Investigating the impact of neo-autotetraploidy on water stress response in Alfalfa

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About me

BSc. Plant Protection
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MSc Agricultural and Environmental Biotech (Expected Graduation- Oct 2024)
University of Perugia

Visiting Researcher
University of California, Davis
Part of UNIPG MSc Program

University of Perugia

- Italian public university established in 1308
- 16 Departments
- Research was conducted in the department of Agricultural, Food and Environmental Sciences (DSA3)
- Multiple research stations in the Umbria region
- Roughly 30,000 students and 12,000 faculty

https://dsa3.unipg.it/en/

http://italypicgallery.com/wp-content/uploads/perugia-italy-map.jpg

dsa3

dipartimento di scienze agrarie, alimentari e ambientali

PRIN Project

Project of national interest in Italy

Collaboration Between 4 Italian Universities: Naples, Perugia, Padova, and Salerno

PRIN 2020

Unravelling the molecular and phenotypic effects of whole genome duplication and its impact on stress adaptation in plants

- Uses Alfalfa and Potato as model organisms
- Currently processing data from preliminary studies to choose and repeat abiotic stress that seems to have largest impact

Project Breakdown

01 **WP1**

Assess the impact of polyploidization on plant stress response

02 **WP2**

Compare, by -omics tools, 2x vs 4x both in standard conditions and following the stress identified in WP1

03 **WP3**

Data validation and translational research in cancer polyploidy

04 **WP4**

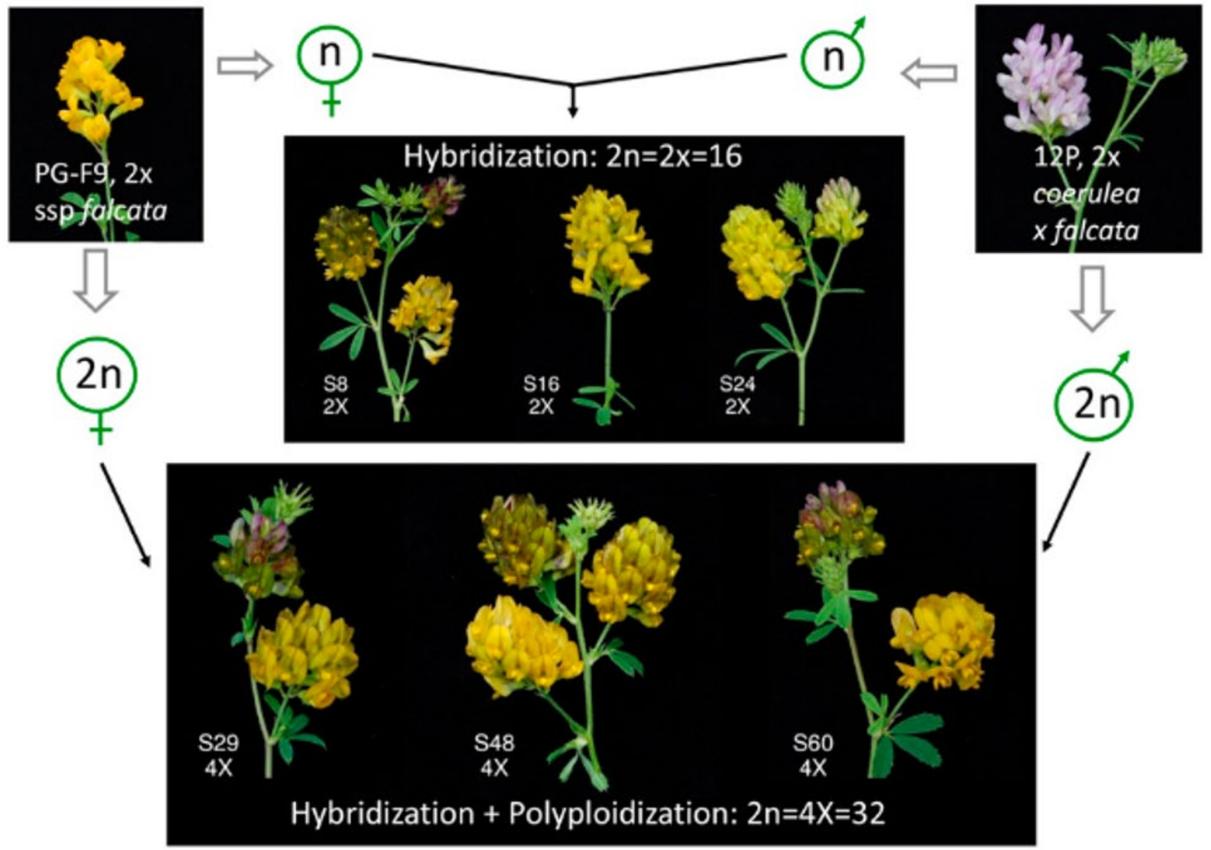
Integrate data from -seq, metabolomics and proteomics analyses and identify potential conservative genomic elements between plants and tumor cancers

Our contribution to the project

- UNIPG contributed unique alfalfa genetic material
  - Neotetraploid and diploid full siblings from meiotic mutant parents studied by Rosellini et al 2016.

- We conducted the drought stress study portion of this work due to our lab's previous experience with drought stress

- Our study was first done in potatoes for proof of concept, but that data will not be shown.



Rosellini et al. 2016

Stress Measurement

1. Daily soil water content measurement
2. Photosynthetic rate
3. Stomatal conductance
4. Transpiration rate
5. Intercellular CO_2 concentration
6. Chlorophyl content
7. Leaf Water Content
8. Proline content
9. MDA content

Photos from Canva

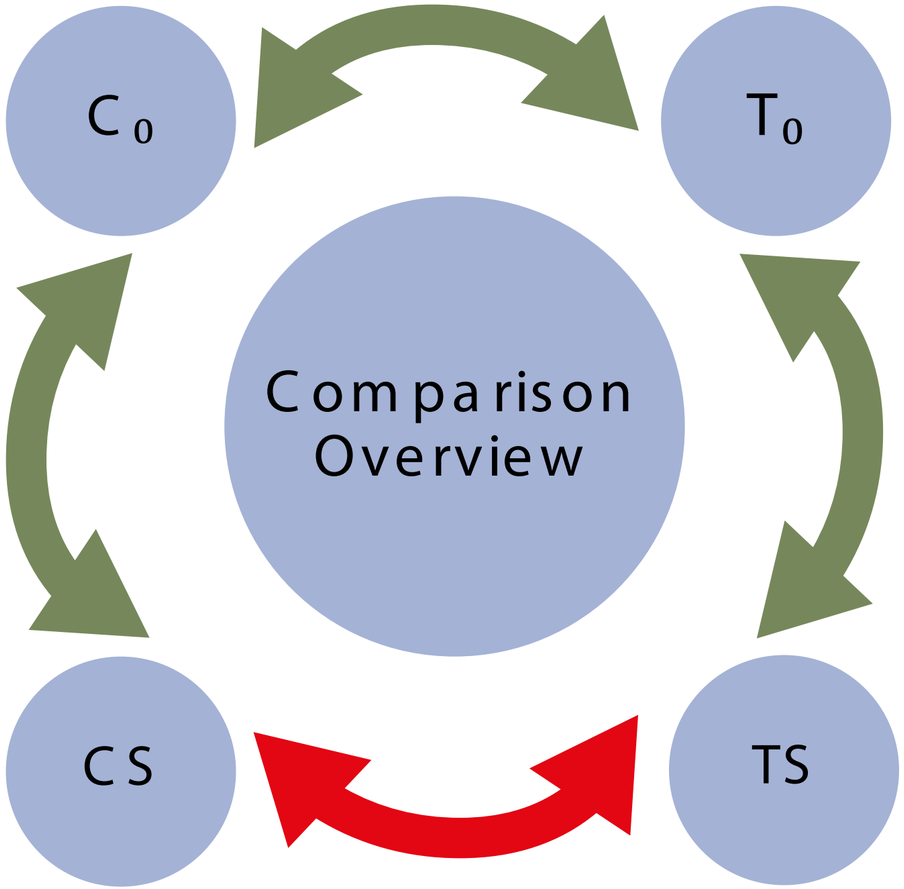
Data analysis

Models have been used

- Linear Regression Model
- Linear Mixed- Effect Model
- ANOVA
- Pairwise Comparison

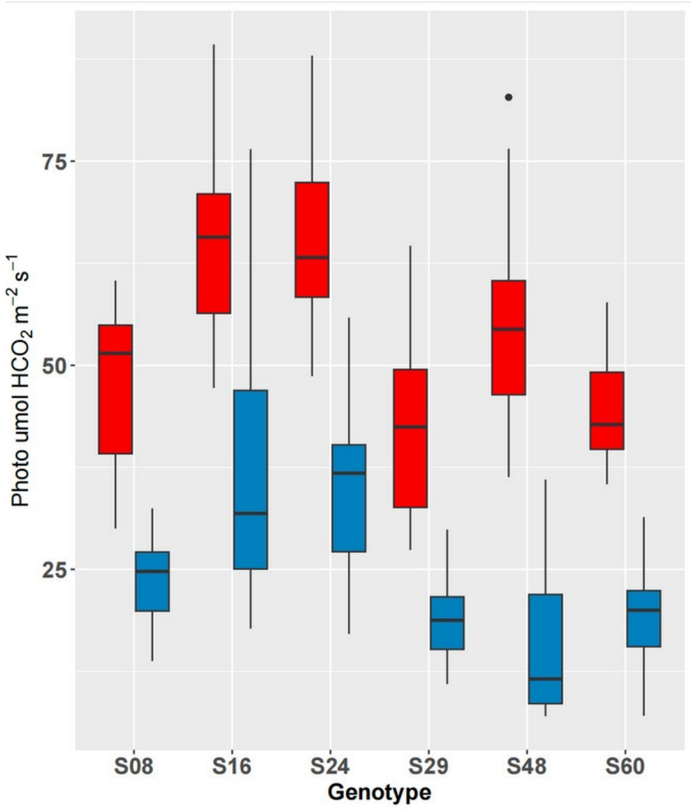
Comparisons have been done

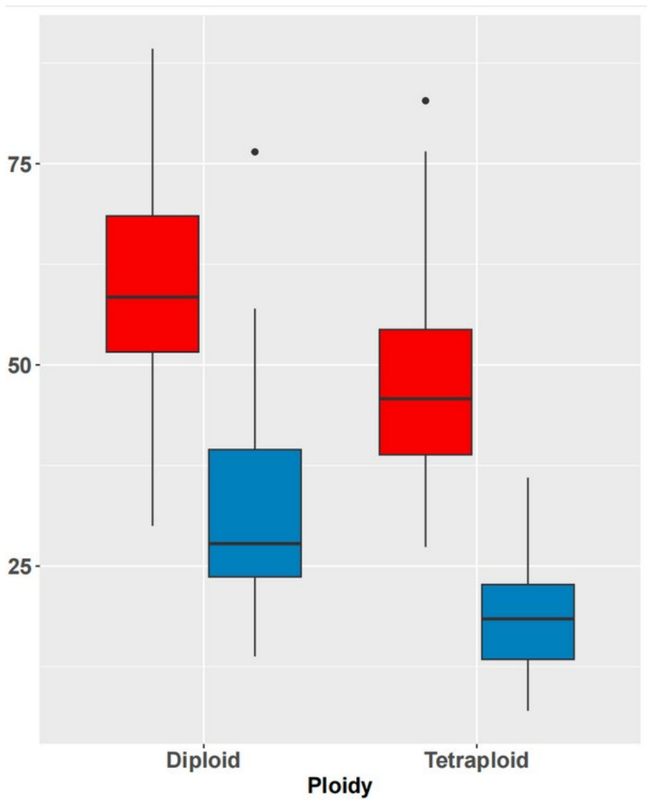
- Comparison between treated (T0) and control (C0) plants at baseline for inherent variation
- Comparison over time to determine normal growth in controls and potential differences in treated plants
- Comparison at stress timepoints (TS and CS)

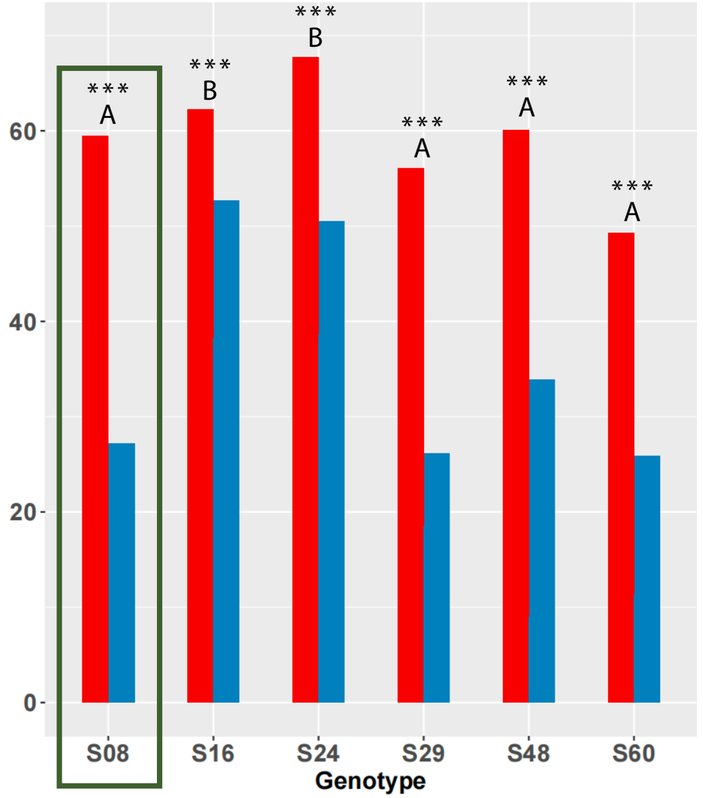


Photosynthetic Rate

Note that significance values are for difference between control and stress for each genotype while pairwise grouping is comparing between stressed values and not controls



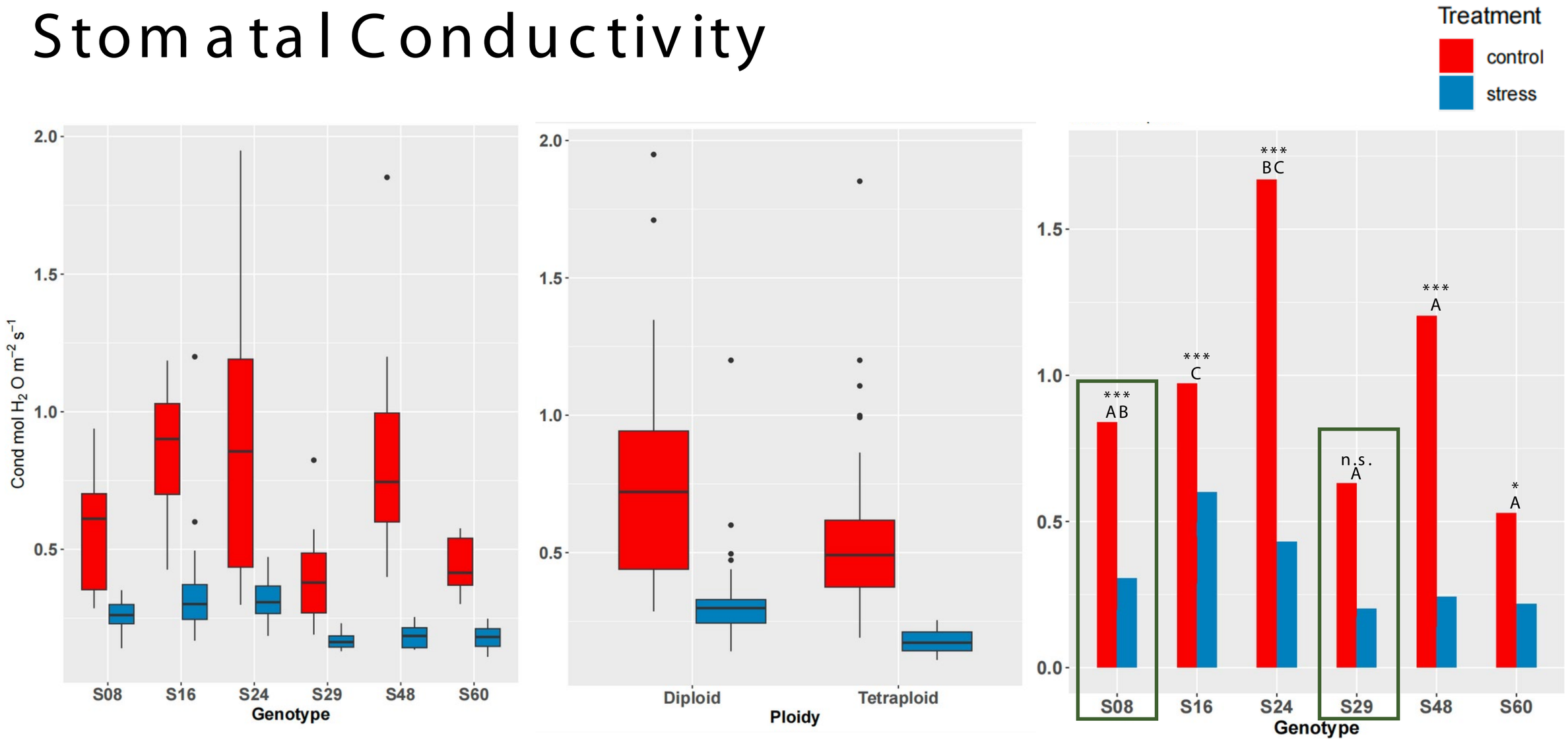




The reduction in photosynthetic rate under stress condition is evident. However, diploid plants seem to have a higher photosynthetic rate under the same conditions compared to tetraploids.

Is there structural differences between s8 and s16-s24 that makes it more like tetraploids?

Stomatal Conductivity

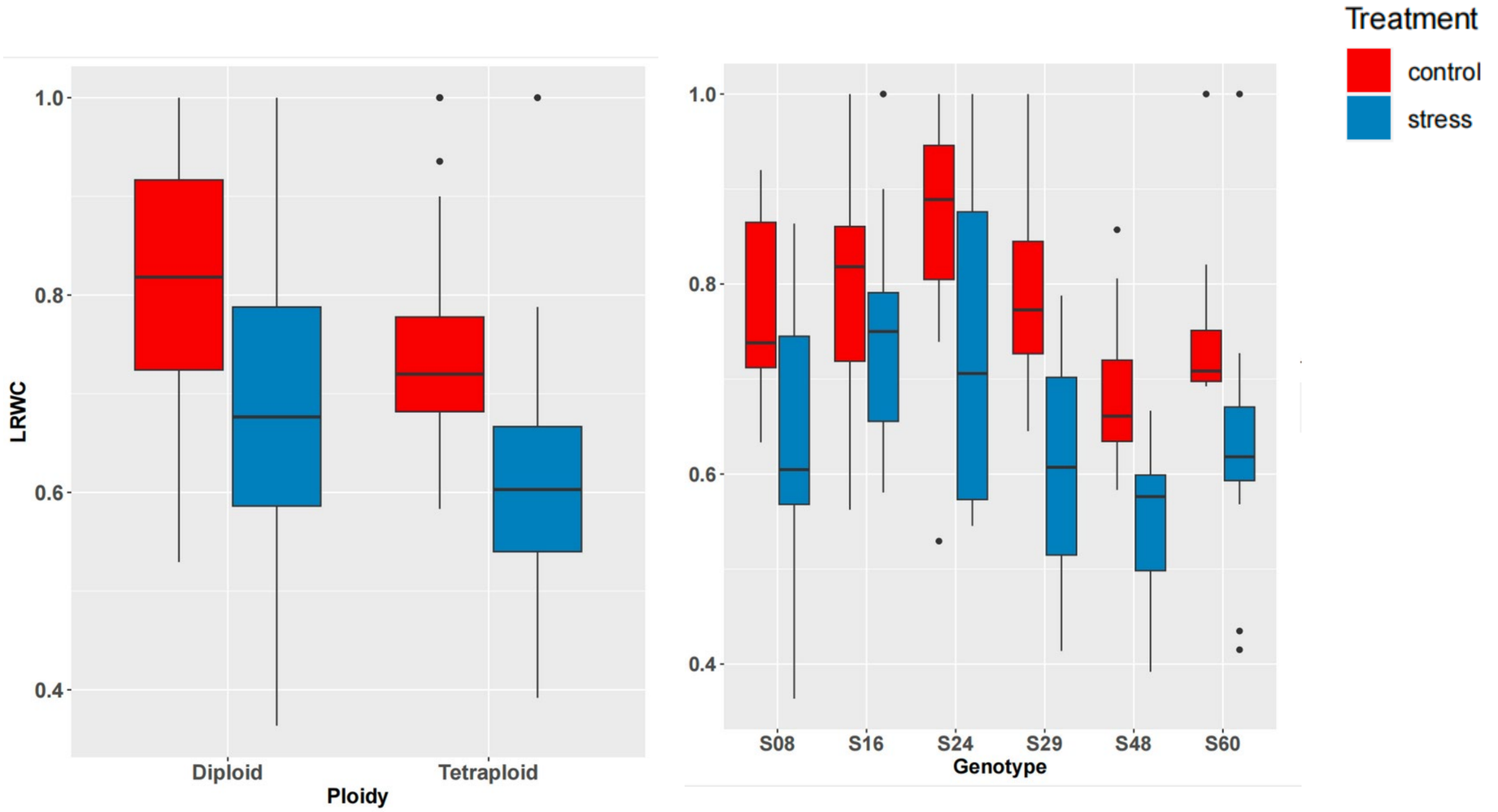


Stomatal conductivity differs between control and treated plants.

Stomatal conductivity decreased in stressed plants compared to control plants.

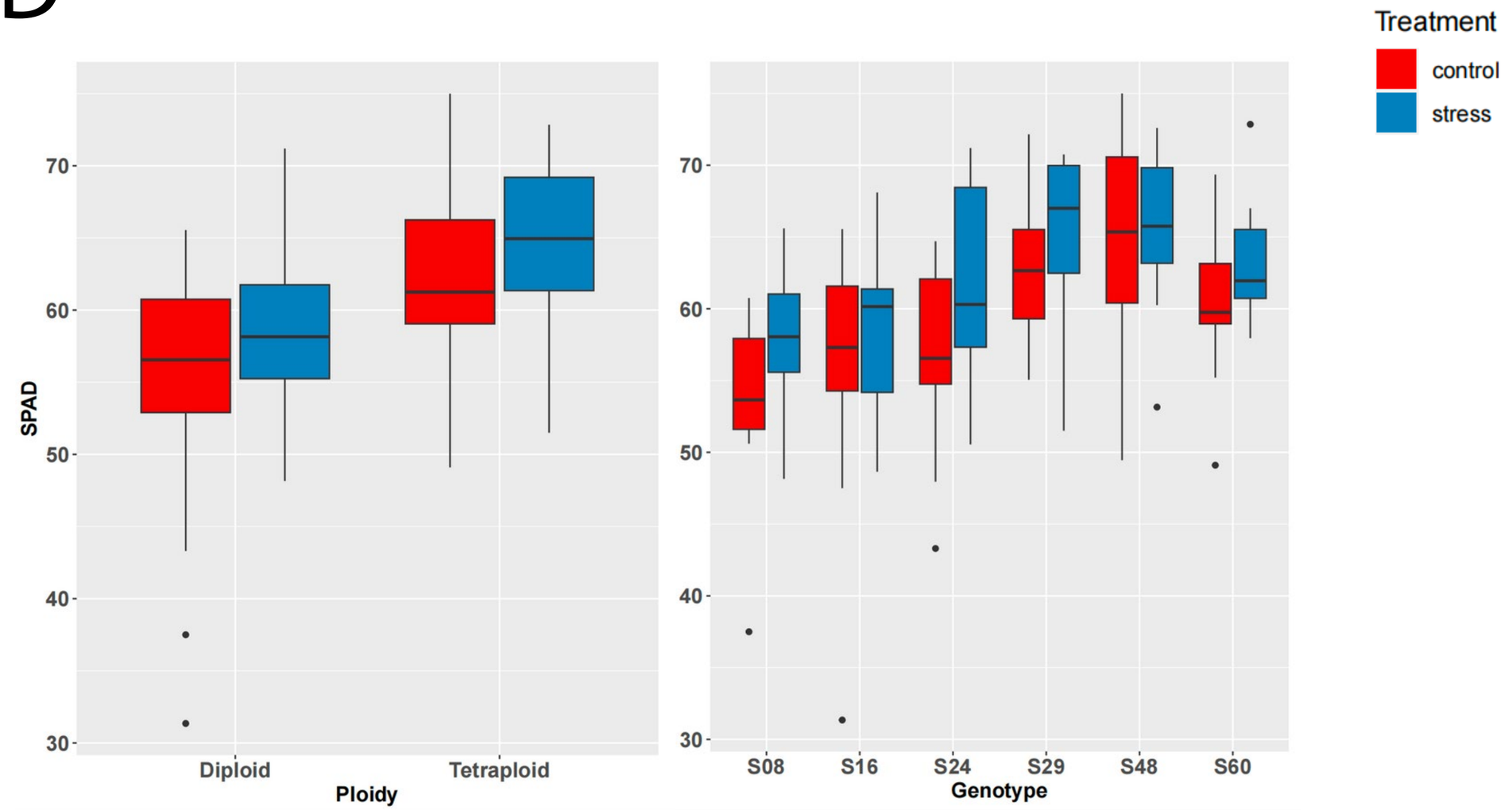
S29 genotype doesn't show any significant different between control and stress.

LRWC



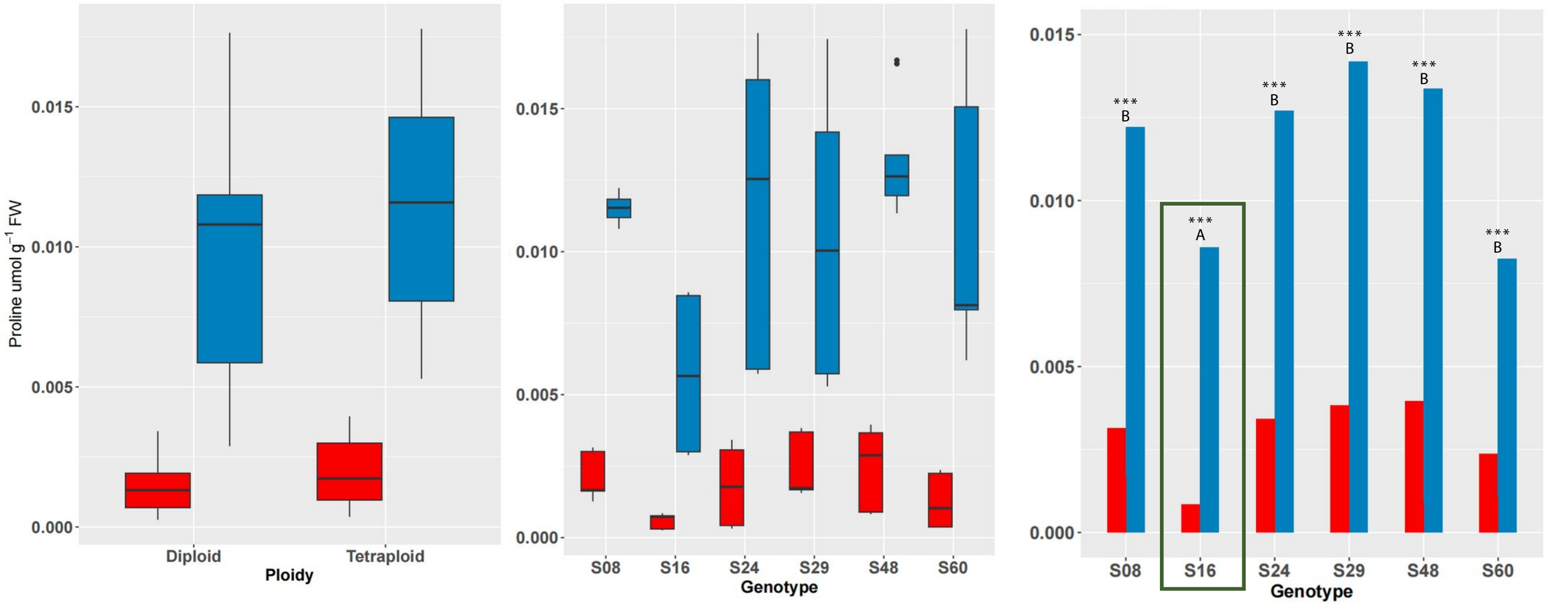
The leaf relative content shows a significant variation among genotypes at the stress timepoint.
The water retention capacity in diploids is higher than in tetraploids.

SPAD



Tetraploids contain more chlorophyll than their diploid counterparts under the same condition.

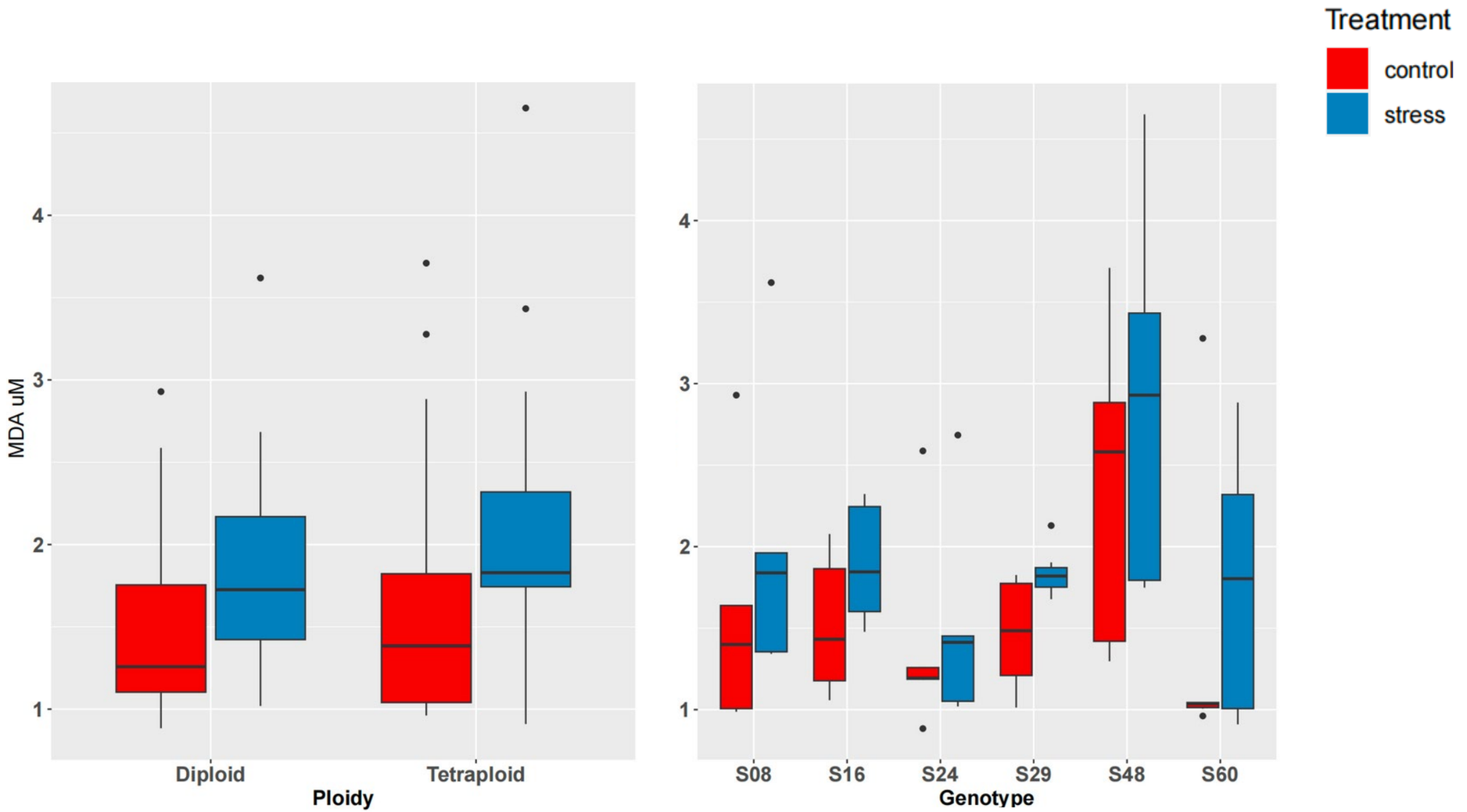
Proline



Proline content increased in all genotypes under stress condition suggesting that the level of response appears to vary between genotypes.

S16 grouped differently from the other genotypes having lowest proline content.

MDA



The MDA level, as an indicator of oxidative stress, varies among genotypes under both stress and non-stress condition.

Discussion

- There is a clear difference between ploidy levels and genotypes and stress for our measured parameters; however, we did not identify a significant genotype by stress or ploidy by stress interaction.
- A single timepoint measurement may not be fully indicative of stress response.
- Variation between genotypes at each ploidy level was pronounced, even though they are full-sibs.

How will this project move forward?

Phase 2 and 3 of the PRIN project uses transcriptomic analysis from RNA-seq data.

- Project by post doc Danilo Fabrizio Santoro
- This is currently being analyzed and will be presented at the 2024 Italian Society of Agricultural Genetics conference

Genomic structure of these plants is being studied in the MSCA-RISE POLYPLOID Project

- Project by PhD Student Aaron Anderson (poster is here)
- Constructing genomes of the parents first, then the offspring from this study to compare structural differences.

Thank you!

- Daniele Rosellini- UNIPG
- Danilo Fabrizio Santoro- UNIPG
- Aaron W. Anderson- UNIPG
- Domenico Carputo-
- Benedetto Battaglia- UNIPG
- E. Charles Brummer- UC DAVIS

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Overall results

Key Take Aways

- Ploidy and Genotype were nearly always significant though their interaction with treatment was not
- Only 3 blocks were used for Biochemical analysis due to tissue handling error and border effects
- Is the ANOVA the final say in this analysis?

Randomized Models with all 5 blocks			
	Model: Ploidy + Treatment + Ploidy :Treatment + (1|Block)		
Variable	Main Effects		Interaction
	P	T	P:T
Photosynthetic Rate	***	***	0.8039
Percent Change in Photosynthesis	***	***	0.67
Stomatal Conductance	**	***	0.5528
LRWC	**	***	0.748
Intracellular CO_2	0.6482	0.2802	0.4494
Transpiration Rate	**	***	0.4807
SPAD	***	*	0.435
Proline	*	***	0.251
MDA	0.103	*	0.759
	Model: Genotype + Treatment + Genotype :Treatment + (1|Block)		
Variable	Main Effects		Interaction
	G	T	G:T
Photosynthetic Rate	**	***	0.7246
Percent Change in Photosynthesis	***	***	0.9505
Stomatal Conductance	***	***	0.1159
LRWC	**	***	0.97
Intracellular CO_2	0.05	0.258	0.834
Transpiration Rate	***	***	0.1164
SPAD	***	*	0.9484
Proline	***	***	*
MDA	***	**	0.956

Experimental Design

Completely Randomized Block

5		S16	S60	S24	S29	S48	S8	R
		85	86	87	88	89	90	
		S16	S24	S29	S48	S60	S8	S
		79	80	81	82	83	84	
		S29	S24	S8	S60	S16	S48	N
		73	74	75	76	77	78	
4		S29	S16	S60	S48	S8	S24	S
		67	68	69	70	71	72	
		S60	S48	S8	S24	S29	S16	R
		61	62	63	64	65	66	
		S48	S29	S24	S8	S16	S60	N
		55	56	57	58	59	60	
3		S24	S29	S60	S48	S16	S8	R
		49	50	51	52	53	54	
		S48	S60	S29	S24	S16	S8	N
		43	44	45	46	47	48	
		S48	S29	S16	S8	S24	S60	S
		37	38	39	40	41	42	
2		S16	S24	S8	S60	S29	S48	N
		31	32	33	34	35	36	
		S29	S16	S48	S60	S8	S24	S
		25	26	27	28	29	30	
		S8	S60	S48	S24	S16	S29	R
		19	20	21	22	23	24	
1		S16	S29	S24	S48	S60	S8	N
		13	14	15	16	17	18	
		S16	S60	S29	S48	S8	S24	R
		7	8	9	10	11	12	
		S60	S16	S29	S24	S48	S8	S
		1	2	3	4	5	6	

Diploid
- S08
- S16
- S24

Polyploid
- S29
- S48
- S60

Temperature
24±1°C

Humidity
80%

Daylength
16/8