Abstract

Plant polygalacturonase-inhibitor proteins (PGIPs) have been shown to be important pathogenesis-related (PR) like proteins involved in host resistance against various biotic and abiotic stress factors. PGIPs contribute to pathogen perception and counter-attack by modulating polygalacturonase (PG) activity of microbial origin, leading to accrual of elicitor active oligogalacturonides (OGs).

Pearl millet \([Cenchrus americanus (L.) Morrone; synonym: Pennisetum glaucum (L.) R. Br.]\) is one of the most drought tolerant staple food crop of the semi-arid and arid tropics, is highly susceptible to downy mildew disease caused by oomycetous pathogen, \(Sclerospora graminicola\) (Sacc.) Schroet. The present study explored the role of PGIPs in pearl millet-downy mildew, host-pathogen system, as a part of our continuous efforts to understand the complete resistance mechanism in this system.

Initial studies using heterologous PGIP probes generated against bean PGIP indicated involvement of PGIPs in pearl millet resistance against the downy mildew pathogen. Temporal transcript and protein accumulation studies showed constitutive expression in resistant cultivar and differential accumulation between the resistant and susceptible seedlings, with the highest accumulation observed at 24 and 48 h post inoculation in the resistant inoculated samples. PGIP staining was intense in epidermal and vascular regions of coleoptiles as observed in tissue blots. The pearl millet cultivars displayed differential inhibitory activities against \(Aspergillus niger\) pectinase. PGIP purification from pearl millet by conventional column chromatography identified two active peaks Peak-A and Peak-B which differed in their inhibition profiles.

Reverse transcriptase-polymerase chain reaction (RT-PCR) using total RNA isolated from 2-day-old pearl millet seedlings resulted in identification of two partial sequences of length 407 bp (\(Capgip1p\)) and 497 bp (\(Capgip2p\)) sharing very good identity with known monocot \(pgip\) genes. Inverse PCR approach based on \(Capgip1p\) and sequence assembly of resulting sequences resulted in a 2295 bp sequence with an uninterrupted open reading frame (ORF) (\(Capgip1\)) of 1014 bp. Southern analysis indicated possible occurrence of \(pgip\) in pearl millet as a small multigene family. \textit{In silico} upstream sequence analysis resulted in the identification of several important DNA motifs with varied functions. \textit{In silico} analysis of deduced CaPGIP1 amino acid sequence made of 337 residues showed the presence of a putative signal peptide of 27 residues. The mature protein (CaPGIP1m) chain comprising of 310 amino acids was found to have a theoretically calculated isotopically averaged molecular weight (\(M_w\)) of 32.9 kDa and a theoretical isoelectric point (pI) value of 6.72, with seven putative glycosylation sites. Conserved domain search analysis showed the presence of a central leucine-rich (LRR) domain, predominantly hydrophobic in nature and in addition, N-and C-terminal domains with conserved cysteine residues. Protein modeling studies found the pearl millet PGIP to have topological appearance similar to bean PGIP2 structure with a solenoid shape and right-handed super helical fold.
Comparative structural studies at the active sites of the two PGIPs identified some differences in key amino acid residues known to be important for interaction with fungal PGs.

Capgip1m was successfully expressed and purified as a recombinant fusion protein (rCaPGIP1m) in Escherichia coli. In addition polygalacturonase III from Fusarium moniliforme isolate PD (FmPGIII) were also expressed and purified as a recombinant protein (rFmPGIII) in E. coli. The PGIP activity assays using rCaPGIP1m against rFmPGIII and Aspergillus niger polygalacturonase II (AnPGII) showed differential activity with partial inhibition against AnPGII in a non-competitive manner and no inhibition against rFmPGIII. Studies on the effect of inhibitor on AnPGII displayed a positive correlation between increase in inhibitor concentration and percent inhibition. Recombinant PGIP preferentially inhibited AnPGII between 4 and 4.5 and not at pH 3.5 and 5. The rCaPGIP1m was found to be stable over a wide pH range of 4 to 9 and relatively thermostable up to 60 °C.

Anti-PGIP polyclonal antibodies (PAb_{pep-CaPGIP1}) were commercially generated against a 15-amino acid custom synthesized peptide from a region of the PGIP. Immuno-affinity purification of PGIP from pearl millet total protein extract using the above mentioned antibody resulted in a major protein spot observed at ~43 kDa with a pI value of 5.9. The protein upon digestion with trypsin was identified to be PGIP by tandem mass spectrometry and homology search of protein databases. Inhibition profile of the immuno-purified PGIP against AnPGII and rFmPGIII was very similar to that observed with purified recombinant PGIP expressed in E. coli.

The role of PGIP in pearl millet resistance against the oomycete pathogen was reconfirmed by state-of-the art techniques such as fluorescence confocal microscopy and quantitative real-time PCR (qPCR) using homologous probes.

The present study has clearly shown PGIP to be an important factor in pearl millet host resistance against the downy mildew pathogen.