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# Use of Some Chemical Inducers to Improve Wheat Resistance to *Puccinia striiformis* f. sp. *tritici*

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**Summary:** The effect of DL-β-aminobutyric acid (BABA), benzothiadiazole (BTH), indoleacetic acid (IAA) and salicylic acid (SA) on induced systemic resistance was investigated in moderately susceptible and susceptible wheat genotypes Tamuz-2 and AL-8/70 against *Puccinia striiformis* f. sp. *tritici*. Resistance was characterized by reduced infection of yellow rust disease (Yrd). Changes in peroxidase, phenylalanine ammonia-lyase activities and in total phenolic compound content demonstrated that the resistance to *Puccinia striiformis* can be induced by BABA, BTH, IAA and SA in these two wheat genotypes. Further studies are needed before a practical method using many analogue compounds, such as potassium phosphate and biotic agent for Yrd resistance in wheat is developed.
**Keywords:** chemical inducers, pathogenesis-related proteins, phenols, *Puccinia striiformis* f. sp. *tritici,* systemic resistance, *Triticum aestivum* L., wheat genotypes, yellow rust disease

## Introduction

Wheat (*Triticum aestivum* L.) is the most important world food crop as it contributes to more than 40% of people's food intake calories and 20% of protein (Austin 1989). Wheat crop is subjected to infection by many important plant pathogens. Among these pathogens, rust fungi are the most important disease causing pathogen which represents serious threat to wheat production. Yellow rust disease (Yrd) caused by *Puccinia striiformis* f. sp. *tritici* West (*Pst*) is the major disease in many wheat growing regions, especially with higher elevation and cooler climate (Stubbs 1985). Yrd in the northern part of Iraq is considered one of the most important wheat diseases in the country. High incidence of Yrd often occurred in Sulaimani province (Al-Maaroof 2003). The complicated aspects involved in chemical control of plant diseases, such as high environmental concerns, cost of fungicides and developed resistance to fungicides by some pathogens are the reason that alternative methods for plant disease control are being developed. The use of disease resistance inducers are among these methods which activate natural plant defence (Andrea et al. 2005). Induced resistance is environmentally friendly, confers to long-lasting protection against a broad spectrum of plant pathogens including viral diseases, bacteria, fungi, oomycetes and nematode (Durrant & Dong 2004). The induction of resistance is usually associated with metabolic and structural changes inside plants (Vladimir et al. 2012). Certain chemicals such as salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA), benzothiadiazole (BTH), and β-aminobutyric acid (BABA) are reported to induce resistance in cereal plants (Stadnik & Buchenauer 1997, Ruess et al. 1997). Pathogenesis related proteins, peroxidase, β-1,4 glucanase and chitinase are reported to be activated in resistant plants (Quiroga et al. 2000).

This study was conducted to determine the effect of BABA, SA, BTH, and indole acetic acid (IAA) on phenols accumulation, peroxidase (PO), and phenylalanine ammonia-lyase (PAL) activity in *Pst* infected wheat cultivars, namely moderately resistant Tamuz-2 and susceptible AL-8/70.

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## Materials and Methods

Field experiment was conducted at Bakrajo Experimental Research station, Sulaimania province, 400 km north of Baghdad, Iraq during the growing season 2012/2013. This region is the major rain fed wheat growing area of Iraq. The field was well prepared for cultivation during December 2012 after adequate rainfalls. Agricultural practices including cultivation time, seed rate, rate and time of fertilization, ploughing, etc. were applied as recommended for the wheat growing in the region. The experimental plots were 3x2 $m^2$. The treatments were arranged in a randomized complete block design (RCBD) with three replications for each treatment.

Two wheat genotypes with different reactions and responses to yellow rust were used: Tamuz 2 (moderately susceptible to susceptible) and AL-8/70 (susceptible). The sowing was performed at the end of December 2012 by 80 g of seed in rows with 3×3 $m^2$ for each genotype. The plots were separated one meter and blocks by two meters path to avoid any kind of interference between plots. The field was entirely surrounded with one meter border plot which was cultivated with mixture of yellow rust susceptible wheat cultivars as a trap and spreader of *P. striiformis* inoculum in the experimental field.

The recommended concentration was prepared for each of the inducer chemicals, BTH: 1 mM, BABA: SA 1000 µg $ml^{-1}$ and IAA: 100 µg $ml^{-1}$. All the test concentrations of the chemical inducers were applied on leaves using 1 l hand sprayer until complete wetness of plants. The plants were sprayed at boot stage. Plots that represented control were similarly sprayed with distilled water only. Artificial inoculation was conducted by spraying the bordered plants with urediniospores suspension at $4.6x10^4$spore/$ml^{-1}$ of *P. striiformis* f. sp. *tritici*. The inoculum was collected from different wheat genotypes at the beginning of March of the current season. This was done to ensure a uniform spread of the inoculum and sufficient disease development in the field.

Activities of PO, PAL and phenol content were estimated at 1, 3, 15 days after spraying inducers. The extraction procedure was based on the method described by Biles and Martyn (1993) to determine the activity of PO as follows: 1 g of leaf tissue was collected, grounded in 2 ml sodium phosphate buffer (pH 6.5, 0.1M) using mortar and pestle. Samples were transferred to Eppendorf tubes, and then centrifuged for 20 min at 12000 rpm at $4^0$C. Supernatant was used in PO assay which represent the crude enzyme. Three replicates were prepared from each treatment. Peroxidase activity was directly determined from the crude enzyme extract according to the procedure proposed by Hammerschmidt et al. (1982). The reaction mixture consisted of 2.9 ml of 100 Mm sodium phosphate buffer (pH 6.0) containing 0.25 % (v/v) guaiacol (2-methoxy-phenol) and 100 Mm $H_2O_2$. The reaction was started by adding 100 µl of the crude enzyme extract. Changes in absorbance at 470 nm using a spectrophotometer were recorded every 30 sec for 3 min. The enzyme activity was expressed as changes in absorbance $min^{-1}$ $gfw^{-1}$.

The determination of PAL was followed as described by Dickerson et al. (1984). Fresh plant material (1 g) was homogenized in 5ml of ice cold 0.1 M sodium borate buffer (pH 7.0) containing 0.1 g of polyvinylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 20000 rpm for 30 min. The supernatant was used to determined PAL activity as the rate of conversion of L- phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer (pH 8.8) and 0.5 ml of 12 Mm L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid was calculated using its extinction coefficient of 9630 M $cm^{-1}$ $gfw^{-1}$.

The method described by Ziesiin and Ben-Zaken (1993) was followed for phenol estimation. One gram of fresh wheat leaves samples were homogenized in 10 ml of 80% methanol and agitated for 15 min at $70^0$C. One millilitre of the methanolic extract was added to 5 ml of sterilize distilled water and 250 µL of Folin–Ciocalteu reagent and the solution was kept at $25^0$C. The absorbance of the developed blue colour was measured using spectrophotometer at 725 nm. Catechol was used as a standard. The amount of phenol was expressed as phenol equivalents in µg $gfw^{-1}$.

To assess the effectiveness of different test inducer treatments in suppressing the disease, infection type and disease severity on the two wheat cultivars were recorded fifteen days after treatments. Infection types were assessed using a scale described by Lewellen et al. (1967) as follows: 0 = no visible infection; R = resistant, necrotic area with or without small pustules; MR = moderately resistant, small pustule surrounded by necrotic area; M = intermediate resistance, pustules of variable size, some necrosis or chlorosis; MS = moderately susceptible, medium sized pustules, no necrosis but some chlorosis and S=susceptible, large pustules, no necrosis or chlorosis. At the same time, disease

severity was estimated by using the modified Cobb scale (Peterson et al. 1948). Data were randomly collected from 15 plants in the second, fourth and sixth row in each plot to avoid any interference and boarder effect. The coefficient of infection (CI) of yellow rust on each cultivar was calculated by the equation:

$$CI = DSxIT$$

Where CI = coefficient of infection DS = disease severity IT = infection type which resemble constant values given to the host response; where 0=0.0, R=0.2, MR=0.4, M=0.6, MS=0.8 and S=1.0 (Roelfs et al. 1992).

**Results**

Coefficient of infection (CI) with Yrd of two genotypes, Tamuz-2 and AL-8/70 was affected by treatments with the applied inducers (Tab. 1). In cultivar Tamuz-2 the disease was significantly (P=0.05) suppressed with CI of 0.81, 0.87, 1.17, and 1.33 for BABA, IAA, BTH, and SA treatment respectively in comparison with 9.44 on Control plants. In cultivar AL-8/70 CI was significantly reduced by IAA, BTH, BABA, and SA treatment respectively in comparison with CI from control plants.

*Enzyme activity*

BTH, BABA, SA, and IAA showed significant differences in their abilities to induce activities in both wheat cultivars (Tab. 2). Treatments of cv Tamuz-2 by IAA, BTH, and SA caused significant increase in PO activity with an average of 81.63, 75.83, and 58.61 respectively compared with 22.09 PO activity in control plants. However, BABA caused the lowest effect with 47.17 PO activities, but still significantly different compared to control treatment. Similarly, in cultivar AL-8/70 all the inducers caused significant effect on PO activity with 70.58, 58.8, and 44.36 for IAA, BTH, and SA respectively and BABA produced the lowest effect compared with the other test inducers with 37.87 compared with 9.24 on control plants. The moderately resistant cultivar Tamuz-2 was significantly superior in response to inducers enhancement for PO activity, recorded average 57.06 compared with average 44.20 in cultivar AL-8/70. Significantly the highest PO activity was recorded 3 days after spraying inducers with 37.28 in cultivar Tamuz-2 and 54.23 in AL-8/70. In Tamuz-2 the significantly highest PO activity was 105.13 caused by IAA 3 days after spraying, while BABA caused the lowest PO activity (52.62). Similarly, the susceptible cultivar AL-8/70 has the highest PO activity (79.44) caused by IAA 3 days after spraying, while BABA produced the lowest PO activity (41.34) after the same period. Generally, PO activity was low in both cultivars after 1 and 15 days and IAA showed significantly the highest gradual excessive PO activity, while BABA caused the lowest one.

The test inducers IAA, BTH, SA and BABA caused significant differences (P=0.05) in PAL activity in both wheat cultivars (Tab. 3). In Tamuz-2, BABA, IAA, BTH and SA caused PAL activity of 94.55, 88.07, 78.93 and 72.16, respectively, compared with 26.23 on control plants. On the other hand, in cultivar AL-8/70 the same inducers caused significant (P=0.05) PAL activity of 52.37, 46.53, 34.22, 28.46 for BABA, IAA, BTH, and SA respectively compared with 11.02 for control plants. BABA caused significantly (P=0.05) the highest PAL activity in the two cultivars, while SA caused the lowest effect. The moderately resistant cultivar Tamuz-2 surpassed cultivar AL-8/70 in response to inducers in recording PAL activity, 71.99 and 55.12 respectively. The highest level of PAL activity was 3 days after treatments (98.56 and

Table 1. Effect of different chemical inducers on coefficient of infection of yellow rust disease in two wheat cultivars after 15 days of spraying

| Cultivar | Coefficient of infection | | | | |
|---|---|---|---|---|---|
| | Treatment | | | | |
| | Control | BABA | BTH | IAA | SA |
| Tamuz-2 | 9.44 | 0.81 | 1.17 | 0.87 | 1.33 |
| AL-8/70 | 9.05 | 2.11 | 1.3 | 1.16 | 2.49 |

LSD (P=0.05) cultivars treatment inducer (inter.) =2.192

Table 2. Changes in peroxidase activity in wheat cultivars at different concentrations of chemical inducers after different period of application

| Cultivar | Treatment | Change in absorption ($min^{-1}gf\,w^{-1}$) Days | | | Mean |
|---|---|---|---|---|---|
| | | 1 | 3 | 15 | |
| Tamuz-2 | BABA | 50.69 | 52.62 | 40.21 | 47.17 |
| | BTH | 67.58 | 98.45 | 61.47 | 75.83 |
| | IAA | 85.61 | 105.13 | 54.17 | 81.63 |
| | SA | 51.73 | 80.32 | 43.84 | 58.61 |
| | Control | 18.69 | 29.9 | 17.70 | 22.09 |
| | Mean | 54.86 | 73.28 | 43.47 | 57.06 |
| AL-8/70 | BABA | 39.96 | 41.34 | 32.31 | 37.87 |
| | BTH | 46.29 | 77.93 | 52.19 | 58.8 |
| | IAA | 65.02 | 79.42 | 67.30 | 70.58 |
| | SA | 39.85 | 60.62 | 32.99 | 44.36 |
| | Control | 8.85 | 12.22 | 7.21 | 9.42 |
| | Mean | 40.0 | 54.23 | 38.4 | 44.2 |

LSD (P=0.05) for cvs=0.218
LSD (P=0.05) for cvs day (inter.) =16.691
LSD (P=0.05) for cvs indu. (inter.) =11.8
LSD (P=0.05) for cvs indu. days (inter.) =0.846

Table 3. Changes in phenylalanine ammonia-lyase activity in wheat cultivars at different concentrations of chemical inducers after different time of application

| Cultivar | Treatment | Cinnamic acid (n M $min^{-1}gfw^{-1}$) Days | | | Mean |
|---|---|---|---|---|---|
| | | 1 | 3 | 15 | |
| Tamuz-2 | BABA | 89.19 | 126.56 | 67.9 | 94.55 |
| | BTH | 78.27 | 108.25 | 50.28 | 78.93 |
| | IAA | 78.53 | 124.97 | 60.72 | 88.07 |
| | SA | 69.47 | 101.91 | 45.1 | 72.16 |
| | Control | 26.36 | 31.14 | 21.19 | 26.23 |
| | Mean | 68.36 | 98.56 | 50.83 | 71.99 |
| AL-8/70 | BABA | 78.92 | 113.11 | 52.37 | 81.46 |
| | BTH | 60.1 | 86.04 | 34.22 | 60.12 |
| | IAA | 69.29 | 90.64 | 46.53 | 68.82 |
| | SA | 48.53 | 66.97 | 28.46 | 47.98 |
| | Control | 17.12 | 23.56 | 11.02 | 17.23 |
| | Mean | 54.79 | 76.06 | 34.52 | 55.12 |

LSD (P=0.05) for cvs=0.224
LSD (P=0.05) for cvs Indu.(inter.)=20.43
LSD (P=0.05) for cvs day (inter.) =18.46, LSD (P=0.05) for cvs indu. days (inter.)=0.867

Table 4. Phenol accumulation in wheat cultivars at different concentrations of chemical inducers after different time of inducers application

| Cultivar | Treatment | Phenol (µg gfw$^{-1}$) Days | | | |
|---|---|---|---|---|---|
| | | 1 | 3 | 15 | Mean |
| Tamuz-2 | BABA | 332 | 451 | 250.66 | 344.55 |
| | BTH | 270 | 415 | 205 | 296.66 |
| | IAA | 310 | 443.66 | 235 | 329.55 |
| | SA | 150 | 300 | 142 | 197.33 |
| | Control | 100.33 | 120.33 | 66 | 95.55 |
| | Mean | 232.46 | 346 | 179.73 | 252.73 |
| AL-8/70 | BABA | 255.33 | 390.33 | 180.33 | 275.33 |
| | BTH | 215.33 | 310.66 | 134.33 | 220.1 |
| | IAA | 240.33 | 360 | 155.33 | 251.88 |
| | SA | 185.66 | 285 | 98.66 | 189.77 |
| | Control | 65 | 90.33 | 40.66 | 65.33 |
| | Mean | 192.33 | 287.26 | 121.86 | 200.48 |

LSD (P=0.05) for cvs=0.343
LSD (P=0.05) for cvs Indu.(inter.)=73.86
LSD (P=0.05) for cvs day (inter.) =67.11LSD (P=0.05) for cvs indu. days (inter.)=1.328

76.06 for Tamuz-2 and AL-8/70 respectively compared with other periods). In both cultivars, BABA caused significantly (P=0.05) the highest PAL activity with 126.56 in cultivar Tamuz-2 and 113.11 in AL-8/70, while SA recorded the lowest effect with 101.91 and 66.97 in Tamuz-2 and AL-8/70, respectively. In both cultivars, the highest gradual increase in PAL activity was caused by BABA, while the lowest one was caused by SA.

*Phenolic content*

BABA, IAA, BTH, and SA treatments caused significantly (P=0.05) higher level of phenol accumulation in cultivar Tamuz-2 with 344.55, 329.66, 296.6 and 197.3 µgfw$^{-1}$ respectively compared with 95.55 µgfw$^{-1}$ for control plants. Similarly, in cultivar AL-8/70 phenol accumulation recorded high level in BABA treated plants (275.33 µgfw$^{-1}$) followed by IAA, BTH, and SA with 251.88, 220.1, 189.77 µgfw$^{-1}$ respectively compared with 65.33 µgfw$^{-1}$ in control plants (Tab. 4). The moderately susceptible cultivar Tamuz-2 was significantly (P=0.05) superior in response to application of inducers in enhancing phenol accumulation with an average of 252.73 µgfw$^{-1}$ compared with 200.48 µgfw$^{-1}$ in the susceptible cultivar AL-8/70. High level of phenol accumulation, 346 µgfw$^{-1}$ was recorded three days after application, which is significantly (P=0.05) higher than the other test periods. Similarly in cultivar AL8/70 the highest phenol accumulation (287.26 µg fw$^{-1}$) was observed 3 days after application. Furthermore, 3 days after application, BABA caused the highest phenol accumulation (461, 0 and 390.33 µgfw$^{-1}$) in Tamuz-2 and AL-8/70 respectively, while SA recorded the lowest level (300 and 285 µgfw$^{-1}$) in Tamuz-2 and AL-8/70 after the same period. Significantly the highest gradual increase in phenol content was caused by BABA while the lowest was recorded by SA 3 days after application.

**Discussion**

The results showed that BABA, IAA, BTH and SA induced resistance against *P. striiformis* f. sp. *tritici* in moderately susceptible Tamuz-2 and susceptible AL-8/70 wheat genotypes by enhanced specific enzymatic activity and phenol content. The protection effect of BABA, BTH, IAA, and SA has previously been reported in number of wheat pathogen interaction systems (Gorlach et al. 1996, Stadnik et al. 2000, Thabit 2008). However, little is known about these inducers in wheat against *P. striiformis* f. sp. *tritici*. The observation of disease reduction was conducted after treatment with 1000 μg $ml^{-1}$ of BABA and SA and with 100 μg $ml^{-1}$ and 1 mM of IAA and BTH inducers respectively. The reduction in disease cannot be attributed to the toxic effect of the inducers on the fungus because no conidial or spore germination inhibition has been occurred after inducers treatment (Jakab et al. 2001). Furthermore the protection effect is caused by triggering defence response, as reported for other plant-pathogen interaction (Lu et al. 2006, Cohen 2001). Our results are in agreement with Thabit (2008) who found that BABA, BTH, IAA, and SA reduced wheat leaf rust infection through the enhanced of pathogenesis related protein, including peroxidase and phenolic compounds. Also, our results are in accordance with Ata et al. (2008) who found a significant reduction in sugar beet rust infection associated with accumulation of high level of peroxidase, PAL, free phenolic compounds and lignin content after treatment with SA and BTH. Barilli et al. (2010) reported that BTH and BABA induced systemic acquired resistance in pea genotypes against rust through the enhancement of enzymatic activity and phenol accumulation. Peroxidases are enzymes involved in the cell wall strengthening through protein and other polymer oxidative cross-linking and have been associated to resistance in several plant-pathogen interactions (Wang et al. 2000). Peroxidase causes the oxidation of wide variety of substrates, using $H_2O_2$, such as phenol, which plays a considerable role in lignin synthesis (Goldberg et al. 1987). In this study BABA caused the lowest induction effect in enhancement of peroxidase activity, and this result was in agreement with Amzalec and Chen (2007) who found that BABA have weak effect in enhancing PO activity against sunflower rust, and also with Barilli et al. (2010) who found that BABA treatment weakly elicits total PO in pea genotypes against *Uromyces pisi* - the causal agent of pea rust. Maximum PO levels were reached three days after the application of inducers and these results are similar to results of Andres et al. (2008) who found that maximum PO levels is reached four days after inoculation of wheat resistant line with the cereal cyst nematode *Heterodera avenae*.

Phenylalanine ammonia-lyase (PAL) catalyses the first reaction of the phenylpropanoid pathway through which different groups of phenolics (e.g. coumarins, flavonoids, and lignin) derives are formed (Southerton et al. 1990). PAL activity in both genotypes, Tamuz and AL-8/70 following treatment of BABA, BTH, IAA, and SA associated with higher accumulation of phenol (Tab. 3 and 4). These results support those of Lin et al. (2008) and Slauquter et al. (2008), in which higher level of phenol compounds was accumulated preceding induction of PAL activity in cucumber-powdery mildew and grapevine mildew interactions.

Phenolics have been shown to play an important role in disease resistance both at the early and late stages of the infection process and they may cause a direct toxic effect on the pathogen and contribute to the cell wall strengthening through cross-linking and lignin formation (Barilli et al. 2010). The increase of total phenol content in both wheat genotypes, Tamuz and AL-8/70 was followed by BABA, BTH, IAA, and SA treatments. Phenolic compounds can play an important role in disease resistance, limiting fungal germ tube development and contributing to cell wall strengthening of lignin, thus preventing plant tissue colonization (Prats et al. 2002). The results of this study were in agreement with previous researches, whereas Thabit (2008) reported the highest level of phenol accumulation at susceptible wheat plant three days after BABA treatment against leaf rust. Sahibani et al. (2011) reported the high accumulation of phenolic compound in tomato plant after BABA treatment and inoculation of root nematode.

## Conclusions

Results of this study indicate the possibility of adopting a practical and friendly environment method for the control of wheat yellow rust through the enhancement of pathogenesis related protein activity and phenol accumulation by inducers application.

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# Upotreba nekih hemijskih jedinjenja u cilju poboljšanja otpornosti pšenice na *Puccinia striiformis f. sp. tritici*

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**Sažetak:** Uticaj DL-β-aminobuterne kiseline (BABA), benzotiadiazola (BTH), indol-sirćetne kiseline (IAA) i salicilne kiseline (SA) na indukovanu sistemičnu otpornost na *Puccinia striiformis* f. sp. *tritici* ispitivan je kod umereno osetljivih i osetljivih pšeničnih genotipova Tamuz-2 i AL-8/70. Otpornost se ispoljila kroz smanjenu infekciju žutom rđom. Promene aktivnosti peroksidaze, fenilalaninske amonijak-lijaze i ukupnog fenolnog sastava pokazale su da otpornost na *Puccinia striiformis* može biti indukovana od strane BABA, BTH, IAA i SA kod ova dva genotipa pšenice. Dalja istraživanja su neophodna pre nego što se ustanovi praktični metod koji bi koristio mnoga analogna jedinjenja, kao što su kalijum fosfat i biotički agens, za otpornost pšenice na bolest žute rđe.

**Ključne reči:** bolest žute rđe, fenoli, genotipovi pšenice, hemijska jedinjenja koja izazivaju otpornost, proteini povezani sa patogenezom, *Puccinia striiformis* f. sp. *tritici,* sistemična otpornost, *Triticum aestivum* L.