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# MOLECULAR BASIS OF ANTIFUNGAL RESISTANCE IN TOMATO VARIETIES

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Tomato has a significant share in human food items and its yield should necessarily be enhanced against a number of its fungal pathogens. Use of plant innate resistance against pathogens is an effective and the cheapest tool of crop protection. Furthermore, proteomic and transcriptomic analyses of plant explore the qualitative and quantitative basis of this resistance, which is helpful to design future agriculture plans. Therefore, constitutive antifungal resistance of tomato varieties commonly cultivated in Pakistan has been evaluated on the basis of their transcriptome and protein profiles. Study disclosed the fact that six pathogenesis related genes belonging to families PR1, PR2, PR3, PR7 and MT2bL exhibited higher transcriptional rate in comparison with gene Chitinase 3 acidic, which showed lower expression in resistant tomato variety. According to the semi quantitative RT-PRCR results, Osmotin-like PR5 doesn't regulate constitutive antifungal resistance and shows equal expression with the varying resistance of plants. Moreover, resistant tomato plants had four additional protein species in their cellular contents, ranging in size 40-52 kDa. Those proteins might be resistance ensuring factors of tomato as they were absent in susceptible plant protein profile. This study demonstrates the molecular basis of tomato resistance against fungal pathogens and will be helpful for researchers to improve such resistance in tomato varieties under development.
**Keywords:** Antifungal resistance, pathogenesis, gene expression, proteomics, proteins of enhanced resistance.

## INTRODUCTION

Determination of bases of phenotypically varied characters in single species has always been a debatable issue in molecular biology (Cameron, 2002; Sanderson, 2004; Aranzana, 2005; Mehmood *et al*., 2013, 2014). Along with the branch of sciences there are many techniques and methods developed for the evaluation of molecular reasons behind such varying characters (Heckman, 2001; Papini, 2007; Fu *et al*., 2009). Two major tools for evaluation of trait expression in any organism are transcriptome analysis and proteome analysis. Both analyses provide sound arguments for the extent of expressed phenotypic character in any organism. These tools are thought to be the most reliable techniques developed in this direction and are also widely used ones (Popesku, 2010; Ghazalpour, 2011; Dyhrman, 2012). One of the advantages of these techniques is that they not only prove the expression of genotypic feature but also give its exact quantification (Gallardo, 2007; Joubert, 2010).

Tomato is an important horticultural food crop of most of the countries of the world including Pakistan (Kamran, 2010; Naz, 2011). Due to its remarkable share in food consumption and industries, it is very necessary to improve its yield against the activities of a number of its devastating pathogens, especially against fungal pathogens (Deckelbaum, 2006; Xie, 2012). The cheapest, the most efficient and ecofriendly way to achieve this goal is the cultivation of resistant varieties on agriculture farms (Chaerani, 2007). For this purpose varieties should be analyzed for their resistance against fungal pathogens. This analysis of innate resistance should be conducted through the most reliable means and technique e.g. molecular techniques. Therefore, in present study, constitutive defense analysis for tomato varieties under routine cultivation in Pakistan has been performed through transcriptome and proteomics analyses. It will be helpful for future cultivation suggestions of tomato varieties under Pakistani environment.

## MATERIALS AND METHODS

Preliminary studies were carried out to determine resistance behavior of different tomato varieties against native fungal pathogens under Pakistani climatic conditions and two varieties were selected i.e. Red Tara & Dinaar (Ahmad *et al*., 2013). Those varieties were denoted as representative varieties (the most susceptible and the most resistant) and subjected to further downstream analysis.

***Pathogenesis related gene expression*:** Expression of eight pathogenesis related PR genes (Table 1) along α-Tubulin were checked by semi-quantitative RT-PCR in both the representative varieties. Primer sequence of [F]: TGAACAACTCATAAGTGGCAAAG; and [R]: TCCAGCAGAAGTGACCCAAGAC was used to target housekeeping gene α-Tubulin. For this purpose total RNA was isolated by using RiboEX (TM) of 'biomol' and treated with Reverse Trancriptase in a single step to produce a convenient quality cDNA. Equal quantities of cDNA of

**Table 1. Detailed primer sequences used to target specific genes in tomato varieties. Accession numbers given against genes, refer to complete gene sequences deposited on online database.**

| Gene family | Specific class | Accession numbers | Primer details | Tm (°C) | References |
|---|---|---|---|---|---|
| PR1 | PR1b, basic PR1 | AJ011520 | F-CCAAGACTATCTTGCGGTTC | 57.3 | |
| | | | R-GAACCTAAGCCACGATACCA | 57.3 | |
| PR2 | PR2a, acidic glucanase | M80604 | F-TATAGCCGTTGGAAACGAAG | 55.3 | Van Kan *et al*. (1992) |
| | | | R-TGATACTTTGGCCTCTGGTC | 57.3 | |
| | PR2b, basic glucanase | M80608 | F-CAACTTGCCATCACATTCTG | 55.3 | |
| | | | R-CCAAAATGCTTCTCAAGCTC | 55.3 | |
| PR3 | Chitinase 3, acidic | Z15141 | F-CAACTTGCCATCACATTCTG | 55.3 | |
| | | | R-CCAAAATGCTTCTCAAGCTC | 55.3 | Danhash *et al*. (1993) |
| | Chitinase 9, basic | Z15140 | F-AATTGTCAGAGCCAGTGTCC | 57.3 | |
| | | | R-TCCAAAAGACCTCTGATTGC | 55.3 | |
| PR5 | Osmotin-like PR5 | AY093593 | F-AATTGCAATTTTAATGGTGC | 49.1 | Rep *et al*. (2002) |
| | | | F-TAGCAGACCGTTTAAGATGC | 55.3 | |
| PR7 | P69A, subtulisin-like | Y17275 | F-AACTGCAGAACAAGTGAAGG | 55.3 | Tornero *et al*. (1996) |
| | | | R-AAC GTGATTGTAGCAACAGG | 55.3 | |
| MT2bL | Metallothionein 2b-like | EF584509 | F-AGTACGCGGGGAGCAAC | 57.3 | |
| | | | R-GGATGAAAGCAGAGGTAGATT | 57.2 | |

representative varieties were added into separate RT-PCR reactions against each separate set of primers (Table 1). Amplifications were obtained under the most suitable reaction conditions for which thee primer sets had already been standardized. Moreover, relative expressions of pathogenesis related genes were evaluated due to unequal amplifications at the end of RT-PCR. Therefore, amplified cDNA were quantified by using spectrophotometer and image analysis was carried out through GELANALYZER (Lazar, Hungary).

***Protein profile analysis*:** To determine the protein profile of subject tomato varieties, total protein contents were isolated from leaf tissues in phosphate buffer saline (PBS) containing NaCl (140mM), KCl (2.5), $Na_2HPO_4$ (10mM) and $NaH_2PO_4$ (1.8mM). Then, two dimensional polyacrylamide gel electrophoresis was performed with "blue native PAGE" in first dimension, and "SDS PAGE" of obtained protein pattern was conducted in the second dimension to get a more precise protein profile of better resolution. Moreover, gels were stained with coomassie blue to visualize protein bands.

## RESULTS

Quantitatively, similar profile of gene expression was found in both tomato varieties but there were drastic qualitative differences as well. All eight genes studied were expressed in both varieties but the band intensities of individual genes obtained in agarose gel were not comparable (Fig. 1). It's a

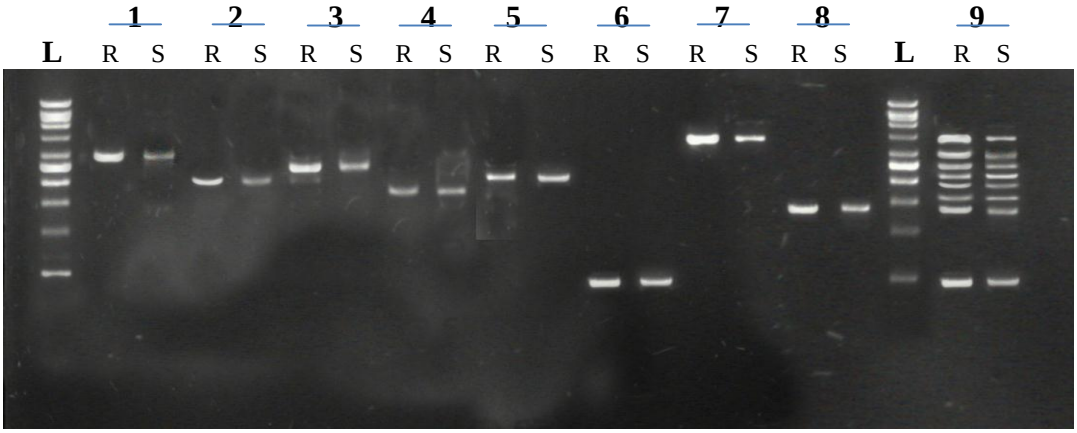


**Figure 1. RT-PCR gel electrophoresis image of tomato varieties for expression of defense related genes.**
1= PR1b, basic PR1; 2= PR2a, acidic glucanase; 3= PR2b, basic glucanase; 4= Chitinase 3, acidic; 5= Chitinase 9, basic; 6= Osmotin-like PR5; 7= P69A, subtulisin-like; 8= Metallothionein 2b-like; R= Resistant; S= Susceptible

widely known phenomenon that all resistance ensuring genes do not undergo high rate of transcription all the times; but some genes are upregulated only under the influence of external stimuli (Klee, 2002; Walther *et al*., 2007). Present Results show that six out of eight genes have enhanced expression in resistant variety except Chitinase 3, acidic and Osmotin like, PR5 (Fig. 2). Products of those six enhanced genes were likely to be responsible for making Dinaar resistant against fungal pathogens (Mahomed and Berg, 2011; Gupta *et al*., 2011). Whereas, it was interesting that gene Chitinase, 3 acidic had a relatively low expression in resistant tomato plants. That was a clear indication of dull behavior of particular gene in constitutive resistance of tomato. Similarly, Osmotin like PR5 gene exhibited equal transcription rate in both extremes of internal resistance, providing a conclusion that this gene do not actively participate in tomato innate antifungal resistance.

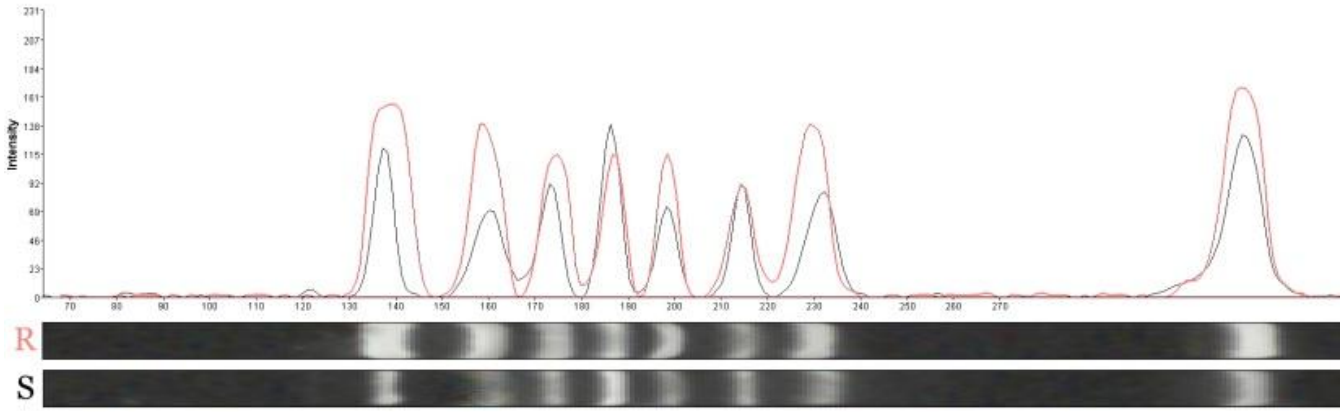


**Figure 2. Pixel intensity based graph of electrophorated bands of lane 9 in Figure 1.**
R: Resistant; S: Susceptible

Protein profiles of two tomato varieties were identical to each other in most of the cases but there were four extra proteins detected in resistant variety (Fig. 3). Three of those four protein species had a size between 40-52 kDa; whereas one was of 65 kDa. First protein species was very low in quantity with comparison to other three proteins but it produced its band of good resolution (Fig. 3B1). Protein 2 and 3 were so close to each other that after blue native PAGE run they were completely coalesced and non-distinguishable. But, after denatured run resolution of their bands was too improved to visualize their identity (Fig. 3B2 and 3). Fourth protein species exhibited highest quantity and lowest size (45kDa) among all four protein species detected (Fig. 3B4).

## DISCUSSION

Plant defenses against fungal pathogens depend upon expression of antifungal genes. A number of studies agree upon the phenomenon that higher pathogenesis related gene expression leads towards stronger antifungal defenses in plants (Houterman, 2008; Li *et al*., 2010a; Liu *et al*., 2012). The same pattern prevailed in this study with enhanced

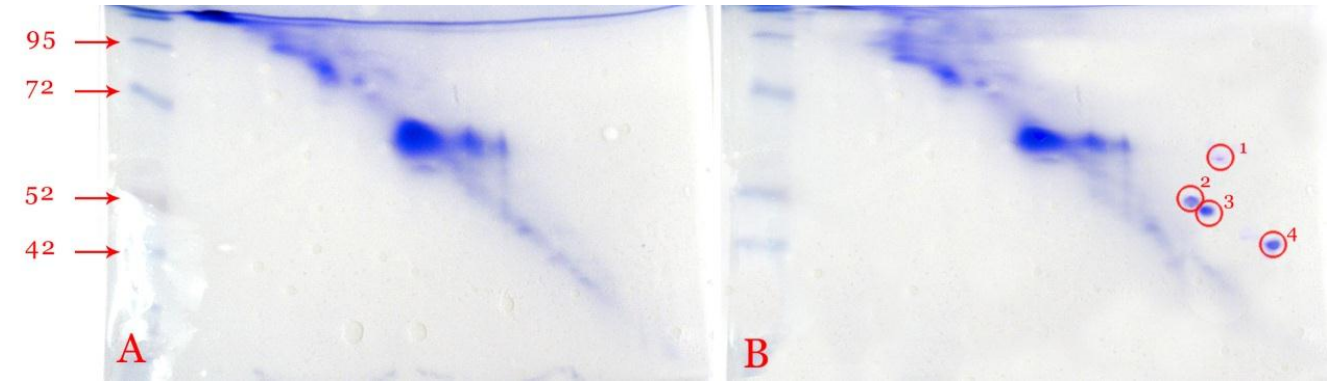


**Figure 3. Two dimensional protein profiles of tomato varieties.**

expression of pathogenesis related genes in varieties Dinaar than Red Tara proving it more resistant against fungal pathogens. Expression of defense genes with reference to plant environment and stimulators is a complex phenomenon that has never been easy to understand (Andersona, 2004; Kidda, 2009; Nzanza *et al*., 2012; Hossain *et al*., 2012). Therefore, no concrete law could be derived regarding quantitative or qualitative expression of defense genes against any genomic or environmental conditions. Therefore, being reminiscent of previous researches, this study also concluded that increased innate resistance is a result of enhanced expression of only some defense genes present. In more simplified terms, higher transcriptional rate of all defense genes present is not necessary for a plant with augmented resistance.

Many researchers including Ellis *et al*. (2007), Fradin *et al*. (2009) and Zhu *et al*. (2009) concluded that all the plants with enhanced resistance exhibited one or more related proteins encoded in their proteomics profile. This phenomenon is strongly supported by current investigation, as resistant tomato variety showed four more protein bands than susceptible variety; which would individually more or less contribute for increased antifungal resistance. These proteins regulate directly or indirectly resistance mechanism of tomato plants. Studies of Yu *et al*. (2001), Li *et al*. (2010a) and Li *et al*. (2010b) fall in this direction. Therefore, it can easily be assumed that all these four proteins regulate resistance potential in tomatoes but still more efforts are required to clarify their mechanism of resistance upregulation in tomato plants.

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