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Enhancement of Wheat Resistant to Diseases by Elicitors

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Abstract: ***Wheat (Triticum aestivum L) is the most important crops in the world and in Egypt. During the last 10 years there has been an increase in the intensity of cereals in the cropping rotation, and an increase in the adoption of stubble retention farming practices. The aim of this study is to investigate the effective use of different elicitors to improve the resistant of wheat to some foliar diseases as leaf blotches, powdery mildew and leaf rust. In bioassay test and artificial inoculation in greenhouse, different elicitors (Acremonium sp., Streptomyces, Bacillus subtilis, B. megaterium, Pseudomonas putida, P. fluorescens, methyl jasmonate (MJ), chitosan (CHI), an antioxidant in the form of ascorbic acid and a polyamine (PA) in the form of putrescein) were used to improve the resistance of wheat to all diseases. Pseudomonas putida, Methyl jasmonate and Chitosan showed a significantly greater decrease the diseases incidence and increased of total phenols, peroxidase, chitinase and total soluble protein in wheat as well as plant growth and yield. The significant increase in grain yield of wheat was observed with the application of different soil and foliar products management practices.***

Keyword: Wheat, Diseases, leaf blotches, powdery mildew and leaf rust, Elicitors

1. Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important crops currently being grown in most parts of the world. It forms more than 40% of the world's staple food and 95% of people in the developing countries eat wheat or maize in form of flour as a main staple food source (Coventry *et al*., 2011). Common bread wheat (*T. aestivum*) and durum wheat contribute a total of 90% of the world's wheat production and they are grown on approximately 17% of the world's cultivatable land, covering over 200 million hectares (Xin *et al.*, 2012). Diseases are primary hazards to wheat production. The most prevalent leaf spotting diseases were tan spot (*Pyrenophora tritici-repentis*) and septoria complex (*Septoria* spp.). The fungal pathogen *Puccinia hordei* Otth is the causal agent of wheat leaf rust, an economically important disease in temperate regions, which can considerably reduce the yield of susceptible cultivars up to about 60% (Cotterill *et al* 1992 and Haggag, Wafaa and Abd-El-Kareem,2009). Powdery mildew, caused by *Blumeria graminis* f. sp. tritici, is an important disease of wheat worldwide, especially in highly productive areas with a maritime or semicontinental climate (Mwale *et al.*, 2014**)**.

In recent years, the induction of the plant defense response by natural products as well as microorganisms as fungi and bacteria that normally colonize living plants without causing visual damage, has received ample attention. Rhizobacteria that are associated with plant roots, are known to promote plant growth, as well as to induce systemic resistance to diseases in plants (Haggag, Wafaa and Salme Timmusk, 2008). Activators of the plant defense response include signaling molecules such as SA, ethylene and jasmonic acid (JA) (Walters *et al*., 2002**,** Haggag Wafaa 2005**,** Haggag, Wafaa and Abd-El-Kareem, 2009). Control of fungal pathogens is based on the use of agronomic practices and pesticides, but widespread application of chemicals inundates the agro-eco systems with toxic compounds that affect the balance of the natural food chain. Biocontrol technologies have gained momentum in disease control of crop plants in recent times as these technologies not only minimize or replace the usage of harmful chemical pesticides but also found to be cheaper and efficient in certain disease control programmes. So, the aim of the research is to study the effect of biotic and abiotic elicitors on the resistance of wheat to diseases.

2. Materials and Methods

2.1 Field Sampling and Fungal Culture

Diseased wheat plants were collected from commercial fields of North Seini. Sporulation of the pathogen was induced by placing sections of infected leaf or stem or root tissue in moist chambers and incubating them for 48 h at 20C. Conidia produced were transferred singly to plates of I0% V-8 juice agar and the resulting colonies subcultured in a similar manner. The suspensions were homogenized for 1-2 min in a Waring Blendor, strained through a layer of cheesecloth, and adjusted to a concentration of 10^3 particles per ml using a hemacytometef. A long-term culture collection was maintained on Potato Dextrose Agar (PDA) slants under paraffin oil. Identity of the isolates was confirmed by the plant host.

2.2 Bioassay

2.2.1 Effects of products on spores germination.

Acremonium, Streptomyces, Bacillus subtilis, B. megaterium, Pseudomonas putida, P. fluorescens, Rhodotorula glutinis, methyl jasmonate, chitosan, an antioxidant in the form of ascorbic acid and a polyamine in the form of putrescein were tested against pathogens growth and germination of fungal spores by glass slide

technique. A 1 ml volume of V-8 juice broth medium containing spores of each fungus (50µl, 10^5 spores ml^{-1}), was mixed with different product, separately on a sterile glass slide. After 24h of incubation at $25C^0$ and 100% RH, spore germination was examined microscopically under an Olympus microscope (BX50+BX-FLA, Olympus Optical, Germany). The percentage of germinated spores within a count of 100 spores was determined on triplicate slides for each sample.

2.2.2 Evaluation of the ability of elicitors to induce the wheat defense response against pathogens under controlled conditions

Seeds of wheat of highly susceptible cultivar to diseases (cv. Giza 160) were grown in 30 cm- diameter plastic pots (2 plants per pots) under a 16 h photoperiod and 15 to 30 ^{0}C. Seeds were sown in 30 cm diameter plastic pots (5 seeds per pot) and grown under a 16 h photoperiod at 22 to 25^0C (Boheria governorate). The seeds were placed into insert in the sterile glass beakers containing a suspension of bioagents cells (1.0 x 10^6 /ml) for 3 h after that sown in soil fortified with biocopmost. Plants were watered every second day with water supplemented with 20N:20P:20K greenhouse fertilizer. The greenhouse experiment was designed as a randomized complete block. Soil and plant were inoculated with pathogens inoculation as follows:

Plants were sprayed with spore suspension 10^5 spores, according to infection stages. Uredospores of *Puccinia recondita* f. sp. *tritici* inoculations were preformed on seedlings at 2-3 leaf stage. Uredospores were suspended in a light mineral oil and inoculated using concentration of 10^5 spores /ml with 0.01% surfactant, using a hand held atomizer (about 0.5 ml per leave). Blotch pathogen i.e. *Pyrenophora teres*, was grown separately, on V-8 juice agar at 20°C[18]. After seven days in culture, conidia were washed from the agar surface, filtered through a 330µm strainer and made up into an aqueous suspension containing 10,000 conidia mL-1 and 0.01% Tween-80. After inoculation, seedlings were incubated overnight in complete darkness and at a relative humidity of 100%. Seedlings were then transferred to a growth chamber at 18–22□C and white fluorescent light (12 h light/12 h dark). Inoculated seedlings and plants 18-21h at greenhouse temperatures in a dark room. Each treatment was replicated three times. Seedling and plants were moved to natural light greenhouse chambers at 22- 24 2^oC, and disease responses were assessed 15 days after inoculation. The severity of rust disease was assessed as the percentage area of leaves infected during growth periods.

Disease index was scored on whole plants with ordinal rating scales. This scale is qualitative and based on lesion size and morphology. Ratings from 1-5 scale were classified as immune (0) to highly susceptible (5) according to Rosielle (Rosielle, 1972). Disease severity (R) was calculated according to the formula, having added the per cent of the affected leaf area of each leaf and having divided the sum by the number of assessed leaves:

$$\frac{R}{N} = \Sigma(n \cdot b)$$

- Where $\Sigma(n \cdot b)$ – sum of product of the number of leaves with the same percent of severity and value of severity, N – number of assessed leaves.

2.3 Chemical Analysis

Ten days after inoculation, three leaves per plant were separately collected, frozen for 36 h, dried and powdered. Generally, 100 mg dried sample were used for analysis.

- **Determination of phenol content**: Free and conjugated phenols were determined in treated leaves, 15 days after plant spraying with chemical elicitors according to A.O.A.C. (1975) using the Folin–Danis reagent. Phenols were identified by HPLC using a reverse phase C8 column and compared with a catecol standard (Sigma chemicals).
- **Peroxidase and chitinase assays**: Peroxidase activity was measured according to the methods described by Allam (1972). The chitinase activity was determined by the colorimetric method of Boller and Mauch (1988). Soluble protein extraction was carried out according to Bollag and Edelstein (1992).
- **Statistical analysis:** Disease assessment results were analyzed using an ANOVA of square- root-transformed data. Data were transformed to acquire the normal distribution necessary for statistical analysis to be carried out. Significant differences were assessed by comparison of sample mean differences with the LSD value.

3. Results and Discussion

3.1 Bioassay

3.1.1 Effects of products on spores germination.

Acremonium, *Streptomyces*, *Bacillus subtilis*, *B. megaterium*, *Pseudomonas putida*, *P. fluorescens*, *Rhodotorula glutinis*, methyl jasmonate (MJ), chitosan (CHI), an antioxidant in the form of ascorbic acid and a polyamine (PA) in the form of putrescein) were effective against pathogens germination isolated from wheat plants as showed in Table (1**).** The most treatment against spot diseases and rust was *Pseudomonas putida*, *P. fluorescens* in both crops. *Bacillus subtlis* was effective against powdery mildew. Methyl jasmonate and Chitosan resulted in a significantly greater decrease in the germination of all pathogens in wheat.

3.1.2 Evaluation of the ability of elicitors to induce the wheat defense response against biotic stress under controlled conditions.

The effects of different compounds on control of diseases of wheat was evaluated and presented in Tables (2-3). Leaf blotches, Powdery mildew, leaf rust are the most important diseases that cause severe losses, Tables (2 &3). Results showed that all compound have potentiality to reduce the diseases incidence in wheat. Significant differences were obtained among treatments and untreated control. Analysis of data indicated that all compounds treatments significantly reduced disease severity under artificial conditions of wheat.

Biocontrol agents were more effective in controlling all the diseases. The most treatment against spot diseases and rust was *P. putida*, *P. fluorescens* in both crops. *Bacillus subtlis* was effective against powdery mildew. *Rhodotorula glutinis* and *Trichoderma harzianum* were

more effective against head blight in wheat. At the same time, Our results clearly show that the polyamines markedly enhanced resistance to diseases control. The control plant developed a strong disease incidence and severity. This may be to increase plant defense compounds against pathogens. Overall, methyl jasmonate holds great promise for protection.

Application of methyl jasmonate significantly increased yield compared with ascorbic acid. Putrescence significantly increased yield in comparison with other compounds. Moreover, data revealed that spraying plants with ascorbic acid, had positive effect on increasing yield / plant (Table 4).Methyl jasmonate, and Chitosan and *Pseudomonas putida* resulted in a significantly greater decrease in the diseases incidence under none and saline soils in addition increased of total phenols, peroxidase, chitinase and total soluble protein in wheat as well as plant growth and yield.

Most sustainable and environmentally acceptable control may be achieved using biocontrol agents (BCAs) (Haggag Wafaa *et al*., 2014).Though bio-control with PGPR is an acceptable green approach, the proportion of registration of biocontrol agents for commercial availability is very slow. This differential effectiveness is clearly illustrated by results from three PGPR strains (Haggag, Wafaa *et al., 2013). Pseudomonas putida*, in different plant–pathogen systems. Bacterial determinants involved in the elicitation of ISR by these strains are the fluorescent siderophore pseudobactin, the outer membrane lipopolysaccharide (LPS), and the flagella of *P. putida* WCS358 (Meziane *et al.*, 2005); the pseudobactin, other iron-regulated metabolites and LPS of *P. putida* WCS374 and the LPS and iron-regulated metabolites other than pseudobactin for WCS417. The genus *Pseudomonas* belongs to the subclass of the Proteobacteria and includes mostly fluorescent Pseudomonads as well as a few non-fluorescent species. *P. putida* was more effective against die back and phomposis. Their mode of action is extremely varied: they may directly start a lethal biological process or only suppress the bio-aggressor by competition. Sometimes they induce resistance factors in the plant. Many studies involve these bacteria. The broad spectrum nature of the isolate, even though targeted could also have potential negative outcomes. *P. putida* as that are able to improve plant growth and plant health and are implicated in the natural supressiveness of plant pathogens (Meziane *et al*., 2005). Because of these changes in the pathogen population, therefore, recent efforts have been directed primarily toward identifying new control measures that could be effective, reliable and safe for the environment. Recently, it has been proposed to reduce Ochratoxin A producing *Asperigillus niger* by means of biocontrol yeast, *Rhodotorula glutinis* Harrison **(**Yuan *et al*., 2004**)**. Several isolates of *R. glutinis* reduced sporulation and established pathogen and disease severity (Haggag Wafaa and, Abdall, 2012). Several yeast species are known to accumulate carotenoid pigments as secondary metabolites. *Trichoderma* spp. is biological control agent for certain fungal plant diseases (Dorner *et al*., 2003). *Trichoderma spp* involves a complementary action of antibiosis, nutrient competition and cell wall degrading enzymes such as 1, 3-glucanases, chitinases and proteases (Choudhary, 2008).Jasmonic acid (3-oxo-2-[2'-*cis*-pentyl]-cyclopentane-l-acetate; JA) and its methyl ester, methyl jasmonate (MJ) are widespread natural regulators involved in many processes during plant development (Thatcher, *et al.,* 2005). Jasmonate, seems to play a dual role in plant development and defense. In fact, jasmonate induced protein include antifungal protein as phenylalanine ammonia lyase, and thionin, hydroxyproline- and proline –rich cell wall proteins. Another line of evidence for the role of jasmonates in disease resistance comes from their stimulatory effect on secondary metabolite production including alkaloids, terpenes, and phenolics and polyamines (Martin *et al*., 2002). In a previous article, a positive relationship between bean rust disease suppress and the over-accumulation of free and acid-soluble polyamine conjugates induced by a precursor of polyamines was proposed (Haggag, Wafaa, 2005). Results in this research indicate that the higher increasing in free and conjugated forms polyamines was obtained with MJ at 20 and 30 mM of both treatments which increased free and conjugated putrescine, spermidine and spermine compared with untreated control.In this respect (Walters *et al*., 2002) Jasmonates are known to enhance the amount of phenolic compounds, the conjugation partners for polyamines, by stimulating the phenylpropanoid pathway. Thus, treatment of wheat from various concentrations led to an increase in total phenol compared with untreated plants. Increase in phenolic content in plants has been correlated with resistance to pathogens.At the same time, all treatments increased the plant height, dry weight and grains yield of wheat plants. Jasmonates are involved in many plant processes such as seed germination, fertility, senescence, and yield, as well as responses to environmental stresses, such as drought and salinity (Martin, *et al*., 2002). In addition, methyl jasmonate can up-regulate the genes that are involved in secondary metabolism, cell-wall formation, and jasmonate biosynthesis, whereas the genes involved in photosynthesis are down-regulated (Cheong and Yang, 2003) reported that the major forms of polyamines are putrescine, spermidine and spermine, in addition to methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings (Haggag 2005). Also, all concentrations of methyl jasmonate treatments increased PR- protein. In this respect, many PR proteins exhibit antifungal activity *in vitro* (Velazhahan, *et al.*, 2000). For instance, ß-1,3-glucanases (PR-2) and chitinases (PR-3) are able to hydrolyze ß-1,3-glucan and chitin, respectively, the major components of fungal cell walls and accumulation of PR-proteins has been correlated with systemic resistance in plants.Our research studies applying bioproducts become more integrated into management strategies in protection and curative of wheat from biotic stress.The results of this study suggested it may be possible to replace conventional chemical fungicides with natural compounds, it is safe for human, environment and thus provided both economic and ecological efficacy.

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Table 1: In vitro, inhibition of wheat fungal growth by natural products

Treatments	Pathogens germination %					
	Necrotrophic fungi (Blotches)				Obligate fungi	
	Net blotch	*Leaf blotch*	*Spot blotch*	*Septoria*	*Powdery mildew (PM)*	*Leaf rust*
Acremonium	0.0	0.0	3.31	0.0	2.54	6.43
Streptomyces,	0.87	0.0	0.93	0.0	2.4 0	0.0
Bacillus subtilis	0.0	2.5	0.0	0.0	0.0	0.0
B. megaterium	4.4	2.7	5.9	2.7	3.0	4.9
Pseudomonas putida	0.0	0.0	0.0	0.0	0.0	0.0
P. fluorescens	0.0	0.0	0.95	0.0	0.88	0.81
Rhodotorula glutinis	0.0	0.0	0.0	0.0	0.0	2.3
Trichoderma harzianum	0.0	0.0	0.0	0.0	0.0	0.89
Methyl jasmonate	0.0	0.0	0.0	0.0	0.0	0.0
Chitosan	0.0	0.0	0.0	0.0	0.0	0.0
Ascorbic acid	5.25	6.5	5.9	5.2	3.25	4.8
Putrescine	2.47	0.94	3.88	1.74	0.90	0.4
LSD	0.057	0.03	0.06	0.06	0.07	1.6

Table 2: Diseases severity and Concentrations of antifungal in Leaves of wheat plants treaded with different products, 10 days after inoculation with pathogenic fungi

Treatments	Necrotrophic fungi (Blotches)							
	Diseases severity %				Phenol content (mg catechol/g F.W.)	Enzymes activities		*Soluble protein (mg g-1 F.W.)*
	Net blotch	Leaf blotch	Spot blotch	Septoria		Peroxidase	*chitinas*	
Acremonium	0.0	0.0	1.35	0.0	21.8	16.5	5.7	21.9
Streptomyces,	0.87	0.0	0.23	0.0	19.9	17.7	4.9	22.3
Bacillus subtilis	0.0	2.0	0.0	0.0	28.9	20.8	5.3	21.8
B. megaterium	4.4	2.0	3.7	2.7	18.8	19.7	5.0	15.6
Pseudomonas putida	0.0	0.0	0.0	0.0	36.8	24.7	7.8	31.5
P. fluorescens	0.0	0.0	0.65	0.0	33.8	22.8	6.5	30.9
Rhodotorula glutinis	0.0	0.0	0.0	0.0	17.8	16.8	5.5	24.5
Trichoderma harzianum	0.0	0.0	0.0	0.0	30.8	21.8	6.8	30.8
Methyl jasmonate	0.0	0.0	0.0	0.0	41.9	26.6	7.8	36.8
Chitosan	0.0	0.0	0.0	0.0	40.8	25.7	7.3	35.5
Ascorbic acid	4.65	3.5	5.0	4.2	23.8	11.8	6.4	27.7
Putrescine	0.97	0.34	2.68	0.96	31.0	18.7	6.6	30.8
Untreated control	3.0	2.0	0.65	0.67	12.8	8.8	4.4	12.7
Infected control	80.87	70.67	90.0	50.98	8.78	4.6	3.2	7.9
LSD	0.07	0.02	0.05	0.06				

Table 3: Diseases severity and Concentrations of antifungal in Leaves of wheat plants treaded with different products, 10 days after inoculation with pathogenic fungi

Treatments	Obligate fungi					
	Diseases severity %		Phenol content (mg catechol/g F.W.)	Enzymes activities (PM)		Soluble protein (mg g-1 F.W.)
	Powdery mildew (PM)	Leaf rust		Peroxidase	chitinase	
Acremonium	2.66	3.36	21.8	16.5	5.7	21.3
Streptomyces,	0.77	0.0	19.9	16.6	4.6	21.0
Bacillus subtilis	0.0	0.0	28.9	20.3	5.1	22.8
B. megaterium	3.0	4.4	18.8	18.7	5.1	16.3
Pseudomonas putida	0.0	0.0	36.8	23.7	7.3	31.5
P. fluorescens	0.88	0.79	33.8	21.8	6.5	31.9
Rhodotorula glutinis	0.0	0.97	17.8	15.3	5.3	25.5
Trichoderma harzianum	0.0	0.65	30.8	20.4	6.8	31.8
Methyl jasmonate	0.0	0.0	41.9	25.4	7.3	36.0
Chitosan	0.0	0.0	40.8	25.2	7.3	35.6
Ascorbic acid	3.25	4.3	23.8	10.8	6.2	28.7
Putrescine	0.60	0.36	31.0	18.7	6.6	31.5
Untreated control	26.8	19.65	12.8	8.3	4.3	12.6
Infected control	80.87	90.0	8.78	4.2	3.2	7.9
LSD	0.07	1.4				

Table 4: Growth and yield of wheat plants treated with natural products

	Mean plant height (cm)	*Mean plant dry weight (g)*	*Grain dry weight of 100 years (g)*
Acremonium	712	28.6	82.5
Streptomyces,	83.4	39.8	88.6
Bacillus subtilis	85.6	47.9	96.4
B. megaterium	80.4	39.6	60.2
Pseudomonas putida	80.3	39.6	88.1
P. fluorescens	90.2	37.6	80
Rhodotorula glutinis	89.5	32.5	88.6
Trichoderma harzianum	85.4	29.5	86.5
Methyl jasmonate	95.3	43.5	104.5
Chitosan	96.5	36.9	92.4
Ascorbic acid	82.3	31.8	80.6
Putrescine	85.4	37	88
Untreated Control	69.7	17.6	51.6
L.S.D	5.42	3.45	6.05

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