A Molecular Insight into the Early Events of Chickpea (*Cicer arietinum*) and *Fusarium oxysporum* f. sp. *ciceri* (Race 1) Interaction Through cDNA-AFLP Analysis

Sumanti Gupta, Dipankar Chakraborti, Rumdeep K. Rangi, Debabrata Basu, and Sampa Das

First, second, third, and fifth authors: Plant Molecular and Cellular Genetics, Bose Institute, Centenary Campus, P1/12 C.I.T. Scheme VII M, Kankurgachi, Kolkata-700054, India; and fourth author: Botany Department, Bose Institute, Main Campus, 93/1, A.P.C. Road, Kolkata-700009, India.

Current address of D. Chakraborti: Department of Biotechnology, St. Xavier's College, 30, Park Street, Kolkata-700016, India.

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ABSTRACT

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Wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the most severe diseases of chickpea throughout the world. Variability of pathotypes of *F. oxysporum* f. sp. *ciceris* and breakdown of natural resistance are the main hindrances to developing resistant plants by applying resistant breeding strategies. Additionally, lack of information of potential resistant genes limits gene-transfer technology. A thorough understanding of *Fusarium* spp.-chickpea interaction at a cellular and molecular level is essential for isolation of potential genes involved in counteracting disease progression. Experiments were designed to trigger

the pathogen-challenged disease responses in both susceptible and resistant plants and monitor the expression of stress induced genes or gene fragments at the transcript level. cDNA amplified fragment length polymorphism followed by homology search helped in differentiating and analyzing the up- and downregulated gene fragments. Several detected DNA fragments appeared to have relevance with pathogen-mediated defense. Some of the important transcript-derived fragments were homologous to genes for sucrose synthase, isoflavonoid biosynthesis, drought stress response, serine threonine kinases, cystatins, arginase, and so on. Reverse-transcriptase polymerase chain reaction performed with samples collected at 48 and 96 h postinfection confirmed a similar type of differential expression pattern. Based on these results, interacting pathways of cellular processes were generated. This study has an implication toward functional identification of genes involved in wilt resistance.

Plants are constantly threatened by a number of pathogens. They face such challenge by inducing cellular reprogramming, which takes place through concerted activities of genes related to the stress-signaling pathways (2). Both biotic and abiotic stress inducers trigger some common signaling pathways in plants and, thereby, have the ability to modulate the effect of each other through some crosstalk (4).

Among many threatened crops, chickpea (*Cicer arietinum* L.) is the most important legume crop, which has failed to earn adequate return due to many biotic and abiotic stress factors. It is the third most important pulse crop in the world (15) and the most important one in India. The crop grows well in tropical, subtropical, and temperate regions of the world (33), including India, Turkey, Canada, Pakistan, Australia, Mexico, Burma, Ethiopia, Nepal, Bangladesh, and countries around the Mediterranean. India, with its neighboring countries, accounts for ≈90% of the total world chickpea production. North and Central America produce ≈5%, with Canada and Mexico being the major producers. European countries such as Spain and Portugal and African countries such as Tunisia and Ethiopia also produce chickpea (26). It consists of ≈25.3 to 28.9% easily digestible and hypocholesteremic protein, plus many vitamins and macroelements, for

which it is considered to be a nutritive dietary product for the mass of the undernourished countries throughout the world. However, yield of this crop is severely curtailed by the wilt causing fungus *Fusarium oxysporum* f. sp. *ciceris*. Annual losses account for 10 to 15% of the yield worldwide, with total loss under specific conditions (23). *F. oxysporum* f. sp. *ciceris* is a soil- or seedborne pathogen that has two different pathotypes. The yellowing pathotype induces progressive foliar yellowing with vascular discoloration while the wilt-causing pathotype induces severe and fast chlorosis, flaccidity, and vascular discoloration (23). The fungus colonizes in the xylem vessels and completely jeopardizes the ascent of sap, thus resulting in wilting of the crop (7). Until now, eight pathogenic races of *F. oxysporum* f. sp. *ciceris* have been identified (races 0, 1, 1B/C, 2, 3, 4, 5, and 6), all derived from a monophyletic origin (25). Races 1, 2, 3, and 4 are known to exist in India (23). All these races induce wilt of chickpea plants (25). Races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean region and the United States (California). Races 0 and 1B/C induce yellowing symptoms, while the rest cause wilt of plants. Unlike the others, only race 1 shows a wide geographical distribution throughout India, California, Morocco, and Spain. As a result, the *F. oxysporum* f. sp. *ciceris* race 1–chickpea interaction received prime investigative concern in India as well as worldwide.

Fusarium wilt of chickpea is primarily managed by the use of resistance breeding programs. However, success in breeding the resistant cultivars is limited due to various reasons, including (i) pathogenic variability amongst natural populations (25); (ii) location-specific occurrence of pathogenic races, due to which resistant cultivars lose their resistance over a period of time and

Corresponding author: S. Das; E-mail address: sampa@bic.boseinst.ernet.in

*The e-Xtra logo stands for "electronic extra" and indicates that the online version contains a table showing the primer combinations that generated distinct polymorphism.

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generations; (iii) susceptibility limits exploitation of other useful agronomic traits in certain cultivars that can otherwise fare well in terms of yield and quality; (iv) existence of specific races and their mutability slows down the progress of natural breeding programs; and (v) breakdown of resistance as a consequence of better natural selection of mutants, recombinants, and immigrants (35). Therefore, a thorough biochemical as well as molecular study of the interactions of resistant and susceptible chickpea cultivars with the pathogen is needed for effective breeding programs and producing cultivars with sustainable resistance. In addition, optimization of gene transfer technology is also an essential prerequisite for developing effective broad-spectrum resistant crops. Several reports have put forth the demonstration of biochemical changes that take place *in vivo* subject to chickpea-*Fusarium* interaction (7,19,35). Additional analytical studies on marker and molecular linkage have also been performed by many research groups (14). However, development of neither a single cultivar nor any biochemical product has been successful in imparting resistance to race 1 of *F. oxysporum* f. sp. *ciceris*. After reviewing the previous investigations performed thus far, it can be stated that many aspects are yet to be unraveled in this plant-pathogen interrelationship. Moreover, instead of emphasizing the role of a particular pathogen-induced product in disease development, it is obviously beneficial and a necessary prerequisite to understand all the defense-signaling reactions that interplay in a plant-pathogen encounter. As a result, the early, progressive, and late events of pathogenesis should be considered sequentially.

In this study, we investigated the changes that occurred in the transcriptome of both *F. oxysporum* f. sp. *ciceris* race 1-induced and -uninduced plants of wilt-susceptible JG62 and wilt-resistant WR315 cultivars by cDNA amplified fragment length polymorphism (cDNA-AFLP) analysis.

The cDNA-AFLP technique has always remained an effective and reliable tool for differential transcriptome analysis. In our present study, we have compared the transcripts of induced and uninduced wilt-susceptible and -resistant cultivars and tried to identify the overexpression and suppression of gene fragments related to the early phasic disease development. We have carried out morphological and anatomical studies to ensure the onset as well as the progression of the pathogen within the host tissue. Attempts have been made to characterize the differentially expressed transcripts detected in susceptible and resistant plant types and find out their correlation with disease development.

MATERIALS AND METHODS

Plant material. Seed of two different cultivars of chickpea (*Cicer arietinum*), JG62 (wilt-susceptible) and WR315 (wilt-resistant), were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India (23). Seed of both cultivars were sown in 15-cm pots filled with sand and synthetic soil at a ratio of 1:1. The seedlings were allowed to grow in natural greenhouse conditions at 22 to 28°C and 35 to 40% humidity. Watering was done on alternate days and biofertilizers nitrogen-phosphorus-potassium (NPK) and diaminopropane (DAP) were applied alternatively after 15 days of initial sowing. Seed were harvested ~150 to 180 days after sowing and used for further analyses.

***F. oxysporum* f. sp. *ciceris* race 1 culture.** *F. oxysporum* f. sp. *ciceris* race 1 culture was obtained from ICRISAT and further purified as outlined by Summerell et al. (47). A single colony was plated at the center of half-strength potato dextrose agar (PDA) plates, (Himedia Laboratories, Mumbai, India) and incubated in darkness at 25 to 28°C for 12 to 14 days (25). After 12 to 14 days of growth, the fungal plate was gently scrapped with a sterile spatula and 10 ml of sterile water added to it. The spore suspension (~7 ml) was collected and stored at -80°C with 30% sterile glycerol.

Sick-soil preparation and chickpea inoculation with race 1.

Seed of both JG62 and WR315 were sterilized using 0.1% HgCl₂, then germinated in autoclaved sand and synthetic soil at a ratio of 1:1 at 22 to 28°C, 35 to 40% humidity, and a photoperiod of 12 to 16 h. Seedlings, 12 to 15 days old, of 15 to 20 cm were used for the infection. Plants were inoculated according to Haware and Nene's sick-soil treatment (23). For sick-soil preparation, 20 to 25 ml of freshly prepared spore solution having 5×10^5 spore/ml was added to 100 g of autoclaved sand:chickpea meal (9:1). The fungus was allowed to sporulate on the sand:chickpea meal for 12 to 14 days, which was finally mixed with 2 kg of sterile sand:synthetic soil (1:1). Earthen pots (15 cm) pretreated with 5% copper sulfate, washed, and sun dried were filled with the prepared sick soil. Seedlings, 12 to 15 days old, were transplanted into the sick-soil-containing pots. The control plants were provided the same growth conditions and transferred to inoculum-free soil. After 2 days of dark incubation (necessary for fungal multiplication), the soil was thoroughly mixed with a sterile glass rod to ensure uniform spread of the fungus throughout the soil. The sets were then transferred to a photoperiod of 12 to 16 h with adequate watering to prevent the plants from being exposed to any other stress other than fungal attack. Serial sections of both infected and uninfected roots of JG62 and WR315 were done after every 24 h of inoculation and visualized under a compound microscope after staining with Trypan blue and Lactophenol (practical grade; Himedia Laboratories, Mumbai, India).

RNA extraction and double-stranded cDNA preparation.

Roots of infected and noninfected plants of both susceptible and resistant lines were collected at 48 and 96 h postinoculation and frozen in liquid N₂. Total RNA was extracted from the collected samples and an RNA pool prepared using TRI reagent kit (Sigma-Aldrich, St. Louis) according to the manufacturer's instructions. The RNA quality, integrity, and quantity were determined by running 2 µl of total RNA in a 1.2% formamide denaturing gel using 3-N-morpholino propane sulfonic acid (MOPS) buffer. The quantity and quality were further verified by spectrophotometric analyses at 260 nm and 260/280 nm (Beckman-Coulter UV-Vis Spectrophotometer). Poly A mRNA was purified from total RNA using the Qiagen mRNA minikit (Qiagen Inc., Valencia, CA). Poly A mRNA (500 ng of each sample) was used to prepare the double-stranded cDNA following the manufacturer's protocol of the SMART PCR-cDNA kit (Clontech-BD Biosciences, Takara Bio Inc., Shiga, Japan). The double-stranded cDNA was then quantitated using the Beckman Coulter UV-Vis Spectrophotometer at 280 nm and its qualitative analysis done by electrophoresing a 2-µl aliquot in 1.1% agarose gel using Tris acetic acid EDTA (TAE) buffer.

cDNA-AFLP analysis. Double-stranded cDNA (~250 ng) was subjected to standard AFLP template preparation according to Bachem et al. (5). The restriction enzymes used were *EcoRI* and *MseI* (10 units/µl) Roche Diagnostics GmbH, Penzberg, Germany). The digested products of cDNA were ligated to adapters of sequences as follows: the *EcoRI* adapter, 5'-CTCGTAGAC-TGCGTACC-3' and 3'-CTGACGCATGGTTAA-5'; and the *MseI* adapter, 5'-GACGATGAGTCCTGAG-3' and 3'-TACTCAGGAC-TCAT-5'.

The ligated products were then preamplified using *EcoRI* 5'-GACTGCGTACCAATTC-3' and *MseI* 5'-GATGAGTCCTGAGT-AA-3' preamplification primers. The restriction digestion, adapter ligation, and preamplification were performed according to the procedure described by Bachem et al. (5). Preamplification was done for 20 cycles at Thermal Cycler (version 1.6032; Bio-Rad, Hercules, CA) using temperatures of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min.

Equal amounts of preamplified products were then amplified with primers having selective nucleotides at their 3' ends. The following primers were used for the study (18): *EcoRI* E-AT, E-CA, E-AAG, E-AGC, E-ACG, E-AAC, E-ACA, E-ACT, E-ACC,

and E-AGG; and *MseI* M-AC, M-AG, M-AT, M-GA, M-GC, M-GT, M-TA, M-TC, M-TG, M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, and M-CTT (Metabion International AG, Deutschland, Germany), generating a total of 82 different combinations.

Radioactive labeling, selective amplification, and polyacrylamide gel electrophoresis analyses of amplified products. Radioactive labeling of the *EcoRI* primers was done using γ 32P ATP (Perkin Elmer Cetus) and T4 kinase (MBI Fermentas, St. Leon-Rot, Germany). Selective *MseI* primer and radiolabeled *EcoRI* primer combinations were then subjected to amplification of preamplified cDNA in the thermal cycler through 13 cycles of touchdown polymerase chain reaction (PCR) of 94°C for 30 s, 65°C for 1 min, and 72°C for 1 min, with a decrease in annealing temperature of 0.7°C in each cycle and an additional 23 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min.

Samples were then boiled after the addition of dye and 50% formamide and separated on 7% urea polyacrylamide gel electrophoresis (PAGE) (Gibco BRL Life Technologies Inc., Gaithersburg, MD). All the gels were run at standard conditions at 55 W. The bromophenol blue and xylene cyanol of gel loading buffer were supposed to move with 40 to 50 and 160 to 180 bp, respectively (41). The gels were run by monitoring the mobility of these two dyes to a good resolution of 70 to 400 bp. Gels were then directly dried on 3 MM Whatmann paper on a slab gel drier (Bio-Rad) after proper marking. Labeled amplified DNA fragments were visualized by autoradiography after one night of exposure at -80°C. In all, $\approx$ 18 to 30 bands were generated per primer combination (5). The autoradiographed film of amplified products was photographed using the Gel Doc System (Bio-Rad).

Isolation and reamplification of transcript-derived fragments. After proper alignment and marking, the differentially expressed polymorphic transcript-derived fragments (TDFs) were cut from the gel with a sharp sterile scalpel. Care was taken to prevent band contamination during cutting. The cut bands were eluted using the protocol as described by Frost et al. (17). The eluted DNA sample (2 μ l) was used with the corresponding same set of primer in a volume of 25 μ l for reamplification. Annealing temperature was 52 to 55°C and the number of cycles used was 40. The PCR products were then resolved in 1.6 to 2% agarose gel and visualized on a UV transilluminator. The product was further purified using a Qiagen PCR purification kit (Qiagen Inc.) and dissolved in 15 to 20 μ l of high-performance liquid chromatography (HPLC)-grade water. The *Taq* polymerase enzyme and buffers were obtained from Fermentas (MBI Fermentas, St. Leon-Rot, Germany).

Cloning and sequencing of TDFs. The eluted TDFs were cloned into the pGEMT Easy vector (Promega Corp., Madison, WI) following the manufacturer's instructions. The single-primer extension sequencing reaction was performed on the recombinant plasmid using Big Dye Terminator Cycle Sequencing Kit v1.1 (Applied Biosystems, Foster City, CA) using both T7 and SP6 promoter primers. The sequencing was carried out on automated ABI Prism 377 Sequencer (Applied Biosystems) at the sequencing facility of Delhi University, South Campus.

Bioinformatic analyses of sequences. The sequences of the TDFs (with vector sequence trimmed off, because recombinant plasmids were used as template) were analyzed for their homology against the publicly available nonredundant genes, expressed sequence tags (ESTs), or transcripts in the National Center for Biotechnology Information (NCBI), DFCI, and TAIR database using the BLASTN and BLASTX algorithms (1,18,38). The sequences were submitted to NCBI and the accession numbers were kept on hold.

Differential expression of TDFs using reverse-transcriptase (RT)-PCR. RNA was collected at 48 and 96 h postinoculation for RT-PCR analyses from both treated and untreated plants of WR315 and JG62. First-strand cDNA was synthesized from 5 μ g

of total RNA using Oligo(dT)12-18 primers supplied with the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) following manufacturer's instructions. PCR was carried out using fragment-specific primers. PCR conditions were as follows: initial denaturation of 2 min at 95°C, followed by 30 cycles of denaturation for 30 s at 95°C, annealing at 52 to 55°C for 30 s, extension at 72°C for 30 s, and a final extension of 8 min at 72°C. An *actin* gene was used as an internal control. Forward and reverse primers for amplification of *actin* gene (6) were 5'CTG GTA TCC ACG AGA CAA C 3' and 5'ATA GTA GAA CCA CCA CTC AAG 3'. PCR products were analyzed on 1.4% TAE agarose gel.

RESULTS

Morphological and anatomical changes. Infected roots of susceptible cv. JG62 showed yellow coloration at 4 days postinoculation (DPI) compared with the control roots. The stem and leaves of the entire plant started drooping and basal leaves exhibited yellowing at 5 DPI. The infected roots showed growth retardation in terms of root length and branching (Fig. 1). At $\approx$ 20 to 30 DPI, 95% of the plants wilted. However, only 5% of the infected wilt-resistant cv. WR315 plants showed slight reddening of the roots even at 20 DPI. The control plants of both the cultivars showed normal growth, ruling out the probability of any type of injury caused during plant transplantation.

Trypan blue and lactophenol staining of the infected JG62 roots confirmed pathogenic invasion at 4 DPI (Fig. 2). Nearly 70% clogging of the xylem vessels with fungal mycelia of race 1 was found at 8 and 10 DPI (Fig. 3). The callose deposition and accumulation of phenolics inside the xylem vessels were also confirmed by the brown ring formation at the root shoot juncture. However, the infected sections of WR315 roots showed no such pronounced anomaly. The root sections of both control sets showed normal root anatomy.

cDNA-AFLP analyses of the TDFs. In all, 82 primer combinations were used, among which 66 combinations (supplementary table) showed a distinct degree of polymorphism (Fig. 4). In total, 1,697 TDFs were generated from both uninfected and infected plant samples of resistant and susceptible chickpea lines using the abovementioned number of combinations. Differential expression (i.e., the intensity of bands or the degree of expression not the same in all the experimental sets of uninduced and induced samples of both JG62 and WR315) was shown by $\approx$ 87% of bands. Among those 1,489 bands, 1,111 (75%) bands showing differential expression were excluded from the study, because they demonstrated the same differences in degree of expression as uninduced JG62 and WR315 cultivars. Such transcripts could not be attributed as over- or underexpressed in response to the pathogen encounter. The remaining 25% of differential TDFs were considered in the present study, which showed differential expression in the fungus-induced susceptible and resistant samples, whereas their corresponding uninduced samples (control JG62 and WR315) showed no difference in degree of expression (Fig. 5). Hence, the differential expression due to difference in their genome was ruled out. Among the 378 pathogen-induced differential bands, 49% were found to show enhanced expression in challenged WR315 compared with challenged JG62, whereas 51% showed less expression or complete suppression in the infected WR315 compared with the infected JG62. The distinctly up- and down-regulated bands were excised from the gel and the same gel was redeveloped to confirm the proper recovery of the bands from the first gel. The eluted TDFs were 70 to 400 bp. In all, 53 differential TDFs were sequenced, of which 11 revealed sequences of similar genes or transcripts and were excluded from Table 1.

Bioinformatic studies of the differential TDFs. The sequence similarity search of the distinct 42 TDFs with that of published NCBI, TAIR, and DFCI sequences identified both upregulation

and downregulation of defense-related gene fragments, wound-responsive fragments, signaling molecules, structural gene fragments, and fragments of unknown functions (Fig. 6). Many fragments were detected having similarity with number of non-annotated clones of *Medicago*, *Arabidopsis*, *Vitis*, *Populus*, *Lycopersicon*, and *Hordeum* spp.; rice; and so on (Table 1). The sequence homology from the BLASTX and BLASTN with best *E* values was considered for further analysis and two pathway maps were generated. The *Arabidopsis* homologues of differential transcripts obtained from the TAIR database were used to generate a map of interacting pathways and cellular function using the software Pathway Studio (version 6.1; Adriadne Genomics, MD) (Fig. 7). Although 17 homologues of differential TDFs were found from the TAIR database, Pathway Studio found the interactions and neighbors of five genes. Additionally, the NCBI homologues of differential transcripts were linked manu-

ally to make a pathway map using the available information in publications (Fig. 8).

Comparison of differentially expressed TDFs. *Defense-responsive TDFs.* Database searches revealed upregulation of many defense-responsive gene fragments such as isoflavonoid biosynthetic pathway-related fragments, drought-stress-related ESTs, phosphoinositide specific phospholipase C, rapid alkalization factors, and cystatin in *Fusarium* spp.-challenged WR315, whereas challenged JG62 showed very low or almost total suppression of expression of the above gene fragments. However, a methylation-sensitive polymorphic fragment was found to be over-expressed in challenged JG62 compared with WR315.

Wound-responsive TDFs. Both challenged WR315 and JG62 showed expression of a wound-responsive arginase-like gene fragment. Challenged WR315 showed enhanced expression of TDF related to arginase of *Prunus armeniaca* whereas JG62

Fig. 1. Morphological changes of chickpea plants 10 days postinfection. **A**, Noninfected WR315; **B**, infected WR315; **C**, noninfected JG62; and **D**, infected JG62.

Fig. 2. Sectional view of infected roots of chickpea plants stained with trypan blue and lactophenol at 4 days postinfection. Root section of infected JG62 at **A**, low and **B**, high magnification. Bars represent 10 μ m.

showed upregulation of a fragment similar to arginase 2 of *Lycopersicon esculentum*.

Structural and signaling TDFs. Pathogenic confrontation showed enhanced expression of many structural and signal-generating molecules such as serine/threonine kinase, nodule-enhanced sucrose synthase, uridylyl transferase, leucine-rich transmembrane protein, photosystem II D1 protein, plastid division regulator, ATPase subunit E and F, oligomycin sensitivity-conferring protein, β -catenin-like protein, cytochrome oxidase subunit 1, cytochrome P450, kinesin heavy chain member 2, 60s ribosomal protein, alkaline invertase, α/β hydrolase, and β amylase-like gene fragments in induced WR315. Induced JG62 showed the presence of moderate to low expression of some of these abovementioned fragments and total suppression of some of the fragments. In contrast, a 14.3.3-like protein kinase-like receptor and tubulin-folding factor were upregulated in induced JG62 compared with WR315.

TDFs of clones and unknown functions. In addition to those mentioned above, up- and down-regulation of many gene fragments related to clones of *Arabidopsis*, *Medicago*, *Populus*, *Hordeum*, *Lycopersicon*, and other species of unassigned functions and many fragments of unknown functions were also found.

RT-PCR analyses of differential TDFs. Gene fragments showing homology with functionally annotated sequences of public database were taken for RT-PCR analyses. Both differentially up- and downregulated gene fragments showed a varied degree of expression at two different time points (48 and 96 h) (Fig. 9). CFb10 (similar to nodule-enhanced sucrose synthase) showed high expression in challenged WR315 at 48 h of infection, which almost remained the same even after 96 h postchallenge, whereas induced JG62 showed low expression at 48 h that was lost at 96 h postinduction. CFb26 (similar to ATPase subunit E) showed enhanced expression in induced WR315 compared with induced JG62 after both 48 and 96 h postinoculation. CFb36 (similar to

cytochrome oxidase subunit 1) expression in infected WR315 was high after 48 h of infection but drastically reduced at 96 h postinduction, whereas infected JG62 showed low expression at 48 h postinfection which was lost at 96 h. CFb14 (cytochrome P450 monooxygenase), -25(2) (phosphoinositide specific phospholipase C) and -45(2) (invertase) showed consistent enhanced expression in challenged WR315 even after 96 h of infection, whereas infected JG62 showed low expression which was lost at 96 h postinfection in the case of CFb45(2). CFb41(2) (cystatin) was only found to be enhanced after 48 h of challenge in WR315. Likewise, CFb12 (DNA methylation sensitive fragment), -13 (arginase), and -21 (tubulin-folding cofactor) were found to be upregulated in challenged JG62 after 48 h of pathogenic treatment compared with WR315, where they were found to be down-regulated. Most of the gene fragments showed variation in degree of expression after 48 h of inoculation and attained saturation or became suppressed after 96 h of inoculation. *Actin*, used as internal control, showed uniform expression in all the experimental sets.

DISCUSSION

F. oxysporum f. sp. *ciceris* race 1, an obligate biotroph, invades the roots of infected plants from contaminated soil or infected seed (40). Assuming the root hairs to be the site of race 1 entry, a better understanding of the plant-pathogen chemistry was accomplished by ensuring the initial establishment of the pathogen within the host. Our studies revealed initial pathogen entry at 4 DPI followed by massive fungal ramification at 8 and 10 DPI in wilt-susceptible JG62 root. The formation of brown ring at the root-shoot juncture suggested the secretion of some fungal toxins or pathogen-induced plant effector molecules leading to necrosis and tissue disintegration. If the site of infection and the resultant tissue decay is in the main nutrition-conducting strand (xylem), it

Fig. 3. Sectional view of infected roots of chickpea plants stained with trypan blue and lactophenol at 8 days postinfection. Root section of infected WR315 at **A**, low and **B**, high magnification, respectively. Root section of infected JG62 at **C**, low and **D**, high magnification, respectively. Bars represent 10 μ m.

causes the wilting of the entire plant. In the case of WR315, no fungal clogging was noticed. This directed us to assume that these events do not follow the same synchrony in a wilt-resistant cultivar. Tissue disintegration occurs at markedly later periods and at distinctly lower intensity in the resistant cultivar compared with its susceptible counterpart. Such variation in pathogenic response directs us to assume that there may be a different signaling pathway diverting the pathogenic effector molecules, thus imparting resistance to the resistant cultivar.

Despite the development of high-throughput full-genome expression systems like microarray, which rely on comparison of two samples and prior knowledge of gene sequences in the form of gene-chip, cDNA-AFLP remains a useful and effective alternative tool for identifying differential transcripts where prior knowledge of sequences is totally unknown (35). Studies conducted by Iruela et al. (24) showed the difference in genetic material between two experimental accessions (JG62 and WR315). Hence, only the differential transcripts derived as an outcome of fungal challenge were taken into consideration. Previous studies of chickpea-*Fusarium* spp. interaction were mainly devoted to the identification of resistant gene analogues and linkage mapping with, a few providing information on salicylic acid- or jasmonic acid-independent pathways (7), whereas the present work exhibited the involvement of several defense-related pathways in this interaction.

Pathway Studio analysis of five TDFs having homology with the TAIR database showed significant roles in defense signaling (Fig. 7). Starch, water, and sucrose are the key small molecules regulating the entire network. β Amylase (BMV7) and α/β hydrolase, sharing homology with upregulated gene fragments CFb39

and CFb59(2), respectively, participates in redox-regulated starch degradation under specific conditions of dark and light (46). Thiol disulphide exchange intermediates are known as activators of such β amylases (46). It can be assumed that fungal attack some

Fig. 5. Percentage of transcript-derived fragments (TDFs) generated due to pathogenic infection.

Fig. 4. Representative cDNA amplified fragment length polymorphism profiles of noninfected and infected JG62 and WR315 samples using different primer combinations. Lanes 1, 5, 9, 13, 17, 21, 25, 29, 33, and 37, noninfected JG62; lanes 2, 6, 10, 14, 18, 22, 26, 30, 34, and 38, noninfected WR315; lanes 3, 7, 11, 15, 19, 23, 27, 31, 35, and 39, infected JG62; lanes 4, 8, 12, 16, 20, 24, 28, 32, 36, and 40, infected WR315. The primer combinations used were: lanes 1 to 4, E-AAC/M-CAA; lanes 5 to 8, E-AAC/M-CAG; lanes 9 to 12, E-AAC/M-CAT; lanes 13 to 16, E-AAG/M-CTG; lanes 17 to 20, E-AAG/M-CTT; lanes 21 to 24, E-AAG/M-CAC; lanes 25 to 28, E-AAC/M-CTA; lanes 29 to 32, E-AAC/M-CTC; lanes 33 to 36, E-AAC/M-CTG; lanes 37 to 40, E-AAC/M-CTT. Arrows indicate some of the bands selected for further analysis.

TABLE 1. Differentially expressed transcript-derived fragments (TDFs) revealed by BLASTN and BLASTX search

Cloned TDFs	Size of TDFs (bp)	Degree of expression ^a		Homology, identities, <i>E</i> score ^b			Type of TDF
		WR315	JG62	NCBI	DFCI	TAIR	
CFb3(2)	186	+++	–	<i>Lotus japonicus</i> genomic DNA clone related to isoflavonoid biosynthesis (AP009237), 83%, 6e-50	<i>L. japonicus</i> homologue to UniRef100_A7Q3C7 Cluster of <i>Vitis vinifera</i> (TC94981), 93%, 3.7e-25	Unknown protein (At3g54190), 82%, 1e-07	Defense responsive
CFb7(2)	190	+++	+	<i>Populus</i> EST from mild drought stressed leaves (CU229655), 60%, 6e-51	<i>Populus</i> homologue to AT3g54190 of <i>Arabidopsis thaliana</i> (TC81900), 61%, 0.20	Unknown protein (At3g54190), 82%, 2e-09	Defense responsive
CFb25(2)	220	+++	+	<i>A. thaliana</i> phosphoinositide specific phospholipase C line (BAA97337) 98%, 2e-26	<i>A. thaliana</i> phosphoinositide specific phospholipase C line (NP225782), 72%, 7.1e-14	Phosphoinositide specific phospholipase C (At2g40116), 98%, 2e-26	Defense responsive
CFb27(2)	395	+++	+	<i>Medicago truncatula</i> chromosome 7 clone mth2-143d17, complete sequence (AC154391), 90%, 9e-116	<i>M. truncatula</i> RAF, complete sequence (TC108336), 84%, 2.8e-54	RAF (At4g15800), 82%, 5e-15	Defense responsive
CFb32(2)	395	+++	–	<i>Populus balsamifera</i> f. sp. <i>trichocarpa</i> × <i>P. deltoids</i> RAF 1 precursor (RALF 1) mRNA complete cds (AY172330), 60%, 6e-112	<i>P. balsamifera</i> f. sp. <i>trichocarpa</i> × <i>P. deltoids</i> RAF 1 precursor (RALF 1) mRNA complete cds (NP12333895), 83%, 2.5e-48	RAF (At4g15800), 82%, 1e-12	Defense responsive
CFb41(2)	144	+++	–	<i>Citrus paradisi</i> cystatin like protein mRNA, complete cds (AF283536), 72%, 2e-29	<i>C. paradisi</i> cystatin like protein mRNA, complete cds (TC15193), 84%, 1.7e-12	Cystatin like protein (At5g47550), 86%, 0.097	Defense responsive
CFb12	52	–	+++	<i>Brassica napus</i> clone MSAP salt 7 methylation sensitive polymorphic fragment (EF061314), 57%, 1e-05	<i>Pinus pinaster</i> induced compression wood cDNA clone PPEM59 (BX294121), 83%, 0.29	No homology found	Defense responsive
CFb13	81	–	+++	<i>Lycopersicon esculentum</i> arginase 2 mRNA, complete cds (AY656838), 61%, 3e-16	<i>L. esculentum</i> arginase 2 mRNA, complete cds (AY656838), 98%, 0.00029	No homology found	Wound responsive
CFb18	75	+++	++	<i>Prunus armeniaca</i> arginase mRNA, partial cds (AY962818), 60%, 2e-13	<i>P. armeniaca</i> arginase mRNA, partial cds (AJ533768), 100%, 0.0010	No homology found	Wound responsive
CFb8(2)	181	+++	–	<i>A. thaliana</i> CDC2b gene for p32 protein serine threonine kinase related protein, complete cds (D10851), 55%, 2e-43	<i>A. thaliana</i> sequence AT3g54180 (TC283126), 93%, 4.1e-16	CDC2b gene for p32 protein serine threonine kinase related protein (At3g54180), 55%, 2e-4	Signal generator
CFb58	164	+	+++	<i>M. truncatula</i> DNA sequence from clone MTH2-21G21 on chromosome 3 complete sequence (CR940307), 82%, 1e-39	<i>M. truncatula</i> receptor kinase-like protein (TC95151), 85%, 4e-16	No homology found	Signal generator
CFb10	118	+++	–	<i>Pisum sativum</i> nodule enhanced sucrose synthase (ness) mRNA, complete cds (AF079851), 71%, 6e-22	<i>M. truncatula</i> root nodule sucrose synthase complete cds (BG582602), 87%, 5.5e-08	Sucrose synthase (At3g43190), 92%, 2e-11	Signal generator
CFb45(2)	141	+++	–	<i>A. thaliana</i> β-fructofuranoside putative alkaline invertase mRNA, complete cds (NM111456), 68%, 1e-38	<i>C. sinensis</i> response regulator aspartate phosphatase, partial sequence (EH17773), 64%, 0.029	β-Fructofuranosidase, putative invertase (At3g05820), 100%, 6e-06	Signal generator
CFb39	108	+++	+	<i>Glycine max</i> β amylase (CAI39244), 51%, 2e-05	<i>Solanum tuberosum</i> β-amylase, partial sequence (TC142269), 83%, 3.1e-06	β-Amylase (At3g23920), 92%, 0.28	Signal generator
CFb59(2)	285	+++	+	<i>A. thaliana</i> hydrolase, α/β fold family protein mRNA (NM119911), 91%	<i>A. thaliana</i> putative hydrolase, partial sequence (91%) TC302987, 8.8e-38	Hydrolase, α/β fold family protein (At4g37470), 100%, 7e-63	Signal generator
CFb33	124	–	+++	<i>M. truncatula</i> chromosome 7 BAC clone mth2-62p5, complete sequence (AC169515), 79%, 4e-19	<i>M. truncatula</i> uridylyl transferase-like, partial cds (TC108971), 84%, 6.7e-10	No homology found	Signal generator
CFb4	223	–	+++	<i>P. sativum</i> mRNA for 14.3.3 like protein (AJ238682), 52%, 7e-59	<i>M. truncatula</i> mRNA for 14.3.3 like protein (TC106310), 92%, 1.6e-12	General regulatory factor 8 (At5g65430), 93%, 1e-11	Structural and signaling component

(continued on next page)

^a Symbols: +++, ++, and + stand for high, moderate, and low expression, respectively, while – refers to total suppression.^b NCBI = National Center for Biotechnology Information, DFCI and TAIR, EST = expressed sequence tag, RAF = rapid alkalization factor.

TABLE 1. (continued from preceding page)

Cloned TDFs	Size of TDFs (bp)	Degree of expression ^a		Homology, identities, <i>E</i> score ^b			Type of TDF
		WR315	JG62	NCBI	DFCI	TAIR	
CFb14	86	+++	+	<i>Helicoverpa zea</i> cytochrome P450 (CYP9A12) gene, complete cds (DQ788839), 54%, 2e-14	<i>Lotus corniculatus</i> var. <i>japonicus</i> nodulating root cDNA clone GNf013a02 3 (BP068279), 72%, 4.0	No homology found	Structural and signaling component
CFb11(2)	372	+++	+	<i>Triticum aestivum</i> vacuolar ATPase subunit F mRNA (DQ486058), 57%, 3e-54	<i>M. truncatula</i> vacuolar ATPase subunit F mRNA (TC102217), 89%, 6.6e-59	Vacuolar ATPase subunit F (At4g02620), 82%, 5e-15	Structural and signaling component
CFb26	140	+++	+	<i>Lycopersicon esculentum</i> putative vacuolar proton ATPase subunit E mRNA, partial cds (AF282970), 76%, 9e-28	<i>M. truncatula</i> vacuolar ATPase subunit E mRNA, complete sequence (TC100855), 91%, 2.1e-14	Vacuolar proton ATPase subunit E (At4g11150), 86%, 4e-16	Structural and signaling component
CFb8	104	+++	+	<i>A. thaliana</i> ATP synthase delta chain mRNA (NM121348), 72%, 2e-14	<i>Phaseolus vulgaris</i> Oligomycin sensitivity conferring protein, partial sequence (TC336), 90%, 1.9e-07	ATP synthase delta chain (At5g13450), 86%, 8e-11	Structural and signaling component
CFb36	86	+++	+	<i>Helicarium masteri</i> cytochrome oxidase subunit 1 (COI) ene, partial cds (EF015412), 54%, 2e-14	No homology found	No homology found	Structural and signaling component
CFb15	86	+++	+	Human DNA sequence clone RP1-107N3 on chromosome X contains kinesin heavy chain member2 (KIF2) pseudogene and a Huntingtin interacting protein K (HYPK) complete sequence (Z75741), 51%, 0.001	<i>Aquilegia</i> sequence similar to UniRef100_A7P101 Cluster, Chromosome chr19 scaffold_4 of <i>Vitis vinifera</i> , partial sequence (TC30361), 77%, 4.1	No homology found	Structural
CFb18(2)	176	+++	–	<i>G. max</i> plastid division regulator MinE mRNA, complete cds (DQ118106), 85%, 7e-56	<i>G. max</i> plastid division regulator MinE mRNA, complete cds (TC229600), 89%, 3.8e-20	No homology found	Structural
CFb22(2)	176	+++	+	<i>M. truncatula</i> chromosome 5 clone mte1-29c13, complete sequence (CU137657), 84%, 2e-55	<i>M. truncatula</i> sequence similar to Plastid division regulator MinE of <i>Glycine max</i> , complete sequence (TC108628), 88%, 5.6e-23	No homology found	Structural
CFb19	75	+++	+	Leucine-rich transmembrane protein of <i>Aedes aegypti</i> (AAEL004777) 86%, 2e-18	No homology found	No homology found	Structural
CFb20	120	+++	+	<i>Pyrus communis</i> photosystem II D1 protein (PsbA) mRNA, partial sequence (AY433956), 62%, 2e-27	<i>Gossypium hirsutum</i> photosystem Q(B) protein, partial sequence (DN826990), 86%, 0.0011	Photosystem Q(B) protein (At2g07688), 91%, 1e-09	Structural
CFb24(2)	220	+++	+	<i>Arabidopsis</i> armadillo/β catenin repeat family protein (NP566136), 98%, 1e-20	<i>Arabidopsis thaliana</i> AT3g01400, armadillo/β catenin repeat family protein (TC286826), 99%, 1.6e-30	Armadillo/β catenin repeat family protein, (At3g01400)100%, 7e-87	Structural
CFB39(2)	334	+++	+	<i>Nicotiana tabacum</i> (TSC40-4) 60s ribosomal protein L34 mRNA, complete cds (L27107), 58%, 5e-49	<i>M. truncatula</i> 60S ribosomal protein L34, complete cds, (TC106474), 82%, 4.1e-44	Ribosomal protein L34 (At1g69620), 82%, 2e-23	Structural
CFb21	100	+	+++	<i>A. thaliana</i> tubulin folding cofactor E, (AAM22962), 53%, 5e-06	<i>A. thaliana</i> tubulin folding cofactor E (AAM22962), 92%, 9.6e-10	Tubulin-folding cofactor E (At1g71440), 100%, 1e-34	Structural
CFb9(2)	186	+++	++	Hypothetical protein of <i>Vitis vinifera</i> related to hexadiploidization (CAN75497), 87%, 2e-16	<i>M. truncatula</i> homologue to UniRef100_A5CB46 Cluster, Putative uncharacterized protein of <i>Vitis vinifera</i> (TC94981), 93%, 3.7e-25	Unknown protein (At3g54190), 82%, 1e-07	Unknown function
CFb17	75	+++	++	<i>Rumex acetosa</i> male enriched Y9 marker sequence (AY433956), 48%, 1e-08	<i>M. truncatula</i> arginase 1, complete cds (TC101600), 93%, 0.077	No homology found	Unknown function
CFb20(2)	255	+++	+	<i>A. thaliana</i> unknown protein mRNA, complete cds (NM102353), 80%, 4e-99	<i>A. thaliana</i> unknown protein mRNA, complete cds (TC286836), 93%, 9.2e-41	Unknown protein (At1g25420), 100%, e-112	Unknown function

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how increases the demand for sugars, resulting in the increased activity of sugar-cleaving enzymes. Overexpression of CFB10, similar to a nodule-enhanced sucrose synthase gene, was also detected in challenged WR315. These nodule-enhanced sucrose synthases (SUS4) preferentially function in the direction of

sucrose cleavage under in vivo conditions where the sucrose is unloaded. However, they also have the capacity to catalyze the synthetic reaction, especially when hexoses are the sole carbon source (34). Hence, their activity is regulated by feedback controls (34). Invertase (similar with upregulated CFB45(2)) also

TABLE 1. (continued from preceding page)

Cloned TDFs	Size of TDFs (bp)	Degree of expression ^a		Homology, identities, <i>E</i> score ^b			Type of TDF
		WR315	JG62	NCBI	DFCI	TAIR	
CFb23(2)	220	+++	+	<i>A. thaliana</i> unknown protein (AAM64910), 73%, 2e-19	<i>A. thaliana</i> cDNA clone GSLTPGH34ZA12 5PRIM, mRNA sequence (BX837618), 65%, 6.9e-07	No homology found	Unknown function
CFb48(2)	148	+++	+	<i>A. thaliana</i> binding mRNA, complete sequence (NM126230), 72%, 2e-18	<i>M. truncatula</i> homologue to UniRef100_A7P3U9 Cluster: Chromosome chr1 scaffold_5 of <i>Vitis vinifera</i> (TC97628), 87%, 2.8e-16	Unknown protein (At2g01690) 84%, 4e-07	Unknown function
CFb4(2)	187	+++	++	<i>M. truncatula</i> chromosome 5 clone mth2-6h11, complete sequence (CR932039), 94%, 1e-71	<i>M. truncatula</i> homologue to UniRef100_A7Q3C7 Cluster of <i>Vitis vinifera</i> (TC95459), 94%, 1.8e-29	Unknown protein (At3g54190), 85%, 1e-14	Clone
CFb5(2)	172	+++	+	<i>Populus trichocarpa</i> clone POPO18-P22, complete sequence (AC210382), 88%, 2e-57	<i>Populus</i> homologue to AT3g54190 of <i>Arabidopsis thaliana</i> (TC81900), 93%, (4.1e-24)	Unknown protein (At3g54190), 83%, 3e-11	Clone
CFb6(2)	185	+++	+	<i>L. esculentum</i> clone 133854F, mRNA sequence (BT014492), 66%, 8e-43	<i>L. esculentum</i> sequence partially similar to AT3g54190 of <i>A. thaliana</i> (TC183360), 90%, 3.6e-18	Unknown protein (At3g54190), 81%, 2e-06	Clone
CFb15(2)	256	+++	++	<i>P. trichocarpa</i> clone WS01211 unknown mRNA (EF146638), 90%, 9e-114	<i>Populus</i> sequence similar to unknown protein of <i>A. thaliana</i> (TC51321), 99%, 7.4e-46	Unknown protein (At1g25420), 83%, 1e-30	Clone
CFb16(2)	255	+++	++	<i>M. truncatula</i> clone mth2-91j8, complete sequence (AC154089), 90%, 4e-61	<i>M. truncatula</i> sequence similar to UniRef100_Q9C6L2 Cluster, Putative uncharacterized protein of <i>A. thaliana</i> (TC99432), 96%, 1e-42	Unknown protein (At1g25420), 79%, 5e-08	Clone
CFb21(2)	257	+++	+	<i>Hordeum vulgare</i> sp. <i>vulgare</i> cDNA clone: Flbaf30d19 mRNA sequence (AK248203), 81%, 5e-98	<i>H. vulgare</i> sequence similar to UniRef100_Q5N7L3 Cluster, Putative uncharacterized protein of <i>Oryza sativa</i> (TC158601), 98%, 6.4e-40	No homology found	Clone
CFb30	75	+++	+	<i>M. truncatula</i> chromosome 2 BAC clone mth2-1733e20, complete sequence (AC195565), 72%, 1e-14	No homology found	No homology found	Clone

Fig. 6. Different types of transcript-derived fragments obtained due to fungal attack.

does the same but requires more energy (22). Sucrose is the key molecule that induces the SUS4 (50). Besides, previous studies showed the anaerobic condition and fungal inoculation-induced expression of sucrose synthase in rice (36) and winter wheat (11), respectively. In some of the legumes such as soybean and *Phaseolus* spp., expression of this gene was found to be reduced along with reduced nitrogenase activity in stressed cells (45). It can be predicted that SUS4 helps in recovering the storage reserves and cell-wall polysaccharides that are affected by pathogenic invasion apart from acting as a signaling molecule. Vacuolar H⁺-ATPase (TUF) enzymes, having homology with CFb26 and CFb11(2), are involved in acidification of intercellular organelles, pumping protons across the plasma membrane, maintaining solute homeostasis, and energizing secondary transport through ATP hydrolysis during stressful conditions (10). TUF acts as a possible target of Ca⁺⁺-activated protein kinase that play a crucial role in defense (43). Serine/threonine kinase is a member of the sucrose nonfermenting 1 (SNF1) kinase family. The CDC2b gene for p32 protein serine threonine kinase (CDC2B) is known to control cell cycle progression and integration of different developmental pathways (8). *Arabidopsis* serine/threo-

nine kinase 1 (ASK1) was overexpressed in response to saline and osmotic shock (32). These kinases were related to carbon metabolism providing cell protection and nutrition (21). CFb8(2), having homology with CDC2b, was overexpressed in infected wilt-resistant cultivars. Abiotic stress and wounding induced the production of ribosomal proteins (r-proteins) in soybean (28) and *Arabidopsis* (31), supporting the stress-responsive alteration in the transcription and translational level. Enhanced expression of fragment CFb39(2), sharing homology with 60S ribosomal protein L34 (RPL34), was identified. RPL34 is regulated by sucrose, octadecanoid, and lipoxygenase pathways (9). The octadecanoid and jasmonic acid pathways are involved in both direct and indirect defense (20). The network also suggests the regulation of RPL34 by the lipoxygenase pathway, which is responsible for the production of oxylipins that are important for plant defense.

Differential TDFs having homology with the NCBI database could predict many important and defense-related probable cellular functions and interactions, which have been illustrated in Figure 8. The overexpression of CFb3(2) and CFb14 was found in infected WR315, sharing homology with the isoflavanoid biosyn-

Fig. 7. Metabolic pathway network constructed using the software Pathway Studio (version 6.1) from differentially expressed transcript homologues from the TAIR database. CDC2B: CDC2b gene for p32 protein serine threonine kinase (At3g54180), TUF: vacuolar proton ATPase subunit E (At4g11150), RPL34: ribosomal protein L34 (At1g69620), BMY7: β amylase (At3g23920), SUS4: nodule enhanced sucrose synthase (At3g43190). Green circle: small molecule, pink oval: gene, blue Star: treatment, orange polygon: functional class, yellow square: cell process, blue arrow: expression, gray arrow: regulation, red arrow: molecule synthesis.

thetic gene of *Lotus* spp. and cytochrome P450, respectively. Isoflavanoids are the branch products of the phenyl propanoid pathway and acetate malonate pathway, having antioxidant property and a profound role in plant defense. Legumes accumulate isoflavanoids, which serve as chemoprotectants during microbial attack. Cytochrome P450 is a member of the hemoprotein superfamily and part of the electron transport chain that catalyzes many aliphatic and aromatic hydroxylations and oxidative deaminations during plant-insect interaction (44). In addition, the downregulation of a DNA methylation-sensitive gene fragment (CFb12) in susceptible infected roots could be observed as a common epigenetic change often found even in response to changes caused during tissue culture treatments (27), which often leads to accumulation of isoflavanoids (3). These studies suggest the probable induction of phenyl propanoid pathway genes leading to accumulation of isoflavanoids, due to race 1 attack.

Enhanced expression of CFb25(2) sharing similarity with phosphoinositide-specific phospholipase C assumes the involvement of secondary messengers in the pathogen-mediated signal transduction process as already documented by a similar type of overinduction of phospholipases in response to the elicitor-driven oxidative burst in soybean (30). The 14.3.3 membrane proteins similar to upregulated CFb4 in challenged JG62 were well known for their wide array of roles, ranging from protein kinase-mediated cell signaling to protein-protein interaction. In a plant-fungus encounter, they have a potential role in acting as receptors of fungal toxins (13). The upregulation of 14.3.3-like molecules and H^+ ATPases strengthens the role of their attachment with

fungal toxins and subsequent irreversible opening of a proton channel of stomata leading to wilt of the susceptible cultivar (49). Cytochrome C oxidase is the key enzyme of aerobic respiration. It is involved in the translocation of protons and has an active role in regulating stress-mediated signals (29). The identification of fragment CFb41(2), similar to cystatin, was an important finding in our study because cystatins are considered as antifungal agents since long and are also used as fungicides (42). Rapid alkalization factor (RALF), a polypeptide hormone of ≈ 49 amino acids, is a plant stress indicator and growth regulator, causing rapid alkalization of the growth medium. RALF was induced in a resistant cultivar of *Brassica rapa* during *Plasmadiophora brassicae* infection (48). RALF also activates serine/threonine kinase that is coupled with a leucine-rich repeat of the extracellular receptor domain. These studies suggest that enhanced expression of RALF-like fragments (CFb27(2) and CFb32(2)) probably has a role in medium alkalization, which may have an adverse effect on the fungal metabolism (39). Uridyl transferases, sharing homology with upregulated CFb33, transfer UMP residues (uridine monophosphates) to the 3' end of newly synthesized RNAs and regulate gene expression by posttranscriptional RNA modification. Studies have shown their upregulation due to viral infection (12).

The expression of arginase in both cultivars showed evidence of plant wounding caused by fungal penetration. Wound-responsive enzyme arginase hydrolyses arginine to urea and ornithine, the latter being the precursor of biosynthesis of polyamine, the well-studied wound healers. Urea gives rise to ammonia that maintains the nitrogen pool during fungal attack (45).

Fig. 8. Metabolic pathway network generated by analyzing differential transcripts obtained from chickpea upon *Fusarium* spp. attack. Pink box: upregulated gene fragment in WR315, blue box: downregulated gene fragment in WR315, yellow box: cell process, deep green box: the effect, arrow: expression and regulation.

Fig. 9. Semiquantitative reverse-transcriptase polymerase chain reaction amplification patterns of selected transcript-derived fragments (TDFs). Lanes 1 and 2, TDFs derived from noninfected JG62 and WR315 samples, respectively, collected after 48 h; lanes 5 and 6, the same, collected after 96 h; lanes 3 and 4, TDFs derived from infected JG62 and WR315 samples, respectively, collected after 48 h of inoculation; lanes 7 and 8, the same, collected after 96 h of inoculation.

Collectively, it can be assumed that the pathogen invasion somehow triggers the signal transduction mediated by sugar sensing as supported by the elevated levels of sucrose synthase, invertase, β amylase, and so on in our study. The sugar's dual function as a nutrient and a signaling molecule is well cited (37). Studies of the *Arabidopsis-Erysiphe* spp. interaction have shown increase in levels of invertases and free sugars (16). Moreover, literature supports the role of 14.3.3 proteins in cleavage of their binding partners in sugar-starved cells (37). In our study, the overexpression of 14.3.3-like proteins in the infected susceptible cultivar suggests a possible sugar starvation in them, while the resistant cultivar can make up for the shortage by overexpression of several sugar-metabolizing genes.

Apart from the above, some fragments sharing quite high similarities with many accessions of the database were also found. Either roles of these fragments need to be further characterized or their functional annotations are still pending. This is the first report of expression of a large number of gene fragments related to the defense response in chickpea upon *F. oxysporum* f. sp. *ciceris* race 1 infection. Molecular characterization of all of the genes would certainly help in better understanding of the *Cicer-Fusarium* spp. interchemistry and the improvement of chickpea cultivation in the near future.

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