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Mechanism of tobacco osmotin gene in plant responses to biotic and abiotic stress tolerance: A brief history

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Abstract: Plants are recurrently exposed to myriads of biotic and abiotic stresses leading to several biochemical and physiological variations that cause severe impacts on plant growth and survival. To overcome these challenges, plants activate two primary defense mechanisms, such as structural response (cell wall strengthening and waxy epidermal cuticle development) and metabolic changes, including the synthesis of anti-microbial compounds and proteins, especially the pathogenesis-related (PR) proteins. PR proteins are members of a super large family of defense proteins that exhibit antimicrobial activities. Their over-expression in plants provides tolerance to many abiotic and biotic stresses. PR proteins have been classified into 17 families, including PR-5–also called thaumatin-like proteins (TLPs) that involve osmotin and osmotin-like proteins (OLPs). Osmotin was first identified in tobacco (*Nicotiana tabacum* var. Wisconsin 38), and then its homolog proteins (OLPs) were reported from the whole plant kingdom. Osmotin and OLPs are ubiquitous in all fruits and vegetables. Their expression has been detected in various plant tissues and organs. The phylogenetic tree studies revealed that the osmotin group originated from spermatophytes. Moreover, the atomic structure of OLP has shown similarity to thaumatin and TLPs from monocot and eudicot species, which determines a strong evolutionary pressure in flowering plants for conserving thaumatin fold. This is associated with the role of these proteins against pathogens as defense molecules and to induce stress tolerance to plants against several biotic and abiotic factors. In this review, we have briefly described the development history of osmotin, including its function and mechanism to induce biotic and abiotic stress tolerance to plants.

Introduction

The growth and productivity of crop plants are mainly determined by their susceptibility to environmental stresses. Throughout their life cycle, plants are continually subjected to several biotic and abiotic stresses, which cause biochemical, morphological, and physiological variations that affect normal plant development, productivity, and even survival (Patade *et al.,* 2013; Kumar *et al.,* 2015). The biotic stress factors include bacteria, fungi, and viruses, while drought, salinity, high or low temperature, ultraviolet radiations, and hypoxia are the abiotic factors that provoke stress conditions in plants (Bashir *et al.,* 2020). To overcome these stresses, plants defend themselves by initiating different stress response mechanisms,

for example, the structural response that involves cell wall strengthening and waxy epidermal cuticle development.

These responses deliver enough strength and rigidity to the plants that enable them to decrease the effects of damage caused by biotic and abiotic stresses (Bashir *et al.,* 2020). Another stress response mechanism that plants activate is the metabolic modifications, such as the generation of reactive oxygen species (ROS), activation of hypersensitive response, synthesis of anti-microbial compounds, and proteins, particularly the synthesis of PR proteins (Das *et al.,* 2011; Li *et al.,* 2015).

PR proteins are members of a super large protein family in plants–related to defense against abiotic and biotic stresses, and the over-expression of these proteins renders plants tolerant to these stresses (Kaur and Kumar, 2020). Based on the primary structure, amino acid composition, biochemical activities, and serological relationship, PR proteins have been classified into 17 families, i.e., β 1,3 glucanases (PR-2), chitinases (PR-3, 4, 8 and 11), thaumatin-like proteins (PR-5), ribosome-inactivating proteins (PR-10), defensins

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(PR-12), thionins (PR-13), and lipid transfer proteins (PR-14) (Hakim et al., 2018; Kaur and Kumar, 2020). Among these, pathogenesis-related protein family 5 (PR-5) in particular, is well-known for its role in osmoregulation, plant development, and for its important antimicrobial activities (Mani et al., 2012). The osmotin and osmotin-like proteins (OLPs) among PR-5 have been considered most important in provoking tolerance to several biotic and abiotic stresses in plants (Chowdhury et al., 2017; Bashir et al., 2020). Since the last decade, several studies have been conducted to explore the functions of osmotin and OLPs in a diverse range of plants (Table 1). The over-expression of these genes provides tolerance to drought, salinity, cold, high/low temperatures, plant development, and tolerance to plant diseases caused by fungi, bacteria, or viruses. Most of the research to date has been conducted on the functions and mechanism of osmotin and OLPs. However, in the present study, we have mainly focused on the development history of osmotin and OLPs and have briefly described their function and mechanism for inducing biotic and abiotic stress tolerance in plants.

Development History of Osmotin

Osmotin protein was first discovered in tobacco (Nicotiana tabacum var. Wisconsin 38) cells adapted to a low osmotic potential environment that exhibited salt stress tolerance (Singh et al., 1987). Thus, it was named 'osmotin' due to its function in lowering osmotic potential under stress (Viktorova et al., 2012). Later, some homolog proteins were also identified throughout the plant kingdom involving various monocot and dicot plants (Viktorova et al., 2019). Osmotin and its homolog proteins, the OLPs are ubiquitous in all vegetables and fruits (Viktorova et al., 2012). In plants, the expression of these genes has been observed in several organs, such as flowers, leaves, trichomes, grape skin, somatic embryos, and olive seed coat and endosperm. Osmotin has also been found in plant tissues like in the epidermis of corolla, stem, and root (Bashir et al., 2020). However, the studies on phylogenetic tree analysis revealed that the osmotin group emerged from spermatophytes (Faillace et al., 2019). The atomic structure of an OLP, CpOsm from Calotropis procera latex has shown that its three-dimensional structure was very similar to the structure of thaumatin and TLPs from eudicot and monocot species. It indicates a strong evolutionary pressure in flowering plants for conserving thaumatin fold associated with the role of these proteins as defense molecules against pathogens and their function in providing tolerance to plants against different stresses (Ramos et al., 2015).

In fact, osmotin is synthesized in the vacuole possessing a molecular weight of 26.4 kDa in its pre-protein form, but its molecular weight decreases to 24 kDa during maturity. Notably, osmotin is a multifunctional protein with 244 residues. In addition to osmoregulation, it plays a significant role in protecting plants against pathogens. Therefore, it has been classified in the family of PR proteins (PR-5 family) (Abdin et al., 2011; Anu et al., 2015; Su et al., 2017). Osmotin and all other members in the PR-5 family share high amino acid sequence similarity to thaumatin (Cao et al., 2016). Typically, the PR-5 family proteins, also known as TLPs, have a molecular mass ranging from 21 to 26 kDa that usually contain 16-cysteines residues. These residues make 8 disulfide linkages, which are associated with the structural stability of these proteins across a diverse pH range, heat-induced denaturation, and accurate folding (Cao et al., 2016).

As a cationic protein, osmotin has two forms–the one possessing isoelectric point (pI) of 7.8 while the other has pI more than 8.2. The molecular weight of both slightly differs, resulting in water-soluble osmotin I and water-insoluble osmotin II (Noori and Sokhansanj, 2008). In addition, the strong amino acid (Glu, Asp, Asp4) residues exist on the grooves of osmotin proteins, which lead to their acidic nature due to the negative charge (Wan et al., 2017). A recent report on osmotin from Kandelia obovate (KoOsmotin) also indicated a negatively charged molecular surface with a hydrophilic nature of the KoOsmotin protein (Fei et al., 2021). Whereas the OLPs are neutral in nature (Wan et al., 2017). The peptide sequence of osmotin is also similar to the tomato NP protein 24, barley thiamine-like proteins, potato PR protein C, the maize trypsin/a-amylase inhibitor, and tobacco antiviral protein gp 22 (Subramanyam et al., 2011). Osmotin comprises three motifs, which exhibit similar folding to PR-5 proteins like zeamatin and thaumatin (Hakim et al., 2018). However, it does not have glycosylation locations, DNA-binding motifs, and introns (Abdin et al., 2011).

The polypeptide structure of osmotin has shown variation in terms of the presence of C-and N-terminal polypeptide extensions. The C-terminal domain is always supposed to be present in osmotin and other PR-5 proteins (Viktorova et al., 2012), for example, the TlOsm. The presence of a C-terminal elongation helps in the localization of the protein in the vacuole (Viktorova et al., 2019). But some of the proteins in the PR-5 family, such as KoOsmotin (Fei et al., 2021), OLP (Onishi et al., 2006), ZLP (Moeller and Tiffin, 2005; Schnable et al., 2009), TLP (Singh et al., 2013), and zeamatin (Sun et al., 2018) lack C-terminal domain. Several PR-5 proteins that lack C-terminal elongation contain only the N-terminal signal peptide–are mainly considered to be secreted into the extracellular matrix (Onishi et al., 2006) and are localized in the apoplastic space of plants (Tachi et al., 2009). However, the subcellular localization studies performed for plant osmotins have reported their localization on multiple subcellular compartments, including cytoplasm, endoplasmic reticulum, chloroplast, vacuole, and plasma membrane (Le et al., 2018; Fei et al., 2021).

Although extensive research has been conducted on the defense function of osmotin, its complex interplay in gene regulation was not clear until the last decade. Initially, various studies hypothesized that osmotin might be a transcription factor for regulating key genes in response to biotic and abiotic stresses (Barthakur et al., 2001; Husaini and Abdin, 2008). However, later this hypothesis was ruled out by the fact that osmotin did not contain any DNA-binding motifs (Abdin et al., 2011; Fei et al., 2021). Further, the osmotin had also been reported to initiate mitogen-activated protein kinase (MAPK) that was essential for most of the regulatory and signaling processes (Yun et al., 1998). Therefore, researchers have recently demonstrated that osmotin is a key regulator responding to different plant stresses (Le et al., 2018). In addition, osmotin plays a vital

TABLE 1

Role of osmotin and osmotin-like proteins (OLPs) in biotic/abiotic stress tolerance in plants

Osmotin/OLP	Gene	Species isolated from	Species validated in	Role	Reference
			Tolerance to abiotic stresses		
Osmotin	N. tabacum Osmotin	Tobacco (N. tabacum L.)	Tomato (S. lycopersicum L.)	Tolerance to salt and drought. Increased chlorophyll contents and leaf area	Goel et al., 2010
Osmotin	N. tabacum Osmotin	N. tabacum L.	Mulberry (Morus indica L. cv K2)	Tolerance to drought and salt	Das et al., 2011
Osmotin	N. tabacum Osmotin	N. tabacum L. cv. Wisconsin 38	Chilli pepper (Capsicum annuum L.)	Salt tolerance, improved level of proline, chlorophyll, and relative water contents, and antioxidative enzymes	Subramanyam et al., 2011
Osmotin	N. tabacum Osmotin	N. tabacum L.	Carrot (Daucus carota L.)	Drought tolerance	Annon et al., 2014
OLP	SnOLP	S. nigrum var. americanum	Soybean (Glycine max L.)	Drought tolerance, higher grain productivity, stomatal conductance, and CO_2 assimilation	Weber et al., 2014
Osmotin	HbOsmotin	Hevea brasiliensis	Arabidopsis thaliana	Osmotic stress tolerance	Tong et al., 2016
OLP, Chitinase (Chi11)	OLP + chi11	–	S. lycopersicum L.	Salinity and drought tolerance. Enhanced plant biomass, water content, and fruit yield.	Kumar et al., 2016
OLP	SindOLP	S. nigrum L.	Sesame (Sesamum indicum)	Tolerance to drought, salt, oxidative stress.	Chowdhury et al., 2017
Osmotin	N. tabacum Osmotin	N. tabacum L.	N. tabacum L.	Salt tolerance and anti-fungal activity	Viktorova et al., 2017
Osmotin	N. tabacum Osmotin	N. tabacum L.	Olive (O. europaea L.)	Drought tolerance	Silvestri et al., 2017
Osmotin	N. tabacum Osmotin	N. tabacum L.	Kiwi (Actinidia deliciosa A. Chev.)	Drought tolerance	Rugini et al., 2018
Osmotin	TlOsm	T. loliiformis	Fiveminute grass (T. loliiformis and Rice (O. sativa)	Drought, salinity, and cold tolerance. Increased survival rate, water content, and membrane integrity in transgenic plants	Le et al., 2018
Osmotin	NtOsm	N. tabacum	Transgenic tomato (S. lycopersicum L. cv. Pusa Ruby)	Cold tolerance, improved seed germination and growth rate	Patade et al., 2018
Osmotin	Osmotin	–	Barley (Hordeum vulgare)	Salt tolerance	Viktorova et al., 2019
Osmotin	–	–	N. tabacum L.	GlcCer tolerance. GlcCer induces resistance in tobacco plants against infection by TMV.	Bernardino et al., 2020
OLP	PhOSM	Petunia hybrida	Petunia hybrida	Secondary cell wall biosynthesis and wood development	Li et al., 2020
Osmotin	N. tabacum Osmotin	N. tabacum L.	O. europaea L.	Salt tolerance	Bashir et al., 2021
Osmotin	KoOsmotin	Kandelia obovata	Escherichia coli	Cold tolerance	Fei et al., 2021
			Tolerance to biotic stresses		
Osmotin	N. tabacum Osmotin	N. tabacum L.	A. deliciosa A. Chev.	Resistance to Botrytis cinerea and Cadophora luteo-olivacea	Rugini et al., 2010
OLP	CaOSM1	C. annuum L.	C. annuum L.	Defense against Xanthomonas campestris pv. vesicatoria by inducing response to cell death and oxidative burst	Choi et al., 2013

(Continued)

Table 1 (continued).

Osmotin/OLP	Gene	Species isolated from	Species validated in	Role	Reference
OLP	ObOLP	Sweet basil (Ocimum basilicum)	O. basilicum	Anti-fungal activity against Pichia pastoris, Candida albicans, and Saccharomyces cerevisiae	Rather et al., 2015
Osmotin	OsOSM1	O. sativa	O. sativa var. Xudao 3	Sheath blight resistance	Xue et al., 2016
OLP, Chitinase (Chi11)	OLP + chi11	–	S. lycopersicum L.	Tolerance to A. solani and F. oxysporum	Kumar et al., 2016
OLP	TcOsm1	Theobroma cacao L.	E. coli	In vitro Growth inhibition of Moniliophthora perniciosa and Pichia pastoris X-33	Falcao et al., 2016
Rice chitinase (chi11), Tobacco osmotin (ap24)	chi11 + ap24	–	O. sativa	Sheath Blight tolerance	Sripriya et al., 2017
OLP	SindOLP	S. nigrum L.	Sesame (Sesamum indicum)	Tolerance to charcoal rot (M. phaseolina) pathogen	Chowdhury et al., 2017
Osmotin	Osmotin	–	H. vulgare	Tolerance to F. oxysporum	Viktorova et al., 2019
OLP	PnOLP1	Panax notoginseng	N. tabacum L.	Resistance to F. solani	Zhao et al., 2020
Osmotin	OsmWS	Withania somnifera	Potato (Solanum tuberosum L.)	Resistance against the early blight of potato	Kaur et al., 2020

Note: GlcCer: Glucosylceramides; TMV: Tobacco mosaic virus.

role in protecting the structure of native proteins and repairing degraded proteins (Patade et al., 2013).

The Role of Osmotin

Role of osmotin in abiotic stress tolerance
Osmotin is a multiple stress-responsive PR-5 protein initially considered as a salt-induced protein in osmotically stressed tobacco cells (Singh et al., 1985; Kumar et al., 2015). Under low water potential conditions, osmotin provides defense to the cell membrane and is involved in osmoregulation and food preservation (Hakim et al., 2018; Bashir et al., 2020). Among all identified PR-5 proteins, osmotin and OLPs are primarily characterized for their function in osmoprotection and anti-fungal activities (Chowdhury et al., 2017). These proteins accumulate under stress conditions that result in the compartmentalization of ions or solutes and initiate anti-pathogen activities (Kumar et al., 2015). To date, the genes that encode osmotins and OLPs have been isolated from various plant species, which are known to exhibit multiple functions to enhance stress tolerance (Le et al., 2018; Li et al., 2020).

After the first discovery from N. tabacum, osmotins and OLPs have now been isolated and cloned in various species of the plant kingdom, as summarized in Table 1. Many studies have reported enhanced tolerance to salinity, cold, drought, as well as some combined stresses by over-expressing osmotin gene in plants (Subramanyam et al., 2012; Le et al., 2018). The ectopic expression of N. tabacum osmotin gene in chilli pepper (Capsicum annuum L.) exhibited improved tolerance to salt stress. In addition, transgenic C. annuum plants showed increased proline, chlorophyll, relative water contents, and antioxidative enzymes (Subramanyam et al., 2011). In a recent report, Bashir et al. (2021) demonstrated salt tolerance in transgenic olive (Olea europaea L.) plants over-expressing the tobacco osmotin gene. Salt stress tolerance in transgenic olive plants was associated with the induction of the S assimilatory pathway, especially due to an increase in the activity of O-acetyl serine(thiol)lyase (OASTL) (Bashir et al., 2021). Under cold treatment (15°C), the over-expression of N. tabacum osmotin gene improved seed germination rate, and the establishment and growth of seedlings in transgenic tomato (Solanum lycopersicum L.) (Patade et al., 2018). Recently, Fei et al. (2021) has reported the characterization of a novel osmotin gene the KoOsmotin–from Kandelia obovata that induces cold tolerance and helps to enhance the growth of Escherichia coli cells under cold stress. The osmotin from Tripogon loliiformis (TlOsm) provided tolerance to Oryza sativa (rice) plants from cold, salt, and drought stresses. This designates the role of osmotin in response to different abiotic stresses in plants, possibly by the signaling pathway (Le et al., 2018).

Drought is another environmental factor that affects yield in crop plants. Several studies have observed drought tolerance in crop plants by expressing osmotin or OLPs, which enhance physiological responses against drought. Along with drought tolerance, higher grain productivity, transpiration rate, stomatal conductance, and net CO_2 assimilation were observed in transgenic soybean (Glycine max L.) expressing OLP gene (SnOLP) (Weber et al., 2014). Tobacco osmotin gene transformation induced drought tolerance in transgenic kiwi (Actinidia deliciosa A. Chev.) and olive plants (Silvestri et al., 2017; Rugini et al., 2018). With higher drought and salt tolerance, over-expressing

tobacco osmotin in Mulberry (*Morus indica* L. cv K2) plants imparted resistance to *Fusarium pallidoroseum*, *Colletotrichum dematium*, and *Colletotrichum gloeosporioides* (Das *et al.*, 2011). The over-expression of *SindOLP* in sesame enhanced the tolerance toabiotic stress (salinity, drought, and oxidative stress), the charcoal rot pathogen (*Macrophomina phaseolina*) (Chowdhury *et al.*, 2017). Moreover, osmotins and OLPs also provide a vital role in plant growth and development. For instance, the accumulation of *PhOSM* in Petunia (*Petunia hybrida*) flowers, leaves, and roots demonstrates the importance of osmotin in plant development (Byoun and Kim, 1997). Recently, Li *et al.* (2020) reported that the downregulation of *PdOLP1* was involved in wood development, secondary cell wall biosynthesis, higher dry weight biomass, and lignin content in poplar (*Populus deltoides* Marsh.).

Role of osmotin in biotic stress tolerance
Many osmotins and OLPs have been studied in plants for their role in biotic stress tolerance as presented in Table 1. These proteins possess anti-fungal activities-related to their potential to induce fungal plasma membrane permeabilization (Ramos *et al.*, 2015). The anti-fungal properties of osmotin and OLPs lead to disease resistance in many crop plants, like tomato, potato, rice, and tobacco (Xue *et al.*, 2016; Hakim *et al.*, 2018; Kaur *et al.*, 2020). The *CpOsm*–an anti-fungal TLP has been reported to inhibit the spore germination of *C. gloeosporioides*, *Neurospora* sp., and *Fusarium solani* (de Freitas *et al.*, 2011). Rather *et al.* (2015) characterized *ObOLP* from sweet basil (*Ocimum basilicum*), which showed anti-fungal activity against *Pichia pastoris*, *Candida albicans*, and *Saccharomyces cerevisiae*. The resistance to potato early blight in susceptible potato plants by over-expressing the *OsmWS* gene has also been reported (Kaur *et al.*, 2020). Osmotin from tobacco was analyzed for anti-fungal activity, which showed significant effects against *Candida tropicalis*, *C. parapsilosis*, *Debaryomyces hansenii*, *Saccharomycodes ludwigii*, *Hyphopichia burtonii*, and *Torulaspora globose* (Viktorova *et al.*, 2017). Osmotin (*OsOSM1*) gene over-expression in rice (*O. sativa* var. Xudao 3) increased resistance to sheath blight, the most destructive rice disease caused by *Rhizoctonia solani*. The study demonstrated the induction of jasmonic acid-responsive marker genes in response to the upregulation of jasmonic acid in transgenic rice plants. It suggests that the jasmonic acid signaling pathway activation may account for the increased resistance in transgenic lines (Xue *et al.*, 2016). In addition, osmotin has been used to evaluate its role in post-harvest fruit damage by fungal pathogens. The long-term stored kiwifruits of transgenic kiwi plants expressing tobacco osmotin gene exhibited significant resistance to post-harvest damage by *Botrytis cinerea* and *Cadophora luteo-olivacea*, which mainly cause gray mould and skin pitting of kiwifruit, respectively (Rugini *et al.*, 2010).

Presently, due to the effects of multiple stress factors in plants, the development of tolerance to individual stresses seems insufficient. Therefore, the introduction of multiple genes is needed to develop biotic and abiotic stress tolerance (Kumar *et al.*, 2016; Sripriya *et al.*, 2017). The combined application of chitinase with osmotin, defensin, PR1, TLP, and glucanase enhanced its anti-fungal activity (Shah *et al.*, 2013; Dolatabadi *et al.*, 2014). Thus, higher resistance to different

stresses can be achieved by the combined use of osmotin and other PR proteins. Thus, a combination of osmotin (*ap24*) and chitinase (*chi11*) gene expression was reported enhancing the tolerance against sheath blight in transgenic rice (Sripriya *et al.*, 2017). Moreover, the transgenic tomato was developed by combinatorial expression of *OLP* and *Chi11* showing increased tolerance to drought, salt, and fungal (*Alternaria solani* and *Fusarium oxysporun*) stresses (Kumar *et al.*, 2016). Thus, the use of multiple genes, such as *osmotin* or *OLPs*, along with other stress-tolerant genes can be more useful to confer multiple stress tolerance in plants. With abiotic and biotic stress tolerance, the recent literature highlights the potential of osmotins and OLPs for engineering crops to increase growth and development, productivity, and survival.

Mechanism of Osmotin

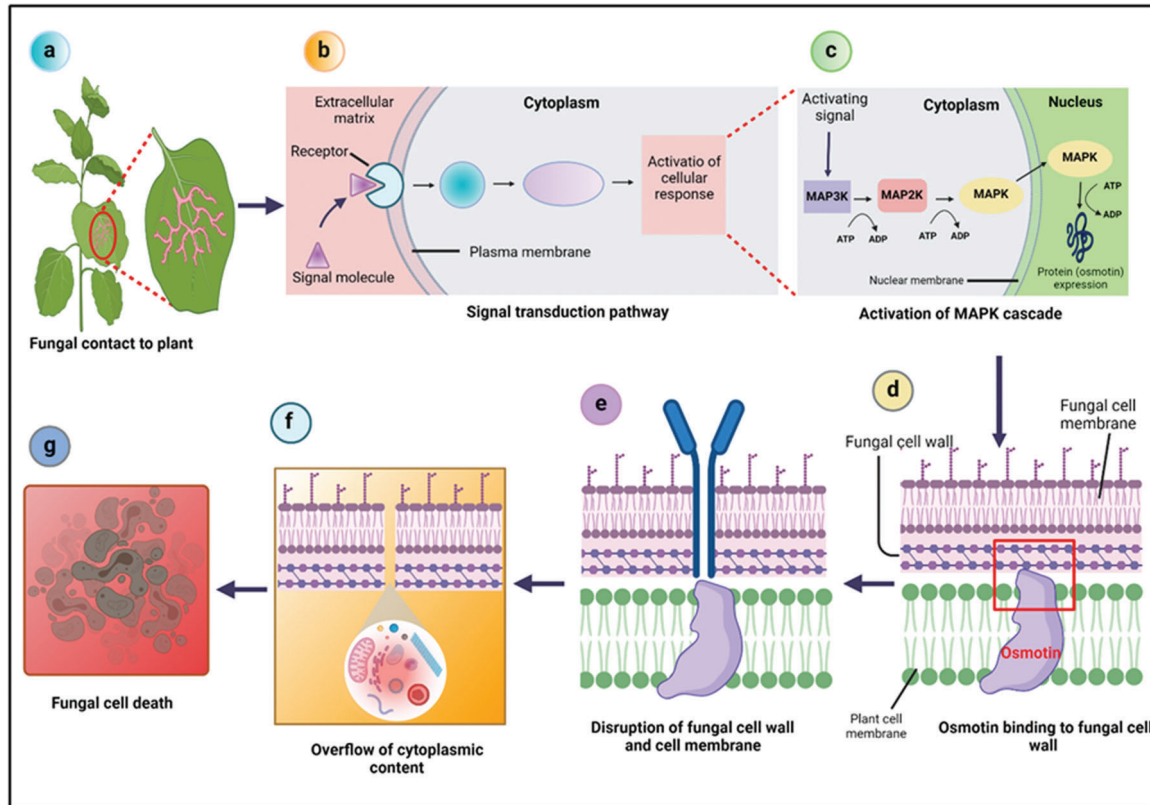*Osmotin mode of action in abiotic stress*
Osmotin and OLPs exhibit tissue-specific activities and are expressed in response to various environmental and hormonal signals, such as drought, wounding, salinity, ABA, ethylene, and pathogenic (fungi, viruses, bacteria) infection. These signals cause transcriptional and post-transcriptional osmotin mRNA regulation (Noori and Sokhansanj, 2008; Parkhi *et al.*, 2009). Under drought or salinity stresses, osmotin or OLPs maintain cellular osmolarity via compartmentalization of solutes in the vacuole and intercellular space or by structural and metabolic alterations, such as changes in Ca^{2+} leading to programmed cell death or by the activation of different signaling pathways (Chowdhury *et al.*, 2017), as shown in Fig. 1. The plants respond to salinity stress through gene expression regulation that eventually results in cellular homeostasis renovation, toxin detoxification, and growth recovery (Subramanyam *et al.*, 2011).

The osmotin gene over-expression triggers downstream gene expression that upregulates the biosynthetic pathway. This pathway is involved in the accumulation of osmolyte proline, which quenches ROS, free radicals, and prevents the breakdown of chlorophyll under higher salt stress conditions (Zhang *et al.*, 2004). The OLP gene is reported to impart increased tolerance to multiple stresses by the integrated activation of multiple components of the defense signaling cascade. For example, the *SindOLP* gene is involved in the expression of *Si-Defensin*, *Si-Apetala2*, and *Si-Ethylene-responsive factor*, the marker genes for the JA/ET pathway, along with *Si-Thaumatin-like protein* and *Si-Chitinase*, the marker genes for the SA pathway (Chowdhury *et al.*, 2017; Pluskota *et al.*, 2019). Moreover, the cell membrane has been considered as the main target of different stresses. Under drought and salt stresses, osmotin provides a defensive role by maintaining cell membrane integrity (Das *et al.*, 2011). For example, OLP-transgenic tomato plants displayed increased thickness of the secondary cell wall of vessels and fibers under salt stress–acting as an important mechanism to improve plant resistance to water stress (Kumar *et al.*, 2016).

Osmotin mode of action in biotic stress
Osmotin has been found to have activities against different phytopathogens, such as bacteria and viruses, but it is well-known for its anti-fungal activity against a broad range of

FIGURE 1. Mechanism of osmotin in abiotic stress tolerance. Osmotin and OLPs confer tolerance to abiotic stresses (salinity, drought, or cold) by compartmentalization of solutes in the vacuole and intercellular space (a), changing Ca^{2+} regulation that eventually results in programmed cell death (b), and by gene expression regulation, such as the activation of biosynthetic pathway that initiates proline accumulation involved in scavenging of reactive oxygen species (ROS). In addition, it also activates the defense signaling cascade, which is involved in the initiation of JA/ET and SA pathways (c). ER: endoplasmic reticulum, JA: jasmonic acid, ET: ethylene, SA: salicylic acid.

fungi. Although the cell wall of fungi has an important role in defining sensitivity to osmotin, the main target position for osmotin and other PR-5 proteins is the fungal plasma membrane (Anžlovar and Dermastia, 2003). To enhance cytotoxicity, osmotin uses an intercellular signal transduction pathway in order to destabilize the protective cell wall barriers (Kumar *et al.*, 2015). After the activation of this pathway, it leads to the stimulation of the MAPK cascade. This causes changes in the cell wall, allowing osmotin to enter the plasma membrane, resulting in the development of a transmembrane pore, which causes membrane leakage and eventually leads to membrane rupture (Singh *et al.*, 2013).

Osmotin is also diffused across the cell wall of fungi by recognizing and binding to the cell wall phosphomannans and enter the cell membrane (Ibeas *et al.*, 2000). The analysis of the interaction between osmotin and the cell surface of pathogens revealed that after treating fungal spores with osmotin the protein is attached to the cell surface components. Where it causes dramatic changes in the permeability of plasma membrane and results in the overflow of cytoplasmic content that shrinks the cell (Ramos *et al.*, 2015), and subsequently it kills the fungus (Roberts and Selitrennikoff, 1990; Sripriya *et al.*, 2017), as presented in Fig. 2. However, the exact mechanism of altering cell membrane permeability by osmotin and other anti-fungal TLPs is unclear. Though, it is considered that due to the negatively charged components in the acidic cleft, these

proteins attach to the positively charged membrane proteins like osmoreceptors, ions, or water channels that ultimately lead to a dramatic increase in the water flow across the membrane (Ramos *et al.*, 2015).

In the case of bacteria, osmotin does not exhibit direct anti-bacterial activity, but indirectly it prevents bacterial infection in host tissues. Some bacterial species like *Agrobacterium tumefaciens* use cytokinins in turning host's activity to their own favor (Viktorova *et al.*, 2012). Osmotin has a close relationship to cytokinin signal transduction (Kobayashi *et al.*, 2000). It has been assumed that osmotin binding to cytokinin results in the deactivation of cytokinins that prevents bacteria from inducing tumors because the elimination of cytokinin suppresses the proliferation of bacterial infection (Viktorova *et al.*, 2012). Choi *et al.* (2013) reported that the overexpression of an OLP (*CaOSM1*) in *A. thaliana* conferred resistance to a bacterial pathogen *Pseudomonas syringae* pv. *Tomato* by accelerating hypersensitive cell death response and accumulating H_2O_2 that prevented the spread of pathogens to healthy cells. Although, osmotin is mainly characterized for its anti-fungal properties, studies have also reported its anti-viral activity. Osmotin was identified in Samsun NN tobacco leaves after infecting with tobacco mosaic virus (TMV) and induced tolerance to viral infection via a hypersensitive response (Stintzi *et al.*, 1991; LaRosa *et al.*, 1992). Infecting tobacco explants with TMV induced the expression of osmotin in infected leaves (Neale *et al.*, 1990). In addition, OLP was

FIGURE 2. Mechanism of osmotin in biotic (fungal) stress tolerance. After the plant is infected by fungi (a), it initiates signal transduction pathway (b), which leads to the activation of MAPK cascade resulting in protein (osmotin) expression (c). The osmotin binds to the fungal cell wall by recognizing phosphomannans (d) and enters the fungal cell membrane where it causes membrane permeability and eventually leads to membrane disruption (e) that results in the overflow of cytoplasmic contents (f) and eventually kills the fungus (g).

accumulated in habanero pepper (*Capsicum chinense*) infected with pepper mild mottle virus (PMMoV) (Elvira *et al.,* 2008). Comprehensive research has been conducted on the anti-fungal activity of osmotin and OLPs; however, further research is needed for their anti-bacterial as well as anti-viral properties.

Conclusions and Future Perspective

Osmotin and OLPs belong to the PR-5 family that offer vital functions in imparting tolerance to various stresses. After the first discovery of osmotin from tobacco plants, osmotin and OLPs are identified from the whole plant kingdom. The similarity of OLPs to thaumatin and TLPs determines a strong evolutionary pressure for conserving the thaumatin fold associated with their role as defense molecules against pathogens and confer plant tolerance to abiotic and biotic stresses. Osmotin is a multi-functional key regulator triggering a response to different stresses. It also protects innate protein structures and is involved in normal plant development. Different environmental and hormonal signals, i.e., salinity, low temperature, wounding, drought, ethylene, ABA, and a pathogenic infection regulate the expression of osmotin and OLP genes. Under salt and drought stresses, these proteins maintain cellular osmolarity via solute compartmentalization, metabolic or structural modification, and by activating different signaling pathways. Importantly, inducing tolerance to individual stresses is insufficient; thus, the introduction of multiple genes can be more beneficial for providing strong tolerance to multiple stresses. Therefore, osmotin or OLPs with other key genes can be cloned in crop plants to confer resistance to different stresses and improve important agronomic traits (Viktorova *et al.,* 2017; Fei *et al.,* 2021). Notably, transgenic plants overexpressing these genes can be used in the remediation of salt-contaminated sites (Viktorova *et al.,* 2017). In addition, the development of disease resistance in plants can not only increase crop yield but also overcome environmental pollution by reducing the use of pesticides.

Due to higher anti-fungal activity, osmotin and OLPs have been widely used in the development of transgenic plants to increase the anti-fungal. It indicates the importance of these proteins in the agricultural, and food industry, where they can be used as preservative agents (Viktorova *et al.,* 2012). Moreover, osmotin also has homology to human adiponectin, which is an anti-diabetic and anti-atherosclerotic protein. Both these proteins provide anti-tumor resistance by preventing the activity of *p53* gene and caspase enzyme (Das and Chakraborty, 2016; Bashir *et al.,* 2020). Thus, due to the similarity of the osmotin receptor to the adiponectin receptor, it can be applied in the pharmaceutical industry to prepare drugs against insulin resistance, atherosclerosis, obesity, and diabetes.

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