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USE OF PROTEIN ELECTROPHORESIS FOR QUALITATIVE AND QUANTITATIVE DIFFERENTIATION OF POWDERY MILDEW (*Blumeria graminis*) DISEASE SEVERITY ON BARLEY GENOTYPES

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ABSTRACT

Ten barley (*Hordeum vulgare* L.) genotypes, five lines originated from International Barley Germplasm Pool (IBGP) and (Nile valley Red Sea Regional Program (NVRSRP), International Center for Agricultural Research in the Dry Area (ICARDA), Aleppo, Syria, and the five commercial varieties, Giza 123, Giza 124, Giza 125, Giza 126 and Giza 2000 were evaluated for (*Blumeria graminis* f.sp. *hordei*) powdery mildew resistance. The genotypes were tested at the seedling stage with 16 differential isolates of Powdery mildew and at adult stage under Egyptian conditions during 2012/2013 growing season. Lines LB-Iran and F6-1-KF showed the resistance to all isolates and lowest ratings of disease severity of 18.75 and 20.31% respectively, while the remaining lines showed intermediate ratings resistance to isolates and disease severity ranging from 39.06 to 46.88%. The commercial varieties showed that about of resistant to all isolates ranging from 6.25 to 43.75% and highest ratings of disease severity ranging from 64.06 to 85.94%. Giza 2000 is the best commercial variety for resistant to isolates and lowest of the diseases severity was 43.75 and 64.06% respectively. Proteins of different genotypes were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) from healthy and diseased plants at adult stage. Two bands with MW 70 and 24 KDa were found only in healthy plants of resistant genotypes, and absent from susceptible genotypes. These protein markers could be used to qualitatively differentiate between resistant and susceptible genotypes. Linear regression analysis constructed one factor model to predict powdery mildew severity. This model indicated that a protein with MW 71 KDa accounted for 63.19% of the total variation in severity ratings. This result indicates that SDS-PAGE of plant proteins may provide a supplementary assay to field tests to distinguish quantitatively between Powdery mildew resistant or susceptible genotypes.
Keywords: *Blumeria graminis*, *Hordeum vulgare*, powdery mildew, barley and electrophoresis.

INTRODUCTION

Powdery mildew (PM) caused by *Blumeria graminis* f.sp. *hordei* Em. Marchal (Bgh) is a widespread fungal disease of many mono and dicotyledonous plant species. In moderate temperate and humid climate, PM fungi cause severe yield loss in a wide range of crops in many countries, including Egypt and Europe as well as, it occurs regularly in Egypt, particularly in the northern parts of the Delta region of the Nile, where humidity and temperature are favorable for both disease incidence and development. Moreover, the asexual conidia are the main sources of the disease Aist and Bushnell (1991). However, the sexual structure ascocarp (cleistothecium) reproduction takes place when condition is unfavorable for conidia formation.

Barley is usually, very susceptible to powdery mildew, and it has been reported that PM causes approximately, 10% yield reduction in cold climate in no-fungicide farming Jorgensen (1988). During strong epidemics, the disease yield loss increases to 25%. Early infection, negatively affects crop density and number of seed per ear. Many strategies are used to control this disease. However, the major component used in integrated pest management (IPM) studies is the chemical fungicides.

Chemical fungicides treatments are generally the most effective, but they are not economically feasibly and might be ineffective when weather conditions are favorable for epidemics. The implication of chemical fungicides pollution has mandated the search for alternative approaches to disease control management. One of these approaches could be the development of resistant cultivars Pandey *et al*. (2003). Therefore, there is a need to know the mechanism behind the resistance reactions such as proteins involved in checking the disease in the plant.

Cultivation of resistant barley cultivars is the best strategy for managing Powdery mildew disease. However, breeding disease resistance genotypes is a continuous process and plant breeders need to add new effective resistance sources to their breeding materials because of the dynamic change of powdery mildew (PM) races, which can breakdown the resistance. Currently, field evaluation is the only reliable method to distinguish barley genotypes with PM resistance. However, the precision of field evaluation of genetic resistance is adversely affected by environmental variation. In addition, field evaluation is expensive and time-consuming. The efficiency and reliability of testing for powdery mildew resistance can be increased significantly by using biochemical markers.

Stresses induce the creation of many types of proteins in plants. A series of novel soluble proteins, defined as pathogenesis-related (PR) proteins, are induced as a result of a hypersensitive reaction of host plants when plants are infected by incompatible pathogens. Higher plants accumulate several types of "pathogenesis-related (PR)" proteins in response to infection by pathogens such as fungi or viruses Kitajima (1999).

PR proteins, which have been studied in many plant species are classified into seventeen families (PR-1 to PR-17), regardless of the original plant species. The sequence similarities, serologic or immunologic relationships, and enzymatic properties are the basis of this classification Van Loon and Van Strien (1999).

The use of gel electrophoresis to analyze plant protein and hence distinguish between and identify cultivars of crop species is a firmly established technique (Cook, 1988). Proteins are primary products of gene expression and reflect gene system specificity in the best manner. Therefore, they are used as very

effective markers for genotype identification and evaluation of the species and cultivar constitution Konarev (1988).

Gel electrophoresis is a powerful technique with numerous application in plant pathology Bonde *et al*. (1993).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is a low cost, reproducible and rapid method for quantifying comparing, and characterizing proteins Bollag and Edelstein (1993).

Dehtyarev (1984) used PAGE to compare protein from inoculated and uninoculated wheat seedling with *Puccinia graminis* f.sp. *tritici*. In little club variety, protein synthesis was affected by infection only up to 40h after inoculation, while in variety khapi greatest changes occurred later than 40h after inoculation.

Fullington and Nityagopal (1986) studied the effect of rust infection by *Puccinia graminis* f.sp. *tritici* on the protein components of wheat. They analyzed total protein extracted from grain samples quantitatively by SDS-PAGE in KSML3 and WG337 varieties. They found that the proteins of KSML3 were less affected by infection than those of the other varieties.

Cattivelli *et al*. (1987) studied the polyacrylamide gel electrophoresis of the grain proteins of 38 (two-rowed) and 40 (six-rowed) Italian barley cultivars. They found that there were 20 bands, only one band was present in every cultivar. There was no distinction neither between 2-rowed and 6-rowed types nor between winter and spring types.

In the period of pathogen fructification, Hlinkova and Sykora (1996) found both quantitative and qualitative differences in the cathodic as well as in the anodic protein patterns in the extra cellular fluid induced by Powdery mildew. New proteins contents depended on host genotype and virulence genes of the pathogens were noticed.

Secalins (Sec-1) are major storage protein of rye, this protein can be detected by polyacrylamide gel electrophoresis can be used as biochemical marker for the identification of rust resistance genes Afshari (2006).

Tan *et al*. (2009) used acid polyacrylamide gel electrophoresis (A-PAGE) and sodium dodecyl sulphat-polyacrylamide gel electrophoresis (SDS-PAGE) to separate endosperm gliadin and glutenin from line 15-3-2, which displays immunity to wheat powdery mildew and stripe rust.

Kakaei *et al*. (2010) studied that the expression of the pathogenesis-related (PR) proteins transcripts in four winter wheat cultivars, differing in resistance to powdery mildew. The results of (SDS-PAGE) showed 25 bands on gel and they had high polymorphism almost in all genotypes. In relatively, tolerant genotype, band 9 of 19 KD differed from susceptible genotypes.

Manikandan (2015) screened five Indian wheat cultivars and their hybrids for seed storage proteins secalin (Sec-1) by the SDS-PAGE. Results revealed that all the hybrids possess the Sec1 band denoting the IBL/IRS translocation and conferring the transfer of gene complex Sr31+ Lr26+Yr9. This study reveals that secalin can be used as biochemical marker for the confirmation of IBL/IRS.

The present investigation, sodium dodecyl sulphat-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was employed as a biochemical technique to differentiate between barley genotypes susceptible and resistant to powdery mildew disease. SDS-PAGE was also employed to develop a regression model to quantify PM resistance in selected barley genotypes.

The aim of this study to find out biochemical marker could be differentiate between resistant and susceptible genotypes. This marker could be useful for predicting of powdery mildew severity at genotypes seedlings.

MATERIALS AND METHODS

Plant materials:

This study was carried out by using ten genotypes (five exotic genotypes) *i.e.* LB Iran, Rihane 8AP, Rihane 3AP, Rihane 7AP and F6-1-KF; obtained from ICARDA 2011/2012 growing season, Aleppo, Syria, and (five indigenous commercial varieties), *i.e*. Giza 123, Giza 124, Giza 125, Giza 126 and Giza 2000, supplied by Barley Section, Field Crop Res. Institute, Agric. Res. Center (ARC), (Table 1).

- **Seedling stage Experiments:**
- **pathogen isolates:**

Sixteen isolates of *Blumeria graminis hordei* (Bgh) were used in this study. Isolates were purified by single pustule isolation. Young seedlings of the cultivar "Giza 123" were used to maintain and propagate all isolates. Isolates were tested frequently on host differentials to assure their purity throughout the experiment.

- **Inoculation and disease assessment:**

Plants were grown in greenhouse at plant Pathol. Res. Inst., Agric. Rec, Center, Giza, Egypt during 2012/2013 growing season. with 16h light and 8h dark at 16-22°C. Greenhouse test included five exotic genotypes and five commercial varieties inoculated with the16 isolates. Plants were grown in plastic pots (5cm upper diameter). Inoculation was carried out when plants were 10-12 days old (two leaf stage) by shaking or brushing conidia from all isolates. After 8-10 days of incubation, the disease reaction types were scored on the primary leaf of the seedling. This scoring was carried out according to a 0-4 scale adapted from Mains and Dietz (1930). Seedlings were classified into susceptible or resistant. Plant with infection types 0-2 were classified as resistant, where plants that scored 3 and 4 were classified as susceptible.

Where: 0: Highly resistant (HR); no visible signs of infection.
1: Resistant; a slight development of mycelium.
2: Moderately resistant (MS); moderate to abundant development of mycelium with slight production of conidia.
3: Moderately susceptible (MR); moderate to abundant development of mycelium accompanied by moderate sporulation.
4: Susceptible (S); large pustules, abundant sporulation.

Table (1): Pedigree and sources of the ten barley genotypes.

No.	Genotypes	Pedigree	Sources
1	(LB Iran) (LB Iran/µna 80//Lignee 640/3/Mja's'	CMB89 A-0138-4 M- 1Y-1M-OY-OAP	Exotic*
2	Rihane-8AP (Rihane-03/3/5604/1025/ Arabi Abiad	ICB89-0145-5AP- IAP- IAP- OTR-OAP- 8AP- OAP	Exotic
3	Rihane-3AP (Rihane-03/3/5601/1025/ Arabi Abiad	ICB89-0145-5AP- IAP- IAP- OTR-OAP- 3AP- OAP	Exotic
4	Rihane-7AP (Rihane-03/3/5604/1025/ Arabi Abiad	ICB89-0145-5AP- IAP- IAP- OTR-OAP- 7AP- OAP	Exotic
5	F6-1-KF Pro/5/Api/CM67/3Apm/Dwarfil-1Y// Por/ Kn 27/4/RM 1508/11012	ICB89-0213-9APP-OAP	Exotic
6	Giza 123	Giza 117/FA086	** indigenous
7	Giza 124	/١١٨Giza 117// Bahteem52 // Giza FA086	indigenous
8	Giza 125	١٢٤Sister line to Giza	indigenous
9	Giza 126	Por -٧٢٩Baladi Bahteem / SD BC-١٢٧٦٢	indigenous
10	Giza 2000	١٢٤Giza 121/ Giza	indigenous

***Genotypes obtained from International Barley Germplasm Pool Nursery (IBGP-011), and Nile Valley Red Sea Regional Program (NVRSRP) ICARDA.**

****Genotypes supplied by Barley Section, Field crop Res. Institute, ARC.**

- **Adult stage experiments:**
- **Electrophoretic studies:**
- **Preparation of plant materials:**

Ten Barley genotypes were used in this study, five exotic genotypes and five indigenous types (Table 1). Grains were germinated into 25-cm pots containing clay soil (3kg per each). Pots were placed in greenhouse with maximum temperature 25°C, falling to a minimum of 18°C at night, under 14 h natural daylight, and a RH ranging between 60 and 80% Sabri & Clark (1995). The experimental plants were inoculated with mixture of conidiospores at growth stage (G.S 30) according to Zadoks scale Zadoks *et al*. (1974), 48 h after inoculation healthy and diseased plants were used to extract proteins.

- **Extraction of soluble protein:**

Plant leaves were taken from inoculated and uninoculated plants. Two g from each sample were ground in 0.05 µ sodium acetate buffer + sea sand with mortar in liquid nitrogen at 4°C. Samples were transferred in Eppendorf tubes, and kept at 0°C over night, centrifuged for 30 min at 18.000 rpm at 4°C. Supernatants containing water soluble protein fraction were subjected for further analysis by SDS electrophoresis Okuno *et al*. (1991).

- **Electrophoresis of dissociated protein (SDS-PAGE):**

Electrophoresis of dissociated protein was carried out by using 10% SDS-PAGE according to Laemmli, (1970). The gel was stained with silver nitrate and distained using 40% methanol, 10% acetic acid and 50% distilled water until the bands were clearly visible Sammons *et al*. (1981). A 0.75 mm thick vertical slab gel was cast protein and electrophoresed using the Bio Rad mini-protein 11 system. Gels were photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphimager 2200), USA. The protein bands in each gel were analyzed by scoring the bands as amount % protein fraction.

- **Statistical analysis of electrophoretic data:**

Simple correlation and regression analyses were used to measure the degree of association between amount of protein (predictor or independent variable) and PM severity (dependent variable). Statistical analysis was performed with the software package SPSS 6.0.

- **Disease index (%):**

Observation on disease incidence was recorded according to the method described by Saari and Prescott (1975). 0-9 at the growth stage, 10.5 Lagrge, (1954). Therefore, 10 leaves of each plant were selected randomly for determining the percentage of disease index, which was calculated using by following equation Towsend and Heuberger (1943):

$P = [\sum(n \times v)/(9N] \times 100$ where:

P: Disease severity (%)

n = Number of leaves within infection grade

v = Value of each grade

N = Total number of samples

Scale of Saari and Prescott (1975) where:

0: free from infection (O).

1:resistant: a few isolated lesions on only the lowest leaves (R).

2: resistant: scattered lesions on the second set of leaves (R).

3:resistant: light infection of lower third of plant (R, MR).

4:moderately resistant: moderate infection of lower leaves (MR).

5:moderately susceptible: severe infection of lower leaves moderate to light infection (MR, MS).

6:moderately susceptible: severe infection of lower third of plant moderate on middle leaves (MS).

7:susceptible: lesions severe on lower and middle leaves (MS, S).

8: susceptible: lesions severe on lower and middle leaves; moderate to severe infection of upper third of plant, flag lead infected in amounts more than a trace (S).

9: highly susceptible: severe infection on all leaves; spike also infected to some degree.

RESULTS

The matching between the ten genotypes and the sixteen tested isolates of powdery mildew disease at seedling stage under greenhouse condition (Table 2) and the evaluation of disease index at adult stage indicated that two resistant genotypes LB-Iran and F6-I-KF expressed resistance to all isolates used with the lowest ratings of disease index of 18.75 and 20.31% respectively, while genotype Giza 125 showed the lowest resistance to all isolates of about 6.25% with the highest rating of disease index of 85.94%. The remaining resistant genotypes showed intermediate ratings ranging from 39.06 to 46.88%. On the other hand, the remaining susceptible genotypes showed the disease index ranging from 64.06 to 73.44%.

Total proteins were extracted from barley genotypes uninoculated and inoculated with Bgh at growth stage 30. When protein was subjected to SDS-PAGE electrophoresis, 64 protein bands were identified among the 10 genotypes (Table 3 and Fig. 1). No single genotype was stained for all the 64 bands, similarly, no single band was common to all genotypes.

Data in Table 3 showed that bands of resistant genotypes decreased when they are inoculated, for example (LB-Iran) and (F6-I-KF) have 28, 26 bands respectively when inoculated their bands decreased to 24 and 18 respectively and the other genotypes showed minor increasing in the number of bands ranged from 1-2 bands. On the other hand, protein bands of the susceptible genotypes increased after inoculation. For example, Giza 2000 and Giza 124 have 20 bands, these bands increased to 28 and 23 respectively after inoculation. Genotype Giza 123 showed little increase, and there was not change in the other genotypes.

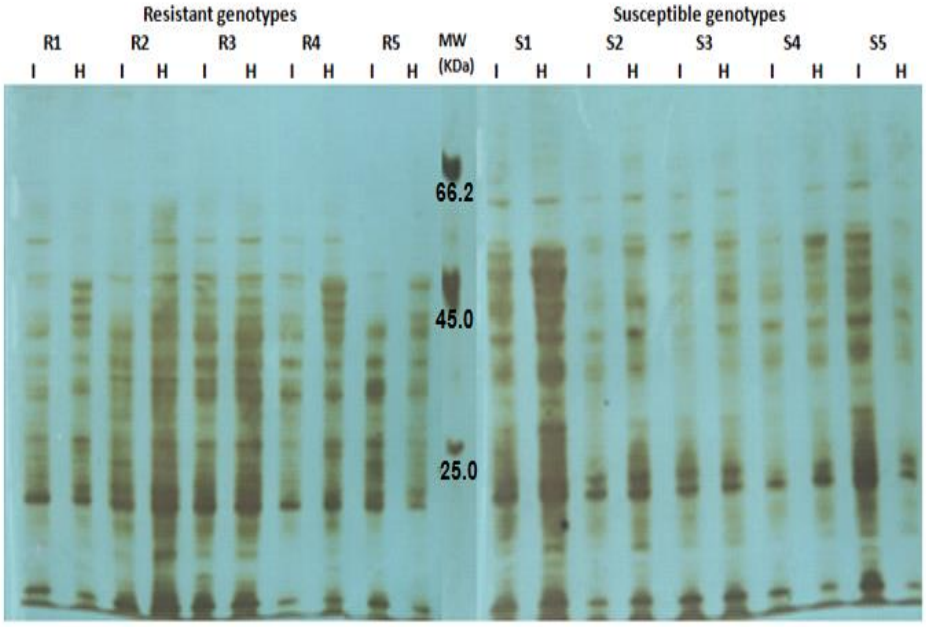

Fig. 1. Protein fraction obtained by PAGE from five resistant (R) and five susceptible (S) genotypes of barely healthy (H) or inoculated (I) with *Blumaria graminis* F. sp *hordei* the causal fungus of powdery mildew.

R1: LB Iran, R2: Rihane 8AP, R3: Rihane 3AP, R4: Rihane 7AP and R5: F6-1-KF.
S1: Giza 123, S2: Giza 124, S3: Giza 125, S4: Giza 126 and S5: Giza 2000

Pearson correlation coefficient was calculated to measure the degree of association between PM severity and the amounts of the separated protein fractions in healthy (Table 4) and infected (Table 5) genotypes. However, few proteins were satisfactory correlated with PM severity. Thus, of the 59 protein fractions separated from healthy genotypes shown in Table 4 only 7 (11.86%) were significantly correlated with PM severity. These protein fraction with molecular mass of 71, 70, 26, 24, 23, 20 and 17 KDa. Of the 60 protein fraction separated from infected genotypes Table 5 only 7 (11.67%) were significantly correlated with PM severity. The molecular mass of these proteins were 78, 70, 26, 24, 23, 20 and 17 KDa.

Table (2): Interaction between barley genotypes and isolates of *Blumeria graminis* f.sp. *hordei* (Bgh) at seedling stage and the estimated disease index at adult stage under greenhouse conditions.

No.	Genotypes	[a]Type of infection to 16 isolates																% resistant to all isolates	% disease[b] index At adult stage (Y)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1	LB-Iran	1	1	0	0	1	0	1	1	1	0	1	1	1	1	1	1	100	18.75
2	Rihane 8AP	1	3	3	2	3	0	3	0	1	1	0	3	2	2	0	1	68.75	39.06
3	Rihane 3AP	2	3	1	0	0	3	3	0	2	2	3	3	2	2	3	1	62.5	46.88
4	Rihane 7AP	2	1	0	1	3	0	3	0	3	2	3	2	3	2	2	0	68.75	42.19
5	F6-I-KF	1	1	0	1	0	1	1	1	0	1	1	1	0	1	2	1	100	20.31
6	G 123	4	3	4	3	4	2	3	3	1	2	3	4	0	4	3	4	25	73.44
7	G 124	3	4	3	4	3	2	2	3	3	4	3	2	0	3	3	2	31.25	68.75
8	G 125	4	3	٤	3	4	3	4	2	3	4	3	3	4	4	3	4	6.25	85.94
9	G 126	3	4	٤	3	4	4	3	2	1	2	3	3	0	2	3	4	31.25	70.31
10	G 2000	4	3	٤	3	4	3	3	1	1	1	4	2	1	2	2	3	43.75	64.06

[a]type of infection 0,1,2 = resistant and 3,4 = susceptible
[b]Disease index was calculated according to the following formula: P = [∑(n×v)/(9N)]×100 where:
P: Disease index (%)
n = Number of leaves within infection grade
v = Value of each grade
N = Total number of samples

Table (3): Electrophoretic pattern of protein bands in leaves of barley resistant and susceptible genotypes un-inoculated (U) and inoculated (I) with *Blumeria graminis* f.sp. *hordei*, the causal fungus of barley powdery mildew disease.

		Amt[c] %																			
		Resistant genotypes										Susceptible genotypes									
No.[a]	Kda[b]	LB-Iran		Rihane 8AP		Rihane 3AP		Rihane 7AP		F6-I-KF		Giza 123		Giza 124		Giza 125		Giza 126		Giza 2000	
		U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I
1	82	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠.٥١	٠	٠	٠	٠.٢٣	٠	٠	٠.٦٤	٠.٦٧
2	81	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠.٩١	٠.٥٢	٠	٠	٠
3	79	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	١.٤	٠	٠	٢.٦٩	٠	٠	٠	١.٨٦	٢.٢٧
4	78	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٢.٠٦	٠.٦٦	٣.١٥	٢.٥٣	٠	١.٨٠	٢.٤٤	٢.٦٦	٠	٠
5	77	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠.٤٤	٠	٠	٠	٠	٠	٠	٠	٠
6	76	٠	١.٩٦	٠	٠	٠.٣٤	٠.٣٩	٠	٠	٠	٠.٥١	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
7	75	٠.٢٧	٠	٠	٠	٠	٠	٠.٣٦	٠	٠	٠	٠	١.٠٩	٠	٠	٠	٠	٠	٠	١.٦	٠
8	74	٠.٧٨	٠	٠	٠	٠	٠	٠	٠	٠	٠	٢.٧٢	٠	١.٣٨	٠	١.٧٥	١.٢٢	٠	٠	٠	٠
9	73	٠	٠	٠	٠	٠	١.٠٨	٠	٠	١.٥٨	٠	٠	١.٨٩	٠	٠	٠	٠	٠	٠	٠	٠
10	72	٠	١.٠٨	٠	٢.٢٩	٠	٠	٠	٠	٠	٠	٠	٠	٠.٨٦	٠	٠	٤.٠٧	٠	٠	٣.٣٧	٢.٧٥
11	71	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٢.٠١	٠	٣.٢٥	٥.٨٢	٤.٩٦	٠	٤.١٢	٠	٠	٠.٧٣
12	70	٢.٥٩	٠	٥.٤٦	٠.٧٦	٣.٠٠	٣.٨٩	٠	٥.٤١	٢.٧١	٤.٣٥	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
13	69	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٣.٠٦	٠	٠	٠	٠	٠	٠	٥.٤٥	٠	٠
14	68	٠.٨٧	٠	٠	١.٤٦	١.٧٨	٠	٦.٢٢	٠	١.١٧	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠.٧٦	٠
15	67	٠.٦٢	٠	٠	٠	٠	٠	٠	٠	٠	١.١٢	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
16	66	٠	١٠.٥٩	٠	٠	٠	٠	٠	٠	٠	٠	٠	١٣.٤٦	٠	٠	٠	٠.٩٩	٠	٠	٠	١.٥٣
17	٦٥	١.٠٣	٠	٠	٠	١.٤٥	١.٤٥	١.٥٧	١.١٩	١.٠٤	١.٠١	١.٣٤	٠	٠	١.٣٩	٠	٠	٠	١.٦	٠	٠
١٨	٦٤	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٣.٨١	٢.١٦

Table (3) cont.

No.[a]	Kda[b]	Amt[c]%																			
		Resistant genotypes										Susceptible genotypes									
		LB-Iran		Rihane 8AP		Rihane 3AP		Rihane 7AP		F6-I-KF		Giza 123		Giza 124		Giza 125		Giza 126		Giza 2000	
		U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I
١٩	٦٣	1.76	1.77	4.73	2.58	3.62	3.37	4.14	2.90	1.52	2.33	3.29	0	4.70	2.84	4.52	1.69	4.89	3.67	0	0
٢٠	٦٢	٠	٠	٠	٠	٠	٠	٠	٠	1.45	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
٢١	٦١	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	0.78	0	1.54	0	0	0.73	0
٢٢	٦٠	1.52	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	0.92	٠	٠	٠	٠	٠
٢٣	٥٩	0	0	2.26	٠	٠	٠	٠	٠	٠	2.63	٠	٠	٠	1.28	0	1.10	2.52	0	2.11	0
٢٤	٥٨	1.40	0	0	0	2.11	0	2.32	0	1.97	0	0	0	2.19	0	1.78	0	0	2.33	0	0
٢٥	٥٦	0	0	0	5.57	٠	٠	٠	٠	٠	٠	5.29	0	0	2.91	0	0	2.74	3.81	0	6.27
٢٦	٥٥	٠	٠	٠	0	3.02	5.73	5.88	5.74	0	5.37	0	9.76	0	0	2.74	3.63	٠	٠	٠	٠
٢٧	٥٤	8.92	7.46	0	1.06	1.82	2.57	0	2.27	0	0	2.26	0	0	4.04	4.11	3.06	5.51	2.97	0	3.09
٢٨	٥٣	0	0	6.58	٠	٠	٠	٠	٠	6.65	٠	٠	٠	7.57	٠	٠	٠	٠	٠	٠	٠
٢٩	٥٢	0	2.52	0	2.32	٠	٠	٠	٠	٠	٠	1.98	٠	٠	٠	٠	٠	٠	٠	٠	١.٦١
٣٠	٥١	٠	٠	٠	٠	4.40	2.05	0	4.05	4.27	٠	٠	٠	3.90	3.38	2.77	2.13	0	1.72	14.13	0
٣١	٥٠	0	1.52	5.28	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠.٩٨
٣٢	٤٩	5.87	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	3.47	0	2.47
٣٣	٤٨	0	15.8	3.27	0	0	3.74	0	0	4.39	0	0	23.57	2.62	3.21	3.64	3.73	٠	٠	٠	٠
٣٤	٤٧	5.64	0	0	0	0	0	8.26	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	١.٤٦	٠	٠
٣٥	٤٦	0	0	6.92	9.52	0	8.12	3.75	0	5	0	11.27	0	7.90	0	4.16	0	9.59	4.87	2.60	5.49
٣٦	٤٥	٠	٠	٠	٠	10.3	0	0	8.74	0	13.04	0	0	0	7.05	٠	٠	٠	٠	٠	٠
٣٧	٤٤	1.09	0	0	1.34	0	0	0	1.82	1.24	٠	٠	٠	٠	٠	2.05	0	1.74	0	3.62	1.21
٣٨	٤٣	1.73	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
٣٩	٤٢	6.38	3.17	0	6.87	0	6.60	0	6.92	5.99	0	6.25	٠	٠	٠	٠	٠	6.93	0	5.79	6.17
٤٠	41	0	0	5.99	0	6.89	0	7.82	0	0	5.65	0	0	5.33	5.99	5.45	6.48	0	5.43	0	0
٤١	40	0	2.58	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
42	39	٠	٠	٠	٠	3.54	0	3.24	2.43	0	3.97	2.63	0	0	0	2.16	0	1.62	2.45	0	1.94

Table (3) cont.

No.[a]	Kda[b]	Amt[c] %																			
		Resistant genotypes										Susceptible genotypes									
		LB-Iran		Rihane 8AP		Rihane 3AP		Rihane 7AP		F6-I-KF		Giza 123		Giza 124		Giza 125		Giza 126		Giza 2000	
		U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I	U	I
43	38	10.24	0	0	11.93	٠	٠	٠	٠	3.91	0	0	1.64	3.28	7.82	0	3.63	0	2.69	8.03	0
44	37	0	0	9.85	0	5.28	10.97	9.13	6.80	0	4.85	2.97	0	6.94	0	2.73	4.64	7.09	0	0	3.83
45	36	0	7.89	0	0	0	0	0	0	0	0	0	5.83	0	0	3.26	0	0	1.56	0	0
46	35	2.35	0	2.30	2.97	6.25	0	0	0	0	5.85	2.73	0	0	0	0	0	0	2.19	0	0
47	34	0	3.06	0	1.45	0	3.37	0	4.81	2.65	0	0	0.99	0	3.65	1.97	3.49	1.88	0	4.54	3.76
48	33	2.75	0	0	0	0	2.22	0	0	0	0	1.83	0.64	0	0.99	1.64	1.31	1.26	0	0	1.47
49	32	4.46	2.20	6.17	7.89	0	5.99	0	0	6.94	0	1.68	1.83	0	0	0	0	0	0	7.20	0
50	31	2.38	0.93	3.69	0	7.50	0	7.63	7.05	3.07	6.71	1.57	1.18	4.37	3.74	3.40	4.06	3.43	5.47	0	3.77
51	30	2.43	0	0	0	0	3.27	0	0	2.49	0	0.95	0	0	2.65	0	1.53	0	0	0	2.61
52	29	0	8.90	1.82	4.05	2.20	0	3.62	2.46	0	5.08	0	6.71	0	0	4	1.93	0	0	0	0
53	28	0	0	0	0	0	2.16	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠
54	27	7.77	2.04	0	4.61	٠	٠	٠	٠	9.46	0	11.36	4.48	0	0	8.97	0	13.82	14.24	0	9.38
55	26	6.31	1.91	14.26	9.76	0	9.89	14.16	14.86	5.52	15.20	0	1.89	٠	٠	٠	٠	٠	7.07	15.57	6.72
56	25	٠	٠	٠	٠	14.92	٠	٠	٠	٠	٠	6.26	0	19.29	16.44	5.42	13.77	6.53	0	0	0
57	24	2.77	3.86	5.78	0	6.62	6.82	0	7.24	4.67	7.82	0	1.71	٠	٠	٠	٠	٠	٠	٠	١٠.٤١
58	23	1.15	1.79	0	4.44	0	0	6.32	0	1.11	0	6.16	1.71	7.86	7.13	4.60	11.4	5.23	6.37	6.52	0
59	22	3.32	1.96	3.48	0	4.37	2.78	6.34	3.87	3.35	4.70	0	2.57	3.52	3.21	3.28	0	4.37	11.1	3.73	0
60	21	0	4.31	2.92	2.76	0	0.62	0	0	3.21	0	5.66	0	0	0	3.41	0	0	0	2.20	1.17
61	20	4.14	2.15	٠	٠	٠	٠	٠	٠	٠	٠	٠	4.53	1.86	2.64	2.42	4.46	2.17	6.37	0	2.10
62	19	0	3.09	7.39	9.55	0	8.73	9.24	0	6.77	0	3.60	5.66	٠	٠	٠	٠	٠	٠	٠	٠
63	18	7.14	1.59	1.87	1.22	10.3	1.14	0	8.13	1.77	9.80	7.10	0	8.26	9.42	9.23	7.02	8.43	11.1	8.75	6.47
64	17	٠	٠	٠	٠	٠	٠	٠	٠	٠	٠	0.40	0	1.60	6.66	2.29	1.84	1.44	2.28	0	2.21

A (No.): number of protein fraction, b (Kda): molecular mass in KDa. c (Amt%): amount of protein fraction, U: uninoculated, I: inoculated

Table (4): Correlation between amount (%) of protein fractions of healthy genotypes and Powdery mildew (PM) disease severity.

No.[a]	KD[b]	r[c](b)[d]	No.[a]	KD[b]	r(b)	No.[a]	KD[b]	r(b)	No.[a]	KD[b]	r(b)
1	81	0.265(0.460)	16	63	0.384(0.274)	31	47	-0.455(0.187)	46	31	-0.179(0.620)
2	79	0.539(0.108)	17	62	-0.499(0.142)	32	46	0.492(0.149)	47	30	0.414(0.235)
3	78	0.515(0.128)	18	61	0.169(0.640)	33	45	-0.093(0.798)	48	29	0.159(0.661)
4	76	-0.093(0.798)	19	60	-0.499(0.142)	34	44	0.236(0.511)	49	27	0.165(0.648)
5	75	-0.096(0.792)	20	59	0.155(0.670)	35	43	-0.523(0.121)	50	26	-0.680*(0.031)
6	74	-0.499(0.142)	21	58	0.359(0.308)	36	42	-0.148(0.663)	51	25	0.442(0.201)
7	73	-0.499(0.142)	22	56	0.419(0.228)	37	41	0.079(0.828)	52	24	-0.650*(0.042)
8	72	0.230(0.523)	23	55	0.015(0.967)	38	39	0.316(0.373)	53	23	0.676*(0.032)
9	71	0.795**(0.006)	24	54	-0.057(0.876)	39	38	-0.428(0.218)	54	22	-0.267(0.456)
10	70	-0.675*(0.032)	25	53	-0.272(0.447)	40	37	0.147(0.686)	55	21	0.255(0.477)
11	69	0.313(0.379)	26	52	0.313(0.379)	41	36	0.504(0.138)	56	20	0.576#(0.082)
12	68	-0.349(0.323)	27	51	0.158(0.663)	42	35	-0.289(0.418)	57	19	-0.442(0.200)
13	67	-0.523(0.121)	28	50	-0.212(0.556)	43	34	0.186(0.607)	58	18	0.017(0.969)
14	65	-0.476(0.165)	29	49	-0.523(0.121)	44	33	0.090(0.804)	59	17	0.772**(0.009)
15	64	0.169(0.640)	30	48	-0.129(0.723)	45	32	-0.533(0.112)			

A (No.): number of protein fraction, b (KDa): molecular mass in KDa.
c (r) = Pearson correlation coefficient (r), which measured the degree of association between the amount (X) of the designated protein fraction and PM disease severity (y).
d (b) = Probability level.
Significant correlation coefficients are shown in bold type (P<0.10 (#), P≤ 0.05(*), and P≤ 0.01 (**)).

Table (5): Correlation between amount (%) of protein fractions of infected genotypes and Powdery mildew (PM) severity.

No.[a]	KD[b]	r(b)	No.[a]	KD[b]	r(b)	No.[a]	KD[b]	r(b)	No.[a]	KD[b]	r(b)
1	82	0.499[c](0.142)[d]	16	66	-0.036(0.922)	31	47	0.238(0.507)	46	31	0.049(0.894)
2	81	0.504(0.138)	17	65	-0.239(0.506)	32	46	-0.046(0.899)	47	30	0.357(0.312)
3	79	0.325(0.360)	18	64	0.169(0.640)	33	45	-0.406(0.245)	48	29	-0.534(0.112)
4	78	0.685*(0.029)	19	63	-0.228(0.527)	34	44	-0.167(0.645)	49	28	-0.093(0.798)
5	77	0.313(0.379)	20	61	0.432(0.212)	35	42	-0.366(0.299)	50	27	0.306(0.390)
6	76	-0.486(0.155)	21	59	-0.158(0.662)	36	41	0.345(0.329)	51	26	-0.576#(0.081)
7	75	0.313(0.379)	22	58	0.265(0.460)	37	40	-0.523(0.121)	52	25	0.535(0.111)
8	74	0.504(0.138)	23	56	0.197(0.580)	38	39	-0.299(0.401)	53	24	-0.585#(0.076)
9	73	0.237(0.510)	24	55	0.065(0.859)	39	38	0.186(0.606)	54	23	0.627*(0.052)
10	72	0.309(0.384)	25	54	-0.133(0.714)	40	37	-0.131(0.718)	55	22	0.030(0.934)
11	71	0.264(0.461)	26	52	-0.457(0.184)	41	36	-0.206(0.567)	56	21	0.357(0.312)
12	70	-0.673*(0.033)	27	51	0.302(0.396)	42	35	-0.412(0.237)	57	20	0.718*(0.019)
13	69	0.265(0.460)	28	50	-0.367(0.298)	43	34	0.106(0.771)	58	19	-0.222(0.538)
14	68	-0.212(0.556)	29	49	0.332(0.349)	44	33	0.449(0.193)	59	18	0.186(0.606)
15	67	-0.499(0.142)	30	48	0.054(0.882)	45	32	-0.309(0.384)	60	17	0.652*(0.041)

A (No.): number of protein fraction, b (KDa): molecular mass in KDa.
c (r) = Pearson correlation coefficient (r), which measured the degree of association between the amount (X) of the designated protein fraction and PM disease severity (y).
d (b) = Probability level.
Significant correlation coefficients are shown in bold type (P<0.10 (#), P≤ 0.05(*), and P≤ 0.01 (**)).

Data for Powdery mildew severity and amounts of protein fractions in healthy genotypes were entered into a computerized linear regression analysis. One-factor model was constructed to predict Powdery mildew severity (Fig. 2). The molecular mass of the protein fraction was 71 KDa. This protein fraction accounted for 63.19% of the total variation in severity ratings.

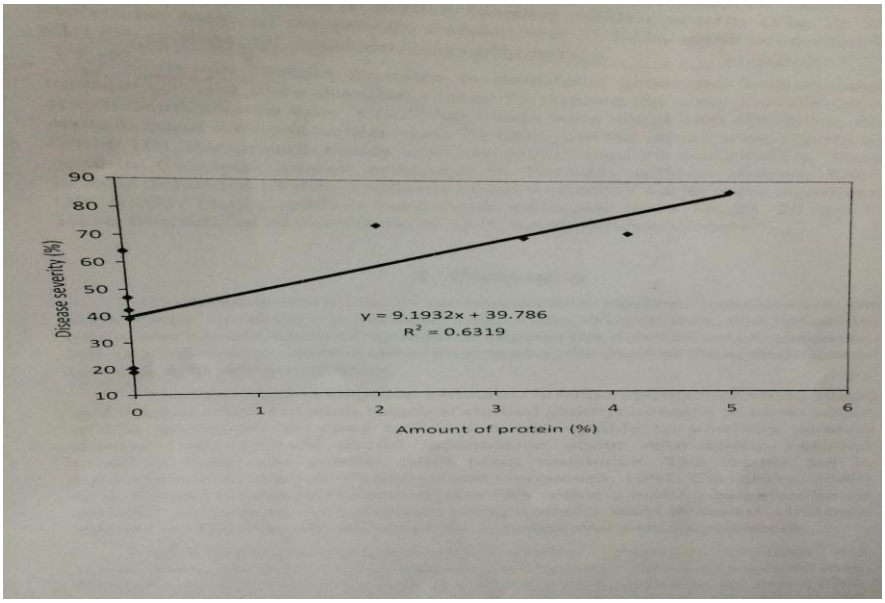

Fig. (2). Regression equation that describes the relationship between the amount of a protein fraction and powdery mildew severity on barley. The molecular mass of the protein fraction was 71 KDa.

DISCUSSION

Electrophoresis is one of the biochemical methods used to study the physiologic variability in different organisms, on this way, the variability of protein pattern has been studied to explain the mechanism of resistance. Many studies were carried out in this respect, the most of these studies were dealing with wheat or barley.

The present investigation estimated infection type (at seedling stage) and disease index (at adult stage) of studied genotypes helps to know which of the genotypes is more resistant or susceptible to powdery mildew disease, and provide useful information about relationship between seedling stage and predict adult plant resistance. The results are in agreement with studies of Brown and Gorgensen (1991); Czembor (2000) they found that determination of barley powdery mildew resistance genes based on tests performed on seedlings using isolates with different virulence spectra is effective and sufficient for breeders and pathologists needs.

SDS-PAGE electrophoresis of barley genotypes resistant and susceptible to barley powdery mildew disease of healthy or diseased revealed that, there were increase or decrease in the protein patterns in susceptible and resistant genotypes, respectively. This is in agreement with Khalil (1981) who found that, following infection of flax with rust, certain changes occurred in the protein patterns of the susceptible cultivars, but not in that of the resistant one. The changes were in the form of a shift in the intensity of some bands and the disappearance of some other bands. Such changes were not evident in the resistant cultivar (Bombay), probably due to the very limited activity of the fungus in that cultivar.

The SDS-PAGE method has the advantages on simplicity high sensitivity and good accuracy, so it is a practical and reliable for barley identification.

SDS-PAGE separated 64 proteins which ranged from 82 to 17 KDa. The most interesting bands, were the bands with KDa 70 and 24 KDa as they appeared in healthy and infected resistant genotypes and completely absent from healthy susceptible genotypes. So, they are consider very interesting, this like PR-protein which inducible defense compounds include reactive oxygen species, phytoalexins, cell wall components. They are distributed, generally, in plants in low amounts but this rate can elevate to much greater concentration in response to pathogen attack Stintzi *et al*. (1993). Likewise, protein band 26 KDa which was significant at 0.05% and 0.01% in healthy and infected resistant genotypes respectively, and no significant effect in healthy and infected susceptible genotypes. This like PR-3 Proteins (chitinases) which are able to degrade fungal cell wall have been, frequently, used in genetic engineering for plant disease resistance Datta and Datta (1999). Most PR-3 proteins have molecular masses of between 26 to 43 KDa. Poulsen (2001) documented that basic isoforms of PR-2 and PR-3 have inhibitory effects on *Blumeria graminis hordei* (Bgh). Finally, there is a positive correlation between 70, 26 and 24 KDa bands expression with powdery mildew resistance. So, they are consider qualitatively resistance expression to powdery mildew disease. This finding could be used to differentiate between susceptible and resistant varieties to powdery mildew disease. Using these protein bands, could be help in detection of gene (s) of resistance for further breeding. So, it could be used as a qualitative marker of resistance.

Few proteins were satisfactory correlated with PM severity in susceptible genotypes at healthy and infected plants, these protein bands with molecular masses 78, 71, 17, 23 and 20 KDa. Protein bands 78, 71 and 17 KDa were appeared in susceptible genotypes and completely absent from resistant genotypes. The results are agreement with Hucklhoven *et al*. (1999). They

recorded that susceptible barley varieties have lower amount of characteristics responsible to penetration resistance, which characterized by formation of cell wall apposition (Papilae) and accumulation of phytoalexins, PR genes transcripts, and hydrogen peroxide. The interesting protein bands are 71 and 17 KDa which were highly significant (P< 0.01), protein 17 KDa like PR-1 with low molecular mass (15-17 KDa). In barley, expression of a gene of the PR-1 family, PR-1b, is frequently used as a reliable marker during challenge with *Blumeria graminis* Agrawal *et al*. (2000). Actually, involvement of PR-1b in penetration resistance of barley to powdery mildew fungus (Bgh) has been demonstrated Schultheiss *et al*. (2003). Protein band with molecular mass 71 which highest significant (P< 0.01) and r value (0.795). This protein constructed linear regression model. Using the predictor supplied by linear regression is constructed to predict parley powdery mildew (PM) severity. This results showed that the comparison of disease severity and protein pattern provide useful information about relationship between resistant or susceptible genotypes and protein expression. So, the SDS-PAGE results are in agreement with powdery mildew seedling and adult stage assay.

Finally, in the present study, satisfactory visualization of banding patterns were obtained by using the silver nitrate staining system for general proteins, it is a robust approach for detecting differentially expressed and potentially important proteins. On one hand, knowledge of the expression patterns of proteins can provide important information with regard to proteins required for resistance. While on the other hand, it might help to develop suitable biochemical markers to identify resistant cultivars and donor varieties. In addition to, using these biochemical markers are a quick method for screening barley germplasm for powdery mildew resistance in different laboratories without any greenhouse facilities in a short time. And the linear regression model, it generated a proved effective in predicting PM severity from banding pattern. Therefore, SDS-PAGE of protein, such as that described herein, may provide a supplementary assay to field trials to distinguish between PM resistant or susceptible genotypes quantitatively.

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استعمال التفريد الكهربائي للبروتينات للتفرقة الوصفية و الكمية في شدة الإصابة بمرض البياض الدقيقي على التراكيب الوراثية للشعير

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قيمت عشرة تراكيب وراثية من الشعير من حيث المقاومة لمرض البياض الدقيقي، خمسة تراكيب وراثية واردة من المنظمة العالمية ((IBGP و NVRSR.P) إيكاردا) وخمسة تراكيب وراثية محلية تجارية هي جيزة ١٢٣، جيزة ١٢٤، جيزة ١٢٥، جيزة ١٢٦، جيزة ٢٠٠٠ في كل من طور البادرة مع ستة عشر عزلة من فطر البياض الدقيقي، وفي طور النبات البالغ تحت الظروف المصرية موسم ٢٠١٢/٢٠١٣. أظهرت التراكيب الوراثية Iran-LB و F6-I-KF مقاومتهم لجميع العزلات في مرحلة البادرة وأقل شدة إصابة في مرحلة النبات البالغ وهي ١٨.٧٥ إلى ٢٠.٣١% على التوالي، أما باقي التراكيب الوراثية أظهرت درجات متوسطة من المقاومة للعزلات وكذلك في شدة الإصابة تراوحت ما بين ٣٩,٠٦ الى ٤٦,٨٨%. بينما في الأصناف التجارية كانت نسبة المقاومة للعزلات تتراوح بين٦.٢٥ إلى ٤٣.٧٥% وإن شدة الإصابة في النباتات البالغة تراوحت بين ٦٤.٠٦ الى ٨٥.٩٤%. الصنف جيزة ٢٠٠٠ من أفضل الأصناف التجارية من حيث المقاومة للعزلات وكذلك أقل نسبة في شدة الإصابة وهي ٤٣.٧٥ و ٦٤.٠٦% على التوالي. استعملت تقنية التفريد الكهربائي لفصل البروتينات من النباتات السليمة والمصابة للتراكيب الوراثية المختلفة في طور النبات البالغ، تم الحصول على حزمتي بروتين ذات كتلة جزيئية ٧٠ و ٢٤ ك. دالتون من أوراق النباتات السليمة للأصناف المقاومة، بينما لا توجد هذه البروتينات في الأصناف القابلة للإصابة. لذا تعتبر هذه البروتينات علامة جيدة للتفرقة الوصفية بين الأصناف المقاومة والقابلة للإصابة، كما أمكن بالتحليل الاحصائي باستخدام أسلوب الانحدار الخطي البسيط التوصل الى نموذج رياضي لوصف العلاقة الكمية بين شدة المرض (متغير تابع) والبروتينات المفصولة من اوراق النباتات السليمة (متغير مستقل)، حيث أظهر هذا النموذج ان ٦٣.١٩% من التباين الكلي في شدة المرض من الممكن أن يعزى الى تأثير البروتين ذو الكتلة الجزيئية ٧١ ك. دالتون. تدل نتيجة الدراسة الحالية على أنه من الممكن استخدام تقنية التفريد الكهربائي للبروتينات كوسيلة مكملة لاختبارات الحقل للتفرقة الكمية او الوصفية بين تراكيب الشعير الوراثية المقاومة والقابلة للإصابة بمرض البياض الدقيقي.