Multi-omics insights into root-driven resource acquisition: understanding responses to water and heat stresses

Corentin Maslard, Mustapha Arkoun, Christophe Salon, Lun Jing, Fanny Leroy, Marion Prudent

Multi-omics insights into root-driven resource acquisition: understanding responses to water and heat stresses

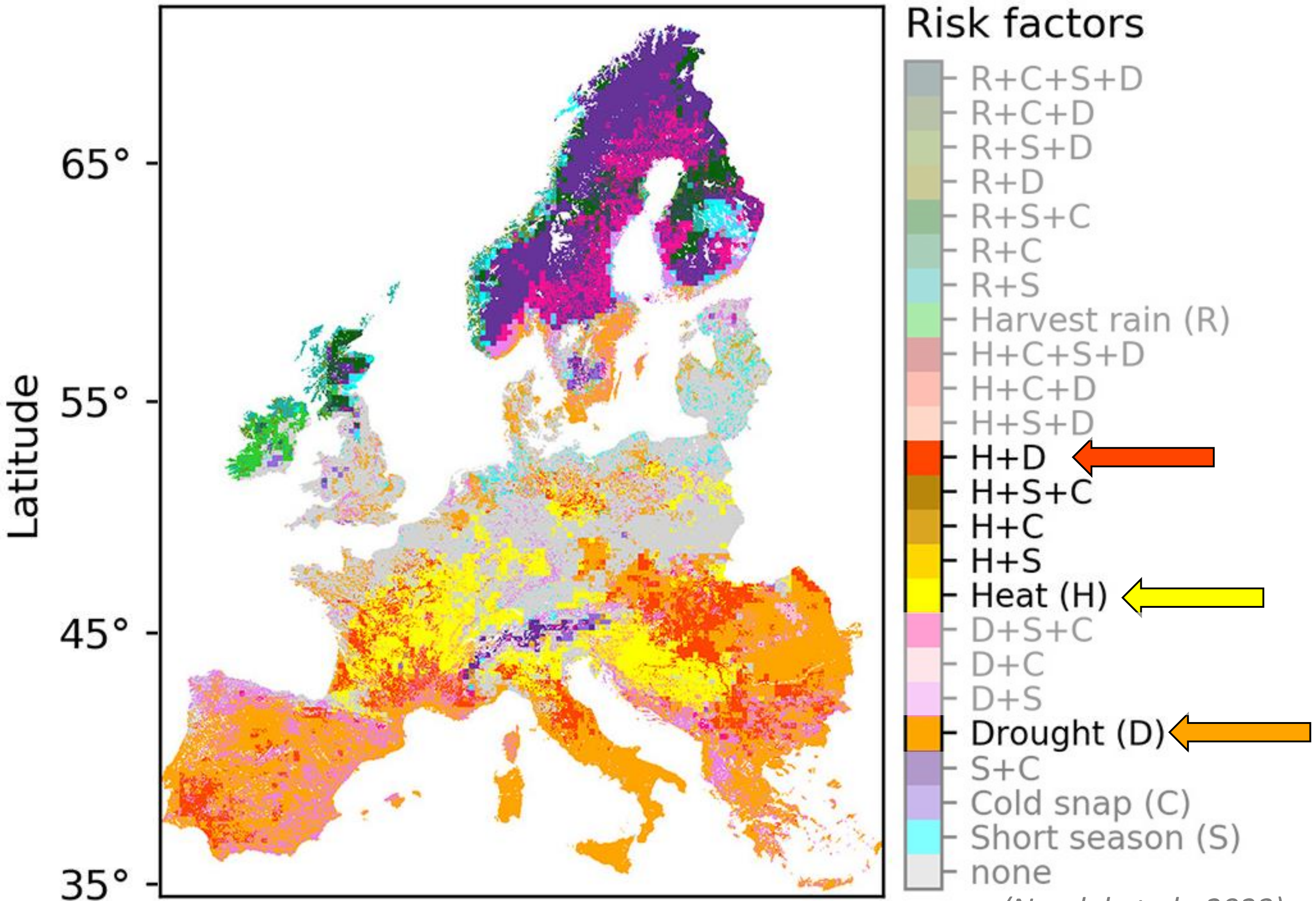
Corentin Maslard

1st meetings of the Rhizophiles network

November 6, 2024

Europe's soybean crops face growing threat from water and heat stresses

Soybean
Glycine max

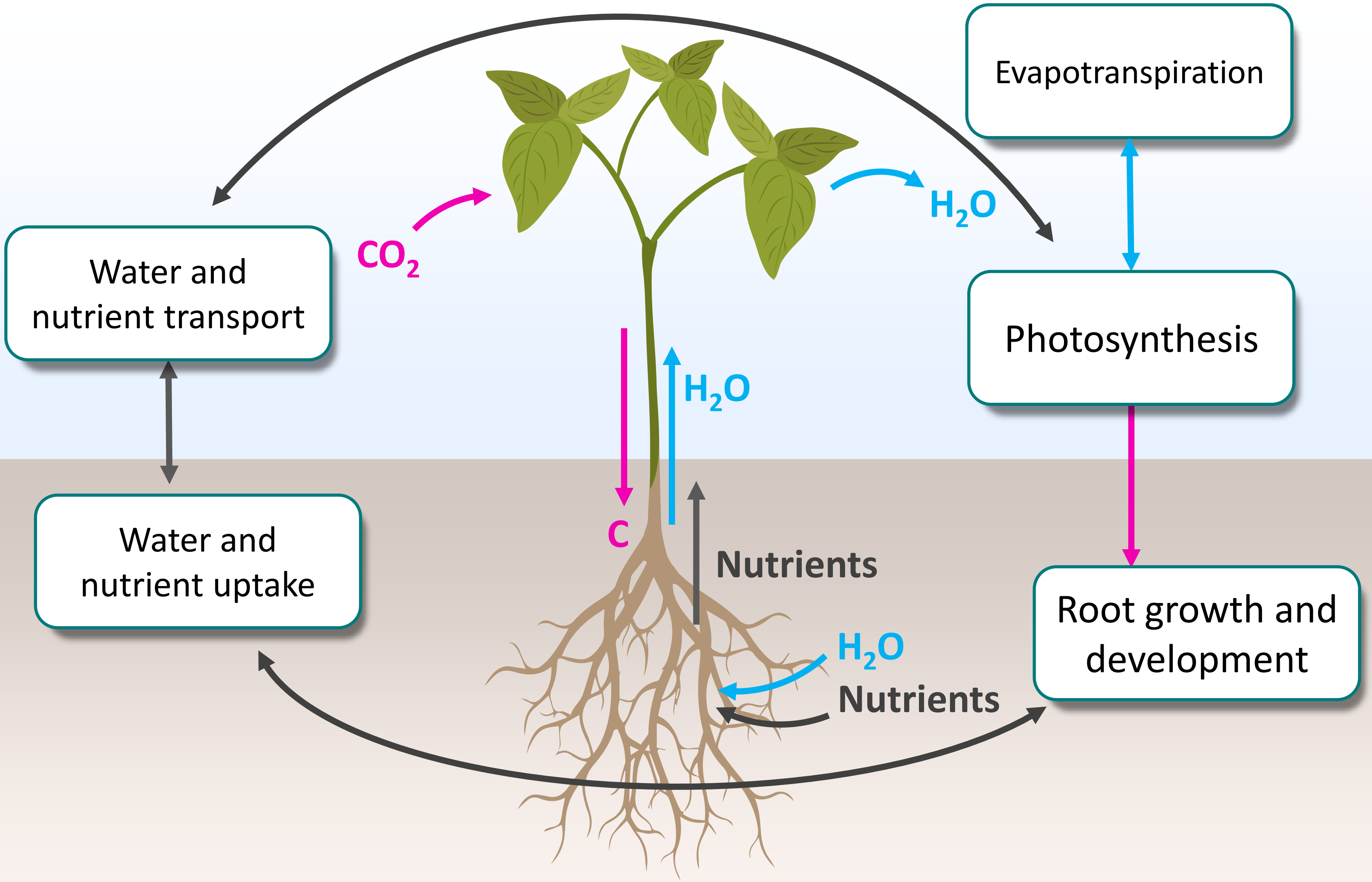


(Nendel et al., 2023)

2040–2069 RCP 8.5 scenario

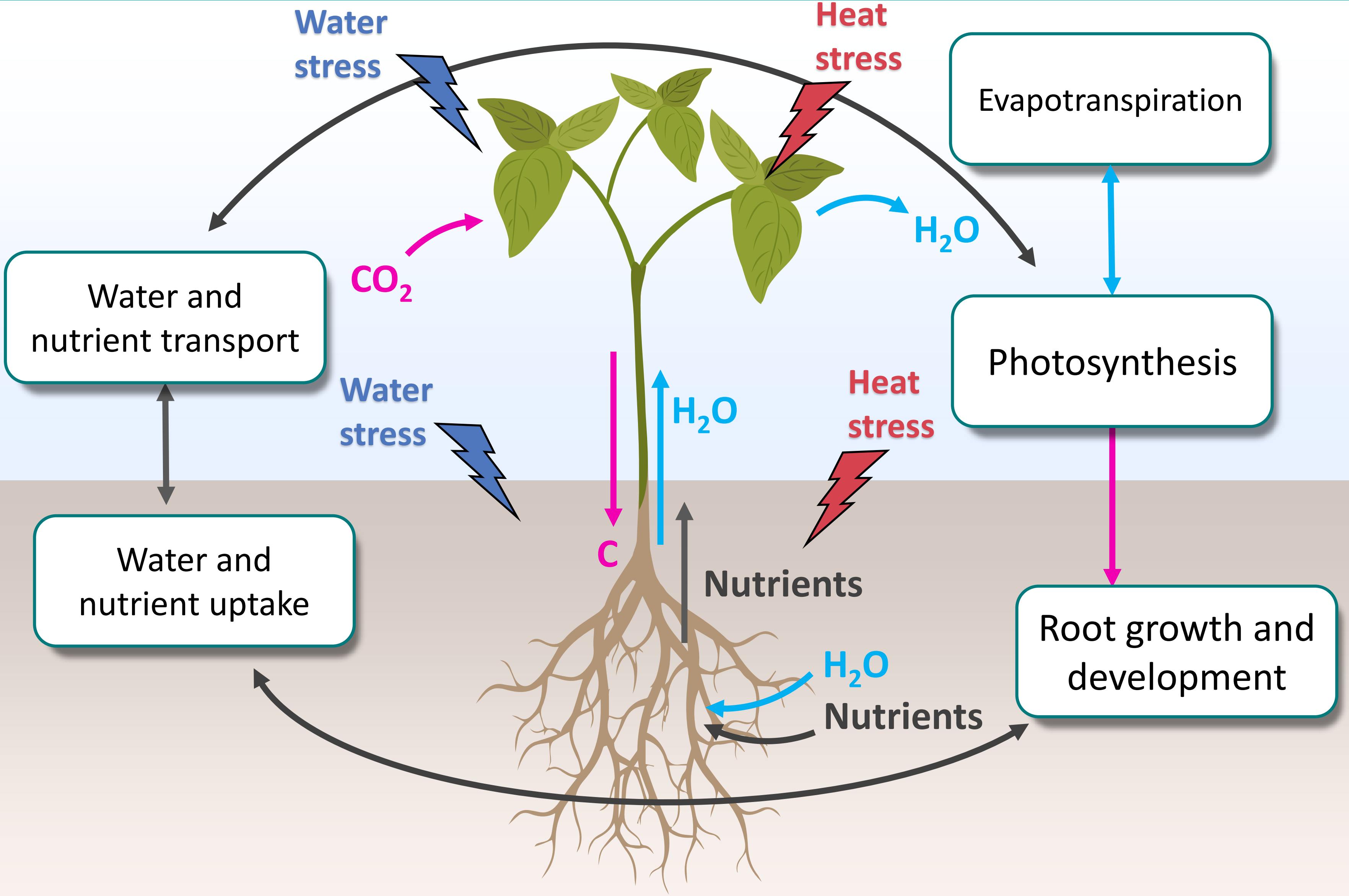
According to several models, in Europe, soybean yields are projected to decrease by approximately **50%**

Conceptual framework for plant nutrition



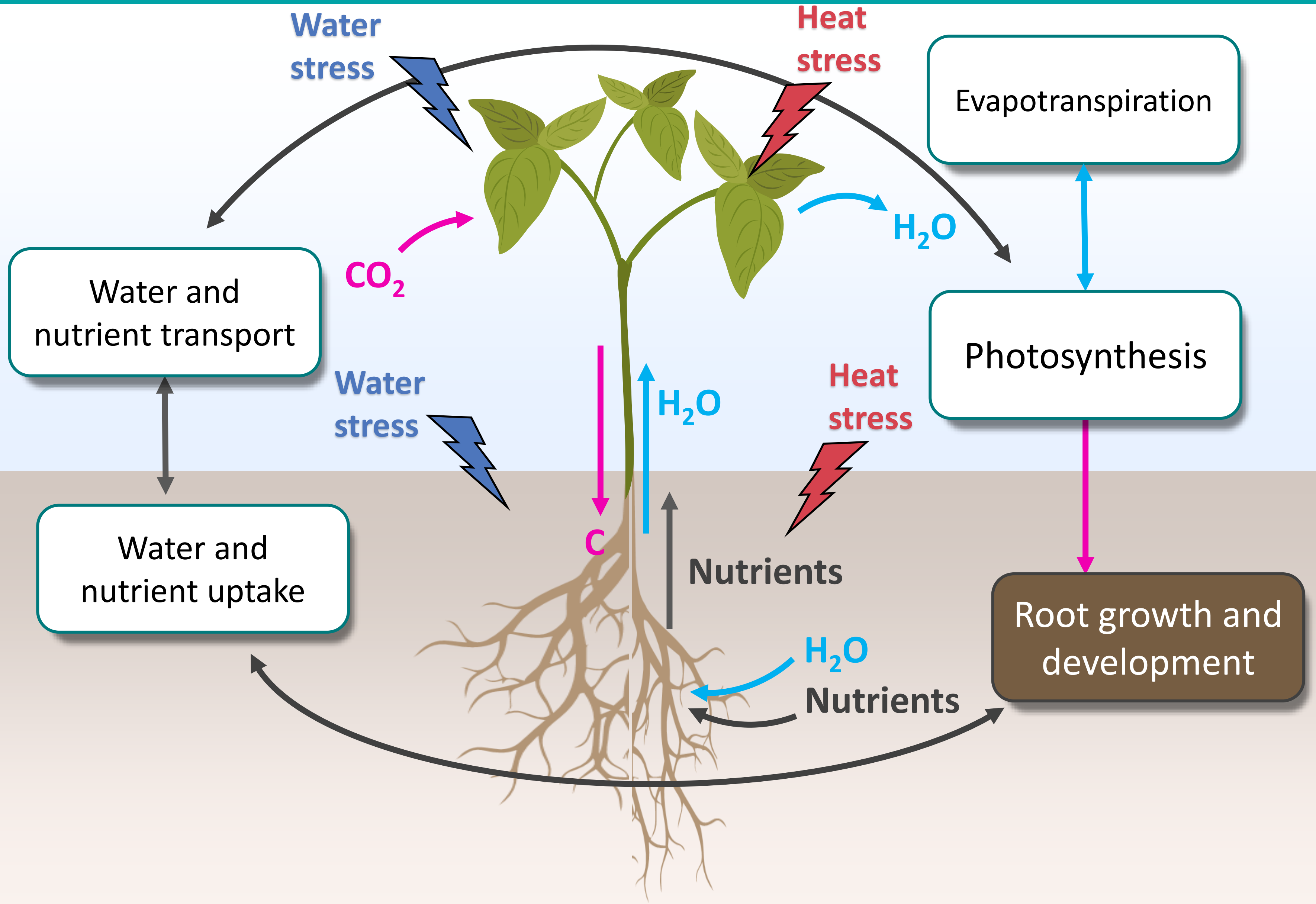
Hypotheses :

- Changes in **water supply** and **temperature** lead to changes in **nutrient uptake**



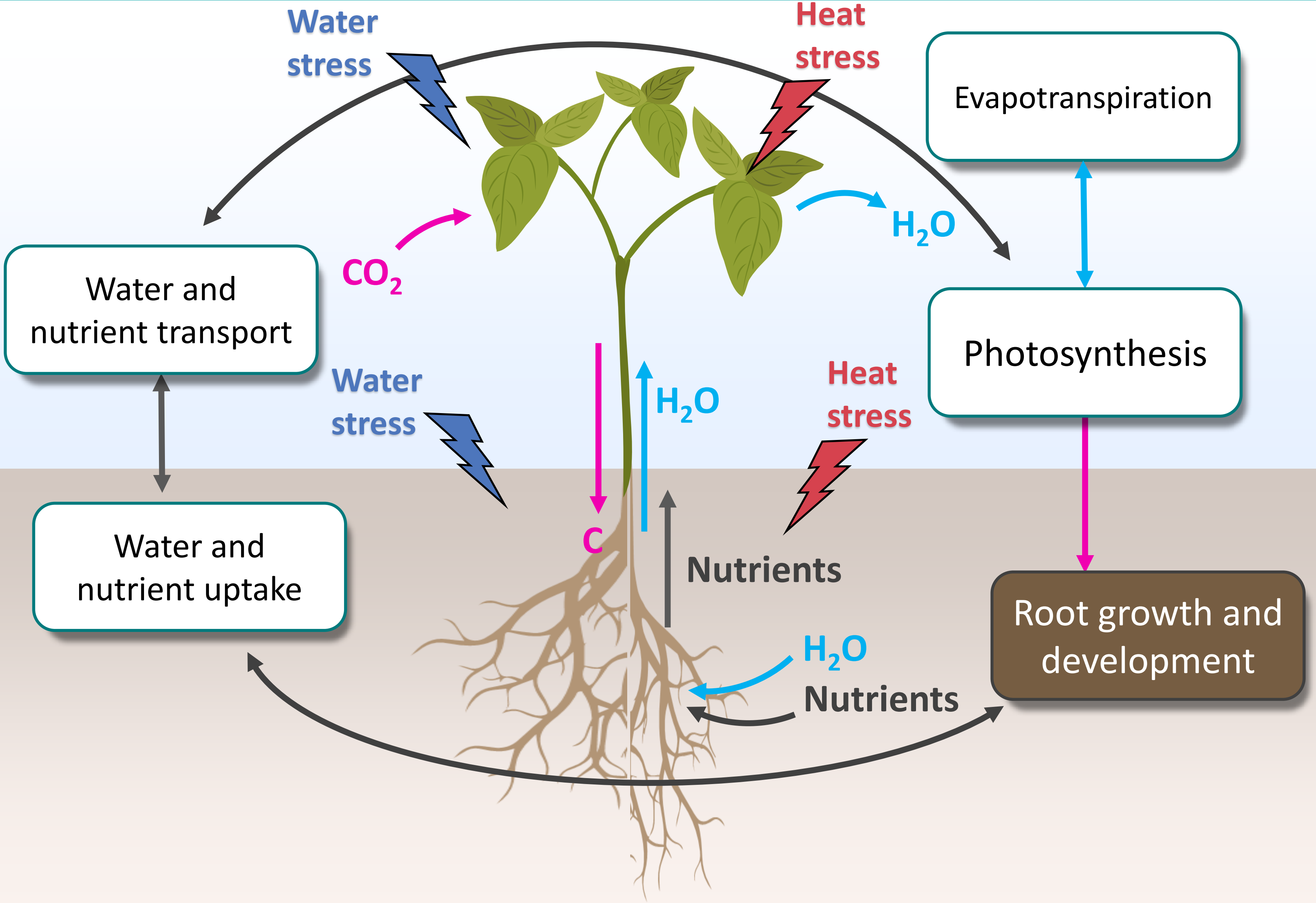
Conceptual framework for plant nutrition

- Changes in water supply and temperature lead to changes in **nutrient uptake**
- Changes in **root architecture** lead to changes in **nutrient uptake**

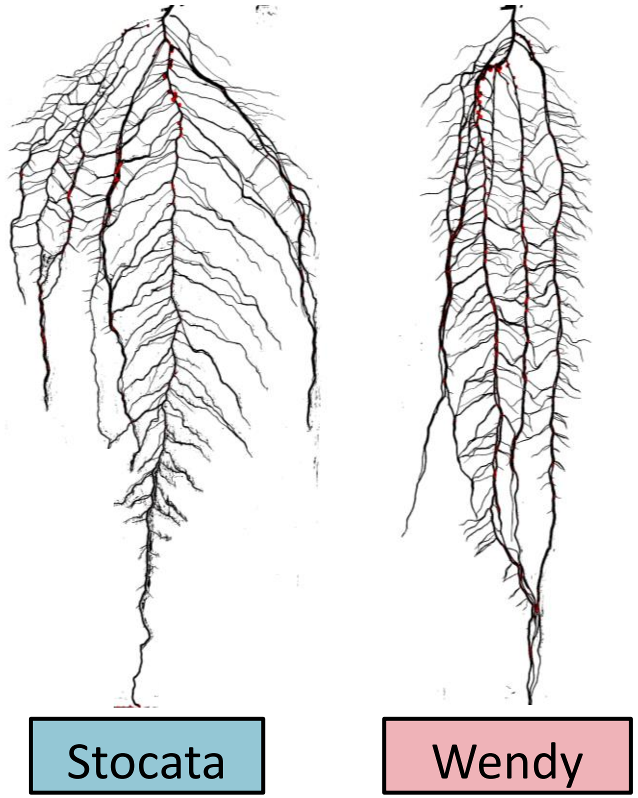


Conceptual framework for plant nutrition

- Changes in **water supply** and **temperature** lead to changes in **nutrient uptake**
- Changes in **root architecture** lead to changes in **nutrient uptake**
- There is a **root architecture** that promotes resistance to **water deficit** and **heat stress**



Experimental design



Two genotypes :
Use of two soybean genotypes with contrasted architecture

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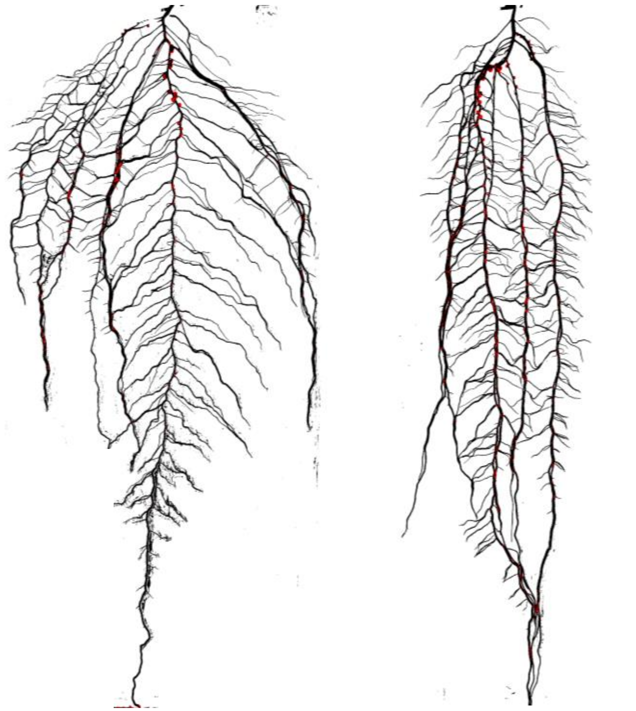

Stocata

Wendy

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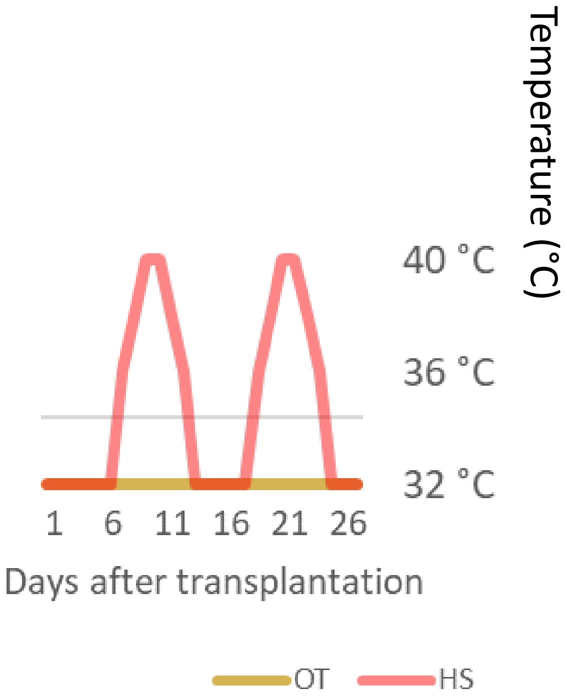
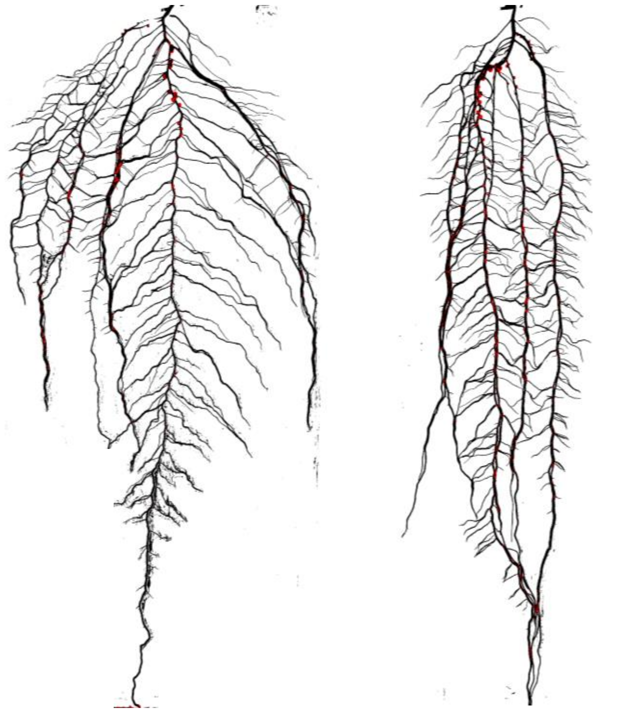


2 temperature conditions :

- **Optimal temperature (OT)**
- **Heat Stress (HS)**



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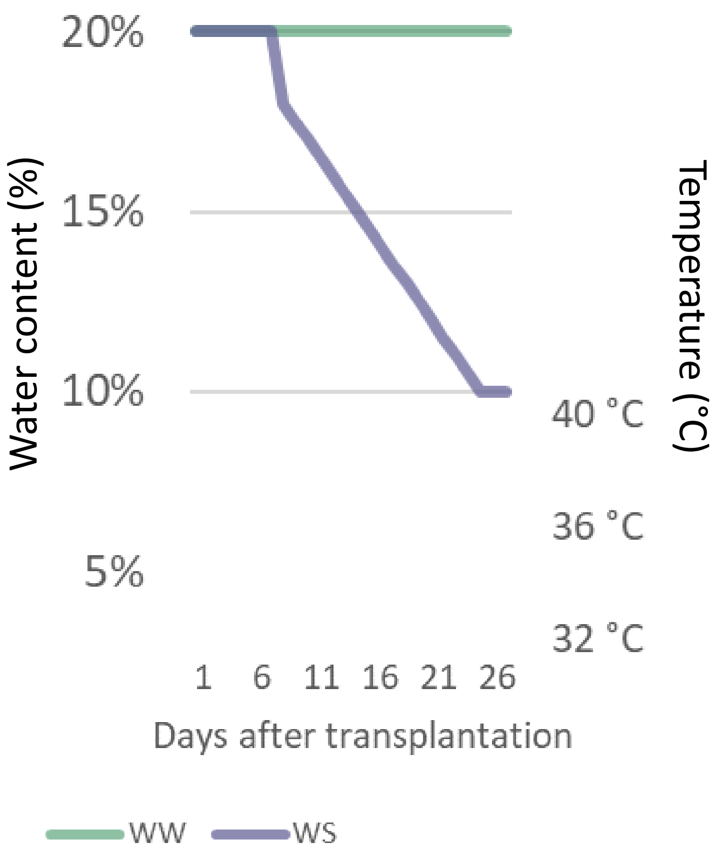
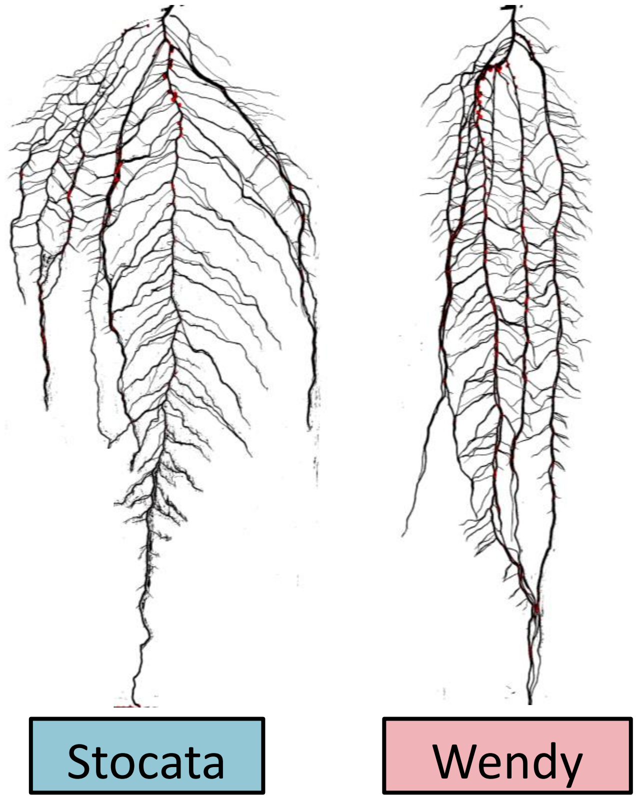


2 water conditions :

- **Well Watering (WW)**
- **Water Stress (WS)**

Experimental design



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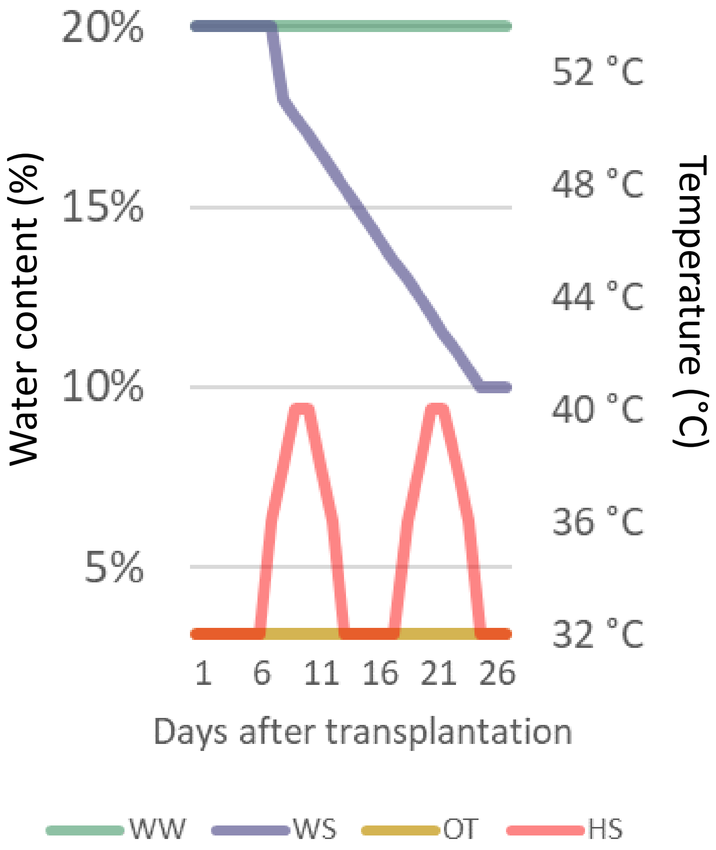

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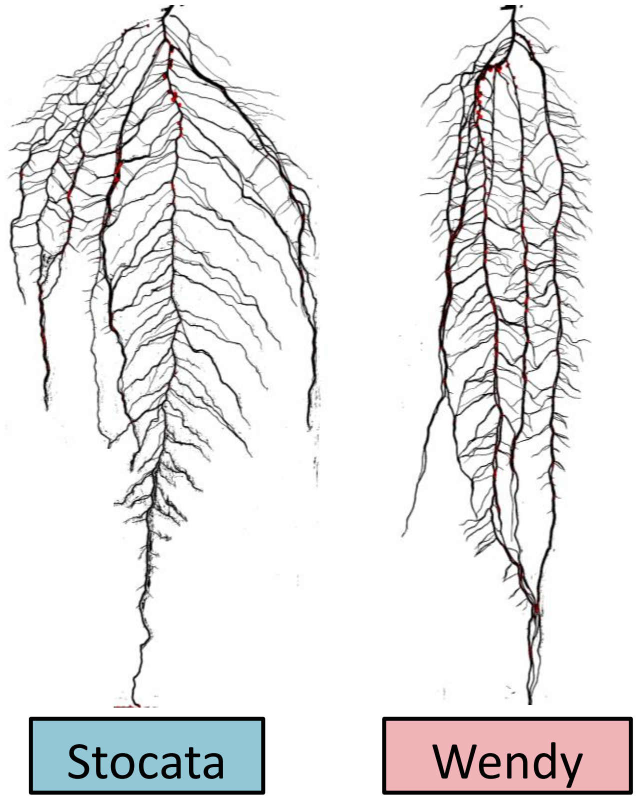

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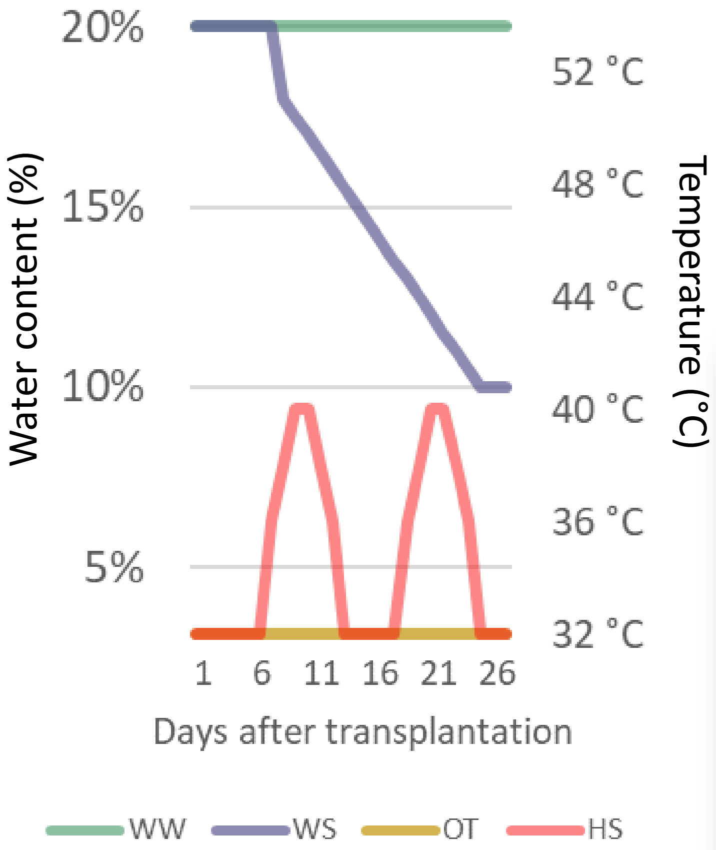

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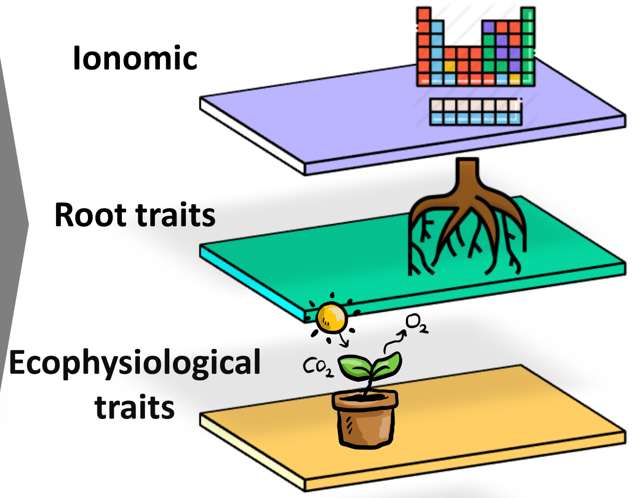

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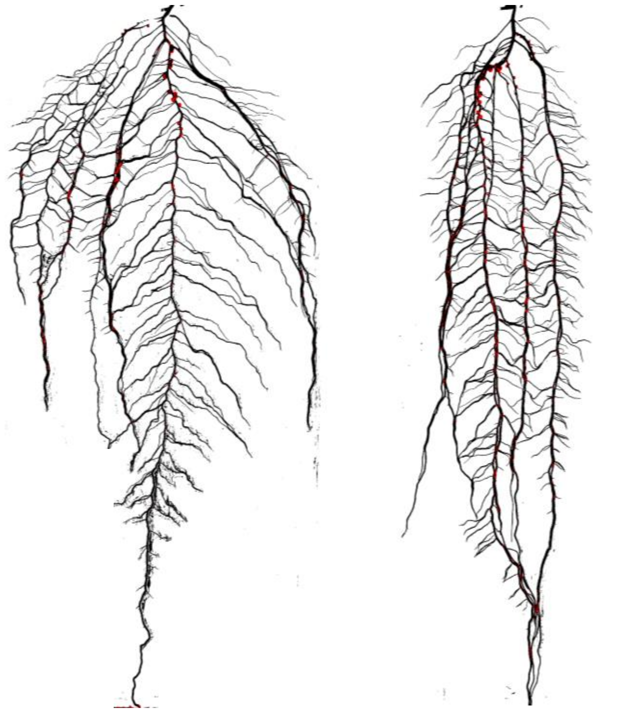

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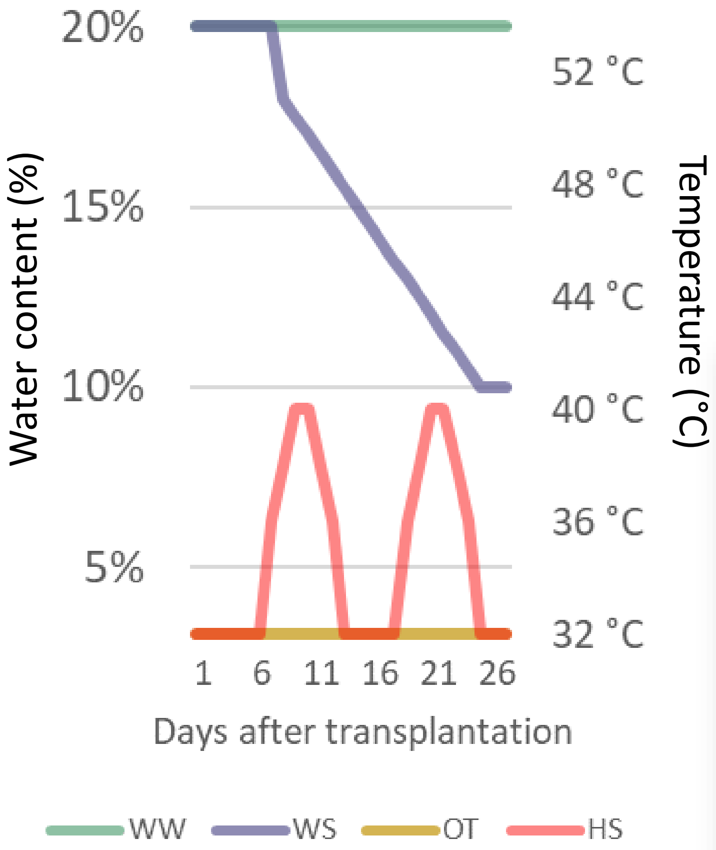

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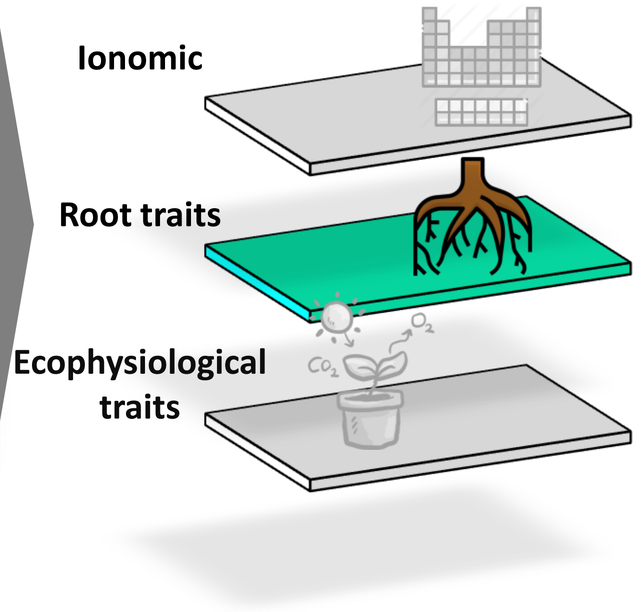
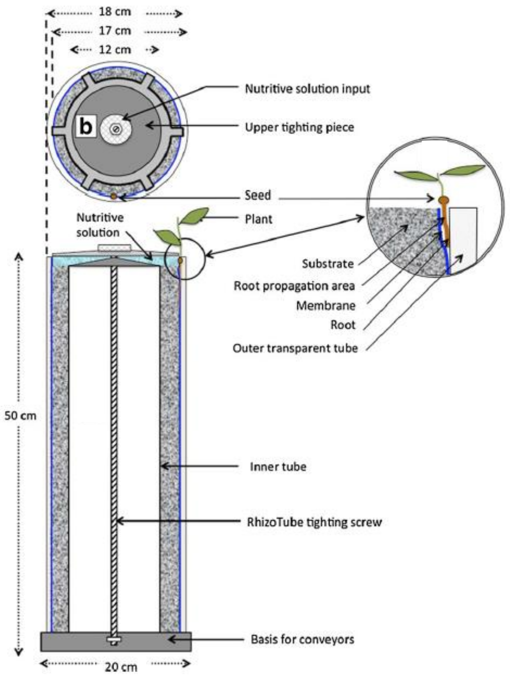


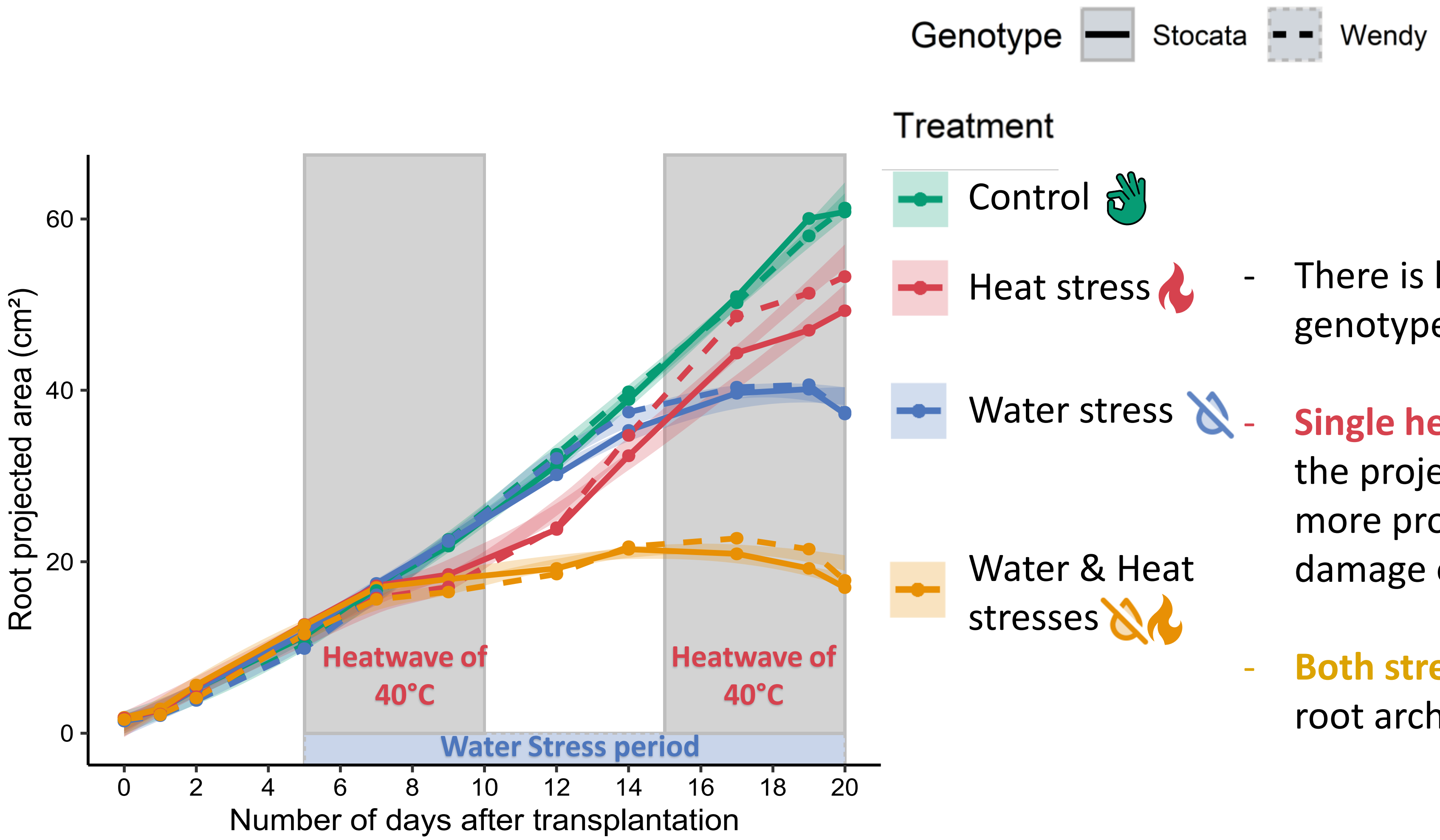
Plant phenotyping with the 4PMI platform

Plant Phenotyping Platform
4PMI
Plant Micro-organisms Interactions



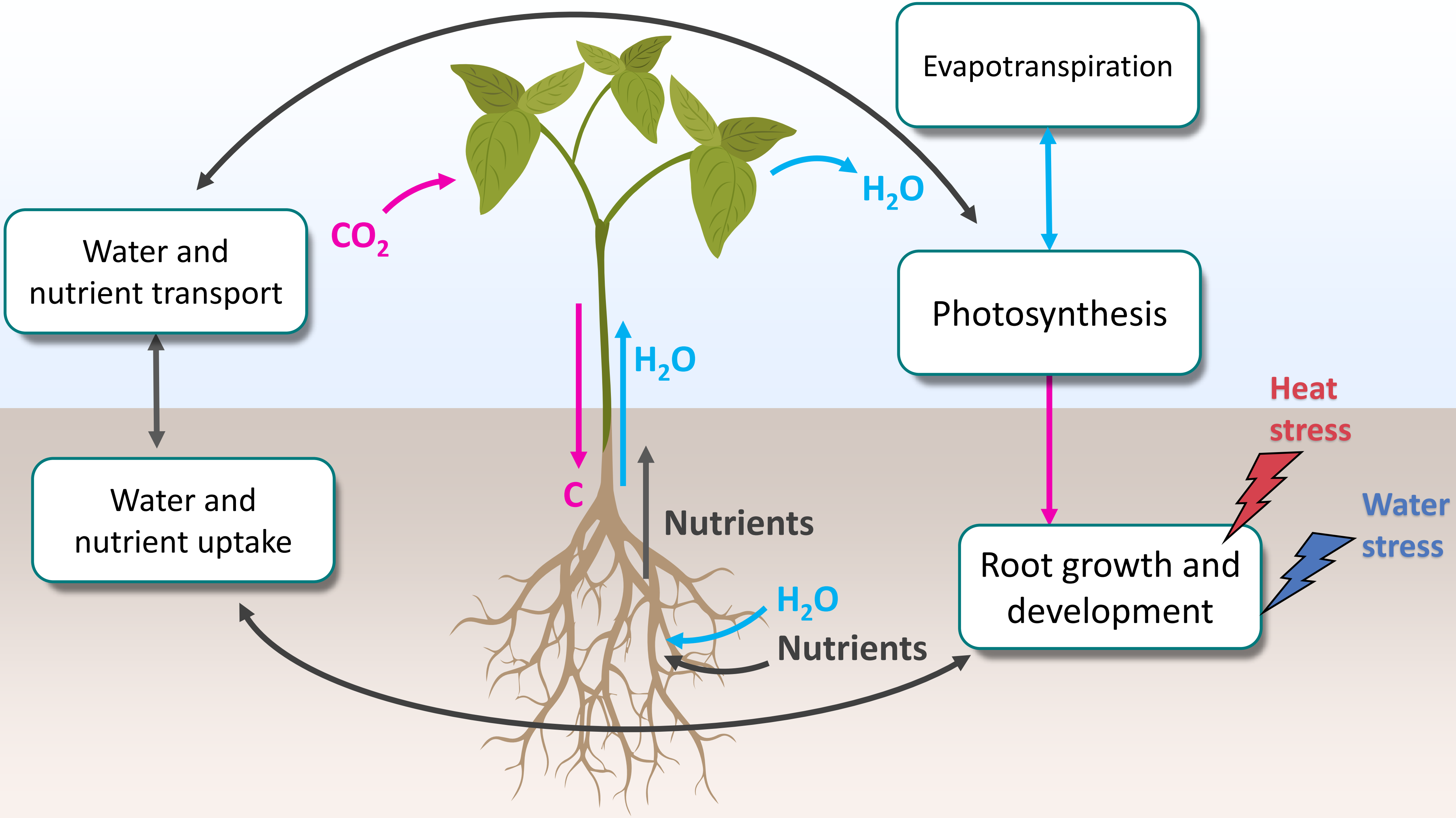
Non-destructive phenotyping of roots over time, precise control of watering and greenhouse climate

How stresses affect the root projected area ?

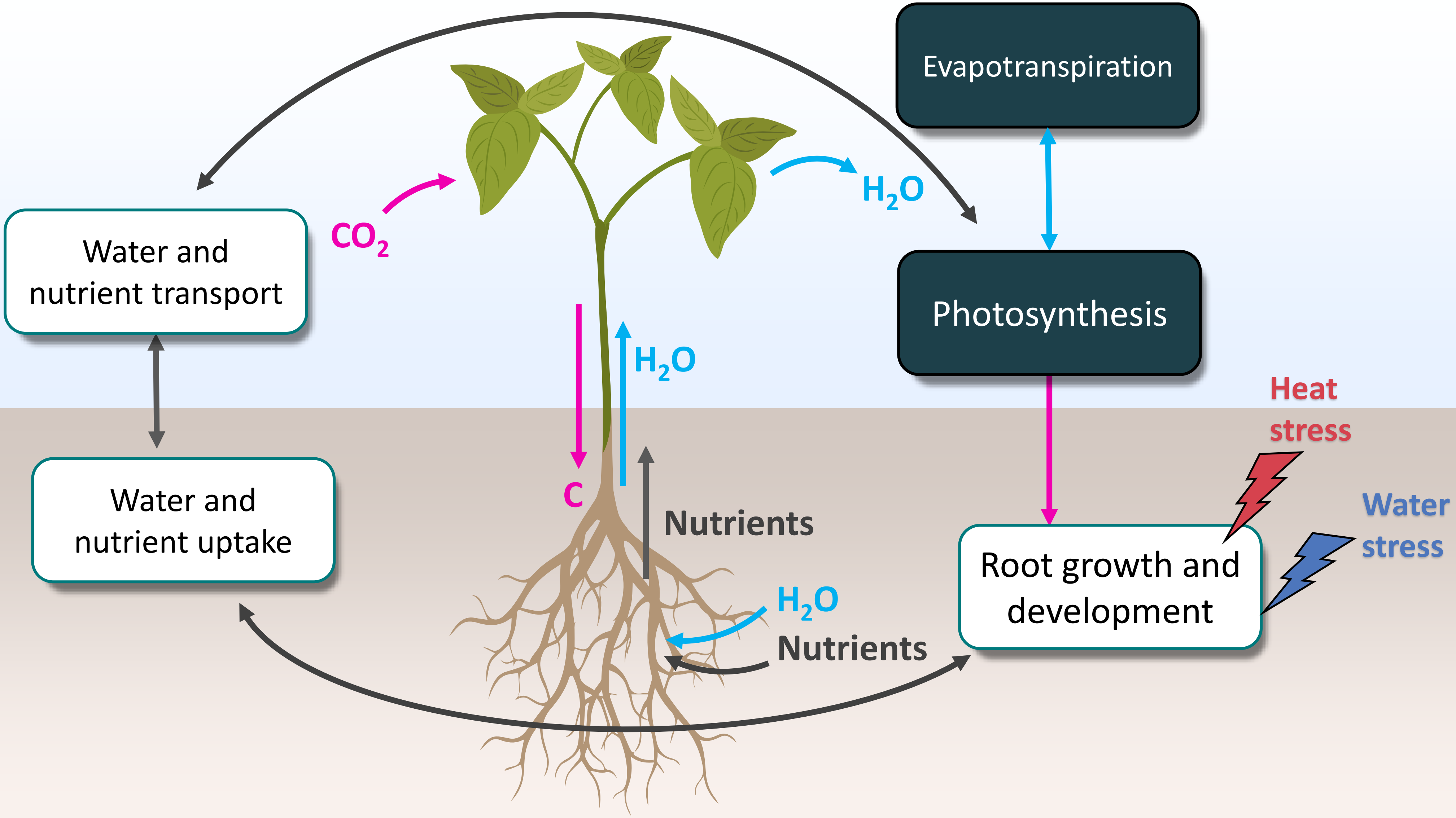


- There is little difference between the two genotypes in terms of root projected area
- **Single heat stress** has a faster impact on the projected root area. **Water stress** is more progressive and causes more damage over the long term
- **Both stresses** have a negative synergy on root architecture

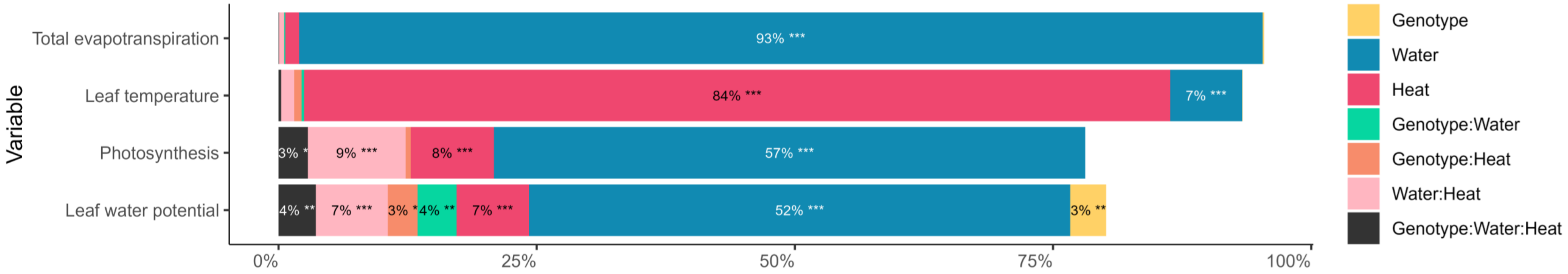
Conceptual framework for plant nutrition



Conceptual framework for plant nutrition



Treatment and genotype effects on variability in key ecophysiological traits

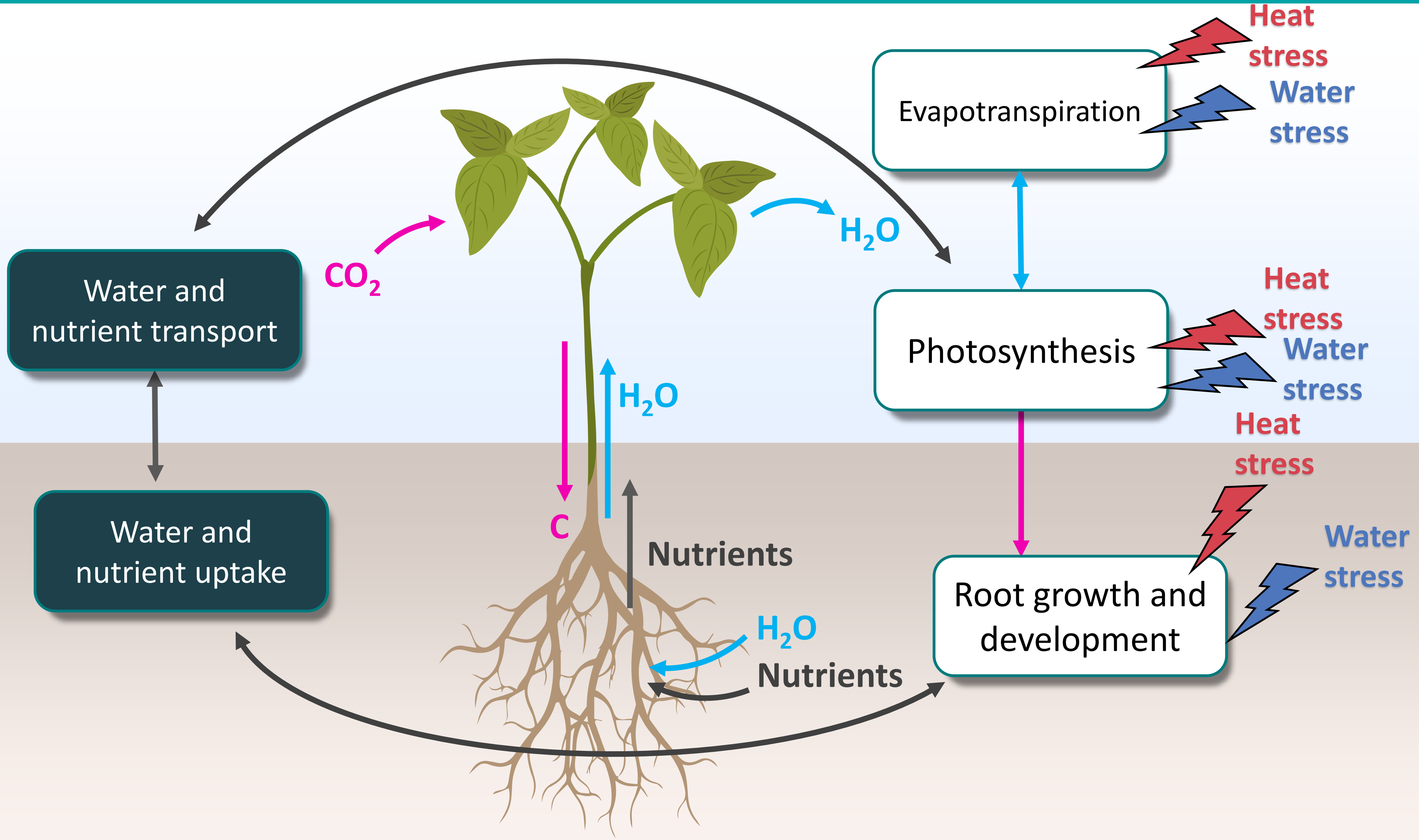


- Variations in certain ecophysiological variables are due entirely to **water stress**.
- Others, on the contrary, are due only to **heat stress** and little to **water stress**.
- There are sometimes **interaction** effects between the two stresses.
- And sometimes there are even **genotypic effects**.

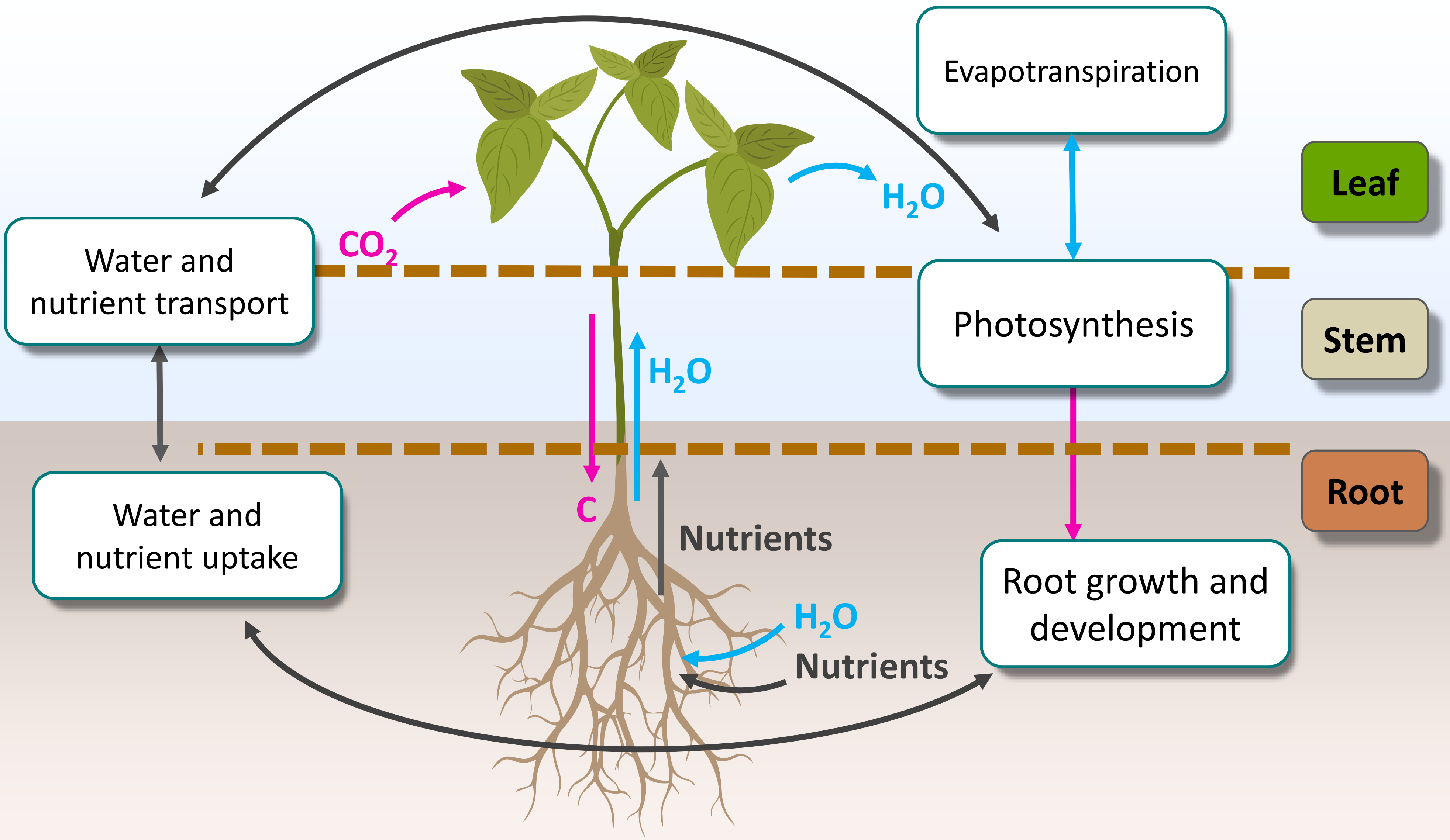
$$V_i = \frac{SS_i \times 100}{\sum_i SS_i + R}$$

V_i: Percentage of variation
SS_i : the sum of square of the factor *I* (genotype, water or heat stress)
R: the Residuals of the model

Conceptual framework for plant nutrition



Conceptual framework for plant nutrition



Essential Macroelement
(C, Ca, K, Mg, N, P, S)

Essential microelement
(B, Cu, Fe, Mn, Mo, Ni, Zn)

Leaf

Stem

Root

Beneficial element
(Co, Na, Se, V)

Other
(As, Ba, Be, Cd, Cr, Rb, Tl)

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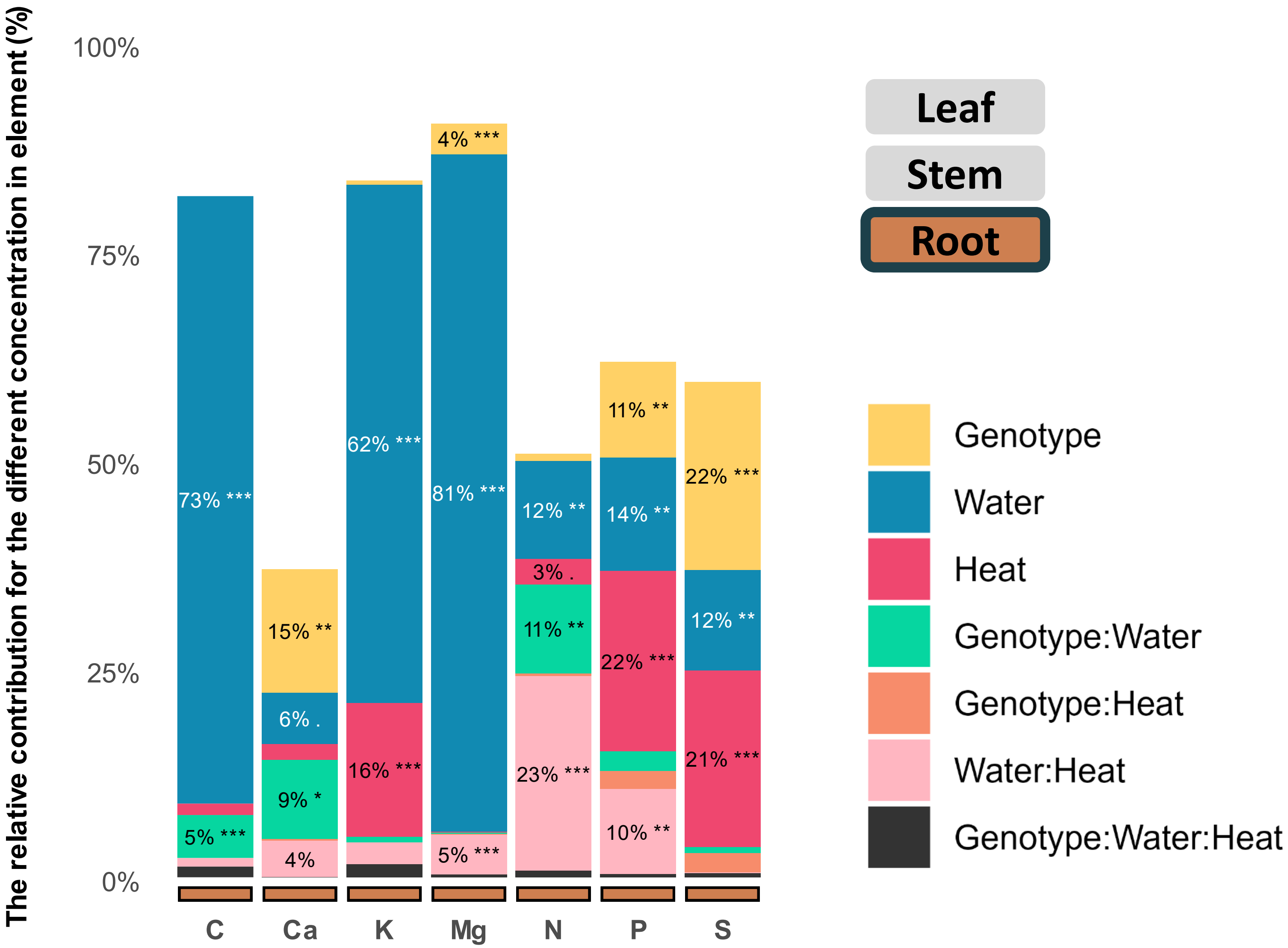
Relative contribution of treatment and genotype to variability in Macroelement concentration in roots



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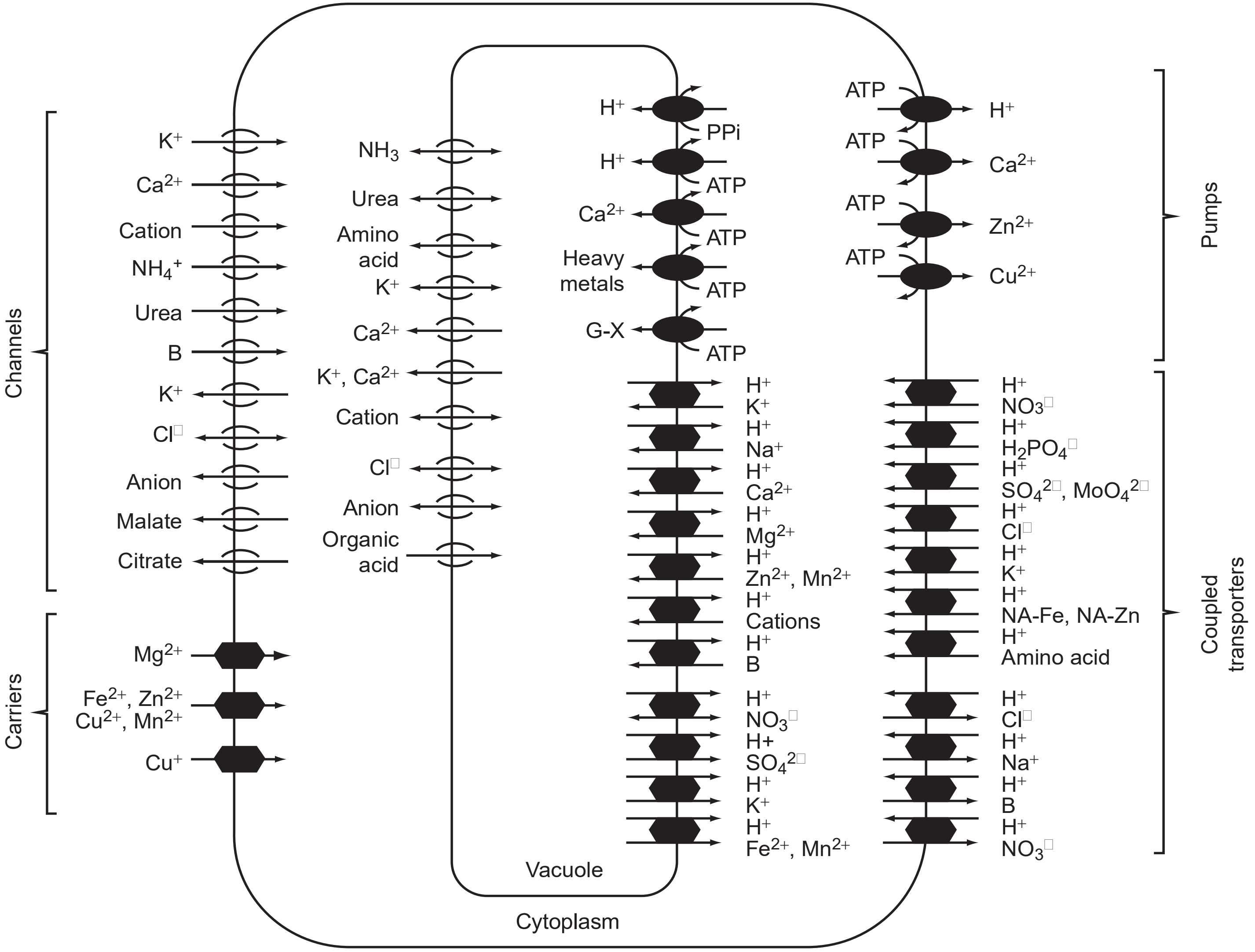
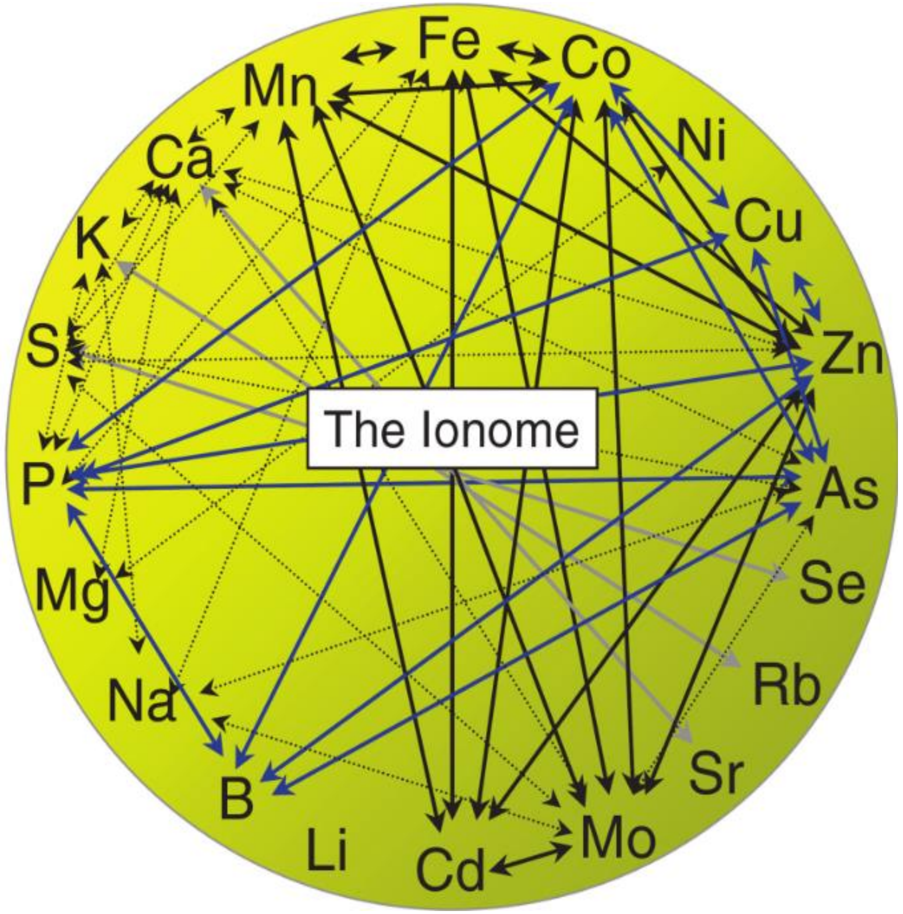
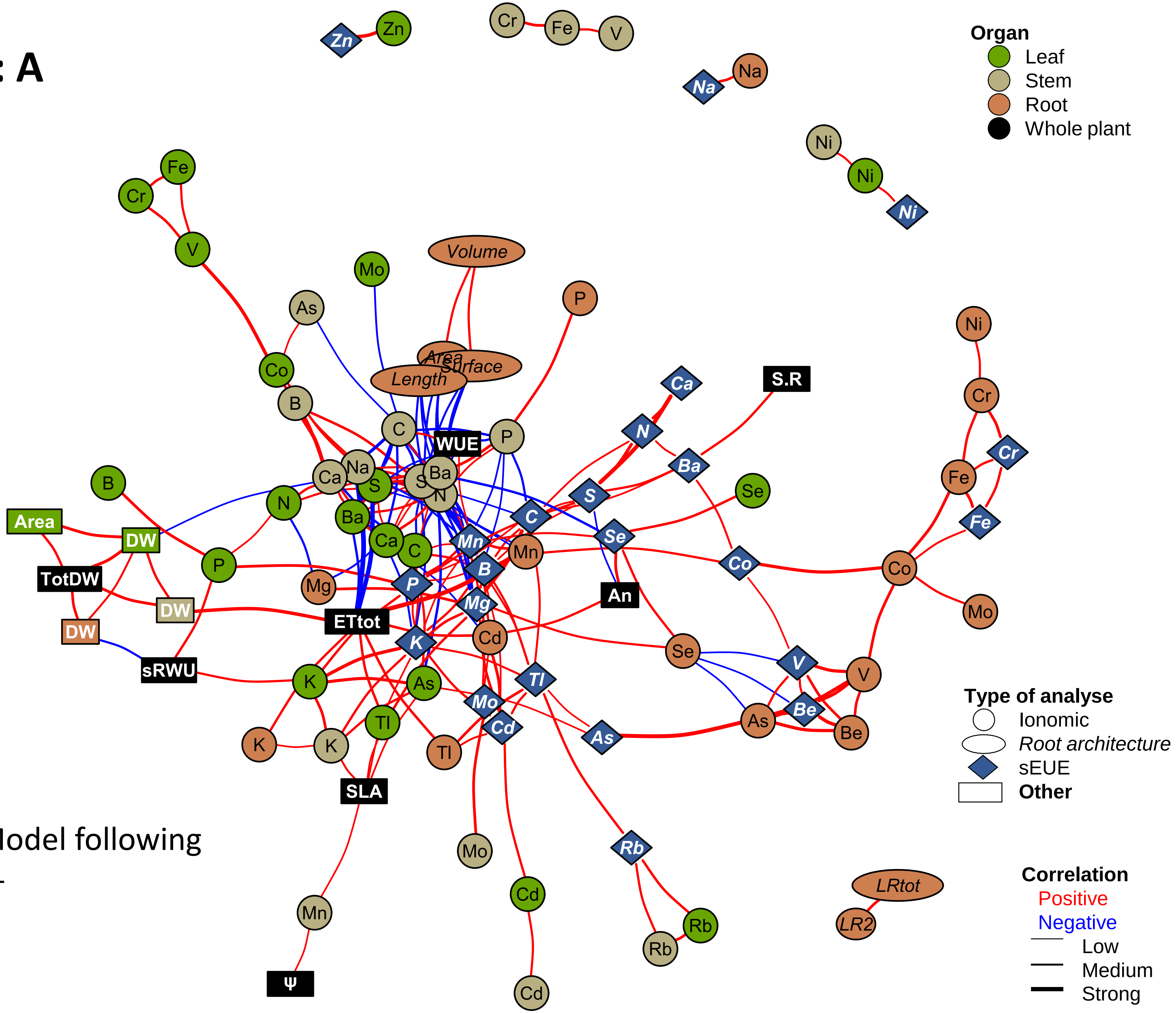


Marschner and Marschner, 2012.



Baxter et al., 2008; Baxter, 2009

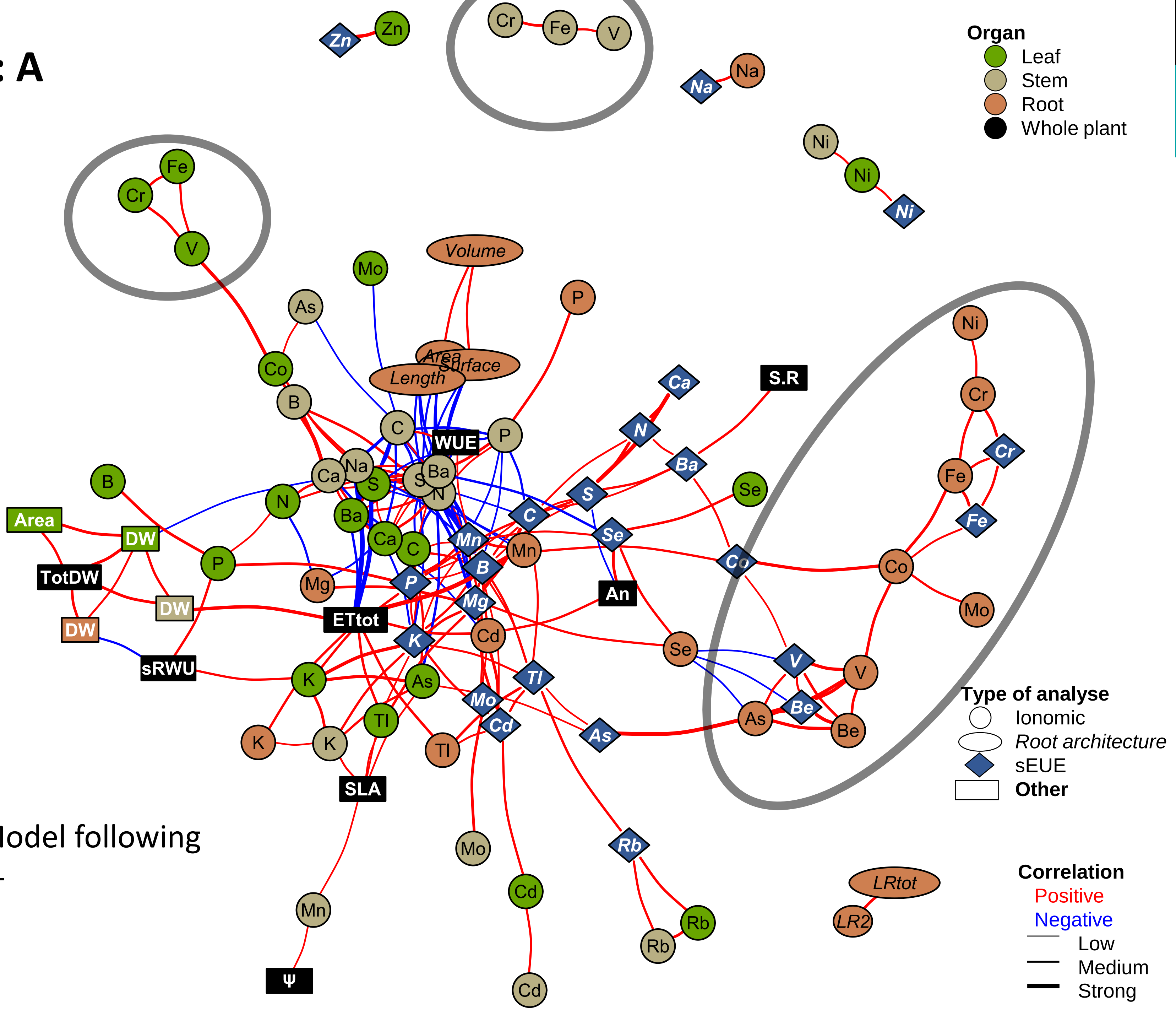
Unraveling Variable Connections: A Network Analysis



Network plot generated with Mixed Linear Model following the equation : $V1_{jklm} \sim V2_j + genotype_k + water\_condition_l + heat\_condition_m + \varepsilon$

Unraveling Variable Connections: A Network Analysis

- Some variable are grouped by compartment



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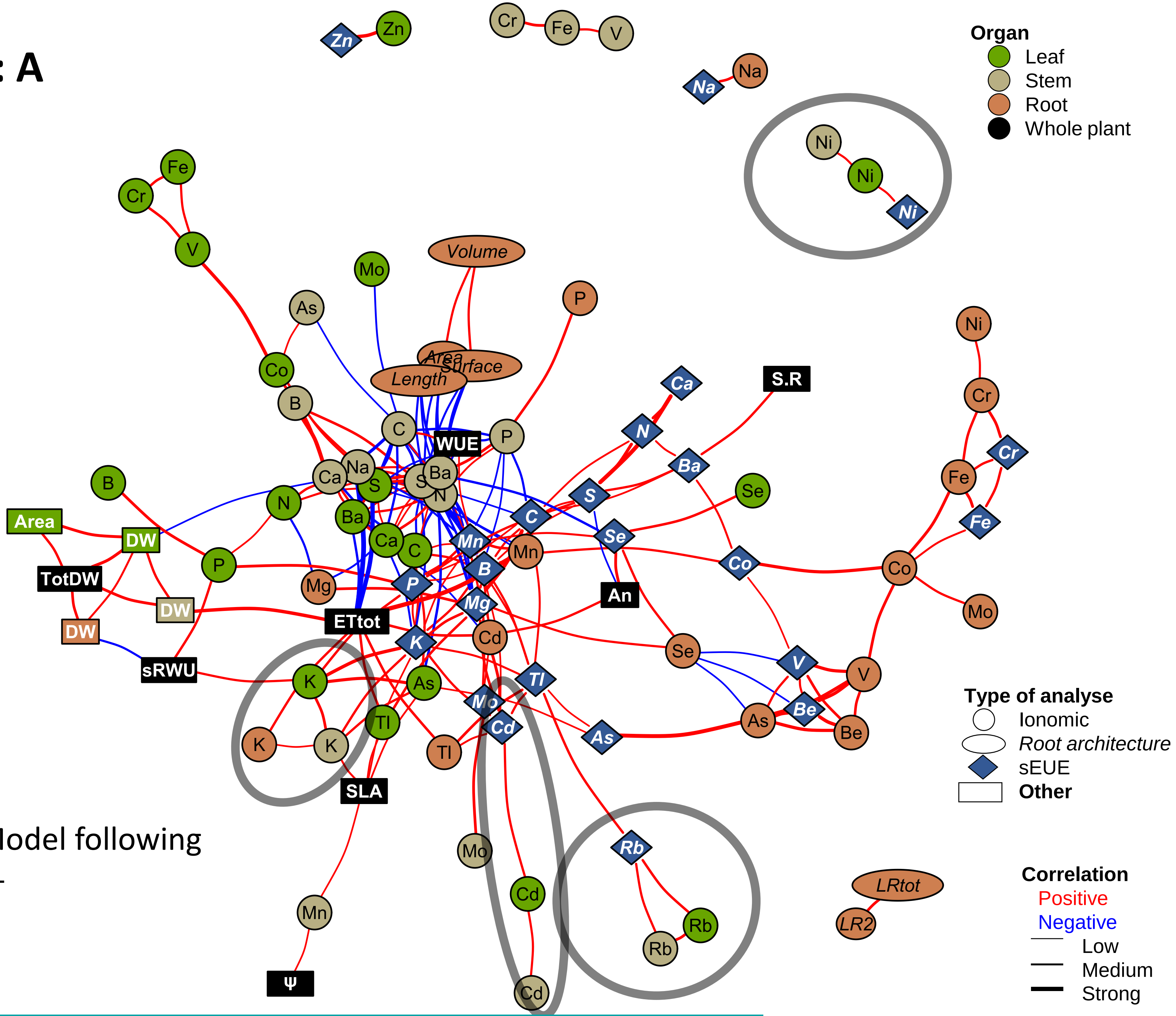
Unraveling Variable Connections: A Network Analysis

- Some variable are grouped by compartment

- Some variables are grouped by element



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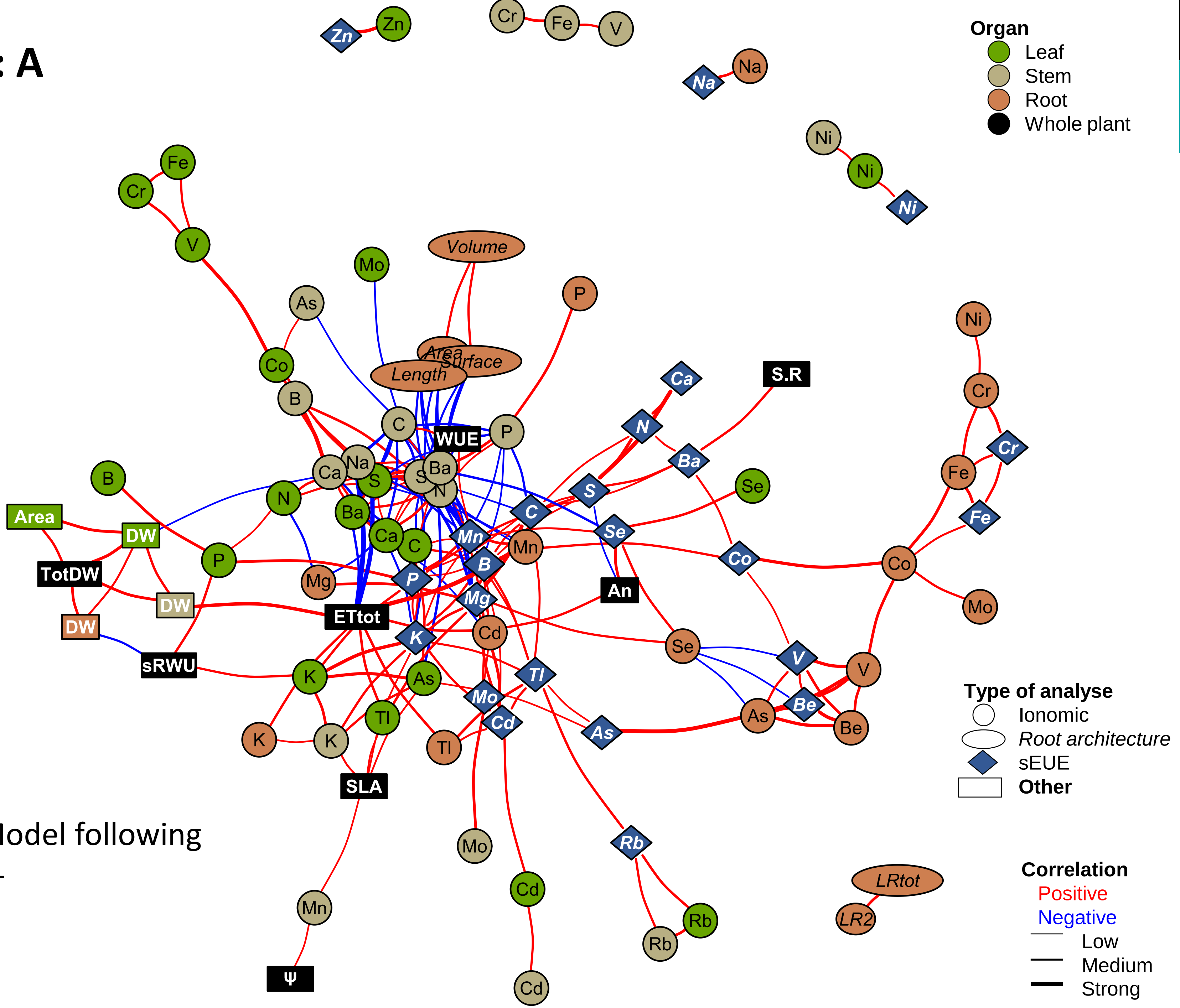
Unraveling Variable Connections: A Network Analysis

- Some variable are grouped by compartment
- Some variables are grouped by element
- Macro-elements and water flows are at the center of the network



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Conclusion

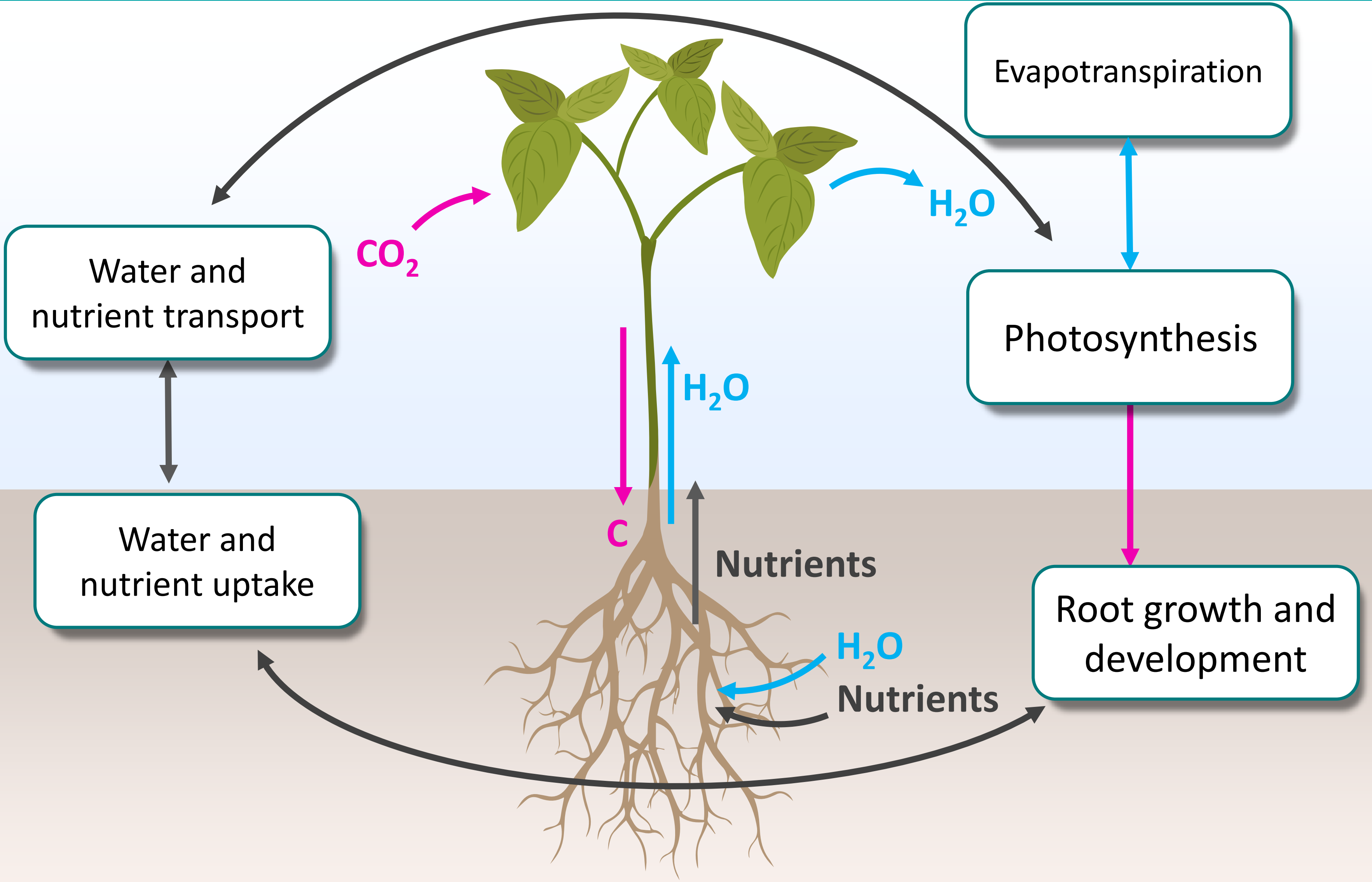
- Minor genotypic effects on root architecture roots but significant differences in element absorption (**Functional instead of structural differences ?**)

- Combined stress is more than just the sum of individual stresses

- Stress effects can be antagonistic, additive, synergistic, or involve other complex interactions.

- Element concentrations show unique responses to each stress condition and compartment, but for many elements, water flow seems to be the main contributing factor

Opening: Conceptual framework for plant nutrition



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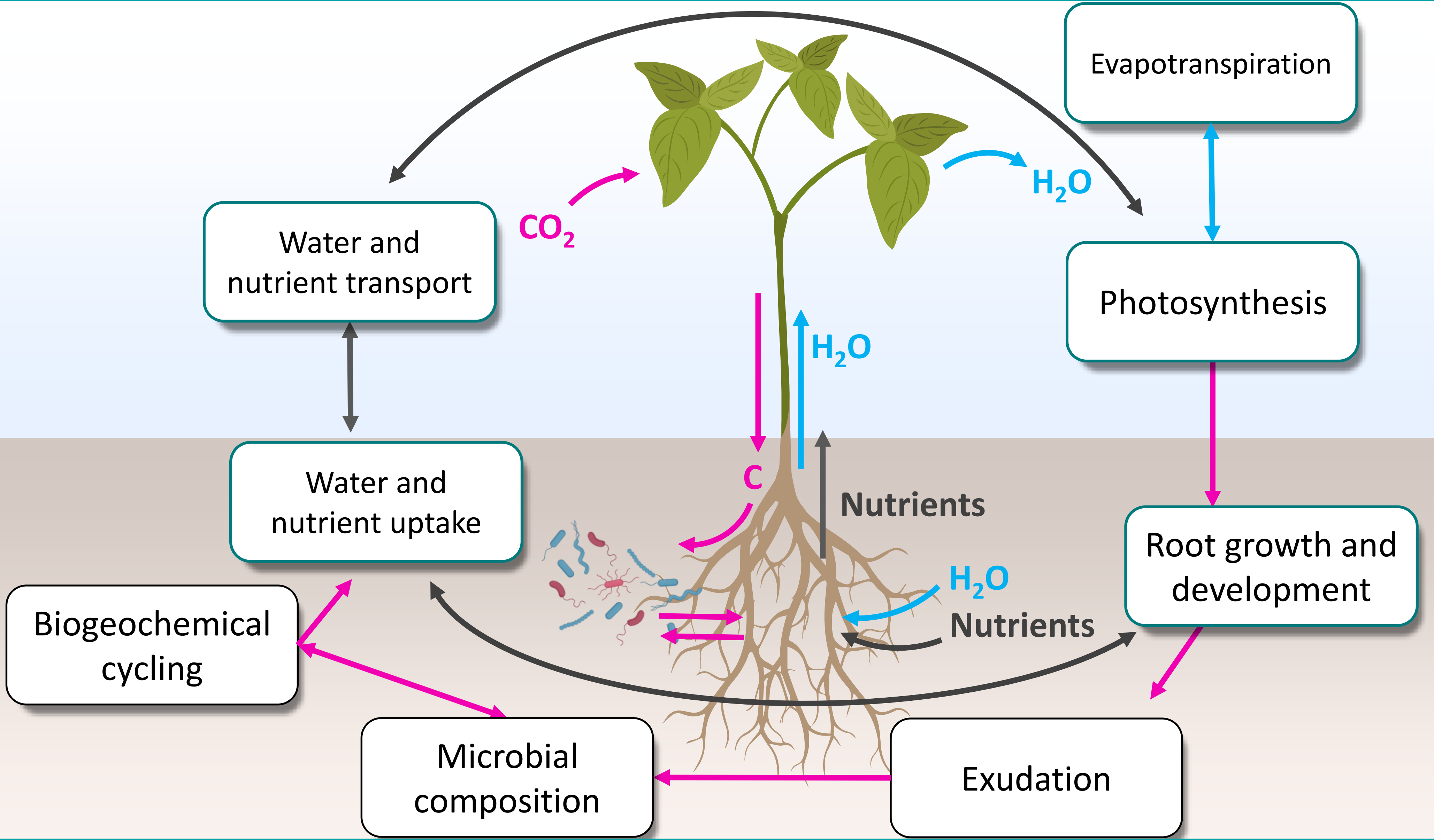
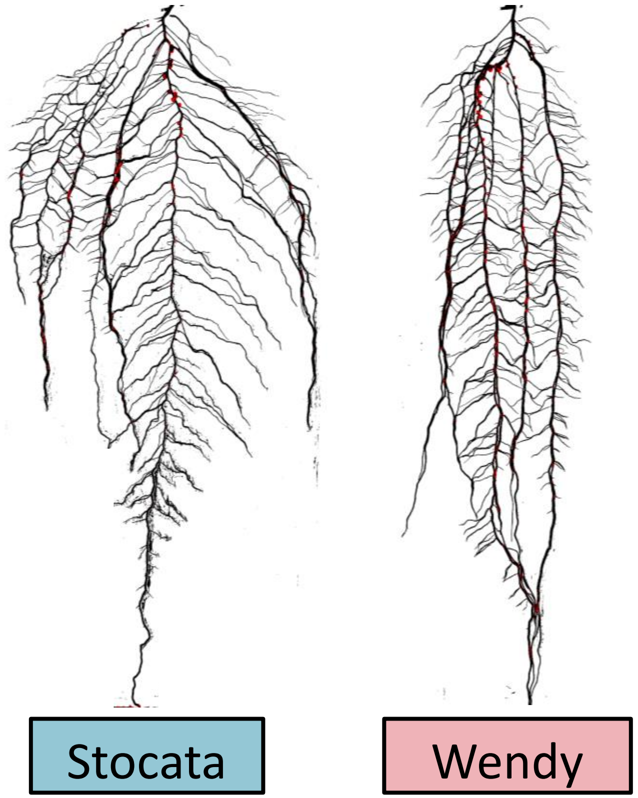


Experimental design



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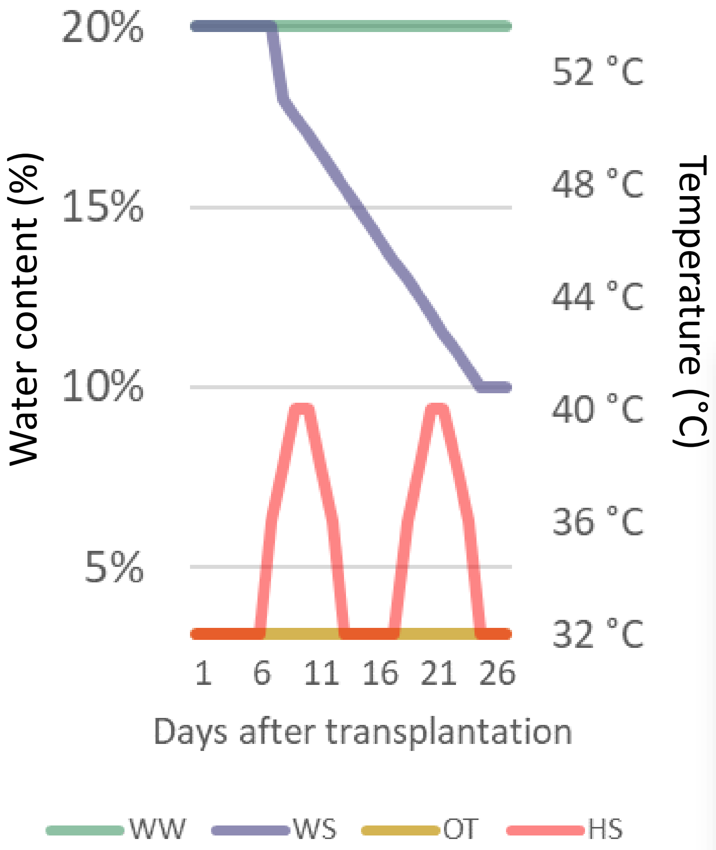

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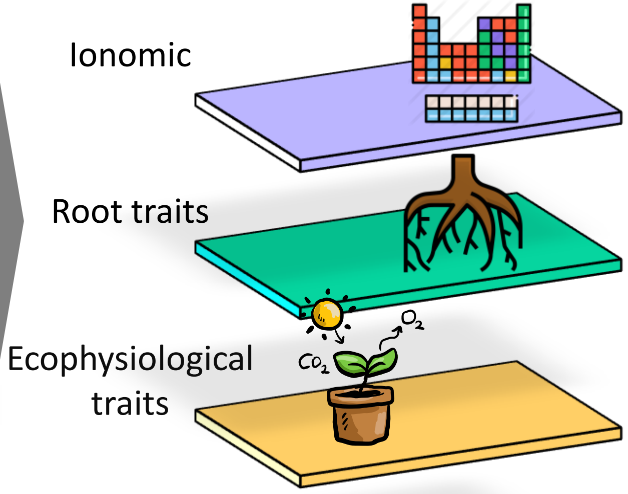
WW_OT / WS_OT / WW_HS / WS_HS



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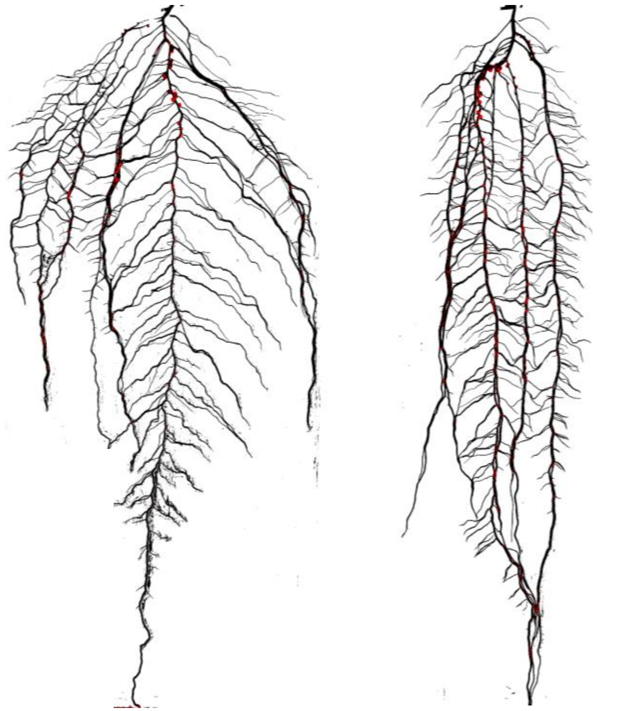

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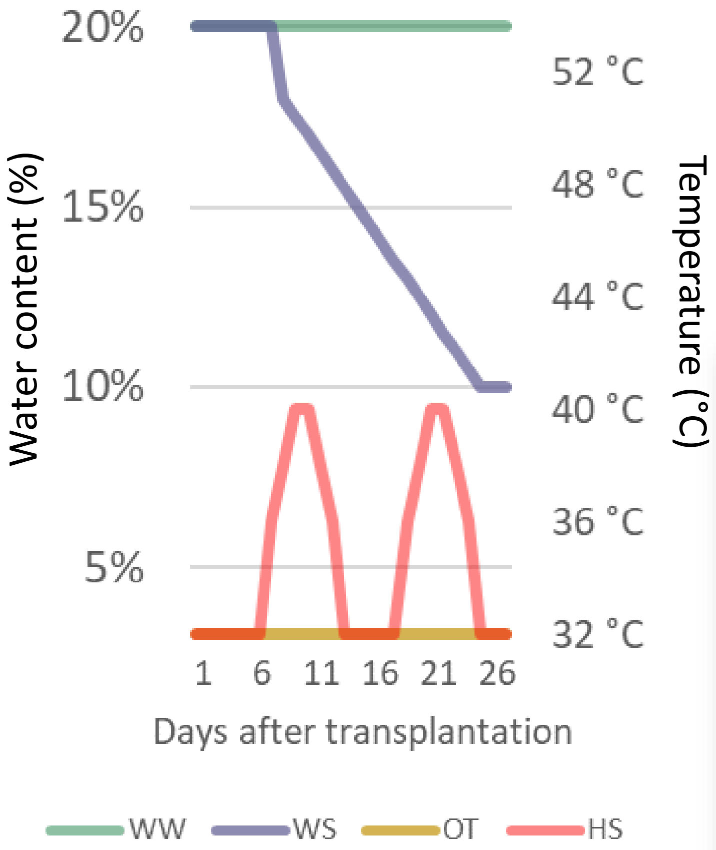

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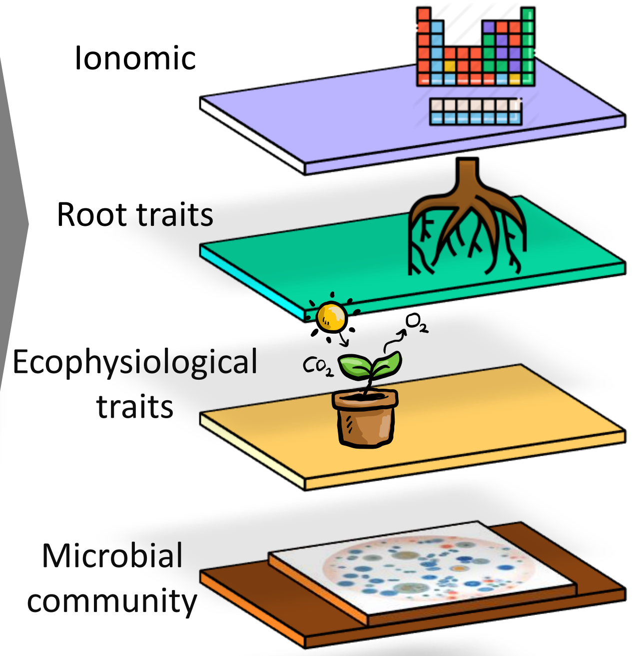

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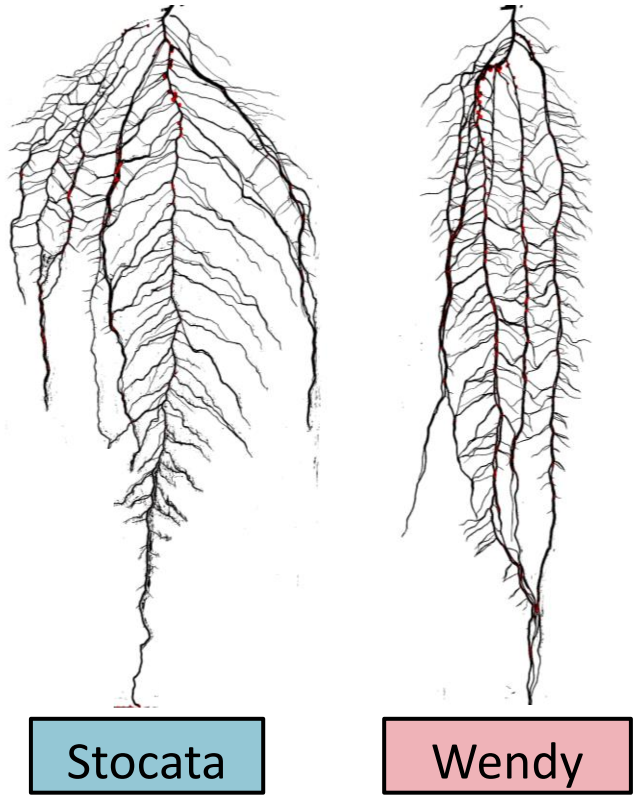

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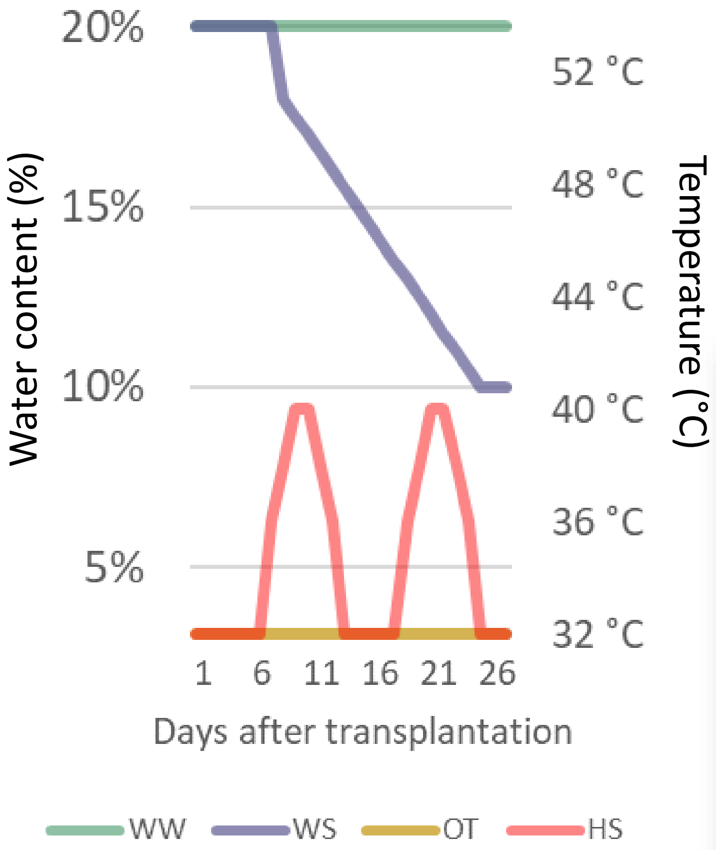

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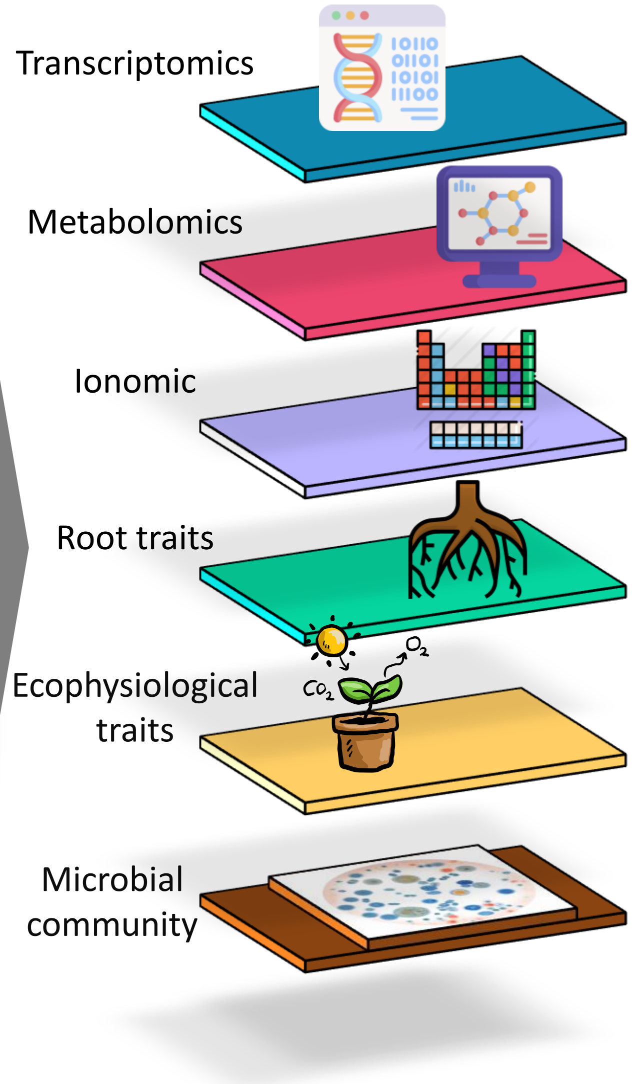


A huge thank you, to all those who participated in the results presented today !!!

M.Prudent M.Arkoun C. Salon P. Jingjing

ANY QUESTION?

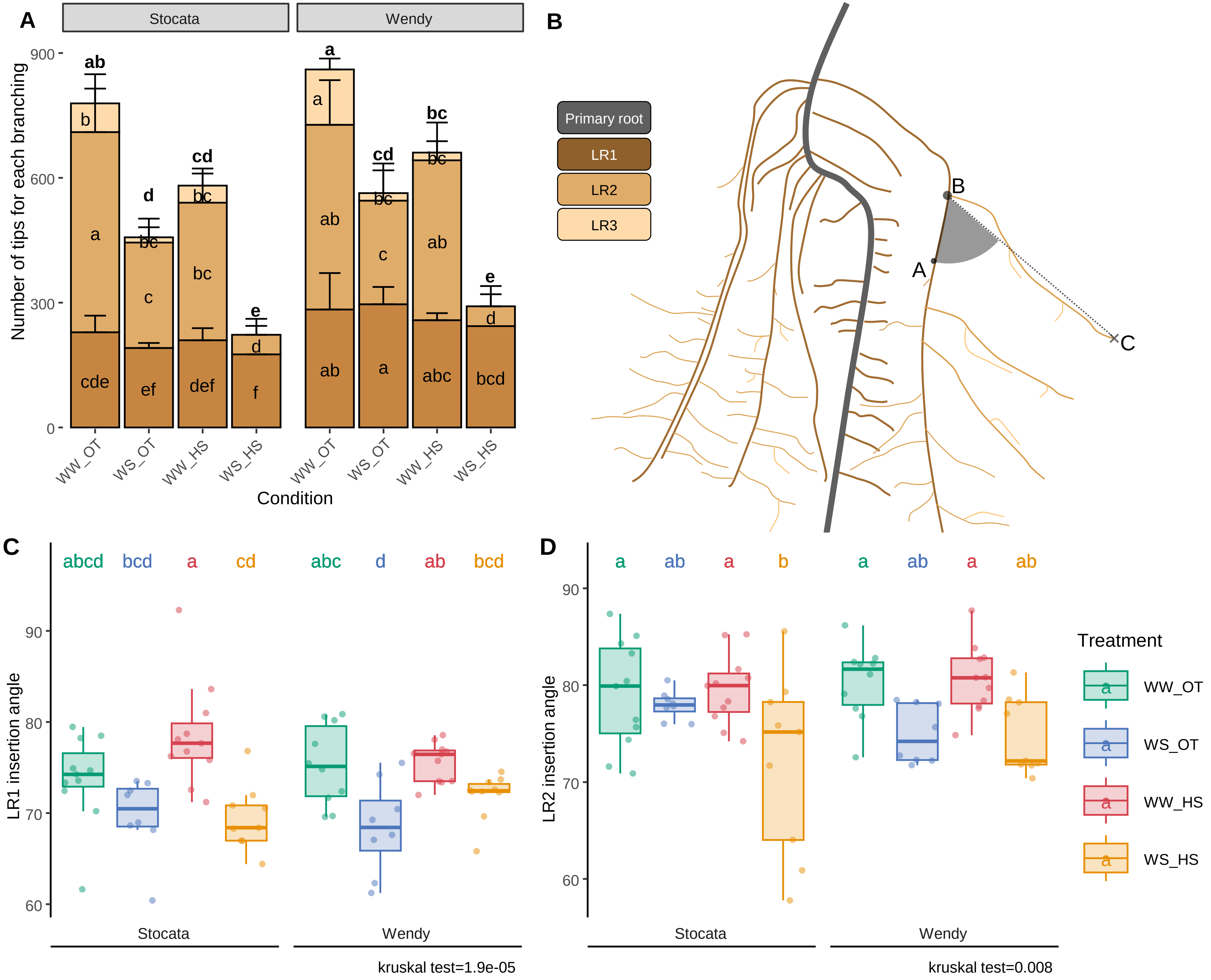


Figure 1: Number of root tips and angles of the two genotypes (Stocata and Wendy) at the second harvest (H2) under each treatment.
(A) Number of root tips as a function of root branching and treatment for both genotypes. (B) Simplified diagram showing the measurements of root angle $\widehat{ABC}$ and length BC.(C:D) Measurements of root insertion angle for order 1 and 2. LR1-3: Lateral Root order 1-3, WW: well-watered condition, WS: water stress condition, OT: optimal temperature, HS: heat stress. In the boxplots, letters indicate significant differences among genotypes (Tukey's HSD test. P < 0.05, n=8).

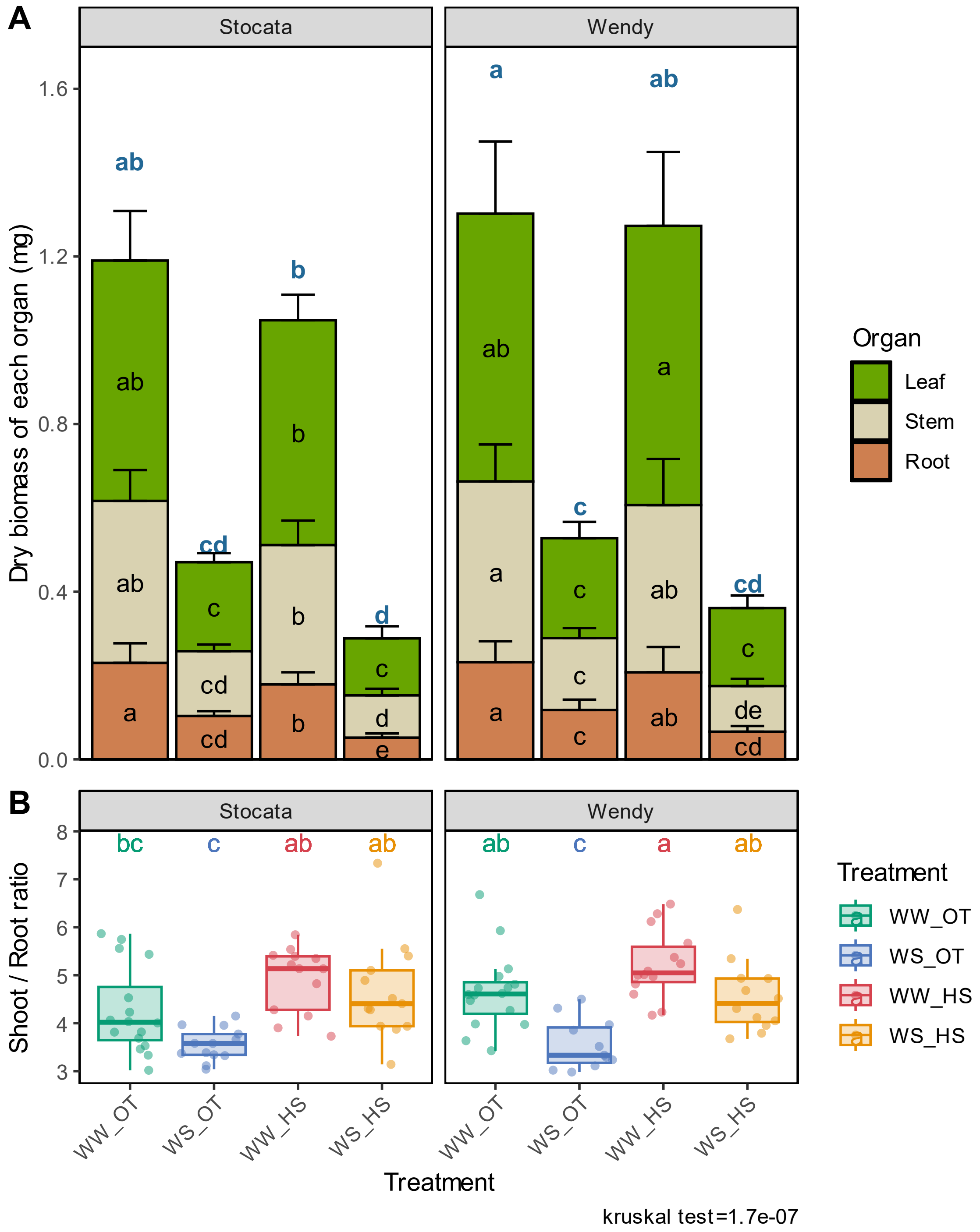


Figure 2: Dry biomass at the second harvest (H2), 20 days after transplantation. (A) Biomass for each organ (mg) according to each treatment. In bar plots, letters indicate significant differences between treatments for the same organ (black), or for plant total biomass (blue). (B) Shoot/root biomass ratio for each genotype and each treatments. In the barplots and the boxplot, letters indicate significant differences among genotypes and treatments (Tukey's HSD test. $P < 0.05$, n=8).WW: well-watered condition, WS: water stress condition, OT: optimal temperature, HS: heat stress.

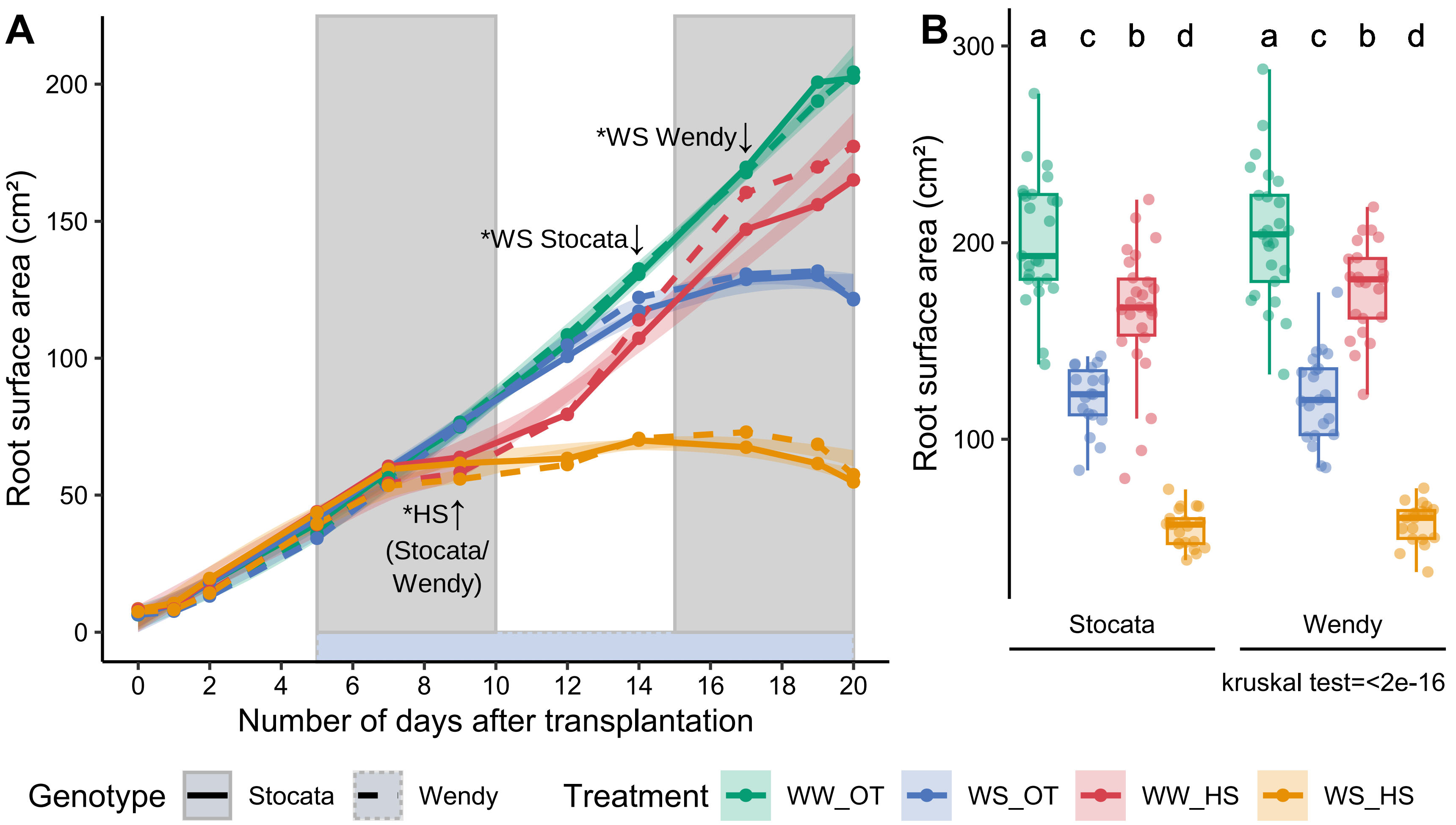


Figure 3 : Root system morphology over time for the two genotypes (Stocata and Wendy) under each treatment.

Root surface area (A) between 0 to 20 days after transplantation and (B) at the second harvest (H2). The 95% confidence interval with local regression adjustment was drawn in transparency. WW: well-watered condition, WS: water stress condition, OT: optimal temperature, HS: heat stress. * and ↓↑ indicates a significant difference between the control condition and the stress condition for each genotype. In the boxplots, letters indicate significant differences among genotypes (Tukey's HSD test. $P < 0.05$)

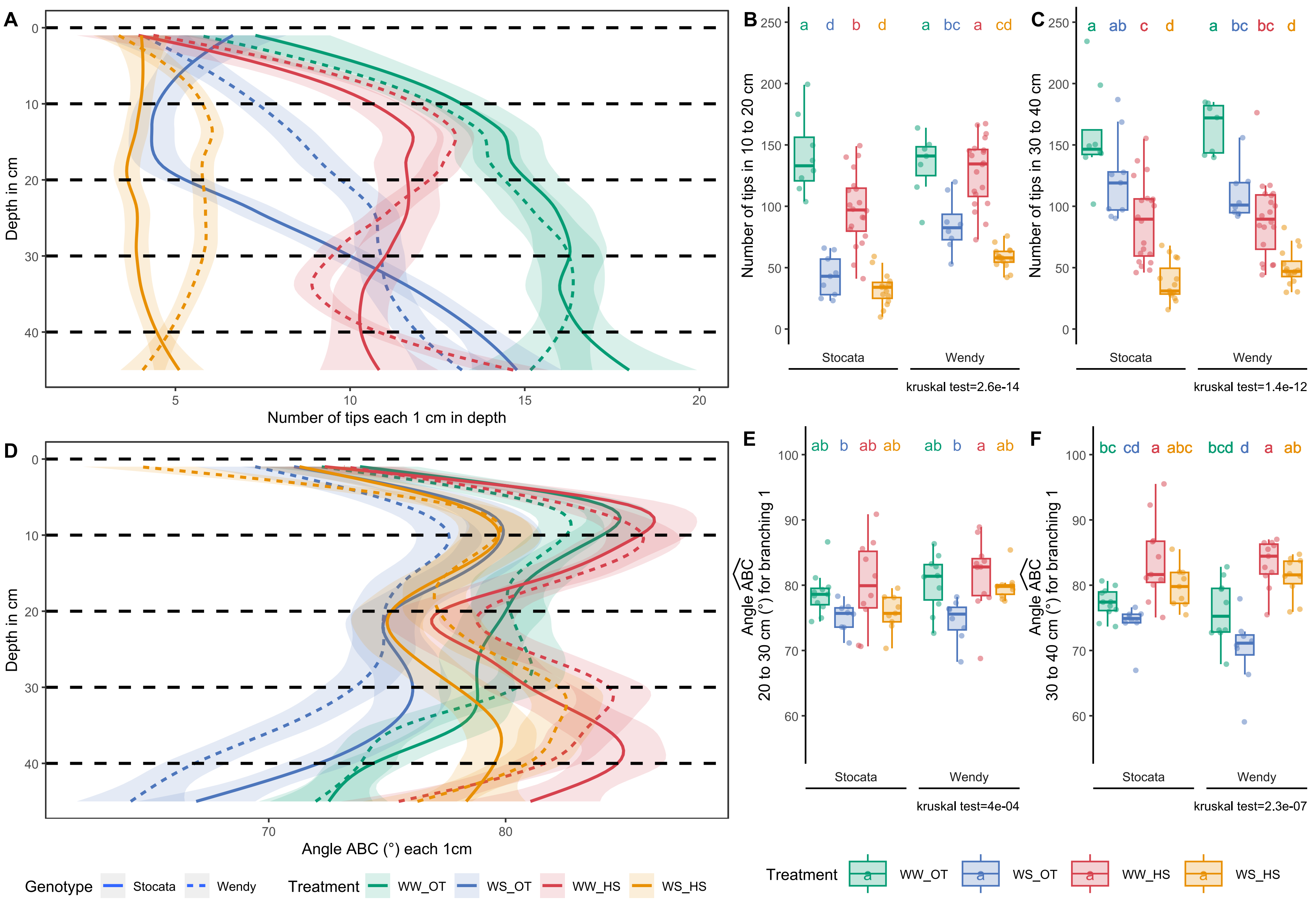


Figure 4: Number of root tips and angle of root insertion as a function of depth. (A) Number of root tips as a function of depth, between 10 and 20 cm deep (B) between 30 and 40 cm deep (C) depending of the treatment. (D) Average angles $\widehat{ABC}$ formed between the primary root and the secondary roots every 1 cm of depth, from 20 to 30 cm deep (E) and from 30 to 40 cm deep (F) for each treatment. The dashed black lines correspond to depth measurements every 10 cm. The 95% confidence interval with local regression adjustment was drawn in transparency. WW: well-watered condition, WS: water stress condition, OT: optimal temperature, HS: heat stress. In the boxplots, letters indicate significant differences among genotypes (Tukey's HSD test. $P < 0.05$, n=8).

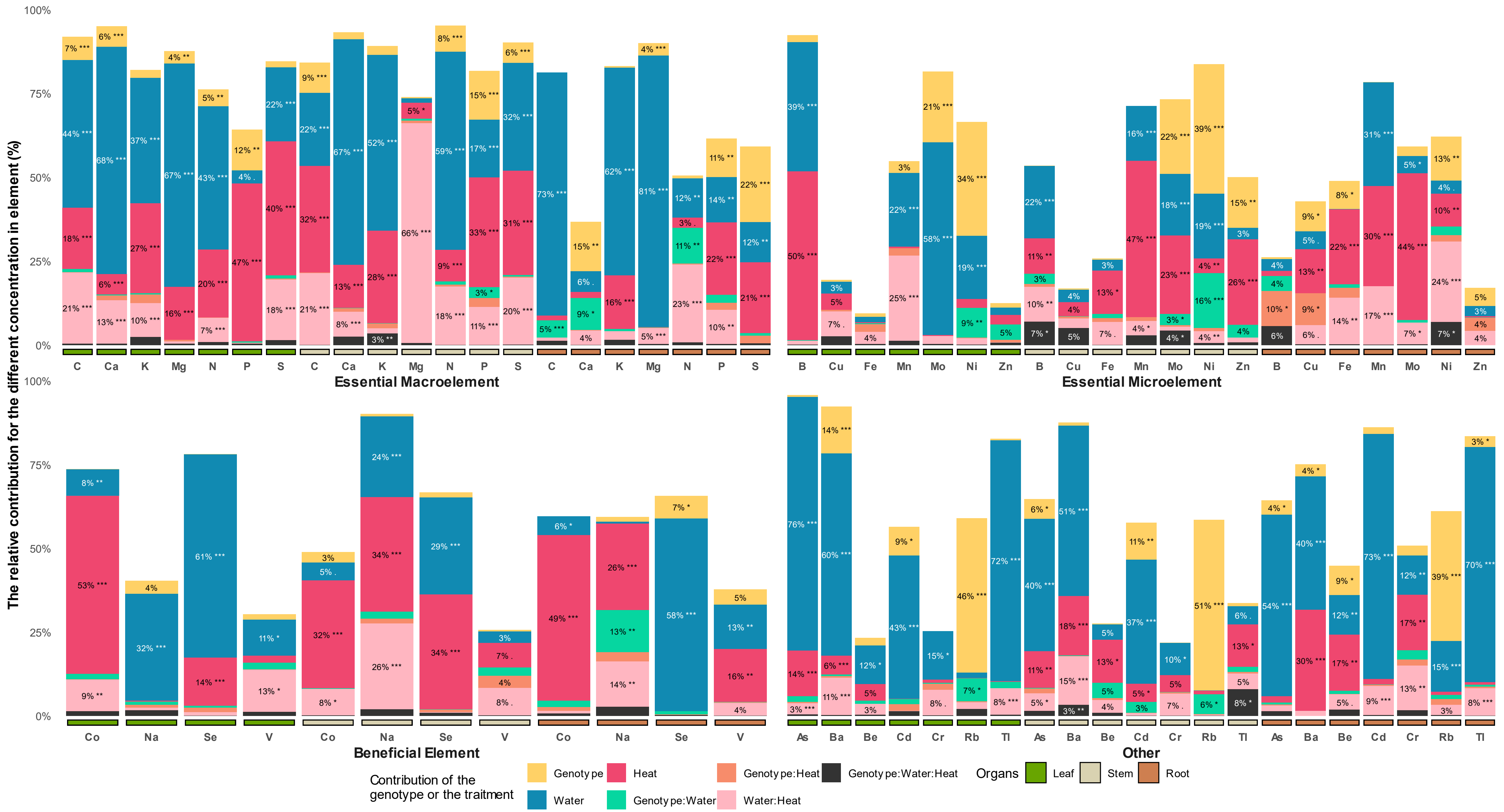


Figure 5: Relative contribution of treatment and genotype to variability in element concentration in each organ (n=8). The asterisks indicate the level of statistical significance of the results as follows: *** p < 0.001, ** p < 0.01, * p < 0.05

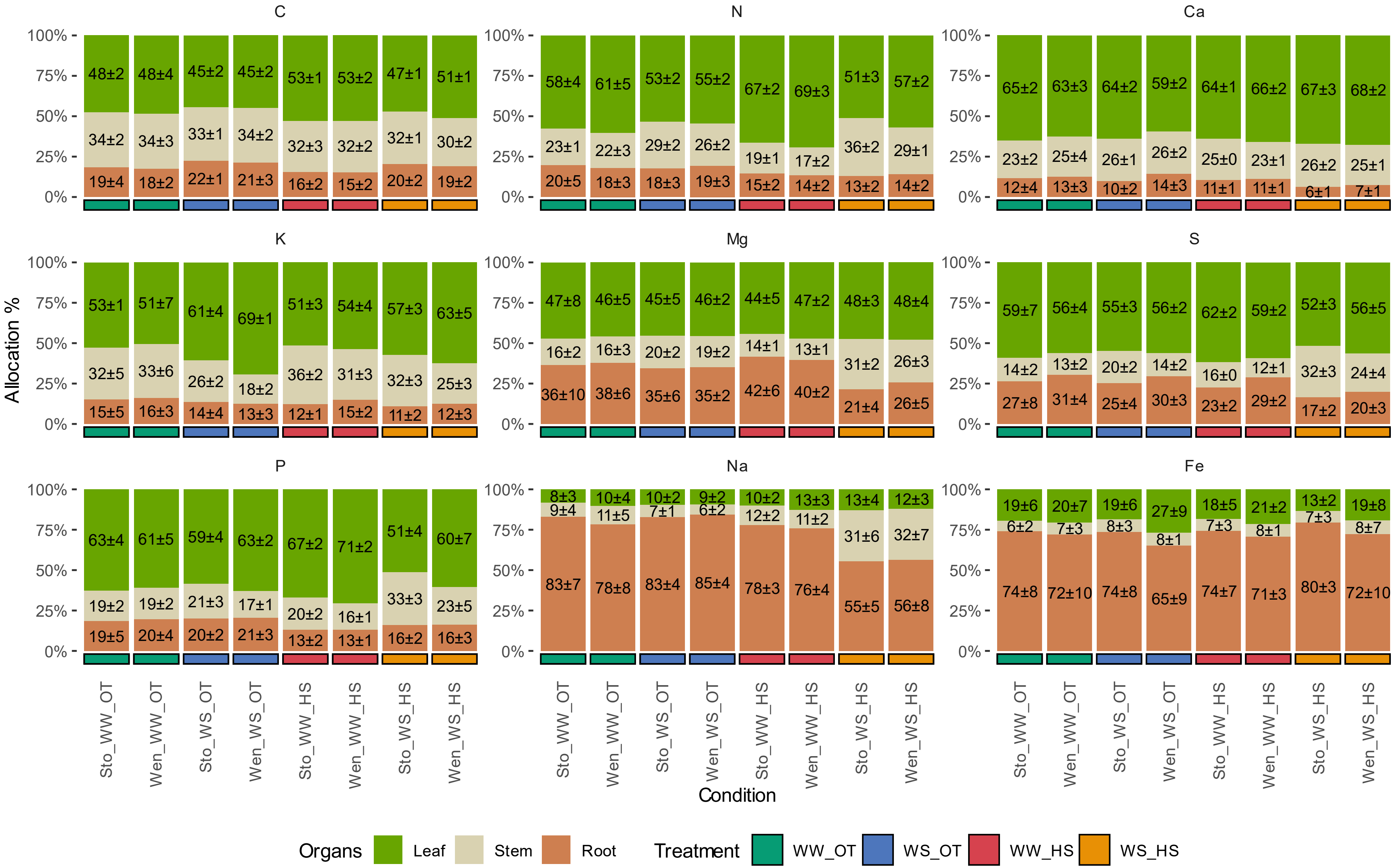
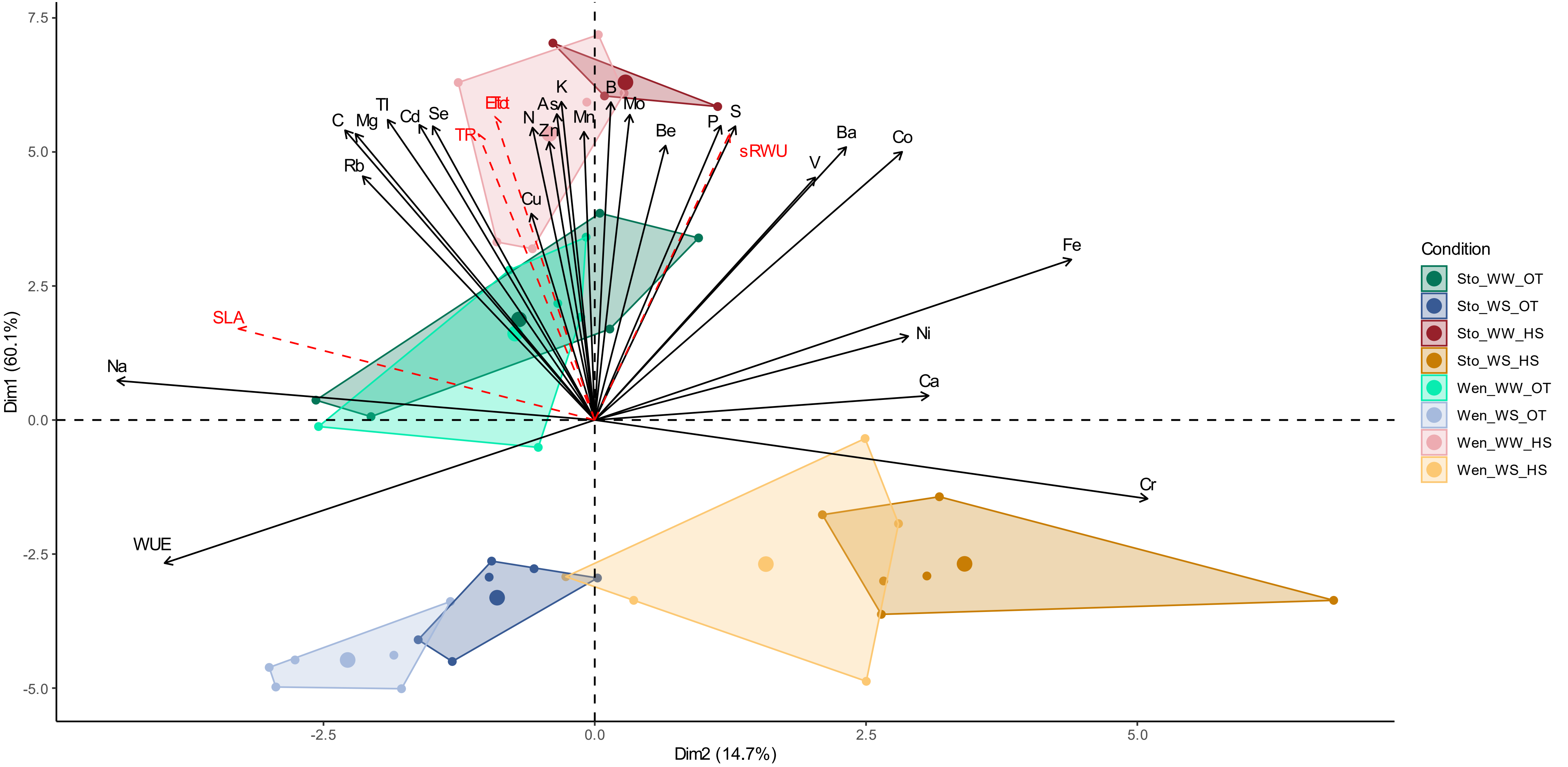
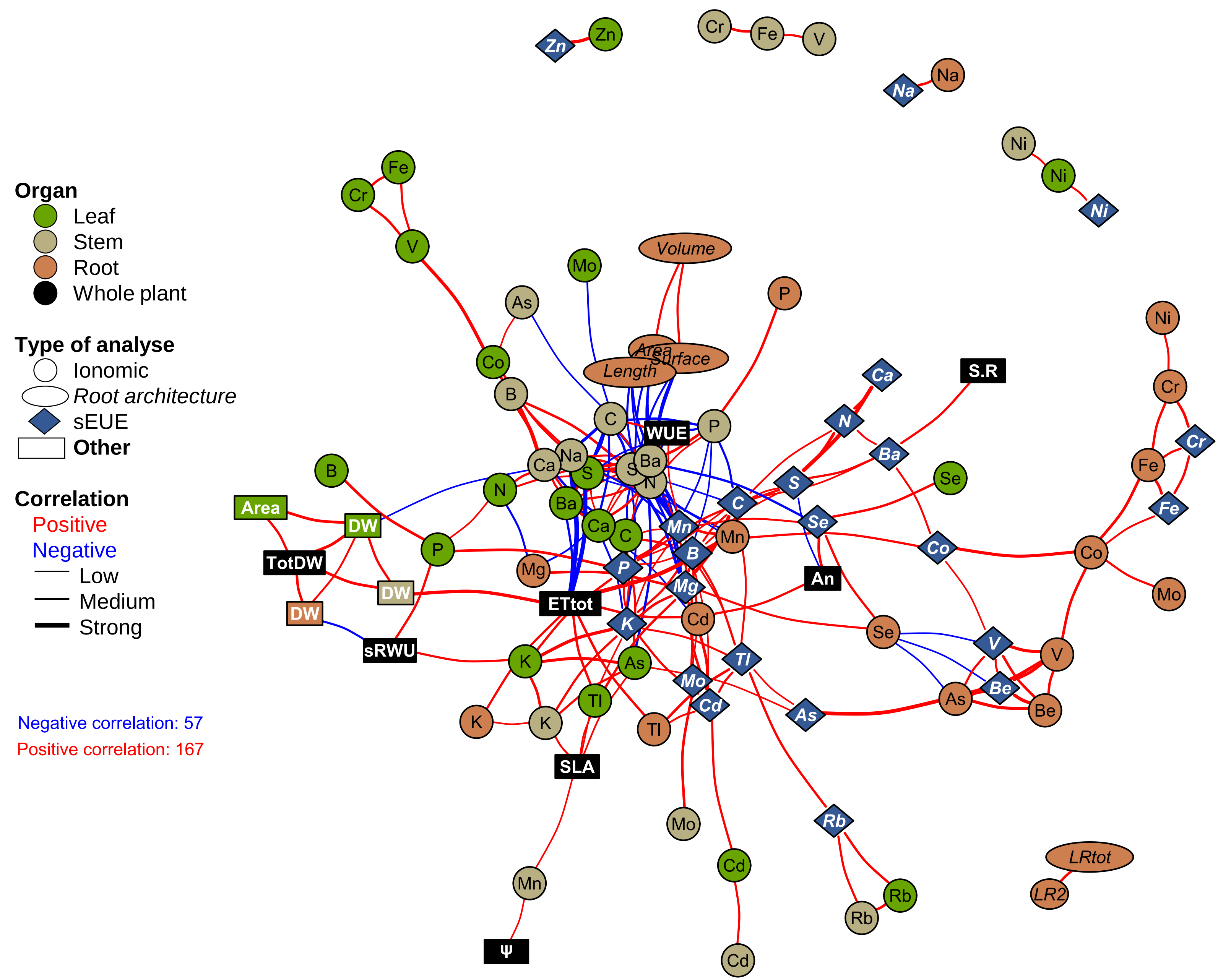


Figure 6: Elemental allocation in plant organs under each treatment. Distribution of the nine most abundant nutrients in the leaves (green), stems (orange), and roots (brown) of plants subjected to well-watered and optimal temperature (WW_OT), well-watered and heat stress (WW_HS), water -stress and optimal temperature (WS_OT), and water stress and heat stress (WS_HS) for Stocata (Sto) and Wendy (Wen) soybean genotypes. Each bar represents the percentage allocation of the indicated element (C, K, P, N, Mg, Na, Ca, S, and Fe). Values within the bars indicate mean percentages of elemental allocation and the standard deviation (n=8).



- **Figure 7: Principal Component Analysis (PCA) of specific element absorption in each treatment.** PCA plot illustrates the variation in specific element uptake efficiency (sEUE) under each treatment, as indicated by the color-coded treatment groups. The vectors show the relationship of each specific element's uptake for each component, while the convex hulls group the samples by treatment. The dashed red vector are additional traits.



- **Figure 8: Network interactions between the different traits.** Each node represents a trait and nodes are connected by edges representing the correlation from MLM model between two traits. Every possible relationships between the two traits are explored, and only the most significant correlations were represented (p-value < 0.05 and R^2 > 0.5). TotDW: Total Dry weight (mg) ; DW: Dry weight (mg); ψ_{leaf}: Leaf Water Potential (Mpa); g_s: stomatal conductance (mol $H_2O.m^{-2}.s^{-1}$) ; TR: Transpiration rate (mmol.$m^{-2}.s^{-1}$). LRtot: Total number of root tips ; LR2: Total number of roots of order2 ; S.R (Shoot / root ratio) ; sRWU: Specific Root Water Uptake (g H_2O. g Root dry weight^{-1}.day^{-1}) ; WUE: Water Use Efficiency (gPlant$_{DW}$.gH_2O^{-1}); ETtot: evapotranspiration total (ml) ; An: Photosynthetic activity (µmol $CO_2.m^{-2}.s^{-1}$).

Table 1: Effects of water and heat stresses on soybean physiological variables. For each trait, values are means (bold) ± SD. Asterisks mean that the values are significantly different from those of the control condition (Welch Two Samples t-test). The asterisks indicate the level of statistical significance of the results as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns not significant. Wen = Wendy genotype; Sto: Stocata genotype, WW: well-watered condition, WS: water stress condition, OT: optimal temperature, HS: heat stress.; WUE: Water Use Efficicency ; RSA: Root system architecture ; sRWU : Specific Root Water Uptake ; An : Photosynthetic activity of the leaf ; g_s Stomtal conductance ; ETtot : Total Evapotranspiration ; TR : Transpiration Rate ; SLA : Specific Leaf Area ; Ψ_{leaf} : Leaf Water Potential

Summary of physiological and structural variables for Stocata

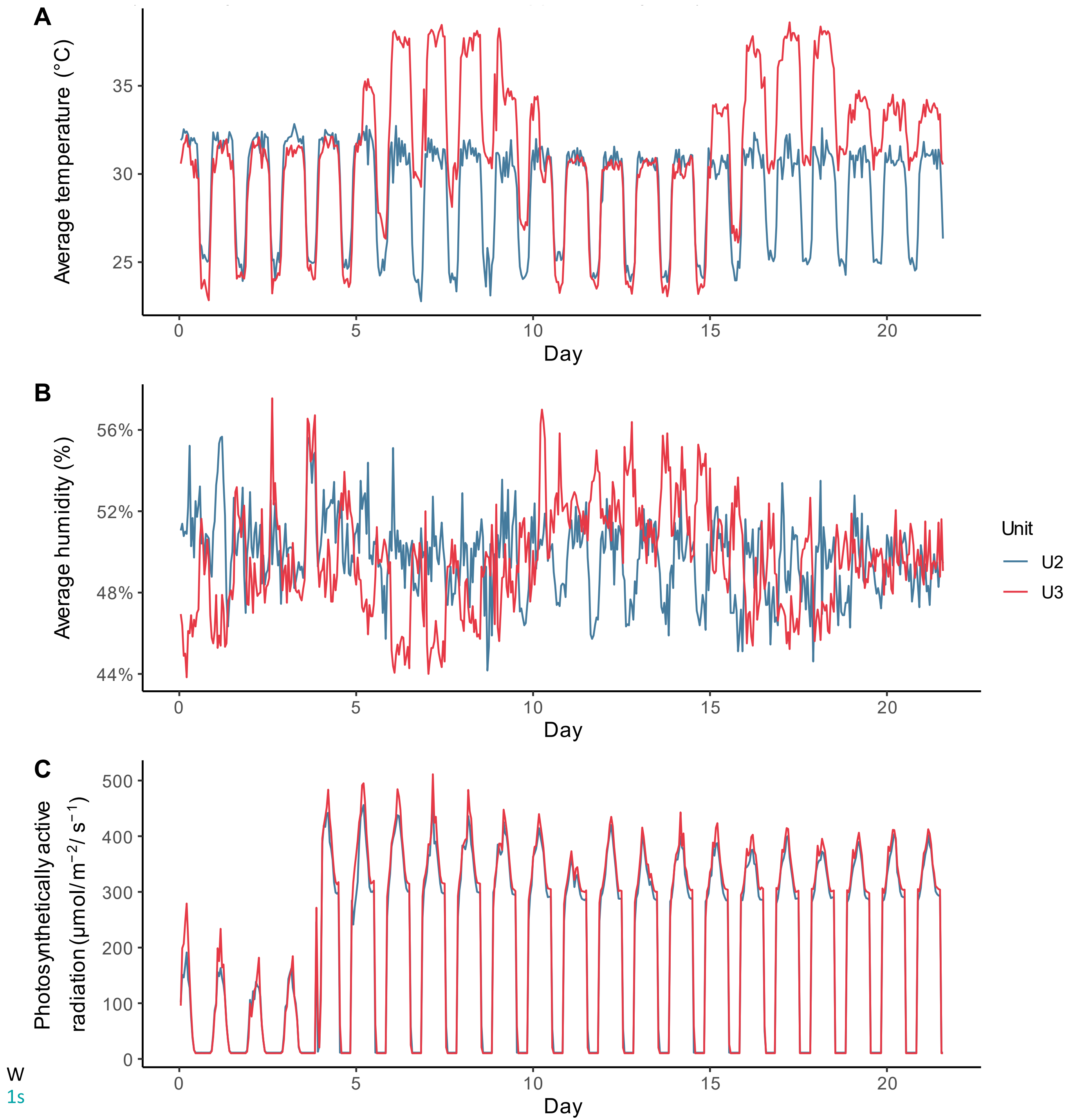
Variable	Sto_WW_OT		Sto_WS_OT			Sto_WW_HS			Sto_WS_HS		
Variable related to carbon flows											
An (μmol $CO_2.m^{-2}.s^{-1}$)	**9.38**	±1	**4.11**	±2.37	***	**7.73**	±2.22	ns	**0.21**	±3.09	**
Leaf area (cm^2)	**187.72**	±35.4	**65.14**	±9.75	***	**164.94**	±28.29	ns	**31.19**	±8.06	***
Variable related to RSA											
Mean Length root of order 1 (cm)	**3.51**	±0.69	**3.43**	±0.54	ns	**3.26**	±0.63	ns	**3.13**	±0.64	ns
Sum Length root of order 1 (cm)	**574.97**	±111.24	**454.55**	±36.55	*	**410.44**	±44.84	**	**388.08**	±73.56	**
Sum Length root of order 2 (cm)	**590.8**	±175.7	**268.18**	±62.7	***	**278.01**	±101.16	**	**20.16**	±10.37	***
Density	**0.3**	±0.04	**0.25**	±0.04	***	**0.23**	±0.05	***	**0.16**	±0.04	***
Root projected area (cm^2)	**60.71**	±10.34	**40.06**	±5.18	***	**46.63**	±8.91	***	**19.74**	±3.16	***
Area of the root convex hull (cm^2)	**205.35**	±34.8	**165.29**	±26.16	***	**205.41**	±37.81	ns	**125.86**	±30.34	***
Root length (cm)	**1.3e+03**	±240.9	**877.14**	±121	***	**976.87**	±175.47	***	**452.54**	±69.73	***
Root surface area (cm^2)	**203.03**	±35.47	**129.83**	±16.96	***	**154.48**	±30.74	***	**63.07**	±10.18	***
Root volume (cm^3)	**960.87**	±217.1	**543.56**	±93.07	***	**713.61**	±198.43	***	**243.63**	±50.19	***
Root Width (cm)	**23.9**	±3.12	**22.42**	±2.64	*	**23.6**	±3.51	ns	**17.14**	±4.06	***
Variable related to water flows											
g_s (mol $H_2O.m^{-2}.s^{-1}$)	**0.1**	±0.1	**-0.01**	±0.03	*	**0.11**	±0.1	ns	**0.00**	±0.02	*
ETtot (mL)	**1.6e+03**	±213.91	**443.00**	±50.34	***	**1.8e+03**	±161.39	***	**501.00**	±42.67	***
Ψ_{leaf} (MPa)	**-0.09**	±0.12	**-0.73**	±0.65	ns	**-0.09**	±0.04	ns	**-2.12**	±0.39	***
sRWU ($gH_2O[gBM_{root}.day^{-1}]^{-1}$)	**0.74**	±0.14	**0.35**	±0.03	***	**1.03**	±0.16	***	**0.66**	±0.11	ns
TR (mmol $H_2O.m^{-2}.s^{-1}$)	**3.51**	±0.44	**1.00**	±0.38	***	**4.31**	±0.61	***	**1.17**	±0.53	***
WUE ($g.gH_2O^{-1}$)	**0.74**	±0.1	**1.03**	±0.1	***	**0.57**	±0.06	***	**0.47**	±0.13	***
Other variable											
SLA (m^2/kg)	**33.58**	±2.62	**30.59**	±2.27	**	**30.39**	±3.18	**	**22.84**	±2.74	***
Leaf temperature (°C)	**31.85**	±0.57	**33.14**	±0.21	***	**35.48**	±0.8	***	**37.26**	±0.5	***

For each trait, values are means ± SD. Asterisks means that the values are considered as significantly different from the values of the control condition (Welch Two Sample t-test). The stars indicate the level of statistical significance of the results as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns not significant.

Summary of physiological and structural variables for Wendy

Wen_WW_OT		Wen_WS_OT			Wen_WW_HS			Wen_WS_HS		
7.39	±1.19	**4.48**	±2.06	**	**9.15**	±2.09	ns	**-1.40**	±0.77	***
218.34	±54.09	**75.06**	±11.35	***	**188.08**	±49.04	ns	**42.83**	±8.12	***
2.74	±0.32	**2.46**	±0.48	ns	**3.53**	±0.32	***	**2.19**	±0.42	**
520.53	±96.37	**536.45**	±147.53	ns	**603.64**	±59.76	*	**386.77**	±81.04	**
557.6	±114.83	**269.80**	±81.88	***	**288.5**	±84.3	***	**30.09**	±27.21	***
0.28	±0.04	**0.26**	±0.05	ns	**0.25**	±0.05	*	**0.18**	±0.03	***
58.02	±9.39	**40.65**	±7	***	**51.33**	±8.42	**	**21.62**	±4.25	***
210.79	±29.39	**157.09**	±30.48	***	**209.04**	±23.5	ns	**122.52**	±24.54	***
1.3e+03	±210.77	**911.48**	±139.09	***	**1.2e+03**	±164.53	**	**506.79**	±81.04	***
193.74	±31.62	**131.81**	±22.98	***	**169.75**	±29.4	**	**69.08**	±13.69	***
839.44	±171.44	**548.83**	±123.94	***	**731.41**	±172.9	*	**265.18**	±69.83	***
25.24	±2.22	**20.41**	±3.85	***	**23.81**	±2.22	*	**15.51**	±3.14	***
0.06	±0.07	**-0.01**	±0.03	*	**0.11**	±0.08	ns	**0.02**	±4.8e-03	ns
1.6e+03	±138.26	**436.71**	±60.24	***	**1.9e+03**	±205.04	***	**473.86**	±74.01	***
-0.07	±0.14	**-0.76**	±0.44	*	**-0.12**	±0.15	ns	**-1.02**	±0.58	**
0.77	±0.21	**0.32**	±0.06	***	**0.98**	±0.24	*	**0.54**	±0.09	**
3.03	±0.67	**0.86**	±0.23	***	**4.12**	±0.95	**	**1.06**	±0.25	***
0.77	±0.18	**1.18**	±0.23	***	**0.65**	±0.14	ns	**0.67**	±0.14	ns
33.82	±3.05	**31.54**	±1.18	*	**29.31**	±3.16	***	**24.07**	±2.54	***
32.63	±0.59	**33.19**	±0.29	ns	**35.27**	±0.54	***	**37.11**	±0.82	***

Asterisks means that the values are considered as significantly different from the values of the control condition (Welch Two Sample t-test). The stars
ınce of the results as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns not significant.



Supporting information 2: **Temperature (A), Humidity (B) and photosynthetically active radiation (C).** The data represents the average each hour derived from four sensors positioned at various locations within each of the two units.

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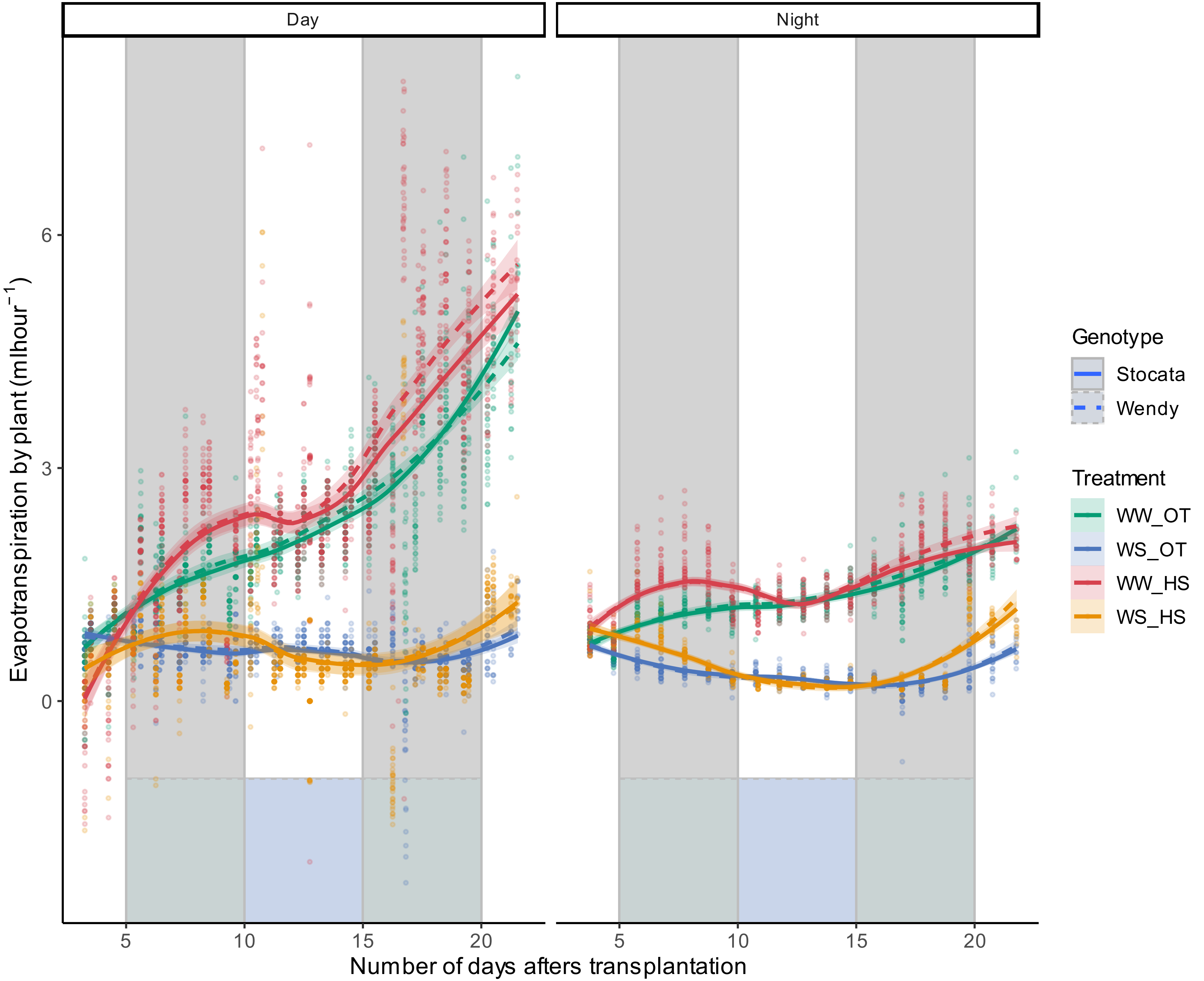
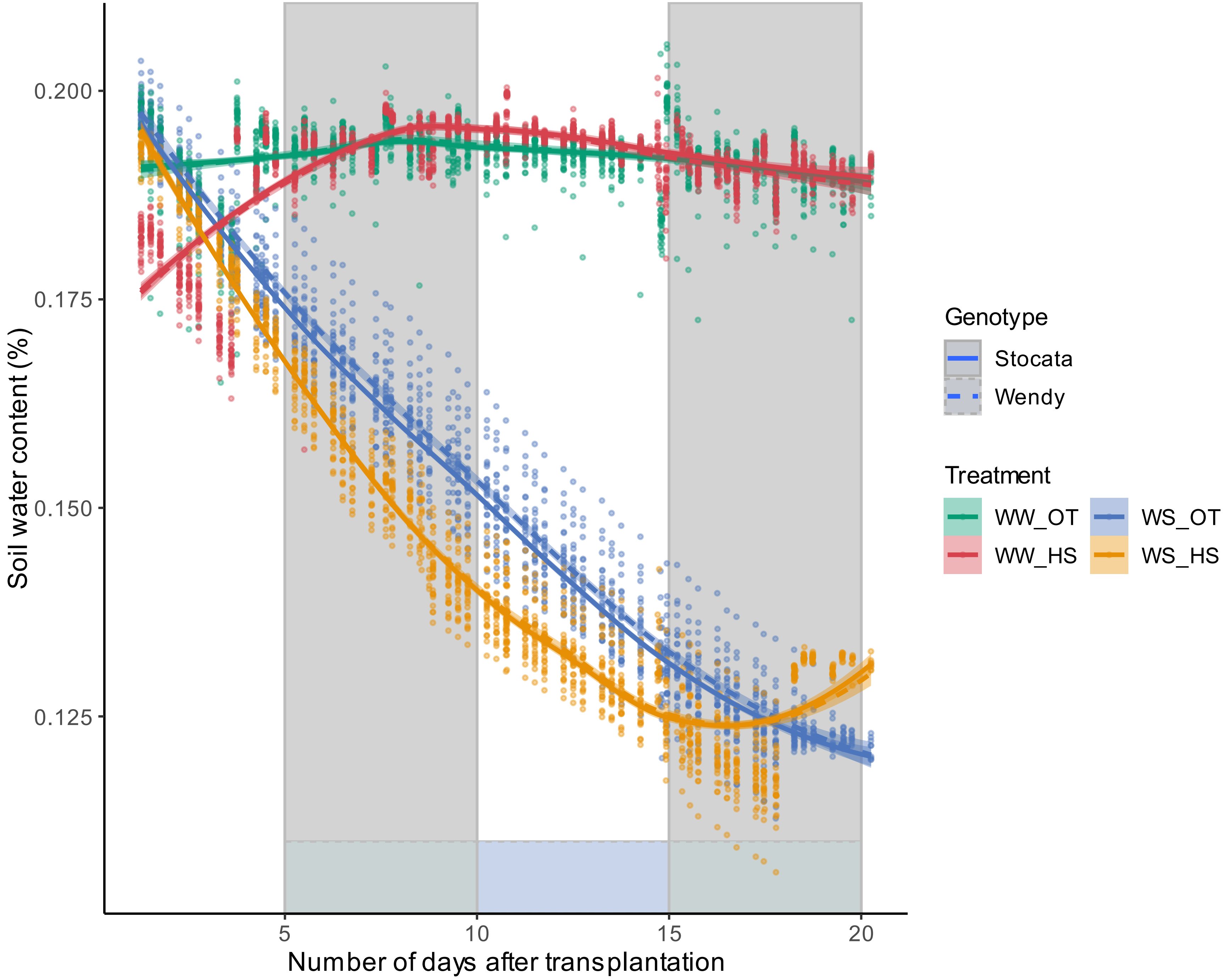
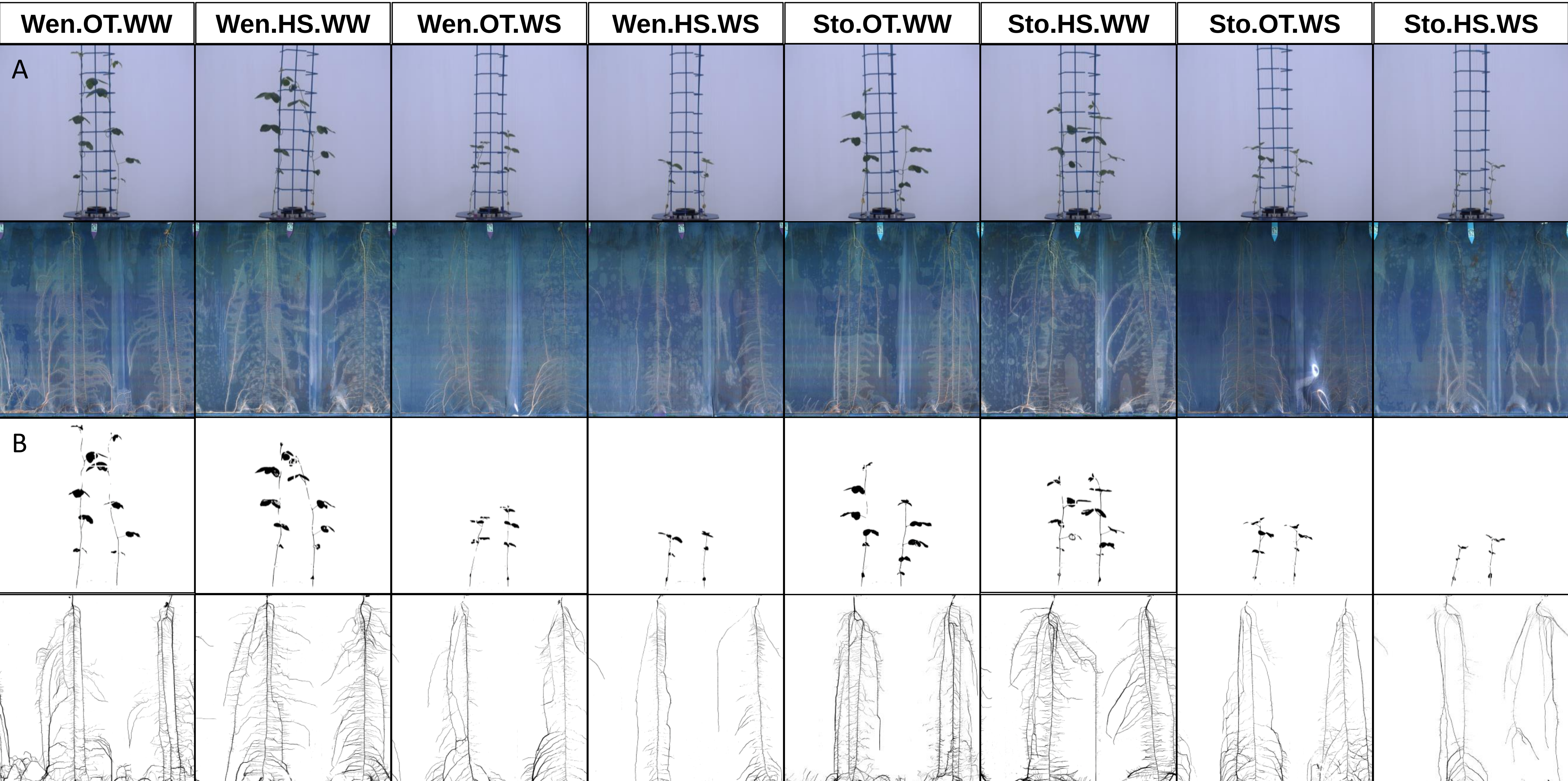


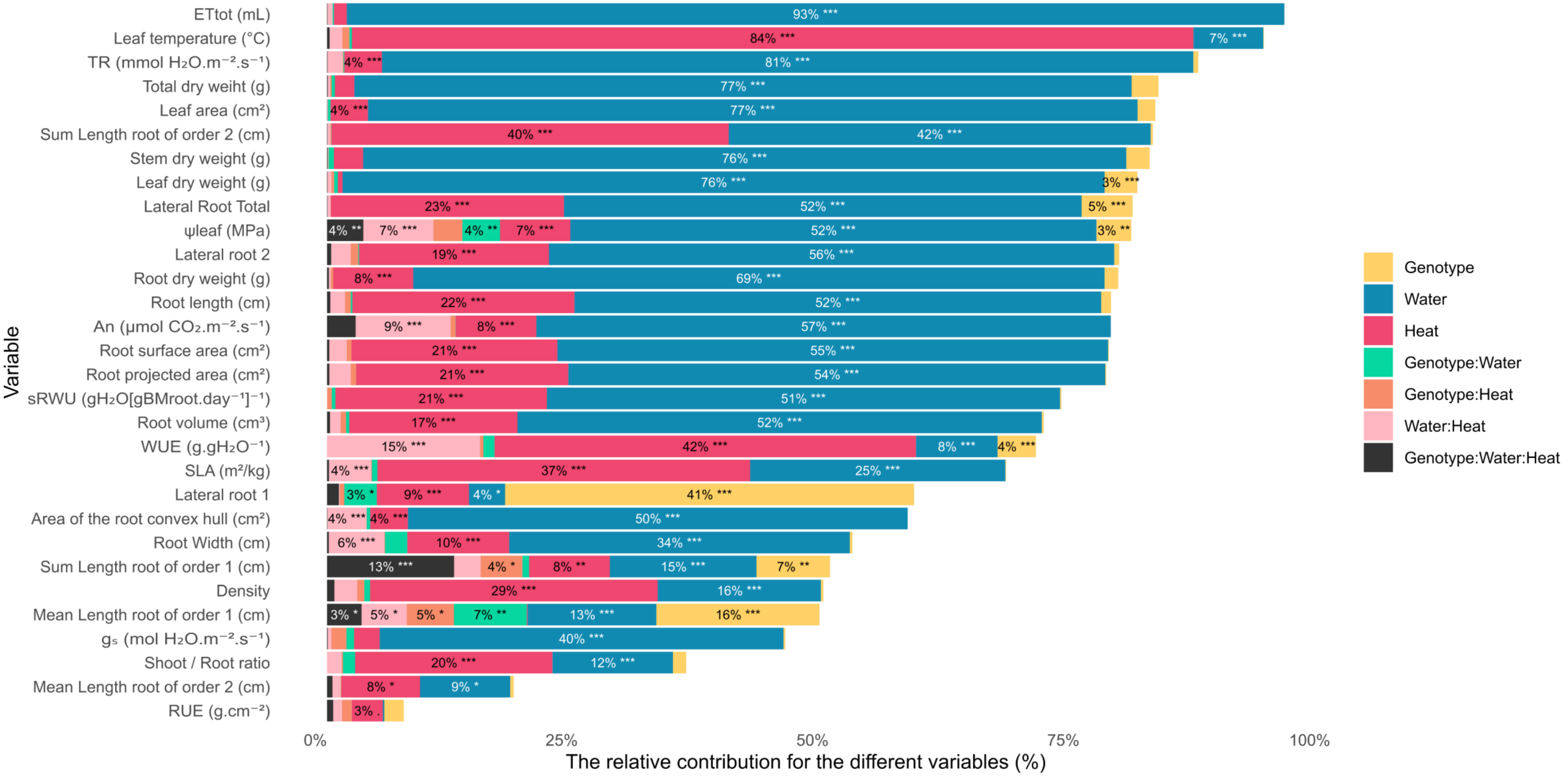
Supporting information 3: Evapotranspiration dynamics for each day-Night post-transplantation. Evapotranspiration rates (ml/hour) of one plant by RhizoTube® for each genotype under each treatment over a period of 20 days after transplantation. The blue shaded areas represent the watering periods, while the gray shaded areas denote the occurrence of heat waves. Each point on the graph corresponds to a measure of evapotranspiration rate. The 95% confidence interval with local regression adjustment was drawn in transparency.



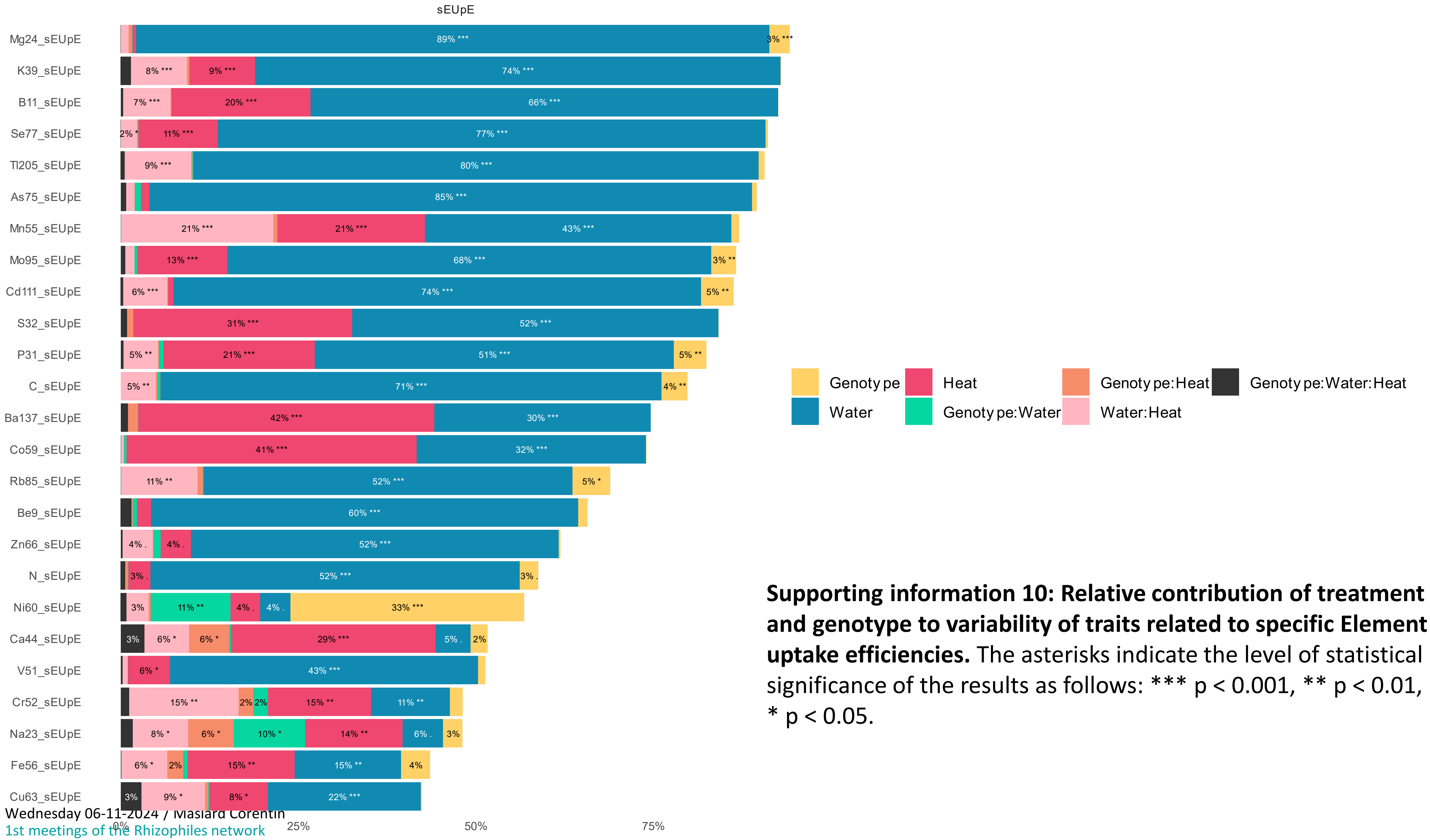
Supporting information 4: Soil water content dynamics for each day post-transplantation.
Soil water content (%) for each RhizoTube® for each genotype under each treatment over a period of 20 days after transplantation. The blue shaded areas represent the watering periods, while the gray shaded areas denote the occurrence of heat waves. The 95% confidence interval with local regression adjustment was drawn in transparency

Supporting information 5 : Pictures of two soybean varieties (Stocata (Sto) and Wendy (Wen)) under different temperature (optimum temperature (OT), heat stress (HS)) and watering (well-watered (WW), water stress (WS)) conditions, 20 days after transplantation, before (A) and after (B) image segmentation by machine learning.

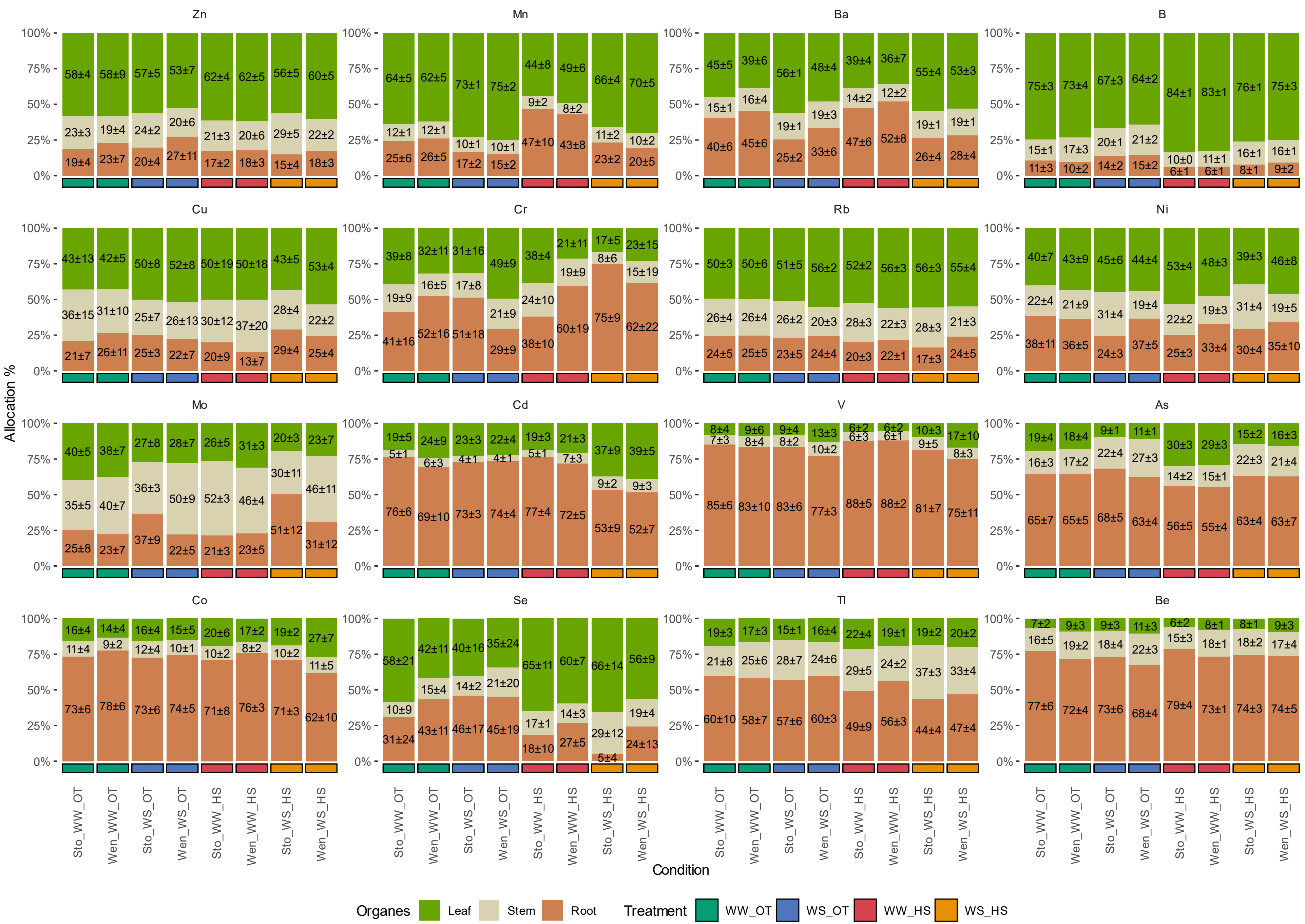




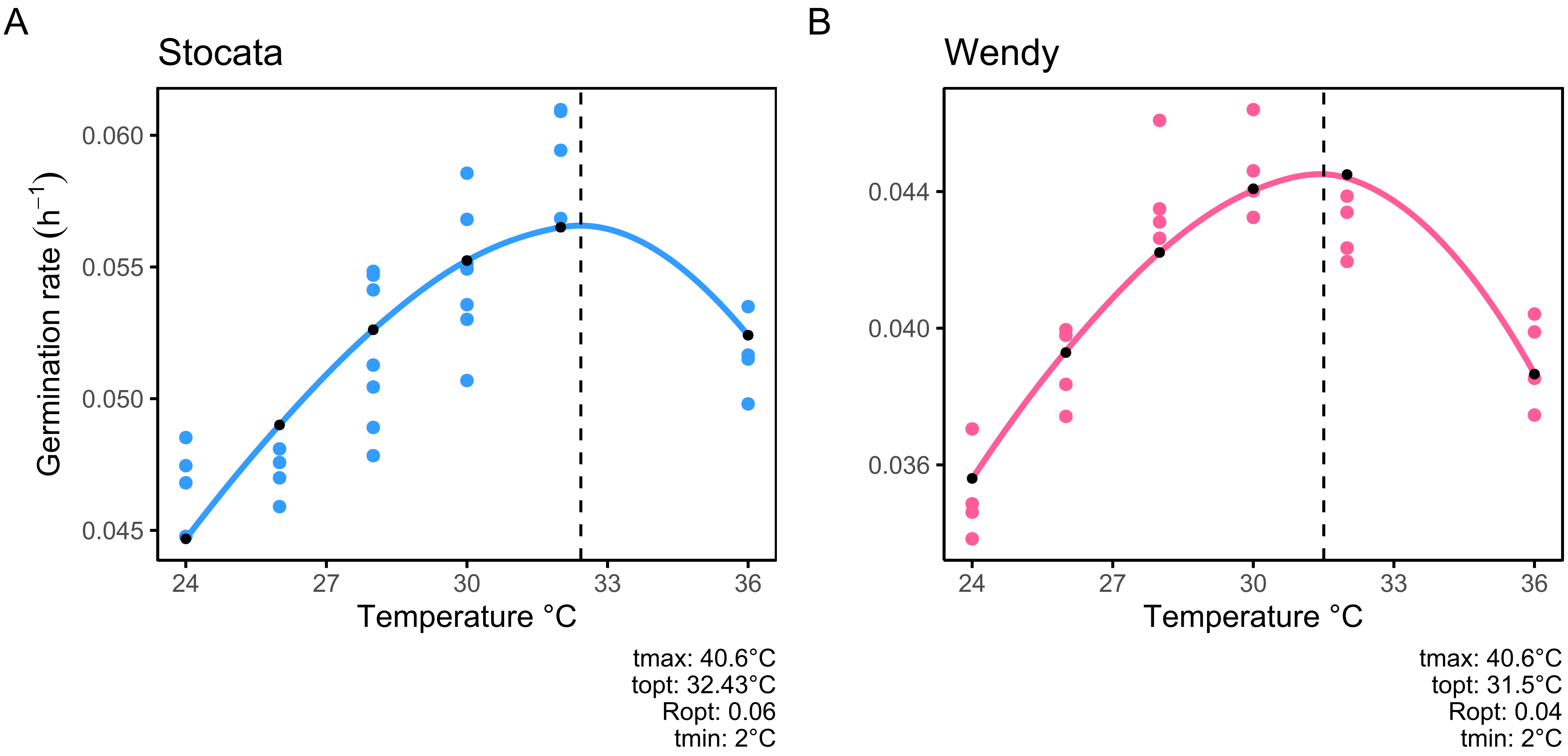
Supporting information 9: Relative contribution of treatment and genotype to variability of each trait related to RSA, water uptake and carbon fixation. The asterisks indicate the level of statistical significance of the results as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.



Supporting information 10: Relative contribution of treatment and genotype to variability of traits related to specific Element uptake efficiencies. The asterisks indicate the level of statistical significance of the results as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.



Supporting information 11: Elemental allocation in plant organs under each treatment. Distribution of nutrients in the leaves (green), stems (orange), and roots (brown) of plants subjected to well-watered and optimal temperature (WW_OT), well-watered and heat stress (WW_HS), water stress and optimal temperature (WS_OT), and water stress and heat stress (WS_HS) for Stocata (Sto) and Wendy (Wen) soybean genotypes. Each bar represents the percentage allocation of the indicated element (Zn, Mn, Ba, B, Cu, Cr, Rb, Ni, Mo, Cd, V, As, Co, Se, Tl and Be). Values within the bars indicate mean percentages of elemental allocation and the standard deviation.



Supporting information 12: Graphical display of the Yin adjustments for the two cultivars at 50% of germination.

Dots represent mean observed data and lines the adjusted Yin function.

CF PDF

Supporting information 13: Effects of water and heat stresses on soybean element concentration. For each trait, values are means (bold) ± SD. Asterisks mean that the values are significantly different from those of the control condition (Welch Two Samples t-test). The asterisks indicate the level of statistical significance of the results as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns not significant. Wen = Wendy genotype; Sto: Stocata genotype, WW: well-watered condition, WS: water stress condition, OT: optimal temperature, HS: heat stress.;

Summary of concentration in element for Stocata in leaf

Variable	Sto_WW_OT		Sto_WS_OT			Sto_WW_HS			Sto_WS_HS		
Essential macroelement											
C (µg/g)	4.4e+05	±3.2e+03	4.3e+05	±4.0e+03	***	4.4e+05	±1.2e+03	ns	4.1e+05	±5.7e+03	***
N (µg/g)	5.1e+04	±2.3e+03	5.6e+04	±1.7e+03	***	5.4e+04	±1.6e+03	*	6.2e+04	±2.3e+03	***
Ca (µg/g)	2.4e+04	±1.6e+03	3.4e+04	±1.3e+03	***	2.1e+04	±1.4e+03	*	3.9e+04	±2.0e+03	***
K (µg/g)	1.6e+04	±1.6e+03	1.5e+04	±688.13	ns	2.3e+04	±438.91	***	1.6e+04	±1.0e+03	ns
Mg (µg/g)	6.3e+03	±223.75	4.1e+03	±173.28	***	4.9e+03	±935.45	*	3.3e+03	±220.31	***
S (µg/g)	2.7e+03	±131.28	2.8e+03	±86.23	ns	2.9e+03	±159.61	ns	3.5e+03	±260.14	***
P (µg/g)	2.6e+03	±244.42	3.0e+03	±349.5	*	3.5e+03	±299.86	**	3.6e+03	±340.8	***
Essential microelement											
Fe (µg/g)	146.36	±22.26	125.29	±14.7	ns	137.41	±6.02	ns	180.27	±74.6	ns
Zn (µg/g)	51.34	±3.43	55.03	±3.64	ns	60.54	±6.7	ns	61	±5.23	**
B (µg/g)	50.15	±2.41	39.01	±3.3	***	73.09	±2.43	***	54.36	±4.27	ns
Mn (µg/g)	39.24	±3.49	49.57	±2.89	***	43.48	±3.79	ns	44.61	±4.73	ns
Cu (µg/g)	11.4	±2.58	13.99	±3.17	ns	14.1	±5.6	ns	14.59	±2.28	ns
Ni (µg/g)	3.84	±0.48	6.27	±2.27	*	4.15	±0.53	ns	7.34	±0.9	***
Mo (µg/g)	0.68	±0.22	0.16	±0.04	**	0.66	±0.11	ns	0.25	±0.02	*
Beneficial element											
Na (µg/g)	258.77	±84.83	347.24	±52.26	ns	215.98	±63.2	ns	325.36	±90.54	ns
Se (µg/g)	0.34	±0.11	0.07	±0.06	**	0.47	±0.15	ns	0.13	±0.07	**
V (µg/g)	0.11	±0.03	0.1	±0.02	ns	0.08	±9.7e-03	ns	0.17	±0.05	*
Co (µg/g)	0.08	±7.7e-03	0.07	±0.01	ns	0.12	±0.03	*	0.15	±0.02	***
Other											
Ba (µg/g)	33.31	±2.89	45.84	±2.25	***	29.66	±1.22	*	54.41	±3.24	***
Rb (µg/g)	14.53	±2.53	18.15	±3.24	ns	14.43	±1.89	ns	18.17	±6.3	ns
Cr (µg/g)	6.87	±2.08	7.4	±3.22	ns	4.44	±1.01	ns	17.78	±12.97	ns
Cd (µg/g)	0.28	±0.07	0.14	±0.03	*	0.36	±0.1	ns	0.15	±0.03	*
As (µg/g)	0.15	±0.02	0.05	±1.0e-03	***	0.25	±0.02	***	0.07	±0.01	***
Tl (µg/g)	0.02	±2.1e-03	0.02	±9.1e-04	**	0.02	±3.9e-03	ns	0.01	±9.4e-04	***
Be (µg/g)	3.5e-03	±1.1e-03	4.3e-03	±1.2e-03	ns	2.7e-03	±5.7e-04	ns	4.0e-03	±7.1e-04	ns

Summary of concentration in element for Wendy in leaf

Variable	Wen_WW_OT		Wen_WS_OT			Wen_WW_HS			Wen_WS_HS		
Essential macroelement											
C (µg/g)	4.5e+05	±2.8e+03	4.4e+05	±4.6e+03	ns	4.5e+05	±2.8e+03	ns	4.1e+05	±7.5e+03	***
N (µg/g)	5.0e+04	±3.2e+03	5.4e+04	±3.0e+03	ns	5.1e+04	±3.7e+03	ns	6.2e+04	±1.3e+03	***
Ca (µg/g)	2.2e+04	±1.3e+03	2.7e+04	±1.9e+03	***	2.1e+04	±1.8e+03	ns	4.0e+04	±2.8e+03	***
K (µg/g)	1.6e+04	±1.7e+03	1.4e+04	±990.7	*	1.9e+04	±1.3e+03	**	1.5e+04	±2.1e+03	ns
Mg (µg/g)	6.3e+03	±679.78	4.4e+03	±423.91	***	5.4e+03	±292.47	*	3.7e+03	±226.75	***
S (µg/g)	2.6e+03	±228.89	2.6e+03	±204.67	ns	2.7e+03	±90.58	ns	3.7e+03	±259.55	***
P (µg/g)	2.4e+03	±451.65	2.5e+03	±281.4	ns	3.1e+03	±306.9	**	3.3e+03	±560.94	*
Essential microelement											
Fe (µg/g)	164.03	±76.24	168.65	±83.82	ns	137.98	±9.62	ns	175.79	±121.09	ns
Zn (µg/g)	59.17	±37.7	45.32	±4.58	ns	58.21	±9.79	ns	51.29	±9.14	ns
B (µg/g)	47.91	±2.72	32.48	±1.83	***	66.94	±1.48	***	48.12	±8.23	ns
Mn (µg/g)	39.46	±5.34	51.93	±5.06	**	48.95	±2.95	**	46.66	±6.03	ns
Cu (µg/g)	9.82	±2.39	16.16	±4.39	*	16.86	±10.56	ns	14.54	±2.57	*
Ni (µg/g)	3.28	±0.93	3.23	±0.62	ns	3.05	±0.47	ns	4.28	±1.7	ns
Mo (µg/g)	0.97	±0.2	0.37	±0.16	***	0.87	±0.1	ns	0.53	±0.2	**
Beneficial element											
Na (µg/g)	245.83	±89	424.1	±87.95	**	295.85	±80.32	ns	386.93	±136.82	ns
Se (µg/g)	0.25	±0.1	0.04	±0.03	**	0.46	±0.1	**	0.17	±0.1	ns
V (µg/g)	0.13	±0.1	0.13	±0.07	ns	0.07	±0.02	ns	0.25	±0.22	ns
Co (µg/g)	0.07	±0.03	0.07	±0.02	ns	0.11	±3.9e-03	*	0.18	±0.05	**
Other											
Ba (µg/g)	27.68	±4.42	35.3	±3.47	**	26.37	±2.76	ns	50.27	±4.08	***
Rb (µg/g)	10.14	±1.06	11.43	±2.27	ns	11.69	±1.62	ns	7.88	±3.28	ns
Cr (µg/g)	8.33	±9.36	12.22	±6.99	ns	3.02	±0.53	ns	16.79	±21.06	ns
Cd (µg/g)	0.48	±0.28	0.19	±0.06	ns	0.39	±0.04	ns	0.18	±0.04	*
As (µg/g)	0.13	±0.01	0.04	±6.7e-03	***	0.19	±0.03	**	0.07	±0.01	***
Tl (µg/g)	0.02	±2.1e-03	0.01	±1.4e-03	**	0.02	±1.3e-03	ns	0.01	±1.2e-04	**
Be (µg/g)	4.3e-03	±1.1e-03	4.3e-03	±1.5e-03	ns	3.3e-03	±4.8e-04	ns	4.4e-03	±1.4e-03	ns

Summary of concentration in element for Stocata in stem

Variable	Sto_WW_OT		Sto_WS_OT			Sto_WW_HS			Sto_WS_HS		
Essential macroelement											
C (µg/g)	4.3e+05	±9.2e+03	4.3e+05	±7.5e+03	ns	4.3e+05	±2.4e+03	ns	4.0e+05	±7.0e+03	***
N (µg/g)	2.8e+04	±1.9e+03	4.0e+04	±2.5e+03	***	2.5e+04	±1.1e+03	**	6.3e+04	±5.8e+03	***
K (µg/g)	1.3e+04	±2.8e+03	8.4e+03	±1.4e+03	*	2.5e+04	±1.1e+03	***	1.3e+04	±1.7e+03	ns
Ca (µg/g)	1.2e+04	±1.2e+03	1.9e+04	±1.2e+03	***	1.3e+04	±1.6e+03	ns	2.2e+04	±1.5e+03	***
Mg (µg/g)	3.0e+03	±94.73	2.4e+03	±190.98	***	2.4e+03	±247.11	*	3.0e+03	±215.37	ns
P (µg/g)	1.0e+03	±108.44	1.5e+03	±410.03	*	1.6e+03	±239.26	*	3.3e+03	±669.76	***
S (µg/g)	893.11	±79.39	1.4e+03	±158.89	***	1.1e+03	±105.11	*	3.1e+03	±507.41	***
Essential microelement											
Fe (µg/g)	64.59	±19.2	69.75	±21.83	ns	81.63	±18.1	ns	134.55	±72.86	ns
Zn (µg/g)	27.66	±4.07	31.32	±4.33	ns	32.77	±5.88	ns	44.88	±10.27	**
Cu (µg/g)	13.98	±8.36	9.36	±2.96	ns	14.98	±11.01	ns	13.09	±1.63	ns
B (µg/g)	13.35	±0.69	15.33	±0.87	**	13.89	±0.81	ns	16.15	±1.36	**
Mn (µg/g)	9.72	±0.67	9.54	±0.59	ns	13.2	±0.89	***	10.37	±0.83	ns
Ni (µg/g)	2.8	±0.39	5.73	±1.59	**	2.69	±0.69	ns	8.24	±1.84	***
Mo (µg/g)	0.8	±0.26	0.32	±0.16	**	2.03	±0.29	***	0.53	±0.18	ns
Beneficial element											
Na (µg/g)	357.07	±80.67	367.57	±56.81	ns	408.15	±116.83	ns	1.1e+03	±179.21	***
V (µg/g)	0.12	±0.03	0.12	±0.02	ns	0.13	±0.03	ns	0.22	±0.12	ns
Se (µg/g)	0.08	±0.07	0.03	±0.02	ns	0.19	±0.04	*	0.1	±0.02	ns
Co (µg/g)	0.08	±0.03	0.07	±0.02	ns	0.09	±0.01	ns	0.12	±0.03	*
Other											
Ba (µg/g)	14.52	±0.64	20.22	±1.33	***	16.54	±0.87	*	26.67	±2.96	***
Rb (µg/g)	10.24	±1.09	12.44	±2.77	ns	11.82	±2.34	ns	13.29	±5.99	ns
Cr (µg/g)	4.39	±2.85	5.64	±3.11	ns	4.47	±2.87	ns	12.26	±10.22	ns
As (µg/g)	0.17	±9.8e-03	0.14	±8.1e-03	**	0.18	±0.01	ns	0.16	±0.01	ns
Cd (µg/g)	0.09	±0.02	0.03	±0.01	**	0.15	±0.07	ns	0.05	±0.01	*
Tl (µg/g)	0.03	±0.01	0.04	±0.01	ns	0.05	±8.7e-03	*	0.04	±4.1e-03	ns
Be (µg/g)	0.01	±2.1e-03	0.01	±2.8e-03	ns	0.01	±9.0e-04	ns	0.01	±1.1e-03	*

Summary of concentration in element for Wendy in stem

Variable	Wen_WW_OT		Wen_WS_OT			Wen_WW_HS			Wen_WS_HS		
Essential macroelement											
C (µg/g)	4.4e+05	±3.9e+03	4.4e+05	±2.9e+03	ns	4.4e+05	±4.2e+03	ns	4.1e+05	±7.3e+03	***
N (µg/g)	2.5e+04	±1.3e+03	3.4e+04	±2.8e+03	***	2.1e+04	±3.0e+03	**	5.3e+04	±4.9e+03	***
K (µg/g)	1.4e+04	±2.6e+03	4.7e+03	±681.44	***	1.8e+04	±3.1e+03	*	1.0e+04	±1.9e+03	*
Ca (µg/g)	1.1e+04	±1.1e+03	1.6e+04	±1.4e+03	***	1.2e+04	±1.3e+03	ns	2.4e+04	±1.7e+03	***
Mg (µg/g)	2.9e+03	±365.85	2.4e+03	±162.45	*	2.5e+03	±182.92	*	3.4e+03	±294.35	*
P (µg/g)	983.81	±170.49	859.29	±101.15	ns	1.2e+03	±163.85	ns	2.2e+03	±807.14	*
S (µg/g)	801.42	±93.5	866.4	±103.48	ns	877.05	±62.85	ns	2.7e+03	±673.24	**
Essential microelement											
Fe (µg/g)	81.81	±51.15	63.6	±20.19	ns	80.7	±12.18	ns	114.69	±80.25	ns
Zn (µg/g)	22.85	±4.72	22.14	±6.55	ns	31.57	±12.08	ns	32.32	±7.65	*
B (µg/g)	14.7	±1.14	13.92	±0.82	ns	14.24	±0.71	ns	17.16	±2.4	ns
Mn (µg/g)	9.9	±1.27	8.73	±0.29	ns	12.7	±1.48	**	11.23	±1.34	ns
Cu (µg/g)	9.57	±3.7	12.69	±12.63	ns	22.44	±20.42	ns	10.04	±1.23	ns
Ni (µg/g)	2.11	±0.95	1.84	±0.3	ns	2.04	±0.55	ns	2.87	±0.79	ns
Mo (µg/g)	1.39	±0.43	0.86	±0.39	ns	2.12	±0.33	**	1.87	±0.98	ns
Beneficial element											
Na (µg/g)	347.11	±31.62	366.22	±95.56	ns	437.43	±84.61	*	1.7e+03	±406.76	**
V (µg/g)	0.15	±0.09	0.12	±0.03	ns	0.13	±0.02	ns	0.17	±0.06	ns
Se (µg/g)	0.11	±0.04	0.03	±0.02	**	0.17	±0.03	*	0.1	±0.06	ns
Co (µg/g)	0.06	±0.03	0.06	±0.01	ns	0.08	±0.02	ns	0.12	±0.03	*
Other											
Ba (µg/g)	15.22	±3.56	17.91	±2.62	ns	14.31	±1.57	ns	29.93	±2.05	***
Rb (µg/g)	6.94	±0.79	5.39	±0.96	*	7.72	±1.67	ns	5.18	±2.61	ns
Cr (µg/g)	5.67	±6.32	6.11	±3.12	ns	4.48	±1.41	ns	11.84	±11.94	ns
Cd (µg/g)	0.17	±0.12	0.05	±0.02	*	0.23	±0.11	ns	0.07	±0.03	ns
As (µg/g)	0.16	±0.01	0.13	±7.2e-03	**	0.16	±9.4e-03	ns	0.15	±0.01	ns
Tl (µg/g)	0.04	±8.6e-03	0.03	±0.01	ns	0.04	±4.0e-03	ns	0.04	±4.1e-03	ns
Be (µg/g)	0.01	±8.0e-04	0.01	±1.6e-03	ns	0.01	±4.6e-04	ns	0.01	±1.0e-03	ns

Summary of concentration in element for Stocata in root

Variable	Sto_WW_OT		Sto_WS_OT			Sto_WW_HS			Sto_WS_HS		
Essential macroelement											
C (µg/g)	4.0e+05	±1.1e+04	4.4e+05	±1.5e+04	***	3.9e+05	±9.9e+03	*	4.5e+05	±5.0e+03	***
N (µg/g)	4.1e+04	±3.2e+03	3.8e+04	±5.5e+03	ns	3.5e+04	±2.3e+03	**	3.9e+04	±5.0e+03	ns
Mg (µg/g)	1.2e+04	±3.0e+03	6.2e+03	±1.3e+03	*	1.4e+04	±426.53	ns	3.7e+03	±402.04	**
K (µg/g)	1.0e+04	±1.8e+03	6.5e+03	±2.0e+03	**	1.7e+04	±3.4e+03	*	7.9e+03	±2.0e+03	ns
Ca (µg/g)	1.0e+04	±2.2e+03	1.0e+04	±2.2e+03	ns	1.1e+04	±1.2e+03	ns	9.5e+03	±1.0e+03	ns
S (µg/g)	2.8e+03	±684.92	2.6e+03	±411.85	ns	3.2e+03	±410.99	ns	2.9e+03	±328.84	ns
P (µg/g)	1.7e+03	±180.32	2.0e+03	±400.32	ns	2.1e+03	±299.87	ns	2.9e+03	±646.59	**
Essential microelement											
Fe (µg/g)	1.5e+03	±832.41	1.1e+03	±474.48	ns	1.8e+03	±518.79	ns	2.7e+03	±1.1e+03	ns
Zn (µg/g)	38.54	±3.92	38.86	±12.44	ns	50.51	±2.35	***	41.18	±11.42	ns
Mn (µg/g)	37.03	±14.41	23.17	±5.2	ns	146.18	±54.05	*	39.61	±3.74	ns
B (µg/g)	16.16	±1.83	15.77	±1.21	ns	15.77	±1.41	ns	14.13	±0.72	ns
Cu (µg/g)	13.26	±3.42	13.76	±1.43	ns	19.31	±14.12	ns	24.78	±5.54	**
Ni (µg/g)	8.98	±3.95	6.47	±1.14	ns	5.94	±0.52	ns	14.1	±3.6	ns
Mo (µg/g)	1	±0.46	0.52	±0.38	ns	1.61	±0.23	*	1.72	±0.7	ns
Beneficial element											
Na (µg/g)	6.5e+03	±2.4e+03	6.2e+03	±1.3e+03	ns	5.1e+03	±955.01	ns	3.5e+03	±479.79	*
V (µg/g)	3.32	±2.16	2.08	±0.88	ns	3.93	±1.2	ns	3.52	±0.45	ns
Co (µg/g)	0.92	±0.37	0.71	±0.22	ns	1.4	±0.29	ns	1.47	±0.27	*
Se (µg/g)	0.42	±0.32	0.1	±0.04	ns	0.41	±0.25	ns	0.03	±0.02	ns
Other											
Ba (µg/g)	71.14	±13.94	40.98	±4.22	**	110.2	±15.5	**	66.02	±8.23	ns
Cr (µg/g)	20.22	±18.44	27.92	±16.97	ns	12.91	±2.4	ns	209.88	±175.23	*
Rb (µg/g)	16.51	±3.86	16.14	±3.1	ns	16.63	±1.62	ns	13.13	±2.86	ns
Cd (µg/g)	2.65	±0.49	0.89	±0.08	**	4.52	±1.12	*	0.57	±0.19	***
As (µg/g)	1.29	±0.56	0.68	±0.23	ns	1.42	±0.12	ns	0.82	±0.09	ns
Tl (µg/g)	0.15	±0.03	0.11	±9.1e-03	*	0.16	±0.02	ns	0.08	±8.2e-03	**
Be (µg/g)	0.1	±0.04	0.07	±0.02	ns	0.12	±0.02	ns	0.1	±0.01	ns

Summary of concentration in element for Wendy in root

Variable	Wen_WW_OT		Wen_WS_OT			Wen_WW_HS			Wen_WS_HS		
Essential macroelement											
C (µg/g)	4.1e+05	±7.0e+03	4.4e+05	±8.0e+03	***	4.0e+05	±8.1e+03	ns	4.3e+05	±9.1e+03	***
N (µg/g)	3.7e+04	±2.0e+03	3.8e+04	±3.4e+03	ns	3.1e+04	±1.9e+03	***	4.3e+04	±3.0e+03	**
Mg (µg/g)	1.2e+04	±1.3e+03	6.7e+03	±242.86	***	1.4e+04	±1.4e+03	ns	5.3e+03	±912.94	***
K (µg/g)	1.2e+04	±1.3e+03	5.0e+03	±529.66	***	1.6e+04	±2.8e+03	*	8.0e+03	±1.6e+03	**
Ca (µg/g)	1.0e+04	±1.8e+03	1.3e+04	±519.25	*	1.1e+04	±1.3e+03	ns	1.2e+04	±1.4e+03	ns
S (µg/g)	3.3e+03	±201.28	2.7e+03	±315.74	**	4.0e+03	±447.88	*	3.6e+03	±452.95	ns
P (µg/g)	1.8e+03	±115.14	1.6e+03	±184.92	ns	1.7e+03	±279.37	ns	2.4e+03	±508.6	ns
Essential microelement											
Fe (µg/g)	1.4e+03	±319.99	784.7	±132.22	**	1.4e+03	±323.84	ns	1.8e+03	±818.46	ns
Zn (µg/g)	48.45	±4.78	48.6	±24.87	ns	50.14	±5.96	ns	40.35	±6.94	ns
Mn (µg/g)	40.15	±8.84	21.01	±3.05	**	133.89	±52.87	**	35.04	±6.89	ns
B (µg/g)	15.22	±0.96	14.74	±0.51	ns	15.17	±1.11	ns	15.82	±1.42	ns
Cu (µg/g)	13.99	±4.57	13.18	±3.89	ns	11.33	±2.55	ns	18	±3.03	ns
Ni (µg/g)	6.74	±1.97	5.41	±1.24	ns	6.35	±1.56	ns	8.37	±2.36	ns
Mo (µg/g)	1.4	±0.42	0.54	±0.08	**	1.92	±0.46	ns	1.94	±0.87	ns
Beneficial element											
Na (µg/g)	4.7e+03	±1.1e+03	7.7e+03	±441.95	***	5.3e+03	±596.11	ns	4.9e+03	±706.75	ns
V (µg/g)	2.91	±0.91	1.48	±0.41	**	3.26	±0.76	ns	2.88	±1.06	ns
Co (µg/g)	1.01	±0.2	0.71	±0.13	*	1.47	±0.31	*	1.18	±0.39	ns
Se (µg/g)	0.61	±0.21	0.14	±0.08	**	0.61	±0.08	ns	0.18	±0.13	**
Other											
Ba (µg/g)	78.22	±6.61	48.1	±4.9	***	117.49	±35.56	*	73.46	±11.73	ns
Cr (µg/g)	25.05	±9.36	14.76	±9.63	ns	31.52	±18.97	ns	113.62	±88.04	ns
Rb (µg/g)	12.26	±1.47	9.73	±1.05	**	13.3	±1.54	ns	8.96	±2.22	*
Cd (µg/g)	3.13	±0.97	1.24	±0.27	**	4.07	±0.98	ns	0.64	±0.13	**
As (µg/g)	1.14	±0.22	0.48	±0.09	***	1.04	±0.13	ns	0.74	±0.19	**
Tl (µg/g)	0.16	±0.02	0.11	±0.01	**	0.18	±0.02	ns	0.08	±0.01	***
Be (µg/g)	0.08	±0.02	0.05	±0.01	*	0.09	±0.01	ns	0.09	±0.02	ns