Food and Agriculture Organization of the United Nations

Salt-affected soils: threats and potentials

Hormonal responses to combined phosphorus deficiency and salinity in soybean (*Glycine max* (L.) Merr.)

Joint meeting of
INSAS and SUSTAIN

CEBAS-CSIC
Centro de Edafología y Biología Aplicada del Segura

South China Agricultural University 1909

Lancaster University

Purificación A. Martínez-Melgarejo, Cristina Martínez-Andújar, Alfonso Albacete, Ian C.Dodd, J. Tian & Francisco Pérez-Alfocea

International Network of Salt-Affected Soils

Funded by the European Union

COST European Cooperation in Science & Technology

SUSTAIN Sustainable use of salt-affected lands

Vniversitat de València

International Union of Soil Sciences

Valencia, Spain
May 27-31, 2024

Global Soil Partnership

Soybeans Crops

Ranking 10 most important crops in the world
in production, harvested area and demand

6%

70-75%

20%

PANORAMA MUNDIAL DE SOYA | PROYECCIÓN 2020/21

Fuente: GCMA con datos del USDA | (Agosto 2021)

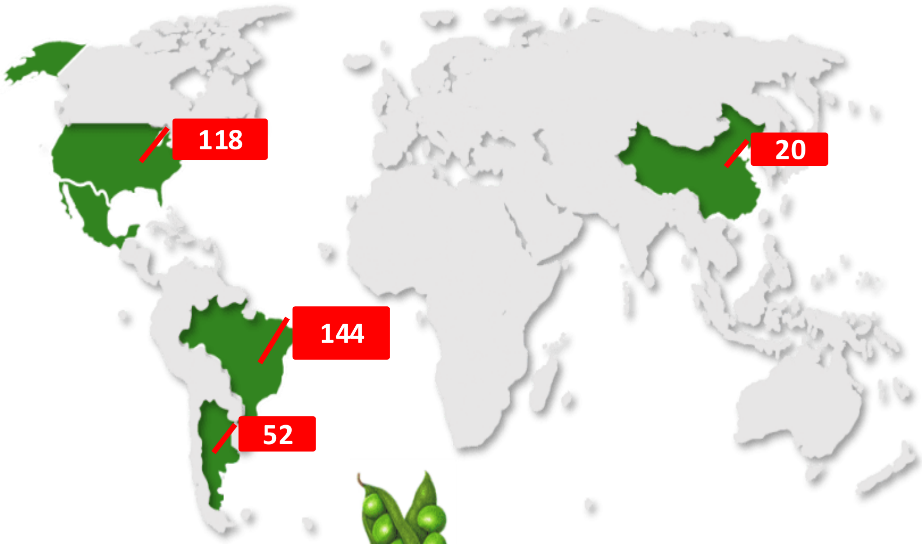
ESTADOS UNIDOS			
PRODUCCIÓN	2°	31%	118.1 MTM
EXPORTACIÓN	2°	32%	55.9 MTM

BRASIL			
PRODUCCIÓN	1°	38%	144.0 MTM
EXPORTACIÓN	1°	54%	93.0 MTM

ARGENTINA			
PRODUCCIÓN	3°	14%	52.0 MTM
EXPORTACIÓN	4°	4%	6.4 MTM
IMPORTACIÓN	5°	3%	4.7 MTM

CHINA			
IMPORTACIÓN	1°	59%	101.0 MTM

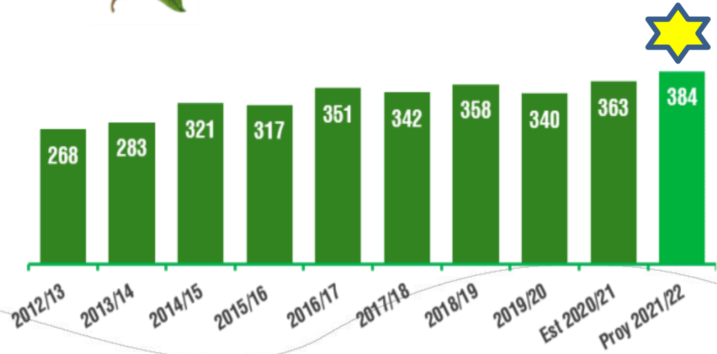
MÉXICO			
PRODUCCIÓN	19°	0.1%	0.3 MTM
IMPORTACIÓN	3°	4%	6.2 MTM



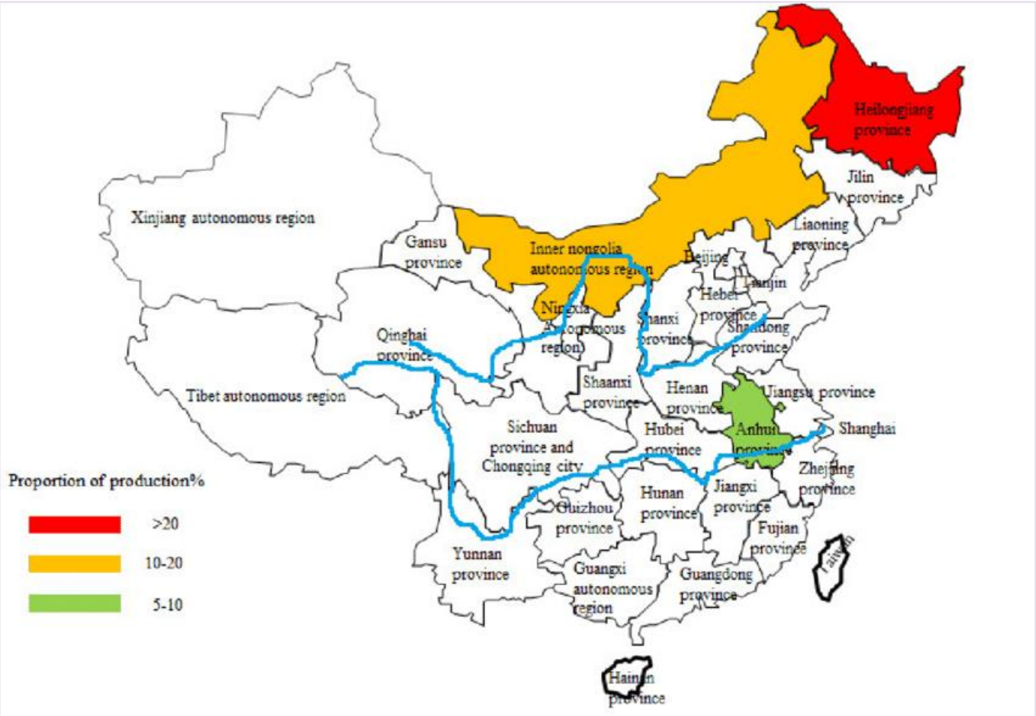
383.6
PRODUCCIÓN
MILLONES DE TM

172.3
COMERCIALIZACIÓN
MILLONES DE TM

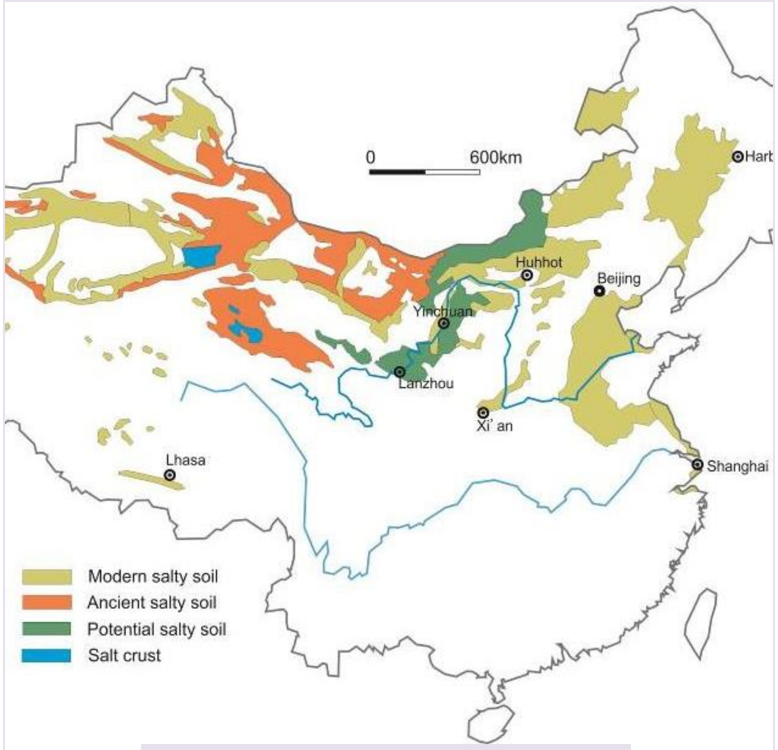
PRODUCCIÓN MUNDIAL
MILLONES DE TONELADAS



Saline soils in Chinese soybean-producing areas !
Significant variation in salt tolerance in Chinese soybean varieties



Chinese soybean-producing areas



Saline soil in China

Salt-tolerant versus salt-sensitive soybean varieties grown with 200 mM NaCl for 18 days (source: Guang et al., 2014)

Soil P deficiency widespread in some Chinese soybean producing regions
Significant variation in P use efficiency in Chinese soybean varieties

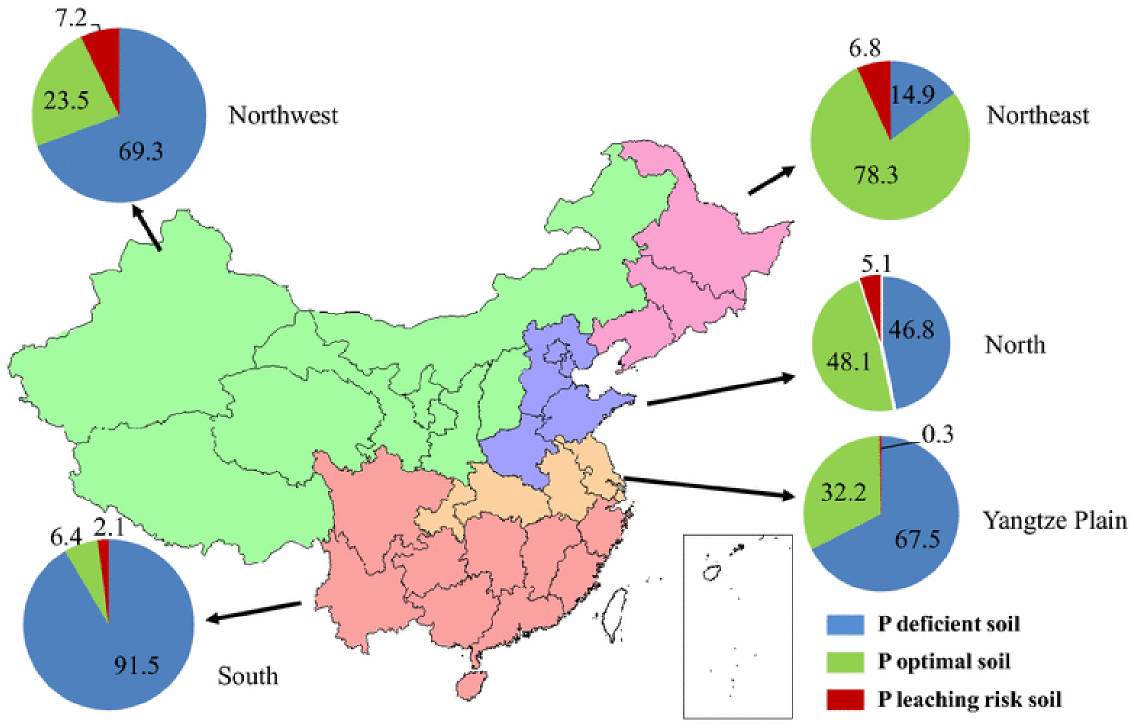
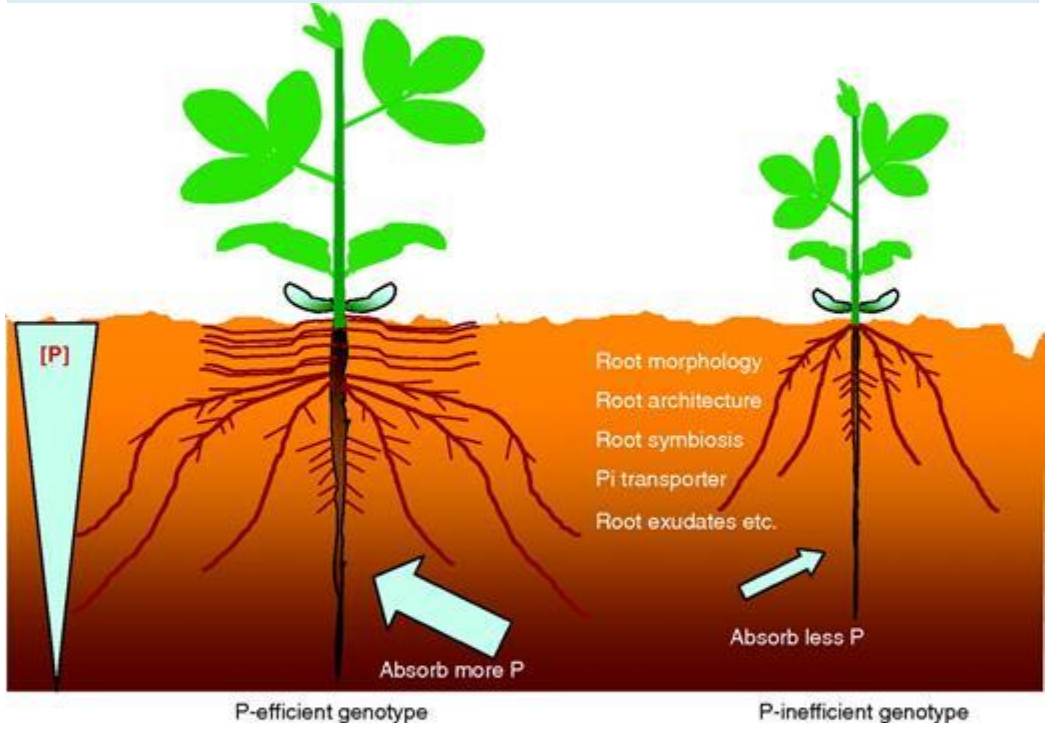


Image 2. Proportions (in %) of arable land with different soil Olsen P status in the Northeast, Northwest, North, Yangtze Plain, and South P management regions of China (Li *et al.*, 2015).

"topsoil foraging" root Ideotype



Main Objetive

Understand the physiological and genetic basis of P use efficiency (PUE) and its interaction with salinity through hormonal changes, with special emphasis on ABA, in soybean

Material and methods

The experiment consisted of a total of 144 specimens:

12 seeds X 2 genotypes X 6 treatments in "hydroponic culture medium"

Contrasting response to P-starvation

- **YCO3-3 P-efficient**
  Shallow RSA
- **BD2 P-inefficient**
  Deep RSA

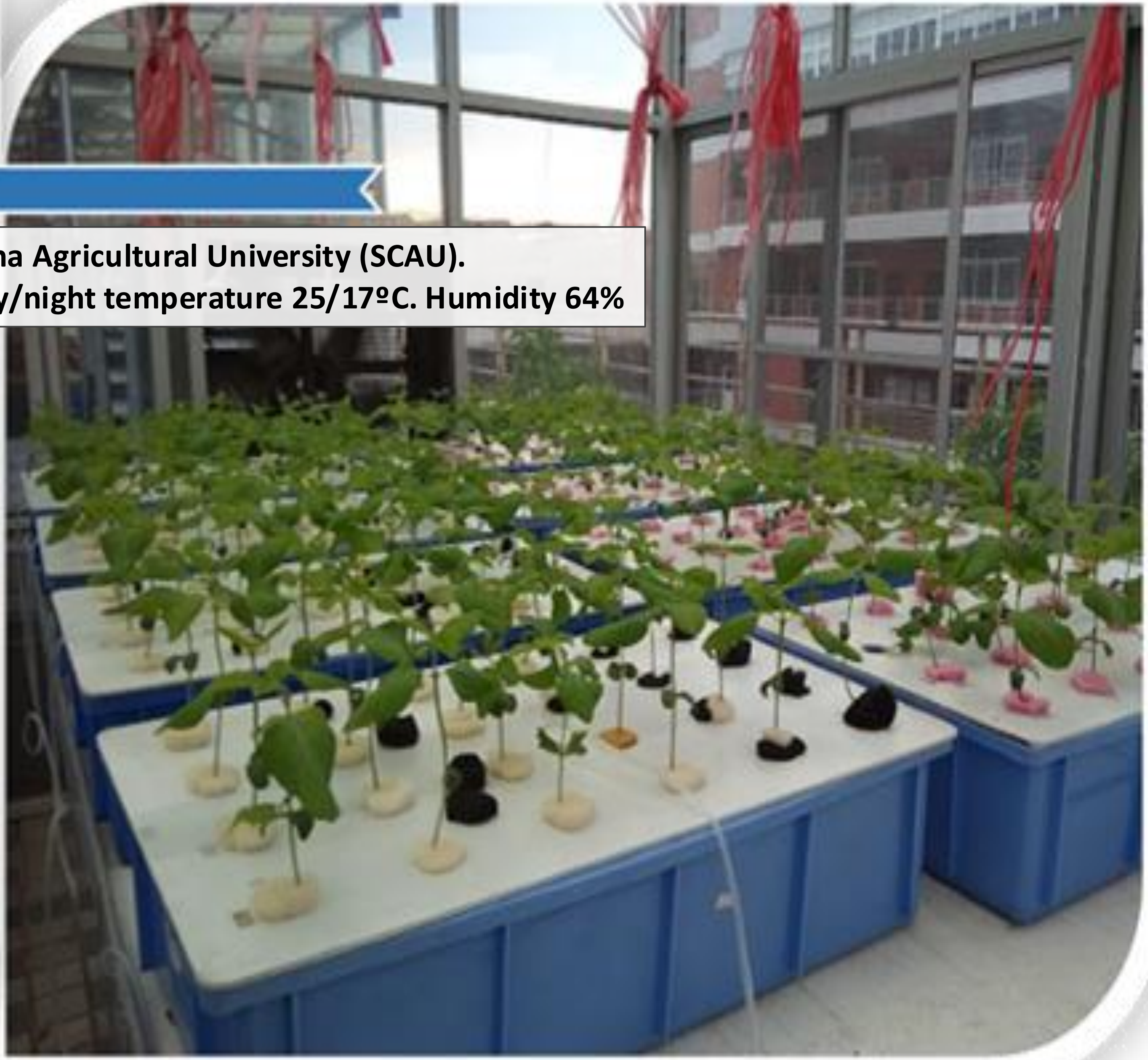
Treatments:

- CONTROL
- Low P (5 μM)
- Moderate Salinity (NaCl 75 mM)
- Severe Salinity (150 mM NaCl) lethal
- Low P (5 μM) + Salinity 75mM NaCl
- Low P (5 μM) + Salinity 150 mM NaCl

FRONTIERS in Plant Science Phosphorus Limitation Improved Salt Tolerance in Maize Through Tissue Mass Density Increase, Osmolytes Accumulation, and Na^{+} Uptake Inhibition Front. Plant Sci., 03 July 2019 https://doi.org/10.3389/fpls.2019.00856

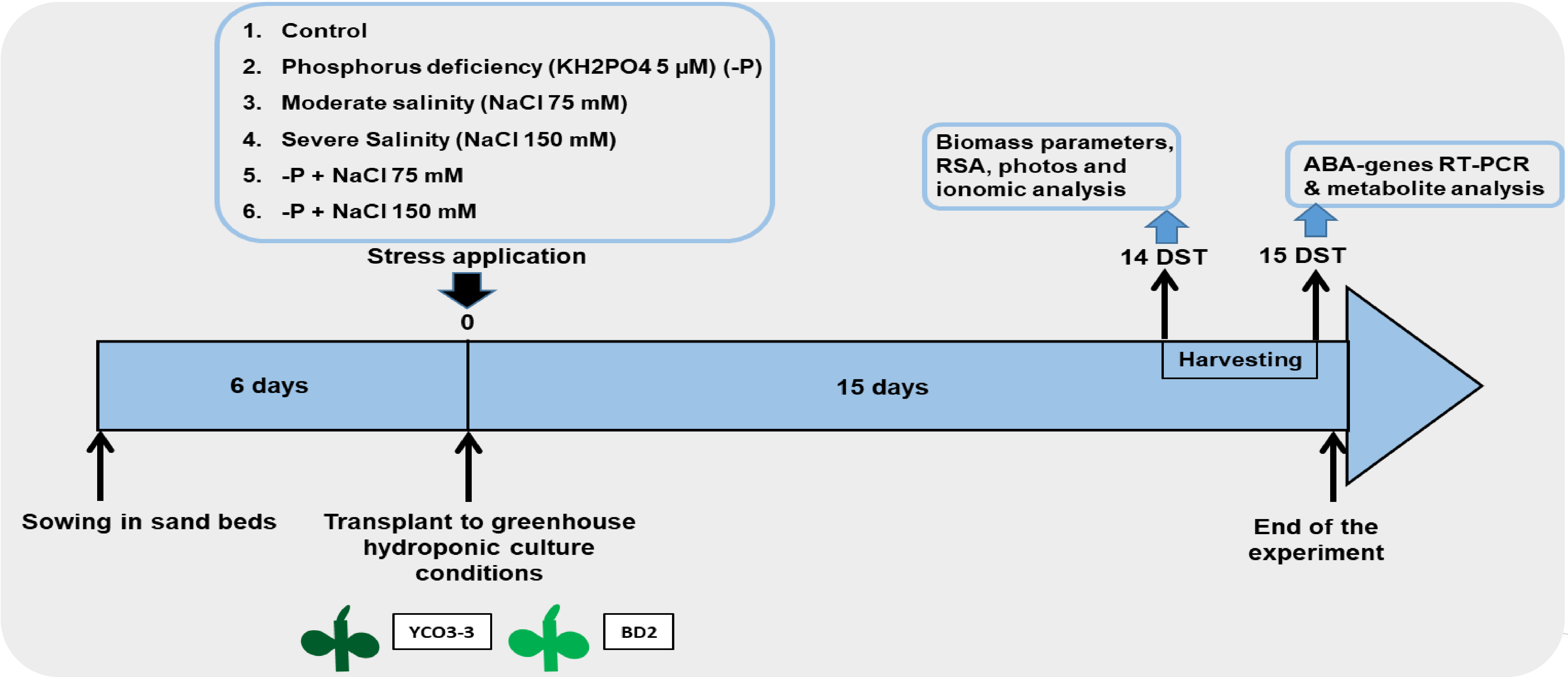
Selecting promising genotypes and understanding the interactions between stress components and underlying adaptive mechanisms, is key to improving crop productivity in P-poor areas affected by salinity and optimizing soil and saline water management.

Experimental location



- Guangzhou, South China Agricultural University (SCAU).
- November, average day/night temperature 25/17ºC. Humidity 64%

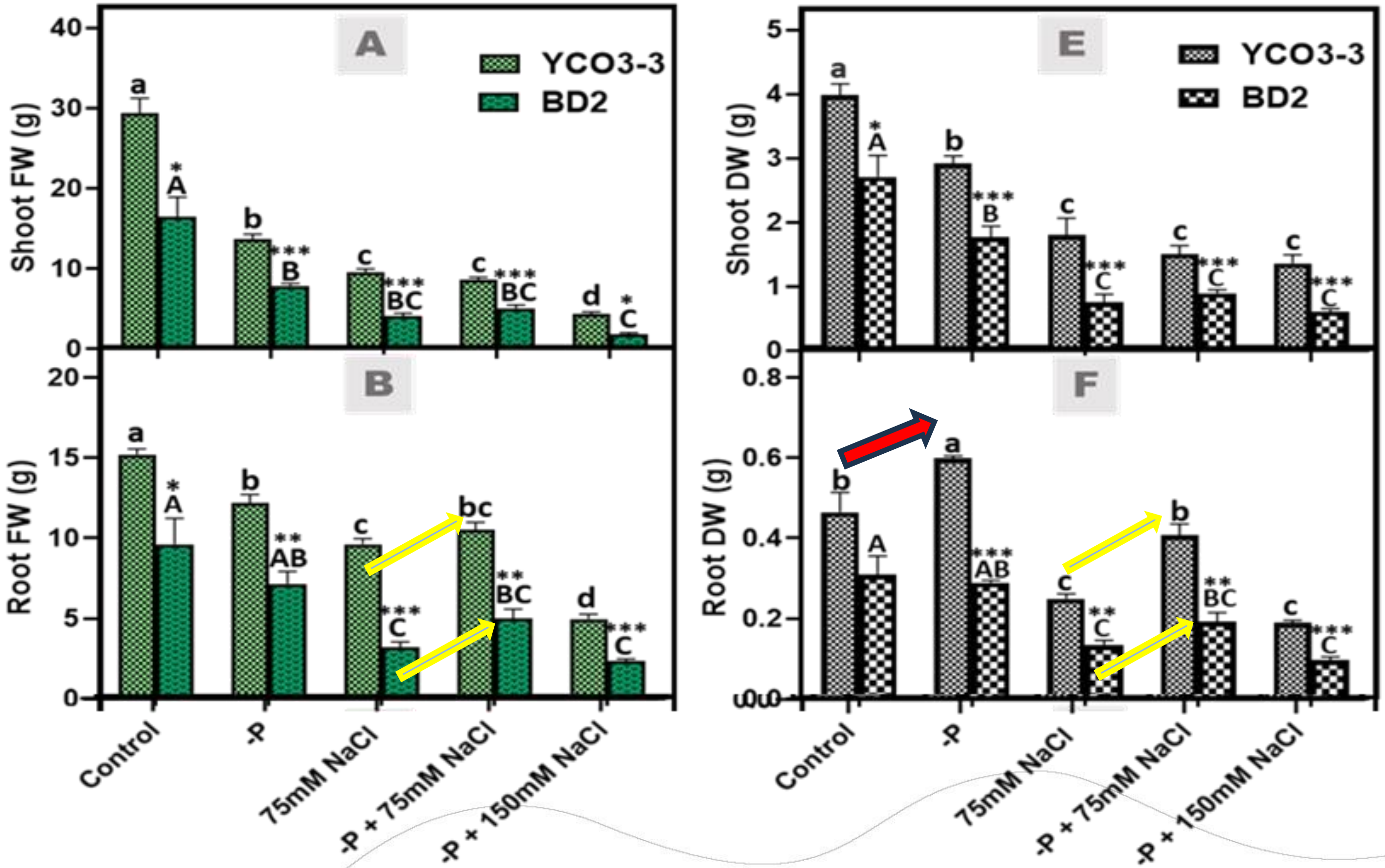
Experimental design



Results

1. Biomass parameters

- YCO33= P-efficient
- BD2= P-inefficient

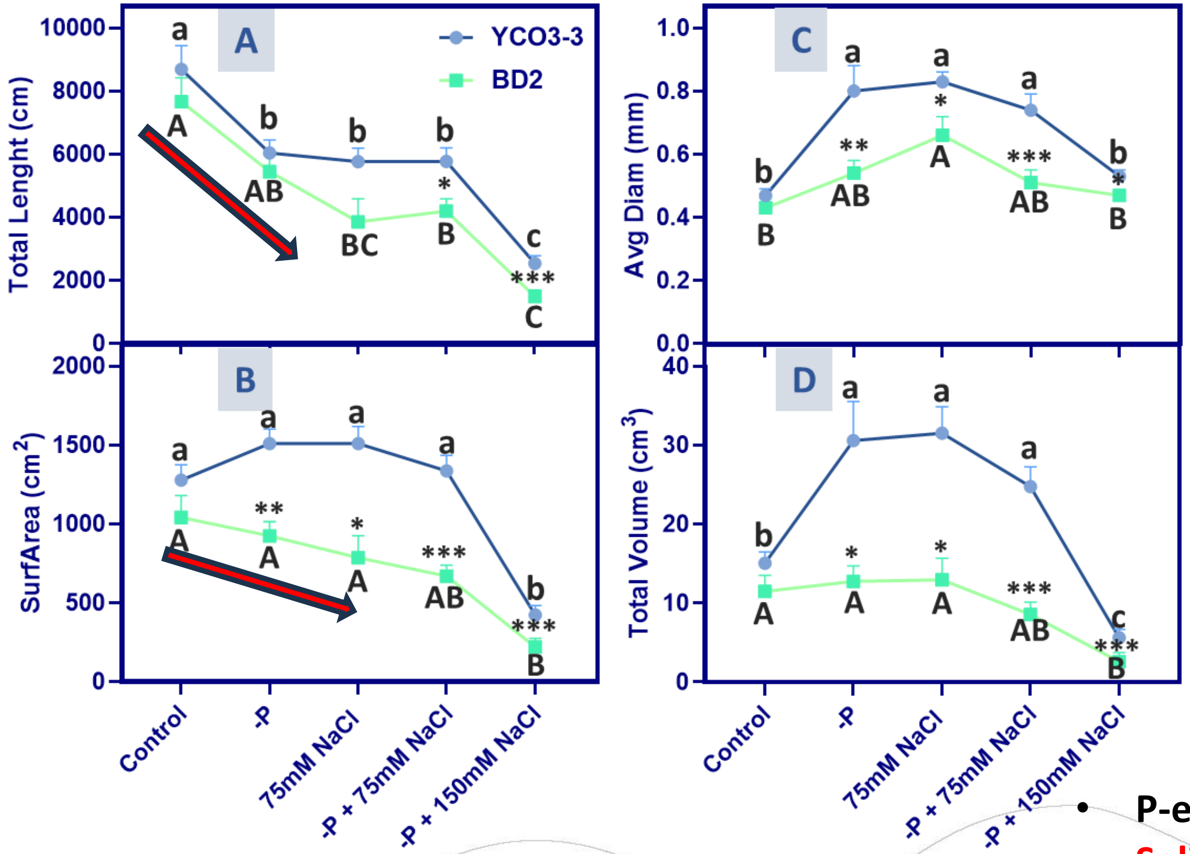


- **In general, P-efficient showed high vigor compared to P-inefficient.**
- **Low P reduced biomass but to a lesser extent than moderate salinity (alone), while its combination alleviated saline stress in both roots.**
- **P-efficient increased RDW in response to –P.**

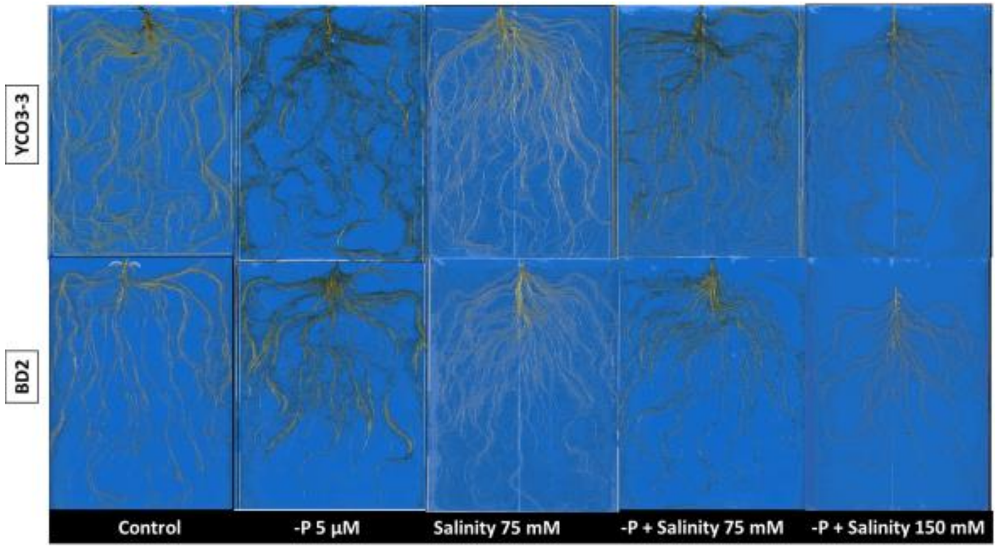
Results

▪ **RSA (Root System Architecture) parameters**

YCO3-3
BD2



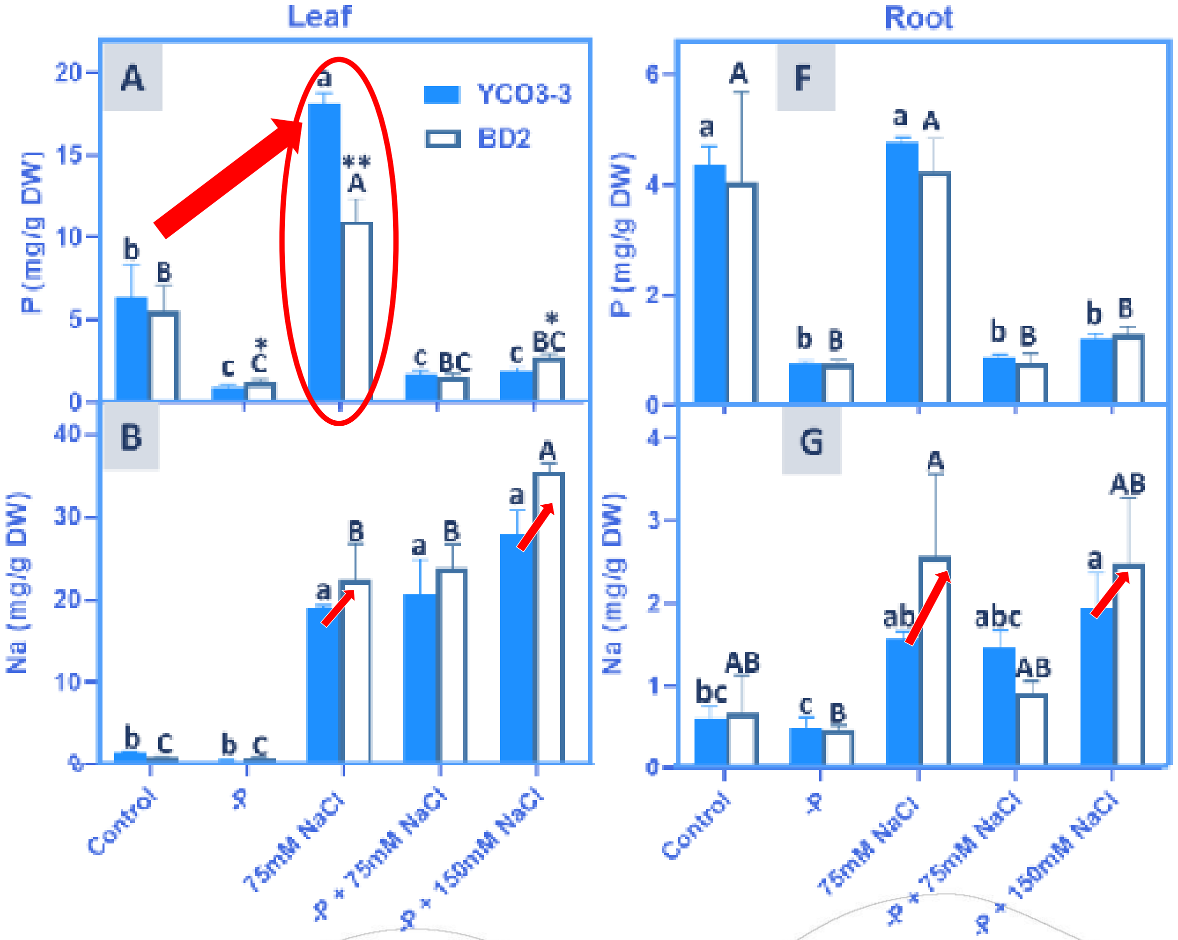
YCO33= P-eficiente
BD2= P-ineficiente



- **P-efficient genotype improved stress adaptation in terms of RSA.**
- **Salinity affected root development more intensely in P-inefficient**

Results

- Na^+ and P concentrations



- **Moderate salinity doubled leaf P in the high vigour YCO3-3 line and less so in BD2.**

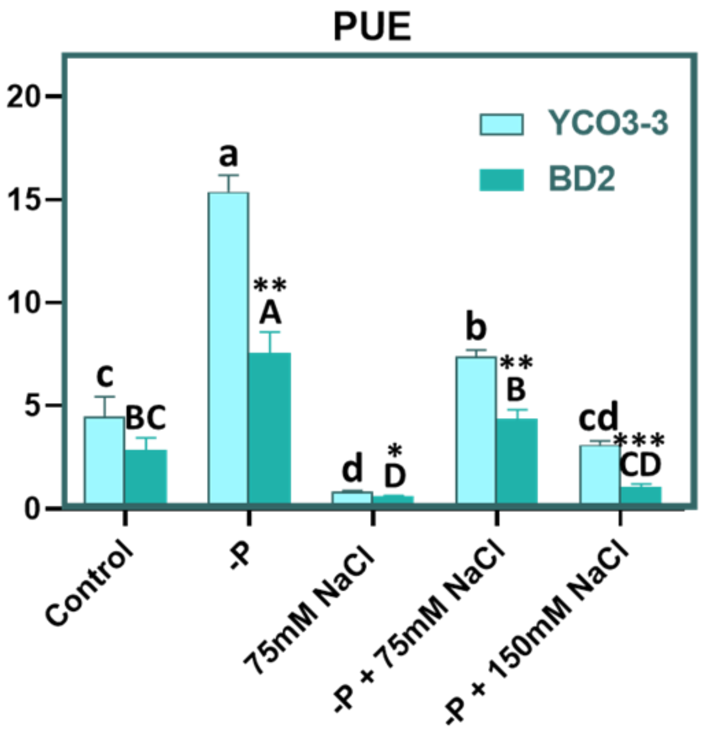
Phosphate transporters (PHTs) are induced in salt-adapted plants (e.g. salt cress, *Eutrema salsugineum)* and confers tolerance to both salinity and P limitation by increasing P uptake and transport to the shoot, and K/Na ratio (Lv *et al.*, 2020).

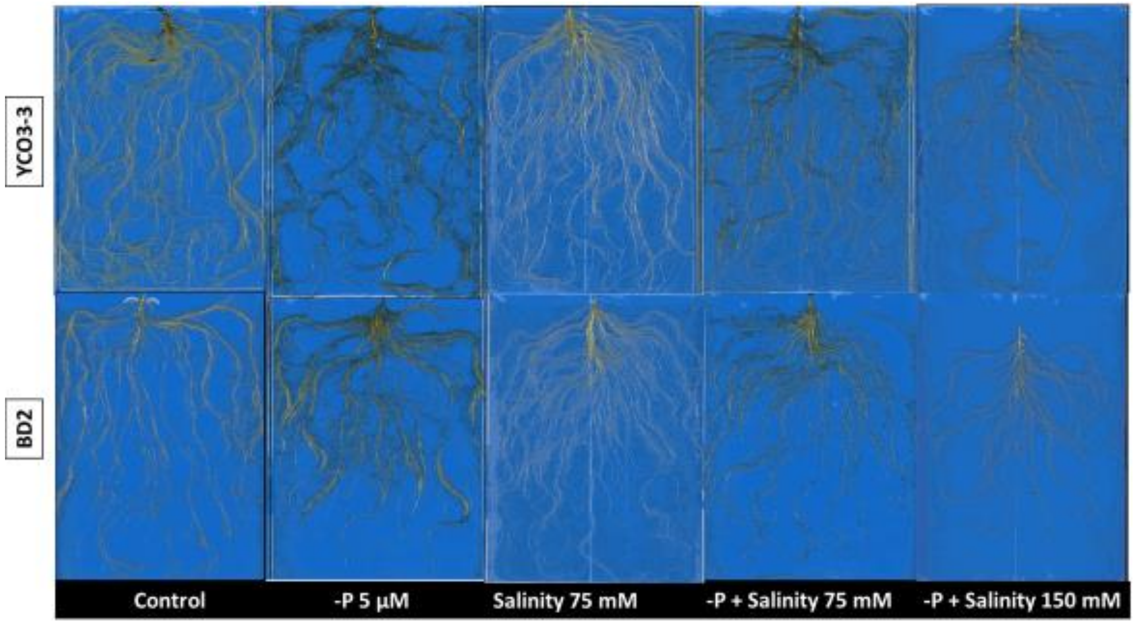
- **Sensible-BD2 tends to accumulate more Na^+ than YCO3-3, although without significant differences**

Results

3. Phosphorus use efficiency (PUE)

YCO33= P-eficiente
BD2= P-ineficiente

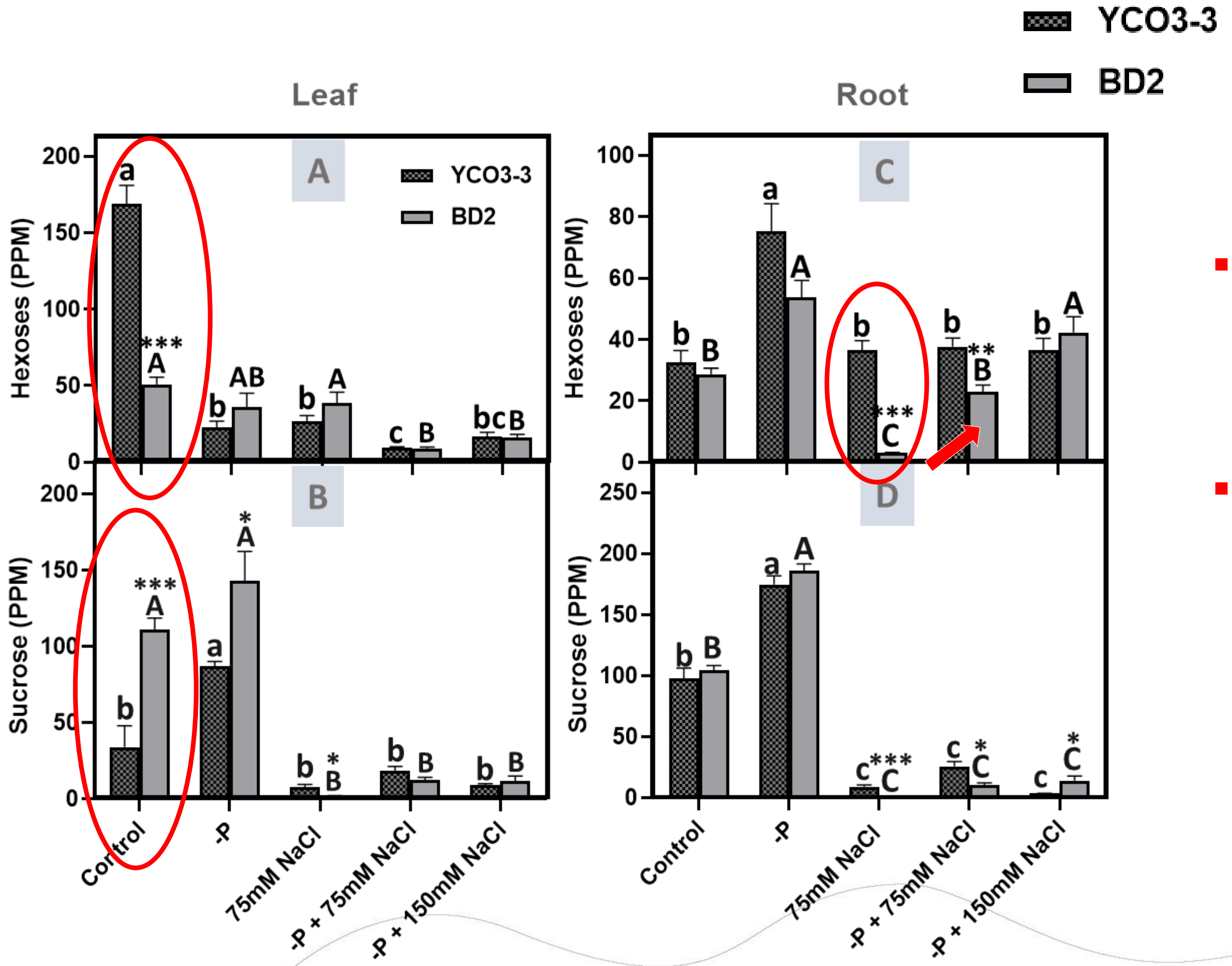




- P-efficient presented a PUE 2-3 times greater than P-inefficient, particularly when P was scarce.
- A high PUE was related to specific root adaptive responses that increased soil exploration volume.

Results

- **photosynthetic assimilates**

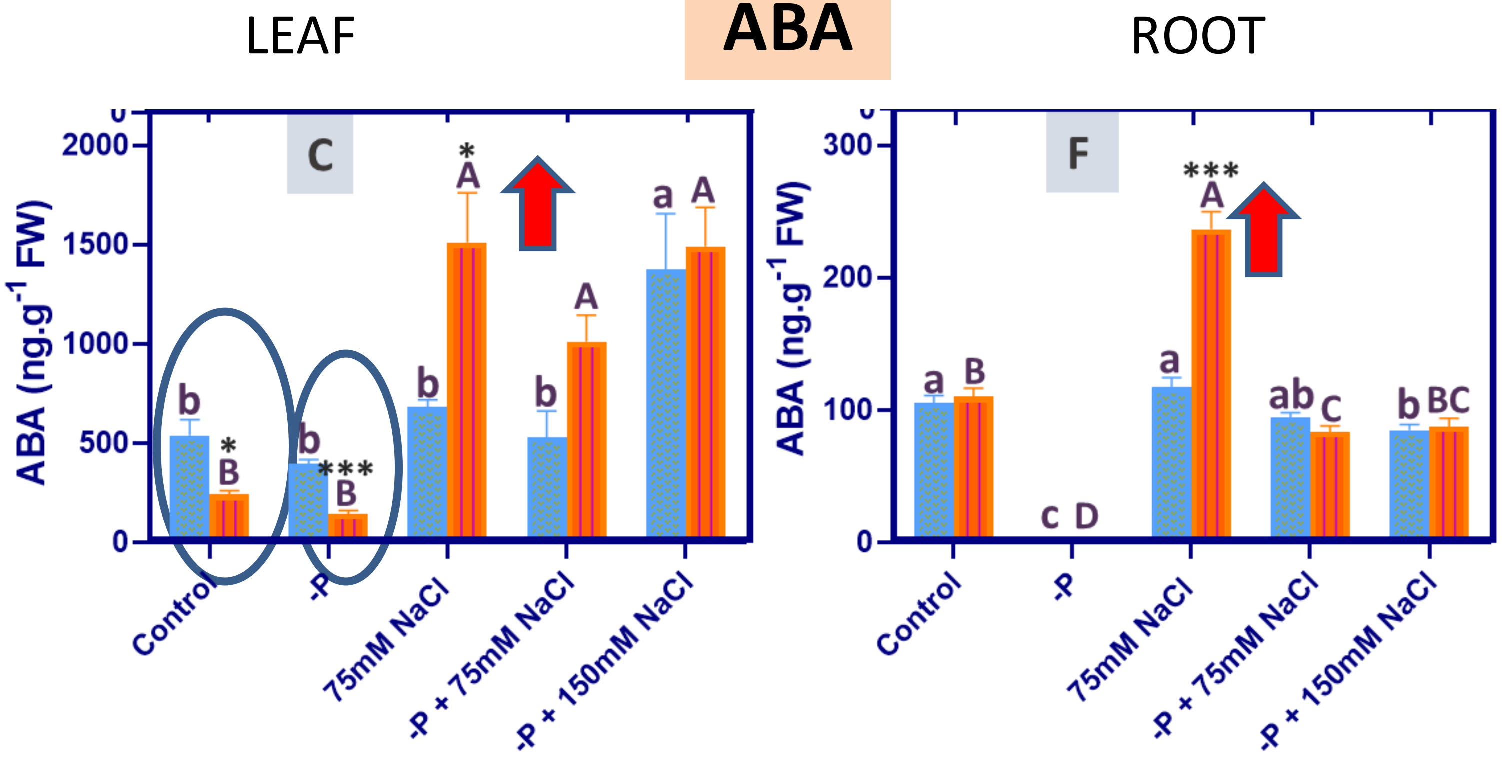


- **In control conditions, YCO3-3 and BD2 accumulate hexoses and sucrose differentially in the leaves.**
- **In roots --> salinity alone does not affect hexoses in YCO3-3, but abolishes them in the sensitive BD2 and is alleviated when combined with low P.**

Results

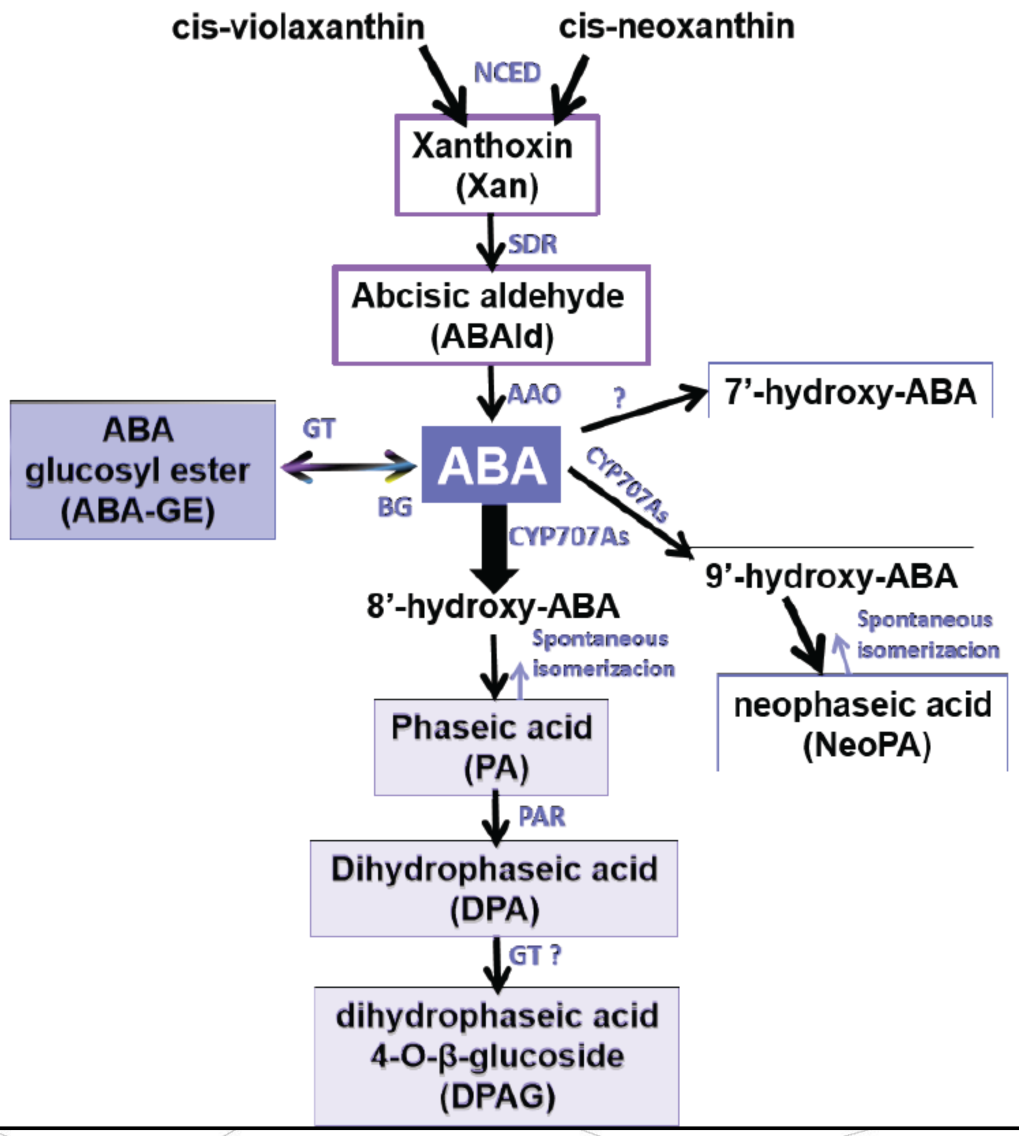
P- efficient vs. P- inefficient

ABA



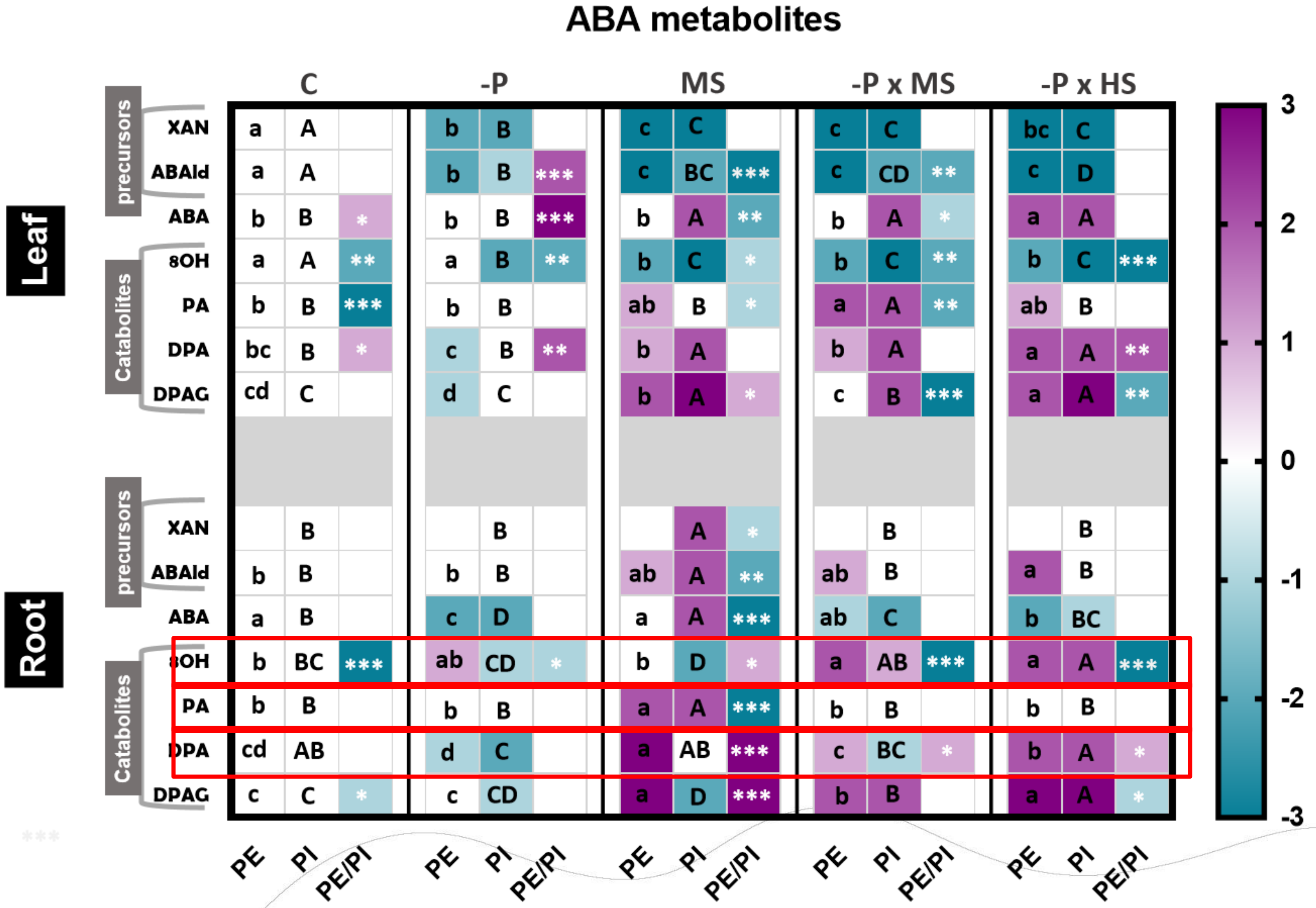
- **Higher constitutive ABA levels in optimal conditions and under low P are related to greater vigor and a more extended root system, observed in P-efficient YCO33.**

- **The strong ABA accumulation in salinity (alone or combined) is related to greater sensitivity to stress, observed in P-inefficient BD2.**


cis-violaxanthin
cis-neoxanthin
NCED
Xanthoxin (Xan)
SDR
Abcisic aldehyde (ABAld)
AAO
?
7'-hydroxy-ABA
ABA glucosyl ester (ABA-GE)
GT
BG
ABA
CYP707As
CYP707As
9'-hydroxy-ABA
8'-hydroxy-ABA
Spontaneous isomerizacion
Spontaneous isomerizacion
neophaseic acid (NeoPA)
Phaseic acid (PA)
PAR
Dihydrophaseic acid (DPA)
GT ?
dihydrophaseic acid 4-O-β-glucoside (DPAG)

Results

P- efficient vs. P- inefficient

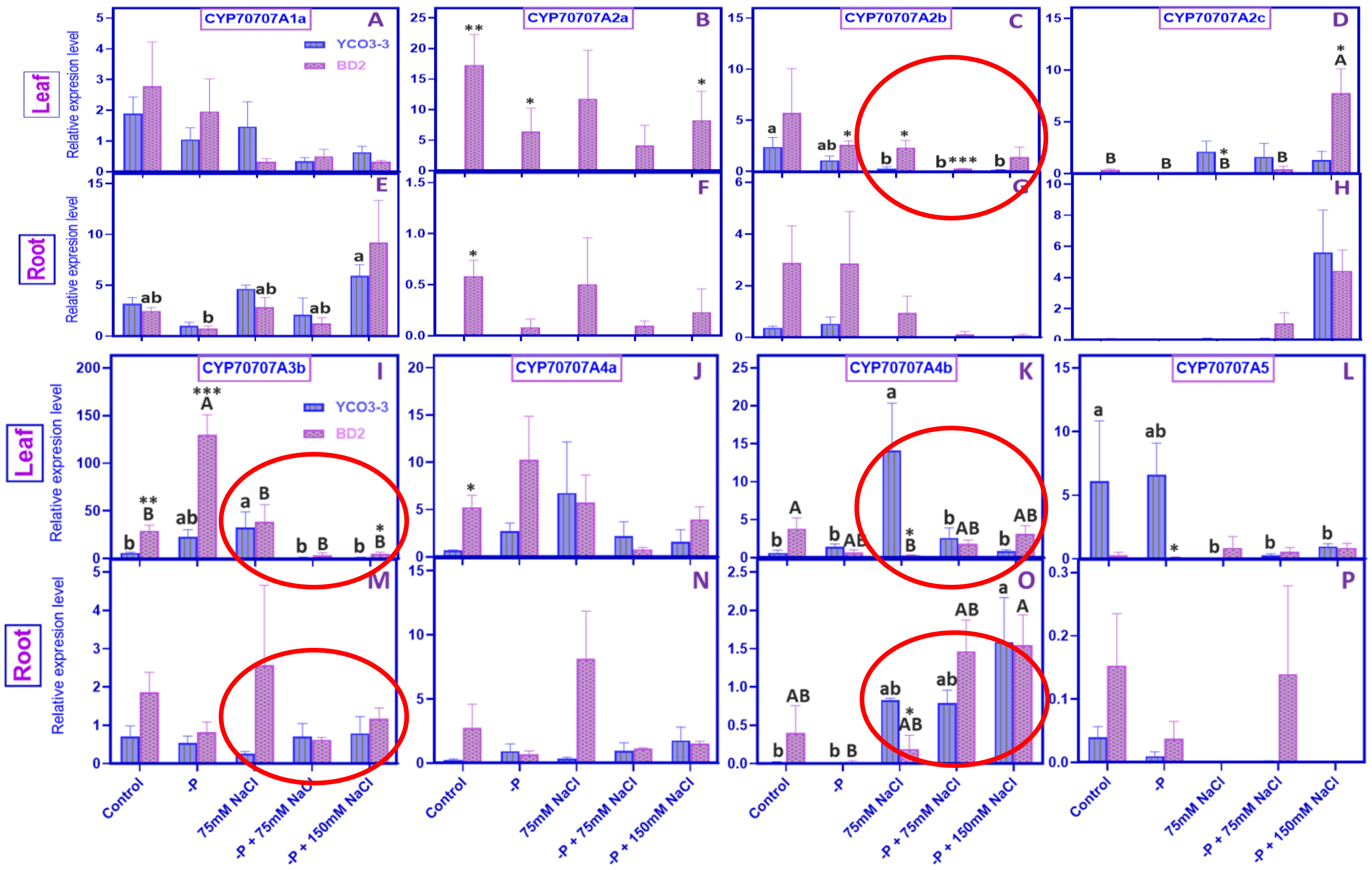


- ABA catabolites are constitutively high in P-inefficient, compared to P-efficient.
- In general, salinity alone or combined reduced ABA precursors and induced ABA catabolism (PA, DPA) although in a higher extent in the P-inefficient genotype.
- Combined stress increases 8-OH-ABA and decreases PA and DPA catabolites in roots, compared to salinity alone.
- ABA hydroxylation is differentially regulated by salinity and salinity-LowP, and between soybean genotypes.

Results

ABA- catabolism gene expression

YCO3-3
BD2

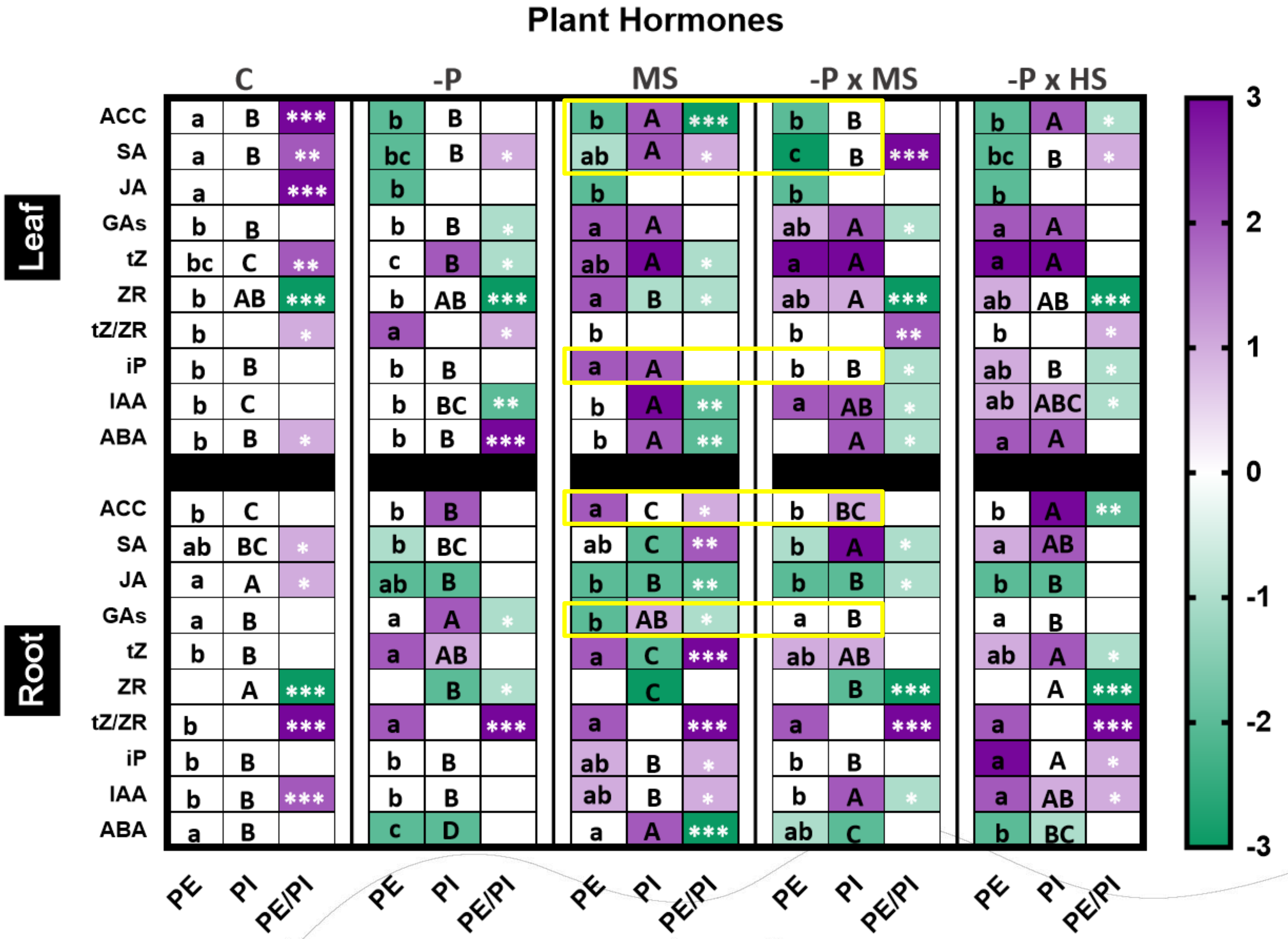


Higher expression of ABA-catabolic genes in P- inefficient explains lower constitutive leaf ABA levels. Salinity induced ABA-catabolic genes, but combined stress reduced some of them compared to salinity alone in a genotype-dependent way.

GLOBAL SOIL PARTNERSHIP

Results

P- efficient vs. P- inefficient



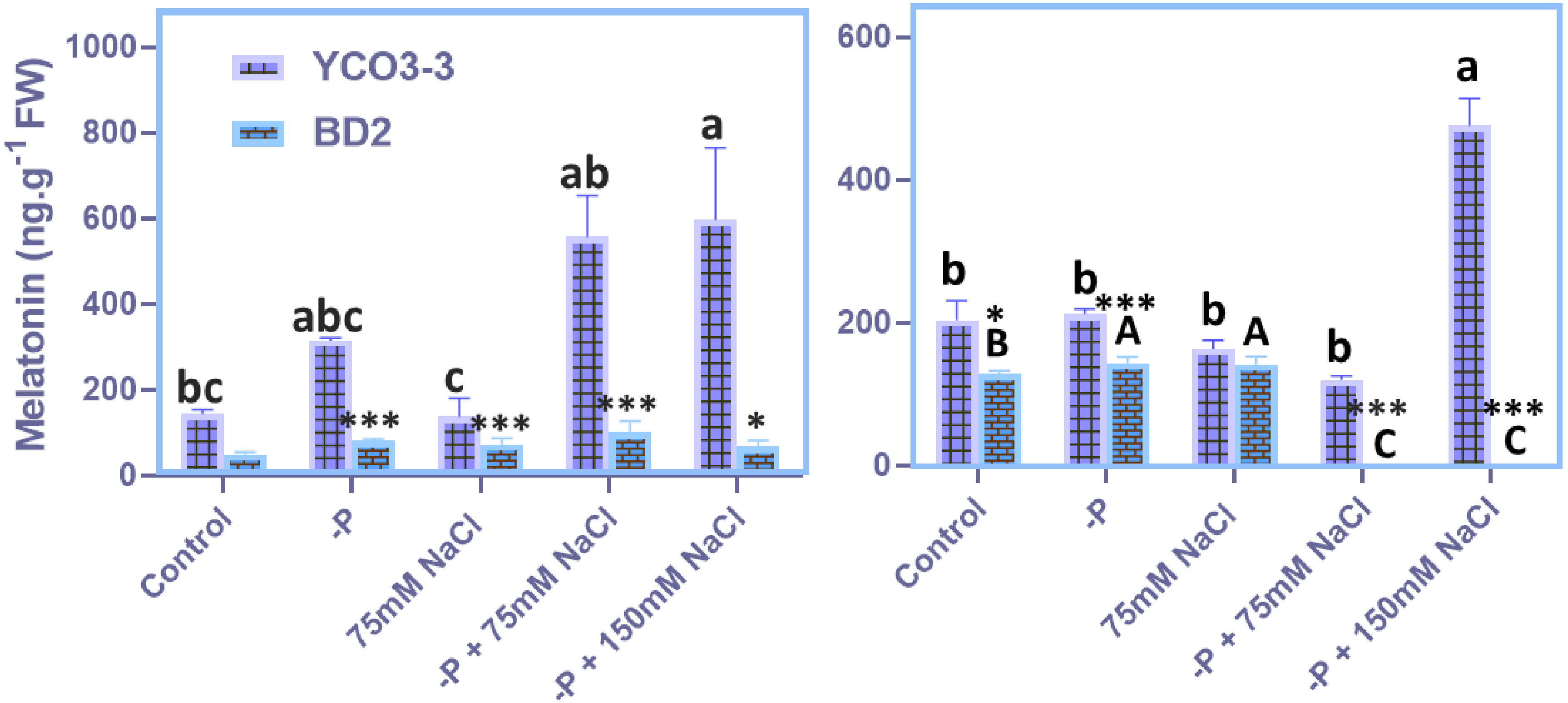
- Combined –PxMS stress alleviates the accumulation of the ethylene precursor ACC, SA and iP compared to MS alone.
- Combined stress recovered GA levels in roots vs salinity.
- The responses are genotype-dependent.

Results

P- efficient vs. P- inefficient

Melatonin

Leaf **Root**



- P-efficient (YCO33) increased melatonin in leaves under Low P and this response was intensified in combination with salinity revealing itself as an important metabolite for adaptation to for adaptation to soil P deficiency.

Conclusions

- **Salinity promoted P uptake, while Low-P alleviates salinity stress, avoiding lethality and recovering growth, initially in the roots.**
- **Low-P x MS reduces ethylene evolution and leaf iP response (likely involved in shoot-to-root communication) and recover GA levels in the roots, therefore alleviating salt stress.**
- **Melatonin is revealed as an important molecule in the adaptive response to low P, which is intensified by salinity, and could improve tolerance to P deficit and alleviate salinity by protecting leaves and promoting root growth through its auxinic effect.**
- **Higher constitutive levels of ABA coincide with greater vigour and a more extended root system in the absence of stress and low P, while under salinity (alone or combined) the strong accumulation of ABA, its metabolites, and the expression levels of the ABA genes are related to an increased sensitivity to stress.**
- **Differential constitutive ABA levels may condition soybean stress sensitivity, which depends on ABA catabolism.**
- **Low-P x MS alters different metabolic ABA traits, particularly ABA oxidation, explaining genotypic responses to stress, which deserve further investigation.**

Project funded by the European Union

CEBAS-CSIC
Centro de Edafología y Biología Aplicada del Segura

Lancaster University

South China Agricultural University 1909

SEW-REAP: a large research community working across the food-agriculture-water-environment nexus

Food and Agriculture Organization of the United Nations

Thank you

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Projects:

With the financial support of

Australian Government
Department of Agriculture, Water and the Environment

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Funded by the European Union

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

SUSTAIN
Sustainable use of salt-affected lands

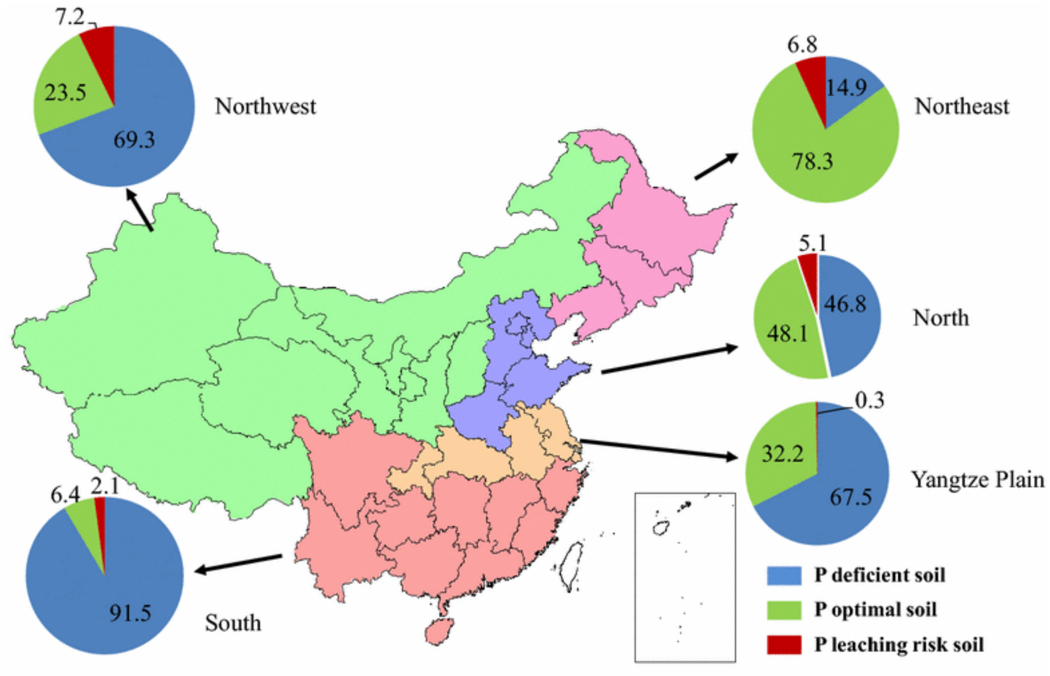
Vniversitat de València

International Union of Soil Sciences

Global Soil Partnership

Ministry of Agriculture, Nature and Food Quality of the Netherlands

Soil phosphorus deficiency combined with salinity is a major threat to food production, especially in soybean-producing areas.

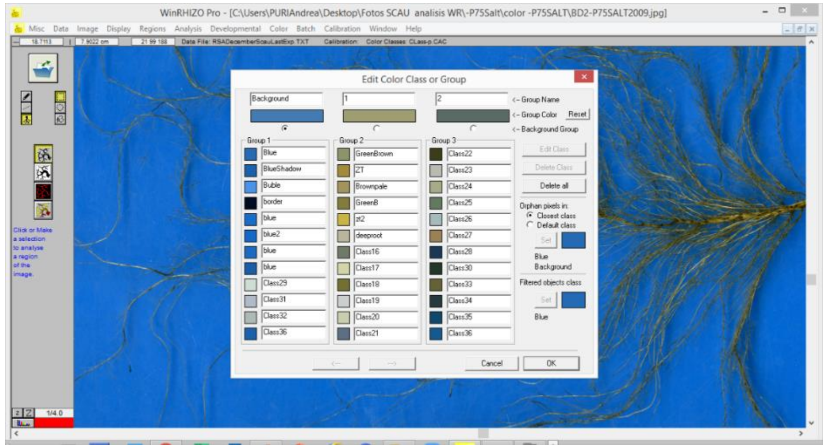


Proportions (in %) of arable land with different soil Olsen P status in the Northeast, Northwest, North, Yangtze Plain, and South P management regions of China

Salt-tolerant soybean variety (top) and salt-sensitive soybean variety (bottom) grown with 200 mM NaCl for 18 days (source: Guang et al., 2014) (source: Guang *et al.,* 2014)

Material and methods

WinRHIZO™



1. Biomass parameters

2. RSA (Root System Architecture) parameters

3. P use efficient (PUE)

- **Phosphorus Use Efficiency of (PUE) was calculated as the total biomass generated per unit of phosphorus assimilated.**

$$PUE = \frac{\mathbf{TFW\ (g)}}{[P]_{root\ (mg/g\ DW)} + [P]_{Leaf\ (mg/g\ DW)}}$$

U-HPLC-HRMS

4. Plant hormones, ABA-metabolites and auxins derivatites levels

Material and methods

5. Relative Expression Level of ABA-genes in the third trifoliate leaf & root samples

Quantitative real-time PCR

Primers Desing

9-*cis*-epoxicarotenoide dioxigenasa (NCED)
ABA Biosynthesis

GmEF-1a

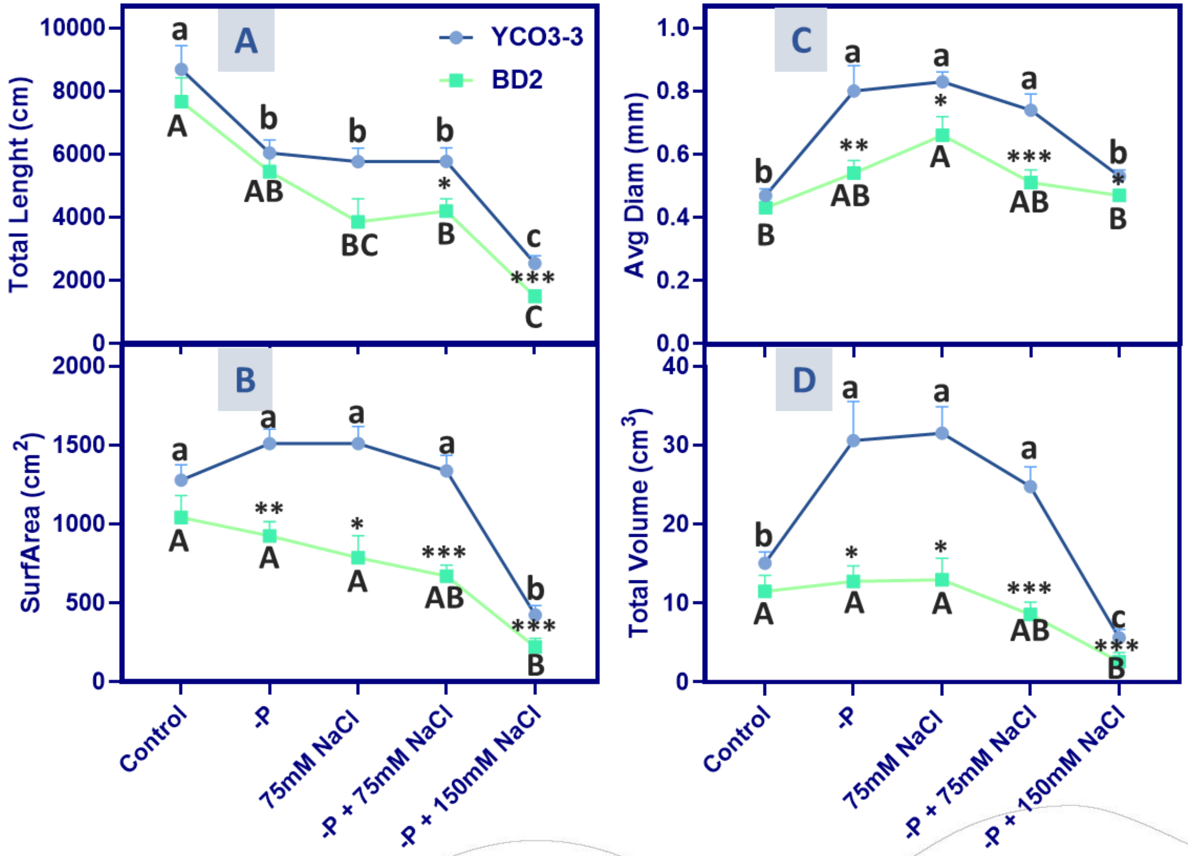
Housekeeping-gene control, to normalize the expression of the corresponding genes in soybeans

GmCYP70A genes
ABA Catabolism

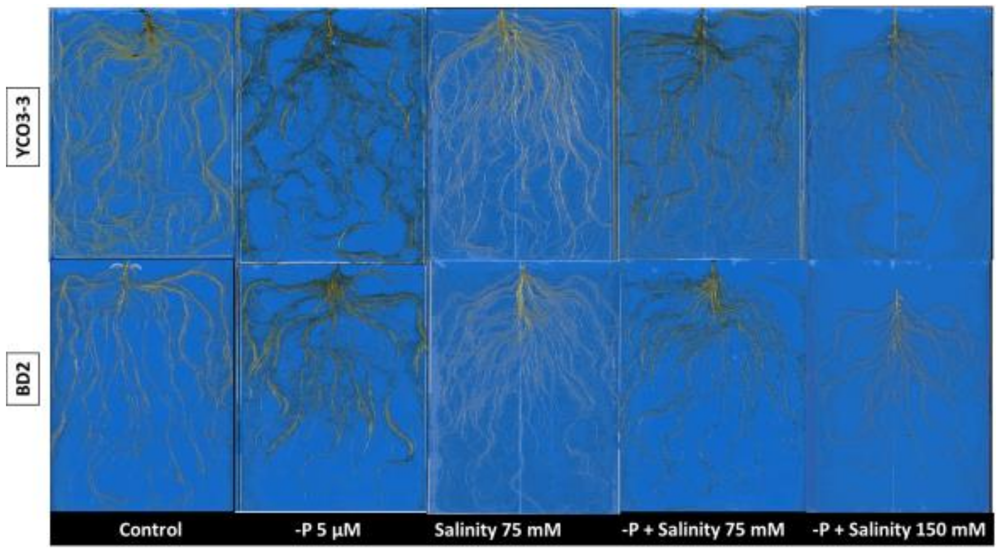
Gene name	Alias (Phytozome website)	Forward (F) & Reverse (R) Primers	Description
Pathway: ABA biosynthesis			
NCED3	Glyma08g18801.1 Glyma.08G176300	F: CTCCTCCTCTTCTACGCCCG R: TAAGTCGCCGTTGGGTGTTA	9-CIS-epoxicarotenoid dioxygenase, NCED3, Chloroplastic-Related
NCED2	Glyma08g28391.1 Glyma.08G255400	F: TCGCTTCTCCCTCTGAAACAA R:TCGTGGGAGACCAAGCCTAT	9-CIS- epoxicarotenoid dioxygenase, NCED2, Chloroplastic-Related
NCED	Glyma05g27250.1 Glyma.01G154900	F: AAACCTCAATTCCATCACCCTCTCT R: TGGAGTGTTTTTGTGAAGGGTTG	9-cis-epoxycarotenoid dioxygenase / VP14 // Zeaxanthin 7,8-dioxygenase
Pathway: ABA catabolism "Phaseic acid biosynthesis" (This family of genes "Glycine max CYP707A" was previously identified and analyzed in response to salinity stress in Zhen *et al.* 2012).			
GmCYP707A1a	Glyma09g35250 Glyma.09G218600	F: ACACACCTATTACAAGTCAAGCCA R: CATCACGCAGGGACAACCTA	
GmCYP707A2a	Glyma16g08340 Glyma.16G076600	F: GATTTGAGGTTGCTCCAAAACCC R: AGTTGCTTCCATGCCTTGATT	
GmCYP707A2b	Glyma16g20490 Glyma.16G109300	F: CCATTGCCCAAAGTTGCCTC R: CATCGCTTCAGATCCTCCCC	Abcisic Acid 8'-hydrolase
GmCYP707A2c	Glyma17g14310 Glyma.17G133900	F: AATCACTCGCCAAATCCTTCC R: CCCCAACATCCTCTCTTTGCT	
GmCYP707A3b	Glyma17g36070 Glyma.17G242200	F: AACAGGGGTGTGGTGCTTTA R: AATTACACGCACACAACTTTACT	
GmCYP707A4a	Glyma02g14920 Glyma.02G132200	F: CCTCCCACACCCATGTTTCT R: CGAGTGCGGGTTATGTGGAA	
GmCYP707A4b	Glyma07g33560 Glyma.07G212700	F: GCCATCGAAGCAAAAACGCA R: CACACACACAAGCGTAGCAA	ABA 8'-hydroxylase; (+)-abscisic acid 8'-hydroxylase
GmCYP707A5	Glyma09g41960 Glyma.09G282900	F: TCGTGGACTTTCGTGGGATG R: CTGCCTGGACAAGAGTGGAC	PTHR24286:SF86 - ABSCISIC ACID 8'-HYDROXYLASE 2

Results

2. RSA (Root System Architecture) parameters



YCO33= P-eficiente
BD2= P-ineficiente

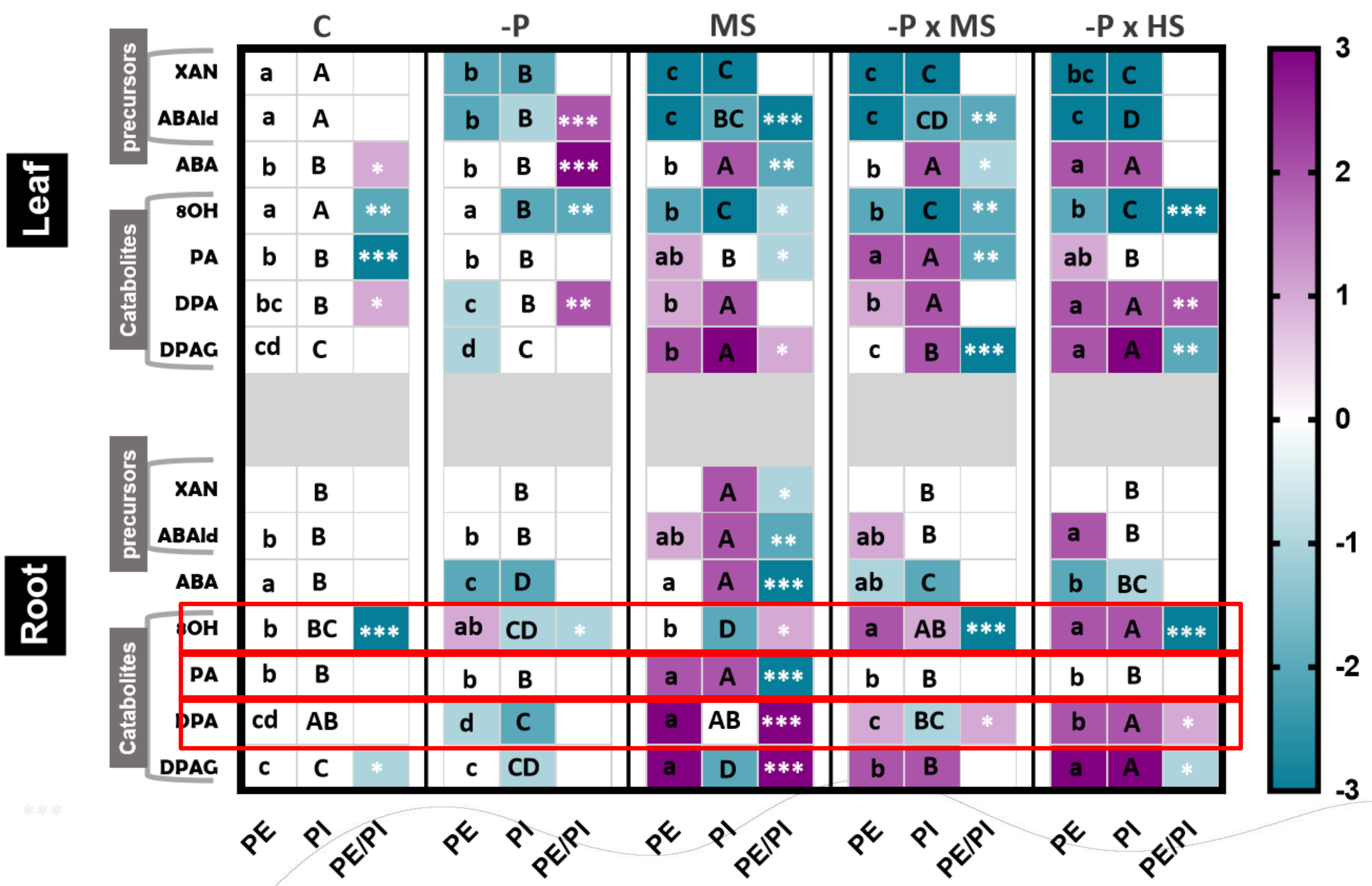


- **P-efficient genotype improved stress adaptation in terms of RSA.**

Results

P- efficient vs. P- inefficient

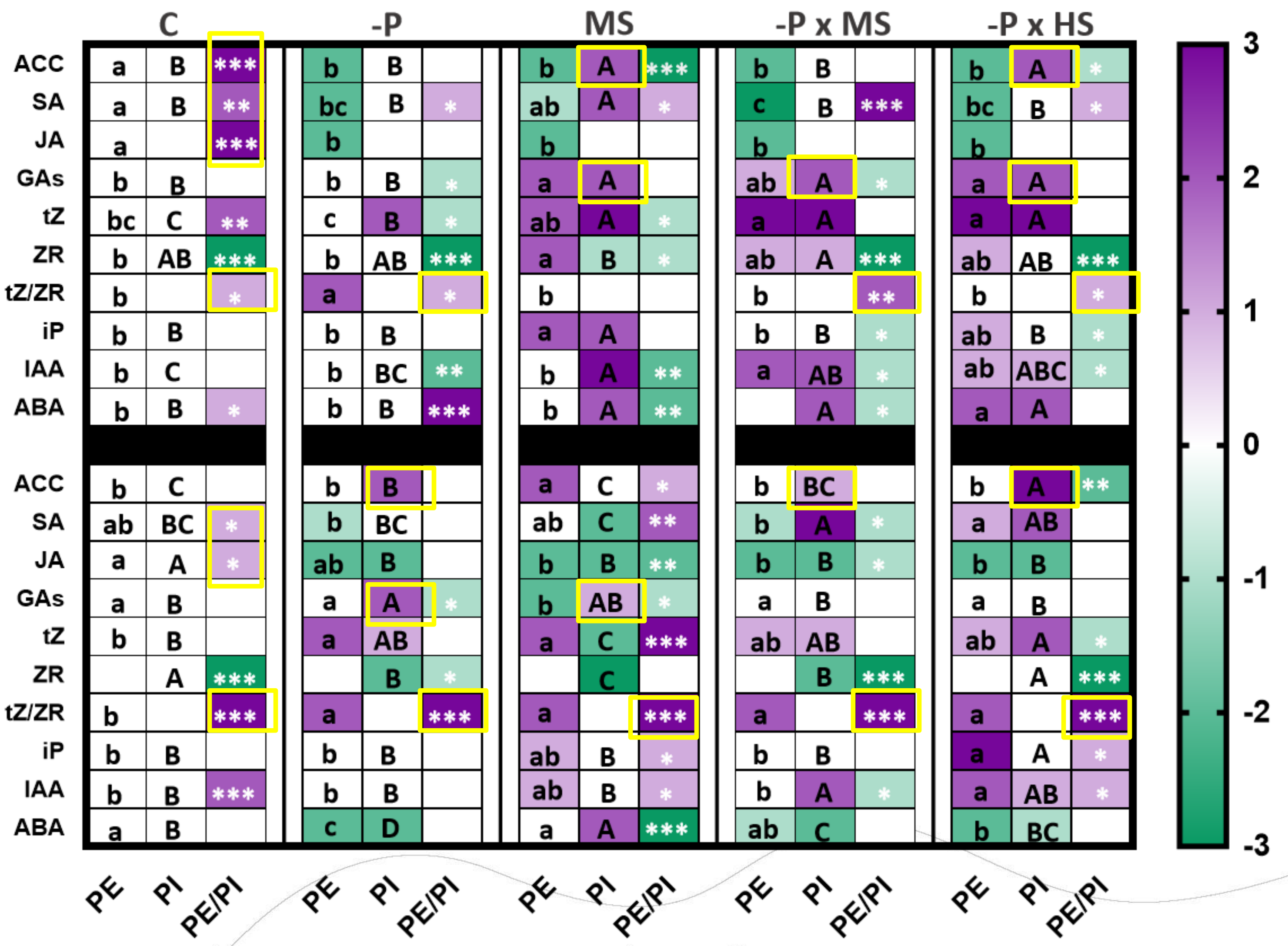
ABA metabolites



- P-efficient presented higher constitutive levels, while in P-inefficient the catabolism of the ABA was constitutively induced.
- Low-P affected both genotypes, although the leaf ABA levels remained higher in the P-efficient line.
- In general, salinity alone or combined reduced anabolism and induced the catabolism of ABA although P-efficient was less sensitive.
- P-inefficient accumulated ABA in response to salinity alone (leaf and root) or combined (sheet) while p-efficient only did it under the high salinity levels.
- ABA levels seem to be controlled by a blockade in ABA oxidation at 8-OH -ABA (main ABA catabolite).
- Combined stress decreases PA and DPA and increases 8-OH-ABA, compared to salinity alone.

Results

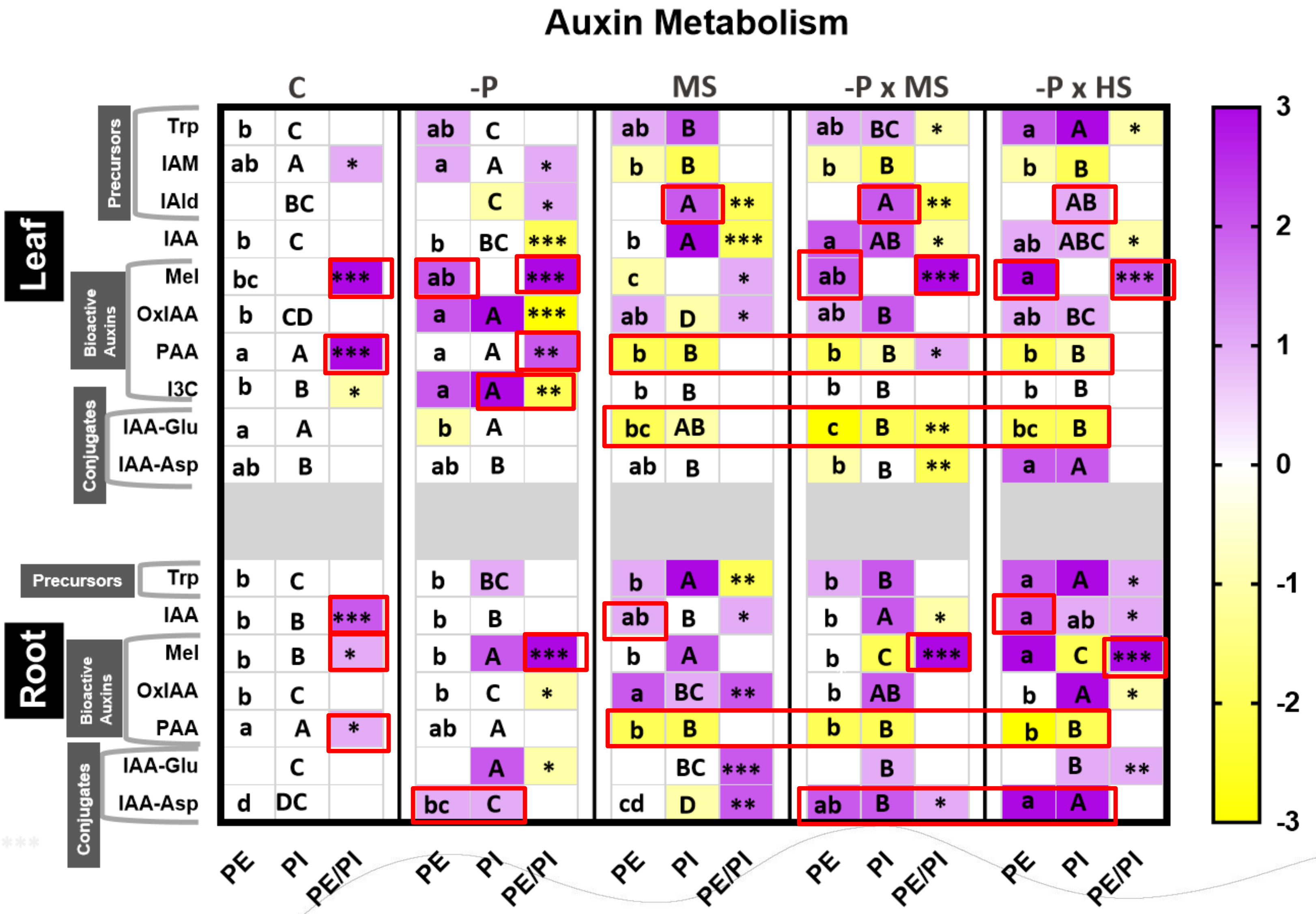
P- efficient vs. P- inefficient



- Higher constitutive levels of ACC, SA and JA in p-efficient could explain the differences in vigor between both genotypes.
- The increase of ACC in the roots and leaves together with the accumulation of gibberellins can help explain the greatest sensitivity of BD2 to the applied stress.
- The high tZ/ZR ratio in p-efficient vs. inefficient is an indicator of the growth of soy plants in any condition.

Results

P- efficient vs. P- inefficient



- Without stress, P-efficient constitutively presented much higher levels of IAA (root), PAA and melatonin (root and leaf) than P-inefficient.
- IAA levels were sensitive to single and combined salinity, accumulating more in the leaves of the more sensitive genotype BD2 and in the roots of the tolerant YCO33.
- I3C was induced by low-P in leaves, but more strongly in the sensitive BD2 genotype.
- Phenylacetic acid (PAA) levels were higher in leaves of the high vigor/high PUE genotype YCO3-3 under control and low P, but were reduced under single and combined salinity to similar levels as BD2.
- In relation to IAA conjugates, in both genotypes IAA-Glu decreased in leaves under salinity alone or combined, while IAA-Asp increased in roots in response to low P and to a greater extent in its combination with salinity.
- P-efficient accumulated more melatonin in leaves and roots than P-inefficient in all conditions. Melatonin levels increased in P-efficient leaves in response to low P and more intensely in combination with salinity, but not under salinity alone.

Conclusions

- High PUE is related to specific root adaptive responses that increase the exploring volume of soil for P uptake, and perhaps also to singular P transport systems.

- The ACC levels in roots and leaves together with the accumulation of gibberellins might be indicators of sensitivity to low P and salinity, while high tZ/ZR is rather an indicator of tolerance and vigour.

- Melatonin is revealed as an important molecule in the adaptive response to low P, which is intensified by salinity, and could improve tolerance to P deficit and alleviate salinity by protecting leaves and promoting root growth through its auxinic effect.

- Differential constitutive ABA levels may condition soybean stress sensitivity or immunity responses. Higher constitutive levels of ABA coincide with greater vigor and a more extended root system in the absence of stress and low P, while under salinity (alone or combined) the strong accumulation of ABA, its metabolites, and the expression levels of the ABA genes are related to an increased sensitivity to stress. Different metabolic ABA traits, particularly in ABA oxidation, could explain the differential responses to stress, which deserve further investigation.