UNIVERSITY "MEDITERRANEA" OF REGGIO CALABRIA
AGRARIA DEPARTMENT
Ph.D. Course in Agricultural, Food and Forestry Sciences
Cycle XXXIV, DSS: AGR/07

Genetic characterization of eggplant (*Solanum melongena* L.) tolerance/resistance to soil-borne diseases

PH.D. THESIS

Ph.D. Candidate
Dott.ssa Maria Rosaria Tassone

Tutor
Prof. Francesco Sunseri

Co-Tutor
Dott. Giuseppe L. Rotino

Ph.D. Coordinator
Prof. Marco Poiana

Sommario

Abstract

The main topic of research was to characterise genes/QTLs associated with the resistance traits against one of the most feared telluric pathogens affecting eggplant: *Fusarium oxysporum* f. sp. *Melongenae* (*Fom*). Different genetic materials and populations available at CREA-GB (Core collections, RIL and Introgressed lines populations) were used for this purpose, and they were submitted to phenotyping for *Fom* tolerance/resistance (artificial inoculation) and genotyping by using different types of molecular markers. These activities were made possible thanks to the availability of information regarding a) QTL loci for the resistance already mapped in a F_2 population obtained from the cross between the two eggplant lines 305E40 x 67/3, b) a high-quality genome sequence of one mapping parent (67/3 line), c) a 35X Illumina sequencing of the other parent 305E40, and d) a 5x Illumina sequencing data of each RIL progeny from the hybrid 305E40 x 67/3. In the present thesis, a mapping study of the QTLs associated with the *Fusarium* tolerance trait previously located on chromosome 11, and *Fusarium* resistance trait previously located on chromosome 2, together with a BSA seq analysis, made it possible to identify 3 and 10 candidate genes respectively within the confidence interval of the QTLs. Chromosome 11 involvement in the partial resistance to *Fom* was confirmed by the high sensitivity of two BC6-BC6S1 progenies of the *S. tomentosum* introgression population carrying introgressed fragments by the wild parent on CH11, which replaced the *S. melongenae* 67/3 resistance QTL. In addition, within the eggplant collection, 13 accessions totally resistant to *Fom* were identified which could represent new sources of resistance traits and be employed into breeding programs of eggplant. Finally, to genetically dissect the positive or negative effect of NO_3 or NH_4 nitrogen supply at the early stage of interaction with *Fusarium oxysporum* in two genotypes was studied a transcriptome analysis was carried out. RNA seq analysis identified a conspicuous number of reciprocal and distinctive genes found in each pairwise comparison from the DEGs, however further analysis is required to better define their biological role.

Riassunto

L'argomento principale della ricerca è stato quello di caratterizzare i geni/QTL associati ai tratti di resistenza contro uno dei patogeni tellurici più temuti che colpiscono le melanzane: *Fusarium oxysporum* f. sp. *Melongenae* (*Fom*). Per questo scopo sono stati utilizzati diversi materiali genetici e popolazioni disponibili al CREA-GB (Core collections, RIL, e Introgressed lines), e sono stati sottoposti a fenotipizzazione per la tolleranza/resistenza a *Fom* (inoculazione artificiale) e genotipizzazione con diversi tipi di marcatori molecolari. Queste attività sono state possibili grazie alla disponibilità di informazioni riguardanti: a) i loci QTL per la resistenza già mappati in una popolazione F_2 ottenuta dall'incrocio tra le due linee di melanzane 305E40 x 67/3, b) una sequenza genomica di alta qualità di un genitore mappante (linea 67/3), c) un sequenziamento illumina 35X dell'altro genitore 305E40, e d) un sequenziamento illumina 5x di ogni progenie RIL dell'ibrido 305E40 x 67/3. Nella presente tesi, uno studio di mappatura dei QTLs associati al tratto di tolleranza al *Fusarium* precedentemente localizzato sul cromosoma 11, e al tratto di resistenza totale al *Fusarium* localizzato sul cromosoma 2, insieme ad un'analisi BSA seq, ha permesso di individuare rispettivamente 3 e 10 geni candidati all'interno dell'intervallo di confidenza dei QTLs. Il coinvolgimento del cromosoma 11 è stato inoltre confermato attraverso l'impiego di due progenie BC6-BC6S1 della popolazione di introgressione da *S. tomentosum*, dove è stata rilevata una risposta di suscettibilità marcata all'infezione in quanto portano frammenti introgressi dal parentale selvatico proprio sul CH11. Inoltre, all'interno di una Core collection di 350 accessioni rappresentative dell'intera variabilità del germoplasma della melanzana ne sono state individuate 13 totalmente resistenti a *Fom* che potrebbero essere impiegate in programmi di breeding. Infine, è stato studiato l'effetto positivo o negativo, rispettivamente, della fornitura di azoto NO_3^- o NH_4^+ nella fase iniziale dell'interazione con *Fusarium oxysporum* in due genotipi. L'analisi RNAseq ha identificato un numero cospicuo di geni condivisi ed esclusivi trovati in ogni confronto a coppie dai DEG, ma sono necessarie ulteriori analisi per definire meglio il loro ruolo biologico.

1. Introduction

1.1 Origin and taxonomy

The large plant family *Solanaceae* contains over 3000 plant species distributed in some 90 genera that are adapted to a wide range of geographic conditions. In the family eggplant (*Solanum melongena* L.), tomato (*S. lycopersicum* L.), potato (*S. tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and pepper (*Capsicum annuum* L.) are included. Eggplant, also known as brinjal or aubergine (*Solanum melongena* L., 2n = 2x = 24), in contrast to many New World-originated vegetables within the family, has probably origin in the Old World, mainly from India (Daunay et al., 2001, 2008; Barchi et al., 2018)

Three distinct cultivated eggplant species are known: the gboma eggplant (*S. macrocarpon* L.), the scarlet eggplant (*S. aethiopicum* L.) and the brinjal eggplant (*S. melongena*). The first two formers are indigenous and of minor economic importance, mainly grown in small scale local crop systems in Africa (Daunay and Hazra, 2012), by contrast, the brinjal eggplant is the most common species cultivated worldwide, in the Mediterranean basin but mainly in Asia, where it ranks among the top five most important vegetable crops (Frary et al., 2007; Syfert et al., 2016).

The three species are included in the *Solanales* order, *Solanaceae* family, *Solanoideae* subfamily, *Solaneae* tribe, *Solanum* genus, *Leptostemonum* subgenus. Members of *Solanum* subgenus *Leptostemonum* are defined by their sharp epidermal prickles and are commonly called “spiny solanums”.

Solanum is one of the largest genera of flowering plants in *Solanaceae*, encompassing some 1,250 to 1,700 species distributed worldwide, furthermore is the top 10 most species-rich seed plant genera (Frodin 2004; Bohs, 2005; Särkinen et al., 2013). *Solanum melongena* possess anthers that open by terminal pores and flowers that lack the specialized calyx found in the related genus *Lycianthes*, which also has poricidal anther dehiscence. *Solanum* species occur on all temperate and tropical continents and exhibit remarkable morphological and ecological diversity (Weese and Bohs, 2007).

1.2 Domestication and wild relatives

All three cultivated eggplants are members of the genus *Solanum* L., which is one of the largest genera of flowering plants and has more than 1000 species distributed worldwide (Bohs, 2005; Särkinen et al., 2013). All eggplants belong to the

Leptostemonum clade, a monophyletic group called the "spiny Solanum" due to the presence of sharp epidermal prickles on stems and leaves (Vorontsova et al., 2013) including almost all Old-World spiny solanums (Stern et al., 2011; Vorontsova et al., 2013) from Africa, Australia, and Asia (including Eurasia and the Middle East).

The progenitor of *Solanum melongena* is considered *S. insanum* L., a species widespread in tropical Asia, where eggplant was domesticated, from Madagascar to the Philippines. According to some authors, recent evidence suggests that there are two centres of domestication in China and/or India (Daunay and Janick, 2007; Wang et al., 2008; Weese and Bohs, 2010), with an additional, independent centre of domestication in the Philippines (Meyer et al., 2012a), whereas Vavilov (1951) previously considered *S. melongena* native from the "Indo-Chinese center of origin" including eggplant in the Indo-Burmese diversity centre. Eggplant is among the handful crops postulated to have multiple centers of domestication (Meyer et al., 2012b).

S. melongena and its wild ancestor *S. insanum* share pollinators and freely interbreed where cultivated and wild forms come together (Meyer et al., 2012a; Davidar et al., 2015).

Despite the eggplant wild relatives are intricate for their taxonomic and phylogenetic relationships (Vorontsova et al., 2013), they have been classified based on the biosystematic data and their cross compatibility with the cultivated species into three genepools (Taher et al. 2017): i) the primary genepool consists in the cultivated eggplant (*S. melongena)* and its wild ancestors which can be crossed easily and produce normal fertile hybrids (Plazas et al., 2016; Ranil et al., 2017); ii) the second genepool includes a large number of wild relatives like *S. dasyphyllum, S. linnaeanum* and *S. tomentosum* L. that can be crossed with eggplant, nonetheless the crosses success and the hybrids viability or fertility may be reduced, until to some interspecific hybrids partially or completely sterile (Rotino et al., 2014; Kouassi et al., 2016); iii) the third genepool includes many distant related species like *S. torvum* Sw., *S. elaeagnifolium* Cav. and *S. sisymbriifolium* Lam., which breeders attempt to use for incorporating their resistance features into the cultivated eggplant, but seeds from sexual crosses are hard to be obtained and the interspecific hybrids are sterile (Kouassi et al., 2016; Plazas et al., 2016; Syfert et al., 2016).

Solanum melongena and the two other cultivated eggplants *S. aethiopicum* and *S. macrocarpon* are related to many wild species (Vorontsova et al., 2013; Syfert et al., 2016) that may serve as sources of genetic variability that can be used in breeding

programs, for traits related to climate change adaptation but also pest and disease resistance (Rotino et al., 2014).

1.3 Production and economic importance

Eggplants global production has been grown year by year during the last two decades (Figure 1) and it is estimated to be around 55 Mt in 2019 on a 1,850,000 Ha harvested area (Figure 1) (FAO data 2019; http://www.faosta.fao.org). In production terms, eggplant is the third most important solanaceous crop species (after potato and tomato) and is cultivated all over the world. The production is mainly concentrated in Asia (94.23% of the world production), followed by Africa (3.36%) and Europe (1,66%) (Table 1). In Europe, there has been a decrease in eggplant production, which is probably due to the reduction in cultivated area in recent years (Figure 2). Italy, with an estimated production of more than 300,000 tonnes over a harvested area of 10,000 ha was the largest European producer in 2019, followed by Spain (Figure 3). Italian production, concentrated in the southern regions (Sicily, Campania, Puglia, and Calabria), is extended all year round thanks to the partial overlap of greenhouse and open field production allowing for local marketing (fresh or processed) or export to Northern Europe (Daunay, 2008).

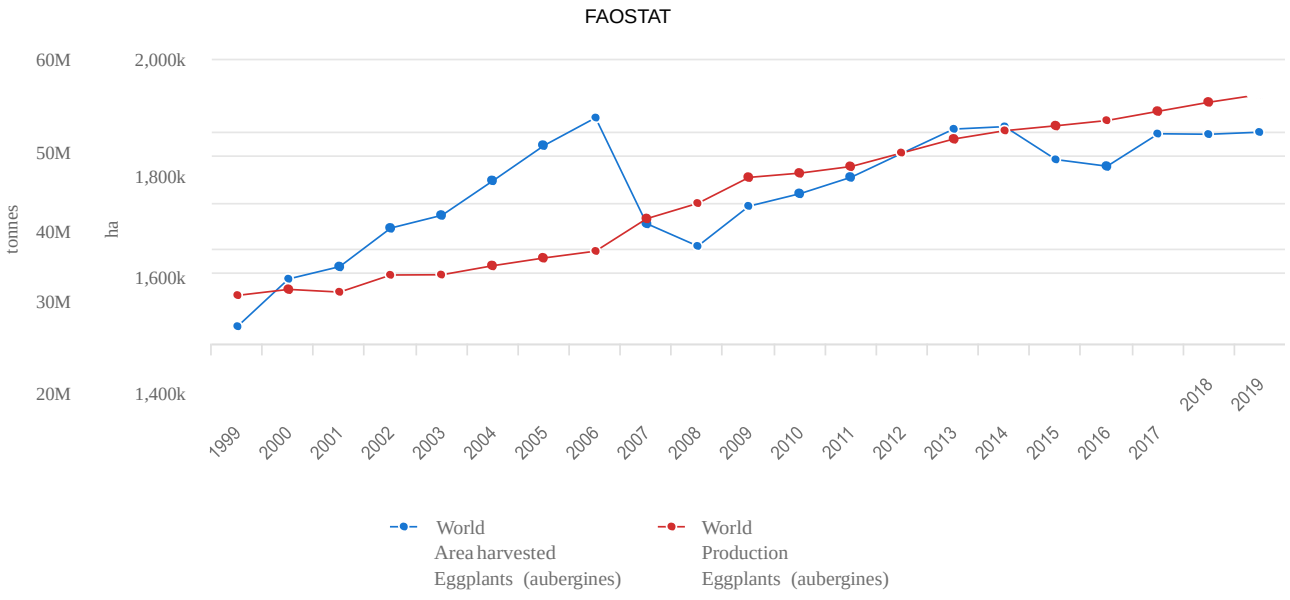


Figure 1 Harvested area and production of Eggplants in World from 1999 to 2019.

Countries	Production Quantity (tonnes)	Area harvested (ha)	Yield (hg/ha)
China	35590700	782998	454544
India	12680000	727000	174415
Egypt	1180240	43818	269350
Turkey	822659	23337	352513
Iran	670158	21350	313891
Indonesia	575392	43954	130908
Japan	301700	8650	348786
Italy	300620	9550	314785
Philippines	249890	21819	114529
Spain	245150	3470	706484

Table 1 Economic data for top 10 countries in eggplant production (FAO, 2019)

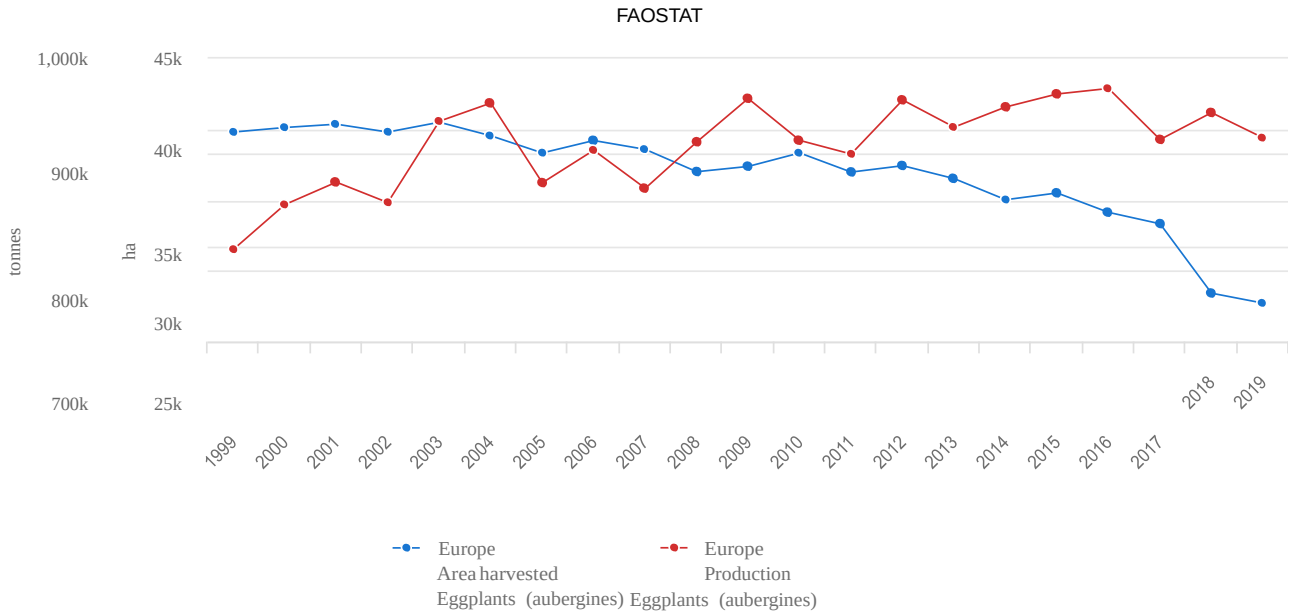


Figure 2 Harvested area and production of Eggplants in Europe

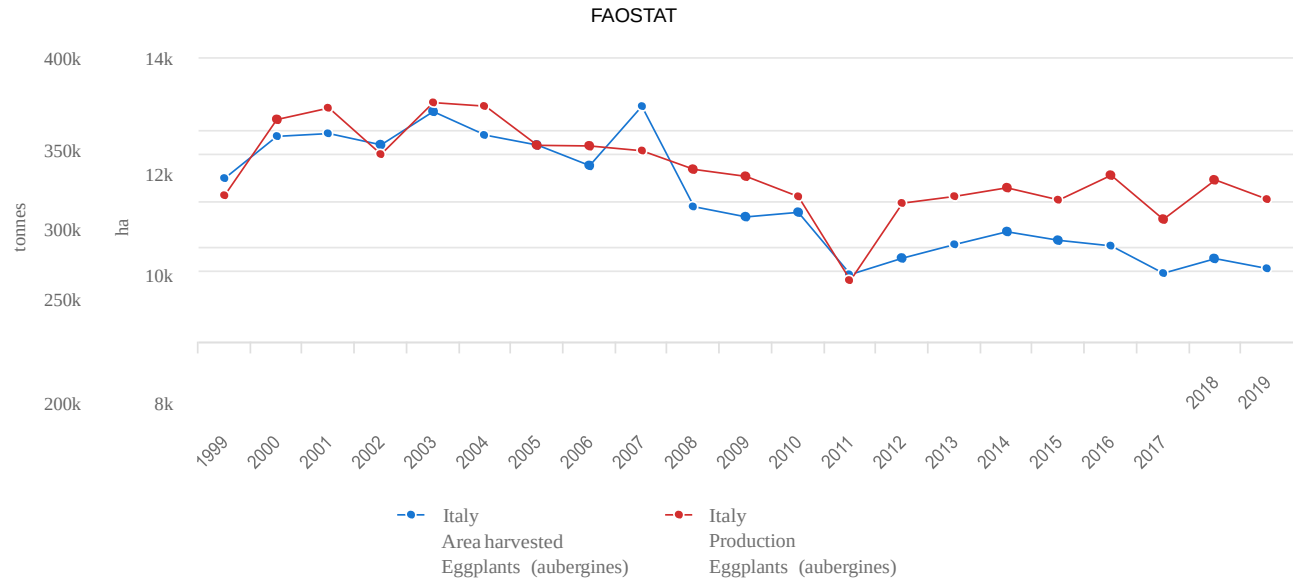


Figure 3 Harvested area and production of Eggplants in Italy

1.4 Botanical framework, cultivation method and requirements

Countries	Production Quantity (tonnes)	Area harvested (ha)	Yield (hg/ha)
China	35590700	782998	454544
India	12680000	727000	174415
Egypt	1180240	43818	269350
Turkey	822659	23337	352513
Iran	670158	21350	313891
Indonesia	575392	43954	130908
Japan	301700	8650	348786
Italy	300620	9550	314785
Philippines	249890	21819	114529
Spain	245150	3470	706484

Germination takes 8-12 days at the optimal range of temperatures (22-28 °C) while at 12-13° C the germination lasts up to 4 weeks and can be reduced by up to 50% (Panero, 1981). Under 12° C the germination capacity is significantly reduced. The expansion of the cotyledons takes a few days, and the first true leaf appears after one week. Depending on the cultivar, the first flowers appear when the plant has developed 5-12 leaves (20-30 cm

tall). Vegetative growth and flowering are then continuous: every 2 developed leaves a new flower appears on each branch. This species is photoperiod neutral (Chen et al., 2002). In general, a temperature range between 15° C and 18° C at night, and between 25° C and 30° C during the day is recommended for the optimal vegetative growth, and 22°C for reproductive phases based on the number and size of fruits produced (Caruso et al., 2017). The optimum relative air humidity ranges from 65 to 70%. Pessarakli (1999) showed that damage caused by 10−15°C temperature range may occur at any stage of eggplant growth and development. Eggplants can tolerate both slight drought and excessive rainfall, but high temperature and abundant rainfall promote the vegetative phase (Chen et al. 2002). Soil must be well drained to avoid water stagnation around the stem. Otherwise, limited water availability leads to the formation of low-quality fruits which grow stringy and bitter, in general, the vegetable crop requires 340-515 mm irrigation per season. Further, it produces a strong root system in soil when is deep, fertile, well drained and high in organic matter with pH values of 5.5–6.8 (Panero, 1981). High clay content and waterlogging should be avoided due to the build-up of root rot (Chen et al., 2002). Climate and soil factors affect eggplant flowering and fruit set as well as the commercial production outcome (Caruso et al., 2017).

The plant is woody and develops several branches according to a roughly dichotomic ramification pattern, it is cultivated as a vegetable that yields immature edible fruits. In temperate climates eggplant is grown as an annual, in tropical climates it is a short-lived perennial (up to 2 years in commercial field). The erect stem is initially herbaceous, then lignifies during the season, becoming resistant to wind and the weight of the fruits. The height of the plant is generally between 60 and 150 centimetres when left to grow freely; under protected cultivation where pruning is generally applied to have plants with 2-3 stems, the height can be more than 200-250 cm; exceptions can be observed among the cultivars. Anthocyanins, prickles and hairiness on vegetative organs and calyx vary quantitatively, as on the leaves, on both leaf sides for the hairs, or on the leaf veins for the prickles.

The leaves are alternate, simple with a petiole ranging from 5 to 10 cm; leaf shape may be straight or dentate often without stipules; leaf blade may be ovate and oblong, sized 3-25 x 2-15 cm long or more. The leaf colour ranges from light green to dark green, but light purple to dark violet leaf pigmentations due to the presence of anthocyanins is also common.

Inflorescences are 1 to 5 andromonoecious cymes, although most modern cultivars display solitary hermaphrodite flowers. Flowers usually are regular, large 3-5 cm in diameter, whit 1-3 cm long pedicel, but up to 8 cm in fruit; the calyx is campanulate, sometimes with prickles; the ovary is superior, 2 or many-celled, the style can be shorter (ipostyle), equal (mesostyle) or longer (longistyle) than stamens; the stigma is green (sometime purple for anthocyanin content), headed and lobed. The basic flower type is 5-merous (5 sepals, 5 petals, 5 stames) but 6,7, and 8-merous flowers are commonly found in globose and round fruited types. The fivelobed corolla shows a broad range of colours, from white to pink, purple or violet; stamens are inserted near the base of the corolla tube and alternate with corolla lobes, filaments are short and thick, the anthers are bicameral, yellow, and opening by terminal pores. The maturation of the male and female organs generally occurs at the same time to favor self-pollination. Eggplant is considered to be prevalently an autogamous species however, in open fields and warm conditions also the allogamy is possible, reaching 70% or more, due to anemophilous and entomophilous pollination facilitated by longistyled flower.

The fruits are berries of highly variable shape (round, intermediate, long, snake-like) and size (tens of grams to more than a kilo), 2-35 cm long (sometimes longer) and 2-20 cm broad, a huge phenotypic variation is observed for calyx coverage and presence of prickles on the calyx, peduncle length, variable firmness of flesh, smoothness shininess and hardness, of skin. Based on the fruit shape, cultivars have been divided into three main botanical varieties: the round, oval or egg-shaped cultivars are grouped under *S. melongena* var. *esculentum* or common eggplant; the long slander types are included under *S. melongena* var. *serpentinum*, or snake eggplant; the small and straggling fruited plants belong to *S. melongena* var. *depressum* or dwarf eggplant (Martin and Rhodes, 1979). The absence or presence as well as the distribution pattern of two kinds of pigments, particularly, chlorophylls and anthocyanins, control a wide diversity of fruit skin colours (Daunay et al., 2004; Frary et al., 2007). In fact, the most popular fruits exhibit different intensities from a unique colour (black, dark purple, dark violet, green, white) to a pigmentation characterized by a prevalent colour combined with the presence of a secondary ones in form of stripes along the fruit or shades around and under the calyx. Seeds are lenticular to reniform, flattened, of about 3 mm x 4 mm. Fruit sets one week after anthesis, and 3-6 weeks are needed to reach commercial ripeness, depending on climatic conditions. The

fruits reach physiological maturity 6-13 weeks after flowering, also depending on the climate, physiologically ripe fruits are brown or yellow.

Eggplant is traditionally grown in open field, but it is also an important greenhouse crop in the Mediterranean basin and in many other countries worldwide. The species can be grown successfully in most commercial soilless culture systems, including cultivation in substrates and nutrient solution. Where intensive and continuous cropping on the soil is quite common, grafting may be a valuable non-chemical alternative, and it is often associated with soil solarization in the greenhouses in Mediterranean area where summer temperatures are too hot to grow plants (Moncada et al.2013). Research on rootstocks for eggplant started in Asia, especially in Japan and Korea, where grafting technique has been used for vegetables since the 1920s (Lee, 1994). The first utilized rootstock was *S. melongena* lines and hybrids tolerant to bacterial and *Fusarium* wilt (Monma et al., 1997; Yoshida et al., 2004 a and b). In Europe, eggplant was grafted mostly into tomato or tomato interspecific hybrids (*L. esculentum* x *L. hirsutum* or *S. lycopersicum* × *S. habrochaites*) which, in addition to their resistance to several soil born pathogen, bring a good tolerance to low soil temperatures (Ginoux and Leterrot, 1991; Miles et al., 2015). In the last decades, *S. torvum* becomes the most common eggplant relative used for grafting; it is a wild species possesses a wide range of soil borne disease resistances, and a valuable rootstock which increase both the vigour and yield of scion even if exhibits long germination time and often show irregular and erratic germination even under favourable growth conditions (Ginoux and Laterrot, 1991; Gisbert et al., 2011). Although *S. torvum* is resistant to *Verticillium* wilt, cases of wilt have been reported in commercial culture of grafted eggplant (Garibaldi et al., 2005). Positive effects on fruit production, eggplant scion growth, and control of *Verticillium* wilt were obtained using *S. torvum*, and similar results were obtained with *S. sisymbriifolium* though control of *Verticillium* wilt was less efficient (Bletsos et al. 2003, Daunay M.C., 2008).

Another utilized rootstock is *S. integrifolium* (i.e., *S. aethiopicum, aculeatum* group) that resists to *Fusarium* and bacteria wilts and present a good graft affinity with *S. melongena* (Yoshida et al., 2004). It is used directly as rootstock or as parent crossed with *S. melongena* varieties for producing interspecific hybrid rootstock [*S. integrifolium* x *S. melongena*] cumulating resistance from both parents (Yoshida et al., 2004b; Mian et al., 1995).

The eggplants yield when grafted on S. *torvum* is better compared to *S. integrifolium* and *S. toxicarium* (i.e., *S. stramonifolium)* (Mochizuki and Yamakawa, 1979a). *Solanum* spp. rootstocks can provide other useful advantages, such as enhanced photosynthesis and transpiration, a lower degree of *Leucinodes orbonalis* infestation, resistance to windfall and chilling tolerance (Alam et al., 1994; MeiXiu et al., 2001; LanChun et al., 2004; Daunay, 2008).

The eggplant is characterised by strong vegetative growth; for this reason, often among the cultivation techniques applied on this species is included the pruning of plants in tunnel or greenhouse. Indeed, optimum spacing and proper pruning increase yield substantially and improve fruit quality in the eggplant (Pessarakli and Dris, 2003). Buczkowska (2010) demonstrated the positive effect of eggplant pruning that leaves 2–4 branches per plant on yield increases up to a certain threshold, which depends also on plant density, and on earliness. However, an excessively high stem density can negatively affect mean fruit size (and thus fruit quality and marketable yield), even though the total yield may still increase. Generally, strong lateral branches are allowed to grow following formation of the first flower, while all lateral shoots below that height are removed. In addition to one main flower, one or more secondary flowers may develop at each node. Most secondary flowers do not set fruit, but may happen, depending on plant vigour, that some of them develop a small fruit but undersized and thus non-marketable and generally removed. Additionally, improving light conditions in the plant profile by pruning increased leaf calcium and phosphorus contents in eggplant (Michałojć and Buczkowska, 2012). The proper balance between plant density range and pruning has not yet identified to allow for increasing field production (Caruso et al., 2017).

Eggplant fruits are harvested at a range of developmental stages depending on the cultivar, but generally when immature, before seeds enlarge and harden, and thus commercially mature and not physiologically mature. Normal quality indices apply, such as size, skin colour, absence of defects, characteristic shape, and presence of a fresh calyx (Baudoin et al., 2013).

The stage at which eggplant should be harvested is not easy to identify without experience. In case of anthocyanin coloured skin, eggplants turn from dark purple when immature, to a brownish colour when fully ripe. In between these two stages is the correct time for harvesting (Mohammed and Sealy, 1986). Indeed, in purple, violet and black eggplant

fruits, the stage of commercial maturation is empirically and visually evaluated based on the peel color intensity and brightness. Eggplant fruit begin to gradually lighten and pale from the tip to calyx. Maynard (1987) indicated that the appropriate time to maximize both yield and quality would be to harvest fruit when they reach about 80% full size. Immature eggplants have a much-reduced shelf life, wrinkling and softening quickly after harvest. As the fruit mature, the flesh softens and becomes spongy (Frary et al., 2007). Fruits are harvested manually by cutting. Fruit of marketable size should be cut from the plant, leaving the calyx attached to the fruit, while overmature fruit should be removed from the plant and discarded in the field to stimulate further flowering and fruit set (Baudoin et al., 2017).

1.4.1 Nitrogen fertilization

Nitrogen is considered one of the essential macronutrients required by plants for growth, development and yield, stimulating growth and photosynthesis (Singh et al., 2003). In addition, nitrogen is the main constituent of amino acids in proteins and lipids that act as structural compounds in the chloroplast (Basela and Mahadeen, 2008). Atmospheric nitrogen (air is 79% N_2 gas) cannot be readily absorbed by plants, with the exception of legumes through the biological fixation of N by *Rhizobium* bacteria located in the nodules that have the ability to convert atmospheric N_2 into a form that the plant can use. The specific uptake of N forms depends mainly on the environmental conditions and soil N availability. In the soil N exists in different forms that change very easily from one form to other. These different N sources can be utilised by plants as the mineral form of nitrate ions (NO_3^-), ammonium (NH_4^+), and to a lesser extent gaseous ammonia (NH_3), nitric oxide (NO), amino acids and organic peptides (von Wiren et al., 1997; Miller et al., 2007). However, nitrate, which is mobile in the soil, limits the uptake of harmful elements, such as chloride, in large quantities, but at the same time synergistically promotes the intake of cations, such as Ca_2^+, K^+ and Mg_2^+, while ammonium competes for uptake with these cations. Otherwise, ammonium is subject to volatilisation when it is not immediately incorporated into the soil; therefore, significant quantities may be lost (Sun et al., 2019). The eggplant yield is highly responsive to N fertilization, frequently, it was observed and reported that eggplant fruit yield increased with increase in N up to 187.5 kg N/ha, as well as increased nitrogen significantly delayed eggplant flowering and increased the number of days required for eggplant fruit set. In addition, nitrogen fertilizers affected seed number, fruit pH, crude protein, total solid and ascorbic acid of eggplant, while nitrogen deficiencies

reduced both physical and chemical properties (Pal et al., 2002; Sat and Saimbhi, 2003; Akanbi et al., 2007). A nutrient solution with a higher N concentration stimulated dry mass accumulation, stem elongation and leaf expansion rate, but decreased the root:shoot dry weight ratio and root whole weight of eggplant (Basela and Mahadeen, 2008). Applying a dosage of 75 kg/ha of fertilized instead of recommended rate, Wange and Kale (2004) observed significant improvement in plant height, number of leaves and yield (74%) of eggplant. Applying instead a higher dosage, 120 kg/ha, Devi et al. (2002) found improved fruit girth, fruit weight and fruit yield level of eggplant. It is a well-known that eggplant need adequate nitrogen for satisfactory growth, development, and high yield; therefore, an adequate level is essential to increase the production and yield of eggplant (Aminifard et al., 2010). The interaction between plants and pathogenic microorganisms is complex being influenced by multiple environmental factors such as temperature, light, humidity, and nutrients. Also consider that N accounts for ~1.5% to 2% of plant dry matter and ~16% of total plant proteins (Scheible et al., 2004). N assimilation is related to key physiological or metabolic processes such as photosynthesis, photorespiration, respiration, amino acid synthesis and the tricarboxylic acid (TCA) cycle (Makino et al., 2011; Foyer et al., 2011). Thus, it might be deduced that plant N status can influence its tolerance/resistance to several abiotic and biotic stresses. In particular, N availability may limit pathogen growth and influence the elicitation and deployment of plant defences. Furthermore, the different N forms (ammonium [NH_4^+] *vs.* nitrate [NO_3^-]) supply showed different effects on plant disease resistance (Tab. 2), also because of diverse assimilation and metabolism pathways (Mur et al., 2017; Bolton et al., 2008; Sun et al., 2020).

Disease Incidence	Effect of Nitrogen in the Form of		
	Unspecified N	NH_4^+	NO_3^-
Cases	73	19	22
Increase in disease	40	9	13
Decrease in disease	25	9	8
No effect or variable	8	1	1

Table 2 Number of published papers reporting the effects of nitrogen nutrition on plant disease incidence. The data were collected from 132 published papers that related to nitrogen nutrition and plant disease ranging from 1944 to 2019 (from Sun et al., 2020).

N availability modulates plant cell structure and composition through its effects on primary and secondary metabolism, thereby influencing plant disease defences by affecting the thickness of the plant physical barrier. As N supply rate increase plant growth is promoted,

but at the same time a delayed deposition of lignin on the xylem cell wall and a reduction in the thickness of the epidermal cuticle were observed (Camargo et al., 2014; Jauset et al., 2000). As a consequence, plants become favourable to the penetration of pathogenic microorganisms and insect herbivores (Kovácik et al., 2007; Sun et al., 2019).

Nitrogen has usually negative effects on structural defenses and the production of anti-microbial phytoalexins whereas it positively affects local protection and systemic response due to defense-related enzymes and proteins. N nutrition may also induce a response through amino acid metabolism and hormone production triggering the downstream defense-related gene expression *via* transcriptional regulation and nitric oxide (NO) production (Sun et al, 2020).

Many studies on plants grown under different N conditions have been performed to identify the expression patterns of N-reactive and Nitrogen Use Efficiency (NUE) related genes through transcriptomic analysis (e.g., Amiour et al., 2012; Gelli et al., 2014; Simons et al., 2014). However, few studies on differential transcriptomic profiling in eggplant have been reported, not focused on N metabolism and NUE trait (Li et al., 2018, 2019; Zhang et al., 2019). More recently, Mauceri et al. (2021) reported a transcriptomics analysis comparing four NUE-contrasting eggplant genotypes following short- and long-term exposure to low N, to identify key NUE-related genes in root and shoot. Genes differentially expressed at low N underlined an involvement of the light-harvesting and receptor complex, a ferredoxin-NADP reductase, a catalase, and the TF *WRKY33*. NUE is a complex trait of great interest in future eggplant breeding programmes because through its improvement, high crop yields can be maintained while N supply is reduced.

2. Eggplant genome and genetic maps

2.1 Breeding objectives

The main breeding objectives, irrespective of the country of cultivation, relate to quantitative and qualitative aspects of the fruit yield. Therefore, one of the main objectives is the adaptation of cultivars to various growing conditions including climate (from temperate to tropical), season of growth and production system (open field or greenhouse). Eggplant breeding programs include improving agronomic traits, such as yield, fruit quality, and postharvest quality, and improving resistance traits to biotic and abiotic stresses (Vilanova, et al., 2012). Quality factors include plant prickliness and postharvest

quality to facilitates transport and extends shelf-life. A long-lasting pigmentation and glossiness of the commercial berries, and a reduced number or absence of seeds (parthenocarpy) are important qualitative traits of commercial importance as well as the fidelity maintenance to the fruit typology when grown in different environments. Recently organoleptic and nutritional properties, bioactive and anti-nutritional compounds, post-harvest (Frary et al., 2007) and processing-related traits of eggplant fruits (flesh consistency and browning, absence of seeds) have been studied in a genetic/genomic perspective with particular attention to the chlorogenic acid and anthocyanin content (Prohens et al., 2007, 2013; Gajewski et al., 2009; Docimo et al., 2016; Lo Scalzo et al., 2016). Drought stress is considered the most significant abiotic stress facing agriculture worldwide and improving crops under drought is a goal of crop breeders in general and eggplant breeders specifically (Cattivelli et al., 2008).

Breeding programs aim to increase pathogen and pest resistance of the cultivated eggplant. The most common diseases include *Fusarium* wilt (*Fusarium oxysporum* f.sp. *melongenae*), *Phytophthora capsici*, and *Verticillium* wilt (*Verticillium dahliae*), fruit anthracnosis (*Colletotrichum gleosporioides*), root knot nematodes (*Meloydogyne* spp.) along with bacterial wilt (*Ralstonia solanacearum*) (Sekara, et al., 2007; Naegele, et al., 2014). The expected and already ongoing climate change will further increase the spread and virulence of plant pathogens, underling the need to find new resistance traits to be used (Pautasso, et al., 2012). This awareness is increasingly encouraging eggplant breeding programs towards immediacy with the need to obtain cultivars with introgressed resistance genes to known and emergent pathogens (Kinsell et al., 2016).

Wild relatives of eggplant have been used as sources of resistances to these diseases, suitable to be transferred into cultivated eggplant by interspecific hybridization. Ano et al. (1991) and later Robinson et al. (2001) used *S. aethiopicum* as a source of bacterial wilt resistance and *S. incanum* as a source of *Verticillium* wilt resistance, respectively. Partial resistance to *Verticillium dahliae* has also been introduced into *S. melongena* from *S. linnaeanum* (syn. *S. sodomeum*) (Acciari et al., 2001). Resistances to *Meloidogyne* nematode species and various insects have been described either in *S. melongena* germplasm or related species, but their use poses difficulties such as insufficient levels of resistance, limited crossability and difficulty of evaluation (Frary et al., 2007). Eggplant wild relatives cannot always be easily crossed with the cultivated eggplant; interspecific crosses between *S. melongena* and the other related *Solanum* species bearing desirable

agronomical traits, have sometimes been limited by sexual barriers (Behera and Singh, 2002; Saini et al., 2019). Therefore, insertion of resistance genes is, along with yield and fruit quality improvement, one of the main concerns of conventional and biotechnological breeding. Till to date, more than 25 wild species of genus *Solanum* were used for crossing with cultivated eggplant with a limited success rate (Rotino et al., 2014). Interspecific hybrids in eggplants can act as rootstocks in order to protect plants from fungal and bacterial infestations (Rotino et al., 1997); for example, it was shown that *S. incanum* can be easily crossed with cultivated eggplant and the resultant interspecific hybrids are fertile (Ranil et al., 2017). Even though various techniques can yield successful fertile hybrids, wild relatives need to be used with caution because they may transfer unfavourable genes or genomic regions (such as high susceptibility to *Colletotrichum gloeosporioides* and bitter taste, because of a high level of steroid saponin, found in *S. torvum* and *S. linneanum* respectively) to hybrids in addition to traits potentially useful for eggplant breeding (Kashyap et al., 2003; Kouassi et al., 2016). These undesirable traits can be eliminated with several generations of backcrossing (Kouassi et al., 2016). Overall, the use of conventional breeding for the improvement of eggplant is limited by several barriers such as crossing issues (pre and post fertilization) linkage drag, etc. (Zulkarnain et al., 2015; Saini et al., 2019; Toppino et al., 2021). To overcome the above-mentioned limitations of conventional breeding, biotechnological approaches such as embryo rescue, *in vitro* selection, anther culture, somatic hybridization genetic transformation and genome editing can be used as for the genetic improvement of eggplant (Collonnier et al., 2001; Sharma et al., 2002; Rotino et al., 2014).

For the economic and nutritional significance of eggplants, breeding efforts focus on developing high-yielding varieties, mostly F_1 hybrids. Studies indicate the successful exploitation of heterosis in the eggplant for a considerable improvement of quantitative traits. Thus, estimating the heterosis for yield-related traits as well as other complex traits of agronomic interest could be useful for examining the most beneficial hybrid combination (Kakizaki et al., 1931; Kumar et al., 2020).

2.2 The eggplant genome

Eggplant is a prevalently selfed diploid species with a basic chromosome number of 12 (2n=2x=24). The eggplant chromosome number is the same in all varieties but shows varied bivalents due to the chiasma frequency during diplonema and diakinesis (Simmonds,

1993). The eggplant nuclear genome contains about 1,14 Gb of DNA and it is larger than tomato (740 Mb) and diploid potato (1,000 Mb) ones, but smaller than the genome of pepper (2,200 – 2,700 Mb) (Arumuganathan and Earle, 1991). The first eggplant genome draft sequence SME_r2.5.1 of the inbred Asian eggplant cultivar Nakate-Shinkuro was determined in 2014 (Hirakawa et al., 2014); although scarcely annotated and only partially (12%) anchored to a genetic map. The non-chromosome-anchored draft sequence of this eggplant genome dataset is composed by 33,873 scaffolds that cover 833.1 Mb, the 74% of the eggplant genome. Recently, a chromosome-anchored draft of eggplant genome (v3.0) was assembled by an Italian consortium (Barchi et al., 2019) using the line '67/3', a breeding line developed at CREA (www.eggplantgenome.org). The hybrid assembly, covering 1.22 Gb, was obtained by merging Illumina sequencing data and optical mapping. The female parent of the RIL mapping population (line '305E40') was also sequenced (coverage of 35X), and thanks to low coverage resequencing (1X) of the F_6 Recombinant Inbred Line (RIL) population a physical map of the assembled genome was obtained. In detail, 469 scaffolds were identified and anchored on chromosomes using the SoiLoCo pipeline (Scaglione et al., 2016) and a linkage map comprising 5,964 markers, developed from an F6 RIL (Barchi et al., 2012; 2019). From the '67/3' assembly and additional RNA-Seq data from 19 tissues of '67/3, obtaining 34,916 high-quality protein-coding gene models (Barchi et al., 2019). Recently, based on Illumina sequencing data, a 1.02 Gb draft genome of *S. aethiopicum* was developed (Song et al., 2019), which contained predominantly repetitive sequences (78.9%), annoting 37,681 gene models, including 34,906 protein-coding genes. The same group resequenced at a high depth (~60×), 60 *S. aethiopicum* genotypes in 2 major groups, "Gilo" and "Shum", and 5 accessions of *S. anguivi*, (the progenitor of *S. aethiopicum);* 18,614,838 single-nucleotide polymorphisms (SNPs) were identified, of which 34,171 were located within disease resistance genes. (Song et al., 2019). In the same period, the resequencing of seven eggplant accessions and one accession of the wild relative *S. incanum,* which are the parents of a MAGIC population, has been performed by Gramazio et al. (2019); a set of identified SNPs polymorphisms has been annotated. In 2020 a new high-quality reference genome for the eggplant inbred line HQ-1315 (*S. melongena*-HQ) using a combination of Illumina, Nanopore and 10X genomics sequencing technologies and Hi-C method for genome assembly was released. The assembled genome has a total size of ~1.17 Gb and 12 chromosomes, with a contig N50 of 5.26 Mb, consisting of 36,582 protein-coding genes

(Wei et al., 2020). The use of genomes is becoming a compulsory tool for the identification of candidate genes for important traits in eggplant.

Finally, Barchi et al. (2021) obtained a novel, highly contiguous genome assembly of the eggplant '67/3' reference line (v4.0), by Hi-C retrofitting of a previously released short read- and optical mapping-based assembly. This has allowed to assign scaffolds to chromosomes using a novel iterative approach which resulted in a large reduction of the size of the 'unknown' chromosome chr. 0 (from 22.5% to 4.4% of the total assembly); compared with v3.0, the percentage of anchored genes in v4.0 increased to 95.6% (Tab 3).

	Smel v1.0	*S. aethiopicum*	Smel v3.0	Smel CL	Smel v4.0
Size of assembly	0.83	1.02	1.21	1.07	1.16
Contig N50 (kb)	14.3	25.2	678.7	5260	702.9
Scaffold N50 (Mb)	0.065	516.1	2.9	89.64	92.1
% Ns	4.75	–	28.23	0.38	9.10
% anchored	–	–	77.5%	92.7%	95.6%
Protein-coding genes	85 446	34 906	34 916	36 582	34 916
% anchored genes	0%	0%	81.4%	97.4%	96.6%
Annotated BUSCO genes	74.8%	77.8%	96.9%	94.2%	96.9%

Table 3 Metrics of publicly available eggplant genome assemblies: Smel v1.0 from Hirakawa et al. (2014), S. aethiopicum from Song et al. (2019), Smel v3.0 from Barchi et al. (2019b), Smel CL from Wei et al. (2020a), and Smel v4.0 (from Barchi et al., 2021)

Furthermore, twenty-four *S. melongena* accessions, representative of the genetic and phenotypic diversity of the species as well as an accession each of the closest wild relatives *S. insanum* and *S. incanum* (non-melongena species [NMSs]) were resequenced to infer an early eggplant pan-genome and pan-plastome. The final genome sizes ranged from 826 to 999 Mb and the N50 value from 3 to 26.8 kb. A total of 816 protein-coding genes with Annotation Edit Distance (AED) score < 0.5 were predicted in the novel sequences. The eggplant pan-genome, including reference and new genome sequences, counted of 1.21 Gbp total size and contained 35,732 protein-coding genes.

2.3 Molecular mapping

In the past, as in several other crops the low level of polymorphism within the cultivated eggplant germplasm required huge efforts for detecting molecular markers exploitable for linkage mapping purposes (Stàgel et al., 2008; Barchi et al., 2019). In eggplant, several inter-specific genetic maps built up by applying pre-NGS (next generation sequencing) techniques (RFLP, AFLP, RAPD, SSR, etc.) were developed, both based on inter and intra-specific populations.

Although the related solanaceous species tomato, potato and pepper have been the focus of several molecular researches, genetic mapping in eggplant has only initiated in the last three decade.

The first molecular map was developed on an intraspecific F_2 population of 168 individuals derived from an intraspecific cross between two *S. melongena* lines: EPL-1, a Japanese commercial-type line, and WCGR112-8, a bacterial wilt-resistant line. The resulting map contained RAPD markers defining 13 linkage groups (Nunome et al., 1998), and later was completed with AFLPs (Nunome et al., 2001) and SSR (Nunome et al., 2003b). This last intraspecific map contained initially 181 markers (93 AFLP, 88 RAPD) in 21 linkage groups spanning 779.2 cM with an average interval of 4.9 cM (Nunome et al., 2001); later, it was improved to a total of 162 markers in 17 linkage groups-encompassing 716.9 cM at an average spacing of 4.9 cM. Nunome et al. (2009) constructed a linkage map, with 236 mapped segregating markers to 14 linkage groups, spanning 959.1 cM, with an average marker distance of 4.3 cM. The discrepancy between the number of LGs and the number of haploid chromosomes of *S. melongena* indicates the low coverage of the genome by markers in these studies.

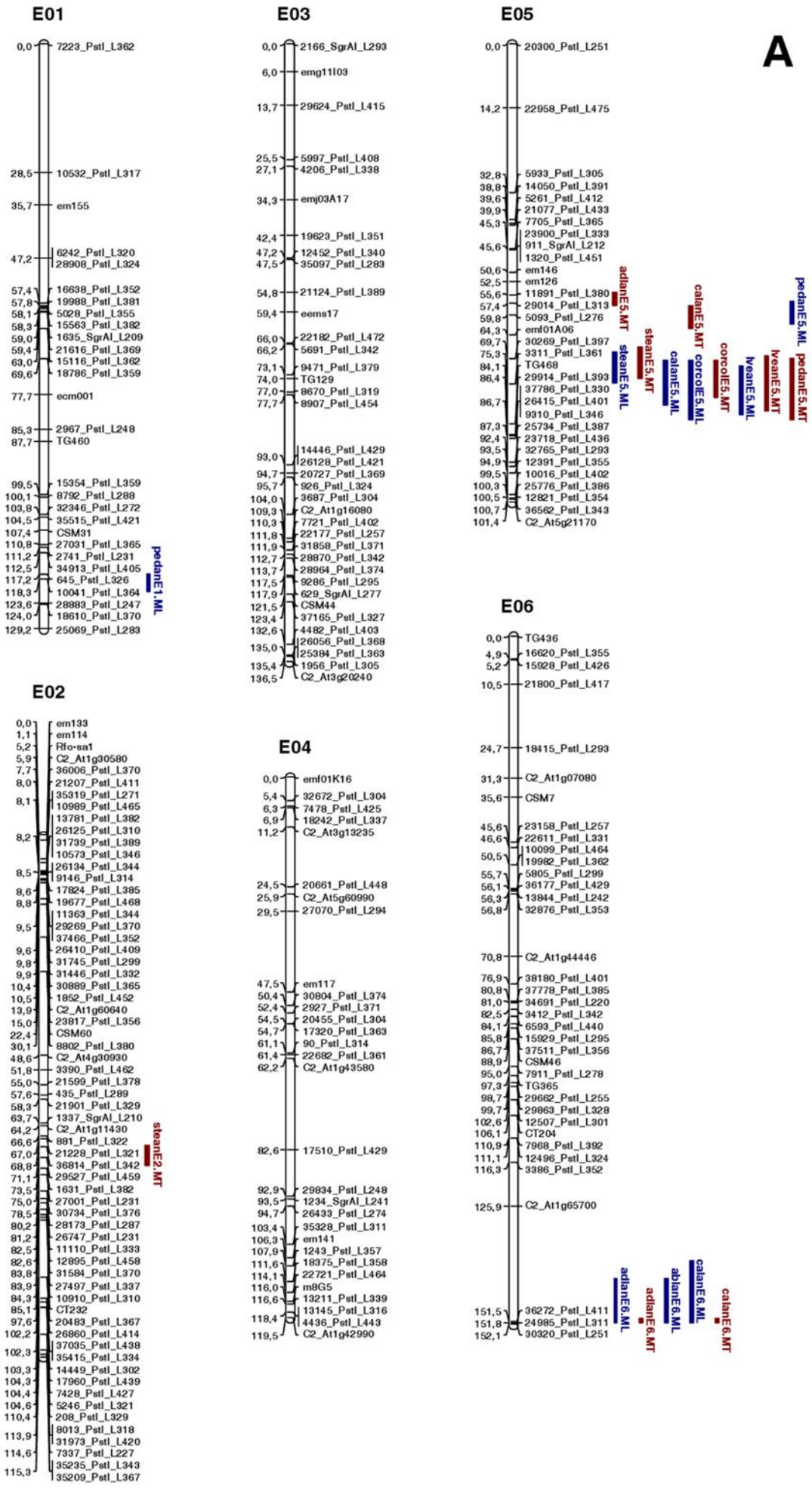
Doganlar et al. (2002) developed a map from an interspecific F_2 population of 58 individuals derived from a cross between the wild species *S. linneanum* Jaegaer & Hepper MM195 and cultivated eggplant *S. melongena* L. MM738; the map based on restriction fragment length polymorphisms (RFLP) contained 12 linkage groups and spanned 1.480 cM with 233 markers at an average distance of 7.6 cM previously mapped in tomato. This map, comparable to the tomato map, showed collinearity between several linkage groups of the two species, and 5 translocations and 23 paracentric inversions for the rest of the linkage groups. The strong conservation between eggplant and tomato chromosomes suggest that the genomic resources developed for tomato may be useful for eggplant genetic

research. A second map using the same segregating population from *S. melongena* and *S. linneanum* interspecific cross was developed in 2009 by Wu et al. This map was improved by adding 110 Conserved Ortholog Set II (COSII) markers and 5 RFLP (Wu et al., 2006, 2009) previously mapped on tomato. This last map contains 347 COS and RFLP markers spanning 1.535 cM.

Another interspecific map from the same interspecific cross was developed using an F_2 population derived from a cross between a *Verticillium* wilt resistant line of *S. linnaeanum* (also called *S. sodomeum*) and *S. melongena* cv. Buia (Sunseri et al., 2003). The map was based on 48 individuals and contains 273 markers (156 AFLP, 117 RAPD) encompassing 736 cM in 12 linkage groups with an average spacing of 2.7 cM. Current progress is being made toward higher density, saturated maps with the addition of more AFLP, SSR and other PCR-based markers to the existing eggplant maps; with the aim to develop a common marker allowing the comparisons between maps (Nunome et al., 2003a, 2009; Frary et al., 2007). Gramazio et al. (2014) developed a new interspecific linkage map between cultivated eggplant and *S. incanum*, based on a first backcross (BC_1) generation of 91 plants towards the cultivated accession of eggplant "AN-S-26", for mapping functional genes for fruit quality as well as for integrating tomato and other eggplant maps using common markers. This linkage map covers 1.085 cM, with linkage groups length comprised between 58.6 and 132.9 cM, and an average marker density of 4.46 cM. A F_2 mapping population of 141 individuals, was developed by Barchi et al. (2010) from an intra-specific cross between the eggplant breeding lines 305E40 and 67/3 and were used for the construction of the genetic map; the pair of parental lines differ markedly from one another for both productive and morphological traits as well as the presence (305E40) or absence (67/3) of *Rfo-sa1* locus which confers resistance to *Fusarium oxysporum*. 348 markers were assigned to 12 main linkage groups. The framework map covered 718.7 cM, comprising 238 markers (212 AFLP, 22 SSR, 1 RFLP and the *Rfo-sa1* CAPS).

The advent of NGS-based marker technologies, by increasing the speed, throughput, and cost effectiveness of genotyping and providing genome-wide marker coverage, allowed the development of the so called 'second generation' maps. Recently, new marker-rich maps were constructed by using NGS-derived molecular markers (Barchi et al., 2012), which sequenced two RAD tag libraries of the two parental lines of the F_2 mapping population 305E40 x 67/3 (Figure 4). 415 markers (*de novo* SNPs and anchoring markers) were assembled into 12 major and 1 minor linkage groups, spanning 1.390 cM, and the

inclusion of established markers allowed each linkage group to be assigned to one of the 12 eggplant chromosomes. Second generation intra-specific genetic maps were also generated for mapping QTLs resistance to *Ralstonia* strains by SNPs developed by intraspecific eggplant population of 132 lines, segregating for resistance (Salgon et al., 2018). Fukuoka et al. (2012) used two intra-specific F_2 mapping populations (LWF2 population 90 individuals and the ALF2 population 93 individuals derived from crosses between *S. melongena* LS1934 and *S. melongena* WCGR112-8 and between *S. melongena* AE-P03 and LS1934, respectively) to build a linkage map which consisted of 12 linkage groups and encompassed 1,285.5 cM in total. 952 DNA markers were mapped, including 313 genomic SSR markers developed by random sequencing of simple sequence repeat (SSR)-enriched genomic libraries, and 623 single-nucleotide polymorphisms (SNP) and insertion/deletion polymorphisms (InDels) found in eggplant-expressed sequence tags (ESTs). Among the used markers, 326 could also be mapped onto the tomato genome. Despite recent efforts, the linkage maps used for identifying the genetic basis of traits of breeding interest are still not saturated, hampering the fine mapping of QTL regions and the identification of candidate genes associated with the phenotypic traits. Nowadays, in addition to the inter- and intra-specific maps developed by Barchi et al. in 2012 (as mentioned above), the only available high-resolution SNP-based linkage map was developed on a F_2 population from the inter-specific cross (*S. melongena* × *S. linneanum*) and was employed to highlight QTLs affecting stem height and fruit and leaf morphology (Wei et al., 2020b). More recently, Toppino et al. (2020) developed a new intraspecific high-resolution linkage map based on the RIL mapping population from the cross 305E40x67/3, including 7,249 SNPs assigned to 12 chromosomes and spanning 2,169.23 cM, with an average distance of 0.4cM between adjacent markers.


A
E01
0,0 7223_PstI_L362
28,5 10532_PstI_L317
35,7 em155
47,2 6242_PstI_L320
28908_PstI_L324
57,4 16638_PstI_L352
57,8 19988_PstI_L381
58,1 5028_PstI_L355
58,3 15563_PstI_L382
59,0 1635_SgrAI_L209
59,4 21616_PstI_L369
63,0 15116_PstI_L362
69,6 18786_PstI_L359
77,7 ecm001
85,3 2967_PstI_L248
87,7 TG460
99,5 15354_PstI_L359
100,1 8792_PstI_L288
103,8 32346_PstI_L272
104,5 35515_PstI_L421
107,4 CSM31
110,8 27031_PstI_L365
111,2 2741_PstI_L231
112,5 34913_PstI_L405
117,2 645_PstI_L326
118,3 10041_PstI_L364
123,6 28883_PstI_L247
124,0 18610_PstI_L370
129,2 25069_PstI_L283
pedanE1.ML
E02
0,0 em133
1,1 em114
5,2 Rfo-sa1
5,9 C2_At1g30580
7,7 36006_PstI_L370
8,0 21207_PstI_L411
8,1 35319_PstI_L271
10989_PstI_L465
13781_PstI_L382
8,2 26125_PstI_L310
31739_PstI_L389
10573_PstI_L346
8,5 26134_PstI_L344
9146_PstI_L314
8,6 17824_PstI_L385
8,8 19677_PstI_L468
11363_PstI_L344
9,5 29269_PstI_L370
37466_PstI_L352
9,6 26410_PstI_L409
9,8 31745_PstI_L299
9,9 31446_PstI_L332
10,4 30889_PstI_L365
10,5 1852_PstI_L452
13,9 C2_At1g60640
15,0 23817_PstI_L356
22,4 CSM60
30,1 8802_PstI_L380
48,6 C2_At4g30930
51,8 3390_PstI_L462
55,0 21599_PstI_L378
57,6 435_PstI_L289
58,3 21901_PstI_L329
63,7 1337_SgrAI_L210
64,2 C2_At1g11430
66,6 881_PstI_L322
67,0 21228_PstI_L321
68,8 36814_PstI_L342
71,1 29527_PstI_L459
73,5 1631_PstI_L382
75,0 27001_PstI_L231
78,5 30734_PstI_L376
80,2 28173_PstI_L287
81,2 26747_PstI_L231
82,5 11110_PstI_L333
82,6 12895_PstI_L458
83,8 31584_PstI_L370
83,9 27497_PstI_L337
84,3 10910_PstI_L310
85,1 CT232
97,6 20483_PstI_L367
102,2 26860_PstI_L414
102,3 37035_PstI_L438
35415_PstI_L334
103,3 14449_PstI_L302
104,3 17960_PstI_L439
104,4 7428_PstI_L427
104,6 5246_PstI_L321
110,4 208_PstI_L329
113,9 8013_PstI_L318
31973_PstI_L420
114,6 7337_PstI_L227
115,3 35235_PstI_L343
35209_PstI_L367
steanE2.MT
E03
0,0 2166_SgrAI_L293
6,0 emg11l03
13,7 29624_PstI_L415
25,5 5997_PstI_L408
27,1 4206_PstI_L338
34,3 emj03A17
42,4 19623_PstI_L351
47,2 12452_PstI_L340
47,5 35097_PstI_L283
54,8 21124_PstI_L389
59,4 eems17
66,0 22182_PstI_L472
66,2 5691_PstI_L342
73,1 9471_PstI_L379
74,0 TG129
77,0 8670_PstI_L319
77,7 8907_PstI_L454
93,0 14446_PstI_L429
26128_PstI_L421
94,7 20727_PstI_L369
95,7 926_PstI_L324
104,0 3687_PstI_L304
109,3 C2_At1g16080
110,3 7721_PstI_L402
111,8 22177_PstI_L257
111,9 31858_PstI_L371
112,7 28870_PstI_L342
113,7 28964_PstI_L374
117,5 9286_PstI_L295
117,9 629_SgrAI_L277
121,5 CSM44
123,4 37165_PstI_L327
132,6 4482_PstI_L403
26056_PstI_L368
135,0 25384_PstI_L363
135,4 1956_PstI_L305
136,5 C2_At3g20240
E04
0,0 emf01K16
5,4 32672_PstI_L304
6,3 7478_PstI_L425
6,9 18242_PstI_L337
11,2 C2_At3g13235
24,5 20661_PstI_L448
25,9 C2_At5g60990
29,5 27070_PstI_L294
47,5 em117
50,4 30804_PstI_L374
52,4 2927_PstI_L371
54,5 20455_PstI_L304
54,7 17320_PstI_L363
61,1 90_PstI_L314
61,4 22682_PstI_L361
62,2 C2_At1g43580
82,6 17510_PstI_L429
92,9 29834_PstI_L248
93,5 1234_SgrAI_L241
94,7 26433_PstI_L274
103,4 35328_PstI_L311
106,3 em141
107,9 1243_PstI_L357
111,6 18375_PstI_L358
114,1 22721_PstI_L464
116,0 m8G5
116,6 13211_PstI_L339
13145_PstI_L316
118,4 4436_PstI_L443
119,5 C2_At1g42990
E05
0,0 20300_PstI_L251
14,2 22958_PstI_L475
32,8 5933_PstI_L305
38,8 14050_PstI_L391
39,6 5261_PstI_L412
39,9 21077_PstI_L433
45,3 7705_PstI_L365
23900_PstI_L333
45,6 911_SgrAI_L212
1320_PstI_L451
50,6 em146
52,5 em126
55,6 11891_PstI_L380
57,4 29014_PstI_L313
59,8 5093_PstI_L276
64,3 emf01A06
69,7 30269_PstI_L397
75,3 3311_PstI_L361
84,1 TG468
86,4 29914_PstI_L393
37786_PstI_L330
86,7 26415_PstI_L401
9310_PstI_L346
87,3 25734_PstI_L387
92,4 23718_PstI_L436
93,5 32765_PstI_L293
94,9 12391_PstI_L355
99,5 10016_PstI_L402
100,3 25776_PstI_L386
100,5 12821_PstI_L354
100,7 36562_PstI_L343
101,4 C2_At5g21170
adlanE5.MT
steanE5.MT
steanE5.ML
calanE5.MT
calanE5.ML
corcoE5.MT
corcoE5.ML
lveanE5.ML
lveanE5.MT
pedanE5.ML
pedanE5.MT
E06
0,0 TG436
4,9 16620_PstI_L355
5,2 15928_PstI_L426
10,5 21800_PstI_L417
24,7 18415_PstI_L293
31,3 C2_At1g07080
35,6 CSM7
45,6 23158_PstI_L257
46,6 22611_PstI_L331
10099_PstI_L464
50,5 19982_PstI_L362
55,7 5805_PstI_L299
56,1 36177_PstI_L429
56,3 13844_PstI_L242
56,8 32876_PstI_L353
70,8 C2_At1g44446
76,9 38180_PstI_L401
80,8 37778_PstI_L385
81,0 34691_PstI_L220
82,5 3412_PstI_L342
84,1 6593_PstI_L440
85,8 15929_PstI_L295
86,7 37511_PstI_L356
88,9 CSM46
95,0 7911_PstI_L278
97,3 TG365
98,7 29662_PstI_L255
99,7 29863_PstI_L328
102,6 12507_PstI_L301
106,1 CT204
110,9 7968_PstI_L392
111,1 12496_PstI_L324
116,3 3386_PstI_L352
125,9 C2_At1g65700
151,5 36272_PstI_L411
151,8 24985_PstI_L311
152,1 30320_PstI_L251
adlanE6.ML
adlanE6.MT
ablanE6.ML
calanE6.ML
calanE6.MT

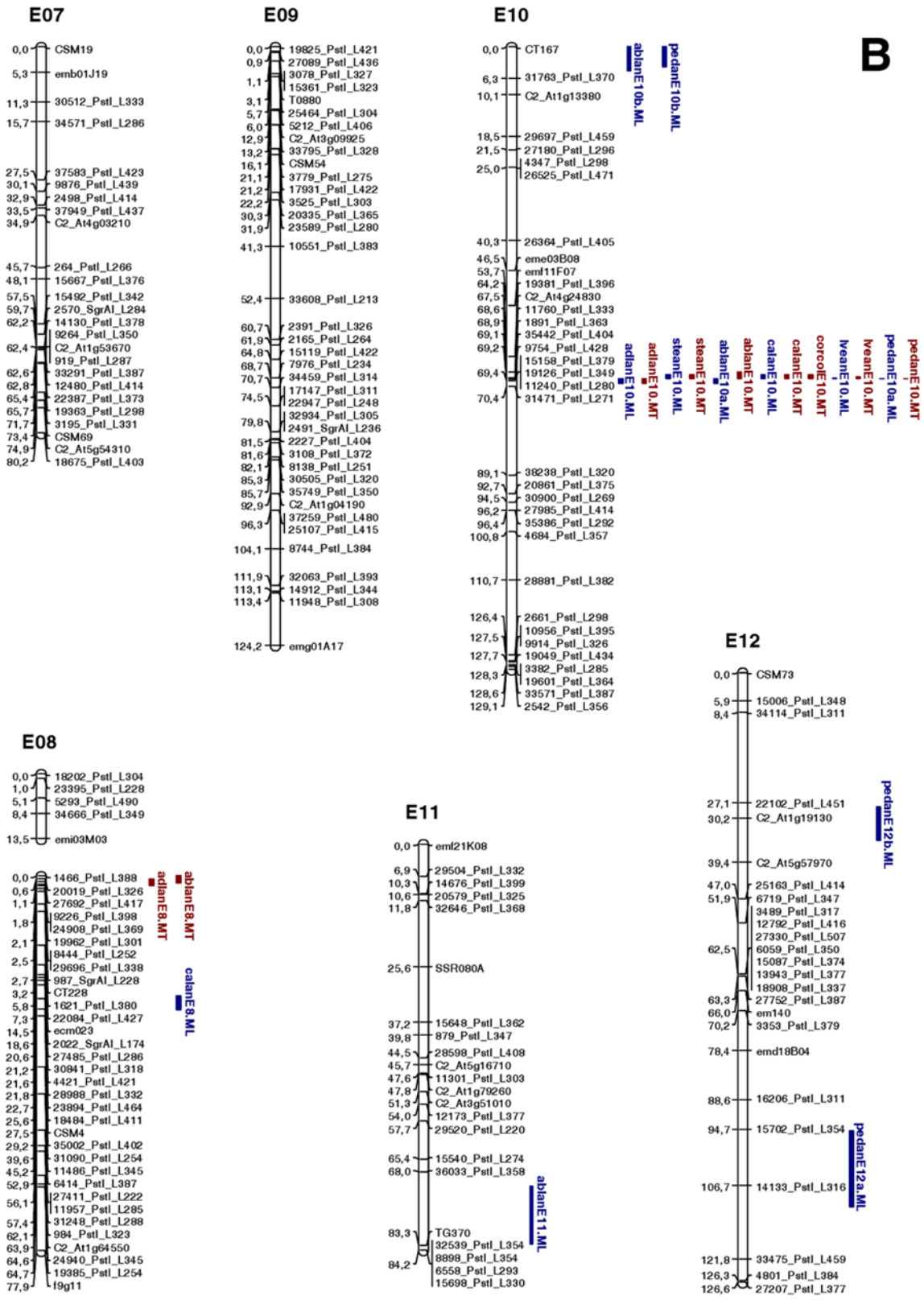


Figure 4 A-B Eggplant molecular map proposed by Barchi et al. (2012)

2.4 Eggplant Quantitative Trait Loci (QTLs) and QTL mapping

In the last decades, both inter- and intra-specific maps have been developed in eggplant, with the former benefitting from an enhanced frequency of marker polymorphism, but also a more limited relevance for marker-assisted crop breeding with respect to the intra-specific ones (Barchi et al., 2019). In parallel with the advances in the genetic linkage maps, the identification of quantitative trait locus (QTLs) associated with agronomic traits has been greatly promoted in eggplant. Among the traits in study, great importance played the identification of QTLs linked to morphological and biological traits, including leaf, flower, plant and fruit characteristics, as well as QTLs associated to parthenocarpy and to resistances to fungal (*Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dahliae*) and bacterial (*Ralstonia solanacearum*) wilts. QTL studies to elucidate the genetic basis of biochemical composition, content in bioactive and antinutritional compounds, as well as other fruit quality traits were also carried out.

The first interspecific eggplant linkage map (eggplant x *S. linnaeanum*) was exploited to detect a total of 125 significant QTLs associated with domestication and several morphological and agronomic traits (Doganlar et al., 2002; Frary et al., 2003; Wu et al., 2009).

The first intra-specific EWF2 genetic map was published by Nunome et al. (2001) using dominant markers (RAPD and AFLP) and several markers were found as associated with fruit shape and color. Miyatake et al. (2012) constructed two linkage maps and identified QTLs linked to parthenocarpy traits using NAF2 and ALF2 populations obtained by crossing two divergent breeding lines; the same populations were recently employed to explore QTL for the resistance trait to *Fusarium oxysporum* (Miyatake et al., 2016) taking advantage from sequence information retrieved from the first eggplant draft genome released (Hirakawa et al., 2014). Barchi et al. (2010) developed a further intra-specific linkage map from an F2 population employed for detecting QTL associated with anthocyanin content (Barchi et al. 2012), key horticultural (Portis et al. 2014) and biochemical (Toppino et al., 2016) traits of great interest for breeding. Major and minor QTLs affecting resistance to *Fusarium* and *Verticillium* in the intraspecific 305E40 × 67/3 map were also detected, and putative orthologous genes from tomato were identified (Barchi et al., 2018). Furthermore, through a GWAs approach, the previously identified

loci were validated, and a number of new marker/trait associations detected (Cericola et al., 2014, Portis et al., 2015). Nonetheless, despite the progress in QTL detection, most of the traits were analyzed in inter-specific populations, and the linkage maps used in genetic mapping are still less saturated. Advances in next generation sequencing (NGS) technologies provide an excellent opportunity to develop a great amount of SNP markers and the construction of a high-resolution linkage map in eggplant which may be fundamental tools for map-based gene mining (Wei et al., 2020). Recently, thanks to NGS-derived molecular markers, new markers-rich maps ("second generation maps") were constructed (Toppino et al., 2020). In addition, the recently more user-friendly high-density map, obtained through a Genotype-by-sequencing (GBS) approach, was used successfully to better define several QTLs associated with plant anthocyanin content and seed quality traits (Toppino et al., 2020) as well as fruit metabolomic content (Sulli et al., 2021) and anthocyanic peel coloration (Florio et al., 2021). Recently, a high-quality genetic linkage Bin map derived from the re-sequencing analysis on a cross of a prickly wild landrace and a cultivated variety has been constructed (Qian et al., 2021). By this map an additional major quantitative trait locus (QTL) controlling the development of prickles on the calyx (explained 30.42% of the phenotypic variation) was identified; candidate genes conferring prickless located in the selective sweep (SS) regions were later characterized by re-sequencing 23 eggplant accessions (Barchi et al., 2021).

3. Biotic stresses

The main diseases affecting eggplant are *Verticillium albo-atrum, Verticillium dahliae, Fusarium oxysporum* f.s. *melongenae, Pyrenocheta lycopersici, Ralstonia solanacearum* and nematodes (*Meloidogyne* spp.). They represent the restricting factor for greenhouse and field cultivation, as they are difficult to control using standard agronomic techniques (Trentini and Montanari, 1996; Moncada et al., 2013)

3.1 *Fusarium oxysporum* f. sp. *melongenae*

Fusarium oxysporum f.s. *melongenae* (*Fom*) is one of the most destructive soil-borne pathogens of eggplant (*Solanum melongena* L.) inducing vascular disorders, vessels browning and subsequent wilting of the plants. This is an economically important pathogen with a worldwide distribution (Armstrong, G. M. and Armstrong J. K.**,** 1981; Beckman, 1987) its occurrence in Europe both in greenhouse and open-field cultivation (Urrutia

Herrada et al., 2004; Altinok, 2005) is rather recent; it was described in the Netherlands (Steekelenburg, 1976), Italy (Stravato et al., 1993), Spain (Urrutia Herrada et al., 2004), Greece (Holevas et al., 2000), and Turkey (Altinok, 2005). This disease was also described in Florida (USA) (Alfieri et al., 1984; 1994), Korea (Cho and Shin, 2004), Spain (Urrutia Herrada et al., 2004) and China (Zhuang, 2005).

The distribution of *Fusarium oxysporum* is known to be cosmopolitan, however, intraspecific variants of *F. oxysporum*, called *formae speciales* (f. sp.), often have different degrees of host distribution causing vascular wilt in 80 botanical species. *Fusarium oxysporum* f. sp. *melongena* (Figure 5) was initially described by Matuo and Ishigami (1958) from *S. melongena* (Solanaceae) plant suffering from a vascular wilt disease. The recovered isolate was so species-specific that in plant inoculation studies it failed to infect other Solanaceae species (including *Lycopersicon esculentum*, *Nicotiana tabacum*, *Solanum tuberosum*, and *Capsicum annuum*). A high degree of variability within *F. oxysporum* has been associated with the complexity of the species (Baayen et al., 2000; Baysal, 2010).

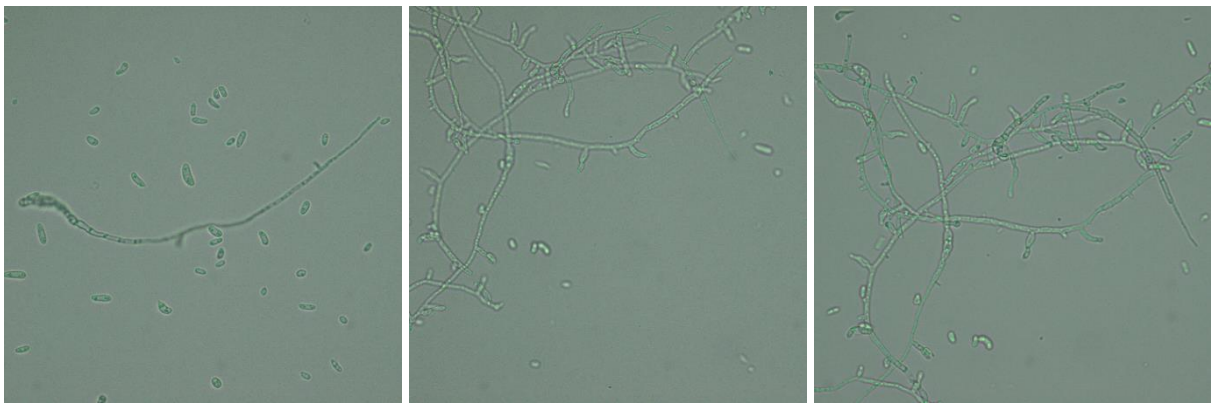


Figure 5 Conidia and conidiophores of F. oxysporum. f.sp. melongenae

F. oxysporum is classified in the subdivision *Deuteromycotina* (Fungi Imperfecti) because it lacks sexual reproduction while *Fusarium* species that have sexual stages are classified in the subdivision *Ascomycotina*. In solid media culture, such as potato dextrose agar (PDA), the different *formae speciales* of *F. oxysporum* can have varying shapes, in addition to an aerial mycelium first appears white, and then may change to a variety of colours ranging from violet to dark purple. *F. oxysporum* is unique in its asexual reproduction: it produces three kinds of asexual spores, macroconidia, microconidia, and chlamydospores (Figure 6) (Nelson, 1981; Nirenberg, 1990.)

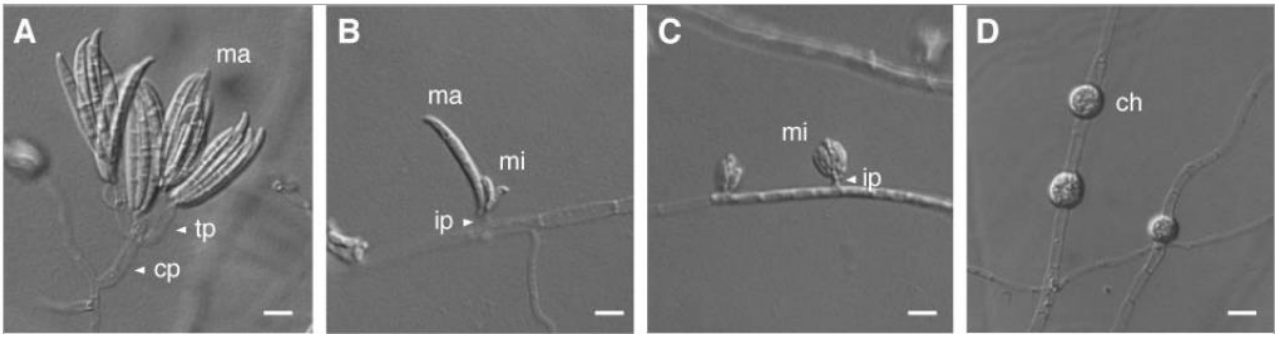


Figure 6 Conidiation of F. oxysporum. Strain Mel02010 was grown on SNA-paper at 25°C for 5 days. (A) Macroconidia (ma) are produced generally from terminal phialides (tp) on conidiophores (cp). (B) Macroconidia are also produced rarely from intercalary phialides (Ohara and Tsuge, 2004)

Macroconidia are falcate and have three- to five septa (Figure 6A and 6B), gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen.

Microconidia are ellipsoidal, have no septa or one septum (Figure 6C), and are the type of spore most abundantly and frequently produced by the fungus under all the conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Conidiogenesis of macroconidia and microconidia is phialidic (Nelson, 1981; Nelson et al.,1983). Macroconidia are produced most often from terminal phialides that arise from conidiophores (Figure 6A) and at low frequencies from intercalary phialides that arise directly from hyphae (Figure 6B). Microconidia are produced from intercalary phialides in false heads (Figure 6C). Moreover, macroconidia and microconidia are produced on the stem surfaces of infected plants and serve as secondary inocula to spread the fungus to neighbouring host plants (Ohara and Tsuge, 2004).

Globose chlamydospores are round, have thick walls (Figure 6D) and are produced on older mycelium or in macroconidia. These spores are either composed by one or two cells. Chlamydospores are generally developed through the modification of hyphal and conidial cells through the condensation of their contents (Figure 6D). Chlamydospores, unlike macro and microconidia, are endurance organs in soil that may remain viable for a long period of time in soil and plant debris, even after rotation with non-host crops (Altinok, 2006; 2007) and act as primary inocula when suitable host plants are planted in soil (Rowe, 1977; Nelson, 1981; Couteaudier and Alabouvette, 1990).

F. oxysporum is an abundant and active saprophyte in soil and organic compounds with the ability to survive in the soil between crop cycles in infected plant debris. The fungus survives either as mycelium, or as any of its three different spore types. Healthy plants grown in soil contaminated by the fungus can be infected by it which can invade a plant via the root either with its sporangial germ tube or its mycelium. The roots can be infected directly through wounds in the roots or at the formation point of lateral roots. Once inside the plant, the mycelium grows through the root cortex between the cells, reaches the xylem, invades the vessels, and as it grows, it branches and produces microconidia, which are transported upwards within the vessel by way of the plant's sap stream. When the microconidia germinate, the mycelium can penetrate the upper wall of the xylem vessel, allowing the production of more microconidia by invading the next or lateral vessel. (Agrios, 1988).

Fusarium oxysporum and its various *formae speciales* have been characterized as causing the following symptoms: vascular wilt, leaf chlorosis, interveinal yellowing of the outer leaflets, then vascular discoloration of the stem, corm rot, root rot, damping-off and finally death of the above ground parts of the plant (Agrios, 1988; Altinok 2005, 2007). The most important of these symptoms is vascular wilt. In general, wilts caused by *Fusarium* first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, defoliation, yellowing of the lower leaves, wilting of leaves and young stems, formation of adventitious roots, marginal necrosis of remaining leaves, and finally death of the entire plant. Browning of the vascular tissue is strong evidence of wilt. *F. oxysporum* is primarily spread over short distances by irrigation water and contaminated farm equipment. The fungus can also be spread over long distances either in soil or in infected transplants. It is also possible that the spores are spread by wind. (Agrios, 1988).

Strategies to control this soil-borne disease have been based, especially in greenhouse cultivation, on soil treatments with methyl bromide, but this compound has officially been phased out in the European Union (Pizano and Banks, 2008; Baysal, 2010). *F. oxysporum* and its many special forms affect a wide variety of hosts, thus the management of this pathogen generally includes disinfestation of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts plants, or by using resistant cultivars (Jones et al.,

1982; Smith et al., 1988). Currently, the efficient method that is favoured for control of soilborne fungal diseases is to take advantage of resistant cultivars. Resistant commercial cultivars yet to be developed, however, some eggplant cultivars susceptible to *Fom* are grafted on resistant eggplant rootstocks to bring them to a good level of resistance against *Fom* (Gisbert et al., 2011; Altinok et al., 2014; 2017). In practice, most of the commercial cultivars display a poor spectrum of disease resistances (Daunay, 2008).

High levels of resistance were identified, in the *S. melongena* germplasm and used successfully in breeding programs (Abdullaeva & Shifman, 1988; Mandare & Patil, 1993; Sakata et al., 1996). A monogenic dominant control was identified in eggplant accessions (LS174, LS1934 and LS2436) (Sakata et al., 1996; Mochizuki et al., 1997; Mutlu et al., 2008; Boyaci et al., 2011). Using two of these resistant germplasms, *Fusarium*-resistant rootstocks, Daitaro and Daizaburou derived from LS1934, and the eggplant line EPL-1 derived from LS174, were developed in Japan (Mochizuki et al. 1997; Monma et al. 1997; Yoshida et al. 2004). Within the germplasm related to eggplant, Yamakawa and Mochizuki (1979) found complete resistance in *S. integrifolium* (i.e., *S. aethiopicum* Aculeatum Group), and *S. indicum* (i.e., probably *S. violaceum*), as well as in *S. incanum* (probably a wild form of *S. melongena*) which however segregated into resistant and susceptible plants. Cappelli et al. (1995) found (i) that *S. torvum* and *S. sisymbriifolium* were resistant, (ii) that *S. aethiopicum* Gilo Group, *S. integrifolium, S. khasianum* - i.e., *S. viarum*- and *S. macrocarpon* segregated for resistance, and (iii) that *S. sodomaeum* (i.e., *S. linnaeanum*) was susceptible. Monma et al. (1996) found that 96% of the 50 accessions of *S. gilo* and *S. aethiopicum* (same species), and 100% of the *S. anguivi* accessions they tested expressed total resistance, thus clearly indicating that *Fusarium* wilt resistance is very frequent in this African species and its close wild relative. They also found resistance sources in *S. macrocarpon* species (Daunay, 2008).

Genetic control of resistance has been demonstrated as monogenic dominant in two *S. aethiopicum* accessions belonging to cultigroups Aculeatum and Gilo (Rizza et al., 2002; Toppino et al., 2008). Progenies derived from anther culture and interspecific somatic hybrids '*S. melongena* X *S. aethiopicum*' (Rotino et al., 2005) allowed Toppino et al. (2008) to identify the locus *Rfo-sa1* which controls the resistance of *S. aethiopicum* to *Fusarium* wilt, as well as its two allelic forms in the Aculeatum and Gilo groups; later, *Rfo-sa1* was mapped on chromosome 2 by Barchi et al. (2018), as well as another QTL derived from the

sequenced line '67/3', located on chromosome 11. Miyatake et al. (2016) mapped *Rfo-sa1* very close to *FM1* with two allelic forms on chromosome 2 and chromosome 4. So far, no interaction has been described between these loci and *Fusarium* strains, unlike monogenic resistances based on different mechanisms and controlling three *Fusarium* races in tomato (Gonzalez-Cendales, 2016; Daunay et al., 2016). Further, in tomato polygenic tolerance has also been described (Crill et al., 1972).

3.2 Other fungal pathogens

Verticillium is another vascular disease, which likewise the *Fusarium* wilt provokes vessels browning and foliar wilting, but it kills rarely the plants. The fungus *Verticillium dahliae* is one of the most destructive and prevalent diseases of eggplant (Bletsos et al., 1997) and its management is similar to that of *F. oxysporum.*

Lesser susceptibility or tolerance (Lin and Xiao, 1995; Robinson et al., 2001) or the so called 'slow wilting' are reported in several varieties, as well as the existence of particularly susceptible accessions (Cirulli et al., 1990). High resistance levels have been reported in *S. torvum, S. sisymbriifolium, S. aculeatissimum, S. anguivi, S. incanum, S. tomentosum, S. linnaeanum,* and *S. scabrum, S.caripense* and *S. persicum* (Sakata et al., 1989; Alconero et al., 1988; Robinson et al., 2001). The resistance of *S. sisymbriifolium* and *S. torvum* could not be so far transferred to eggplant because their sexual cross with eggplant either did not yield progenies or yielded sterile progenies. Somatic crosses were realized for both species, with so far limited success in terms of breeding for resistance since the interspecific hybrids are sterile. Acciarri et al. (2001) and then Liu et al (2015) reported the introgression of the resistance from *S. sodomaeum* (= *S. linnaeanum*) into *S. melongena* through sexual hybridization and advanced back cross material was screened for resistance in the field conditions (Acciarri et al., 2004). A gene specific marker for the *Vd* homolog in PI388846 was developed to detect *Verticillium* wilt resistance in the backcross population (Liu et al., 2015); in addition, Sunseri et al. (2003) developed AFLPs for tagging the resistance factors of these progenies. Finally, Barchi et al. (2018) identified two major and one minor QTL involved in *Vd* resistance, mapped on CH8, CH5 and CH9, respectively.

Resistance to anthracnosis, caused by *Colletotrichum gloeosporioides* f. sp. *melongenae*, is quite common within *S. melongena* germplasm, controlled by a single gene (Kaan, 1973; Messiaen, 1975). The resistance to *Phomopsis* blight and fruit rot within *S. melongena*

germplasm was shown as polygenic and recessive (Kalda et al., 1977). Chauhan and Duhan (1980) report resistance against *Alternaria melongenae*, *A. solani*, *Phomopsis* sp., *Cercospora solani-melongenae*, and *C. solani* within eggplant germplasm. Immunity through hypersensitivity to *Cercospora solani* has been found in eggplant as well as in *S. macrocarpon* by Madalageri et al. (1988). Wild species were found resistant to this fungus such as *S. indicum* (probably *S. violaceum*) and *S. khasianum* (= *S. viarum*) (Vadivel and Bapu, 1989), *S. torvum*, *S. khasianum* as well as *S. xanthocarpus* (Datar and Ashtaputre, 1988) High level of resistance against powdery mildew (*Oidiopsis* [*Leveillula*] *taurica*, consisting of 0-5% of infected leaf surface has been reported (Mahrshi et al., 1980). High as well as moderate resistance levels of eggplant lines against *Sclerotinia sclerotiorum* (local rotting of stems and subsequent wilting) have been revealed by artificial inoculation (Kapoor et al., 1989/1990) and only moderate resistance against *S. rolfsii* were found (Begum and Ahmed, 1990; Daunay, 2008).

3.3 Plant responses to pathogens

During its life, a plant is often exposed to a wide range of pathogens in the air and/or soil that pose a threat to normal growth and seed production. Therefore, pathogens can have considerable impacts on agricultural productivity and have important economic consequences (Sun et al., 2019). When a plant and a pathogen come into contact, there is close communication between the two organisms (Hammond-Kosack and Jones, 1996). The activities of pathogens focus on colonising the host and utilising its resources, whereas plants are adapted to detect the presence of pathogens and respond with antimicrobial and other defence responses. Throughout millions years of evolution, plants have formed multi-layered defence systems to sense and resist the invasion of many pathogenic microorganisms, which include, among others, bacteria, fungi nematodes or viruses (Sun et al., 2019). Plant and pathogen species are often highly co-evolved, which means, for example, that standard plant barriers to microbial infection can be circumvented by particular pathogen species. In the course of an infection, plant metabolism often represents a variable mixture of resistance and susceptibility responses to disease. Interactions between plants and pathogens induce a range of plant defence responses (Hammond-Kosack and Jones, 1996). Plants rely on mechanisms of innate immunity, which can be present in two forms: basal (or horizontal) resistance and R gene-based (or vertical)

resistance. The first one (horizontal resistance) is based on the recognition of a pattern-recognition receptor (PRR) and a pathogen-associated molecular pattern (PAMP), which trigger basal or non-cultivar specific defence responses in plants. The second (also called R gene-based resistance) is based on the highly specific interaction between pathogenic effectors and plant R-gene products according to the gene-for-gene theory. This recognition event leads generally to hypersensitive response, characterized by rapid apoptotic cell death and local necrosis (Boller and Felix, 2009). Danger signals are perceived in the immediate surroundings of pathogen invasion sites. Plant species and plant cultivar-specific resistance represent evolutionarily linked types of immunity that are collectively referred to as the plant innate immune system. Signal transduction cascades that mediate activation of innate immune responses include elements that are common to both forms of plant immunity, such as alterations in cytoplasmic calcium levels, mitogen activated protein kinase activities or the production of reactive oxygen species. Not surprisingly, host transcriptional activity is substantially modulated and redirected during such defence responses (Scheideler et al., 2002). When a pathogen-derived avirulence (*avr*) protein of a virus, bacterium, fungus, nematode or insect is recognized directly or indirectly by the corresponding resistance (R) protein in the plant, the R protein typically activates defence response to make the infection unsuccessful (Dangl and Jones, 2001). Thus, R genes form an important "front end" of the plant immune system and are widely exploited for disease control in crop plants. The pathogen surveillance system mediated by R genes allows particularly rapid activation of defence responses. Hypersensitive response (HR), a programmed plant cell death response at the site of pathogen infection, is often associated with gene-for-gene disease resistance. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are related but distinct versions of systemic host response and share the high production of antimicrobial compounds are activated more strongly and rapidly in response to subsequent infections (Glazebrook et al., 2005; Noman et al., 2020).

The term "pathogenesis-related protein" (PR protein) was introduced in the 1970s to refer to proteins that are newly synthesized or present at substantially increased levels after a plant has been infected (Van Loon et al., 2006). A number of classically defined PR genes encode proteins such as chitinases, glucanases or defensins that have been shown to have antimicrobial activity. However, individual PR proteins apparently make only a small quantitative contribution to defence, and the contribution will depend on the pathogen target.

3.3.1 PTI or PAMP-Triggered Immunity

PAMPs (pathogen-associated molecular pattern) constitute highly conserved determinants typical of entire classes of pathogens that are not found in potential host organisms and that are indispensable for the microbial lifestyle, such as chitin for fungi or peptidoglycan for bacteria. PAMPs can also be divided into microbe-associated molecular patterns (MAMPs) derived only from pathogen, and damage-associated molecular patterns (DAMPs), derived from the plant itself due to damage caused by the microbe. However, plants possess pattern recognition receptors (PRRS) able to perceive both MAMPS and DAMPS. The perception of PAMPS by PRRS initiates an active defence response, called basal immunity.

Well-adapted microbial pathogens, however, have found ways to breach this first active line of defence. Plants have evolved a second line of defence, called R-gene-based resistance which is more specific than the basal line of defence (Andersen et al., 2018).

3.3.2 ETI or Effectors-Triggered Immunity

ETI, also called R-gene-based resistance, is based on direct or indirect interaction between pathogen effectors and the products of plant R genes according to the gene-for-gene theory. This recognition events leads to a vigorous type of defence reaction called hypersensitive response, characterized by rapid apoptotic cell death and local necrosis (Martin et al., 2003). The genetic basis for disease-specific resistance of plant cultivars is determined by gene pairs called pathogen-derived avirulence genes (*Avr)* and plant-derived resistance genes (R). *Avr* gene-encoded proteins are likely effectors (sometimes dispensable) that contribute to host infection. It is important to consider the defence program induced by PTI or ETI: plants do not seem to discriminate between PAMPs and elicitors. The perception of all these signals appears to trigger the same defence responses, although with differences in kinetic and amount of induction. The response induced by ETI seems to be stronger and longer than the response induced by PTI (Tao et al., 2003). Although plants have two levels of defense mechanisms namely PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI), CRISPR/Cas9 technology is being widely exploited to modify the genome to enhance the plant immune system to achieve long-lasting resistivity against pathogen, as described by Tyagi et al. (2020) and Ghosh and Dey (2022).

3.3.3 Resistance (R) proteins

Innate immunity is based on specialised receptors that can be divided into two groups: the PRRS and the R proteins. PPRs recognize PAMPs, which are highly conserved molecules,

and allow plants to recognize distinct invaders using a limited set of receptors (Van Ooijen et al., 2007). In contrast to PPRS, R proteins respond to molecules called avirulence proteins (avr) or elicitors, that are generally not conserved between species or isolates of given pathogen. R proteins are encoded by large gene families, numbering several hundred of genes per genome (Meyers et al., 2003). R protein-mediated resistance is often associated with hypersensitive response. R genes confer resistance to very different pathogens, but the encoded proteins share only a limited number of conserved domains. Based on this, R proteins can be divided in four classes. Most of these contain a central nucleotide-binding (NB) domain as part of a larger entity called NB ARC domain. C-terminal to the NB-ARC domain lies a leucine-rich repeat (LRR) domain, which is sometimes followed by an extension of variable length. Thus, this group of R proteins is collectively referred to as NB-LRR proteins. On the basis of their N-terminal region, TNL and CNL proteins can be classified. If the N-terminal region shows homology with a protein domain found in the *Drosophila* Toll and human Interleukin-1 Receptor (IL-1R), it is called the TIR domain and this protein referred to as TIR-NB LRR or TNL protein (Wise et al., 2007). Because some non-TIR proteins contain predicted coiled-coil structures (CC) in their N-terminal domain, non-TIR NB-LRR proteins are referred to as CC-NB-LRR or CNL proteins. A limited number of R proteins are extracellular, and they contain predicted a extracellular LRR (eLRR) domain at their N terminus. This eLRR is connected via a transmembrane domain to a variable cytoplasmic C terminal region. When the cytoplasmic domain contains a protein kinase domain the protein belongs to the RLK class (Receptor Like Kinase), if no such domain is present, the protein is placed in the RLP class (Receptor Like Protein). A schematic representation of the typical members of the four R protein classes in show in Figure 7.

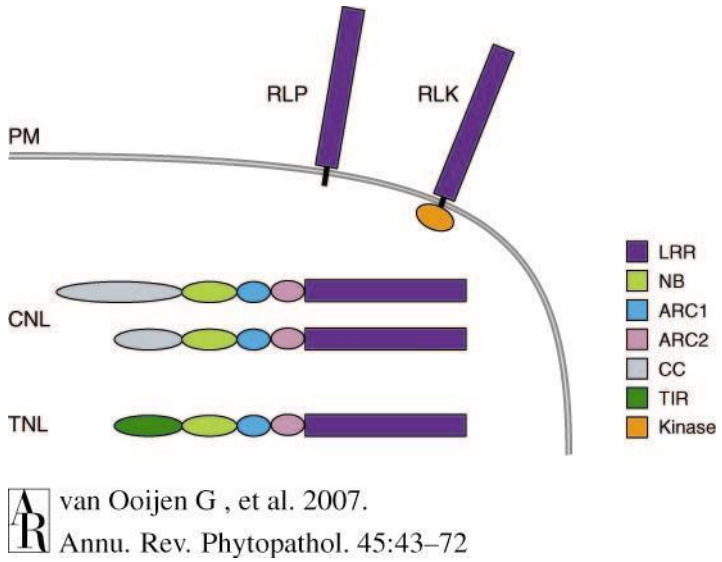


Figure 7 Schematic representation of typical members of the four R protein classes. Protein domains and putative cellular localization are indicated. The Receptor-Like Protein (RLP) and the Receptor-Like Kinase (RLK) classes of R proteins span the plasma membrane (PM) and contain an extracellular Leucine Rich Repeat (LRR) domain. The CNL and TNL classes of R proteins are located intracellularly (cytoplasmic, nuclear, or membrane-bound) and contain a central NB-ARC domain (consisting of NB, ARC1 and ARC2 subdomains) coupled to an LRR domain. TNLs carry an N-terminal TIR domain, while CNLs contain either a CC or an extended CC domain (from van Ooijen G. et al., 2007).

In the Solanaceae, the larger class of R protein is the CNL class (Van Ooijen et al., 2007). We can find indirect and direct *Avr*/R interaction; for most R protein, this mechanism is still unknown. Activation of NB-LRR proteins likely requires a series of conformational changes, mediated via nucleotide hydrolysis by the central nucleotide binding site. Delineation of the 3-D structure of NB LRR and RLP proteins will improve the understanding of molecular mechanisms underlying their function.

3.3.4 Active Oxygen Species, Lipoxygenases, and Disruption of Cell Membranes

The plant cell membrane consists of a phospholipid bilayer in which many kinds of protein and glycoprotein molecules are incorporated. The cell membrane is also an active site for the induction of defence mechanisms; it serves as an anchor of R gene-coded proteins that recognize the elicitors released by the pathogen and subsequently trigger the hypersensitive response. The most important membrane-associated defence responses include the release of important molecules in signal transduction within and around the cell and, possibly, systemically through the plant and the release and accumulation of reactive oxygen "species" and of lipoxygenase enzymes (Sharma, 2004).

The first events in the defence response are perturbations in ion fluxes and the pattern of protein phosphorylation, which precede the accumulation of ROS (mainly O_2- and H_2O_2) and NO as well as the transcriptional activation of defence-related genes (McDowell and

Dangl, 2000). Indeed, pathogen recognition is linked to the perception of microbe/pathogen-associated molecular patterns triggering a specific and transient accumulation of reactive oxygen species (ROS) at the pathogen attack site (Camejo et al., 2019). The attack of cells by pathogens, or exposure to pathogen's toxins and enzymes, often results in structural and permeability changes of the cell membrane. In many plant pathogen interactions, one of the first events detected in attacked host cells is the rapid and transient generation of activated oxygen species, including superoxide (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). Generation of superoxide and other reactive oxygen species as defence response occurs most dramatically in localized infections, but also in general and systemic infections, as well as in plants treated with chemicals that induce acquired systemic resistance. These highly reactive oxygen species are thought to be released by the multisubunit NADPH oxidase enzyme complex of the host cell plasma membrane and appear to be released into affected cells within seconds or minutes of the cell's contact with the pathogen. Specifically, one NADPH oxidase, RBOHD, associates with PRRs EFR and FLS2, and is phosphorylated by BIK1, triggering ROS production (Andersen et al., 2018). The activated oxygen species trigger the hydroperoxidation of membrane phospholipids, producing mixtures of lipid hydroperoxides. The latter are toxic, as their production destroys plant cell membranes, and appear to be involved in normal or HR-induced cell collapse and death. The presence of active oxygen species, however, also affects the membranes and the cells of the pathogen that advances directly or indirectly through the hypersensitive response of the host cell. The production of reactive oxygen species in affected but surviving nearby cells is controlled by the radical scavenger enzymes superoxide dismutase, catalase, ascorbate peroxidase, etc. Several isoenzymes of each of these molecules are produced, with different ones appearing at different stages after inoculation. The oxygenation of membrane lipids also seems to involve various lipoxygenases. These are enzymes that catalyze the hydroperoxidation of unsaturated fatty acids, such as linoleic acid and linolenic acid, which have previously been released from membranes by phospholipases. The lipoxygenase-generated hydroperoxides formed from these fatty acids, in addition to disrupting cell membranes and leading to HR-induced cell collapse of host and pathogen, are also converted by the cell into several biologically active molecules, such as jasmonic acid, which play a role in the plants' response to wounding and other stresses (Sharma, 2004). Transgenic plants that lack the ability to detoxify ROS compounds were found to have more intense responses to pathogens that trigger HR (Mittler et al., 1999; Andersen et al., 2018).

3.3.5 Transduction of Pathogen Signals in Plants

Plants are able to recognize pathogen-derived elicitor molecules that trigger a series of induced defences in plants. Recognition of a potential pathogen results the activation of intracellular signalling events including ion fluxes, phosphorylation-dephosphorylation cascades, kinase cascades, and generation of reactive oxygen species (ROS) (Radman et al., 2003). Intercellular signalling system involves ROS, nitric oxide (NO), salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Two major pathways in defence signalling are recognized, one SA dependent and the other SA-independent but involving JA and ET (Kunkel and Brooks, 2002). These signalling events lead to reinforcement of plant cell walls and the production of defence proteins and phytoalexins. These events proceed in both susceptible and resistant interactions, probably with different speed and intensity. Pathogens also produce suppressor molecules to counteract the action of elicitors, resulting in susceptibility.

3.3.6 Nitric oxide (NO) in signal transduction

Nitric oxide (NO), together at hydrogen sulphide (H_2S) are the two pivotal gaseous messengers diffuses easily through biomembranes and involved in growth, germination and improved tolerance in plants under stressed and non-stress conditions (Bethke et al., 2004; Singh et al., 2020). It is well established that NO is involved in the plant defence signalling (Delledonne et al., 2001). NO production in tobacco cells within 5 min after treatment with the cryptogein elicitor and reaches the maximum within 30 min has been observed (Lamotte et al., 2004). Plants synthesize NO from nitrite and nitrate reductase catalyzes the NAD(P)H dependent reduction of nitrite to NO (Morot-Gaudry Talarmain et al., 2002). Nitrate reductase reduces nitrate to nitrite and can further reduce nitrite to NO. Nitrite-dependent NO production has been observed in soybean and sunflower (Delledonne et al., 1998; Rockel et al., 2002). NO induces defence gene expression via signalling pathways probably involving cyclic GMP and CADPR.

3.3.7 Salicylic acid (SA) in signalling defence response in plants

SA is a phenolic compound commonly present in the plant kingdom. Plants synthesize SA (O-hydroxybenzoic acid) by the action of PAL (phenylalanine ammonia lyase), which is a key regulator of the phenylpropanoid pathway and produces a variety of phenolics with structural and defence related functions. SA has been reported as one of the most important signal molecules, acting locally in intracellular signal transduction and also systemically in intercellular signal transduction (Raskin, 1992). SA accumulates in plants inoculated with

pathogens, its level increasing in both proximal and distal tissue from the point of infection. Increased levels of SA led to in induction of various defence-related genes (Dorey et al., 1997). The importance of SA-signalling system in induction of host defences has been investigated by developing transgenic plants expressing the bacterial NahG gene. This gene encodes for the enzyme salicylate hydroxylase, which inactivates SA by converting it to catechol. Some of the NahG transgenic plants were unable to accumulate SA and consequently unable to develop HR, indicating that SA accumulation is required for HR to occur (Delaney et al., 1994). Disease resistance is also induced in plants by spray treatments with SA (Navarre and Mayo, 2004).

3.3.8 Jasmonate signalling (JAs) in induction of defence response

JAs, which were first detected in essential oils of *Jasminum grandiflorum* (Demole et al., 1962), are ubiquitously present in all plant tissues, and they are a major group of signalling compounds in inducing host defence JA and its cyclic precursors and derivatives are collectively referred to as JAS (Li et al., 2005; Ruan et al., 2019). JAs, derived from peroxidized linolenic acid, are members of a large class of oxygenated lipids called oxylipins (Hamberg and Gardner, 1992). Oxylipins are acyclic or cyclic oxidation products derived from fatty acids catabolism (Creelman and Mulpuri, 2002). JA, MeJA, 12-oxo-phytodienoic acid (OPDA), and other oxylipins act as defence signals against pathogens (Krumm et al., 1995). The accumulation of JAs is followed by the activation of JA-mediated defence responses (Wasternack and Hause, 2002). The importance of JA in signalling induction of defence genes has been demonstrated by using plant mutants deficient in JA synthesis and perception. Constitutive JA production in an *Arabidopsis* mutant was accompanied by constitutive expression of defensin PDF1.2, thionine Thi2.1, and chitinase CHI genes (Ellis et al., 1999), and this mutant showed increased resistance against *E. cichoracearum* and a bacterial pathogen *Pseudomonas syringae*.

3.3.9 Ethylene-dependent signalling pathway

The increased ET production is one of the first chemically detectable events in pathogen-infected or elicitor-treated plants (Toppan and Esquerre-Tugaye, 1982). The role of ET in plant-pathogen interaction is complex (Geraats et al., 2003). ET stimulates defence mechanisms against several pathogens, and it also induces susceptibility to several other pathogens (Boller and Felix, 1991). ET applied as pre-treatment induces resistance against *Botrytis cinerea* (gray mold) in tomato (Diaz et al., 2002), and exogenous application of ET improves *B. cinerea* incidence in tomato, pepper, cucumber, bean, rose, and carnation

(Boller and Felix, 1991). The ET-insensitive mutant of tomato showed increased resistance to *Fusarium oxysporum* (Lund et al., 1992), and soybean mutants with reduced ET sensitivity were less susceptible to *Phytophthora sojae* (Hoffman et al., 1999). By contrast, ET insensitivity increased susceptibility to various pathogens in different plants, e.g., *Arabidopsis* mutant ein2-1 (ethylene-insensitive 2-1) showed increased susceptibility to *B. cinerea* (Thomma et al., 2001a). After its synthesis, ET is perceived, and its signal is transduced through transduction mechanism to trigger specific biological responses. The signalling system consists of two proteins, a histidine kinase and a response regulator. The histidine kinase acts as a sensor that autophosphorylates an internal histidine residue in response to signals, and the response regulator activates the downstream components when it receives a phosphate from the sensor histidine residue on its aspartate residue (Pirrung, 1999). Iqbal et al. (2021) confirmed that SlETR3 (better known as Mai mature-Nr) plants, which is a receptor in tomato capable of binding ET with high affinity, were more sensitive to FA phytotoxicity suggesting the key role of ethylene in activating of defense responses.

3.3.10 Abscisic acid signalling (ABA)

Abscisic acid (ABA), an isoprenoid phytohormone, is a critical signalling mediator that regulates diverse biological processes in various organisms (Kumar et al., 2019); in addition to regulating plant development and response to abiotic stress, also plays a role in the regulation of innate immunity (Adie et al., 2007, Berrocal-Lobo et al., 2002). Meta analysis of pathogen-inducible genes in *Arabidopsis* reveals that a significant subset of ABA-regulated genes is activated upon pathogen infection (Adie et al., 2007). In stress and developmental responses in plants, ABA signaling largely depends on the SnRK family of protein kinases (Kumar et al., 2019). In some plant-pathogen interactions, such as that between *Arabidopsis* and the vascular bacterium *Ralstonia solanacearum*, ABA signalling plays a direct role in the activating the defensive response (Hernandez-Blanco et al., 2007). In contrast, in other plant-pathogen interactions, ABA appears to play a negative regulatory function by inactivating other defence signalling pathways, such as those mediated by SA or JA/ET (Anderson et al., 2004). This negative function of ABA has been proposed as a mechanism used by some pathogens to suppress basal plant resistance (Melotto et al., 2006).

3.3.11 Pathogenesis-related proteins (PRs)

Pathogenesis-related proteins have been discovered in tobacco hypersensitive to *Tobacco mosaic virus* (TMV) and later in other plant species. The recognized PRs currently

comprise 17 families (Van Loon et al., 2006). A type of the member, usually the first, was chosen and families were defined on the basis of their common biochemical and biological properties. A role of different families in limiting the activity, growth and spread of pathogens fits the identification of family members PR-2 as B-1,3-endonucleases, PR 3, 4, 8, and -11 as endochitinases and PR-6 as proteinase inhibitors. Members of PR-8 family also play an important role against bacteria with their lysozyme activity, while PR-12 (defencins) and PR 13 (thionins) have both antibacterial and antifungal activities (Lay and Anderson, 2005; Epple et al., 1997). PR-14 family also includes lipid transfer proteins with antibacterial and antifungal activity (Garcia-Olmedo et al., 1997), while member of PR-1 and PR-5 (thaumatin-like) families have been associated with activity against oomycetes. PR-7 is an endoproteinase that could contribute to the dissolution of the microbial cell wall (Jorda et al., 2000). PR-9 is a specific type of peroxidase that could act in reinforcement the cell wall by catalyzing lignification (Passardi et al., 2004). The families PR-15, -16, and -17 have been added recently. PR-15 and -16 are typical of monocots and comprise families of germin-like oxalate oxidases and oxalate oxidase-like proteins with superoxide dismutase activity (Bernier and Berna, 2001), respectively. These proteins generate hydrogen peroxide that can be toxic to different types of attackers or could directly or indirectly stimulate plant-defence responses. PR-17 proteins have been found as an additional PRS family in infected tobacco, wheat, and barley and contain sequences similar to the active site of zinc-metalloproteinases (Christensen et al., 2002), but have remained uncharacterized to date. A putative novel family (PR 18) includes fungus- and SA-inducible carbohydrate oxidases, as exemplified by proteins with hydrogen peroxide-generating and antimicrobial properties from sunflower (Custers et al., 2004). PR proteins, through their specific hydrolytic activities, can also be expressed during plant development at specific stages or organs and contribute to the generation of signal molecules that can act as morphogenetic factors. However, their widespread induction upon pathogen attack and their regulation by the defence regulatory hormones SA, JA, and ET suggest that they play an important role in alleviating the effects of pathogens and insects attack, as well as some forms of abiotic stress (Andersen et al., 2018). In several instances, quantitative resistance against pathogens has been shown to be associated with constitutively expressed PRs (Liu et al., 2004). In SAR, the presence of induced PR-type proteins is likely to contribute to some extent to the enhanced defensive capacity. In contrast, in ISR, no defence-related proteins are present in induced leaves prior to challenge, but upon infection activation of

JA responsive genes in particular is accelerated and enhanced, a phenomenon known as priming (Conrath et al., 2002).

Aim of the thesis

The aim of Ph.D. research project focused on plant-pathogen interaction between eggplant and the telluric pathogen *Fusarium oxysporum f. sp. melongenae* to achieve durable resistance against this disease by accumulating resistance traits. Here, we characterized already known resistance genes, identifying also novel source of resistance using germplasm collections and RIL, IL, F_2 populations already available. Depending on the level of knowledge, we employed long-read sequencing techniques through Oxford Nanopore Technology and *de novo* assembly, genotypic analyses by using high density SPET and GWAS analyses and fine mapping to isolate genes involved in resistance, as well as functional genomics techniques for their characterization. With the aim to a more sustainable crop management, the influence of N fertilization on *Fusarium* resistance expression has been also evaluated.

In detail, the general objectives of this thesis are:

A) The identification of potential new resistance source to *Fusarium oxysporum* f. sp. *melongenae*. Phenotyping an international eggplant collection and some introgression lines with *S. tomentosum* for resistance to *Fom* wilt by artificial inoculation under greenhouse conditions.

B) The identification of allelic variants and the validation of the resistance loci to fungal diseases. Molecular analyses on full and partial resistant accessions to look for allelic variants of the resistant gene/locus (i.e., *Rfo-sa1* for *Fom*) and molecular markers linked to the QTLs for *Fom*-resistance.

C) The identification and characterization of putative genes involved in *Fom*-resistance. Identification of candidate genes through sequence analysis of resistant regions, with the prospect of performing expression analysis and functional analysis (qRT-PCR, RNAi or CRISPR/CAS9) to confirm their involvement in the resistance trait.

D) The nitrogen effect on defence reaction towards fungal wilts. Evaluation of phenotypic and molecular response by RNAseq of two *Fom*-tolerant genotypes to fungus inoculation under NO_3^- or NH_4^+ supply.

Chapter 1

A genomic BSAseq approach for characterization of QTLs underlying resistance to *Fusarium oxysporum* in eggplant

Abstract

Eggplant (*Solanum melongena* L.), as many other crops, suffers for soil borne diseases, including *Fusarium oxysporum* f. sp. *melongenae* (*Fom*), causing wilting and heavy yield loss. To identify the genetic factors underlying plant responses to *Fom*, a genomic mapping approach was set up using a Recombinant Inbred Lines (RILs) population segregating for resistance, which was previously developed to anchor the high-quality genome sequence of the male parent '67/3' and being the female parent '305E40' fully resistant to *Fom.* The RILs population was assessed for the responses to *Fom,* and two major QTLs on chromosomes CH02 and CH11 were identified, associated to fully and partial resistance to the fungus, respectively. A targeted BSAseq procedure, based on Illumina reads bulks of RILs grouped according to their resistance score and aligned to the suitable reference genomes, highlighted differentially enriched regions between resistant/susceptible progenies in the genomic regions underlying both QTLs whose characterization allowed us to identify the most reliable candidate genes for the two resistance traits. A draft *de-novo* assembly of available Illumina sequences of the '305E40' parent was performed and employed as reference to shed light on the non-recombining genomic region on its CH02 carrying the resistance locus to *Fom* introgressed from *S. aethiopicum.* Through the comparison to the '67/3' genome, private species-specific contigs and scaffolds inherited from this wild species and associated with the resistance trait were revealed.

Keywords *Solanum melongena*, *Fusarium oxysporum* f. sp. *Melongenae*, Recombinant Inbred Lines (RILs), BSAseq, Biotic stress, Resistance genes

1.1 Introduction

The large family of the Solanaceae includes over 3000 plant species adapted to a wide range of geographic conditions, including the cultivated eggplant (*Solanum melongena* L.), tomato (*S. lycopersicum* L.), potato (*S. tuberosum* L.), pepper (*Capsicum annuum* L.), and tobacco (*Nicotiana tabacum* L.). In contrast with many species belonging to this family, eggplant originated from the Old World, firstly in Africa and then domesticated in Asia

(Weese et al. 2010). The common eggplant, also known as “brinjal” or “aubergine”, represents the third most important crop of the Solanaceae family after potato and tomato. It is cultivated worldwide, with a global production of 54 Mt in 2018 (http://faostat.fao.org). Asia represents the main area of cultivation, (93% of both the world production and harvested area with China and India main producers), followed by Africa and the subtropical regions. In Europe, the crop is mainly cultivated in Egypt, Turkey and Italy (http://www.fao.org/faostat/en/#data/QC). Two allied species of common eggplant, scarlet (*S. aethiopicum* L) and gboma (*S. macrocarpon* L.) eggplants, are native and commonly cultivated in Africa, while locally cultivated in other countries including Italy.

Eggplant is susceptible to many diseases and especially to some soil borne fungal wilts caused by *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) (Stravato et al. 1993; Sihachakr et al. 1994; ; Urrutia Herrada et al. 2004; Altinok 2005). *Fom* is a major soil-borne pathogen, responsible for one of the most devastating vascular wilt diseases in eggplant. The fungus penetrates through the roots and proliferates in the vascular tissue. Wilting progresses from lower to upper leaves, causing from yellowing to necrosis, followed by the collapse of the plant, especially when young plantlets are attacked. *Fom* has been identified both in the open field and greenhouse cultivation in several countries, affecting eggplant production causing heavy yield losses (Steekelenburg 1976; Stravato et al. 1993; Altinok 2005). Despite the anthropogenic selection caused a drastic reduction of the genetic variation in the cultivated germplasm (Daunay et al. 1991; Rotino et al. 2014), partial resistances/tolerances to most pathogens were found within the eggplant gene pool, with degree of resistance often scarce for an effective employment in breeding programs (Daunay et al. 1991). Nevertheless, some promising resistance traits to *Fusarium* wilt have been identified in *S. melongena* (Abdullaheva and Shifman 1988; Komochi et al. 1996; Monma et al. 1997; Mandhare and Patil 1993; Barchi et al. 2018), successfully transferred into breeding lines with the development of associated molecular markers (Mutlu et al. 2008; Myatake et al. 2016).

It is well known that *S. melongena* progenitors, allied and wild relatives are important reservoir of potential genetic variability for many agronomic and qualitative traits, as well as a source of valuable resistance to diseases and pests (Daunay et al. 2001; Plazas et al. 2016; Syfert et al. 2016; Toppino et al. 2021). For this reason, conventional (sexual crosses) and unconventional (protoplast fusion, embryo rescue) strategies to introgress traits of interest into the genetic background of cultivated eggplant (Rotino et al. 2014; Kaushik et

al. 2016), including the full resistance to *Fusarium oxysporum* (Rizza et al. 2002; Rotino et al. 2005; Toppino et al 2008), have been employed.

Some studies aimed to characterize the eggplant defence responses and signalling pathways activated upon *Fom* infection as well as identifying loci, QTLs and genes involved in the resistance to fungal wilts have been carried out. Eggplant breeding lines resistant to *Fom* by a cross with Asian accession (Monma et al. 1997), characterized with different molecular markers associated to the resistance trait (Mutlu et al. 2008) were developed, proving to be useful to assist breeding through MAS (Boyaci et al. 2010; 2011). Toppino et al. (2008) demonstrated that the *Fom* resistance trait introgressed into eggplant from protoplast somatic hybridization between *S. aethiopicum* and *S. integrifolium* is controlled by a single dominant locus (named *Rfo-sa1*) and developed codominant molecular markers associated to the resistant and/or the susceptible phenotype through a Bulked Segregant Analysis (BSA) (Michelmore et al., 1991)

The *Rfo-sa1* locus was localized on the eggplant chromosome CH02, using a RAD-tag derived markers map (Barchi et al. 2012) based on the intra-specific segregant population from the cross '305E40'x'67/3', in which the male line, '67/3' was recently employed to develop a high-quality genome sequence (Barchi et al., 2019), while the female parent, '305E40', is an introgressed double-haploid line developed by somatic hybridization with *S. aethiopicum* (Rizza et al., 2002; Toppino et al., 2008) and is fully resistant to *Fom*. The genotypic characterization of '305E40' line revealed a haplotype identical to that from *Solanum aethiopicum* in the upper portion of chromosome CH02 (0-10.4 cM) which included the locus *Rfo-Sa1* (Portis et al. 2014), this "alien portion" being probably inherited from this wild genotype during the production of the somatic hybrid through protoplast fusion. Two major QTLs for the resistance trait to *Fom* were identified in the same F_2 population: a first on chromosome CH02, *FomE02,* derived from the resistant parent '305E40' lying in the genomic region of the *Rfo-Sa1* locus inherited from *S. aethiopicum,* and a second, *FomE11.1*, on chromosome CH11, inherited from the male parent '67/3' which carries a source of partial resistance to *Fom* never spotted before (Barchi et al., 2018). Moreover, candidate genes involved in early defence responses or signalling pathways activated upon infection were identified in the *Fom*-resistant advanced breeding line '305E40' (Barbierato et al. 2016). Two *Fusarium* semi-dominant inherited resistance loci (Fukuoka et al. 2012; Hirakawa et al. 2014) were also mapped on chromosomes CH02 and CH04 in a linkage map developed from Asian *Fom*-resistant lines (with the QTL on CH02

orthologous to the *Rfo-sa1* locus), and a set of orthologous candidate genes were suggested within the confidence interval of the discovered QTLs by exploiting the syntenic relationships with tomato (Miyatake et al. 2016).

More recently, a F_6-F_7 RIL population was developed from the same cross '305E40'x'67/3', whose 5X Illumina sequencing data, together with an Illumina 35X sequencing of '305E40' line were exploited to anchor the scaffolds of the sequenced line '67/3' to the 12 chromosomes to obtain the first anchored eggplant genome sequence (Barchi et al. 2019). A more user-friendly high-density map, based on Genotype-by-sequencing (GBS), was recently developed on the same population, and successfully used to better define several QTLs associated with plant anthocyanin content and seed quality traits as well as fruit metabolomic content and anthocyanin peel coloration (Toppino et al. 2020; Florio et al. 2021; Sulli et al. 2021). A more continuous Hi-C based assembly of line '67/3', together with the first the first pangenome of eggplant obtained by resequencing of 23 additional accessions of *S. melongena* representative of the worldwide phenotypic, geographic, and genetic diversity of the species was also recently released (Barchi et al., 2021).

In the present work, to better localize the QTLs and understand the genetic mechanisms of the full and partial resistance to *Fusarium oxysporum* f. sp. *melongenae* traits inherited from '305E40' and '67/3', this last high-density GBS-Based map was exploited.

For a finer characterization of the regions underlying both QTLs, we took advantage of the availability of the high-quality annotated genome sequence of the '67/3' parent, of 35x resequencing data of the other parent '305E40', as well as of the resequencing data of each RIL to apply a targeted BSA-seq approach.

With this purpose, to detail the fine characterization of the locus on CH02 inherited from *S. aethiopicum,* the available 35X reads of line '305E40'were employed to build a draft *de-novo* genome assembly which allowed to recover and reconstruct unique portions of introgressed allied genome more likely responsible of the full resistance trait to *Fom,* which are absent in the reference line '67/3'.

Two independent rounds of BSA-seq were performed, in which bulked reads of different subsets of RILs, grouped according to their resistance score, were then aligned to the more suitable reference genome for each QTL, thus enabling to highlight differentially enriched

portions and to identify the most reliable candidate genes responsible of both resistance traits.

1.2 Materials and Methods

1.2.1 Plant material

A population of 168 $F_{6–7}$ Recombinant Inbred Lines (RIL), recently characterized by GBS was employed in this work (Toppino et al., 2020). The RIL population was developed from the cross between the two eggplant breeding lines '305E40' and '67/3', contrasting for a wide number of key agronomic and metabolic traits (Barchi et al. 2012; Portis et al. 2014; Toppino et al. 2016; Barchi et al., 2018). The line '305E40' (female parent) is a double haploid derived from an interspecific somatic hybrid *Solanum aethiopicum* gr. *gilo* (+) *S. melongena* cv. Dourga (Rizza et al. 2002), which was repeatedly backcrossed with the recurrent lines 'DR2' and 'Tal1/1', before selfing and anther culture. This breeding line carries the locus *Rfo-sa1* introgressed from *S. aethiopicum*, which confers complete resistance to the soil-borne fungus *Fom* (Toppino et al. 2008; Barchi et al 2018). Otherwise, the line '67/3' is a F_8 selection from the intra-specific cross between *cvs* 'Purpura' and 'CIN2', that showed a partial resistance trait to *Fom* (Barchi et al 2018).

1.2.2 Eggplant/*Fusarium oxysporum* f. sp. *melongenae* resistance assessment

To assess both the full and partial *Fom* resistance traits, the RILs, together with parental lines, their F_1 hybrid and the full susceptible (line 'Tal1/1') and resistant (*S. aethiopicum*) control lines were sawn in plastic trays filled with pasteurized peat and grown in greenhouse at CREA-GB, Montanaso Lombardo (45°20'N, 9°26'E). For each progeny/accession, a 104 holes tray was sawn. A total of 8135 plantlets were employed to assess resistance to *Fom.* The inoculation was conducted according to the dip-root method as reported by Cappelli et al. (1995). Plantlets, at the 2–3 true leaf stage, were gently removed from the tray and their roots washed under running tap water, then immersed for 15 min in a conidial suspension of *Fusarium oxysporum* f.sp. *melongenae* at a concentration of 1.5×10^6 conidia/ml. All the plantlets (min 22, max 74 plants) for each progeny were divided in two blocks and then inoculated with the *Fom* conidial suspension. After dipping, the two blocks of plants were transplanted in 54-hole trays and randomized in two different greenhouses until symptoms evaluation. For each line and progeny, 9 plants were mock inoculated with water and kept in greenhouse as negative control.

Evaluation of symptoms was assessed on each plant 30 Days After Inoculation (DAI), according to a scale (compared with mock inoculated controls) ranging from 1 to 0, where 1 corresponds to ‘‘fully resistant plant with complete absence of symptoms’’, 0 to ‘‘dead plant’’ and with the intermediate values as follows: 0.9 = some yellowing spots in basal leaves, absence of symptoms in intermediate and upper ones; 0.8 = extended yellowing in basal leaves; 0.7 = extended yellowing in basal leaves and some yellowing spots in intermediate ones; 0.6 = extended yellowing in both basal and intermediate leaves; 0.5 = some necrosis spots in basal leaves, extended yellowing in basal and intermediate ones and some spots of yellowing in upper ones; 0.4 = partial necrosis in basal leaves, extended yellowing in intermediate and upper ones; 0.3 = necrosis in basal leaves and some necrosis spots in intermediate ones; 0.2 = necrosis in basal and intermediate leaves, falling of basal ones; 0.1 = complete necrosis in all the leaves, falling of basal and intermediate ones (Figure 1.1).

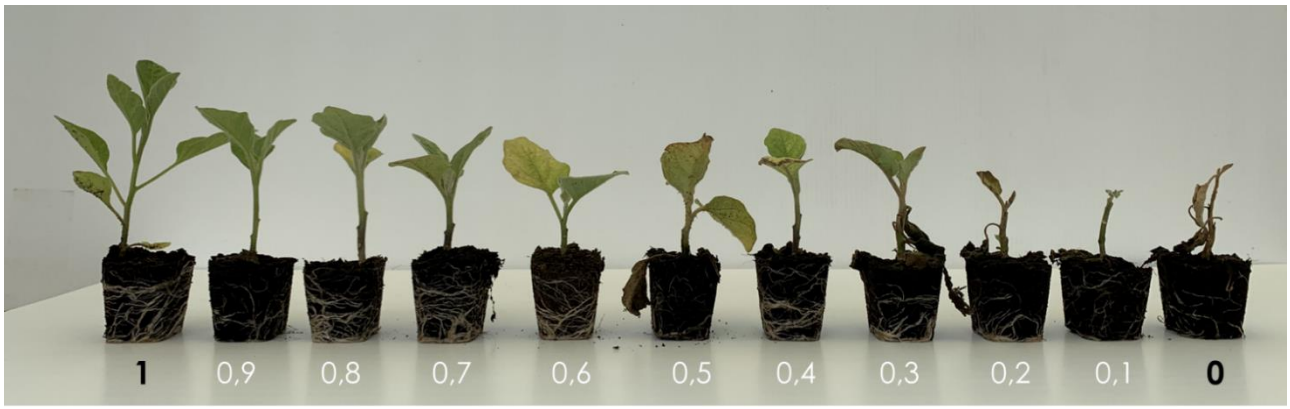


Figure 1.1: Visual representation of the degree of symptoms assigned on each single plant ranging from 1 to 0.

For each block, the resistance ratio (%) was calculated as

$$R = \frac{\sum (\text{plant} * \text{score assigned})}{\text{total n°of inoculated plants}} * 100$$

1.2.3 Statistical Analyses and QTL Detection

Analysis of variance (ANOVA) of resistance trait was performed to test the significance of differences between RILs and replications using JMP v. 7 software (SAS Institute, Milano, Italy). The effects of replications and genotypes were accounted in the model. Broad-sense heritability values were given by σ2G/([σ2G+σ2E]/n), where “σ2G” represented the genetic variance, “σ2E” the residual variance and “n” the number of replicates.

Normality, kurtosis and skewness were assessed with the Shapiro-Wilks test (α=0.05). Segregation was considered as transgressive when at least one RIL recorded a trait value higher or lower by at least two standard deviations than the higher or lower scoring parental line.

QTL mapping was conducted using the recently published high density genetic map (Toppino et al., 2020) with the R/qtl package of the R statistical computing software (Broman et al., 2003). For each trait, an initial QTL scan was performed using simple interval mapping with a 1 cM step (Lander and Botstein, 1989), and the position of the highest LOD was recorded. A genome-wide significance level of 5% was calculated after 1000 permutations (Churchill and Doerge; 1994), and the LOD threshold was used to identify a QTL. Then, QTL location and effect were determined using the multiple imputation method by executing the "sim.geno" command, followed by the "fitqtl" command (Sen and Churchill; 2001). To search for additional QTLs, the "addqtl" command was used. If a second QTL was detected, the "fitqtl" was used to test a model containing both QTLs and then the interaction effect. If both QTLs remained significant, the "refineqtl" command was used to re-estimate the QTLs' positions based on the full model including both loci. QTLs interactions were studied, and the significant locus combinations are reported based on F-measure. The additive effects of QTLs were estimated as half of the difference between the phenotypic values of the respective homozygotes. The confidence interval (CI) of each QTL was determined as proposed by Darvasi and Soller (1997).

1.2.4 Composition of the bulks of 5X Illumina sequences

Three bulks (RR, resistant; SS, susceptible; PR partially resistant) of available 5x Illumina sequences for each RIL (Barchi et al., 2019 submitted to the NCBI Sequence Read Archive under the accession number SRP078398) were clustered according to their disease symptoms score:

- BULK RR: includes 28 fully resistant RILs with a calculated disease ratioof 100% and harboring the resistance locus *Rfo-Sa1*.
- BULK SS: includes 18 fully susceptible RILs, in which the molecular marker for the locus *Rfo-Sa1* is lacking and with a disease resistance ratio of 0;
- BULK PR: includes 17 Partially resistant RILs, in which the locus *Rfo-Sa1* is lacking, but with a resistance ratioe ranging from 30 to 100%.

1.2.5 Alignment of bulked sequences to the reference '67/3'

The two bulks of reads PR (Partially Resistant) and SS (fully susceptible) were aligned with Bowtie2 (Langmead et al., 2012; tolerance of max 2 mismatches per reads) to the '67/3' eggplant reference Version3 (V3) genome (Barchi et al., 2019 available at http://www.solgenomics.net). Furthermore, forward reads from '67/3' V3 genome and 35X reads from '305E40' were mapped on the reference genome as positive and negative control, respectively. GFF3 files generated with Augustus annotation was loaded in Integrative Genomic Viewer (IGV) (Robinson et al., 2013) for read and gene visualization in the genomic region and reads alignment context were integrated for further confirmation. Regions of interest were estimated based on a combination of cues including, in case of virtually identical reads, ratio of PR *versus* SS reads, or, in case of read heterogeneity (as evidenced by SNP abundance in mapped reads), coherence with the PR reads set. The QTL confidence intervals on CH11 was analyzed with the SnpEff v4.3 program (Cingolani et al., 2012), to infer the potential effect of SNP/Indel identified on candidate genes for the resistance trait. The effect of each polymorphism in 305E40 with respect to the reference 67/3 was classified into four classes: 1) modifier effect, as variants located outside genes (non-transcribed regions or introns); 2) low effect, as synonymous variants in coding regions; 3) moderate effect, as variants altering the aminoacidic sequence and 4) high effect, as variants changing frameshift thereby introducing/eliminating stop codons or modifying splice sites. Finally, to identify the best candidate genes, functional annotations pinpointing high confidence genes with a defense role, as well as evidence from expression levels found in the previously published '67/3' RNA-seq data (Barchi et al., 2019), of 16 tissues including roots were considered.

1.2.6 *De novo* assembly of 305E40

The 35X Illumina sequencing reads submitted to the NCBI Sequence Read Archive are available under the accession number SRP078398. Further information, including the '67/3' genome assembly, pseudomolecules, annotations and tracks for the genome browser are available, in downloadable form, on the Solanaceae Genome Network. Draft *de novo* assembly (hereafter named "asm_305") of the 35x Illumina sequences was performed using the software soapdenovo2 (Luo et al., version 2.04) in multi-kmer mode (kmers: 43-91) and average insert size 200. The draft assembly resulted in a total of 1,667,559 contigs (N50 value 7,937) for a total of 1.155 Gb; of these, 141,312 contigs longer than 1 Kb where subjected to further BSA-seq analysis.

1.2.7 Alignment of bulked sequences alignment to *asm_305*

The two bulks of reads RR (Fully Resistant) and SS (Fully Susceptible) were aligned with Bowtie2 (tolerance of max 2 mismatches per reads) to the *de novo* assembled reference (*asm_305*). Forward reads from '67/3' and 35x '305E40' sequencing were mapped on the *asm_305* reference as negative and positive control, respectively. To prevent artefacts-related issues and false-positive covered regions, contigs longer than 1000 bp were further filtered based on the following criteria: a minimum of 50 reads/kb in at least one of RR or SS mapping bulks and mapping ratio (RR vs SS) of at least 16. Differentially represented contigs were subjected to gene model identification implementing Augustus version 3.1 (Hoff and Stanke, 2019) trained with tomato.

As the region putatively containing the QTL *FomCH02* was not physically localizable, the entire sequence of CH02 was considered for the BSA-seq analysis as above described, and in this case, being the 305E40 line totally resistant to *Fom,* the identification of differentially represented regions was based on a combination of cues relating to RR vs SS abundance ratio and heterogeneity.

1.2.8 Expression analysis of candidate genes

The expression levels of the best candidate genes identified on chromosome 11 were retrieved from in the previously published '67/3' RNA-seq data (Barchi et al., 2019), of 16 tissues including roots.

The primer sequences to amplify candidate genes on chromosome 02 were retrieved by *in silico* analysis and CDS sequences predictions within the induced contigs and are detailed in Table 1. For the molecular analysis, the experimental dataset available was the same already described in Barbierato et al., 2016. More in detail, samples of inoculated and mock-inoculated (dipping in water) roots of the line '305E40' harvested at 0, 4 (T0+4h) and 8 hours (T0+8h) after the artificial inoculations with *Fom* were employed for RNA extraction, cDNA synthesis and RT-qPCR analysis. Three biological replicates of root samples, each consisting of a pool of 8 inoculated plantlets, were harvested for each inoculation treatment and timing. RT-qPCR analysis was performed in 72-Well Rotor with Rotor-Gene RG-6000 (Corbett Research) using GoTaq® RT-qPCR Master Mix by PROMEGA. The reaction containing 1.0 µl of previously diluted cDNA (1:20), from 0.2 µl to 1.0 µl of primers (1 µM each), 5 µl of GoTaq® RT-qPCR Master Mix and RNase-Free water up to the final volume of 10 µl. All the reactions in triplicate with three biological replicates were performed, and no-template samples in all the analyses as negative controls

were included. Standard curves for each primer pair across a 5-fold dilution series of pooled diluted cDNA amplified in technical triplicate were calculated. Primers, based on the available '305E40' sequences by Primer 3 software (https://bioinfo.ut.ee/primer3-0.4.0/primer3/) were designed (Table 1.1).

GENE ABBREVIATION	Oligo sequences for RT-qPCR
RES_1	Gene1RTFor:5'_TGGCAGAATCTCCACAACCT_3' Gene1RtRev:5'_GATGATGAAGGACTGCTCGC_3'
RES_2	RES_2_FW 5'_ACCAGCACTGATCTGTCTCC_3' RES_2_RV 5'_TATGACCGGTCCCTTTTCCC_3
RES_3	RES_3_FW 5'AGTACAAGGGAAGCCGTGAG 3' RES_3_RV 5'GAGCAGCATCAGATCAGCAC 3'
RES_4	RES_4_FW 5' ACGGAACTAGAGCGACAACA 3' RES_4_RV 5' TAGCCTTGCCTCTATCCTGC 3'
RES_5	RES_5_Fw 5' CCGCCAATTCACTGCGTAG 3' RES_5_RV 5'TTGTATCCTCCTCCTCGCTG 3'
RES_6	RES_6_FW 5'TTTGAGCTGTTGGGCCAATC 3' RES_6_RV 5'CCGTGGTGCATTATAGCCAC 3'
RES_7	RES_7_FW 5'AATGGGAAGTGCAGTGGAGA3' RES_7_RV 5'_GGGGAAGTTGGCAGCATAAG_3'
RES_8	RES_8_FW 5'ACCAGGTTAAGTTACAGCTCTGA3' RES_8_RV 5'ACCCCTTTCCAGACACATCA3'
RES_9	RES_9_FW 5'TCTCCCTTTCTCCGTTCACC3' RES_9_RV 5'TCTGGTTGTTGGCGATGTTG3'
RES_10	RES_10_FW 5'CCCATCTCTTCCACCAGTGT3' RES_10_RV 5'AGAATAGGGGTGGCTGGTTC3'

Table 1.1: List of candidate genes and primers for RT-qPCR analysis (detailed information are reported in Tab.6)

1.3 Results

1.3.1 Phenotypical score and Statistical analysis

The plantlets of the line '305E40' and of the F_1 hybrid '305E40x67/3', carrying the locus *Rfo-Sa1,* exhibited a complete resistance against *Fom* (Figure 1.2a and 1.2b, respectively) as well as the wild donor species *S. aethiopicum*, whose plantlets were completely symptomless at 30 DAI after inoculation (Table 1.2).

Figure 1.2: plantlets of line 305E40 (a), of the F1 hybrid (b), of 67/3 (c) and of two full sensitive lines DR2 and TAL1/1 (d) at 30 DAI after inoculation with F. oxysporum.

Conversely, the *Fom*-susceptible control line 'Tal1/1', which is one of the recurrent parents for selecting the '305E40' breeding line after the somatic hybridization [*S. melongena*+*S. aethiopicum*], confirmed its full susceptibility to the pathogen, so that all the inoculated plantlets were completely wilted and died (figure 1.2d). All the plantlets belonging to the '67/3' (figure 1.2c) parental line survived to *Fom* inoculation although they showed an average resistance score value of 60% due to reduced growth and yellowish leaves with respect to mock inoculated plantlets of the same line (Table 1.2). Unfortunately, eleven progenies of the 168 total RILs used to build the GBS based map did not germinate. The distribution of the resistance ratio to *Fom* among the 157 RIL progenies is displayed in Figure 1.3. Fifty-eight RILs were completely resistant (Score =100) while 42 were found to be susceptible (score =0) after *Fom* inoculation. Most of the remaining 57 lines displayed a resistance score above 50 (Figure 3), with an average disease score of RIL population of about 60%. Highly significant genotypic effect was detected for the *Fom* resistance trait (Table S1), also confirmed by a very high value of hereditability. Transgressive segregation was observed only respect to the most sensitive parent '67/3' in 66 RILs (Table 1.2).

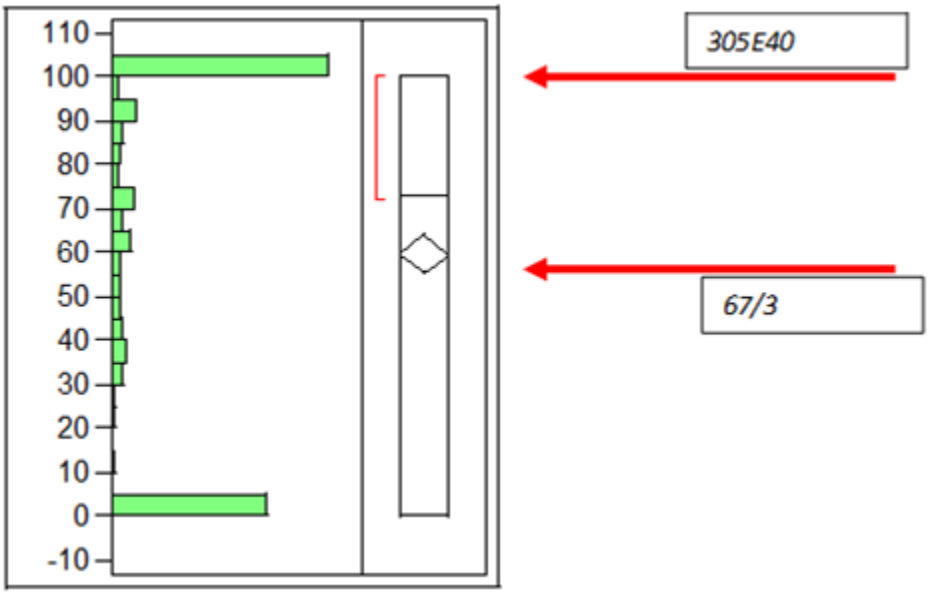


Figure 1.3. Distribution of the resistance score among the RIL population. The scores values of the two parental lines '67/3' and '305E40' are highlighted with red lines

Quantiles

100.0%	maximum	100,00
99.5%		100,00
97.5%		100,00
90.0%		100,00
75.0%	quartile	100,00
50.0%	median	72,96
25.0%	quartile	0,00
10.0%		0,00
2.5%		0,00
0.5%		0,00
0.0%	minimum	0,00

Moments

Mean	59,962144
Std Dev	41,935881
Std Err Mean	2,3297712
upper 95% Mean	64,545586
lower 95% Mean	55,378703
N	324

Oneway Anova
Summary of Fit

Rsquare	0,998668
Adj Rsquare	0,997344
Root Mean Square Error	2,161174
Mean of Response	59,96214
Observations (or Sum Wgts)	324

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
genotype	161	567277,00	3523,46	754,3791	<.0001
Error	162	756,65	4,67		
C. Total	323	568033,65			

Trait code	Parents mean ±SD '305E40'	'67/3'	*S.aeth*	Tal1/1	F1	F2 population mean ±SD	Skewness	SE	Kurtosis	SE	Heritability	Transgressive respect '305E40'	Transgressive respect '67/3'
Fom	100±0	60±0	100±0	100±0	100±0	59.83±41.99	-0.455	0.193	-1.502	0.384	0.98	-	66

*Table 1.2: Disease score of the parental lines, of the two control lines for complete susceptibility ('Tal1/1') and resistance (*S. *aethiopicum) the F1 hybrid (67/3 x 305E40) and the mean value in the RIL populations. Skewness, kurtosis, Broad sense heritability and transgressive genotypes for the trait in study are also reported.*

1.3.2 QTL mapping

The analysis of *Fusarium oxysporum* resistance allowed the identification of two associated region on chromosomes 2 and 11 named as *FomCH02* and *FomCH11*, respectively. (Table 1.3).

QTL code	chr	cM	LOD	PVE (%)	A	IC (cM)	
						start	end
FomCH02	2	222.7	29.03	46.47	-30.9	221.66	223.74
FomCH11	11	88.5	18.98	25.72	14.61	86.61	90.39
	Interaction 2*11		7.42	8.37	13.15	0	5.80

Table 1.3: QTLs associated to the Fusarium *resistance trait. Chromosomes (Chr), peak marker position (cM), LOD scores, percentages of phenotypic variance explained (PVE), estimated additive effects, and the confidence interval (CI) were also provided.*

The strongest QTL, *FomCH02*, explained ~47% of PVE and the female parental line '305E40' contributed the allele with the positive effect The second major QTL (*FomCH11*) is located on CH11, explains about 26% of variance (PVE) with a LOD of 19. In this region, whose centre is determined by two colocalizing markers (physically positioned at CH11_65173656 and CH11_65191109, respectively), the male parent '67/3' contributed the allele with the positive effect. Finally, 8% of the variance was explained by the interactions between the two QTLs detected.

To confirm the involvement of *FomCH02* and *FomCH11* in the *Fom* resistance trait, the haplotype distribution of all the GBS markers belonging to the genomic regions underlying the two QTLs was assessed in all the RILs after a manual ordering in accordance with their resistance score. For *FomCH11*, a region spanning between 80 and 90cM on chromosome 11, corresponding to a physical extension between 64.5 and 69.5 Mb was considered. The physical extension of the CI of the *FomCH02* QTL was difficult to be determined, due to a highly discordant ordering of the molecular markers according to their physical mapping along the V3 sequence of 67/3compared to their position in the genetic map, therefore we decided to include in this investigation all the GBS markers belonging to the entire CH02. This choice allowed us to highlight a highly conserved and nearly-entire region of CH02 spanning from 0 to 310 cM out of the total 326 cM, where the haplotype resulted continue and identical to that of "305E40" (Figure 1.4) in forty-seven out of 58 (47/58) completely resistant RILs, plus 2/58 RILs in which the same region was fully heterozygous. 5/58 full-resistant RILs exhibited a slightly fragmented haplotype in CH 02, which will deserve a

better characterization. Last, 4/58 RILs harboured a "67/3" haplotype on CH02, but, concurrently, showed the haplotype of "67/3" within the genomic region underlying the *QTL Fomch11,* therefore their resistance score could likely be due to a transgressive effect of this QTL, mimicking the "full resistant" phenotype.

Among the RILs with R score less than 100%, 4 still displayed a slightly fragmented haplotype of '305E40' in CH02 (which would deserve a better characterization). All the remaining RILs with a score ranging between 98% and 28% exhibited a "67/3" haplotype both in CH02 and in the region underlying the *FomCH11,* therefore inherited the partial resistance trait harboured from the 67/3 line. while all the RILs with resistance score below 28% showed a "67/3" haplotype in CH02 and a "304E40" haplotype within *QTL FomCH11,* therefore seeming to have inherited neither of favourable traits haplotypes from the parents.

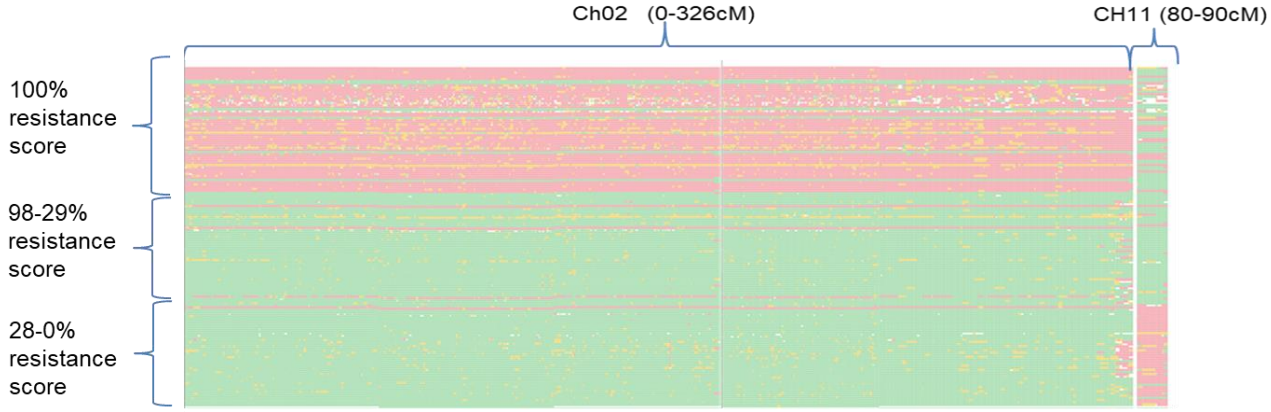


Figure 1.4. Haplotype distribution in the RIL population of all GBS markers mapped on CH02 (0-236cM) and CH11 (80-90cM) ordered according to their resistance score (on the left panel, descending from 100% to 0). Each line represents a RIL progeny. In red, haplotype of '305E40'; in green haplotype of '67/3'; in yellow heterozygous markers. In white, missing data

1.3.3 QTL *FomCH11*- BSAseq and candidate genes identification

The genomic region within the CI of the major QTL *FomCH11* was subjected to a finer characterization and revealed minor inversions and other embedded structures still present in the V3 genome (Barchi et al. 2019), evidenced by slight discrepancies between the GBS markers order and their physical location along the chromosome. For the BSA-seq analysis, two bulks of reads from selected PR and SS RILs were created and aligned to the '67/3' eggplant V3 reference genome. In addition, reads from '67/3' and '305E40' were mapped on the reference V3 genome as positive and negative control, respectively. default V3 GFF3, plus an additional Augustus annotation not subjected to masking of TE and repeat regions were loaded on IGV browser for gene and read mapping visualization (Figure 1.5) focusing on the region on CH11 spanning 4Mb between 64.5 and 69.5 Mb, which contains

278 annotated genes. The differentially represented genomic regions of interest were estimated based on a combination of cues including in most cases a differential (higher) ratio of mapping PR versus SS reads within a predicted region as CDS, but also the presence of differential mismatches of PR *vs.* SS reads in a region covered by comparable amount of mapping reads between the two bulks. Indeed, such poor coherence between the reads would be consistent with, e.g., SS reads arising from close paralogous genes mapping artefactually solely due to mismatch tolerance. Also, of course, the presence of a CDS was among the cues considered to select the most interesting regions. IGV Visual inspection led to the identification of five differentially enriched genomic regions underlying the QTL characterized by a differential coverage in reads from RR compared to the SS bulk (table 1.4). Among these 5 regions nine candidate genes, annotated as resistance genes, were highlighted.

Figure 1.5: Example of IGV Visual score in the region spanning 65 and 66 MB on chromosome 11. At the top of the image, the entire region within the CI of the FomCH11 QTL is represented. The yellow box depicts the region zoomed in the box below. Read alignments of the two bulks PR (on the top) and SS (bottom) against the reference sequence of '67/3' are shown, together with the annotated genes according to V3 genome sequence (Barchi et al., 2019, top) and the Augustus annotation (bottom) also including TE-related genes. The two yellow ovals highlight two differentially enriched regions between bulks of PR and SS (i.e., with different read coverages detectable when comparing upper and lower panel), while the pink ones highlight the position of two non-enriched ones.

Diff. enriched region	Physical interval in V3	Number of annotated genes	Number of Resistance genes	Gene ID according to gene annotation by Barchi et al., 2019
A	65,030-65,120Mb	2	2	**SMEL_011g374890.**1Similar to At1g58602: Probable disease resistance protein At1g58602 (*A. thaliana*)
				SMEL_011g374900.1 Similar to RPP13: Disease resistance protein RPP13 (*A. thaliana*)
B	65,532-65,553Mb	2	**2**	**SMEL_011g375310.1** Similar to RPP13: Disease resistance protein RPP13 (*A. thaliana*)
				SMEL_011g375320. 1: Similar to RPP13: Disease resistance protein RPP13 (*A. thaliana*)
C	66,920-67,557Mb	31	0	
D	68,093-68,171Mb	7	3	**SMEL_011g376860.1** Similar to XA21: Receptor kinase-like protein Xa21 (*O. Sativa* subsp. Indica)
				SMEL_011g376900.1 Similar to FLS2: LRR receptor-like serine/threonine-protein kinase FLS2 (*A. thaliana*)
				SMEL_011g376910.1 Similar to FLS2: LRR receptor-like serine/threonine-protein kinase FLS2 (*A. thaliana*)
E	69,366-69,410Mb	7	2	**SMEL_011g377340.1** Similar to R1A: Late blight resistance protein R1-A (S. *demissum*)
				SMEL_011g377380.1; Similar to R1C-3: Putative late blight resistance protein homolog R1C-3 (*S. demissum*)

Table 1.4: List of differentially enriched regions within the confidence interval of the QTL. For each region, extension, number of annotated genes, number of genes annotated as resistance genes and details for each best candidate gene according to the annotation in eggplant genome V3 (Barchi et al., 2019) is reported

To infer functional annotation and the potential deleterious effect of the candidate genes responsible for the *Fom*-resistance trait in the "305E40" compared to the reference "67/3", all the annotated genes within the QTL confidence interval were analyzed by SnpEff v4.3 program (Cingolani et al. 2012, Supp File S2). All the candidate genes highlighted within the 5 differentially represented regions exhibited a SNP effect from "moderate" to "high" in the "305E40" variants (Table 5); moreover, two additional genes, SMEL_011g374910 and SMEL_011g374920, annotated as 'Similar to RPP13: Disease resistance protein RPP13 from *A. thaliana*' and proximal to the Diff. region A, (thus named "A+", from 65.150Mb to 65.168Mb) were included to the list of best candidates according to their putative function and the high SnpEff score. The expression levels of the best eleven candidate identified genes in sixteen '67/3' tissues according to previously published RNA-seq data (Barchi et al. 2019) are reported in Table 1.5.

			expression level (Barchi et al., 2019)					
DIFF REGION	**gene_ID**	SnpEff	**Roots**	**Expanded leaves**	**Open flowers**	**Fruits 2-4 cm**	**Fruit stage B**	**Fruit stage C**
A	**SMEL_011g374890.1**	Mod	0	0	0	0	0	0
A	**SMEL_011g374900.1**	High	0.716154	12.2523	0	8.27647	3.05559	2.95479
A+	**SMEL_011g374910.1**	High	0	5.38543	0	4.35919	4.00833	16.8497
A+	**SMEL_011g374920.1**	High	26.9772	25.087	31.93	9.58168	16.8583	50.3871
B	**SMEL_011g375310.1**	High	0	0	0	0	0.221595	0.474865
B	**SMEL_011g375320.1**	Mod	0	0	0	0	0.636017	0

D	**SMEL_011g376860.1**	High	0	0	0	0	0	0
D	**SMEL_011g376900.1**	High	0	0	0	0	0	0
D	**SMEL_011g376910.1**	High	0	0	0	0	0	0
E	**SMEL_011g377340.1**	High	1.31895	0	0	0.761885	1.12618	0
E	**SMEL_011g377380.1**	ND	0	0	0	0	0	0

Table 1.5 SNPEff and RNA-seq expression levels of the most reliable candidate genes in different tissues of '67/3' (Barchi et al., 2019).

1.3.4 QTL *FomCH02- De novo* assembly of '305E40', BSAseq and candidate genes identification

For *FomCH02,* a similar BSA-seq characterization to that performed for *FomCH11* QTL using the '67/3' V3 genome as reference sequence was not conceivable due to the wild (*S. aethiopicum)* origin of the genomic region underlying this QTL*,* which is exclusive of the '305E40' parent and entirely lacking in '67/3' (Portis et al. 2014). better resolve the CH02 region introgressed from the allied species *S. aethiopicum* in '305E40', a new genome assembly of the available 35X Illumina reads of this line was performed, leading to the generation of a *de novo* assembly more suitable as reference (Hereafter named *asm_305*), including 1,667,559 contigs (CG) with a N50 of 7,937bp, a N90 of 159bp, respectively, covering 1.155 Gb, with a total number of 263.683 contigs having length > 300 bp, 202.945 contigs > 500 bp, and 141.312 contigs > 1000 bp.

To identify scaffolds and contigs highlighting differentially covered regions in which genes responsible for full resistance trait to *Fom* might be included, the *305_asm* was employed as reference for the BSAseq mapping of bulked reads from 18 and 28 SS and RR RILs, respectively. Among the contigs with length> 1000 bp and a tolerance of >50 reads/kb in at least one RR or SS mapping bulks, a subset of 1838 contigs/scaffolds with *log2*>4 RR *vs*. SS was identified, with a total scaffold length of 9.36 Mb. Among the contigs with length > than 3Kb, 691 (7.43 Mb) with *log2*>4 of which 306 (3.01Mb) with *log2>4.90*, equivalent to a 30-fold enrichment in RR *vs*. SS reads, have been identified (Figure 1.6). Among this last dataset, the scaffold #67320 contains the sequence amplified by the *Rfo-Sa1* markers. The *log2*>4 over-represented subgroup of 1838 contigs >1Kb was employed for the *in silico* analysis and CDS prediction. BLASTn comparison between all the identified CDS in the enriched contigs *versus S. aethiopicum* assembled transcripts (Gramazio et al., 2014) led to the identification of nine candidate genes (RES2-RES10) with complete or nearly complete match with *S. aethiopicum* and a tolerance of $1e^{-100}$. BLASTn search of the contigs/scaffolds belonging to the *asm_305* containing these genes

led to the identification within the *S.aethiopicum* draft genome (Song et al., 2019) of corresponding scaffolds with complete or nearly complete match (Table 1.6).

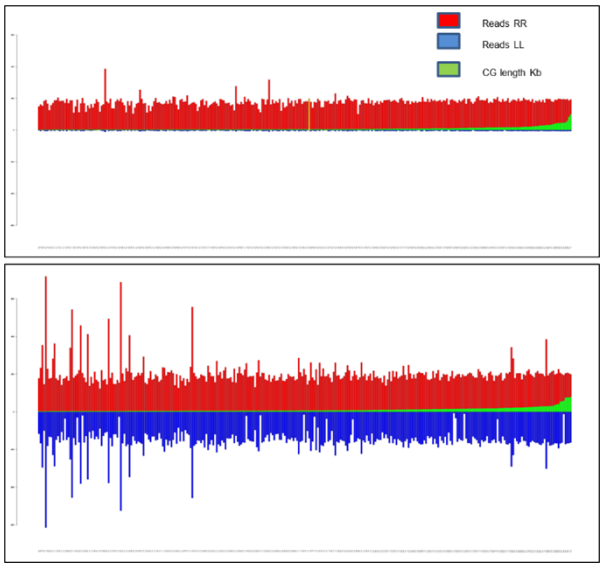


Figure 1.6: Graphical representation of selected contigs coverage in the asm_305 assembly. Upper panel: Contigs exhibiting a ratio of mapped RR vs SS reads of a least 30 (log2>4.90 are shown. Lower panel: contigs exhibiting a ratio of RR vs SS reads<30 are shown. For both panels, contigs are ordered by increasing length along X axis. RR, SS and contig length are represented as red, blue and green bars, respectively

Candidate genes	Query	Scaffold on *S.aethiopicum transcriptome*	identities	Predicted function based on domains analysis performed via NCBI platform	Scaffold on S. aethiopicum pangenome
RES 1	SMEL_002g157480.1 (Miyatake et al., 2016, orthologous of SOLYC02G032200.2	SAUC48279_TC01 Length = 3745	3392/3418(99%) Strand = +/+	Encoding a putative TMV resistance protein N-like LOC102604931, transcript variant X2	scaffold3814_cov65 (978531-977633) lenght:898 strand (+/-)
RES 2	C7021905__121_4977 (348 letters)	SAUC67459_TC01 Length = 578	348/348(100%) Strand = +/-	chaperonin	scaffold150403_cov62 (904702-904125) lenght:577 strand (+/-)
RES 3	C7104747__218_9061 (1767 letters)	SAUC05724_TC02 Length = 1311	915/915 (100%) Strand = +/+	cysteine-rich RLK (RECEPTOR-like protein kinase) 8	scaffold150406_cov62 (122904-121605) lenght:1299 strand (+/-)
RES 4	C7123897__247_10432 (1176 letters)	SAUC85719_TC01 Length = 1281	194/194(100%)+ 807/807(100%) Strand: +/+	Putative late blight resistance protein homolog R1A-3	scaffold149207_cov61 (473206-471925) lenght:1281 strand (+/-)
RES 5	scaffold131120__543_23320 630 letters)	SAUC54187_TC01 Length = 581	312/312(100%) Strand: +/+	-	scaffold4400_cov64 (319724-320299) lenght:575 strand (+/+)
RES 6	scaffold151247__813_35520 (1935 letters)	SAUC60998_TC03 Length = 2636	986/986(100%)+ 719/721(99%), Strand = +/+	-	scaffold149470_cov62 (278029-278886) lenght:857 strand (+/+)
RES 7	scaffold161031__1035_45870 (1362 letters)	SAUC62185_TC01 Length = 517	517/517(100%) Strand = +/+	Protein transparent testa 12-like	scaffold149494_cov61 (246517-247031) lenght:514 strand (+/+)
RES 8	scaffold4270__1160_51181 (3702 letters)	SAUC68094_TC01 Length = 4264	2697/2697(100%) + 974/974(100%) Strand = +/-	Putative late blight resistance protein homolog R1B-14-like [Solanum lycopersicum]	scaffold872_cov63 (353791-349798) lenght:3993 strand (+/-)
RES 9	scaffold160330__1010_44805 (327 letters)	SAUC18225_TC01 Length = 741	327/327(100%) Strand = +/-	-	scaffold149207_cov61 (183171-183700) lenght:529 strand (+/+)
RES 10	scaffold83272__1378_60779 (375 letters)	SAUC44781_TC01 Length = 543	364/364(100%) Strand = +/-	-	scaffold150551_cov61 (32877-32417) lenght:460 strand (+/-)

Table 1.6: List of best candidate genes identified among the overrepresented bulks of reads from resistant RILs and with the best match with transcriptome data of S. aethiopicum. For each gene, the 305_asm scaffold or contig, the matching S. aethiopicum scaffold (from Gramazio et al., 2016), the percentage of homology and a putative gene prediction is indicated.

Recently, twenty-five tomato candidate genes within the confidence interval of the QTL for *Fom*-resistance on CH02 by a synteny analysis have been identified (Miyatake et al. 2016). We searched for these 25 orthologues in the eggplant V3 genome finding that four were annotated on CH02, among which one gene showed 99% homology with a *S. aethiopicum*

transcript which was added to the list of candidate genes as RES1. The comprehensive list of the 10 best candidate genes is reported in table 1.6.

1.3.5 Expression analysis of the best candidate genes

Primers for qRT-PCR were designed on the consensus predicted CDS sequence (Table1) of the 10 genes (RES1-RES10) with highest match with the *S. aethiopicum* transcript. For 7 out the total 10 candidate genes, preliminary expression data through real-time qPCR analysis was successfully obtained in '305E40' cDNA samples of roots at different timepoints after inoculation with *Fom*, while for RES7, RES9, RES10 RT-qPCR always led to multiple peaks in melting or was not reliable at all. These 7 genes are always expressed at T0 both in *Fom*- and mock-inoculated roots (Figure 1.7) and therefore we can exclude they are modulated in response to the fungal inoculation.

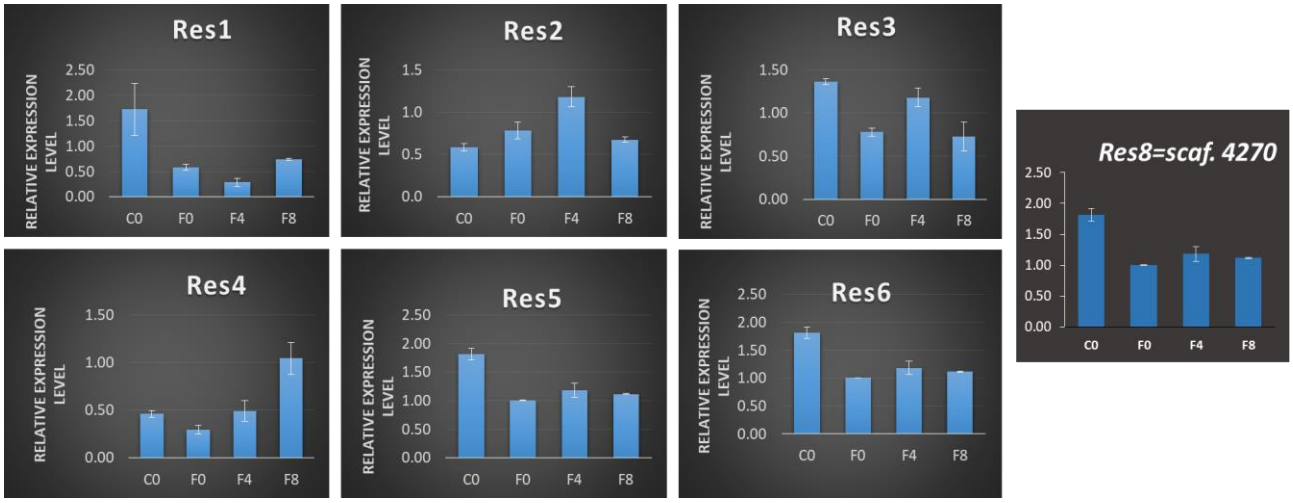


Figure 1.7: RT-qPCR analysis of S. melongena candidate transcripts with RT-qPCR. Relative quantification of 7 candidate RES genes. Values are expressed as relative to the GAPDH used as reference gene and are the averages of three biological replicates (n = 3). Mock control indicates not inoculated samples, fom T0-T8 indicates inoculated samples at 0, 4 and 8 days post infection.

1.4 DISCUSSION

Fusarium oxysporum f. sp. *melongenae* (*Fom*) is one of the most serious and widespread diseases in eggplant, because of the fungus can persist in the soil for many years. Crop rotation, fumigation and fungicide applications can reduce the risk of infection; however, they are not highly effective as well as being environmentally impactful (Fradin et al. 2009; King et al. 2010). Therefore, the best available alternative is represented by the development of *Fom*-resistant cultivars by exploiting the natural diversity of the cultivated eggplant including the sources of resistance existing within the related species of cultivated *S. melongena* (Toppino et al., 2021). Likewise, the identification of QTLs and candidate genes playing a key role in the plant response to the infection, combined with the

development of molecular markers strictly linked to the resistance trait, is a valuable approach that can substantially speed up breeding programs to obtain new varieties with improved resistance to *Fom*.

In eggplant, several 'first generation" inter-specific and intra-specific maps were developed, with the former exploiting a higher genetic polymorphism, but being of minor relevance for marker-assisted breeding (Barchi et al., 2019). An intraspecific F2 segregating population, obtained from the intraspecific cross between the breeding lines '305E40' (female parent) and '67/3' (male parent), two eggplant lines highly contrasting for many key agronomic traits, was employed for the development of a RAD-TAG based intraspecific map which already proved to be a highly efficient tool for the detection of more than 140 QTLs associated to leaf, flower, plant and fruit traits, fruit biochemical composition and resistances to fungal wilts (Barchi et al., 2012, 2018; Portis et al., 2014; Toppino et al., 2016). The first high quality eggplant genome sequence of the breeding line '67/3' was recently released (Barchi et al., 2019b), and even more recently an improved version of this genome together with a first pangenome obtained through resequencing of 23 eggplant accessions was released (Barchi et al., 2021). The intraspecific RILs population developed from the cross between '305E40'x'67/3' was recently used for developing a high-quality GBS-based map and identification of QTLs affecting key breeding traits (Toppino et al. 2020). In this paper, the same population was assessed for the response after artificial inoculation with *Fusarium oxysporum* (*Fom*) to spot and better characterize the QTLs associated with the resistance trait. Both the two parents of the RIL population carry a resistance trait to *Fom* (Barchi et al 2018): the breeding line '305E40', in fact, carries the locus *Rfo-sa1* introgressed from *S. aethiopicum,* which confers complete resistance to the soil-borne fungus *Fom* (Toppino et al. 2008), while the breeding line '67/3' carries a source of partial resistance trait almost unexpected within the cultivated eggplant germplasm. All the '305E40x67/3' F1 plantlets revealed a full resistance to *Fom,* further confirming the dominant behavior of the full resistance trait introgressed from the wild species and associated with the *Rfo-Sa1* locus with respect to the partial resistance one triggered by '67/3'. Data of replicated inoculations of each RIL with *Fom* were successfully employed to identify major QTLs associated with the resistance trait to *Fom* on chromosomes CH02 (*FomCH02*) and CH11 (*FomCH11*) associated with complete and partial resistance, respectively. The greatest QTL *FomCH02*, lies at the edge of chromosome 02, in the same position in which Barchi et al. (2018) already spotted a major

QTL associated with the full resistance trait employing the F2 population from the same cross and the RAD-Tag-based map. Despite the availability of an ultra-dense genetic map and of a sequenced reference genome, it was still difficult to determine the true physical extension of this QTL as in this region on CH02 the GBS-based molecular markers order along the chromosome according to the map is still extremely discordant with the physical position along the V3 sequence of 67/3, probably due to the presence of the introgressed wild fragment in 305E40 which hampered a correct pairing of chromosomes during meiosis and therefore led to distorted segregation ratio of markers and, as consequence, to assign a wrong marker position along the map or an uncorrected chromosome scaffolding. The other major QTL *FomCH11*, is in the same position in which Barchi et al., (2018) already spotted (despite with a broader Confidence interval) a major QTL associated with the partial resistance trait using the F2 population developed from the same cross. The development of next-generation sequencing (NGS) and reliable bioinformatics tools boosted segregant populations analyses (including BSA), expression profiling, and the construction of polymorphism databases to assist QTLs identification. Moreover, thanks to the availability of genotyping data from thousands of markers in a unique segregant population, gene mapping and discovery of the causative genes of many traits of interest can be easily addressed (Le Nguyen et al., 2019). The Bulked-Segregant Analysis coupled to Whole Genome Sequencing (BSA-Seq) technique was successful exploited in narrowing the CI of QTLs and developing molecular markers more strictly linked to several traits of interest including the late leaf spot resistance in peanut (Clevenger et al., 2017) providing a baseline for candidate gene discovery and map-based cloning of candidate genes (Kayam et al., 2017; Klein et al., 2018). An even more localized or targeted approach can be set up if QTLs and genomic regions controlling a trait of interest are already available. A strategy to combine BSA-Seq and linkage mapping approach proved to be efficient in identifying and deeply characterizing the genomic regions associated with several traits of interest as reported for *Phytophthora* root and crown rot resistance in squash (Vogel et al., 2021). This combined approach was also successfully adopted to identify the genomic interval containing a mutant gene of interest, up to the mutation itself, in case of sufficient coverage of reads available (Zou et al., 2016). Therefore, only a high-quality assembled genome can serve as the reference sequences in genotype calling, an essential step in BSA-Seq data analysis. For most species, however, such high-quality assembled sequences are available only for a single or a limited number of breeding lines in many plant species. So that, the parent included in a BSA-Seq experiment that lack for a high-quality assembled genome

might be sequenced *via* NGS for determining the allelic variants compared to a reference genome.

In this paper, to deeply investigate the genomic regions underlying two *Fom*-resistance QTLs in eggplant, a targeted BSA-Seq approach by exploiting Illumina sequencing data available for '305E40', '67/3' and each RIL progeny was applied. This strategy allowed us to spot differential genomic regions between the parents putatively involved in controlling either the partial and complete resistances on chromosomes CH11 and CH02, respectively. As the two resistance traits are carried by different parental lines, and considering that the '305E40 lines' carries unique portions associated with the resistance trait that are not present into the 67/3 reference genome, two different BSA-seq analysis were performed, in which the RILs sequences, bulked according to their disease resistance ratio, were aligned to the more suitable reference genome to identify differentially enriched regions within the confidence intervals of each QTL. The alignment to the '67/3' (itself a parent of the cross adopted) reference genome of bulked sequence reads from partially resistant (PR) and fully susceptible (SS) RILs in the region within the QTL CI allowed us to identify five differentially enriched regions containing eleven putative candidate genes for *FomCH11* QTL. Among them, best candidates may be selected according to SnpEff analysis and expression data to be deserving for a functional characterization through gene silencing or overexpression to identify the gene(s) underlying the PR trait inherited from '67/3'. Furthermore, from comparison of PR and SS reads in the regions carrying the candidate genes, molecular markers may be developed for a finer mapping of the QTL *CH11Fom* locus and for MAS.

The other parent '305E40' of the RIL employed is a stable double-haploid line derived from the androgenetic progeny of a somatic hybrid consisting of the fusion between protoplasts of eggplant and *S. aethiopicum*. It bears a segment derived from the allied parent, which includes the locus *Rfo-sa1*, located in the distal portion of chromosome CH02. The haplotype in this chromosomal region is identical to that from *S. aethiopicum* (Portis et al., 2014). This genomic region did not or very hardly show genetic recombination, making mapping and cloning extremely difficult, probably due to genes megaclusters with many other inactive copies of the same.

Thus, the short Illumina read sequences already available for '305E40' (Barchi et al., 2019) were *de novo* assembled and coupled with a targeted BSA-Seq technique. The RILs

sequences, pooled according to their disease resistance score, were aligned to the *de novo* assembly *305_asm* to identify exclusive chromosomal segments from '305E40' differentially enriched by comparing reads from resistant (RR) and susceptible SS bulks of the RILs. This analysis enabled the identification of 1838 contigs/scaffolds containing differentially-enriched regions which were crucial in search of unique candidate genes associated with the resistance trait introgressed from the wild species. As a whole, combining the results obtained from genes annotation within the differentially enriched regions in the *asm_305 de novo* assembled genome together with the comparison to *S. aethiopicum* transcriptome (Gramazio et al., 2016) and draft genome (Song et al., 2019) and genes expression analysis, two best candidate genes for further functional analysis were identified:

- RES1, SMEL_002g157480.1, encoding a putative TMV resistance protein N-like LOC102604931, transcript variant X2 (TIR_2), with ID=99% to a *S. aethiopicum* sequence
- *RES8*, Scaffold 4270__1160_51181 (*305_asm* assembly), matching with SAUC44781_TC01 from *S. aethiopicum* transcriptome, encoding for a putative late blight resistance protein homolog R1B-14-like [*S. lycopersicum*] with ID=100% to a *S. aethiopicum* sequence

However, a fine identification of the genomic region involved in the *Fom*-resistance from *S. aethiopicum* might need an effective long read sequencing strategy of the '305E40' line, coupled with an additional enrichment stage of the genomic regions with repeated sequences and resistance genes in tandem as well as a targeted novel RNA-seq experiment. Moreover, the genomic characterization of RILs revealed that five of the *Fom*-resistant (RR) harbour reduced portions of the QTL on CH2 which might be re-sequenced with the aim to narrow down the genomic region easing the cloning of resistance gene(s). Likewise, the RILs miming a fully resistant phenotype despite they did not harbour *FomCH02* QTL but the '67/3' haplotype of the *FomCH11* QTL could represent the lines carrying the most effective partial resistance trait to be subjected to re-sequencing for identification and isolation of the underlying genes. Finally, the best performing RILs for full and/or partial resistance traits will be employed for breeding purposes to introgress and pyramiding the two *Fusarium* resistance QTLs into élite eggplant germplasm.

Chapter 2

Functional characterization of the candidate gene RES8, *de novo* ONT sequencing of line 305E40 and new BSA-seq analysis to identify new candidate genes associated with the *Fom* resistance trait

2.1 Silencing of the candidate gene *RES8*

2.1.1 Introduction

As described in the first chapter, one of main objects of this thesis was the identification of candidate genes through sequence analysis of the differentially enriched regions associated with the resistance trait (BSASeq), their expression analysis and functional analysis (silencing through RNAi or CRISPR/CAS9 strategies) to confirm their true involvement in the resistance trait.

Basing on annotation, preliminary comparison with the *S. aethiopicum* draft genome and transcripts, amplification in Resistant-Susceptible genotypes and expression data, the *RES8* gene was selected as the more reliable candidate gene responsible of the *Fom* resistance trait carried by *S. aethiopicum*. Therefore, the activity was focused on the isolation of the *RES8* full genomic and CDS sequence and preparation of constructs for silencing its expression in '305E40' line to confirm its role in the resistance trait.

Further activity reported in this chapter is the long-read sequencing of the line 305E40 through Oxford Nanopore Technology (ONT) (Michel et al., 2018; Dumshott et al., 2020) coupled with Illumina cleaning and a new BSAseq through bulked reads alignment as performed in chapter one using this more reliable reference to identify additional candidate genes, to be further validated with the aid of RNA-seq analysis.

2.1.2 Materials e methods

2.1.2.1 Isolation of the CDS sequence of gene *RES8*

The complete genomic sequence of gene *RES8* was depicted by comparison between the sequences in the scaffold s4270__850_38331 from *asm-305* and the sauc68094 scaffold from the transcriptome of *S. aethiopicum.* CDS prediction was performed with Fgenesh (Salamov A. and Solovyev V., 2000). The list of primers utilized for sequencing of the

genomic region of *RES8*, for full cloning of the CDNA sequence, and for cloning the fragment in the construct for RNAi are reported in Table 2.1

Primer name	Primer seq	use
RES8genomicseqfw1	5'-GTCTGGAAAGGGGTAGGTCC-3'	Full seq
RES8genomicseqrev2	5'-TGAACCTCACTCGACTTCCC-3'	Full seq
RES8genomicseqfw3	5'-TCACCTGAAGTACCTCGCTG-3'	Full seq
RES8genomicseqrev4	5'-TATGGTCGGTGGTGGAAGTTGCT-3'	Full seq
RES8fullFor	5'-caccATGAACCTAATTCCAAGA-3'	CDNA amp-cloning
RES8fullRev	5'-CGGTCACATCCTTCAATGGC-3'	CDNA amp-cloning
RES8INTER-fw	5'-caccTCCCTGGGTATATGGGATTCC-3'	Interference-cloning
RES8INTER-rev	5'-acccCTTTCCAGACACATCA-3'	interference-cloning
NPTII-FW	5'-TGCTCCTGCCGAGAAAGTAT-3'	PCR
NPTII-Rev	5'-AGAACTCGTCAAGAAGGCGATAG-3'	PCR
SmGAPDH -Fw	5'-GGTGCCAAGAAGGTTGTGAT-3'	qRT-PCR
SmGAPDH -Rev	5'-CCAATGCTAGTTGCACAACG-3'	qRT-PCR
RES8RTFor:	5'_ACCAGGTTAAGTTACAGCTCTGA_3'	qRT-PCR
RES8RtRev:	5'_ACCCCTTTCCAGACACATCA_3'	qRT-PCR

Table 2.1 List of primers utilized, their sequences and employment for isolation and characterization of RES8-gene.

2.1.2.2 DNA extraction and sequence analysis

DNA samples were extracted from young leaves, using the GenEluteTM Plant Genomic DNA Miniprep kit (Sigma, St. Louis, MO), following the manufacturer's protocol. Amplification was performed with Master Mix G2 (Promega). Amplification conditions were as follows:

95x2’

95x30’’
60x1’ } x 38 cycles
72x4’

72x5’

4x ∞

PCR products were checked on agarose 1% before being purified and sent out for sequencing. The complete genomic sequence of gene *RES8* was then reconstructed.

2.1.2.3 RNA extraction, cDNA synthesis and primer design

Total RNA was extracted from root samples of ‘305E40’ as well as from all the transformed T1 plantlets using the Spectrum™ Plant Total RNA Kit (www.sigma.com); each sample was resuspended in a final volume of 30 µl. The cDNA was synthesised from 1 µg of RNA using the High-Capacity cDNA Reverse Transcription Kits (www.appliedbiosystems.com) after treating the samples with Dnase (RQ1 RNase-Free DNase, www.promega.com).

The single strand cDNA was synthesised. The standard procedure was changed by adding both 0.5 µg/reaction of Oligo(dT) and 0.5 µg/reaction of Random Primers to 1 µg of DNase treaty RNA and heating at 70 °C for 5 min. The RNA mix was cooled to 25 °C and 15 µl of RT Master mix (according to the manufacturer’s instructions) was added, followed by heating to 42°C up to 1hrs and 75 °C for 10 min to inactivate the Reverse transcriptase. Primers for cDNA full length and RT- expression analysis of *RES8* in ‘305E40’ and the T1 transformed plants, detailed in Table 2.1, were designed basing on the available sequence of *305_asm* utilising the Primer 3 software (https://bioinfo.ut.ee/primer3-0.4.0/primer3/).

2.1.2.4 Cloning of the *RES8* gene and creation of the RNAi construct

Both Full CDS sequence of *RES8* and the interference fragment (highlighted in bold within the full *RES8* CDS sequence) were amplified from total cDNA of 305E40 roots using the “Phusion High-Fidelity DNA Polymerase” (Thermo Fisher Scientific, Waltham, MA,

United States) according to the manufacturer's protocol using the primers detailed in Table 3.1. The PCR products were cloned into pENTR/D-TOPO vector (Thermo Fisher Scientific) according to the manufacturer's protocol and were sequenced for confirmation. The entry clones containing the interference fragment were recombined with destination vectors pK7GWIWG2 containing the constitutive 35S promoter via Invitrogen TM Gateway TM recombination cloning technology (Thermo Fisher Scientific). The binary vector containing a NPTII/SPEC/STREP selection cassette flanked by T-DNA border sequences containing the *RES8* hairpin, was used to transform *Agrobacterium tumefaciens* strain GV2260

2.1.2.5 Eggplant Transformation

The procedure for eggplant transformation of cotyledon from in vitro grown plantlets of '305E40' was essentially as described in Rotino et al., 1990 and 1997. Cotyledons explants were pre-cultured for two days in a pre-culture medium (Arpaia et al., 1997). For explants infections, an overnight *Agrobacterium tumefaciens* liquid culture was centrifuged and the pellet resuspended at 0.1 OD600 in MS basal medium, 2% glucose, 200mM acetosyringone pH 5.5. All the explants were infected by dipping in bacteria suspension for 5 min, blotted dry onto sterile filter paper and placed back in the same plates. After 48h the explants were transferred to a selective medium without acetosyringone and supplemented with 30mg/L kanamycin and 500mg/L cefotaxime. Calli with compact green nodules were then transferred to the regeneration medium. Regenerated shoots were rooted and propagated in V3 medium (Chambonnet, 1985) without antibiotics. Plants material for transformation, calli and transformed sprouts were grown in in vitro conditions in a growth room chamber with 16hr of daylight at ~50 μE m−2 s−1 intensity and day/night temperature regimes on ~ 25/20 °C. Kanamycin-resistant transgenic plantlets were confirmed by PCR for the presence of the insert, using the primers NPTIIfw and NPTIIrev (Table 2.1). The PCR-positive transformed plants regenerated from the "in vitro" culture (T0), after ex vitro adaptation, were potted and grown under glasshouse condition and self-pollinated; the seeds obtained (T1) were sown at the beginning of summer season 2019 in plateau of 104 holes and seedlings were sprayed with kanamycin (Sunseri et al 1993) (Figure 2.1). The resistant T1 plants were checked for the presence of an active copy of NRTII gene and by PCR for the presence of the construct, respectively.

Figure 2.1 Chlorotic spots on leaves of T1 seedlings sprayed with kanamycin

2.1.2.6 qRT-PCR setup

Quantitative polymerase chain reaction (qPCR) analyses were performed to measure the expression of the *RES8* gene, putatively involved in resistance to *Fom*, in the silenced plants compared to the wild type. qPCR analyses were performed using three biological replicates for each T1 transformation event considered (3 indipendent events) and for the two lines '305E40' and 'DR2' employed as positive and negative control, respectively. All samples were run in three technical replicates, and no-template controls were included in all analysis. The qRT-PCR reactions were carried out according to the following PCR parameters: 95°C for 5 min, followed by incubation for 15s at 95°C and denaturation for 15s at 95°C, annealing for 60s at 59°C for 40 cycles, followed by elongation at 72°C for 20s. The reaction was performed using GoTaq® qRT-PCR Master Mix by PROMEGA. The reaction containing 1.0 µl of previously diluted cDNA (1:20), from 0.2 µl to 1.0 µl of primers (1 µM each), 5 µl of GoTaq® qRT-PCR Master Mix and RNase-Free water up to the final volume of 10 µl. Standard curves for both *RES*8 and two housekeeping primer pair were calculated across a 5-fold dilution series of pooled diluted cDNA amplified in technical triplicate. The PCR efficiency was calculated by Rotor-Gene 6000 Series Software and it was optimised to be in the range 90-100%. The expression levels of the target genes were determined in all samples in triplicates, and relative expression ratio was calculated using the "Delta-delta method" using SmelGADPH (Glyceraldehyde 3-phosphate dehydrogenase) and Smel18S as housekeeping genes (Barbierato et al 2017) with the following formula described by Pfaffl MW 2001:

ratio = 2 –ΔCP sample - ΔCP control

Specificity of amplifications was assessed first by PCR for the presence of a single band and then through the melt curves analysis.

2.1.3 RESULTS

2.1.3.1 Isolation of the *RES8* full genomic and CDS sequence

Basing on the comparison between the sequences in the scaffold s4270__850_38331 from *asm-305* and the sauc68094 scaffold from the transcriptome of *S. aethiopicum* and combining results with CDS prediction performed with Fgenesh we retrieved the complete CDS sequence of gene *RES8* and the tentative aminoacidic conversion below reported:

> *RES8* CDS sequence

ATGATATTGAAACAC**ATG**AACCTAATTCCAAGATTTGGTGAACTTGCAGGAA
TGTTTCCACAGAAAATGGCTCAAAATGAAATTGAGAAAATGTTACATCACCT
AAGAAGGATAAAGAGTGGAGGTAATCCGAGTGACCTCAAGTATTTTCGAATT
AAGGAACTGGAAATGGTGCTAAGAGTTTTTAGAACCTTTCTGAAGTATCATCA
TGTTCTTTTGCCTGATTCCTTTGTCAAACTCAGAAAGAATGTCAAATATATTGT
GCAAAGACT**TCCCTGGGTATATGGGATTCCAGATGAATGTAAAACTAACC**
TTAATCTGGAAAGCCTAGAATCACATTTATTAGAATTCTTTGAAGGTAAT
ACCAGGTTAAGTTACAGCTCTGAGTTGAATGATTTTGATTTGTCAAAATA
TATGGATTGCCTCGGAAAACTTCTAAATGATGTACTGATGATGTCTGG
AAAGGGGTAGGTCCTGCCATCCCAAAGAAAACTTTGCAATACATCAGTTGAT
AAAGAAACTGAAAACTATTCAAAAGAAAATGAGATTTTTGAGATACTTATAT
GCCACAGAGATAAAGGGTAATGTCAACCATGAAAAGTTGGAATGTTTGGAGA
CTCGAATTCAGTTCATGGCTAACAATGTGGAACACTTTTGTCTTTCTGTATTTG
ATTATCTTGCTGCAATTAATGTAGTTGAGGATGAAAATGATATGTTTAATAAA
CCTCCTTTTCTATTATCATTGATTGTGTTTGTGGAGCTGGAAATGAAGAAGAT
TTTTCTTGGTGAACTAAAGGCTTCAATGTTTACTCAATCAAAAATTTTCAAGA
AATTACCAGAAGGATTTTCACATCATCTCCACAGTCTGCTGATGTATCTCAGA
AACAAAAAGCTCGAGAACTTTCCTAATAATATCTCTGCTCAAAATATTGATGT
CGCAATAGACTTCTTGTTGGTTTTCCTTGATGCTGATGTGTCAAATCATGTTGT
TAATGGTAACTGGTTAACTGAGGCTATGGAAAAGGTTGGAGCTATAGCAGGT
GATATTCTATATGTAATTCAAAAGCTTCTTCCTAGCTCTATAAACAAAGGTGA
CACTAGCAAAATAAGTCTTTGCTCGATACAGATATTGGAGAAAACAAAAGAT
CTGAAGGCACAAGCGGAGACGTACTACAAATCCTTAAAATTCACTCCATCTC
AATTCCCCACATTTGGTGTATTGAGCTTTCTGGATTCTCTTTTTCGGAAACTGA
ATGAGATGTCGAAATCTAAATCTGGTTTAGATTTCTTGATGAAACCTCTTTTA
GGGAACTTGGAGAAAGAACTATCAGCTCTTACATCCATTTTAGCGAAGGAGC
TGTCATCCATTTTCAGAGATGTCTCAAAGGTGCACTATGAACATAAAATTCTT
AAAGATCTTCAGAGACGTGCCCTCAATTTGGCATATGAAGCTGAGGTGGCCA
TTGACTCTATTCTTGCTCAGTATAATGCTTTTTTGCATATCTTTTGCTCACTTCC
TACAATCTTAAAAGAGATCAAGCAAATTAATGCAGAGGTGACAGAGATATGG
TCGGTGGAAGTTGCTCTTAAGCCTCACTATGTGGTAGAGCCATCTAAACATCT

GCCAACTCGACATAGCAATCCAGTGCCTGATGAGGAGATAATTGGTTTTGGG
AACGACACAGAAAAGCTGATTCAGTATCTAATTAGAGGTACAAATGAGCTAG
ATATCGTCCCAATTGTAGGCATGGGGGGACAAGGGAAAACGACAATTGCTAG
AAAGGTGTACAACAGTGATAACATTGTTTCTCGTTTTGATGTTCGAGCATGGT
GCATTGTTTCCCAAACATATAACCGGAGGGATCTATTACAAGATATTTTTAGT
GACGTTACCGGTTCCAAGGACAAGGAAGATAGGGATGACGACGTTCTTGCTG
ACATGTTGAGGAAAAGACTAATGGGAAGGAGATATCTCATTGTTTTGGATGA
TATGTGGGATGGTATGGCATGGGATGACTTAAGGCTTTGTTTTCCAGATGTTG
GAAATAGAAGCAGAATAATCATAACGACTCGACTTGAGAAAGTGGGTGAGC
AAGTCAAGTACCAAACTGATCCTTATTCTCTTCCATTCCTCACAACAGAAGAG
TGTTGCCAATTGTTGCAGAAGAAAGTGTTTCAAAAGGAATATTTCCCGCCTGA
ACTACAAGATGTGAGTCTAGCAGTTGCAGAAAAATGCAAAGGACTGCCCCTA
GTGGTTGTCTTGGTAGCTGGAATAATCAATAAAAGGAAAATGGAAGAATCTT
GGTGGAATGAGGTGAAAGATGCTTTATTTGACTATCTTGATCGTGAGTCAGAA
GAATATAGTCAGATGACTATGAAGTTGAGTTTTGATAACTTACCCCATCTTTT
AAAGCCTTGTCTTCTTTATATGGGGATGTTTCCAGAGGATGCAAGAATTCCAG
CGCCTAAATTGATAAGTTTATGGATTGCAGAAGGATTTGTGGAGAACACTGA
ATCTGGGAGATTACTGGAAGAGGAAGCTGAAGGTTACTTGATGGATCTCATT
AGCAGTAATGTGGTAATGCTTTCAAAGAGAAGTTATAATGGTAAAGTCAAAT
ACTGTCAGGTTCATGATGTTGTGCATCACTTTTGCTTGGAGAAGAGTAGAGAA
GAAAAGTTTATGCTGGCAGTGGGGTCAATATATCCAGTTTCAACCTTTGGATT
GGAAGGGAAGTCGAGTGAGATAAGTGAATTGCGACTTCTTAAGGTCTTGGAT
TTGAGTTCTCACACTATGGAATTTCTGTCGTTAGCTACATTCAAACAACTATA
TCACCTGAAGTACCTCGCTGTTTGGGCAGATAAATTCTATTTTCATCCAGAAT
CACATCTGCCCCATCTAGAAACTCTAATTGTGAAGAATTTTCCTTATATAGTG
GTGTTACCAACATCTTTTTGGAAAATGGAAAAATTAAGGCATGTTCATTTTGG
TAAGGCTGTTTTTGATTTGGAAGAGGATAAGCAGGGGATGTTTGAAGAATCC
TCTAAATTGGAAAACTTGAGGATATTAAGGAATGTTATATTTCGAATTCGCAA
TGCTGATAAGGTGGATGTGTTATTAAGGAGGTGTCCTAATCTTCAACAACTTG
AAATTACTTTTGAGGACAATAAAGATTCTGTAGAGTCTTTTTATCTCACACTG
GAGAATCTTACCCAGCTTCAAAAACTTCAACTTTCCTTTAAGTGTCCCCACAT
TCTATCCGGGTTAGAGTTGCCTTCAAATTTAAAGAAGTTGGTACTACAAGGGA
TTCATATAGAAAGCGCAATTCCCTTCATCGCAAGACAACCAAATCTGGAGCA
TCTACATTTACGGTACTCCAATTATTTTCAATCCCAAGAGTGGTGTATTGGAG
ATATCACGTTCCATAAACTTAAGTTGTTGAAACTGGTGCAGTTACGTATCCCA
AGGTGGGATGCCTCAGACGAATCCTTTCCCCTGCTTGAAACACTTGTTATAAA
AAAGTGCGATGAGCTTGAGGAAATCCCCCTTAGCTTTGCAGATATTCCAACTC
TGAAACAGATTAAGTTGATTGGATCCTGGAAAGAATCTCTGGAGGTTTCAGCT
ACAAGAATTGAGGAAGAAATCGAAGCCATTGAAGGATGTGACCGTGTTAAGC
TCATCAAGAATTATCAGATTGGGGATAGTTTTGTTAGCTAA

>_RES8_prot
MILKHMNLIPRFGELAGMFPQKMAQNEIEKMLHHLRRIKSGGNPSDLKYFRIKEL
EMVLRVFRTFLKYHHVLLPDSFVKLRKNVKYIVQRLPWVYGIPDECKTNLNLES
LESHLLEFFEGNTRLSYSSELNDFDLSKYMDCLGKLLNDVLMMCLERGRSCHPK
ENFAIHQLIKKLKTIQKKMRFLRYLYATEIKGNVNHEKLECLETRIQFMANNVEH
FCLSVFDYLAAINVVEDENDMFNKPPFLLSLIVFVELEMKKIFLGELKASMFTQSK
IFKKLPEGFSHHLHSLLMYLRNKKLENFPNNISAQNIDVAIDFLLVFLDADVSNHV
VNGNWLTEAMEKVGAIAGDILYVIQKLLPSSINKGDTSKISLCSIQILEKTKDLKA
QAETYYKSLKFTPSQFPTFGVLSFLDSLFRKLNEMSKSKSGLDFLMKPLLGNLEK
ELSALTSILAKELSSIFRDVSKVHYEHKILKDLQRRALNLAYEAEVAIDSILAQYN
AFLHIFCSLPTILKEIKQINAEVTEIWSVEVALKPHYVVEPSKHLPTRHSNPVPDEEI
IGFGNDTEKLIQYLIRGTNELDIVPIVGMGGQGKTTIARKVYNSDNIVSRFDVRAW
CIVSQTYNRRDLLQDIFSDVTGSKDKEDRDDDVLADMLRKRLMGRRYLIVLDD
MWDGMAWDDLRLCFPDVGNRSRIIITTRLEKVGEQVKYQTDPYSLPFLTTEECC
QLLQKKVFQKEYFPPELQDVSLAVAEKCKGLPLVVVLVAGIINKRKMEESWWN
EVKDALFDYLDRESEEYSQMTMKLSFDNLPHLLKPCLLYMGMFPEDARIPAPKLI
SLWIAEGFVENTESGRLLEEEAEGYLMDLISSNVVMLSKRSYNGKVKYCQFQPL
DWKGSRVRFSFNEELSKFASLGSKTQKPFHQHLRSLITNRGRSIHLIPFCQISELRL
LKVLDLSSHTMEFLSLATFKQLYHLKYLAVWADKFYFHPESHLPHLETLIVKNFP
YIVVLPTSFWKMEKLRHVHFGKAVFDLEEDKQGMFEESSKLENLRILRNVIFRIR
NADKVDVLLRRCPNLQQLEITFEDNKDSVESFYLTLENLTQLQKLQLSFKCPHILS
GLELPSNLKKLVLQGIHIESAIPFIARQPNLEHLHLRYSNYFQSQEWCIGDITFHKL
KLLKLVQLRIPRWDASDESFPLLETLVIKKCDELEEIPLSFADIPTLKQIKLIGSWKE
SLEVSATRIEEEIEAIEGCDRVKLIKNYQIGDSFVS

The deduced amino acid sequence of *RES8* was employed as a query against the flowering plant database using the pBLAST function tool in NCBI and gave the best match with the *Solanum demissum* R8 gene cluster, partial sequence GenBank: KU530153.1. Basing on comparison with this sequence, a highly conserved and unique region of 199 bp (highlighted in bold within the sequences reported) was selected within the CDS sequences to be utilized in the development of a *RES8*_RNAi construct. As already above described, primers for complete CDS cloning and qRT-PCR were designed on the consensus predicted CDS sequence after comparison of the overrepresented scaffold from *asm_305* with the *S. aethiopicum* transcript. cDNA end point amplification of *RES8* led to the amplification of a unique fragment of expected length in root samples of '305E40' and *S. aethiopicum*, while no amplicon was yielded in cDNA samples from '67/3' (Figure 1.6).

2.1.3.2 Eggplant transformation with the RNAi_*RES8* construct

The fragment of 199 bp was successfully cloned into the entry TOPO vector, then recombined into the interference binary vector. Eggplant line '305E40' transformation with

the construct led to the regeneration of several plantlets from independent transformation events which were characterized for the presence of an active NPTII gene through spraying with kanamycin and PCR amplification of the NPTII fragment. 16 T0 plants from 9 independent events were assessed as Kan^R, maintained in growth chamber and selfed to obtain T1 progenies.

2.1.3.3. Evaluation of T1 progenies for expression of the *RES8* gene and resistance to *Fom*

The first three T1 progenies were obtained from 3 different T0 Kan^R transformed lines and subjected to phenotypic and molecular characterisation. For each T1 progeny, 54 seedlings were initially sprayed with kanamycin. All the kanamycin-resistant seedlings were artificially inoculated with *Fom* and characterised for response to *Fusarium* at 30 DAI but gave little or no symptom following inoculation.

Quantitative polymerase chain reaction (qPCR) analyses were performed to measure the reduction in expression of the *RES8* gene in the silenced plants compared to the wild type. For the 3 T1 progenies, DNA was extracted from the root samples of each seedling to verify the actual presence of the silencing construct, then partial root samples were taken from 3 Kan^R and 3 Kan^S seedlings per progeny at 0 and 4 hours after being singularly inoculated with *Fom*. The plants were then grown in the greenhouse and singularly evaluated at 30 DAI for symptom onset.

Gene expression analysis (Figure 2.2) confirmed that gene silencing has occurred, as the expression of *RES8* in all the KAN^R T1 plants is statistically lower than the untransformed control lines, but the reduced expression was not accompanied by a phenotype congruent with the hypothesis as none of the KAN^R plants showed any symptom in response to *Fom* (Figure 2.3).

In spring 2020, 11 additional T1 progenies developed during the previous year were inoculated with *Fom*, but all seedlings showed no symptoms in response to the inoculation, and it was considered not appropriate to proceed with gene expression analysis.

Thus, although the expression analysis shows that the *RES8* gene is silenced, the plants do not show any expected symptom; therefore, it can be concluded that the putative candidate gene *RES8* is not responsible for conferring the resistance trait, or that it is not the unique gene in charge of it.

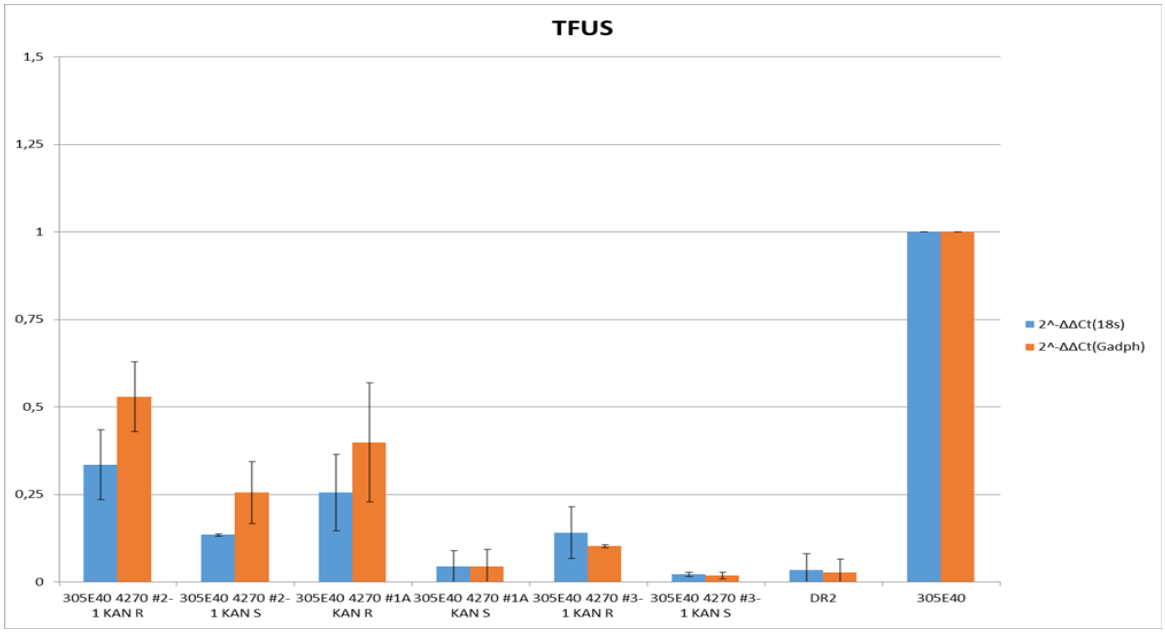


Figure 2.2 RT-qPCR expression analysis of candidate genes RES8 involved in the early phases of response to Fusarium oxysporum f. sp. melongenae (Fom) infection

Figure 2.3 T1 plants Kan+ shows no symptoms in response to Fom

2.2 DE NOVO SEQUENCING OF EGGPLANT LINE '305E40'

2.2.1 Introduction

An alternative strategy of characterising the portions of the genome putatively associated with the resistance trait by long read resequencing of the '305E40' line through ONT sequencing technique was therefore pursued. Analysis of the sequencing data and a differential BSA-seq mapping of reads from susceptible and resistant RILs was again performed to identify more reliable enriched regions associated with the resistance trait introgressed by *S. aethiopicum*.

The ONT strategy coupled with Illumina cleaning followed by a new BSA-seq analysis was carried out and a new Bulked reads alignment was performed utilizing this even more reliable reference in order to select more reliable candidate genes for functional characterization. The ONT sequencing of line '305E40' was commissioned as an external service to the group of Prof. Delledonne at the University of Verona, department of Bioscience. The aim of the activity was the generation of *de novo* genome assembly of the Solanum melongena line '305E40' to identify the genes located in the introgressed region containing the *Rfo-Sa1* locus (distal position of CH02 from 0 till position 10.4 cM) associated to *Fusarium oxysporum* f.sp. *melongenae* resistance.

2.2.2 Materials e methods

2.2.2.1 DNA isolation

Nuclear High molecular weight DNA samples were extracted starting from 1g of young leaves, using Gravity columns (Gtip – www.sigmaaldrich.com) following the manufacturer's protocol. A DNA quality control was performed through the Bioanalyzer High sensitivity DNA kit (Agilent). Extraction yielded 25.6 ug of DNA size ranging 30-300kb, with 260/280 ratio of 1.89 260/230 ratio of 2.38 and a concentration of 128ng/ul. A size selection for fragments >25Kb was also performed before library preparation.

2.2.2.2 Library preparation, genome assembly and comparison with the '67/3' V3 reference

ONT library were prepared and sequenced through PromethION (1 flow cell). Paired-end libraries for Illumina sequencing were prepared with the corresponding kits (Illumina) following the manufacturer's instructions and sequenced on Illumina platforms (HiSeq 2000) using standard protocols. From the total ONT reads, those shorter than 200bp were removed. Reads correction was performed using Canu (Koren et al., 2017). ONT reads were assembled with Wtdbg2 (Ruan &Li, 2020), and the assembly polishing was performed with both Racon (https://github.com/isovic/racon) and Medaka (https://github.com/nanoporetech/medaka) pipelines. The ONT contigs were therefore submitted to 3 rounds of error polishing with 35X Illumina 100PE reads through Pilon (https://github.com/broadinstitute/pilon/wiki).

ONT-Illumina polished contigs were super-scaffolded according to a Reference-guided scaffolding (RagTag) using the V3 *S. melongena* line '67-3' genome as reference. Mapping of '305E40' unplaced contigs to the '67/3' chromosomes was performed with Minimap2

with asm20 option (asm-to-ref mapping, for ~5% sequence divergence). For comparative purposes, the *de novo* 305E40 assembly (hereafter named "305_RagTag") was aligned against the V3 reference genome of 67/3 using MUMmer4, keeping alignments longer than 1kbp with identity > 70% and plotted with dotPlotly (hhttps://github.com/tpoorten/dotPlotly)

2.2.2.3 Annotation

A species-specific-trained Augustus with the use of external evidence (RNA-seq and proteins from correlated species) was employed to annotate the 305_RagTag genome as part of the service at the university of Verona. Functional annotation on predicted genes was performed using a custom script which uses InterProscan annotation together with the homology information from proteins of correlated species.

An additional gene model identification was performed with a combination of Augustus (*parameters: pre-trained tomato, gene complete; Both strands; allow in-frame stop codons)* and CPAT (coding potential assessment) trained with coding and non-coding *S. melongena* genes (parameters: ORF size; ORF coverage; Fickett TESTCODE; Hexamer usage bias) which led to the prediction of 81,975 predicted genes (mainly transposon-related). Functional annotation on predicted genes was performed using a combination of UniProtKB/Swiss-Prot and eggNOG 5.0, a database of biological information hosted by the EMBL based on the original idea of **COGs** (clusters of orthologous groups) and expands that idea to non-supervised orthologous groups constructed from numerous organisms.

2.2.2.4 Composition of the bulks of 5x Illumina sequences

Two bulks (RR, resistant; SS, susceptible) of available 5x Illumina sequences for each RIL (Barchi et al., 2019 submitted to the NCBI Sequence Read Archive under the accession number SRP078398) were clustered according to their resistance ratio to disease:

- BULK RR: made by 28 fully resistant RILs (and namely RIL3, 9, 10, 16, 18, 19, 21, 28, 34, 37, 38, 53, 55, 61, 74, 85, 100, 102, 104, 110, 111, 114, 121, 126, 127, 129, 151, 158) all with a resistance ratio of 100 and possessing the resistance locus *Rfo-Sa1*.

· BULK SS: composed by 18 fully susceptible RILs (RIL2, 5, 7, 23, 50, 52, 58, 65, 71, 84, 90, 103, 113, 119, 125, 131, 149, 168), in which the molecular marker for the locus *Rfo-Sa1* was absent and with a resistance ratio of 0;

2.2.2.5 Alignment of Bulks to the 305_RagTag genome assembly

The two bulks of reads RR (Fully Resistant) and SS (fully susceptible) were aligned with HISAT2 (with a tolerance of max 1 mismatches per reads) to the *de novo* assembled 305_RagTag genome. In order to prevent artefacts-related issues and false-positive covered regions, it was arbitrary chosen to maintain an alignment tolerance of at least 100 reads/kb in at least one of RR or SS mapping bulks.

Forward reads from '67/3' and 35x '305E40' sequencing were mapped on the 305RagTag reference as negative and positive control, respectively. Putative regions of interest were screened within the entire sequence of CH02, as already described in Chapter1 for the regions on chromosome CH11, based on a combination of cues relating, in this case, being the line totally resistant to *Fom*, to RR vs SS abundance ratio and heterogeneity.

2.2.3 RESULTS

2.2.3.1 ONT sequencing, de novo assembly and annotation of the '305E40' genome

A total of 2,272,787 long reads with average length of 18,507bp were generated in the PromethION instrument, with a N50 of 20496bp, a N90 of 14,247bp and the longest read of 134,260bp, for a total of 42 Gb and a 40x coverage. Reads shorter than 200bp were removed. From Illumina PE sequencing 193,107,005 reads were generated, for a total of 39.6Gbp and an expected fold genome coverage of 35X. The total assembly size, number of contigs, length and N50-N90 after each round of cleaning (pipeline as described in mat e met) is detailed in table 2.2.

	Raw assembly	**Long read polished assembly**	**Long + Short read polished assembly**
Software	Wtdbg2	Racon + Medaka	Pilon (x3)
Total assembly length (bp)	980,827,571	988,051,012	980,682,365
Number of contigs	4,360	3,883	3,855
Contigs average length	224,960	254,455	254,392
Contigs N50 (bp)	591,501	597,803	598,403

Contigs N90 (bp)	123,664	127,374	126,192
Longest contigs (bp)	4,495,668	4,532,887	4,509,281
BUSCO stats (solanales_odb10) *	Ongoing	Ongoing	C:91.2%[S:88.8%,D:2.4%],F:1.1%,M:7.7%

*Table 2.2 genome assembly size after each round of polishing. * C=complete; S=complete and single copy; D=complete and duplicated; F=fragmented; M=missing*

The *de novo* genome assembly of 305E40 (hereafter *ragtag_305*) is composed by 1.007.875.867bp, of which 882.136.482bp assembled in 12 scaffolds (chromosomes) plus 963 un-anchored contigs, for a total of 125.739.385bp (Table 2.3). Total length of the assembly is in line with the V3 67/3 genome even if a higher portion of unanchored scaffolds is reported, but on the other size the number of Gaps and Gaps length is significantly reduced. The size of individual chromosomes, although reduced with respect to the 67/3 V3 is comparable with what reported by Barchi et al., 2019.

	67/3 chromosomes	**Long + Short read polished assembly scaffolded to 67/3**
Software	-	RagTag
Total assembly length (bp)	1,142,799,992	1,007,875,867
Total scaffolds length (bp)	1,142,799,992	882,136,482
Number of scaffolds	12	12
Scaffolds N50 (bp) **	106,644,908	77,071,593
Scaffolds average length (bp)	95,233,332.67	73,511,373.5
Longest scaffold (bp)	142,382,611	109,270,332
Shortest scaffold (bp)	36,100,499	29,405,140
Number of Gaps	103,067	2,880
Gaps size (bp)	317,286,259	27,193,502
Contigs in scaffolds	103,077	2,892
Remaining contigs		963
Remaining contig total length (bp)	332.106.472	125,739,385
Remaining contigs N50 (bp) ***	-	400,278

Remaining contigs average length (bp)	-	130,570.49
Longest remaining contig (bp)	-	4,030,182
Shortest remaining contig (bp)	-	146
BUSCO stats (solanales_odb10) *	Ongoing	C:91.2%[S:88.8%,D:2.4%],F:1.1%, M:7.7%
Chromosome 01	136.534.347	109.270.332
Chromosome 02	83.340.400	62.600.880
Chromosome 03	97.014.425	73.173.976
Chromosome 04	105.621.276	77.071.593
Chromosome 05	43.853.053	35.007.526
Chromosome 06	108.971.373	81.838.092
Chromosome 07	142.382.611	107.051.798
Chromosome 08	109.575.448	90.296.474
Chromosome 09	36.100.499	29.405.140
Chromosome 10	106.644.908	86.359.469
Chromosome 11	72.290.491	53.675.510
Chromosome 12	100.421.161	76.385.692
Chromosome 0 unanchored	332.106.472	124.883.876

Table 2.3 metrics and chromosome length of the 305RagTag genome assembly compared with the '67/3' V3 by Barchi et al., 2019

The multiple mapping of 127.74Mb of unplaced '305E40' sequences and contigs on the '67/3' V3 anchored chromosomes as also to the unanchored '67/3' contigs and scaffolds (CH0) allowed to resolve the position of great part of the sequences (Table 2.4), leaving only 10.9Kbp of unplaced sequences orphans of a counterpart.

305E40 unplaced contigs (963 contigs, 125.74 Mbp)	67/3 chromsomes				67/3 unplaced contigs and scaffolds			
	Num. alignments	Average alignment length	Mapped bases	Mean sequence identity	Num. alignments	Average alignment length	Mapped bases	Mean sequence identity
Primary alignments	897	8.2 kbp	7.39 Mbp	92.1%	894	26.8 kbp	23.98 Mbp	96.7%
Primary + Supplementary alignments	29,756	2.9 kbp	86.59 Mbp	87.3%	12,030	10.9 kbp	130.92 Mbp *	95.8%

Table 2.4 results of multiple mapping of unplaced 305E40 sequences against both 67/3 chromosomes and CH0. Supplementary alignments may include up to 50% of the same portion of a read

Alignment between the *ragtag _305* and 67/3_V3 (Figure 2.4) revealed a high collinearity between the two eggplant genomes in almost all chromosomes with the exception of CH02 and CH12 in which a great amount of interchromosomal rearrangements can be highlighted (figure 2.4), thus confirming the hypothesis of the presence of introgressed portions from the wild genome *S. aethiopicum* in these regions which hampered the correct chromosome pairing during meiosis in the process which led to generation of the F2 and RIL progenies from the cross 305E40x67/3. The presence of alien genome portions is a reasonable cause of the wrong recombination frequencies and false map position of the markers present in these regions which led, as a consequence, to a less reliable reconstruction of their genome assembly as the scaffolds were anchored according to a putatively incorrect map.

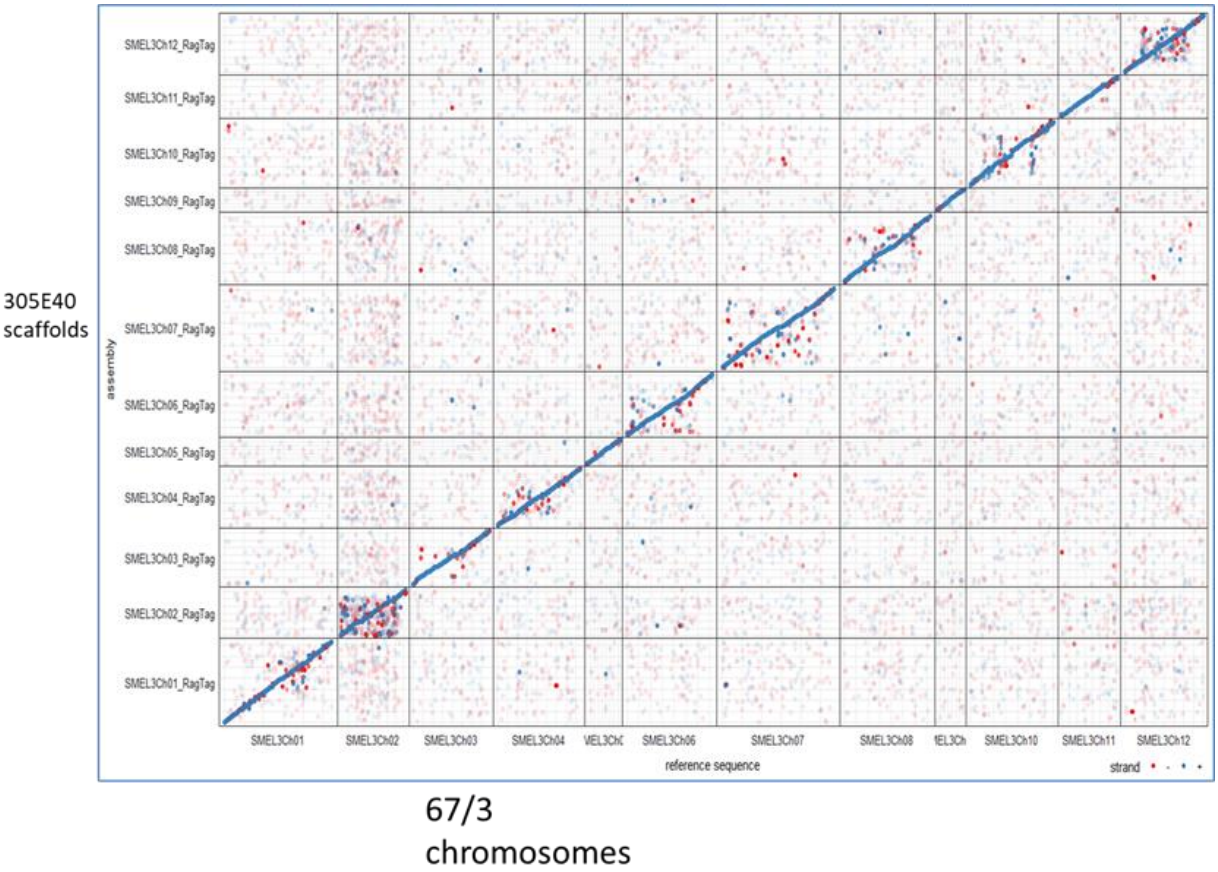


Figure 2.4 305E40 scaffolds aligned to 67/3 chromosomes. Alignment performed with MUMmer4, keeping alignments longer than 1kbp with identity > 70%

2.2.3.2 Gene annotation

The number of annotated genes in the *ragtag _305* genome as reported in table 3.4 is in line with the number of genes found in other Solanaceae genomes (34,899 genes in *Capsicum annuum*, 34,688 genes in *Solanum lycopersicum*). Mean CDS length (Table 2.5) is comparable with the annotation of *Solanum melongena* 67/3 (Barchi et al., 2019) and HQ (Wei et al., 2020).

	***S. melongena* HQ, Wei et al,2020**	***S. melongena* 67/3, Barchi et al.,. 2019**	***S.melongena* 305E40** RagTag
BUSCO annotation completeness	95.4%	92.1%	93.5 %
Predicted genes	36,568	34,916	34,806
Mean CDS length (bp)	990.73	1102.48	1069.09
Median CDS length (bp)	732.00	861.00	825.00
% of repetitive elements	70.09%	72.89%	74.78 %

Table 2.5: metrics of gene annotation in the 305_RagTag assembly

Thanks to the combined interproscan annotations + homology information from proteins of correlated species, 95,53% (33,253 genes) of total predicted genes was reconducted to an assigned function. Within the entire 305E40 genome, a total of 1880 resistance genes (128 on CH0 among the 11000 total) were annotated according to a combination of all utilised annotations (Non-redundant + Uniprot7Swissprot + eggNOG 5.0) 1880 genes (plus 128 genes on CH0) were annotated as Resistance-associated of which 126 are localised on CH02.

2.2.3.1 Bulked reads alignment against the QTL02 in the *ragtag _305*

The *ragtag _305 de novo* assembly was therefore employed as reference for a new round of BSAseq mapping of bulked reads of 18 completely susceptible (SS) and of 28 fully resistant (RR) RILs to identify chromosomal regions in which the reads alignment highlighted differentially mapped regions between the RR vs SS bulks, therefore containing genes more likely involved in the full resistance trait to *Fom*. The differentially represented regions were defined both as with a high ratio RR/SS, (therefore the gene or some domains are probably absent in SS progenies), or as with a similar ratio RR/SS but with extremely heterogeneous reads (therefore the gene is present also in SS progenies, but with a highly different sequence with respect to the RR version).

The entire chromosome 02 was scanned manually to highlight differentially enriched regions also considering the annotation (Figure 2.5a). Regions on other chromosomes than CH02 were also considered for the reads mapping as “negative control” (Figure 2.5b).

Figure 2.5: A) example of a region of 2.5Mb on CH02 in which 2 regions (blue boxes) covering different genes are highlighted with differential representation in mapping reads SS vs RR (also reads 305E40 and 67/3 are reported as

control). B) example of casual sampling of a region of 0.5Mb on CH03 in which no differential representation in mapping reads SS vs RR (also reads 305E40 and 67/3 are reported as control) is evidenced

Considering a tolerance of at least 100 reads/kb in at least one of RR or SS mapping bulks, a great portion between 0 and 57 Mb (over a total length 62.5Mb of CH02) was depicted as "differentially enriched" in bulked reads of resistant RILs with respect to the susceptible ones. Visual scoring and was conducted on each gene assigned as resistance gene both on CH02 and CH0 (all 124Mb). Regarding CH0, none among the 128 genes annotated as resistance genes was scored as convincingly differentially represented in RR/SS reads. Among the 1880 genes in the entire 305E40 genome annotated as resistance genes, 126 were localised on CH02 a visual scoring was performed on each gene, and 33 were considered as valuably differentially represented (Tab 2.6).

	IDGENE	chrom	V2	tipo	start	end	score	strand	frame	IGV	V9	commenti	PRIORITA	Tags	mRNA.size	ORF.size	Coding.probability
1	SMEL3Ch02_RagTag.g335	SMEL3Ch02_RagTag	AUGUSTUS	gene	3362756	3370951	0.01	-	.	SMEL3Ch02_RagTag:3362756-3370951	ID=SMEL3Ch02_RagTag.g335;seqName=SMEL3Ch02_RagTag.g335;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance** protein___TAXA:Poales___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	20x reads_incoerenti	3	[CODING]	3927	3927	1
2	SMEL3Ch02_RagTag.g567	SMEL3Ch02_RagTag	AUGUSTUS	gene	5735103	5737985	0.08	-	.	SMEL3Ch02_RagTag:5735103-5737985	ID=SMEL3Ch02_RagTag.g567;seqName=SMEL3Ch02_RagTag.g567;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: disease resistance protein___TAXA:Poales___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	20x reads abb simili	3	[CODING]	1584	1584	0,999992
3	SMEL3Ch02_RagTag.g1279	SMEL3Ch02_RagTag	AUGUSTUS	gene	12913631	12917931	0.08	-	.	SMEL3Ch02_RagTag:12913631-12917931	ID=SMEL3Ch02_RagTag.g1279;seqName=SMEL3Ch02_RagTag.g1279;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: disease resistance protein___TAXA:Poales___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	20x reads_abb_incoerenti	2	[CODING]	3432	3432	1
4	SMEL3Ch02_RagTag.g1559	SMEL3Ch02_RagTag	AUGUSTUS	gene	15963596	15974156	0.01	+	.	SMEL3Ch02_RagTag:15963596-15974156	ID=SMEL3Ch02_RagTag.g1559;seqName=SMEL3Ch02_RagTag.g1559;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	30x reads total incoerenti	5	[CODING]	4197	4197	1
5	SMEL3Ch02_RagTag.g1586	SMEL3Ch02_RagTag	AUGUSTUS	gene	16284779	16299927	0.01	+	.	SMEL3Ch02_RagTag:16284779-16299927	6;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: CG-1___TAXA:asterids___GOs: **P:defense response to bacterium;** F:sequence-specific DNA binding; P:multi-organism process; P:leaf senescence; P:defense response to fungus; P:positive regulation of defense response to insect; C:nucleus; P:positive regulation of transcription by RNA polymerase II; P:cellular response to cold; F:DNA-binding transcription activator activity, RNA polymerase II-specific; F:calmodulin binding BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Calmodulin-binding transcription activator 2; Short=AtCAMTA2; AltName: Full=AtER66; AltName: Full=Ethylene-induced calmodulin-binding protein c; Short=EICBP.c; AltName: Full=Signal-responsive protein 4;	20x reads_total_incoerenti	3	[CODING]	1347	1347	0,9999453
6	SMEL3Ch02_RagTag.g1920	SMEL3Ch02_RagTag	AUGUSTUS	gene	20074477	20087394	0.01	-	.	SMEL3Ch02_RagTag:20074477-20087394	ID=SMEL3Ch02_RagTag.g1920;seqName=SMEL3Ch02_RagTag.g1920;Note:Type: ENOG___gene_name: MLO___EGGNOG_DESCR: May be involved in modulation of pathogen defense and leaf cell death___TAXA:asterids___GOs: P:multi-organism reproductive process; P:defense response; C:cytoplasm; C:endomembrane system; P:pollen tube reception; C:plasma membrane BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=MLO protein homolog 1; AltName: Full=OsMLO1	2x ma reads eterogenei	2	[CODING]	1608	1608	0,9999921

7	**SMEL3Ch02_RagTag.g2004**	SMEL3Ch02_RagTag	AUGUSTUS	gene	20954436	20958473	0.01	-	.	SMEL3Ch02_RagTag:20954436-20958473	ID=SMEL3Ch02_RagTag.g2004;seqName=SMEL3Ch02_RagTag.g2004;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	30x reads molto dissimili	3	[CODING]	3024	3024	1
8	**SMEL3Ch02_RagTag.g2019**	SMEL3Ch02_RagTag	AUGUSTUS	gene	21094775	21095233	0.31	-	.	SMEL3Ch02_RagTag:21094775-21095233	9;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Belongs to the disease resistance NB-LRR family**___TAXA:asterids___GOs: BLASTP: BLASTP: [NO-BLAST]_descr: ---NA---	2x reads dissimili	1	[CODING]	234	234	0,6775876
9	**SMEL3Ch02_RagTag.g2020**	SMEL3Ch02_RagTag	AUGUSTUS	gene	21095493	21100483	0.05	-	.	SMEL3Ch02_RagTag:21095493-21100483	0;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: Belongs to the disease resistance NB-LRR family___TAXA:asterids___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Putative disease resistance RPP13-like protein 1	20x reads totalm dissimili	2	[CODING]	2418	2418	1
10	**SMEL3Ch02_RagTag.g2022**	SMEL3Ch02_RagTag	AUGUSTUS	gene	21102297	21105453	0.04	-	.	SMEL3Ch02_RagTag:21102297-21105453	2;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Belongs to the disease resistance NB-LRR family**___TAXA:asterids___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=**Putative disease resistance RPP13-like** protein 1	30x_reads_totalm_dissimil	4	[CODING]	1326	1326	0,9999548
11	**SMEL3Ch02_RagTag.g2035**	SMEL3Ch02_RagTag	AUGUSTUS	gene	21218599	21227420	0.02	-	.	SMEL3Ch02_RagTag:21218599-21227420	5;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Belongs to the disease resistance NB-LRR family**___TAXA:asterids___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=**Putative disease resistance protein RGA4;** AltName: Full=RGA4-blb	30x_reads_totalm_dissimil	4	[CODING]	1509	1509	0,9999812
12	**SMEL3Ch02_RagTag.g2137**	SMEL3Ch02_RagTag	AUGUSTUS	gene	22221733	22248070	0.01	+	.	SMEL3Ch02_RagTag:22221733-22248070	ID=SMEL3Ch02_RagTag.g2137;seqName=SMEL3Ch02_RagTag.g2137;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	30x_reads_tabb_simili	3	[CODING]	4191	4191	1
13	**SMEL3Ch02_RagTag.g2142**	SMEL3Ch02_RagTag	AUGUSTUS	gene	22289233	22293460	0.06	+	.	SMEL3Ch02_RagTag:22289233-22293460	2;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Belongs to the disease resistance NB**-LRR family___TAXA:asterids___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Putative late blight resistance protein homolog R1B-16	40X no reads in SS **EX SCAFF 4270 !!**	5	[CODING]	3702	3702	1
14	**SMEL3Ch02_RagTag.g2286**	SMEL3Ch02_RagTag	AUGUSTUS	gene	23533686	23542533	0.01	+	.	SMEL3Ch02_RagTag:23533686-23542533	ID=SMEL3Ch02_RagTag.g2286;seqName=SMEL3Ch02_RagTag.g2286;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	50x reads divesi	4	[CODING]	1143	1143	0,9995285
15	**SMEL3Ch02_RagTag.g2293**	SMEL3Ch02_RagTag	AUGUSTUS	gene	23608380	23616306	0.02	-	.	SMEL3Ch02_RagTag:23608380-23616306	ID=SMEL3Ch02_RagTag.g2293;seqName=SMEL3Ch02_RagTag.g2293;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	40x reads divesi	4	[CODING]	2943	2943	1

16	**SMEL3Ch02_RagTag.g2573**	SMEL3Ch02_RagTag	AUGUSTUS	gene	26663291	26672310	0.01	+	.	SMEL3Ch02_RagTag:26663291-26672310	ID=SMEL3Ch02_RagTag.g2573;seqName=SMEL3Ch02_RagTag.g2573;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	10 x reads diversi	3	[CODING]	2721	2721	1
17	**SMEL3Ch02_RagTag.g2626**	SMEL3Ch02_RagTag	AUGUSTUS	gene	27259182	27263483	0.02	-	.	SMEL3Ch02_RagTag:27259182-27263483	ID=SMEL3Ch02_RagTag.g2626;seqName=SMEL3Ch02_RagTag.g2626;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein**___TAXA:Poales___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	2x reads abb simili	2	[CODING]	2664	2664	1
18	**SMEL3Ch02_RagTag.g2779**	SMEL3Ch02_RagTag	AUGUSTUS	gene	28929358	28944236	0.01	+	.	SMEL3Ch02_RagTag:28929358-28944236	ID=SMEL3Ch02_RagTag.g2779;seqName=SMEL3Ch02_RagTag.g2779;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	40x reads diversi	4	[CODING]	5718	5412	1
19	**SMEL3Ch02_RagTag.g2781**	SMEL3Ch02_RagTag	AUGUSTUS	gene	28955873	28960330	0.01	+	.	SMEL3Ch02_RagTag:28955873-28960330	ID=SMEL3Ch02_RagTag.g2781;seqName=SMEL3Ch02_RagTag.g2781;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	2x reads diversi	3	[CODING]	2997	2997	1
20	**SMEL3Ch02_RagTag.g2782**	SMEL3Ch02_RagTag	AUGUSTUS	gene	28975313	28978751	0.05	+	.	SMEL3Ch02_RagTag:28975313-28978751	2;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: Belongs to the disease resistance NB-LRR family___TAXA:asterids___GOs: P:defense response; P:signal transduction; C:plasma membrane BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Probable disease resistance protein At4g27220	0x reads diversi possibili SNP	2	[CODING]	3042	3042	1
21	**SMEL3Ch02_RagTag.g2791**	SMEL3Ch02_RagTag	AUGUSTUS	gene	29074253	29080274	0.01	+	.	SMEL3Ch02_RagTag:29074253-29080274	ID=SMEL3Ch02_RagTag.g2791;seqName=SMEL3Ch02_RagTag.g2791;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: disease resistance protein___TAXA:Poales___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	20x reads diversi	3	[CODING]	3204	3204	1
22	**SMEL3Ch02_RagTag.g2935**	SMEL3Ch02_RagTag	AUGUSTUS	gene	30797959	30805083	0.04	-	.	SMEL3Ch02_RagTag:30797959-30805083	5;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: Protein kinase domain___TAXA:asterids___GOs: P:cell surface receptor signaling pathway; P:protein phosphorylation; F:protein serine/threonine kinase activity; C:plasma membrane BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=LEAF RUST 10 DISEASE-RESISTANCE LOCUS RECEPTOR-LIKE PROTEIN KINASE-like 1.5; AltName: Full=Probable receptor-like serine/threonine-protein kinase LRK10L-1.5; Flags: Precursor	50x reads diversi(no reads per cfr)	4	[CODING]	477	477	0,9017602
23	**SMEL3Ch02_RagTag.g2945**	SMEL3Ch02_RagTag	AUGUSTUS	gene	30884410	30887506	0.01	-	.	SMEL3Ch02_RagTag:30884410-30887506	ID=SMEL3Ch02_RagTag.g2945;seqName=SMEL3Ch02_RagTag.g2945;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales___GOs:** BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	50x reads diversi	4	[CODING]	1614	1614	0,9999946

24	**SMEL3Ch02_RagTag.g3114**	SMEL3Ch02_RagTag	AUGUSTUS	gene	32882262	32887772	0.01	+	.	SMEL3Ch02_RagTag:32882262-32887772	4;Note:Type: ENOG___gene_name: ___EGGNOG_DESCR: **TMV resistance protein N**-like___TAXA:asterids___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Disease resistance protein RRS1; AltName: Full=Disease resistance protein RCH2; AltName: Full=Disease resistance protein SLH1; AltName: Full=Probable WRKY transcription factor 52; AltName: Full=Protein RPS4-homolog; AltName: Full=Protein SENSITIVE TO LOW HUMIDITY 1; AltName: Full=Resistance to Colletotrichum higginsianum 2 protein; AltName: Full=Resistance to Ralstonia solanacearum 1 protein; AltName: Full=WRKY DNA-binding protein 52	0x reads diversi	1	[CODING]	3261	3261	1
25	**SMEL3Ch02_RagTag.g3959**	SMEL3Ch02_RagTag	AUGUSTUS	gene	41564951	41570931	0.02	+	.	SMEL3Ch02_RagTag:41564951-41570931	9;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: Belongs to **the disease resistance NB-LRR family___TAXA:asterids**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Putative disease resistance RPP13-like protein 1	50 x reads incomparab	5	[CODING]	2541	2541	1
26	**SMEL3Ch02_RagTag.g4078**	SMEL3Ch02_RagTag	AUGUSTUS	gene	42718867	42722868	0.01	+	.	SMEL3Ch02_RagTag:42718867-42722868	ID=SMEL3Ch02_RagTag.g4078;seqName=SMEL3Ch02_RagTag.g4078;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	50 x reads incomparab	4	[CODING]	2877	2877	1
27	**SMEL3Ch02_RagTag.g4098**	SMEL3Ch02_RagTag	AUGUSTUS	gene	42867834	42870891	0.07	-	.	SMEL3Ch02_RagTag:42867834-42870891	ID=SMEL3Ch02_RagTag.g4098;seqName=SMEL3Ch02_RagTag.g4098;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	40x__reads_ dversi	4	[CODING]	1236	1236	0,9998976
28	**SMEL3Ch02_RagTag.g4259**	SMEL3Ch02_RagTag	AUGUSTUS	gene	44354430	44359034	0.05	+	.	SMEL3Ch02_RagTag:44354430-44359034	ID=SMEL3Ch02_RagTag.g4259;seqName=SMEL3Ch02_RagTag.g4259;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	30x reads diversi	4	[CODING]	3570	3570	1
29	**SMEL3Ch02_RagTag.g4273**	SMEL3Ch02_RagTag	AUGUSTUS	gene	44535918	44539376	0.07	-	.	SMEL3Ch02_RagTag:44535918-44539376	ID=SMEL3Ch02_RagTag.g4273;seqName=SMEL3Ch02_RagTag.g4273;Note:Type: COG,KOG,ENOG___gene_name: ___EGGNOG_DESCR: **disease resistance protein___TAXA:Poales**___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	30x reads diversi	5	[CODING]	2883	2883	1
30	**SMEL3Ch02_RagTag.g4323**	SMEL3Ch02_RagTag	AUGUSTUS	gene	44998287	45000024	0.39	+	.	SMEL3Ch02_RagTag:44998287-45000024	ID=SMEL3Ch02_RagTag.g4323;seqName=SMEL3Ch02_RagTag.g4323;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Resistance protein**___TAXA:asterids___GOs: BLASTP: BLASTP: [NO-BLAST]_descr: ---NA---	50x incomparab	4	[CODING]	669	669	0,9841044
31	**SMEL3Ch02_RagTag.g4324**	SMEL3Ch02_RagTag	AUGUSTUS	gene	45000517	45010496	0.01	+	.	SMEL3Ch02_RagTag:45000517-45010496	ID=SMEL3Ch02_RagTag.g4324;seqName=SMEL3Ch02_RagTag.g4324;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Resistance protein**___TAXA:asterids___GOs: BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Retrovirus-related Pol polyprotein from transposon TNT 1-94; Includes: RecName: Full=Protease; Includes: RecName: Full=Reverse transcriptase; Includes: RecName: Full=Endonuclease	20x reads in parte dissimili	3	[CODING]	5364	5364	1

32	**SMEL3Ch02_RagTag.g4438**	SMEL3Ch02_RagTag	AUGUSTUS	gene	46198827	46199841	0.14	-	.	SMEL3Ch02_RagTag:46198827-46199841	8;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: Zinc finger, C3HC4 type (RING finger)___TAXA:asterids___GOs: P:protein ubiquitination; P:defense response to bacterium; F:ubiquitin-protein transferase activity; P:multi-organism process; P:defense response to fungus; P:negative regulation of programmed cell death; P:negative regulation of cysteine-type endopeptidase activity; C:nucleus; P:response to acid chemical; P:response to gibberellin BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=BOI-related E3 ubiquitin-protein ligase 1; AltName: Full=RING-type E3 ubiquitin transferase BRG1	40x no reads comparabili	3	[CODING]	564	564	0,9665829
33	**SMEL3Ch02_RagTag.g4539**	SMEL3Ch02_RagTag	AUGUSTUS	gene	47240179	47243927	0.02	+	.	SMEL3Ch02_RagTag:47240179-47243927	9;Note:Type: KOG,ENOG___gene_name: ___EGGNOG_DESCR: **Belongs to the disease resistance NB-LRR family**___TAXA:Brassicales___Gos: P:defense response to Gram-negative bacterium; P:multi-organism process; F:protein self-association; P:signal transduction; C:plasma membrane BLASTP: BLASTP: [BLASTED, MAPPED, ANNOTATED]_descr: RecName: Full=Disease resistance RPP13-like protein 4; AltName: Full=**Disease resistance protein ZAR1**; AltName: Full=Protein HOPZ-	20x reads dissimili	4	[CODING]	459	459	0,8651382

Table 2.6 list of 33 best candidates according to differential mapping between RR vs SS reads.

A detailed characterization of each gene was performed to highlight regions in which differential coverage of reads was depictable and its effect on the gene translation (example in figure 2.6).

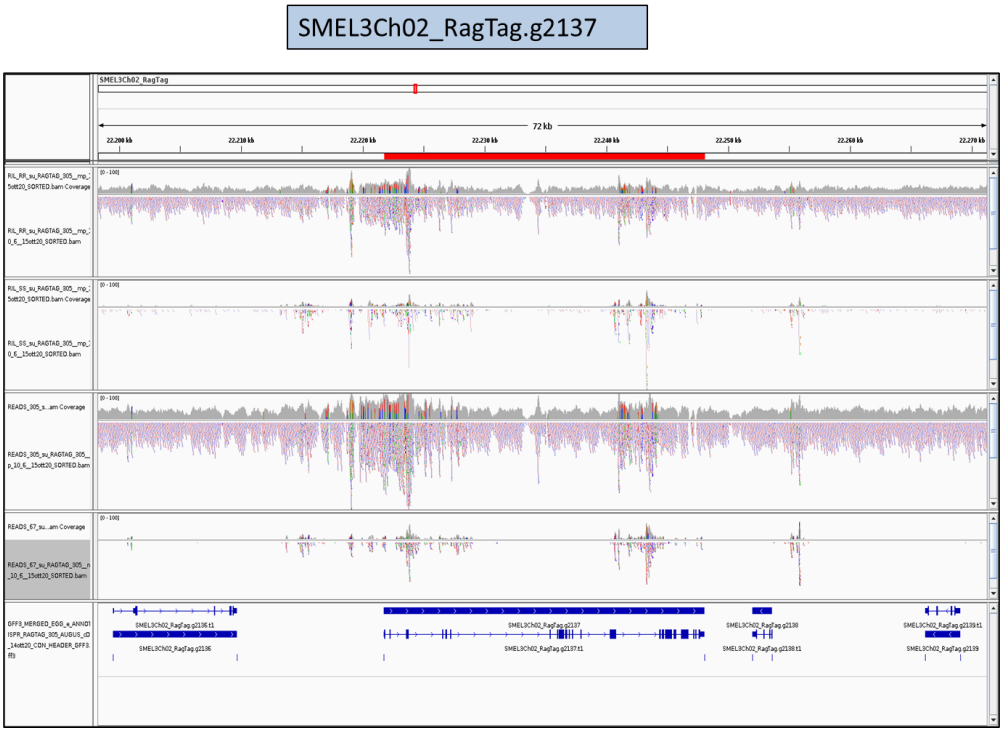


Figure 2.6 Detail of resistance gene with underneath reads RR (present), SS (absent), 305 (present) and 67/3 (absent)

Comparing this list of 33 genes with all the genes highlighted in chapter 1 as best candidates, 9 genes are in common between the two lists (Tab 2.7). These genes are mainly localized in two regions (20-30 Mb e 40-45 Mb), and those belonging to the latter one being more reliable as candidate as the *Rfo-Sa1* locus was localized at 37Mb. A tentative degree of priority assigned to each gene basing on coherence and reliability between different annotations (*EGGNOG –swissprot, score augustus and CPAT*), mapping patterns (RRvsSS) and reads incoherence allowed to identify 7-8 of them are eligible as best candidates for future studies. Moreover, two more genes were added to the panel of best candidates which were not highlighted by this analysis because, according to recent literature or other analyses, they have been assessed involved in the resistance to *Fusarium.*

The first added gene was highlighted from GWAS utilising genetic information and disease score data of the eggplant core collection as described in chapter 3 and corresponds to one of the four genes of the 25 already highlighted by Miyatake et al. (2016) whose orthologues mapped on CH02, while the other, is a chitin-binding lectine retrieved from literature as isolated from roots exudates in a wild *solanum* species in response to *Fom* inoculation (Chen et al., 2018). For both genes, constructs for knock-out through gene editing are under development.

n gene	33+2 GENES (Query)	POSITION IN ch02	ANNOTATED AS	MATCHING WITH	GENE NAME CHAPTER 1	ANNOTATION	evalue	% identities
9	*SMEL3Ch02_RagTag.g2020*	21095493-21100483	Belongs to the disease resistance NB-LRR family	*scaffold157917__682_31063 in 305_asm*	*RES 13*	disease resistance rpp13-like protein 1-like	0.0	85.109
10	*SMEL3Ch02_RagTag.g2022*	21102297-21105453	Belongs to the disease resistance NB-LRR family	*scaffold157917__682_31063 in 305_asm*	*RES 13*	disease resistance rpp13-like protein 1-like	$1.64e^{-68}$	86.207
11	*SMEL3Ch02_RagTag.g2035*	21218599-21227420	Belongs to the disease resistance NB-LRR family	*scaffold157917__682_31063 in 305_asm*	*RES 13*	disease resistance rpp13-like protein 1-like	0.0	83.788
13	*SMEL3Ch02_RagTag.g2142*	22289233-22293460	Belongs to the disease resistance NB-LRR family	*scaffold4270__1160_51181 in 305_asm*	*RES 8*	SAUC68094_TC01	0.0	100.000
14	*SMEL3Ch02_RagTag.g2286*	23533686-23542533	disease resistance protein	*scaffold88978__1014_45579 in 305_asm*	*RES 16*	tir-nbs-lrr type disease resistance protein	$1.71e^{-47}$	88.816
20	*SMEL3Ch02_RagTag.g2782*	28975313-28978751	Belongs to the disease resistance NB-LRR family	*SMEL_002g156420.1 (Miyatake et al., 2016)*	*MIYA3*	Cc-nbs-lrr%2C resistance protein	0.0	98.274
24	*SMEL3Ch02_RagTag.g3114*	32882262-32887772	TMV resistance protein N-like	*SMEL_002g157480.1 (Miyatake et al., 2016)*	*RES 1*	Tir-lrr%2C resistance protein fragment Encoding a putative TMV resistance protein N-like	0.0	99.666
25	*SMEL3Ch02_RagTag.g3959*	41564951-41570931	Belongs to the disease resistance NB-LRR family	*scaffold157917__682_31063 in 305_asm*	*RES 13*	disease resistance rpp13-like protein 1-like	0.0	100.000
32	*SMEL3Ch02_RagTag.g4438*	46198827-46199841	Zinc finger, C3HC4 type (RING finger)	*scaffold131120__543_23320 in 305_asm*	*RES 5*	SAUC54187_TC01	0.0	100.000

Table 2.7 List of 9 common genes located mainly in two regions (20-30 Mb and 40-45 Mb) obtained by comparing the list of 33 genes with all the genes highlighted in Chapter 1 as best candidates

Formally, any gene manifesting alteration in RR vs SS reads patterns should be considered as a candidate, (not only genes annotated as associated with resistance as considered at this stage) therefore, due to the vastness of CH02 regions altered in RR vs SS patterns, any element aimed at selecting CH02 subregions more plausibly associated with resistance would reduce the number of candidate genes. For this reason, resequencing of those RIL progenies highlighted in chapter 1 whose haplotype was not in accordance with the resistance score will be performed to reduce the length of the chromosomal region involved in the resistance trait. Moreover, RNA-SEQ of eggplant roots of the lines '305E40', '67/3' and the fully susceptible line 'Tal 1/1' (the recurrent parent of 305E40) has been planned to better clarify the expression levels of these candidates and the networks of genes in these different lines after inoculation with the fungus.

Chapter 3

Search of a new sources of resistance

3.1 Introduction

Susceptibility to soil-borne diseases and especially to fungal wilts is one of the main factors affecting the eggplant production. An eco-friendlier alternative to the extensive use of chemical treatments would be the improvement of the eggplant natural resistance through the development of resistant breeding lines by exploiting the sources of resistance present within the cultivated germplasm or among its allied and wild relatives. To this end, a wide screening of the eggplant germplasm available as well as the characterization of the intraspecific variability could play an essential role as it would allow to uncover new interesting sources of resistances exploitable for breeding. Extensive studies on this topic within the germplasm of cultivated eggplant are still rather limited and the findings obtained so far did not provide a great amount of valuable material for usage in breeding programs (Toppino et al., 2021). Wild species of eggplant already proved potential invaluable sources of resistance genes towards diseases and insects represent a great source of variation for many traits of agronomical interest including the resistances to pests and diseases, fruit quality traits and, more recently, the nutritional value of the fruits (Rotino et al., 2014; Toppino et al., 2021). Many wild and allied species have been reported to carry traits of resistance to most diseases and pests affecting eggplant, including fungi (*Leveillula taurica, Phomopsis vexans, Fusarium spp., Verticillium spp, Colletotrichum coccodes, Phytophthora spp, Cercospora solani)*, bacteria (*Ralstonia solanacearum*), Nematodes (*Meloidoygine* spp.), pests (*Leucinodes orbonalis, Epilachana vigintioctopunctata, Aphis gossypii, Tetranychus cinnabarinus, T. urticae*), viruses (*potato virus Y, eggplant mosaic virus*) and mycoplasma (Daunay and Hazra, 2012; Rotino et al., 2014). Introgression breeding is a strategy widely used to enlarge the genetic background of a crop species by exploiting the source of variation residing in donor allied species (Prohens et al., 2017); however, with a few exceptions (Rotino et al., 2014; Liu et al., 2015), eggplant breeders have largely neglected the potential of wild species for breeding purposes aimed at developing new eggplant cultivars with improved features. Although interspecific hybrids between eggplant and wild relatives have been obtained through sexual crosses using 27 species (Daunay et al., 2012; Rotino et al., 2014; Devi et al., 2015; Plazas et al., 2016), most of the studies have been conducted for taxonomic purposes and preliminary breeding works and have not undertaken the development of backcross generations. Crosses of wild species to with cultivated eggplant are generally sterile or yield low fertile hybrids (Rakha et al., 2021, Plazas et al., 2020, Toppino et al., 2021) but few of them gave rise to fertile progeny from partially fertile F_1 thus allowing the development of backcross generations. Efforts have been made to transfer alien genes controlling important traits from wild and allied relatives into the cultivated gene pool of eggplant, also using unconventional (somatic hybridization, embryo rescue, and genetic transformation) breeding methods (Rotino et al., 2014, Toppino et al., 2021). Different kinds of introgression materials have been obtained with eggplant relatives, including breeding

lines from the somatic hybrid with *S. aethiopicum* gr. *gilo* and *aculeatum* carrying an introgressed trait of resistance to *Fusarium oxysporum* (Toppino et al., 2008). Backcrossed progenies from sexual cross with *S. linnaeanum* and *S. aethiopicum* displayed an improved resistance to *Verticillium* (Acciarri et al., 2004; Liu et al., 2015) and *Fusarium* (Zhuang and Wang, 2009) wilts, respectively. Backcross progenies (BC_1) derived from the cross between *S. incanum* and eggplant were used to develop introgression lines (Vilanova et al., 2010; Prohens et al., 2012; 2013) with improved tolerance to abiotic stresses. The characterization of wild species and the obtained interspecific hybrids for traits of interest is a crucial step for the efficient utilization of crop wild relatives in breeding. Combined phenotypic and genotypic data on the cultivated and wild species and the interspecific hybrids obtained from their crosses, not only would allow to identify sources of variation and materials of potential interest, but also provide information on the inheritance of interesting traits and for the identification of genetic regions and genes involved in them.

In the current chapter, a wide panel of eggplant accessions, wild species from the primary and secondary genepool of plants and the introgressed population from the interspecific cross between *S. melongena* line 67/3 and *S tomentosum* were investigated for the responses against the artificial inoculation with *Fom* to support their future employment in eggplant breeding programs and with the aim to identify new genetic regions associated with the *Fom* resistance traits.

3.2 Core collection

3.2.1 Introduction

A core collection of nearly 425 cultivated *S. melongena* accessions and 25 *Solanum* wild species was developed in the framework of the H2020 G2P-SOL project to be characterized both at genotypic and phenotypic level finalized at GWAS analysis for all relevant traits. Analyses of Resequencing data of the core collection, selection of the panel of SNPs and GWAS analyses are activities performed by other partners of the G2P-SOL project and are not directly part of this thesis. As GWAS analyses are still in progress, we will report only the preliminary results obtained for *Fom* resistance trait by the partner which is performing all analyses, as conclusion of this chapter.

3.2.2 Materials and methods

3.2.2.1 Plant material and Fom inoculation

Figure 3.1 Variation of different eggplant fruit types from the core collection observed in the field trial.

In May 2020, seeds of 396 *S. melongena* and 25 wild accessions of the core collection were sown in plastic tray with 104 holes (13x8, Figure 3.3) filled with peat and placed over an electric warmed carpet (24°C) and maintained in heated glasshouse (minimum air temperature of 15°C ensured). Specifically, two blocks of 6 plants per accession were composed, and independently inoculated with a conidial suspension of *Fusarium oxysporum* f.s. *melongenae.*

The inoculation was conducted according to the dip-root method as reported by Cappelli et al. (1995). Plantlets, at the 2–3 true leaf stage, were gently removed from the tray and their roots washed under running tap water, then immersed for 10 min in a conidial suspension of *Fom* at a concentration of 1.0×10^6 conidia/ml.

Figure 3.2 The eggplant core collection were sown in seed plates

Plantlets of the two inoculated blocks (biological replicates) were transplanted in 54 holes plastic trays filled with peat and arranged in randomized positions of in two different greenhouses until phenotypic evaluation.

Evaluation of symptoms was assessed on each single plant 30 Days After Inoculation (DAI), according to a degree scale of evaluation of symptoms (compared with mock inoculated controls) ranging from 1 to 0, as described in Chapter 1, figure 1.1

2.2.2 DNA isolation

For each accession of the core collection phenotyped in the field, approximately 100 mg of fresh tissue was sampled from a single plant and collected in racks of 96 samples (plus two Eppendorf backup replicates) with the purpose of extracting genomic DNA for the subsequent sequencing to be conducted as service by an external partner (BGI). Given the large quantities of material to be extracted, a low-cost extraction protocol (Silex, Vilanova et al. 2020) was used and optimised to extract material directly into 96 plates rather than into individual Eppendorf (figure 3.2). The results were excellent in terms of both yield and quality, verified with NanoDrop-One at CREA, and with Qubit Fluorometric (www.thermofisher.com) at the partner's uniTo facilities before sending them to BGI for sequencing.

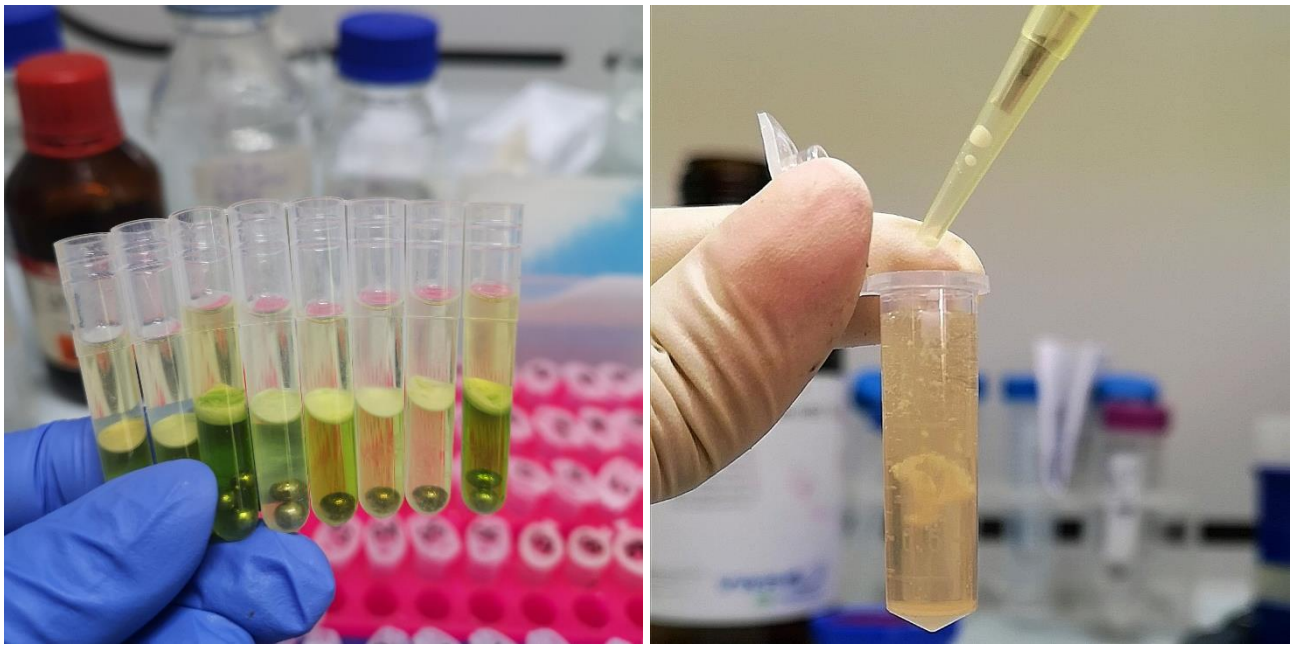


Figure ***Errore. Per applicare 0 al testo da visualizzare in questo punto, utilizzare la scheda Home.*** *3.3 DNA extraction steps, in accord to Vilanova et al. 2020*

The protocol includes the following main steps:

-Take samples in 96 plates, add tungsten marbles and store at -80°;

-freeze the plates in liquid nitrogen and grind the material using a plate grinder (TissueLyser II, Qiagen);

-Add to the sample, using a multichannel pipettor, a master mix consisting of an extraction buffer, ß-Mercaptoethanol or TRI Reagent, and RNase;

- Vortex for 30 secs or until complete homogenization and incubate in the thermo-block for 30 to 60 min at 65 °C.

- move the samples to ice for 5 minutes, add chloroform: isoamyl alcohol (24:1), and after vortexing pass through centrifuge to obtain the division into the two phases separated by interphase;

- Take the supernatant, without disturbing the interphase and transfer it to a new 2 ml Eppendorf tube (to allow optimal resuspension of the silica powder which would otherwise be hampered by the small volume of the tubes);

- first add a buffer solution and silica matrix, and following shake gently by hand, carry out two washes with ethanol;

- after allowing the silica to dry for 10 min and then resuspended with TE, incubate for approximately 10 minutes at 65°, proceed with a centrifugation step to deposit the silica, and transfer the supernatant into new Eppendorf tube;

DNA is thus ready for measure the concentration and quality with Nanodrop, and by gel running of the samples.

3.2.2.3 Data analysis

Trait distribution, basic stats, and Analysis of variance (ANOVA) of resistance trait was performed to test the significance of differences between Accessios and replications using JMP v. 7 software (SAS Institute, Milano, Italy). The effects of replications and genotypes were accounted in the model.

3.2.3 Result

3.2.3.1 Disease score on Core Collection

Evaluation of symptoms from *Fom* inoculation was performed on 377 germinated accessions (of which 20 wilds) in the first block and 373 accessions (of which 20 wilds) in the second one.

The Frequency distribution of *Fom* resistance score (average of all data collected for each accession) is shown in Figure 3.4. Evaluation of the *Fom* symptoms on the entire core collection (Figure 3.4) revealed that more than 120 accessions were totally susceptible (score assigned 0, dead plants) and about 127 accessions showed highly severe symptoms (leaves with large necrotic areas and prolonged yellowing), while 13 accessions were totally resistant and therefore with no or mild symptoms; the rest of the collection proved to be intermediate tolerant, with a wide range of yellowing on the basal and intermediate leaves.

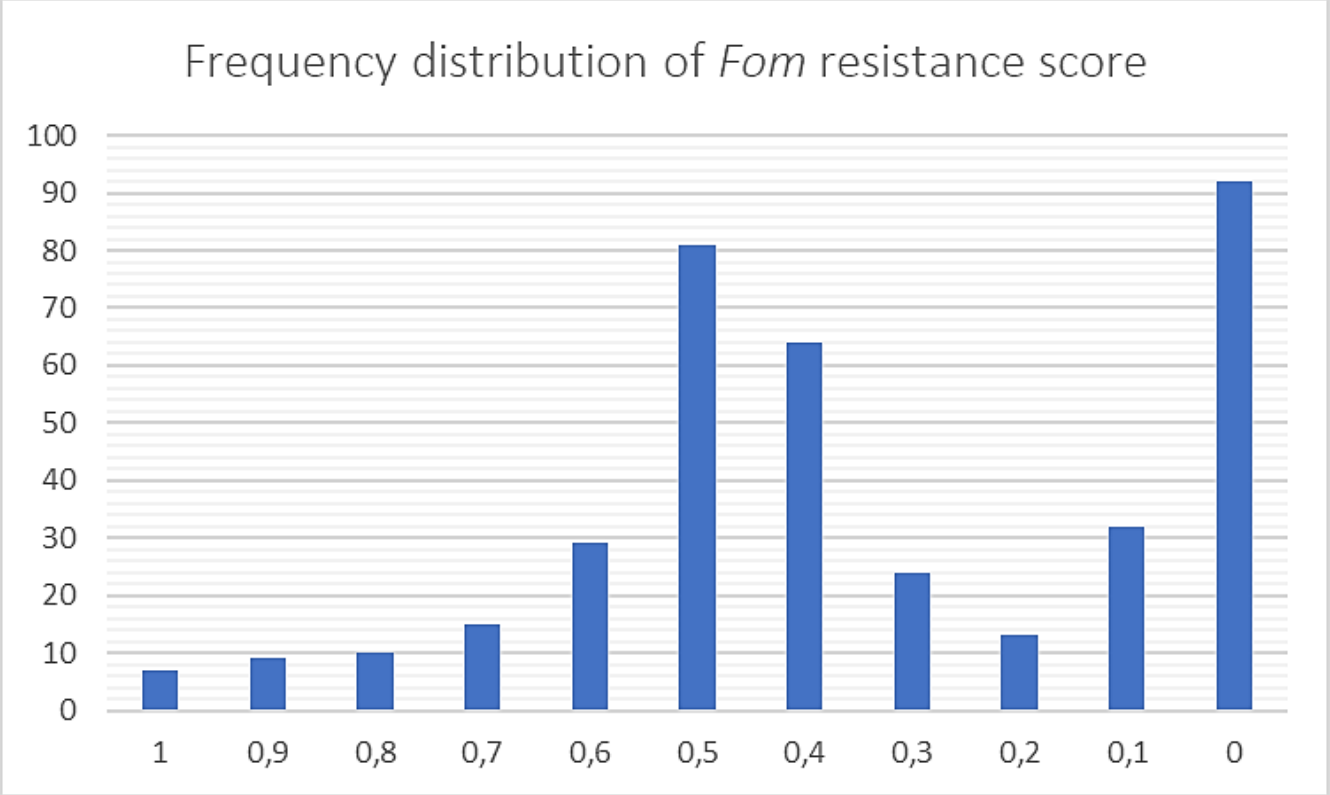


Figure 3.4. Frequency distribution of accessions according to their average symptom score following Fusarium oxysporum inoculation (average symptoms of all tested plants for each accession)

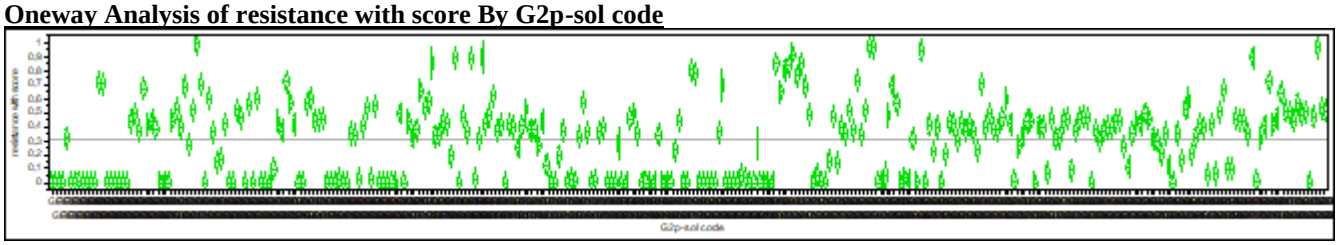
Quantiles

100.0%	maximum	1,0000
99.5%		0,9833
97.5%		0,9000
90.0%		0,6600
75.0%	quartile	0,4667
50.0%	median	0,3500
25.0%	quartile	0,0000
10.0%		0,0000
2.5%		0,0000
0.5%		0,0000
0.0%	minimum	0,0000

Moments

Mean	0,3066614
Std Dev	0,2650287
Std Err Mean	0,0105674
upper 95% Mean	0,3274131
lower 95% Mean	0,2859097
N	629

Oneway Analysis of resistance with score By G2p-sol code



Summary of Fit

Rsquare	0,977236
Adj Rsquare	0,954033
Root Mean Square Error	0,056822
Mean of Response	0,306661
Observations (or Sum Wgts)	629

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
G2p-sol code	317	43,106739	0,135983	42,1170	<.0001
Error	311	1,004128	0,003229		
C. Total	628	44,110867			

Table 3.1 Distribution, basic stat data, analysis of variance of the Fom resistance trait

The 20 best resistant genotypes from the core collection were selected and were screened, in biological triplicate, for the presence of the *Rfo-sa1* gene locus already identified by Toppino et al., 2008 and for the resistance locus identified by Mutlu et al., 2008.

For the *Rfo-Sa1* locus, the 20 accessions were amplified using the CAPS markers in combination with the restriction enzymes XmiI, PstI and Eco47III (as described in Toppino et al., 2008). All genotypes tested, despite their excellent resistance to the fungus, seemed do not carry the Rfo-Sa1 locus, with the only exception of line 305E40 (the *Fom* resistant mapping parent, also present in the collection), and its hybrid with 67/3, *S. aethipicum* and 5 other RILs (described in chapter 1) which were used as positive controls.

The resistant accessions were amplified with both the SRAP and SCAR-type markers developed by Mutlu et al., (2008) without evidencing a correspondence between the presence of these markers and the *Fusarium* resistance trait. We can conclude that the *Fom* resistance traits brought by these

assessions seem to be novel or at least not detectable with the available markers already developed and would deserve a deeper characterization.

Figure 3.5 response to artificial inoculation with Fom of resistant and susceptible accessions

3.2.3.2 GWAS analysis

After comparison of Resequencing data of all accessions in the core collection, a panel of 1.32M biallelic Single Nucleotide Polymorphisms (SNPs) with a read depth > 10, missing data (NA) < 20%, heterozygosity < 20% and minor allele frequency (MAF) greater than 5% was retained for GWAS analyses.

Twenty-two accessions from the core collection displayed a NA > 20% and heterozygosity > 5% and were therefore excluded from further analyses, the final core collection being therefore composed by 309 *S. melongena* accessions. To identify population stratification, a Principal Component Analysis (PCA) was performed in which the first three principal components accounted, respectively, for 13%, 8.7% and 5.6% of the genetic variation.

The SNP matrix was thinned to retain one SNP every 4K bp. LD was estimated as pairwise squared allele-frequency correlation (r^2) between SNP markers, correcting for relatedness using a kinship matrix by the R package LdcorSV(Mangin et al., 2012). The kinship matrix was estimated as covariance between genotypes, based on a LD-pruned set of SNPs. LD pruning was performed to

exclude SNP pairs with LD (r^2) greater than 0.98. A sliding window of 4 Mb was used to estimate local LD for each chromosome, with the main aim of identifying and exclude heterochromatic regions. GWAS analyses were carried out using the R package "Genomic Association and Prediction Integrated Tool" (GAPIT, Lipka et al., 2012). Different single-trait association models were used, including Mixed Linear Model (MLM, Yu et al., 2006), Multiple Locus Mixed Linear Model (MLMM, Segura et al., 2012), FarmCPU (Liu et al., 2016) and BLINK (Huang et al., 2019). The significance threshold was adjusted for multiple testing using the Bonferroni method.

Quantitative trait nucleotides (QTNs) identified using three different methods (MLMM, FarmCPU and BLINK) on up to three different environments or across all environments were merged in QTLs according to the LD decay value identified in our two steps procedures. For each chromosome, heterochromatic regions were identified and excluded

GWAS

Overall, more than 900 QTLs were identified for the field categorical traits, and more than 600 were identified for the metabolic dataset, considering both single and multi-environments analyses. Regarding fusarium resistance trait, 23 QTLs were identified among 10 chromosomes. A strong QTL was highlighted on chromosome 02, and within this interval a candidate gene already described in chapter 2 was evidenced.

Chr	*Fusarium*
0	
1	
2	3
3	1
4	1
5	1
6	2
7	4
8	
9	5
10	3
11	2
12	1
Total	23

Table 3.2 Summary table reporting the number of QTLs detected per chromosome according to trait type

3.3 ILs with S. tomentosum

3.3.1 Introduction

Solanum tomentosum L. is a wild species belonging to the secondary genepool of eggplant, representing a valuable source for key breeding traits like resistances to pathogens, plant/fruit features, and fruit biochemical composition. Its short internodes and red colored fruits at ripe, as well

as its high and species-specific glycoalkaloids content and its resistance to several pests including *Fusarium oxysporum* and nematodes make this species suitable to be employed in breeding programs aimed at the genetic improvement of eggplant for agronomic performance, fruit quality, nutraceutical value or ornamental purposes. From the cross between eggplant line 67/3 x *S. tomentosum* (used as male in order to maintain the cytoplasmic background of *S. melongena*) some F1 plants were obtained and were subsequently backcrossed to eggplant line 67/3. From the unique 5 BC1 plants obtained, 72 BC2 plants were developed and evaluated for the presence of introgressed wild fragments through molecular analysis. Nearly 150 molecular markers (SSR, COS and above all HRM) with known position in the molecular map 305E40x67/3 of Barchi et al., 2019, were selected as discriminating between the two parentals and used to identify in each plant the presence and position of fragments introgressed from the allied species. Genotypic characterization of the BC2 plants revealed that nearly 80% of *S. tomentosum* genome was represented across the BC2 population. To recover the missing regions, a further backcross of a BC2 plant with the wild species to recover the lost fragment was planned (Retro-backcross progenies or RBC). At each cycle of backcross, 12 plants of each progeny were submitted to molecular characterization through HRM markers to select the plants containing a single introgressed fragments from the wild donor as much cleaned as possible of other undesired introgressed regions. A population of advanced backcrossed lines was then developed through several cycles of backcross each followed by HRM molecular characterization of the introgressed regions of interest. From BC2 to BC6, nearly 100 plants were selected after genotypic analysis to be further backcrossed with the recurrent parent so that after 5 cycles of backcross the introgressed population was composed by 90 BC5-BC6 progenies, which were fully characterized through HRM covering 88% of the wild genome, plus another 9% of genome coverage which can be still recovered employing plants at a lower backcross level. BC5-BC6 selected plants were then selfed to start fixing the introgressed fragments at homozygous level. Final aim of the project is to develop a population of introgressed lines (IL) each harboring a unique fragment from the wild species inserted at homozygous level into the genome of eggplant line 67/3, representing a living library of the *S. tomentosum* genome introgressed into the genetic background of *S. melongena*. The IL population will be usefully exploited for both basic and applied research.

During the first year of my PHD, the two parents of the population (belonging to the panel of CREA accessions included in the G2P-SOL eggplant collection) were genotyped with the SPET “low”-density panel of markers of nearly 5000 markers (Barchi et al., 2019c). After selection of more than 5000 markers polymorphic between the two parental lines, a maximum of 12 plants of nearly 100 BC6-BC7, BC6S1, BC7S1 lines were characterized with the purpose to select more stabilized plants

owning introgressed regions from the wild parent at homozygous level and to generate advanced IL through selfing or backcross.

The same population was assessed for the response to *Fom* after artificial inoculation in order to select useful introgressed lines to be utilized for breeding and to identify genomic regions putatively associated with the resistance trait.

3.3.2 Materials and methods

3.3.2.1 plant material and *Fom* inoculation

A population of 108 advanced lines from the cross between *S. melongena* line '67/3' and *S. tomentosum* (3BC4, 10BC4s1, 14BC5, 21BC5S1, 21BC6, 19BC6S1, 12BC7, 6RBC3, 1RBC3S1,1RBC4 progenies), the parental lines *S. tomentosum*, 67/3, plus the sensitive line DR2 and the fully resistant one 305E40 were sawn in peat in 104 hole plastic trays in greenhouse for leaf sampling and to be submitted to the artificial *Fom* inoculation.

Plantlets of each progeny (3 blocks of 10 plants each), grown until the 2–3th true leaf stage, were gently removed, the roots washed under tap running water and immersed in the suspension of *Fom* conidia with the same procedure already described both in Chapter 1. For the mock inoculation, sterile distilled water was used in place of the conidial suspension. After inoculation, the plantlets were gently dried and transferred to 54-hole plastic trays filled with sand: peat 3:1 (v/v) and placed in growth chamber. The three replicated blocks were transferred randomly in the hole trays and kept in different greenhouse position. Determination of disease index was performed as already above described.

3.3.2.2 DNA extraction

For each progeny, leaves samples from a max 12 un-inoculated plantlets were collected for DNA extraction according to the optimized Silex method already described in this chapter. DNA samples were sent to IGA technology for SPET genotyping (about 5000 markers) as external service. The un inoculated plants were transplanted in field for selfing/backcrossing and for phenotypic evaluation beyond the scope of this thesis. SPET genotyping results were employed to select the plants for each progeny to be selfed or backcrossed for the next season.

3.3.2.3 Data analysis

Trait distribution, basic stats and Analysis of variance (ANOVA) of resistance trait was performed to test the significance of differences between progenies and replications using JMP v. 7 software (SAS Institute, Milano, Italy).

3.3.3 Results

Evaluation of symptoms from *Fom* inoculation was performed on all 108 progenies in triplicate. Plantlets of both *S. tomentosum* and eggplant line 67/3 displayed a resistance score around 6-7, thus confirming to possess a partial resistance trait. All 305E40 plantlets displayed a full resistant phenotype, and all control DR2 plantlets died. The distribution of *Fom* resistance score, assessed as described previously, is reported in Figure 3.6.

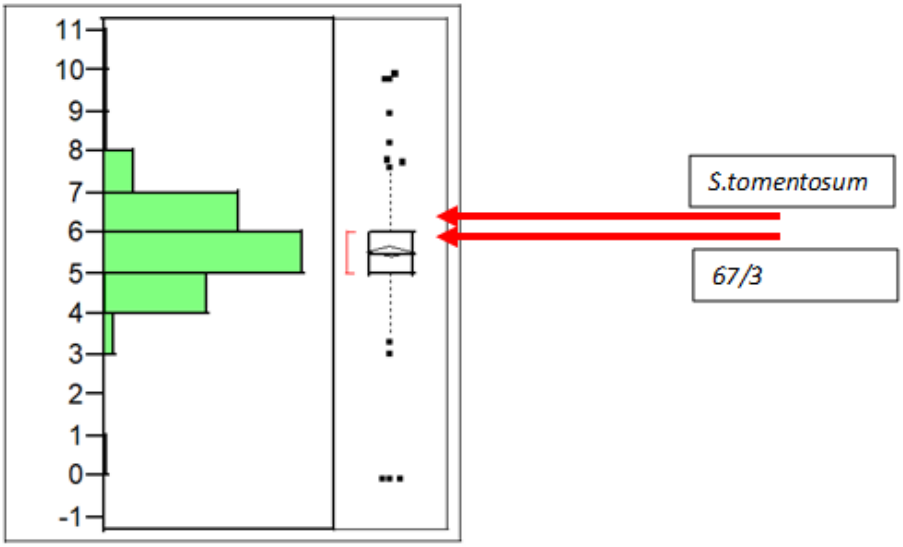


Figure 3.6 Distribution of the resistance score among the BC population. The scores values of the two parental lines '67/3' and S. tomentosum are highlighted with red lines.

Overall, great part of the progenies displayed a good level or resistance to *Fom*, ranging from 5 to 7, with an average of 5.5 and a 75% percentile of 6. Only few progenies displayed low values of resistance, including the presence of completely sensitive plants which died; an explanation to this performance can be found when considering the SPET genotypic data regarding these lines.

Quantiles

100.0%	maximum	10,000
99.5%		9,931
97.5%		7,657
90.0%		6,830
75.0%	quartile	6,050
50.0%	median	5,500
25.0%	quartile	5,000
10.0%		4,400
2.5%		3,843
0.5%		0,000
0.0%	minimum	0,000

Moments

Mean	5,5361243
Std Dev	1,1015058
Std Err Mean	0,0600921
upper 95% Mean	5,6543297
lower 95% Mean	5,417919
N	336

Table 3.3 a,b: General stats and Quantiles distribution of the Fom resistance trait among the introgressed progenies as output of the JMP software.

The Tukey test performed on all progenies revealed that most part of the progenies are not statistically divergent from the parental lines. However, few progenies can be highlighted as the best performing lines as they reached high levels of resistance ranging 8. (Highlighted in green in Table 3.4). By combining SPET genotypic data with the performance of these two lines, we may suggest that an

additional genetic trait derived from *S. tomentosum* could be associated with resistance to *Fom* lies on the lower part of chromosome 12, as both progenies display an introgression fragment in this region.

On the lower part of the table 3.4, many progenies accounted as worst performing ones (highlighted in red). After comparison of SPET data, these progenies showed to possess an introgressed fragment on chromosome 11. The position of the introgressed fragments carried by each line is colocalizing with the *CH11Fom* QTL position. All the considered progenies were segregating for the introgressed fragments, and the presence of completely susceptible plants to F*om* which died after inoculation with a segregation ratio fitting 1:3 could be explained by considering that an introgressed wild portion at homozygous level in this region (again, with a 1:3 segregation) could completely replace the *Fom* resistance trait carried by the line 67/3 in that region therefore leading to a loss of function of the candidate gene(s).

Level																															Mean
305E40 RR	A																														9,9333333
67si18 CH12-2A ⊗		B																													8,3000000
67SI18 CH9-4 ⊗		B	C																												7,8000000
67Si15_CHR11_1A BC			C	D																											7,0666667
67Si_14 CHR6-2C BC			C	D	E																										7,0166667
67SI18 CH11-3 BC			C	D	E	F																									7,0000000
67SI17 CH6 2A BC			C	D	E	F																									7,0000000
67SI17 CH10 4B BC			C	D	E	F	G																								6,9666667
67SI17 CH7 1A BC			C	D	E	F	G	H																							6,9333333
67SI17 CH9 2A BC			C	D	E	F	G	H	I																						6,8666667
67SI18 CH10-3 BC			C	D	E	F	G	H	I																						6,8666667
67SI17 CH9 1A BC				D	E	F	G	H	I	J																					6,7000000
67SI18 CH9-5 ⊗				D	E	F	G	H	I	J																					6,7000000
67Si18 CH6-3 ⊗				D	E	F	G	H	I	J	K																				6,6666667
67-SI18 CHR5-3 ⊗				D	E	F	G	H	I	J	K																				6,6333333
67Si18 CH7-3 BC				D	E	F	G	H	I	J	K	L																			6,5000000
67SI17 CH6 1B ⊗				D	E	F	G	H	I	J	K	L	M																		6,4333333
67Si18 CH6-4 ⊗				D	E	F	G	H	I	J	K	L	M	N																	6,3000000
67SI18 CH9-1 BC				D	E	F	G	H	I	J	K	L	M	N																	6,3000000
67-SI_15 CHR8-2° BC				D	E	F	G	H	I	J	K	L	M	N	O																6,2500000
67SI18 CH9-6 ⊗				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1333333
67SI17 CH5 3A BC				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1037037
67SI17 CH3 1B BC				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1000000
67SI17 CH2 5A BC				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1000000
67SI17 CH3 5A BC				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1000000
67SI17 CH4 2B BC				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1000000
67SI17 CH5 3B BC				D	E	F	G	H	I	J	K	L	M	N	O	P															6,1000000
67SI17 CH2 2A BC				D	E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0333333
67SI17 CH9 3B BC				D	E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0333333
67Si18 CH3-4 BC					E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0000000
67SI17 CH1 1B BC					E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0000000
67SI17 CH6 4B ⊗					E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0000000
67-SI_14 CHR8-1B ⊗					E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0000000
67SI17 CH5 1A BC					E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0000000
67SI17 CH1 2A BC					E	F	G	H	I	J	K	L	M	N	O	P	Q														6,0000000
67-SI_15 CHR8-3° ⊗					E	F	G	H	I	J	K	L	M	N	O	P	Q														5,9981481
67si18 CH12-2 BC						F	G	H	I	J	K	L	M	N	O	P	Q	R													5,9666667
67Si18 CH7-2 ⊗							G	H	I	J	K	L	M	N	O	P	Q	R	S												5,9333333
67-SI_14 CHR8-1A BC							G	H	I	J	K	L	M	N	O	P	Q	R	S												5,9333333
67SI17 CH3 2B ⊗								H	I	J	K	L	M	N	O	P	Q	R	S	T											5,9000000
67/3								H	I	J	K	L	M	N	O	P	Q	R	S	T											5,9000000
67SI17 CH7 1B ⊗									I	J	K	L	M	N	O	P	Q	R	S	T	U										5,8333333
67SI17 CH10 5B ⊗									I	J	K	L	M	N	O	P	Q	R	S	T	U										5,8333333
67Si18 CH1-2 BC										J	K	L	M	N	O	P	Q	R	S	T	U	V									5,8000000
67SI17 CH3 2A ⊗										J	K	L	M	N	O	P	Q	R	S	T	U	V									5,8000000
67SI17 CH3 1A ⊗										J	K	L	M	N	O	P	Q	R	S	T	U	V									5,8000000
67-SI18 CHR5-2 ⊗										J	K	L	M	N	O	P	Q	R	S	T	U	V									5,8000000
67SI18 CH10-3A ⊗										J	K	L	M	N	O	P	Q	R	S	T	U	V									5,8000000
67Si18 CH1-5 BC										J	K	L	M	N	O	P	Q	R	S	T	U	V									5,8000000
67-SI_15 CHR8-2A ⊗										J	K	L	M	N	O	P	Q	R	S	T	U	V	W								5,7666667
67Si18 CH5-1 BC										J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X							5,7333333
67-SI15_CHR5_3B ⊗											K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y						5,6533333
67Si15 CH5-3A ⊗											K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y						5,6240741
67Si18 CH3-1 ⊗												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z					5,5333333
67SI17 CH4 1D ⊗												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z					5,5333333
67SI18 CH11-1 BC												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z					5,5333333
67SI17 CH9 3C BC												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[				5,5000000
67Si18 CH7-1 ⊗												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,4666667
67SI17 CH10 2A BC												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,4666667
67Si18 CH1-6⊗													M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,4333333
67SI17 CH1 3B BC													M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,4000000
67SI18 CH9-6 BC													M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,4000000
67Si18 CH1-5 ⊗													M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,4000000
s.tomentosum														N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,3666667
67SI17 CH9 2B BC														N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,3666667
67Si18 CH4-6 ⊗														N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\			5,3333333
67SI17 CH11 1A BC															O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]		5,2333333
67SI17 CH8 1A BC															O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]		5,2333333
67SI17 CH5 2B ⊗																P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	5,2000000
67SI18 CH9-8 BC																P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	5,2000000
67-SI15_CHR5-2° ⊗																P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	5,2000000
67Si16 CH9-1° BC																P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	5,1666667
67SI17 CH2 3A BC																P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	5,1333333

Level																																Mean
67Si18 CH6-1 BC															P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_					5,1333333
67SI17 CH4 2A BC																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0333333
67si18 CH12-8 ⊗																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0333333
67SI17 CH4 1C BC																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0333333
67SI17 CH4 1C ⊗																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0000000
67Si18 CH4-4 ⊗																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0000000
67SI17 CH1 1A BC																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0000000
67SI18 CH11-4 BC																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0000000
67Si18 CH2-4 BC																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0000000
67SI17 CH1 2A ⊗																Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				5,0000000
67SI18 CH9-3 ⊗																	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				4,9333333
67Si15 CH2-3B ⊗																		S	T	U	V	W	X	Y	Z	[	\	]	^	_	`				4,9166667
67SI18 CH10-2 ⊗																			T	U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8666667
67Si18 CH1-1 ⊗																			T	U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8666667
67SI17 CH4 3B BC																				U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8333333
67Si18 CH4-1 BC																				U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8333333
67-SI15_CHR5-3B ⊗																				U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8166667
67Si15_CHR11_1B ⊗																				U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8000000
67SI17 CH10 5B BC																				U	V	W	X	Y	Z	[	\	]	^	_	`	a			4,8000000
67Si18 CH4-5 ⊗																					V	W	X	Y	Z	[	\	]	^	_	`	a			4,7666667
67SI18 CH9-9 ⊗																					V	W	X	Y	Z	[	\	]	^	_	`	a			4,7666667
67Si18 CH3-2 ⊗																					V	W	X	Y	Z	[	\	]	^	_	`	a			4,7666667
67Si18 CH4-3 ⊗																						W	X	Y	Z	[	\	]	^	_	`	a			4,7333333
67SI17 CH9 1B BC																							X	Y	Z	[	\	]	^	_	`	a			4,7000000
67SI18 CH10-1 BC																								Y	Z	[	\	]	^	_	`	a			4,6666667
67SI17 CH12 3A BC																									Z	[	\	]	^	_	`	a	b		4,5666667
67Si18 CH2-7 BC																									Z	[	\	]	^	_	`	a	b		4,5000000
67SI17 CH11 1B BC																									Z	[	\	]	^	_	`	a	b		4,5000000
67Si18 CH2-5 BC																									Z	[	\	]	^	_	`	a	b		4,5000000
67SI17 CH3 3A ⊗																										[	\	]	^	_	`	a	b		4,4666667
67SI17 CH3 3B ⊗																											\	]	^	_	`	a	b		4,4333333
67Si18 CH2-1 BC																												]	^	_	`	a	b		4,2000000
67SI18 CH11-2 ⊗																													^	_	`	a	b		4,1666667
67Si15_CHR11_1A ⊗																														_	`	a	b		4,1000000
67SI18 CH11-2 BC																															`	a	b		4,0333333
67SI17 CH11 1B ⊗																																a	b		3,8666667
67Si_15 CHR6-1B BC																																a	b		3,8333333
67SI17 CH3 4A ⊗																																	b		3,5666667
DR2 ss CTR																																		c	0,0000000

Table 3.4 Outcomes of resistance score of the IL progenies, S. tomentosum (donor) and S. melongena line '67/3' (recipient). Rows with at least a common letter are not significantly different according to Tukey test (p=0.05)

3.4 Conclusion

As a general consideration, these progenies are a good starting point for characterization of the *Fom* resistance/tolerant trait carried by *S. tomentosum*, as the effect of this trait is frequently masked by the one carried by 67/3. Moreover, great part of the progenies still revealed the presence of several introgression fragments at heterozygous level, whose segregation could have led to less accurate evaluation of symptoms and, consequently, to an underestimation of the best performing progenies. More solid data will be gathered when stable introgression lines will be available. Finally, a detailed characterization of the regions underlying the introgressed fragments on chromosome 11 may allow a finer mapping of the resistance locus carried by 67/3. In addition, the large collection of eggplant genotypes has demonstrated the wide variability in pathogen response, leading to increased curiosity for future investigations into these sources of resistance, as well as their simultaneous use in genetic breeding programmes.

Chapter 4

Nitrogen fertilization and response to soil-borne fungal diseases

4.1 Introduction

Mineral nutrition and the different fertiliser forms can affect the development of different pathogens. The mechanisms leading to these nutrient forms induced changes in disease development are complex, including effects of mineral nutrients directly on the pathogen, plant growth and development, as well as plant resistance mechanisms. Mineral nutrition has long been recognised as an important component of disease control practices (Huber & Wilhelm, 1988); but so far, these mechanisms are not well understood, considering that mineral nutrition has not been fully exploited in disease control (Walters & Bingham, 2007).

Nitrogen (N) is a key element for plant metabolism, it is adsorbed by plants from the soil as nitrate or ammonium; in addition, legumes are also able to fix N *via* bacteria symbiosis (Lea & Azevedo, 2006; Lea et al., 2007). Nitrate taken up by the plant is firstly converted into ammonia by nitrate and nitrite reductases before amino acid biosynthesis can occur (Lea & Azevedo, 2006). In higher plants, the major route for amino acid biosynthesis is the glutamine synthetase–glutamate synthase pathway, which converts ammonia into glutamine. Finally, a range of amino acids and amides are formed, including glutamine, glutamate, asparagine and aspartate, which represent the major N-carrying molecules in higher plants (Lam et al., 1996).

Depending on plant species, nitrate reduction can take place in the root, shoot or both (Marschner, 1995; Camargos et al., 2006). Therefore, an invading pathogen will encounter different N forms in both the apoplast and symplast of plant tissues, ranging from organic N-like glutamine through to inorganic N-like nitrate.

Once the pathogen entered into the plant, the different plant responses to the disease development depend on the pathogen type, N forms, supply and application time, but also on the host plant, in particular the organ and tissue attacked (Hoffland et al., 2000; Lecompte et al., 2010; Fagard et al., 2014). Likewise, available N sources also depend on the mode of pathogen nutrition: pathogens that kill host tissues access to a wider range of N sources than biotrophic pathogens, restricted to N available in the apoplast or the haustorial matrix (Farrar et al., 1995).

Nitrogen (N) has long been demonstrated to influence plant-microbe interactions and plant disease development (Walters et al., 2007; Fagar, et al, 2014). It is well known that different N forms can affect the physiological process of plants, such as photosynthetic rate (Horchani et al., 2010), enzyme activity (Engelsberger et al., 2012), respiration rate (Guo et al., 2007), signaling pathway (Patterson

et al, 2010), and water balance (Yang et al., 2012), thus affecting plant diseases. Moreover, different N forms could also regulate disease tolerance by influencing signals controlling virulence factor activation than pathogen metabolic adaptation (Solomon et al., 2003, López-Berges et al., 2010). Several studies have shown that a high N rates supply could decrease plant resistance to diseases (Stout et al., 1998) by increasing nutrients for pathogen development (Jensen et al., 1997; Neumann et al., 2004; Walters et al., 2007). Otherwise, N limitation appear to be a key signal for triggering the expression of virulence genes in plant pathogens, thus influencing plant diseases (Snoeijers et al., 2000); therefore, N supply levels seem also related to host resistance.

However, a decreased plant resistance to diseases was observed with a lower N supply (Hoffland et al., 1999) resulted from a higher C/N ratio and lower N compounds (e.g., protein), which are involved in plant resistance to disease (Dietrich et al., 2004).

Furthermore, NO_3^--fed cucumber plants were reported more tolerant to *Fusarium* wilt compared to NH_4^+-fed plants; the disease index decreased as NO_3^- supply increased by contrast increased when NH_4^+ was the main N form (Zhou et al., 2017).

In the present study, to identify the phenotypic effects and the molecular changes caused by N forms supply in response to *Fusarium oxysporum* f. sp. *melongenae* on eggplant tolerant genotype, an assay for disease index under NO_3^- or NH_4^+ together with a RNAseq experiment were conducted.

4.2 Materials and methods

4.2.1 Plant material

Two eggplant (*Solanum melongena* L.) accessions, C45 and 67/3, were selected from a large germplasm collection available at CREA-GB which include genotypes characterized by a wide variability for fruit size, shape, colour and plant growth habit, as well as different levels of tolerance to telluric pathogens including *Fusarium* wilt. In detail, four tolerant genotypes to *Fusarium oxysporum* (67/3, C22, C40, C45), which respond to the fungal attack with a symptomatology ranging from 0.4 to 0.6 on our scale of symptoms described above (Figure 1.1) were tested under different N supply (from 6 to 250 units per NO_3^- and NH_4^+ form). The nitrogen fertilization was carried out on these eggplant genotypes, until identifying C45 and 67/3 as those that showed a higher different behaviour following nitrogen treatment in combination with artificial inoculation with *Fusarium.*

4.2.2 Growth conditions and fertilization

The trial was carried out in growth chamber with a day/night temperature of 27/20 ± 2°C and 65% relative humidity. The seedlings were sown on peat substrate in 104-hole trays. When the second true

leaf was reached, they were transferred into 54-hole trays 80 cm^3 volume, filled with sand: peat 3:1 (v/v). Then, ammonium and nitrate fertilization started, by dissolving ammonium bicarbonate $(NH_4)_2SO_4$ and calcium nitrate tetrahydrate $Ca(NO_3)_2$ in water, respectively. N supply was divided into 5 treatments of increasing concentration. In detail, the total input of 20 nitrogen units was divided into 5 doses of 10%, 15%, 20%, 25% and 30% total rate, respectively. Fertilisation was carried out every 7 days, pouring 8ml of solution per seedling with the help of an electronic pipette controller using plastic pipettes.

At least 5 days after the second treatment, and thus when 25% of the total planned nitrogen units have been supplied, the plants at the 2-3 true leaf stage were inoculated with *Fom* or mock-inoculated with water and placed again in 54-hole trays filled with the same substrate [sand: peat 3:1 (v/v)].

4.2.3 Pathogen Incubation and Infection

The *Fusarium oxysporum* f. sp. *melongenae* isolate is available at CREA-GB. The *Fom* isolate was first incubated on potato dextrose agar medium (PDA) in Petri dishes at 28 °C in the dark for 7 days. Then, cubes of this agar containing fungus mycelium were removed from the culture margins and inoculated into 1-liter Erlenmeyer flasks containing Czapek's medium. The Erlenmeyer flasks were incubated at 28 °C for 4 days with orbital shaker. The resulting cultures were filtered through gauze to remove mycelium fragments. Then, the conidia were counted using a Bürker Counting Chambers, and resuspended in sterile water until a concentration of 1.5 x 10^6 conidia/ml was reached.

Plantlets at 2–3^{th} true leaf stage, grown in peat in 104 holes plastic trays, were gently removed, the roots washed under tap running water and, immersed in the suspension of *Fom* conidia for 10 min. Sterile distilled water was used instead the conidial suspension in the control plantlets. After inoculation, the plantlets were gently dried and transferred to 54-hole plastic trays filled with sand: peat 3:1 (v/v) and placed in growth chamber.

4.2.4 Determination of the Disease Index

Throughout the period, plants were inspected for wilt symptoms weekly for up to 4 weeks after inoculation. According to the method described by Barchi et al. (2019), the disease index of infected plants was rated on a scale 0–1 (Figure 1.1).

The disease index was calculated according to the formula already reported in Chapter 2.

$$R = \frac{\sum (\text{plant} * \text{score assigned})}{\text{total n}^{\circ}\ \text{of inoculated plants}}$$

4.2.5 RNA extraction and library prep

Roots of selected genotypes were collected at 0, 4 hours, and 15 days after *Fom* inoculation. Fifteen samples per genotype were collected (three independent biological replicates for each sample, where each replicate was a pool of three plants) and immediately frozen in liquid nitrogen. Total RNA was extracted and purified from 20mg of grinded not-fibrous root tissue using ReliaPrep™ RNA Tissue Miniprep System (www.promega.com) following the technical manual (TM394) for Illumina (IL) library preparations. The protocol included an incubation step with DNase I enzyme, the amount and purity of RNA were measured in agarose gel (Figure 4.1) and by using Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometers.

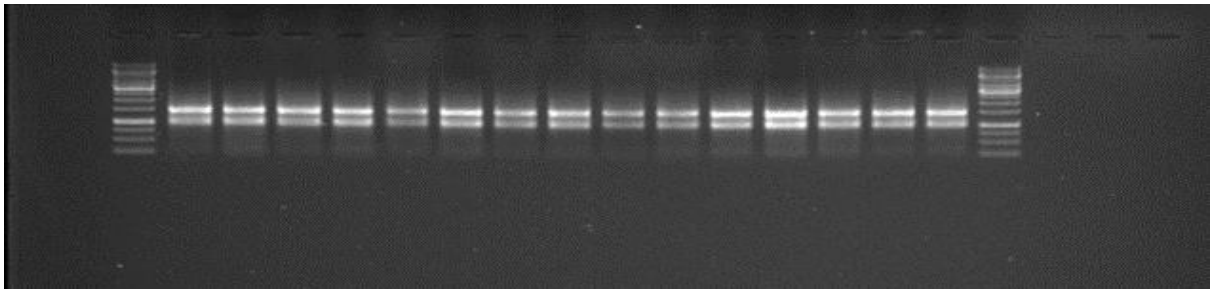
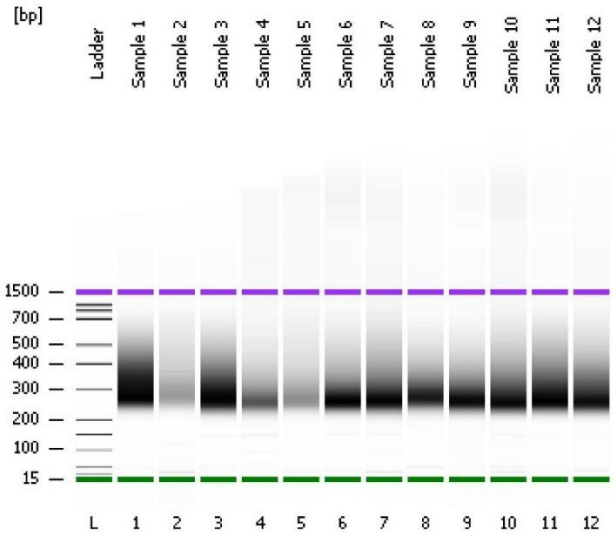


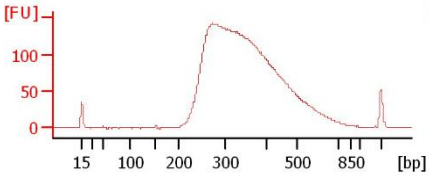
Figure 4.1 Quality control of RNA sample by agarose gel electrophoresis

In addition, RNA quality by using an Agilent 2100 bioanalyzer that allows the calculation of an RNA integrity number (RIN) was checked.

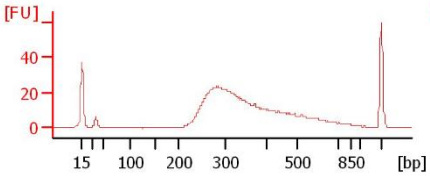
Library prep was performed using Illumina® Stranded mRNA Prep, Ligation kit (www.illumina.com) that converts the messenger (mRNA) in total RNA into up to 384 dual-indexed libraries. Oligo(dT) magnetic beads purify and capture the mRNA molecules containing polyA tails. The purified mRNA is fragmented and copied into first strand complementary DNA (cDNA) using reverse transcriptase and random primers. In the second strand cDNA synthesis, dUTP replaces dTTP to achieve strand specificity. The final steps add adenine (A) and thymine (T) to fragment ends and ligate the adapters. The resulting products are purified, checked with an Agilent 2100 bioanalyzer (figure 4.2) and Qubit fluorometer, and selectively amplified for sequencing on Illumina NovaSeq6000 Sequencing System through a S4 flow cell.



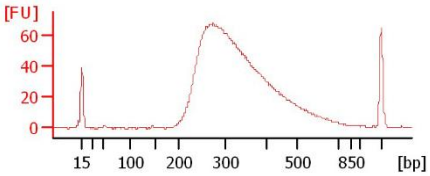
Sample 1



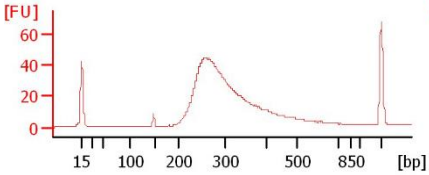
Sample 2



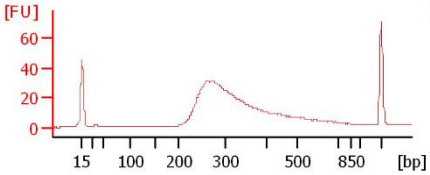
Sample 3



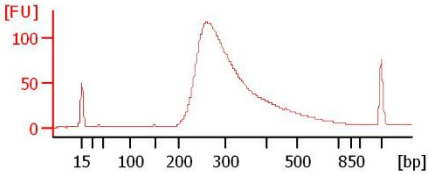
Sample 4



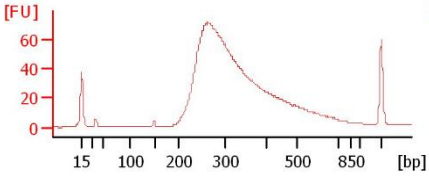
Sample 5



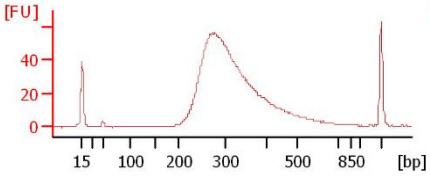
Sample 6



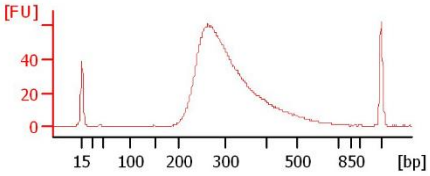
Sample 7



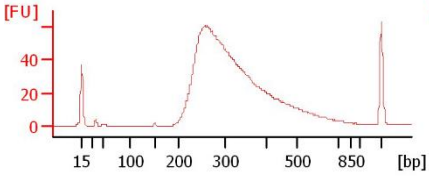
Sample 8



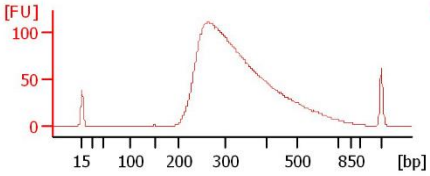
Sample 9



Sample 10



Sample 11



Sample 12

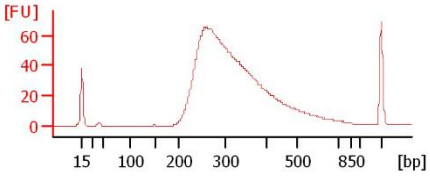


Figure 4.2 Electrophoresis File Run Summary

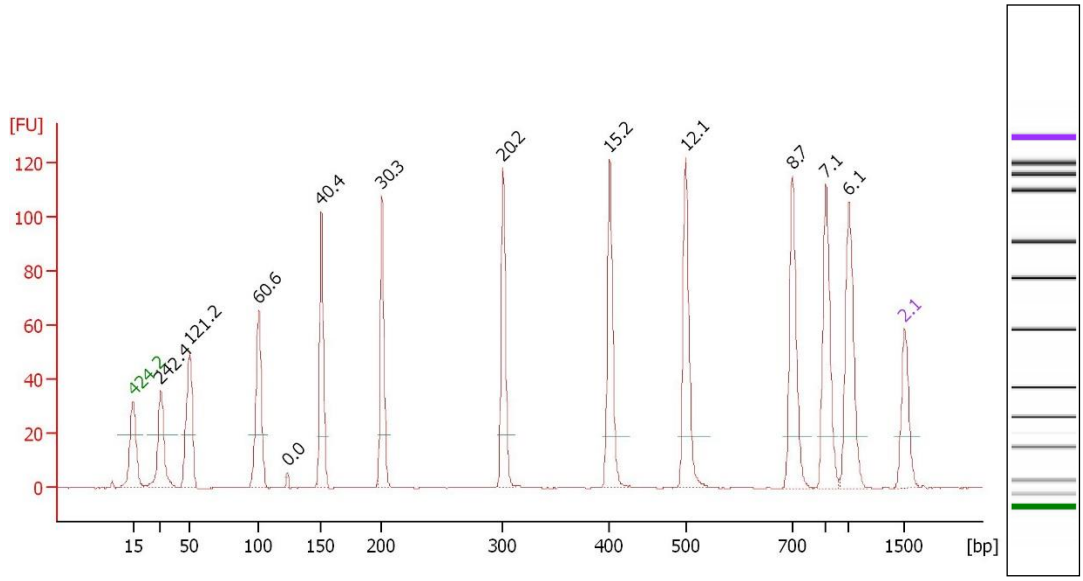


Figure 4.3 Electropherogram Summary

Peak	Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
1	15	4,20	424,2	Lower Marker
2	25	4,00	242,4	Ladder Peak
3	50	4,00	121,2	Ladder Peak
4	100	4,00	60,6	Ladder Peak
5	123	0,00	0,0	
6	150	4,00	40,4	Ladder Peak
7	200	4,00	30,3	Ladder Peak
8	300	4,00	20,2	Ladder Peak
9	400	4,00	15,2	Ladder Peak
10	500	4,00	12,1	Ladder Peak
11	700	4,00	8,7	Ladder Peak
12	850	4,00	7,1	Ladder Peak
13	1.000	4,00	6,1	Ladder Peak
14	1.500	2,10	2,1	Upper Marker

Table 4.1 Peak table for Ladder

4.2.6 Bioinformatics analysis RNA-Seq gene expression analysis

RNA-Seq paired-end libraries through Illumina Stranded mRNA Prep protocol, according to manufacturer's instructions were produced and sequenced through NovaSeq 6000 S4 by AMES Group SRL. The resulting high-quality reads were quality-checked through FASTQC (Andrews, 2010) sequences were demultiplexed through bcl2fastq, removing the adapters during the same process. The tool Trimmomatic version 0.39 (Bolger et al., 2014) was finally used to trim reads, clipping the first nucleotide, characterized by a very low quality in all the libraries. The final output of this pipeline is a pair of "FASTQ" files, very large in size, for each sample.

The libraries were then aligned to the novel eggplant reference genome, available at the Solanaceae web portal Solgenomics.net (https://www.solgenomics.net), and the latest 4.1 version recently released was used (Barchi et al., 2021). Reads mapping was performed through the function Rsubread in the Bioconductor R package (Liao et al., 2019), allowing a maximum of 7 mismatches and multiple

alignments, and reads counts were assigned to genetic features through featureCounts. The annotation file.gff3 containing gene features identities and coordinates was also downloaded from Solgenomics.net. BAM alignment files, obtaining a large count matrix.

Gene expression analysis was carried out through a quasi-likelihood edgeR pipeline (Robinson et al., 2010; Chen et al., 2016) and a DESeq2 workflow (Love et al., 2014) to compare and validate results using two statistical methods. However, both methods rely on a negative binomial model, are considered a golden standard approach for RNA-Seq analysis. Counts were filtered by expression, excluding genes with a value lower than 10 (in edgeR) or a sum of counts < 10 (in DESeq2). Normalization was performed through TMM (Trimmed Mean of M Values) in the edgeR pipeline, and DESeq2 in-built normalization methods. A Multidimensional scale Analysis (MDS), similar to a Principal Component Analysis (PCA), was performed and then plotted through the R Bioconductor function *plotMDS*, designed for microarray/RNA-Seq data.

Twenty-five pairwise comparisons were performed and the Differentially Expressed Genes (DEGs) showing a p-value < 0.01, a Fold Change (FC) > 2 or < -2 and a False Discovery Rate (FDR) (edgeR) / p-adjusted (DESeq2) value < 0.05 for each comparison were considered significant. DEGs analysis was performed through the glmQLFTest and makeContrasts functions in the edgeR quasi-likelihood pipeline, and DESeq and results in the DESeq2 pipeline. This filtering allowed us to focus on biologically relevant differences in gene expression by a significant reduction of false positive rate among samples, in order to estimate the genes subjected to expression change after exposure to different treatments. However, it is likely expected that genes responsible for phenotypical differences in disease resistance are constitutive, and thus not differentially expressed, but DEG analysis could identify the down-stream activated transcription factors and genes involved in the activated cellular changes, such as secondary and cell wall metabolism.

GO enrichment analysis and WGCNA are still ongoing, preliminary results are here reported but they will be analysed in more detail in future experiments. A Gene Ontology (GO) enrichment analysis was performed through the R package ‘topGO’, with the aim to identify over-represented GO terms within pairwise comparisons. The default method ‘weight01’, particularly balanced by reducing the redundant terms within GO hierarchy and false positive rate, was chosen. A Weighted Gene Co-expression Network Analysis (WGCNA) was carried out as well, through the homonymous R package. The input was a matrix of all the normalized expression levels of DEGs from the pairwise comparisons. Relevant DEGs modules, positively or negatively correlated to the experimental variables (time sampling, genotype, *Fom* inoculation and N treatment) were detected and merged to reduce the number, a heatmap was then depicted.

Finally, the modules correlated only to time sampling were excluded ($p < 0.05$), the resulting 14 modules networks were visually plotted by the software Cytoscape v.3.9.1, hub genes (showing the highest degree) were identified and, in such cases, their first neighbours in the network were plotted as well.

4.3 Result

Nitrogen play an important role in plant responses to disease being affected by N fertilization management and N-form employed. In cucumber, NO_3^- supply was related to the improvement of plant defence response, decreasing the disease index, while NH_4^+ determine a reduction of resistance response, increasing the disease index (Zhou et al, 2017; Wang et al, 2018). These results was confirmed in our trial, where eggplants infected with *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) differed significantly in their disease symptoms 15 days after *Fom*-inoculation.

4.3.1 Nitrogen fertilization and response to soil-borne fungal diseases

The effects of different N forms supply in the resistance to the vascular fungal attacks *Fusarium oxysporum* f. sp. *melongenae* (*Fom*), based on the contrasting behaviour of eggplant genotypes grown under low N supply, have been investigated. In detail, the phenotypic and molecular responses of the *Fom*-tolerant eggplant lines 67/3 and C45 to *Fom*-inoculation supplied with different NO_3^- or NH_4^+ were evaluated.

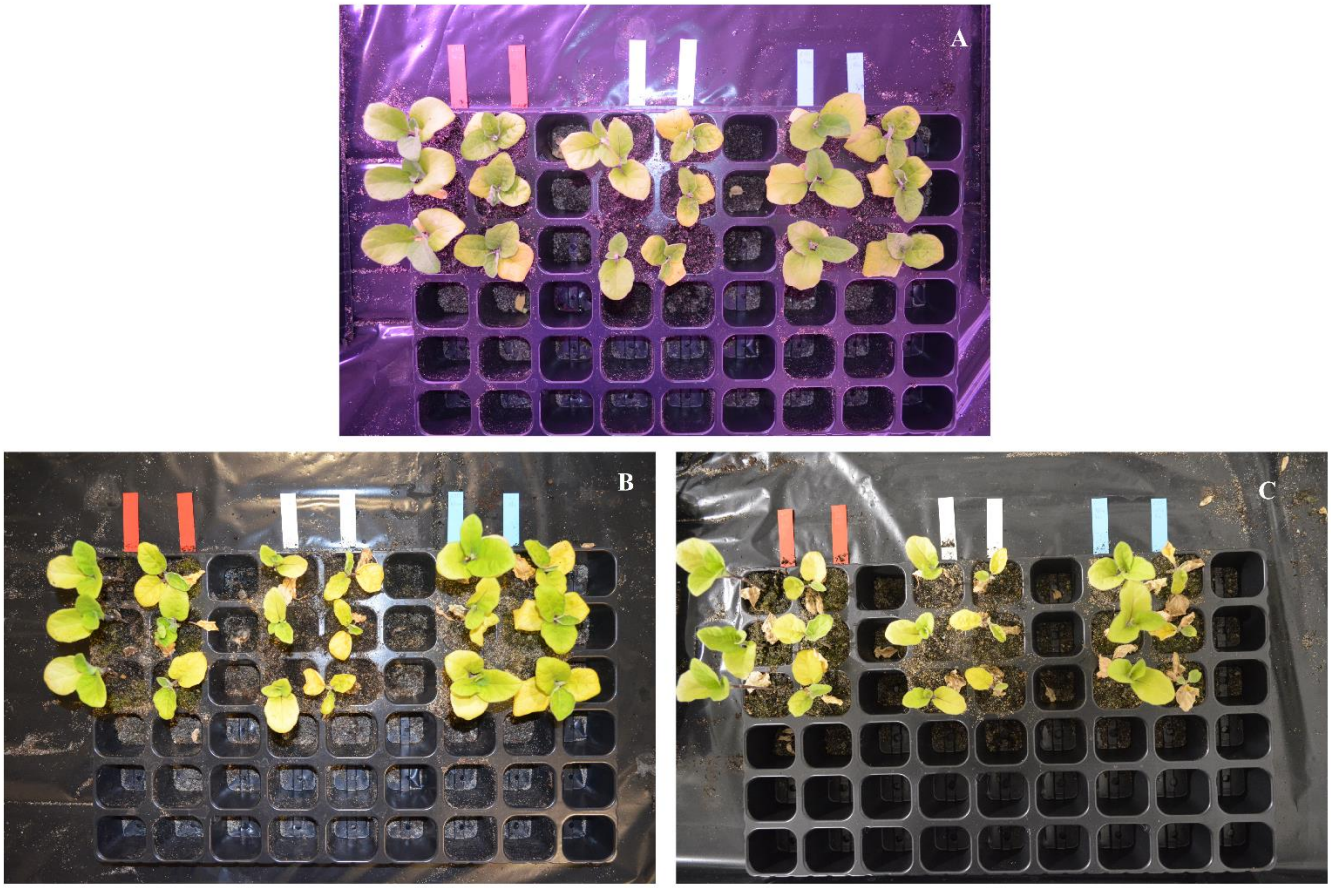


Figure 4.4 Monitoring of infection symptoms: -A: Plants just before artificial inoculation with Fom; -B: Mock-inoculated plants at 15 days; -C: Plants at 15 days after inoculation with conidial solution. From left to right in the plastic trays: 67/3, C45, fertilized with NO_3^- ; 67/3, C45 not fertilized; 67/3, C45, fertilized with NH_4^+

67/3 line did not show different responses between N forms; only the control plants that have not been N-fed showed severe symptoms. In genotype C45, the first symptoms such as discolouration and leaf drop start to appear after the first 7 DAI. At 14 DAI, a significant contrasting responses between N forms was observed; NO_3^--fed plants show mild symptoms compared to NH_4^+-fed plants, which are more severe than unfertilised plants. At 21 DAI a gradual recovery of the NH_4^+-fed plants started with the production of new small deep green leaves.

A higher leaf loss in C45 than 67/3 was observed, particularly when fertilised with ammonium, while NO_3^--fed plants shows a smaller drop, differing slightly from unfertilised plants.

Plant height, frequently reduced following a fungus infection, was also monitored. The impact of the infection on plant growth and height was evident in 67/3 fed with NO_3^- compared to NH_4^+, reduced growth was also observed in the unfertilised plants (control). Otherwise, C45 height was firstly higher in the control and NH_4^+ compared to NO_3^--fed plants, during the 7-21 DAI interval the plant height became similar among treatments, with a significant increase in the last week of monitoring.

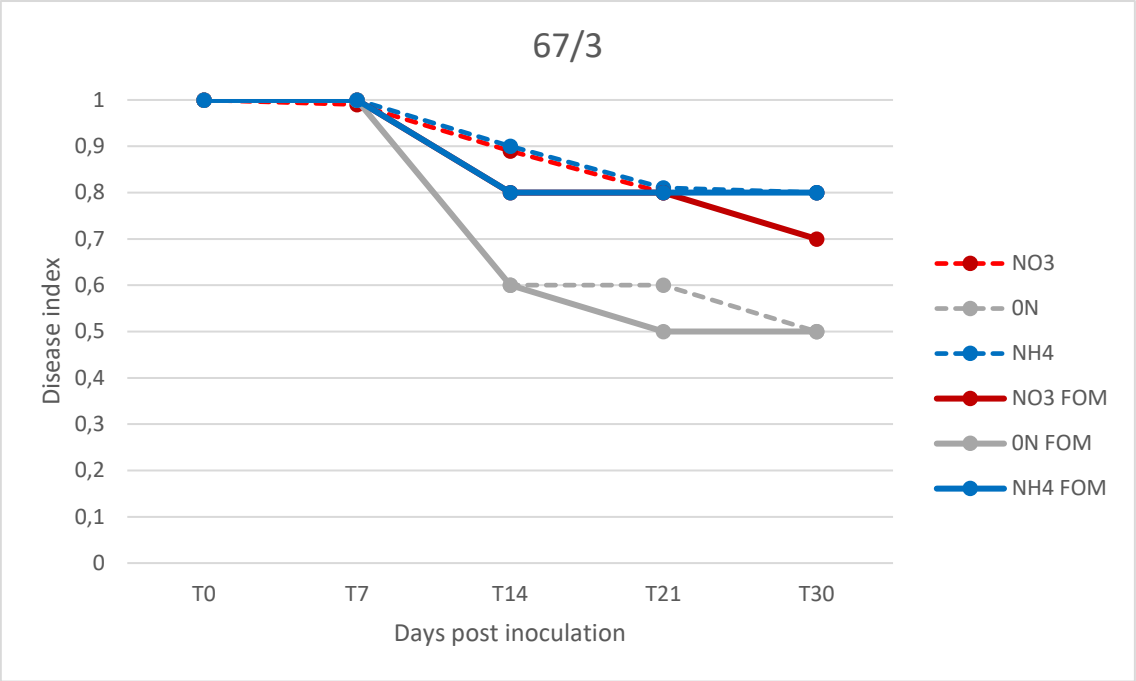


Figure 4.5 a: ease index of Fusarium wilt of eggplant plants -67/3 line- caused by Fusarium oxysporum f. sp. melongenae with different nitrogen sources. The disease index was calculated from 7 to 30 days post inoculation. Experiments were repeated more than three times with similar results.

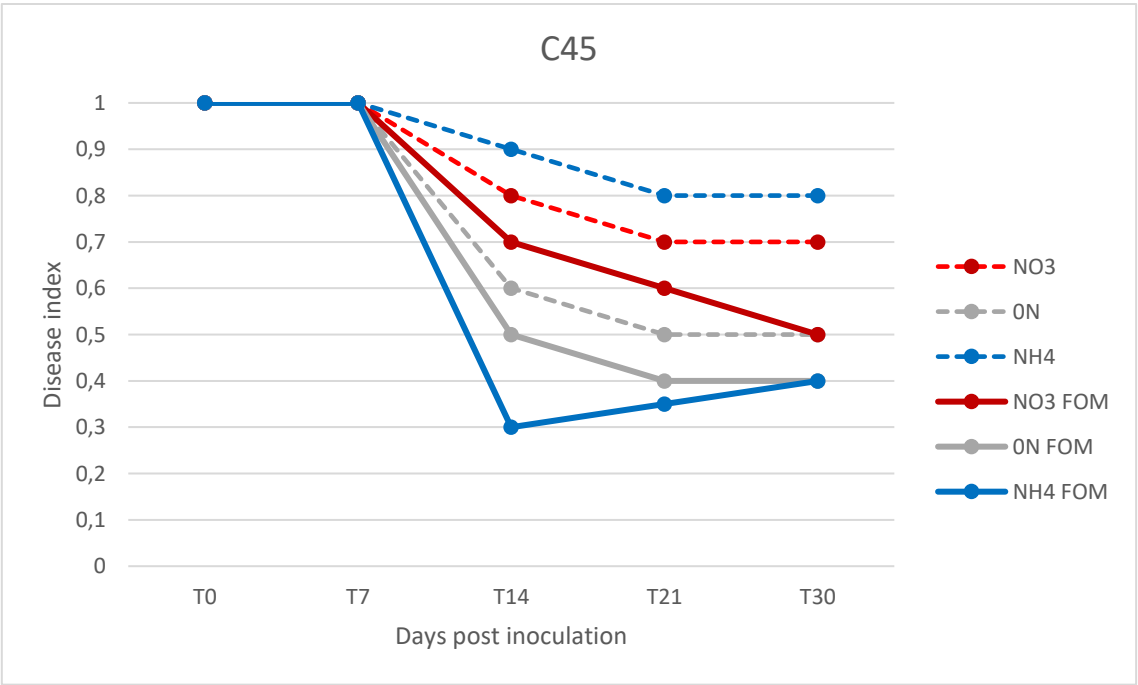


Figure 4.5 b: ease index of Fusarium wilt of eggplant plants -C45- caused by Fusarium oxysporum f. sp. melongenae with different nitrogen sources. The disease index was calculated from 7 to 30 days post inoculation. Experiments were repeated more than three times with similar results.

4.3.2 PCA plotting and samples gene counts analysis

DEGs analysis was performed and used for a PCA analysis in order to analyze the distinctness of treatments and the biological replicates.

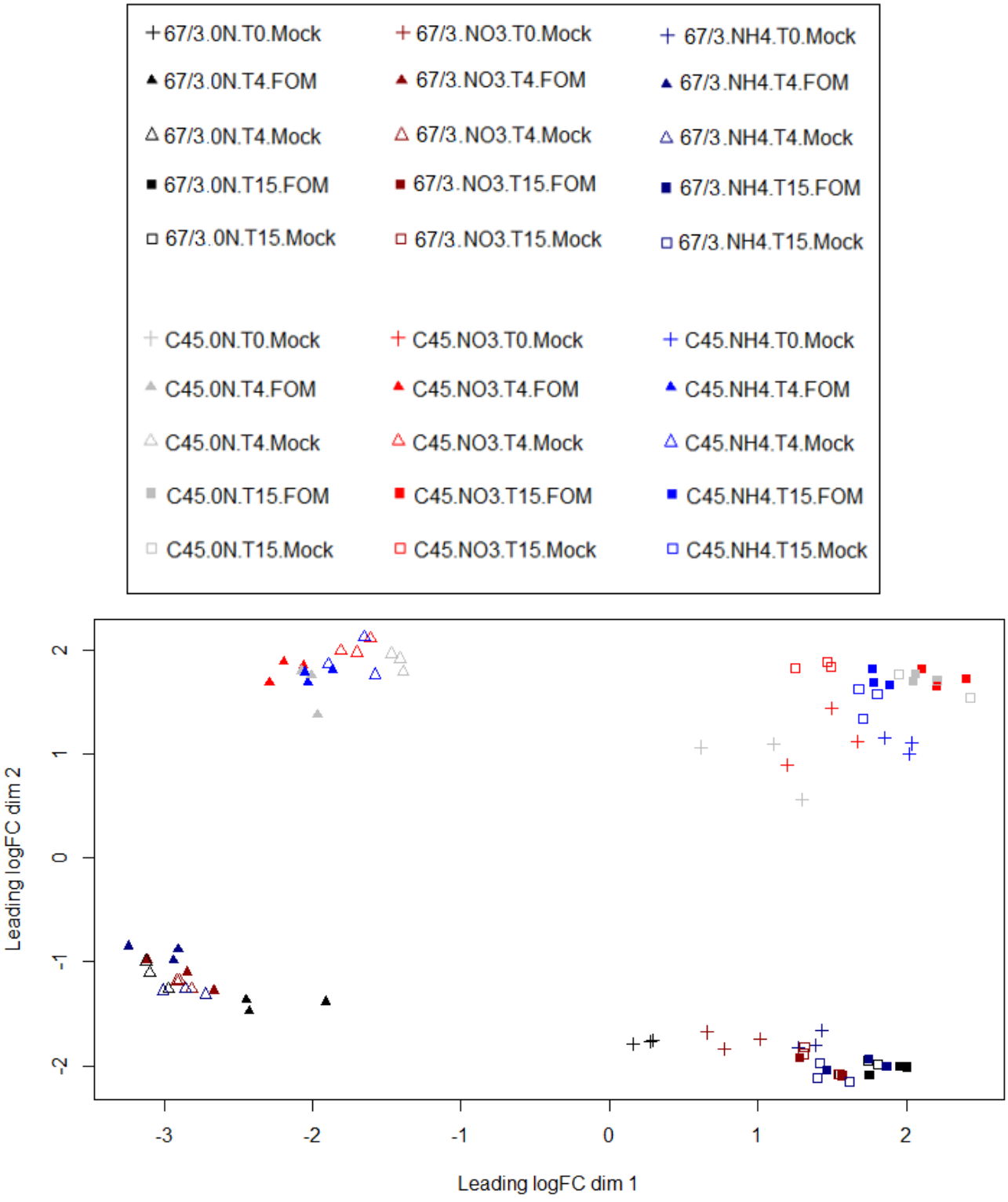


Figure 4.6 Principal component (PC) analysis of gene expression in roots of two eggplant genotypes artificially inoculated with the Fusarium fungus, at different N-fed.

In Figure 4.6, the PCA was presented, and each variable described as follows: - Different colour distinguish among N treatments: grey/black for 0N, red/burgundy for NO_3^- and blue/navy for NH_4^+.

- Darker shades were chosen for 67/3, 0N (black), NO_3^- (burgundy) and NH_4^+ (navy), respectively, while lighter (grey, bright red and electric blue) were picked for C45 samples subjected to the same treatments, respectively.

- Full symbols represent *Fom*-inoculated samples, while empty ones are Mock libraries.

- T0 samples are represented by a plus symbol, T4 and T15 by triangle and square, respectively.

The Principal Component Analysis (dim 2) discriminates between genotypes, C45 and 67/3 samples appearing distinct (four major clusters) in the upper (C45) and lower (67/3) panels (left part), respectively. As a whole, all the biological replicates behave coherently and always clustered together. All the T4 samples (triangles) appeared in the left panel of the plot, regardless N and *Fom* treatments. Interestingly, both *Fom* and Mock NO_3^- and NH_4^+ samples appeared shifted on the left when compared to 0N. A similar behaviour can be observed in the other two clusters of the plot, on the right, with 0N samples appearing in the middle of the plot as a whole.

Surprisingly, T15 and T0 samples appeared in the same group for both lines, again, regardless N and *Fom* treatments (right part). However, T4 samples seemed to behave differently and were distinct by dim 2.

4.3.3 Pairwise comparisons in DEGs analysis

The complex experimental design included 4 different variables: the genotype (C45 and 67/3), N treatment (NO_3^-, NH_4^+ and the untreated control - 0N), pathogen treatment (*Fom* and Mock control) and time samplings (T0, T4 and T15). Thirty different conditions were obtained by combining the variables, taking into account that T0 samples make no distinction between *Fom* inoculation and Mock control, and this particular classification only applies to T4 and T15 samples.

Particularly, we chose to focus on twenty-five comparisons which were able to identify DEGs, according to the filtering criteria for both edgeR and DESeq2 statistics, summarized in Table 4.2. DESeq2 statistics (for the chosen threshold), was able to identify a higher number of DEGs. Remarkably, the order of magnitude of DEGs appeared coherent in all cases and also for the same conditions between genotypes. Moreover, the DEGs analysis by pairwise comparisons was integrated by VENN diagrams to identify distinctive genes shared by different pairwise comparisons.

Ten thousand five hundred and four (10,504) and 10,928 DEGs (out of 34,916 annotated eggplant genes) was found according to edgeR and DESeq2 pipeline results, respectively.

Pairwise comparisons were organised as follows:

Within the same genotype:

- Pairs 1 and 2 compared NH_4^+ *Fom* to its control NH_4^+ Mock samples at T4 and T15, respectively, on C45. Similarly, pairs 9 and 10 focused on 67/3.
- Pairs 3 and 4 compared NO_3^- *Fom* to its control NO_3^- Mock samples at T4 and T15, respectively, on C45. Similarly, pairs 11 and 12 focused on 67/3.
- Pairs 5 and 6 compared 0N *Fom* to its control 0N Mock samples at T4 and T15, respectively, on C45. Similarly, pairs 13 and 14 focused on 67/3.
- Pair 7 compared NH_4^+ T15 Mock to the control 0N T15 Mock on C45. Similarly, 15 does the same on 67/3.
- Pair 8 compare NO_3^- T15 Mock to the control 0N T15 Mock on C45. Similarly, 16 does the same on 67/3.

Between the genotypes:

- Pairs 17 and 18 compared 67/3 NH_4^+ *Fom* to C45 NH_4^+ *Fom* samples, at T4 and T15, respectively.
- Pairs 20 and 21 compared 67/3 NO_3^- *Fom* to C45 NO_3^- *Fom* samples, at T4 and T15, respectively.
- Pairs 23 and 24 compared 67/3 0N *Fom* to C45 0N *Fom* samples, at T4 and T15, respectively.
- Pair 19 compared 67/3 and C45 NH_4^+ T15 Mock samples.
- Pair 22 compared 67/3 and C45 NO_3^- T15 Mock samples.
- Pair 25 compared 67/3 and C45 0N T15 Mock samples.

In detail, the second list of pairwise comparisons will help to highlight key differences in gene expression during the disease progression stages between genotypes.

Pairwise comparisons	edgeR	DESEQ2

Pair	**1**	C45 NH4+ T4 FOM	**vs**	C45 NH4+ T4 Mock	797	889
Pair	**2**	C45 NH4+ T15 FOM	**vs**	C45 NH4+ T15 Mock	521	523
Pair	**3**	C45 NO3- T4 FOM	**vs**	C45 NO3- T4 Mock	1169	1172
Pair	**4**	C45 NO3- T15 FOM	**vs**	C45 NO3- T15 Mock	3165	3175
Pair	**5**	C45 0N T4 FOM	**vs**	C45 0N T4 Mock	2515	2595
Pair	**6**	C45 0N T15 FOM	**vs**	C45 0N T15 Mock	1180	1171
Pair	**7**	C45 NH4+ T15 Mock	**vs**	C45 0N T15 Mock	3068	3087
Pair	**8**	C45 NO3- T15 Mock	**vs**	C45 0N T15 Mock	2966	2954
Pair	**9**	67/3 NH4+ T4 FOM	**vs**	67/3 NH4+ T4 Mock	872	948
Pair	**10**	67/3 NH4+ T15 FOM	**vs**	67/3 NH4+ T15 Mock	1133	1126
Pair	**11**	67/3 NO3- T4 FOM	**vs**	67/3 NO3- T4 Mock	942	957
Pair	**12**	67/3 NO3- T15 FOM	**vs**	67/3 NO3- T15 Mock	255	283
Pair	**13**	67/3 0N T4 FOM	**vs**	67/3 0N T4 Mock	1169	1203
Pair	**14**	67/3 0N T15 FOM	**vs**	67/3 0N T15 Mock	28	52
Pair	**15**	67/3 NH4+ T15 Mock	**vs**	67/3 0N T15 Mock	1927	1894
Pair	**16**	67/3 NO3- T15 Mock	**vs**	67/3 0N T15 Mock	747	779
Pair	**17**	67/3 NH4+ T4 FOM	**vs**	C45 NH4+ T4 FOM	2340	2414
Pair	**18**	67/3 NH4+ T15 FOM	**vs**	C45 NH4+ T15 FOM	2394	2468
Pair	**19**	67/3 NH4+ T15 Mock	**vs**	C45 NH4+ T15 Mock	2176	2232
Pair	**20**	67/3 NO3- T4 FOM	**vs**	C45 NO3- T4 FOM	2818	2979
Pair	**21**	67/3 NO3- T15 FOM	**vs**	C45 NO3- T15 FOM	2424	2474
Pair	**22**	67/3 NO3- T15 Mock	**vs**	C45 NO3- T15 Mock	3018	3046
Pair	**23**	67/3 0N T4 FOM	**vs**	C45 0N T4 FOM	3203	3287
Pair	**24**	67/3 0N T15 FOM	**vs**	C45 0N T15 FOM	3439	3528
Pair	**25**	67/3 0N T15 Mock	**vs**	C45 0N T15 Mock	2340	2435

Table 4.2 The 25 DEGs comparisons among sample conditions and the number of filtered (FC < -2 or FC > 2, FDR < 0.05 and p-value < 0.01) resulting from the two edgeR and DESeq2 methods.

4.3.4 Candidate resistance genes behaviour

As discussed here in Chapter 1 (section 1.5), a list of 11 candidate resistance genes could be identified on chromosome 11. To check their gene expression in our experimental design and to assign a genomic region to each gene, the latest 67/3 genome release was chosen as reference (Barchi et al., 2021). In the other sections of this PhD thesis, we referred to the genes found in the version 3 of this nomenclature. Barchi et al. research team also released a table showing the correspondence between the releases, here, we have used the version 3 names.

To further validate the candidate genes, and to confirm their presence in a recent genes annotation, a gene expression analysis could be useful. All the candidate resistance genes were expected to be expressed at low levels, regardless treatments. Moreover, as mentioned previously, these genes should not be differentially expressed, but they could be constitutively expressed, albeit they might be responsible of down-stream responses whose effects can be related to DEGs. According to edgeR

and DESeq2 results, all the candidate genes were expressed either at low or constitutive levels among conditions or in some cases were even DEGs. The exception is SMEL_011g374890.1 gene, which was differentially expressed according to DESeq2, but not using edgeR counts filtering criteria, as a consequence SMEL_011g374890.1 did not result as DEG in any comparison.

4.3.5 VENN diagrams between significant pairwise comparisons

Six VENN diagrams among the most impactful were depicted by the online tool available at the URL https://bioinformatics.psb.ugent.be/webtools/Venn. In addition, the results of 12 additional VENN diagrams are shown in Table 5.3.

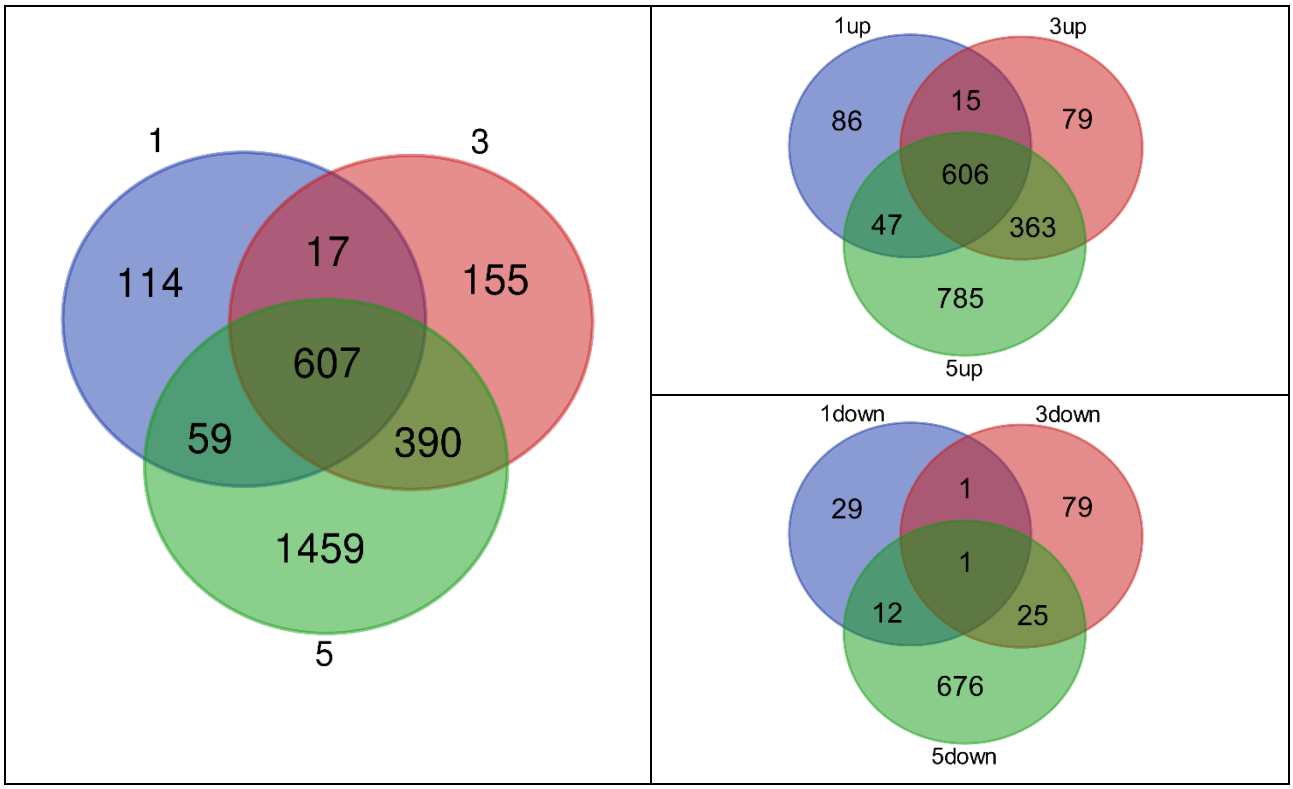
For each comparison, 3 different sub-diagrams were provided. VENN diagrams showed the shared and distinctive genes as a whole, and up- and down-regulated DEGs. A gene referred to 'up-regulated' showed a positive FC and its expression resulted higher in treated compared to its control. In this context, the mutual and distinctive genes in each pairwise comparison were discussed.

A further Gene Ontology (GO) analysis is still ongoing, aiming to identify the enriched functional terms belonging to the three domains Cellular Component (CC), Molecular Function (MF) and Biological Process (BP) in each comparison of interest, providing an exhaustive functional overview of the individuated genes.

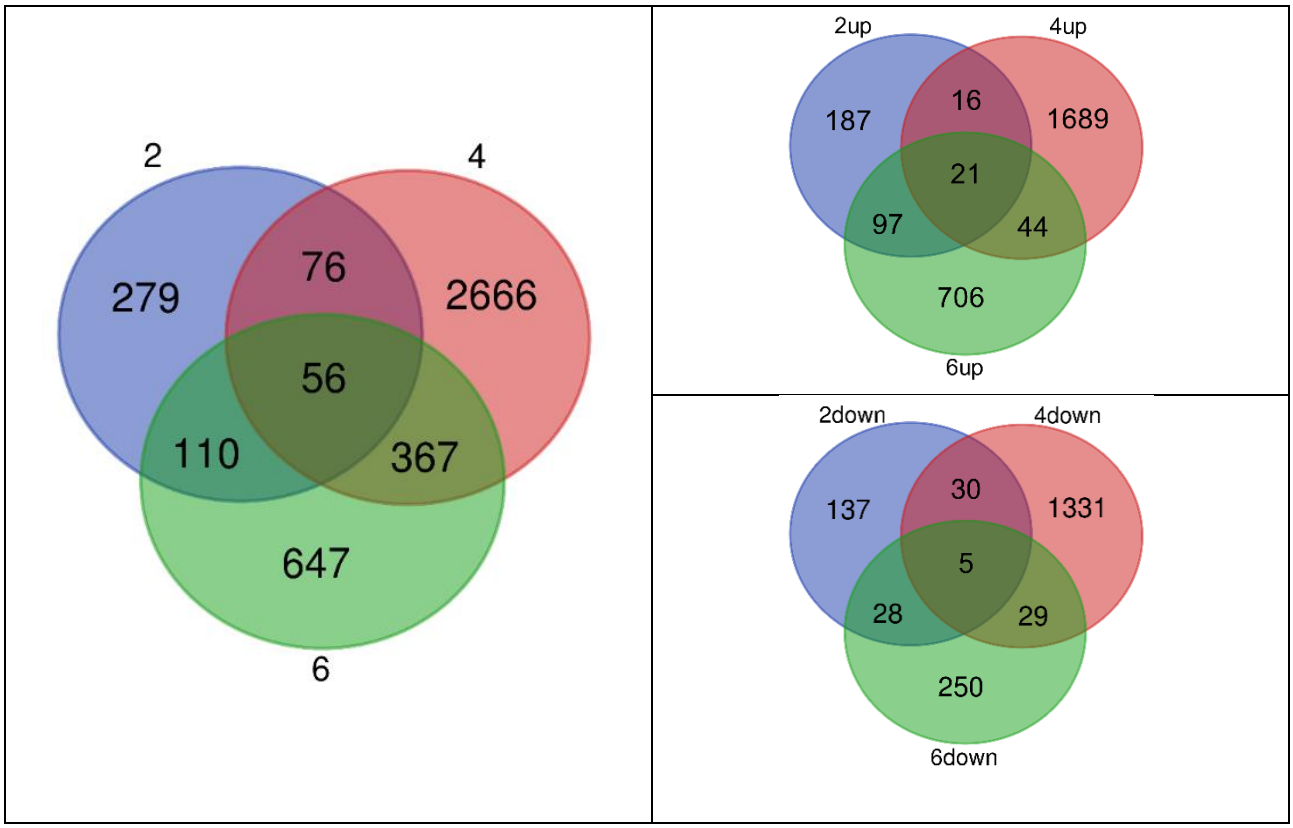
Furthermore, a Weighted Gene Co-Expression Network Analysis (WGCNA) will re-organize DEGs in functional modules associated to a similar expression pattern, and, as a consequence, biological function.

In detail, the VENNs were defined between the pairwise comparisons:

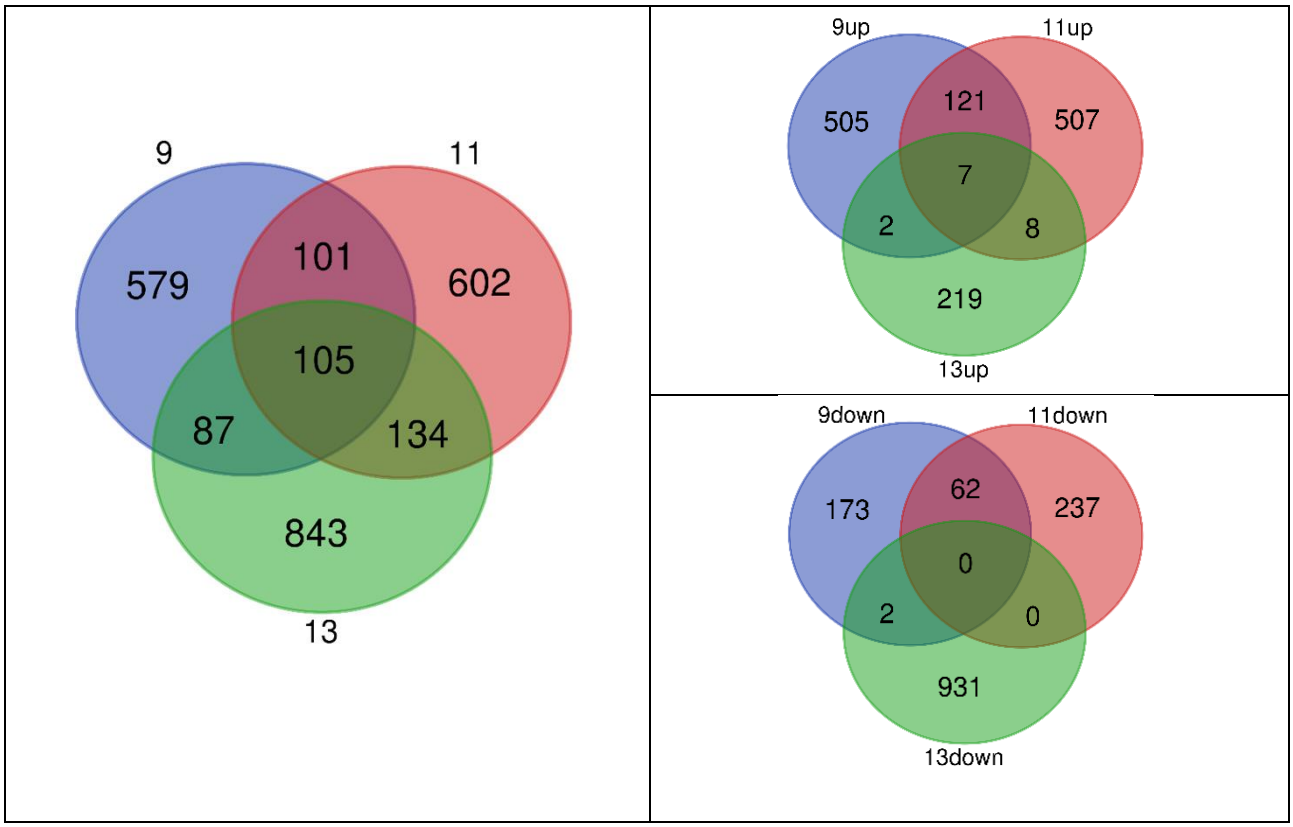
- 1 (C45 NH_4^+ T4 *Fom* vs. C45 NH_4^+ T4 Mock), 3 (C45 NO_3^- T4 *Fom* vs. C45 NO_3^- T4 Mock) and 5 (C45 0N T4 *Fom* vs. C45 0N T4 Mock) aimed to identify DEGs between T4 on C45, *Fom* treatments and among N treatments.



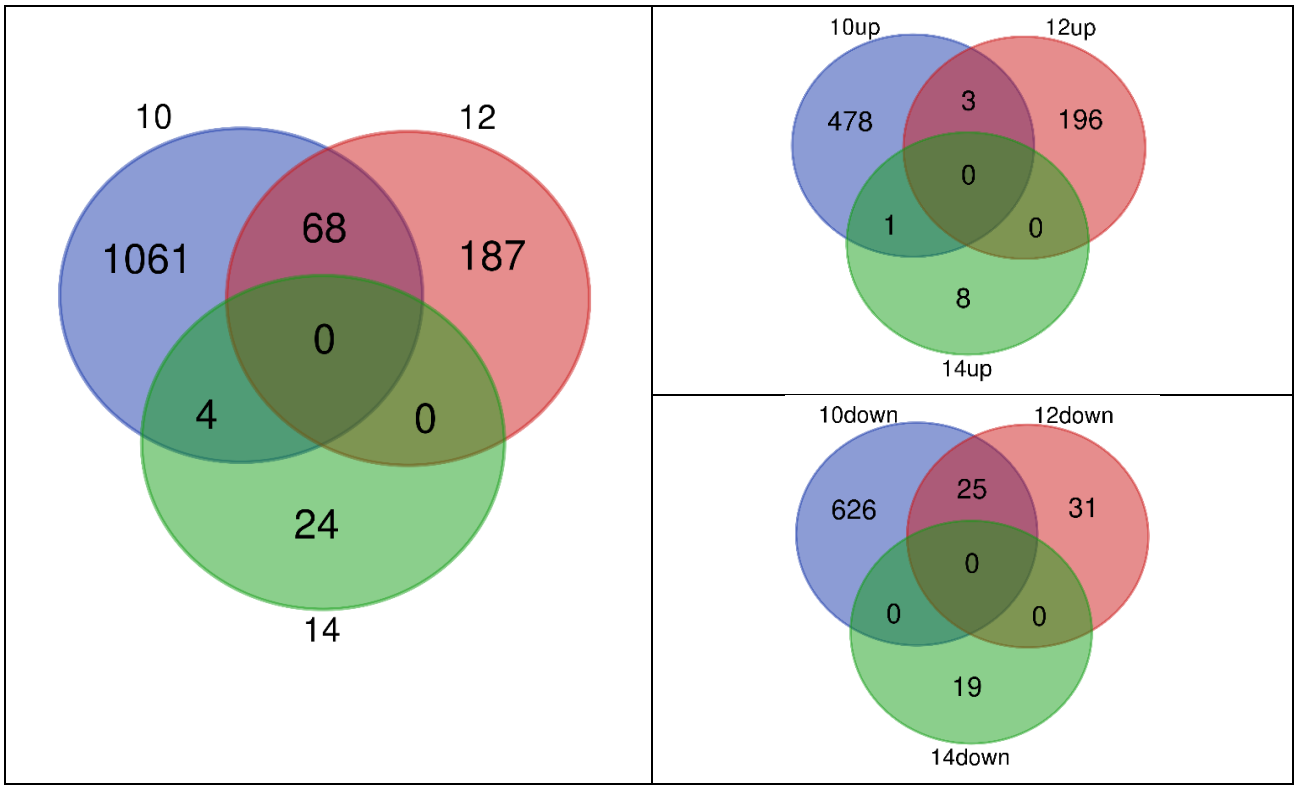
- 2 (C45 NH_4^+ T15 *Fom* vs. C45 NH_4^+ T15 Mock), 4 (C45 NO_3^- T15 *Fom* vs. C45 NO_3^- T15 Mock) and 6 (C45 0N T15 *Fom* vs. C45 0N T15 Mock) aimed to identify DEGs between T15 on C45, *Fom* treatments and among N treatments.



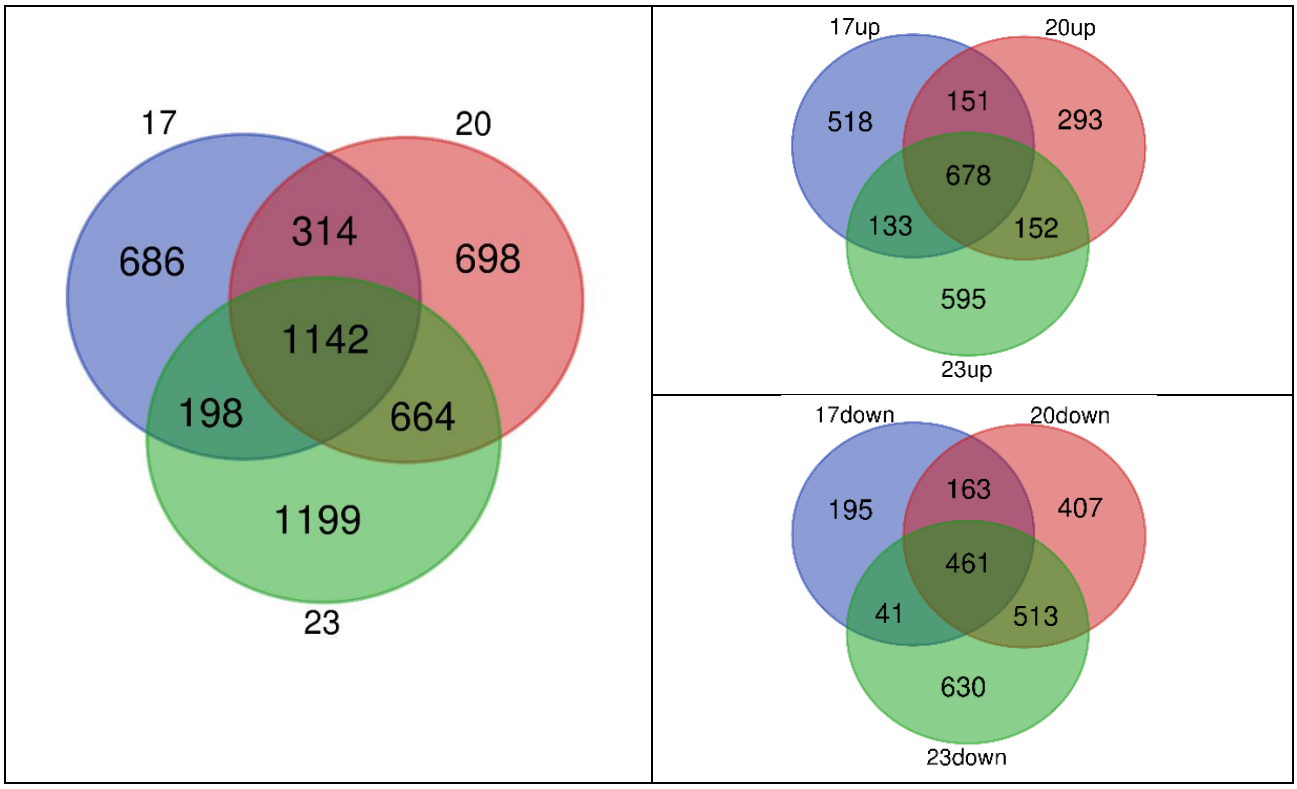
- 9 (67/3 NH_4^+ T4 *Fom* vs. 67/3 NH_4^+ T4 Mock), 11 (67/3 NO_3^- T4 *Fom* vs. 67/3 NO_3^- T4 Mock) and 13 (67/3 0N T4 *Fom* vs. 67/3 0N T4 Mock) aimed to identify DEGs between T4 on 67/3, *Fom* treatments and among N treatments.



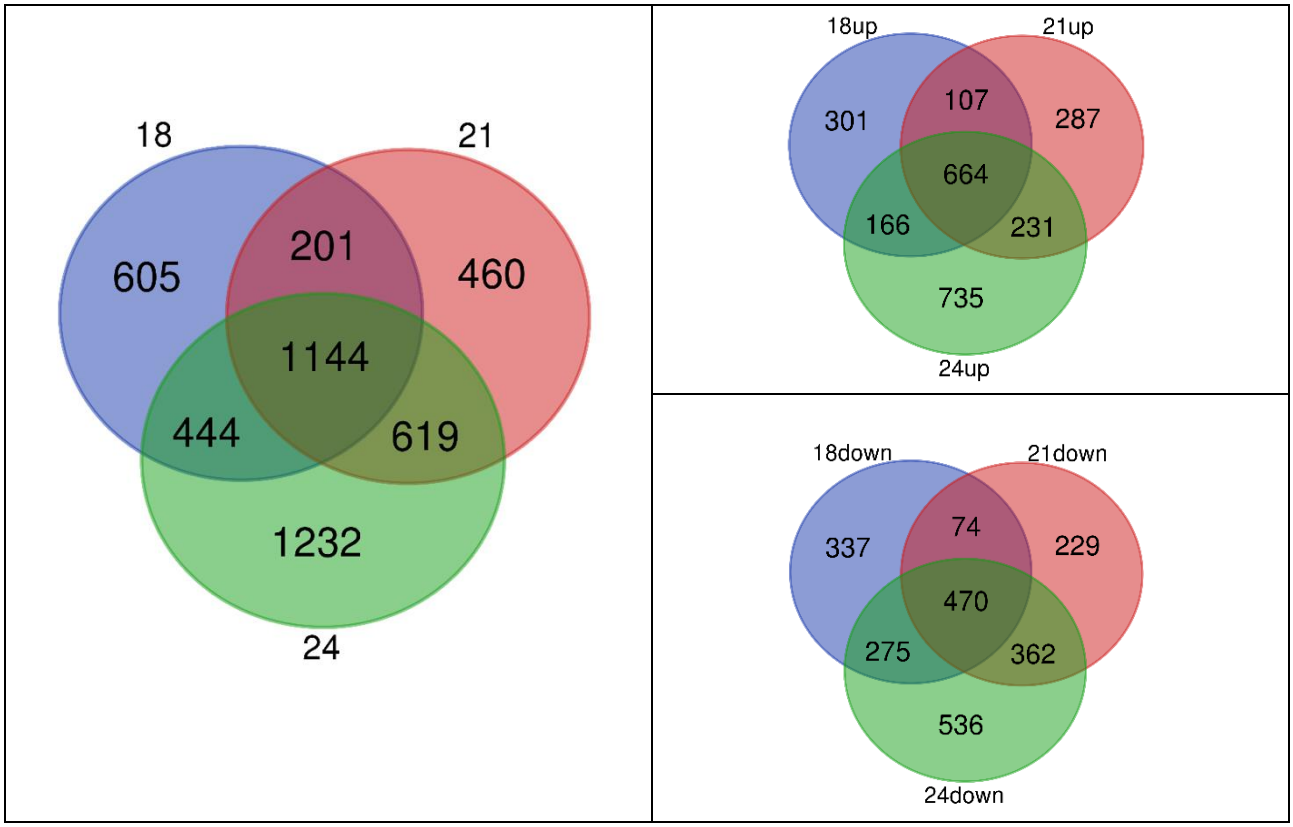
- 10 (67/3 NH_4^+ T15 *Fom* vs. 67/3 NH_4^+ T15 Mock), 12 (67/3 NO_3^- T15 *Fom* vs. 67/3 NO_3^- T15 Mock) and 14 (67/3 0N T15 *Fom* vs. 67/3 0N T15 Mock) aimed to identify DEGs between T4 on 67/3, *Fom* treatments and among N treatments.



- 17 (67/3 NH_4^+ T4 *Fom* vs. C45 NH_4^+ T4 *Fom*), 20 (67/3 NO_3^- T4 *Fom* vs. C45 NO_3^- T4 *Fom*) and 23 (67/3 0N T4 *Fom* vs. C45 0N T4 *Fom*) aimed to identify shared and distinctive DEGs between genotypes among N treatments, at T4 and after *Fom* treatment.



- 18 (67/3 NH_4^+ T15 *Fom* vs. C45 NH_4^+ T15 *Fom*), 21 (67/3 NO_3^- T15 *Fom* vs. C45 NO_3^- T15 *Fom*) and 24 (67/3 0N T15 *Fom* vs. C45 0N T15 *Fom*) aimed to identify shared and distinctive DEGs between genotypes among N treatments, at T15 and after *Fom* treatment.



Comparison	Number of DEGs								
1 *(C45 NH4+ T4 FOM vs C45 NH4+ T4 Mock)* and **2** *(C45 NH4+ T15 FOM vs C45 NH4+ T15 Mock)*	total 1 only	total shared	total 2 only	1 up only	shared up	2 up only	1 down only	shared down	2 down only

	776	*21*	*500*	*748*	*6*	*315*	*42*	*1*	*199*
3 *(C45 NO3- T4 FOM vs C45 NO3- T4 Mock)* and 4 *(C45 NO3- T15 FOM vs C45 NO3- T15 Mock)*	total 3 only	total shared	total 4 only	3 up only	shared up	4 up only	3 down only	shared down	4 down only
	683	*486*	*2679*	*1036*	*27*	*1743*	*105*	*1*	*1394*
5 *(C45 0N T4 FOM vs C45 0N T4 Mock)* and **6** *(C45 0N T15 FOM vs C45 0N T15 Mock)*	total 5 only	total shared	total 6 only	5 up only	shared up	6 up only	5 down only	shared down	6 down only
	2185	*330*	*850*	*1555*	*246*	*622*	*664*	*59*	*262*
9 *(67/3 NH4+ T4 FOM vs 67/3 NH4+ T4 Mock)* and **10** *(67/3 NH4+ T15 FOM vs 67/3 NH4+ T15 Mock)*	total 9 only	total shared	total 10 only	9 up only	shared up	10 up only	9 down only	shared down	10 down only
	474	*398*	*735*	*298*	*337*	*145*	*203*	*34*	*617*
11 *(67/3 NO3- T4 FOM vs 67/3 NO3- T4 Mock)* and 1**2** *(67/3 NO3- T15 FOM vs 67/3 NO3- T15 Mock)*	total 11 only	total shared	total 12 only	11 up only	shared up	12 up only	11 down only	shared down	12 down only
	919	*23*	*232*	*629*	*14*	*185*	*297*	*2*	*54*
13 *(67/3 0N T4 FOM vs 67/3 0N T4 Mock)* and **14** *(67/3 0N T15 FOM vs 67/3 0N T15 Mock)*	total 13 only	total shared	total 14 only	13 up only	shared up	14 up only	13 down only	shared down	14 down only
	1165	*4*	*24*	*235*	*1*	*8*	*931*	*2*	*17*
17 *(67/3 NH4+ T4 FOM vs C45 NH4+ T4 FOM)* and **18** *(67/3 NH4+ T15 FOM vs C45 NH4+ T15 FOM)*	total 17 only	total shared	total 18 only	17 up only	shared up	18 up only	17 down only	shared down	18 down only
	1295	*1045*	*1349*	*933*	*547*	*691*	*446*	*414*	*742*
20 *(67/3 NO3- T4 FOM vs C45 NO3- T4 FOM)* and **21** *(67/3 NO3- T15 FOM vsC45 NO3- T15 FOM)*	total 20 only	total shared	total 21 only	20 up only	shared up	21 up only	20 down only	shared down	21 down only
	1461	*1357*	*1067*	*706*	*568*	*721*	*804*	*740*	*395*
23 *(67/3 0N T4 FOM vs C45 0N T4 FOM)* and **24** *(67/3 0N T15 FOM vs C45 0N T15 FOM)*	total 23 only	total shared	total 24 only	23 up only	shared up	24 up only	23 down only	shared down	24 down only
	1640	*1563*	*1876*	*793*	*765*	*1031*	*918*	*727*	*916*
18 *(67/3 NH4+ T15 FOM vs C45 NH4+ T15 FOM)* and **21** *(67/3 NO3- T15 FOM vs C45 NO3- T15 FOM)*	total 18 only	total shared	total 21 only	18 up only	shared up	21 up only	18 down only	shared down	21 down only
	1049	*1345*	*1079*	*467*	*771*	*518*	*612*	*544*	*591*
18 *(67/3 NH4+ T15 FOM vs C45 NH4+ T15 FOM)* and **24** *(67/3 0N T15 FOM vs C45 0N T15 FOM)*	total 18 only	total shared	total 24 only	18 up only	shared up	24 up only	18 down only	shared down	24 down only
	806	*1588*	*1851*	*408*	*830*	*966*	*411*	*745*	*898*
21 *(67/3 NO3- T15 FOM vs C45 NO3- T15 FOM)* and **24** *(67/3 0N T15 FOM vs C45 0N T15 FOM)*	total 21 only	total shared	total 24 only	21 up only	shared up	24 up only	21 down only	shared down	24 down only
	661	*1763*	*1676*	*394*	*895*	*901*	*303*	*832*	*811*

Table 4.3 Number of shared and distinctive DEGs between some relevant pairwise comparisons shown in Table X. VENN diagrams (pairwise) were made starting from lists of total DEGs, and, separately, up- and down-regulated ones.

- 1 (C45 NH_4^+ T4 *Fom* vs. C45 NH_4^+ T4 Mock) and 2 (C45 NH_4^+ T15 *Fom* vs. C45 NH_4^+ T15 Mock) aimed to identify DEGs between T4 and T15 samplings after NH_4^+ treatment and pathogen inoculation on C45.

- 3 (C45 NO_3^- T4 *Fom* vs. C45 NO_3^- T4 Mock) and 4 (C45 NO_3^- T15 *Fom* vs. C45 NO_3^- T15 Mock) aimed to identify DEGs between T4 and T15 samplings after NO_3^- treatment and pathogen inoculation on C45.

- 5 (C45 0N T4 *Fom* vs. C45 0N T4 Mock) and 6 (C45 0N T15 *Fom* vs. C45 0N T15 Mock) aimed to identify DEGs between T4 and T15 samplings without N treatments and pathogen inoculation on C45.

- 9 (67/3 NH_4^+ T4 *Fom* vs. 67/3 NH_4^+ T4 Mock) and 10 (67/3 NH_4^+ T15 *Fom* vs. 67/3 NH_4^+ T15 Mock) aimed to identify DEGs between T4 and T15 samplings after NH_4^+ treatment and pathogen inoculation on 67/3.

- 11 (67/3 NO_3^- T4 *Fom* vs. 67/3 NO_3^- T4 Mock) and 12 (67/3 NO_3^- T15 *Fom* vs. 67/3 NO_3^- T15 Mock) aimed to identify DEGs between T4 and T15 samplings after NO_3^- treatment and pathogen inoculation on 67/3.

- 13 (67/3 0N T4 *Fom* vs. 67/3 0N T4 Mock) and 14 (67/3 0N T15 *Fom* vs. 67/3 0N T15 Mock) aimed to identify DEGs between T4 and T15 samplings without N treatments and pathogen inoculation on 67/3.

- 17 (67/3 NH_4^+ T4 *Fom* vs. C45 NH_4^+ T4 *Fom*) and 18 (67/3 NH_4^+ T15 *Fom* vs. C45 NH_4^+ T15 *Fom*) aimed to identify, among DEGs between genotypes, shared DEGs between T4 and T15, after NH_4^+ treatment and *Fom* inoculation.

- 20 (67/3 NO_3^- T4 *Fom* vs. C45 NO_3^- T4 *Fom*) and 21 (67/3 NO_3^- T15 *Fom* vs. C45 NO_3^- T15 *Fom*) aimed to identify, among DEGs between genotypes, shared DEGs between T4 and T15, after NO_3^- treatment and *Fom* inoculation.

- 23 (67/3 0N T4 *Fom* vs. C45 0N T4 *Fom*) and 24 (67/3 0N T15 *Fom* vs. C45 0N T15 *Fom*) aimed to identify, among DEGs between genotypes, shared DEGs between T4 and T15, without N treatment and *Fom* inoculation.

- 18 (67/3 NH_4^+ T15 *Fom* vs. C45 NH_4^+ T15 *Fom*) and 21 (67/3 NO_3^- T15 *Fom* vs. C45 NO_3^- T15 *Fom*) aimed to identify, among DEGs between genotypes, shared DEGs between NO_3^- and NH_4^+ treatments, at T15 and after *Fom* inoculation.

- 18 (67/3 NH_4^+ T15 *Fom* vs. C45 NH_4^+ T15 *Fom*) and 24 (67/3 0N T15 *Fom* vs. C45 0N T15 *Fom*) aimed to identify, among DEGs between genotypes, shared DEGs between NH_4^+ and the control (0N treatment), at T15 and after *Fom* inoculation.

- 21 (67/3 NO_3^- T15 *Fom* vs. C45 NO_3^- T15 *Fom*) and 24 (67/3 0N T15 *Fom* vs. C45 0N T15 *Fom*) aimed to identify, among DEGs between genotypes, shared DEGs between NO_3^- and the control (0N treatment), at T15 and after *Fom* inoculation.

4.3.6 Ongoing analyses: Gene Ontology enrichment and WGCNA

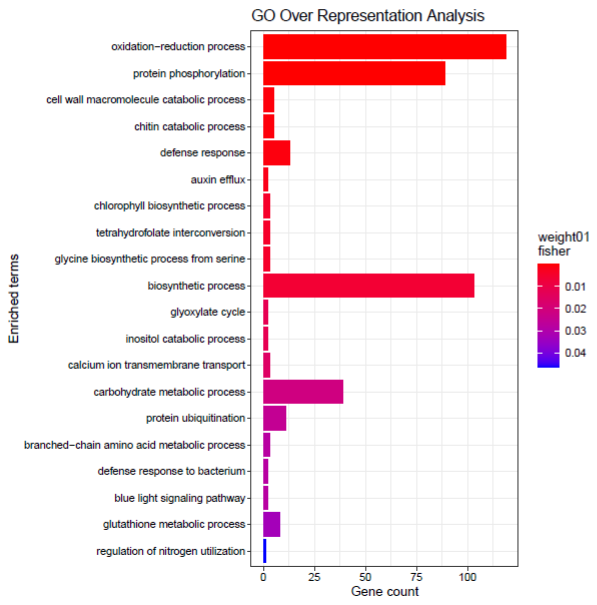
A further Gene Ontology (GO) analysis is still ongoing, to detect the enriched functional terms belonging to the three domains Cellular Component (CC), Molecular Function (MF) and Biological Process (BP) in each comparison of interest and providing an exhaustive functional overview of the identified genes. Here, only some GO enriched terms plots in relevant comparisons are shown. In detail, GO enriched terms within the comparisons 17, 20 and 23 were highlighted:

Pair	**17**	67/3 NH4+ T4 FOM	**vs**	C45 NH4+ T4 FOM
Pair	**20**	67/3 NO3- T4 FOM	**vs**	C45 NO3- T4 FOM
Pair	**23**	67/3 0N T4 FOM	**vs**	C45 0N T4 FOM

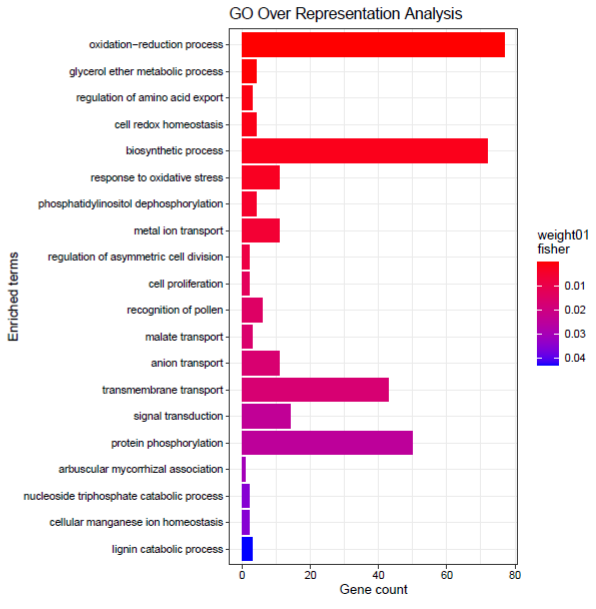
The differences in GO enriched terms within each genotype at T4, *Fom* inoculated and under different N treatments were presented. The results related to the Molecular Function (MF) and the Biological Process (BP) macro-categories are shown in the following barplots:

BP

67/3 NH4+



C45 NH4+



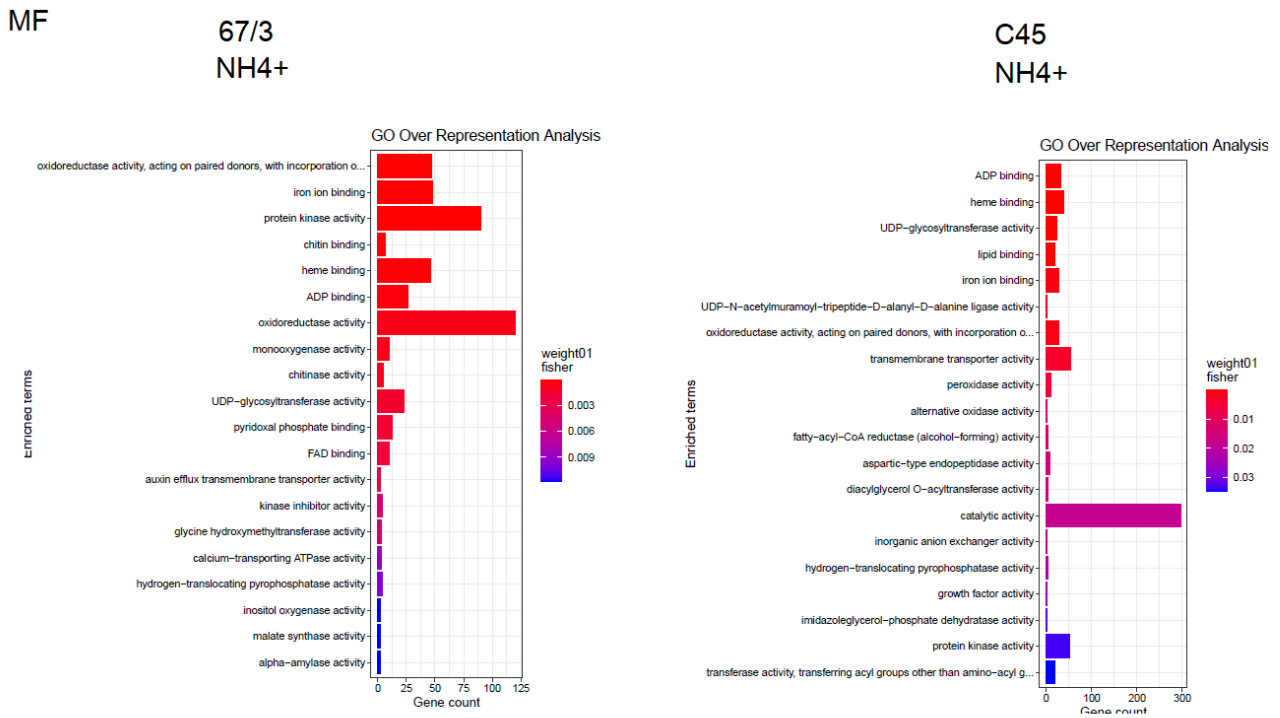


Figure 4.7 GO enriched terms within the two lines, at T4, after pathogen exposure and NH4+ treatment. The two macro-categories Biological Process and Molecular Function were analysed. Top 20 terms are shown, ranked by p-value, from the most to the least significant.

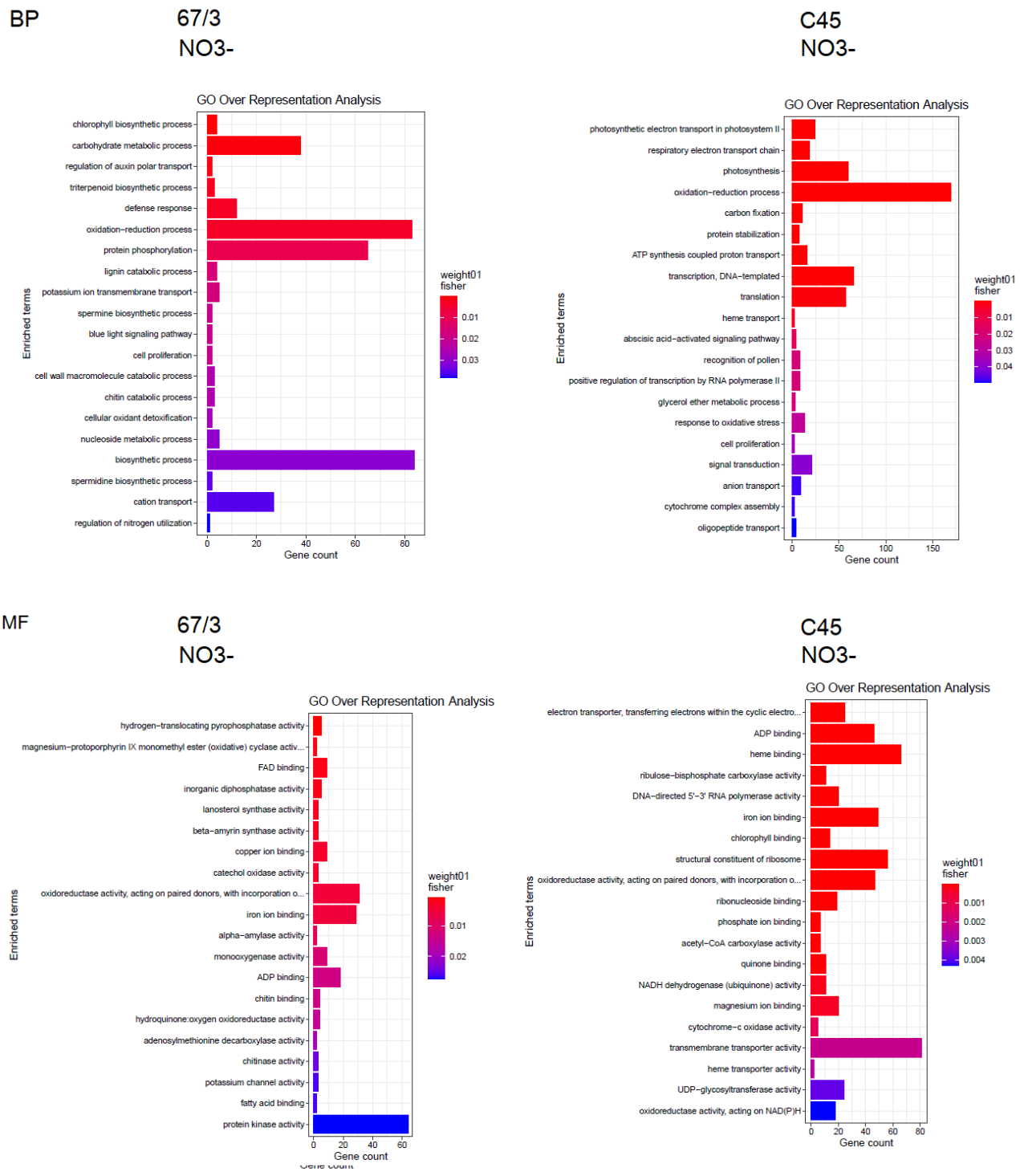


Figure 4.8 GO enriched terms within the two lines, at T4, after pathogen exposure and NO3- treatment. The two macro-categories Biological Process and Molecular Function were analysed. Top 20 terms are shown, ranked by p-value, from the most to the least significant.

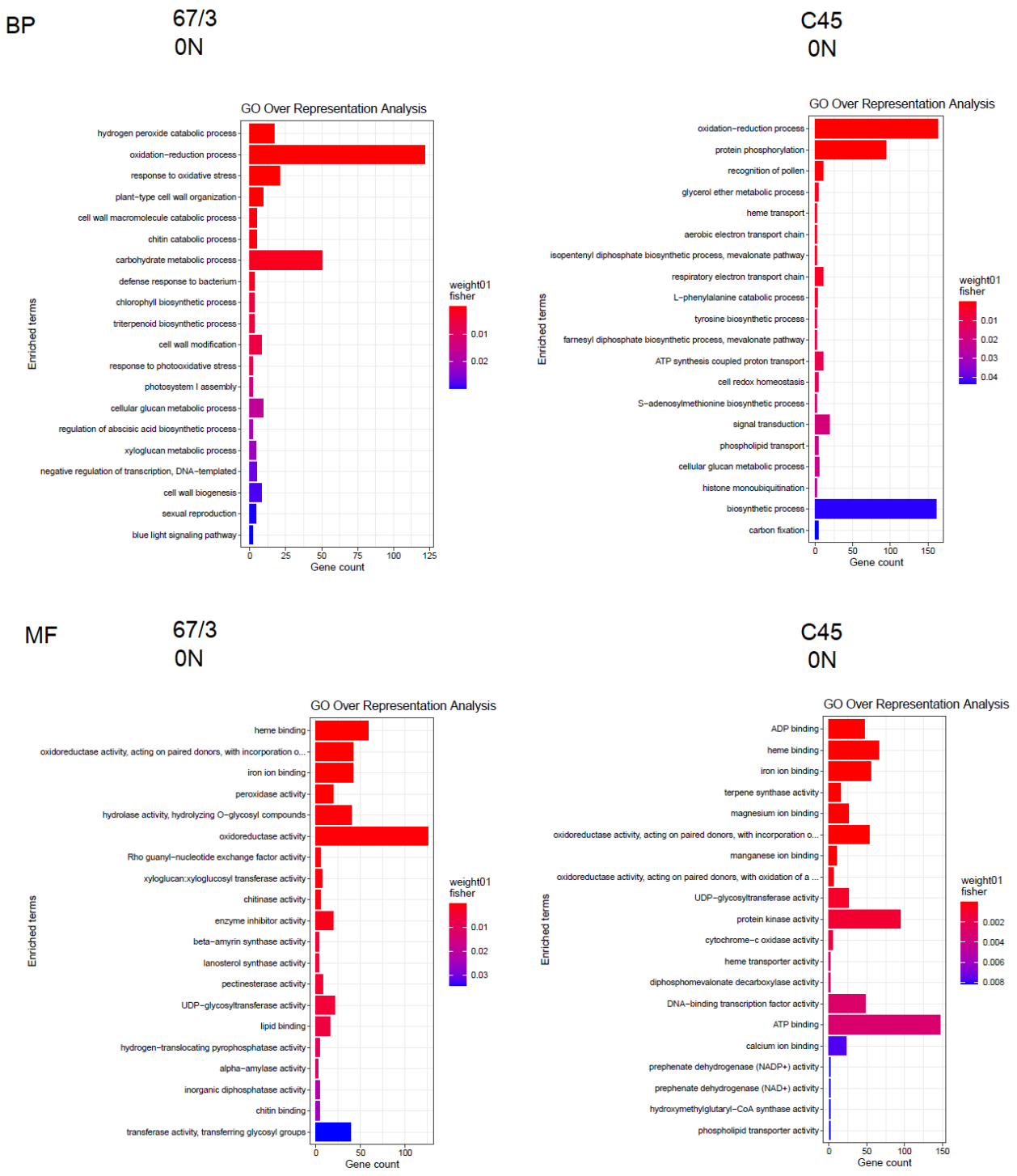


Figure 4.9 GO enriched terms within the two lines, at T4, after pathogen exposure with no nitrogen treatment. The two macro-categories Biological Process and Molecular Function were analysed. Top 20 terms are shown, ranked by p-value, from the most to the least significant.

From a preliminary analysis, some responses are common to all treatments, and that some relevant differences have been found within each genotype between different treatments. Among the most enriched terms, oxidation and oxidoreductive processes occurred regardless N treatment and in both genotypes. In agreement, defence response terms have been not found in the samples without N

treatment, and chitin degradation terms occurred in 67/3 after both N treatments, but not in C45. GO terms related to phosphorylation were over-represented in response to NH_4^+ and NO_3^- in 67/3, suggesting that key downstream signal transduction pathways may be involved and regulated. Conversely, biosynthetic processes seemed commonly found in response to NH_4^+, as well as several terms involved in photosynthesis in NO_3^- treated samples in C45. A significant gene expression regulation seemed involved, since many GO terms related to translation and transcription, were found among the most relevant. C45 after NH_4^+ treatment seemed more active in re-locating both amino acids and ions and transmembrane transport, even though ion transport occurs also in the control (0N treatment).

A Weighted Gene Co-Expression Network Analysis (WGCNA) re-organized the DEGs in functional modules including genes with similar expression pattern, and, as a consequence, biological function, as detailed before. This analysis has been carried out as well, but, like Gene Ontology Enrichment, data still requires further attention, and their interpretation is still ongoing.
After merging the modules showing similar correlations with experimental conditions, 23 modules were identified.

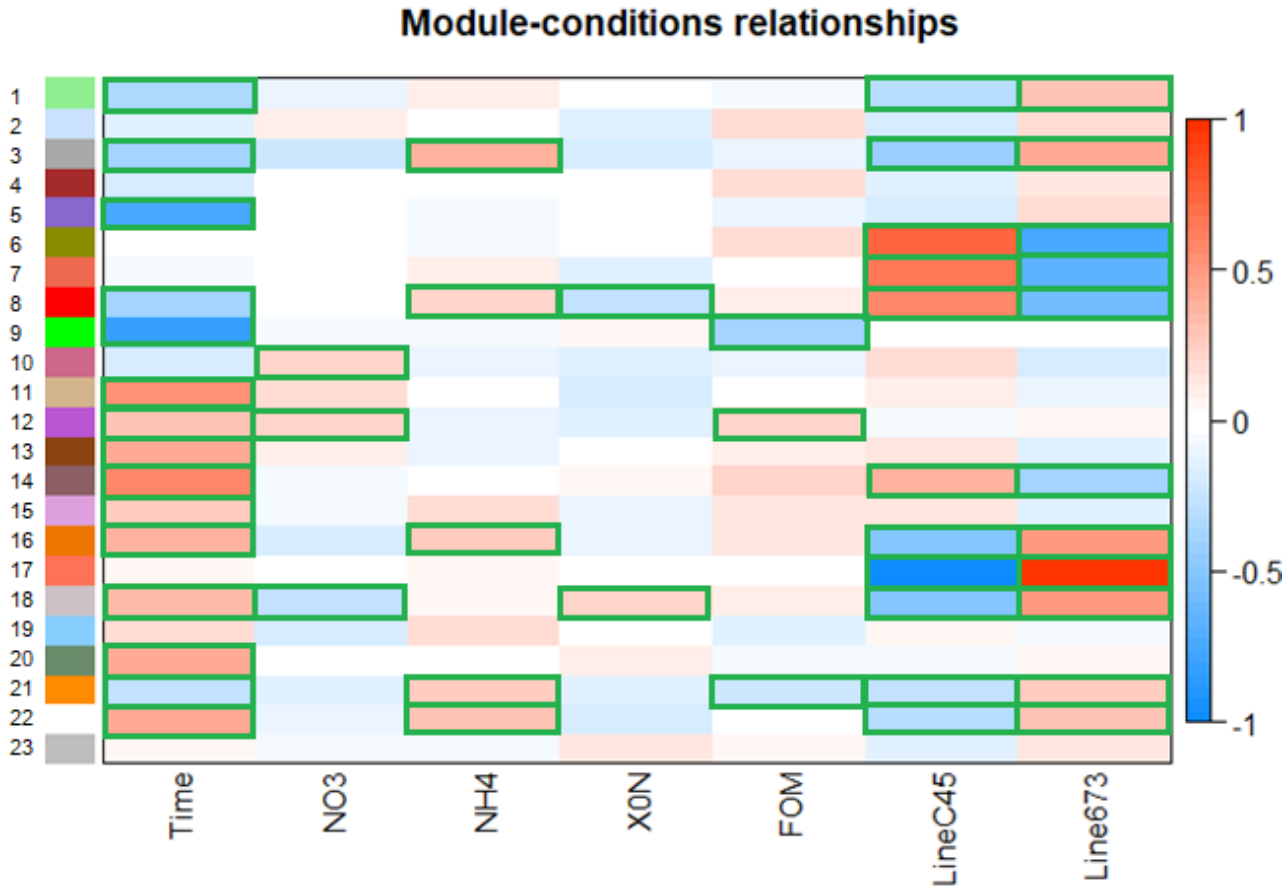


Figure 4.10 Heatmaps showing the correlations between merged modules individuated in WGCNA analysis and experimental variables. The green borders indicate statistically significant ($p < 0.05$) correlations.

pvalue							
	Time	NO3	NH4	X0N	FOM	LineC45	Line673
1	0.00119	0.40138	0.27944	0.8039	0.6033	0.00365	0.00365
2	0.25862	0.38276	0.79801	0.21474	0.09846	0.06731	0.06731
3	0.00021	0.05013	0.00033	0.06843	0.41877	1.60E-05	1.60E-05
4	0.0691	0.94016	0.91441	0.8907	0.0686	0.14038	0.14038
5	1.75E-17	0.98706	0.56039	0.70842	0.36497	0.12222	0.12222
6	0.87057	0.87903	0.63244	0.87763	0.0876	2.62E-17	2.62E-17
7	0.62953	0.9704	0.28366	0.19868	0.944	9.16E-13	9.16E-13
8	0.00016	0.87106	0.04782	0.01531	0.3037	7.76E-10	7.76E-10
9	2.73E-22	0.6602	0.68294	0.47059	0.00033	0.8678	0.8678
10	0.08449	0.02324	0.27532	0.20726	0.38006	0.08461	0.08461
11	3.27E-08	0.08437	0.97627	0.08011	0.78985	0.42485	0.42485
12	0.00699	0.04942	0.35327	0.25983	0.02964	0.56493	0.56493
13	6.32E-05	0.28282	0.26516	0.99486	0.38847	0.1566	0.1566
14	2.28E-09	0.61183	0.9081	0.55801	0.05504	0.00022	0.00022
15	0.00795	0.63875	0.12401	0.26424	0.15982	0.14726	0.14726
16	0.00012	0.0913	0.01004	0.43689	0.20428	1.70E-07	1.70E-07
17	0.55361	0.72687	0.64873	0.87469	0.98176	2.07E-69	2.07E-69
18	0.00168	0.00994	0.51942	0.02699	0.42231	1.16E-06	1.16E-06
19	0.10219	0.07032	0.06776	0.99795	0.17505	0.65752	0.65752
20	3.91E-05	0.80538	0.76966	0.35305	0.64846	0.70645	0.70645
21	0.01987	0.21108	0.00993	0.17127	0.03798	0.01804	0.01804
22	6.67E-05	0.26166	0.00216	0.06962	0.93661	0.00349	0.00349
23	0.47822	0.70445	0.49539	0.22637	0.53526	0.22113	0.22113

Pearson							
	Time	NO3	NH4	X0N	FOM	LineC45	Line673
1	-0.33639	-0.08953	0.11524	-0.02654	-0.05551	-0.30341	0.30341
2	-0.12033	0.09311	0.02735	-0.13205	0.17527	-0.19373	0.19373
3	-0.38174	-0.20713	0.37032	-0.19296	-0.08628	-0.43772	0.43772
4	-0.1925	0.00803	-0.01149	-0.01469	0.19284	-0.15664	0.15664
5	-0.75014	-0.00173	-0.06219	0.03996	-0.09662	-0.1641	0.1641
6	-0.01742	0.01627	-0.0511	0.01646	0.1811	0.74748	-0.74748
7	-0.05153	0.00397	0.11424	-0.13676	0.00751	0.66461	-0.66461
8	-0.38766	0.01735	0.20921	-0.25494	0.10962	0.59235	-0.59235
9	-0.81202	-0.04697	-0.04364	0.07702	-0.3702	-0.01779	0.01779
10	-0.18287	0.23908	-0.11622	-0.13421	-0.09363	0.1828	-0.1828
11	0.54276	0.18294	-0.00318	-0.18546	-0.02848	0.08516	-0.08516
12	0.28247	0.20776	-0.09899	-0.12002	0.22939	-0.06147	0.06147
13	0.40881	0.11444	-0.1187	0.00069	0.092	0.15058	-0.15058
14	0.57891	-0.05421	-0.01234	0.06256	0.20296	0.37972	-0.37972
15	0.27812	-0.05015	0.16333	-0.11893	0.14943	0.15401	-0.15401
16	0.39346	-0.17905	0.27008	-0.08297	0.13509	-0.51801	0.51801
17	0.06326	-0.03733	0.04867	0.01686	0.00244	-0.98537	0.98537
18	0.32662	-0.27043	0.06879	0.23316	0.08562	-0.48656	0.48656
19	0.17339	-0.19168	0.19342	0.00027	-0.14422	0.04737	-0.04737
20	0.41922	-0.02634	-0.0313	0.09904	-0.04871	-0.04025	0.04025
21	-0.24515	-0.1331	0.27046	-0.14548	-0.21912	-0.24883	0.24883
22	0.40762	-0.11956	0.31927	-0.19215	0.0085	-0.30484	0.30484
23	0.0757	-0.04053	-0.07279	0.12879	0.06621	-0.13024	0.13024

Table 4.4 and Table 4.5 Tables showing (on the left) p-values of correlations between modules and experimental conditions and (on the right) Pearson coefficients. Green boxes refer to statistically significant values (p-value < 0.05), while red and light blue ones to positive and negative correlations, respectively.

However, only some modules were selected for further network visualization. The modules correlated only to time samplings were excluded, as well as those not correlated to any variable. The modules showing both correlations to genotypes and pathogen exposure/N treatment (modules 8 and 18) appeared of particular interest.

Ongoing analysis will focus on the identification of significant hub genes through the software Cytoscape v. 3.9.1. In each module, hubs represent the genes correlated to the highest number of genes. They often codify for biologically significant protein or factors implied in a specific biological phenomenon, such as transcription factors or genes involved in hormone signalling, regulating the expression of many other genes within the same module.

	0.1	0.2	Unfiltered
1	85	37	168
3	74	21	131
6	198	118	265
7	46	42	50
8	498	473	543
9	1201	927	1403
10	54	44	64
12	25	2	50
14	451	144	594
16	66	60	83
17	1312	937	1587
18	25	0	57
21	1142	573	1315
22	437	78	549

Table 4.6 Table showing the number of DEGs with different filtering significance thresholds while exporting networks to Cytoscape. 0.1 was selected for further analysis, significantly reducing the number of genes in many cases.

4.4 DISCUSSION

Nitrogen (N), besides necessary for the normal plant growth, can influence disease development and plant resistance. Several studies have shown that N form, as well as the N amount, are important in determining the severity of symptoms, indeed different N sources could change plant physiological and biochemical processes, thus influencing plant-pathogen interactions (Fagard et al., 2014). Nitrate (NO_3^-) decreased black root rot of sugar beet induced by *Rhizoctonia solani* where the number of diseased plants was doubled with NH_4^+ compared to NO_3^- supply (Afanasiev & Carlson, 1942).

The same effect was observed with root rot caused by *Fusarium oxysporum* in bean and wheat (Huber and Watson, 1974); furthermore, Wang et al (2016), demonstrating that NO_3^- increased the resistance of cucumber to *Fusarium* wilt compared to NH_4^+ supply, proved that the disease index decreased with the increased of NO_3^- supply, on the contrary with NH_4^+ supply. NO_3^- increased also tobacco resistance to *Pseudomonas syringae* pv. *phaseolicola* through the accumulation of SA and NO, as well as increased HR-mediated defence (Gupta et al., 2013).

In agreement, our experiments on eggplant showed a worsening of symptoms when treated with NH_4^+, and an improvement of tolerance when treated with NO_3^-. In detail, this was evident in C45, not consistent with the results on 67/3 as well as its parent which did not show changes in fungal attack responses under different N-forms fertilization.

Different fertiliser rates, ranging from 6 to 60 N units, were tested in preliminary trials until a discriminating dose of 20 was found. This dosage was chosen for the following trials with 20 N units supplied during 45 days of experiment.

Phenotypic measurements for *Fom* symptoms were carried out on 3 seedlings (6 seedlings in all preliminary trials) per genotype and treatment, inoculated and mock-inoculated. The evolution of the infection was monitored by scoring the symptoms once a week. In addition, the height and number of lost leaves was measured weekly on each seedling. This was done to quantify the effects on the growth and the response of the two genotypes to the FOM attack.

After *Fom* infection, 67/3 did not show evident differences between treatments, except the intensity of leaf colour. The control plants (0N treatment) showed significant differences as well as C45. Our results indicated that the disease index of eggplant plants decreased under NO_3^- - fed but increased under NH_4^+ fed, mainly after 15 days artificial inoculation (DAI); while at 30 DAI a recovery of vigour in the NH_4^+ -fed seedlings is evident, by growing new small leaves of an intense green colour. After 3 fertilisations, the inoculated mock seedlings showed an intense green colour of plant biomass in the NH_4^+- fed compared to NO_3^- -fed plants. Root samples were taken from 45 seedlings per

genotype during and after the inoculation for RNA-Seq analysis carried out as a service by AMES S.r.l.

Although data analyses are still being processed, a conspicuous number of reciprocal and distinctive DEGs genes have been found in each pairwise comparison. However, completion of analysis is required to identfy their biological role.

General Conclusions

The Ph.D. thesis focused on the molecular characterization of genes involved in eggplant (*S. melongena* L.) tolerance/resistance to the telluric pathogen *Fusarium oxysporum f. sp. melongenae*.

- To deeply investigate the genomic regions underlying two *Fom*-resistance QTLs in eggplant, a targeted BSA-Seq approach was applied using the Illumina sequencing data available from the parents '305E40' and '67/3' as well as their RIL opulation. This strategy allowed us to spot differential genomic regions between the parents putatively involved in controlling either the partial and complete resistances on chromosomes CH11 and CH02, respectively. In detail, the alignment to the '67/3' reference genome identified five differentially enriched regions containing eleven putative candidate genes for *Fom*CH11 QTL. The alignment to the *de novo* 305_asm assembly permitted to identify two best candidate genes for further functional analysis by combining the results obtained from the latter annotation to the *S. aethiopicum* transcriptome, draft genome, and gene expression analysis.

- The genomic characterization of segregant population revealed that five RILs harbour the *Fom*-resistance (RR) in a reduced portions containing the QTL on CH2 which might be re-sequenced with the aim to narrow down the genomic region easing the cloning of resistance gene(s). Likewise, other RILs that mimick a fully resistant phenotype, despite they did not harbour *Fom*CH02 QTL rather the '67/3' haplotype of the *Fom*CH11 QTL, could represent the lines carrying the most effective partial resistance trait useful to identify and isolate the underlying genes by re-sequencing. Finally, the best performing RILs for full and/or partial resistance traits will be employed for breeding purposes to introgress and pyramiding the two *Fusarium* resistance QTLs into élite eggplant germplasm.

- The backcross progenies (BC_6-BC_6S_1) between eggplant and *S. tomentosum* was a further introgression population for characterizing the *Fom* resistance/tolerance trait carried by the wild parent, albeit this other source of resistance is often masked compared to that carried by 67/3. A marked tolerance to *Fom*-infection was detected in some progenies showing an introgression on CH11, although still segregating. This confirms that the tolerance resulting from 67/3 is found on

CH11, as when progenies where *S. tomentosum* completely covers the area of CH11, the plants do not resist the fungal attack and die. Moreover, many backcross progenies revealed the presence of several chromosome with introgressed fragments at heterozygous level, whose segregation could led to an inaccurate evaluation of symptoms and, consequently, an underestimation of the best performing progenies. More solid data will be gathered when stable introgression lines will be available.

- In the last chapter, the phenotypic and some molecular effects of N-form supply on the response to *Fusarium oxysporum* f. sp. *melongenae* of tolerant eggplant were evaluated and identified. A *Fom*-tolerant genotype namely C45 was identified, and interestingly it showed a different response to fungal attack when fertilised with nitrate compared to ammonium. Thus, these different responses were investigated by RNA seq analysis, and the data are still being processed. Gene expression analysis was carried out through a quasi-likelihood edgeR pipeline and a DESeq2 workflow. Twenty-five different pairwise comparisons between significant experimental conditions were performed, and quantitatively described for the relevant differentially expressed genes (DEGs). Further analyses are still ongoing: more in detail, a Gene Ontology (GO) analysis aiming to identify the enriched functional terms in each comparison of interest gave an exhaustive functional overview of the individuated genes was performed. At the same time, a Weighted Gene Co-Expression Network Analysis (WGCNA) will be carried out to re-organize DEGs in functional modules related to similar expression pattern of biological function.

References

Abdullaheva K, Shifman IA (1988) Resistance of eggplant to fusarium wilt. Selektsiya i Semenovodstvo 1:29–31

Acciarri, N., Rotino, G.L., Sabatini, E., Valentino, D., Sunseri, F., Mennella, G., and Tamietti, G. (2004). Improvement of eggplants for resistance to *Verticillium*. Proceedings of the 12th Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant: 178.

Acciarri, N., Rotino, G.L., Valentino, D., Vitelli, G., Sunseri, F., Martelli, G., and Tamietti, G. (2001). Genetic improvement of eggplant for resistance to *Verticillium* through interspecific hybridization with *Solanum sodomeum* L. Proceedings. of the 11th Eucarpia meeting on Genetics and Breeding of *Capsicum* and Eggplant: 298-302.

Adie, B.A.T. and Perez-Perez, J. (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis.. Plant Cell 19, 1665-1681

Afanasiev, M. M., Carlson, W. E. (1942). The relation of phosphorus and nitrogen ratio to the amount of seedling diseases of sugar beets. Ann. Am. Soc. Sugar Beet Tech., pp. 1-5

Agrios, G. N. (1989). Plant Pathology, 3rd Edition Academic Press, Inc.: New York. 803pp. Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman, eds. (1989). Fungi on plants and plant products in the United States. APS PRESS: St. Paul. 1252pp.

Akanbi W.B., Togun A.O., Olaniran O.A., Akinfasoye J.O., Tairu F.M., (2007) Physico-chemical properties of eggplant (solanum meloongena L.) fruit in response to nitrogen fertilizer and fruit size. Agr. J. 2(1):140-148

Alam, M.Z., Ali, M., Akanda, A.M., Choudhury, D.A.M., Haque, N.M.M., Hossain, M.M., and Ogata, K. (1994). Grafting technology: an integrated pest management component for eggplant and tomato. Bull. of the Inst. of Trop. Agric., Kyushu Univ. 17: 85-91.

Alconero, R., Robinson, R. W., Dicklow, B., and Shail, J. (1988). Verticillium wilt resistance in

Alfieri Jr., S.A., Langdon, K.R., Kimbrough, J.W., El-Gholl, N.E., and Wehburg, C. (1994). Diseases and Disorders of Plants in Florida. Florida Dept. Agric. and Consumer Serv., Div. Plant Ind. Bull. 14 : 1114.

Alfieri Jr., S.A., Langdon, K.R., Wehlburg, C., and Kimbrough, J.W. (1984). Index of Plant Diseases in Florida (Revised). Florida Dept. Agric. and Consumer Serv., Div. Plant Ind. Bull. 11 : 1-389.

Altinok HH (2005) First report of fusarium wilt of eggplant caused by Fusarium oxysporum f. sp. melongenae in Turkey. Plant pathology 54:577.

Altınok, H. H., & Can, C. (2010). Characterization of Fusarium oxysporum f. sp. melongenae isolates from eggplant in Turkey by pathogenicity, VCG and RAPD analysis. Phytoparasitica, 38(2), 149-157.Cericola F, Portis E, Toppino L, Barchi L, Acciarri N, Ciriaci T, Sala T, Rotino GL, Lanteri S (2013). The population structure and diversity of eggplant from Asia and the Mediterranean Basin. PLoS ONE 8: e73702.

Altinok, H. H., Can, C., Boyaci, H. F., & Topcu, V. (2014). Genetic variability among breeding lines and cultivars of eggplant against Fusarium oxysporum f. sp. Melongenae from Turkey. Phytoparasitica, 42(1), 75–84.

Altinok, H., Canan C., Altinok M. (2017). Characterization of Fusarium oxysporum f. sp. melongenae isolates from Turkey with ISSR markers and DNA sequence analyses. European Journal of Plant Pathology. 150. 10.1007/s10658-017-1305-7

Altinok, H.H. (2006). Abundance of eggplant wilt (Fusarium oxysporum schlecht. f. sp. melongenae Matuo and Ishigami) in eastern mediterranean region, molecular characterization of agent and induced resistance of plant against the disease. PhD thesis, Çukurova University, Turkey

Aminifard M.H., Aroiee H., Fatemi H., Ameri A. and Karimpour S. (2010). Responses of eggplant (Solanum melongena L.) to different rates of nitrogen under field conditions. J. Central Eur. Agric. 11(4):453-458

Amiour N, Imbaud S, Clément G, Agier N, Zivy M, Valot B, Balliau T, Armengaud P, Quilleré I, Cañas R. 2012. The use of metabolomics integrated with transcriptomic and proteomic studies for identifying key steps involved in the control of nitrogen metabolism in crops such as maize. Journal of Experimental Botany 63, 5017–5033.

Andersen EJ, Ali S, Byamukama E, Yen Y, Nepal MP (2018). Disease Resistance Mechanisms in Plants. *Genes (Basel)*. 2018;9(7):339. Published Jul 4. doi:10.3390/genes9070339

Anderson, J.P., Badruzsaufari E., Schenk P. M., Manners J. M.. Desmond O. J., Ehlert C., Maclean D. J., Ebert P. R. and Kazan K. (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell 16, 3460-3479

Andrews S. FastQC A Quality Control Tool for High Throughput Sequence Data. [(accessed on 4 April 2021)];2010 Available online: https://www.scienceopen.com/document?vid=de674375-ab83-4595-afa9-4c8aa9e4e736

Ano G, Hebert Y, Prior P, Messiaen CM (1991) A new source f resistance to bacterial wilt of eggplants obtained from crossSolanum aethiopicum L. ×Solanum melongena L. Agronomie 11:555–560

Armstrong, G. M., and J. K. Armstrong. (1981). Formae speciales and races of *Fusarium oxysporum* causing wilt diseases, p. 391–399. *In* P. E. Nelson, T. A. Toussoun, and R. J. Cook (ed.), *Fusarium*: diseases, biology, and taxonomy. The Pennsylvania State University Press, University Park

Arpaia S, Mennella G, Onofaro V, Perri E, Sunseri F, Rotino GL. (1997) Production of transgenic eggplant (Solanum melongena L.) resistant to Colorado Potato Beetle (Leptinotarsa decemlineata Say). Theor Appl Genet 1997; 95: 329–334.

Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter 9: 208-218

Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L. P. N. M., Roebroeck, E. J. A., et al. (2000). Gene genealogies and AFLP analyses in the Fusarium oxysporum complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. Phytopathology, 90, 891–900

BaiQing Lin, and YunHua Xiao. (1995). Sources of resistance to Verticillium wilt in Solanum melongena and its affinities identified by improved root dip method. Capsicum and Eggplant Newsl. 14: 81-84.

Barbierato V, Toppino L, Rinaldi P, Sala T, Bassolino L, Valè G, Ferrarini A, Delledonne M, Bagnaresi P, Rotino GL (2016). Phenotype and gene expression analyses of the Rfo-sa1 resistant aubergine interaction with Fusarium oxysporum f. sp. melongenae and Verticillium dahliae. Plant Pathology 5(8):1297-1309. DOI: 10.1111/ppa.12518

Barbierato V., Sala T., Rinaldi P., Bassolino L., Barchi L., Rotino G.L., Toppino L. (2017). A spiking strategy facilitates housekeeping selection for RT-qPCR analysis under different biotic stresses in eggplant. DOI 10.1007/s00709-017-1111-2

Barchi L, Acquadro A, Alonso D, Aprea G, Bassolino L, Demurtas O, Ferrante P, Gramazio P, Mini P, Portis E, Scaglione D, Toppino L, Vilanova S, Díez MJ, Rotino GL, Lanteri S, Prohens J, Giuliano G. (2019). Single Primer Enrichment Technology (SPET) for high-throughput genotyping in tomato and eggplant germplasm. Frontiers in plant science, 10, 1005.

Barchi L, Lanteri S, Portis E, Stagel A, Valè G, Toppino L, Rotino GL (2010). Segregation distortion and linkage analysis in eggplant (Solanum melongena L.). Genome 53: 805-815.

Barchi L, Lanteri S, Portis E, Valè G, Volante A, Pulcini L, Ciriaci T, Acciarri N, Barbierato V, Toppino L, Rotino G., (2012). A RAD Tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. PLoS ONE 7: e43740.

Barchi, L., Pietrella, M., Venturini, L., Minio, A., Toppino, L., Acquadro, A., Andolfo, G., Aprea, G., Avanzato, C Bassolino, L. Comino, C., Comino, C., Molin, A., Ferrarini, A., Maor, L., Portis, E., Reyes-Chin-Wo, S., Rinaldi, R., Sala, T., Scaglione, D., Sonawane, P., Tononi, P., Almekias-Siegl, E., Zago, E., Ercolano, M., Aharoni, A., Delledonne, M., Giuliano, G., Lanteri, S., & Rotino, G.L. (2019). A chromosome-anchored eggplant genome sequence reveals key events in Solanaceae evolution Scientific Reports, 9, 11769 (2019). https://doi.org/10.1038/s41598-019-47985-w

Barchi, L., Rabanus-Wallace, M.T., Prohens, J., Toppino, L., Padmarasu, S., Portis, E., Rotino, G.L., Stein, N., Lanteri, S. and Giuliano, G. (2021), Improved genome assembly and pan-genome provide key insights into eggplant domestication and breeding. Plant J, 107: 579-596. https://doi.org/10.1111/tpj.15313.

Barchi, L., Toppino, L., Valentino, D., Bassolino, L., Portis, E., Lanteri, S., & Rotino, G. L. (2018). QTL analysis reveals new eggplant loci involved in resistance to fungal wilts. Euphytica, 214(2), 20.

Basela O., Mahadeen A., (2008) Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (Brassica oleracea). J. Agr. Biol 10–6:627–632

Baudoin W,; Nersisyan, A.; Shamilov, A.; Hodder, A.; Gutierrez, D.; Pascale S, DE .; Nicola, S.; Gruda, N.; Urban, L.; Tany, J (2017) Food and Agriculture Organization of the United Nations. Good Agricultural Practices for greenhouse vegetable production in the South East European countries - Principles for sustainable intensification of smallholder farms

Baudoin W., Nono-Womdim R., Lutaladio N, HodderA., (2013). Food and Agriculture Organization of the United Nations. Good agricultural practices for greenhouse vegetable crops: Principles for Mediterranean climate areas

Baysal O, Siragusa M, Gümrükcu E, Zengin S, Carimi F, Sajeva M, Teixeira da Silva JA, (2010) Molecular characterization of *Fusarium oxysporum* f. sp. *melongenae* by ISSR and RAPD markers on eggplant. Biochemical Genetics 48, 524-537 [http://dx.doi.org/10.1007/s10528-010-9336-1]

Beckman, C. H. (1987). The nature of wilt diseases of plants. The American Phytopathological Society Press, St. Paul, Minn.

Beevers, H. (1961). Respiratory Metabolism in Plants. Evanston, I11.: Row, Peterson. 232 pp

Begum, S.N., and Ahmed, H.U. (1990). Reactions of eggplant lines to *Sclerotium rolfsii*. Bangladesh J. of Plant Pathol. 6 (1-2): 39-40.

Behera, T.K., Singh, N., (2002) Inter-specific crosses between eggplant (Solanum melongena L.) with related Solanum species. Scientia Hortic. 95, 165–172.

Ben-Yephet, Y.; Reuven, M.; Zviebil, A.; Szmulewich, Y.; Lavkovits, I.; Markovits, T.; Soriano, S.; Bar-Yosef, B (2006). Effect of nutrition on deformation disease in Gypsophila paniculata mother plants. Phytopathology 2006, 96, 771–776.

Bernier F, Berna A. (2001) Germins and germin-like proteins: plant do-all proteins. But what do they do exactly?, Plant Physiology and Biochemistry, 2001, vol. 39 (pg. 545-554)

Berrocal-Lobo, M. et al. (2002) Constitutive expression of ETHYLENE- RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant J. 29, 23-32

Bethke, P. C., Badger, M. R., & Jones, R. L. (2004). Apoplastic synthesis of nitric oxide by plant tissues. *The Plant cell*, *16*(2), 332–341. https://doi.org/10.1105/tpc.017822

Bhat RG, and Subbarao KV, (1999). Host range specificity in Verticillium dahliae. Phytopathology, 8912:12181225

Bletsos, F., Thanassopoulos, C., and Roupakias, D. (2003). Effect of grafting on growth, yield and *Verticillium* wilt of eggplant. HortScience 38: 183-186

Bletsos, F.A., Stavropoulos, N.I., Papadopoulou, P.P., and Antonopoulou, P.D. (2004). Evaluation of eggplant (*Solanum melongena* L.) germplasm for resistance to *Verticillium* wilt. Adv. Hort. Sci. 18 (1): 33-37.

Bohs L (2005) Major clades in Solanum based on ndhF sequence data. In: Keating R, Hollowell VC, Croat TB (Eds) A festschrift for William G. D'Arcy – The legacy of a taxonomist. Missouri Botanical Garden Press, St. Louis, U.S.A., 27–49.

Boiteux, L.S., and Charchar, J.M. (1996). Genetic resistance to root-knot nematode (Meloidogyne javanica) in eggplant (Solanum melongena). Plant Breeding 115: 198-200

Bolger A.M., Lohse M., Usadel B. Trimmomatic (2014) A Flexible Trimmer for Illumina Sequence Data. Bioinformatics; 30:2114–2120. doi: 10.1093/bioinformatics/btu170.

Boller T., & Felix G. (2009). A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. Annual review of plant biology. 60. 379-406. 10.1146/annurev.arplant.57.032905.105346.

Boller, T., & Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annual review of plant biology, 60, 379-406. doi:10.1146/annurev.arplant.57.032905.105346

Bolton, M.D.; Thomma, B.P.H.J. (2008) The complexity of nitrogen metabolism and nitrogen-regulated gene expression in plant pathogenic fungi. Physiol. Mol. Plant Pathol. 72, 104–110.

Boyaci F, Unlu A, Abak K (2010) Screening for resistance to Fusarium wilt of some cultivated eggplants and wild Solanum accessions. In XXVIII International Horticultural Congress on Science and Horticulture for People IHC2010: International Symposium on New, pp 23–27

Boyaci HF, Unlu A, and Abak K (2011) Genetic analysis of resistance to wilt caused by Fusarium (Fusarium oxysporum melongenae) in eggplant (Solanum melongena). The Indian Journal of Agricultural Sciences, p 819

Broman, K.W.; Wu, H.; Sen, S.; Churchill, G.A. (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 2003, 19, 889–890

Buczkowska H. (2010): Effect of plant pruning and topping on yielding of eggplant in unheated foil tunnel. Acta Scientiarum Polonorum, Hortorum Cultus, 9: 105–115.

Camargo, E.L.O.; Nascimento, L.C.; Soler, M.; Salazar, M.M.; Lepikson-Neto, J.; Marques,W.L.; Alves, A.; Teixeira, P.J.P.L.; Mieczkowski, P.; Carazzolle, M.F.; et al. (2014) Contrasting nitrogen fertilization treatments impact xylem gene expression and secondary cell wall lignification in Eucalyptus. BMC Plant Biol., 14, 256.

Camejo D., Guzmán-Cedeño A., Vera-Macias L., Jiménez A., (2019) Oxidative post-translational modifications controlling plant-pathogen interaction, Plant Physiology and Biochemistry, Volume 144, Pages 110-117, ISSN 0981-9428, https://doi.org/10.1016/j.plaphy.2019.09.020.

Cappelli C, Stravato VM, Rotino GL, Buonaurio R (1995) Sources of resistance among Solanum spp., to an Italian isolate of Fusarium oxysporum f. sp. melongenae, In: Andràsfalvi A, Moòr A, Zatykò (eds) EUCARPIA, 9th Meeting on Genet Breed Capsicum Eggplant, SINCOP, Budapest, pp 221–224

Caruso G., Pokluda R., Sękara A., Kalisz A., Jezdinský A., Kopta T., Grabowska A. (2017): Agricultural practices, biology and quality of eggplant cultivated in Central Europe. A review. Hort. Sci. (Prague), 44: 201-212

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: An integrative view from breeding to genomics. Field Crop. Res., 105: 1-14.

Cericola F, Portis E, Lanteri S., Toppino L, Barchi L, Acciarri N, et al. (2014) Linkage disequilibrium and genome-wide association analysis for anthocyanin pigmentation and fruit color in eggplant. BMC Genomics 15:896 doi:10.1186/1471-2164-15-896

Cericola F, Portis E, Toppino L, Barchi L, Acciarri N, Ciriaci T, Sala T, Rotino GL, Lanteri S (2013). The population structure and diversity of eggplant from Asia and the Mediterranean Basin. PLoS ONE 8: e73702

Chambonnet D. (1985) Culture d'antheres in vitro chez trois Solanacees maraicheres: le piment (Capsicum annuum L.), l'aubergine (Solanum melongena L.), la tomate (Lycopersicon esculentum Mill.) et obtention de plantes haploides

Chauhan, M.S., and Duhan, J.C. (1980). Screening of different cultivars of brinjal for resistance against leaf spots, *Phomopsis* fruit rot and little leaf virus diseases. MACCO Agricultural Digest 5 (2): 13-14.

Chen CS, Chen CY, Ravinath DM, Bungahot A, Cheng CP, You RI. (2018) Functional characterization of chitin-binding lectin from Solanum integrifolium containing anti-fungal and insecticidal activities. BMC Plant Biol. 2018 Jan 3;18(1):3. doi: 10.1186/s12870-017-1222-0. PMID: 29298668; PMCID: PMC5751800.

Chen N.C. Kalb T., Talekar N.S., Wang J.F. Ma C.H. (2002): Suggested cultural practices for eggplant. AVRDC Training Guide. Asian Vegetable Research and Development Center, Shanhua, Taiwan. Available at http://avrdc.org/wpfb-file/culti_practices-pdf-2/

Chen, Y., Lun, A. T., & Smyth, G. K. (2016). From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. F1000Research, 5, 1438. https://doi.org/10.12688/f1000research.8987.2

Cho W.D. and Shin H.D. (2004) List of plant diseases in korea. 4th ed. Lorean society of plant pathology, 779

Christensen AB, Cho BH, Næsby M, Gregersen PL, Brandt J, Madriz-Ordeñana K, Collinge DB, Thordal-Christensen H. (2002) The molecular characterization of two barley proteins establishes the novel PR-17 family of pathogenesis-related proteins. Mol Plant Pathol. 2002 May 1;3(3):135-44. doi: 10.1046/j.1364-3703.2002.00105.x. PMID: 20569319.

Churchill, G.A.; Doerge, R.W. (1994) Empirical threshold values for quantitative trait mapping. Genetics 1994, 138, 963–971.

Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 2012 Apr-Jun;6(2):80-92. doi: 10.4161/fly.19695. PMID: 22728672; PMCID: PMC3679285.

Cirulli, M., Ciccarese, F., and Amenduni, M. (1990). Progress in the search for *Verticillium* wilt-resistant eggplant. Phytopathologia Mediterranea 29 (3): 184-190.

Clevenger, J., Chu, Y., Chavarro, C., Botton, S., Culbreath, A., Isleib, T. G., ... & Ozias-Akins, P. (2018). Mapping late leaf spot resistance in peanut (*Arachis hypogaea*) using QTL-seq reveals markers for marker-assisted selection. Frontiers in plant science, 9, 83

Collonnier, C., Fock, I., Kashyap, V. *et al.* (2001). Applications of biotechnology in eggplant. *Plant Cell, Tissue and Organ Culture* 65, 91–107 https://doi.org/10.1023/A:1010674425536

Conesa A, Gotz S (2008) Blast2GO: A comprehensive suite for functional analysis in plant genomics. Int J Plant Genomics 2008:619832.

Conrath U, Pieterse CM, Mauch-Mani B. (2002) Priming in plant-pathogen interactions. Trends Plant Sci. 2002 May;7(5):210-6. doi: 10.1016/s1360-1385(02)02244-6. PMID: 11992826.

Couteaudier, Y., and C. Alabouvette. (1990). Survival and inoculum potential of conidia and chlamydospores of Fusarium oxysporum f. sp. lini in soil. Can. J. Microbiol. 36:551–556.

Creelman RA, Mulpuri R. (2002) The oxylipin pathway in Arabidopsis. Arabidopsis Book. 2002;1:e0012. doi: 10.1199/tab.0012. Epub 2002 Aug 12. PMID: 22303193; PMCID: PMC3243350.

Crill P., Jones JP., Burgis D.S, Woltz SS, (1972). Controlling Fusarium wilt of tomato with resistant varieties. Plant Disease Reporter 56(8): 695-699.

Custers J., Harrison S., Sela-Buurlage M., Deventer E., Lageweg W., Howe P., Meijs P. Ponstein A., Simons B., Melchers L., Stuiver M. (2004). Isolation and characterisation of a class of carbohydrate oxidases from higher plants, with a role in active defence. The Plant journal : for cell and molecular biology. 39. 147-60. 10.1111/j.1365-313X.2004.02117.x.

Dangl, J., Jones, J. (2001). Plant pathogens and integrated defence responses to infection. *Nature* **411,** 826–833 https://doi.org/10.1038/35081161

Darvasi, A.; Soller, M. (1997) A simple method to calculate resolving power and confidence interval of QTL map location. Behav. Genet. 1997, 27, 125–132

Das, A. C., Western, J. H. (1959). The effect of inorganic manures, moisture and inoculum on the incidence of root disease caused by Rhizoctonia solani Kuhn in cultivated soil. Ann. ,4ppl. Biol. 47:37-48

Daunay M.C., Chaput M.H., Sihachakr D., Allot M., Vedel F., Ducreux G. (1993) Production and characterization of fertile somatic hybrids of eggplant (*Solanum melongena* L) with *Solanum aethiopicum* L. Theor Appl Genet 85:841–850

Daunay M.C., Hennart J., Salgon S., Dintinger J. (2016). Eggplant resistance to bacterial wilt and to *Fusarium* wilt: Is there a link?. 16. Eucarpia Capsicum and Eggplant Meeting, Sep 2016, Kecskemét, Hungary. 14 p. ⟨hal-02801124⟩

Daunay Marie-Christine (2008) Eggplant. In: Prohens J., Nuez F. (eds) Vegetables II. Handbook of Plant Breeding, vol 2. Springer, New York, NY. 163-220 https://doi.org/10.1007/978-0-387-74110-9_5

Daunay MC, Aubert S, Frary A, Doganlar S, Lester RN, Barendse G, van der Weerden G, Hennart JW, Haanstra J, Dauphin F, Jullian E (2004) Eggplant (Solanum melongena) fruit color: pigments, measurements and genetics. In: Proceed-ingsofthe12thEucarpiaMeetingonGeneticsandBreeding of Capsicum and Eggplant, Noordwijkerhout, The Netherlands, 2004, pp 108–116

Daunay MC, Lester RN, Laterrot H (1991) The use of wild species for the genetic improvement of Brinjal (eggplant) (Solanum melongena) and tomato (Lycopersicon esculentum). In: Hawks JC, Lester RN, Nee M, Estrada N (eds) Solanaceae III, taxonomy, chemistry, evolution, vol 27. Royal Botanic Gardens Kew and Linnean Soc, London, pp 389–413.

Daunay, M. C., and Hazra, P. (2012). "Eggplant," in Handbook of Vegetables, eds K. V. Peter and P. Hazra (Houston, TX: Studium Press), 257–322.

Daunay, M.-C., and J. Janick. (2007). History and iconography of eggplant. *Chronica Horticulturae* **47**: 16– 22. Dhawan SC & Sethi CL (1976) Observations on the pathogenicity of

Meloidogyne incognita to eggplant and of relative susceptibility of some varieties to the nematodes. Ind. J. Nematod. 6: 39-46

Daunay, M.C., Lester, R., Gebhardt, C., Hennart, J., and Jahn, M. (2001). Genetic resources of eggplant (Solanum melongena) and allied species: a new challenge for molecular geneticists and eggplant breeders. In Solanaceae V: advances in taxonomy and utilization. Edited by R.G. van den Berg, G.W.M. Barendse, G.M. van der Weerden, and C. Mariani. Nijmegen University Press, Nijmegen, the Netherlands. pp. 251–274.

Davidar, P., A. A. Snow, M. Rajkumar, R. Pasquet, M.-C. Daunay, and E. Mutegi. (2015). The potential for crop to wild hybridization in eggplant (*Solanum melongena*; Solanaceae) in Southern India. *American Journal of Botany* **102**: 129– 139.

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J. (1994) A central role of salicylic Acid in plant disease resistance. Science. 1994 Nov 18;266(5188):1247-50. doi: 10.1126/science.266.5188.1247. PMID: 17810266.

Delledonne M., Zeier J., Marocco A., and Lamb C. (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response, Nature, 394:585-588.

Demole E., Lederer E., Mercier D. (1962) Isolement et détermination de la structure du jasmonate de méthyle, constituant odorant caractéristique de l'essence de jasmin, Helvetica Chimica Acta, 45:675-685

Devi H.H., Maity T.K., Paria N.C., Thapa U., (2002) Response of brinjal to different sources of nitrogen. J.Veg. Sci. 29(1):45-47

Di Vito M, Zaccheo G & Catalano F (1992) Source for resistance to root knot nematodes Meloidogyne spp in eggplant. In: Proc. VIIIth Eucarpia meeting on Genetics and Breeding of Capsicum and Eggplant (pp 301303). Rome, Italy

Diaz, J., ten Have, A., and van Kan, J.A.L. (2002). The role of ethylene and wound signaling in resistance of tomato to Botrytis cinerea, Plant Physiol., 129:1341-1351.

Dietrich, R.; Ploss, K.; Heil, M. (2004) Constitutive and induced resistance to pathogens in Arabidopsis thaliana depends on nitrogen supply. Plan Cell Environ. 27, 896–906.

Diwan N, Fluhr R, Eshed Y, Zamir D, Tanksley SD (1999). Mapping of Ve in tomato: a gene conferring resistance to the broad-spectrum pathogen, Verticillium dahliae race 1, Theor Appl Genet, 982:315-319

Docimo, T., Francese, G., Ruggiero, A., Batelli, G., De Palma, M., Bassolino, L., ... & Tucci, M. (2016). Phenylpropanoids accumulation in eggplant fruit: characterization of biosynthetic genes and regulation by a MYB transcription factor. Frontiers in plant science, 6, 1233.

Dodman, R. L. (1970). Factors affecting the prepenetration phase of infection by Rhizoctonia solani. See Ref. 6, pp. 116-21 eggplant, related Solanum species, and interspecific hybrids. HortScience 23(2): 388-390.

Doganlar S, Frary A, Daunay M-CC, Lester RN, Tanksley SD (2002) A comparative genetic linkage map of eggplant (Solanum melongena) and its implications for genome evolution in the Solanaceae. Genetics 161:1697–1711

Dorey S., Baillieul F., Pierrel M., Saindrenan P., Fritig B., Kauffmann S. (1997). Spatial and Temporal Induction of Cell Death, Defense Genes, and Accumulation of Salicylic Acid in Tobacco Leaves Reacting Hypersensitively to a Fungal Glycoprotein Elicitor. Molecular Plant-Microbe Interactions. 10. 646-655. 10.1094/MPMI.1997.10.5.646.

Dumschott, K. , Schmidt, M. H. , Chawla, H. S. , Snowdon, R. , & Usadel, B. (2020). Oxford nanopore sequencing: New opportunities for plant genomics? *Journal of Experimental Botany*, 71, 5313–5322. 10.1093/jxb/eraa263

eggplant, related Solanum species, and interspecific hybrids. HortScience 23(2): 388-390.

Ellis J., Lawrence G., Luck J., Dodds P. (1999) Identification of Regions in Alleles of the Flax Rust Resistance Gene *L* That Determine Differences in Gene-for-Gene Specificity, *The Plant Cell*, Volume 11, Issue 3, March 1999, Pages 495–506, https://doi.org/10.1105/tpc.11.3.495

Engelsberger,W.R.; Schulze,W.X. (2012) Nitrate and ammonium lead to distinct global dynamic phosphorylation patterns when resupplied to nitrogen-starved Arabidopsis seedlings. Plant J. 69, 978–995.

Epple, P., Apel, K., & Bohlmann, H. (1997). Overexpression of an endogenous thionin enhances resistance of Arabidopsis against Fusarium oxysporum. *The Plant cell*, *9*(4), 509–520. https://doi.org/10.1105/tpc.9.4.509

Fagard, M.; Launay, A.; Clément, G.; Courtial, J.; Dellagi, A.; Farjad, M.; Krapp, A.; Soulié, M.C.; Masclaux-Daubresse, C. (2014) Nitrogen metabolism meets phytopathology. J. Exp. Bot. 65, 5643–5656

Fernández-Crespo, E.; Scalschi, L.; Llorens, E.; García-Agustín, P.; Camañes, G. (2015) NH4+ protects tomato plants against Pseudomonas syringae by activation of systemic acquired acclimation. J. Exp. Bot. 66, 6777–6790

Florio FE, Gattolin S, Toppino L, Bassolino L, Fibiani M, Lo Scalzo R, Rotino GL. (2021) A *SmelAAT* Acyltransferase Variant Causes a Major Difference in Eggplant (*Solanum melongena* L.) Peel Anthocyanin Composition. Int J Mol Sci. 2021 Aug 25;22(17):9174. doi: 10.3390/ijms22179174. PMID: 34502081; PMCID: PMC8431300.

Foyer, C.H.; Noctor, G.; Hodges, M. (2011) Respiration and nitrogen assimilation: Targeting mitochondria-associated metabolism as a means to enhance nitrogen use effciency. J. Exp. Bot., 62, 1467–1482.

Fradin EF, Zhang Z, Juarez Ayala JC, Castroverde CD, Nazar RN, Robb J, et al. (2009) Genetic dissection of Verticillium wilt resistance mediated by tomato Ve1. *Plant Physiol.* 2009;150:320–332

Frary A, Doganlar S, Daunay MC, Tanksley SD (2003) QTL analysis of morphological traits in eggplant and implications for conservation of gene function during evolution of solanaceous species. Theor Appl Genet 107:359–370

Frary A., Doganlar S., Daunay M.C. (2007). Eggplant. DOI 10.1007/978-3-540-34536-7_9. In: Kole C(ed) Vegetables II: genome mapping and molecularbreeding in plants. Springer, Heidelberg, pp 287–313

Frodin, D. G. (2004). History and concepts of big plant genera. Taxon 53, 753–776. doi: 10.2307/4135449

Fukuoka H, Miyatake K, Nunome T, Negoro S, Shirasawa K, Isobe S, Asamizu E, Yamaguchi H, Ohyama, A. (2012). Development of gene-based markers and construction of an integrated linkage map in eggplant by using Solanum orthologous (SOL) gene sets. Theor Appl Genet. 125(1):47–56. http://doi.org/10.1007/s00122-0121815-9.

Gajewski M, Katarzyna K, Bajer M (2009). The influence of postharvest storage on quality characteristics of fruit of eggplant cultivars. Not Bot Horti Agrobo 37(2):200-205.

García-Olmedo F, Molina A, Segura A, Moreno M. (1995) The defensive role of nonspecific lipid-transfer proteins in plants. Trends Microbiol. 1995 Feb;3(2):72-4. doi: 10.1016/s0966-842x(00)88879-4. PMID: 7728389.

Garibaldi, A., Minuto, A., and Gullino, M.L. (2005). *Verticillium* wilt incited by *Verticillium dahliae* in eggplant grafted on *S. torvum* in Italy. Plant Dis. 98 (7): 777.

Gelli M, Duo Y, Konda AR, Zhang C, Holding D, Dweikat I. 2014. Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genomics 15, 179.

Geraats, B.P.J., Bakker, P.A.H.M., Lawrence, C.B., Achuo, E.A., Ho fte, M., and van Loon, L.C. (2003). Ethylene-insensitive tobacco shows differentially altered susceptibility to different pathogens, Phytopathology, 93:813-821

Ghosh, S., Dey, G. Biotic and abiotic stress tolerance through CRISPR-Cas mediated genome editing. *J. Plant Biochem. Biotechnol.* (2022). https://doi.org/10.1007/s13562-021-00746-1

Ginoux, G., and Laterrot, H. (1991). Greffage de l'aubergine: reflexions sur le choix du portegreffe. PHM Revue Horticole 321: 49-54.

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD. (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res. 1991;19(23):6553–6568. Doi: 10.1093/nar/19.23.6553.

Gisbert C., Prohens J., Raigon M.D., Stommel J.R., Nuez F. (2011): Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. Scientia Horticulturae, 128: 14−22.

Glazebrook J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol.;43:205-27. doi: 10.1146/annurev.phyto.43.040204.135923. PMID: 16078883.

Gleddie, S., Fassuliotis, G.; Keller, W. & Setterfield, G. (1985) "Somatic hybridization as a potential method of transferring nematode and mite resistance into eggplant." Zeitschrift fur Pflanzenzuchtung= Journal of plant breeding. 94: 352-355

Gomez-Vazquez J., Aguilera-Lirola A., and Martin-Bretones G. (2020)- First Report of Verticillium Wilt on Eggplant in Almerıa Province, Spain- Plant Disease / Vol. 104 No. 12 https://doi.org/10.1094/PDIS-11-19-2456-PDN

Gonzales-Cendales Y, Catanzariti A.M, Baker B., McGrath Des.J., Jones D.A., (2016). Identification of 1-7 expends the repertoire of genes for resistance to Fusarium wilt in tomato to three resistance gene classes. Mol. Plant Pathol. 17(3): 448-463.

Gousset C, Collonnier C, Mulya K, Mariska I, Rotino GL, Besse P, Servaes A, Sihachakr D (2005) Solanum torvum, as a use¬ful source of resistance against bacterial and fungal dis¬eases for improvement of eggplant (S. melongena L.). Plant Sci 168:319–327

Gramazio P, Prohens J, Plazas M, Andújar I, Herraiz FJ, Castillo E, Knapp S, Meyer RS, Vilanova S (2014) Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant. BMC Plant Biol 14:350 . doi: 10.1186/s12870-014-0350-z

Gramazio, P., Yan, H., Hasing, T., Vilanova, S., Prohens, J., & Bombarely, A. (2019). Whole-Genome resequencing of seven eggplant (Solanum melongena) and one wild relative (S. incanum) accessions provides new insights and breeding tools for eggplant enhancement. Frontiers in plant science, 10, 1220.

Guo, S.W.; Chen, G.; Zhou, Y.; Shen, Q.R. (2007) Ammonium nutrition increases photosynthesis rate under water stress at early development stage of rice (Oryza sativa L.). Plant Soil 296, 115–124.

Gupta, K.J.; Brotman, Y.; Segu, S.; Zeier, T.; Zeier, J.; Persijn, S.T.; Cristescu, S.M.; Harren, F.J.; Bauwe, H.; Fernie, A.R. (2013) The form of nitrogen nutrition affects resistance against Pseudomonas syringae pv. phaseolicola in tobacco. J. Exp. Bot. 64, 553–568

Hamberg, M. and Gardner, H.W. (1992). Oxylipin pathway to jasmonates: biochemistry and biological significance, Biochim. Biophys. Acta, 1165:1-18.

Hammond-Kosack KE, Jones JD. (1996) Resistance gene-dependent plant defense responses. Plant Cell. 1996 Oct;8(10):1773-91. doi: 10.1105/tpc.8.10.1773. PMID: 8914325; PMCID: PMC161314.

Hernández-Blanco C, Feng DX, Hu J, Sánchez-Vallet A, Deslandes L, Llorente F, Berrocal-Lobo M, Keller H, Barlet X, Sánchez-Rodríguez C, Anderson LK, Somerville S, Marco Y, Molina A. (2007) Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. Plant Cell. 2007 Mar;19(3):890-903. doi: 10.1105/tpc.106.048058. Epub 2007 Mar 9. PMID: 17351116; PMCID: PMC1867366.

Hirakawa H, Shirasawa K, Miyatake K, Nunome T, Negoro S, Ohyama A, Yamaguchi H, Sato S, Isobe S, Tabata S, Fukuoka H (2014). Draft genome sequence of eggplant (Solanum melongena L): the representative solanum species indigenous to the old world. DNA Research 216:649-660.

Hoffland, E.; Jeger, M.J.; van Beusichem, M.L. (2000) Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. Plant Soil 218, 239–247.

Hoffland, E.; van Beusichem, M.L.; Jeger, M.J. (1999) Nitrogen availability and susceptibility of tomato leaves to Botrytis cinerea. Plant Soil 210, 263–272.

Hoffman, T., Schmidt, J.S., Zheng, X., Bent, A.F. (1999). Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance, Plant Physiol., 119:935-950.

Holevas, C. D., Chitzanidis, A., Pappas, A. C., Tzamos, E. C., Elena, K., Psallidas, P. G., Alivizatos, A. S., Panagopoulos, C. G., Kyriakopoulou, P. E., Bem, F. P., Lascaris, D. N., Velissariou, D. E., Vloutoglou, I., Analytis, S. C., Paplomatas, E. J., Aspromougos, J. S., Varveri, C., (2000). Annales de l'Institut Phytopathologique Benaki 19(1), 1-96. Benaki Phytopathologique Institute, Athens, Greece. English language

Horchani, F.; Hajri, R.; Aschi-Smiti, S. (2010) Effect of ammonium or nitrate nutrition on photosynthesis, growth, and nitrogen assimilation in tomato plants. J. Plant Nutr. Soil Sci. 173, 610–617.

Huang, M., Liu, X., Zhou, Y., Summers, R. M. & Zhang, Z. (2019) BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. GigaScience 8, giy154 https://doi.org/10.1093/gigascience/giy154

Huber, D.; Watson, R. (1974) Nitrogen form and plant disease. Annu. Rev. Phytopathol. 12, 139–165.

Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, Von Mering C, Bork P (2019): EggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res 2019, 47:D309–D314.

Iqbal, N. and Czékus, Z. and Ördög, A. and Poór, P. (2021) *Ethylene-dependent effects of fusaric acid on the photosynthetic activity of tomato plants.* PHOTOSYNTHETICA, 59 (2). pp. 337-348. ISSN 0300-3604, DOI 10.32615/ps.2021.029

Jauset, A.M.; Sarasua, M.J.; Avilla, J.; Albajes, R. (**2000)** Effect of nitrogen fertilization level applied to tomato on the greenhouse whitefly. Crop Prot., 19, 255–261.

Jensen, B.; Munk, L. (1997) Nitrogen-induced changes in colony density and spore production of Erysiphe graminis f. sp. hordei on seedlings of six spring barley cultivars. Plant Pathol. 46, 191–202.

Jones, J.P., J.B. Jones, and W. Miller (1982). Fusarium wilt on tomato. Fla. Dept. Agric. & Consumer Serv., Div. of plant industry. Plant Pathology Circular No. 237

Jordá, L., Conejero, V., & Vera, P. (2000). Characterization of P69E and P69F, two differentially regulated genes encoding new members of the subtilisin-like proteinase family from tomato plants. *Plant physiology*, *122*(1), 67–74. https://doi.org/10.1104/pp.122.1.67

Jupe, F., Witek, K., Verweij, W., Śliwka, J., Pritchard, L., Etherington, G. J., ... & Cardle, L. (2013). Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. The Plant Journal,76(3), 530-544.

Kaan, F. (1973). Etude de l'hérédité de la résistance de l'aubergine (*Solanum melongena* L.) à l'anthracnose des fruits (*Colletotrichum gloeosporiodes* f.sp. *melongenae* Penzig Fournet). Ann. Amélior. Plantes 23 (2): 127-131.

Kakizaki Y. (1931) Hybrid vigor in egg-plants and its practical utilization. *Genetics.* ;16:1–25.

Kalda, T.S., Swarup, V., and Choudhury, B. (1977). Resistance to *Phomopsis* blight in eggplant. Vegetable Sci. (India) 4 (2): 90-101.

Kalloo G, Berg BO (1993) Genetic improvement of vegetable crops. Pergamon Press Ltd, Oxford, pp 587–604

Kapoor, K.S., Sharma, S.R., and Gangopadhyay, S. (1989/1990). Assessment of resistance in eggplant against Sclerotinia wilt with a new screening technique. Capsicum Newsl. 8-9:70.

Karagiannidis N, Bletsos F, Stavropoulos N. (2002). Effect of Verticillium wilt (Verticillium dahliae Kleb.) and mycorrhiza (Glomus mosseae) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. Sci Hort. 94: 145–156.

Kashyap V, Kumar SV, Collonnier C, Fusari F, Haicour R, Rotino GL, Sihachakr D, Rajam M (2003) Biotechnology of eggplant. Scientia Horticulturae 97: 1-25

Kaushik P, Prohens J, Vilanova S, Gramazio P, Plazas M (2016) Phenotyping of eggplant wild relatives and interspecific hybrids with conventional and phenomics descriptors provides insight for their potential utilization in breeding. Front Plant Sci 7:677

Kawashima, C. G., Guimarães, G. A., Nogueira, S. R., MacLean, D., Cook, D. R., Steuernagel, B., ... & de Oliveira, J. C. (2016). A pigeonpea gene confers resistance to Asian soybean rust in soybean. Nature biotechnology, 34(6), 661.

Kayam, G., Brand, Y., Faigenboim-Doron, A., Patil, A., Hedvat, I., & Hovav, R. (2017). Fine-mapping the branching habit trait in cultivated peanut by combining bulked segregant analysis and high-throughput sequencing. Frontiers in plant science, 8, 467.

Keller O, Kollmar M, Stanke M, Waack S (2011): A novel hybrid gene prediction method employing protein multiple sequence alignments. Bioinformatics 2011, 27:757–763.

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL (2013): TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 2013, 14:R36.

King, M. D., Fountain, C., Dakhlallah, D., & Bearman, P. S. (2010). KING ET AL. RESPOND. *American Journal of Public Health*, *100*(3), 390. https://doi.org/10.2105/AJPH.2009.184168

Kinsell S. (2016) Characterizing climatic niches and environmental properties of crop wild relatives of the brinjal eggplant (Solanum melongena L.; Solanaceae)- Thesis submitted in partial fulfillment for the MSc in the Biodiversity and Taxonomy of Plants. Royal Botanic Garden Edinburgh

Klein, H., Xiao, Y., Conklin, P. A., Govindarajulu, R., Kelly, J. A., Scanlon, M. J., ... & Bartlett, M. (2018). Bulked-segregant analysis coupled to whole genome sequencing (BSA-Seq) for rapid gene cloning in maize. G3: Genes, Genomes, Genetics, 8(11), 3583-3592.

Komochi S, Monma S, Narikawa T, Sakata Y (1996). Evaluation of resistance to bacterial wilt and verticillium wilt in eggplants (Solanum melongena L.) collected in Malaysia. J Jpn Soc Hortic Sci 65:81–88.

Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. (2017) Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. Genome Res. 2017 May;27(5):722-736. doi: 10.1101/gr.215087.116. Epub 2017 Mar 15. PMID: 28298431; PMCID: PMC5411767.

Kouassi, B., Prohens, J., Gramazio, P., Kouassi, A. B., Vilanova, S., Galán-Ávila, A., ... & Plazas, M. (2016). Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (Solanum melongena). Scientia Horticulturae, 213, 199-207.

Kováˇcik, J.; Klejdus, B.; Baˇckor, M.; Repˇcák, M. (2007) Phenylalanine ammonia-lyase activity and phenolic compounds accumulation in nitrogen-deficient Matricaria chamomilla leaf rosettes. Plant Sci., 172, 393–399.

Krumm, T., Bandemer, K., and Boland, W. (1995). Induction of volatile biosynthesis in the Lima bean (Phaseolus lunatus) by leucine and isoleucine conjugates of 1-oxo- and 1-hydroxyindan-4 carboxylic acid: evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signaling pathway, FEBS Lett., 377:523-529.

Kumar A, Sharma V, Jain BT, Kaushik P. Heterosis Breeding in Eggplant (*Solanum melongena* L.) (2020) Gains and Provocations. Plants (Basel). Mar 24;9(3):403. doi: 10.3390/plants9030403. PMID: 32213925; PMCID: PMC7154857.

Kumar, M.; Kesawat, M.S.; Ali, A.; Lee, S.-C.; Gill, S.S.; Kim, H.U (2019). Integration of Abscisic Acid Signaling with Other Signaling Pathways in Plant Stress Responses and Development. *Plants* 2019, *8*, 592. https://doi.org/10.3390/plants8120592

Kunkel BN, Brooks DM. (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol. Aug;5(4):325-31. doi: 10.1016/s1369-5266(02)00275-3. PMID: 12179966.

Lamotte O, Gould K, Lecourieux D, Sequeira-Legrand A, Lebrun-Garcia A, Durner J, Pugin A, Wendehenne D. (2004) Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein. *Plant Physiology.* 2004;135:516–529.

LanChun N., GuiLin C., and HongBo G.. (2004). Study on the chilling tolerance of rootstocks and grafting seedlings of eggplant. China Vegetables 1: 4-6.

Lander, E.S.; Botstein, D. (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics, 121, 185–199.

Langmead B, Salzberg SL (2012): Fast gapped-read alignment with Bowtie 2. Nat Methods 2012, 9:357–359

Lassaletta L., Billen G., Garnier J., Bouwman L., Velazquez E., Mueller N. and Gerber J. (2016). Nitrogen use in the global food system: past trends and future trajectories of agronomic performance, pollution, trade, and dietary demand. Environ. Res. Lett. 11 095007

Lay FT, Anderson MA. (2005) Defensins--components of the innate immune system in plants. Curr Protein Pept Sci. 2005 Feb;6(1):85-101. doi: 10.2174/1389203053027575. PMID: 15638771.

Le Nguyen, K., Grondin, A., Courtois, B., & Gantet, P. (2019). Next-generation sequencing accelerates crop gene discovery. Trends in plant science, 24(3), 263-274.

Lecompte, F.; Abro, M.A.; Nicot, P. (2010) Contrasted responses of Botrytis cinerea isolates developing on tomato plants grown under different nitrogen nutrition regimes. Plant Pathol. 59, 891–899.

Lee, J.M. (1994). Cultivation of grafted vegetables. I. Current status, grafting methods, and benefits. HortScience 29 (4): 235-239.

Lemmens, M.; Buerstmayr, H.; Krska, R.; Schuhmacher, R.; Grausgruber, H.; Ruckenbauer, P. (2004) The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grains. Eur. J. Plant Pathol. 110, 299–308.

Lester RN and Hasan SMZ (1991). Origin and domestication of the brinjal eggplant, Solanum melongena, from S. incanum, in Africa and Asia., in Hawkes JG, Lester RN, Nee M, Estrada N ed Solanaceae III: Taxonomy, chemistry, evolution. Roy. Bot. Gard.: Kew & Linnean Soc.: London. pp. 369–387.

Li J, Gao Z, Zhou L, Li L, Zhang J, Liu Y, Chen H. (2019) Comparative transcriptome analysis reveals K+ transporter gene contributing to salt tolerance in eggplant. BMC Plant Biology 19, 67.

Li J, He YJ, Zhou L, Liu Y, Jiang M, Ren L, Chen H. (2018) Transcriptome profiling of genes related to light-induced anthocyanin biosynthesis in eggplant (*Solanum melongena* L.) before purple color becomes evident. BMC Genomics 19, 201.

Li, C., Schilmiller, A. L., Liu, G., Lee, G. I., Jayanty, S., Sageman, C., Vrebalov, J., Giovannoni, J. J., Yagi, K., Kobayashi, Y., & Howe, G. A. (2005). Role of beta-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. *The Plant cell*, *17*(3), 971–986. https://doi.org/10.1105/tpc.104.029108

Liao Y, Smyth GK, Shi W. (2019) The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res.;47(8):e47. doi:10.1093/nar/gkz114

Lin B., and Xiao Y. (1995). Sources of resistance to Verticillium wilt in Solanum melongena and its affinities identified by improved root dip method. Capsicum and Eggplant Newsl. 14: 81-84.

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z. (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics. 2012 Sep 15;28(18):2397-9. doi: 10.1093/bioinformatics/bts444. Epub 2012 Jul 13. PMID: 22796960.

Liu J, Zheng Z, Zhou X, Feng C, Zhuang Y (2015) Improving the resistance of eggplant Solanum melongena to Verticillium wilt using wild species Solanum linnaeanum. Euphytica 2013:463–469

Liu S.Y., Chen J.Y., Wang J.L., Li L., Xiao H.L., Adam S.M., Dai X.F., (2013) Molecular characterization and functional analysis of a specific secreted protein from highly virulent defoliating Verticillium dahlia Gene 529:307–316

Liu, B., Zhang, S., Zhu, X., Yang, Q., Wu, S., Mei, M., Mauleon, R., Leach, J., Mew, T. and Leung, H. (2004) Candidate defense genes as predictors of quantitative blast resistance in rice. Mol. Plant-Microbe Interact. 17: 1146–1152

Liu, J., Zheng Z., Zhou X., Feng C., Zhuang Y. (2015). Erratum to: Improving the resistance of eggplant (Solanum melongena) to Verticillium wilt using wild species Solanum linnaeanum. Euphytica. 201. 10.1007/s10681-014-1234-x.

Liu, X., Huang, M., Fan, B., Buckler, E. S. & Zhang, Z. (2016) Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. PLOS Genetics 12, e1005767.

Lo Scalzo R, Fibiani M, Francese G, D'Alessandro A, Rotino GL, Conte P, Mennella G. (2016) Cooking influence on physico-chemical fruit characteristics of eggplant (Solanum melongena L.). Food Chem. Mar 1;194:835-42. doi: 10.1016/j.foodchem.2015.08.063. Epub 2015 Aug 20. PMID: 26471625.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology, 15(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Lund P, Dunsmuir P. (1992) A plant signal sequence enhances the secretion of bacterial ChiA in transgenic tobacco. Plant Mol Biol. 1992 Jan;18(1):47-53. doi: 10.1007/BF00018455. PMID: 1731977.

Madalageri, B.B., Sharmatti, P.R., Madalageri, M.B., and Padaganur, G.M. (1988). Reaction of eggplant genotypes to *Cercospora solani* and *Leucinodes orbonalis*. Plant Pathology Newsletter 6 (1-2): 26-27.

Mahrshi, R.P., Mathur, A.K., and Singh, G. (1980). Note on the evaluation of resistance to powdery mildew in eggplant. Indian J. Agric. Sci. 50 (3): 280-281.

Makino, A. (2011) Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. Plant Physiol. 155, 125–129.

Mandhare VK, Patil PL (1993) Varietal screening and efficacy of fungicides against Fusarium wilt of brinjal. J Maharashtra Agric Univ 18:34–36

Mangin, B., Siberchicot, A., Nicolas, S., Doligez, A., This, P., & Cierco-Ayrolles, C. (2012). Novel measures of linkage disequilibrium that correct the bias due to population structure and relatedness. Heredity, 108(3), 285–291. https://doi.org/10.1038/hdy.2011.73

Markakis, E. A., Fountoulakis, M. S., Daskalakis, G. C., Kokkinis, M., & Ligoxigakis, E. K. (2016). The suppressive effect of compost amendments on Fusarium oxysporum f. sp. radicis-cucumerinum in cucumber and Verticillium dahliae in eggplant. Crop Protection, 79, 70-79.

Martin G., Bogdanove A., Sessa G. (2003). Understanding the Functions of Plant Disease Resistance Proteins. Annual review of plant biology. 54. 23-61. 10.1146/annurev.arplant.54.031902.135035.

Martin, C., & Paz-Ares, J. (1997). MYB transcription factors in plants. Trends in Genetics, 13(2), 67-73.

Martin, F. W., & Rhodes, A. M. (1979). Subspecific grouping of eggplant cultivars. Euphytica, 28(2), 367-383.

Matuo T and Ishigami K. (1958). On the wilt of *Solanum melongena* L. and its causal fungus *Fusarium oxysporum* f. *melongenae* n. f. *Jpn J Phytopathol*23:189–192.

Mauceri A, Abenavoli MR, Toppino L, Panda S, Mercati F, Aci MM, Aharoni A, Sunseri F, Rotino GL, Lupini A. (2021) Transcriptomics reveal new insights into molecular regulation of nitrogen use efficiency in Solanum melongena. J Exp Bot. May 28;72(12):4237-4253. doi: 10.1093/jxb/erab121. PMID: 33711100.

Mauceri A., Bassolino L., Lupini A., Badeck F., Rizza F., Schiavi M., Toppino L., Abenavoli M., Rotino GL., Sunseri F. (2019) Genetic variation in eggplant (Solanum melongena L.) for Nitrogen Use Efficiency (NUE) under contrasting NO3- supply. Journal of integrative plant biology. DOI: 10.1111/jipb.12823

Maynard, D.N. (1987). Commercial vegetable cultivars for Florida. Univ. of Florida Coop. Ext. Serv., IFAS, Circular 530.

McDowell JM, Dangl JL. (2000) Signal transduction in the plant immune response. Trends Biochem Sci. 2000 Feb;25(2):79-82. doi: 10.1016/s0968-0004(99)01532-7. PMID: 10664588.

McKeen CD (1972). Observations on the invasion and subsequent development of Verticillium albo-atrum and Verticillium dahliae in eggplant, tomato and potato. Proceedings of the Canadian phytopathological Society 39,21.

MeiXiu G., ShuHe L., YuQin L., ShiHai S., RenShun Z., FengMei B., and XiuFeng Y.. (2001). Effects of different stocks on resistance, physical activity and output of eggplant. Acta Hort. Sinica 28 (5): 463-465.

Melotto M, Underwood W, Koczan J, Nomura K, He SY. (2006) Plant stomata function in innate immunity against bacterial invasion. Cell. 2006 Sep 8;126(5):969-80. doi: 10.1016/j.cell.2006.06.054. PMID: 16959575.

Messiaen, C.M. (1975). L'aubergine. P 231-249. In: *Le Potager tropical, 2. Cultures spéciales*. Presses Universitaires de France, Coll. Techniques vivantes.

Meyer, R. S., A. E. DuVal, and H. R. Jensen. (2012b). Patterns and processes in crop domestication: An historical review and quantitative analysis of 203 global food crops. New Phytologist 196: 29– 48.

Meyer, R. S., K. G. Karol, D. P. Little, M. H. Nee, and A. Litt. (2012a). Phylogeographic relationships among Asian eggplants and new perspectives on eggplant domestication. Molecular Phylogenetics and Evolution 63: 685– 701.

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW. (2003) Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell. Apr;15(4):809-34. doi: 10.1105/tpc.009308. Erratum in: Plant Cell. 2003 Jul;15(7):1683. PMID: 12671079; PMCID: PMC152331.

Mian, I.H., Ali, M., and Akhter, R. (1995). Grafting on *Solanum* rootstocks to control root-knot of tomato and bacterial wilt of eggplant. Bull. of the Inst. of Trop. Agric., Kyushu Univ. 18: 41-47.

Michael, T. P. , Jupe, F. , Bemm, F. , Motley, S. T. , Sandoval, J. P. , Lanz, C. , Loudet, O. , Weigel, D. , & Ecker, J. R. (2018). High contiguity *Arabidopsis thaliana* genome assembly with a single nanopore flow cell. *Nature Communications*, 9, 541. 10.1038/s41467-018-03016-2

Michałojć Z., Buczkowska H. (2012): The nutritional status of eggplant (*Solanum melongena* L.) depending on plant training method and nitrogen fertilization. Acta Scientiarum Polonorum, Hortorum Cultus, 11: 109–119.

Michelmore, R. W., Paran, I., & Kesseli, R. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the national academy of sciences, 88(21), 9828-9832.

Miles, C., Wimer, J. and Inglis, D. (2015). GRAFTING EGGPLANT AND TOMATO FOR VERTICILLIUM WILT RESISTANCE. Acta Hortic. 1086, 113-118 DOI: 10.17660/ActaHortic.2015.1086.13

Miller ND, Parks BM, Spalding EP (2007) Computer-vision analysis of seedling responses to light and gravity. Plant J 52: 374–381

Mittler R., Herr E.H., Orvar B.L., Van Camp W., Willekens H., Inzé D., Ellis B.E. (1999) Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc. Natl. Acad. Sci. USA.;96:14165–14170. doi: 10.1073/pnas.96.24.14165.

Miyatake K, Saito T, Negoro S, Yamaguchi H, Nunome T, Ohyama A, Fukuoka H (2012) Development of selective markers linked to a major QTL for parthenocarpy in eggplant (Solanum melongena L.). TAG Theor Appl Genet 124:1–11 . doi: 10.1007/s00122-012-1796-8

Miyatake K, Saito T, Negoro S, Yamaguchi H, Nunome T, Ohyama A, Fukuoka H (2016). Detailed mapping of a resistance locus against Fusarium wilt in cultivated eggplant Solanum melongena. Theor Appl Genet 129(2):35767. doi: 10.1007/s00122-015-2632-8.

Miyatake S, Koshimizu E, Hayashi YK, Miya K, Shiina M, Nakashima M, et al. (2014) Deep sequencing detects very-low-grade somatic mosaicism in the unaffected mother of siblings with nemaline myopathy. Neuromuscul Disord; 24(7):642-7.

Mochizuki, H., and Yamakawa, K. (1979a). Potential utilization of bacterial wilt resistant *Solanum* species as rootstocks for commercial eggplant production. Bull. Veg. Ornamental Crop Res. Stn. A. 6: 18.

Mochizuki, H., and Yamakawa, K. (1979b). Resistance of selected eggplant cultivars and related wild *Solanum* species to bacterial wilt (*Pseudomonas solanacearum*). Bull. Veg Ornamental Crop Res. Stn. A. 6: 10.

Mochizuki, H., Sakata, Y., Yamakawa, K., Nishio, T., Komochi, S., Nariakawa, T., and Monma, S. (1997). Eggplant parental line 1, and eggplant breeding line resistant to *Fusarium* wilt. Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea 12: 85-90.

Mohamed, Moawad. (2018) Integrated control of the nematode Meloidogyne incognita infecting eggplant and effects on associated weeds and crop yield; a field study. Agricultural Engineering International: CIGR Journal 19.5: 125-131.

Mohammed, M., and Sealy. (1986). Extending the shelf life of melongene using polymeric films. Trop. Agr. 63:36-40.

Moncada A., Miceli A., Vetrano F., Mineo V., Planeta D., D'Anna F. (2013) Scientia Horticulturae 149 108–114

Monma, S., Akazawa, S., Simosaka, K., Sakata, Y., and Matsunaga, H. (1997). 'Diataro', a bacterial wilt- and *Fusarium* wilt-resistant hybrid eggplant for rootstock. Bulletin of the National

Research Institute of Vegetables, Ornamental Plants and Tea. Series A: Vegetables and Ornamental Plants (12): 73-83.

Monma, S., Sato, T., and Matsunaga, H. (1996). Evaluation of resistance to bacterial, *Fusarium* and *Verticillium* wilt in eggplant and eggplant-related species collected in Ghana. *Capsicum* and Eggplant Newsl. 15: 71-72.

Morot-Gaudry-Talarmain Y, Rockel P, Moureaux T, Quilleré I, Leydecker MT, Kaiser WM, Morot-Gaudry JF. (2002) Nitrite accumulation and nitric oxide emission in relation to cellular signaling in nitrite reductase antisense tobacco. Planta. 2002 Sep;215(5):708-15. doi: 10.1007/s00425-002-0816-3. Epub 2002 Jul 9. PMID: 12244435.

Mur, L.A.; Simpson, C.; Kumari, A.; Gupta, A.K.; Gupta, K.J. (2017) Moving nitrogen to the centre of plant defence against pathogens. Ann. Bot., 119, 703–709.

Mutlu N, Boyaci FH, Göçmen M, Abak K (2008). Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. Theor Appl Genet. 117:1303-1312.

Naegele RP, Boyle S, Quesada-Ocampo LM, Hausbeck MK (2014) Genetic Diversity, Population Structure, and Resistance to *Phytophthora capsici* of a Worldwide Collection of Eggplant Germplasm. PLoS ONE 9(5): e95930. https://doi.org/10.1371/journal.pone.0095930

Navarre D.A. and Mayo D. (2004). Differential characteristics of salicylic acid-mediated signaling in potato, Physiol. Mol. Plant Pathol., 64:179-188

Nelson, P. E. (1981). Life cycle and epidemiology of Fusarium oxysporum, p. 51–80. In M. E. Mace, A. A. Bell, and C. H. Beckman (ed.), Fusarium wilt diseases of plants. Academic Press, Inc., New York, N.Y.

Nelson, P. E., T. A. Toussoun, and W. F. O. Marasas. (1983). Fusarium species: an illustrated manual for identification. The Pennsylvania State University Press, University Park.

Neumann, S.; Paveley, N.; Beed, F.; Sylvester-Bradley, R. (2004) Nitrogen per unit leaf area affects the upper asymptote of Puccinia striiformis f. sp. tritici epidemics in winter wheat. Plant Pathol. 53, 725–732.

Nirenberg, H. I. (1990). Recent advances in the taxonomy of Fusarium. Stud. Mycol. **32:**91–101.

Nirenberg, H. I. (1990). Recent advances in the taxonomy of Fusarium. Stud. Mycol. 32:91–101.

Noman A., Aqeel M., Qari SH., Al Surhanee AA., Yasin G., Alamri S., Hashem M:, Al-Saadi AM., (2020) Plant hypersensitive response vs pathogen ingression: Death of few gives life to others, Microbial Pathogenesis, Volume 145, 104224, ISSN 0882-4010, https://doi.org/10.1016/j.micpath.2020.104224.

Nunome T, Ishiguro K, Yoshida T, Hirai M (2001) Mapping of fruit shape and color development traits in eggplant (Solanum melongena L.) based on RAPD and AFLP markers. Breeding Science 51: 19-26

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A, Fukuoka H (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (Solanum melongena L.). Theoretical and Applied Genetics 119: 1143-1153

Nunome T, Suwabe K, Iketani H, Hirai M (2003a) Identification and characterization of microsatellites in eggplant. Plant Breeding 122: 256-262

Nunome, T., Suwabe, K., Ohyama, A., Fukuoka, H. (2003b) Characterization of trinucleotide microsatellites in Eggplant Breeding Science 53: 77-83

Ohara, T. & Tsuge, T. (2004) FoSTUA, encoding a basic helix- loop- helix protein, differentially regulates development of three kinds of asexual spores, macroconidia, microconidia, and chlamydospores, in the fungal plant pathogen Fusarium oxysporum. Eukaryotic Cell,3, 1412– 1422

Pal S., Saimbhi M.S., Bal S.S., (2002). Effect of nitrogen and phosphorus levels on growth and yield of brinjal hybrid (Solanum melongena L.). J.Veg. Sci. 29, 90–91

Pandey A. and Dubey S. (2018). Inheritance and Molecular Mapping of Fusarium Wilt Resistance Gene in Brinjal (Solanum melongena). Int. J. Pharm. Life Sci., 9(4):5772-5778.

Panero M. (1981). Peperone e Melanzana. La tecnica di coltivazione e la difesa antiparassitaria, 109-203.

Papasolomontos, A. & Baudoin, Wilfried & Lutaladio, N. & Castilla, N. & Baeza, Esteban & Montero, J.I. & Teitel, M. & Lopez, J.C. & Kacira, Murat & Kittas, Constantinos & Katsoulas, Nikolaos & Bartzanas, Thomas & Bakker, S. & Leonardi, Cherubino & Maggio, Albino & Gallardo, M. & Thompson, R.B. & M.D.FernЈndez, & Abou Hadid, Ayman & Patsalos, K.. (2013). Good Agricultural Practices for Greenhouse Vegetable Crops: Principles for Mediterranean Climate Areas Volume 217 of FAO plant production and protection papers, ISSN 0259-2517

Passardi F, Penel C, Dunand C. (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci. 2004 Nov;9(11):534-40. doi: 10.1016/j.tplants.2004.09.002. PMID: 15501178.

Patterson K.; Cakmak T.; Cooper A.; Lager I.; Rasmusson A.G.; Escobar M.A. (2010) Distinct signalling pathways and transcriptome response signatures differentiate ammonium-and nitrate-supplied plants. Plant Cell Environ. 33, 1486–1501.

Pautasso, M., Doring, T. F., Garbelotto, M., Pellis, L., and Jeger, M. J. (2012) Impacts of climate change on plant diseases-opinions and trends. Eur. J. Plant Pathol. 133:295-313.

Pessarakli M. (1999): Handbook of plant and crop stress. New York, Marcel Dekker Inc.

Pessarakli M., Dris R. (2003): Effects of pruning and spacing on the yield and quality of eggplant. Food, Agriculture and Environment, 1: 215−216.

Pietro A.D.; Madrid M.P.; Caracuel Z.; Delgado-Jarana J.; Roncero M.I.G. (2003) Fusarium oxysporum: Exploringthe molecular arsenal of a vascular wilt fungus. Mol. Plant Pathol. 4, 315–325.

Pirrung MC. (1999) Histidine kinases and two-component signal transduction systems. Chem Biol. 1999 Jun;6(6):R167-75. doi: 10.1016/S1074-5521(99)80044-1. PMID: 10375545.

Pizano M, Banks J (eds) (2008) Phasing out methyl bromide in Turkey. Final report

Plazas M., Vilanova S., Gramazio P., Rodríguez-Burruezo A., Fita A., Herraiz F. J., ... & Kouassi B. (2016). Interspecific hybridization between eggplant and wild relatives from different genepools. Journal of the American Society for Horticultural Science, 141(1), 34-44.

Portis E., Barchi L., Toppino L., Lanteri S., Acciarri N., Felicioni N.,et al. (2014).QTL Mapping in eggplant reveals clusters of yield-related loci and orthology with the tomato genome. *PLoSONE* 9:e89499. doi: 10.1371/journal.pone.0089499

Portis E., Cericola F., Barchi L., Toppino L., Acciarri N., Pulcini L., et al. (2015). Association Mapping for Fruit, plant and leaf morphology traits in eggplant *PLoSONE* 10:E0135200. doi:10.1371/journal.pone.0135200

Prohens J, Rodríguez-Burruezo A, Raigón MD, Nuez F. (2007) Total Phenolic Concentration and Browning Susceptibility in a Collection of Different Varietal Types and Hybrids of Eggplant: Implications for Breeding for Higher Nutritional Quality and Reduced Browning. J Am Soc Hortic Sci; 132: 638–646.

Prohens J, Whitaker BD, Plazas M, Vilanova S, Hurtado M, Blasco M, Gramazio P, Stommel JR (2013). Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, Solanum melongena, and its wild ancestor (S. incanum). Ann Appl Biol 162:242-257

Qian Z, Zhang B, Chen H, Lu L, Duan M, Zhou J, Cui Y, Li D. (2021) Identification of Quantitative Trait Loci Controlling the Development of Prickles in Eggplant by Genome Re-sequencing Analysis. Front Plant Sci. Sep 8;12:731079. doi: 10.3389/fpls.2021.731079. PMID: 34567042; PMCID: PMC8457335.

Radman R, Saez T, Bucke C, Keshavarz T. (2003) Elicitation of plants and microbial cell systems. Biotechnol Appl Biochem. Feb;37(Pt 1):91-102. doi: 10.1042/ba20020118. PMID: 12578556.

Rakha M., Prohens J., Taher D., Wu T.H., Solberg S.Ø. (2021): Eggplant (Solanum melongena, S. aethiopicum and S. mac rocarpon) breeding. In: Advances in Plant Breeding Strategies: Vegetable Crops. Cham, Springer: 163–203.

Ranil R. H. G., Prohens J., Aubriot X., Niran H. M. L., Plazas M., Fonseka R. M., ... & Knapp S. (2017). Solanum insanum L. (subgenus Leptostemonum Bitter, Solanaceae), the neglected wild progenitor of eggplant (S. melongena L.): a review of taxonomy, characteristics and uses aimed at its enhancement for improved eggplant breeding. Genetic resources and crop evolution, 64(7), 1707-1722.

Raskin I. (1992). Salicylate, a new plant hormone. *Plant physiology*, *99*(3), 799–803. https://doi.org/10.1104/pp.99.3.799

Rizza F, Mennella G, Collonnier C, Shiachakr D, Kashyap V, Rajam MV, Prestera M, Rotino GL (2002). Androgenic dihaploids from somatic hybrids between Solanum melongena and S. aethiopicum group gilo as a source of resistance to Fusarium oxysporum f. sp. melongenae. Plant Cell Reports 2011:1022-1032.

Robinson J.T, Thorvaldsdóttir H, Winckler W, Guttman M, Lander E.S., Getz G, Mesirov J.P. (2011). Integrative Genomics Viewer. Nature Biotechnology 29, 24–26 .

Robinson M.M., Shah S., Tamot B., Pauls K.P., Moffatt B.A. and Glick B.R. (2001) Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase Mol. Plant Pathol. 2, 135–145.

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics (Oxford, England), 26(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Rotino GL, Gleddie S. (1990) Transformation of eggplant (Solanum melongena L.) using a binary Agrobacterium tumefaciens vector. Plant Cell Rep; 9. doi:10.1007/BF00232129.

Rotino GL, Mennella G., Fusari F., Vitelli G., Tacconi M.G., D'Alessandro A., Acciarri N., (2001). Towards introgression of resistance to Fusarium oxysporum F. sp. melongenae from Solanum integrifolium into eggplant. In: Proceedings of the 11th Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant, Antalya, Turkey, pp. 303–307

Rotino GL, Rizza F., Mennella G., Tacconi M.G.,Alberti P., D'Alessandro A., Acciarri N., Toppino L., (2004). Production and utilization of sexual "doubled hybrid" between the somatic hybrids S. melongena (+) S.integrifolium and S. melongena (+) S. aethiopicum gr.gilo. EUCARPIA, XIIth Meeting on Genetics and Breeding of Capsicum & Eggplant. Noordwijkerhout-The Netherlands, 17-19 May 2004; pp 203-209

Rotino GL, Sala T, Toppino L, (2014). Eggplant book chapter 16, In A, Pratap and J, Kumar eds, Alien Gene Transfer in Crop Plants, Volume 2: 381, Achievements and Impacts, DOI 10,1007/978-1-4614-9572-7_16, © Springer Science+Business Media, LLC 2014.

Rotino GL, Sihachakr D, Rizza F, Vale' GP, Tacconi MG, Alberti P, Mennella G, Sabatini E, Toppino L, D'alessandro A, Acciarri N (2005) Current status in production and utilization of dihaploids from somatic hybrids between eggplant (Solanum melongena L) and its wild relatives. Acta Physiol Plant 27(4B):723–733

Rotino GL; Lanteri S; Sala T; Toppino L; Acquadro A; Barchi L; Portis E; Rinaldi R; Scaglione D; Dal Molin A; Minio A; Ferrarini A; Tononi P; Zamperin G; Fantini E; Pietrella M; Giuliano G; Delledonne M. (2017) An Eggplant (Solanum melongena L.) High Quality Genome Draft. Proceedings of the Plant and Animal Genome XXIInd Conference. 10-15 January PAG San Diego California USA.

Rotino, G. L., Perri, E., Zottini, M., Sommer, H., & Spena, A. (1997). Genetic engineering of parthenocarpic plants. Nature biotechnology, 15(13), 1398-1401.

Rowe R. C., Farely J. D., and Coplin D. C. (1977). Airborne spore dispersal and recolonization of steamed soil by Fusarium oxysporum in greenhouses. Phytopathology 67:1513–1517.

Ruan J, Li H. (2020) Fast and accurate long-read assembly with wtdbg2. Nat Methods. 2020 Feb;17(2):155-158. doi: 10.1038/s41592-019-0669-3. Epub 2019 Dec 9. PMID: 31819265; PMCID: PMC7004874.

Ruan, J.; Zhou, Y.; Zhou, M.; Yan, J.; Khurshid, M.; Weng, W.; Cheng, J.; Zhang, K. Jasmonic (2019) Acid Signaling Pathway in Plants. *Int. J. Mol. Sci.* 2019, *20*, 2479. https://doi.org/10.3390/ijms20102479

Saini D. K. and Kaushik P. (2019)Visiting eggplant from a biotechnological perspective: A review[J]. Scientia Horticulturae, 2019, 253 : 327-340.

Sakata Y, Monma S, Narikawa T, Komochi S.. (1996). Evaluation of resistance to bacterial wilt and Verticillium wilt in eggplants (Solanum melongena L.) collected in Malaysia. J Jpn Soc Hort Sci. 65:81–88

Sakata, Y., Nishio, T., and Monma, S. (1989). Resistance of *Solanum* species to *Verticillium* wilt and bacterial wilt. Proceedings of the 8th Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant: 177-181.

Salamov AA, Solovyev VV (2000). Ab initio gene finding in Drosophila genomic DNA. Genome Res. 2000 Apr;10(4):516-22. doi: 10.1101/gr.10.4.516. PMID: 10779491; PMCID: PMC310882.

Salgon S, Raynal M, Lebon S, Baptiste JM, Daunay MC, Dintinger J, Jourda C (2018) Genotyping by sequencing highlights a polygenic resistance to Ralstonia pseudosolanacearum in eggplant (Solanum melongena L.). Int J Mol Sci 19:357 . doi: 10.3390/ijms19020357

Särkinen TS, Bohs L, Olmstead RG, Knapp S (2013) A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. BMC Evolutionary Biology 13: 214. doi: 10.1186/1471-2148-13-214

Sat P., Saimbhi M.S., (2003) Effect of varying levels of nitrogen and phosphorus on earliness and yield of brinjal hybrids. J. Res. Crops. 4(2):217-222

Scaglione D, Reyes-Chin-Wo S, Acquadro A, Froenicke L, Portis E, Beitel C, Tirone M, Mauro R, Lo Monaco A, Mauromicale G, et al. (2016). The genome sequence of the outbreeding globe artichoke constructed de novo incorporating a phase-aware low-pass sequencing strategy of F_1 progeny. *Sci Rep* 6: 19427

Scheible W.-R.; Morcuende R.; Czechowski T.; Fritz C.; Osuna D.; Palacios-Rojas N.; Schindelasch D.; Thimm O.; Udvardi M.K.; Stitt M. (2004) Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. Plant Physiol., 136, 2483–2499.

Scheideler M, Schlaich NL, Fellenberg K, Beissbarth T, Hauser NC, Vingron M, Slusarenko AJ, Hoheisel JD. (2002) Monitoring the switch from housekeeping to pathogen defense metabolism in Arabidopsis thaliana using cDNA arrays. J Biol Chem. Mar 22;277(12):10555-61. doi: 10.1074/jbc.M104863200. Epub 2001 Dec 17. PMID: 11748215.

Segura V, Vilhjálmsson BJ, Platt A, Korte A, Seren Ü, Long Q, Nordborg M. (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat Genet. 2012 Jun 17;44(7):825-30. doi: 10.1038/ng.2314. PMID: 22706313; PMCID: PMC3386481.

Sekara A, Cebula S, Kunicki E. (2007) Cultivated eggplants – origin, breeding objectives and genetic resources, A Review, Folia Horticulture Ann, 19(1): 97-114.

Sen, S.; Churchill, G.A. (2001) A statistical framework for quantitative trait mapping. ´ Genetics 2001, 159, 371–387.

Sharma P. D. (2004) Plant pathology. New Delhi, India. Rastogi Publication. ISBN 81-7133-700-7.

Sharma, H.C., Crouch, J.H., Sharma, K.K., Seetharama, N., Hash, C.T., (2002). Applications of biotechnology for crop improvement: prospects and constraints. Plant Sci. 163, 381–395.

Shephard M. C., Wood R. K. S. (1963). The effect of environment and nutrition of pathogen and host, in the dampingoff of seedlings by Rhizoctonia solani. Ann. Appl. Biol. 51:389-402

Sihachakr D, Daunay MC, Serraf L, Chaput MH, Mussio I, Haicour R, Rossignol L, Ducreux G (1994). Somatic hybridization of eggplant (Solanum melongena L), with its close and wilt relatives, In: Bajaj, Y,P,S, (ed), Biotechnology in Agriculture and Forestry, Somatic Hybridization in Crop Improvement, Springer, Berlin.

Simmonds NW (1993) G. Kalloo and B. O. Bergh (Editors) Genetic improvement of vegetable crops. Pergamon Press, Oxford and New York, 1993, £85, 833 pp. ISBN 0 08 040826 5. Phytochemical Analysis 4: 293-293

Simons M, Saha R, Guillard L, Clément G, Armengaud P, Cañas R, Maranas CD, Lea PJ, Hirel B. 2014. Nitrogen-use efficiency in maize (*Zea mays* L.): from 'omics' studies to metabolic modelling. Journal of Experimental Botany 65, 5657–5671.

Singh S.S., Gupta P., Gupta A.K., (2003) Handbook of Agricultural Sciences. Kalyani Publishers, New Delhi, India. p. 184-185

Singh, S., Kumar, V., Kapoor, D., Kumar, S., Singh, S., Dhanjal, D.S., Datta, S., Samuel, J., Dey, P., Wang, S., Prasad, R. and Singh, J. (2020), Revealing on hydrogen sulfide and nitric oxide signals co-ordination for plant growth under stress conditions. Physiol Plantarum, 168: 301-317. https://doi.org/10.1111/ppl.13002

Smith, I.M., J. Dunez, D.H. Phillips, R.A. Lelliott, and S.A. Archer, Eds. (1988). European Handbook of Plant Diseases. Blackwell Scientific Publications: Oxford. 583pp.

Snoeijers S.S.; Pérez-García A.; Joosten M.H.; DeWit P.J. (2000) The effect of nitrogen on disease development and gene expression in bacterial and fungal plant pathogens. Eur. J. Plant Pathol., 106, 493–506.

Solomon P.S.; Tan K.C.; Oliver R.P. (2003) The nutrient supply of pathogenic fungi; a fertile field for study. Mol. Plant Pathol. 4, 203–210.

Song B., Song Y., Fu Y., Kizito E. B., Kamenya S. N., Kabod P. N., ... & Maina S. (2019). Draft genome sequence of Solanum aethiopicum provides insights into disease resistance, drought tolerance, and the evolution of the genome. GigaScience, 8(10), giz115.

Stagel A., E. Portis, L. Toppino, G. Rotino and S. Lanteri (2008) Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9:

Steekelenburg NV (1976) Fusarium wilt of eggplant in the Netherlands. Netherlands J Plant Pathol 82:191–192

Stout M.J.; Brovont R.A.; Duffey S.S. (1998) Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, Lycopersicon esculentum. J. Chem. Ecol. 24, 945–963.

Stravato VM, Cappelli C, Polverari A (1993). Attacchi di Fusarium oxysporum f, sp, melongenae agente della tracheofusariosi della melanzana in Italia centrale, Informatore Fitopatol, 4310:51-54.

Sulli M, Barchi L, Toppino L, Diretto G, Sala T, Lanteri S, Rotino GL, Giuliano G. (2021) An Eggplant Recombinant Inbred Population Allows the Discovery of Metabolic QTLs Controlling

Fruit Nutritional Quality. Front Plant Sci. 2021 May 17;12:638195. doi: 10.3389/fpls.2021.638195. PMID: 34079565; PMCID: PMC8166230.

Sun Y.; Wang M.; Mur L.A.J.; Shen Q.; Guo S. (2020) Unravelling the Roles of Nitrogen Nutrition in Plant Disease Defences. *Int. J. Mol. Sci.* 2020, *21*, 572. https://doi.org/10.3390/ijms21020572

Sunseri F., Sciancalepore A., Martelli G., Acciarri N., Rotin G.L, Valentino D., and Tamietti, G. (2003). Development of RAPD-AFLP map of eggplant and improvement of tolerance to Verticillium wilt. In Hammerschlag FA, Saxena P. (eds.). Acta Hort. 625:107-110.

Swati Tyagi, Robin Kumar, Vivak Kumar, So Youn Won & Pratyoosh Shukla (2021) Engineering disease resistant plants through CRISPR-Cas9 technology, GM Crops & Food, 12:1, 125-144, DOI: 10.1080/21645698.2020.1831729

Syfert M. M., Castañeda-Álvarez N. P., Khoury C. K., Särkinen T., Sosa C. C., Achicanoy H. A., et al. (2016). Crop wild relatives of the brinjal eggplant (Solanum melongena): poorly represented in genebanks and many species at risk of extinction. Am. J. Bot. 103, 635–651. doi:

Taher D., Solberg S. Ø., Prohens J., Chou Y. Y., Rakha M., & Wu T. H. (2017). World Vegetable Center Eggplant Collection: Origin, Composition, Seed Dissemination and Utilization in Breeding. *Frontiers in plant science*, *8*, 1484. https://doi.org/10.3389/fpls.2017.01484

Tao Y., Xie Z., Chen W., Glazebrook J., Chang H., Han B., Zhu T., Zou G., Katagiri F., (2003) Quantitative Nature of Arabidopsis Responses during Compatible and Incompatible Interactions with the Bacterial Pathogen *Pseudomonas syringae* , *The Plant Cell*, Volume 15, Issue 2, February 2003, Pages 317–330, https://doi.org/10.1105/tpc.007591

Thomma BP, Penninckx IA, Broekaert WF, Cammue BP. (2001) The complexity of disease signaling in Arabidopsis. Curr Opin Immunol. 2001 Feb;13(1):63-8. doi: 10.1016/s0952-7915(00)00183-7. PMID: 11154919.

Toppan A., Roby D., Esquerré-Tugayé M. (1982), Cell Surfaces in Plant-Microorganism Interactions : III. *In Vivo* Effect of Ethylene on Hydroxyproline-Rich Glycoprotein Accumulation in the Cell Wall of Diseased Plants, *Plant Physiology*, Volume 70, Issue 1, July 1982, Pages 82–86, https://doi.org/10.1104/pp.70.1.82

Toppino L., Barchi L.; Mercati F.; Acciarri N.; Perrone D.; Martina M.; Gattolin S.; Sala T.; Fadda S.; Mauceri A., Ciriaci T., Carimi F., Portis E., Sunseri F., Lanteri S., Rotino G.L. (2020) A New Intra-Specific and High-Resolution Genetic Map of Eggplant Based on a RIL Population, and Location of QTLs Related to Plant Anthocyanin Pigmentation and Seed Vigour. *Genes*. 2020; 11(7):745. https://doi.org/10.3390/genes11070745.

Toppino L., Barchi, L., Scalzo, R. L., Palazzolo, E., Francese, G., Fibiani, M., Pulcini, L. Sala, T., Acciarri, N., Portis, E., Lanteri, S., Mennella, G., Rotino, Gl. (2016). Mapping Quantitative Trait Loci Affecting Biochemical and Morphological Fruit Properties in Eggplant (*Solanum Melongena L.*). Frontiers In Plant Science, 7:256. Doi: 10.3389/Fpls.2016.00256

Toppino L., Prohens J., Rotino G.L., Plazas M., Parisi M., Carrizo-Garcia C., Tripodi P. (2019) Pepper and eggplant genetic resources. In: The Wild Solanums Genomes. Domenico Carputo; Riccardo Aversano; Maria Raffaella Ercolano Editors.

Toppino L., Ribolzi S., Bassolino L., Rotino G.L. (2017) Development of an introgression population of eggplant from the wild species S. indicum. Proceedings of the joint congress SIBV-

SIGA (Italian Society of Agricultural Genetics) Annual Congress. Pisa, 19-22 Settembre 2017. Poster communication 6.41

Toppino L., Ribolzi S., Shaaf S., Rossini L., Boyaci HF., Caliskan S., Unlu A., Bassolino L., Irdani T., Fadda S., Rotino G.L. (2019) Introgression breeding in eggplant from S. tomentosum and genetic mapping of novel key agronomical traits. In: Proceedings of the 17th Eucarpia meeting on Genetics and Breeding of Capsicum and Eggplant; Avignon (France) 10-13 September 2019, Lefebvre and Daunay ed. pp 232-233.

Toppino L., Valè GP., Rotino GL. (2008). Inheritance of Fusarium wilt resistance introgressed from Solanum aethiopicum Gilo and Aculeatum groups into cultivated eggplant S. melongena and development of associated PCR-based markers. Molecular Breeding. 222:237-250, DOI 10,1007/s11032-008-9170-x.

Trentini L., Montanari V., 1996. L'innesto nelle solanacee e nelle cucurbitacee. L'Informatore Agrario 29, 69–75

Urrutia Herrada MT, Gomez Garcia VM, Tello Marquina J (2004). Fusarium wilt on eggplant in Almeria (Spain). Boletin de Sanidad Vegetal, Plagas, 30:85–92.

Vadivel, E., and Bapu, J.R.K. (1989). Evaluation and documentation of eggplant germplasm. *Capsicum* Newsl. 7: 80.

van Loon LC, Rep M, Pieterse CM. (2006) Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol.;44:135-62. doi: 10.1146/annurev.phyto.44.070505.143425. PMID: 16602946.

van Ooijen G., Mayr G., Kasiem M., Albrecht M., Cornelissen B., Takken F. (2007) Structure–function analysis of the NB-ARC domain of plant disease resistance proteins, *Journal of Experimental Botany*, Volume 59, Issue 6, April 2008, Pages 1383–1397, https://doi.org/10.1093/jxb/ern045

Vavilov, N. I. (1951). The origin, variation, immunity and breeding of cultivated plants (translated from Russian by K. Starr Chester). *Chronica Botanica* **13**: 1– 366.

Vilanova S, Manzur JP, Prohens J (2012) Development and characterization of genomic simple sequence repeat markers in eggplant and their application to the study of diversity and relationships in a collection of different cultivar types and origins. Mol Breed: doi 10.1007/s11032–011–9650–2.

Vilanova S., Alonso D., Gramazio P., Plazas M., García F., Ferrante P., Schmidt M., Díez M., Usadel B., Giuliano G., Prohens J. (2020). SILEX: a fast and inexpensive high-quality DNA extraction method suitable for multiple sequencing platforms and recalcitrant plant species. Plant Methods 16, 110 https://doi.org/10.1186/s13007-020-00652-y

Vogel, G., LaPlant, K. E., Mazourek, M., Gore, M. A., & Smart, C. D. (2021). A combined BSA-Seq and linkage mapping approach identifies genomic regions associated with Phytophthora root and crown rot resistance in squash. Theoretical and Applied Genetics, 134(4), 1015-1031

von Wirén, N., Gazzarrini, S. & Frommer, W.B. (1997) Regulation of mineral nitrogen uptake in plants. *Plant and Soil* 196, 191–199 https://doi.org/10.1023/A:1004241722172

Vorontsova,M. S., Stern, S., Bohs, L., and Knapp, S. (2013). African spiny Solanum subgenus Leptostemonum, Solanaceae): a thorny phylogenetic tangle. Bot. J. Linn. Soc. 173, 176–193. doi: 10.1111/boj.12053

Walters, D.; Bingham, I. (2007) Influence of nutrition on disease development caused by fungal pathogens:implications for plant disease control. Ann. Appl. Biol. 151, 307–324.

Wang J.-X., T.-G. Gao, and S. Knapp. (2008). Ancient Chinese literature reveals pathways of eggplant domestication. *Annals of Botany* **102**: 891– 897.

Wang M., Gu Z., Wang R., Guo J., Ling N., Firbank L. G., & Guo S. (2018). Plant Primary Metabolism Regulated by Nitrogen Contributes to Plant–Pathogen Interactions. Plant and Cell Physiology, 60(2), 329-342.

Wange S.S., Kale R.H., (2004) Effect of bio fertilizers and nitrogen levels on brinjal crop. J. Soils. Crops. 14(1):9-11

Wasternack, C. and Hause, B. (2002). Jasmonates and octadecanoids: signals in plant stress responses and development, Prog. Nucleic Res. Mol. Biol., 72:165-221.

Weese T. L., and L. Bohs. (2010). Eggplant origins: Out of Africa, into the Orient. *Taxon* **59**: 49– 56

Weese, T. L., and L. Bohs. (2007). A three-gene phylogeny of the genus *Solanum* (Solanaceae). *Systematic Botany* 32: 445– 463.

Wei Q., Wang J., Wang W., Hu T., Hu H. & Bao C. (2020a) A high-quality chromosome-level genome assembly reveals genetics for important traits in eggplant. Horticulture Research, 7, 1–15. https://doi.org/10.1038/s41438-020-00391-0.

Wei Q.; Wang W.; Hu T.; Hu H.; Wang J.; Bao C. (2020b) Construction of a SNP-based genetic map using SLAFSeq and QTL analysis of morphological traits in eggplant. Front. Genet, 11, 178, doi:10.3389/fgene.2020.00178.

Weinhold A. R., Bowman T., Dodman R. L. (1969). Virulence of Rhizoctonia solani as affected by nutrition of the pathogen. Phytopathology 59: 1601-5

Weinhold A. R., Dodman R. L., Bowman T. (1972). Influence of exogenous nutrition on virulence of Rhizoctonia solani. Phytopathology 62:278-81

Wise RP, Moscou MJ, Bogdanove AJ, Whitham SA. (2007) Transcript profiling in host-pathogen interactions. Annu Rev Phytopathol.;45:329-69. doi: 10.1146/annurev.phyto.45.011107.143944. PMID: 17480183.

Wu F, Eannetta N, Xu Y, Tanksley S (2009) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theoretical and Applied Genetics 118: 927-935

Wu F, Mueller LA, Crouzillat D, Pétiard V, Tanksley SD. (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. *Genetics*;174(3):1407-1420. doi:10.1534/genetics.106.062455

Yamakawa K, Mochizuki H (1979) Nature and inheritance of Fusarium wilt resistance in eggplant cultivars and related wild Solanum species. Bull Veg Orn Crops Res Sta 6:19–27

Yang X.X.; Li Y.; Ren B.B.; Ding L.; Gao C.M.; Shen Q.R.; Guo S.W. (2012) Drought-induced root aerenchyma formation restrains water uptake in nitrate-supplied rice seedlings. Plant Cell Environ., 53, 495–504.

Yemm E. W., Willis A. J. (1956). The respiration of barley plants. IX: The metabolism of roots during the assimilation of nitrogen. New Phytol. 55:229-52

Yoshida T., Monma S., Matsunaga H., Sakata Y., Sato T., and Saito T. (2004b). Development of a new rootstock eggplant cultivar 'Daizaburou' with high resistance to bacterial wilt and Fusarium wilt. Bull. Natl. Inst. Vegetable and Tea Science 3: 199-211.

Yoshida T., Monma S., Matsunaga H., Sakata Y., Sato T., Saito T., Saito A., and Yamada T. (2004a). New rootstock 'Eggplant Ano 2' with highly resistance to bacterial wilt and Fusarium wilt. Proceedings of the 12th Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant: 98.

Yoshida, T., Matsunaga, H., Saito, T., Yamada, T., and Saito, A. (2004c). Verticillium wilt resistance and cytoplasmic male sterility in progenies of eggplant rootstock variety 'Taibyo VF'. Proceedings of the 12th Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant: 97.

Yu J, Pressoir G, Briggs WH, Vroh Bi I, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet. 2006 Feb;38(2):203-8. doi: 10.1038/ng1702. Epub 2005 Dec 25. PMID: 16380716.

Zhang S, Zhang A, Wu X, Zhu Z, Yang Z, Zhu Y, Zha D. (2019) Transcriptome analysis revealed expression of genes related to anthocyanin biosynthesis in eggplant (*Solanum melongena* L.) under high-temperature stress. BMC Plant Biology 19, 387.

Zhou J.; Wang M.; Sun Y.; Gu Z.; Wang R.; Saydin A.; Shen Q.; Guo S. (2017) Nitrate Increased Cucumber Tolerance to Fusarium Wilt by Regulating Fungal Toxin Production and Distribution. Toxins, 9, 100. doi: 10.3390/toxins9030100

Zou C., Wang P., Xu Y., (2016) Bulked sample analysis in genetics, genomics and crop improvement. Plant Biotechnol. J. 14: 1941–1955. doi:10.1111/pbi.12559

Zulkarnain, Z., Tapingkae, T., Taji, A., (2015). Applications of in vitro techniques in plant breeding. Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. Springer, pp. 293–328.

List of figures

Figure 4.5 a: ease index of Fusarium wilt of eggplant plants -67/3 line- caused by Fusarium oxysporum f. sp. melongenae with different nitrogen sources. The disease index was calculated from 7 to 30 days post inoculation. Experiments were repeated more than three times with similar results.

Figure 4.5 b: ease index of Fusarium wilt of eggplant plants -C45- caused by Fusarium oxysporum f. sp. melongenae with different nitrogen sources. The disease index was calculated from 7 to 30 days post inoculation. Experiments were repeated more than three times with similar results.

Figure 4.6 Principal component (PC) analysis of gene expression in roots of two eggplant genotypes artificially inoculated with the Fusarium fungus, at different N-fed.

Figure 4.7 GO enriched terms within the two lines, at T4, after pathogen exposure and NH4+ treatment. The two macrocategories Biological Process and Molecular Function were analysed. Top 20 terms are shown, ranked by p-value, from the most to the least significant.

Figure 4.8 GO enriched terms within the two lines, at T4, after pathogen exposure and NO3- treatment. The two macrocategories Biological Process and Molecular Function were analysed. Top 20 terms are shown, ranked by p-value, from the most to the least significant.

Figure 4.9 GO enriched terms within the two lines, at T4, after pathogen exposure with no nitrogen treatment. The two macrocategories Biological Process and Molecular Function were analysed. Top 20 terms are shown, ranked by p-value, from the most to the least significant.

Figure 4.10 Heatmaps showing the correlations between merged modules individuated in WGCNA analysis and experimental variables. The green borders indicate statistically significant ($p < 0.05$) correlations.

List of tables