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Identification of Differentially Expressed Genes During *Pseudomonas fluorescens* Mediated Systemic Resistance in Cabbage

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Abstract: The study of microbial ecology and the microbial interactions with plants provides an insight into the biocontrol of plant diseases using antagonistic microbes. *Pseudomonas fluorescens* was used as a biological control agent against black rot disease caused by *Xanthomonas campestris* pv. *campestris*. The Suppression subtractive hybridization (SSH) was used to elucidate the differentially expressed genes in cabbage (*Brassica oleracea* var. *capitata*) upon the application of *Pseudomonas fluorescens.* A total of 140 expressed sequence tags (EST) were obtained. The analyses of these ESTs showed that many defense related genes like peroxidase, heat shock proteins, were upregulated. Many transcripts related to signalling pathways and pathogen recognition were identified. The important finding of the study is the identification of the unigene belonging to the SWEET protein family in cabbage. The study also resulted in the identification of 10 unigenes which possibly depict the interaction of *Pseudomonas fluorescens* in combating disease. These unigenes have been submitted to dbEST. The results show that those genes which are upregulated during pathogen attack are also induced upon application of *Pseudomonas fluorescens* indicating the possible mechanism of systemic resistance induced by *P. fluorescens* to combat disease.

Keywords: *Xanthomonas campestris* pv. *campestris*, RT-PCR, *Pseudomonas Fluorescens,* Suppression Subtractive Hybridization

1. Introduction

Cabbage (*Brassica oleracea* var. *capitata*) Pammel Dowson is an important vegetable grown worldwide. Black rot of cabbage has been a disease of global importance (Williams 1980). *Xanthomonas campestris* pv. *campestris* the causative agent of black rot disease, commonly enters the cabbage leaf through the hydathodes, and under favorable conditions spreads systemically in the xylem vessels throughout the plant. The intercostal's regions become chlorotic, and desiccated after they become surrounded by blackened veins (Sutton and Williams 1970). Biological control of plant diseases makes management of plant diseases less dependent on the use of high-risk chemicals, and it is environmentally friendly. Fluorescent pseudomonads, non-pathogenic rhizobacteria, are among the most effective biological control agents against soil-borne plant pathogens (Mishra and Arora 2012). *Pseudomonas fluorescens* is one of the most important biocontrol agents against certain seed and soil-borne plant pathogens. Several isolates of *Pseudomonas fluorescens*, *P. putida*, and *P. aureofaciens* suppress the soil-borne pathogens through rhizosphere colonization, antibiosis and iron chelation by siderophore production (Mishra and Arora 2012). Fluorescent pseudomonads promote plant growth by production of plant growth-promoting substances and thus are called plant growth promoting rhizobacteria (PGPR). PGPR are known to cause resistance against fungal, bacterial and viral diseases (Kurkcuoglu et al. 2007; Vanitha et al. 2009). Plant growth-promotion and activation of defense genes by PGPR application is an important strategy in plant protection. The potential of *P. fluorescens* in providing disease resistance and plant growth promotion has been proved in a variety of crops and pathogen interaction, as in sheath blight, sheath rot, blast of rice and bacterial blight of cotton (Nandakumar et al. 2001);

groundnut (Meena et al. 2000) and wilt disease of tomato (Ramamoorty et al. 2002). The induction of systemic resistance by the *Pseudomonas* strains was demonstrated in bean, carnation, rice, and cucumber (Alstrom 1991; Nandakumar et al. 2001). The strains of *Pseudomonas* spp. were found to cause resistance against various pathogens in cucumber (Wei et al. 1991) and radish (Hoffland et al. 1996). The use of *P. fluorescens* and its effect has been studied at the rhizosphere level, but very little is known about its effect in the phyllosphere of the host plant. In general, the phyllosphere of plants is a biocoenosis of different non-pathogenic micro-organisms, such as bacteria and fungi including yeasts, that colonize the host plant without causing significant morphological changes in the appearance of the plant. Positive results were achieved with *P. fluorescens*, which controlled bacterial wilt and also bacterial blight on potato in both field and laboratory trials (Beattie and Lindow 1995; Burr et al. 1996). A study by Singh et al. (2004) showed that the non-pathogenic bacterium *Pseudomonas fluorescens* Bk3 can suppress the conidial germination and the in vitro the mycelium growth of the pathogen *Venturia inaequalis* (Singh et al. 2004). The black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris* is difficult to manage. Black rot management includes the use of pathogen-free seed and transplants along with the use of resistant varieties, cultural practices and physical and chemical treatment. But in recent years the control of black rot in brassicas has been experimentally demonstrated with antagonistic bacteria (Massomo et al. 2004). *Pseudomonas* and *Bacillus* species are known to produce a variety of antimicrobial substances, and these may be involved in disease suppression (Schmidt et al. 2001). The potentiality of rhizobacteria in management of black rot was studied by using the *Pseudomonas fluorescens* KA19 strain isolated from soil and the secondary metabolites produced by them which control the disease development (Mishra and Arora 2012).

The present study describes the possible machinery of the host- *Brassica oleracea* var. *capitata* - *P. fluorescens* interaction. In order to accomplish the task both black rot resistant and susceptible cultivars of cabbage were selected, and gene expression studies were conducted upon challenge inoculation with black rot pathogen. Suppression subtractive hybridization was performed to target the differentially expressed genes before and after application of *P. fluorescens* upon challenge inoculation with *Xanthomonas campestris* pv. *campestris*.

2. Materials and Methods

2.1. Isolation of P. Fluorescens from Soil

The strains of *P. fluorescens* were isolated from native soil where cabbage were grown. The bacteria were isolated by adding approximately 1g of soil to 9 cm^3 of sterile distilled water. The solution was vortexed, allowed to settle for at least 20 min, and then vortexed again. The bacterial fraction was collected from the supernatant following centrifugation at 8000*g* and aliquots were plated onto King's B medium (King et al. 1956). The isolated *P. fluorescens* strains were further evaluated for their antagonistic activity by the dual culture technique. The identity of the isolate based on inhibition zone with the best antagonistic activity was confirmed by performing various tests specific to *P. fluorescens*. The isolate which showed very high inhibition zone was chosen for further studies (UOMPfu-5). The 48 h-old-cultures grown on King's medium B broth was centrifuged at 8000 *g* for 10 min. Inoculum was prepared by adjusting the bacterial concentration with sterile distilled water to 1×10^8 cfu cm^{-3} (Vanitha et al. 2009).

2.2. Plant Material

The black rot susceptible and resistant cultivars were chosen for the study based on our previous experiments. The seeds of black rot susceptible cultivar *Golden acre* and resistant cultivar *Pusa mukta* were sown in earthern pots (9cm dia) and seedlings were maintained under 80% relative humidity at 25°C and under 16 hour light/8 hour dark period before being inoculated with *P. fluorescens* (UOMPfu-5). Leaves were collected at 48 hours post inoculation (hpi). The leaves were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.3. Inoculation Studies

The seeds of the susceptible cultivar *Golden acre* were used in the present investigation. The 48h old culture of *P. fluorescens* grown on King's medium B broth was centrifuged at 8000 *g* for 10 min using bench top centrifuge. Inoculum was prepared by adjusting the bacterial concentration with sterile distilled water to 1×10^8 cfu cm^{-3} (Vanitha *et al*. 2009). The seeds were then pretreated with *P. fluorescens* (UOMPfu-5) by placing the seeds in a 100ml solution containing 20µl Tween 20 and the 1ml of *P. fluorescens* (UOMPfu-5) inoculum for 5h on a rotary shaker at 37°C and then the seeds were air dried and used for further experiments. The four week old seedlings were kept under 80% relative humidity, at 25°C and under 16 hour light/8 hour dark period before being inoculated with *Xanthomonas campestris* pv. *campestris* (UOMBT-6 isolate). The inoculum was prepared by growing the bacteria in nutrient broth incubated at 28±2°C for 36 h on a rotary shaker at 100 rpm. The final inoculum was prepared by adjusting the bacterial concentration with sterile distilled water to 1×10^8 CFU ml^{-1} at A610 nm using a UV-visible spectrophotometer (Hitachi U-2000, Tokyo, Japan). Four-week-old seedlings were inoculated by spraying the bacterial suspension. Plants were sampled 10 days after inoculation with the pathogen. Leaves were collected 48 hours post inoculation (hpi) inoculation. The leaves were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.4. RNA Isolation

Total RNA was isolated from the cabbage leaves harvested 48 h post inoculation (hpi) using the RNeasy Plant kit (Qiagen, France). RNA yield was determined by measuring the absorbance at 260nm and RNA integrity was checked by electrophoresing 3µg of total RNA through 1.2% agarose gel. Poly (A)+ RNA was purified from total RNA using mRNA purification kit (Bangalore Genei, Bangalore, India).

2.5. Suppression Subtractive Hybridization Studies

2.5.1. cDNA Library Construction and Amplification of cDNA Inserts

Suppression subtractive hybridization was performed as described by Diatchenko et al. (1996) using PCR select cDNA subtraction kit (BD biosciences Clonetech, Palo Alto, CA, USA) according to the manufacturer's instructions. The cDNA obtained from the susceptible cultivar *Golden acre* after the application of *P. fluorescens* 48 hpi was used as "tester" population and cDNA from the mock inoculated *Golden acre* cultivar was used as "driver" population. The efficiency of subtraction was analyzed by comparing cDNA abundance before and after subtraction by PCR using specific primers for the constitutively expressed cabbage specific 18S rRNA gene (Table 1). PCR amplification was performed using *Taq* DNA polymerase (Bangalore Genei, Bangalore, India) and 5µl aliquots were removed following determined numbers of PCR cycles. The amplified products were examined in 2% agarose gel. The differences in the number of cycles, which were needed to generate an approximately equal amount of the corresponding PCR product in subtracted and unsubtracted samples, served to indicate the subtraction efficiency.

2.5.2. Cloning of the Subtracted cDNA

The secondary PCR products were purified using the QIAquick PCR purification kit (Qiagen, France). The subtracted cDNA fragments were cloned into the pGEMT-easy using a pGEMT –easy cloning kit (Promega, USA) and transformed into *Escherichia coli* JM109 cells. Individual colonies containing recombinant plasmids were inoculated into 100µl Luria Bertani broth in 96 well microtitre plates. Cultures were grown overnight at 37°C with gentle shaking (100rpm). 100µl of 15 % glycerol was added to each of the wells and the microtitre plates were stored at -80°C until use.

2.5.3. Identification of Insert Size of cDNA by PCR Amplification

Sequencing of cDNA clones was performed using M13 primer. In order to correct the sequencing ambiguities, the sequences were edited by removing the plasmid and SSH adaptor sequences. The edited sequences were used to query the NCBI (National centre for Biotechnology Information, USA) databases using the blastX, blastN and dbEST algorithms. The cDNA were classified according to the E-values generated in the searches. Sequences were checked for stop codons to ensure that cDNA fragments represented a position of open reading frame.

2.6. Enzyme Assay

2.6.1. Preparations of Crude Enzyme Extract

The cabbage seeds of the susceptible cv. *Golden acre* were treated with *P. fluorescens* and subjected to seed germination (ISTA, 2014). The four week old seedlings were challenge inoculated with *X. campestris* pv. *campestris*. The seedlings were harvested at different time intervals of 6, 12, 24, 48 and 72 hpi respectively. One gram of cabbage seedlings was macerated to a fine paste in a pre-chilled mortar with 50 mM buffer (pH 8.8) (w/v; 1:1). One gram of cabbage seedlings was homogenized in 10 mM phosphate buffer (pH 6.0) in a prechilled mortar and pestle on ice (w/v; 1:1). The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C and the supernatant served as enzyme source for peroxidase (POX).

2.6.2. Peroxidase Assay

POX activities were measured at room temperature according to the standardized procedure of He et al. (2001). The POX assay reaction mixture contained 7.5 µL of 10 mM guaiacol in 50 mM sodium phosphate buffer (pH 6.0), 100 µL of crude extract, 792.5 µL of 10 mMsodium phosphate buffer (pH 6.0), and 100 µL of 600 mM $H2O_2$. The change in optical density at 470 nm was measured for 1 min using UV-Visible spectrophotometer (Hitachi U-2000, Tokyo, Japan). POX activity was calculated as change in absorbance units min^{-1} mg^{-1}.

2.7. Semiquantitative RT-PCR

Total RNA was isolated from the seedlings of susceptible cv. *Golden acre* using plant RNeasy kit (Qiagen). The mRNA was purified using mRNA purification kit (Bangalore Genei, Bangalore, India). One-step reverse transcription-PCR (RT-PCR) was performed using *M-MuLV* reverse transcriptase (BangaloreGenei, Bangalore, India) according to the manufacturer's instructions. First strand cDNA was diluted (1: 5) with RNase free water and used as a template for PCR. The RT-PCR primers were designed from the peroxidase unigene obtained from the analysis of EST library using primer3 software version 0.4.0 (Table 1).

Table 1. *Primers used for semi-quantitative PCR analysis.*

Gene	Forward Primer	Reverse Primer
18S rRNA	GCTACGCAGAAGACAG TTGAT	TGGGCACACGGAAG GACATAC
Peroxidase	ATGGCTGAGGAGTCTC CTC	TCCAGTAGAGTATCC TTCTCG

The primers were designed from the unigene of peroxidase and 18S rRNA obtained from the SSH library using primer3 software.

18S rRNA was used as endogenous control. Semiquantitative RT-PCR was carried out and the PCR cycling parameters consisted of 94°C for 2 mins, annealing at 59°C and 60°C (depending on the primer) for 1 min and extension at 72°C for 2 mins for 22 and 18 cycles respectively. The PCR products were checked on 1% agarose gel.

3. Results

3.1. Construction of Subtracted Library

Cabbage leaf samples were harvested 48 hpi and used to identify differentially expressed genes upon inoculation with *P. fluorescens* to susceptible cultivar *Golden acre*. A cDNA subtractive library was constructed wherein *P. fluorescens* inoculated leaf samples acted as the tester and uninoculated leaf samples as driver. The subtraction efficiency was evaluated by expression of the 18S rRNA between subtracted and unsubtracted cDNA's. The amount of 18S rRNA decreased significantly after subtraction and could be detected in agarose gel at 28 PCR cycles whereas in the unsubtracted samples 18S rRNA was detected at 18 PCR cycles. This indicated that the subtraction had worked well. Finally 1000 clones were obtained and 155 clones were randomly selected from the library and the insert size was detected by PCR with SSH primer provided in the subtraction kit. The 120 positive clones carrying single exogenous fragment were detected and the length of the inserted fragments ranged from 200bp to 700bp.

3.2. Analysis of EST Sequences

Table 2. *Identification of ESTs from the SSH library obatined from the cabbage-susceptible cultivar treated with the P. fluorescens*

Clone no.	BLAST/Similarity	Related Accession no
Metabolism and structural proteins		
Ga57	Ribulose phosphate	GQ184377.1
Ga14	Photosystem I	P06512
Ga74	Photosystem II	AY185358.2
Ga100	Sucrose synthase	AA41682
Ga092	4-hydroxy phenyl pyruvate	AF251665
Ga25	40S ribosomal proteins	AF144752.1
Ga19	Membrane proteins	U13631.1
Ga09	60S Ribosomal proteins	L21897.1
Defense related		
Ga99	WRKY transcription factor	GQ168839.1
Ga64	Super oxide dismutase	AF071112.1
Ga62	Cytochrome P450	AY029178.1
Ga19	Heat shock proteins	AAB97316.1
Ga 4	Glutathione Peroxidase	AF411209.1
Ga 1	Beta glucosidase	NP199277
Ga 99	MAP- kinase like protein	AAX96170
Ga 50	Peroxidase	GR723799.1
Signalling proteins		
Ga 3	Pyruvate dehydrogenase	JF682847.1
Ga 7	Phosphatase	FJ346565.1
Ga 5	Zinc finger protein	HM579881.1
Ga 24	ABC transporter like protein	DQ296184.1
Ga 45	Serine/threonine kinase gene	DQ375116

The ESTs obtained from SSH library were subjected to BLAST analysis and the functions were assigned based on homology.

The 120 unigenes obtained were classified into three major categories: Metabolism and structural proteins, Defense related, and Signalling pathway related. The metabolism related unigenes accounted for 32%, defense related (42%), Signalling pathway related (26%) (Fig. 1).

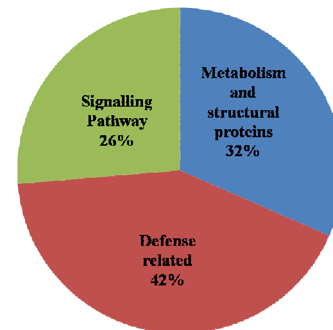

Fig 1. *Classification of ESTs obtained from the SSH library of the susceptible cultivar and assignment of putative biological functions based on homologies to sequences of known function identified by BLAST N analysis.*

All the unigenes were subjected to similarity search using BLASTX, BLASTN dbEST databases. Sequences showing the E-value < 10^{-3} were considered as significant. The most abundant group consisted of the defense related genes peroxidase, HSP70, superoxide dismutase were prominent which are predicted to play an important role in the resistance process upon pathogen attack followed by the metabolism and structural proteins category (Table 2).

Some of the unigenes like Jasmonate O-methyltransferase (Ga8), S-adenosylmethionine (Ga89), novel protein (Ga33) were found. The unigene Ga33 upon analysis was found to belong to SWEET class of protein. 10 unigenes have been submitted to dbEST with the accession numbers JZ585524 to JZ585533 (Table 3).

Table 3. *List of upregulated ESTs from the cabbage-susceptible cultivar after treatment with the P. fluorescens*

Clone No	dbEST id	Putative id
Ga10	JZ585524	Transcription factor
Ga8	JZ585525	Jasmonate O-methyltransferase
Ga89	JZ585526	S-adenosylmethionine
Ga5	JZ585527	Thionin
Ga21	JZ585528	Glutathione-S-transferase
Ga33	JZ585529	Novel protein (SWEET Protein)
Ga91	JZ585530	Histone
Ga122	JZ585531	Lipase
Ga1	JZ585532	Disease Resistance Protein
Ga6	JZ585533	Disease Resistance Protein

The ESTs obtained from the SSH library have been submitted to dbEST database

Since the peroxidase was very prominent in the study, the peroxidase assay was performed followed by the transcript accumulation study by semiquantitative RT-PCR.

3.3. Semiquantitative RT-PCR

The defense related unigene, peroxidase, showed high homology percentage and were selected to study their expression in resistant and susceptible cultivars during infection with *Xanthomonas campestris* pv. *campestris.* The transcript level was investigated at 48hpi. The genes were compared with the internal control being 18S rRNA. The 18S rRNA expressed in both cultivars. The expression of 18S rRNA was constitutively expressed as compared to peroxidase. The susceptible cultivar showed that upon treatment with *P. fluorescens* showed higher transcript accumulation showing which is evident in the enzyme assay (Fig 2).

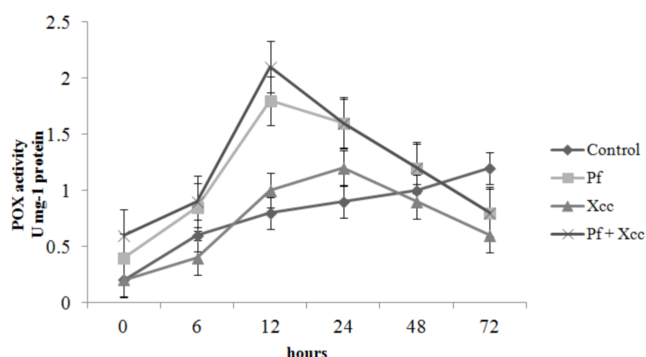

Fig 2. *Peroxidase activity of the susceptible cultivar. Pf+Xcc - P. fluorescens pre-treated cabbage seelings challenge inoculated with X. campestris pv. campestris; Xcc –cabbage seedlings inoculated with X. campestris pv. campestris; Pf - seedlings treated with P. fluorescens; C - control seedlings treated with distilled water. The means of three independent experiments, bars indicate SE.*

The resistant cultivar at 48 hpi showed better transcript accumulation upon challenge inoculation of the pretreated cabbage seedlings compared to the susceptible cultivar (Fig. 3).

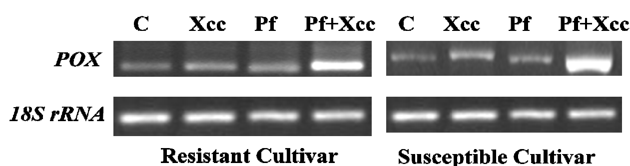

Fig 3. *Comparison of induced gene expression pattern of peroxidase gene and 18S rRNA in cabbage cultivars. Resistant cultivar – Pusa mukta. Pf+Xcc - P. fluorescens pre-treated seeds challenge inoculated with X. campestris pv. campestris; Xcc - seeds inoculated with X. campestris pv. campestris; Pf - seeds treated with P. fluorescens; C - control seeds treated with distilled water.*

4. Discussion

The phyllosphere represents a niche with great agricultural and environmental significance. The significant interactions of phyllosphere microbial inhabitants affect the fitness of natural plant populations, quality and productivity of agricultural crops. Phyllosphere bacteria can promote plant growth and both suppress the colonisation of infection of tissues by plant pathogens (Lindow and Brandl 2003).

The surfactants released by some of the epiphytic pseudomonads increase the wettability of leaf surfaces making it easier for microorganisms to use water and increasing solubilisation and diffusion of nutrients, thereby increasing substrate availability to epiphytic bacteria. The increased permeability of the cuticle enhancing water and nutrient availability in the phyllosphere have been shown by some bacteria colonizing the phyllosphere. (Hutchison et al. 1995).Bacteria multiplying inside the intercellular spaces of tissues can hardly be reached with chemicals or antibacterial substances, which have been used until now and caused hazard to human beings, animals, plants and the environment. When the disease occurs in clusters, overcoming it is in most cases unsuccessful. Therefore, we must not depend entirely on defensive elements but preventive measures should be taken first so that the pathogen can be kept far from the plant either by quarantine regulations or using healthy seeds. A considerable advantage of using antibiotics as opposed to other plant protective agents is that they have a real absorbing ability, and they translocate in tissues, which are considered significant with bacterial diseases.Bacteria being the most abundant inhabitants of the phyllosphere have received attention on the interactions occuring between bacteria on the leaves.. Studies of the composition of bacterial communities on leaves have been numerous but rather limited in scope. The phyllosphere is both scientifically and economically a critical habitat to study microbial ecology. Because of the importance of many phyllosphere microbial inhabitants to plant health, there will likely be many practical applications that result from a better understanding of the interactions of microbes with plants and among themselves. Thus, knowledge of phyllosphere offers to the field of microbial ecology that contribute to more efficient and less environmentally damaging means of plant protection. Lindow and Brandl (2003) suggested that presence of a functional type III secretion pathway in *Pseudomonas fluorescens* provides the capacity to modify the local habitat needed for growth and survival in the phyllosphere.

In the present study, majority of genes identified in the subtractive analyses were pathogen, disease or stress-related. For instance, S-adenosylmethionine (Ga89) has been shown to be involved in stress responses (Chen et al. 2010). Some of the other genes found in our study have been reported to encode pathogenesis related (PR) proteins. The DNA-binding protein WRKY reportedly plays an important role in defense responses to pathogens. The study suggests that a significant amount of genes have overlapping functions in both pathogen responses. Besides the well-characterized proteins with a function in plant defence, more recently, a few additional ones have been described. Amongst these are phospholipid-derived molecules with a function as novel, second messengers in

signal transduction pathways for plant defence. The SWEET family of plant transmembrane proteins was a significant finding of the present study. SWEET proteins were shown to transport glucose and sucrose across cell membranes, thus contributing to phloem unloading (Chen et al. 2010). A subset of SWEET genes in Arabidopsis and rice are transcriptionally induced during bacterial and fungal infection (Chen et al. 2010). The importance of these sugar transporters for disease susceptibility has been revealed in rice. The occurrence of the SWEET protein which is a transmembrane protein probably helps in inducing the interaction of the host phyllosphere upon application of *P. fluorescens*.In summary, application of the non-pathogenic bacterium *P. fluorescens* to the phyllosphere of cabbage cv. Golden acre leads to the up-regulation of a large number of transcripts. Several of these transcripts encode proteins/enzymes that are also implicated during infection with the pathogen black rot pathogen. Thus, it can be concluded that the expression of these proteins, initiated by the non-pathogenic bacterium, perhaps helps to cope against infection with a pathogen. The molecular mechanism of signal perception of *P. fluorescens* by the host plant is still elusive. A possible candidate for an elicitor of the plant defence reaction could be flagellin that is released by *P. fluorescens* (Singh et al. 2004) and which is well known as a signal molecule (Zipfel et al. 2004)

In the very early stages of the plant defence, reactive oxygen species (ROS) are formed to prevent or inhibit pathogen infection. This oxidative burst requires a plasma membrane-located NADPH oxidoreductase that generates hydrogen peroxide, which can destroy the infected tissue and avoids the spreading and multiplication of pathogens. On the other hand, it is important that the uninfected tissues are protected from damage by ROS. Therefore, it is not surprising that the results of the experiments undertaken reveal that a substantial number of transcripts that encode enzymes or proteins, which have a role in the adaptation process to oxidative or more general stress, are up-regulated after the application of the non-pathogenic bacterium to keep the fungus under control. It is clear from the present studies that the defense-related enzymes are up-regulated. Hence, the regulated levels of defense-related enzymes can be effectively used to screen for host resistance.

POX is a key enzyme in the biosynthesis of lignin. Increased activity of cell wall bound peroxidases have been elicited in different plants due to pathogen infection. In our study, POX activity increased. Increased activity of POX has been elicited by *P. fluorescens* in various plants such as rice (Nandakumar et al. 2001), tomato (Ramamoorthy et al. 2002) and mulberry (Ganeshmoorthi et al. 2008). In conclusion, the present study proved that P. fluorescens induced resistance against *X. campestris* pv. *campestris* in cabbage seedlings is associated with the enhanced expression of genes for defense-related protein. The significant finding of the study was the identification of the SWEET protein unigene in cabbage upon interaction with *P. fluorescens*.

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