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SEARCHING FOR RESISTANCE SOURCES TO WHEAT COMMON BUNT DISEASE AND EFFICIENCY OF BT GENES AGAINST *TILLETIA TRITICI* AND *T. LAEVIS* POPULATIONS

SUMMARY

Common bunt disease incited by Tilletia tritici and T. laevis, is one of the most important disease of wheat in Iraqi Kurdistan region. The disease can cause severe yield losses when the susceptible cultivars are grown without chemical treatment. This study was carried out to search for resistance sources to common bunt disease in wheat and also to determine the efficiency of Bt genes against the pathogen to be introduced in the future breeding program to improve disease resistance. Seeds of different wheat genotypes including the released cultivars and the differential varieties set were artificially inoculated with a mixture of the pathogen teliospores and planted at Bakrajo, Sulaimania for three successive generations. Disease scoring for each genotype was conducted at maturity stage. Results revealed that most of the tested wheat cultivars showed susceptible and intermediate reaction to the disease while the local wheat cultivars Ashor, Acsad, Farris1, Hasad, Waha, Simmetto and the promising advance lines Shaho, Hamada and Charmo2 showed high resistance level to the disease. The international resistance sources Nadro, carbidit, Togano, tillet and Firsal were highly resistant to the local pathogen population. The known resistant genes Bt₁ Bt₃, Bt₅, Bt₉, Bt₁₁ and Bt₁₂ were highly effective against the races of *Tilletia tritic*i and T. laevis under Sulaimania conditions.

Keywords: Cover smut, Triticum aestivum, Resistant genes, Host response, Iraq

INTRODUCTION

Fungal diseases are the most important biotic constraints for wheat production in Iraq. Several diseases, particularly rust and smuts, have drastically decreases grain yield and quality of wheat. Among the smuts, common bunt incited by *Tilletia tritici* and *T. laevis* is the most important disease. Occurrence and distribution of the disease was formerly limited in Iraqi Kurdistan region and the northern parts of Iraq. Yield losses reached up to 70% when the susceptible cultivars grown without chemical treatment (2, 4). Recently the disease was observed for the first time in the central and southern parts of Iraq. High disease

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incidences were detected in most wheat fields in the region during the last decade, which causes potential hazard to wheat production in the country (6).

Chemicals treatment of seed is regularly used to control the disease. Organic mercury compounds were widely used in the past (26). Historically, many poisoning problems were reported due to the direct or indirect consumption of treated seeds by farmers. Application of ethyl mercury (Cerasan) was the principle cause of human and livestock poisoning in 1956 and 1960 in Iraq, while seed treatment with methyl mercury compounds (Panogen and Methyl Mercury Acetate) caused severe poisoning in 1972, where 459 people dead and other 6530 were poisoned in Iraq (9). Later on Diathane M-45 was widely used to protect wheat from the infection (5). Other previous studies confirmed efficacy of some other chemical compounds and fungicides (1, 13, 20). Recently Al-Maaroof et al., (2004, 2011) indicates the high efficiency of Mancozeb, Diathane, Dividend and Lamardor in wheat protection from bunt diseases (5, 8). Chemical seed treatment is not safe and not allowed in organic agriculture and several active ingredients will be banned from the Phytosanitary register in near future (32). The deployment of genetic resistance is one promising option to efficiently control the disease in an environmentally friendly manner. Specific gene for resistance to common bunt are tabulated by McIntosh, 1983 (33), while the occurrence of bunt races with specific virulence that can overcome of the specific resistance of the host greatly increase the difficulty of attaining long term effective control (22, 34). Major breeding efforts have been made to develop multiple gene resistance. Plant breeders mainly depend on the cultivars Martins containing resistant gene Bt1, Turkey (Bt4) and Redit (Bt13) as a source for resistance to improve disease resistance against cover bunt and dwarf bunt diseases (28). The resistant source PI178383 contain resistance genes Bt8, Bt9, Bt10 confer durable resistance for more than 20 years in USA, this was due to the lack of virulence against Bt8 in the pathogen races, while virulence's against this gene was found in European races (16, 31). Many studies were conducted in Iraq to improve wheat resistance to the disease using different breeding program. Ibrahim et al. (1988), able to get 42 resistant mutants by irradiation Saberbeg and it hybrid with Maxipak by different doses of gama rays and fast neutrons (24), two mutant cultivars with moderate resistance to the disease were also developed (23). Al-Maaroof et al., (1993) obtained 15 variants resistant to covered smut for four successive generations (M4-M7) by irradiating Saberbeg hybrids with Maxipak, Ajeeba, Araz and Abu Ghraib with different doses of fast neutrons (3). Furthermore, two mutant wheat cultivars resistant to covered smut and Septorial leaf blotch were developed by mutation teqniuges (4). Many Advance resistant lines of wheat against common bunt, leaf rust and yellow rust diseases were developed from the resistant sources and their hybridization with the local cultivars (7, 10). The new resistant cultivars Farris, Hamada, Alaa, Hsad and

Maaroof were recently registered by the Ministry of agriculture and released to the farmers as new sources for resistant (34).

The current study conducted to search for resistance sources to common bunt disease in the international wheat sources for disease resistance and the local wheat cultivars. Also, to determine the efficiency of Bt genes against the bunt pathogen population under Iraqi environmental conditions to introduce them in the commercial susceptible wheat cultivars to improve bunt disease resistance program and grain quality of wheat in Iraq.

MATERIAL AND METHODS

Three separate experiments were conducted at Faculty of Agricultural Sciences Field, Sulaimani University (N 35°32`351``, E 45°21`978``), about 15 km north west of Sulaimania province in the Iraqi Kurdistan region. The first experiment include 36 registered and released wheat cultivars, 30 bread wheat and 6 durum wheat cultivars obtained from the state board for seed testing and certification, Iraqi Ministry of Agriculture, The second experiment include 22 resistant wheat cultivars to common bunt disease from the international wheat collections introduced from the European Tilletia Ringtest (ETR). It has been initiated involving several international breeding and research institutes including Sulaimani University based on a Memorandum of Understanding (MoU). The exchange of material was governed either by MTAs (material transfer agreement) or by the breeders' privilege according to the UPOV convention. The third experiments include differential variety set for common bunt disease including 15 genotype that contains resistant genes Bt1 to Bt15 for identification of T. tritici and T. laevis races. The winter wheat Red Bobs were used as universally susceptible lines (designated in previous work as Bt0) in field nurseries to indicate disease pressure in the experiment. Seed of common bunt differential set was obtained from Dr. Blair Goates (USDA-ARS, Aberdeen, Idaho, USA). Seeds of each wheat genotype from the above mentioned experiments were artificially inoculated with a bulk population of T. tritici, T. laeves and T. intermedia teliospores collected from different locations in the previous season at a rate of 0.5 g/100 g seeds. Inoculated seeds were mechanically mixed for 15 minutes by shaker at 80 rpm/minute (19). Each genotype was planted by hand at a depth of 5 cm in two 1.5 m rows (5 gm seed/line) with 30 cm apart between lines and 60 cm between treatments. Planting were done by the mid of December for two successive season (2011-12 and 2012-13) when the soil temperatures were 5-10°C. Plots were arranged in Randomized Complete Block Design (RCBD) with three replicates and two meters space between blocks. Field management and all the agricultural practices were conducted according to Ministry of Agriculture recommendations. Infection percent of each genotype from the first two experiments was calculated at dough stage by counting number of healthy and infected spike per each meter according to Dodoff and Todorova, 1974 modified method (15) where R= Resistant (Infection percent 0-10%), I= Intermediate resistant (Infection Percent 11-30%), S= Susceptible (Infection Percent 31-50%),

HS= Highly Susceptible (Infection Percent 51-100%). Data were statistically analyzed by using ANOVA table at L.S.D. 0.05.

Data from each differential line were averaged to determine a virulent-resistant (0-10% spike infection) or virulent-susceptible (11-100% spike infection) in accordance with Hoffman and Metzger method (21).

RESULTS AND DISCUSSION

Table 1 shows that there are a wide range of differences in the response of the tested wheat cultivars against T. tritici and T. laevis population under artificial inoculation condition in Sulaimania. The registerd and released wheat cultivars can be divided into four groups according to their average resistance to the disease. The first group includes the cultivars (Farris 1, Ashoor, Hasad, Hamada, Charmo 2, Cimmeto and Acsad), which are characterized by their resistance to the disease and significantly surpassed the other groups during both seasons. The mean infection percent of this group was between 2.36% in cv. Acsad to 9.73% in cv. Waha. The second group included cultivars (Iratom, Tamuz 2, Tamuz 3, Furat, Al-Iraq, Sham 6, Alaa, Maaroof, Shahoo, Azmar, Azad, Um Rabia and IPA'99) characterized by their intermediate resistance to the disease ranging from 12.7% in cv. IPA'99 to 27.5% in cv. Maaroof, while the third group included the susceptible cultivars to the disease (AbuGhraib, Araz, Rabia, Babel, Rasheed, Latifia, Sali, Hashmia, Tahedi, Fateh, IPA'95 and Al-Ize with mean infection rate of 31.98% in cv. Rasheed to 44.10% in cv. Sali. The fourth group was represented by the highly susceptible wheat cultivar SaberBeg and Al-R.V23 with means infection percent of 57.38 and 50.55 % respectively.

Combined analyses results revealed that the mean infection percent of the cultivars with common bunt disease in 2011-12 significantly surpassed the mean infection percent in 2012-13. This is significantly reflected on the response of Cvs SaberBeg, Babel, Tamuz 3, Furat, Sali, Hashmia, Fateh, IPA'95, Sham6, Al-Ize and Rasheed in 2011-12 season. The infection percent was higher between 23% in cv. IPA'95 to 68% in cv. Tamuz 3 in the first season comparing with the second season. The high infection incidence in the first season my be attributed mainly to the favorable environmental conditions for infection and disease development during 2011-12 particularly temperature, humidity and light. This also led to significant variation in host response of some genotypes "Babel, Tamuz 3, Al-Ize, Maaroof, Azad and Whaha" to the disease between seasons. Results of the study revealed change in resistance catagories of some wheat cultivars in comparing with the previous studies results. This was clear in response of cv. Tamuz 2 and cv. Iratom wich changed from intermediat resistant to resistant and from intermediate resistance to susceptible in cv. Al-Ize (38). Resistant changes of this cultivars may be attributed to the appearance of new

races in the pathogen population as it is also was confirmed in previous studies (6, 7).

The high level of resistance in Durum wheat cultivars may be correlated to the inoculum component of the pathogen species used in the artificial inoculation in the study. Frequency incidence of *T. tritici* was more prevalent than *T. laevis* and T. *intermedia* in the mixed population inoculum, which is more virulent on bread wheat cultivars than durum wheat cultivars (38).

Results of Table 2 revealed that most of the introduced international resistant sources showed resistant to moderately resistant reaction to the local T. tritici and T. laevis populations under artificially inoculation conditions. The genotypes Nadro, Carbidit, Tillet, Togano, Casan, Frisal, Skagen, Torrild, Tirone, Tommi, Rehti, Ridit and Rio showed resistant reaction to common bunt disease. The mean infection percent of the resistant genotypes varied from 0.0% in the genotypes Casan, Frisal, Tommi and Rio to 7.75% in the resistant genotype Tillet which was significantly less than all other intermediat resistance and susceptible genotypes. The genotypes Fiorina, Lorenzo, Surita and Urho showed intermediat resistant to the disease. The mean infection percent in this group were from 16.91% in Surita to 29.20% in Urho, which were significantly less than the infection percent of the susceptible group except Urho. The susceptible group include the genotypes, Grenia, Sertori, Lona 59 and Segor. No significant differeces were found in the infection percent of this group with the local wheat cultivars. On the other hand, the genotype Runal showed highly susceptible reaction to the disease with infection percent of 62.53%, which were significantly, surpassed all other genotypes including the local cultivar Araz. No significant differences were found betwen the seasons although infection percent was higher in the first season. The high resistance level in the resistant sources Casan, Frisal, Tommi and Rio encourage their introduce in breeding program for common bunt disease improvement in Iraq.

Host parasite interaction of common bunt resistant genes (Bt genes) with the prevalent populations of *T. tritici* and T. *laevis* under artificial inoculation conditions in Sulaimania are presented in Table 3. Results revealed that Bt1, Bt2, Bt4, Bt7, Bt10, Bt113, Bt14 and Bt15 genes showed high infection type to the pathogen population, while Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 genes showed low infection type to the pathogen population at both seasons. Therefore, it turns out the possibility of exploiting Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 in breeding programs for improving common bunt disese resistance in Iraq.

Cultivar	Wheat Type ¹	2011/12 season		2012/13 season		Mean	
		Infection ² %	Infection Type	Infection%	Infection Type	Infection%	Infection Type
SaberBeg	BW	67.42	HS	47.33	S	57.38	HS
AbuGhraib	BW	36.76	S	43.07	S	39.92	S
Araz	BW	43.29	S	39.02	S	41.12	S
Rabia'	BW	32.30	S	45.40	S	38.85	S
Iratom	BW	16.20	Ι	13.12	Ι	14.66	Ι
Babel	BW	47.40	S	22.03	Ι	34.72	S
Tamuz2	BW	17.65	Ι	22.93	Ι	20.29	Ι
Tamuz3	BW	23.35	Ι	07.33	R	15.34	Ι
Furat	BW	25.60	Ι	15.03	Ι	20.32	Ι
Al-Iraq	BW	15.30	Ι	13.87	Ι	14.59	Ι
Farris1	BW	5.10	R	1.57	R	3.34	R
Rasheed	BW	39.13	S	24.83	Ι	31.98	S
Latifia	BW	35.77	S	29.60	Ι	32.67	S
Sali	BW	53.80	HS	34.40	S	44.10	S
Hashmia	BW	47.40	S	35.47	S	41.44	S
Tahedi	BW	37.80	S	32.50	S	35.15	S
Ashoor	BW	9.10	R	3.10	R	6.10	R
Fateh	BW	43.33	S	31.40	S	37.37	S
IPA'95	BW	47.66	S	36.93	S	42.30	S
Sham6	BW	26.78	Ι	17.33	Ι	22.06	Ι
Al-Ize	BW	48.40	S	27.17	Ι	37.79	S
Alaa'	BW	26.09	Ι	18.20	Ι	22.15	Ι
Maaroof	BW	31.33	S	23.66	Ι	27.50	Ι
Hsad	BW	9.66	R	5.30	R	7.48	R
Hamada	BW	4.86	R	9.20	R	7.03	R
Shaho	BW	20.60	Ι	14.8	Ι	17.70	Ι
Azmar	BW	14.96	Ι	10.50	Ι	12.73	Ι
Charmo 2	BW	7.40	R	8.90	R	8.15	R
ALR.V23	BW	60.40	HS	40.70	S	50.55	HS
Azad	BW	36.6	S	13.2	Ι	24.9	Ι
Um Rabee'	DW	26.78	Ι	14.8	Ι	20.79	Ι
Cimmetto	DW	3.71	R	5.30	R	4.51	R
IPA'99	DW	22.26	Ι	14.96	Ι	18.61	Ι
Acsad	DW	2.02	R	2.70	R	2.36	R
Sawa	DW	43.66	S	38.33	S	40.99	S
Waha	DW	2.66	R	16.80	Ι	9.73	R
Mean		28.68		21.69		25.18	
L.S.D 0.05 C S Cult	Cultivars easons t. * Seas.	9.73	-	8.55		9.03 6.75 9.35	

Table 1. Host reaction of registered and released wheat cultivars with common bunt disease under artificial inoculation conditions during 2012-2013 seasons at Bakrajo, Iraq

	201	1/12	201	2/13	Mean	
Genotype/ Cultivar	Infection ¹ %	Infection ² Type	Infection %	Infection Type	Infection %	Infection Type
Greina	40.73	S	36.57	S	38.65	S
Runal	65.06	HS	60.00	HS	62.53	HS
Nadro	0.00	R	6.20	R	3.10	R
Carbedit	1.64	R	6.50	R	4.07	R
Fiorina	27.19	Ι	23.9	Ι	25.54	Ι
Sertori	39.90	S	34.13	S	37.02	S
Lona 59	24.60	Ι	35.70	S	30.15	S
Tillet	13.10	Ι	2.40	R	7.75	R
Lorenzo	23.46	Ι	20.36	Ι	21.91	Ι
Togano	0.00	R	11.20	Ι	5.60	R
Surita	18.93	Ι	14.90	Ι	16.91	Ι
Casan	0.00	R	0.00	R	0.00	R
Segor	43.10	S	30.80	S	36.95	S
Frisal	0.00	R	0.00	R	0.00	R
Skagen	0.00	R	3.20	R	1.60	R
Torrild	0.00	R	5.40	R	2.70	R
Tirone	33.7	S	31.0	S	2.35	R
Tommi	0.00	R	0.00	R	0.00	R
Rehti	0.00	R	3.60	R	1.80	R
Urho	37.10	S	21.30	Ι	29.20	Ι
Ridit	3.60	R	1.90	R	2.75	R
Rio	0.00	R	0.00	R	0.00	R
Araz	45.35	S	43.66	S	44.51	S
Mean	18.15		17.07		16.31	
L.S.D 0.05 genotypes Seasons Genot *Seas	13.10		11.35		11.66 n.s 12.31	

Table 2. Mean infection percentage of different wheat genotypes with common bunt disease under artificial inoculation conditions during 2012-2013 at Bakrajo, Iraa

Table 3. Host parasite interaction of common bunt differential varieties (Bt genes) with a mix population of the pathogens under artificial inoculation conditions at faculty of agricultural sciences field, bakrajo, sulaimania, Iraq.

Entry	Differential Variety [*]	Resistant	Infection Type **		
No.	Differential vallety	genes	2012	2013	
1	M84-504 to 510, Red Bobs	Bt0	Н	Н	
2	M84-512 to 520, RB/WF 8	Bt1	L	Н	
3	M84-522 to 530, RB/SEL 1403	Bt2	Н	Н	
4	M84-532 to 538, RB/RDT.	Bt3	L	L	
5	M82-542 to 550, RB/TK 3055	Bt4	Н	Н	
6	M82-34, Promose	Bt5	L	L	
7	M84-552 to 560, RDT.	Bt6	L	L	
8	M82-562 to 570, RB/TK 3055	Bt7	Н	Н	
9	M78-9496, RB/PI 178210	Bt8	L	L	
10	M84-597 to 605, RB/CI 7090	Bt9	L	L	
11	M84-625, SEL M83-162	Bt10	Н	Н	
12	M82-2123	Bt11	L	L	
13	P.I. 119333(M82-2141), BW	Bt12	L	L	
14	Thule III; P.I. 181463, BW Bt 13	Bt13	Н	Н	
15	Doubi, DW	Bt14	Н	L	
16	Carlton, DW	Bt15	Н	Н	
	Number of virulence's		7	7	

*. Source of differential varieties seeds is Dr. B. Goates (USDA-ARS), Aberdeen, Idaho, USA.

**. Infection Type: L= Infection less than 10%, H= Infection more than 10% according to Hoffman and Metzger, 1974

Results of this study confirms efficiency of the known resistance genes Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 in resistance of most preavelent *T. tritici* and *T.leavis* races in most of wheat fields in Iraq according to what has been referred

by many other researchers in previous studies in the world (12, 26, 28, 37 and 39). The high level of resistance in the known resistant genes Bt9, Bt11 and Bt12 against the pathogen population at both seasons encourage their implementation in the breeding programs for common bunt disease resistance in wheat through their use as parents in hybridization with the high yielding susceptible commercial wheat cultivars, particularly most of Iraqi wheat cultivara are susceptible to the disease (6 and 24). Mamluk and Nachit (1994 and 199), (28, 29) confirmed the effectiveness of the resistance genes Bt5, Bt8, Bt9, Bt10 and Bt11 in differential varieties in Syria. On the other hand, some previous studies referred that the pathogen races overcome resistance of Bt1, Bt2, B3, Bt4, Bt6 and Bt7 in Syria and resistance genes Bt1, Bt2 and Bt7 in Lebanon (25 and 29). In Turkey pathogen isolates has possessed virulence against the resistance genes Bt1, Bt2, Bt3, Bt4 and Bt7, while the Iranian isolates showed virulence against resistance genes Bt4, Bt7 and BtP (14, 17, 36). It was found that virulence against the resistance genes Bt2, Bt5, Bt7, Bt8, Bt9 and Bt10 were prevalent in pathogen communities in India (12). The resistant genes Bt9 and Bt10 showed high efficiency in resistance of pathogen races in Europe followed by genes Bt5, Bt6 and Bt8 (11, 16, 30 and 37). Breeding for common bunt disease resistance in the USA depend mainly on the genotype PI 178383 of Turkish origin which possesses resistance genes Bt8, Bt9 programs, Bt10 and one of undefined resistance genes. These genes also adopted in breeding programs for disease resistance in both former Russia and Australia (18).

CONCLUSIONS

The current study results refer to changes in resistance categories of some commercial wheat cultivars due to appearance of new pathogen races, While wheat Cvs Farris 1, Ashoor, Hasad, Hamada, Charmo 2, Cimmeto and Acsad are characterized by their resistance to the disease. It can be also concluded that the high levels of resistance in the international resistant sources Casan, Frisal, Tommi and Rio and the known resistance genes Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 turns out the possibility of exploiting them in breeding programs for improving common bunt disease resistance in Iraq. In conclusion, there are urgent needs to use all the technological possibilities to control bunt disease resistance and improve grain quality of wheat.

- 1. BW= Bread Wheat, DW= Durum Wheat,
- 2. Each number is representing the mean of three replicates,
- 3. R= Resistant (Infection percent 0-10%),
- I= Intermediate resistant (Infection Percent 11-30%),
- S= Susceptible (Infection Percent 31-50%),

HS= Highly Susceptible (Infection Percent 51-100%) according to Dodoff and Todorova, 1974 modified method.

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