Colodete CM, Ruas KF, Barbirato, JO, Barroso ALO e Dobbs, LB (2015) Caracterização bioquímica da defesa de plantas contra estresse oxidativo. Natureza on line 13 (4): 195-204.

Submetido em: 20/05/2015 Revisado em: 02/07/2015 Aceito em: 08/08/2015

Natureza on line
ISSN 1806–7409

Biochemistry characterization of proteins defense against oxidative stress in plants and their biosynthetic pathways of secondary metabolites

Caracterização bioquímica das proteínas de defesa contra o estresse oxidativo em plantas e suas rotas biossintéticas dos metabólitos secundários

Carlos Moacir Colodete[1, 2 *]; Katherine Fragas Ruas[1, 3]; Juliano de Oliveira Barbirato[1, 4]; André Luiz P. Barroso[1, 5] e Leonardo Barros Dobbss[1, 6]

1. Organic Matter Ecology Laboratory (LEMO), Universidade Vila Velha (UVV), Rua Comissário José Dantas de Melo 21, Boa Vista, Vila Velha, ES, Brazil; 2. Doctoral student at the Pos-Graduate Program in Ecosystem Ecology (PPEE); 3. Master student at PPEE; 4. Doctoral student at PPEE; 5. Master student at PPEE; 6. Full Professor, UVV/PPEE

*Correpondent author: carloscolodete@gmail.com

Abstract The purpose of this present review is to characterize the new proteomic approaches in the study of the regulatory mechanisms of defense against oxidative stress in plants. Plant and pathogens establish relationships, resulting in biochemical information exchanges. Accordingly, certain cellular compartments develop various defense mechanisms. The first mechanism is pre-existing, using structural and/or preformed antimicrobial compounds. The second are induced as hipersensitive response (HR), accumulation of secondary metabolites by phytoalexins, the synthesis of signaling molecules, induction of hydrolytic enzymes, lignin deposition in cell wall, biosynthesis of proteins related to pathogenesis (PRs) and generation of reactive oxygen species (ROS). The main ROS are hydrogen peroxide (H_2O_2), superoxide ions ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$). These chemicals act as direct toxic action against pathogens. However when in excess, they can lead to oxidation of proteins, nucleic acids and unsaturated fatty acids. To avoid such damage, plants activate efficient antioxidant systems as the enzymes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11). Here we unraveled several proteins defense plant cell wall as well as the antimicrobial activity. It is supported in this work, that jasmonic acid (JA) substantially induces plant defense. In addition, we propose models for the conversion route of linolenic acid in JA. We approach in a simplified, but consistent way, the divisions of secondary metabolites as well as its ecological and agricultural attributes. Finally, we present a schematic model summarizing the main biosynthetic routes of secondary metabolites and their interrelations with the primary metabolism.

Keywords: hydrogen peroxide, superoxide ions, hydroxyl radical, jasmonic acid.

Resumo O objetivo desta presente revisão é caracterizar as novas abordagens proteômicas dos mecanismos de regulação da defesa contra o estresse oxidativo em plantas. Plantas e patógenos estabelecem relações, resultando em trocas de informações bioquímicas. Nesse sentido, certos compartimentos celulares destas plantas desenvolvem variados mecanismos de defesa. O primeiro mecanismo é pré-existente, através de barreiras estruturais e/ou compostos antimicrobianos pré-formados. O segundo são induzidos, como respostas hipersensitivas (HR), acúmulos de metabólitos secundários, por meio das fitoalexinas, síntese de moléculas sinalizadoras, indução de enzimas

ISSN 1806-7409 – www.naturezaonline.com.br

hidrolíticas, deposição de lignina na parede celular, biossíntese de proteínas relacionadas à patogênese (PRs) e geração de espécies reativas de oxigênio (EROs). As principais EROs são peróxido de hidrogênio (H_2O_2), íons superóxido ($O_2^{\bullet -}$) e radicais hidroxilas ($^{\bullet}OH$). Estes compostos químicos funcionam como ação tóxica direta contra patógenos. Entretanto, quando em excesso, podem levar à oxidação de proteínas, ácidos graxos insaturados e ácidos nucleicos. Para evitar tais danos, as plantas ativam eficientes sistemas antioxidantes como às enzimas dismutase de superóxido (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) e peroxidase do ascorbato (APX, EC 1.11.1.11). Aqui, discutimos várias proteínas de defesa da parede celular vegetal, bem como as de atividade antimicrobiana. É corroborado neste trabalho, que o ácido jasmônico (AJ) induz substancialmente a defesa vegetal. Além disso, propomos modelos sobre a rota de conversão do ácido linolênico em AJ. Abordamos de forma simplificada, mas consistente, as divisões dos metabólitos secundários, bem como seus atributos ecológicos e agrícolas. Finalmente, apresentamos um modelo esquemático sumarizado das principais rotas de biossíntese dos metabólitos secundários e suas inter-relações com o metabolismo primário.

Palavras-chave: peróxido de hidrogênio, íons superóxido, radical hidroxila, ácido jasmônico.

Introduction

During the course of their co-evolution, plants and pathogens have established intrinsic relations, as a result of continuous exchange of biochemical informations (Jones 2006). Nowadays due to this chemical dialogue, pathogens use several strategies to parasitize plants, and these, in turn, have developed various defense mechanisms, including constitutive and induced cellular events (Coninck *et al*. 2014).

The pre-existing defense mechanisms involve structural barriers such as waxes, callose, cutin, lignin and preformed antimicrobial compounds, such as fitoanticipinas that prevent tissue colonization (Coninck *et al*. 2014). Plants also have an active defense response that can be induced by all plant pathogens classes and eliciting molecules both from the host as the pathogen. The response involves induced oxidative burst mechanisms like, when it occurs, fast and transient increase in hypersensitive response (HR) characterized by rapid and localized cell death at the infection site of accumulation of secondary metabolites, such as phytoalexins, the production of signaling molecules such as salicylic acid, jasmonic acid (JA), ethylene, induction of hydrolytic enzymes, lignin deposition to enhance the cell wall biosynthesis of proteins related to pathogenesis (PRs) and reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ions ($O2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$) (Collinge 2009).

Therefore, in this summary are discussed some of the mechanisms by which plants protect themselves from herbivores and pathogens. At first we have considered the defense oxidative reactions of plants and their detoxification mechanisms by the enzymes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11. 1.11). Additionally, various report proteins composing the cell wall in plants, as well as antimicrobial activity of the proteins. Finally, we outline the structure and biosynthetic routes of the three major classes of secondary metabolites: terpenes, phenolic compounds and nitrogen compounds.

Oxidative reaction as plant defense mechanism

In the oxidative burst, excess of ROS production is an important defense mechanism in plants (Torres *et al*. 2006). In fact, when attacked by pathogens, it activates mechanisms including the rapid accumulation of these chemical species. The ROS can function in plant defense by direct toxic action against the pathogen, lignin formation, phytoalexin production and hypersensitive reaction, which restricts the development of the pathogen (Tománková *et al*. 2006). When in excess, these ROS can lead to oxidation of proteins, unsaturated fatty acids and DNA, causing cellular damage and eventual cell apoptosis (Shulaev and Oliver 2006). However, in order to to avoid such damage, these plants developed efficient antioxidant systems. Participating these systems, we highlight the activities of SOD and CAT as a first line of defense and APX as the second one (Sharma *et al*. 2012).

Superoxide dismutase (SOD, EC 1.15.1.1)

The SOD are metalloenzymes that catalyze the hydrogen peroxide formation from superoxide ion as a first line of cellular defense against ROS. The H_2O_2 excess should also be quickly removed from the cells (Foyer and Noctor 2005). Furthermore, they can react with superoxide radicals in presence of transition metals, thus resulting in more reactive hydroxyl radical $^{\bullet}OH$ via Fenton oxidative routes and Haber-Weiss (Figure 1).

 ISSN 1806-7409 – www.naturezaonline.com.br

Fenton-reaction:

A) $H_2O_2 + Cu^+/Fe^{2+} \longrightarrow \bullet OH + OH^- + Cu^{2+}/Fe^{3+}$

$Cu^{2+}/Fe^{3+} + \bullet O_2^- \longrightarrow Cu^+/Fe^{2+} + O_2$

Haber-Weiss-reaction:

B) $H_2O_2 + \bullet O_2^- \longrightarrow \bullet OH + OH^- + O_2$

Figure 1 Routes of oxidative reactions of Fenton and Haber-Weiss, showing the role of iron in the metabolism of hydroxyl radical •OH. A) The •OH radical is formed when the H_2O_2 reacts with Fe^{2+} or Cu^+. B) The •OH radical is formed when the H_2O_2 reacts with superoxide to becatalyzed by transition metal ions like iron or copper. Modified (Gill and Tuteja 2010).

Catalase (CAT, EC 1.11.1.6)

The CAT enzyme activity protects cells from oxidative damages arising from the excessive accumulation of hydrogen peroxide. Several isoforms of CAT were found in plant peroxisomes, glioxissomos, cytosol and mitochondria (Jaleel *et al*. 2009). CATs have the main role in H_2O_2 detoxification in plants and can dismutate them, directly or oxidising H_2O_2 substrates, such as methanol, ethanol, formaldehyde, formic acid (Breusegem *et al*. 2001). Although, compared to peroxidases ascorbate, the CAT are low affinity enzymes for H_2O_2 substrate, they have high catalytic activity. This difference in kinetic property is attributed to the need of connecting two simultâmea H_2O_2 molecules to the catalytic site of the CAT for the occurrence of reaction (Youssef and Azoo 2013).

The expression of the CAT gene is subject to various environmental factors (Table 2). These factors, as physicists and chemists are also known to cause oxidative stress and induction of antioxidant defense system in different plant species (Youssef and Azoo 2013). A (Table 2) shows the physical factors that affect the catalytic activity of CAT.

Ascorbate peroxidase (APX, EC 1.11.1.11)

The APX are important oxidoreductases, antioxidants system components of higher plants. They are found mainly in chloroplasts, mitochondria, cytosol and peroxisomes; Although they occur on various isoforms, all APX use ascorbate as a specific electron donor (Gill and Tuteja 2012). These oxiredutases play an important role in ROS scavenging system and the ascorbate-glutathione cycle (Figure 2) (Noctor and Foyer 1998; Zhang 2013).This cycle (Figure 2) involves four enzymes: APX, the glutathione reductases (GRs), the desidroascorbato reductases (DHAR) and monodesidroascorbato reductase (MDHAR) (Noctor and Foyer 1998). The GRs provide reduced glutathione to be used as a substrate for the enzyme to regenerate DHAR ascorbate. Furthermore, this compound may be regenerated by MDHAR using NADPH as a reducing agent. Thus, in the presence of ascorbate, the APX can remove H_2O_2 into cells. So this cycle (Figure 2) is considered an important defense mechanism against ROS (Noctor and Foyer 1998; Zhang 2013).

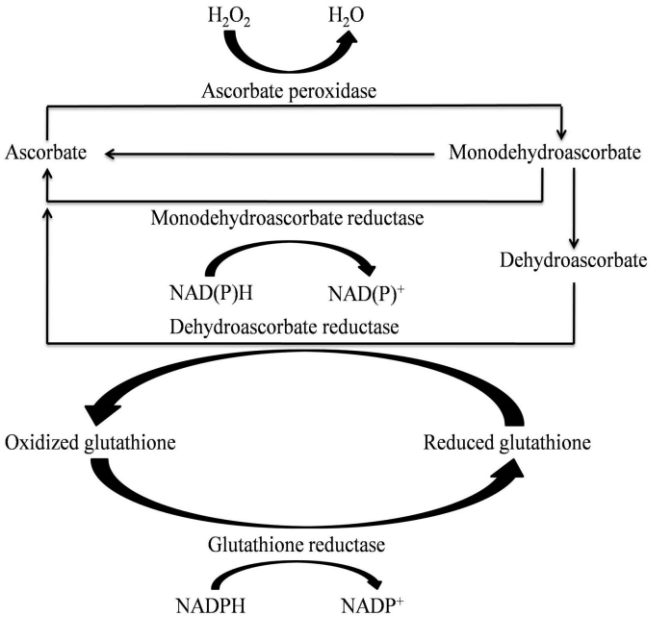

Figure 2 Scheme summarized ascorbate-glutathione cycle. Modified (Noctor and Foyer 1998).

Defense proteins in plant cell wall

Various proteins that make up the extracellular matrix in plants also serve as defense mechanisms against stress and these defense mechanisms are represented mainly by changes in cell wall composition and changes in associations between its polymers (Mittler *et al*. 2004).

The HRGPs play a central role in the organization of primary cell wall and are accumulated in response to pathogens invasion and mechanical damages. All of them are highly glycosylated protein. The polypeptides are synthesized within cells and exploited for the matrix in the form of soluble monomers, which are interconnected to form a real network that makes them insoluble phase (Zhu 2002). These are encoded usually by multigene families, although cases have been described alternative splicing of messenger RNA from a single gene. Considerable evidence has been obtained indicating that the genes encoding the HRGPs are regulated by developmental and environmental cues,

 ISSN 1806-7409 – www.naturezaonline.com.br

and in some cases by chemical treatment with elicitors (Zhu 2002).

The GRPs wall are proteins characterized by high glycine content (above 60%). Its regulation takes place by developmental and environmental cues. It has been observed an increased synthesis in the presence of chemical elicitors of defense mechanisms, such as salicylic acid (AS) (Mittler *et al*. 2004).

Peroxidase enzymes use H_2O_2 as substrate; its activities and its isoenzyme patterns may be correlated with a large number of growth processes, development, differentiation and protection in higher plants. His performance in the metabolism of extracellular matrix has been widely proven (Qu *et al*. 2013; Nahakpam and Shah 2012) for example, the elimination of H_2O_2 in the synthesis of suberin; the lignin synthesis and construction of intermolecular bonds. There is some evidence that peroxidases act also in the inactivation of pathogenic organisms by oxidation of phenolic compounds (Shinozaki and Yamaguchi-Shinozaki 2000).

Table 1 Description of SOD isoenzymes, with its isolated, cellular localization, sensitivity and most representative authors.

SOD isoenzymes	Species isolated	Cellular localization	Sensibility	References
Cu/Zn-SOD	*Photobacterium leiognathi* *Caulobacter crescentus* *Avena sativa* *Citrullus lanatus* *Pisum sativum* *Spinacia oleracea* *Zea mays*	Cytosol Chloroplast Mitochondria Peroxisome	of KCN and H_2O_2	Sharma *et al*. 2012
Mn-SOD	*Cyanobacteria* *Pisum sativum* *Spinacia oleracea* *Vigna mungo* *Zea mays*	Mitochondria Peroxisome	not KCN and H_2O_2	Giannopolitis e Ries 1977
Fe-SOD	*Nicotiana plumbaginifolia* *Escherichia coli* *Thiobacillus denitrificans* *Euglena gracilis* *Nuphar luteum* *Arabidopsis thaliana* *Glycine max* *Oryza sativa* *Zea mays* *Brassica campestris* *Ginkgo biloba*	Chloroplast Cytosol Mitochondria Peroxisome	H_2O_2, but not KCN	Arora *et al*. 2002

Table 2 Description of physical and chemical factors that affect the catalytic activity of CAT.

Factors	Description	References
Osmolytes	Sucrose induces a characteristic stimulator for CAT;	Rosas-Rodríguez *et al*. 2010
Presence inhibitor	Strong binding inhibitors with the structure of heme catalase and stop the action of the enzyme; Non-competitive inhibitor: for example, cations cobre^{2+} and sulfate; Competitive inhibitor: for example, cyanide;	Teng *et al*. 2014
pH	6,8 e 7,5;	Pastsart *et al*. 2013
Temperature	Should be less than 40-50C°;	Shadmani *et al*. 2015
Substrate concentration	The H_2O_2 count defines the type of catalytic activities and peroxidative reaction.	Xu *et al*. 2015

ISSN 1806-7409 – www.naturezaonline.com.br

Proteins with antimicrobial activity

The lectins are proteins that exhibit multiple non-catalytic sites linked to carbohydrates (Vandenborre *et al*. 2011). These proteins represent more than 30% of the total proteins content in seeds. They show high specificity for oligosaccharides and are very efficient informational transmitters of concentration of these molecules, which makes them good candidates as recognition molecules mediators (Michiels *et al*. 2010). In the vegetative organs of the plants, several genes encoding proteins very similar to lectins are found and their activation, it is believed, is related to the presence of the inducing stimulus defense mechanisms against stress (Michiels *et al*. 2010).

The main hydrolases related to defense against stress in plants are those with catalytic function of glucanases and kinases (Fong-Chin Huang and Wilfried Schwab 2013). These proteins have been found in angiosperms and are accumulated in response to the developmental and environmental stimuli (Newman *et al*. 2005).

The serine proteinase inhibitors are well studied classes of proteins involved in defense against biotic and abiotic stresses in plants like soybean *Glycine max* (Blée and Schuber 1992a,b), *Arabidopsis* (Kiyosue *et al*. 1994) and potato *Ipomoea batatas* L. (Morisseau *et al*. 2000). The encoded proteins have a strong detrimental effect on the development of herbivorous insects and are able to control the growth and sporulation of plant pathogenic fungi *in vitro* (Fong-Chin Huang and Wilfried Schwab 2013).

The PRs appeared significantly in various plant species, following pathogen infection or treatment with chemical elicitors. These proteins are known as additional bands on extracts of leaves or roots after induction and submitted to the vertical polyacrylamide gel electrophoresis and specific dyes (Linthorst 1991). Once discovered PRs at first in tobacco *Nicotiana tabacum*, many other proteins with physico-chemical properties and similar induction have been isolated in several species, including mono and dicotyledonous (Stintzi *et al*. 1993).

In general, all PRs biochemically characterized by their enzymatic activity have been shown to be the group of hydrolases (Garcia-Breijo *et al*. 1990). Many of them have been described as having glucanase activity, enzymes able to degrade the cell wall of fungus and inhibit their growth indicating a protective activity against the attack of such microorganisms (Van Loon 1984; Van Loon and Geritsen 1989).

The defenses of proteins, not only PRs, activated are systematically transported via phloem and its activation the distance seems to be related to growth regulators effect (Metraux *et al*. 1990). It has been reported that the natural mediator of PRs accumulation is ethylene. Physiological stresses that are associated with ethylene, such as flowering or high levels of growth regulators, induce the synthesis of PRs (Van Loon 1984), the same may occur with other proteins of defense against pathogenic microorganisms attack.

Other growth regulators may be involved in the induction or regulation of the defenses of proteins, but the evidence on that fact confined to isolated cases or certain species can not yet be generalized. One of these is jasmonic acid (JA) that, as well as ethylene, is rapidly produced when the plant is attacked by pathogens, especially when necrotic lesions are established. In these circumstances, JA levels rises in a systematic way, in all plant tissues. Exogenous application of JA promotes PRs of expression and the establishment of other defense reactions in healthy plants (Maucher *et al*. 2000).

Jasmonic acid: phytohormone activator of plant defense responses

The level of JA increases rapidly in response to the damage caused by insect herbivores and triggers the production of many proteins involved in plant defense (Cenzano *et al*. 2007). In plants, JA is synthesized from linoleic acid which is released from the plasma membrane lipids, and then is converted into JA trough a pathway known as signalization route of octanoids (Figure 3) (Kolomiets *et al*. 2013). Two organelles participate in the JA biosynthesis: the chloroplast and the peroxisome. In the chloroplast, a derivative of linoleic acid intermediate is cyclized and, then, transported into the peroxisomes where the β-oxidation route enzymes complete the conversion to JA (Figure 3) (Kolomiets *et al*. 2013).

The JA induces transcription of many genes involved in plant defense metabolism. According to van der Fits and Memelink (2000) with the perwinkle of Madagascar, *Catharanthus roseus* identified a transcription factor activated by JA, that induces the expression of several genes encoding enzymes of the biosynthetic pathway of alkaloids. This also activates transcription factor genes in certain primary metabolic pathways (secondary metabolites) that provide precursors for the formation of alkaloids and appears to be a key regulator of this kind of metabolism (Kolomiets *et al*. 2013).

Secondary metabolites: plant defense against herbivory and pathogens

Plants produce a large variety of organic compounds that seem to have no direct role in their growth and development. These substances are known as secondary metabolites or byproducts (Arimura *et al*. 2000).

The secondary metabolites also differ from primary metabolites (amino acids, nucleotides, sugars and lipids), having a distribution in the plant kingdom limited to a species or to a group of related species while primary metabolites are found in throughout the plant kingdom. Furthermore, secondary metabolic

ISSN 1806-7409 – www.naturezaonline.com.br

products have important ecological functions as the protection against herbivores and infection by pathogenic microorganisms; they also attract (odor, color or taste) animal pollinators and seeds dispersors and act as agents in the plant-plant competition and plants-microorganisms symbioses (Engelberth *et al*. 2004). All the functions described above are usefull in agriculture too is in agriculture, because they increase the plants reproductive performances, defending them from fungi, bacteria and herbivores (Sanchez and Demain 2011).

These secondary metabolites can be divided into three chemical groups: terpenes, phenolic compounds and nitrogen compounds. The (Figure 4) shows in a simplified manner, the pathways involved in the biosynthesis of secondary metabolites and their interconnections with the primary metabolism (Sanchez and Demain 2011).

Terpenes and terpenoids: five-carbon isoprene units

The various substances of this class are generally insoluble in water and synthesized from acetyl CoA or glycolytic intermediates. All terpenes are formed by the merge of five-carbon isoprene units (pentacarbons) (Lichtenthaler 2009).

Terpenes are synthesized from primary metabolites by at least two different routes. On the route of mevalonic acid three acetyl CoA molecules are linked in a sequence of reactions to form the mevalonic acid. This six-carbon intermediate is then pirophosforilated, decarboxylated and dehydrated, producing isopentenyl diphosphate (IPP^2). The IPP (isopentenyl pyrophosphate) is the basic unit active in the formation of terpenes. The PPI may also be formed from intermediates of glucose or by the reducing photosynthetic carbon cycle through a series of reactions called route of metileritritol phosphate (MEP), which occurs in chloroplasts and other plastids (Lichtenthaler 1999; 2009).

Certain terpenes have well-characterized role in plant growth and development and can be considered as primary metabolites rather than secondary. Additionally, toxins may be foraging inhibitors for many insects, so that they possibly serve important functions in the defense of the plant kingdom (Lichtenthaler 2009).

Phenolic compounds: chemically heterogeneous group

Plants synthesize a wide variety of secondary products which contain a phenol group and a hydroxyl group on an aromatic ring. Such substances are classified as phenolic compounds. The plant phenols constitute a chemically heterogeneous group, with approximately 10.000 compounds, some of which are soluble only in organic solvents , others carboxylic acids are soluble in water glycosides and others which are large insoluble polymers (Armelle *et al*. 2014). Many of these compounds act in defense against herbivores and pathogens, while others attract pollinators and fruit dispersers, protect against ultraviolet radiation, give mechanical support or reduce the growth of competing plants (Armelle *et al*. 2014).

After the cellulose, lignin, a highly branched polymer of phenylpropanoid groups, is the most abundant organic substance in plants. The lignin is present in the cell walls of various kinds of supporting vascular tissue and especially in the tracheids and vessels elements (Loqué *et al*. 2014). In addition to providing mechanical support, lignin plays important role in plant protection. Its mechanical resistance discourage herbivores and its chemical stability makes it indigestible by these animals. The lignification blocks the growth of pathogens and is a frequent response to infection or injury (Loqué *et al*. 2014).

Flavonoids are the major class of plant phenolics. Among these there are anthocyanins which are colored flavonoids that attract animals (plant-animal interaction) and flavones which has the function to protect against damage caused by ultraviolet light. Flavones absorb light at shorter wavelengths (280-320nm - not sensitive to the human eye) than the anthocyanins (Agati *et al*. 2012). Furthermore, flavones are not restricted to the flowers as they are present in the leaves of all green plants. Finally isoflavones may also be related to antimicrob activity (Agati *et al*. 2012).

Nitrogen compounds: alkaloids, cyanogenic glycosides and glucosinolates

A wide variety of plant secondary metabolites have nitrogen in their structure. Alkaloids and cyanogenic glycosides, having a role in plant defense against herbivores, are included in this category. The majority of nitrogen containing secondary metabolites is synthesized from the common amino acids (Mansour *et al*. 2000).

The alkaloids are synthesized from few common aminoacids, especially lysine, tyrosine and tryptophan. However, the carbon skeleton of some alkaloid shows between its compounds a derivative one of the route of terpenes. A (Table 3) shows the main types of alkaloids and their precursor aminoacids (Guggisberg and Hesse 2003).

 ISSN 1806-7409 – www.naturezaonline.com.br

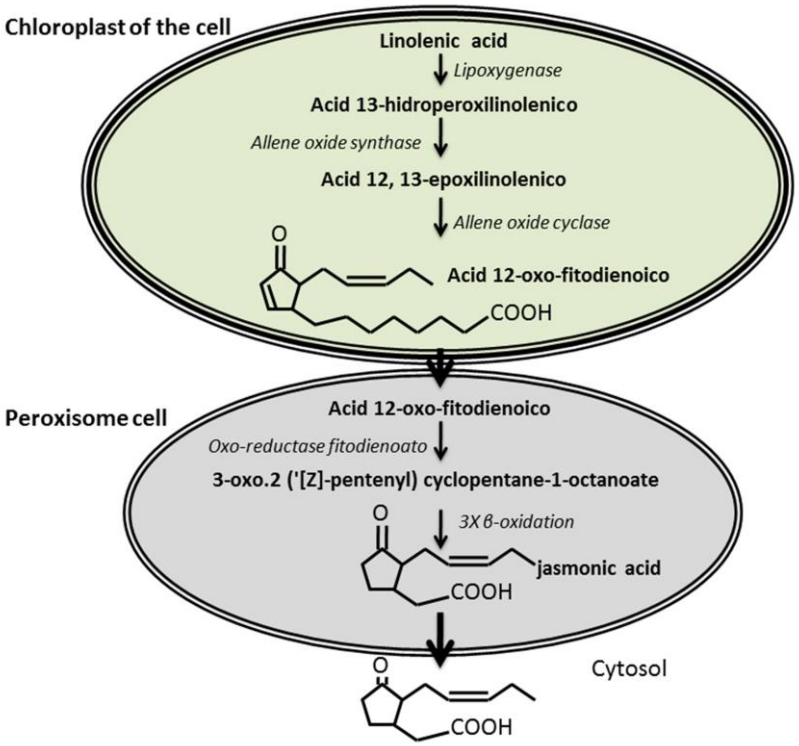

Figure 3 Stages of conversion route of linolenic acid in JA. The first three enzymatic steps occurring in the chloroplast, resulting in a cyclic product, the 12-oxo-fitodienóico acid. The intermediate is transported to the peroxisome, where it was initially reduced and, after converted into β-oxidation by JA. Modified (Kolomiets *et al*. 2013).

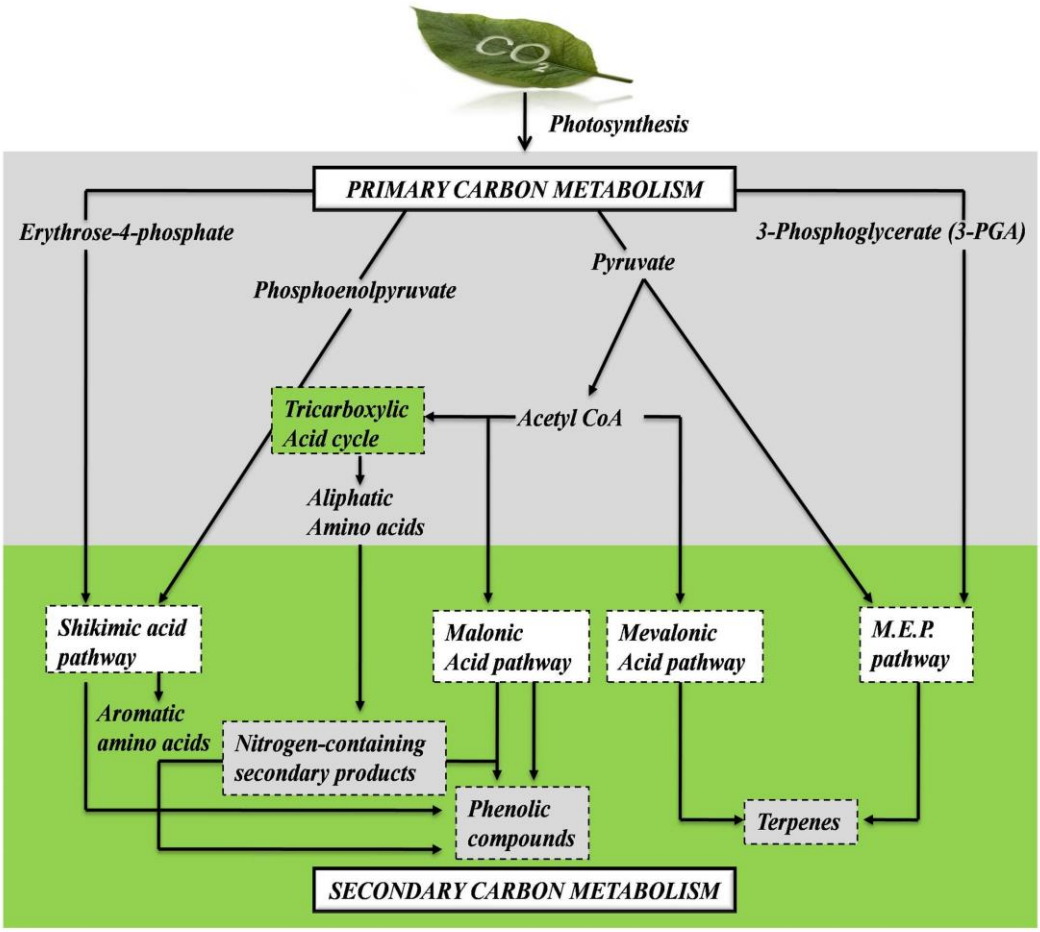

Figure 4 Simplified model of the main routes of biosynthesis of secondary metabolites and their interrelations with the primary metabolism. Modified (Sanchez and Demain 2011).

 ISSN 1806-7409 – www.naturezaonline.com.br

Table 3 Top alkaloids, amino acids and their precursors, the most representative examples.

Alkaloids	Precursor amino acids	Examples
Indole	Tryptophan	Psilocybin
		Reserpine
		Strychnine
Isoquinolínico	Tyrosine	Codeine
		Morphine
Quinolizidínico	Tysine	Lupinine
Pyrrolizidine	Ornithine	Retrorsina
Piperidinic	Acetate [1]	Coniine
Tropânico	Ornithine	Antropina
Pirrolidínico	Aspartate [2]	Nicotine

[1] Precursor also called lysine biosynthetic; [2] Precursor biosynthetic also called ornithine. Modified: (Guggisberg and Hesse 2003).

In addition to the alkaloids, plants contain other nitrogen compounds with protective function. Two groups of these substances, cyanogenic glycosides (Figure 5) and glucosinolates (Figure 5), are not toxic as such, but they rapidly decompose when the plant is injured, producing volatile poisons (Williams *et al*. 2013).

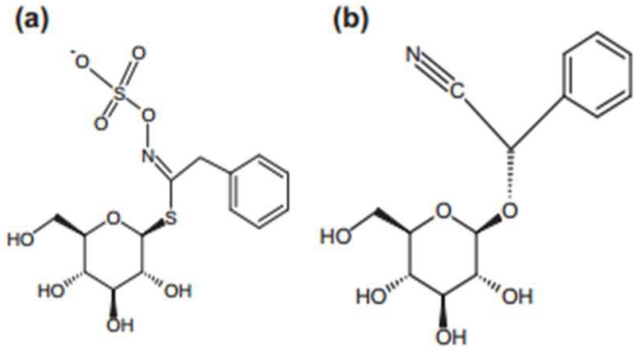

Figure 5 Chemical structure of the glucosinolates (a) and cyanogenic glycosides (b). Modified: (Williams *et al*. 2013).

Conclusions

Many researches on mechanisms of defense in plants have provided great interest currently and significant advances have happened in proteomic knowledge and innovative potential in biotechnological applications. We suggest using appropriate techniques like genomics, it will be important in evaluating responses of plants on stress, signaling the production of more tolerant plants.

Several researches have speculated that the secondary metabolites protect plants from predators, based on their toxicity and ability to repel herbivores and microorganisms. The important results presented confirm that the plant defense against insect herbivore involves the induction of various secondary metabolites and protein inhibitors of digestion, through signaling pathways that include the JA.

We evaluated the three major groups of secondary metabolites, such as the terpenes consisting of five-carbon isoprene units which may be toxic and foraging inhibitors of herbivores; phenolic components specially synthesized from the product of route specialized shikimic acid in plant protection; nitrogenous compounds synthesized by aromatic amino acids are classified into compounds alkaloids, glycosides, cyanogenic.

Acknowledgments

The FAPES Doctoral students Carlos Moacir Colodete and Juliano de Oliveira Barbirato and Master student Katherine Fragas Ruas. The CAPES by Master student André Luiz P. Barroso. Dr.Marco Pittarello for reviewing the manuscript Dipartimento di Biotecnologie Agrane the Università di Padova (Italy). This work was supported by FAPES (Process #546879852011), CNPq (Process#475436/2010-5, Process#312399/2013-8 and Process#483518/2013) and the Confederation of Agriculture and Livestock of Brazil (CNA) - Biomes project (Activity-21).

References

Agati G, Azzarello E, Pollastri S, Tattin M (2012) Flavonoids as antioxidants in plants: Location and functional significance. **Plant Science** 196: 67-76.

Arimura G, Ozawa R, Shimoda T, Nishioka T, Boland W, Takabayashi J (2000) Herbivory-induced volatiles elicit defence genes in lima bean leaves. **Nature** 406: 512–515.

Armelle T, Mbaveng G, Zhao Q, Kuete V (2014) Harmful and Protective Effects of Phenolic Compounds from African Medicinal Plants. **Toxicological Survey of African Medicinal Plants** 33: 577-609.

Arora A, Sairam RK, Srivastava GC (2002) Oxidative stress and antioxidative system in plants. **Journal Science** 82: 1227-1238.

Blée E, Schuber F (1992a) Occurrence of fatty acid epoxide hydrolases in soybean: purification and characterization of the soluble form *Glycine max* L. **Biochemical Journal** 282: 711-714.

Blée E, Schuber F (1992b) Region and enantioselectivity of soybean fatty acid epoxide hydrolase *Glycine max* L. **Biology Chemical** 267: 11881-11887.

Bowless DJ (1990a) Signal in the wounded plant. **Nature** 343: 314-315.

Breusegem F, Vranova E, Dat JF, Inze D (2001) The role active oxygen species in plant signal transduction. **Plant Science** 161: 405-414.

Cenzano A, Abdala G, Hause B (2007) Cytochemical immuno-localization of allene oxide cyclase, a jasmonic acid biosynthetic enzyme, in

developing potato stolons. **Journal of Plant Physiology** 164: 1449-1456.

Collinge DB (2009) Cell wall appositions: the first line of defence. **Journal of Experimental Botany** 60: 351-352.

Coninck B, Timmermans P, Vos C, Bruno PA, Kazan K (2014) What lies beneath: belowground defense strategies in plants. **Cell Press** 11: 1-11.

Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH (2004) Airborne signals prime plants against insect herbivore attack. **Proceedings of the National Academy of Sciences** 101: 1781-1785.

Fong-Chin Huang F, Wilfried Schwab S (2013) Transformation of terpenes into fine chemicals. **European Journal of Lipid Science and Technology** 115: 3-8.

Foyer CH, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in physiological context. **Plant Cell Enviromental** 28: 1056-1071.

Garcia-Breijo FJ, Garro R, Conejero V (1990) C7 (P32) and C6 (P34) PR proteins induced in tomato leaves by *Citrus exocortis* viroid infection are chitinases. **Physiological and Molecular Plant Pathology** 36: 249-260.

Giannopolitis CN, Ries SK (1977) Superoxide dismutases. Occurrence in higher plants. **Plant Physiol** 59: 309-314.

Gill SS, Khan NA, Tuteja N (2012) Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.) **Plant Science** 182: 112-120.

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiol Biochem** 48: 909-930.

Guggisberg A, Hesse M (2003) Alkaloids in plant. **Sciences and Chemical Engineering Encyclopedia of Physical Science and Technology** 22: 477-493.

Jaleel CA, Riadh K, Gopi R, Manivannan P, Ines J, Al-Juburi HJ (2009) Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. **Acta Physiology Plant** 31: 427-436.

Jones JDG, Dangl JL (2006) The plant immune system. **Nature** 444: 323-329.

Kiyosue T, Beetham JK, Pinot F, Hammock BD Yamaguchi-Shinozaki K (1994) Isolation and characterization of cDNA that encodes a soluble epoxide hydrolase from *Arabidopsis thaliana* L. **Journal Plant** 6: 259-269.

Kolomiets MV, Yan Y, Borrego E (2013) Jasmonate Biosynthesis, Perception and Function in Plant Development and Stress Responses. **Intech Journal** 06: 394-441.

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. **Annual Review of Plant Physiology and Plant Molecular Biology** 50, 47–65.

Lichtenthaler HK (2009) Biosynthesis and Accumulation of Isoprenoid Carotenoids and Chlorophylls and Emission of Isoprene by Leaf Chloroplasts. **Bulletin of the Georgian National Academy of Sciences** 3: 81-94.

Linthorst HJM (1991) Pathoneses-related proteins of plant. **Critcal Reviews in Plant Sciences** 10: 123-150.

Loqué D, Eudes A, Liang Y, Mitra P (2014) Lignin bioengineering in plant. **Current Opinion in Biotechnology** 26: 189-198.

Maldonado MT, Hughes MP, Rue EL, Wells ML (2002) The effect of Fe and Cu on growth and domoic acid production by (*Pseudo-nitzschia multiseries*) and (*Pseudo-nitzschia australis*). **Limnology and Oceanography** 47: 515-526.

Mansour MMF (2000) Nitrogen Containing compounds and adaptation of plants to salinity stress. **Biologia Plantarum** 43: 491-500.

Maucher H, Hause B, Feussner I, Ziegler J, Wasternack C (2000) The allene oxide synthases of barley (*Hordeum vulgare* cv. Salome)-tissue specific regulation in seedling development PR. **Plant Journal** 21: 199–213.

Metraux JP, Signer H, Ryals J, Wards E, Benz MW, Gaudin J, Raschdorf K, Shid E, Blum W, Inverardi B (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. **Science** 250: 1004-1006.

Michiels K, Van Damme EJM, Smagghe G (2010) Plant-insect interactions: Wat can we learn from plant lections? **Archives of Insect Bichemistry and Physiogy** 73: 193-212.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. **Trends Plant Science** 7: 405-410.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. **Trends Plant Sciences** 9: 490–498.

Morisseau C, Beetham JK, Pinot F, Debernard S, Newman JW, Hammock BD (2000) Cress and potato soluble epoxide hydrolases: Purification, biochemical characterization, and comparison to mammalian enzymes. **Archives of Biochemistry and Biophysics** 378: 321-332.

Nahakpam S, Shah K (2012) Expression of key antioxidant enzymes under combined effect of heat and cadmium toxicity in growing rice seedlings. **Plant Growth Regulation** 63: 23-35.

Newman M, Thompson C, Roberts AP (2005) Helping practitioners understand the contribution of qualitative research to evidence-based practice**. Journal Evidence Nursing** 9: 4-7.

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. **Plant Physiology** 49: 249-279.

Pastsart U, Boever MD, Claeys E, Smet SD (2013) Effect of muscle and post-mortem rate of pH and temperature fall on antioxidant enzyme activities in beef. **Meat Science** 93: 681-686.

Qu AL, Ding YF, Jiang Q, Zhu C (2013) Molecular mechanisms of the plant heat stress response. **Biochemical and Biophysical Research Communications** 432: 203-207.

Rosas-Rodríguez JA, Valenzuela-Soto EM (2010) Enzymes involved in osmolyte synthesis: How does oxidative stress affect osmoregulation in renal cells. **Life Sciences** 87: 515-520.

Sanchez S, Demain AL (2011) Secondary Metabolites plant. **Elsevier Journal** 12: 155-167.

Shadmani N, Ahmad SH, Saari N, Ding P, Tajidin NE (2015) Chilling injury incidence and antioxidant enzyme activities of (*Carica papaya*

L.) 'Frangi' as influenced by postharvest hot water treatmentand storage temperature. **Postharvest Biology and Technology** 99: 114-119.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **Journal Botany** 33: 1-26.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **Journal of Botany** 34: 1-26.

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. **Current Opinion in Plant Biology** 3: 217-223.

Shulaev V, Oliver DJ (2006) Metabolic and proteomic markers for oxidative stress. New Tools for Reactive Oxygen Species Reseach 1. **The International Journal on the Biology of Stress** 141: 367-372.

Stinzi A, Heitz T, Prasad V, Wiedemann S, Geoffroy P, Legrand M, Friting B (1993) Plant PR proteins and their role in defense against pathogens. **Biochimie** 75: 687-706.

Teng Y, Zou L, Huang M, Zong W (2014) Molecular interaction of 2-mercaptobenzimidazole with catalase reveals a potentially toxic mechanism of the inhibitor. **Journal of Photochemistry and Photobiology Biology** 141: 241-246.

Tománková K, Luhová L, Petricalský M, Pec P, Lebeda A (2006) Biochemical aspects of reactive oxygen formation in the interaction between *Lycopersicon* spp. and *Oidium neolycopersici*. **Physiological and Molecular Plant Pathology** 68: 22-32.

Torres MA, Jones JDG, Dangl JL (2006) Reactive oxygen species signaling in response to pathogens. **Plant Physiology** 141: 373-378.

van der Fits L, Memelink J (2000) ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. **Science** 289: 295-297.

Van Loon LC (1984) **Regulations of Pathogenesis and Symptom Expression in Diseased Plant by Ethylene**. In: Fuches Y; Chalutz E. (Eds). Ethylene: biochemical, physiological and applied aspects. Netherlansds.

Van Loon LC, Geritsen YAM (1989) Protease activy and PR proteins in virus-infected samsun NN tobacco leaves. **Elsevier Scientific Publishers** 18: 25-32.

Vandenborre G, Smagghe G, Van Damme EJ (2011) Plant lectins as defense proteins against phytophagous insects. **Phytochemistry** 72: 1538-1550.

Williams DJ, Pun S, Chaliha M, Scheelings P, O'Hare T (2013) An unusual combination in papaya (*Carica papaya*): The good (glucosinolates) and the bad (cyanogenic glycosides). **Journal of Food Composition and Analysis** 29: 82-86.

Xu Q, Cai L, Zhao H, Tang J, Shen Y, Hu X, Zeng H (2015) Forchlorfenuron detection based on its inhibitory effect towards catalase immobilized on boron nitride substrate. **Biosensors and Bioelectronics** 63: 294-300.

Youssef, MM, Azooz MM (2013) Biochemical studies on the effects of zinc and lead on oxidative stress, antioxidant enzymes and lipid peroxidation in Okra *Hibiscus esculentus* cv. Hassawi. **Science International** 1: 12-16.

Zhang Y (2013) Ascorbic Acid in Plants: **Springer Briefs in Plant Science** 22: 365-387.

Zhu JK (2002) Salt and drought stress signal transduction in plants. **Annual Review of Plant Biology** 53:247-273.