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*Review*

# Recent Advances in Understanding the Function of the PGIP Gene and the Research of Its Proteins for the Disease Resistance of Plants

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**Abstract:** Polygalacturonase-inhibiting protein (PGIP) is an important plant biochemical anti-disease factor. PGIP has a leucine-rich repeat structure that can selectively bind and inhibit the activity of endo-polygalacturonase (endo-PG) in fungi, playing a key role in plant disease resistance. The regulation of PGIP in plant disease resistance has been well studied, and the effect of PGIP to increase disease resistance is clear. This review summarizes recent advances in understanding the PGIP protein structure, the PGIP mechanism of plant disease resistance, and anti-disease activity by PGIP gene transfer. This overview should contribute to a better understanding of PGIP function and can help guide resistance breeding of PGIP for anti-disease effects.

**Keywords:** polygalacturonase-inhibiting protein; polygalacturonase; anti-disease



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## 1. Introduction

In the long-term survival battle against pathogenic microorganisms, plants have evolved a series of complex but effective defense systems that can rapidly respond to infection and disease [1]. Plants can produce structural anti-disease factors such as lignins, hydroxyproline-rich glycoproteins (HRGPs), glucose-regulated proteins (GRPs), and biochemical anti-disease factors, such as polygalacturonase-inhibiting protein (PGIPs) and plasticity-related genes (PRGs), to deal with potential threats and disease. The rapid activation of defense responses is critically important for plants to resist infection by pathogenic bacteria [2,3]. To understand the mechanism of anti-disease systems in plants, relevant genes need to be identified and characterized. Anti-disease genes are also important for the selection and cultivation of anti-disease varieties of plants, so the study of these systems can facilitate the breeding and development of plants with improved anti-disease properties.

The plant cell wall is the first barrier to resist infection by pathogenic bacteria. Pathogens can secrete plant cell wall-degrading enzymes (PCWDEs) to infect the host, and in response, the host can produce several inhibitory proteins, such as polygalacturonase-inhibiting protein (PGIP) to resist the hydrolysis caused by these enzymes [4]. PGIP is mainly concentrated in the cell wall and the intimal system, and its ability to inhibit PG activity is positively correlated with plant disease resistance [2]. The oligogalacturonic acids may activate plant defense responses such as synthesis of phytoalexins, lignin, and ethylene, expression of proteinase inhibitor I, and production of reactive oxygen species [5]. PGIP is considered a key component of the plant immune response, inhibiting pathogen infection by preventing the activation of endo-polygalacturonase (PG) and subsequent cell wall degradation to limit the growth and colonization of fungi [6,7].

This review focuses on and discusses recent work to characterize the function of PGIP, the mechanism of PGIP-related disease resistance, and the use of genetic integration of PGIP for anti-disease practice.

## 2. Discovery of PGIP and Its Variety and Diversity

In 1971, Albershein and Anderson [8] discovered PGIP in protein extracts from cell cultures of pea, bean, tomato, and *Acer platanoides*, and presented evidence that PGIP is located in the plant cell wall. In 1993, Williamson et al. [9] purified the 38.5 kDa molecular weight PGIP from raspberry fruit. In 1994, Favaron et al. [10] separated and purified PGIP from germinated soybean seeds and successfully cloned the soybean PGIP gene for the first time. More recently, scientists have identified PGIP in paddy rice, European rape, *Arabidopsis thaliana*, alfalfa, mung bean, citrus, and sisal [11–17]. The NCBI database has published 400 full or partial sequences of PGIP from both monocotyledons and dicotyledons, but there have been few investigations into the function of these proteins to inhibit PG protein. Plants can have multiple PGIP genes. For example, *Brassica napus* L. has the most PGIPs found in a plant, with 17 BnPGIPs on different chromosomes [13]. *Phaseolus vulgaris* has at least five PGIPs on chromosome 10. *Arabidopsis thaliana* has two PGIPs, AtPGIP1 and AtPGIP2, on chromosome 5, encoding proteins that are 76.1% similar in amino acid sequence [11].

The expression level of PGIP varies among different plant species, different organs, and different stages of development. The expression level of PGIP protein is proportional to the plant's anti-disease ability. Ruan et al. [18] used Western blotting to show a higher PGIP level in a wheat variety with good disease resistance, SW89-2598, than the level in a variety with poor resistance, 3626. Similarly, Chen et al. [19] showed via qRT-PCR that good resistant wheat variety YSBR1 has higher PGIP expression than a variety with poor resistance. Tobacco genetically integrated with the PGIP gene from *Hypericum perforatum* exhibited increased anti-disease ability [20]. A study of fungal resistance on two varieties of wheat found PGIP expression level proportional to the anti-disease ability [21]. For the same variety of wheat, different organs have different levels of PGIP, with the maximum in stems, a moderate amount in roots, and the lowest levels in leaves [22]. In the late period of plant development, the expression level and activity of PGIP in fruits are negatively correlated with maturity [23].

## 3. Structural Characteristics of PGIP Protein

The PGIP gene is universally present in animals, plants, and microorganisms and belongs to the LRR (leucine-rich repeat) protein superfamily. PGIP proteins have an LRR domain rich in cysteine, forming a pattern of LxxLxxLxxLxLSxNxGxIPxx [6,24]. The number of tandem duplications varies among plant species. There are 9 tandem duplications in PGIP from rice and wheat [25] and 11 in PGIP from sugar beets [24]. In a single plant, different PGIPs can have different functions. It is reported that 7 OsPGIPs share a common signaling peptide, and 9 to 11 LRR fragments contain a characteristic domain of xxLxLxx of PGIP. After folding to α-helix β-sheet and random curling secondary structure, it forms a coiled-like structure, making up a specified concave following the right-hand screw rule, responsible for the interaction between OsPGIP and PG from harmful pests. The seven OsPGIPs are all hydrophobic proteins, with good fat solubility, transmembrane structure, extracellular localization, and multiple N-glycosylation sites, making these proteins insoluble when expressed in *E. coli* [26]. The exposed LRR motif is critical for the affinity and specificity of PGIP [25–28].

## 4. Biological Function and Mechanism of Disease Resistance by PGIP

### *4.1. Biological Function of Disease Resistance by PGIP*

Plants have evolved different types of PGIPs to defend against pathogen infection. PGIP is mainly concentrated in the cell wall and endomembrane system, where it can specifically recognize different PGs released by fungi and inactivate or lower the activity of

PGs by protein–protein interaction [7]. PGIP has different inhibitory effects on pathogens that secrete different PGs [29]. The alignment and structural prediction of the amino acid sequences of PGIP and PG suggest the binding of PGIP and PG can be altered by changing single amino acids of PGIP, suggesting that PGIP has a high specificity and selectivity to various hosts [30]. The PvPGIP1/2 and PvPGIP3/4 of kidney bean inhibit fungi infection and pest damage, respectively, although they share 10 tandem duplications of LRR. The different inhibitory effects of these proteins are due to a change in a single amino acid, leading to a dramatic change in the interactive PG domain [27,31,32]. The interaction of PGIP and PG prevents plant cell wall degradation to maintain the integrity of the cell, thus limiting access to nutrients for pathogens and, finally, inhibiting pathogen growth and proliferation.

### 4.2. Regulation of PGIP Expression

PGIP gene expression is affected by pathogen infections, ethylene, injury, oligogalacturonides (OGs), and induction by jasmonic acid and salicylic acid, indicating the complex molecular regulation of PGIP [33–35]. Most PGIPs are expressed under the stimulation of pathogens [36,37], and different PGIPs have specific stimulating conditions. In rape, insect and mechanic damage can stimulate the high expression of Bnpgip1, with little effects of fungi and cold induction. The expression of *BnPGIP2* responds to fungi infection and injury but not insects, and jasmonic acid but not salicylic acid stimulates both genes [13]. In strawberry, there is high PGIP induction after inoculation with *B. cinerea,* indicating that PGIP plays a role in defense [38] (Table 1). Under the stimulation of fungi, the expression of OsPGIP7 decreases, but *OsPGIP4* gradually increases and lasts a relatively long time [36]. Devoto et al. [39] cloned the promoter of PGIP-1 in soybean and found it is activated by injury but not chemicals or pathogen infection [39]. In *Arabidopsis thaliana*, *AtPGIP1* is regulated by OGs, but not by ethylene, salicylic acid, or methyl jasmonate. *AtPGIP2* is regulated by methyl jasmonate, COI1, and JARI gene product but not by OGs [11]. Treatment of strawberry (*Fragaria chiloensis* L.) with chitosan and methyl jasmonate for more than 48 h effectively inhibited grey mold incidence and was accompanied by the up-regulation of *FcPGIP1* and *FcPGIP2* [40]. Proteomics analysis revealed a high expression of *AcPGIP* after infection of kiwifruit *(Actinidia deliciosa* "Hayward") by *B. cinerea* [41]. After biological and abiotic stress treatment, the expression level of different *OsPGIPs* in rice showed significant changes with an overall significant increase in expression level, suggesting rice can improve its stress resistance by regulating the expression of *OsPGIPs* under stress conditions [26].

**Table 1.** PGIP regulatory factors tested in different systems against PGs.

| | Gene | System for Expression | Mediated Factor | Enhanced Resistance |
|---|---|---|---|---|
| *Arabidopsis thaliana* | *AtPGIP1* | *Arabidopsis thaliana* | Oligogalacturonides (OGs) [11] | *Botrytis cinerea* |
| | *AtPGIP2* | | Jasmonate [11] | *Botrytis cinerea* |
| Kidney bean (*Phaseolus vulgaris* L.) | *PvPGIP1* | | Wounded [6] | *Aspergillus niger* [42] |
| | *PvPGIP3* | *Nicotiana benthamian* | Oligogalacturonides (OGs) [6] | |
| Rapeseed (*Brassica napus*) | *BnPGIP1* | | Jasmonate, insect herbivory, mechanical wounding [43] | *Sclerotinia sclerotiorium* |
| | *BnPGIP2* | | Jasmonate, defense hormones, and *Sclerotinia sclerotiorium* infection | |
| *Solanum torvum* | *StPGIP* | | *Verticillium dahliae* | *Verticillium dahliae* [44] |
| Wheat (*Triticum aestivum* L.) | *TaPGIP3* | | IAA, MeJA, SA, and ABA | *Fusarium graminearum* [25] |
| | *TaPGIP2* | | | |
| Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) | *BrPGIP1* | Cabbage | JA [45] | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) |
| | *BrPGIP2* | | JA [45] | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) |
| | *BrPGIP5* | | JA [45] | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) |

**Table 1.** *Cont.*

| | Gene | System for Expression | Mediated Factor | Enhanced Resistance |
|---|---|---|---|---|
| Grape (*Vitis vinifera* L.) | *VvPGIP1* | Tobacco | Auxin, salicylic acid and sugar treatment, wounding, and pathogen infection [46,47] | *Botrytis cinerea* |
| Soybean (*Glycine max* L.) | *GmPGIP1* | PVX/*Nicotiana benthamiana* | PVX/Nicotiana benthamiana | [48] |
| | *GmPGIP2* | | | [31] |
| | *GmPGIP3* | PVX/*Nicotiana benthamiana* | PVX/*Nicotiana benthamiana* | *Botrytis cinerea*, *Botrytis aclada* [31,49] |
| | *GmPGIP4* | | | [31] |
| | *GmPGIP7* | | | [50] |
| Potato leaves (*Solanum tuberosum* L. cv. *Spunta*) | | | Salicylic acid, wounding, and infection | *Phytophthora infestans* [51] |
| Kiwifruit (*Actinidia chinensis*) | *AcPGIP* | | *Botrytis cinerea* infection [41] | *Botrytis cinerea* [52] |
| Strawberry (*Fragaria* × *ananassa* Duch.) | *PGIP* | *B. cinerea or wounding* | *Botrytis cinerea* wounding [38] | |

During pathogen infection, PGIPs can interact with pathogen PGs, eventually leading to the accumulation of OGs [29]. OGs function as a damage-associated molecular pattern (DAMP) that is recognized by the receptor wall-associated kinase 1 (WAK1) to induce host immunity [53]. This can activate the defense system of plants in the model of pathogen-related resolution-associated molecular patterns (RAMPs), such as the accumulation of anti-disease factors, deposition of callose, and increased NO/ROS [54]. Benedetti et al. [29] detected a high level of OGs in the PGIP-PG transgene family by the negative ion exchange chromatography pulse method, with the resistance of transgene material toward various types of fungi. Studies have reported that the application of trimeric OGs activates the immune response to resist necrotrophic pathogens and nematodes [5]. These results indicate that OGs can act as RAMPs and initiate the PTI immune pathway to regulate gene expression and defend against pathogenic fungi and other diseases. PGIPs inhibit the endogenous PGs of *Sclerotinia sclerotiorum* but also stimulate programmed death dependent on calcium [55] When a plant is infected with pathogens, plant cells can rapidly release and accumulate ROS, leading to a change in cellular pH and the production of $Ca^{2+}$ ion, which can stimulate the synthesis of defense-related proteins and plant antitoxins as second messengers and cause the death of adjacent cells [56].

## 5. Application of PGIP in Plant Disease Resistance Genetic Engineering

The PGIP anti-disease effect has been confirmed in a series of in vivo experiments. PGIP-mediated fungi resistance has been shown in various important cash crops, such as grapes [57,58], papaya [59], potatoes [51], alfalfa [15], cotton [60], green beans [6,28,61], tomatoes [62], peas [63], mandarin orange [64], tobacco [65], and Arabidopsis [66]. PGIP expression does not affect plant phenotype or inhibit plant growth in sugar beet [67] or tobacco [67]. Thus, PGIP should be able to be used to improve plant disease resistance by overexpressing active PGIPs or by strengthening the specific recognition and inhibitory effect of native PGIPs by altering amino acid residues at recognition sites [68]. Consistently, the overexpression of *GhPGIP1* enhanced the resistance of cotton (*Gossypium hirsutum* L.) to Verticillium and Fusarium wilts, and resistance was severely reduced when the gene was silenced [60]. The overexpression of *HpPGIP* led to the upregulation of key genes involved in hormone signaling, microRNA-based gene silencing, homeostasis of reactive oxygen species, and the phenylpropanoid pathway [20]. There were increased expression levels of disease resistance-related genes, including *VvPGIP* in fruit inoculated with *B. cinerea* infection [47].

*PvPGIP* exhibits strong inhibition toward most PGs from different pathogens [69]. *PvPGIP2* can be transferred to *Brassica napus* via *Agrobacterium*, and in vitro experiments show that crude extract from transgenic strains can inhibit 29–37% PG activity in rhizoctonia, with an increased effect seen with the progression of infection and delayed lesion expansion in the transgenic strain [70]. Wang et al. [71] overexpressed soybean GmPGIP3 in wheat and found the transgenic strain exhibited elevated resistance to wheat root rot. The overexpression of *OsPGIP1* that can inhibit the PG from sheath blight allowed rice to efficiently limit the expansion of *Rhizoctonia solani*, with no effect on agronomic characteristics [19,72,73]. Similarly, the expression of *PGIP1* from cotton in *Arabidopsis* strengthened its resistance to fungal disease caused by *Verticillium dahliae* and *Fusarium*, and silencing *PGIP1* in cotton increased its sensitivity to these fungi [60]. *AcPGIP* silencing enhanced kiwifruit's susceptibility to *B. cinerea* [52]. The expression of PGIP from sugar beet (*BvPGIP1*) can protect transgenic tobacco against *Rhizoctonia solani*, *Fusarium,* and *Botrytis cinerea* [24]. The overexpression of *OsPGIP2* can efficiently inhibit *Sclerotinia* disease and enhance resistance to *Sclerotinia* in *Brassica napus* [74]. Overall, many studies show that increasing the protein levels of PGIPs can allow plants to effectively resist many common pathogenic fungi [75] (Table 2).

**Table 2.** PGIP tested in different systems against fungal PGs.

| | Gene | System for Expression | PG Inhibition |
|---|---|---|---|
| St. John's wort (*Hypericum perforatum*) | *HpPGIP* | | *Agrobacterium tumefaciens* [20] |
| Pepper (*Capsicum annuum* L.) | *CaPGIP1* | | *Alternaria alternate, Colletotrichum nicotianae* [76] |
| | *CaPGIP2* | | *Alternaria alternate, Colletotrichum nicotianae* [76] |
| Rice (*Oryza sativa* L.) | *OsPGIP2* | Rapeseed | *Sclerotinia sclerotiorium, SsPG3 and SsPG6* [74] |
| | *OsPGIP1* | Rice | Sheath blight, *Rhizoctonia solani* [73,77] |
| | *OsPGIP4* | Rice | *Xanthomonas oryzae* pv. *oryzicola* [78] |
| Pear (*Pyrus bretschneideri*) | *pPGIP* | Tomato | *Botrytis cinerea* [79] |
| Mungbean (*Vigna radiata* (L.) R. Wilczek) | *VrPGIP1* | Mungbean | *Callosobruchus chinensis* [14] the polygalacturonase in *Callosobruchus maculatus* [80] |
| | *VrPGIP2* | Mungbean | The polygalacturonase in *Callosobruchus maculatus* [14,80] |
| Alfalfa (*Medicago sativa*) | *MsPGIP2* | *Medicago sativa* | *Pseudopeziza medicaginis* (Lib.) Sacc. [15] |
| Mulberry (*Morus alba* L.) | *MPGIP* | | [81] |
| Apple (*Malus domestica*) | *MdPGIP1* | Tobacco and potato | *Fungal polygalacturonases* [82] |
| Cotton (*Gossypium hirsutum*) | *GhPGIP1* | *Gossypium hirsutum, Arabidopsis thaliana* | *Verticillium dahliae, Fusarium oxysporum* [60] |
| Sugar beet (*Beta vulgaris*) | *BvPGIP1* | *Nicotiana benthamiana* | *Fusarium solani*, *Botrytis cinerea*, and *Rhizoctonia solani* [24] |
| | *BvPGIP2* | *Nicotiana benthamiana* | *Fusarium solani, Botrytis cinerea* [24] |
| Tobacco (*Nicotiana tabacum*) | *NtPGIP* | *Escherichia coli* (DE3) | *Phytophthora capsica* [65] |

PGIPS can also inhibit bacterial diseases in different crops [45,73,78,83] (Table 3). Expressing PGIP from pear in transgenic grape can improve its resistance against Peel's disease caused by *Xylella fastidiosa* [84]. The overexpression of PGIP2 from Chinese cabbage in transgenic cabbage or tobacco can enhance the resistance to soft rot bacteria [45]. PGIP from tomato stem extract can inhibit PGs from *Ralstonia solanacearum* [83]. A recent study on rice showed that the PGIP4-containing chromosome is related to the resistance of bacterial stripe disease, with increased susceptibility to bacterial stripe disease in rice when the *PGIP4* is silenced by RNAi [78]. RNA-seq analysis revealed that the *OsPGIP1*-mediated resistance of bacterial leaf streak is induced by PG of *Xoc* and other pathogenicity factors,

and is primed by the activated expression of *PR* genes, cell wall defense-associated genes and regulators, and an accumulation of *JA* [85]. Increased PGIP in potato improved the resistance of *Ralstonia solanacearum* [86]. Overall, it is clear that PGIPs contribute to defense responses and inhibit bacterial disease [83].

**Table 3.** PGIP genes studied in various systems against bacterial stresses.

| | Gene | System for Expression | Enhanced Resistance |
|---|---|---|---|
| Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) | *BrPGIP1* | Cabbage | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) [45] |
| | *BrPGIP2* | | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) [45] |
| | *BrPGIP3* | | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) [45] |
| | *BrPGIP4* | | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) [45] |
| | *BrPGIP5* | | *Pectobacterium carotovorum* ssp. *Carotovorum* (Pcc) [45] |
| Pear (*Pyrus bretschneideri*) | *pPGIP* | Grape | *Xylella fastidiosa* [84] |
| Tomato (*Lycopersicon esculentum*) | *PGIP* | Tomato stem | *Ralstonia solanacearum* [83] |
| Rice (*Oryza sativa* L.) | *OsPGIP1* | Rice | Bacterial leaf streak [85] |
| Potato (*Solanum tuberosum* L. cv. *Spunta*) | *PGIP* | Potato | *Ralstonia solanacearum* [86] |

## 6. Future Prospects

Although there has been progress in cloning PGIP genes and investigating PGIP structure, function, expression, and regulation, future work is required to determine how to effectively integrate the heterologous PGIP gene into a plant genome for stable expression to improve plant disease resistance. In addition, more studies are required to characterize differences in the mechanism of action, expression conditions, expression effects, and antimicrobial effects for different PGIPs. The sequence similarity of PGIP genes in homologous plants is relatively high, and there is almost no difference in the protein sequence of coding products. Therefore, the study of PGIP gene sequence difference is not enough to explain the expression difference among PGIP gene family members, nor to reveal the difference of PGIP activity among varieties. More and more scholars believe that the most important thing is the difference in gene expression regulation. In addition, the same PGIP may have different activities to inhibit PG. Changing a single amino acid of PGIP can change the specificity of the PG–PGIP interaction. Therefore, the overexpression of PGIP genes can improve plant disease resistance; for example, the overexpression of active PGIPs genes or changing the amino acid residues of inhibition recognition sites will make PGIP produce stronger specific recognition and inhibition intensity [27,31,32]. This increases plant resistance [68]. Traditional disease resistance genetic breeding is not enough to solve the problem of plant disease resistance, but with the continuous improvement of genetic engineering, molecular biology, and transformation system, transgenic breeding can be used to transform a wide range of resistance genes into plants, so as to effectively improve plant disease resistance.

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