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CORRELATION AND GENETIC ANALYSES OF DIFFERENT CHARACTERISTICS IN SAUDI ARABIAN WHEAT REVEAL CORRELATION NETWORKS AND SEVERAL TRAIT-ASSOCIATED MARKERS

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ABSTRACT

Thirty-six different morpho-agronomic traits in 40 different accessions of Saudi Arabian wheat were investigated through marker-trait association analysis, which could provide a single PCR marker. A total of 25 different correlations were retrieved, among which, days to heading (DH) was negatively correlated with flag leaf anthocyanin (FLA) (r = –0.73) and positively correlated with plant height (PH) (r = 0.63) and leaf length (LL) (r =0.62). Additionally, seeds count on spike (SCS) was positively correlated with awn color (AC) (r = 0.67) and spike color (SC) (r = 0.61) and negatively correlated with grain shape (GS) (r = –0.6). Additionally, 19 PCR primers belonging to three different types of markers (ISSR, SSR, and SCoT) were used to study the population structure and diversity and to investigate the association between different agronomic traits. The total number of bands (TNB) produced by different molecular marker assays was 158. The number of polymorphic bands (PB) ranged from 1 (SCoT3 and SSR14) to 10 (SCoT5, and SCoT35), with a mean of 4.7 bands per primer. The polymorphism percentage (PP) for primers ranged from 14% (SCoT3) to 100% (SSR9, SSR10, and SSR2), with an average of 60% per primer. Thirty-seven molecular markers (7 SSRs, 26 SCoTs, and 4 ISSRs) manifested significant associations with 29 wheat plant traits. Some markers were associated with more than one agronomic trait. These findings could support Saudi Arabian wheat breeding programs by providing several markers associated with agronomic traits that could be used in marker-assisted selection in local wheat accessions.

Keywords: *Wheat, SSR, SCoT, ISSR, Saudi wheat, traits correlation, marker-assisted selection.*

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INTRODUCTION

Wheat is a fundamental nutritional source of calories and proteins for a variety of human populations, which makes it a strategic crop and one of the most important cereal crops (Curtis *et al*., 2002). Global wheat production exceeded 700 million tonnes in 2015/2016, valued over 145 billion dollars per year (Figueroa *et al*., 2018). With distinctive characteristics of cultivars and different end-use quality traits, wheat is adapted to divergent production environments and can fulfill different types of human needs. Additionally, wheat is a major industrial crop and a source of raw material in feed mills, livestock feeds, and human consumptions (Westendorf, 2000). Grain yield is a complicated trait which is mainly controlled by several yield component traits such as spikes per unit area, grains per spike and grain weight. It seems, however, that heavier grains have less chance for genetic improvement in wheat yield (Fischer, 2011). In order to design a well-constructed breeding program by implementing selection strategies for grain yield, important pieces of information regarding the diversity of cultivars and the association between grain yield components must be retrieved. Correlation studies of agronomic traits give a better view toward comprehending the association of different traits with grain yield that could be of great help to wheat breeders (Muhammad *et al*., 2011). Moreover, wheat hypersensitivity to pathogens, pests, and high temperature affect its sustainability, cultivation, and production in areas where it is continuously threatened by environmental changes (Muhammad *et al*., 2011; Skoracka *et al*., 2018). Molecular markers are an irreplaceable tool for genetic improvement of crops and they could be used to investigate the genetic diversity and population structure in the studied genotypes. Additionally, they are efficient tools for marker-assisted selection as well as saving labor, time and financial resources in breeding programs (Ibrahim *et al*., 2016).

Marker-trait association analysis is based on the statistical connection between the specific PCR marker and the observed phenotype. It measures the redundancy of co-occurrence of the observed phenotype in a population, which is nonrandom and more than expected (Munshi and Osman, 2010). This association analysis plays an important part in population genetics in understanding the genetic etiology of complex diseases and traits (Lowe *et al*., 2015). Successful application of this analysis in salt- (Munir *et al*., 2013) and drought- (Quarrie *et al*., 2003) tolerant and disease-resistant crops (Qi *et al*., 2008) has confirmed its incomparable advantages. Advanced molecular marker analysis could be used to investigate the relationship between genes and traits and the correlation between PCR-based molecular markers and gene networks in plants, thus giving crop breeders more control over plant genetic resources with less effort (Alsamman *et al*., 2019; Habib *et al*., 2019). Start codon targeted (SCoT) assay targets the start codon (ATG) and its primer design reduces the efficiency of unwanted and random amplification of genomic regions. Both annealing temperature and primer length do not affect the reproducibility of dominant markers like SCoT (Collard and Mackill, 2009).

The integration of SCoT molecular markers in breeding different plant species such as coconut (Rajesh *et al*., 2015), jojoba (Heikrujam *et al*., 2015), tomato (Abdein *et al*., 2018) and olive (Alsamman *et al*., 2017) has been reported. Inter-simple sequence repeat (ISSR) markers are used to target nucleotide repeats in regions of plant genome containing simple sequence repeats (SSRs). ISSR technique has high repeatability and polymorphism and is suitable for studying intra and inter populational genetic variability in different plant species. Additionally, it has been used to study the genetic diversity in plant species such as barley (Fernandez *et al*., 2002), durum wheat (Alireza *et al*., 2016), tomato and garlic (Abdein *et al*., 2018). The aim of the current paper is to identify those traits which most strongly influence the variation in final yield and the correlation networks of different agronomic traits by means of the analysis of traits in wheat. Furthermore, the identification of PCR markers which are linked to the observed phenotypic variation and could be used for marker-assisted selection provides a better understanding of the genetic structure of wheat plant traits. Moreover, the present study investigates whether the genetic diversity in wheat can be partly explained by ISSR, SSR and SCoT polymorphic markers in order to establish the relationships between different wheat genotypes.

MATERIALS AND METHODS

Plant material: Forty wheat genotypes were collected from the Saudi Arabia region. A total of 36 different morpho-agronomic traits measured were as follows: awn color (AC), awn direction of ear (AD), awn length (AL), awn presence (AP), anthocyanin stain (AS), cross section thickness of the plant leg (CSPL), day to heading (DH), flag leaf anthocyanin (FLA), flag leaf bud (FLB), flag leaf hair (FLH), flag leaf length (FLL), flag leaf width (FLW), grain color (GC), growth rate (GR), grain shape (GS), grain wrinkle (GW), hair density on glume (HDG), leaf color (LC), length of glume lower peak (LGLP), leg length (LL), one thousand seed weight (OTSW), plant height (PH), spike color (SC), seed count on spike (SCS), shape of glume (SG), shape of glume lower peak (SGLP), shape of glume shoulder (SGS), seeds hair (SH), spike length (SL), seeds phenolic color degree (SPCD), spike shape (SS), wax layer of flag leaf (WLFL), wax layer of flag leaf blade (WLFLB), wax layer of leg neck (WLLN), wax layer on the spike (WLS), and wheat seasonal mode (WSM) (Table 1).

DNA extraction and PCR-based molecular marker analysis: DNeasy Plant Mini Kit (Qiagen, New York, NY, USA) was used to extract total DNA. DNA quality and quantity were estimated using gel electrophoresis and DNA samples were stored at –20 °C. A total of two ISSR, twelve SCoT and five SSR primers were used in this study (Table 2). The PCR amplifications were performed in reactions with templates having GC content during the PCR cycles according to Ibrahim *et al*. (2016) and Ahla *et al*. (2019). The final PCR products were stored at 4 °C. The ethidium bromide-stained agarose gel (1.5%), which was used to separate DNA fragments, was documented using the Gel Doc XR system (Bio-Rad, Hercules, CA, USA).

Statistical and genetic analyses: Correlation analysis between agronomic and morphological traits was performed using the R package "Hmisc" (Harrell and Harrell, 2019) and the correlation coefficients (r) were estimated using the "corrplot" (Taiyun *et al*., 2017). The marker-trait association analysis based on F-test statistics was conducted using PowerMarker software (Liu and Muse, 2005). Correlations and associations were considered statistically significant if the p-value was lower than 0.05 ($P < 0.05$). In order to identify the markers linked with different agronomic traits, an online web tool called ClustVis was used to visualize a heat map from the similarity matrix (Metsalu and Vilo, 2015). PCR fragments were scored as present (1) or absent (0). Dice similarity coefficients between different samples were calculated using the unweighted pair group method with arithmetic averages (UPGMA) and they were used for the construction of phylogenetic tree or dendrogram using the Paste software. The population was studied using the STRUCTURE software with burn-in and 100,000 MCMC iterations (Pritchard *et al*., 2000). The results were assessed using STRUCTURE HARVESTER to determine the number of populations (Earl and others, 2012).

RESULTS AND DISCUSSION

Correlation analysis of morpho-agronomic traits: Grain production is a complex phenomenon, involving many contributing parameters, which control grain production, both indirectly and directly. Breeders are naturally interested in exploring the size and type of association between the morpho-agronomic traits (Saima *et al*., 2018). Correlations among plant traits mainly indicate potential trade-offs or allometric relationships in biological processes like carbon gain, support, water uptake, and reproduction that are related to different plant organs (Saima *et al*., 2018). In this study, the correlations among 36 different morpho-agronomic traits in wheat were investigated. Among a total of 25 different correlations that were retrieved, the maximum positive correlation was between PH and LL (r = 0.92), while the maximum negative correlation was observed between GS and GW (r = –0.93). Days to heading (DH) was negatively correlated with flag leaf anthocyanin (FLA) (r = –0.73) and positively correlated with plant height (PH) (r = 0.63) and leaf length (LL) (r = 0.62). Additionally, seed counts on spike (SCS) showed positive correlation with awn color (AC) (r = 0.67) and spike color (SC) (r = 0.61), whereas it was negatively correlated with grain shape (GS) (r = –0.6) (Table 3, Fig. 1). The performance, correlation, and cluster analysis for various quantitative traits including grain yield among the wheat material exotic to Pakistan showed that days to heading (DH) had significant positive correlations with days to maturity (r = 0.7995), spikelets per spike (r = 0.4391), plant height (r = 0.3168), and spike length (r = 0.2696) (Saima *et al*., 2018). Moreover, the study on the heritability and variance components of root traits in wheat under drought stress revealed that the highest correlation between days to heading and grain yield was observed under non-stressed conditions (r = 0.72) (Alsamman *et al*., 2017). Figure 2 shows the correlation coefficients (r) higher than 0.5 among studied traits. The results of correlation network analysis demonstrated the correlations between GS, SH, GW, SC, AC, SS, SCS, and HGD and between PH, LL, DH, and FLA. Furthermore, it showed that the changes in some traits of wheat would affect most traits directly or indirectly.

Molecular marker analysis: All 19 PCR primers produced scorable bands (Figure 3 and Table 3). The total number of bands (TNB) produced by different molecular marker assays was 158. The total number of bands ranged from 2 (SSR14, SSR9, and SSR10) to 14 (SCoT16), with a mean of 8.3 bands per primer. The number of polymorphic bands (PB) varied between 1 (SCoT3 and SSR14) and 10 (SCoT5 and SCoT35) with an average of 4.7 bands per primer. The polymorphism percentage (PP) ranged from 14% (SCoT3) to 100% (SSR9, SSR10, and SSR2), with a mean of 60% per primer. The minor allele frequency (MAF) ranged from 0.383 (SCoT13 and SCoT35) to 0.85 (SSR14 and SSR10) with a mean of 0.383 per primer. The polymorphism information content (PIC) ranged from 0.22 (SSR14) to 1 (SCoT13). In order to study the genetic diversity in some durum wheat genotypes using six SCoT primers (Alireza *et al*., 2016), 54 polymorphic bands with 100% PP were obtained. SCoT markers whose moderate potential to detect genetic variation compared to other molecular markers has been reported, were used for detecting allelic variation among different olive genotypes (Alsamman *et al*., 2017). Moreover, to examine the genetic diversity and population structure of several *Jatropha curcas* L. genotypes using the ISSR assay, 11 ISSR primers were tested and they generated a total number of 307 bands (TNB) and 294 polymorphic bands (PB) (Gomes *et al*., 2018). In barley, the PIC mean value was 0.636, and it ranged from 0.351 to 0.874 (Yong-Cui *et al*., 2005), which confirmed the results of the present study on the high efficiency of ISSR markers in exhibiting high PIC values.

Population structure and genetic diversity: Population structure analysis involves assigning each individual in a population to a cluster and then reporting the number of clusters. This analysis has many applications in genetic studies including grouping individuals, identifying immigrants and inferring the demographic history of populations. There are several approaches to population structure inference, such as principal component analysis (PCA), detrended correspondence analysis (DCA) and allele frequency-based analyses (Lee *et al*., 2009).

Detrended correspondence analysis (DCA) is a multivariate statistical method mainly used by researchers to identify the main factors in large and sparse data matrices that represent the structure of ecological community data (Hill and Gauch, 1980). Figure 4 demonstrates the population structure based on detrended correspondence analysis (DCA). A total of eight accessions including 23, 24, 25, 26, 13, 15, 16, and 36 were separated from the other wheat accessions, forming two separate groups (clusters). Accession 37 was somewhat different from most of the accessions. Principal component analysis (PCA) is a method for reducing the dimension, which uses an orthogonal transformation in exploratory data analysis to visualize genetic distances and relatedness between individuals belonging to different populations (Lee *et al*., 2009). PCA analysis confirmed the predication about the population structure of the wheat accessions based on the detrended correspondence analysis (DCA) (Fig. 5). Additionally, the population structures of two clusters became more condensed and the eight accessions formed one population separated from the other accessions. Population structure analysis by STRUCTURE software is commonly used to infer population structure and to

assign individuals to different clusters given the allele frequencies using Markov Chain Monte Carlo (MCMC) (Pritchard *et al*., 2000). Population structure inference using STRUCTURE software confirmed results obtained using population structure analysis. A set of wheat accessions was divided into two different populations, where 23, 24, 25, 26, 13, 15, 16 and 36 belonged to one population with 85% similarity and the other accessions were clustered into another population. Additionally, the population containing accession 37 with a significantly high score was separated from the others (Fig. 6). The studies of genetic diversity based on the molecular markers were conducted and data, which were transformed into binary form, were used for phylogenetic analysis. Accession 31 with the similarity of 86% was separated from the main group (Figure 7).

Marker-trait association analysis: Statistical analysis of breeding systems coupled with genetic data is an efficient method for the use of a variety of crop species in professional breeding programs (Danail *et al*., 2010). In this study, SSR, SCoT, and ISSR assays produced markers associated with several traits in wheat. Only markers with association scores higher than 0.05 ($-\log_{10}$ (p-value) = 1.3) were considered. A total of 37 molecular markers (7 SSRs, 26 SCoTs, and 4 ISSRs) manifested significant associations with 29 traits in wheat. Association of marker $SCoT3_{170}$ (marker $_{\text{band size}}$) with PH had the highest significance score ($-\log_{10}$ (p-value) = 6.9). Additionally, several markers were associated with more than one trait. The $SCoT33_{650}$ was linked to SCS, SL, AC, HDG, GS, GW, and FLB. However, the $SCoT16_{580}$ was linked to FLL, PL, SGLP, LL, and FLA and the $SCoT2_{600}$ was associated with AC, HDG, GS, GW, and FLA. These markers could be used to target more than one trait and to follow the trait-trait correlation network (Fig. 8, Table 4). Marker-trait association analysis was used to identify SSR markers associated with salt tolerance in chickpea genotypes (Shaimaa *et al*., 2019). By using SSR markers, 40 PCR-based markers associated with 13 different agronomic traits have been identified and reported in *Lotus japonicus*, which could explain the phenotypic variation in stem color from genotypes (Gondo *et al*., 2007). Moreover, 14 traits related to salt-tolerance and 46 trait-associated markers were detected in barley (Elakhdar *et al*., 2016).

Association analysis of different traits in wheat was conducted based on shared trait-associated molecular markers. Figure 9 shows that some traits in wheat formed linked groups, where several traits were clustered into one block with a large number of shared markers. Traits such as SCS, SL, FLB, AC, HDG, DH, AL, GW and GS were grouped into one block, while WLLN, SS, WLS, and LC were clustered into another. Furthermore, GR, FLA, SGLP, FLL, PL, and LL were divided into one cluster, whereas the cluster containing PH and LL had the highest number of shared markers.

Table 1. Plant number (PN), local name (LN) and region (Reg.) for the forty wheat accessions used in this study.

PN	LN	Reg.	PN	LN	Reg.
1	Helba	Barida	21	Baladi	Koara-Elkaseem
2	Maiaa	Barida	22	Maiaa Baladi	Taimaa-Tabok
3	Lokami	Barida	23	Lokami	Koara-Elkaseem
4	Samaa	Tamir	24	Unknown	Abhaa-Aseer
5	Soariak	Tamir	25	Unknown	Aseer
6	Samaa Baladi	Tamir	26	Shokia	Elbaha
7	Lokami Abiad	Tamir	27	Unknown	Tabok
8	Henta Asmr	Tamir	28	Unknown	Tabok
9	Baal	Tamir	29	Unknown	Tabok
10	Bor Baladi	Aldalim	30	Bor Samraa	Najran
11	Molloaha Mokaom	Alehsaa	31	Bor Zarai	Najran
12	Samaa Baladi	Elkharag	32	Bor Henta	Elkaseem
13	Asmr	Najran	33	Hab Bar Atrali	Aseer
14	Somiraa	Elnamas	34	Hab Bar Saib	Aseer
15	Kias	Belsamr-Aseer	35	Unknown	Najran
16	Saib	Elnamas	36	Lokami	Khobraa-Elkaseem
17	Maiaa Baladi	Elnamas	37	Bor	Khobraa-Elkaseem
18	Khobraa-Elkaseem	Khobraa-Elkaseem	38	Kawara	Barida
19	Maiaa	Elbakiria	39	Morgan	Barida
20	Ymani Baladi	Tabok	40	Kasim	Barida

Table 2. Primers name (PN), Sequences were used in this study.

PN	Sequence	PN	Sequence
ISSR-14	5'-CTCCTCCTCCTCCTCTT-3'	SCoT-2	5'-CAACAATGGCTACCACCC-3'
ISSR-15	5'-CTCTCTCTCTCTCTCTRG-3'	SCoT-3	5'-CAACAATGGCTACCACCG-3'
SSR-2	F. 5' TCATTGGTAATGAGGAGAGA-3'	SCoT-4	5'-CAACAATGGCTACCACCT-3'
	R. 5' GAACCATTCATGTGCATGTC-3'	SCoT-5	5'-CAACAATGGCTACCACGA-3'
SSR-9	F. 5' AATTTCAAAAAGGAGAGAGA-3'	SCoT-11	5'-AAGCAATGGCTACCACCA-3'
	R. 5' AACATGTGTTTTTAGCTATC-3'	SCoT-12	5'-ACGACATGGCGACCAACG-3'
SSR-10	F. 5' AAGATGGACGTATGCATCACA-3'	SCoT-13	5'-ACGACATGGCGACCATCG-3'
	R. 5'GCCATATTTGATGACGCATA-3'	SCoT-14	5'-ACGACATGGCGACCACGC-3'
SSR-14	F. 5' CGACCCGGTTCACTTCAG 3'	SCoT-16	5'-ACCATGGCTACCACCGAC-3'
	R. 5' AGTCGCCGTTGTATAGTGCC 3'	SCoT-20	5'-ACCATGGCTACCACCGCG-3'
SSR-16	F.5 GCGGGTCGTTTCCTGGAAATTCATCTAA 3'	SCoT-33	5'-CATGGCTACCACCGGCCC-3'
	R. 5' GCGAAATGATTGGCGTTACACCTGTTG 3	SCoT-35	5'-CCATGGCTACCACCGCAG-3'

Table 3 The significant correlation scores (r) between different wheat traits.

Trait	Trait	R	Trait	Trait	R	Trait	Trait	r
SG	SGS	0.63^{*}	SCS	AC	0.67^{**}	GS	SS	-0.67^{**}
LL	DH	0.62^{*}		SC	0.61^{*}		SCS	-0.6^{*}
	PL	0.92^{***}		GS	-0.6^{*}		AC	-0.84^{***}
PL	FLA	-0.61^{*}	HDG	AC	0.82^{***}		HDG	-0.68^{**}
	DH	0.63^{*}		GS	-0.68^{**}		SC	-0.9^{***}
	LL	0.92^{***}		GW	0.63^{*}		GW	-0.93^{***}
GW	SS	0.62^{*}	SGS	SG	0.63^{*}	AL	AP	0.6^{*}
	AC	0.79^{***}	AC	SCS	0.67^{**}	SH	SC	-0.6^{*}
	HDG	0.63^{*}		HDG	0.82^{***}			
	SC	0.83^{***}		SC	0.73^{***}			
	GS	-0.93^{***}		GS	-0.84^{***}			
WLFLB	WLLN	0.61^{*}		GW	0.79^{***}			
SS	SC	0.75^{***}	DH	FLA	-0.73^{***}			
	GS	-0.67^{**}		PL	0.63^{*}			
	GW	0.62^{*}		LL	0.62^{*}			
SC	SS	0.75^{***}	AP	AL	0.6^{*}			
	SCS	0.61^{*}	WLS	WLLN	0.67^{**}			
	AC	0.73^{***}	FLA	DH	-0.73^{***}			
	GS	-0.9^{***}		PL	-0.61^{*}			
	GW	0.83^{***}	WLLN	WLFLB	0.61^{*}			
	SH	-0.6^{*}		WLS	0.67^{**}			

* significant

Table 4. primer name (PN), total number of PCR bands (TB), monomorphic bands (MB), polymorphic bands (PB), marker allele frequency (MAF) and polymorphism information content (PIC).

PN	TB	MB	PB	PP	MAF	PIC
ISSR14	8	5	3	0.38	0.55	0.62
ISSR15	9	3	6	0.67	0.28	0.87
SCoT11	11	5	6	0.55	0.2	0.88
SCoT12	11	5	6	0.55	0.18	0.92
SCoT13	11	2	9	0.82	0.08	0.96
SCoT14	13	9	4	0.31	0.55	0.65
SCoT16	14	8	6	0.43	0.23	0.86
SCoT2	11	5	6	0.55	0.18	0.89
SCoT20	11	6	5	0.45	0.33	0.82

SCoT3	7	6	1	0.14	0.7	0.33
SCoT33	7	3	4	0.57	0.18	0.89
SCoT35	12	2	10	0.83	0.08	0.95
SCoT4	6	3	3	0.5	0.43	0.63
SCoT5	13	3	10	0.77	0.13	0.95
SSR10	2	0	2	1	0.85	0.24
SSR14	2	1	1	0.5	0.85	0.22
SSR16	5	1	4	0.8	0.28	0.82
SSR2	3	0	3	1	0.75	0.37
SSR9	2	0	2	1	0.45	0.55
Total	158	67	91	11.8	7.28	13.4
Mean	8.3	3.5	4.7	0.62	0.38	0.70

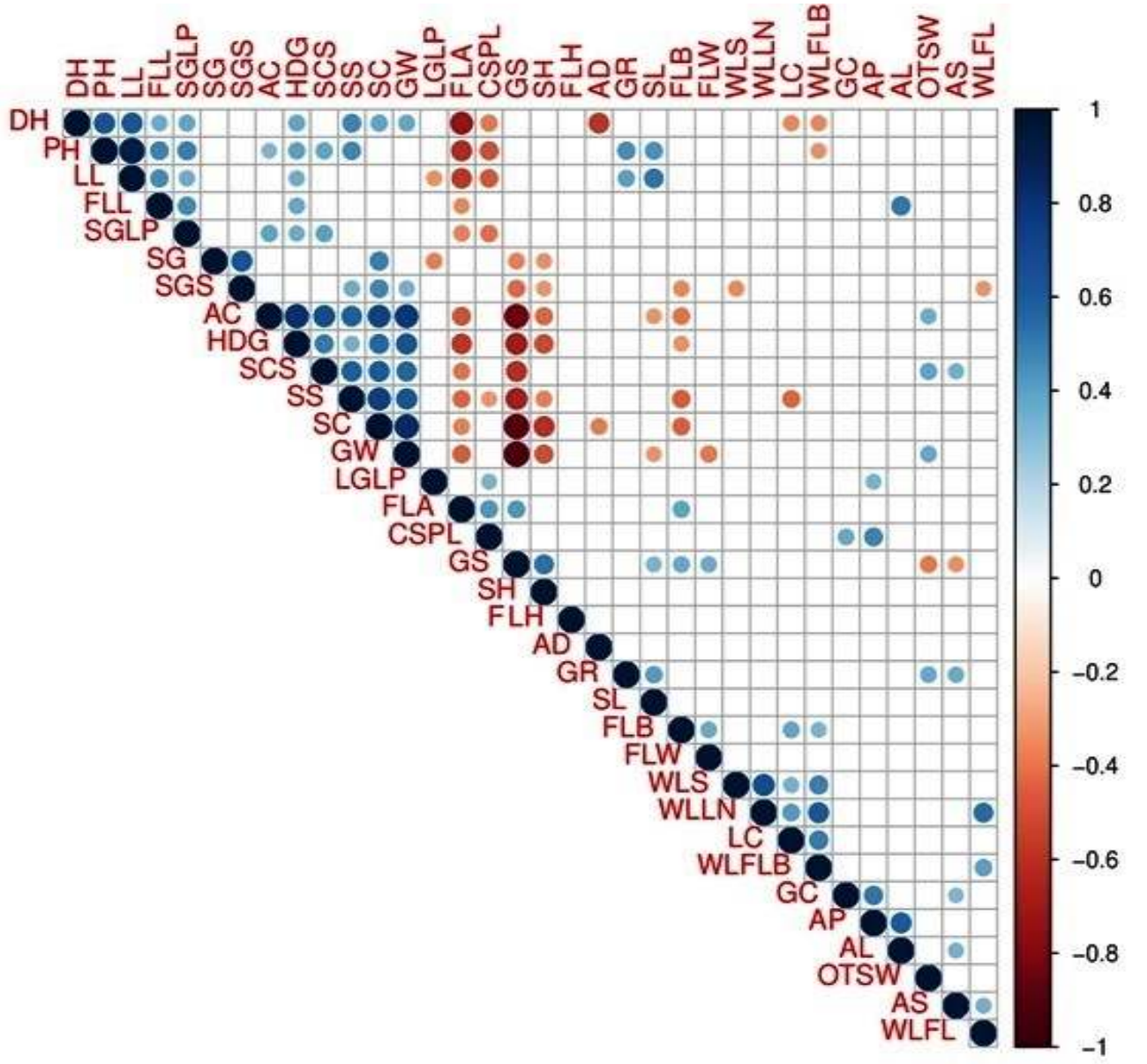

Fig. 1. The significant correlation analysis of r between different morpho-agronomic traits of wheat accessions. The blue and red circles indicate positive and negative correlation, respectively. The circles size is relative to correlation values.

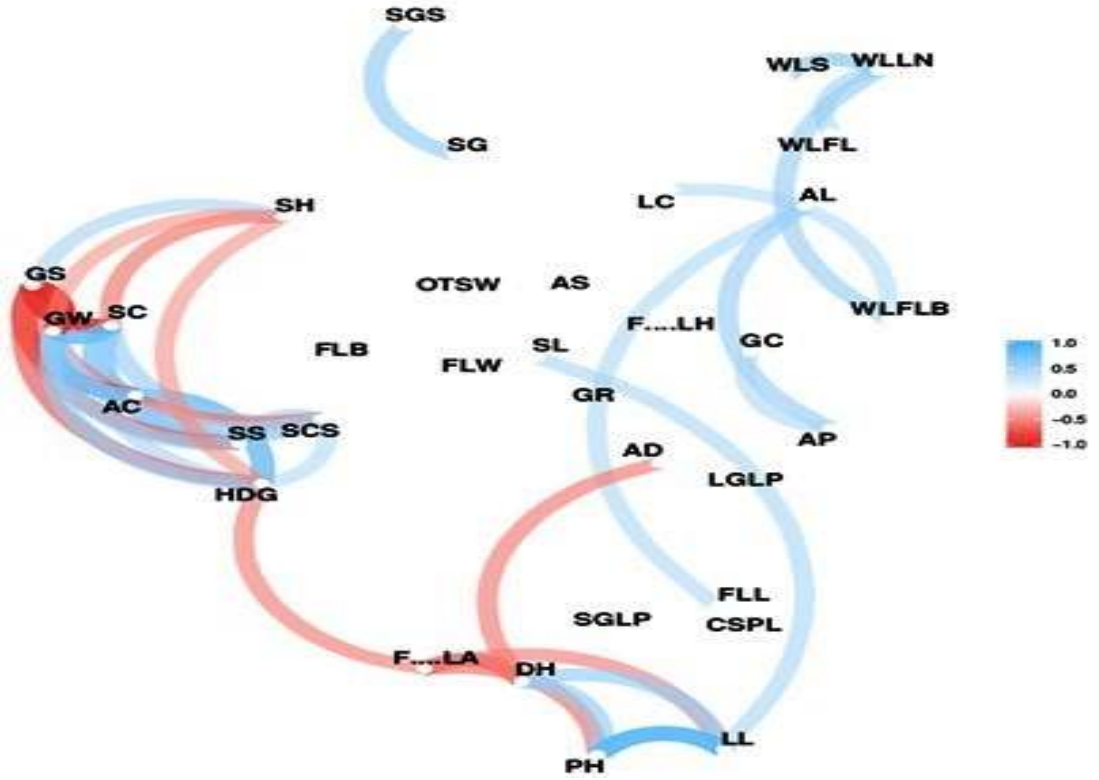

Fig. 2. The correlation network between different wheat morpho-agronomic traits, where of r > 0.5. The blue and red links indicates positive and negative correlation, respectively.

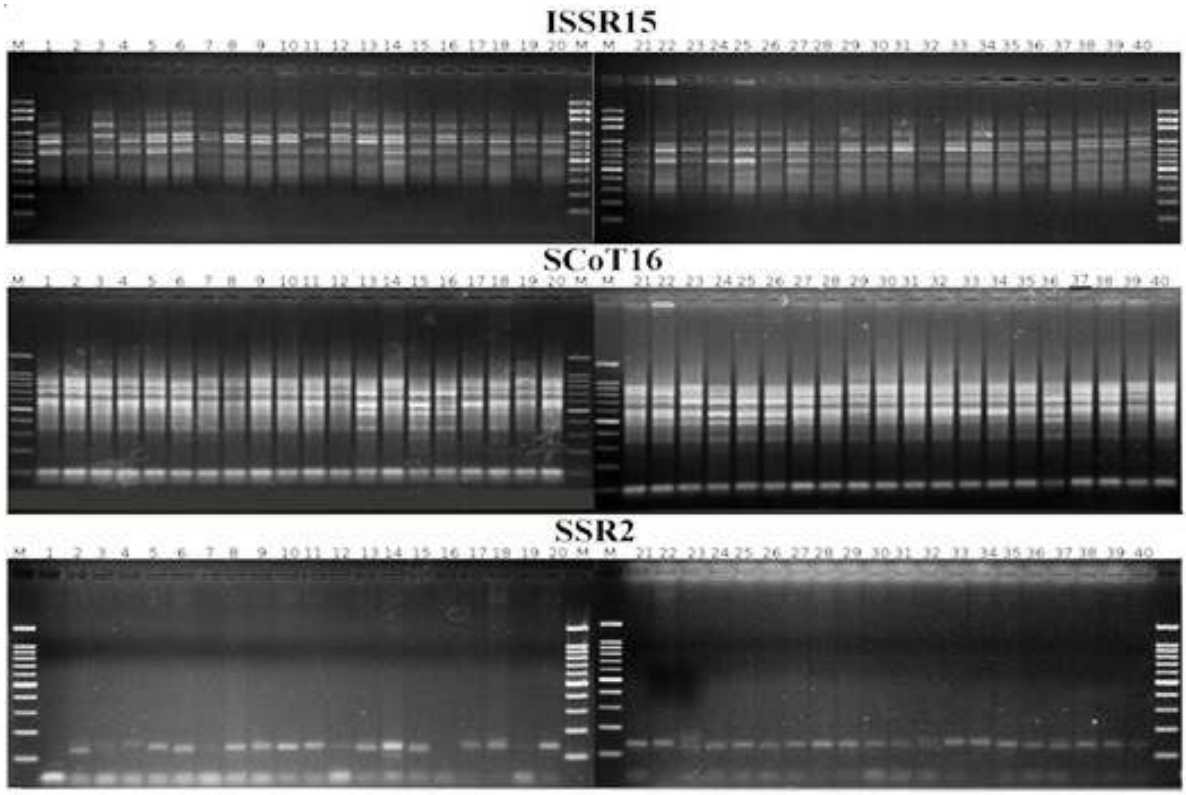

Fig. 3. The gel electrophoresis for the 40 wheat accessions analyzed using three different PCR primers.

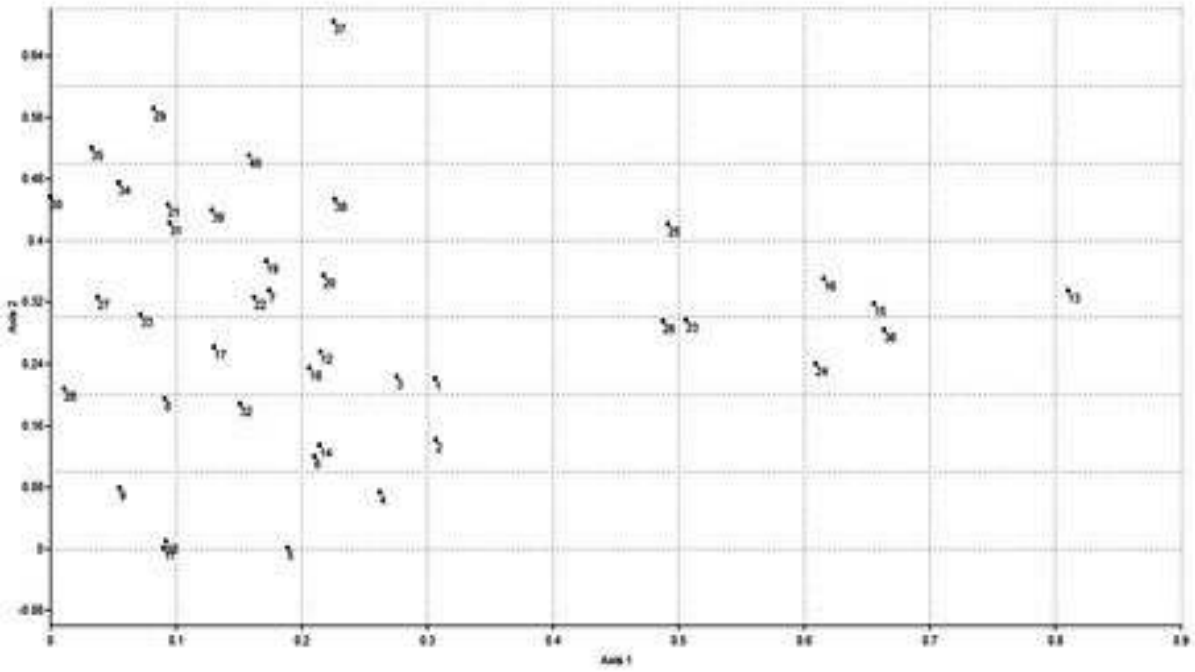

Fig. 4. The DCA-based population structure analysis for the 40 wheat accessions used in this study.

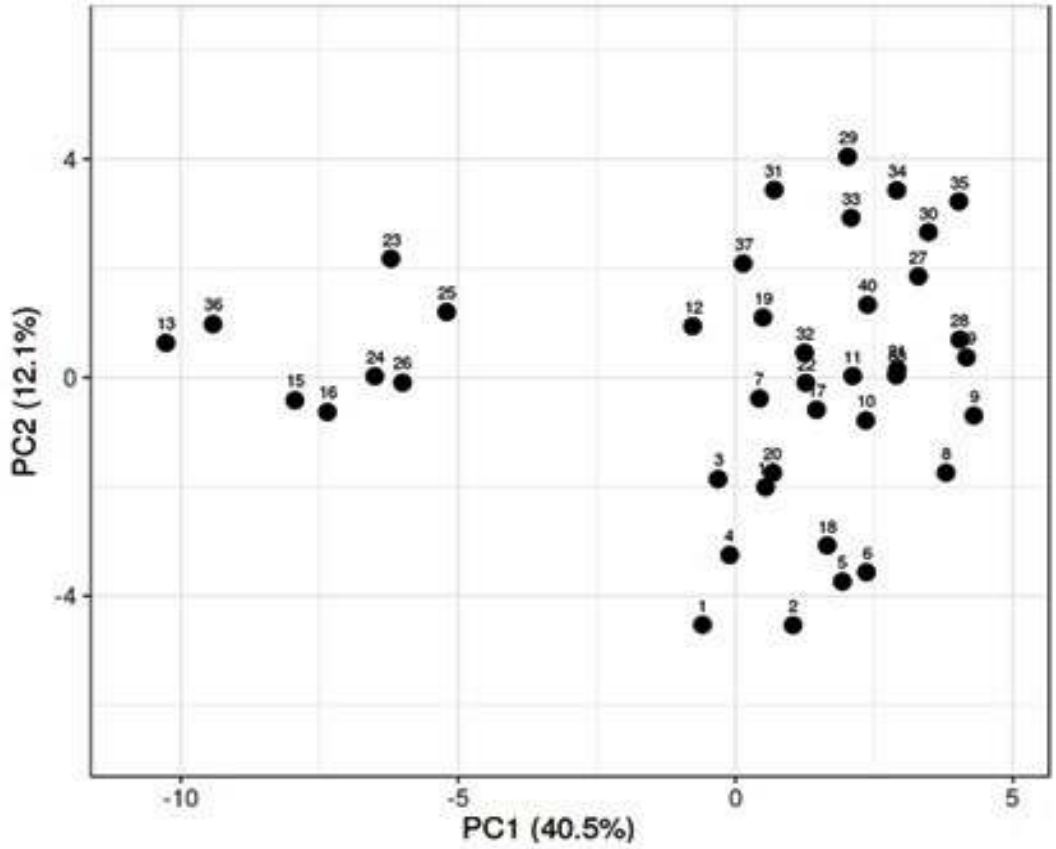

Fig. 5. The PCA-based population structure analysis for the 40 wheat accessions used in this study.

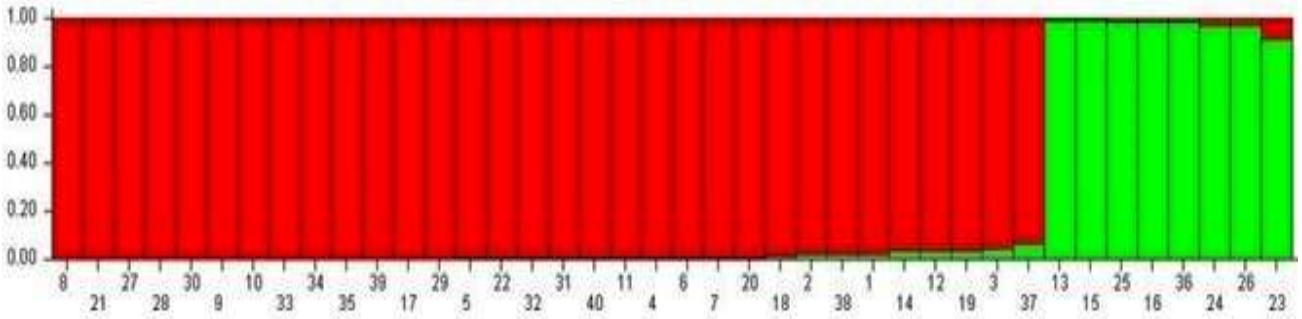

Fig. 6. The alleles frequencies-based population structure analysis using STRUCTURE software for the 40 wheat accessions used in this study.

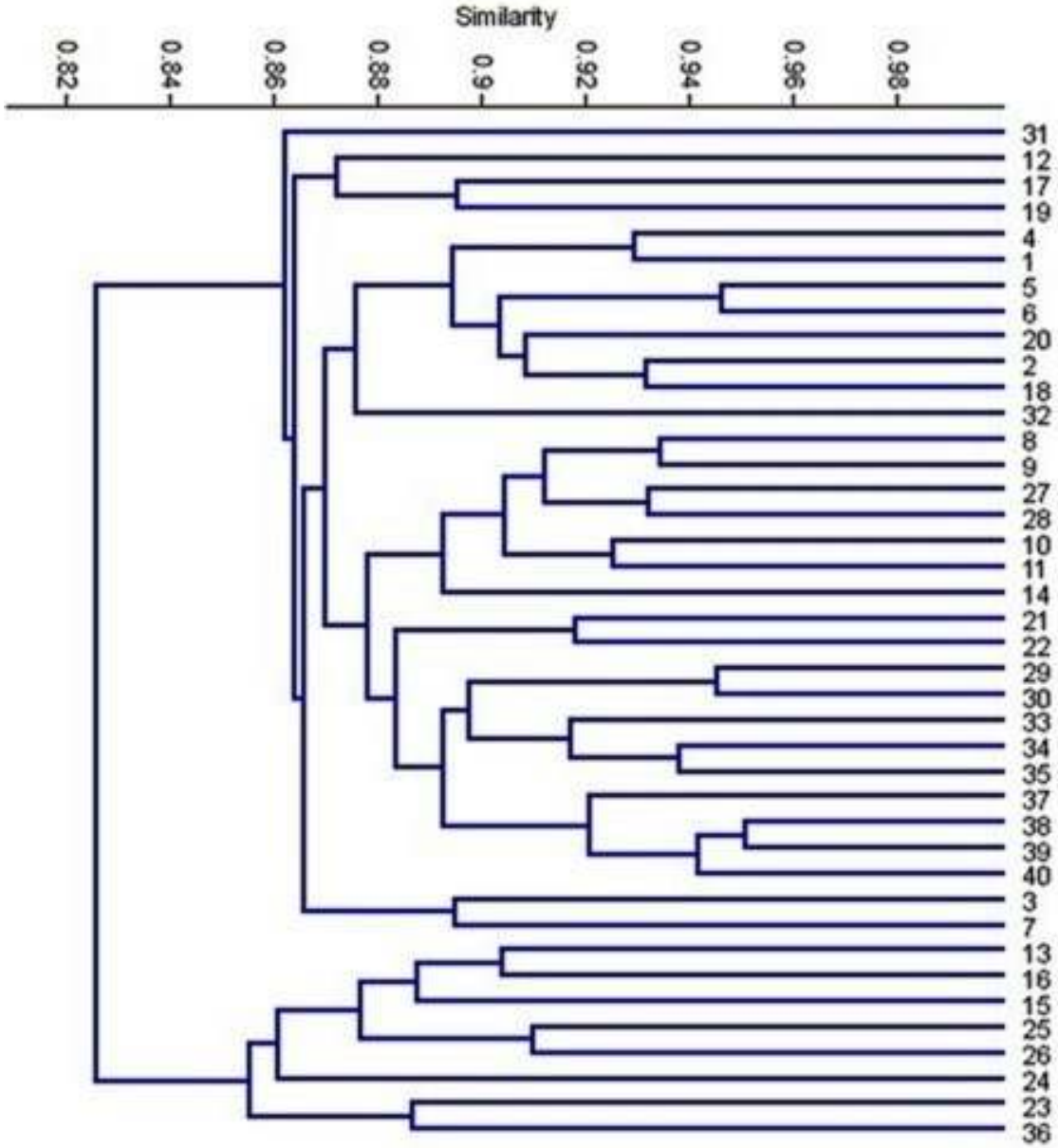

Fig. 7. The phylogenetic tree of the 40 wheat accessions constructed using binary data retrieved from ISSR, SSR and SCoT marker assays.

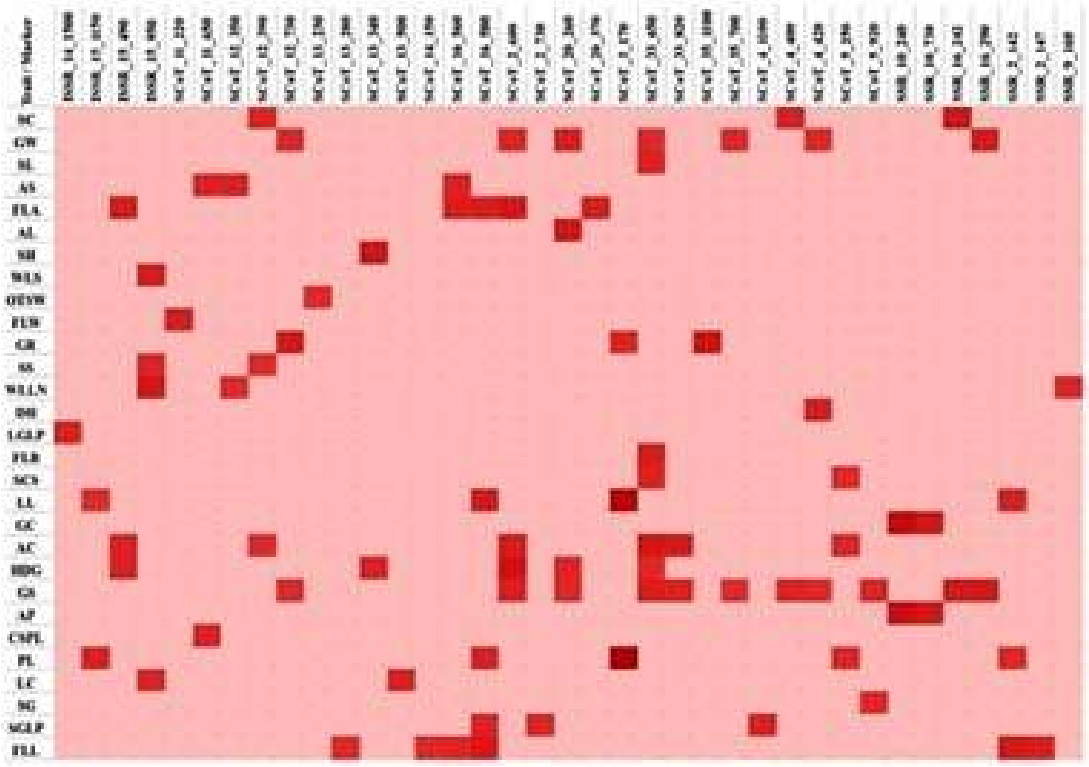

Fig. 8. The multiple traits controlling markers, the red color concentration is relative to the p-value score (highest scores have darker red colors).

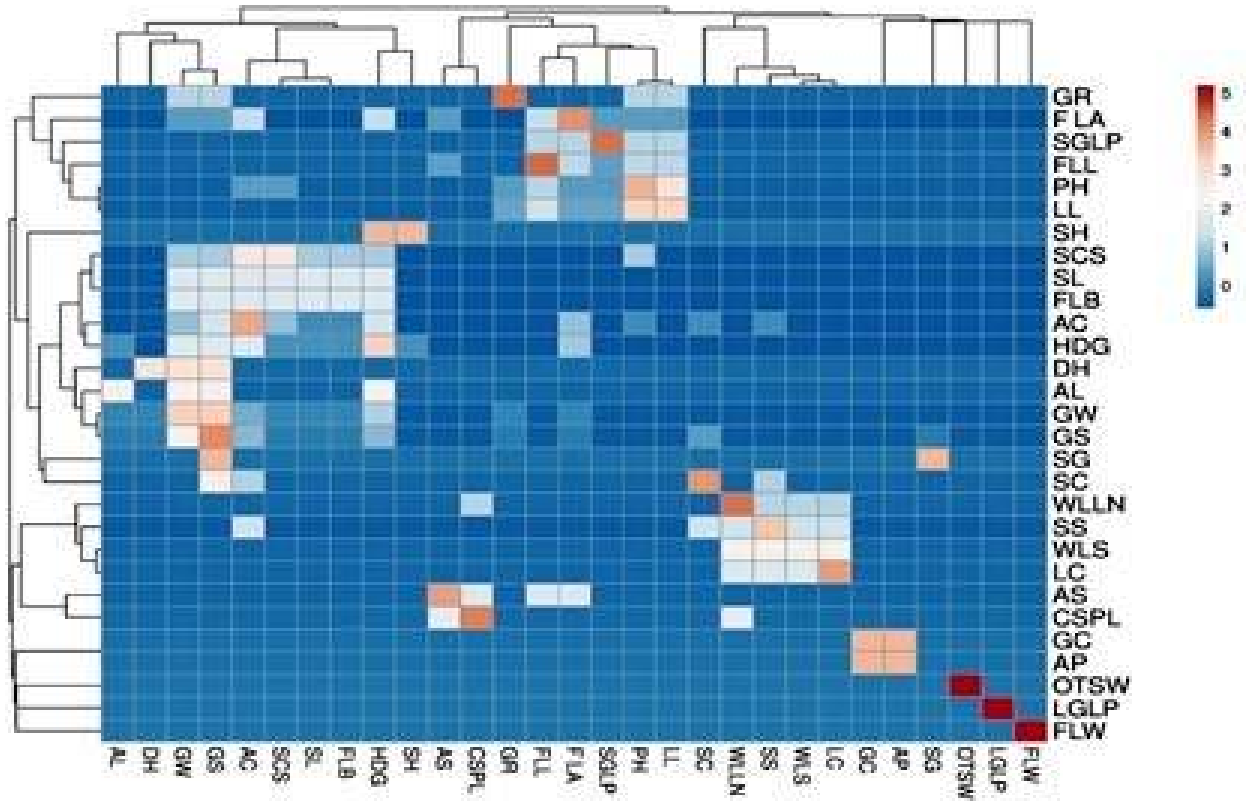

Fig. 9. Heatmap for significant shared markers between traits, the blue and red color scale (left side) is relative to the number of shared markers.

Conclusion: Statistical associations between different morpho-agronomic traits of some wheat genotypes grown in Saudi Arabia were studied in order to estimate the hidden correlation network and to detect few trait-associated markers which could be used in marker-assisted selection. The studies on associations between traits in wheat revealed a robust correlation between DH and FLA, PH and LL, SCS and AC, and SC and GS. These correlations could be used to study the impact of traits on wheat yield and the effect of the genotypic variation in wheat. Moreover, by using different molecular markers, several markers associated with different wheat traits were identified. These markers could be used in national wheat breeding programs for developing and selecting the most adaptive and productive genotypes.

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