Evaluation of F₅ Individuals Obtained from B28×Kunduru-1149 Reciprocal Cross Population by Functional Markers

Gülan AYDEMİR¹, Ziya DUMLUPINAR², Ilker YÜCE³, Tuğba BAŞKONUŞ⁴, Selçuk SUNULU⁵

Hüseyin GÜNGÖR⁶

¹,²KSU Agricultural Biotechnology Department, Kahramanmaraş, Turkey, ³-⁴KSU Field Crops Department, Kahramanmaraş, Turkey, ⁵Düzce University Field Crops Department, Düzce, Turkey

https://orcid.org/0000-0001-6054-8893, ³https://orcid.org/0000-0003-3119-6926, ⁴https://orcid.org/0000-0002-9761-3561, ⁵https://orcid.org/0000-0002-1806-2547, ⁶https://orcid.org/0000-0001-6708-6537

Ebeveynler gluten dayanıklılığı (Bx7[08], Yellow rust (Sun104, Xgwm18, Xwp115 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 Xwp115 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_F₅₄ (Sun479) and B28/Kunduru-1149_F₅₁ (Sun209) genotypes. One of the yellow rust resistance alleles Yr15 (Xgwm18) was detected in B28/Kunduru-1149_F₅₂ and B28/Kunduru-1149_F₅₃ genotypes, while Yr51 (Sun104) was identified in B28/Kunduru-1149_F₅₃, B28/Kunduru-1149_F₅₆, B28/Kunduru-1149/B28_F₅₂ and Kunduru-1149/B28_F₅₆ genotypes. A dendrogram was created to determine kinship of the individuals with the parents. The highest genetic similarity was observed between B28 / Kunduru-1149_F₅₆ and Kunduru-1149 / B28_F₅₂ genotypes with 0.714, while the most diverse ones were Kunduru-1149 and B28/Kunduru_F₅₇ with 0.10.

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 F₅ stage. The cross combinations and the parents were screened with 10 DNA markers to determine alleles of gluten strength (Bx7[08]), Yellow rust (Sun104, Xgwm18, Xwp115 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 Xwp115 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_F₅₄ (Sun479) and B28/Kunduru-1149_F₅₁ (Sun209) genotypes. One of the yellow rust resistance alleles Yr15 (Xgwm18) was detected in B28/Kunduru-1149_F₅₂ and B28/Kunduru-1149_F₅₃ genotypes, while Yr51 (Sun104) was identified in B28/Kunduru-1149_F₅₃, B28/Kunduru-1149_F₅₆, B28/Kunduru-1149/B28_F₅₂ and Kunduru-1149/B28_F₅₆ genotypes. A dendrogram was created to determine kinship of the individuals with the parents. The highest genetic similarity was observed between B28 / Kunduru-1149_F₅₆ and Kunduru-1149 / B28_F₅₂ genotypes with 0.714, while the most diverse ones were Kunduru-1149 and B28/Kunduru_F₅₇ with 0.10.

ÖZET
Bu çalışmada, B28 ve Kunduru-1149 makarnalık buğday genotipi olarak melezlenmiş ve elde edilen 13 B28/Kunduru-1149-Kunduru-1149/B28 F₅ kademesindeki hat materyal olarak kullanılmıştır. Melez kombinasyonları ve ebeveynler gluten dayanıklılığı (Bx7[08]), sari pas (Sun104, Xgwm18, Xwp115 ve Xgwm47), karas pas (Sun209 ve Sun479), yüksek protein oranı (UHW89), küllene (Xgwm66) ve kahverengi pas (Xgwm130) alcelleri bakımından 10 DNA markörü ile taramıştır. Çalışmada, ortalama polimorfizmi bilgi içeriği (PIC) 0.98 olarak hesaplanmıştır ve en düşük PIC değeri 0.96 ile Xwp115 marköründen elde edilirken, diğer markörler 0.99 PIC değerine sahip olmuştur. B28/Kunduru-1149_F₅₄ (Sun479) ve B28/Kunduru-1149_F₅₁ (Sun209) genotiplerinde sari pasa dayanıklılık alleles Sr49 tespit edilmiştir. Sarı pas dayanıklılık alcellerinden Yr15 (Xgwm18) ve B28/Kunduru-1149_F₅₂ ve B28/Kunduru-1149_F₅₃ genotiplerinde bulunurken, Yr51 (Sun104) geni B28/Kunduru-1149_F₅₃, B28/Kunduru-1149_F₅₆, B28/Kunduru-1149_F₅₇, Kunduru-1149/B28_F₅₂ ve Kunduru-1149/B28_F₅₆ genotiplerinde tanımlanmıştır. Ebeveynler...
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 Karacadag location of South-East Anatolia Region (Anonymous, 2016).

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 F5 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 (Bx70E), 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-∑P^2, where Pi 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

| No. | Primer Name (Primer Ad) | Primer Sequence (Primer Dizisi) | Reference (Referans) | Loci (Lokus) | Expected Fragment Size (Bant Uzunluğu (bp)) | Marker Type (Markör Tipi) |
|-----|-------------------------|---------------------------------|----------------------|-------------|----------------------------------|--------------------------|
| 1   | Bx708_F, Bx708_R        | CCTCAGCATGCAAACATGCAGCCTGAAACCTTTGGCCAGTCATGCT | Butow et al., 2003  | Glutent Strength | 563 | Co-dominant |
| 2   | SUN104_F, SUN104_R      | TGCTATGTCGATGATGAAATTACATGCTCCAGCGACTTG | Randhawa et al., 2014 | Yellow Rust Yr51 | 225 | Dominant |
| 3   | SUN209_F, SUN209_R      | AGCTATGACCCCGCTATGF66GTGATGTTATCATTA | Bansal et al., 2015 | Stem Rust Sr49 | 148 | Co-dominant |
| 4   | SUN479_F, SUN479_R      |CAAATGAAATGTGATCCTGTGTCATGGTCATGGTCATGCT | Bansal et al., 2015 | Stem Rust Sr49 | 200 | Co-dominant |
| 5   | UHW89_BF, UHW89_R       | TCTCCAAAGAGGGGAGAGACA| Distelfeld et al., 2006 | High Protein Content Gpc-B1 | 122 | Co-dominant |
| 6   | XGWM18_F, XGWM18_R      | TTGCTACCATGCGATACTGGGTGAGTGCTCCT | Roder et al., 1998 | Yellow Rust Yr15 and Yr26 | 186, 190 | Co-dominant |
| 7   | XGWM47_F, XGWM47_R      | TTGCTACCATGCAATGGCTGAGTGCTCCT | Roder et al., 1998 | Yellow Rust Yr64 and Yr66 | 190 | Co-dominant |
| 8   | XGWM66_F, XGWM66_R      | TTGCTACCATGCGATGGCTGAGTGCTCCT | Roder et al., 1998 | Powdery Mildew | 137 | Co-dominant |
| 9   | XGWM180_F, XGWM180_R    | AGCTCTCCGCTACGAGGAGCCTCTCTCTATATCGATGCCCTCCT | Roder et al., 1998 | Leaf Rust Lr34 | 121,126 | Co-dominant |
| 10  | XGWM115_F, XGWM115_R    | AGTGCTTCTTTAGGGATATTCGAGGGTGGATCTGAAATAT | 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 allele sayıları

Figure 1 Allele numbers of the primers screened for the durum wheat reciprocal segregation populations

Şekil 1 Makarnalık buğday resiprokal melez popülasyonunda görüntülenen primerlerin allele sayıları
Figure 2 Visualization of Xgwm18 (Yr26) DNA marker which produced alleles on B28/Kunduru-1149_F₅₀₂ and B28/Kunduru-1149_F₅₀₃ genotypes on Fragment Analyzer.

Şekil 2 Fragment analiz cihazında, B28 / Kunduru-1149_F₅₀₂ ve B28 / Kunduru-1149_F₅₀₃ 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 külemeye 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 ağacı

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 kombinasyonlarının allelik varyasyonları

**Markers (Markörler)**

| Genotypes (Genotipler) | SUN 104 | UHW89 | SUN209 | BX708 | SUN479 | XWGP115 | XGWM18 | XGWM130 | XGWM466 | XGWM47 |
|------------------------|---------|-------|--------|-------|--------|---------|--------|---------|---------|--------|
| Kunduru-1149           |         |       |        |       |        |         |        |         |         |        |
| B28                    |         |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_1 | +       |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_2 | +       |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_3 | +       |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_4 | +       |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_5 | +       |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_6 | +       |       |        |       |        |         |        |         |         |        |
| B28/Kunduru-1149_F_5_7 | +       |       |        |       |        |         |        |         |         |        |
| Kunduru-1149/B28_F_5_1 | +       |       |        |       |        |         |        |         |         |        |
| Kunduru-1149/B28_F_5_2 | +       |       |        |       |        |         |        |         |         |        |
| Kunduru-1149/B28_F_5_3 | +       |       |        |       |        |         |        |         |         |        |
| Kunduru-1149/B28_F_5_4 | +       |       |        |       |        |         |        |         |         |        |
| Kunduru-1149/B28_F_5_5 | +       |       |        |       |        |         |        |         |         |        |
| Kunduru-1149/B28_F_5_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, Bx7OE 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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