International Journal of Plant and Environment
Vol. 4, No. 1 (January, 2018), 76-90

Polyamines Metabolism and their Relation with Reactive Oxygen Species and other Cellular Molecules during Plant Interactions with Pathogens

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Publication Info

***Article history*:**
Received: 19.11.2017
Accepted: 01.01.2018
DOI: https://doi.org/10.18811/ijpen.v4i01.12420

***Key words*:**
Biotic stress
H_2O_2
Plant defense
Polyamines
Spermine
Thermospermine

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Abstract

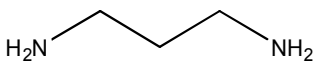
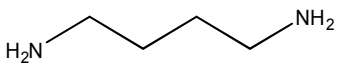
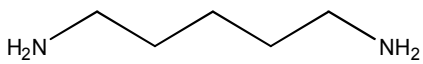
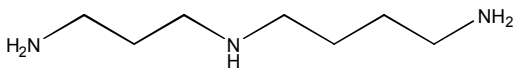
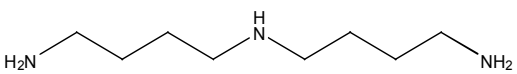
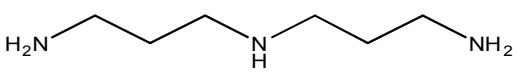
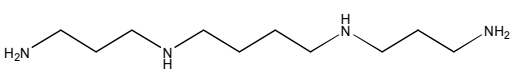
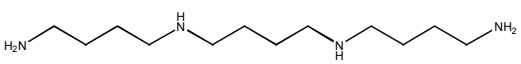
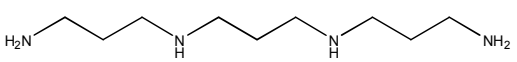
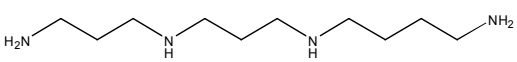
Polyamines are considered as essential molecules for plant growth and development. They are also implicated in abiotic and biotic stress responses. The roles of polyamines in abiotic stress regulation have been well understood, whereas their roles in response to biotic stress are poorly characterized in plants. However, various recent reports have acknowledged that polyamines and their catabolism generated H_2O_2 play important roles during biotic stress in plants. Polyamines and polyamines dependent signalling mediate reactive oxygen species (ROS) scavenging as these molecules accumulate in response to biotic stress. Polyamine catabolism also results in generation of H_2O_2 which act either by directly killing the pathogens or by acting as signalling molecules that mediate defense responses in plants. The H_2O_2 also interacts with the plant cell wall components and provides strength to the cell wall after infection and wounding. Polyamine catabolism generated H_2O_2 also play a role in signalling events post infection, which leads to hypersensitive cell death. They also interact with calcium dependent signalling cascade, phytohormones and secondary metabolites and provide resistance to plants during biotic stress. In this review we discussed the possible roles of polyamines and polyamines catabolism generated H_2O_2 and metabolites during plant host and pathogen interactions.

1. Introduction

Plants are challenged by variety of microbes throughout the life cycle and can eventually be infected by the pathogens. In order to protect themselves, plants have evolved a defense system, and several studies indicated that polyamines and polyamine dependent signalling make notable contributions to plant defense. Polyamines are low molecular weight organic molecules having at least one primary amino group (Takahashi and Kakehi, 2010). The most common polyamines in all organisms are diamine putrescine, triamine spermidine and tetraamine spermine (Kumar *et al*., 1997). In addition, there are some uncommon polyamines like thermospermine and norspermine that have been reported to occur in some microorganisms and plants (Oshima, 2007) (Table 1). Thermospermine was first identified in the extreme thermophilic bacterium *Thermus thermophilus*, and was also reported in oomycetes and plants (Oshima, 1979, 2007; Takano *et al*., 2012). Thermospermine was observed to protect thermophiles under extreme growth conditions by protecting and stabilizing their nucleic acids (Oshima, 2007). In plants, polyamines are present in free as well as in conjugated forms, mainly with phenolic compounds, proteins and nucleic acids (Yatin, 2002). Because of the cationic nature, polyamines are able to bind with polyanions and thus involved in a various cellular processes, including transcription and translation regulation, membrane stabilization, chromatin organization, ion channels maintenance, cell differentiation and cell signalling (Tabor and Tabor, 1984; Igarashi and Kashiwagi, 2000). Polyamines are also implicated in regulating programmed cell death (PCD) and autophagy (Thomas and Thomas, 2001; Seiler and Raul, 2005). In plants, they are reported to regulate embryogenesis, vascular tissues development, senescence, and abiotic stress (Evans and Malmberg, 1989; Galston and Sawhney, 1990; Kusano *et al*., 2008).

Apart from their role in plant development and abiotic stress, polyamines are reported to have protective role during biotic stress (Walters, 2003; Sagor *et al*., 2012; Jiménez-Bremont *et al*., 2014). Polyamines levels are reported to vary at the time of pathogen attack (Walters, 2003). Accumulation of H_2O_2 due to polyamine catabolism and induction in the synthesis of nitric oxide (NO) by polyamine activity plays an important role in plant-pathogen interactions (Romero-Puertas *et al*., 2004; Tun *et al*., 2006; Yamasaki and Cohen, 2006; Wimalasekera *et al*., 2011; Tiburcio *et al*., 2014). The ROS molecule H_2O_2 is the essential component of hypersensitive response (HR) reaction and play a key role in plant defense as it acts as an antimicrobial agent and also as a signal molecule (Peng and Kuc, 1992; Chen *et al*., 1995; Oshima, 2007).

Table 1: Structure of common and uncommon polyamines.

Sl. No.	Name	Structural Representation
A.	**Diamines**	
1.	1,3-Diaminopropane	
2.	Putrescine	
3.	Cadaverine	
B.	**Triamines**	
1.	Spermidine	
2.	Homospermidine	
3.	Norspermidine	
C.	**Tetraamines**	
1.	Spermine	
2.	Homospermine	
3.	Norspermine	
4.	Thermospermine	

However, despite of the extensive work done on polyamines, the roles of polyamines and polyamine-dependent signalling during biotic stress responses are poorly known in plants. In this review, we have addressed the metabolism of polyamines and their relation with H_2O_2 and some other cellular molecules during biotic stress responses. We also tried to elaborate the possible underlying mechanisms.

2. The Metabolism of Polyamines during Biotic Stress Responses

The metabolism of polyamines in plants includes their synthesis, transport and degradation according to the cellular needs, and these processes have extensively been reviewed (Bagni and Tassoni, 2001; Martin-Tanguy, 2001; Yatin, 2002; Illingworth *et al*., 2003; Kusano *et al*., 2007; Minguet *et al*., 2008; Tiburcio *et al*., 2014).

2.1. Polyamine biosynthesis and their accumulation

The precursor of all the polyamines is putrescine, which is synthesized via ornithine by the enzyme ornithine decarboxylase (ODC) (EC4.1.1.17) in all the organisms, but in plants and in some microorganisms, there exist another route for putrescine synthesis, i.e. via arginine by the activity of arginine decarboxylase (ADC) (EC4.1.1.19) (Fig. 1). Further putrescine is converted to triamine spermidine by spermidine synthase (SPDS) (EC2.5.1.16) and subsequently to tetraamines spermine or thermospermine by spermine synthase (SPMS) (EC2.5.1.22) or thermospermine synthase (TSPMS) (EC2.5.1.79), respectively. All SPDS, SPMS and TSPMS use an aminopropyl group from decarboxylated *S*-adenosyl methionine (dcSAM) which is produced by the activity of *S*-adenosylmethionine decarboxylase (SAMDC) (EC4.1.1.50) from S-adenosyl methionine (SAM).

The ODC, ADC and SAMDC are considered main enzymes of polyamines biosynthetic pathway, and it has been reported that during biotic stress, activity of these enzymes increases and resulting in accumulation of polyamines in plants (Jiménez-Bremont *et al*., 2014). As shown in Table 2; several other reports have also showed higher accumulation of polyamines in plants at

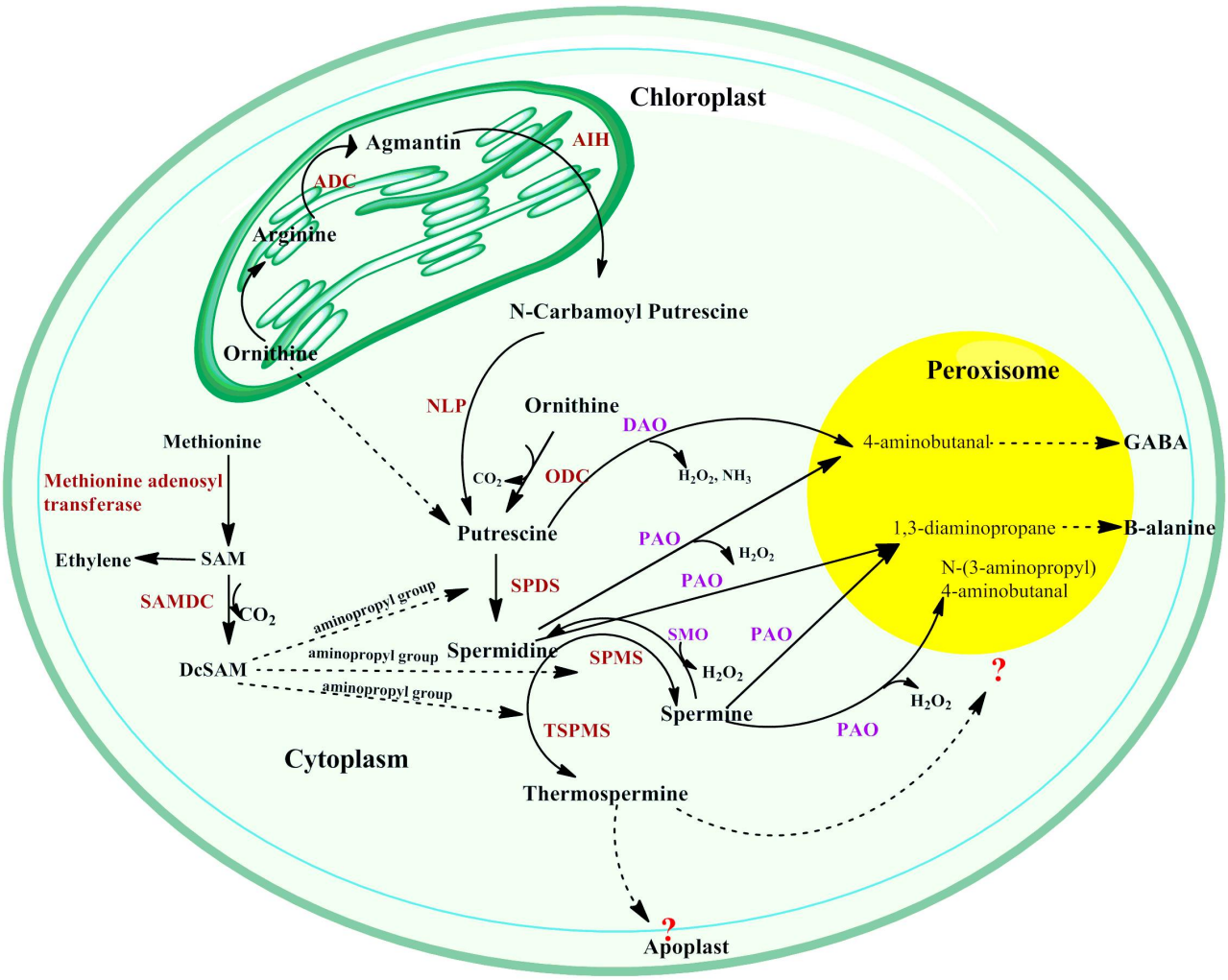


Fig. 1: Metabolic pathways of polyamines in plants: Biosynthesis of all the polyamines takes place in chloroplast, nucleus as well in the cytoplasm, whereas catabolism involves peroxisomes and apoplast. ADC: Arginine decarboxylase, AIH: Agmantine iminohydrolase, NLP: N-Carbamoyl Putrescine amidohydrolase, ODC: Ornithine decarboxylase, SPDS: Spermidine synthase, SPMS: Spermine synthase, TSPMS: Thermospermine synthase, SAMDC: S-adenosyl methionine decarboxylase, SMO: Spermine oxidase, PAO: Polyamine oxidase, DAO: Diamine oxidase.

the time of pathogen attack. Moreover, an increase in accumulation of free polyamines also leads to accumulation of polyamine conjugates in plants (Martin-Tanguy, 2001). These polyamine conjugates were observed mainly during hypersensitive reactions towards the plant pathogens (Flores and Martin-Tanguy, 1991; Martin-Tanguy, 2001). Interestingly, accumulated polyamine conjugates Hydroxycinnamic acid amides (HCAAs), which are a conjugated form of hydroxycinnamic acids with putrescine, spermidine or spermine, have been shown to have antifungal effects as reported in case of sugarcane infected with fungus *Ustilago scitaminea,* (Walters *et al*., 2001). So, accumulation of polyamines and polyamine conjugates in plant cells during biotic stress seems to be a widespread phenomenon and have some conserved roles in controlling pathogens.

In addition to the accumulation of polyamines and polyamine conjugates, acetylation of polyamines also interferes with plant defense. Expression of N-acetyltransferase activity 1 (NATA1) gene which encode putrescine acetyltransferase was strongly induced by an effector molecule coronatine produced by *Pseudomonas syringae* DC3000 and plant defense signalling phytohormone jasmonic acid (JA) suggesting

Table 2: Different types of plant-pathogen interactions and their effects on polyamine metabolism : The mechanism how these interactions bring about these changes in polyamines metabolism is not completely known.

Pathogen	Host Plant	Polyamine Enzyme activity	Polyamines Level	References
Viruses				
Tobacco mosaic virus (TMV)	Tobacco	-	HCAA including Feruloyltyramine accumulation	Martin-Tanguy *et al*. (1973, 1976)
TMV	Tobacco	ODC activity increased	Putrescine increased	Negrel *et al*. (1984)
TMV	Tobacco	-	Free and conjugated Putrescine and Spermidine increased	Torrigiani *et al*. (1997)
TMV	Tobacco	SPMS activity increased	Spermine increased	Yamakawa *et al*. (1998)
TMV	Tobacco	DAO activity increased	Putrescine decreased	Marini *et al*. (2001)
Cauliflower mosaic virus (CaMV)	Arabidopsis	SPMS activity increased	Spermine increased	Mitsuya *et al*., (2009)
Citrus exocortis viroid (CEVd)	Tomato	ODC activity increased	Putrescine decreased	Bellés *et al*. (1991, 1993)
Bacteria				
Pseudomonas cichorii	Tobacco	DAO and PAO activity increased	Putrescine and Spermidine oxidation increased	Yoda *et al*. (2009)
Pseudomonas syringae	Arabidopsis	DAO and PAO activity increased	Putrescine and Spermidine oxidation increased	Yoda *et al*. (2009)
P. syringae	Tobacco	Apoplastic PAO enzyme activity increased	Spermine and H_2O_2 accumulation	Moschou *et al*. (2009)
P. syringae	Arabidopsis	SAMDC1 activity increased	Spermine increased	Marco *et al*. (2014)
Pseudomonas viridiflava	Arabidopsis	TSPMS activity increased	Thermospermine increased	Marina *et al*. (2013)
P. viridiflava	Arabidopsis	SPMS activity increased	Spermine accumulation	Gonzalez *et al*. (2011)
Rhodococcus fascians	Arabidopsis	ADC	Putrescine accumulation	Stes *et al*. (2011)
Xanthomonas campestris	Arabidopsis	TSPMS activity increased	Thermospermine increased	Marina *et al*. (2013)
Fungi				
Alternaria brassicicola	Arabidopsis	-	Conjugated putrescine increased	Muroi *et al*. (2009)
Alternaria tenuis	Tobacco	-	Putrescine and Spermidine decreased	Edreva (1997)

Pathogen	Host Plant	Polyamine Enzyme activity	Polyamines Level	References
Ascochyta rabiei	Chickpea	DAO activity increased	Putrescine decreased	Angelini *et al*. (1993, 2010)
Blumeria graminis	Barley	ODC and SPMS activity increased	Free and conjugated forms of Putrescine and Spermine increased	Cowley and Walters (2002a)
Botrytis cinerea	Grapevine	CuAO and PAO activity increased	High accumulation of Polyamine related amino acids, Arg,Glu, Gln and Pro and GABA	Hatmi *et al*. (2015)
Erysiphe cichoracearum	Tobacco	-	Putrescine and Spermidine decreased	Edreva (1997)
Fusarium	Flax	Increased expression of PA biosynthesis genes	Increase in polyamine content	Wojtasik *et al*. (2015)
Peronospora tabacina	Tobacco	-	Putrescine and Spermidine decreased	Edreva (1997)
Phoma exigua	Potato	-	Feruloylputrescine accumulation	Malmberg (1984)
Scerotinia sclerotiorum	Tobacco	ADC activity increased	Putrescine and Spermine accumulation	Marina *et al*. (2008)
Ustilago maydis	Maize	ADC & SAMDC	free and conjugated Polyamines	Rodriguez-Kessler *et al*. (2008)
Verticillium dahlia	Cotton	ACL5 expression increased	Thermospermine increased	Mo *et al*. (2015)
Oomycetes				
Phytophthora parasitica	Tobacco	Apoplastic PAO enzyme activity increased	Spermine and H_2O_2 accumulation	Moschou *et al*. (2009)
Nematodes				
Heterodera schachtii	Arabidopsis	SPDS activity increased	Spermidine increased	Hewezi *et al*. (2010)

that acetylation of putrescine to N- acetylputrescine have suppressed antimicrobial defense in plants (Lou *et al*., 2016). Acetylated form of other polyamines, such as, N1-acetylspermine, N1-acetylspermidine, and N8-acetylspermidine have also been reported in plants (Mesnard *et al*., 2000; Dufeu *et al*., 2003; Fliniaux *et al*., 2004; Hennion *et al*., 2012). Therefore, suggesting that different acetylated form of polyamines possibly be part of microbial defense.

2.2. Polyamine catabolism

Polyamine catabolism is mostly carried out by two types of enzymes, diamine oxidase (DAO) (EC1.4.3.22) and polyamine oxidase (PAO) (EC1.5.3.14). DAO has a broad range of substrate specificity. It acts on the primary amino group of putrescine, spermidine and spermine to form aminoaldehydes and release ammonia and H_2O_2. It is usually found to be loosely attached with cell wall so that it can be easily released in apoplastic fluids (Federico and Angelini, 1991; Smith, 1985). In contrast, PAO has relatively specific substrate specificity, which is restricted to spermidine and spermine only. Arabidopsis genome encodes five PAO, where PAO1 and PAO5 are localized to cytosol; PAO2, PAO3, and PAO4 are found specific to the peroxisomes (Kamada-Nobusada *et al*., 2008; Moschou *et al*., 2008;

Kim *et al*., 2014). Polyamine oxidation by PAOs is found to be major source of stress induced H_2O_2 accumulations in plants (Yoda *et al*., 2006, 2009). Higher production of H_2O_2 molecule is known to mediate cross-linking of cell wall components that lead to cell wall stiffening in response to damage and during defense action against pathogens (Apel and Hirt, 2004; Torres and Dangl, 2005). A higher apoplastic PAO activity has shown to induce tolerance mechanisms in plants against the *P. syringae* pv *tabaci*. As a result of this infection, spermine gets excreted into the apoplast and catabolized by the increased activity of apoplastic PAO, resulting in higher accumulation of H_2O_2 (Moschou *et al*., 2009). Incompatible interaction between barley and powdery mildew fungus *Blumeria graminis* also resulted in increased activity of catabolic enzymes DAO and PAO, which leads to the production of H_2O_2 that was responsible for lignification and rigidity of cell walls (Cowley and Walters, 2002b; Walters, 2003). A similar DAO activity in the cell wall of chickpea was also reported in response to wounding (Cona *et al*., 2006). Therefore, polyamine catabolism directly contributes in H_2O_2 production, which play key role in defense response against biotic stress (Yoda *et al*., 2006). Generally, production of ROS is one of the early responses induced during biotic stress (Bolwell, 1999; Pottosin *et al*., 2014). The ROS act directly as antimicrobial molecules or may act as signalling molecules in the regulation of defense response against pathogens (Camejo *et al*., 2016).

In addition to catabolism of polyamines, both DAO and PAO may also participate in plant defense through the generation of various secondary metabolites, like, tropane, nicotine and some other alkaloids (Facchini, 2001; Martin-Tanguy, 2001). These metabolites are known to interact with pathogens and provide resistance to plants (Sudha and Ravishankar, 2002).

3. Plant Pathogens Interactions

In this section, we focused on the role of polyamines in different types of plant pathogen interactions (Table 2).

3.1. Viral pathogens

Viral infection leads to increase in the polyamine content in plant cells. Drastically increased level of free spermine and increased activity of ODC have been reported during the *Tobacco mosaic virus* (TMV) infection of tobacco, mainly in the necrotic cells (Negrel *et al*., 1984; Yamakawa *et al*., 1998). Also, ODC activity increases HCAA conjugates accumulation in tobacco cells, which prevent further TMV infection (Martin-Tanguy, 1985). In Arabidopsis, spermine has shown to suppress *Cauliflower mosaic virus* (CaMV) multiplication by activating signalling cascade leading to activation of defense responses (Mitsuya *et al*., 2009). Spermine activate the expression of several transcription factors including ZAT7, ZAT12 and WRKY40, which are components of H_2O_2 signalling pathway suggesting that spermine signalling pathway play important role in defense against viruses. Similar to spermine, exogenous application of thermospermine also activates genes that encode components of spermine signalling pathway followed by activation of mitochondrial alternative oxidase (AOX), Mitogen activated protein kinase 3 (MAPK3), and many transcription factors like bZIP60, WRKY40 and ZAT7, thus suppressing the viral infection up to the same extent as spermine does (Sagor *et al*., 2012). These reports indicates that spermine or thermospermine responses are also mediated by H_2O_2 signalling pathway. In addition to these, longer uncommon polyamines, caldopentamine, caldohexamine, homocaldopentamine and homocaldohexamine were also shown to have antiviral activity. When infected Arabidopsis leaves were treated with these polyamines they repressed CMV multiplication more efficiently than spermine (Sagor *et al*., 2013).

3.2. Bacterial pathogens

Polyamine metabolism also gets altered in plants in response to bacterial pathogens. Overexpression of Arabidopsis SAMDC1 has been reported to elevate the level of spermine, which resulted in increased tolerance of plants against *P. syringae* (Marco *et al*., 2014). Also, SPDS overexpressing sweet orange plants were reported to have more tolerance against the citrus canker pathogen *Xanthomonas axonopodis* pv *citri* (Marco *et al*., 2014). Furthermore, accumulation of thermospermine has been shown to reduce *P. viridiflava* and *X. compestris* multiplication in Arabidopsis (Marina *et al*., 2013), which shows that thermospermine plays a role in providing tolerance against a range of bacteria. However, this improved resistance was observed to be due to the increased catabolism of thermospermine. Similarly, spermidine and putrescine also showed to provide resistance to tobacco and Arabidopsis against *P. chichorii* and *P. syringae,* respectively (Yoda *et al*., 2009). All these reports demonstrated that accumulation of polyamines in plants is important in protecting the plants against pathogenic bacteria. In addition to accumulation of spermine, infection of *P. syringae* and *P. viridiflava* in tobacco plants also resulted in its subsequent catabolism to H_2O_2 which act as antimicrobial molecule (Moschou *et al*., 2009; Marina *et al*., 2008). Higher H_2O_2 accumulation leads to increased

expression of defense-related genes, such as PR1 (Pathogenesis-related protein 1), PR2 and PR5 and reduced growth of the pathogen *P. syringae* (Lou *et al*., 2016). These results suggested that polyamine catabolism also contribute to resistance of plants against bacterial pathogens.

3.3. Fungal pathogens

Fungal pathogens also influence polyamine metabolism in plants. During powdery mildew infection, induced activity of polyamine biosynthetic enzymes ODC, ADC and SAMDC was observed that resulted in increased level of putrescine, spermidine and spermine in barley plants (Walters *et al*., 2001). In another report, when the barley plants reacts to *Blumeria graminis* infection hyper-sensitively, a rigorous increased concentration of free as well as conjugated putrescine and spermidine has been observed in plants (Cowley and Walters, 2002a). Increased expression of the polyamine biosynthetic genes also resulted in an increase in polyamine content in flax plants infected with *Fusarium* (Wojtasik *et al*., 2015). Expression of polyamine biosynthetic gene ACL5 has also been induced in cotton by vascular wilt fungal pathogen *Verticillium dahliae* (Mo *et al*., 2015).

In addition to biosynthetic enzymes, an increase in the activity of their catabolic enzymes DAO and PAO have also been reported post fungal infection leading to the generation of H_2O_2 (Cowley and Walters, 2002b; Walters, 2003). Similar enhanced activity of DAO has been observed during chickpea plants interaction against *Aschyta rebie* infections (Angelini *et al*., 1993, 2010).

3.4. Oomycete pathogens

Polyamine metabolism is also shown to influence oomycete growth and colonization in host plant cells. Higher apoplastic activity of PAO plays an important role in providing defense to tobacco plants against *Phytophthora parasitica* var. *nicotianae* (Moschou *et al*., 2009). Use of oomycetes elicitor cryptogein was also reported to elevate the activity of apoplastic PAO in tobacco (Yoda *et al*., 2006).

3.5. Parasitic nematodes

Nematodes are sedentary endoparasites of plants. Parasitic nematodes secrete effector proteins into the host root cells, which convert normal cells to specialized feeding sites called syncytia, required for their successful endoparasitism. Secreted effector proteins are observed to alter polyamine signalling during the host defense response (Hewezi and Baum, 2013). Nematode effector protein "10A06" has been shown to interact with Spermidine Synthase 2 (SPDS2), causing the higher spermidine content as well as increased PAO activity in Arabidopsis cells. Moreover, overexpression of SPDS2 in plants was resulted to enhance host susceptibility to the nematode *Heterodera schachtii* (Hewezi *et al*., 2010). Thus, nematodes alter polyamine biosynthesis in plants, disrupt the defense signalling, causing various physiological and morphological changes suitable for their survival as endoparasite.

4. Polyamines Mediated Plant Defense Responses

In this section as summarized in Figure 2, we elaborated the interactions of polyamines with various components of the plant defense system during biotic stress responses.

4.1. The role of Polyamine catabolism generated H_2O_2 molecule

Polyamine catabolism is one of the sources of H_2O_2 production during plant defense responses and PAOs are the main enzymes involved in its production (Yoda *et al*., 2009; Lou *et al*., 2016). In addition to PAO, DAO is also involved in the H_2O_2 production by oxidizing putrescine (Moschou *et al*., 2014). During defense responses, increased production of H_2O_2 is known to mediate cross-linking of cell wall components that lead to cell wall stiffening in response to damage caused by the pathogens (Apel and Hirt, 2004; Torres and Dangl, 2005). H_2O_2 also induce Phenylalanine ammonia lyase (PAL) which is a key enzyme of phenylpropanoid pathway responsible for providing resistance to the plants against the diseases (Dorey *et al*., 1997). Phenylpropanoid pathway provides intermediates for the synthesis of flavonoids (anthocyanins, proanthocyanidins, flavonols, flavones, flavanones, isoflavonoids, and phlobaphenes), phytoalexins, stilbenes, various phenolic acids and signalling molecule Salicylic acid (Dixon and Paiva, 1995; Liu *et al*., 2015).

The Ca^{2+} signalling plays an important role in plant defense responses, which includes HR (Lecourieux *et al*., 2006). The H_2O_2 also activate Ca^{2+} ion channels that results in induction of signal transduction cascade related to defense responses. ROS generated in the apoplast due to catabolism of polyamines induces Ca^{2+} influx and amplifies defense-related signals after pathogen attack to the plants (Garcia-Brugger *et al*., 2006). It can be suggested that polyamine catabolism generated H_2O_2 cause increase in Ca^{2+} ion which is involved in the progression of HR cell death. Furthermore, elevated Ca^{2+} also predicted to activate

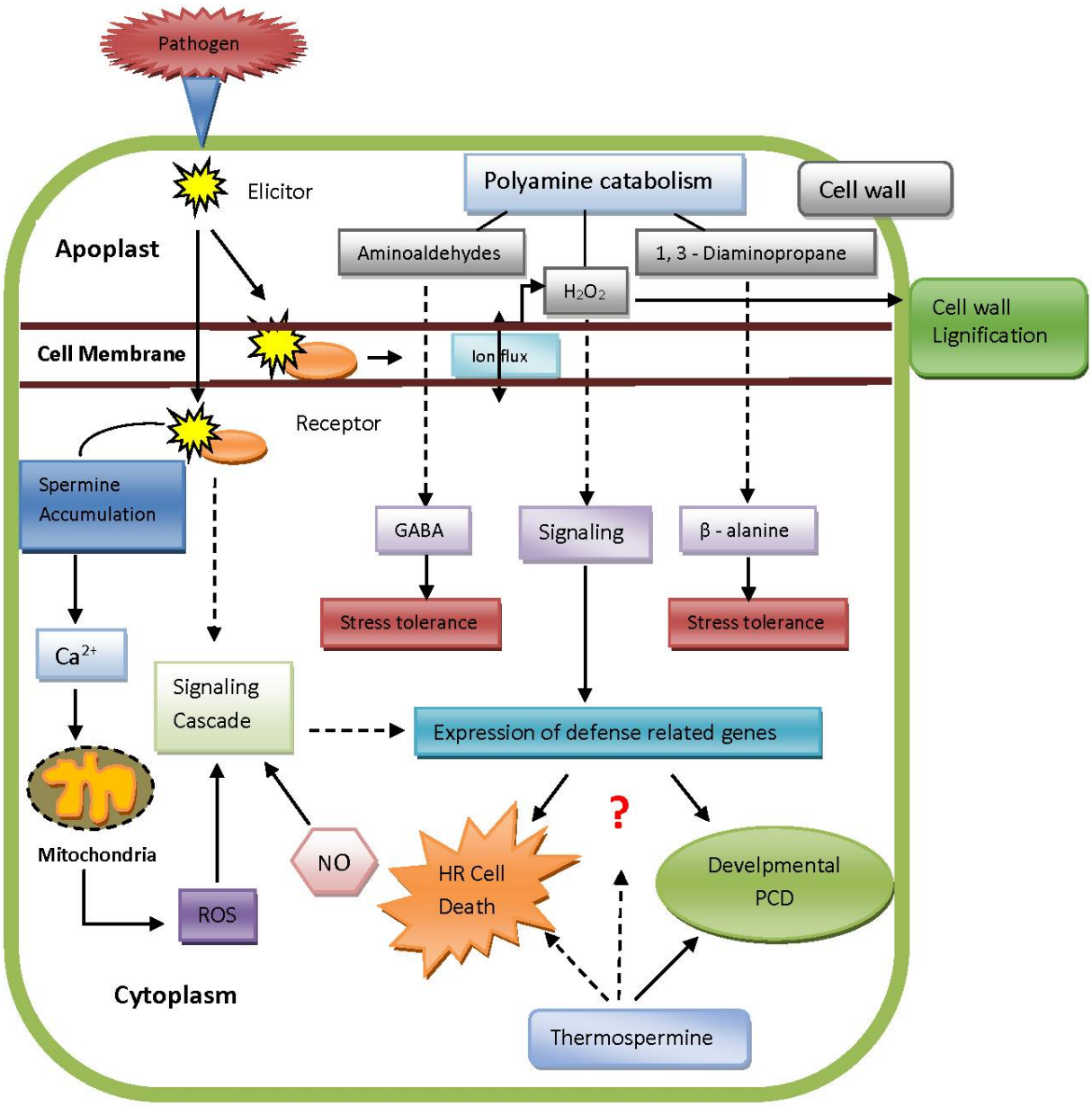


Fig. 2: Response of polyamines during biotic stress: After pathogen challenge catabolism of polyamines results in release of H_2O_2 which activate signalling cascade leading to expression of defense-related genes. Other signalling molecules resulted from polyamines degradation like, GABA also play role in providing stress tolerance. Accumulation of polyamines, such as spermine causes mitochondrial dysfunction ultimately results in HR cell death and developmental cell death. Role of thermospermine in defense responses is yet to be elucidated.

Metacaspases which are cell death executioner proteins in plants (Watanabe and Lam, 2011).

4.2. The role of Polyamine catabolic product GABA

Polyamine catabolism dependent product Gamma-aminobutyric acid (GABA), a non-protein amino acid is also found to accumulate during biotic stress in plants. One of the pathways for GABA formation is via γ-aminobutyraldehyde intermediate from polyamine degradation by DAO (Wakte *et al.*, 2011). An increase in level of GABA was observed in tomato apoplast during *Cladosporium fulvum* infection (Solomon and Oliver, 2001, 2002). The GABA shunt pathway also activated in rice plant infected with blast

fungus (Wu *et al*., 2006). Due to its rapid increase during biotic stress in plants, it is predicted that it act as an osmoregulator or intracellular signalling molecule (Roberts, 2007). In addition to these, GABA is also suggested to involve in acquisition of minerals such as Manganese that activates lignin biosynthetic pathway enzymes, as lignin is responsible for disease resistance by providing physical barrier for the penetrating fungal hyphae in cereals (Hammerschmidt, 1984). Another proposed function of GABA is to mediate interactions between plants and its pathogens (Shelp *et al*., 2006).

4.3. Polyamine as a signalling molecule

Polyamines also proposed to act as signalling molecule. One hypothesis is that polyamines act via signalling molecules H_2O_2 and NO, as both induced by the activity of polyamines at the time of biotic stress (Romero-Puertas *et al*., 2004; Tun *et al*., 2006; Yamasaki and Cohen, 2006; Wimalasekera *et al*., 2011).

It is also suggested that increased level of spermine stimulates Ca^{2+} influx in mitochondria, which may alter functions of mitochondria leading to more ROS generation and triggers alternative oxidases (AOXs) which cause activation of MAP kinases and subsequent transcription of certain sets of genes associated with HR induced cell death (Takahashi *et al*., 2003).

4.4. Polyamine interaction with defense signalling phytohormones

The plant hormones, salicylic acid (SA) and jasmonic acid (JA) signalling pathways play key roles in plant defense against pathogens. Many studies have demonstrated that polyamine metabolism is also linked to these defense signalling hormones. Exogenous application of methyl jasmonate (MeJA) on barley leaves reported to increase concentration of free as well as conjugated putrescine and spermidine (Walters *et al*., 2002). Similarly, exogenously supplied MeJA to loquat fruits caused polyamine accumulation and decreased the chances of rot caused by *Colletotrichum accutatum* (Cao *et al*., 2014). An increased activity of both polyamine biosynthetic and catabolic enzymes was also observed when MeJA exogenously supplied to wheat, which induces PR proteins and cause reduction in the infection by *Puccinia recondite* (Haggag and Abd-El-Kareem, 2009).

The plant hormone SA is also reported to interact with polyamines during biotic stress. The SA has been shown to induce the activity of ODC and ADC causing accumulation of polyamines in maize, tobacco and tomato tissues (Németh *et al*., 2002; Jang *et al*., 2009). In addition to these, SA has also been reported to acts synergistically with H_2O_2 to trigger defense responses in plants (Neuenschwander *et al*., 1995; Sharma *et al*., 1996; Mukherjee *et al*., 2010). Arabidopsis NATA1 which mediates polyamine acetylation and interferes with H_2O_2 production is also suggested to have a role in the cross-talk between SA and JA during plant defense signalling (Lou *et al*., 2016). These reports indicated that polyamines and defense responsive plant hormones interact during biotic stress, however exact mode of their interaction is still not well-known.

4.5. Polyamine as mediator of PCD during biotic stress

Polyamines also contribute directly or indirectly in pathways regulating PCD (Moschou and Roubelakis-Angelakis, 2014). Indirectly, ROS (H_2O_2) generated by catabolism of polyamines contributes to HR induced death of infected plant cells (Yoda *et al*., 2003). Polyamines also contribute directly to PCD through their regulatory effects on ion channels in the absence of H_2O_2 and aminoaldehydes (Weisell *et al*., 2014). For instance, spermine regulates K^+ channels and low K^+ activates metacaspases and nucleases in plants (Demidchik *et al*., 2010). The Ca^{2+} signalling also plays a significant role in protecting plants after pathogen attack, mainly through HR mediated cell death (Lecourieux *et al*., 2006). Catabolism of polyamines in apoplast induces Ca^{2+} influx and its involvement in the progression of HR, suggesting that polyamine dependent increase in the Ca^{2+} level is necessary for HR execution post pathogen infection (Moschou and Roubelakis-Angelakis, 2014).

In Arabidopsis, thermosperime has been demonstrated to regulate developmental PCD during the xylem formation (Muñiz *et al*., 2008; Vera-Sirera *et al*., 2010). Silencing of thermospermine biosynthetic gene ACL5 in Arabidopsis cause premature cell death of xylem cells which resulted in defective xylem specification (Muñiz *et al*., 2008).

Polyamines have also been shown to delay cell death. Increased level of spermidine and spermine by the ectopic overexpression of yeast SAMDC gene has been shown to increase the lifespan of tomato fruits (Mehta *et al*., 2002). Spermidine has been found to induce autophagy pathway in yeast and reduces age related oxidative damage in mice which resulted into increased their lifespan (Eisenberg *et al*., 2009). Autophagy, which is a recycling mechanism, is known to removes unwanted and damaged molecules, and cell organelles generated due to various abiotic and biotic stresses, disease and ageing, thus increases life span

and delays or inhibit PCD (Chaabane *et al*., 2013; Moschou *et al*., 2014). Given the importance of polyamines in the PCD and autophagy in animals, it is most likely that polyamines also act during pathogens induced plant immune response which resulted in induction of autophagy and other PCD pathways for removal of pathogens and damaged molecules. However, how the polyamines act in these pathways is largely unknown in plants.

4.6. Polyamine and chromatin compaction and epigenetic modifications

Chromatin compaction is known to protect DNA from damages thereby maintaining the integrity of genome (Takata *et al*., 2013). Some reports suggest that polyamines also interact with DNA and stabilizes chromatin structure (Ivan *et al*., 1988). Induced chromatin compaction due to oxygen and nutrient deprivation (OND) environment has also been shown to resulted in redistribution of cellular polyamines to the nucleus from cytoplasm there by restricting access to histones (Kirmes *et al*., 2015). The polycationic nature of polyamines is essential for these interactions.

Some recent work indicated that the epigenetic modifications play important roles in regulating plant defense responses during biotic stress (Alvarez *et al*., 2010; Ding and Wang, 2015). The epigenetic modifications include DNA methylation, histone methylation and histone acetylation often involves modifications and remodeling of chromatin, transcriptional reprogramming and regulation of defense-related genes in plants in response to the pathogen challenges. There are some evidence to support that polyamines also influence these epigenetic modifications (Fiori *et al*., 2012). We hypothesize that when challenged by pathogens, polyamines are involved in protecting DNA and chromatin and in fine-tuning expression of defense responsive genes in plants. However, how polyamines mediate such modifications are largely unknown in plants. Further work is needed to support this hypothesis.

5. Targeting Polyamines for Pathogen Control

Polyamines are essential molecules for normal developmental homeostasis of plants and its pathogens. Therefore, inhibitors of polyamine biosynthetic enzymes, such as α-Difluoromethylornithine (DFMO) and α-difluoromethylarginine (DFMA) which selectively inhibit the activities of polyamine biosynthetic enzymes, ODC and ADC, respectively, have effectively been used to inhibit growth of several pathogenic fungi (*Botrytis* sp*., B. cinerea*, *Rhizoctonia solani* and *Monilinia fruticola*) *in vitro* and also provides systemic protection to bean plants against infection by fungal pathogen, *Uromyces phaseoli* (Rajam and Galston, 1985; Rajam *et al*., 1985). However, such inhibition can be completely reversed by addition of the putrescine and spermidine to the growth medium resulted in a promotion of growth of fungi. Similar exogenous application of DFMO to leaves of Pinto beans has completely inhibited the growth of pathogen *U. phaseoli* (Rajam *et al*., 1986).

In another strategy, host induced RNAi approach was also used to control the pathogenic fungus *Aspergillus nidulans* by silencing the expression of fungal polyamine biosynthesis gene ODC which resulted in significant reduction in fungal growth (Khatri and Rajam, 2007). Using same approach, wilt pathogen fungus *Verticillum dahliae* infection has been controlled by developing pathogen specific ODC gene silencing lines in tobacco (Rajam, 2012). These results demonstrated that key polyamine biosynthetic genes of pathogens could be targeted to improve crop protection against various plant pathogens.

6. Conclusions

Recent research work has increased our understanding of how polyamines and polyamine metabolism affects defense responses in plants. As summarized in Figure 2, polyamines implicated to have both direct and indirect roles during plants interaction with pathogens. The moment pathogens attack plants, genes for polyamine metabolism get activated, which results in synthesis and accumulation of free as well conjugated polyamines, providing resistance to the plants. The accumulation is often accompanied by polyamine catabolism resulting in generation of H_2O_2 leading to HR mediated cell death. Accumulated polyamines and H_2O_2 also act as signalling molecules, which ultimately lead to activation of defense pathways.

Despite the extensive research work done on polyamines, our understanding of polyamine signalling during biotic stress is still very obscure. Further investigations are needed that will elucidate how polyamines activate the plant defense signalling cascade and how accumulated polyamines cross-talk with varied pro-survival, autophagy, PCD-related genes and defense signalling hormones at the time of biotic stress. How the levels of both polyamine metabolic enzymes, and the polyamines, are regulated in the cells attacked by pathogens are also largely unknown. All these works might provide the basis to develop innovative disease control strategies for plants against their pathogens.

Acknowledgements

The work in the laboratory of SKS was funded by grants from DBT and SERB. MF thank the DST for INSPIRE Fellowship (No. DST/ INSPIRE Fellowship/2012/729). Authors also thank DST-FIST and UGC-SAP programs for financial support.

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