Brussels, 4 June 2019

COST 027/19

DECISION

Subject: **Memorandum of Understanding for the implementation of the COST Action "Oxygen sensing a novel mean for biology and technology of fruit quality" (Roxy-COST) CA18210**

The COST Member Countries and/or the COST Cooperating State will find attached the Memorandum of Understanding for the COST Action Oxygen sensing a novel mean for biology and technology of fruit quality approved by the Committee of Senior Officials through written procedure on 4 June 2019.

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MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA18210
OXYGEN SENSING A NOVEL MEAN FOR BIOLOGY AND TECHNOLOGY OF FRUIT QUALITY (Roxy-COST)

The COST Member Countries and/or the COST Cooperating State, accepting the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action (the Action), referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any new document amending or replacing them:

a. "Rules for Participation in and Implementation of COST Activities" (COST 132/14 REV2);
b. "COST Action Proposal Submission, Evaluation, Selection and Approval" (COST 133/14 REV);
c. "COST Action Management, Monitoring and Final Assessment" (COST 134/14 REV2);
d. "COST International Cooperation and Specific Organisations Participation" (COST 135/14 REV).

The main aim and objective of the Action is to understand how ethylene, hypoxia, NO and ROS signals are integrated to impact fruit ripening and over-ripening processes. This signaling network will be deciphered in different contexts: tomato to understand how it controls ripening and over-ripening, and pome fruit in order to reduce post-harvest damages. This will be achieved through the specific objectives detailed in the Technical Annex.

The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 76 million in 2018.

The MoU will enter into force once at least seven (7) COST Member Countries and/or COST Cooperating State have accepted it, and the corresponding Management Committee Members have been appointed, as described in the CSO Decision COST 134/14 REV2.

The COST Action will start from the date of the first Management Committee meeting and shall be implemented for a period of four (4) years, unless an extension is approved by the CSO following the procedure described in the CSO Decision COST 134/14 REV2.

OVERVIEW

Summary

It is widely accepted that consumption of fruit and vegetable is beneficial to human health due to their content of essential nutrients such as vitamins and antioxidants. Any strategy aimed at increasing fruit consumption must necessarily improve the organoleptic qualities of these commodities since fruit quality is judged by the consumer not at the time of harvest but after a post-harvest period that can be long due to the complexity of the distribution channels. Fruits continue to evolve during their post-harvest shelf life which results in substantial deterioration. Postharvest losses are 30% of total fruit and vegetables production in Europe. Therefore, the control of the ripening process is instrumental to maintaining high nutritional and sensory values and to reducing post-harvest losses. Post-harvest management of fruits relies on controlled or modified atmosphere and on packaging. The recent discovery that factors involved in sensing low oxygen and oxidative stress are involved in ripening opens new research avenues for controlling fruit quality via innovative breeding strategies and new dedicated technologies. By bringing together researchers from different disciplines, the action is anticipated to bring major breakthroughs in the understanding of fruit physiology, thus providing new targets to control fruit quality and post-harvest shelf life. The research will implement advanced methodologies and concepts and will significantly enhance European competitiveness through promoting training of early stage researchers in cutting-edge technologies. By combining studies on different models this Action will lead to advances that will translate into novel practices and technologies to improve fruit sensory and nutritional qualities.

Areas of Expertise Relevant for the Action	**Keywords**
● Agriculture, Forestry, and Fisheries: Agriculture related to crop production, soil biology and cultivation, applied plant biology, crop protection ● Biological sciences: Plant biology, Botany	● Tomato ● oxygen ● ethylene ● ripening ● Post-harvest

Specific Objectives

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination

- Establish a road map detailing how a multidisciplinary approach by means of genomics, transcriptomics, proteomics, fluxomics and applied technologies used can apply to crops for which post-harvest storage is a challenge.
- Extending basic knowledge to open new avenues towards improving crop tolerance to long-time storage using cutting-edge technologies to control low oxygen sensing and associated ROS signalling.
- Combining complementary concepts and methodologies to decipher the mode of action of low O2 sensing and ROS signaling allowing the setup of novel technologies for the control of fruit quality.
- Bringing together expert in fruit biology and post-harvest physiology with experts in O2 sensing in order to develop a common understanding of complex processes occurring in different contexts where plant organs face hypoxia.
- Developing new theories reflecting the control mechanisms of fruit physiology ripening that leads to the development of novel technologies in post-harvest storage
- Facilitation of the transfer of knowledge related to ripening and over-ripening to develop new technologies to maintain fruit quality, postharvest.
- Facilitation of the transfer of knowledge from hypoxia and low oxyen sensing during submergence to explore new concepts in fruit ripening and over-ripening physiology.
- Bringing new insight into fruit development to broaden our
understanding of the metabolic networks operating in fleshy fruit species, post-harvest, thus

strengthening European research in fruit crops which constitute a large proportion of ‘healthy food’

Capacity Building

- Enhance communication and exchange of information between research groups to avoid duplication of work in different COST countries.
- Allow faster decision making on research priorities and on scientific questions to be addressed. Use the latest tools and resources to bridge data from genomics, proteomics, metabolomics, fluxomics, reverse genetics, bioinformatics and physiology, so that important horticultural processes can be studied using an integrated approach.
- Transfer of research techniques and concepts between the participating groups.
- Provision of easy access to research infrastructure facilities for partners via Short-Term Scientific Missions (STSMs) and promote wide diffusion of advanced technologies among different groups
- Sharing ‘know-how' and provision of advanced graduate and postdoctoral training in state-of the-art stress adaptation, ripening physiology, over-ripening and shelflife and post-harvest technologies.

EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

TECHNICAL ANNEX

1 S&T EXCELLENCE

1.1 SOUNDNESS OF THE CHALLENGE

1.1.1 DESCRIPTION OF THE STATE-OF-THE-ART

The rapidly growing world population makes it challenging to ensure global food security and to provide sufficient production of highly nutritious and high sensory quality food in sustainable ways. In addition, climate change and global warming are predicted to result in reduced crop productivity and quality leading to higher food prices and global food insecurity (FAO, 2014, The state of food Insecurity in the world 2014), a scenario anticipated to create social unrest and famine. Fruits and vegetables contribute massively to healthy human diets (Martin et al., 2013; Martin, 2018; Tucker, 2009) and represent the major source of vitamins and antioxidants. However, due to their high water content fruits are prone to postharvest deterioration and rapid loss of organoleptic traits. Preserving organoleptic qualities after harvest depends on the control of ripening/senescence processes and storage conditions. Current technologies are efficient in reducing fruit deterioration and losses and extending the commercial shelf through slowing down the ripening process. However, in most cases these treatments are not suitable for maintaining high nutritional and sensory properties. Modified Atmosphere Packaging (MAP) and Controlled Atmosphere (CA) are commonly used to prolong the shelf life of fresh fruits. Most of these technologies are detrimental to the sensory quality of fruits, reducing their appeal to the consumer, as well as being costly and environmentally unfriendly. For prolonged storage and long-distance transportation common methods are based on low oxygen levels (from slightly lower air concentration to less than 0.5%, depending on the techniques used and the type of commodity) and high (1.5-3.0%) CO2 concentrations, combined with low temperature, which can create physiological changes such as chilling injuries or hypoxic/high CO2-related metabolic alterations.

Fleshy fruit ripening is controlled by a complex network of interacting endogenous (hormones) and exogenous factors (environmental cues). The ripening of climacteric fruits such as apple, tomato and peaches is characterized by a sharp increase in respiration marked by elevated CO2 production, the so-called “climacteric crisis”, that is associated with a rise in autocatalytic production of the plant hormone ethylene known to be instrumental to climacteric ripening. The oxygen consumption associated with the respiration peak and the reduced gas exchange fluxes between fruit tissues and the surrounding atmosphere are likely to create hypoxic conditions in the fruit tissues undergoing ripening. Hypoxia, *a fortiori* anoxia stress, represents an adverse and stressful environment for the fruit organ since less energy is produced and higher amounts of Reactive Oxygen Species (ROS) are generated. Indeed, exposure to low oxygen leads to increased ROS production which has deleterious effects on cell and tissue integrity (Pucciariello and Perata, 2016; Sasidharan et al., 2018), thus when facing such adverse conditions, plants set up adaptive pathways to allow normal development. Hypoxia related events during fruit ripening and CA/MA storage are probably similar to those occurring during flooding and water logging. While, the role of hypoxia and ROS in postharvest physiology has not been considered so far as a major topic, the novelty of the COST Action (RoxyCOST) is to determine to what extent and at what level these new factors impact ripening and post-harvest behavior. Because during ripening and postharvest life fruit experience low oxygen conditions, the main idea underlying the network is to bring together two scientific communities that were not previously interconnected, those dealing with fruit ripening and vegetative growth under

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flooding-induced anoxia, in order to promote exchange and to uncover common and different features between the two hypoxia-related processes.

Amongst the biggest challenges for the tomato industry is postharvest deterioration, which accounts for huge economic losses averaging over 25% of fresh produce every year. Shelf life of tomato is determined by the rate of fruit softening during over-ripening (which is the stage that follows commercial maturity, when the fruit softens and loses its organoleptic properties including its characteristic taste and flavour). In tomato fruit, the levels of ROS increase as fruit undergo different metabolic and physiological changes following completion of ripening, possibly signalled by ethylene (Jimenez et al., 2002; Mondal et al., 2004; Zhang et al., 2013). Once oxidative stress reaches a level that exceedes the scavenging systems capacity the oxidative damage begins. As oxidative damage reaches this critical point, it can directly cause physiological disorders or indirectly (via ROS signalling) trigger downstream pathways which accelerate over-ripening (Zhang et al., 2013). For fruit from varieties with low antioxidant capacity, free radicals accumulate rapidly post-breaker (Mondal et al., 2004), thus over-ripening proceeds strongly and quickly. By contrast, fruit with higher hydrophilic antioxidant capacity, have a greater ability to scavenge free radicals and to reduce oxidative stress (Bassolino et al., 2013; Mehta et al., 2002; Nambeesan et al., 2010; Zhang et al., 2013) thus leading to lesser oxidative damage and delayed overripening.

Both waterlogging and flooding conditions create anaerobic environments which in turn lead to hypoxic and anoxic conditions for plants and as a result produce ROS in transition which are many fold higher than under normal conditions causing severe damage to plant cells (Ashraf, 2009; Irfan et al., 2010). Different traits, adaptive to flooding have been described in many different crops (Bailey-Serres and Voesenek, 2008; Jackson, 2002; Jackson and Campbell, 1976; Visser and Voesenek, 2005; Voesenek and Blom, 1989; Voesenek et al., 2006). In Arabidopsis model plant tolerance to submergence is dependent on target genes of RAP2.12, a transcription factor that belongs to group VII Ethylene Response Factors (ERFs) (Loreti et al., 2016). In rice, engineering ERF expression improved tolerance to submergence (Xu et al., 2006, Hattori et al., 2009), highlighting the key role of ERFs in adaptation to hypoxia. Group VII ERFs are also involved in the responses of apple fruit to extreme hypoxic conditions applied in Dynamic Controlled Atmosphere (DCA) technique (Cukrov et al., 2016). Genes activated under O2 shortage include heat shock transcription factors, heat shock proteins and ROS-related transcription factors (Pucciariello and Perata, 2016).

Together with ROS, Nitric Oxide (NO) is considered as major component of plant responses to stress. NO accumulation occurs during hypoxic conditions (Gupta et al., 2011a; Sasidharan et al., 2018). In plants, NO regulates a wide range of processes such as seed dormancy, vegetative growth, flowering, stomatal aperture, leaf senescence and responses to pathogens (Mur et al., 2013), and NO concentrations are higher in unripe than in ripe fruit (Leshem et al., 1998). Moreover, recent studies indicate that NO treatments delay climacteric ethylene production and ripening in several fruit species. Exogenous NO treatment has been used successfully to extend shelf-life of many fruit likely via decreasing ethylene production (Simontacchi et al., 2013).

Strikingly, despite the importance of oxygen levels, ROS and NO during fruit ripening and post-harvest storage, the regulatory mechanisms taking place *in planta* that involve these factors remain elusive and the molecular mechanisms connecting ethylene, ROS, low O2 and NO in fruit ripening are totally unknown. Identification of the role of these factors as well as understanding the role of the molecular mechanism linking ethylene and hypoxia would have major consequences, not only in fundamental research but also in laying new avenues for innovative postharvest technologies with significant commercial applications. In particular, the Action expects to reveal shared and divergent mechanisms of fruit ripening, *in-* and *ex-planta*. The innovative aspect of this COST Action resides in the application of new concepts, such as the interplay of oxygen and ethylene during the climacteric crisis, that would likely translate into new technologies aimed at improving nutritional quality and storage life of fruit.

1.1.2 DESCRIPTION OF THE CHALLENGE (MAIN AIM)

The main objective of this COST Action is to understand how ethylene, low oxygen, NO and ROS signals are integrated to impact the ripening and over-ripening processes of fruit. This signaling network will be deciphered in two different contexts, (i) in tomato to understand how it controls ripening and overripening and, (ii) in pome fruit such as apple, with the goal to reduce post-harvest damage. By addressing the way low oxygen is perceived by plant tissues, the COST Action will decipher the molecular mechanisms underlying the role of oxygen sensing in triggering ripening and the adaptation to hypoxia during fruit storage.

While in the present paradigm the plant hormone ethylene is the key factor in fruit ripening, the novelty of the new working hypothesis is that the crosstalk between ethylene and hypoxia is instrumental in controlling ripening and postharvest physiology. On the other hand, productivity of fruit and vegetables is threatened by global climate change which is expected to induce higher rainfall, more waterlogging and postharvest losses. In addition, with world population expected to reach a peak in 2050 and greater urbanization in many developing countries, higher-value food will have to be moved over longer distances and greater efforts are needed to reduce food losses.

Overall, the new insight to be gained by the Action's networking activities on the physiology, biochemistry and genetics of low oxygen sensing and ROS is expected to optimize storage, transport and shelf life of crops. In addition, the training of researchers, and particularly young scientists, on cutting-edge methodologies and the implementation of bioinformatics tools for data integration will significantly enhance the competitiveness of European research in this field.

1.2 PROGRESS BEYOND THE STATE-OF-THE-ART

1.2.1 APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE-OF-THE-ART

It is known that ethylene plays a role in post-harvest loss, but new insights in O2 sensing have placed O2 at the center of this regulation. Recent studies suggest that members of subclass E tomato ERFs, orthologs to Arabidopsis group VII, are involved in fruit ripening (Liu et al., 2016). Because the stability of group VII ERFs in Arabidopsis depends on O2 and Nitric Oxide (NO), these ERF types are central to the mechanism sensing changes in O2/NO concentrations during hypoxic stress and development (Gibbs et al., 2011; Licausi et al., 2011). Therefore, these ERFs emerge as best candidate to integrate ethylene, oxygen and NO signals during fruit ripening. Tomato (*Solanum lycopersicum*) is a very important horticultural crop and a major source for nutrients worldwide (Klee and Giovannoni, 2011). Tomato fruits are enriched in water making them sensitive to rapid degradation after harvesting. While keeping the yield issue among their objectives, scientific programs nowadays have also to discover new means for maintaining fruit quality during post-harvest storage by enlarging our knowledge of the biological processes underlying fleshy fruit over-ripening. It is well known that the organoleptic and nutritional quality of fruit, post-harvest, depends on the control of over-ripening process known to be impacted by storage conditions. In addition to its agronomical and economic importance, tomato is a reference species for Solanaceae and a model plant for studying the development, ripening, and metabolism of climacteric fruits. Climacteric fruit generate an ethylene burst at the onset of ripening concomitantly with an increase in respiration. Most of our academic knowledge on the molecular basis of fleshy fruit development has been achieved using tomato as a model system, in particular the role of the plant hormone ethylene in controlling the ripening of climacteric fruits. In tomato, a number of mutants disturbed in fruit ripening (the so-called ripening mutants) have been collected by breeders and geneticists such as never-ripe (*nr*), ripening-inhibitor (*rin*), nonripening (*nor*) and Colorless nonripening (*Cnr*). Although ideal for transport and long-term shelf life these mutants are less suitable for consumption due to lack of tissue softening, lack of taste and absence of the typical red color (Hobson, 1980). The levels of ROS increase as fruit undergo different metabolic and physiological changes associated with ripening starting at breaker stage and possibly signalled by ethylene (Jimenez et al., 2002; Mondal et al., 2004; Zhang et al., 2013). Once oxidative stress reaches a certain level, the scavenging systems can no longer work effectively, and oxidative damage begins. As oxidative damage reaches this critical point, it can cause cell damage and leakage directly, with accompanying fruit softening (via lipid peroxidation and membrane damage) or indirectly via ROS signalling trigger downstream pathways which accelerate over-ripening (Zhang et al., 2013). In fruit varieties with low antioxidant capacity, free radicals accumulate rapidly, post-breaker (Mondal et al., 2004), resulting in fast and strong over-ripening process.

Tomato is the best model for addressing the main chalenges of the COST Action, although the new knowledge gained will be also applied to other economically important climacteric fruit species such as apple, peach and pear. Deciphering the oxygen and NO signalling pathway in tomato fruit tissue will yield breakthrough knowledge in molecular mechanisms and genetic factors controlling different aspects of the ripening processes. In Europe, fruit such as apples, pears and peaches are harvested before or at the early stages of ripening, and then stored until selling time, whereas the best nutritional and sensory quality attributes for these fruits are reached when ripening is completed on the tree. In addition, to prolong their storage life, pome fruit are stored under controlled atmosphere conditions (CA).

By controlling the levels of O2 and CO2 the quality of apples can be maintained almost all year round. However, this technology is expensive because it necessitates CO2 scrubbers, N2 generators with concomitant reduction of O2, ethylene absorbers, the control of humidity and installation of low temperatures. Apple will be choosen as a model to decipher the role of low oxygen sensing during postharvest storage. Because tomato and apple display different behaviour in response to CA storage, the two networks will be compared.

Submergence is the stress for which low oxygen signalling is the best understood. During floods, plants endure restricted access to atmospheric O2 and CO2, and hampered outward diffusion of plant evolved ethylene (C2H4) (Voesenek and Sasidharan, 2013). A fundamental advance was made with the description in *A.thaliana* of the O2-regulated localization and turnover of the type VII ERFs, and their role in transcription of genes encoding enzymes of anaerobic metabolism (Gibbs et al., 2011; Licausi et al., 2011). ERF-VIIs and N-end rule pathway genes are highly conserved in plants suggesting that the whole mechanism is conserved across higher plants. At times where plants experience hypoxia and invoke ROS signalling and when fruit experience low oxygen levels during post-harvest treatments efforts to improve fruit quality can take advantage of knowledge acquired from studying submergence responses in other plants.

This COST Action will take advantage of two different models to address the role of low O2/NO and ethylene
signalling interplay in development of fruit quality traits *in planta* and alteration of these traits, postharvest. The role of hypoxia sensing in the regulation of ripening in these two models will benefit from knowledge from flooding stress model systems.

1.2.2 OBJECTIVES

1.2.2.1 Research Coordination Objectives

The ultimate goal of this COST Action is to open new avenues towards improving the crop tolerance to long-time storage using the cutting-edge of knowledge related to sensing of low O2 and subsequent ROS signalling. The Action will also focus on increasing nutritional and sensory quality attributes of fleshy fruit during post-harvest storage, that will decrease the huge waste due to the deterioration of fruit and vegetables. Up to now, researchers in fruit ripening physiology, post-harvest and O2 sensing have not belonged to the same community, but this COST Action will bring them together in order to develop a common understanding of complex processes occurring in different contexts. The RoxyCOST Action aspires to develop new theories in fruit physiology leading to the development of new technologies in post-harvest conservation. To reach this objective, RoxyCOST aims to coordinate an international group of researchers, fruit processors, growers and industry. The current Action will bring together researchers working on sensing of low O2, submergence stress, *in*-planta fruit physiology, post-harvest physiology and technology. In doing so, these different topics will take advantage of new concepts to maintain productivity and ensure quality once fruit have been harvested. Because the most advanced research on sensing low O2 comes from the study of the effects of crop submergence, deciphering how plants sense low O2 during submergence is requested to complete this Action, successfully. This Action also aims at enhancing EU competitiveness in basic and applied sciences by promoting the training of young scientists in new concepts and cutting-edge technologies such as functional genomics and by providing access to the latest resources in the field of fruit biology and hypoxia research.

In addition to these overall goals, the COST Action aims to meet the following objectives:

- Establish a road map detailing how a multidisciplinary approach by means of genomics, transcriptomics, proteomics, fluxomics and applied technologies can be transferred to other crops for which post-harvest storage is a challenge .
- Facilitation of the transfer of knowledge related to ripening and over-ripening to develop new technologies to maintain fruit quality, postharvest.
- Facilitation of the transfer of knowledge from hypoxia and low oxyen sensing during submergence to explore new concepts in fruit ripening and over-ripening physiology.
- Bringing new insight into fruit development to broaden our understanding of the metabolic networks operating in fleshy fruit species, post-harvest, thus strengthening European research in fruit crops which constitute a large proportion of ‘healthy food’.

1.2.2.2 Capacity-building Objectives

To reach these objectives and to structure a strong European Research Network on fruit ripening, low oxygen physiology and ROS signalling, the following capacity building objectives are targeted: Facilitate transfer of knowledge and flow of information between researchers working on sensing low O2, flooding stress, ROS signalling, fruit ripening and over-ripening and post-harvest physiology by combining complementary concepts and methodologies in order to decipher the mode of action of low O2 sensing and ROS signaling in the control of fruit quality.

- Enhance communication between research groups to avoid duplication of work in different COST countries.
- Allow faster decision making on research priorities and on further questions to be addressed. Use the latest tools and resources to bridge data from genomics, proteomics, metabolomics, fluxomics, reverse genetics, bioinformatics and physiology, so that important horticultural processes can be studied using an integrated approach.
- Transfer of research techniques and concepts between the participating groups.
- Provision of easy access to research infrastructure facilities for partners via Short-Term Scientific Missions (STSMs) and promote wide diffusion of advanced technologies among different groups
- Sharing ‘know-how' and provision of advanced graduate and postdoctoral training in state-of the-art stress adaptation, ripening physiology, over-ripening and shelflife and post-harvest technologies.

2 NETWORKING EXCELLENCE

2.1 ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1 ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

The COST Action will gather some of the best research groups studying O2 sensing and oxygen stress in one hand, and fruit biology and post-harvest physiology on different plant models (tomato and apple) in the other hand. In addition geneticist will be involved to explore natural diversity to uncover and map QTLs impacting submergence tolerance and fruit shelf-life, in relation to oxygen and NO metabolism. Their research is internationally recognized as reflected by the high-impact publications and patents issued by the partners of this research network. This will ensure future joint publications and the emergence of research projects as well as standardized methods to be shared between participating groups, accelerating the overall research effort. The main focus of this COST Action is a joint initiative between groups working on sensing low O2, fruit ripening and storage physiology which will offer important added value because, so far, these research communities have worked separately. There is no current ongoing project addressing this challenge by combining the different approaches considered in this Action. In addition, RoxyCOST aims to bring together research groups working on different fruit models, gathering the broader fruit science community. The RoxyCOST Consortium will organize common workshops on fruit ripening, post-harvest physiology, and low O2/NO physiology aiming to provide a tailored framework for cross-field interactions between these research communities. Because of the importance of integration of “omics” data during the program, special attention will be paid to the organization of systems biology symposia where the participation of young European scientists will be promoted strongly.

Several scientific meetings in the fields of fruit and O2 research take place internationally almost every year. However, cross-interaction between groups working on these topics are very limited or non-exsistent and the fruit research community is generally divided into two sub-communities specialised either in fleshy fruit ripening physiology or in post-harvest physiology. In addition, except for the tomato community, the fruit physiology community is still spread out. This COST Action will provide an excellent opportunity to bridge the gaps between researchers and allow exchange of knowledge and know-how between European fruit research groups. The fruit community will take advantage of participation of low oxygen and ROS/NO experts to improve their knowledge of ripening and post-harvest physiology.

Workshops open to non-COST partners will be organised once a year. The Action will approach a broad impact scientific journal about a special issue with joint research articles on the roles of low O2 and NO signalling in crop plants.

The "RoxyCOST" Action will seek active collaborations with other COST Actions and other European and international research programmes. Initially, these will be established by personal as many members of this COST Action are already members of other European programmes and international consortia. The Management Committee (MC) will look at further expansion of contacts with these international research programmes by inviting the leading scientists dealing with fruit development and quality to participate in the general conferences and focused workshops. The originality of this Action is that storage practioners, post-harvest physiologists and fruit ripening/hypoxia scientists will be gathered together to improve storage efficiency by extending postharvest shelflife and quality of fruit. Moreover, where appropriate this COST Action will seek interaction with research groups from non-EU Mediterranean countries, given the importance of fruit production in these regions. "RoxyCOST" will specially encourage the access to modern technologies and training of young researchers and female gender from less developed European countries, southern Mediterranean and north African countries.

2.2 ADDED VALUE OF NETWORKING IN IMPACT

2.2.1 SECURING THE CRITICAL MASS AND EXPERTISE

The COST Action request expertise in low O2 signalling, fruit ripening and over-ripening biology, post-harvest physiology and technology. Because the biggest advances in understanding low O2 signalling have been made in submergence RoxyCOST will recruit experts in submergence stress. To achieve the objectives of RoxyCOST, the network will include experts from different domains. Inclusion of participants from different areas of Europe will provide a consistent network able to address the COST Action's stated challenges and objectives. Following the kick off meeting the core group will be open to expand the network in low O2 signalling, fruit ripening and over-ripening and post-harvest physiology. Because the appreciated organoleptic traits of fruit are dependent of the country of origin (Causse et al., 2010), RoxyCOST will take advantage of the wide geographical distribution of partners, to ensure that the technological ouput of the Action corresponds to our expectations.

2.2.2 INVOLVEMENT OF STAKEHOLDERS

By generating new fundamental knowledge that paves the way for future innovations, the European research community in plant sciences will contribute to the prosperity of society, the promotion of sustainable agriculture and related industrial activities. The groups involved in the COST Action are strongly committed to this objective and would all benefit from the increased scientific interaction promoted by this initiative. In that regard, the COST Action fulfils the goals of the Plants for the Future Technology Platform, including key aspects of the Strategic Research Agenda by bringing together key research groups from both public and private sectors. Moreover, when considering its mid- /long-term objectives, the knowledge generated in this COST Action will provide enabling technologies that can be applied by industry through the establishment of pioneering Small and Medium Enterprises (SMEs).

The target groups/end users of "RoxyCOST" Action are the following:

- Basic scientists dealing with O2 sensing, flooding stress, fruit ripening and over-ripening and post-harvest storage. The structuring of the research efforts dedicated to post-harvest physiology of fruit will be of great importance to the researchers in this field mainly by preventing duplication of initiatives or experiments in different laboratories/countries thus avoiding wastage of time and resources. In this regard, the COST Action will allow progress towards a set of standardised conditions for experimentation and comparison between results obtained from different labs in different European member states.

- National government and European policy makers. Documents and guidelines prepared as a result of this COST Action will be valuable to policy makers both at the level of national governments and the European level. These guidelines can be used for decision making regarding research funding activities at national and European levels. In addition the results can be further used in planning and directing breeding programmes in Europe.

- Breeders will drive innovative breeding strategies and improve current technologies using the resources and knowledge generated through this Action.

- Companies involved in post-harvest fruit storage will be fully involved as experts in fruit storage. They will be in charge of testing new storage protocols and the behaviour of new varieties, postharvest.

- Consumers, because the results obtained will contribute to improved sensory and nutritional fruit traits and promote fruit consumption and reduction in waste, post purchase.

2.2.3 MUTUAL BENEFITS OF THE INVOLVEMENT OF SECONDARY PROPOSERS FROM NEAR NEIGHBOUR OR INTERNATIONAL PARTNER COUNTRIES OR INTERNATIONAL ORGANISATIONS

To reach these stated objectives and structure a strong European research network on fleshy fruit and low oxygen, the following actions will be undertaken:

- Bring together different research groups and link European research to the most advanced non-EU groups.

- Facilitate transfer of knowledge and flow of information between researchers working with different fruit models and on low oxygen and ROS signalling by combining complementary concepts and methodologies.

- Allow rapid decisions on research priorities and identify relevant questions to be addressed.

- Use novel resources and skills to build bridges between genomics, proteomics, metabolomics, fluxomics reverse genetics, bioinformatics, physiology and eco-physiology, so that low oxygen and ROS signalling can be studied together with fruit quality, using integrated approaches.

- Provide easy access to research infrastructure facilities for partners via Short-Term Scientific Missions (STSMs) and promote diffusion of advanced technologies between different groups and particularly to the Near Neighbour Countries.

- Organize training schools for sharing ‘know-how' and advanced research technologies involving non-European researchers.

- Give access to cutting edge of “know-how” to researchers from Near Neighbour Coutries .

- Provide easy access to research infrastructure facilities from the European country to the involved Near Neighbour Coutries .

Several scientific meetings on fruit research take place internationally almost every year. It is expected that this COST Action will bridge the gap between fruit research and low oxygen research and allow exchange of knowledge and know-how between European fruit research groups and the low oxygen signalling community. Workshops open to non-COST partners will be organised once a year.

3 IMPACT

3.1 IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAK-THROUGHS

3.1.1 SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

The major impact of this COST Action will be the creation and the advancement of dormant research with the long term objective to diminish food losses, to increase tolerance of crops to climate change conditions of flooding in a sustainable way by boosting the advantages of EU-based industries in the postharvest sector and EU-based breeding companies and Institutions. Specifically, the main benefit expected from this COST Action is to increase the long term competitiveness of the European plant scientific community through the promotion of innovative interactions and the development of new state-of-the-art techniques to store fruit without decreases in quality. Coherent programmes without duplication are essential to the development of synergistic science, especially in research on molecular physiology. One of the goals of this COST Action is to tune genetics, transcriptomics, proteomics, metabolomics and fluxomics research efforts and create synergy among European research groups dealing with low O2 , ROS signalling and fruit physiology.

More specifically, this COST Action will facilitate the identification of endogenous and environmental cues that control fruit ripening *in*- and *ex*-planta in tomato and apple. This knowledge will be transferred to other fruit species such as apricot, peaches or pears, and non-climateric fruit like melon and grape that use components of ethylene and ROS signalling in ripening and over-ripening. It will also allow the exploration of natural diversity to uncover and map QTLs impacting fruit shelf-life. The physiological significance of selected target genes will be revealed via reverse genetics strategies such as Genome Editing and disruptive DNA technologies to increase or reduce gene expression levels. The impact of the mechanisms and regulatory factors identified will be evaluated under low oxygen conditions in a post-harvest context to assess their relevance under storage conditions. It is expected that their identification will greatly improve storage technology, and provide benefits for the food industry and consumers through the supply high organoleptic quality fruits.

The collaboration will benefit from the wide range of expertise available within the participating laboratories and companies expert in fruit and vegetables storage, which will be harnessed towards establishing links between the genetic/phenotypic variability observed in natural species, cultivars and RILs. The integrated approach to be implemented in this COST Action relies on the development of a range of novel bioinformatics tools aiming to reveal transcriptomic, proteomic and metabolomic networks underlying sensing of low O2 during hypoxia stress during fruit ripening and over-ripening (*in planta* and during storage). Special attention will be paid to training in bioinformatics, which will benefit to most partners through the crucial role of these methodologies in modern biology.

While most participants have ongoing projects supported by their national funding bodies, the scientific added value of the COST Action resides on the fact that, for the first time, fruit shelf life and quality, post-harvest, including under modified/controlled atmospheres will be studied through focus on the molecular mechanisms that control the ripening and over-ripening processes and the post-harvest effects of these signalling mechanisms. This COST Action will benefit from pooled expertise in fruit physiology
and low oxygen signalling. The development of new knowledge on fruit storage by the COST Action will ultimately open new avenues to provide “enabling technologies” for maintaining fruit sensory and nutritional qualities.

Linking European and international research groups will ensure standardization of methods and protocols and lead to joint publications and common research projects.

Long-term technological and socio-economic impacts is dual :

1. Deciphering of molecular mechanism linking ethylene, low oxygen and NO during ripening and submergence will enable use of natural mutants, affected in these pathways, in classical breeding programs to create new varieties with better storage and resilience traits.

2. Knowledge of the mechanisms involved in over-ripening and post-harvest physiology is very important for companies involved in storage of fruit, and also for manufacturers of storage infrastructure and storage devices. Optimisation of storage protocols and identification of markers for hypoxia-related storage disorders is one of the main goals of these companies. Reaching these objectives is linked strongly to understanding the molecular mechanisms.

3.2 MEASURES TO MAXIMISE IMPACT

3.2.1 KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

Whereas the molecular mechanisms that underlie responses to hypoxia in a submergence context are well known, the role of hypoxia in fruit ripening is completely unknown. The role of hypoxia in the development of fruit quality traits and the description of the molecular mechanism underlying hypoxia signalling in fruit ripening will be an important cutting-edge outcome at the end of the RoxyCOST Action. This new knowledge will serve as base to improve conservation of fruit, post-harvest. Indeed, postharvest storage is critical to selling, because in many cases off-flavours develop and the worst cases result in fruit loss. Different fruit react differently when transfered back to air after a CA storage period. Indeed apples and pears respond nicely to low O2 and keep their metabolism low and upon return back to air, they ripen properly, whereas fruit like tomato, peach and avocado do not respond favorably to low O2 strorage.

3.2.2 PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

The target groups/end users of RoxyCOST are the following:

1. National governments and European level policy makers. Documents and guidelines prepared as a result of this COST Action will be valuable to policy makers both at the level of national governments and at the European level. These guidelines can be used for decision making about research funding in member states at both national and European levels. The results could be used further in directing breeding programmes and formulating post-harvest recommendations in the European community.

2. Consumers- the results obtained will contribute to improvement of nutritional and sensory properties of fruit and thereby promote fruit consumption.

3. CA/MAP-related industries interested to enhance their capabilities in producing innovative products based on the outcomes and deliverables of the WGs.

4. Breeding companies interested to better adapt their cultivars to climate change conditions such as flooding and additional abiotic stresses.

The activities of RoxyCOST will be disseminated as widely as possible to a broad range of different groups along the entire value chain from basic scientists as well as breeders, post-harvest processing industries, supermarkets, policy makers and the general public. Since RoxyCOST will address the health-promoting content of fruits, it is expected that Institutions and Associations working towards improving public health will also be a target audience of this COST Action. European level policy makers, government policy makers, regional planners and policy makers will be targeted by this Action too.The most important target group of RoxyCOST will be the general public as final customers. Consumers are interested in knowing about improved nutritional and sensory qualities of new fruit varieties. First, a mailing list will be prepared for the general public, policy makers and scientists interested to be kept up-to-date with developments regarding this Action. The main purpose of this e-mail network will be to periodically (every 6 months) send digest e-mails in the form of text and hyperlinks to inform them of the latest developments regarding the Action and direct them to the relevant articles or news stories regarding such developments. A dedicated public website will provide information to the international scientific community and will facilitate communication flow between the partners. The website will be kept active by a web-coordinator appointed at the kick-off meeting. This website will be open to the general public and target all audience groups of the COST Action. It will be used to communicate the latest results of the Action to specialised and non specialised audiences. This will be achieved by producing articles that are understandable by the general public and delivering them to sections of the web site that are accessible to the internet community. In addition to contact details for RoxyCOST participants, the website will contain: (i) online courses, proceedings of meetings, talks and posters from meetings, (ii) STSM Calls and Reports, (iii) teaching tools (e.g. slides, course notes, protocols), (iv) links to the website of participating institutions, websites related to fleshy fruit development and quality traits and (v) job announcements.

The main aim of RoxyCOST will be to better harness existing knowledge and research on fleshy fruit development and the molecular basis to fruit quality traits, post-harvest so that it can be used more effectively for crop improvement in Europe. The coordination of this COST Action to establish better communication, standardised conditions and guidance will potentiate these research activities. It is expected that the dissemination of the results of this Action will result in changes to the practices of post-harvest storage in Europe. A Technology Transfer Platform will be established to optimise the diffusion and exploitation of results deriving from RoxyCOST. The activities will be tuned to those partners that lack sufficient resources to exploit their discoveries.

The research published from this COST Action and the application of guidelines that produced this research will allow RoxyCOST to be evaluated and its success in realizing dissemination of the data and outcomes, determined. Representatives of each country will be responsible for disseminating the activities of RoxyCOST to research groups, industrial partners and representatives of society in their own countries. To these ends, a study will be made of the innovative activities of RoxyCOST as a social scientific study of the impacts of genomic science on society. Results will be made available for internal interest and information.

Opportunities also exist for making results available to policy makers, and for publication in journals in fields such as innovation studies and the social sciences. This will assist dissemination to technical specialists working in other fields of technology development, and associated end-users. Expertise will also be available on wider dissemination of findings from the COST Action. It is important to note that the dissemination plan will be updated during the course of RoxyCOST taking into account the progress of the Action as well as the results of its evaluation.

4 IMPLEMENTATION

4.1 COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1 DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

The goal of this Action represents part of the effort to propose new responses to the challenge of food security. The specific contribution of RoxyCOST is to identify and decipher the mode of action of factors controlling fruit ripening and over-ripening and to define their effects during post-harvest.

The participants have identified the following Working Groups (WG) as priorities to facilitate research collaborations and to address the overall COST Actionct objectives:

WG1: Data management and dissemination

WG2: Deciphering the mechanisms of low oxygen sensing in fruit crops

WG3: Impact of low oxygen, ROS and NO on fruit crop productivity and quality

WG4: Data integration and systems analysis of role of hypoxia and ROS signalling in crops

WG5: Applications and developments in postharvest

WG1 will provide informatics tools allowing storage and analysis of data generated during the COST Action.
WG2 identifies the main factors involved in sensing low oxygen and the generation of molecular tools for the other WGs. Using these tools, WG3 will decipher the role of ROS and NO during hypoxia stress, during ripening and over-ripening and during the storage of fruit in CA. WG4 will focus on the development and integration of data generated in previous WGs to produce a consistent model of fruit ripening in different contexts. WG5 will transfer knowledge from other WGs to new field applications.

WG1: Data management and dissemination

To exploit the genomic data generated within this COST Action efficiently, this Working Group will coordinate data management and integration and analysis tools. This includes databases for archiving and management of data, versatile tools for comprehensive analysis, connectivity technology for integrating distributed data and analysis resources and standards for data representation and processing. The overall objective is to give biological meaning to the data obtained in other WGs. Some existing websites contain information concerning transcriptomic data mining in tomato during ripening (http://gbf.toulouse.inra.fr/tomexpress). They will enable comparative mapping to identify similarities and differences between species and major candidate genes underlying QTLs, to conduct genome comparisons to view comparative genetic maps and to identify synteny between tomato and apple genomes. In addition, this WG will coordinate the set up of friendly interfaces for users to access all databases (transcriptomics, metabolomics, genotyping and phetotyping databases). Significant dissemination efforts will be conducted comprising training of young scientists through STSM and specific workshops and practicals sessions.

Task 1.1. Processing and user-friendly availability of transcriptome, metabolome, genotyping and phenotyping datasets. These data will be centralised and made accessible from a “one stop shop”. Easy access to these data will be essential to identify candidate regulatory and biosynthetic genes underlying hypoxia and fruit ripening and over-ripening processes and post-harvest. This will involve development, operation and maintenance of computational infrastructure for handling experimental data from transcriptome and metabolome analyses. It will require development and use of tools for raw data processing, preparatory data analysis for assessment of data quality, set up of Laboratory Information Management Systems, and building functional genomics databases. This will be achieved, firstly, by bringing data managers from the distributed laboratories where large-scale functional genomics datasets will be generated, together to ensure consistent data storage and standards-based data processing. Secondly, by working out transparent data integration requirements in association with the formulation of data format standards, data exchange and database connectivity technologies.

Task 1.2 Organization of Workshops and Training Schools on new technologies. Workshops will be organized to train young scientists on the new omics and bioinformatics tools and database analysis in genomics and Genome Wide Association Studies (GWAS) in the field of low oxygen and flooding tolerance in various crop species. Moreover, practicals will be organized on the use of various pipelines for low oxygen and bioinformatics related datasets.

Task 1.3 Construction of interactive Webpage. A webpage will be constructed which is going to link all the databases which will be created providing easy, real time access to low oxygen and flooding omics data. This webpage will be linked to SOL site and sites of other crops to enhance visibility of the COST Action and facilitate scientific interactions.

WG2: Deciphering the mechanisms of low oxygen sensing in fruit crops

Task2.1 Generation of molecular tools. Transient and stable transformation systems used to assess gene function are routinely used in tomato. Stable transformation will be used in tomato to validate candidate genes identified in the next task. The CRISPR/Cas9 genome-editing technology offers unprecedented tools to edit DNA sequences precisely in plants. To overcome difficulties in transforming fruit tree species, WG2 will develop a direct-delivery protocol that combines injection of in vitro synthesized and assembled Cas9-crRNA (CRISPR RNA)-tracrRNA (trans-activating crRNA) ribonucleprotein complex. Key regulators of fruit ripening are likely transcription factors (TF) controlling metabolic pathways, as well as components of the low O2 sensing pathway. To improve post-harvest storage, it is first important to determine the target genes of these TFs. Chromatin Immuno Precipitation (ChIP) is the most efficient method to identify target genes of transcription factors. Although this method works in tomato, in tree species, very few research groups have succeeded to use this method. This WG would implement ChIP experiments to identify target genes of TFs in tomato and apple fruit. Identification of target genes involved in low O2 sensing and fruit ripening in- and ex-planta during storage require high/mid throughput facilities for phenotyping fruit quality traits. This WG will be in charge of identifying and organising platforms dedicated to high throughput fruit phenotyping based on nondestructive methodologies. These methods will allow monitoring fruit growth, shape, size, color, sugar/organic acid content, texture and hypoxia sensing. Genotyping is an important issue to establish gene to trait association. Access to high throughput genotyping facilities and training in the associated methodologies will be coordinated within this task.

Task 2.2 Identification of key factors of the low oxygen sensing pathway. In Arabidopsis and rice, it has been recently demonstrated that regulation of the responses to hypoxia and to NO can be regulated by the N-end-rule pathway. This pathway is involved in the degradation of group VII Ethylene Response Factor (ERF) in presence of oxygen. Notably, RAP2.12 is involded in this regulation and has been identified as a major low O2 sensor in Arabidopsis. Two main enzymes are associated to the Nend rule pathway in Arabidopsis: ATE (Arginine Transferase) and PCO (Plant Cysteine Oxidase). Existence and expression of these enzymes in tomato and apple is not yet clear. Putative redundancy between ERF homologues is also unknown and will be studied within this WG. The Action will use a combination of gene discovery tools; transcriptome analysis, genome wide association studies and forward (and reverse) genetic screens, to unravel the low O2 sensing pathway in plants.

WG3: Impact of low oxygen and NO on crop productivity and quality

Recent results suggest an interconnection between ethylene, low oxygen and NO, but the role of this pathway in hypoxic stress in crops is unknown. Regulation of ERFs during ripening and the fact that hypoxia related genes are regulated during ripening suggest the importance of low O2 and NO in this process. The role of this pathway will be explored in fruit in- and ex-planta using reverse genetic targeting the different factors of the N-end rule pathway identified in the previous WG.

***Task 3.1 Effects on ethylene and NO signalling during climacteric ripening*.** For a long time the climacteric crisis has been characterized through the increase in CO2 levels (Herner and Sink, 1973), but O2 consumption in different tissues during ripening is still unknown. Within this task this WG will be in charge to better characterized the climacteric crisis at the tissue level *in*- and *ex-planta*. In plants, O2 can be scavenged by class I Hb, which is induced by hypoxia and sequestration of O2 by nsHb facilitates NO scavenging, reducing the NO levels produced in hypoxia. Oxygenated class 1 Hbs react with NO to produce nitrate which represents the main mechanism for NO scavenging in plants (Gupta et al., 2011b). Moreover NO has been reported to act as a signal molecule involved in fruit ripening and senescence (Leshem et al., 1998). Therefore, along with the determination of oxygen levels in different fruit tissues, the Action also plans to measure the NO concentration in the same samples using the same device. Because ROS can be generated during hypoxia as well as upon transfer back to air and may be responsible for alterations in fruit, their production in fruit will be followed during ripening and over-ripening in different tissues. The work planned in this WG will provide for the first time a full characterization of climacteric ripening including O2 and NO concentrations. Special emphasis will be on quantifying microstructural changes occurring during fruit ripening, affecting tissue transport, contributing to the development of steep gas gradients within a single fruit. These impacts will be quantified through gas transport models and used to interpret gas measurements using microsensors, and to understand associated metabolic changes. In the model plant, Arabidopsis, hypoxia results in the regulation of the ethylene pathway through the degradation and subcellular localization of ERF proteins. A particular focus of RoxyCOST will be to follow the regulation of ERF proteins using specific antibodies. The effects of low O2 levels on fruit ripening and ethylene production will also be assessed. Fruit ripening will be assessed at the physiological and molecular levels by following ripening-associated traits in lines misexpressing genes involved in N-end rule protein turnover.

***Task 3.2 Effects on regulatory networks*.** This task will coordinate the global transcriptomic profiling of different phases of fruit ripening and over-ripening *in*- and *ex-planta* under low oxygen conditions. This is essential for the identification of regulatory features that are common or specific between the two kinds of ripening (*in*- and *ex-planta*). An interesting point is that fruit reacts differently when transferred back to air after hypoxia. Indeed apples and pears respond nicely to low O2 and keep their metabolism low and upon return back to air ripen normally, whereas fruit like tomato, peach avocado did not respond favorably to low O2 strorage. For over-ripening mutants affecting ROS sensing have been generated in tomato using CRISPR/Cas9. Transcriptomic analysis will identify which genes are modulated in their expression by ROS signalling, and how these signalling pathway determines shelf life of fruit, under normal and CA/MA conditions. Transcriptomic experiments will be performed to study the modification of the regulatory networks occurring when fruit are transferred to low oxygen, and also when they are transferred back to the air after storage. Moreover, it will be necessary to identify functional orthologues of genes between species of interest. Transcriptomic profiling will be performed using RNA-seq technology, taking advantage of tomato and apple genome sequences. Task 2.1 will identify the best platforms for transcriptomic profiles and will organise access and training in these cutting-edge technologies for the partners of the COST Action. Using tools generated in task 2.1. Transcriptomics data will be used to elucidate the regulatory networks that govern long term changes in the respiration metabolism, interaction with sensing of low O2, ethylene and NO signalling.

Task 3.3 Effects on quality attributes. Metabolic profiling can identify pathways leading to the accumulation of metabolites of interest in fruit ripened *in-planta* and harvested fruit. The main focus will be on compounds important for sensory and health promoting substances (e.g. organic acid and sugar, volatiles, vitamins, carotenoids and polyphenols) which also impact shelf life. Metabolic profiling will be used to follow the dynamics of hormone content and distribution in the fruit tissue throughout fruit ripening. Particular focus will be given to comparing metabolic profiles of fruit ripened *in*- and *ex-planta* for each fruit type.

The availability of large collection of mutants affecting in ripening (*rin*, *nr*, *nor*), and transgenic lines affected in low O2, ethylene and ROS sensing, will allow measurement of metabolic fluxes in these fruit where signalling has been perturbed. Analysis of fluxomes *in*- and *ex-planta* associated with transcriptomic changes will help to identify modifications of the gene networks active during storage under CA and MA conditions. In ripening fruit, metabolism can be considered to be dynamic and occurring at multiple regulatory levels that impact metabolic responses. For this reason, dynamic approaches will be applied, both starting from 13C labelling experiments. Various metabolic profiling techniques will be combined to measure the 13C tracer accumulating in the compounds of interest applying both classical steady state meatbolic flux analysis and state of the art dynamic flux modelling.

WG4: Data integration and Systems biology for low O2 role in crops

The interactions and activities within the COST Action and national and international pertinent industries, and climate change-related regional stakeholders and EU policymakers will build up a vibrant community through data integration and exploitation.

Task 4.1. Metabolic pathway models of respiration (based on fluxomics data) The objective of this WG task is to integrate experimental data collected at the various organisation levels using quantitative models. The experimental fluxomics data (WG3) will be used to develop and calibrate a metabolic pathway model of central carbon metabolism of fruit encompassing detailed reactions of glycolysis and the Krebs cycle linking to the electron transport chain where O2 plays a crucial role as the final electron acceptor. As central metabolism is very well conserved throughout the plant kingdom it is reasonable to expect that the model structure of central metabolism in various fruits will be very similar. Secondly, the model structure of the respiratory pathway will be extended with an extra layer of genetic regulation. The information for this will be derived from the transcriptional results from WG2 and WG3 revealing which pathways will be up or down regulated either in response to low O2 stress or as a function of climacteric fruit ripening. Depending on the nature of the transcriptome changes observed and their impact on the behaviour of the model, subsequent efforts will be made to develop sub-models for the most critical stages. This will be done using either statistical relationships or by developing semi-mechanistic sub-models. The most important point in this regard is to capture the involvement of the possible low O2 sensors from the transcriptomics data. While fruit ripening is known to modify the balance of the central metabolism it is not the aim of this task to model fruit ripening in further detail (for this see task 4.2), but mainly to identify where it might have discernible effects in central carbon metabolism.

Task 4.2: Modeling of regulatory networks involved in fruit ripening The objective of this WG is to maximize the knowledge gain from experimental data through consistent data management, standardization and integration to perform comprehensive and in-depth analysis of (comparative) genome data together with transcriptomics, metabolomics, proteomics, genetic and phenotype data. The systems approach should lead to the inference of information / targets associated with major fruit ripening processes shared by tomato and apple as seen in Phenomene (http://phnserver.phenome-networks.com/). In addition to such explorative ‘big data’ approaches, special efforts will be made to develop mechanistic models for selected regulatory networks by integrating data from different omics levels to create qualitative and quantitative insight into mechanisms underlying low O2 sensing in fruit crops and postharvest fruit quality.

Task 4.3. Integrated modelling, including gas gradients Based on *in silico* experiments it has been shown that concentration gradients and anoxic zones may occur in fruit. Microstructural features, such as the volume and interconnectivity of the intercellular space, affect O2 and CO2 diffusivity through tissues and partially explain why some fruit are more susceptible to low O2 stress related disorders than others. Firstly, numerical approaches will be developed to resolve the spatial distribution of diffusivity resulting from differences in porous structure across fruit. The spatial distribution of porosity and diffusivity will be calculated from 3D X-ray computed tomography (CT) images and correlated to tissue microstructure. Microscale diffusion models will be used to calculate effective gas diffusivity of tissues for the different gasses involved in hypoxia. Secondly, diffusion models for transport of O2, CO2, N2 and the fermentation metabolites, ethanol and acetaldehyde will be combined with kinetic pathway models for other metabolites and transcripts. An integrated model will allow prediction of the changes in the spatial distribution of O2, CO2, N2, and all intermediate metabolites and transcripts during ripening. The integrated model wil be benchmarked against the available macroscale reaction diffusion model based on homogeneous gas transport and simple Michaelis-Menten respiration kinetics. Finally, the model will be used to test hypotheses relating to hypoxia, taking into account limitations on diffusion and the spatial heterogeneity of fruit tissue. The combination of fruit microstructural properties and the regulation of central carbon metabolism at the metabolic and transcriptomic levels, will inform us of adaptations to central metabolism that go beyond the straightforward effects of temperature and gas composition.

Through a better quantitative understanding of these complex interactions, alternative storage approaches will be developed for the postharvest conditioning of fruit.

WG5: Applications and developments in postharvest

The various outcomes and deliverables by the other WGs will be communicated to the EU Dynamic Controlled Atmosphere (DCA) and CA/MAP industries through workshops in the pertinent conferences such as the CA/MAP Conference, the Postharvest Unlimited Conference and the International Postharvest Conference which are under the Aegis of ISHS (International Society for Horticultural Science). It is critical for this COST Action to involve and interact creatively with this industry considering that postharvest losses account for the 30% of the total food losses around the world. Particularly the DCA technology can provide a comparative advantage for EU in terms of postharvest quality preservation. Protocols based on the application of low oxygen levels are used widely for storage of pome fruits such as apples and pears (Controlled Atmosphere, CA) and tomatoes (Modified Atmosphere, MA). Optimization of these protocols, improved logistical management, introduction of innovative and sustainable packaging materials, reduction of wastes and losses need to be based on elucidation of the responses of fruit to hypoxic conditions over prolonged periods of time (> 6-9 months).

Task 5.1 (D)CA storage of pome fruit**.** Pome fruit are harvested mature but unripe, stored at low temperature (typically -1.0 to 3°C) in combination with a marked reduction of O2 and increases of CO2 partial pressure (CA storage). This delays climacteric ripening, and extends fruit storage life. The optimal storage gas composition is critical, because O2 levels that are too low combined with too high CO2 levels may induce fermentation in fruit, causing off-flavours (e.g., ethanol) and storage disorders (e.g., flesh browning). Levels of oxygen and carbon dioxide for CA storage are determined by cultivar and region, based on trial and error. More recently, Dynamic Controlled Atmosphere (DCA) storage, in which gas conditions are determined based on the stress responses of fruit has been used. Several factors affect the responses of apple and pear fruits to CA/DCA storage: including genotype, pre-harvest (field) factors, ripening stage at harvest, and technical parameters such as the rate of oxygen decrease. This WG will be related to WGs 2, 3 and 4. Fruit samples collected in commercial facilities and at lab level will be analysed for their physiological, phenological and molecular parameters (as described in the previous WGs) together with the technological parameters of the imposed CA/DCA treatments. Interactions between hypoxia and ethylene, ethylene inhibitors (1-MCP), and NO (exogenously applied) will be defined for a better elucidation of the fundamental mechanisms and responses of apple fruit to extreme and/or prolonged low oxygen conditions. Comparative analyses within the same fruit (from different cultivars) and between different species (tomato vs Apple) will be performed by combining results from this task and task 2.2. The identification of key elements of the low O2 sensing and primary responses to hypoxia in fruit is the main aim of this task: together with metabolic fingerprinting which will advance the development of sensors that can be used in CA/DCA rooms. Fruit storage companies will be involved in the activities of this task.

Task 5.2 MAP storage of tomato fruit**.** Controlling storage atmosphere is a key factor for delaying loss of tomato quality, postharvest. Tomatoes benefit from the use and application of MAP technology, resulting in prolonged shelf-life and ease of handling, postharvest and commercialization. Since tomatoes respond differently to MA (and associated decreases in temperature) depending on genotype and the stage of ripening when detached (e.g., Mature green (MG) vs Red Ripe (RR)), the events and mechanisms underlying this differential behavior need to be elucidated. In addition to the physiological, molecular and metabolic responses to changes in atmosphere occurring within the package, the effects of and the implementation of new packaging materials, produced more sustainably that is more efficient in preserving quality and prolonging the commercial life of tomatoes will be investigated. Films from different biological sources (e.g. starch) with differential performance and gas permeability are now available. Evaluation of their efficacy in MA will be undertaken using both technological and physiological/molecular parameters. Packaging material companies will be involved. This task is connected with both tasks 2.2. and 4.3. The use of packaging will be exploited for the study of the interactions beween hypoxic conditions, ethylene inhibitors and NO for possible practical applications in reducing chilling injury in tomatoes stored at low temperature (4°C).

4.1.2 DESCRIPTION OF DELIVERABLES AND TIMEFRAME

The COST Action will involved in 5 Working Groups, each group has different objectives, serving the RoxyCOST Action objectives. All these objectives are SMART (Specific, Measurable, Achievable, Relevant, Timely).

WG1: Data management and dissemination

D1.1: Databases for archival and management of data (Year 1 to 4) : data will be centralised and made accessible from a “one stop shop”.

D1.2: Versatile analysis tools for comprehensive analysis by all partners (Year 1 and 2) including development and use of tools for raw data processing, preparatory data analysis for assessment of data quality

D1.3: Setup of web-based database linked to other plant science databases (Year 1) : A webpage will be constructed to gather all the data related to response to hypoxia in different contexts in order to enable easy and real time access.

D1.4: Publication in appropriate scientific journals of the results obtain in the frame of RoxyCOST Action

WG2: Deciphering the mechanisms of oxygen sensing in fruit crops

D2.1: Development and implementation of direct-delivery protocol that combines injection of *in vitro*-synthesized and assembled Cas9-crRNA (CRISPR RNA)-tracrRNA (trans-activating crRNA) ribonucleoprotein complex. (Year 1 and 2)

D2.2: List of target genes of TFs in tomato and apple fruit (Year 2 and 3)

D2.3: Platform for non-destructive high throughput phenotyping (Year 1): These methods will allow monitoring fruit growth, shape, size, color, sugar/organic acid content, texture and hypoxia sensing

D2.4: List of genes involved in the low O2 sensing pathway (Year1 and Year 2) ; using a combination of gene discovery tools: transcriptome analysis, genome wide association studies and forward (and reverse) genetic screens, to unravel the low O2 sensing pathway in plants.

WG3: Impact of oxygen and NO on crop productivity and quality

D3.1: A non-destructive device allowing to monitor Oxygen and NO levels during tomato and apple fruit ripening/over-ripening (Year 1)

D3.2: Generation of antibodies allowing to assess the levels and tissues localization of regulatory proteins involved in the mechanisms underlying low O2 sensing (Year 1) : RoxyCOST will particularly focus on the regulation of ERF proteins using specific antibodies

D3.3: Transcriptomic profiling of genes involved in low O2 sensing after CA and MA storage (Year 2 and 3)

D3.4 : Metabolic profiling of fruits ripened *in-planta* and stored at low oxygen. The main focus will be on compounds important for sensory and health promoting substances (e.g. organic acid and sugar, volatiles, vitamins, carotenoids and polyphenols) which also impact shelf life.

WG4: Data integration and Systems biology for role of low O2 in crops

D4.1: Metabolic pathway models of respiration (Year 2): metabolic pathway model of central carbon metabolism of fruit encompassing detailed reactions of glycolysis and the Krebs cycle linking to the electron transport chain

D4.2: Model for regulation of respiratory metabolism (Year 4): mechanistic models for selected regulatory networks by integrating data from different omics levels

D4.3: Integrated model (Year 4) : model for spatial distribution of porosity and diffusivity for the different gas involved in hypoxia within fruit.

WG5: Applications and developments in postharvest

D5.1: Optimized CA/DCA guidelines for different pome fruit cultivars (Years 2 and 3) : Identification of key and regulatory elements of the low O2 primary responses to hypoxia of pome fruit and optimized guidelines for CA/DCA storage for different cultivars.

D5.2: Innovative protocols in MAP technology for tomatoes (Years 2 and 3): Description of technological, physiological, molecular and metabolic alteration of tomato fruit in response to different MAP techniques.

4.1.3 RISK ANALYSIS AND CONTINGENCY PLANS

This COST Action offers an unprecedented opportunity to establish the mechanisms underlying climacteric fleshy fruit ripening, over-ripening and deterioration during storage. It may also shed light on these processes in non-climateric fruit which use some ethylene signals during ripening. It will bring new insight into the factors regulating fruit ripening through the identification of new factors in the initiation of the ripening such as NO and Hb. It is based on a new working hypothesis that fruit ripening is impacted by low O2 sensing and that understanding the molecular mechanisms of ripening and over-ripening will improve organoleptic traits and shelf-life in flesy fruit. The main risk that can be identified at this stage is the use of molecular tools in fruit trees. However, several steps have been taken to overcome this issue. In this context, molecular markers will be used to validate the functionality of candidate genes.

4.1.4 GANTT DIAGRAM

	Year1				**Year2**				**Year3**				**Year4**			
Coordination	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Kick-off meeting	x															
Website generation	x	x														
Website update			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Reporting				x				x				x				x
MC meeting	x		x				x				x				x	
Core group meeting	x		x		x		x		x		x		x		x	
WG1		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		
Task 1.1	x	x	x	x				x				x		x		
Task 1.2	x	x			x	x			x	x						
Task 1.3	x	x	x	x				x				x		x		
WG1 meeting		x	x			x	x			x	x			x	x	
WG2		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	
Task 2.1		x	x	x	x	x	x	x	x	x						
Task 2.2			x	x	x	x	x	x	x	x	x	x	x	x		
WG2 meeting		x	x			x	x			x	x			x	x	
WG3	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	
Task 3.1	x	x	x	x	x	x	x	x	x	x						
Task 3.2					x	x	x	x								
Task 3.3								x	x	x	x	x	x	x		
WG3 meeting		x	x			x	x			x	x			x	x	
WG4		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Task 4.1		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Task 4.2		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Task 4.3		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
WG4 meeting		x	x			x	x			x	x			x	x	
WG5		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Task 5.1			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Task 5.2		x	x			x	x			x	x	x	x	x	x	x
WG5 meeting		x	x			x	x			x	x			x	x	
Workshops					x				x				x			
Final Conference																x

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