Yuzuncu Yil University
Journal of the Institute of Natural & Applied Sciences
https://dergipark.org.tr/en/pub/yyufbed

Research Article

The Screening of Black Point in Commercial Bread Wheat Cultivars Grown in Turkey, and The Effect of Black Point on Thousand Grain Weight

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Article Info

Received: 02.09.2022
Accepted: 22.11.2022
Online April 2023

DOI:10.53433/yyufbed.1170102

Keywords
Black point,
Bread wheat,
Thousand grain weight,
Triticum aestivum

Abstract: The black point is a discoloration that shows up on the embryos of wheat and barley, and it is becoming an important problem in many wheat-growing areas. In this study, 200 commercially important bread wheat varieties that were cultivated between 1931 and 2017 in Turkey were screened in terms of black point, and the effect of black point was observed on thousand grain weight in all cultivars. The trials were conducted in accordance with the augmented block trial design in three environments during the 2016-17 growing season. A high variation was observed among the cultivars for black point and thousand grain weight. Twelve varieties (6% of populations) showed no discoloration (0.00%) in any environment, whereas 44 varieties (22% of population) showed discoloration rate more than 10.00%. The first three most susceptible cultivars showed black point percentages of 38.76, 32.08, and 27.5, respectively. In the remaining cultivars, eighty-eight showed discolorations up to 5.00%, whereas the rest demonstrated discoloration from 5.00% to 24.00%. Interestingly, a positive correlation was observed between black point damage and thousand grain weight (r=0.32). The reasonable interpretation is that the larger seed surfaces may have increased the effect of black point causes, such as pathogens and oxidative reactions. This screening enabled us to determine black point susceptible and tolerant cultivars in well-known bread wheat cultivars growing in Turkey. Hence, the results will be useful for wheat production and wheat breeding approaches in Turkey and around the world.

Türkiye'de Yetiştirilen Ticari Ekmeklik Buğday Çeşitlerinde Embriyo Kararması Taraması ve Embriyo Kararmasının Bin Dane Ağırlığına Etkisi

Makale Bilgileri

Geliş: 02.09.2022
Kabul: 22.11.2022
Online Nisan 2023

DOI:10.53433/yyufbed.1170102

Anahtar Kelimeler
Bin dane ağırlığı,
Ekmeklik buğday,
Embriyo kararması,
Triticum aestivum

Öz: Embriyo kararması, buğday ve arpa embriyosunda ortaya çıkan bir renk değişikliğidir ve buğday yetiştirilen birçok bölgede önemli bir sorun haline gelmektedir. Bu çalışmada, Türkiye'de 1931 ve 2017 yılları arasında tarımı yapılan 200 adet ticari ekmeklik buğday çeşidi embriyo kararmasına karşı taranmış ve tüm çeşitlerde embriyo kararmasının bin dane ağırlığına etkisi gözlemlenmiştir. Denemeler, 2016-17 yetiştirme sezonunda üç çevrede augmented deneme desenine uygun olarak gerçekleştirilmiştir. Embriyo kararması ve bin dane ağırlığı bakımından çeşitler arasında yüksek bir varyasyon gözlenmiştir. On-iki adet çeşit (popülasyonun %6'sı) hiçbir ortamda (%0.00) renk değişikliği göstermemişken, 44 adet çeşit (popülasyonun %22'si) %10.00'dan fazla renk değişikliği göstermiştir. En duyarlı ilk üç çeşit sırasıyla 38.76, 32.08

ve 27.5 embriyo kararma yüzdeleri göstermiştir. Kalan çeşitlerin seksen-sekizinde %5.00'e kadar renk değişimi görülürken, diğer çeşitlerde %5.00'den %24.00'e kadar renk değişimi görülmüştür. İlginç bir şekilde, embriyo kararması hasarı ile bin dane ağırlığı arasında pozitif bir ilişki tespit edilmiştir (r=0.32). En makul yorum, daha iri tohumların daha geniş tohum yüzeylerine sahip olmalarından dolayı patojenler ve oksidatif reaksiyonlar gibi embriyo kararması etmenlerinin etkisini artırmış olabileceğidir. Bu tarama, Türkiye'de iyi bilinen ekmeklik buğday çeşitlerinde embriyo kararmasına duyarlı ve dayanıklı çeşitleri belirlememize olanak sağlamıştır. Bu nedenle, sonuçlar Türkiye’de ve dünya çapında buğday üretimi ve buğday ıslah çalışmaları için faydalı olacaktır.

1. Introduction

Wheat is one of the most important food crops in the world, especially bread wheat (*Triticum aestivum*) provides approximately 20% of the world's food. Maintaining food security is a crucial factor to ensuring enough food for people in the face of a growing human population and the adverse effects of climate change (Abberton et al., 2016; Batley & Edwards, 2016).

The black point is a dark discoloration that shows up on the embryo of cereal seeds such as bread wheat, durum wheat and barley, and it may reach around the adaxial side of the seeds as well (Fernandez & Conner, 2011). It appears in many wheat-growing areas where a high amount of wheat is produced (Sissons et al., 2010; Fernandez & Conner, 2011; Busman et al., 2012). Black point decreases the end-use quality of the grain (Dexter & Matsuo, 1982), the germination percentage, and produce weak seedlings (Li et al., 2014). In addition, it affects many other agronomic and quality parameters of wheat (Dexter & Matsuo, 1982). It can also influence the plant vigour and growth (Hudec, 2007; Fernandez et al., 2014). Besides that, it may lead to the production of harmful metabolites like Alternaria mycotoxin and Alternariol monomethyl ether (Logrieco et al., 2003; Desjardins et al., 2007), which are the potential causes of esophageal cancer (Liu et al., 1992). The severity of black point rises crucially when the available seed moisture exceeds 20% and the air relative humidity ratio is more than 90%. Also, late irrigation, excessive nitrogen fertilization, and plant lodging could increase the effect of black point damage (Conner et al., 1992). Black-pointed seeds cause blighted seedlings and rotten roots. All these reasons eventually affect the seed quality and hence the food products because the infected seeds have the undesired smells and colours. The wheat markets demand disease-free grains, and the marketing experts defined some upper limits in the wheat trade (Rees et al., 1984; Lehmensiek et al., 2004).

Some fungus species, such as *F. proliferatum*, *B. sorokiniana*, and frequently *A. alternata,* are the main reasons of black point in wheat (Kumar et al., 2002; Perelló et al., 2008; Davis & Jackson, 2009; Busman et al., 2012). The other potential reasons of the black point are the enzymatic reactions by phenolic complexes (Fernandez et al., 2014). The colour changes on plant tissue is mostly followed by enzymatic reaction that occurred by some kind of biotic and abiotic stresses factors and mechanical injuring that possibly cause oxidation events by polyphenol oxidase (Fuerst et al., 2014), peroxidases (Fernandez et al., 2014), and lipoxygenase (Porta & Rocha-Sosa, 2002). Also, some of the pigments like quinines (Fernandez & Conner, 2011) and melanins (Fuerst et al., 2014) can affect the seed colour.

Wheat cultivars demonstrate a high variation in black point susceptibility (Waldron, 1934; King et al., 1981; Ellis et al., 1996). The wide range of black point incidence (0.3% to 66.7%) was reported in wheat (Ellis et al., 1996; Li et al., 2014; El-Gremi et al., 2017). The varying black point incidence was explained by the responses of the genotypes to various environmental and fungal factors, and the genetic basis of black points (Walker, 2012). Many approaches are used to decrease this problem, such as cultivating resistant cultivars and other basic standard applications during the growing stages (El-Gremi et al., 2017). Cultivating resistant cultivars is a more important and profitable way compared to other standard protecting procedures, such as chemical applications or biocontrol agents. Therefore, discovering resistant cultivars is the most important way to struggle with this problem.

This study aimed to 1) screen for naturally occurring black point damage in 200 commercially important bread wheat cultivars growing in Turkey from 1931 to 2017, and 2) determine the effect of black point on thousand grain weight (TWG) in bread wheat.

2. Material and Methods

2.1. Materials

Seed material consists of 200 bread wheat cultivars that were cultivated and somehow included in wheat breeding approaches between the years 1931 and 2017 in Turkey. The materials were retrieved from the collections of Research Institutes, Private Sector, and Çukurova University Field Crops Department. The variety names and registered years were demonstrated in Table S1 and were ordered depend on their registration years. Four well-known cultivars in the trial regions, Ceyhan-99, Adana-99, Pandas, and Osmaniyem, were used as control and nominated as Ceheck-1, Check-2, Ceheck-3, and Check-4 in blocks, respectively.

2.2. Phenotyping

The trials were established in the 2016-2017 growing season in three different locations, Cukurova University (Adana), Dogankent (Adana) and Çumra (Konya) in Turkey. Hereafter, the environments will be referred as E1, E2, and E3, respectively. E1 has 29-m altitude (37°00'45" N and 35°21'020" E), E2 has 100-m altitude (36°51'09" N and 35°20'41" E), and E3 has 119-m altitude (37°03'06" N and 35°21'45" E).

The trials were conducted in an augmented trial design that consisted of 10 blocks, each comprising 20 test entries (cultivars) and 4 controls. The needed seed materials were determined depending on thousand grain weight and germination percentage. The seeds were planted in two 2-m rows, with a 10-cm seed spacing in a row and a 20-cm spacing between rows. The sowing times were determined according to the optimum climatic conditions of the trial regions. Precipitation, humidity, and temperature values of growing areas were demonstrated in monthly basis in Figure 1 and Table1.

Standard agricultural practices (such as irrigation, fertilization, and disease and pest control) were employed through the plant development periods. The weeds in the plots were removed manually, whereas those among the blocks were removed by a hand-operated mini-hoeing machine. Fully matured plants were harvested by using a mini combine harvester (Hege-125C). This is corresponding to the Zadoks Scale GS93 (Zadoks et al., 1974)

Table 1. Precipitation, humidity, and temperature values of environments were shown in monthly basis, as well as total and average

Precipitation mm=kg÷m²										
Env.	November	December	January	February	March	April	May	June	July	Total
E1	22.50	6.00	186.50	108.50	2.50	90.00	77.50	67.00	10.50	571.00
E2	0.20	11.90	216.30	52.00	0.80	65.40	65.90	45.90	17.30	475.70
E3	0.00	19.40	101.20	27.80	0.80	93.00	60.40	58.60	14.00	375.20
Temperature °C										
Env.	November	December	January	February	March	April	May	June	July	Average
E1	21.40	13.30	3.30	7.50	9.30	14.00	17.20	20.60	24.80	14.60
E2	17.70	10.30	5.30	5.10	5.60	10.30	13.00	17.00	21.30	11.70
E3	14.00	6.00	-1.90	-4.40	-0.60	7.20	11.40	15.80	20.30	7.50
Humidity %										
Env.	November	December	January	February	March	April	May	June	July	Average
E1	61.50	54.70	85.40	72.10	56.20	71.70	69.10	74.70	75.90	69.00
E2	56.40	52.20	64.80	62.10	50.70	62.80	60.70	68.80	69.10	60.80
E3	49.10	54.80	84.30	86.30	74.80	64.80	51.90	56.70	53.80	64.00

Adana Meteorological Service, 2017

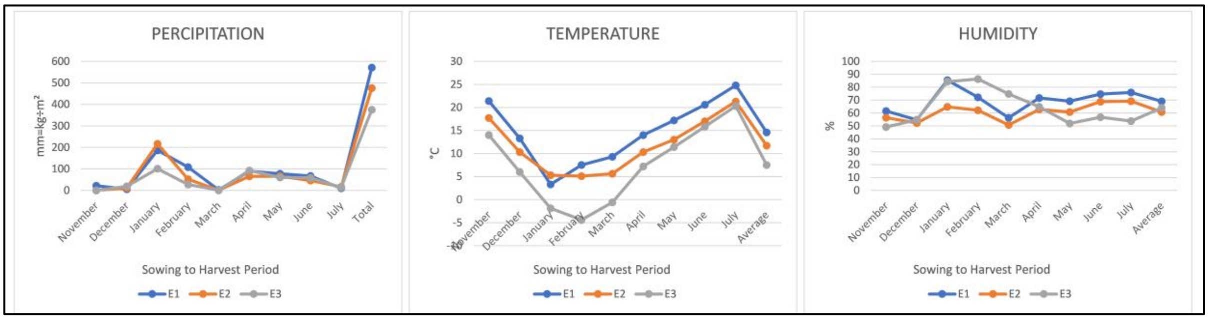

Figure 1. The graphical demonstration of climate factors in three environments.

2.2.1. Black point evaluation

The harvested samples were brought to the laboratory, sifted, and broken seeds were removed from the samples. Three replicated 100 seeds were selected from each plot to determine the black point damage rates. The seeds with black point (if black or brown discoloration is > 1 mm^2) were counted for each replicate and averaged (Figure 2a and 2b). The black point percentage was calculated with the number of discoloured seeds divided by the total seeds in one replication and multiplied by 100 to convert the percentage. The mean of three replications was used for variance analysis according to the augmented block trial design.

2.2.2. TGW measurements

The thousand grain weights were also accounted with the same seed samples. Three replicates, with 250 grains were weighted by analytic balance, and the results were multiplied by four to convert to 1000 grain weight. The replications were averaged, and mean values were used for variance analysis in accordance with the augmented block trial design.

2.2.3. Statistical analyses

The analysis of variance was implemented by "augmentedRCBD" R package (Aravind et al., 2020) in RStudio 2022.02.0. The distribution plots were also created by this package. The Pearson's correlation coefficient between black point and TGW was calculated by JASP software Version 0.11.1 (Team, 2019). The Student's multiple comparison test was used to compare the means of every entry across three environments with a <0.05 significance level for black point and TGW performed with JMP Genomics v.9.0 (SAS Institute Inc., Cary, NC, USA). Broad sense heritability was calculated manually from the descriptive statistics output table for both traits in each environment using the formula $H^2 = V_G/V_P$, where V_G is genotypic variance and V_P is phenotypic variance.

3. Results

3.1. Black point evaluation

Black point symptoms were evaluated in 200 bread wheat cultivars that were cultivated between 1931 and 2017 years in Turkey. A near normal distribution was observed for black point in a combined three environments, however, distribution was skewed to the right side (Figure 3a). Significant skewness (1.83) and kurtosis (7.85) was observed for this combined data (Table 2). This is a highly skewness and moderated kurtosis for black point distribution in the population. ANOVA results indicated that the black point symptoms in different environments varied considerably among the cultivars (Table 3). The means of black point percentage of all genotypes were 14.85%, 4.71.%, and 0.39% in E1, E2, and E3, respectively. These results were expected when considering the weather conditions that prevailed throughout the growing season (Table 1 and Figure 1). The prevailing precipitation, temperature, and humidity, especially in the grain filling periods, created a suitable environment to produce black point factors, which may be due to fungal pathogens or oxidation reactions. The broad sense heritability for black point susceptibility over three environments and combined data was 0.91 (E1), 0.81 (E2), 0.99 (E3), and 0.91. Although black point susceptibility demonstrated high heritability values, environmental percentages and their parallelism to weather conditions clearly show that black point damage was considerably affected by environment (Table 1, Table 2, Figure 1). The coefficient of variation showed pretty high values across environments (E1: 26.07%, E2: 95.20%, E3: 47.09%, and Mean: 29.20%). These results were expected and corresponded to widely varied black point damage ratios in the population.

The Student's multiple comparison test grouped the varieties into a few wide ranges depending on discoloration rates (Table S2). The focus here is the most susceptible and black point-free cultivars specifically. Twelve varieties, which are corresponding to 6% of the populations (Altindane, Libellula, Lancer, Chinespring, Sakarya-75, 4-22 (landraces), Alpu-2001, Veery's, Irnerio, Altay 2000, Seri 2013, and Uzunyayla) showed no discoloration (0.00%) in any environment, whereas 44 varieties, which are corresponding to 22% of the population demonstrated discoloration rate more than 10.00% (Table S2). The first three most susceptible cultivars were Yoruk, S-24, and Cemre, with black point percentages of 38.76, 32.08, and 27.5, respectively, whereas the last five most resistant cultivars, Kirkpinar-79, Aldane, Eswyt-103, Scirocco, and Yildirim, had a range between 0.08% and 0.25% among discoloured genotypes (Table S2). In the remaining cultivars, eighty-eight showed discolorations till 5.00%, whereas the rest demonstrated discoloration rate from 5.00% to 24.00%.

Figure 2. The healthy and damaged seed symptoms (a). The ranges of symptom severity within a cultivar, from brown to fully black-coloured embryo (b).

3.1. TGW

TGW values were calculated for all cultivars in each individual environment and in combined environments. The mean of three environments showed a normal distribution for TGW traits (Figure 3b). Skewness and kurtosis values were not significant with -0.31 and 2.75, respectively (Table 1). Variance analysis showed significant differences among cultivars (Table 3). Averaged TGW values for three environments were 46.44 g, 40.50 g, and 44.28 g. The broad sense heritability for TGW in three environments and combined environments was 0.89, 0.90, 0.57, and 0.91, respectively. The coefficient of variation values were 4.06% (E1), 5.20% (E2), and 8.50% (E3) for environments separately, and 3.66% for the combined environments. As such as black point results, the Student's multiple comparison test grouped the cultivars into a couple of large ranges. Here, the highest TGW was obtained in Cumhuriyet-75 cultivar with 57.26 g, whereas the lowest in Alora cultivar with 28.81 g (Table S3).

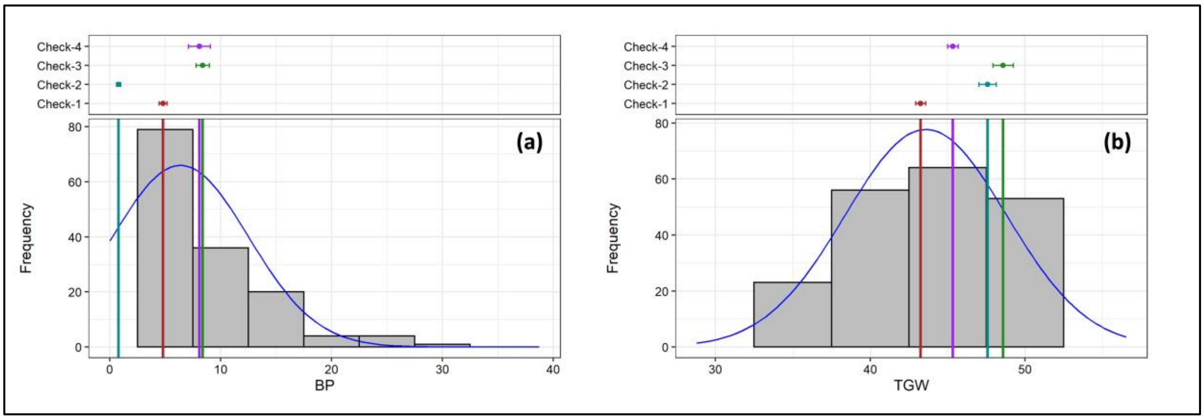

Figure 3. Distribution plots of black point (a) and thousand grain weight (b) in a combined three environments. The control cultivars were demonstrated as vertical-coloured lines on the bars. BP: Black Point, TGW: Thousand Grain Weight.

Table 2. Basic statistic parameters for black point effect and TGW in bread wheat cultivars in three environments and combined environments

Env.	Trait	Mean	Std.Er	Std.Dev.	Min	Max	Skewness	Kurtosis	CV
E1	TGW	46.44	0.41	5.90	30.32	61.03	-0.17ns	2.75ns	4.06
	BP	14.85	0.87	12.45	0.00	59.84	1.06**	3.83*	26.07
E2	TGW	40.05	0.47	6.75	21.78	53.31	-0.54**	2.82ns	5.20
	BP	4.71	0.57	8.09	0.00	48.46	2.78**	11.32**	95.92
E3	TGW	44.28	0.41	5.90	29.77	60.65	-0.02ns	2.85ns	8.50
	BP	0.39	0.20	2.82	0.00	38.03	12.08**	159.14**	47.09
Combined	TGW	43.59	0.37	5.21	28.79	56.53	-0.31ns	2.75ns	3.66
	BP	6.36	0.43	6.14	0.00	38.76	1.83**	7.85**	29.20

ns P > 0.05; * P <= 0.05; ** P <= 0.01, BP: Black point, TGW: Thousand Grain Weight

Table 3. Augmented block design variance analyses for black point effect and TGW in bread wheat cultivars in three environments and combined environments

		E1		E2		E3		Combined	
Source of Variation	Df	BP	TGW	BP	TGW	BP	TGW	BP	TGW
Treatment (Ignoring Block)	200	165.25**	35.28**	74.55**	48.80**	8.04**	34.90**	41.31**	28.87**
Treatment: Check	3	691.43**	40.60**	74.49**	71.42**	0.02ns	86.60**	125.47**	56.54**
Block (Eliminating Treat.)	9	25.67ns	9.21*	47.46**	4.33ns	0.03ns	18.17ns	6.79 ns	2.97ns
Residuals	30	13.82	3.60	14.00	4.47	0.02	14.34	3.23	2.59

ns P > 0.05; * P <= 0.05; ** P <= 0.01, BP: Black point, TGW: Thousand Grain Weight

4. Discussion and Conclusion

The black point damage shows up with different causatives in seed and other parts of wheat. Depending on the effect factors involved, such as fungal pathogens or oxidation reactions, it could result in a low rate of germination, low seedling establishment, weak seedlings, seedling blight, leaf spot, head blight, root decay, and a drop in wheat yield and quality (Kumar et al., 2002; Toklu et al., 2008; Li et al., 2014). Conversely, in this study, black point did not affect the TGW negatively. Interestingly, a positive correlation was identified between the black point and TGW among genotypes at level of $p<0.01$ ($r=0.325$). Thus, the discoloured seeds were heavier than the black point-free or light discoloured ones. It is thought that larger seed surface areas may be more exposed to the damage factors than smaller seed surfaces (Fernandez et al., 1994; Fernandez et al., 2014). The positive correlation between black point and TGW reported in several previous studies as well (Ellis et al., 1996; Toklu et al., 2008; Li et al., 2019). Besides that, it was reported that the grain filling period is longer in larger genotypes which can contain more moisture content that leads to more sub-epidermal mycelium (if the cause is pathogens) compared to small seeds (Waldron, 1934; Evans et al., 1975; Li et al., 2019). In this study, the grain filling durations were not calculated.

In this study, the rate of black point varied among the cultivars and locations, demonstrating that black point was affected by both genetic and environmental factors. Li et al. (2019) reported the enormous effect of environment on black point incidence, as well. However, the high broad-sense heritability values (Table 2) showed that genetic factors played a more crucial role in black-point than the environment. For example, Li et al. (2014) identified 58.6% broad sense heritability for black point in a population involved 403 wheat genotypes. In present study, the higher skewness in the distribution of black points across genotypes indicated that black points could be controlled by a few genes, whereas TGW showed a normal distribution that could be influenced by many genes. High variation of black point was reported for wheat in many studies (Beniwal et al., 2005; Gul, 2005; Toklu et al., 2008; Draz et al., 2016).

High precipitation, humidity, and temperature during the seed formation stages contributed to high black point damage, whereas the low values in this period decreased the black point damage level. This is an expected result that has been reported previously (Kumar et al., 2002; Clarke et al., 2004; Mak et al., 2006; Moschini et al., 2006; Toklu et al., 2008; Jain et al., 2012). For instance, Moschini et al. (2006) reported that the extreme precipitation throughout the milk formation to dough formation phase brings about high levels of black point damage in seeds. In the current study, the climatic data showed that there is a rise in rainfall between April and the end of June, which corresponds to the filling period for those environments where the trials were conducted (Table 1 and Figure 1). The average black point rates of all cultivars in separate environments were 14.85% (E1), 4.71% (E2), and 0.39% (E3), which support this suggestion. Li et al. (2019) also reported similar results for their study.

The high level of black point especially decreases the seed quality, and this directly affects the end-use products. Seed appearance (such as colour and brightness) is one of the important quality parameters that determine the wheat market price. The deterioration in this parameter led to a decrease in wheat prices in the market. Solanki et al. (2006), reported a price reduction in wheat from 3.71% to 12.49% depending on seed black point damage levels. Additionally, the seeds that have black point damage may carry toxic matters that are worsening the wheat quality (Fernandez & Conner, 2011; Amatulli et al., 2013).

Various degrees of black point and black point-free seeds were identified among the cultivars and across environments. This will enable the use of the black point-free (resistant or tolerant) genotypes in direct wheat production or wheat breeding research. As a result, the cultivars having low TGW were more tolerant to black point. It is shown that these results will offer a preliminary selection of black point-free genotypes from mass populations for wheat breeders to use in breeding studies. However, the black point is considerably affected by environmental conditions, so the correlation analyses between black point and other agronomical traits, especially over different environments, will uncover the resistant and susceptible genotypes effectively. In the conclusion, more experiments, such as artificial inoculation or oxidation reaction tests, are required to detect the exact resistant cultivars from such collections. Here, molecular marker techniques also will provide a solid confirmation if used in experiments.

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