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PERSPECTIVE

Smart selection of soil microbes for resilient and sustainable viticulture

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SUMMARY

The grapevine industry is of high economic importance in several countries worldwide. Its growing market demand led to an acceleration of the entire production processes, implying increasing use of water resources at the expense of environmental water balance and the hydrological cycle. Furthermore, in recent decades climate change and the consequent expansion of drought have further compromised water availability, making current agricultural systems even more fragile from ecological and economical perspectives. Consequently, farmers' income and welfare are increasingly unpredictable and unstable. Therefore, it is urgent to improve the resilience of vineyards, and of agro-ecosystems in general, by developing sustainable and environmentally friendly farming practices by more rational biological and natural resources use. The PRIMA project PROSIT addresses these challenges by characterizing and harnessing grapevine-associated microbiota to propose innovative and sustainable agronomic practices. PROSIT aims to determine the efficacy of natural microbiomes transferred from grapevines adapted to arid climate to commonly cultivated grapevine cultivars. In doing so it will test those natural microbiome effects on drought tolerance. This multidisciplinary project will utilize *in vitro* culture techniques, bioimaging, microbiological tests, metabolomics, metabarcoding and epigenetic analyses. These will be combined to shed light on molecular mechanisms triggered in plants by microbial associations upon water stress. To this end it is hoped that the project will serve as a blueprint not only for studies uncovering the microbiome role in drought stress in a wide range of species, but also for analyzing its effect on a wide range of stresses commonly encountered in modern agricultural systems.

Keywords: grapevine, endophytes, drought stress, metabarcoding, metabolomics, epigenetics, abscisic acid, calcium signaling.

INTRODUCTION

The International Organization of Vine and Wine (www.oiv.int) estimated that in 2021, the global grape production was in the range of 72.7 million tons, of which more than half (52%) was destined for pressing for wine production, and only a small percentage for the production of musts and juices. Of the top 10 countries under vine, five are located around the Mediterranean area, where grapevine cultivation for wine production is deeply bound to the territory and the cultural and historical traditions of its people. In the past

50 years, climate variables associated with high temperatures and low precipitation rates significantly contributed to improving the quality of grapes and wines (Chaves et al., 2010; Neethling et al., 2012). In response to high temperature and low precipitation rates, the content of sugars and the acidity scores of grape berries rise and decrease, respectively, facilitating spontaneous fermentation of musts and the production of quality wines. Moreover, as berry fruits accumulate specialized metabolites to contrast moderately harsh weather conditions, wine color, taste, and aromas generally improve. However, in the most recent decade, the continued rise in temperature and prolonged droughts overturned the favorable agronomic advantage of the Mediterranean and Mediterranean-like areas for grape production (Bernardo et al., 2018). Therefore, while the world vineyard surface area has remained relatively stable over the past few years, world wine production has been witnessing a slight decrease in all wine-producing regions of the Northern and Southern hemispheres (Roca, 2022). An analysis of the causes of this decrease revealed that adverse climatic conditions, and the grape diseases associated with weather variables, were the main factors affecting production. Moreover, the expected world harvest for 2023 is estimated to be the smallest of the past 60 years (OIV, 2023). *Vitis vinifera* evolution, domestication, and further dispersal all occurred across a temperate climatic zone in Western Asia and the Caucasus (Dong et al., 2023). Therefore, strong deviations from conditions of mild and wet winters and warm and dry summers, have the potential to hamper grapevine standards of quality and production. The situation is further aggravated by the complex topography of the Mediterranean regions, as the vicinity of the coastal line and the mountainous territory can further amplify the adverse effects of weather variables on a local scale. Indeed, the concept of *terroir*, which is mostly associated with high-quality wines, depends on the distinctive environmental variables of local territory, including topography, climate, soil physicochemical features and soil microbiome, which are intertwined with local traditions of oenology and viticulture. As the disturbance of this complex equilibrium hampers local wine production and its cultural heritage, solutions to contrast adverse weather conditions associated with climate change are urgently needed. In this respect, the risk that inoculation of endophytes could be altering the resident soil biota equilibrium is considered negligible as countless reports of microbial ecology have shown that the impact of released microorganisms on autochthonous ones is practically absent as the survival and multiplication odds of the former are largely prevented by the homeostatic competitivity of the latter.

GRAPEVINE RESPONSE TO WATER SCARCITY AND APPROACHES TO INCREASE TOLERANCE TO STRESS

The operational range of grapevine water potential (Ψ) is beyond the margins of physiological safety, as determined for most Eudicots species from temperate climates. Therefore, it is said that grapevine is moderate to highly tolerant to drought stress (Gambetta et al., 2020). Nonetheless, the negative effect of drought on grapevine plants is undeniable. Curled and wilted leaves and drooping shoots are macroscopic symptoms that may appear even after a relatively short period of water shortage when temperature and solar radiation pass the threshold of stress, whereby. typically, photorespiration and thermal dissipation via non-photochemical quenching are promptly activated in grapevine (Beis & Patakas, 2012). However, the appearance of leaf discoloration and brown spots, accompanied by reduced content of photosynthetic pigments, is clear evidence that the enzymatic and non-enzymatic quenching machinery cannot fully cope with the burst of reactive oxygen species (ROS) produced in response to drought. As such, degradation of the cellular endomembrane system and increased autophagy are often reported when plants are exposed to water stress (Zeng et al., 2022). An even more severe symptom is cavitation, the presence of water vapor (air) in the xylem vessels. Cavitation may eventually lead to embolism, when air bubbles trapped in the vessels reduce the hydraulic conductivity and, in extreme cases, can induce plant mortality when it occurs repeatedly over several years (Vuerich et al., 2023). Still, transcriptional activation of abscisic acid (ABA) biosynthetic genes, increased enzymatic ROS quenching, and accumulation of protective molecules such as proline and putrescine are common responses shared across drought-tolerant cultivars, currently targeted for breeding and treatment trials. Largely unknown and partially unexplored is the relative contribution of these molecules to the conferral of the drought-tolerant phenotype at the whole plant level. That a cross-regulatory interplay exists between multiple pathways is also a possibility as shown by the crosstalk between putrescine and other polyamines and the activation of the ABA response (González-Hernández et al., 2022). Agricultural and breeding practices brought in by centuries of viticulture and winemaking work are being developed to improve grapevine tolerance to water scarcity. Below, we provide a historical overview of these approaches alongside a discussion of more recent and sustainable implementations.

Historical overview of the agricultural and breeding practices aimed at enhancing grapevines resistance to abiotic stressors

Grapevine breeding programs were initiated to find solutions to biotic stressors, and in particular to defeat the "Grape Phylloxera", the epidemic plague that in the late nineteenth century devastated most of the European vineyards (Eibach & Töpfer, 2015). However, attempts to breed French grapevines for resistance to the pest were unsuccessful, while American hybrids, which took advantage of crosses with sturdy American *Vitis* spp., were neglected because of the poor quality of their berries for wine production. Given the poor outcomes of these attempts, viticulturists turned their attention to the ancient practice of grafting and by combining scions of *Vitis vinifera* onto American *Vitis* spp. rootstocks. In doing so they succeeded in integrating traits of berry quality and resistance by the union of two species. Since then, the practice of grapevine grafting has been adopted for all cultivated plants destined for wine production. Therefore, although breeding and selection are successfully applied to modern viticulture, physiological and biochemical parameters contributing to berry quality and drought tolerance are bred separately (Eibach & Töpfer, 2015). In particular, to address the need for drought-tolerant grapevines, breeding focuses attention on American *Vitis* spp., which are already characterized by an extensive root apparatus that can penetrate deeply into the soil and extract water and can be improved even further. By contrast, tailored selection of the grafted scion focuses on reducing evapotranspiration while maintaining traits of berry quality. Still, practices of orchard management have traditionally been applied along with breeding to secure yields. Indeed, since grapevines are perennials and new plants require, on average, 3 years before becoming productive, orchard dismissing and replanting of new clones is performed sporadically or after many decades, while regular planting is mostly carried out to replace individual plant dieback. Therefore, agricultural practices including low vine training, which reduces sunlight exposure, are pursued in hot regions, while spur pruning and goblet training are adopted to contrast drought stress. Mulching, defoliation and treatments with protective compounds, such as ABA and kaolin are also commonly applied to contrast abiotic stress in grapevines (Bernardo et al., 2018). Despite these multiple and integrated approaches, the reduced water availability consequent to the intensification of drought and heat waves associated with climate change (Santos et al., 2020), represent a real threat to viticulture. As a consequence, viable and sustainable approaches to ameliorate plant resilience and tolerance to these stresses are required.

Sustainable approaches to increase grapevine tolerance to abiotic stress

The practices of orchard management mentioned above have been used for centuries to increase tolerance to abiotic stresses and secure grapevine yield and high-quality berries. Yet, prolonged and recurring periods of drought that occurred in the last decade determined the need to find alternative, sustainable, and long-lasting approaches to face climate change in agriculture (Markova et al., 2018). Given the positive impact of bacteria and fungi on plant health and performance, scientists have recently turned their attention to the soil microbiome as a tool to improve resistance (Bettenfeld et al., 2022).

In their natural environment, grapevine plants build close relationships with microorganisms of the phyllosphere, anthosphere, and rhizosphere. Mutualistic and commensal relationships are also established with microorganisms that enter plants and colonize their tissues, also referred to as the endosphere. These microbial communities are composed of a wide range of prokaryotes (bacteria and archaea) and eukaryotes (e.g., fungi, algae, protozoa), which all together form the plant microbiota (Choi et al., 2021; Trivedi et al., 2020). As these interactions progress, the plant host and its associated microbiome develop into a single biological macro-entity known as the plant holobiont (Figure 1; Vandenkoornhuyse et al., 2015; Zilber-Rosenberg & Rosenberg, 2008). Hence, the microbiome does not simply provide the plant with a supplementary set of genes which are microbe-borne and contribute to the holobiont functions. Instead, as integrated molecular signaling arises between plant and microbial genomes, a third genetic entity, the hologenome, becomes established (Ravanbakhsh et al., 2021; Zilber-Rosenberg & Rosenberg, 2008). The plant root is the main entry point for microbes that later establish endophytic relationships with the plant host and contribute traits of abiotic and biotic stress resilience. Without any doubt, soil bacteria that form biofilms of polymeric substances surrounding plant roots provide a physical barrier to water loss in the soil (Ali et al., 2022). Nonetheless, microbial-mediated triggering of plant-responsive genes through the transcriptional activation of the hologenome appears particularly effective at boosting tailored tolerance and resilience to many stresses. A recent analysis of the vineyard microbiome from around the world revealed that rhizosphere microbes contribute the most to regional wine characteristics, also referred to as the *terroir* (Gobbi et al., 2022). Of particular interest was the observation that microbiome data can successfully discriminate vineyards from a specific continent and, within a continent, vineyards from a specific wine region. As the vineyard microbiome is shaped by climate (temperature, humidity, and altitude) and soil (primarily pH, but also the soil elemental composition), the observations brought by this study can find practical applications in viticulture. Indeed, given the tremendous microbial diversity measured across vineyards with distinctive edaphic and climatic characteristics, microbes associated with plants from dry areas have the potential to confer cross-tolerance to grapevines from temperate areas. Research on this topic has already been performed in herbaceous plants, which revealed microbial-induced drought tolerance in *Arabidopsis thaliana* (Thale cress) and *Medicago sativa* (alfalfa) following root inoculation with bacteria isolated from desert plants (Alwutayd et al., 2023).

Grapevine endophytes

Endophytes, as mentioned, are a class of non-pathogenic microorganisms present in the internal tissues of plants. The endophytic portion of the microbiota is particularly interesting for grapevine resilience to abiotic stress as it can elicit local and systemic responses, leading to

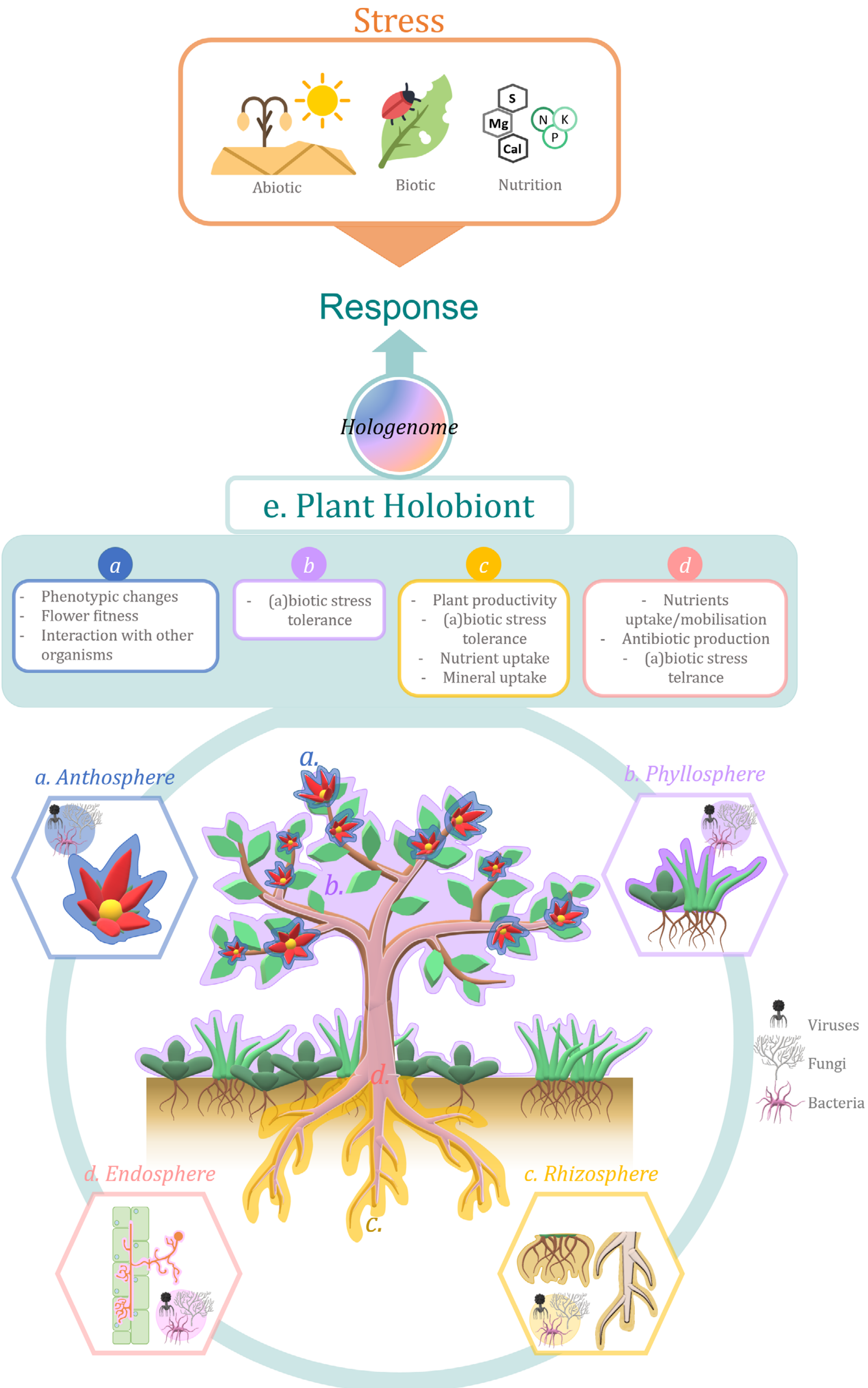
Stress
Abiotic
Biotic
S
Mg
Cal
N
K
P
Nutrition
Response
Hologenome
e. Plant Holobiont
a
- Phenotypic changes
- Flower fitness
- Interaction with other organisms
b
- (a)biotic stress tolerance
c
- Plant productivity
- (a)biotic stress tolerance
- Nutrient uptake
- Mineral uptake
d
- Nutrients uptake/mobilisation
- Antibiotic production
- (a)biotic stress telrance
a. Anthosphere
b. Phyllosphere
a.
b.
d.
c.
d. Endosphere
c. Rhizosphere
Viruses
Fungi
Bacteria

Figure 1. Plant holobiont.
In their natural environment, plants established close relationships with microorganisms of anthosphere (a; flowers), phyllosphere (b; aerial parts), rhizosphere (c; roots), and endosphere (d; apoplast, intracellular). Microbiomes in association with the plant host forms a single biological macro-entity known as the (e) plant holobiont. The integrated molecular signaling resulting from the combination of microbiome and plant genes sets constitute a third genomic entity called the hologenome, whose activation can promote plant stress resilience.

increased tolerance to heat, drought, and high salinity (Pacifico et al., 2019; Salvi et al., 2022). It is generally assumed that endophytes enter the plant from the root (Nigris et al., 2018). Indeed, Deyett and Rolshausen (2020) showed that most grapevine endophytes are localized in root tissues, which thus represents the first barrier to microbial colonization. Thereafter, endophytes diffuse systemically to aboveground plant tissues. The taxonomic composition of microbial communities found in the plant aerial organs segregates from the root bacteria, revealing that specific plant tissues exert differential selective pressure on colonizing microbes (Deyett & Rolshausen, 2020; Zarraonaindia et al., 2015). Interestingly, grapevine endophytes are equally distributed in aboveground and belowground tissues, showing that the grafting union does not hamper the systemic diffusion of microbes from root to shoot (Deyett & Rolshausen, 2020).

The benefits provided by endophytes are direct, through the induction of stress-related genes, or indirect, by priming plants to further exposure (Pacifico et al., 2019). Moreover, as endophytes colonize intercellular spaces and depolymerize non-structural carbohydrates to sustain their growth, they could favor the creation of the osmotic potential that refills embolized xylem vessels. For example, short-chain carbohydrates and monomers produces from the breakdown of larger polymers, such as starch, may contribute to the formation of hydrogels which could contribute to stabilize the vessels (Vuerich et al., 2023). In grapevine, mortality of the main water conduits is relatively uncommon as the plant sacrifices petioles and leaves to maintain long-distance stem water flow (Gambetta et al., 2020). Still, different cultivars adopt alternative strategies for conduit recovery, such as the formation of hydrogels or sucrose mobilization (Vuerich et al., 2023). However, whether endophytes have a role in the process of hydraulic recovery has yet to be explored.

The molecular mechanisms associated with increased grapevine tolerance to abiotic stress include the modulation of plant active response to abiotic stimuli and the accumulation of active microbial compounds. These responses are mainly activated by the microbial release of specialized metabolites and hormone-like molecules, which regulate plant growth and development in response to stress. Bacteria can reduce drought damage in many ways. In addition to promoting the development of roots and shoots, endophytes modulate the biochemistry of plants, enhancing ROS quenching, the synthesis of plant-specialized metabolites, as well as modulating synthesis and response to auxins and ABA (Pacifico et al., 2019). The plant growth-promoting activity of endophytes depends upon phosphate solubilization and the production of ammonia via peptone mineralization. Moreover, endophyte-mediated synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme, which degrades the ACC precursor of ethylene, indirectly promoting plant development during drought and salinity stresses (Gamalero & Glick, 2015; Miliute et al., 2015). Intriguingly, endophyte regulation of plant metabolism also depends on tissue and stage of development, as transcript and metabolite abundance significantly differ in leaves and berries (Yang et al., 2016). Along with better fitness, grapevine inoculation with beneficial bacteria is associated with a higher quality of berries due to enhanced synthesis of volatile organic compounds (VOCs) and phenols such as resveratrol, flavonoids, and anthocyanins. As the synthesis of compounds related to wine quality, such as pigments, tannins, and VOCs, increases as a means of plant adaptation to the stress, we emphasize that mild stress is purposely sought for, especially by growers of red grapevine varieties. Therefore, inoculation with endophytes that maintains the plant in a mild-stress condition could promote the synthesis of these metabolites (Otoguro & Suzuki, 2018).

Often, dry and hot climates are associated with high salinity in the soil (Zhou-Tsang et al., 2021). It was shown that halotolerant plant growth-promoting bacteria (HT-PGPBs) promote plant growth under saline conditions (Teo et al., 2022). Therefore, applying HT-PGPBs consortia may represent a new strategy to develop sustainable agricultural production in salty environments. Navarro-Torre et al. (2023) found that HT-PGPB isolated from salt tolerant *Limonium* spp. could promote grapevine tolerance to salt stress. Fast sprouting mediated by HT-PGPB-induced synthesis of auxins, accompanied by the release of siderophores, increased nitrogen fixation and solubilization of phosphates, were reported in inoculated plants (Navarro-Torre et al., 2023). In addition to bacteria, the potential application of endophytic fungi has also been reported (Qu et al., 2022; Yu et al., 2020). Pan et al. (2023) showed that fungal species increase anthocyanin accumulation in berries, which ameliorates the quality of red wines and offers protection against UV radiation (Pan et al., 2023).

In conclusion, endophytes can potentially increase grapevine tolerance to abiotic stress. Nonetheless, while root inoculation with mixtures of arbuscular mycorrhizae (AM) and other fungi (e.g., *Trichoderma* spp.) is a well-accepted and broadly commercialized technique to prevent wood diseases, the use of soil microbes to contrast abiotic stress has yet to receive attention. Moreover, as molecular mechanisms mediating enhanced drought tolerance upon endophyte inoculation of grapevine plants are poorly understood, transdisciplinary teams of investigators are better suited to answer these questions and find sustainable solutions for practical application in agriculture.

PROSIT: A COMPLETE PHENOTYPING OF GRAPEVINE PLANTS FOLLOWING INOCULATION WITH SELECTED ENDOPHYTES

The challenges described above will be addressed by the recently funded PRIMA project, Plant Microbiome in Sustainable Viticulture (PROSIT). In particular, PROSIT aims to assess whether the tolerance to drought stress displayed by *V. vinifera* cultivars adapted to dry areas depends on the soil microbiome and whether microbes associated with plants from dry regions have the potential to confer cross-tolerance to cultivars from temperate areas. The molecular mechanisms underlying the enhanced drought tolerance in the final host will also be investigated (Figure 2). To achieve this goal, grapevine plants with superior resilience to drought will be selected from dry areas around the Mediterranean Basin and utilized as microbiome donors. Microbial endophytes that had traveled across the grafting junction into the scion will be harvested from those selected mother plants, cultured, and utilized for direct application to *V. vinifera* cultivars from temperate areas, which will be later subjected to drought experiments and comprehensive phenotypic evaluation. Non-culturable endophytes, which are microbe's refractory to *in vitro* cultivation, will be inoculated by direct grafting. Endophyte-free *V. vinifera* scions will be grafted onto the mother plants and later detached and rooted before performing the drought experiments. It has been observed that successful plant-microbial inoculation often depends upon the compatibility between the plant and microbial species (Zhang et al., 2020). Therefore, by selecting endophytes already adapted to a grapevine host, PROSIT will increase the chance of successful microbial inoculation. The endophyte-inoculated plants generated in this experiment will be subjected to comprehensive phenotypical analysis, which includes the assessment of physiological, biochemical, and transcriptional parameters via transcriptomics and metabolomics. Non-coding small RNA will be sequenced to evaluate their possible involvement in drought stress response either via Post Transcriptional Gene Silencing or Transcriptional Gene Silencing, and determine whether small RNA exchanges between graft partners could be influenced by the endophyte (Rubio et al., 2022). At the same time, changes in DNA methylation profile will be determined to evaluate eventual effects on gene expression and transposon activity.

Plants have developed several mechanisms that allow rapid responses to adverse environmental conditions, including hormones, secondary messengers, transcription factors, and epigenetic regulators that lead to cellular responses and changes in gene expression (Gallusci et al., 2022; VanWallendael et al., 2019). Epigenetic regulations are now known to play essential roles in the responses and adaptation of plants to stresses, even though most work so far was performed on herbaceous and annual plants (Ashapkin et al., 2020). As far as grapevine is concerned, genes encoding enzymes controlling histone methylation and/or acetylation are differentially regulated in response to abiotic stresses suggesting that epigenetic regulations are important in the response to stresses in this plant as well. The importance of DNA methylation in grapevine stress responses is also shown by changes in DNA methylation patterns induced after *Flavescence dorée* infection (Pagliarani et al., 2020), or by the application of elicitors (Gao et al., 2020), and pieces of evidence are accumulating that it could play important roles in abiotic stress response and memory as well (reviewed in Berger et al., 2023). In Arabidopsis plants inoculated with microbes isolated from desert plants, histone methylation in the promoter regions of aquaporin genes was revealed to increase the tolerance to drought stress (Alwutayd et al., 2023). Grapevine plants, which are represented by the union of two species (American and *V. vinifera* spp.) through the grafting union, may represent a great model to understand how the signaling induced by endophyte inoculation travels systemically to increase resilience to drought stress. *V. vinifera* plants harboring ABA and Ca^{2+}, a key second messenger in drought response (Kim et al., 2010), fluorescent reporters will be generated and utilized to investigate hormonal and cellular signaling activated in response to drought stress in endophyte-inoculated and control plants. With the need to understand if and how endophytes have a positive role in plant responses to drought, it is worth investigating whether they modulate transpiration by modulating the levels of ABA or impinging on its signal transduction pathway, consequently inducing stomata closure. Before the development of genetically encoded biosensors, chromatography-coupled mass spectrometry was utilized to measure ABA levels in plant tissues. However, this analytical approach required extraction of ABA from plant tissue and, therefore, provided limited spatial resolution (Balcerowicz et al., 2021; Wang et al., 2019). In 2014, two independent groups reported the development of two Förster resonance energy transfer-based fluorescence ABA biosensors, called ABAleon and ABACUS1 (ABA Concentration and Uptake Sensor), respectively (Jones et al., 2014; Waadt et al., 2014). Within PROSIT, genetically encoded biosensors will be exploited to study non-invasively and in a natural context different parameters such as ABA, Ca^{2+} at first

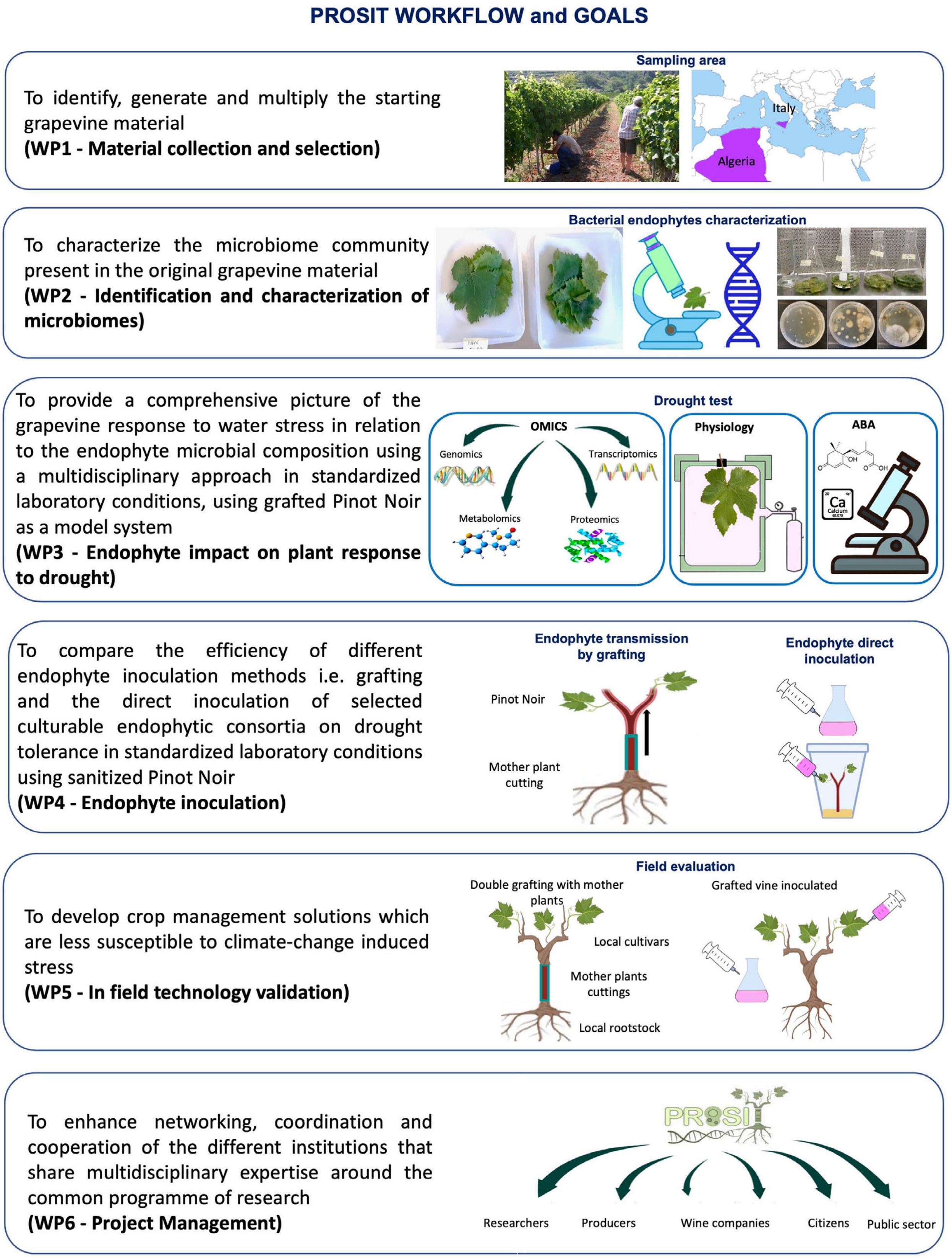

Figure 2. Organization and outputs of the PROSIT project.

but also pH, ROS, and redox status of antioxidant redox buffers (e.g., glutathione) (Walia et al., 2018). In particular, we aim to measure ABA levels in guard cells of control and water-stressed grapevine plants, before and after endophyte inoculation. In addition to measuring ABA levels, we will evaluate the potential effects of endophyte inoculation on the different components of the ABA signal pathway. In doing so, we will disentangle the role of Ca^{2+} signaling and modulation of the redox status of stomata cells (Liese et al., 2023; Zhang et al., 2020). For this purpose, different genetically encoded fluorescent biosensors, already available and successfully used in Arabidopsis (Grenzi et al., 2021; Walia et al., 2018), will also be utilized in grapevine.

The extensive grapevine phenotyping proposed by PROSIT will provide a means to correlate metabolic responses to transcript abundance and epigenetic regulation of gene expression. Concurrently, ABA and Ca^{2+}-mediated signaling responses will be visualized in reporter plants to understand how endophytes modulate and affect ABA synthesis and transport. Concurrently, we will evaluate the effect of the endophytes on the Ca^{2+}-triggered responses induced by drought (Jones et al., 2014; Waadt et al., 2014). At the completion of this project, a comprehensive model of endophyte-induced drought acclimation, including epigenetic silencing, or activation, of targeted genes, as well as its dependence on the ABA and Ca^{2+} signaling pathways, will be presented. The multidisciplinary approach, proposed in PROSIT based on omics, imaging and epigenetics analyses, will allow to understand to which extent different *V. vinifera* varieties adaptation to water deficit is influenced by the microbiome, and whether such a beneficial microbiome is transmissible to other varieties. Beside the scientific merit represented by the mechanistic understanding of the endophyte-induced tolerance to drought in crops, the outcomes of PROSIT are of a much broader impact in scope. Indeed, the PROSIT-generated list of endophyte-cultivar associations correlated by scores of their effectiveness in inducing acclimation to drought will be made available for more applied studies in grapevine nurseries and viticulture practice. Concurrently, implemented strategies by these latter will bridge novel product discovery to market. In the same way soil microbes are utilized as a preventive practice against grapevine fungal and wood diseases, we foresee the commercialization of pre-selected endophytes, or inoculated vine plants, for more resilient agriculture. With this approach, PROSIT hopes to open new avenues to exploit and optimize natural resources for sustainable agriculture. The outcomes of PROSIT will allow wine producers to value their products and, at the same time, respond to the requests of consumers who are increasingly responsive to wine quality obtained from organic and environmentally friendly vineyards.

CONCLUDING REMARKS

There is an urgent need to find sustainable solutions to climate change and the worsening water scarcity conditions affecting most of the vine areas globally. Recently funded national and international projects show a growing interest in finding avenues to improve crop resilience by exploiting the plant microbiome. While still in line with this general direction, PROSIT differs in its approach. Selection and application of endophytes already adapted to a grapevine host from dry areas hold the promise to increase the chance of successful outcomes. Combined with a comprehensive phenotyping approach, we believe that this strategy will shed light on the mechanistic processes that increase drought tolerance in inoculated vines. Whether increased tolerance depends on the grapevine genotype and the transcriptional activation induced by the microbes will be revealed. Alternatively, whether microbes induce epigenetic modifications will also be unveiled. Visualization of signal induction and translocation within the plant body will furthermore allow us to understand whether microbes enhance pre-existing mechanisms already used by the plant or enforce alternative signaling routes. We will compare and contrast evidence in support of any of these mechanisms as well as outlining approaches by which to evaluate their relative importance. Thus, while providing mechanistic insights in a crop of economic relevance, PROSIT also proposes the transfer of knowledge for readily applicable solutions to be adopted in viticulture as well as attempting to provide a blueprint which can be used more generally in other fields of horticultural and agricultural research. The implementation of the use of endophytes will contribute to the opening of new perspectives for agriculture, optimizing beneficial natural resources in order to set up an integrated agro-biological system. The use of endophytes for stress management is envisaged as a valuable nature-compliant way to optimize crops capabilities to cope with increasing drought, and plant diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ali, S., **Tyagi, A.**, **Park, S.**, **Mir, R.A.**, **Mushtaq, M.**, **Bhat, B.** ***et al.*** (2022) Deciphering the plant microbiome to improve drought tolerance: mechanisms and perspectives. *Environmental and Experimental Botany*, **201**, 104933.

Alwutayd, K.M., **Rawat, A.A.**, **Sheikh, A.H.**, **Almeida-Trapp, M.**, **Veluchamy, A.**, **Jalal, R.** ***et al.*** (2023) Microbe-induced drought tolerance by ABA-mediated root architecture and epigenetic reprogramming. *EMBO Reports*, **24**(8), e56754. Available from: https://doi.org/10.15252/embr.202256754

Ashapkin, V.V., **Kutueva, L.I.**, **Aleksandrushkina, N.I.** & **Vanyushin, B.F.** (2020) Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses. *International Journal of Molecular Sciences*, **21**(20), 7457. Available from: https://doi.org/10.3390/ijms21207457

Balcerowicz, M., **Shetty, K.N.** & **Jones, A.M.** (2021) Fluorescent biosensors illuminating plant hormone research. *Plant Physiology*, **187**(2), 590–602. Available from: https://doi.org/10.1093/plphys/kiab278

Beis, A. & **Patakas, A.** (2012) Relative contribution of photoprotection and anti-oxidative mechanisms to differential drought adaptation ability in grapevines. *Environmental and Experimental Botany*, **78**, 173–183. Available from: https://doi.org/10.1016/j.envexpbot.2011.12.038

Berger, M.M.J., **Stammitti, L.**, **Carrillo, N.**, **Blancquaert, E.**, **Rubio, B.**, **Teyssier, E.** ***et al.*** (2023) Epigenetics: an innovative lever for grapevine breeding in times of climatic changes. *OENO one*, **57**(2), 265–282. Available from: https://doi.org/10.20870/oeno-one.2023.57.2.7405

Bernardo, S., **Dinis, L.-T.**, **Machado, N.** & **Moutinho-Pereira, J.** (2018) Grapevine abiotic stress assessment and search for sustainable adaptation strategies in Mediterranean-like climates. A review. *Agronomy for Sustainable Development*, **38**(6), 66. Available from: https://doi.org/10.1007/s13593-018-0544-0

Bettenfeld, P., **Cadena, I.**, **Canals, J.**, **Jacquens, L.**, **Fernandez, O.**, **Fontaine, F.** ***et al.*** (2022) The microbiota of the grapevine holobiont: a key component of plant health. *Journal of Advertising Research*, **40**, 1–15. Available from: https://doi.org/10.1016/j.jare.2021.12.008

Chaves, M.M., **Zarrouk, O.**, **Francisco, R.**, **Costa, J.M.**, **Santos, T.**, **Regalado, A.P.** ***et al.*** (2010) Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Botany*, **105**(5), 661–676. Available from: https://doi.org/10.1093/aob/mcq030

Choi, K., **Khan, R.** & **Lee, S.-W.** (2021) Dissection of plant microbiota and plant-microbiome interactions. *Journal of Microbiology*, **59**(3), 281–291. Available from: https://doi.org/10.1007/s12275-021-0619-5

Deyett, E. & **Rolshausen, P.E.** (2020) Endophytic microbial assemblage in grapevine. *FEMS Microbiology Ecology*, **96**(5), 53. Available from: https://doi.org/10.1093/femsec/fiaa053

Dong, Y., **Duan, S.**, **Xia, Q.**, **Liang, Z.**, **Dong, X.**, **Margaryan, K.** ***et al.*** (2023) Dual domestications and origin of traits in grapevine evolution. *Science*, **379**(6635), 892–901. Available from: https://doi.org/10.1126/science.add8655

Eibach, R. & **Töpfer, R.** (2015) 1 – Traditional grapevine breeding techniques. In: Reynolds, A. (Ed.) *Grapevine breeding programs for the wine industry*. Sawston, UK: Woodhead Publishing, pp. 3–22. Available from: https://doi.org/10.1016/B978-1-78242-075-0.00001-6

Gallusci, P., **Agius, D.R.**, **Moschou, P.N.**, **Dobránszki, J.**, **Kaiserli, E.** & **Martinelli, F.** (2022) Deep inside the epigenetic memories of stressed plants. *Trends in Plant Science*, **28**, 142–153. Available from: https://doi.org/10.1016/j.tplants.2022.09.004

Gamalero, E. & **Glick, B.R.** (2015) Bacterial modulation of plant ethylene levels. *Plant Physiology*, **169**(1), 13–22. Available from: https://doi.org/10.1104/pp.15.00284

Gambetta, G.A., **Herrera, J.C.**, **Dayer, S.**, **Feng, Q.**, **Hochberg, U.** & **Castellarin, S.D.** (2020) The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *Journal of Experimental Botany*, **71**(16), 4658–4676. Available from: https://doi.org/10.1093/jxb/eraa245

Gao, S., **Ma, W.**, **Lyu, X.**, **Cao, X.** & **Yao, Y.** (2020) Melatonin may increase disease resistance and flavonoid biosynthesis through effects on DNA methylation and gene expression in grape berries. *BMC Plant Biology*, **20**(1), 231. Available from: https://doi.org/10.1186/s12870-020-02445-w

Gobbi, A., **Acedo, A.**, **Imam, N.**, **Santini, R.G.**, **Ortiz-Álvarez, R.**, **Ellegaard-Jensen, L.** ***et al.*** (2022) A global microbiome survey of vineyard soils highlights the microbial dimension of viticultural terroirs. *Communications Biology*, **5**(1), 241. Available from: https://doi.org/10.1038/s42003-022-03202-5

González-Hernández, A.I., **Scalschi, L.**, **Vicedo, B.**, **Marcos-Barbero, E.L.**, **Morcuende, R.** & **Camañes, G.** (2022) Putrescine: a key metabolite involved in plant development, tolerance and resistance responses to stress. *International Journal of Molecular Sciences*, **23**(6), 2971. Available from: https://doi.org/10.3390/ijms23062971

Grenzi, M., **Resentini, F.**, **Vanneste, S.**, **Zottini, M.**, **Bassi, A.** & **Costa, A.** (2021) Illuminating the hidden world of calcium ions in plants with a universe of indicators. *Plant Physiology*, **187**(2), 550–571. Available from: https://doi.org/10.1093/plphys/kiab339

Jones, A.M., **Danielson, J.A.**, **Manojkumar, S.N.**, **Lanquar, V.**, **Grossmann, G.** & **Frommer, W.B.** (2014) Abscisic acid dynamics in roots detected with genetically encoded FRET sensors. *eLife*, **3**, e01741. Available from: https://doi.org/10.7554/eLife.01741

Kim, T.H., **Böhmer, M.**, **Hu, H.**, **Nishimura, N.** & **Schroeder, J.I.** (2010) Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. *Annual Review of Plant Biology*, **61**, 561–591.

Liese, A., **Eichstädt, B.**, **Lederer, S.**, **Schulz, P.**, **Oehlschläger, J.**, **Matschi, S.** ***et al.*** (2023) Imaging of plant calcium-sensor kinase conformation monitors real time calcium-dependent decoding in planta. *The Plant Cell*, **36**, 276–297. Available from: https://doi.org/10.1093/plcell/koad196

Markova, G., **Baas, S.**, **Conforti, P.** & **Ahmed, S.** (2018) *2017: The impact of disasters and crises on agriculture and food security*. Rome, Italy: FAO.

Miliute, I., **Buzaite, O.**, **Baniulis, D.** & **Stanys, V.** (2015) Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. *Zemdirbyste*, **102**(4), 465–478. Available from: https://doi.org/10.13080/z-a.2015.102.060

Navarro-Torre, S., **Ferrario, S.**, **Caperta, A.D.**, **Victorino, G.**, **Bailly, M.**, **Sousa, V.** ***et al.*** (2023) Halotolerant endophytes promote grapevine regrowth after salt-induced defoliation. *Journal of Plant Interactions*, **18** (1), 2215235. Available from: https://doi.org/10.1080/17429145.2023.2215235

Neethling, E., **Barbeau, G.**, **Bonnefoy, C.** & **Quénol, H.** (2012) Change in climate and berry composition for grapevine varieties cultivated in the Loire Valley. *Climate Research*, **53**(2), 89–101. Available from: https://doi.org/10.3354/cr01094

Nigris, S., **Baldan, E.**, **Tondello, A.**, **Zanella, F.**, **Vitulo, N.**, **Favaro, G.** ***et al.*** (2018) Biocontrol traits of bacillus licheniformis GL174, a culturable endophyte of *Vitis vinifera* cv. Glera. *BMC Microbiology*, **18**(1), 133. Available from: https://doi.org/10.1186/s12866-018-1306-5

OIV. World Wine Production Outlook. (2023) *OIV first estimates*. Available from: https://www.oiv.int/sites/default/files/documents/OIV_World_Wine_Production_Outlook_2023.pdf [Accessed 7th November 2023].

Otoguro, M. & **Suzuki, S.** (2018) Status and future of disease protection and grape berry quality alteration by micro-organisms in viticulture. *Letters in Applied Microbiology*, **67**(2), 106–112. Available from: https://doi.org/10.1111/lam.13033

Pacifico, D., **Squartini, A.**, **Crucitti, D.**, **Barizza, E.**, **Lo Schiavo, F.**, **Muresu, R.** ***et al.*** (2019) The role of the endophytic microbiome in the grapevine response to environmental triggers. *Frontiers in Plant Science*, **10**, 1256. Available from: https://doi.org/10.3389/fpls.2019.01256

Pagliarani, C., **Gambino, G.**, **Ferrandino, A.**, **Chitarra, W.**, **Vrhovsek, U.**, **Cantu, D.** ***et al.*** (2020) Molecular memory of Flavescence dorée phytoplasma in recovering grapevines. *Horticulture Research*, **7**, 126. Available from: https://doi.org/10.1038/s41438-020-00348-3

Pan, X.-X., **Chen, C.-X.**, **Wang, Y.-T.**, **Zhu, Y.-Y.** & **Yang, M.-Z.** (2023) Exposure to the endophytic fungi regulates the anthocyanin profiles in the post-veraison grape berries of "Cabernet Sauvignon". *Horticulturae*, **9**(2), 237. Available from: https://doi.org/10.3390/horticulturae9020237

Qu, J.-Z., **Liu, F.**, **Pan, X.-X.**, **Liao, C.-M.**, **Li, T.**, **Zhang, H.-B.** ***et al.*** (2022) Coordinative changes in metabolites in grape cells exposed to endophytic fungi and their extracts. *Molecules*, **27**, 5566.

Ravanbakhsh, M., **Kowalchuk, G.A.** & **Jousset, A.** (2021) Targeted plant hologenome editing for plant trait enhancement. *The New Phytologist*, **229**(2), 1067–1077. Available from: https://doi.org/10.1111/nph.16867

Roca, P. (2022) *State of the world vine and wine sector*. OIV Press Conference. https://doi.org/10.1787/9789264244047-44-en

Rubio, B., **Stammitti, L.**, **Cookson, S.J.**, **Teyssier, E.** & **Gallusci, P.** (2022) Small RNA populations reflect the complex dialogue established between heterograft partners in grapevine. *Horticulture Research*, **9**, 67. Available from: https://doi.org/10.1093/hr/uhab067

Salvi, P., **Mahawar, H.**, **Agarrwal, R.**, **Kajal**, **Gautam, V.** & **Deshmukh, R.** (2022) Advancement in the molecular perspective of plant-endophytic interaction to mitigate drought stress in plants. *Frontiers in Microbiology*, **13**, 981355. Available from: https://doi.org/10.3389/fmicb.2022.981355

Santos, J.A., **Fraga, H.**, **Malheiro, A.C.**, **Moutinho-Pereira, J.**, **Dinis, L.-T.**, **Correia, C.** ***et al.*** (2020) A review of the potential climate change impacts and adaptation options for European viticulture. *NATO Advanced Science Institutes Series E: Applied Sciences*, **10**(9), 3092. Available from: https://doi.org/10.3390/app10093092

Teo, H.M., **Aziz, A.**, **Bhubalan, K.**, **Nordahliawate, S.**, **Cl, M.S.** & **Ng, L.C.** (2022) Setting a plausible route for saline soil-based crop cultivations by application of beneficial halophyte-associated bacteria: a review. *Microorganisms*, **10**(3), 657. Available from: https://doi.org/10.3390/microorganisms10030657

Trivedi, P., **Leach, J.E.**, **Tringe, S.G.**, **Sa, T.** & **Singh, B.K.** (2020) Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews. Microbiology*, **18**(11), 607–621. Available from: https://doi.org/10.1038/s41579-020-0412-1

Vandenkoornhuyse, P., **Quaiser, A.**, **Duhamel, M.**, **Le Van, A.** & **Dufresne, A.** (2015) The importance of the microbiome of the plant holobiont. *The New Phytologist*, **206**(4), 1196–1206. Available from: https://doi.org/10.1111/nph.13312

VanWallendael, A., **Soltani, A.**, **Emery, N.C.**, **Peixoto, M.M.**, **Olsen, J.** & **Lowry, D.B.** (2019) A molecular view of plant local adaptation: incorporating stress-response networks. *Annual Review of Plant Biology*, **70**, 559–583. Available from: https://doi.org/10.1146/annurev-arplant-050718-100114

Vuerich, M., **Petrussa, E.**, **Boscutti, F.**, **Braidot, E.**, **Filippi, A.**, **Petruzzellis, F.** ***et al.*** (2023) Contrasting responses of two grapevine cultivars to drought: the role of non-structural carbohydrates in xylem hydraulic recovery. *Plant & Cell Physiology*, **64**(8), 920–932. Available from: https://doi.org/10.1093/pcp/pcad066

Waadt, R., **Hitomi, K.**, **Nishimura, N.**, **Hitomi, C.**, **Adams, S.R.**, **Getzoff, E.D.** ***et al.*** (2014) FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in Arabidopsis. *eLife*, **3**, e01739. Available from: https://doi.org/10.7554/eLife.01739

Walia, A., **Waadt, R.** & **Jones, A.M.** (2018) Genetically encoded biosensors in plants: pathways to discovery. *Annual Review of Plant Biology*, **69**, 497–524. Available from: https://doi.org/10.1146/annurev-arplant-042817-040104

Wang, X., **Cheng, M.**, **Yang, Q.**, **Wei, H.**, **Xia, A.**, **Wang, L.** ***et al.*** (2019) A living plant cell-based biosensor for real-time monitoring invisible damage of plant cells under heavy metal stress. *The Science of the Total Environment*, **697**, 134097. Available from: https://doi.org/10.1016/j.scitotenv.2019.134097

Yang, M.-Z., **Ma, M.-D.**, **Yuan, M.-Q.**, **Huang, Z.-Y.**, **Yang, W.-X.**, **Zhang, H.-B.** ***et al.*** (2016) Fungal endophytes as a metabolic fine-tuning regulator for wine grape. *PLoS One*, **11**, e0163186.

Yu, M., **Chen, J.C.**, **Qu, J.Z.**, **Liu, F.**, **Zhou, M.**, **Ma, Y.M.** ***et al.*** (2020) Exposure to endophytic fungi quantitatively and compositionally alters anthocyanins in grape cells. *Plant Physiology and Biochemistry*, **149**, 144–152.

Zarraonaindia, I., **Owens, S.M.**, **Weisenhorn, P.**, **West, K.**, **Hampton-Marcell, J.**, **Lax, S.** ***et al.*** (2015) The soil microbiome influences grapevine-associated microbiota. *mBio*, **6**(2), 14. Available from: https://doi.org/10.1128/mBio.02527-14

Zeng, G., **Gao, F.**, **Li, C.**, **Li, D.** & **Xi, Z.** (2022) Characterization of 24-epibrassinolide-mediated modulation of the drought stress responses: morphophysiology, antioxidant metabolism and hormones in grapevine (*Vitis vinifera* L.). *Plant Physiology and Biochemistry: PPB*, **184**, 98–111. Available from: https://doi.org/10.1016/j.plaphy.2022.05.019

Zhang, S., **Wang, Y.**, **Chen, X.**, **Cui, B.**, **Bai, Z.** & **Zhuang, G.** (2020) Variety features differentiate microbiota in the grape leaves. *Canadian Journal of Microbiology*, **66**(11), 653–663. Available from: https://doi.org/10.1139/cjm-2019-0551

Zhou-Tsang, A., **Wu, Y.**, **Henderson, S.W.**, **Walker, A.R.**, **Borneman, A.R.**, **Walker, R.R.** ***et al.*** (2021) Grapevine salt tolerance. *Australian Journal of Grape and Wine Research*, **27**(2), 149–168. Available from: https://doi.org/10.1111/ajgw.12487

Zilber-Rosenberg, I. & **Rosenberg, E.** (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews*, **32**(5), 723–735. Available from: https://doi.org/10.1111/j.1574-6976.2008.00123.x