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Research Article

Study of *Puccinia striiformis* -related proteins in wheat using two-dimensional gel electrophoresis

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Abstract: Wheat is one of the strategic products and has rich nutritional value. Plant diseases are major limiting factors that reduce the yield and quality of wheat, and yellow rust *Puccinia striiformis* f. sp. *tritici* causes considerable damage to wheat production. The most reliable way to control this disease is the use of resistant varieties. Plants have different mechanisms to defend against pathogens, one of which being proteomics employed to examine defense mechanisms in both sensitive and resistant plants. In this research, in order to identify the expression pathways and proteins involved in the mechanisms, resistant (Gaspart) and sensitive (Morvarid) wheat varieties were inoculated with *P. striiformis*, followed by the examination of peroxidase, catalase enzymes, intracellular osmoprotectants. The results showed that the amount of protein, carbohydrates, proline concentration, and peroxidase and catalase activity significantly increased by rust in the resistant variety. Also, results of the electrophoresis of two-dimensional proteins with stripes of IPG = 17 cm and pH = 4-7 showed that expression changes of proteins could be divided into two groups in response to *P. striiformis*. The first group included the proteins that directly reacted to pathogens such as peroxidase and PR10 proteins. The second group consists of the resistance proteins as transcription factors or proteins involved in signaling pathways or chaperons in determining proteins' partial structure such as molecular chaperone protein Hsp90.

Keywords: 2D gel electrophoresis, Carbohydrates, Catalase, Peroxidase, Proline

Introduction

Wheat *Triticum aestivum* is a basic food for the majority of people and is widely cultivated around the World. According to FAO, the production of wheat was about 730.2 million tons during 2018 which is less than the demand (FAO, 2019). By some estimates, in order to meet the wheat requirement, global wheat production must be increased to 760 million tons in 2020 (Solh *et al.,* 2012). However, pests and diseases annually cause a significant reduction in the yield of wheat.

Yellow rust (stripe rust) is a serious wheat disease around the world caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriksson (Wellings, 2011). *P. striiformis* causes great loss to global wheat production every year. Stripe rust can reduce the yield and quality of grain and forage. In many areas10% and 70% yield losses have been reported (Chen, 2005). It

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also causes poor seed germination. There are different methods to control this disease, including agronomic and chemical control. Utilization of resistant varieties is the most reliable and efficient method for yellow rust management (Chen, 2007). In environmental stresses, plants respond to stress by storing osmotic regulators. As an amino acid, proline has a major role in osmotic pressure, reduces cell water loss in plants, regulates osmotic pressure, and accumulates in response to biotic and abiotic stresses (Venkateswarlu *et al.,* 2012). Stripe rust has different effects on the amount of carbohydrates, protein and antioxidant enzymes. Plants resist against diseases by accumulating a large amount of proline (Hong *et al.,* 2000), yellow rust is no exception. The production of adaptive metabolism and resistance need carbon skeleton. Metabolism of carbohydrates is a factor that has a direct relevance to the plant defense response against pathogens. Carbohydrates are important intermediate compounds in photosynthesis, respiration, structural units of nucleic acids, and some key components of electron transport chain, such as nicotine amide and nucleotides (Hopkins *et al.,* 2008).

Studying defense mechanisms (genes, proteins, and osmoprotectants) in plants against diseases is needed for producing resistant varieties. Plant defense responses are regulated using new proteins synthesis and a complex network of interconnected signaling pathways. Signal transduction pathways lead to the strength of cell walls, production of antimicrobial metabolites (phytoalexins), and production of reactive oxygen species (ROS) (Almagro *et al.,* 2009). Resistance and susceptibility to diseases are related to the host and genotype of pathogens. Plants have developed mechanisms against pathogens, some of which have been designed during the production of chemical and physical barriers to prevent infections**.** Interactions of coding pathogen elicitors with host receptors seemingly activate a signal transduction cascade consisting of protein phosphorylation, ion fluxes, ROS, and other signaling occurrences. Endogenous pathogen signals have the ability of activating different arrays of plant defense genes and make products such as glutathione S-transferase, peroxidases, cell wall proteins, proteinase inhibitors, hydrolytic enzymes (e. g. chitinases and β-1,3-glucanases), pathogenesis-related (PR) proteins, and phytoalexins; biosynthetic enzymes such as phenylalanine ammonia-lyase and chalcone synthase (Manichavelu *et al.,* 2010). Indeed, stress is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense in different parts of the plant (Liu *et al.,* 2010). ROS can potentially interact with many cellular compounds and cause damage through responding to them in the membrane and other essential macromolecules such as photosynthetic pigments, proteins, nucleic acids, and lipids. Accordingly, the level of ROS is critical in cells (Blokhina *et al.,* 2003). Plants have anti-oxidation mechanisms to reduce the effects of free radicals, which include the rate of plant defense enzymes such as peroxidase and catalase (Staskawicz *et al.,* 1995). Peroxidase is an antioxidant enzyme that cleans hydrogen peroxide, a toxic compound, from the environment. Peroxidase belongs to a large multigene family and is involved in a wide range of physiological processes such as lignin formation, phytoalexin synthesis, and ROS metabolism. It plays a role in plants' hypersensitive response (HR) and programmed cell death (PCD) in infected hosts (Almagro *et al.,* 2009) .

Proteomics addresses total proteins available in a biological unit (cells, tissues, and organs) at each developing stage under particular environmental conditions. Interaction of protein-protein, protein modifications, and protein function can be studied under proteomics (Zhang, 2007). Thus, it has research potentials for identifying the mechanisms of plant defense. Many of the early methods adopted based on two-dimensional gel electrophoresis are valuable for rapid identification of major proteomic differences

between healthy and infected plants. Through proteomics, many economically important diseases are studied to determine the pathogenicity and immune complex aspects of plants (El-Hadrami *et al.,* 2012).

Ngadze *et al.* (2012) studied higher polyphenol oxidase activity in potatoes in resistant varieties because of soft rot pathogens, and found that this enzyme has an important and effective role in resistance against potato soft rot disease. Proline accumulation in plants causes resistance against diseases so that it increases proteins synthesis and prevents their modification. Because of their protein structure, Enzymes are influenced by this mechanism and are protected by proline. Scott *et al.* (2005) also reported higher amount of carbohydrates in the interaction of the fungus *Phytophthora nicotianae* and tobacco leaves. Herbers *et al.* (2000) studied the tobacco affected by Potato virus Y and showed greater amount of carbohydrate after applying the pathogen.

In this paper, proteomics and spectrophotometric studies were performed for scrutinizing proteins expression and activity level of some enzymes involved in the resistance of wheat against *P. striiformis*.

Materials and Methods

This study was conducted in the Greenhouse of University of Mohaghegh Ardabili. Two wheat varieties including resistant (Gaspard) and sensitive (Morvarid) were planted based on a completely randomized design (CRD) in 2012. Wheat was cultivated at 70% relative humidity and 20-25 °C. Soil mixture was 50% Peat moss, 30% manure, 10% sand, 5% Perlite, and 5% peat. Plants were inoculated with *P. striiformis* at three leaf stage and the leaves were collected 1, 3, and 5 days post inoculation. Leaf samples were immediately frozen in liquid Nitrogen and placed at –70 °C. To inoculate plants, 0.1 g of *P. striiformis* spores, 500 ml distilled water and a drop of Tween-20 were mixed in a container. The pots were inverted so that the leaves of the seedlings were in complete contact with the suspension of inoculum produced.

Compatibility metabolites measurements

Omokolo's *et al.* (1996) method was adopted with some modifications to extract soluble carbohydrates. A total of 0.1 g inoculated leaf sample was abraded using 5 ml 80% Ethanol and placed in a water bath at 70 °C, and the obtained extract was centrifuged for 10 min at 10,000 rpm. The supernatant was used to measure the content of soluble carbohydrates. The concentration of soluble carbohydrates was measured using a spectrophotometer at 620 nm. Bates (1973) method was applied to measure proline; leaf tissue (0.5 g) was crushed in 3% Sulfosalicylic acid (5 ml) and then centrifuged. Ninhydrin reagent (2 ml) and Glacial acetic acid (2 ml) were added to the extract and incubated in water at 100 °C for 1 h, which was then mixed with Toluene (4ml). The absorbance was measured in a spectrophotometer at 520 nm.

Protein was extracted via Damerval *et al.* (1986) method with some modifications. The obtained supernatant contains protein extracts. Bradford's (1976) method was adopted to investigate protein concentration, based on the chemical binding (Coomassie Brilliant Blue G-250 to protein) in an acidic environment calculated at 595 nm. The concentration of lysine and methionine was measured by O'Farrell (1969) method with some modifications. Absorption of lysine and methionine was measured at 570 nm and 510 nm.

Antioxidant enzyme activity measurements

The enzyme activity was measured at the hypocotyl stage (after 14 days). The supernatant of centrifuge was used for enzyme activity measurements (Sudhakar *et al.,* 2001). Catalase activity was assayed according to Kar and Mishra (1976) where 60 µl protein extract was added to Tris buffer (50 mM, pH =7) H_2O_2 5 mM in the ice bath, and then the absorbance was considered at a wavelength of 240 ‘nm. Enzyme activity was obtained for OD/mg protein fresh tissue. Peroxidase activity was

measured as described by Kar and Mishra (1976): 50 µl protein extract was added to 2.5 ml extraction buffer containing 100 µM Tris buffer 100 mM and absorbance changes was read at a wavelength of 425 nm. Polyphenol oxidase activity assay was performed by tyrosine: The tyrosine PPO activity was measured according to the method of Anderson and Morris (2001). The change in absorbance at 475nm was compared with the control sample. One unit of PPO activity was defined for OD/mg protein fresh tissue.

Protein electrophoresis

For studying changes of protein expression by two-dimensional electrophoresis, Lamly *et al*.'s method was employed where 750 µg protein extract was added to the buffer Rehydration (4.8 g urea, 2g CHAPS, Bromophenol blue, 350 µl 2-Mercaptoethanol, and 200 µl Ampholyte) and dumped in Bio Rad IEF CELL apparatus tray wells. Then, IPG strips (4-7 pH, 17 cm) were placed on Bio Rad IEF CELL apparatus. Then, for the second dimension separation, the sample containing the protein was placed in equilibration buffer (36.01 g Urea, 34.5 g Glycerol, 2 g SDS, 3.35 ml Tris, and Bromophenol blue) for 15 min. Afterwards, IPG stripes were washed with SDS- PAGE 1x running buffer and put between the two glass plates of SDS-Page and run on the gel with 30 mA.

Statistical Analysis

Data analysis was performed using SPSS (Ver. 23) software and charts were drawn by Sigma plot (Ver. 13). Comparisons were made by LSD at level 5%. Also, the results of electrophoresis were analyzed by Total Lab software.

Results

Compatibility metabolites

According to the results of the experiment, Yellow rust disease caused by *P. striiformis* had a significant effect on the compatible metabolites (protein, lysine, methionine, proline and carbohydrate) in different wheat varieties. *P. striiformis* decreased the amount of protein in Morvarid (sensitive variety) compared to the control, while in the Gaspard variety (resistant variety), *P. striiformis* increased its amount from 40 to 60 mg per g FW^{-1}. Also, lysine amino acid was lightly reduced in Morvarid variety (from 5 to 4.5 mg per Fw^{-1}) and elevated in Gaspard variety (from 2.5 to 5mg per Fw^{-1}). Amount of methionine in both resistant and sensitive variety diminished by *P. striiformis* compared to the control. Proline and carbohydrate in both varieties rose in the treatment group compared to the control, but the increase in Morvarid was sharper than that in Gaspard. However, the gradient of Carbohydrate in Gaspard (from 0.05 to 0.28 mg per Fw^{-1}) was stronger than Morvarid (from 0.05 to 0.15 mg per Fw^{-1}) (Fig. 1). Plants deal with biotic stress by saving osmotic regulators.

Antioxidants enzyme activity

According to the results, the activity of antioxidant enzymes such as catalase, peroxidase and polyphenol oxidase in both Gaspard and Morvarid were affected by Yellow rust disease (*P. striiformis*). The disease elevated the activity of Catalase and peroxidase and reduced polyphenol oxidase activity. In Morvarid variety, the *P. striiformis* caused a sharp increase in Catalase and Peroxidase activity compared to Gaspard; the activity of Catalase and Peroxidase in Morvarid was about 2.5 and 4, which rose after the treatment to 6 and 23 respectively. However, in Gaspard, their activity was 1, 5 and obtained 5 and 6.5 OD per mg protein Min^{-1}. Polyphenol oxidase activity fell and then rose due to yellow rust disease. Its activity increased from 23 to 25 OD per mg protein.min^{-1}in Morvarid variety and decreased from 18 to 13 OD per mg protein.min^{-1}in Gaspard variety. *P. striiformis* causes an increase in Reactive Oxygen Species (ROS) and H_2O_2 and while the level of ROS and H_2O_2 in cellular environment is high, the activity of antioxidant enzymes along with their effects were reduced (Fig. 2). The results of these findings are consistent with the research of Magbanua *et al*. (2007).

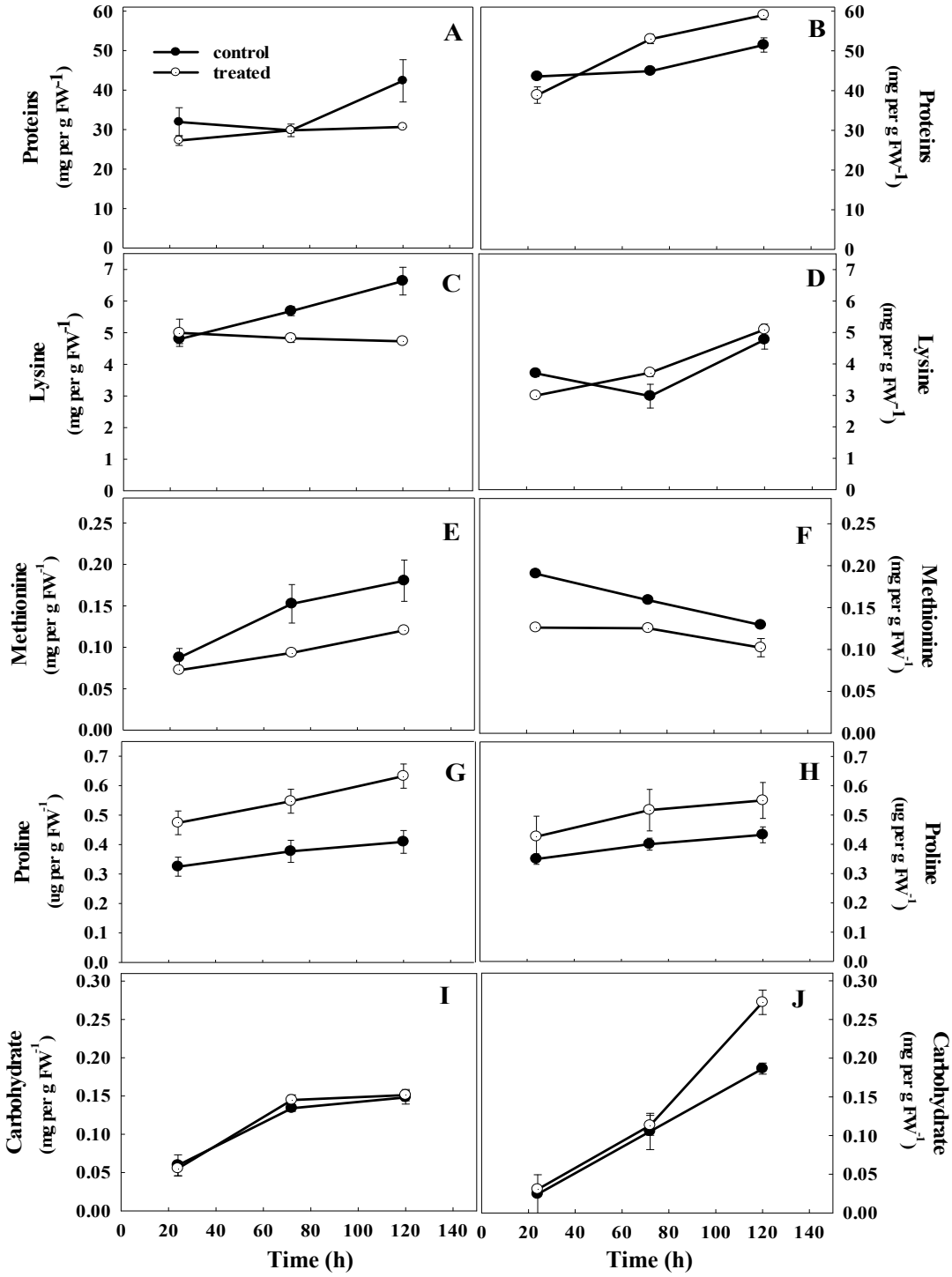

Figure 1 Effect of yellow rust *Puccinia striiformis* on compatibility metabolites. A, C, E, G and I for Morvarid variety and B, D, F, H and J for Gaspard variety.

Two-dimensional gel electrophoresis

By comparing 2D-PAGE gel of inoculated wheat leaves with controls, 14 proteins were identified (Fig. 3). These spots belonged to ribosomal P0 protein (spot 1), alpha2 tubulin (spot 3), heat shock protein 70 (spot 6), Cyclophilin-type PeptidylprolylCis-trans isomerase (spot 8), and Blr0690 protein resistance multidrug (spot 9) in the sensitive variety (Table 1). The expression of these proteins was higher in sensitive than resistant varieties but none were observed in the control samples. Comparison of 2D-PAGE gel spots (2D-PAGE) in both varieties of treated samples on the fifth day after inoculation (Fig. 3) showed that spots 4 (TCP-1/cpn 60 chaperon in hypothetical protein (UM05831.1)), 5 (CNBA306 (similar to HSP70) hypothetical protein), 7 (CNBF4120 (CD: F1 hypothetical protein ATP synthase b subunit domains), and 14-10 (Kunitz trypsin protease inhibitor, peroxidase, PR10 protein, Pprg2 protein, molecular chaperone Hsp90) were expressed in both inoculated plants and controls (Table 1). These proteins showed higher expression in both varieties in the treatment group than the control (Fig. 3).

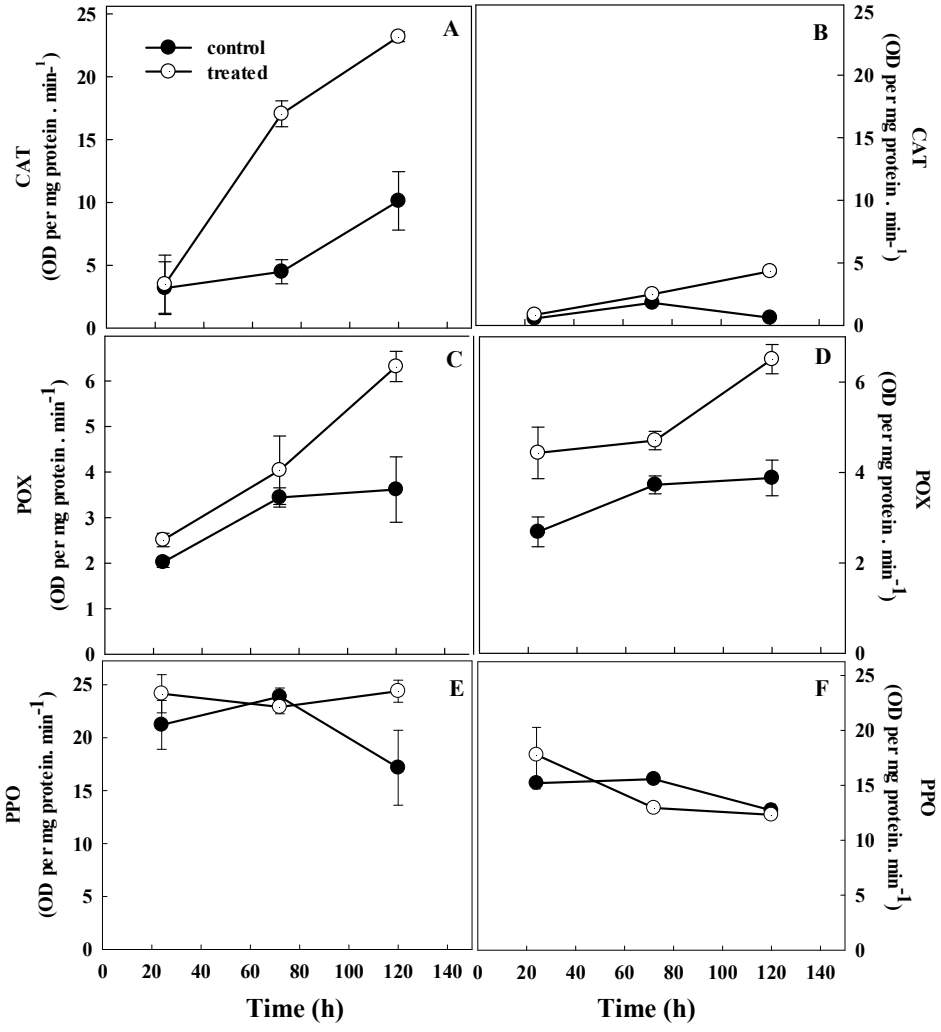

Figure 2 Effect of yellow rust *Puccinia striiformis* on antioxidant enzymes activity. A, C, E, for Morvarid variety and B, D, F, for Gaspard variety.

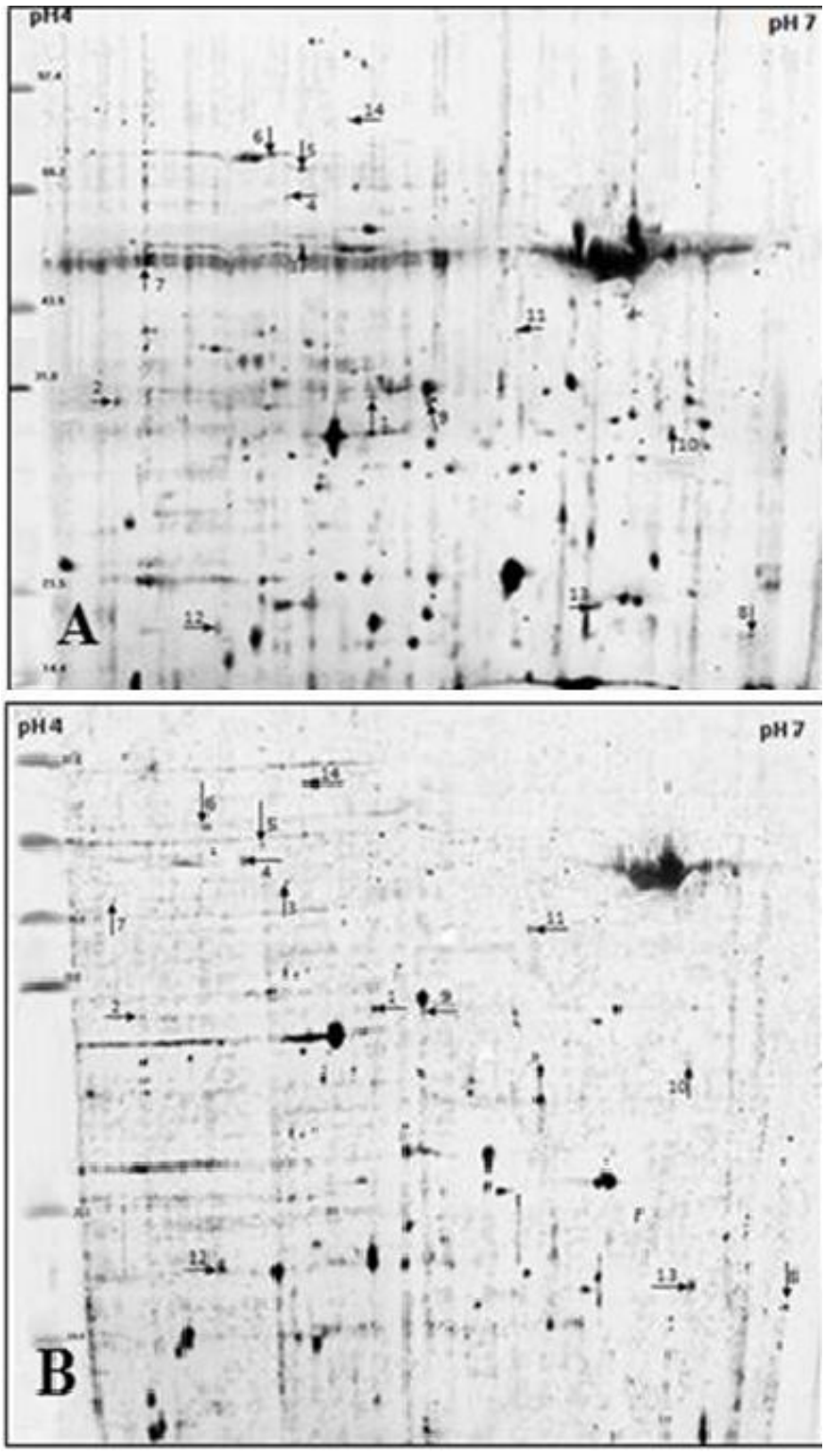

Figure 3 Electrophoresis of wheat leaf tissue on the 2D-PAGE gel five days after inoculation of leaves: (A) resistant variety and (B) sensitive variety.

Table 1 Protein spots detected in sensitive and resistant wheat varieties against *Puccinia striiformis*.

Spot[1]	Protein ID[1]	Mw (kDa)[3]	pI[4]	Predicted ID[5]
1	Ribosomal P0 protein	33.7	4.56	CAD29995.1gi:37359627
2	14–3-3 protein	29.0	4.50	EAK80778.1gi:46095545
3	Alpha2 tubulin	50.5	4.73	gi:763393
4	Hypothetical proteinUM05831.1 (CD: TCP-1/cpn 60 chaperoninfamily)	60.0	5.70	EAK86776.1gi:46101543
5	Hypothetical proteinCNBA306(similar toHSP70)	70.0	5.50	EAL23659.1gi:50261009
6	Heat shock protein 70	70.0	5.86	CAA70695.1gi:3114952gi:18482472
7	Hypothetical proteinCNBF4120 (CD: F1ATP synthase b subunitdomains)	50.0	4.98	EAL20086.1gi:50257377
8	Cyclophilin-type peptidylprolyl*cis-trans*isomerase	19.2	6.22	NP_595664.1gi:19112456F-49373
9	Blr0690 protein (putativemultidrugresistanceprotein)	34.5	5.90	gi:27348939
10	Kunitz trypsinprotease inhibitor	23.0	5.10	CU019604_15
11	Peroxidase	38.2	5.80	gi|537317
12	PR10 protein	6.2	4.50	gi|1616609
13	Pprg2 protein	5.2	5.80	gi|22266001
14	Molecular chaperone Hsp90	80.5	4.90	CT573078_38

1; Numbered protein spots on two-dimensional gels, 2; Name of identified proteins, 3; Molecular weight of predicted proteins, 4; Isoelectric point of predicted proteins, 5; Accession number of proteins in databases.

Expression levels of peroxidase protein increased in both varieties after inoculation and its value was higher in resistant than Sensitive varieties. High defensive capability in resistant varieties was probably due to the over-expression of this enzyme. In all the process of physiological and phenological changes, hydrogen peroxide produced a poisonous substance in the plant tissues.

Discussion

Amino acids are osmotic regulators, and plants resist stress by proline, lysine, methionine and carbohydrate (Hong *et al.,* 2000). Plants confront diseases and their subsequent damage by boosting concentration of compatibility metabolites in order to prevent cellular weakness. Stress conditions lead to accumulation of reactive oxygen species (ROS), change in protein structure, inactivation of enzymes, and disruption of proteins; plants resist stress by producing more proteins (Chisholm *et al.,* 2006).

Plant cells produce Hydroxyl radical (OH) under the stress conditions of superoxide O2 and hydrogen peroxide H_2O_2 (Turkan *et al*., 2005, Sairam and Saxena, 2000). These reactive oxygen species (ROS) cause damages such as lipid oxidation, and membrane deformation, discoloring or destroying pigments such as chlorophyll and may cause molecular damage as in the DNA (Mohanty, 2003). Plant can resist by proline accumulation (Hong *et al.,* 2000) whose molecules have hydrophilic and hydrophobic regions and can affect the solubility of proteins. By growth propagation, plants produce more proteins to fight stress. In our research, the concentration of protein was higher in resistant variety than the susceptible. It seems that plants synthesize protein to fight pathogens in stress conditions; hence, the rate of protein synthesis and total protein concentration are higher in resistant variety. There is an interaction between proline and hydrophobic proteins, which leads to higher stability and prevents them from changing their nature due to the increase in the total level of

hydrophobic protein molecules. Enzymes are affected and protected by this proline mechanism because of their protein structure (Venkateswarlu *et al.,* 2012).

Yellow rust disease increased carbohydrate level of cells and, because it is the source of carbon and energy supply in plant cells, the amount of carbohydrates increases in order to provide energy for combating pathogens. The presence of carbohydrates in cells causes the activation of resistance genes (Bolton and Thomma, 2008). Production of compatibility and resistance metabolites requires a carbon skeleton, which necessitates the availability of carbohydrates. When a tissue is contaminated with a pathogen, photosynthesis is lessened and cells need more energy for defensive activities and transporting it from source to the infected tissue; this transition is generally associated with higher invertase gene expression in the cell wall. After pathogen inoculation, the concentration of apoplectic sucrose increases for several hours. Increased activity in the cell wall breaks sucrose down into glucose and fructose. These are hexoses that are transported into the cell by hexose transporters to provide the energy and carbon required to initiate resistance to pathogen. Also, the concentration of hexose reduced in apoplast, which leads to more nutrients (to be used in apoplast pathogen). In addition, increased carbohydrate acts as a metabolic message and causes higher defensive gene expression and reduced photosynthesis (Kocal *et al.,* 2008). Furthermore, hexoses are involved in photosynthesis gene silencing, and Hexokinases in the regulation of programmed cell death in plants, which indicates that plant carbohydrate metabolism is directly associated with the plant defensive response (Bolton and Thomma, 2008). In this study, the concentration of carbohydrates was increased as a result of yellow rust disease attack (Venkateswarlu *et al.,* 2012). Methionine is the essential metabolite in plant cells and, irrespective of its role as a key component of the protein and mRNA translation, indirectly regulates the different cellular processes through the precursor, MmS, which is the first biological methyl group. It seems that under stress conditions, the plant converts more methionine to other defense metabolites to cope with stress, thereby increasing its resistance to disease if and when the concentration of methionine in the infected plant is lower than that in the control cultivar. Methionine levels initially differ noticeably from sensitive cultivars and begin to decline during the process of disease development in resistant cultivars and possibly counteract the disease by converting to defense metabolites.

Lysine is the major precursor of the three most important stress-related metabolites including proline, a potent osmolyte (Hare and Cress, 1997), Aminobutyric acid, a stress-related signaling molecule (Baum *et al*., 1996), and Arginine, a potential precursor to polyamines and nitric oxide (Klessig *et al*., 2000).

Peroxidase enzyme removes toxin materials from the environment (Milavec *et al.,* 2008). Pathogenesis related (PR) proteins are members of host proteins that are triggered in the pathogen attack and similar requirements (Domg and Beer 2000). PR10 is an intracellular type of PRs have typical physicochemical properties that enable plants to resist stress. Biological activity of some groups of PRs are unknown, which have antifungal properties by hydrolyzing structural components of fungal cell wall (Van Loon and Pieterse 2006). In the present study, the expression of this protein was increased in both varieties after inoculation, which seems to be related to the plant's defense response against the invasion of yellow rust agent.

The results of this study agree with those of Omranzadeh *et al*. (2011) and Tyagi *et al*. (2000), who found higher polyphenol oxidase enzyme activity in resistant wheat cultivar (ACC-8226) than the susceptible cultivar (MP-845) inoculated with the fungus *Alternaria triticina* as well as the important role of peroxidase and polyphenol oxidase in plant defense mechanisms.

Plants usually respond to biotic and abiotic stresses by the diffusion of reactive oxygen species (Turkan *et al*., 2005). Hydrogen peroxide plays an important role in defensive

mechanisms and signal transfer pathways, which leads to activating other defensive mechanisms (Magbanua *et al*., 2007). Antioxidant enzymes clean hydrogen peroxide toxic compound from the environment. Peroxidase enzyme reduces free radicals of oxygen and associate in the plant's defenses. Peroxidase is an induced defense protein against pathogens in host plants (Almagro *et al*., 2009). ATP synthase is composed of F1 external catalytic complex and proton pathway of F0 (Nishio *et al*., 2002). Belonging to the family of Pr 10 genes, Pprg2 protein has ribonuclease and defense activities in the cell (Borsics and Lados, 2002).

A crucial defense mechanism of plants against various pathogens is the expression of defense-related genes as encoding pathogenesis-related (PR) proteins. PRs are involved in lignin production (Van Loon and Pieterse, 2006). It seems that plants increase the expression level of these proteins when facing pathogens.

Under stress, resistant plants spend all their energy to curb the development of the disease to synthesize all the factors needed in defense mechanisms. Meanwhile, the concentration of Lysine and Methionine amino acids decreases while the amount of Carbohydrate and proline rises. Activity of antioxidant enzymes in plants elevates their resistance to diseases and their activity is enhanced in the resistant compared to the sensitive variety. Both respond to pathogens and express the same proteins to improve the defense system, but the expression level is lower in the sensitive variety.

Conclusion

In this study, it was found that the plant increased the expression of this enzyme as a result of disease, for decreasing accumulation of oxygen free radicals in tissues. There is ample evidence suggesting polyphenol oxidase in plants, especially regarding stress and pathogen attacks (Mayer 2006). Increased activity in resistant cultivars protects the plant against damage caused by the disease.

According to the results of proteomics studies, spots 1, 3, 6, 8, and 9 were possibly related to the proteins of pathogens that were synthesized intercellularly and in plant cells as a result of infection. Therefore, low defensive capability in the sensitive variety led to increased growth of pathogens and more protein synthesis. Hypothetical protein UM05831.1 (CD: TCP-1/cpn 60 chaperon in family) and hypothetical protein CNBA306 (similar to HSP70) are members of heat shock proteins (Table 1) which interacted with other cellular proteins, facilitated the proper folding of target proteins and their transport to the cell organelles, and protected cells against the damage caused by environmental stresses (Ohtsuka *et al*., 2005). Hypothetical protein CNBF4120 (CD: F1ATP synthase b subunit domains) belonged to one of the subunits of the mitochondrial F0F1 complex. Higher expression of this protein in the leaves is probably due to providing the energy required for the defense reactions against the development of disease; it is also higher in defensive activities in resistant varieties.

Kunitz trypsin protease inhibitor has trypsin inhibitory properties, which prevents protein decomposition of plants by inhibiting pathogenic proteases that are an essential amino acid for their growth. Increasing the expression of these proteins in wheat prevents pathogen growth and development in the leaf tissue, because the fungi contain trypsin. As, the amount of its expression in the resistant variety was more than the sensitive variety.

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مطالعه پروتئین‌های مرتبط با قارچ *Puccinia striiformis* در گندم با استفاده از الکتروفورز ژل دوبُعدی

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چکیده: گندم یکی از محصولات راهبردی و از نظر ارزش غذایی بسیار مهم است. یکی از مهم‌ترین بیماری‌های گندم در سراسر جهان زنگ زرد گندم است. این بیماری توسط *Puccinia striiformis* f. sp. *tritici* ایجاد می‌شود. مطمئن‌ترین روش برای کنترل زنگ زرد، به‌کارگیری صحیح مقاومت ژنتیکی و استفاده از ارقام مقاوم می‌باشد. گیاهان سازوکارهای مختلفی برای دفاع در برابر حمله بیمارگر دارند که یکی از آنها پروتئومیک است که به‌طور هم‌زمان برای بررسی سازوکارهای دفاعی در گیاهان حساس و مقاوم استفاده می‌شود. در این تحقیق به‌منظور شناسایی مسیرهای بیان و پروتئین‌های مربوط به مقاومت به *P. striiformis* در یک رقم مقاوم (گاسپارد) و یک رقم حساس (مروارید)، میزان فعالیت آنزیم‌های پراکسیداز، کاتالاز، کربوهیدرات‌های داخل سلولی، غلظت پرولین و انواع پروتئین مورد بررسی قرار گرفتند. نتایج این مطالعه نشان داد که میزان پروتئین، کربوهیدرات، غلظت پرولین و فعالیت پراکسیداز و کاتالاز به‌طور معنی‌داری در اثر تیمار بیمارگر افزایش یافتند. هم‌چنین نتایج الکتروفورز دو بُعدی پروتئین‌ها با نوارهای IPG = 17 سانتی‌متر و pH 4-7 نشان داد که پروتئین‌ها در پاسخ به *P. striiformis* به دو گروه تقسیم می‌شوند: گروه اول شامل پروتئین‌هایی مانند پروتئین‌های پرکسیداز و PR10 بود که به‌طور مستقیم به بیمارگرها واکنش نشان می‌داد. گروه دوم پروتئین‌هایی مانند Hsp90 که به‌عنوان پروتئین‌های رونویسی یا پروتئین‌های مربوط به مسیرهای انتقال پیام یا پایشگرهای مولکولی در تعیین ساختار فضایی پروتئین‌های دیگر دخالت می‌کنند و عامل مقاومت به شمار می روند.

واژگان کلیدی: الکتروفورز ژل دوبُعدی، کربوهیدرات‌ها، کاتالاز، پراکسیداز، پرولین، گندم، *Puccinia striiformis*