The Most Important Fruit Crops in the Mediterranean Basin

Position Paper

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Milano University Press

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Contributors are listed, by crop, from page 139

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FREECLIMB
FRUIT CROPS RESILIENCE TO CLIMATE CHANGE IN THE MEDITERRANEAN BASIN

Contents

Foreword

The overall aims of this book, other than presenting an overview of the main fruit crops industry grown in the Mediterranean basin (MB), are: i) describing the most interesting features of their cultivation (state of the art), ii) enlighting the possible solutions in order to tackle the negative impacts of climatic extremes on the industry, iii) reporting the main findings of the 'Freeclimb' international project (https://primafreeclimb.com/) funded in the framework of PRIMA, a EU initiative meant to foster MB cooperation (https://prima-med.org/).

The work is structured into nine chapters, other than an Introduction and Conclusion sections.

Introduction

The climatic scenario predicted for Mediterranean areas poses specific challenges for agriculture, fruit crops in particular. The vulnerability of agricultural sectors to the modification of agro-climatic conditions depends on both the expected regional climate extremes and the sectors' adaptation ability. For their perennial status, fruit tree crops are particularly exposed to environmental extremes, i.e. yield and quality of fruit production are strongly affected by genotype x environment interactions. The FREECLIMB project (https://primafreeclimb.com/) was built for developing smart and sustainable tools for fruit crops systems to spare natural resources and to increase agriculture efficiency. This major goal was pursued by advancing knowledge on mechanisms of plant environmental adaptation and biotic/abiotic stress resilience. The project targeted the major fruit tree crops in the Mediterranean basin with the aim of improving the availability of breeding and germplasm material adapted to limited external resources and future climatic scenarios predicted for the region, through the characterization and exploitation of local biodiversity. The project focused on key ideotypes elaborated in collaboration with breeders, nurseries, growers, etc, with the core objective of providing a toolkit (diverse germplasm, tools and methods) to accelerate exploitation, breeding and selection of resilient varieties in key traditional fruit crops of the Mediterranean fruit crops industry (stone fruits such as almond, apricot and peach, *Citrus* spp., grape and olive). To these ends, the project has pursued the following specific objectives:

i) applying protocols (e.g. phenotyping methods) and integrated tools (e.g. genotyping methods, data analysis) to support the characterization, exploitation and selection of varieties adapted to a range of agro-ecological and field management conditions;

ii) dissecting the genetic bases of traits/processes linked to sustainability and plant resilience to biotic and abiotic stresses, with particular focus on disentangling genotype-by-environment-by-management (GxExM) interactions;

iii) unraveling the molecular, biochemical and physiological basis of plant adaptation to different environmental (soil and climate) and field conditions (particularly for water management) and to biotic/abiotic stresses;

iv) developing and applying genomics-based breeding methods to improve introgression and selection efficiency;

v) devising adaptation strategies to cope with the combined effects of multiple stresses possibly co-occurring under field conditions (e.g heat waves and drought, pests and diseases);

vi) exploiting germplasm resources, by identifying and characterizing wild, feral and domesticated sources of biodiversity;

vii) transferring project results through training and dissemination activities dedicated especially to breeders (focusing on young scientists), nurserymen, growers and stakeholders, particularly in those countries where the fruit crops industry is less developed.

Considering the current southern countries climate of the Mediterranean basin as representative of the future climatic scenarios predicted for the northern ones, the Freeclimb project strongly benefited from cooperation between the south and north Mediterranean shores: for each species targeted by the project, partners from at least two countries were involved - one from north and one from south.

1. Scope

The Freeclimb project targeted major issues for fruit tree breeding through well-integrated and complementary actions that ultimately widened the range of varieties (scion and/or rootstock) adapted to a changing climate scenario. This has been achieved by a broad-spectrum approach involving the implementation of tools and germplasm resources, in turn enabling breeders, nurseries and growers to identify and access the most appropriate varieties for each climatic context and farming system. FREECLIMB has focused on key adaptive traits suited to Mediterranean farming conditions and related to the resistance and resilience to abiotic and biotic stresses, implementing efficient methods and tools to phenotype the relevant traits facilitating screening and characterization of diverse genetic materials to rapidly access sources of genetic variation for specific target traits.

The project has taken advantage of genetic resources by acting at various levels: (i) providing open access to extensive genetic and phenotypic information about diverse germplasm collections for key fruit tree species; (ii) implementing innovative genomics-based approaches to predict plant performance and accelerate breeding and selection of superior genotypes in constrained environments; (iii) integrating multi-omics (transcriptomic, proteomic and metabolomic) approaches for dissecting plant response to different environments and management practices and increasing knowledge about mechanisms of adaptation to abiotic and biotic stresses; iv) laying the foundation for the establishment of multi-site collections and progeny field testing in MB.

Advantage was taken of the availability of large germplasm collections, some in multiple locations, covering a range of Mediterranean environments. The project partnership included geneticists, breeders, physiologists, biochemists, bioinformaticians and pathologists, and also defined priority areas for research and breeding activities through the engagement of stakeholders and end-users.

2. Background

Since a long time, the MB has been a region of temperate and subtropical fruit production, among which crops such as *Citrus* spp., grape, olive, and stone fruits stand out (Figure 1). In the MB, total production of grapes was 28.3 million tons in 2021, with Italy accounting for the largest share (8.1 million tons) followed by Spain (6 million tons) and France (5 million tons). In the same year total MB production for Citrus spp. was about 27,6 Mt (Spain and Turkey as major producers) (FAOSTAT, 2023). The MB accounts for more than 90% of global olive and oil production, which has reached 23 million tons. Spain is the largest olive producer with the largest invested areas (2.6 million ha). A sharp increase of olive orchards has affected North Africa regions, particularly Tunisia which has reached 1.2 million ha. Mediterranean countries as a whole contribute to about 48% worldwide apricot production, with Turkey, Algeria and Italy as main producers. Almond production is almost equally shared among Northern and Southern Mediterranean countries, accounting for about 28% of worldwide production, while peach and nectarines are mainly cultivated in the

Northern countries with Spain and Italy as top producers followed by Turkey, Greece and Egypt).

The MB is a trademark of high-quality agricultural products, representing an excellent example of typicality, vocation, and biodiversity. Maintaining this heritage transcends specific national agricultural policies, assuming a transnational dimension that brings together Europe, North Africa and Middle-East Asia. The MB climate is characterized by infrequent rainfall (less than 100 days per year) that is unevenly distributed over time (long periods of summer drought) and sometimes quite sparse (about 300 to 500 mm per year in some semi-arid regions). Most climate change scenarios for the MB predict a decrease of rainfalls and rising temperatures. IPCC forecasts indicate a yearly temperature increase between 2 and 4° C and a decrease in rainfall between 4 and 30% by 2050, even to more than 50% in the most pessimistic scenario for North Africa, Middle East and South of Spain (IPCC, 2013). These environmental constraints pose additional challenges for a 'sustainable intensification' and an efficient use of resources, exacerbating the incidence of biotic and abiotic stresses (Pretty et al., 2014). Due to their perennial biology, fruit tree crops are highly vulnerable to environmental extremes. Temperature increase will strongly affect plant phenology and physiology. Therefore, determination of functional traits enabling adaptation to current and future climatic conditions is essential to improve plant resilience (e.g. 'resistance' and 'recovery' ability of the plant).

Variability in functional traits can be the result of either genetic change or phenotypic plasticity, the latter occurring when a genotype adjusts its phenotype according to environmental conditions (e.g. by epigenetic mechanisms). Genetic variation plays a pivotal role for plant adaptability, being the basis for the evolutionary potential of a species (Anderson et al., 2011). Genomics represent a promising tool for deciphering the stress responsiveness of crop species to environmental constraints (Badenes et al., 2016). Genomic-based breeding approaches, such as marker-assisted breeding (MAB) and introgression (MAI), and genomic selection (GS) have revolutionized plant breeding in the last decades and are the most suitable in terms of acceptance to the public opinion, farming associations and government authorities. The adoption of molecular markers is instrumental for (i) defining the genetic diversity underlying the germplasm available to breeders; (ii) identifying genes, alleles and Quantitative Trait Loci (QTLs) controlling the traits; (iii) aiding breeders in the creation of new varieties matching the desired ideotypes. With respect to adaptation and resilience to variable climatic conditions, breeding requires a systematic analysis of GxExM interactions and a better understanding of the underlying molecular and physiological mechanisms. To realize the varietal improvements needed in the coming decades for addressing the above challenges and obtain plants with optimized features for the MB, appropriate breeding strategies have to be adopted (Lammerts van Bueren et al., 2010) since, depending on the length of generation cycles, it often takes 15-20 years or more from the initial crosses to develop a new fruit tree variety. Modern breeding principles, especially those addressing plant-biotic/abiotic interactions, are based on two key points: (i) the search for diversity through the extensive characterization of germplasm resources; (ii) the exploitation of such diversity in pre-breeding and breeding programs. Such activities must take into account the possible multi-factorial control of relevant traits and GxExM interactions (Rigby et al., 2001).

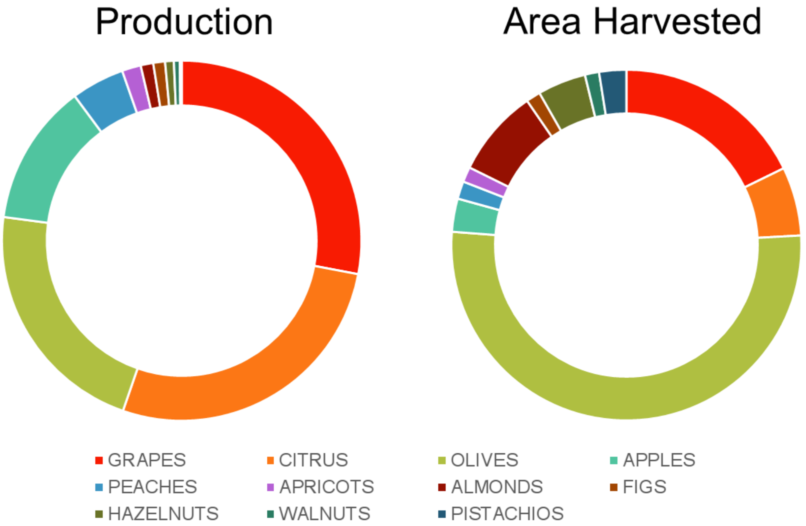

Figure 1. Tree crops production and harvested area in the MB (FAOSTAT, 2023). Production (million tons): grapes 28.3, Citrus 27.6, olives 22.1, apples 12.9, peaches 4.8, apricots 1.7, almonds 1.1, figs 1.1, hazelnuts 0.8, walnuts 0.5, pistachios 0,2. Area harvested (million hectares): olives 9.9, grapes 3.4, almonds 1.5, Citrus 1.2, hazelnuts 0.9, apples 0.6, pistachios 0.5, peaches 0.3, apricots 0.3, figs 0.3, walnuts 0.2.

In recent years the EU has funded several projects with the aim of promoting innovation in fruit breeding towards more sustainable farming systems, especially apple (e.g. Hydras, FruitBreedomics) where major advances have been achieved in key resistance traits. Regarding the fruit species targeted by ‘Freeclimb’, progress has been mainly made in fruit quality traits while breeding for resilience traits has been largely neglected or focused on crucial specific biotic challenges only, such as resistance to Sharka in apricot (MARS and SharCo) and to Xylella in olive (XYL-EID).

3. Methodology

The Mediterranean environment is characterized by hot and dry summers, with high daily irradiance and evaporative demand, and significant, long-term soil water deficit (Flexas et al., 2002). Indeed, a general decrease in annual precipitation and an increase in drought intensity have been observed in the MB (Capra et al., 2013) and predicted scenarios of climate extreme events suggest increasing vulnerability to global warming and drying (Giorgi et al., 2008). Also, rising temperatures and decreasing precipitations promote soil degradation and desertification around the MB. A direct consequence of desertification is soil salinization due to the increase in evapotranspiration. Soil salinization is exacerbated by water irrigation likely having an even higher salt content (Bultot et al., 1988). About 25% of irrigated cropland in the Mediterranean area is estimated to be affected by moderate to high salinization (Mateo-Sagasta and Burke, 2011). For these reasons, drought and salinity stress are overarching themes across species considered by the project.

Over the last decades, fruit tree breeding efforts have been mainly focused on fruit quality traits and productivity in a context of non-limiting inputs while limited resources were allocated for the study of plant adaptation to environment and resilience to biotic and abiotic stresses. As a consequence, cultivated varieties have limited adaptability beyond cultivation niche, being highly vulnerable to climatic change and less productive under suboptimal conditions, particularly regarding water and nutrient availability. The base of the project was built on the following actions and tools:

I) Crops ideotypes

The crops ideotypes were set up, meant to include the most important traits that ideally should be incorporated in a variety for more sustainable farming systems. Pre-breeding activities were focused on pyramiding both abiotic and biotic resilience traits, as recommended by fruit stakeholders.

Citrus, *rootstock*: resistance/tolerance to *Phytophthora* spp., soil salinity, calcareous soils and sub-optimal soil water availability; *scion*: tolerance to 'Mal secco'.

Grape, *rootstock*: phylloxera (*D. vitifoliae*, Fitch 1855) resistance, adaptation to a range of soil type and water availability. *Scion*: downy and powdery mildew resistance.

Olive, *rootstock* (or self-rooting): resistance to Verticillium wilt, soil salinity and sub-optimal water availability; *scion*: tolerance/resistance to olive fruit fly (*B. oleae*); early flowering to avoid heat-stress; adaptation to high air temperature during flowering.

***Stone fruits* (almond, apricot and peach)**, *rootstock*: adaptation to sub-optimal water supply and different soil type (in particular calcareous soils); nitrogen use efficiency; root-knot nematode resistance.

Almond, *scion*: resistance/tolerance to late frost damage; Sharka (Plum Pox Virus, PPV) resistance.

Apricot, *scion*: resistance/tolerance to late frost damage; low-chilling requirements; resistance to blossom brown rot (*Monilinia* spp.); PPV resistance.

Peach, *scion*: low-chilling requirements; high water use efficiency; resistance to blossom and fruit brown rot (*Monilinia* spp.), powdery mildew, leaf curl and PPV.

II) Resistance to biotic and abiotic stresses

The crucial priority goals to increase resilience to biotic and abiotic stresses, and adaptability to climatic scenarios predicted for MB, are discussed within each of the crops targeted by the project (following chapters).

III) Tools and methods for efficient dissection and selection of relevant traits

Phenotypes notoriously result from the interactions between genomes and the environment. As a consequence, by jointly analyzing several individuals in different environments, the total phenotypic variation can be partitioned into genotypic, environmental and genotype x environment interaction (GxE) components, helping in selecting individuals whose interesting traits are more heritable and, therefore, more resilient to a range of environmental conditions. In order to boost the selection process, marker-trait association represents a powerful tool to facilitate genome-assisted breeding and an essential preliminary step to further GxE interaction studies. Next generation sequencing (NGS) technologies have led to the rapid accumulation of genome sequences (Varshney et al., 2021). Genomic resources provide the opportunity for a revolution in breeding by facilitating the dissection of complex traits relevant to sustainable farming systems. Among fruit crops treated in the present volume, reference genomes have been assembled for peach (as a model for stone fruits) (Verde et al., 2017), grape (Jaillon et al., 2007), Citrus (Xu et al., 2013; Wu et al., 2014), and olive (Unver et al., 2017). These reference genomes are available on public browsers and have been leveraged for re-sequencing of diverse germplasm collections to explore genome-wide sequence variations. The use of genome-wide markers and high-throughput genotyping methods has led to the development of new strategies to analyze quantitative traits, such as genome-wide association studies (GWAS) and genome-wide selection (GS) (Zahid et al., 2022). In addition, the use of large germplasm panels and of multi-parental populations helps to increase the accuracy of quantitative/Mendelian trait loci (QTL, MTL) detection (Varshney et al., 2020). During past years, a number of QTLs have been discovered for pests and disease resistance traits, with relatively high mapping resolution. This is the case of the loci linked to resistance to lea powdery mildew, green aphid and brown rot in peach (Oliveira-Lino et al., 2016), to PPV (sharka disease: Mariette et al., 2016), powdery mildew (Salazar et al., 2016) and bacterial spot in apricot (Socquet-Juglard et al., 2013), to leafminer in *Citrus* spp. (Bernet et al., 2005), to downy and powdery mildew in grape (Zyprian et al., 2016). All previously identified genomic regions could be targeted by fine mapping and marker-linked selection, together with other poorly studied resistance traits, although relevant for the MB. Indeed, the molecular markers for these QTLs represent a useful resource for enhancing selection efficiency via marker-assisted selection (MAS) in breeding programs as was already done for fruit traits (De Mori and Cipriani, 2023). However, depending on the traits, specific breeding strategies are required. While MAS is suitable for mono-/oligogenic traits, tools to exploit loci with moderate to small effect are needed to further take advantage of the existing genetic variability, and for addressing polygenic traits as well. One of the most promising approaches is Genome-Wide Selection (GS), which uses the entire genomic information to estimate future phenotypes or unobserved genetic/breeding values (Crossa et al., 2017). Compared to MAS, GS can better accommodate multiple-trait prediction models, when the selection objective is on several traits simultaneously. In order to run GS studies, genotypic and phenotypic data are collected on a reference population to train a model for the estimation of individual breeding values (genomic-estimated breeding values, GEBV) which is then applied to select superior candidates based only on genotypic data (Lorenz et al., 2011; Meuwissen et al., 2001). In recent years, GS has been extensively adopted and validated in a range of crop species (Desta and Ortiz, 2014). Initial studies demonstrated the validity of this approach in fruit trees where GS has been mostly used to predict fruit quality traits, e.g. in apple, peach, grape and Citrus (Biscarini et al., 2017; Fodor et al., 2014; Kumar, 2014; Minamikawa et al., 2017; Viana et al., 2016). In long-generation species such as fruit trees, the breeding cycle can be significantly shortened by selecting young, non bearing seedlings, based exclusively on genotypic information. This would accelerate genetic progress and increase the efficiency of fruit breeding. Efforts in the US RosBREED project (funded under the USDA-ARS Specialty Crop Research Initiative) aimed at combining disease resistance with horticultural quality in new Rosaceous cultivars by using both MAS and GS. However, there is no systematic work until now that used genome-wide approaches in breeding for resilience traits in fruit trees, or accounted for the GxE interaction, a crucial factor in abiotic stress and disease development. Using peach as a model, this can be filled by developing GS approaches for high-priority resilience traits that incorporate also GxExM interactions.

IV) Germplasm resources and pre-breeding materials for pyramiding of multiple traits

Notwithstanding the introduction and extensive spread of high-input agriculture, local accessions and landraces have often been maintained in cultivation niches, since offering two key advantages: adaptation to specific environments and/or agronomic or cultural values for farmers and local communities. Genetic diversity, maintained in the fields at different levels (inter- and intra-specific, at spatial and temporal levels), provides a number of recognized environmental and genetic values (Jarvis et al., 2016). Breeding for the production of resilient cultivars adapted to a more sustainable farming is only at its early infancy and may benefit from phenotypic and genetic characterization of the above materials. A number of interesting traits (mostly related to biotic stress) have been investigated and, sometimes, integrated into a number of cultivars in different fruit species. When sources of desirable traits and markers associated to major loci are available, the next step involves introgression/pyramiding of target loci to develop new cultivars assembling durable resistance/resilience and quality traits. Initially this involves crossing selected parents and marker-assisted selection of progenies to combine the desired loci. Existing and newly acquired genome-wide information make this strategy possible.

In **grape**, downy and powdery mildew resistant cultivars were obtained from interspecific cross between *V. vinifera* and North American and Asian *Vitis* spp., such as *V. riparia*, *V. rupestris*, *V. amurensis*. MAS strategy combined with multiple backcrossing with *V. vinifera* cultivars allowed the development of resistant cultivars with a significant percentage of *V. vinifera* genome. The main genetic markers related to plant resistance genes include markers for downy mildew (*rpv1*, *rpv3*, *rpv10*, *rpv12*) and for powdery mildew (*ren1*, *ren3*, *run1*) (Zini et al., 2015; Di Gaspero et al., 2012). Pyramiding of resistant haplotypes from different grape species into new cultivars should prevent pathogens bypassing resistance mechanisms, making the resistance more durable (Di Gaspero and Foria, 2015).

In **stone fruits**, valuable data concerning many of the above described biotic stresses (sharka, powdery mildew, peach green aphid, root-knot nematode) are already available for peach and, partly, for apricot (Pascal et al., 2010; Decroocq et al., 2014; Pascal et al., 2017; Lambert and Pascal, 2011; Pacheco et al., 2014), making feasible the incorporation of two or more resistance genes in a single individual that would be one or a few generations away from a commercial variety, e.g. combining favorable alleles of resistance to pest and pathogens together with resilience with abiotic stresses (chilling requirements, tolerance to spring frost, drought, salinity, calcareous soils and iron chlorosis).

In **citrus**, sources of resistance were established against *Citrus tristeza virus* (CTV) which caused the loss of about 100 million trees a few decades ago (Gómez-Muñoz et al., 2017). Through breeding works the selection of several *Citrus* hybrid rootstocks tolerant to this disease was accomplished. However, breeding works aimed at introgressing resistance to other diseases such as *Malsecco* or *Phytophthora* in *Citrus* elite accessions are very limited.

In **olive**, considerable levels of tolerance to the highly virulent defoliating pathotype (D) of *Verticillium dahliae* have been reported for a limited number of traditional cultivars only, such as 'Frantoio', 'Changlot Real' and 'Empeltre' (Arias-Calderon et al., 2015). Accessions with low susceptibility and good horticultural characteristics have been recently selected. Resistant/tolerant accessions to anthracnose have been found in olive germplasm (Moral et al., 2015). In contrast, breeding activities towards the improvement of traits linked to abiotic stress resilience are scarce, hampered by the very limited knowledge at both phenotypic and genetic levels.

V) Access to genetic resources for the fruit industry in the MB

Tailored cultivars and pre-breeding materials must be easily available for growers and breeders in order to implement a more sustainable fruit production in MB. No repository is available where cultivars adapted to MB agriculture are maintained as starting material (mother plants), complying with phytosanitary regulations and nursery qualitative standards. As a result, growers usually recur to cultivars that were selected for high-input farming management; therefore, low yields are often experienced when unfavorable environmental conditions occur (poor rainfall, extreme temperatures) or under high pests and diseases pressure. Another major limitation is the lack of a cultivar evaluation and testing network dedicated to low-input agriculture; the only existing case at EU level is the EUFRIN network, although subscribed on a voluntary basis (http://eufrin.org/index.php?id=1), and not specifically addressing the improvement of sustainability of fruit crops system in the MB.

VI) Stakeholders survey

A survey has been carried on by a voluntary bases among many stakeholders (agronomists, growers, growers organizations, extensionists) from almost all the interested countries, in order to collect the needs and opinions of the components of the tree fruit crops industry in the Mediterranean Basin; the questionnaire has been reported in the **annex 2**, together with the list of the contributors (**annex 3**).

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Citrus spp.

1. Importance in the Mediterranean Basin: a summary of the project outcomes

Citrus production worldwide increased by around 20% in the last 10 years, from 134 to 161 Mt (FAOSTAT, 2023). The most important producers are China (46.2 Mt), Brazil (18.9 Mt), India (14.3 Mt) and Mexico (8.8 Mt). The United States were the third citrus producers in the world, but in recent years the production declined mostly due to Huanglongbing (HLB) disease, also known as citrus greening. The sweet orange production in the US has dropped from 12.5 Mt in 1997-1998 to 3.5 Mt in 2021/2022, the lowest level in over 55 years, mostly because of HLB (USDA, FAS. Citrus: World markets and trade. 2022, https://usda.library.cornell.edu/concern/publications/w66343603?locale=en). This indicates that the occurrence of devastating diseases, together with the effects of climate changes, might threaten the existence of the citrus industry of a whole country. The major citrus producers in the Mediterranean basin are Spain (6.7 Mt), Turkey (5.4 Mt), Egypt (4.4 Mt) and Italy (3.1 Mt). The production trend of the main Mediterranean citrus producing countries is reported in Figure 2.

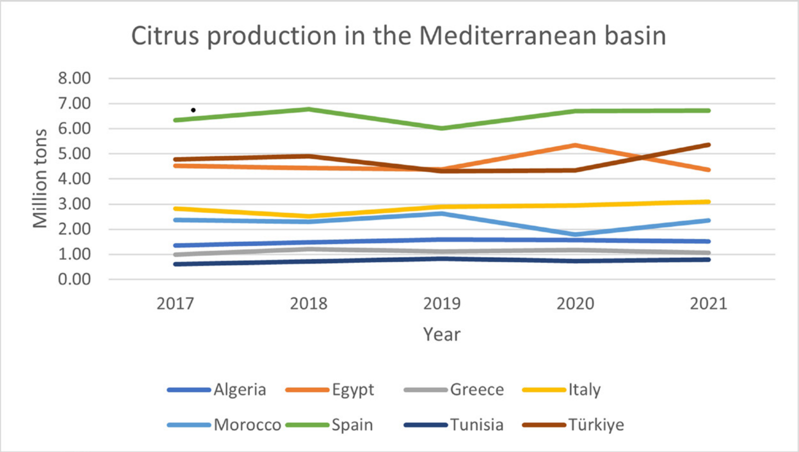

Figure 2. Citrus production in the Mediterranean basin (FAOSTAT, 2023)

2. Outline of the crop evolution until present: cultivars and orchard management

Citrus cultivars derived from several cycles of hybridization among pure (mainly mandarin, pummelo, and citron) and hybridized species (Talon et al., 2020). Most of the recent mandarin cultivars derived from controlled crosses or chance seedlings, while in the case of sweet orange, lemon, clementine, and grapefruit, almost all cultivars are clonal selections that resulted from somatic mutations that accumulated over time from a single ancestral genotype. This narrow genetic base exposes these cultivated species to higher risks when they are threatened by new biotic or abiotic stresses (Stover et al., 2020). Although clonal selection is still the most used method for lemon and sweet orange improvement, there are examples of lemon-like and sweet orange-like cultivars generated though hybridization (Caruso et al., 2020). The Mediterranean countries produce fruits mostly for the fresh market. This implies that besides productivity, fruit quality (appearance, taste, post-harvest attitude) has a huge importance for the competitiveness of the citrus industry. Fruits are produced for the domestic market and for export in EU and non-EU countries, with different proportions in each country. The main challenge of the citrus growers is to find the most suitable rootstock-scion combinations to guarantee high productivity and fruit quality under sub-optimal pedoclimatic conditions and using less external inputs, and in the presence of more frequent extreme climatic events, such as frequent drought or heat waves (Vincent et al., 2020).

2.1 Cultivars

The most important scion varieties have been recently reported by Barry et al., (2020). The most cultivated citrus species in the Mediterranean areas are sweet orange, mandarins and lemon. Among Sweet oranges, Navel clonal selections are the most widespread and adapted to several environments, and their harvesting period is extended from late October (‘Navelina’, ‘Newhall’, ‘Fukumoto’) until June (‘Lane Late’, ‘Chislett’, ‘Powell’). Blood oranges are mostly cultivated in Italy and their importance is increasing in other countries, although they require suitable and very specific climatic areas for the development of anthocyanin pigmentation in pulp and peel. Among mandarins, several clonal selections of Clementine are harvested from late September to mid-January. Other varieties (hybrids between mandarins, tangors and tangelos) cover the mid and late season (from January on): 'Nova', 'Afourer', 'Tango', 'Orri', and different selections of 'Murcott'. All these cultivars are seedless or low-seeded 'Avana' (also called 'Willowleaf' or 'Mediterranean') mandarin is the only seedy mandarin that still has a niche market in some countries, due to its peculiar, intense flavor. Regarding lemons, 'Fino' and 'Verna' are the most diffused clones in Spain, which is the largest producer, while in Italy the several clones of 'Femminello' represent the most cultivated and the best in terms of qualitative features. 'Kutdiken' is one of the main varieties in Turkey. 'Interdonato' is cultivated in Italy and Turkey for its earliness and tolerance to *mal secco* disease. 'Meyer' lemon is a cold tolerant and *mal secco*-resistant lemon hybrid, mostly cultivated in Turkey but not comparable to the quality of true lemons. Other varieties of foreign origin, like 'Lisbon' or 'Eureka' have a limited diffusion due to their sensitivity to *mal secco*. Moreover, in recent years several seedless selections have been released.

2.2 Rootstocks

The major citrus rootstocks have been recently described by Bowman and Joubert, (2020). The most diffused ones in the Mediterranean area are 'Carrizo', 'Troyer' and 'C35' citranges (hybrids between sweet orange and *Poncirus trifoliata*), *Citrus macrophylla*, 'Volkamer' lemon and 'Swingle' citrumelo. More recently, the citrandarin 'Forner Alcaide 5' (a hybrid between 'Cleopatra' mandarin and *P. trifoliata*) is gaining importance in Spain and Italy, mainly for its tolerance to salinity. Another rootstock that is now spreading in different countries is the citrandarin 'Bitters' ('C22'), released by the University of California, due to its tolerance to calcareous soils. Sour orange is one of the preferred rootstocks for lemon, due to its adaptability to different soil conditions and tolerance to Phytophthora root rot, but it cannot be used in combination with sweet orange or mandarins due to its susceptibility to CTV.

2.3 Environment and orchard management

Citrus cultivated species are native to subtropical and tropical climates, although adapted to different climatic areas (from 40° south to 40° north latitude). Despite their adaptation to different climates and geographical regions, their cultivation is mainly limited by low temperatures and drought sensitivity. Moreover, citrus cultivated species are considered salt-sensitive (Colmenero-Flores et al., 2020). In Mediterranean regions, which are characterized by low rainfall and dry summers, citrus species need irrigation to guarantee plant growth and productivity. The rise in temperatures caused by climate extreme events coupled with long periods of drought will likely lead to higher evapotranspiration, and, consequently, higher water supply for this crop that already has high irrigation demand. Water deficit can be applied in some phenological stages, even if during fruit set and development it leads to reduced fruit size and quality, increasing fruit drop (Romero et al., 2006). Moreover, the quality of irrigation water is low in many citrus growing areas of the Mediterranean, and the increase in the use of saline waters is leading to soil degradation and salinity (Vincent et al., 2020). Therefore, citrus cultivation will have to cope with a generalized increase in the frequency and intensity of drought periods, salinization of available soils and with a more frequent use of low-quality water. On the other hand, the uneven distribution of rainfall might lead to flooding, which creates direct damages to the trees and favors the spread of soilborne diseases. Using rootstocks more tolerant to drought, flood and salinity is one of the strategies to cope with such abiotic stresses. However, combining tolerance to multiple stresses in a single rootstock genotype remains challenging. Planting the trees over raised beds is an effective choice to reduce excess water, especially in the presence of heavy soils. Rootstock choice, and the vigor of rootstock scion-combination, should be also taken into consideration for the choice of tree spacing. Several choices are adopted in the Mediterranean. The classical 6 x 4 m spacing has been sometimes replaced by higher densities, which guarantee higher incomes in the first years of plantation, but also require adequate pruning management (topping and hedging) to avoid the detrimental effect of shading on tree productivity.

3. Abiotic and biotic challenges

The challenge of the citrus industry in the Mediterranean basin is to cope with several biotic and abiotic stresses, while at the same time ensuring productivity and the production of high-quality fruits. Here we report some of the most important biotic and abiotic stresses with high economic impact on the Mediterranean citrus industry.

3.1 Abiotic challenges

Soil salinity. Salinity has become a major threat for Citrus production in the coastal regions of the MB as a consequence of increasing fertilizers and decreasing precipitations. Salinity in the calcareous soil can lead to major problems in terms of fruit yield and quality. Salinity reduces plant growth due to osmotic effects, and to the accumulation of toxic levels of Cl−, Na+ or B+. Salinity plays a role in nitrogen uptake, decreasing chlorophyll content, root hydraulic conductivity and plant transpiration. Moreover, citrus salinity stress may affect plant sensitivity to other biotic and abiotic stresses (Syvertsen, 2014). Uptake and/or transport of saline ions to the scion is controlled by the rootstock, therefore rootstock choice represents a strategy to avoid yield losses when low quality water is used for irrigation. Various degrees of tolerance to water and soil salinity have been observed in citrus and citrus relatives, although only a few relatively salt-tolerant rootstocks have been obtained by breeding, due to a rather limited existing genetic pool and knowledge. In many studies Cl− exclusion from leaves served as a reliable criterion for salt tolerance leading to a decreasing order of salinity tolerance in rootstocks: mandarin > sour orange > sweet orange > rough lemon > *Poncirus trifoliata* (Christensen et al., 2007). In recent years, different salt tolerant rootstocks have been released, such a 'Forner Alcaide 5' (Forner Alcaide et al., 2009; Sykes, 2011; Othman et al., 2023), and breeding efforts are underway to release others (Mahmoud et al., 2020). Tetraploid rootstocks showed a higher tolerance to salinity (Khaid et al., 2020). Differences in salt tolerance also depend on the nature of the scion. QTL analysis for salt tolerance in an intergeneric BC1 population from *C. grandis* x *P. trifoliata* suggests a complex polygenic inheritance (Tozlu et al., 1999; Tozlu et al., 2000).

Drought. Sustainability of citriculture has to face the general decreasing trend of water resources in the MB through the adoption of optimized water management strategies. Rootstock choice has a great influence on the plant tolerance to drought (Rodríguez-Gamir et al., 2010). Citrus rootstocks have differential capacities for supplying shoot tissues with water and carbon, affecting plant water status and photosynthesis. Water relations have been well studied in citrus trees, showing that rootstocks alter the physiological performance under water deficit through variations in plant hydraulic conductance, leaf water potential and stomatal conductance. In addition, citrus rootstocks showed different performances when exposed to drought. Some studies also indicate that using tetraploid rootstocks increases drought tolerance in comparison to their diploid clones (Allario et al., 2013).

3.2 Biotic challenges

Citrus tristeza Virus (CTV). CTV is the most devastating virus for Citrus because it causes the most damaging and economically important disease. It is called *tristeza* which means "sadness, melancholy" in Portuguese and Spanish. The causal agent of this disease is a virus that has one of the largest plant RNA viruses (Falaki, 2023). Depending on the strain it can cause stem pitting in the grafting region and a reduction in fruit size and yield (Hilf et al., 2005). This virus can cause many side effects, as this virus infects phloem-associated cells. Affected trees show a general decline, leaves yellowing included, until tree death. It is transmitted by infected propagation material and aphids. The use of tolerant rootstocks (usually Poncirus and its hybrids) is a prerequisite for new plantings where CTV is endemic, however several resistance breaking CTV strains, overcoming Poncirus resistance, are threatening citrus orchards in different countries (Catara et al., 2021).

Mal secco. This vascular disease is caused by the fungus *Plenodomus tracheiphilus*, which leads to dieback of twigs and branches, defoliation, and in many cases to the death of the tree. The disease is ubiquitous in many Mediterranean countries, including Italy, Greece, Turkey, and Tunisia. It is particularly destructive on lemon, but has been reported in other citrus species. In years with severe damages, especially after heavy rains, hail storms and frost (that favor pathogen penetration), the disease may cause yield losses of up to 50% (Migheli et al., 2009). Recently, *P. tracheiphilus* has been occasionally detected in Spain (EPPO, 2023). Due to the high susceptibility of the Spanish lemon clones, this disease may represent a major threat for lemon cultivation in that region, and may potentially spread to other lemon-producing places. Sources of tolerance to the disease can be found in the genus *Citrus* and in *Citrus* relatives (Russo, 1977; Russo et al., 2020) .

Phytophtora root rot, is caused by different species of *Phytophthora* oomycete and is recognized as a major disease in all citrus growing countries. In addition to the root system, it may infect all parts of the tree. The most common and important *Phytophthora* spp. are *P. parasitica* and *P. citrophthora*. The rot is particularly severe under rainy or flooded conditions. Sensitivity of the tree depends on tree age (young trees are more affected) and rootstock. *Phytophthora* spp. may also cause damages in the nurseries where up to 80% of young seedlings in seed beds may be killed (Cacciola et al., 2008). Together with *Penicillium* spp., *Phytophthora* spp. has been considered the most important diseases in the MB (Tena et al., 2011).

Alternaria brown spot (ABS), caused by the fungus *Alternaria alternata*, is characterized by necrotic lesions surrounded by a yellow halo in twigs, young leaves, and fruits, inducing defoliation and fruit drop. Damaged fruits are not marketable. Many treatments with fungicides are needed from flowering until harvest to control the disease. Several mandarin cultivars are susceptible, such as ‘Nova’ and ‘Murcott’, but sources of resistance are available in citrus germplasm, and molecular markers associated with resistance have also been developed (Cuenca et al., 2016).

4. ‘Freeclimb’ contribution: a summary of the project outcomes

The project focused on the generation of new breeding material and the characterization of local germplasm tolerant to the major biotic and abiotic constraints of the MB. The Algerian germplasm was subjected to a screening for salinity tolerance, providing information that could be used in future breeding programs. New rootstocks (‘Sunki’ mandarin × *P. trifoliata*) with tolerance to drought, salinity, iron chlorosis and Phytophthora root rot were generated and phenotyped and will be soon field-tested in combination with different scions. Regarding *mal secco*, new segregating populations and pre-breeding materials were obtained. For the first time, a segregating population (*Citrus latipes* × lemon) was subjected to field phenotyping and genotyped by Single-Primer Enriched Technology (SPET), identifying a QTL associated with *mal secco* resistance. This represents a prerequisite for further work aimed at generating molecular markers associated with resistance that could be efficiently used for MAS. New phenotyping protocols are available to screen populations in the field subjected to the natural pressure of the pathogen, as well as to screen young seedlings in greenhouse subjected to artificial inoculation, to rapidly discard highly susceptible hybrids before field evaluation. These protocols were essential to select new promising *mal secco*-resistant lemon hybrids that will be backcrossed with true lemons in the next few years, laying the groundwork for a new breeding program based on hybridization. Moreover, Freeclimb gave the opportunity to establish a network among different citrus countries of the Mediterranean for the implementation of three multi-site collections including pre-breeding material, rootstocks and lemon germplasm that could be used for future breeding programs.

5. Stakeholders survey

Eight stakeholders from Turkey and Italy were asked to rank the major challenges of the citrus industry in the Mediterranean countries. The stakeholders who participated in the survey are growers, nurserymen, consultants, and field technicians. Half of them indicated the need both to exploit existing cultivars to cope with biotic and abiotic stresses and to breed new varieties. Regarding abiotic stresses, stakeholders indicated water deficit (30.2%) and high temperatures as the most dangerous ones (22.7%): Figure 3. This is probably due to the frequent cases of heat waves in the Mediterranean area occurring in the last two decades, which severely affected plant growth and yield. It is likely that these extreme events will be more frequent in the next decades (Molina et al., 2020). The risk of damages caused by high temperatures is followed by water deficit (19.9%) and salinity (18.7%). Both stresses represent a major limitation for Mediterranean citriculture and are partly related. *Ceratitis capitata* (Mediterranean fruit fly) represents the major threat among citrus pests, it causes several damages in all citrus areas of the Mediterranean and it is not easy to control especially in early-maturing cultivars. Med fly is followed by scales and by the leaf miner *Phyllocnistis citrella*. It is important to underline that the psyllids *Tryoza etritreae* and *Diaphorina citri*, both vectors of HLB, were not included in the survey because they are not still diffused in the Mediterranean. However, both insects were recently detected in two countries (*Tryoza eritreae* in Spain and *Diaphorina citri* in Israel), and they will likely become major pests for the MB citrus industry. Regarding viruses, viroids, and diseases caused by fungi, oomycetes, and bacteria, the fungus *Plenodomus tracheiphilus* was considered the most harmful, likely because it represents the major threat for lemon cultivation in Italy and Turkey. Mal secco is followed by Phytophthora root root and by CTV. Other diseases such as Citrus Variegated Chlorosis (CVC), citrus black spot and HLB, despite not being present in the Mediterranean basin, are already considered as threats because they are causing severe damages worldwide.

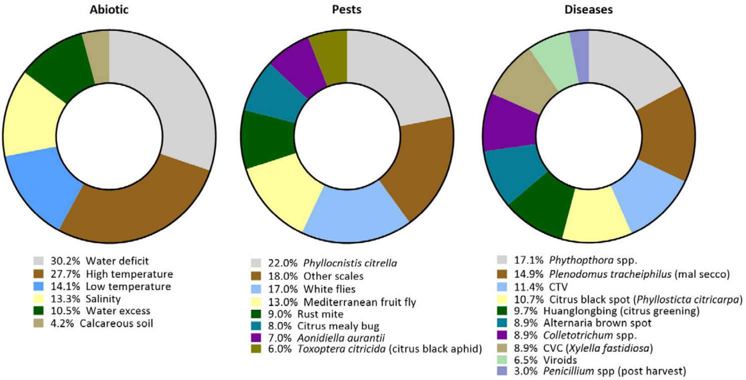

Figure 3. Stakeholders' opinion for abiotic, pest and disease stresses with the highest risk for Citrus growing. Combined stress risk index (CSRI) is presented based on the risk evaluation on a 1-10 scale and on the frequency of each stress suggested by the respondents.

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Grape
Vitis vinifera L.

1. Crop importance in the Mediterranean Basin

Viticulture and winemaking play a significant socioeconomic role for many countries worldwide. These industries involve cultivating vines of the Eurasian species *Vitis vinifera* L., which comprises around 6,000 cultivars despite approximately 12,000 names being reported. 'Kyoho' (*Vitis* interspecific crossing), 'Cabernet-Sauvignon,' and 'Sultanina' are the most abundant cultivars grown across the globe, according to OIV (International Organisation of Vine and Wine, 2020). As of 2019, the global area planted for various purposes, including young vines not yet in production, is estimated to be 7.4 Mha, as reported by EUROSTAT (2019). In 2019, wine production worldwide, excluding juices and musts, amounted to an estimated 260 Mhl. According to the OIV, the largest wine producing countries are Italy (47.5 Mhl in 2019 with over 700,000 ha of vineyards), France (42.1 Mhl), Spain (nearly 1 million ha, 33.5 Mhl), the United States (24.3 Mhl in 2019) and Argentina (13 Mhl) (OIV, 2020). The wine export market, which includes exports from all countries, saw a growth in both volume and value compared to 2018. Notably, there was a substantial increase in exports from Italy, Spain, Canada, and Chile, while France remained the largest world exporter in terms of value. Bottled wines (<2 l.) accounted for 53% of the trade volumes globally, similar to 2018, whereas sparkling wines saw significant growth in terms of volume and value (OIV, 2020). The European Union (EU) is the world's leading wine producer, with an average annual production of 167 Mhl between 2014 and 2018. It accounts for 45% of world wine growing areas, 65% of production, 60% of global consumption and 70% of exports. Around 75% of grape production is meant for wine making, of which 20-30% is waste, known as "grape pomace," made up of skins, remaining pulp, seeds, and stalks. Grape juice production is much less common, with only 21% and 5.1% of the world production taking place in Europe and Asia, respectively, and 73.7% concentrated in the Americas. The top grape juice producers are the USA, Spain, Argentina, Chile, and Brazil.

2. Outline of the crop evolution until today

Grapevine, which is believed to have originated in Eurasia, has a long evolutionary history that dates back around 65 million years ago, according to Olmo et al. (1995). The *V. vinifera* species includes both wild (*V. vinifera* subsp. *sylvestris*) and cultivated (*V. vinifera* subsp. *sativa*) subspecies, with the former being the progenitor of the latter (This et al., 2006). The domestication of grapevine has raised major questions about whether it occurred as a single or multiple events, and where these events took place. It is reasonable to assume that such conditions may have arisen in several areas, differing in chronology and level of development. If until recently, the most widely accepted hypothesis suggests that the domestication of *V. vinifera* occurred in the South Caucasus region between the Caspian and Black Seas around 6,000-5,800 BC, before spreading to Europe and the Mediterranean Basin, through the spread of civilizations, according to McGovern et al. (2017). However, it was recently pointed out that the domestication occurred concurrently about 11,000 years ago in Western Asia and the Caucasus (Dong et al., 2023). The domestication of grapevine was largely driven by the superior taste and alcoholic qualities of its fermented juice, i.e. wine, in comparison to other fruit wines, although the exact sequence of events leading to its domestication is not entirely clear (Terral et al., 2010). The key changes that occurred during the domestication process were related to the morphology of flowers (cultivated accession are hermaphroditic), berry size (larger in cultivated germplasm), sugar content (higher in cultivated germplasm), a wide range of berry colors and aromatic content, all of which contributed to higher yield, better quality, and improved fermentation (This et al., 2006). The domestication process led to bred thousands of diverse cultivars, adapted to a wide range of climates.

2.1 Cultivars

Grapevine domestication has led to the development of wine and table cultivars (Dong et al., 2023). The main differences between wine and table cultivars are related to the fruit traits. Wine cultivars have been selected for sugar content, acidity, and other chemical properties, such as anthocyanin and volatile compounds. They generally have smaller berries, thicker skins, and higher tannin content than table grapes, which are characterized by large, seedless berries with a thinner skin and a firm flesh.

Although each Mediterranean Country grow its own wine cultivars, such as ‘Sangiovese’, ‘Nebbiolo’, ‘Aglianico’ in Italy, ‘Tempranillo’, ‘Monastrell’, ‘Bobal’ in Spain, there are a number of varieties, which are often referred to as ‘international’ cultivars, i.e. those that have been widely cultivated in many wine-producing regions, mainly due to their adaptability to different environmental conditions, the quality of the resulting wine, and their popularity with consumers. Some of the most common international grapevine varieties include: i) ‘Cabernet Sauvignon’, red grapevine originally from Bordeaux, France; it gives full-bodied wines with high tannins, ‘black fruit’ flavors, and herbal notes; ii) ‘Chardonnay’, white grapevine from Burgundy, France, which gives wines with a wide range of styles, from light and crisp to full-bodied and oaky, with flavors of apple, citrus, and tropical fruits; iii) ‘Merlot’, red grapevine from Bordeaux, France, which gives wines with medium body, with flavors of black cherry, plum, and chocolate; iv) ‘Pinot Noir’, a red grapevine from Burgundy, France, which gives wines with medium body and high acidity, with flavors of ‘red’ fruit, earth, and spices; vi) ‘Sauvignon Blanc’, a white grapevine from the Loire Valley, France, which gives wines with high acidity and flavors of grapefruit, lime, and grass; vi) ‘Syrah’/’Shiraz’, a red grapevine that gives full-bodied wines with high tannins and flavors of ‘black’ fruit, pepper, and spice.

Table cultivars are mainly cultivated in Turkey (3 Mt), Egypt (2 Mt), Italy (1.7 Mt), Spain (1.6 Mt), Greece (1.3 Mt) and Morocco (0.8 Mt), based on FAOSTAT (2014). Spain is one of the main producers of table grapes in the world, with varieties such as ‘Thompson Seedless’, ‘Crimson Seedless’ and ‘Italia’. In Italy, table grape production is mainly concentrated in southern regions, such as Apulia and Sicily, and includes varieties such as ‘Italia’, ‘Victoria’ and ‘Red Globe’. Turkey is one of the largest exporters of table grapes in the world, with varieties such as ‘Sultana’ (‘Thompson Seedless’) and ‘Razaki’. In Greece, table grapes are mainly concentrated in the Macedonia region, and include varieties such as ‘Crimson Seedless’ and ‘Thompson Seedless’. In Morocco, the most cultivated table grape cultivars are ‘Italia’, ‘Crimson Seedless’ and ‘Red Globe’. While in Egypt, the most cultivated table grape cultivars are ‘Thompson Seedless’ and ‘Crimson Seedless’. ‘Thompson Seedles’s is a green oval-shaped grape with a sweet, delicate flavor and a crisp texture. It is seedless, making it a popular choice for both fresh consumption and raisin production. The skin is thin and can easily break, so it is not suitable for long-term storage or transportation. ‘Crimson Seedless’ has a deep red color, round shape, and crisp texture. It has a sweet and slightly tangy flavor with a hint of berry. It is seedless and has a firm skin, making it a popular choice for fresh consumption and export. ‘Italia’ is a large, oval-shaped grape with a yellow-green color and a sweet, musky flavor. The flesh is firm and juicy, and the skin is thick and tough, making it suitable for long-term storage and transportation. ‘Victoria’ is a red grape with a round shape and a sweet, floral flavor. It has a thin skin and is seedless, making it a popular choice for fresh consumption and export. ‘Red Globe’ is a large, round grape with a deep red color and a crisp texture. It has a sweet and slightly tart flavor, with a hint of musk. The skin is thick and tough, making it suitable for long-term storage and transportation. ‘Razaki’ is a medium-sized grape with a light red color and a slightly tart flavor. It has a firm texture and a thick skin, making it suitable for long-term storage and transportation. It is often used for making raisins.

2.2 Rootstocks

Since the first decade of the 19th century, grapevine has been grown on rootstocks, represented by a mix of non-*vinifera* grapevine species, such as *Vitis rupestris*, *Vitis riparia* and their hybrids. Rootstocks contribute to control soil borne pests (e.g., phylloxera and nematodes) and some environmental conditions (e.g., drought and salinity) (Gale, 2002). A wide range of works have been done in relation to the influence of rootstocks on vine water uptake, mineral nutrition, and on its adaptability to soil conditions (salinity, iron deficiency and water deficit) (Keller et al., 2001). The scion/rootstock interaction strongly impacts on shoot development and grape quality, bud fertility, phenology, leaf area and canopy development, vigor and yield (Stevens et al., 2008). The rootstocks used in the MB exhibit a relatively narrow genetic background, because their selection was essentially based on only a few traits, such as phylloxera resistance, rooting ability and scion-induced vigor. No more than ten rootstocks are used in MB and worldwide, as well (Keller et al., 2010). The most used are: i) ‘101-14’, a hybrid of *V. rupestris* and *V. riparia*; it is considered a moderately vigorous rootstock with good resistance to phylloxera and drought; it is also tolerant to lime-induced chlorosis and is popular for calcareous soils; ii) ‘1103 Paulsen’, a hybrid of *V. berlandieri* and *V. rupestris* species; it is a vigorous rootstock that has good resistance to phylloxera, drought, and lime-induced chlorosis; it is also known for its high tolerance to salinity and wet soils; it has moderate resistance to nematodes and some fungal diseases; iii) 'SO4', a hybrid of *V. berlandieri* and *V. riparia*; it is known for its excellent resistance to phylloxera and it is used in acid soils; iv) '140RU', a hybrid of *V. berlandieri* and *V. rupestris* ; it shows good resistance to phylloxera, high tolerance to lime-induced chlorosis, moderate resistance to root rot, and a moderate vigor; v) 'Kober 5BB', a hybrid between *V. berlandieri* and *V. riparia*; it shows high vigor, good tolerance to lime-induced chlorosis, and a moderate level of resistance to root knot nematodes; it is widely used in calcareous soils. Since 1985, the DiSAA Department of the University of Milan has been working on the selection of new grapevine rootstocks; the 'M' series ('M1', 'M2', 'M3', and 'M4') rootstocks was developed, registered at the National Register of Vitis varieties in Italy; these rootstocks have shown tolerance to iron-limited conditions ('M1' better than 'M3'), moderate resistance to salinity ('M2' and 'M4'), and high tolerance to drought ('M4') (Meggio et al., 2014; Vannozzi et al., 2017; Corso et al., 2015; Corso et al., 2016). However, these rootstocks may not be sufficient to handle the emerging challenges in viticulture related to climate extremes.

2.3 Environment and orchard management

Grapevines are cultivated in a range of different environments, from cool, humid regions to hot, dry climates. The regions where grape cultivation takes place are generally situated between the 35th and 55th parallels in the northern hemisphere, and the 25th and 35th parallels in the southern hemisphere. Currently it is present in all continents except Antarctica, so it is one of the most cosmopolitan plant species. At low latitudes, the availability of solar radiation is sufficient to ensure its production, except in vintages with very rainy summers, causing a reduction of sugar content. At higher latitudes, photoperiod and temperature negatively affect the maturation and sugar content. Generally, grapevines require warm temperatures for successful growth and fruit ripening, as well as adequate sunlight and water. The typically average annual temperature ranges from 10 to 20° C. The most suitable cultivation regions are characterized by the alternation of a favorable growing season and a less favorable cold season, with relatively mild winters (minimum temperatures between -10 and 15° C) and a growing season lasting more than 200 days, with average temperatures higher than 10 degrees Celsius, which is sufficient for grapes to mature and ripen properly (Gladstones, 1992). Water availability is also crucial for grape cultivation, and it is often necessary to irrigate vineyards to ensure that vines receive sufficient water during the growing season. Soil quality is also an important factor, with well-drained soils being preferred as grapevines do not tolerate waterlogged conditions (Keller et al. 2010).

Grapevine planting density refers to the number of grapevine plants per unit area of land in a vineyard. The density can vary depending on the desired quality and quantity of grape production, the grape variety, and the soil and climate conditions of the vineyard. In general, higher plant densities result in yields of higher quality grapes, while lower plant densities result in yields of lower quality grapes. The ideal plant density can vary between grape varieties and growing regions. The following classes of planting density can be distinguished: i) low density, with less than 3000 plants per hectare; ii) medium density, from 3000 to 6000 plants per hectare; iii) high density, above 6000 plants per hectare. There are several training systems used by grape growers worldwide. Regardless of their complexity, training systems can be simplified into four basic combinations: i) head/spur, which consists of a short trunk and several two-node bearing units, such as bush vine; ii) head/cane, which includes a short trunk with one or more longer bearing units, such as Guyot; iii) cordon/spur, which involves horizontal extensions of the trunk with several two-node spurs, such as midwire cordon; iv) cordon/cane, which is similar to head/spur but with longer bearing units, such as Sylvoz (Reynolds and Van den Heuvel, 2009). Although each training system has its own advantages and disadvantages, in most cases, training systems as well as planting density are determined by the specification for the production of wines with denomination of controlled and guaranteed origin.

For table grapes, the most common training system used is the overhead trellis system, also known as the pergola system. In this system, the grapevines are trained to grow upward on a series of overhead wires supported by posts. The vines are trained to develop a horizontal canopy that provides shade to the fruit and allows for easier harvesting. The pergola system is popular for table grape production because it allows for high plant densities, efficient use of space, and good air circulation around the fruit.

3. Abiotic and biotic challenges

3.1 Abiotic challenges

The wine industry faces one crisis: the ongoing challenge of climate extreme events. Climate extreme events are a serious threat to the wine industry. Addressing climatic extremes now would be more cost-effective than dealing with the disruption and damage caused by global warming in the future. Over the past century, Europe has experienced a significant rise in temperatures, ranging from 2 to 5 °C. In addition, Southern Europe has seen a decrease in precipitation. According to the latest report by the Intergovernmental Panel on Climate Change, global temperatures are expected to increase between 1 and 5° C by the end of the 21st century, depending on different scenarios. Recent research suggests that modern global temperatures are the highest they have been in the past 12,000 years, approaching the warmth of the last interglacial period. Abrupt changes in climate greatly affect grapevine growth, development, and production, leading to changes in wine quality. Weather patterns, such as temperature, precipitation, and solar radiation, can vary annually, leading to changes in productivity. Extreme weather events such as hail, late frost spells, and excessive rainfall have been found to negatively impact grape yield and quality (Fraga et al., 2019; Botton et al., 2022; Meggio et al., 2022). These events have already caused tremendous losses, and the expected changes in climate will further increase the incidence of biotic threats. Although grapevine is well adapted to arid and semi-arid environments, water stress can impair some vine physiological traits, ranging from mild impact to irreversible vine death. The predicted more frequent and severe water shortages represent a substantial risk for viticulture (Schultz, 2000). Consequently, in areas with limited water resources low quality irrigation water (e.g. salty groundwater) is increasingly used to overcome drought stress threatening cropping systems due to salt accumulation in the root-zone soil (Mariani and Ferrante, 2017). Hence, improving knowledge on potential rootstocks able to cope with multiple stresses (e.g. drought and salinity) is highly desirable.

3.2 Biotic challenges

Biotic factors such as fungi, oomycetes, bacteria, phytoplasmas, viruses, and nematodes, as well as pests such as insects and mites, can cause diseases and disease-like symptoms, affecting grapevine physiological development and a lower yield. The main diseases affecting grapevine, in the current climatic conditions and in scenario projections for 2050, are downy and powdery mildews, caused by fungi (Bois et al., 2017) Together, these two diseases are responsible for about 20 fungicide sprays in a relatively short period (April-September), from sprouting to harvest.

Downy mildew, caused by the oomycete *Plasmopara viticola*, is the most important disease in intermediate to hot climates with sub-humid to humid conditions, such as many regions in Europe. Powdery mildew, caused by the ascomycete *Erysiphe necator*, is found also in dry areas. Both *P. viticola* and *E. necator* are biotrophic pathogens and obligate parasites. They infect all green tissues of grapevine, causing both quantitative and qualitative reductions, due to the damages on bunches (reduced harvest) and leaves (reduced sugar content in berries) (Merdinoglu et al., 2018). Grapevine is highly susceptible to these pathogens. The disease control is mainly performed through the frequent (every one/two weeks) applications of fungicides from grapevine receptivity to the start of bunch maturation. The use of chemical pesticides in agriculture to protect crops contributes to soil, water and air pollution and biodiversity loss, affecting non-target organisms. Also, the massive use of some fungicide increases the risk of resistance development (Massi et al., 2021). Fungicides are the largest category sold in Europe, with the inorganic fungicides (copper and sulfur, in organic farming) representing more than half (53.1%) of the fungicide sales. Spraying against grape diseases represents 65% of the total amount of fungicides employed in agriculture, in the face of a cultivated area of only 3.3% (Gessler et al., 2011). This makes the mitigation of fungicide impact in viticulture a priority in Europe. Indeed, one of the "Farm to Fork Strategy" objectives for a fair, healthy and eco-friendly food system, is to reduce the overall use and risk of chemical pesticides by 50% and the use of more hazardous pesticides by 50% by 2030. In the current scenario, it is crucial to develop customized grapevine ideotypes that can cope with the main abiotic and biotic stresses, safeguarding the environment. The cultivation of resistant *V. vinifera* varieties would be the most effective way to reduce the damage caused by the pathogens and the impact of disease management. *V. vinifera* does not show a specific response to infection compared to the American grapevines species. Nevertheless, recent studies showed that *V. vinifera* sources of high tolerance are indeed present in Georgian wild and cultivated germplasm (Toffolatti et al., 2016; Bitsadze et al., 2015).

4. 'Freeclimb' contribution: a summary of the project outcomes

In the FREECLIMB project, the activities were focused to identify loci associated with the resistance to drought, downy and powdery mildew and via GWAS. Two different sets of genotypes were used to identify loci associated with drought resistance in grapevine rootstock: i) core collection; ii) F1 population (M1 x *Vitis rupestris* 'Thyres'). Core collection samples have been phenotyped for resistance to drought and with Vitis18kSNP chip array. Genotypic data of grapevine core collection have been published in Bianchi et al. (2020). GWAS identified two loci as associated with the resistance to drought, one on chromosome 7 and one chromosome 14. The locus on chromosome 7 is in a gene encoding for a RPP13-like protein 1, while the one on chromosome 14 is located in a gene encoding for a peroxisome biogenesis protein 12. F1 population genotypes have been phenotyped for resistance to drought and with Vitis18kSNP chip array as well and GWAS identified one locus associated with the resistance on chromosome 6, located on a gene U-box family gene. For downy mildew, a group of Georgian-based accessions were phenotyped for susceptibility to *P. viticola* and genotyped with Vitis18kSNP chip array. Three newly significant loci on chromosomes 14 (*Rpv29*), 3 (*Rpv30*), and 16 (*Rpv31*) were identified as being linked to a reduced level of pathogen sporulation. These loci were observed to be associated with plant defense genes against biotic stresses, such as genes involved in pathogen recognition and signal transduction. These results present the initial proof of resistant loci against *P. viticola* in *V. vinifera* germplasm and pinpoint potential target genes for the development of *P. viticola* resistant grapevine varieties through breeding. The data were published in Sargolzaei et al. (2020). For powdery mildew, a group of grapevine accessions coming from Caucasus, Iran and Uzbekistan were phenotyped for susceptibility to *E. necator* and genotyped with Vitis18kSNP chip array. Only one locus has been identified as associated with the powdery mildew resistance. The locus is located on chromosome 17. The gene where this locus is located is a cyclic nucleotide-gated ion channel.

5. Stakeholders survey

Thirteen stakeholders from Algeria and Italy were asked to rank the major abiotic and biotic challenges of viticulture in the Mediterranean countries, being growers of both wine and table grapes (Figure 4).

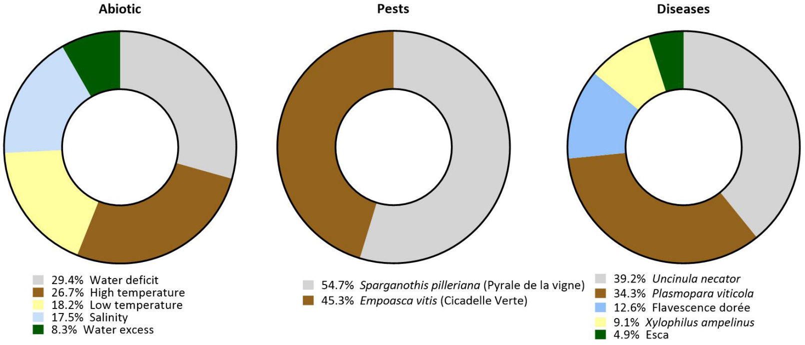

Figure 4. Stakeholders' opinion for **abiotic and biotic** stresses with the highest risk for grape growing. Combined stress risk index (CSRI) is presented based on the risk evaluation on a 1-10 scale and on the frequency of each stress suggested by the respondents.

Regarding abiotic stresses, the stakeholders indicated water deficit, salinity and high and low temperatures as the major challenges in the vineyard. The damage due to the water deficit was considered highly risky for both Countries (around 30%), followed by high temperature (around 27%), low temperature (18%), salinity (17%) and water excess (around 8%). Regarding biotic stresses, the stakeholders showed *Sparganothis pilleriana*, *Empoasca vitis*, among pest agents, and *E. necator*, *P. viticola*, *Xylophilus ampelinus*, fungi causing 'Esca' disease and *Candidatus Phytoplasma vitis*, among disease agents, as the major challenges in the vineyard. Pests damage is most felt by Algerian growers, particularly for *S. pilleriana* (vine leafroller tortrix), around 55% of the respondant. *E. necator* (causing powdery mildew) and *P. viticola* (causing downy mildew) are considered the most highly risky for both Countries, with around 39 and 34%, respectively, followed by *Candidatus Phytoplasma vitis*, a phytoplasma causing 'flavescence dorée' (12%), *X. ampelinus,* a gram-negative bacterium causing necrosis (9%), and fungi causing 'Esca' disease (5%).

When growers were questioned about whether they would suggest exploitation of existing cultivars or breeding for new ones, all of them replied that both strategies have to be pursued.

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Olive

1. Crop importance in the Mediterranean Basin

Olive *(Olea europaea* L.) is an iconic fruit tree species for human societies in the Mediterranean area (Kaniewski et al., 2012). It is considered as a structural element of many Mediterranean agroecosystems and is of significant socio-economic, cultural, nutritional and ecological importance (Loumou and Giourga, 2003). More than 10 million ha are devoted to olive cultivation worldwide, 90% of which is in the Mediterranean area (FAOSTAT, 2023). The tree has been a staple food in the Mediterranean region for thousands of years, and the cultivation of olives and its use have been an integral part of the region's cultural heritage (Bombarely et al., 2021). Olive oil is also an essential ingredient in the Mediterranean diet due to its nutritional values: high levels of monounsaturated fats and antioxidants, making it a valuable source of a healthy diet. Additionally, the species is well-adapted to the Mediterranean climate, characterized by hot and dry summers, making it an essential crop for the region's agriculture and a vital element in preventing soil erosion and maintaining soil fertility (Gomez et al., 2008).

Olive markets are highly diversified ranging from a mass market with standard products to niche markets for high value products. The crop is a crucial source of income, providing jobs and livelihoods for thousands of farmers, processors, and traders, and its industry generates significant revenues for the producing countries. Olive oil production has tripled in the last 60 years to reach an average of 3.25 Mt annually with the European Union representing 3.0 Mt (International Olive Council, IOC, 2023; Figure 5). Spain is the largest producer country with over 1.4 Mt, followed by Italy (0.286 Mt), Greece (0.242 Mt), Tunisia (0.240 Mt) and Turkey (0.213 Mt) (IOC, 2023). Significant amounts of olive oil are produced also in Morocco (0.176 Mt), Portugal (0.137 Mt), and Algeria (0.096 Mt). For the table olives, the global annual production averages 2.91 Mt annually (IOC, 2023; Figure 6). Egypt is the top producer country with 0.6 Mt followed by Spain 0.56 Mt, Turkey 0.41 Mt, Algeria 0.3 Mt and Greece 0.21 Mt. Morocco and Syria are also important table olive producer countries with an annual average production of 0.13 and 0.12 Mt, respectively.

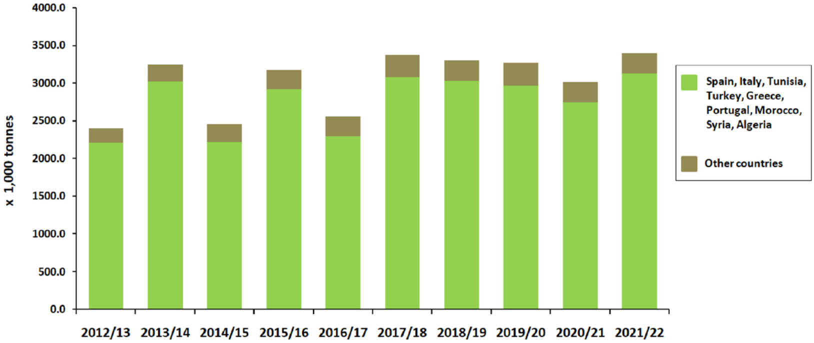

Figure 5. Evolution of olive oil production during the last ten years (source: IOOC, 2023)

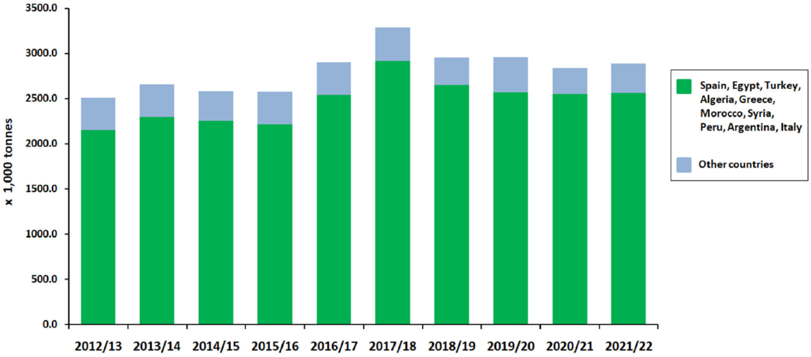

Figure 6. Evolution of table olives production during the last ten years (source: IOOC, 2023)

2. Outline of the crop evolution until today: cultivars and orchard management

Six subspecies of olive are currently recognized and supported by genetic data (Green, 2002; Besnard et al., 2018). Within the Mediterranean olive [*O. europaea* L.], two forms are recognized: wild olive or oleaster [*O. europaea* var. *sylvestris*] and the cultivated olive (*O. europaea* var. *europaea*; Green, 2002). Over the last two decades, a wealth of paleobotanical, archaeological, historical, and molecular data have been accumulated on olive and tits domestication (Besnard et al., 2013; Newton et al., 2014; Diez et al., 2015; Khadari and El Bakkali, 2018; Terral et al., 2021). However, the question remains unclear as to whether cultivated olives derived from a single primary domestication center followed by secondary diversification events or whether they are the result of independent primary selection events. Genetic, archaeological and palaeontological sources, are supporting the hypothesis of one center of primary domestication in the Levant, where olive cultivation first emerged around 6,500 yrs. ago (Zohary et al., 2012; Kaniewski et al., 2012; Besnard et al., 2018; Terral et al., 2021). From this center, there was a slow dissemination of domesticated forms, cultivation practices, and technical skills, first to the Aegean in the 3rd millennium B.C. and then to central and western areas (Newton et al., 2014; Margaritis et al., 2021; Terral et al., 2021). The expansion of cultivated olive in the Mediterranean basin occurred mostly during the Roman Empire and later during the Arabic period. Early domesticated forms were probably disseminated during successive human migrations from east to west and introgressed with local wild olives, in turn giving rise to local cultivated forms through selection by farmers (Baldoni et al., 2006; Belaj et al., 2007; Diez et al., 2015; Khadari and El Bakkali, 2018). Olive then spread from Mediterranean areas throughout the world. This crop is now of increasing commercial interest beyond the MB, including countries such as Australia, Chile and USA (IOC, 2023).

2.1 Cultivars

Over the long history of olive domestication, cultivated forms have been selected, propagated and disseminated by farmers. An international initiative was conducted to pool olive germplasm information in a single database. The 2008 web-based edition (http://www.oleadb.it/) is currently the largest database (Bartolini, 2008). There are currently more than 1,200 cultivars with over 3,000 synonyms reported in 54 different countries and maintained in almost 100 separate collections at international, national and regional levels for conservation and evaluation purposes (Bartolini et al., 2005), including three worldwide olive germplasm banks in Cordoba, Spain (Trujillo et al., 2014), Marrakech, Morocco (El Bakkali et al., 2019) and

Izmir (Turkey). The available genetic resources have been evaluated using many morpho-agronomical descriptors and diverse molecular markers (AFLP, SSR, SNPs, DArt) as well as Whole Genome Sequencing and results revealed high diversity of the Mediterranean olive germplasm (Haouane et al., 2011; Koehmstedt et al., 2011; Trentacoste et al., 2011; Diez et al., 2012; Kaya et al., 2013; Muzzalupo et al., 2014; Koubouris et al., 2019; Avramidou et al., 2020; Bazakos et al., 2023). As a clonally propagated tree, olive has been widely disseminated worldwide, with many cases of synonymy, homonymy and molecular variants, i.e. intra-cultivar, or clonal variation (Khadari et al., 2008; Trujillo et al. 2014; El Bakkali et al., 2019). These issues could be overcome via cultivar characterization and identification, thus avoiding varietal confusion, and providing authenticated cultivars (Trujillo et al., 2014).

Socioeconomic changes in most olive producing countries have led to significant improvements in olive growing, including the establishment of modern orchards based on a few high-yielding and low-vigor cultivars, such as 'Arbequina', 'Arbosana' and 'Koroneiki' cultivars. In the current setting of climatic extreme events (Ponti et al., 2014; Cramer et al., 2018) and the emergence of new diseases, such as *Xylella fastidiosa*, olive cropping systems based solely on a few cultivars may be less resilient than conventional systems and may potentially lead to the erosion of local olive germplasm because several minor traditional cultivars are being replaced by a few cultivars. Despite the large genetic diversity preserved in ex-situ collections worldwide, cultivated olive is not much diversified since less than sixty varieties are widely used worldwide (Figure 7). Some of these cultivars are widely diffused, as the case of 'Picholine marocaine' which represents more than 90% of olive germplasm in Morocco (Khadari et al., 2008; El Bakkali et al., 2013) and 'Galega vulgar' with more than 80% in Portugal (Cordeiro et al., 2008). Furthermore, 'Arbequina' is still one of the most widely cultivated worldwide, with a significant adaptation capacity for oil yield in different environments (Mousavi et al., 2019).

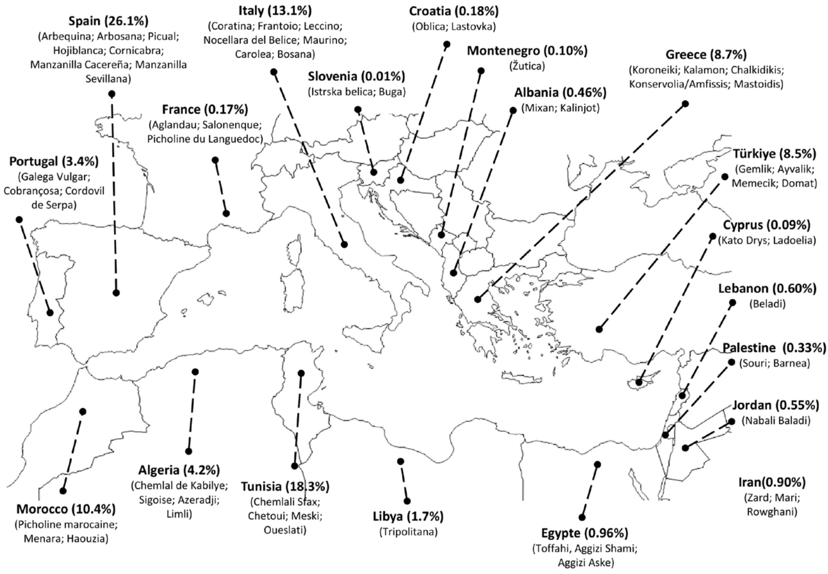

Figure 7. Distribution of olive areas per Country in the Mediterranean Basin and the main cultivars in each country (Source: IOOC, 2023)

2.2 Environment and Orchard management

Olive is characterized by a diversity of cropping systems, ranging from extensive and diversified agroecosystems to very high density orchards. Three major production systems could be identified: (i) extensive olive production system with a few varieties and oriented for competitive mass markets; ii) local cultivars addressing niche market requests; and iii) mono-varietal orchards optimized for very early and high yields. In recent decades, traditional olive orchards have gradually been replaced by modern ones wherein one or two cultivars are grown at a planting density up to 400 trees/ha (Rallo et al., 2013). Presently high density orchards featuring from 600 two 2,000 irrigated trees/ha, highly mechanized management, and increased use of synthetic chemical inputs is spreading worldwide. The major transition that is under way has given rise to a diversification of the cropping systems, raising a variety of biological, economic, environmental, and social issues (Russo et al., 2016). During the 20th century, significant advancements have marked the olive industry, e.g. the adoption of mechanical pruning and harvesting and the optimization of irrigation systems (e.g. smart irrigation), fertilizers and spraying programs. Overall, these practices have led to an increase in terms of yield and oil quality (Camposeo et al., 2022). Additionally, there has been a growing interest in organic olive farming relying on 'natural' methods to control pests and soil fertility while minimizing the use of synthetic chemicals. Moreover, a growing interest in sustainable olive farming, in terms of carbon footprint, has been observed to minimize the environmental impact of olive cultivation through an efficient use of water and land (soil and biodiversity) (Michalopoulos et al., 2020). The olive cultivars choice depends on climatic conditions and the intended final use of the fruit. The choice of high-yielding olive cultivars that are more disease-resistant and tolerant to various environmental conditions is becoming more important. The focus on cultivars with unique flavors or properties with distinctive olive oil quality is another trend in the olive market. This market segment, based on 'designation of origin' (recognized by the European Union legislation), is usually opposed to the development of a mass product based on a few high yielding cultivars, not well identified in terms of oil quality attributes, and aims to preserve and promote local, peculiar, high quality olive cultivars. Modern olive groves management relies on a variety of tools and technologies to optimize olive production while minimizing environmental impact and could be summarized as follows.

-Precision Agriculture: involving technologies such as satellite imagery, drones, and soil sensors to map and monitor the olive groves; this information is used to optimize irrigation, fertilization, and phytosanitary spraying strategies, resulting in an optimal use of inputs and therefore a higher profit with lower cost and less environmental impact (Roma and Catania, 2022).

-Integrated Pest Management (IPM): combining biological, cultural, and chemical inputs to manage plant nutrition, diseases and pests, even recurring to natural predators and biopesticides, and minimizing the use of synthetic pesticides (Gómez-Guzmán et al., 2022).

-Sustainable Agriculture: reducing the environmental impact of agriculture while keeping satisfactory yields; practices such as crop rotation, cover cropping, and reduced tillage are employed, to improve soil health and reduce its erosion (Kourgialas et al., 2022).

-Organic Farming: relying on natural inputs such as compost and cover crops; organic farming can improve soil health, reduce pesticide residues in food, and promote biodiversity conservation (Gomez et al., 2008).

-Irrigation Management: reducing water waste and optimizing its use through smart technologies such as drip irrigation techniques (Kourgialas et al., 2019).

3. Abiotic and biotic challenges

The olive is a long-lived tree with a relatively slow growth rate, which makes it particularly vulnerable to various biotic and abiotic stress factors that can significantly affect its growth, yield, and overall health.

3.1 Abiotic challenges

Among the constraints linked to climate change in the Mediterranean area, increased winter temperatures and a longer and hotter summer dry period are two key-factors that threaten olive crop because they affect the flowering phenology and cause severe drought stress.

Temperatures. In the context of climate warming, olive growers have to cope with two main constraints: (i) phenological asynchrony of flowering time among compatible varieties and increased temperatures that impair pollination success (Koubouris et al., 2010; El Yakoubi et al., 2014; Saumitou-Laprade et al., 2017); and (ii) deficit of cold winter temperatures leading to a substantial reduction in the flowering rate (Haberman et al., 2017; Koubouris et al., 2019). It was reported that high temperatures (above 16 °C) during the floral initiation prevented the development of flower buds and induced an abnormal reproductive cycle; temperature is strictly associated with the tree chill requirements (Haberman et al., 2017) and can also drastically reduce the pollen germination rate above 30° C during pollination (Koubouris et al., 2009). Olive production in the Mediterranean Countries often suffers huge economic losses due to heat stress during flowering; for example olive oil production in Greece was reduced by 60% compared to the annual average due to hot southern wind storms during May 2013 (USDA, 2014). Additionally, in regions with high summer temperatures, excessive heat can reduce plant growth, yield, and oil quality. In a recent study, more than 300 olive cultivars were classified into four groups according to their chill requirements and results indicated that flowering dates of most of these cultivars (92%) is governed by both chilling and heat requirements which provide first insights to select cultivars adapted to global warming (Abou-saaid et al., 2022). Many practices are performed by farmers to manage heat stress, by planting heat-tolerant cultivars, adopting appropriate irrigation techniques, and applying shading, such as netting or other tree covers.

Drought. The increasing demand of olive oil has led to the intensification and expansion of olive cultivation in the MB and to the shift from traditionally rainfed to irrigated olive orchards (Guzmán et al., 2022). Annual olive oil production in the MB is directly affected by precipitation levels and significant yield losses have been reported e.g. in Spain, Italy and Greece following seasons with poor rainfall. Even reduced irrigation may result in 30-50 % yield reduction (Arampatzis et al., 2018). Variability in terms of drought tolerance or WUE among cultivars has been reported in several studies, although this knowledge has not been widely used in olive growing management. Olive cultivars can be classified into three categories according to their susceptibility to drought - high, medium and low -, while several studies have found quantitative differences in phenotypic traits among varieties differing in drought resistance (Bosabalidis and Kofidis, 2002; Trentacoste et al., 2018). However, such classification does not associate drought resistance with any quantitative traits, and most studies involving phenotypic traits have compared only a few varieties simultaneously, with a limited focus on leaf traits and phenology (Trentacoste et al., 2018). Therefore, a broad perspective as to how olive copes with drought across the MB is lacking.

Salinity. Although olive is regarded as a moderately salt tolerant tree (Moula et al., 2020), salinity may cause significant damages in regions with high soil levels of salinity. Many areas devoted to olive cultivation are becoming salty, because of the increasing utilization of poor-quality irrigation water, mostly supplied during the warm summer period (Boussadia et al., 2023). Salinity has a negative influence on shoot growth, photosynthetic rate, inflorescence development and pollen germination (Koubouris et al., 2015). Farmers can manage salinity stress by planting salt-tolerant cultivars, e.g. ‘Koroneiki’, by appropriate irrigation techniques, and applying soil amendments, such as gypsum and organic matter.

Frost. It is a major abiotic stress factor affecting olive trees in regions with low winter temperatures. Frost damage can cause wilting, leaf necrosis, and even death of the tree. Farmers can manage frost stress by planting frost-resistant olive tree cultivars, e.g. 'Arbequina' and 'Cornicabra'; Comez-del-Campo and Barranco, 2005, Mougiou et al., 2020), by windbreaks to reduce wind chill, and applying appropriate irrigation techniques.

3.2 Biotic challenges

The phytosanitary problems of olive crop are one of the major constraints for the development of the olive industry worldwide due to its sensitivity to many biotic stresses, such as the olive leaf spot (*Fusicladium oleagineum*), the verticillium wilt (*Verticillium dahliae*) and the anthracnose (*Gloeosporium olivarum*); pests such as the olive fly (*Bactrocera oleae*), the olive psyllid (*Euphyllura olivina*) and the black cochineal (*Saissetia oleae*); and diseases of bacterial origin such as the Pierce disease (*Xylella fasitidiosa*), the crown gall (*Agrobacterium tumefaciens*) and the olive tuberculosis caused by *Pseudomonas savastanoi*p.

Olive fruit fly**.** In most producing regions, olive fruit fly (*Bactrocera oleae*, Gmelin) is considered the major biotic threat of the crop (Burrack et al., 2009). Adult females lay eggs inside the fruit where larvae feed on the mesocarp, grow and exit as a new generation adults after one to few weeks (Malheiro et al., 2015). *B. oleae* is a pest that feeds exclusively on *O. europaea* fruit (Daane et al., 2010) and therefore population burst may cause devastating losses in olive production through massive fruit drop and quality loss of the resulting oil. Economic losses are estimated at 15% of production with value around €1624 million annually. Farmers manage this pest by monitoring the adult population through traps and by various insecticides; such as spinosad, deltamethrin, and phosmet. To improve the sustainability of fruit fly management Decision Support Systems (DSS) have been also implemented (Miranda et al., 2019). Also, strong genotype-dependent variation has been reported, pointing to the significance of selecting more tolerant varieties (Garantonakis et al., 2016).

Verticillium wilt**.** It is a fungal disease that affects the olive tree's vascular system, causing wilting, yellowing of the leaves, and eventual death of trees. Olive is regarded as the most vulnerable tree host of *Verticillium dahliae*. Recent estimates of disease prevalence in Andalusia, Spain, the most important olive tree area in the world, showed that more than 50% of olive orchards were affected by verticillium wilt (Ruiz Torres, 2010). Farmers are managing this disease by planting resistant cultivars, e.g. 'Frantoio', by planting healthy trees, and avoiding contaminated soil.

Olive fruit moth (*Prays oleae*). The larvae of this moth feed on the fruit, causing the fruit to drop prematurely, or reducing oil quality. Farmers usually use pheromone traps to monitor the population and apply insecticides, such as methoxyfenozide and spinosad.

Olive leaf spot. Also called 'repilo', olive leaf scab or peacock's eye, is a devastating fungus developing at the epidermis of the leaf, resulting in circular lesions surrounded by yellow halos on the upper side (Buonario et al., 2023). Affected leaves become partially chlorotic, then necrotic, and drop prematurely, leading to a complete defoliation of the tree in the worst cases, resulting in a general weakening of the tree with subsequent remarkable yield reduction. The disease causes significant damage in most olive-growing regions in terms of both yield and oil quality. Management of the disease is based on the use of 'integrated' control measures including cultural practices (e.g. choice of the cultivation site, plant density, etc.), resistant cultivars and chemical control (e.g. copper compounds; Buonario et al., 2023).

Anthracnose (*Colletotrichum spp.*) is a fungal disease that affects the fruit, causing dark spots and lesions. Farmers can manage this disease by using fungicides, such as azoxystrobin and copper-based products, and by practicing orchard sanitation.

***Xyllela fastidiosa*.** The initial outbreak in the MB of the disease on olive trees, oleanders and almond trees was reported in Puglia, Italy, in 2013. The situation evolved very rapidly into an epidemic, expanding exponentially in olive groves. The strain responsible belongs to the *pauca* subsp. of *X. fastidiosa*. It is a quarantine bacterium that can infect more than 600 species, including major agricultural crops such as grapevines, citrus fruits, almonds and olives, as well as herbs and ornamental, forest and wild plants (Morelli et al., 2021). There are currently no curative measures against this bacterium, and only containment measures could be applied. However, cases of cultivar resistance have been reported recently.

4. 'Freeclimb' contribution: a summary of the project outcomes

The Mediterranean Basin was used as a living lab to determine the resilience of current olive cultivars to the major stresses and to advance the breeding work towards superior genotypes with combined traits of yield, fruit and oil quality, and stress tolerance. A rich pool of cultivars from four countries (Algeria, Greece, Morocco, and Tunisia) along with some cultivars of international importance (Spanish and Italian) was evaluated in multiyear experiments to classify the available germplasm for suitability in areas with unfavorable conditions such as heat stress, drought, and salinity. We also gained information regarding the olive cultivars that could better coexist with biotic stresses such as the olive fruit fly and *Verticillium* wilt. The Freeclimb project focused on genotyping and phenotyping of many olive cultivars maintained in several olive collections. A total of 28,135 SNPs, generated by the 'genotyping by sequencing' approach, were applied to study the olive genetic diversity of 187 cultivars: results showed a large genetic diversity. Similar results regarding synonymous cases were obtained with SNPs compared to SSR markers, as reported in previous studies. Also, results revealed a low linkage disequilibrium extent (almost 180 bp) resulting from the long history of olive and the high heterozygosity level of the species. Applying GWAS on traits related to leaves revealed a set of six loci on chromosomes 2, 5 and 9 as associated with leaf shape (length to width ratio) and leaf weight. The phenological study allowed clarifying the impact of the interannual variation of temperatures on flowering date and classifying mediterranean varieties according to their flowering date and chill requirements, while G x E interactions showed a variability of the flowering date according to the variation of temperatures between the two sites studied (Morocco and Greece). The effect of the high temperatures on pollen germination was also studied and both the germination rate and the pollen tube length were revealed to be highly cultivars-dependent. For drought and salinity tolerance, efforts were made to characterize several cultivars in different collections and results revealed some tolerant cultivars to both abiotic stresses with the aim of a possible use by farmers of these cultivars in the current climate change settings. At the end of the project, an informative document is available for stakeholders, ranking the olive cultivars studied for their tolerance to all these major stresses.

5. Stakeholders survey

Based on a survey with the participation of stakeholders from Mediterranean Countries the major needs in olive tree cultivars were for high fruit and olive oil yields combined with tolerance to local environmental stresses. In fact, there was an impressive variety of responses regarding the desired stress tolerance. Heat stress, frost, drought, and salinity were suggested in the case of abiotic factors. Olive fruit fly, *Verticillium* wilt, olive fruit moth, *Xylella fastidiosa, Cycloconicum oleaginym, Calocoris trivialis, Gloesporium olivarum, Zeuzera pyrina L.,* and *Pseudomonas savastanoi* were considered as the biotic factors for which the most desired tolerance is requested by the olive industry. When questioned about whether they would suggest exploitation of existing varieties or breeding for new genotypes, the respondents declared that all the available genetic pools for tolerance should be used to achieve the highest resilience to climatic challenges.

A core aim of this stakeholder survey was to identify which are the major stresses in each crop. In abiotic stresses, water deficit tolerance was the major risk (36.5 Combined stress risk index - CSRI) followed by heat stress (27.6 CSRI). Salinity and low temperature were the two subsequently important stresses with 14.2 CSRI and 13.07 CSRI, respectively. Water excess in the form of floods was also reported as a high risk with 8.6 CSRI (Figure 8). Olive fruit fly was suggested as the major biotic stress (50.7 CSRI), followed by *Prays oleae* with 35.5 CSRI, *Euphyllura olivina* (7.4 CSRI), and *Calocoris trivialis* with 4.2 CSRI. Other important pests were *Phoeotribus scarabeoides* (1.1 CSRI), *Saissetia oleae* (0.6 CSRI), and *Palpita vitrealis* (0.3 CSRI). *Verticillium dahliae* was identified as the major plant disease risk (33.6 CSRI), followed by *Cycloconicum oleaginym* (22.6 CSRI), and *Pseudomonas savastonoi* (15.3 CSRI). Other important disease risks were *Gloesporium olivarum (12.4 CSRI), root rot pathogens (7.9 CSRI), Pseudocercospora cladosporioides (6.3 CSRI), and* Xylella fastidiosa (1.9 CSRI): Figure 8.

There is a strong geographical variation between the priorities in olive tree genetic resources regarding resilience to stresses. For example, in Spain and some areas in Central and Northern Greece, *Verticillium* wilt is considered the most devastating disease while for Italy, *Xylella* is certainly the major threat. In the majority of Greek olive growing areas, olive fruit fly is by far the top concern for farmers while for Southern Mediterranean countries like Tunisia this is of minor importance. Regarding abiotic stresses, drought tolerance is certainly the top priority. This finding is reinforced by the fact that olive yield fluctuations during the recent decade are mainly driven by water deficiency in years with reduced rainfall. It is very encouraging that most stakeholders would be willing to participate in promoting/facilitating the evaluation of plant varieties for suitability in different environments. Through the project activities, a network of stakeholders was established for long-term collaboration in the search and exploitation of olive genetic resources with resilience to current and future challenges.

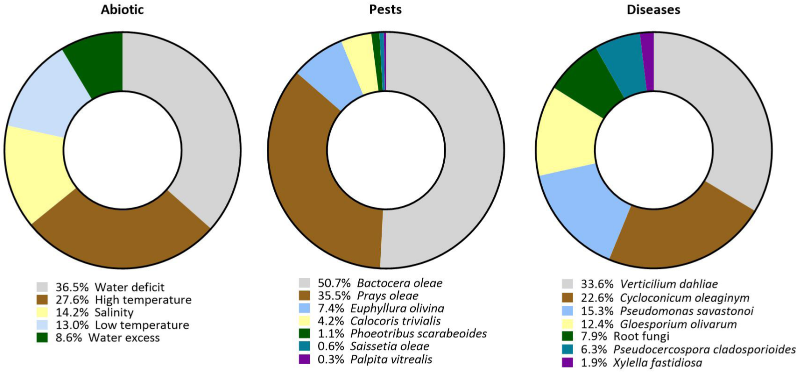

Figure 8. Stakeholders' opinion for abiotic, pest and disease stresses with the highest risk for olive growing. Combined stress risk index (CSRI) is presented based on the risk evaluation on a 1-10 scale and on the frequency of each stress suggested by the respondents.

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Prunus spp.

1. Main abiotic stresses

Among the environmental challenges affecting *Prunus* industry in the MB, the tree thermal requirements and the water requirement/management are the most crucial.

Chilling and heat requirement. Low-temperature damages on flower buds due to late Spring frost is one of the most serious risks for stone fruits production, particularly almond and apricot. In perennial temperate trees, lateral buds gradually shift into an endo dormant state in autumn, to protect against low winter temperatures. Endodormancy is characterized by growth inhibition after shoots stop growing and terminal buds set (Lang, 1987), although several studies have demonstrated that dormancy is an active phase in which structural and anatomic changes occur (Canton et al., 2022). After exposure to a certain amount of chilling the endo dormant buds shift to an ecodormancy status and are able to burst in response to growth-promoting factors, such as warm temperatures (Faust et al., 1997). Climate extremes predicted for MB will strongly affect tree phenology and physiology, particularly the increase of temperature. Winter warming is presently causing an advancing of blooming date and an acceleration of developmental processes (leafing and fruit maturity). Stone fruits enclose genetic material with a wide range of chilling requirements (CR). Nevertheless, the introduction of low-chill cultivars is particularly problematic for early flowering species, such as apricot and almond, exposing flowers to the risk of late frost damage, a major issue in North Mediterranean countries. The selection of late-blooming cultivars combining low CR and high-heat requirements (HR) to overcome ecodormancy is one of the most promising escaping strategies. In contrast to the well-established genetic component of CR and its well-known relationship with flowering time, the genetic control of HR has been controversial in stone fruits. However, the existence of a potential contribution of genetic factors to the HR trait has been suggested (Ruiz et al., 2007; Romeu et al., 2014). In almond, a major QTL regulating flowering time (the Lb locus) explains part of the variability for CR (Kester, 1965). In other *Prunus, e.g.* in peach and apricot, quantitative inheritance of CR for breaking endodormancy was also observed (Fan et al., 2010; Olukolu et al., 2009). In peach, a non-dormant mutant linked to a single recessive gene called Evergrowing (EVG) was mapped in a region containing six tandem-arrayed DAM (dormancy-associated MADS-box) genes, representing prime candidates for regulating growth cessation and terminal bud formation (Rodriguez et al., 1994). Seasonal expression of DAM genes in lateral buds of high-chill and low-chill peaches in field conditions shows that these genes are differentially regulated during endodormancy release (Li et al., 2009). Also, epigenetics factors seem to play a role in the control of dormancy, although still largely unknown (Rios et al., 2014).

Drought. Stone fruit cultivation largely occurs under irrigation regimes, requiring up to 7000 m^3/ha per year and weighing heavily on water reserves. Pursuing a more sustainable water use is a fundamental objective, particularly for peach in the most important production areas (Spain and Italy), located in semi-arid environments. Horticultural approaches based on ‘deficit irrigation’ are valuable water-saving strategies, although obtaining fruits with a non-commercial size represents a strong limiting factor, particularly in peach. Several *Prunus* species, including almond, are considered drought-tolerant, being able to tolerate water restriction. Among them, hybrid selections from almond (such as ‘GF677’) or other complex hybrids are commonly used as rootstocks in stone fruits due to their adaptability to heavy soils or drought conditions. However, in *Prunus*, knowledge on genetic and phenotypic variability associated with root functional traits as well as its relationship with whole-tree strategies to increase productivity under different drought conditions is still scarce. Recently, overexpression of peach gene *PpeDRO1* in plum led to deeper-rooting phenotypes, suggesting a potential role for *DRO1*-related genes to alter root architecture and improve drought avoidance and water use (Guseman et al., 2017). At scion level, responses to drought are reflected in physiological-adaptive mechanisms such as stomatal closure, reduction of cellular growth and photosynthesis deprivation (Rouhi et al., 2007). Several parameters have been established to assess drought tolerance, including water status, leaf hydraulic conductivity, stomata features and water use efficiency (WUE), although scarce information is available on scion performance at the orchard level (Rieger and Dummel, 1992).

2. Main biotic stresses

Brown rot. Among the plethora of pathogenic agents striking *Prunus* spp. crops, brown rot caused by *Monilinia* spp. is the major limiting factor (Hu et al., 2011; Oliveira-Lino et al., 2016), affecting either bloom and/or fruit: yearly value of worldwide losses hover around 1.7 thousand million euros. Identifying sources of resistance, always quantitative, is presently a major objective for breeding programs in different countries. Advances in terms of phenotyping and development of genetic studies are useful to foster breeding of more tolerant/resistant cultivars.

Powdery mildew. The causal fungus of powdery mildew (PM), *Sphaerotheca pannosa*, can affect leaves, twigs, and fruit of many stone fruit species. Various sources of resistance have been identified, including a single dominant locus, *Vr2* (Pascal et al., 2010; Pascal et al., 2017). However, PM resistance seems to be often quantitative and polygenic in *Prunus* spp., opening the opportunity of pyramiding major genes and QTLs already reported (*Vr3*, *qPM.SP-G6* and *qPM.PF-G7*) from *P. dulcis*, *P. davidiana* and *P. ferganensis* (Donoso et al., 2016; Foulongne et al., 2003; Verde et al., 2002) for durable resistance.

Sharka (*Plum Pox Virus*) is the most devastating viral disease on stone fruits, causing significant losses and no resistant cultivars have been found yet in peach (Rubio et al. 2012), except for a few promising tolerant selections currently under evaluation. Field tolerance could be regarded as a possible practical solution for the peach industry, due to the heritability of the trait found in its germplasm (Cirilli et al., 2016; Cirilli et al., 2017). Some sources of resistance to PPV in apricot were identified in wild material and are extensively exploited in breeding programmes (Decroocq et al. 2014). Also in almond reliable sources of resistance are available (Martínez-Gómez et al., 2004), other than an interesting mechanism of intraspecific transfer of resistance to peach by grafting, a possible tool to be further elucidated, in order to exploited by the industry (Rubio et al., 2013).

Capnodium (*Capnodis tenebrionis*) affects almost all *Prunu*s species cultivated under dry conditions, causing damage to the root system, leading to the death of the tree, or part of it.

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Almond
Prunus dulcis L.

1. Crop importance in the Mediterranean Basin

Almond (*Prunus dulcis* Mill. = *Prunus amygdalus* Batsch) is the most important fruit nut produced and consumed throughout the world. The crop is cultivated in more than 50 countries and its acreage and production is growing consistently every year, recording an increase of 196% in the last decades (25% only from 2017 to 2021), with a global production of 3.0 Mt to 2021 with 4 Mt (FAOSTAT, 2021). USA is leading the production with 54.8% of the global share, followed by the countries of the MB with 24.8%, Australia 7.2%, and other countries, mainly from Asia. It is important to highlight that in some countries, e.g. in the USA, almonds are cultivated under irrigation reaching productions of around 4 t/ha, while in most countries of the MB almond has been traditionally grown in drylands, yielding around 0.5 t/ha. In the Mediterranean area the crop has undergone significant changes in recent decades. Whilst in some countries production has substantially decreased, in others a new paradigm of almond growing is taking place, thanks not only to new cultivars, but to new rootstocks and training systems as well (Rubio and Cabetas, 2016).

2. Outline of the crop evolution until present: cultivars and orchard management

During almond domestication humans have been selecting for almond trees with sweet and large kernels. Early human communities collected almonds from more than 30 different species of Asian origin. These first pre-domesticated almonds were disseminated through trade routes from Asian countries to the Mediterranean Basin. During this period the sweet-kernel almond appeared, initially known as the 'Greek nut' (Gradziel, 2017). Subsequently, the cultivated almond was further spread to the East and to the West, representing the local cultivars or landraces that have been the ground for most of the modern almond breeding programs. The ancestor of the cultivated almond is still not identified. Nowadays, wild populations of almond species are spread geographically in south-west and central Asia, Turkey, Syria, Caucasus, Iran, Tajikistan, Uzbekistan, and Afghanistan (Browicz and Zohary, 1996; Denisov, 1988; Kester et al., 1991). Browiz and Zohary (1996) reported 26 species, clustered in different ways. Groups: *Communis* (nine species), *Orientalis* (six species); Sections: *Chamaeamygdalus* (four species), *Spartioides* (two species); and Subgenus: *Dodecandara* (five species). Some of the Mediterranean regions such as Turkish Anatolian are rich in wild almonds species as *Prunus nana, Prunus orientalis, Prunus turcomenica, Prunus arabica and Prunus webbii* (Ozbek, 1978; Kuden and Kuden, 2000; Ak et al., 2001). The protection of these genetic resources requires not only preserving action, but also their use in breeding programs. Besides, the proficient use of these genetic resources requires a clear understanding of their genetic diversity (Sehirali and Ozgen, 1987). *P. webbii* and *P. bucharica* show late blossoming and therefore, they are putatively resistant to late spring frost. *P. webbii, P. persica and related species* (e.g. *P. ferganensis*, *P. mira, etc.*) can be used as a source of alleles for self-compatibility. *P. webbii* and *P. argentea* can be used to modify canopy growth and structure (Kester et al., 1991). Therefore, almond wild species, some of them prone to extinction, should be collected for preservation as a source of useful genes that can help to cope with present and future challenges.

2.1 Cultivars

Between the middle of the 19th and the second part of the 20th century most cultivating-almond regions in the world that were had selected their preferred local cultivars, most of them coming from the Mediterranean Basin, e.g.: 'Desmayo Largueta' or 'Marcona' in Spain, 'Princess' or 'Ardechoise' in France, 'Cristomorto', 'Tuono' or 'Pizzuta d'Avola' in Italy, and finally 'Nonpareil' in California and later also in Australia. Although most of these local cultivars have been progressively abandoned, some of them are still grown and highly appreciated from the market, or have been used in modern breeding programs. In the last years almond breeding programs have focused on the development of self-compatible and late blooming cultivars. Self-compatibility, the main donor being the Italian cultivar 'Tuono', reduces the dependency from bee pollinators. Late blooming has been introduced to mitigate late spring frosts, the main donor of the trait has been the Italian cultivar

'Cristomorto', other than 'Primorsky', from the former USSR. However, since 'Nonpareil' has been largely used as a parent in USA and Australia breeding programs, the genetic variability of modern cultivars is being reduced as well (Pérez de los Cobos et al., 2021). 'Nonpareil' has been, and continues to be, the most important cultivar in California, while in the Mediterranean area the outlook is changing. Today, almond super high density (SHD) orchards mainly employ four recently bred cultivars: 'Soleta', 'Lauranne® Avijor', 'Penta', and 'Guara-Tuono' (Iglesias et al., 2021).

2.2 Rootstocks

Initially, due to the non-irrigated conditions of most almond orchards, almond seedlings, often bitter almonds, have been the dominant rootstocks for centuries, because their roots were more efficient in the extraction of nutrients and water from the soil. In the 1970s, the wide genetic diversity within the *Prunus* genus and the sexual compatibility between some species enabled the development of rootstocks via intraspecific or interspecific crosses. Although they were initially developed for peach, they also showed a very good performance with almond, and they started to substitute the almond seedlings as the main rootstocks in the Mediterranean regions (Rubio-Cabetas et al., 2017). The main rootstocks have been 'GF677' and 'Garnem' (Rubio-Cabetas et al., 2016; Felipe, 2009). In California and Australia, the main almond rootstock has been the peach seedling 'Nemaguard', mainly due to its resistance to root-knot nematodes (RKNs) in sandy soils. Nowadays, 'Guardian' and 'Viking' are also used in regions affected by bacterial canker and saline soils, respectively. Recently, modern almond orchards in the Mediterranean basin are moving towards intensification (high-density plantings), new cultivars, and drip irrigation systems, in order to achieve low harvesting costs, better use of water and fertilizers, and the effectiveness of phytosanitary sprays. High-density planting systems require smaller and more efficient canopies, with a significant impact on light interception and distribution of the canopy. To achieve these goals, new cultivars but also new rootstocks are essential (Socias i Company et al., 2009).

2.3 Environment and orchard management

Almond is well adapted to mild winter and dry and hot summer conditions, being early spring frosts and adaptation to different soilsl are the main limitations for its cultivation (Rubio Cabetas, 2016). The main production areas in the world are characterized by a Mediterranean climate and include, other than the Mediterranean countries, the central valley of California, the Middle east, central Asia, the Himalayan slopes and some regions from Australia, Argentina, Chile, and South Africa (Gradziel et al., 2017). In the Mediterranean and Asian countries, the most common production systems are characterized by low yield because of water scarcity, adverse soil and climatic conditions, and low-input crop management. However, in the Mediterranean countries, the rising profitability in recent years, due to the increase of the global demand, is leading to dramatic changes in the orchard management, by increasing irrigated areas, and the adoption of new training systems. Almond growing is facing many constraints that limit the extension of its cultivation and the appropriate scion-rootstock combination is an important factor for its adaptation to local conditions and specific training systems (DeJong et al., 2004). Understanding the mechanism resulting from the interactions among rootstock × scion × environments will contribute to the development of new cultivars and rootstocks able to withstand climate extreme events, but also diseases and pests and their possible increased aggressiveness (Vahdati et al., 2021). The most important factors influencing the planting design are the environmental conditions, the cultivar growth habit and vigor and the type of machinery that will be used for the orchard management (Arquero et al., 2017). In the past, the traditional low-density and the medium-high systems were the most popular (Felipe et al., 2017). The traditional system has a density of around 150 trees per hectare, with tree spacings from 8 × 8 m to 6 × 6 m. It is usually characterized by the use of vigorous rootstocks, no irrigation and almost no mechanization (Felipe et al., 2017; Sottile et al., 2014). In the medium to high density systems, instead, the density is between 250 to 700 trees per hectare, with tree spacings from 6 × 4 m to 5 × 3.6 m. Rootstocks must not be very vigorous and medium to high levels of mechanization are applied in order to carry on pruning and harvesting (pruners and trunk shaker machines) (Felipe et al., 2017; Sottile et al., 2014). Recently, very high-density planting systems with more than 1000 trees per hectare have been adopted. In these systems low vigor rootstocks are crucial and the degree of mechanization is very high.

3. Abiotic and biotic challenges (please also refers to the chapter 'Prunus spp')

Almond is exposed to several abiotic (drought, salinity and cold) and biotic (bacteria, viruses, and fungi) stresses (Yildirim et al., 2021), negatively affecting tree growth and yield. However, almond is commonly considered as tolerant to many of the stresses above, although climate extreme events could enhance its susceptibility.

3.1 Abiotic stresses

Almond is regarded as a good performer under environmental constraints, possibly due to the inheritance of traits from its wild ancestors. Because almond is one of the earliest flowering *Prunus* species, one of the main abiotic stresses is spring late frosts, and the main strategy to avoid it is the selection of late flowering cultivars. Another important abiotic stress is drought: although almond is considered a tolerant species its yield is strongly affected by the available water. Therefore, the selection of high tolerant rootstocks (and cultivars) and the application of strategies to increase water use efficiency is crucial in determining its profitability. Finally, low water availability or availability of low-quality water can increase soil salinity leading to a yield reduction, making tolerance to salinity an important selection factor in rootstock breeding programs (Doll, 2017).

3.2 Biotic stresses

Almond is affected by several pests and diseases, according to the different cultivation environments with different environments and/or agricultural practices. Some of them have been well known since a long time, while others are emergent diseases in some areas (Palacio-Bielsa et al., 2017). Some of the most common diseases are caused by fungi and can affect different organs like leaves, flowers, fruits, or roots and include brown rot blossom blight (*Monilinia* spp.), green fruit rot (*Botrytis cinerea*), almond anthracnose (*Colletotrichum acutatum*), red leaf blotch (*Polystigma amygdalinum*) or root and crown rot (*Phytophtora* spp.), among others. Regarding bacterial diseases the most common are bacterial spot (*Xanthomonas arboricola* pv *pruni*) and almond leaf scorch (*Xylella fastidiosa*). In the virus group some are transmitted by grafting, as prune dwarf virus (PDV), and others by insect vectors, such as the plum pox virus (PPV). Finally, the most important pests include root-knot nematodes (RKNs) (*Meloidogyne* spp.) that affect the rootstock, and the almond seed wasp (*Eurytoma amygdali*).

4. 'Freeclimb' contribution: a summary of the project outcomes

4.1 Genetic variability and association mapping

Almond and wild related species are native from the mountainous regions of Central Asia. In FREECLIMB, a collection of *P. dulcis* of 156 samples from fourteen countries maintained at INRAE Bordeaux and Avignon (France), IRTA (Spain), HAO (Greece) and CU (Turkey) was genotyped. The above collection was significantly extended by sampling from *Amygdalus* species, including accessions of wild relatives from Turkey, Caucasia, and Central Asia: *Prunus communis, P. orientalis*, *P. fenzliana* and *P. spinosissima*. Based on the fingerprinting of 23 microsatellite loci, the genetic diversity and subdivision of *P. dulcis* at the intraspecific level was documented, highlighting four differentiated clusters with contrasting geographical distributions, from Central Asia to North America, Europe included. One of the clusters, corresponding to *P. dulcis* from Akdamar island (Turkey), is particularly distinct from the others. The origin of this population is puzzling. It might have diverged a long time ago, first by population isolation followed by local adaptation. In Europe, two distinct genetic clusters were identified for cultivated almonds: one specific to Southern Spain and North-Africa and the second one, being present along the Northern shores of the Mediterranean Basin. About the almond wild related species, by Bayesian clustering with one of the four *P. dulcis* populations, we showed that *P. communis* is a feral form of *P. dulcis*. Our phylogenetic study also illustrated the relationship between the *P. dulcis* and its wild relatives: *P. orientalis, P. spinosissima* and *P. fenzliana*. This comprehensive analysis of almond genetic diversity will pave the way for the building of a worldwide core-collection. In addition to the study based on SSRs and including almond cultivars and wild accessions, we also explored the genetic structure and non-additive genotype-phenotype associations in a collection of 243 different almond accessions (including the collections from IRTA-GAFL and CRAG) that were genotyped with the new almond 60K SNP array (Duval et al., 2023). The genetic structure analysis strongly supported the subdivision of the accessions into 5 ancestral groups. All these groups were formed by accessions with a common origin. One of them was formed exclusively by Spanish accessions and the others were mainly formed by accessions from China, Italy, France, and the USA, respectively. These results agree with the archaeological and historical evidence that separate the modern almond dissemination into four phases: Asiatic, Mediterranean, Californian, and southern hemisphere. In total, we found 13 independent QTLs for blooming time, nut weight, crack-out percentage and double kernels percentage. This information can enhance the application of marker assisted selection in almond breeding programs.

5. Stakeholders survey

Half of the 11 respondents underlined the need for spring frost resistant cultivars, and most are in favor of breeding for new cultivars, although taking into account the existing cultivar array. One mentioned the need for resistance to *Fusicoccum* spp. When asked to detail the most important abiotic challenges, temperature extremes were the most cited, being Spring frost the most important one (31.8%), followed by high temperature waves in Summer, together with water scarcity (22.3 and 21.6%, respectively), while only 13.6% were worried for soil salinity (Figure 9).

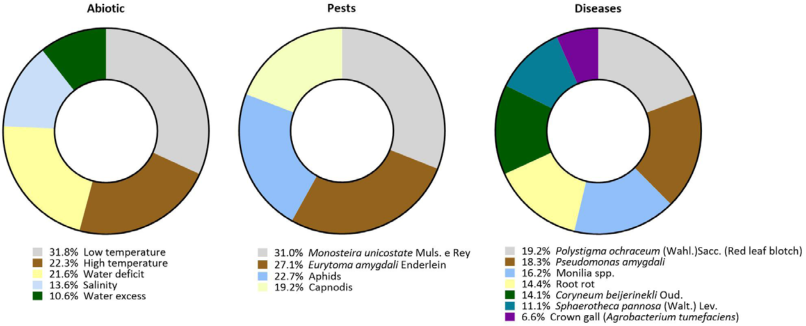

Figure 9 Stakeholders' opinion for abiotic, pest and disease stresses with the highest risk for almond growing. Combined stress risk index (CSRI) is presented based on the risk evaluation on a 1-10 scale and on the frequency of each stress suggested by the respondents.

Pointing out the most important challenging pests, about one third (31%) pointed out *Monosteira unicostate* Muls. e Rey, closely followed by *Eurytoma amygdali* Enderlein (27.1%), followed by aphids (22.7%) and *Capnodis* beetle (19.2%). About diseases, the most cited was red leaf blotch [*Polystigma ochraceum* (Wahl.)Sacc.] (19.2%), closely followed by *Pseudomonas amygdali* (18.3%), followed by *Monilinia*spp., root rot and *Coryneum beijerinekli* Oud. (16.2, 14.4 and 14.1%, respectively). Finally, all the stakeholders would be willing to participate in promoting/facilitating the establishment of cultivar field evaluation tests.

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Apricot
Prunus armeniaca L.

1. Crop importance in the Mediterranean Basin

In 2021, world apricot production amounted to about 3.65 Mt, with Turkey (0.80), Uzbekistan (0.42) and Iran (0.32) being the main producers, followed by Algeria, Italy, Pakistan, and Spain (FAOSTAT, 2023). In the MB, there are about 1,065 Mha of apricot cultivated areas, most of which are divided between Turkey (55%), Algeria (11%), Spain (8%) and Italy (7%), corresponding to 49%, 11%, 7% and 11% the of the MB total production, respectively (FAOSTAT, 2023): Figure 10. Worldwide, apricot is mainly intended for fresh consumption in national markets and only a small part (about 10%) is exported. Apart from fresh consumption, dried apricots are an important product in some Asian countries, Turkey being the first world producer with 0.1 Mt in 2016, of which 90-95% exported (covering about 60% of the world market). Interestingly, the production of dried apricots is covered by a few local varieties, the main one being 'Hacihaliloglu'. Processing to juice (or other processed products such as jams) has a role only in Italy, where apricot nectar (juice) covers about 8.1% of the European supply.

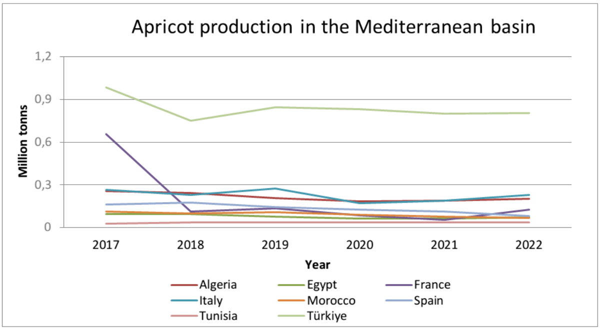

Figure 10. Production trend of the main Mediterranean apricot producing countries (FAO, 2023). Turkey production is divided by ten for for reasons of graphic representation

2. Outline of the crop evolution until present: cultivars and orchard management

Apricot (*Prunus armeniaca* L.) is a deciduous tree belonging to the Rosaceae family, which can reach 8-10 meters in height; erroneously believed to originate from Armenia by Linnaeus, the origins of the apricot date back to the Cenozoic, in central-northern China. Wild apricot species are still endemic to the Tian Shan Mountains in Central Asia. According to Kostina (1960), the Ice Age played a crucial role in the processes of natural selection of the apricot, differentiating cold-resistant wild species, such as *P. sibirica*, *P. mandshurica* and *P. mume*. Morphological studies suggest that the domestication of apricot began about 5000 years ago, in the Fergana valley (Central-Western Asia). The ease of obtaining new genetic material via seed, combined with vegetative propagation methods, have played a key role in the selection process. From Central Asia, the apricot would have been transferred to the Iranian-Caucasian areas and then to Europe, through the commercial routes (Vavilov, 1951), in particular the so-called 'silk road'. The processes of selection and diffusion have caused a loss of genetic diversity, according to a decreasing gradient from the center of origin to the areas of recent cultivation (Europe and North America). Recent molecular studies suggest the presence of two other secondary centers of origin, one Iranian-Caucasian (Iran and Turkey), and one Chinese, probably associated with hybridization with wild species such as *P. sibirica*. On the basis of some peculiar morphological characteristics, such as the size and composition of the fruit and the floral biology, Kostina (1960) distinguished four main eco-geographic groups:

(I) The Central Asian group (Xinjiang, Afghanistan, Pakistan and Northern India), more diversified, with self-incompatible accessions and high chilling requirements; in particular, the Central Asian and Xinjiang cultivars are very similar to the wild forms of apricot;

(II) the "Dzhungar-Zailig" group, mainly corresponding to the Dzhungarsky and Zailisky National Parks located in Tien Shan, between Kazakhstan, Kyrgyzstan and the northern part of Xinjiang Province of P.R. China, which includes self-incompatible accessions with small fruit size, between 15-30g, down to 3g in wild forms;

(III) The Iranian-Caucasian group, which includes accessions with low chilling requirements;

(IV) The European group, the most recent, which includes self-compatible accessions ranging in size from 10-160g and beyond.

In particular, the expansion of the apricot in the Mediterranean basin would seem to be attributable to two main routes, the first due to the Arabs, in the Middle East and North Africa, and the secon, through Hungary and Central Europe. However, the distinction between Northern and Southern Mediterranean accessions is not very clear, both pools being very similar at the genetic level (Bourguiba et al., 2020).

2.1 Cultivar

Apricot is a diploid species (n = 8), highly susceptible to varietal improvement, in particular for adaptability to the environment, disease and pest and fruit quality (size, firmness and flavor). The prospects for obtaining cultivars suitable for specific industrial uses (purées, dried, canned) are also very promising, also taking into account that in some countries, e.g. Italy, 30 to 50% of the entire production could be sent for juice, depending on the season. The genetic variability to be exploited is still large and many sources of potentially usable germplasm are already known and described, above all those coming from different areas of the Asian continent. For the fresh market there are very interesting reference accessions to be exploited for breeding. These accessions, often of North American origin (or derived from them) and widespread at the end of the last century, have highly colored fruits (intense orange, with a more or less extensive bright red overcolour), very attractive, sometimes even very firm. However, this material is not without limits, such as the skin astringency, a predisposition to fruit drop and, often, floral self-incompatibility. Furthermore, these fruits are unlikely to lend themselves to processing due to an excess of polyphenols or acidity, in particular from the skin. The most demanding challenge for breeding is disease resistance. For brown rot a double problem arises for flowers (and shoots) and for fruit, possibly not regulated by the same genes; still little is known about the sources of resistance, also due to the strong interference with the environment, and reliable markers have yet to be identified. About PPV, the present situation is much more positive, due to the availability of sources of resistance and the easy genetic heritability: the presence of only two loci meant to ensure resistance (Decroocq et al., 2014). In addition, reliable protocols are available for indexing and ascertaining virus resistance/

susceptibility, thus the introduction of resistant cultivars into the market is rather frequent. Ultimately, a considerable increase in the availability of new cultivars in the short and medium term (5-10 years) appears to be foreseeable, with improved characteristics as regards fruit quality (for both fresh market and processing) and yield reliability. However, the cultivation outside the currently known suitable areas appears more difficult, since the strong influence of environmental conditions on flower differentiation are difficult to control from a genetic point of view, and require that the selection phases be carried out in conditions similar to the environment where the new cultivar will then be adopted. Even the well known syndrome called ‘apricot decline’ seems closely linked to environmental adaptability.

2.2 Rootstocks

The development of *Prunus* rootstocks specifically meant for apricot has been relatively modest so far. While a relatively large number of rootstocks are potentially available, almost none are perfectly compatible with all cultivars. The main ones belong to a wide range of species, e.g. apricot, almond, myrobalan, peach, plum (several species) or their interspecific hybrids, e.g.: ‘Ishtara - Ferciana’, ‘Puebla 101’ and ‘Adesoto’. This diversity allows the cultivation of apricot in different soils, even marginal: from calcareous to rocky, from sandy to lime, from arid to humid. However, each rootstock should be previously tested on each cultivar before commercial introduction. Furthermore, since grafting incompatibility is very frequent in apricot and can show even many years after planting, the evaluation of a new rootstock should take place through field tests, which can last from 15 to 30 years. Bearing in mind the cost of these procedures, rapid methods have been developed to accelerate the selection process (Errea and Borruey, 2004), and the most effective are of histological or biochemical nature, even by the help of molecular markers. However, above all, the field test remains the most reliable one.

2.3 Environment and orchard management

Apricot species prefer hilly areas that allow cold and/or humid air to drain, to avoid damage from spring frosts or brown rot during flowering. Given its resistance to winter cold, higher than that of the peach and Chinese plum tree (*Prunus serotina*), it can also be cultivated in the plains or low mountains. However, in order to lower the Spring hazards, hilly regions are preferred, while at southern latitudes it can be cultivated in the plains, provided that the cold requirement is not limited, otherwise yield could be heavily affected by Spring drop of the buds and/or very prolonged flowering. The accumulation of chilling and heat requirements is mandatory in order to overcome endo- and eco-dormancy, respectively. Incomplete satisfaction of these physiological requirements in short or mild winters, can cause the inability to complete the flower buds differentiation, or their drop, also accompanied by poor or prolonged blooming (Fadòn et al., 2020).

A common tree growth habit classification in apricot identifies three main groups (Neri and Massetani, 2011): I) very vigorous tree, with open crown that bears fruit mainly on spurs, brindles and sylleptic branches; II) trees of medium vigor and upright or regular growth habit that bears fruit on shoots and vigorous fruiting branches; III) trees with diverse diverse growth habits bearing fruit on all types of branches. Therefore, specific training and pruning approaches should be taken for each growth habit. During the first year after planting, no interventions are envisaged on the scion, except for the elimination of vigorous shoots, competing with the main branches. The 3-D, or 'in volume' training system, i.e. the 'vase' and subsequent modifications ('late' and 'Catalan' vase), are to be preferred in the hilly area in order to facilitate operations from the ground. They develop on 4-5 primary branches, on which second order branches are inserted, leading to fruiting branches. In the 3-4th year, at the end of summer (August-September), the central part of the stem will be eliminated, heading back to the main branches. The 2-D, or hedgerow training systems, among which the 'palmette' should be mentioned, are to be adopted in the more fertile areas, both in the foothills or in plain areas, where late frost in Spring are more frequent. Being very developed in height, they require poles and wires and are therefore also compatible with roofing structures, to prevent damage from hail and/or pests that are difficult to control (e.g. *Halyomorpha halys*). They are prone to mechanization of pruning and thinning operations, their training being facilitated by the use of medium-low vigor rootstocks (e.g.: 'Ishtara Ferciana', 'Puebla 101' and 'Adesoto'). In all training systems, it is advisable to adopt the 'full top' pruning management (not heading back to the top of the stem). Summer pruning should be preferred in order to favor the development of the branches which will form the skeleton of the tree, other than lowering the risk of dieback due bacterial diseases favored by pruning woundings, very difficult to heal in winter. About the orchard tree density, it could be referred to the Peach chapter (*Orchard management*).

3. Abiotic and biotic challenges (please also refers to the chapter 'Prunus spp')

3.1 Abiotic challenges

In the temperate-continental regions, the main problems are due to low winter temperatures (a relatively rare phenomenon in this species), and to late frosts, to which the species is particularly sensitive due to its early flowering. Flower buds can stand up to -8/-12° C during endo-dormancy, while as bud development progresses, lethal temperatures rise from -5 (swollen buds) to -2° C in full bloom.

3.2 Biotic challenges

Other than unsatisfied thermal requirements of floral buds, yield can be affected by floral and grafting incompatibilità. In addition, floral anomalies such as the abortion of the pistil and/or the necrosis of the entire flower bud. Floral necrosis is not necessarily related to unsatisfied chilling, as it can also occur between the end of end-dormancy and the beginning of flowering. Other possible causes responsible for floral anomalies could be related to the availability of nutrients or the fruiting habit, linked to the position of the bud and/or the type of fruiting branch. The most important diseases are bacterial decay (caused by *Pseudomonas syringae*, pv *Syringae* and pv *Morsprunorum*) and yellow stone fruit (syndrome caused by the phytoplasma *European Stone Fruit Yellow Phytoplasma*: ESFY). Both lower yields from the first years after planting and must be faced with orchard management criteria aimed at preventing measures, starting from healthy propagation material and avoiding hìgh vigor due to excessive watering and nitrogen supply. Even the pruning season is critical, and must not be carried out in cold, humid and rainy seasons, i.e. possibly avoiding winter. Genome-wide association mapping approaches have been conducted to identify sources and components of resistance to the bacterial canker (*Pseudomonas syringae*) (Omrani et al., 2019).The brown rot due to *Moniliania spp*. blossoms still remain a limiting factor in many areas, especially for organic crop regimes (also in this case the counter measures are only preventive). Projections in future climate scenarios carried out by simulations made it possible to evaluate the interest of delaying the flowering in future cultivars to limit the impact of the disease on apricot (Tresson et al., 2020). Sharka caused by *Plum Pox Virus* (PPV): the use of nursery healthy material, and resistant cultivars (available today), are to be regarded as the main means for the effective containment of the virus. Being cultivated in very diversified environments, problems related to pests and diseases are often specific to the cultivation site.

4. 'Freeclimb' contribution: a summary of the project outcomes

The project focused on the characterization of germplasm for traits linked to disease resistance and environmental adaptation, also for the generation of new breeding material. Materials included both cultivated and wild apricots. Considering late frost damage and chilling satisfaction crucial threats for the apricot Mediterranean industry, Italian, Algerian and Greek cultivated apricot germplasms were screened for early/late flowering, thermal requirements (chilling and heat), self-compatibility and fruit quality; also, local Turkish materials even for tolerance to lime-induced chlorosis and drought (to be used as rootstocks); sources of tolerance/resistance to Sharka, bacterial canker and shot hole disease were identified in wild or cultivated apricots from a French collection, representing important pre-breeding sources for future programs. Available germplasm was genotyped by different approaches: whole-genome re-sequencing (wild and cultivated apricot from the French collection), Single-Primer Enriched Technology (the Italian collection) and SSR markers (the Greek and Algerian collections). QTLs controlling bud breaking, early flowering, maturity date and Sharka disease resistance have been found in both cultivated and wild materials, providing important insight on their genetic bases. A marker assisted selection (MAS) approach has been validated for selecting flower self-compatibility, a crucial trait to mitigate late frost damage. Finally, several crosses have been made to combine low chill and high heat requirements and self-compatibility or to transfer disease resistance in cultivars with high horticultural and pomological value, representing important pre-breeding sources for future programs.

The project has launched the design and construction of an ApricotRefPop multi-site collection to be shared among Italy, Greece and Algeria, to be used for the future dissection of Genotype-by-Environment interactions for crucial traits.

5. Stakeholders survey

Most of the nine stakeholders responding to the survey underlined the need for disease resistance cultivars (Sharka disease and brown rot on bloom), followed by summer rainfall tolerance (causing fruit cracking) and frost hardiness, and above all needed cultivar requirements. Indeed, when asked about whether they would suggest exploitation of existing accessions or breeding for new varieties, they were in favor of either solution in order to tackle present and future needs. When asked to detail the most important abiotic challenges, temperature extremes were the most cited, being freezing the most important one (31.8%), followed by heat waves and excessive summer rainfall (23.6 and 23.2%, respectively: Figure 11). Water deficit and salinity followed.

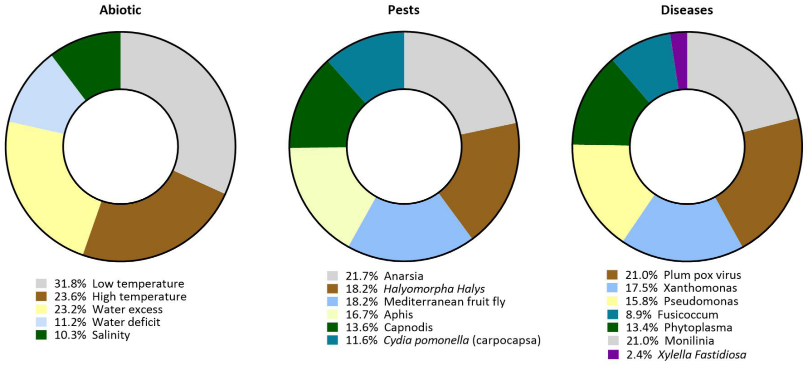

Figure 11. Stakeholders' opinion for abiotic, pest and disease stresses with the highest risk for **apricot** growing. Combined Stress Risk Index (CSRI) is presented based on the risk evaluation on a 1-10 scale and on the frequency of each stress suggested by the respondents.

Among the most challenging pests, the most cited were Anarsia lineatella (21.7% of the questionnaires), shortly followed by Halyomorpha Halys and

Mediterranean fruit fly (both at 18.2%): Figure 11. Then aphids, Capnodis tenebrionis and Cydia pomonella were the other not less important pests reported.

About diseases, the most cited were *Monilinia* spp (on bloom) and Sharca (PPV): 21.0% (Figure 11), followed by *Xanthomonas* spp., *Pseudomonas* spp. and phytoplasma (17.5, 15.8 and 13.4, respectively). *Fusicoccum* spp. and *Xylella fastidiosa* were reported as well. Finally, all the stakeholders would be willing to participate in promoting/facilitating the establishment of cultivars field tests.

6. References

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Peach
Prunus persica L. (Batsch)

1. Crop importance in the Mediterranean Basin

Currently, there are approximately 24,6 million metric tons of peaches produced yearly worldwide, with China accounting for almost 60%, followed by the EU (Spain, Italy, and Greece) at 15% and the USA with approximately 3%. A significant growth of production over the last 20 years was monitored due to advancements on fruit production systems. The peach production in the Mediterranean Basin has been relatively steady, since Greece and Spain showed a gradual increase in production (FAO, 2023) compensating for Italy's decline. Spain comes first in the MB, with 1.2 Mt on 72,000 hectares, primarily in the more northern areas of Aragon, Murcia, and in the southern areas of Catalonia. Italy follows, with 0.99 Mt produced on approximately 56,000 hectares, mostly in the northern regions, especially Emilia-Romagna (the country's top producing region), and Piedmont, other than in more southern areas, primarily Campania. Turkey is third, with 0.89 Mt on 50,000 hectares. Greece is in fourth position with 0.591 Mt on about 38,000 hectares; the bulk of the production is in the northern part of the country. Egypt comes fifth with 0.24 Mt on 13,000 hectares (Figure 12).

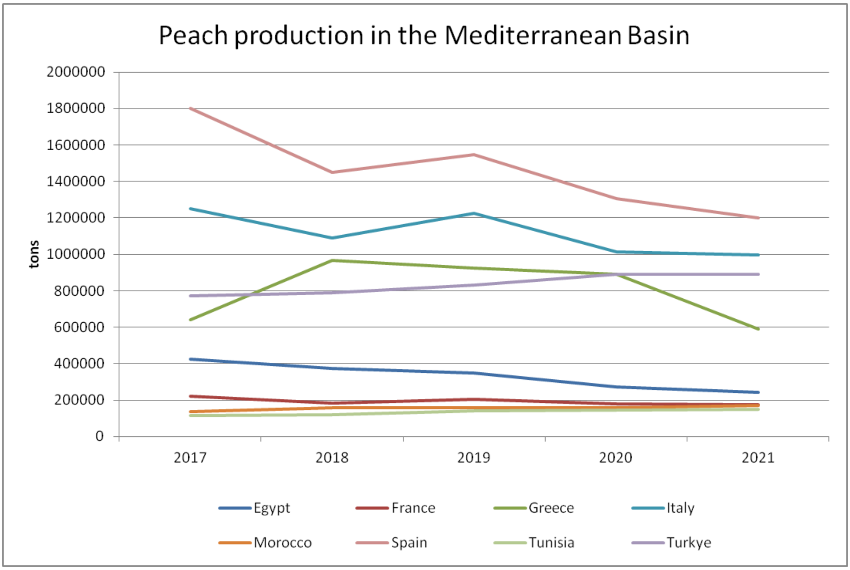

Figure 12. Production trend of the main Mediterranean peach producing countries (FAOSTAT, 2023).

2. Outline of the crop evolution until present: cultivars and orchard management

2.1 Cultivars

The domestication of the peach tree dates back to around 3000 BC in China. Its arrival in Europe may have occurred through the Silk Road, where it spread throughout the Ancient Greek and Roman Empire, as reported by early Latin authors (Faust and Timon, 1995). After the fall of the Roman Empire, in the Middle Ages peach tree cultivation mainly survived in monastery gardens, probably saved by king Charlemagne, among others unknown amateurs. It reappeared during the Renaissance period in the noble *horti* of France, England and Italy: from the end of the sixteenth century, pomologists and painters began describing the cultivars preserved in the '*pomari*', Italian old style for 'gardens', of noble villas. Later on it was exported to the New World by Hispanic colonizers. But it was at the beginning of the 20th century the peach 'modern' breeding began (in the

USA first), with the establishment of thousands of cultivars, at a rate of over a hundred per year in the last few decades. The two main objectives pursued since the middle of the 20th century, in addition to improving the aesthetic and commercial characteristics of the fruit, were i) the widening of the ripening calendar, given the low correlation between flowering and ripening times, and a fruit development period between 55-60 and over 250 days from full bloom to maturity; and ii) the low chilling requirement, which made it possible to expand the crop to increasingly wider climatic regions, e.g. from Ontario (Canada) to the subtropics (Sansavini et al., 2006). The improvement of fruit appearance, particularly the extent of skin color, size and shape, is evident when observing the modern large-sized, symmetrically-round, fully red colored peach and nectarine cultivars (Bassi and Monet, 2008). For flesh color, other than yellow and white (the latter being dominant), there are two independent 'blood flesh' traits, one being dominant (Shen et al., 2013) and the other recessive (Werner et al., 1998), characterized by the abundant accumulation of anthocyanins in the mesocarp, associated with either white or yellow flesh. However, the 'blood flesh' has currently no commercial relevance, except in some niche markets.

At the end of the 20th century, a real milestone was achieved to facilitate harvest, handling and postharvest operations with the introduction in the market of a new fruit texture. In addition to the already well known three flesh texture phenotypes, i.e.: *melting* (M), *non-melting* (NM) (Bailey and French, 1932 and 1949) and *stony hard* (SH) (Yoshida, 1976), the so called '*slow softening*' (SwS) flesh was introduced and then described as a putative mutation of the M type (Ciacciulli et al., 2017). The M-type flesh is characterized by an initial slow firmness decrease followed by a rapid softening (melting phase). The NM flesh, unlike the M, retains a firm flesh even at full maturity and does not soften, although it becomes rubbery when losing water. The SH is a monogenic recessive trait and is regulated by the *Hd* locus (Scorza and Sherman, 1996). The trait is epistatic (non-reciprocal influence between non-allelic genes) to the *M* locus, the allelic status of the latter in heterozygous genotypes being detectable only after treatment with ethylene (Haji et al., 2005). The SH texture is very firm and crunchy but in practice not easily distinguishable from the NM type. The real difference found so far is of a biochemical nature since this fruit, unlike NM, does not produce ethylene and can hang on the tree for several weeks after physiological ripening (stage not easy to be assessed). Finally, the SwS type (putative *SwS* locus), is a genetically dominant variant of the M type (Ciacciulli et al., 2018), behaving similarly to the M phenotype, but softening at a slower pace, thus facilitating field and postharvest handling, and shelf life as well.

One relevant market success in terms of cultivar development, at least in Europe (notably in Spain), was the commercial exploitation of the fruit flat shape, genetically dominant over the round or elongated one (the latter, dominant over the round). Breeding programmes for flat peaches only started at the end of 1980s with 'Stark Saturn' (released in the USA), followed by France (noteworthy releases were 'Platina' and 'Mesembrine'), Italy ('UFO' series), China ('Ruipan' series).

The weight of the fruit can vary from less than 50 g in wild peach relatives to 80-110 g for very early cultivars, and up to around 700 g for the late ripening accessions. Although the commercial success of a peach is related to (large) fruit size, there might be some commercial interest in small sizes, like those developed in apple, which are easy to handle, show an attractive appearance, are very high flavored with a firm flesh, and could be ideal as 'snack' fruit.

Innovating companies that are reducing production cost, selecting more flavorful cultivars, selling pre-ripened fruit for improved eating quality, are still profiting from producing fresh peaches. The current approach for increased consumption includes releases of white and yellow-fleshed peach cultivars with improved flavor, including high total soluble solids (TSS), high aromatic profile, desirable texture, attractive appearance. Low-acidity (dominant genetic trait) combined with high TSS and flat shape (easy to eat) cultivars, have been launched from active breeding programs around the world (Iezzoni et al., 2020). These new cultivars represent the continuing efforts in providing improved flavor with enhanced nutritional and eating qualities, with recent consumer's feedback being very promising.

2.2 Rootstocks

About rootstocks, until the introduction of the clonal ones, the dominant was the peach seedlings, selected or of unknown origin. In terms of grafting compatibility and regula yield, it is the ideal stock (some of them are even resistant to some nematodes) but it is extremely sensitive to iron chlorosis, root asphyxia and problems associated with replanting.

Therefore, several alternatives have been proposed over time, including those from different species of plum. However, despite lowering the vigor of the scion and being tolerant to the replanting syndrome and/or to root asphyxia, may show problems such as high suckering or insufficient yield. A further genetic source of rootstocks is coming from hybrids (even complex ones) between peach and related species: the one that has achieved by far the greatest success is the hybrid peach × almond tree 'GF 677'. Other interesting hybrids stocks are: Adesoto® 101 Puebla*, Garnem and Ishtarà® Ferciana*.

2.3 Environment and Orchard management (Bassi et al., 2022)

Peach is one of the most studied fruit crop species where the innovation of the training systems and of the orchard design has led to a profound transformation over time. This can be explained both by its plasticity and early fruiting, which has stimulated technicians and researchers to study new solutions, both to anticipate the achievement of the full yield of the tree, thus increasing the orchard profitability. The evolution at the nursery and training techniques allows economically viable productions even in the year of planting, a possible goal which however should not be a priority, as it is necessary first to take care of obtaining an adequate scaffold structure. In fact, since very often its tree shows high vigor, the fructification in the year of planting is used as a natural 'brake' to lower vigor, to avoid unnecessary pruning that could stimulate the vegetative growth ('recall to wood') to the detriment of fruiting, as well as increasing the production costs. When considering the orchard design, it goes from very low density (400 trees/ha), to medium (400-800), high (800-1,500) and very high (1,500-2,500).

Given the great plasticity of the species, the choice between the various solutions is essentially linked to the mechanical equipment of the farm, other than to the soil type, the availability of manpower and the technical knowledge of the farmer. As a general reference, about the most common training systems, it can be referred to the Apricot chapter (*Planting and training systems*). The exploitation of the peach growth habit diversity has not parallelled what has been done in other fruit crops, e.g. with the spur habit in apple and sweet cherry in modern orchards. In peach, other than the standard habit (the only one commercially exploited), other growth types are known, the most diverse being pillar, compact, weeping and dwarf

(Bassi et al., 1994). While some attempts may have been made, there are no reports of using any of these growth forms to create novel orchard designs, and very few plant physiology studies have been carried out on them. One exception was a columnar growth habit cultivar 'Alice Col', which was used in the 'Asymmetric Peach Orchard', an experimental site at the University of Bologna that lasted over a decade (Losciale et al., 2010). This orchard featured rows of varying orientations: N-S, NE-SW, NW-SE. In addition, some of the rows were also inclined by 35° from vertical. This design allowed to attain three profiles of daily light interception which differed as to the time of day when the canopies were at highest light interception, hence the name 'asymmetric'. 'Alice Col' was selected specifically for its growth habit, which was expected to simplify canopy management, in particular pruning, to maintain the rows in their expected shape. Intercrossing phenotypes with different crotch branch angles and internode length can be used as a strategy to produce tree forms adapted to specific training systems, thus allowing for better orchard management.

Continued hybridization and development and testing of peach tree growth habits may increase productivity of commercial peach orchards and even expand use of the peach as a garden fruit crop or as an ornamental species (Scorza et al., 2002). The development of commercially novel tree growth forms could combine other interesting morphological traits, i.e. the 'narrow' (or 'willow') leaf (Okie and Scorza, 2002), that allow better light penetration within the canopy with obvious advantages for bud differentiation and increased fruit quality. In addition, the 'narrow' leaf has higher water use efficiency than the standard width leaf phenotype (Glenn et al., 2000).

3. Abiotic and biotic challenges (please also refers to the chapter 'Prunus spp.')

3.1 Abiotic challenges

Environmental adaptation, together with yield stability, are major concerns for the peach industry and have been cornerstones of all breeding programmes, taking advantage of the remarkable plasticity of the peach species. In the subtropics, yield performances have been improved due to a century of low-chill cultivar selections. The ‘honey’ and *peento* (i.e. flat) peaches from southern China (e.g. ‘Peento’ and ‘Lukens Honey’, probably from seedstocks imported from Australia) were the main source of low- or medium-chill traits in the early breeding programmes started in California in 1907 (Hume, 1902). The first important release of the Californian programme was ‘Babcock’ in 1933, a 350 Chill Units, white-fleshed, low-acid and freestone peach still cultivated until 1990. Since 1907, about 475 low and medium chill cultivars have been released from various programs with a trend of about 15 per year since 2010 (Byrne, 2014). These efforts have led to the availability of cultivars adapted for a wide range of low to medium chill zones. Interesting, the longest running low-chill programs of UF/IFAS (Florida), ARC-Infruitec (South Africa), EMBRAPA (Pelotas) and IAC (São Paulo) share a few founding accessions in their genetic background, such as ‘Peento’, ‘Okinawa’ (a nematode-resistant rootstock introduced in Florida from the Ryukyu Islands in 1953), ‘Hawaii’, and in Brazilian programs some local cultivars originating from non-melting, yellow-fleshed clingstones peaches introduced to the New World by Spanish and Portuguese explorers in the 16th century (Byrne and Bacon, 1999). To be mentioned, successes from the University of Florida (USA) included ‘Flordaprince’ (1982) and ‘Tropic Beauty’ (1988) peaches with less than 150 chilling hours (CH) and the UF series of non-melting peaches, with a range of 100 - 200 CH. Despite the huge improvement of low-chill cultivars, breeding activities are still hindered by the scarce knowledge on the physiological, biochemical and molecular mechanisms behind the processes of flower bud dormancy, particularly endo-dormancy to eco-dormancy transition and blooming that are dependent on chilling requirement (CR) and heat (HR) requirement satisfaction. In low-chill regions, daytime winter temperatures are often in the chill negation range, suggesting the dynamic model is more effective (Allan et al., 1995). Also, HR measurement through GDHs has been questioned, since it assumes that basal temperature and the response curves do not vary across cultivars/species, while there is evidence of genetic diversity for both traits (Bielenberg and Gasic, 2022). The increasing incidence of late frosts places more emphasis on the characterization and eventual selection of HR-associated traits to delay bloom without increasing CR. Moreover, CR and HR measurements require expensive and time-consuming ‘forcing’ tests, which are not feasible to be incorporated into a breeding programme, though the use of molecular tools (MAS, GS, etc.) is a promising solution. However, progress in the characterization of genetic bases of CR and HR (Romeu et al., 2014; Bielenberg et al., 2015; Cirilli et al., 2021) is still insufficient to be routinely incorporated in assisted selection of these traits.

3.2 Biotic challenges

Less advancement has been achieved in breeding for disease and pest resistance. Despite the renowned Mendelian sources of resistance for green aphid (Pascal et al., 2002; Lambert et al., 2011; Pascal et al., 2017), nematodes, powdery mildew (Monet and Bassi, 2008) and leaf curl (Ritchie and Werner, 1981), others disease are still major threats for the industry, e.g. bacterial leaf spot (*Xanthomonas* spp.), brown rot (*Monilinia* spp.) and Sharka (*plum pox virus*, PPV), depending on the cultivation region. For brown rot, sharka (PPV) and powdery mildew, see chapter *Prunus* spp)

Monilinia spp. causing fruit brown rot (BR) is, among the pathogenic fungi of fruit trees, particularly worrying in peach. BR can only be controlled by chemical treatments (Oliveira Lino et al., 2016, 2020 and 2022). Efforts have been made to i) develop phenotyping methods (reviewed in Mustafa et al., 2021) to study progenies and collections to try to map resistance factors, ii) to progress in plant-pathogen interactions and iii) to identify fruit defense mechanisms and compounds with antifungal potential (Dini et al., 2023) further tested by in vitro experiments (Mustafa et al., 2023). In addition, horticultural levers, such as fruit thinning and modulation of irrigation, must be explored (Bellingeri et al., 2018) to be associated with non-complete cultivar resistance. Using models to simulate the progression of brown rot epidemics in stone fruit orchards can help to identify efficient practices (Bevacqua et al., 2018).

Bacterial spot, also known as bacterial leaf spot (BLS) and bacterial shot hole, infects most *Prunus* species but causes significant economic losses on peaches, although not so common in the Mediterranean Basin. BLS is caused by *Xanthomonas arboricola* (syn. *campestris*) pv. *pruni* (Smith) and affects leaves, fruits and twigs (Ritchie et al., 2008). Lesion centers become purple and necrotic and, if centers abscise, leaves develop a shot-hole, tattered appearance. On highly susceptible cultivars, multiple years of severe premature defoliation result in reduced numbers of fruit buds, reduced fruit crops and weakened trees. Fruit symptoms are first visible as small, angular, water-soaked lesions 3–5 weeks after petal fall. BLS lesions on fruit may be confused with peach scab lesions, which are darker in color, circular and usually more restricted to the surface of the peach skin. Peach cultivars vary greatly in susceptibility and the most effective control is through the use of host plant resistance (Werner and Ritchie, 1986). Chemical controls have shown limited efficacy but are most effective when management programmes are started before the infection of newly emerged leaves and fruits occurs, with success depending on weather conditions and disease pressure.

Peach constriction canker, also known as *Fusicoccum* canker, is a disease caused by the fungus *Phomopsis amygdali* (Del.) Tuset & Portilla, previously known as Fusicoccum amygdali Del. (Adaskaveg et al., 2008). Symptoms develop primarily on twigs and leaves. Typically, stem infections develop on 1-year-old shoots as reddish-brown or pale brown, sunken, elliptical lesions around buds or nodes. This is in contrast to cankers caused by brown rot blossom blight, which develop from infected blossoms. Lesions of constriction canker also exude some gum and sometimes can be confused with brown rot infections, but the latter disease causes more profuse gumming. The fungus produces a toxin (fusicoccin) that is distally translocated from twig cankers and contributes to leaf wilting and yellowing on blighted twigs. Peach cultivars differ widely in susceptibility to constriction canker. Thus, planting of less-susceptible cultivars is crucial in establishing an orchard in areas where the disease is a problem.

Peach leaf curl is caused by *Taphrina deformans* (Berk.) Tul. Symptoms occur mainly on new leaves in Spring. Leaves, first developing discolored areas that thicken and become wrinkled, causing the leaves to curl. Infected leaves can have a range of colors from light green to yellow, red and purple (Adaskaveg et al., 2008). Treatments after infection or symptom development are ineffective. Sanitation and cultural practices do not provide control against this disease. Most cultivars are susceptible to the disease, but there is a wide range of susceptibility. Although peach leaf curl can be rather easily managed with one well-timed preventive fungicide application, either in late autumn after 90% of the leaves have fallen or in spring before bud swell, could be a problem for organic cultivation. The genetic resistance, likely of Mendelian origin (Monet, 1985; Svetaz et al., 2017), is a useful tool for the breeders.

4. 'Freeclimb' contribution: a summary of the project outcomes

The most crucial limitation to further improves the environmental adaptation and biotic stress tolerance is the lack of knowledge on many traits linked to resilience (water availability, extreme temperatures etc.) or the complex introgression of disease resistances from wild or obsolete materials. The multi-site peach reference collection (*PeachRefPop*) across different European countries has been the first effort to build this knowledge and to facilitate the dissection of G x E (and/or by management interaction) (Cirilli et al., 2020). In FREECLIMB, activities were focused on application of this powerful tool (the PeachRefPop) to dissect traits linked to reproductive phenology and adaptation to drought conditions, providing a first example of its potential and usefulness to build novel knowledge and improve breeding and exploitation of germplasms resources. Efforts were also made to characterize traits linked to disease resistance (such as brown rot), although its complex quantitative inheritance (affected by environmental conditions and year-to-year variation) remains a strong limitation for breeding exploitation. Local Turkish materials have also been screened for tolerance to lime-induced chlorosis and drought, with the aim of a possible use as rootstocks.

5. Stakeholders survey

Most of the 15 stakeholders underlined the need for cultivars improved for resistance to both abiotic and biotic stresses, while very few highlighted yield or fruit quality as major requirements. When questioned about whether they would suggest exploitation of existing accessions or breeding for new varieties, most of them were in favor of both solutions in order to tackle present and future needs. When asked to detail the most important abiotic challenges, temperature extremes were the most cited, being Spring freezing the most important one, followed by heat stress (27.5 and 24.8, respectively: figure 17. Water excess was shown as important as drought (18.6 vs 17.7, respectively), while salinity was at the last place (11.5% of the questionnaires): Figure 13.

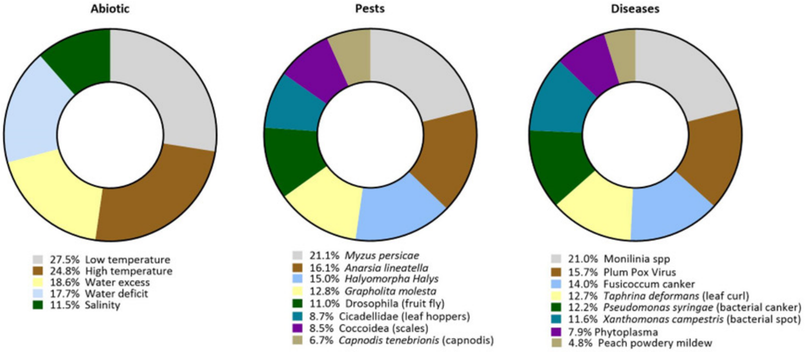

Figure 13. Stakeholders' opinion for abiotic, pest and disease stresses with the highest risk for peach growing. Combined Stress Risk Index (CSRI) is presented based on the risk evaluation on a 1-10 scale and on the frequency of each stress suggested by the respondents.

About the most challenging pests, surprisingly *Myzus persicae* was the most cited (23.2%), showing the extent of this aphid's spread across peach growing regions, even more than leafhoppers (*Cicadellidae*), *Anarsia lineatella*, *Halyomorpha halys* and *Grapholita molesta* (20%, 17.6, 16.5, and 14.0, respectively). Scales and *Capnodis tenebrionis* followed. About diseases, the most cited were brown rot caused by *Monilinia* spp, followed by Sharka (Plum Pox Virus): 21.0% and 15.7%, respectively, followed by *Fusicoccum* spp., *Taphrina deformans* and *Pseudomonas* spp. *Phytoplasma spp.* and powdery mildew were the least cited. Finally, all stakeholders would be willing to participate in/promoting/facilitating the establishment of variety field tests.

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Peach x *Almond* hybrids

1. Crop importance in the Mediterranean Basin

Interspecific crosses between different *Prunus* species have been very important for almond, peach, apricot and plum in the Mediterranean Basin. In fact, as we have seen in the previous chapters, many commercial rootstocks have been obtained crossing different *Prunus* species. Some examples include the rootstock 'GFF677', developed from an interspecific cross between peach and almond, widely planted in Europe, or 'Cadaman' and 'Barrier', that were obtained from an interspecific cross between peach and its wild relative *P. davidiana*. Rootstock breeding has been traditionally incorporating new traits for biotic and abiotic stress tolerance for a long time, allowing the development of many stocks adapted to different climatic conditions and soil types. On the other hand, the impact of interspecific crosses for cultivar development has been almost null, except for the development of many plum cultivars and from interspecific crosses between apricot and plums. Most commercial plum cultivars are, or carry in their background, interspecific hybrids between *P. salicina* and at least other 12 diploid plum species that were obtained by Luther Burbank between the late 19th and early 20th centuries. Plums have also been crossed with apricots obtaining different fruit typologies as the 'black apricots', a cross between apricot and *P. cerasifera* traditionally grown in southwest Asia, or the most recent 'pluots', 'plumcot' or 'apriums', that are interspecific crosses between apricot and *P. salicina,* developed in the USA, now being introduced in some countries in the Mediterranean Basin as Italy Spain (Guerrero et al., 2022).

The underutilization of wild *Prunus* species in stone fruit breeding is mainly explained by the fact that many interesting genes are found in germplasm with a very low fruit quality, and linkage drag of undesired traits makes the breeding process long and resource consuming. That is a very important limitation because many resistance genes to biotic and abiotic stress are not found in cultivars but in wild species (Marimon, 2020; Gradziel et al., 2022). In addition as source of biotic and abiotic stress resistance genes, *Prunus* wild species could also hide other interesting genes that could have an impact on the yield, fruit quality or fruit diversification, and could also help to mitigate the problem of the narrow genetic base of commercial cultivars present in peach and that is also a problem in almond (Pérez de los Cobos et al., 2021).

The development of new technologies and strategies derived from genomics and biotechnology are allowing the introduction of new genetic variability coming from other species or from germplasm with low fruit quality, more efficiently and faster. One example is the marker assisted introgression (MAI), a new strategy based on the selection of the whole genome using molecular markers, designed to rapidly introgress genes from exotic germplasm into elite peach cultivars in an efficient way (Serra et al., 2016). This strategy is based on the marker assisted selection, from a large BC1 population, of few individuals (15-30) with few introgressions from the exotic donor into the elite cultivar background. The introgressions obtained in those individuals should cover the whole genome of the donor species. These plants, called pre-introgression lines (or prILs), are then phenotyped for the traits of interest, while genetic analyses are carried out to identify the genomic regions carrying the genes of interest. Finally, lines with only one introgression carrying the gene of interest are developed to be used as pre-breeding material or directly as new cultivars. Alternatively, a whole collection of ILs covering the whole genome of the donor species could be developed to study quantitative traits.

2. Abiotic and biotic challenges

Wild species have been adapted to very different environments, including several biotic and abiotic stresses. For this reason, interspecific hybridization has been an important tool for crop diversification, even during domestication (Purugganan, 2019). Thus, trying to recover all this available genetic variability is a very promising breeding approach in a scenario of climatic extreme events.

The first interspecific hybridizations in *Prunus* focused on resistance to pest and diseases, especially for the root knot nematodes resistance genes identified in *P. cerasifera*, *P. davidiana*, *P. dulcis*, *P. kansuensis*, *P. mira* and *P. salicina*. Other pest and diseases tolerant or resistant genes identified in wild *Prunus* species and that affect the scion include, between others, peach powdery mildew (*P. davidiana*, *P. dulcis*, *P. mira*), brown rot (*P. dulcis*), sharka (*P. davidiana*, *P. dulcis*) and green peach aphid (*P. davidiana*) (Marimon 2020; Gradziel, 2022).

For abiotic stress no major genes have been already identified but several interspecific rootstocks or wild *Prunus* species are known to be more tolerant to some problems as calcareous soil, as the peach x almond interspecific rootstock ('GF677'), or to high drought stress as *P. petunnikowii*. Recently, a major gene related to the photosynthetic stem capability (PSC), that could help to better cope with high winter temperatures, has been identified in the wild almond *P. arabica* (Brukental et al., 2021).

3. 'Freeclimb' contribution: a summary of the project outcomes

In the FREECLIMB project, two main activities related to interspecific crosses have been developed. On one side, an F2 population from a cross between peach (cultivar 'Honey Blaze') and almond (cultivar 'Del Cid'), has been studied for its resistance to sharka disease (PPV), resulting in the identification of two main QTLs located in linkage group 3 and 8. Other sources of sharka resistance have also been identified in other species as *P. fenzliana* (Tricon et al., 2023). On the other side, several interspecific progenies of peach by almond and peach by *P. davidiana* crosses were developed for the application of MAI strategy in peach. In the first case the first collection of ILs ever in a tree species has been developed (Kalluri et al., 2022) and a peach powdery mildew resistant gene (*Vr3*) coming from the almond cultivar 'Texas' is being introgressed into different commercial peach cultivars (Marimon et al., 2020). Furthermore, a collection of prILs with introgressions from *P. davidiana* and another peach powdery mildew resistant gene called *Vr4* has been identified (Zaracho et al., 2022). Those preliminary results opened the path to the pyramiding of those genes to obtain, in a close future, peach cultivars carrying resistance to powdery mildew and sharka.

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Conclusions

Among the most important outputs of the Freeclimb project, to the profit of the Mediterranean Basin tree fruit crop industry, some are highlighted below.

i) Survey and exploitation of plant materials

Since the very beginning of the project all partners shared the list of the germplasm materials kept in their repositories and open field collections. Part of this invaluable treasure was assessed for the aims of the project, while the vast majority is now part of the common legacy of the project and could be exploited to the benefit of all Mediterranean Basin fruit industry. One example is explained below.

ii) Multisite Reference Collections

For each fruit crop under study, the road has been paved in order to build multi-site reference collections. This is the first case ever of an international cooperation initiative on multi-site collections for fruit tree crops in the Mediterranean Basin. Integration of genome-wide marker data with replicated phenotypic data collected from the same genetic material (core collections), grown for years in different environments allows building of robust GWAS and genomic prediction models to support genetic analysis and breeding of complex traits. Indeed, many traits of horticultural importance, e.g. disease resistance or adaptability to climatic extreme events, are regulated by complex gene networks, in turn affected by environmental factors: powerful statistical tools and datasets collected from multi-site experimental designs are needed to disentangle these genotype x environment interactions.

iii) Breeding activities to match stakeholders expectations

Breeding activities were launched for several crops, in particular as pre-breeding (crosses with low commercial value accessions, bringing resistance to diseases or to abiotic stresses), in some cases sharing the resulting seed progenies among partners. This matches the wish of many stakeholders, who were asking for the development of new varieties integrating the positive traits of the old accessions (e.g. fruit quality attributes) with resistance/tolerance to abiotic and biotic stresses. In addition, the vast majority of stakeholders would be willing to participate in/promote/facilitate the evaluation of plant varieties for suitability in different environments. Through the project activities, a network of stakeholders was established for long-term collaboration in search and exploitation of genetic resources with resilience to current and future challenges.

iv) Perspectives

All the milestones above would be meaningless without strong human ties among scholars. Solid foundations for long-term cooperation among the Freeclimb partners were established, in order to pave the road for the full exploitation of the actions started during the formal course of the project. As tree crops need many years in order to reach maturity and fully display sound outcomes, so far only preliminary results have been collected. Furthermore, since during the project several young scholars had the chance to visit some other partners, this could be seen as a very promising legacy for the cooperation to be continued.

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Annexes

Annex 1. Partners of the 'Freeclimb' Consortium

- Università degli Studi di Milano (La Statale), Italy (UMIL,DiSAA)

- Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria , Italy (CREA, OFA)

- Di3A - University of Catania - Department of Agriculture, Food and Environment, Italy (UNICT)

- Consiglio Nazionale delle Ricerche Italy (CNR, IBBA)

- Ecole Nationale Supérieure Agronomique, Algeria (ENSA)

- UNIVERSITE FRERES MENTOURI CONSTANTINE 1 , Algeria (UFMC1)

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- Institute of Olive tree, Subtropical crops and Viticulture, Greece (ELGO-DIMITRA, IOSV)

- Olive Institute, Tunisia (IO)

- Institut National de la Recherche Agronomique, Morocco (INRA-Morocco)

Annex 2. Stakeholders Questionnaire Template

POSITION PAPER ON THE MOST IMPORTANT FRUIT CROPS IN THE MEDITERRANEAN BASIN (MB)

Stakeholders' opinion

Blue text needs respondant input

Freeclimb project summary

The FREECLIMB project is built to match topic 1.2.1 of the PRIMA (Sect. 2) framework in developing smart and sustainable farming systems in Mediterranean countries, to preserve natural resources (water and land use) by increasing production efficiency. This will be pursued by advancing knowledge on mechanisms of plant environmental adaptation and biotic/abiotic stress resilience. The project targets major fruit tree species with the aim of improving the availability of breeding and germplasm material adapted to limited external resources (input) and future climatic scenarios predicted for the Mediterranean area, through the characterization and exploitation of local biodiversity. The project will focus on key ideotypes elaborated in collaboration with Fruit Farming Actors (FFAs, breeders, nurseries, growers) with the core objective of providing a toolkit (diverse germplasm, tools and methods) to accelerate exploitation, breeding and selection of resilient varieties in key traditional fruit crops of Mediterranean agriculture: stone fruits such as peach, apricot and almond trees; Citrus spp. such as orange, lemon, mandarin trees; olive trees, and grape).

Please use one questionnaire for each crop

Your opinion is kindly requested on the following aspects:

1. Which fruit tree crops do you handle?
2. For each fruit tree crop that you handle, what are the major needs in plant varieties?
3. Would you suggest exploitation of existing varieties or breeding for new genotypes?
4. Which are the major stresses in each crop that you handle? Please fill in the table below.
5. Would you be willing to participate in/promote/facilitate the evaluation of plant varieties for suitability in different environments?

Stress	Rate risk (1 is the lowest and 10 is the highest risk)	Comments and specific suggestions
water deficit		
water excess		
salinity		
high temperature		
low temperature		
Pest 1 (please name it)		
Pest 2 (please name it)		
Add lines if necessary		
Disease 1 (please name it)		
Disease 2 (please name it)		
Add lines if necessary		

Annex 3. Stakeholders list by country

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Mouhamed Hmaidia	Tree farm, Benchicao - Medea
Mouloud Hmaidia	Tree farm, Medea
Mohamed Laalam	Tree farm, Boufarik - Blida
Mohamed Sellami	Tree farm, Oued El Allayeugue- Blida
Mouloud Sellami	Tree farm, Chebli- Blida
Ameur Torki	Tree farm, Medea
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France

Name	Activity
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Greece

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Grape	Osvaldo FAILLA
Olive	Daniele BASSI
Peach	Daniele BASSI
Prunus	Daniele BASSI

The Most Important Fruit Crops in the Mediterranean Basin

Position Paper

Editors
Daniele Bassi, Marco Cirilli, Laura Rossini

Climatic scenarios predicted for Mediterranean areas pose specific challenges for agriculture. The vulnerability of the fruit crops industry to the modification of agro-climatic conditions depends on both the expected regional climate extremes and the sectors' adaptation ability. For their perennial status, fruit tree crops are particularly exposed to environmental extremes, i.e. yield and quality of fruit production are strongly affected by genotype x environment interactions. The overall aims of this book, other than presenting an overview of the main fruit crops industry in the Mediterranean basin (Almond, Apricot, Citrus spp., Grape, Olive, Peach), are: i) describing the most interesting features of their cultivation (state of the art), ii) enlighting the possible solutions in order to tackle the negative impacts of climatic extremes on the industry, iii) reporting the main findings of the 'Freeclimb' international project (https://prima-freeclimb.com/) funded in the framework of PRIMA, a EU initiative meant to foster MB cooperation (https://prima-med.org/).

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