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Oligoglucan Elicitor Effects During Plant Oxidative Stress

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1. Introduction

Molecular oxygen is essential for the existence of life of aerobic organisms including plants. However, Reactive Oxygen Species (ROS), which include the superoxide anion (O_2•$^-$), hydroxyl radical (•OH), perhydroxyl radical (•O_2H) and hydrogen peroxide (H_2O_2), are generated in all aerobic cells as byproducts of normal metabolic processes. In general, under various conditions of environmental stress, plant cells show an increase in ROS levels leading to oxidative stress. Indeed, oxidative stress is a major cause of cell damage in plants exposed to environmental stress. Plants under the effect of biotic (senescence, pathogen attack) and/or abiotic factors (heat, chilling, drought, salinity, chemical compounds, mechanical damage) may increase ROS levels, and their accumulation produce a disruption of the redox homeostasis.

Plants employ an efficient ROS scavenging system based on enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX) and non-enzymatic antioxidants (carotenoids, tocopherols, glutathione, phenolic compounds) to counteract ROS adverse effects against important macromolecules like lipids, proteins and nucleic acids, which are necessary for cell structure and function. However, the catalytic activity of these antioxidant systems could be negatively affected by several stress conditions due to abiotic and biotic factors; a very common situation for plants in fields or commercial stocks. The efforts of farm growers to bring up healthy crops and sufficient yields could be reinforced with the scientific experience and development of novel techniques focused in plant physiology and crop protection by means of the elicitation of plant defense responses against any kind of stress.

Multiple biological responses in plants including controlled ROS overproduction during phytopathogen attack, changes in ionic fluxes across lipid membranes, phosphorylation of proteins, transcription factors activation and up-/down-regulation of defense related genes have been demonstrated when using oligogalacturonides and some oligoglucans derivatives from plants and fungi cell wall. The study of these elicitors is essential for designing strategies to reduce negative effects of oxidative stress in plants. Therefore, the objective of this chapter was to review the oxidative stress generated in plants and its relationship with the elicitation of defense responses carried out by oligosaccharides, and particularly, by oligoglucans.

2. Oxidative stress and reactive oxygen species

Oxidative stress is defined as the rapid production of $O_2^{\bullet -}$ and / or H_2O_2 in response to various external stimuli (Wojtaszek, 1997) therefore their disturbance between production and elimination of the host cell. The decrease in catalytic activity of the plant antioxidant system is also a reason for oxidative stress to appear (Shigeoka et al., 2002). The balance of the antioxidant system may be disturbed by a large number of abiotic stresses such as bright light, drought, low and high temperatures and mechanical damage (Tsugane et al., 1999). The presence of heavy metals in the field, like pollution by lead (Pb) induces oxidative stress that damages cells and their components such as chloroplasts, in addition to altering the concentration of different metabolites including soluble proteins, proline, ascorbate and glutathione, and antioxidant enzymes (Reddy et al., 2005). On the other hand, processes related to the deterioration of fruits and vegetables, either by attack of pathogens, senescence or changes in the storage temperature are factors that increase ROS levels, leading to further economic losses (Reilly et al., 2004).

In plants, ROS are byproducts of diverse metabolic pathways localized in different cell compartments (chloroplasts, mitochondria and peroxisomes, mainly). Under physiological conditions, ROS are eliminated or detoxified by different components of enzymatic or non-enzymatic antioxidant defense system (Alscher et al., 2002). However, when plants are under the effect of single or multiple biotic and/or abiotic factors, the catalytic action of various antioxidants is negatively affected, allowing ROS accumulation that turns oxidative stress into an irreversible disorder (Qadir et al., 2004).

A common feature among different types of ROS is their ability to cause oxidative damage to proteins, lipids and DNA. However, depending on its intracellular concentration, ROS can also function as signaling molecules involved in the regulation and defense responses to pathogens, but mainly at very low concentrations (Apel & Hirt, 2004). It is proposed that ROS affect stress responses in two different ways. ROS act on a variety of biological molecules, causing irreversible damage leading to tissue necrosis and in extreme cases, death (Girotti, 2001). On the other hand, ROS affect the expression of several genes and signal transduction pathways related to plant defense (Apel & Hirt, 2004).

3. Antioxidant system in plants

The chloroplast is the cellular compartment associated with photosynthetic electron transport system and is a generous provider of oxygen, which is a rich source of ROS (Asada, 1999). In a second place, peroxisomes (glyoxisomes) and mitochondria are another ROS generating places inside the cell. A large number of enzymatic and non-enzymatic antioxidants have evolved to detoxify ROS and/or prevent the formation of highly reactive and damaging radicals such as hydroxyl radical (•OH). Non-enzymatic antioxidants include ascorbate, glutathione (GSH), tocopherol, flavonoids, alkaloids, carotenoids and phenolic compounds. There are three key enzymatic antioxidants for detoxification of ROS in chloroplasts, superoxide dismutase (EC 1.15.1.1, SOD), ascorbate peroxidase (EC 1.11.1.11, APX) and catalase (EC 1.11.1.6, CAT). SOD catalyzes the dismutation of two molecules of $O_2^{\bullet -}$ in O_2 and H_2O_2. On the other hand, using ascorbate as electron donor, the enzyme APX reduces H_2O_2 to H_2O. The formation of hydroxyl radicals by $O_2^{\bullet -}$ and H_2O_2 can be controlled by the combination of dismutation reactions carried out by enzymes SOD, APX and CAT (Tang et al., 2006) (Figure 1).

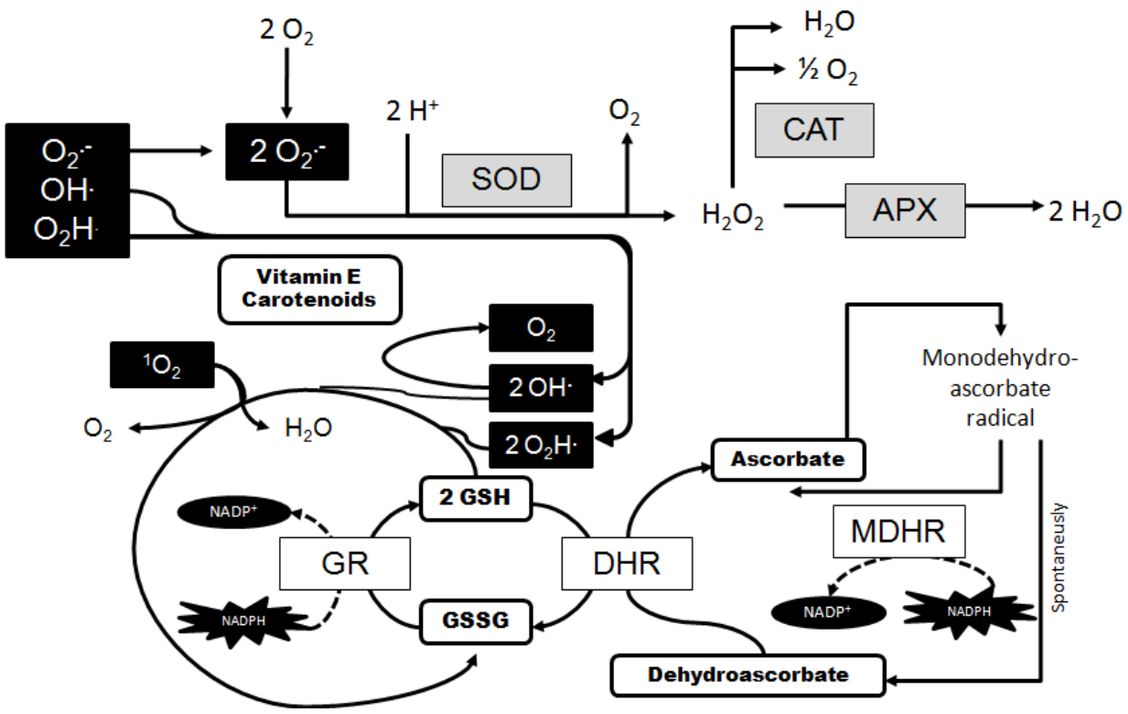

Fig. 1. Enzymatic and non-enzymatic antioxidant system in plants. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are the proteins responsible for eliminating ROS. While the elimination of ROS by non-enzymatic processes is carried out by vitamin E, carotenoids, ascorbate, oxidized glutathione (GSH) and reduced (GSSG). Enzymes that promote the elimination of ROS via the ascorbate-glutathione cycle are monodehydroascorbate reductase (MDHR), dehydroascorbate reductase (DHR) and glutathione reductase (GR) (Modified from Halliwell, 2006).

Superoxide Dismutase is a major ROS scavenging enzyme found in aerobic organisms. In plants, three types of SOD were distinguished on the basis of its active site cofactor: manganese SOD (MnSOD), copper / zinc SOD (Cu / ZnSOD) and iron SOD (FeSOD) (Reilly et al., 2004). CAT is a tetramer containing 4 heme groups, located mainly in peroxisomes (Apel & Hirt, 2004) and eliminates H_2O_2. It is proposed that CAT plays a role in mediating signal transduction where H_2O_2 acts as second messenger, possibly via a mechanism related to salicylic acid (Leon et al., 1995). On the other hand, APX enzyme has been found in higher plants, algae and some cyanobacteria, but not in animals. It is necessary for plants to have high levels of ascorbate to maintain functionally viable the endogenous antioxidant action of this enzyme (Shigeoka et al., 2002). APX activity in plants has increased in response to various stress conditions such as drought, ozone, chemicals, salinity, heat, infection (López et al., 1996; Mittler & Zilinskas, 1994). The sequencing of *Arabidopsis thaliana* genome has revealed the presence of 9 genes of APX (The Arabidopsis Genome Initiative, 2000). This fact shows how relevant the antioxidant enzymes-coding genes are in plants, as well as their down or up-regulated expression during stress conditions.

Different APX isoenzymes have been identified in plant cells: cytosolic (Ishikawa et al., 1995), peroxisomal (Ishikawa et al., 1998), two chloroplasmatic APX (in the stroma and thylakoid) (Ishikawa et al., 1996) and mitochondrial (De Leonardis et al., 2000). Each one,

with a specific role as antioxidant enzyme, being activated or inhibited in response to different cellular signals as a consequence of biotic or abiotic stresses. The cytosolic APX isoenzyme has been considered one of the most important enzymes in defense against H_2O_2. Because of its cellular localization is the first to receive the signals produced during stress, acting very quickly to prevent severe damage to the cell and/or whole tissue. It has been reported the characterization of cDNAs encoding for cytosolic APX from various plants such as pea (Mittler & Zilinskas, 1992), Arabidopsis (Jespersen et al., 1997), rice (Morita et al., 1999), spinach (Webb & Allen, 1995) , tobacco (Orvar & Ellis, 1995) and potato (Kawakami et al., 2002; Park et al., 2004). However, the information about the genomic organization of the cytosolic APX is scarce, since there is only complete information of APX genes for tomato (Gadea et al., 1999) and pea (Mittler & Zilinskas, 1992).

4. Defense responses in plants during oxidative stress

During oxidative metabolic processes, ROS are generated at controllable levels and they play a key role in facilitating the defense of plants. This can be summarized in the following points: (1) strengthening the cell wall by structural carbohydrate modifications in linkages, (2) the induction of defense-related genes encoding protein-related proteins like glucanase, chitinase or protein inhibitors, and (3) causing cell death in a particular region of the plant (Reilly et al., 2004). During the defense response against pathogens, ROS are produced by the plant cell by increasing the activities of NADPH oxidase enzymes bound to plasma membranes, peroxidase attached to the cell wall and amino oxidase in the apoplast (Hammond-Kosack & Jones, 2000). The strengthening of the cell wall plays an important role in defense mechanisms against penetration by fungal pathogens (Bolwell et al., 2001). During defense responses by the attack of pathogens, plants produce higher levels of ROS while decreasing the detoxifying capacity, then the accumulation of ROS and activation of programmed cell death (PCD) happens. The suppression of ROS removal mechanisms is crucial for the establishment of the PCD. The production of ROS in the apoplast alone without the detoxification of ROS does not result in the induction of PCD (Delledonne et al., 2001).

Reactive Oxygen Species are among the major signaling molecules in the cell. These molecules are small and can diffuse a short distance, and there are several mechanisms for its production, many of which are fast and controllable. H_2O_2 generation occurs locally and systemically in response to mechanical damage or wounding (Orozco-Cardenas & Ryan, 1999). Other research shows that H_2O_2 acts as a second messenger mediating the systemic expression of several defense-related genes in tomato plants (Orozco-Cardenas et al., 2001).

5. Biological active elicitors

An elicitor can be defined as a molecule which, when introduced in low concentrations in a biological system, initiates or promotes the synthesis of biologically active metabolites (Radman et al., 2003). The type and structure of elicitors varies greatly, so there is no universal elicitor (Radman et al., 2003). Various elicitors have been purified: oligosaccharides, proteins, glycoproteins and lipophilic compounds (Coté & Hahn, 1994). The oligosaccharides are the most studied elicitors today. There are four types of oligosaccharides: oligoglucans, oligochitin, oligochitosan (predominantly from fungal source) and oligogalacturonides from plants (Coté & Hahn, 1994) (Figure 2). In the same way that the fungal and plant

oligosaccharides have been studied, the oligosaccharides obtained from algae and animals have presented a great potential as signaling molecules (Delattre et al., 2005).

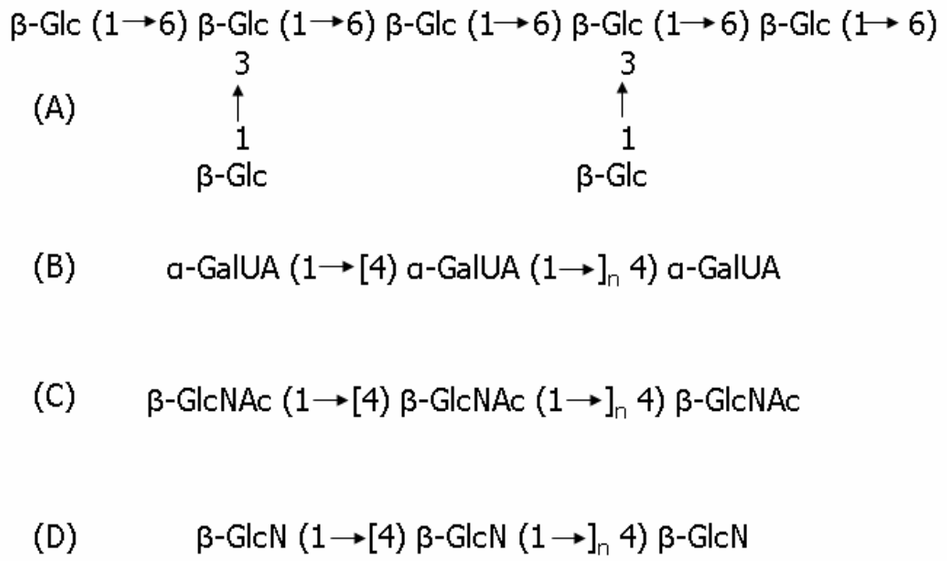

Fig. 2. Major oligosaccharides recognized by plants: (A) oligoglucans, (B) oligogalacturonide, (C) chitin-oligomer (D) chitosan-oligomer. Glc, glucose; GalUA, galacturonic acid; GlcNAc, N-acetyl glucosamine; GlcN, N-glucosamine.

5.1 Biochemical responses elicited by oligosaccharides

Of the major biochemical responses (Radman et al., 2003) that occur when a plant or cell culture is confronted with an elicitor are:

- Elicitor recognizing by plasma membrane receptor
- Changes in the flow of ions across the membrane
- Rapid changes in protein phosphorylation patterns
- Activation of NADPH oxidase enzyme complex responsible for ROS production and cytosolic acidification
- Reorganization of the cytoskeleton
- Accumulation of defense-related proteins
- Cell death at the site of infection (hypersensitive response)
- Structural changes in the cell wall (lignification, callose deposition)
- Transcriptional activation of defense related genes
- Synthesis of jasmonic acid and salicylic acid as second messengers
- Systemic acquired resistance

5.2 Oligoglucans

In the search for active oligosaccharides, at first it was considered the fungi kingdom, and specially biotrophic or necrotrophic fungi such as pests, because they cause important damage in plants, fruits and vegetables. But these organisms are the cue to reinforce the defense mechanisms of plants. When the plant-pathogen interaction occurs, several signaling receptor are activated by

fungi or plant cell wall fragments, and then a biological response could be the main factor determining the survival or decline of plants. Many fungal pathogens have β-glucans as major components of their cell walls, which are recognized by different plant species (Yoshikawa et al., 1993). The Albersheim working group, at the middle of 70's, was the first to extract glucans elicitors of phytoalexins (a natural antimicrobial compound) in soybean from the mycelial walls of *Phytophthora megasperma* by heat treatment. These fungal wall structures were analyzed by Sharp et al., (1984) detailing the primary structure of an active glucan from *Phytophthora megasperma* f. sp. *glycinea* (Pmg) obtained by partial acid hydrolysis, finding that the hepta-β-glucoside elicitor was the active subunit.

Partial characterization of the fraction with elicitor activity from Pmg walls showed β-glucans with terminal residues 1-3 (42%), 1-6 (2%) and 1-3, 1-6 (27 %) glycosidic bonds (Sharp et al., 1984; Waldmüller et al., 1992). They observed that the obtention method of the cell wall fragments influenced the type of links present in the fungal elicitor. If the elicitor is released naturally or by heat treatment, then elicitors differ greatly from those glucans obtained by partial acid hydrolysis. While naturally released glucans have β-(1-3, 1-6) ramifications, β-(1-6) links are in greater proportion when glucans are released from acid hydrolysis (Waldmüller et al., 1992).

5.3 Oligoglucan receptors in plants

The recognition of elicitors by plants could be possible if the oligoglucan-receptor interaction occurs (Yoshikawa et al., 1993). In plants, receptors of fungal elicitors are found on the cell surface, while bacterial receptors are found within the cell (Ebel & Scheel, 1997). Other binding sites for oligosaccharides, glycopeptides, peptides and proteins are located on the cell surface and in the membranes (Cosio et al., 1990). Hence, many defense responses could be activated against pathogens, if the correct single or complex mixtures of elicitors are applied in healthy or unhealthy plants.

Binding proteins have been reported in soybean membranes for the hepta-β-glucosides (1-3, 1-6) and their branching fractions (Cosio et al., 1992). Other binding sites for yeast glycopeptides have been reported in tomato cells (Basse et al., 1993), for chitin-oligosaccharides these binding proteins have been found in tomato, rice (Baureithel et al., 1994) and parsley cells (Nürnberger et al., 1994). On the other hand, induction of phytoalexins by fungal β-glucans showed good correlation with the presence or absence of high affinity binding sites in several Fabaceae family plants (Cosio et al., 1996). A key method for assessing the presence of receptors on the membranes is through homogeneous ligand binding assays in isolated membranes (Yoshikawa et al., 1993). The radiolabeled ligand competition experiments using non-derivatized hepta-β-glucan as a competitive agent showed the existence of specific binding in at least four (alfalfa, bean, lupin and pea) of six species of Fabaceae family plants analyzed (Cosio et al., 1996).

The active oligoglucans can be isolated from the cell wall of algae and phytopathogenic fungi (Shinya et al., 2006). The oligoglucan laminarin is a β-(1-3)-glucan branching β-(1-6) glucose, which significantly stimulates defense responses in various crops including tobacco. The best known fungal elicitor is the heptaglucan (penta-β-(1-6) glucose with two branches β-(1-3) glucose) that was isolated from the cell walls of *Phytophthora megasperma*. This oligoglucan elicits defense responses in soybean cell cultures but not in cell cultures of tobacco or rice (Cheong & Hahn, 1991; Klarzinsky et al., 2000, Yamaguchi et al., 2000). A branched

oligoglucan isolated from *Pyricularia oryzae* induces phytoalexins in rice but not in soybean (Yamaguchi et al., 2000). Linear oligoglucans were active in tobacco (Klarzinsky et al., 2000), but not in rice (Yamaguchi et al., 2000) or soybean plants (Cheong & Hahn, 1991). Another oligoglucans obtained from the cell walls of *Colletotrichum lindemuthianum* produce oxidative damage, common plant response to the invasion of pathogens, has been extensively studied in cell cultures of *Phaseolus vulgaris* (Sudha & Ravishankar, 2002). This clearly explains the great diversity of oligoglucans and the various biological effects that can be generated in the plant or crop to be evaluated. Clearly these facts show that the successful recognition for this kind of elicitor depends on specific plant receptors among plant species, even within families.

5.4 Oligoglucans action mechanism in plants

At the present time, only few reports about the action mechanisms of oligoglucans have been described. These reports focused in the final steps of the defense response, mainly during fungal attack, while other abiotic factors such as stress by uncontrollable temperatures (heat or cooling) have been less addressed. In order to address these issues, Doke *et al.,* (1996) proposed a mechanism of oxidative damage in plant cells in response to elicitors derived from fungal cell wall. The invasive fungal elicitor molecule (oligoglucan or, if the elicitation is mediated by pectic oligogalacturonic from plants) is recognized by the plasma membrane receptor (peripherial or transmembrane proteins), this recognition stimulates Ca^{2+} influx through Ca^{2+} channels. The increase in free Ca^{2+} in the cell acts as a second messenger, together with the activation of calmodulin (CaM) to activate protein kinases and protein factors by phosphorylation. Then the activated NADPH oxidase provides electrons through the oxidation of NADPH, and the electron transport system reduces O_2 molecules generating the radical $O_2^{\bullet -}$ (Figure 3).

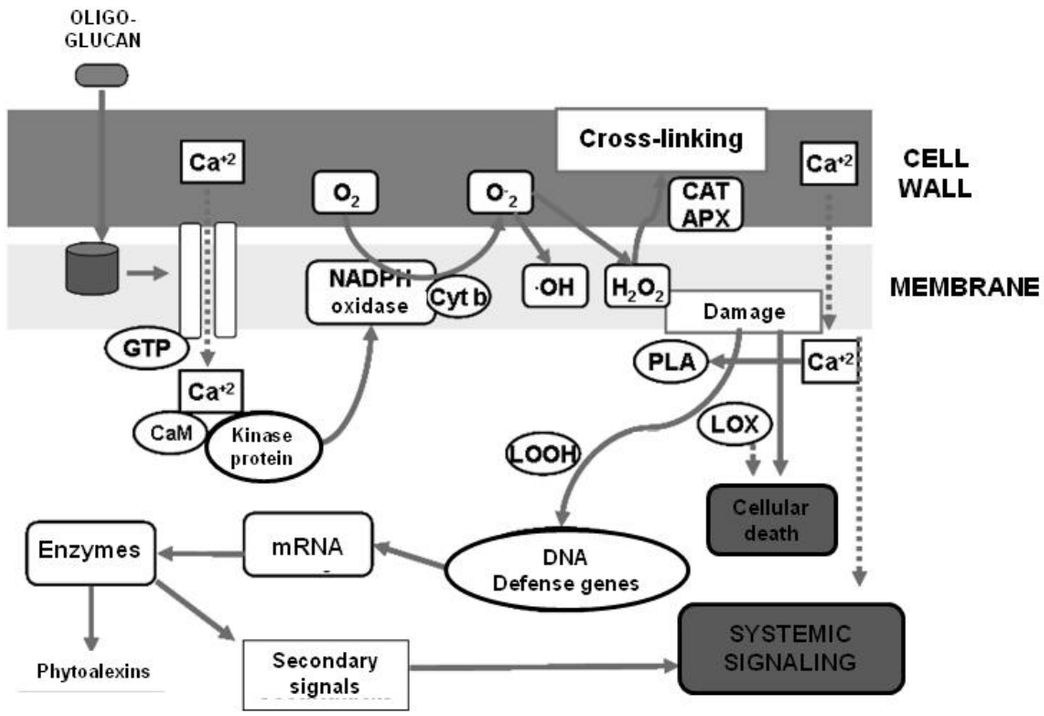

Fig. 3. Oligoglucans action mechanism in plants (modified Doke *et al.,* 1996).

6. Fungal glucans and their relationship with the enzymatic antioxidant system in cold stressed plants

Every day, the non-desirable climate change effects are present in our agriculture and the worldwide food production suffers the adverse consequences. Therefore, crop yields fell around fifty percent for several crops (Wahid et al., 2007). Several environmentally agencies report increments or reductions in temperature along the year. It is crucial to find an environmental friendly solution to challenge against low crop yields.

Under thermal stress (heat or chilling temperatures), important metabolic and physiologic plant processes are interrupted. As a consequence, protein aggregation and denaturalization in chloroplasts and mitochondria, destruction of membrane lipids, production of toxic compounds and the ROS overproduction (Howarth, 2005) are the most common responses of plant cells. Those are some reasons of the destructive effects of this kind of abiotic stress.

There are several pre- and postharvest treatments to deal with thermal stress like genetic modifications, thermal conditioning treatments of seeds and fruits or triggering early defense systems in plants by exogenous elicitation (Falcón-Rodríguez et al., 2009; Islas-Osuna et al., 2010). Our work team, evaluated the triggering of some important antioxidant enzymes in squash (*Cucurbita pepo* L.) seedlings at low temperature by the spraying of a novel mixture of fungal glucans isolated from *Trichoderma harzianum* by chemical and/or enzymatic fungal cell wall hydrolysis (Cerón-García et al., 2011). Two of the most active antioxidant enzymes, catalase and ascorbate peroxidase, were triggered by the exogenous elicitation with fungal oligoglucans in cold-stressed squash seedlings. Both antioxidant enzymes are the main active H_2O_2 detoxificant elements in the plant cell. Antioxidant enzymatic system in plants became unstable under thermal stresses, mainly by the inhibition of the catalytic activities during extreme temperatures. However, the elicitation with fungal glucans restored the deficiency of the antioxidant enzymatic system.

7. Conclusion

Biotic and abiotic factors may have a negative effect on plants, favoring the accumulation of ROS to generate further oxidative stress. Multiple biochemical responses are clearly generated by the use of oligoglucans as elicitors of defense responses against oxidative stress. The recognition of elicitors may vary depending on their characteristics, on the plant species or even for a particularly tissue, where specific receptors enables the generation of secondary signals that promote the most active plant defense against various biotic and/or abiotic factors by strengthening the antioxidant system, the accumulation of antimicrobial compounds such as phytoalexins and the activation of plant defense-related genes. Since there is little research on plant-oligoglucan interactions, so many questions remain unanswered.

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