**Tropical Agricultural Research Vol. 25 (2): 240 – 251 (2014)**

# Identification of a Set of Up-regulated Genes due to Infection by *Colletotrichum musae* in a Dessert Banana Cultivar, Moderately-Resistant to Anthracnose

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***ABSTRACT*:** *Understanding the mechanisms of host plant resistance at molecular level is useful towards designing effective disease management programmes against infections of plant pathogens. The present study was conducted to identify host plant genes expressed in response to infection by Colletotrichum musae using a moderately-resistant cultivar of banana (Seenikesel) to anthracnose. The expression profile of a selected defense-related gene over the infection process in the banana cultivar Seenikesel was also determined. Identification of genes was done by differential hybridization of a cDNA library constructed from peel tissues of banana inoculated with C. musae. Up-regulated genes due to C. musae infection were predicted through DNA homology search. Among the identified putative defense related genes, expression profile of Germin-like protein was studied by relative RT-PCR over a period of 144 h after inoculation. The set of up-regulated gene transcripts identified in the present study were responsible for plant defense, stress response, growth, development, protein synthesis, protein folding and stabilization. Expression of Germin-like protein gene homologue was comparatively high at the late phase of inoculation (96 h after inoculation) of C. musae in banana cultivar Seenikesel.*

***Keywords:*** *cDNA library, differential hybridization, Germin-like protein, relative RT-PCR*

## INTRODUCTION

Banana including plantain (*Musa* spp.) have been ranked as the fourth important food crop and the second important fruit crop in the world (FAO Stat, 2007; Lassois *et al*., 2010). In Sri Lanka, banana and plantains are widely consumed as a dessert fruit and a vegetable fruit, respectively. Although it is predominantly produced for the local market of Sri Lanka, banana has been identified as a crop with a high potential to contribute to the national economy through export earnings (De Costa & Kalpage, 2006).

Bananas are subjected to numerous infectious diseases which can lead to severe pre- and postharvest losses in terms of quality and quantity (Ma *et al*., 2009). Anthracnose caused by *Colletotrichum musae* (Berk. and Curt.) is one of the most important diseases infecting banana at postharvest stage (Abd-Elsalam *et al*., 2010; Wardlaw, 1934). Symptoms of anthracnose on banana occur as peel blemishes and black or brown sunken spots of various

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sizes on fruits. The symptoms initially develop on the fruit peel and later can get extended into the fruit pulp of ripe banana. However, previous investigations have revealed that the dessert type banana cultivars available in Sri Lanka have a variation in the degree of symptom development (De Costa *et al*., Unpublished data). This variation could be due to incompatibilities between the host and the pathogen, which cause a failure in the pathogen to become successful. There could be three reasons for the failure of the pathogen, *i.e*. the plant is unable to fulfill the niche requirements of the potential pathogen hence it is a non-host, the plant possesses preformed structural or biochemical defense mechanisms that prevent colonization of the pathogen (Shibuya & Minami, 2001) or plant defense mechanisms are activated upon the recognition of the pathogen to contain infection (Hammond-Kosack & Jones, 1996). A detailed understanding of the basis of host resistance at genetic and molecular level would be an essential initial step towards effective disease management and crop improvement against plant pathogens.

The objectives of the present study were to identify genes expressed in host plant in response to infection by *Colletotrichum musae* using a moderately-resistant cultivar of banana and to determine the expression profile of a selected defense-related gene over the infection process in the banana cultivar moderately-resistant to anthracnose development.

## MATERIALS AND METHODS

### Collection of banana fruits and inoculation by *C. musae*

Well-grown and fully-matured, (*i.e*. at Maturity Index 1 according to the standard charts by Kader, 2005) banana bunches of cultivar *Seenikesel* (ABB), which were free from external blemishes were collected randomly from three semi-commercial plantations in Kandy, Sri Lanka. In the field, the banana bunches were covered with plastic sleeves to avoid natural inoculation of *C. musae*. The fruits were detached from different hands or bunches were randomly assigned to two sets of treatments, namely 'inoculated' and 'non-inoculated' (control). Peel of banana fruits of 'inoculated' sample were injected with an aliquot of 10 µl ($1 \times 10^5$ spores $ml^{-1}$) of *C. musae* spore suspension. Spore suspension of *C. musae* was prepared by vortexing spore masses obtained from a pure culture of the fungus grown on PDA and enumerating the spore concentration by a haemocytometer. Two spots per fruit were inoculated in thirty fruits and inoculated fruits were incubated in incubation chamber at room temperature (28 $^{o}$C) and 90% RH. The banana fruits kept as 'control' were inoculated with 10 µl of sterilized distilled water and incubated as given above.

### Preparation of cDNA library

### Harvesting of the banana fruit peel tissues

Peel tissues were collected from the immediate surroundings of the point of inoculation one hour before inoculation with *C. musae* spores suspension. Similarly, peel tissues were collected from the inoculated fruits 1 h, 24 h, 48 h, 96 h, 144 h, and 192 h after the inoculation. Three fruits were used at each harvesting time. Peel tissues were collected at different time intervals before and after inoculation of *C. musae* to determine the sequence of gene expression along different time intervals, just before and also from the onset of inoculation till symptoms were developed. Peel tissues collected at different time intervals were ground separately into a fine powder, with an adequate amount of liquid nitrogen in a

0.1% Diethyl-Phyrocarbonate (DEPC) treated motor and pestle and stored at -80 $^{o}$C until further use.

## Construction of cDNA library

Total RNA was extracted separately from peel tissues of banana collected at different time intervals (*i.e*. 1 h before inoculation and 1 h, 24 h, 48 h, 96 h and 144 h and 192 h after inoculation of *C. musae)* using the method as follows. Extraction buffer contained 100 mM Tris-Borate (pH 8.2), 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB and 0.1% β mercaptoethanol. 0.1% β mercaptoethanol was added into the extraction buffer prior to the pre-warming of the buffer at 65 $^{o}$C for 30 min. An aliquot of 1 ml of pre-warmed extraction buffer was added to each of the eppendorf tube containing powdered banana peel tissues, mixed thoroughly by vortexing and incubated at 65 $^{o}$C for one hour. Samples were cooled to room temperature (28 $^{o}$C) and centrifuged at 12000 rpm for 15 min at room temperature. The supernatant was collected into new eppendorf and extracted twice with chloroform:isoamyl alcohol (24:1). After centrifuging at 12000 rpm for 15 min at room temperature, the supernatant was collected into new eppendorf tube and mixed with 10 M LiCl to a final concentration of 3 M. Then, the content was incubated overnight at 4 $^{o}$C to precipitate total cellular RNA which was recovered by centrifuging at 14000 rpm for 20 min at 4 $^{o}$C. The supernatant was decanted carefully and the pellet was dissolved in 500 µl of DEPC treated water and extracted thrice with water saturated phenol, phenol: chloroform (1:1) and chloroform:isoamyl alcohol (24:1) respectively. The supernatant was then mixed with 1/30 volume of 3 M sodium acetate (pH 5.2) and 0.1 volume of absolute alcohol, and kept on ice for 30 min before centrifuging at 14000 rpm for 25 min at 4 $^{o}$C. The supernatant was collected carefully without disturbing the white jelly like precipitate, and mixed with 3 M sodium acetate (pH 5.2) to a final concentration of 0.3 M and 3x volume of absolute alcohol. The mixture was incubated at -80 $^{o}$C overnight and RNA was recovered by centrifuging at 14000 rpm for 20 min. at 4 $^{o}$C. The RNA was rinsed with 70% ethanol and then recovered by centrifuging at 10000 rpm 5 min at 4 $^{o}$C. Ethanol was removed by air drying. Then the pellet of RNA was dissolved in 10 µl of DEPC treated water and was treated with DNAse (1u/µl) (Promega, RQ-1, DNAse).

Dynal beads and the paramagnetic particle technique (Dynal Biotech ASA, Norway) was used to extract mRNA from the total RNA samples. Those mRNA obtained from different time intervals after inoculation of the pathogen were pooled together and used for cDNA library construction. Pooling of mRNA was done to collect mRNA transcripts covering a range of time interval starting immediately after inoculation until a later stage of the symptom development. Construction of cDNA library was done using pDONR$^{TM}$222 vector of CloneMiner$^{TM}$ cDNA library construction kit (Catalog No. 18249-029, Invitrogen, USA) based on Gateway$^{R}$ technology.,

## Obtaining of cDNA colonies

After electroporation to *E. coli* host cells (ElectroMAX $^{TM}$ DH10B), 25 µl of the cDNA library was diluted with 175 µl of sterilized distilled water and plated on LB agar medium supplemented with Kanamycin (50 µg/ml) and incubated at 37 $^{0}$C for overnight. Well-isolated single colonies were transferred on to a grid pattern on LB agar plate supplemented with Kanamycin (50 µg/ml). Each clone was designated with a number and incubated at 37 $^{0}$C overnight.

**Preparation of colony blots**

Pieces of nylon membranes (Amersham Hybond $N^+$, GE Healthcare,UK) were laid on LB solid medium supplemented with Kanamycin (50 µg/ml) under aseptic conditions. A set of cDNA clones (*i.e*. 384 cDNA clones) were arrayed on four pieces of sterilized Nylon membranes and incubated overnight at 37 $^0$C.

**Differential hybridization**

Differential hybridization was done using Digoxygenin-labeled total cDNA probes to determine up- and down-regulated genes in cultivar *Seenikehel* due to *C. musae* infection. Two different probes were prepared from total cDNA synthesized from the peel tissues of banana inoculated with *C. musae* (*i.e*. Treated probe) and non-inoculated with *C. musae* (*i.e*. Control probe). Labeling of the total cDNA was done according to instruction of the manufacturer (Dig DNA labeling and detection kit - Catalog No. 11 093 657 910, Roche, Germany).

Detection of the signals after nucleic acid hybridization was done by chemiluminesence method on X-ray films (DIG Luminescent Detection Kit-Cat No.11 363 514 910, Roche, Germany). cDNA clones with up and down-regulated genes were identified by comparing the signal intensity developed on X-ray films. Briefly, cDNA clones which were having an up-regulated gene in response to *C. musae* inoculation were shown higher signal intensity when hybridized with the treated probe (*i.e*. prepared from banana peel tissues inoculated with *C. musae*) than the signal intensity given by a control probe (*i.e*. non-inoculated). In contrast, cDNA clones which were having down-regulated genes in response to *C. musae* inoculation were shown lower signal intensity when hybridized with the treated probe than the signal intensity given by that hybridized with control probe.

**DNA sequencing and homology search**

cDNA clones consist with up and down-regulated genes were subjected to PCR amplification using M13 universal primers. The vector pDONR$^{TM}$222, which was used for the cDNA library construction contained M13 forward and reverse priming sites proximal to *att*$L_1$ and *att*$L_2$ regions, respectively. These amplified PCR products were sequenced and subjected to homology search using available DNA/Protein data bases (*i.e*. NCBI-BLAST and Esttik-BLAST- CIRAD) to predict putative genes and protein products of the cloned transcripts.

**Relative RT- PCR**

Relative RT-PCR was selected as a method of confirming gene expression (Dean *et al*., 2002; Chelly & Kahn, 1994). RPS-2 gene was selected as an internal control for relative RT-PCR as recommended by Chen *et al.,* (2011) for gene expression studies of banana fruit. In addition to RPS-2, EBF-1 gene was also used to normalize the gene expression in banana fruit peel (Binder *et al*., 2007). One of the up-regulated gene transcripts, cloned in cDNA and designated as Ma SINI 1407 in the present study, was selected for confirmation of gene expression by relative RT-PCR. According to DNA sequencing and subsequent homology search, the transcript harboured by Ma SINI 1407 was identified as a gene homologous to Germin-Like-Protein (GLP) produced in *Musa acuminata*. Primer pair for amplifying cDNA fragment contained in Ma SINI 1407 clone was designed by Primer 3 software based on sequence information obtained for the gene transcript of Ma SINI 1407 in the present study.

The base pair sequences of the forward and reverse primers were 5'-TGCTTGTCCAAAGACAGTGC-3'and 5'- GCTTGTCTAAGCCGGTGAAG-3', respectively. The forward and reverse primers used for amplification of the internal control/reference gene of RPS-2 were 5'-TAGGGATTCCGACGATTTGTTT-3' and, 5'-TAGCGTCATCATTGGCTGGGA-3', respectively and the forward and reverse primers used for amplification of the internal control/ reference gene of EBF-1 were 5'-CCTCAATAAGCGATTACGGTG-3' and 5'- TCAGACTCCCAAGAGATTCC-3' respectively (Chen *et al*., 2011). The template cDNA having the same concentration for control and treated samples (1000 ng/ µl) was used for the PCR amplification. PCR amplification was performed separately targeting the reference genes (*i.e*. RPS-2 and EBF-1) and Germin Like Protein Gene (GLP) homologue using cDNA samples prepared from inoculated and non-inoculated peel tissues collected at different time intervals after inoculation of *C. musae* as described earlier. PCR reaction conditions for the two reference genes (*i.e*. RPS-2 and EBF-1) and gene of interest (*i.e*. GLP) included an initial denaturation at 95 $^0$C for 2 min, denaturation at 95 $^0$C for 30 sec, annealing at 60 $^0$C for 30 seconds elongation at 72 $^0$C for 2 min and final extension at 72 $^0$C for 5 min with 40 reaction cycles. The expected sizes of the PCR products of RPS-2 and EBF-1 are 84 bp and 280 bp, respectively.

**Analysis of RT-PCR products and quantification of relative gene expression of GLP gene homologue**

RT-PCR products were separated on 2 % agarose gel and gel documentation was done by model Canon Utilities Zoom browser EX (Version 5.2 Copyright CANON INC 1996-2005). Gel photos were scanned and band intensity of each RT-PCR product was measured using an image digitizing software (Gel UN-Scan-IT software Silk Scientific Inc., USA). Relative GLP gene expression level was calculated by dividing the expression of gene of interest (*i.e.* GLP) by the expression of reference gene (RPS-2 or EBF-1) according to the method described by Dean *et al*. (2002). The relative intensity was determined based on the intensities of the reference gene and the gene of interest given by the gel image. The average pixel values for band intensities for the reference gene and the gene of interest were obtained by the Gel UN-SCAN-IT software.

## RESULTS AND DISCUSSION

**Identification of differentially-expressed genes, responsive to *C. musae* infection in banana (cultivar *Seenikesel*)**

Identification of differentially-expressed genes was performed according to the intensity of the signal intensity on X-ray films (Fig. 1) after subjecting to the differential hybridization with treatment probes (inoculated with *C. musae*) and control probes (not-infected with *C. musae*). In the present study, 20 cDNA clones were identified and selected as up-regulated clones since they gave high signal intensities by treatment probe in comparison to the control probe. Differential hybridization resulted in 21 down-regulated cDNA clones which showed high signal intensities by control probe in comparison to treatment probe. However, focus in the present study was only on the up-regulated clones.

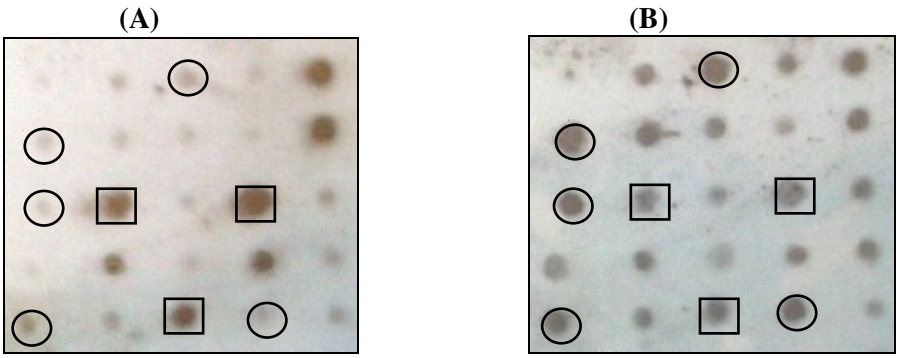


**Fig. 1. Signals marked with ▭ shape are up-regulated clones according to the signal intensity; signals marked with ◯ shapes are down-regulated clones according to the signal intensity**

**Analysis of DNA sequence of PCR products and homology search**

As depicted by the size of the PCR products, it was clear that the selected cDNA clones were carrying cDNA inserts of heterogeneous lengths (Fig. 2) which could be considered as a desirable feature of the cDNA library constructed in the present study.

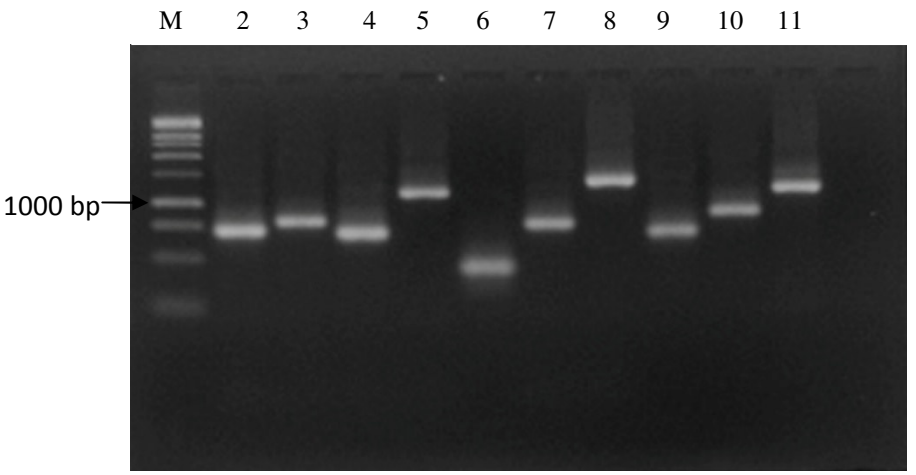


**Fig. 2. PCR products amplified by M13 primers separated on 2% agarose gel and stained with Ethidium Bromide. Lane M: 1kb DNA size marker (Promega, USA), lanes 2-11: PCR products amplified from cDNA clones 1093, 1101, 1233, 1247, 1259, 1272, 1336, 1340, 1354 and 1407, respectively.**

Moreover, the cDNA inserts were larger than 500 bp. Therefore, these cDNA fragments were suitable for sequencing and subsequent homology search for identification of putative genes. The amplified PCR products were subjected to DNA sequencing (Macrogen, Korea) and homology search was done using available DNA databases to predict putative genes and protein products after vector screening by Vecscreen software (NCBI, USA). The sequence

information for 20 cDNA clones were deposited in EMBL Gene Bank (EMBL Gene Bank, European Nucleotide Archive-ENA, EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK) according to the EMBL Gene Bank instructions. Accession numbers obtained for the sequences from EMBL Gene Bank are given in Table 1.

**Table 1. DNA homology search results of the selected up-regulated genes in response to *C. musae* infection of banana (cultivar *Seenikesel*)**

| cDNA Clone Code | Gene/protein homology | Homologous host | EMBL accession number | Identity | E-value |
|---|---|---|---|---|---|
| Ma SINI 1093 | Stress induced proteins | *Musa* spp. (ABB) | HF567786 | 95.70% | 1.80E-46 |
| Ma SINI 1101 | Stress induced proteins | *Musa* spp. (ABB) | HF567787 | 99.80% | 3.70E-38 |
| Ma SINI 1233 | Putative 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase | *Ginkgo biloba* | HF567788 | 81% | 7.00E-59 |
| Ma SINI 1247 | Pectate lyase 1 | *Musa acuminate* | HF567789 | 94.20% | 7.60E-72 |
| Ma SINI 1259 | Eukaryotic elongation factor 1A | *Bruguiera sexangula* | HF567790 | 97% | 0 |
| Ma SINI 1272 | Stress induced protein | *Musa* spp. (ABB) | HF567791 | 96.90% | 7.30E-61 |
| Ma SINI 1336 | Germin-like protein | *Musa acuminata* | HF567793 | 94 .1% | 3.00E-124 |
| Ma SINI 1340 | Aspartic protease | *Hordeum vulgare* sub spp. *vulgare* | HF567794 | 79% | 1.00E-126 |
| Ma SINI 1354 | â,1,3-Glucanase | *Musa* (ABB) | HF567797 | 95.40% | 2.80E-78 |
| Ma SINI 1407 | Germin-like protein | *Musa acuminata* | HF567801 | 94% | 6.00E-103 |

Homology search with available DNA/ Protein databases indicated that the EST sequence information were highly homologous with *Musa* genome with high percentage identities and E- values.

Fig. 3 illustrates the percentage composition of the up-regulated genes according to functional categories of the genes.

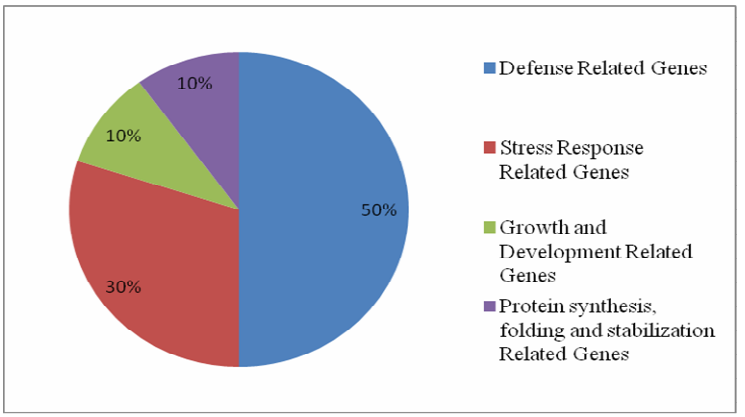


**Fig. 3. Percentage distribution of up-regulated gene transcripts based on functional categories**

Majority of the up-regulated transcripts identified (50%) due to *C. musae* infection in banana cultivar *Seenikesel* belonged to defense related genes which are members of the general plant defense responses induced in many different plant-pathogen systems (Patricia *et al*., 2009). The second major group (30%) of up-regulated transcripts were stress induced protein (SIP) related genes in *Musa* ABB genome. Moreover, some of the other up-regulated transcripts were homologous with growth and development related genes, and protein synthesis, folding and stabilization related genes (Fig. 3).

Transcripts β-1, 3 glucanase mRNA complete DNA sequence (cds), GLP [*Musa acuminata*] and putative 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase and aspartic protease identified in the present study were categorized under the functional group of 'Defense'. Germins and GLPs participate in many processes that are important for plant development and defense (Patricia *et al*., 2009; Patnaik & Khurana, 2001). Defense mechanisms also include production of lytic enzymes such as chitinases and β-1, 3-glucanases which degrade chitin and glucan present in the cell wall of fungi (Attia *et al*., 2011). By degrading the cell wall it will reduce the growth of fungal hyphe of infecting fungal pathogens, within the host plant cell.

Stress induced proteins (SIPs) play a major role in defending plants from different stress conditions due to various biotic and abiotic stress factors. It is now well established that virtually all biotic and abiotic stresses induce or involved in oxidative stress to some degree, and the ability of plants to control oxidant levels is highly correlated with stress tolerance (Cheeseman, 2007). In contrast, growth and development related genes and protein synthesis, folding and stabilization related genes also mediate and enhance the plant defense mechanism to protect the plant against pathogenic invasions (Wegener *et al*., 1996).

### Analysis of RT-PCR products and quantification of relative gene expression of GLP gene homologue

RT-PCR amplification of the GLP gene homologue and reference gene (EBF-1) under inoculated and non-inoculated conditions after different inoculation time intervals are demonstrated by Fig. 4.

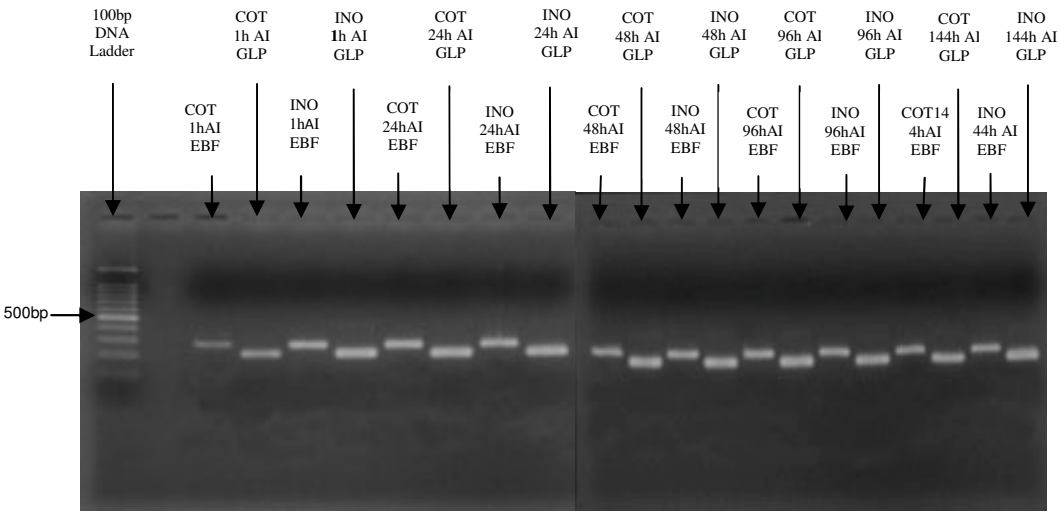


**Fig. 4. RT-PCR amplified products of the GLP gene homologue and reference gene (EBF-1) under inoculated and non-inoculated (control) conditions after different time intervals of inoculation (COT= control (non-inoculated), INO= (inoculated with *C. musae*), AI = after inoculation)**

The RPS -2 and EBF-1 genes were used to normalize the GLP gene expression in the present study. It is essential to normalize the gene expression analyzed by RT-PCR using one or more internal controller genes. RPS-2 gene was validated as the most suitable reference genes for RT-PCR analysis in banana (Chen *et al*., 2011). Relative gene expression of GLP gene at different time intervals were calculated based on EBF-1 and RPS-2 gene expression at each time interval after inoculation (Tables 3 and 4).

**Table 3. Relative expression levels of homologous gene encoding Germin Like protein (GLP) under control and inoculated conditions at different time periods with reference to EBF-1 gene**

| Time after inoculation (h) | Average pixel values | | | | Relative expression | |
|---|---|---|---|---|---|---|
| | EBF- 1 gene (control) | EBF-1 gene (inoculated) | GLP homologue (control) | GLP homologue (inoculated) | GLP homologue (control) | GLP homologue (inoculated) |
| 1 | 20.87 | 44.36 | 20.5 | 42.48 | 0.98 | 0.95 |
| 24 | 47.92 | 46.78 | 41.83 | 41.55 | 0.87 | 0.88 |
| 48 | 22.51 | 26.96 | 23.19 | 23.41 | 1.03 | 0.86 |
| 96 | 28.04 | 24.34 | 14.88 | 11.5 | 0.53 | 0.47 |
| 144 | 20.66 | 19.64 | 17.06 | 21.42 | 0.82 | 1.09 |

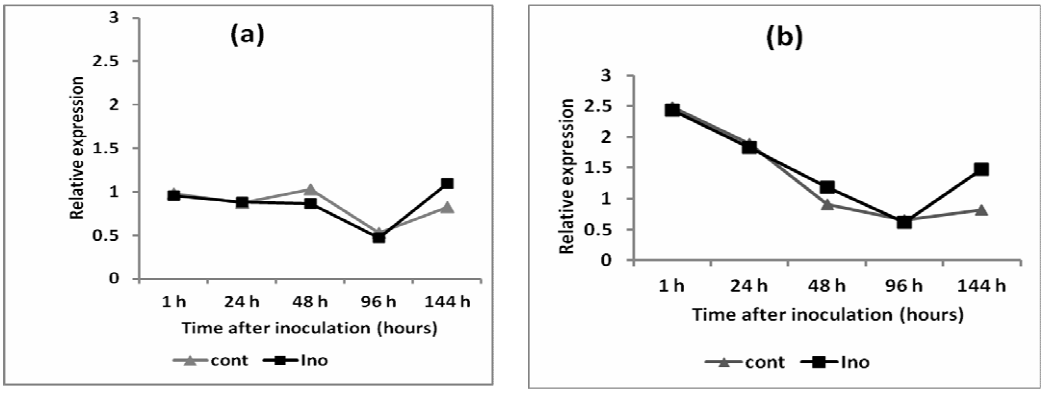


**Fig. 5. Relative expression levels of Germin Like protein (GLP) gene homologue under control and inoculated conditions at different time periods with reference to, (a) EBF-1 and (b) RPS-2**

**Table 4. Relative expression levels of homologous gene encoding Germin Like protein (GLP) under control and inoculated conditions at different time periods with reference to RPS-2 gene**

| Time after inoculation (h) | Average pixel values | | | | Relative expression | |
|---|---|---|---|---|---|---|
| | RPS-2 gene (control) | RPS-2 gene (inoculated) | GLP homologue (control) | GLP homologue (inoculated) | GLP homologue (control) | GLP homologue (inoculated) |
| 1 | 8.26 | 17.47 | 20.5 | 42.48 | 2.48 | 2.43 |
| 24 | 22.13 | 22.6 | 41.83 | 41.55 | 1.89 | 1.83 |
| 48 | 25.26 | 19.63 | 23.19 | 23.41 | 0.91 | 1.19 |
| 96 | 22.68 | 18.37 | 14.88 | 11.5 | 0.65 | 0.62 |
| 144 | 20.78 | 14.49 | 17.06 | 21.42 | 0.82 | 1.47 |

Based on relative RT-PCR analysis, it was clear that relative expression of GLP homologue was slightly equal to the relative expression of the GLP under non-inoculated conditions, especially at the early phase of infection. However, after a gradual decrease of the level of relative expression, a marked increase of the gene homologue could be identified in inoculated plants especially during the late phase of infection of *C. musae* (*i.e.* 96 h after inoculation) (Fig. 5).

Several research evidence suggest that GLPs are involved in general plant defense responses (Lane, 2002; Patnaik & Khurana, 2001), including the observation that expression of certain GLPs is enhanced after infection with pathogens, feeding of insects, or application of chemicals such as salicylic acid, hydrogen peroxide ($H_2O_2$), or ethylene (Zimmermann *et al*., 2006; Godfrey *et al*., 2007). Transient over expression of certain barley GLP subfamilies resulted in enhanced resistance to the powdery mildew fungus, and for some subfamilies, silencing resulted in enhanced susceptibility to the pathogen (Zimmermann *et al*., 2006). Accordingly, though GLP homologue is not the only reason for host plant resistance against anthracnose development in cultivar *Seenikesel*, it is clear that it could have a contributory

effect on the lower spreading rate of the symptoms which is generally found in cultivar *Seenikesel.*

## CONCLUSION

The present study identified 20 up-regulated and 21 down-regulated cDNA clones by differential hybridization of a cDNA library of banana (cultivar *Seenikesel*) infected with *C. musae*, the causal organism of anthracnose. Among the selected up-regulated cDNA clones, transcripts responsible for defense, stress response, growth, development, protein synthesis, protein folding and stabilization related genes were identified. Expression of GLP gene homologue (one of the defense related gene homologue) was comparatively higher in cultivar *Seenikesel* at the late phase of inoculation (96 h after inoculation) of *C. musae*.

## REFERENCES

Abd-Elsalam, K.A., Roshdy, S., Amin, O.E. and Rabani, M. (2010). First morphogenetic identification of the fungal pathogen *Colletotrichum musae* (Phyllachoraceae) from imported bananas in Saudi Arabia. Genetics and Molecular Research. *9(4),* 2335-2342.

Attia, M., Awad, N.M., Azza-Sh. and Hamed, T.H.A. (2011). Induction of defense responses in soybean plants against *Macrophomina phaseolina* by some strains of plant growth promoting rhizobacteria. Journal of Applied Sciences Research. *7(11),* 1507-1517.

Binder, B.M., Walker, J.M., Gagne, J.M., Emborg, T.J., Hemmann, G., Bleecker, A.B. and Vierstra, R.D. (2007). The *Arabidopsis* EIN3 binding F-box proteins EBF1 and EBF2 have distinct but overlapping roles in ethylene signaling. The Plant Cell. *19(2)*, 509-523.

Cheeseman, J.M. (2007). Hydrogen peroxide and plant stress: a challenging relationship. Plant Stress. Global Science Books. 4-15.

Chelly, J. and Kahn, A. (1994). RT-PCR and mRNA quantitation. In: Mullis, D.B., Ferre, F. and Gibbs, R.A. (Eds), The Polymerase Chain Reaction. Birkhauser, Boston, MA. pp97-109.

Chen, L., Zhong, H., Kuang, J., Li, J., Lu, W. and Chen, J. (2011). Validation of reference genes for RT-qPCR studies of gene expression in banana fruit under different experimental conditions. Planta. *234*, 377-390.

Dean, J.D., Goodwin, P.H. and Hsian, T. (2002). Comparison of relative RT-PCR and northern blot analyses to measure expression of -1, 3-Glucanase in *Nicotiana benthamiana* infected with *Colltotrichum destructivum.* Plant Molecular Biology Reporter. *20*, 347-356.

De Costa, D.M. and Kalpage, M.D. (2006). Differential response of *Colletotrichum* isolates infecting banana to synthetic fungicide and a biological control agent. Sri Lankan Journal of Agricultural Sciences. *43*, 50-65.

FAO Stat. (2007). [online]. [Accessed on 06.07.2012]. Available at http://faostat.fao.org/. htm.

Godfrey, D., Able, A. and Dry, I. (2007). Induction of a grapevine germin-like protein (VvGLP3) gene is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? Molecular Plant Microbe Interaction. *20*, 1112-1125.

Hammond-Kosack, K.E. and Jones, J.D.G. (1996). Resistance gene-dependent plant defense responses. Plant Cell. *8*, 1773-1791.

Kader, A.A. (2005). Banana: Recommendations for maintaining postharvest quality. [On line]. [Accessedon 06.02.2013]. Available at <http://postharvest.ucdavis.edu/Produce /ProduceFacts/Fruit/full_banana_ripening chart.shtml>.

Lane, B. (2002). Oxalate, germins, and higher-plant pathogens. IUBMB Life. *53*, 67-75.

Lassois, L., Jijakli, M.H., Chillet, M. and de Lapeyre de Bellaire, L. (2010). Crown rot of banana- Pre harvest factors involve in postharvest disease development and integrated control methods. The American Phytopathological Society. Plant Disease. *94(6),* 648-658.

Ma, B.C., Tang, W.L., Ma, L.Y., Li, L.L., Zhang, L.B. and Zhu, S.J. (2009). The role of chitinase gene expression in the defense of harvested banana against anthracnose disease. Journal of American Society of Horticultural Science. *134(3),* 379-386.

Patnaik,D. and Khurana,P.(2001). Wheath Biotechnology: A minireview. Electronic Journal of Biotechnology ISSN:0717-3458.

Patricia, M., Manosalva, P.M., Davidson, R.M., Xiaoyuan- Zhu, B.L Hulbert, S.H., Leung, H. and Leach, J.E. (2009). A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. Plant Physiology. *149(1),* 286-296.

Shibuya, N. and Minami, E. (2001). Oligosaccharide signalling for defence responses in plant. Physiological and Molecular Plant Pathology. *59*, 223-233.

Wardlaw, C.W. (1934). The nature and occurrence of pitting diseases and fruit spots. Journal of Tropical Agriculture. *11*, 8-13.

Wegener, C., Bartling, S., Olsen, O., Weber, J. and Wettstein, D. von (1996). Pectate lyase in transgenic potatoes confers pre-activation of defense against *Erwinia carotovora.* Physiological and Molecular Plant Pathology. *49(6),* 359-376.

Zimmermann, G., Baumlein, H., Mock, H., Himmelbach, A. and Schweitzer, P. (2006). The multigene family encoding germin-like proteins of barley: regulation and function in basal host resistance. Plant Physiol. *142*, 181-192.