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Plant Defensins : Tissue Specific Expression Leading to Distinctive Functions

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Abstract

Plant defensins are small, cysteine-rich cationic antimicrobial peptides that possess biological activity towards a broad range of pathogenic organisms. These defense peptides are ubiquitous within the plant kingdom and acts as the first line of plant defense. Plant defensins are expressed in several plant tissues, such as seedlings, leaves, tubers, ûowers, pods, roots and fruits. They are mainly secreted at peripheral layers of cells and play an integral role in protecting storage, developmental and reproductive parts of the plants, against pathogen attack or injury as part of a systemic defense response. The expression of plant defensins might be constitutive or can be induced in response to pathogenic attack, abiotic stress or downstream to hormone signaling pathways. Moreover, most defensins are localized and expressed in particular tissues, performing very specific functions, thereby bestowing various benefits in respective hosts. From past few years plant defensins have become interesting and important candidates in transgenic technology, owing to their multifunctional but specific biological roles, especially for their broad-spectrum antifungal activity. This review summarizes about the biological roles displayed by plant defensins when constitutively over expressed in targeted tissues of transgenic plants, under the control of tissue specific promoters, and the predominant role exhibited by plant defensins in defense and developmental processes of plants.

Key words : Plant defensins, tissue specific, constitutive, floral organs, fruit specific, antifungal activity, promoter induced, genetic engineering, transgenic plants.

1. Introduction

Plant defensins are endogenous antimicrobial polypeptides that form an important component of the plant innate immune system. They are produced as the first line of defense in response to invading pathogens (1, 2, 3). In addition, some plant defensins are also induced in response to environmental stress such as drought, salinity (4, 5, 6), and signaling molecules, including methyl jasmonate (MJ), ethylene (ET) and salicylic acid (SA). These plant defensins have multifarious functions such as antifungal, antibacterial and antiviral activities. They also act as protease inhibitors, leading to insecticidal activity (7, 8). The multifunctional roles exhibited by many plant defensins include growth inhibitory effects against microbial pathogens such as bacteria (gram positive and gram negative bacteria), virus, fungi, protozoa and yeast (9, 2, 10, 11) inhibitors of digestive enzymes like α-amylases and serine proteases, anti-herbivore (12, 13), in abiotic stress tolerance (14, 15), heavy metal tolerance (16), plant development, protection of storage and reproductive organs (17, 18, 19, 8), ion channel blockers in mammalian and microbial cell walls (20, 21), antiproliferic activity (22, 7), boosting the herbicide property of

BAR gene (23, 24), antiparasitic activity (25) and root growth inhibition activities (26). The most widely studied and reported biological role of plant defensins is their antifungal role.

Plant defensins form a small gene family comprised of around 15 to 50 defensins per plant species (27). So far more than 1200 plant defensins have been identified from plant species such as *Arabidopsis thaliana, Medicago truncatula, Brassica rapa, Vitis vinifera*, many legumes and grass species (28, 3). The occurrence of multiple copies of defensins across the genome can be attributed to gene duplication events (29). However, sub-functionalization and neo-functionalization of these duplicate genes over the year lead to vast functional diversity on the defensin family. Though most plant genomes have multiple defensin genes, it is intriguing how only few members of the family are responsible for a specific function (30). For example, two defensins *MtDef1* and *MtDef2* identified from *M. truncatula* show difference in antifungal activity (31), suggesting that different defensins may be play specific functional roles.

The functional specificity of defensins can be reviewed at three levels, (i) tissue specific expression of defensin genes in response to particular conditions (ii) distinct subcellular localization of the protein and (iii) structure-dependent activity with respect to target molecules. There are numerous reports that describe the structures of various plant defensins, and their interactions with potential target molecules (17). Moreover, the mode of action of defensins and related pathways has also been studied. The specificity in biological roles of individual plant defensins can be attributed not only to the large structural disparity in the patterns of interconnected cysteine loops and disulphide bridges (10, 32), but also to their distinct spatio-temporal expression patterns. Although members of the defensin family are expressed ubiquitously throughout the plant organs such as seeds, leaves, tubers, flowers, pods, roots and fruits, individual members are usually expressed in specific organs or in response to particular stimuli (33, 30). For example, defensins play an integral role in protecting storage, developmental and reproductive parts of plants, through high expression in the epidermal cells and stomatal cells, which are likely to be the initial points of pathogen attack or injury (34, 3). Expression of most plant defensins is tissue-specific and developmentally regulated, thereby allowing them to perform specific biological functions (35, 36). Although the protein structures and their contribution to the mode of action of defensins have been well reported (37, 30, 28, 38, 39, 3), a detailed account on tissue specific expression of defensins are lacking. This review summarizes how the tissue specific expression imparts more specificity to the function of individual defensins.

2. Structure of plant defensins

Plant defensins were initially identified in the seeds of wheat and barley and were grouped as distant members of the thionin family due to homogeneity in molecular mass, amino acid sequence and the number of cysteine residues (40, 17, 10, 3). However, later studies revealed that these proteins differed in structure, pattern of disulfide bridges and spacing of cysteine residues, demonstrating that they were not a part of thionins, but an independent family (17, 2, 41). In subsequent years these peptides were termed as plant defensins after the identification and characterization of two novel antifungal proteins from *Raphanus sativus Rs-AFP1* and *RsAFP2* (40). Plant defensins are small, globular, cysteine rich cationic peptides with molecular masses between 5-7 kDa (37, 42, 43, 38, 44). The three-dimensional structure of plant defensins is highly conserved with a pattern of eight cysteine residues stabilized by four disulphide bonds, interconnected with three antiparallel beta-sheets and one alpha-helix which is in turn stabilized by a structural motif CS-$\alpha\beta$ (28, 45).

Plant defensins can be classified in to two groups based upon the structure of the mature transcript. The first consists of a signal peptide with size 25-30 amino acid residues, an acidic rich precursor protein (except *Ha-DEF1*, *Lm-def*, *PCP-A1* and *TAD1*) and a mature peptide, basic

in nature with about 45-54 amino acids (37, 30). The signal peptide helps in targeted subcellular localization and mitigates the biological activity of mature peptide when required. The mature peptide is composed of eight strictly conserved cysteine residues that are intended in four intrachain disulfide bridges responsible for the stabilization of the typical defensin structure. These intra-connected disulfide bridges form the CS-$\alpha\beta$ motif that is responsible for typical antimicrobial activity exhibited by plant defensins (28, 45, 46). Although most plant defensins contain four disulphide bridges in its structure, some peptides *PhD1* and *PhD2* from *Petunia hybrid*, contain the fifth disulphide bridge interconnecting the α-helix and the β1-strand, further improves stability of the defensin peptide structure (47). The second group of defensins has an additional carboxy-terminal pro-domain, observed especially in solanaceous species.

X- ray crystallography studies of certain defensins such as *R. sativus* (*RsAFP1*), *Nicotiana alata* (*NaD1*), *Pachyrrhizus erosus* (*SPE10*), *P. hybrida* (*PhD1*), *Pisum sativum* (*Psd1*) and *Saccharum officinarum* (*Sd5*) (48, 49, 47, 50, 51, 52) revealed that carboxy-terminal domain is composed of high content of acidic and hydrophobic amino acids (33 amino acids) along with signal peptide and mature defensin domain (30). This acidic nature of the pro-domain is used to neutralize the basic nature of the mature defensin domain leading to neutrally charged peptide. In addition, carboxy terminal domain also acts as a targeting sequence for sub-cellular sorting, post-translational proteolytic processing and intermolecular steric chaperone (47, 30). Another highly conserved motif found in the plant defensin structure is the γ-core. This motif comprises of two antiparallel β-sheets with an interposed turn region called the β2β3 loop. The β-core is cationic amphipathic motif contains specific residues proline and cysteine, that contributes to the secondary structure and amphipathicity of the motif (53). This motif plays an important role in the antifungal activity of defensin peptides, by inducing effective membrane permeabilization in susceptible fungi (54, 55, 2).

Multifunctional roles and mechanisms of action displayed by plant defensins is been illustrated in detail, along with the signaling cascades and pathways using case studies *RsAFP1* and *RsAFP2* from *R. sativum*, Psd1 from *P. sativum* pods, *MsDef1* from *M. sativa*, and *MtDef4* from *M. truncatula*, and *NaD1* from *N. alata*, *DmAMP1* from the seed of *Dahlia merkii*, *HsAFP1* antifungal peptide *Heuchera sanguinea* (28, 2, 3). The proposed mechanisms include three steps, first is receptor-mediated internalization- defensins specifically interacts with the lipid rafts of fungal plasma membrane composed of sphingolipids and phospholipids, the most common spingolipids is glucosylceramide (GlcCer) (56, 2). Different plant defensins have been shown to interact with different classes of sphingolipids, for example the plant defensin *RsAFP2* from *R. sativam* interacts with GlcCer (57), whereas the plant defensin *DmAMP1* from *D. merkii* interacts with mannosyl di-inositol phosphoryl ceramide ($M(IP)_2C$) (58) . In contrast, the plant defensins *NaD1* from *N. alata* was recently shown to interact with a variety of phospholipids, including phosphatidyl inositol mono-/bis-/tri-phosphates, phosphatidyl serine and phospatidic acid, but not with sphingolipids (59). Second is membrane translocation- upon interaction plant defensins are either internalized in to the fungal cell and interact with intracellular targets, or they stay at the cell surface and induce alteration of membrane integrity and distorts the membrane permeability (60, 61). The third is membrane permeabilization thus results in an increased Ca^{2+} uptake and K+ efflux and ultimately leads to cell death through induction of signaling cascades (62, 63). Kushmerick et al. (1998) have described the ability of plant defensins *1-zeathionin* and *2-zeathionin*, isolated from *Zea maize* kernels in block Na^+ ion-channel on fungal membrane, which leads to fungal membrane impermeability followed by fungal death. Likewise the ability of *MsDef1* isolated from *M. sativus* seed tissue to block L-type Ca^{2+} channels of fungal membranes. A specific γ-core motif (RGFRRR) is been identified in the *MtDef4* sequence acts as translocation

signal required for fungal cell entry (64). Alternatively, ROS production and oxidative stress, most often play a role in defensin-mediated cell death, as has been reported in *RsAFP2, HsAFP1, DmAMP1*, and *NaD1* defensins (52, 65, 66, 67).

3. Tissue specific localization and expression of plant defensins:

Plant defensins are widely distributed in various tissues across the plant. At least one defensin gene is expressed in each plant tissue and some tissues show expression of two or more defensins. The tissue specific localization and expression patterns of these peptides unfold the critical roles they play in defense and development of plants (68). Plant defensins have been identified in leaves, tubers, flowers, pods, seeds, germinating seeds, seedlings and also localized in other peripheral sites like xylem, stomata, and stomata cells, parenchyma cells, where they are expressed either constitutively or upon pathogenic infection, by mechanical wounding and other stress responses (69) Fig. 1. Overall, most of plant tissues constitutively express two or more defensin genes, implying that each defensin is expressed under specific conditions or in specific tissues and display target-oriented functions (Table-1).

Amongst the numerous plant defense peptides isolated from a variety of plant species certain deliver tissue specific expression, for instance four defensin genes isolated and characterization from *Heliophila coronopifolia* (*Hc-AFP1-4*), have a tissue-specific expression patterns confirmed by differential gene expression studies in the native host. The peptides *Hc-AFP1* and *3* expressed in mature leaves, stems and flowers, whereas *Hc-AFP2* and *4* are exclusively expressed in seed pods and seeds. All four peptides were active against two test pathogens *Botrytis cinerea, Fusarium solani,* but displayed different levels of antipathogenecity and modes of action. The expression patterns of the peptides suggests role in protecting vegetative and reproductive structures against pathogen attack, but their roles in plant developmental and physiological processes have not been clearly distinguished yet (8).

3.1 Seedlings : *SPI1*defensin (PR-12)-like protein from *Picea abies*, was found to be expressed only in the radicles, roots, stem, and aerial part of seedlings, but was not detectable in the embryo (70). In more mature plants, expression was observed in leaves most predominantly in epithelial cells such as guard cells of stomata (71), since stomata are the main entryway used by many

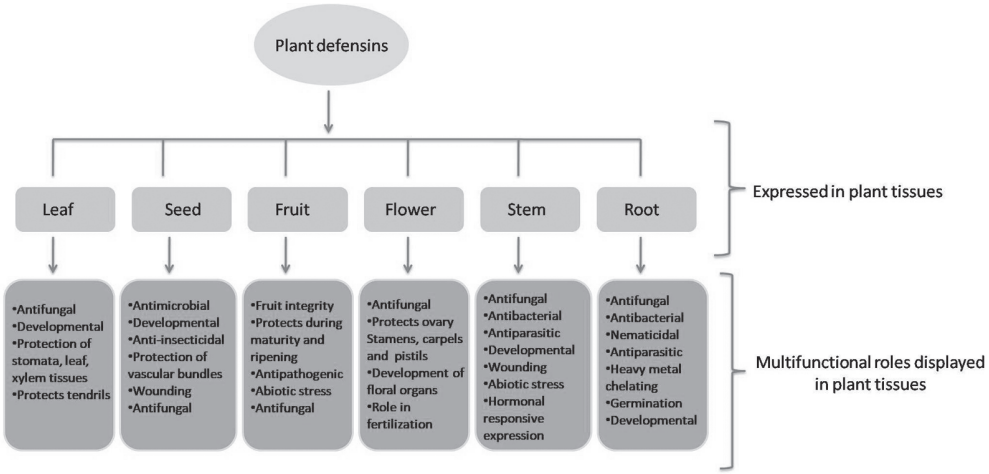

Fig.1. Schematic representation of multifunctional roles displayed by plant defensins in various tissues.

Table 1. Overview of defensin genes from various plant sources, there tissue specific expression leading to characteristic biological activity and transgenic applications.

Plant species	Defensin gene	Expressed in Tissues	Induced by signaling compounds	Transgenically expressed in	Biological role	References
Arabidopsis thaliana	*Pdf2.2, Pdf2.3, Pdf 1.2, Pdf2.1* *Thi2.1*	seedlings, roots, leaves, stems, flowers. flower	MJ, ET JA		antifungal activity, nematicidal activity developmental role,	(99, 100, 72) (87)
Brassica campestris, *Brassica pekinensis*	*BSD1*	flowers			protecting reproductive organs	(82)
Brassica juncea	*Bjdefensin*	leaf, roots	JA		induced by biotic and abiotic stresses	(96)
Brassica oleracea	A1(*PCP-A1*)	flower			development of floral organs	(88)
Capsicum annuum	*J1-1* *CaDef*	fruit leaf		*Solanum esculentum*	antifungal activity	(91, 108)
Citrullus lanatus	*Cldef2.2*	leaves, roots, stems	MJ, SA, ET		antifugal activity	(95)
Carica papaya	*pdf1.1*, *pdf1.2*	fruit	MJ		protects vegetative and reproductive tissues	(101)
Fragaria ananassa	*FaDef1*	fruit			antifungal activity	(92)
Heliophila coronopifolia	*Hc-AFP2,4* *Hc-AFP1*, 3	seed leaves, stems , flowers			protecting vegetative, reproductive organs, antifungal activity	(8)

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Medicago truncatula	*DEFLs MtDef1.1*, *MtDef2.1 MtDef4.2*	root, seeds	MJ	*Triticum aestivum Arachis hypogeae*	antipathogenic activity antifungal activity	(77, 78, 79, 80)
Medicago sativus	*MsDEF1*	seed	MJ		antifungal activity	(31)
Nicotiana alata	*NaD1*	flowers			protects of the reproductive organs	(47)
Nicotiana megalosiphon	*NmDef*	leaf		*Nicotiana tobacum, Solanum tuberosum*	inhibits oomycetes	(112)
Oryza sativa	*CAL1* *PRP1* promoters	root Leaf, stem, grain		*Triticum aestivum, Oryza sativum*	chelating Cd ions protecting storage and reproductive organs	(81) (36)
Picea abies	*SPI1*	radicles, roots, stem, seedlings, leaves			antifungal activity	(70, 71)
Pisum sativum	*Psd1*	pods			antipathogenic role	(62)
Pisum sativum	*DRR230-a*, *DRR230-b, DRR230-c*	leaves, stems, flowers			antipathogenic activity	(74)
Prunus persica	*PpDfn1*	bark			antifungal activity	(93)
Picea glauca	*PgD PgD1 promoters*	leaf, roots	JA	*Arabidopsis thaliana*	antifungal activity	(102, 114)
Pinus sylvestris	*Sp-AMP2*	leaf		*Nicotiana tobacum*	inhibits necrotrophic pathogens	(113)

Raphanus sativus	*RsAFPs*	seeds		*Niotiana tobacum, Oryza sativum*	antifugal activity	(40, 109)
Solanum lycopersicum	*DF1*, *DF2* *DEF2*	leaf leaf , seed			antifungal activity, protection of developmental and storage organs	(73)
Sorghum bicolor		leaf, roots			insecticidal activity, antifungal activity	(90)
Triticum aestivum	*PDF3, PDF5, PDF30* *PRP1*	seed leaf, stem, grain		*Oryza sativum*	antipathogenic activity, protecting storage, reproductive organs	(75, 36)
Torenia fournieri	LURE	flowers			role in pollen tube attractants during fertilization	(18)
Vitisvinifera	*VvAMP2*	flowers			antifungal activity, protects the reproductive organs	(86)
Vigna unguiculata	*VuDEF*	seed			insecticidal activity	(12)
Vigna radiate	*VrD1*	seed			insecticidal activity	(9, 94)
Wasabi japonica	*WD*	root		*Nicotiana tobacum, Lycopersicum esculentum*	antifungal activity	(107)
Zea Maize	*1-zeathionin, 2-zeathionin*	kernels			antifungal activity	(20)
Zea mays	ZmES4	flowers			role in pollen tube attractants during fertilization	(19)

tissue specific expression leading to distinctive functions

leaf infecting fungal pathogens. Likewise, *A. thaliana* defensins *Pdf2.2* and *Pdf2.3* were expressed in seedlings, roots, leaves, stems, and flowers. Besides *Pdf2.1* gene was strongly expressed in syncytia region of roots in host plants, which is a feeding site of beet cyst nematode *Heterodera schachtii,* apart from the feeding site it was expressed only in siliques but *not in other healthy tissues*. Hence the promoter of the *Pdf2.1* gene turned out to be an interesting candidate to drive root specific expression of nematocidal products that would subsequently inhibit syncytium development (72). In addition, *A. thaliana* defensin *Pdf1.2* may be induced in response to ET and MJ further protects the host by minimizing attack of phytopathogenic fungus *Verticillium dahlia.*

3.2. Shoots and leaves : Defensins and defensin-like peptides are functionally diverse and are commonly presented as an immune reaction between plant and pathogen. High expression levels of the defensin (*DF1* and *DF2*) transcripts were observed in *Solanum lycopersicum* leaf tissues collected from the plants grown in soil treated with *Trichoderma viridae* and *Bacillus subtilis* as biological control agents to suppress the activity of the pathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani* (73). Lai and colleagues studied about the expression levels of three homologous *Pisum sativum* defensin genes *DRR230-a*, *DRR230-b, DRR230-c* in various *P. sativum* tissues under biotic stress. Relatively high levels of *DRR230-a* and *DRR230-c* transcripts are present in mature leaves and stems, with intermediate expression levels in young leaves, tendrils and flowers, and low levels in roots and pods (1, 74). Three specific defensin genes *PDF3, PDF5,* and *PDF30* expressions were investigated in shoot tissues of seven commercial Egyptian *Triticum aestivum* varieties: Misr1, Giza168, Sakha94, Sids1, Gemmiza7, Gemmiza11, and Shandawel1 during seed germination, showed that there was difference in defensin gene expression among the seven varieties. This included absence of *PDF5* expression in Sids1and *PDF30* expression in Gemmiza7, Misr1 showed lowest and Shandawel1 gave the highest expression levels of the three studied genes. Other varieties represented various degrees of expression for the three genes (75). The observations can be related to the resistance of *T. aestivum* varieties to diseases and abiotic stresses, would certainly contribute information for wheat breeding programs and variety evaluation.

3.3 Roots Mitra and Long, (2004) reported that majority of defensins and defensin like proteins (*DEFLs*) were expressed in root nodules and seeds in *M. truncatula*, since they are the nutrient rich sources, composed of large amounts of protein, polysaccharides, and lipids that provide energy and raw materials for germination and development of the seedling, and also most vulnerable sites for attack of multitude soil pathogens to attack (77). Therefore nodule-speciûc *DEFLs* are engage in complex synergistic interactions with other AMPs to increase their efficiency against broad spectrum microbial population invitro and in field conditions as well (78, 79, 80). Defensins and defensin like proteins also play heavy metal remediating role, by accumulating toxic metal in edible plant parts while producing safe and nutritious edible by-products. Similarly defensin-like protein *CAL1* (cadmium (Cd) accumulation in leaf 1) is expressed preferentially in root exodermis and xylem parenchyma cells of *Oryza sativa*. *CAL1* acts by chelating Cd in the cytosol and facilitating Cd secretion to extracellular spaces, hence lowering cytosolic Cd concentration while driving long-distance Cd transport via xylem vessels. *CAL1* does not allow Cd or other heavy metals accumulation in rice grains, thus providing an efficient molecular tool to agriculture biotechnology, to develop *O. sativa* varieties that produce safe grains while remediating paddy soils (81).

3.4 Flower Several plant defensins and other *DEFLs* are highly expressed in flowers (Lay et al., 2003). These flower abundant antimicrobial peptides were shown to be crucial for plant reproduction, playing different functions during flower fertilization. In *Brassica campestris*

and *Brassica pekinensis* defensin 1 (*BSD1*) was expressed only in stamens of flowers (82). Flower-specific expression of defensin genes was also observed in solanaceous plants like, *N. tabacum* (83), *N. alata* (47), and *N. paniculata* (84). This suggests that flower specific defensin genes are more likely to protect the reproductive organs from effective pathogenic attack. The expression patterns of *N. alata* plant defensin (*NaD1*) was observed in floral organs like anthers, pistils, ovaries and petals of ornamental *N.tobaccum* flowers, and barely expressed in any other organs. *NaD1* expression was highest in young floral buds and decreased significantly as the flower matures. It is noteworthy that this peptide was expressed in the outermost layers of the sepals and petals and in tissues that surround the pollen or pollen tubes. The location of *NaD1* is consistent with its defense role as it protects the germ cells against possible damage by invading pathogens (47). Similar expression patterns were observed in two other floral defensins *FST*, *TPP3* (83, 85). According to Lay et al. (2003), floral defensins are of two types in solanaceous plants. One with C-terminal pro-domain which is deposited in the vacuoles this type is present only in floral buds, and the other type that does not have the C-terminal pro-domain is produced in epithelial layers of cells (47). *V. vinifera* defensin like peptide *VvAMP2* is highly conserved peptides with 10 cysteine residues, and active against the fungal pathogen *Botrytis cinerea*. Quantitative expression analysis revealed that *VvAMP2* and related *DEFLs* are specifically expressed in *V. vinifera* inflorescences, highly expressed in pollen/ stamen, and weak expression was observed in calyptrae and carpels suggesting a role in *V. vinifera* fertilization (86). Similarly *LURE* and *ZmES4*, *DEF* like genes from *Torenia fournieri* and *Zea mays* are highly expressed in the gametophyte synergid cells and functions as pollen tube attractants during fertilization (18, 19).

Plant defensins are also induced in response to plant hormones in floral tissues. For example, the flower defensin *Thi2.1* in *A. thaliana* can be induced by abiotic stress mediated by the activation of SA induction within the systemic acquired resistance pathway (87). In flowers the induction of defensins may also be correlated with flower development suggesting that other factors may be involved in flower defensin gene transcription. An intriguing defensin transcript, Pollen coat protein class A1(*PCP-A1)*, from *B. oleracea*, accumulate in microspores in flower and associated with self-incompatibility systems, further studies are required to elucidate its exact role (88). Certain transcriptional reprogramming like inverse regulation or antisense suppression occurs in host tissues occurs during plant defense activation against pathogenic attack. Stotz et al. (2009) reported the defensin gene *DEF2* expression was observed in developing flowers tissues in *S. lycopersicum*, constitutive over expression of *DEF2* enhances foliar resistance against *B. cinerea* and displayed inversely regulations like reduces pollen viability and seed production, alterations in various developmental and storage organs (73).

3.5 Seed and fruit Recently, microarray analysis in two model plants *A. thaliana* and *M. truncatula* showed a set of defensins and defensin-like genes were expressed specifically in seeds or fruits (89). Plant defensins play a very important role in protection of seed and seedlings from soil borne pathogens (40) *R. sativum* seeds with pathogens infected or mechanically damaged seed coats showed 30 folds increased expression of defensin genes. Various experiments on the location of plant defensins within the seed revealed that they are located in high levels in the peripheral cell layers and in the spaces between different seed organs, middle lamellae of the cell walls of the different seed tissues. Like the other defensins *RsAFPs* is localized in seeds organs where the first contacts with invading fungal pathogens occur. Furthermore, defensin peptides (*Psd1*) isolated from the seed of *P. sativum*, was shown to be localized primarily in vascular bundles and epidermal tissues of *P. sativum* pods, which are the first barriers to pathogen invasion (62). Plant defensins has an important activity like anti-insecticidal inhibition (12). They could interfere

with α-amylase enzyme secreted in the insect gut and seize the insect energy derived from the starch degradation activity. Three defensin peptides $SI\alpha_1$, $SI\alpha_2$ and $SI\alpha_3$ isolated and characterized from these seed tissue of plant *Sorghum bicolor* inhibited the amylase activity of insects *Periplaneta americana* and *Locusta migratoria migratorioides* and attributes weak antifungal activity against fungus *Aspergillus oryzae* (90).

Fruits are especially vulnerable to pathogen infection at the fully ripe stage due to significantly high amount of nutrient rich material are stored in fruits, therefore, the putative extracellular localization of antimicrobial proteins like plant defensins enhances the chances of the maintenance of fruit integrity and seed maturation (91). The defensin peptides *J1-1* isolated from *Capsicum annum* is associated with fruit specific expression, but not in other tissues such as leaf, stem, root, flower. Protein levels of *J1-1* were gradually increased in the fruits from the early stage of the ripening to maturity, because this stage is more prone to the infection of anthracnose pathogen, *Colletotrichum gloeosporioides.* Furthermore *J1-1* defensin gene expression levels were likely increased both transcriptional and translationally in infected fruits during ripening. This peculiar characteristic of the *C. annum* defensin was further exploited in developing transgenic *C. annum* plants overexpressing *J1-1*, as expected the products showed increased tolerance to anthracnose fungus (91).

Semi quantitative expressions of defensin genes from *Fragaria ananassa* (*FaDef1*) were analyzed in root, stem, leaf, flower, and fruit tissues in three cultivars namely, Queenelisa, Camarosa, and Paros. The results revealed that higher amount of *FaDef1* expression was observed in developed fruits compared to that of immature fruit, and there was no observable expression in the root. Moreover, *FaDef1* is responsive to biotic and abiotic stress signal compounds and showed significant resistance against *B. cinerea* (92). Hence these peptides may be used as a candidate gene for engineering plants against gray mold. *Prunus persica* defensin gene (*PpDfn1*) is expressed in bark tissues of an year-old shoots, and is also expressed in early fruit development stages. A recombinant version of *rDFN1* was expressed in the yeast, *Pichia pastoris*, the obtained protein inhibited germination of the fungal pathogens *Penicillium expansum* and *B. cinerea*, but not the Gram-negative bacterium *Erwinia amylovora* (93).This study clearly indicated that both physiological role and antifungal potential exhibited by plant defensins in specific tissues. Defensins *VuDEF* expressed in seeds of *Vigna unguiculata* and defensin *VrD1* from *Vigna radiata* expressed in the germinating seed exhibited anti-insecticidal activity against α-amylase enzyme activity in insects *Acanthoscelides obtectus*, *Callosobruchus maculates, Zabrotessub fasciatus*, *Tenebrio molitor* (12, 9, 94).

3.6 Hormone-responsive constitutive expression Defensin-like protein from *Citrullus lanatus Cldef2.2*, had high amino acid homology with the *A. thaliana PDF2* cluster and is close to *AtPDF2.5.* The expression profiles revealed that expression was observed in all the examined tissues, including leaves, roots, and stems, the highest expression level was observed in roots. The protein abundance was observed in various tissues especially when subjected to SA, MJ and ET, also to *F. oxysporum* challenge (95). Similarly, the gene expression studies of *Bjdefensin* gene from source *B. juncea* revealed that the transcript levels of *Bjdefensin* gene increased significantly upon *Alternaria* infection, Jasmonic acid and wounding treatments but was not induced by SA. Consequently, the *Bjdefensin* promoter (2.5 kb) was isolated and cloned upstream of GUS gene in pORER2 vector. In silico studies of *Bjdefensin* promoter showed many important conserved cis-elements, responsive to biotic and abiotic stresses. Histochemical GUS assay showed pathogen-inducible expression of *Bjdefensin* promoter after fungal infection and also induced by JA and wounding (96).

Effect of fungal infection, wounding, various plant hormones and chemicals induces the accumulation of plant defensin transcripts in various tissues (97). As per the literature

chemicals such as mercuric chloride, MJ, ET and paraquat led to the induction of defensin gene expression (97). In *M. truncatula* defensin genes *MtDef1.1* and *MtDef2.1*are highly expressed in dry mature seed and are strongly induced by exogenous MJ application in young seedlings but not by ET or SA (98). Interestingly in closely related *M. sativa*, defensin gene expression is not observed by treatment with MJ, and down-regulated expression was observed by ET treatment (98). The *Arabidopsis* defensin gene *PDF1.2,* has been shown to be induced strongly in leaves by MJ and ET, but not by SA (99, 100). The data presented here suggest that some aspects such as induction of defensin genes via hormones applications or chemicals may not be uniform in inter and interspecific plant species. Similarly, *pdf1.1* and *pdf1.2* is induced in fruit, peel and leaf tissues of papaya upon cold stress and MJ treatment, which suggests the presence of analogous defense mechanisms in the vegetative and fruit tissues of plants (101, 102). Pervieux et al. (2004) demonstrated that *Picea glauca* Defensin 1 (*PgD1*) is up-regulated by wounding and JA in leaf and root tissues, more importantly, that recombinant *PgD1* displays antifungal activity against *Cylindrocladium floridanum*, *F. oxysporum,* and *Nectria galligena* (102).

4. Tissue specific expression of defensin genes in transgenic plants Certain attempts have been made by deploying heterologous defense peptides in many susceptible plants as tools to enhance their disease-resistance capability (103). Although most of them were not so successful, few of them were inspiring in the search for new alternatives (79, 104). The reasons behind might be low expression levels, or low half-life of the transgene or transgene product inactivation by host proteolytic enzymes (105, 106). Numerous studies have demonstrated the efficient role of plant defensins when cloned and expressed in different host plants and assayed against various pests and pathogen exists, most of them were efficacious in invitro and field conditions (2). As already discussed, plant defensin genes are induced by biotic, abiotic factors, during seed germination, flowering and hormonal treatments. They might be constitutively expressed, or show tissue-specific and developmentally regulated expression patterns (35, 36, 17, 19, 47). Plant defensins have been recognized as prominent candidates for generating transgenic crops due to their multifunctional role to pave ways for generating durable resistance against broad range phytopathogens. To validate the presumed role, plant defensins from distinctive plant sources have been cloned and transgenically expressed in various hosts (97, 1, 79). The first attempt was made to evaluate transgenic tobacco plants expressing antifungal defensin genes *Rs-AFP2* source from radish, high levels of peptide expression in leaf and root tissues was observed in transgenic plants, and showed an increasing resistance towards *Alternaria longipes* in invitro assays (40).

Wasabi defensin gene (0.5 kb) gene expression driven by the root-specific *LjNRT2* and *AtNRT2.1* promoters were overexpressed in the roots of transgenic *N. tobaccum* and *S. esculentum* plants showed stable integration and expressed in the root tissues but not in the leaf tissues. In fungal bioassays all transgenic plants showed increased resistance towards *F. oxysporum* compared to non-transformed plants. The study suggests that LjNRT2 and AtNRT2.1 promoters triggered the antifungal gene expression in the roots tissues and conferred increased resistance to the root pathogen *Fusarium oxysporum*. The transgenic products are safe in terms of biosafety issues since the roots of *Solanum esculentum* are not edible (107). Similarly, transgenic *Solanum esculentum* plants expressing the *Capsicum annum* defensin gene (*CaDef*) under the control of CaMV 35S promoter, accumulated defensin peptide in the leaf tissue showed enhanced ability in effective growth inhibition of fungi *Fusarium* sp. and *Phytophthora infestans* in vitro (108).

Jha and Chatoo, (2009) performed a successful attempt of generating transgenic *O. sativa* plants expressing cleavable chimeric gene

constructs consists of a leader peptide and two *Dm-AMP1* and *Rs-AFP2*, defensin genes from the seeds of *D. merckii* and *R. sativus*, driven by control of single maize ubiquitin promoter, peptides were targeted to express at the extracellular spaces of leaf and root tissues. Plants transformed with polyprotein construct showed 70-90% significant disease resistance against *Magnaporthe oryzae* and *Rhizoctonia solani* pathogens (109). Similarly, transgenic *Triticum aestivum* genotypes expressing a chimeric gene encoding an apoplast-targeted antifungal plant defensin *MtDef4.2* from *M. truncatula*, displayed resistance leaf rust pathogens without affecting the root colonization of a beneûcial arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Histo-pathological analysis suggested the presence of both pre- and post-haustorial resistance to leaf rust in these transgenic lines expressing plant defensin *MtDef4.2* can provide substantial resistance to leaf rust disease in transgenic *T. aestivum* without negatively impacting its symbiotic relationship with the beneûcial mycorrhizal fungus (110). Similarly transgenic *Arachis hypogeae* genotypes expressing *Medicago* defensin genes *MtDef4.2, MsDef1* in seed tissues showed enhanced resistance against *Aspergillus flavus* infection and low to non existence levels of aflatoxin accumulation (111). Constitutive expression of *NmDef02* gene derived from *N. megalosiphon*, in leaf tissues of transgenic *N. tobaccum* and *S. tuberosum* plants delivered enhanced resistance against various plant microbial pathogens, including the oomycete *Phytophthora infestans*, causal agent of potato late blight disease, under greenhouse and in field conditions (112).

In addition plant defensins isolated from forest tree species contribute to sustainable forestry practices and the improvement of commercially grown trees to combat many microbial pathogens (113). These AMPs elevate host defense and can be used as molecular markers for resistance breeding. Transgenic *N. tobaccum* plants expressing the gene encoding *Pinus sylvestris* antimicrobial protein *Sp-AMP2,* gene showed enhance resistance and reduced lesions size caused by the necrotrophic pathogen *B. cinerea*. The transcript of *Sp-AMP2* was abundantly secreted in extracellular spaces of leaf and root tissues in most transgenic lines. This study provides an insight into the role of *Sp-AMP2* and its functional and ecological significance in the regulation of plant–pathogen interactions (113). The characterization of tissue-specific and pathogen-inducible promoters is essential for localized expression of defense-related genes. Transgenic *T. aestivum* and *O. sativa* plants were developed through the stable transformation with four defensin promoters pathogen responsive and resistance genes (*PRPI) promoter* from *T. aestivum* and *O. sativa* source, along with GUS reporter gene as fusion constructs. The promoters were active before and at anthesis in both transgenic *T. aestivum* and *O. sativa* plants with activity mainly concentrated in the ovary. In transgenic *O. sativa*, GUS activity was also observed in vascular tissue of lemma and anthers. After fertilization, GUS was strongly expressed in the outer cell layers of the pericarp and in vascular bundle of the grain. *T. aestivum* promoters were active in transgenic rice embryos, roots and coleoptiles. All *T. aestivum* and *O. sativa* promoters were strongly induced by wounding in leaf, stem and grain of transgenic *O. sativa* plants. These results suggest that *PRPI* promoters will be useful for tissue specific targeting and accumulation of proteins for resistance towards pathogens in vulnerable tissues of developing and germinating grains (36). Furthermore, *P. glauca* Defensin 1 (*PgD1*) promoter fragment fused to the uidA gene (GUS) was cloned, characterized in *A. thaliana* and *P. glauca* to analyse spatio-temporal promoter activity. The transgenic plants were subjected JA, wounding and infection by the hemibiotrophic pathogen *Pseudomonas syringae, Ceratocystis resinifera*, showed an up-regulation of both endogenous defensin and PgD1:GUS transgene, in transgenic spruce embryos, expression was clearly restricted to the shoot apical meristem. In *Arabidopsis*, leaves, flowers, guard cells and trichomes showed upregulation of transgene, and

also resistance against infection with the necrotrophic pathogen *Ceratocystis resinifera* and wounding (114). This study demonstrated that inspite of being expressed in evolutionarily divergent hosts *A. thaliana* and *P. glauca*, the promoter fragment appears relatively conserved and fully functional in regulatory mechanism and the defence signaling pathways. A defensin like ORF from *Mytilusedulis chilensis* driven by 35S promoter transformed in to *N. tobacum* plants, showed reasonably good transgene expression in leaf tissues not in other tissues, further offered detectable resistance to *N. tobacum* leaves when challenged with *Pseudomonas syringae* tissues (115).

Conclusions

Plant defensins are important components of the plants innate immunity, and exhibit protective antimicrobial role in various plant tissues and organs. Plant defensins are ubiquitous among different plant species, and are localized in wide range of plant organs, including seeds, leaves, pods, flowers and tubers. The tissue specific localization of plant defensins play a vital role in protection and development of plants, where they are expressed either constitutively or induced upon fungal infection, abiotic stress conditions or mechanical wounding. Plant defensins are mostly secreted in the periphery layers of plant organs, since these locations are consistently prone to stress, they are activated in the initial defense response against pathogens and inturn activate other antimicrobial pathways. Furthermore, plant defensins display an array of biological activities including protein translation inhibition activities and enzyme inhibitors of α-amylases and proteases, antiproliferic, antiparasitic and heavy metal remediation and many more. Considering the broadspectrum antipathogenic activity, tissue specific expression and various developmental roles of plant defensins, they are considered as prominent candidates in agricultural and pharmaceutical biotechnology. For last two decades tremendous scientific efforts were made and progress has been achieved, by using genetic engineering technology in plants. Expression of antimicrobial peptides in specific tissues towards fungal pathogens and their role in enhanced resistance to combat the infection attracted the scientific community. Engineering tissue-specifically expressed plant defensins or pathogen-inducible promoters, to develop the transgenic traits that are effective against a broad range of pathogens. Utilization of chimeric defensin peptides and polypeptide construct shows double impact to enhanced disease resistance. Successful evaluation of transgenic plants for their efficacy against pathogenic attack invitro and in field conditions is a prerequisite to augment in on-going disease management practices. Transgenic plants with targeted expression of defensin genes with enhanced disease resistance can become an integral component of food security and disease management programs in the future.

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