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Pseudomonas putida BP25 alters root phenotype and triggers salicylic acid signaling as a feedback loop in regulating endophytic colonization in *Arabidopsis thaliana*

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ABSTRACT

Endophytic *Pseudomonas putida* BP25 (PpBP25) triggered density dependent alterations on *Arabidopsis thaliana* Col-0 growth. Endogenous colonization of PpBP25 was found regulated within *Arabidopsis* that caused induction and repression of 131 and 74 plant genes, respectively. Induced genes like *WRKY33, AtRLP19, ATL2, ATEXO70B2, pEARLI, RPS2, CBP60G, PLA2, CRK18, ATFBS1, DREB2A, TIR, RAP2.4*, and *MOS1* were components of defense and salicylic acid (SA) signaling. Development associated genes were found significantly repressed. Biased activation of phytohormone signaling with their associated fitness costs on plant growth was observed. The data suggests that PpBP25 colonization triggered expression of defense genes that restricted its own population in a feedback loop besides causing altered root phenotype.

1. Introduction

Endophytic bacteria inhabit the interior of plants, especially in the intercellular spaces and have been isolated from all plant parts including seeds [1–3]. Plant endophytic bacteria are unique as they are known to colonize the plant interiors and contribute to sustained biological control through triggering plant resistance or prevent plant disease through antibiosis [4–6]. The endophyte-mediated *de novo* synthesis of antimicrobial compounds and their role within the plant is one of the exciting areas in plant endophyte research. Recently, we have reported that endophytic *Pseudomonas putida* secrete broad spectrum bacterial volatile compounds (BVC) that suppressed a broad range of plant pathogens at microgram concentrations [7].

In fact the intimate association of endophytes with plants is believed to trigger a phenomenon called induced systemic resistance (ISR) which primes the host plant to resist a wide range of stresses including biotic stress instigated by plant pathogens. The endophytic multiplication in plant interiors by endophytic bacteria is one of the key traits that make them unique among the prokaryotes. The endogenous population level of endophytes is limited and regulated in contrast to plant colonizing vascular pathogens like *Ralstonia solanacearum* that multiply unlimitedly and often unregulated within the xylem element of the vascular system [8]. Such an uncontrolled multiplication leads to death of plantlets owing to symptoms such as wilt. Therefore, the delicate balance between endogenous population level of endophytes and plant growth is crucial for not only the bacterial survival, but also the host plant's growth. It is not known how endogenous populations of endophytes are regulated for the benefit of the plants. Recently a study by Millet et al. [9], found that MAMPs (microbe-associated molecular pattern) elicit callose deposition on roots and exudation of the antimicrobial compound camalexin. It is believed that the suppression of MAMPs signaling is necessary for successful root colonization by beneficial bacteria which protect the bacteria against MAMPs-elicited antimicrobial exudates. Nevertheless, one of the key questions still surrounding endophytic microorganisms is how they are able to maintain their population density within the

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plant interiors without significantly harming the plant growth and development.

Development of microarray technology has allowed monitoring of expressional changes in thousands of genes simultaneously and this technology has now become an important tool in stress genomics for understanding plant stress biology [10–11]. Most of these studies have adopted *A. thaliana* as a model plant organism because of the vast amount of genomic information available for this species with the publication of the whole genome sequences and advanced annotation of *A. thaliana* genes [12]. Plant endophytic *P. putida* BP25 (PpBP25) was originally isolated from stem tissues of apparently healthy cultivar Panniyur-5, that inhibited broad range of plant pathogens through antimicrobial volatiles belong to dimethyl trisulphide and several diverse pyrazine compounds [7]. The present investigation was conducted primarily to understand the consequence of endophytic colonization of PpBP25 on plant growth using the model plant *A. thaliana* Col 0. Genetically tagged PpBP25 expressing green fluorescence protein (GFP) was used to localize the endophytic bacterium in plant tissue. We, further, conducted microarray based gene expression profiling in *A. thaliana* Col 0 in order to understand the endophyte induced alteration in gene(s) expression vis-a-vis plant growth and development.

2. Material and methods

2.1. Bacterial strain and culture conditions

The endophytic bacterium *P. putida* BP25 (PpBP25) isolated from stem tissues of black pepper was used in the study. PpBP25 was originally isolated from stem tissues of black pepper collected from Western Ghats of Kerala, India as an endophytic bacterium. The bacterium suppressed black pepper foot rot causing oomycetes, *Phytophthora capsici in vitro*. Unless stated otherwise PpBP25 was routinely cultured in Luria Bertani's broth (gL^{-1} Tryptone 10; Yeast Extract 5; NaCl 10) at 28 °C for 24 h in a rotary shaker at 200 rpm. Bacterial cells were pelleted after 24 h at 6000 rpm for 5 min and washed pellets were used for inoculating the *A. thaliana* plants after re-suspending in sterile distilled water.

2.2. Effect of Pseudomonas putida BP25 on growth of Arabidopsis thaliana

2.2.1. Plant growth conditions

The model plant *A. thaliana* Columbia 0 (At-Col-0) ecotype was used in the experiment. Seeds of *A. thaliana* (Col-0) were surface-sterilized by 70% ethanol and 0.4% sodium hypochlorite (NaClO) and were washed three times with distilled water. Thus obtained disinfected seeds were placed on half strength Murashige and Skoog (MS) medium and varying concentrations of PpBP25R::gfp suspension (10 μL per seed) were spot inoculated on seeds. Prior to that the plates sown with seeds were incubated at 4 °C for two days as dormant releasing, and then, the MS petri dishes were placed, and allowed to germinate and grow at 22/20 °C (day/night) temperature, 24-h light period and 40% relative humidity in a growth chamber.

2.2.2. Bacterial inoculation and plant growth

Effect of PpBP25 on growth and development of *A. thaliana* was assessed under aseptic *in vitro* conditions. To study the endophytic bacterial colonization, a genetically tagged strain of PpBP25::gfp expressing GFP was used [7]. PpBP25R::gfp was cultured in LB amended with rifamycin (50 μg mL^{-1}) and gentamycin (75 μg mL^{-1}) at 28 °C with constant agitation of 150 rpm. Bacterial suspensions were prepared from mid log phase growth of PpBP25::gfp in sterile deionized water. Decimal dilutions of cell suspension with each concentration were prepared so as to obtain 10^{10}, 10^{9}, 10^{8} and 10^{7} cells per mL and 10 μL of cell suspension was drop inoculated on seeds of *A. thaliana* Col-0. The control set were inoculated with same amount of sterile water. The plant phenotype was assessed and endogenous bacterial population size was determined at 21 days post inoculation (dpi). Plant growth parameters such as root length, number of roots, shoot length, and number of leaves was counted. Statistical analysis of data was done using online data analysis programme (http://hau.ernet.in/about/opstat.php).

2.2.3. Estimation of endophytic Pseudomonas putida BP25

The endogenous population size of the bacteria was estimated by serial dilution plating on antibiotics amended Luria Bertani's Agar (g L^{-1} Tryptone 10; Yeast Extract 5; NaCl 10; Agar 16). The whole plantlet of *A. thaliana* harvested on 21 dpi was used for the estimation of endogenous population. Plantlets were surface sterilized with NaClO (0.5%) + tween 20 (0.01%) for 20 min, ethyl alcohol (70%) for 1 min followed by rinsing with sterile distilled water 2–3 times. Each sample was ground aseptically with 2 mL of Phosphate Buffered Saline [PBS, g L^{-1} NaCl 8; KCl 0.2; Na_2HPO_4 1.44; KH_2PO_4 0.24; pH-7.4] and serially diluted up to 10^{-6}. One mL of serially diluted samples was pour plated on LBA amended with rifamycin (50 μg mL^{-1}) & gentamycin (75 μg mL^{-1}) and incubated at 28 °C for 48 h. Colonies were counted and expressed as colony forming unit (CFU) per gram of fresh tissue. Statistical analysis of data was done using online data analysis programme (http://hau.ernet.in/about/opstat.php).

2.2.4. Bio-PCR assay to detect endogenous Pseudomonas putida BP25

Bio-PCR was adopted to detect bacterial cells in *Arabidopsis* endosphere. The plantlets were surface sterilized with 1% NaClO for 1 min followed by three washes 1 min each with distilled water. The plant samples were then ground with a pestle in sterile micro centrifuge tubes containing 1 mL of distilled water for approximately 1 min. The extract was inoculated in LB broth amended with rifampicin (50 μg mL^{-1}) +gentamycin (75 μg mL^{-1}) and incubated at 28 °C for 48 h. Broth culture (2 μL) was used as a template for PCR confirmation using *P. putida* specific primers tpiA_F:5′-CGAATTCGTCTGCGTTCAG-3’; tpiA_R: 5′-GAGCTGACCAAAGGCTTGAG-3′ that resulted in PpBP25 specific 667 bp amplicon.

2.2.5. Localization of P. putida BP25 in A. thaliana using confocal laser scanning microscopy (CLSM)

In order to localize PpBP25::gfp in plant tissues, confocal laser scanning microscopy was used. Plantlets of three week old At-Col-0 ecotype from seeds inoculated with PpBP25::gfp (OD600: 1.0) were washed in sterile water and thin sections fixed in paraformaldehyde (4%) for 12 h at 4 °C. The tissues were scanned and imaged in CLSM (DM6000, Leica microsystems) at multiple sites on the sections.

2.3. Gene expression analysis using microarray

2.3.1. RNA extraction, cDNA preparation and microarray analysis

Three weeks old seedlings emerged on MS medium from seeds inoculated with PpBP25::gfp (OD600: 1.0) were carefully detached from agar plates, rinsed with sterile distilled water and frozen in liquid nitrogen. Plants were macerated with a sterile mortar-pestle and total RNA was extracted using Qiagen RNAeasy kit, Germany as per the manufactures' instructions. RNA quality and quantity were checked spectrophotometrically (Eppendorf Biophotometer, Germany). Quality of RNA was further assessed by agarose gel electrophoresis. Two hundred nanograms of total RNA were converted

to cDNA using a cDNA synthesis kit from Affymetrix, USA. IVT labeling was done for synthesized cDNA strand followed by quantification, purification and fragmentation of aRNA using GeneChip® 3′ IVT Express Kit, USA according to the manufacturers' instructions. Fragmented aRNA was hybridized on Affymetrix ATH1 Genechip probe arrays, USA representing approximately 22500 genes. The standard wash and double-stain protocols were applied using an Affymetrix GeneChip Fluidics Station 450, USA as per instrument protocol. The arrays were scanned on an Affymetrix GeneChip scanner 3000, USA. Raw data files generated through Affymetrix Gene chip instrument were analyzed using Agilent's Gene Spring software, United States (Gene Spring 12.6 version).

2.3.2. Data analysis

The 22500 probes on the *A. thaliana* ATH1 Gene chip were used to compare across samples. The data was analyzed by one way analysis of variance (ANOVA) using the student's T-test with $P \leq 0.05$. Differentially expressed genes were identified by outlier detection of a contaminated bivariate distribution. To determine the differentially expressed candidate genes from these outliers, the fold-change values were calculated providing up and down regulated genes across the samples as compared to mock. The biological significance and functional classification of differentially expressed genes was performed using the Gene Ontology programme (https://www.arabidopsis.org/tools/bulk/go/index.jsp).

2.3.3. Quantitative RT-PCR validation

To confirm and extend the results obtained in the genome-wide microarray analyses, quantitative Real Time PCR analysis was performed on RNA isolated from *Arabidopsis* plants grown *in vitro* for 21 days as described above. Two biological replicates containing 10 to 12 plants each were used. The plant tissues were frozen in liquid N_2 and stored at −80 °C. RNA was isolated from the frozen tissues with the Qiagen RNAeasy kit followed by DNase I treatment. Two hundred nanograms of total RNA were used for copy DNA synthesis using oligo-dT primer and ImProm-II™ Reverse Transcription System (Promega, USA) kit according to the manufacturer's protocol. The cDNA was quantified using Nanodrop 2000 spectrophotometer, ThermoScientific, USA. Validation of the transcriptome profiles was performed by RT-PCR on selected candidate genes identified in the microarray experiments. Quantitative PCR was conducted on Light Cycler 96 system with 2X SYBR Mix (Roche Diagnostics, Germany) having a final concentration of 3.0 mm $MgCl_2$. Gene specific RT-PCR primers were designed using primer 3 plus software and synthesized (IDT, USA). The primers used for the quantitative PCR are listed in Supplementary Table 1. The primer concentrations were optimized and a dissociation curve was obtained to check the specificity of the primers. PCR amplification was done using 1 μM each of gene specific forward and reverse primers and 10–15 ng of cDNA as template. One of the housekeeping genes, PP2A was also included as a reference gene to check the efficiency of RT-PCR amplification and to compare the gene expression level of randomly selected genes for validation. The genes were amplified using Light cycler 96 PCR machine (Roche diagnostics) with the following PCR cycling conditions: 95 °C for 5 min followed by 40 cycles of 95 °C for 10 sec, 15 sec at the annealing temp. and 72 °C for 15 sec followed by one cycle of 95 °C for 10 sec, AT + 5 °C for 60 sec, 97 °C for 1 sec and the final cooling of 37 °C for 30 sec. RT-PCR data generated was analyzed using LightCycler® 96 SW 1.1 software. Five microlitres of amplified RT-PCR reaction mixture were visualized on a 2.5% agarose gel.

Table 1
Endophytic *Pseudomonas putida* BP25 induced phenotypic changes in 21 days old *Arabidopsis thaliana* plantlets.

Bacterial titer Log 10 CFU mL^{-1}	Root length (cm)	Shoot length (cm)	Number of roots
Mock	3.17	2.23	3.00
7	2.63	1.80	3.33
8	1.47	1.20	4.67
9	0.73	1.03	7.33
10	0.37	0.63	7.67
CD (P = 0.05%)	**0.24**	**0.22**	**0.95**
SE(m)	**0.08**	**0.07**	**0.30**
SE(d)	**0.11**	**0.10**	**0.42**

3. Results

3.1. Effect of P. putida BP25 on growth of Arabidopsis thaliana

A. thaliana plantlets emerged from PpBP25::gfp inoculated seeds showed a phenotype characterized by shortened roots and tuft like growth pattern as compared to elongated and few roots observed in untreated plants. The bacteria challenged plants

Fig. 1. A. Endophytic bacterium, PpBP25 induced changes in phenotype of *Arabidopsis thaliana* Col 0 roots; 1. Plantlet emerged from seed inoculated with 10^{9-10} cells of PpBP25::gfp; 2. Plantlet emerged from seed inoculated with 10^{8-9} cells of PpBP25::gfp; 3. Plantlet emerged from seed inoculated with 10^{7-8} cells of PpBP25::gfp; 4. Plantlet emerged from seed inoculated with 10^{6-7} cells of PpBP25::gfp; 5. Mock (Water). Note: The root length was significantly reduced with concomitant increase in number of roots. B. Colonies of PpBP25 on 2,3,5-triphenyl tetrazolium chloride amended media.

Table 2
Significantly up-regulated genes (over 2 fold changes) in *Arabidopsis* upon endophytic colonization *by Pseudomonas putida* BP25.

Gene symbol/Gene name	Fold change	Probe set ID	AGI number	Functional details	Broad role
pEARLI 1: EARLI1, EARLY ARABIDOPSIS ALUMINUM INDUCED 1, pEARLI 1	7.2	254805_at	At4g12480	A putative lipid transfer protein, vernalization-responsive and cold-induced	Electron transport or energy pathways; Cell organization and biogenesis; Signal transduction; Transport; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
ATPRX71: ATPRX71, PEROXIDASE 71, PRX71	5.4	247327_at	At5g64120	Encodes a cell wall bound peroxidase that is induced by hypo-osmolarity and is involved in the lignification of cell walls.	Cell organization and biogenesis; Signal transduction; Transport; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
CRK18: CRK18, CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 18	3.3	254247_at	At4g23260	Encodes a cysteine-rich receptor-like protein kinase.	Protein metabolism; Signal transduction; Other biological processes; Other metabolic processes; Other cellular processes
ATSTP14: ATSTP14, STP14, SUGAR TRANSPORT PROTEIN 14	3.3	264482_at	At1g77210	AtSTP14 belongs to the family of sugar transport proteins (AtSTPs) involved in monosaccharide transport. Heterologous expression in yeast revealed that AtSTP14 is the transporter specific for galactose and does not transport other mono saccharides such as glucose or fructose.	Transport; Other cellular processes
CBP60G: CAM-BINDING PROTEIN 60-LIKE G, CBP60G	3.2	246821_at	At5g26920	Encodes a calmodulin-binding protein CBP60g (calmodulin binding protein 60-like.g). The calmodulin-binding domain is located near the N-terminus; calmodulin binding is dependent on Ca (2+). Inducible by both bacterial pathogen and MAMP (microbe-associated molecular pattern) treatments. Bacterial growth is enhanced in cbp60g mutants. Cbp60g mutants also show defects in salicylic acid (SA) accumulation and SA signaling.	Electron transport or energy pathways; Transcription, DNA dependent; Cell organization and biogenesis; Protein metabolism; Signal transduction; Transport; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
UCP5: ATPUMP5, DIC1, DICARBOXYLATE CARRIER 1, PLANT UNCOUPLING MITOCHONDRIAL PROTEIN 5, UCP5, UNCOUPLING PROTEIN 5	3.0	264000_at	At2g22500	Encodes one of the mitochondrial dicarboxylate carriers	Transport; Other biological processes; Other cellular processes
XBAT34: XB3 ORTHOLOG 4 IN ARABIDOPSIS THALIANA, XBAT34	2.8	245329_at	At4g14365	XB3 ortholog 4 in *Arabidopsis thaliana* (XBAT34); FUNCTIONS IN: zinc ion binding	Cell organization and biogenesis; Protein metabolism; Signal transduction; Transport; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
DIN11: DARK INDUCIBLE 11, DIN11	2.7	252265_at	At3g49620	Encodes a protein similar to 2-oxoacid-dependent dioxygenase. Expression is induced after 24 hours of dark treatment, in senescing leaves and treatment with exogenous photosynthesis inhibitor. Induction of gene expression was suppressed in excised leaves supplied with sugar. The authors suggest that the gene's expression pattern is responding to the level of sugar in the cell.	Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
ATFBS1: ATFBS1, F-BOX STRESS INDUCED 1, FBS1	2.6	264758_at	At1g61340	Encodes a F-box protein induced by various biotic or abiotic stress.	Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
AT-HSFB2B: AT-HSFB2B, HEAT SHOCK TRANSCRIPTION FACTOR B2B, HSF7, HSFB2B	2.5	254878_at	At4g11660	Member of Heat Stress Transcription Factor (Hsf) family	Transcription, DNA dependent; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
DREB2A: DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2, DRE-BINDING PROTEIN 2A, DREB2, DREB2A	2.5	250781_at	At5g05410	Encodes a transcription factor that specifically binds to DRE/CRT cis elements (responsive to drought and low-temperature stress). Belongs to the DREB subfamily A-2 of ERF/AP2 transcription factor family (DREB2A). There are eight	Transcription, DNA dependent; Protein metabolism; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes

Table 2 (*continued*)

Gene symbol/Gene name	Fold change	Probe set ID	AGI number	Functional details	Broad role
				members in this subfamily including DREB2B. The protein contains one AP2 domain. Over expression of transcriptional activation domain of DREB2A resulted in significant drought stress tolerance but only slight freezing tolerance in transgenic Arabidopsis plants. Microarray and RNA gel blot analyses revealed that DREB2A regulates expression of many water stress–inducible genes.	
ATL31: ARABIDOPSIS TOXICOS EN LEVADURA 31, ATL31, CARBON/NITROGEN INSENSITIVE 1, CNI1	2.5	246777_at	At5g27420	Encodes CNI1 (Carbon/Nitrogen Insensitive1) (also named as ATL31), a RING type ubiquitin ligase that functions in the Carbon/Nitrogen response for growth phase transition in Arabidopsis seedlings	Protein metabolism; Signal transduction; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
TIR: ATTN10, TIR, TIR-NUCLEOTIDE BINDING SITE FAMILY 10, TN10, TOLL/INTERLEUKIN-1 RECEPTOR-LIKE	2.5	262374_s_at	At1g72930	Toll/interleukin-1 receptor-like protein (TIR) mRNA	Signal transduction; Response to stress
ATPUP18: ATPUP18, PUP18, PURINE PERMEASE 18	2.4	245866_s_at	At1g57990	Member of a family of proteins related to PUP1, a purine transporter. May be involved in the transport of purine and purine derivatives such as cytokinins, across the plasma membrane.	Transport; Other cellular processes
TG: TG, TRANSLUCENT GREEN	2.4	263194_at	At1g36060	Encodes a member of the DREB subfamily A-6 of ERF/AP2 transcription factor family. The protein contains one AP2 domain. There are 8 members in this subfamily including RAP2.4. Over expression results in increased drought tolerance and vitrified leaves. Binds to DRE/GCC promoter elements and activates expression of aquaporin genes AtTIP1; 1, AtTIP2; 3, and AtPIP2; 2.	Transcription, DNA dependent; Response to abiotic and biotic stimulus; Other metabolic processes; Other cellular processes
CYP707A3: "CYTOCHROME P450, FAMILY 707, SUBFAMILY A, POLYPEPTIDE 3″, CYP707A3	2.4	248964_at	At5g45340	Encodes a protein with ABA 8'-hydroxylase activity; involved in ABA catabolism. Mutant analyses show that disruption in the gene results in more drought tolerance whereas over expression results in increased transpiration rate and reduced drought tolerance. Gene involved in post germination growth. Plant P450 CYP707A3, ABA 8'-hydroxylase, binds enantio selectively (+)-ABA but not (−)-ABA, whereas the enzyme binds both enantiomers of AHI1 (a structural ABA analogue used as ABA 8'-hydroxylase competitive inhibitor).	Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
SQE6: SQE6, SQUALENE MONOOXYGENASE 6	2.3	249775_at	At5g24160	squalene monooxygenase 6 (SQE6); FUNCTIONS IN: squalene monooxygenase activity, oxidoreductase activity, FAD binding; INVOLVED IN: sterol biosynthetic process	Other metabolic processes; Other cellular processes
ADP1: ACTIVATED DISEASE SUSCEPTIBILITY 1, ADP1, ADS1, ALTERED DEVELOPMENT PROGRAM 1	2.3	253732_at	At4g29140	Encodes Activated Disease Susceptibility 1 (ADS1), a putative MATE (multidrug and toxic compound extrusion) transport protein that negatively regulates plant disease resistance.	Transport; Other biological processes; Response to stress; Other cellular processes
QQS: QQS, QUA-QUINE STARCH	2.2	256940_at	At3g30720	QQS, QUA-QUINE STARCH	Other metabolic processes; Other cellular processes
ATHSFA2: ATHSFA2, HEAT SHOCK TRANSCRIPTION FACTOR A2, HSFA2	2.2	266841_at	At2g26150	Member of Heat Stress Transcription Factor (Hsf) family. Involved in response to mis-folded protein accumulation in the cytosol. Regulated by alternative splicing and non-sense-mediated decay.	Transcription, DNA dependent; Protein metabolism; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
MBF1C: ATMBF1C, MBF1C, MULTIPROTEIN BRIDGING FACTOR 1C	2.2	258133_at	At3g24500	One of three genes in A. thaliana encoding multiprotein bridging factor 1, a highly conserved transcriptional co-activator. May serve as a bridging factor between a bZIP factor and TBP. Its expression is specifically elevated in response to pathogen infection, salinity, drought, heat, hydrogen peroxide, and application of abscisic acid or salicylic acid. Constitutive expression enhances the	Transcription, DNA dependent; Protein metabolism; Signal transduction; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes

(*continued on next page*)

Table 2 (*continued*)

Gene symbol/Gene name	Fold change	Probe set ID	AGI number	Functional details	Broad role
				tolerance of transgenic plants to various biotic and abiotic stresses.	
PLA2A: PATATIN-LIKE PROTEIN 2, PHOSPHOLIPASE A 2A, PLA IIA, PLA2A, PLAII ALPHA, PLP2	2.2	245038_at	At2g26560	Encodes a lipid acyl hydrolase with wide substrate specificity that accumulates upon infection by fungal and bacterial pathogens. Protein is localized in the cytoplasm in healthy leaves, and in membranes in infected cells. Plays a role in cell death and differentially affects the accumulation of oxylipins. Contributes to resistance to virus	Cell organization and biogenesis; Protein metabolism; Signal transduction; Transport; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
ATL2: ATL2, TL2, TOXICOS EN LEVADURA 2	2.2	258436_at	At3g16720	RING-H2 protein induced after exposure to chitin or inactivated crude cellulase preparations.	Cell organization and biogenesis; Protein metabolism; Signal transduction; Transport; Other processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
GTE8: GLOBAL TRANSCRIPTION FACTOR GROUP E8, GTE8	2.1	257146_at	At3g27260	Kinase like protein with similarity to yeast BDF1 and human RING3 protein, which have two bromo domains GTE8 has a single bromo domain	Transcription, DNA dependent; Other metabolic processes; Other cellular processes
CYP705A12: "CYTOCHROME P450, FAMILY 705, SUBFAMILY A, POLYPEPTIDE 12″, CYP705A12	2.0	249202_at	At5g42580	A member of the cytochrome P450 family	Developmental processes; Other biological processes; Other metabolic processes; Other cellular processes
RAP2.4: RAP2.4, RELATED TO AP2 4	2.0	255926_at	At1g22190	The gene encodes a putative transcription factor belongings to the abiotic stress-associated DREB A-6 clade	Transcription, DNA dependent; Signal transduction; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
WRKY33: ATWRKY33, WRKY DNA-BINDING PROTEIN 33, WRKY33	2.0	267028_at	At2g38470	Member of the plant WRKY transcription factor family. Regulates the antagonistic relationship between defense pathways mediating responses to *P. syringae* and necrotrophic fungal pathogens. Located in nucleus. Involved in response to various abiotic stresses - especially salt stress.	Transcription, DNA dependent; Cell organization and biogenesis; Protein metabolism Signal transduction; Transport; Other biological processes; Response to abiotic and biotic stimulus; Response to stress; Other metabolic processes; Other cellular processes
AtGH9B13: ATGH9B13, GH9B13, GLYCOSYL HYDROLASE 9B13	2.0	255517_at	At4g02290	Glycosyl hydrolase 9B13 (GH9B13); FUNCTIONS IN: hydrolase activity, hydrolyzing O-glycosyl compounds, catalytic activity; INVOLVED IN: carbohydrate metabolic process	Cell organization and biogenesis; Other biological processes; Other metabolic processes; Other cellular processes
PHI-1: EXL1, EXORDIUM LIKE 1, PHI-1, PHOSPHATE-INDUCED 1	2.0	245757_at	At1g35140	EXL1 is involved in the C-starvation response. Phenotypic changes of an exl1 loss of function mutant became evident only under corresponding experimental conditions. For example, the mutant showed diminished biomass production in a short-day/low light growth regime, impaired survival during extended night, and impaired survival of anoxia stress.	Other biological processes; Response to abiotic and biotic stimulus; Response to stress
MOS1: MODIFIER OF SNC1, MOS1	2.0	254143_at	At4g24680	Encodes MOS1 (MODIFIER OF snc1). MOS1 contains a BAT2 domain that is conserved in plants and animals. MOS1 regulates the expression of SNC1, a TIR-NB-LRR-type of R protein.	Other metabolic processes; Other cellular processes

showed significantly more number of roots with characteristic branching and reduction in root length and shoot length was also observed as compared to untreated plants (Table 1). Interestingly the root phenotype alteration was found to be bacterial density dependent (Fig. 1).

3.2. Endophytic colonization of *P. putida* BP25 in *A. thaliana*

An endogenous population of 7 log cfu gram^{-1} was enumerated in plantlets emerged from bacteria inoculated seeds on 21 dpi. The plant endogenous population size did not increase with increasing concentration of cells used for seed inoculation (Table 2). Bio-PCR using PpBP25R specific primers yielded the specific amplicon of 667 bp that further confirmed the endogenous presence of the bacteria (Data not shown). PpBP25R::gfp inoculated on seeds could be observed in plant interiors in CLSM imaging further confirming its endophytic behavior (Fig. 2).

3.3. Gene expression analysis using microarray

Microarray based gene expression profiling enabled to obtain a global view on transcript modification during the *A. thaliana*–PpBP25::gfp interaction and a consequent altered plant phenotype. A total of 205 genes were differentially expressed with

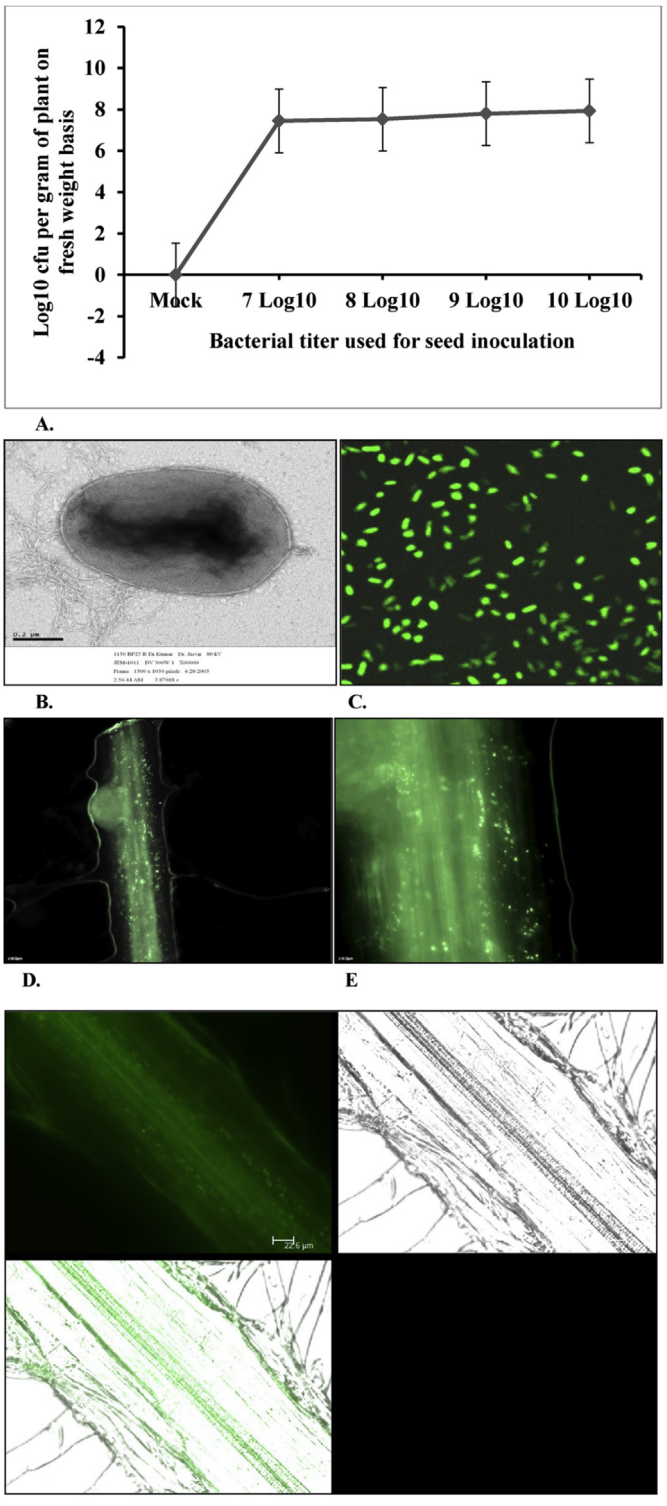

Fig. 2. A. Endophytic population size and localization of *Pseudomonas putida* BP25::gfp in A. thaliana Col-0. A. Graph showing endophytic population size of PpBP25::gfp in *Arabidopsis* Col-0; Error bar indicate standard error; B. Transmission Electron Microscopic image of PpBP25; C. Image showing green fluorescence of PpBP25::gfp; D. Endophytic cells of PpBP25::gfp in roots of *Arabidopsis* Col-0; E. Close up view of tissue localization of PpBP25::gfp in *Arabidopsis* Col-0; F. Confocal Laser Scanning Microscopic images showing endophytic colonization and localization of PpBP25::gfp in *Arabidopsis thaliana*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fold change of $\geq$ 1.5 that included 131 and 74 genes up or down regulated respectively, in endophyte inoculated *Arabidopsis* plants at 21 dpi as compared with mock inoculated plants. Complete list of up regulated/down regulated genes are furnished in Supplementary Tables 2 and 3. Functional categorization of differentially expressed genes based on their involvement in various processes is shown Figs. 3 and 4. Genes participating in other cellular and metabolic process (38%) followed by stress responsive genes and genes expressed due to biotic and abiotic stimuli (29.2%) were significantly induced upon endophytic colonization (Fig. 2). Genes such as *pEARLI ATPRX71, CRK18, ATSTP14, XBAT34, DIN11, UCP5, CBP60G, ATFBS1, AT-HSFB2B, DREB2A, TIR, ATL31, ATPUP18, CYP707A3, TG, SQE6, ADP1, QQS, ATHSFA2, MBF1C, PLA2A, ATL2, GTE8, CYP705A12, RAP2.4, WRKY33, AtGH9B13, PHI-1* and *MOS1* were significantly induced over two folds in *A. thaliana* upon endophytic bacterial colonization (Table 2). Among them *pEARLI, CBP60G, TIR, PLA2A, ATL2* and *WRKY33* are well known defense related genes in plants. Many other defense related genes especially, *AtMC2, PDF1.4, AtRLP19, ANAC036, NHL3, ATEXO70B2, WAKL2, AtRLP22, RPS2* and *LUG* were also found up-regulated at 1.5–2.0 folds in bacteria challenged plantlets. Apart from defense related roles, some of the induced genes such as *WRKY33, AtRLP19, ATL2, ATEXO70B2, pEARLI, RPS2, CBP60G, RAP2.4* and *PLA2A* are reported to directly participate in the SA mediated signaling pathway and SA biosynthetic process.

Among the repressed genes, significant ones were associated with cellular and metabolic processes in plants (44.4%). Nearly 15% of the genes participating in stress responses and expressed upon biotic and abiotic stimuli were down regulated. Several developmental and biological processes related genes (11%) were repressed upon endophytic colonization (Fig. 4). Down regulated genes participating in cellular processes in *A. thaliana* Col-0 upon endophytic colonization by *P. putida* BP25 are furnished in Supplementary Table 3. Many of the genes associated with developmental processes, transport of nutrient through transmembranes, transcription, cell organization and biogenesis, protein metabolism, growth regulating factors were found to be down regulated. Significant among them were *SLAH1, PROPEP4, OFP2, RBCX1,* and *KAT1* as they were down regulated over 2 folds in bacteria challenged plantlets (Table 3). Others such as *ACO1* (participate in ethylene biosynthesis); *AtGRF5, MEE23, MEE3, OFP2, iqd21* (involved in developmental processes); *AtbZIP3, HAT3, HSFB4, MYB28, NRPB6A, ZFHD1, ZFP3* (transcription related genes); *GDU1, NRT1.5* (play role in transport mechanisms) were down regulated at 1.5–2.0 folds in endophyte inoculated plantlets (Supplementary Table 3) (see Table 4).

3.4. Quantitative RT-PCR validation

To verify the Gene Chip results, a semi quantitative RT-PCR analysis was performed for selected up and down regulated genes identified in the microarray analysis. The relative visible intensity of PCR amplicons clearly indicated the elevated expression level of up regulated genes as compared to mock inoculated plants. Similarly for down regulated genes the visible intensity was less than mock (Fig. 5). RT-PCR results further authenticated the gene expression data obtained from microarray analysis.

4. Discussion

Bacteria mediated biological control activity against plant diseases is exerted either directly through antagonism on plant pathogens or indirectly by eliciting a plant resistance response [40–41] or both. To gain insights in the plant physiological, biochemical and molecular changes triggered by endophytic bacterial colonization, a detailed understanding of all biological

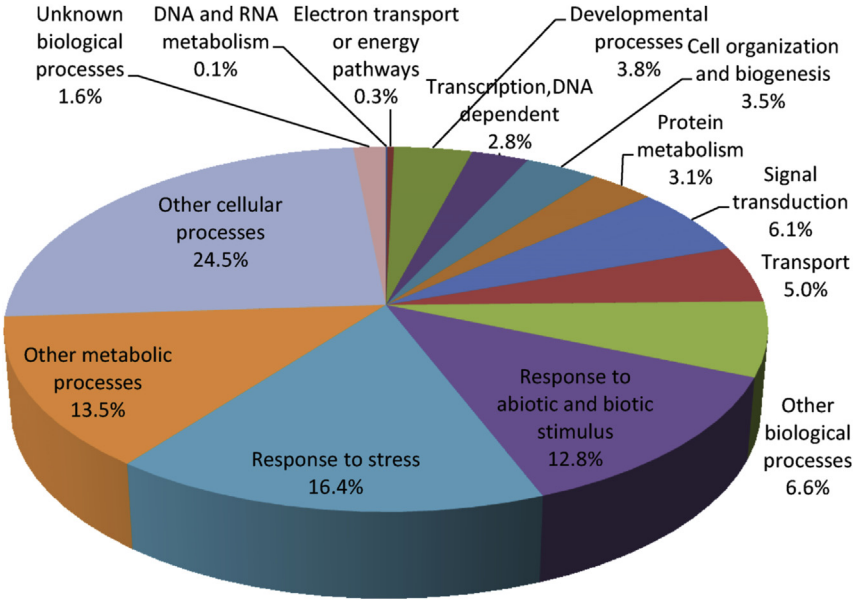

Fig. 3. Functional classification of endophytic *Pseudomonas putida* BP25 induced *Arabidopsis thaliana* genes.

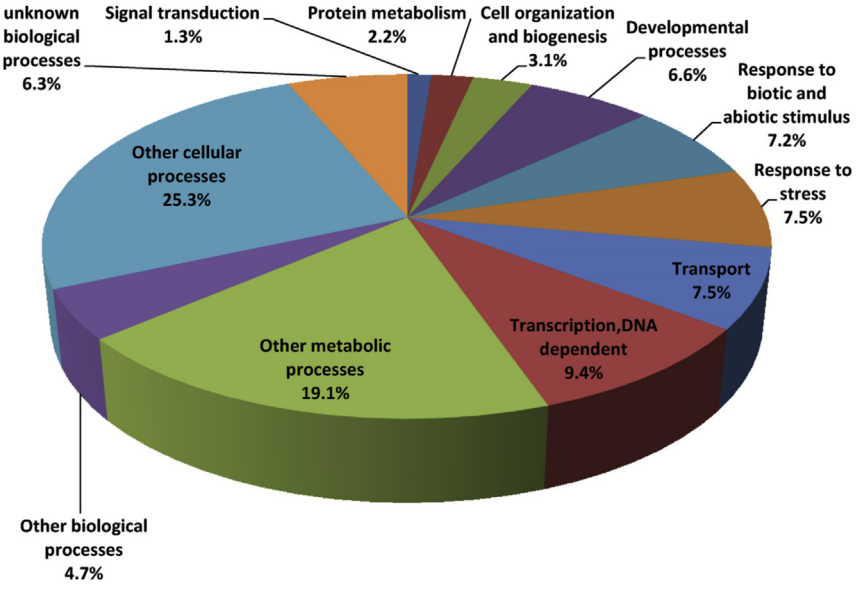

Fig. 4. Functional classification of endophytic *Pseudomonas putida* BP25 repressed *Arabidopsis thaliana* genes.

processes including gene expression changes and altered biosynthetic pathways is essential. In this study, consequences of endogenous colonization of PpBP25 on plant growth and development, especially the root development were investigated in a model plant, *A. thaliana* Col 0. We showed that colonization of *Arabidopsis* plantlets by an endophytic bacterium Pp*BP25* caused significant morphological changes albeit without any harm to plant survival. PpBP25 inoculated *Arabidopsis* seedlings showed altered shoot and root development and increased lateral root formation. *Arabidopsis* plantlets emerged from PpBP25 inoculated seeds showed a unique phenotype that is characterized by proliferation of shortened root growth in contrast to elongated few roots observed in uninoculated plants. Characteristically the bacterium displayed density dependent alternations on the root phenotype that we termed as “tuft-root phenotype”. Plantlets inoculated with high populations of PpBP25 triggered significant changes in root growth and also showed significantly high number of roots with reduced root length. The bacterized plantlets maintained its green appearance and remained healthy. The endogenous population estimation revealed that population size of PpBP25 remained constant at 7 log CFU per gram of plant tissue at all bacterial titers tested. Bio-PCR using PpBP25R specific primer and CLSM imaging of gfp-signals further confirmed its endophytic presence in plants. This internal regulation of endogenous bacterial population facilitated the controlled colonization of endophytic bacterium PpBP25 with a concomitant beneficial plant–microbe interaction. The stunted phenotype was not unexpected since morphological changes are often associated with plants in elevated defense [17].

Table 3
List of SA signaling and plant immunity associated genes induced upon colonization by plant endophytic *Pseudomonas putida* BP25 in *Arabidopsis thaliana* Col 0.

Gene name/other name	AGI number	Remarks	Reference
1. *WRKY33*, WRKY DNA-BINDING PROTEIN 33; transcription factor	At2g38470	Play a direct role in SA signaling in plants	[13] and [14]
2. *AtRLP19* (Receptor Like Protein 19); kinase/protein binding	At2g15080	Putative disease resistance protein in *Arabidopsis*	[16]
3. *ATL2*, TL2, TOXICOS EN LEVADURA 2; protein binding/zinc ion binding	At3g16720	ATL2 is a member of multigene family coding highly related RING-H2 zinc-finger proteins that may function as E3 ubiquitin ligases. ATL2 mRNA accumulation occurs rapidly and transiently after incubation with elicitors of pathogen response.	[17]
4. *ATEXO70B2* (exocyst subunit EXO70 family protein B2); protein binding	At1g07000	Exo70B2 is required for both immediate and later responses triggered by PAMPs, suggestive of a role in signaling. Exo70B2 plays an important role in immunity against bacterial and oomycete pathogens; Exo70B2 contributes to the activation of PTI during pathogen invasion	[18–20]
5. *pEARLI 1*; EARLI1, EARLY ARABIDOPSIS ALUMINUM INDUCED 1; lipid binding	At4g12480	EARLI1, a hybrid proline-rich protein of *Arabidopsis*, displayed inhibition effect to the growth of fungal pathogens and *Saccharomyces cerevisiae*.	[21] and [22]
6. *RPS2* (RESISTANT TO *P. SYRINGAE* 2); protein binding	At4g26090	*RPS2 moderates* salicylic acid accumulation in resistant plants	[23] and [24]
7. *CBP60G* (CAM-BINDING PROTEIN 60-LIKE.G); calmodulin binding	At5g26920	Positive regulator required for SA synthetic enzyme gene, *ICS1*, induction and SA accumulation upon pathogen infection	[25] and [26]
8. *PLA2A*, PATATIN-LIKE PROTEIN 2 (PHOSPHOLIPASE A 2A); lipase/nutrient reservoir	At2g26560	The Arabidopsis Patatin-Like Protein 2 Plays an Essential Role in Cell Death Execution and Differentially Affects Biosynthesis of Oxylipins and Resistance to Pathogens	[27–29]
9. *CRK18*, CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 18	At4g23260	Stress responsive protein kinases	[30]
10. *ATFBS1*, F-BOX STRESS INDUCED 1	At1g61340	*AtFBS1* responds to biotic and abiotic stress and is subjected to a complex regulation. *AtFBS1* interacts with ASK1, the component of the SCF complex that binds the F-box	[31]
11. *AT-HSFB2B*, HEAT SHOCK TRANSCRIPTION FACTOR B2B, HSF7	At4g11660	HSFB2B positively regulate the acquired thermotolerance	[32]
12. *DREB2A*, DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2, DRE-BINDING PROTEIN 2A, DREB2, DNA binding/transcription activator/transcription factor	At5g05410	DREB2A regulates drought stress–responsive gene expression, which enhances drought stress tolerance in plants	[33] and [34]
13. *TIR*, ATTN10, TIR-NUCLEOTIDE BINDING SITE FAMILY 10, TN10, TOLL/INTERLEUKIN-1 RECEPTOR-LIKE; transmembrane receptor	At1g72930	Play a role in R- gene mediated resistance in plants	[35–37]
14. *RAP2.4*, RELATED TO AP2 4; transcription factor family	At1g22190	Constitutive over expression of RAP2.4 results in defects in multiple developmental processes regulated by light and ethylene, including hypocotyl elongation and gravitropism, apical hook formation and cotyledon expansion, flowering time, root elongation, root hair formation, and drought tolerance	[38]
15. MODIFIER OF SNC1, *MOS1*	At4g24680	MOS1 epigenetically regulates the expression of plant Resistance gene SNC1	[39]

4.1. Genes participating in plant defense were induced

To identify plant genes involved in the associative interaction between endophytic PpBP25 and *Arabidopsis*, microarray based gene expression analysis was performed to monitor changes in the transcriptome. Plant defense related plant genes were found over represented among the up-regulated genes which includes *pEARLI ATPRX71, CRK18, ATSTP14, XBAT34, DIN11, UCP5, CBP60G, ATFBS1, AT-HSFB2B, DREB2A, TIR, ATL31, ATPUP18, CYP707A3, TG, SQE6, ADP1, QQS, ATHSFA2, MBF1C, PLA2A, ATL2, GTE8, CYP705A12, RAP2.4, WRKY33, AtGH9B13, PHI-1* and *MOS1*. Substantial number of genes such as *WRKY33, AtRLP19, ATL2, ATEXO70B2, pEARLI, RPS2, CBP60G, PLA2, CRK18, ATFBS1, DREB2A, TIR, RAP2.4* and *MOS1* were involved in number of defense pathway especially SA mediated phytohormone signaling and its biosynthetic pathway. Plant WRKY transcription factors are key regulatory components of plant responses to microbial colonization [13–14]. In particular, *WRKY33* regulates expression of genes encoding camalexin biosynthetic enzymes [14] and [42]. *CYP705A12*-an important gene participating in the biosynthesis of camalexin is found to be induced upon PpBP25 colonization. In addition to regulating the expression of defense-related genes, WRKY transcription factors have also been shown to regulate cross-talk between jasmonate- and salicylate-regulated disease response pathways in plants [15]. Plant endophytic PpBP25 induced *ATRLP19*- a Receptor like Protein 19, in *Arabidopsis* Col 0 is a putative disease resistance protein [16]. *ATL2* is a member of RING-H2 zinc-finger proteins that may function as E3 ubiquitin ligases. Our results are in agreement with previous studies that reported that mRNA of *ATL2* rapidly and transiently increased after incubation with elicitors of pathogen response [17]. Induction of plant immune pathway by endophytic PpBP25 is best exemplified in up regulation of *ATExo70B2*. *Exo70B2* plays an important role as PTI (PAMP-triggered immunity) inducer against bacterial and oomycete pathogens [18–20]. *Exo70B2* gene is required for full activation of early immune signaling events such as the ROS (reactive oxygen species) burst and MPK3 activity, as well as later responses, such as reduced root growth inhibition [18] and [20]. Regulated multiplication of endophytic population can be attributed to induction of *pEARLI 1*. *EARLI1*, a hybrid proline-rich protein of *Arabidopsis*, displayed inhibitory effect on growth of fungal pathogens and *Saccharomyces cerevisiae* [21–22]. The *Arabidopsis RPS2* gene encodes nucleotide-binding site–leucine-rich repeat (NBS–LRR) class of plant resistance proteins and its over expression apparently leads to activation of the defense response [43]. Our results indicate a

Table 4
Significantly down-regulated genes (over 2 fold changes) in *Arabidopsis* upon endophytic colonization *by Pseudomonas putida* BP25.

Gene Symbol/Gene name	Fold change	Probe set ID	AGI number	Specific function	Broad role
SLAH1: SLAC1 HOMOLOGUE 1, SLAH1	−2.2	264734_at	At1g62280	Encodes a protein with ten predicted transmembrane helices. The SLAH1 protein has similarity to the SLAC1 protein involved in ion homeostasis in guard cells. Although it is not expressed in guard cells, it can complement a slac1-2 mutant suggesting that it performs a similar function. SLAH1: GFP localizes to the plasma membrane.	Transport; Other cellular processes;
RBCX1: ATRBCX1, HOMOLOGUE OF CYANOBACTERIAL RBCX 1, RBCX1	−2.1	255331_at	At4g04330	Encodes a chloroplast thylakoid localized RbcX protein that acts as a chaperone in the folding of Rubisco.	Protein metabolism; Response to biotic and abiotic stimulus; Other metabolic processes; Other cellular processes
OFP2: ARABIDOPSIS THALIANA OVATE FAMILY PROTEIN 2, ATOFP2, OFP2, OVATE FAMILY PROTEIN 2	−2.1	267493_at	At2g30400	Ovate family protein 2 (OFP2); INVOLVED IN: N-terminal protein myristoylation	Developmental processes; Transcription, DNA dependent; Other biological processes; Other metabolic processes; Other cellular processes;
PROPEP4: ELICITOR PEPTIDE 4 PRECURSOR, PROPEP4	−2.1	250455_at	At5g09980	Elicitor peptide 4 precursor (PROPEP4); INVOLVED IN: response to jasmonic acid stimulus	Other biological processes
KAT1: ATKAT1, KAT1, POTASSIUM CHANNEL IN ARABIDOPSIS THALIANA 1	−2.0	248888_at	At5g46240	Encodes a potassium Channel protein (KAT1). ABA triggers KAT1 endocytosis both in epidermal cells as well as guard cells. KAT1 belongs to the Shaker family K + channel.	Transport; Other cellular processes

typical molecular feedback circuitry wherein the endogenously multiplied *P. putida* driven plant defense could be attributed for restricting the same bacterium that triggered the defense.

CBP60g [CAM (calmodium)-BINDING PROTEIN 60-LIKE G] was demonstrated to play role in microbe-associated molecular pattern (MAMP)-triggered SA accumulation [44]. Endophyte induced *CBP60g* gene may have contributed to the shortened root phenotype in *Arabidopsis* as dwarfed growth of *CBP60g* over expressing lines is attributed to ABA (Abscisic acid) hypersensitivity triggered by elevated level of SA biosynthesis [45]. *CBP60g* gene regulates early seedling growth, is also a component of abiotic stress induced ABA signaling. Induction of *PLA2A* by plant endophytic PpBP25 could have played its role in triggering plant resistance as reported that the *Arabidopsis* Patatin-Like Protein 2 plays an essential role governing resistance to pathogens [27–29]. Other significant defense related genes induced upon endophytic bacterial colonization are *CRK18* and *ATFBS1*. While *CRK18* is a well reported stress responsive protein kinase [30], the *ATFBS1* responds to biotic and abiotic stress. *AtFBS1* interacts with SCF (Skp1− Cullin−F-box protein) complex that binds the F-box [31]. Interestingly, well known R-gene belonging to TIR-NUCLEOTIDE BINDING SITE FAMILY 10 was found induced upon PpBP25 indicating elevated level of plant defense triggered due to bacterial colonization [35–37]. Plant transcription factors such as *RAP2.4* was found induced due to endophytic colonization by PpBP25. Constitutive over-expression of *RAP2.4* results in defective plant developmental processes such as hypocotyl elongation and gravitropism, apical hook formation and cotyledon expansion, flowering time, root elongation, and root hair formation [38]. Yet another candidate gene found up-regulated upon endophytism is *MOS1*, the MODIFIER OF SNC1, that

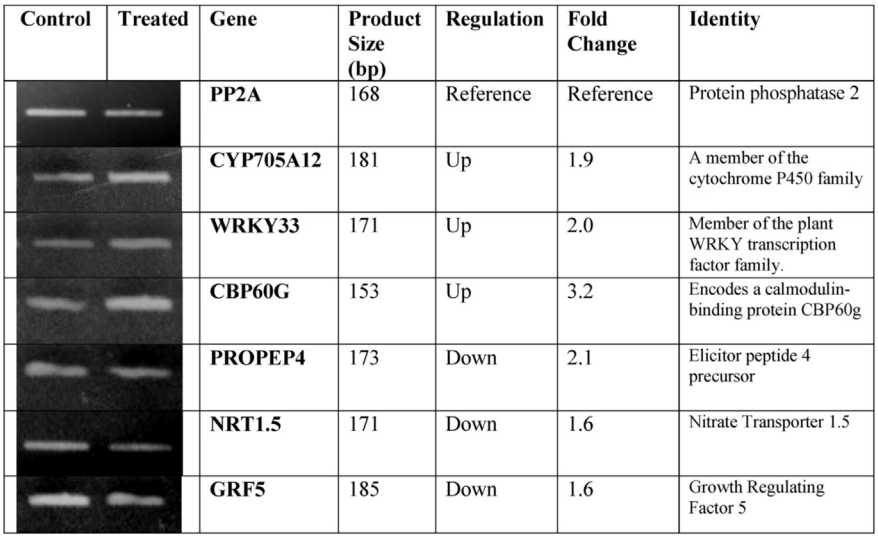

Fig. 5. Quantitative RT-PCR validation of genes showing differential expression in *Arabidopsis thaliana* Col 0 upon endophytic colonization. Note: Relative increase or decrease in expression of selected genes as observed in the intensity of amplicon confirmed the microarray data.

epigenetically regulates the expression of plant resistance gene SNC1 [39]. *ANAC036*, a member of the *Arabidopsis* NAC transcription factor family, when over-expressed in transgenic plants showed semi dwarf phenotype. It is possible that over expression of *ANAC036* gene leads to excessive stress responses, subsequently compromising cell growth [46]. Over expression of the OsNAC6-gene induced up-regulation of PR genes and growth retardation of plants [47]. The endophytic colonization seems to trigger other plant stress responsive genes including certain abiotic stress responders as evident from up regulation of *AT-HSFB2B* and *DREB2A*. While *HSFB2B* positively regulates acquired thermo tolerance [32], *DREB2A* is reported to regulate drought stress–responsive gene expression, which enhances drought stress tolerance in plants [33–34].

Collectively these data suggest that over expression of defense related genes & their pathways and a consequent skewed metabolic shift towards plant immunity could be a possible explanation for seedling growth inhibition triggered by PpBP25. The phytohormones SA and jasmonic acid are major players in the regulation of induced plant defenses and their associated fitness costs on growth and development. Constitutively active SA or jasmonic acid pathways exhibit a stunted phenotype in *Arabidopsis* [48]. Induction of defense responses requires energy [49], and photoassimilates serve as a carbon source in the synthesis of defense compounds [50]. This causes an increased demand for photosynthesis in the plant [51]. It is further reported that activation of defense by PAMPs leads to a rapid decrease in non-photochemical quenching [52]. Colonization of PpBP25 not only triggered induction of several defense genes in the SA signaling pathway, but also repressed several defense related genes such as *AtbZIP3, BGLU34, HSFB4, MYB28* and *ZFHD* which further indicates that PpBP25 might have partially blocked certain immune responses for initial colonization and establishment [53–54]. Our data is clearly in agreement with several recent reports on the observation that hormone-controlled signaling pathways cross-communicate, providing the plant with a finely balanced molecular regulatory network that can contribute to both defense and growth.

4.2. Genes participating in growth and developmental processes were repressed

Genes participating in the developmental processes, transport of nutrient through transmembranes, transcription, cell organization and biogenesis, protein metabolism, growth regulating factors were found to be repressed. Repression of a number of development associated plant genes (*SLAH1, RBCX1, OFP2, AtGRF5, MEE23, MEE3, iqd21)* upon endophytic colonization by PpBP25 suggest that the physiological functions of the plants were affected and compromised. *SLAH1* (SLOW ANION CHANNEL-ASSOCIATED HOMOLOGUE 1) is known to participate in ion homeostasis in guard cells [55]. *ATRBCX1* encodes a chloroplast thylakoid localized RbcX protein that acts as a chaperone in the folding of Rubisco was found down regulated [56]. Transcriptional repressor like *OFP2* that regulate multiple aspects of plant growth and development was also found down regulated upon endophytic colonization [57]. Similarly, *PROPEP4* is a part of systemic defense response and specifically expressed in the tips of primary and lateral roots [58]. Significantly *AtGRF5* which positively influence cell division, chloroplast proliferation and photosynthetic capacity of the plant is found to be down regulated in plants colonized by PpBP25 which probably resulted in phenotypic alteration of *A. thaliana* especially reduction in leaf size [59]. Similarly expression of genes playing a role in nutrient transport such as *GDU1,* and *NRT1.5* were found affected. Repression of *GDU1* participating in nutrient transportation across the membranes is reported to show stunted plant growth [60]. Down regulation of *NRT1.5* might have enabled nitrate retention in roots and contributed to the stress tolerance [61–62]. The observation on repression of *ACO1* gene has been earlier reported to induce *Pseudomonas* mediated ISR by reduced ethylene (ETH) signaling [63].

5. Conclusions

Our gene expression data along with other published reports strongly suggest that PpBP25 mediated alteration in plant phenotype and internal regulation of endogenous population of PpBP25 in a feedback loop could be a consequence of elevated plant defense. Taken together it may be concluded that the interaction of plants with diverse microorganisms at rhizosphere, phyllosphere and endosphere during their growth and development can have a profound impact on the metabolic process. Each one of these interaction is potentially able to trigger a multitude of immune responses in plants wherein the metabolic balance between "**induced defense mediated survival**" and "**growth & development mediated productivity**" is vital for a plant's success.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pmpp.2016.01.008.

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