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Induced Resistance to Wheat Yellow Rust by Chemical Inducers

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Abstract
Four chemical inducers, DL-*β*-aminobutyric acid (BABA) and salicylic acid (SA) at 0, 250, 500 and 1000 µgml^{-1}, Benzothiadiazole (BTH) at 0, 0.3, 0.6 and 1mM and Indole acetic acid (IAA) at 0, 25, 50 and 100 µg ml^{-1} were applied as foliage spray on two genotyps of wheat,Tamuz-2 and AL-8/70 to assess their ability to induce resistance against stripe (Yellow) rust disease (Srd) caused by *Puccinia striiformis* west. f.sp. *tritici*. Field trail was conducted during 2012/13 growing season in Bakrago experimental research station, Sulumani Province under natural infection conditions. All the test chemicals significantly (p=0.05) reduced disease severity (DS), coefficient of infection (C.I) and rate of infection (r). BABA and SA at 1000 µgml^{-1}, IAA at 100 µgml^{-1} and BTH at 1 mM were the most effective treatments for induction of rust resistance in cv. Tamuz-2. Similar reduction in the above mentioned disease parameters were observed in cv. AL-8/70 when plants were treated with the high test concentrations of IAA, BTH, BABA and SA. However, the two cultivars reacted differently toward the chemical inducers in terms of rust disease development. Further research work is needed before effective and practical management of wheat stripe rust disease can be achieved by chemical inducers.
Keywords: Induced resistance, *Puccinia striiformis* f.sp. *tritici*, Chemical inducer, Wheat, Disease severity.

1. Introduction

Wheat crop is affected by three important types of rust diseases; stem (black) rust, leaf (brown) rust and stripe (yellow) rust. Because of their ability to move for long distance and form new virulent races, these pathogens are capable of causing serious losses in wheat (Zhensheng, 2010). *Puccinia striiformis* west. f.sp. *tritici*, the causal agent of stripe rust is one of the most important diseases of wheat worldwide inflecting high economic yield looses in most of the wheat growing areas of the worldwide (Stubbs, 1985 and Hovmollers *et al.*, 2002). In Iraq, yellow rust caused 25-50 % loss in commercial wheat production cultivars in the northern part and up to 33 % in the middle region of the country under natural field conditions (Al-Maarof, 1997). Growing resistant cultivars is considered as the most effective, low cost, and environmentally safe approach to control strip rust (Chen, 2005). Because, yellow rust is able within a relatively short time to form new races capable of overcoming plant resistance of the newly produced commercial cultivars, it is very essential to continuously search and strength a strategic disease control measures like induced disease resistance in commercial wheat cultivars. A type of induced resistance known as systemic acquired resistance (SAR) was applied and by which the plant can be induced to develop and utilize its own defense mechanisms without altering plant genome (Van Loon, 1997). SAR was induced in response to pathogens by synthetic chemical compounds causing localized necrotic lesions as a result of hypersensitive reaction associated with accumulation of pathogenesis related proteins and the activation of many key enzymes (Andrea and Ray, 2005). Many known chemicals and biological elicitors are commercially available and are used to induce resistance against many plant pathogens (Gary and Robert, 2004). Different inducers such as Benzothiadiazole(BTH), salicylic acid (SA) and *β* -amino butyric acid (BABA) were used to induce SAR in plants against fungi , viruses , bacteria and nematodes (Andrea and Ray, 2005). The application of induced resistance in wheat against pathogens is well known in the literature (Gorlach, *et al.*, 1996; Stadnik, *et al.*, 1999; Thabet, 2003). Recent biochemical studies have shown increased activity of a number of key enzymes in wheat leaves during the expression of resistance against rust fungi (Southerton and Deverall, 1996). However, induced resistance in wheat against stripe rust pathogen by chemical inducers has not been reported before.

Therefore, the present study was conducted to evaluate the efficiency of four chemical inducers, BABA, BTH, SA and IAA to induce resistance wheat against Srd under natural infection conditions in Iraq.

2. Materials and methods

2.1. Field Experiment

Field experiment was conducted at Bakrajo Experimental Research station, Sulaimania province, 400Km north of Baghdad, Iraq during the growing season 2012/2013. This region is the major rain fed wheat growing area of Iraq.

2.2. Experimental Units and Design

The field was well prepared for cultivation during Dec. 2012 after enough rainfalls during this period. Agricultural practices including cultivation time, seed rate, rate and time of fertilization, rowing etc. were applied as recommended for wheat growing in the region. The experimental units were 3×2 m^2 plots. The treatments were arranged as a randomized complete block design (RCBD) with three replications for each treatment.

2.3. Wheat Cultivars

In this experiment two wheat genotypes with different reaction and response to stripe rust; cv Tamuz 2 (moderately susceptible) and cv Al8/70 (susceptible) were used.

2.4. Sowing

Seeds of the two genotypes (80 g) were sown in 7 rows within the experimental plots in Dec.2013 using RCBD with three replications and one meter interval between plots and two meter between blocks. The field was entirely surrounded with one meter border plot which was cultivated with mixture of yellow rust susceptible wheat cultivars to surf as a trap and spreader of *P. striiformis* inoculums in the experimental field.

2.5. Preparation and Application of Chemical Inducer

Three concentrations were prepared for each of the inducer chemicals, BTH: 0.3 and 0.6 mM, BABA: 1 mM, SA: 250, 500, and 1000 µg ml^{-1} and IAA: 25, 50 and 100 µg ml^{-1}.

2.6. Application of Chemical Inducers

All the test concentrations of the test chemical inducers were applied on leaves of wheat by spraying plant using 1L hand sprayer until complete wetness of plants. The plants were sprayed when they were at their booting stage. Control plants were similarly spread with distilled water only. The plants were sprayed once more after fifteen days. All plants in the experiment were left for natural infection by *P. striiformis* f.sp. *tritici*

2.7. Disease Assessment

To determine the effectiveness of the different test inducer treatments in suppressing the disease, infection type , diseases severity and rates of infection (r) , the development of disease on the two wheat cultivars at two intervals of the disease development were recorded after fifteen and thirty days after the first inducers spraying. Infection types were assessed using a scale described by Lewellen, *et al.*, (1967): 0 = no visible infection; R = resistant, necrotic area with or without small pustules; MR = moderately resistant, small pustule surrounded by necrotic area; M = intermediate, pustules of variable size, some necrosis or chlorosis; MS = moderately susceptible, medium sized pustules, no necrosis but some chlorosis. At the same time, disease severity was estimated by using the modified cobb scales (Peterson *et al.*, 1948) which depends on comparing the infected wheat leaves with a theoretical diagram showing the frequency of uredia for particular percentage disease severity. Data were randomly collected from 15 plants in line No.2, 4 and 6 in each plot. The coefficient of infection (C.I) of yellow rust on each cultivar was calculated by the equation:

CI = DS× IT

Where CI = coefficient of infection

DS = disease severity

IT = infection type which resemble constant values given to the host response; where immune 1= 0.0, R= 0.2, MR= 0.4, M= 0.6, MS= 0.8 and S= 1.0 (Roelfs *et al.*, 1992) .

The infection rates (r) of yellow rust disease on each of the genotypes were calculated at the first and second period of disease development by using Vander plank equation (Vander plank, 1963) as indicated in the formula:

$r = 2\text{-}3 / (t_2\text{-}t_1) \text{ Log } \frac{x2\,(1-x1)}{x1\,(1-x2)}$ per unit per day

t_1 and t_2 = the time of first and second reading of disease severity

x1 and x_2 = disease severity of first and second reading for each time

3. Results

3.1. *Effect of BTH, BABA, SA and IAA on DS and CI in Tamuz 2 and AL8/70 cultivars*

3.1.1. *Direct Effect*

Both wheat cultivars, Tamuz 2 and AL8/70 showed significantly (p=0.05) similar responds to treatments with BTH, BABA, SA and IAA (Table 1). However, in cv Tamuz 2 all treatments reduced both DS and CI to 5.12, 4.74; 5.83, 5.18; 6.70, 5.34 and 6.74, 5.83 and in cv AL8/70 to 5.94, 5.00; 7.86, 7.30; 8.65, 7.67 and 9.19, 8.59 for SA, ,IAA, BABA and BTH treatments respectively compared with their respective control treatments (Table 1).

3.1.2. Cultivars Response

Tamuz 2 cultivar response significantly (P=0.05) surpassed cv AL/70 in its response to the inducers, BTH, BABA, SA and IAA by decreasing the value of both DS and C.I to 6.10 and 5.27 respectively compared with 7.91 and 7.14 in cv AL/70 (Table1).

3.1.3. Effect of Inducers Concentrations

All the test concentrations of BABA, BTH, SA and IAA significantly (P=0.05) reduced both DS and CI compared with control plants for the two cultivars (Table 1). In cv Tamuz-2, the highest average reduction in DS and CI, 1.83 and 1.05 respectively, was observed at highest concentration of the test inducers compared with 12.18 and 11.51 respectively in control plants. Similarly, in cv AL8/70 the highest concentration of inducers produced significantly (P=0.05) the highest reduction in DS and CI, 2.66 and 1.76 respectively compared with 18.17 and 17.86 in control plants 15 days after the first inducers spraying (Table 1). In cv Tamuz 2 the highest

reduction in DS and CI, 1.70 and 0.81 was recorded when the highest concentration of BABA (1000 µg ml^{-1}), IAA (100µg ml^{-1}), 1.72 and 0.87, BTH (1Mm), 2.01 and 1.17, SA (1000µg ml^{-1}) respectively. In cv AL8/70 the significantly (P=0.05) highest reduction in DS and CI was respectively, caused by IAA, 1.84 and 1.16, BTH, 2.10 and 1.30, BABA, 3.31and 2.11 and SA, 3.40 and 2.49.

3.2. Effect of BTH, BABA, SA and IAA on DS and CI in Tamuz 2 and AL8/70 cultivars

3.2.1. Direct Effect

BTH, BABA, SA and IAA treatments showed no significant (P=0.05) differences in their reducing effects on DS and CI in both wheat cultivars (Table2). Treatment of cv Tamuz 2 with BABA, BTH, IAA and SA caused an average DS and CI of 12.50 and 10.27, 14.64 and 10.94, 17.96 and 15.64, 18.34 and 16.21 respectively. Similarly, cv AL 8/70 also responded to these chemical inducers with no significant differences in their effects on DS and CI which scored, 14.11and 12.96, 14.77 and 12.42, 15.85 and, 14.12, 28.42 and 25.97 for BTH, BABA, IAA and SA respectively.

3.2.2. Cultivars Response

Tamuz 2 cultivar was significantly (P=0.05) superior in response to inducers treatments, recorded average DS of 15.82 and CI of 13.22 compared with average DS, 18.29 and CI, 16.30 in cv AL 8/70 (Table 2).

3.2.3. Inducers Concentrations

Similarly, inducer's concentrations significantly (P=0.05) affected both DS and CI treated Tamuz 2 and AL 8/70 compared with control plants (Table2). In cv Tamuz 2, the lowest average DS and CI were 2.98, 1.89 respectively, when plants were treated with the test highest concentrations of inducers compared with DS, 39.69 and CI, 35.14 in control plants. Similarly, in cv Al 8/70 the highest concentrations of inducers produced significantly (P=0.05) the highest reduction in both DS and CI, 3.74 and 2.41 respectively compared with 46.32 and 43.90 in untreated control plants. In cv Tamuz 2 the reduction in DS and CI were 2.08 and 1.17, 2.30 and 2.18, 2.71 and 1.47, 4.28 and 3.75 at the highest test concentrations of BABA, IAA, BTH and SA respectively. However, in cv AL 8/70 all the test high concentrations of inducers caused significantly (P=0.05) less reduction both of DS and CI, 2.35 and 1.26, 2.66 and 1.67, 4.14 and 2.93, 5.53 and 3.78 in IAA, BTH, BABA and SA teratments respectively.

3.3 Effect of BTH, BABA, SA and IAA on development of infection(r) in cv Tamuz 2 and cv AL 8/70 thirty day after spraying

3.3.1. Direct Effect

BTH, BABA, SA and IAA significantly (P=0.05) affected development strip rust infection(r) in the test treated wheat cultivars (Table 3). When cv Tamuz 2 was treated with BABA, infection rate was low, 0.0364 compared with 0.0438, 0.0672 and 0.0798 in BTH, IAA and SA treated plants respectively. The highest reduction in r, however, 0.0455 was recorded in cv AL 8/70 treated with BTH compared with, 0.0455, 0.566 and 0.082 in IAA, BABA and SA treated plants respectively.

3.3.2. Cultivars Response

Both cultivars, Tamuz 2 and AL 8/70 responded similarly with no significant (P=0.05) differences toward the test inducers. The average r of the disease was 0.0568 and 0.0538 in cv Tamuz 2 and cv AL 8/70 respectively (Table 3).

3.2.3. Inducers Concentrations

Most of the test concentrations of the test chemical inducers produced significant (P=0.05) decrease in r in both wheat cultivars (Table 3). 2 The lowest average r was 0.0220 when the highest concentration of inducers were used compared with r, 0.1077 in control plants. Similarly, in cv AL 8/70 the highest concentration of inducers caused the highest reduction in r, 0.0218 compared with r, 0.0931 in control plants. In cv Tamuz 2 the lowest reduction in r, 0.0156 was recorded at the highest concentration of BABA (1000 ^{1}µg ml) followed by 0.0213, 0.0224 and 0.0289 at the highest concentrations, 100µg ml^{-1}, and 1Mm, 1000 µg ml^{-1} for IAA, BTH and SA respectively. However, In cv AL 8/70, the significantly highest reduction in r, 0.0168, 0.0174, 0.0202 and 0.0329 was recorded at the test highest concentration of BTH , IAA, BABA and SA treatments respectively compared with the other test concentrations of inducers and control plants.

4. Discussion

Four chemical compounds (DL-β-amino butyric acid (BABA), Salicylic acid (SA) , Benzothiadiazole(BTH) and indole acetic acid (IAA) were used to induce systemic resistance in wheat plant against stripe (yellow) rust disease caused by *Puccinia striiformis* west. f.s.p *tritici*. These inducers markedly reduced wheat Srd on Tamuz 2 and AL 8/70 wheat cultivars. Chemicals inducers such as SA, BTH and BABA were reported to induce resistance in cereals against wheat disease and pathogens such as stem rust (EL-Najar,1998), common bunt (Lu etal., 2006), leaf rust (EL-Deeb, 1999), *Fusarium graminearum* (Mohammodi etal., 2002), powdry mildew caused by *Blumaria graminis* f.sp *tritici* (Stadnik and Buchnauer,2000) and root knot nematode (Oka and Cohen, 2001), and against barely diseases such as net blotch and powdery mildew (Aly et al., 1989). Systemic acquired

resistance (SAR) was attributed to a signal transduction at sight of application to non-treated sites (Kessmann et al., 1994). Tamuz 2 cultivar showed more ability to induce its own defense mechanism by clearly decreasing disease severity and coefficient of infection caused by *P.striiformis* f.s.p *tritici* (Table 1&2). Thabit (2003) reported on the high effectiveness of BTH, IAA, SA and BABA in inducing resistanse against leaf rust disease of wheat caused by *Puccinia triticina*, and that cv Sids-1 was more able to be induced than cv Giza-168 gainst the causal fungus. Various studies have reported on the active role of chemical inducers such as BTH and SA in reducing the impact of sugar beet rust (Ata et al., 2008), BABA on sunflower rust (Amzalak and Cohen, 2007). Treatment of wheat with acetyl SA inhibited the development of uridino spores of *Puccinia* spp (Abdela, 1983). It seems that BABA and SA required more time and high dosage before induction of resistance to Srd can be observed. This was indicated by the significantly similar values of DS and CI, 6.46 and 5.24 , 7.60 and 5.80, 6.39 and 5.96 when 250, 500 250 µg ml^{-1} BABA and 250 µg ml^{-1} SA was used respectively and were not significantly(P=0.05) different compared with control plants. Similar results have been observed at low concentration of SA for induction of wheat leaf rust of wheat (Thabit, 2003). Furthermore, Erland, et al.,(2010) reported on the weak effect of BABA at low concentrations especially with epidemic diseases and higher concentrations of BABA gave stronger disease protection effects. As seen in table 3, reduced r is considered as a consequence of application of the chemical inducers BABA, BTH, IAA and SA, thus indicating that the low infection rate (r) reflect that the isolate of Pst in this study became less producing of spores and had longer latent periods. Reduction in r causes the velocity of Srd to increase more slowly, so that r is associated positively with rate of disease velocity in both time and space (Cowger, et al., 2005). Reduction of r can be considered as a suitable management strategy to control epidemic diseases like Srd in which the disease speared is usually initiated from focal spots over large area.

Pathogenesis related proteins, peroxidase, B-1, 4-glucanase and chitinase were activated in resistant plants (Quiroga etal., 2000). Strong relationship between lignifications in wheat appear to be of special importance in induced resistance mechanisms (Sherwood,1980). Lignin biosynthesis in wheat is related to two enzymes, PAL and ciannymyl alcohol dehyrogenase (Bruno,1990). Cohen (2001) found increased lignifications rate through accomplished hypersensitive reaction due to foliar application of BABA. Thabet, (2003) reported higher lignifications rate in the vascular bundles as a result of treating wheat plant with BABA and IAA against leaf rust. Application of IAA increased SAR in wheat plants infected by *P. recondita* f.sp. *tritici* was related to increase of peroxidase activity , meanwhile, the number of pustules cm^{-2} of leaf and infection percent were decreased (Sallam, 2001). Local and systemic increases in chitinase and peroxidase activity have been observed in response to BTH and SA treatments (Catherine, et al., 2004). Exogenous application of BTH on wheat has been tried to activate numbers of SAR associated genes which led to enhanced plant protection against various pathogens through increased synthesis and activity of oxidative enzyme especially PAL leading to production of phytoalexines and pathogenesis related proteins ,the molecular markers of SAR (Gorlach,etal.,1996). Furthermore, SA plays an important role in signal transduction in SAR in various plant pathogen interactions (Gaffney et al., 1993; Ryais et al., 1996). Several evidences indicated that one mode of action of SA in defense against pathogen is to activate various defense reactions in plants (Christian and jean, 1999; Shirasu, et al.,1997). SA induces rapid transient-generation of reactive O2 through oxidative burst in incompatible interaction (Rao et al., 1997). Significant increases in the activity of peroxidase and polyphenoloxidase were found after spraying wheat (Thabet, 2003) and sugar beet plants (Ata etal. 2008) with SA. Because of the ability of Pst pathogen to continuously produce new races that are able to overcome the resistance genes in wheat plant, it is essential beside other strategies to use effective inducers to reduce the impact of Srd. It is important to use such chemicals to induce SAR through the elevation of the level of resistance in susceptible wheat cultivars within an integrated management strategy of strip rust disease. Further research is needed before a sustainable management program to control this important disease can be achieved.

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Table 1. Disease severity (DS) and coefficient of infection (CI) in two wheat cultivars treated with different concentrations of chemical inducers against stripe rust 15 days after spraying (2012/2013 growing season)

Cultivar	Inducer	Concentration								Mean	
		1		2		3		4			
		DS	CI	DS	CI	DS	CI	DS	CI	DS	CI
Tamuz 2	BABA	11.05	9.44	6.46	5.42	7.60	5.87	1.70	0.81	6.70	5.34
	BTH	17.81	17.12	4.93	3.75	2.24	1.27	2.01	1.17	6.74	5.83
	IAA	11.29	10.93	7.40	6.85	2.95	2.08	1.72	0.87	5.83	5.18
	SA	8.61	8.54	6.39	5.96	3.57	3.14	1.92	1.33	5.12	4.74
	Mean	12.18	11.51	6.29	5.45	4.08	3.09	1.83	1.05	6.10	5.27
AL8/70	BABA	9.24	9.05	7.37	6.04	3.87	2.97	3.31	2.11	5.94	5.00
	BTH	25.46	25.00	3.61	2.35	3.45	2.04	2.10	1.30	8.65	7.67
	IAA	23.95	23.65	2.93	2.33	2.73	2.07	1.84	1.16	7.86	7.30
	SA	14.06	13.75	10.45	9.89	8.68	8.22	3.40	2.49	9.19	8.59
	Mean	18.17	17.86	6.08	5.15	4.72	3.78	2.66	1.76	7.91	7.14
LSD (P=0.05) for cvs, DS = 1.149*, CI = 1.153* LSD (P=0.05) cvs. indu.(inter.), DS = 5.396*, CI = 5.518* LSD (P=0.05) cvs. conc.(inter.), DS = 3.308*, CI =3.297* LSD (P=0.05) cvs. indu. conc.(inter.), DS = 4.596*, CI = 4.612*											

Each number is a mean of three replicates fifteen plant each.*indicate significant difference .Wheat plant was sprayed twice at booting stage. BABA=*β*-aminobutyric acid,SA=Salicylic acid. 1=Control,2=250µgml^{-1},3=500µgml^{-1}and4=1000µgml^{-1}Benzothiadizole(BTH)1=Control,2=0.3mM,3=0.6mM and4=1Mm, Indole acetic acid (IAA),1=Control,2=25µgml^{-1},3=50µgml^{-1}and4=100µgml^{-1}.

Table 2. Disease severity (DS) and coefficient of infection(CI) in two wheat cultivars treated with different concentrations of chemical inducers against stripe rust 15 days after spraying (2012/2013 growing season)

Cultivar	Inducer	Concentration								Mean	
		1		2		3		4			
		DS	CI	DS	CI	DS	CI	DS	CI	DS	CI
Tamuz 2	BABA	28.40	25.94	9.42	6.12	10.11	7.84	2.08	1.17	12.50	10.27
	BTH	43.32	35.34	8.20	5.00	3.63	1.96	2.71	1.47	14.64	10.94
	IAA	46.17	42.25	18.74	15.26	4.65	3.14	2.30	1.18	17.96	15.46
	SA	40.86	37.04	19.22	17.06	8.44	7.01	4.82	3.75	18.34	16.21
	Mean	39.69	35.14	13.89	10.86	6.71	4.98	2.98	1.89	15.82	13.22
AL8/70	BABA	31.34	28.31	16.59	13.31	6.74	5.11	4.41	2.93	14.77	12.42
	BTH	43.31	42.15	5.83	4.15	4.66	2.80	2.66	1.67	14.11	12.69
	IAA	50.95	48.13	5.78	4.35	4.33	2.77	2.35	1.26	15.85	14.12
	SA	59.68	57.01	27.32	23.97	21.14	19.13	5.53	3.78	28.42	25.97
	Mean	46.32	43.90	13.88	11.44	9.22	7.45	3.74	2.41	18.29	16.30
LSD (P=0.05) DS, cvs. =1.776*C.I, cvs. = 1.623* LSD (P=0.05) DS, cvs. indu.(inter.) =14.137*C.I, cvs. indu. (inter.) =13.419* LSD (P=0.05) DS, cvs. conc. (inter.) =6.183*C.I, cvs. indu. (inter.) =5.868* LSD (P=0.05) DS, cvs. indu. conc. (inter.) =7.105* C.I cvs. indu. conc. (inter.) =6.492*											

Each number is a mean of three replicates and fifteen plant each .*indicate significant difference .Wheat plant were sprayed twice at booting stage. , BABA=β-aminobutyricacid,SA=Salicylicacid,2=250µgml^{-1},3=500µgml^{-1},4=1000µgml^{-1}.BTH=Benzothiadizole,2=0.3mM,3=0.6mM,4=1Mm.IAA=Indoleaceticacid,2=25µgml^{-1} ,3=50µgml^{-1},4=100µgml^{-1}.

Table 3. Development of stripe rust infection(r) in two wheat cultivars treated with different concentrations of chemical inducers against stripe rust 15 days after spraying (2012/2013 growing season)

Cultivar	Inducer	Concentration				Mean
		1	2	3	4	
		r	r	r	r	r
Tamuz 2	BABA	0.0765	0.0270	0.0265	0.0156	0.0364
	BTH	0.0848	0.0349	0.0330	0.0224	0.0438
	IAA	0.1375	0.0732	0.0369	0.0213	0.0672
	SA	0.1323	0.0888	0.0691	0.0289	0.0798
	Mean	0.1077	0.0560	0.0414	0.0220	0.0568
AL8/70	BABA	0.1010	0.0623	0.0413	0.0202	0.0566
	BTH	0.0536	0.0330	0.0216	0.0168	0.0312
	IAA	0.0812	0.0496	0.0338	0.0174	0.0455
	SA	0.1368	0.0851	0.0735	0.0329	0.0821
	Mean	0.0931	0.0575	0.0430	0.0218	0.0538
LSD (P=0.05), cvs. =0.079* LSD (P=0.05) cvs. indu. (inter.) =0.0295* LSD (P=0.05) cvs. conc. (inter.) =0.0221* LSD (P=0.05) cvs. indu. conc. (inter.)=0.0315*						

Each number is a mean of three replicates and fifteen plant each .*indicate significant difference .Wheat plant were sprayed twice at booting stage. 1= control, BABA=β-aminobutyricacid,SA=Salicylicacid,2=250µgml^{-1},3=500µgml^{-1},4=1000µgml^{-1}.
BTH=Benzothiadizole,2=0.3mM,3=0.6mM,4=1Mm.IAA=Indoleaceticacid,2=25µgml^{-1},3=50µgml^{-1},4=100µgml^{-1}.