Genetic Characterization of Rainfed Durum Wheat Genotypes Based On Functional Markers Associated With Grain Quality Proteins

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Abstract

Analysis of genetic diversity provides helpful information necessary to develop the breeding and conservation strategies of crops. In this study, the genetic diversity and population structure of 90 durum wheat genotypes maintained at Sararud Dryland Agricultural Research Institute, Kermanshah, Iran, were evaluated by using 23 gene-specific markers (functional markers, FMs) encoding high and low molecular weight glutenin and gliadin alleles. Results showed that 12 out of the 23 FMs used were polymorphic and amplified 52 polymorphic loci. Primer *Ax2** had the highest discriminatory power. The population structure analysis classified the durum wheat collection into four populations. On average, population 4, consisting of 8 genotypes, had the highest allele number as well as genetic variation. Analysis of molecular variance indicated that 82% of the total variation was distributed among populations. The diversity among populations and gene flow were 0.14 and 3.03, respectively. The Jaccard distance coefficient revealed that genetic dissimilarities ranged from 0.031 between G62 and G65 to 0.725 between G36 and G51. Neighbor-joining method clustered individuals into six main groups. Results showed a remarkable level of genetic diversity among studied durum wheat genotypes which can be of interest for future breeding programs.

Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum*, 2n = 4x = 28; AABB) is the only tetraploid species of commercial wheat cultivated over almost 17 million ha of the Mediterranean basin and other semi-arid areas globally, with 38.1 million t (Aslan-parviz et al. 2020; Etminan et al. 2018; Xynias et al. 2020). This plant is the primary source of semolina, often used in food products such as pasta, couscous, bulgur, various bread, and other local products (El Haddad et al. 2021; Magallanes-López et al. 2017).

Genetic variation is an essential component of plant genetics, breeding, conservation, and evolution programs that provides a basis for selecting superior parent compounds and predicting offspring performance (Fattah and Tayib 2020). The study of genetic variation leads to discovering new alleles or genes in the population under investigation and thus maximizes the efficiency of breeding programs (Alemu et al. 2020). So far, numerous breeding programs have been designed and implemented to study and measure genetic variation in durum wheat germplasm (Aslan-parviz et al. 2020). Molecular markers are among the tools that have been successfully used to dissect many plant populations' genetic architecture and structure (Fayaz et al. 2019). During the past decades, the use of molecular markers at the gene level, such as SSR, RFLP, RAPD, AFLP, DArT, etc., has become common in wheat genetic analysis (Kumar et al. 2021). However, these markers are neutral, which can be somewhat distant from the genes, and are often relevant to a particular population or parents (Kage et al. 2016). Another class of DNA molecular markers is called gene-specific or functional molecular markers (FMs), based on polymorphisms within genes and are therefore directly related to the desired trait allele. Hence, it has been suggested that FMs are preferable to random markers, especially in marker-assisted selection projects (Li et al. 2020).

After yield, quality is one of the most critical aspects of wheat breeding programs. The quality of wheat-based food products is determined by the grain quality characteristics (Magallanes-López et al. 2017), including high protein content, high gluten strength, and the tenacity, strength, and extensibility of dough (Regina and Guzmán 2020). The genetic control of these properties depends mainly on the structure of glutenin and gliadin proteins. Glutenins are divided into two subunits of High Molecular Weight (HMWGs) and Low Molecular Weight subunits (LMWGs). In durum wheat, HMWGs are encoded by *Glu-A1* and *Glu-B1* loci on the long arms of the group-1 homoeologous chromosomes, whereas LMWGs are encoded by *Glu-A3*, *GluB3*, and *Glu-B2* genes on the short arms of group-1 homoeologous chromosomes (Chegdali et al. 2020). The majority of γ-gliadin and ω-gliadin genes are encoded by the *Gli-1* loci on the short arm of chromosome 1, whereas the α-gliadins encoded on the group-6 chromosomes (Hsia and Anderson 2001).

In Iran, the production and consumption of pasta are widespread. However, due to a shortage of durum semolina, bread wheat flour is often used in pasta production resulting in technological difficulties and reduced quality (Irani 2000). Therefore, several projects were started to select suitable and high-quality durum wheat genotypes. Due to different alleles, it seems possible to study genetic diversity based on specific markers related to grain proteins responsible for quality. In this regard, this research aimed to investigate the genetic variation and population structure of a set of durum wheat genotypes using functional markers associated with grain quality proteins.

Materials And Methods

Plant materials and DNA extraction

In this study, 90 durum wheat genotypes provided by Sararud Rainfed Research Center, Kermanshah, Iran, were used (Table 1). All genotypes were rainfed and showed good performance in various experiments. Molecular experiments and genotyping of samples were performed at the Islamic Azad University of Kermanshah. For this purpose, three seeds of each genotype were planted in the greenhouse, and the genomic DNA was extracted by the CTAB method from plant leaves at the five-leaf stage (Murray and Thompson 1980). The DNA quality of the samples was then examined using 1% agarose gel.

Table 1
Specifications of 90 durum wheat geno

Genotype	Pedigree
G1	MÂALI/10/ALTAR 84/CMH82A.1062//ALTAR 84/3/YAZI_10/4/SNITAN/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/H
G2	MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/6/PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//CREX/ALLA/3/SOM
G3	GUAYACAN INIA/2*SNITAN/3/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO_2
G4	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/6/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO
G5	PLATA_7/ILBOR_1//SOMAT_3/3/CABECA_2/PATKA_4//BEHRANG/10/1A.1D 5+1–06/2*WB881//1A.1D 5+1–06/3*MOJO/3/SOOTY_9/RASC
G6	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/SOOTY_9/P 12Y-1M-06Y-0B
G7	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/SOOTY_9/P 18Y-1M-06Y-0B
G8	GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/7/CAMAYO//HYDRANASSA30/SILVER_5/3/SOOTY_9/RASCON_37/5/DUKEM_15/3/BISU_1/P 84/ALD/4/POD_11/YAZI_1/5/VANRRI
G9	CNDO/VEE//PLATA_8/3/6*PLATA_11/6/PLATA_8/4/GARZA/AFN//CRA/3/GTA/5/RASCON/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV
G10	RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/9/CBC 509 CH 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHA
G11	CIRNO C 2008
G12	CBC 509 CHILE/5/2*AJAIA_16//HORA/JRO/3/GAN/4/ZAR/6/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/CHEN_1/TEZ/3/GUIL//CIT
G13	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOT D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11
G14	GEN/4/D68.1.93A.1A//RUFF/FGO/3/MTL_5/5/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/4/CANELO_8//S
G15	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOT 0BINIA/3/DUKEM_12/2*RASCON_21
G16	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOT 0BINIA/3/DUKEM_12/2*RASCON_21
G17	CBC 514 CHILE/3/AUK/GUIL//GREEN/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/
G18	GUAYACAN INIA/2*SNITAN/5/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
G19	PLATA_7/ILBOR_1//SOMAT_3/3/CABECA_2/PATKA_4//BEHRANG/10/1A.1D 5+1–06/2*WB881//1A.1D 5+1–06/3*MOJO/3/SOOTY_9/RASC
G20	GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN/5/CAMAYO/GUANAY/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_ 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_
G21	CAMAYO/2*KUCUK/3/SOOTY_9/RASCON_37//GUAYACAN INIA/5/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_3/PHAX_1//TILO_1/LOTUS
G22	AINZEN_1//PLATA_6/GREEN_17/5/TATLER_1/TARRO_1/3/CANELO_8//SORA/2*PLATA_12/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
G23	DACK/KIWI//OSTE/3/CHEN84_1/4/MEXI75/5/NIGRIS_4/6/CANELO_8//SORA/2*PLATA_12/7/SOMAT_4/INTER_8/8/GEDIZ/FGO//GTA/3/SRN_
G24	SELIM/6/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMBRA_20/4/SNITAN/5/SOMAT_4/INTER_8/7/NASR 99/4/BCRIS/BICUM//L
G25	SELIM/3/CF4-JS 21//TECA96/TILO_1/4/SORA/2*PLATA_12//SRN_3/NIGRIS_4
G26	ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/4/TOSKA_26/RASCON_37//SNITAN/5/PLAYERO
G27	WID22241/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_4/INTER_8/4/ARMENT//SRN_3/N
G28	ALAMO:DR/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/PLATA_6/GREEN_17//SNITAN/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREE
G29	E90040/MFOWL_13//LOTAIL_6/3/PROZANA/ARLIN//MUSK_6/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV
G30	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/10/CMH79.959/CHEN//SOOTY_9/RASCON_37/9/USDA595/3/D67.3/RABI//CR
G31	ZENIT/5/SORA/2*PLATA_12//RASCON_37/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/6/MINIMUS_4/GRO_2/3/PROZANA/ARLIN//MUSK_6
G32	SIMETO/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/5/TOSKA_26/RASCON_37//SNITAN/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
G33	P91.272.3.1/3*MEXI75//2*JUPARE C 2001/5/ARTICO/AJAIA_3//HUALITA/3/FULVOUS_1/MFOWL_13/4/TECA96/TILO_1/6/RISSA/GAN//POHC
G34	P91.272.3.1/3*MEXI75//2*JUPARE C 2001/5/ARTICO/AJAIA_3//HUALITA/3/FULVOUS_1/MFOWL_13/4/TECA96/TILO_1/6/RISSA/GAN//POHC
G35	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/10/CMH79.959/CHEN//SOOTY_9/RASCON_37/9/USDA595/3/D67.3/RABI//CR
G36	ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/4/STOT//ALTAR 84/ALD/3/PATKA_7/YAZI_1/5/HUALITA

Genotype	Pedigree
G37	ODIN_15/WITNEK_1//ISLOM_1/6/MINIMUS/COMB DUCK_2//CHAM_3/3/FICHE_6/4/MOJO/AIRON/5/SOMAT_3.1
G38	TRIDENT/3*KUCUK
G39	PLANETA/AMIC//BERGAND/TRILE/3/KNIPA
G40	ATIL/3/KNIPA/TAGUA//PLANETA/TRILE
G41	ATIL/HELLER #1 CDSS09Y00298S-099Y-043M-27Y-0M-04Y-0B
G42	ATIL/BAIRDS CDSS09Y00386S-099Y-058M-23Y-0M-04Y-0B
G43	ATIL/BAIRDS CDSS09Y00388S-099Y-047M-19Y-0M-04Y-0B
G44	ATIL/3/KNIPA/TAGUA//PLANETA/TRILE
G45	CIRNO C 2008/HELLER #1 CDSS09Y00771T-099Y-040M-10Y-0M-04Y-0B
G46	CIRNO C 2008/BAIRDS CDSS09Y00795T-099Y-024M-29Y-0M-04Y-0B
G47	CIRNO C 2008/BAIRDS CDSS09Y00449S-099Y-011M-10Y-0M-04Y-0B
G48	CIRNO C 2008/3/KNIPA/TAGUA//PLANETA/TRILE CDSS08B00149T-099Y-056M-16Y-0M-04Y-0B
G49	CIRNO C 2008/3/KNIPA/TAGUA//PLANETA/TRILE CDSS09Y00970T-099Y-063M-11Y-0M-04Y-0B
G50	CIRNO C 2008/3/KNIPA/TAGUA//PLANETA/TRILE CDSS09B00077S-099Y-014M-4Y-3M-06Y-0B
G51	CIRNO C 2008/3/KNIPA/TAGUA//PLANETA/TRILE CDSS10Y00288S-099Y-038M-17Y-1M-06Y-0B
G52	ATIL*2/HELLER #1 CDSS10Y00291S-099Y-044M-5Y-2M-06Y-0B
G53	ATIL*2/BAIRDS CDSS10Y00491T-099Y-040M-7Y-1M-06Y-0B
G54	ATIL*2/DUNKER CDSS10Y00493T-099Y-035M-9Y-4M-06Y-0B
G55	ATIL*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS10Y00500T-099Y-028M-1Y-4M-06Y-0B
G56	ATIL*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS10Y00504T-099Y-037M-5Y-2M-06Y-0B
G57	ATIL*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS10Y00517T-099Y-055M-13Y-4M-06Y-0B
G58	CIRNO C 2008*2/HELLER #1 CDSS09B00261T-099Y-046M-7Y-2M-06Y-0B
G59	CIRNO C 2008*2/HELLER #1 CDSS09B00268T-099Y-050M-3Y-4M-06Y-0B
G60	CIRNO C 2008*2/BAIRDS CDSS09B00347T-099Y-033M-13Y-3M-06Y-0B
G61	CIRNO C 2008*2/BAIRDS CDSS10Y00539T-099Y-025M-29Y-1M-06Y-0B
G62	CIRNO C 2008*2/BAIRDS CDSS10Y00550T-099Y-014M-7Y-3M-06Y-0B
G63	CIRNO C 2008*2/BAIRDS CDSS10Y00550T-099Y-014M-8Y-3M-06Y-0B
G64	CIRNO C 2008*2/BAIRDS CDSS10Y00553T-099Y-068M-8Y-3M-06Y-0B
G65	CIRNO C 2008*2/BAIRDS CDSS10Y00556T-099Y-031M-16Y-2M-06Y-0B
G66	CIRNO C 2008*2/BAIRDS CDSS09B00483D-099Y-032M-2Y-1M-06Y-0B
G67	CIRNO C 2008*2/BAIRDS CDSS09B00490D-099Y-032M-11Y-3M-06Y-0B
G68	CIRNO C 2008*2/BAIRDS CDSS10Y00572T-099Y-030M-13Y-1M-06Y-0B
G69	CIRNO C 2008*2/BAIRDS CDSS10Y00573T-099Y-056M-4Y-4M-06Y-0B
G70	CIRNO C 2008*2/BAIRDS CDSS09B00128S-099Y-057M-1Y-1M-06Y-0B
G71	CIRNO C 2008*2/DUNKER CDSS09B00150S-099Y-023M-4Y-1M-06Y-0B
G72	CIRNO C 2008*2/DUNKER CDSS10Y00017S-099Y-034M-7Y-1M-06Y-0B
G73	CIRNO C 2008*2/DUNKER CMSS08B01003S-099B-099Y-22B-0Y
G74	CIRNO C 2008*2/DUNKER CDSS07Y00746T-099Y-099M-5Y-3M-04Y-0B
G75	CIRNO C 2008*2/DUNKER CDSS07Y00079S-099Y-099M-4Y-2M-04Y-0B
G76	CIRNO C 2008*2/DUNKER CDSS06B00053S-099Y-099M-12Y-2B-04Y-0B
G77	CIRNO C 2008*2/DUNKER CDSS08Y00401S-099Y-028M-9Y-4M-0Y
G78	CIRNO C 2008*2/DUNKER CDSS09Y00029S-099Y-020M-9Y-0M-04Y-0B
G79	CIRNO C 2008*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS09Y00241S-099Y-022M-10Y-0M-04Y-0B

Genotype	Pedigree
G80	CIRNO C 2008*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS09Y00286S-099Y-026M-24Y-0M-04Y-0B
G81	CIRNO C 2008*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS09Y00762T-099Y-024M-20Y-0M-04Y-0B
G82	CIRNO C 2008*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS09Y00771T-099Y-040M-3Y-0M-04Y-0B
G83	CIRNO C 2008*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS10Y00498T-099Y-018M-12Y-1M-06Y-0B
G84	CIRNO C 2008*2/3/KNIPA/TAGUA//PLANETA/TRILE CDSS10Y00498T-099Y-018M-18Y-1M-06Y-0B
G85	PLATINUM*2/3/KNIPA/TAGUA//PLANETA/TRILE
G86	Dehdasht/IC-142070 IRD2010-11-003-OMAR-OMAR-OSAR-OSAR-OSAR-1SAR
G87	Saji
G88	Zahab
G89	SRN-1/KILL//2*FOLTA-1
G90	Imren

Genotyping assays

In this study, a set of 23 gene-specific primers encoding alleles gliadin, HMWGS, and LMWGS (high and low molecular weight glutenin subunits, respectively), made by Sinaclon Co., Tehran, Iran, were initially tested (Table 2). The polymerase chain reaction was performed using a thermocycler (Techne model TC-5000) in a volume of 15 µl consisted of 2 µl template DNA from each sample, one µl of each primer (0.5 forward and 0.5 reverse), 4.5 µl double distilled water, and 7.5 µl master mix. The PCR reaction was carried out as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 56°C for 30 s and primer elongation at 72°C for 1 min; the final extension at 72°C was held for 5 min. The PCR products were electrophoresed on a 1.5% agarose gel with TBE 1X buffer, stained with Safe View, and finally photographed under UV light. Of the above 23 markers mentioned, only 12 were polymorphic. Therefore, the experiment was continued based on the 12 markers (Table 2).

Table 2
Locus name and sequences of 12 gene-specific markers related to grain quality used.

	Marker/gene	Forward Sequences (5' → 3')	Backward Sequences (5' → 3')	Reference
α/β-Gliadin	gli-AS_3	TCACCGCTACAACGACCAAACCATGTTT	GCAACCATTTCTGCCACAACTACCAT	Kawaura et al. (2006)
	gli-AS_4	CCTAGGCCTATGGGTTCTGCTGAGA	GCAACCACAGTATCCGCAACCAC	Kawaura et al. (2006)
LMW-GS	glu-AS_2	GGCACAGGGTACCTTTTTGCATC	ATACAAGGGCACATTGACACGGC	Kawaura et al. (2006)
	glu-A1	CGAGACAATATGAGCAGCAAG	CTGCCATGGAGAAGTTGGA	Li et al. (2008)
	glu3A.1	GCCGTTGCGCAAATTTCACAG	AACAGATGGATGAATAACTGGTAT	Long et al. (2005)
	glu3A.2	AGTGCCATTGCGCAGATGAAT	AACGGATGGTTGAACAATAGA	Long et al. (2005)
	glu3A.3	ATGGAGACTAGCTGCATCC	CTGCAAAAAGGTACCCTTTT	
	glu-A3ac	AAACAGAATTATTAAAGCCGG	GTGGCTGTTGTGAAAACGA	Wang et al. (2010)
	glu-A3f	AAACAGAATTATTAAAGCCGG	GCTGCTGCTGCTGTGTAAA	Wang et al. (2010)
	glu-A3g	AAACAGAATTATTAAAGCCGG	AAACAACGGTGATCCAACTAA	Wang et al. (2010)
HMW-GS	*Ax2 **	ATGACTAAGCGGTTGGTTCTT	ACCTTGCTCCCCTTGTCTTT	Ma et al. (2003)
	ZSBy9aF1/R3	TTCTCTGCATCAGTCAGGA	AGAGAAGCTGTGTAATGCC	Lei et al. (2006)

Data analysis

First, bands formed on the gel were scored as absent (0) and present (1), compiling the data as a binary matrix. To compare the banding patterns of primers, some genetic parameters were calculated by using PowerMarker 3.25 software (He et al. 2020). The Polymorphic Information Content (PIC) of each locus was calculated as $PIC = 1 - \sum_i^n p_i^2$, where p_i is the frequency of the ith allele. The Resolving power (Rp) of each locus was calculated as $Rp = \Sigma\ 1\text{-}\ (2 \times |0.5 - pi|)$. The heterozygosity (He) value was computed according to Nei's formula as $He = 1 - \sum_{i=1}^{n} p_i^2$. The Shannon's Information index was determined as $I = -\Sigma\ pi \times \ln pi$. The genetic variability indices, including total amplified bands (TAB), the number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), the effective number of alleles (Ne), Nei's gene diversity (h), diversity among populations (Gst), and the estimate of gene flow (Nm) were estimated using POPGENE version 1.32 (Negisho et al. 2021). The distribution of genetic variation within and among populations (AMOVA) and principal coordinate analysis (PCoA) was carried out using the GenAlex 6.5 software (Peakall and Smouse 2012). The model-based STRUCTURE version 2.3.4 was used to recognize subpopulations of genetically similar individuals. The optimal number of subpopulations was determined by using Structure Harvester software (Earl and

vonHoldt 2012) according to the *ΔK* approach proposed by Evanno et al. (2005). Cluster analysis was performed using MEGA 6.0 software. First, the Jaccard's dissimilarity distance matrix was calculated using DARWIN software version 6.0 (Shaygan et al. 2021). Afterward, the cluster dendrogram was obtained using the neighbor-joining method based on the distance matrix.

Results

Of the 23 initial FMs tested on 90 durum genotypes, only 12 (52.2%) were polymorphic. These 12 primers created a total of 52 polymorphic bands. On average, 4.33 bands were amplified per primer of which, four primers had only two bands, whereas glu3A.1 and glu-A3ac produced seven bands (Table 3). The mean number of effective alleles (Ne) was 1.56 per locus. Markers gli-AS_4 and glu-A1 with 1.98 and 1.01 alleles showed the highest and lowest Ne, respectively. The polymorphism information content (PIC) values varied from 0.011 (by glu-A1) to 0.287 (by *Ax2 **), with an average of 0.139. The highest and lowest values of marker index (MI) were observed for primer *Ax2 ** (1.72) and primer glu-A1 (0.011), respectively, with an average of 0.68 (Table 3). Furthermore, the mean values for resolving power (Rp) was 4.51 and ranged from 2.38 to 8.97 for primers gli-AS_3 and *Ax2 **, respectively (Table 3).

Table 3
Discriminating power statistics and the amplification results of 12 gene-specific markers related to grain quality used in the study.

	Marker/Locus	TAB	NPB	PPB%	Ne	PIC	MI	Rp
α/β-Gliadin	gli-AS_3	2	1	50	1.81	0.153	0.306	2.38
	gli-AS_4	6	5	83.3	1.98	0.045	0.271	5.88
LMW-GS	glu-AS_2	5	4	80	1.74	0.261	1.306	4.20
	glu-A1	2	1	50	1.01	0.011	0.011	3.97
	glu3A.1	7	6	85.7	1.60	0.216	1.516	3.80
	glu3A.2	6	6	100	1.60	0.104	0.625	3.16
	glu3A.3	3	1	50	1.72	0.060	0.180	4.20
	glu-A3ac	7	7	100	1.83	0.150	1.050	5.55
	glu-A3f	4	3	75	1.78	0.225	0.900	4.44
	glu-A3g	2	1	50	1.14	0.123	0.247	3.71
LMW-GS	*Ax2 **	6	6	100	1.50	0.287	1.720	8.97
	ZSBy9aF1/R3	2	2	100	1.04	0.043	0.086	3.91
	Mean	4.33	3.58	77.00	1.56	0.139	0.680	4.51

TAB total amplified bands, *NPB* number of polymorphic bands, *PPB* percentage of polymorphic bands, *Ne* number of effective alleles, *h* Nei's gene diversity, *I* Shannon's information index, *PIC* polymorphism information content, *MI* marker index, *Rp* resolving power.

The results of the population structure analysis have been shown in Figs. 1 and 2. First, to detect the optimal number of subpopulations that best fit the data, the likelihood values of partitioning (*ΔK*) were plotted across multiple values of *K* (Fig. 1), assuming that the loci are independent in Hardy-Weinberg equilibrium and *K* is the number of populations. As shown in Fig. 1, the maximum likelihood value was obtained when the initial population was divided into four subpopulations. Accordingly, the durum wheat collection was classified into four subpopulations, containing 30, 19, 33, and 8 members, respectively (Fig. 2). The summary of the genetic diversity parameters of the four detected populations has been shown in Table 4. The percentage of polymorphic loci were 42.31, 44.23, 42.31, and 75.00 for subpopulations 1 to 4, respectively. The number of different alleles ranged from 1.13 for subpopulation 1 to 1.69 for subpopulation 4. Similarly, subpopulations 1 and 4 with 1.18 and 1.38 had the lowest and the highest number of effective alleles, respectively. Also, the Shannon's index were 0.18, 0.21, 0.19, and 0.37, for subpopulations 1 to 4, respectively. Furthermore, the Nei's gene diversity values were 0.11, 0.14, 0.12, and 0.24, respectively (Table 4). These results revealed that subpopulation 4 genotypes had the highest genetic variation than others while subpopulation 1 had the lowest.

Table 4
Summary of genic variation statistics estimated using gene-specific primers related to grain quality for the four subpopulations studied durum wheat genotypes.

Sub pop No.	Size	PPL%	Na	Ne	I	h
1	30	42.31	1.13	1.18	0.18	0.11
2	19	44.23	1.23	1.22	0.21	0.14
3	33	42.31	1.21	1.20	0.19	0.12
4	8	75.00	1.69	1.38	0.37	0.24

PPL percentage of polymorphic loci, *Na* number of different alleles, *Ne* number of effective alleles, *I* Shannon's Information index, *h* Nei's gene diversity

The allele distribution statistics across the 4 populations have been presented in Fig. 3. Population 4 showed the highest numbers of different bands (49) whereas population 1 had the lowest (37). There were 2 and 7 bands unique to populations 2 and 4, respectively. Also, no bands were found at less than or

equal to 25% of the population. The number of less common bands presented at less than or equal to 50% of the populations was reported in subpopulations 2, 3, and 4. Likewise, the mean diversity ranged from 0.112 (population 1) to 0.238 (population 4).

The analysis of molecular variance (AMOVA) showed that the variation between the populations was 18% of the total variation while 82% of the variations were among individuals within the population, indicating that most amplified fragments were informative for separating the genotypes. In addition, the diversity among populations and gene flow were 0.14 and 3.03, respectively (Table 5).

Table 5
Analysis of molecular variance (AMOVA) based on Gene-specific markers related to grain quality for four populations of durum wheat

Source	df	SS	MS	Est. Var.	Variation (%)	Gst	Nm
Among Pops	3	61.441	20.480	0.796	18%		
Within Pops	86	309.259	3.596	3.596	82%		
Total	89	370.700		4.392	100%	0.14	3.03

df degree of freedom, *SS* sum of squares, *MS* means of squares, *Est. Var* estimated variance components, *Gst* diversity among populations, *Nm* estimate of gene flow.

Calculation of the Jaccard distance coefficient revealed that genetic dissimilarities ranged from 0.031 between G62 and G65 to 0.725 between G36 and G51 (Data not shown). These results showed that the primers used in the study had an appropriate potential for calculating genetic distances and detecting relationships among durum wheat genotypes.

Cluster analysis based on the neighbor-joining method grouped the 90 individuals into four main clusters (Fig. 4). According to the subpopulations, the clustering pattern was different from that detected by structure classification results.

The results of the Principal coordinates analysis revealed that genotype G51 had a significant load on the first coordinate. Besides, due to their relatively large distance from other genotypes, G51, G70 had the highest degree of dissimilarity compared to different genotypes (Fig. 5).

Discussion

Genetic diversity stems from the natural selection of wild ancestors and mediation through farmers and breeders. The study of genetic diversity is essential in understanding the structure of germplasm. So far, genetic diversity analysis in crops has been applied for breeding programs related to the marker-trait association, marker-assisted selection, creation of artificial variation to find specific hybrids, and crop germplasm conservation (Negisho et al. 2021). In the past decades, different molecular tools such as neutral and functional DNA markers were introduced to plant breeding programs. Compared with neutral DNA markers, FMs have the advantage of being entirely associated with target genes. Therefore, FMs have been preferred in biodiversity studies (van Tienderen et al. 2002; Wu et al. 2020).

In this study, polymorphisms derived from FMs related to grain quality proteins were used to evaluate the genetic diversity of a collection of 90 durum wheat genotypes for future selective breeding programs. The results showed a relatively high level of molecular variability among the studied genotypes. Compared to neutral markers, the markers used in this study have been occasionally used in genetic diversity assays because gene-specific primers are often used in marker-assisted selection programs (Liu et al. 2012). Therefore, this is the first-of-its-kind report which shows the applicability of FMs, associated with wheat grain quality proteins, in assessing the genetic diversity of durum wheat. Significant polymorphisms and the formation of more than one band were among the practical factors in obtaining acceptable results. Some other kinds of FMs, such as CAAT box-derived polymorphism primers, have been used successfully to evaluate the genetic diversity of durum wheat (Shaygan et al. 2021; Etminan et al. 2019; Khodaee et al. 2021; Ghobadi et al. 2021).

According to our results, FMs had an acceptable percentage of polymorphic bands. Also, Marker information parameters showed the differentiation power of these primers and thus the appropriate efficiency of this type of DNA marker to investigate the genetic diversity in the germplasm of durum wheat. Of the primers used, four primers had PIC values above 0.20, indicating that these primers were informative in determining polymorphism. AMOVA results revealed that there was a higher percentage of genetic variation within durum populations than among populations. This result is also confirmed by the Gst value (diversity among populations). Zang et al. (2018) proposed that Gst is an important parameter to measure whether genetic differentiation exists among populations. When the Gst value falls between 0.05 and 0.15, the genetic differentiation is considered as the medium. Therefore the identified subpopulations were estimated to have the medium value of genetic differentiation. The Nm index, the number of migrants per generation, is an estimation of the gene flow. The distribution of genetic diversity between and within populations is a consequence of the amount of gene flow between them. Gene flow rate, in turn, is affected by the number of seeds or pollen that move between populations (Etminan et al. 2018). Nm values higher than 1 indicate that a weak possibility of genetic drift inhibits differentiation among populations whereas Nms less than 1 specify that local populations tend to differentiate. Hence, the higher Nm the less genetic differentiation among populations (Chen et al. 2020). Accordingly, in our study, the existence of large gene flow (Nm = 3.03) among populations decreased the degree of genetic differentiation among populations.

The Nei genetic diversity index and the Shannon Index are known as the most genetic diversity parameters to show diversity and differentiation between and within populations (Carvalho et al. 2010). The higher values of these indices denote the greater the genetic diversity (Que et al. 2014). In the current study, the level of variability between h and I indices revealed a high level of genetic diversity among subpopulations 2 and 4. The former was rich in α/β-Gliadin alleles while the latter showed a high percentage of bands associated with HMW-GS (data not shown). These results indicated that these populations of durum wheat may be a good source of grain quality-related protein subunits that can be used for a variety of purposes including breeding programs related to quality improvement especially in crosses with elite durum wheat germplasm.

As it can be seen, the clustering pattern (Fig. 4) was not consistent with that obtained from the population structure algorithm (Fig. 2). STRUCTURE identifies subsets by detecting allele frequency differences within the data and assigns individuals to those sub-populations based on analysis of likelihoods (Porras-Hurtado et al. 2013) while cluster analysis subdivides cases into clusters according to a specific measure of similarity and arranges cases in a hierarchy. Therefore, the observed results may be due to differences in the algorithm of the two methods. For example, in cluster analysis, a relatively large genetic distance between genotype G51 and other genotypes left this genotype in a single group while such a thing was not observed in grouping by STRUCTURE analysis (Figs. 2 and 4). This result is also supported by PCoA analysis (Fig. 5)

Breeding targets will have an advantage when the focus is on the cross of distant genotypes, or to increase diversity and identify new genes to further improve wheat. Hence, the classification and characterization of wheat germplasm is a prerequisite stage in the selection and improvement of wheat. The results of this study could be useful to select parents to be crossed for obtaining new appropriate populations intended for transgressive segregation of some important agronomic characters in the descendant populations. Our results confirmed a high level of genetic diversity in the studied genotypes. In addition, the results revealed that the FMs were reproducible, efficient, and powerful to assess genetic diversity among durum wheat genotypes.

Abbreviations

AMOVA Analysis of molecular variance

FM Functional marker

HMWGs High molecular weight glutenin subunits

I Shannon's Information Index

LMWGs Low molecular weight glutenin subunits

MI Marker index

Ne Number of effective alleles

Nm The estimate of gene flow

NPB The number of polymorphic bands

PCoA Principal coordinate analysis

PIC Polymorphism information content

PPB Percentage of polymorphic bands

Rp Resolving power

TAB Total amplified bands

UPGMA Unweighted pair group method with arithmetic mean

Declarations

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Author contributions

Conceptualization: ZMKh, RA, AMM, MK, AE; Data curation: RA, AMM, MK, AE; Formal analysis: ZMKh, RA; Investigation: ZMKh, RA, AMM, MK, AE; Methodology: RA, AMM, MK, AE; Validation: RA, AMM, MK, AE; Writing original draft: ZMKh, Review, and editing: RA, AE

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Data availability Data related to the results obtained in the present study are available from the corresponding author by request.

Ethics approval Not applicable

Conflict of interest The authors of this article have no conflicts of interest.

Consent for publication All authors have given their consent for publication.

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Figures

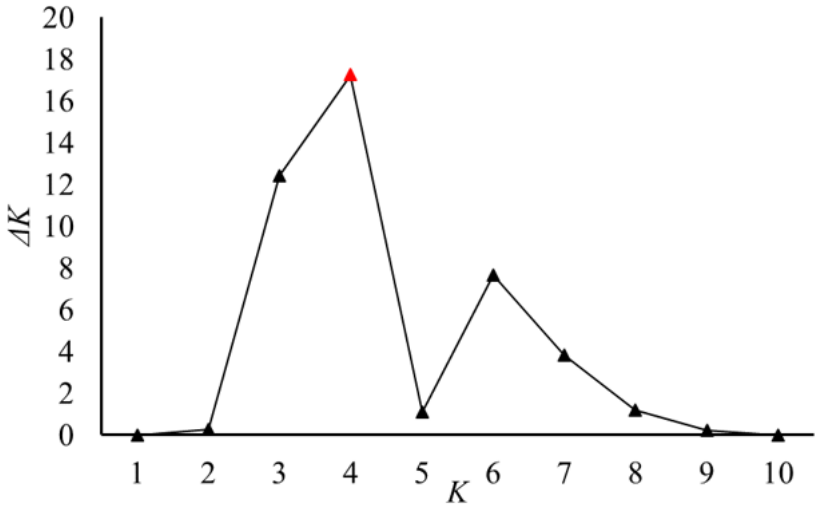

Figure 1

Detection of the optimal number of K subpopulations of 90 durum wheat genotypes based on data obtained by 12 gene-specific primers related to grain quality traits (Evanno et al. 2005).

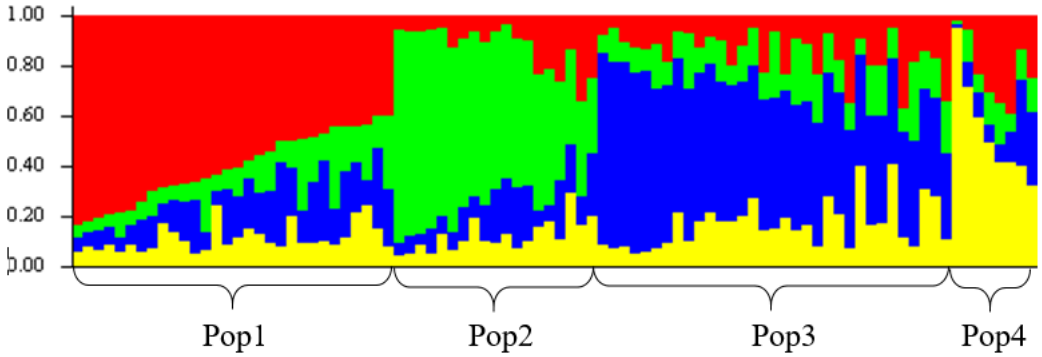

Figure 2

Bayesian model-based population structure based on data obtained by 12 gene-specific markers related to grain quality traits in 90 durum wheat genotypes.

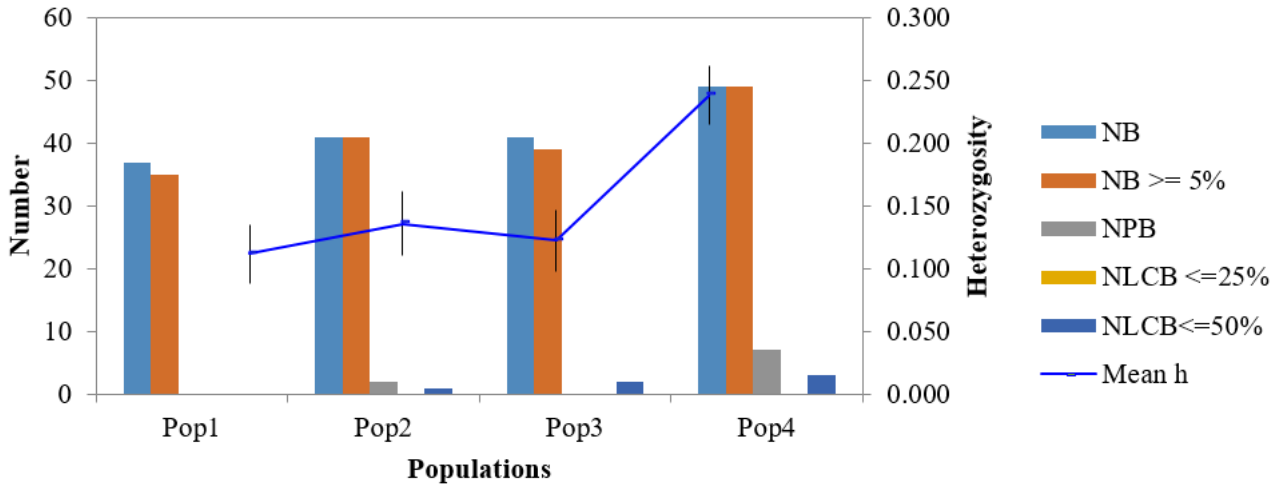

Figure 3

Allele distribution statistics across populations of 90 durum wheat genotypes NB No. of different bands, NB >= 5% No. of different bands with a frequency >= 5%, NPB No. of bands unique to a single population, NLCB <=25% No. of locally common bands (Freq. >= 5%) found in 25% or fewer populations, NLCB <=50% No. of locally common bands (Freq. >= 5%) found in 50% or fewer populations h Diversity (1 - (p^2 + q^2))

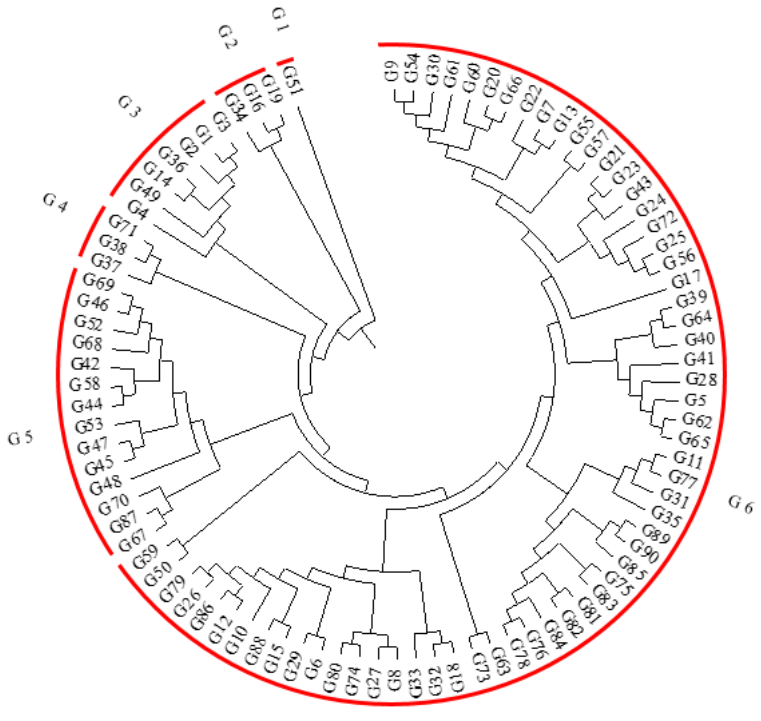

Figure 4

UPGMA phylogenetic dendrogram according to Jaccard's distance coefficient based on data obtained by 12 gene-specific markers related to grain quality traits on 90 durum wheat genotypes.

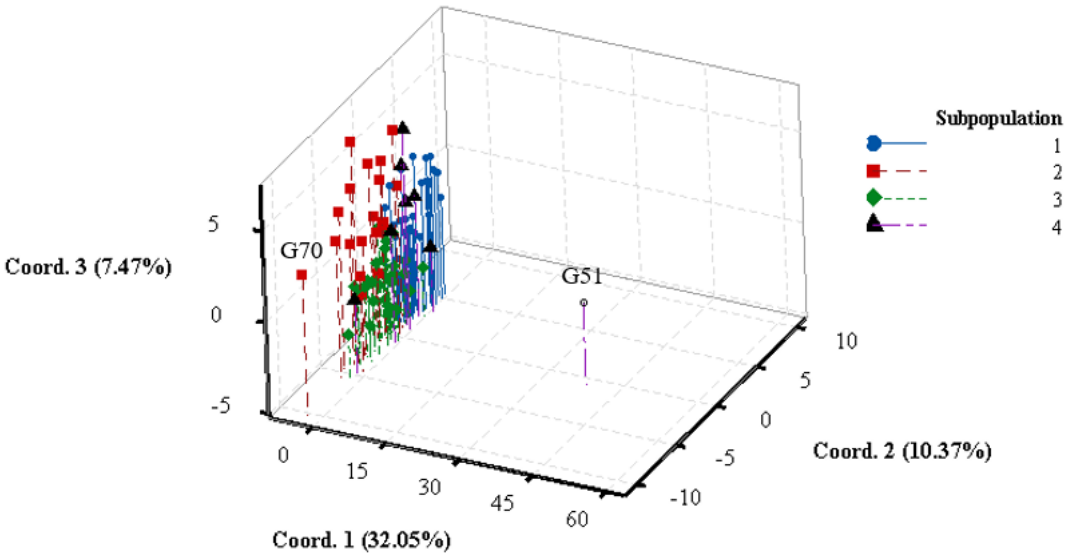

Figure 5

principal coordinate analysis based on data obtained by 12 gene-specific markers related to grain quality traits on 90 durum wheat genotypes.