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National University of Córdoba

### LECTURE 1-PLANT ENVIRONMENT. PLANT STRESS RESPONSES: OXIDATIVE STRESS AND SENESCENCE

LECTURE 2-PLANT ENVIRONMENT. LEGUME-RHIZOBIA SYMBIOTIC INTERACTION

LECTURE 3-PLANT ENVIRONMENT. AUTOPHAGY IN PLANTS: ONE PROCESS, MULTIPLE PHYSIOLOGICAL FUNCTIONS







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### LECTURE 1-PLANT ENVIRONMENT. PLANT STRESS RESPONSES: OXIDATIVE STRESS AND SENESCENCE

- Argentina's agriculture
- National Institute of Agricultural Technology (INTA)
- Plant stress responses: oxidative stress and senescence



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### Argentina



### NATIONAL UNIVERSITY OF CÓRDOBA (1613)



Faculties	15
High Schools	2
Institutes and Research Centers	145
Libraries	25
Museums	17
Radios / TV channels	4
Hospitals / Laboratories / Blood Bank	4



### Argentina: 2.78 millions km<sup>2</sup> Population: 45 millions





#### **PRODUCTION OF THE AGRICULTURAL SECTOR (2014/2015)**

Product	Production	
Grains	122,4 million tonnes	
Citrics	2,6 million tonnes	
Wine	13,4 million hectoliters	
Beef	3 million tonnes	
Poultry	2 million tonnes	
Pig	441 thousand tons	
Bovine Milk	11 billion litres	
Biofuels	3.900 million litres	

21.170.000 ha oilseed crops 16.120.000 ha cereals



#### **PRODUCTION OF THE AGRICULTURAL SECTOR**

Argentina produces enough *food* for 450 million people (10 times the Argentine population)

However, almost four million of our own citizens face serious food insecurity...



#### **PRODUCTION OF THE AGRICULTURAL SECTOR (2014/2015)**

Journal of Social and Development Sciences (ISSN 2221-1152) Vol. 7, No. 2, pp. 79-87, June 2016

Organics Olympiad 2016: Global Indices of Leadership in Organic Agriculture

MEDAL	Country	Statistic
Gold	Australia	17,150,000 hectares
Silver	Argentina	3,061,965 hectares
Bronze	USA	2,178,471 hectares

Table 1: Organic agriculture hectares(Willer & Lernoud, 2016).

#### **Organic agriculture is reported as just 0.99% of world agriculture**

John Paull, 2016. Journal of Social and Development Sciences



Japan International Research Center for Agricultural Sciences



"Promote innovation and contribute to the sustainable development of the agri-food system"

3.00% 4.72% The second of the second second

### **National Institute of Agricultural Technology (INTA)**



### **National Institute of Agricultural Technology (INTA)**







# How do plants respond to stress conditions, and how do these affect plant growth and yield?



of Córdoba





#### PLANT ENVIRONMENT WHY STUDING THE RESPONSES OF PLANTS TO STRESS?

Stress conditions are the main factor affecting plant growth and productivity

**Plants are sessile organisms** 







#### Even in the most adverse conditions...







#### **PLANT ENVIRONMENT**

#### **HOW DO PLANTS RESPOND "PROPERLY" TO THE ENVIRONMENT?**



### HOW DO PLANTS RESPOND TO THE ENVIRONMENT? FROM CELL BIOLOGY TO PLANT PHYSIOLOGY

Environmental conditions are sensed and transformed by the plants into biochemical signals. For example, hervivore attack...



Toyota et al. 2018. Science

### HOW DO PLANTS RESPOND TO THE ENVIRONMENT? FROM CELL BIOLOGY TO PLANT PHYSIOLOGY

Environmental conditions are sensed and transformed by the plants into biochemical signals.



#### OXIDATIVE STRESS AND ACCELERATED SENESCENCE: A COMMON FEATURE OF STRESS CONDITIONS



#### **REACTIVE OXIGEN SPECIES (ROS)**



Different energy and reduction states of the oxygen molecule

Because of their oxidative capacity, ROS are toxic molecules



Stage of the ontogeny of the plant that culminates in the death of a part of the plant (leaves, roots, flower parts, tissues of ripening fruits) or the whole organism





Stage of the ontogeny of the plant that culminates in the death of a part of the plant (leaves, roots, flower parts, tissues of ripening fruits) or the whole organism

<u>Highly regulated process</u> characterized by the disassembly and remobilization of components of cellular structures



Pottier et al. (2014) Frontiers in Plant Science

Stage of the ontogeny of the plant that culminates in the death of a part of the plant (leaves, roots, flower parts, tissues of ripening fruits) or the whole organism

Highly regulated process characterized by the disassembly and remobilization of components of cellular structures

#### **SENESCENCE IS A YIELD LIMITING FACTOR**



Stage of the ontogeny of the plant that culminates in the death of a part of the plant (leaves, roots, flower parts, tissues of ripening fruits) or the whole organism

Highly regulated process characterized by the disassembly and remobilization of components of cellular structures

### SENESCENCE IS A YIELD LIMITING FACTOR

- Accelerated Senescence

Reduction of the total capacity to assimilate CO<sub>2</sub>



Stage of the ontogeny of the plant that culminates in the death of a part of the plant (leaves, roots, flower parts, tissues of ripening fruits) or the whole organism

Highly regulated process characterized by the disassembly and remobilization of components of cellular structures

### SENESCENCE IS A YIELD LIMITING FACTOR

- Delayed Senescence

Maintenance of CO<sub>2</sub> assimilation, but reduction of nutrient remobilization



#### **REACTIVE OXIGEN SPECIES (ROS) AND ANTIOXIDANT SYSTEM**



### Non-enzimatic components

- Glutathione
- Ascorbate
- Tocoferol
- Carotenoid

### Enzimatic components

Enzymes that degrade ROS
superoxide dismutase (SOD), peroxidases (PX),
catalase (CAT)

 Enzymes that regenerate non enzimatic antioxidants
gluthatione reductase (GR), dihydro- and monodihydro-ascorbate peroxidase (DHAR, MDHAR)

#### **REACTIVE OXIGEN SPECIES (ROS) AND ANTIOXIDANT SYSTEM**



### ANTIOXIDANT SYSTEM: KEY ELEMENT IN TOLERANCE TO STRESSFUL SITUATIONS



### Positive correlation between antioxidant enzyme activity and tolerance to different stress conditions

#### Antioxidant system response of different wheat cultivars under drought: field and *in vitro* studies

Hernán R. Lascano<sup>AC</sup>, Gerardo E. Antonicelli<sup>A</sup>, Celina M. Luna<sup>A</sup>, Mariana N. Melchiorre<sup>A</sup>, Leonardo D. Gómez<sup>A</sup>, Roberto W. Racca<sup>A</sup>, Victorio S. Trippi<sup>A</sup> and Leonardo M. Casano<sup>B</sup>



Osmotic pressure (MPa)

Lascano et al. (2001) Australian Journal of Plant Physiology

### ANTIOXIDANT SYSTEM: KEY ELEMENT IN TOLERANCE TO STRESSFUL SITUATIONS



Positive correlation between antioxidant enzyme activity and tolerance to different stress conditions



INDUCED CROSS-TOLERANCE: Priming of resistance through previous exposure to another biotic, abiotic, or chemical stress inducer SHARED TRANSCRIPTIONAL RESPONSE: Overlap of differentially expressed gene networks between stresses

Barrios Perez and Brown (2014) Frontiers in Plant Science

### ANTIOXIDANT SYSTEM: KEY ELEMENT IN TOLERANCE TO STRESSFUL SITUATIONS



- Positive correlation between antioxidant enzyme activity and tolerance to different stress conditions
- Cross-tolerance phenomena
- Overexpression of antioxidant enzymes



Wild-type SOD

Transgenic cotton plants expressing chloroplast-localized SOD have increased tolerance to chilling-induced oxidative stress

#### HOWEVER... ENHANCED ROS, ENHANCED TOLERANCE?

Superoxide dismutase and glutathione reductase overexpression in wheat protoplast: photooxidative stress tolerance and changes in cellular redox state

Mariana Melchiorre · Germán Robert · Victorio Trippi · Roberto Racca · H. Ramiro Lascano



Melchiorre et al. (2009) Plant Growth Regulation

#### REACTIVE OXIGEN SPECIES (ROS) AND ANTIOXIDANT SYSTEM: OXIDATIVE SIGNALING



Robert et al. (2009) Plant Science

### REACTIVE OXIGEN SPECIES (ROS) AND ANTIOXIDANT SYSTEM: SYSTEMIC OXIDATIVE SIGNALING



ORIGINAL RESEARCH published: 15 February 2019 doi: 10.3389/fpls.2019.00141

Redox Systemic Signaling and Induced Tolerance Responses During Soybean–*Bradyrhizobium japonicum* Interaction: Involvement of Nod Factor Receptor and Autoregulation of Nodulation

Tadeo F. Fernandez-Göbel<sup>1</sup>, Rocío Deanna<sup>2</sup>, Nacira B. Muñoz<sup>1,3</sup>, Germán Robert<sup>1,3</sup>, Sebastian Asurmendi<sup>4</sup> and Ramiro Lascano<sup>1,3\*</sup>



wounding, heat, cold, high-intensity light, salinity stresses, and symbiosis

Baxter et al. (2014) Journal of Experimental Botany Fernandez Göbel et al. (2019) Frontiers in Plant Science

### REACTIVE OXIGEN SPECIES (ROS) AND ANTIOXIDANT SYSTEM: <u>SYSTEMIC OXIDATIVE SIGNALING</u>



ORIGINAL RESEARCH published: 15 February 2019 doi: 10.3389/fpls.2019.00141

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wounding, heat, cold, high-intensity light, salinity stresses, and symbiosis



**Reduced Senescence symptoms** 

Fernandez Göbel et al. (2019) Frontiers in Plant Science

#### SUMMARIZING...

### THE DARK SIDE OF ROS

## ROS are highly reactive and induced OXIDATIVE DAMAGE = OXIDATIVE STRESS



### ROS are highly reactive and induced OXIDATIVE CHANGES = OXIDATIVE SIGNALING

**THE BRIGHT SIDE OF ROS** 

#### SUMMARIZING...

#### **OUR HYPOTHESIS**

**Controlling the processes associated with senescence** (i.e. ROS production, disassembly/remobilization and cell death) increases stress tolerance and yield


## KNOWLEDGE CONSTRUCTION IS A DYNAMIC AND COLLECTIVE PROCESS OUR TEAM

Ramiro Lascano (Group Leader) Germán Robert (Researcher) Laura Saavedra (Researcher) Santiago Otaiza (Postdoc) Alejandro Enet (PhD student) Tadeo Fernández (PhD student) Sofía Andreola (PhD student) Georgina Pettinari (PhD student) Tatiana Bellagio (undergraduate student) Juan Finello (undergraduate student)

#### Collaborators:

Sebastián Arsumendi (INTA Castelar) Claudia Vega (INTA Manfredi) Constanza Carrera (INTA Manfredi) Vanina Davidenco (FCA, UNC)



Overseas Collaborators: Kohki Yoshimoto (Meiji University) Céline Masclaux (INRA Versailles)





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# THANK YOU FOR YOUR ATTENTION!



#### LECTURE 2-PLANT ENVIRONMENT. BIOLOGICAL NITROGEN FIXATION: LEGUME-RHIZOBIA SYMBIOTIC INTERACTION

#### Germán Robert - robert.german@inta.gob.ar















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#### PLANT ENVIROMENT PLANT GROWTH AND DEVELOPMENT



#### Agroecosystems:

Nitrogenous fertilizer



- Contributes to greenhouses emissions

- Highest cost in crop management

# NITROGEN

#### **OVERVIEW OF NITROGEN UPTAKE IN PLANTS**

#### Natural ecosystems:

**80–90%** of the N available to plants originates from reduction of atmospheric N<sub>2</sub> to NH<sub>4</sub><sup>+</sup>



Biological Nitrogen Fixation (BNF)

≈ 80% is produced in symbiotic associations



#### **OVERVIEW OF NITROGEN UPTAKE IN PLANTS**

#### Legume-rhizobia interaction

#### Agroecosystems:

Nitrogenous fertilizer

Highest cost in crop management
Contributes to greenhouses emissions

-including legumes in crop management (crop rotation)



#### LEGUME – RHIZOBIA INTERACTION: BIOLOGICAL NITROGEN FIXATION

#### However, the benefits of nitrogen fixation are not without cost...



#### **LEGUME NODULE PROVIDES:**

- \* Carbohydrates
- \* Microaerobic conditions



#### High %BNF played a pivotal role in determining neutral soil N balance

Vessey (2004) Crop Management Santachiara et al. (2017) Plant Soil LEGUME – RHIZOBIA INTERACTION: BIOLOGICAL NITROGEN FIXATION

However, the benefits of nitrogen fixation are not without cost...

#### ENZYMATIC REDUCTION of N<sub>2</sub> to NH<sub>4</sub> $\rightarrow$ <u>NITROGENASE</u>

Legumes suppress nodule formation and function when nitrate or ammonia is available.

Symbiotic interaction is strictly controlled.

NODULES

6P,

#### STRICT NODULATION CONTROL MECHANISM FROM ROOT INFECTION TO NODULE DEVELOPMENT

## Ros and cell death modulating the nodulation process



#### **STRICT NODULATION CONTROL MECHANISM Molecular dialog between plant and bacteria**



Nodulation shares features of signaling pathways involved in other symbioses, and in plant-pathogen interactions

#### **STRICT NODULATION CONTROL MECHANISM** Very early host responses



#### STRICT NODULATION CONTROL MECHANISM Very early host responses



DPI  $\rightarrow$  NADPH oxidase-inhibitor LY294002  $\rightarrow$  PI3K-inhibitor FM4-64  $\rightarrow$  plasma membrane staining





Muñoz et al. (2012) Environmental and Experimental Botany Robert *et al*. (2018) Journal of Experimental Botany

#### **STRICT NODULATION CONTROL MECHANISM** Very early host responses: a very early control of nodulation



Tube 1: Pre-treatment (30 min) DPI or LY294002

Tube 2: Inoculation (30 min) *B. japonicum* 

Hydroponic medium: (24 days) Nodule formation

DPI  $\rightarrow$  NADPH oxidase-inhibitor LY294002  $\rightarrow$  PI3K-inhibitor



Robert et al. (2018) Journal of Experimental Botany

#### **STRICT NODULATION CONTROL MECHANISM** Early host responses: root hair curling and infection thread formation



2 h post-inoc

Fournier *et al.* (2015) Plant Physiology Santos *et al.* (2001) MPMI Muñoz *et al.* (2012) Environmental and Experimental Botany Robert *et al.*, (2018) Journal of Experimental Botany

# **STRICT NODULATION CONTROL MECHANISM** Early host responses: root hair curling and infection thread formation



Fournier *et al.* (2015) Plant Physiolog Santos *et al.* (2001) MPMI Muñoz *et al.* (2012) Environmental ar. Robert *et al.,* (2018) Journal of Experimental Botany

# Early control of nodule number by infection thread abortion

#### **STRICT NODULATION CONTROL MECHANISM** Bacterial infection (epidermis) and nodule development (cortex)



Oldroyd and Downie. Annual review of plant biology (2008)

#### **STRICT NODULATION CONTROL MECHANISM** Local responses involved on nodulation



Fournier *et al.* (2015) Plant Physiology Estrada-Navarrete *et al.* (2016) The Plant Cell

#### STRICT NODULATION CONTROL MECHANISM

Autoregulation of nodulation: root-to-shoot-to-root signaling



Ferguson et al. (2018) Plant, Cell & Environment

#### STRICT NODULATION CONTROL MECHANISM Autoregulation of nodulation: root-to-shoot-to-root signaling





Redox Systemic Signaling and Induced Tolerance Responses During Soybean–*Bradyrhizobium japonicum* Interaction: Involvement of Nod Factor Receptor and Autoregulation of Nodulation

Fernandez-Göbel et al. (2019) Front. in Plant. Sci.

#### STRICT NODULATION CONTROL MECHANISM Autoregulation of nodulation: root-to-shoot-to-root signaling



Fernandez-Göbel et al. (2019) Front. in Plant. Sci.

#### LEGUME – RHIZOBIA INTERACTION How is it affected under stress conditions?



Legume host would suppress nodulation under stressful conditions to conserve resources

What are the underlying molecular mechanisms?

#### LEGUME – RHIZOBIA INTERACTION UNDER SALINE STRESS Effects on the very early responses and root hair curling



Muñoz *et al*. (2012) Environmental and Experimental Botany Robert *et al*. (2014) Plos One

#### LEGUME – RHIZOBIA INTERACTION UNDER SALINE STRESS Effects on the very early responses and root hair curling



Muñoz *et al.* (2012) Environmental and Experimental Botany Robert *et al.* (2014) Plos One

#### LEGUME – RHIZOBIA INTERACTION UNDER SALINE STRESS Induced root hair death



#### **Evans Blue staining**



#### Does rhizobium perception trigger root hair death?

Muñoz *et al.* (2012) Environmental and Experimental Botany Robert *et al.* (2014) Plos One

#### LEGUME – RHIZOBIA INTERACTION UNDER SALINE STRESS Induced root hair death

#### **Evans Blue staining**



Muñoz *et al*. (2014) Functional Plant Biology Robert *et al*. (2018) Journal of Experimental Botany

#### TAKE HOME MESSAGE - CONCLUSION Root hair death as a very early control of nodulation



Rhizobia

(B. japonicum)

- ROS plays key role in the very early control of nodulation
- Saline stress affects very early host responses impacting on nodule formation
- Rhizobium perception under sublethal saline stress induces root hair death (as if it were a pathogen?)
- Root hair death prevents the allocation of resources/energy for nodule formation under unfavorable environmental conditions

#### TAKE HOME MESSAGE - CONCLUSION Root hair death as a very early control of nodulation



Early control of the number of nodules under control and stress conditions by cell death process

#### EXPRESSION OF ANIMAL CELL DEATH SUPPRESSORS IN PLANTS Ced-9 and BcL-xL $\rightarrow$ NO HOMOLOGS IN PLANTS!

# Animal cell-death suppressors $\operatorname{Bcl-x}_L$ and Ced-9 inhibit cell death in tobacco plants

Ichiro Mitsuhara\*<sup>†</sup>, Kamal A. Malik\*<sup>‡</sup>, Masayuki Miura<sup>§</sup> and Yuko Ohashi\*<sup>†</sup>

Plant Cell Physiol. 43(9): 992-1005 (2002) JSPP © 2002

#### Enhanced Resistance to Salt, Cold and Wound Stresses by Overproduction of Animal Cell Death Suppressors Bcl-xL and Ced-9 in Tobacco Cells — Their Possible Contribution Through Improved Function of Organella

Jingbo Qiao<sup>1,2,3</sup>, Ichiro Mitsuhara<sup>1,2</sup>, Yosiaki Yazaki<sup>1</sup>, Katsuhiro Sakano<sup>1</sup>, Yoko Gotoh<sup>1</sup>, Masayuki Miura<sup>4</sup> and Yuko Ohashi<sup>1,2,5</sup>

Journal of Experimental Botany, Vol. 55, No. 408, pp. 2617–2623, December 2004 doi:10.1093/jxb/erh275 Advance Access publication 8 October, 2004



**RESEARCH PAPER** 

Bcl-2 family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides

#### EXPRESSION OF ANIMAL CELL DEATH SUPPRESSORS IN SOYBEAN Ced-9-expressing hairy roots

Expression of Animal Anti-Apoptotic Gene Ced-9 Enhances Tolerance during *Glycine max* L.– *Bradyrhizobium japonicum* Interaction under Saline Stress but Reduces Nodule Formation





Expression of Ced-9 in transgenic hairy roots by *Agrobacterium rhizogenes* infection



Robert et al. (2014) Plos One

#### EXPRESSION OF CED-9 IN HAIRY ROOTS: ENHANCED TOLERANCE TO STRESS CONDITIONS

Expression of Animal Anti-Apoptotic Gene Ced-9 Enhances Tolerance during *Glycine max* L.– *Bradyrhizobium japonicum* Interaction under Saline Stress but Reduces Nodule Formation



#### EXPRESSION OF CED-9 IN HAIRY ROOTS: INHIBITION OF NODULATION

Expression of Animal Anti-Apoptotic Gene Ced-9 Enhances Tolerance during *Glycine max* L.– *Bradyrhizobium japonicum* Interaction under Saline Stress but Reduces Nodule Formation



# **TO BE CONTINUED...**

Robert et al. (2014) Plos One

## KNOWLEDGE CONSTRUCTION IS A DYNAMIC AND COLLECTIVE PROCESS **OUR TEAM**

Ramiro Lascano (Group Leader) Germán Robert (Researcher) Nacira Muñoz (Researcher) Laura Saavedra (Researcher) Santiago Otaiza (Postdoc) Alejandro Enet (PhD student) Tadeo Fernández (PhD student) Sofía Andreola (PhD student) Georgina Pettinari (PhD student) Tatiana Bellagio (undergraduate student) Juan Finello (undergraduate student)

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**Overseas Collaborators:** Kohki Yoshimoto (Meiji University) Céline Masclaux (INRA Versailles)





## THANK YOU FOR YOUR ATTENTION!

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### LECTURE 3-PLANT ENVIRONMENT. AUTOPHAGY IN PLANTS: ONE PROCESS, MULTIPLE PHYSIOLOGICAL FUNCTIONS

New advances in the role of autophagy on Nitrogen Use Eficiency (NUE)

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#### AUTOPHAGY IN EUKARIOTES: SELF-EATING PROCESS



Lysosomal/vacuolar degradation pathway of self-digestion

#### AUTOPHAGY IN PLANTS: MACRO- AND MICRO-AUTOPHAGY


### **AUTOPHAGY IN EUKARYOTES**



Christian de Duve → proposed the term Autophagy to denote the function of lysosomes in self-eating (Nobel Prize in Physiology or Medicine in 1974)



How could autophagy functions be proved by morphological observations?

## AUTOPHAGY IN EUKARYOTES ATG, AUTOPHAGY-RELATED GENES



Analysis of mutant-yeast (1993)  $\rightarrow$  identification of ATG genes (1995)

## AUTOPHAGY IN EUKARYOTES AUTOPHAGIC AND ENDOCYTIC PATHWAYS CROSSTALK



## MONITORING AUTOPHAGY IN PLANTS atg MUTANT AND GFP-ATG8 EXPRESSING PLANTS



#### Inhibition of vacuole degradation (Conc A)



**Autophagic bodies** 



abGFP

Yoshimoto et al. (2004) Plant Cell

## NITROGEN RECYCLING >> NITROGEN USE EFFICIENCY PHYSIOLOGICAL FUNCTIONS OF AUTOPHAGY

The Plant Cell 2015

#### Autophagic Recycling Plays a Central Role in Maize Nitrogen Remobilization

Faqiang Li,<sup>a</sup> Taijoon Chung,<sup>a,1</sup> Janice G. Pennington,<sup>b</sup> Maria L. Federico,<sup>c</sup> Heidi F. Kaeppler,<sup>c</sup> Shawn M. Kaeppler,<sup>c</sup> Marisa S. Otegui,<sup>b</sup> and Richard D. Vierstra<sup>a,2</sup>

#### Journal of Experimental Botany 2016 Nitrogen remobilisation during leaf senescence: lessons from Arabidopsis to crops

Marien Havé<sup>1</sup>, Anne Marmagne<sup>1</sup>, Fabien Chardon<sup>1</sup> and Céline Masclaux-Daubresse<sup>1,\*</sup>

Guiboileau et al (2013). New Phytologist Masclaux-Daubresse et al. (2018) Curr Opinion in Plant Biol Wang et al. (2017) Seminars in Cell & Developmental Biology

## PHYSIOLOGICAL FUNCTIONS OF AUTOPHAGY NH<sub>4</sub><sup>+</sup> TOLERANCE >> NITROGEN USE EFFICIENCY

# NH<sub>4</sub><sup>+</sup> accumulation >>> reduced plant growth

### **DOES AUTOPHAGY HAVE A ROLE IN NH<sub>4</sub><sup>+</sup> TOLERANCE RESPONSES?**



Sarasketa et al. (2014) Journal of Experimental Botany

# PERSPECTIVES

#### **AUTOPHAGY: ONE PROCESS, MULTIPLE PHYSIOLOGICAL FUNCTIONS**



Wang et al. (2017) Seminars in Cell & Developmental Biology

## **KNOWLEDGE CONSTRUCTION IS A DYNAMIC AND COLLECTIVE PROCESS ACKNOWLEDGEMENTS**

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