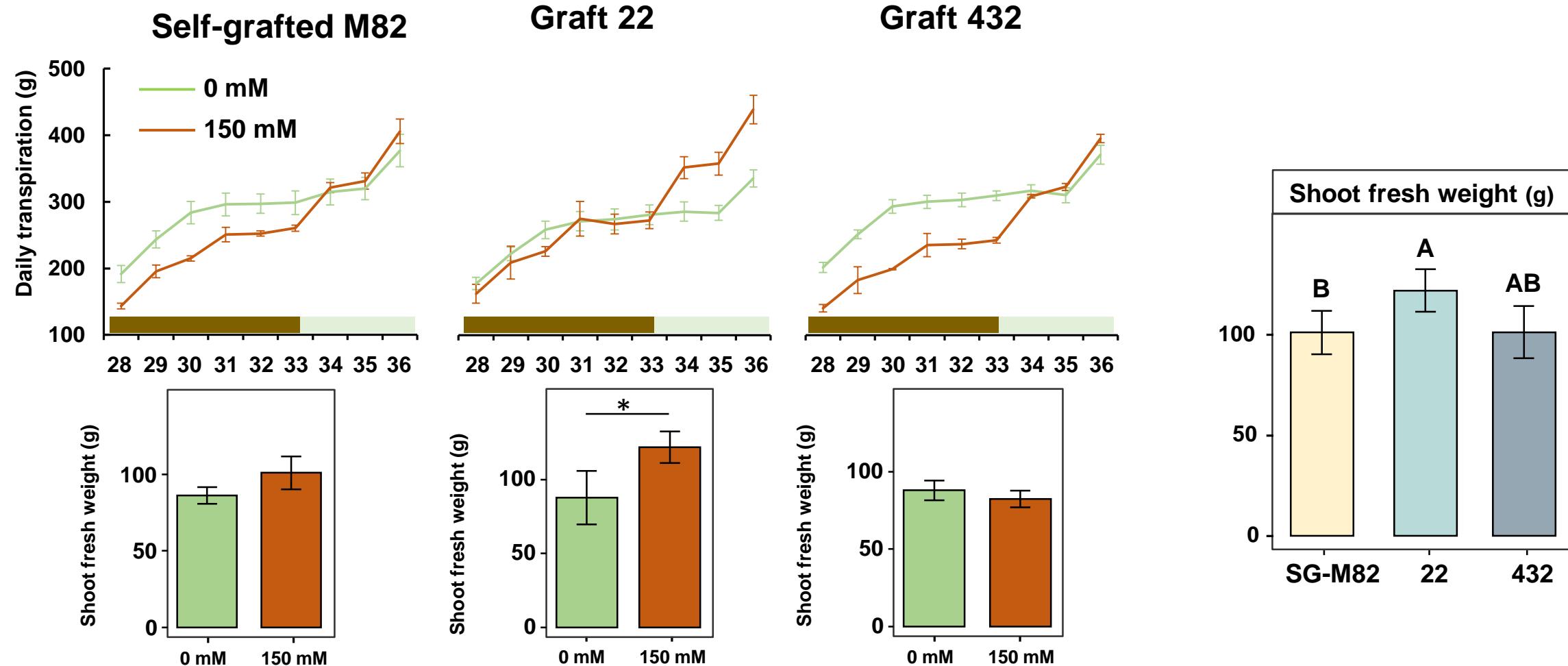


The daily transpiration did not vary between grafts under the freshwater condition



* Data was shown as mean \pm SE ($n = 4-6$)

The changes in daily transpiration and shoot weight of plants under 150 mM NaCl, followed by recovery with freshwater



Group	Metabolite	Fold change of 150 mM / 0 mM		
		SG-M82	22	432
Organic acid	Ascorbate	1.79	1.46	0.59
	Caffeate	0.66*	0.88	1.28
	<i>Cis</i> -5-O-caffeooyl-D-quinate	1.58	1.05	0.95
	Citrate	3.4*	5.30*	10.07*
	Dehydroascorbate dimer	1.16	1.84*	1.54
	Erythronate	1.48*	2.13*	2.48*
	Fumarate	0.87	1.26	2.04*
	Gluconate	1.16	1.12	0.49*
	Glucuronate	0.95	0.51	0.15*
	Glycerate	1.55*	2.13*	1.47*
	Malate	1.42*	2.16	2.14*
	Maleate	0.76	0.87	1.86
	Nicotinate	1.23	1.25	0.78
	Pyruvate	1.03	1.32	1.75
	Quinate	0.66	0.54	0.56*
	Ribonate	1.39*	1.26	1.15
Amino acid	Saccharate	1.59	1.90	0.49*
	Shikimate	1.22	1.05	1.00
	Threonate	0.9	0.89	0.68
	<i>Trans</i> -3-O-caffeooyl-D-quinate	0.97	0.71*	0.50*
	<i>Trans</i> -5-O-caffeooyl-D-quinate	0.76	1.35	1.91
	Alanine	1.19	0.81	0.54
	Aspartate	1.45	2.21	1.51
	GABA	0.99	0.95	0.50*
	Glutamate	1.41*	2.04*	1.88*
	Glycine	1.74	1.44	0.73
	Isoleucine	1.38	0.85	0.58
	Leucine	1.23	1.95	1.97*
	Phenylalanine	0.82	1.58	1.19
	Proline	48.57*	29.48*	120.01*
	Pyroglutamate	1.35	1.75	1.50*
	Serine	1.92*	4.19*	2.57
Sugar	Threonine	2.05	1.47	1.17
	Valine	1.51	1.18	0.99
	1,6-anhydro- β -glucose	0.92	0.64*	0.91
	Fructose	1.51	1.91*	1.79*
	Fucose	1.01	1.16	1.38
	Galactose	1.3*	1.73*	1.26
	Glucopyranose	7.13	7.31	0.52
	Glucopyranose [-H2O]	1.05	1.24	1.29
	Maltose	0.9	1.09	1.44*
	Rhamnose	1.13	1.30	1.60*
Amine	Sucrose	1.47*	1.75*	1.47*
	5-hydroxytryptamine	1.39	1.32	0.49
	Dopamine	0.66	0.94	1.31
	Ethanolamine	1.35	1.36	0.90
Amine	Tyramine	1.71*	2.03	1.47

The sever salt stress (150 mM) resulted in the significant alterations on:

Organic acids

Citrate, Erythronate, glycerate, malate

amino acids

Glutamate, proline, serine

Sugars

Fructose, galactose, and Sucrose

The fold change of **150 / 0 mM** for each metabolite in graft combinations. The sample was collected on **day 28 (two days under final con. of 150mM)**. The values highlighted in bold and color indicate a significant difference ($*p < 0.05$) using a Student t-test between stressed plants and the corresponding control ($n = 3-7$).

Group	Metabolite	Self-grafted M82		22		432		
		S	R	S	R	S	R	
Organic acid	Ascorbate	1.19	1.47	1.30	1.12	1.37	1.18	
	Cis-5-O-caffeooyl-D-Quinate	1.50	1.00	1.36	1.43	1.15	0.63	
	Citrate	2.17	0.44*	8.87*	1.06	4.69*	0.50*	
	Dehydroascorbate dimer	1.34	1.52	1.03	1.17	1.03	1.02	
	Erythronate	1.58	1.18	2.84*	1.56*	1.85*	1.05	
	Fumarate	1.01	0.98	2.24	1.44	2.65*	1.08	
	Gluconate	0.99	0.92	1.09	1.43	1.08	1.13	
	Glucuronate	0.66	0.27*	0.42*	0.47	0.65	0.85	
	Glycerate	1.70*	0.93	2.87*	1.42	1.85*	1.21	
	Maleate	0.96	2.31*	0.80	3.47	1.70	0.80	
	Malate	1.00	0.5*	2.29*	0.78	1.70	0.80	
	Nicotinate	1.24	1.35	1.49*	1.83*	1.19	1.73*	
	Pyruvate	1.19	1.12	0.65	1.14	0.72	1.02	
	Quinate	0.63	0.35*	0.48*	0.52*	0.54*	0.82	
	Ribonate	1.41*	1.27	1.69*	1.22	1.34	1.17	
	Saccharate	1.23	1.14	1.04	1.08	3.11*	2.7*	
	Shikimate	1.61	0.94	1.11	0.90	1.30	0.85	
	Threonate	1.33	1.01	2.40*	0.91	1.91*	0.74	
	Trans-3-O-caffeooyl-D-quinate	1.44*	0.72*	0.85	0.65	0.67*	0.64*	
	Trans-5-O-caffeooyl-D-quinate	1.36	1.71*	1.51	2.59*	2.70*	1.28	
Amino acid	Caffeate	1.16	1.26	1.54	0.94	1.67	0.86	
	Alanine	1.01	0.98	0.67*	0.98	1.48	1.61	
	Aspartate	1.55	0.88	2.46*	1.88*	1.86*	2.18	
	GABA	1.44	1.77*	1.32	1.45	1.04	1.67*	
	Glutamate	1.35	1.12	2.75*	1.50	2.04*	1.04	
	Glycine	1.15	1.17	2.12	2.63*	1.37	1.63	
	Isoleucine	1.28	1.64*	2.32*	1.93	1.96*	1.97*	
	Leucine	2.12*	1.51	1.70	1.55	1.76	2.20*	
	Phenylalanine	0.76	1.05	0.82	1.62	1.05	1.58	
	Proline	33.4*	0.93	58.96*	4.09	50.21*	1.61	
	Pyroglutamate	1.79*	1.45*	2.65*	1.70*	2.30*	1.81*	
	Serine	2.12*	1.85*	2.92*	1.90	3.08*	1.51	
	Threonine	1.93*	1.29	2.30*	1.97*	1.74	1.99*	
	Valine	1.28	1.32*	1.74	1.57	1.09	1.60*	
Sugar	1,6-anhydro-β-glucose	1.00	1.14	1.01	1.20	1.34	1.16	
	Fructose	1.08	1.41*	1.18	1.38	0.94	1.42	
	Fucose	1.05	1.20	1.12	1.11	0.84	1.14	
	Galactose	1.04	1.14	0.86	1.25	0.91	1.14	
	Glucopyranose	0.97	0.81	1.18	1.24	1.09	0.58	
	Glucopyranose [-H2O]	0.86	1.05	1.25	1.30	0.95	0.90	
	Maltose	1.19	1.28	1.32	1.12	1.02	1.09	
	Rhamnose	1.46*	1.31	1.73*	1.48*	1.33	1.22	
Amine	Sucrose	1.41*	1.22	1.66*	1.22	1.31*	1.19*	
	5-hydroxytryptamine	1.27	0.74	1.13	1.17	0.95	1.52	
	Dopamine	0.72	1.35	0.57	1.02	0.73	0.73	
	Ethanolamine	1.41*	1.14	1.42	1.62*	1.01	1.30	
	Tyramine	1.18	0.73*	0.89	0.93	1.15	0.86	

After recovered with freshwater, the **FC of citrate, glycerate, and proline greatly reduced**, as compared with that under saline condition.

The fold change of each metabolite in salt-stressed (S) (**day 32**) or recovered (R) (**day 36**) plants to the corresponding control. The values highlighted in bold and color indicate a significant difference (* $p < 0.05$) using a *Student t-test* between stressed or recovered plants and the corresponding control (n = 3–7).