Egyptian Journal of Biological Pest Control, 25(1), 2015, 23-31

Postulation and Efficiency of Leaf Rust Resistance Genes of Wheat and Biological Control of Virulence Formulae of *Puccinia triticina* Races

Ghoneem[*], K. M.; W. I. A. Saber[**]; I. A. M. Youssef[***]; M. R. Mohamed[****] and A. A. Al-Askar[*****]

[*]Seed Pathology Research Dept., Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt.
[**]Microbial Activity Unit, Microbiology Dept., Soils, Water and Environ. Res. Ins., Agric. Res. Center, Giza, Egypt.
[***]Wheat Diseases Research Dept., Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt.
[****]Plant Nutrition Dept., Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt.
[*****]Botany and Microbiology Dept., Faculty of Science, King Saud University, Riyadh, Saudi Arabia.
(*Received: August 11, 2014 and Accepted: September 8, 2014*)

ABSTRACT

Rust-infected wheat leaves caused by *Puccinia triticina* Eriks. were collected throughout the survey of nurseries at Tag El-Ezz Agricultural Research Station, Dakahlia Governorate, Egypt, in order to evaluate and test the efficiency of leaf rust resistance genes and then determine suitable biological control agents for such pathogen. Seven formulae of *P. triticina* were derived by single uredinial isolates. The TKTT pathotype was the most virulent formula. KKTT and PKTT pathotypes showed the most common frequencies (23.08 % each). The leaf rust resistance gene (Lr9) was the most efficacy one. Postulation of rust genes in the commercial cultivars proposed that Giza 168 and Sakha 93 probably did not contain any common genes. Sakha 93 probably exhibited 6 resistance genes (1, 2c, 3, 26, 3ka and 30). Biological control of such rust showed that combined application of arbuscular mycorrhizal fungi and *Azospirillum amazonense* improved wheat plant growth, yield and quality, in addition to the reduction in rust disease severity, compared with untreated ones.

Key words: *Puccinia triticina*, *Azospirillum amazonense*, Arbuscular mycorrhizal fungi, Leaf rust, Gene postulation.

INTRODUCTION

Rusts are the most devastating fungal diseases that threaten wheat (*Triticum aestivum* L.) production worldwide. Leaf and stripe rust, caused by *Puccinia triticina* Eriks., is a major disease in most of the wheat growing areas, resulting in economic losses and affects the longevity of wheat cultivars (Park *et al.*, 2007). In Egypt, leaf rust is the most common and important wheat disease. It caused severe losses in grain yield reaches more than 23% and in epidemic years they may reach up to 50% (Kassem *et al*., 2011). The most environmentally sound and low cost method for controlling leaf rust is breeding and grows resistant varieties. So far, over 60 leaf rust resistance genes have been identified and localized on the wheat chromosomes (McIntosh, 2009; Samsampour *et al*., 2010). Certain resistance genes are very important for breeding because they proved to confer durable resistance over a long period in different environments against diverse pathotypes of the fungus (Schnurbusch *et al*., 2004). Effectiveness of resistance genes depends on the composition of the pathogen populations, as the later changes dynamically, new pathotypes virulent to the given resistance genes multiply periodically, so the resistance of a variety is not a constant trait. Therefore, continuous detection of the new virulent pathotype and postulation of resistance genes are traditionally carried out under various environmental setup conditions.

The increased concern about the impact of agrochemicals on the environment and food safety is leading to the development of alternative approaches for crop production, including biological and ecological strategies (Romero *et al*., 2003). Certain strains of rhizosphere bacteria, called plant growth promoting rhizobacteria (PGPR), stimulate plant growth mainly by directly affecting plant metabolism and/or the availability of nutrients (Saikia *et al*., 2012). Other PGPR strains promote plant growth indirectly by suppressing soil-borne pathogens, or by stimulating plant natural defenses, by a mechanism called induced systemic resistance (Tortora *et al*., 2012). Members of the genus *Azospirillum* are good example for such mission. Arbuscular mycorrhizal fungi are ubiquitous soil inhabitants forming symbiosis associating with 80% of the plant families in the world (Giovannetti and Sbrana, 1998). Colonization of roots by such fungi has been shown to improve growth and productivity of several field crops by increasing nutrient element uptake (El-Amri *et al*., 2013) and resistance to abiotic and biotic stress factors(Abdel-Fattah *et al.*, 2010), as well as, decrease diseasing severity (De la Pena *et al*., 2006).

Association of the two beneficial microorganisms (*Azospirillum* and mycorrhizal fungi) plays a vital role in supplying N and P and enhances growth and yield. Registered effect of the dual inoculation of both bioinoculants might be due to the provision of nitrogen and growth promoting substances by *Azospirillum*, and phosphorus by mycorrhizal fungi, creates sustainable growth of the crop (Sridevi and Ramakrishnan, 2010).

The present study focused mainly on postulation and characterization of virulence formulae of *Puccinia triticina* races, as well as determination of highest efficacy genes against this pathogen. *Azospirillum amazonense* and/or mycorrhizal fungi were also evaluated as control bioagents of leaf rust disease and inducers of growth, yield and quality of wheat.

MATERIALS AND METHODS

Wheat leaves samples, having the symptoms of leaf rust disease caused by *Puccinia triticina* Eriks, were collected from Sakha 93, Giza 168, Sids1 and Beni-Sweif 5 wheat varieties, grown in nurseries at Tag-El-Ezz Agricultural Research Station, Dakahlia Governorate, Egypt. Obtained specimens were used in identification of pathotypes and genes' efficacy in some Egyptian wheat cultivars in 2010/11 growing season. The infected specimens were transferred on the susceptible wheat cultivars, *i.e*. Morocco and *Triticum spelta saharrensis* in seedling stage. The method of inoculation was carried out as described by Stakman *et al*. (1962). After developing the rust cultures, three single pustules were separately isolated from each specimen for reproduction on the highly susceptible to leaf rust Morocco wheat cultivar to obtain enough purified urediospores multiplication. Scoring infection type was carried out according to Mains and Jakson (1926).

Race identification using infection type of rust

Race identification was assigned as described method by Long and Kolmer (1989), using a four letter code of leaf rust resistance genes (Lr's) to describe the low or high infection type of each rust isolate to the 16 North American differential host lines, each letter corresponds to the infection types of four differentials (Table 1). The differential host lines were kindly obtained from the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

The scale of Mains and Jackson (1926) and Long and Kolmer (1989) was used to determine the infection type by the rust isolates at seedling stage of wheat plants, in which: 0 = no visible symptoms of the leaf, 0; = hypersensitive necrosis, 1 = minute uredia surrounded by necrotic area, 2 = medium uredia surrounded by necrotic or chlorotic area, 3 = large uredia surrounded by chlorotic area, 4 = large uredia without any chlorosis or necrosis area and X = resistant and susceptible reactions are present together on the leaf blade. Infection type from 0 to 2 were considered low, infection type (L), 3 and 4 were considered high infection type (H) (Kolmer and Oelke, 2006).

Gene postulation of the commercial varieties

Genes were postulated according to the method of Browder and Eversmeyer (1980) based on the reactions between the tested unknown host (B) and the known gene host (A). The presence of such gene in the tested host exhibited the symbol (-0). On the other hand, when host B proved to have high infection type (H) versus low infection type (L) in host A, this behavior would indicate the absence of such gene in host B (-). The presence of L (in host B): H (in host A) indicated the presence of such gene in host B and it may additionally have another ones (0). With especial reference to the absence of L: H or H:L, the presence of pathotypes having H:L and L:H in the comparison indicates that either of hosts did not have the same gene (+), as described in diagram (1).

Field evaluation of microbial bioagents on leaf rust

Inocula preparation

The non-symbiotic nitrogen-fixing *Azospirillum amazonense* NRRL B-23163 was kindly, obtained from the ARS Culture Collection (NRRL), Peoria, Illinois USA, in lyophilized preparation. The strain was reactivated on TGY medium contained (g/L) 5.0 tryptone, 5.0 yeast extract, 1.0 glucose and 1.0 K_2HPO_4 with pH 7.0) at 28 °C for 48 h to obtain inoculum containing 3×10^7c.f.u. ml^{-1}, approximately. The strain was checked for nitrogenase activity before use. Suspension mixture (10^6 units L^{-1}) of multi-arbscular mycorrhiza fungi (AM) consisted of equal proportions of spores of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus intraradices* Schenck & Smith, *Glomus clarum* Nicol. & Schenck, *Gigaspora gigantean* (Nicol. & Gerd.) Gerd. & Trappe, and *Gigaspora margarita* (Becker & Hall), was kindly provided by Prof. S. El-Haddad, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. The mass production of AM inoculum was carried out by spores of the previous formula using the pot culture technique and Sudan grass as a host plant.

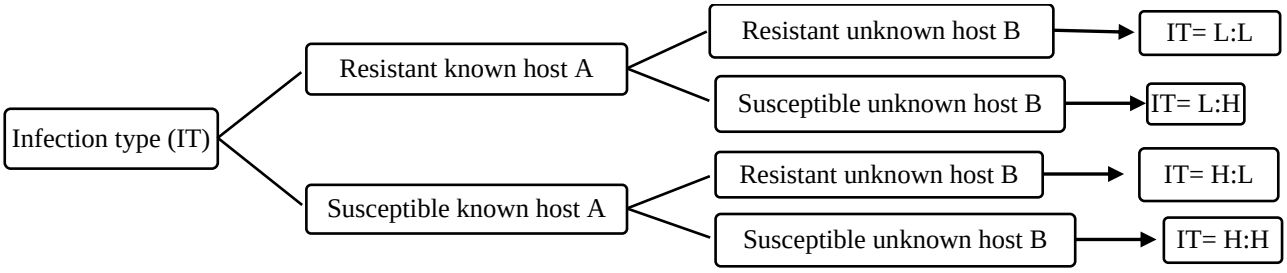

Diagram (1): The possible gene pathotypes of the resistant and susceptible hosts.

Table (1): Code of 16 North American differential hosts of *P. triticina* in ordered sets of four differentials applied for race identification (based on Long and Kolmer 1989)

*P. triticina*code	Subset	Infection types produced on Near-isogenic lines Lr's			
	Host set: 1	1	2a	2c	3
	Host set: 2	9	16	24	26
	Host set: 3	3k	11	17	30
	Host set: 4	10	18	21	2b
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

L =Low infection type H =High infection type

Plantation of the commercial cultivars

The field experiment was carried out at Tag El-Ezz Research Station, Dakahlia Governorate, Egypt. Two commercial cultivars (Giza 168 and Sakha 93) were sown during 2010/11 growing season in 2 m long rows and 30 cm apart. Each raw was sown by 40 seeds with a distance 10 cm. The experimental unite included 3 rows for each cultivar. Prior to sowing, wheat grains were inoculated by soaking in TGY medium culture of *A. amazonense* NRRL B-23163 and/or mixing with AM, in the presence of Arabic gum as adhesive agent. Inoculated grains were air dried by spreading over a plastic sheet for short time before planting. The control treatment was done using uninoculated grains. To ensure the presence of effective mycorrhizal infection, fresh wheat roots inoculated with AM, single or combined with *A. amazonense*, were evaluated for mycorrhizal colonization. After coloration with trypan blue (Phillips and Hayman, 1970), root samples were investigated at 40× by the gridline (Giovannetti and Mosse, 1980), which was found to be positively ranged from 80–100% colonization.

Disease severity

Field evaluation for rust intensity in the nursery consisted of taking a severity reacting (% of tissue of tiller of flag leaf infected) and host response (size of lesion). Present disease severity and host response were combined in a single value and an average coefficient of infection type. The disease severity was multiplied by a numerical notation for host response where; immunity = 0, nearly immunity = 0;, resistant = 0.2, moderately resistant= 0.4, mixed (moderately resistant - moderately susceptible) = 0.6, moderately susceptible = 0.8, and susceptible = 1.0. The area under disease progress curve (AUDPC) was calculated for each treatment of variety to evaluate adult plant resistance character and to compare tested treatments of varieties with susceptible one. The following equation of Pandey *et al*. (1989) was used:

$$\text{AUDPC} = \text{D}((Y1 + Yk)/2) + Y2 + Y3 + \cdots Yk1)$$

Where: D is number of days within the interval, Y_1, Y_2 …*Yk* are disease records at constant interval, and *Yk*1 is the disease recorded before the last record (*Yk*).

Growth and yield characteristics

Samples of wheat plants, 60 and 90 days from sowing, were taken to determine plant height, tillers and leaves numbers per plant and flag leaf area. At the end of wheat life cycle, the yield components *i.e*. spike No/cm^2, spikelet's number/ spike, spike length and grains number/ spike as well as wheat yield, expressed as 1000-grain weight were determined.

Nitrogen and phosphorus determinations

Total nitrogen content in wheat grains was determined, following the procedure described by AOAC (1995), and then the protein was calculated by multiplying N × conversion factor. Phosphorus content of the seeds was determined by the method of Jackson (1967) after digestion with H_2SO_4.

Statistical analysis

Treatments were arranged in completely randomized block design. Means were compared by

Duncan multiple range test at the probability level (P)≤ 0.05, using the statistical analysis software CoStat (version 6.4).

RESULTS AND DISCUSSION

Rust race formulae (phenotype) and their frequencies

Thirteen single uredinial isolates of *P. triticina*, derived from ten collections (samples), were tested for virulence to the Thatcher near-isogenic lines at the Wheat Disease Research Dept., ARC, Giza, Egypt. Seven avirulence/ virulence formulae of *P. triticina* were found. Data in table (2) show infection types produced by the seven *P. triticina* pathotypes and their frequency. The letters B through T, excluding the vowel letters (Table 1) were used for race identification. The 4-letters code of *P. triticina* consisted of the designation for subset, followed by that for subset 2 … etc. Race TKTT was virulent, *i.e.* high infection type (H) at all of differential hosts, except Lr9 only. TKTR was virulent on all of differential hosts, except Lr9 and Lr21. On the other hand, CSRS exhibited virulent on most of leaf rust resistance genes (Lr's), except 1, 2a, 2c; 26; 17; 2b. The rest of pathotypes lied in between. Low and high infections indicated an incompatible and compatible host-pathogen interaction, respectively. Each of the phenotypes KKTT and PKTT was represented by 23.08%. Their phenotypes avirulence/ virulence were the most common frequency in nurseries at the experimental station. CSRS, TKJS and TKTT showed the lowest frequencies (7.69%). On the other hand, MDTT and TKTR exhibited avirulance/ virulence phenotypse lied in between, being15.38%. Frequencies of virulence differed among populations of *P. triticina* at the experimental station (Table 3). Among different monogenic wheat lines, the most effective gene, *i.e*. highly efficacy gene was recorded at Lr9 against leaf rust, followed by Lr2a. The low efficacy gene was observed at Lr2a, 1, 26 and 2c. However, the rest genes exhibited relatively high virulent. All the obtained results, recorded during seedling stage, gave evidence to the presence of seven virulence formulae of *P. triticina*, originated from thirteen leaf rust isolates. TKTT pathotype was the most virulent formula, while KKTT and PKTT were the most common frequencies. This may reflect the variability and the dynamic genetic nature of the leaf rust pathogen and its response to the changing of environmental conditions (Long *et al*., 2000; McCallum and Seto-Goh, 2006).

Postulated genes in the commercial cultivars

Postulation, frequency and efficiency of Lr's during seedling stage of wheat were tested. Qualitative resistance genes were expressed at seedling stage than quantitative resistance genes at adult plant (Schnurbusch *et al*., 2004). Data represented in table (4) indicate the response of 13 pathotypes of *P. triticina* against twenty monogenic lines Lr's and two commercial Egyptian wheat cultivars, based on low infection type (L) and high infection type (H). The cultivar Giza 168 showed the categories (+) or (-) and this indicated that the cultivar and the monogenic lines did not carry the same resistance genes or at least this cultivar did not contain the gene in the tested Lr's. On the other hand, the cultivar Sakha 93 probably exhibited 6 Lr's, *i.e.* 1, 2c, 3, 26, 3ka and 30, while Giza 168 likely had not any gene of tested Lr's, at least against the isolated rust pathotypes. Also, both wheat cultivars, probably had not exhibited any common gene between each other although the comparison among the tested commercial wheat cultivars, infected with the 13 isolates of *P. triticina*, was carried out in all possible combinations. The results indicated that the cultivars probably did not contain any common genes, based on L: H and H: L, at least against the tested pathotypes, recording plus symbol (+). The presence of this symbol indicated that both compared cultivars did not contain the same Lr's. These results proved that the Egyptian wheat cultivars lack Lr9, but they are not considered an indication to the lack of Giza 168 and Sakha 93 to the resistance genes, but they may have genes other than those tested, at least against these 13 rust isolates. However, wheat cultivars, with combinations of genes, conferred nonspecific resistance and display highly effective levels of durable resistance (Kolmer and Oelke, 2006 and Zhang *et al*., 2008).

Biological control of rust under open field conditions

Rust severity development

Although many methods of control are possible, such as use of inheritance resistance, modified cultural practices and chemicals (fungicides), leaf rust remains a serious threat to wheat production due to appearance of virulent isolates (Park *et al*., 2007 and Kassem *et al*., 2011). In addition to the genetic base methods, biological control programs remain inevitably alternatives. Data in table (5) show the leaf rust development(S) on two wheat cultivars and their response to bioagent treatments in term of area under disease progress curves (AUDPC) during 2010/11 season. Data of AUDPC showed that the highest values were recorded at both controls of Giza 168 and Sakha 93 (370 and 280, respectively). In contrast, the lowest values were recorded at the combined application of *A. amazonense* + AM in both wheat cultivars (72.5, for each). The rest treatments of single application by *A. amazonense* or AM exhibited moderate values lied in between of the combined and control applications in both cultivars.

Table (2): Infection type produced by seven pathotypes of *P. triticina* on 16 near-isogenic lines and frequencies of avirulence/ virulence formulae against Lr's and differential sets samples under greenhouse conditions at seedling stage

Rust pathotype	Infection type on near-isogenic lines																Avirulence/ virulence formula	Frequency	
	1	2a	2c	3	9	16	24	26	3ka	11	17	30	10	18	21	2b		No.	%
CSRS	L	L	L	H	H	H	H	L	H	H	L	H	H	H	H	L	1, 2a, 2c; 26; 17; 26/	1	7.69
KKTT	L	H	H	H	L	H	H	H	H	H	H	H	H	H	H	H	1; 9; /	3	23.08
MDTT	H	L	L	H	L	L	H	L	H	H	H	H	H	H	H	H	2a, 2c; 9, 16, 26; /	2	15.38
PKTT	H	L	H	H	L	H	H	H	H	H	H	H	H	H	H	H	2a; 9; /	3	23.08
TKJS	H	H	H	H	L	H	H	H	L	H	H	L	H	H	H	L	9; 3ka, 30; 26;	1	7.69
TKTR	H	H	H	H	L	H	H	H	H	H	H	H	H	H	L	H	9; 21; /	2	15.38
TKTT	H	H	H	H	L	H	H	H	H	H	H	H	H	H	H	H	9; /	1	7.69

Table (3): Percentage of virulence frequency of wheat leaf rust pathotypes and Lr's genes efficacy at seedling stage under greenhouse conditions, Egypt

Lr's	No. of pathotype			Frequency %	
	Avirulent	virulent	Total	Avirulence	Virulence
1	4	9	13	30.8	69.2
2a	6	7	13	46.2	53.8
2c	3	10	13	23.1	76.9
3	0	13	13	0.0	100.0
9	12	1	13	92.3	7.7
16	2	11	13	15.4	84.6
24	0	13	13	0.0	100.0
26	4	9	13	30.8	69.2
3ka	1	12	13	7.7	92.3
11	0	13	13	0.0	100.0
17	1	12	13	7.7	92.3
30	1	12	13	7.7	92.3
10	0	13	13	0.0	100.0
18	0	13	13	0.0	100.0
21	2	11	13	15.4	84.6
2b	0	13	13	0.0	100.0
14b	0	13	13	0.0	100.0
15	0	13	13	0.0	100.0
35	0	13	13	0.0	100.0
42	0	13	13	0.0	100.0

Table (4): Comparison between 20 monogenic lines of Lr's and 2 Egyptian wheat cultivars against 13 isolates of *P. triticina* Eriks at seedling stage under greenhouse conditions

Cultivar	Leaf rust resistance genes																			
	1	2a	2c	3	9	16	24	26	3ka	11	17	30	10	18	21	2b	14b	15	35	42
Giza 168	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sakha 93	0	+	0	0	+	+	+	0	0	+	+	0	+	+	+	+	+	+	+	+

Table (5): Response of two Egyptian wheat cultivars to bioagent treatments against leaf rust (*P. triticina*) under field conditions, one-week interval after onset of leaf rust in 2010/11 growing season

Cultivar	Inoculation treatment	Disease severity				AUDPC
		16 week	17 week	18 week	19 week	
Giza 168	Control	30 S	40 S	50 S	50 S	370
	A. amazonense	20 S	20 S	30 S	30 S	225
	AM	10 S	10 S	20 S	20 S	135
	Combination	5 S	10 S	10 S	10 S	72.5
Sakha 93	Control	20 S	30 S	40 S	40S	280
	A. amazonense	20 S	20 S	30 S	30 S	225
	AM	10 S	10 S	20 S	20 S	135
	Combination	5 S	10 S	10 S	10 S	72.5

Table (6): Effect of various treatments with tested bioagents on vegetative growth of two wheat cultivars under field conditions in 2010/11 wheat growing season

Cultivar	Inoculation treatment	Plant height (cm)		Tillers no. per plant		Leaves no. per plant		Flag leaf area (cm^2)	
		60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Giza 168	Control	70.7 a	81.0 a	3.0 e	4.2 d	9.1 f	11.3 f	13.2 f	24.1 e
	A. amazonense	90.0 a	96.0 a	5.5 ab	6.6 ab	12.2 b	16.5 c	16.5 c	26.8 b
	AM	92.0 a	98.0 a	5.8 ab	6.7 ab	12.7 a	17.0 b	17.0 b	27.2 b
	Combination	96.0 a	100.0 a	6.2 a	6.9 a	13.1 a	18.2 a	17.9 a	27.8 a
Sakha 93	Control	57.0 a	64.7 a	4.2 d	4.6 cd	8.2 g	10.6 g	11.2 g	23.3 f
	A. amazonense	75.0 a	82.0 a	4.5 cd	5.2 c	10.3 e	13.9 e	13.9 e	24.8 d
	AM	78.0 a	84.7 a	4.9 bcd	6.2 b	10.8 d	14.2 e	14.2 e	25.2 d
	Combination	81.0 a	88.3 a	5.2 bc	6.6 ab	11.3 c	14.9 d	14.9 d	25.9 c

Means followed by the same letter(s) within each column do not significantly differed ($P \leq 0.05$).

Table (7): Yield and its attributes of wheat cultivars due to the treatment with tested bioagents under field conditions in 2010/11 wheat growing season

Cultivar	Inoculation treatment	Spike No/cm^2	Spikelets No./spike	Spike length (cm)	Grains No/Spike	1000-grain wt. (g)
Giza 168	Control	325 d	13.7 a	8.7 e	40.3 d	40.8 f
	A. amazonense	329 b-d	19.5 a	10.6 ab	59.5 a	46.8 bc
	AM	333 ab	19.7 a	10.7 a	59.9 a	48.3 a
	Combination	337 a	20.6 a	10.9 a	60.7 a	47.6 ab
Sakha 93	Control	312 f	12.2 a	9.2 d	38.7 d	43.7 e
	A. amazonense	317 e	17.4 a	9.9 c	55.0 c	44.6 de
	AM	328 cd	17.5 a	10.2 bc	55.2 c	45.4 cd
	Combination	332 a-c	18.7 a	10.7 a	57.2 b	45.7 cd

Means followed by the same letter(s) within each column do not significantly differed ($P \leq 0.05$).

Disease severity of wheat rust was reduced vigorously by *A. amazonense* and/ or AM, since *Azospirillum* strains are able to stimulate plant growth indirectly by suppressing soil-borne pathogens and other deleterious microorganisms in various systems. For instance, *Azospirillum* was reported to reduce the incidence and severity of damping off caused by *Rhizoctonia solani*, possibly by bacterial colonization of the sclerotia (Gupta *et al*., 1995). Tortora *et al.* (2012) reported that *A. brasilense* REC3 reduced anthracnose symptoms on pathogen-challenged strawberry plants. The mechanism of plant protection from disease may involve pathogen displacement, in a process called pre-emptive competitive exclusion (Wilson *et al*., 2002), induction of systemic host response (Ramos Solano *et al*., 2008), transient accumulation of salicylic acid, induction of defense-related genes and/or structural cell wall modifications as consequence of the observed increase in phenolic compounds and callose deposition (Tortora *et al*., 2012). Resistance and tolerance against plant pathogens due to mycorrhizal fungi may be attributed to physiological, biochemical and morphological alterations in the host induced by the mycorrhizal fungi, such as thickening of cell wall through lignification and production of other polysaccharides in mycorrhizal plants (Abdel-Fattah *et al*., 2011). Another explanation based on genetic mechanism proposed that root colonization by the arbuscular mycorrhizal fungus *Glomus intraradices* was accompanied by the systemic induction of genes involved in regulating host defenses, as well as genes involved in signal transduction and calcium-mediated signaling. mycorrhizal plants, also exhibited stronger induction genes that response to infection, suggesting that the protective effect of the AM fungi symbiosis dependent on both systemic activation of defense regulatory genes and enhanced expression of defense-related genes (Campos-Soriano *et al*., 2012). Comparing disease severity at the control treatments of both varieties, led to the conclusion that Sakha 93 recorded lower AUDPC than Giza 168. This might be back to the genetic structure of Sakha 93, which contains 6 Lr's, so recorded lower AUDPC. However, dual inoculation with *A. amazonense* and AM reduced the AUDPC to equal values in both varieties (72.5), indicated positive effectiveness of this kind of inoculation on various genetic structures of both tested varieties, irrespective to the presence of Lr's or not.

Vegetative growth, yield and yield components of wheat

Growth parameters of the wheat cultivars Giza 168 and Sakha 93 showed different responses to the tested bioagents' applications (Table 6). In this

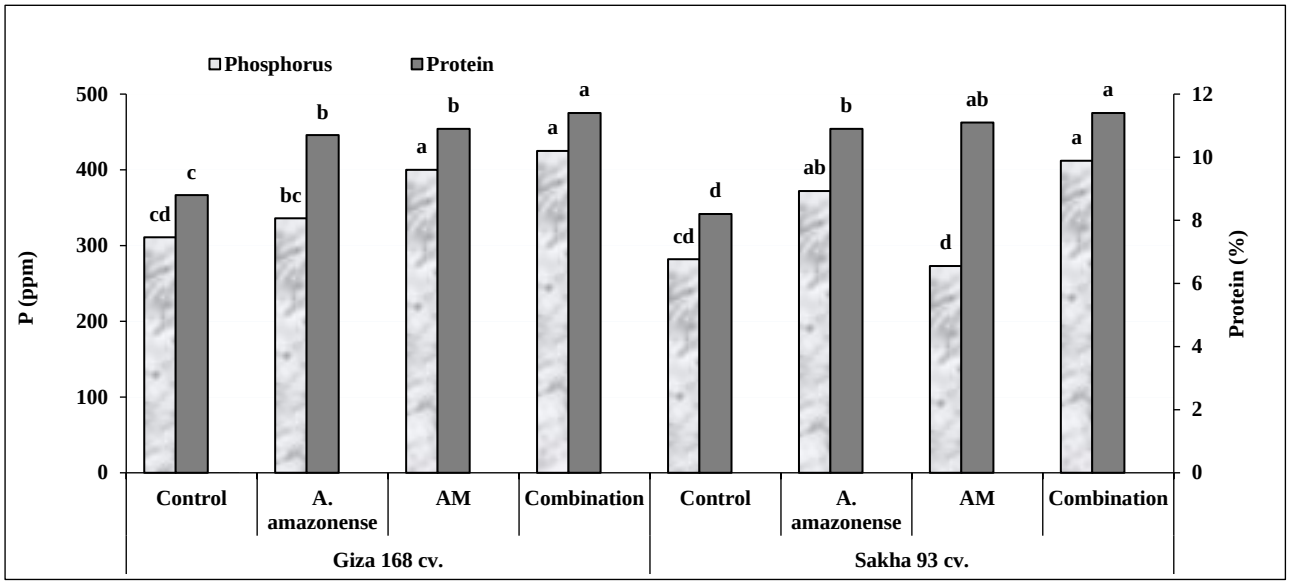

Fig. (1): Grain quality of wheat due to the treatment with tested bioagents under field conditions in 2010/11 wheat growing season. Each group (phosphorus or protein) of columns designated with different letter(s) differ significantly ($P \leq 0.05$)**.**

respect, no significant differences were recorded in plant height when treated with any of the bioagents in both wheat cultivars. On contrast, application of *A. amazonense* + AM had a pronounced positive effect on numbers of tillers and leaves per plant and flag leaf area at 60 and 90 days in comparison with other treatments in both tested wheat cultivars. Single inoculation with AM or *A. amazonense* came in the second order for increasing growth parameters of both wheat cultivars. Regarding the yield and its components (Table 7), highest spike number, length, number of grains per spike and weight of 1000-grain was obtained from applications of *A. amazonense* + AM, in comparison with both wheat cultivars controls. Single application came next in this respect. However, the only exception was the treatment of Giza 168 with AM alone, in which weight of 1000-grain reached its maximum. Spikelets number per spike did not reach the level of significance by any of the tested treatments for both varieties. The marked improvements in growth and yield of wheat plants that were received *A. amazonense* and/or AM may be attributed to the secretion of various PGPR by *Azospirillum*, *viz*. N-fixation, hormonal interaction, improvement in root growth, solubilization of nutrients, alleviation of salinity and biocontrol against phytopathogens in the host rhizosphere (Nadeem *et al*., 2006; Gholami *et al*., 2009 and Saikia *et al*., 2012). Besides, mycorrhiza enhance plant growth through positive influence of certain substances produced by fungi such as auxins and gibberellins (Barker and Tagu, 2000). All these factors together positively induced the growth, subsequently improved the yield.

Grain quality

Quality of wheat grains was evaluated in terms of protein and phosphorus contents as response of inoculation with *A. amazonense* and/or AM for both wheat varieties. Results presented in Fig. (1) showed that the combined inoculation with *A. amazonense* and AM increased significantly the protein and phosphorus contents. Grain protein content recorded 11.4 % for both varieties, followed by the single inoculation with AM. The same trend was observed at P content for the dual inoculation, in which grain P content recorded 425 and 412 ppm for Giza 168 and Sakha 93, respectively. Obviously, single inoculation of Sakha 93 with AM recorded lowest value of P content. The increment in protein and phosphorus contents of wheat grains may be due to additional fixation of N_2 and P caused by *A. amazonense* and AM, respectively. Rabie *et al*. (2005) suggested that mycorrhizal fungi may improve P nutrition, enhance N uptake, as well as improve disease resistance in their host plants. N-fixing bacteria such as *A. amazonense*, may synergistically interact with mycorrhizal fungi and thereby benefit growth development, yield and its quality.

Generally, the dual inoculation with *A. amazonense* and AM came first in all tested parameters. Interaction between mycorrhizal fungi and *Azospirillum*, showed a stimulation of plant growth, root colonization and disease suppression in a positive manner. In this respect, dual inoculation of mycorrhizal fungi and *Azospirillum* suppressed damping off in chilli, caused by *Pythium aphanidermatum* by 72.7% (Kavitha *et al*., 2003). Co-inoculation of tomato with mycorrhizal fungi and *Azospirillum* expressed good growth, nutritional characteristics, better yield and quality fruits (Guru *et al*., 2011). Useful fungi and bacteria can also act as plant growth-promoting or root-associated microbes in the rhizosphere of many plant species that increase plant growth and suppress plant disease (Chung and

Sa, 2012), by secretion of antibiotics, some of these microbes can also elicit induced systemic resistance against a broad range of pathogens, nematodes and insects (Ngoma *et al*., 2012).

In conclusion, all the previous proposed modes of actions may be attributed to the synergism, complementary and the non-competitive living nature of both inoculants, where, *A. amazonense* is a free living N-fixing bacterium, where as mycorrhizal fungi is living associatively, with the plant roots, each one has its unique role, place, nutritional requirements and growth nature, which balanced the ecosystem of living organisms. This, in turn, motivates the recruitment of such bioagent in both controlling the rust as well as increment the quality and the final gain of wheat

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research through the Research Group Project No. RGP-VPP-327.

REFERENCES

Abdel-Fattah, G. M., El-Dohlob, S. M., El-Haddad, S. A., Hafez, E. E. and Rashad, Y. M. 2010. An ecological view of arbuscular mycorrhizal status in some Egyptian plants. J. Environ. Sci., 37: 123-136.

Abdel-Fattah, G. M., El-Haddad, S. A., Hafez, E. E. and Rashad, Y. M. 2011. Induction of defense responses in common bean plants by arbuscular mycorrhizal fungi. Microbiological Research, 166(4): 268-281.

AOAC.1995. Official Methods of Analysis. Arlington VA: AOAC International.

Barker, S. J. and Tagu, D. 2000. The roles of auxins and cytokinins in mycorrhizal symbiosis. Journal of Plant Growth Regulation, 19(2): 144-154.

Browder, L. E. and Eversmeyer, M. G. 1980. Sorting of *Puccinia recondita*: *Triticum* infection-type data sets towards the gene for-gene model. Phytopathology, 70: 666-670.

Campos-Soriano, L., Garcia-Martinez, J. and San Segundo, B. 2012. The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defense-related genes in rice leaves and confers resistance to pathogen infection. Mol. Plant. Path., 13: 579-592.

Chung, J. and Sa, T. 2012. Effect of *Azospirillum brasilense* and *Methylobacterium oryzae* inoculation on growth of red pepper (*Capsicum annuum* L.). Korean J. Soil Sci. Fert., 45(1): 59-65.

De la Pena, E., Echeverria, S. R., Put ten, W. H., Freitas, H. and Moens, M. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytol., 169(4): 829-840.

El-Amri, S. M., Al-Whaibi, M. H., Abdel-Fattah, G. M. and Siddiqui, M. H. 2013. Role of mycorrhizal fungi in tolerance of wheat genotypes to salt stress. African Journal of Microbiology Research, 7(14): 1286-1295.

Gholami, A., Shahsavani, S. and Nezarat, S. 2009. The effect of plant growth rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Acad. Sci. Eng. Technol., 49:19-24.

Giovannetti, M. and Sbrana, C. 1998. Meeting a non-host: the behavior of AM fungi. Mycorrhiz a, 8: 123-130.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. The New Phytologyst 84: 489-500.

Gupta, S., Arora, D. K. and Srivastava, A. K. 1995. Growth promotion of tomato plants by rhizobacteria and imposition of energy stress on *Rhizoctonia solani*. Soil Biol. Biochem., 27: 1051-1058.

Guru, V., Tholkappian, P. and Viswanathan, K. 2011. Influence of arbuscular mycorrhizal fungi and *Azospirillum* co-inoculation on the growth characteristics, nutritional content and yield of tomato crops grown in South India. Indian Journal of Fundamental and Applied Life Sciences, 1(4): 84-92.

Jackson, M. L. 1967. Soil Chemical Analysis. Prentice Hall of India, New Delhi.

Kassem, M., El-Ahmed, A., Hakim, M. S., El-Khaliefa, M. and Nachit, M. 2011. Identification of prevalent races of *Puccinia triticina* Eriks. in Syria and Lebanon. Arab Journal Plant Protection, 29(1): 7–13.

Kavitha, K., Meenakumari, K. S. and Sivaprasad, P. 2003. Effect of dual inoculation of native arbuscular mycorrhizal fungi and *Azospirillum* on suppression of damping off in chilli. Indian Phytopath., 56 (1): 112-113.

Kolmer, J. A. and Oelke, L. M., 2006. Genetics of leaf rust resistance in the spring wheat's 'Ivan' and 'Knudson' spring wheat. Canadian Journal of Plant Pathology, 28(2): 223-229.

Long, D. L. and J. A. Kolmer. 1989. A North American System of Nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopath., 79: 525-529.

Long, D. L., Leonard, K. J. and Hughes, M. E. 2000. Virulence of *Puccinia triticina* on wheat in the United States from 1996 to 1998. Plant Disease, 84(12): 1334-1341.

Mains, E. B. and Jackson, H. S. 1926. Physiologic specialization in the leaf rust of wheat *Puccinia triticina* Erikss. Phytopathology, 16: 89-120.

McCallum, B. D. and Seto-Goh, P. 2006. Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust in Canada in 2004. Can. J. Plant Pathol., 28: 566-576.

McIntosh, R. A. 2009. History and status of the wheat rusts. In RA McIntosh (ed.) Proc. Borlaug Global Rust Initiative 2009 Technical Workshop BGRI Cd Obregon, Mexico, pp.11-23.

Nadeem, S. M., Zahir, Z. A., Naveed, M., Arshad, M. and Shahzad, S. M. 2006. Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress. Soil Environ., 25: 78-84.

Ngoma, L., Babalola, O. O. and Ahmad, F. 2012. Ecophysiology of plant growth promoting bacteria. Scientific Research and Essays, 7(47): 4003-4013.

Pandey, H. N.; Menonm T. C. M. and Rao, M. V. 1989. A single formula for calculating Area under Rust Progress Curve. Rachis, 8: 38-39.

Park, R. F., Wellings, C. R. and Bariana, H. S. 2007. Preface to global landscapes in cereal rust control. Australian Journal of Agricultural Research, 58(6): p. 469.

Phillips, J. M. and Hayman, A. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for assessment of infection. Trans. British Mycolog. Soc., 55: 158-161.

Rabie, G. H., Aboul-Nasr, M. B. and Al-Humiany, A. 2005. Increased salinity tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungus *Glomus clarum* and a nitrogen-fixer *Azospirillum brasilense*. Mycobiology, 33(1): 51-60.

Ramos Solano, B., Barriuso Maicas, J., Pereyra de la Iglesia, M. T., Domenech, J. and Gutierrez Manero, F. J. 2008. Systemic disease protection elicited by plant growth promoting Rhizobacteria strains: relationship between metabolic responses, systemic disease protection, and biotic elicitors. Phytopathology, 98: 451-457.

Romero, A. M., Correa, O. S., Moccia, S. and Rivas, J. G. 2003. Effect of *Azospirillum*-mediated plant growth promotion on the development of bacterial diseases on fresh-market and cherry tomato. Journal of Applied Microbiology, 95: 832-838.

Saikia, S. P., Bora, D., Goswami, A., Mudoi, K. D. and Gogoi, A. 2012. A review on the role of *Azospirillum* in the yield improvement of non-leguminous crops. African Journal of Microbiology Research, 6(6): 1085-1102.

Samsampour, D.; Zanjani, B. M.; Pallavi, J. K.; Singh, A.; Charpe, A.; Gupta, S. K. and Prabhu, K. V. 2010. Identification of molecular markers linked to adult plant leaf rust resistance gene *Lr48* in wheat and detection of *Lr48* in the Thatcher near isogenic line with gene *Lr25*. Euphytica, 174(3): 337–342.

Schnurbusch, T., Bossolini, E., Messmer, M. and Keller, B. 2004. Tagging and validation of a major quantitative trait locus for leaf rust resistance and leaf tip necrosis in winter wheat cultivar Forno. Phytopathology, 94(10): 1036–1041.

Sridevi, S. and Ramakrishnan, K. 2010. Effects of combined inoculation of AM fungi and *Azospirillum* on the growth and yield of onion (*Allium cepa* L.). Journal of Phytology 2(1): 88-90.

Stakman, E. C., Stewart, P. M. and Loegering, W. Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. US Department of Agriculture, Agricultural Research Service, Publ. E617.

Tortora, M. L.; Díaz-Ricci, J. C. and Pedraza, R. O. 2012. Protection of strawberry plants (*Fragaria ananassa* Duch.) against anthracnose disease induced by *Azospirillum brasilense*. Plant and soil, 356(1-2): 279-290.

Wilson, M., Campbell, P. J., Jones, J. B. and Cuppels, D. A. 2002. Biological control of bacteria speck of tomato under field conditions at several locations in North America. Biological Control, 92: 1284-1292.

Zhang, J. X., Singh, R. P.,Kolmer, J. A., Huerta-Espino, J., Jin, Y. and Anderson, J. A. 2008. Genetics of leaf rust resistance in Brambling wheat. Plant Dis., 92:1111-1118.