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Candidate gene expression profiling during wilting in chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* in race 2 and race 4

Sujata N Dandale, SS Mane, ST Ingle, AN Patil, RS Nandanwal and PV Jadhav

Abstract
The Chickpea wilt, one of the most important fungal pathogen of chickpea, is a constant threat to this crop plant. In the present study gene expression analysis of chickpea roots during Foc infection was performed using various approaches *viz.* cDNAs derived from total mRNA during infection process of susceptible (JG-62) and moderately resistant (Jakki-9218) and resistant (WR-315) cultivars, were amplified using random oligonucleotides. The sequence characterization of differentially expressed transcripts revealed their homology with many plant genes essential for various metabolic functions including defense. Further, expression patterns of specific candidate gene transcripts were analyzed in the Foc inoculated and uninoculated resistant moderately resistant and susceptible chickpea cultivars, on day 7th and 14th days of infection. The semiquantitative RT-PCR analysis of defense related genes was performed using gene specific oligonucleotides in resistant moderately resistant and susceptible chickpea cultivars. The expression of fungal pathogenesis related genes and their race specific response was determined throughout the course of chickpea- FOC interaction. Temporal expression and race specific response of plant defense related and fungal virulence genes were studied in the resistant and susceptible cultivars of chickpea inoculated with three races of Foc highlighting the host-pathogen interactions. Few genes, involved in chickpea defense against Fusarium wilt which was not reported previously were unveiled in this study.

Keywords: *Cicer arietinum* L., *Fusarium oxysporum* f. sp. *ciceri*, Chitin synthase, 18s r RNA, Fgb1, Betv1 genes

1. Introduction
Chickpea (*Cicer arietinum* L.) is an important pulse crop**.** The chickpea has diploid genome (2n) comprising 8 pairs of chromosomes (2n=16) with genome size approximately ~738 Mbp (Jain *et al*., 2013; Varshney *et al*., 2013) [10, 16]. A range of biotic and abiotic stresses affect stable and high yields of chickpea crop worldwide. The *Fusarium* wilt of chickpea has the potential to devastate the entire crop and cause tremendous losses.
The *F. oxysporum* f.sp. *ciceri* is a seed borne (Erwin and Snyder, 1958) [7] and present in the helium of the diseased seeds (Haware *et al*., 1978) [9]. It has the low saprophytic ability, is capable of colonizing subtracts precolonized by a saprophytic or antagonistic micro-organism (Biswas and Gupta, 1981; Satyaprasad *et al*., 1989) [6, 13]. It could survive on crop residues in soil for more than six years in the form of resting spore *viz.* chlamydospores.
The morphological identification of *F. oxysporum* f. sp. *ciceri* isolates / races is difficult because of varied morphological variations. Individual pathogenic strains within the species; having an ability to infect a particular host range have been assigned to intraspecific groups called forma specials. Some of the forma specials are further divided into races based on differential virulence to a set of cultivars e.g. *F. oxysporum* f. sp. *ciceri* (FOC) infecting chickpea. Similar to other soil borne diseases, various strategies have been employed for controlling Fusarium wilt, such as, use of chemical fungicides, biological control, etc., but most of these strategies have proven ineffective or have hazardous effect. Widely accepted and cost effective strategy is to develop and use wilt resistant cultivars. A complex interaction between plant and its fungal pathogen is an outcome of expression of both, plant defense genes as well as fungal pathogenesis related genes. The result of such a relationship is projected as either resistance or disease development in the plant. There are multiple events involved that lead to successful plant defense during pathogen attack.

Further, these defense mechanisms are governed by an array of genes, which are either singly or synergistically, involved in plant resistance traits. Many defense related genes have been cloned and characterized in an attempt to elucidate the mechanism of defense upon *F. oxysporum* attack in various plant species, including chickpea. In our earlier studies, enzymes like glucanases, chitinases and proteases have been shown to be probably involved in chickpea defense against FOC infection. However, exact molecular mechanisms involved in chickpea resistance are still unexplored. Understanding the pathogenicity mechanism of fungi, on the other hand, demands knowledge of the virulence factors, which are active in the host environment. Till date, many pathogens have been studied in context to their virulence and various genes with a prime role in fungal pathogenesis have been identified. Chitin synthase genes and transcription factors like Ftf1 are essential for virulence of Fusarium. Since then, contribution of few more genes in pathogenesis of *F. oxysporum* has been established. Race specificity is an important criterion in fungal pathogenesis, and plant defense response is dependent on it. In this study, we have analyzed the race specific expression of various plant and fungal genes and identified various up-regulated transcripts using several approaches. Crop plants are constantly exposed to their pathogens in the field which has directed us to consider a wide time-scale (7^{th} – 14^{th} dai) for the expression analysis of plant and fungal genes involved in the chickpea- Foc interaction. An attempt has also been made to analyze the expression pattern of four important fungal genes previously reported to be essential for growth and pathogenesis, namely Fgb 1 (G protein subunit), Gas1 (glucanosyltransferase), chs7 (chitin synthase chaperonin) and Fow 1 (mitochondrial carrier protein). The host-pathogen interaction using chickpea- Foc system has been unveiled in this study.

The plant-pathogen interaction is complex phenomenon which accomplished by chain of events of plant defense genes as well as pathogenesis related genes. The end result of such a relationship projects either as host resistance or disease development in the plant. This massive reprogramming of gene expression in plants may varied with the different biotype, which gives an inside idea of expression of genes in relation to different biotype against the different cultivars.

2. Materials and Methods

The present investigation entitled "Molecular variability amongst the races of *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea*"* was carried out at Department of Plant Pathology, Dr. P.D.K.V., Akola during academic year 2016-2021.

2.1. Fungal Cultures

The standard races of FOC namely, 1. Race-2 *Viz*. FOC-60 [Kanpur, UP- Northern plain hot sub humid (dry) eco-region (AER 9.2)], 2. Race-4 *Viz.* FOC-7692 [Jabalpur, MP- Central Highlands, Hot sub-humid (dry) ecoregion (AER 10.1)]. Molecular diversity and race specificity of these cultures were established in the laboratory. The cultures were maintained on Potato Dextrose Agar (PDA) slants with timely sub-culturing and infection to a susceptible cultivar, JG-62.

2.2. Plant Material

The *Cicer arietinum* (L.) seeds of cultivars, susceptible cultivar – JG-62, Moderately resistant cultivar- Jaki-9218 and WR-315 (Warangal) as a resistant cultivar to Fusarium wilt, used in this study were obtained from Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra, India.

2.3. Seed Germination and Inoculation

The seeds were wrapped in wet sterile muslin cloth and stored at room temperature (24˚C to 26˚C) till sprouting. The sprouted seeds were transferred to trays containing sterile water with macro-and micro-nutrients (half strength Hoagland's nutrient medium) and kept at 22˚ C and 60% relative humidity under normal day conditions (14 hrs light /10h dark). Freshly prepared spore suspension (1×10^6 spores / mL) of FOC races 2 and 4 was added individually to the sterile sand trays containing seven days old chickpea plants. Seedlings grown in similar trays with no pathogen (un-inoculated plants) served as control.

2.4. Sample Collection and RNA Extraction

Total RNA was extracted from chickpea roots collected at time intervals such as 7 and 14 days after inoculation (DAI) and immediately frozen in liquid nitrogen (N_2) until further use. Later, the tissue was crushed and 100mg of tissue was used for RNA extraction using TRIzol reagent (Invitrogen, USA) or TRI Reagent (Sigma Aldrich, USA) as described by Nimbalkar *et al*. (2006). The DNase treated RNA was used for the first strand cDNA sysnthesis using RT-PCR kit (Promega, Madison, WI, USA). Undiluted or 1:100 diluted cDNA was used for PCR using RAPD oligonucleotides (OPAD and OPAE series, Operon technologies, Huntsville, AL, USA) or gene specific oligonucleotides.

2.5. RNA extraction and cDNA synthesis

The chickpea roots tissue 200 mg was pulverized in liquid nitrogen (N_2) and total RNA was extracted by Trizol reaction method. The homogenized root tissues 200µg were mixed in 1µl of trizol placed at room temperature for 5min. Then 200µl chloroform (2M) was mixed. The tubes were inverted and placed at room temperature for 2 min. The ependrof tube were centrifuge at 12000rpm for 20min at 4°C.The supernatant were collected and placed in fresh ependrof tube and 250µl of 2M Nacl and 200 µl of Isopropanol was added. The tubes were then inverted and incubated for 1 hr. at room temperature. The samples were centrifuge at 12000 rpm for 10 min. at 24°C.The supernatant were discarded and the pellets were washed with ice cold 70% ethanol (40 µl) to remove the traces of plant debris. The supernatant were removed and pellets were air dried in laminar airflow. The nuclease free water 25 µl were added and placed at 4°C to dissolve the pellets. The RNA samples were quantified by spectrophotometer at 260-280 nm or stored at -80^0 C until used also the m-RNA's were checked on 0.8% agarose gel.

2.5.1. Cloning of Amplified cDNA Fragments and Their Sequence Characterization: The cDNAs isolated from FOC inoculated and uninoculated JG-62, Jaki-9218 and WR-318 plant roots were used as templates for amplification with RAPD oligonucleotides (OPAD and OPAE series, Operon technologies, USA). The amplified product from cDNA of resistant inoculated cultivar, WR-315 was cloned in pGEM-t easy vector (Promega, USA). Cloned cDNA fragments were sequenced by di-deoxy termination method (Sanger method) and the sequences were determined using an automated DNA sequencer (NCBI, U.S.National Library of Medicine, USA). The nucleotide sequences were compared with the reported nucleotide sequences in Genbank non-redundant database using the BLAST sequence alignment program.

Oligonucleotides designed from amplified sequences were used for expression analysis of transcripts in inoculated and un-inoculated cDNA pool of WR-315, Jaki-9218 and JG-62 root tissues.

2.5.2. cDNA synthesis (MBT076)

The first strand cDNA was synthezed as per the protocol given in the HIMEDIA script kit (MBT-076). Initially the kit solutions were kept at room temperature, the cDNA reaction was made with following steps. For synthesis of cDNA (Table 1), a total of 4µl RNA sample, 1µl Oligo (dT) and 9µl sterile nuclease free water were short spin in PCR tube and incubated at 65^0C for 5 minutes. The samples were chilled in ice for 5 min. After incubation the reaction mixture, a total volume of 20 µl, Template RNA primer mixture (From 2 step) 10 µl, 4 µl RT buffer for MMuLV,10X solution for MMuLV 2µl, 1µl RNase H- and 0.5 µl ribonuclease Inhibitor, 2 µl dNTP mix, added in same PCR tube and the volume was made 20µl. The reaction mixtures were gently mix and ensured that all the components were at the bottom of the amplification tube and it was centrifuge briefly. For preparation of cDNA following PCR cycle was used as follows.

Table 1: Preparation of cDNA with Hi-media (MBT076) kit

Sr.	Oligo (dT)	temperature	No. of cycles
1.	42 °C	60 minutes	1 cycle
2.	70 °C	5 minutes	1 cycle
3.	Hold at 4 °C	Optional	Optional

The cDNAs isolated from Foc inoculated and uninoculated JG62, Jaki-9218 and WR-315 plant roots tissues were used as templates for amplification with RAPD oligonucleotides (OPAD and OPAE series, Operon technologies, USA). The amplified product from cDNA of susceptible, moderately resistant and resistant inoculated cultivar and uncontrolled cultivar, was cloned in pGEM-t easy vector (Promega, USA). Cloned cDNA fragments were sequenced by dideoxy termination method (Sanger method of sequencing) and the sequences were determined using an automated DNA sequence (MegaBACE1000, Amersham Biosciences, NJ, USA). The nucleotide sequences were compared with the reported nucleotide sequences in Genbank non redundant database using the BLAST sequence alignment program. The oligonucleotides designed from amplified sequences were used for expression analysis of transcripts in inoculated and un-inoculated cDNA pool of WR-315, Jaki-9218 and JG62 root tissues. Plant specific cDNA pool was normalized using 18s rRNA oligonucleotides while fungal specific cDNA pool was normalized using ITS oligonucleotides.

2.6. Expression Analysis of Known Specific Genes

Gene specific oligonucleotides were designed from the conserved regions of plant defense related genes (Table 3) and fungal virulence related genes (Table 2) using sequences available in the NCBI Genbank database (database-fungi and database- Fabaceae) (http://www.ncbi.nlm.nih.gov).

Table 2: Pathogenesis related primers used for expression analysis of chickpea- Foc transcriptome.

Sr.	Gene	Primers (5'- 3')
1	Tri	F: CTGTCGGTAGCCGAAGACTC R: CGTGAGTTTGCGACAAAGAA
2	Gas1	F: GACTCCGACCTCTGCGACT R: CTTTTACAGAAGGACCATACCG
3	Chs7	F: GCTGGGCGTTATGATGGT R: GCGAGTAAGGCAGATCATAG
4	Fgb1	F: GTGATGAGCATCAGCCTCAA R: CAACGAGAGAGCCAACCTTC
5	Fo-transposase	F: TTGGCATGAGATTGTACCCAG R: GGGGTTTTACGTCTTGGC

Table 3: Defence related primers used for expression analysis of chickpea- Foc transcriptome.

Sr.	Gene	Primers (5'- 3')
1	H2A	F: CTGCTACAACCAAGGGAGGA R: AGATCCCAAAAGCTTGCTGA
2	Betv1	F: TCACGATGTGCAAAACCATT R: TCCATGTATCCATTTGGAGGA
3	60srp	F: GATCAGGGGAATCGGAAAAT R: GACTGATTCACACGCCTCAA
4	glyt	F: CTGCACTTGACCGGTGTG R: TCAAAGCATGAAGTCGCATC
5	CHS	F: GAGCTGCTGCATTGATTGTTGG R: CACCCCATTCAAGTCCTTCTCC
6	18sRNA	F: AAACGGCTACCATCCAAG R: TCATTACTCCGATCCCGAAG

2.7. Genbank Accession Numbers: BankIt 2563415,

3. Results and Discussion

3.1. Pathogenicity Assays

The seven days old chickpea plants were inoculated with fungal spores of FOC race 2 and race 4 individually and uninoculated plants served as control. In general, WR-315 cultivar was found to grow slower as compared to Jaki-9218 and JG-62 cultivar. Upon infection JG-62 showed wilting symptoms by 10 DAI, while no wilting was observed in WR-315 even by 25 DAI (till the plants were allowed to grow hydroponically). Within FOC infected JG-62; race 2 infected JG-62 showed wilting symptoms earlier followed by race 4. Figure 1 shows the chickpea cultivars, JG-62, Jaki-9218 and WR-315, uninoculated (control) and race 2 and 4 inoculated (showing wilting symptoms). Root tissues of JG-62, Jaki-9218 and WR-315 cultivars (uninoculated and inoculated with FOC races) were further used for gene expression studies from several biological as well as technical replicates. For RAPD analysis Foc-2 infected tissue at 7 dai, was used while for candidate gene expression studies Foc 2 and 4 inoculated tissues were analyzed at different time points from 7^{th} – 14^{th} dai.

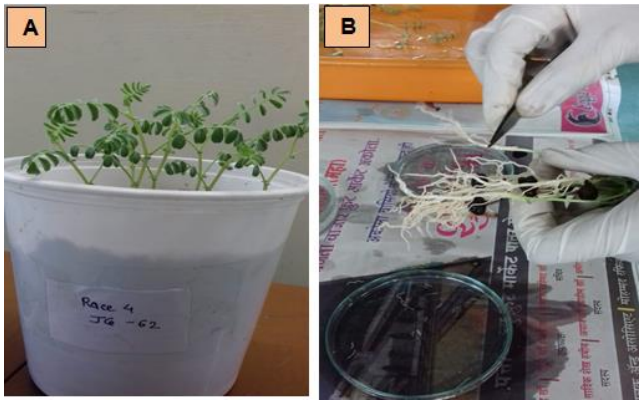


Fig 1: A. Chickpea plants inoculated with *F. oxysporum* f. sp. *ciceri.* B. Collections of chickpea root tissue for isolation of mRNA inoculated with races of *F. oxysporum* f. sp. *ciceri.*

3.2. cDNA-RAPD of inoculated resistant, moderately resistant and susceptible chickpea cultivars

Previously, Nimbalkar *et al*. (2006) reported cDNA- RAPD and cDNA-AFLP analysis with Foc 2 inoculated susceptible (JG-62), moderately resistant (Jaki-9218) and resistant (WR-315) chickpea varieties from our lab. In this study, the expression of transcription factors such as Extracellular calcium-sensing receptor proteins, Nitric oxide reductase proteins, Growth harmone releasing hormone receptor protein, Cytochrome C oxidase Cbb-3 type subunit I protein, Hydroxynitrite lyase protein, Tir chaperone protein, Glutamate receptor ionotropic, kainite 3 protein, endo-1,4-beta mannanase protein, 60 S ribosomal protein L2-A and L3 protein, Formate-Tetrahydrofolate ligase protein, Mitochondrial calcium uniporter protein and 40S ribosomal S27 protein gene sequences and other genes during chickpea-Foc2 interaction was analyzed.

To explore these interactions further, transcript profiling of root cDNAs obtained from Foc 2 inoculated JG-62,Jaki-9218 and WR-315 (more resistant to Fusarium wilt than cultivar Jaki-9218 and JG-62) cultivars at 7th dai, was accomplished, using RAPD oligonucleotides of OPAD and OPAE series (20 oligonucleotides each) in the present study. Differentially expressed and reproducible 134 transcripts exhibiting >200 bp size were selected for cloning and 117 clones thus obtained, were further sequence characterized.

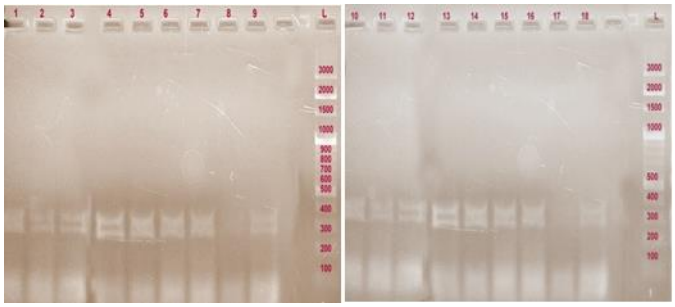
3.3. Involvement of important fungal and plant specific genes during Foc wilt

All the oligonucleotides designed from the sequence information obtained as above were used for semi-quantitative RT-PCR in case of JG 62, Jaki-9218 and WR-315 inoculated with Foc race 2 and race 4. However, only eight of these oligonucleotides pairs could reveal consistently reproducible and differential amplification profiles and were used for further analysis shows the differential expression pattern of plant defense related and fungal specific transcripts of WR-315, Jaki-9218 and JG62 at 7 DAI. Although resistant cultivar WR-315 showed no wilting symptoms upon Foc inoculation, fungal genes were found to be expressed; indicating the fungal growth and proliferation in the resistant cultivar. In this study, fungal Tri101 gene was found to be unregulated during Foc-chickpea interaction, although not all the species of the genus Fusarium express trichothecene. However, some species have been reported to harbour both, functional and non- functional copies of Tri genes. Semi quantitative RT-PCR revealed the expression of transposase gene during fungal invasion of chickpea roots. Transcription of fungal transposons is known to occur during carbon or nitrogen starvetion condition; which mimics the pathogenesis conditions in fungi. Apart from the above mentioned fungal genes, several plant genes were found to express in WR-315 upon Foc inoculation. Histone proteins are involved in chromosome duplication of eukaryotic genome and are shown to be over expressed in plants during pathogen attack

3.4. Plant Defense Related Gene Expression in Chickpea upon Inoculation with FOC Races 2 and 4

3.4.1. Betv1

The Pathogenesis Related proteins (PR proteins) are reported to be extensively involved in chickpea defence against various pathogens. The BetvI which is known to show significant homologies is a major pollen allergen of several plant species, reproducible, race specific and prolonged expression (7th – 14th DAI) in all chickpea cultivars. In our findings, susceptible cultivar JG-62 at both 7th and 14th DAI it was expressed but not in uninoculated at 7th DAI. In Jakki-9218 (moderately resistant) at 7th DAI showed no expression in race 2 and 4 at, but expressed at 14 DAI. In the same manner WR-315 cultivar found expressed at 7th and 14th DAI but it was not expressed at 14th DAI in race4In inoculated plants (Fig. 2), The BetvI expression was pronounced around early stages of infection. This is in accordance with the previous studies conducted by Foster-Hartnett *et al*. (2007) where, during infection by *Erysiphe pisi*, resistant and susceptible cultivars of *Medicago truncatula* showed transcript accumulation.



1. A1-(JG-62, R2, 7DAI), 2. A2-(JG-62, R-4, 7DAI), 3.A3-(JG-62, Uninoculated, 7DAI), 4.B1-(Jaki-9218, R-2, 7DAI), 5.B2-(Jaki-9218, R-4, 7DAI), 6.B3-(Jaki9218, Uninoculated, 7DAI), 7.C1-(WR-315, R-2, 7DAI), 8.C2-(WR-315, R-4, 7DAI), 9.C3-(WR-315, Uninoculated, 7DAI), L-Ladder, 10.A4-(JG-62, R-2, 14DAI), 11.A5-(JG-62, R-4, 14DAI), 12.A6-(JG-62, uninoculated, 14DAI), 13.B4-(Jaki-9218, R-2,14DAI), 14.B5-(Jaki-9218, R-4, 14DAI), 15.B6-(Jaki-9218, uninoculated, 14DAI), 16.C4-(WR-315, R-2, 14DAI), 17.C5-(WR-315, R-4, 14DAI), 18.C6-(WR-315, uninoculated, 14DAI).

Fig 2: The banding pattern of Betv 1 gene in gene profiling

3.4.2. H2A

The gene H2A expressed in all three cultivars with both the races except JG-62 inoculated with race 4 of *F. oxysporum* f. sp. *ciceri* at 14th DAI, Jaki-9218 inoculated with race4 of *F. oxysporum* f. sp. *ciceri* at 7th DAI and in uninoculated control of cultivar Jaki-9218 at 14th DAI (Fig. 3).

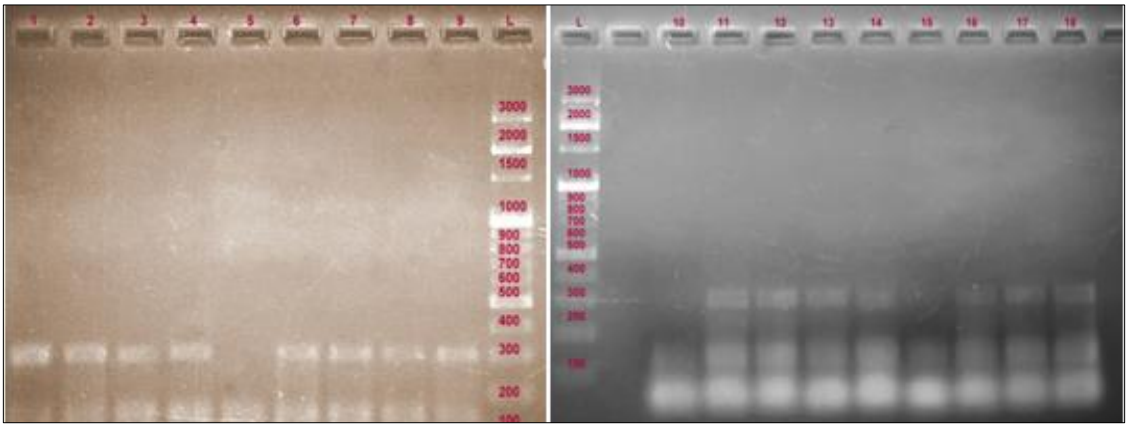
Nucleosomes are a distinguishing feature of eukaryotes. They consist of two copies of each of the four core histones H2A, H2B, H3, and H4. Each nucleosome prevents accessibility to ~147 bp of DNA (Luger *et al*., 1997) [11]. As a consequence,

nucleosomes act as general repressors of transcription. Posttranslational modifications of core histones have been associated with transcriptional regulation but the extent to which chromatin directly controls the transcriptional machinery remains a matter of controversy. Histone variants are isoforms of core histones that acquired comparable properties and functions through convergent evolution (Talbert *et al*., 2012; Talbert and Henikoff, 2017) [14-15]. Bingkun Lei and Frederic Berger (2020) [5] studied the genome-wide distribution patterns and deposition of H2A. Z in plants as well as its association with histone modifications and roles in plant chromatin regulation. Eukaryotic nucleosome prevents access to the genome. Convergently evolving histone isoforms, also called histone variants, form diverse families that are enriched over distinct features of plant genomes. Among the diverse families of plant histone variants, H2A.Z exclusively marks genes.

3.4.3. 18s rRNA

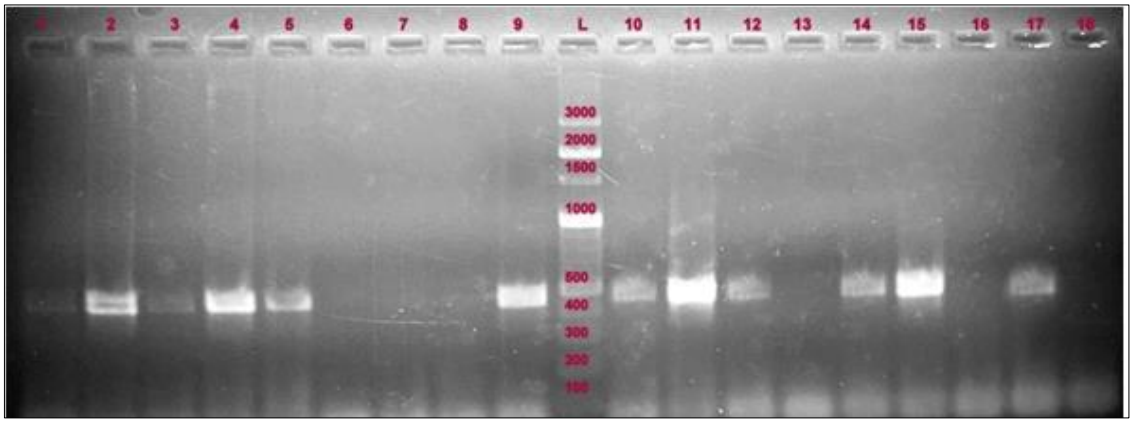
In present investigation showed that at 7th DAI in JG-62 cultivar found expression in race 2, 4 and un-inoculated control, Jakki-9218 expressed at race 2 and 4 but not in controlled condition, and WR-315 cultivar expressed on in controlled condition but not found expression in race 2 and 4. In the same way 14 DAI, JG-62 expressed at race2, 4 and control condition. Jakki-9218 expressed in race 4 and un-inoculated but no expression found in race 2 and resistant cultivar WR-315 expressed only in race 4 but no expression of gene found in race 2 and uninoculated (Figure 4).

Molecular taxon identification is mainly based on marker gene sequencing whose sensitivity, resolution and throughput are controlled by the choice of the marker gene and sequencing platform. The most prominent fungal phylogenetic markers are the 28S and the 18S rRNA gene sequences. 18S rRNA gene sequence primers have been designed as fungi-specific, however, characteristics, overall fungal and group-specific coverage rate, and possible co-amplification with non-fungal eukaryotic taxa are rarely reported and comparisons among primer pairs are generally lacking.



1. A1-(JG-62, R2, 7DAI), 2. A2-(JG-62, R-4,7DAI), 3. A3-(JG-62, Uninoculated, 7DAI), 4. B1-(Jaki-9218, R-2,7DAI), 5. B2-(Jaki-9218, R-4,7DAI), 6. B3-(Jaki 9218, Uninoculated, 7DAI), 7.C1-(WR-315, R-2,7DAI), 8. C2-(WR-315, R-4,7DAI), 9. C3-(WR-315, Uninoculated, 7DAI), L-Ladder, 10. A4-(JG-62, R-2,14DAI), 11.A5-(JG-62,R-4,14DAI), 12. A6-(JG-62, uninoculated, 14DAI), 13. B4-(Jaki-9218, R-2,14DAI), 14. B5-(Jaki-9218, R-4,14DAI), 15. B6-(Jaki-9218, uninoculated, 14DAI), 16.C4-(WR-315, R-2,14DAI), 17. C5-(WR-315, R-4,14DAI), 18. C6-(WR-315, uninoculated, 14DAI).

Fig 3: The banding pattern of H2A gene in gene profiling



1.A1-(JG-62, R2, 7DAI), 2.A2-(JG-62, R-4, 7DAI), 3.A3-(JG-62, Uninoculated, 7DAI), 4.B1-(Jaki-9218, R-2, 7DAI), 5.B2-(Jaki-9218, R-4,7DAI), 6.B3-(Jaki9218, Uninoculated, 7DAI), 7.C1-(WR-315, R-2, 7DAI), 8.C2-(WR-315, R-4, 7DAI), 9.C3-(WR-315, Uninoculated, 7DAI), L-Ladder, 10.A4-(JG-62,R-2,14DAI), 11.A5-(JG-62,R-4,14DAI), 12.A6-(JG-62, uninoculated,14DAI), 13.B4-(Jaki-9218, R-2,14DAI), 14.B5-(Jaki-9218,R-4,14DAI), 15.B6-(Jaki-9218,uninoculated,14DAI), 16.C4-(WR-315, R-2, 14DAI), 17.C5-(WR-315, R-4, 14DAI), 18.C6-(WR-315,uninoculated,14DAI).

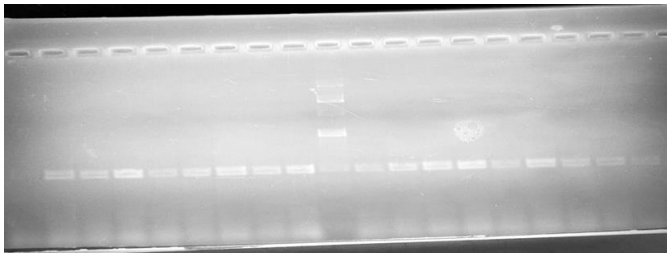
Fig 4: The banding pattern of H2A gene in gene profiling

4. Fungal pathogenesis related gene expression in *F. oxysporum* f. sp. *ciceri* inoculated chickpea during wilt progression.

F. oxysporum G protein subunit: Fgb 1 Gene:

In the present investigation, race specific response of Fgb1gene was studied by using the primer (F:GTGATGAGCATCAGCCTCAA, R:TGATGAGCATCAGCCTCAA) on the cDNA of challged and unchallenged cultivars chickpea JG-62 (susceptible), Jakki-9218(moderately resistant) and WR315 (resistant) with in race 2 and 4 of *F. oxysporum* f.sp. *ciceri*. A specific monomorphic band of 500 bp was observed on 1.5 agarosel gel.

The results indicated (Figure 5) showed that, the race 2 and race 4 showed progressively intense expression of Fgb1 gene till 14 DAI infections. In the susceptible cultivar JG-62 no expression of Fgb1 gene was observed at 7th DAI with race 2. In moderately resistant cultivar (Jakki-9218) and resistant (WR-315) it expressed at 7th and 14th day after infection.



1.A1-(JG-62, R2, 7DAI), 2.A2-(JG-62, R-4, 7DAI), 3.A3-(JG-62,Uninoculated, 7DAI), 4.B1-(Jaki-9218, R-2, 7DAI), 5.B2-(Jaki-9218, R-4, 7DAI), 6.B3-(Jaki9218, Uninoculated, 7DAI), 7.C1-(WR-315, R-2,7DAI), 8.C2-(WR-315, R-4, 7DAI), 9.C3-(WR-315, Uninoculated,7DAI),L-Ladder, 10.A4-(JG-62, R-2,14DAI), 11.A5-(JG-62,R-4, 14DAI), 12.A6-(JG-62, uninoculated,14DAI), 13.B4-(Jaki-9218,R-2,14DAI), 14.B5-(Jaki-9218,R-4,14DAI), 15.B6-(Jaki-9218,uninoculated,14DAI), 16.C4-(WR-315, R-2, 14DAI), 17.C5-(WR-315, R-4,14DAI), 18.C6-(WR-315, uninoculated, 14DAI).

Fig 5: The banding pattern of Fgb 1 gene in gene profiling

5. Chitin synthases 7: Chs7

In the present investigation, race specific response of Chs 7gene was studied by using the primer (F: GCTGGGCGTTATGATGGT, R:GCGAGTAAGGCAGATCATAG) on the cDNA of challged and unchallenged cultivars chickpea JG-62 (susceptible), Jakki-9218 (moderately resistant) and WR315 (resistant) with in race 2 and 4 of *F. oxysporum* f. sp. *ciceri*. A specific monomorphic band of 200 bp was observed on 1.5 agarosel gel the specific band of 200bp was eluted and sequenced (Annexure II).

The results showed in (Figure 6), Chs7 gene expression was initially weak, however, its prolonged expression was observed in case of race2 with JG 62 (susceptible) and no expression of gene was observed with race 4. In case of race 2 and 4 both were found expressed in moderately resistant cultivar Jakki-9218. Where as at 14 DAI susceptible cultivar JG-62 showed non of expression in controlled condition. In challenged condition with race 2 and race 4 was found expressed, in Jakki-9218 moderately resistant cultivar was found no expression and inoculated WR-315 (resistant) with race 2 and 4 showed expression, while in control didn't showed any expression.

6. References

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