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Implication of Phenolic Compounds and Amino Acids in Tolerance Against Net Blotch Disease in Barley (*Hordeum vulgare* L.)

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Abstract: Net blotch disease is one of the most serious foliar diseases that attack barley and reduce economic crop. This study investigates the effect of chemical inducers, *i.e.* salicylic acid (100, 200 and 400 ppm), ethephon (100, 200 and 400 ppm), indole acetic acid (100, 150 and 200 ppm), hydrogen peroxide (8, 16 and 24 ppm), phenylalanine (20, 40 and 60 ppm) and silicon (200, 400 and 600 ppm) on tolerance against net blotch disease and its relation to some biochemical constituents in two barley cultivars under Maryout conditions. Application of chemical inducers (especially HP, PA and Si) had a positive effect on reducing disease incidence and severity. Also, Giza 132 is lower than Giza 2000 in pathological parameters, which associated with decrease in malondialdehyde content and increase in peroxidase activity and yield components. These treatments had an active role in increasing phenolic compounds content (total, free and conjugated). Treatments had an important role in increasing phenolic acids content (folic, fulvic, ferulic, ellagic, syringic and coumaric), which associated with tolerance of net blotch disease. Data showed the presence of 16 acids and classified into four groups. Neutral amino acids group was predominant (about 51%), followed by acidic group and basic group, while aromatic and imine amino acids group came in the latter arrangement. These results draw attention to neutral acids group (contain 8 acids) may implicate a clear role in enhancing barley plants to tolerance against net blotch disease. Electrophoretic behaviour of soluble proteins showed the presence of 18-23 bands with 12-96 kDa. Bands of 90 and 53 kDa were presented in two barley cultivars after treatment with chemical inducers and thus can be used as biomarker to tolerance of net blotch disease. This study confirms on the importance of tolerance net blotch disease in barley and recommends using disease tolerant Giza132 under Maryout conditions as well as using some chemical inducers; hydrogen peroxide, phenylalanine, silicon to enhance barley plants to disease tolerance. Also, we can benefit from biochemical and pathological indicators to enhance sensitive genotypes for tolerance of net blotch disease.

Key words: Net blotch disease · Barley · Phenolic compounds · Amino acids

INTRODUCTION

Barley, *Hordeum vulgare* L., is one of the principal cereal crops in the world. It is the fourth most important cereal crop both in terms of quantity produced and global area of cultivation [1]. In Egypt, barley is the main crop and widely grown in the rainfed areas of the north coastal region and in the newly reclaimed lands with saline soils [2] and a little is grown irrigated in the Nile Valley. It is mainly used as animal feed and recently as human food because of its nutritional and health properties. The major use of barley in Egypt is for animal feed and malt production. The grain is used in blends with other feed materials for most farm animals. Barley malt is used in making malted milk, barley foods and in brewing beer. Attempts to improve nutritional value of the barley grains include increasing protein content and improving the amino acid balance in the protein through higher lysine

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content. Barley is nutritious and it has the dietary fiber that is important for intestinal function and lowering cholesterol content in the blood. It can be used in many forms, such as barley flour, barley grits and barley flakes. Net blotch disease is one of the most widely distributed foliar diseases of barley and it caused by *Pyrenophora teres f. teres* [3]. It activates in the rainfed areas of the north coastal region (high rainfall and humidity) and that leads to loss in economic crop reached 10-40% [4]. Symptoms of net blotch infection can occur on leaves, leaf sheaths, flowers and grain, but are most common on leaves.

The lesions usually have a characteristic netted appearance and four types of such net-type lesions can be distinguished. When leaves of young shoots are infected by conidia or ascospores during winter or early spring, small brown lesions appear within two days. As the lesion expands, a dark brown reticulation develops on a light brown background. A zone of chlorosis usually surrounds the lesion. Net blotch is considered one of the most important diseases of barley in all major barley growing areas of the world [4] and causes considerable yield loss [5]. Induced resistance play an important role in the preservation of plants in nature. Thus, the induced resistance can be defined as an increased expression of natural defense mechanisms of plants against different pathogens provoked by external factors [6]. There are many researches which showed the importance of use chemical inducers in push the plants to diseases resistance. In this regard, salicylic acid plays a key role in plant disease resistance and hypersensitive cell death [7]. Moreover, ethephon can produce a defense against foliar diseases [8]. Additionally, there are many studies that emphasize the importance of induced resistance in plant diseases resistance using some chemical inducers such as indole acetic acid [9], hydrogen peroxide [10] and silicon [11, 12]. In resistant plants, phenolic based defense responses are characterized by the early and rapid accumulation of phenolics at the infection site resulting in the effective isolation of the pathogen [13]. Phenolics present in healthy, uninfected plant tissues, as preformed antimicrobial compounds, that inhibit the growth of fungi may include simple phenols and phenolic acids [14] and they are considered secondary metabolites, primarily synthesized through the shikimate metabolic pathway and have been identified in many crops such as barley and wheat [15]. In addition, eight phenolic acids (trans cinnamic, salicylic, ferulic, chlorogenic, p-hydroxybenzoic, protocatechuic, coumaric and vanillic) were identified in barley tissue [16]. Therefore, the present work was conducted to study the implication of phenolic compounds and amino acids in tolerance against net blotch disease in barley under Maryout conditions.

MATERIALS AND METHODS

Field Experiment: Two field experiments were carried out during 2010/2011 and 2011/2012 seasons at Agricultural Experimental Station of Desert Research Center (DRC) located in Maryout station, Egypt. The grains of two barley cultivars (Giza 2000 and Giza 132) were obtained from the Field Crop Institute, Agriculture Research Center, Giza, Egypt. Barley grains were sown in November at rate of 70 kg/faddan (one faddan=0.42ha). The experiments were designed in split plot design with three replicates. The plot was $2x3m^2$ and recommended fertilization for this type of soil was applied according to Desert Research Center. Chemical analysis of soil and water are presented in Table 1 and the results were determined according to Richards [17] and Jackson [18]. While, the meteorological data for the Maryout location is presented in Table 2. Six chemical treatments each with three concentrations were applied to the two barley cultivars as follows:

- Control (Tap water).
- Salicylic acid (SA) at 100, 200 and 400 ppm.
- Ethephon (Eth) at100, 200 and 400 ppm.
- Indole acetic acid (IAA) at 100, 150 and 200 ppm.
- Hydrogen peroxide (HP) at 8, 16 and 24 ppm.
- Phenylalanine (PA) at 20, 40 and 60 ppm.
- Silicon (Si) at 200, 400 and 600 ppm.

The salicylic acid was prepared from potassium salicylate and silicon was prepared from magnesium silicate. Each treatment was sprayed on plants at rate of 400 liter/faddan after 20 days (1^{st} net blotch infection stage) and 30 days (2^{nd} net blotch infection stage) from sowing. All treatments were applied in the morning. The samples of fresh plants were collected after 40 days from sowing. Fresh samples were tested for malondialdehyde content, peroxidase activity, phenolic compounds and soluble proteins. Then, drying samples until have constant weight at oven 70°C. Dry samples were ground to fine powder and tested for amino acids content. Also, fresh samples of naturally infected barley leaves were collected in paper bags and transferred to the laboratory for isolation, purification and identification of the pathogenic fungus (*Pyrenophora. teres. f. teres*) the causing agent of net blotch disease of barley and determination of disease incidence and disease severity.

Table 1: Chemical analysis of the experimental soil and underground irrigation water at Maryout station.

Chemical analysis of the experimental soil									
		Cations (meq/l)				Anions (meq/l)			
EC (dS/m)	pH	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{-}
1.0	8.1	1.6	4.4	4.8	0.47	--	1.4	3	6.87
Chemical analysis of irrigation water									
		Cations (meq/l)				Anions (meq/l)			
EC (dS/m)	pH	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{--}
4.44	7.2	12	11.4	37.56	0.9	--	4.2	32.5	25.16

Table 2: The meteorological data at Maryout site.

	Avg. Temp (°C)		Min Temp (°C)		Max Temp (°C)		Humidity (%)		Wind (km/day)		Rad (MJ/m^2/day)		Rain (mm)	
Month	F	S	F	S	F	S	F	S	F	S	F	S	F	S
November	20.7	18.1	15.9	13.9	25.6	22.2	69.0	70.0	9.0	7.0	13.3	13.0	1.0	90.0
December	16.9	15.3	11.9	10.7	21.8	20.0	72.0	74.0	12.0	9.0	12.2	11.7	31.0	15.0
January	15.0	12.9	10.8	9.1	19.2	16.7	71.0	74.0	10.0	14.0	12.3	12.3	47.6	80.2
February	15.5	13.3	11.1	8.8	19.9	17.7	65.0	71.0	14.0	13.0	14.3	14.3	13.5	26.2
March	16.1	15.8	11.3	11.7	21.0	20.0	71.0	68.0	12.0	15.0	17.2	17.2	15.0	37.9
April	19.1	19.6	14.1	14.1	24.0	25.1	63.0	65.0	14.0	11.0	19.6	19.6	4.1	0.0
May	22.0	22.9	17.0	17.5	27.1	28.3	66.0	67.0	14.0	12.0	21.6	21.6	0.0	0.0

F= The first season (2010/2011) and S= The second season (2011/2012).

Plants were harvested after 150 days from sowing. The following yield components were recorded: plant height (cm), grain yield (kg/faddan) and straw yield (kg/faddan).

Pathological Analysis: Determination of disease incidence and severity: Disease incidence (%) was estimated by determination of the number of infected plants among random samples of barley divided on their numbers multiplying by 100. However, disease severity (%) was estimated as percentage from 0 to 100 as described by Tekauz [19] and Khan and Boyd [20].

Biochemical Analysis

Malondialdehyde Content: The level of lipid peroxidation in barley leaves was quantified by determination of malondialdehyde content according to Health and Packer [21] and modified by Zaho *et al.* [22].

Antioxidant Enzyme Activity (Peroxidase): Peroxidase activity (POD) was measuring in barley leaves according to the method described by Worthington Biochemical Corp [23].

Soluble Proteins Content: Soluble proteins in barley leaves were determined according to Lowry *et al.* [24].

Phenolic Compounds

Total, Free and Conjugated Phenols: Phenol contents (total, free and conjugated) were determined in ethanolic extract of barley leaves according to Snell and Snell [25].

Phenolic Acids by HPLC: Phenolic acids (ellagic, folic, syringic, ferulic, fulvic and coumaric acids) were determined in methanolic extract of barley leaves using High Performance Liquid Chromatography (HPLC) at Al Azhar University. The analytical column was HYOPERCARB. Separation was performed with acetonitrile and water (85: 15 v/v) as the elusion solvent at flow rate of 1 ml/min and the detection wavelength was 254 nm.

Amino Acids Content: Amino acids in leaves were determined by amino acid analyzer apparatus model (Sykam). Hydrolysis was carried out according to the method of Pellet and Young [26].

Electrophoretic Behaviour of Soluble Proteins (SDS-PAGE): SDS-PAGE gel electrophoresis was performed according to the method of Laemmli [27] and modified by Studier [28] to identify and separate the patterns of soluble proteins in barley leaves.

Statistical Analysis: Data were analyzed statistically according to the procedure outlined by Snedecor and Cochran [29]. Combined analysis over growing seasons was done when the homogeneity test was insignificant according to Gomez and Gomez [30]. Duncan's multiple range test was used for the comparison between means [31].

RESULTS AND DISCUSSION

Pathological Analysis

Disease Incidence and Severity: In Table 3 and Fig. 1 and 2, salicylic acid (SA) showed a positive effect on decreasing the disease incidence and disease severity, the minimum value was recorded at 400 and 100 ppm, respectively. Comparison between the two cultivars, Giza 132 explored lower value of disease severity than Giza 2000. Considering the interaction effect between foliar application and barley cultivars, data indicated that Giza 132 gave the lowest values of disease incidence and severity at 400 ppm. These findings were supported by Vaitkuniene *et al.* [32] on barley. The positive effect of SA on decreasing disease incidence and severity may be serving as a signaling molecule for activation of several plant defense responses against biotic and abiotic stress including systemic acquired resistance to pathogens by inducing a range of defense genes [33]. In addition, SA able to systemically protect many plants from pathogen attacks including fungal, bacterial and viral infection [34]. Also, SA has been found to be active as antimicrobial as disease resistance inducer against several plant pathogens including the fungal foliar diseases [35]. Ethephon (Eth) recorded a positive effect on decreasing the disease incidence and severity; the lowest values were recorded at 400 ppm. Giza 132 was less than Giza 2000 in disease severity. Regarding the interaction effect, the minimum percentages of disease incidence and severity were obtained by Giza132 at rate 400 ppm. The effect of Eth on decreasing disease incidence and severity is well documented by Belhadi *et al.* [8]. The decrease of pathological parameters after treatment with Eth may be ascribed to it acts *via* liberation of ethylene and interferes in the growth process [36]. Also, it is assumed to play a role in the development of disease resistance [8]. In the same direction, ethylene has been identified as an important signaling component in plant pathogen interactions [37].

Application of indole acetic acid (IAA) at 200 ppm gave the lowest value of disease incidence, but disease severity at 150 ppm. Comparison between cultivars,

Fig. 1: Asexual spores (conidia) of *Pyrenophora teres f. teres*

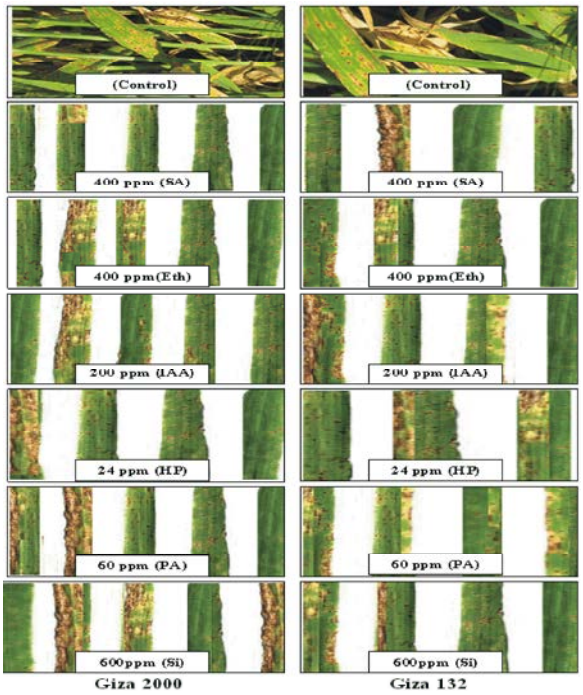

Fig. 2: Net blotch symptoms on barley cultivars after foliar applications

Giza 132 is lower than Giza 2000 in pathological parameters. Concerning the interaction effect, Giza 132 recorded the minimum percentage of disease incidence at all doses, also the same cultivar recorded the minimum value of disease severity at 150 ppm. These results are in agreement with those obtained by Petti *et al*. [38] on barley. The positive role of IAA against net blotch disease may be ascribed to it considered as plant activator, which contributes to develop new plant activators for disease control [39] as well as, it have many biological functions, including plant responses to biotic and abiotic stressors [38]. Hydrogen peroxide (HP) had a positive role on decreasing pathological parameters. In this regard, Giza 132 was less than Giza 2000 in disease incidence and severity. Considering the interaction effect, Giza 132 gave the minimum percentage of disease incidence when HP was applied at rate 16 ppm, but the minimum percentage of disease severity were achieved by

Table 3: Disease incidence and disease severity as affected by foliar applications (SA, Eth, IAA, HP, PA and Si), barley cultivars and their interaction under Maryout conditions.

	Disease incidence (%)			Disease severity (%)		
Foliar applications	Giza2000	Giza132	Mean	Giza 2000	Giza 132	Mean
	Salicylic acid (ppm)					
Control	65.00 a	60.00 a	62.50 A	17.33 a	13.33 c	15.33 A
100	50.00 b	50.00 b	50.00 C	12.71 c	12.66 c	12.69 B
200	50.00 b	60.00 a	55.00 B	18.00 b	12.91 c	15.46 A
400	50.00 b	40.00 c	45.00 D	22.00 a	10.00 d	16.00 A
Mean	53.75 A	52.50 A	--	17.51 A	12.23 B	--
	Ethephon (ppm)					
Control	65.00 a	60.00 b	62.50 A	17.33 a	13.33 d	15.33 AB
100	55.00 c	50.00 d	52.50 C	21.00 a	14.50 c	17.75 A
200	50.00 d	65.00 a	57.50 B	14.50 c	12.08 d	13.29 C
400	50.00 d	40.00 e	45.00 D	14.00 c	10.00 e	12.00 D
Mean	55.00 A	53.75 A	--	16.71 A	12.48 B	--
	Indole acetic acid (ppm)					
Control	65.00 a	60.00 a	62.50 A	17.33 a	13.33 d	15.33 A
100	60.00 a	40.00 c	47.50 B	18.33 a	13.33 d	15.83 A
150	50.00 b	40.00 c	50.00 B	15.50 c	10.00 e	12.75 B
200	50.00 b	40.00 c	35.00 C	15.50 c	15.83 bc	15.66 A
Mean	52.50 A	45.00 B	--	16.67 A	13.12 B	--
	Hydrogen peroxide (ppm)					
Control	65.00 a	60.00 b	62.50 A	17.33 a	13.33 b	15.33 A
8	45.00 c	35.00 e	40.00 C	12.49 bc	10.00 d	11.25 C
16	65.00 a	30.00 f	47.50 B	13.66 b	14.16 b	13.91 B
24	40.00 d	40.00 d	40.00 C	10.00 d	11.25 cd	10.63 C
Mean	53.75 A	41.025 B	--	13.37 A	12.19 B	--
	Phenylalanine (ppm)					
Control	65.00 a	60.00 ab	62.50 A	17.33 a	13.33 bc	15.33 A
20	40.00 c	20.00 d	30.00 D	15.00 b	10.00 d	12.50 B
40	50.00 bc	45.00 c	47.50 B	14.16 bc	12.49 c	13.33 B
60	40.00 c	40.00 c	40.00 C	12.50 c	10.00 d	11.25 C
Mean	40.75 B	41.25 A	--	14.75 A	11.46 B	--
	Silicon (ppm)					
Control	65.00 a	60.00 a	62.00 A	17.33 a	13.33 b	15.33 A
200	60.00 a	40.00 c	50.00 B	12.49 bc	10.00 d	11.25 C
400	50.00 b	40.00 c	45.00 C	13.66 b	14.16 b	13.91 B
600	50.00 b	40.00 c	45.00 C	10.00 d	11.25 cd	10.63 C
Mean	56.25 A	45.00 B	--	13.37 A	12.19 B	--

Values followed by the same letter (s) are not significantly different at $P < 0.05$

Giza 2000 at 24 ppm and Giza 132 at 8 ppm. This finding was supported by Hafez *et al.* [10]. The important role of HP in resistance against net blotch disease may be due to: i) Prevent the increase of oxidative stress and endogenous HP concentration in plants and enhancing the production of enzymatic and non enzymatic antioxidants [40]. ii) It is involved in cell walls cross linking, induction of gene expression, phytoalexin production and induced systemic resistance [41]. iii) The role of HP in increasing of lignin and suberin contents and phenolic acids [42]. The results showed that the treatment of phenylalanine (PA) had an effective impact on reducing disease incidence and severity. Comparison between the two cultivars, Giza 132 is lower than Giza 2000 in disease severity. The reverse was true in disease incidence.

Regarding to the interaction effect, Giza132 recorded the lowest value of disease incidence at 20 ppm. While, it was gave the minimum value of disease severity at 20 and 60 ppm. These results are in agreement with those obtained by Silue *et al*. [43]. The positive effect of PA on reducing of disease incidence and severity may be due to:

Table 4: Malondialdehyde content in leaves as affected by foliar applications (SA, Eth, IAA, HP, PA and Si), barley cultivars and their interaction under Maryout conditions.

	Malondialdehyde content (ηmol / g FW)						
Foliar applications	Giza 2000	Giza 132	Mean	Foliar applications	Giza 2000	Giza 132	Mean
	Salicylic acid (ppm)				Hydrogen peroxide (ppm)		
Control	222.8 a	153.9 c	180.3A	Control	222.8 c	153.9 g	188.3C
100	118.1 e	64.44 f	91.25D	8	251.3 a	168.9 f	210.1B
200	192.0 b	141.7 d	166.8B	16	231.1 b	191.2 e	211.2A
400	141.4 d	141.1 cd	143.8C	24	98.30 h	198.6 d	148.4D
Mean	168.6 A	126.5 B	--	Mean	200.9 A	178.1 B	--
	Ethephon (ppm)				Phenylalanine (ppm)		
Control	222.8 a	153.9 c	188.3A	Control	222.8 a	153.9 c	188.3AB
100	125.0 d	95.00 f	110.0C	20	119.3 d	236.6 a	178.0B
200	73.89 h	101.9 e	87.92D	40	229.5 a	170.2 bc	199.8A
400	80.5 g	198.4 b	139.5B	60	90.91 e	180.0 b	135.5C
Mean	125.6 B	137.3 A	--	Mean	165.6 B	185.2 A	--
	Indole acetic acid (ppm)				Silicon (ppm)		
Control	222.8 a	153.9 e	188.3A	Control	222.8 a	153.9 de	188.3 A
100	142.3 f	167.2 c	154.7B	200	148.0 e	163.0 bc	155.5C
150	175.2 b	131.1 g	153.1B	400	159.5 cd	169.0 b	164.2 B
200	162.1 d	106.8 h	134.4C	600	159.0 cd	125.0 f	142.0 D
Mean	175.6 A	139.8 B	--	Mean	172.3 A	152.7 B	--

Values followed by the same letter (s) are not significantly different at $P < 0.05$

FW = fresh weight

i) Amino acids induced a hypersensitive like reaction (HR) in plants and PR proteins [44] also, amino acids can induce resistance in many plant species against fungal diseases [45]. ii) Amino acids effects on plant protection against pathogens and function as biosynthesis of other organic compounds such as protein, amines, purins, pyrimidines, vitamins, enzymes and terpenoids [46]. Application of silicon (Si) recorded a positive effect on decreasing disease incidence and severity. Comparison between the two cultivars, Giza 132 explored lower value of disease incidence and severity than Giza 2000. Considering the interaction effect, the minimum percentage of disease severity was achieved by Giza 2000 and Giza 132 at 600 and 200 ppm, respectively. While, the minimum percentage of disease incidence was achieved by Giza132 at all doses. These results are in agreement with those obtained by Chen *et al.* [11]. The positive effect of Si on enhancement plant resistance against pathogen infection is probably due to: i) The insoluble silicon layer is deposited in epidermal cells preventing penetration by the pathogen. ii) Silicon affects the response of the plant at the biochemical and molecular level, in other words increases in plant defensive compounds such as phenolics, phytolexins and plant resistance proteins. Also, it appears to affect a number of components of host plant resistance, by delaying the incubation and latent period, reduces lesion expansion rates, lesion size, lesion number, the number of sporulating lesions and the number of conidia produced per lesion. As a consequence, disease progress or final disease severity is dramatically reduced [12]. iii) This resistance appears to be associated with the increased enzyme activities and gene expression related to defense response [11].

Chemical Analysis

Lipid Peroxidation Product (Malondialdehyde Content): Data in Table 4 clearly demonstrated that foliar application of SA significantly alleviated the malondialdehyde (MDA) toxic product in barley cultivars. In this connection, Giza 132 gave the lowest value of this content compared with Giza 2000. Concerning the effect of interaction, Giza 132 recorded the lowest value of MDA when SA applied at rate 100 ppm. The effect of SA on alleviating MDA content is well documented by Agarwal *et al.* [47]. The positive effect of SA on alleviating MDA content may be attributed to switch on pathways that result in preventing of oxidative damage or repair that damage [48]. Also, SA molecule acts as plant growth regulator, which plays number of plant physiological processes [49]. Foliar application of Eth decreased MDA content in barley leaves. The lowest value was recorded by 200 ppm. Comparison between the two cultivars, Giza 2000 achieved the lower value than Giza 132. Concerning the interaction effect, Giza 2000 achieved the lowest value when Eth applied at rate 200 ppm. These results are in harmony and

agreement with those obtained by Larkindale and Knight [48]. The positive effect of Eth on decreasing MDA content may be attributed to ethylene has been implicated in a number of stresses induced pathways [50] as well as application of ACC (ethylene precursor) alleviated the thiobarbituric acid reactive substances and increased plant survival [48]. IAA decreased production of MDA in barley leaves. The minimum value was recorded at rate 200 ppm; also Giza 132 achieved the lower value than Giza 2000. Taking the interaction effect in consideration, data showed that the minimum value was obtained by Giza 132 at rate 200 ppm. The effect of IAA on alleviating MDA content is well documented by Agami and Mohamed [51]. The positive effect of IAA on decreasing MDA content may be attributed to its important role which acts as a plant hormone that controls processes like growth [52], also IAA decreased the build-up of active oxygen species, enhanced the growth parameters and metabolic activities [53].

Foliar application of HP in some levels decreased MDA content in barley leaves. The lowest value was recorded by 24 ppm. Regarding barley cultivars, Giza 132 recorded the lower MDA level than Giza2000. Concerning the interaction effect, the minimum value of MDA was obtained when Giza 2000 was treated with 24 ppm. These results are in agreement with those obtained by Li *et al.* [54]. The positive effect of HP on decreasing MDA content may be due to it improves stress tolerance by enhancing antioxidants (enzymatic and non enzymatic which can quench the ROS) and reduced peroxidation of membrane lipids in plants. Application of PA in some levels decreased MDA content, the minimum value was achieved when applied at 60 ppm. In this connection, Giza 2000 achieved the lower value than Giza 132. Concerning the interaction effect, Giza 2000 recorded the minimum value of MDA content at rate 60 ppm. The positive effect of PA on decreasing MDA content may be due to it is an important precursor for many biosynthetic pathways. Application of Si reduced the accumulation of MDA; the lowest value was recorded at 600 ppm. Comparison between the two cultivars, Giza 132 achieved the lower value than Giza 2000. The interaction effect showed that, spraying of Giza 132 with 600 ppm recorded the lowest amount of MDA. These results are in harmony and agreement with that obtained by authors [55, 56]. The positive effect of Si on decreasing MDA content may be due to Si could alleviate oxidative stress on plants by increasing activity of antioxidant enzymes as well as alleviating oxidative damage of cellular functional molecules induced by over produced reactive oxygen species and maintaining many physiological processes of stressed plants [56].

Antioxidant Enzyme Activity (Peroxidase Activity): Data in Fig. 3 indicated that SA treatment (400 ppm) enhanced the enzymatic defense mechanisms of plant i.e. peroxidase (POD) in barley leaves compared with the control. These results are compatible with that obtained by authors [57, 58]. In this concern, Larkindale and Knight [48] suggested that SA may switch on pathways that result in preventing of the oxidative stress damage or repair that damage. In addition, Agarwal *et al.* [47] attributed the ability of SA to cause the increase in antioxidant enzyme (peroxidase) to the increase in contents of chlorophyll, carotenoids and relative water content, which increases the total biomass of plant. As a consequence, the effect of SA leads to a marked enhancing in the integrity of photosynthetic apparatus and decrease the toxic product formation also, preservation of water content inside plant. This may give an account to the preserving to structures of proteins and gave higher antioxidant activity. Foliar application of Eth improved the activity of POD in samples of Giza 132 at 200 ppm compared with the control, but had a negative effect on POD level of Giza 2000. These results are well established by De and De [59] and Larkindale and Huang [60]. The positive effect of Eth in enhancing antioxidant enzymes is due to it is a precursor of ethylene which is closely related to other plant hormones including auxins, cytokinin [61]. In addition, Larkindale and Knight [48] suggested that ethylene may switch on pathways that result in preventing the oxidative damage or repair that damage. De and De [59] suggested that there was a relation between Eth concentration and catalase and peroxidase activities. Also, Kwak *et al*. [62] showed that ethylene is a gaseous plant hormone that is involved in a wide spectrum of important physiological processes in plants. In addition, Liu *et al*. [63] found that Eth treatment at different concentrations increased antioxidant activity.

Application of IAA also improved the POD activity in barley cultivars (expect Giza 2000 with 150 ppm and Giza 132 with 200 ppm). These results are well documented by authors [58, 64]. The positive effect of IAA in enhancing antioxidant enzymes is due to its ability to regulate the growth which will improve the antioxidant enzymes content [58]. In addition, exogenous of IAA provides an attractive approach to counter the stress conditions [65].

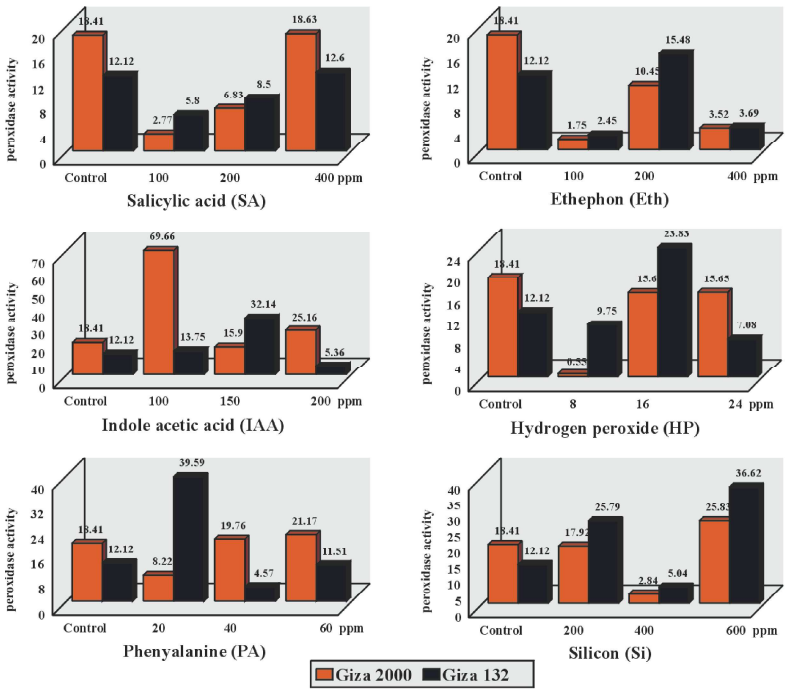

Fig. 3: Effect of foliar applications of SA, Eth, IAA, HP, PA and Si on peroxidase activity (Ä 460 / mg protein/3 min) in leaves of barley cultivars

Regarding the hydrogen peroxide treatment, it was found that HP improves the POD activity of Giza 132 at 16 ppm, but had a negative effect on Giza 2000. These results were documented by Gondium *et al.* [66] and Liu *et al.* [67]. The positive effect of HP is due to it accelerated the germination percentage of seeds that induce a pronounced increase in antioxidant enzymes [66]. Foliar application of PA induced the activity of POD in barley cultivars (expect Giza 2000 with 20 ppm and Giza 132 with 40 and 60 ppm). Concerning the effect of PA, there is no previous work which can clarify mode of action of PA on peroxidase in plants, but PA is the precursor of SA biosynthesis in plants, in this concern, SA had an important role in activating antioxidant enzyme as mentioned previously. Also, application of Si enhanced POD activity in barley cultivars (expect Giza 2000 with 200 & 400 ppm and Giza 132 with 400 ppm). These results are in agreement with Sara and Haddad [68]. In addition, Si had been extensively researched in relation to the response of plants to biotic stress, as an element defense which activates the antioxidant system [69].

Phenolic Compounds

Total Phenolic Compounds (Total, Free and Conjugated): Data presented in Table 5 showed that treatment of barley plants with SA had a clear role in increasing the content of phenolic compounds (total, free and conjugated). Moreover, Giza132 significantly exceeded Giza 2000 in phenolic compounds. Considering the interaction effect, data indicated that treatment of Giza 132 with SA at all doses led to a clear increase in phenol compounds content compared with the control. In this regard, SA plays a key role in establishing resistance to pathogens in many plants so, the content of soluble phenolics will increases after treatment with SA [70]. Data showed that Eth enhanced total, free and conjugated phenols at 100 and 400 ppm. Comparison between the two cultivars, Giza 2000 significantly exceeded Giza 132 in total and conjugated phenols, while it took the reverse trend in free phenols. As to the effect of interaction, data exhibited that the highest values of total and conjugated phenols were achieved by Giza 2000 at 400 ppm. While, Giza132 recorded the highest value of free phenols at rate 100 ppm. Application of IAA showed an enhancing effect on phenolic compounds, but the maximum values of total and conjugated phenols were obtained at 200 ppm. Moreover, Giza 2000 significantly exceeded Giza 132 in total and conjugated phenols, while it took the opposite trend in free phenols. Concerning the interaction, the maximum values of total and conjugated phenols were achieved by Giza 2000 when IAA applied at rate 200 ppm, while Giza 132 recorded the superior mean value of free phenols at rates 150 and 200 ppm.

Table 5: Total, free and conjugated phenols in leaves as affected by foliar applications (SA, Eth, IAA, HP, PA and Si), barley cultivars and their interaction under Maryout conditions.

	Total phenols (mg/g DW)			Free phenols (mg/g DW)			Conjugated phenols (mg/g DW)		
Foliar applications	Giza 2000	Giza 132	Mean	Giza 2000	Giza 132	Mean	Giza 2000	Giza 132	Mean
Salicylic acid (ppm)									
Control	2.020 c	2.030 c	2.025 B	0.560 de	0.546 e	0.553 C	1.450 c	1.483 bc	1.467 B
100	2.277 b	2.610 a	2.443 A	0.610 cd	0.780 b	0.695 B	1.660 ab	1.827 a	1.743 A
200	2.160 bc	2.610 a	2.385 A	0.643 c	0.810 ab	0.726 AB	1.510 bc	1.797 a	1.653 A
400	2.313 b	2.610 a	2.462 A	0.650 c	0.850 a	0.750 A	1.653 ab	1.750 a	1.702 A
Mean	2.193 B	2.465 A	--	0.615 B	0.746 A	--	1.568 B	1.714 A	--
Ethephon (ppm)									
Control	2.020 e	2.030 e	2.025 C	0.560 d	0.546 d	0.553 C	1.450 d	1.483 d	1.467 C
100	2.707 d	3.070 b	2.888 B	0.506 d	0.853 a	0.680 B	2.167 b	2.217 b	2.192 B
200	2.673 d	1.510 f	2.092 C	0.526 d	0.793 b	0.660 B	2.147 b	0.713 e	1.430 C
400	3.267 a	2.820 c	3.043 A	0.656 c	0.833 ab	0.745 A	2.607 a	1.987 c	2.297 A
Mean	2.667 A	2.358 B	--	0.562 B	0.756 A	--	2.092 A	1.600 B	--
Indole acetic acid (ppm)									
Control	2.020 d	2.030 d	2.025 D	0.560 e	0.543 e	0.553 D	1.450 c	1.483 c	1.467 C
100	3.453 a	2.590 c	3.022 B	0.840 c	1.310 b	1.075 A	2.607 b	1.273 d	1.940 B
150	1.827 e	2.920 b	2.373 C	0.553 e	1.400 a	0.976 C	1.260 d	1.520 c	1.390 C
200	3.510 a	2.907 b	3.208 A	0.626 d	1.407 a	1.017 B	2.883 a	1.493 c	2.188 A
Mean	2.703 A	2.612 B	--	0.645 B	1.165 A	--	2.050 A	1.442 B	
Hydrogen peroxide (ppm)									
Control	2.020 d	2.030 d	2.025 C	0.560 c	0.546 c	0.553 B	1.450 d	1.483 d	1.467 C
8	4.083 a	2.603 c	3.343 B	1.200 a	0.766 b	0.983 A	2.886 a	1.837 c	2.360 B
16	4.037 a	2.607 c	3.322 B	1.233 a	0.806 b	1.020 A	2.803 ab	1.800 c	2.302 B
24	3.813 a	3.450 b	3.632 A	1.293 a	0.810 b	1.052 A	2.520 b	2.640 ab	2.580 A
Mean	3.488 A	2.672 B	--	1.072 A	0.732 B	--	2.414 A	1.940 B	--
Phenylalanine (ppm)									
Control	2.020 c	2.030c	2.025 C	0.560 b	0.546 b	0.553 B	1.450 d	1.483 cd	1.467 D
20	3.057 a	3.083 a	3.070 A	0.986 a	1.060 a	1.023 A	2.070 ab	2.023 b	2.047 B
40	3.123 a	3.127 a	3.125 A	1.027 a	0.993 a	1.010 A	2.097 ab	2.133 a	2.115 A
60	2.523 b	3.037 a	2.780 B	0.973 a	0.993 a	0.983 A	1.550 c	2.043 b	1.797 C
Mean	2.681 B	2.819 A	--	0.886 A	0.898 A	--	1.792 B	1.922 A	--
Silicon (ppm)									
Control	2.020 c	2.030 c	2.025 C	0.560 e	0.546 e	0.553 D	1.450 d	1.483 cd	1.467 B
200	2.660 b	2.580 b	2.620 B	0.846 c	0.760 d	0.803 C	1.813 ab	1.820 ab	1.817 A
400	2.580 b	2.587 b	2.583 B	1.047 a	0.923 b	0.990 B	0.533 cd	1.653 bc	1.593 B
600	2.970 a	2.660 b	2.812 A	1.033 a	1.060 a	1.047 A	1.937 a	1.600 cd	1.768 A
Mean	2.557 A	2.464 B	--	0.871 A	0.825 B	--	1.683 A	1.639 A	--

Values followed by the same letter (s) are not significantly different at $P < 0.05$

DW= dry weight

Foliar application of HP had a positive effect on total, free and conjugated phenols. The maximum value of phenolic compounds was recorded when HP applied at rate 24 ppm. Comparison between the two cultivars, Giza 2000 exceeded Giza 132 in all fractions of phenols. Regarding to interaction effect, treatment of Giza 2000 with 8 ppm gave the highest values of total and conjugated phenols, while it gave the highest value of free phenols at 24 ppm compared with the control. Data demonstrated that PA recorded a positive effect on total, free and conjugated phenols compared with the control. Comparison between the two cultivars, Giza 132 exceeded Giza 2000 in total and conjugated phenols. Concerning the interaction effect, Giza132 reached the superior values of total and conjugated phenols when PA applied at rate 40 ppm, while it recorded the maximum value of free phenols at 20 ppm. The results showed that treatment with Si had an effective impact on the accumulation of phenolic compounds. The highest values of total and free phenols were recorded in barley plants at 600ppm. Comparison between the two cultivars, Giza 2000 exceeded Giza 132 in total and free phenols.

Table 6: Phenolic acids in leaves of barley cultivars as affected by foliar applications (SA, Eth, IAA, HP, PA and Si) under Maryout conditions.

		Phenolic acids (µg/g FW)					
Treatments		Ellagic	Folic	Syringic	Ferulic	Fulvic	Coumaric
				Control			
Giza 2000		1.88	97.70	n.d.	2.28	18.11	0.02
Giza 132		n.d.	16.42	n.d.	n.d.	n.d.	n.d.
				Salicylic acid (ppm)			
100	Giza 2000	1.28	25.05	0.66	1.30	7.27	0.02
	Giza 132	1.54	79.29	n.d.	2.28	16.62	0.03
200	Giza 2000	1.66	n.d.	n.d.	n.d.	22.55	0.03
	Giza 132	3.03	n.d.	1.08	3.80	n.d.	0.03
400	Giza 2000	2.80	33.22	1.50	2.62	25.97	0.03
	Giza 132	2.80	n.d.	n.d.	2.40	n.d.	0.02
				Ethephon (ppm)			
100	Giza 2000	0.89	n.d.	0.716	n.d.	n.d.	0.02
	Giza 132	0.80	n.d.	0.52	n.d.	10.84	n.d.
200	Giza 2000	n.d.	31.78	n.d.	2.00	14.53	0.07
	Giza 132	n.d.	28.10	n.d.	n.d.	n.d.	n.d.
400	Giza 2000	2.52	n.d.	n.d.	16.88	n.d.	0.52
	Giza 132	n.d.	18.15	0.58	3.17	n.d.	n.d.
				Indole acetic acid (ppm)			
100	Giza 2000	0.05	n.d.	n.d.	8.30	81.56	0.24
	Giza 132	0.02	n.d.	n.d.	17.38	n.d.	n.d.
150	Giza 2000	n.d.	0.58	n.d.	18.04	n.d.	0.55
	Giza 132	n.d.	0.36	n.d.	n.d.	86.63	0.29
200	Giza 2000	n.d.	15.29	0.46	n.d.	11.50	0.02
	Giza 132	1.12	n.d.	0.86	n.d.	8.62	0.02
				Hydrogen peroxide (ppm)			
8	Giza 2000	1.07	37.10	1.46	n.d.	n.d.	0.01
	Giza 132	0.85	44.36	0.35	n.d.	3.29	n.d.
16	Giza 2000	2.43	n.d.	0.34	1.19	n.d.	0.02
	Giza 132	0.67	n.d.	0.17	0.65	4.62	n.d.
24	Giza 2000	0.80	30.59	0.54	1.71	n.d.	0.01
	Giza 132	0.64	26.76	0.36	1.22	7.94	0.01
				Phenylalanine (ppm)			
20	Giza 2000	0.40	n.d.	0.43	n.d.	4.04	n.d.
	Giza 132	0.54	28.42	0.44	n.d.	n.d.	n.d.
40	Giza 2000	0.41	n.d.	0.15	n.d.	1.94	n.d.
	Giza 132	0.64	n.d.	0.12	0.32	n.d.	n.d.
60	Giza 2000	0.93	33.43	0.64	n.d.	8.94	0.01
	Giza 132	0.95	58.05	0.82	3.38	n.d.	0.02
				Silicon (ppm)			
200	Giza 2000	0.02	n.d.	n.d.	13.89	n.d.	0.37
	Giza 132	0.74	n.d.	n.d.	n.d.	15.85	0.02
400	Giza 2000	0.80	n.d.	1.00	n.d.	n.d.	0.02
	Giza 132	n.d.	27.74	n.d.	n.d.	21.03	0.07
600	Giza 2000	0.63	n.d.	0.01	3.76	13.05	0.01
	Giza 132	0.03	n.d.	n.d.	13.20	n.d.	0.41

n.d = not detectable, FW = fresh weight

Concerning the interaction effect, the maximum values of total and conjugated phenols were obtained by Giza 2000 when Si applied at rate 600 ppm, but the highest value of free phenols was recorded by Giza132 at rate 600 ppm. These results well documented by many authors [70, 71, 72]. The positive effect of Si was demonstrated by Hanafy Ahmed *et al.* [71], who noticed the favorable effects of Si in decreasing the harmful effects of abiotic and biotic stress through increasing the ability of plants under stress to condensate simple organic compounds like

sugars, phenols and amino acids. Also, Shetty *et al*. [72] found that application of Si increased the concentration of antimicrobial phenolic acids and flavonoids in response to infection. The increase in phenolic compounds correlated with 46% reduction in disease severity. Furthermore, Si application without pathogen inoculation induced gene expression and primed the accumulation of several phenolics.

Phenolic Acids: Phenolic acids are very important for tolerance against biotic stress. Data in Table 6 indicated six phenolic acids arranged according to their concentration in a descending order: folic, fulvic, ferulic, ellagic, syringic and coumaric that detected in two barley cultivars. In control samples, it was noticed that syringic acid is not detected in two cultivars, while the phenolic acids (ellagic, ferulic, fulvic and coumaric) are not detected in Giza132. In this regard, folic acid was increased when Giza132 treated with SA, Eth, HP, PA and Si at 100 ppm, (200 and 400 ppm), (8 and 24 ppm), (20 and 60 ppm) and 400 ppm, respectively compared with the control. On the other hand, application of IAA with Giza132 showed a negative effect on folic acid; also all foliar applications had a negative effect on Giza 2000. Pushpalatha *et al.* [73] found that folic acid used for the development of host resistance is a sustainable approach for plant disease management which used to treat pearl millet to test their ability to induce resistance to downy mildew disease. Concerning fulvic acid, data indicated accumulation of such content in Giza 2000 when SA and IAA applied at rates (200 and 400 ppm) and 100 ppm, respectively. Also, it was increased in samples of Giza 132 after treatment with SA, Eth, IAA and Si at rates 100 ppm, 100 ppm, (150 and 200 ppm) and (200 and 400 ppm), respectively compared with the control. Also, it was responded positively in samples of Giza 132 after treated with HP, while it took opposite effect when Giza132 treated with PA at all doses.

Data showed more accumulation of ferulic acid in Giza 132 after treated with SA, Eth, IAA and Si at 400 ppm, 400 ppm, (100 and 150 ppm) and (200 and 400 ppm), respectively compared with the control. In the same direction, ferulic acid was increased in Giza 132 when Eth, IAA, HP, PA and Si were applied at 400 ppm, 100 ppm, (16 and 24 ppm), (40 and 60) and 600 ppm, respectively. In this regard, foliar application of SA had a positive effect on accumulation of ferulic acid in samples of Giza132. Data in Table 6 also showed that coumaric was found in low quantities compared with the other phenolic acids. Other identified phenolic acids (ellagic and syringic) had concentrations in between those extremes and decreased or increased depending on type and dose of foliar application treatments as well as the interaction between these foliar applications and environmental conditions at Maryout. These results well documented by Kovacik *et al.* [70], Hanafy Ahmed *et al.* [71] and Shetty *et al.* [72]. In addition, Zhou *et al.* [74] showed that syringic acid inhibited cucumber growth and altered rhizosphere microbial communities; also it plays a role in the communication between plants and soil microorganisms. Salicylic acid plays a key role in establishing resistance to pathogens in many plants. So, the treatment with SA led to increase soluble phenolics content [70] also application of Si increased the concentration of antimicrobial phenolic acids and flavonoids in response to infection [72]. In the same manner, Si reduces the harmful effects of biotic and abiotic stress by increasing the ability of plants to the accumulation of sugars, amino acids and phenols [71].

Amino Acids: Data in Table 7 and Fig. 4 showed that 16 amino acids were detected in samples of Giza132 and classified into four groups according to their chemical composition.

Acidic Amino Acids Group: The major acidic amino acid was glutamic followed by aspartic in descending order. Data clearly demonstrated that foliar application of IAA, PA (except 20 and 60 ppm) and Si (except 600 ppm) had a promotive role in accumulation of glutamic acid compared with the control. While, treatments with SA, Eth and HP negatively affected on the accumulation of glutamic acid. Concerning aspartic acid, data showed that all treatments enhanced the increase of aspartic acid (except SA with 100 ppm, Eth with 100 ppm, IAA with all doses, PA with 40 ppm and Si with 200 & 400 ppm) compared with the control. In general, the results showed that acidic amino acids recorded the highest values with all foliar application treatments and mostly higher than other amino acids possibly due to their being precursors for synthesis of most amino acids. These results were compatible with those obtained by Hanafy Ahmed *et al.* [71], Caputo and Barneix [75], Hussein *et al.* [76] and Mahdi [77]. In this regard, Caputo and Barneix [75] found that the amino acids composition of phloem sap is different in variable plant species, in barley glutamic acid accounts for approximately 50% of the total amino acids, while aspartic acid accounts for roughly 20% and in wheat glutamic amounted to 30% of the total amino acids and aspartic acid to 20%, with these proportions changing with plant age.

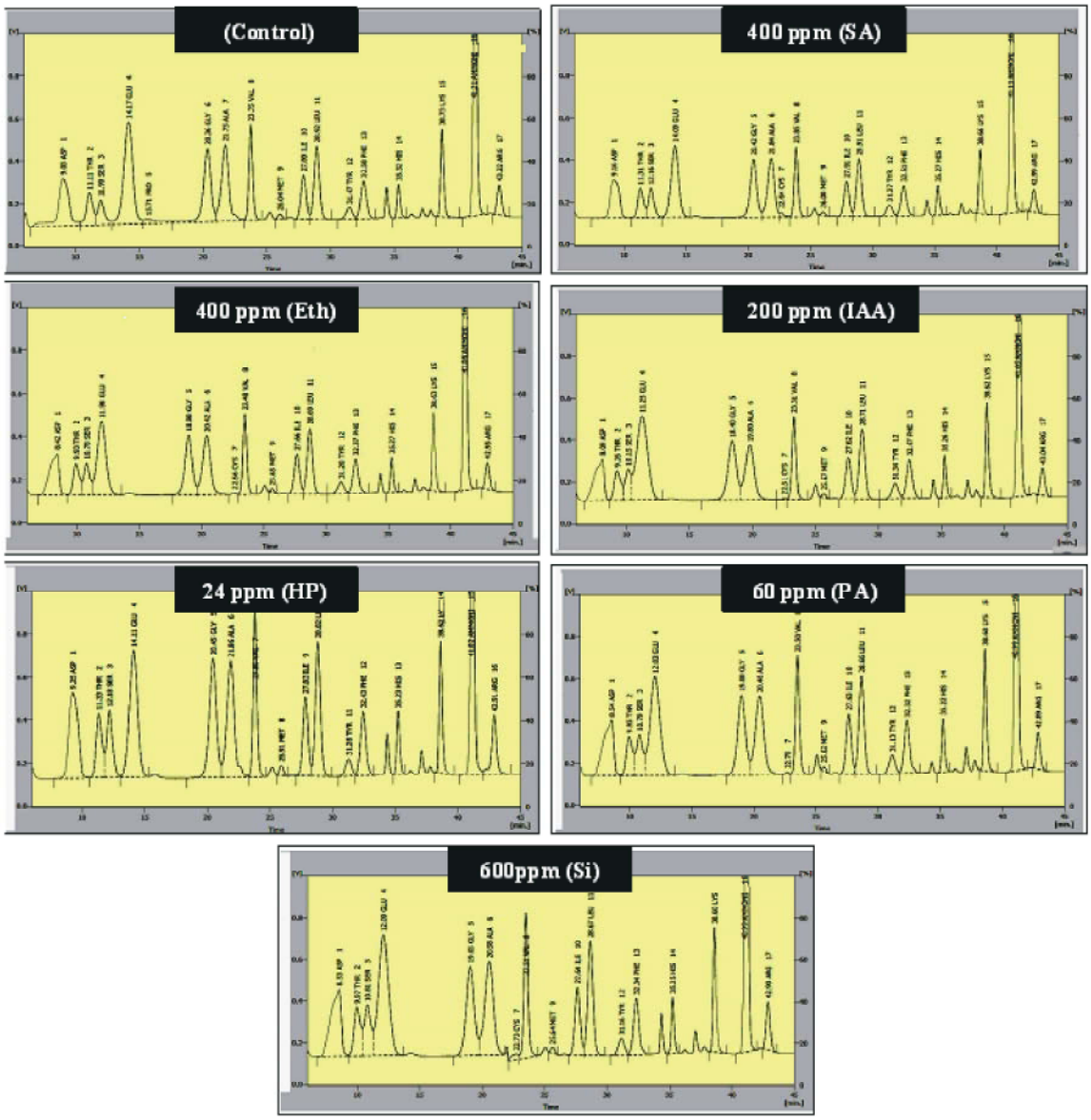

Fig. 4: Effect of foliar applications of SA, Eth, IAA, HP, PA and Si on amino acids in leaves of Giza132

Basic Amino Acids Group: Basic amino acids varied in their ability to accumulate in Giza132 under different foliar application treatments. It could be ranked according to their concentrations in the descending order: lysine, arginine and histidine. Data showed that all foliar applications increased histidine content (except HP at 8 ppm) compared with the control. On the other hand, foliar applications with Eth, HP and PA showed a negative effect on lysine acid, while it was increased after treatment with SA (100 ppm), IAA (200 ppm) and Si (200 and 400 ppm). Concerning arginine acid, it was decreased in samples of Giza132 after application of all treatments (except HP at 8 and 24 ppm, PA at 20 ppm and Si at 600 ppm). These results are in harmony and agreement with those obtained by Hussein *et al.* [76] and Mahdi [77].

Neutral Amino Acids Group: Neutral amino acids arranged according to their concentration in the descending order: alanine, glycine, leucine, valine, isoleucine, threonine, serine and methionine. Alanine was increased when Giza 132 treated with SA (100 and 200ppm), Eth (400 ppm), HP (8 ppm), PA (40 ppm) and Si (600 ppm) compared with control. The results also showed that treatments had a positive effect on the accumulation

Table 7: Amino acids in leaves of Giza 132 as affected by foliar applications (SA, Eth, IAA, HP, PA and Si) under Maryout conditions

		Amino acids (%)							
Treatments		Acidic		Basic			Aromatic and imine		
Foliar applications	Doses (ppm)	Aspartic	Glutamic	Histidine	Lysine	Arginine	Proline	Tyrosine	Phenylalanine
Control		9.44	18.12	2.39	6.64	2.80	1.38	1.70	4.89
Salicylic acid	100	9.05	17.30	2.59	6.79	2.76	1.16	1.95	4.89
	200	9.69	16.5	2.78	5.68	2.59	1.21	1.83	4.85
	400	9.66	15.90	2.78	6.10	2.75	1.37	1.92	4.85
Ethephon	100	9.20	17.96	2.48	6.61	2.71	1.47	1.85	4.83
	200	9.77	17.02	2.76	5.74	2.60	1.38	1.84	4.94
	400	9.67	16.10	2.65	6.08	2.77	1.50	1.84	4.80
Indole acetic acid	100	8.89	20.36	2.43	6.51	2.75	1.40	1.83	4.81
	150	9.12	19.30	2.82	6.58	2.46	1.39	1.99	4.83
	200	8.26	20.06	2.79	6.67	2.61	1.46	2.15	4.91
Hydrogen peroxide	8	10.30	14.40	2.27	5.66	3.22	1.75	1.46	4.69
	16	9.50	17.55	2.61	6.63	2.73	1.42	1.72	4.99
	24	10.30	13.80	2.49	5.52	3.93	1.53	1.36	4.66
Phenylalanine	20	9.80	16.20	2.64	5.70	3.47	1.42	1.69	4.72
	40	9.08	18.40	2.41	6.57	2.73	1.40	1.71	4.74
	60	9.71	16.7	2.77	6.60	2.65	1.34	1.94	5.07
Silicon	200	9.01	19.20	2.86	6.65	2.49	1.26	1.98	5.13
	400	8.96	18.52	2.71	6.67	2.77	1.11	1.91	5.05
	600	9.84	17.70	2.46	5.69	2.93	1.54	1.50	4.57

% = Area % of amino acids

Table 7: Continued

		Amino acids (%)							
Treatments		Neutral							
Foliar applications	Doses (ppm)	Threonine	Serine	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine
Control		4.57	3.84	10.62	11.74	7.72	0.32	5.08	8.75
Salicylic acid	100	4.81	4.37	10.60	12.00	7.74	0.42	5.00	8.60
	200	4.91	4.69	11.30	11.80	7.71	0.36	5.36	8.78
	400	4.90	4.78	11.20	11.60	7.75	0.42	5.19	8.86
Ethephon	100	4.68	4.53	10.55	11.53	7.71	0.31	5.02	8.56
	200	4.80	4.68	11.00	11.31	7.74	0.26	5.36	8.80
	400	4.80	4.77	11.00	11.90	7.80	0.30	5.20	8.83
Indole acetic acid	100	4.45	4.5	10.24	10.71	7.55	0.34	4.69	8.54
	150	4.41	4.48	11.10	10.90	7.23	0.27	4.86	8.35
	200	4.19	3.86	11.11	10.84	7.34	0.32	4.93	8.50
Hydrogen peroxide	8	4.29	3.16	11.40	13.50	8.72	0.47	5.36	9.36
	16	4.53	4.74	10.83	11.03	7.63	0.33	5.05	8.71
	24	5.00	5.23	11.40	11.60	8.05	0.54	5.33	9.29
Phenylalanine	20	4.73	4.79	10.70	11.70	7.77	0.47	5.45	8.83
	40	4.60	4.51	10.20	12.10	7.74	0.37	4.96	8.57
	60	4.22	4.51	10.80	11.60	7.78	0.23	5.29	8.83
Silicon	200	4.50	4.48	10.90	10.70	7.23	0.38	4.95	8.35
	400	4.61	4.41	10.44	11.50	7.38	0.46	4.94	8.57
	600	4.49	4.86	10.40	11.80	8.20	0.32	5.04	8.72

% = Area % of amino acids

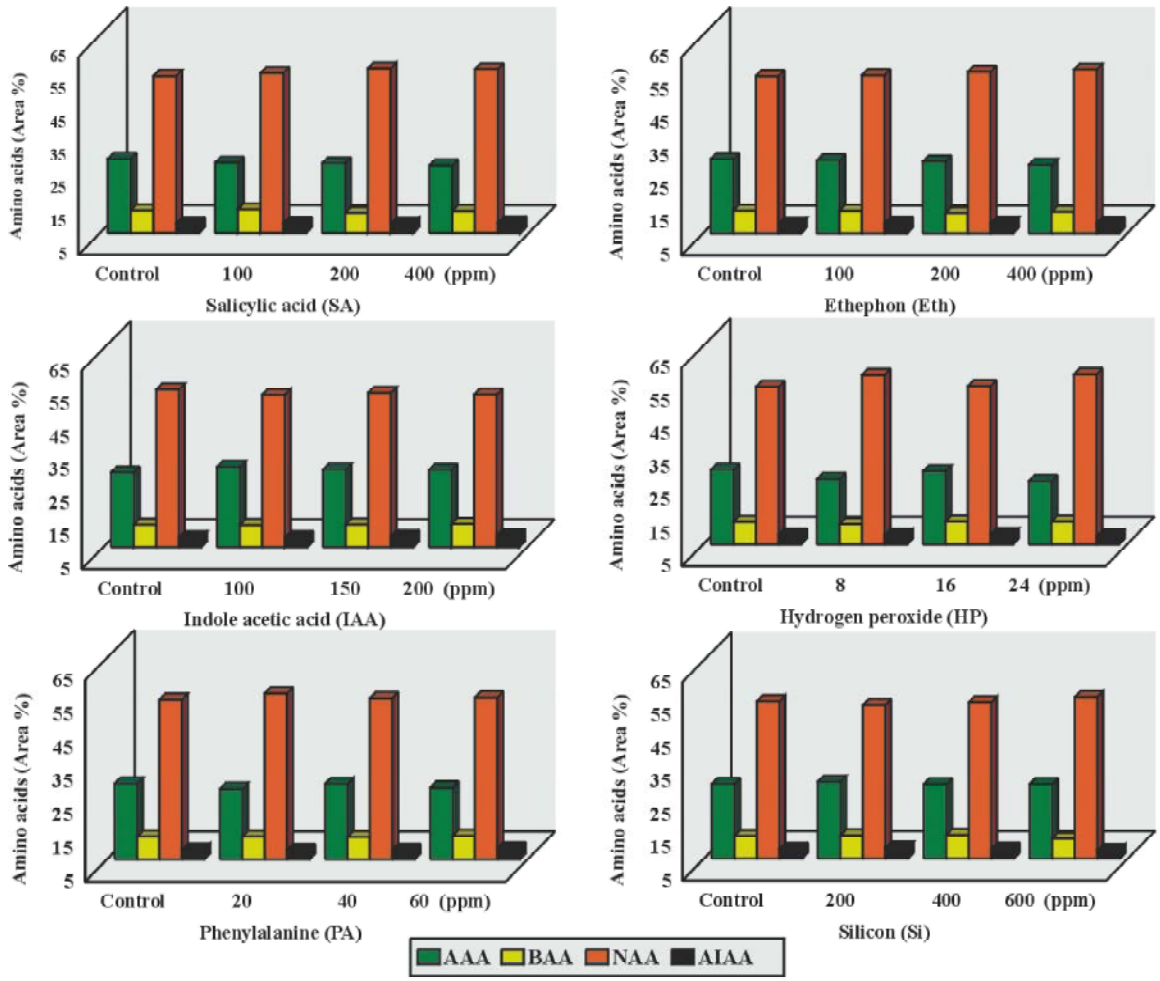

Fig. 5: Effect of foliar applications of SA, Eth, IAA, HP, PA and Si on acidic amino acids (AAA), basic amino acids (BAA), neutral amino acids (NAA) and aromatic and imine amino acids (AIAA) in leaves of Giza 132

of glycine acid (except SA, Eth and IAA with 100 ppm as well as PA at 40 ppm and Si at 400 & 600 ppm). Methionine was present in low quantities compared with the other amino acids. Other identified neutral amino acids had concentrations in between those extremes and decreased or increased depending on the type and dose of treatments as well as the interaction between these treatments and environmental conditions at Maryout. These results were documented by Hussein *et al.* [76] and Mahdi [77].

Aromatic and Imine Amino Acids Group: Aromatic and imine amino acids group ranked according to their concentration in the descending order: phenylalanine, tyrosine and proline. Phenylalanine is the major amino acid in this group and it was increased when Eth, IAA, HP, PA and Si applied at rates 200, 200, 16, 60 and (200 & 400) ppm, respectively. Also, all foliar applications positively affected on the accumulation of tyrosine except HP (at 8 and 24 ppm), PA at 20 ppm and Si at 600 ppm. Concerning proline acid, it was increased after treated with all foliar applications except Eth at 200 ppm, PA at 60 ppm and Si at 200 & 400 ppm compared with the control. Moreover, treatment with SA negatively affected on the accumulation of proline acid. These results are in agreement with those obtained by Hussein *et al.* [76], Mahdi [77] and Abd El Aziz *et al.* [78].

Comparing between the groups of amino acids (Fig. 5), the results showed that the group of neutral amino acids was predominant (about 51%), followed by acidic amino acids group and basic amino acids group in descending order, while the group of aromatic and imine amino acids came in the latter arrangement. These results draw attention to the group of neutral amino acids

(contain 8 acids) may implicate a clear role in pushing barley plants in tolerance against net blotch disease under Maryout condition. There are many interpretations, which describe the positive role of some chemical inducers on amino acids. In this concern, the positive effect of foliar application with SA in increasing amino acids in plant might be due to its effects on the enzymatic activity and translocation of the metabolites [79] as well as SA enhances the defensive compounds like betaine, glycine and proline [80]. Also, the role of Eth on enhancing amino acids may be due to that Ethephon's mode of action acts *via* liberation of ethylene, which is absorbed by the plant and interferes in the growth process [36] and ethylene is a potent regulator of plant growth and maturity. In this concern, ethylene had been implicated in a number of stresses induced pathways [50]. Concerning hydrogen peroxide, it plays an important role in activation of any array of host defense mechanisms including induced activity of enzymes and proteins [42] as well as it is considered stress signal molecule, which enhances in the seedlings to overcome the ion induced oxidative damage. However, the effect of foliar application of amino acids on plant biochemical constituents could be through plant protection from ammonia toxicity as they remove amide formation, serving as a source of carbon and energy as well as biosynthesis of other organic compounds such as protein, amines, purins, pyrimidines, vitamins, enzymes and terpenoids [46]. It is noticed by Hanafy Ahmed *et al.* [71] that the favorable effects of silicon in decreasing the harmful effects of a biotic and biotic stress through increasing the ability of plants under stress to condensate simple organic compounds such as sugars, phenols and amino acids.

Electrophoretic Behaviour of Soluble Proteins (SDS-PAGE): The data presented in Tables 8 and 9 showed that the number of bands found in barley cultivars ranged from 18 to 23 with molecular weight ranged between 12 to 96 kDa. Band of molecular weight 96 kDa was absent in control and foliar sprayed plants of Giza 2000, however sometimes accumulated in Giza 132. Bands of molecular mass 63 and 90 kDa were present after treatment with SA at rate 200 and 100 ppm, respectively compared with the control of Giza 2000. Also, bands of molecular mass 51 and 76 kDa were accumulated with all doses of SA. In the same direction, bands of molecular weight 45 and 53 kDa were found after application of SA at rates 200 and 400 ppm compared with the absent bands in control. Concerning bands of Giza 132, the molecular masses of 96 and 69 kDa were accumulated when SA was applied at rate of 100 ppm. Also, bands of molecular weights 34, 53 and 56 kDa took the same trend after treatment with SA at all doses. Band of 90 kDa was present in samples of Giza132 after treatment with 100 and 200ppm. Also, band at molecular weight 47 kDa took the same trend with 200 and 400 ppm compared with the control. These results were closely related to results obtained by Abd El Rady [81] and Hendawey and Hassany [82].

Data in Tables 8 and 9 also illustrated the effect of ethephon on patterns of soluble proteins in barley cultivars. In Giza 2000, molecular masses of 90 and 63 kDa were absent from the control, but accumulated after spraying with Eth at rates of 100 and 400 ppm. Band of molecular weight 76 kDa was present when Eth was applied at 100 and 200 ppm. Also, bands of molecular masses 53, 51 and 45 kDa were accumulated after spraying with all doses of Eth compared with the control. In Giza 132, bands of molecular weights 90, 69 and 53 were appeared at rates of 100 and 400 ppm. Bands of molecular weights 56 and 47 kDa also accumulated after application of 100 and 200 ppm. Also, bands of molecular weight 96 and 34 kDa were accumulated after application of all doses of Eth. These results were similar of the findings obtained by Vera and Conejero [83].

Concerning the effect of IAA on the bands patterns in Giza2000, bands of molecular weights 90, 63 and 51 kDa were absent in control, but accumulated at all IAA doses. Band of molecular weight 76 kDa took the same trend in control, but appeared at 100 and 200 ppm. Also, bands of molecular weights 53 and 45 kDa were found after application of IAA at rates of 100 and 150 ppm compared with the control. In Giza 132, bands of molecular weights 96 and 69 kDa were accumulated after spraying of IAA at all doses and disappeared from control. Bands of molecular masses 56 and 34 kDa were present at 150 and 200 ppm. In addition, bands of molecular masses 90 and 53 were accumulated after treatment with IAA at 100 and 200 ppm. The results also showed that HP had a clear effect on patterns of soluble proteins. In Giza 2000, bands of molecular weights 90, 76 and 53 kDa were absent from control, however accumulated at all doses of HP. Also, band of molecular weight 63 kDa was found at rates of 8 and 24 ppm. Band of molecular weight 51 kDa took the same trend when Hp was applied at 24 ppm compared with the control. In this regard, band of molecular weight 45 kDa was accumulated only after treatment with HP at rates of 8 and 16 ppm. In Giza 132, treatment with HP at all concentrations led to appearance of bands with molecular weight 90, 47 and 34 kDa compared with the control.

Table 8: Soluble proteins pattern in leaves of Giza 2000 as affected by foliar applications (SA, Eth, IAA, HP, PA, Si) under Maryout conditions.

			Soluble proteins pattern								
			Salicylic acid (ppm)			Ethephon (ppm)			Indole acetic acid (ppm)		
No.	M. wt	Control	100	200	400	100	200	400	100	150	200
1	96	0	0	0	0	0	0	0	0	0	0
2	93	1	0	1	0	0	1	0	0	0	0
3	90	0	1	0	0	1	0	1	1	1	1
4	86	1	0	1	1	1	1	0	1	0	1
5	81	1	1	1	1	1	1	1	0	1	1
6	76	0	1	1	1	1	1	0	1	0	1
7	72	1	0	1	1	1	1	0	1	1	1
8	69	1	1	1	0	1	1	1	0	0	1
9	67	1	1	0	1	0	1	1	1	1	0
10	63	0	0	1	0	1	0	1	1	1	1
11	60	1	1	1	1	1	1	0	1	0	0
12	56	1	1	0	0	1	0	1	1	1	1
13	53	0	0	1	1	1	1	1	1	1	0
14	51	0	1	1	1	1	1	1	1	1	1
15	47	1	1	1	1	1	1	1	1	1	1
16	45	0	0	1	1	1	1	1	1	1	0
17	42	1	1	1	1	1	1	1	0	1	1
18	38	1	1	1	1	1	1	1	0	1	1
19	34	1	1	1	1	1	0	1	1	1	1
20	31	1	1	1	1	1	1	1	1	1	1
21	27	1	1	1	1	1	1	1	1	1	1
22	24	1	1	1	1	1	1	1	1	1	1
23	20	1	1	1	1	1	1	1	1	1	1
24	17	1	1	1	1	1	1	1	1	1	1
25	14	1	1	1	1	1	1	1	1	1	1
26	12	1	1	0	0	1	1	1	1	1	0
Total	--	19	18	21	19	23	21	20	20	20	19

1= The presence of band, 0 = The absence of band and M.wt= Molecular weight

Bands of 96 and 53 kDa were present in samples of Giza132 after treatment with 8ppm. Also, band at molecular weight 69 kDa took the same trend when HP was applied at rates 8 and 16 ppm. In addition, band of molecular weight 56 kDa was absent from control, but accumulated at 16 and 24 ppm.

Data demonstrated that bands of molecular weights 90, 53, 51 and 45 kDa were absent in control of Giza 2000, but accumulated after application of PA at all doses. Also, bands of molecular weights 76 and 63 kDa were present when Giza 2000 was treated with PA at (40 & 60 ppm) and 20 ppm, respectively. In Giza 132, it is evident that PA (all doses) had a clear effect on accumulation of proteins with molecular weight 69, 47 and 34 kDa compared with the control. Also, bands of molecular masses 90 and 56 kDa were accumulated only at 40 and 60 ppm, while bands of molecular weights 96 kDa was accumulated after spraying with PA at rates of 20 and 40 ppm. In the same direction, band of 53 kDa was absent in control, but accumulated at rates of 20 and 60 ppm. Goss [46] found that amino acids work as buffer in plants and biosynthesis of other organic compounds such as proteins. Schnitzer [84] stated that amino acid is absorbed by the cells and is fed into the metabolic machinery of the cell.

The effect of silicon on protein patterns for barley plants could be followed from data presented in Tables 8 and 9. Bands of molecular weights 90, 76, 53 and 45 kDa were absent in control of Giza 2000, but accumulated after application of Si at all doses. Band of molecular weight 63 kDa was accumulated at 200 and 600 ppm, however bands of 51 kDa was accumulated at 200 and 400 ppm. In addition, bands of 90, 69 and 34 kDa were present in samples of Giza132 after treatment with all doses of Si compared with the control. Also, bands of molecular weights 53 and 47 kDa were found at (200 & 400 ppm) and (400 & 600 ppm), respectively. Also, band at molecular weight 56 kDa took the same trend when Si applied at 400 ppm compared with the control. In this concern, Sara and Haddad [68] showed that treatment with Si increased the tolerance of wheat by increasing soluble protein content.

Table 8: Continued

			Soluble proteins pattern								
			Hydrogen peroxide (ppm)			Phenylalanine (ppm)			Silicon (ppm)		
No.	M. wt	Control	8	16	24	20	40	60	200	400	600
1	96	0	0	0	0	0	0	0	0	0	0
2	93	1	0	1	0	0	0	0	0	0	0
3	90	0	1	1	1	1	1	1	1	1	1
4	86	1	1	0	1	1	0	0	1	1	1
5	81	1	1	1	1	1	1	1	1	1	1
6	76	0	1	1	1	0	1	1	1	1	1
7	72	1	1	1	1	1	1	1	0	1	0
8	69	1	0	0	0	0	1	1	1	0	1
9	67	1	1	1	0	0	0	1	0	1	0
10	63	0	1	0	1	1	0	0	1	0	1
11	60	1	1	1	0	1	1	1	1	1	1
12	56	1	1	1	1	0	0	0	1	0	1
13	53	0	1	1	1	1	1	1	1	1	1
14	51	0	0	0	1	1	1	1	1	1	0
15	47	1	1	1	0	0	1	1	0	1	1
16	45	0	1	1	0	1	1	1	1	1	1
17	42	1	1	1	1	1	1	0	1	0	0
18	38	1	1	1	1	1	1	1	1	1	1
19	34	1	1	1	1	1	1	1	1	1	1
20	31	1	1	1	1	1	1	1	1	1	1
21	27	1	1	1	1	1	1	1	1	1	1
22	24	1	1	1	1	1	1	1	1	1	1
23	20	1	1	1	1	1	1	1	1	1	1
24	17	1	1	1	1	1	1	1	1	1	1
25	14	1	1	1	1	1	1	1	1	1	1
26	12	1	0	0	1	0	0	0	1	0	1
Total	--	19	21	20	19	18	19	19	21	19	20

1= The presence of band, 0 = The absence of band and M.wt= Molecular weight

Yield: Data presented in Table 10 indicated that plant height and grain yield significantly increased after treatment with SA at rates of 200 and 400 ppm compared with the control. Moreover, Giza132 significantly exceeded Giza 2000 in plant height and grain yield. Considering the interaction effect, Giza 2000 and Giza 132 recorded the maximum values of plant height and grain yield when SA applied at rate of 200 and 400 ppm, respectively. These results are in harmony and agreement with those obtained by Khalilaqdam and Mir-Mahmoodi [85].The positive effect of SA on enhancing yield may be due to: i) The capability of SA to increase fresh and dry weights [86]. ii) Reduction of lipid peroxidation and H_2O_2 accumulation [87]. iii) SA improved antioxidant reactions that protect the plants from damaging [51], photosynthetic capacity [88], the activity of leaf hill reaction [89] and enhanced the nutrients contents [88]. iv) Plays a key role in suppressing pathogens and disease resistance [90] also, it causes increase in lignin of cell wall [91].

Data showed that Eth enhanced plant height at all doses and grain yield at 100 and 400 ppm, but straw yield at rate 200 ppm. There was no significant difference between the two cultivars. As to the effect of interaction, data exhibited that the highest values of plant height and straw yield were achieved by Giza 2000 at rate of 200 ppm. Also, Giza132 recorded the highest of grain yield when applied at rate 400 ppm. The effect of Eth in increasing barley yield is documented by Turk *et al.* [92]. The positive effect of Eth on enhancing yield may be due to: i) It increases the number of tillers [93]. ii) Eth accelerates the transition from vegetative to reproductive growth [94]. iii) Eth mode of action acts *via* liberation of ethylene which interferes in growth process [36]. Application of IAA had a positive effect on plant height and enhanced grain and straw yields at rate 200 and 100 ppm, respectively. Moreover Giza 132 significantly exceeded Giza 2000 in plant height while, Giza 2000 exceeded Giza 132 in grain yield. Concerning the interaction, the

Table 9: Soluble proteins pattern in leaves of Giza 132 as affected by foliar applications (SA, Eth, IAA, HP, PA, Si) under Maryout conditions.

			Soluble proteins pattern								
			Salicylic acid (ppm)			Ethephon (ppm)			Indole acetic acid (ppm)		
No.	M. wt	Control	100	200	400	100	200	400	100	150	200
1	96	0	1	0	0	1	1	1	1	1	1
2	93	1	1	1	1	0	0	0	0	1	0
3	90	0	1	1	0	1	0	1	1	0	1
4	86	1	0	1	1	1	1	0	0	1	1
5	81	1	1	1	0	0	1	1	1	0	1
6	76	0	1	1	1	1	0	1	1	1	0
7	72	1	0	1	1	1	1	1	0	1	1
8	69	1	1	0	0	1	0	1	1	1	1
9	67	1	1	1	1	0	1	1	1	1	1
10	63	0	1	0	0	1	1	0	1	0	1
11	60	1	0	1	1	0	0	1	1	1	0
12	56	1	1	1	1	1	1	0	0	1	1
13	53	0	1	1	1	1	0	1	1	0	1
14	51	0	1	1	1	1	1	1	1	1	0
15	47	1	0	1	1	1	1	0	0	0	0
16	45	0	1	1	1	0	1	1	1	1	1
17	42	1	1	1	1	1	1	1	1	0	1
18	38	1	1	1	1	1	1	0	1	1	1
19	34	1	1	1	1	1	1	1	0	1	1
20	31	1	0	1	1	1	1	0	1	1	1
21	27	1	1	1	1	1	1	1	1	1	0
22	24	1	1	0	0	1	1	1	1	1	1
23	20	1	1	1	1	1	1	1	1	1	1
24	17	1	1	1	1	1	1	0	1	1	1
25	14	1	0	1	1	1	1	1	1	1	1
26	12	1	0	1	1	0	1	1	1	1	0
Total	--	19	19	22	20	20	20	18	20	20	19

1= The presence of band, 0 = The absence of band and M.wt= Molecular weight

maximum values of plant height and grain yield were achieved by Giza 2000 when applied at rate 200 ppm and 100 ppm, respectively. The maximum value of straw yield was achieved by Giza 132 at rate 100 ppm. These results are in agreement with those obtained by Petti *et al.* [38]. The positive effect of IAA on enhancing yield and yield components may be due to: i) IAA play a major role on regulating plant growth [95]. ii) IAA enhances the antioxidant defense activities [51], photosynthetic activities in plants [96] also activates the translocation of carbohydrates during their synthesis [97]. iii) IAA induces sugar and mineral accumulation at the site of application and stimulates flower initiation [98].

Data showed that plant height and straw yield were increased after treatment with all doses of HP. The maximum value of grain yield was obtained when applied at rate 8 and 24 ppm. Comparison between the two cultivars, Giza 2000 exceeded Giza132 in grain yield. Regarding to the interaction effect, Giza 2000 gave the maximum values of plant height and grain yield at rate 16 and 24 ppm, respectively. While, Giza 132 gave the highest value of straw yield when treated with 24 ppm. These results are in agreement with that obtained by Abd El Monaim *et al.* [99]. The positive effect of HP on enhancing yield and yield components is may be due to: i) It increases the activity of antioxidant enzymes and proline content [64]. ii) The important role of HP in host-pathogen systems and causes inhibition of biotrophic pathogens and has a direct antimicrobial effect and is involved in cell walls cross-linking, induction of gene expression, phytoalexin production and induced systemic resistance [41]. iii) The role of HP in activation of any array of host defense mechanisms as well as reduced both disease incidence and disease severity [42]. iv) HP improves shoot and root lengths, which was associated with higher superoxide dismutase, growth parameters, protein contents, photosynthetic pigments and nutrients contents [99]. v) Treatment with HP at low level reduces peroxidation of membrane lipids in plants [100].

Table 9: Continued

			Soluble proteins pattern								
			Hydrogen peroxide (ppm)			Phenylalanine (ppm)			Silicon (ppm)		
No.	M. wt	Control	8	16	24	20	40	60	200	400	600
1	96	0	1	0	0	1	1	0	0	0	0
2	93	1	0	1	1	1	1	1	0	1	1
3	90	0	1	1	1	0	1	1	1	1	1
4	86	1	1	0	1	1	1	0	1	0	0
5	81	1	1	1	1	1	1	1	0	1	1
6	76	0	0	0	1	1	1	1	1	1	1
7	72	1	1	1	1	1	0	1	1	1	1
8	69	1	1	1	0	1	1	1	1	1	1
9	67	1	0	1	1	1	1	1	1	1	0
10	63	0	1	0	1	1	1	0	1	1	1
11	60	1	1	0	0	1	1	1	1	0	1
12	56	1	0	1	1	0	1	1	0	1	0
13	53	0	1	0	0	1	0	1	1	1	0
14	51	0	0	1	1	0	0	0	1	0	1
15	47	1	1	1	1	1	1	1	0	1	1
16	45	0	0	1	1	1	0	0	0	1	1
17	42	1	1	0	1	0	1	1	1	1	1
18	38	1	1	1	0	1	1	1	1	0	1
19	34	1	1	1	1	1	1	1	1	1	1
20	31	1	1	1	1	1	1	1	1	1	1
21	27	1	1	1	1	1	1	1	1	1	1
22	24	1	1	1	1	1	1	1	1	1	1
23	20	1	1	1	1	1	1	1	0	1	1
24	17	1	1	1	1	1	1	1	1	1	1
25	14	1	1	1	1	1	0	1	1	1	1
26	12	1	0	1	1	0	1	1	1	0	1
Total	--	19	19	19	21	21	21	21	19	20	21

1= The presence of band, 0 = The absence of band and M.wt= Molecular weight

Data demonstrated that PA enhanced plant height and grain yield at rate 20 ppm, but straw yield at 60 ppm. Giza 132 exceeded Giza 2000 in plant height and grain yield while Giza 2000 exceeded Giza 132 in straw yield. Concerning the interaction effect, the maximum values of plant height and grain yield were achieved by Giza 132 when treated with 20 ppm. Also, Giza 2000 recorded the maximum value of straw yield at rate 60 ppm. These results are in agreement with those obtained by Abd El Samad *et al*. [101]. The positive effect of PA on enhancing yield and yield components may be due to: i) It increases photosynthetic pigments and leaf area [101] and vegetative and flowering [102]. ii) Protection of plants against ammonia toxicity and pathogens, functioning as buffer and biosynthesis of other organic compounds such as protein, amines, purins, pyrimidines, vitamins, enzymes and terpenoids [46]. Data showed that the maximum values of plant height, grain and straw yields were obtained after treatment with Si at rate of 600 ppm. Moreover, Giza 132 exceeded Giza2000 in plant height. Concerning the interaction effect, the maximum values of plant height and grain yield were obtained by Giza 132 when applied at rate 600 ppm. Also, Giza 2000 recorded the maximum value of straw yield at 600 ppm. These results are in agreement with those obtained by Karmollachaab *et al.* [103]. The positive effect of Si on enhancing yield may be due to: i) Si increase the activities of some antioxidant enzymes and the contents of photosynthetic pigments and soluble proteins [55]. ii) Si increase resistance of plants to diseases [104].

Table 10: Plant height, grain yield and straw yield as affected by foliar applications (SA, Eth, IAA, HP, PA and Si), barley cultivars and their interaction under Maryout conditions.

	Plant height (Cm)			Grain yield (kg/fad)			Straw yield (kg/fad)		
Foliar applications	Giza2000	Giza132	Mean	Giza2000	Giza132	Mean	Giza2000	Giza132	Mean
				Salicylic acid (ppm)					
Control	38.05 e	44.00 ab	41.03B	1231 b	1318ab	1275AB	3842 a	4054 a	3948 A
100	34.50 f	41.15 cd	37.83C	662.4c	1350ab	1006 B	3772 a	5475 a	4624 A
200	45.60 a	43.00 bc	44.30A	1466ab	1263 b	1364 A	4853 a	3675 a	4264 A
400	42. 50 bc	39.87 de	41.18A	1243 b	1702 a	1473 A	3803 a	6789 a	5296 A
Mean	40.16 B	42.00 A	--	1151B	1409 A	--	4067A	4998A	--
				Ethephon (ppm)					
Control	38.05 d	44.00 ab	41.03B	1231 d	1318cd	1275 C	3842 b	4055b	3948 B
100	44.53 ab	42.25 c	43.39A	1920ab	1689abcd	1804AB	4479ab	4598ab	4539 B
200	45.55 a	43.00 bc	44.28A	1362bcd	1684 abc	1523BC	6569 a	5942ab	6269 A
400	44.25 ab	44.50 ab	44.38A	1807abc	2110 a	1959 A	5374ab	4510ab	4942AB
Mean	43..10 A	43.44 A	--	1580 A	1700 A	--	5073A	4776A	--
				Indole acetic acid (ppm)					
Control	38.05c	44.00 a	41.03C	1231bcd	1318 abc	1275 B	3842 c	4054bc	3948 B
100	41.25 b	44.10 a	42.67B	1598 a	973.6 b	1286 B	4271bc	6016 a	5144 A
150	44.25 a	44.05 a	44.15A	1582 a	1183 cd	1382AB	4917 b	3849 c	4383 B
200	44.80 a	44.00 a	44.40A	1566 a	1535 ab	1551 A	3978bc	4384bc	4181 B
Mean	42.09 B	44.04 A	--	1494 A	1253 B	--	4252A	4576A	--
				Hydrogen peroxide (ppm)					
Control	38.05 e	44.00 d	41.03B	1231 c	1318 bc	1275 B	3842 b	4054 b	3948 B
8	46.93 bc	48.30abc	47.62A	1638 b	1656 b	1647 A	5698 a	4954ab	5326 A
16	50.30 a	46.35 cd	48.33A	1534 bc	1218 c	1376 B	5617 a	4415ab	5016 A
24	49.75 ab	48.50abc	49.13A	1985 a	1546 bc	1766 A	4761ab	5785 a	5273 A
Mean	46.26 A	46.79 A	--	1597 A	1435 B	--	4979A	4802A	--
				Phenylalanine (ppm)					
Control	38.05 c	44.00 b	41.03B	1231 c	1318 bc	1275 B	3842 c	4054bc	3948BC
20	48.07 a	48.98 a	48.53A	1197 c	2084 a	1640 A	5234ab	3981 c	4608AB
40	33.25 d	34.25 d	33.75C	1444 bc	1380 bc	1412AB	3674 c	3708 c	3691 C
60	42.50 b	42.00 b	42.25B	1218 c	1704 ab	1461AB	5872 a	4598bc	5235 A
Mean	40.47 B	42.31 A	--	1273 B	1622 A	--	4656A	4085 B	--
				Silicon (ppm)					
Control	38.05 e	44.00 b	41.03C	1231 c	1318 c	1275 B	3842 c	4054 c	3948 C
200	40.50 d	42.00 cd	41.25C	1290 c	1446 bc	1368 B	5208 b	5099 b	5204 B
400	43.50 bc	43.50 bc	43.50B	1674 b	1120 c	1397 B	3157 d	3886 c	3521 C
600	46.50 a	47.75 a	47.13A	1396 bc	2120 a	1758 A	7320 a	5436 b	6378 A
Mean	42.14 B	44.31 A	--	1398 A	1501 A	--	4907A	4619A	--

Values followed by the same letter (s) are not significantly different at $P < 0.05$

CONCLUSION

The present study confirms on the importance of tolerance net blotch disease in barley and recommends using disease tolerant Giza132 under Maryout conditions as well as using some chemical inducers; hydrogen peroxide, phenylalanine, silicon to enhance barley plants to disease tolerance. Also, we can benefit from biochemical and pathological indicators to enhance sensitive genotypes for tolerance of net blotch disease.

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