RESISTANCE OF IRAQI WHEAT CULTIVARS TO COMMON BUNT DISEASE AND MOLECULAR DIAGNOSIS OF THE AVIALABLE BT GENES IN EACH CULTIVAR

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ABSTRACT

This study was conducted to identify resistance sources in the Iraqi wheat cultivars using pathological and molecular approaches. Results revealed that forty-four reeleased wheat cultivars were divided into four groups according to their mean infection percentage to common bunt disease. Resistant group consists of wheat cultivars Farris 1, Ashur, Tamuz 3, AlMadian, AlBaraka, Latiffiya, and Rabia. The mean infection percentage of this group ranged from 0.28-3.98% and significantly was higher than that of all other groups during two growing seasons. Moderately resistant cultivars included cv's Bura, Iratom, Charmo, Maaroof, and AlRasheed with mean infection range of 10.39- 21.10%. The majority of the tested cultivars (63%) explored high susceptibility to the disease (52.14-91.97%) while the mean infection of the susceptible group ranged from 31.51% in Buhoth 22 to 47.47% in Wafia. Molecular analysis using SSR markers GWM7433, XGWM114, and XGWM264 revealed that Charmo and Hsad- possess the resistance gene Bt9 at 296bp, while Buhoth 10 possesses the known resistance gene Bt12 at 175bp. All the tested cultivars not contain the known resistance genes Bt8, Bt10, and Bt11. The high resistance level of the resistant group could be attributed to the presence of additional known resistance Bt genes or other unidentified resistance genes.

Key words: *Triticum aestivum*, covered smut disease, disease resistance, molecular markers, fungal diseases.

مقاومة أصناف القمح العراقية لمرض التفحم المغطى والتشخيص الجزيئي لمورثات المقاومة المتاحة في كل صنف

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المستخلص

أجريت الدراسة الحالية لتحديد مصادر المقاومة في أصناف القمح العراقية باستعمال الطرائق المرضية والجزيئية. أسفرت النتائج عن تصنيف أربعة وأربعين صنفاً معتمدا من القمح إلى أربع مجاميع وفقا لمتوسط إصابتها بمرض التفحم الشائع. تآلفت المجموعة المقاومة من أصناف القمح فارس 1، عاشور، تموز 3، المدائن، البركة، اللطيفية، والربيع. تراوحت معدل نسبة إصابتها بين 0.28–3.98٪ وتفوقت معنوياً على بقية المجاميع خلال موسمي النمو. ضمت مجموعة الأصناف معتدلة المقاومة كل من الأصناف بورا، إيراتوم، جرمو، معروف، والرشيد، بمتوسط إصابة بين 10.39– 21.10٪. تميزت غالبية الأصناف المختبرة (63٪) بحساسيتها العالية للإصابة بالمرض (52.14–91.97٪) بينما تراوح متوسط إصابة المجموعة الحساسة بين 31.51٪ في الصنف بحوث 22 إلى 47.47٪ في الصنف وفية. أظهر التحليل الجزيئي باستخدام واسمات ال SSR، GWM7433 و XGWM 114 و XGWM 264 امتلاك أصناف القمح جرمو و حصاد على مورث المقاومة Bt9 عند 296 زوج قاعدي ، بينما امتلك الصنف بحوث ١٠ مورث المقاومة المعرف Bt12 عند 175 زوج قاعدي. بينما افتقرت جميع الأصناف المختبرة على مورثات المقاومة المعرفة Bt8 و Bt10 و Bt11. قد يُعزى مستويات المقاومة العالية لمجموعة الأصناف المقاومة إلى وجود مورثات مقاومة إضافية أخرى غير مشمولة بالبحث أو مورثات مقاومة أخرى غير محددة.

كلمات مفتاحية: *Triticum aestivum*، مرض التفحم المغطى، مقاومة العائل، واسمات جزيئية، امراض فطرية.

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INTRODUCTION

Wheat "*Triticum aestivum"* is a common food worldwide (13, 20, 21), It is the primary source of income for 35% of the population and accounts for 20% of global spending (14, 15, 25, 38). Common bunt disease is a major biotic impediment to wheat production across the world. It could cause 75%losses, particularly in wheat-growing areas where the seeds did not treat with fungicides (27). During the epiphytotic years, the fungus can cause the greatest yield losses (30). The disease also called stinking smut due to synthesis of trimethylamine in the teliospores, which gives the disease a distinctive fishy odor even at contamination levels as low as 0.1 percent by volume (16). Seed treatment with fungicides is widely used to prevent the disease spread and obtain uncontaminated agricultural products (29). Modern fungicides successfully eliminate spores on seeds and in the soil, which reduces losses in agricultural output. The use of seed dressing protectants, on the other hands, has negative impact on the environment and human health. This method of protection is improper for organic farming because it is not profitable (1, 31). However, since synthetic pesticides are prohibited in organic farming, growers must mostly rely on genetic disease resistance (30). The disease is widespread and serious in most of Asian countries, including Al-Jezera and northern Iraq (4). Most of the low input or organic fields that used untreated seeds had yield losses of up to 70%. A new epidemic of the disease was recently affected the central and southern parts of Iraq. This posed a threat to the country wheat production because the majority of wheat cultivars were highly susceptible to the new races and had a high disease incidence (6). The frequency of bunt contamination in the field is directly related to embryonic infection. Despite the fact that fungicidal seed treatment has minimal impact on the disease severity, it is still necessary to manage common bunt disease since it can still infect crops. According to numerous studies, combating common bunt disease should be diverse and complicated involving chemical application, agronomic, and biological methods, as well as the cultivation of resistant cultivars, particularly in the organic wheat crop (32). Bt genes are responsible for resistance to the common races of bunt pathogen. The main resistance genes that have been identified and added to the gene banks are Bt1 to Bt15 and Btp (24, 37). Genetically, the Bt8, Bt10, and BT11 genes are tied to the 6DS chromosome's distal end, while the Bt12 is linked to chromosome 7Ds. Meanwhile, Bt9 was discovered to be a distinct component on chromosome 6DL's terminal end (40, 41). The pathogen population in Iraq were able to overcome the known resistance genes Bt2, Bt4, Bt7, Bt10, Bt13, Bt14, and Bt15. However, under artificial inoculation conditions in the field, the resistance genes Bt3, Bt5, Bt6, Bt9, Bt11, and Bt12 effective against the pathogen (8). Using molecular markers in addition to conventional methods is one strategy to improve the success of breeding programs for resistance. The capacity to assess genetic resistance to plant diseases and pests will increase with the use of molecular markers (10, 33). The development of long-term resistant wheat cultivars ensures field safety, quality, cost and yield stability, particularly during epiphytic years. The most cost-effective strategy to protect plants while reducing the pesticide load on agroecosystem is to cultivate common bunt resistant wheat cultivars, given rise the expense of seed protectants fungicides and their environmental hazard (5). The aim of this study is to identify common bunt disease resistant cultivars and molecular diagnosis of the available known Bt resistance genes in the genetic background of bread wheat and triticale cultivars for the first time in Iraq.

MATERIALS AND METHODS

This research was conducted at the experimental research fields of the college of Agricultural Engineering Sciences, Bakrajo (N35˚ 32.351, E45˚ 21.978), about 15km northwest of Sulaimania province in the Iraqi Kurdistan region, and plant biotechnology lab. of the biotechnology and crop science dept. College of Agricultural Engineering Sciences, University of Sulaymaniyah, IKR, Iraq, during 2020 to 2022. Forty-two Iraqi registered and released bread wheat (*Triticum aestivum* L.) and two triticale cultivars (*Triticosecales*) were used in this study (Table 1).

Host response of wheat cultivars to common bunt disease: Seeds of all wheat cultivars were artificially inoculated with a bulk teliospores of (*Tilletia tritici, T. laevis,* and *T. intermedia*) collected from various infected fields with common bunt disease in the previous season in Sulaimani. The inoculum was prepared by gently grinding the bunt balls to teliospore powder and sieving it through a 500-mesh. Artificial inoculation was conducted at a rate of 0.5g teliospores/100g seeds. contaminated seeds were shaken gently by hands in all directions then placed in an electric shaker and mixed further for 15min at a rate of 80 rpm/minute to guarantee the homogenous mixing and well distribution of the teliospores on the surface of the contaminated seed (6). Each cultivar sown in three rows (1.5m length and 5cm depth) at a seed rate of 5g/1m length using RCBD with three replications. The first two rows were sown with inoculated seeds and the third one used as control. Row to row space was maintained 30cm, and the distance between blocks was one meter with 3m distance between experiments. All the agricultural managements were conducted as it is recommended. Disease scoring was conducted at dough stage, all the spikes in one-meter length center of the plant stands were manually harvested and labeled then placed in plastic bags and transferred to the lab. Infection percent of each genotype to common bunt was calculated by counting the number of healthy and infected spike per meter length as below.

$$\%\text{Infection} = \frac{\text{Number of Infected Spikes}}{\text{Total Number of Spikes}} * 100.$$

Response of each genotype to bunt disease was evaluated according to Dodoff and Todorova, (19) modified method where R= Resistant (Infection percent 0-10%), I= Intermediate resistant (Infection Percent 11-30%), S= Susceptible (Infection Percent 31-50%), and HS = highly susceptible (infection percent 51–100%) (9). All the data were statistically analyzed using analyses of variance (ANOVA). Least significant differences at 5% level were used to compare the mean of treatments.

Postulating of Bt resistance genes in wheat cultivars: Forty-four wheat cultivars were assessed for possessing five Bt genes. Saberbeg was used as negative control and the Bt differential stock lines 554120 Bt8, 554098 Bt11, WESTON Bt10, 554099 Bt9, and 16160 Bt12 were used as positive control. Genomic DNA was extracted using the kit approach from 20 days old wheat cultivars. Each cultivars fresh leaves were placed in liquid nitrogen container and ground with a pestle and mortar, 100mg of this product was used for DNA extraction in accordance with the Sinaclon procedure (www.Sinaclon.com). Amplified DNA fragments were separated in 2% Agarose gel using a TBE buffer 1x solution supplemented with 4µl of ethidium bromide. PCR protocol used for amplifying Bt genes in SSR markers (30). Bt8, Bt10 and Bt11 genes were discovered using the Xgwm114 (forward: ACAAACAGAAAATCAAAACCCG, reverse ATCCATCGCCATTGGAGTG) (18), Bt9 was discovered using the Xgpw7433 (forward: GTACATGGAAAGAGACCAACA CCA, reverse CGCTGAGCAAGGACGATAG) (40), and Bt12 was discovered using the Xgwm264 marker (forward: GAG AAA CAT GCC GAA CAA CA, reverse: GCA TGC ATG AGA ATA GGA ACTG) (36). PCR amplification was carried out in a total reaction volume of 25µl, which contain1µl of F and 1µl R primers, 2µl DNA material, 12.5µl ready-to-use Taq master mix, and 8.5µl of free nuclease water packed in 0.2ml PCR strip tubes. A Maan amplifier (M1000-G Thermal cycler) with the following programming was used for the amplifications: Initial denaturation at 94C for three minutes; 45 cycles of one minute each at 94C°, 58C°, and 72C°; and a final elongation for seven minutes at 72C°. The PCR programs were modified depending on the identified gene (18, 40).

Table 1. Name, pedigree and origin of the registered and released bread wheat and triticale cultivars and other introduced genetic materials used in the Experiments

Cultivar	Synonym	Wheat Type	Pedigree	Year of release	Origon
AlRasheed	Al-Mansur Bellah	BW	Max.(Rad.)	2001	Iraq
Saberbeg (White)	Qandeharia	BW	Land race	n.a	Iraq
Iba'a 99	Al-Farris	BW	Ures/Boww/oowwJup/ Biyiyiy Biyiy	1997	CIMMYT
Latiffiya	None	BW	Australian Line/Aras	1995	Iraq
Abu Ghraib	None	BW	Ajeeba/Inia66/ Mexico24	1973	Iraq
Bura	None	BW	H31/Trapf21/Enesco	2014	Italy
Buhoth 158	None	BW	118//S2/57-S2-CR7-S2	2012	Iraq
AlBaraka	hh	BW	IARI*STD	2013	Iraq
Tamuz 2	None	BW	Sab./Max. (Rad.)	2012	Iraq
Uruk	None	BW	Inia 66 (Rad.)	2013	Iraq
Saberbeg (Red)	Qandeharia	BW	Land race	n.a	Iraq
Iba'a 95	None	BW	Veery eer	1995	CIMMYT
AlFateh	None	BW	CI8224/CI6833/Conz/7IIC/Top-Swm6328/9AP-OAP//Max.	2001	Iraq
Buhoth 10	None	BW	Ibaeh 10 -Swm63	2013	Iraq
Adana 99	None	BW	PFAU/SERI-M-82/BOBWHITE	2012	Turkey
Baghdad 3	None	BW	-	2014	Iraq
Cham 6	Sham 6	BW	PLC"S"-Ruff"S"/Gta"S"-RTTCM-12904-1M-3M-1Y-1Y-OSK-OAP	2000	ICARDA
Buhoth 22	None	BW	CMSS96Yo3204F-PJN/Bow//OPATA	2011	CIMMYT
Iratom	Iratom 1	BW	Sab. (Rad.)	1992	Iraq
Hawler 4	None	BW	Baj#1/3/KirijAI/A*2/PastorCMSSOYOO288S-OS8-099Y-99M-099Y3M-OWGY	2018	CIMMYT
Baghdad 1	None	BW	MX105-6MVLT40/BNSN	2012	CIMMYT
Ashur	AlTahadi	BW	Sab./Max.	1992	Iraq
Rabia	1610	BW	Sab./HD (Rad.)	1994	Iraq
Farris	Farris	BW	STAR/TR771773/SLM	2012	Iraq
Hsad	None	BW	SNB//CMH79A955/3*CNO79/3/ATTILA	2014	Iraq
Slemani 2	Kauz*2	BW	Kauz*2	2016	CIMMYT
Aras	None	BW	Sonora 64/ Lerma (Rojo 64) // Sentaclena	2016	Iraq
Alaa	None	BW	ESDA/VEE*10	2014	Iraq
Maaroof	None	BW	KTN M12/DAMANxADL//TAMUZ2	2014	Iraq
Babel 113	None	BW	Max./R23	2000	Iraq
Bingal	None	BW	BISU/3/YAV79/ALO1/ALTARS4/CD93683.7Y.040M-03OY-LPAP.B	2013	Spain
Hawler 2	None	BW	Florkwa2/6/Sakers/5//YMH/TOB/4/BOWS-LC96-0180-030APS-2AP-DAPS-OPA	2018	CIMMYT
Al-Ize	AlIze 66	BW	Najah/Maxipak(Rad.)	1997	Iraq
AlMadian	AlNeda'	BW	Sab./Max. (Rad.)	1992	Iraq
Barcelona	None	BW	-	-	Spanish
Al-Iraq	AlKaed278	BW	Max.(Rad.)	1999	Iraq
Tamuz 3	None	BW	Sab./Max.*AbuGh.(Rad.)	1992	Iraq
Wafia	None	BW	-	2015	France
Al Nur	None	BW	Car853/Coc/Vee/3/Bow	1997	ICARDA
Azmar	None	BW	CMH 83/ ELVIRA// CMH 79/ AGA/ INIA (Rad.)	2020	Iraq
Sawa	AlNakhwa	BW	SaberBeg/Maxipak (Rad.)	2000	Iraq
Charmo	None	BW	CMH79A/AGA/CNO67//INIA66/NAC/BABAX (Rad.)	2020	Iraq
Rezan	None	T	MILAN/2*ERIZO11/4/ERIZO9//TOPO1419/ARDI1/3/6HBIDA	2018	Iraq
Sarah	None	T	ASAD/ELK54//ERIZO10/3/CHAKAL-5/4/ARD-1/TOPO	2020	Iraq

RESULT AND DISCUSSION

Host response of wheat cultivars to common bunt disease: Host response of the registered and released wheat cultivars to common bunt disease under artificial inoculation conditions during two growing seasons (2020-21and 2021-22), is shown in Table (2). A wide range of variations in the responses of the tested wheat cultivars against the populations of *T. tritici* and *T. laevis* were found in both seasons

under artificial inoculation conditions at Bakrajo, Sulaimani. Bread wheat cultivars could be divided into four groups according to their mean infection percentage to common bunt disease. Goup one consists of the disease-resistant wheat cultivars Farris 1, Ashur, Tamuz 3, AlMadian, AlBaraka, Latiffiya, and Rabia. The mean infection percentage of this group was significantly higher than that of all other groups during both growing seasons. No significant differences were detected among group one members. The mean infection percentage of group one ranged from 0.28% in Farris 1 to 3.98% in Rabia' (Table 2). Farris 1 significantly outperformed all other groups cultivars in terms of resistance except cv.'s Iratom and Bura. Group two included only five bread wheat cultivars "Bura, Iratom, Charmo, Maaroof, and AlRasheed that were characterized by their intermediate resistance to common bunt disease. The mean infection percentage of this group ranged from 10.39% for cv. Bura to 21.10% for cv. AlRasheed. No significant differences were found in the mean infection percent among group two members. Group three represents the susceptible cultivars including wheat cultivars Buhoth 22, Iba'a 99, Azmar, and Wafia. The mean infection percentage of this group ranged from 31.51% in Buhoth 22 to 47.47% in Wafia which explore higher significant mean infection than others. The remaining wheat cultivars were placed in the fourth group of highly susceptible cultivars. This group which makes the majority of the tested cultivars (63%), is represented by the following cvs: AlFateh, Uruk, Baghdad 3, Iba'a 95, Adana 99, Saberbeg white, Saberbeg red, Hsad, Al-Ize, Kalar 1, Hawler 4, Baghdad 1, Bingal, Kalar 2, Alaa, Buhoth 10, Slemani 2, Aras, Abu Ghraib, Cham 6, Hawler 2, Al-Iraq, Tamuz 2, Babel 113, AlNur, Barcelona, and Buhoth 158 (Table 2). The mean infection percent of the highly susceptible group ranged between 52.14% in wheat cultivar Buhoth 158 to 91.97% in cv. AlFateh. No significant differences were detected in the mean infection percent of the cultivars in both seasons. Host-parasite interaction of the high susceptible cultivars with common bunt disease was stable in both agricultural seasons with no significant differences except in the cv.'s Al-Ize, Sawa, Aras, Hawler 2, Al-Iraq, Tamuz 2 and Babil 113 that explore higher infection percentage in 2022, while infection types of AlNur and Barcelona were significantly changed from Susceptible in 2021 to highly susceptible in 2022. On the other hand, the infection type of Buhoth 158 was greatly changed from a highly susceptible reaction in 2021 to an Intermediate reaction in 2022 with significant differences in their infection percentages. Significant differences were found in the infection percent of the susceptible wheat cultivars Wafia and Buhoth 22 between seasons, while Azmar exhibits a stable reaction in both seasons and infection types of Iba'a 99 and Buhoth 22 were changed from Susceptible in the first season to Intermediate in the second season (Table 2). All the intermediate group cultivars' infection types shifted from Intermediate reaction in 2021 to resistant in 2022 with no significant differences, except for Charmo, which shown a stable reaction in both seasons. While the resistant group cultivars displayed a stable reaction in both seasons with no significant differences among them (Table 2). According to Shams-Allah *et al*., (39), Tamuz 2 explored high susceptible reaction and Iratom showed moderate resistance to the disease which agrees with the current study results, while Al-Iraq was moderately resistant in 2002-03 and 2013-14, but it explored highly susceptible reaction in both 2020-21 and 2021-22 growing seasons. The reaction of Al Ize was changed from moderate resistance in 2002-03 to highly susceptible in the 2020-21 and 2021-22 growing seasons. In contrast, the host-parasite interaction of Tamuz3 and Rabia was changed from susceptible in 2002–03 to resistant in 2013-14 and remains resistant during the growing seasons 2020-21and 2021-22. Previous reports refer to the high susceptible reaction of SaberBeg to common bunt disease, in contrast, the response of Farris, Maaroof and Ashur to the disease remains stable and explores resistant reaction (8, 9). Baenziger *et al*., (12) explain the difficulties in finding resistant cultivars to CB disease due to the high genetic variations in the pathogen population and the lack of stability in the genetic behavior of wheat cultivars towards the pathogen as well as the instability of the

ideal conditions for infection and disease development. Therefore, conducting subsequent experiments may give different results due to the variations in environmental condition factors which are usually out of control in the field experiments. This will reflect the reliable results in the reaction of the tested cultivars to the disease and come in line with the conclusions of many researchers in the field of searching for resistance sources to the disease (2, 11). The high disease percentage levels of common bunt disease on the susceptible cultivars at both seasons which ranges from 92.4% in the high susceptible wheat cultivar Saberbeg in 2021/22 to 93.8% in the high susceptible wheat cultivar AlFateh in 2020/21 could be attributed to the favorable environmental conditions to bunt disease development beside the availability of the susceptible host and the virulent pathogens. The mean temperature ranged from 10-15C° during the time of seed cultivation and germination between November and December in both seasons (Fig. 1) which is suitable for germination of the teliospores, According to Kumar *et al*., (27) *T. laevis* and *T. tritici* teliospores have different germination rates depending on the isolate and/or pathogen race, and they do so throughout a wide range of temperatures. At 14-16ºC, teliospores germinate uniformly and most quickly at 18-20C^{o}. Under ideal laboratory conditions, germination happens after 4-5 days at 15ºC and after 10-14 days at 5ºC. Production of primary and secondary sporidia and development of the infection mycelium, fewer of them are produced at 5 ºC than at 15 ºC. seeding at a depth of 7cm as opposed to 4cm increases the incidence of CB. Temperatures are more favorable to infection as soil depth increases; infection is most effective at soil temperatures of 5 to 10ºC, with just a small infection occurring at 22ºC. Compared to seed borne inoculum, natural soilborne inoculum causes infection at higher soil temperatures. This is because while soil may contain spores that have already germinated before planting, with seed borne inoculum, few spores will have done so by the time the plants are in a vulnerable growth stage (23). Light has relatively little effect on the teliospore germination of CB fungus. Photoperiod can also affect bunt infection; greater incidence of smut is found in plants that receive 14.5 to 16 hours of light each day. The appearance of resistance could be also be influenced by additional unidentified environmental factors. Depending on the region, a certain race/cultivar combination can exhibit either virulence or a virulence, while other race/cultivar combinations are unaffected in the same test. High teliospore germination percentages occur when the pH is neutral to acidic, however, germination is lowered at pH 7.8–8.2. Teliospores of *T. laevis* and *T. tritici* may remain viable for more than 20 years when held in a dry atmosphere at room temperature in the laboratory, proving that soil type is not a crucial factor in the disease development (27), although a minimal portion of the teliospores might survive in the soil for ten years in its natural environment. The relative humidity and the amount of precipitation at the time of cultivation also highly enhance the high the infection levels in the susceptible cultivars. Figure1 shows that the precipitation average in cultivation season 2020-21 was 408mm and in 2021-22 was 432mm, and the maximum average of air humidity was78% and the minimum average was 27% in both cultivation season, those factors also effect on the plant growth and the bio-mass in the field, also dry condition effect on the size of the teliospores. Delay in the formation of the crown node also promotes

Table 2. Mean Infection percentage and types of the Iraqi wheat cultivars with common bunt disease under artificial inoculation conditions during the growing seasons 2020/21 and 2021/22 at Bakrajo, Sulaimania, Iraq.

Cultivar	2020/21 season		2021/22 season		Mean	
	Infection %	Infection Type	Infection %	Infection Type	Infection %	Infection Type
AlFateh	93.78	HS	90.16	HS	91.97	HS
Uruk	89.64	HS	86.40	HS	88.02	HS
Baghdad 3	84.47	HS	89.70	HS	87.09	HS
Iba'a 95	86.49	HS	86.80	HS	86.65	HS
Adana 99	86.27	HS	84.60	HS	85.44	HS
Saberbeg (White)	78.37	HS	91.96	HS	85.17	HS
Saberbeg (Red)	76.68	HS	92.40	HS	84.54	HS
Hsad	89.60	HS	78.60	HS	84.10	HS
Al-Ize	77.22	HS	90.53	HS	83.88	HS
Kalar 1	82.6	HS	84.6	HS	83.6	HS
Hawler 4	84.66	HS	81.70	HS	83.18	HS
Baghdad 1	85.81	HS	80.00	HS	82.91	HS
Bingal	82.19	HS	74.00	HS	78.10	HS
Kalar 2	76.86	HS	78.86	HS	77.86	HS
Alaa	81.57	HS	70.90	HS	76.24	HS
Sawa	86.43	HS	65.20	HS	75.82	HS
Buhoth 10	76.74	HS	73.40	HS	75.07	HS
Slemani 2	74.07	HS	74.26	HS	74.17	HS
Aras	65.89	HS	79.86	HS	72.88	HS
Abu Ghraib	76.06	HS	69.06	HS	72.56	HS
Cham 6	66.50	HS	68.60	HS	67.55	HS
Hawler 2	57.14	HS	71.30	HS	64.22	HS
Al-Iraq	57.89	HS	70.06	HS	63.98	HS
Tamuz 2	69.12	HS	52.83	HS	60.98	HS
Babel 113	51.93	HS	67.46	HS	59.70	HS
AlNur	46.82	S	62.23	HS	54.53	HS
Barcelona	43.71	S	62.63	HS	53.17	HS
Buhoth 158	74.48	HS	29.80	I	52.14	HS
Wafia	38.12	S	56.82	HS	47.47	S
Azmar	36.22	S	39.03	S	37.63	S
Iba'a 99	37.50	S	27.86	I	32.68	S
Buhoth 22	42.22	S	20.80	I	31.51	S
AlRasheed	18.33	I	23.86	I	21.10	I
Maaroof	19.32	I	8.64	R	13.98	I
Charmo	15.40	I	11.20	I	13.30	I
Iratom	14.06	I	8.37	R	11.22	I
Bura	15.90	I	4.88	R	10.39	I
Rabia	3.75	R	4.02	R	3.98	R
Latiffiya	0.93	R	3.76	R	2.35	R
AlBaraka	3.17	R	1.11	R	2.14	R
AlMadian	1.44	R	1.61	R	1.53	R
Tamuz 3	0.00	R	2.08	R	1.04	R
Ashur	0.00	R	1.55	R	0.78	R
Farris 1	0.55	R	0.00	R	0.28	R
Mean	53.41		52.81		53.11	
L.S.D 0.05 cultivars	11.15		13.63		11.59	
Seasons					n.s	
Cul.* Seasons					12.36	

***. Each number represents an average of three replicates.**

****. Disease Scoring was conducted according to Dodoff and Todorova (1974), where R= Resistant, infection percent range from 0-10%., I= Moderate resistant, infection percent ranges from 11-30%., S= Susceptible, with infection percent, ranging from 31-50%., and HS= Highly susceptible, with infection percentage ranging from 51-100%.**

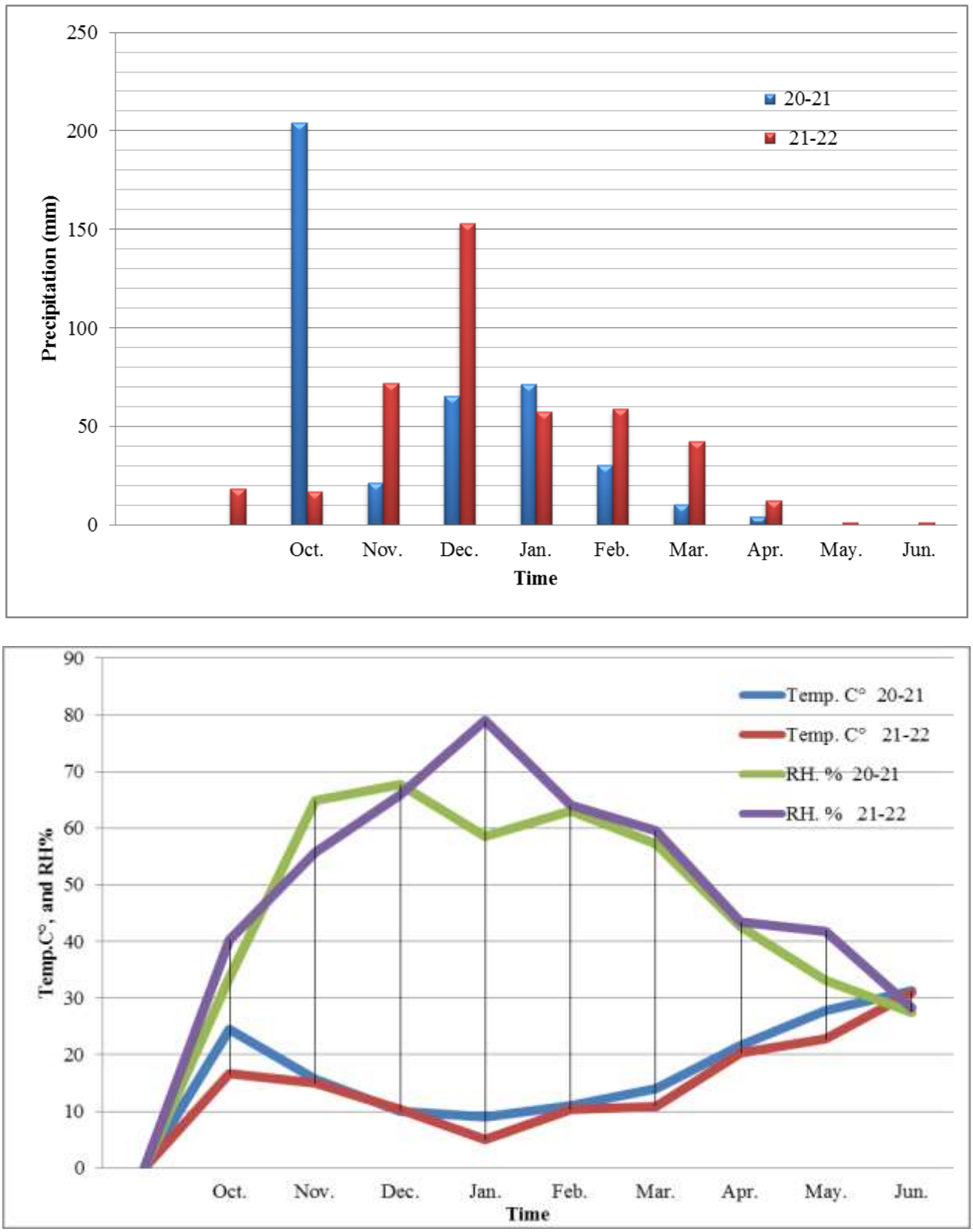
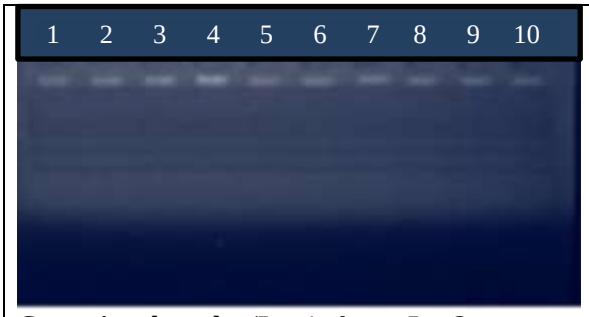


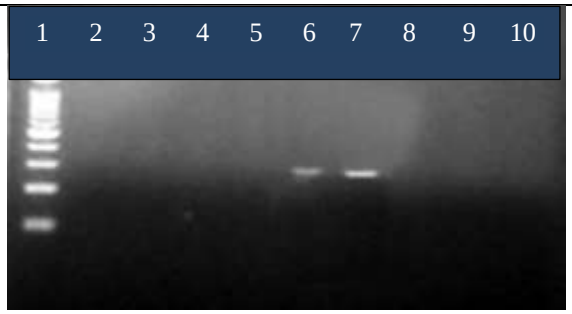
Figure 1. Average of temperature, humidity, and precipitation during the growing seasons of 2020/21 and 2021/22 at college of agricultural engineering sciences field, Bakrajo, Sulaimania.

infection and hyphal establishment in the apical meristem. CB incidence is highest when soil moisture levels are between field capacity and the permanent wilting point (27). The low soil water content in the dry season increases the infection level with common bunt disease since the emergency of the seedlings decrease and the seedling stays longer time in the soils and exposed longer time to the infectious mycelium of the pathogen. The infection period with common bunt pathogen is limited and occurs only at the germination of wheat seeds in the soil at dark conditions (25).

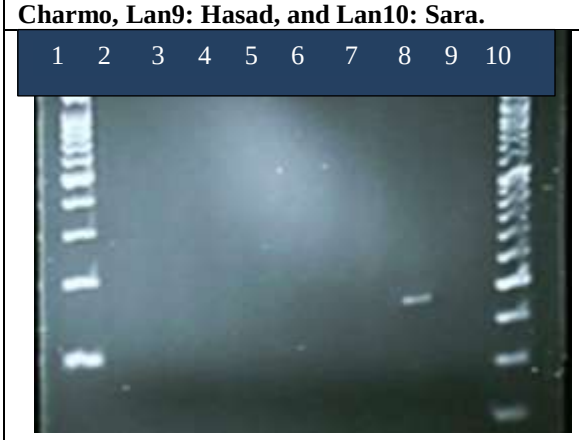
Postulating of Bt resistance genes in wheat cultivars: The current study was conducted to identify the probability of presence of five known Bt resistance genes in 42 registered and released bread wheat and two Triticale cultivars using three specific SSR markers GWM7433, XGWM 114, and XGWM 264 compared to the stock culture of Bt8, Bt9, Bt10, Bt11 and Bt12 that exhibits high levels of resistance in the fields to the available pathogen population under Sulaimani conditions (7, 9). Results revealed that only two bread wheat cultivars -Charmo and Hsad- possess the resistance gene Bt9 at 296bp, and only one bread wheat cultivar -Buhoth 10- possesses the known resistance gene Bt12 at 175bp, while no any of the tested cultivars contain the known resistance genes Bt8, Bt10, and Bt11 (Figure 2). The processes were repeated twice with two different types of Taq master mix, and both of them produced the same results, confirming the existence of the tested known resistance Bt genes in the local wheat cultivars and the positive control. The current study findings showed that the genetic pool of the Iraqi bread wheat cultivars is extremely poor with the known Bt resistance genes particularly Bt8, Bt9, Bt10, Bt11, and Bt12. Out of 44 tested wheat cultivars, only Charmo and Hsad have the known resistance gene Bt9 and Buhoth10 possesses the known resistance gene Bt12 which represents 7% of all tested wheat cultivars (Table 2). The moderate resistance of the bread wheat cultivar Charmo could be attributed to the Bt9 resistance gene, while despite that Hsad possessing the Bt9 resistance gene, it explored high infection percentage to *Tilletia* species populations in the field. This means that this gene alone is not responsible for the moderate resistance of Charmo. Although Buhoth 10 possesses resistance gene Bt12, it explores high susceptible reactions to common bunt disease. This could be due to the absence of virulence against Bt12 gene in the pathogen populations in Iraq, which is well represented in Table 2. The highest resistance of wheat cultivars Farris1, Rezan, Ashur, Tamuz3, AlMadian, Baraka, and Latiffiya to common bunt disease, and the moderate resistance of Rabia, Sarah, Bura, Iratom, and Charmo may be attributed to the presence of additional known resistance Bt genes like Bt1, Bt3, Bt5, Bt6, Bt10, Bt13, Bt15, and Btp, which showed high efficiency against *Tilletia* populations in the field and not included in the current study. Or it might be due to the presence of different unidentified resistance genes in the genetic pool of these cultivars. The efficiency of the known resistance genes under Iraqi environmental conditions was confirmed before in previous studies (10). Molecular markers linked to bunt resistance genes could help in developing of resistant wheat cultivars by enabling resistance screening and bunt resistance gene introgression into wheat cultivars with good agronomic traits (17). Developing disease-resistant cultivars is a good strategy to control common bunt disease. The study by Steffan *et al.,* (41) using the SSR technique with the XGPW7433 marker in comparison of marker sequences linked to Bt9 and Bt10 on physical maps of chromosome 6D confirmed that Bt9 and Bt10 are two distinct resistance factors located at the distal (6DL) and proximal (6DS), respectively, of chromosome 6D. While Stefan *et al*., (41), identified two significant marker-trait associations (MTA) for common bunt resistance, named QCbt.cph-2B and QCbt.cph-7A, located on wheat chromosomes 2B and 7A, respectively. According to McIntosh *et al*., (33) the Bt8, Bt10, and Bt11 genes were linked to 180, 160, and 120bp segments by Xgwm114 primers in the Turkish genotype PI178383. The isogenic



Genomic gel smples (Lan1: Aras, Lan2: Sulaimani 2, Lan3: Hawler 2, Lan4: Hawler 4, Lan5: Azmar, Lan6: Allaa, Lan7: Baraka, Lan8: Charmo, Lan9: Hasad, and Lan10: Sara.



PCR gel smples (Lan1: Ladder100bp, Lan6: Charmo Bt9, Lan7: Hsad Bt9).



PCR gel smples (Lan1: Ladder100bp, Lan8: Buhoth10 175bp, Lan10: Ladder 50bp).

PCR gel smples (Lan1: Buhoth10, Lan3: Ladder100bp, Lan4: Charmo, Lan5: Hsad)

PCR gel smples (Lan1: Buhoth10 Bt12, Lan2: Ladder100bp, Lan3: Charmo Bt9, Lan4: Hsad Bt9, Lan5: Ladder100bp, and Lan6: 554120 BT8, Lan7: 554099 BT9, Lan8: WESTON BT10 , Lan9: 554098 BT11, and Lan10: 16160 BT12 are introduced varieties used as a positive control.

Figure 2. a. Genomic gel samples (Lan1: Aras, Lan2: Sulaimani 2, Lan3: Hawler 2, Lan4: Hawler 4, Lan5: Azmar, Lan6: Allaa, Lan7: Baraka, Lan8: Charmo, Lan9: Hasad, and Lan10: Sara., b. Locus of Bt9 gene in both local bread wheat cultivars Charmo and Hsad, c. Locus of Bt12 gene in the local bread wheat cultivar Bohuth10, d. PCR gel samples (Lan1: Buhoth10, Lan3: Ladder100bp, Lan4: Charmo, Lan5: Hsad), and e. Band size of the local wheat cultivars compared to the positive control var. using SSR Markers GWM7433, XGWM 114, and XGWM 264

line M82-2098 possessing Bt9 resistance gene was employed as a positive control for detection of Bt9 gene in Kazakhs wheat cultivars using the Gwm7433 SSR marker. The DNA segment for Bt 9 gene is 296 bps in size was found in Dinara, Yegemen 20, Zhalyn, Kazakstanskaya 75, Karasai, Mereke75, Matai, Naz, Sultan 2, Sultan 95, Sanzar 8, Steklovidnaya 24, Raminal, Farabi, and Yubileynaya 75 (30). Muellner *et al., (*35) mentioned that Bt12 is resistant to all CB races and Kompetitve Allele-Specific PCR (KASP) marker could be used to detect this gene on chromosomal location. This gene could be

useful for hybridization with the susceptible cultivars. Under artificial inoculation conditions, the tested wheat cultivars explored wide range of responses against *T. tritici* and *T. laevis* populations in Sulaimani (Table 2). The highest infection levels of the highly susceptible cultivars compared to control treatment of each cultivar is a good proof of the inoculation success. Other researchers also tried to control the disease without seed dressing with chemical (32). Genetic resistance to common bunt disease is a highly desired for grain-grade protection that is environmentally sustainable. The identification of race-specific resistance genes is just one example of the significant breeding advancements made to induce wheat cultivars with higher disease resistance (3, 11, 22).

Conclusions

The expression of certain resistance genes could be influenced by temperature during the early stages of plant development (34). The best way to reduce the amount of chemicals and protect the ecosystem and public health could be achieved by using resistant cultivars or resistant sources as a donor of resistant genes in a breeding programs (28). In conclusion, the genetic constitutions of the Iraqi local wheat cultivars are poor in the identified Bt resistance genes, that is why 73% of the tested cultivars explored high levels of susceptibility to the disease under artificial inoculation conditions at bakrajo conditions, Sulaimani, while only 27% explored different levels of resistance. Incorporating the identified Bt resistance genes in the susceptible cultivar or using the resistance cultivars or other resistance sources as a donor of resistant genes in a breeding program might improve resistance levels of wheat and considered as a good strategy to prevent common bunt disease. Molecular markers linked to bunt resistance genes may also help in improving resistance levels in wheat cultivars by allowing screening for bunt resistance gene and introgression them into wheat cultivars with good agronomic genotypes (17). To take advantage of the genetic gain of the resistant cultivars and control the disease, it is essential to speed up the multiplication of the resistant cultivars seeds and provide them to farmers and small holders.

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