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Plant Senescence

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The 10th International Symposium on Plant Senescence

The International Symposium on Plant Senescence is a biennial symposium that gathers participants from all over the world to discuss recent advances in different aspects of plants and their organs (leaf, flower, and fruit), aging, crop senescence, and stress biology research, including postharvest-induced senescence.

Senescence is a complex, genetically programmed phenomenon that affects plant longevity, and in agricultural systems, it affects the yield and quality of crops. Natural senescence is controlled by developmental age and environmental stressors. Understanding the senescence process in plants is essential for improving agriculture by increasing the yield of agronomic plants. In their research, the biologists attending the conference will focus on a fundamental understanding of the influences on the senescence of plant cells, such as genetic or environmental factors, and how it is possible to manipulate the process of senescence to obtain desirable results, such as making crops more resistant to abiotic stresses or improving the postharvest quality of produce.

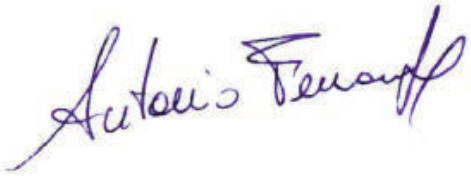
The International Symposium on Plant Senescence returns after five years of interruption due to COVID-19. The last edition was held in Berlin, Germany in 2019. The 10th International Symposium on Plant Senescence was organised by the Institute of Crop Science of the Sant'Anna School of Advanced Studies under the aegis of the Italian Society for Horticultural Science (SOI).

At this 10th edition of the Symposium, six keynote lecturers from invited speakers were planned. Moreover, the scientific committee selected from the abstracts received 10 oral presentations and 10 short oral presentations.

I hope that you enjoy the symposium and stay at Pisa.

Pisa 17 July 2024

The Convener/Presidente of SOI
Prof. Antonio Ferrante



Book of abstracts

10th International Symposium Plant Senescence

Molecular mechanisms involved in the senescence regulation

Metabolic and biochemical changes under senescence

Natural and induced postharvest senescence

Biotic and Abiotic stresses in the senescence regulation

Natural and induced postharvest senescence

Autophagy, cell death, and senescence

From leaf senescence to crop resilience

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Leaf senescence represents the terminal stage of leaf development. It is regulated developmentally and environmentally. In addition to age and endogenous hormones (such as ABA, ethylene, SA, JA), all forms of abiotic stresses such as drought, salinity, extreme temperature, strong radiation, shading and darkness can readily induce leaf senescence. Therefore, inhibiting leaf senescence under abiotic stresses enhances crop resilience. It is believed that leaf senescence is driven by a subset of senescence-associated genes (*SAG*s) that account for approximately 10% of genes in a plant genome. Among *SAG*s are transcription factor genes and those involved in signal transduction. My lab has focused on deciphering the molecular regulatory mechanisms underlying leaf senescence and devising ways to manipulate/delay senescence for enhanced crop resilience. Specifically, we have identified *AtNAP* (a NAC family transcription factor) as a master regulator of senescence of leaves, fruits, and flowers. Chip and CHIP (chromatin immunoprecipitation) analyses revealed ~180 potential target genes of *AtNAP*, among which is *SAG113*, a protein phosphatase 2C gene. We have identified SAG114 SnRK3.25 that is dephosphorylated by SAG113 in vitro and in planta. By utilizing proteomics and phosphoproteomics analyses of *sg114* and WT we have further identified hundreds of genes that are targets of SAG114 SnRK3.25 that are under investigation. We have also performed translational research on leaf senescence. The future direction will also be discussed.

The ubiquitin E3 ligase UPL5 controls cold-induced leaf senescence in *Arabidopsis*

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Introduction

The catabolism of macromolecules is a significant process in senescent cells, particularly involving the proteolysis of organelles and abnormally aggregated proteins, nutrient circulation, and precise control of intracellular environmental balance. Proteasomes are distributed in the nucleus and cytoplasm; however, their presence in organelles is limited.

Method and Materials

In this study, multi-omics proteomic analyses were conducted to investigate global changes in protein levels and ubiquitination modification levels of *upl5* mutant relative to wildtype plant using an antibody against "di-Gly-Lys" *via* a free labeling assay. Subcellular localization analysis revealed that UPL5 is located in the nucleus, cytoplasm, and plastid within the cell; furthermore, the direct lysine site pattern of UPL5 was screened by H89R substitution in the tagged ubiquitinated assay. Among putative candidates, WHIRLY2 protein was selected as it is triply located in mitochondria, plastid, and nucleus affecting leaf senescence and silique development.

Results

The results revealed HECT-type ubiquitin E3 ligase UPL5's ability to interact with WHIRLY2 in cytoplasm, plastid, and nucleus. It can selectively ubiquitinate various Kub-sites of WHIRLY2 to alter its distribution between plastid and nucleus both in vivo and vitro. The UPL5-WHIRLY2 module maintains plastid genome stability by responding to $[Ca^{2+}]_{cyt}$ induced by cold stress or $CaCl_2$ stress.

Conclusions

This study demonstrates that integration of UPL5 mediated 26S proteasome or ubiquitin-proteasome system with regulation of WHIRLY2 protein allocation affects organelle genome stability during cold-induced leaf senescence; thus suggesting that UPL5 acts as a candidate for organelle E3 ligase either in the nucleus or cytoplasm or plastid modifying numerous targets related to nuclear transcription and plastid photosynthesis involving Ca^{2+} signaling pathway during plant senescence response to (a)biotic stress protection.

Key Words: HECT-type E3 ligase UPL5; protein allocation; cold-induced cell senescence; *Arabidopsis*

The WRKY25 can act as redox switch to drive the expression of *WRKY53* during leaf senescence in *Arabidopsis thaliana*

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Introduction

Senescence is a process requiring high plasticity and, therefore, has to be coordinated and regulated by a complex molecular network. Transcriptional regulation by WRKYs and NACs factors plays a central role in many plant species. In Arabidopsis, WRKY53 functions as one of the central hubs within this network. Interestingly, WRKY18 acts as a strong negative regulator of *WRKY53* expression, while WRKY25 serves as positive regulator, creating a small complex subnetwork. We aim to characterize the interactions within this subnetwork, their specificity and selectivity.

Method and Materials

We investigated the molecular mechanisms driving interactions within the WRKY18/WRKY25/WRKY53 subnetwork by dissecting their protein structures. Through domain-swapping experiments between WRKY18 and WRKY25, we examined whether exchanging the DNA-binding domains of these proteins alters their regulatory functions. Transactivation potential under normal and oxidizing conditions was analyzed by transient reporter gene analyses in Arabidopsis protoplasts Homo- and heterodimerization were characterized using Bimolecular Fluorescence Complementation (BiFC) in planta, employing the efficient tobacco leaf system. Transgenic plant complementation lines were created to reveal the impact of domain swapping on senescence. Protein structure was modeled using “Alpha Fold”.

Results

Our transactivation assays suggest that the N-terminal domain of WRKY25 is crucial for its activator effect on *WRKY53* expression. BiFC assays revealed that WRKY25 forms heterodimers but not homodimers, with the N-terminal domain essential for protein-protein interactions. The impact on WRKY53 and senescence regulation was validated *in planta* using transgenic complementation lines. Under oxidative conditions both *in vitro* and *in planta*, WRKY25 specifically downregulates *WRKY53* expression. Modeling WRKY25 indicated a putative covalent lysine-cysteine redox switch in both DNA-binding domains.

Conclusions

WRKY25 acts as a redox switch, balancing the expression and interactions of the WRKY53/WRKY25/WRKY18 network to ensure progressive senescence induction. This enhances our understanding of plant senescence regulation and highlights the significant role of redox conditions in plant regulatory processes.

Key Words: *Arabidopsis*; senescence regulation; WRKY53/WRKY18/ WRKY25 subnetwork; ROS

Assessing salt tolerance through senescence markers in *Solanum lycopersicum* and *Solanum pimpinellifolium*

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Introduction

Tomato is a crucial vegetable crop and is considered moderately salt tolerant (FAOSTAT, 2024). The negative impact on crop productivity is intensifying since available soil and water resources are becoming increasingly salinized (Munns, 2002; Al-Busaidi et al, 2009). Salinity triggers physiological, metabolic and morphological events causing senescence and reducing yields (Ghanem et al, 2008). In this scenario, it is valuable to identify senescence markers that could be useful in assessing tolerance to salt stress. An interesting approach is to study salt stress in wild relatives of *Solanum lycopersicum*, such as *Solanum pimpinellifolium*, which are adapted to harsh environments and have high levels of tolerance to salinity stress (Bohra et al, 2022) In this work, *in vitro* and *in vivo* greenhouse screening protocols have been developed to identify senescence markers useful to improve salt tolerance by avoiding or delaying senescence. The usefulness of the *in vitro* screening was to identify in a small space, such as a growth chamber, and in a limited time frame of 12 days, the accessions to be studied later *in vivo*.

Method and Materials

Two trials were performed. In the first *in vitro* screening trial, 1 variety of *S. lycopersicum* and 21 accessions of *S. pimpinellifolium* were grown on plates with sucrose-free agar medium containing two different salt concentrations corresponding to 30% and 60% seawater in the medium. In the second greenhouse trial, five accessions were compared to a tomato cultivar in a closed-loop system using two increasing salt treatments (EC = 11 mS/cm and 21 mS/cm, corresponding to 18% and 33% of seawater). These two concentrations were identified in a preliminary experiment as the best for identifying the different behavior in response to salt in the two species. In both experiments, biochemical, physiological and morphological parameters related to senescence were collected.

Results

In both experiments, senescence markers were useful in identifying the most salt-stress tolerant accessions of *S. pimpinellifolium* (Cialli et al., 2024). In the first experiment it was possible studying the chlorophyll content and the salt injury level parameters (S.I.L.). S.I.L. is based on senescence sign on the aerial part, such as yellowing of the leaves, and on the roots, such as root necrosis. Moreover, in the *in vivo* trial, the higher resistance of the accessions evaluated *in vitro* was confirmed by observing delayed leaf senescence symptoms as lower ethylene production and loss of cell membrane permeability.

Conclusions

With regard to the *in vitro* experiment, in all *S. pimpinellifolium* accessions the chlorophyll content was higher than in the cultivar, while the S.I.L. was lower, even when grown on T60. Concerning the *in vivo* greenhouse trial all S. pimpinellifolium accessions recorded lower ethylene levels when grown under high salt stress conditions compared to *S. lycopersicum*. *S. pimpinellifolium* recorded higher EL values, compared to the cultivar, possibly related to the efflux of K+, which is abundant in plant cells (Hniličková et al., 2019). In conclusion, we can state that senescence markers can provide useful infor-

mation for the study of *Solanum* spp. response to salt stress. Furthermore, the *in vivo* trials validated the robustness of the *in vitro* screening, confirming the high salinity tolerance of *Solanum pimpinellifolium*.

Acknowledgment

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Key Words: *in vitro*; greenhouse; *S. lycopersicum*; *S. pimpinellifolium*

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NtMYB1 and NtNCED1/2 control abscisic acid biosynthesisand tepal senescence in Chinese narcissus (*Narcissus tazetta*)

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Introduction

Chinese narcissus (*Narcissus tazetta*) is one of the 10 most famous traditional flowers of China, having a beautiful and highly ornamental flower with a rich fragrance. The flower longevity affects its commercial appeal. Petal senescence features hormone imbalance such as altered levels or ratios of abscisic acid (ABA) and ethylene. However, ABA-induced ethylene release is not responsible for petal senescence in all plants. While petal senescence in Narcissus is ethylene-independent and abscisic acid-dependent, the regulatory mechanism has yet to be determined.

Method and Materials

In this study, we identified a R2R3-MYB gene (NtMYB1) from *Narcissus tazetta* and generated *oeNtMYB1* and *ntmyb1* RNA interference mutants in Narcissus as well as an *oeNtMYB1* in *Arabidopsis*. Meanwhile, we isolated the promoter sequences of *NtNCED1* and *NtNCED2* by using a genome walking assay. A dual-luciferase assay and chromatin immunoprecipitation–quantitative PCR revealed that NtMYB1 controls *NtNCED1*/ *NtNCED2* expression both *in vivo* and *in vitro*. We comparatively analyzed alterations of metabolites in tepals and leaves of *Narcissus* and leaves of *Arabidopsis* caused by an ABA biosynthesis inhibitor.

Results

Overexpressing *NtMYB1* in *Narcissus* or *Arabidopsis* led to premature leaf yellowing, an elevated level of total carotenoid, a reduced level of chlorophyll b, and a decrease in photosystem II fluorescence (Fv/Fm). NtMYB1 directly binds to the promoter of *NtNCED1* or *NtNCED2* and activates *NtNCED1/2* expression. Moreover, overexpressing *NtMYB1* accelerated abscisic acid biosynthesis, up-regulated the content of zeatin and abscisic acid, and down-regulated the level of β-carotene and gibberellin A1, leading to petal senescence and leaf yellowing in *Narcissus*.

Conclusions

A NtMYB1–NtNCED1/2 module redirects metabolism flux of GA, ABA, and β-carotene biosynthesis to cause petal senescence and leaf yellowing in *Narcissus* and *Arabidopsis*, revealing a regulatory process that is fundamentally different between leaves and petal.

Key Words: ABA biosynthesis; metabolic flux; *Narcissus*; NtMYB1; *NtNCED*; tepal senescence

Small peptides in plant senescence: big pictures to be unveiled

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Plant peptides are a category of small signaling molecules that are expressed, secreted and post-translationally processed from precursor proteins. By binding to receptor proteins on the cell membrane and thereby initiating downstream signal transduction cascades, plant peptides play essential roles in intercellular signal communication, governing processes like plant stem cell maintenance, defense responses, pollen-stigma recognition, organ shedding, and several stress responses. In recent years a number of plant peptides have been characterized to be key regulators of plant senescence and complex regulatory networks involving peptides from different protein families are getting unveiled. The CLE14 peptide functions as a ”brake signal” of leaf senescence at late developmental stages via activating the expression of NAC family transcription factor JUB1 which plays a role in suppressing ROS accumulation during senescence. The IDL6 peptide on the other hand, promotes senescence of Arabidopsis leaves. More interestingly, SCOOP10 and SCOOP12, two peptides from the same family, compete for the same receptor in regulating leaf senescence: SCOOP10 promotes senescence at early stages and SCOOP12 expression is activated at late stages to suppress senescence. Both SCOOP peptides bind and function through the receptor-like kinase MIK2.The fine-tuned coordination of signaling cascades initiated by different peptides ensures an orderly senescence process which is critical for remobilizing nutrients from senescing leaves to sink tissues. Crosstalk between peptide signals and traditional phytohormones and potential application of plant peptides in agriculture will be discussed.

Key Words: leaf senescence; plant peptide; receptor-like kinase; signal transduction

Molecular regulation and hormonal signaling for heat-induced leaf senescence in perennial grass species

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Introduction

Leaf senescence is a typical characteristic of heat stress, which attributes to the reduction of photosynthesis and plant growth. Molecular and metabolic factors regulating heat-stress induced leaf senescence are beginning to be unraveled with the advancement of biotechnology and omics technology. This presentation will discuss mechanisms underlying heat-induced leaf senescence in perennial grass species by overviewing and integrating information from multiple studies on cool-season species.

Methods and Materials

Effects of heat stress on leaf senescence were examined and compared among different cultivars or transgenic plants of perennial ryegrass or creeping bentgrass. Metabolites, hormones, transcriptional factors and candidate genes involved in heat-induced leaf senescence or stay-green traits were analyzed.

Results and conclusions

Heat-induced leaf senescence in cool-season turfgrass was mainly due to accelerated chlorophyll degradation. Heat-activated activity of chlorophyll degrading enzymes and genes contributed to breakdown of chlorophyll degradation and accelerated leaf senescence. Through genetic analysis, RNAi transformation, and transcriptomic profiling of perennial ryegrass, several genes (SGR, PPH, NYC1, PAO, and NOL) related to chlorophyll degradation were found to regulate heat-induced leaf senescence in cool-season grass species. Several molecules, including salicylic acid, cytokinins (CK), ethylene, abscisic acid (ABA), and Ca were identified as key signaling molecules of those senescence-related genes. Several of these senescence-related genes were direct downstream target transcription factors in CK, ethylene or ABA signaling pathways. The metabolic and molecular factors for signaling and transcription factors that may activate or deactivate expression of chlorophyll-degrading genes controlling heat-induced leaf senescence will be further discussed in the presentation.

Key Words: perennial grass species; heat stress; leaf senescence

Decoding the intricate metabolic and biochemical changes in plant senescence

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Plant organ maturation and senescence are crucial, irreversible processes that transition organs from nutrient assimilation to nutrient redistribution, significantly impacting the quality and value of agricultural crops. The initiation and progression of these processes rely on complex genetic programs influenced by both internal and external signals [1]. During leaf senescence, photosynthetic activity is notably reduced, primarily due to the breakdown of chlorophyll and the dismantling of chloroplast structures. In particular, a reduction in chlorophyll a, and associated pigments like pheophytin and violaxanthin, accompanied by a decrease in chloroplast lipids, including mono galactosyl diacylglycerols (MGDGs) and digalactosyl diacylglycerols (DGDGs), signal the onset of senescence, with reduced photosynthetic capacity ad overproduction of reactive oxygen species (ROS) playing a key role in increasing oxidative stress and regulating ageing. Primary metabolites such as sugars and amino acids are closely linked with photosynthesis to meet the plant's energy and metabolic demands, aiding stress tolerance [2]. Sugars, in particular, play critical roles in plant growth and aging. In Arabidopsis, sugar signalling influences the transition from the juvenile to the adult phase; lower sugar levels increase miR156 levels, restricting this transition, whereas exogenous sucrose promotes adult leaf growth by repressing miR156 genes [3]. Sugars also affect the timing of leaf senescence; lower sugar levels delay senescence, while high glucose and low nitrogen levels induce it [4]. A continuous increase in sugars such as fructose, glucose, sucrose, and sugar derivatives like galactinol and raffinose happens during senescence [5]. Mutations in sugar-sensing genes such as Hexokinase-1 (HXK-1) and sucrose nonfermenting1 (SNF1)/SnRK1 also affect senescence timing [2]. Other metabolites involved in ROS and stress regulation also influence plant ageing. In Arabidopsis, mutants of GABA transaminase exhibit higher ROS accumulation, early flowering, and senescence [6]. Accumulation of GABA and other stress-related amino acids like proline and alanine occurs at the same time as branched-chain amino acids (BCAAs) and aromatic amino acids [5]. Overexpression of arginine decarboxylase and polyamine uptake transporters delayed flowering and senescence. The Polyamine Oxidase 4 mutant shows delayed senescence, reduced ROS levels, and increased redox-regulating metabolites [6]. The spotted leaf32 mutation in rice affects glutamate biosynthesis, leading to early senescence due to higher ROS levels [2]. Changes in ratios of glutamine/glutamate and asparagine/aspartate reflect the complex regulation of nitrogen transport and utilisation [5].

The complex interplay between hormones, transcription factors, and abiotic stresses is crucial in regulating senescence. Ethylene, abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) are known to accelerate senescence by upregulating ethylene-responsive genes in mature leaves. Ethylene, in particular, interacts with oxidative stress pathways, influencing the timing and progression of senescence by regulating oxidative enzymes such as peroxidases. The upregulation of energy metabolism pathways, especially the tricarboxylic acid cycle and nitrogen recycling, is critical during leaf senescence [1].

Recent findings highlight a new role for alternative oxidase (AOX) in ripening and/or senescence. AOX modulates respiration during the acceleration of the respiratory phase by providing an alternative pathway for electron flux, thus preventing over-reduction of the mitochondrial electron transport chain and enhancing ripening and/or senescence-associated phenotypes [7]. Autophagy is upregulated during senescence, helping to break down macromolecules such as proteins and releasing amino acids that can be transported to other parts of the plant. Proteolysis also plays a significant role in nutrient recycling

during senescence. However, senescence is not merely a passive process leading to death but an active process where nutrients, particularly nitrogen, are mobilised from the senescing leaves to other parts of the plant. This remobilisation is crucial for developing reproductive organs such as seeds and fruits. For instance, in crops like wheat and rice, a significant portion of the nitrogen in the grains is remobilised from the leaves [8].

The timing and regulation of senescence can significantly affect crop yield and quality. Delaying senescence (functional stay-green phenotype) can maintain photosynthetic activity for longer, potentially increasing biomass and yield. However, promoting senescence at the right time can enhance nutrient remobilisation, improving the nutritional quality of seeds and fruits [3]. Understanding these complex metabolic and biochemical changes during plant senescence is essential for improving horticultural quality and yield.

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Key Words: chlorophyll degradation; oxidative stress; sugar signalling; nutrient remobilization; GABA

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Whole plant resource integration and function during flooding induced senescence

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Introduction

Complete submergence of plants causes a strong reduction in the gas diffusion rate and lowers light availability due to murky water. This reduces oxygen availability and photosynthesis causing a carbon and energy crisis. Subsequently, leaves start to senesce in order of age, the oldest first, then the youngest. The shoot apex (meristem and primordia) dies last. Maintaining the shoot apex is imperative to plant survival, since it is crucial for regrowth. We hypothesized that old leaves sacrifice themselves to provide metabolites to sustain the essential shoot apex. Since phloem loading transport is energetically demanding combined it remains unclear whether resources yielded by senescence can be transported during flooding and whether this would improve flood stress tolerance.

Method and Materials

We studied small and large Arabidopsis plants, which differ in source strength when senescence starts. Additionally, we consider *Rorippa sylvestris*, a close relative and highly tolerant to flooding. With 13C labelling, we efficiently enriched the structural fractions and with LC-MS we tracked the fluxes of senescence released metabolites.

Results

Irrespective of species or developmental stage, metabolites are transported to the shoot apex. These are primarily carbon rich amino acids. Interestingly, the presence of movement does not aid survival, partly because the rate of breakdown is independent from resource demands at the shoot apex.

Conclusions

Senescence is a key aspect of flooding responses, where the metabolic signature of amino acid mobilization is distinct from developmental senescence. Despite functionally moving resources, senescence does not contribute to survival during flooding.

Key Words: flooding; 13C flux analysis; metabolite movement; resource integration

Postharvest metabolic changes in *Brassica rapa* L. subsp. *sylvestris*

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Introduction

Upon harvest, leafy vegetables experience significant alteration of metabolism, triggering oxidative stress and senescence. This cascade of physiological changes adversely affects nutritional, nutraceutical, and organoleptic properties. This study examined postharvest metabolic changes in the leaf blades of *Brassica rapa* L. subsp. Sylvestris (friariello Napoletano) ecotype Sessantino.

Method and Materials

Leaf blades were harvested and frozen in liquid nitrogen for subsequent analyses at commercial maturity or after storage at 10 °C or 4 °C for 2 or 20 days. Proteins, free amino acids, and proline, soluble sugars and starch, ions were estimated according to Dell'Aversana [1]. Glucosinolate according to Nacca [2]. Ascorbate and dehydroascorbate, α- and γ-tocopherol, chlorophylls, and carotenoids were determined according to Annunziata [3].

Results

The control (Ctrl) shows significantly higher levels (by over 50%) of sucrose, fructose, starch, proteins, chlorophylls, reduced ascorbate, and RWC compared to postharvest samples. Storage caused a decrease in sucrose and starch (by 75%), while several amino acids, including Asn and Gln, increased by more than 100% in stored samples. The increase in amides is due to amino acid catabolism, which releases carbon skeletons (acetate and oxalacetate) but also ammonia that must be re-organicated to avoid further metabolic disturbances. At 10 °C for 20 days, samples showed a significant decrease in chlorophylls (by 80%) and ascorbate (by 80%) and an increase in tocopherols (by over 200%). Tocopherols are likely synthesised from phytol release from chlorophyll catabolism, providing oxidative stress protection (Figure 1).

Conclusions

Prolonged cold storage, especially at 10°C, induces significant metabolic changes, reflecting stress responses and alterations in primary and secondary metabolites. These changes are largely driven by the plant's efforts to protect tissues from oxidative stress.

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Key Words: *Brassica rapa* L.; amino acids; tocopherols; oxidative stress

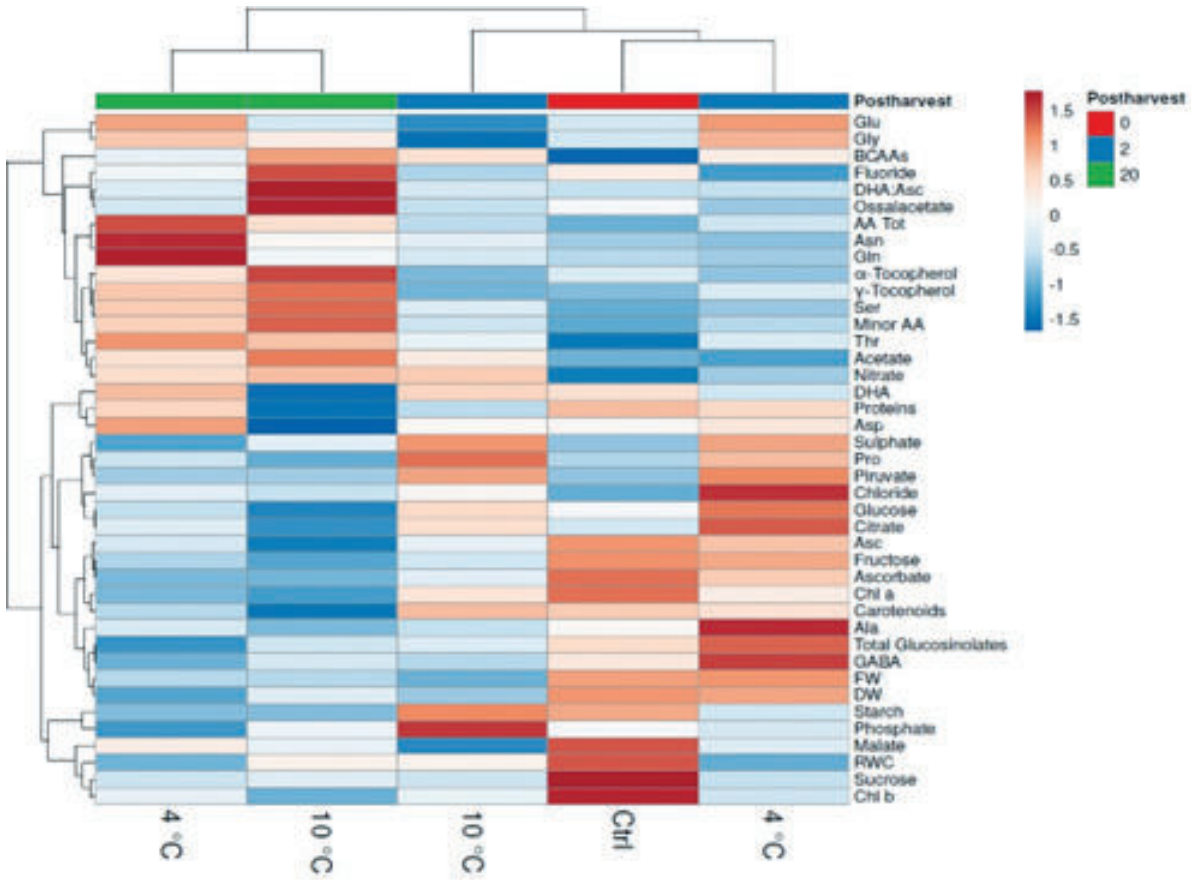

Figure 1. Heatmap showing the metabolite and ion changes in frarielli leaves during post-harvest for 2 and 20 days at 4 °C and 10 °C.

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Omics and epigenetics of plant organ senescence post-harvest

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When plant organs are detached from the plant, a suite of biochemical and molecular processes are initiated which culminate in the death of the organ. To delay this process for fresh produce, the plant organs are typically refrigerated in the supply chain. They are also typically stored under low light or dark conditions. This alters the progression of the natural developmental senescence, superimposing additional stress responses. A range of plant organs are used as fresh produce including notably leaves, fruit and flowers. These respond differently to the post-harvest conditions. Their response can be charted though a range of omics techniques that reveal different aspects of this process. We have been using the volatile organic compound (VOC) profile to report on metabolic changes and find that the shift in volatilome profiles mirrors that of the whole metabolome. In addition the VOC profile is also important post-harvest for providing the flavour to the produce and a loss of flavour results in increased waste and reduced consumption of health-promoting fresh fruit and vegetables. In strawberries we have used ChIP seq to show that some of the metabolic changes are likely due to an epigenetic repression of key genes involved in aroma production as well as probably affecting post-harvest ripening and senescence. Another important aspect of post-harvest senescence is the effect of pre-harvest stress. We have shown in rocket salad that pre-harvest stress affects the volatilome, metabolome and transcriptome, again with indications that epigenetic processes may be important in modulating the response of the leaves to post-harvest conditions. Flowers present an even more complex multi-organ tissue, and in lilies an effect of postharvest handling is that young buds enter senescence instead of developing into open flowers. We have been using metabolomic, and transcriptomic studies to assess the key processes involved, showing changes an interplay of gene expression changes related to hormone signalling and resource allocation may be important in this process.

The dark side of leaf senescence

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Leaf senescence is a complex and regulated process that can be induced by dark conditions, as demonstrated in various studies. Dark-induced leaf senescence (DIS) mimics natural senescence by triggering typical symptoms such as chlorophyll and protein degradation. Interestingly, the presence of high soil water content has been shown to induce non-programmed cell death (non-PCD) and increase pheophorbide a (Pheide a) content in senescent leaves under darkness, which can be alleviated by overexpressing *7-hydroxymethyl Chl a reductase (HCAR)* (Hu et al., 2021). Additionally, the *SUBMERGENCE1A (SUB1A)* gene in rice plays a role in delaying dark-induced senescence by maintaining chlorophyll and carbohydrate reserves, and by modulating hormone responsiveness. Contradictory to the general acceleration of senescence by darkness, mild shading has been found to induce photosynthetic acclimation rather than senescence, suggesting that the degree of light reduction is a critical factor. Moreover, the regulatory mechanisms of DIS are complex, involving plastid signaling, hormonal regulation, and transcriptional changes. The transcription factor *SlWRKY37* has been identified as a key regulator of both jasmonic acid and dark-induced leaf senescence in tomato. The setup of DIS experiments and the measurement of senescence-related parameters are well described in the literature. Additionally, light receptors such as *GmCRY1s* have been implicated in the regulation of leaf senescence in response to reduced blue light. Furthermore, *JAZ7*, a regulator of jasmonate signaling, has been shown to suppress dark-induced leaf senescence by modulating *MYC* transcription factors. Lastly, treatments with cytokinins, gibberellins have been found to prevent rapid leaf senescence induced by dark, low-temperature storage in Lilium. In summary, DIS is a valuable model for studying the senescence process, revealing the involvement of water content, genetic factors such as *SUB1A* and *SlWRKY37*, light receptors, and hormonal pathways in its regulation. These findings underscore the complexity of the senescence process and the potential for genetic and environmental manipulation to delay senescence, with implications for crop yield and shelf life.

Understanding senescence delay of colour and firmness traits in CO_2-treated strawberries through transcriptomics

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Introduction

To maintain fruit quality after harvest, attempts to delay senescence are necessary. However, the molecular understanding of the control of postharvest senescence in strawberries remained unclear. In addition, senescence may be accelerated or delayed by treatments during storage. To identify the metabolic and molecular markers associated with colour and texture changes directly or indirectly related to the development of senescence, we performed a transcriptomic and metabolomic study of strawberries at 20 ºC at the time of consumption after storage under different storage conditions.

Method and Materials

A comparative study was carried out analysing strawberries (*Fragaria vesca* L. cv. Mara des Bois) after harvest (AH), cold stored with high CO_2 (CCS), cold stored with air (ACS) or directly exposed to 20 ºC without previous cold storage (NCS). Colour (CIE Lab), antioxidant capacity, flavonoid content by HPLC-QTOF-MS/MS, firmness and transcriptomic analysis (RNAseq) were analysed.

Results

Strawberry senescence at 20°C, as observed in NCS fruits, is accompanied by a change in red colour with a significant decrease in Chroma, accumulation of P-3-G and increased expression of several *UDP-glucosyltransferase* genes. Furthermore, the activation of the senescence process in NCS is consistent with the increase in H_2O_2 levels and with the results of the SEA, showing a functionally enriched category closely related to oxidative stress response processes. In terms of texture, we provide an overview of genes that control cell adhesion in CO_2-treated strawberries, as revealed by the clustering and localisation of genes generated by WGCNA.

Conclusions

High CO_2 pre-treatments preserve the colour and firmness quality characteristics of strawberries at the time of consumption. The genes (FvH4_3g42900, CslE1; FvH4_5g37950, β-glc; FvH4_6g38170, XTH15 and FvH4_1g00290, PL) were significantly repressed by high CO_2 treatment. Our results also provide new insights into the regulation of postharvest senescence in strawberries.

Keywords: transcriptoma; firmness; oxidative stress; colour

Computer vision system for non-destructive and contactless evaluation of quality traits in fresh rocket leaves (*Diplotaxis tenuifolia* L.)

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Introduction

The quality loss of fresh-cut rocket leaves, during the postharvest storage, is mainly due to senescence, strictly related to chlorophyll degradation that, therefore, is the most common index used to evaluate quality and freshness of this product. Another important indicator of leaves senescence in fresh-cut rocket is ammonium accumulation in plant tissues. It is reported that ammonium is a product of protein catabolism, thus it is considered an indicator of freshness, when it is detected in low amount in the vegetal tissues.

Traditional approaches for chlorophyll and ammonia content measurements in leafy vegetables include destructive methods, based on spectrophotometric assays. These approaches require specific laboratory equipment and destructive sampling, and they are considered expensive and time consuming. While for the ammonium analysis the destructive method is widely applied, for chlorophyll evaluation, modern, handheld sensors have received a considerable attention in the last decades because of their high accuracy and real time measurement in a non-destructive way directly on field or on minimally processed products. Many researchers developed various types of chlorophyll meters (e.g. multispectral and hyperspectral sensors) that measures the spectral reflectance of leaves to assess the total chlorophyll content. Many of these techniques are costly and complex and require the presence of specialised personnel. Although such instruments are simpler, faster and cheaper than chemical analysis, they need to touch the leaf to measure the chlorophyll content of a limited area of the leaf surface. Therefore, their use in industrial lines is limited, also because the estimation of the chlorophyll content depends on the quality of sampling.

Recently, image analysis based on common digital RGB cameras has proved to be a promising approach for the assessment of chlorophyll content of leafy vegetables in smart agriculture and postharvest quality assessment. Imaging systems proved to be more robust than area-based instruments as they work at pixel level considering the entire visible surface of the product.

The success of Computer Vision Systems (CVS) is due to the possibility of establishing relationships between spectral reflectance indices and chlorophyll absorbance, and RGB (red, green and blue) components of an image. On the other hand, few applications regarding the use of CVS for the detection of ammonia content in leafy vegetables, are reported.

The aim of the present investigation was to verify and assess the capability of the non-invasive contactless CVS in assessing the visual quality changes during postharvest storage of packaged fresh-cut rocket leaves and in estimating some of their internal characteristics (chlorophyll and ammonia contents): experiments have been made on samples coming from soil and soilless growing systems.

Method and Materials

Fresh-cut rocket leaves (*Diplotaxis tenuifolia* L. 'Dallas'), from three consecutive harvest time, separated per cultivation system (soil or soilless) were packed in open PP bags (dimensions 50 ×30 cm, Orved, Musile di Piave, Italy) of about 600 g each one, and stored at 10 °C for 16 and 18 d for soilless and soil system, respectively. At harvest and during storage samples were observed to attribute the visual quality level (QL) according to the 5–1 rating scale, where 5 = very good (very fresh, no signs of yellowing, bright, dark and uniform green, no defects), and 1 = very poor (unacceptable quality due to decay, severe wilting and yellowing, complete loss of texture and other evident defects). Then, images of packaged and unpackaged fresh-cut rocket leaves were acquired by CVS equipped with a Random Forest model used as classifier and the total chlorophyll and ammonium content of the same samples was evaluated through destructive conventional methods.

Results

CVS exploits the combination of image processing techniques and machine learning models (Random Forests) to assess the visual quality and predict the internal traits on unpackaged and packaged rocket leaves. Its performance did not depend on the cultivation system (traditional soil or soilless).

Moreover, the same CVS, exploiting its machine learning components, was able to build effective models for either the classification problem (visual quality level assignment) and the regression problems (estimation of senescence indicators such as chlorophyll and ammonia contents) just by changing the training data. The experiments showed a negligible performance loss on packaged products (Pearson's linear correlation coefficient of 0.84 for chlorophyll and 0.91 for ammonia) compared to unpackaged ones (0.86 for chlorophyll and 0.92 for ammonia). Thus, the non-destructive and contactless CVS represents a valid alternative to destructive, expensive and time-consuming analyses in the lab and can be effectively and extensively used along the whole supply chain, even on packaged products that cannot be analyzed using traditional tools.

Conclusions

The research activities developed a CVS for continuous monitoring the freshness level and the quality of rocket leaves from harvest to final consumers even through a plastic packaging. This technology worked in an objective and consistent way on non-destructive and contactless measurement of biological markers (such as chlorophyll and ammonia) which are strongly related to leaf senescence. The proposed CVS was able to automatically select, without human intervention, the most relevant color traits using the Random Forest machine learning model.

Results could have a significant impact on advanced applications of the traditional CVS commonly used for the inspection of fruit and vegetables. In particular, CVS may represent a valid alternative to destructive, expensive and time-consuming analyses in the laboratory and may be effectively and extensively used along the whole supply chain, even on packaged rocket leaves which could not be analysed by traditional tools.

Key Words: contactless quality level assessment; *Diplotaxis tenuifolia* L.; image analysis packaged vegetables; senescence indicators prediction

RIPLESS: a natural solution to improve the shelf life of stone fruits

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Introduction

One of the groups of fruits with the highest demands on the market are stone fruits, which include sweet cherries, plums, peaches and nectarines, and whose commercial price depends largely on the hedonic appreciation determined by the visual appearance of the fruits and its firmness. However, these market expectations greatly condition its post-harvest life, which is mostly short because symptoms of deterioration appear very early in the distribution chain, even in local markets.

Method and Materials

In this framework, RIPLESS has been developed as a patented formulation based on an oil-in-water nano-emulsion containing melatonin and α-tocopherol as active ingredients. Firmness and incidence of pathogens have been measured to assess the RIPLESS effects on the increase of the post-harvest life of stone fruits including plums and sweet cherries.

Results

It has been shown that RIPLESS displays effective properties to prevent post-harvest deterioration of stone fruits, maintaining the firmness of the fruit and reducing the incidence of pathogens by up to 5 days at room temperature, thus avoiding fruit waste. Furthermore, RIPLESS is a solution that offers several advantages over other existing technologies, since it can be implemented in current distribution systems, it can be used in organic agriculture, and it can be an alternative to refrigerated systems that reduce nutritional quality and induce fruit disorders. Most importantly, it simplifies postharvest handling to provide high quality fruits.

Conclusions

In conclusion, RIPLESS, currently in the TLR4 state of development, is an innovative alternative to reduce post-harvest deterioration of fruit and constitutes a triple impact model since its application guarantees maximum use of natural resources, provides nutritious and safe food to society and reduces economic losses from food waste.

Key Words: stone fruits; postharvest; shelf life; fruit decay

Tissue-differential response to long-term postharvest cold storage and short-term high CO_2 treatments in table grape

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Introduction

The aim of postharvest handling is to maintain optimal fruit quality for longer by slowing down the rate of senescence-associated processes. The breakdown of cell structures leads to water loss, softening of fruit and increased susceptibility to fungal attack. It has been previously highlighted the efficacy of a short-term high CO_2 pretreatment in maintaining table grape quality, avoiding postharvest losses.

Method and Materials

This paper examines the relationship between ultrastructural integrity of membranes (transmission electron microscopy), oxidative state at cellular (H_2O_2 generation) and membrane level (malondialdehyde accumulation) and postharvest quality of table grapes (*Vitis vinifera* L.) cv. Autumn Royal. The response of berry tissues at the level of membrane fatty acid composition (polar lipid fraction), organic osmolytes and stress responsive proteins after 41 days of storage at 0 °C and the residual effect of the application of a single or dual 3-days 20 kPa CO_2 treatment are also evaluated.

Results

Dual CO_2 treatment was very effective in delaying the decline in harvested cluster quality caused by postharvest senescence-related disorders. At structural level, high CO_2 treatments maintained the integrity of cell microstructure and energy-related organelles, reduced oxidative damage in cells and membranes, and increased the unsaturation of 18-carbon fatty acids and the lipid unsaturation ratio in polar membrane lipids. In exocarp tissues up-regulate the expression of VviDHN isoforms and activate trehalose and GABA synthesis, both functionally involved in cold tolerance. In mesocarp tissues, the predominant active defence response to CO_2, in a dose dependent manner, was the up-regulation of VviOsmo, VviDHN4, VviDHN1a and proline, preventing the dehydration disorders.

Conclusions

Short-term high CO_2 treatments counteracting structural and cellular damage prompted by dehydration and oxidative senescence-related disorders and contribute to prolonging fruit fresh-like quality. The cold-drought defence strategies activated by CO_2 are dose-dependent, tissue-specific and transcriptionally timing-regulated.

Key Words: CO_2; postharvest; senescence

Oxidative pinking discolouration in *Lactuca sativa* across leaf parts and lettuce types

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Introduction

Fresh-cut lettuce quality is reduced by oxidative pinking discolouration, lowering consumer acceptance. The pink-coloured compounds synthesis *via* phenylpropanoid pathway are mediated by phenylalanine-ammonia lyase (PAL) and polyphenol oxidase (PPO) enzymes. Due to differences in substrate and enzyme activity, it is hypothesised that different leaf parts (LP) and lettuce types (LT) would differ in biochemical reactions towards wounding. We aim to elucidate the occurrences and biochemistry of pinking in fresh-cut lettuce during cold storage (5^{o}C) of different LP (lamina and midrib) of two lettuce types (LT), Romaine and Iceberg.

Method and Materials

Midrib and lamina of Romaine and Iceberg lettuce were minimally-processed to initiate cell-wounding and stored in cold storage (5^{o}C) for 0, 2, 4, 6, 8 days. Pink scoring was done using quadrat-scoring with a 5-point scale. PAL and PPO assays were done and phenols associated with pinking were analysed using HPLC-DAD.

Results

Pinking indices were affected by LT and LP, with the highest observed in Romaine midrib. Leaf lamina for both LT showed negligible increase of pinking across storage period. PAL activity in midrib and lamina were significantly higher than those of Iceberg on Day 4, though both showed no significant difference across storage period and decreased after Day 6. The activity of PPO in lamina was higher than those in midrib in Romaine.

HPLC-DAD analysis showed chlorogenic acid as the dominant phenol and increased markedly during storage (13.51-40.60 μmol.g^{-1}FW; 7.47-22.58 μmol.g^{-1}FW in the midrib of Iceberg and Romaine, respectively). PCA showed that chlorogenic acid, caffeic acid, and coumaric acid were positively correlated with pinking index.

Conclusion

Both LT and LP affected pinking of fresh-cut lettuce with midrib tissues showing higher pinking index than leaf lamina, especially for Romaine. Pinking correlated positively with coumaric, caffeic, and chlorogenic acid, indicating these as pinking substrates. PPO, although increased during storage, was negatively associated with pinking.

Key Words: Oxidative discolouration; phenolic compounds; enzymes; leaf parts

Drought stress-induced leaf senescence in plants: how to detect it and why

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Two decades ago, I wrote a review paper with my former PhD supervisor Prof. Leonor Alegre entitled "Die and let live: leaf senescence contributes to plant survival under drought stress" (Funct Plant Biol [2004] 31: 203-216) where we emphasized the importance of leaf senescence for the survival of drought-tolerant plants. Here, I am aiming not only at revisiting the most important points described in this paper (still of relevance today), but most importantly focus on how we can detect drought-stress induced leaf senescence and why for. Emphasis will be placed on examples on how this is useful not only to understanding fundamental aspects of biology, but also on how this knowledge is important for the management of invasive plants and biodiversity. Drought stress-induced leaf senescence contributes to nutrient remobilization during stress, thus allowing the rest of the plant (i.e. the youngest leaves, fruits or flowers) to benefit from the nutrients accumulated during the life span of the leaf. This process needs, however, to be clearly differentiated from leaf abscission, which although sometimes occurring in the same organ, they must not be confounded because their physiological role and spatiotemporal regulation is completely different. Aside from showing some examples to differentiate these two processes, I will discuss here the importance of leaf (and even whole plant) senescence for the success of some invasive plants, such as *Carpobrotus* spp. in Mediterranean-type ecosystems. This example showcases how leaf senescence is not only important for understanding plant responses to the environment better, but also for our knowledge on fundamental features sustaining the invasion success of some species and how we can mitigate their effects if we really aim at reaching the biodiversity goals set by the European Union for 2030.

Key Words: fruit over ripening; fruit senescence; hormonal regulation; leaf abscission

Non-destructive analyses of *Populus alba* L. provide a valuable indicator of the status of leaves senescence under salt stress

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Introduction

Leaf senescence is a natural phenomenon that occurs during the aging process of plants and is influenced by various internal and external factors. Salt stress is one of the most severe abiotic stresses that accelerates this process. During senescence, loss of photosynthesis efficiency, in terms of leaf gas exchanges, and chlorophyll a fluorescence are the most affected physiological mechanisms, resulting in reduced plant growth, yield, and quality. Moreover, a decrease in the secondary metabolite flavonoids, which have a role in protecting cells from reactive oxygen species damage, is widely recorded when plants are subjected to salt stress.

Method and Materials

Two-month-old plantlets of Poplar (*Populus alba* L. "Villafranca") clones were exposed for four weeks to 60, 120, and 180 mM NaCl and compared to control plants. Plant performance was followed by non-destructive measurements twice per week using leaf gas exchanges (CIRAS 2), chlorophyll a fluorescence (Handy PEA, Hansatech, UK), relative total chlorophyll content (SPAD), and flavonols (MPM-100, ADC BioScientific, Ltd, UK) to understand the physiological responses under different salt stress levels.

Results

After one month of NaCl treatment, noticeable phenotypic changes were observed on poplar "Villafranca" clone plants. Leaf yellowing indicated that the effect of saline stress was stronger on the basal than on the apical part of the plants. The most obvious symptoms were observed in the group treated with the highest saline concentration (180 mM NaCl), followed by the group treated with 120 mM NaCl. The 60 mM NaCl plants did not show any notable variations from the control plants. The Non-destructive methods showed a decrease in the total flavonol contents, and an increase in the active reaction center for cross-section (RC/CSo) at the end of the first week of treatment in higher-stressed plants. Significant effects of salt stress on photosynthetic parameters such as stomatal conductance (gs) and net photosynthetic rate (Pn) were observed 11 days after the treatments. The relative chlorophyll content of the leaves showed that the treatments did not significantly affect the chlorophyll content (Chl a+b) until the 25th day of treatment.

Conclusions

In this study, we demonstrated how nondestructive parameters can be a useful tool to predict the onset of leaf senescence induced by long-term exposure to salt stress in poplar plants.

Key Words: gas exchange; chlorophyll a fluorescence; relative total chlorophyll content; flavonols

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Stress as the mold of ageing mechanisms in mountain pines

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Introduction

The lifespan of very old tree species entails complex mechanisms that minimize senescence and ultimately postpone the death of the whole organism. Among different strategies, meristem indeterminacy, protective mechanisms operating in meristematic tissues, and a vast set of whole- plant plastic and adaptive mechanisms serve to defy ageing by delimiting senescence to independent modules. Furthermore, very old scarce trees are of special interest in ecosystem dynamics and play crucial ecological and biodiversity roles in forests.

Method and Materials

Growth and senescence events, at various organization levels, were monitored in several mature forests of mountain pine (*Pinus uncinata*) in the Pyrenees. Various physiological stress markers (water contents, hormone and antioxidant contents, and PSII integrity) in both somatic and meristematic tissues (needles and buds, respectively) were measured through the complete developmental stages of mountain pine lifespan, under the influence of different stresses. Site- specific environmental events and other living associated species were also monitored to link their dynamics to tree decay and senescence events.

Results

Size, age and their resulting cumulative stresses modulate the tear and wear of time and strongly influence various development and senescence processes at different organization levels. Ancient trees suffer indefectible age-related constraints and deploy extreme plasticity in hormonal, antioxidant and dormancy processes by enhancing resilient phenotypes at the expense of growth. Meristematic dysfunctions trigger modular senescence and gradual decay mechanisms that mold the limits of tree life and establish unique ecological niches for other living species.

Conclusions

Ancient trees reveal extraordinary divergence in both senescent and developmental processes that defy ageing at various structural levels. Slow and cumulative stresses mold exceptional lifespans but can also be understood as a gradual senescence triggering factor limiting essential developmental processses. This study not only reveals that very old trees display singular physiological traits that enable them to attain extreme longevities, but also shows that these same sets of resilient tools are the base of unique reservoirs of biological diversity in high-mountain ecosystems.

Key Words: ancient trees; modular senescence; tree ageing; whole-plant senescence

Anthocyanin-mediated resistance and senescence modulation in Potatoes against *Rhizoctonia solani*: insights from two contrasting varieties

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Introduction

Anthocyanins, renowned for their antioxidant properties, significantly contribute to plant defense against various environmental stresses. However, their role in countering necrotrophic fungal infections in potatoes remains unclear. This study investigated the molecular mechanisms underpinning anthocyanin-mediated resistance to *Rhizoctonia solani* in cultivated *Solanum tuberosum*, with a particular focus on senescence-related processes.

Method and Materials

Two potato varieties with differing anthocyanin contents were examined: Musica, featuring white tubers and shoots, and Blue Star, which accumulates anthocyanins in all tissues.

Results

Upon Rhizoctonia infection, Musica exhibited a 75% reduction in tuber yield, whereas Blue Star exhibited complete resistance. Biochemical analyses indicated that Blue Star maintained higher levels of photosynthetic pigments, unlike Musica, which saw a reduction post-infection. RNA-seq data revealed distinct transcriptomic responses between the two varieties upon infection. In Blue Star, four differentially expressed genes (DEGs) were identified, including *StSGR*, which is linked to chlorophyll degradation and senescence and was downregulated two-fold post-infection. The downregulation of *StSGR* in Blue Star suggests a suppression of senescence processes, contributing to its enhanced resistance. Conversely, Musica showed 156 DEGs associated with cell wall modification and hormone signaling, indicating an unsuccessful defense attempt and potential premature senescence. Interestingly, the anthocyanin-related R2R3 MYB transcription factor gene *StAN2* was significantly upregulated in Musica, despite the lack of anthocyanins in the infected tissues. Functional assays demonstrated that transgenic tobacco overexpressing *StAN2* had elevated levels of phenolamides, compounds involved in biotic stress signaling, along with anthocyanins. These transgenic plants also showed enhanced tolerance to *Botrytis cinerea*, another necrotrophic pathogen, with notable changes in vascular tissues observed through promoter localization experiments.

Conclusions

These findings suggest that the absence of anthocyanins in Musica correlates with increased susceptibility, a pronounced transcriptomic response, and potential premature senescence upon Rhizoctonia attack compared to Blue Star. Ongoing research aims to elucidate the molecular mechanisms by which anthocyanins modulate reactive oxygen species bursts and senescence processes using genome editing techniques.

Key Words: R2R3 MYBs; phenolamides; transcriptomic; tobacco

Plant senescence in tissue-cultured ornamental plants

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Introduction

The work was conducted to improve the quality of *in vitro* propagated shoots of *Passiflora incarnata* L. and *Citrus australasica*. *In vitro* culture techniques are useful tools to improve the production and marketing of ornamental species. Micropropagation allows rapid clonal propagation of plants selected for their ornamental value and for other characteristics: medicinal for Passiflora and nutraceutical for Finger Lime.

Method and Materials

In vitro culture of the two selected species was performed according to species-specific protocols previously validated. Ethylene accumulated in the culture vessels was measured by withdrawing air samples by a modified stainless-steel joint through a hole in the vessel closure. Samples were analyzed by a gas chromatograph using a flame ionization detector and a stainless-steel column (150 x 0.4 cm ø packed with Hysep T); column and detector temperatures of 70 °C and 350 °C respectively and using Helium as carrier gas (30 mL min^{-1}). Quantification was performed using an external standard, and the results were expressed as volume (pL mL^{-1}).

Results

During the active shoot proliferation of *Passiflora incarnata* and *Citrus australasica*, leaf senescence symptoms occurred impairing the successful outcome of their *in vitro* cultivation. The observed senescence can be related to the high level of ethylene inside vessels, in part due to the physical characteristics of the container (volume, type of closure) and to the chemical composition of the culture medium. To avoid using unsustainable compounds such as silver salts, we adopted two different approaches using on one side inhibitors of ethylene action as 1-MCP, while on the other side promoting not forced vessel ventilation. The number of gas exchanges (h^{-1}) was determined to compare the gas losses from the different types of vessels employed for the two species.

Conclusions

When plant species sensitive to ethylene are cultured in vitro, senescence can represent an obstacle for regular plant development. Our results show that it is possible to apply eco-friendly molecules to avoid the occurrence of senescence.

Key Words: ethylene; *in vitro*; ethylene inhibitors; micropropagation

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The UK Food Aid sector - where postharvest senescence becomes a huge problem

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Postharvest quality of fruit and vegetables is highly dependent on the supply chain it moves through. The commercial supply chain makes great efforts to operate just-in-time systems that have good temperature controls embedded within them. Despite imperfections, the vast majority of fresh produce reaches the consumer in good condition. However, the fresh produce supply chain generates over 170,000 tonnes of food waste every year, which is in addition to the c.500,000 tonnes of fresh produce which is wasted in hospitality and food service. Some of this produce finds its way into the Food Aid sector. The Food Aid sector operates in a very different way to the commercial sector, receiving goods from a variety of retail and wholesale routes, often when fresh produce is already near end of its life. Whilst Food Aid has to date been regarded as an emergency support mechanism in the UK, it is clear from our work in the FoodSEqual project that for many citizens the Food Aid sector represents a core route for them to obtain at least part of their diet. Clearly the optimal situation is that our food system is transformed so that people do not need to access food aid as consigning people who are economically challenged to eating poor quality, end of life food further exacerbates the inequities leading to health inequalities. This paper considers current postharvest supply chains that characterise the present state of the Food Aid sector and explores developments that could secure improved quality for citizens who currently obtain fresh produce through the Food Aid sector.

Fluorescent light with a background of sunlight delays yellow tomato postharvest senescence by a re-accumulation of chlorophylls

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Introduction

During postharvest, fruit coloration is used as an important index for assessing the commercial and nutritional value of fruits, as well as evaluating the fruit postharvest senescence. As fruits ripen, chlorophylls, which predominantly accumulate in the immature stage, start to degrade while carotenoids accumulate. This is generally accompanied by the differentiation of chloroplasts into chromoplasts in ripe mature fruits. However, some fruits, such as citrus and cucurbits, have shown to regain the green coloration due to a re-accumulation of chlorophylls and re-differentiation of functional chloroplasts, a process known as fruit regreening. In this study, we aimed to evaluate if the regreening process can also occur in tomato fruits, where it has not been described to date.

Materials and methods

Different independent experiments testing various light treatments, including fluorescent light under different photoperiods, blue LED light, and fluorescent light with a background of sunlight, were performed to induce regreening in various tomato varieties during postharvest. Fruit color variations were assessed by measuring periodically the color of the exocarp with a colorimeter evaluating the CIE L*a*b* and CIE L*C*h color spaces. Furthermore, chlorophyll and carotenoids content were assessed spectrophotometrically to confirm results on color variation.

Results

Results showed that fruits under fluorescent light with a background of sunlight induced regreening of the SummerSun variety. Neither fluorescent or sunlight alone nor blue LED light, reverted the degreening associated to fruit over-ripening and senescence during postharvest. Results of changes in color were confirmed by chlorophyll contents. The regreening of tomatoes exposed to fluorescent light with a background of sunlight occurred after 20 days of exposure to the light treatment. This green coloration was accompanied by a re-accumulation of chlorophylls in the tomato fruits. Results also showed that regreening does not result in an improvement of shelf life, since once they are exposed to conditions promoting ripening again, they quickly deteriorate in a few days, leading to rapid fruit decay.

Conclusions

It is concluded that the regreening process can also occur in some varieties of tomato fruits during postharvest exhibiting a clear re-accumulation of chlorophylls. It is discussed why this phenomenon occurs in some tomato varieties, but not in others.

Keywords: Regreening; chloroplast; ripening; *Solanum lycopersicum*

Study the mechanisms of ethylene action during postharvest senescence in Broccoli

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Introduction

Postharvest longevity of perishable produce remains a challenge in the global fresh market supply chain. Postharvest longevity is determined by the rates of ripening and senescence, which are influenced by harvest time and storage conditions. Ripening and senescence are predominantly regulated by ethylene, which produces a plethora of metabolic effects within the harvested produce, leading to physiological and developmental changes during postharvest. Broccoli (Brassica oleracea L. var. italica) is prone to yellowing and wilting due to the relatively high respiration rate and tissue senescence during postharvest handling, transportation, and storage, which greatly affects the quality and reduces market value that led to the problem of food waste and loss. Broccoli florets treated with hydrocooling, 1-MCP (ethylene inhibitor) and controlled atmosphere (CA) can delay the senescence. However, little is known about the mechanisms on how those treatments worked at the molecular level.

Method and Materials

The study involved physiological assessments to investigate the broccoli senescence patterns as influenced by temperature, and the effects of CA, 1-MCP, and ethylene on the shelf life of broccoli. Concurrently, RNA sequencing, and subsequent CRISPR gene editing analysis yielded a general understanding of the intricate molecular mechanisms behind postharvest senescence.

Results

We combined a physiological, biochemical, and genomics analyses on the postharvest broccoli and identified a core gene regulatory network governing senescence-associated developmental events, ethylene-regulated signaling pathways, and activation of stress responses. Additionally, we developed genome-editing toolkits by CRISPR/Cas9 system to understand deterioration of broccoli as well as through machine learning approaches to aid development of an innovative and easy-to-use accessibility tool to accurately estimate the freshness of produce.

Conclusions

The findings give insights into ethylene biosynthesis and signal transduction at the tissue-specific level in broccoli and provide guidance on how to extend broccoli shelf life and reduce its economic losses, which also generate genetics and molecular recourses for marker-assistant breeding and expand the general scientific knowledge of regulating senescence of Brassicaceae family.

Key Words: SAGs; RNA-seq; CRISPR

Exogenous postharvest application of abscisic acid: effect on quality in tomato fruit senescence

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Introduction

Abscisic acid (ABA) is a fundamental hormone that influences the development, maturation, and senescence of tomato berries. The biosynthesis of ABA utilizes carotenoids (C 40) as precursors; therefore, it is hypothesized that these compounds play a role in the completion of ripening and senescence of tomato fruits. The aim of the study was to investigate the effect of a post-harvest treatment with ABA on tomato fruits.

Method and Materials

The industrial tomato plants "Leader F1" (*Solanum lycopersicum* L.) were grown in pots at the University of Milan. The fruits were harvested at commercial ripening, selecting berries with a homogeneous level of ripening (colour). Half of the fruits were immersed in a 100 µM ABA solution for 30 minutes (T0), while another group (control) was treated with distilled water. Subsequently, berries were maintained at 20 °C for up to thirteen days, to evaluate the effects of the treatment on qualitative and physiological traits. Analyses were conducted on the berries after 5 (T1), 8 (T2), and 13 days (T3) of shelf life. At each time point, analyses included berry colour, soluble solids, titratable acidity, pH, respiration rate, content of phytoene, lycopene, β-carotene, and volatile organic compounds (VOCs).

Results

The treatment did not cause changes in the colour of the fruits, which remained constant during storage, as shown by the analysis of the L*, a*, b*, C, and h parameters. Similarly, pH and soluble solids content did not significantly change in response to ABA application. Given the correlation between these parameters and sensory attributes, this could suggest that the ABA treatment did not negatively affect the commercial quality of fruits. Regarding the respiration, the trends of O_2 and CO_2 were significantly influenced by the shelf-life period at 20 °C but not by the treatment. At harvest, the β-carotene concentration in the ABA-treated tomato fruits was lower compared to the controls. This effect was temporary and was not observed in subsequent sampling timepoints. The VOCs analysis allowed identifying different compounds present in the berries at specific time points or following ABA treatment. The number and concentration of VOCs changed during time with an average value of compounds about 25 (T0), 11 (T1), 13 (T2), and 34 (T3). Among the most abundant compounds we found Nerylacetone and 6 methyl 5 hepten 2 one, responsible of the typical tomato aroma.

Conclusions

The application of ABA did not cause alterations to most of the qualitative parameters analysed (aesthetic quality, colour, soluble solids, titratable acidity, pH). On the other hand, the treatment induced a temporary decrease carotenoids concentration, affected the aromatic profile and the content of some aromatic compounds, especially esters.

Key Words: ABA; VOCs; shelf life; *Solanum lycopersicum* L.

Throwing away to survive: senescence of rhizome as a mechanism adopted by clonal plants to remain young forever

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Introduction

Senescence is a stage of development in plants that occurs from cells to whole organisms. In clonal species, senescence of the clonal growth organ (e.g. rhizome or stolon) is a strategy that ensures a long-lived genetical individual (genet) is composed of young independent rooting units (ramets). Those plants shed old tissues and organs rather than preserving them. To structurally understand this little-explored process of programmed death, we assessed the belowground system of *Rumex alpinus* L., a rhizomatous perennial clonal species native to Central and Southern Europe.

Method and Materials

Twenty individuals located in the Krkonoše Mountains, Vrchlabí - Czech Republic were collected and divided the rhizomes, that persisted for about 8 years, into young, middle, and old segments, and submitted for standard anatomical and histochemical protocols.

Results

The senescence process of rhizomes in *R. alpinus* starts from the oldest segment and progresses to intermediate parts, with changes in pigmentation from yellowish to dark brown or black as the first morphological symptom. A significant shrinkage in the black parts was also observed. Internally, signs of senescence do not present a regular pattern, with loss of turgor and accumulation of phenolic compounds occurring randomly in spots of the covering tissue, the vascular system, and the medullary parenchyma. However, there is an increase in the occurrence of calcium oxalate crystals in parenchyma cells and vessel elements sealing with phenolic and lipid compounds in the middle and old segments compared to the young parts.

Conclusions

Our study is the first attempt to anatomically examine the senescence process in rhizomes. This helps us to understand how diverse the growth strategies are in clonal species, since senescence is essential for the longevity of these plants and for ecosystem functions once a senescent rhizome becomes part of organic matter in the soil.

Key Words: Autophagy; clonal growth; longevity

Cathepsin B cleaves BiP during ER-stress induced PCD

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Introduction

Programmed cell death (PCD) is a genetically controlled biological process that results in the targeted destruction of unwanted or diseased cells. PCD in plants is induced by prolonged ER-stress. To prevent this, ER-stress triggers an Unfolded Protein Response (UPR) designed to restore cellular homeostasis. Among the UPR genes, Binding Immunoglobulin Protein (BiP) is the most abundant chaperone protein within the ER. It belongs to a multi-gene family with diverse roles in biotic and abiotic stress response. This protein plays a key role in some plant PCD processes and can function as a negative or positive modulator. In soybean, BiP overexpressing lines had reduced ER-stress and delayed leaf senescence. This shows that BiP can regulate developmental PCD (dPCD) implicated in senescence. Our research has shown that BiP is a substrate for cathepsin B (CathB), a key protease in plant PCD, which plays a role in senescence. A greater understanding of the molecular mechanisms underlying the processes downstream of CathB is the next important step for new discoveries in cell biology and applications in biotechnology. To achieve this goal, it is of fundamental importance to discover and test specific CathB substrates in plants.

Method and Materials

To induce ER-stress and the resulting PCD, tunicamycin was injected into *Arabidopsis* leaves and BiP cleavage was monitored using western analysis. Subsequently, cleavage assays were conducted in *vitro* using recombinant BiP2-GFP and CathB2 fusion proteins and in *vivo* by co-agroinfiltration of BiP2-GFP and CathB2-RFP DNA constructs into tobacco leaves.

Results

During tunicamycin-induced PCD, BiP is cleaved in *Arabidopsis*. Moreover, our results show that BiP is cleaved by CathB following co-expression of BiP2-GFP with CathB2-RFP in *Nicotiana* leaves.

Conclusions

During ER-stress-induced PCD caused by tunicamycin, CathB cleaves BiP. The significance of this cleavage is being investigated.

Key Words: PCD; ER-stress; CathB; BiP