Polyamine: Role in Fungal Resistance

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Abstract—*Polyamines (putrescine, spermidine and spermine) have been shown to be important in stress tolerance. They are small basic molecule play important role in plant response (or defense) to both abiotic and biotic stresses. They are small polycationic compounds that are present in all living organisms. They also play an important role in the regulation of various cellular and molecular processes including growth and development etc. Polyamines also play important role in the interactions between plants and their pathogens like fungi, viruses, bacteria etc. Although many reports on polyamines and there genes (ADC, ODC, SAMDC) shows resistance to various fungal pathogens. After interaction there is an increases in free polyamines, diamine oxidase (DAO) and polyamine oxidase (PAO). The activities of these enzymes produces hydrogen peroxide (H2O2), which may act defense mechanism. These H2O2 produced might trigger the hypersensitive response (HR), thought to be a form of programmed cell death. Therefore, the accumulation of polyamine in the plants make the plants for resistance to fungal pathogens. Advances in the use of polyamines and there genes used for the research to make the plants for fungal resistance are summarized.*

Keywords*: Polyamines, diamine oxidase (DAO), Polyamine oxidase (PAO), Hydrogen peroxide (H2O2)*

1. INTRODUCTION

Crop plants are affected with various environmental stresses like biotic and abiotic stresses, it will reduces crop yield and quality. Biotic stresses like insects, fungi, bacteria and viruses and abiotic stresses like high, low temperature, salinity, drought, flooding, heavy metals and oxidative stresses [20-21], [4]. Among the biotic stresses, fungal pathogens are responsible for large-scale damage to many cultivated species. Most of the cases, fungal diseases can completely destroy the crops. To over come this problem, transgenic or plant breeding approaches, especially the engineering of biosynthetic pathways associate with stress responses has emerged as a promising way to improve tolerance in crop plants [4],[14]. There are wide variety of low molecular weight molecule such as pathogenesis-related proteins (PR), chitinases, mannitol, glucanases and polyamines (PAs) [18],[22] ,[32] have been studied for their role in defense mechanism against fungal pathogens and are potential source for genetic manipulation (13],[19].

2. POLYAMINES AND BIOSYNTHETIC PATHWAY

The polyamines, diamine Putrescine, triamine Spermidine and tetra-amine Spermine (Fig. 1) are low molecular weight, aliphatic amines that are positively charged at physiological $_{p}$H. These small molecules, which are ubiquitous in occurrence, are involved in maintaining membrane integrity, synthesis and function of macromolecules such as DNA, RNA and protein, regulation of cell proliferation and differentiation, senescence and various stress responses [1],[12][14][15],[32], [34-35].

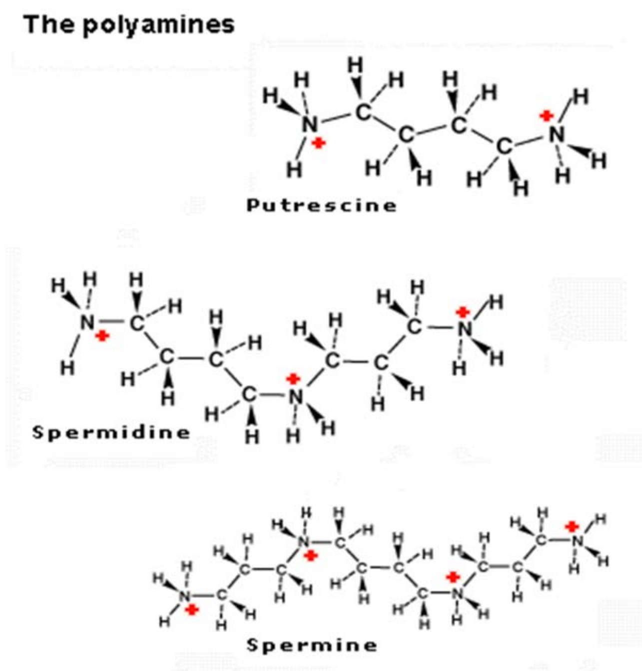

Fig. 1: Structure of Common Polyamines

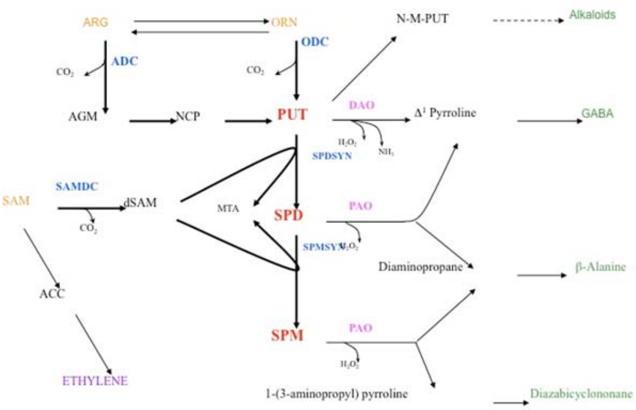

Fig. 2 : Polyamine biosynthetic pathway

Polyamines exist in three forms in the cell, as free cations, covalently bound to low molecular weight phenolic compounds like hydroxycinnamic acids (conjugated form of PAs) and bound to macromolecules or membranes (bound form of polyamines). Though the major form is the free cationic form of polyamines, there are instances when the amounts of conjugated form exceed the free form and these are known to be critical in certain physiological processes including seed germination, flower development, and abiotic stress responses [22],[37].

Polyamine biosynthetic pathway in plants has been well elucidated and is similar to those in animals and microorganisms. The common polyamines Put, Spd, and Spm are synthesized by the decarboxylation of the amino acids ornithine and or arginine and their further aminopropylations [22],[29]. The diamine Put is the obligate precursor for the higher polyamines Spd and Spm, and is synthesized either directly from ornithine, by the action of the enzyme ornithine decarboxylase (ODC) or indirectly, from arginine by arginine decarboxylase (ADC). Agmatine, the immediate product of ADC activity, is further converted into N-Carbamoyl-Put by agmatine iminohydrolase and ultimately to Put by N-Carbamoyl-Put aminohydrolase (Figure.2).

Except for bacteria, plants and some fungi, which utilize both pathways for the synthesis of Put, all other organisms posses only the ODC pathway for polyamine biosynthesis. In higher plants, however owing to the higher cellular pools of arginine and its involvement in transport in the plants, the ADC pathway is the major pathway for the synthesis of Put [22]. In bacteria, ODC pathway is more predominant than ADC pathway [22]. The ADC and ODC pathways are connected by the inter-conversion of arginine and ornithine through the urea cycle [29] Put is further converted to Spd and Spm. Spd is synthesized by the addition of an aminopropyl group derived from decarboxylated SAM (dcSAM) through the action of Spermidine Synthase (Spd Synthase). The addition of another aminopropyl group to Spd by Spermine Synthase (Spm Synthase) leads to the synthesis of Spm. The decarboxylation of SAM is catalysed by S-adenosylmethionine decarboxylase (SAMDC) [30]. SAM in turn, is formed from methionine by the action of SAM Synthase [22]. SAM is shared between the polyamine and ethylene pathways, as it is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase that is further catalysed by ACC Oxidase to form ethylene.

3. POLYAMINE CATABOLISM

The catabolic pathway for polyamines involves their oxidative deamination, catalyzed by amine oxidases, which mainly acts on the free protonated forms of polyamines [11]. These pathways mediate polyamine involvement in cellular processes by providing the degraded products for further utilization (Rea *et al.* 1998). Involvement of catabolic pathway has been implicated in cell wall lignifications as well as for defense responses [27],[28]. The copper containing diamine oxidase (DAO) is a dimeric glycoprotein and acts primarily on diamines (Put and Cad) although they may also act on higher polyamines to form pyrroline, ammonia and H_2O_2. The polyamine oxidases (PAOs) are monomeric glycoproteins containing flavin, which act on the secondary amino groups of Spd and Spm forming pyroline, diaminopropane (DAP) and H_2O_2 [31]. The pyrroline (4-aminobutylaldehyde) obtained by the action of DAO on Put and PAO on Spd is further oxidized by an NAD-dependent dehydrogenase to γ-aminobutyric acid (GABA) [10]. GABA in turn is transaminated to form Succinic semialdehyde, which is further oxidized to Succinate. Succinate is finally connected to the TCA cycle. NH_3 produced in the pathway is further connected to amino acid metabolism through ammonia detoxification cycle. Thus, this pathway helps in the recycling of the polyamine carbon skeleton and part of the nitrogen. The H_2O_2 produced by diamine and polyamine oxidation is used for the polymerisation of lignin and suberin precursors or in the cross linking of polysaccharide bound phenols [11]. Diaminopropane, a major product of oxidation of Spd and Spm is further converted to β-alanine or utilized for the synthesis of the higher polyamines like norspermidine, norspermine and cladopentamine by successive aminopropylations [6]. By producing intermediates and products further utilized by other pathways, polyamine metabolism is a connecting link between amino acid and carbon metabolism. The recycling pathway i.e. the conversion of Spd into Put and Spm into Put and Spd has been known to regulate the polyamine levels in animals and fungi. This is a common occurrence in animals; no substantial evidence for the occurrence of the same in plants is available [7].

4. POLYAMINES - FUNGAL RESISTANCE

Polyamines play important role in controlling diseases at multiple levels. At the cell wall level, they prevent the entry of the different pathogen by lignifications and suberization.

Conjugated polyamines, especially hydroxyl cinnamic acid amides (HCA amides) and comouryl-agmatine, have been known to have anti microbial properties [22], [26],[32]. Polyamines particularly Spm has been shown to be an endogenous precursor for pathogenesis related (PR) proteins, PR-1, PR-2, PR-3 and PR-5 [34]. Polyamine, mainly Put acts as a substrate and precursor for many tropane alkaloids such as hyosyanine and scopolamine, which are well known for their anti- microbial properties.

Plant system has two pathways for Put synthesis, one from ornithine involving ODC and another from arginine involving ADC. Fungi have only ODC pathway for polyamine formation [25]. The involvement of polyamines in resistance against fungal disease is well documented. In barley leaves, inoculation of *Puccinia*, results in an increase in the level of Put and Spd [22]. Put, Spd and Spm levels increased in the 'clubbed' region of turnip roots infected with plasmodiophora, where as regions of roots not showing disease symptoms did not show increased polyamines [32]. Walters *et al.*2003 [32], found similar polyamine increase in barley leaves infected with powdery mildew and correlated to respective increase in ADC, ODC and SAMDC activities. Therefore, polyamines were suggested to be involved in "green island" formation [32]. Further work, using a detached leaf system for the generation of Green Island on powdery mildew infected barley leaves showed that several-fold increase in polyamines occurred in these green islands [8]. Similarly polyamine increase was reported in wheat infected with *Puccinia* [38]. The growth of the phytopathogenic fungi can be controlled by using DFMO, the inhibitor of ODC effectively [23]; [32]. Rajam and Galston, [25] for the first time, demonstrated the control of the growth of several phytopathogenic fungi *in vitro* by DFMO. Later, [25], reported the complete prevention of *Uromyces* infection on *Phaseolous Vulgaris* by DFMO treatment either pre or post inoculation. Broad bean could be protected from the rust and barley against powdery mildew by DFMO treatment. Reported, that the DFMO treatment inhibited uredospore germination and restricted germ-tube development and also the effect of DFMO on pathogenesis control was persistent for about two weeks [24]. Near isogenic lines of wheat differing in susceptibility to stem rust were tested for their polyamine levels and it was found that resistant lines have a higher endogenous polyamine level which does not show any change on infection where as susceptible and moderately resistant lines showed significant increase in Put and Spd levels on infection. [5], [16]. Recent work on barley reacting hypersensitively to the powdery mildew fungus, [9] showed an increase in conjugated polyamines and the polyamine biosynthetic enzymes ODC, ADC and the catabolic enzymes activity following inoculation. Further, that in an incompatible interaction between barley and powdery mildew where the resistance was penetration based, level of Put, Spd and catabolic enzyme activity was found to increase after inoculation [9]. Tomato accumulate high amount of Feraloyl-Put as a result of tobacco mosaic virus (TMV) infection, and the amount was highest after particle multiplication [39],[40]. Negrel *et al.* [17] reported, a 20-fold increase in ODC activity, which parallels the phenylalanine ammonia lyase (PAL) activity during hypersensitive response in tobacco. Hiraga *et al.* [12] reported that the HR of tobacco to TMV, a HR-induced peroxidase gene (tpoxc$_1$) is responsive to spermine but not to salicylate. Belles *et al.* [2], reported that increase in free Put in tomato plants infected with C*itrus exocortis* viroid was found to be due to decrease in ODC rather than ADC activity. Hypersensitive tobacco plants showed an increased Put and Spd levels and also ADC and ODC activities on TMV infection whereas DAO activity was negligible. Rabiti *et al.* [26] reported that susceptible plants however showed low Put and Spd levels and no change in ADC, ODC or DAO levels showing the involvement of polyamine in viral infection. There is information that polyamine plays a role in stress responses, and there are some reports on transgenic approach.

However, reports of fungal resistance by Over- expression of Polyamine pathway gene are limited. Polyamine genes, like *Samdc* in tobacco and tomato [19],[33] and *adc* in eggplant [18] were developed. These plants showed resistance to various fungal pathogens.

REFERENCES

[1] Ahmad P, Ashwani Kumar, Aditi Gupta, Xiangyang Hu, Khalid ul Rehman Hakeem, Mohamed Mahgoub Azooz, and Satyawati Sharma. Polyamines: Role in Plants Under Abiotic Stress. Crop Production for Agricultural Improvement., M. Ashraf et al. (eds.), Crop Production for Agricultural Improvement. 2012.491-512

[2] Belles JM, Perez-Amador MA, Carbonell J & Conejero U. Correlation between ornithine decarboxylase and putrescine in tomato plants infected by citrus exocortis vioid or treated with ethephon. Plant Physiol. 1993.102: 933-937

[3] Bhatnagar P, Glasheen BM, Bains SK, Long SL, Minocha R, Walter C & Minocha SC. Transgenic manipulation of polyamine metabolism in poplar (Populus Nigra x maximoviczii) cells. Plant Physiol. 2001.125: 2139-2153

[4] Bhatnagar-Mathur P,Vadez V, Sharma KK. Transgenic approaches for abiotic stress tolerance in plants; retrospect and prospects. Plant Cell Rep. 2008. 27;411-424

[5] Bharthi, Rajam MV & Kaur-Sawhney R. Involment of polyamines in resistance of wheat to puccinia recondite. Phytochem. 1996. 43: 1009-1013

[6] Bouchereau A, Aziz A, Larher F & Martin-Tanguy J. Polyamines and environmental challenges: recent developments. Plant Sci.1999. 140: 103-125

[7] Bhatnagar P, Glasheen BM, Bains SK, Long SL, Minocha R, Walter C & Minocha SC. Transgenic manipulation of polyamine metabolism in poplar (Populus Nigra x maximoviczii) cells. Plant Physiol. 2001.125: 2139-2153

[8] Coglan SE & Walters DR. Polyamine metabolism in green-islands on powdery mildew-infected barley leaves: possible interaction with senescence. New Phytol. 1990. 116: 417-424

[9] Cowley T & Walters DR. Polyamine metabolism in barley reacting hypersensitively to the powdery midew fungus Blumria graminis f.sp. hordei. Plant, cell and Environ.2002a. 25: 461-468

[10] Flores HE & Filner P. Polyamine catabolism in higher plants: characterization of pyrroline dehydrogenase. Plant Grow. Regul. 1985. 3: 277-291

[11] Federico R & Angelini R. Polyamine catabolism in plant. In: Biochemistry and physiology of polyamines in plant (Sloccum RD and Flores HE eds.) CRC Press, Boca Raton. 1991. Pp41-56

[12] Groppa MD.Benavides MP. Polyamines and abiotic stress. Recent advances. Amino Acids. 2008. 34;35-45.

[12] Hiraga SI, Yamakawa H, Ohtsubo N, Seo S, Mitsuhara I, Matsui H, Honma M & Ohashi Y. An HR-induced tobacco peroxidase gene is responsive to spermine, but not to salicylate, methyl jasmonate, and ethephon. Molecular Plant- Microbe Interactions. 2000. 13: 210-216

[13] Islam A. Fungus resistant transgenic plant; strategies, progress and lessons learnt. Plant Tissue Cult Biotechnol .2006. 16;117-138

[14] Kumar SV, Sharma ML, Rajam MV.Polyamines Biosynthetic pathway as a novel target for potential applications in plant biotechnology. Physiol Mol Biol Plants.2006. 12;13-18.

[15] Martin-Tanguy J. Metabolism and function of polyamines in plants; recent development (New approaches). Plant Growth Regul . 2001.34:135-148

[16] Machatschke S, Kambrowski C, Moerschbacher BM & Reisener HJ. Polyamine levels in stem rust infected wheat leaves and effects of alpha-difluoromethylornithine on fungal infection. Physiol. Mol. Pathol.1990. 36 : 451-459

[17] Negrel J, Vallee JC & Martin C. Ornithine decarboxylase activity and the hypersensitive reaction of tobacco mosaic virus in Nicotiana tobacum. Phytochem. 1984.23: 739-741

[18] Prabhavathi VR, Rajam MV. Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. Plant Biotechnol 2007.24:273–282

[19] Pranjal H. and Rajam MV. Biotic and abiotic stress tolerance in transgenic tomatoes by constitutive expression of S-adenosylmethionine decarboxylase gene. physiol Mol Biol Plants. 2011.17(2): 115–128

[20] Punja ZK. Genetic engineering of plants to enhance resistance to fungal pathogens-a review of progress and future prospects. Can J Plant Psthol 2001. 23:216-23

[21] Rao G, Kaur M, Verma A, Sihachakr D, Rajam MV. Genetic engineering of crop plants for resistance to fungal pathogens. J Plant Biol.1999. 26:31-42

[22] Rajam MV. Polyamines. In: Prasad M.N.V. (ed.) Plant Ecophysiology. John Wiley & Sons. Inc., New York., pp.1997. 343-374.

[23] Rajam MV.. Polyamine biosynthesis inhibitors: New protectants against fungal plant disease. Curri. Sci. 1993.65: 461-469.

[24] Rajam MV. Restriction of pollen germination and tube growth in lily pollen by inhibitors of polyamine metabolism. Plant Sci. 1989. 59: 53-56

[25] Rajam MV, Weinstein LH & Galston AW. Prevention of a plant disease by specific inhibition of fungal polyamine biosynthesis. Proc. Natl. Acad. Sci. USA. 1985. 82: 6874-6878

[26] Rabiti AL, Betti L, Bortolotti C, Marini F, Canova A, Bagni N & Torrigiani P. Short – term polyamine response in TMV-inoculated hypersensitive and susceptible tobacco plants. New Phytol. 1998.139: 549-553

[27] Rea G, Laurenzi M, Tranquilli E, D'Ovidio R, Federico R & Angelini R. Developmentally and wound regulated expression of the gene encoding a cell wall copper amine oxidase in chick pea seedlings. FEBS Lett. 1998. 437: 177-182

[28] Rea G, Metoui O, Infantino A, Federico R & Angellini R. Copper amine oxidase expression in defense response to wounding and Ascochyta rabiei invasion. Plant Physiol. 2002. 128: 865-875.

[29] Slocum RD, Kaur-Sawhney R & Galston AW. The physiology and biochemistry of polyamines in plants,. Arch. Biochem. Biophys. 1984. 235: 283-303

[30] Smith TA Plant polyamines-metabolism and functions. In: Ploymines and ethylene: Biochemistry, physiology, and interactions. (Flores HE, Arteca RN & Shannon JC. eds) American society of plant physiologists. Rockville, 1990. pp 1-23

[31] Smith TA, Davies PJ & Reid JB. Role of polyamines in gibberellin-induced internode growth in peas. Plant Physiol. 1985. 78: 92-99

[32] Walters DR. Polyamines and plant disease. Phytochem. 2003. 64: 97-107

[33] Waie B & Rajam MV. Effect of increased polyamine biosynthesis on stress response in transgenic tobacco by introduction of human S-adenosyl-methionine gene. Plant Sci. 2003. 164: 727-734

[34] Yamakawa H, Kamada H, Satoh M, Ohashi Y. Spermine is a salicylate – independent endogenous inducer for both tobacco acidic pathogenesis- related proteins and resistance against tobacco mosaic virus infection. Plant Physiol. 1998.118: 1213-1222

[35] Ines Ben Rejeb, Victoria Pastor, Brigitte Mauch-Mani. Plant Responses to Simultaneous Biotic and Abiotic stress; Molecular Mechanisms. Plants 2014. 3,458-475.

[36] Hussain S.S, Ali. M, Ahmad, Siddique. K.H. Polyamines;Natural and engineered abiotic and biotic stress tolerance in plants. Biotechnol. Adv. 2011. 29.300-311.

[37] Martin-Tanguy J. 1985. The occurrence and possible function of hydroxycinnamoyl acid amides in plants. *Plant Growth Regul.* 1985. **3**: 381-399

[38] **Foster SA & Walters DR.** 1992. Polyamine concentration and activities of ornithine and arginine decarboxylase in wheat infected with the stem rust fungus**.** ***J. Plant Physiol*.** **140:** 134-136

[39]Martin-Tanguy J, Martin C. & Gallet M. Presence de composes aromatiques lies ala putrescine dans divers *Nicotiana virous.* CR Acad Sci (Paris) Ser D. 1973. 276: 1433-1444. (In French)

[40]Martin-Tanguy J, Martin C, Gallet M & Vernoy R. Sur de puissants inhibiteurs de multiplication du virus de la mosaique de tabac. *CR Acad Sci.*1976. 282: 2231-2234 (In French)