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Evaluation of F_5 Individuals Obtained from B28×Kunduru-1149 Reciprocal Cross Population by Functional Markers

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ABSTRACT

In the study, B28 and Kunduru-1149 durum wheat genotypes were crossed as reciprocal in 2012-2013 cropping season. 13 (B28/Kunduru-1149 and Kunduru-1149/B28) reciprocal crosses were obtained and were used as materials at F_5 stage. The cross combinations and the parents were screened with 10 DNA markers to determine alleles of gluten strength ($Bx7^{OE}$), Yellow rust (Sun104, Xgwm18, Xwgp115 and Xgwm47), stem rust (Sun209 and Sun479), high protein ratio (UHW89), powdery mildew (Xgwm66) and leaf rust (Xgwm130). In the study, the average polymorphism information content (PIC) was calculated as 0.98 and the lowest PIC value was obtained from Xwgp115 marker with 0.96, while the rest of the markers had 0.99 PIC values. Stem rust resistance allele *Sr49* was detected in B28/Kunduru-1149_F_5_4 (Sun479) and B28/Kunduru-1149_F_5_1 (Sun209) genotypes. One of the yellow rust resistance alleles *Yr15* (Xgwm18) was detected in B28/Kunduru-1149_F_5_2 and B28/Kunduru-1149_F_5_3 genotypes, while *Yr51* (Sun104) was identified in B28/Kunduru-1149_F_5_3, B28/Kunduru-1149_F_5_6, B28/Kunduru-1149_F_5_7, Kunduru-1149/B28_F_5_2 and Kunduru-1149/B28_F_5_6 genotypes. A dendrogram was created to determine kinship of the individuals with the parents. The highest genetic similarity was observed between B28 / Kunduru-1149_F_5_6 and Kunduru-1149 / B28_F_5_2 genotypes with 0.714, while the most diverse ones were Kunduru-1149 and B28/Kunduru_F_5_7 with 0.10.

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B28×Kunduru-1149 Resiproklu Melez Popülasyonundan Elde Edilen F_5 Bireylerinin Fonksiyonel Markörlerle Değerlendirilmesi

ÖZET

Bu çalışmada, B28 ve Kunduru-1149 makarnalık buğday genotipleri 2012-2013 ürün sezonunda resiproklu olarak melezlenmiş ve elde edilen 13 B28/Kunduru-1149-Kunduru-1149/B28 F_5 kademesindeki hat materyal olarak kullanılmıştır. Melez kombinasyonları ve ebeveynler gluten dayanıklılığı ($Bx7^{OE}$), sarı pas (Sun104, Xgwm18, Xwgp115 ve Xgwm47), kara pas (Sun209 ve Sun479), yüksek protein oranı (UHW89), külleme (Xgwm66) ve kahverengi pas (Xgwm130) allelleri bakımından 10 DNA markörü ile taranmıştır. Çalışmada, ortalama polimorfizmi bilgi içeriği (PIC) 0.98 olarak hesaplanmış ve en düşük PIC değeri 0.96 ile Xwgp115 marköründen elde edilirken, diğer markörler 0.99 PIC değerine sahip olmuştur. B28/Kunduru-1149_F_5_4 (Sun479) ve B28/Kunduru-1149_F_5_1 (Sun209) genotiplerinde kara pasa dayanıklılık alleli *Sr49* tespit edilmiştir. Sarı pas dayanıklılık allellerinden *Yr15* (Xgwm18) B28/Kunduru-1149_F_5_2 ve B28/Kunduru-1149_F_5_3 genotiplerinde bulunurken, *Yr51* (Sun104) geni B28/Kunduru-1149_F_5_3, B28/Kunduru-1149_F_5_6, B28/Kunduru-1149_F_5_7, Kunduru-1149/B28_F_5_2 ve Kunduru-1149/B28_F_5_6 genotiplerinde tanımlanmıştır. Ebeveynler

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Anahtar Kelimeler
Makarnalık buğday
Fonksiyonel markörler
Sarı pas
Kara pas
Gluten mukavemeti

ile genotiplerin akrabalıklarını belirlemek için bir dendrogram oluşturulmuştur. En yüksek genetik benzerlik oranı B28 / Kunduru-1149_F_5_6 ve Kunduru-1149 / B28_F_5_2 genotipleri arasında 0.714 olarak tespit edilirken, birbirlerine en uzak genotipler 0.10 benzerlik oranı ile Kunduru-1149 ve B28/Kunduru_F_5_7 olmuştur.

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INTRODUCTION

Wheat (*Triticum* spp.) is an annual cereal crop consumed as major food source for centuries (Sevinç, 2010). Turkey is one of the centers of origin of the durum wheat which is originated in Karacadağ location of South-East Anatolia Region (Anonymous, 2016).

Wheat is grown in 219.3 mil. ha and 757.7 mil. ton produced world-wide, while 7.2 mil. ha grown and 20 mil. ton produced in Turkey (TUIK, 2018). World durum wheat production is approximately 40.2 mil. ton, while 3.6 mil. ton in Turkey (TUIK, 2018). Turkey is one of the durum wheat producers in the world especially in South Anatolia Region.

Durum wheat (2n=4x=28, AABB) differs for its utilization from bread wheat (2n=6x=42, AABBDD) as pasta, bulgur and couscous were made from durum wheat, while bread, noodle etc. were made from hexaploid bread wheat.

Plant breeders have put effort on developing high quality and high yielding cultivars for many years and different breeding techniques are used to improve crop-plants. Crossing is one of the breeding techniques used to combine traits from both parents.

Landraces have been sources to expand genetic diversity for disease resistance, drought tolerance, quality traits and many more traits. Besides favorable characteristics, they have some negative traits such as lodging, low grain yield etc. which must be eliminated by improving the traits.

Molecular marker technology is used widely in breeding programs with recent developments in biotechnology. Markers assisted selection (MAS) has been used to detect alleles related to the traits such as disease, quality parameters and agronomic traits. It provides earlier and precise selection especially for quantitative traits. Functional markers have been developed after many efforts on quantitative trait locus (QTL) studies and available for many genes and traits. Marker assisted selection have now been used widely due to its accurate, rapid, reproducible and cost effective solutions for the breeding programs.

Kunduru-1149 is a cultivar developed from selection of landraces. It has many advantages besides disadvantages such as lodging and lower quality parameters. On the other hand B28 is a landrace obtained from USDA National Small Grains Collection, Aberdeen, USA gene bank and has higher quality traits and disease resistance. Those genotypes crossed as reciprocal and 13 genotypes at F_5 stages selected from both combinations (B28 × Kunduru-1149 and Kunduru-1149 × B28). In the study it was aimed to identify some disease and quality traits by allele specific markers to determine genotypes related with those gluten strength, high protein ratio, yellow rust, stem rust, powdery mildew and leaf rust diseases genes. For this purpose, the genotypes were screened with 10 allele specific DNA markers.

MATERIALS and METHOD

In the study, 13 genotypes obtained from reciprocal crosses of B28 and Kunduru-1149 and the parents were used as plant material. Seeds of the genotypes were planted and two leaves seedling were harvested to extract DNAs (Dumlupinar, 2016). The DNA content and purity were determined by spectrophotometer (Thermo-Scientific Nanodrop 2000 spectrophotometer).

Allele specific markers of gluten strength ($Bx7^{OE}$), Yellow rust (Sun104, Xgwm18, Xwgp115 and Xgwm47), stem rust (Sun209 and Sun479), high protein ratio (UHW89), powdery mildew (Xgwm66) and leaf rust (Xgwm130) were screened on Qiagen Qiaxcel Fragment Analyzer (Table 1). The data obtained from fragment analyzer were scored and the genetic similarity of the genotypes were determined by Dice index (Dice, 1945) using NTSYSpc 2.21q software (Rohlf, 2005). Polymorphism information content (PIC) was determined by using the formula described by Weir (1996), where $PIC=1-\sum P_i^2$, where P_i is the frequency of the i^{th} allele in the 15 durum wheat genotypes studied.

RESULTS and DISCUSSION

Marker assisted selection (MAS) studies in wheat have been accomplished in many plant breeding programs worldwide and succeeded on many agronomical traits. Based on genotypic data obtained from durum wheat segregation populations was investigated. The allele numbers of the primers, figures of the primers Xgwm18 and Xgwm66 were shown in Figures 1, 2 and 3 respectively. The dendrogram was generated from the marker data of durum wheat genotypes is shown in Fig. 4. The allele specific markers interrogated on the durum wheat genotypes were indicated in Table 2.

Table 1 DNA Primers Used in the Study
Çizelge 1 Çalışmada kullanılan DNA Primerleri

No (*Numara*)	Primer Name (*Primer Adı*)	Primer Sequence(5'-3') (*Primer Dizisi (5'-3')*)	Reference (*Referans*)	Loci (*Lokus*)	Expected Frqagment Size (bp) (*Beklenen Bant Uzunluğu (bç)*)	Marker Type (*Markör Tipi*)
1	Bx7^{OE}_F Bx7^{OE}_R	CCTCAGCATGCAAACATGCAGC CTGAAACCTTTGGCCAGTCATGT C	Butow et al., 2003	Glutent Strength	563	Co-dominant
2	SUN104_F SUN104_R	TGCTATGTGCGTGATGATGA TTACATGCTCCAGCGACTTG	Randhawa et al., 2014	Yellow Rust *Yr51*	225	Dominant
3	SUN209_F SUN209_R	AG CTATGAGCTTCGCTATTG GTGATTGGTTCGGATTACTTA	Bansal et al., 2015	Stem Rust *Sr49*	148	Co-dominant
4	SUN479_F SUN479_R	CAAATGAAATGTGATCCTGTT TCATCTAACCAGCAATGGTAT	Bansal et al., 2015	Stem Rust *Sr49*	200	Co-dominant
5	UHW89_BF UHW89_R	TCTCCAAGAGGGGAGAGACA TTCCTCTACCCATGAATCTAGCA	Distelfeld et al., 2006	High Protein Content *Gpc-B1*	122	Co-dominant
6	XGWM18_F XGWM18_R	TTGCTACCATGCATGACCAT TTCACCTCGATTGAGGTCCT	Roder et al., 1998	Yellow Rust *Yr15 and Yr26*	186, 190	Co-dominant
7	XGWM47_F XGWM47_R	TTGCTACCATGCATGACCAT TTCACCTCGATTGAGGTCCT	Roder et al., 1998	Yellow Rust *Yr64* and *Yr66*	190	Co-dominant
8	XGWM66_F XGWM66_R	TTGCTACCATGCATGACCAT TTCACCTCGATTGAGGTCCT	Roder et al., 1998	Powdery Mildew	137	Co-dominant
9	XGWM130_F XGWM130_R	AGCTCTGCTTCACGAGGAAG CTCCTCTTTATATCGCGTCCC	Roder et al., 1998	Leaf Rust *Lr34*	121,126	Co-dominant
10	XWGP115_F XWGP115_R	AGTGTCTTGTAGGGTATC TCAGGCCGTGAAAAATAT	Roder et al., 1998	Yellow Rust *Yr45*	492	Co-dominant

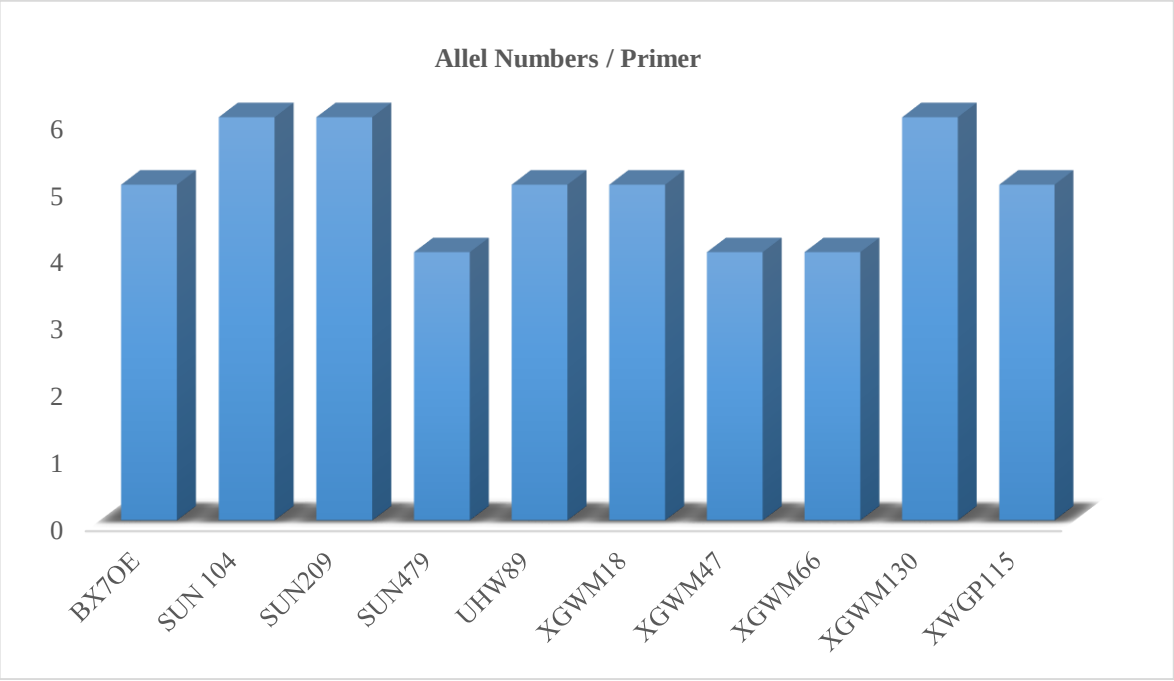

Figure 1 Allele numbers of the primers screened for the durum wheat reciprocal segregation populations
Şekil 1 Makarnalık buğday resiprokal melez popülasyonunda görüntülenen primerlerin allel sayıları

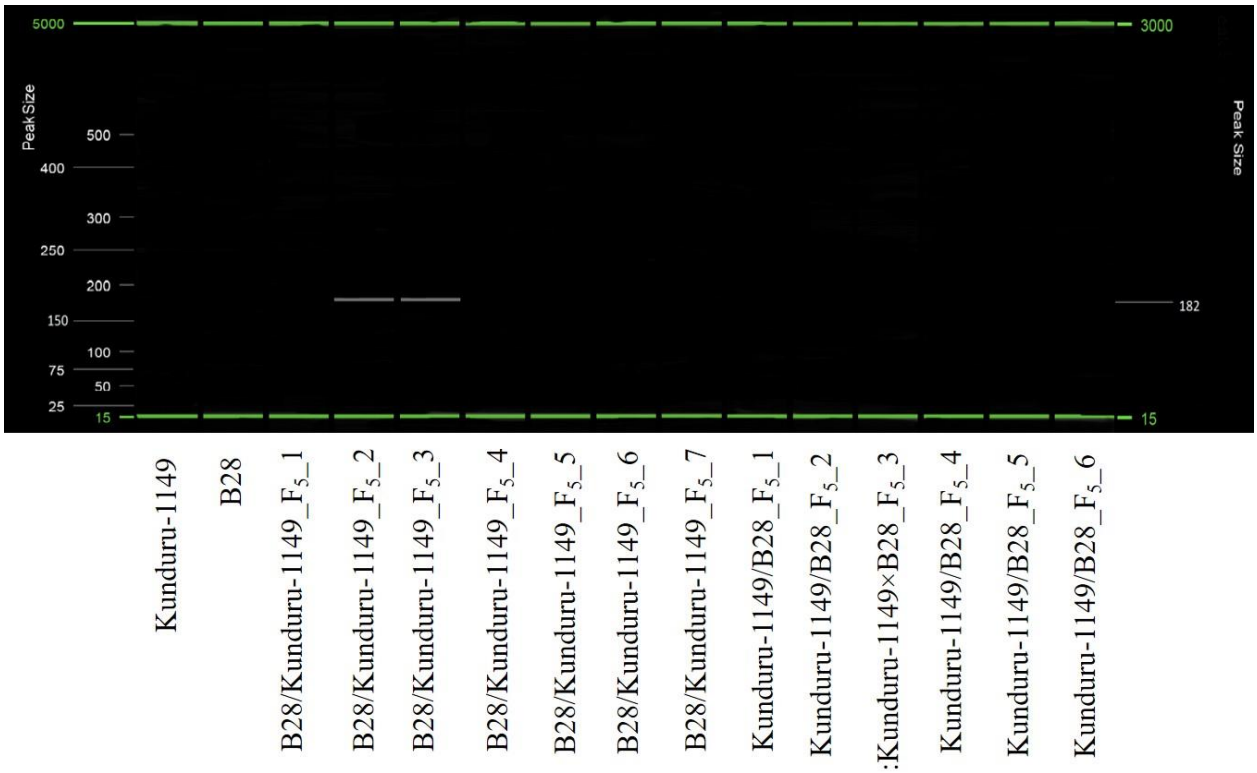

Figure 2 Visualization of Xgwm18 (Yr26) DNA marker which produced alleles on B28/Kunduru-1149_F5_2 and B28/Kunduru-1149_F5_3 genotypes on Fragment Analyzer

Şekil 2 Fragment analiz cihazında, B28 / Kunduru-1149_F5_2 ve B28 / Kunduru-1149_F5_3 genotiplerinde alleller üreten Xgwm18 (Yr26) DNA markörünün görüntülenmesi

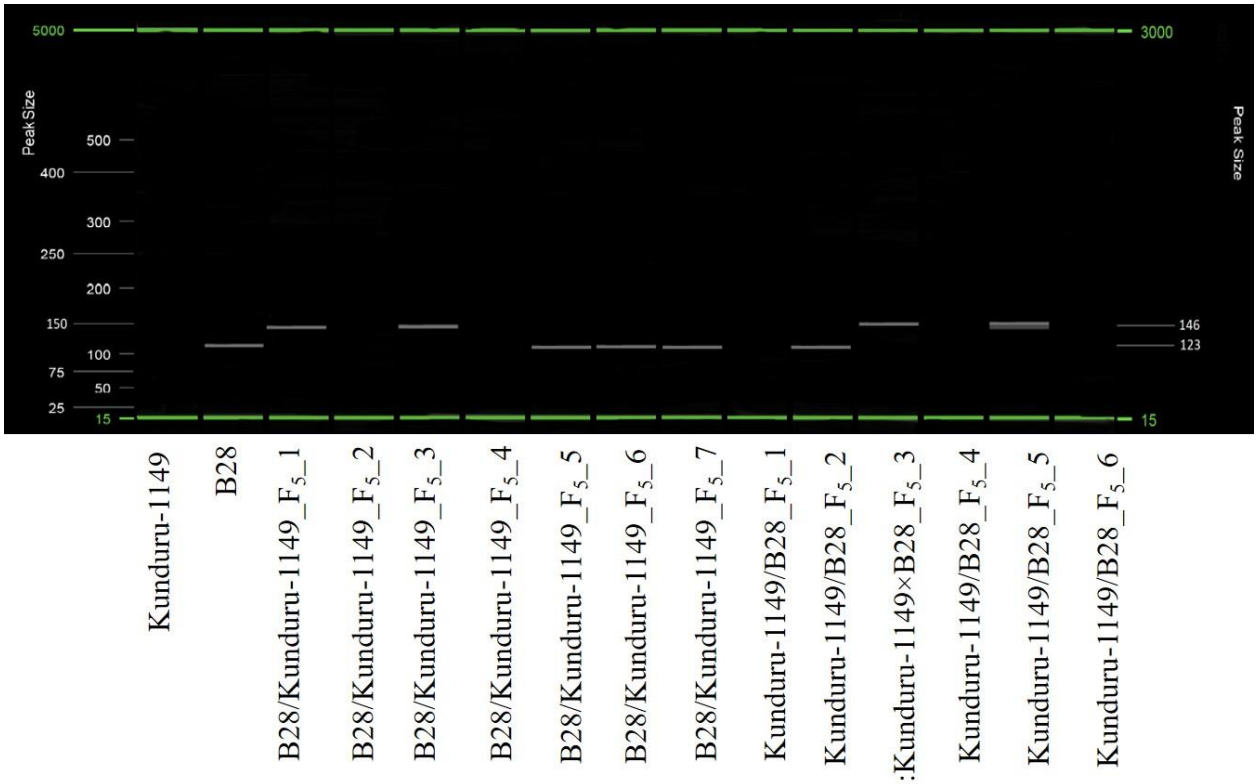

Figure 3 Visualization of Xgwm66 DNA marker which could not produce powdery mildew resistance gene along the durum wheat genotypes on Fragment Analyzer

Şekil 3 Fragment Analiz cihazında makarnalık buğday genotiplerinde küllemeye dayanıklılık genini üretemeyen Xgwm66 DNA markörünün görüntülenmesi

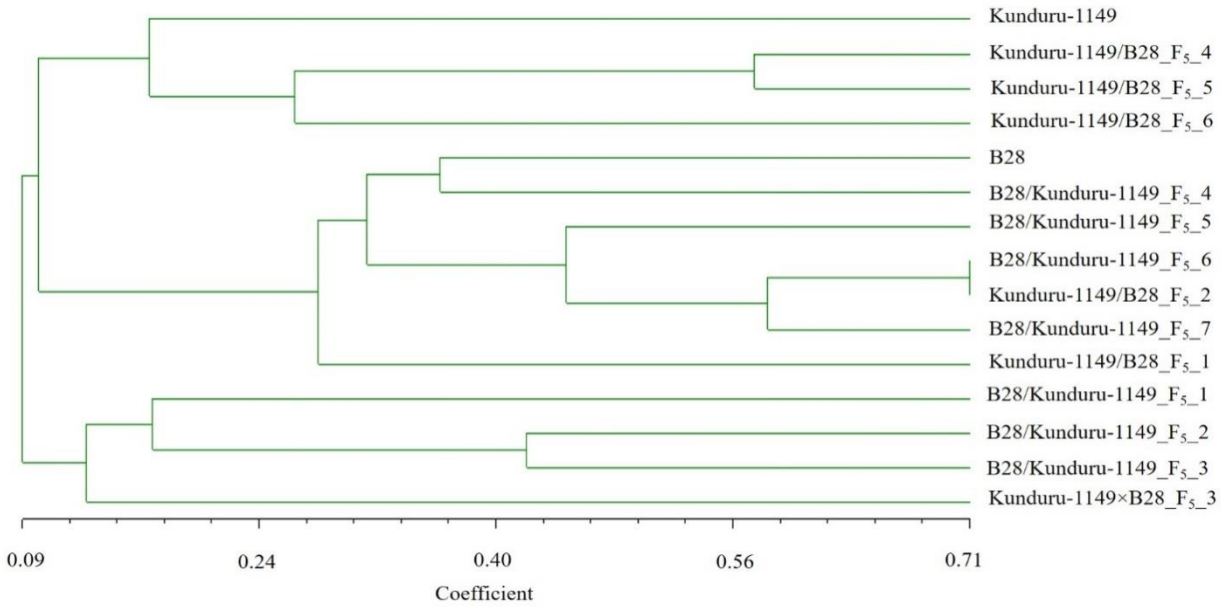

Figure 4 A dendogram was created using genetic similarity index of the durum wheat genotypes
Şekil 4 Makarnalık buğday genotiplerinin genetik benzerlik indeksi kullanılarak oluşturulmuş filogenetik ağaç

Table 2 Allelic variation of B28, Kunduru-1149, Kunduru-1149/B28 and B28/Kunduru-1149 genotypes
Çizelge 2 B28, Kunduru-1149, Kunduru-1149/B28 ve B28/Kunduru-1149 melez kombinasyonlarınun allellik varyasyonları

Markers (Markörler) / ***Genotypes (Genotipler)***	SUN 104	UHW89	SUN209	BX7OE	SUN479	XWGP115	XGWM18	XGWM130	XGWM66	XGWM47
Kunduru-1149										
B28										
B28/Kunduru-1149_F5_1			+							
B28/Kunduru-1149_F5_2							+			
B28/Kunduru-1149_F5_3	+						+			
B28/Kunduru-1149_F5_4					+					
B28/Kunduru-1149_F5_5										
B28/Kunduru-1149_F5_6	+									
B28/Kunduru-1149_F5_7	+									
Kunduru-1149/B28_F5_1										
Kunduru-1149/B28_F5_2	+									
Kunduru-1149/B28_F5_3										
Kunduru-1149/B28_F5_4										
Kunduru-1149/B28_F5_5										
Kunduru-1149/B28_F5_6	+									
PIC Values (%)	99	99	99	99	99	96	99	99	99	99

In terms of expected primer sizes, the allele specific markers data evaluated on segregation durum wheat populations.

In the study the functional markers for gluten strength (Bx7OE), Yellow rust (Sun104, Xgwm18, Xwgp115 and Xgwm47), stem rust (Sun209 and Sun479), high protein ratio (UHW89), powdery mildew (Xgwm66) and leaf rust (Xgwm130) were used. According to the results a total number of 50 alleles were produced by 10 DNA primers and, average allele number per primer was 5. The average polymorphism information content of the study was determined as 98%, and the lowest PIC value was obtained from Xwgp115 marker with 96%, while the rest of the primers had a 99% PIC values (Table 2). In a previous study Maccaferri et al. (2003) conducted a research on genetic diversity of a durum wheat set derived from Mediterranean basin using microsatellites and reported a mean diversity index (DI) of 56%. Moragues et al. (2007) investigated the genetic variation of 63 durum wheat landraces and indicated an average PIC value of 24% for AFLP and

70% for SSR markers. Also Gungor (2019) reported an average PIC value of 72.5% on a durum wheat cultivar panel derived from different breeding programs.

B28/Kunduru-1149_F5_3, B28/Kunduru-1149_F5_6, B28/Kunduru-1149_F5_7, Kunduru-1149/B28_F5_2 and Kunduru-1149/B28_F5_6 genotypes carried alleles for yellow rust resistance gene *Yr51* as reported and expected size of 225 bp (Randhawa et al., 2014). Gungor (2019) also detected 225 bp alleles using the Sun104 marker for yellow rust on a durum wheat panel. Yan et al. (2003) reported a marker-trait relation for Sun104 marker and yellow rust resistance gene *Yr51*. In addition, B28/Kunduru-1149_F5_2 and B28/Kunduru-1149_F5_3 genotypes had alleles for Xgwm18 markers related with yellow rust resistance gene *Yr15* and *Yr26*. However, the other markers related with yellow rust resistance such as Xwgp115 and Xgwm47 had no alleles among the genotypes, though Cowger et al., 2012) reported a marker-trait relation for Xgwm47 for Yr64 and Yr66 yellow rust resistance and Gungor (2019) indicated an allele for Xwgp115 marker in durum wheat cultivars (Table 2). Of the Sun209 and Sun479 markers linked to stem rust resistance gene *Sr49,* Sun209 had allele on B28/Kunduru-1149_F5_1 genotype at 148bp, while Sun479 amplified allele on B28/Kunduru-1149_F5_4 genotype at 200 bp. Bansal et al. (2015) and Gungor (2019) reported relation for the Sun209 and Sun479 markers with *Sr49* stem rust gene at 148 bp and 200 bp respectively, which is consistent with our findings.

On the other hand, some of the markers such as Xgwm130 marker linked to the leaf rust resistance gene, Bx7^{OE} marker for gluten strength, UHW89 marker which is carrying high protein content gene *Gpc-B1* and Xgwm66 marker which is involved with powdery mildew resistance gene used in the study had no alleles on the genotypes. However, Butow *et al.* (2003), Cho *et al.* (2017), Liang *et al.* (2010) and Gungor (2019) indicated that Bx7OE marker produced allele on their genotype panel and related with gluten strength and improved dough strength. In addition, Distelfeld *et al.* (2006) indicated marker-trait relation for UHW89 and high protein content, consistent with Gungor (2019).

A dendrogram was created using the whole alleles produced by DNA markers. According to the dendrogram two main groups obtained. The first one was consisted of B28/Kunduru-1149_F5_1, B28/Kunduru-1149_F5_2, B28/Kunduru-1149_F5_3 and Kunduru-1149/B28_F5_3 genotypes, while the parents were took place on the other group. The second group divided into two groups including parents in each group. The most similar genotypes were found as Kunduru-1149/B28_F5_2 and B28/Kunduru-1149_F5_6 genotypes with the 71% genetic similarity (Figure 4). Ren *et al.* (2013) indicated a narrow genetic base on a worldwide germplasm accession of durum wheat released in 1960s and 1970s, though a rapid increase after 1970s. Gungor (2019) reported a broad genetic variation in durum wheat cultivars derived from different collections and breeding programs.

CONCLUSION

Two reciprocal cross combinations of B28/Kunduru-1149 and Kunduru-1149/B28 were screened for the 10 allele specific DNA markers. It is determined that B28/Kunduru-1149_F5_4 and B28/Kunduru-1149_F5_1 genotypes had stem rust resistance allele *Sr49*. B28/Kunduru-1149_F5_2 and B28/Kunduru-1149_F5_3 genotypes had yellow rust resistance alleles *Yr15,* while yellow rust resistance allele *Yr51* was detected on B28/Kunduru-1149_F5_3, B28/Kunduru-1149_F5_6, B28/Kunduru-1149_F5_7, Kunduru-1149/B28_F5_2 and Kunduru-1149/B28_F5_6 genotypes. On the other hand, according to the dendrogram B28 / Kunduru-1149_F5_6 and Kunduru-1149 / B28_F5_2 genotypes were found the most similar genotypes with 0.714, while Kunduru-1149 and B28/Kunduru_F5_7 with 0.10 combinations were found the most diverse ones.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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