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Polyamines are common players in different facets of plant programmed cell death

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Abstract Programmed cell death (PCD) is a process that occurs throughout the life span of every plant life, from initial germination of the seed to the senescence of the plant. It is a normal physiological milestone during the plant's developmental process, but it can also be induced by external factors, including a variety of environmental stresses and as a response to pathogen infections. Changes in the morphology of the nucleus is one of the most noticeable during PCD but all the components of the plant cell (cytoplasm, cytoskeleton and organelles) are involved in this fascinating process. To date, relatively little is known about PCD in plants, but several factors, among which polyamines (PAs) and plant growth regulators, have been shown to play an important role in the initiation and regulation of the process. The role of PAs in plant PCD appears to be multifaceted acting in some instances as pro-survival molecules, whereas in others seem to be implicated in accelerating PCD. The molecular mechanism is still under study. Here we present some PCD plant models, focusing on the role of the enzyme responsible for PA conjugation to proteins: transglutaminase (TGase), an enzyme linked with the process of PCD also in some animal models. The role of PAs and plant TGase in the senescence and PCD in flowers, leaf and the self-incompatibility of pollen will be discussed and examined in depth.

Abbreviations

PAs	Polyamines
PCD	Programmed cell death
DCD	Developmental cell death
PU	Putrescine
SD	Spermidine
SM	Spermine
TGase	Transglutaminase
TG2	Tissue transglutaminase type 2

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Introduction

In plants, as well as in animal pluricellular organisms, the developmental and growth processes require the selective elimination of single or groups of cells. The organism actively controls this process termed programmed cell death (PCD). In plants, this process can involve the elimination of entire organs which no longer have a useful role (like leaves, petals, stamen, roots, branches, buds, etc.) or of useful living organs, such as fruits. In this case, only a thin layer of cells of the pedicel dies in order to allow the detachment of the fruit. The dead parts fall after abscission or remain in situ after a mummification process. In some cases, like in monocarpic plants, the entire organism undergoes death after the production of seeds.

PCD might occur in cells which are no longer useful in order to conserve energy and nutrients, or to produce

specialised dead cells, for example, for the sap transport (e.g. vessels), mechanical support or to provide defence from the environment (van Doorn et al. 2011). To complete terminal differentiation and become functional, some cells must digest the cytoplasm and undergo PCD. This occurs for example in vessels, tegumental tissues or fibres, whose function is passively exerted by the modified cell wall, which remains in situ.

Programmed cell death represents a natural process occurring during morphogenesis of the embryo or of the adult plant and may conclude the senescence of vegetative or reproductive organs. In the literature, there is sometimes confusion on the application of the terms senescence and PCD which may be considered separate, partially overlapping or even identical events (Thomas et al. 2003; Rogers 2006). Senescence is not necessarily a stage of all types of PCD; when senescence takes place, it is not a steady state but a gradual evolution of the entire cell (even though sometimes it can be delayed or reversed) preliminary to cell death.

The term Developmental Cell Death (DCD) concerns a PCD process driven by an internal programme of species-specific development (even though it might also be triggered by adverse environmental factors) (Wu et al. 2012) and, in this case, the term DCD could be used more appropriately. In this review, the general term PCD will be used if not otherwise specified; as senescence concerns the last part of development it is considered a PCD process (examples are senescence of leaves and petals ending with the cell death and falling of the organs). In addition to the developmental events of PCD, other accidental PCD events can occur, in a defence response to biotic (pathogen and toxin attack) or abiotic stresses triggered by adverse environmental factors (temperature, salinity, hypoxia, anoxia and drought due to failure of oxygen or water, wound, ROS, NO and other chemical/physical factors) (Van Doorn et al. 2011; Del Duca et al. 2014a). The crosstalk between nitric oxide (NO) and ROS in plant PCD (Wang et al. 2013) as well as catabolic products of polyamines (PAs) have been recently reviewed (Moschou and Roubelakis-Angela-kis 2013). Generally, PCD (or DCD) can be accompanied by nuclear condensation, membrane blebbing, cysteine proteases activity and occasionally DNA fragmentation (Serafini-Fracassini et al. 2002; Ye et al. 2013).

At the subcellular level, mitochondria may play a central role in PCD, retaining their function during senescence, perpetuating respiration by the use of an alternative oxidase (Vanlerberghe 2013). An increase in reactive oxygen species (ROS) production and protease and nuclease activities have been reported during leaf senescence (Rubinstein 2000). In green tissues, chloroplasts swell and re-differentiate into gerontoplasts characterised by the dismantling of thylakoidal membrane; thus, proteins, chlorophylls, lipids as well as nucleic acids are degraded, and the photosynthetic activity decreases. While mitochondria catabolise lipids deriving from thylakoids, vacuoles (which represent the lytic compartment of the plant cell) play relevant roles in the autophagic degradative metabolisms, as exemplified by chlorophyll degradation; finally rupture of tonoplast membrane takes place causing the release of degradative enzymes (van Doorn and Woltering 2010). Increasingly evidence shows that cytoskeleton modifications are prominently involved in PCD in plants (Thomas et al. 2003). Cell walls of some specialised cells frequently undergo second-ary modifications, such as lignification, suberification and gelification, before the cell dies.

Models of PCD

Programmed cell death in plants may follow models which are well defined in animal cells e.g. apoptosis, regulated necrosis or in some cases, the process is quite different. The Greek word *apoptosis* describes the fall of flow-ers or leaves, but in ancient medicine it was also used to describe the breakage of bones and scabs detaching from flesh wounds. Some typical characteristics of animal "apoptosis"(a term which is not used to describe any equiv-alent process in plant) are not detected in plants due to dif-ferences at the cellular, tissue and organism level. Moreo-ver, the cell death strategies between plants and animals can be different in accordance with differences in organ-ism and cytological organisation. For example, in plants, no apoptotic bodies are formed and macrophages are not present. In contrast, plants possess chloroplasts that can furnish energy to the dying plant cells. However, some of the plant and animal PCD metabolisms and some inducing factors seem to be functionally conserved during evolution. For example, caspases of animal origin and caspase-like proteases from plants perform similar functions but show little homology at the molecular level. This is also true for transglutaminases (TGases) from animals and plants which show scarce homology, but similar functions.

In common with animal PCD, PAs exert a role, which very frequently consists of a juvenilation effect by retarding senescence (Serafini-Fracassini et al. 2010) and PCD; nevertheless, PAs sometimes seem to accelerate this process (Del Duca et al. 2014a; Seiler and Raul 2005). Among the different plant PCD models described in literature, some may be classified as "vacuolar cell death", others as"necrotic cell death" or, finally, to a mixture of both as proposed by van Doorn et al. (2011). During vacuolar cell death, the cell contents are removed by a combination of autophagy-like process and the release of hydrolases from collapsed lytic vacuoles. Vacuolar cell death is common during tissue formation and elimination, whereas necrosis

is mainly found after abiotic stress. However, this classification is based only on morphological observations, which are not always available. The proper diagnosis of vacuolar cell death should be obtained by combining electron microscopy observations together with the analysis of autophagic activity of the vacuolar processing enzymes and with changes of the cytoskeleton. Morphological events occurring during vacuolar cell death include the assembly of actin bundles, breakdown of the nuclear envelope and nuclear segmentation. According to van Doorn et al. (2011), vacuolar cell death has been observed in plant tracheary cells, in pollen as well as in petals (DNA laddering and nuclei fragmentation were observed, Serafini-Fracassini et al. 2002). Necrosis is characterised by early rupture of the plasma membrane, shrinkage of the cytoplasm, mitochondria swelling, production of ROS and the absence of vacuolar cell death features. Necrosis in plants is no longer considered an unprogrammed process, but remains poorly characterised at the biochemical and genetic levels. Although there are no molecular markers for necrosis in plants, it can still be defined as PCD. In humans, the multiple pathway of regulated necrosis has been better characterised and is considered the most prominent mode of cell death (Vanden Berghe et al. 2014). Regulated necrosis consists of a controlled cell death process resulting in cellular leakage morphologically characterised by cytoplas-mic granulation as well as organelle and/or cellular swell-ing. The diversity and redundancy in the molecular mecha-nisms of the multiple pathways of regulated necrosis have been recently reviewed by Vanden Berghe et al. (2014).

A mixture of necrotic and vacuolar-related cell death also occurs during the self-incompatibility (SI) response, possibly even in the models presented in this review.

The term programmed cell death implies control exerted at the transcriptional, translational and post-translational levels. In mammals, the incorporation of PAs into proteins by TGase linked to PCD events has been studied in detail. Aspects of the involvement of PAs and of TGase in some of the more known models of plant PCD are presented in this review.

Polyamines and plant PCD

Polyamines are recognised to exert important regulatory roles in PCD of both animals and plants (see recent reviews on PAs and PCD in plants when compared to ani-mals: Della Mea et al. 2007a; Moschou and Roubelakis-Angelakis 2013; Del Duca et al. 2014b). Research on PAs in plants has dealt with the growth stimulatory effect of the three aliphatic PAs (putrescine, PU; spermidine, SD; spermine, SM) and more recently also of thermospermine; the latter is a structural isomer of spermine, first discovered in

thermophilic bacteria, but of increasing relevance also for higher plants. In addition, other aspects of the role of PAs in plants, related to PA transport, metabolism, stress tolerance, growth, senescence, chemoprevention and conjugated PAs were reviewed in plants (by Various authors 2010, in a special issue of Plant Physiology and Biochemistry dedicated to PAs). Polyamines also regulate differentiation, organogenesis, reproduction and cell proliferation in higher plants and algae, as well as senescence, PCD and homeostatic adjustments in response to external stimuli and stresses.

The majority of the PA data have been obtained by meas-uring the concentration of free PAs in tissue extracts, but this provides only a "snap shot" picture of a continuously changing environment. The cellular level of PAs arises from a balance of different metabolisms involving their synthesis, catabolism, and attachment to other molecules. These attachments may occur by both electrostatic linkages causing conformational stabilisation/destabilisation of DNA, RNA, chromatin, proteins (enzymes, receptors, onco-proteins), fluidity of membranes and by covalent link-ages to proteins giving rise to formation of hypusine and TGase-mediated 'cationisation' or cross-link formation of many proteins. A more indicative measure of the cellular levels of PAs is to follow their level in both free and bound forms during a physiological event, like for example leaf development until death. Polyamines were found localised in all the cellular organelles, thereby exerting roles related to the organelle's specific functions.

In plants, data available mainly suggest that PAs allow a prolonged survival of excised organs or senescent organs in vivo, like leaves, flowers and fruits. Nevertheless, as in animals, some contradictory data are reported (Legocka and Zajchert 1999; Lester 2000; Hanzawa et al. 2000; Bagni and Tassoni 2006; Muñiz et al. 2008; Kusano et al. 2008; Nambeesan et al. 2010; Serafini-Fracassini et al. 2002, 2010). Much data on the PA effect were obtained through the exogenous supply of PAs and PA analogues or from loss-of-function, over-expression or mutants in PA metab-olism genes. However, the complex metabolism of PAs sometimes can minimize, compensate and blur the effects in the modified plant cells.

The review of Seiler and Raul (2005) represents a mile-stone on PA involvement in apoptosis of animal cells. It has been evidenced that both activation and prevention of apop-tosis due to PA depletion are reported for several cell lines and that elevation of PA concentrations may lead to apoptosis or to malignant transformation. The authors concluded that 'contradictory results and incomplete information blur the picture and complicate the interpretation'. Contrarily to animal cells, plant cells can be less affected by excess PAs, at least in some plant families, by binding them to TCA-soluble conjugates, such as cinnamic acids, or, in general, by storing them in the vacuole (Bagni and Tassoni 2001).

Examples of the different types of plant PCD studied in relationship with PAs are excised or senescing leaves and protoplasts (Galston and Kaur-Sawhney 1990; Bes-ford et al. 1993), aged leaf discs (Legocka and Zajchert 1999; Serafini-Fracassini et al. 2010), as well as vessels (Muñiz et al. 2008; Vera-Sirera et al. 2010; Imai et al. 2006), incompatible pollen/style system (Del Duca et al. 2010; Gentile et al. 2012) and senescing flowers (Serafini-Fracassini et al. 2002; Bagni and Tassoni 2006; Della Mea et al. 2007a, b). Thermospermine has been shown to be critical for proper vascular development and xylem cell specification, in preventing premature maturation and death of the xylem vessel elements (Vera-Sirera et al. 2010). Overexpression of S-adenosylmethionine (SAMDC) caused an increase of SD and SM and suppression of senescence in tomato fruits (Mehta et al. 2002). During senescence, the levels of PAs are not constant showing peaks especially at its beginning, but thereafter PAs usually decrease (Galston and Kaur-Sawhney 1990; Paschalidis et al. 2009). This pattern, however, depends on the type of senescence model, if induced by natural or external factors. Programmed cell death can be induced also by biotic and abiotic stresses, in which especially the oxidation of PAs in the apoplast seems to play a relevant role even though the proposed model is rather correlative (reviewed by Moschou and Roubelakis-Angelakis 2013).

The functions of intracellular PAs in PCD appear to be related to the prevention of membrane damage, the retardation of nucleic acid and protein degradation (including the chloroplast photosystems), probably by the direct effect of the binding to these molecules. It is also known from many years that PAs also activate transcription and translation in plants (Tong et al. 2014; Alcázar and Tiburcio 2014).

In addition, PAs can act as free radical scavengers, which are produced in plant cells undergoing PCD. Poly-amines also regulate ion channels such as those involved in K^+ efflux and Ca^{2+} influx during the progression of hypersensitive response, which are relevant for the activation of metacaspases and for ROS production (Watanabe and Lam 2011). The formation of hydrogen peroxide and cytotoxic products via PA catabolism is considered to be one of the possible mechanisms of PA involvement in PCD (Yoda et al. 2006), and the ability of plants to control stress is related to their capacity to metabolise PAs (Alcázar et al. 2010; Pottosin and Shabala 2014).

Polyamine conjugation to proteins

The post-translational modification is identified as one of the most important, rapid and precise methods by which eukaryotic cells respond to environmental stresses or developmental changes. The process of transamidation is part of a set of post-translational modifications to which proteins can be subjected and include a number of efficient regulatory strategies, such as phosphorylation/dephosphorylation, proteolytic degradation or activation, interaction with partner proteins. Polyamines can be involved in the modifications of proteins by their covalent conjugation to proteins catalysed by the enzyme family of TGase (R-glutaminyl-peptide-amine γ-glutamyltransferase; E.C. 2.3.2.13), which has been discovered and studied in animals since many years (Folk 1980). Transglutaminases, present in eukary-otic and prokaryotic organisms are, with few exceptions, Ca^{2+}-dependent; in animals they fulfil different enzymic functions as summarized in a book edited by Mehta and Eckert (2005) and reviewed (Griffin et al. 2002; Lorand and Graham 2003; Beninati et al. 2009; Gundemir et al. 2012). While their activity level in growing animal cells is believed to be low, TGase gene expression, protein accu-mulation and/or protein activity are associated with animal cell death (Griffin and Verderio 2000; Lorand and Graham 2003). It is becoming evident that the multifunctional roles of the extensively studied animal tissue, TGase (TG2), has both a cytosolic and nuclear location, and its involve-ment in cell death processes (apoptosis and or autophagy) is dependent upon the cell type, stimuli, subcellular locali-sation and conformational state of the TG2 enzyme (Gun-demir et al. 2012). A clear role for TG2 was revealed in forming cross-links between proteins participating in the formation of apoptotic bodies (Fesus et al. 1989; Griffin et al. 2002; Lorand and Graham 2003). Although in many mammalian systems TG2 is a downstream effector in the later stages of apoptosis, TG2 expression has also been connected with an early apoptotic event (Griffin and Verde-rio 2000) even though TG2 role is still discussed (Piacen-tini et al. 2005).

Transglutaminase 2 is the most widely distributed member of the transglutaminase family; it is an extremely versatile protein exhibiting transamidating, protein disulphide isomerase and guanine nucleotide binding and hydrolysing activities even if only in vitro. Transglutaminase 2 can also act as an extracellular protein scaffold or linker. It should be considered that the biological function of TG2 is dependent on whether transamidation or GTP binding is switched on, in a mutually exclusive way (Griffin et al. 2002). An inter-esting paper about the different functional properties of TG2 in connection to the binding of Ca^{2+} or GTP has been published recently in neuroblastoma cell differentiation in which two structurally distinct TG2 protein isoforms, the full-length (TGase2-L), able to bind GTP, and the short form (TGase2-S, about 62 kDa) not able to bind GTP, exert opposing effects on cell differentiation (Tee et al. 2010). In addition, over-expression of TG2-S in response to a cyto-toxic stimulus, such as tumour necrosis factor A, leads to PCD, while over-expression of TG2-L blocks cytotoxicity.

In human neuroblastoma cells, TG2-L blocks, and TG2-S induces cell differentiation (Ling et al. 2012).

One of the TGase activities, namely the transamidation catalysis, consists in the covalent conjugation of PAs and other amine-donors (lysyl residues among them) to γ-carboxamide groups of protein endo-glutamine residues (Folk 1980). The two terminal amino-groups of PAs conjugate to one or two glutamyl residues giving rise to PA-derivatives, either *mono*-(γ-glutamyl)-PAs (*mono*-PAs) or *bis*-(γ-glutamyl)-PAs (*bis*-PAs). The additional positive charges introduced by protein-bound PAs due to their internal iminic- or free terminal aminic group (*mono*-PAs) may induce protein conformational changes. *Bis*-PA-derivatives can form both inter- and intramolecular cross-links in proteins. The backbone length of the PAs determines the length of the cross-link it forms: *bis*-(γ-glutamyl)-SD (*bis*-SD) bridges and even more so those involving bis(γ-glutamyl)-SM (*bis*-SM), span greater distances than those formed by *bis*-(γ-glutamyl)-PU (*bis*-PU). The link formed between glutamyl and lysyl residues is much shorter that those involving PAs. The binding is highly specific and is probably primarily dependent on substrate conformation (Griffin et al. 2002). *Mono*-PA production is affected by PA concentration since high levels of PAs saturate the acyl donor residues of the substrate proteins limiting the formation of *bis*-derivatives. In this sense, the levels of PAs have a critical role in the modulation of the number of protein cross-links formed. Since several PA molecules can cross-link more proteins simultaneously, complexes with a high molecular mass may form. In addition, the free terminal amino-group of *mono*-PAs can interact by additional linkages, for example, with negatively charged groups of other types of molecules, thus forming heterogeneous complexes. The supramolecular nets of linked proteins are resistant to mechanical stress and proteolysis and are observed fre-quently as a product of TGase cross-linking activity. This enzymatic activity suggested to Griffin and co-workers to entitle their review: "Transglutaminases: nature's biological glues" evidencing the peculiar role of this enzyme (Griffin et al. 2002). Transglutaminases have been extensively stud-ied in plants (reviewed by Del Duca and Serafini-Fracassini 2005; Serafini-Fracassini and Del Duca 2008; Del Duca et al. 2014a, b). A family of TGases of different molecular masses is located in various organs of higher plants, like seeds, pollens, meristems, mature vegetative organs, flow-ers and petals. The enzymes are very active in chloroplasts where they react to external stimuli, including light, but they are active also in the cytoplasm, in a relationship with the cytoskeleton, chloroplast and mitochondria.

Research on plant TGase has been hampered by the difficulties encountered in their purification and by the lack of significant amino acid sequence homologies between ani-mal TGases and the polypeptides reported in the available plant databanks (Serafini-Fracassini et al. 2009). A computational analysis has shown the presence in *Arabidopsis thaliana* of only one gene, *AtPng1p*, which contains the TGases catalytic domain with the Cys-His-Asp triad. This gene encodes a putative N-glycanase, active at least in vitro in heterologous systems (Diepold et al. 2007; Masahara-Negishi et al. 2012), but its product also acts as a TGase, having a Ca^{2+}- and GTP-dependent transamidase activ-ity and forming glutamyl-PA-derivatives (Della Mea et al. 2004b). This was the first plant protein, isolated and char-acterised at the molecular level, displaying a TGase activ-ity, whose biochemical parameters and 3D structure agree with those typically exhibited by animal TGases. Recently, a sequence with high homology degree to *AtPng1p*, called *HvPng1-like* gene has been identified also in barley (Sobieszczuk-Nowicka, personal communication). Other TGases of different molecular masses of chloroplasts origin were sequenced (Villalobos et al. 2004; Campos et al. 2013), and the homology of the amino acidic composition of three TGases of *Helianthus tuberosus* meristems with mammal TGases was reported (Beninati et al. 2013).

An increasing number of reports on plant TGase in senescence and PCD, studying both reproductive and veg-etative organs, suggest a strict correlation of TGase and PA physiological effects. Studies have been mainly focused on senescence and death of the leaf (Serafini-Fracassini et al. 2010; Sobieszczuk-Nowicka et al. 2007, 2009; Sobieszc-zuk-Nowicka and Legocka 2014) and of the flower petals (Serafini-Fracassini et al. 2002; Della Mea et al. 2007a, b) or on the growing or abiotic stress-induced dying pollen (Iorio et al. 2012a) as well as self-incompatible (SI) pol-lination system (Del Duca et al. 2010; Gentile et al. 2012) and on the hypersensitive response (IR) to pathogens (Del Duca et al. 2007).

Polyamines in leaf senescence

The leaf ageing/senescence can be divided into three stages: initiation/induction, degradation and terminal phase of ageing/senescence programme that leads to cell death. The initiation phase starts with changes in gene expres-sion profile, first with a group of genes that encoding pro-teins are responsible for the chlorophyll degradation. One of the first morphological symptoms of ageing/senescence within the leaf is its yellowing. Other important changes that take place within the ageing/senescing cell of leaf are ultra-structural modifications that include decay of the cytoskeleton, fragmentation of the endoplasmic reticulum, degradation of ribosomes and of course structural changes within chloroplasts. Structural changes of the chloroplasts, eventually leading to chloroplasts degradation, mark the first phase of a sequential process of leaf senescence, both

developmental one as well as induced by stresses (Sarwat et al. 2013).

The transformation of mature chloroplasts of green leaves to senescing chloroplasts, term gerontoplast, exhibits extensive structural modifications of the thylakoid membranes, which are reported to unstick at the initial stages. This is followed by a gradual loss of membranes with concomitant formation of a large number of plastoglobuli with lipophilic materials. The envelope of the plastid, however, remains intact. Formation of gerontoplasts is synchronized with a functional shift of the leaf from a photosynthetic organ to the organ responsible for mobilisation of nutrients during senescence. Gerontoplasts in senescing leaves export nutrients, primarily nitrogen, to other growing and reproductive parts of the plant. Gerontoplasts develop only from fully mature chloroplasts without any further growth and they gradually lose biosynthetic potential (Thomas et al. 2003). Chlorosis and transition of colour from green to yellow in senescing leaves may indicate variation in the developing status of gerontoplasts. However, it is difficult to define the onset of leaf senescence or initiation of gerontoplast formation. Equally fuzzy is its end-point i.e. death when necrosis takes over followed by collapse of the entire organelle (Thomas et al. 2003).

Within an ageing leaf, the individual cells are usually at many different stages of the process with the distal parts of the leaf ageing first and the cells surrounding the veins tending to remain active longer to maximize transport from the leaf. Thus, the study of ageing has been complicated by the lack of coordinated development of the cells within an individual leaf. Dark-induced senescence has been used frequently as a useful method to induce synchronous senescence as many typical senescence symptoms such as those described above are in common with the ontogeny ageing (Buchanan-Wollaston et al. 2003, 2005; Gregersen et al. 2008). Moreover, in monocotyledonous plants such as cereals, ageing/senescence appears to be regulated mainly at the level of the individual leaf. The leaves of cereals have a basal meristem and the leaf tip consists of the oldest cells, whereas the youngest cells are found at the leaf base (Gregersen et al. 2008). Due to this, cereal leaves are a useful model for studies on the progress/development of the process.

However, the dark-induced senescence has also many features that significantly differ in many key processes that regulate it (Buchanan-Wollaston et al. 2003, 2005); for example, in developmental senescence, photosynthesis continues, although at a reduced rate, and this presumably provides some energy for the process to take place. However, leaves undergoing senescence in the light have to contend with oxidative stress resulting from the degradation products of chlorophyll and other macromolecules. Genes related to flavonoid synthesis show increased expression in developmental senescence only although an alternative pathway of unknown function might be involved in dark-induced senescence. In this model, the main signal for the process could be a rapid reduction in sugar levels, leading to a more significant switch to lipid degradation to supply an energy source. The differences in the C:N balance or the source of carbon skeletons in the light grown tissues compared with the dark treatment may be the reason why these cells synthesize glutamine instead of asparagine for export. Figure 1 presents a model showing the common and alternative processes involved in the events leading to the induction and progress of the ageing and dark-induced senescence.

Leaf senescence is a highly regulated process that concern different transformations, both biochemical and cytological, involving the cessation of photosynthesis, decomposition of chloroplast and disruption of other organelles (nucleus and mitochondria). Moreover, internucleosomal fragmentation of chromatin and condensation of nuclear DNA in the senescent mesophyll leaf indicate that dark-induced senescence is a genetically defined process involving mechanisms associated with PCD (Buchanan-Wollaston et al. 2003). Chloroplasts are the organelles in which senescence changes appear first. A decrease in the levels of endogenous-free PAs has been observed in senescing/ageing leaves, so it is believed that they might have a key role in controlling leaf senescence.

A number of reports show the role of exogenously applied PAs (Cohen et al.1979; Mizrahi and Galston 1989; Besford et al. 1993; Legocka and Zajchert 1999; Serafini-Fracassini et al. 2010) or over-expressed ones (Nambeesan et al. 2010) in delaying senescence of leaf. In par-ticular, Mizrahi and Galston (1989) observed that PAs delayed senescence in oat and *Petunia* leaves and found PAs strongly bound to proteins of high molecular weight, suggesting a role for bound PAs. In oat leaves exposed to osmotic stress, it was found that exogenously applied PAs play an important role in chloroplast degradation (Besford et al. 1993). In this condition, chlorophyll underwent rapid senescence and accumulated large amounts of PU. The exogenous addition of SD or SM inhibited protein degradation, chlorophyll loss and stabilized thylakoid proteins such as D1, D2, cyt f and a large subunit of ribulose-bis-phosphate carboxylase (RuBisCO) essential enzyme for carbon fixation (Besford et al. 1993) as more recently confirmed by Legocka and Zajchert (1999) and Serafini-Fracassini et al. (2010). Cohen et al. (1979), in one of the first reports on PAs' role on senescing leaves wrote: "The polyamines putrescine, spermidine, and spermine prevent the loss of chlorophyll normally associated with senescence of excised leaf tissue maintained in darkness on water. In contrast, the loss of soluble protein was hastened with spermidine and spermine treatments but it was retarded by putrescine.
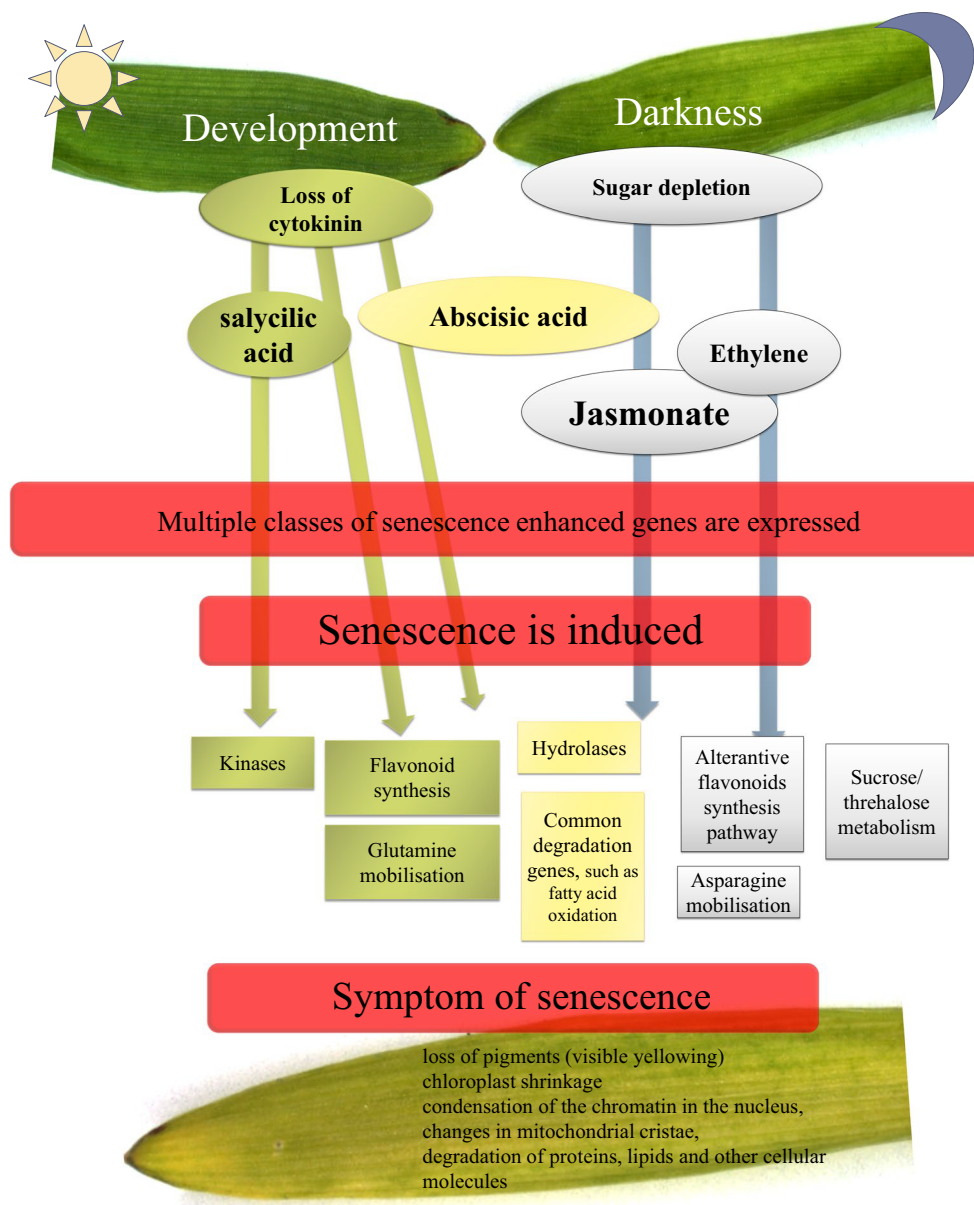
Fig. 1 Model illustrating the similar and alternative pathways that operate in the developmental and dark-induced senescence that lead to symptoms of senescence. Example of genes that are expressed in the different types of senescence are illustrated in *coloured boxes*: *green* for developmental senescence only, *grey* for dark-induced one and *yellow* for the genes that are expressed in both types of senescence. The genes upregulated in each category are also shown (modified from Buchanan-Wollaston et al. 2005)

Photosystem I and II activities from polyamine-treated leaf discs declined more rapidly as compared to the control. Chloroplast ultra-structural changes resulting from the polyamine treatments included the apparent destruction of the envelope, preservation of thylakoid membrane structure, and reduced accumulation of osmiophilic bodies. The influence of polyamines on senescence-related processes may be due to their cationic nature". Treatment of senescing leaves with SD and SM that inhibited the degradation of thylakoids and chlorophyll was described by Legocka and Zajchert (1999) and Serafini-Fracassini et al. (2010).

Like SM, SD added to cut leaves of barley, senescing in darkness, inhibited RNase activity, degradation of chlorophyll and of LHCII protein (Legocka and Zajchert 1999). This effect can be due to the conjugation of PU, SD and SM to the isolated light-harvesting complex ofphotosystem II (LHCII) with different degrees of efficiency, SM being the PA most efficiently conjugated. This is attributed to a light-stimulated activity of thylakoidal Ca^{2+}-dependent TGase identified in the photosystem II-enriched fraction of chloroplasts of maize and *Helianthus* leaves (Della Mea et al. 2004a; Dondini et al. 2003). In the *Helianthus*

chloroplast stroma, also a 150-kDa TGase immunorecognised protein catalysed the conjugation of ^{14}C-polyamines to RuBisCO (Dondini et al. 2003). An indication of the presence of TGase in chloroplasts was at first suggested by Cohen et al. (1982) and detected in leaves by Signorini et al. (1991). Later on, in isolated chloroplasts of H. tuberosus, Ca^{2+}-dependent TGases have been reported to catalyse the conjugation of PAs to both stromal and thylakoid proteins (LHCII, CP29, CP26, CP24, RuBisCO)(Del Duca et al. 1994). It has been suggested that remodel-ling of grana may be possible through over-expression of a TGase and polyaminylation of antenna proteins, and this might play a functional role in the formation of the grana stacks and cause an imbalance between capture and use of light energy (Ortigosa et al. 2010; Ioannidis et al. 2012). Polyamines have been reported to operate on the structure and function of the photosynthetic apparatus during photoadaptation and photoprotection against factors such as UV-B, ozone, etc. (Novakoudis et al. 2007). Ioannidis and Kotzabasis (2014) propose that the physiological role of uncharged PAs could be crucial in chemiosmosis. The authors explain the theory behind PA pumping and ion trapping in acidic compartments (i.e. the lumen of chloroplast) and how this regulatory process could improve either photochemical efficiency and the synthesis of ATP or fine tune antenna regulation and make plants more tolerant to stress.

Exogenous SM caused a delay in degradation of chlorophyll b, of some plastidial proteins and an increase in Ca^{2+}-dependent TGase activity in excised lettuce leaf discs (Serafini-Fracassini et al. 2010). In already senescing Lactuca leaves, left to senesce on the mother plant, SM had a similar effect: it reactivated TGase activity to the level in young leaves following SM treatment, producing more PA-protein conjugates and maintained the leaf in a visible younger state. Spermine was not only conjugated to LHCII but also to proteins of the isolated PSI fractions, whose light-harvesting complexes (LHCI) sub-fractions contained an immunodetected TGase (Serafini-Fracassini et al. 2010). Spermine thus protected senescing Lactuca leaves from the decay of their chloroplast photosystem complexes. The protective effect of SM on chlorophyll degradation could be related to non-enzymatic binding of SM (Dondini et al. 2003) by its free primary amino-group. As chlorophyll b is also linked to a glutamine of LHCII (to which SM is covalently linked by TGase), this binding could further enhance the complex protein-chlorophyll stability and delay its degradation. Immunodetection of TGase revealed multiple proteins in young leaves, possibly representing different TGase isoforms when TGase activity was high, whereas in senescing leaves at the latest stages, when its activity decreased, one high molecular mass band was found, possibly because of enzyme polymerisation (Serafini-Fracassini et al. 2010).

Polyamine biosynthesis in ageing and senescing leaves of Avena sativa L., expressed as activities of arginine decarboxylase (EC 4.1.1.19), ornithine decarboxylase (EC 4.1.1.17), S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) and PA content, showed that arginine decarboxylase activity decreased progressively in ageing as well as in senescing in darkness leaves. Ornithine decarboxylase activity was high and constant in ageing leaf, but it decreased in that kept in the dark. S-adenosyl-L-methionine decarboxylase showed no correlation with age or senescence. Levels of PU, diaminopropane, agmatine and SM were high in young leaves and the levels declined with age. The best single indicator of senescence was SM, which decreased in excised leaves incubated in the dark. Spermine had an inverse relationship with PU, indicating, as the authors concluded, that spermidine synthase, which converts PU to SD, may exert an important physiological role (Kaur-Sawhney et al. 1982). Very recently, Ioannidis et al. (2014) added extra information to this model on up-regulation of mRNA for di- and polyamine oxidases, which point to the participation of PAs' catabolism in ageing.

In the model in which senescence in barley leaves is dark-induced, the activity of TGases and the fate of chloroplast-associated PAs in particular changes in the level of PAs bound to thylakoids in this process are reported (Sobieszczuk-Nowicka et al. 2009). Bound PU and SD showed an increase throughout senescence, whereas bound SM decreased. A different role of PU/SD and SM in the process may be then suggested. As mentioned above, senescence can be delayed by an exogenous sup-ply of PAs, with SM being particularly effective. In gen-eral, SM is known as the most abundant PA conjugated to isolated LHCPII (Della Mea et al. 2004a) and the reac-tion centre of photosystem II (Kotzabasis et al. 1993). The decrease in bound SM during thylakoid degradation could be related to the breakdown of chloroplasts, degradation of LHCPII as well as other proteins of the chlorophyll a/b antenna complexes. In contrast, the increase in bound PU and SD appears to be related to dark-induced senescence and might be partly related to the increase in TGase activ-ity during this process. Using an anti-TGase antibody (Ab-4) three polypeptides were detected in the chloroplasts of senescing barley leaves with the following masses: 33, 58 and 78 kDa (Sobieszczuk-Nowicka et al. 2009). Numer-ous studies have shown that within a single cell and even one organelle, several isoforms of plant TGase can occur (Dondini et al. 2003; Della Mea et al. 2004a; Beninati et al. 2013). The 58 kDa protein was previously identified in the cells of the corolla of tobacco, where its level of expres-sion (as in the case of the barley leaves) decreased over the course of senescence (Della Mea et al. 2007b). However, the characteristics of the 58 kDa form is different from annotated animal TGases, Arabidopsis AtPng1 (Della Mea

et al. 2004b) and two larger isoforms described by Beninati et al. (2013). This isoform of TGase is the most wide-spread throughout the entire cell. Outside of the chloroplasts, it was localised in the microsomes and in the cell wall of flower petals (Della Mea et al. 2007b). The low molecular mass proteins ranging from 33 to 39 kDa have until now only been localised in plastids (Della Mea et al. 2004a, 2007b; Sobieszczuk-Nowicka et al. 2007). With respect to the Western blotting result, the 78-kDa band could be similar to the 77-kDa band cited as a native form of a mammalian TGase, which is cleaved into one fragment containing the active site (about 58 kDa) and another inac-tive fragment around 30 kDa (Griffin et al. 2002). In green control leaves, the levels of 58 and 78 kDa TGases were very low and increased significantly throughout the initiation and progress of the dark-induced senescence. The levels of the TGases were lower when the samples were incu-bated with cytokinin, a PGR known for its anti-senescence properties (Sobieszczuk-Nowicka et al. 2009). Moreover, a colorimetric TGase activity assay, performed in the pres-ence of N',N'-dimethylcasein, as the exogenous substrate, throughout the different stages of senescence, showed that the differences in TGase activity were expected to reflect the changes of the enzyme level. Transglutaminase activity increased continuously through senescence. The data show that there is a positive correlation between the immuno-detectable TGase protein level and its enzymatic activity. Thus, both the level and activity increased throughout the senescence process.

The assay in barley leaves showed changes of TGase activity similar to those in plastid fraction of tobacco flow-ers, where the activity increased in the terminal stage of DCD. Formation of hydrogen peroxide and cytotoxic prod-ucts via PA catabolism is considered as one of the possible mechanisms of PAs' involvement in PCD (Moschou and Roubelakis-Angelakis 2013). Furthermore, Novakoudis et al. (2007) reported on a mechanism that regulates the plasticity and adaptation status of the photosynthetic appa-ratus and found that modification of the structure of LHCII and CPs by any of the PAs can drastically affect their con-formation either stabilising them or exposing them to pro-tease activity.

As a possible hypothesis, enzymes such as proteases, nucleases, those involved in lipid and carbohydrate metab-olism and in nitrogen mobilisation are also activated during senescence. Then, TGases might alter these enzymes' function by "cationisation" and/or cross-linking with PAs, with obvious structural consequences that contribute to the stability of protein structures and changes in their solubility and ability to interact with other molecules (Serafini-Fra-cassini and Del Duca 2008). Conversely, the modification or change in the protein availability elucidated by initiated organelle degradation could affect the PU and SD linkage,

with a possible impact on the protein activity and senescence progress.
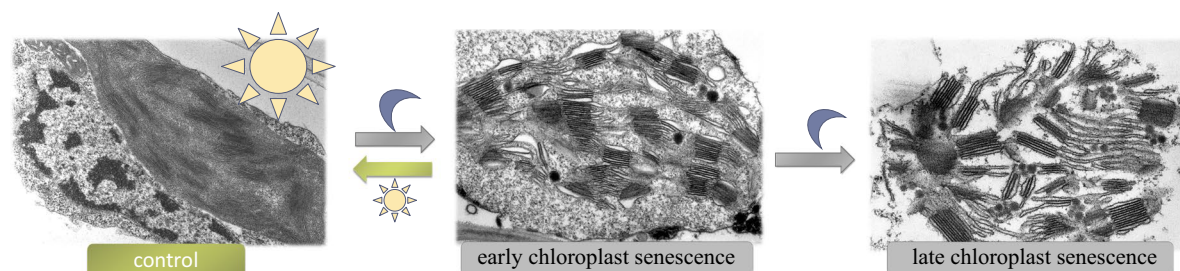
The activity of chloroplast TGases through senescence can be controlled not only by post-translational regulation but also by transcription. It has been shown that the expres-sion pattern for the *HvPng1-like* gene, which was computa-tionally identified because of its high sequence homology to *AtPng1p*, in conditions of undisturbed growth was at a low level. Its expression profile during plant senescence showed that it increased early as the process was dark-induced (Sobieszczuk-Nowicka personal communication). A model summarizing the contribution of PAs in executing the tightly regulated scenario of leaf senescence and their likely contribution in this process is shown in Fig. 2; TGase might be involved in some steps of this scenario.

In conclusion, physiological and structural changes of chloroplasts, associated with the dark-induced senescence of barley leaves and natural or induced senescence of let-tuce leaves, involve PA conjugation and modification of chloroplast proteins, accompanied by modulation of barley chloroplast TGases expression and possibly aggregation of TGases in lettuce leaves. Several other studies, mentioned above, also have shown that the accumulation or reduction of PAs is one of the senescence/PCD symptoms in leaf, but it is not clear whether PAs act as mediators or regulators in this process. Do PAs regulate the leaf senescence by selec-tive change in the level of their free or conjugated form? A possible contribution of transglutaminases in the process should be considered. It is also assumed that, in senescence, PAs may act as free radical scavengers but it has been pro-posed that in a result of oxidative deamination PAs them-selves are a source of free radicals and toxic aldehydes.

The flower corolla PCD (DCD)

A model of DCD which is of great interest is the flower, in which reproductive organs and petals undergo highly reproducible morphological and physiological modification during their life span. Petals, which are modified leaves, have in general a vexillary role in attracting insects for pol-lination and, once this role has been completed, they enter senescence and fall; in some cases, e.g. *Nicotiana*, they remain in situ, become rigid and papyraceous to protect the initial growth of the ovary. Petals consist of parenchyma, thin veins and a protecting layer of epidermis.

Flower petal senescence and death is a highly regu-lated developmental phase, controlled by PAs (Lee et al. 1997; Serafini-Fracassini et al. 2002; Bagni and Tassoni 2006; Della Mea et al. 2007b) and PGRs, including ethyl-ene, cytokinins and abscisic acid (Rubinstein 2000; Rog-ers 2006, 2012; van Doorn and Woltering 2008; Amasino and Michaels 2010). Polyamine modulation of flower

senescence has been studied in models, which are differently sensitive to ethylene (the PGR more related to induction of senescence) including carnation, *Nicotiana* and gerbera. Aliphatic PAs share with ethylene the common precursor *S*-adenosylmethionine (SAM).

In the 'ethylene sensitive' carnation flower, treatment with exogenous 10 mM SD was effective in delaying the progression of senescence: this was marked by a clear-cut peak of free SD, PU and TCA-soluble PAs, suggesting that the PA could then bind to and stabilize essential molecules (e.g. DNA fragmentation was delayed) of the corolla cells (Bagni and Tassoni 2006). Polyamines were also found conjugated to proteins as shown in *Nicotiana* by Serafini-Fracassini et al. (2002). This is a good flower corolla model, whose senescence and death were studied *in planta* or in flowers excised at different growing stages (Serafini-Fracassini et al. 2002; Della Mea et al. 2007a, b) and will be treated here more in detail.

The senescence of *Nicotiana* corolla follows a visible acropetal gradient, completed by the death of the entire corolla that concludes with the teeth curling in the distal part of the flower. The stages of maturation, senescence and death were established macroscopically and the timing and localisation of the most characteristic events were evaluated by biochemical and physiological analyses as well as by cytological observations. Even though precocious signs can be detectable also before, flower petal senescence was characterised by the appearance at its base of a 'ring', named abscission zone (AZ) of dying cells, which detaches from each other and blocks the sap transport. This event is concomitant with nuclear blebbing,

DNA laddering, cell wall modification, a peak of protease activity, decline of protein, water and pigment (anthocyanins, chlorophylls) content, decrease in membrane integrity and increase in Ca^{2+}-dependent TGase activity, detected as increase especially in *mono*-glutamyl-PU and decrease of *bis*-derivatives production (Serafini-Fracassini et al. 2002). The maximum of TGase activity coincides with the appearance of high polymers immunorecognised by TGase antibody and with the flower senescence (Della Mea et al. 2007b). When PAs are present in physiological concentrations, both the glutamine–lysine and glutamine-PA-glutamine cross-links are permitted; if PAs are at a high concentration, the cross-links can be inhibited due to the prevalent formation of mono-glutamyl-PAs' derivatives. These data support the hypothesis that the formation of more cross-linkages among proteins possibly increased the dimension and strength of the protein net. This could be relevant for structural substrates, like cytoskeleton or cell walls. Calcium ions could exert an important regulatory role of the enzyme activity. It is known that in senescing tissues this cation increases in concentration especially because of its release from the vacuole, caused by tonoplast rupture (Van Doorn et al. 2011).

The acropetal gradient of tobacco corolla senescence and DCD was temporally preceded by a maximum of total TGase activity, which shifted from the proximal to the dis-tal part of the corolla, (which was sectioned in three parts, proximal, medial and distal), during the developmental stages ranging from stage 1 (the corolla, still closed, is green) to stage 8 (the corolla is further senescing, losses turgidity and colour; it exhibits an enlarging brown ring
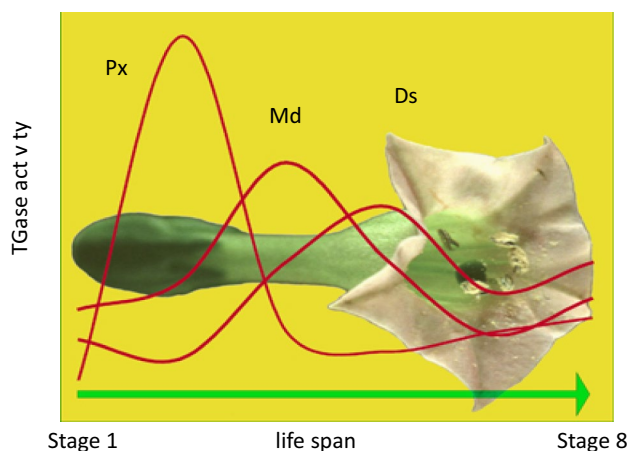
Fig. 3 Acropetal wave of TGase activity during corolla life span. The activity was studied at different developmental stages (1–8) by incubating corolla extracts in the presence of 0.2 mM [^{14}C] SM as tracer and the labelled conjugates were measured in three parts of the sectioned corolla: proximal (Px), medial (Md), and distal (Ds). Modified from Della Mea et al. (2007b)

proceeding acropetally from the AZ) (Fig. 3) (Della Mea et al. 2007b). This activity modified either the endogenous substrates alone or a specific recombinant mammal TGase exogenous substrate (His6-X Press-green fluorescent protein, GFP); the modifications are revealed by changes in their electrophoretic migration, thus of their molecular mass and the PA glutamyl-derivatives produced. The recombinant GFP is a good substrate for TGase, because its electrophoretic shift changes in a similar way after modification by animal and plant TGases (Della Mea et al. 2007a).

The TGase protein bands were immunorecognised by three antibodies raised against mammalian, nematode and *Arabidopsis thaliana* TGases. The fact that the antibody raised against *Arabidopsis* TGase recognises some proteins of *Nicotiana*, also immunodetected by two animal antibodies, and that plant and animal TGases present similar molecular weights and modify GFP in a similar way, would suggest a similarity among these enzymes. The localisation

of TGase in the *Nicotiana* petal cells could suggest new and different roles of this enzyme in PCD in addition to those detected in animal cells. As reported by Della Mea et al. (2007b), a 58-kDa band immunodetected by anti-TGase antibodies (either antibodies developed against the plant TGase AtPng1p or antibodies developed against TGase of animal origin), representing also the prevalent form in leaves, decreased during corolla life and was present in the soluble, microsomal, plastidial and cell wall fractions (Table 1). By contrast, the peak location of a 38-kDa band, mainly a plastidial form, moved progressively from basal to distal parts of the corolla, where it was exclusively present. This 38-kDa putative enzyme could match with a TGase isolated from maize (*Zea mays*) thylakoids (Della Mea et al. 2004a) and with a 39-kDa enzyme detected in chloroplasts of *Medicago sativa* (Kuehn et al. 1991). In senescing barley leaves, similar values for TGase were reported (Sobieszczuk-Nowicka et al. 2007) and treated in the paragraph on leaves in this review. In the soluble fraction, a 52 kDa immuno-positive band was decreasing with age and in late senescence a high (>250 kDa) molecular mass band appeared (Della Mea et al. 2007b), suggesting a binding of the enzyme with a cytoplasmic component (possibly cytoskeleton proteins) or possibly because of enzyme polymerisation as suggested in animals (Lorand and Graham 2003).

Transglutaminase activities were detected in different cell compartments (Della Mea et al. 2007b); activity pre-vailed in the microsome fraction (Table 1), where it is, in general, higher in the proximal part of petals, peaking at the corolla opening, and in the plastids, where it shows an increasing trend. In particular stages of senescence, a low activity was detected also in the cell walls prevailing in the distal part and progressively increasing as well as in the soluble fraction, where it is present only in the proxi-mal part at senescence. The intracellular TGase, possibly released into the cell wall as in pollen (as reported in the present review), was hypothesized to co-operate with cell wall strengthening or modification by protein cross-linking, especially either at the basal abscission zone or distally

Table 1 Transglutaminase immunodetection and activity in *Nicotiana tabacum* flowers

	Micrososomal fr.	Soluble fr.	Plastid fr.	Cell wall fr.
TGase kDa				
>250		X late stages		
58	X		X	X
52		X		
38			X	
TGase max activity (^{14}C SM nmol/h mg prot)	0.45 middle stage	0.15 late stages	0.43 late stages	0.28 late stages

Transglutaminase forms immunodetected with the antibody raised against *Arabidopsis* TGase (AtPng1p) in different cell fractions isolated from *Nicotiana tabacum* corolla during its life span and maximum enzymatic activity (^{14}C SM nmol/h mg prot)

where the teeth curl, outwards during differentiation and then refold at the later stages to protect the developing ovary.

During these morphological events, cytoskeleton and turgor changes play a major role, but are presumably supported by cell wall local strengthening. The walls of the corolla parenchyma cells during senescence undergo modifications evidenced by an increased auto-fluorescence, indicative of its suberification/lignificaton (Serafini-Fracassini et al. 2002) and by the rigid/papyraceous-like aspect of the corolla. Relevant cell wall modifications occur also in cells located in the AZ to prevent the release of toxic substances, desiccation and pathogen attack after corolla abscission; in fact, the tissues around the AZ must be pro-tected by the waterproofing features of the scar. Cell wall could be assimilated to extracellular matrix of senescing animal cells, where TGase stabilize the dying cell and sur-rounding matrix thus maintaining both cellular and tissue integrity or remodelling. Some data on the TGase in plant cell walls are discussed and revised by (Del Duca et al.2014a).

Overall, these data suggest a relationship between DCD and TGase, whose roles are probably different, depending on the function and modification of the compartments in which the enzyme is located. Unfortunately, there are no data on TGase in petal mitochondria; the only data available of a covalent binding of PAs to proteins, tentatively via TGase, in plant mitochondria was obtained in potatoes and mung beans (Votyakova et al. 1999).

In order to evaluate the anti-senescence effects of PAs, detached *Nicotiana* flowers were treated with exogenous SM and with silver thiosulphate (an inhibitor of ethylene action); they showed senescence delay, retarded DNA fragmentation and vacuole damage, prolonged chloroplast viability with visible preservation of chlorophyll content (Serafini-Fracassini et al. 2002). Spermine taken up was also converted back to PU and SD, found either in free or TCA-soluble form. In *Nicotiana,* these conjugates are mainly hydroxycinnamoyl-derivatives, which are known to increase during flowering (Martin-Tanguy et al. 1996), but no evidence is reported of their involvement in senescence.

The anti-DCD effect could be mediated, at least in part, by SM covalent binding to TGase substrates. The supply of PAs causes the formation of high molecular mass products, especially in the presence of an excess of PAs, which cannot be separated by electrophoresis, in addition to different protein bands of lower mass. In animals, many protein substrates were detected among them actin, beta-tubulin, annexin, fibronectin and core and H1 histones and others, which could easily be involved in PCD (Piacentini et al. 2005). These proteins could also be substrates of *Nicotiana* TGase; currently, those identified in plants are actin and tubulin, and photosystem proteins, like LHCII, as well as some cell wall proteins (Serafini-Fracassini and Del Duca 2008; Del Duca et al. 2014a); thus these substrates are located in different cell compartments, exactly like TGase.

The function of exogenous PAs in the regulation of DCD depends on their concentration, precise stage of life of the treated cells and by the duration of the supply. All these factors are determinant in the DCD prevention or promotion, which, at least in part, can be mediated by flower TGases. The flower model also evidences the presence of TGases in different cell compartments and a general increase of their activity according to the progression of the acropetal DCD along the petals.

Polyamines in the pollen tube PCD

The self-incompatibility (SI) process is a mechanism that prevents growth of self-pollen tubes in order to impede self-fertilization and to increase genetic variability. As anticipated above, self-incompatibility is characterised by a mixture of necrotic and vacuolar events. Because of the importance of pollen-pistil interactions for plant reproduction and because of the ease by which pollen tubes can be studied, this cell is an excellent model to study processes related to stress and cell death. The pollen tube is a cell predestined to expire as it dies after transporting the sperm cells to the embryo sac. Once the pollen tube successfully reaches the ovule, it bursts and releases the sperm cells close to the embryo sac allowing fertilization.

The pollen tube grows through the stigma and style by a process known as "tip growth" (Cole and Fowler 2006). During tip growth, secretory vesicles accumulate in the apical area and then fuse with the plasma membrane thereby releasing all the molecules required for cell growth. Because pollen tubes grow following a precise pathway in the pistil, a signal transduction system is required to allow pollen tubes to grow directionally. Inside the pollen tube, a cytoskeleton network implements the information from the signal transduction system and promotes the accumulation of secretory vesicles. The cytoskeleton is a highly dynamic structure whose organisation is timely and spatially controlled by several factors, such as an oscillation in Ca^{2+} concentration at the apex (Cole and Fowler 2006). The process of tip growth is a delicate equilibrium between different factors; therefore, any physical, chemical or biological agent capable of altering this delicate mechanism can affect the growth mechanism with the most dramatic consequence of blocking the fertilization process.

This series of events takes place when the pollen tube is not self, that is when its genetic features are different from the female counterpart. Conversely, when the pollen tube shares genetic characteristics with the pistil, the pollen tube is prevented to grow (gametophytic self-incompatibility).

The so-called sporophytic self-incompatibility requires the genetic features of pollen-surrounding cells be similar to those of pistils. When the SI takes place, plants are prevented to auto-fertilize thereby allowing them to interbreed increasing genetic variability. Therefore, the SI process is controlled at genetic level and is consequently selective (Rea and Nasrallah 2008). When SI occurs, it can be considered a kind of PCD because it is controlled at genetic level and because it takes place in a timely and spatially controlled manner. During the last 20 years of research, several molecular, biochemical and cellular steps have been characterised that are involved in pollen tube rejection. Of the three main mechanisms of SI that are characterised in Angiosperms, two of them operate at the level of pollen tubes, while the third mechanism works at the level of non-germinated pollen.

In the poppy, the SI response requires the recognition between stigmatic S proteins (PrsS) and receptor proteins (PrpS) on the plasma membrane of pollen tubes (Poulter et al. 2010) (Fig. 4a). This process triggers a series of events within the pollen tube such as the increase of Ca^{2+} and the acidification of the apical cytoplasm. These changes inhibit tip growth by affecting the trafficking of organelles and by altering the dynamics of actin filaments (Bosch et al. 2008). The recognition between S proteins induces the activation of at least two pollen proteins, p26 (an inorganic pyrophosphatase) and p52 (a MAP kinase) (Rudd and Franklin-Tong 2003); both proteins are likely responsible for the activation of profilin, an actin-binding protein that regulates actin filament dynamics (Staiger et al. 2010). Profilin is also regulated by Ca^{2+} and by the Ca^{2+}-activated ABP80, a protein related to the gelsolin/villin family that might have a regulatory effect on the assembly of actin fringe (Huang et al. 2004). Other proteins, such as actin-depolymerizing factors (ADF) and CAP (cyclase-associated proteins) might also regulate the dynamics of actin filaments during the SI response in poppy. These proteins are likely important in the production of the so-called "actin foci" (Poulter et al. 2011), irregular aggregates of actin dispersed in the tube cytoplasm that may have a function during SI. Although actin filaments are an important target during SI in poppy, microtubules might also be another potential target (Poulter et al. 2008). The process of SI in poppy proceeds with the activation of caspase-like protease activities.

In the *Solanaceae*, *Rosaceae* and *Plantaginacee*, SI is based on the presence of S-RNases, small proteins with RNAse activity that are produced by the pistil and internalized in the pollen tube by either direct absorption or endocytosis (Wang et al. 2003). An example of this mechanism of self-incompatibility is shown in the *Maloideae*. In pear pollen tubes, SI starts with the internalization of specific S-RNases (Liu et al. 2007) (Fig. 4b). During incompatible pollination, internalized S-RNases are likely released from vacuoles in the cytoplasm where they degrade mRNA (Goldraij et al. 2012). Blocking the growth of incompatible pollen tubes would not depend exclusively on the degradation of mRNA because the SI response also affects the activity of mitochondria thereby changing the production of ROS (reactive oxygen species) (Wang et al. 2010). Reactive oxygen species may in turn affect the levels of Ca^{2+} causing changes to actin filaments (Liu et al. 2007). Disorganisation of actin filaments would lead to the breakdown of vacuoles thereby releasing S-RNase in the cytoplasm. The role of microtubules in the SI response is unclear but data obtained in apple indicate that microtubules could drive endocytotic membranes towards the vacuoles in order to release S-RNase in the pollen tube cytoplasm (Meng et al. 2014).

Within the molecular network controlling SI, PAs and their metabolizing enzyme TGase are known to be involved although the molecular details are only partially known. Transglutaminase (especially the extracellular form) is involved in the apical growth of pollen tubes by taking place in the construction of the cell wall and possibly in the interaction between pollen tubes and pistils (Di Sandro et al. 2010). Apart from the extracellular form, the *Malus domestica* pollen contains cytosolic variants of TGase that can cross-link actin and tubulin thereby generating products with a higher molecular mass (Del Duca et al. 2009). This Ca^{2+}-dependent enzymatic activity may destabilize the cytoskeleton affecting considerably many processes of the pollen tube, such as organelle and vesicle trafficking.

In the pollen of *M. domestica*, two polypeptides with a mass of 70 and 75 kDa were identified by immunoblot-ting with monoclonal antibodies (ID-10, ABIII monoclonal antibody and the AtPng1p polyclonal antibody directed to *Arabidopsis* TGase (Della Mea et al. 2004a, b; Di Sandro et al. 2010; Del Duca et al. 2013b) against heterologous TGase. These proteins are able to cross-link both actin and tubulin thereby generating a number of products with a higher molecular mass (from 90 to 160 kDa). An addi-tional 55 kDa immunoreactive polypeptide of the cell wall fraction has a similar molecular mass as an active TGase extracted from the *Nicotiana* petal cell wall as reported above (Del Duca et al. 2009). The extracellular TGase is involved in the apical growth of pollen tubes; as both spe-cific inhibitors and monoclonal antibodies against TGase can block the growth of pollen tubes. Pollen tube TGase may play a role in the construction of the cell wall and in the interaction between pollen tubes and styles during ferti-lization (Di Sandro et al. 2010).

Data on the relationship between TGase and SI have been also obtained in *Citrus* (Gentile et al. 2012). In this species, the level of conjugated PAs and the TGase activity increase during incompatible pollination coinciding with the appearance of SI (Gentile et al. 2012). In pear, TCA-insoluble PAs increase after fertilization, while SM and PU
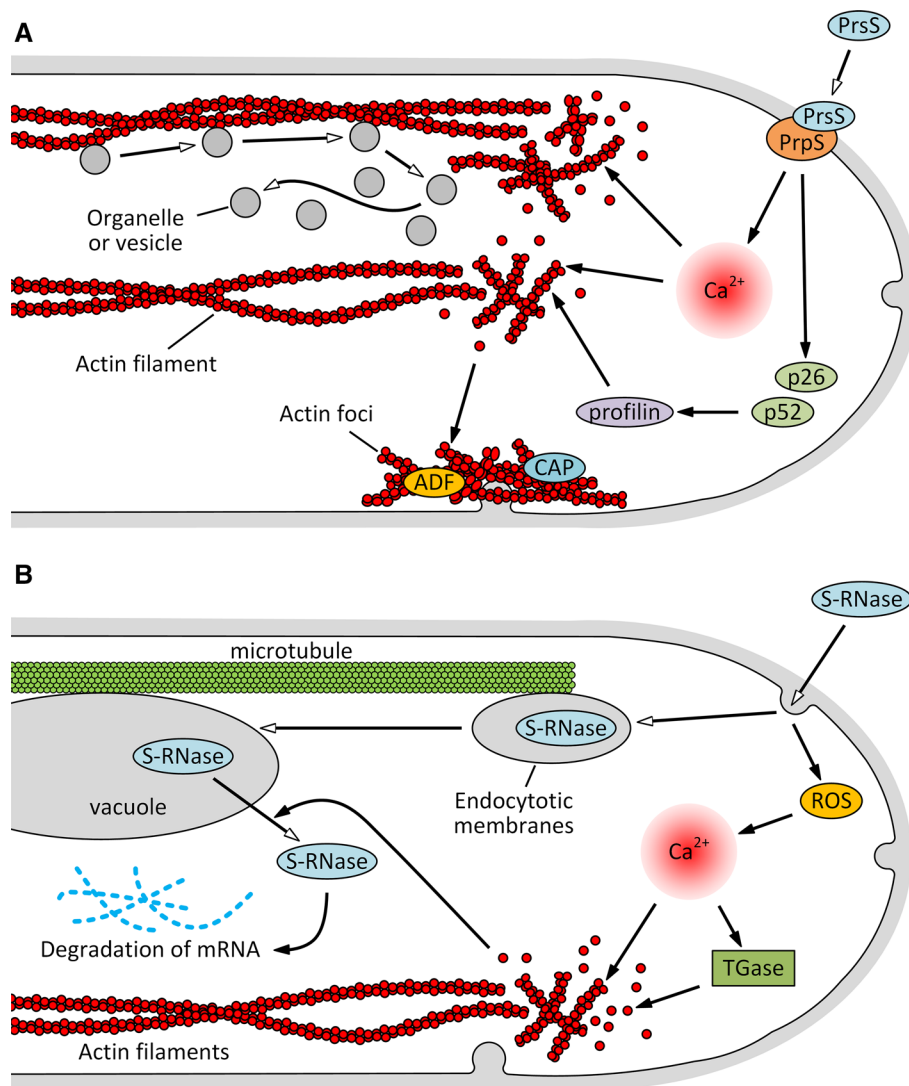
Fig. 4 Schematic illustration of the main components involved in the incompatibility response in poppy (**a**) and pear (**b**). In the poppy **a**, the incompatibility response starts with the recognition between female S proteins (PrsS) and pollen receptor-like proteins (PrpS). The recognition event triggers different effects in the pollen tube, from the increase of Ca^{2+} concentration to the activation of specific proteins (p26, p52). Combination of Ca^{2+} and p26/p52-activated profilin alters the dynamics of actin filaments by inducing depolymerization and the formation of actin foci. The latter also contains additional actin-binding proteins such as ADF and CAP. These injuries likely affect the dynamics of organelles and vesicles leading to tube arrest. In the pear **b**, the incompatibility process requires S-RNases, which are likely incorporated into the pollen tube by endocytosis. The microtubular cytoskeleton is apparently important in delivering S-RNases to the vacuolar compartment. Once S-RNases are released into the cytosol by the breaking of vacuole membrane, S-RNases can degrade the mRNA. In parallel with RNA degradation, the entry or the recognition of S-RNases can alter the level of ROS and consequently of Ca^{2+}, thus leading to changes of actin dynamics. Those changes can be also critical for releasing S-RNases from vacuoles. Transglutaminases might be part of this mechanism as they are Ca^{2+}-activated enzymes that can post-translationally modify actin and tubulin (the latter not shown). *Open* (*white*) *arrows* indicate movement while *solid* (*black*) *arrows* indicate either activation or inhibition

are more abundant in incompatible pollination (Del Duca et al. 2010) comparably to the increase of TGase activity observed in *Citrus*. The increase of TGase activity during incompatible pollination in pear is likely dependent on the increase of intracellular Ca^{2+} concentration rather than on a higher gene expression (Iorio et al. 2012b; Wang et al. 2010). This finding is in line with the evidence that

TGase is a Ca^{2+}-dependent enzyme; therefore, SI-induced changes of Ca^{2+} concentration may significantly alter the enzymatic activity of TGase. A further indication that TGase may function during SI response is also exemplified by evidence that cytoplasmic TGase of apple pollen can post-translationally modify actin and tubulin by conjugating them with PAs (Del Duca et al. 1997). Such activity

produces high molecular weight aggregates (Del Duca et al. 2009) that alter the dynamic properties of cytoskeletal filaments and their interaction with motor proteins. We may speculate that TGase actively takes part during SI response by reorganizing the cytoskeleton. The cytoskeleton-mod-ifying activity of TGase may also depend on changes in Ca^{2+} concentration occurring at the onset of SI (Liu et al. 2007). The above-mentioned steps are partially hypotheti-cal but they suggest that the rejection of self-incompatible pollen may share molecular mechanisms among different families, such as poppy and pear.

Transglutaminase may have an additional role during SI response. In incompatible pollen tubes of pear, TGase forms a "cap" in the apex of incompatible pollen tubes (Del Duca et al. 2010); this feature also occurs in *Citrus*. Hypothetically, TGase might increase the rigidity of the apical cell wall thereby counteracting turgor pressure and preventing pollen tubes to grow further. TGase also accumulates occasionally in the form of aggregates in the pollen tube cell wall (Iorio et al. 2008; Di Sandro et al. 2010; Del Duca et al. 2013b). These aggregates could putatively represent "reaction centers" involved in the modification of specific pollen cell wall glycoproteins and polysaccharides. This putative role may indirectly be supported by co-localisation between TGase and other specific cell wall components such as arabinogalactans and pectin (Del Duca et al. 2013b). The process of TGase secretion in the cell wall is not known at the molecular level and it may require non-canonical mechanisms (Del Duca et al. 2013a). Once secreted in the cell wall, TGase could modify the cell wall texture during SI leading to growth arrest of incompatible pollen tubes.

Conclusions

In all models of plant PCD described here, TGase appears to be involved in a way to some extent similar to some of those described during apoptosis in animal cells, in particular when TGase catalyses the post-translational modification of proteins by transamidation and formation of protein cross-links in which PAs may be involved. As this enzymatic activity is Ca^{2+}-dependent, it might be regulated, like in animal cells, by the changes of this cation occurring during plant senescence and PCD progression (Van Doorn et al. 2011); Ca^{2+} increase was shown to occur also in pollen tube during SI (Liu et al. 2007).

The effect of TGase is probably related to the type of plant PCD, but mostly to the substrate to be modified in order to achieve a specific PCD programme. TGase activity increases during natural senescence of the papyraceous petals of *Nicotiana,* whose cell walls become probably lignified or suberified, as well as during the dark-induced

senescence in leaves in which chloroplast became gerontoplast and the photosynthetic apparatus is progressively dismantled. In the model represented by SI pollination, the TGase activity increased when compared to compatible pollination and its action concerns mainly the post-translational modification of cytoskeleton proteins such as microfilaments and microtubules. Moreover, during the SI, the pollen apex is covered by a thick cap in which TGase localises thereby building a protein network that contributes to arrest pollen tube elongation; then, the pollen tube is driven towards a PCD pathway. As reported in the models of PCD here presented, differential subcellular localisations of TGases were highlighted; it was shown that different TGase isoforms were present in distinct subcellular compartments, suggesting either different roles or different regulatory mechanisms of enzyme activity (Della Mea et al. 2004a, b; Del Duca et al. 2013a, b). On the basis of the current literature in plant PCD, we cannot state, like in animals, that differential localisation, conformation and activities of TG2 may distinctly mediate cell death pro-cesses (Gundemir et al. 2012).

In parallel with the TGase activity, the type of PA, as substrate of TGase and involved in PCD, needs to be considered. For example, in dark-induced senescing leaves, a different role of PU/SD and SM in the process is suggested. The decrease in bound SM during thylakoid degradation could be related to the breakdown of chloroplasts, whereas the increase in bound PU and SD appears to be related to dark-induced senescence and might be partly related to the increase in TGase activity during this process. The modification of protein substrates by the conjugation of PAs via TGase can drastically affect their conformation—either stabilising or exposing them to protease activity. What about the effects of exogenous supply of PAs? The function of exogenous PAs in the regulation of PCD depends on their concentration, on precise life stage of treated cells and on the duration of supply. All these factors are determinant in the prevention or promotion of PCD.

Finally yet importantly, an aspect to be considered is the possible role that PAs could exert in modulating gene expression. In recent years, more and more works report on the regulatory role of PAs in gene expression during plant growth and development (Mehta et al. 2002; Imai et al. 2006; Various authors 2010, Ioannidis et al. 2014), the rea-son why they are attractive subjects of senescence research.

Conflict of interest
The authors declare that they have no conflict of interest.

References

Alcázar R, Tiburcio AF (2014) Plant polyamines in stress and development: an emerging area of research in plant sciences. Front Plant Sci. 5:319. doi:10.3389/fpls.2014.00319

Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta 231:1237–1249

Amasino RM, Michaels SD (2010) The timing of flowering. Plant Physiol 154:516–520

Bagni N, Tassoni A (2001) Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants. Amino Acids 20:301–317

Bagni N, Tassoni A (2006) The role of polyamines in relation to flower senescence. In: Floriculture Ornamental, Teixeira da Silva JA (eds) Floriculture, ornamental and plant biotechnology. Global Science Books, Ltd, Isleworth, pp 88–95

Beninati S, Bergamini CM, Piacentini M (2009) An overview of the first 50 years of transglutaminase research. Amino Acids 36:591–598

Beninati S, Iorio RA, Tasco G, Serafini-Fracassini D, Casadio R, Del Duca S (2013) Expression of different forms of transglutaminases by immature cells of Helianthus tuberosus sprout apices. Amino Acids 44:271–283

Besford RD, Richardson CM, Campos JL, Tiburcio AF (1993) Effect of polyamines on stabilization complexes in thylakoid membranes of osmotically stressed oat leaves. Planta 189:201–206

Bosch M, Poulter S, Vatovec S, Franklin-Tong VE (2008) Initiation of programmed cell death in self-incompatibility: role for cytoskeleton modifications and several caspase-like activities. Mol Plant 146:1358–1367

Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpou S, Page T, Pink D (2003) The molecular analysis of plant senescence—a genomics approach. Plant Biotechnol J 1:3–22

Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T, Pink D (2005) The molecular analysis of plant senescence—a genomic approach. Plant Biotechnol J 1:3–22

Campos N, Castañón S, Urreta I, Santos M, Torné JM (2013) Rice transglutaminase gene: identification, protein expression, functionality, light dependence and specific cell location. Plant Sci 206:97–110

Cohen AS, Popovic R, Zalik S (1979) Effects of polyamines on chlorophyll and protein content, photochemical activity, and chloroplast ultrastructure of barley leaf discs during senescence. Plant Physiol 64:717–720

Cohen SS, Marcu D, Balint R (1982) Light-dependence fixation of polyamines into chloroplasts of chinese cabbage. FEBS Lett 141:93–97

Cole RA, Fowler JE (2006) Polarized growth: maintaining focus on the tip. Curr Opin Plant Biol 9:579–588

Del Duca S, Serafini-Fracassini D (2005) Transglutaminases of higher, lower plants and fungi. In: Metha K, Eckert R (eds) Transglutaminases: the family of enzymes with diverse functions. Karger Publishers, Basel, pp 223–247

Del Duca S, Tidu V, Bassi R, Serafini-Fracassini D, Esposito C (1994) Identification of transglutaminase activity and its substrates in isolated chloroplast of Helianthus tuberosus. Planta 193:283–289

Del Duca S, Bregoli AM, Bergamini C, Serafini-Fracassini D (1997) Transglutaminase-catalyzed modification of cytoskeletal proteins by polyamines during the germination of *Malus domestica* pollen. Sex Plant Reprod 10:89–95

Del Duca S, Betti L, Trebbi G, Serafini-Fracassini D, Torrigiani P (2007) Transglutaminase activity changes during the hypersensitive reaction, a typical defense response of tobacco NN plants to TMV. Physiol Plant 131:241–250

Del Duca S, Serafini-Fracassini D, Bonner PL, Cresti M, Cai G (2009) Effects of post-translational modifications catalyzed by pollen transglutaminase on the functional properties of microtubules and actin filaments. Biochem J 418:651–664

Del Duca S, Cai G, Di Sandro A, Serafini-Fracassini D (2010) Compatible and self-incompatible pollination in *Pyrus communis* displays different polyamine levels and transglutaminase activity. Amino Acids 38:659–667

Del Duca S, Serafini-Fracassini D, Cai G (2013a) An unconventional road for the secretion of transglutaminase in pollen tubes? Plant Signal Behav 8:e24446

Del Duca S, Faleri C, Iorio RA, Cresti M, Serafini-Fracassini D, Cai G (2013b) Distribution of transglutaminase in pear pollen tubes in relation to cytoskeleton and membrane dynamics. Plant Physiol 161:1706–1721

Del Duca S, Verderio E, Serafini-Fracassini D, Iorio R, Cai G (2014) The plant extracellular transglutaminase: what mammal analogues tell. Amino Acids 46:777–792

Del Duca S, Serafini-Fracassini D, Cai G (2014) Senescence and programmed cell death in plants: polyamine action mediated by transglutaminase. Plant metabolism and chemodiversity. Front Plant Sci 5:120. doi:10.3389/fpls.2014.00120

Della Mea M, Di Sandro A, Dondini L, Del Duca S, Vantini F, Bergamini C, Bassi R, Serafini-Fracassini D (2004a) A *Zea mays* 39-kDa thylakoid transglutaminase catalyses the modification by polyamines of light-harvesting complex II in a light-dependent way. Planta 219:754–764

Della Mea M, Caparros-Ruiz D, Claparols I, Serafini-Fracassini D, Rigau J (2004b) AtPng1p. The first plant transglutaminase. Plant Physiol 135:2046–2054

Della Mea M, Serafini-Fracassini D, Del Duca S (2007a) Programmed cell death: similarities and differences in animals and plants. A flower paradigm. Amino Acids 33:395–404

Della Mea M, De Filippis F, Genovesi V, Serafini-Fracassini D, Del Duca S (2007b) The acropetal wave of developmental cell death (DCD) of tobacco corolla is preceded by activation of transglutaminase in different cell compartments. Plant Physiol 144:1211–1222

Di Sandro A, Del Duca S, Verderio E, Hargreaves A, Scarpellini A, Cai G, Cresti M, Faleri C, Iorio R, Hirose S (2010) An extracellular transglutaminase is required for apple pollen tube growth. Biochem J 429:261–271

Diepold A, Li G, Lennarz WJ, Nürnberger T, Brunner F (2007) The Arabidopsis AtPNG1 gene encodes a peptide: *N*-glycanase. Plant J 52:94–104

Dondini L, Del Duca S, Dall'Agata L, Bassi R, Gastaldelli M, Della Mea M, Di Sandro A, Claparols I, Serafini-Fracassini D (2003) Suborganellar localisation and effect of light on *Helianthus tuberosus* chloroplast transglutaminase and their substrates. Planta 217:84–95

Fesus L, Thomazy V, Autuori F, Ceru MP, Tarcsa E, Piacentini M (1989) Apoptotic hepatocytes become insoluble in detergents and chaotropic agents as a result of transglutaminase action. FEBS Lett 245:150–154

Folk JE (1980) Transglutaminases. Annu Rev Biochem 49:517–531

Galston AW, Kaur-Sawhney R (1990) Polyamines in plant physiology. Plant Physiol 94:406–410

Gentile A, Antognoni F, Iorio RA, Distefano G, Las Casas G, La Malfa S, Serafini-Fracassini D, Del Duca S (2012) Polyamines and transglutaminase activity are involved in compatible and self-incompatible pollination of *Citrus grandis*. Amino Acids 42:1025–1035

Goldraij A, Roldán JA, Rojas HJ (2012) Early F-actin disorganization may be signaling vacuole disruption in incompatible pollen tubes of *Nicotiana alata*. Plant Sign Behav 7:1695–1697

Gregersen P, Holm PB, Krupinska K (2008) Leaf senescence and nutrient remobilisation in barley and wheat. Plant Biol 10:37–49

Griffin M, Verderio E (2000) Tissue transglutaminase in cell death. In: Bryant JA, Hughes SG, Garland JM (eds) Programmed cell death in animals and plants. BIOS Scientific, Oxford, pp 223–240

Griffin M, Casadio R, Bergamini CM (2002) Transglutaminases: nature's biological glues. Biochem J 368:377–396

Gundemir S, Colak G, Tucholski J, Johnson GV (2012) Transglutaminase 2: a molecular Swiss army knife. Biochim Biophys Acta 1823:406–419

Hanzawa Y, Takahashi T, Michael AJ, Burtin D, Long D, Pineiro M, Coupland G, Komeda Y (2000) ACAULIS5, an Arabidopsis gene required for stem elongation, encodes a spermine synthase. EMBO J 19:4248–4256

Huang S, Blanchoin L, Chaudhry F, Franklin-Tong VE, Staiger CJ (2004) A gelsolin-like protein from *Papaver rhoeas* pollen (PrABP80) stimulates calcium-regulated severing and depolymerization of actin filaments. J Biol Chem 279:23364–23375

Imai A, Hanzawa Y, Komura M, Yamamoto KT, Komeda Y, Takahashi T (2006) The dwarf phenotype of the Arabidopsis acl5 mutant is suppressed by a mutation in an upstream ORF of a bHLH gene. Development 133:3575–3585

Ioannidis NE, Kotzabasis K (2014) Polyamines in chemiosmosis in vivo: a cunning mechanism for the regulation of ATP synthe-sis during growth and stress. Front Plant Sci 5:71. doi:10.3389/fpls.2014.00071

Ioannidis NE, Lopera O, Santos M, Torné JM, Kotzabasis K (2012) Role of plastid transglutaminase in LHCII polyamination and thylakoid electron and proton flow. PLoS One 7:e41979

Ioannidis NE, Zschiesche W, Barth O, Kotakis C, Navakoudis E, Humbeck K, Kotzabasis K (2014) The genetic reprogramming of polyamine homeostasis during the functional assembly, maturation, and senescence—specific decline of the photosynthetic apparatus in *Hordeum vulgare*. J Plant Grow Regul 33:77–90

Iorio RA, Di Sandro A, Scarpellini A, Del Duca S, Serafini-Fracassini D, Verderio EA (2008) Visualisation of transglutaminase-mediated cross-linking activity in germinating pollen by laser confocal microscopy. Plant Biosys 142:360–365

Iorio R, Di Sandro A, Paris R, Pagliarani G, Tartarini S, Ricci G, Serafini-Fracassini D, Verderio E, Del Duca S (2012a) Simulated environmental criticalities affect transglutaminase of *Malus* and *Corylus* pollens having different allergenic potential. Amino Acids 42:1007–1024

Iorio RA, Serafini-Fracassini D, Aloisi I, Del Duca S, De Franceschi P, Dondini L, Sansavini S, Cai G, Faleri C (2012b) Post-translational modification by transglutaminase of proteins involved in pear self-incompatibility. Acta Hort 967:141–148

Kaur-Sawhney R, Shih LM, Flores HE, Galston AW (1982) Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol 69:405–410

Kotzabasis K, Fotinou C, Roubelakis-Angelakis KA, Ghanotakis D (1993) Polyamines in the photosynthetic apparatus. Photosynth Res 38:83–88

Kuehn GD, Sotelo M, Morales T, Bruce-Carver MR, Guzman E, Margosiak SA (1991) Purification and properties of transglutaminase from *Medicago sativa* L. (alfalfa). FASEB J 5:A1510

Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines:essential factors for growth and survival. Planta 228:367–381

Lee TM, Lur HS, Chu C (1997) Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings.: II. Modulation of free polyamine levels. Plant Sci 126:1–10

Legocka J, Zajchert I (1999) Role of spermidine in the stabilization of the apoprotein of the light-harvesting chlorophyll a/b-protein complex of photosystem II during leaf senescence process. Acta Physiol Plant 21:127–132

Lester GE (2000) Polyamines and their cellular anti-senescence properties in honey dew muskmelon fruit. Plant Sci 160:105–112

Ling D, Marshall GM, Liu PY, Xu N, Nelson CA, Iismaa SE, Liu T (2012) Enhancing the anticancer effect of the histone deacetylase inhibitor by activating transglutaminase. Eur J Cancer 48:3278–3287

Liu Zq XuGh, Sl Zhang (2007) *Pyrus pyrifolia* stylar S-RNase induces alterations in the actin cytoskeleton in self-pollen and tubes in vitro. Protoplasma 232:61–67

Lorand L, Graham RM (2003) Transglutaminases: crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol 4:140–156

Martin-Tanguy J, Sun LY, Burtin D, Vernoy R, Rossin N, Tepfer D (1996) Attenuation of the phenotype caused by the root-inducing, left-hand, transferred DNA and its rolA gene (correlations with changes in polyamine metabolism and DNA methylation). Plant Physiol 111:259–267

Masahara-Negishi Y, Hosomi A, Della Mea M, Serafini-Fracassini D, Suzuki T (2012) A plant peptide: *N*-glycanase orthologue facilitates glycoprotein ER-associated degradation in yeast. Biochim Biophys Acta 1820:1457–1462

Mehta K, Eckert RL (2005) Transglutaminases: family of enzymes with diverse functions. In: Bertino JR (ed) Progress in experimental tumor research. Karger Publishers, Basel, pp 1–247

Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality and vine life. Nat Biotechnol 20:613–618

Meng D, Gu Z, Yuan H, Wang A, Li W, Yang Q, Zhu Y, Li T (2014) The microtubule cytoskeleton and pollen tube Golgi-vesicle system are required for in vitro S-RNase internalization and gametic self incompatibility in apple. Plant Cell Physiol 55:977–989

MizrahiY Applewhite PB, Galston AW (1989) Polyamine binding to proteins in oat and petunia protoplasts. Plant Physiol 91:738–743

Moschou PN, Roubelakis-Angelakis KA (2013) Polyamines and programmed cell death. J Exp Bo 3:1061–1066

Muñiz L, Minguet EG, Singh SK, Pesquet E, Vera-Sirera F, Moreau-Courtois CL, Carbonell J, Blázquez MA, Tuominen H (2008) ACAULIS5 controls Arabidopsis xylem specification through the prevention of premature cell death. Development 135:2573–2582

Nambeesan S, Datsenka T, Ferruzzi MG, Malladi A, Mattoo AK, Handa AK (2010) Overexpression of yeast spermidine synthase impacts ripening, senescence and decay symptoms in tomato. Plant J 63:836–847

Novakoudis E, Vrentzou K, Kotzabasis K (2007) A polyamine- and LHCII protease activity-based mechanism regulates the plasticity and adaptation status of the photosynthetic apparatus. Biochim Biophys Acta 1767:261–271

Ortigosa SM, Díaz-Vivancos P, Clemente-Moreno MJ, Pintó-Marijuan M, Fleck I, Veramendi J, Santos M, Hernandez JA, Torné JM (2010) Oxidative stress induced in tobacco leaves by chloroplast over-expression of maize plastidial transglutaminase. Planta 232:593–605

Paschalidis KA, Moschou PN, Toumi I, Roubelakis-Angelakis KA (2009) Polyamine anabolic/catabolic regulation along the woody grapevine plant axis. J Plant Physiol 166:1508–1519

Piacentini M, Amendola A, Ciccosanti F, Falasca L, Farrace M, Mastroberardino P, Nardacci R, Oliverio S, Piredda L, Rodolfo C, Autuori F (2005) Type 2 transglutaminase and cell death. Prog Exp Tumor Res 38:58–74

Pottosin I, Shabala S (2014) Polyamines control of cation transport across plant membranes: implications for ion homeostasis and abiotic stress signaling. Front Plant Sci 5:154. doi:10.3389/fpls.2014.00154

Poulter NS, Vatovec S, Franklin-Tong VE (2008) Microtubules are a target for self-incompatibility signaling in Papaver pollen. Plant Physiol 146:1358–1367

Poulter N, Wheeler M, Bosch M, Franklin-tong V (2010) Self-incompatibility in *Papaver*: identification of the pollen S-determinant PrpS. Biochem Soc Trans 38:588–592

Poulter NS, Bosch M, Franklin-Tong VE (2011) Proteins implicated in mediating self-incompatibility-induced alterations to the actin cytoskeleton of *Papaver* pollen. Ann Bot 108:659–675

Rea AC, Nasrallah JB (2008) Self-incompatibility systems: barriers to self-fertilization in flowering plants. Int J Dev Biol 52:627–636

Rogers HJ (2006) Programmed cell death in floral organs: how and why do flowers die? Ann Bot 97:309–315

Rogers HJ (2012) From models to ornamentals: how is flower senescence regulated? Plant Mol Biol 82:563–574

Rubinstein B (2000) Regulation of cell death in flower petals. Plant Mol Biol 44:303–318

Rudd JJ, Franklin-Tong VE (2003) Signals and targets of the selfincompatibility response in pollen of *Papaver rhoeas*. J Exp Bot 54:141–148

Sarwat M, Naqvi AR, Ahmad P, Ashraf M, Akram NA (2013) Phytohormones and microRNAs as sensors and regulators of leaf senescence: assigning macro roles to small molecules. Biotechnol Adv 31:1153–1171

Seiler N, Raul F (2005) Polyamines and apoptosis. J Cell Mol Med 9:623–642

Serafini-Fracassini D, Del Duca S (2008) Transglutaminases: widespread cross-linking enzymes in plants. Ann Bot 102:145–152

Serafini-Fracassini D, Del Duca S, Monti F, Poli F, Sacchetti G, Bregoli AM, Biondi S, Della Mea M (2002) Transglutaminase activity during senescence and programmed cell death in the corolla of tobacco (*Nicotiana tabacum*) flowers. Cell Death Differ 9:309–321

Serafini-Fracassini D, Della Mea M, Tasco G, Casadio R, Del Duca S (2009) Plant and animal transglutaminases: do similar functions imply similar structures? Amino Acids 36:643–657

Serafini-Fracassini D, Di Sandro A, Del Duca S (2010) Spermine delays leaf senescence in *Lactuca sativa* and prevents the decay of chloroplast photosystems. Plant Physiol Biochem 48:602–611

Signorini M, Beninati S, Bergamini C (1991) Identification of transglutaminase activity in the leaves of silver beet (*Beta vulgaris* L.). J Plant Physiol 137:547–552

Sobieszczuk-Nowicka E, Legocka J (2014) Plastid-associated polyamines: their role in differentiation, structure, functioning, stress response and senescence. Plant Biol 16:297–305

Sobieszczuk-Nowicka E, Di Sandro A, Del Duca S, Serafini-Fracassini D, Legocka J (2007) Plastid-membrane-associated polyamines and thylakoid transglutaminases during etioplast-to-chloroplast transformation stimulated by kinetin. Physiol Plant 130:590–600

Sobieszczuk-Nowicka E, Wieczorek P, Legocka J (2009) Kinetin affects the level of chloroplast polyamines and transglutaminase activity during senescence of barley leaves. Acta Biochim Pol 56:255–259

Staiger CJ, Poulter NS, Henty JL, Franklin-Tong VE, Blanchoin L (2010) Regulation of actin dynamics by actin-binding proteins in pollen. J Exp Bot 61:1969–1986

Tee AE, Marshall GM, Liu PY, Xu N, Haber M, Norris MD, Iismaa SE, Liu T (2010) Opposing effects of two tissue transglutaminase protein isoforms in neuroblastoma cell differentiation. J Biol Chem 285:3561–3567

Thomas H, Ougham HJ, Wagstaff C, Stead AD (2003) Defining senescence and death. J Exp Bot 54:1127–1132

Tong W, Yoshimoto K, Kakehi JI, Motose H, Niitsu M, Takahashi T (2014) Thermospermine modulates expression of auxin-related genes in Arabidopsis. Front Plant Sci 5:94. doi:10.3389/fpls.2014.00094

van Doorn WG, Woltering EJ (2008) Physiology and molecular biology of petal senescence. J Exp Bot 59:453–480

van Doorn WG, Woltering EJ (2010) What about the role of autophagy in PCD? Trends Plant Sci 15:1361–1362

Van Doorn WG, Beers EP, Dangl JL, Franklin-Tong VE, Gallois P, Hara-Nishimura I, Jones AM, Kawai-Yamada M, Lam E, Mundy J (2011) Morphological classification of plant cell deaths. Cell Death Differ 18:1241–1246

Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P (2014) Regulated necrosis: the expanding network of non-apoptotic cell death pathways. Nat Rev Mol Cell Biol 15:135–147

Vanlerberghe GC (2013) Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. Int J Mol Sci 14:6805–6847

Various authors (2010) Special issue on polyamines dedicated to the memory of Nello Bagni. Plant Physiol Biochem 48(7):487–634

Vera-Sirera F, Minguet EG, Singh SK, Ljung K, Tuominen H, Blázquez MA, Carbonell J (2010) Role of polyamines in plant vascular development. Plant Physiol Biochem 48:534–539

Villalobos E, Santos M, Talavera D, Rodriguez-Falcon M, Torné JM (2004) Molecular cloning and characterization of a maize transglutaminase complementary DNA. Gene 336:93–104

Votyakova TV, Wallace HM, Dunbar B, Wilson SB (1999) The covalent attachment of polyamines to proteins in plant mitochondria. Eur J Biochem 260:250–257

Wang Y, Wang X, Skirpan AL, Kao TH (2003) S-RNase-mediated self-incompatibility. J Exp Bot 54:115–122

Wang CL, Wu J, Xu GH, Gao YB, Chen G, Wu JY, Wu HQ, Zhang SL (2010) S-RNase disrupts tip-localized reactive oxygen species and induces nuclear DNA degradation in incompatible pollen tubes of *Pyrus pyrifolia*. J Cell Sci 123:4301–4309

Wang Y, Loake GJ, Chu C (2013) Cross-talk of nitric oxide and reactive oxygen species in plant programed cell death. Front Plant Sci 4:314

Watanabe N, Lam E (2011) Arabidopsis metacaspase 2d is a positive mediator of cell death induced during biotic and abiotic stresses. Plant J 66:969–982

Wu X, Kuai B, Jia J, Jing H (2012) Regulation of leaf senescence and crop genetic improvement. J Int Plant Biol 54:936–952

Ye Y, Li Z, Xing D (2013) Nitric oxide promotes MPK6-mediated caspase-3- like activation in cadmium-induced Arabidopsis thaliana programmed cell death. Plant Cell Environ 36:1–15

Yoda H, Hiroi Y, Sano H (2006) Polyamine oxidase is one of the key elements for oxidative burst to induce programmed cell death in tobacco cultured cells. Plant Physiol 142:193–206