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Genetic Variation in Turkish Bread Wheat (*Triticum aestivum* L.) Varieties for Resistance to Common Bunt

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Abstract: Common bunt, caused by *Tilletia laevis* and *T. caries*, is one of the major wheat diseases in Türkiye and in many countries in the world. To control this disease, chemical seed treatment is commonly used; however, it may cause harm to human and environmental health. Therefore, genetic resistance to control common bunt in an environmentally friendly, cost-effective, and sustainable manner is the best choice. This study was conducted to determine the reactions of 102 bread wheat (*Triticum aestivum* L.) varieties with regard to their resistance to common bunt in field conditions over three consecutive years. Additionally, these varieties were molecularly screened with linked markers to *Bt8*, *Bt9*, *Bt10*, and *Bt11*. The infection rates ranged from 3.17 to 91.49%, 5.41 to 91.41%, 5.29 to 94.06%, and 6.85 to 90.30% in the growing seasons 2019–2020, 2020–2021, and 2021–2022 and overall, respectively. In molecular screening, *Bt8* was detected in 2 of the varieties, *Bt10* in 10 of them, and *Bt11* in 15 of them. There was no variety carrying only *Bt9*. However, many gene combinations, such as *Bt8* + *Bt9*, *Bt8* + *Bt11*, *Bt9* + *Bt10*, *Bt9* + *Bt11*, *Bt8* + *Bt9* + *Bt10*, and *Bt8* + *Bt9* + *Bt11*, were determined. The varieties with a gene combination of *Bt8* + *Bt9* + *Bt11* had the lowest infection rates. As a result, 65.68% of the varieties were very susceptible. Only 3.92% of them had moderately resistant reaction. These varieties could be used in breeding programs conducted for resistance to common bunt.

Keywords: bread wheat; common bunt; *Bt* genes; variety; variation

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1. Introduction

Common bunt, caused by *Tilletia laevis* (syn. *Tilletia foetida*) and *Tilletia caries* (syn. *Tilletia tritici*), is a seed-borne wheat (*Triticum* L.) disease which has the potential to cause huge yield and quality losses. Wheat (*Triticum* L.) is a primary food source for millions globally, and due to its adaptation to many environments, it covers about 215.9 million ha of farmland [1]. Wheat is also very important for Türkiye; the country is not only a leading producer and consumer of wheat but also the top exporter of flour and bulgur and the second-largest macaroni exporter [2]. Therefore, it is crucial to mitigate the potential economic loss caused by common bunt.

In plants infected with one of these pathogens, teliospores fill the wheat kernels with an odor containing trimethylamine; therefore, harvested grains are mostly rejected by millers due to their odor in wheat flour [3]. It is known that this is one of most devastating wheat diseases, especially in North America (the USA and Canada), European countries, and the semi-arid areas of the CWANA region [4]. It is also huge problem in Türkiye [5,6].

Compared to other wheat diseases, such as rust diseases, powdery mildew, etc., the number of studies conducted on common bunt resistance is quite low both in Türkiye and in the world. The primary reason for this is the chemical seed treatments [7]. Without chemical seed treatments, the damage caused by common bunt can reach 75–90% in a few years [8]. Since the advent of effective seed treatments, wheat breeding studies in Türkiye and elsewhere that were conducted to consider resistance to common bunt have been given a less important place. However, this chemical treatment increases the cost of wheat production [4], and its side effects on environmental and human health cannot be ignored [9].

On the other hand, in recent years the trend in European countries has shifted towards organic, sustainable, and cost-effective agriculture, emphasizing minimal chemical inputs. In a similar way, organic farming has also been increasing in Türkiye [10,11]. Crop quality and stable grain yields are much more important in this agricultural system than they are in intensive farming. Both organic and conventional farming face challenges in managing common bunt. However, since the use of synthetic pesticides is prohibited in organic farming, chemical seed treatment cannot be applied for resistance to common bunt in the way that it can in conventional agriculture. The identification and development of genetic resistance to this disease are the best means of managing the disease with an environmentally friendly, cost-effective, and sustainable approach. Due to the European Green Deal objectives, this process has gained even more speed [12]. However, many studies have shown that most of the wheat varieties cultivated in different regions of the world are susceptible to common bunt. In the United Kingdom, scrutinizing wheat seed lots for seed-borne diseases like common bunt is crucial. Occasionally, common bunt contamination is reported during seed certification processes and seeds infected over a maximum level are destroyed [13]. Common bunt is also one of the most dangerous diseases in Australia; however, most of the wheat varieties cultivated in Australia are susceptible to common bunt. Therefore, there are strict quarantine regulations in Australia to prevent common bunt contamination [14]. Additionally, wheat varieties cultivated in the United States have not been particularly resistant to common bunt [15]. Dumalasova et al. [16] screened the Czech and European winter wheat cultivars for resistance to common bunt in a three-year field test, and they also reported that the mean infection rate was found 39%.

Until now, sixteen race-specific resistance genes against common bunt have been designated in wheat [17]. Unfortunately, only a limited number of loci have been mapped which can be used for marker-assisted selection (MAS). While the *Bt1*, *Bt4*, *Bt5*, *Bt6*, and *Bt11* are located on chromosome B [18–21], *Bt7* and *Bt10* are located on chromosome D [22,23]. Of these sixteen genes, *Bt10*, due to its effectiveness against most races of *T. laevis* or *T. caries*, is widely used in breeding programs worldwide [23]. Akcura and Akan [5] reported that one of the most common races of *T. foedita* in Türkiye was virulent on *Bt0*, *Bt2*, *Bt3*, *Bt4*, *Bt6*, and *Bt7*. Mourad et al. [6] also reported that the resistance genes *Bt10*, *Bt11*, *Bt12*, and *Btp* have been effective against the common bunt races in USA and Türkiye.

Many studies have shown that the genetic diversity in the modern wheat gene pool is limited for this trait [24]. Together with this, Türkiye is known as one of the gene centers for wheat worldwide and many genetic resources, including varieties, landraces, and wild relatives, for resistance to other diseases have been characterized [24,25]. Unfortunately, Turkish wheat varieties have not been adequately subjected to research on common bunt resistance. A few studies have been reported on the common bunt resistance of landraces and some varieties in Türkiye. In one of these studies, Aydogdu and Kaya [3] reported that 77.41% of 32 spring wheat varieties registered in Türkiye were very susceptible to common bunt. In another study, 19 out of 200 pure lines selected from landraces collected from different regions of Türkiye had resistant reactions to the most common race of *T. foedita* [5].

The objectives of this present study are (1) to test the reactions of 102 bread wheat varieties to common bunt under Antalya conditions and (2) to molecularly detect the common bunt resistance genes (*Bt8*, *Bt9*, *Bt10*, and *Bt11*) in these varieties.

2. Materials and Methods

2.1. Plant Materials and Inoculum Source

A total of 102 bread wheat (*Triticum aestivum* L.) varieties were used in this study. These varieties were registered from 1937 to 2005 in Türkiye. The pedigree information on the varieties was given in Table S1. In addition, the variety "Red Bobs" identified as susceptible was used as the control. Both the susceptible variety and the inoculum source (*T. foedita* [syn: *T. laevis*]) used in the field trials were supplied from the Department of Plant Protection of Kırşehir Ahi Evran University. The virulence formula (*Vr*: *Bt0*, *Bt2*, *Bt3*, *Bt4*, *Bt6*, *Bt7*) of this inoculum was determined by Akcura and Akan [5] using a differential

set, including the varieties Red Bobs (*Bt0*), Sel2092 (*Bt1*), Sel1102 (*Bt2*), Ridit (*Bt3*), Turkey 1558 (*Bt4*), Hohenheimer (*Bt5*), Rio (*Bt6*), Sel50077 (*Bt7*), M78-9496 (*Bt8*), M82-2098 (*Bt9*), M82-2102 (*Bt10*), P.I. 178383 (*Bt8*,*9*,*10*), M82-2123 (*Bt11*), P.I. 119333 (*Bt12*), P.I. 181463 (*Bt13*), Doubi (*Bt14*), and Carlton (*Bt15*).

2.2. Field Trials

To evaluate the resistance of bread wheat varieties to common bunt (CB), field trials were conducted at the experimental sites of Akdeniz University in Antalya, Türkiye, in three consecutive growing seasons (2019–2020, 2020–2021, and 2021–2022). All the varieties were planted using a randomized complete block design with two replications in all three seasons. Additionally, the susceptible check "Red Bobs" was added to the field trials in all the seasons to confirm the effectiveness of inoculation. The seeds of each variety were separately placed in a paper envelope and inoculated with teliospores by shaking seeds in an envelope for 5 min [26]. This was reported as a very effective method to inoculate small amount of seeds. Then, the seeds of each variety were manually sown in one-meter-long rows at a 5–7 cm depth in mid-October in each growing season. The monthly meteorological data, such as average temperature and rainfall over three growing seasons (2019–2020, 2020–2021, and 2021–2022) are given in Figure 1. The meteorological data were obtained from the agroclimatology archive of NASA (https://power.larc.nasa.gov/data-access-viewer/) (accessed on 3 September 2023).

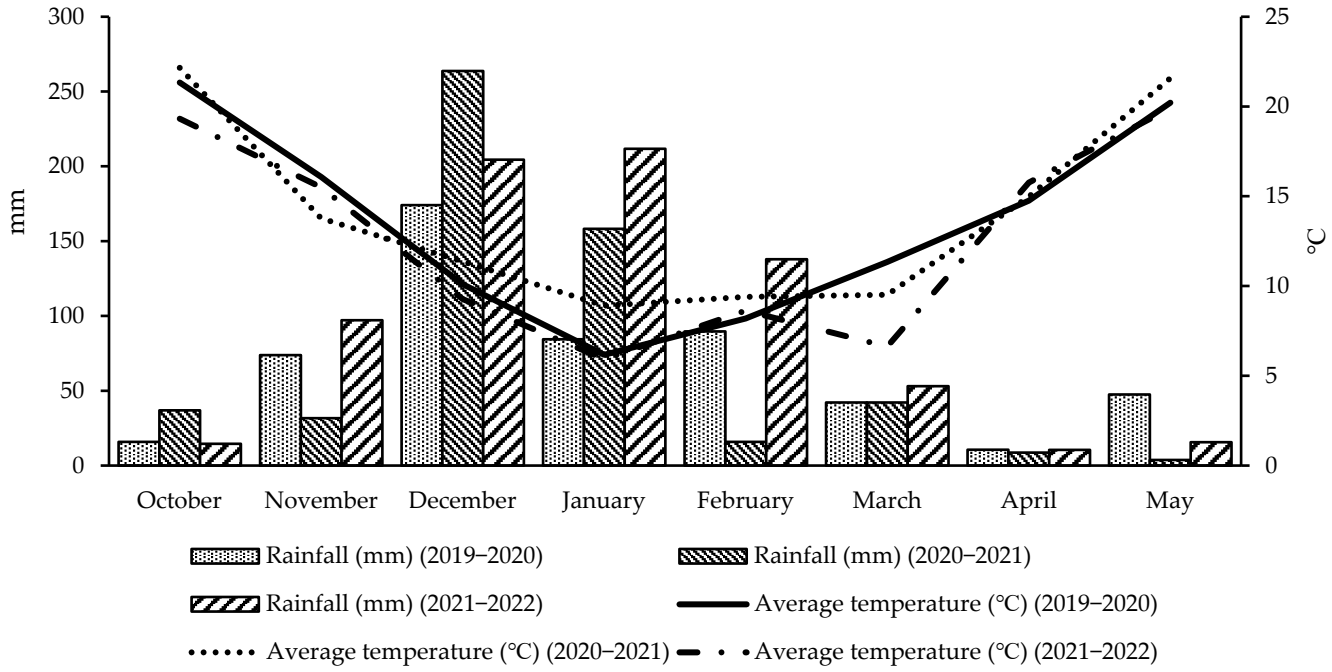


Figure 1. Data of average temperature and rainfall over three growing seasons in Antalya.

CB resistance was evaluated for all the varieties as the percentage of infected spikes, using the following equation:

$$\text{CB} = (\text{number of infected spikes / total number of infected spikes per variety}) \times 100 \quad (1)$$

Disease resistance was determined using a classification described by Mourad et al. [6]. According to this, varieties with a percentage of infected spikes of 0.1–5.0%, 5.1–10%, 10.1–30.0%, 30.1–50.0%, and 50.1–100.0% were considered as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and very susceptible (VS), respectively.

2.3. Extraction of Genomic DNA

To extract genomic DNA, at least three seeds of each variety were first sown in trays, and fresh leaves of each variety were obtained at the two-leaf stage. Genomic DNA was extracted from the fresh leaves of each variety using the NucleoSpin® Plant II Extraction Kit (Macherey-Nagel, Hœrdt, France). The concentration and quality of the extracted genomic DNA were checked by 0.5% agarose gel electrophoresis with a DNA ladder. To adjust the final concentration of 50 ng/µL for PCR analyses, the genomic DNA was diluted with Tris-EDTA (TE) buffer and then stored at −20 °C until use.

2.4. Molecular Detection of CB Resistance Genes

The genomic DNA of each variety was genotyped using different molecular markers linked to the resistance genes *Bt8*, *Bt9*, *Bt10*, and *Bt11* to detect the resistance gene(s) carriers (Table 1). Additionally, the genotypes M78-9496 (*Bt8*), M82-2098 (*Bt9*), M82-2102 (*Bt10*), and M82-2123 (*Bt11*) were used as positive controls to confirm the molecular results. The information about these markers is given in Table 2. The total volume of the PCR reaction mixture was 15 µL, containing a 50 ng DNA template, 1× PCR buffer, 1.5 mM MgCI2, 0.2 mM of dNTPs, 1 µM forward–reverse primer, and 1 U Taq DNA polymerase. A thermal cycler (Bio-Rad, Hercules, CA, USA) was used for amplifications under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 44–60 °C (Table 1) for 30 s, extension at 72 °C for 1 min, and a final extension of 10 min at 72 °C. After amplifications, the PCR products were separated in a 2% agarose gel, and the gels were visualized under UV light in a gel imaging system after staining with ethidium bromide.

Table 1. The information on molecular markers used to detect the common bunt resistance genes.

Genes	Marker Name	Marker Type	Sequence (5′→3′)	Annealing Temperature (°C)	Reference
Bt8 *Bt10* *Bt11*	Xgwm114	SSR	ACAAACAGAAAATCAAAACCCG ATCCATCGCCATTGGAGTG	58 °C	[27]
Bt9	Xgpw7433	SSR	GTACATGGAAAGAGACCAACACCA CGCTGAGCAAGGACGATAG	60 °C	[28]
Bt10	FSD RSA	SCAR	GTTTTATCTTTTTATTTC CTCCTCCCCCCA	44 °C	[29]

Table 2. Number of bread wheat varieties carrying either each *Bt* gene or a gene combination.

Number of Varieties	Resistance Gene			
	Bt8	***Bt9***	***Bt10***	***Bt11***
2	+ [a]	-	-	-
10	-	-	+	-
15	-	-	-	+
10	+	+	-	-
2	+	-	-	+
18	-	+	+	-
30	-	+	-	+
6	+	+	+	-
9	+	+	-	+
Total	29	73	34	56

[a] Presence (+) or absence (-) of the *Bt* genes according to the obtained molecular data.

2.5. Statistical Analyses

Basic statistical parameters such as mean, minimum/maximum, standard deviation, coefficient of variation, kurtosis, and skewness were first calculated using XLSTAT (Addin-

soft Co., New York, NY, USA). Combined analysis of variance (ANOVA) was performed in JMP using the following model.

$$Y_{ijk} = \mu + l_j + r_k + g_i + lg_{ik} + e_{ijk} \quad (2)$$

where Y_{ijk} is the observation of variety i in replication k at year j; μ is the general mean; l_j, r_k, and g_i are the mean effect of the year, replication, and varieties, respectively; lg_{ik} is the interaction between the varieties and the years; and e_{ijk} is the error. The means were compared with the least significant difference (LSD) test. Additionally, broad-sense heritability was calculated using META-R software version 6.0 [30]. The correlation analysis and *t*-test were performed in Minitab software version 20.0 (Minitab Inc., State College, PA, USA).

3. Results

3.1. Evaluation of Bread Wheat Varieties for Resistance to Common Bunt

The analysis of variance (ANOVA) showed the statistically important differences between the varieties and years as well as the genotype × year of interaction. Broad-sense heritability was calculated as 0.92 ($p < 0.01$) (Table 3). High correlations between the reactions of all the varieties in all the three seasons were determined (Figure 2).

Table 3. Combined ANOVA results and broad-sense heritability of bunt resistance of the varieties in all three years.

Source of Variance	d.f.	Mean Squares	F
Genotype	101	4224.49	90.42 **
Year	2	15,541.00	332.66 **
Replication	1	27.30	0.58 ^{ns}
Genotype × year	202	306.95	6.57 **
Error	611	46.72	
Broad-sense heritability		0.92 **	

** p <0.01, ^{ns}: non-significant.

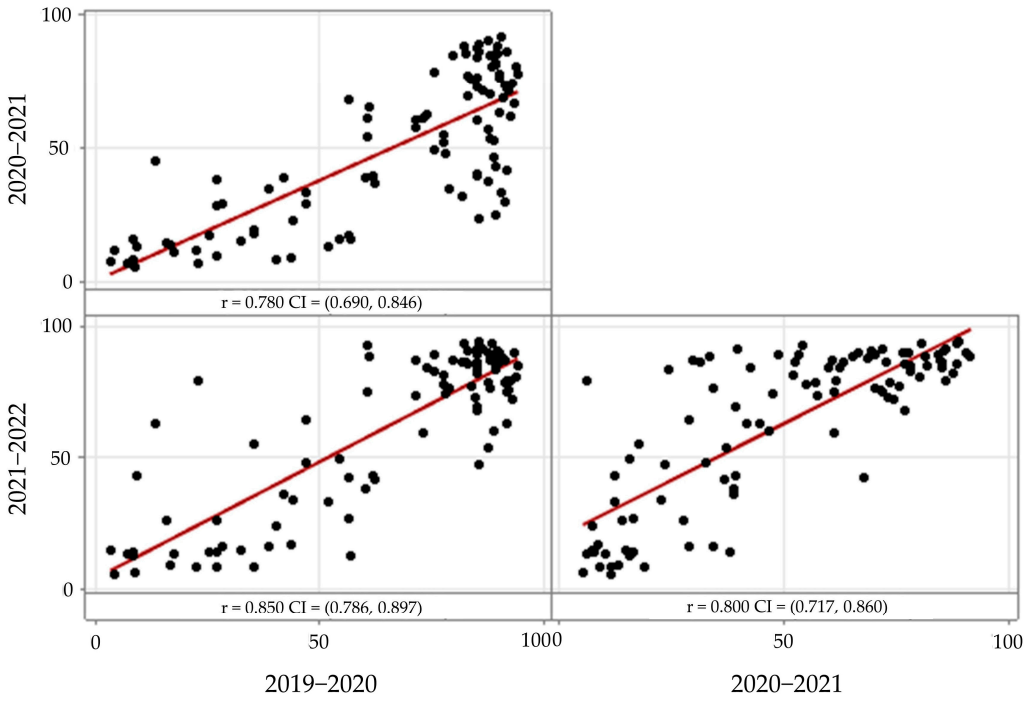


Figure 2. Correlations between percentage of infected spikes of all varieties under Antalya conditions in three growing seasons.

Among the varieties, the infection rates ranged from 3.17 to 91.49%, 5.41 to 91.41%, and 5.29 to 94.06% in the growing seasons 2019–2020, 2020–2021, and 2021–2022, respectively (Table 4). The least significant difference between the years was calculated as 1.33%. The highest mean of disease severity was calculated in 2019–2020 (66.44%), followed by 2021–2022 (62.94%), and 2020–2021 (49.88%), respectively. Additionally, skewness and kurtosis values also confirmed normal distribution for disease severity among the varieties in all three years (Table 4).

Table 4. Basic statistical parameters of reactions of all varieties overall and in three years.

Season	N	Mean	Minimum	Maximum	CV (%) *	SD **	Kurtosis	Skewness
2019–2020	102	66.44	3.17	94.49	42.69	28.36	−0.59	−0.92
2020–2021	102	49.88	5.41	91.41	54.63	27.25	−1.38	−0.15
2021–2022	102	62.94	5.29	94.06	46.93	29.53	−0.99	−0.77
Overall	102	59.75	6.85	90.30	44.41	26.53	−1.01	−0.68

* CV: coefficient of variation; ** SD: standard deviation.

The susceptible check 'Red Bobs' showed a very susceptible reaction to common bunt with an infection rate of at least 90% in all three seasons. In total, only two bread wheat varieties, Karahan 99 (3.17%) and Zencirci-2002 (4.07%), showed a resistance reaction to common bunt in the 2019–2020 season (Table S2). Tosun 21 (6.91%), Çetinel 2000 (8.33%), Müfitbey (8.15%), Ekiz (8.82%), and Yayla 305 (8.95%) were other prominent varieties with a moderately resistant reaction to common bunt in this season. On the other hand, 72.54% of the 102 varieties showed a very susceptible reaction (Figure 3).

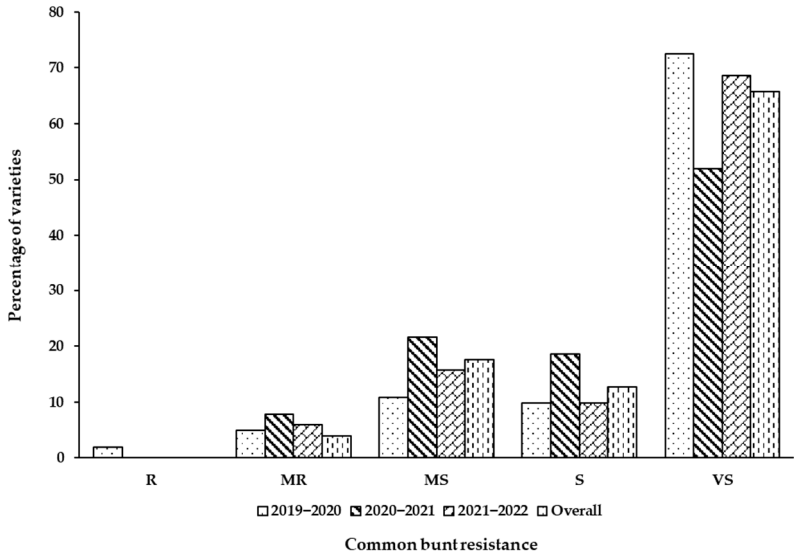


Figure 3. Reactions of bread wheat varieties to common bunt in 2019–2020, 2020–2021, and 2021–2022 and overall.

In the 2020–2021 season, no variety showed a resistant reaction to common bunt. The minimum infection rate was determined in the variety Ekiz (5.41%) with a moderately resistant reaction, followed by Tosun 21 (6.45%), Selimiye (6.48%), Karahan-99 (7.39%), Aytın 98 (7.69%), Çetinel 2000 (8.00%), Hanlı (8.82%), and Porsuk-2800 (9.15%) (Table S2). The highest infection rates were determined in the varieties Karatopak and Gökkan, with 91.41% and 90.58%, respectively. In this growing season, 51.96% of all the varieties showed a very susceptible reaction to common bunt (Figure 3).

In the 2021–2022 season, no variety showed the same resistant reaction to common bunt as in the 2020–2021 season. Zencirci-2002 (5.29%), Ekiz (6.31%), Nota (8.42%), Nacibey (8.59%), Porsuk-2800 (8.67%), and Atay-85 (8.95%) were the prominent varieties with

moderately resistant reactions to common bunt. A total of 68.63% of the varieties showed a very susceptible reaction (Figure 3), and the highest infection rates were recorded in the varieties Seri 2013 (94.06%), Alibey (93.53%), and Cumhuriyet 75 (93.52%), respectively (Table S2).

Overall, while 65.68% and 12.75% of the varieties were very susceptible and susceptible to common bunt, 17.65% and 3.92% of them showed moderately susceptible and moderately resistant reactions, respectively (Figure 3). The least significant difference (LSD) value for the varieties was calculated as 7.76%. According to this, the most resistant varieties to common bunt were Ekiz (6.85%) and Zencirci 2002 (6.99%), with moderate resistant reactions overall. Karahan 99 (8.33%) and Tosun 21 (8.85%) were the other prominent varieties with low infection rates (Table S2). On the other hand, Karatopak, with the infection rate of 90.30%, was the most susceptible variety overall.

3.2. Molecular Detection of Common Bunt Resistance Genes

The linked markers for *Bt8*, *Bt9*, *Bt10*, and *Bt11*, which were used to detect the R genes in 102 bread wheat varieties, were also tested with the susceptible check 'Red Bobs' to confirm the obtained data. After molecular screening of the related R genes in these varieties, the results were obtained and are given in Table 2. According to these results, *Bt8* was detected in 2 of the varieties, *Bt10* in 10 of them, and *Bt11* in 15 of them. There was no variety carrying only *Bt9*. However, many gene combinations, such as *Bt8* + *Bt9*, *Bt8* + *Bt11*, *Bt9* + *Bt10*, *Bt9* + *Bt11*, *Bt8* + *Bt9* + *Bt10*, and *Bt8* + *Bt9* + *Bt11*, were determined (Table 2).

The contribution of each *Bt* gene or gene combination to resistance to common bunt infection was also interpreted by comparing the infection rates of the susceptible check 'Red Bobs' without R genes. According to this, the lowest infection rate (37.14%) was determined on average in varieties with a gene combination of *Bt8* + *Bt9* + *Bt11*, followed by those with *Bt8* + *Bt9* (52.36%), *Bt8* + *Bt9* + *Bt10* (53.93%), *Bt9* + *Bt10* (57.21%), *Bt8* (62.16%), *Bt11* (63.75%), *Bt9* + *Bt11* (65.25%), *Bt10* (69.57%), and *Bt8* + *Bt11* (74.77%), respectively. The mean infection rates of the varieties with each gene or gene combinations, except for those with *Bt8* and *Bt8* + *Bt11*, were significantly lower ($p < 0.05$, *t* test) than the infection rates of 'Red Bobs' without R genes.

4. Discussion

In this study, highly significant differences were determined between 102 Turkish bread wheat varieties for resistance to common bunt (Table 3). This indicates that there is a high level of variation for this trait. The broad-sense heritability calculated for the infection rates of the varieties was 0.92 ($p < 0.01$) (Table 3), and this reveals the accuracy of the results of the study over three years. Additionally, the correlation analyses performed to decipher the relationships between the years show that the years (2019–2020, 2020–2021, and 2021–2022) were found to be highly correlated (Figure 2).

In this study, the mean infection rates of Turkish bread wheat varieties were found to be 66.44%, 49.88%, 62.94%, and 59.75% in 2019–2020, 2020–2021, and 2021–2022 and overall, respectively (Table 4). These results are consistent with those of Aydogdu and Kaya [3]. They reported that 77.41% of 32 spring wheat varieties in Türkiye were very susceptible to common bunt. Additionally, some varieties in the present study and their study are the same, and these varieties had very susceptible reactions to common bunt in both studies. Except for that of Aydogdu and Kaya [3], there has been no study to identify the registered varieties with resistance to common bunt in Türkiye. Limited studies have been conducted with wild relatives and landraces. In one of these studies, Mamluk and Van Slageren [31] tested the resistance of 450 accessions of *T. dicoccoides* and *T. boeticum* to many diseases, such as *Septoria tritici* blotch, common bunt, leaf rust, and stripe rust. They found that while most *T. boeticum* accessions had good resistance to common bunt, the majority of *T. dicoccoides* accessions were susceptible. In another study, Akcura and Akan [5] determined that 19 of 200 lines developed from landraces collected from different regions of Türkiye were resistant to common bunt. Only 19% of these landraces had very susceptible

reactions. In the present study, no variety was very resistant to common bunt except in the first growing season where only two varieties, Karahan-99 and Zencirci-2002, had a very resistant reaction. Bhatta et al. [32] already used the variety Karahan-99 as a resistant check in the study they carried out to find favorable alleles associated with bunt resistance in synthetic wheats. The results of both studies [5,31] are indicators of genetic erosion from wild relatives to landraces and then to modern varieties. In the present study, bread wheat varieties registered from 1931 to 2015 were used. One of these varieties is Yayla-305, which is a composite variety developed from landraces (Table S1). This variety has been cultivated in Anatolia for many years due to its good resistance to common bunt [33]. Then, it fell into disfavor when higher-yielding varieties replaced it. In this study, it also had very low infection rates compared to many varieties (Table S2).

As mentioned above, the infection rates of the varieties observed in the first growing season were higher compared to those in the second and third growing seasons (Table 4). Common bunt infection can be affected by environmental conditions as well as genetic resistance. Many studies have indicated that air and soil temperature during the first weeks after sowing are critical and directly correlated with the infection level of common bunt. Some studies reported that higher air temperature after sowing was negatively correlated with common bunt infection [34,35]. However, in the present study, it was determined that higher infection rates during the first weeks after sowing were in the first and third growing seasons with higher air temperature (Figure 1). These findings are consistent with the findings of the study reported by Liatukas and Ruzgas [36]. They claimed that higher air temperature during the first six weeks after sowing was positively correlated with common bunt infection. To prove the effectiveness of air temperature on common bunt infection, some researchers have also conducted studies in controlled environments [29,37,38]. In one of these studies, Laroche et al. [38] used an air temperature of 16 °C and obtained the infection rate of up to 100%. In the present study, the mean of the air temperature during the first weeks (from mid-October to the end of November) after sowing was higher in the first growing season than those of the other growing seasons. Therefore, it can be concluded that higher infection rates in the first growing season may be caused by higher air temperature during the first weeks after sowing.

Common bunt resistance in wheat is expressed with the *Bt* genes, and sixteen R genes (*Bt1*-*Bt10* and *Btp*) have been designated until now [17]. Unfortunately, a limited number of loci have been mapped which can be used for marker-assisted selection (MAS) or pyramiding. However, some studies have been conducted to find favorable alleles to be used in MAS studies in recent years [32,39,40]. In this study, three linked markers (Xgwm114, Xgpw7433, and FSD/RSA) with *Bt8*, *Bt9*, *Bt10*, and *Bt11* [27,28,38] were used to detect carriers of these genes (Table 1). In molecular detection, *Bt8* was detected in 2 of the varieties, *Bt10* in 10 of them and *Bt11* in 15 of them, whereas there was no variety carrying only *Bt9*. However, many gene combinations were determined (Table 2). Madenova et al. [41] conducted a study of the screening of wheat genotypes for the presence of resistance genes (*Bt8*, *Bt9*, *Bt10*, and *Bt11*) and reported that 15 out of 60 wheat genotypes had *Bt8*, *Bt9*, and *Bt11* resistance genes. In addition, a gene combination consisting of *Bt8*, *Bt9*, *Bt10*, and *Bt11* was detected only in the Karasai variety. Many of the gene combinations in the present study were also detected in the study reported by Madenova et al. [41].

Mourad et al. [6] conducted a field study in the USA and Turkey with 41 genotypes, including checks, synthetic wheat varieties, and crosses between synthetics and some Turkish varieties. They reported that the genotypes carrying a combination of *Bt10*, *Bt11*, *Bt12*, and *Btp* had a resistance reaction in both countries. In the present study, the varieties with a gene combination of *Bt8* + *Bt9* + *Bt11* had the lowest infection rates. The resistance gene *Bt11* was common in both studies; however, in the present study, only *Bt11* was not sufficiently effective against the inoculum source of *T. laevis*. Therefore, a marker-assisted pyramiding strategy could be the most sensible choice to manage this disease. Bhatta et al. [32] also reported that the identification and pyramiding of resistance genes are critical in breeding programs to obtain durable resistance to common bunt. As many

resistance genes have been identified and advanced differential sets consisting of near isogenic lines have been used, this strategy has been used for resistance to many foliar diseases, including stripe rust, leaf rust, stem rust, etc., in wheat for many years [42–44].

The resistance/susceptibility status of a wheat germplasm to a disease may differ in theory and practice. Even though *Bt* genes can enhance the resistance of a wheat germplasm experimentally, successful practices using a single major gene or a combination of genes are uncommon. In this study, it was found that some bread wheat varieties with *Bt* gene(s) had a susceptible reaction to common bunt (Table S2). The reason for this can be explained by the fact that the R genes are not expressed equally in all germplasm [45] because none of them have the same genetic background. In order to achieve this, it is necessary to develop near-isogenic lines (NILs) using a marker-assisted backcrossing strategy.

5. Conclusions

In summary, the common bunt infection rates of the bread wheat varieties ranged from 6.85 to 90.30% over three years. Unfortunately, 65.68% and 12.75% of the varieties had very susceptible and susceptible reactions. The varieties that were the most resistant to common bunt were determined to be Ekiz and Zencirci-2002, while Karahan-99 and Tosun 21, with very low infection rates, were other prominent varieties. According to the molecular detection, many gene or gene combinations were determined in the varieties. In the resistant varieties, Ekiz and Zencirci-2002, gene combinations of *Bt8* + *Bt9* + *Bt10* and *Bt8* + *Bt9* + *Bt11* were detected, respectively. The varieties with low infection rates could be used in breeding programs conducted for resistance to common bunt. However, to obtain more concrete data on the resistance status of these varieties, it is necessary to test them with more pathotypes in future studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102491/s1, Table S1: Information about the bread wheat varieties used in this study, Table S2: Infection rates and infection types of 102 bread wheat varieties in all three years and overall and molecular detection results with linked markers of *Bt8*, *Bt9*, *Bt10*, and *Bt11*.

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