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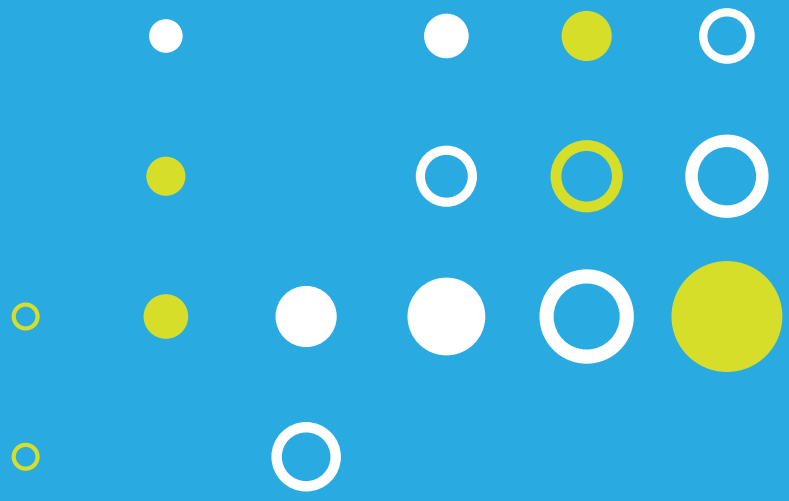

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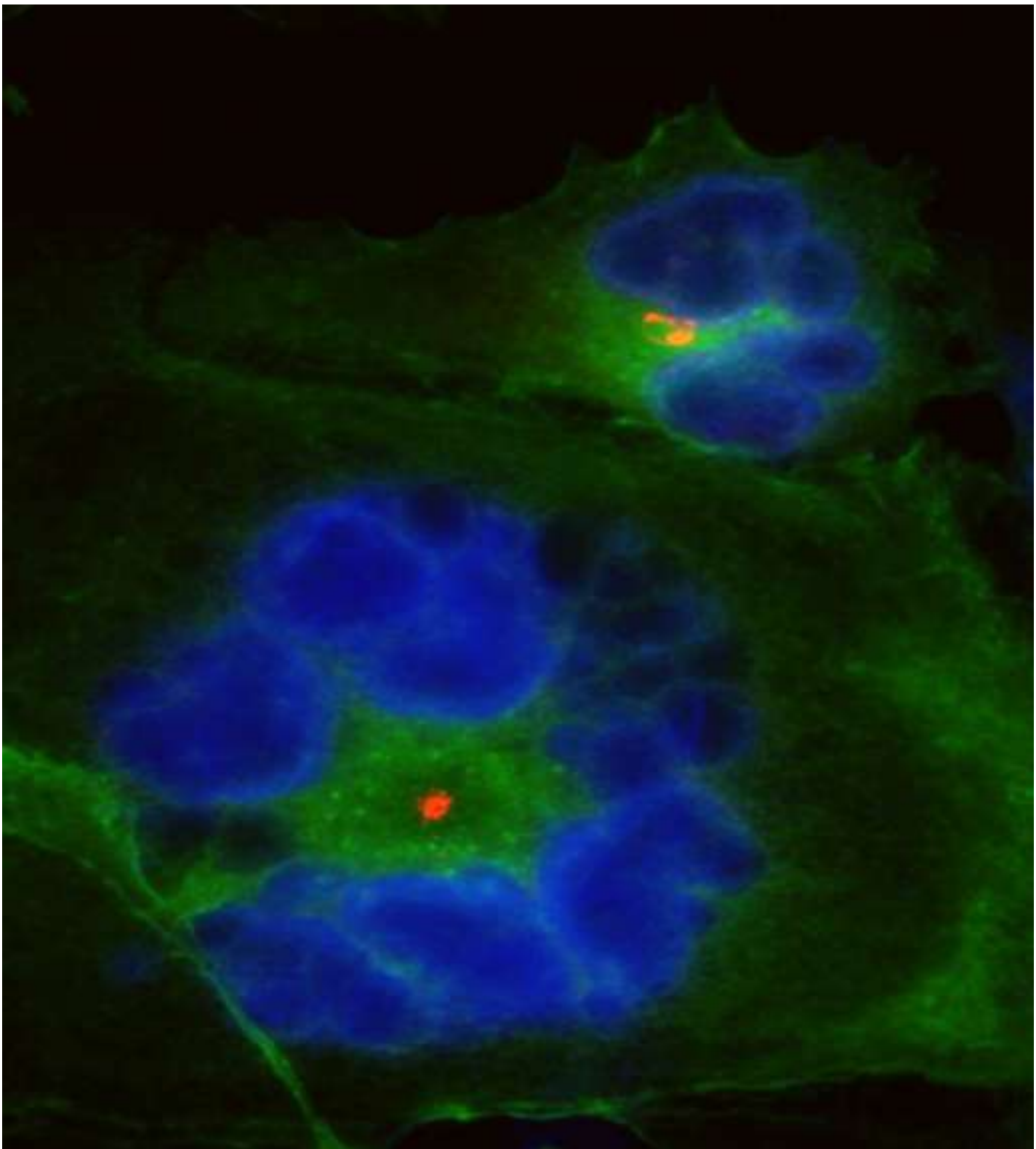

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Cover page: The Synthetic Lethal Rosette

Aberrant mitotic phenotype found in BRCA1-deficient cells treated with the PLK1 inhibitor Volasertib. Cells become giant and multinucleated and acquire a flower shape, with nuclei arranging in a circular disposition around a cluster of centrosomes. Blue (DAPI: nuclei), Green (FITC-phalloidin: actin cytoskeleton), Red (γ-Tubulin: centrosomes).

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ATG1 ROLE IN THE IMMUNITY OF TOMATO AGAINST *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

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The bacteria *Pseudomonas syringae* pv. t*omato* DC3000 (*Pst*) is widely recognized as a model to study plant immunity. Plants have evolved a two-layered immunity system to defend themselves from pathogens. Pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) are detected by host's pattern recognition receptors (PRRs) during pattern-triggered immunity (PTI) activation. This leads to changes in the intracellular calcium concentration, production of ROS, activation of MAPK cascades and transcriptional alterations. However virulent pathogens as *Pst* are able to bypass PTI through the delivery of effector proteins. Resistant plants recognize some of them activating effector-triggered immunity (ETI) that mainly leads to localized programmed cell death (PCD), limiting pathogen growth. Tomato Pto protein kinase interacts with the effectors AvrPto and AvrPtoB and together with Prf lead to activation of ETI response. Using previously generated RNA-seq data we identified two tomato genes (Solyc10g084930 and Solyc09g011320) whose expression is induced during ETI activation. Sequence and phylogeny analysis allowed us establishing that they encode for proteins belonging to the autophagy-related protein 1 (ATG1) group and that each of them has two orthologs in *Nicotiana benthamiana*, the species we use to test the role of candidate genes in immunity through virus-induced gene silencing (VIGS). Plants silenced with a construct that targets all 4 *N. benthamiana* orthologs (*NbATG1*) showed a delay in the development of PCD due to co-expression of Pto and AvrPto, as compared to non-silenced *Ec1* control plants. To confirm this result, we challenged *N. benthamiana 35S::pto* silenced plants with *Pseudomonas syringae* pv. *tabaci* (*Pstab*) expressing AvrPto or an empty vector (EV). We did not observe an increase in disease symptoms with *Pstab-AvrPto* strain. However, we found a delay in the development of *Pstab*-EV disease symptoms. To test whether silencing the 4 *N. benthamiana* orthologs is required for the observed phenotype, we generated two constructs (6008 and 1011) to target them by pairs. We found a similar overall phenotype trend, but with a more marked delay in symptoms when silencing with 1011 and *NbATG1* constructs. It is worth noticing that we have not observed growth or development abnormalities in plants silenced with any of the constructs used. We chose a well-established transcriptional marker of autophagy (*ATG8a*) to investigate if silencing of our candidates affects this process. Using qPCR we did not observe differences in *ATG8a* transcript level between *Ec1*- and *NbATG1*-silenced plants, suggesting that at least in unchallenged plants, autophagy is not affected by knocking-down the genes under study. Based on our results, we believe that this group of autophagy-related proteins would be involved in two different pathways, playing a role during ETI-associated PCD and also in the development of disease symptoms.

PL-P04
COLD STORAGE INDUCES DIFFERENT METABOLIC RESPONSES IN PEACH FRUITS WITH DIFFERENT HARVEST TIME

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Peaches ripen and deteriorate quickly at room temperature. Therefore, refrigeration is used to slow these processes and to extend fruit market life; however, several fruits can develop chilling injury (CI) during storage at low temperature. CI symptoms mainly develop during fruit ripening after cold storage, so this problem is not perceived until the fruit reaches consumers. Hence, the molecular reconfiguration that takes place during cold storage impacts on the way fruits ripen during the following shelf-life, situation that limits commercialization of these fruits. In this study, a metabolite profiling study of six peach varieties with different agronomic characteristics was performed after ripening, either following cold treatment or not, in order to evaluate the effects of refrigeration on the levels of metabolites involved in organoleptic properties and protection against stress. By using GC-MS, 51 polar metabolites were detected in Flordaking (FD), Rojo 2 (R2), Springlady (SL), Red Globe (RG), Elegant Lady (EL) and Limón Marelli (LM) varieties when fruits were stored at 20ºC until reaching firmness and organoleptic characteristics suitable for consumption (SL), and after cold storage at 0ºC for 21 days followed by ripeness at 20ºC (CS+SL). The identified metabolites were divided into sugars, sugar alcohols, organic acids, amino acids, fatty acids and miscellaneous compounds. Interestingly, xylose was the only metabolite that increased in CS+SL fruits, in relation to SL samples, in all the varieties analyzed, indicating a particular reconfiguration of the cell wall after cold storage. PCA analysis revealed interesting results. The first principal component (PC1, 38.7% of the variance) separated the samples depending on harvest time, with mid & late varieties on the positive and early varieties on the negative side, independently to whether the fruits were resistant or susceptible to CI. Among the metabolites that most contribute to PC1 separation, higher levels of maltose, maltitol and fructose 6-P were found in mid & late varieties, while higher levels of Thr, Ile and Val were found in early varieties. PC2 and PC3 did not contribute to separate the samples in any biologically meaningful group. HCA showed similar results, although a clear separation of SL and CS+SL samples in EL variety could be observed, which were fruits characterized by a large increase in sugars like sucrose, glucose and fructose, and organic acids such as citrate, malate, and quinate after refrigeration. Overall, the results showed a differential restructuration of peach fruit metabolism following exposure to cold in varieties with different harvest time. In particular, the identification of the molecular basis of the particular response of EL to cold is a future challenge, since it could aid in defining strategies for the improvement of the organoleptic quality of peach fruits by increasing sugar and organic acids levels while fruits are stored at low temperatures.

PL-P05

ATG1 role in the immunity of tomato against *Pseudomonas syringae* pv. *tomato*

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INFIVE CONICET UNLP

Abstract

The bacteria *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) is a model to study plant immunity. Microbe-associated molecular patterns (MAMPs) are detected by host's pattern recognition receptors (PRRs) during pattern-triggered immunity (PTI) activation. However virulent pathogens as *Pst* are able to bypass PTI through the delivery of effector proteins. Resistant plants recognize some of them activating effector-triggered immunity (ETI) that mainly leads to localized programmed cell death (PCD), limiting pathogen growth. Tomato Pto protein kinase interacts with the effectors AvrPto and AvrPtoB and together with Prf lead to activation of ETI response. We identified two tomato genes (Solyc10g084930 and Solyc09g011320) whose expression is induced during ETI activation. They encode for proteins belonging to the autophagy-related protein 1 (ATG1) group and each of them has two orthologs *in Nicotiana benthamiana*. Plants silenced with a construct that targets all 4 *N. benthamiana* orthologs (*NbATG1*) showed a delay in the development of PCD due to co-expression of Pto and AvrPto, as compared to non-silenced *Ec1* control plants. To confirm this result, we challenged *N. benthamiana* (*35S*::pto) silenced plants with *Pseudomonas syringae* pv. *tabaci* (*Pstab*) expressing AvrPto or an empty vector (EV). We found a delay in the development of *Pstab*-EV disease symptoms. To test whether silencing the 4 *N. benthamiana* orthologs is required for the observed phenotype, we generated two constructs (*NbATG1-a* and *NbATG1-b*) to target them by pairs. We found a similar overall phenotype trend, but with a more marked delay in symptoms when using *NbATG1* construct. Employing a well established transcriptional marker of autophagy (*NbATG8a*) did not observe transcriptional changes affected by gene silencing. Based on our results, we believe that this group of ATG1 proteins would be involved in two different pathways, playing a role during ETI-associated PCD and also in the development of disease symptoms.

RNA-seq experiments using tomato infiltrated with *Pst* DC3000 mutants

Our group previously generated RNA-seq data from resistant tomato leaves challenged using *Pst* DC3000 mutants (Table 1).

Table 1. Detail of the treatments performed for RNA-seq analysis

Plant	Strain	Concentration	Sampling time
RG-PtoR tomato	*Pst* DC3000 Δ*fliC*	5 x 10^6 cfu/mL	6 h
	Pst DC3000 Δ*avrPto* Δ*avrPtoB*		
	Pst DC3000 Δ*avrPto* Δ*avrPtoB* Δ*fliC*		

Solyc10g084930 and Solyc09g011320 are induced during the ETI-response

From 3370 ETI-induced genes we identify two of them (Solyc10g084930 and Solyc09g011320) as induced specifically during the ETI-response (Fig. 1).

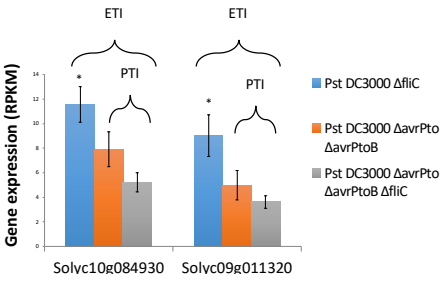

Figure 1. Transcript abundance (RPKM) of candidates genes. RNA-seq expression analysis in tomato RG-PtoR plants infiltrated with different DC3000 strains.. Bars represent the mean of three biological replicates with their corresponding standard deviation. Asterisks indicate significant differences (q<0.05) with raw p-values corrected for multiple testing using the false discovery rate.

Phylogenetic analysis of ATG1 family for the design of silencing constructs.

Solyc10g084930 and Solyc09g011320 belong to Autophagy-Related Gene 1 (*ATG1*) protein family. To perform a functional characterization we designed three constructs based in *N. benthamiana* (Fig. 2) gene sequences to perform virus-induced gene silencing (VIGS)

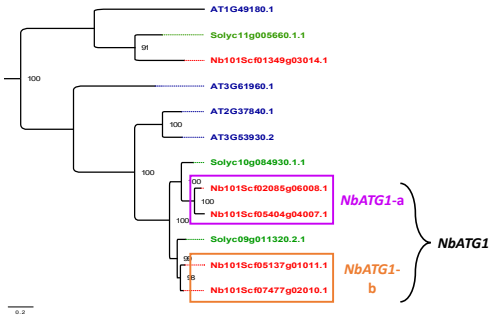

Figure 2. Phylogenetic tree of ATG1-related genes from *A. thaliana* (blue), tomato (green), *N. benthamiana* (red). The curly bracket indicates the *N. benthamiana* members silenced by the *NbATG1* construct. The purple and orange rectangules indicate the members silenced by the *NbATG1-a* and *NbATG1-b* respectively.

Silenced plants have a normal development

Atg mutant plants usually present abnormal development, it is worth noticing that *NbATG1* silenced plants exhibit a normal phenotype (Fig. 3).

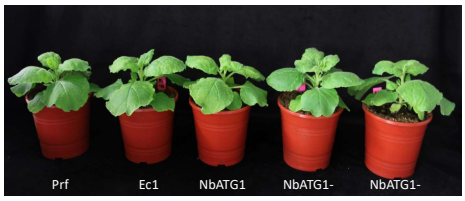

Figure 3. Photography of 15-week old silenced plants

NbATG1 silenced plants have a marked reduction of PCD symptoms

In *NbATG1* silenced plants we observed a significant reduction of PCD symptoms. Using individual constructs (*NbATG1-a* and *NbATG1-b*) we observed a similar, but less consistant trend.

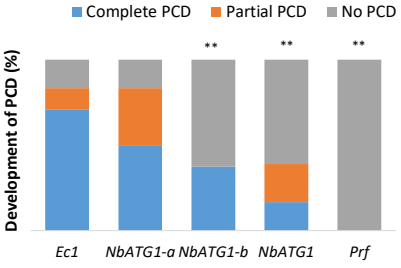

Figure 4. Programmed cell death (PCD) in silenced plants. *N. benthamiana* leaves silenced for the indicated genes, were infiltrated with a mix of *A. tumefaciens* expressing Pto and AvrPto proteins. Controls included were: *Ec1* (fragment from *E. coli* that does not target any *N. benthamiana* gene) and *Prf* (NB-LRR required for Pto/AvrPto mediated PCD). Percentage in each category is shown at day 5 post inoculation. ** indicate significant differences (p<0.01) compared with *Ec1* using Fisher's exact test.

NbATG1 silenced plants show a reduction in disease symptomps

To further confirm the previous result (Fig. 4), we challenged silenced plants with Pstab expressing avrPto. We did not observe the expected increase in disease symptoms using any of our constructs (Fig. 5A). However a clear decrease in symptoms was observed in NbATG1-silenced plants, compared with the Ec1 control (Fig 5B). A less marked decrease was found when silencing with the individual constructs.

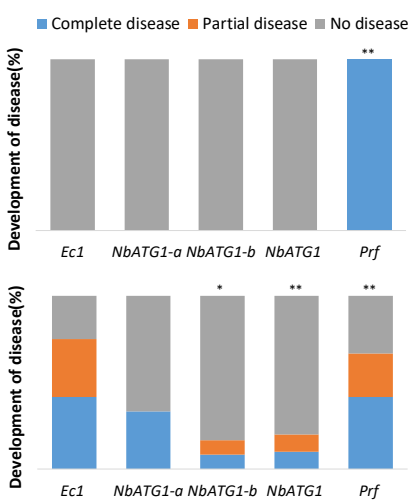

Figure 5. Disease assay in silenced plants. *N. benthamiana* 35S::*pto* plants were silenced for the indicated genes and syringe inoculated with 5x10^4 cfu/mL **(A)** pDSK519::*avrPto* (under *avrPto* promoter) or **(B)** *Ps tab* carrying empty pDSK519 vector (EV). Control genes used were the same as described in Fig. 4. Disease symptoms were visually evaluated. Percentage in each category is shown at day 5 post inoculation. * and ** indicate significant differences (p<0.05 or p<0.01) compared with *Ec1* using Fisher's exact test.

Silencing *NbATG1* does not affect *NbATG8a* transcript levels

To test whether silencing NbATG1 affects autophagy we analyzed NbATG8 transcript levels and found no difference between silenced and control plants (Fig. 6).

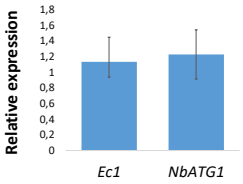

Figure 6. *NbATG8a* relative expression (qRT-PCR) in *Ec1 and NbATG1* silenced plants

Conclusions

- **Silencing *NbATG1* does not affect plant growth**
- ***NbATG1, NbATG1-a* and *NbATG1-b* silenced plants posses a decreased PCD and disease symptoms.**
- **The fact that the expression of NbATG8a is not affected by silencing NbATG1 suggests that probably basal autophagy is not altered.**

Figure 7. Proposed model. NbATG1 coding proteins could play a role in PCD and disease symptoms with *P. syringae*.