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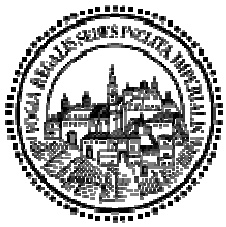

Study of biotic and abiotic stresses in Solanaceae by metabolic and proteomic approaches

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Abstract

Changes in the global climate have shown an increase in extreme temperature related events, such as drought, salinization, contamination and flooding of vast areas of the planet. As for farming, these uncertain climate scenarios are likely to lead to multiple biotic and abiotic stresses. These environmental stress factors negatively influence various aspects of plant growth, such as development and productivity of the crops. Over the last few decades, advances in plant physiology, genetic, and molecular biology have greatly improved our knowledge about plant responses to biotic and abiotic stress conditions.

The emergence of the novel “omics” technologies, such as genomics, proteomics, and metabolomics, is now allowing researchers to identify the genetic to the base of plant stress responses. These omics technologies enable a direct and unbiased monitoring of the factors affecting plant growth and development and provide the data that can be directly used to investigate the complex interaction between the plant, its metabolism, and also the stress caused by the environment or the biological threats (insects, fungi, or other pathogens).

In this research activity, a study on the response of two species of Solanaceae either to the growth in salt stress conditions (abiotic stress) or in fungal infection conditions (biotic stress) was carried out, by using "omics"approaches. In particular, the present PhD thesis is divided into two sections, respectively related to:

1) the study of the response to abiotic stress in pepper (*Capsicum annuum* L.) by metabolomics approaches;
2) the study of the response to biotic stress in eggplant (*Solanum melongena* L.) by proteomic approaches.

Study of abiotic stress in pepper using metabolomic approaches.

Salt content in soil and water is one of the most damaging abiotic stress limiting crops production, especially in Mediterranean basin area. Salinity can cause adverse effects on plant development at molecular and biochemical levels and can have a negative impact on fruit quality. Among horticultural crops, pepper is consideredas a species moderately sensitive to

salinity. In literature a few information is available about the genetic variability responsible for salt tolerance in pepper and the identification of genotypes characterized by high tolerance to salt stress. In this study, we investigated the response of pepper plants to saline stress, applying irrigation water with increasing NaCl level via drip irrigation, in a closed soilless system available at CREA-ORT (Pontecagnano, SA). In the course of the study, two different pepper genotypes were used such as:"Quadrato D'Asti Giallo", a commercial variety, and "Cazzone Giallo", a local ecotype cultivated in Campania region. Metabolomic and biochemical analyses, at different plant growth stages, on different tissue types, including leaves and fruits, were carried out. The work has had the following two objectives:

a) characterization and biochemical analysis of the main secondary metabolites of pepper fruit (ascorbic acid, flavonoids, carotenoids) grown in presence of different NaCl concentrations;

b) characterization and analysis of volatile compounds of pepper fruit and their modulation in salt stress conditions.

The results obtained may be integrated with molecular and morpho-physiological studies for a better understanding of the mechanisms of salt stress response and for the identification of useful genes for the genetic improvement for tolerance/resistance to this abiotic stress in pepper varieties.

Study of biotic stress in eggplant by proteomic approaches.

Plant wilting caused by *Fusarium oxysporum* and *Verticillium dahliae* are among the most serious soil-borne diseases threatening eggplant production, both in greenhouse and in open field cultivations. The objective of this research was the characterization of proteins involved in first plant pathogen interaction occurring in eggplant, when infected with these pathogens. We used innovative eggplant genotypes obtained by conventional and unconventional breeding methods. Young plants of the susceptible cultivar Tal 1/1 and resistant/tolerant cultivar 305E40 were artificially inoculated with one or both the two pathogens. Root proteomes, extracted after 0, 8 and 24 hours from the artificial inoculation, were analyzed using proteomic approaches. Several proteins have been identified through this procedure, and marked differences were

evidenced with respect to the root extracts of mock-inoculated samples. Moreover, quali-quantitative differences were also identified among the three timings studied (T0, T0+8h and T0+24h). The results indicated the presence of proteins putatively newly and differentially synthesized by the plant in response to the infections. Analysis of the major proteins allowed to understand the molecular mechanisms that give an evolutionary advantage to resistant/tolerant genotypes. This analysis, carried out at a molecular level, will contribute to the improvement of knowledge about the mechanism of plant response against these two fungal pathogens. These results will allow to better exploit the source of resistance in plant and that will determine a reduction of the damage caused by tracheomycotic fungi and an increased eggplant production.

1. Introduction

1.1 Plants and environmental stresses

Plants are linked to their habitat, where they are able to conclude the growing cycle arriving at production. However, they are often exposed to several changes in the environmental conditions and this represents a threat for their natural growth. There are many situations of stress, know as external factors which, depending on their intensity and duration, result in a reduction of plant vitality and a negative impact on their productivity. If induced by other living organisms, such as bacteria, viruses, fungi, nematodes, insects and herbivorous animals, the stresses to which the plants are subjected are called biotic (1); if caused by lack or excess of elements which define the physical or chemical characteristics of the natural environment, such as nutrient imbalance, low / high temperatures, drought, salinity, etc., the stresses are defined as abiotic. The exposure of plants to biotic and abiotic stresses induces a disruption of their metabolism, which implies physiological costs (2,3), thus resulting in a reduction of fitness and ultimately of productivity (4)which can also reach 50%, if compared with plants growing in optimal environmental conditions (5).

In the course of evolution process, in response to stress signals, the plants have developed accurate and sophisticated adaptation forms and defense mechanisms able to confer them the ability to tolerate or resist to stress conditions (6, 7, 8). Vegetable organisms are therefore able to detect the presence of a given stress factor, to translate the stress signal and to put into action a wide range of responses including clear signs, such as changes of growing and development rates and less clear signs, such as changes of gene expression, cellular metabolism or new proteins synthesis.

All these changes are crucial for plant acclimation from which its ability to survive the occurred stressful conditions depends. Acclimation phenomena occur every time the plant is faced with non-lethal changes in environmental conditions causing physiological changes, thus allowing the plant to survive severe stress situations. In response to stress, some genes are overexpressed. Overexpression is intended to reduce the effect

of stress and induce in the plant the fundamental changes to overcome the non-optimal environmental conditions. Changes occurring in the plant can be defined as: “elastic” if, once eliminating the stress cause, the vegetable organism resumes its original condition, and “plastics” if some plant conditions remain permanently modified over the time. When a plant is able to survive unfavorable environmental conditions, it is defined ”stress-resistant“, a characteristic which can become stable in the course of evolution process.. When a plant is able to avoid the unfavorable conditions by preventing cells from the stress effect, , ”stress elution“ mechanism occurs. Finally, if a plant survives tolerating the unfavorable environmental conditions, the so-called “stress tolerance” mechanism occurs.

The increasing food demand by world population, together with the natural resources and farmlands decline, addressed scientific and commercial interests in the elucidation of plant response mechanisms to stresses and the interventions to carry out in order to modify these mechanisms and ultimately increase plant productivity under non-optimal environmental conditions.

In recent years, due to the great interest both in basic and applied research, a significant progress in understanding defense mechanisms and processes regulating the adaptations to biotic and abiotic stresses of several plant species has been observed (9, 10).

1.2 Salt Stress and its effect on plant

In the last few decades, research has focused on the use of agronomic techniques that not only provide the quantity and quality of the products but also contribute to environmental sustainability by promoting the preservation of natural resources and lowering the environmental impact. One of the most widely studied aspects, particularly in the arid and semi-arid regions of the planet, but also in developed and developing countries where water resources limit agricultural production, is the salinisation of irrigated lands. This results in a significant loss of agricultural production, as saline and sodium soils reduce the value and productivity of large areas around the world (11). Although salinized soils are more common in desert and semi-desert areas, they can also be found in areas

where all natural conditions are favorable to crop production. In the Mediterranean, the salinisation of the cultivated soil is often associated with poor irrigation management (12), consequently, for many vegetable species saline stress has become a serious problem. Over the past decade, experimental tests of soil salt-load in the soil have shown that salinity has a strong impact on crop yield and therefore is one of the most relevant environmental factors limiting plant productivity (13, 14). The presence of salts in the soil is the source of a series of responses and symptoms manifested by the plant in an extremely unspecific and variable from species to species and between varieties. Therefore, it is not possible to outline a common response or symptom to all cultivated plants. The effect of salt on plants is a result of combined morphological, physiological and biochemical adaptation processes to stress. Salt stress affects the plant causing a reduction in yield, leaf surface, size and weight of the fruit, and ultimately leads to necrosis and death of the plant (15, 16, 17, 18, 19).

1.2.1 Salinity effect on plant growth and water relations

Most cultivated plants exhibit remarkable hypersensitivity to saline environments, causing intracellular build up of Na^+ cations. This cation is toxic to cellular metabolism and, for many salt-sensitive plants, excess Na^+ in the soil plays an important role in inhibiting growth. The response most commonly manifested by the plant to saline stress is a growth and production reduction (20). These effects are the combination of nutritional problems, ionic imbalance or nutrient deficiency, and degraded soil physical properties, such as the permeability decrease to water and air (16). It has been widely observed that in a longer time period, saline stress results into a decrease of fresh and dry weight of leaves, roots and stems (21, 22, 23). It has also been demonstrate that a prolonged saline stress decreases the average leaf area surface, which is directly proportional to the saline concentration (24). Leaf area reduction can be quantified through a leaf index (or LAI, Leaf Area Index). Reduced radical growth is partly attributable to the reduction of the aerial plant part, characterized by a smaller LAI and subjected to a saline stress altering the normal activity, and partly to the toxic action of high-sodium

concentration. The first effect of the salinisation is observed outside the plant roots, with a reduction of the osmotic water potential in the circulating soil solution and the consequent radical absorption decrease (25). The plant growth reduction consists of two phases; in the first phase, the reduction is apparent and is due to the presence and osmotic effect of ions outside the roots, the second phase leads instead to a vegetable tissue damage. Based on these results, the initial reduction of plant growth is due to the osmotic effect of salts in the root zone, and what distinguishes a sensitive species from a tolerant one to salinity is the plant ability to prevent salts reach toxic levels in the leaves (26). Water moves in the soil-plant system following a potential gradient, from high (low salt concentration) to low (high salt concentration) potential , and, when soil solution has a low osmotic potential than the vegetable cells, the plant is not capable to draw water from soil. As soil salinity increases, the osmotic potential of the circulating solution decreases, so the plants lower their total potential (27, 28, 29). Under saline stress, the water and osmotic potential of the plant decreases and this results in a lower availability of free water utilized in the biochemical reactions but also in the adjustment of plant osmotic equilibrium. The water stress induced by salinity causes a water activity decrease due to solute increase, so the relative content of water within the plant, the transpiration and the water use efficiency undergo to a high limitation.

1.2.2 Salinity effect on plant anatomy and photosynthetic activity

Under salinity stress, the reduced plant growth is partly caused by unproductive photosynthesis due to stomata closure and limited CO_2 absorption. The associated decrease of turgescence potential reduces stomatal conductance, which results in reduced transpiration and increased leaf temperature. Closure of stomata induced by stress also decreases the flow of CO_2 in the leaves limiting the photosynthetic activity (29, 30). Reduction of photosynthetic rate under saline stress was observed on a wide variety of species. The decrease of photosynthetic activity due to saline stress can be attributed to two effects; 1) a "short term" effect occurring after a few hours / day from the NaCl supply and

resulting in a lower CO_2; 2) a "long-term" effect causing a reduction of plant fresh weight and a interruption of new leaf growth. A decrease in PSII activity has also been observed with the salinity increasing (31). According to Iyengar and Reddy (32), there are several causes of photosynthetic rate decrease, and they are not attributable to a single aspect of saline stress, such as:

1. decreased cellular membrane hydration and CO_2 permeability;
2. saline toxicity;
3. reduced CO_2 concentration as a result of stomatal closure ;
4. senescence induced by salinity;
5. change of enzymatic activity;
6. imbalance of sink-source activity.

The salt absorbed by the plant does not directly inhibit the growth of new leaves but accelerates the senescence of old leaves. In the long term, the continuous transport of ions into the transpiring leaves involves a very high concentration of both Na^+ and Cl^-, leading to the leaf death. The percentage of dead leaves is critical to the survival of the plant. If the production of new leaves exceeds the senescence of old leaves, the plant performs the photosynthesis process and produces flowers and seeds, otherwise the plant do not reach the full production. The rate at which old leaves die depends on the rate at which the ions accumulate. The control is carried out at the level of root cortex, xylematic tissues and at the contact point between these two sites. These three processes serve to reduce the amount of ions transported to the top of the plant. The control at foliar level is via the exclusion of salts transported by sap flow (33). Saline stress can cause leaf morphological changes including adaptation responses, such as increased leaf thickness and burns. Salinity can directly or indirectly inhibit cellular division and relaxation (34). The growth interruption occurs in new tissues and not involves mature and photosynthetic cells. Sodium toxicity is responsible of leaves edges necrosis and its accumulation causes dehydration, reduced turgor and death of the cells. Chlorine toxicity causes necrotic areas extending from

the leaf apex to the stalk/ branch (25). Salinity generally increases the plant dry matter percentage (16, 34, 35) and also causes root density reduction (27), root / crown ratio increase (34), leaf area and size reduction (26, 29), weight, size and number of fruits decrease (27), and lower production (34, 36). In conclusion, under saline conditions, leaves and branches of plants show a limited growth (34).

1.2.3 Salinity effect on plant ionic composition

Saline water has a much more ion content than an ideal solution and a high amount of not essential ions for the plant. The competition of these ions (usually Na^+ and Cl^-) with the essential ones reduces nutrient plant uptake. Indeed, Na^+ and Cl^- reduce the absorption of K^+ and NO_3^-, respectively (25, 26, 29, 37), causing nutritional imbalances and deficiencies in the plant. The Na^+ excess reduces the Ca_2^+ availability as well as its translocation and mobility towards younger parts of the plant, thus affecting the quality of both vegetative and reproductive organs. It has been shown that in alkaline soil, the solubility of zinc and iron is significantly reduced, thus limiting the availability to the plant. The changes in other macro and microelements plant uptake is difficult to evaluate since the experimental results, based on nutrients / salinity interaction trials, are often contradictory among them (38). If the plant indistinctly absorbs all the ions, it would easily meet toxicity phenomena due the excess of Na^+ and Cl^-. At radical and then at the cellular level the plant operates a selective ion absorption. The selection is not total and part of the ions, are absorbed at several degree into the cellular environment. Ion accumulation is much greater in leaves than roots, due to the water flow within the plant which quickly transports the ions upward with the xylematic flow and much more slowly downward with the floematic flow. The different transport speed originates a unidirectional Na^+ accumulation in the leaves. Na^+ and K^+ imbalances cause disturbances to several classes of enzymes in which the potassium is a cofactor. Potassium is needed for the enzymes involved in protein synthesis. The presence of Na^+ replacing K^+, may be a possible cause of protein synthesis decrease during saline stress (39). Also the nitrate reductase (NR), a key enzyme in the metabolism of nitrogen, which catalyzes the transport of 2 electrons from NADH to nitrate, is affected by ionic imbalance due to saline excess. Many saline stressed plants, show a decreased NR activity in the leaves (40), with the

consequence of nitrogen uptake reduction and a deficiency of this essential macro-element for the plant. The decrease in nitrogen uptake can be mainly due to the competition between Cl^- and NO_3^- and, therefore, to a substrate deficiency.

1.2.4 Salinity effects on macro-micronutrients plant content and fruit quality

The excess of salts can cause specific deficiencies of nutrients and build up of toxic ions in the plant. Regarding nutritional deficiencies, they are due to more or less specific competitions between toxic ions and essential macronutrients (such as phosphorus and nitrogen). Consequently, salinized plants show severe effects of malnutrition. High soil salinity reduces essential biological processes (limiting flora and fauna) and limits or blocks the release of important nutrients from organic matter by limiting their availability (41). Salinity is also cause of the low quality of the crop products (27), even if quality improvement can be recorded (42), as in the case of tomatoes in which salinity increases the synthesis and accumulation of citric acid, sugars, and potassium responsible of a high berries quality (14).

1.2.5 Salinity effect on reactive oxygen species (ROS) formation

The osmotic stress induced by salinity excess inevitably leads to the formation of reactive oxygen species (ROS). ROS are a class of highly reactive compounds which exhibit an unpaired electron, able to tear an electron to other molecules by oxidizing them. Among the most dangerous and commonly radicals formed in the cell, there are the superoxide (O_2^-), the hydrogen peroxide (H_2O_2) and the hydroxyl radical (**• OH**). The presence of these species in the cells causes damage due to lipids, proteins and nucleic acids oxidation. ROS is produced as a result of several mechanisms: during the cellular respiration, some electrons can deviate from the main transport system and interact directly with molecular oxygen (O_2) to give superoxide anion (O_2^-). The deregulation of two superoxide anions, due to both enzymatic and non-enzymatic mechanism, produces hydrogen peroxide (H_2O_2). Hydrogen peroxide is

also produced by other reactions, such as β-oxidation of fatty acids in glyoxysome and oxidation of glyoxylate in peroxisomes. Hydrogen peroxide is very harmful to plants as it is a strong inhibitor of Calvin cycle. If the Calvin cycle slows down, the reducing power, as NADPH, can accumulate in chloroplast, which can be subject to oxidative damage. NADPH reductioncauses an increase of several ROS species, in particular: superoxide, hydrogen peroxide, single oxygen and hydroxyl radical. It is important to remember that with the increase in reducing power there is an "increase in ROS" because these species are in any case generated in chloroplast, even when stress conditions do not occur. Superoxide anion does not directly interact with cell macromolecules, but its protonated form, as hydroxyl radical, causes lipid peroxidation; it is also able to accelerate the production of hydrogen peroxide and hydroxyl radical. Hydrogenated water, in the presence of enzymes with Fe catalysts (Fe_3^+ or Fe_2^+), can form the hydroxyl radical and also can inactivate the detoxifying enzyme Cu / Zn SOD, by modifying the copper site. The hydroxyl radical can be produced by reactions involving superoxide anion and hydrogen peroxide. Since there are no specific detoxifying enzymes in plants, it can damage cellular macromolecules.

1.3 Biochemical mechanisms of plant response to salt stress

Saline stressed plant responds with numerous processes which need to work in synergy to relieve both hyperosmolarity and cellular disequilibrium (14). Plants use genetic, biochemical and physiological mechanisms to overcome the adverse effects of salt stress, including rearrangement of carriers, control of toxic ions by accumulating them in vacuums or mobilizing them in old leaves destined to fall, and metabolic processes (14, 43). In addition, four strategies of high salinity tolerance have essentially evolved in the plants: (i) ion-homeostasis; ii) osmolites biosynthesis; iii) transcription and regulation of salt-related genes; iv) hormonal regulation. Many compounds are known to play a role in osmoprotection, by preserving the structure of enzymes and proteins while maintaining membrane integrity and *scavenging* of reactive oxygen species (ROS). Generally plants become more tolerant during the last stage of growth (20) but there are exceptions (20, 44). In literature, salt tolerance is not given by a single or a few genes. Indeed, it has been shown that the adaptation to salinisation is given at least by three fundamental physiological mechanisms, which involve ionic homeostasis (synthesis and activation of ionic carriers), water homeostasis (regulation of waterborne functions) and neutralization of free radicals (synthesis of molecules with antioxidant properties). These mechanisms are generally under the control of stress hormones such as ABA, regulatory molecules and metabolic flows of stress-associated components (44, 45). Ionic homeostasis is one of the most responsive to salt stress (26). To prevent salt concentration in sprouts, the roots block 98% of the salts in solution, allowing 2% of transport to sprout through the xilema (46). Thus, plants that can limit the absorption of toxic ions and maintain a normal level of nutrients are better resistant to salt than those that do not block ion build-up and can not balance nutrients (44). The ability to reduce the absorption of ions into salt tolerant species is one of the major differences with respect to susceptible plants (44, 46). The plant response to the decrease in external water potential is a decrease in osmotic potential in the plant through an increase in solutes. The active accumulation of inorganic solids before and of organic molecules after allow the plant to maintain a high turgor. Adjusting the osmotic potential in plants exposed to saline or water stress is one of the key mechanisms for countering saline stress (16, 34).

Sugars, proline, glycinebetaine and other organic solvents contribute to preserving osmotic balance and enzymatic activity in the presence of toxic ions (47).

1.3.1 Internal ion reorganization

Salt ionic imbalance creates great difficulties for plant organisms. High amounts of salts inside the cytosol, especially low useful salt such as NaCl, are not tolerated by plants. This means that in order to perform normal biochemical processes in the cells, also tolerant plants need to eliminate excess ions. Thus, most plants, both glycophytes and halophyte, accumulate salt particularly in vacuums, interstitial compartments, leaves or senescent branches (47). The ability of plants to compartmentalize salts in mesophilic cell vacuoles is another important strategy to minimize or reduce the toxic effects of high salt concentrations. The control of salts transport to and within the plant can take place at three plant points. The first one is at the cortical root level, the second consists of the xilema loading, the third is the translocation from the xilema to the top of the roots. In these three loci, the transport of ions can be reduced or controlled. Some controls mechanisms occur in the sprouts through the exclusion of salt from the floema flowing towards the meristematic sprout regions. An additional mechanism of some halophytes is the accumulation of toxic ions in specialized glands (46). Storage inside the vacuole is possible thanks to a specific transport enzyme, the Na^+ / H^+ antibody enzyme which permits sodium storage inside the vacuole and the resulting salt concentration decrease in the cytosol. In salinity conditions, the plants, and especially the most tolerant ones, maintain a high K^+ and reduced Na^+ concentration within the cytosol. Compartmentation is useful in case of stress and seems to play a central role in salinity resistance, although this is a mechanism essential to the plant, both in salinity and under normal conditions (48). Another saline regulation mechanism is saline secretion. Saline secretion allows a unique specialized cellular structure, which takes the name of a saline gland, to secrete excess salts through the leaves. They directly secrete the salt outside the plant, thus making possible to tolerate cell concentrations (49).

1.3.2 Synthesis of metabolites and organic solutes

Stressed plants need to synthesize and then accumulate in the cytosol molecules, called metabolites, not interfering negatively with the activities in it carried out (14, 50, 51). The primary function of these metabolites is to allow water to enter the cell or plant or, in extreme situations, to prevent water coming out from the cell, thus re-establishing an osmotic balance (14). Their chemical nature varies, but a key characteristics is to interfere as little as possible with the normal activities that are carried out in the cytosol, and to contribute to cellular retention during saline stress. The most abundant and most studied molecules are amino acids, proteins, polyamines and quaternary nitrogen compounds. In addition to the classic osmoprotector function, these compounds act as a reserve of nitrogen, pH balancing, and furthermore, a role in reducing ROS (52) has been proposed over the last few years. The accumulation of these compounds is frequently associated with saline tolerance (53). Increasing proteins as a response to salinity is very controversial. In many plant species a positive correlation has been observed between osmotic leaf potential and glycinebetaine whose increase has been documented in many species. Glycinebetain is a molecule with a quaternary, methylated nitrogen, and it is found in some plant groups, in some animals and in various microorganisms (54, 55). Its location is mainly in chloroplast, where it plays a key role in the adjustment of osmotic potential within the tilacoidal membrane, maintaining the membrane intact and a high photosynthetic efficiency (56). In particular, the maintenance of photosynthetic efficiency is linked to the protection of PSII under saline conditions. The leaf application of this compound is able to promote an increase in salinity tolerance in rice (57) and tomato (58). At high concentrations does not affect cytoplasmic functions and stabilizes the structure and function of many macromolecules. Glycinebetain appears to be a critical determinant of stress tolerance in plants. A test of the amino acids involvement in salinity response, in addition to a higher accumulation of some of them with respect to the normal concentration, is the change of quantity ratios between them. About 50% of the free aminoacids in the wheat plant is cysteine, methionine and arginine. After treatment with NaCl, the

prevalent amino acids become valine, proline, aspartic acid and isoleucine, and the total amount increases with respect to the control (59). The peculiar characteristic of the amino acids which makes them good osmolytic, lies in their zwitterionic nature, as they have the ability to preserve the pH of the cytosol and thus to alter as little as possible the enzymatic metabolism. Having two charges, the amino acids are able to perform a real buffer effect (60).

1.3.2.1 Proline

Also for proline, the attitudes listed above for other amino acids, namely that the accumulation acts as a nitrogen storage and promotes pH maintenance, are valid. Proline is defined as a "multifunctional amino acid"; its production is stimulated by a considerable number of abiotic and biotic stress conditions, as it is able to protect protein integrity, retain the activity of numerous enzymes, and exercise the role of molecular chaperon both in case of extreme temperatures under conditions of osmotic stress or oxidative stress induced by heavy metals. Several studies attribute antioxidant properties to proline, acting on the single oxygen level. Proline treatment decreases the development of ROS in fungi and yeasts (60), and limits the harmful effect of ROS on photosystem II membranes (PSII) (61). Gene silencing studies for proline metabolism have shown a greater tendency to accumulate oxygen radicals; therefore,its "indirect" role in ROS protection has been observed: when stress condition occurs, proline specifically stabilizes some of the enzymes involved in the detoxification of radical forms such as peroxidase, glutathione transferase and superoxide dismutase (Figure 1).

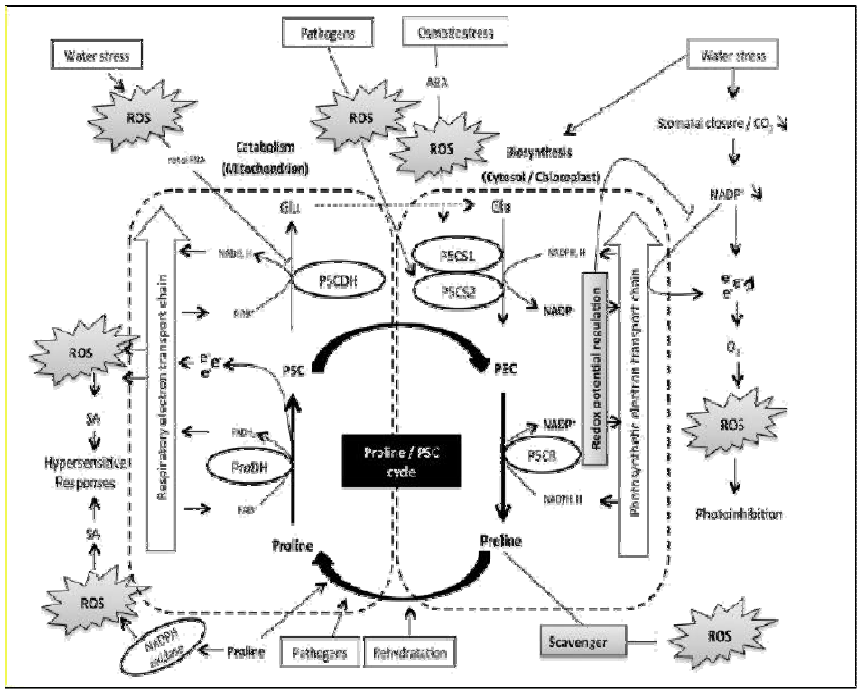


Figure 1. The role of proline in stress response.

Finally, the proline regulates the redox balance of the cell. In fact, studies on plants, whose growth conditions were characterized by high irradiance, showed an increase of proline-dependent glutamate synthesis in plastids. This process assures a high amount of proline able to stabilize chloroplast proteins, and especially re-oxidize NADPH to $NADP^+$, which is used as the end acceptor of electrons from the photosystem I (PSI), thus avoiding the formation of ROS (62). Glutamate phosphorylation consumes ATP, resulting in the formation of a new substrate for ATP synthesis during photosynthesis, so that the proline synthesis in the chloroplast ensures a low NADPH / $NADP^+$ ratio, limiting the phenomenon of photoinhibition and allowing the fundamental maintenance of the integrity of the membranes even under stress. Proline metabolism can influence the metabolic pathway of phosphate pentoses, where the first two reactions, starting with glucose-6-phosphate, lead to the formation of two NADPH molecules and release carbon dioxide. Under stress conditions, namely of stomata closure, the low concentration of carbon dioxide can be used in the Calvin cycle while NADPH, as seen above, can be used for proline synthesis by recapturing $NADP^+$ and preventing the formation of ROS (63). The biosynthetic pathway of proline, which is located in cytosol and chloroplast (64, 65),

can occur through two metabolic pathways, from ornithine or glutamate. The biosynthetic pathway of glutamate, which is converted into proline by two successive reductions, catalyzed by "pyrroline-5-carboxylate synthase" (P5CS, EC 2.7.2.11) and subsequently by "pyrroline-5-carboxylate reductase" (P5CR, EC 1.5.1.2). P5CS, is an enzyme which catalyzes two different reactions: a first activation of glutamate by phosphorylation, followed by reduction of the reaction intermediate, γ-glutamyl phosphate, in semi-aldehyde glutamate (GSA); the second biosynthetic pathway sees the ornithine as a precursor which can be transaminated to pyrroline-5-carboxylate (P5C) from "ornithine-δ-aminotransferase (δ-OAT, EC 2.6.1.68), a reaction that is located in the mitochondria. In most plants, two P5CS isoforms, one constituent and one induced by stress conditions, are identified, while P5CR is usually coded by one gene. The main pathway during a saline stress is that of glutamate, although studies conducted on Arabidopsis have shown a contribution from the pathway of the ornithine (66). Recent studies on Arabidopsis thaliana have highlighted the presence of two genes related to proline synthesis: the P5CS1 and P5CS2 genes, both linked to proline metabolism, but only the P5CS1 gene is activated under saline stress (68, 69). The P5CS1 gene has also been found to be induced by abscissic acid (ABA) (70). The role of ABA in determining accumulation of proline has been well investigated by Verslues and Bray (71). These authors, experimenting with mutants in the biosynthesis and perception of ABA, have highlighted not only the dependence of proline accumulation on ABA levels, but also that this depends on the sensitivity or ability of the plants to respond to this hormone. Other results support the idea that H_2O_2 is an integral part of the signal and responses regulated by the ABA. During osmotic stress, the availability of atmospheric CO_2 is limited due to stomata closure. The resulting accumulation of NADPH, which is not used in the Calvin cycle, can lead to an overload of the chloroplastic transport that in turn induces the production of ROS. In this context, the ability of the enzyme P5CS to use NADPH oxidizing it to $NADP^+$ may decrease the possibility of ROS formation in chloroplast. In this context also the pathway of phosphate pentoses (PPPs) using NADP

$^{+}$ is inhibited by NADPH excess and a linkage to proline metabolism linked to the use of NADPH seems obvious.

1.3.2.2 Hormonal activity

In the adaptation mechanism of plants to saline stress, the implication of various hormonal classes such as cytokines, gibberellins, auxins and ethylene is known. However, the hormone for which more evidence has been collected is abscisic acid (ABA), which plays a key role in plant tolerance also in other types of stress such as cold, freezing, high temperature and water deficit. In salinity conditions, an increase in endogenous ABA levels is observed and, if the cells are treated with ABA before being exposed to salinity, their tolerance increases as it is involved in many aspects of plant stress response, including induction of gene expression responses to salt (72). The lack of ABA seems instead to be responsible for blocking the expression of some genes responsive to saline stress (73). As it is now known, ABA is able to promote stomata closure by altering ionic flow in guard cells. Its action, involved in modifying the expression of some genes, is therefore a very complex action. It can be shown that, in the same plant, the ABA inhibitor hormone increases as a result of exposure to saline stress; indeed, it has been noted that plants treated with ABA for a few days are able to tolerate more saline stress, thus indicating an active role of hormone in the resistance mechanisms (72). ABA-mediated response capabilities are therefore very varied, and its involvement is now ascertained as reported in the literature. Unfortunately, the poor knowledge of the signal transduction of the ABA and, very often, of the response to the ABA does not allow to outline a clear picture, also considering that the nature of the receptor for ABA is still unknown.

1.3.3 Plant Protection against ROS and antioxidant enzymes

ROS formation in the cell is a normal process and the plant, in order to defend itself, produces antioxidants both in the cytosol, chloroplast and other organelles. Superoxide dismutase (SOD, EC 1.15.1.1) are enzymes found in all plant cells, with a subcellular localization involving

chloroplast, mitochondria, peroxisome and cytosol. The superoxide dismutase catalyzes the following dimming reaction:

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

It leads to the conversion of a highly reactive oxidant, such as superoxide, into a less reactive and deactivable by other antioxidants one. Catalase promotes reduction to H_2O, but it is not present in chloroplast, therefore, H_2O_2 is detoxified through a series of reactions involving various enzymes and antioxidant molecules, known as Halliwell and Asada cycle (74) (Figure 2).

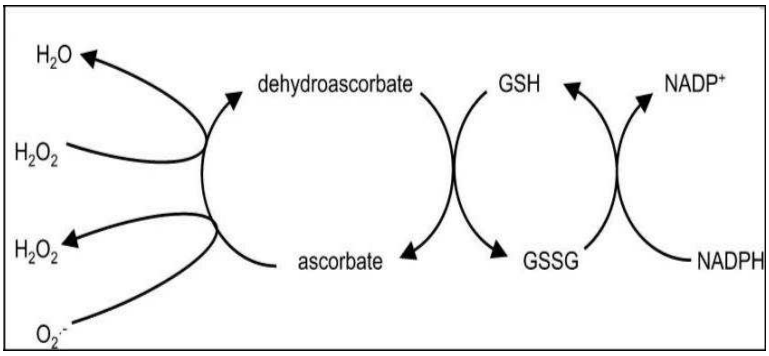


Figure 2. Halliwell-Asada cycle responsible for chloroplasty, superoxide anion disintegration and hydrogen peroxide.

Halliwell and Asada cycle also utilize NADPH for the regeneration of glutathione in reduced form, thus contributing to the elimination of excessive reducing power. The interdependence between increased activity of antioxidant enzymes and stress is now known.

1.3.4 Plant protection and synthesis of volatile organic compounds (VOCs)

In addition to the above listed mechanisms, plants have developed sophisticated defense mechanisms through the synthesis of Volatile Organic Compounds (VOCs). VOCs are used by plants to communicate with other plants or other organisms. VOCs are involved in a wide range of ecological functions including: pollinator attraction, plant-plant communication, plant-pathogenic interactions, ROS removal, thermo-tolerance and adaptation to environmental stresses. Their evolution is rather complex and is influenced by the interactions of plants with biotic and abiotic factors in ever-evolving environments at local and global level (75). However, the emission of VOCs

may also be in response to the perception of abiotic stress and they can play a role in adapting to stress or responding to environmental stresses such as physical damage, nutrient deficiency, salinity, and drought. There is a wide chemical variability between VOCs: alkenes, alkanes, higher carboxylic acids and nitrogen containing alcohols, but the dominant compounds tend to be isoprene, terpene and volatile C6 compounds, indicated as Green Leaf Volatiles (GLVs) (76). There are also a large variety of volatile organic compounds belonging to the class of terpenoids and fatty acid derivatives (Figure 3) (77).

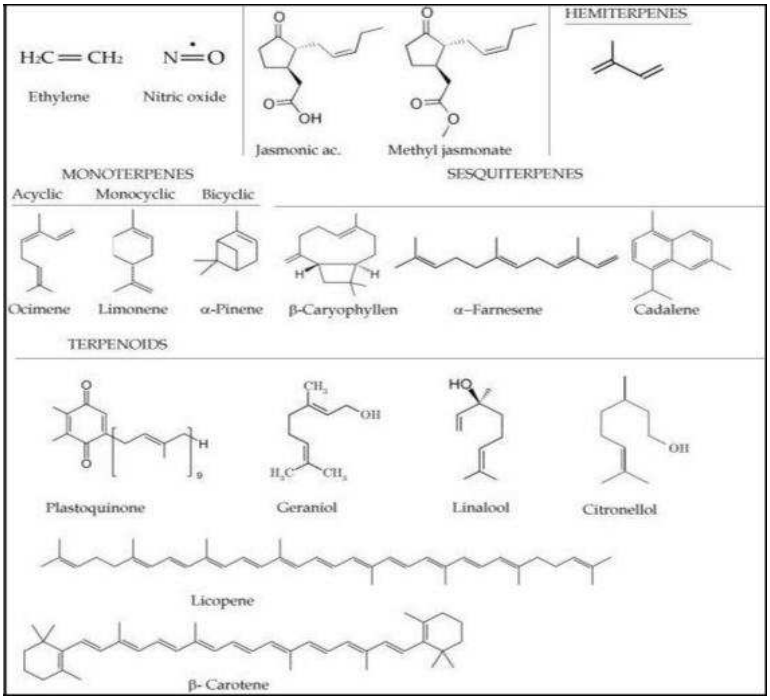


Figure 3. Chemical diversity of the different VOCs and their compounds present in the plant

The production of VOCs by plants varies widely depending on the species, organ, development phase and environmental conditions (76). GLVs are produced via the pathway of lipoxygenase (LOX), and can account for more than 50% of the emissions from damaged parts of plants. Chemically, GLVs are mainly aldehydes, saturated or mono-unsaturated alcohols and esters, and may have different configurable isomers with different sensory properties (78). The great diversity of VOCs suggests that plants are able to actively change their growth conditions by using them (79). Several studies have highlighted the role of VOCs in tritrophic interactions (plant-insect-environment) and in communication between plants, proving that these compounds, whether

they are constitutively emitted or induced by biotic attacks, can attract specific parasitoids and predators as well as constitute a direct deterrent against pathogens and herbivores (80). In addition, VOCs have been shown to be induced as a result of abiotic stress and their synthesis, in quantitative terms, is closely regulated by the type of abiotic stress (80). Several researchs have shown that some VOCs signals are potentially capable of generating abiotic stress responses in the plant and this is important in order to minimize stress-related damage (76, 81). VOCs act with a common mechanism, or they play a protective role against oxidative stress (82). A recent study on the effects of salinity on Arabidopsis thaliana shows that the emissions of saline stressed VOCs such as terpenes, isoprene, mono and sesquiterpenes, lipoxygenase (LOX) and methanol, are relevant to activate stress tolerance and tinduce salt resistance to near plants (83). However, the biological relevance and the mechanism of communication in the presence of an imminent environmental stress are still largely unknown.

1.4 Phytopathogenic biotic stress and effects on plant

In their habitat, plants are constantly exposed to several adversities which may negatively affect development, growth and reproduction, and also cause the death. In the varieties of agricultural interest they can lead to significant decrease of productivity and product quality, resulting in depreciation and economic damage. Diseases affecting plants, when caused by the competitive action of other living organisms such as herbivorous insects, nematodes, viruses, bacteria, pathogenic fungi, etc., are called phytopathies (biotic stresses). In particular, pathogens act through mechanisms promoting their proliferation and inducing changes in the physiological activities and basic metabolic processes of plants. Their control has always been based on the application of chemical compounds characterized by a wide spectrum action. However, the extensive use of pesticides with reduced selectivity is causing serious environmental consequences, development of resistance in pathogenic populations, toxicity to non-target organisms with the consequent reduction of biodiversity, increase of environmental pollution and public

health problems. In recent years, the increased interest in environmental issues and attention of consumer to nutritional and health aspects of agricultural products have stimulated the scientific world to extensively study plant-pathogen relationships in order to develop operational strategies able to protect the agroecosystems and improve the quality of vegetable products.

1.4.1 Mechanisms of protection from biotic stresses

Plants have developed sophisticated defense mechanisms, characterized by a specific or wide spectrum action, which allow to detect the pathogen at a molecular level and control the propagation of infection. Normally, the development of a plant disease represents a rare occurrence, appearing when the plant is infected by a virulent pathogen, which does not activate or suppress the plant resistance or repress the defenses activated by the plant. When a plant and a pathogen come into contact, the main pathogens activity is the host colonization and the host resources utilization, while the plants detect the presence of pathogens and activate antimicrobial and other defense responses. Plant and pathogenic organisms are often highly co-evolved, thus the plant barriers to microbial infections may be bypassed by specific pathogenic species (84). Plants recognize pathogens through innate immunity mechanisms, which can be present in two forms: basal resistance or R gene-based resistance. The former is based on the recognition of a *pattern recognition* receptor (PRR) and a *pathogen-associated molecular pattern* (PAMP), which activate basal responses or non-specific defense mechanisms in the plant. The latter is based on the highly specific interaction between pathogen and R gene plant genotype, according to gene-by-gene theory. The recognition pathogen-plant leads to a hypersensitive response (HR) causing the rapid and programmed plant cells death (apoptosis) to contain local tissues invasion and necrosis (85). The perception of danger signs occurs in the immediate proximity of the pathogen invasion sites. Plant species with specific resistance are evolutionarily characterized by a type of immunity, which is referred as the innate immune system of the plant. A series of signal transduction cascades mediate the activation of the innate immune response and

include common elements to both forms of plant immunity, such as modified cytoplasm calcium levels, reactive oxygen species production. During those defense responses activation, host activity is substantially modulated and redirected (86). When a virus, bacterium, mushroom, nematode or insect avirulence protein (Avr) is directly or indirectly recognized by the corresponding plant protein resistance (R), R protein activates a defense response to suppress the infection (87). So, the R genes constitute an important "barrier" of plants immune system and are widely used to control disease in cultivated plants. The pathogen monitoring system mediated by R gene allows a rapid activation of plant defense responses. The Hypersensitive Response (HR), a programmed plant cell response to the pathogen-infected site, is often associated with resistance to the disease. The term "pathogenesis-related protein" (PR protein) was introduced in 1970 and indicates proteins of new synthesis or present at remarkably high levels after the plant has been infected (88). A number of PR genes encode proteins such as chitinase, glucanase or defensine, which have been shown to possess antimicrobial activity.

1.4.1.1 PAMP-triggered immunity (PTI)

Immediately after the first contact with pathogen, the plants activate defense responses against non-self molecules. Such responses are caused by the M / PAMPs (microbe / pathogen associated molecular patterns). microbial molecular structures playing an essential role in the life of pathogenic microorganism, termed "general elicitors". The variability of these molecular structures is limited, consequently, the possibility of evading their recognition by the plant is very limited (89). During the early stages of infection, the pattern recognition receptors (PRRs) recognize the M / PAMPs and activate a set of physiological responses from which the defense response to the pathogen, called *PAMP triggered immunity* (*PTI*) or *basal immunity*, originate. The activation of these receptors stimulates several physiological reactions at the cell membrane level and intracellular signalling pathways that are chronologically described below. Microbial pathogens have found the way to break this first plant defense line, so the plants have developed a second defense

line, the *base resistance of R gene* which is more specific than the basal immunity.

1.4.1.2 Effector triggered immunity (ETI)

In response to host-pathogen competition, some populations within the same plant species have evolved a set of proteins capable of directly or indirectly perceiving the activity of pathogenic agents and activating a new, rapid and very intense response, called *Effector Triggered Immunity* or *ETI* (traditionally termed *R gene resistance*). This allows the plants to overcome the PTI suppression, operated by pathogens, reinforcing the defense response. The virulence factors recognized by this second defense system are called avirulence factors, and the encoding genes "*avr genes*"; the host-pathogen interaction is defined as "incompatible" and the plant is resistant. Typically, the ETI response is associated with a number of mechanisms very similar to PTI but characterized by a more robust and rapid response, sucha as: a second, moreintense and prolonged peak of oxidative burst, massive activation of cellular ionic flows, salicylic acid accumulation, phitoalessine and PR proteins production, and finally hypersensitivity response (HR). The latter represents a type of programmed cell death which leads to the visible manifestation of typical lesions or necrosis, whose function is to isolate and quickly kill the pathogen. In addition, once ETI defense is activated, a series of systemic signals able to establish the acquired systemic resistance (SAR), is generated. This resistance is characterized by a wide spectrum action and can last for a long time (90). Moreover. the response induced by ETI seems to be stronger and longer than the response induced by PTI (91).

1.4.1.3 Resistance and R proteins

Innate immunity relies on specialized receptors divided into two groups: the PRRs and the R proteins. PPRs recognize PAMPs, highly conserved molecules which allow plants to recognize pathogens using a limited set of receptors (92). In R proteins respond to the avirulence proteins (avr) or elicitors, that are generally not conserved among pathogen species or

pathogens isolated . R protein are encoded by gene families, consisting in several hundreds of genes per genome (93). Only in *Arabidopsis thaliana* gene family includes about 150 genes, among which genes with highly conserved domains, such as nucleotide binding sites (NBS) -LRRs. NBS domains share sequence similarity zones with the NB regions of apoptosis regulators, such as CED 4 of *Caenorhabditis elegans* and Apaf-1 of humans, whereas LRR domains are involved in protein-protein interactions (94). In turn, NBS-LRR gene family (or NB-ARC-LRR) can be further subdivided into: coiled-coil (CC) -NBS-LRR and Toll-interleukin-1 receptor (TIR) -NBS- LRR. The different N-terminal extremity affects the protein behavior in their interaction with the downstream signaling members, activated by the transducer recognition (95). In the NBS-LRR family, the better characterized members are Arabidopsis RPS2, RPM1, and RPS5 that determine resistance to *P. syringae* and expressing, respectively, the effectors AvrRpt2, AvrRpm1 / AvrB, and AvrPphB (96, 97, 98). This pairing association, describing the recognition of effectors within the plant cell, was genetically characterized by Flor in 1942 and is known as "gene-per-gene" hypothesis. This theory, however, postulated the interaction between the virulence determinant and the resistance determinant (avr / R) according to the typical key-lock model of direct physical interaction.

The "gene-per-gene" theory was overcome by guard hypothesis and integrated with the decoy model, since it has been shown that in most cases the recognition not occur through a direct effector / protein R molecular interaction, but resistance proteins perceive the tertiary structure modification induced by effector on specific endogenous proteins, , crucial for plant defense response (guard). In addition, in the cytoplasm of plant cell there are bait proteins (decoy) which mime the domains structure of that proteins directly involved in the resistance response on order to attract the effector whose activity is detected by the resistance protein associated with bait (99). Within the Solanaceae botanical family, the largest class of R protein is the CNL class (100). We can find direct or indirect Avr / R interaction, however, for most R proteins, this mechanism is still unknown. The study of NB-LRR 3-D

structure and *Receptor-like proteins* (RLPs) will contribute to better understanding the molecular mechanisms underlying their function (Figure 4).

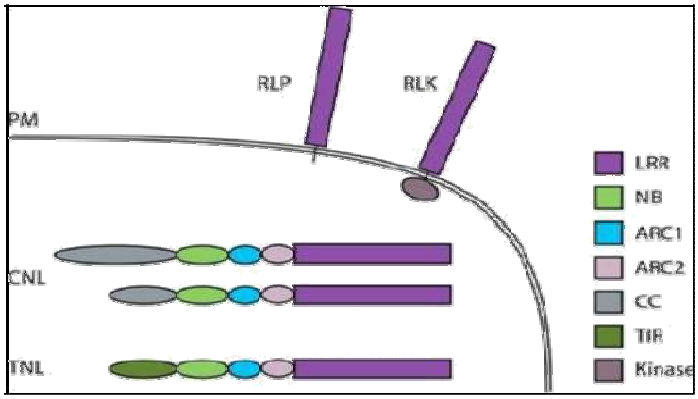


Figure 4. Schematic representation of typical components of the four R protein classes. Protein domains and putative cellular localization are indicated. The Receptor-Like Protein (RLP) and the Receptor-Like Kinase (RLK) classes of R proteins pass through the plasma membrane (PM) and contain an extracellular Leucine Rich Repeat (LRR) domain. The CNL and TNL classes of R proteins are located in the intracellular space (cytoplasmic, nuclear, or membrane-bound space) and contain the central NB and the ARC domains (the latter consisting of NB, ARC1 and ARC2 subdomains) coupled to a LRR domains. TNLs contain a N-terminal TIR domain, while CNLs contain either a CC or an extended CC domain (100).

1.4.2Reactive Oxygen Species, Lipoxygenases, and disruption of Cell Membranes

Plant cell membrane consists of a phospholipidic bilayer in which many different kinds of protein and glycoprotein are incorporated. Cell membrane is also an active site for the defense mechanisms induction because it serves as anchoring of R gene-codified proteins which recognize the elicitors released by the pathogen and subsequently activate the hypersensitive response. The most important membrane-associated defence responses include the release of molecules involved in signal transduction within and around the cell, the release and the accumulation of reactive oxygen "species" and lipoxygenase enzymes. The first defence response events are perturbations of ionic fluxes changes in protein phosphorylation, accumulation of ROS (mainly O_2^- and H_2O_2) and NO, as well as transcriptional activation of defence-

related genes (101, 102). Cell attack by pathogens, or cell exposure to pathogen toxins and enzymes, often results in structural and permeability changes of the cell membrane. In many plant-pathogen interactions, one of the first events observed in host attacked cells is the rapid and transient generation of activated oxygen species, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). The generation of superoxide and other reactive oxygen species as defence response occurs in localized infections, but also in general and systemic infections, as well as in plants treated with chemicals that induce systemic acquired resistance. These highly reactive oxygen species are probably released by subunits of the enzyme complex of the host cell plasma membrane NADPH oxidase. They appear to be released within few seconds or minutes from the contact of the cell with the pathogen. The activated oxygen species stimulate the hydroperoxidation of phospholipidic membrane, producing mixtures of hydroperoxide lipids. The latter are toxic, as their production breaks the plant cell membranes, and they seem to be involved in the HR-induced cell collapse and death. The presence of active oxygen species, however, also affects the membranes and the cells of the pathogen through the hypersensitive response of the host cell. The production of reactive oxygen species in affected but surviving nearby cells is kept under control by the superoxide dismutase, catalase, ascorbate peroxidase enzymes. The oxygenation of membrane lipids seems to involve various lipoxygenases, enzymes catalyzing the hydroperoxidation of unsaturated fatty acids, such as linoleic and linolenic acids, which have been released previously from membranes through phospholipases action. The lipoxygenase-generated hydroperoxides, formed from such fatty acids, in addition to breaking the cell membranes and leading to HR-induced host and pathogen cell collapse, are also converted by the cell into several biologically active molecules (such as jasmonic acid) playimg a role in the response of plants to wounding and other stresses.

1.4.3 Transduction of Pathogen Signals in Plants

Plants are able to recognize pathogen-derived elicitor molecules acting most of the induced defense responses. The recognition of a potential pathogen results in activation of intracellular signaling events including ion fluxes, phosphorylation/dephosphorylation cascades, kinase cascades, and generation of reactive oxygen species (ROS) (103). Intercellular signaling system involves ROS, nitric oxide (NO), salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) production. Two major pathways in defence signalling are recognized: 1) SA-dependent pathway; 2) SA-independent, but involving JA and ET, pathway (104). These signalling events lead to the reinforcement of plant cell walls and the production of defence proteins and phytoalexins.

1.4.3.1 Nitric oxide in signal transduction

Nitric oxide (NO) is a gaseous free radical that diffuses readily through biomembranes (105). It is now well established that NO is involved in the plant defence signalling (106). Plants synthesize NO from nitrite. Nitrate reductase has been found to catalyze the NAD(P)H-dependent reduction of nitrite to NO (107). Nitrate reductase reduces nitrate to nitrite and can further reduce nitrite to NO. Nitric oxide induces defense gene expression via signaling pathways that likely involve cyclic GMP and cADPR.

1.4.3.2 Main defense pathways and phytohormones involved in plant biotic stress response

Plants produce many types of hormone-like molecules, some of which can be of great importance in defense reactions to biotic stress: ethylene, salicylic acid and jasmonic acid (108). Recent studies have shown that other hormones, already regulating several processes of plant growth and development (108, 109), are involved in these protective responses: abscisic acid (ABA), gibberellin (GA), auxin, cytokin (CK) and brassinosteroids (BR) (Figure 5).

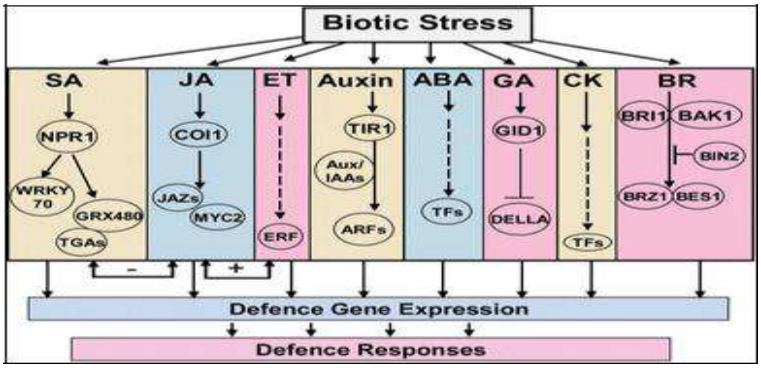


Figure 5. Main defense pathways and phytohormones involved in plant biotic stress response in (109)

1.4.3.3 Ethylene-dependent signalling pathway

The increased production of ET is one of the earliest chemically detectable events in pathogen-infected plants or in plants treated with elicitors .The role of ET in plant–pathogen interaction is complex (110). ET stimulates defence mechanisms against several pathogens, and it also induces susceptibility to several other pathogens (111). ET applied as pre-treatment induces resistance against *Botrytis cinerea* in tomato (112), the ET-insensitive mutant of tomato showed enhanced resistance to *Fusarium oxysporum* (113) and soybean mutants with reduced sensitivity to ET were less susceptible to *Phytophthora sojae* (114). By contrast, ET insensitivity enhanced susceptibility to various pathogens in different plants, for example *Arabidopsis* mutant ein2-1 (ethylene/insensitive 2-1) showed enhanced susceptibility to *B. cinerea* (115) After its synthesis, ET is perceived and its signal is transduced through transduction machinery to trigger specific biological responses. The signalling system consists of two proteins, a histidine kinase and a response regulator. The histidine kinase acts as the sensor that autophosphorylates an internal histidine residue in response to signals, and the response regulator activates the downstream components upon receiving a phosphate from the histidine residue of the sensor on its aspartate residue (116).

1.4.3.4 Salicylic acid in signalling defence response in plants

Salicylic acid (SA) is a phenolic compound commonly present in the plants. Plants synthesize SA (O-hydroxybenzoic acid) by the action of PAL (phenylalanine ammonia lyase), which is a key regulator of the phenylpropanoid pathway and yields a variety of phenolic compounds with structural and defense-related functions. SA accumulates in plants inoculated with pathogens, its level increases both in proximal and distal tissue with respect to the infection. The increased levels of SA resulted in induction of various defence-related genes (117).

The importance of SA-signalling system in induction of host defences was studied by developing transgenic plants expressing the bacterial gene NahG. 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 incapable of developing HR, indicating that SA accumulation is required for HR to occur (118).

1.4.3.5 The jasmonic acid in defense response to plants

The jasmonic acid is a 12-carbon molecule derived from linoleic acid (119). He is involved in various aspects of plant biology, such as pollen and seeds development, defense against physical and chemical stresses (wounds, ozone) and biotic stress (insect and pathogen attack). The use of non-producing A. thaliana mutants has allowed the high level of susceptibility to various fungal and bacterial pathogens including *Botrytis cinerea*, *Pythium spp.* and *E. carotovora*. Many genes are activated by jasmonic acid and their products are linked to resistance induction: among them, defensin (plant defensin 1.2, PDF 1.2), thionin 2.1 (thion 2.1, THI 2.1) can be cited as a protein similar to evein (hevein-like protein, HEL) and chitinase B (CHT B); These products are commonly used to track JA-dependent defense responses (120).

1.4.4 PR proteins

PR proteins are encoded by guest host genes (PR genes) and synthesized de novo following induction by various pathogens or related situations. PR proteins all work together to inhibit the growth, multiplication and spread of the pathogen. Pathogenesis-related proteins were discovered in tobacco reacting hypersensitively to Tobacco mosaic virus (TMV) and later in other plant species. The recognized PRs currently comprise 17 families described in Table 1, based on structural or functional similarities (122). Within each family of PR proteins, there are also acidic isoforms, secreted within the apoplast, and basics, with vacuolar localization. Molecular mechanisms that confer antimicrobial activity on many PR proteins in some cases are still unclear. The existence of similar proteins in animals could help clarify its functions and it is eloquent that the innate immunity of plants has much in common with that of animals.

Classification of pathogenesis related proteins

Families	Type member	Properties
PR-1	Tobacco PR-1a	Antifungal
PR-2	Tobacco PR-2	β-1,3-glucanase
PR-3	Tobacco P, Q	Chitinase type I, II, IV, V, VI, VII
PR-4	Tobacco 'R'	Chitinase type I, II
PR-5	Tobacco S	Thaumatin-like
PR-6	Tomato Inhibitor I	Proteinase-inhibitor
PR-7	Tomato P69	Endoproteinase
PR-8	Cucumber chitinase	Chitinase type III
PR-9	Tobacco 'lignin forming peroxidase'	Peroxidase
PR-10	Parsley 'PR1'	Ribonuclease like
PR-11	Tobacco 'class V' chitinase	Chitinase, type I
PR-12	Radish Rs AFP3	Defensin
PR-13	Arabidopsis THI2.1	Thionin
PR-14	Barley LTP4	Lipid-transfer protein
PR-15	Barley OxOa (germin)	Oxalate oxidase
PR-16	Barley OxOLP	Oxalate oxidase-like
PR-17	Tobacco PRp27	Unknown

Van Loon & Van Strien (1999), Physiological and Molecular Plant Pathology 55: 85-97.

Table 1. Recognised families of PRs proteins

1.5 Fusarium oxysporum

Fusarium oxysporum f. melongenae is a major soil-borne pathogen of eggplant (*Solanum melongena* L.). The drying caused by *Fusarium* occurs on plants both in greenhouse and open-field cultivation, at any stage of vegetation and in any phenological phase, although the infections contracted early cause the most significant economic damage (123, 124). *F. oxysporum* is one of *Fusarium* species of most phytopathological and economic interest. Various forms are responsible for vascular diseases (tracheofusariosis) and parenchymal rotting of many economically important crops (125). Vegetable guests include tomato (126), lettuce (127), melon (128), banana (129), as well as several ornamental plants such as carnation (130). Under the name of tracheomycosis, there are diseases characterized by the prevalent localization of the fungal pathogen in the woody vascular system of plants. Typical symptoms of the disease include: nanism, drying, chlorosis, vascular discoloration, necrosis, and plant death. The *F. oxysporum* forms spread their propagules by air and usually penetrate the host through the radical system. During the guest colonization process, fungi produce degradative enzymes, predominantly pectinolytic responses to cellular maceration and vascular blockage (131, 132). The *F. oxysporum* forms have a high intraspecific variability, besides physiologically and genetically, which is also manifested in the morphology and pigmentation of the colonies, which can take on a very different aspect depending on the culture medium used. In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying shapes. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple-according to the strain (or special form) of *F. oxysporum. Figure 6.*

Figure 6. *F. oxysporum in solid media culture*

F. oxysporum produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. Microconidia are composed by one- or twocelled, 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. Macroconidia are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen. Chlamydospores are round, thick-walled spores, produced on older mycelium or in macroconidia. These spores are either by one or two cells. *F. oxysporum* is an abundant and active saprophyte in soil and organic compounds. Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant residues. The fungus can survive either as mycelium, or as any of its three different spore types. Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant either with its sporangial germ tube or its mycelium by invading the plant roots. The roots can be infected directly through the root tips (133), 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. When the mycelium reaches the xylem, it invades the vessels through the xylem pits. At this point, the mycelium remains in the vessels, where it usually advances upwards toward the stem and crown of the plant. As it grows, the mycelium branches and produces microconidia, which are carried upward within the vessel by way of the plant sap stream. When the

microconidia germinate, the mycelium can penetrate the upper wall of the xylem vessel, enabling more microconidia to be produced in the next vessel. The fungus can also advance laterally as the mycelium penetrates the adjacent xylem vessels through the xylem pits. Adhesion or close contact of the fungal spores with plant surface appears to be important in sensing the plant signals. The recognition process is initiated almost at the first contact of the plant surface by pathogen. Initiation of the signaling process has been demonstrated even within 20 s of the first. 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. As *F. oxysporum* and its many special forms affect a wide variety of hosts, the management of this pathogen includes: disinfestation of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts plants, or by using resistant cultivars (134, 135).

1.6 *Verticillium dahliae*

Over 300 woody and herbaceous plant species are known to be susceptible to *Verticillium dahliae* including tomato, eggplant, pepper, potato, peppermint, chrysanthemum, cotton, asters, fruit trees, strawberries, raspberries, roses. *V. dahliae* occurs worldwide but is more important in temperate zones, and naturally occurs at low levels in soils and grows better at slightly higher temperatures 25 -28° C. The fungus belongs to the fungal class Deuteromycetes, a group of fungi which do not have a known sexual stage. The vegetative mycelium is septate and multinucleate; the nuclei are haploid in culture. Conidia are ovoid or ellipsoid and usually singlecelled. Symptoms vary among hosts, and none is absolutely diagnostic. Premature foliar chlorosis and necrosis and a tan to brown colored discoloration or streaking of the vascular system, however, are characteristic of all hosts. Symptoms of wilting are most evident on warm, sunny days. The fungus can overwinter as mycelium in perennial hosts, plant residues, and vegetative propagative parts. The fungus can survive for many years (10 years or more) in soil in the form of tiny, black, seed-like structures called microsclerotia that are stimulated to germinate by root exudates of both host and non-host plants. The fungus penetrates a root of a susceptible plant in the region of elongation and the cortex is colonized. From the cortex, the hyphae penetrate the endodermis and invade the xylem vessels where conidia are formed. Vascular colonization occurs as conidia are drawn up into the plant along with water. As the diseased plant senesces, the fungus reached the cortical tissue and produces microsclerotia, which are released into the soil with the decomposition of plant material.

The management of this fungal disease is similar to *F. oxysporum*: disinfestation of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (134,135)

1.7 Innovative methodologies for studying plant stresses

The emergence of "omic" disciplines, such as genomics, proteomics and metabolomics, has led to a transition from a reductive analysis of biological complexity, where the representation of a biological system was based on a hierarchical vision, from genes, to transcripts, proteins, metabolites, to a global or integrated approach, where control over the functioning of a biological system is exerted by a rich structure of genetic, protein and metabolic inheritance. Such methodologies have the ability to produce extremely high data and information, and this feature completely altered the approach of analysis which, until a few years ago, was based on the study of a small number of genes. This mode of study has created a new science, called "Systems Biology". That is, the science of discovering, reducing to model, understanding and eventually acting molecularly on the dynamic relationships existing between the biological molecules that define living organisms (136). This science is therefore a way of understanding a biological system in its entirety, studying its genes, mRNAs, proteins, metabolites, their roles in the cell and their concentrations (then goes to quantitative science). "Systems Biology" is a biological discipline that studies living organisms as systems evolving over time, that is, in the dynamic interaction of the parts of which they are composed. In particular, this goal is achieved by integrating the results of different experiments, combining in practice, for example, the knowledge of genomics, transcripts, proteomics and metabolomics. It is no longer necessary to look at a single aspect of an organism's life, but to study the whole living organism. The aim is therefore to come up with a more complete model of the functioning of biological systems. Through the study of systems, it is possible to see how individual pathways or metabolic networks are interconnected. This approach is based on solid theoretical structures and the use of computer models useful to explain experimental observations; Such studies are commonly referred to transcripts, proteomics and metabolomics (137)

Transcripts, proteomics and metabolomics reflect complex functions of different cellular components. The transcription of a certain gene is a function of the concentration of transcription factors and kinase activity and upstream activators. Likewise, the concentration of a protein is determined by the concentration of the corresponding mRNA and by the

activity of the translation apparatus consisting of kinase, phosphatase and protease proteins. As for the concentration of metabolites, it has a much more complex function because it depends on gene activation, transcript activity, and flows in the different metabolic pathways. Figure 7 shows the interactions mentioned.

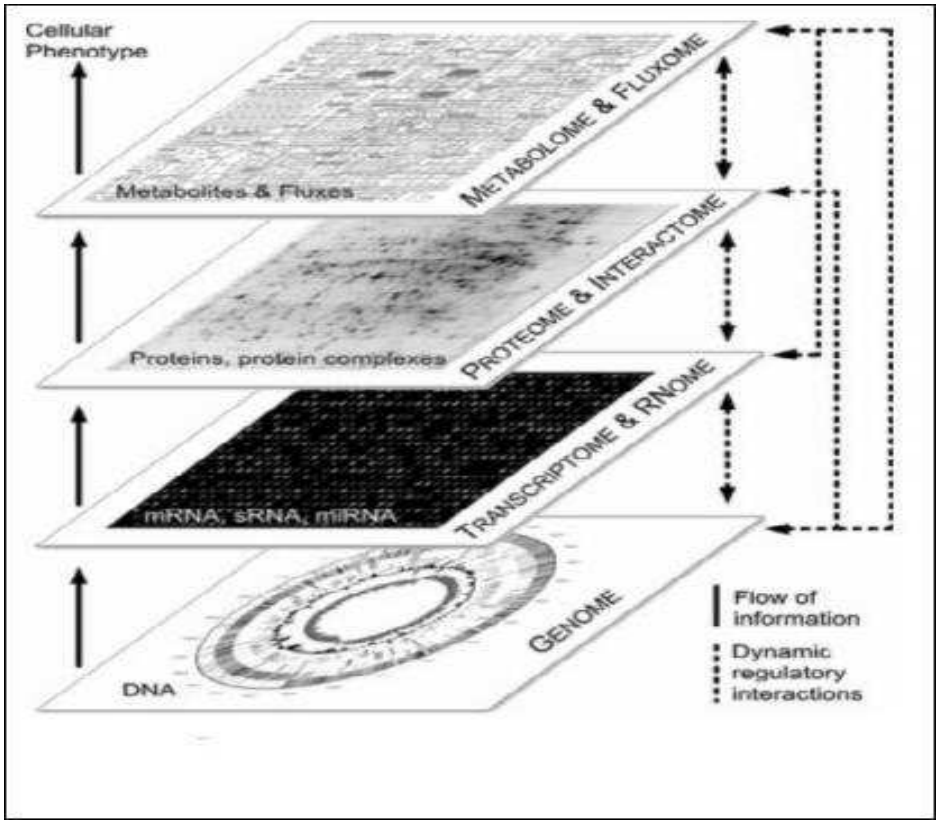


Figure 7. Flow of biological information.

“Systems Biology", with its "omic" technologies, can be used perfectly in order to carry out a direct and impartial monitoring of the factors that influence plant growth and development by providing data that can be used directly to investigate the complex Interaction between the plant, its metabolism and stress caused by the environment or biological threats (insects, mushrooms, or other pathogens) (Figure 8).

Plant responses to stress are mediated through profound changes in gene expression that give rise to changes in the composition of transcripts, proteomas and metabolomas of the plant (138).

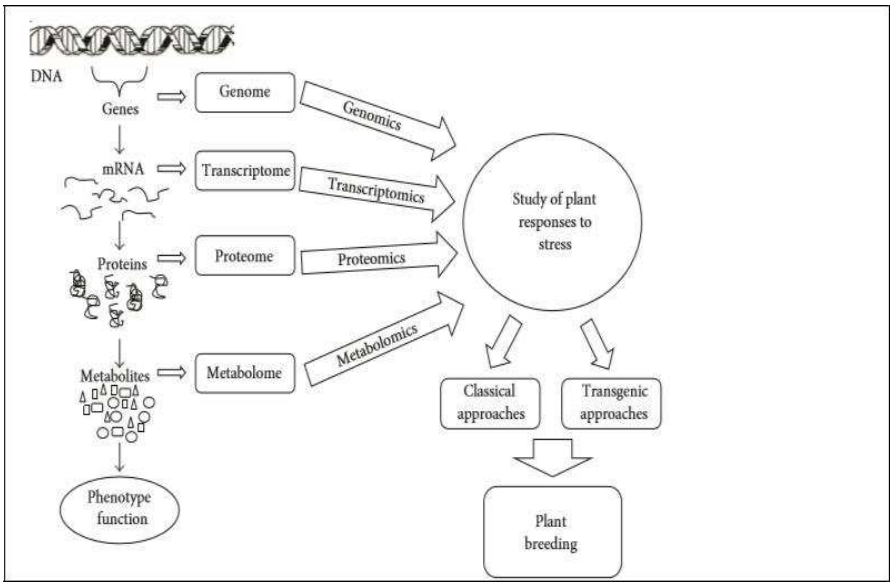


Figure 8. Genomics, transcripts, proteomics, metabolomics allow to increase knowledge about plant responses and adaptation to stress conditions and to improve plant cultivation.

1.7.1 Proteomics and mass spectrometry

The term proteomic was first proposed in Siena in 1994 during the Conference on Genome and Protein Maps, as "PROTEins expressed by completing a genome" (139, 140)

Currently, proteomic studies focus on two main areas: 1) expression proteomics, which tends to qualitatively and quantitatively define the increase and / or decrease in protein levels depending on appropriate stimuli and / or physiopathological conditions ; 2) functional proteomics, which attempts to identify cell compartment components, multiprotein complexes, and signal transduction pathways.

In addition, to the primary amino acid sequence, other protein properties, such as relative amount, specific activity, subcellular localization, and three-dimensional structures, represent crucial information for the description of biological systems. Significant progress has been made in this area in recent years, even in plant biology. In fact, it deals with complex and dynamic plant proteomics, choosing key proteomic approaches that promote protein identification and the modification that can help to improve crops. In recent years, high-resolution quantitative proteomics studies and mass-precision instruments have contributed to important information for understanding the growth of plants, their

development, and their interactions with the environment. This study opportunity is particularly useful for crops as it can not only help to increase nutritional value and yield, but also to understand crop adaptation mechanisms in response to environmental stresses (141). Such studies are considerably increasing, thanks to a number of methodological improvements concerning the manipulation and fragmentation of biological samples, the development of public databases and computer prediction programs.

Significant progress has also been made towards identifying and cataloging proteins from plant tissues and organelles. The growing number of proteomic studies in plant species shows increasing interest in the analysis of plant proteomics, specific tissues or subcellular compartments. Despite the great advances in proteomic technology, the effectiveness of this strategy is firmly based on the quality of the biological sample to be analyzed.

Sample preparation and manipulation are the most critical points of proteomics. Additionally, building protein lists is just a step towards knowledge of proteoma dynamics within a given cell or body. Finally, a proteoma is much more dynamic than a genome, as it changes suddenly during plant growth or in response to different stimuli to which it is subjected and proteins form large interaction and regulation networks(142).

Proteomics is one of the tools that can help you understand protein components within functional complexes and is a key prerequisite for understanding cellular functioning and to reveal the complex molecular signalling pathway network. However, in order to fully understand the complex strategies, it is not enough to make a simple list of proteins, but it is necessary to make a quantitative profile of the same and to illustrate all existing interactions (143). Today using mass spectrometry as a basic protein-based technique, one can have completely reliable information on the amino acid sequence of a polypeptide and also obtain quantitative data on expression levels in different biological samples. A previously purified protein or protein complex blends, usually separated by electrophoresis on single- (1D) and two-dimensional (2D) SDS-PAGE gel, are subsequently subdivided proteolytically into smaller peptides,

which are then analyzed by spectrometry mass. It is an analytical technique that produces ions, separates them in the gas phase (fragmentation) according to their different mass / charge ratio (m / z) and that, unlike other techniques, is a destructive analysis method How much the molecule does not remain intact after the analysis. The molecular ion formed by the spectrometer, partially fragmented by giving molecules and / or neutral radicals that the instrument does not detect, in part generates cations. The molecular ion formed by the spectrometer, partially fragmented by giving molecules and / or neutral radicals that the instrument does not detect, in part generates cations. The molecular ion and the various ions that originate by fragmentation are discriminated based on their mass / charge ratio and detected by a detector. The proteomic analysis procedure comes in simplicity schematized in the following steps:

- **pre-fractionation of the sample (ie separation of cellular organs);**
- **fractionation of molecules of interest by chromatography or 1D / 2D gel;**
- **qualitative / quantitative differential analysis by mass spectrometry;**
- **bioinformatic analysis qualitative / quantitative data.**

When the protein is drawn from a mono- or two-dimensional gel (1D or 2D), the band or spot is generally digested with trypsin (specific for arginine and lysine amino acids) which fragment the protein. Fragmentation necessary for the protein to "exit" from the gel jets to be introduced into the mass spectrometer. Figure 9 shows the processes occurring within the mass spectrometer, that is:

- **ionization of molecule;**
- **separation of ions;**
- **signal measurement**

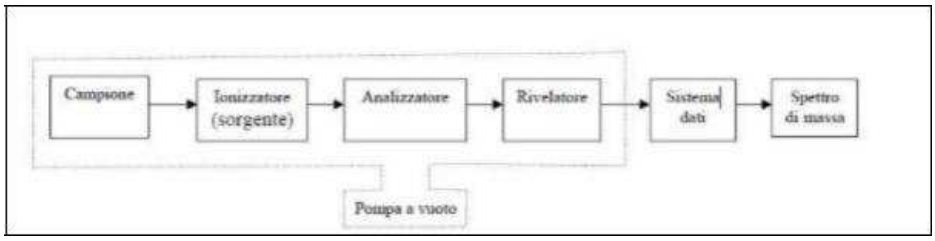


Figure 9. Parts forming a mass spectrometer. The analyte molecules are ionized in the source. The analyzer separates the ions from the source based on their mass / charge ratio (m / z). For each m / z ratio value, the detector detects the amount of ions that come from the analyzer.

The peptide mixtures produced by chemical or enzymatic hydrolysis are separated by capillary HPLC / UPLC and the column eluted fractions are directly introduced into the ionization source of the spectrometer. The most used are: i) electronic impact (E.I.); ii) chemical ionization (C.I.); iii) electrospray (E.S.I.). In particular, in the electrospray ionization (E.S.I.) the sample is dissolved in a polar solvent and atomized at atmospheric pressure inside the ionization chamber through a needle held at high electrical potential. Spray droplets, positively charged by the action of the electric field, are attracted to an "ion extraction lens", which coarsely consists of a vacuum capillary and a negative potential; So the solvent evaporates and the charged ions are accelerated to the analyzer. Ions isolated from a first analyzer pass into a collision chamber. Product fragment ions are selected by a second analyzer. The loaded load fragments give information about the primary structure of the selected peptide. The instrumentation chosen for this type of investigation involves the use of a pair between two analyzers separated by a collision chamber or an ion trap. By this method, the peptides are fragmented by collision with inert gas (Collision-Induced Dissociation, CID); In appropriate conditions, the peptides are fragmented at the bonds between the amino acids (peptide bonds). The measurement is done through an analyzer that differentiates the generated ions based on their mass / charge ratio. Signal measurement is carried out through an ion collector and detector, usually an electronic multiplier is used, consisting of a series of cascade electrodes and as a result there is a strong amplification of the signal which is then digitized and processed by the spectrometer calculator for The presentation of the mass spectrum. Subsequently

spectra are analyzed by specific software that can do qualitative analysis, useful for protein identification, and quantitative analysis. The fundamental principle on which they are based exploits the idea that the proteolytic pattern of each protein is uniquely dictated by the specific amino acid sequence and that it is therefore identifiable as the protein in question (144).The collection of collected data is compared with a "virtual" digestion, made by software, of all proteins contained in genomic databases. Accurate mass determination of a sufficient number of peptides and their sequence will identify one and only one protein among those in the databases. In other words, only one protein can give rise to all of the peptides having the observed masses. The answer can be ambiguous and equivocal if a set of parameters are not satisfied such as: insufficient number of peptides, presence of peptide contaminants due to sample manipulation, poor electrophoretic resolution. The application of proteomics to the study of plants is still in a primordial phase compared to proteomic research aimed at studying animals and yeasts (145, 146, 147), also because there are few databases that report plant protein sequences.

1.7.2 Metabolomic and mass spectrometry

The term metabolomic was coined by Oliver (148), and refers to the complete set of low molecular weight metabolites present in a biological system: cells, tissues, organs, biological fluids or an entire organism.

The metabolome consists of endometaboloma, the set of intracellular metabolites and esometaboloma, the whole of the metabolites that are secreted in the growth medium or in the extracellular fluid (149). The metabolomic term was coined to indicate the techniques capable of identifying and quantifying the metabolomic and consists in the global analysis of all metabolites present in a sample and dependent on the pathophysiological state and cell development (150, 151).

Plants produce a large number of diversified structure and concentration metabolites that play an important role in growth, development and response to environmental conditions. These metabolites with different molecular weight are also a valuable source of nutrition and energy for humans (152). Metabolites are classified into primary and secondary. Primers are indispensable to the growth and development of a plant,

while secondary ones are not essential but are crucial for a plant to survive in stressful conditions while maintaining a delicate balance with the environment. In addition, primary metabolites are highly conserved in their structure and concentration, while secondary metabolites have a wide variability in plant kingdom (153). Although metabolomics are downstream of other functional genomic techniques (transcriptional and proteomic), however, the metabolism of a species, unlike transcripts and proteomics, cannot be hypothesized directly from available genomic information. Therefore, metabolomics are used to obtain a great deal of information, useful for the discovery of genes There seems to be a complicated network of regulation between these small molecules in plants, therefore metabolic analysis can significantly contribute to understanding the relationship between the genotype and the metabolic product (155). However, the study of plant metabolomics has become a powerful tool for exploring various aspects of plant and biology physiology, which significantly enhances our knowledge of metabolism and molecular mechanisms of plant growth regulation, developmental responses and stress , The improvement of crop productivity and product quality (156). The metabolites to be identified are often hundreds of small molecules with different physical-chemical properties (pKa, polarity, size) and are present in a wide range of concentrations in complex matrices (157). Currently in the study of natural extracts, mass spectrometry, coupled with chromatographic techniques, and in particular gas chromatography (GC), is the most effective technique to fully describe the whole metabolome. This technique offers a set of tools that can be used for the chemical and structural characterization of the various compounds, such as:

- accurate measurement of mass / charge ratio (m / z) of molecular ions and fragments
- comparison of data obtained experimentally with those reported in databases and databases containing chemical-physical properties or mass spectra and chromatographic data (158).

Metabolic studies in general are performed using targeted (target) and non-targeted (untarget) approaches. Targeted approaches identify a list of metabolites that have a preliminary knowledge, such as mass characteristics, fragmentation spectrum, retention time; The identification is then validated with a reference standard (159). The fundamental limitation of this approach is that standards are not always available and the analysis focuses on a limited number of compounds that standards are available or that have already been identified and validated. The non-target metabolic approach is instead used to try to locate all the analytes present in a sample, although many chemical compounds are not immediately identifiable. In the non-ambiguous identification of many chemical compounds an important role is represented by web-based databases containing lists of experimental and bibliographic data, of metabolites that can be expected in different arrays. In view of the complexity of biological systems, these databases, while containing thousands of metabolites, appear incomplete and must be continually updated and implemented.

1.8 Aim of the thesis

Solanaceae, a family of dicotyledonous angiosperm among the most important in the vegetable kingdom, are characterized by a high economic importance (160). The highest concentration of diversity lies in South America where it is believed to have originated (161). In addition to a wide variety of other uses (eg traditional medicine, pharmacology, ornamental horticulture). Solanaceae species are of very high importance as food plants around the world (162). For example, only in 2010, 28 million hectares of Solanaceae species were cultivated globally, producing about 540 million tonnes of fruits (163). However, this only refers to the four best known species (potato, tomato, eggplant and pepper) and does not take into account the less cultivated species. The Solanaceae play a major role from a scientific point of view, for the large number of species that the family is composed of, and for their genetic plasticity, their environmental adaptability due to their genome that allows their development in more disparate environments and has allowed a big differentiation in sub-families. Solanaceae are important also from a ocial point of view, due to their high nutraceutical, economic and health value. For these reasons, they have gained a significant place in the scientific environment, becoming a model for the study of plant diseases, response to disease and environmental stress.

The general objective of the PhD thesis is to study the response to biotic and abiotic stress in *Solanaceae* by means of proteomic and metabolomic approaches.

- As regard to the abiotic stress study, pepper response to high salinity conditions was investigated, by means a metabolomic approach. Pepper (*Capsicum annuum* L.) is widely cultivated in the Mediterranean basin where is often irrigated with water containing high salinity levels, as it is considered a moderately resistant saline species (164, 165). The available informations about salt tolerance mechanism of pepper, due to the genetic and physiological complexity of the studies (166), is derived from experiments carried out during a brief plant exposure to salt.

  In the present study, two different genotypes of pepper were considered: “Quadrato D'Asti Giallo” e “Cazzone Giallo”. The research activity had the following two objectives:

a) biochemical characterization and analysis of the main secondary metabolites (ascorbic acid, flavonoids, carotenoids)produced by pepper fruit cultivated in the presence of several NaCl concentrations;

b) biochemical characterization and analysis of the volatile compounds produced by pepper fruit and their modulation under saline stress conditions.

- As regards to the biotic stress study, a proteomic approach wasapplied to identify the proteins involved in the resistance / tolerance mechanism to tracheomycotic pathogenic fungi, such as *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dalhiae Kleb*, in eggplant. These pathogens are responsible for extensive damage to eggplant cultivation both in greenhouse and in the open field, in Europe and above all in Asia. In the present study, two eggplant genotypes, obtained by means of non-conventional breeding techniques, were considered:

1) **Tal 1/1**, *S. melongena* susceptible (S) to *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dalhiae Kleb*;
2) **305E40,** *S. melongena* resistant (Rfo-sa1 resistance locus) to *Fusarium oxysporum* f. sp. *melongenae* and tolerant to *Verticillium dalhiae Kleb*.

The key objective of research activity was to identify the proteins expressed by eggplant in response to *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dalhiae* infection, in order to delineate the molecular pathways through which resistance or tolerance mechanisms are implemented. The knowledge at a molecular level of these mechanisms will contribute to improve the current informations about eggplant response to fungal pathogens. This will allow to exploit the sources of resistance and tolerance in order to reduce damages by tracheomycotic fungi on eggplant crops and obtain higher yield.

2. Materials and e Methods/pepper

2.1.Historical reference, morpho-physiological characteristics and production

Pepper, originating in the tropico-equatorial area of Central and South America, is widely cultivated worldwide in temperate-warm countries as well as in tropical and sub-tropical areas. Already between 5200 and 3400 a.C there is evidence of its presence, while in Europe the cultivation was introduced by Christopher Columbus, from Europe spread all over the world to China in 1700.

The edible part of the plant is represented by the fruit, a berry, which can be consumed fresh, transformed or as a condiment in the form of powders.

The genus Capsicum is classified according to the structure of its flower, its fruit and the number of chromosomes (2n = 2x = 24; 2n = 2x = 26, 2n = 4x = 48) (167).

On the basis of the geographic origin, morphological traits, reproductive behavior, karyotype analysis and biochemical and molecular marker information, Capsicum species are grouped into three main complexes: *C. annuum*, *C. baccatum*,and *C. pubescens* (168, 169). These complex groups are organized in two main phylogenetic branches: the white-

flowered group (including *C. annuum* and *C. baccatum*) and the purple-flowered group (Figure 10).

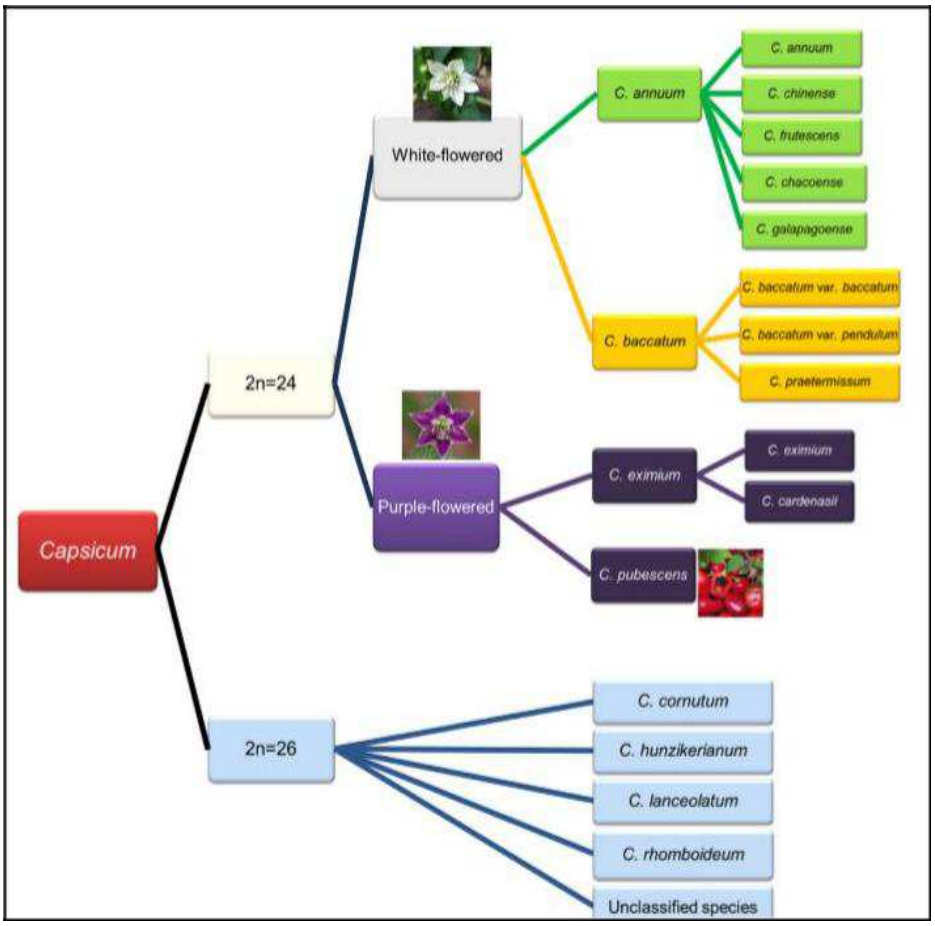


Figura 10. Phylogenetic relations between species of the genus *Capsicum*

The most cultivated species, *Capsicum annuum L.*, finds its ideal environment in the tropical regions, where it grows and produces fruits in all seasons, while with our climate the plant is annual. Temperature has a fundamental role and may be deleterious to survival, below 4-5 ° C and above 42-44 ° C. In addition, C. annuum is a longitudinal plant and heliophila plant, so for flowering and planting it prefers high luminous intensity and a 16-hour photoperiod. The fruit produced is a fleshy berry and during development, the esocarpus first has a bright green coloration or, in some cases, ivory, then change with ripening to varying shades of yellow, red, purple and brown. The shape of the berry is variable and characterizes the various types of commodities. Inside the berry there are logs, empty spaces, in varying numbers and are divided by white placental placentas that start from the peduncular attack, forming a thick spongy fabric until it reaches the end of the fruit. On the placenta there

are round shaped seeds, flattened and in proximity to the seeds and along the placentary seals can be, in the spicy species, the glands secreting the capsaicin, a phenylethylamine alkaloid, which determines the spiciness of the berry. The species Capsicum annuum L. currently has seven botanical varieties described and divided according to the characteristics of the berry produced and are (170):

C. annuum var. *abbreviatum* (Fingerh), ovoid shaped berries, medium small and irregular

C. annuum var. *cerasiferum* (Irish), fruity cherry, spicy and compact pulp

C. annuum var. *conoides* (Irish), conical fruits of about 3 cm, spicy with uptight

C. annuum var. *fasciculatum* (Irish), very spicy, herbaceous fruit, about 7cm in length, grouped in small bouquets

C. annuum var. *acuminatum* (Fingerh), berries of oblong and pointed shape, the size is over 9 centimeters and are generally spicy

C. annuum var. *longum* (Sendt), elongated berries bent down and tapered at the apex

C. annuum var. *grossum* (Sendt), large berries, bend down and are generally sweet with a thick pulp.

Fruits are a good source of health-related compounds such as ascorbic acid (vitamin C), carotenoids (provitamin A), tocopherols (vitamin E), flavonoids and capsaicinoids (171) and mineral salts such as potassium, calcium and phosphorus (Figure 11).In the world spice trade, the market for Capsicum is expanding. Peppers production has also increased over the past 30 years and in 2010 reached 29 million tonnes with an area of 1.8 million hectares (163). The world surface for the cultivation of the various edible species of the Capsicum gene is 3,904,349 hectares (FAOSTAT, 2014). Asia is at the forefront of both fresh and powdered peppers, followed by Africa and the Americas. Among the countries contributing most to world production are China (15,823,000 t), Mexico (2,294,400 t), Turkey (2,072,132 t), Indonesia (1,726,382 t) United States (889,269 t). In Europe, the country with more product production is fresh and processed than Spain (999,600 tonnes) followed by Holland

(345,000 tonnes), Romania (227,690 tonnes), Italy (212,425 tonnes) and Greece (205,900 tonnes). Data from FAOSTAT 2014 show that 9,036 hectares of open field and greenhouse were cultivated in Italy and total production was 191,351 tonnes.

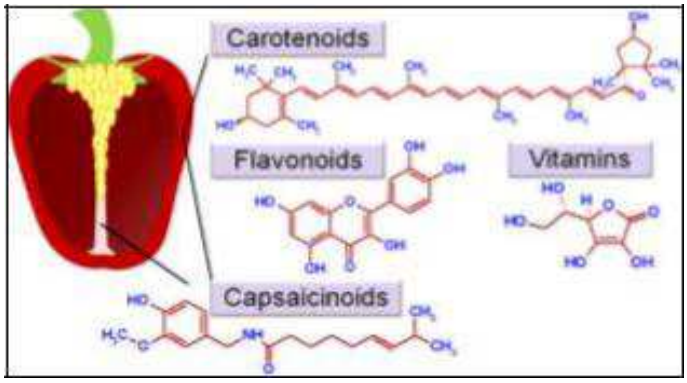


Figure 11. Localization of high nutraceutical compounds in the fruit of pepper.

2.2 Plant materials and experimental plan

The experiment was carried out in a heated greenhouse located at the CREA-Research centre for vegetable crops in Pontecagnano (SA), Italy, and equipped with an automatic, computer-controlled drip irrigation soilless system. Pepper plants, cv. "*Quadrato D'Asti*" (QA) and cv. "*Cazzone Giallo*" (CG), were cultivated in protected environment by applying the soilles cultivation technique, in a plant consisting of four independent canals with recirculation of the nutrient solution and an automated drop irrigation system. Plants were transplanted in pots (diameter 27 cm, capacity 10 liters) filled with coconut fiber as growing substrate and were subjected to saline stress 13 days after transplantation. Stress was gradually applied by adding to the nutrient solution 30 mM NaCl, increasing by 30 mM NaCl every 12 hours until the final concentrations of 30, 90, and 120 mM NaCl were obtained for three (T30 , T90 and T120) of the four canals used , respectively. The fourth canal, used as a control, had a NaCl concentration of 0 Mm (T0).

The composition of the basic nutrient solution adopted was (meq/l):

Na^+, 0.2; N-NH_4^+, 0.5; K^+, 5.0; Ca^{++}, 10.8; Mg^{++}, 4.0; Cl^-, 0.5; N-NO_3^-, 14.6; P-$H_2PO_4^-$, 1.2; S-$SO4^{--}$, 3.5; HCO_3^-, 0.5.

Micronutrient concentrations were applied according to Hoagland and Arnon (172). Saline treatment started after 13 days from transplanting ('*shoot development*' stage) and continued until the end of the experiment ('fruiting' stage, 100 days of stress).

Sixty plants were distributed among the saline treatments (15 plants for each treatment). The experiment was laid out in a randomized complete block design with the four irrigation treatment each replicate three times (5 plants per treatment). Each nutritive solution was pumped from an independent tank to the corresponding channel where each of the 15 pots was provided with two emitters (emitter flow rate: 2 litres/h). After application, the solution drained from the pot substrate and returned to its tank for later recirculation (closed system). The electrical conductivity (EC) of the four solutions was 2.6 - 5.7 - 12.0 and 15.6 dS / m (pH 5.6) for T0, T30, T90 and T120, respectively. Both the pH and EC of each solution were daily controlled and maintained constant over the whole cycle: the pH value was maintened within the range of 5.5 to 6.0 by

adding HNO_3, while, the EC was stabilized, with oscillations within the 10% of its value, with a fresh nutritional solution.

2.2.1 Sampling

For the two pepper varieties , plant sampling was carried out by harvesting leaves and fruits. The leaves were collected in several stages of plant growth (vegetative phase; breeding phase; green berry, berry breaker and yellow berry phases), while fruits in the three different maturation stages (green berry, berry breaker, yellow berry). The biological material, leaves and fruits, was sampled on three different plants for each of the three biological replicates per treatment and was immediately frozen in liquid nitrogen and stored at -80 ° C until preparation of the extracts for analysis. Each biological replicate was analyzed in double for all the parameters.

2.2.2 Consumables

The salts used for the preparation of metabolite extraction buffers, the standards used for the creation of calibration curves, solvents and materials used are Sigma-Aldrich's products (Sigma-Aldrich, St. Louis, MO, USA).

Pepper ecotype CG under salt stress

0 mM **30 mM** **90 mM** **120 mM**

Fruits of the two pepper genotypes obtained from the four different levels of saline stress

2.3 Methods of Analysis

2.3.1. Proline Extraction

The proline content was determined in accordance with the method of Chandrachood et al. (173), with some minor changes. The leaves (0.5 g) were homogenized in a mortar with 2 ml of water. After centrifugation (14,000 rpm for 10') applied in order to sediment the material for faster filtration, the suspension was filtered using 0.2 μm polytetrafluoroethylene (PTFE) filters.

2.3.2 Determination of proline by UHPLC-MS

The proline extracts have been quantificated though an ultra high resolution liquid chromatograph (UHPLC) mod. UltiMate 3000, coupled to a photodiode series detector and an ion trap system and mass spectrometry (LC-PDA-MS, ThermoFisher Scientific, Sunnyvale, CA, USA). UHPLC analysis was performed using a Kinetex column (75x4.6 mm, 100 Å, 2.6 μm particle size), (Phenomenex, Torrance, CA, USA), and a binary solvent was used. It was composed of: A) 10mM ammonium formate in water + 0.1% formic acid and B) Acetonitrile + 5% solvent A + 0.1% formic acid. The elution program was as follows: from 5% B to 100% B in 3.5 minutes, 100% B for 0.5 min, from 100% B to 5% for B in 5.0 min; Flow 0.2 ml / min; Volume injected 2 μl. The LC-PDA-MS system was set in the positive electrospray ionization (ESI) mode, in the range m / z 100-1400. Capillary voltages were fixed at -5.96 V and the source temperature was 205 ° C. The amino acid standard was analyzed, at several and known concentrations, to obtain the mass spectrum and the retention time, and to construct a calibration curve. Subsequently, by comparison, the concentration of the amino acid present in the samples was obtained. The data were expressed in μg / g of fresh weight (fw).

2.3.3 Abscisic Acid (ABA) extraction

The Abscisic Acid content was determined according to the method of Forcat et al. (174), with some modifications. The vegetable material (1 g of leaves) was homogenized in a mortar with 1.5 ml of a solution of acetone / water / acetic acid (80: 19: 1, v / v). After centrifugation at 15,000 rpm for 10 ', the material was filtered with 0.2 μm filters in PTFE.

2.3.4 Determination of Abscisic Acid (ABA) by UHPLC–MS

The extracts were subsequently analyzed and quantified, using the same column and LC-PDA-MS system as for proline analysis, with some modifications, such as the ESI set in negative mode. The data were expressed in μg / g of fresh weight (fw).

2.3.5 Ascorbic acid extraction (AsA)

AsA extraction has been obtained from an aqueous extract of pepper pericarp, the latter obtained in this way: 1 g of pepper pericarp in 3 ml of 6% metaphosphoric acid in distilled water, homogenized for 30 s using an Ultra-Turrax homogenizer, then centrifuged at 3000 rpm for 15 min. The extraction was repeated twice on the pellet, the supernatant was collected each time and brought to a final volume of 10 ml with the extract solvent. The extracts were filtered through 0.2 μm PTFE filters.

2.3.6 Determination of Ascorbic acid by UHPLC

The extracts were analyzed by a chromatographic column and by the UHPLC system previously indicated for proline analysis. The mobile phase consisted of 0.02 M aqueous phosphoric acid solution at a flow rate of 0.35 ml / min, the injection volume was 5 μl. The quantification of the AsA was carried out at 254 nmusing a calibration curve obtained with an appropriately diluted standard. The data were expressed in mg / 100 g of fresh weight (fw).

2.3.7 Carotenoid Extraction

Carotenoids (α-tocopherol, β-carotene, violaxanthin, neoxanthin and lutein) were extracted and determined according to the method of Rotino et al. (175). 10 g of pepper pericarp were homogenized with 2 ml of hydroxytoluene butylate (BHT) in 0.1% methanol (v / v) for 3 minutes an added with 15 ml isoottane. Samples were agitated to vortex and incubated at 4 ° C for 1h; 5 ml of extract was dried in a rotary evaporator at 45 ° C and the dry residue, dissolved in 1 ml mobile phase, was filtered through a 0.22 μm PTFE filter.

2.3.8 Determination of carotenoid by reverse phase HPLC

The extracts were analyzed and quantized using high resolution reverse phase liquid chromatography (FI-HPLC).

The FI-HPLC analysis was performed using a Waters E-Alliance system consisting of a mod. 2695 with quaternary pump and self-sampler and a photodiode series detector mod. 2996. FI-HPLC analysis was performed by injecting 20 μl of sample on a C18 Partisil 5 ODS-3 column (250 × 4.6 mm) connected to an appropriate pre-column (Whatman). Isocratic at 40 ° C was used as mobile phase: acetonitrile / water / ethyl acetate / ethyl acetate: tetrahydrofuran 1: 1 (42: 10: 18: 30) containing 0.2% glacial acetic acid A flow of 0.8 ml / min. Each separation lasted 25 min and quantization was made at 502 nm using a calibration curve. The data were expressed in μg for 100 g of fresh weight (fw).

2.3.9 Extraction of glucoside flavonoids

The glucoside flavonoids were extracted and determined using the method described by De Vos et al. (176) with small modifications: 10 g of pepper pericarp was homogenized in a mortar with 1.0 ml of 70% methanol acidified with 0.125% formic acid and mixed immediately for 10 s. The extracts were sonicated for 15 minutes and filtered using 0.22 micron PTFE filters.

2.3.10 Determination of glucoside flavonoide byUHPLC–MS

The filtered extracts were analyzed using the LC-MS system already above indicated. A 4 µl aliquot of sample was injected on a Luna C18 (100x2.0 mm, 2.5 µm particle size) column equipped with a SecurityGuard guard column (3.0x4.0 mm) from (Phenomenex, Torrance, CA, USA).. The separations were carried out using a binary gradient of ultrapure water/0.1% formic acid (A) and acetonitrile/0.1% formic acid (B) with a flow rate of 0.2 ml/min. The initial solvent composition consisted of 97% (v/v) of A and 3% (v/v) of B; decreased linearly to 75% A and 25% B in 4.5 min, 65% A and 35% B in 3.5 min, 10% A and 90% B in 3 min, maintained for 0.5 min and returned to 97% A and 3% B for 1 min. The column was equilibrated to 97% A and 3% B for 5.5 min before the next injection. The analysis lasted for 18 min and the column temperature was set to 40 °C. The LC-PDA-MS system was set up in electrospray negative mode over the range *m/z* 100–1400. The capillary voltages were set at -5.96 V and the source temperature was 205 °C. Quercetin and luteolin glycoside identification was carried out by means of the UV spectra, molecular weight and MS–MS fragmentation profile in negative ion mode at 25 eV collision energy. Peak areas of quercetin and luteolin glucosides were obtained by integration at 340 nm. The concentration of quercetin and luteolin glucosides were calculated and expressed as µg of quercetin 3-glucoside equivalents/100 g fw.

2.3.11 Extraction of Volatile compounds (VOCs)

The volatile compounds were extracted and determined as described by Tikunov et al. (177) with some modifications. 1 g of pepper pericarp was homogenized in a mortar, then incubated at 30 ° C for 10 min. To the test sample was added 1 ml of a 50 mM EDTA-NaOH solution of pH 7.5 and then $CaCl_2$ to a final concentration of 5 M; The extract was then sonicated for 5 min. A 1 ml aliquot of pepper pulp was transferred to a 10 ml glass vial and used for solid-phase micro-extraction (SPME) analysis.

2.3.12 Volatile compound analysis by(VOCs) SPME-GC-MS

The volatile compounds were monitored using the gas-chromatography technique combined with mass spectrometry (GC-MS) and coupled to solid phase micro-extraction (SPME). At the time of the analysis, the sample was incubated at 50 ° C for 10 minutes (conditioning phase). Immediately after this phase, volatile molecules were adsorbed by the use of a silica fiber (65-micron polydimethylsiloxane-divinylbenzene, Supelco, Bellefonte, PA, USA). The fiber was left exposed to the head space for 20 minutes under continuous stirring and heating at 50 ° C. The volatile compounds adsorbed on the surface of the fiber were subsequently desorbed in the capillary column BR-5 FS (50 m × 0.32 mm X 1.0 μm, Bruker) for a period of 1 minute to 250 ° C. A gas chromatograph with a mass spectrometer (Scion SQ, Bruker Daltonics Inc., Billerica, MA, USA) was used for the separation, identification and quantification of the various metabolites. The GC interface and MS temperatures are 260 ° C and 250 ° C, respectively. The applied GC temperature program was as follows: 45 ° C (2 minutes) was then brought to 250 ° C at a rate of 5 ° C / min and finally kept at 250 ° C for 5 min. The total running time, including the cooling of the oven, was 60 min. The transport gas used was helium, with a flow of 1.0 ml / min. The molecules have been identified by comparing spectra The molecules have been identified by comparing mass spectra with those of pure compounds contained in the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA, http://www.nist.gov). Forty two standards have been used as authentic standards for optimizing the SPME-GC-MS method and for identifying VOCs. For volatile compounds, chromatography and spectral data were evaluated using MSWS 8.0 software (http://www.bruker.com).

2.3.13 Statistical analysis

Biochemical and metabolic analyses were performed in triplicate and all the measured data were statistically processed.

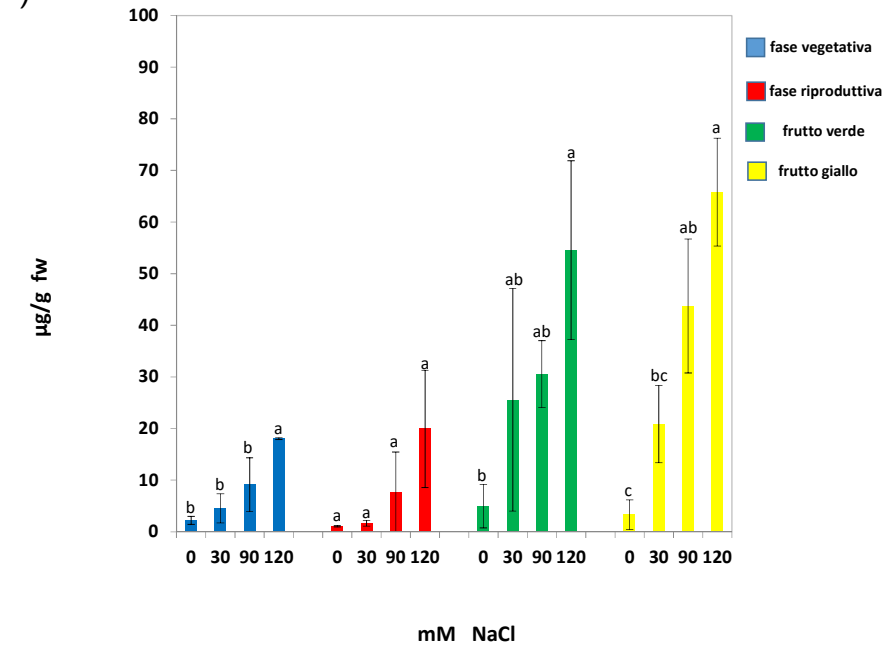
All the statistical analyzes were performed using JMP version 9.0 software (SAS Institute, NC, USA). Significant differences between the treatments were evaluated uniquely through ANOVA. The Tukey HSD test was used for separating the media. Multivariate analysis of volatile compounds was carried out using the Genesis software version 1.7.6 (http: // www .gene.tugraz.at). A log2 transformation was applied to data before multivariate analysis.

3. Results / pepper

3.1 Content of proline and ABA in the leaves

Content of proline and ABA was measured in both genotypes, Quadrato d'Asti (QA) and and Cazzone Giallo (CG), by analyzing foliar tissue samples at different phases of phenological growth of plants (vegetative phase, reproduction phase, green berry, yellow berry). The results obtained show no statistically significant differences between the different phenological phases under consideration, but it is noted that the content of proline and ABA increases significantly, depending on the concentration of NaCl applied (Figures 12-13). These two metabolites accumulate predominantly in the leaves, as these data confirm; rarely such metabolites were measured in other organs (preliminary analyses carried out on the fruit showed, however, a non-detectable concentration). The molecular results (data not shown) obtained for P5CS (Delta 1-pyrroline-5-carboxylate synthetase), a gene involved in proline biosynthesis, indicated that the stage of plant development and salt concentration influenced gene expression. In particular, a slight increase in gene expression was observed at higher saline concentrations. Interestingly, transcriptional modifications of the P5CS gene, as noted in metabolic analyses, are related to proline buildup.

a) Proline



b) ABA

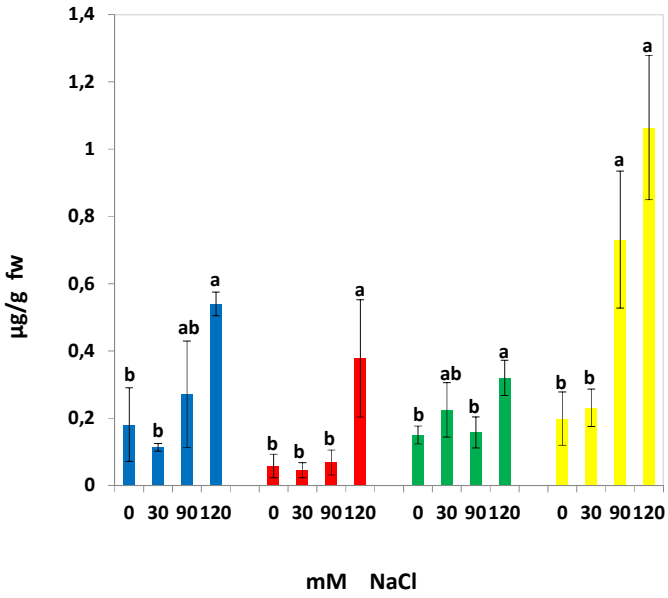
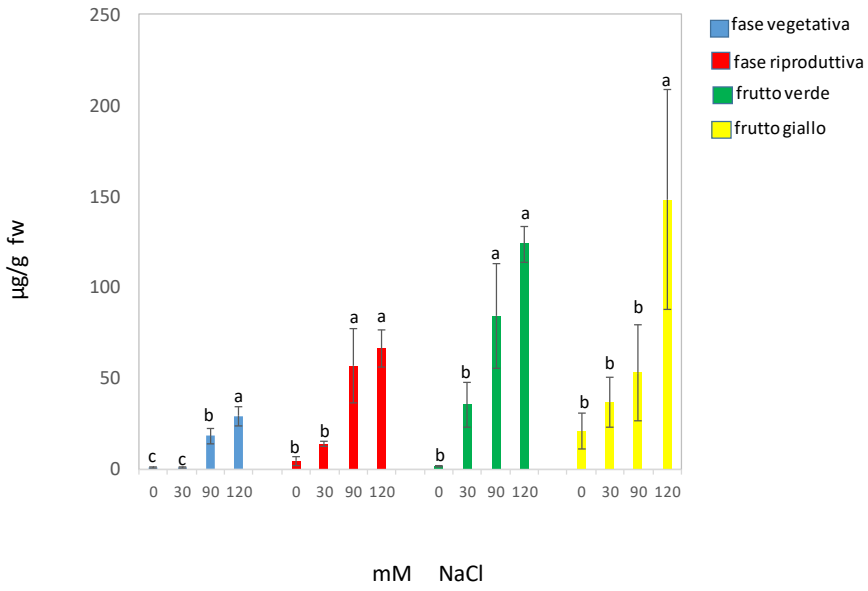


Figure 12. Proline levels a) and ABA b) measured in the pepper leaves of 'QA' genotype cultivated under saline stress conditions (T0-T30-T90-T120), during the 4 main development phases. Values with different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

a) Proline



b) ABA

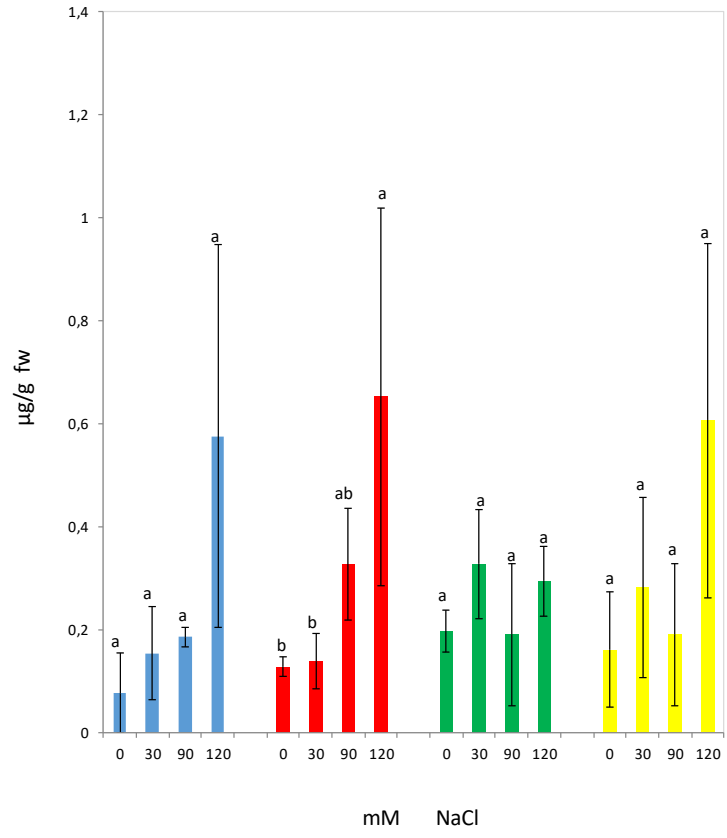


Figure 13. Proline levels a) and ABA b) measured in the pepper leaves of 'CG' genotypes cultivated in saline stress conditions (T0-T30-T90-T120) during the four main development phases. Values with different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

3.2 Content of Ascorbic Acid (ASA) in fruits

AsA content was measured in pepper fruits at three different maturation stages (green berry, berry breaker and yellow or ripe berry) in both genotypes, Quadrato d'Asti (QA) and Cazzone Giallo (CG). The average content of AsA was not significantly different among control (0 mM NaCl) and stressed plants (30, 90, 120 mM NaCl) during maturation (Figure 14). Comparing the average concentration of AsA at beginning of maturation with that at advanced maturation, a significant decrease was observed in both genotypes.

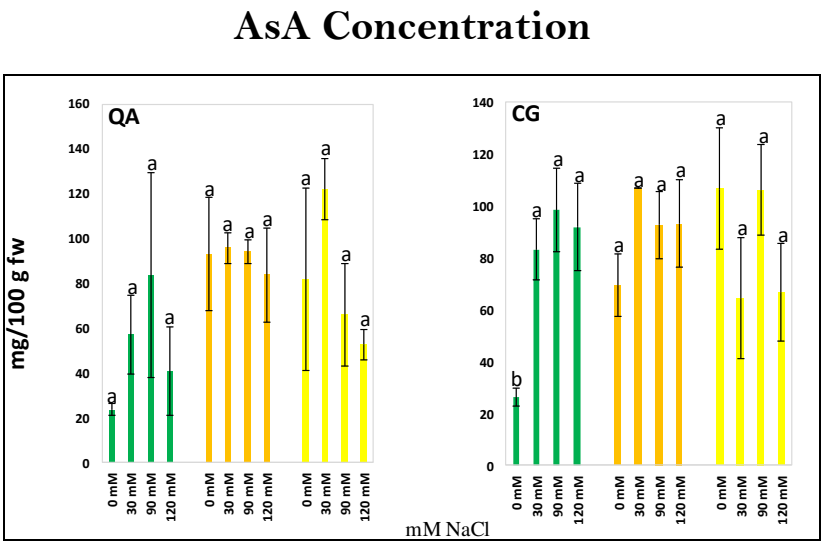


Figure 14. Concentration of Ascorbic Acid (AsA) in the pepper fruit of 'QA' and 'CG' genotypes grown under saline stress conditions (0-30-90-120 mM NaCl), during the maturation phase. Values with different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

3.3 Carotenoid content in fruits

Carotenoid content analysis was performed in both genotypes, Quadrato d'Asti (QA) and and Cazzone Giallo (CG), at different maturation stages (green, breaker and yellow berry). In particular, the content of **β-carotene, violaxanthin, lutein, neoxanthin and α-tocopherol** has been evaluated. Figures 15 and 16 show the graphs of mean concentrations of the various metabolites for the two genotypes according to the four experimental saline treatment. In both genotypes there was an increase in carotenoid levels with fruit ripening (0 mM theses), also a remarkable increase in the concentration of some different carotenoids was observed in relation to the increase in NaCl concentrations. It was particularly observed for **violaxanthin** and **lutein** metabolites.

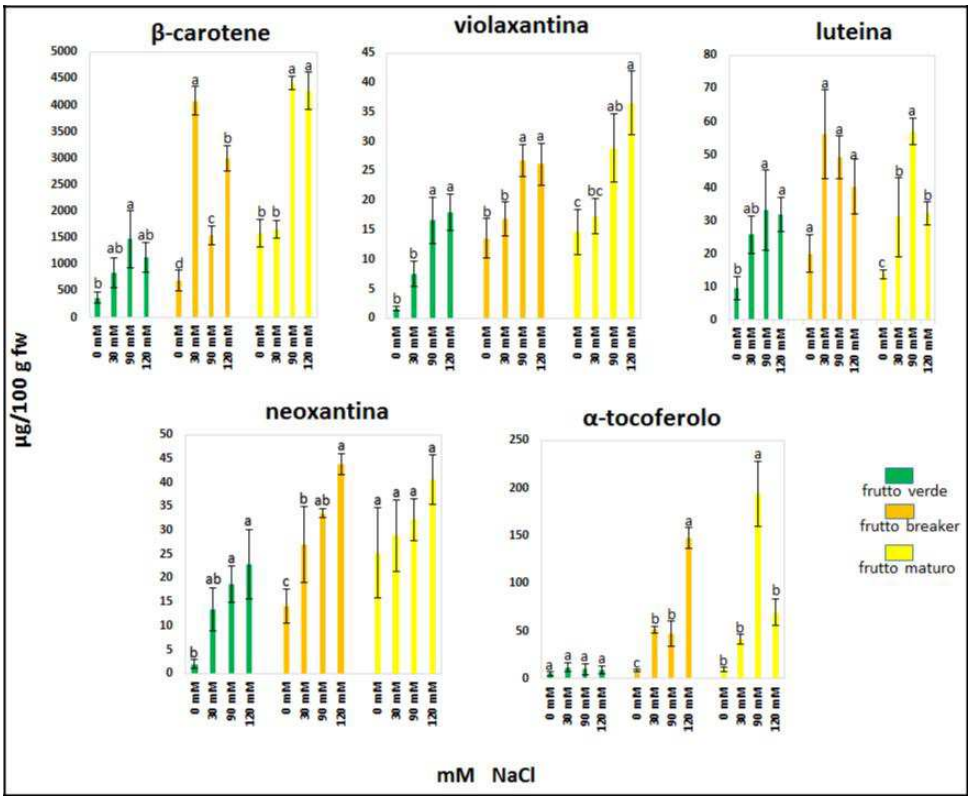


Figure 15. Concentration of some carotenoids contained in the pepper fruit of 'QA' genotype cultivated under saline stress conditions (0-30-90-120 mM NaCl) during maturation phase.. Values with different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

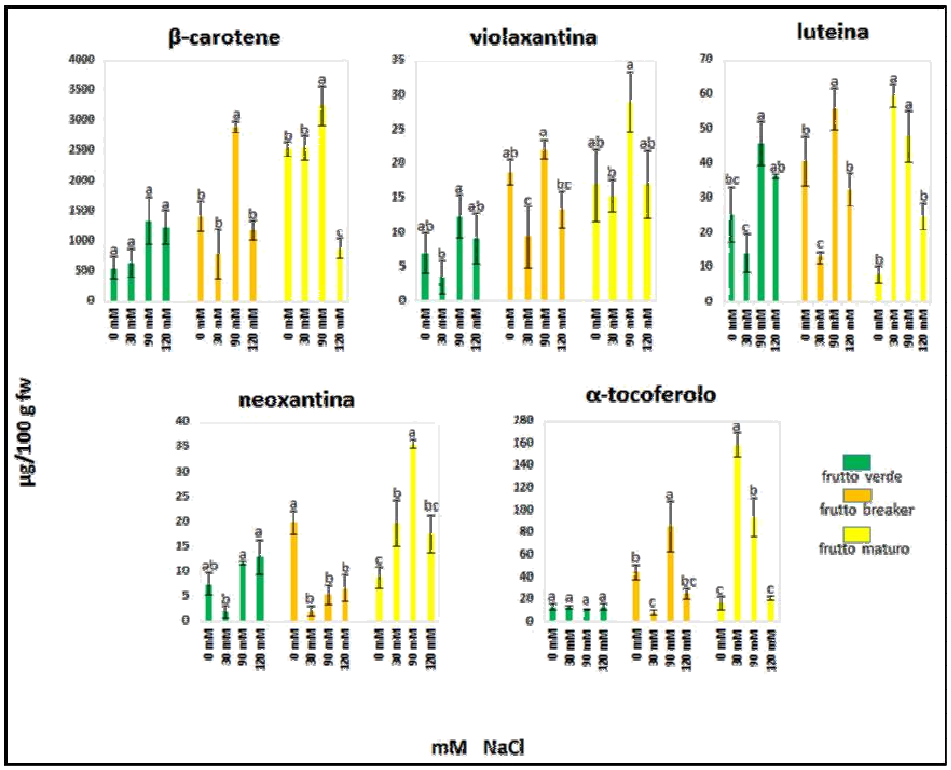


Figura 16. Concentration of some carotenoids contained in the pepper fruit of 'GC' genotype cultivated under saline stress (0-30-90-120 mM NaCl) during maturation phase. Values with different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

3.4 Content of flavonids in fruits

The flavonoid content was analyzed in both genotypes, Quadrato d'Asti (QA) and and Cazzone Giallo (CG), in different maturation stages (green, breaker and yellow berry). In particular, the content of total quercetin and lutein was evaluated since these are the most representative flavonoids in the fruit of pepper. For both genotypes, generally, an increase in concentration of such compounds was observed with maturation. A significant increase in the levels of these substances was observed, as a result of increased saline concentration, for almost all the considered phenological phases. In particular, the fruits from the 120 mM NaCl treatment showed higher levels of the two flavonoids in both genotypes (Figure 17).

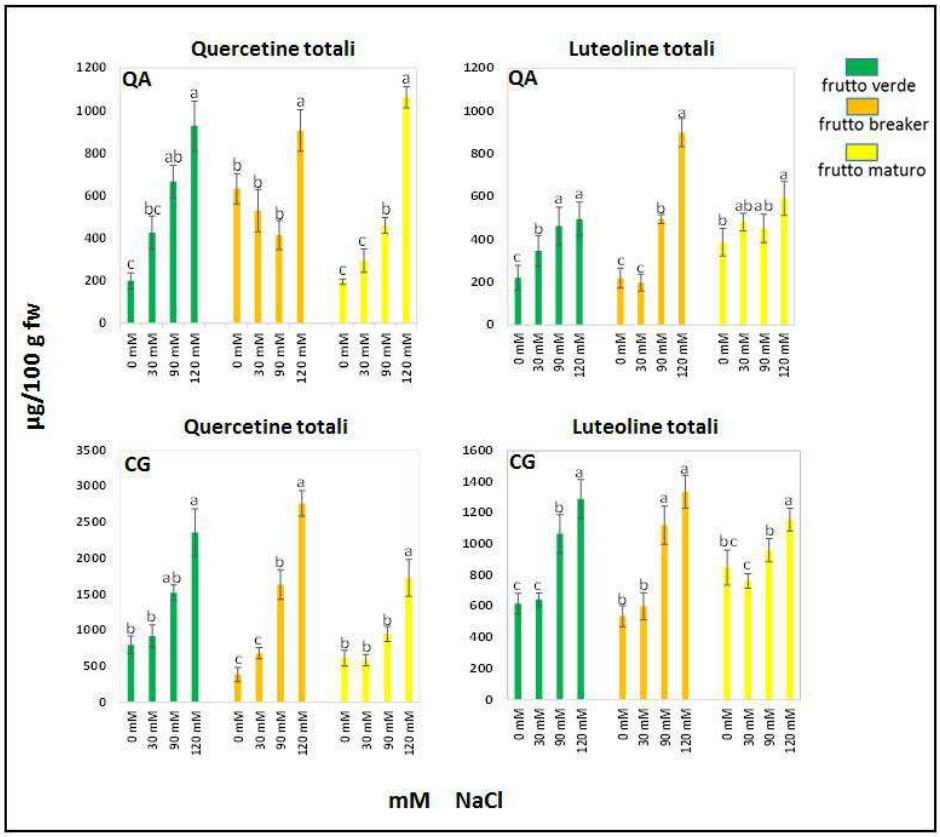


Figure 17. Concentration of total flavonoids (Quercetin + Luteolin) in the pepper fruit of 'QA', and 'CG' genotypes grown under saline stress conditions (0-30-90-120 mM NaCl), during maturation phase.. Values with different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

3.5 Content of Volatile Organic Compounds (VOCs) in Fruits

Volatile organic compounds (VOCs) of pepper fruits in both genotypes, Quadrato d'Asti (QA) and and Cazzone Giallo (CG), was studied by static head space analysis above the level of pepper extracts (in sealed vial) and identified through GC-MS.

The use of authentic standards has allowed to carry out a qualitative and quantitative analysis of the compounds present in the samples. Samples of fruits of both genotypes were analyzed at different maturation stages (green berry, breaker and yellow). Figures 18 and 19 show target volatiles (identified by comparison with a known standard) and untargeted volatiles (identified by comparison with profiles in databases), identified in the fruits of QA and GC genotypes. In the QA genotype, 83 volatile metabolites were uniquely identified and quantified. As shown in Figure 18, from the analysis of hierarchical clustering VOCs were grouped into 6 different groups (are they belonging to common pathways of the VOCs of each group?) and the VOCs profile in each thesis allowed the clear differentiation of the 12 experimental treatments in two main groups: plants grown under low (0-30 mM NaCl) and high (90-120 mM NaCl) salinity conditions.

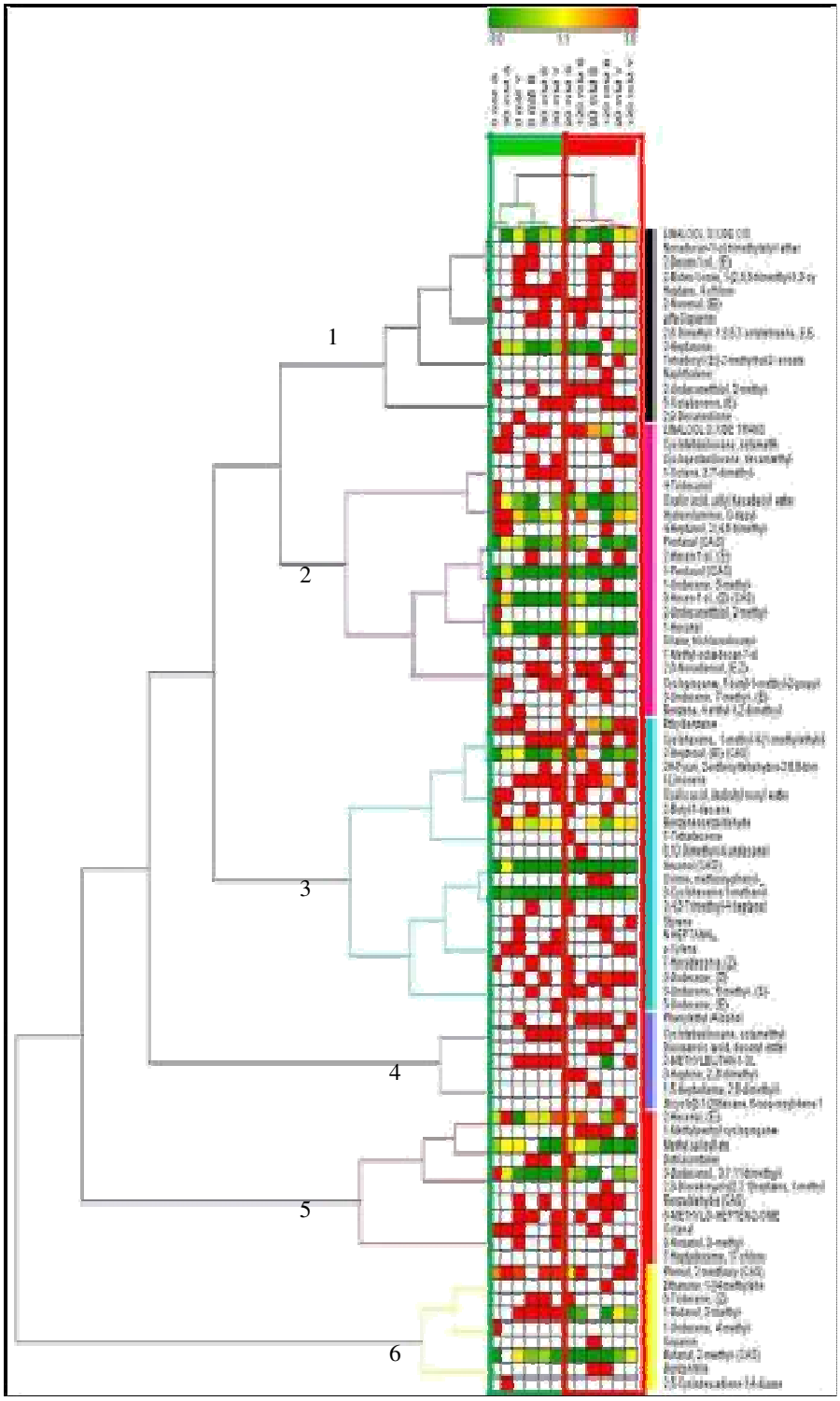


Figure 18. Heap map of 83 VOCs in the three pepper fruit maturation stages, green (G), breaker (B) and yellow (Y), and four stress levels (0-30-90-120 mM NaCl), identified in the genotype 'QA'. The color matrix represents the mean values of the intensity of the metabolites in the three biological replicates of pepper

In the GC genotype, from targeted and untargeted volatile metabolites analysis, 104 volatile metabolites were uniquely identified and quantitfied (Figure 19). However, from the analysis of hierarchical clustering, contrary to what was observed for the fruits of QA, no relationship was found between volatile metabolites and low or high NaCl levels. Only in the breaker stage, at the 90 and 120 mM levels of NaCl, an independent grouping is reported, probably at a more active metabolic phase, during the fruit ripening process (Figure 19). In addition, the main characteristic aromatic profiles of the two pepper genotypes have been identified.

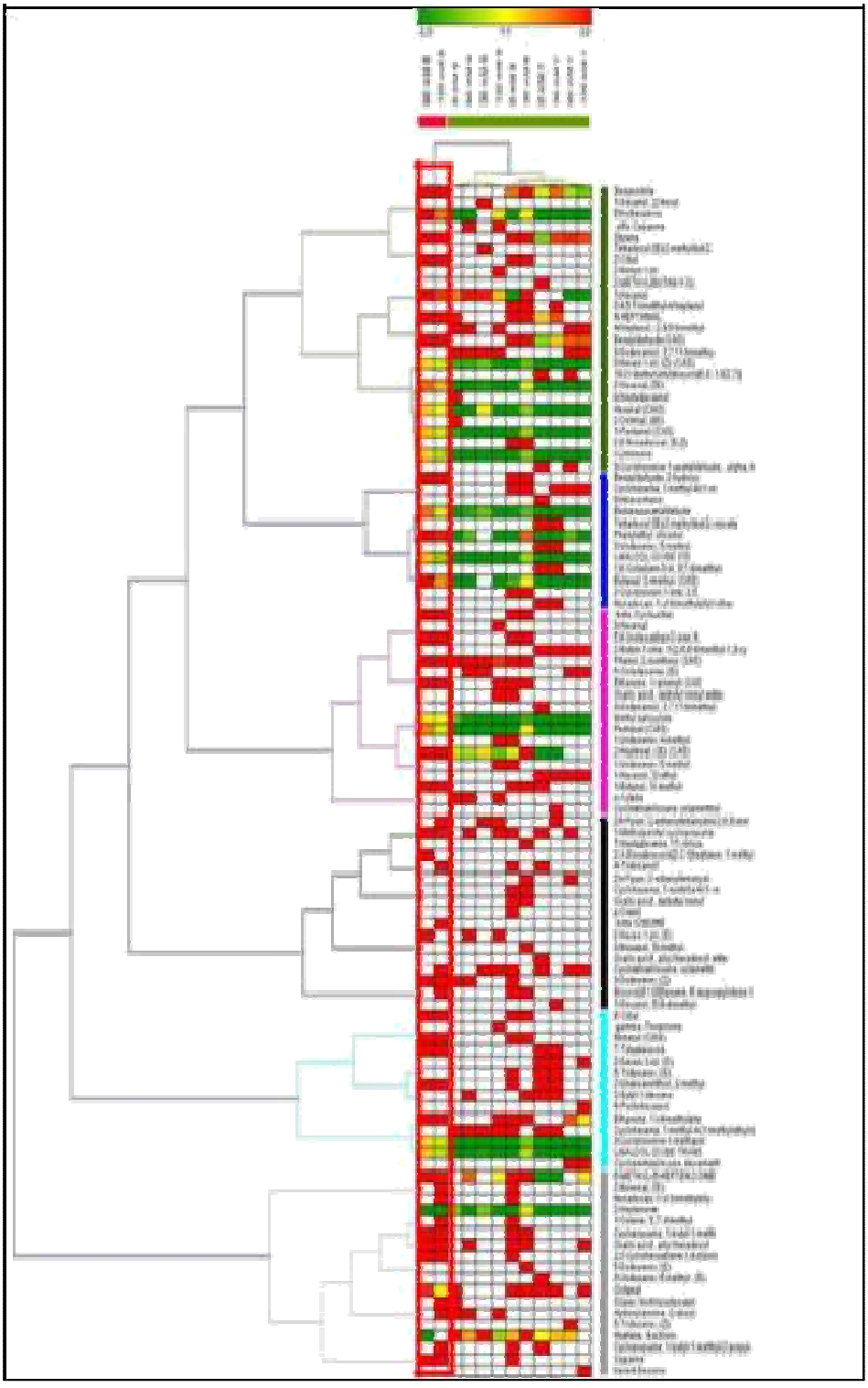


Figure 19. Heap map of 104 VOCs in the three pepper fruit maturation stages, green (G), breaker (B) and yellow (Y), and four stress levels (0-30-90-120 mM NaCl), identified in the genotype 'CG '. The color matrix represents the mean values of the intensity of the metabolites in the three biological replicates of pepper samples. Labels above the dendrogram correspond to stress levels and maturation stage.

4. Discussion / pepper

Both in natural and in agricultural systems, plants, are often subjected to abiotic stress, such as high salinity conditions. Abiotic stresses are able to reduce crop production by more than half with respect to the yields obtained in optimal plant growth conditions. Among the several abiotic stresses, water salinity is one of the main environmental factor affecting the productivity of plants and their distribution on the territory. The response of plants to saline stress is therefore of great importance for the implications it may have on plant productivity, particularly for those of agronomic interest. From an agronomic point of view, salinity is a complex phenomenon that implies short and long-term effects both on the plant and on the soil.

Currently, given the global climate change affecting our planet, it is necessary to pay attention to new characters for the genetic improvement of plant species. Indeed, in the near future, there will be a decrease in available food resources, an increase in temperatures and a lower availability of water resources, and therefore a greater use of saline waters. In this context, research activities carried out in last decades, has been significantly oriented towards the study of saline stress mechanisms affecting plant growth, with the aim to identify the feasible interventions. The prevalent approach is to identify the physiological processes underlying plant response to saline stress conditions and to define the possible strategies aimed at improve plant salt tolerance. The knowledge of genetic, biochemical and metabolomic mechanisms of plant response to salinity, may highly contribute to thecontrol of the plant growth and particularly the fruit production.

4.1 Biochemical response to saline stress in the studied pepper genotypes

In the present work a metabolic approach was used in order to study the biochemical mechanisms involved in saline stress response and tolerance of pepper plants and to specifically identify the biochemical responses

occuring during the main plant growth and fruit ripening stages. The aim was to collect useful experimental data for improving management and harvesting of this crop and obtaining fruits with a higher nutritional value. Two pepper cultivars were subjected to a comparative test in a controlled system and were cultivated in a protected environment, by applying the soilles cultivation technique, and irrigated with water at four different salinity levels (0-30- 90-120 mM NaCl). Leaves and fruits of both genotypes were collected at different growth stages and were analized for: osmolytic (proline), signal molecules (ABA), antioxidant activity molecules (carotenoids, ascorbic acid and flavonoids). Furthermore, since the aromatic component of the pepper fruit represents a very important organoleptic characteristic, the aromatic profiles of the pepper fruit from the four experimental treatments were determined to verify any changes in the levels of volatile metabolites, during the ripening stage. If compared with the studies related to the response mechanisms of pepper to other types of abiotic stress, only few researches has been carried out to verify the molecular mechanisms underlying the resistance of this plant to salinity. Such mechanisms present a complex character and implications at different levels of organization (organ, tissue, cell and organelles). Multiple biochemical pathways facilitate water storage and / or acquisition, the protection of chloroplast functions, and the maintenance of ionostasis of the ionic component. The negative effects on plants of high salinity conditions can be observed at level of the entire plant, such as productivity decreases and / or plant death. Many plants develop mechanisms to exclude salt from their cells and to tolerate their presence within the cells. During the onset and development of saline stress within a plant, the main metabolic processes, such as photosynthesis, protein synthesis, and lipid metabolism, are affected. The first response is a reduction in the rate of leaf surface expansion. The photosynthetic process is generally lower in plants exposed to salinity, especially to NaCl, and pathways, including the synthesis of osmotically active metabolites, specific proteins and enzymes which control the flow of ions and water or are implicated in the oxygen scavenging support, are activated. The ability of plants to detoxify oxygen radicals under saline stress is probably the most critical requirement in order to defend against oxidants. Plants have evolved specific protection mechanisms that involve antioxidant molecules and

enzymes that protect against active and potentially cytotoxic species of oxygen. It has been reported that plants with high levels of antioxidants, either constitutive or induced, have greater resistance to this oxidative damage. While the interpretation of the important contribution of inorganic ions such as osmolytes that can maintain turgidity and growth by plant distension in the saline environment remains valid, the present study investigated the accumulation of organic osmolite proline. Proline accumulation in saline stressed plant cells is a common phenomenon that can be used as a biochemical marker for the selection of salt tolerant varieties (179). Prolin accumulation in the leaves of both pepper genotypes resulted dose-dependent on NaCl, confirming that this amino acid is one of the main osmotic proteins under saline conditions (Figures 12 and 13). Molecular analysis of P5CS gene expression levels, which specify for the enzyme involved in prolactin metabolism, confirm this data. On the other hand, the enzymatic activity of P5CS was significantly higher in saline stressed plants than control. Many plants accumulate proline as non-toxic and protective osmolite in saline conditions (180, 181). In saline stressed plants, proline is involved in osmotic adjustments, membrane protection and cytoplasmic pH regulation. The increase in proline concentration is a sign of the strong stress which pepper plants have been undergone during the 87 days of saline treatment, but it also depends on genetic factors. In fact, by comparing the accumulation of proline in the two genotypes, it resulted higher in the leaves of the GC genotype in the considered development phases than the QA genotype (Figures 12 and 13). This result was also observed by Gharsallahe coll. in two genotypes of tomato, where a different accumulation of proline was observed. It was higher in the tolerant genotype and occurred mainly in leaves, than the more sensitive genotype (182). Proline, preferably accumulated in the leaves to maintain the level of chlorophyll and cellular turgor needed to protect the photosynthetic activity under saline stress (183), also has a potential role in the scavenging of ROS products (184).

The plant stress response was also monitored by evaluating the levels of abscisic acid **(ABA),** a hormone that plays a very complex physiological role and whose action invests many of the biological functions of the plant. It is defined as the stress hormone, as it increases the ability of the plant to adapt to stress of different origin, even to saline. Studies on the role of ABA

have shown that the increase in Ca_2^+ ion in saline stress is associated with the increase in ABA, which therefore contributes to the integrity and functionality of the membrane, and allows plants to regulate absorbing and transporting ions, especially during long periods of stress (184). In saline-stressed citrus plants, ABA has been reported to reduce ethylene release and leaf fall, probably decreasing the accumulation of C^{l-}toxic ionsin the leaves (185). In addition, ABA regulates the transcription of a multitude of genes associated with abiotic stress (186). The present study showed an higher accumulation of ABA, which was significant in the case of the Quadrato d'Asti genotype, in the leaves of stressed plants of both genotypes, if compared with the control (0 mM NaCl) (Figures 12 and 13).

Salinity does not only act at the morphological level of the plant, but its effects are also reflected in fruits that are generally reduced by weight, size, and number, resulting in a decrease in production (43, 187). Salinity is generally a cause of the qualitative fall in the products obtained (43). However, exceptions can be recorded with quality improvement (42), as in the case of tomatoes where the synthesis of some compounds that define berry quality is activated. In addition, since stress stimulates the production of antioxidant molecules (187), moderate stress can induce increases in carotenoid content and ascorbic acid with a consequent improvement in the nutritional value of pepper berries. Petersen et al. (42) have in fact observed a higher content of carotenoids in berries of tomato plants irrigated with saline waters. A further objective of this thesis project was to evaluate the effects of salinity on the qualitative aspects of plant production, as they are almost always negative at high levels of stress, but under moderate stress conditions the positive effects can also largely offset those negative. In order to evaluate the response to saline level of pepper fruit, analysis of the pericarp of both genotypes at different maturation stages (green berry, breaker, mature) was carried out. Ascorbic acid and carotenoids are important compounds in peppers, the first chelating heavy metal ions (188), reduces the risk of arteriosclerosis, cardiovascular disease and some forms of cancer (189), while both the two compounds react with single oxygen (* O2) and with free radicals and suppress peroxidation (188, 190). In addition, an important carotenoid in pepper is β-carotene, which was studied in vitro for the interaction with free radicals (191, 192). Ascorbic acid levels in both pepper genotypes increase at the beginning of ripening and then decrease

with advanced maturation (yellow berry) and this is probably due to the antioxidant role of ascorbic acid, which increases with the respiration rate in climatic fruits (193). The data collected in this work therefore confirm that the concentration of ascorbic acid has an accumulation independent of the applied saline stress level (Figure 14). With respect to β-carotene levels, generally there was an increase in levels with fruits maturation in both genotypes (Figures 15 and 16), as reported in the literature (194). The concentrations of xanthophylls, violaxanthin and lutein was influenced in both genotypes by the increase in salinity (Figures 15 and 16). The increase in concentrations of these compounds is probably due to a photoprotection mechanism that may be essential to confer tolerance under saline stress (195). The main flavonoids of pepper are quercetin and luteolin, which are present in conjugated forms. Flavonoids are involved in the organoleptic properties of fruits and vegetables, such as taste (196). Flavonoids are often stress-induced, and their glycosylation and / or methylation may affect their antioxidant properties, so they can help inhibit lipid peroxidation in stressed plants. The results of this study show that, for both genotypes, total quercetin decreases with maturation while luteins show an upward trend. The total amount of flavonoids increases with salinity increase for both genotypes (Figure 17).

Ultimately, the VOCs metabolome, a potent indicator of plant health status under saline stress, applied throughout the fruit ripening stage, was characterized. From the VOCs metabolome analysis of QA genotype pepper fruit, 83 metabolites were identified in a unique way. From the analysis of hierarchical clustering was observed that the identified VOCs are grouped into 6 different clusters and this allowed the differentiation of the 12 experimental theses into two main groups: 1) fruits of poorly cultivated plants (0-30 mM) And 2) high (90-120) saline concentration (Figure 18). This type of pooling is in line with the biochemical data and most likely confirming that the 30 mM NaCl concentration does not have a high metabolic effect on the plant, unlike the two highest saline concentrations (90 and 120 MM of NaCl) which activated a more active biochemical defense response from the plant. For the CG genotype a completely different trend was observed. Indeed, a higher number of VOCs (104) was identified, and from the hierarchical clustering analysis any relationship between volatile metabolite concentrations and low or high NaCl levels (Figure 19) was obtained. Only at the breaker stage, at 90 and 120 mM NaCl

respectively, there was a separate clustering. This is probably linked to a more active metabolic phase during the fruit ripening process in this genotype. The type of biochemical response highlighted in CG would suggest a lower susceptibility to high saline concentration than QA genotype plants. In addition, by carrying out a more accurate analysis of volatile metabolites, the presence of certain compounds belonging to the class of terpenes, mono and sesquiterpenes, produced by the lipoxygenase activity (LOX), which have a significant role in saline stress tolerance (82), was observed. Terpene emission, stimulated through a wide range of biotic and abiotic stresses (83), has the role of neutralizing reactive oxygen species (ROS) and protecting plants from negative effects caused by environmental stresses. The activity of these compounds is probably linked to the presence of double bonds in the molecule, which assumes important antioxidant properties (197, 198). Because all the environmental stresses involve the accumulation of ROS, the terpene class can play a role in alleviating the damage caused by abiotic stress such as excess salinity.

5. Materials and Methods / eggplant

5.1 Historical reference, morpho-physiological characteristics and production

The origin of eggplant is rather uncertain; The most credited hypotheses refer to Indies as the center of origin and to China as a secondary diversification center. About 2000 years ago, however, there were traces of the existence of this vegetable-fruit in Chinese writings. In Europe, the species came through Asia Minor and Egypt to the fourteenth century, imported from the Arabs, in the following century it was introduced to Italy and cultivated as curiosity and ornamental purposes also because fruit consumption was considered to be harmful to health . The edible part of the plant is represented by fruit, a berry, which can be consumed after cooking.

Eggplant belongs to the Solanaceae family, many species of eggplant, belonging to the sub-genus *Leptostemonum* which includes the three main cultivated species: *S. melongena*, *S. macrocarpon* and *S. aethiopicum.* Eggplant has 12 chromosomes, such as tomato (*Solanum lycopersicum L.*) and pepper (*Capsicum annuum* L.), and is a diploid plant with 2n = 24. 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). In temperate climates eggplant is grown as an annual, in tropical

climates it is a short lived perennial (up to 2 years in commercial fields). The eggplant is a shrub that grows up from 20 cm to over 2 m in height, often much-branched, with long taproot; stems and leaves could be with or without prickles. Flowers usually are bisexual, the calyx is campanulate. Eggplant is autogamous but with a fairly high rate of cross pollination. Pollination occurs mostly by insects mostly bumble bees or bees such as Exomalopsi (Figure 20 a) and corolla shows a broad range of colours, from white to pink and violet.(Figure 20 b).

Figure 20. a) Flower pollination; b) Eggplant flowers.

The fruit is a globose or snake-shaped, furrowed or smooth berry, 2–35 cm long (sometimes longer) and 2–20 cm broad, whit smoothness and shininess variable. The colours at commercial stage are white, green, violet-purple or black, sometimes striped, many-seeded (Figure 21). Seeds are lenticular to reniform, flattened, 3 mm × 4 mm. Fruit sets one week after anthesis, and 3–6 weeks are needed to reach commercial ripeness, depending on climatic conditions. Fruits reach physiological maturity 6–13 weeks after flowering, also depending on the climate.

Figura 21. Eggplant fruits.

Fruits have a reasonable nutritional value and can be compared to tomato values.The chemical composition and texture of berry makes it attractive for human consumption around the world. Eggplant fruits contain important phytochemicals that have antioxidant activity, include phenolic compounds, with predominant compounds such as caffeic acid and chlorogenic acid; Anthocyanins such as nasunin or delphinidine-3-(cumaroylrutinosinide) -5-glucoside and 3-rutinoside delphinide (D3R), which are the major eggplant skin metabolites. Nasunin metabolite is responsible for purple pigment found in eggplant peel, violet radish, red radish, and red cabbage (199, 200), black color is due to the D3R. Eggplant fruits also contain several other antioxidants including flavonoids, myricetin and kaempferol (201, 202), and are also an excellent source of some minerals such as P, K, Ca or Mg (203, 204). Many studies have shown that environmental conditions and cultivation techniques can affect the content and build up of eggplant phenolic and mineral compounds (205, 206, 207).

Over the last ten years, eggplant has greatly increased its economic importance with its world production over more than 50 million tonnes in 2014 (FAOSTAT, http://faostat.org). In production terms, eggplant is the third most important solanaceous crop species after potato and tomat, and is cultivated all over the world, but most intensively in China production stands at 29.5 million tonnes per year for an area of 800,000 hectares; India occupies second place after China with 13.5 million tonnes and 711,000 hectares of land invested. Italy is the first in terms of eggplant

productivity, which is about 380.6 quintals / hectare, while the area invested is 10.331 hectares.

5.2 Plant material and experimental plan

The materials studied were obtained by non-conventional breeding techniques (somatic hybridization between different cultivated parental species and wild species *S. aethiopicum* or *S. integrifolium*) followed by androgenis and reincrocians. The genotypes object of study were:

- **Tal 1/1,** line of *S. melongena* suscettibile (S) to *Fusarium* and *Verticillium*;
- **305E40,** introgressed resistance trait (R) (locus of resistence *Rfo-sa1)* resistant to *Fusarium oxysporum* f.sp. *melongenae* and tollerant to *Verticillium dahliae.*

Four artificial inoculations were carried out using a conical suspension (1,5 x 10^6/ml) of an Italian isolate of *Fusarium oxysporum* f. sp. *melongenae* (Fom), *Verticillium dahliae* (Vd), a suspension containing both pathogenic fungi (Mixta) and water control inoculation (Cont).

5.2.1 Sampling

The roots of the seedlings (at the stage of 4 true leaves) were immersed in the respective solutions for 10 minutes and then re-transplanted into the plastic alveoli trays. Afterwards, the roots from both the inoculated and not inoculated plants of the two genotypes, at predetermined time intervals after inoculation, were collected. The roots were taken at the time of inoculation (T0), after 8 hours (T0 + 8h) and after 24 hours (T0 + 24h), frozen in liquid nitrogen and stored at -80 ° C.

5.2.2 Consumables

Iodoacetamide (IAM), ammonium bicarbonate, dithiothreitol (DTT) and trypsin are produced by Sigma-Aldrich (St. Louis, MO, USA). All materials used for electrophoretic techniques: 30% Acrylamide / Bis, N, N, N ', N'-tetramethylenediamine (TEMED), Sodium dodecylsulphate (SDS), Ammonium persulfate (APS), Mixture of known molecular weight proteins "Total blue" used as standard in electrophoretic

migration are produced by BIO-RAD (Hercules, CA, USA) and colloidal Blue Coomassie by PIERCE (Waltham, MA, USA).

Effect of infection with *Fusarium oxysporum f. sp. Melongenae* (Fom)on genotypes 305E40 and Tal 1/1

Effect of infection with V*erticillium dahliae* (*Vd*) on genotypes 305E40 and Tal 1/1

5.3 Methods of analysis

5.3.1 Extraction of protein from eggplant roots

The eggplant root proteinss were obtained from 1 gram of radical tissue, powdered in a mortar with liquid nitrogen and homogenized with 1 ml buffer (25 mM Tris-HCl pH 7.5, 5 mM EDTA, 1 mM DTT), agitated for 15 minutes and centrifuged for 15 minutes at 14,000 rpm. The supernatant, represented by the protein extract, was filtered with filters of 0.45 μm, transferred to a microplate and stored at -80 ° C. This extract was determined by Bradford's protein concentration using the kit of Bio Rad containing bovine albumin as the reference protein to build the calibration line.

5.3.2 SDS-PAGE

SDS-PAGE (Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis) is a method of proteins separating by their size and relative molecular weight. This technique is carried out under denaturing conditions using an anionic detergent such as SDS ($CH_3(CH_2)_{10}$-CH_2O-SO_3^-). This proteic denaturing confer a net, almost uniform, charge to the protein chains to be separated, whose migration to the gel will consequently depend only on the molecular weight. The mixture to be separated into SDS-PAGE is first boiled for 5 minutes in a buffer (loading buffer, 100 mM Tris-HCl pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% blue bromofenol, 20% glycerol) containing DTT which reduces any sulphide bridges that together hold the protein tertiary structure.

The SDS strongly binds to it so denaturing it; on average, a molecule of SDS binds every two amino acid residues. The native protein charge is then definitively eliminated by the SDS. All protein-SDS complexes will move to the anode and, due to the molecular sieve property of the gel, the proteins will separate. The molecular weight of protein mixture is obtained by running the sample with standards of known molecular weight. Electrophoretic running is carried out thanks to a ionizable tracer colorant, usually bromophenol blue, and the glycerol present in the loading buffer makes the density of the sample solution such that the

sample stratify through the electrophoretic swab at the bottom when injected into the well loading.

Once prepared the 2x loading buffer, the samples were denatured at 98 ° C for 5 min and quickly loaded onto a gel (15x15 cm) at 12%. The electrophoretic running was carried out at constant voltage. The proteins were visualized by a colloidal blue coomassie colouration. Protein display required immersion of the gel into a coomassie solution for an entire night, after which the excess of colorant was removed by extensively washing with deionized water.

5.3.3 Hydrolysis in situ

After electrophoretic separation, the bands of interest were cut from the gel with a scalpel, crushed and placed in microprovette eppendorf. They were then treated with 50 µl of acetonitrile; after 15 minutes the solvent was removed and 50 µl of a 50 mM NH_4HCO_3 solution pH 8.0 was added. After 5 minutes the gel particles were centrifuged, the solvent removed and then eliminated.

The protein samples in the cut-off gel strips were subjected to treatment with reducing agents and finally the present cysteines were alkylated. To this purpose, the gel bands were treated with 50 µl of a 10 mM dithiothreitol solution in NH_4HCO_3 50 mM and incubated for 45 minutes at 56 ° C. At the end of the reduction, the excess of reducing solution was removed and the pieces of gel were dehydrated with acetonitrile; subsequently they are rehydrated with a 50 mM NH_4HCO_3 solution containing 55 mM of iodoacetamide. The reaction was carried out in the dark and at room temperature to alkylate the present thiol groups. After 30 minutes excess of iodoacetamide was removed and the particles were again treated with acetonitrile to dehydrate them. They were finally washed with NH_4HCO_3 50 mM / acetonitrile as described above.

5.3.4 Digestion in situ and peptide extraction

The dehydrated gel bands were treated with 10 µl of a trypsin solution (10 ng / µl) in 50 mM NH_4HCO_3 pH 8 and placed at 4 ° C for 1 hour. At this point, NH_4HCO_3 50 mM was added, in a minimum volume but enough to cover the gel pieces. The eppendorf microprovetes were then set at 37 ° C for 12 hours. The hydrolysis mixture was centrifuged at maximum speed for a few seconds and the supernatant containing the peptide mixture harvested in eppendorf and acidified with 25 µl TFA (trifluoroacetic acid) at 20% (1 µl TFA at 20% per µl of supernatant). To the remaining gel pieces, a small amount of acetonitrile was added, leaving it at room temperature for about 15 minutes in order to fully extract any peptides still retained in the gel jets. At the end of the incubation, the extract was centrifuged and recovered, which was combined with the first supernatant and then freeze-dried.

5.3.5 Proteome analysis by LC-MS / MS

Peptide mixtures were analyzed by UPLC / MS-MS using a mass spectrometer, LTQ XL, equipped with a Z-spray source and equipped with an UPLC system. The sample, 2 µl, was then fractionated on a column Atlantis T3 (100 Å, 3 µm, 4.6 x 150 mm) and a flow of 0.250 ml / min with a linear gradient of B in A from 10 to 45% In 60 min. The eluate was injected into the mass spectrometer programmed in data-dependent MS / MS mode, wherein each mass spectrum (range 400-2000 m / z) was followed by one or more tandem mass spectra obtained from More intense relay ions eluted into each chromatographic fraction. A ramp of energy collisions between 30 and 60 eV was applied, based on the mass and charge of the ion precursor. The fragmentation spectra thus obtained were analyzed using the Proteome Discoverer 1.1 software (ThermoFisher Scientific, Sunnyvale, CA, USA). Such software generates peak lists that are placed in databases available on the network, thus leading to the identification of the protein.

5.3.5.1 Identifying Proteins by SEQUEST

The mass values of the peptides obtained following the analysis by UPLC / MS-MS were inserted into the SEQUEST database. The research program mimics the experiment by computing the possible theoretical m / z values obtained by the fragmentation of peptides, derived from proteolytic digestion, for each protein whose sequence is present in the database. The mass values of the fragments found in the spectrum are then compared to the theoretical ones.
The search was done by setting the following parameters:

• **Database**: a) obtained from home by mRNA using functional genomic experiments; b) A. thaliana.
• **Enzyme**: Trypsine.
• **Fixed modifications**: methylation carbossamide.
• **Variable modifications**: oxidation of methionines and cyclization of Glutamine at the N-terminal.
• **Mass of peptide mass** error: 600 ppm.
• **Mass of the fragment ion mass error:** 0.6 Da.
• **Ion charge:** +2, +3.
The output report from the software contains information about identified proteins. The access number of the protein to the database allows to get its sequence, which is then analyzed with the Blast software, which aligns protein sequences present in different databases. For each analysed protein, Blast allows to obtain the identification code of the same protein in the Swiss Prot database. This operation was carried out in order to obtain as much functional and not functional information on the identified proteins; indeed, the Swiss Prot is a database very annotated, but not updated at the same frequency as NCBI, and therefore referring only to it, there would be a risk of losing information. Alignments of protein sequences were performed by consulting protein databases in Viridiplantae (Green Plants).

6. Resultat/eggplant

6.1 Identification of proteins in genotypes 305E40 and Tal 1/1

To investigate the response mechanisms triggered by the resistant / tolerant genotype and the susceptible genotype, following the three different inoculations and at different times, the roots of eggplant seedlings were immersed in 4 solutions: 3 solutions containing the fungus (*Fom, Vd , Mixta*) and a control inoculation solution containing only water. To perform the proteomic experiment, radical extracts containing about 1 mg / ml protein were used and the extracts thus obtained were prepared for single-dimensional electrophoretic analysis by resuspending them directly in the buffer loading. The proteins were fractionated on a 12% monodimensional gel. The gel, colored with colloidal coomassie, is shown in Figures 22 and 23.

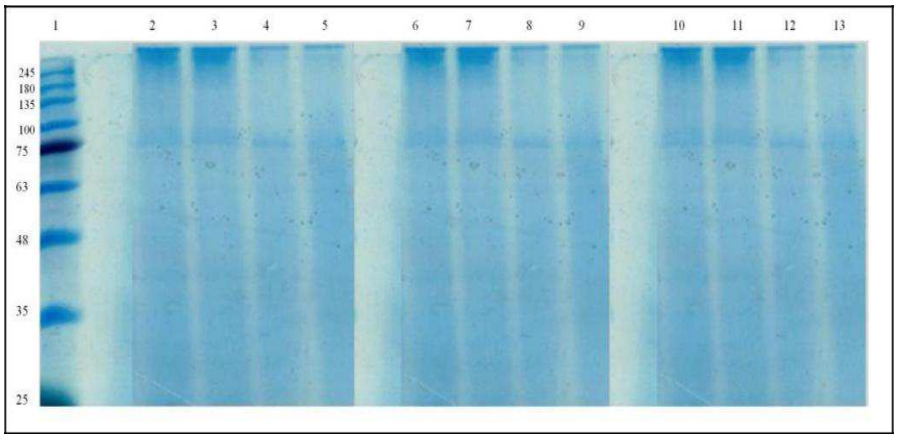


Figura 22. Preparative gel of the genotype 305E40, at different times and inoculations, colored with colloidal coomassie.
Lane 1 *Marker*, **Lane 2** 305E40 *contr* T0, **Lane 3** 305E40 *Fom* T0, **Lane 4** 305E40 *Vd* T0, **Lane 5** 305E40 *Mixta* T0, **Lane 6** 305E40 *contr* T8, **Lane 7** 305E40 *Fom* T8, **Lane 8** 305E40 *Vd* T8, **Lane 9** 305E40 *Mixta* T8, **Lane 10** 305E40 *contr* T24, **Lane 11** 305E40 *Fom* T24, **Lane 12** 305E40 *Vd* T24, **Lane 13** 305E40 *Mixta* T24.

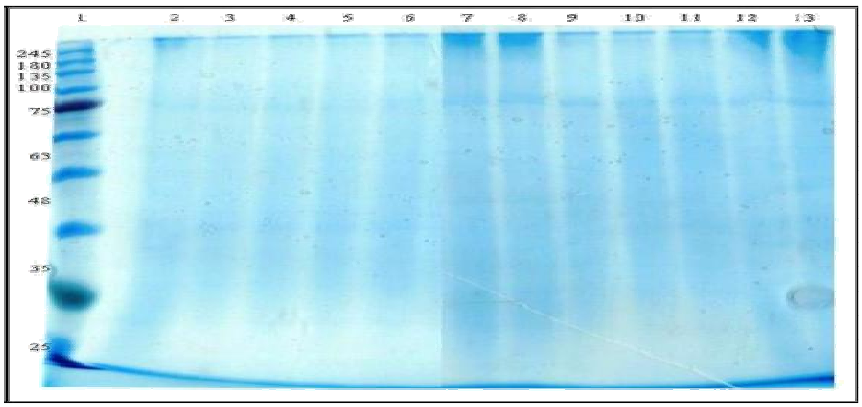


Figura 23. Preparative gel of the genotype Tal 1/1, at different times and inoculations, colored with colloidal coomassie.
Lane 1 *Marker*, **Lane 2** Tal 1/1 *contr* T0, **Lane 3** Tal 1/1 *Fom* T0, **Lane 4** Tal 1/1 *Vd* T0, **Lane 5** Tal 1/1 *Mixta* T0, **Lane 6** Tal 1/1 *contr* T8, **Lane 7** Tal 1/1 *Fom* T8, **Lane 8** Tal 1/1 *Vd* T8, **Lane 9** Tal 1/1 *Mixta* T8, **Lane 10** Tal 1/1 *contr* T24, **Lane 11** Tal 1/1 *Fom* T24, **Lane 12** Tal 1/1 *Vd* T24, **Lane 13** Tal 1/1 *Mixta* T24.

From the comparison between the control lanes and those of the samples, discriminant bands are observed. However, one-dimensional gel does not solve proteins with a molecular weight or similar electrophoretic migration. It was therefore not possible to rely on image analysis for the identification of discriminating stripes between the lanes, but it was necessary to fully analyze the control lanes and samples. All bands, both of the sample and of the control, were excised, subjected to in situ hydrolysis with trypsin and the peptide mixtures obtained were analyzed by LC-MS / MS. This method combines reverse phase UPLC chromatographic procedures with tandem mass spectrometric analysis, not only achieving accurate mass values of the individual peptides but also, as chromatography progresses, to isolate the corresponding ions of the peptides and fragment them. From fragmentation spectra, information on peptide sequences was obtained. Thus the identification of proteins in the database was carried out using two types of informationthus resulting more stringent because it was based not only on the use of accurate molecular weights, but also on the sequences of the peptide species analyzed. The so-obtained peak lists was introduced into the Sequest program, allowing to search for eggplant proteins.

6.2 Analysis of the proteome extracted from roots of infected and uninfected plants

By proteomic analysis the complete proteoma of the two different genotypes in the four experimental conditions studied were identified. The total number of identified proteins is given in Table 2.

Genotipe	**305E40**				**Tal 1/1**			
Inoculation	*Cont*	*Fom*	*Vd*	*Mixta*	*Cont*	*Fom*	*Vd*	*Mixta*
N. protein identifid	83	89	127	90	88	40	48	35

Table 2. Number of radical proteins identified in the two genotypes in the 4 different inoculations.

The numerical difference of the proteins identified in the two non-inoculated eggplant root samples may be due to a difference in the cultivars. In the genotype 305E40, after artificial inoculation with Fom, Vd and Mixta, respectively, 89, 127 and 90 radicals total proteins were identified. While, in the genotype Tal 1/1, after the artificial inoculations, were respectively identified 40 48 and 35 proteins in total. Among the proteins detected in the infected samples, 12, 27 and 17 proteins were common among 305E40 (respectively 13.8% of 89, 21.3% of 127 and 18.9% of 90) and Tal 1/1 (respectively 30, 0% 40, 56.3% 48 and 48.6% of 35). Subsequently, the common proteins identified in both the samples and the controls were discarded whereas those present exclusively in inoculated samples were considered as *Fom, Vd* and *Mixta* infection response proteins. Thanks to the information in the *Swiss Prot* database or in the literature, they were classified into nine functional categories: 1) defense proteins, 2) ROS and regulatory elimination proteins, 3) stress-related proteins, 4) metabolism proteins, 5) synthesis proteins, folding and degradation proteins, 6) structural proteins, 7) signal proteins, 8) transport and channel proteins, 9) uncharacterized proteins. In the first three categories, which are the most important from the point of view of plant response to fungal infection, there are the proteins listed in table 3.

6.2.1 Inoculation of 305E40 and Tal 1/1 with Fusarium and proteoma analysis at different times

Proteoma analysis of 305E40 roots, infected by Fom at three different times (T0, T0 + 8h, T0 + 24h), compared with that of the same uninoculated genotype roots, allowed to identify 21, 11 and 12, differentially expressed in three times proteins, respectively. The most represented functional protein categories were: defense proteins and ROS proteins and elimination regulators (Figure 24). The proteins belonging to the first category are 7, of which 5 were identified at T0. In the second category, a number of 11 proteins were identified (Table 3).

On the other hand, the analysis of the proteins from the roots of Tal 1/1 infected with Fom and compared to that of non-inoculated control, allowed to uniquely identify, in three times, 5, 16 and 12 distinctly expressed proteins, respectively. The most represented protein functional categories were: i) defense proteins and ii) metabolism proteins (Figure 25). The identified defense proteins were 9, most of which were identified at time T0 + 8h and T0 + 24h.

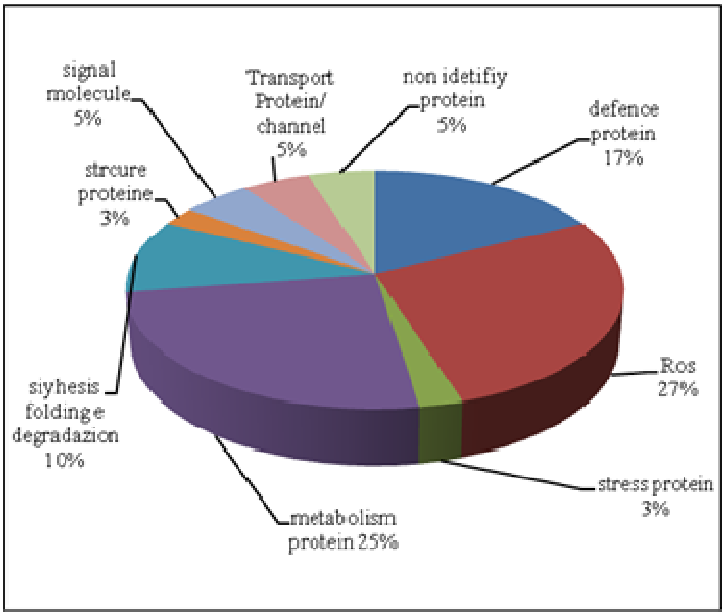


Figure 24. Functional classification of differential expressed radical proteins, identified in 3 time in 305E40 after inoculation with Fom

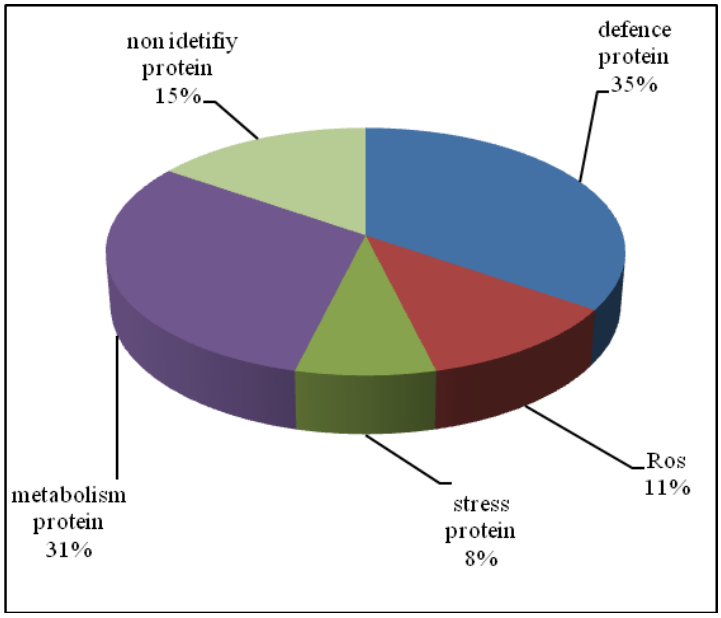


Figura 25. Functional classification of differential expressed radical proteins, identified in 3 time in Tal 1/1 after inoculation with Fom

Number	Protein name	Swiss prot code	Gene Ontology - Biological process	Protein name	Swiss prot code	Gene Ontology - Biological process	Timing
	305 E 40			**Tal 1/1**			
	Defense related proteins						
1	Xyloglucan specific endoglucanase inhibitor	D6QUQ0					**T0, T8**
2	β-1,3-glucanase 22	K9MBH7	defense response to fungus	β-1,3-glucanase 22	K9MBH7	defense response to fungus	**T0-305 E40, T24-Tal 1/1**
3	Low-temperature-induced cysteine proteinase	K4BUT9	defense response to fungus				**T0**
4	Cyprosin-like	K4CF87	protein catabolic process				**T0**
5	Syntaxin-124	M1B2R5	defense response to fungus				**T0**
6				Chaperone protein ClpB3, mitochondrial	Q0E3C8		**T0**
7				CYP1	O49877	defense response to fungus	**T0**
8	Kirola-like	M1ACY1	defense response	Kirola-like	M1ACY1	defense response	**T8**
9				Stress-associated protein 10	C4NAM2		**T8**
10				Repression of shoot growth	G4WG39		**T8**
11				NBS-LRR resistance protein-like protein	A1Y9R1		**T8**
12				Uncharacterized protein	K4DFC8		**T8, T24**
13	MLP-like protein 28	K4CQ40	response to biotic stimulus				**T24**
14				Calcium-dependent protein kinase 1	K4CQ45	abscisic acid-activated signaling pathway	**T24**
	ROS–scavengers/ Regulators						
15	Uncharacterized protein	M0ZRV6					**T0**
16	Uncharacterized protein	M0ZSA2	cell redox homeostasis				**T0**
17	Peroxidase	M1A251	response to oxidative stress				**T0**

18	Monodehydro ascorbate reductase	Q49B52					**T0, T8**
19	Catalase	P55311	response to oxidative stress				**T0, T24**
20	Minor allergen	B9T876					**T8**
21	Ribonucleoside-diphosphate reductase	M1BQQ1					**T8**
22				Homogentisate 1,2-dioxygenase	Q9M6U1		**T8**
23				Aldehyde oxidase 1 homolog	Q7DM89		**T8,T24**
24	Uncharacterized protein	K4BBQ6	oxidation-reduction process				**T24**
25	Receptor-like protein kinase HAIKU2	M1A1Q3	response to oxidative stress				**T24**
26	Glutathione S-transferase. class-phi	O22330	defense response to fungus				**T24**
27	Allene oxide syntase	Q0H7R9					
28				Ascorbate oxidase	Q4VDN6		**T24**
	Stress related proteins						
29				Probable prolyl 4-hydroxylase 7	Q8L970		**T0**
30	Molecular chaperone Hsp90-2	Q53Z32	response to stress	Molecular chaperone Hsp90-2	Q53Z32	response to stress	**T8**

Table 3. Distinctly expressed radical proteins (related to the 3 main categories), identified in the 3 timing genotypes 305E40 and Tal 1/1 inoculated with Fom.

6.2.2 Inoculation of 305E40 eT at 1/1 with Verticillium and proteome analysis at different times

The proteomic analysis of 305E40 radicals extracted with *Vd* in three different times (T0, T0 + 8h and T0 + 24h) compared to that of non-inoculated root extracts showed the presence of 16, 37 and 55 proteins respectively differentially expressed (Table 4). The most representative protein functional categories were: defense proteins and folding synthesis proteins and protein degradation (Figure 26). In particular, the two main categories of stress response, ie defense and elimination proteins of ROS, were respectively 15 and 16, identified at T0 + 8h and T0 + 24h. However, considering the proteoma of Tal 1/1 infected with *Vd* and compared to that of non-infected root, the presence of 12, 4 and 12 distinctly expressed proteins in the three studied times, were unequivocally identified. The most functional protein categories were: defense proteins and folding synthesis proteins and protein degradation (Figure 27). Among the protein protection categories, ie ROS elimination proteins and stress-regulating proteins, were respectively identified: 4 proteins, 3 of which at T0 + 24h, and 6 proteins, 4 of which at T0 + 8h (Table 4).

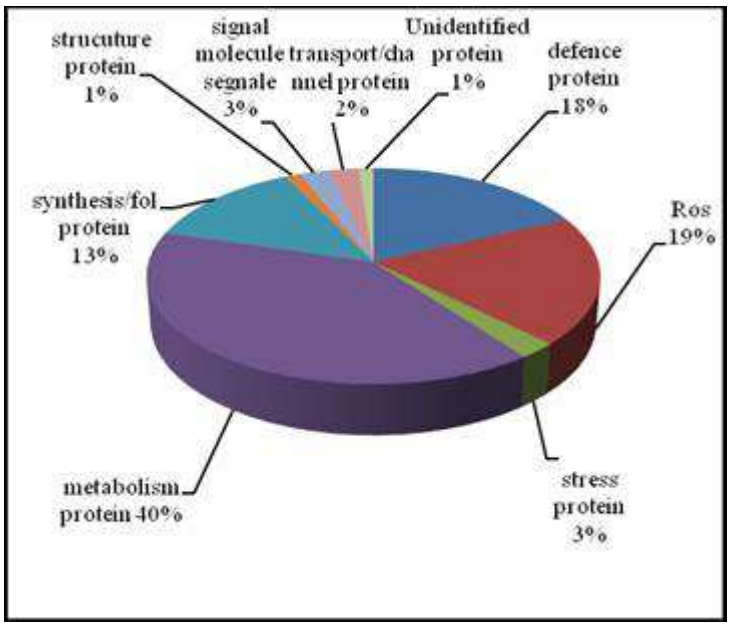


Figure 26. Functional classification of differential expressed radical proteins, identified in 3 time in 305E40 after inoculation with *Vd.*

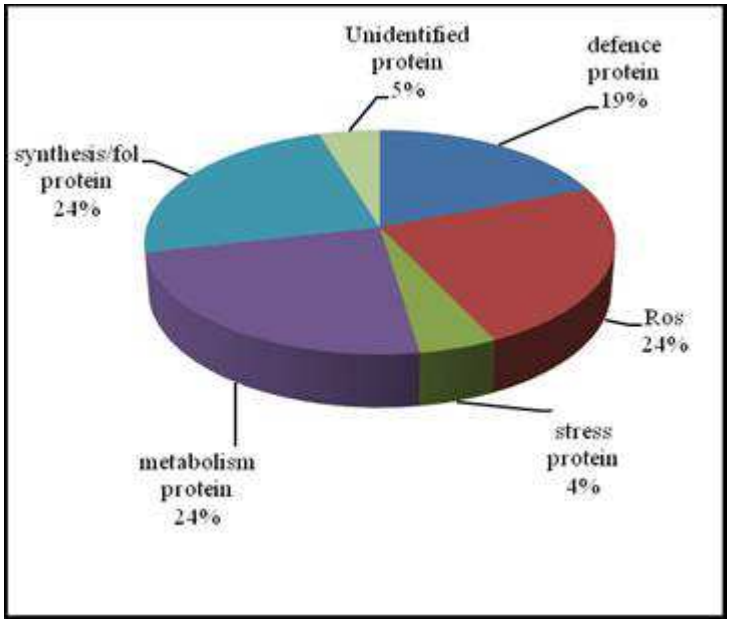


Figura 27. Functional classification of differential expressed radical proteins, identified in 3 time in Tal 1/1 after inoculation with Vd.

Number	Protein name	Swiss prot code	Gene Ontology-Biological process	Protein name	Swiss prot code	Gene Ontology-Biological process	Timing
	305 E 40			**Tal 1/1**			
	Defense related proteins						
1	Tubby-like protein	M1B4D5					**T0**
2	Low-temperature-induced cysteine proteinase	K4BUT9	defense response to fungus	Low-temperature-induced cysteine proteinase	K4BUT9	defense response to fungus	**T0 Tal 1/1; T24 305 E 40**
3	Probable carboxylesterase 120	M1BMB5					**T8**
4	Probable linoleate 9S-lipoxygenase 4	Q43190					**T8**
5	Probable aspartyl aminopeptidase	K4BJY1					**T8**
6	Transcription factor MYB30	M1CQ00					**T8**
7	Kirola-like	M1ACY1	defense response				**T8**
8	Pathogenesis-related protein STH-2	P17642					**T8**
9	Light-inducible protein ATLS1	K7VK53					**T8, T24**
10	CYP1	O49877	defense response to fungus				**T8, T24**
11	Xyloglucan specific endoglucanase inhibitor	D6QUQ0					**T8, T24**
12	E3 ubiquitin-protein ligase RHF1A-like	M1AI48					**T24**
13	Uncharacterized protein	K4BJY2					**T24**
14	MLP-like protein 28	K4CQ40	response to biotic				**T24**
15	eEF53 protein	Q9SLQ1					**T24**
16				Glucan endo-1,3-β-glucosidase 14-like isoform X1	M1CHV3	regulation of plant-type hypersensitive response	**T24**
17				Uncharacterized protein	K4DFC8	Probable inactive ATP-dependent zinc metalloprotease FTSHI 2, chloroplastic	**T24**
18				β-1,3-glucanase 22	K9MBH7	defense response to fungus	**T24**
	ROS –scavengers/ Regulators						
19	Fatty acid 9/13-hydroperoxide lyase	Q06BI1					**T0**
20	Annexin	Q9M3H3	cellular oxidant deto				**T0**

21	Aldehyde oxidase 1 homolog	Q7DM89		Aldehyde oxidase 1 homolog	Q7DM89		**T0, T8 - 305 E 40; T8 Tal 1/1**
22				Probable linoleate 9S-lipoxygenase 4	Q43190		**T0**
23				Ascorbate peroxidase	A0A060N2B3	response to oxidative stress	**T0**
24	Monodehydroascorbate reductase	Q49B52		Monodehydroascorbate reductase	Q49B52		**T0 Tal 1/1; T8 305 E 40**
25				Lactoylglutathione lyase	M1BCZ9	lactoylglutathione lyase activity	**T0**
26	Minor allergen	B9T876					**T8**
27	Peroxidase	M1A251	response to oxidative stress				**T8, T24**
28	Catalase	P55311	response to oxidative stress				**T8, T24**
29	Glutaredoxin	B3F8F4					**T8**
30	UDP-glucose 6-dehydrogenase	M1DDR8					**T8**
31	Glutathione S-transferase. class-phi	O22330	defense response to fungus				**T8, T24**
32	Dehydroascorbate reductase-like protein	Q3HS01	cellular oxidant detoxification				**T24**
33	Allene oxide syntase	Q0H7R9					**T24**
34	AOS3	Q32ZJ4					**T24**
35	Uncharacterized protein	K4C977					**T24**
36	Uncharacterized protein	M1BWS8					**T24**
37	Glutathione S-transferase T1	K4DFV6	Detoxification				**T24**
38				Ascorbate oxidase	Q4VDN6		**T24**
	Stress related proteins						
39	Uncharacterized protein	K4BEL1	response to stress				**T0**
40				Uncharacterized protein	K4AXM0	stress-activated protein kinase signaling	**T0**
41	Molecular chaperone	Q53Z32	response to stress				**T8, T24**

Table 4. Expressed radical proteins (related to the 3 main categories), identified in the 3 timing genotypes 305E40 and Tal 1/1 inoculated with *Vd.*

6.2.3 Inoculation of 305E40 and Tal 1/1 with Fusarium + Verticillium (Mixta) and proteome analysis at different times

The proteoma extracted from the roots of the genotype 305E40 inoculated with Mixta in the three different times, compared to that extracted from the roots of the same non-inoculated genotype, allowed to identify 10, 19 and 40 differentiated proteins, respectively. The most representative functional categories were: defense proteins, Ros/ regulators and protein synthesis, *folding* and protein degradation (Figure 28). For the two most important functional categories, 10 and 9 proteins respectively, mainly detected at T0 + 24 hours were identified (Table 5). Likewise, the root proteoma of Tal 1/1 infected with Mixta in the three times, compared with those extracted from non-inoculated roots, allowed to identify respectively 7, 11, and 12 distinctly expressed proteins. The most represented categories were the eliminating proteins of Ros / regulators and metabolism proteins (Figure 29); as for the first, 5 proteins were identified in T0 + 8h and T0 + 24h (Table 5).

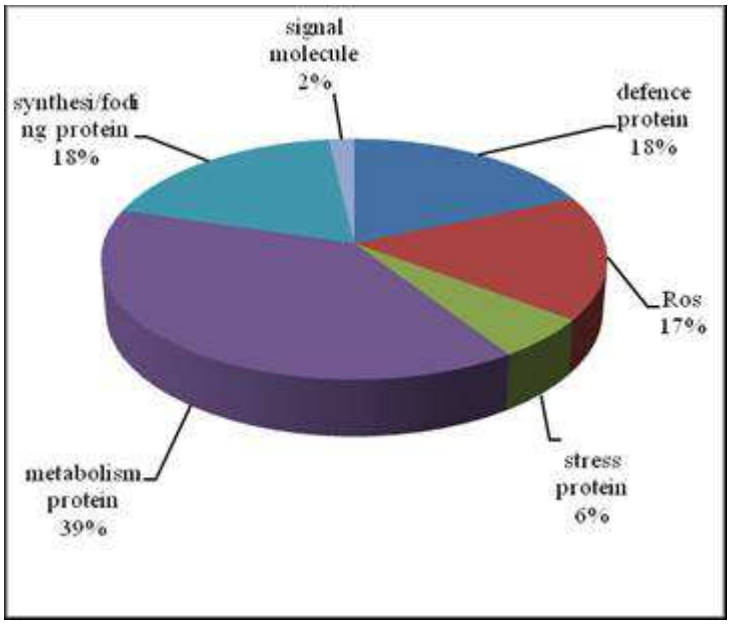


Figure 28. Functional classification of differential expressed radical proteins, identified in 3 time in 305E40 after inoculation with *Mixta.*

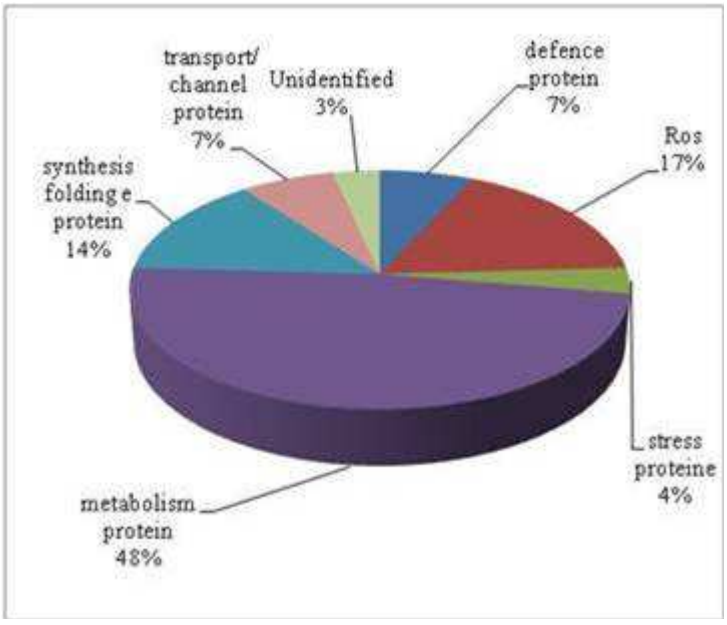


Figure 29. Functional classification of differential expressed radical proteins, identified in 3 time in Tal 1/1 after inoculation with *Mixta.*

Number	Protein name	Swiss prot code	Gene Ontology-Biological process	Protein name	Swiss prot code	Gene Ontolog Biologic al process	Timin g
	305 E 40			**Tal 1/1**			
	Defense related proteins						
1	Kirola-like	M1ACY1	defense response				**T0, T8**
2	RSI3	R4HGL0					**T8**
3	β-1,3-glucanase 22	K9MBH7	defense response to fungus				**T8, T24**
4				NBS-LRR resistance protein-like protein	A1Y9R1		**T8**
5				Oligopeptidase B	A0A0G2RJN8		**T8**
6	Light-inducible protein ATLS1	K7VK53					**T24**
7	Miraculin-like	M1AG06					**T24**
8	Uncharacterized protein	M1ASH9					**T24**
9	Low-temperature-induced cysteine proteinase	K4BUT9	defense response to fungus				**T24**
10	MLP-like protein 28	K4CQ40	response to biotic stimulus				**T24**
11	EEF53 protein	Q9SLQ1					**T24**
12	Prf	Q9LLD0	Plant defense				**T24**
	ROS –scavengers/ Regulators						
13	Glutathione S-transferase. class-phi	O22330	defense response to fungus	Glutathione S-transferase. class-phi	O22330	defense response to fungus	**T0, T8, T24 305 E40 T0, Tal 1/1**
14	Minor allergen	B9T876					**T8**
15	Cytokinin oxidase/dehydrogenase 2	C0LPA7					**T8**
16	Catalase	P55311	response to oxidative stress	Catalase	P55311	response to oxidative stress	**T8 Tal 1/1; T24, 305 E 40**
17				Aldehyde oxidase 1 homolog	Q7DM89		**T8**
18	Annexin	Q9M3H3	cellular oxidant detoxification				**T24**

19	Uncharacterized protein	K4BBQ6	oxidation-reduction process				**T24**
20	Uncharacterized protein	M0ZSA2	cell redox homeostasis				**T24**
21	Peroxidase	M1A251	response to oxidative stress				**T24**
22	Uncharacterized protein	K4DFV6	Detoxification				**T24**
23				Sorbitol related enzyme	Q3C2L6		**T24**
24				Ascorbate oxidase	Q4VDN6		**T24**
	Stress related proteins						
25	Heat shock 70 kDa protein	B6U4A3		Heat shock 70 kDa protein	B6U4A3		**T0**
26	Uncharacterized protein	K4BEL1	response to stress				**T8**
27	Molecular chaperone Hsp 90-2	Q53Z32	response to stress				**T24**

Table 5. Distinctly expressed radical proteins (related to the 3 main categories), identified in the 3 timing genotypes 305E40 and Tal 1/1 inoculated with *Mixta*.

7. Discussion/eggplant

The aim of this work was to identify the proteins involved in the defense response to inoculation with pathogenic *Fusarium oxysporum* f. *sp melongenae* and *Verticillium dahliae Kleb* of resistant / tolerant (305E40) and susceptible (Tal 1/1) eggplant genotypes. The changes over the time of compatible and incompatible interaction in root, where occurs the penetration and the first plant response to the above-mentioned pathogens, was monitored. the results of the study show that a discrete array of proteins accumulates in the roots following infection either by the two tracheomycotic fungi or both (mixed infection).

The time intervals (T0, T0 + 8h, T0 + 24h) were chosen in order to study the very first responses following the plant / fungal interaction. The proteoma analysis of non-inoculated plants over the time revealed a marked / quantitative difference in the proteins identified in the three different times between Tal 1/1 and 305E40.

Similar results were also reported by Mandelc (208) in a study carried out on hops (Humulus lupulus L.). The authors highlighted differences in the dynamics and flexibility of the radical proteoma between susceptible and resistant genotypes, also evidenced by the different trend of concentrations of total protein in control samples.

The next step was to analyze all the proteins involved in the oxidative stress response resulting from the release of ROS which is known to be one of the earliest responses implemented by plants following the attacks by pathogens. ROS plays a central role in plant defense response to pathogens. The production and storage of reactive oxygen species in plants as a defense response to pathogen attacks are well documented (105, 209). Proteins differentially expressed in the resistant genotype in the three different inoculations were: 11 for Fom inoculation, 16 for inoculation with Vd and 9 for Mixta inoculation, many proteins of which are common to the 3 different inoculations and more expressed at T0 + 8 and T0 + 24h (Tables 3, 4 and 5). Particularly interesting was the enzyme protein Allene Oxide Syntase, identified in both Fom and Vd infections. This protein is involved in the 9-LOX pathway (210) and many experimental evidences suggest that this pathway plays an important role in the defense response to pathogens. The activity of 9-

LOX and its metabolites has been described as essential for initiating programmed cell death (PCD) and hypersensitive (HR) response induced by the *Ralstonia solanacearum* pathogenic bacterium in tobacco (211).

In Tal 1/1, the eliminator and regulatory ROS proteins identified were in significantly less number: 3, 6 and 5 respectively for *Fom, Vd* and *Mixt*a inoculations, and some of them were common to the 3 different inoculations. Another interesting protein was lipoxygenase 4, identified only in the inoculation with Vd at T0 (Table 4). Several authors report the involvement of this protein in programmed cell death and HR response in different types of plants (212, 213, 214, 215). Most of the eliminator ROS proteins have been identified at T0 + 8h and T0 + 24h in the resistant genotype, unlike the susceptible genotype where they were identified above all at T0 and in few number at T0 + 8h (Tables 3, 4, 5). Similar results have been highlighted in other species including *Arabidopsis*, rice and soy. Indeed, in these species, early ROS production was observed both in resistant and susceptible genotypes; this production was followed by a subsequent explosion of scavenger proteins only in resistant genotypes. This has suggested that ROSs can contribute to the activation of R genes, and thus induce changes in gene expression (216).

As for the functional category of proteins related to the defense response, it was observed that in the inoculation with the fungus *Fusarium oxysporum* f.sp. *melongenae* defense proteins contribute to 17% and 35% of the total differentiated proteins expressed in the genotype 305E40 and in the genotype Tal 1/1 (Figures 24, 25) respectively.

In the genotype 305E40 inoculated with *Fom*, this category includes 7 differentially expressed proteins, 3 of which are common to *Vd* and *Mixta* inoculations, such as: i) *Low-temperature-induced cysteine proteinase*, ii) *Kirola-like* (a member of the PR family 10) and iii) *MLP-like protein 28*. As reported in the literature, these proteins are involved in the defense response to fungus or stimuli. A *Xiloglucan specific endoglucanase inhibitor*, common to inoculation with *Vd*, has been

purified for the first time by a tomato culture suspension and has been shown to inhibit the hydrolysis activity of a xyloglucan-endoglucanase (XEG) of the *Aspergillus aculeatus* fungus *(*217). A common to *Mixta* inoculation *β-1,3-glucanase 22*, belonging to a class of proteins known for their antifungal activity, has been reported by several authors as a protein with a strong antifungal activity against *Fusarium oxysporum* in transgenic tomato plants that overexpress the class β-1,3-glucanase I (218). The *Cyprosin-like*, identified at T0, belongs to the group of Aspartic proteinases that are involved in programmed cell death and / or PR protein protein turnover (219). Finally, *Syntaxin-122*, present only in plant inoculation with *Fom*, appears to be involved in defense mechanisms. In fact, genetic analyzes carried out on *Arabidopsis mutants* for the PEN1 gene (Penetration 1) have showed an increased penetration by the pathogen *Blumeria graminis f.sp. Horde* (Bgh). This gene encodes for a sintaxin located on the plasma membrane (220). In the PEN 1 mutants there is a greater penetration by the fungus, suggesting that in the wt (wild) the fungus encounters effective barriers against penetration. There are at least three potential barriers to fungus penetration; the first two barriers are thought to be constituted by the cuticle and the primary cell wall. The papillae, whose formation is induced before the infection occurs, represent the third barrier. Being a membrane syntaxin presumably involved in the secretion, PEN1 may be implicated in the deposition of the precursors of the cuticle, primary cell wall, and papille.

In the Tal 1/1 genotype, 11 differently expressed proteins were detected: 2 proteins (*β-1,3-glucanase 22* and a non-characterized protein) was in common with *Vd* inoculation; 1 protein (*NBS-LRR-resistant protein-like*) was in common with *Mixta* inoculation; 6 proteins were found to be specific for inoculation with *Fom.* Among the 7 proteins identified in the resistant genotype, 6 were found to be accumulated at T0 and T0 + 8h, probably due to an immediate response of the plant to the pathogen attack: In the susceptible plants, 7 out of 9 proteins were identified at T0 + 8 and T0 + 24h, suggesting a slower response to the recognition of the attack by the pathogenic fungus.

One of these proteins, the *β-1, 3 glucanase* protein, also known as PR-2, is an enzyme that acts primarily at the level of glycoside bonds β (1-3) of the fungi cell wall and is thus known to provide resistance to plants. Chatterjee et al. (221), in studies carried out on radical extracts of susceptible and resistant to *Fusarium oxysporum* f. sp. *ciceri* Race 1 (Foc1) chickpeas found that *β-1, 3 glucanase* accumulates in response to fungal attack in both genotypes. However, the susceptible genotype showed a higher accumulation after 24 hours of inoculation with the pathogen.

In the inoculation with *Verticillium dahliae Kleb*, defense proteins account for 18 and 19% of the differentially expressed proteins identified in the genotype 305E40 and Tal 1/1 respectively. In 305E40, 15 distinctly expressed proteins were identified: 3 proteins are common to inoculation with *Fom* and *Mixta* (see above); 2 proteins are common only to inoculation with *Mixta*, such as the *light-inducible ATLS1 protein*, involved in potato resistance to abiotic stress, the *EEF53*, and the *Xiloglucan specific endoglucanase inhibitor* protein which is common to inoculation with *Vd*,. All these proteins were identified within 8 to 24 hours after infection. Another distinctly expressed protein was *CYP1* (identified at T8 and T24). It is a member of the papain-like cysteine proteases family, involved in plant protection against several pathogens. Literature data indicates that pathogenic proteins can interact with members of the family of papain-like cysteine proteases (PLCPs) which are involved in plant defense against pathogens by triggering the response (HR). In addition, a PR, *pathogenesis-related protein STH-2*, was also identified at T0 + 24h by the radical extract of the genotype 305E40. A gene of the STH-2 family has been rapidly induced in potato during a response (HR) and it has been shown that pathogenesis-related protein STH accumulated after infection in tubers, stem, petiole and at significant levels also after a wound occurred (222).

It is not yet clear if the accumulation of *pathogenesis-related protein STH* is required for resistance to pathogens, however, it has been observed that a low concentration of spores induced STH-2 faster

accumulation and higher levels has been observed in the resistant genotype.

Finally, a *MYB 30 transcription factor* was identified as a differentiated protein expressed in the radical extract of the genotype 305E40 at T0 + 8h. In studies carried on *Arabidopsis thaliana*, several authors, have reported that MYB 30 may be a positive regulator of the cell death pathway programmed following a HR response. In the susceptible genotype the differentially expressed proteins were 4: 2 proteins (*β-1,3-glucanase 22* and a non-characterized protein) was common to inoculation with *Fom* and identified at T0 + 24h. In addition, a *low temperature-induced cysteine proteinase*, which plays a role in the response to pathogenic defenses in tomato (222, 223), was detected at T0; finally, *glucan endo-1,3-beta-glucosidase*, a *PR-protein*, resulted more pronounced after 24 hours from inoculum (224).

In the *Mixta* inoculation, defense-related proteins represented 18% and 7% of the total differentiated expressed proteins, identified in 305E40 and Tal 1/1 respectively. With respect to the resistant genotype, 10 distinctly expressed proteins were identified: 3 proteins were common to inoculation with *Fom* and *Vd*: 2 proteins were only common to *Vd* inoculation; 1 protein was onlycommon to inoculation with *Fom.* The other 4 identified proteins appear to be involved in resistance mechanisms to bacterial and fungal pathogens in different plant species (225; 226).

In Tal 1/1, 2 proteins differentially expressed at T0 + 8h were identified: an *NBS-LRR resistance protein-like protein* and a *Oligopeptidase B.* These proteins have already been described by other authors for their role in cell death and response to virulent and avirulent pathogens (227, 228).

8. Conclusions

The aim of the present research work was to study:

1) the biochemical mechanisms underlying the saline stress tolerance responses of pepper;
2) the tolerance / resistance responses to infection with tracheomycotic fungi of eggplant.

In the first case, a metabolomic approach was applied, while in the latter a proteomic approach was used.

In the "pepper / saline stress system", the carried out research activities and the obtained results allowed to achieve:

- a detailed analysis of the complex response mechanism of plant to saline stress conditions. In particular, biochemical parameters (accumulation of ABA and proline) was studied;
- a detailed characterization of the quality of pepper berry under saline stress conditions;
- a detailed analysis and a detailed characterization of the volatile metabolome, a potent indicator of the health status of the plant in response to saline stress conditions.

The obtained results will be integrated with molecular and morpho-physiological studies for a better understanding of saline stress response mechanisms and an identification of useful genes for genetic improvement of pepper with reference to the *abiotic stress tolerance / resistance* character.

In the "eggplant / pathogenic fungi system", the carried out research activities and the obtained results allowed to achieve:

- a detailed identification of the proteins putatively involved in the mechanism of plant resistance / tolerance to tracheomycotic fungi
- a detailed analysis of the strategy implemented by the plant following a fungal infection.

Since there are few molecular and genetic information about resistance / tolerance mechanism (it is a mono- or a polygenic resistance?), the next step will be to implement molecular and functional experiments to

validate the function of the proteins identified in the resistant genotype, which will contribute to improve the knowledge about the mechanisms of plant response. This will enable to genetically exploit the source of resistance in order to reduce damages caused by tracheomycotic fungi to the eggplant crops thus reaching higher product yields.

9.References

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