



Comparative expression profile of some putative resistance genes of chickpea genotypes in response to ascomycete fungus, *Ascochyta rabiei* (Pass.) Labr.

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Abstract

Chickpea (*Cicer arietinum* L.) is one of the most important legumes infected with many pathogens including fungal pathogens. One of the most important devastating fungal pathogens in chickpea is *Ascochyta rabiei* (Pass.) Labr. which causes up to 100% reduction in yield. In the present work, the expression patterns of *AFP-ca*, *CaD₂*, *LRR* and *PGIP* genes were studied in response to *Ascochyta rabiei* in two susceptible and resistant chickpea genotypes. The experimental system was conducted in greenhouse, for both inoculated and mock-inoculated plants (control). RNA was isolated from Icc 12004 (resistant) and FLIP 82-150c (susceptible) genotypes in 0, 6, 12, 24, 48, 72 and 96 h after inoculation. The expression of the genes was measured in susceptible and resistant plants via semiquantitative RT-PCR. Results showed higher expression of all three genes in resistant genotype compared with susceptible one. Results showed that the candidate genes from antimicrobial families (*CaD₂* and *AFP-ca*) were up-regulated in resistant genotypes at early hours after inoculation (6–24 hpi), and also for *PGIP* from galacturonase-inhibiting protein families, the maximum expression was observed at early hours of inoculation to 48 hpi. In general, we concluded that all genes studied in this investigation contribute to plant–pathogen interaction and all of them can increase the resistance responses to *Ascochyta* blight disease.

Keywords Antimicrobial peptides · *Ascochyta* blight · Gene expression · Polygalacturonase-inhibitor proteins · RT-PCR

1 Introduction

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop after beans (14776827 tonnes/annually) that has been grown and consumed all over the world especially in developing countries (FAOSTAT 2016), because of its high levels of carbohydrates and proteins with good quality (Jukanti et al. 2012), and also it has significant amounts of

the essential amino acids (Jukanti et al. 2012). Chickpea is rich in unsaturated fatty acids and nutrients including Ca, Mg, P, K and important vitamins such as riboflavin, niacin and thiamin (Jukanti et al. 2012). Consumption of chickpea has good impact on weight control (Papanikolaou and Fulgoni 2008), cardiovascular disease (Augustin et al. 2015), cancer (Cummings et al. 1981; Mathers 2002), glucose and insulin response (Pittaway et al. 2008) and gastrointestinal tract health (Murty et al. 2010; Wallace et al. 2016).

Fungal plant pathogens are a real threat to agriculture and cause economical yield losses in most of the agricultural crops. *Ascochyta* blight (AB) caused by necrotrophic ascomycete fungus *Ascochyta rabiei* (Pass.) Labr. is a widespread chickpea foliar disease that causes extensive crop losses (up to 100%) in most commonly grown regions of the world (Pande et al. 2005) and produces several phytotoxins (Chen and Strange 1991). The occurrence and severity of *ascochyta* blight depend on environmental conditions such as humidity (> 60%) and temperature (15–25 °C) (Pande et al. 2011a, b). This fungus can directly penetrate and invade all the aerial parts of

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chickpea (Chen and Strange 1991). This disease can cause serious damage and necrosis on plant, and finally, the dark pycnidium grow on the necrosis spots (Santra et al. 2001; Pande et al. 2005, 2011a, b). Few natural sources of stable resistance to *A. rabiei* have been reported (Singh and Reddy 1996; Chen et al. 2004), although resistance using irradiation of chickpea seeds was reported (Mabrouk et al. 2018). Two main genotypes of chickpea have been identified as susceptible and resistant genotypes, the kabuli type as a susceptible genotype and desi type as a resistant genotype (Haware et al. 1995; Kaiser 1997).

The important way for control of ascochyta blight is using resistant genotypes, but mutations in *A. rabiei* may cause generation of new races that can break resistance. However, increment knowledge about transcript expression of resistance genes can help us on development of durable resistance (Coram and Pang 2006). Therefore, one way for studying defense reactions is a differential screening of cDNA library and studying of gene analogues (Rajesh et al. 2002).

The ability of plants to detect pathogens is essential in plant response and defense. Both active and passive defense responses in chickpea can prevent initial pathogenic attacks and spread to neighboring cells (Coram and Pang 2006). *R* genes play a key role in active defense systems and might be employed to recognize pathogen-specific effectors encoded by the *Avr* genes (McDonald and Linde 2002).

Plants produce a high number of toxic molecules, including antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs), as a part of defense response (Nawrot et al. 2014). Plant resistance to pathogenic fungi involves multiple response pathways including hydrolytic enzymes and antimicrobial proteins (Nehra et al. 1994; Singh et al. 2003). Most of the known AMPs causing formation of membrane pores result in ion and metabolite leakage, depolarization, interruption of the respiratory processes and cell death (Pellegrini and Franco 2005). This small antimicrobial peptides as antibiotic peptides have been considered as a new generation peptides that can maintain plants against pathogens (Bowdish et al. 2005). The early perception of pathogens, signal induction and activation of transcription factors are vital for the level of resistance and defense (Yang and Pedersen 1997). Plant defensins, one of the AMPs families, are small basic proteins of 45 to 54 amino acids (Nawrot et al. 2014). Plant antifungal defensins cause reduction in hyphal growth and increase in hyphal branching, or may cause reduced hyphal elongation without morphological distortion (Broekaert et al. 1995; Segura et al. 1998). Polygalacturonase-inhibiting proteins (PGIPs) are leucine-rich repeat (LRR) protein families that inhibit fungal endopolygalacturonases (PGs) (D'ovidio et al. 2006). These genes produce proteins that can detect pathogen virulence proteins via both direct and indirect mechanisms (Jones and Dangl 2006; Eitas and Dangl 2010).

Transcriptome analysis of host plants has been utilized to study different abiotic and biotic stress responses; this strategy has been used for some pathogen attacks in chickpea (Raju et al. 2008; Leo et al. 2016), but it is not enough to understand the real mechanisms of resistance and susceptibility. The need to identify resistance mechanisms and improve resistance of chickpeas against fungal pathogens is a desired aim of the breeders. One strategy to survey the resistance mechanism against pathogens is to assess and compare putative resistance gene transcripts between resistant and susceptible genotypes. The expression profiles of several host genes related to defense mechanisms have been studied within the chickpea-*A. rabiei* pathosystem (Kombrink and Schmelzer 2001; Edereva 2005; Coram and Pang 2005a, b, 2006; Leo et al. 2016). Some defensins and antimicrobial peptides were identified to perform defense against plant pathogenic fungi, but they are not studied yet for chickpea encounter with *A. rabiei*. This study aimed at determining the transcription level on some other putative resistance genes that may play important role in ascochyta-chickpea interaction including LRR (NBS-LRR gene family), PGIP (*pgip* gene families), AFP-ca and CaD₂ (antimicrobial protein families).

2 Materials and methods

Plant materials and pathogens isolates – Two chickpea genotypes, desi (Icc 12004) as a resistant genotype and kabuli (FLIP 82-150c) as a susceptible genotypes, were used in this research based on the previous studies (Kaiser 1997; Haware et al. 1995). *Ascochyta rabiei* isolate (Shw24) was provided from the Department of Plant Protection, University of Kurdistan, Sanandaj, Iran. The pathogen was plated out on potato dextrose agar (PDA) at 18 °C for 14–18 days. For the preparation of inoculum, the *A. rabiei* was cultured on liquid media (PD) at 60 rpm shaking at 25 °C. After 3 days, the *A. rabiei* conidia were precipitated using centrifuge at 3000 rpm for 10 min. The liquid media was removed, and then, the conidia were re-suspended in sterile water to 1.2×10^6 conidia per milliliter.

Bioassay – All 14-day-old chickpea plantlets were sprayed with *A. rabiei* using 1.2×10^6 spore suspension until runoff on leaves. Control plants were sprayed with sterile water, and then, plants were covered by plastics and sprayed 3 times each day with water for the preparation of required humidity up to 90% for 3 days at 20–25 °C. Plant stem and leaves from three plants for each time points (0, 6, 12, 24, 48 and 72 hpi) were collected and used for RNA extraction. However, after sampling, the plants were kept at greenhouse condition for showing symptoms and to make sure that plant infection has happened.

RNA extraction – RNA extraction was carried out from inoculated plant leaves and stems in bioassay experiment. RNA extraction was performed using the RNX-Plus solution (CinnaGen, Tehran, Iran) according to the manufacturer's instructions. Around 0.1 gr of leaf and stem of each plant was ground using liquid nitrogen, and 1 ml RNX-Plus solution was added. Samples were vortexed for 15 s and kept at room temperature for 5 min. 200 µl of chloroform was added, and samples were shaken gently for 5 min. Samples were centrifuged for 15 min at 14,000 rpm at 4 °C. The supernatant was transferred into another tube. Cold isopropanol (600 µl) was added, and samples were inverted gently and incubated at –20 °C for 20 min. The tubes were centrifuged at 4 °C at 12,000 rpm for 15 min. The supernatant was removed, the precipitated pellet was washed using 70% ethanol, and pellet was dried and re-suspended in 30 µl of DEPC water.

cDNA synthesis and PCR – For cDNA synthesis, the RNAs were equaled to the same concentration using BioPhotometer Plus (Eppendorf, Germany). The reverse transcription was performed using 2X HyperScript RT premix (GeneAll, South Korea) as described by the manufacturer. The cDNA was synthesized in 10-µl reactions containing 3.6 µg ml⁻¹ of RNA and 1 µl of 40 mM of oligo dT. Microtubes were kept at 65 °C for 5 min and put on ice for 5 min. 10 µl of the 2X HyperScript RT premix was added to the tubes and kept at 55 °C for 60 min. At the end of reaction, the samples were kept at 95 °C for 5 min to stop the reactions. PCR reactions for all genes were carried out using specific primers, and actin gene (XM012716420.1) was used as a reference gene (Table 1). For PCR reactions, the sequences of selected genes were obtained from NCBI and specific primers were designed using Web-based software Primer3 and Oligo analyzer v3. The primers sequences were blasted against chickpea sequences available in NCBI to check probable off-targets. Selected genes were *LRR* (Aj609275), *PGIP* (loc101504619), *AFP-ca* (DQ288897.2) and *PvD2* orthologue (HM240259.1). PCR was conducted in 25 cycles,

using thermal cycler (Biorad, USA), in 14 µl final volume including 0.7 µl cDNA, 7 µl 2X master mix, 0.5 µl from each primers (10 pm) and 5 µl nuclease-free water. The *PvD2* orthologue was isolated and sequenced from chickpea genome first and named CaD₂.

Gel analysis – The PCR products were run on 1.2% agarose gel and 1X TAE buffer, and for semiquantitative analysis, the gel picture was analyzed using GelQuantNET software. By comparing the intensity of interest gene with reference gene (actin), the relative gene expression was measured.

Analysis – Factorial experiment was conducted based on completely randomized design (CRD) with three replicates. Significant difference and mean comparison was carried out using Student's *t* test and Duncan test. Data were analyzed using the statistical software SAS 8.2.

3 Results

Fourteen-day-old chickpea plants were inoculated with *A. rabiei* conidia as a treatment plants, and mock-inoculated plants were considered as control. Upon infection, the desi (resistant) genotype showed necrotic spots by 10 days after inoculation (dai), while no symptoms were observed in kabuli (susceptible) genotype until 25 dai. Leaves and stems of each time point and genotypes were further used for gene expression studies. RT-PCR was carried out for *LRR*, *PGIP*, *AFP-Ca*, *CaD₂* genes using specific primers (Table 1), and gene expression measured during *A. rabiei*-chickpea interactions for these putative resistance genes.

Using specific *PvD2* primers, the expected band (172 bp) was amplified and sequenced. The sequence of chickpea *PvD2* analog showed 100% similarity to *Phaseolus vulgaris* L. D2 gene (*PvD2*, HM240259), and it was named CaD₂ (Fig. S1). The expression profile of CaD₂ gene at different time points in resistant and susceptible genotypes showed that this gene involves in chickpea-*A. rabiei* interaction.

Table 1 List of primer sequences used for RT-PCR in this study

Name	Sequence	Annealing T_m (°C)	Fragment length (bp)	NCBI sequence number
Actin-F	TTGGGATGGGTCAAAGG	56	398	XM-012716420.1
Actin-R	ACGAAGGATGGCATGAGG			
RR-F	ATTAGCTGCGACTCCACCTC	58	366	Aj609275.1
LRR-R	GGTGTGAGAGAACCGAGG			
PGIP-F	GCTTCAGAAACCGACTCTC	58	317	Loc101504619
PGIP-R	CGATGGAATCTGACCACAC			
AFP-Ca-F	TGGCTTGCTTCCTCTTC	57	184	DQ288897.2
AFP-Ca-R	ACAACGAAAGTCATCCCTGC			
CaD2-F	TATAGGTCGCGCCTTTAG	57	172	–
CaD2-R	ATGAACCGGAAGCTGGGT			

Statistical analysis showed that the expression level between genotypes was significantly different at $P < 0.01$ (Figs. 1a, 2a, Tables S1, S5) and CaD_2 had significantly higher expression in resistant genotype (desi) compared with susceptible genotype (kabuli) after treatment with *A. rabiei* spores (Figs. 1a, 2a, Table S1). The expression profile showed high level of expression at 12 h after inoculation (hai) in resistant genotype (Icc 12004). The gene expression in this genotype showed two phases, the expression profile from 0 to 12 hai showed increase; after 12 hai, the gene expression decreased, and at 24 hai, the gene expression reached the lowest level. The second phase started after 24 hai, and the gene expression increased slowly until the end of experiment (96 hai) (Fig. 1a). The expression profile in susceptible genotype (FLIP 82-150c) showed no change in expression until 12 hai, and minor increase was observed after that time (Fig. 1a). The results based on Student's *t* test and Duncan test showed significant difference between treatments (Fig. 2a).

AFP-ca gene was studied in resistant and susceptible genotypes using specific primers (Table 1). The expression profile for this gene in chickpea after inoculation by *A. rabiei* showed different patterns for resistant and susceptible genotypes and was significantly different at $P < 0.01$ (Fig. 2b, Tables S2, S5). The results showed increment expressions in desi genotype (resistant), the relative gene expression

showed two peaks at 6 and 24 hai, and the maximum level of expressions was observed at 24 hai. The lowest gene expression level in desi genotype ($RGE = 0.7$) was showed at 48 hai, and after that, the expression was increased with low rate. In kabuli genotype (susceptible), the expression was decreased immediately after inoculation, the lowest expression was showed in 6 hai, and the highest expression ($RGE = 0.43$) was shown in 12 hai, but it was lower than kabuli (Fig. 1b) after 24 hai. The expression profile showed almost no change at 48 and 72 hai, but at 96 hai, the expression was increased again in resistant genotype (Figs. 1b, 2b).

In the present work, *LRR* gene (Aj609275.1) was amplified from chickpea genome using specific primers. This gene has 83% nucleotide and 85% amino acid similarity to DRT100-like gene in alfalfa. The expression profile for this gene at different time points in resistant and susceptible genotypes was compared. The expression profile in susceptible genotype showed a significant increase compared with resistant genotype at $P < 0.01$ at different time points (Fig. 2c, Table S3, S5), the gene expression profile showed the highest differences at early time after inoculation (6 hai), and the resistant genotype (desi) showed delay in expression (Fig. 1c). At 48 hai, desi and kabuli genotypes showed the same level of gene expression, and at 72 hai, the gene expression level in susceptible genotype increased (Fig. 1c).

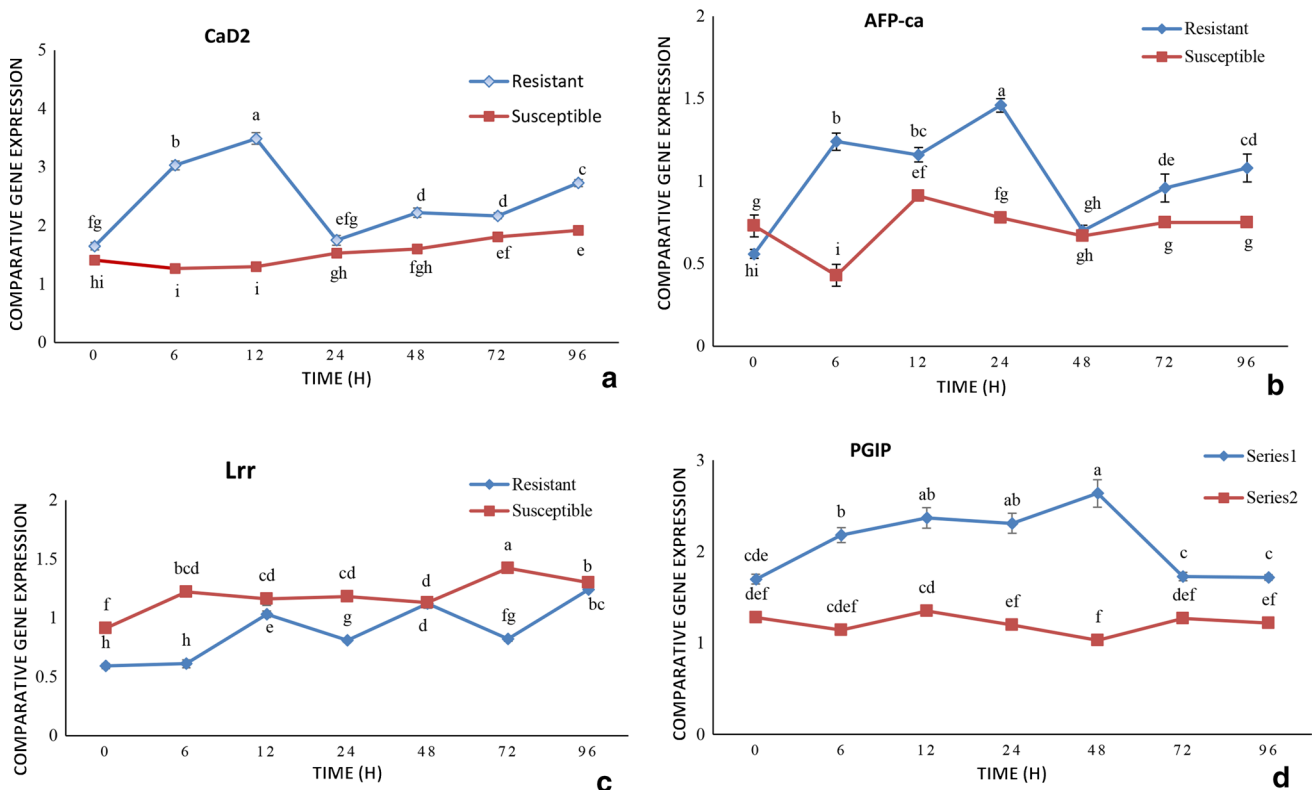


Fig. 1 Gene expression of CaD_2 (a), AFP-Ca (b), LRR (c) and PGIP (d) for desi (resistant) and kabuli (susceptible) genotypes over the time course of infection with *A. rabiei* isolate. Different letters show significant difference based on Duncan's test ($P < 0.01$) ($n = 3$)

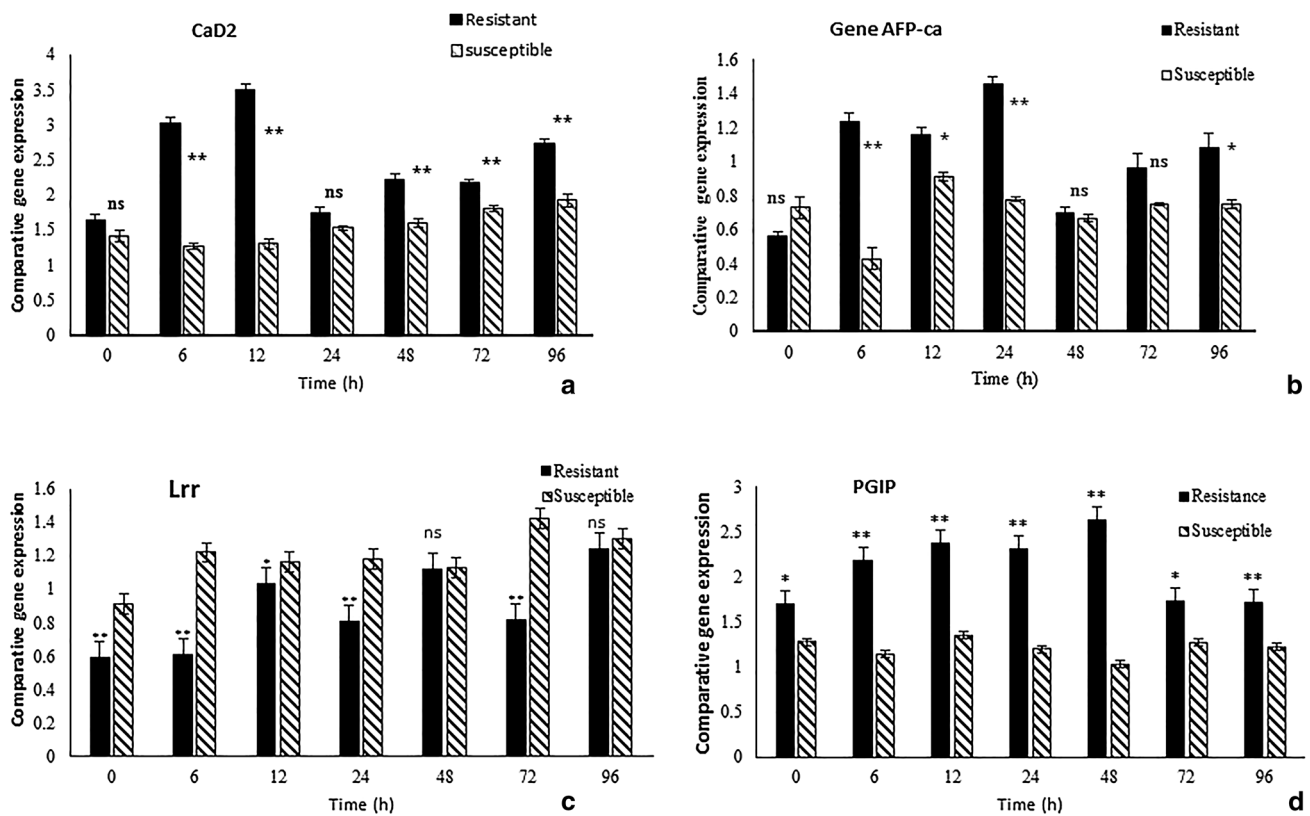


Fig. 2 Relative gene expression of CaD₂ (a), AFP-Ca (b), LRR (c) and PGIP (d) at different time points. Bars with (**) shows significant difference at ($P < 0.01$), (*) at ($P < 0.05$) according to Duncan's test, and (ns) represents no significant difference based on t test ($P < 0.05$) ($n = 3$). Gene expression levels were normalized using actin gene as reference gene

PGIP expression was measured in resistant and susceptible genotypes at different time points after inoculation. Results showed the differences in *PGIP* expression in Icc 12004 and FLIP 82-150c genotypes at $P < 0.01$ (Figs. 1d, 2d, Tables S4, S5). Results showed that the expression of *PGIP* in resistant genotype (Icc 12004) is higher than in susceptible genotype (FLIP 82-150c) at all times examined (Fig. 1d). The expression level for resistant genotype ($RGE = 2.64$) reached the highest amount at 48 hai, and after that, the gene expression was reduced. Results showed that no change was observed in gene expression level for susceptible genotype during all time points after inoculation (Fig. 1d).

4 Discussion

Resistance and susceptibility to pathogens controlled by genetic background of both plant and pathogens and the level of plant defense responses depend on the interactions between plant hosts and pathogens. Perception of pathogen-associated molecular patterns using plant receptors activates plant defense reactions via signal transduction cascades and activates the transcription of some defense genes (Coram

and Pang 2006). Different studies showed difference between resistant and susceptible genotypes for the perception of pathogens, reactions and defense signaling in response to different pathogen isolates (Elliott et al. 2011).

Chickpea possesses both active and passive defense against pathogens (Coram and Pang 2006), and in the present work, complex defense signaling and kinetics of differential expression of studied genes were observed in the chickpea after inoculation with *A. rabiei*. The rate of pathogen perception, signal transduction and activation of some transcriptional factors are vital for defense and resistance efficiency (Yang and Pedersen 1997). Genetic resistance to ascochyta blights depends on chickpea genotype, biological type of ascochyta, dominant and recessive resistance gene and also monogenic or polygenic resistance (Cho and Muehlbauer 2004). Previous studies showed that chickpea resistance to *A. rabiei* is a quantitative and polygenic resistance which is related to several defense mechanisms (Iruela et al. 2007).

Studies showed that chickpea has defense mechanisms that might induce after inoculation by *A. rabiei* and this mechanism causes race nonspecific resistance. However, our information about resistance to *A. rabiei* is not enough and

we need more research, especially when plants are infected by different pathogen isolates (Elliott et al. 2011). A research in Australia showed the lack of genome diversity in chickpea because of selection of a narrow gene pool and subsequent inbreeding (Leo et al. 2016). However, it might decrease the potential of diversity of defense mechanisms as it was shown by studying resistance gene homologues in chickpea compared to other legume species (Varshney et al. 2013).

Several reports exist about the transcriptome analysis of resistance genes of chickpea in interaction with *A. rabiei* using qRT-PCR or microarray which show the differences in gene expressions patterns at different time points in resistant and susceptible genotypes (Leo et al. 2016; Coram and Pang 2005a, b, 2006). In the present work, chickpeas infected by *A. rabiei* show reduction in some genes expression such as housekeeping gene and genes that are involved in plant metabolism in some genotypes in order to increase the expression of defense genes (Coram and Pang 2006). In this research, the expression level of studied genes showed that most of up-regulation in resistant genes has happened in early time points after inoculation (6–24 hai) (Fig. 1a, b) and it shows that maybe these genes are involved in pathogen perception and activation of other resistance mechanisms. In consistent with this, in another research, Coram and Pang (2006) showed that the highest differential expression of some defense genes between resistant and susceptible genotypes happened at 6–12 hai, and they interpreted that these genes might involve in the perception of pathogens and faster up-regulation of these genes in resistant genotypes causes fast perception and consequently restricts the pathogen penetration. It has been reported that *A. rabiei* spores germinate 12 hai (Pandey et al. 1987), and after 24 h, it can form appressorium and produce mucilage products that help attachment to the surface of plants for penetration (Kohler et al. 1995). As PGIP is a polygalacturonase inhibitor and fungal pathogens need polygalacturonase to degrade plant cell wall and penetration, and also up-regulation of this protein at 24–48 hai in resistant genotype has been observed, it can be concluded that this gene might inhibit pathogen penetration and recognition of fungal pathogens in resistant genotype as it was showed for soybean *pgip3* (GmPGIP3) (D'ovidio et al. 2006). In previous studies, it has been shown that structural PR proteins in plant cell wall prevent pathogen penetration (Otte and Barz 2000). As mentioned above, higher PGIP expression happened in resistant genotype at 48 hai, and it was also reported that up-regulation of PRs at 48 hai cannot inhibit pathogen penetration, but inhibit the infection of adjacent cells (Coram and Pang 2006). Based on this, PGIP might prevent pathogen penetration and it might also prevent expansion of infection to surrounding cells. It has been reported that that maximum expression of defense-related genes takes place at 48 hai (Moy et al. 2004). One of the reasons for differential expression between resistant and

susceptible genotypes at different time points is the production, deletion or selection of fungal effectors by pathogen that impact on pathogen recognition and this may trigger different host defense mechanisms (Leo et al. 2016). However, significant differences in expression levels and timings of the four defense-related genes in resistant and susceptible genotypes were studied in this research. The results showed the significant differences between susceptible and resistant genotypes in expression profile of some resistant genes.

PvD2 is a sulfur-rich protein highly expressed in the absence of phaseolin and major lectins (Yin et al. 2011). In this research, it was concluded that CaD₂ is not affected by *A. rabiei* attack in kabuli genotype (susceptible) at early time of inoculation and this might be related to the delay in defense reactions in susceptible genotypes as reported by Yang and Pedersen (1997). CaD₂ expression profile for desi genotype (resistance) showed up-regulation at early time points after infection (6–12 hai), and this might show that this gene has important role in pathogen perception and activation of defense cascades as it has been reported for some PR proteins (Coram and Pang 2006). It has been also reported that the level of defense-related gene expressions is higher in resistant genotype compared with susceptible genotypes (Rea et al. 2002). Based on the low expression of CaD₂ at 24 hai in resistant genotype compared with early time of inoculation, it might be concluded that this gene has no key role in the prevention of pathogen penetration and colonization. CaPGIP showed higher expression in resistant genotype compared with susceptible genotype in the present work over all time points, and the maximum of gene expression was observed at 48 hai in resistant genotype. The chickpea PGIP studied in this research has 73% nucleotide similarity to *Glycine max* (L.) Merr. PGIP (NP001304551) which was previously identified as a resistance protein against fungal (D'ovidio et al. 2006). D'ovidio et al. (2006) showed that PGIP expression in soybean in response to *Sclerotinia sclerotiorum* (Lib.) de Bary after infections changed, and they showed that GmPGIP3 up-regulated at early times after infection, but PGIP2 showed delay in gene expression. CaPGIP has 73% nucleotide identity with bean leucine-rich PGIP (PvPGIP2); it was reported that all variants of this gene can inhibit fungal PGs (Farina et al. 2009). PvPGIP2 has some variants with conserve regions and some regions with minor differences that can play role in recognition and specification of different PGs from different fungi (Farina et al. 2009). AFP-Ca is an antifungal gene that has 83% similarity to of *Trigonella foenum-graecum* Linn. defensin2 (TFgd2) and *Raphanus sativus* L. antifungal protein 2(RsAFP2). These genes were isolated from *T. foenum-graecum* and *R. sativus* and were transformed and expressed in bacteria (Karri and Bharadwaja 2013). Biological assay of this protein showed fungal inhibition broadly against some fungus such as *Rhizoctonia solani* JG Kühn, *Fusarium*

oxysporum (E.F. SM) W.C. Snyder & H.N Hansen Schlecht: Fr, *Botrytis cinerea* Pers ex Fr. *Phytophthora parasitica* var. *nicotianae* (Breda de Haan) Tucke. in a low concentration (Karri and Bharadwaja 2013). In another study, it has been reported that RsAFP2 causes induction of apoptosis in *Candida albicans* (C.P. Robin) Berkhout. (Mello et al. 2011). Consistent with this, in our research, we observed the higher expression of AFP-Ca at early time after inoculation in resistant genotype compared with susceptible genotype and at 48 hai, it was decreased and reached the same level of susceptible genotype. However, it has been reported that AFP-Ca might have role in perception and induction of resistance reactions in resistant genotypes, or even might have role in the induction of apoptotic reaction as has been reported for RsAFP2.

Our results showed that studied chickpea defense-related genes encounter with *A. rabiei* are differentially expressed between resistant and susceptible genotypes, and it shows that the resistance to *A. rabiei* might involve the genes from known resistance gene families. The important role of *CaD₂*, *CaPGIP* and *AFP-Ca* in *A. rabiei*–chickpea pathosystem was described, and it was revealed that the earlier expression of these defense genes can define the level of resistance to *A. rabiei*. Our results provided us more information about *A. rabiei*–chickpea interaction and contributed to molecular breeding programs.

Author contributions AA: Researcher who was carried out the experiment. AA: Designed and performed experiments. MM: Designed and performed experiments. JA: Mycologist and advisor who provided critical revision of the article, Associate professor, Department of Plant Protection, University of Kurdistan, Iran.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest regarding the publication of this article.

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