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Research Article/Araştırma Makalesi

Molecular screening of registered durum wheat (*Triticum durum*) varieties and landraces to common bunt disease in Türkiye

Türkiye 'de tescilli makarnalık buğday (Triticum durum) ve yerel çeşitlerin sürme hastalığına karşı moleküler taraması

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ABSTRACT

Common bunt caused by the basidiomycetes fungi *Tilletia caries* and *Tilletia foetida* is significant wheat disease, particularly following rust (*Puccinia* spp.) diseases. Seed treatment with fungicides has traditionally been the primary method for disease control. However recently its effectiveness has reduced. Growing resistant plant materials are therefore preferred to management of common bunt disease. In this regard, the current study was conducted to detect common bunt resistance genes (*Bt8*, *Bt10*, and *Bt11*) in a total of 61 registered durum wheat varieties and landraces using molecular techniques. In PCR assay, these plant materials were screened with SSR marker Xgwm114 to identify whether they carried any of the *Bt* resistance genes. According to the molecular results, most of the tested varieties and landraces were found in the *Bt8* resistance gene (15 registered varieties and 5 landraces). Additionally, the results showed that none of the tested varieties and landraces had gene combinations. To sum up, this is the first molecular study to identify common bunt resistance genes in durum wheat varieties and landraces in Türkiye. Furthermore, these findings can be used in breeding programs to management with common bunt disease.

Key Words: Durum wheat, common bunt, *Bt* genes, resistance genes, marker

ÖZ

Basidiomycetes fungusu *Tilletia caries* ve *Tilletia foetida*' nın neden olduğu sürme hastalığı buğdayda pas (*Puccinia* spp.) hastalıklarından sonra önemli bir hastalıktır. Bu hastalığı kontrol etmek için genellikle tohum ilaçlaması yapılır, ancak son zamanlarda etkinliği azalmıştır. Bu nedenle, hastalık ile mücadelede dayanıklı bitki materyalleri tercih edilmektedir. Bu kapsamda, mevcut çalışmada toplam 61 tescilli makarnalık buğday çeşidinde ve yerel çeşitlerde sürme dayanıklılık genlerini (*Bt8*, *Bt10* ve *Bt11*) moleküler tekniklerle belirlemek amacıyla gerçekleştirilmiştir. PCR analizinde, kullanılan bitki materyallerinde *Bt* dayanıklılık genlerini tanımlamak için SSR belirteci Xgwm114 kullanılmıştır. Moleküler sonuçlara göre, test edilen tescilli çeşit ve yerel çeşitlerin çoğunda *t8* dayanıklılık geninin bulunduğu tespit edilmiştir (15 tescilli çeşit ve 5 yerel çeşit). Ayrıca, sonuçlar test edilen materyallerin gen kombinasyonu içermediği belirlenmiştir. Sonuç olarak, Türkiye'deki tescilli makarnalık buğday ve yerel çeşitlerde sürme dayanıklılık genlerinin tanımlandığı ilk moleküler çalışmadır. Ayrıca, bu bulgular buğdayda sürme hastalığı ile ilgili ıslah programlarında kullanılabilir.

Anahtar Kelimeler: Makarnalık buğday, sürme, *Bt* genleri, dayanıklılık genleri, belirteç

Introduction

Common bunt (CB) caused by *Tilletia caries* and *T. foetida* can cause dramatically grain yield losses in wheat. Generally, grain yield decreases on infected plant with CB disease compared to the non-infected plants and their quality is also low which due to the infected heads filled with dark colore bunt balls spores (Goates et al. 1996; Mourad et al. 2018). Seed treatment with fungicides is an effective strategy for managing common bunt disease. However, genetic resistance is a more suitable control method to the disease and also it mitigates the need for chemical seed treatments and can be particularly advantageous in organic farming systems.

Marker-Assisted Selection (MAS) has been widely used in the resistance breeding studies to fungal pathogens. Molecular markers, when associated with bunt resistance genes, can assist in the development of resistant varieties. They enable the determination of resistance and the introgression of resistance genes into varieties with good agronomic traits (Matanguihan et al. 2011). Since symptoms of bunt disease become apparent when the plant matures, screening for resistant varieties is time-consuming. In addition, when disease incidence is low, it is difficult to detect on common bunt disease in wheat since the evaluation of the variety reaction varies according to environmental effects. For these reasons, to identify for common bunt resistance, it is necessary to have information about the genes that confer resistance to common bunt (Mourad et al. 2018). The resistance is often controlled by a single gene with complete or incomplete dominance effect (Knox et al. 1998). To date, 16 race-specific resistance genes, ranging from *Bt1* to *Bt15*, and *Btp*, have been identified for common bunt (Goates, 2012). Similarly, mapping studies has been conducted for some of these 16 resistance genes (Menzies et al. 2006). These genes are determined by employing the race differential set (Matanguihan et al., 2011). The study of Mamluk (1998) emphasized the *Bt5*, *Bt6*, *Bt8*, *Bt9*, *Bt10*, and *Bt11* resistance genes are effective in common bunt pathogen race/races.

In Türkiye, a study conducted by Mamluk and Nachit (1994), durum wheat genotypes were tested against nine common bunt isolates obtained from the West Asia and North Africa (WANA) region. According to the obtained results, it was reported that 26 genotypes showed resistance reactions. In another study, to identify the common bunt resistance in durum wheat varieties, resistance tests were conducted against disease races virulent on 10 *Bt* resistance genes, as well as local races in the Mediterranean Region from 1988 to 1991. In the reaction analysis, it was determined that five out of 29 durum wheat varieties showed resistance reactions (Ataç and Çetin 1995). A review study reported by Mamluk et al. (1997) evaluated the prevalence of the dominant common bunt pathotypes in Türkiye, Egypt, Syria, Tunisia, Lebanon, Iran, and Morocco. In Türkiye, 37 and 88 races were reported in 1981 and 1983 respectively. it was also stated that five races were similar to North American races. As mentioned above, it can be seen that the conducted studies were mainly based on the use of a race differential set and their reactions against to common bunt disease. According to the literature, there is no study currently published information available regarding the common bunt resistance genes using the molecular techniques in Türkiye. To sum up, the objective of this study is to molecular screening of the durum wheat varieties and landraces for resistance to common bunt disease in Türkiye.

Materials and Methods

Plant materials

A total of 61 durum wheat including 51 registered varieties and 10 landraces were used in this study. Information about these varieties and landraces were given in Table

DNA extraction, PCR analysis and gel

electrophoresis

At least three seeds of each variety were planted in plastic pots (with mixture of seat and peat in 1:1 and grown up to the ZGS 12-13 of seedling (Zadoks et al., 1974). . Later, the leaves of each variety were collected and transferred to 1.5 ml Eppendorf tubes for the DNA isolation. NucleoSpin Plant II (Macherey-Nagel) DNA isolation kit was used for total genomic DNA extraction. To quality of the isolated DNAs was control by agarose gel electrophoresis and stored at -20^{o}C until used. To identify *Bt8*, *Bt10* and *Bt11* resistance genes carries of each variety, SSR marker Xgwm114 (F: 5'-ACAAACAGAAAATCAAAACCCG-3', R: 5'-ATCCATCGCCATTGGAGTG-3') (Goates and Mercier, 2009) which is located on chromosome 3B was used in the current study.

Table 1. Information about the durum wheat varieties/landraces used in this study

No	Variety	Release year	Pedigree
1	Kunduru 414/44	1963	Landrace
2	Berkmen 469	1967	Landrace
3	Çakmak 79	1979	UVEYIK-162/ND-61-130
4	Kızıltan 91	1991	UVEYIK-162/61-130//BARRIGON-YAQUI-ENANO*2/TE
5	Altın 40/98	1998	BARRIGON-YAQUI-ENANO/2*TEHUACAN-60//2B//LONGSHANKS/3/BERKMEN-469
6	Yılmaz 98	1998	DF-9-71/3/V-2466//ND-61-130/414-44/4/ERGENE
7	Ankara 98	1998	KOBAK-2916/LEEDS//6783/3/BERKMEN-469/7/CRANE/GANSO//APULICUM/3/DF-17-72/4/DI-165137/GEDIZ-75/5/ANHINGA/6/CASTELPORZIANO/G2//2*TEHUACAN-60/TEHUACAN-60
8	Çeşit-1252	2000	61-130/KUNDURU-414-44//377-2
9	Mirzabey 2000	2000	GD-2/D-1184528
10	Eminbey	2009	CMK79//14-44/OVIACHIC-65/3/BERKMEN/OVIACHIC-65/4/KUNDURU-1149/5/LEEDS//DWARF-MUTANT/SARIBASAK
11	İmren	2009	DF-21-72/GERARDO-VZ-466//ND-61-130/414-44/3/ERGENE/4/DF-21-72//ND-61-130/UVEYIK-162/3/128-3
12	Kunduru 1149	1967	Landrace
13	Altıntaş 95	1995	KUNDURU//D-68111/WARD
14	Kümbet 2000	2000	ND-61-130//414-44/377-2/3/DF-15-72
15	Yelken 2000	2000	ZF/LEEDS//FORAT/3/ND-61-130/LEEDS/4/AU-107/5/GERARDO
16	Dumlupınar	2006	BERKMEN/G-75-T-181
17	Fata Sel	1961	Landrace
18	Selçuklu-97	1997	073-44*2/OVIACHIC-65/3/DF-21-72//ND-61-130/UVEYIK-162
19	Meram-2002	2002	ND-61-130/414-44//CAKMAK-79
20	Tunca 79	1979	FATA(SEL.181-1)/ND-61-130//LEEDS
21	Gökgöl 79	1979	BUCK-BALCARCE//BARRIGON-YAQUI-ENANO*2/TEHUACAN-60
22	Diyarbakır-81	1987	LD-393//BELADI-116-E/2*TEHUACAN-60/3/COCORIT-71
23	Ceylan 95	1995	STORK/RABICORNO
24	Sarı çanak 98	1998	DACKIYE/GEDIZ-75//USDA-575

25	Altın toprak 98	1998	ALTAR-84/ARAOS
26	Aydın-93	2002	JORI-C-69/HAURANI
27	Fırat-93	2002	SNIPE/3/JORI-C-69/CRANE/GANSO/ANHINGA
28	Artuklu	2008	LAHN//GANSO/STORK
29	Eyyubi	2008	MORUS//ALTAR-84/ALONDRA
30	Şahinbey	2008	Unknown
31	Zühre	2010	SN-TURK-M-183-84-375/NIGRIS-5//TANTLO-1
32	Güney Yıldızı	2010	RASCON-39/TILD-1
33	Gediz-75	1976	LD-357-E/2*TEHUACAN-60//JORI-69
34	Ege 88	1988	JORI-C-69/ANHINGA//FLAMINGO
35	Salihli 92	1992	SHWA//21563/ANHINGA/3/EGE-88
36	Tüten 2002	2002	ALTAR-84/AVETORO/3/GANSO/FLAMINGO//CANDO
37	GAP	2004	GEDIZ-75)/FLAMINGO//TEAL
38	Turabi	2004	CRESO/CRANE
39	Sham-1	1991	PELICANO/RUFF//GAVIOTA/ROLETTE
40	Amanos-97	1997	OSTRERO//CELTA/YAVAROS,
41	Fuatbey 2000	2000	Unknown
42	Sarı Başak	2013	Unknown
43	Akçakale-2000	2002	SCHELLENTE//CORMORANT/RUFFOUS/3/AJAIA
44	Özberk	2005	FLAMINGO,MEX/GARZA//CANDEAL-1/GREBE/3/CENTRIFEN/FLAMINGO,MEX/PETREL,MEX/5/AKBASAK-073-44/YERLI/6/CAR
45	Pınar-2001	2001	Unknown
46	Zenit	2001	VALRICCARDO/VIC
47	Svevo	2001	CIMMYT-SELECTION/ZENIT
48	Levante	2011	G-80/PICENO//IONIO
49	Saragolla	2011	IRIDE/LINEA-PSB-0114
50	Maestrale	2012	IRIDE/SVEVO
51	Bisante	2012	Unknown

Landraces			
No	**Name**	**No**	**Name**
1	Landrace 1	6	Landrace 6
2	Landrace 2	7	Landrace 7
3	Landrace 3	8	Landrace 8
4	Landrace 4	9	Landrace 9
5	Landrace 5	10	Landrace 10

Polymerase chain reaction (PCR) was carried out in a thermal cycler (T100: BioRad, USA). Total reaction volume was 15 µL with 1X PCR buffer (50 mmol KCl, 10 mmol Tris-HCl, pH 8.3), 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1U Taq DNA polymerase, each of forward and reverse primers (10 µM), 100 ng template DNA and double distilled water. The PCR reaction was performed an initial denaturation at 94 °C for 3 min, 35 cyles of denaturation at 94 °C for 1 min, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min and last extension at 72 °C for 5 min. The amplified PCR products were separated on 2.5% agarose gel staining with ethidium bromide at 80 V for 2 h. UVP UVsolo touch gel imaging system (Analytik Jena, Germany) was used to visualize the PCR bands under UV. The sizes of the PCR products were determined by using the 100 bp DNA ladders as reference standard.

Data analysis

For data analysis, the obtained band with a different size of each *Bt* resistance gene was scored as presence (1) or absence (0) and the data was recorded in Excel software.

Results and Discussion

Molecular markers associated with resistance genes provide facilities for the development of resistant varieties and the transfer of these resistance genes to cultivars with good agronomic traits (Muellner et al. 2021). Randhawa et al. (2013) stated that marker-assisted selection in resistance to common bunt disease in wheat is crucial. In accordance with this, the usage of molecular markers has been rapidly increasing in recent years (Wang et al. 2019; Aboukhaddour et al. 2020; Mourad et al. 2023; Amangeldikyzy et al. 2023). One of the most effective methods to manage common bunt disease is the use of resistant cultivars in production. Therefore, the aim is to transfer existing *Bt* genes or new resistance genes that provide resistance to common bunt into wheat (Madenova et al. 2021). To detect the presence of the *Bt8*, *Bt10*, and *Bt11* genes, PCR analysis was conducted by using the Xgwm114 marker. This primer amplified fragments of 180, 160, and 120 bp linked to resistance genes *Bt8*, *Bt10*, and *Bt11* respectively (Goates and Mercier, 2009). In the present study, the Xgwm114 marker was used and it was determined that common bunt resistance genes namely *Bt8*, *Bt10* and *Bt11* are found in durum wheat varieties and landraces (Figure 1).

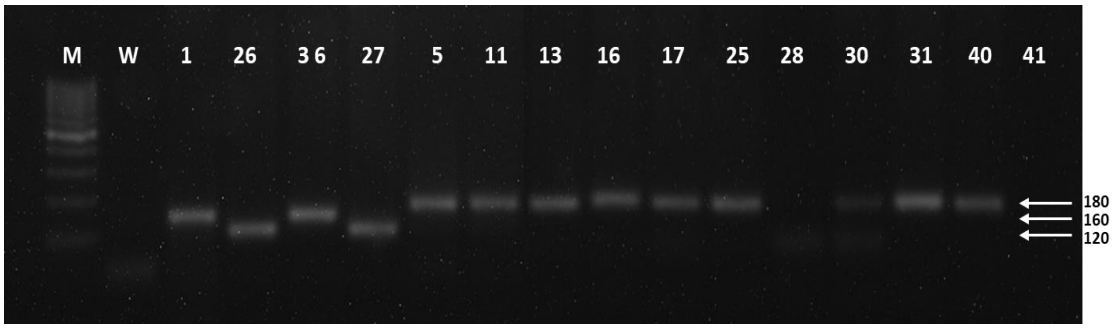

Figure 1. Agarose jel electrophagrame of *Bt* genes. M: DNA ladder (100 bp), W: Water and the numbers of lanes corresponding to the durum wheat materials as stated in Table 1. The arrow shows resistance fragments *Bt8*, *Bt10* and *Bt11* genes 180, 160 and 120 respectively.

Among the *Bt* resistance genes, the resistance gene *Bt8* was detected in 15 durum wheat varieties (Altın 40/98, İmren, Kunduru 1149, Altıntaş 95, Dumlupınar, Fata Sel, Altın toprak 98, Şahinbey, Zühre, Amonos-97, Sarı Başak, Levante, Saragolla, Maestrale and Bisante) and five

landraces (Table 2).

Table 2. Molecular screening of registered and landraces durum wheat varieties.

No	Varieties	Resistance genes			No	Varieties	Resistance genes		
		Bt8	*Bt10*	*Bt11*			*Bt8*	*Bt10*	*Bt11*
1	Kunduru 414/44	-	+	-	27	Fırat-93	-	-	+
2	Berkmen 469	-	-	-	28	Artuklu	-	-	-
3	Çakmak 79	-	-	-	29	Eyyubi	-	-	-
4	Kızıltan 91	-	-	-	30	Şahinbey	+	-	-
5	Altın 40/98	+	-	-	31	Zühre	+	-	-
6	Yılmaz 98	-	-	-	32	Güney Yıldızı	-	-	+
7	Ankara 98	-	-	-	33	Gediz-75	-	-	-
8	Çeşit-1252	-	-	-	34	Ege 88	-	-	-
9	Mirzabey 2000	-	-	-	35	Salihli 92	-	-	-
10	Eminbey	-	-	-	36	Tüten 2002	-	+	-
11	İmren	+	-	-	37	GAP	-	-	-
12	Kunduru 1149	+	-	-	38	Turabi	-	-	-
13	Altıntaş 95	+	-	-	39	Sham-1	-	-	-
14	Kümbet 2000	-	-	-	40	Amanos-97	+	-	-
15	Yelken 2000	-	-	-	41	Fuatbey 2000	-	-	-
16	Dumlupınar	+	-	-	42	Sarı Başak	+	-	-
17	Fata Sel	+	-	-	43	Akçakale-2000	-	-	+
18	Selçuklu-97	-	-	-	44	Özberk	-	-	-
19	Meram-2002	-	-	-	45	Pınar-2001	-	-	-
20	Tunca 79	-	-	-	46	Zenit	-	-	-
21	Gökgöl 79	-	-	-	47	Svevo	-	-	-
22	Diyarbakır-81	-	-	-	48	Levante	+	-	-
23	Ceylan 95	-	-	-	49	Saragolla	+	-	-
24	Sarı çanak 98	-	-	-	50	Maestrale	+	-	-
25	Altın toprak 98	+	-	-	51	Bisante	+	-	-
26	Aydın-93	-	-	+					
1	Landrace 1	+	-	-	6	Landrace 6	-	-	+
2	Landrace 2	+	-	-	7	Landrace 7	-	-	+
3	Landrace 3	+	-	-	8	Landrace 8	-	+	-
4	Landrace 4	+	-	-	9	Landrace 9	-	+	-
5	Landrace 5	+	-	-	10	Landrace 10	-	-	+

The CB resistance gene *Bt10* was only detected in two varieties (Kunduru 414/44 and Tüten 2002). Among the tested landraces, the *Bt10* gene was found in two landraces. Menzies et al. (2006) stated that *Bt10* is commonly employed in wheat breeding programs due to its efficacy against the majority of common bunt races. In line with this, these genotypes carrying the *Bt10* gene can be used to control of the pathogen. In the present work, the resistance gene *Bt11* was molecularly detected in four varieties namely, Aydın-93, Fırat-93, Güney Yıldızı and Akçakale-2000). In addition, this resistance gene was found in three landraces (Table 2). In a similar way to the current study, several studies have been conducted to determine the presence of common bunt resistance genes in wheat varieties. As for these studies, 43 Kazakh and foreign winter wheat cultivars were tested to determine resistance genes, *Bt9* and *Bt10*, and their results showed that four and seven varieties contained *Bt9* and *Bt10* respectively (Madenova et al. 2019). Similarly, it was shown to be an effective resistance gene to common bunt disease and as a result of these studies, different *Bt* genes effective in Syria (*Bt5, Bt8, Bt9, Bt10,* and *Bt11)*, Iraq (*Bt1*, *Bt3*, *Bt9*, *Bt11*, and *Bt12*), America (*Bt6*, *Bt9*, *Bt11*,

Bt12, *Bt13*, *Bt15*, and *Btp*), Australia (*Bt8, Bt9, and Bt10)* and Kazakhstan (*Bt8, Bt9, Bt10 and Bt11*) were identified (Mamluk and Nachit, 1994; Al-Maaroof et al., 2016; Hagenguth, 2016; Mourad et al., 2018: Madenova et al. 2021; Moruad et al. 2023). When examining the previous works conducted in Türkiye, common bunt isolates have been tested on differential sets, the virulence phenotypes of existing races and their reactions on wheat genotypes have been revealed (Finci, 1981; Akçura and Akan, 2018; Morgounov et al. 2018; Moruad et al. 2023). On the other hand, based on the molecular findings, none of the tested varieties and landraces had two or three gene-based resistance in the present work. Contrary to the results obtained in this study, Madenova et al. (2021) identified four *Bt* resistance genes (*Bt8, Bt9, Bt10, and Bt11)* in the one variety "Karasai" in their study. In summary, molecular research focusing on the identification of the specific *Bt* resistance genes present in existing varieties have been performed recently but there is no molecular study in Türkiye related with this. Therefore, with this conducted research, the presence of *Bt* genes in durum wheat genotypes has been revealed at the molecular level for the first time.

Conclusion

The presence of resistance genes *Bt8*, *Bt10*, and *Bt11*, which provide resistance against common bunt disease, has been detected in the tested varieties in the current study. According to molecular results, it was determined that the tested material contains different *Bt* genes, and most of them had *Bt8* resistance gene. In addition, two or three combinations of each resistance gene were not found., The varieties identified to contain resistance genes can be used as parents in the wheat breeding program to control of the common bunt disease.

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