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Scald-Cold: Joint Austrian-Italian consortium in the Euregio project for the comprehensive dissection of the superficial scald in apples

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Abstract

After harvest, apples are stored at low temperature to slow down the ripening physiological processes. This strategy can, however, also promote the development of superficial scald, a chilling injury-related disorder showing brown-discoloured areas on the fruit skin, totally compromising its marketability. To examine thoroughly the underlying physiological mechanisms and genetic control of superficial scald, the "Scald-Cold" project, a three-year Interegional Project Network, was granted by the European Region Tyrol, South Tyrol and Trentino (EGTC). The project is centred on a comprehensive approach, integrating into a joint effort different scientific disciplines, ranging from genetics and transcriptomics to NIR spectroscopy and hyperspectral imaging, together with the employment of distinct postharvest storage technologies distinguished by a specific regime of low oxygen. The core of this project aims to disclose novel regulatory processes of this disorder, providing new series of tools important for both the scientific and technical communities interested in apple breeding and postharvest. The "Scald-Cold" project will identify new molecular markers suitable for the selection of new apple varieties genetically resistant to superficial scald, while developing tools for an early detection of this phenomenon.

Keywords

Superficial scald, apple, postharvest disorder, genetics, food, NIR technologies

Introduction

Apples, after harvest, are cold stored to enable a continuous year-round availability, and to retain the freshness of the fruit in the market. The near-freezing temperature applied to control the ripening process, avoiding therefore important fruit loss, can also trigger the development of a series of chilling injury disorders, such as superficial scald (Figure 1). The main symptoms typical of this phenomenon are characterized by the development of brown-discoloured area on the fruit skin, resulting from the oxidation of the chlorogenic acid in the skin tissue, compromising, in the end, the overall fruit marketability. To comprehensively dissect the underlying physiological mechanisms and genetic control of superficial scald, a multidisciplinary approach has been designed and presented within the framework of "Scald-Cold", an Interregional Project Network (IPN) granted by the European Region Tyrol-South Tyrol and Trentino (EGTC). The "Scald-Cold" consortium is represented by three scientific Institutes located in the North Italian-Austrian area: the Fondazione Edmund Mach, the Laimburg Research Center and the University of Innsbruck (Table 1). The aims of this project are addressed to the global deciphering of the superficial scald physiology, achieved through the employment of several technologies. Genetics and systems genetics approaches will be initially employed to

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Figure 1. Fruit of 'Granny Smith' apple cultivar asymptomatic (left panel) and showing superficial scald symptoms (right panel).

Table 1. Partners involved in the Euregio project "Scald-Cold".

Institute	Location	Group leader
Fondazione Edmund Mach (FEM)	Italy	Fabrizio Costa (project coordinator)
Laimburg Research Centre	Italy	Angelo Zanella
Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck	Austria	Christian Huck

target the genomic regions involved in the control of several phenotypic entities related to scald. Within this task, quantitative trait locus (QTL) regions involved in the genetic control of the symptoms and metabolites related to scald would be targeted and located on the apple genome. The investigation of the genetic control of the gene expression associated to scald would instead provide useful information to understand the regulatory network involved into this physiological mechanism. To shed light on the protecting mechanisms involved in the physiological response against this disorder, the fruit transcriptomes will be also investigated using the RNA-seq technology, in an attempt to elucidate the specific effects of different postharvest technologies in preventing the occurrence of the superficial scald. In the end, the development of this disorder will be also monitored with innovative non-destructive methods implemented for the definition of predicting tools. The different disciplines representing the core of this project aim to disclose novel regulatory processes of this disorder, gaining new knowledge important for apple breeding and postharvest activities. For a more efficient and sustainable horticulture, the need of varieties naturally resistant to important disorders or diseases is compelling. The breeding of horticultural crops is however a laborious and time-consuming activity, which can take advantage to the use of modern biotechnologies. The selection process can be nowadays assisted by the availability of molecular markers associated to important traits (such as the superficial scald), identifying in early stage the most performing individuals distinguished by superior traits, therefore, guaranteeing a valuable economic success. The information acquired through the analysis of the variation of the transcriptome profiling following different postharvest technologies, and the early detection of superficial scald development with non-destructive and on-line systems, would improve the fine tuning of the postharvest strategies towards the valorisation of the fruit quality in apples during storage.

Motivation

Fresh fruits play a major role in the human diet as natural source of beneficial and valuable compounds, such as organic acids, sugars, fibres, vitamins and secondary metabolites. The external aspect of a fruit drives the consumers' preference, guaranteeing the economic success of a cultivar. To date, fruits are also considered essential dietary nutraceutical supplements, providing protection against important chronicle diseases.[1] For these reasons, it is therefore important to have a continuous access to fresh fruit, which is nowadays ensured by postharvest storage technologies that up to now efficiently reduced the fruit loss of about 40% worldwide.[2] The low temperature, however, can also induce the development of important disorders, such as the superficial scald in apples.[3,4] To control this disorder, several strategies have been implemented in the past. Initially, the antioxidant diphenylamine (DPA) was largely used by dissolving in water or

atomising in the storage chambers to control the oxidation process at the base of superficial scald. Due to its toxicity, the use of this molecule has been prohibited in the European Union since 2011. As an alternative, 1-MCP (1-methylcyclopropene), normally employed to control the progression of fruit ripening through an interfering process at the level of ethylene receptors, turned out to be very effective in the prevention of superficial scald. To this end, the role of ethylene and its effect on α-farnesene were thought to be a primary event in this physiological mechanism.[4–6] Although efficient and safe, the use of 1-MCP is not permitted in the organic production, a growing cultivation practice in the South Tyrol area. The incidence of superficial scald would also probably increase in a close future, since it is stimulated by high temperatures before harvest, which are in slow but continuous increase (more in the Alpine area with regard to the valley) due to the effect of climate change and global warming. Within the framework of the "Superficial-Cold" project we aimed to achieve two important goals. The first aspect is to shed light on the physiological mechanisms of superficial scald, disclosing the actual cause of this disorder, since other processes, such as the role of polyphenol oxidase (PPO) in the reaction with the chlorogenic acid, has been also recently proposed and confirmed (Figure 2).[7–9] In addition to this, the development of apple varieties naturally resistant to the development of this disorder would also represent a valid and crucial alternative to prevent this phenomenon in a sustainable fashion. Due to the necessity of an environmentally friendly horticulture, the investigation of the genetic variability and its exploitation to create new varieties are compelling. The genetic amelioration of horticultural crops is to date mostly achieved through classical breeding technologies, implying the cross and selection of the most favourable individuals and the subsequent selection of the individuals distinguished by superior performances or with pyramided traits. The selection process can be nowadays supported by the use of molecular markers enabling the identification at 2–3 leaves stage of the individuals characterized by a natural resistance to this disorder. This tool, together with the knowledge generated by the transcriptomics investigation of the apples stored with different storage conditions (defined by a diminished level of oxygen), and the creation of innovative and early non-destructive tool for the monitoring of the superficial scald, would step forward into the improvement and maintenance of the fruit quality in apples.

Indeed, the dynamic characterization of the physical and biochemical properties of the fruit in a time efficient manner will open new possibilities for the technological implementation of the storage facilities, enabling the protection from scald and the maintenance of high-quality standard. For the long-term storage, the dynamic controlled atmosphere

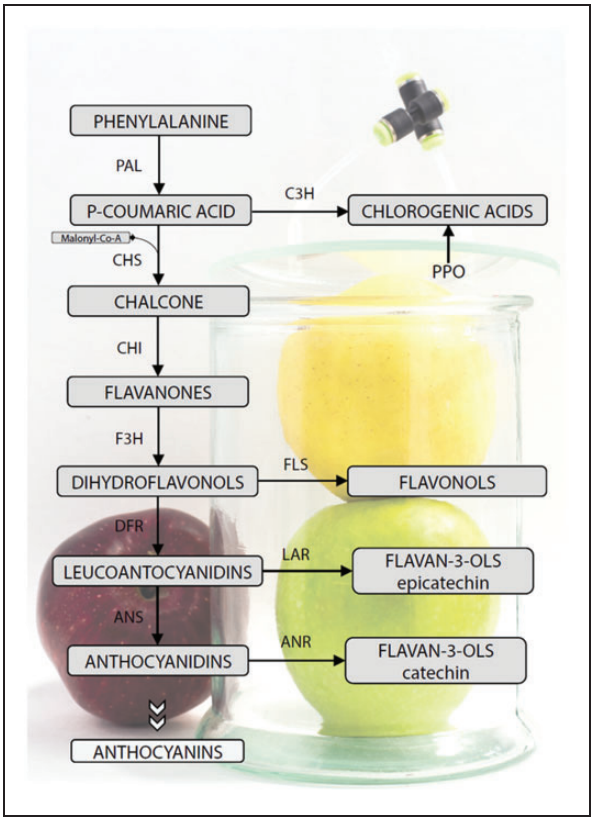

Figure 2. Visual representation of the phenolic pathway in apple. The reactivation of its first branch during the onset of superficial scald leads to the oxidation of chlorogenic acid due to the activity of the enzyme PPO, resulting in the end to the browning phenomenon. The main polyphenolic classes are highlighted in grey boxes. For each step, the committed enzyme is indicated. PAL: phenylalanine ammonia lyase; CHS: chalcone synthase; CHI: chalcone isomerase; MdF3H: flavone 3-hydroxylase; DFR: dihydroflavonol 4-reductase; ANS: anthocyanidin synthase; C3H: p-coumarate 3-hydroxylase; FLS: flavonol synthase; LAR: leucoanthocyandin reductase; ANR: anthocyanidin reductase; PPO: polyphenol oxidase.

(DCA) by the means of chlorophyll fluorescence (DCA-CF) proved to be an efficient tool in preventing scald.[10] Due to the DCA-CF storage technology the oxygen level in the storage atmosphere can be lowered close to the lowest level tolerated by the fruit (anaerobic compensation point). Hereby, very low O_2 levels can be maintained continuously during the whole storage period, resulting in an improved scald control.[10]

NIR spectroscopic methods are an essential aid enabling high-throughput and cost-effective analysis of apple skin. This is achieved through a non-destructive, rapid, chemical-free analysis, with minimal or no sample preparation. In hyphenation with data-analytical methods, quantitative information on various properties of the apple skin may be processed toward desired knowledge, and associated with the physiological symptoms of cold scald disorder. Additionally, NIR hyperspectral imaging will be used for further deepening the spectroscopic insight into the

spatial distribution of chemical information related to the changes ongoing in the apple skin. This technique offers a unique ability to elucidate spatial, spectral, and multi-constituent information of high chemical specificity from the skin of apples in a non-invasive manner. Various properties of the sample may be unravelled, trimmed in redundancy, processed toward desired knowledge, and presented in the form of an easily accessible image. The spectral dimension captured in such experiment is largely superior with regard to an electronic eye, let alone a naked eye. The spectral profile associated with the development of superficial scald may be followed over the time-dimension and/or the spatial dimension, granting high completeness of the captured information. Moreover, advanced data-analytical methods also enable to elucidating quantitative information from hyperspectral images.

Project aims

The main goal of the "Scald-Cold" project is a comprehensive physiological and genetic dissection of the superficial scald disorder in apple, through the elucidation of the responsible metabolites and network of genes together with the identification of valuable molecular markers suitable for DNA-informed breeding programs. These tasks will be assisted by newly developed strategies for non-destructive analytical assessment of superficial scald by means of NIR spectroscopy and hyperspectral imaging.

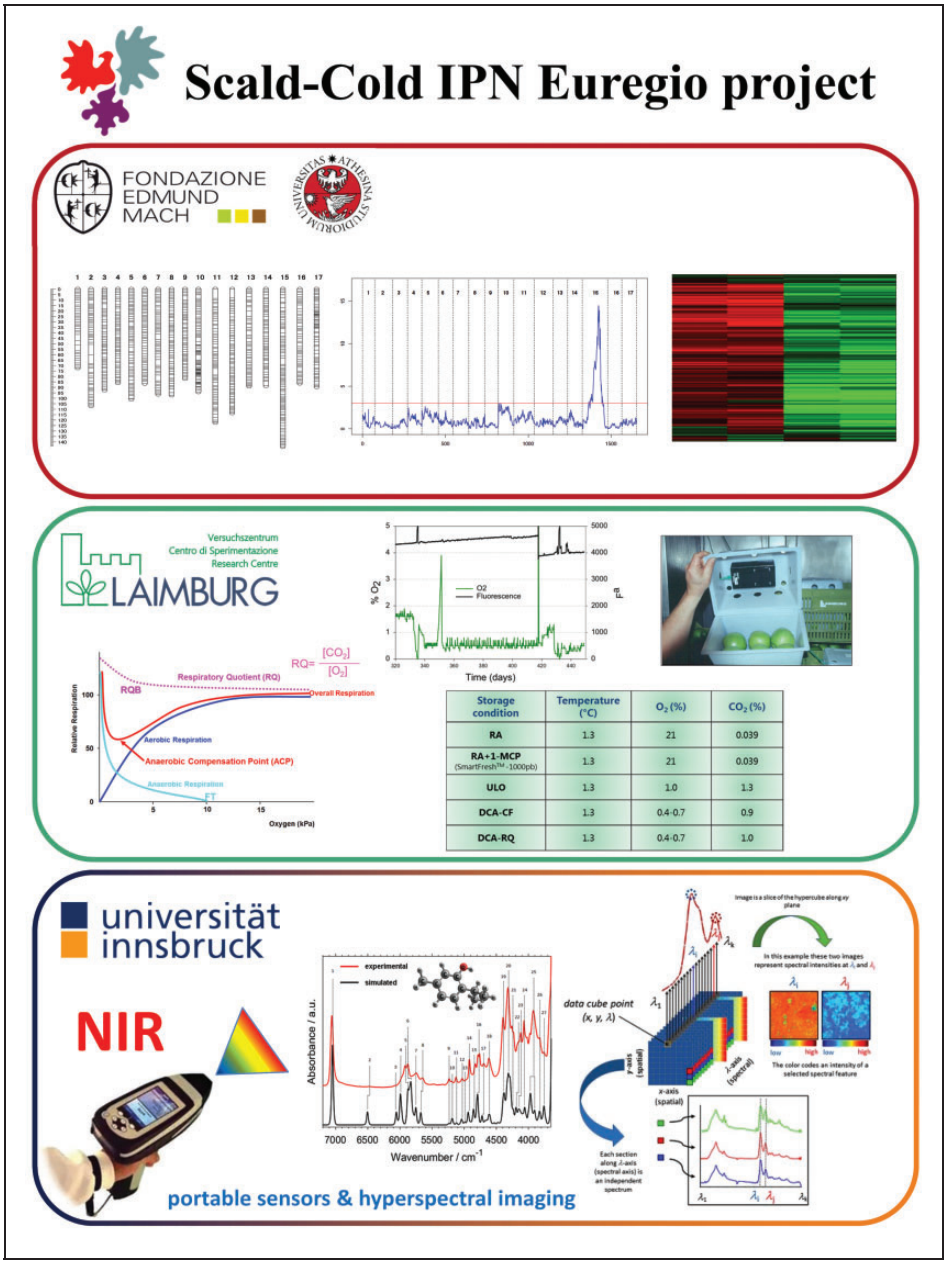

Figure 3. Synergy of methodologies being used in the 'Scald-Cold' project.

The "Scald-Cold" project is a step forward into the physiological regulation of superficial scald in apple, taking benefit from both basic and practical studies, integrating multiple disciplines to achieve its main goal. OMIC approaches have been to date mostly performed as independent entity, and until now very few works presented a multidisciplinary integration, mostly focused on model organisms only. The expected outcomes of this initiative include understanding of the mechanisms underlying the development of scald, e.g. identification of the genetic networks and biochemical pathways, the ability to monitor and predict the development of the disorder using non-invasive analytical methods (NIR spectroscopy, hyperspectral imaging),[11–13] as well as potentially establishing the optimal storage method to maintain the fruit quality. Novel miniaturized NIR spectroscopic sensors offer the possibility of high-throughput and cost-effective monitoring of the condition of apple skin. Moreover, NIR hyperspectral imaging technique will form a substantial aid in elucidating the chemical profile associated with the physiological changes occurring in apple skin upon the development of superficial scald. The principal methodologies that would be employed within the framework of the "Scald-Cold" project are illustrated in Figure 3.

Summary

Apple is a leading fruit crop of the North of Italy and South Tyrol area, and a more informative horticultural production management with reduced chemical input (also in postharvest) is certainly mandatory, especially to improve the sustainability of horticultural fruit crops. The superficial scald compromises the fruit marketability and the associated critical factors necessary to understand, predict and control this phenomenon are not yet adequately explored. The "Scald-Cold" project carried out by joint Austrian-Italian consortium of three research institutes is oriented to investigate the fundamental aspects underlying this postharvest disorder. The first goal is focused on disclosing the metabolite and transcription variation in apple fruit stored with different atmospheres with decreasing level of oxygen concentration. In addition to the comprehensive analysis of the different storage strategies, a more dynamic investigation will be also carried out to unravel the change of particular aspects during the ongoing process of the superficial scald.

Declaration of conflicting interests

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