Hapres | Crop Breeding, Genetics and Genomics

cbgg.hapres.com

Review

An Overview on Citrus Mal Secco Disease: Approaches and Strategies to Select Tolerant Genotypes in *C. limon*

Loredana Abbate, Francesco Mercati *, Sergio Fatta Del Bosco

Institute of Biosciences and Bioresources (IBBR), National Research Council,
Corso Calatafimi 414, 90129, Palermo, Italy
* Correspondence: Francesco Mercati, Email: francesco.mercati@ibbr.cnr.it.

ABSTRACT

Citrus is an economically important fruit crop that is seriously afflicted by several biotic stress, caused by both viruses and fungi, thus, new sustainable strategies to manage these diseases are needed. Lemon (*Citrus limon* (L.) Burm. f) is a tree in the Rutaceae family and after orange and mandarin, is the third most important Citrus species. Lemon fruits contain important bio-compounds and have central role in prevention of diseases of a balanced diet. Among *C. limon* diseases, "mal secco" is a highly destructive tracheomycotic fungal infection spread over the coasts of the Mediterranean basin and the Black Sea areas. The causal agent is the mitosporic fungus *Phoma tracheiphila* (Petri) Kantschaveli & Gikachvili (syn. *Deuterophoma tracheiphila* Petri). The pressure of disease has led to a marked decline of the Mediterranean's lemon crop acreage in the last 30 years. The present review explores the state of art in the struggle against mal secco disease (MSD), summarizing main results obtained from both conventional and unconventional breeding approaches. In addition, the strategies used to study the mechanisms involved in *P. tracheiphila*/lemon interaction and the results obtained to isolate key genes activates in tolerant genotypes are also described in depth. The overview of emergent approaches and technologies is focused to highlight the progress obtained using advanced lemon-breeding methods and suggest guidelines for future research.

KEYWORDS: *Citrus limon*; *Phoma tracheiphila*; conventional breeding; biotechnology

Received: 04 July 2019
Accepted: 16 September 2019
Published: 18 September 2019

HISTORY, GEOGRAPHICAL DISTRIBUTION, FEATURES AND ECONOMIC IMPACT OF *Citrus limon*

Citrus is the most economically important fruit tree crop in the world with an annual production of approximately 125 million tons of fruits harvested from more than 9 million hectares of cultivated field [1]. The term "citrus" indicates a complex of species and hybrids of the genera *Citrus, Eremocitrus, Fortunella, Microcitrus and Poncirus*, subfamily *Aurantioideae* (family Rutaceae). It is assumed that all currently cultivated citrus species have been developed from three ancestral “true” species, *Citrus* medica (citron), *Citrus reticulata* (mandarin) and *Citrus maxima* (pummelo)[2]. Recently using genomic, phylogenetic and biogeographic information, ten natural citrus species were described [3]. The authors hypothesized that Citrus species were developed from a possible first diversification in the late Miocene epoch (10.4–10.5 million years ago) through a rapid southeast Asian radiation that correlates to a marked weakening of the monsoons, followed by a second radiation enabled by migration across the Wallace line that gave rise to the Australian limes in the early Pliocene epoch [3].

Lemon (*Citrus limon* (L.) Burm. f) is a tree in the Rutaceae family, originally grown probably in Asia (Northern India and South East China or in northern Myanmar) [4]. Nicolosi *et al.* [5] proposed that lemons come from a direct cross between *C. aurantium* L. (sour orange) as the female parent and *C. medica* L. (citron) as the male parent. This hypothesis was confirmed by molecular studies [4,6–8]. Two lemon groups, one from direct hybridizations between *C. reticulata* and *C. medica* and another one coming from crosses between *C. maxima* × *C. reticulata* and *C. medica* were proposed [4,9]. Gulsen and Roose [9] also suggested that the most commercially important lemon cultivars originated by mutations, including prevalent grown lemons, such as “Eureka”, “Monachello”, “Villafranca”, “Lisbon” lemons, and their selected clones.

After orange and mandarin, lemon is the third most important *Citrus* species, with a very difficult and complex taxonomy [9]. Although lemon plants are grown all over the world in more than 80 countries, most of the crop grows on either side of a belt around the equator covering tropical and subtropical areas of the world 35° N and 35° S latitudes. It is currently cultivated commercially worldwide in tropical and subtropical countries [9], especially in China, United States, Israel, Argentina, Spain, Lebanon, Turkey, Brazil, South Africa, Australia and Japan, respectively [10]. Global sales from exported lemons and limes by country totaled US$3.6 billion in 2018 [11]. The Mediterranean Basin is a major area of lemon production, accounting for 48% of production worldwide [12], with Turkey the most important producing country in this area (annual production > 1,000,000 tons), followed by Spain (900,000 tons) and Italy (500,000 tons)[13].

Lemon fruit has a strong commercial value for the fresh consumption and for food industry. It contains many important natural chemical components including phenolic compounds (mainly flavonoids) and other nutrients and non-nutrients (vitamins, minerals, dietary fiber, essential oils and carotenoids)[14]. Their health-promoting effects and properties have been associated with their contents, namely vitamin C and flavonoids, due to their natural antioxidant characteristics [15]. Overall, lemon fruits, rich in flavonoids, are a very important part of a balanced diet, particularly for their role in prevention of diseases, such as obesity, diabetes, blood lipid lowering, cardiovascular diseases, and certain types of cancer [16–18].

Seedless lemons with high nutraceutical and organoleptical proprieties and resistance to important diseases, such as mal secco, are in high demand by growers and consumers [19–21]. Several lemon-breeding programs are focused to reach these goals [19,21–24], despite the difficulties imposed by the high heterozygosity and the low genetic variation of this species [25,26]. Hence, mal secco tolerant and seedless lemons cultivars, with improved yields and longer storage periods, may have great potential for the expansion of lemon market because they are preferred by farmers and consumers, by lowering the production and conservation costs.

“MAL SECCO” DISEASE (MSD)

The term “fungal” disease referred to citrus pathologies includes both diseases caused by “true” fungi or fungi sensu stricto (eumycota) and those caused by oomycetes. Lemon trees are affected by pests, diseases, and environmental factors that limit the quantity and quality of their production. In particular, the lemon industry in the Mediterranean basin is strongly threatened by a highly destructive vascular disease called “mal secco” (MSD), caused by the mitosporic fungus *Phoma tracheiphila* (Petri) Kantschaveli & Gikachvili (syn. *Deuterophoma tracheiphila* Petri). *P. tracheiphila* is a quarantine pathogen included on the list A2 quarantine pests of the European and Mediterranean Plant Protection Organization (EPPO), and the lists of most other regional plant protection organization, worldwide, such as Asia and Pacific Plant Protection Commission (APPPC), Caribbean Plant Protection Commission (CPPC), Comité Regional de Sanidad Vegetal del Cono Sur (COSAVE), North American Plant Protection Organization (NAPPO) and Inter-African Phytosanitary Council (IAPSC) which provide detailed information for avoiding or restraining the spread of the pathogen [21,27]. Moreover, *P. tracheiphila* was included in a list of microorganisms that have to be regarded as potential biological weapons as they cause destructive diseases of economically relevant crops [28].

The disease firstly appeared on the Greece islands of Chios and Poros at the end of the nineteenth century then, rapidly spread over the coasts of the Mediterranean basin. Currently, the geographical distribution of MSD comprises the east coast of the Black Sea (Georgia) and mainly all citrus-growing countries of the Mediterranean Basin, except for Morocco, Portugal and Spain [29] with variable incidence and severity of plant damages [27].

According to Ruggieri [30], MSD destroyed in Sicily (Italy) 12,000 ha of lemon groves between 1918 and 1953, while in Turkey, the disease killed about 20,000 lemon plants in 15 years [31]. In the same years, in Greece an average loss of 56% has been recorded [32]. In Tunisia, MSD was first observed in 1960 in restricted areas in the northern part and, later, the infection has progressively spread to the major citrus producing regions of the country [33]. We can estimate that the pressure of disease has led to a decline of more than 80 percent of lemon crop acreage in the last 30 years [21].

The economic losses linked to mal secco disease have been dramatic for the Mediterranean's citrus industry in terms of job's decline, lost in industry output and labor income. Thus far, the disease has not been detected in the citrus-growing countries of the Americas or Oceania, even though there are no obvious environmental factors limiting the establishment of mal secco in such un-infested areas [21].

Despite relatively the large diffusion of the disease, analysis of fungal isolates collected from various citrus species and growing areas confirmed high homogeneity in *P. tracheiphila* populations [27,34] and no significant variation in the pathogenicity of *P. tracheiphila* [35,36], as well pathogen spread. The primary source of inoculum is represented by the fungal conidia that differentiate in late autumn and winter at relatively low temperatures. Pathogen dissemination, in favorite by wind and rainfalls, are strictly correlated with infection rate [37,38]. The optimum temperature for pathogen growth and symptom expression is between 20 and 25 °C. At temperature above 28 °C fungal growth ceases and symptoms are not expressed.

P. tracheiphila penetrates the host through wounds and leaf scars and invades the vascular tissue causing a typical reddish discoloration of the xylem [39,40]. Additionally, *P. tracheiphila* usually infects frost-damaged trees by entering through the damaged bark [41] and affects the branches that eventually die out, as the fungus is growing toward the lower parts of the plant [40].

The erratic field behavior of MSD, mainly caused by alternation of seasons with high incidence with others, in which infections are less severe or not detectable, makes difficult to estimate the incidence of damage. Moreover, in addition to the direct damage to the plants, there are indirect loss due to both the presence of plants showing different age and susceptibility because of the replacement of dead trees, causing a heterogeneous and lower qualitative production, and higher costs due to the disease monitoring [27].

Measures for effective infection control are implemented with the use of tolerant rootstocks and healthy propagation material. If the symptoms are already evident pruning, combustion of cutting residues and chemical treatments (fungicides) are carried out [42]. The biological control of plant pathogens and pests using endophytic microorganisms exhibiting antagonistic activity and colonizing the same ecological niche as pathogens has been applied in several pathosystems [43,44]. The biological control as a possible option against citrus mal secco disease has been only preliminarily explored [21]. Endophytic bacteria have been demonstrated to induce plant resistance against several diseases [45]. In particular, *Bacillus* species are the most common endophytic bacteria that can be effective biocontrol agents of vascular plant pathogens [46,47]. Kalai-Grami *et al.* [48] demonstrated the antagonistic effect of an endophytic strain of *B. amyloliquefaciens* against *P. tracheiphila* both *in vitro* and in planta.

MSD IN CITRUS GENUS

Lemons are the principal host of *P. tracheiphila*, however, the host range includes also citron *(C medica* L.), bergamot (*C. bergamia* Risso), chinotto (*C. myrtifolia* Raf.), lime (*C. aurantifolia* Christ.), and Rangpur lime (*C. limonia* Osbeck), that are considered very susceptible to natural infections of MSD. Though considered tolerant, also mandarin (*C. deliciosa* Tenore) is affected sporadically by the disease [49–51]. Among rootstocks, sour orange (*C aurantium* L.), the most prevalent lemon rootstock in the Mediterranean citrus countries, rough lemon (*C. jambiri* Lush), citrus alemow (*C. macrophilla* Wester) and volkameriana lemon (*C. volkameriana* Ten. & Pasq) are considered very susceptible. The fungus has been reported on many other citrus species and also on several genera of the *Rutaceae* family, such as *Fortunella, Poncirus and Severina* [52], as well as on intergeneric and interspecific hybrids [27]. MSD affects the agronomically best performant lemon cultivar “Femminello”, causing severe damages and plant death. Disease symptoms usually appear in spring as leaf vein and shoot chlorosis followed by both shedding of leaves and phylloptosis; wilt and dieback of apical twigs and branches rapidly occur. Although most of the symptoms of malsecco are not specific, the syndrome of the disease is characteristic since typical salmon-pink or brown-reddish discoloration of the wood can be observed into the affected withered twigs or after peeling off the bark of branches or trunk of infected trees. On the contrary, if *P. tracheiphila* is present in an asymptomatic and latent infection, an apparently healthy plant may suddenly develop the disease and collapse [53]. Consequently, diagnosis of infection based on typical phenotypic symptoms is not suitable to estimate disease development and should be supported by molecular approaches for an earlier and certain detection in still symptomless plants [54,55]. The progress of the disease is basipetally, indeed *P. tracheiphila* invades the xylem progressively starting from the site of infection, proceeding slowly downward from the infected young shoots to the branches and main limbs, seizing the whole stem massively before foliar symptoms appeared [53–55]. When the pathogen infects trunk and roots the tree dies. This movement is supported by phialoconidia, which move in the xylem by utilizing the transpiration flow of plants [55]. Since extensive virulence variability in *P. tracheiphila* causing MSD on citrus was detected [29], the pathogen may kill a lemon plant within a few months or years, depending on type/pathogenicity of isolate and, also, the age of tree. The disease course is strictly related to the rate of colonization of the vascular system by the fungus. Despite being less frequent than foliar infections, root infections may also occur; they accelerate the progress of the disease since the inoculum spreads more efficiently through the vascular system. This form of the disease may kill the plant within a few weeks [21,56].

There are no curative measures to eradicate MSD. Once a tree is infected, the only option is to remove rapidly withered twigs, shoots, suckers and, eventually, branches, by synchronizing pruning time with low susceptibility of wound to infection (end of vegetative season and dry weather) and low risk period (low inoculum production)[44]. Complete eradication of the tree is necessary when the systemic invasion of the functional xylem by the pathogen occurs. Chemical control through application of copper-based fungicides during the rainy season (every 20 days, from October to March) may reduce but not eradicate infection by *P. tracheiphila*. In addition, long-term use of copper-based fungicides led to an accumulation of this heavy metal in citrus soils. The soil copper accumulation may induce phytotoxicity, root and shoot growth reduction as well as leaf number decrease and leaf edge chlorosis. Besides, heavy metal accumulation in soils negatively affects the agroecosystem sustainability, causing decreases of earthworm populations and alteration of microbiological parameters and enzymatic activities [57]. As reported in several pathosystems [43,44], the biological control using antagonist endophytic microorganisms could be an alternative strategy against MSD [21,48,58,59]. In particular endophytic bacteria, such as Bacillus species, the most common endophytic bacteria, induce plant resistance against several diseases [45], including vascular plant pathogens [47]. Indeed, an isolate of *B. amyloliquefaciens*, named TEB1, showed in *C. arantium* antagonist effect against *P. tracheiphila*, reducing conidia germination by 55%. These results suggest that lipopeptides produced by TBE1 drive the pore formation in the fungus cytoplasmic membrane, proving to be a potential candidate for the biological control of citrus MSD [48].

However, the control measures listed above are not completely effective. Real approaches in the control of MSD on a large scale are (a) the early diagnosis of *P. tracheiphila* infections in nurseries through molecular methods [53,54], or (b) the use of tolerant lemon cultivars. Unfortunately, the latter is currently limited due to the lack of lemon-tolerant cultivars with satisfactory production characteristics [21].

HOST-PATHOGEN INTERACTION AND MECHANISMS OF TOLERANCE/RESISTANCE

When vascular tissues are infected, *P. tracheiphila* spreads within the xylem vessels and colonize the neighboring vascular tissues. Phoma colonization compromises water and nutrient transport due to fungal hyphae and gum production, showing the typical wilting associated to MSD. The disease course is related to the rate of colonization of the vascular system by the pathogen [42,53] which, in turn, is related to the virulence of the different fungal strains. The time course of depression of plant water status and gas exchange is faster when the stem is infected, as compared with the values observed in inoculated leaves [40,60]. As mentioned, young infections confer a typical pink-salmon discoloration to the wood. This discoloration is associated with the gums accumulating in xylem tissues caused by the alteration of the primary cell wall and middle lamella complex [61,62]. The high hydrolytic enzyme activity observed in infected xylem induces electrolyte leakage [63]. *P. tracheiphila* produces several phytotoxic substances [64–66] that alter cell membrane permeability causing the water syndrome. The main phytotoxic compound associated to the presence of symptoms is a specific complex of glycopeptides named "malseccina", whose major phytotoxic fraction, denoted Pt60, shows a sequence homology not found with any known protein [67,68].

Penetration and diffusion of the pathogen within the host plant are affected by climatic trends, inoculum density and juvenility [69]. Many species, following biotic or abiotic stress, produce one or more proteins termed PR (pathogenesis-related) proteins [70]. Many of these proteins belonging to diverse chitinase or glucanase play a direct role in the host plant's defense mechanisms through their lytic activity on chitin, the principal constituent of the cell wall of *P. tracheiphila* and many other fungal pathogens. The presence of these proteins in mal secco-resistant genotypes suggests that PR-proteins could have a specific role in activating plant defense mechanisms [71,72]. Russo [73], observing the development of natural infection in Citrus hybrids, proposed that resistance was polygenically inherited. Tuzcu [74], analyzing the number of plant deaths following artificial inoculation of a progeny from a lemon intraspecific cross, proposed that the resistance trait was encoded by some recessive genes and that environmental conditions play an important role in the levels of trait expression. No studies aimed at identifying the hereditary basis of mal secco resistance via genetic analysis of a sufficiently large progeny have been reported [69].

As concern, mechanism of resistance can be determined by a structural modification after infection with the formation of new wood, as seen by Somma *et al.* [75] in "Monachello" lemon. Bassi *et al.* [76] and Perrotta *et al.* [77–79] studied cytological and histological changes in leaves of different susceptible citrus species after infection. They showed that the most frequent alterations were: hyperplasia and xylem hypertrophy parenchyma cells, plasmolysis, vessel gumming, changes in the cell wall, crushing of the vessels, imbalance of the plastids, increase in the number of mitochondria [65]. In the resistant "Monachello" lemon the pathogen progressed for a short distance from the site of infection and only within the injured tissues. The cambium was unaffected and remained active. In 1958, Egorova [80] highlighted different chemical profiles and activities (persoxidase, acid and alkaloid content, and the amount of nitrogen and soluble alkalis) comparing the behavior of resistant and sensitive lemons after infection. However, according to

Pacetto and Davino [81], the activity of oxidative enzymes does not appear to be related to the degree of susceptibility to MSD.

Reverberi *et al.* [82] conducted studies in planta and *in vitro* focused to verify the role of oxidative stress in lemon/*P. tracheiphila* interaction, using the cvs "Monachello", "Interdonato" and "Femminello", which are considered respectively as resistant, partially resistant and susceptible to fungus infections. The experiments indicated that extracellular proteins of fungal injected into leaves induced lipoperoxide formation in cvs "Interdonato" and "Femminello", while the presence of lyophilized twigs and leaves of cv. "Monachello" in the fungal growth medium, stimulated the production of superoxide dismutases, glutathione peroxidase, and catalase in the mycelium. When lyophilized twigs and leaves of cv. "Femminello" were added, the pathogen produced a higher quantity of hydrolytic enzymes. In summary, the authors highlighted that the coordinated presence of hydrolytic enzymes, toxic compounds, oxidative stress inducers and membrane transporters in the fungus, and the differential capacity to regulate the lipoperoxidative pathway in the host, can play a key role for MSD in *C. limon*.

Complete control of MSD with chemicals and other control measures such as sanitary applications is not possible yet. Mechanisms involved in the MSD tolerance are unknown, therefore the development of cultivars tolerant or resistant to this disease is hard but it is essential in order to plan future successful breeding strategies [19,74,83].

CONVENTIONAL BREEDING TO ISOLATE *C. limon* TOLERANT GENOTYPES TO MSD

The selection of tolerant and/or resistant genotypes is the main and most desirable goal among the measures to control MSD [27]. Therefore, the most important objective in lemon breeding throughout the Mediterranean and Black Sea areas is to reduce the impact of MSD, developing cultivars that combine the excellent agronomical and productive characteristics of "Femminello" and the resistance or tolerance to "mal secco", and grafting them onto resistant rootstocks. Use of tolerant cultivars and rootstocks would double the lemon production in lemon-producing countries [83].

Despite the considerable losses due to fungal pathogen, very few publications have reported development of enhanced disease tolerant/resistance in lemon. Since the transfer of desirable traits to commercial cultivars by interspecific hybridization in Citrus is difficult because of several factors (pre- and post-fertilization barriers, nucellar embryony, high level of heterozygosis, extended juvenility, limited knowledge on trait inheritance), the oldest breeding method for lemon cultivar improvement has been based on clonal selection of genotypes obtained by spontaneous or induced mutagenesis. The approach involves the field selection of lemon trees with desirable phenotype and no mal secco symptoms, followed by their integration into breeding programs.

However, despite the strong efforts spent in the last 40 years in search of a lemon selection alternative to “Femminello”, the clonal selection approach did not give the expected results. “Monachello” and “Interdonato”, two spontaneous hybrids between lemon and citron highly resistant to “mal secco”, have reduced yield, do not bloom several times and their juice has low acidity, as compared to “Femminello” lemon, which has reduced their diffusion. Two promising mal secco tolerant selections, named “Femminello Zagara Bianca” and “Continella”, producing high-quality fruits, have been proposed as alternatives to “Femminello”, but their tolerance to mal secco is not comparable to that of “Monachello” and their adoption is limited. Foreign lemon cultivars such as “Lisbon”, “Eureka”, “Mesero”, “Verna”, *etc*., have shown susceptibility similar to that of common “Femminello”, when grown in areas strongly affected by “mal secco”. Only “Meyer lemon” (probably, a natural hybrid between lemon and sweet orange) shows levels of resistance comparable to those of “Monachello” and “Interdonato”, but this lemon is totally unsatisfactory as a commercial variety for the fresh-fruit trade.

In 1975, a wide sanitary and clonal selection breeding program for lemon was started in Italy, at the National Research Council of Italy (CNR), Institute of Biosciences and BioResources (IBBR), in endeavor to combine agronomic/productive properties and MSD tolerance. Lemon plants selected were planted in performance plots and evaluated by breeders through visual assessment of morphological, anatomical, and physiological characteristics in heavily MSD infested environments. The breeding program allowed identification of several “Femminello” clones, overall characterized by MSD field tolerance and adequate horticultural performance. The impact of MSD, evaluated in terms of infection intensity on the lemon clonal lines, was determined over a period of 15 years. Next, selected plants were also tested through artificial stem and leaf inoculation tests and by analysis of propagule number of *P. tracheiphila* in the xylem of stem-inoculated plants. One of studied lines, called “LCNR58”, showed over the time slighter progression of the disease and marked reduced symptoms associated to high and constant levels of lemon fruit yield and productivity.

Finally, polyploidy is one of the main forces that drive the plants evolution, providing great advantages for breeding. Ploidy increase by conventional methods has been attempted. In *Citrus* genus, most species are diploids ($2n = 18$). However, citrus plants with a different number of chromosomes, such as triploids ($2n = 3x = 27$) and tetraploids ($2n = 4x = 36$), occur naturally. Citrus polyploids hold great value for seedless triploid breeding, rootstock breeding and relevant basic researches on genomic variation, environmental adaptability and biotic/abiotic stress resistances. In citrus scion improvement, tetraploid plants are valuable since crossings with diploid plants (interploid hybridization) can produce triploid varieties that are seedless [84]. Lemon interploid hybridization between tetraploid Lisbon lemon and diploid varieties allowed the obtainment of several triploid lemon genotypes. However, such lemon triploids were low yielding and their fruits had a poor commercial quality [85] or were susceptible to *P. tracheiphila* [86]. Clonal selection programs aiming of obtaining a well performing and mal secco resistant lemon variety have been conducted intensively in all the citrus Mediterranean countries and are still in course [74,87]. However, lemon varieties derived via clonal selection have very low-level of polymorphism, therefore, at present this strategy has been nor successful.

BIOTECHNOLOGY APPROACH TO DEVELOP AND SELECT MSD TOLERANT/RESISTANT GENOTYPES IN *C. limon*

Induced mutagenesis is a valuable tool in altering only few genes while conserving plant genetic background [88–90]. Gamma irradiation (Co60) and chemical mutagens are used to induce mutations on seeds or buds, producing several times more mutants than spontaneous mutations.

Since the first attempts performed on citrus seeds using X-rays in 1935 [91] many projects were conducted to improve some properties of citrus, such as seedlessness, fruitfulness and shape of canopy, fruit peel color, early bearing, all through artificial mutation [92–94]. Lemon cultivars were subjected to a mutation-inducing process by exposing buds to Co60 gamma irradiation [88,95] to obtain seedless and malsecco tolerant varieties [19,83,96]. Although mutation breeding is a useful method to develop new varieties, it takes several disadvantages. Mutagenesis is not targeted to a specific locus but acts randomly on the genome. Moreover, most of the important traits, such as mal secco resistance, are quantitatively inherited. It implies screening of large populations to select plants expressing the desired traits. In addition, establishment of a stable mutant genotype requires considerable time due to long juvenility and needs long field trials to ascertain the acquired resistance or tolerance to biotic and abiotic stress. This approach allowed the obtainment of an excellent highly productive nucellar mutant, the 2Kr “Femminello” Siracusano, but this mutant did not behave toward mal secco infection any better than the wild type. An induced mutation breeding program aiming to obtain seedless and mal secco tolerant lemons through budwood irradiation allowed the recovering of several mutant “Kutdiken” lemons [83]. One of them, “Eylul” lemon, showed no disease symptoms after controlled inoculation with *P. tracheiphila* [96], but long field trial performance of this new mutant has not been published yet. Recent advances in genomic science and molecular biology techniques will support in the development of faster, targeted and more efficient tools to expedite lemon breeding [97]. For instance, DNA markers for traits such as disease resistance could be used as tools for rapid selection and directed breeding. An alternative biotechnological approach is to recover disease-resistant plants by selecting cellular lines resistant to a toxin produced by the pathogen [98]. However, no one of the plants regenerated from the treated cell lines was tolerant to the pathogen [99,100]. Using tissue culture techniques, *i.e.*, exposure of the sensitive “Femminello” lemon cells to partially-purified toxin (PPT), a cell line was established that was tolerant to the *P. tracheiphila* toxin and labelled “Femminello-S” [71,72]. Plants regenerated from the tolerant cell lines were grafted to rootstock sour orange showed mild symptoms of disease [101]. Considering the contrasting results reported in literature, it seems that a reliable relationship between susceptibility to mal secco and sensitivity to the culture filtrates of the pathogen does not exist [27].

Genetic engineering provides a fast and effective way to introgress specific genes in plants aiming to improve targeted traits. In the Citrus genus this method has opened the way to induce specific genetic changes within a shorter period of time than using classical genetic approaches [102–108]. Genetic transformation has been used to obtain lemon plants tolerant to mal secco. Knowing that chitinases from different organisms had successfully been used to induce transgenic resistance to pathogenic fungi, the *Chit42* gene of *Trichoderma harzianum*—a fungal genus able to inhibit plant pathogens—was inserted into the “Femminello” lemon genome by *Agrobacterium tumefaciens* mediated transformation [73]. Foliar extract of transgenic plants inhibited conidial germination *in vitro* and mycelian growth of *P. tracheiphila* [109,110]. However, due to EU restriction on field experiments with transgenic plants, to our knowledge the research on induced transgenic resistance has not been further developed.

Somatic cell hybridization through protoplast fusion is an effective tool to enhance the genetic diversity of citrus. This approach was introduced more than 30 years ago in citrus improvement programs [111–117]. It offers the possibility to circumvent some of the impedimental reproductive features of citrus (sexual incompatibility, polyembryony and diffuse pollen/ovule sterility) by combining nuclear, chloroplast and mitochondrial genomes in novel and unobtainable somatic arrangements [113,118,119]. The obtainment of inter-specific and inter-generic somatic hybrids (also between sexually incompatible species) offers several useful opportunities for the development of improved cultivar [120–123]. In order to obtain lemon types tolerant to *P. tracheiphila* through somatic hybridization, two approaches have been adopted. The first one is to combine simmetrically nuclear allotetraploid somatic hybrid plants between mal secco susceptible “Femminello” and tolerant citrus types. The production of interspecific somatic hybrids between sensitive and resistant varieties is important for introduction of the resistant traits of the latter species. To eliminate undesirable traits and introgress “mal secco” tolerance traits, backcrosses with “Femminello” lemon are performed by using somatic hybrids as pollen parents in interploid backcrosses, in efforts to produce triploid cultivars with desirable horticultural traits (seedlessness, *in primis*) and disease tolerance [114]. Following that strategy we have obtained several lemon triploid populations [119,124] on which an extensive field survey for evaluation of agronomic performances and “mal secco” tolerance is actually in course. The second approach goes through the obtainment of cybrids or cytoplasmic hybrids, individuals harboring only one parental nuclear genome and either the cytoplasmic genome of the other parent or a combination of both parents [123,125–127]. Cytoplasmic hybrids are usually obtained by asymmetric fusion between irradiated donor protoplasts whose nuclei have been destroyed, and recipient protoplasts whose organelle genomes have been metabolically inhibited by iodoacetate (IOA). As a result, the heterokaryons combine vital cytoplasms from donor parent with wholesome nucleus, from recipient parent [125]. Since they have particular characters, their potential in plant improvement is high. Plastids and mitochondria, in fact, fulfil important metabolic functions that greatly affect plant growth and productivity. Many metabolic and developmental pathways involved in plant adaptation intersect within the plastid [128], such as phytohormone- and lipid-centred processes and plant biotic defence. Likewise, heat tolerance, cytoplasmic male sterility and oxidative stress response [129] are controlled by mitochondria. In some species, especially in tobacco and citrus, cybrids arise spontaneously from intraspecific, interspecific and intergeneric symmetric hybridization procedures. To date, citrus cybrids from more than 40 combinations were unexpectedly produced from among over 300 fusion combinations simply via symmetric fusion. These cybrids usually have their nuclear genome from the mesophyll parent and mitochondrial genome from the embryogenic callus parent, while chloroplast genome is randomly inherited [125]. In Citrus, the transfer of organelle-encoded traits into a particular genetic back-ground (*i.e.*, male sterile cytoplasm from Satsuma mandarin to seedy mandarin cultivars) allowed the obtaining of potential seedless varieties [130]. To understand if specific mechanisms of resistance to *P. tracheiphila* invasion into the xylem of lemon types could be activated as an effect of nuclear/cytoplasmic DNA interactions, we tested a wide population of diploid and tetraploid lemon cybrids, carrying stem and leaf artificial inoculation tests [131]. A slower development of the disease and a lower mortality was observed in diploid lemon cybrids, as compared with the susceptible “Femminello” control. However, compared with the behavior of the resistant “Monachello” lemon, cybrids showed more symptomatic at the end of the various tests [131].

MOLECULAR APPROACHES TO STUDY CITRUS/*P. tracheiphila* INTERACTION

Quantitative and population genetics have a key role for germplasm conservation and genetic improvement in the breeding programs. The knowledge of genes' action and their regulation in important agronomic traits, such as diseases tolerance, are crucial to improve plant breeding efficiency. The advent of Next Generation Sequencing (NGS) technologies has radically increased the number of whole-genome sequences available for a plethora of organisms belonging to all kingdoms of life, including citrus genus. NGS combined with bioinformatics tools can generate extensive data in a very cost-effective way [132–134], providing significant insights into genes and transcription factors involved in key mechanism, including phytopathogen emergence and niche specialization [135–139]. In conjunction to other approaches, such as metabolomics and proteomics, and the development of high-throughput phenotyping platform and phenomics facilities may greatly help to understand the gene function and their response to stress, thereby enabling efficient association between genotypic and phenotypic records [140,141].

Table 1. An overview of the main publications available in the last six years related to citurs/pathogen interaction investigated through molecular approaches.

Disease	**Molecular approach**	**Reference**
Huanglongbing (HLB)	Proteomics	[142]
	Proteomics; nutrient analysis	[143]
	Proteomics; enzyme activity; reverse genetic	[144]
	Transcriptomics (Microarray)	[145]
	Transcriptomics (RNASeq)	[137,138,146]
	Transcriptomics (RNASeq); proteomics; FISH	[147]
	Transcriptomics (qPCR); physiology; hormone content	[148,149]
Phytophthora spp	Metabolomics	[150]
	Transcriptomics (RNASeq)	[151]
Xanthomonas citri	Metabolomics	[152]
	Proteomics	[153,154]
	Proteomics; structural study; enzyme activity; reverse genetic	[155]
Citrus Tristeza Virus	Metabolomics; physiology; hormone content	[156,157]
	Proteomics; enzyme activity	[158]
	Reverse genetic	[159,160]
	Transcriptomics (qPCR)	[161,162]
	Transcriptomics (RNASeq)	[146,163]
Mal secco	Proteomics; metabolomics; physiology	[164]
	Transcriptomics (SSH)	[40]

Unlike to other citrus diseases, such as *Phytophthora* spp., *Xanthomonas citri* ssp., Citrus Tristeza Virus (CTV) and, more recently, Huanglongbing (HLB), to date, only few information are reported about mechanisms involved in the MSD disease, with limited value for breeding programs aimed to select tolerant genotypes in *C. limon* (Table 1). Indeed, several transcriptome investigations on citrus focused on citrus greening

(HLB) disease are reported [137,138,142,145], sometimes together with multidisciplinary approaches [139,165,166], identifying many candidates and regulated genes. Other works are focused to identify potential genotypes tolerant to CTV by qPCR [162] or to highlight the differentially expressed transcripts (DETs) after combined infection with different virus (HLB and CTV)[146]. Chiesa *et al.* [167] characterized for the first time a type I NHR (Non Host Resistance) in a non-model citrus plant. Through physiological, biochemical and molecular approaches the authors investigated responses of *C. limon* associated with the NHR triggered by *X. campestris* pv. *campestris* (*Xcc*), and compared these with the responses associated with the HDR (Host Defence Response) triggered by *X. fuscans* ssp. *aurantifolii* (*Xfa*). Interesting, *Xcc* induced type I NHR in *C. limon*, a response sharing several components with the HDR triggered by *Xfa*. However, differences between the two induced responses in the maintenance of stomatal closure and the accumulation of ROS and secondary metabolites were observed.

As reported above, in contrast to other citrus diseases there is a lack of molecular works on *P. tracheiphila* finalized to highlight genes and mechanisms activated after infection. Currently, the studies were mainly focused on (i) epidemiology disease, or (ii) the development of methods for disease management through both an early detection and the molecules isolation with antifungal activity and/or (iii) to investigate the toxins involved in the pathogenicity [36,37,42,48,54,59,68,168–170]. The first studies on *P. tracheiphila/*citrus interaction suggested that phoma produced phytotoxic complex, called malseccin, carried though plant transpiration stream to leaf and shoot parenchyma tissues, showing the characteristic symptoms of disease [66,171]. At the end of the last century, an extracellular phytotoxic 60 kDa glycoprotein (Pt60) was isolated from stationary cultures of *P. tracheiphila*. Since Pt60 induced chlorosis followed by necrosis into the mesophyll of sour orange and lemon leaves, this glycoprotein was considered as the most toxic component of the malseccin complex [68] responsible of MSD. The toxic effects of the malseccin complex have been shown on citrus leaves under controlled illuminated conditions, suggesting the possible key role of the light in the toxin activity. The artificial injection of phytotoxic glycoprotein induced chloroplast degeneration, and the toxic effects were avoided when the leaves were shaded [64,68]. Light drive the activity of many phytotoxic complexes [172,173] able to damage cellular structures through reactive oxygen species (ROS) induction. ROS cause lipid membrane peroxidation leading and afterward the loss of membrane integrity followed by cell death. Therefore the oxidative stress can drive the metabolism of both host and pathogen during their interaction [82,174]. Starting from peptides identified by Fogliano [68], gene sequence coding for malseccin was obtained by PCR [175] showing significant homologies with iron membrane transporter, NADPH-dependent reductase, and a monoammine oxidase, suggesting that more than one toxic component is involved in the genesis of MSD. Physiological experiments disclosed the activity of different enzymes related to the oxidative burst in different *P. tracheiphila* filtrates, confirming that, in the tolerant “Monachello” lemon, this fungus undergoes oxidative stress that affects its ability to invade the host [175]. The evidences underlined that oxidative stress occurs in the *C. limon/P. tracheiphila* interaction [82], however Zun *et al.* [176] showed a general increasing of total peroxidase activity (TPX) in both mal secco-resistant and susceptible citrus rootstocks in response to *P. tracheiphila* infection, as well as ascorbate peroxidase activity (APX), but no correlation was found between catalase activity (CAT) and MSD resistance. Therefore, which pathways were correlated to mal secco resistance remained unknown still.

Recently, Koutsioumari *et al.* [40] employed a Suppression Subtractive Hybridization (SSH) to isolate differentially expressed genes (DEGs) between two very closely related elite lemon cultivars that exhibit different behaviour to MSD (tolerant and susceptible). The authors identified 56 ESTs (Expressed Sequence Tags) but only 16 showed significant similarity with sequences in GenBank and most of them are implicated in metabolic procedures related to biotic stress responses. In particular SSH technique underlined in the tolerant genotype (cv. “Adamopoulou”) the presence of enzyme involved in the allantoin hydrolysis. In mutants of *Arabidopsis thaliana* for the allantoinase gene, an increased level of abscisic acid (ABA) was shown [177]. Therefore the higher expression level of allantoinase in cv. “Adamopoulou” could be related positively to disease tolerance due to low ABA levels. In addition in cv. “Adamopoulou” higher level of 4-coumarate-CoA ligase gene (4CL) and cytochrome P450 monooxygenase was also highlighted. The expression of genes encoding 4CL plays a key role in the content and composition of lignin in plant cell walls [178], which have an important function in the disease resistance of different plant species [179]. Similarly, cytochrome P450 monooxygenase is involved in several biochemical pathways, including lignin biosynthesis, defense compounds, fatty acids, signaling molecules, and regulators of plant growth, such as gibberellins, jasmonic acid, and brassinosteroids [180]. In *A. thaliana*, Cytochrome P450 monooxygenase plays a key role in the biosynthesis of important compounds, such as camalexin and alkaloid phytoalexin, related to necrotrophic fungal pathogens resistance [181].

A multidisciplinary approach was carried out by Faddetta *et al.* [164] to study a MSD tolerant diploid cybrid (C2N)[131], resulting from protoplasts fusion between “Valencia” sweet orange (*Citrus sinensis* L. Osbeck)(V) and “Femminello” lemon (*Citrus limon* L. Burm)(F). Proteomic, metabolomics and physiological profiles of C2N were compared to their parents and the corresponding somatic allotetraploid hybrid (V + F). Cybrid genotype showed an improved photosynthetic capability and, interestingly, some molecular effectors that may explain the tolerance to *P. tracheiphila* infection [131] were isolated. In this context, a peroxidase 3-like protein, an enzyme associated to syringyl lignin biosynthesis was upregulated in C2N. As reported above, lignin plays a crucial role in the disease tolerance of several species [182–186] and a defense-induced enrichment of lignin, through a triggering of peroxidase and phenylalanine-ammonia lyase (PAL) activities, was also observed in wheat, tomato and cotton [182,183,185].

To date, only few molecular studies are available, however they are the first pivotal works and the starting point to better understanding the mechanisms involved in the onset of MSD tolerance in lemon. Using NGS approaches and the recent genetic transformation technologies (e.g., CRISPR-CAS9), further efforts are needed in order to full explain the interaction between lemon and *P. tracheiphila*, to improve cultivar selection and cultural practices to minimize losses due to this disease.

CONCLUSIONS AND PROSPECTS

Although several researches aimed to explain different aspects of biology, epidemiology, and control of MSD were carried out, there are still a number of gaps that should be filled to have a whole and effective disease control. Some goals are related to the knowledge of the host/pathogen interaction and related mechanism involved. Indeed only limited information on the pathways and key genes related to MSD tolerance are available. This point is crucial in order to develop and/or select tolerant genotypes for future breeding programs. Therefore molecular markers identification linked to quantitative trait loci (QTLs) driving the mechanism of susceptibility/resistance to MSD of *C. limon* and other Citrus species are a challenge that in the future must take on and overcome, using also the new high-throughput technologies available.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Antonio Motisi (IBBR-CNR, Palermo) for his support during the drafting of the present review.

REFERENCES

1. FAO 2017. Available from: http://www.fao.org/3/a-i8092e.pdf. Accessed 2019 May 9.
2. Barrett HC, Rhodes AM. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. Syst Bot. 1976;1:105-36.
3. Wu GA, Terol J, Ibanez V, López-García A, Pérez-Román E, Borredá C, et al. Genomics of the origin and evolution of Citrus. Nature. 2018;554:311-16.

4. Curk F, Ollitrault F, García-Lor A, Luro F, Navarro L, Ollitrault P. Phylogenetic origin of limes and lemons revealed by cytoplasmic and nuclear markers. Ann Bot. 2016;117:565-83. doi: 10.1093/aob/mcw005
5. Nicolosi E, Deng ZN, Gentile A, La Malfa S, Continella G, Tribulato E. *Citrus* phylogeny and genetic origin of important species as investigated by molecular markers. Theor Appl Genet. 2000;100:1155-66.
6. Froelicher Y, Mouhaya W, Bassene J, Costantino G, Kamiri M, Luro F, et al. New universal mitochondrial PCR markers reveal new information on maternal citrus phylogeny. Tree Genet Genomes. 2011;7:49-61.
7. Ollitrault P, Terol J, Garcia-Lor A, Bérard A, Chauveau A, Froelicher Y, et al. SNP mining in *C. clementina* BAC end sequences; transferability in the *Citrus* genus (Rutaceae), phylogenetic inferences and perspectives for genetic mapping. BMC Genomics. 2012;13:13.
8. Garcia-Lor A, Curk F, Snoussi-Trifa H, Morillon R, Ancillo G, Luro F, et al. A nuclear phylogenetic analysis, SNPs, indels and SSRs deliver new insights into the relationships in the 'true citrus fruit trees' group. (*Citrinae*, Rutaceae). and the origin of cultivated species. Ann Bot. 2013;111:1-19. doi: 10.1093/aob/mcs227
9. Gulsen O, Roose ML. Lemons: diversity and relationships with selected citrus genotypes as measured with nuclear genome markers. J Am Soc Hortic Sci. 2001;126:309-17.
10. FAO. Food and agriculture organisation of the United Nations. Available from: http://www.fao.org/faostat. Accessed 2016 Dec 12.
11. Workman D. Lemons and limes exporters by country. Available from: http://www.worldstopexports.com. Accessed 2019 Mar 14.
12. Duportal M, Jorda E, Sanchez C, Imbert E, Loeillet D, Vannière H. FruiTrop FOCUS. Montpellier (France): CIRAD-FLHOR; 2013. p. 1-140. French.
13. Martín JJH, González JC. La fruticultura del siglo XXI en España. Cajamar Caja Rural Editions. Almería (Spain): Cajamar; 2014. Spanish.
14. Tripoli E, La Guardia M, Giammanco S, Di Majo D, Giammanco M. Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. Food Chem. 2007;104:466-79.
15. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med. 2002;113(9):71-88.
16. Ram L, Singh S. Medicinal importance of citrus products and by-products—a review. Agric Rev. 2006;27:170-80.
17. Barreca D, Bellocco E, Caristi C, Leuzzi U, Gattuso G. Flavonoid profile and radical-scavenging activity of Mediterranean sweet lemon (*Citrus limetta* Risso) juice. Food Chem. 2011;129:417-22.
18. Hamdan D, Ashour ML, Mulyaningsih S, El-Shazly A, Wink M. Chemical composition of the essential oils of variegated pink-fleshed lemon (Citrus × limon L. Burm. f.) and their antiinflammatory and antimicrobial activities. Z Naturforsch C. 2013;68:275-84.
19. Uzun A, Gulsen O, Kafa G, Seday U. "Alata", "Gulsen" and "Uzun" seedless lemons and "Eylul" early-maturing lemon. Hort Sci. 2008;43:1920-1.

20. Migheli Q, Cacciola SO, Balmas V, Pane A, Ezra D, Magnano di San Lio G. Mal secco Disease Caused by *Phoma tracheiphila*: A Potential Threat to Lemon Production Worldwide. Plant Dis. 2009;93:9. doi: 10.1094/PDIS-93-9-0852
21. Pérez-Tornero O, Córdoba F, Moreno M, Yuste L, Porras I. Classic methods and biotechnical tools in lemon breeding, preliminary results. Acta Hortic. 2012;928:259-63. doi: 10.17660/ActaHortic.2012.928.32
22. Calabrese F, De Michele A, Barone F. New seedless lemon varieties for Sicily. In: Proceedings of the ninth Congress of the International Society of Citriculture; 2000 Dec 3-7; Orlando, FL, USA.
23. Recupero GR, Russo G, Recupero S. New promising citrus triploid hybrids selected from crosses between monoembryonic diploid female and tetraploid male parents. Hort Sci. 2005;40:516-20.
24. Spiegel-Roy P, Vardi A, Yaniv Y, Fanberstein L, Elhanati A, Carmi N. ‘Ayelet’ and ‘Galya’, New seedless lemon cultivars. Hort Sci. 2007;42:1723-4.
25. Krueger RR, Navarro L. Citrus germplasm resources. In: Khan A, editor. Citrus Genetics, Breeding, and Biotechnology. Wallingford (US): CAB International; 2007. p. 45-140.
26. Rouiss H, Cuenca J, Navarro L, Ollitrault P, Aleza P. Unreduced Megagametophyte Production in Lemon Occurs via Three Meiotic Mechanisms, Predominantly Second-Division Restitution. Front Plant Sci. 2017;8:1211. doi: 10.3389/fpls.2017.01211
27. Nigro F, Ippolito A, Salerno MG. Invited Review: Mal secco disease of *Citrus*: a Journey through a century of research. J Plant Pathol. 2011;93(3):523-60.
28. Lillie SH, Hanlon E Jr, Kelly JM, Rayburn BB. Army Knowledge Online. 2005. Available from: https://www.us.army.mil. Accessed 2019 May 30.
29. Ziadi S, Chebil S, Melki I, Ippolito A, Mliki A. Virulence spectra and geographical distribution of Mal Secco disease of citrus caused by *Phoma tracheiphila* in the Mediterranean countries: Tunisia and Italy. Eur J Plant Pathol. 2014;138:123-31.
30. Ruggieri G. Periodicità nelle infezioni di “mal secco” e fondamentali orientamenti di lotta. Giornale di Agricoltura. 1953;34:1-8. Italian.
31. Karel G. The Lemon disease, kurutan (mal secco) and its control. Ziraat Vekal Mucad Enstitu Mudurlugu. 1956;9:1-30.
32. Thanassoulopoulos CC, Manos BD. Current status prognosis and loss assessment of Mal secco (*Phoma tracheiphila*) of citrus in Greece. In: Proceedings of the International Society of Citriculture: VII International Citrus Congress; 1992 Mar 8-13; Acireale, Italy. Catania (Italy): International Society of Citriculture; 1994. p. 869-72.
33. Crossa-Raynaud P. Le Mal secco. Extrait de Vignobles, Jardins et Vergers de Tunisie. Tunis (Tunisia): Imprimerie El Assia; 1960. p. 1-7. French.
34. Ezra D, Kroitor T. Molecular characterization of *Phoma tracheiphila*, causal agent of ‘mal secco’ disease of citrus, in Israel. Eur J Plant Pathol. 2007;118:183-91.
35. De Cicco V, Luisi N. Variabilità della patogenicità e di alcuni caratteri colturali in isolati di *Phoma tracheiphila*, provenienti da aree e ospiti diversi

del Mediterraneo. Annali della Facoltà di Agraria, Università di Bari. Brindisi (Italy): Università di Bari; 1977. Italian.

36. Luisi N, De Cicco V, Cutuli G, Salerno M. Analisi della patogenicità di Phoma tracheiphila (Petri) Kanc. Et Ghik. su alcune specie e cultivar di agrumi. Phytopathologia Mediterranea. 1979;18:162-5. Corsican.
37. Solel Z. Epidemiology of Mal secco disease of lemons. Phytopathology. 1976;85:90-2.
38. Tuttobene R. Monitoring of *Phoma tracheiphila* inoculum. La Difesa delle Piante. 1994;17:69-74.
39. Nachmias A, Barash I, Solel Z, Strobel GA. Purification and characterization of a phytotoxin produced by *Phoma tracheiphila*, the causal agent of mal secco disease of citrus. Physiol Plant Pathol. 1977;10:147-57. doi: 10.1016/0048-4059(77)90018-2
40. Koutsioumari EM, Voloudakis AE. Isolation and expression analysis of differentially expressed genes in stem tissue of the Greek lemon cv. Adamopoulou. J Hortic Sci Biotechnol. 2016;92:48-56.
41. Solel Z, Salerno M. Mal secco. In: Timmer L, Garnsey S, Graham J, editors. Compendium of Citrus diseases. 2nd ed. St. Paul (US): APS Press; 2000. p. 33-5.
42. Timmer LW, Garnsey SM, Graham JH. Compendium of citrus diseases. 2nd ed. Saint Paul (US): The American Phytopathological Society; 2000. p. 19-21.
43. Narisawa K, Tokumasu S, Hashiba T. Suppression of clubroot formation in Chinese cabbage by the root endophytic fungus, *Heteroconium chaetospira*. Plant Pathol. 1998;47:206-10.
44. Hudson O, Ahmad NH, Van Buren R, Savka MA. Sugarcane and grapevine endophytic bacteria: isolation, detection of quorum sensing signals and identification by 16S v3 rDNA sequence analysis. In: Mendez-Vilas A, editor. Current research technology and education topics in applied microbiology and microbial biotechnology. Badajoz (Spain): Formatex Research Center; 2010. p. 801-6.
45. Cho KM, Hong SY, Lee SM, Kim YH, Kahng GG, Lim YP, et al. Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. Microb Ecol. 2007;54:341-51.
46. Mahaffee WF, Kloepper JW. Temporal changes in the bacte-rial communities of soil, rhizosphere, and endorhiza associated withfield-grown cucumber (*Cucumis sativus* L.). Microb. Ecol. 1997;4:210-23.
47. Soad AA, Xie G, Coosemans J. Delivery methods for introducing endophytic *Bacillus* into tomato and their effect on growth promotion and suppression of tomato wilt. Plant Pathol J. 2005;4:69-74.
48. Kalai-Grami L, Ben Slimane I, Mnari-Hattab M, Rezgui S, El Aouani MEA, Hajlaoui MR, et al. Protective effect of *Bacillus amyloliquefaciens* against infections of *Citrus aurantium* seedlings by *Phoma tracheiphila*. World J Microbiol Biotechnol. 2014;30:529-38.
49. Palm ME. Pests not known to occur in the United States or of limited distribution no 91: *Phoma tracheiphila*. Vol 81-50. Riverdale (US): U.S. Dep. Agric. Animal and Plant Health Inspection Service (USDA APHIS); 1986.

50. Karapapa V, Doudoumis V, Tsiamis G. First report of *Phoma tracheiphila* causing severe malsecco disease on a mandarin hybrid (cv. Ortanique) grafted onto Citrumelo rootstock in western Greece. New Dis Rep. 2015;31:20. doi: 10.5197/j.2044-0588.2015.031.020
51. Hajlaoui MR, Kalai L, Mnari Hattab M, Guermech A, Ben Abdelaal N. Occurrence of mal nero disease on mandarin and orange trees in Tunisia. Plant Pathol. 2008;57:784.
52. EPPO CABI. Deutherophoma tracheiphila. Quarantine Pests for Europe. 2nd ed. Wallingford (UK): CAB International; 1997. p. 733-6.
53. Demontis MA, Cacciola SO, Orrù M, Balmas V, Chessa V, Maserti BE, et al. Development of real-time PCR systems based on SYBR® Green I and TaqMan® technologies for specific quantitative detection of *Phoma tracheiphila* in infected Citrus. Eur J Plant Pathol. 2008;120:339-51.
54. Kalai L, Mnari Hattab M, Hajlaoui MR. Molecular diagnostics to assess the progression of phoma tracheiphila in citrus aurantium seedlings and analysis of genetic diversity of isolates recovered from different citrus species in Tunisia. J Plant Pathol. 2010;92:629-36.
55. Kroitor-Keren T, Liarzi O, Gat T, Skovorodnikova J, Belausov E, Ezra D. Identification and characterization of *Phoma tracheiphila* mutants impaired in pathogenicity following Agrobacterium-mediated mutagenesis. Phytoparasitica. 2013;41:491-502.
56. Khanchouch K, Pane A, Chriki A, Cacciola SO. Major and Emerging Fungal Diseases of *Citrus* in the Mediterranean Region. In: Gill H, Garg H, editors. Citrus Pathology. London (UK): IntechOpen; 2017.
57. Zhou X, He Z, Liang Z, Stoffella PJ, Fan J, Yang Y, et al. Long-Term Use of Copper-Containing Fungicide Affects Microbial Properties of *Citrus* Grove Soils. Soil Sci Soc Am J. 2011;75:898-906.
58. Kalai-Grami L, Saidi S, Bachkouel S, Ben Slimane I, Mnari-Hattab M, Hajlaoui MR, et al. Isolation and characterization of endophytic bacteria antagonistic to *P. tracheiphila* and *V. albo-atrum*. Appl Biochem Biotech. 2014;174:365-75.
59. Kalai-Grami L, Karkouch I, Naili O, Ben Slimene I, Elkahoui S, Ben Zekri R, et al. Production and identification of iturin A lipopeptide from *Bacillus methyltrophicus* TEB1 for control of *Phoma tracheiphila*. J Basic Microbiol. 2016;56:1-8.
60. Raimondo F, Raudino F, Cacciola SO, Salleo S, Lo Gullo MA. Impariment of leaf hydraulics in young plants of *Citrus aurantium* (sour orange) infected by *Phoma tracheiphila*. Funct Plant Biol. 2007;34:720-9.
61. Magnano di San Lio G, Lo Giudice L. Role of cell wall in gum production in “Citrus”. Caryologia. 1982;35:388-9.
62. Cacciola SO. Attività pectolitica di *Phoma tracheiphila* (Petri) Kanc. & Ghik [Ph.D. Thesis]. Bari (Italy): University of Bari; 1989. Italian.
63. Cacciola SO, Natoli M, Pane A, Perrotta G, Petrone G. Characterization of polygalacturonase activities from *Phoma tracheiphila*. Ital J Biochem. 1990;39:3.
64. Nachmias A, Barash I, Solel Z, Strobel GA. Effects of Mal secco toxin on lemon leaf cells. Phytoparasitica. 1980;8:51-60.

65. Magnano di San Lio G, Cacciola SO, Pane A, Grasso S. Relationship between xylem colonization and symptom expression in mal secco infected sour orange seedlings. In: Proceedings of the International Society of Citriculture: VII International Citrus Congress; 1992 Mar 8-13; Acireale, Italy. Catania (Italy): International Society of Citriculture; 1994. p. 873-6.
66. Barash I, Pupkin G, Koren L, Ben-Hayyim G, Strobel GA. A low molecular weight phytotoxin produced by *Phoma tracheiphila*, the cause of mal secco disease in citrus. Physiol Plant Pathol. 1981;19:17-29.
67. Fogliano V, Graniti A, Marchese A, Ritieni A, Randazzo GE, Visconti A. Purification of a phytotoxic glycoprotein from the malseccin complex produced in culture by *Phoma tracheiphila*. Theor Appl Genet. 1994;86: 527-32.
68. Fogliano V, Marchese A, Scaloni A, Ritieni A, Visconti A, Randazzo G, et al. Characterization of a 60 kDa phytotoxic glycoprotein produced by *Phoma tracheiphila* and its relation to malseccin. Physiol Mol Plant Pathol. 1998;53:149-61.
69. Reforgiato Recupero G, Gentile A, Russo MP, Domina F. Genetic analysis of resistance to *Phoma tracheiphila* in three *Citrus* and *Poncirus* progenies. Plant Breed. 1997;116:198-200.
70. Linthorst HJM, Danhash N, Brederede FT, Van Kan JAL, De Wit PJGM, Bol JF. Tobacco and Tomato PR Proteins Homologous to win and Pro-Hevein Lack the “Hevein” Domain. Mol Plant-Microbe Interact. 1991;4(6):586-92.
71. Gentile A, Tribulato E, Continella G, Vardi A. Differential responses of citrus calli and protoplasts to culture filtrate and toxin of *Phoma tracheiphila*. Theor Appl Genet. 1992;83:759-64.
72. Gentile A, Tribulato E, Deng ZN, Galun E, Fluhr R, Vardi A. Nucellar callus of “Femminello” lemon, selected for tolerance to *Phoma tracheiphila* toxin, shows enhanced release of chitinase and glucanase into the culture medium. Theor Appl Genet. 1993;86:527-32.
73. Russo F. Portinnesti e propagazione. In: Spina P, editor. Trattato di Agrumicoltura. Vol. 1. Bologna (Italy): Edagricole; 1985. p. 221-89.
74. Tuzcu O, Kaplankiran M, Yesiloglu T, Erkilic A. Preliminary results on breeding lemon cultivars tolerant to ‘mal secco’ (*Phoma tracheiphila* Kanc. et Ghik.) disease in Turkey. Derim. 1992;9:99-107.
75. Somma V, Cutuli G, Salerno M. Ricerche sul saggio della resistenza al mal secco di giovani limoni innestati. Atti del II Seminario di Studio sul Miglioramento Genetico del Limone; 1979 Apr 05; Bari, Italy. Italian.
76. Bassi M, Magnano di San Lio G, Perrotta G. Morphological observations on the host parasite relations in sour orange leaves infected with *Phoma tracheiphila*. Phytopath Zeitschrift. 1980;98:320-30.
77. Perrotta G, Magnano di San Lio G, Bassi M. Some anatomical and morpho-functional aspects of resistance to *Phoma tracheiphila* in citrus plants. Phytopath Zeitschrift. 1979;98:346-58.
78. Perrotta G, Magnano di San Lio G, Lo Giudice L, Bassi M. Ultrastructural modifications induced by *Phoma tracheiphila* in sour orange. Rivista di Patologia Vegetale. 1979;14:25-33.

79. Perrotta G, Magnano di San Lio G, Lo Giudice V. Histology and cytology of citrus mal secco diseased tissues induced by *Phoma tracheiphila*. In: Proceedings of the 4th International Citrus Congress; 1981 Nov 9-12; Tokyo, Japan.
80. Egorova GN. Distinguishing features of citrus forms resistant to mal secco. In: Biochemistry of fruits and vegetables. Moskva (Russia): Akadmya Nauk SSSR; 1958. p. 112-17.
81. Pacetto M., Davino M. Indagine sull'attività perossidasica e polifenolossidasica di piante di agrumi resistenti e suscettibili a *Phoma tracheiphila*. Tecnica Agricola Catania. 1980;32:279-90. Italian.
82. Reverberi M, Betti C, Fabbri AA, Zjalic S, Spadoni S, Mattei B, et al. A role for oxidative stress in the *Citrus limon/Phoma tracheiphila* interaction. Plant Pathol. 2008;57:92-102. doi: 10.1111/j.1365-3059.2007.01699.x
83. Gulsen O, Uzun A, Pala H, Canihos E, Kafa G. Development of seedless and 'mal secco' tolerant mutant lemons through budwood irradiation. Sci Hortic. 2007;112:184-90.
84. Singh S, Rajam MV. Citrus biotechnology: achievements, limitations and future directions. Physiol Mol Biol Plants. 2009;15(1):20.
85. Geraci G. Osservazioni su alcuni ibridi di *Citrus limon* × *Citrus sinensis* (Lisbon 4*n* × Trovita 2*n*) e di *Citrus reticulata* × *Citrus deliciosa* (Fortune × Avana). Atti del Convegno: Il recente contributo della ricerca allo sviluppo dell'agrumicoltura italiana. Sassari (Italy): Università di Sassari; 1986. p. 45-8. Italian.
86. Cacciola SO, De Patrizio A, Raudino F, Pane A, Lo Giudice V, Magnano di San Lio G. High susceptibility of the triploid hybrid Lemox to mal secco disease caused by *Phoma tracheiphila*. J Plant Pathol. 2010;92:S4.76.
87. Solel Z, Spiegel-Roy P. Methodology of selection of lemon clones for tolerance to mal secco (*Phoma tracheiphila*). Phytoparasitica. 1978;6(3): 129-34.
88. Spiegel-Roy P. Economic and agricultural impact of mutation breeding in fruit trees. Mut Breed Rev. 1990;5:215-35.
89. Shu QY. Induced plant mutations in the genomics era. Rome (Italy): Food and Agriculture Organization of the United Nations; 2009.
90. Van Harten AM, Broertjes C. Induced mutations in vegetatively propagated crops. Plant Breed Rev. 1989;6:55-91.
91. Cameron JW, Frost NB. Genetic, breeding and nucellar embryony. In: Reuther W, Batchelor LD, editors. The citrus industry. Berkeley (US): University of California Press; 1968. p. 325-79.
92. Bermejo A, Pardo J, Cano A. Murcott seedless: influence of gamma irradiation on citrus production and fruit quality. Span J Agric Res. 2012;3:768-77.
93. Williams TE, Roose ML. Tango-a new, very low-seeded, late-season irradiated selection of 'W. Murcott' mandarin from the University of California Riverside. Proc Int Soc Citricult. 2010;1:202.

94. Huang JC, Xiao Y, Zhao CX, Chen ZS, Wang XY. Induction of superior seedless mutation of *C. grandis* Osbeck cv. Sshatianyou by irradiation. Acta Agric Nucleatae Sin. 2003;17:171-4.
95. Spiegel-Roy P, Vardi A, Elhanati A. Seedless induced mutant in lemon (*Citrus limon*). Mutat Breed Newsl. 1985;26:1.
96. Uzun A, Gulsen O, Kafa G, Seday U, Tuczu O, Yesiloglu T. Characterization for yield, fruit quality, and molecular profiles of lemon genotypes tolerant to ‘mal secco’ disease. Sci Hort. 2009;122(4):556-61.
97. Polat I. Advanced Innovative Tools in Lemon (*Citrus limon* L.) Breeding. In: Al-Khayri JM, Jain SM, Johnson DV, editors. Advances in Plant Breeding Strategies: Fruits. Volume 3. Cham (Switzerland): Springer; 2018. p. 437-63.
98. Deng ZN, Gentile A, Domina F, Nicolosi E, Tribulato E. Selecting lemon protoplasts for insensitivity to *Phoma tracheiphila* toxin and regenerating tolerant plants. J Am Soc Hortic Sci. 1995;120:902-5.
99. Cacciola SO, Magnano di San Lio G. Susceptibility to mal secco of lemon clones selected *in vitro*. Petria. 1994;4:276.
100. Bas B, Koc NK. *In vitro* selection of Kutdiken lemon 20b to candidate for resistance to *Phoma tracheiphila*. Plant Pathol J. 2006;5:35-40.
101. Gentile A, Deng ZN, Tribulato E, Vardi A, Albanese G, Grimaldi V, et al. Evaluation of lemon somaclones for tolerance to mal secco disease by artificial inoculation. In: First International Citrus Biotechnology Symposium. ISHS Acta Horticulturae 535. Eilat (Israel): ISHS; 2000. p. 259-63.
102. Çevik B, Lee RF, Niblett CL. Genetic transformation of *Citrus paradisi* with antisense and untranslatable RNA dependent RNA polymerase genes of *Citrus tristeza closterovirus*. Turk J Agric For. 2006;30(3):173-82.
103. Donmez D, Simsek O, Izgu T, Kacar YA, Mendi YY. Genetic transformation in *Citrus*. Sci World J. 2013;2013:491207.
104. Machado MA, Cristofani-Yaly M, Bastianel M. Breeding, genetic and genomic of citrus for disease resistance. Rev Bras Frutic. 2011;33(1):158-72.
105. Pons E, Peris JE, Peña L. Field performance of transgenic citrus trees: assessment of the long-term expression of *uidA* and *nptII* transgenes and its impact on relevant agronomic and phenotypic characteristics. BMC Biotechnol. 2012;12:41.
106. Huang XS, Wang W, Zhang Q, Liu JH. A basic helix-loop-helix transcription factor, PtrbHLH, of *Poncirus trifoliata* confers cold tolerance and modulates peroxidase-mediated scavenging of hydrogen peroxide. Plant Physiol. 2013;162:1178-94.
107. Velázquez K, Agüero J, Vives MC, Aleza P, Pina JA, Moreno P, et al. Precocious flowering of juvenile citrus induced by a viral vector based on *Citrus leaf blotch virus*: a new tool for genetics and breeding. Plant Biotechnol J. 2016;14:1976-85.
108. Moore GA, Rogers KL, Kamps TL, Pajon M, Stover J, Febres VJ, et al. Rapid cycling plant breeding in citrus. Citrograph. 2016;7(3):80-5.
109. Gentile A, Deng Z, La Malfa S, Distefano G, Domina F, Vitale A, et al. Enhanced resistance to *Phoma tracheiphila* and *Botrytis cinerea* in

transgenic lemon plants expressing a *Trichoderma harzianum* chitinase gene. Plant Breed. 2007;126:146-51.

110. Di Stefano G, La Malfa S, Vitale A, Lorito M, Deng Z, Gentile A. Defence-related gene expression in transgenic lemon plants producing an antimicrobial *Trichoderma harzianum endochitinase* during fungal infection. Transgenic Res. 2008;17:873-9.
111. Koç NK, Kayım M, Çınar A, Küsek M. Investigations on the possibility to obtain mal secco (*Phoma tracheiphila* Kanc. et Ghik.) resistant varieties via protoplast fusion (somatic hybridization) in lemon. Trop J Agricult Forest. 1999;23(1):157-68.
112. Ohgawara T, Kobayashi S, Ohgawara E, Uchimaya H, Ishi S. Somatic hybrid plants obtained by protoplast fusion between *Citrus sinensis* and *Poncirus trifoliata*. Theor Appl Genet. 1985;71:1-4.
113. Grosser JW, Gmitter FG Jr. Protoplast fusion and citrus improvement. Plant Breed Rev. 1990;8:339-74.
114. Grosser JW, Gmitter FG Jr. Protoplast fusion for production of tetraploids and triploids: applications for scion and rootstock breeding in citrus. Plant Cell Tiss Org. 2011;104:343-57.
115. Grosser JW, Ollitrault P, Olivares-Fuster O. Somatic hybridization in citrus: an effective tool to facilitate variety improvement. Vitro Cell Dev Biol-Plant. 2000;36:434-49.
116. Guo WW, Deng XX. Wide somatic hybrids of citrus with its related genera and their potential in genetic improvement. Euphytica. 2001;118:175-83.
117. Ruiz M, Pensabene-Bellavia G, Quiñones A, García-Lor A, Morillon R, Ollitrault P, et al. Molecular Characterization and Stress Tolerance Evaluation of New Allotetraploid Somatic Hybrids Between Carrizo Citrange and *Citrus macrophylla* W. rootstocks. Front Plant Sci. 2018;9:901. doi: 10.3389/fpls.2018.00901
118. Tusa N, Grosser JW, Gmitter FG Jr. Plant regeneration of 'Valencia' sweet orange, 'Feminello' lemon and the interspecific somatic hybrid following protoplast fusion. J Am Soc Hort Sci.1990;115:1043-6.
119. Tusa N, Grosser JW, Gmitter FG, Louzada ES. Production of tetraploid somatic hybrid breeding parents for use in lemon cultivar improvement. Hort Sci. 1992;27:445-7.
120. Grosser JW, Gmitter FG Jr, Chandler JL. Intergeneric somatic hybrid plants of *Citrus sinensis* cv. Hamlin and *Poncirus trifoliata* cv. Flying Dragon. Plant Cell Rep. 1988;7:5-8.
121. Grosser JW, Gmitter FG Jr, Chandler JL. Intergeneric somatic hybrid plants from sexually incompatible woody species: *Citrus sinensis* and *Severinia disticha*. Theor Appl Genet. 1988;75:397-401.
122. Ohgawara T, Uchimiya H, Ishii S, Kobayashi S. Somatic Hybridization Between *Citrus sinensis* and *Poncirus trifoliata*. In: Bajaj YPS, editor. Biotechnology in Agriculture and Forestry. Volume 27. Berlin (Germany): Sprinter-Verlag; 1994. p. 439-54.
123. Grosser JW, Gmitter FG Jr, Tusa N, Reforgiato Recupero G, Cucinotta P. Further evidence of a cybridization requirement for plant regeneration

from citrus leaf protoplasts following somatic fusion. Plant Cell Rep. 1996;15:672-6.

124. Tusa N, Fatta Del Bosco S, Nardi L, Lucretti S. Obtaining triploid plants by crossing *Citrus limon* cv 'Femminello' 2*N* × 4*N* allotetraploid somatic hybrids. Proc Int Soc Citricult. 1996;1:133-6.
125. Guo WW, Prasad D, Cheng YJ, Serrano P, Deng XX, Grosser JW. Targeted cybridization in citrus: transfer of satsuma cytoplasm to seedy cultivars for potential seedlessness. Plant Cell Rep. 2004;22:752-8.
126. Guo WW, Cheng YJ, Chen CL, Deng XX. Molecular analysis revealed autotetraploid, diploid and tetraploid cybrid plants regenerated from an interspecific somatic fusion in *Citrus*. Sci Hortic. 2006;108:162-6.
127. Guo WW, Xiao SX, Deng XX. Somatic cybrid production via protoplast fusion for citrus improvement. Sci Hortic. 2013;163:20-6.
128. Rolland N, Curien G, Finazzi G, Kuntz M, Maréchal E, Matringe M, et al. The biosynthetic capacities of the plastids and integration between cytoplasmic and chloroplast processes. Annu Rev Genet. 2012;46:233-64.
129. Jacoby RP, Li L, Huang S, Lee CP, Millar AH, Taylor NL. Mitochondrial composition, function and stress response in plants. J Integr Plant Biol. 2012;54:887-906.
130. Xiao SX, Biswas MK, Li MY, Deng XX, Xu Q, Guo WW. Production and molecular characterization of diploid and tetraploid somatic cybrid plants between male sterile Satsuma mandarin and seedy sweet orange cultivars. Plant Cell Tissue Organ Cult. 2014;116(1):81-8.
131. Tusa N, Fatta Del Bosco S, Nigro F, Ippolito A. Response of cybrids and a somatic hybrid of lemon to *Phoma tracheiphila* infections. Hort Sci. 2000;35:125-7.
132. Ekblom R, Galindo J. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity. 2011;107:1-15.
133. Harkess A, Zhou J, Xu C, Bowers JE, Van der Hulst R, Ayyampalayam S, et al. The asparagus genome sheds light on the origin and evolution of a young Y chromosome. Nat Commun. 2017;8:1279.
134. Harkess A, Mercati F, Abbate L, McKain M, Pires JC, Sala T, et al. Retrotransposon Proliferation Coincident with the Evolution of Dioecy in *Asparagus*. G3 (Bethesda). 2016;6:2679-85.
135. Gardiner DM, McDonald MC, Covarelli L, Solomon PS, Rusu AG, Marshall M, et al. Comparative pathogenomics reveals horizontally acquired novel virulence genes in fungi infecting cereal hosts. PLoS Pathog. 2012;8:e1002952.
136. Klosterman SJ, Subbarao KV, Kang S, Veronese P, Gold SE, Thomma BPHJ, et al. Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. PLoS Pathog. 2011;7:e1002137.
137. Wang Y, Zhou L, Yu X, Stover E, Luo F, Duan Y. Transcriptome Profiling of Huanglongbing (HLB) Tolerant and Susceptible *Citrus* Plants Reveals the Role of Basal Resistance in HLB Tolerance. Front Plant Sci. 2016;7:933. doi: 10.3389/fpls.2016.00933

138. Yu Q, Chen C, Du D, Huang M, Yao J, Yu F, et al. Reprogramming of a defense signaling pathway in rough lemon and sweet orange is a critical element of the early response to '*Candidatus* Liberibacter asiaticus'. Hortic Res. 2017;4:17063. doi: 10.1038/hortres.2017.63
139. Yao L, Yu Q, Huang M, Hung W, Grosser J, Chen S, et al. Proteomic and metabolomic analyses provide insight into the off-flavour of fruits from citrus trees infected with '*Candidatus* Liberibacter asiaticus'. Hortic Res. 2019;6:31.
140. Yin X, Struik PC, Kropff MJ. Role of crop physiology in predicting gene-to-phenotype relationships. Trends Plant Sci. 2004;9:426-32.
141. Leakey ADB, Ferguson JN, Pignon CP, Wu A, Jin Z, Hammer GL, et al. Water Use Efficiency as a Constraint and Target for Improving the Resilience and Productivity of C_3 and C_4 Crops. Ann Rev Plant Biol. 2019;70(1):781-808.
142. Martinelli F, Reagan RL, Uratsu SL, Phu ML, Albrecht U, Zhao W, et al. Gene regulatory networks elucidating huanglongbing disease mechanisms. PLoS One. 2013;8:e74256.
143. Nwugo CC, Duan Y-P, Lin H. Study on citrus response to huanglongbing highlights a down-regulation of defense-related proteins in lemon plants upon '*Ca*. Liberibacter asiaticus' infection. PLoS One. 2013;8(6):e67442.
144. Clark K, Franco JY, Schwizer S, Pang Z, Hawara E, Liebrand TWH, et al. An effector from the Huanglongbing-associated pathogen targets citrus proteases. Nat Commun. 2018;9:1718. doi: 10.1038/s41467-018-04140-9
145. Mafra V, Martins PK, Francisco CS, Ribeiro-Alves M, Freitas-Astúa J, Machado MA. *Candidatus* Liberibacter americanus induces significant reprogramming of the transcriptome of the susceptible citrus genotype. BMC Genomics. 2013;14:247.
146. Fu S, Shao J, Zhou C, Hartung JS. Transcriptome analysis of sweet orange trees infected with '*Candidatus* Liberibacter asiaticus' and two strains of *Citrus Tristeza Virus*. BMC Genomics. 2016;17:349.
147. Kruse A, Fattah-Hosseini S, Saha S, Johnson R, Warwick E, Sturgeon K, et al. Combining 'omics and microscopy to visualize interactions between the Asian citrus psyllid vector and the Huanglongbing pathogen *Candidatus* Liberibacter asiaticus in the insect gut. PLoS One. 2017;12(6):e0179531.
148. Boava LP, Cristofaniyaly M, Machado MA. Physiologic, anatomic, and gene expression changes in *Citrus sunki*, *Poncirus trifoliata* and their hybrids after After '*Candidatus* Liberibacter asiaticus'. Infect Phytopathol. 2017;107:590-9.
149. Zou X, Bai X, Wen Q, Xie Z, Wu L, Peng A, et al. Comparative Analysis of Tolerant and Susceptible Citrus Reveals the Role of Methyl Salicylate Signaling in the Response to Huanglongbing. J Plant Growth Regul. 2019. doi: 10.1007/s00344-019-09953-6
150. Blaya J, Marhuenda FC, Pascual JA, Ros M. Microbiota Characterization of Compost Using Omics Approaches Opens New Perspectives for *Phytophthora* Root Rot Control. PLoS One. 2016;11(8):e0158048.

151. Naveed ZA, Huguet-Tapia JC, Ali GS. Transcriptome profile of Carrizo citrange roots in response to *Phytophthora parasitica* infection. J Plant Interact. 2019;14(1):187-204.
152. Matsukawa T, Asai T, Kajiyama S. Metabolic Changes during Defense Responses against Wound Stresses in Citrus Plants. In: Gill H, Garg H, editors. Citrus Pathology. London (UK): IntechOpen; 2017.
153. Rani TS, Podile AR. Extracellular matrix-associated proteome changes during non-host resistance in citrus–*Xanthomonas* interactions. Physiol Plant. 2013;150(4):565-79.
154. Facincani AP, Moreira LM, Soares MR, Ferreira CB, Ferreira RM, Ferro MIT, et al. Comparative proteomic analysis reveals that T3SS, Tfp, and xanthan gum are key factors in initial stages of *Citrus sinensis* infection by *Xanthomonas citri* subsp. *citri*. Funct Integr Genomics. 2014;14:205.
155. Alexandrino AV, Goto LS, Novo-Mansur MTM. treA Codifies for a Trehalase with Involvement in *Xanthomonas citri* subsp. *citri* Pathogenicity. PLos One. 2016;11(9):e0162886.
156. Hančević K, Radić T, Pasković I, Urlić B. Biochemical and physiological responses to long-term *Citrus tristeza* virus infection in Mexican lime plants. Plant Pathol. 2018;67(4):987-94.
157. Clemente RMP, Montoliu A, Peris VV, Arbona V, Gómez-Cadenas A. Hormonal and metabolic responses of Mexican lime plants to CTV infection. J Plant Physiol. 2019;238:40-52.
158. Dória MS, de Sousa AO, de Jesus Barbosa C, Costa MG, da Silva Gesteira A, Souza RM, et al. Citrus tristeza virus (CTV) Causing Proteomic and Enzymatic Changes in Sweet Orange Variety "Westin". PLoS One. 2015;10(7):e0130950.
159. Cheng CZ, Yang JW, Yan HB, Bei XJ, Zhang YY, Lu ZM, et al. Expressing p20 hairpin RNA of *Citrus tristeza* virus confers *Citrus aurantium* with tolerance/resistance against stem pitting and seedling yellow CTV strains. J Integr Agric. 2015;14(9):1767-77.
160. Gómez-Muñoz N, Velázquez K, Vives MC, Ruiz-Ruiz S, Pina JA, Flores R, et al. The resistance of sour orange to Citrus tristeza virus is mediated by both the salicylic acid and RNA silencing defence pathways. Mol Plant Pathol. 2017;18:1253-66. doi: 10.1111/mpp.12488
161. Bowman KD, Albrecht U. Comparison of gene expression changes in susceptible, tolerant and resistant hosts in response to infection with Citrus tristeza virus and huanglongbing. In: Proceedings of the 19th Conference of the International Organization of Citrus virologists; 2013 Jul 28-Aug 2; Skukuza, South Africa.
162. Abbate L, Panno S, Mercati F, Davino S, Fatta Del Bosco S. Citrus rootstock breeding: response of four allotetraploid somatic hybrids to *Citrus tristeza* virus induced infections. Eur J Plant Pathol. 2019;153:837.
163. Cheng C, Zhang Y, Zhong Y, Yang J, Yan S. Gene expression changes in leaves of *Citrus sinensis* (L.) Osbeck infected by *Citrus tristeza* virus. J Hortic Sci Biotechnol. 2016;91:466-75.
164. Faddetta T, Abbate L, Renzone G, Palumbo Piccionello A, Maggio A, Oddo E, et al. An integrated proteomic and metabolomic study to evaluate the effect

of nucleus-cytoplasm interaction in a diploid citrus cybrid between sweet orange and lemon. Plant Mol Biol. 2018;98:407.

165. Fan J, Chen C, Yu Q, Brlansky RH, Li ZG, Gmitter FG. Comparative iTRAQ proteome and transcriptome analyses of sweet orange infected by '*Candidatus* Liberibacter asiaticus'. Physiol Plant. 2011;143:235-45.
166. Zhong Y, Cheng CZ, Jiang NH, Jiang B, Zhang YY, Wu B, et al. Comparative transcriptome and iTRAQ proteome analyses of citrus root responses to *Candidatus* Liberibacter asiaticus infection. PLoS One. 2015;10(6):e0126973. doi: 10.1371/journal.pone.0126973
167. Chiesa MA, Roeschlin RA, Favaro MA, Uviedo F, Campos-Beneyto L, D'Andrea R, et al. Plant responses underlying nonhost resistance of *Citrus limon* against *Xanthomonas campestris* pv. *campestris*. Mol Plant Pathol. 2019;20(2):254-69.
168. Solel Z, Pinkas J, Loebenstein G. Evaluation of systemic fungicides and mineral oil adjuvants for the control of Mal secco disease of lemon plants. Phytopathology. 1972;62:1007-13.
169. Graniti A, Perrota G. *Phoma tracheiphila* (Petri) Kanchaveli & Gikashvili in European handbook of plant diseases. London (UK): Blackwell Scientific publications; 1988.
170. Licciardello G, Grasso FM, Bella P, Cirvilleri G, Grimaldi V, Catara V. Identification and detection of *Phoma tracheiphila*, causal agent of citrus mal secco disease, by real-time polymerase chain reaction. Plant Dis. 2006;90:1523-30.
171. Tringali C, Parisi A, Piattelli M, Magnano di San Lio G. Phomenin A and B, bioactive polypropionate pyrones from culture fluids of *Phoma tracheiphila*. Nat Prod Let. 1993;3:101-6.
172. Daub ME, Ehrenshaft M. The photoactivated *Cercospora* toxin cercosporin: contributions to plant disease and fundamental biology. Ann Rev Phytopathol. 2000;38,461-90.
173. Choquer M, Dekkers KL, Chen HQ, Cao L, Ueng PP, Daub ME, et al. The *CTB1* gene encoding a fungal polyketide synthase is required for cercosporin biosynthesis and fungal virulence of *Cercospora nicotianae*. Mol Plant-Microbe Interact. 2005;18,468-76.
174. Fedoroff N. Redox regulatory mechanisms in cellular stress responses. Ann Bot. 2006;98:289-300.
175. Betti C, Reverberi M, Zjalic S, Mattei MB, Fabbri AA, Fanelli C. Different approaches to study the interaction citrus/phoma tracheiphila reveal an important role played by oxidative stress. J Plant Pathol. 2005;87:275.
176. Zun A, Seday U, Canihos E, Gulsen O. Oxidative enzyme responses of six citrus rootstocks infected with *Phoma tracheiphila* (petri) Kantschaveli and Gikashvili. Exp Agric. 2012;48(4):563-72. doi: 10.1017/S0014479712000464
177. Watanabe S, Matsumoto M, Hakomori Y, Takagi H, Shimada H, Sakamoto A. The purine metabolite allantoin enhances abiotic stress tolerance through synergistic activation of abscisic acid metabolism. Plant Cell Environ. 2014;37:1022-36. doi: 10.1111/pce.12218

178. Kajita S, Katayama Y, Omori S. Alterations in the biosynthesis of lignin in transgenic plants with chimeric genes for 4-coumarate: Coenzyme A ligase. Plant Cell Physiol. 1996;37:957-65. doi: 10.1093/oxfordjournals.pcp.a029045
179. Cristofani-Yaly M, Berger IJ, Targon MLPN, Takita MA, Dorta SDO, Freitas-astúa J, et al. Differential expression of genes identified from *Poncirus trifoliata* tissue inoculated with CTV through EST analysis and in silico hybridization. Genet Mol Biol. 2007;30:972-9. doi: 10.1590/S1415-47572007000500025
180. Pan Y, Michael TP, Hudson ME, Kay SA, Chory J, Schuler MA. Cytochrome P450 monooxygenases as reporters for circadian-regulated pathways. Plant Physiol. 2009;150:858-78. doi: 10.1104/pp.108.130757
181. Nafisi M, Goregaoker S, Botanga CJ, Glawischnig E, Olsen CE, Halkier BA, Glazebrook J. Arabidopsis cytochrome P450 monooxygenase 71A13 catalyzes the conversion of indole-3-acetaldoxime in camalexin synthesis. Plant Cell. 2007;19:2039-52. doi: 10.1105/tpc.107.051383
182. Menden B, Kohlhoff M, Moerschbacher BM. Wheat cells accumulate a syringyl-rich lignin during the hypersensitive resistance response. Phytochemistry. 2007;68:513-20. doi: 10.1016/j.phytochem.2006.11.011
183. Gayoso C, Pomar F, Novo-Uzal E, Merino F, De Ilárduya OM. The Ve-mediated resistance response of the tomato to *Verticillium dahliae* involves H_2O_2, peroxidase and lignins and drives PAL gene expression. BMC Plant Biol. 2010;10:232. doi: 10.1186/1471-2229-10-232
184. Tronchet M, Balagué C, Kroj T, Jouanin L, Roby D. Cinnamyl alcohol dehydrogenases-C and D, key enzymes in lignin biosynthesis, play an essential role in disease resistance in *Arabidopsis*. Mol Plant Pathol. 2010;11:83-92. doi: 10.1111/j.1364-3703.2009.00578.x
185. Xu L, Zhu L, Tu L, Liu L, Yuan D, Jin L, et al. Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahliae* as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. J Exp Bot. 2011;62:5607-21. doi: 10.1093/jxb/err245
186. Skyba O, Douglas CJ, Mansfield SD. Syringyl-rich lignin renders poplars more resistant to degradation by wood decay fungi. Appl Environ Microbiol. 2013;79:2560-71. doi: 10.1128/AEM.03182

How to cite this article:
Abbate L, Mercati F, Del Bosco SF. An Overview on Citrus Mal Secco Disease: Approaches and Strategies to Select Tolerant Genotypes in *C. limon*. Crop Breed Genet Genom. 2019;1:e190018. https://doi.org/10.20900/cbgg20190018