Biological Forum – An International Journal 13(3): 255-261(2021)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Expression of *CAT* gene Resistance against Fusarium Wilt in Pigeonpea

***Prashant Gigaulia*[1], *Keerti Tantwai*[2*], *Sushma Nema*[3] *and Sanjay Kumar Singh*[4]**
[1]*Ph.D. Scholar, Biotechnology Centre, JNKVV, Jabalpur, (Madhya Pradesh), India.*
[2]*Assistant Professor, Biotechnology Centre, JNKVV, Jabalpur, (Madhya Pradesh), India.*
[3]*Professor, Biotechnology Centre, JNKVV, Jabalpur, (Madhya Pradesh), India.*
[4]*Senior Scientist, Department of Plant Breeding and Genetics,*
College of Agriculture, Jabalpur, (Madhya Pradesh), India.

(Corresponding author: Keerti Tantwai)*
(Received 31 May 2021, Accepted 02 August, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Wilt is most common and serious disease in pigeonpea. Many published reports have indicated 30-100% yield loss due to wilt in pigeonpea. Study of molecular markers and expression level of associated genes are very important to overcome the problem of wilt disease in pigeonpea. The causal organism of Fusarium wilt in pigeonpea is *Fusarium udum*. We report the efficacy of SSR markers among 14 pigeonpea varieties and expression analysis of Fusarium wilt resistance gene done by qRT-PCR. 14 varieties of pigeonpea were collected and analyzed using 13 SSR primers. *Fusarium udum* was isolated from infected plants of pigeonpea. Three varieties ICP-2376, Asha, and TJT-501 were grown in infected and control condition for expression analysis. Inoculation was done by root dipping into fungal spore suspension culture at four different time intervals *viz.* 0, 48, 72 and 120 hours. Total RNA was extracted from root parts. The expression level of *CAT* gene was tested in all the selected variety at four different stages after infection along with a housekeeping gene *TUBA5*. The data of relative ratio revealed that the concentration of *CAT* gene was found higher in two Asha and TJT-501 resistant varieties and lowest in ICP-2376 variety under wilt infected condition.

Keywords: Pigeonpea, SSR Markers, wilt resistance gene, *Fusarium udum*, qRT-PCR.

Abbreviations: CAT: Catalase; cDNA: Complementary DNA; DNA: Deoxyribonucleic Acid, FW: Fusarium Wilt; PCA: Principle Component Analysis; PCR: Polymerase Chain Reaction; PDA: Potato Dextrose Agar; PIC: Polymorphism Information Content; qRT-PCR: Quantitative Real Time Polymerase Chain Reaction; RAPD: Random Amplified Polymorphic DNA; RNA: Ribonucleic Acid; SMD: Sterility Mosaic Disease; SNP: Single Nucleotide Polymorphism; SSR: Simple Sequence Repeats; UPGMA: Unweighted Pair Group Method with Arithmetic Mean

INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millsp.) belongs to the genus *Cajanus*, and family *Fabaceae*. It is the second most important pulse crop after gram and major perennial pulse crop grown in India as well as in the world. The center of origin is the eastern part of peninsular India; it is an oldest cultivated crop of India. It is a diploid species ($2n = 2x = 22$) comprising a genome of 833.1 Mbp arranged into 11 linkage groups (Varshney *et al.,* 2012). Pigeonpea crop is affected by both biotic and abiotic stresses. In abiotic stress, plants are affected mostly by temperature (drought condition) and salinity. In biotic stress, plants are attacked by various microbial organisms; soil borne fungal diseases severely affect the plant metabolism. The most prominent disease of pigeonpea is 'wilt'. It is most wide spread and caused by the fungus *Fusarium udum* (Butler, 1906). It is one of the serious and oldest known diseases and causes 30-100% yield loss reported in susceptible genotypes (Pande *et al*., 2013). The main symptom of disease is characterized, in steady phase, by yellowing, withering and drying of leaves subsequently leading to drying of whole plant: internally xylem vessels get blocked and the whole xylem vessel get discoloured from brown to black. *Fusarium udum* is a facultative saprophyte and necrotrophic type of pathogen, it can easily get transferred from soil. When it grows on PDA culture media, the colour of fungus changes from white to pink and later it turns to dark purple.

This research takes place on the basis of economic impact of Fusarium wilt on pigeonpea crop production; the disease is endemic and continues to be responsible for greater losses. Hence, to reduce yield losses, it is necessary to challenge this problem at molecular level for developing resistant or tolerant varieties against biotic stress. Biswas *et al*., (2020) reported molecular characterization of antioxidant (AO) enzyme [*APX* and *SOD*] and pathogenesis-related (PR) protein

[*CHS* and -*1, 3-glucanase*] families performed in five distinct pigeonpea genotypes, with resistant (ICP2894) and susceptible (ICP2376) controls to identify a superior pigeonpea genotype that was resistant to FW across a major biogeographic region. In the plant genome, various defensive or resistance (R) genes are present that get expressed at the time of wilt pathogen attack. Molecular techniques offer a latest approach for detection and identification of such defensive genes. Molecular markers, such as RAPD, SSR, SNP, *etc*. are used to evaluate genetic variations at DNA level. Among these, simple sequence repeats (SSR) play an important role towards identification and detection of genetic diversities in the plant genome. Another, important molecular technique is Real Time PCR (qRT-PCR), it gives access to precisely quantify a specific gene within a host plant, to evaluate the genes assay on diverse samples such as, artificially infected plantlets and agricultural field plant samples. The qRT-PCR has reformed the field of molecular diagnostics.

MATERIALS AND METHODS

Plant material: Planting material of 14 varieties of pigeonpea crop collected from three districts of Madhya Pradesh namely Narsinghpur, Sehore, and Jabalpur districts. Some promising varieties commonly in farmer's cultivation program and resistant germplasm reported by the earlier workers were collected from College of Agriculture, Sehore and Department of Plant Breeding and Genetics, College of Agriculture, Jabalpur as shown in Table 1.

Table 1: Location of pigeonpea varieties collected from different part of India.

Sr. No.	Varieties	Type	Place	Location
1.	IPCL-20114	Resistant	Sehore	23.20°N 77.08°E
2.	IPCL-20124	Resistant	Sehore	23.20°N 77.08°E
3.	IPCL-99010	Resistant	Sehore	23.20°N 77.08°E
4.	IPCL-99048	Resistant	Sehore	23.20°N 77.08°E
5.	IPCL-99099	Resistant	Sehore	23.20°N 77.08°E
6.	Durga	Resistant	Narsinghpur	23.27°N 79.13°E
7.	TJT-501	Resistant	Jabalpur	23.10°N 79.56°E
8.	IPCL-87 (Pragrati)	Resistant	Jabalpur	23.10°N 79.56°E
9.	IPCL-151 (Jagrati)	Resistant	Jabalpur	23.10°N 79.56°E
10.	ICP-2376 (Bahar)	Susceptible	Sehore	22.81°N 77.62°E
11.	ICPL-87119 (Asha)	Resistant	Narsinghpur	22.93°N 79.06°E
12.	Pallavi	Resistant	Narsinghpur	22.94°N 79.19°E
13.	ICPL-85063 (Laxmi)	Resistant	Narsinghpur	22.92°N 78.78°E
14.	Richa 2000	Resistant	Narsinghpur	23.23°N 79.29°E

DNA Extraction from pigeonpea varieties: Genomic DNA of 14 pigeonpea varieties was collected from apical leaf samples by using DNA extraction protocol proposed by Saghai-Maroof *et al*., (1984) with some modifications. Chemical composition used for DNA extraction buffer was 10 ml of 100mM Tris HCl (pH 8.0), 8 ml of 20mM EDTA (pH 8.0), 28 ml of 1.4M NaCl, 2% CTAB, 2% PVP and 400 µl of 0.4% mercaptoethanol. Genomic DNA was quantified using Nanodrop by measuring the absorbance ratio A260/A280 and adjusted between 1.7-1.9.

PCR amplification using Simple Sequence Repeats (SSR) marker: The amplification of genomic DNA was carried out by using 13 Simple sequence repeats primers listed at Table 2.

Table 2: List of Simple Sequence Repeats (SSR) markers.

Sr.No.	SSR Primer	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
1.	PFW-13	GCAAGTGTTCCCTACGTTGC	CTCCAACGGCCATAGTAGGA
2.	PFW-24	TCTTAGCATGTCCTCTATTTTCGT	AGTACATTTCAAATCCACACATCC
3.	PFW-25	TCACAGAGGACCACACGAAG	TGGACTAGACATTGCGTGAAG
4.	PFW-35	TGGGCATGGTAGAGGAAGTT	CGTCATGAAGCAACAGGAGA
5.	PFW-37	AGGCTTTCTCCCTTCAATCC	GCCTTTTCAAACTTTTCTCACA
6.	PFW-38	ACATGTGTGGCGTAGTGTGA	GCAAAACCGTTCCATAAAAA
7.	PFW-43	TCGTGGGAATGCTCTACAAC	AACCACAAGTACACCCACACC
8.	PFW-52	ATCCCAGACTTCATAGGGAGATAG	GTCTAGTCCCAGGTACAAAGAGGT
9.	PFW-56	ATCCTCCAAAAGTTCCACCA	CAAAGGAGGATTTCCACCAA
10.	PFW-64	CAGGTCTGCTACTGCCATCA	AGCCCACTTCTGCATCACTC
11.	PFW-67	GGGAAACAAAATATCCCCTAATC	TAATCACACACATCACACCTAGCA
12.	PFW-70	ACAAATCCGGTGACCCATAA	CCGAGAACAAAAACATTGAACA
13.	PFW-72	TCTTTCAGACGCAATGACCTT	CACTTATTTGTGGGGACCATC

The components and their concentration used in the PCR reaction was 50ng plant DNA sample, 1µl of 1X PCR buffer, 0.7µl of 2.5mM $MgCl_2$, 0.7µl of 200µM dNTPs, 0.5µl of 10pM forward primer and 0.5 µl of 10pM reverse primer, 0.2µl of Taq Polymerase, Molecular grade water to makeup the total volume of 10µl. PCR programme was optimized for amplification by using primers of unique sequence with higher GC ratio at high stringency. The optimized conditions of PCR for 45 cycles were Denaturation at 95°C for 1 min, Annealing at 57-60°C for 1 min and Elongation at 72°C for 1 min. Amplified PCR products using SSR markers were resolved on 4.0% agarose gel electrophoresis. The products were observed by Ethidium Bromide staining and final gel picture was documented with gel documentation system.

Scoring of amplified product and data analysis was performed using available NTSYS software. Distinction of varieties of wilt resistance and wilt susceptibility was accomplished on the basis of SSR primers. Scoring of alleles was done on the basis of presence (1) and absence (0) of bands in the gel of all the varieties.

Isolation and screening of fungal culture to study gene expression against *Fusarium* wilt: Infected roots of pigeonpea were collected from the field and *Fusarium udum* was isolated from the wilted plant. Infected parts between the root and shoot segments (2 cm long) of wilted plant were taken and washed with running tap water to remove the soil from external surface. The outer surface of segments was removed, and these segments are used to obtain nearly 0.5 cm size transfer sections. These small pieces were sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for two minute and washed three times consequently with sterile distilled water, and then dried on blotter paper. The small sections were aseptically shifted under laminar air flow into sterile petri plates containing 10 ml Potato Dextrose Agar (PDA) medium (HiMedia ready to use, 39gm in one liter distilled water). The plates were kept in incubator at 25°C temp for 48 hr. After two days white mycelial growth of fungus was seen in PDA media containing plates. The fungal culture was sub cultured on fresh PDA medium to obtain pure culture. The pure culture of *F. udum* was prepared by single spore isolation technique on potato dextrose agar (PDA) medium at 25°C temp. Sub-culturing was repeated thrice to obtain pure culture of fungus. Isolated pure culture was preserved on same medium and condition.

Raising of pigeonpea seedling: Pigeonpea seeds of three varieties ICPL-87119, TJT-501 and ICPL-2376 were grown in pots filled with the mixture of sterilized sand, compost and soil under specific condition. These pots were placed in a net house. Before sowing, seeds were treated with 2% sodium hypochlorite, and grown in polyhouse for 20 days.

Inoculum preparation, inoculation and transplanting: Inoculum was prepared by culturing *Fusarium udum* on PDA medium. The culture was kept in incubator at 25±1°C temperature for 10 days with alternate period of 12 hr light and dark. Spore suspension of *F. udum* was diluted with distilled water to maintain the threshold level of inoculum (7×10^6 spores/ml) using a haemocytometer. The 20 days old seedlings were carefully uprooted from the pots and roots washed under running water to remove soil. One cm long root tips were cut and dipped into agitated inoculum suspension for 2:30 hr. Inoculated seedlings were transplanted into 15 cm pre-irrigated pots containing sterilized soil and sand (3:1) with compost. 20 inoculated seedlings were transplanted per pot and three replications were maintained for each treatment (resistant and susceptible genotype) with a positive control. All treated plants were kept in the poly house. Disease incidence was observed and recorded at four different interval of time (0, 48, 72 and 120 hours).

RNA extraction and cDNA synthesis: Total RNA was extracted from pigeonpea root tissues of infected and uninoculated seedlings as control sample collected at different time interval after infection. DEPC (diethylpyrocarbonate) treatment was carried out to all the plastic ware that were used in the experiment. Samples were frosted in liquid nitrogen and immediately processed for RNA extraction using RNeasy® Plant Mini Kit (QIAGEN®) according to manufacturer's instructions. Before starting the process chemical for RNA isolation was prepared. Quantification and quality assessment of total RNA was carried out by NanoDrop/spectrophotometer using software. RNA sample of 1µl concentration and purity of the total RNA extraction was determined in the 260/280 nm and 260/230 nm ratio, calculated by the software. RNA samples with A260/280 and A260/230 ratios between 1.8 to 2.2 and 2.0 to 2.2 respectively retained for cDNA synthesis. cDNA synthesis was performed in duplicates for every RNA sample immediately after its extraction, which passed the quality control measures. Thermo Scientific Maxima First Strand cDNA Synthesis Kit for qRT-PCR was used for cDNA synthesis. Chemical composition for cDNA synthesis was 4µl of 5X Reaction Mix, 2µl of Maxima Enzyme Mix, 5µg of Template RNA 1pg, made up the total volume 20µl with nuclease-free water. The cDNA was directly used in PCR or stored at -20°C up to one week.

Quantitative real time PCR (qRT-PCR) and data analysis: Validation of internal control gene and relative quantification of defense related genes in pigeonpea was carried out using qRT-PCR. Roche Light Cycler® 96 System real time PCR using USB®VeriQUEST™ SYBR® Green qRT-PCR with fluorescein kit (Affymetrix). Thawing of 2x VeriQUEST™ SYBER Green PCR master mix, template cDNA, primers as shown in Table 3, and RNase-free water were done on ice. Reaction mixture was prepared as 2.0µl of Template cDNA, 0.5µl each of

Primer Forward and Reverse (10 pmole/µl), 10µl of SYBER Green PCR master mix, made up to 20µl per reaction volume with RNase-free water. This Master Reaction was mixed thoroughly after that, 18µl master mix dispensed into individual wells of PCR plates. 2µl of template cDNA were added to the individual wells. Plates were then placed in real time cycler. As the reaction starts, the data were analyzed by the inbuilt software (Roche Light Cycler 96). Relative quantification was applied for the target cDNA relative to *TUBA5* Housekeeping (endogenous control). The quantification of gene was expressed as "n-fold up/down regulation of transcription" in relation to the endogenous control. The expression of selected genes was calibrated by the reference gene *TUBA5* at pointed time and converted into the relative expression.

Table 3: Primer used for real time PCR.

Gene	Forward/ Reverse primers (5'-3')	Amplicons	Description
CAT	F- GTTCCCATTCCTCCTCGTATCC R- AGAGGCCCAGGATCGGTATC	105	Catalase
TUBA5	F- GACCAACCTTGTCCCCTACC R- CAGGCTCGAACACAGCATTG	128	Tubulin alpha-5

RESULTS AND DISCUSSION

Data analysis of SSR markers: During this experiment a total number of 14 different pigeonpea varieties were analyzed by 13 SSR primers. These 13 SSR primers and their sequences are shown in Table 2. These SSR primers were showing polymorphism between wilt susceptible and resistant varieties on the basis of clear and sharp banding pattern. A total of 18 alleles were amplified using 13 SSR primers from all the varieties of pigeonpea. The band size of amplified primers ranged from 140-240bp. Maximum numbers of 3 alleles was scored in PFW56 and PFW67. Altogether seven loci were found to be polymorphic. The average number of bands and polymorphism per primer was recorded 1.34 and 0.53 respectively. Five SSR primers were showing polymorphism as shown in Fig. 1.

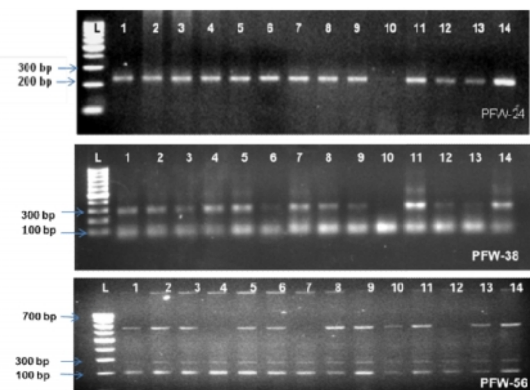
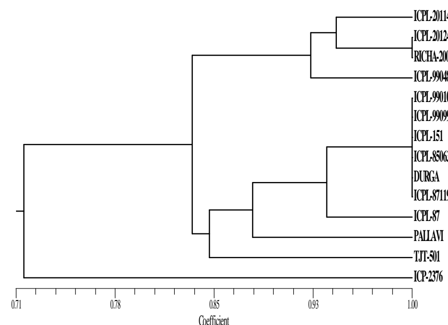


Fig. 1. PCR amplification pigeonpea varieties using SSR markers PFW-24, PFW-38, PFW-56.

On average the polymorphism percentage was 0.53%. Polymorphic information content (PIC) of SSR primers ranged between 0 to 0.265 and an average percentage of 0.056 per marker. PIC value was estimated for all 13 SSR markers. Higher PIC value indicates higher polymorphism and it helps to select the best SSR marker in phylogenic analysis. Highest PIC value was 0.31 found in PFW67 marker having three alleles among 14 varieties. In the PFW24, PFW38, PFW56 and PFW72 markers estimated PIC value was found 0.036, 0.265, 0.087 and 0.036 respectively.

Lowest PIC value (0) was obtained in PFW13, PFW25, PFW35, PFW37, PFW43, PFW52, PFW64, and PFW70 marker. According to banding pattern of amplification with 13 SSR markers, the cluster analysis revealed that the pigeonpea varieties divided into two groups, major group and minor group. Major group contained 13 varieties that divided into two sub groups, subgroup 'A' and 'B'. Subgroup 'A' contained 4 varieties and sub group 'B' contained 9 varieties. Minor group contained single pigeonpea variety. Major group contained all wilt resistance varieties and minor group contain single wilt susceptible variety as shown in Fig. 2 (Gigaulia, 2019).



1. IPCL-20114, 2. IPCL-20124, 3. IPCL-99010, 4. IPCL-99048, 5. IPCL-99099, 6. Durga, 7. TJT-501, 8. IPCL-87 (Pragrati), 9. IPCL-151 (Jagrati), 10. ICP-2376 (Bahar), 11. ICPL-87119 (Asha), 12. Pallavi, 13. ICPL-85063 (Laxmi), 14. Richa 2000

Fig. 2. Phylogenetic tree showing similarity coefficient amongst pigeonpea varieties.

Similarly, Sousa *et al.*, (2011) found 16 polymorphic markers out of 43 SSR markers in 77 pigeonpea genotypes. The PIC value ranged from 0.11 to 0.80 (average 0.49) a total of 83 putative alleles were obtained from the 16 polymorphic microsatellite markers. Mayomba, (2015) used the phenotypic and genotypic characterization together to analyse Fusarium wilt markers correlated to FWD resistance in pigeonpea and suggested that the correlated markers to wilt diseases will apply more for selection of resistant genotypes rather than phenotypic screening which is expensive in terms of time and resources.

Khoiriyah *et al*., (2018) studied the genetic diversity of pigeonpea based on molecular characterization using randomly amplified polymorphic DNA (RAPD) markers. Hullur *et al.,* (2018) also found only four out of 12 SSR primers showing polymorphism in pigeonpea varieties. Saxena *et al*., (2021) have diagnosed SNPs/Indels for Fusarium wilt (FW) and sterility mosaic diseases (SMD) resistance in pigeonpea. Comprehensive data analysis successfully identified 9 robust markers for FW resistance and 10 robust markers for SMD resistance in pigeonpea. These markers have been converted into a diagnostic kit for their routine use in crop improvement programs focusing on the development of FW and SMD resistant genotypes. Further, identified two genes for FW and four genes for SMD resistance that offers new opportunities for assigning functional role and understanding their participation in molecular mechanisms underlying diseases resistance in pigeonpea. Likewise, these molecular markers are proved to be useful for identification of resistance against *Fusarium wilt* disease in pigeonpea or other pulse crops.

Morphological characters of *Fusarium udum:* Fungal culture grown on PDA medium for 10 days at 25±2 °C. The mycelium was reddish in colour, hyaline and slender in nature. Culture was showing regular growth pattern at margin and whitish mycelium at margin on PDA medium plate as shown in Fig. 3. Microconidia were fusiform to reniform or oval and had 0-1 septa as shown in Fig. 4 (Gigaulia, 2019). The above studies on the morphological and cultural characters of isolated *Fusarium* sp. showed the close identity with *F. udum* as described by Patel *et al*., (2012); Pande *et al*., (2012 and 2013).

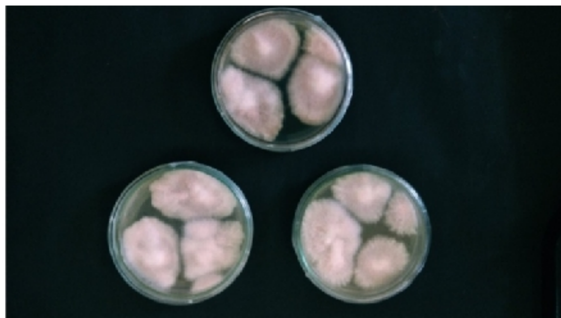


Fig. 3. Pure culture of *F. udum.*

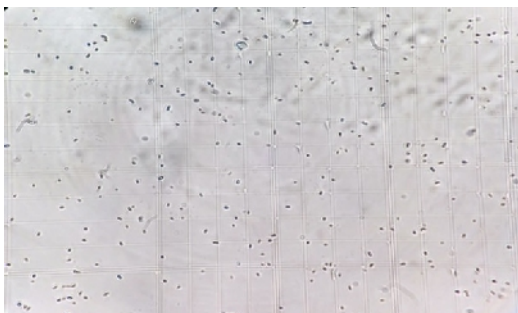


Fig. 4. Spore suspension of *F. udum.*

Gene expression in Real Time PCR analysis: Fresh lateral root tissues of 20 days old seedlings of pigeonpea were collected after inoculation of fungal spore suspension culture at four different time interval stages *viz.* 0, 48, 72 and 120 hours after infection along with uninfected plant as control sample. Okiror, (1998) reported that root dipping led to start of wilting from four weeks after inoculation. Wilted plant roots were trimmed and dipped in the most concentrated inoculum suspension. Wilting increased with time and in all cultivars and root treatments. Mishra and Dhar (2005) found that under water culture techniques, observations on number of wilted seedlings recorded at 3, 5, 7, 9, 11 and 15 days after inoculation. First wilting was recorded at five days after inoculation. Final wilt counts taken at 15 days after inoculation, the control seedlings did not show any wilting symptoms.

Relative expression analysis of *CAT* gene in Asha variety: Relative expression of *CAT* gene in Asha variety was found maximum in all infection stages. At 0 hours *CAT* expression was raised two folds in both control and diseased plants. At 48 hours gene expression was increased by 3 folds in control plants and increased by 11 folds in diseased plants. At 72 hours *CAT* expression was increased 4 folds in control plant and it raised 16 folds in diseased plant. In the last 120 hours after infection *CAT* expression in control plant was increased by 4 folds and 24 folds found in diseased plant.

Relative expression analysis of *CAT* gene in Bahar (ICP-2376) variety: The expression level of the *CAT* gene tested in ICP-2376 variety with different infection hours. Though ICP-2376 was wilt susceptible variety and the *CAT* activity did not increased as compare to wilt resistant varieties. Relative expression was obtained in all infected stages. At 0 hours expression was raised by 2 folds in both diseased and control plants. At 48 hours expression was increased by 2 folds in control plants and increased by 4 folds in diseased plants. At 72 hours expression was enhanced by 3 folds in control plants and 8 folds in diseased plants. After 120 hours of infection stage, expression in control plants increased by 5 folds and 13 folds was found in diseased plants.

Relative expression analysis of *CAT* gene in TJT-501 variety: The expression level of the gene tested in TJT-501 variety at four different stages after infection. Though TJT-501 variety was wilt resistant and the *CAT* activity goes upsurges when disease occurred in plants. Relative expression was found maximum in all infection stages. At 0 hours expression was raised 2 folds in control plants and 3 folds in diseased plants. At 48 hours expression was increased by 4 folds in control plants and increased by 10 folds in diseased plants. At 72 hours expression was enhanced by 4 folds in control plants and it raised 16 folds in diseased plants. After 120 hours, gene expression in control plants increased by 5 folds and 25 folds found in diseased plants.

The data of relative ratio revealed that the concentration of *CAT* gene was higher in resistant varieties (Asha and TJT-501) and lowest in ICP-2376 variety (susceptible) under wilt infected condition as shown in Fig. 5. The relative expression of *CAT* gene in three varieties Asha, Bahar and TJT-501 have been analyzed and graphical representation shown in Fig. 6 (Gigaulia, 2019). Likewise, Li *et al*., (2011) also observed that, the expressions of H_2O_2-related genes were quickly reprogrammed after inoculation in banana, when they determined *SOD* and *CAT* transcript levels. Unexpectedly, the *CAT* transcripts were likely to be kept at its initial level in each line and only a little statistical significant difference was observed between the two lines during the whole process. In the present investigation, resistant varieties of pigeonpea also express the genes which increases there activity at the time of severe pathogen infection. The gene makes certain types of proteins that participate against the pathogen attack. These genes are present in whole genome and that can be screened out by molecular markers, and their expression can be studied by molecular techniques like qRT-PCR. Gene expression can be understood by comparing these specific genes to housekeeping genes. Housekeeping genes are those genes which always activate in all types of condition either favorable or unfavorable. By comparing these wilt resistance genes with housekeeping genes, the expression of wilt resistant genes can be understood.

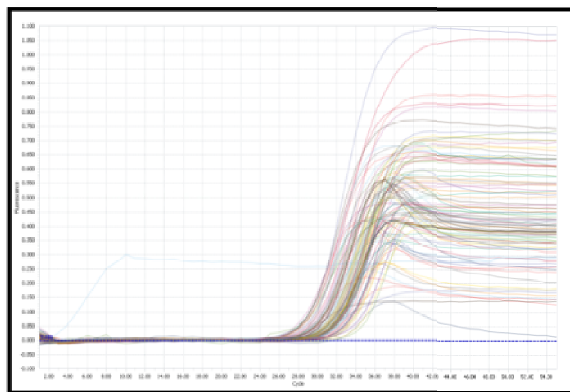


Fig. 5. Amplification Curve of *CAT* gene expression.

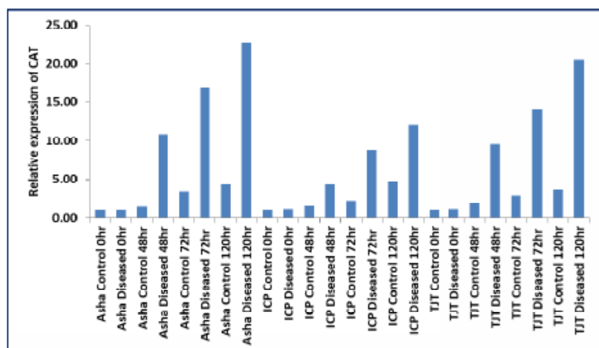


Fig. 6. Relative expression analysis of *CAT* gene in pigeonpea.

CONCLUSION

Identification of different wilt resistant and wilt susceptible pigeonpea varieties was achieved by the use of SSR markers. SSR marker helps in the selection of diverse pigeonpea varieties for crop improvement programme and also useful for efficient screening of the germplasm. Total 18 different alleles were generated using 13 SSR primers. It is also concluded that *Fusarium udum* can easily and rapidly grow in Potato Dextrose Agar (PDA) media at optimum temperature and light. The inoculation procedure for causing wilt disease in plants by spore suspension method was also optimized. Wilt resistant gene present in wilt resistant plant expresses when fungal attack or biotic stress occurs. The expression of genes correlates with infection levels in response to changes in environmental conditions even in control condition. The qRT-PCR technique has validated its potential for further use in gene expression analysis of other targeted genes involved in pigeonpea against wilt disease. The polymorphic bands of SSR markers can be converted into SCAR markers for better results of genetic diversity in pigeonpea. Varieties of different agronomical, morphological characters and geographical origin may be involved in the analysis with broad genetic base and for finding varietal identification. Morpho-physiological, biochemical, and molecular parameters were successfully used during gene expression against wilt resistant and tolerant pigeonpea genotypes. Some other important gene associated with wilt resistant and tolerant pigeonpea genotypes can be analyzed at different time interval after infection. These genes can be used for many other crops having problem of wilt disease.

FUTURE SCOPE

Due to limited studies reported so far in pigeonpea (*Cajanus cajan*) the genome wide SSR markers would facilitate assessment of genetic distance in the genetic resources that would be useful for diversity studies, genome mapping, trait mapping and assessment of gene flow between populations in pigeonpea. Housekeeping genes such as *EF1 , UBQ10, 18SrRNA, 25SrRNA, ACT1, UBC, GAPDH, HSP90 etc.* can be studied in pigeonpea for expression analysis. Study on plant parts such as root, stem and leaves tissues of pigeonpea will also be useful for gene expression in pigeonpea especially under biotic/abiotic stress conditions.

Conflict of Interest. Nil.

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How to cite this article: Gigaulia, P., Tantwai, K., Nema, S. and Singh, S.K. (2021). Expression of *CAT gene* Resistance against Fusarium Wilt in Pigeonpea. *Biological Forum – An International Journal*, *13*(3): 255-261.