International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03Issue 8
April 2016

Expression of Cercosporin Toxin Biosynthetic Gene Cluster in Wild Type and Cercosporin Deficient Mutant Isolates of *Cercospora Canescens*

Pratima Singh [1*], Ramesh Chand[2], Chattarpal[2], Shweta Singh[1], Priyanka Maurya[1], Navaneet Singh[3] and Sadhana mishra[3].

Department of Genetics and plant Breeding Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India.

Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi 221005

Abstract:

Many phytopathogenic cercospora spp. synthesize the photoactivated phytotoxin cercosporin which are couses many plant disease. Cercosporin is a photosensitizing compound (M.E. Daub, 1982). When activated by light, it absorbs light energy, converting it to an electronically activated (triplet) state, which reacts with oxygen molecules via either electron or energy transfer to generate reactive oxygen species such as superoxide and singlet oxygen. Cercosporin has been shown to produce both singlet oxygen and superoxide in vitro, but the major toxicity of cercosporin to the cells is dependent on the production of singlet oxygen (Daub and Hangarter, 1983) that destructively reacts with lipids, proteins, and DNA. Exposure of plant cells and tissues to cercosporin results in peroxidation of the membrane lipids, leading to membrane breakdown and death of the cells (Daub and Briggs, 1983) and this membrane damage allows for leakage of nutrients into the leaf intercellular spaces, allowing for fungal growth and sporulation.

Key words: Cercospora, inoculation, inoculum, mung bean, spores.

Author for correspondence: Pratima Singh, e-mail: pratimasinghpbt@gmail.com

Introduction:

A large number of fungal genera belong to class Dothideomycetes and *Cercospora* is one of them which are pathogenic to plants, causing leaf spot and blight diseases. It is a relatively well-studied genus of fungi and has wide host range such as tobacco, corn, rice, sugar beet, soya bean, banana and many ornamental and weed *spp*. Symptoms of the *cercospora* can appear on any aerial plant part, although initial symptoms may appear on cotyledon as circular to

irregularly shaped with grayish white Centre and reddish brown to dark brown margins. *Cercospora species* are known for the production of the non-host specific toxin Cercosporin**.**

Cercosporin is a light-activated perylenequinone phytotoxin, it has deep red colour when dissolved in acetone and has the molecular formula $C_{29}H_{26}O_{10}$. Cercosporin is structurally related to several photosensitizing compound. Cercosporin has been shown to produce both singlet oxygen and superoxide ions *invitro*, but the major toxicity of cercosporin to the cells is dependent on the production of singlet oxygen, (Daub and Hangarter, 1983). It causes fatty acid peroxidation of plasma membrane lipids, and is considered important for fungal pathogenicity and symptom development by *Cercospora species,* cercosporin also destructively reacts with proteins, and DNA. Thus, cercosporin is not only highly toxic to plants, but it is also toxic to bacteria, many fungi and cultured human tumors cells.

Chand *et al*., (2012) reported a new strain of *C. canescens* (isolate NFCCI-2370), on the recently released mung bean (*Vigna radiata*) cv. Meha. The disease severity caused by the new strain of *C. canescens* varied from 60 to 80% at the farm of the Indian Institute of Pulses Research Kanpur, India. Symptoms of the new strain were dry necrotic, grey irregular sporulating spots without chlorotic margins. A morphological comparison of the new strain with the typical strain was done using mycelium from infected leaves. The new strain was distinguished from the typical strain by 1-2 scars on the conidiophores, whereas the number of scars was 2-7 in the typical strain. The conidiophores and conidia were also shorter in the new strain. The new strain bears a single conidium at the tip of conidiophores with a prominent scar. The radial growth, colony morphology and pigment production of the new strain on PDA at 25°C was faster and colony colour was creamy without production of cercosporin or spores. The identity of the new strain was further confirmed by comparison of ITS (Internal Transcribed Spacer) sequence data with reference isolates. BLASTn analysis of the sequence obtained showed 98-99% homology with reference isolates of *C. canescens*.

Material & Method:

Fungal isolation and culture conditions:

Wild and mutant strain of *Cercospora canescens* was obtained from the Department of Mycology and Plant Pathology and maintained on potato

dextrose agar (PDA) media at 25˚C. Mutant was screened for the lack of red pigment (cercosporin) production on thin PDA media plates. Thin PDA plate inoculated by wild strain (less than 15 ml medium in a 90 mm diameter Petri dish) supported the highest production of cercosporin.

Fifteen days old mycelium plug were used for extraction of cercosporin. Cercosporin was extracted in 30 ml acetone from mycelial plugs (6 mm diameter) of *C. Canescens* (MTCC 10835) in the dark for 4 hours. Absorbance of the soaking solution was measured at 473 nm (maximum visible absorbance of cercosporin in Acetone) by using ELICO double beam SL 191 UV VIS spectrophotometer.

Treatment details:

T_1: Leaves of cv. Kopergaon were treated with isolated cercosporin T_2: Mycelia plugs (6 mm dia.) of wild type isolate T_3: Mycelia plugs (6 mm dia.) of mutant isolate T_4: Inoculation of Acetone T5: Control (only plain water).

The inoculated plants were incubated in a dew chamber in the polyhouse at >98% relative humidity and expression of symptoms was observed. Disease data were recorded after appearance of symptoms (after 7-8 days) on 0-9 scale (Ahmad, 1985). These numerical ratings were used to calculate the percent disease index as follows:

$$\text{Disease Severity (Area) \%} = \frac{\text{Area of the plant tissue affected by the disease}}{\text{Total Area}} \times 100$$

Isolation of Genomic DNA:

0.5-1g Mycelia was grounded in liquid N_2 using pre cooled pestle and mortar. Ground mycelium was suspended in 10 ml pre warmed CTAB buffer (1M Tris base, 0.5M EDTA, 5M Nacl, CTAB 10%, β-mercaptoethonol, distilled water) and shaken gently for 5-10 min. 2.5ml SDS (Sodium dodisile sulphate) was added and further shaken well and incubated at 65°C in water bath for 1 hr. After incubation samples were kept at room temperature for few minutes to cool down and then centrifuged at 12000 rpm (23182.56g) for 15 min. After centrifugation, supernatant were taken in fresh sterilized micro centrifuge tube. RNase solution was added in each sample and incubated at 37°C for

45 min. Equal volume of chloroform: isoamyl alchohol (C: I, 24:1) was added. Samples were mixed gently by inverting the microfuge tubes for a period of 10 minutes at shaker. After shaking, samples were centrifuged at 12000 rpm (23182.56g) for 15 min at room temperature. Supernatant was taken and transferred to new micro centrifuge tube (1.5ml) and added 0.6 volumes of isopropanol (chilled) and 0.1 volume sodium acetate. Samples were placed at -20˚C for overnight and centrifuged at 12000 rpm (23182.56g) for 12 min at 4˚C. Supernatant was discarded and 200 µl ethanol 70% was added and centrifuged at 12000 rpm (23182.56g) for 15 minutes for washing the DNA pellets. The supernatant was discarded and tubes were inverted for overnight on blotting paper for drying the DNA pellets. DNA pellets were dissolved in 50 µl distilled water and stored at -20°C.The extraction buffer and final concentration and volume used in the experiment is given below:

Polymerase chain reaction:

Polymerase chain reaction was performed to selectively amplify *in vitro* a specific segment of the total genomic DNA to a billion fold (Mullis et al., 1986). The most essential requirement of PCR is the availability of pair of short (typically 20-25nucleotides) primers having sequence complementary to either end of the target DNA segment (called template DNA) to be synthesized in large amount. The components of the PCR reaction were first added in a sterilized micro centrifuge tube thoroughly in a sequence as mentioned in above table no. 3.1 and then mixed thoroughly by vortaxing. To each PCR tubes (0.2 ml), 13 µl of reaction mixture was distributed and finally template DNA of individual isolates was added. The tubes containing reaction mixture were placed in the wells of the thermocycler block and amplification reaction was carried out with the thermocycler programme summarized in the next table no. 3.2. For PCR programming all the steps were kept as such except the annealing temperature. For adjustment of concentration of various chemicals, amount of $MgCl_2$ was changed keeping other PCR components as constant. Annealing temperature was determined based on the GC content of the primer using the formula given below:

$$Tm = [2\times (A+T) + (G+C)] - 4$$

This formula gave preliminary information but not the exact annealing temperature. Therefore, the correct annealing

temperature was determined based on best PCR amplification. All the amplifications were performed in the Eppendorf Master Cycler gradient (Germany). After the completion of the PCR, the products were stored at -20 ºC until the gel electrophoresis was done.

The amplified DNA fragments generated through primers were resolved through agarose gel electrophoresis in 1.5% .Agarose gel was prepared in TAE (242 g Tris-base; 57.1 ml glacial acetic acid and 100 ml 0.5M EDTA (P^H 8.2) bring final volume to 1000ml) buffer. Ethedium bromide solution at a final concentration of 0.03 ng/ µl was added to the agarose solution.

For electrophoresis, 15 µl of the PCR product was mixed with 2 µl of 6× loading dye (0.25% bromophenol blue in 30% glycerol) and loaded in the wells of the agarose gel. In order to determine the molecular size of the amplified products, one well was loaded with 100 bp DNA size marker. Gel electrophoresis was performed at a constant voltage of 65V for appropriate time. Finally, the gels were visualized under a UV light source in a gel documentation system (Gel DocTM XR+, BIO-RAD, USA) and the images of amplicons were captured and stored in a computer for further analysis and use.

RNA Isolation:

Sample for RNA isolation were collected from the above method. One gm fungal mycelial mat was used for RNA isolation using Sure prep^{TM} RNA purification kit. Mycelial mat was ground in liquid nitrogen using pre cooled mortar and pestle to make fine powder. 600µl of lysis solution was added to the sample and further ground until the sample has been homogenized. The lysate was transferred into RNase-free micro centrifuge tube by using the pipette. The lysate was centrifuged for 2 minute for sedimentation of cell debris and then supernatant was transferred to another RNase free micro centrifuge tube. Equal volume of 70% ethanol was added and then vortexed to mix. Assembled a spin column with one collection tube and clarified lysate (600 µl) with the ethanol added into the column and centrifuged for 1 min at 12500 rpm (25154.68g). RNA wash solution (400 µl) was added to the column and centrifuged for 1 min at 12500 rpm (25154.68g). The flow through was discarded and the column was reassembled with the collection tube. Column was washed a second time by adding another 400 µl of

RNA wash solution and centrifuged for two min in dry column. The collection tube was discarded with the flow through and the column was placed into fresh centrifuge tube. 50 µl of RNA elution solution was added and centrifuged for 1 minute at 12000 rpm (23182.56g). If the entire volume has not been eluted, the column was Spinned at 12000 rpm (23182.56g) and the purified RNA sample was stored at -20ºC for few days.

To assess integrity and purity of the RNA, sample were analyzed by formaldehyde agarose gel electrophoresis (1.3% agarose, 5% formaldehyde in 1× MOPS) buffer (20 mM 3-[n-morpholino] propanole-sulphonic acid, 5 mM sodium acetate, 1 mM EDTA). Five µg of total RNA was mixed with 3.5 µl formaldehyde solution (40% w/v), 10 µl formamide, 2 µl of 10 × MOPS buffer and 1 µl of ethidium bromide (1 mg/ml) in a total volume of 20 µl, then heated to 95°C for 5 min and 5 µl RNA loading dye (8% ficoll, 0.02% bromophenol blue, 0.04% xylenecyanol FF) added prior to loading.

cDNA construction:

1 µl Oligo (dT), 2 pmole gene-specific primer ,2 ng total RNA ,1 µl dNTP Mix and 12 µl distilled water were added in a nuclease-free microcentrifuge tube. Mixture was heated at 65°C for 5 min and quickly chilled on ice and centrifuged for 1 minute at 12000 rpm. The supernatant was collected in fresh nuclease-free microcentrifuge tube. 4 µl 5X First-Strand Buffer and 0.1 M DTT 2 µl was added in the tube. Contents of the tube were mixed gently and incubate at 42°C for 2 min. 1 µl of SuperScript™ II RT was added and mix by pipetting gently up and down and heated at 70°C for 15 min for inactivation of reaction. H RNase was added in the tube for the denaturation of RNA. The cDNA can now be used in the further experiment and stored at -20ºC.

Real Time-Polymerase Chain Reaction:

Quantitative RT-PCR is performed to quantify mRNA in both relative and absolute terms. It can be applied for the quantification of mRNA expressed from endogenous genes. It is the most sensitive method as yet in quantitative analysis of mRNA.

PCR amplification was performed using 10µl 2× Master mix, 2 µl cDNA as a template, 1 µl Gene specific forward and reverse primer and 6µl DEPC water was added in the 32 optical RNase-free microcentrifuge tube. A negative control (DEPC water) was added in the two RNase-free microcentrifuge tubes and

Standard primers (Actin) were added in 8 RNase-free microcentrifuge tubes at the room temperature and then all the tubes were mixed thoroughly by vortaxing. The tubes containing reaction mixture were placed in the wells of the Light cycler® 96 Coche block and amplification reaction was carried out with the Light cycler RT-PCR programme for 26 cycles of: 94°C for 50 s, 55°C for 50 s, and 72°C for 50 s. Real-time qRT-PCR was performed for 40 cycles using 2 µl cDNA as a template and Light cycler® 96 Coche. Real time system with SYBR-2 (Bio-Rad). For PCR programming all the steps were kept as such except the annealing temperature.

After the completion of the RT-PCR, the fluorescence and amplification curve was prepared by RT-PCR (Light cycler® 96 Coche) of the products Quantitative RT-PCR data were analyzed with Light cycler® 96 Manager (Coche). Data was normalized to *actin* levels.

Result and Discussion:

The present experiment consisted of wild strain (MTCC-10835) and the mutant strain (NFCCI-2370) of *Cercospora canescens*. The disease severity of these strains has been estimated on mung bean cultivar Kopergaon. Through the morphological characterization, cercosporin production assay and symptom production, the identity of isolates (mutant and wild type) was further confirmed (Chand et. al. 2012). Finally an attempt was also made to study the Identification and characterization of genes involve in cercosporin biosynthesis pathway in *C. canescens*. The results of the present investigation are presented under the following heads.

1. Role of cercosporin in disease development on host.
2. Identification and characterization of genes involve in cercosporin biosynthesis pathway in *Cercospora canescens*.
3. Gene expression through RT PCR analysis.

***Isolates of C. canescens and their characterization*:**

In culture, wild Strain of *C. canescens* was produced red pigment (cercosporin) whereas mutant strain was completely inhibited in production of cercosporin. A morphological comparison of the mutant strain (NFCCI-2370) with the wild strain (MTCC-10835) was done by using mycelium from petri plate. On host the mutant strain bears a single conidium at the tip of conidiophores whereas abundant

conidia with minimal mycelia were produced in the wild strain.

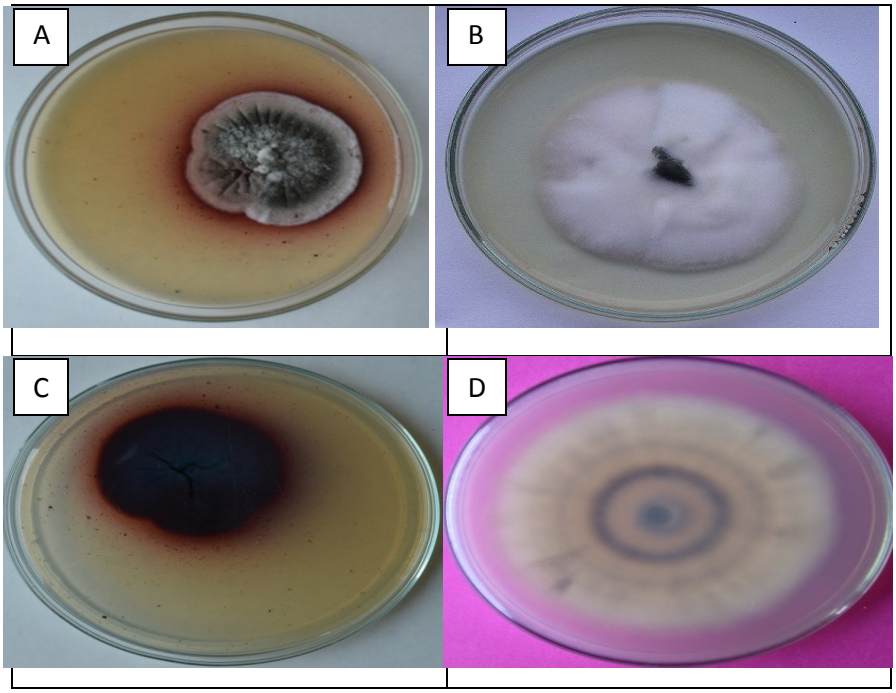


Fig.1: Culture plates of wild type and Mutant;

A) Front view of wild strain (MTCC-10835),

B) Mutant strain (NFCCI-2370),

C) Inverted view of wild (MTCC-10835),

D) Mutant strain (NFCCI-2370) strain

Pathogenicity test:

Both isolates were pathogenic to mung bean plants. The symptoms were similar to those observed in the polyhouse (Fig.4.2).Inoculation on plants of cv Kopergaon of the mutant strain produced the atypical symptoms, whereas inoculation with the wild strain of *C. canescens* produce grayish white Centre and reddish brown to dark brown margins appeared on inoculated leaves in 7-8 days after inoculation. The spots later enlarged to angular-to-irregular lesions. Percentage of leaf surface showing symptoms was estimated visually 21 days after inoculation.

Fig.2: Symptoms produced by *C. canescens* on mung bean leaves in polyhouse. Pathogenicity test: A-Cercosporin + Acetone; B-Acetone; C-Wild strain; D-Mutant strain; E- Control (water).

Treatment	Mean of disease severity (%)
Cercosporin + Acetone	32.607
Acetone	4.937
Plugs of wild strain	25.539
Plugs of mutant strain	18.567
Control (water)	0.000
LSD(0.05)	7.4

Table.1: Percent Disease severity of CLS on mung bean with different treatments:

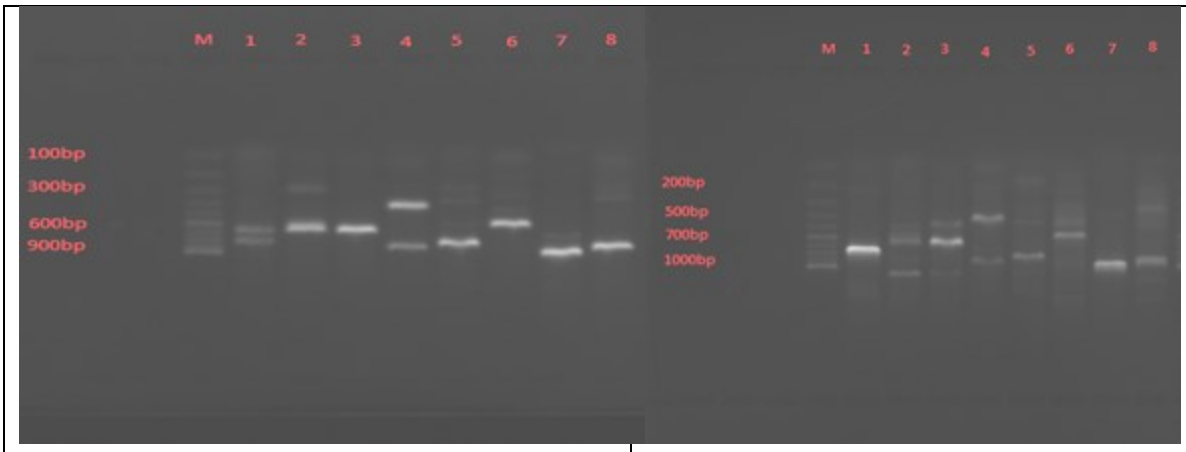
The results indicated that the extracted cercosporin in the acetone (T_1) had highest % disease severity (32.60) followed by wild strain and mutant (25.53 and 18.56 respectively) whereas the acetone alone caused 4.93 % damage to leaves. These data suggests possible role of cercosporin in disease development.

Amplification of genes involved in Cercosporin Toxin Biosynthesis (CTB):

The agarose gel electrophoresis after polymerase chain reaction revealed the presence of amplicons related to gene (CTB1-8) involve in cercosporin biosynthesis pathway in both Wild strain (MTCC-10835) and the mutant strain (NFCCI-2370) of *C. canescens*. The amplicons present in gel images fig 4.1 confirmed the presence of CTB1-8 gene in both the strains.

Genes	Amplicon size	Anneling temperature
CTB1	891bp	55.30
CTB2	779bp	56.30
CTB3	601bp	58.35
CTB4	741bp	53.70
CTB5	365bp	57.35
CTB6	666bp	57.00
CTB7	602bp	57.30
CTB8	771bp	55.20

Table.2: Genes involves in cercosporin biosynthesis:

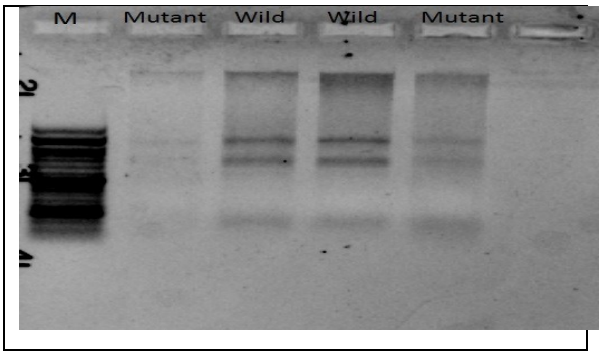


Wild strain (MTCC-10835) | **Mutant strain (NFCCI-2370**

Fig.3: Amplicon indicating the CTB1-8 genes involves in cercosporin biosynthesis

The (CTB1-8) gene involves in cercosporin biosynthesis pathway in *Cercospora spp.* (Chen *et.al,* 2007). CTB1 are responsible for condensation of acetyl-CoA and malonyl-CoA units (step 1), chain elongation to form a pentaketide molecule (step 2), and ring closure (step 3) in the early steps of cercosporin

biosynthesis. During ring closure, oxidation and hydration reactions take place to form aromatic rings. The oxidation reactions are likely completed by a monooxygenase domain encoded by *CTB3*, followed by hydration steps. Once the ring is closed, methylation steps (step 4) are operated by methyltransferases domains encoded by *CTB3*, resulting in polyketomethylene units. Cercosporin has a bilateral symmetric structure; thus, cercosporin is likely formed by fusing two identical polyketomethylene units (step 5, dimerization). This step can likely be completed enzymatically or nonenzymatically. Once it is synthesized, cercosporin must be exported out of fungal cells, presumably by the function of a



major facilitator superfamily (MFS) transporter encoded by *CTB4* (Daub, 2007). The expression of the *CTB* genes identified to date correlate with environmental conditions that regulate cercosporin production, and that this regulation was controlled through a transcription factor (CTB8).

A new pathogenic variant of *Cercospora canescens* were observed without production of cercosporin Chand *et al*., (2012). The *CTB1* to *CTB8* genes present in the wild strain of *C. canescens* during the cercosporin biosynthesis. The roles of these eight genes are well studied in other *Cercospora* spp. (ref.). However, it is clear from present experiment that *CTB1* to *CTB8* genes have been also produced in the mutant strain, but it could not produce cercosporin.

Extraction of total RNA and visualization:

The formaldehyde agarose gel electrophoresis after RNA isolation revealed the presence of higher amount of RNA in wild strain of *C. canescens* where as in mutant strain the amount of RNA was very low (fig.4.4).

Fig.4: Total RNA of wild and Mutant strain.

Gene Expression analysis

The difference of expression of the genes, involved in cercosporin toxin biosynthesis (CTB) of wild strain and the mutant strain of *C. canescens* were studied by the Real Time- Polymerase Chain Reaction (RT-PCR) by using Light cycler® 96 (Roche Diagnostics Corporation, USA).

After the analysis Fluorescent curve, amplification curve and ΔCt (cycle threshold) value were generated.

Fluorescence curve:

Fluorescent curve correlates the emitted fluorescence (C_T value) with the initial concentration of the standards used and the final result was achieved by interpolation of the produced fluorescence (C_T value) during the amplification of the sample in fluorescent curve.

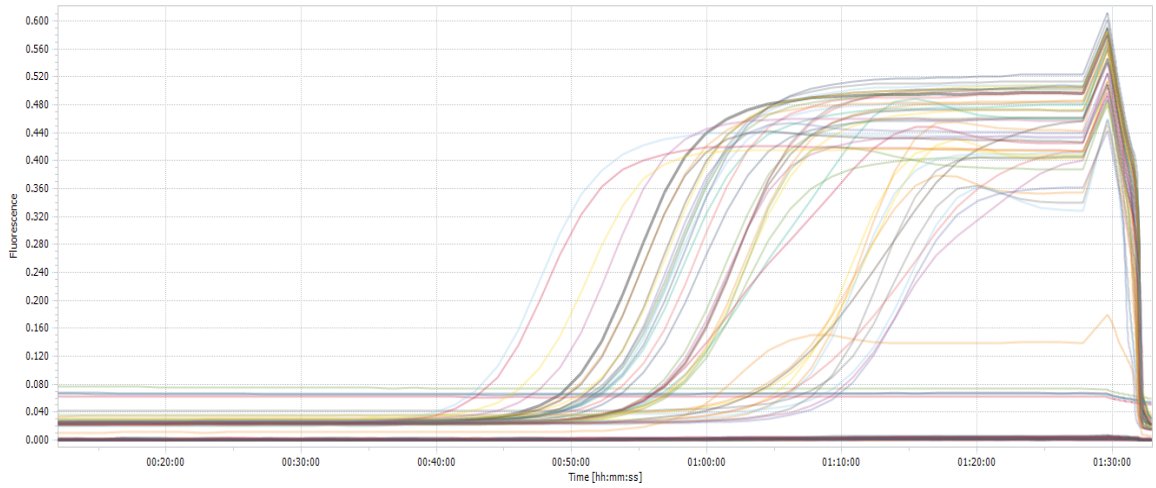


Fig.5: SYBR green fluorescence chart for eight samples, each having four replicates, generated as a result of quantitative PCR (qPCR).

Amplification curve:

The dotted line in the amplification curve represents the "threshold" above which the product of a reaction was deemed to be significant. The Cycle-Threshold (C_T) is the numeric value for the cycle at which the product crossed the threshold. Reactions with lower C_T values contain more of the gene of interest since they took less time to amplify. In the same way, samples with a higher C_T contain less of the gene of interest.

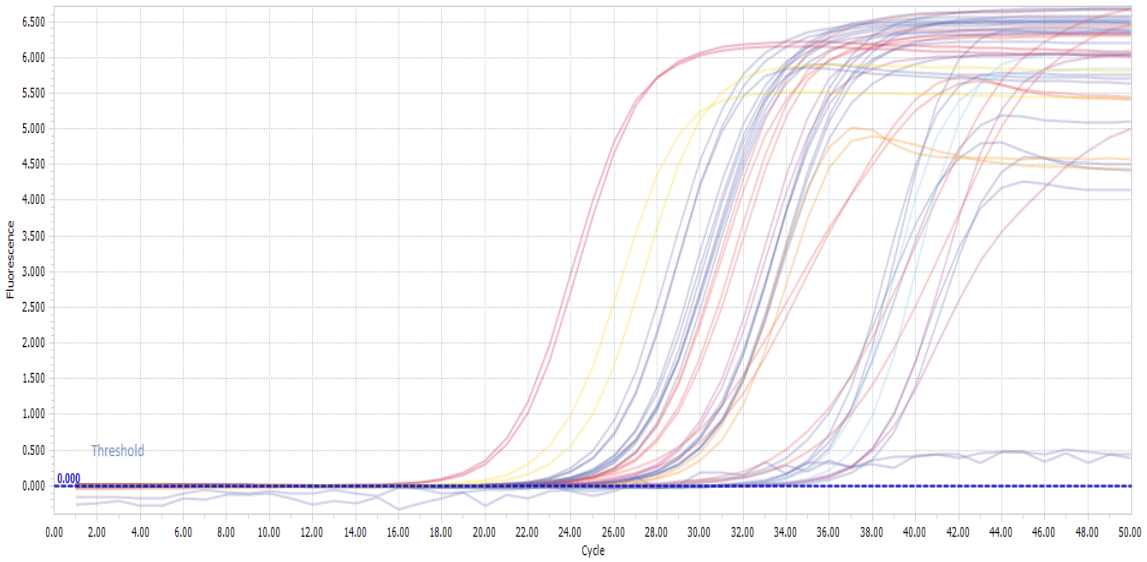


Fig.6: Amplification curve for eight samples, four replicates each, which is a result of annealing temperature analysis of quantitative PCR results (qPCR).

To quantify gene expression, the C_q for cDNA from the gene of interest is subtracted from the C_q of cDNA from a housekeeping gene in the same sample to normalize for variation in the amount and quality of RNA between different samples. This normalization procedure is commonly called the *ΔC_t-method* and permits comparison of expression of a gene of interest among different samples. However, for such comparison, expression of the normalizing reference gene needs to be very similar across all the samples. Choosing a reference gene fulfilling this criterion is therefore of high importance, and often challenging, because only very few genes show equal levels of expression across a range of different sample. Although cycle threshold analysis is integrated with many commercial software systems, there are more accurate and reliable methods of analyzing amplification profile data that should be considered in cases where reproducibility is a concern.

Table.3: ΔCt value of wild strain of *Cercospora canescens*

Gene	Isolate 1	Actin	ΔCT
CTB1	28.21	24.61	3.6
CTB2	36.73	24.61	12.12
CTB3	37.4	24.61	12.79
CTB4	27.4	24.61	2.79
CTB5	25.43	24.61	0.82
CTB6	28.29	24.61	3.68
CTB7	27.93	24.61	3.32
CTB8	34.93	24.61	10.32

Data of ΔCt value of Mutant strain of *Cercospora canescens*:

Gene	Isolate 2	Actin	ΔCT
CTB1	25.12	19.22	5.90
CTB2	32.66	19.22	13.44
CTB3	33.88	19.22	14.66
CTB4	25.94	19.22	4.77
CTB5	23.99	19.22	0.82
CTB6	25.83	19.22	6.61
CTB7	25.00	19.22	5.78
CTB8	26.50	19.22	7.28

ΔΔCT	Fold changes
-2.3	4.924578

-1.32	2.496661
-1.87	3.655326
-3.93	15.24221
-3.95	15.45498
-2.93	7.621104
-2.46	5.502167
3.04	0.121582

Table 3 showed that the ΔCT value of the gene (CTB1-CTB8) of wild and mutant strain of *C. canescens*.

In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater the amount of target nucleic acid in the sample).

ΔCT value of the wild strain indicated that the greater amount of cDNA was produced in the wild strain and the gene was expressed very high whereas in mutant strain amount of cDNA was low and expression of gene was low than wild strain.CTB4 and CTB5 were expressed very high in both the strain.

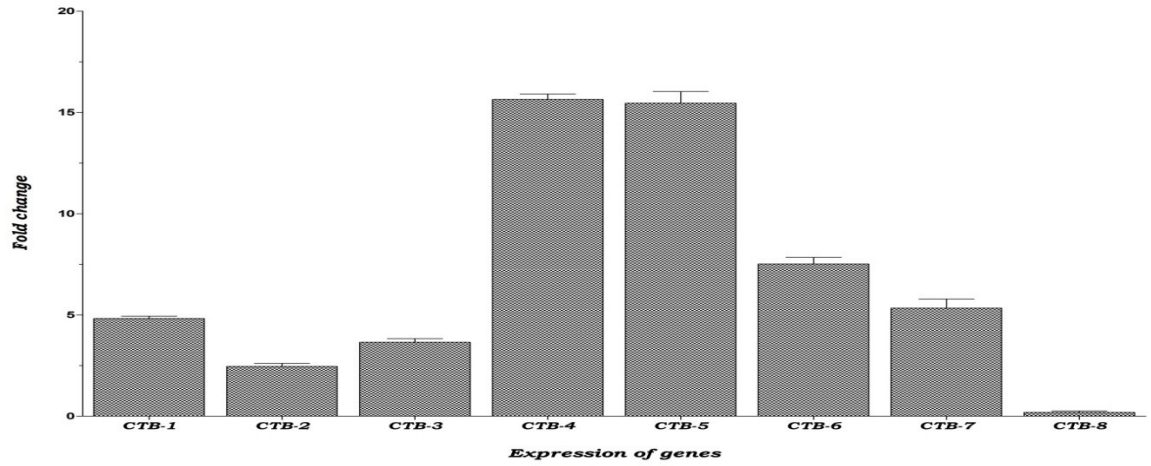


Fig 7: Bar chart of fold change

Bar chart with bars to visualize the expression fold change of target genes in samples. Each panel represents one target gene (CTB1-8) and the expression level in each sample is indicated by the height of bars. In the present study bar chart showed higher expression (CTB4 and CTB5) gene in both wild and mutant strain whereas the CTB8 gene expression was very low in both the strain, but not yet undetectable. All the genes of wild and mutant stain were upregulated because of their pathogenic nature.

In the present study, according to the ΔCt value the expression of the gene (CTB1 to CTB8) in wild strain was higher accepting CTB8 gene whereas in mutant strain the genes were expressed very low but the expression of CTB8 gene was higher than wild strain. Therefore wild strain produced cercosporin that caused high level of disease severity and mutant strain could not produce cercosporin and varied from 50 to 70% in disease severity. According to the graph of real time polymerase chain reaction genes of the both strain were up-regulated because of their pathogenic nature.

Cercosporin, a toxin produced by *Cercospora species*, rapidly kills plant cells in the light and causing leaf spot and blight diseases (Daub and Briggs, 1982). A new pathogenic variant of *Cercospora canescens* were observed without production of cercosporin or spores on the recently released mung bean (*Vigna radiata*) cv. Meha Chand *et al*., (2012). So an experiment was conducted to find out the role of cercosporin in CLS development and genes involve in cercosporin biosynthesis pathway in *C. canescens*. Cercosporin is a compound that sensitizes cells to visible light and converting it to an electronically activated

state, which reacts with oxygen molecules via either electron or energy transfer to generate reactive oxygen species such as superoxide and singlet oxygen (Daub, 1981). Cercosporin has been shown to produce both singlet oxygen and superoxide ions *in vitro*, but the major toxicity of cercosporin to the cells is dependent on the production of singlet oxygen, (Daub and Hangarter, 1983).Their findings indicated that the cercosporin play an important role in the development of leaf spot.

Conclusion:

Leaf spot caused by *Cercospora Canescens* is a destructive disease of mung bean (*Vigna radiata*) in warm and humid mung bean growing regions of the world and reduce the production. *C. Canescens* are known for the production of the non-host specific toxin Cercosporin. It is structurally related to several photosensitizing compound major toxicity of cercosporin to the cells is dependent on the production of singlet oxygen that destructively reacts with lipids, proteins, and DNA.

The present study was under taken using the wild-type *C. canescens* strain (MTCC-10835) and the mutant strain (NFCCI-2370) with the objective.

4. Role of cercosporin in disease development on host.
5. Identification and characterization of genes involve in cercosporin biosynthesis pathway in *Cercospora canescens.*
6. Gene expression analysis through RT PCR.

Wild strain of *C. canescens* produce grayish white Centre and reddish brown to dark brown margins appeared whereas mutant strain produced the atypical symptoms. The spots later enlarged to angular-to-irregular lesions. Isolated cercosporin toxin and plugs of wild strain were highly severed to causing disease in mung bean plant whereas the disease severity caused by the mutant strain of *C. canescens* (isolate NFCCI-2370, DS-18.567) varied from 50 to 60% at the poly house.

After polymerase chain reaction the agarose gel electrophoresis revealed the amplicons of 891bp, 779bp 601bp, 741bp, 365bp, 666bp 602bp 771bp in gel doc system confirmed the presence of the (CTB1, CTB2, CTB3 CTB4, CTB5, CTB6, CTB7, CTB8) gene in both wild and mutant strains of *C. canescens*.

Smaller ΔCT (Cycle threshold) value of the wild strain indicated that the greater

amount of cDNA was produced in the wild strain and the gene was expressed very high whereas in mutant strain amount of cDNA was low and expression of gene was also low than wild strain. Therefor cercosporin is produced in wild strain.

REFERENCES:

1. Daub, M. E., 1982 Cercosporin, a photosensitizer toxin from *cercospora* species phytopathology 77:1515-1520.
2. Daub, M. E., and Briggs, S. P. 1983. Changes in tobacco cell membrane composition and structure caused by the fungal toxin, cercosporin. Plant Physiol. 71:763-766.
3. Daub, M. E., and R. P. Hangarter**.** 1983. Light-induced production of singlet oxygen and superoxide by the fungal toxin, cercosporin. Plant Physiol. **73:**855–857.
4. Daub, M. E., and Ehrenshaft, M. 2000. The photoactivated *Cercospora* toxin cercosporin: Contributions to plant disease and fundamental biology. Ann. Rev. Phytopathology. 38:461-490.
5. Kuyama, S., and T. Tamura. 1957. Cercosporin. A pigment of *Cercospora kikuchii* Matsumoto et Tomoyasu. I. Cultivation of fungus, isolation and purification of pigment. J. Am. Chem. Soc. 79:5725.
6. Venkataramani, K. 1967. Isolation of cercosporin from *Cercospora personata*. Phytopathology. Z. 58:379-382.
7. Upchurch R. G. Walker, D. C., Rollins, J. A., Ehrenshaft, M., and Daub, M. E. 1991Mutants of *Cercospora kikuchii* altered in cercosporin synthesis and pathogenicity Apple Environ. Microbiol.57: 2940-2945.