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THE CORRELATION BETWEEN OXIDATIVE STRESS AND LEAF SENESCENCE DURING PLANT DEVELOPMENT

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Abstract: In plants, besides being the final step leading to the death of the whole organism, senescence has a developmental function involving the coordinated degradation of macromolecules and the mobilization of nutrients out of senescing tissues into developing parts of the plant. Free radicals are thought to play an essential role in senescence, especially those derived from oxygen. Since these molecules are extremely toxic, the levels of the different reactive oxygen species have to be tightly regulated. However, at low concentrations, hydrogen peroxide may also serve as a signalling molecule. Therefore, a coordinated regulation of the free radical scavenging system, which comprises enzymatic components such as catalase, superoxide dismutase and ascorbate peroxidase, and non-enzymatic molecules such as ascorbate and glutathione is essential. The increased radical levels displayed during senescence are not only caused by the elevated production of radicals but also by a loss in antioxidant capacity.

Key Words: Leaf Senescence, Oxidative Stress, Free Oxygen Radicals, Senescence Regulation

INTRODUCTION

Aging and death are essential aspects of the development of all living organisms, and naturally, there is much scientific research focused on understanding the molecular mechanisms underlying these processes. Many questions remain unanswered in this area, though there are some hints concerning the triggers and progression of senescence in plants. There is research being done in this field using almost all the established model systems. The information gained might be

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Abbreviations used: ABA – abscisic acid; APX – ascorbate peroxidase; CAT – catalase; HR – hypersensitive response; JA – jasmonic acid; PCD – programmed cell death; ROS – reactive oxygen species; SA – salicylic acid; SAG – senescence-associated gene; SOD – superoxide dismutase.

exploited to prevent losses during the storage of fruits, legumes, flowers, and crops, to increase the yields of various economically important plants [1-3], and to strengthen plant stress tolerance [4]. The manipulation of senescence processes, such as prolonging the photosynthetic activity of leaves, can be achieved through breeding programmes and via genetic engineering [5].

WHAT IS SENESCENCE?

In plants, senescence refers to both the final step leading to the death of the whole organism, and a complex, highly regulated process with a crucial developmental function ongoing throughout the life of the plant. It results in the coordinated degradation of macromolecules and the mobilization of regained nutrients like nitrogen, carbon and minerals from senescing tissues into other parts of the plant [6, 7]. In addition, senescence is characterized by the loss of chlorophyll and a decrease in the total RNA and protein contents [8]. Different kinds of senescence are distinguished: leaf, flower and fruit senescence, and post-harvest senescence. The focus of this paper is leaf senescence.

During leaf senescence, there are observable changes in cell structure, metabolism and gene expression [9]. Since nutrients have to be transported out of senescing tissues into young tissues and reproductive organs (flowers, seeds, siliques) as a kind of recycling, the tissue of the vascular system is maintained until the very late stages of senescence [10]. Proteins are degraded to amino acids, RNA is broken down to low molecular weight nitrogen compounds, and membrane lipids are metabolized to sugars [6]. By contrast, the DNA content remains largely constant, and DNA fragmentation can only be observed in the very late stages of the process [11].

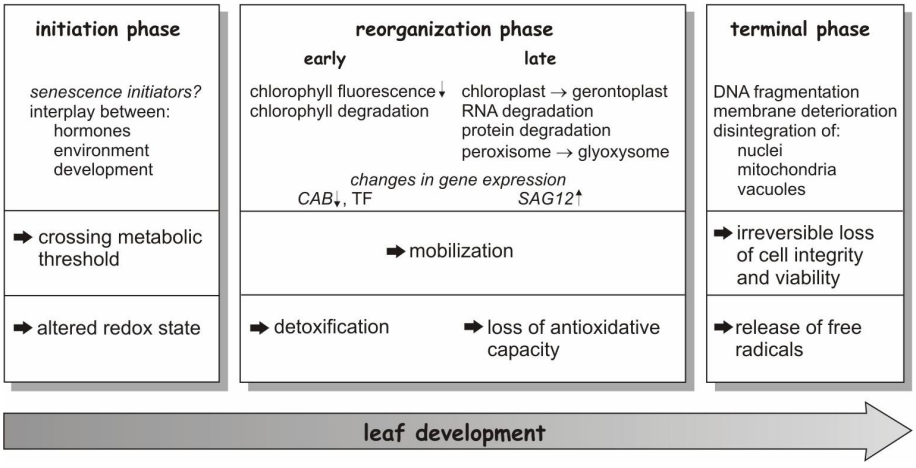

Fig. 1. Different phases of leaf development and their characteristic features.

Leaf senescence can be induced by external and internal signals. The external factors include extreme heat or cold, drought, shading, elevated ozone content, nutrient deprivation, pathogen attack or wounding [12]. The internal triggers include the age of the plant and the developmental stage of the reproductive organs [7, 10]. An overview of the different phases of leaf development is shown in Fig. 1.

Senescence processes at the cellular level

The degradation of the nucleus is a relatively late event in senescence. The conservation of a functional nucleus is very important, since the degradation of the other organelles is controlled by the nucleus [10]. The process can be blocked by inhibitors of RNA and protein synthesis, so nuclear gene expression appears to be necessary until the process is complete [7].

The first visible sign of senescence is the onset of chloroplast degradation [10, 12, 13]. This coincides with a decrease in the amount of chlorophyll, the degradation products of which are transported into the vacuole [14]. The loss of chloroplast integrity can be observed in the very early stages of senescence. Electron microscopy revealed that the chloroplasts of senescing leaves show an increased number of enlarged plastoglobuli, a disorientation of the grana stacks and a swelling of the thylacoids [13]. It is assumed that the formation of plastoglobuli is associated with the degradation of the thylacoids [15]. The term "gerontoplast" was established to describe the organelle of a senescing, formerly green tissue [16]. The conversion of chloroplasts to gerontoplasts in leaves is reversible in some, possibly all higher plants [17, 18]. For example, the removal of the stem of *Alstroemeria* leads to a regreening of the lower leaves [19]. From a physiological point of view, the activity of the membrane-associated electron transport of photosystem I and II decreases continuously during senescence [12], while the composition and fluidity of the thylacoid membrane is not changed. Therefore, the loss of photochemical capacity (F_V/F_M), which reflects the functional integrity of photosystem II, can be used as a parameter to describe senescence (Fig.1) [20-22].

The mitochondria play an important role in the regulation of programmed cell death (PCD) in animal systems by retrieving the general status of the cell. In this case, the loss of mitochondrial membrane integrity leads to the release of elicitors, which can induce cell death processes [23]. During plant senescence, the function of the mitochondria is maintained during the gradual breakdown of the cell up to a very late point (Fig. 1). This is essential for the cell to gain energy via ATP synthesis during respiration [12]. It is therefore unlikely that plant mitochondria trigger plant senescence. The chloroplasts may play a regulatory role during leaf senescence, similar to that of the mitochondria during animal PCD [24, 25]. Aging or stress which leads to the loss of photosynthetic capacity or membrane integrity might produce a signal for initiating the senescence program [5].

Another characteristic of senescence is the loss of membrane integrity [reviewed in 26, 27]. Membranes provide storage for lipid molecules which can be released and mobilized to provide energy for the senescence processes [6]. Dhindsa *et al.* [28] described a correlation between lipid peroxidation and increased membrane permeability. The decrease in the proportion of unsaturated fatty acids leads to a decline in membrane fluidity (Fig. 1).

The vacuole also plays an important role in the terminal phase of senescence. As mentioned above, it assimilates the end products of chlorophyll degradation [29, 30]. Only in the very late phase of development does the vacuole release its content, particular proteolytic enzymes, into the cytosol (Fig. 1) [12].

Regulation of senescence

Drought and nutrient deficiency, especially nitrogen deprivation, are among the main factors inducing senescence. Under such stresses, plants are forced to transport nutrients to their reproductive organs and to reduce water consumption by old, less productive leaves. Other factors include shading and extreme darkness. However, there are differences in gene expression between artificial dark-induced and natural senescence [10, 31].

The most important senescence-inducing endogenous factors are the ages of the leaves and the age of the plant. The leaves of annual plants show a continuous decrease in their photosynthesis rate after full expansion [32, 33]. This rate may differ from species to species. An extreme example of a plant with fast-aging leaves is *Arabidopsis thaliana*. If the plant is kept under continuous light, its photosynthetic capacity decreases by 50% within four to six days of full expansion of the leaves [32]. It is assumed that a decline in photosynthetic activity under a certain threshold may act as a senescence-inducing signal [13, 34]. Although this is still an open question, there is some evidence to support this theory. It is known that elevated sugar content represses photosynthesis-related genes [35].

It can be observed that the number of senescing leaves is strongly elevated during flowering and seed development. This correlates with the hypothesis that aging leaves serve as a source of nutrients during reproduction. The ubiquitin-dependent proteolysis during developmentally dependent nitrogen mobilization plays an important role [36]. The mobilized nutrients are stored in the developing seeds for the next generation [37]. By removing the flowers or siliques of soybean plants, leaf senescence can be retarded [7, 38]. The removal of the stem even results in regreening of the lower leaves. Similarly, removing young flower buds leads to a prolonged flowering time [19]. This kind of experiment reveals the reversibility of senescence, which can also be observed during fruit ripening in some plants [19]. There are two putative models to explain this phenomenon: either leaf senescence is induced by the huge demand for nitrogen by the reproductive organs or the reproductive tissues release a kind of “senescence hormone” which is transported into the leaves to start the process [37].

Senescence-associated genes

Leaf senescence is accompanied by large changes in gene expression (Fig. 1). Differential screening and subtractive hybridization techniques of cDNA libraries revealed that the expression of many genes is downregulated during senescence, while other genes are upregulated. These upregulated genes are called senescence-associated genes (SAGs). They can be classified into two groups: senescence-specific genes (class I) and genes which are already expressed during early development but whose expression increases strongly during senescence (class II) [10]. Many SAGs have already been identified [39]. They comprise genes for degrading enzymes, like RNases [40], proteinases [8, 32, 41] and lipases [42], and genes for products involved in nutrient transport. One example for a class I SAG is *SAG12* [44], which encodes a cysteine protease, the expression of which is senescence dependent, but not leaf specific; it is also expressed in other aging tissues, like stems and different flower organs [45, 46]. Since *SAG12* is expressed exclusively during senescence, it is often used as a molecular marker for senescence [47]. In addition, Chen *et al.* [43] identified senescence-specific expression for more than 40 different transcription factors. Recently, microarray analyses revealed that NAC and WRKY factors constitute the two largest groups of transcription factors (TF) of the senescence transcriptome [48].

Genes whose expression is downregulated during senescence are called senescence-downregulated genes (SDGs). Genes encoding photosynthesis-related proteins such as chlorophyll-*a/b*-binding protein (*CAB*) are in this group [10, 32].

Of the many isolated genes with senescence-dependent expression, not all could have a function assigned. This is one aim of current research in the field of senescence and its regulation.

It has not yet been possible to determine the exact moment of onset of senescence. The most obvious sign of senescence is the bleaching of the leaves. However, at the point when a leaf becomes light green, the chlorophyll content has already declined to 50% [49]. Chlorophyll degradation and the fall in the photosynthetic rate are currently used to define the beginning and mark the progression of senescence [50].

Phytohormones

Phytohormones are natural organic compounds which influence growth, development and physiological processes in plants [reviewed in 51]. Besides the classic phytohormones cytokinin, ethylene, auxin, gibberellic acid and abscisic acid, several substances were identified as chemical signal molecules in plants. The brassinosteroids, salicylic acid and jasmonic acid are newly characterized phytohormones.

Delayed senescence of detached leaves after exogenous application of cytokinin which can even lead to regreening of yellow leaves [53] was described by Richmond and Lang [52] in 1957. To further examine the action of cytokinin,

Gan and Amasino [54] produced transgenic tobacco plants expressing the bacterial isopentenyltransferase gene (*ipt*), a key enzyme in cytokinin production, under the control of the senescence-specific promoter *SAG12*. They thereby built up an autoregulatory system. As soon as senescence starts, the *SAG12* promoter is activated. Via the expression of *ipt*, high cytokinin levels are generated; this in turn delays senescence and leads to the inactivation of the *SAG12* promoter. Some time later, the cycle starts anew. Phenotypically, these tobacco plants live much longer than the wild type plants, and the senescence-delayed leaves are photosynthetically active for a longer period [54, 55]. Additionally, an increase in the biomass and number of flowers was observed, and the plants had a longer flowering time [54].

Ethylene plays an important role during leaf senescence. In many species, the exogenous application of ethylene promotes senescence-characteristic processes like chlorophyll, protein and starch degradation and an increase in the activity of many hydrolytic enzymes [56]. Plants exposed to ethylene show an early senescence phenotype with yellowing of the lower leaves [39]. If ethylene synthesis is blocked by the introduction of an antisense construct of ACC-oxidase, a key enzyme of ethylene biosynthesis, senescence is delayed [20, 57]. Different studies of ethylene-insensitive mutants have shown that ethylene is neither necessary nor on its own sufficient to induce leaf senescence. Leaves should reach a certain age to be susceptible to the ethylene signal [39]. Ethylene's involvement in senescence appears to be related to the timing of the process [2, 58].

Brassinosteroids also have a leaf senescence-promoting role [59], as does jasmonic acid (JA). It was found that several genes involved in JA biosynthesis are upregulated during leaf senescence in Arabidopsis, and that the JA level in senescing Arabidopsis leaves increases [59]. Since JA induces the expression of several senescence-enhanced genes [31, 60], JA appears to be an important senescence-promoting factor. Salicylic acid (SA), involved in pathogen defence, has also been shown to be required for the expression of some SAGs [31, 61]. Abscisic acid (ABA) has been implicated in the regulation of stress-induced senescence and the expression of several SAGs can be induced upon treatment with ABA [31].

By contrast, auxin and gibberellic acid have a negative effect on leaf senescence [62]. However, proper plant development is accomplished by a correct interaction of all the hormones at specific concentrations acting in synergistic or antagonistic ways.

OXIDATIVE STRESS

In 1956, in his "free radical theory of aging", Harman [63] postulated a correlation between the formation of free radicals and aging. It is known that the production of free radicals increases during senescence. Due to their toxic nature, it is supposed that the accumulation of these radicals might cause the senescence processes and the associated degradation events.

Formation of reactive oxygen species in the cell

Aerobic organisms use molecular oxygen as a terminal oxidant during respiration as it is relatively harmless and not very reactive. However, it has the potential to be reduced incompletely to toxic intermediates, like singulett oxygen ($^1O_2$), the superoxide radical ($O_2^{\cdot-}$), the hydroperoxyl radical ($HO_2^{\cdot}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (·OH). These molecules are called reactive oxygen species (ROS). All ROS are extremely reactive and are able to oxidize biological molecules, such as DNA, proteins or lipids [overview in 64].

In the plant system, many reactions which lead to ROS production are known. Some of these reactions are involved in the normal metabolism, like photosynthesis and respiration. This correlates with the traditional opinion that reactive oxygen species are inevitable by-products of an aerobic metabolism [65]. Other sources of ROS formation are reactions which are induced during abiotic stress, such as the photorespiration in the peroxisomes. In recent years, new ROS-producing enzymes were identified, such as NADPH oxidases, aminooxidases and cell wall-bound peroxidases [66].

The chloroplasts are the main source of ROS in plants. During photosynthesis, light energy is absorbed by a series of redox reactions and transferred to the reaction centres of the photosystems. Thereby, the electrons are transmitted to CO_2. Since only in some plants is the rate of CO_2 fixation high enough to convert more than 50% of the light energy [67], alternative electron acceptors like molecular oxygen are used, leading to the formation of superoxide radicals ($O_2^{\cdot-}$). In addition, the chloroplasts can form significant amounts of singulett oxygen ($^1O_2$): chlorophyll is the main source of this ROS. Normally, the excited singulett status of the chlorophyll serves the transfer of energy or electrons. To emit energy, chlorophyll uses fluorescence or conversion to the triplett status, which leads to the formation of singulett oxygen [68]. Another source of ROS formation, especially H_2O_2, is the photorespiration in the peroxisomes. During CO_2 fixation, ribulose-1,5-bisphosphate-carboxylase (RubisCO) uses CO_2 to carboxylate ribulose-1,5-bisphosphate. This enzyme can also use molecular oxygen to oxygenate ribulose-1,5-bisphosphate [69]. During this reaction, glycolate is formed and transported from the chloroplasts into the peroxisomes. The glycolate is then oxidized, and H_2O_2 is formed as a byproduct.

The mitochondria are also an important source of reactive oxygen. The mitochondrial electron transport chain consists of several dehydrogenase complexes which reduce a common pool of ubiquinone [70]. Cytochrome-*c*-oxidase or alternative oxidase serves as a terminal electron acceptor. Here, the superoxide radical is mainly produced by ubiquinone and the NADH-dehydrogenases [71], specifically by autooxidation of the reduced components of the respiration chain.

Mode of action of ROS

Oxygen radicals are extremely reactive and cytotoxic in all organisms, since they can react with unsaturated fatty acids and thus cause the peroxidation of

essential membrane lipids in the plasmalemma or the intracellular organelles. Peroxidation of the plasmalemma leads to leakage from the cells, rapid dehydration and finally to cell death. Damage to intracellular membranes may influence the respiration activity of the mitochondria, and cause the degradation of pigments and a loss of the CO_2 fixation ability. Very high concentrations of ROS can damage the photosynthetic apparatus leading to photoinhibition. Additionally, bleaching of leaves occurs due to oxidation of pigments [72]. The most reactive ROS is the hydroxylradical, which affects macromolecules leading to massive impairments of all cellular components and to DNA lesions and mutations. This damage often results in irreparable metabolic misfunction and cell death. Specific amino acids, like histidine, methionine and tryptophane, may be oxidized by superoxide radicals [73]. Hydrogen peroxide oxidizes SH groups and is particularly significant because of its relatively long half life and its diffusibility. At high concentrations, it may trigger programmed cell death. The hypersensitive response (HR) is the fastest defence reaction after contact with an incompatible pathogen. One of the first reactions is the rapid accumulation of H_2O_2 (oxidative burst) leading to local cell death around the infection site, thereby preventing the spread of the pathogen [74]. In addition, hydrogen peroxide plays a role in systemic acquired resistance (SAR), where signals are transmitted from the infection site to distal, uninfected parts of the plant [75]. This occurs via a non-toxic increase in H_2O_2 concentration inducing the expression of pathogenesis-related genes (PR) [76] and allowing a systemic pathogen resistance to be attained.

Low concentrations of H_2O_2 may act as signal molecules transmitting the plant reactions to a number of biotic and abiotic stresses [77, 78]. MAPK cascades and calmodulin are elements of these signal transduction pathways [79, 80], which lead to the activation or inhibition of different transcription factors. Vandenabeele ***et al.*** [81] identified a set of genes induced by high light treatment of catalase-deficient tobacco plants. These genes code for transcription factors and components of various signal transduction pathways, like pathogen defence and cell death.

This means that the reactive oxygen species might act as a signal for the activation of gene expression in response to pathogens [82] and various stresses, like wounding [83] and high light [84]. Presumably, there are a lot more signal pathways involving ROS.

The antioxidative system

The excessive formation of ROS is called “oxidative stress”. Since these radicals are very toxic, organisms have developed different systems to detoxify these radicals involving various enzymes, like catalase, superoxide dismutase and ascorbate peroxidase. A strong regulation of these enzymes is essential to keep the content of superoxide and hydrogen peroxide under tight control [66]. Besides the antioxidative enzymes, there is also a nonenzymatic component of

the scavenging system consisting of antioxidative molecules, like ascorbate (vitamin c), glutathione, α-tocopherol (vitamin e) and carotinoids [65].
Superoxide dismutases (SOD; EC 1.15.1.1) are metalloenzymes and ubiquitous in all oxygen-consuming organisms. They catalyze the dismutation of superoxide radicals ($O_2^{\cdot-}$) to molecular oxygen and hydrogen peroxide, the substrate of catalases and peroxidases.

$$2\ O_2^{\cdot-} + 2\ H^+ \rightarrow H_2O_2 + O_2$$

Since the superoxide radicals are removed from the cell by this reaction, the formation of the very toxic hydroxyl radicals by the Haber-Weiss reaction is prevented [68]. Takahashi and Asada [85] showed that phospholipid membranes are impermeable for superoxide radicals. Therefore, it is necessary for SOD to be available at the site of superoxide formation. The family of superoxide dismutases can be divided into three classes [86]. One class comprise SODs with Cu^{2+} and Zn^{2+} at the active binding site. They are located in the cytosol, chloroplasts and mitochondria of eukaryotic cells. The second class uses Mn^{2+} as a cofactor. The manganese-dependent SODs are found in prokaryotes and in the mitochondria of eukaryotes. Additionally, there is a speculated peroxisomal and cytosolic location for MnSODs [68]. The third group has Fe^{3+} at the active site. These enzymes are found in prokaryotes and mainly in the chloroplasts of some plants. Independent of their cellular location, all superoxide dismutases are encoded by the nucleus and then transported to the respective cell compartments. Hydrogen peroxide can be produced in all cell compartments. In the peroxisomes, catalases are responsible for its detoxification. In other compartments, the cells have developed another system containing ascorbate peroxidase and glutathione reductase, which remove H_2O_2 via the ascorbate-glutathione cycle. Here, H_2O_2 is reduced to H_2O using $NADPH+H^+$. Ascorbate and glutathione play a very important role in these reactions. Ascorbate peroxidase (APX; EC 1.11.1.11) uses ascorbate to reduce H_2O_2 to water, whereby dehydroascorbate (DHA) is produced. To maintain a certain level of ascorbate, it has to be regenerated by dehydroascorbate reductase (DHA; EC 1.8.5.1), which reduces dehydroascorbate to ascorbate by oxidizing reduced glutathione (GSH). Glutathione reductase (GR; EC 1.6.4.2) regenerates GSH via the reduction of oxidized glutathione (GSSG) using $NADPH+H^+$ [87]. APX is the most important enzyme scavenging H_2O_2 produced in the chloroplast. Eight types of APX have been described for Arabidopsis [88, 89]. These genes are regulated differentially in response to stress and developmental processes [88, 90, 91]. APX has a high affinity for H_2O_2 and is able to detoxify low concentrations of H_2O_2, whereas CAT has a high reaction rate, but a low affinity for H_2O_2. However, besides its role in the elimination of peroxisomal H_2O_2, catalase action appears to be critical for maintaining the redox balance during oxidative stress. It is also indispensable for stress defence in some C_3 plants [92]. Catalase is a tetrameric heme-containing enzyme (H_2O_2:H_2O_2 oxidoreductase) which can be found in all aerobic organisms. It catalyzes the conversion of H_2O_2

into water and molecular oxygen, whereby hydrogen peroxide acts simultaneously as an acceptor and donor of hydrogen molecules.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Catalase is the only enzyme that scavenges H_2O_2 without the need for reducing equivalents [93]. Plants contain several isoforms of catalases, presumably with different functions for the different isoforms. Normally, catalases form homotetramers, but under certain conditions they are able to form heterotetramers of the different isoforms. Other than the mitochondrial CAT-3 of maize [94], all the catalases thusfar characterized are located in the peroxisomes, where hydrogen peroxide is mainly generated during photorespiration and fatty acid β-oxidation. H_2O_2 may also diffuse into the peroxisomes from other sites of ROS synthesis [66]. The catalases can be divided into three classes depending on their expression and physiological parameters [95]. The first class of catalases is characterized by its strong, highly light-dependent expression in leaves. It is very abundant in photosynthetic active cells, and therefore presumably involved in the scavenging of H_2O_2 generated during photorespiration. Class II catalases are also found in mature plants, with a strong expression in the vascular tissue. They are mainly located in the vasculature of the stem and the mesophyll of the leaves, and their expression is light independent. In addition, they might play a role in lignification or the stress response [72, 96]. However, the precise function of this class is still unknown. The third group comprises catalases mainly expressed in seeds and young seedlings; they are in some cases also present in the late phases of senescence [96]. The function of this class is the removal of H_2O_2 produced during fatty acid degradation in the glyoxysomes [reviewed in 64, 95].

FREE RADICALS AND SENESCENCE

Oxidative processes are an important component of senescence. The degradation of chlorophyll and the membranes causes an increase in the production of free radicals. In addition, the amount of reduced oxygen, e.g. hydrogen peroxide, increases greatly during senescence. Plants react to this by activating a series of antioxidative enzymes, like the catalases, superoxide dismutases and components of the ascorbate-glutathione cycle, which can scavenge these toxic metabolites [97].

The excessive formation of reactive oxygen species is induced by several processes occurring during senescence, such as the activation of peroxidases in the peroxisomes, the activation of membrane-bound lipoxygenases and the misleading of electrons in the electron transport chains. Lipid peroxidation leads to the generation of free radicals, which in turn initiates an increase in ethylene formation leading to the promotion of senescence [68]. The high abundance of oxygen radicals during senescence is also observable in animal systems. In transgenic *Drosophila melanogaster*, the influence of the overexpression of

different radical scavenging enzymes was investigated. The overexpression of superoxide dismutase or catalase alone had no effect, whereas the combined overexpression of both enzymes led to a clear prolonged lifespan [98]. In plant systems, there is evidence that increased radical levels during senescence are not only caused by the elevated formation of radicals, but also by the loss of the compensatory function of the antioxidative enzymes (Fig. 1) [99, 100]. Antioxidative capacity and potential lifespan seem to be correlated in many organisms [101]. For example, Prochazkova ***et al.*** [102] reported on a maize line (cv. X 3342) with early senescence, the symptoms of which are induced by increased H_2O_2 formation and lipid peroxidation and by a decline in the activity of different antioxidative enzymes at the time of ripening.

Different stressors, including ozone and UV-B radiation, may cause an increase in the amount of internal free radicals, to which the plant reacts with symptoms of early senescence [103]. UV-B exposure causes dramatic increases in jasmonic acid levels and ethylene production, with ROS upstream of JA and ethylene in the signal transduction pathway [104]. Intact JA and ethylene signalling pathways are required for maximum defence against UV-B-induced tissue damage [104]. Furthermore, eight of twelve senescence-associated genes (SAGs) of Arabidopsis were induced by ozone [105], and the expression of many other SAGs was also enhanced by increased levels of ROS [103]. Stress-response pathways such as those involving salicylic acid and jasmonic acid have been shown to have a role in controlling gene expression during plant senescence [60, 61]. Hydrogen peroxide acts as a second messenger for the induction of defence genes in tomato plants in response to wounding and methyl jasmonate, suggesting an overlap between the pathogen and wounding response [106]. Therefore, jasmonates are rather part of the stress signal transduction pathway than a direct causative agent of senescence [107]. The level of the senescence-promoting phytohormone ABA also increases under conditions of stress such as drought, low temperature and high salt, which predispose a plant to senescence [107]. A number of studies indicate that H_2O_2 is synthesized in response to exogenous ABA and that H_2O_2 mediates ABA responses, including stomatal closure [108]. Thereby, the alkalization of the cytoplasm precedes ROS production, which in turn activates Ca^{2+} channels in the plasma membrane of the guard cells. Furthermore, the ABA-induced elevation of the cytosolic Ca^{2+} leads to an upregulation of outward K^+ channels, leading to potassium efflux and a loss of turgor in the guard cells [109]. Moreover, the ascorbate deficient Arabidopsis mutant ***vtc1*** shows the induction of some SAGs and symptoms of premature senescence [110], indicating that oxygen free radicals might be a signal to promote senescence.

There are also some indications of a direct correlation of senescence and mitochondrial respiration. The inactivation of the subunit V of the cytochrome-***c***-oxidase complex in the fungus ***Podospora anserina*** leads to the exclusive use of the alternative respiration pathways and to a decline in ROS formation in

these mutants. This inactivation results in an extraordinary longevity of the fungus [111].

PROSPECTS

Abiotic stress is the primary cause of crop loss worldwide, with the potential to cause a reduction of more than 50% in the average yield of the main crops [103]. Focusing on the detoxification mechanisms of the reactive oxygen species produced during oxidative stress is an adequate approach to produce plants that are tolerant to various stresses [112]. As pointed out above, oxidative stress may provoke an early senescence of individual leaves or whole plants. Vegetables harvested before full adolescence are exposed to enormous stress by the sudden interruption of the energy and nutrient supply. Products, like asparagus and broccoli, show a very fast post-harvest senescence during storage and have a very short shelf life. Many changes observed during the storage of green vegetables, like the loss of chlorophyll, damage to cellular structures and finally cell death, exhibit similarities with the changes during developmental-dependent senescence. It could be shown that genes which are induced during leaf senescence are also expressed in stored broccoli [39]. Moreover, the timing of senescence affects many different agriculturally important traits like the timing of seed set, the number and quality of seeds, and fruit ripening. Increased longevity can have an important impact on human health, as fungi and other micro-organisms thrive on senescing tissue. Therefore, the understanding of senescence mechanisms provides new tools for the further improvement of agricultural plants.

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