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SENESCENCE ASSOCIATED PROTEASES: THE KEY PLAYERS IN PLANT SENESCENCE

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ABSTRACT

Senescence is the final stage of the plant development which leads to cell death. This process consists of a highly regulated, ordered series of events involving loss of photosynthetic capability, breakdown of proteins, loss of chlorophyll, disintegration of chloroplasts and export of all solubilised nutrients. The regulation and control of gene expression of senescence is governed by a set of Senescence Associated Genes (SAGs). They encode the enzymes which degrade the biomolecules during the leaf senescence and thus strictly associated with senescence in different plants. The enzymes involved in protein degradation for recycling of peptides are known as proteases. There are various categories of reported proteases, include cysteine, serine, aspartic, threonine and metalloprotease. Most of the proteases undergo the processing for their maturation and then activate the senescence process. The multiple roles of these proteases in plant defense include hypersensitive response (HR), a form of programmed cell death (PCD) that is associated with resistance to pathogens. Cysteine proteases are a major family of proteases which has been found to be associated with senescence thus playing a key role in plant defense and development.

INTRODUCTION

Supply of food remained always a concern to meet the hunger of the growing population. Development of various new high yielding varieties and improved cultivation practices has increased the food production in world but it is still insufficient to meet the demands of increasing population (Fischer *et al.*, 2000; Sasaki and Burr, 2000). So there is an urgent need to increase the yield from the fixed land area without degrading environment. One avenue for yield increase is to delay leaf senescence as a means of extending the available time for photosynthesis (Liu *et al.*, 2010; Zhang *et al.*, 2010). Transcriptomic studies have been used for identification of genes involved in leaf senescence but still the gene expression profile of rice in senescence is under investigation. Therefore, genome-wide identification of leaf senescence genes is important for understanding the mechanisms underlying senescence which can be helpful for improving stress resistance, pathogen defense and yield increase. Thus, studying leaf senescence will not only improve our understanding of a fundamental biological process, but may also may provide way to control leaf senescence which will further help to improve agricultural traits of crop plants.

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Senescence

Senescence is the last stage of plant development and is a complex, developmental phase in the life of a leaf that results in the co-ordinated degradation of macromolecules and the subsequent mobilization of components to other parts of the plant and is critical for plants fitness as massive mobilization of nitrogen, carbon and minerals from the mature leaf to reproducing seeds occurs through this process (Lim *et al.*, 2007; Fischer, 2012). Senescence of the leaves is associated with sequential programmed death of matured cells, tissues, organs or sometimes the entire plant (Liu *et al.*, 2008; Gan, 2014). The senescence in perennial evergreen plants is a continuous process in which the older leaves undergo this programmed death. In contrast, annual plant like rice undergo a process known as reproductive senescence during which the entire plant under goes this death process after completion of the reproductive phase of life (Fig. 1).

Senescence and its association with plant development

Senescence is a highly regulated process involving photosynthetic decline, chloroplast degeneration and protein degradation, chlorophyll degradation, lipid peroxidation, deduction of amino acids and reduction of total RNA due to which expression of many genes is switched off (Bate *et al.*, 1991; Hensel *et al.*, 1993; Jing and Nam, 2012). It regulates

the expression of a set of genes associated with the various developmental phases of plant such as seedlings, mature/young leaves, old leaves, either they are retarding the growth of early tissues or targeting other genes involving in phosynthetic process mainly RUBISCO (Hanaoka *et al.,* 2002). Leaf yellowing is the first symptom of cell death, in which chlorophyll and chlorophyll-protein complexes start to degrade and show senescence (Lim *et al.,* 2007) (Fig. 1). Process of senescence is natural but influenced by several external and internal stimuli. The nutritional status of soil, temperature, light, humidity, water availability, soil, ionic concentrations, pest and pathogen invasion affect the process of senescence (Lim *et al.,* 2007; Liu *et al.*, 2008). Both under natural and stressed condition, process of senescence is regulated mostly at transcriptional level. The hormones play critical role in transcriptional reprogramming for controlled cell death during senescence. Plant hormones, prolonged darkness, age, seasonal responses and biotic/abiotic stresses are some of the external and internal factors which can induce senescence while changing various factors such as plant phytohormones, photosynthetic efficiency or level of metabolite can regulate senescence by delaying it before it reaches the critical level (Liu *et al.,* 2008). Delayed leaf senescence, or stay-green is related with the high photosynthetic capacity and increased yield (Thomas and Howarth, 2000; Zhang *et al.,* 2010). In addition to internal age-dependent factors, external factors such as auxin, cytokinin and gibberellin (GA1/3) delay leaf senescence, while salicylic acid, jasmonic acid, abscisic acid (ABA) and ethylene speed up leaf senescence. Addition of nitrogen to the growth media or direct spraying of leaves with cytokinins (CKs) at the seeding stage proved to be useful for delaying senescence (Gan, 1995). However, with the advent of new biotechnological developments and more efficient molecular tools, CK has been utilized to delay leaf senescence (Gan and Amasino, 1995). Over-expression of cytokinin has been reported to be a very successful technique to delay senescence in several plants including tobacco, maize, *Arabidopsis*, lettuce, petunia etc. (McCabe *et al.,* 2001; Masferrer *et al.,* 2002; Chang *et al.,* 2003; Robson *et al.,* 2004; Pineda Rodo *et al.,* 2008; Rivero *et al.,* 2010). CKs control various processes such as cell division and differentiation, nutrient mobilization, and plant growth and CKs can act to inhibit the transcription of Cysteine proteases, delaying degradation of nucleic acids and proteins (Sakakibara *et al.,* 2005; Werner, 2006). Auxin is another hormone that negatively regulates senescence both in cytokinin dependent and independent manner (Ellis *et al.,* 2005; Okushima *et al.,* 2005; Guilfoyle and Hagen, 2007; Lim *et al.,* 2010). Ethylene hormone function has been mostly associated as positive regulator for both natural and stress induced senescence. Ethylene concentration increases during senescence and the mutants that are defective in biosynthesis or signal transduction of ethylene are delayed in senescence (Gepstein and Thimann, 1981; Agarwal *et al.,* 2012; Wang *et al.,* 2013). Temperature, shading and pathogen attack can activate leaf senescence as well (Ohet *et al.,* 1996; He *et al.,* 2005).

Leaf senescence is one of the key stages of plant development and its understanding is very important for biomass production. It is a highly complex but ordered process involving expression of large scale senescence associated genes, and its molecular mechanisms still remain unclear. Major breakthroughs in the molecular understanding of leaf senescence were achieved through characterization of various senescence mutants and senescence-associated genes revealing the nature of regulatory factors and a highly complex molecular regulatory network underlying leaf senescence (Liu *et al.,* 2008). Delayed leaf senescence, or stay-green is related with the high photosynthetic capacity and increased yield (Zhang *et al.,* 2010; Thomas, 2013). In rice, membrane localized short chain dehydrogenase/reductase (*NYC1, NOL*) is responsible for chlorophyll degradation while mutants (*nol-1/2, nyc-1*) show 'stay-green' phenotypes (Sato *et al.,* 2009). The knockout insertion mutants in *Arabidopsis thaliana L* speed up senescence and (reduce tolerance to abiotic stress) but still pathway is not clear, it might be due to mutation in transcriptional regulators like HSFB1 for hsp70 (Breeze *et al.,* 2008). AtNAP, a NAC family N-termini of the petunia NAM (NO APICAL MERISTEM) and *Arabidopsis* ATAF1 and CUC2 (CUP-SHAPED COTYLEDON2) transcription factor, has an important role in leaf senescence (Guo and Gan, 2006). Similarly, a rice NAC gene has been reported to be involved in the regulation of leaf senescence (Zhou *et al.,* 2013; Liang *et al.,* 2014).

Senescence associated genes (SAGs)

The process of senescence is coupled with transcriptional reprogramming, during which expressions of many genes are switched off (Bate, 1991; Hensel, 1993; Guo, 2013). Expression of genes associated with the various developmental phases of plant such as seedlings, mature/young leaves, old leaves, either they are retarding the growth of early tissues or targeting other genes involving in photosynthetic process mainly RUBISCO (Hanaoka *et al.,* 2002). Senescence associated genes (SAGs) are those genes which control and regulate gene expression of leaf senescence. In a senescencing leaf, many genes that are expressed in green leaves, including those genes involved in photosynthesis, are down regulated, while a subset of genes, generally referred to as senescence-associated genes (SAGs), are up regulated. A variety of SAGs have been isolated from *Arabidopsis*, brassica, barley, maize and soybean (Otegui *et al.,* 2005; Brusslan *et al.,* 2012; Liu *et al.,* 2008). Their gene products comprised of transcription factors, lipoidases, proteases, RNases, kinases etc. (Gan, 1995; Hinderhofer *et al.,* 2001; Guo and Gan, 2006). Although numerous SAGs have been reported for different plants, a few are reported to be strictly related with senescence (Table 1), such as *Sweet Potato Cysteine Protease 3* (*SPCP3*) from sweet potato (Chen *et al.,* 2006), *Senescence associated protein 15* (*SPA15*) of sweet potato and its homologue from rice (Yap *et al.,* 2003), *SAG12* from *Arabidopsis* and its rice homologues (Grbic, 2003; Otegui *et al.,* 2005; Singh *et al.,* 2013, 2016), SAG39 from rice (Liu *et al.,* 2010) etc. Early responsive to dehydration ERD1/(*SAG15*), late embryogenesis -abundant gene (*SAG21)*, glutamine synthase (*Atgsr2*), ACC synthase (*ACS6*), lipases, glyoxylate cycle enzymes, and polyubiquitin which may be involved in protein degradation and nitrogen remobilization, catalase (*LSC 650*), metal binding proteins (*SAG14*), metallothionins (*SAG17*), and cytochrome P450, likely to scavange and detoxify reactive oxygen species (Weaver *et al.,* 1998) are some of the other SAGs which have also been reported. Wang *et al*. (2015) have reported that functional inactivation of UDP-N-acetylglucosamine pyrophosphorylase 1 (UAP1) induces early leaf senescence and defense responses in rice. But, the physiological roles played by most of them are not known (Lim *et al.,* 2007; Park *et al.,* 2007; Fischer-Kilbienski *et al.,* 2010).

Table 1. Senescence associated genes encoding proteases expressed in different plants

Gene Name	Function	Plant	References
SAG2	Cysteine protease	*Arabidopsis*	Hensel *et al.,* 1993
SAG12	Cysteine protease	Rice, *Arabidopsis*	Singh *et al.,* 2013,2016; Lohman *et al.,* 1994
See1	Cysteine protease	Maize	Smart *et al.,* 1995
See2	Cysteine protease	Maize	Smart *et al.,* 1995
LSC7	Cysteine protease	*B. napus*	-
LSC790	Cysteine protease	*B. napus*	Buchanan-Wollaston and Ainsworth., 1997

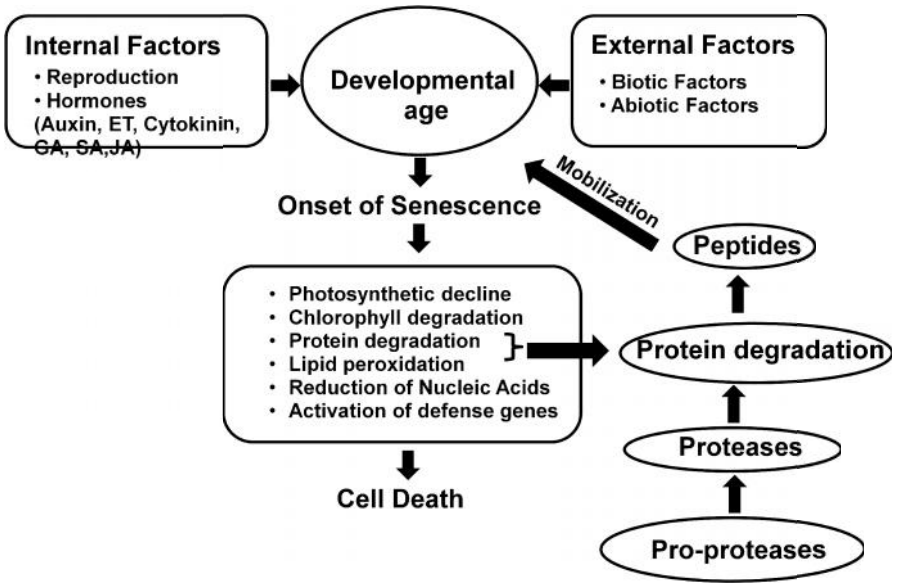

Figure 1. Mechanism of senescence and factors involved

Arabidopsis SAG12 gene (*AtSAG12*) is the best characterized gene amongst the SAGs reported from plant species (Millar *et al.,* 1999; Noh and Amasino 1999a; Otegui *et al.,* 2005; Ay *et al.,* 2009; Brusslan *et al.,* 2012). It belongs to a papain-like (C1A family) and encodes cysteine-type peptidase (Beers *et al.,* 2004; Cambra *et al.,* 2012; Martinez *et al.,* 2012). Approximately about 800 SAGs expressed in *Arabidopsis* senescent leaves were detected by suppression subtracted hybridization which could be involved in recycling of metabolites, degradation of macromolecules, oxidative metabolism, detoxification of oxygen reactive species, secondary metabolite synthesis, pathogens response and regulation of senescence (Gepstein *et al.,* 2003; Otegui *et al.,* 2005). The plant hormone cytokinin negatively regulates senescence process. Expression of cytokinin biosynthesis gene isopentenyltransferase (ipt) appreciably delayed natural and stress induced senescence in *Arabidopsis* under the *AtSAG12* promoter, wheat and lettuce (McCabe *et al.,* 2001; Liu *et al.,* 2005; Sykorova *et al.,* 2008). *Bn*SAG12-1 and *Bn*SAG12-2, the two AtSAG12 homologues from *Brassica napus* have been predicted by screening the cDNA library (Noh and Amasino, 1999a). These two genes showed senescence specific induction in transgenic *Arabidopsis* under their native promoters demonstrating the presence of evolutionarily conserved expression regulation system for the *OsSAG12* genes (Noh and Amasino, 1999b). Expression of *AtSAG12* is limited to the senescence associated vacuoles (SAV) (Otegui *et al.,* 2005) but still its physiological role is not known. The homozygous *atsag12* mutants reveal normal senescence and usual development of SAVs (Otegui *et al.,* 2005). Studies on senescence in rice are in progress in addition to the establishment of *Arabidopsis* senescent leaf transcriptome using microarray analysis (Guo *et al.,* 2004; Lin *et al.,* 2004).

Expression of SAG39 gene of rice, encoding a cysteine protease, having 55% identity with AtSAG12 has been found to be associated with senescence (Liu *et al.,* 2010). SAG39-ipt transgenic rice plant also shows the delayed senescence suggesting expression regulation similar to that of AtSAG12 (Liu *et al.,* 2010). However, like the *Arabidopsis* its physiological role is also unclear. About 14 rice SAGs were identified using suppression subtracted hybridization and out of them, 11 were associated with natural senescence and responded to induction after dark treatment (Lee *et al.,* 2001). Jiang *et al.* (2004) studied a population of 190 doubled haploid lines from the cross between an indica parent Zhenshan 97 and a stay-green japonica parent Wuyujing 2 and detected more than 70 correlated QTLs for the genetic basis of stay-green.

Proteases

Protein degradation occurs during senescence leading to recycling of nutrients and many of the genes up-regulated during senescence are proteases (Bhalerao *et al.,* 2003; Guo *et al.,* 2004) (Fig. 1). Proteases and proteasomes are comprised of plant proteolytic systems, which are involved in a variety of cellular metabolic processes including senescence and defense responses (Beers *et al.,* 2000; Van der Hoorn and Jones., 2004). Proteolytic enzymes are generally termed as proteases, peptidases or proteinases. They are distinctively expressed in time and space and accumulate in different subcellular compartments (Van der Hoorn, 2008; Van der Hoorn and Jones, 2004). Plant proteolysis is a metabolic pathway that involves broad metabolic networks, different subcellular compartments and types of proteases (Van der Hoorn, 2008). They are divided into five classes based on the amino acid determinants of their catalytic site or their required metal

cofactors, such as serine protease (EC 3.4.21), cysteine protease (EC 3.4.22), aspartic endopeptidase (EC 3.4.23), metalloprotease (EC 3.4.24), and threonine protease (EC 3.4.25) (Powers *et al*., 2002; Barrett *et al*., 2004). The catalytic classes are subdivided into Clans, and the Clans are further subdivided into Families based on the evolutionary relationship. The Ser proteases (SPs) is the largest class with about 200 members, and the Cys, aspartic, and metalloprotease classes each contain about 100 members (http://merops. sanger.ac.uk; Van der Hoorn and Jones, 2004; Van der Hoorn, 2008). Among the largest protease families in *Arabidopsis* are subtil like Ser proteases (58 members in family S8 of clan SB) and papain-like Cys proteases (30 members in family C1 of clan CA Beers *et al.,* 2004; Van der Hoorn *et al.,* 2004). Most proteases are produced as pre-proproteases having a signal sequence, a mature protease domain and an auto inhibitory prodomain. Tripathi and Sowdhamini, (2006) identified 206 and 222 serine proteases and serine protease-like proteins in *Arabidopsis* and rice, respectively. SPCP2 in sweet potato (Chen *et al.,* 2010) and GMCP3 in soybean (Esteban-Garc´ıa *et al.,* 2010) have been recognized as papain-like CP genes. In addition to constitutive expression of SPCP2 in transgenic *Arabidopsis* plants, it is also involved in developmental process such as earlier flowering time (Chen *et al.,* 2010).

Plant genomes encode for about 500 to 800 proteases (Garcı´a-Lorenzo *et al.,* 2006; Van der Hoorn., 2008, Diaz and Martinez, 2013). Among them 140 correspond to cysteine proteases (CysProt) which can be grouped in 15 families in 5 clans (Rawlings *et al.,* 2010). The best-known cysteine-proteinases are caspase-like proteins, vacuolar-processing enzymes (VPEs), papain-like peptides, and cathepsin-type proteases (Palma *et al.,* 2002). Most plant cysteine proteinases belong to the legumain (C13) and papain (C1) families. The members of cysteine proteinases: caspases (family C14) and calpains, the calcium- dependent proteinases (family C2), have recently been found in plants (Grudkowska and Zagdanska, 2004; Rawlings *et al.,* 2014). Vacuolar processing enzyme (VPE, family C13), also called legumain has caspase 1-like activity and are involved in vacuolar proteins maturation and cell death responses during stress response in different organs (Hara-Nishimura *et al.,* 2005). Plant metacaspases (MCs, family C14) are CPs showing different substrate specificity. Caspases cleave after Asp residues and MCs after Arg and Lys. *A. thaliana* has nine genes for MCs, AtMCP1a-c/MC1-3 and AtMCP2a-f /MC4-9 which play role in controlling cell death (Coll *et al.,* 2010, Watanabe and Lam, 2011), flowers (MC3, MC9) and gene upregulation in senescent leaves (MC6, MC9) (Sanmartın *et al.,* 2005, Breeze *et al.,* 2011). The *Arabidopsis* genome encodes about 800 proteases which are dispersed over almost 60 families belonging to 30 different clans. About 955 putative protease genes have been known in the *Populus* genome (Garcia-Lorenzo *et al*., 2006). Three of the senescence-enhanced protease genes, isolated from maize, *Arabidopsis* and *B. napus* show sequence similarity to a class of cysteine protease represented by seed-specific proteases from cereals such as the oryzain protease from rice (Watanabe *et al.,* 1991). Proteases can act at various levels such as pathogen recognition, induction of defense responses, signals leading to the release of positive regulators, the degradation of negative regulators or execution of defense responses (Van der Hoorn and Jones, 2004). Ubiquitin-dependent proteolysis is also found to be involved in leaf senescence regulation. Cysteine proteinases being labeled with the prefix C are also referred as thiol proteases. They consist of more than 40 families of peptidases and further grouped into six super families or clans. Besides, an essential role in plant growth and development they are also found to be associated with senescence and programmed cell death, in accumulation of storage proteins such as in seeds, storage protein mobilization. In addition, they are involved in signaling pathways and in the response to biotic and abiotic stresses, regulation of epidermal cell fate, flowering time, and inflorescence architecture and pollen or embryo development. (Grudkowska and Zagdanska., 2004). The *Arabidopsis thaliana* genome encodes 32 papain-type (C1 family) cysteine proteinases which can be classified into eight main groups (senescence- and stress-induced, aleurain, cathepsin-b like, bromelain-like, KDEL, telo sequences, actinidain-like) based on the sequence similarity to other cysteine proteinases (Simpson 2001; Diaz-Mendoza *et al.,* 2014). Plant papain type enzymes (C1) are synthesized as small preproproteins of 40-50 kDa prepropeptides which undergo proteolytic processing of the pre and pro peptides to yield mature, fully active enzymes (22-35 kDa). Two additional families of cysteine proteinases: ubiquitin C-terminal hydrolases (family C12) and ubiquitin-specific proteinases (C19) have been reported. The components of the ubiquitin-proteasome-dependent pathway involved in deubiquitination of proteins have also been determined in plants (Vierstra, 2003).

Cysteine proteinases are synthesized as large precursors having short N-terminal and much longer C-terminal propeptides at membrane bound polysomes in the cytoplasm. Firstly, the inactive proenzymes enter the lumen of the endoplasmic reticulum (ER) and later are transported to the vacuole or cell wall. A KDEL or HDEL tetrapeptide sequence is present at C-terminal in most of the soluble plant proteins which is recognized by the ERD2-KDEL receptor on the Golgi apparatus (Okamoto *et al.,* 2003) and further transported to the *trans*-Golgi network. The KDEL/HDEL sequences acts as protein retention signals in the endoplasmic reticulum and also regulate the delivery of proteins to other compartments. Sequential removal of the N- and C-terminal propeptides in vacuoles leads to production of mature form (Yamada *et al.,* 2001). Papain-like cysteine proteinases have the ER retention signal KDEL (Okamoto and Minamikawa, 1999) at the C-terminus of their cDNA-deduced amino-acid sequences removed post-translationally (Fischer *et al.,* 2000). The potential receptors of the KDEL tetra peptide are coded by more than one gene in *Arabidopsis* (Frigerio *et al.,* 2001). There are different receptors for HDEL and KDEL which can be separately distributed in the Golgi complex (Frigerio *et al.,* 2001). KDEL has been found in SH-EP (sulfhydrylendopeptidase) which is a vacuolar proteinase playing role in degradation of seed storage proteins accumulated in protein storage vacuoles (Okamoto and Minamikawa, 1998). SH-EP is synthesized as an inactive zymogen of 43 kDa with a carboxy-terminal KDEL motif (Okamoto and Minamikawa, 1998) and further processed to form active enzyme of 33 kDa. It is later sequestered into endoplasmic reticulum-derived electron dense vesicles reaching the vacuole through a pathway that bypasses the Golgi complex (Toyooka *et al.,* 2000; Okamoto *et al.,* 2003). KDEL motif is removed prior to translocation during the post-translational processing of SH-EP and leads to activation of the mature protein (Okamoto *et al.,* 1999). The KDEL-containing proteinase is a different plant-specific enzyme subset related to the endoplasmic reticulum-derived precursor protease vesicles (Chrispeels and Herman, 2000) and has been

recognized exclusively in higher plants (Toyooka *et al.,* 2000; Okamoto *et al.,* 2003). The C-terminal extension sequences are comprised of two domains: a Pro-rich domain and a domain of high homology to animal proteins of the epithelin/granulin family (Bhandari *et al.,* 1992). Epithelins and granulins are small proteins of about 6 kDa which regulate the intensity of growth of animal cells, while plant granulins have a 4 kDa insertion with two cysteine residues and the function of these proteins is still unknown (Bhandari *et al.,* 1992; Yamada *et al.,* 2001). The induction of at least four vacuolar CPs has been observed in wheat leaves senescing in continuous darkness (Martinez *et al.,* 2007). Similarly, formation of small vacuolar compartments senescence-associated vacuoles (SAVs) containing high CP activity has been reported in leaves of *Arabidopsis* and soybean (Otegui *et al.,* 2005). The CP SAG12 highly expressed in naturally senescing tissues (Guo *et al.,* 2004; Parrott *et al.,* 2007; Ruuska *et al.,* 2008) have also been found in SAVs (Otegui *et al.,* 2005). Cys-EPs has been established to play role in various processes such as senescence (Drake *et al.,* 1996; Ueda *et al.,* 2000), degradation of stromal proteins (Thoenen *et al.,* 2007), pathogen linked programmed cell death (Beers *et al.,* 2000), wounding (Ueda *et al.,* 2000), pollen or embryo development, mobilization of proteins during germination (Schlereth *et al.,* 2000). A unique 33-kD cysteine proteinase encoded by *mir1 gene* accumulates in the maize whorl in response to larval feeding. It was expressed in response to wounding and was found in senescent leaves and it may be a marker of programmed cell death (Pechan *et al.,* 2000). A unique group of papain-type cysteine endopeptidases, characterized by a C-terminal endoplasmic reticulum (ER) retention signal (KDEL Cys EP), was established to be involved in plant PCD in *Arabidopsis*, *Ricinus* and *Trifolium repens* (Hierl *et al.,* 2012; Mulisch *et al.,* 2013).

Role of plant proteases in defense

Proteases have great role in defense and can act at various levels like perception, signalling and execution (Van der Hoorn and Jones, 2004; Baek and Choi, 2008).They can directly degrade proteins from the invader, release peptide-based toxins or activate enzymes from their precursor proteins. Proteases such as the papain and papain-like proteases Mir1 and RD21, metalloprotease LapA, the subtilisin-like P69 and many other proteases which accumulate at high levels at the site of the invasion are mainly responsible for defense. Plant genomes encode hundreds of proteases, but little is known about their roles in the life of a plant. Functions for only a few of the 550 proteases of *Arabidopsis* (http://merops.sanger.ac.uk) have been determined genetically (Adam and Clarke., 2002; Beers *et al.,* 2004). A reported new family of *P. infestans*, EPIC1 to EPIC4 which secreted proteins with similarity to cystatin-like protease inhibitor domains. These proteases of diverse catalytic families and other pathogen inhibitors are involved in plant-pathogen interactions and defense response. C1A proteases play important role in local and systemic defense in response to pathogen and pest attacks (Shindo and Van der Hoorn, 2008, McLellan *et al*., 2009). Several apoplastic proteases have also been associated with defense responses. Rcr3, a secreted Cys protease from tomato *(Lycopersicon esculentum*), is required for resistance mediated by the Cf-2 receptor-like protein against strains of the fungus *Cladosporium fulvum* that carry the Avr2 avirulence gene (Kruger *et al*., 2002). The multiple roles of proteases in plant defense also include hypersensitive response (HR), a form of programmed cell death (PCD) that is associated with resistance to pathogens (D'Silva *et al.,* 1998; Solomon *et al*., 1999; Mosolov *et al*., 2001; Chichkova *et al*., 2004). Proteasome complexes involved in the ubiquitin-mediated protein degradation pathway have been implicated in PCD and disease resistance (Suty *et al*., 2003; Tor *et al*., 2003; Zeng *et al*., 2004).

Avr4 and Avr9 factors from *C. fulvum* are processed proteolytically indicating the participation of host apoplastic proteases in modification of these Avr proteins (Joosten and De Wit 1999). Several tomato pathogenesis-related (PR) proteins (P69A, P69B, P69C, P69D) are subtilisin-like serine proteases that are accumulated in the apoplast following pathogen infection (Jorda *et al*., 1999). *Phytophthora infestans*, the oomycete species that causes late blight disease of potato and tomato, secretes an extracellular protease inhibitor (EPI1), which inhibits proteolytic activity of P69B (Tian *et al*., 2004). Recently, an *Arabidopsis* gene *CDR1* that encodes an apoplast aspartic protease has been identified to activate the defense response (Xia *et al*., 2004).

Conclusion

Senescence is a complex phenomenon for nutrient recycling in the plants. It is associated with various coordinated stages of plant development starting from seed germination to maturity. Senescence is regulated by both internal (reproduction, hormones) as well as external factors (biotic and abiotic stresses). The senescence-associated proteolysis includes different sub-cellular compartments, several types of proteases and regulators and a complex trafficking of proteins that leads to a massive protein turnover with a crucial role in nutrient recycling. The identification of many *SAGs* has provided a foundation for further molecular analysis of gene regulation during senescence. Using both molecular and genetic approaches to decipher the regulatory mechanisms underlying leaf senescence will be a very important tool for future studies of senescence.

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Conflict of Interest

The authors declare that they have no competing interest.

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