Plant-Environment Interaction

Responses and Approaches to Mitigate Stress

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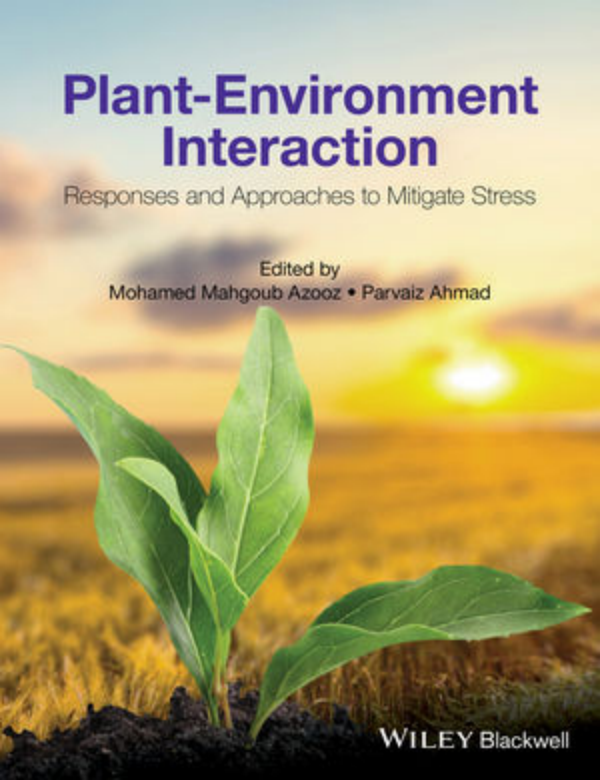

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CHAPTER 11

Lipid metabolism and oxidation in plants subjected to abiotic stresses

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11.1 Introduction

Plant lipids include fats, waxes, steroids, phospholipids and hydrocarbons. The free higher fatty acids and their salts (soaps) also belong to this category. Below are detailed the main classes of plant lipids.

11.1.1 Membrane lipids

These are important chemical components of all cell membranes, where they are represented mainly by amphipathic phospholipids (the more abundant) and sterols (particularly stigmasterol), spontaneously forming bilayers in an aqueous environment (López *et al.*, 2011). The exception is the thylakoid membranes of chloroplasts, which contain primarily galactolipids, that are also amphipathic and mostly stable in a bilayer configuration (Robinson & Mant, 2005; Tetlow *et al.*, 2010). The amphipathic nature of membranes permits the formation of membranous sheets that self-anneal their edges into a sealed compartment. The inner and outer surfaces of a membrane, both plasma and organelle membranes, differ considerably in chemical composition (Evert, 2006).

11.1.2 Carotenoids

These are yellow to red-coloured non-polar lipids with a terpenoid structure containing eight isoprene units arranged in a symmetrical linear pattern. Their conjugated double bonds are responsible for the typical visible light absorption of this class of compounds. Carotenoids are responsible for protecting the chlorophylls from photodynamic destruction (Cutriss & Pogson, 2004). They exist in many flowers and fruits where they are valuable in terms of providing signals to other species on the availability of food sources, and in this way ensure pollination and the spread of seeds.

The two major classes of carotenoids are the carotenes and their oxygenated derivatives, the xanthophylls. The most abundant xanthophylls, lutein and violaxanthin, are key components of the light-harvesting complex (LHC) of leaves (Foyer & Shigeoka, 2011; Singh & Tuteja, 2010).

11.1.3 Oil bodies

Oil bodies are spherical structures, also called spherosomes or oleosomes, particularly concentrated in fruits and seeds, arising by the accumulation of triacylglycerol molecules. Seeds contain high amounts of lipids, together with proteins and carbohydrates, accumulated as a nutrient source for seedling growth. Approximately 45% of the weight of sunflower, peanut, flax and sesame seeds is composed of oil (Bradford & Nonogaki, 2007). Lipids of seed cells have been suggested to be sensitive to temperature, thus functioning as a 'temperature probe' able to detect the signals for stopping dormancy. Aleurone grains within endosperm store abundant reserves of lipids that are broken down to sugar by beta-oxidation and the glyoxylate cycle to fuel hypocotyl elongation in the dark (Golovina & Hoekstra, 2003). The outer layers of seed integument are covered and/or impregnated with more or less solid lipids which are primarily responsible for seed waterproofing properties. When the seeds of various species of Sapindaceae are injured by pathogens, specific lipids (cyanogenic

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lipids) are rapidly cleaved by an esterase, liberating HCN as a defence response (Selmar, 2010). The essential oils, present mainly in the leaves of some species (e.g. Lamiaceae), are volatile oils that contribute to the essence and odour of plants. They are made by special cells in the form of oil bodies and then excreted into intercellular cavities. Oil bodies are also common storage products and cryoprotectants of dormant cambial cells (Evert, 2006).

11.1.4 Waxes

Waxes are long-chain (20–25 C) lipid compounds (mixtures of hydrocarbon molecules, predominantly paraffins and related alcohols, ketones, acids and esters), solid at normal temperatures, constituting part of the protective coating (cuticle) on the epidermis of the aerial parts of the primary plant body and on the inner surface of the primary wall of cork cells in woody roots and stems. They constitute a major barrier to water loss from the surface of the plant by reducing the wetability of leaves. They also reduce the ability of fungal spores to germinate and of bacteria to grow, thereby reducing the ability of these agents to cause diseases (Zentgraf, 2007). Cuticle components are made in the epidermal cells and are extruded through the outer walls to the exterior, where they take up their final form. In the nectaries of many flowers, the nectar is released by rupture of the wall and cuticle of each epidermal cell (Evert, 2006). The nectar has high sugar content, mainly sucrose, and also contains lipids and proteins.

The smooth endoplasmic reticulum (SER) is involved in the synthesis of different types of lipidic compound (Evert, 2006). The role of the SER labyrinths is not fully understood but it is present in a variety of plant glands, secreting fats, oils and fragrant essential oils, and is also abundant in epidermal cells that are making lipid molecules to be deposited in the external cell wall waxes, cutin and suberin (Robinson & Mant, 2005). In general, the SER has been found to process the enzymes necessary for making complex lipids, given the ingredients of fatty acids (made in plastids) and lipid head groups (made by cytoplasmic enzymes). On the basis of the model best supported by experimental evidence, these raw materials come together in the cytoplasmic face of the SER membrane to produce a variety of products. Depending on their nature, they may accumulate until they form lipid droplets, initially still in the membrane but then liberated into the cytoplasm for storage or transport around the cell. Special enzymes are able to 'flip' lipids from the cytoplasmic face of the ER membrane (where they are made) to the luminal face, correcting the imbalance that arises from asymmetrical synthesis. In this way, the ER membrane grows in surface area. Expanses of membrane may then be mobilized to other systems in the form of vesicles – especially to the Golgi apparatus and from there to the plasma membrane or other cell parts (Tetlow *et al.*, 2010).

Whereas most membranes are composed primarily of phospholipids, galactolipids account for over 80% of thylakoid membranes but they are also present in outer and inner chloroplast envelopes. Galactolipids are synthesized at the inner envelope of the chloroplast and these lipids are furthermore highly unsaturated. It is concluded that an intraorganellar lipid transport system must exist that transfers lipids from their site of synthesis to the thylakoids. Thylakoid membranes are thought to be derived from invaginations of the inner membrane, as maturing chloroplasts sometimes exhibit a continuum between the inner membrane and internal membrane structures, although this continuum is not present in mature chloroplasts. It has been suggested that vesicle trafficking from the inner membrane to the thylakoids allows maintenance and regeneration of these structures in the mature chloroplast (Robinson & Mant, 2005). Furthermore, an ATP-dependent factor involved in vesicle fusion within pepper chromoplasts has been isolated and the gene cloned.

Such a 'budding' mechanism of thylakoid biogenesis would explain how other hydrophobic membrane components (e.g. carotenoids and galactolipids) are synthesized on the chloroplast envelope, and are able to reach the thylakoid membranes themselves. The interaction between specific proteins and galactolipids could be important for distinguishing the chloroplast from other potential target membranes inside the cell. Leucoplasts are colourless plastids that are distinct from proplastids because they have lost their progenitor function. Within this group are elaioplasts/oleoplasts, that are the sites of lipid synthesis (Tetlow *et al.*, 2010).

The biosynthetic pathway of carotenoids involves a series of desaturations, cyclizations, hydroxylations and epoxidations, commencing with the formation of phytoene and typically terminating in lutein and neoxanthin accumulation (Cutriss & Pogson, 2004). In brief, phytoene is formed by the condensation of geranylgeranyl diphosphate by phytoene synthase.

Phytoene is subjected to four desaturation reactions by phytoene desaturase and zeta-carotene desaturase to produce tetra-cis-lycopene, which is isomerized by the carotenoid isomerase to produce all-trans-lycopene. Lycopene is cyclized twice to produce beta-carotene or once to produce alpha-carotene. The two carotenes are hydroxylated by the beta- and epsilon-hydroxylases to produce zeaxanthin and lutein, respectively. Zeaxanthin is epoxidated by zeaxanthin epoxidase to form violaxanthin, which is further modified by neoxanthin synthase to produce neoxanthin.

Carotenoids and their biosynthetic enzymes are placed in the plastids, although carotenoid biosynthetic genes are within the nuclear genome. The pathway is at least in part regulated via changes in transcription. As a consequence, the transcriptional regulation of carotenoids and also chloroplast-nuclear signalling are probably induced by various environmental stimuli, oxidative stress, redox balance and metabolite feedback regulation (Foyer & Shigeoka, 2011).

11.2 Lipid vulnerability to reactive oxygen species and mechanism of lipid oxidation

Lipid peroxidation starts when activated oxygen species react with the double bonds present in lipid hydrocarbon chains. On the basis of the lipids involved, type of oxidant compounds and oxidation severity, different products of lipid peroxidation are produced, such as compounds containing hydroperoxyls, hydroxyls, ketones, aldehydes, caroxylic acids and trans double bonds (Borchman & Sinha, 2002; Hameed *et al.*, 2013; Sharma *et al.*, 2012).

Lipid peroxidation in plants is mainly due to ROS activity. According to Browne & Armstrong (2002), the primary target on lipids of ROS is the 1,4-pentadiene structure of a polyunsaturated fatty acid (PUFA), either free or esterified to cholesterol or glycerol. The process starts (initiation) when a ROS removes a methylene hydrogen from PUFA. In this reaction, the ROS is quenched and a PUFA-centred alkoxyl radical (L•) is formed, with a consequent spontaneous rearrangement of its double bonds and the formation of a conjugated diene. Reaction of L• with oxygen in its molecular form produces a PUFA-centred peroxyl radical (LOO•). The reaction continues (propagation) when either L• or LOO• acts as initiating ROS, so attacking a neighbouring PUFA of the lipidic bilayer structure of a membrane or within a lipoprotein. Thus, a new L•, which can further propagate the reaction and form a lipid hydroperoxide (LHP), is produced. The reaction ends (termination) when an antioxidant molecule able to absorb the intermediate free radicals, or free radical scavengers, interrupts this chain reaction.

The mechanism described above can be summarized in the following phases: activation, distribution and cleavage. In brief, the activation of an unsaturated fatty acid by one radical causes the cleavage of one H^+ from the methyl vinyl group present in the fatty acid:

$$OH\bullet + RH \rightarrow L\bullet + H_2O$$

The resonance structure of this reaction reacts with triplet oxygen, a biradical with two unpaired electrons, producing a peroxide radical:

$$L\bullet + O_2 \rightarrow ROO\bullet$$

This latter reacts with a hydrogen atom from a second fatty acid, with the formation of a lipid hydroxide; the free carbon can participate in the secondary assimilation of hydrogen:

$$LOO\bullet + RH \rightarrow LOOH + L\bullet$$

The high reactivity of hydroxide radicals in any given lipid system is due to their ability to initiate a chain reaction at very low levels (Hinojosa *et al.*, 2010).

Little is known about the ability of plants to repair the effects of stress-induced oxidative damage in cell membranes. Particularly, lipid peroxidation occurring during abiotic stresses generates changes in fatty acid composition that in turn influence both structural and functional properties of cell membranes (Lyubenova & Schröder, 2010). It has been clearly demonstrated that lipoxygenase isoenzymes (LOX; EC 1.13.11.12) catalyse the dioxygenation of polyunsaturated fatty acids containing a cis,cis-1,4-pentadiene structure, with the consequent production of highly reactive and toxic hydroperoxy fatty acids (Figure 11.1). Subsequently, they are degraded into metabolites, such as jasmonates, conjugate dienoic acids and volatile aldehydes, such as malondialdeyde (MDA). LOX activity, nearly ubiquitous in the plant kingdom, is involved in stress responses, flowering, seed germination, pigment bleaching, flavour and aroma formation in plant products, and plant growth and development.

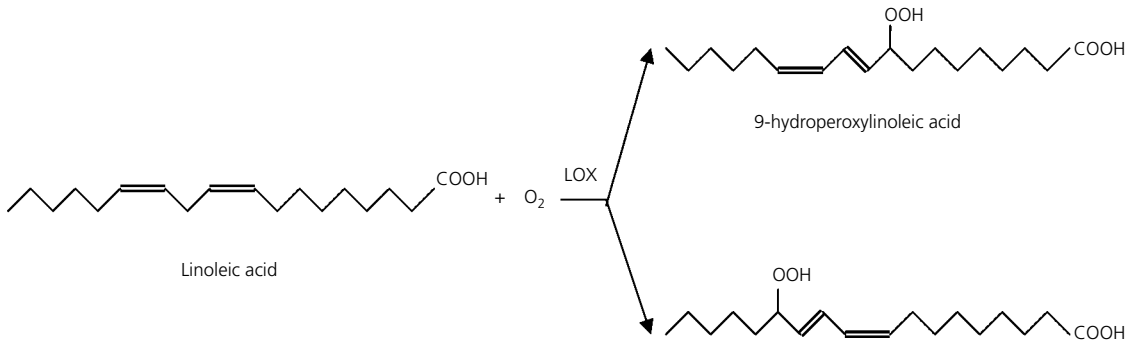

Figure 11.1 The reaction catalysed by lipoxygenase indicates two possible products of linoleic acid degradation.

Despite numerous studies, LOX's physiological role in plants is not yet completely understood. In plants, there are mainly three isoenzymes: LOX-1, LOX-2 and LOX-3. The LOXs are globular proteins, soluble in water, constituted by a single polypeptide associated with an atom of Fe (III) that is essential for the catalysis. LOX isoenzymes, having different substrate specificity and optimum pH, are located in the cytosol, microsomes, plasma membrane and oil bodies. LOX-3 is the most abundant isoform and is the one that has the greatest activity (Sofo *et al*., 2004a, and references within). For instance, the LOXs of olive trees are divided into soluble and membrane associated, the latter found mainly, but not exclusively, in the plastid fraction. Both in callus and in fruit of the olive tree, the main products of LOX are 13-hydroperoxides. The soluble isoforms are located in most of the tissues of olive trees, while the LOXs with acidic pH are associated with the chloroplast membranes of the fruit (Sofo *et al*., 2004a, b).

Malondialdehyde has been utilized as a reliable biomarker of lipid peroxidation in plants (López *et al*., 2011; Sofo *et al*., 2004a, b; Sorkheh *et al*., 2012a). Interestingly, MDA is also able to damage DNA, so enhancing the damage to cellular components and biomolecules.

The ROS produced in peroxisomes play a key role in plant ROS scavenging during abiotic stress conditions and senescence. Different experimental reports have indicated that peroxisomes are involved in cell signalling (by NO, $O_2\bullet^-$, H_2O_2 and S-nitrosoglutathione) during leaf senescence and in stress situations induced by xenobiotics and heavy metals. The activity of enzymes participating in the ascorbate-glutathione cycle in the peroxisomes is deeply influenced by abiotic stresses and senescence, when peroxisomal glutathione levels, H_2O_2 concentration and lipid peroxidation rate are considerably increased (Zentgraf, 2007). Furthermore, the peroxisomal NADH-dependent production of $O_2\bullet^-$ radicals is enhanced by the reverse transition of leaf peroxisomes to glyoxysomes occurring when plants experience adverse environmental conditions (Hameed *et al*., 2013; Sharma *et al*., 2012).

11.3 Methodologies for lipid oxidation estimation

The hydroperoxide moiety of a LHP is reduced by divalent metal ions or glutathione-dependent peroxidases to an alcohol, with the production of a hydroxy derivative (LOH). In plants, many different products of lipid peroxidation can vary in the length of carbon chain and level of unsaturation of the hydroperoxy unoxidized PUFA composition. For this reason, simultaneous determination of both the substrate and its derivative oxidation products has been suggested (Browne & Armstrong, 2002).

Reverse-phase high-performance liquid chromatography (RP-HPLC) is an analytical technique capable of separating regioisomeric species of LHP and LOH derived from plant PUFA. Following total lipid extraction, alkaline hydrolysis and re-extraction of the liberated fatty acids, two separate systems with different mobile-phase conditions and analytical columns are usually used, one for LOH and LHP and the second for the native unoxidized PUFA. It was also reported (Browne & Armstrong, 2002) that a change of this methodology, allowing simultaneous determination of LHP, LOH and PUFA on a single chromatographic separation, using diode-array detection, allows determination of

the PUFA at 215 nm and the conjugated diene of LHP and LOH at 236 nm. This methodology sacrifices a small amount of resolution of LHP and LOH for inclusive determination of PUFAs in a single isocratic run but is useful for the determination of total LHP and LOH relative to their precursor PUFA within 20 min after injection.

Infrared spectroscopy (IS) can detect the major products of lipid peroxidation and is sensitive in detecting both hydroxyl and hydroperoxyl groups (Borchman & Sinha, 2002). This discrimination is especially useful for quantifying the oxidation of monounsaturated lipids, where secondary products of lipid oxidation are not promptly produced. Indeed, many plant lipids are highly unsaturated and so they are strongly subjected to lipid oxidation, particularly under stress conditions.

According to many authors (Sofo *et al*., 2004a; Sorkheh *et al*. 2012a, b, and references within), MDA is usually extracted from plant tissues using trichloroacetic acid, and the supernatant resulting from centrifugation is added to thiobarbituric acid in 20% (w/v) trichloroacetic acid. The mixture obtained after heating (100°C) is subsequently cooled and centrifuged, and then absorbance values of the supernatant are recorded at 532, 600 and 440 nm. A series of compounds, including MDA, react with thiobarbituric acid, and for this they are called thiobarbituric acid-reactive substances (TBARS). When measuring MDA levels, especially in green tissues, it is very important to carry out a correction of the high sucrose content and also for the presence of anthocyanins or other interfering compounds. Thus, the values for a specific absorption at 600 nm are subtracted from the sample reading at 533 and 440 nm. Moreover, a standard curve of sucrose is used to correct the results from the interference of soluble sugars in samples.

11.4 Lipid oxidation in abiotic-stressed plants

Different types of abiotic stresses cause increase of ROS in plants, with consequent damage to lipids that ultimately results in oxidative stress (Foyer & Shigeoka, 2011; Gill & Tuteja, 2010; Miller *et al*., 2010; Nishida and Murata, 1996; Sharma *et al*., 2012). Plants under abiotic stresses must intercept photosynthetic light and at the same time avoid oxidative damage due to a specific stressor or the combination of them. It is becoming increasingly clear that not only antioxidant enzymes and phenolic compounds but also soluble sugars (such as disaccharides, raffinose family, oligosaccharides and fructans), other compatible solutes (such as betaines and proline) and their associated metabolic enzymes are strong protective compounds against lipid peroxidation in stressed plants (Chen & Murata, 2002; Cruz *et al*., 2013; Keunen *et al*., 2013; Kotchoni *et al*., 2006; Szabados & Savouré, 2010).

11.4.1 Drought and salinity

Membranes are considered to be a primary target of desiccation injury, as the ability of desiccation-tolerant organisms to avoid membrane damage during a dehydration-rehydration cycle is related to changes in membrane fluidity. Membrane bilayer structure in dried desiccation-tolerant organisms is thought to be stabilized as a result of interactions of the polar groups with sugars and proteins. Such interactions create space between phospholipids and prevent membrane phase changes. Membranes thus remain in the liquid-crystalline phase when the hydration shell is lost (Golovina & Hoekstra, 2003). Drought stress, especially if at a high degree and prolonged, is the main cause of photoinhibition, resulting in a light-dependent inactivation of the primary photochemistry associated with photosystem II. Though many species show a high tolerance to drought stress, net photosynthesis and transpiration rates generally decrease with increasing drought stress, whether for stomata closure or non-stomatal oxidative effects. Damage to lipid structure and functioning in plants subjected to various degrees of drought stress was recently found in cereals (Campo *et al*., 2014; Csiszár *et al*., 2012; Fukao *et al*., 2011; Hameed *et al*., 2011, 2013), various tree species (Štajner *et al*., 2013), horticultural crop and forage plants (Abbas *et al*., 2014; Slama *et al*., 2011) and medicinal plants (Tian *et al*. 2012). Interestingly, exogenous cinnamic acid and derivatives of jasmonic acid were effectively used in improving plant drought stress tolerance by modulating the membrane lipid peroxidation and antioxidant activities (Anjum *et al*., 2011; Sun *et al*., 2012). In addition, Zhu *et al*. (2011) recently demonstrated that arbuscular mycorrhiza are able to alleviate the detrimental effect of drought by reducing MDA content and membrane permeability, and increasing proline content and antioxidant enzyme activities.

Drought, osmotic and salt stresses enhance the production of ROS, causing oxidative injury to lipids (Miller *et al*., 2011, and references within). Salt stress, also at mild levels, is able to cause lipid peroxidation in cereals (Ashraf *et al*., 2010; de Azevedo Neto *et al*., 2006), herbs and vegetables (Sergio *et al*., 2012; Tayebimeigooni *et al*., 2012) and tree species (Ahmad *et al*., 2010; Ayala-Astorga & Alcaraz-Meléndez, 2010). Tomato seedlings exposed to exogenous ascorbic acid show enhanced resistance against salt stress and decreased lipid peroxidation (Shalata & Neumann, 2001).

11.4.2 Metals

Elevated metal concentrations in the environment coincide with an oxidative stress-related constraint on platidial and mitochondrial electron transport, which increases lipid peroxidation in these two compartments and in the whole cell (Dresler *et al*., 2014; Keunen *et al*., 2011; Nagajyoti *et al*., 2010; Pospíšil, 2014; Yadav, 2010). Copper (Cu), iron (Fe), nickel (Ni), selenium (Se) and zinc (Zn), even if at relative low concentrations, are essential for plant physiological and biochemical processes. However, cadmium (Cd), aluminium (Al) and lead (Pb) are considered to be non-essential or toxic for plants (Cuypers *et al*., 2010; Sofo *et al*., 2013).

Transition metals (M) are very good catalysts of oxygen reduction due to their unpaired electrons. Among these, Fe and Cu are the most important for plant metabolism.

$$M^n + O_2 \rightarrow M^{n+1} + O_2\bullet^-$$

In turn, $O_2\bullet^-$ in aqueous solutions at neutral pH leads to the production of H_2O_2, subsequently decomposed to produce •OH by the Haber–Weiss reaction, as follows:

$$M^{n+1} + O_2\bullet^- \rightarrow M^n + O_2$$

$$M^n + H_2O_2 \rightarrow M^{n+1} + OH^- + \bullet OH$$

Furthermore, transition metals lead to the formation of reactive alcohol radicals by the production of OH^- via the Fenton reaction:

$$LOOH + Fe^{2+} \rightarrow OH^- + LO\bullet + Fe^{3+}$$

Among metals, Cd alters the functionality of membranes by inducing lipid peroxidation and disturbs chloroplast metabolism by inhibiting chlorophyll biosynthesis and increasing photoinhibition (Ahmad *et al*., 2011; Cuypers *et al*., 2010, 2011; Gallego *et al*., 2012; Gill *et al*., 2013; Pérez-Chaca *et al*., 2014). On the other hand, Liptáková *et al*. (2013) showed that the upregulation of LOXs is important for barley root response to toxic Cd but it is not responsible for the Cd-induced harmful lipid peroxidation. Also, Cu (Opdenakker *et al*., 2012; Thounaojam *et al*., 2012), Ni (Gajewska *et al*., 2012; Kazemi *et al*., 2010), Se (Malik *et al*., 2012) and Pb (Maldonado-Magaña *et al*., 2011), and other heavy metals and transition metals (Sytar *et al*., 2013), are implicated in dose-specific and species-specific lipid peroxidation processes, and biological membranes are extremely susceptible to the presence of these metals into the soil.

11.5 Conclusion and future prospects

The status of membrane lipids can be perturbed by abiotic stressors, such as excess UV radiation, temperature extremes, nutrient deficiency and pollution of soil and air (Gill & Tuteja, 2010; Li *et al*., 2012; Szarka *et al*., 2012, and references within; Takshak & Agrawal, 2014; Tripathi *et al*., 2011; Wyrwicka & Skłodowska, 2014; Yan *et al*., 2010). The contribution of membrane lipids in protecting the photosynthetic machinery from photoinhibition during cold stress has been intensively discussed for many years (Nishida & Murata, 1996) and recently confirmed by Karabudak *et al*. (2014). In an important paper, Welti *et al*. (2002) conducted studies on membrane lipid profiles and on the role of some types of phospholipases in freezing-induced lipid change in *Arabidopsis*, demonstrating the crucial role of these enzymes in plant tolerance under cold conditions. Exposure of plants to herbicides appeared to be sigificantly related to the increase in lipid peroxidation (Dias *et al*., 2014; McCarthy-Suárez *et al*., 2011; Pazmiño *et al*., 2011, Spoljaric *et al*., 2011).

From the results of these researches, it appears clear that in the future the degree of lipid peroxidation in plants could be effectively used as a reliable biomarker of plant stresses, and of soil water and air pollution, with evident benefits for both agriculture and the enviroment.

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