WORLD PETUNIA DAYS

Rome, 3-5 june 2026

Book of Abstract

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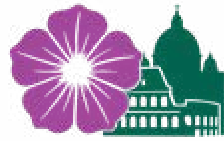

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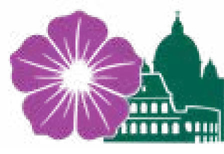

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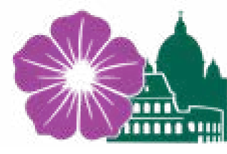
Scientific session I

Organ development



Divergent Evolution of WOX1&3 homeodomain transcription factor function during diversification of the core eudicot plant species

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Costanzo E.
Morel P.
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Rodrigues Bento S.
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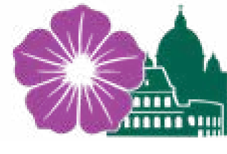
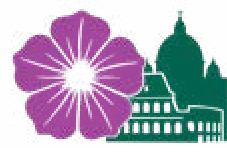
Abstract

WOX genes play major developmental roles in plant development, ranging from embryo till flower development, often involved in maintaining stem-cell niches in different plant meristems. In petunia (Asteridae), the MAW (MAEWEST) gene from the WOX1 subfamily plays an important role in blade outgrowth in leaf and floral organs. In Arabidopsis (Rosidae), WOX1 performs a comparable function, but in a largely redundant fashion with PRS (PRESSED FLOWER), a member of the structurally distinct WOX3 subfamily, and with WOX5. To provide more insight in the evolutionary developmental fate of WOX1, WOX3 and WOX5 genes in higher eudicots, we have functionally analysed all members of these subfamilies in petunia, allowing full comparison with Arabidopsis. We found that MAW together with MAE (MAE EAST), a second petunia WOX1 member, together are required for medio-lateral polarity of all lateral organs, leading to complete disruption of petal and carpel fusion in



flowers of mae maw mutants, as well as a severe reduction of blade outgrowth in all lateral organs. Furthermore, the extent of blade outgrowth appears to be quantitatively affected by MAW/MAE gene dosage. In addition, we show that MAW/MAE play an important role in ovule identity. Unexpectedly, we found that the two petunia WOX3 genes, called DREV (DOCTOR EVIL) and MIME (MINI ME) do not function in blade development. Instead, they are required for multicellular trichome development, thus performing a completely different role compared to Arabidopsis WOX3 (PRS). Introduction of Petunia WOX1 and WOX3 genes in Arabidopsis recapitulate Petunia expression patterns, suggesting that Petunia WOX3 genes acquired a novel role through acquisition of specific cis elements in their regulatory sequences. In conclusion, our results further illustrate the evolutionary plasticity of the WOX family to be recruited in different plant developmental processes, across evolution and species borders.



Form and function of fused-petal flowers

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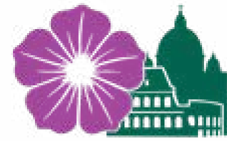
Abstract

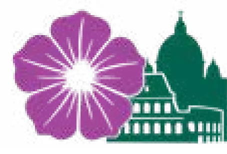
Among ~300 000 flowering plant species, about 80,000 produce flowers with petals fused into a tubular structure. This structural innovation has allowed plants to exploit different pollinators and has contributed to the rapid diversification of floral shapes. The trumpet-shaped corolla of Petunia, with its petals fused into a tube expanding into a colourful limb, has become a key model for studying how petal tube morphology influence pollinator interactions. By restricting access to nectar located at the base of the tube, corolla shape can favour pollinators with matching tongue lengths.
Although some of the genetic pathways involved in petal fusion have been identified, the cellular events that initiate and build the fused corolla remain poorly understood. Major gaps persist in our understanding of how the tube forms and grows, and how molecular regulation and mechanical forces act together to shape its development. The team has recently developed a quantitative imaging pipeline to visualise



and analyse early petal development at cellular resolution. Using a multidisciplinary approach, we will investigate how cellular dynamics drive the initiation and growth of the fused corolla tube, and identify the molecular mechanisms underlying petal fusion. Finally, we will examine how fused floral traits, particularly corolla geometry, shape pollinator responses.

Keywords:
Petal fusion
Shape
Cell behaviour
Pollinators
Quantitative imaging

back to program



How is tubular corolla architecture regulated? Understanding the molecular mechanisms of petal fusion in Petunia flowers

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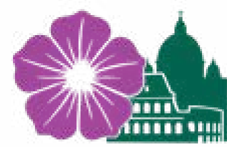
Abstract

Within the plant kingdom, Angiosperms (flowering plants) comprises over 300,000 species representing about 80% of known terrestrial plants diversity1. Angiosperms stand out for their unique reproductive structure: the flower, which has undergone extensive diversification over the past 135 million years particularly in terms of floral architecture2. This diversity is partly the result of coevolutionary interactions between plants and pollinators, underscoring the importance of understanding how floral organs are organised and how they have evolved through these interactions.

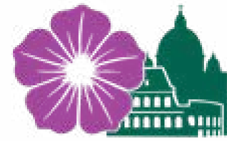
The fusion of the petals (sympetaly) is a key morphological innovation that has contributed to the diversification of many angiosperm lineages by enhancing the efficiency of animal mediated-pollination. Sympetaly occurs in more than 80,000



Angiosperms species, representing about one third of them2,3. Sympetalous flowers present a considerable developmental diversity, including fusion with other floral organs or/and the degree and timing of fusion4. Within the Asterales group, which includes Petunia (Solanaceae family), sympetaly likely appeared multiple times independently during evolution2,3. Interestingly, studies have shown that variations in the length and diameter of the tube formed by petals within the genus Petunia have been associated with distinct pollinators, suggesting that sympetaly may have evolved as an important component of pollination syndromes5–8. The resulting tube shape acts as a barrier to pollinators, restricting access to the nectaries at the tube base, and allowing for the selective recruitment of specialized pollinator species. In species with free petals such as Arabidopsis thaliana (Rosids, Brassicaceae family), the classical plant model, the molecular basis of boundaries between separated organs is relatively well characterised. On the contrary, the pathways controlling petal fusion remain poorly understood, despite Its importance in diversification of flowering plants.
The main objective of this PhD project is to advance our understanding of the molecular mechanisms of petal fusion. This question is addressed through the characterization of gene regulatory networks (GRNs) involved in petal fusion in Petunia as well as a detailed cellular analysis of petal development.

Keywords

Petal fusion
Development
Petunia



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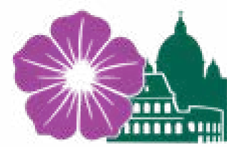
Scientific session II

Flower traits



OpenFlower: Designer Flowers for Art & Education

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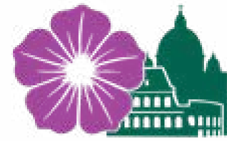
Abstract

Flowers have long been objects of admiration, symbolism, and selective breeding, yet their rational design as an artistic medium remains largely inaccessible in practice, constrained by institutional barriers and a lack of enabling tools. Here, I introduce *OpenFlower*, a non-profit organization dedicated to enabling flower design as a platform for both artistic expression and biotechnology education. Using the model systems *Arabidopsis thaliana* and *Petunia hybrida*, I present a framework for the rational design of floral traits, spanning flower architecture and organogenesis (“genetic sculpting”) as well as pigmentation and patterning (“genetic painting”). I focus in particular on *Petunia* as a tractable system for aesthetic engineering, highlighting ongoing work that enables programmable modifications of floral form and the implementation of novel coloration and patterning strategies.

Together, this work positions flower design as an emerging practice at the intersection of developmental biology, genetic engineering, and art, and outlines a path toward making this medium more accessible to researchers, educators, and artists.



Keywords:
Development
Pigmentation
Patterning
Biotechnology
Synthetic Biology

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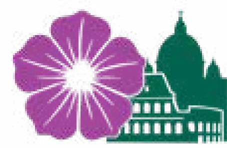

Benzyl alcohol biosynthesis and its subcellular compartmentalization

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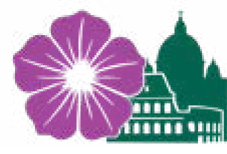
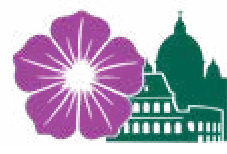
Abstract

Benzyl alcohol is a component of floral scents in at least 50 plant families, but typically accumulates at very low, often undetectable levels outside scent and flavor-producing organs. This simple aromatic alcohol plays important roles in plant-environmental interactions and serves as a precursor of benzyl benzoate, an intermediate in the phenylalanine-dependent biosynthesis of the phytohormone salicylic acid. Despite its importance, the enzyme responsible for benzyl alcohol formation in plants has remained unknown. We took advantage of the high levels of benzyl alcohol production in petunia flowers, and via a combination of classical biochemical, proteomic and genetic approaches, identified two NADPH-dependent benzaldehyde reductases (PhBR1 and PhBR2) responsible for the formation of benzyl alcohol and its downstream derivatives, benzyl benzoate and methyl salicylate. Both petunia enzymes, which differ by only three amino



acids exhibit substrate promiscuity, preferentially reducing benzaldehyde and cinnamaldehyde with residue 97 (Trp in PhBR1 and Cys in PhBR2) being responsible for their slightly different substrate specificities. Interestingly, the developmental expression profile of *PhBRs* does not coincide with that of scent biosynthetic genes or benzyl alcohol emission, as transcript levels peak during flower opening. Suppression of *PhBRs* expression in petals reduced the emission and internal pools of benzyl alcohol and benzyl benzoate as well as the emission of methyl salicylate. Moreover, downregulation of *PhBRs* expression substantially reduced salicylic acid levels in petunia stems upon pathogen infection providing direct evidence that PhBRs are involved in benzyl alcohol formation and subsequently in SA biosynthesis via benzyl benzoate. In petunia, PhBRs exhibit cytosolic localization unlike to benzaldehyde synthase and the enzymes responsible for the side-chain shortening of cinnamic acid to benzoyl-CoA, all of which are localized in the peroxisomes. Analysis of the subcellular localization of PhBR homologs across flowering plants revealed that it is species-specific, occurring either in peroxisomes or cytosol. These results suggest that different species employ distinct compartmental organization for phenylalanine-dependent salicylic acid biosynthesis.

Keywords:
Petunia
Benzyl alcohol
Volatiles
Benzaldehyde reductases
Salicylic acid



How Petunia flowers gained yellow colour

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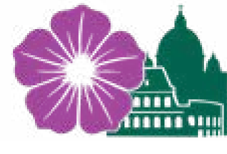
Abstract

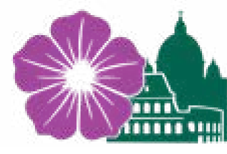
To understand how novel traits arise during evolution, we investigated how plants acquired on multiple occasions yellow flowers coloured by carotenoids. Until recently Petunia species lacked bright yellow pigmentation; however, a variety with vibrant yellow petals arose spontaneously in a breeding population about two decades ago. We show that this new trait is caused by a dominant mutation at a single locus: *CAROTENOID* (*CAR*) and identified the gene. *CAR* originated through transposition of a GARP-type transcription factor gene on chromosome 5 (*PARENT OF CAR, PAC*) to chromosome 6. In its new genomic context, *CAR* is strongly expressed in petals where it activates genes involved in carotenoid biosynthesis genes and chromoplast development. In *Calibrachoa*, yellow petal evolution also involved upregulation of a CAR homolog—here by changes in an upstream regulatory gene— indicating evolutionary convergence. These findings show how spontaneous mutations can rewire gene regulatory networks to



generate novel traits during evolution.

Keywords:

Carotenoids
GARP
Transcription Factor
Petunia



Carotenoids effect on Petunia flower colour and chromoplast organization

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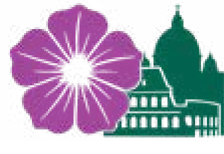
Abstract

Flower pigmentation is the result of the accumulation of secondary metabolites, such as anthocyanins, which are responsible for reddish/blue colours. Another class of pigments, carotenoids, contributes to a range of shades from yellow to orange. While the water soluble anthocyanins are stored in the central vacuole (Mackon *et al*., 2021), carotenoids can be stored in different kinds of chromoplasts (crystalline, globular, membranous, tubular o fibrillar), which can coexist in a single cell (Oleszkiewicz *et al*., 2024). Chromoplast differentiation is strongly associated with carotenoid production. The formation of new and distinct types of chromoplasts correlates with the accumulation of specific carotenoids (Lado *et al*., 2015). While it is clear that carotenoids induce chromoplast substructure changes, whether and how these metabolites also initiate chromoplast biogenesis remain to be elucidated. In wild Petunia species carotenoids are not present in petals. Yellow



flower colours are however available in some commercial petunia varieties since the end of 1900, as result of breeding. We recently showed that the CAR1 regulator is at the basis of the acquisition of carotenoids in the petals of these varieties (manuscript in preparation).
We are now testing two hypothesis: 1) Carotenoids induce the maturation of these organelles; 2) CAR controls the maturation of plastids into globular chromoplasts and in their absence carotenoids are not accumulated. I will present the results of this investigation.

Keywords:
Petunia
Carotenoids
Chromoplasts

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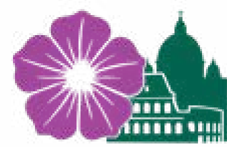
Scientific session III

Regulation of gene expression and epigenetics



Leveraging the Petunia Model to Develop Approaches for Discovering Regulatory Networks for Traits and Metabolites

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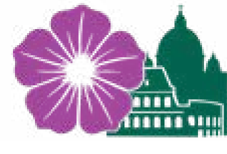
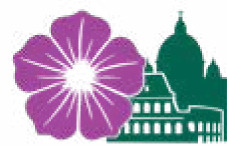
Abstract

Decades of research into pollination syndromes in the petunia model system have developed a well-described network of genes contributing to pollinator-linked floral traits. These networks are made up of genes encoding enzymes and transporters which participate in biochemical pathways to produce floral flavonoid pigments and volatiles as well as their associated transcriptional regulators. Broadly, however, most specialized plant metabolites which contribute to unique traits remain poorly characterized, both at the level of enzymes involved in biosynthetic pathways and transport as well as their transcriptional regulation. The ability to obtain or generate large-scale sets of gene expression data, together with advances in computing approaches which can decipher underlying patterns in biological data, creates opportunities to work toward filling these knowledge gaps by using network-based analyses to understand the relationships between genes and traits by harnessing natural variation. As proof-of-concept, I leveraged knowledge in the petunia model system by applying a two-phase machine learning approach to determine underlying genes and regulators contributing to



floral pigment variation across a sample of petunia and related species. I was able to recapitulate essential genes and key regulatory networks for anthocyanin biosynthesis among the top predicted candidate genes from the analysis pipeline, using only metabolite measurements and RNA-Seq data as inputs. Similar results could be obtained using only visible trait information in the form of flower color in place of metabolite measurements with minimal loss of predictive power. Connectivity-based prioritization which combines machine learning predictions together with gene regulatory network information has highlighted potential genes of interest for further evaluation which may contribute to floral color traits. The success of this proof-of-concept project supports the use of this approach to study other plant traits and metabolic pathways for which pathways and/or regulation are poorly understood.

Keywords:
Specialized metabolism
Machine learning
Gene regulatory networks



Decoding Regulatory Information in Solanaceae Genomes Using Large Language Models

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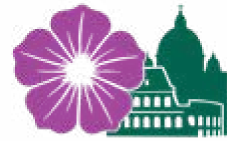
Abstract

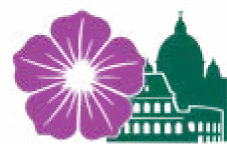
Specialized metabolism is central to crop reproduction, environmental adaptation, and stress resilience. The Solanaceae family, which includes tomato, potato, pepper, eggplant, tobacco, and petunia, provides an outstanding system for studying the evolution and regulation of specialized metabolism because it contains many lineage-specific compounds with major ecological, agricultural, and sensory impacts. A defining feature of specialized metabolism is that it is often restricted to particular cell types or tissues, developmental context, and/or environmental conditions. Many specialized metabolic genes arose through duplication of genes from primary metabolism, followed by divergence in both coding and regulatory regions. While changes in the coding region can alter enzyme activity and substrate specificity, changes in non-coding regulatory DNA are essential for activating these genes in the correct developmental, tissue, or environmental context. However, comparing regulatory motifs among plant genomes remains a major challenge, especially in crops, where intergenic regions are often dominated by repetitive sequences that obscure short but informative cis-regulatory elements.
This talk will present a framework for using large language models (LLMs) to decode hidden regulatory



information in crop genomes. Recent advances in DNA language models such as DNABERT and related architectures have led to plant-specific LLMs trained to recognize informative regulatory patterns buried within noisy non-coding DNA. Our goals are to develop foundational DNA language models for representative Solanaceae species and to use these models to identify cis-regulatory regions associated with tissue-specific gene activation in specialized metabolic pathways. By comparing promoters within gene families, across germplasm collections, and among homologous genes across species, this work aims to reveal how regulatory sequence variation shapes context-dependent gene expression. Ultimately, this approach will provide new tools for understanding crop genome function and for engineering agriculturally important traits through improved transcriptional control.

Keywords:

Specialized metabolism
Large language model
AI
Gene regulatory motifs



Identifying the regulatory mechanisms of complex pigmentation patterns // Using floral pigmentation patterns to study how complex cell identities are regulated.

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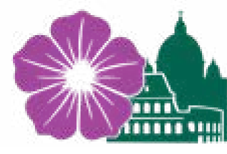
Abstract

Cells differentiate through changes in gene expression driven by internal and external cues. These processes are regulated by mechanisms such as epigenetic modifications, transcription factors, and post-transcriptional control by small RNAs. However, how these regulatory layers generate and maintain complex spatial patterns of cell identity remains poorly understood.
To address this, we will use *Petunia* flower pigmentation patterns as a model system to study the establishment and maintenance of cell identity patterns. Pigmentation provides a direct and visually accessible readout of spatially restricted gene expression, making it an ideal system for dissecting pattern formation mechanisms. In specific *Petunia* cultivars, pigmentation patterns arise from Post-Transcriptional Gene Silencing (PTGS) of *CHALCONE SYNTHASE* (CHS), a key enzyme in anthocyanin



biosynthesis. This silencing is mediated by small RNA–driven RNA interference (RNAi), leading to distinct patterns of pigmented and unpigmented cells. While the role of small RNAs and epigenetic regulation in gene silencing is established, how these mechanisms produce precise spatial patterns remains unclear.

We will address this question through three complementary work packages:

WP1.1 Identify genomic regions underlying colour pattern variation by crossing lines with different pigmentation patterns and tracking loci that co-segregate with these traits.

WP1.2 Identify candidate genes involved in CHS silencing using transposon (*dTph1*) tagging to compare mutation profiles between patterned lines and pattern mutants. Candidate genes will be validated using CRISPR-Cas by testing whether targeted mutations can rescue pigmentation patterns.

WP1.3 Refine candidate gene selection and reconstruct the sequence of events underlying pattern formation by performing spatial single-cell sequencing of young flower primordia before, during, and after pattern establishment, enabling identification of transcriptional changes that initiate and shape these patterns.

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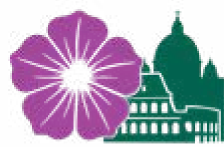

That's Random; exploring the mechanism behind stochastic transposon excision in Petunia W138

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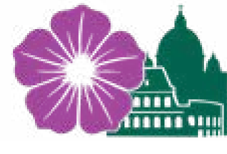
Abstract

Transposable elements (TEs) are major drivers of genetic diversity, yet their uncontrolled activation can compromise genome integrity and plant fitness. Epigenetic mechanisms such as DNA methylation typically suppress TE activity, but how plants fine-tune this balance between adaptive flexibility and stability remains unclear.
This study investigates TE regulation using *Petunia hybrida* W138, a model that exhibits high-frequency TE excision and a distinct speckled flower pigmentation pattern reporting transposon activity. The flower pattern results from excision of the non-autonomous *dTph1* element from the *AN1* gene. *dTph1* mobilization depends on a trans-acting factor, hypothesized to be the uncharacterized *Activator 1 (ACT1)*. Although dTph1 is found in all Petunia lines, transposition occurs at a much higher rate in Petunia W138, suggesting a species-wide conservation but differential activation of ACT1.
To dissect the epigenetic basis of this variation, we

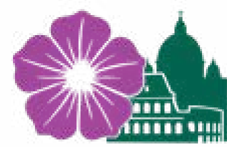


generate whole-genome assemblies for the high-mobilization line W138 and the transposition-deficient line W1007 using Oxford Nanopore Technologies (ONT). Long-read data provide both high-resolution genome assemblies and native DNA methylation profiles, enabling direct comparison of methylation states at TE loci. Integrating these data with whole-genome bisulfite sequencing and insertion mapping will reveal epigenetic signatures associated with TE suppression and clarify ACT1's regulatory role. We will also demonstrate a method for applying abiotic stresses (UV-A and salt) to Petunia W138 and assessing their effects on TE-dependent pigmentation patterns.

Keywords:
Transposable Elements
Epigenetic regulation
(ONT) long read sequencing
Abiotic stress
Petunia W138

Scientific session IV

Protein expression and sorting



Fading and pollination syndrome

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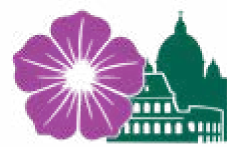
Abstract

While most plant cells contain a single central vacuole (CV), some specialized cells possess multiple vacuoles with distinct functions. This is the case for epidermal petal cells of several species. In petunia, additional vacuoles have been identified and termed "vacuolinos." Vacuolinos act as intermediate stations for proteins traveling to the CV. As proteins transit through vacuolinos, they encounter a checkpoint: some proteins are permitted to proceed to the CV, whereas others are retained and do not reach it. One protein that is halted by vacuolinos is FADING (FA), a vacuolar membrane protein. When FA is present on the tonoplast of the CV, it can trigger anthocyanin degradation in the vacuolar lumen.

In wild-type petunia petals, this degradation does not occur because FA does not reach the CV; instead, it is stopped and degraded in vacuolinos. In mutants deficient in vacuolinos, FA reaches the tonoplast of the CV, leading to anthocyanin degradation and resulting in petal decoloration.

The formation of vacuolinos is regulated by the MBWW (MYB, bHLH, WDR, WRKY) transcription factor complex, which is known to control several aspects of epidermal cell differentiation in plants.



In petals with a non-functional MBWW complex (due to mutation in any of its components), vacuolinos are absent, and vacuolar proteins are delivered directly to the CV.
Although anthocyanin fading is observed in several plant species, its biological role in petals has remained unclear. FA homologues are present in all species examined and are mostly expressed at low levels in leaves. In some species, however, FA is also expressed in petals. These include wild Petunia accessions with white, scented flowers that are pollinated by moths at dusk. In these species, FA is highly expressed in petals during the final phase of flower life. This elevated expression is accompanied by mutations that reduce, but do not completely eliminate, pigmentation (e.g. in AN2).
We will present experimental evidence that petals of these wild Petunia species have defective vacuolinos, allowing FA to reach the CV and degrade anthocyanins. These findings support a role for FA in the evolution of the moth pollination syndrome within the Petunia genus.

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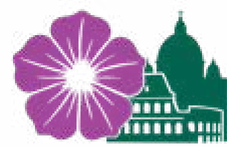

PHOX as a Rab5a Effector in Cargo-Specific Vacuolar Trafficking

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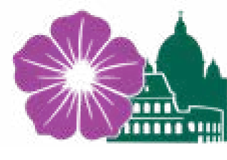
Abstract

Some plant cells contain different vacuolar types with distinct functions[1]. Protein trafficking to this different compartment is essential for the specialization of these cells, yet the mechanisms conferring cargo specificity to vesicles moving towards one or the other vacuole remain understood[2]. In Petunia hybrida, petal epidermal cells contain a specialized vacuolar system that supports processes such as pigmentation and ion homeostasis[3]. Previous work has identified the small GTPase Rab5a as a central regulator of endosomal trafficking toward the vacuole[4]; however, the downstream effectors mediating cargo-specific transport steps remain largely unknown.
In this study, we identify and characterize PHOX, a PX-domain–containing protein, as a regulator of vacuolar trafficking. PHOX was initially identified through a forward genetic screen of a population plants of the high-transposition line W138 line. In this population, a mutant exhibiting a vacuolar



phenotype led to the discovery of the petunia PHOX gene. Using fluorescent protein markers and confocal microscopy, we show that PHOX localizes to endosomal membranes in a Rab5a-dependent manner. Functional analysis of phox loss-of-function mutants reveals a selective delay in the trafficking of specific vacuolar cargo proteins, including the tonoplast proton pump PH5 and the vacuolar marker ALEU, while the FADING protein remains unaffected. This indicates that PHOX has a role in determining the cargo-specificity of the vacuolar sorting pathway rather than in general endomembrane trafficking. Complementation by ectopic PHOX expression restores normal trafficking.
Together, these findings identify PHOX as a Rab5a-dependent effector that mediates cargo-specific transport to the vacuole in petunia petal cells.
This work provides new insight into the molecular mechanisms underlying specificity in plant vacuolar trafficking and highlights the diversification of Rab5-associated pathways in specialized cell types.

Keywords:

PX domain proteins
Vacuolar trafficking
Petunia hybrida

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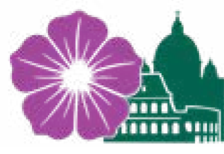

Endomembrane transport through the vacuolinos pathway, characterising key players in transport, function and delivery

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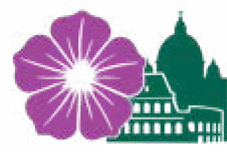
Abstract

Vacuoles are a group of very diverse organelles in plant cells that determine much of the cell function. In Petunia petal epidermal cells, specialized vacuoles, called vacuolinos, acts as intermediate station for proteins on their way to the central vacuole (CV). In petal epidermal cells of wild type plants, vacuolinos are formed by the fusion of pre-vacuolar compartment (PVCs). The vacuolinos pathway is an alternative protein sorting route for vacuolar proteins, as compared to the canonical pathway to the CV in other cell types.
We set out to identify the genes controlling the genesis and function of vacuolinos.
Candidate genes involved in the vacuolinos pathway are being investigated by means of transposon and CRISPR generated mutants, which we further characterize by transient localization assays in protoplasts. A group of candidate genes encode Rab-GTPases and their effectors. For RAB5a we have shown the direct involvement in the genesis of vacuolinos, for others we have mutants and preliminary data. Other candidates are instead



possibly involved in vacuolinos function as gatekeeper. This means that specific vacuolar membrane proteins (e.g. FADING) gets stuck in vacuolinos where they get degraded and do not reach the CV. One possible key player in this role of vacuolinos is CAC16.5, a cysteine protease highly accumulated in the lumen of these organelles: could it be responsible for protein degradation in vacuolinos?

Keywords:

Endomembrane trafficking
Vacuolinos
Rab-GTPase
Protease
Effectors

Literature:

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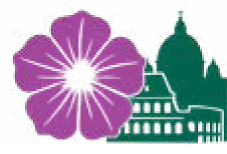

Closing the loop: from anthocyanin synthesis to degradation

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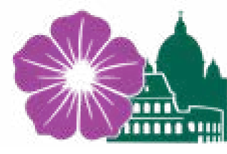
Abstract

The balance between synthesis and degradation of anthocyanins determines plant pigmentation patterns. The mechanisms that govern when and where anthocyanins are synthesized are well understood, not least through the analysis of flower color mutants in petunia. However, much less is known about the processes underlying anthocyanin turnover *in vivo.* Over the past 100+ years, countless studies have shown that anthocyanins are chemically unstable and prone to discoloration under in vitro conditions,



and that plant extracts (e.g. juices) and processed products (e.g. canned fruits) contain numerous oxidative enzymes—such as polyphenol oxidases, peroxidases, and laccases—that can decolor anthocyanins *in vitro*[1–3]. To address whether these or other enzymes are involved in anthocyanin turnover i*n vivo*, we studied petunia mutants described more than 50 years ago, in which purple flower color fades after bud opening[4,5].

We isolated *FADING* (*FA*) and found that it encodes a ferric reductase oxidase (FRO) that is targeted to the tonoplast of the central vacuole[6], where it post-translationally activates one or more H_2O_2-dependent anthocyanin-degrading enzymes (ADEs). We will present evidence that FA homologs contribute to the fading of flower and fruit color in other nightshades and discuss potential mechanisms by which FA may activate ADEs.

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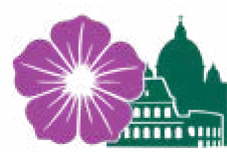
Scientific session V

Biotic and Abiotic Stress



From Gerbera to Petunia: Engineering Polyketide-Based Defense

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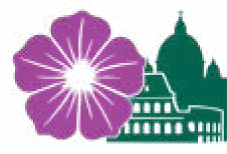
Abstract

Tissues of the ornamental plant *Gerbera hybrida* accumulate structurally distinct polyketide-derived defense metabolites, the triketide-derived -lactone glucosides gerberin and parasorboside, and the rare pentaketide derived 5-methylcoumarin glucoside gerberinside. Gerberin and parasorboside function as bitter-tasting insect antifeedants, while gerberinside, or its aglycone, exhibits strong inhibitory effects on the growth of pathogenic fungi.
The biosynthesis of these compounds is initiated by chalcone synthase-like polyketide synthases. GERBERA 2-PYRONE SYNTHASE 1 (G2PS1) is responsible for the formation of gerberin and parasorboside [1], whereas G2PS2 and G2PS3 are involved in gerberinside biosynthesis [2]. Both pathways require reductive tailoring of the polyketide backbone, followed by glucosyltransferase (GT) activity to yield the final glycosylated products. Reductases involved in gerberin and parasorboside biosynthesis have been identified [3,4], and candidate enzymes for coumarin reduction and glycosylation are



currently under investigation. As these biosynthetic pathways become increasingly well characterized, their transfer to heterologous hosts, such as tobacco or petunia, can be explored. Co-expression of G2PS1 with selected reductases results in the accumulation of gerberin and/or parasorboside in tobacco, likely facilitated by endogenous GT activity in the host plant.

Although achieving sufficient levels of metabolite accumulation remains a challenge, the successful transfer of complete defense metabolite pathways with functional protective effects appears increasingly feasible.

Keywords:

Gerberа
Petunia
Secondary metabolism
Defense

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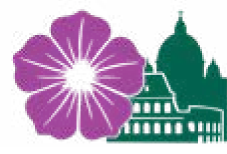

Screening Petunia for tobacco mosaic virus resistance

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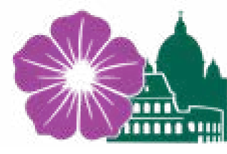
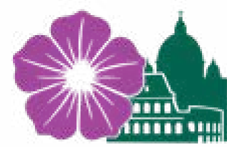
Abstract

Already hundred years ago the significant impact of tobacco mosaic virus (TMV) infection on Nicotiana tabacum has been reported resulting in 33 % yield reduction (Valleau and Johnson, 1927, Phytopathology 17:523-527). Since then, more than 100 experimental hosts have been described, among those many belong to the Solanaceae comprising global crop plants like tomato, pepper and Petunia (Zamfir et al, 2023, https://doi.org/10.1094/PHYTO-11-22-0439-V). In the later a wide range of symptom expression has been observed reaching from no apparent signs of infection to strong mosaic patterns and deformations in leaves. We inoculated seedlings of P. axillaris, P. exerta, P. inflata, P. violaceae, P. hybrida cv. Rose du Ciel with equal amounts of purified TMV virions of the JKI isolate Le98-1870, propagated in P. hybrida, for investigation of virus abundance in new developed leaves and associated phenotypes using electron microscopy (EM) and SYBR Green real time RT-PCR. Degenerated primers spanning the 3' part of the movement protein (MP), the complete coat protein (CP) and 300 nt from the viral genome 3' end synthesized a one kb fragment (Letschert et al. 2002, J Virol Methods 106,1-10).



All plants tested negative for TMV in EM before inoculation. Weekly EM monitoring for three weeks revealed a complex and dynamic pattern of TMV presence and spread in the investigated petunia species. Sampling timepoints at 7 dpi and 21(24) dpi seemed most suitable. The preliminary data identified distinct responses in P. inflata S6 as a host facilitating onset of TMV infection by showing highest number of virions and deformed leaves already 7dpi and P. axillaris S1 as a host supporting viral replication illustrated by highest virus titers in EM 21dpi and by the lowest ct values at 24 dpi. After 24 dpi all inoculated petunia species were TMV positive but displayed distinct virus titers. P. violaceae, P. hybrida and interestingly P. axillaris S26 showed lower TMV concentration (ct around 20) while P. axillaris S1 promoted viral replication (ct 8) followed by P. axillaris S21 (ct 11), P. inflata (ct 12) and P. exerta (ct 14). The real time RT-PCR results 24 dpi were consistent with phenotyping and EM observations 21dpi. In a second step we will design Taqman probes out of the small subunit of the viral replicase (p126), the movement protein (MP) and the coat protein (CP) since their counterplay determines the course of infection in the host (Spiegelman and Dinesh-Kumar, 2023, doi.org/10.1146/annurev-virology-111821-122847) and will allow us to decipher if viral movement and/or replication are driving TMV infection in the tested Petunia.



Epigenetic Priming of defense gene expression by repeated Botrytis cinerea infection in Petunia

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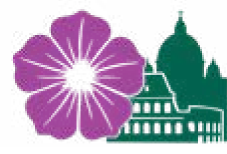
Abstract

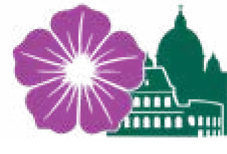
Climate change is affecting all aspects of horticulture, including infection with various pathogens. Here, we studied the effect of repeated Botrytis cinerea infection, identifying inherited biotic stress induced changes in gene expression and phenotypes. The focus of this work is on in-plant infections of the leaves of Petunia hybrida 'Mitchell' as well as their cuttings. First, the infected leaves as well as non-infected leaf material were harvested and the regulation of selected immune genes was checked by RT-qPCR. Interestingly, the induction of gene expression after four consequtively botrytis infected generations was much higher compared to the first infection. The presented results show that implementation of this assay is possible and a plant immune system response can be "trained" as well as passed on to follow-on generations.

In parallel, the plants after 4 botrytis infection generations were used for generative propagation, in order to investigate if this "trained" effect could be transferred to the offspring generation or not. In the preliminary data, we observed botrytis-induced



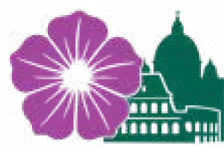
change of leaf phenotype (length, width of the 1st 5er constellation site) on “naïve” plants lines (which their parental plants were not contacted with botrytis at all). This effect was observed also in sister lines which at least one of the parental lines ever contacted with botrytis. With the exception of “pollen (botrytis-contacted) x carpel (mock)” lines, in which the botrytis-induced leaf phenotype was attenuated back to the “naïve” plant level upon the botrytis infection.
This brought us to consider a putative priming effect via a pollen-inherited manner. It will be interesting to further investigate whether the expression level of immune genes is changed in these candidate lines.

In order to identify corresponding epigenetic modifications related to the observed effects, we started to analyze DNA methylation in a genome wide manner to identify differentially methylated regions first. Since genomes of petunia are still not fully available and annotations are scarce, we established the state of the art third generation sequencing in FGK to tackle this issue. One of those platforms, namely Oxford Nanopore Technology (ONT), was being established. In comparison to previous sequencing technologies, ONT produces long reads which enables us to overcome several structural obstacles during Genome analysis. We generated data consisting of sequence length spanning several Kilo-base-pairs ranges. In addition to the plain sequence information, ONT could also be used for direct Methylome analysis. With this capability, the step of chemical or enzymatic treatment for Methylome analysis, which is notorious for huge loss of materials, could be totally skipped.



During the last years, the methylation detection of 5mC on several experimental combinations could be established, highlighting the overlap with previous whole genome bisulfite sequences. Finally, we found a correlation between the “trained” genes and corresponding changes in the DNA methylation states of the respective gene promoters. Thus, the analysis of epigenetic states at these promoters might furthermore be used to identify plants with a higher resilience against Botrytis infection.

Keyword:
Botrytis cinerea
Nanopore
Direct Methylome
Phenotypic variation



From wheat to Petunia: development of a plant model for high-resolution cellular tracking of fluorescent nanoplastics

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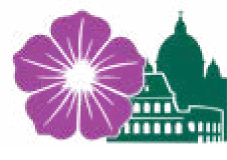
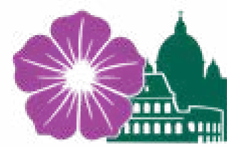
Abstract

Nanoplastics are emerging contaminants of increasing concern, owing in part to their potential uptake, translocation, and accumulation in plant systems. While crop species such as wheat are widely used to investigate the effects of nanoplastic exposure [1,2], their complex tissue architecture often limits high-resolution cellular imaging and localization studies. In this work, we developed a white-flowered Petunia hybrida model to investigate the uptake, transport, and cellular localization of fluorescent polystyrene nanoplastics (PSNPs) using confocal microscopy and quantitative image analysis. Plants were exposed to fluorescent PSNPs via both stomata infiltration and hydroponic uptake experiments over multiple exposure times (2, 24, 48, and 72 h). For both exposure approaches, tissues were stained with fluorescein diacetate (FDA), a vital fluorescent probe widely used to visualize the cytosol and nuclei of living cells and intracellular organization.



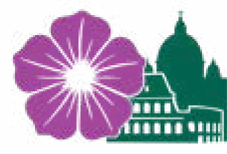
Following forced infiltration, confocal z-stack imaging revealed that PSNPs were predominantly localized in cell wall-like compartments after 2 h. At 24 and 48 h, fluorescent puncta progressively appeared within cytosol-like regions, while at 72 h the signal was additionally associated with vacuole-like structures, suggesting a time-dependent intracellular redistribution of PSNPs. Petunia plants grown hydroponically in contaminated solutions displayed a similar localization trend, with clear cellular uptake detected after 72 h. However, compared with infiltrated tissues, hydroponically exposed plants showed substantially lower PSNPs abundance in cell wall- and cytosol-like compartments at 24 and 48 h, consistent with the slower kinetics expected from root uptake and systemic translocation processes. Quantitative image analysis performed in Fiji/ImageJ included particle segmentation, fluorescent-positive area quantification, fluorescence intensity analysis, and colocalization measurements using Pearson's correlation and Manders' coefficients. Control samples showed negligible fluorescent signal, whereas treated tissues displayed increased particle abundance and intracellular spatial association, as supported by FDA-assisted cellular visualization. Whole z-stack analyses demonstrated that fluorescent signals were present across the entire floral tissue not only in the superficial layers. In addition, metabolic analyses revealed treatment-associated physiological alterations, linking nanoplastics accumulation with biological responses at the cellular and metabolic levels. Overall, our results demonstrate that white-flowered Petunia represents a powerful model system for high-



resolution visualization and quantitative cellular tracking of PSNPs derived fluorescence in plants. The combined use of controlled stomata infiltration and hydroponic exposure approaches provides new insights into the temporal dynamics of PSNPs uptake, intracellular trafficking, and tissue accumulation in plant systems. This represents a first approach for an in-depth study of the putative mechanisms for PSNPs uptake and translocation in plant tissues and organelles.

Keywords:
Polystyren
Nanoplastics
Co-localization
Cell

1. Benedetta Pizziconi, Giuliana Bruno, Samuela Palombieri, Vittoria Locato, Francesco Sestili, Sara Cimini, Laura De Gara, Transcriptomic and metabolic reprogramming of durum wheat under nanoplastic exposure, Plant Nano Biology, https://doi.org/10.1016/j.plana.2026.100274.
2. Benedetta Pizziconi, Giuliana Bruno, Samuela Palombieri, Francesco Sestili, Sara Cimini, Laura De Gara, From soil to shoot plant responses to polystyrene nanoplastics and relevance for sustainable food systems, Plant Nano Biology, https://doi.org/10.1016/j.plana.2025.100230



Establishing Mycorrhizal Marker Lines in Transgenic Mutator Petunia hybrida W138 for Forward Genetics Research

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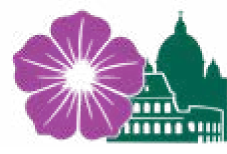
Abstract

The *Petunia hybrida* W138 line, carrying a *dTph1* insertion in the *AN1* gene for anthocyanin biosynthesis, along with 150–200 additional insertions elsewhere in the genome, represents a powerful resource for forward genetics. However, its use has been limited by the absence of a widely adopted and well-documented genetic transformation protocol. To circumvent this limitation, earlier approaches relied on transgenic GUS reporter lines in the W115 Mitchell background, which were subsequently crossed with W138 mutator populations[1]. While effective, this strategy is time- and labor-consuming and restricts experimental flexibility. Moreover, crossing with the Mitchell background reduces the effective transposon copy number, thereby potentially decreasing mutagenesis efficiency.
Here, we report progress toward the direct genetic transformation of W138. Using *de novo* meristem induction from shoot tissues, we established a transformation pipeline that enables the introduction



of reporter constructs into W138. In parallel, we generated transgenic lines of *Nicotiana benthamiana* expressing the betalain-based RUBY reporter to evaluate its suitability as marker in the context of arbuscular mycorrhizal (AM) symbiosis.
Our results show that RUBY expression in *N. benthamiana* provides a robust visual marker without major detrimental effects on plant growth. Importantly, preliminary analyses indicate that RUBY expression does not impair AM colonization, suggesting that it can serve as a non-invasive reporter for symbiotic studies. In contrast, GUS-based assays require destructive sampling and are less suitable for dynamic analyses.
In W138, we demonstrate successful regeneration of transformed flowering shoots following meristem induction, representing a critical step toward stable transformation of this genotype. Ongoing work focuses on improving transformation efficiency and validating reporter expression in the context of AM symbiosis.
Together, these results establish a foundation for the use of visual reporter systems in W138 and open new avenues for forward genetic screens targeting mycorrhizal traits in *Petunia*.

Keywords:

Petunia hybrida W138
dTph1
RUBY reporter
Genetic transformation
Arbuscular mycorrhiza

1Wegmüller S, Svistoonoff S, Reinhardt D, Stuurman J, Amrhein N, Bucher M (2008) A transgenic dTph1 insertional mutagenesis system for forward genetics in mycorrhizal phosphate transport of Petunia. Plant J https://doi.org/10.1111/j.1365-313x.2008.03474.x

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