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Evaluation of Agronomic Traits and Allele Specific DNA Markers Related to Some Disease and Quality Traits in Mutant Karakılçık M_4 Individuals

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ABSTRACT
Karakılçık (KK) is a landrace, of which both bread and durum wheat forms exist. This is the first study that reports mutation induction and detection with agronomic traits and allele specific markers on KK durum wheat landrace. In the study, KK landrace was induced with chemical mutation using sodium azide (NaN_3) (3 mM) mutagen to improve agronomic traits. In the research, KK genotype and the 13 M_4 individuals (KK-1 to KK-13) were used as plant materials. According to the results, the shortest mutant genotype was KK-10 (125.80 cm), with the highest protein ratio (18.50%) and wet gluten ratio (37.10%), while KK-9 genotype had the highest grain yield (4285.6 kg ha^{-1}). The average polymorphism information content (PIC) was calculated as 0.97, while the average allele number was 15.2 per marker. *Glu-B1* ($Bx7^{OE}$ primer) allele was determined on KK-11 and KK-13 genotypes. *Wx-A1* allele was found on KK-1, KK-2, KK-4, KK-5 and KK-7 genotypes. *Sr49* allele (Sun209) was determined on KK-5 genotype, while *Yr45* allele (Xwgp118) was detected on KK-10 genotype. In addition, the high protein content allele *Gpc-B1* (UHW89 primer) was found on KK-1, KK-2, KK-3 and KK-4 genotypes. Based on the principal component biplot analysis, it was determined that there was a positive relationship between grain yield (GY) with test weight (TW), grain number and weight per spike (GNS and GWS), and negative relationship with the other traits.

Field Crops

Research Article

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Mutant Karakılçık M_4 Bireylerinde Agronomik Özellikler, Bazı Hastalık ve Kalite Özellikleri ile İlişkili Allellerin DNA Markörleri ile Saptanması

ÖZET
Karakılçık (KK), hem ekmeklik hem de makarnalık buğday formları bulunan yerel bir çeşittir. Bu, yerel KK makarnalık buğday çeşidinde agronomik özellikler ve allel spesifik markörler ile mutasyon tespitini bildiren ilk çalışmadır. Çalışmada, agronomik özellikleri iyileştirmek için sodyum azid (NaN_3) (3 mM) mutajeni kullanılarak kimyasal mutasyon ile yerel KK çeşidi muamele edilmiştir. Araştırmada bitki materyali olarak KK genotipi ve 13 M_4 bireyi (KK-1 ila KK-13) kullanılmıştır. Elde edilen sonuçlara göre, en yüksek protein oranı (%18.50), yaş gluten oranı (%37.10) ile en kısa mutant genotip KK-10 (125.80 cm) olurken, KK-9 en yüksek tane verimine (4285.6 kg ha^{-1}) sahip genotip olmuştur. Ortalama polimorfizm bilgi içeriği (PIC) 0.97, ortalama allel sayısı markör başına 15.2 olarak hesaplanmıştır. *Glu-B1* ($Bx7^{OE}$) alleli, KK-11 ve KK-13 genotiplerinde, *Wx-A1* alleli ise KK-1, KK-2, KK-4, KK-5 ve KK-7 genotiplerinde belirlenmiştir. KK-5 genotipinde *Sr49* alleli (Sun209), KK-10 genotipinde ise *Yr45* alleli (Xwgp118) tespit edilmiştir. Ayrıca KK-1, KK-2, KK-3 ve KK-4 genotiplerinde yüksek protein içerikli allel olan *Gpc-B1* alleli (UHW89) bulunmuştur. Temel bileşen biplot analizine göre, tane verimi (TV) ile hektolitre ağırlığı (HL), başakta tane sayısı ve başakta tane ağırlığı (BTS ve BTA) arasında pozitif, diğer özelliklerle negatif ilişki olduğu belirlenmiştir.

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INTRODUCTION

Cereals are cultivated 41% of the cultivated products and wheat constitutes 48% of the cereals (FAO, 2020). Durum wheat is an important component of the human diet. In the world, the cultivation of durum wheat constitutes about 8% of the total wheat planting, which is limited to a small number of countries, including Turkey, which produces about 3% of the world wheat production. Turkey is one of the most important countries in the world in pasta production. It is one of the gene center of durum wheat, which is the basic raw material of pasta production. In Turkey, primarily the South-Eastern Anatolia and Middle Anatolia are the most suitable regions for the production of high-quality durum wheat (Yildirim & Atasoy, 2020).

In Turkey, durum wheat is used in the bulgur, semolina and pasta sector. Although Turkey's durum wheat production is adequate to meet the needs of industry as quantity, quality raw materials are needed. The quality of durum wheat is affected by the environmental conditions as well as the genetic influence. For this reason, the development and production of high yielding and quality durum wheat varieties suitable for different ecological conditions will make an important contribution to meeting the quality durum wheat demands of industry (Yildirim & Atasoy, 2020).

Karakılçık is an important genetic resource of Turkey and one of the most preferable landrace for bulgur production in East-Mediterranean region due to its high protein ratio resulting in taste on foods made with its bulgur. On the other hand, it has some disadvantages such as lodging due to plant height and low grain yield. Therefore, its farming is not common in the region.

Based on plant genetic diversity, there are hybridizations, naturally occurring selections and mutations. Mutagenesis is one of the breeding tools used to improve agronomic traits. Mutagenesis with chemical and physical mutagens cause random changes in an organism's DNA (Durland & Ahmadian-Moghadam, 2022). Mutagen agents with well-defined mutagen dose and duration can positively affect the traits such as disease resistance, earliness, plant height, yield and quality in cultivated plants (Kiraz et al., 2019). Since gene mutations are mostly recessive, thus they may be detected in the M_2 generation, while chromosome mutations may be detected in M_1 and later generations. However, mutagenesis may be detected with molecular markers.

Principal component biplot analysis is used to enable constructing graphics indicates more than one parameter of the genotypes and ensures a comparison between genotypes and investigated characteristics (Aslan et al., 2017; Bai et al., 2018; Al-Ashkar et al., 2019; Güngör et al., 2019).

In this study, it was aimed to (i) evaluate agronomic traits of 13 M_4 mutant lines, induced by chemical mutation using NaN_3 and Karakılçık local genotype, and (ii) detect alleles of some disease and quality traits with molecular markers.

MATERIAL and METHOD

Plant material

In this study, 13 M_4 mutant lines induced by chemical mutagenesis and original Karakılçık local durum wheat genotypes were used as plant material.

NaN_3 induced mutation

The M_4 mutant genotyped derived from a mutagenesis assay, in short; the seeds of Karakılçık durum wheat landrace (5000 seeds) were used soaked over-night (shaked for aeration) and rinsed before treating two hours with 3 mM sodium azide (NaN_3) mutagenesis agent, in a pH 4.5 and 100 mM potassium phosphate monobasic (KH_2PO_4) buffer (shaked for aeration). The seeds were rinsed with tap water three times (each 30 minutes and the last one for one hour) and planted as M_1 plants (Kiraz et al., 2019). Selections were made on M_1 and M_2 generations and M_4 plants were generated from M_3 single plant rows.

Field trial

The field trial was carried out in 2020-2021 cropping season in Kahramanmaraş, which is located in East-Mediterranean and with the coordinates of 37° 35' 4.9" north latitude and 36°55'35" east longitude. The elevation of the experiment site is 568 m, and the climate data of the experiment year is given in Table 1 (Anonymous, 2021).

The research was arranged in a randomized complete block design with three replications. Sowing was done with a six-rowed special experiment drill with a 20 cm row space and 6 m long plot size (6 × 1.2 m) 7.2 m^2 at planting and 6 m^2 at harvest (5 × 1.2 m) with 550 seeds per m^2. Fertilizer was applied at planting as 80 kg ha^{-1} N and 80 kg ha^{-1} P_2O_5 and 70 kg ha^{-1} N as top dressing at jointing stage. Herbicide (Mesosulfuron-methyl + Thiencarbazone-methyl + Iodosulfuron-methyl-sodium + Mefenpyr-diethyl) was used for weed control.

Table 1. Average climatic data from experiment years
Çizelge 1. Deneme yılına ait iklim verileri

	Month									Total or average *Toplam veya Ortalama*
	Year *Yıl*	November *Kasım*	December *Aralık*	January *Ocak*	February *Şubat*	March *Mart*	April *Nisan*	May *Mayıs*	June *Haziran*	
Precipitation (mm), *Yağış (mm)*	2020–2021	52.0	56.8	204.3	29.5	137.1	16.2	8.2	0.0	504.1
	Long term *Uzun yıllar*	87.5	116.6	125.4	108.3	93.4	69.8	41.2	8.4	650.8
Average temperature (°C), *Ortalama Sıcaklık (°C)*	2020-2021	12.4	8.2	6.9	9.3	10.4	16.6	23.5	25.5	14.1
	Long term *Uzun yıllar*	11.5	6.8	4.9	6.4	10.6	15.5	20.3	25.3	12.6
Relative humidity (%), *Ortalama Nem (%)*	2020-2021	65.9	74.7	70.2	59.8	61.2	57.5	43.3	49.0	60.2
	Long term *Uzun yıllar*	66.7	79.9	69.9	65.6	60.0	57.6	54.9	49.7	63.0

In the research, agronomic traits such as plant height (cm) (PH), spike length (cm) (SL), spikelet number (number) (SN), grain number per spike (grains) (GNS), grain weight per spike (g) (GWS), thousand kernel weight (g) (TKW) and grain yield (kg ha^{-1}) (GY) were evaluated as described by Güngör and Dumlupinar (2019) and test weight (kg) (TW), protein ratio (%) (PR) and wet gluten (%) (WG) were measured by Near Infrared (NIR) spectroscopy (Thermo Fisher Scientific).

DNA isolation and PCR

DNA isolation was performed using cetyl trimethyl ammonium bromide (CTAB) method (Oliver et al., 2010) and all genotypes were screened using five allele-specific DNA markers (Table 2). PCR; on 96 PCR plates with a volume of 0.02 ml; 1 µl dNTP mix (10 mM mix (A+T+G+C)), 3 µl 10x buffer, 1.2 µl $MgCl_2$, DNA primer pair (1 µl F and 1 µl R), 3 µl (50 ng) genomic DNA, A total of 20 µl of PCR solution was prepared with 9.5 µl of dH_2O, and 0.3 µl of Taq DNA polymerase (5 U μl^{-1}, Fermantes). PCR reactions are in "eppendorf" brand thermal cycler device; after running at 95°C for 5 minutes, at 95°C for 1 minute, at 55°C for 1 minute and at 72°C for 1 minute and 35 cycles at 72°C and at 95°C in the last stage, it has been completed by running it at 72°C for 5 minutes. The products obtained after PCR process for fragment analysis were carried out in the "QIAxcel Advanced System" fragment analyzer of Qiagen company and DNA bands of genotypes were obtained.

Statistical analysis

The data obtained from the experiment was subjected to the variance analysis and the mean data comparisons were made by Duncan test and determined as 1% likelihood if not else showed. Principal component (PC) biplot analysis was performed over mean data of the investigated traits with JMP, version 15.1 (SAS Institute Inc. USA). Genetic similarities of durum wheat genotypes were calculated using the Dice index (Dice, 1945) in the NTSYSpc 2.21q (Rohlf, 2005) program. The DNA bands of each genotype were coded as "0" or "1" and a binary data matrix was created, and with the help of this matrix, a dendrogram was formed showing the similarities of the genotypes using UPGMA (Unweighted Pair Group Method Arithmetic Average). Polymorphism information content (PIC) was determined by using the formula described by Weir (1996), $PIC = 1-\sum Pi^2$, where Pi is the frequency of the i^{th} allele in the 14 Karakılçık durum wheat genotypes studied.

RESULT and DISCUSSION

Agronomic traits

In the study, the differences among genotypes related to plant height were found to be statistically significant at the level of 5%. The average plant height of the genotypes varied 125.8 to 148.27 cm (Table 3). The shortest plant height was determined in KK-10 genotype and the highest plant height was determined in KK-2 genotype. Plant height depends on the genetic structure of the genotypes and changes with climatic and growing conditions. In tall genotypes, the lodging rate increases as the stem becomes thinner, and the energy accumulated in the grain decreases with the use of the products obtained as a result of photosynthesis in the stem and leaf development, resulting in yield losses (Shah et al., 2019). In a previous work, different plant height values depending on genotypes were indicated as 95-135 cm (Wolde et al., 2019), which is in agreement with our findings. Differences among genotypes in terms of spike length were found to be statistically significant ($P < 0.05$) and SL varied between 8.42-10.69 cm. The KK-13 had the shortest SL, while KK-2 had the longest spike length. Alemu et al. (2020) reported a positive and significant relationship between spike length and grain yield. Wang et al. (2021) indicated a variation from 4.5 to 17.0 cm among genotypes in terms of spike lengths. Spikelet number of the genotypes were found

significant (P < 0.01), and SN of the genotypes ranked between 20.60 (KK-9) and 26.87 (KK-2). In similar studies, Philipp et al. (2018) and Wolde et al. (2019) determined variation on spikelet number (16.51 to 25.68 and 18.8 to 21.8, respectively). Differences among genotypes regarding the grain number per spike were found to be statistically significant at the level of 5% (Table 3).

Table 2. DNA primers used
Çizelge 2. Kullanılan DNA primerleri

No.	Primer name *Primer Adı*	Primer sequence (5'-3') *Primer dizisi (5'-3')*	Reference *Referans*	Loci *Lokus*	Expected fragment size (bp) *Beklenen bant uzunluğu (bp)*	Marker type *Markör tipi*
1	Bx7^{OE}_F Bx7^{OE}_R	CCTCAGCATGCAAACATGCAGC CTGAAACCTTTGGCCAGTCATGTC	Butow et al., 2003	Gluten strength (*Glu-B1*)	563	co-dominant
2	Xwgp118_F Xwgp118_R	AAGTGGAACAAGGTTACG ACACTGGTCCATGAGGTT	Li et al., 2011	Yellow rust (*Yr45*)	411	dominant
3	Sun209_F Sun209_R	AGCTATGAGCTTCGCTATTG GTGATTGGTTCGGATTACTTA	Bansal et al., 2015	Stem rust (*Sr49*)	148	co-dominant
4	UHW89-BF UHW89-R	TCTCCAAGAGGGGAGAGACA TTCCTCTACCCATGAATCTAGCA	Distelfeld et al., 2006	High protein content (*Gpc-B1*)	122	co-dominant
5	Sun1_F Sun1_R	CGCTCCCTGAAGAGAGAAAGAA ATAGGCACAACCCCTAAC	Shariflou & Sharp, 1999	Waxy (*Wx-A1*)	Xsun-7A, 219,233, 260, 271, 275, 285 and 289	co-dominant

Table 3. Mean data belong to Karakılçık genotypes of investigated traits
Çizelge 3. İncelenen özelliklere ait Karakılçık genotiplerinin ortalama verileri

Genotype	PH	SL	SN	GNS	GWS	TKW	GY	TW	PR	WG
KK-1	144.02ab	10.00ab	25.67ab	49.28ab	1.99 ab	39.89 ab	3208.2 ab	77.0 j	16.53 f	32.60 f
KK-2	148.27a	10.69a	26.87a	54.47 ab	2.29 a	42.07 a	3119.4 ab	76.8 k	17.2 d	34.10 d
KK-3	146.60ab	10.25a-c	24.80 a-c	49.67 ab	2.15 ab	43.20 a	3355.0 ab	79.0 f	16.9 e	33.40 e
KK-4	135.53ab	10.32ab	26.73 a	45.07 b	1.39 cd	29.98 b	2588.6 b	74.5 m	17.4 c	34.60 c
KK-5	145.67ab	10.03a-c	24.07 a-d	47.93 ab	2.15 ab	44.30 a	3770.0 ab	77.7 ı	14.6 m	28.30 l
KK-6	131.80ab	8.81bc	21.07 ef	53.47 ab	1.80a-d	33.70 ab	4118.1 a	80.9 c	14.1 n	27.20 m
KK-7	133.80ab	9.48a-c	21.80 d-f	45.73 ab	1.56b-d	34.10 ab	3413.3 ab	78.8 g	16.5 g	32.60 f
KK-8	136.73ab	9.50a-c	21.40 d-f	42.40 b	1.33d	31.46 b	3275.8 ab	77.8 h	18.2 b	36.30 b
KK-9	132.00ab	8.61bc	20.60 f	50.07 ab	1.78a-d	35.61 ab	4285.6 a	80.6 d	15.4 j	30.10 ı
KK-10	125.80b	9.06a-c	23.73 b-e	49.67 ab	1.85a-d	37.16 ab	3386.7 ab	75.9 l	18.5 a	37.10 a
KK-11	137.00ab	8.80bc	21.20 ef	55.07 ab	1.96a	35.60 ab	4023.6 a	80.2 e	16.2 h	31.90 g
KK-12	133.67ab	9.21a-c	23.20 b-f	59.40 a	1.79a-d	30.16 b	4246.4 a	81.9 a	14.8 l	28.70 k
KK-13	133.67ab	8.42c	22.27 c-f	50.33 ab	1.95a-d	39.15 ab	3565.6 ab	81.4 b	15.4 k	30.00 j
KK	140.27ab	9.13a-c	23.20 b-f	54.87 ab	2.15ab	39.13 ab	3780.8 ab	78.8 g	16.1 ı	31.60 h
Mean	**137.49**	**9.47**	**23.33**	**50.53**	**1.87**	**36.82**	**3581.2**	**78.66**	**16.27**	**32.04**
CV (%)	**5.12***	**6.56***	**4.03****	**9.10***	**17.78***	**14.70***	**11.10***	**6.48****	**1.5****	**16.11****

* – p < 0.05, ** – p<0.01, PH – plant height (cm), SL – spike length (cm), SN – spikelet number, GNS – grain number per spike, GWS – grain weight per spike (g), TKW – thousand kernel weight (g), GY – grain yield (kg ha^{-1}), TW – test weight (kg hl^{-1}), PR – protein ratio (%), WG – wet gluten (%).

The lowest grain number per spike was 45.07 (KK-4) while, KK-12 genotype was upmost (59.40). Grain number is positively affected in favourable conditions, while exposed to high temperature and low humidity during flowering period causes problems in fertilization resulting in a lower number of florets causes less grain number in the spike. In previous studies, Philipp et al. (2018) indicated a number of 32.97 to 71.31 grains varying for wheat genotypes. Grain weight per spike values of the genotypes were also found significant (P<0.05). The lowest grain weight was determined in KK-4 (1.33 g) and the

highest grain one was determined in KK-2 genotype with 2.29 g. Similar findings were reported from the previous works in grain weight on genotypes (Mehrabi et al., 2020; Woźniak, 2020). In terms of thousand-kernel weight, the differences among the genotypes were found to be statistically significant at the level of 5% (Table 3). Thousand kernel weights were between 29.98 (KK-4) to 44.30 g (KK-5). In addition to genotypic influence, climate, soil structure and cultivation techniques during flowering and grain filling period are effective on thousand-kernel weight. In addition, Yildirim and Atasoy (2020) reported different TKW for genotypes as 47.1-53.8 g.

Genotypes were differed significantly for grain yield ($P < 0.05$). The mean data for grain yield of the genotypes are given in Table 3. It was observed that the grain yield of the genotypes ranked between 2588.6 (KK-4) to 4285.6 kg ha^{-1} (KK-9) while the mean grain of the experiment was 3581.2 kg ha^{-1}. It is reported that grain yield was affected by genetic influence and climatic conditions with cultural practices that in agreement with our results and the previous works (Güngör & Dumlupinar, 2019). Test weight is one of the basic factors in the classification for quality in wheat. It is reported that the test weight, which is an observation of kernel density, varies depending on the genetic structure of the genotype, environmental conditions and kernel structure, (Howarth et al., 2021). Differences in genotypes for test weight were found statistically significant ($P < 0.01$). It was observed that the lowest test weight was determined in the KK-4 genotype with 74.50 kg hl^{-1}, while the highest one was determined on KK-12 with 81.90 kg hl^{-1}. Yildirim and Atasoy (2020) reported different test weights for genotypes as 81.7-84.7 kg hl^{-1}. Protein ratio is one of the most important criteria for determining the quality of wheat (Fu et al. 2018), influenced by environment more than genotype (Nehe et al., 2019). It varies according to the variety, environmental conditions and growing techniques. Differences among the genotypes for protein ratio were significant ($P < 0.01$). Protein ratio of the genotypes ranked between 14.10-18.50%. The lowest protein ratio was found in the KK-6 genotype, and the highest protein ratio was found in the KK-10 genotype. Nehe et al. (2019) determined a mean protein ratio of 13.6%, while Yildirim and Atasoy (2020) indicated a variation of 13.8-16.7%. The wet gluten values of the genotypes are given in Table 3. Differences in genotypes for wet gluten ratio were statistically significant at the level of 1%. The lowest wet gluten ratio was determined in KK-6 genotype (27.20%), and the highest wet gluten ratio was found in KK-10 genotype (37.10%). Nehe et al. (2019) reported the mean wet gluten ratio as 28.4% in a modern and historical spring wheat set.

Principal component (PC) biplot analysis

Principal Component (PC) biplot analysis was accomplished to lay out a powerful exhibition of the results with clarification of the relationships between Karakılçık genotypes and investigated characteristics. Based on the results of the present study showed that PCA explained 77.8% of the total variation where 47.4% was shown by PC1 and 30.4% by PC2 on biplot (Figure 1).

The correlation among the examined characteristics and the genotypes that were acted superior for those characteristics are demonstrated in Figure 1. It was determined that there was a positive relationship between GY with TW, GNS and GWS, and a negative relationship with other traits (PH, SL, SN, TKW, PR and WG). It has been observed that there was a positive relationship between protein ratio and wet gluten, which are among the most important quality traits. KK-6, KK-9, KK-11, and KK-12 were concluded as promising genotypes for GY and TW, while KK-4, KK-7, KK-8 and KK-10 were for PR and WG. On the other hand, KK-5 genotype was featured for TKW.

Molecular analysis

The kinship of the 14 Karakılçık durum wheat genotypes used in the study was screened with five DNA markers. Allele numbers and polymorphism information content (PIC) values of DNA markers used in screening genotypes are shown in Table 4. According to the results, five DNA markers amplified 76 polymorphic bands, while the average number of alleles was found 15.2. Where the average PIC value was calculated as 0.97, the highest PIC value was determined as 0.99 from the Xwgp118, Sun209 and Sun1 markers, while the lowest PIC value was calculated as 0.94 from $Bx7^{OE}$ marker. Tsonev et al. (2021) reported 8.14 allele number per marker, while Vanzetti et al. (2013) indicated 3.26 allele number per marker. Kiraz et al. (2019), Aydemir et al. (2020) and Koçyiğit et al. (2021) found the PIC values to be 79%, 98% and 52% respectively, in their study.

The $Bx7^{OE}$ primer is a marker used to identify genes for gluten strength. KK-11 and KK-13 genotypes had the allele for gluten strength with 563 bp DNA band. Butow et al. (2004) reported that the $Bx7^{OE}$ marker is a co-dominant marker corresponding to the 750 bp portion of the encoded region, and lines lacking *Glu-B1al* (520 bp) obtained a 563 bp long allele with an excess of 43 bases to the gene (Figure 3). Xwgp118 primer is used to recognize alleles to yellow rust resistance (*Yr45*) disease. KK-10 genotype produced 411 bp DNA band, which was related to the yellow rust resistance gene (*Yr45*) (Figure 2). Li et al. (2011) reported that the Xgwp118 marker amplified a DNA band with a length of 411 bp in spring and winter bread wheat genotypes and was associated with yellow rust disease.

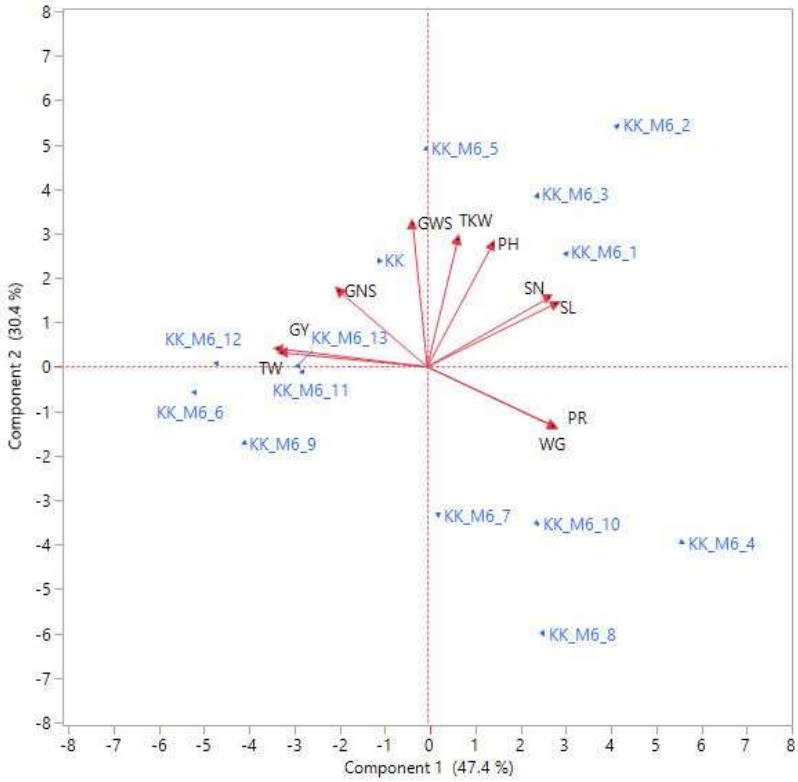

Figure 1. Principal component biplot analysis of the relationships between Karakılçık genotypes and investigated traits

Şekil 1. Karakılçık genotipleri ve incelenen özellikler arasındaki ilişkiyi gösteren temel bileşenler biplot analiz grafiği

Table 4. Allele numbers and PIC values of primers
Çizelge 4. Primerlere ait allel sayısı ve PIC değerleri

No	Primer Name *Primer Adı*	Allele Number *Allel Sayısı*	PIC Value *PIC Değeri*
1	Bx7^{OE}	18	0.94
2	Xwgp118	9	0.99
3	Sun209	15	0.99
4	UHW89	20	0.98
5	Sun1	14	0.99
Average *(Ortalama)*		15.2	0.97

The Sun1 primer is used to identify alleles for the Waxy (*Wx-A1*) gene. It was determined that KK-1, KK-2, KK-4, KK-5 and KK-7 genotypes had *Wx-A1* alleles with producing 271, 275, 271, 271 and 275 bp DNA bands, respectively (Figure 3). Shariflou and Sharp (1999) reported 219, 233, 260, 271, 275, 285 and 289 bp bands for the *Wx-A1* gene. The Sun209 primer is used to detect allele for resistance to stem rust (*Sr49*) disease. Among the Karakılçık genotypes, the only *Sr49* resistance allele was found in the KK-5 genotype with producing 148 bp DNA band. Bansal et al. (2015), indicated that 148 bp DNA band amplified by the Sun209 marker was associated with the *Sr49* stem rust resistance gene.

The UHW89 primer is used to determine high protein content alleles (*Gpc-B1*). In current panel, KK-1, KK-2, KK-3 and KK-4 genotypes produced alleles related to high protein *Gpc-B1* gene amplifying 125, 122, 124 and 125 bp alleles (Figure 3). Distelfeld et al. (2006) detected 122 and 126 bp long bands using the UHW89 marker, and that 4 bp polymorphism was formed as a result of ACTT duplication.

CONCLUSION

In current study, Karakılçık mutant genotypes developed using chemical agent varied for investigated traits especially for plant height and protein ratio. Allele specific DNA markers also detected the variation among mutant lines. An increase in protein ratio with decreasing plant height by chemical mutagenesis, is crucial for Karakılçık cultivation and consumption. Based on results, KK-10 genotype is

concluded superior with stripe rust resistance gene (*Yr45* allele), short plant height, and high protein and wet gluten ratio. KK-5 genotype was the only one that stem rust allele Sr49 was determined. On the other hand, KK-1, KK-2, KK-3 and KK-4 genotypes were also found promising due to having high protein content allele (*Gpc-B1*). Those genotypes developed by chemical mutagenesis might be used in future studies as parents to improve agronomic traits.

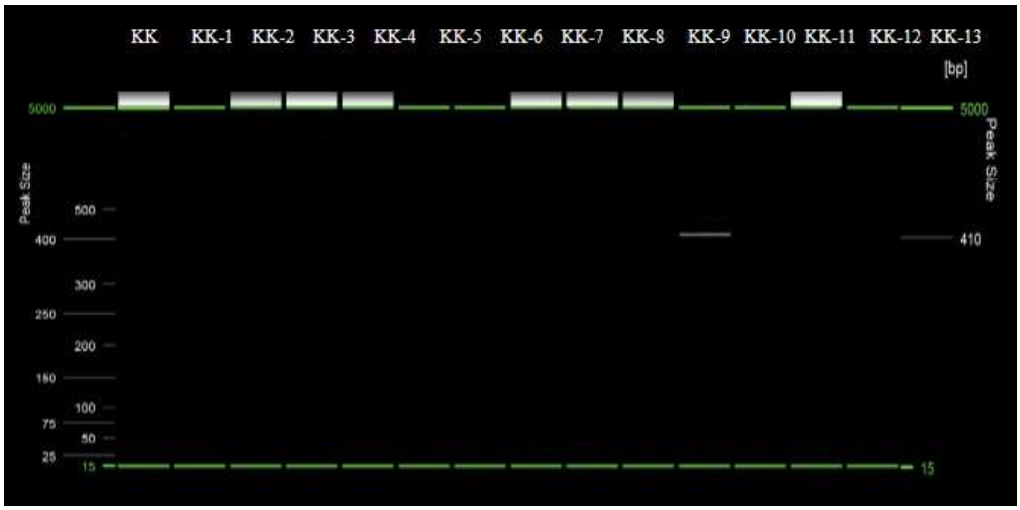

Figure 2. Gel image of Xwgp118 primer for Karakılçık genotypes
Şekil 2. Karakılçık genotiplerinin Xwgp118 primerine ait jel görüntüsü

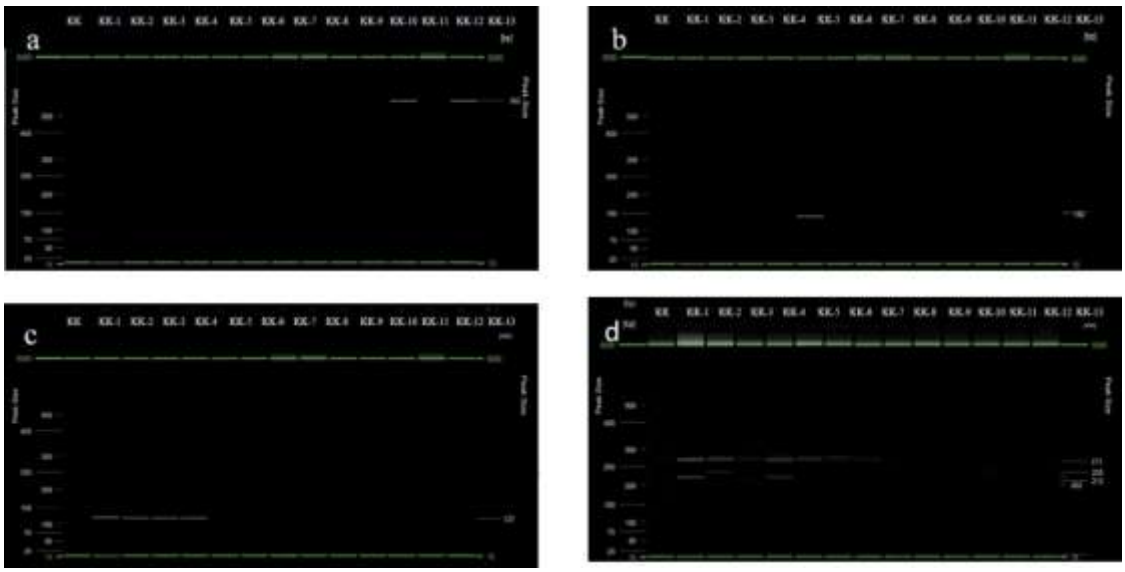

Figure 3. Gel images for DNA markers a- $Bx7^{OE}$, b- Sun209, c- UHW89 and d- Sun1
Şekil 3. a- $Bx7^{OE}$, b- Sun209, c- UHW89 and d- Sun1 DNA markörlerine ait jel görüntüleri

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

The contribution of the authors is equal.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no competing interests.

REFERENCES

Al-Ashkar, I., Alderfasi, A., El-Hendawy, S., Al-Suhaibani, N., El-Kafafi, S. & Seleiman, M.F.

(2019). Detecting Salt Tolerance in Doubled Haploid Wheat Lines. *Agronomy*, *9*, 211. Doi: 10.3390/agronomy9040211

Alemu, Y.A., Anley, A.M. & Abebe, T.D. (2020). Genetic Variability and Association of Traits in Ethiopian Durum Wheat (*Triticum turgidium* L. *var. durum*) Landraces at Dabat Research Station, North Gondar. *Cogent Food & Agriculture*, *6* (1), 1778604. Doi: 10.1080/23311932.2020.1778604

Anonymous, (2021). Turkish State Meteorology Service. https://www.mgm.gov.tr/ ?il=Kahraman maras. Accessed on 15.09.2021

Aslan, D., Aktaş, H., Ordu, B. & Zencirci, N. (2017). Evaluation of Bread and Einkorn Wheat under *in vitro* Drought Stress. *The Journal of Animal and Plant Sciences*, *27* (6),1974-1983.

Aydemir, G., Dumlupinar, Z., Yüce, I., Baskonus, T., Sunulu, S. & Güngör, H. (2020). Evaluation of F_5 Individuals Obtained from B28 × Kunduru-1149 Reciprocal Cross Population by Functional Markers. *KSU Journal of Agriculture and Nature*, *23* (4), 1005-1011. Doi: 10.18016/ksutarimdoga.vi. 687935

Bai, J., Yan, W., Wang, Y., Yin, Q., Liu, J., Wight, C. & Ma, B. (2018). Screening Oat Genotypes for Tolerance to Salinity and Alkalinity. *Frontiers in Plant Science*, *9*, 1302. Doi: 10.3389/fpls.2018.01302

Bansal, U.K., Muhammad, S., Forrest, K.L., Hayden, M.J. & Bariana, H.S. (2015). Mapping of A New Stem Rust Resistance Gene *Sr49* in Chromosome 5B of Wheat. *Theoretical and Applied Genetics*, *128* (10), 2113-2119. Doi: 10.1007/s00122-015-2571-4

Butow, B.J., Ma, W., Gale, K.R., Cornish, G.B., Rampling, L., Larroque, O., Morell, M.K. & Bekes, F. (2003). Molecular Discrimination of Bx7OE Alleles Demonstrates That A Highly Expressed High-Molecular-Weight Glutenin Allele Has A Major Impact on Wheat Flour Dough Strength. *Theoretical and Applied Genetics*, *107* (8), 1524–1532. Doi: 10.1007/s00122-003-1396-8

Dice, L.R. (1945). Measures of The Amount of Ecologic Association Between Species. *Ecology*, *26*, 297- 302.

Distelfeld, A., Uauy, C., Fahimaand, T. & Dubcovsky, J. (2006). Physical Map of The Wheat High-Grain Protein Content Gene *Gpc-B1* and Development of A High Throughput Molecular Marker. *New Phytologist*, *169*, 753–763. Doi:10.1111/j.1469-8137.2005.01627.x

Durland, J. & Ahmadian-Moghadam, H. (2022). Genetics, Mutagenesis. [Updated 2021 Sep 21]. In: StatPearls [Internet]. Treasure Island (FL), StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560519/

FAO, (2020). FAO Statistical Databases. http:// www.fao.org/faostat/en/#data. Accessed 15.06.2021

Fu, B.X., Wang, K., Dupuis, B., Taylor, D. & Nam, S. (2018). Kernel Vitreousness and Protein Content: Relationship, Interaction and Synergistic Effects on Durum Wheat Quality. *Journal of Cereal Science*, *79*, 210-217. Doi: 10.1016/j.jcs.2017.09.003

Güngör, H., Çikili, Y. & Dumlupinar, Z. (2019). Evaluation of Morpho-Physiological Traits of Turkish Rice Genotypes in Response to Salt Stress under *in vitro* Conditions. *Journal of Animal and Plant Sciences*, *29*, 556-567.

Güngör, H. & Dumlupınar, Z. (2019). Evaluation of Some Bread Wheat (*Triticum aestivum* L.) Cultivars for Yield, Yield Components and Quality Traits in Bolu Conditions. *Turkish Journal of Agricultural and Natural Science*, *6* (1), 44-51. Doi: 10.30910/turkjans.515346

Howarth, C.J., Martinez-Martin, P.M.J., Cowan, A.A., Griffiths, I.M., Sanderson, R., Lister, S.J., Langdon, T., Clarke, S., Fradgley, N. & Marshall, A.H. (2021). Genotype and Environment Affect The Grain Quality and Yield of Winter Oats (*Avena sativa* L.). *Foods*, *10*, 2356. Doi: 10.3390/foods10102356

Kiraz, H., Yüce, İ., Kekilli, Ö., Ocaktan, H., Topsakal, M., Gürocak, N.Y., Osanmaz, H., Kılınç, F.M., Başkonuş, T. & Dumlupınar, Z. (2019). Characterization of M_3 Mutants of Seri 82 Bread Wheat Cultivar Using Functional Markers. *Black Sea Journal of Agriculture*, *2* (4), 194-202.

Koçyiğit, B.K., Yüce, İ., Başkonuş, T., Dokuyucu, T., Akkaya, A. & Dumlupinar, Z. (2021). Evaluation of F_4 Individuals Belong to Seri 82 × B35 Bread Wheat (*Triticum aestivum* L.) Cross Population Using Functional DNA Markers. *KSU Journal of Agriculture and Nature*, *24* (3), 586-593. Doi: 10.18016/ksutarimdoga.vi.752972

Li, Q., Chen, X.M., Wang, M.N. & Jing, J.X. (2011). *Yr45*, A New Wheat Gene for Stripe Rust Resistance on The Long Arm of Chromosome 3D. *Theoretical and Applied Genetics*, *122* (1), 189-197. Doi: 10.1007/s00122-010-1435-1

Mehrabi, A.A., Pour-Aboughadareh, A., Mansouri, S. & Hosseini, A. (2020). Genome-wide Association Analysis of Root System Architecture Features and Agronomic Traits in Durum Wheat. *Molecular Breeding*, *40*, 55. Doi: 10.1007/s11032-020-01136-6

Nehe, A., Akin, B., Sanal, T., Evlice, A.K., Unsal, R., Dincer, N., Demir, L., Geren, H., Sevim, I., Orhan, Ş., Yaktubay, S., Ezici, A., Guzman, C. & Morgounov, A. (2019). Genotype x Environment Interaction and Genetic Gain for Grain Yield and Grain Quality Traits in Turkish Spring Wheat Released between 1964 and 2010. *PLoS ONE 14*(7), e0219432. Doi: 10.1371/journal. pone.0219432

Oliver, R.E., Obert, D.E., Hu, G., Bonman, J.M., O'Leary-Jepsen, E. & Jackson, E.W. (2010). Development of Oat-Based Markers from Barley and Wheat Microsatellites. *Genome*, *53* (6), 458-471. Doi: 10.1139/G10-021

Philipp, N., Weichert, H., Bohra, U., Weschke, W., Schulthess, A.W. & Weber, H. (2018). Grain Number and Grain Yield Distribution Along The

Spike Remain Stable Despite Breeding for High Yield in Winter Wheat. *PLoS ONE*, *13* (10),e0205452. Doi: 10.1371/journal.pone.0205452

Rohlf, F.J. (2005). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.2.

Shah, L., Yahya, M., Shah, S.M.A., Nadeem, M., Ali, A., Ali, A., Wang, J., Riaz, M.W., Rehman, S., Wu, W., Khan, R.M., Abbas, A., Riaz, A., Anis, G.B., Si, H., Jiang, H. & Ma, C. (2019). Improving Lodging Resistance: Using Wheat and Rice as Classical Examples. *International Journal of Molecular Sciences*, *20*(17), 4211. Doi: 10.3390/ijms20174211

Shariflou, M.R. & Sharp, P.J. (1999). A Polymorphic Microsatellite in The 3'end of '*Waxy*' Genes of Wheat, *Triticum aestivum*. *Plant Breeding*, *118*, 275 -277.Doi:10.1046/j.1439-0523.1999.118003275. x

Saal, B. & Wricke, G. 1999. Development of Simple Sequence Repeat Markers in Rye (*Secale cereale* L.). *Genome*, *42*, 964–972. Doi: 10.1139/g99-052

Tsonev, S., Christov, N.K., Mihova, G., Dimitrova, A. & Georgieva Todorovska, E. (2021). Genetic Diversity and Population Structure of Bread Wheat Varieties Grown in Bulgaria Based on Microsatellite and Phenotypic Analyses. *Biotechnology & Biotechnological Equipment*, *35*(1), 1520-1533. Doi: 10.1080/13102818.2021. 1996274

Vanzetti, L.S., Yerkovich, N., Chialvo, E., Lombardo, L., Vaschetto, L. & Helguera, M. (2013). Genetic Structure of Argentinean Hexaploid Wheat Germplasm. *Genetics and Molecular Biology*, *36*, 391-399. Doi: 10.1590/S1415-47572013000300014

Yildirim, A. & Atasoy, A. (2020). Quality Characteristics of Some Durum Wheat Varieties Grown in Southeastern Anatolia Region of Turkey (GAP). *Harran Journal of Agriculture and Food Science*, *24*(4), 420-431. Doi: 10.29050/harran ziraat.738505

Wang, Y., Wang, S., Jia, X., Tian, Z., Wang, Y., Wang, C., Zhang, H., Liu, X., Zhao, J., Deng, P. & Ji, W. (2021). Chromosome Karyotype and Stability of New Synthetic Hexaploid Wheat. *Molecular Breeding*,*41*, 1–12.Doi:10.1007/s11032-021-01253w

Weir, B.S. (1996). Genetic Data Analysis II. Sinauer Associates Inc., Sunderland, England.

Wolde, G.M., Mascher, M. & Schnurbusch, T. (2019). Genetic Modification of Spikelet Arrangement in Wheat Increases Grain Number without Significantly Affecting Grain Weight. *Molecular Genetics and Genomics*, *294* (2), 457-468. Doi: 10.1007/s00438-018-1523-5

Woźniak, A. (2020). Effect of Cereal Monoculture and Tillage Systems on Grain Yield and Weed Infestation of Winter Durum Wheat. *International Journal of Plant Production*, *14* (1), 1-8. Doi: 10.1007/s42106-019-00062-8