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REVIEW ARTICLE

Leaf senescence: an overview

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Abstract Senescence constitutes the last phase of plant growth and is characterized by a series of degenerative events that decrease metabolic activities and cause the death of cells, tissues and organs. Yellowing of leaves is a morphological indicator of senescence, which results due to loss of green pigments, i.e., chlorophylls. Alteration in mitochondrial and thylakoid structure, shrinkage of chloroplast and nucleus are prominent effects observed during senescence. Essential macromolecules like carbohydrates, proteins, lipids, nucleic acids, and photosynthetic pigments such as chlorophyll and carotenoids are degraded during senescence and these degraded constituents play important role in nutrient recycling mechanisms, which are mediated by senescence associated genes (SAGs). Sugar senescing mechanism, down regulation of photosynthetic genes, phytohormones, reactive oxygen species and SAGs play important role in signal transduction pathways to initiate progress and terminate the senescence process. The levels of heavy metals have increased in the environment due to various anthropogenic activities, which produce phytotoxic effects and reduce productivities. This review paper describes various biochemical/metabolic changes associated with leaf senescence.

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Introduction

Senescence in plants is defined as regulated, degenerative and terminal phase in development, which leads to death of single cells, organs or even whole plant during their life cycle (Nooden and Penny 2001). Leaf passes through three different phases of development. During early developmental phase, the leaf is a sink receiving nutrients from the rest of the plant and as soon as it reaches full photosynthetic capacity, it becomes the main source organ of the plant, i.e., the productive phase. After this productive period, the leaf enters the senescence phase, during which most compounds present in it are removed and reused (Hortensteiner and Feller 2002; Buchanan-Wollaston et al. 2003). Leaf senescence is a programmed process as evidenced by genetic studies. Senescence involves a series of cytological and biochemical changes, which cause degradation of macromolecules and remobilization of nutrients from senescing tissues to reproductive, young or even storage tissues. During senescence, there are considerable changes in cell structure, metabolism and gene expression (Barth et al. 2006). Leaf senescence is accelerated due to various environmental stresses such as extreme temperatures, drought, nutrient deficiency, ozone, insufficient light, darkness and pathogen attack (Buchanan-Wollaston 1997). The factors that affect the senescence are shown in Fig. 1.

Prominent symptoms of leaf senescence includes yellowing, which is a reflection of physiological and biochemical changes such as decline in chlorophyll content decrease in photosynthetic activity, degradation of RNA and proteins (Barth et al. 2006). During senescence,

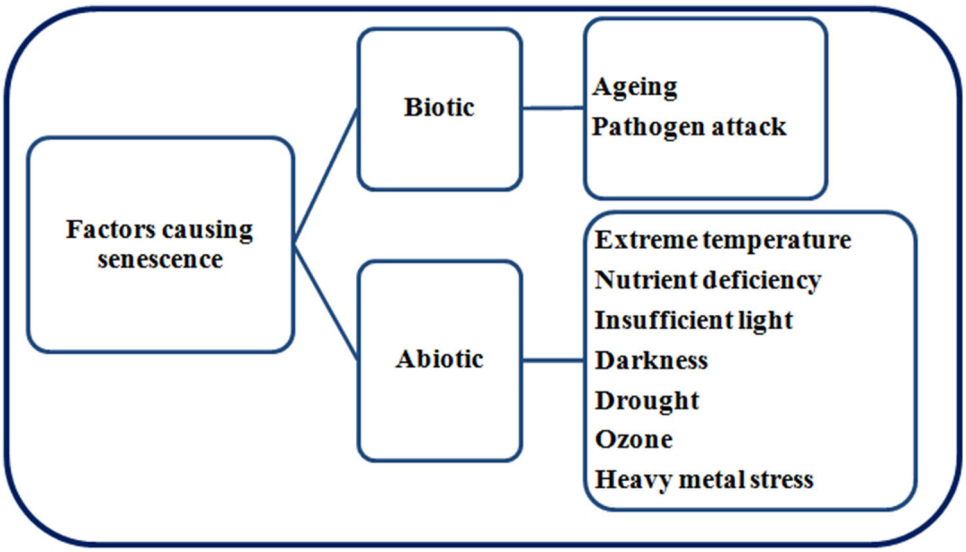

Fig. 1 Factors affecting senescence

anthocyanins are formed in vacuoles of some leaves imparting them red and purple shades. Programmed cell death (PCD) plays an important role in plants mainly during development and differentiation. Formation of xylem elements that die and lose their content in order to conduct water and solutes is an example of PCD in plants. The autolysis of cells in roots, cell death during pollination, embryo development and seed maturation are also related to PCD. According to Barlow (1982) leaf senescence has three criteria, which are necessary for plant PCD process. First, the cells die at a predictable time and location; second, death has some beneficial effect on plant development; and third, cell death is encoded in the hereditary material. However, this suggestion excludes necrotic cell death due to accidental damage or injury as a result of exposure to a toxic environment (Barlow 1982). Programmed cell death (PCD) in plants is described by two ways namely, senescence and cell death associated with hypersensitive response (HR). There are large differences in the rate of senescence in plant systems. Senescence is particularly very rapid in some cases, *e.g.*, flower petals of *Ipomoea tricolor*. In this case, the petals begin senescing just after the flower has opened. In other cases, the senescence takes longer time period for completion. For example, *Pinuslongaeva*leaves have a life span of 45 years.

Genes regulating senescence

Genes, which regulate the senescence process by affecting the system of plant tissues, are called senescence associated genes (SAGs) (Barth et al. 2006). Senescence associated genes (SAGs), transcription factors are mentioned in Table 1. Senescence associated genes identified from various plants includes genes involved in synthesis of protease, protease regulators, 1-amino cyclopropane-1-carboxylase (ACC) oxidase, ribonucleases, glutamine synthetase, lipases, metallothioneins, malate synthase and xanthin dehydrogenase, which are responsible for carbohydrate, lipids, protein and nucleic acid degradation (Barth et al. 2006; Buchanan-Wollaston 1994). More than 30 SAGs have been isolated, cloned and characterized in different plant systems (Hensel et al. 1993; Gan and Amasino 1997), i.e., SAG13, SAG21, early responsive to dehydration (ERD1), blue copper-binding protein (BCB), SAG18, SAG20, ACS6 (ACC synthase), copper chaperone (CCH) gene transcripts, small subunit of rubisco (rbcs), chlorophyll a/b-binding protein (cab), SAG12, SAG19, metallothionein (MT1), ethylene-insensitive 3 (EIN3), glutaminesynthetase (Atgsr2) and terminal flower 1 (TFL1) gene. Terminal flower 1 (TFL1) gene has been used to regulate the length of juvenile period in perennial plants. Some senescence associated genes encode transcription factors of WRKY (having conserved WRKYGQK sequence, followed by one of the two types of zinc finger motifs, the C_2H_2 and C_2–HC types) (Eulgem et al. 2000), NAC, AP_2, and MYB families, which have been used to enhance the leaf senescence by promoting transcription regulation.

The NAC acronym is derived from three genes that were initially discovered to contain a particular domain (the NAC domain): NAM (for no apical meristem), ATAF1 and −2, and CUC2 (for cup-shaped cotyledon) (Souer et al. 1996; Aida et al. 1997). Activating Protein 2 (AP-2) is a family of closely related transcription factors (Williams and Tjian (1991a, b) which plays a critical role in regulating gene expression during early development. MYB proto-oncogene protein is a member of the MYB (myeloblastosis) family of transcription factors. The protein contains three domains, an N-terminal DNA-binding

Table 1 Senescence associted genes and transcription factors

Name of gene/transcription factor	Symbol	Function	References
Red chlorophyll catabolite reductase	*Arabidopsis* RCCR	Chlorophyll degradation	Pruzinska et al. (2007)
Stay green	*Arabidopsis* Sgr1	Chlorophyll degradation	Rodoni et al. (1997)
Terminal flower 1	TLF1	Juvenile period in perrinials	Bergonzi and Albani (2011)
Sucrose non-fermenting related kinase 1	SnRK1	Sugar mediated regulation of ageing	Thelander et al. (2004)
Hexokinase 1	HXK1	Sugar mediated regulation of ageing	Smeekens et al. (2010), Parrott et al. (2007)
Telomerase reverse transcriptase	*Arabidopsis* TERT	Telomere attrition	Watson and Riha (2010)
Rice telomere binding protein 1	RTBP1	Telomere attrition	Hong et al. (2007)
Autophagy genes	ATGs	Cell degradation	Thompson et al. (2005)
Target of Rapamycin	TOR	Growth and development	Blagosklonny and Hall (2009)
Arabidopsis AtMYB2	AtMYB2	Bud outgrowth during monocarpic senescence	Guo and Gan (2011)
Responsive-to-antagonist1	RAN1	Vital for ethylene response pathway	Himelblau and Amasino (2000)
Early responsive to dehydration1	ERD1	Protein degradation, senescence	Weaver et al. (1999)
Senescence associated genes 12	SAG12	Senescence specific, proteolysis	Lohman et al. (1994)
Yellow leaf specific gene	YLS	Nutrient remobilization	Yoshida et al. (2001)
Yellow leaf specific gene3	YLS3	Lipid transfer activity	Yoshida et al. (2001)
Yellow leaf specific gene7	YLS7	Leaf senescence-related protein	Yoshida et al. (2001)
	SARK	Intiate senescence	Hajouj et al. (2000)
Pheophorbide A oxygenase	PAO	Positive regulator in leaf senescence	Sakuraba et al. (2012)
Chitinase	CHI	Senescence-enhanced gene	Masclaux-Daubresse et al. (2006)
Organic cation/carnitine transporter1	OCT1	Strong senescence up-regulation	Brusslan et al. (2012)
Hydroxymethylglutaryl COA reductase1	HMG1	Negative regulator in leaf senescence	Suzuki et al. (2004)
Cytokinin receptor1/Arabidopsis histidine kinase4	CRE1/AHK4	Programmed cell death	Vescovi et al. (2012)
CCH copper chaperone	CCH	Role in metal remobilization during senescence	Himelblau et al. (1998)
Blue copper-binding protein	BCB	Role in metal remobilization during senescence	Van Gysel et al. 1993
Bifunctional nuclease1	BFN1	Nucleic acid degradation during senescence	Buchanan-Wollaston et al. (2005)
1-aminocyclopropane-1carbocylic acid (ACC) synthase	ACS6	Senescence	Vahala et al. (1998)
Glutamine synthetase (cytosol)	Atgsr2	Nitrogen mobilization	Bernhard and Matile (1994)
Metallothionein	MT1	Scavenging free ions and remobilization of nutrients	Lohman et al. (1994)
Soybean (Glycine max) senescence-associated receptor-like kinase	GmSARK	Leaf senescence	Xu et al. (2011)
Senescence-associated ubiquitin ligase1	SAUL1	Negative regulator of plant senescence	Raab et al. (2009)
Beta glucosidase 16	BGLU16	Leaf senescence associated genes	Graaff et al. (2006)
MAP Kinase 6	MPK6	Positive regulator of leaf senescence	Zhou et al. (2009)
Transcription factors			
NAC (NAM, ATAF and CUC)	NAC family	Control organ development and response to pathogens	Souer et al. (1996), Aida et al. (1997)
NAC-like activated by AP3	AtNAP	Delay leaf senescence	Guo and Gan (2006)
VND-interacting 2	VNI2	Negative regulator of leaf senescence	Yang et al. (2011)
ORESARA1	ORE1	Regulator of leaf senescence	Kim et al. (2009)
No apical meristem	NAM-B1	Positive role in leaf senescence	Uauy et al. (2006)

Table 1 continued

Name of gene/transcription factor	Symbol	Function	References
Jungbrunnen1	JUB1	Negative regulator of leaf senescence	Wu et al. (2012)
WRKY transcription factor genes			
	WRKY53	Leaf senescence	Miao et al. (2004)
	WRKY6	Leaf senescence	Miao et al. (2004)
Zn fingerfamily			
Solanumlycopersicum Zinc Finger2	SlZF2	Negative role in leaf senescence	Hichri et al. (2014)
MYB family			
Myeloblastosis-related protein R1/Myb-related protein 44	MYBR1/ MYB44	Negative role in leaf senescence	Jaradat et al. (2013)
Apetala2/Ethylene response factor	AP2/ERF family		
Glycine max related to ABI3 and VP1	GmRAV	Positive role in leaf senescence	Zhao et al. (2008)
Related to ABI3 and VP1	RAV1	Positive role	Woo et al. (2010)
Ethylene response DNA binding protein1	EDF1	Negative role	Chen et al. (2011)
Ethylene response DNA binding protein2	EDF2	Negative role	Chen et al. (2011)
Cytokinin response factor6	CRF6	Negative role	Zwack et al. (2013)
Ethylene-insensitive3	EIN3	Accelerates age-dependent leaf senescence	Zhonghai et al. (2013)

domain, a central transcriptional activation domain and a C-terminal domain involved in transcriptional repression. This protein plays an essential role in the regulation of hematopoiesis and may play a role in tumorigenesis, including the regulation of miR-155 in B-cells (Vargova et al. 2011). MYB factors represent a family of proteins that include the conserved MYB DNA-binding domain. Plants contain a MYB-protein subfamily which ischaracterized by the R2R3-type MYB domain (Stracke et al. 2001). A schematic representation of genes and transcription factors involved in senescence, and abiotic and biotic stresses responses in plants is presented in Fig. 2.

For example, in *Arabidopsis*, specific WRKY protein (plant specific Zinc-finger type transcription factor) regulates the gene expression during senescence (Miao et al. 2004; Guo and Gan 2006). Genetic variants, which help to delay senescence, are called as "stay-green" mutants. They have proved useful in elucidation of mechanisms associated with leaf senescence. With the help of molecular biological techniques, a large range of cDNA clones representing genes have been isolated which increase the expression of senescing leaves. Genes of degradative enzymes like proteases and nucleases, enzymes of lipid and carbohydrate metabolism, and enzymes which participate in nitrogen mobilization are characterized as senescence enhanced genes. It means that several internal and external signals are known to induce the senescence (Buchanan-Wollaston 1997).

Abiotic stresses and senescence

Plants experience an array of biotic and abiotic stresses during their life cycle. Common abiotic stresses that adversely affect the plant growth and productivity include drought, temperature changes, soil salinity and heavy metals. Internal factors such as reproductive structures also influence the rate of leaf senescence. Earliest responses of plant cells under abiotic stress condition are generation of reactive oxygen species (ROS) and senescence. Chloroplasts are the main targets of ROS-linked damage during various environmental stresses and natural senescence, as ROS detoxification systems decline with age. These stresses affect the crop yield and growth rate by inducing changes in gene expression and cellular metabolism. Plant responses to various stresses depend upon the duration, severity, developmental stage, genotype and intensity of stress. Different abiotic stresses have been reported to influence the patterns and magnitude of senescence of plant organs including leaves. Suppression of blue radiation in shaded wheat (*Triticumaestivum* L.) leaves triggered senescence, and leads to increase in oxidative stress. Blue radiation altered the catalase (CAT) activity both at the transcriptional and post-transcriptional levels (Causin et al. 2015). Melatonin is an antioxidant in plants, which is actively involved in extending the longevity and enhancing abiotic stress resistance. Melatonin significantly reduced chlorophyll degradation, suppressed the transcripts of

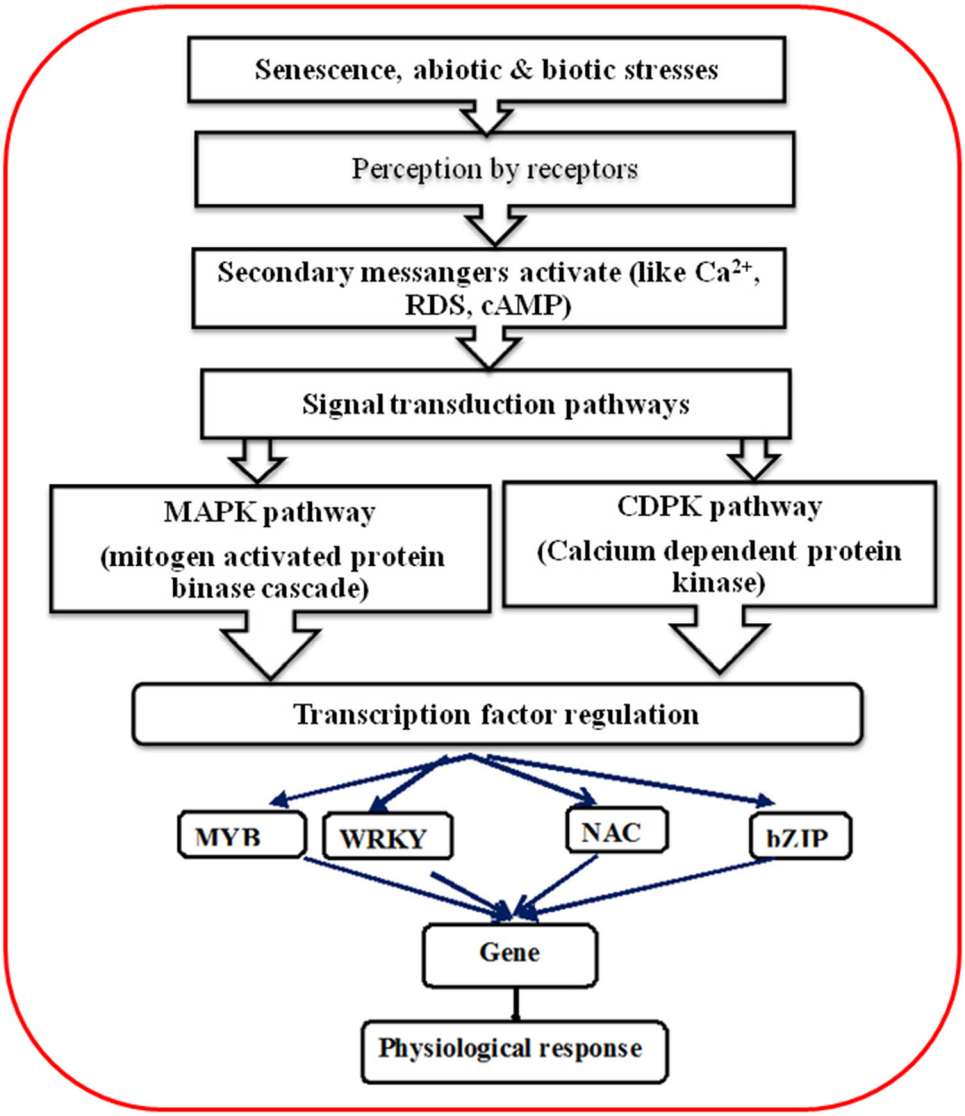

Fig. 2 Signal transduction pathways during senescence, and abiotic and biotic stresses

senescence-associated genes, delayed the leaf senescence and enhanced salt stress tolerance by directly or indirectly counteracting the cellular accumulation of H_2O_2 (Chu et al. 2015).

Temperature extremes also affect various biochemical and physiological traits in plants. When sunflower (*Helianthus annuus* L.) plants were grown under control or at high temperature for consecutive days, the plants exposed to high temperature exhibited stunted growth and reduced leaf area and decrease in soluble protein content as compared to control (Zhang et al. 2012). High temperature also reduces net photosynthetic rate by decreasing the content of photosynthetic pigments, stomatal conductance, activity of nitrate reductase and glutamine synthetase, while deaminating activity of glutamate dehydrogenase increased (Lam et al. 1996; Lea and Miflin 2003). High temperature induced early senescence in sunflower leaves was reported to be due to the accumulation of soluble sugars and the decrease in starch level (Buchanan-Wollaston et al. 2003; Lim et al. 2007). Elevated level of H_2O_2 accumulation resulted in oxidative damage and a decline in antioxidant activity as well as accelerated senescence of primary leaves at high temperature (De la Haba et al. 2014). High temperature induced early senescence in sunflower leaves was reported to be due to the accumulation of soluble sugars and the decrease in starch level (Srivastava et al. 2012)

Heavy metals adversely affect the rate of photosynthesis and photosystem II activity. Lead ions caused increase in O_2 uptake in dark-treated leaves and increased malondialdehyde content, an indicator of lipid peroxidation (Parys et al. 2014). Exposure of soyabean to different concentrations of cadmium caused severe reduction in photosynthetic pigments, increase in lipid peroxidation and

hydrogen peroxide levels, a oxidative stress response in leaves, increase the activities of superoxide dismutase, catalase and alteration in glutathione content (Hashem 2014).

Biochemical/metabolic changes associated with leaf senescence

Degradation of chloroplasts

The chloroplasts in green leaves are the first and major target of senescence (Biswal and Biswal 1988; Nooden et al. 1997). Both qualitative and quantitative changes occur in the pigments, macromolecules, molecular structures, thylakoid organization and enzymes participating in the CO_2 fixation in stroma of chloroplasts (Biswal and Biswal 1999; Biswal et al. 1994). The leaf proteins, mainly the photosynthetic proteins of plastids are highly decreased during senescence. Chloroplasts have active proteases which help to break the DI and LHCII proteins of photosystem II. The vacuoles also appear to participate in senescence. In senesced leaves, the senescence associated vacuoles (SAVs) have high proteolytic activity (Martinez et al. 2008). Senescence associated vacuoles have been detected in soybean, *Arabidopsis* and tobacco. The number of SAVs increased as the senescence progresses, reaching relatively large numbers (40–60 SAVs per cell in a 1 μm thick optical section) during the period when chloroplast proteins are lost intensively. Senescence associated vacuoles have been detected in chloroplast-containing cells, i.e., mesophyll and guard cells but absent in the rest of the epidermis. They are acidic and contain vacuolar H^+pyrophosphatase in their limiting membrane and have high peptidase activity due to cysteine-proteases (Carrion et al. 2013). In the senescing leaves both the PSI and PSII are affected (Biswal 1997). Microarray technology has been used to study the role of 800 genes in case of senescence in *Arabidopsis* (Guo and Gan 2006; Lim et al. 2007; Gregersen et al. 2008). Chloroplast is the major site for protein degradation, and ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) is known to be rapidly and selectively degraded during senescence and stress (Khanna-Chopra 2012). The processes of protein degradation are initiated by reactive oxygen species (ROS) and have active participation of proteolytic enzymes, such as cysteine and serine proteases (Gregersen et al. 2008). During senescence, the breakdown of chloroplasts results in disorganization of the thylakoid membranes with a decrease in grana stacking and dilation of thylakoids (Guiamet and Luquez 2005; Gepstein 1988). Chloroplast structure becomes completely disrupted at late stages of senescence. Structural changes in thylakoids include gradation of photosystem proteins, thylakoid galactolipids and loss of chlorophylls, which result in unbalanced photosynthetic electron transport (Krupinska and Humbeck 2004). The stromal proteins of plastids, including rubisco and other components of the C_3 photosynthetic pathway, glutamine synthetase II and even sulfur assimilating enzymes are also degraded(Schmutz et al. 1983). The rate of degradation of stromal and thylakoid proteins and chlorophyll may vary which helps to adjust the remaining photosynthetic capacity of a leaf during senescence and environmental stresses (Hortensteiner and Krautler 2011).

Reactive oxygen species causes deterioration of membrane lipids, which results in leakage of solutes and ions from membrane. Malondialdehyde (MDA) is a degradation product of polyunsaturated fatty acids, and it is used as a biomarker for lipid peroxidation (Dai et al. 2011). With advancement of leaf senescence, MDA contents increases gradually. Leaf senescence initiate from lower internodes and move towards the upper internodes after flowering of plants. Yielding varieties are negatively associated with MDA accumulations at the later stage of growth. The effective inhibition of leaf senescence or prolonging the functional period of leaves at the late growing stage plays an important role in raising yield (Feng et al. 2013).

Effect of senescence on pigment metabolism

Symptoms of senescence are first seen in chloroplast; however, till end it retains some level of organization when all other organelles are completely damaged (Biswal and Biswal 1988). During senescence the chloroplast of green leaves changed to gernotoplast (senescing chloroplast), which gradually loses the biosynthetic potential. Destacking of thylakoid membrane and accumulation of plastoglobuli occurs during early stages of senescence. As the size of plastoglobuli increased, there is decrease in thylakoid membrane (Thomas et al. 2003). During senescence chlorophyll is converted to chlorophyllide in the presence of enzyme chlorophyllase, which is also responsible for phytol formation and accumulation of lipid globules of gerontoplasts in the form of esters. Degradation of chlorophyll in leaves unmasked the underlying carotenoids, thus imparting yellow or orange colorations. Degradation of chloroplast pigments occurs by vacuolar enzymes. The pigments and compounds, which accumulate in the senescing cells, are produced from phenylpropanoid metabolism. Phenylpropanoid metabolism produces phytochemicals, such as phenols, tannins, flavonoids and lignins. Factors like ethylene treatment, O_3, pathogen attack alter the synthesis of phenolic compounds (Dixon and Paiva 1995). Degradation process of chloroplast and protein is shown in Fig. 3.

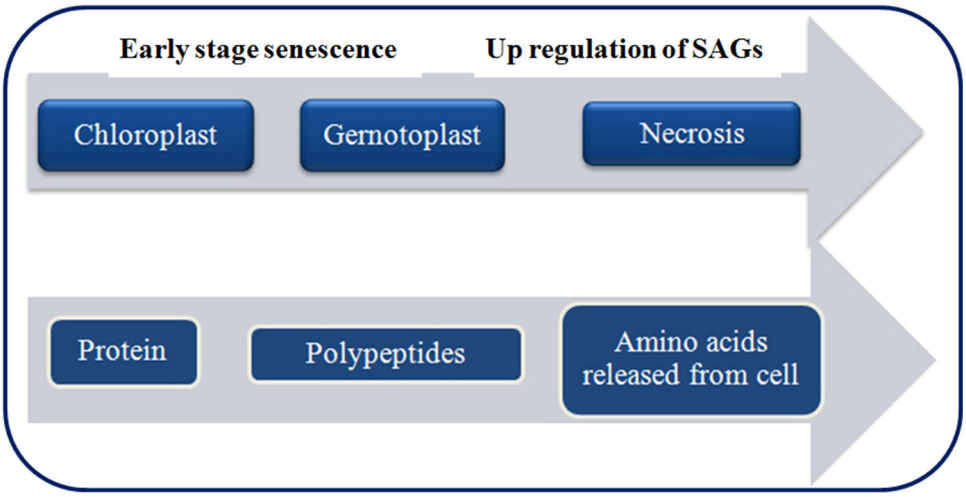

Fig. 3 Degradation of chloroplast and proteins during senescence

The genes involved in gluconeogenesis and chlorophyll breakdown are related to senescence enhanced cDNAs (Smart et al. 2006). In artificially induced senescence of barley, the gene expression was studied by in vitro translation and mRNA hybridization with cDNA clones. There was a rapid chlorophyll loss when detached barley leaves were incubated in dark. Six copy DNA (cDNA) clones were derived from three different transcripts, which were classified according to the expression of mRNAs in dark incubated barley leaves. Two of the three transcript showed similar expression in detached leaves, the transcripts were induced by abscisic acid and inhibited by kinetin. They were also induced by osmotic stress and injuries but could not detect in naturally senesced leaves. Only one mRNA was detected in naturally senescenced leaves and it concluded that other two related to stress (Becker and Apel 1993).

Organic nitrogen and sulphur transportation

The breakdown of proteins during leaf senescence leads to the release of amides like glutamine and asparagines, which play an important role in export of organic nitrogen (Buchanan-Wollaston 1997). Proteins and amino acids are the important sources of mobilization of not only nitrogen but also sulphur, which is translocated predominately in the form of homoglutathione. Ubiquitin serves as a molecular tag, which marks the protein for degradation. The expression of glutamine synthetase (GS1), responsible for the synthesis of glutamine has been studied during the senescence of radish cotyledons. Three cDNA clones for GS1 enzyme have been characterized and two of them namely, Gln 1:1 and Gln 1:3 were expressed both during natural and dark-induced senescence (Watanabe et al. 1994). During leaf senescence there is degradation of proteins and recycling of nitrogen to developing grain and leaves. Protein degradation is marked by an oxidative modification, i.e., carbonylation (Leitao et al. 2015). A fluorescence-based method has been used to detect protein carbonyl derivatives by using fluorescein-5-thiosemicarbazide (FTC) as a probe for detecting protein carbonyl derivatives in flag leaf of winter wheat during natural senescence. It was the best way to measure qualitative and quantitative modifications in protein carbonyl levels during the last developmental stages of wheat flag leaves. There was increase inprotein carbonylation and decrease in protein content duringstimulation of endoproteolytic activity in senescing leaf. It supports the relationship between protein oxidation and proteolysis during natural leaf senescence (Leitao et al. 2015).

Synthesis of sugars and secondary metabolites, and their transportation

During senescence the photosynthetic activity decreases that cause sugar starvation. This triggers the conversion of lipids released by thylakoid breakdown into sugars by gluconeogenesis (Kim and Smith 1994a, b). Sugars are important factors in the regulation of plant metabolism and development. They are known to accumulate during stress and leaf senescence. Herbivorous attack, bacterial and fungal pathogen infection could influence the leaf senescence by activating sugar starvation by directly affecting the primary carbon metabolism or by regulating the status of plant hormones (Buchanan-Wollaston 1997). The interaction between sugars and biotic and abiotic stresses leads to the induction of senescence. For example, the biotic interaction induces the extracellular invertase activity, and in turn the enzyme associated with phloem unloading pathway increases the hexose/sucrose ratio (Wingler and Roitsch 2008).

During leaf senescence, the nucleic acids get broken down to the constituent purines and pyrimidines, which are further, degraded to low molecular weight transportable forms of carbon and nitrogen compounds. Flavonoids and phenylpropanoids are carbon-based secondary metabolites. Their concentrations fluctuate in response to high-light stress, especially UV-B light, and biotic stress, during the defense against herbivores and pathogens (Agati and Tattini 2010; Løvdal et al. 2010; Samanta et al. 2011; Wu et al. 2012) or abiotic stresses such as mineral nutrient depletion (Watanabe et al. 2010). Before chlorophyll breakdown there is accumulation of anthocyanin in senescing leaves (Field et al. 2001). This is a protective mechanism, since the absence of chlorophyll affects the susceptibility to light-induced reactive oxygen damage in leaf cells caused by decreasing the photosynthetic capacity (Nooden et al. 1996).Anthocyanins are a group of water-soluble flavonoids (glycosides of phenolic aglycons with flavan C6-C3-C6 skeleton) which produced in the cytoplasm and transported to the vacuole (Shirley 1996) In addition to the important roles of flavonoids and phenylpropanoids under light stress, nutrient conditions such as nitrogen, sulfur and phosphorus deficiency as well as high-carbon stress can also cause anthocyanin synthesis (Bonguebartelsman and Phillips 1995; Park et al. 2007). Many studies concerning the relationship between nitrogen and the rate of CO_2 assimilation and photosynthesis have suggested that anthocyanin accumulation is a metabolic marker for carbon/nitrogen imbalance under stress conditions (Zheng et al. 2009).

Role of plant hormones in senescence

Plant hormones play important regulatory roles in either promoting or delaying the leaf senescence. For example ethylene, abscisic acid and jasmonic acid are regarded as promoters of senescence, whereas cytokinins (CK) delay the senescence. Up or down regulation of genes associated with various hormones showed differential expression during senescence (Thomas and Stoddart 1980). Major role of cytokinin is as senescence-delaying hormone. During leaf senescence the endogenous CK level declined, while exogenous application of CK delayed senescence in a large variety of monocotyledonous and dicotyledonous species (Hwang et al. 2012). Transcription of genes, which participate in CK biosynthesis and signaling was repressed, while the activity of enzymecytokinin oxidase, which is responsible for CK degradation was elevated during senescence (Buchanan-Wollaston et al. 2005). Over expression of isopentyl-transferase (IPT) under control of a senescence-inducible promoter in tobacco (*Nicotianatabacum*) caused the leaf longevity and delayed senescence (Gan and Amasino 1995). Similar results have been reported in case of other plants like lettuce (*Lactuca sativa*) (McCabe et al. 2001), broccoli (*Brassica oleracea* var. *italica*) and bokchoy (*Brassica rapachinensis*) (Chen et al. 2001; Yuan et al. 2002). Cytokinins (CKs) signaling utilizes three histidine protein kinases, i.e., *AHK2*, *AHK3*, and *AHK4*, which act as CK receptors in *Arabidopsis* (Higuchi et al. 2004). After CK binding, the receptors autophosphorylate and transfer CK signals to nuclear localized *Arabidopsis* response regulators (ARRs) by histidine phosphate transfer proteins, which regulates transcription of CK target genes (Hwang et al. 2012). Cytokinin receptor *AHK3* also functions as an important component for leaf longevity. Gain of function mutation of *AHK3* delayed leaf senescence, while a loss-of-function mutation in *AHK3* reduced the sensitivity to CK in leaf senescence assays (Kim et al. 2006). *AHK3*-mediated regulation of senescence depends on its phosphorylation activity. The extracellular invertase, an enzyme involved in the apoplastic phloem unloading pathway was helpful in mediating CK-induced leaf longevity (Lim et al. 2007).

The *ipt* gene has been isolated from *Agrobacterium tumefaciens* that encodes the enzyme isopentenyltransferase (Akiyoshi et al. 1984). It has an important role in cytokinin biosynthesis. Gene *Ipt*has been employed to generate transgenic plants for overproduction of cytokinins (Chang et al. 2003). Kinetin strongly retarded leaf senescence, which was influenced by naphthalene acetic acid. Response towards kinetin was more in young leaves than in old (Abrams and Pratt 1966). Senescence is delayed in those parts of plant where the concentration of cytokinins is high. Cytokinins counter the apical dominance induced by auxins; and in conjunction with ethylene promote abscission of leaves, flower parts and fruits (Deborah and John 1983). Stresses induce common responses such as enhancement of plant hormones. For example, wounding can induce production of ethylene, auxin and ABA, i.e., plant stress hormones.

Biologically active auxins are indole acetic acid (IAA) and indole butyric acid (IBA), which are synthesized from tryptophan (Kelley and Estelle 2012). SAUR36, auxin inducible gene is highly upregulated in its expression in senescing leaves. Loss of SAUR36 activity results in delayed senescence and gain in its activity leads to premature leaf senescence. It shows that SAUR36 is a positive regulator of senescence (Hou et al. 2013). Effects of auxin on senescence are similar to those of gibberellic acid (GA) and in most cases, treatment with exogenous auxins delayed senescence. Auxins are known to promote the production of other hormones, and in conjunction with cytokinins they control the growth of stems, roots and fruits (Daphne and Michael 2005). Leaf abscission initiated by the growing point of a plant ceasing to produce auxins, and they regulate protein synthesis in seeds (Alexander et al.

2002). Auxins are toxic to plants in large concentrations; they are more toxic to dicots than monocots.

Senescence-associated receptor-like kinase,GmSARK from soybean, was identified as another positive regulator of leaf senescence. Arabidopsis plants expressing GmSARK under control of a glucocorticoid-inducible promoter showed acceleration in leaf senescence, while impairment in the expression of a GmSARK delayed the leaf senescence and its expression was found to be auxin-inducible (Xu et al. 2011). Flavin-containing mono-oxygenases are a family of enzymes that catalyzes the rate-limiting step in auxin biosynthesis and are encoded by the YUCCA gene family in *Arabidopsis*. Interestingly, an overexpression of YUCCA6 in the dominant yuc6-1D mutant led to increased levels of free IAA, and also caused delayed dark-induced senescence (Kim et al. 2011). When detached leaf assays was performed in the dark, it was observed that yuc6-1D delayed dark-induced senescence, which correlated with elevated auxin levels and a reduced expression of the senescence marker gene SAG12 (Kim et al. 2011). It thus indicates that auxin delayed dark-induced leaf senescence.

Ethylene is produced at a faster rate in rapidly growing and dividing cells especially in darkness. Ethylene is considered as the strongest promoter of senescence (Gepstein and Thimann 1981). In most of the plant species exogenous ethylene induces senescence in leaves, flower and fruits (Wang et al. 2007). Ethylene is involved in the regulation of detached rice leaf senescence, which proceeds by chlorophyll degradation. But various treatments such as light, cycloheximide, α, α-dipyridyl, Ni^{2+} and cold temperature retarded the chlorophyll degradation and inhibited ethylene production (Kao and Yang 1983). In yellow leaves, there is a decrease in the activity of photosynthesis related genes and increase in SAGs. The concentration of endogenous ethylene is related to leaf senescence. As the leaf becomes older or is induced to senescence by dark treatment, increase in ethylene production is observed. *Arabidopsis etr1* mutant and tomato *never ripe mutant* are insensitive to ethylene (Lanahan et al. 1994). These plants showed delay in leaf senescence. 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase are two enzymes that play important role in ethylene biosynthesis (Kao and Yang 1983; Wang and Shang 2004). The nuclear protein *ethylene insensitive 2* (*EIN2*) is regulated by ethylene, and enhanced production of other hormones, including abscisic acid (ABA) and other stress hormones (Wang et al. 2007). Indole acetic acid on the other hand repressed the effect of ACC (1-amino cyclo propane-1-carboxylic acid) and ethylene on oat leaf senescence (Gepstein and Thimann 1981).

Role of abscisic acid (ABA) in the regulation of leaf senescence has been established and well documented (Zeevaart and Creelman 1988). In plants under water stress condition, ABA plays important role in closing of stomata (Ren et al. 2007). Abscisic acid exists in all parts of the plant and its concentration within any tissue seems to mediate its effect, and as a hormone its degradation or catabolism within the plant affects metabolic reactions, cellular growth and production of other hormones. Abscisic acid is involved in diverse physiological processes like stomatal closure, embryo morphogenesis, synthesis of storage proteins and lipids, and most prominently in leaf senescence. Exogenous application of ABA is associated with increased plant adaptive responses to various environmental conditions. Addition of ABA (5 mg l^{-1}) to callus of *Croterostigmaplantagineum* (resurrection plant) induced tolerance to desiccation (Bartels et al. 1990). Though exogenous ABA generally causes yellowing of leaves, but there is not any definite correlation between the concentrations of endogenous ABA and tissue senescence. It is suggested that ABA affects the senescence through its action with other growth regulators. Interaction between sugar and hormone signaling also plays a role in response to abiotic stresses. For example, interaction between sugar and ABA signaling is responsible for induction of leaf senescence during drought stress. While cold treatment delayed senescence despite sugar and ABA accumulation (Wingler and Roitsch 2008). Effect of ABA is light-dependent and in the presence of light it delayed protein break down but could not prevent yellowing effect (Rao and Khan 1983). Abscisic acid and jasmonate showed chemical and physiological similarities. Methyl jasmonate (MeJA) induced senescence in detached rice leaves was mediated through an increase in endogenous ABA levels (Wang and Kao 2004).

Interrelations between the effects of cytokinin and gibberellic acid (GA) in retarding leaf senescence have been investigated. Leaf discs from plants of *Taraxacum, Rumex* and *Tropaeolum*were floated on solutions of cytokinin and GA to which ABA was added. After 5 days, ABA was found to reduce the senescence retarding effect of GA more than cytokinin. Higher concentration of cytokinin nullified senescence enhancing effect of ABA but it was not possible in case of GA (Back and Richmond 1971). Gibberellins (GAs) promote seed germination; stem elongation, flowering, cone production and retards leaf and fruit senescence. Gibberellins have been reported to antagonize ABA because they reversed the inhibition of shoot growth and dormancy induced by ABA (Tsai et al. 1997).

Polyamines are considered as leaf senescence inhibitors (Kaur-Sawhney and Galston 1991). The unpollinated ovaries of tomato, which underwent senescence have high polyamine content. In short, the exogenous hormones like cytokinin, GA and auxin retard senescence, whereas

ethylene, ABA and jasmonic acid speed it up (Mayak and Halevy 1972; Lamattina et al. 1987; Creelman and Mullet 1997). Jasmonic acid (JA) is an oxylipin, one of the important signaling molecules in plants. It affects number of processes in plants, like biotic and abiotic stresses, seed germination, flower development, fruit ripening and embryogenesis. First evidence for a senescence-promoting role of JA came from the observation, where a compound isolated from wormwood (*Arthemisiaabsinthum*) caused rapid chlorophyll loss in oat (*Avena sativa*). This compound was identified as methyl jasmonate (MeJA), a volatile JA derivative, and further research showed that application of external MeJA induces leaf senescence in a number of plant species. Jasmonic acid induced the expression of several key enzymes involved in chlorophyll breakdown (Reinbothe et al. 2009).

Phenolic compound salicylic acid (SA) is not only known for triggering defense reactions against biotrophic pathogens but also play role in leaf senescence. It has been reported that SA levels increased approximately fourfold in senescing leaves, and SA controls the expression of the WRKY transcription factors like WRKY53, -54, and -70 that are involved in regulation of senescence (Morris et al. 2000; Besseau et al. 2012).

Conclusion

Senescence is a highly complex process, which includes expression of multiple genes and signaling pathways that integrates age information, and various endogenous and exogenous signals. Many senescence associated genes have been isolated but the exact mechanism of gene regulation and expression is not clearly known. Different signal transduction pathways increase the complexity, and it needs further research. Complete analysis of hormonal mechanism will help to increase the shelf life of fruits, vegetables and crops. Many nutrients like amino acids, amides, sugars, minerals are released during senescence, which are beneficial for the growth and development of newly growing parts of plant. Shoot senescence is a best mechanism of plants perennation under unfavorable conditions. Senescence reduces the transpiration rate, which increases the survivability of plant under adverse conditions. Senesced plant parts are used as important source of minerals and humus to increase soil fertility. Excessive use of pesticide and insecticide causes premature senescence due to inculcation of harmful heavy metals into environment. Pollution is also a major cause of abiotic stress and senescence, which reduces the productivity and yield of crops.

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