Genetic variation of Saudi Wheat Genotypes through ISSR and SCoT Assays

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ABSTRACT

Agriculture is responsible for food security against the massive increase in the global population, where wheat stands as a prime source of calories and protein in human nutrition. A well-designed selection breeding program for grain yield depends on the retrieved information of the genetic diversity and agronomic traits association. In this study, thirteen Saudi local wheat genotypes have been collected from Al-Qassim Region in Saudi Arabia. About 34 different morpho-agronomic traits were measured. The correlation analysis between different wheat agronomic traits was observed to study the trait-trait network. Also, 21 SCoT and ISSR primers have been used for fingerprinting of selected wheat genotypes. The marker-trait association analysis was used to detect trait-linked PCR markers. In this research, we noticed a strong negative correlation with grain shape and spike color (SC) while, plant length (PH) had a strong positive correlation with day to heading. Both ISSR and SCoT assays were also adopted and eighty-five PCR markers were significantly linked with 32 local Saudi wheat traits. The SCoT assay was produced 44 markers, while ISSR produced 41 markers respectively. The GW had the highest number of linked markers (17) followed by GR and FLA. Concerning, marker-trait association analysis, the p-value (-log10) of significance associated markers ranged from 1.32 to 4.13. This study could provide fundamental information for local breeding programs of agronomically important traits in Saudi kinds of wheat.

Key Words: *Wheat; morpho-agronomic traits; SCoT and ISSR assays.*

eIJPPR 2019; 9(6):57-65

HOW TO CITE THIS ARTICLE: Mohammed Ali Alshehri (2019). "Genetic variation of Saudi Wheat Genotypes through ISSR and SCoT Assays", International Journal of Pharmaceutical and Phytopharmacological Research, 9(6), pp.57-65.

INTRODUCTION

Wheat is one of the most important economic cereals in the worldwide and a cheap source of multiple nutrientsfor human consumption [1]. More than 700 million tons of wheat have been produced globally in 2015/2016, which could be translated in approximately 145 billion $ [2]. In addition to human consumption, wheat is an important industrial by-product, life stock feeds and its stalks are used in mulch and construction material [3]. Wheat breeding for different end-use quality traits and a wide range of adaptive characteristics developed distinctive cultivars for a divergent production environment [4].

Agriculture is responsible for food security against the massive increase in the global population. Wheat grain yield is a complex trait and highly effected by a variety of genetic and environmental factors [5]. A well-designed selection of a breeding program for grain yield depends on the retrieved information of the genetic diversity and agronomic traits associated with grain yield [5]. For instance, Ali et al. [6] stated that correlation studies provide a better way that helps wheat breeders to understand the association of different traits with grain yield. Additionally, wheat sensitivity to high temperature, pests, and pathogens affecting its production and sustainability and its cultivation is steadily threatened by environmental changes [7, 8]. Concerning, spike characteristics, including total spikelet number, fertile spikelet number per spike, spike compactness, and grains per spikelet determine the yield potential [9]. These characteristics are quantifiable traits and subject to ecological [10]. All parts of the wheat spike, such as the glume, awn, lemma, pericarp, palea, and peduncle, are capable of photosynthesis and are a considerable part of grain mass [11].

There are growing appeals for genetic yield improvement in wheat, where molecular markers stand as an efficient tool for providing information about the genetic diversity

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 10 July 2019; **Revised:** 28 November 2019; **Accepted:** 02 December 2019

and structure [12]. Moreover, its successful application in a marker-trait association such as drought [13], salinity [14]; tolerance and disease resistance [15]. Additionally, the association between plant gene network and molecular markers could be detected through advanced bioinformatics analysis that could help in the understanding of trait-gene relationship [16, 17].

Start Codon Targeted (SCoT) markers are PCR-based marker assay targets the gene start codon (ATG), where these nucleotides are included in the primer structure. This design system provides the ability to reduce random and unintended amplification for untranslated genomic areas. SCoT assay PCR profiles revealed dominant markers, where the primer length and temperature of annealing are not the main factors influencing reproducibility [18]. This marker assay has been successfully integrated into different plant species e.g. jojoba [19]; coconut [20]; olive [21]; and tomato [22].

Moreover, the plant genome richness with simple sequence repeats (SSR) provides the ability to design PCR markers that could be used to target the genomic regions with high variability. Inter-simple sequence repeats (ISSR) marker was designed to target nucleotide repeats inside SSRs, and it has high polymorphism and repeatability. ISSR is a suitable tool for studying the genetic variability of different plant species [23]; barley [24]; garlic [25]; and tomato [22].

In this research, e investigated whether the wheat agronomic trait variability can be partly explained by ISSR and SCoT markers polymorphism. We used ISSR and SCoT marker assays to study the diversity of selected wheat genotypes, a relationship between these markers and wheat morpho-agronomic traits and the relationship among different agronomic traits. Overall, his study could make a significant contribution to wheat programs,particularlyin developed countries.

MATERIAL AND METHODS

Plant material

Thirteen seeds of wheat were collected from the different eco-geographical region of Al-Qassim, Kingdom of Saudi Arabia and then it was conserved under associated code numbers at the Plant Gene bank in National Agriculture & Animal Resources Research Center, Ministry of Environment Water & Agricultures (Riyadh, Saudi Arabia) (Table 1). About 34 different morpho-agronomic trait have been taken as follow; anthocyanin stain (AS), growth rate (GR), leaf color (LC), flag leaf anthocyanin (FLA), flagleaf hair (FLH), flag leaf bud (FLB), day to heading (DH), wax layer of flag leaf (WLFL), wax layer of flag leaf blade (WLFLB), wax layer on the spike (WLS), wax layer of leg neck (WLLN), flag leaf length (FLL), flag leaf width (FLW), plant height (PH), cross section thickness of the plant leg (CSPL), spike shape (SS), seeds count on spike (SCS), spike length (SL), awn presence (AP), awn length (AL), awn color (AC), awn direction of ear (AD), hair density on glume (HDG), spike color (SC), shape of glume (SG), shape of glume shoulder (SGS), length of glume lower peak (LGLP), shape of glume lower peak (SGLP), leg length (LL), grain color (GC), grain shape (GS), grain wrinkle (GW), seeds hair (SH), and the one thousand seed weight (OTSW).

Table 1: List of thirteen seeds of wheat were collected from Al-Qassim Region in Saudi Arabia and their associated code numbers

S. No	Sample code	Popular name
1	6	Helpa
2	7	Maia1
3	8	Lukaimi1
4	317	Kasim1
5	323	Maia2
6	553	Baladi
7	569	Lukaimi2
8	839	Hinta
9	977	Lukaimi3
10	978	Bor
11	983	Kuara
12	984	Mrgan
13	988	Kasim2

DNA extraction and PCR-based molecular marker analysis

The total DNA was retrieved by using the DNeasy Plant Mini Kit (Qiagen, New York, NY, USA). The quality and quantity of the DNA were detected using gel electrophoresis and stored at –20°C. In this research, Ten SCoT primers were used (Table 2). The PCR reaction and amplification program was done as outlined by Ibrahim et al. [12]. Eleven ISSR markers were applied during this study, the PCR reaction content and ISSR amplification program were done according to the method ofAwad et al. [26]. The PCR reactions were stored at 4°C. The ISSR and SCoT PCR fragments were separated on an 8% agarose gel. Afterward, the gel was stained with ethidium bromide and then was photographed using the Gel Doc XR system (Bio-Rad, Hercules, CA, USA).

Table 2: The primers and sequence for ISSR and SCoT primers

Primer Name	Sequence	Primer Name	Sequence
S1	5'-AGAGAGAGAGAGAGAGYC-3'	SCoT-7	5'-ACAATGGCTACCACTGAC-3'
S2	5'-AGAGAGAGAGAGAGAGYG-3'	SCoT-8	5'-CAATGGCTACCACTACAG-3'
S3	5'-ACACACACACACACACYT-3'	SCoT-9	5'- ACAATGGCTACCACTGCC-3'
S4	5'-ACACACACACACACACYG-3'	SCoT-10	5'-ACAATGGCTACCACCAGC-3'
S5	5'-GTGTGTGTGTGTGTGTYG-3'	SCoT-15	5'-CCATGGCTACCACCGGCT-3'
S6	5'-CGCGATAGATAGATAGATA-3'	SCoT-17	5'-CCATGGCTACCACCGGCA-3'
S7	5'-GACGATAGATAGATAGATA-3'	SCoT-19	5'-CCATGGCTACCACCGGCG-3'
S8	5'-AGACAGACAGACAGACGC-3'	SCoT-20	5'-ACCATGGCTACCACCGCG-3'
S9	5'-GATAGATAGATAGATAGC-3'	SCoT-22	5'-CCATGGCTACCACCGCAC-3'
10	5'-GACAGACAGACAGACAAT-3'	SCoT-23	5'-CATGGCTACCACCGGCCC-3'
S11	5'-ACACACACACACACACYA-3'		

Statistical and genetic analyses

The R packages "Hmisc" [27] has been used for agronomic and morphological traits correlation analysis and R^2 visualization [28]. The F-test was also conducted for the marker-trait association through power marker software [29]. The *p*-value for significant correlation and association was $p< 0.05$. The online web tool ClustVis was used to visualize shared linked-markers between different agronomic and morphological traits through a heat map.

PCR fragments were counted as a present (1) or absent (0) for all samples. Dice's similarity matrix coefficients were calculated between different samples using the unweighted pair group method with arithmetic averages (UPGMA) and this matrix was used to construct a phylogenetic dendrogram using Dendro UPGMA (http://genomes.urv.es/UPGMA/).

RESULTS AND DISCUSSION

Morpho-agronomic traits correlation analysis

The present results showed that the correlation studies of different plant traits have a pivotal role in indirect genotypes selection for higher yield [30]. The correlative effects among yield components were misled consideration between simple and component yield breeding programs [31]. Indeed, high yielding cultivar selection using specific traits demands information of several compensation mechanisms between yield counterparts developed by changing management, genotypic and environmental factors [32]. The different wheat organ coloration has an adaptive significance and can be used in taxonomy, varietal certification and as a suitable model for molecular genetic researches [33]. The present results highlighted that spike color (SC) had a strong negative correlation with grain shape meanwhile positive correlation with growth rate (GR), awn color (AC), seeds count on spike (SCS) and spike shape (SS) respectively. On the other hand, it was noticed that a weak positive correlation with important traits such as day to heading (DH), seeds count on spike (SCS) and one thousand seeds weight (OTSW) (**Figure 1**). The SC and AC trait is an efficient descriptor for a varietal identity for farmers and the principal component analysis (PCA) as they can be used as quantitative parameters [34]. The significant positive or negative correlation of SC with other traits could be used as morphological markers for the identification of different wheat cultivars [35].

Figure 1 shows a plant length (PH) had a strong positive correlation with day to heading [36] (**Figure 1**). Li et al. [36]. Additionally, some quantitative trait loci (QTLs) control the interaction between PH and HD, where it could improve grain yield, this has been reported in rice [37].

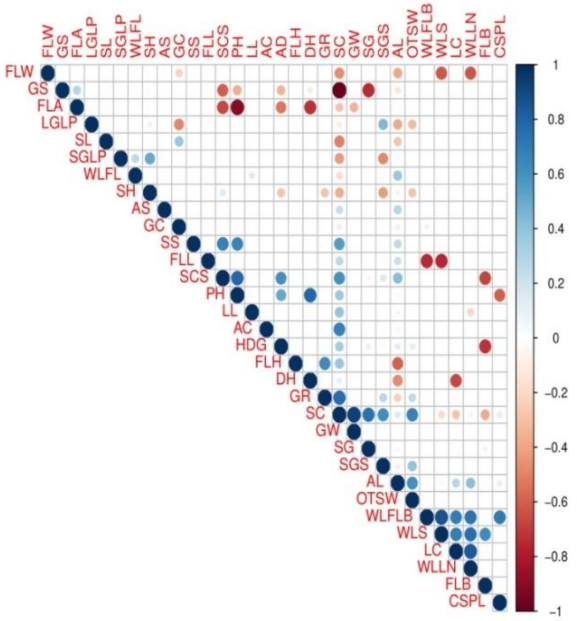


Fig. 1: The significant correlation analysis of R^2 between different morpho-agronomic traits of wheat genotypes;the blue and red circle indicates a positive and negative correlation. The circles' size is relative to correlation values.

Molecular marker analysis

The results showed thatall used primers exhibited visible and scorable fragments (**Figure 2 and Table 3**). SCoT marker assays target genomic sequences with the "ATG" nucleotide sequence. Ten SCoT primers were used and it elucidated 158 total number of bands (TNB) ranged from 12 (SCoT22 and SCoT23) to 19 (SCoT8 and SCoT20) with an average of 15.8 bands/primer. For example, fifteen SCoT primers were used to assess the genetic diversity among Iranian wheat germplasm and the TNB was 116 with an average of 11.06 bands/primer respectively [38].

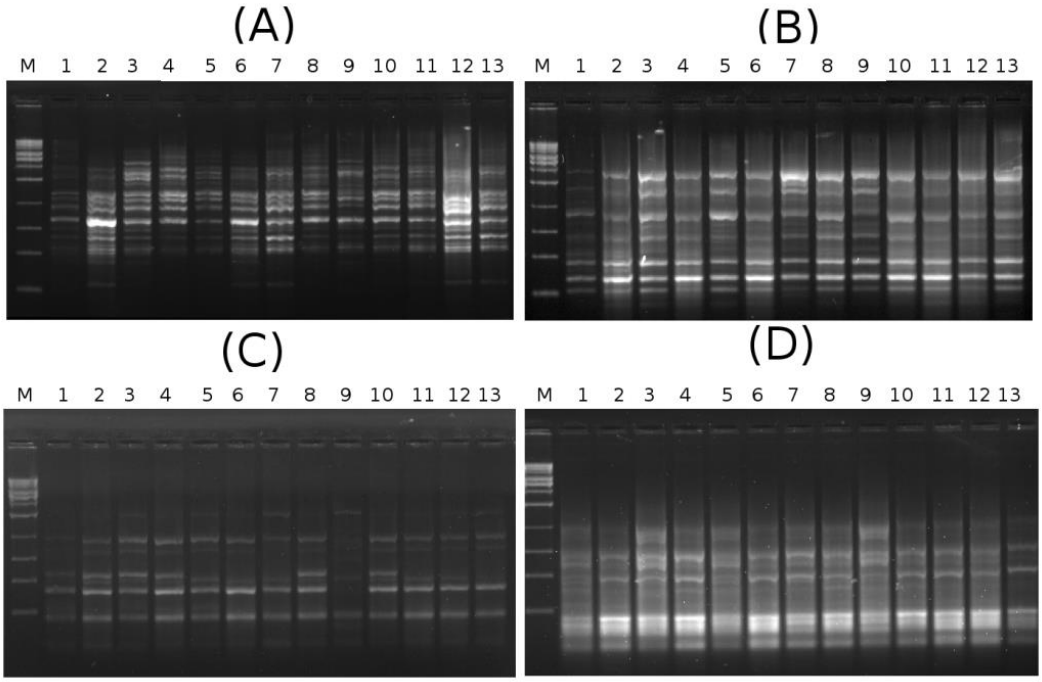


Fig. 2: SCoT and ISSR profiles of the 13 wheat cultivar as revealed by primers SCoT-7 (A), SCoT-8 (B), S-2 (C) and S-6 (D); Lanes 1 to 13; M: DNA molecular weight marker (1kb Ladder)

The present investigation showed that SCoT primers were generally produced polymorphic PCR bands demonstrated the genetic variation among different wheat cultivars, the number of polymorphic bands (PB) ranged from 0 (SCoT22) to 11 (SCoT20) with an average of 5.6 bands/primer. The ratio of polymorphic bands (PP) ranged from 0 (SCoT22) to 57.9% (SCoT20) with an average of 35.4%. Similarly, Etminan *et al.* [23] tested SCoT primers for genetic diversity of durum wheat genotypes and they found out 54 polymorphic bands with 100%.

Concerning polymorphism information content (PIC) score measures the ability of PCR primers to detect polymorphism among different individuals with ranges from 0 to 1.The SCoT primers PIC values ranged from 0.1319 (SCoT22) to 0.9176 (SCoT20) with a mean of 0.7354 indicating a high ability to detect allelic diversity among different wheat genotypes. For instance, Alsamman *et al.* [21] reported the use of SCoT assay to detect allelic variation among different genotypes of olives. Further, Etminan *et al.* [23] noted that the effective number of alleles (Ne) and Nei's gene diversity (He) were higher in landraces when compared to breeding lines.Also, we noticed the marker allele frequency (MAF) ranged from 0.0769 (SCoT20) to 0.9231 (SCoT22), indicating the effectiveness of SCoT to detect genotypes divergence. Moreover, some unique bands were also successfully detected using SCoT assays and the number of unique bands (UB) was ranged from 0 (SCoT15, SCoT20, SCoT7, SCoT19,and SCoT8) to 2 (SCoT10) respectively (**Table 3**).

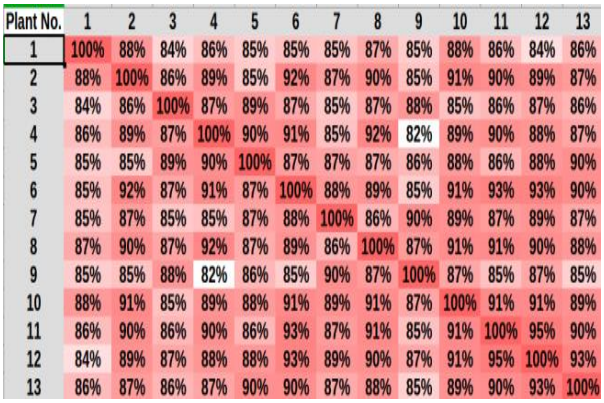
In this present research, ISSR assays were successfully used to detect the divergence among wheat genotypes. ISSR primers produce TNB ranged from 4 (S11) to 17 (S9), whereas the PB was 58 bands, ranged from 0 (S5, S11, S4) to 11 (S1, S9) respectively. For example, ISSR assay was used to assist the population structure and genetic diversity of *Jatropha curcas* and 307 bands TNB and 294 bands of PB were generated by ISSR primers [39]. The ISSR PIC ranged from 0 (S5 and S4) to 0.9176 (S1) with a mean of 0.65 and MAF ranged from 0.0769 (S1) to 1 (S5 and S4) with a mean of 0.41, indicating a high ability of ISSR to detect allelic polymorphism among different wheat genotypes. Hou et al. [40] studied the genetic diversity of barley by using ISSR assay with the mean of PIC was 0.636. Additionally, the ISSR assay was also produced UBs ranged from 1 (S1, S2, S9) to 2 (S8) (**Table 3**).

In the present study, we observed the genetic similarity analysis among 13 wheat genotypes. The results of combined binary data of SCoT and ISSR assays, indicating

the genetic similarity ranged from 82% (Lukaimi3 and Kasim1) to 95% (**Figure 3**). The phylogenetic tree was constructed using SCoT and ISSR combined data showed the genetic diversity relationship among selected wheat genotypes (**Figure 4**). Similarly, Manivannan et al. [41] highlighted the genetic diversity and phylogenetic relationship of *Dianthus caryophyllus* germplasm using ISSR and RAPD markers. The phylogenetic tree represents Helpa separated as one cluster. Then a revolutionary level, the genotypes Lukaimi2 and Lukaimi3 were grouped as another cluster, followed by Lukaimi1 and Maia2. Finally, the other genotypes such as Kasim1, Hinta, Kasim2, Baladi, Kuara, Morgan, Maia1 and Bor were separated as one group.

Table 3. The primer name (PN), major allele frequency (MAF), polymorphism information content (PIC), total number of bands (TNB), polymorphic bands count (PB), polymorphism (%) (PP %), monomorphic bands count (MB) and unique bands (UB) for ISSR and ScoT molecular markers

PN	MAF	PIC	TNB	MB	PB	UB	PP
S1	0.0769	0.9176	13	2	11	1	84.6
S10	0.2308	0.8348	13	3	10	0	76.9
S11	0.3077	0.7791	4	0	0	0	0
S2	0.2308	0.8496	10	4	6	1	60
S3	0.3077	0.8092	9	4	5	0	55.6
S4	1	0	8	8	0	0	0
S5	1	0	10	10	0	0	0
S6	0.3077	0.7791	14	9	5	0	35.7
S7	0.5385	0.5758	9	6	3	0	33.3
S8	0.1538	0.8769	12	5	7	2	58.3
S9	0.3846	0.7727	17	6	11	1	64.7
Mean	0.41	0.65	10.82	5.18	5.27	0.45	48.7
SCoT10	0.3846	0.6938	17	10	5	2	29.4
SCoT15	0.6154	0.5686	13	10	3	0	23.1
SCoT17	0.3077	0.8395	18	8	9	1	50
SCoT19	0.3846	0.7727	14	10	4	0	28.6
SCoT20	0.0769	0.9176	19	8	11	0	57.9
SCoT22	0.9231	0.1319	12	11	0	1	0
SCoT23	0.3846	0.7563	12	8	3	1	25
SCoT7	0.1538	0.9043	16	10	6	0	37.5
SCoT8	0.2308	0.8781	19	12	7	0	36.8
SCoT9	0.1538	0.8907	18	9	8	1	44.4
Mean	0.3615	0.7354	15.8	9.6	5.6	0.6	35.4



Plant No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	100%	88%	84%	86%	85%	85%	85%	87%	85%	88%	86%	84%	86%
2	88%	100%	86%	89%	85%	92%	87%	90%	85%	91%	90%	89%	87%
3	84%	86%	100%	87%	89%	87%	85%	87%	88%	85%	86%	87%	86%
4	86%	89%	87%	100%	90%	91%	85%	92%	82%	89%	90%	88%	87%
5	85%	85%	89%	90%	100%	87%	87%	87%	86%	88%	86%	88%	90%
6	85%	92%	87%	91%	87%	100%	88%	89%	85%	91%	93%	93%	90%
7	85%	87%	85%	85%	87%	88%	100%	86%	90%	89%	87%	89%	87%
8	87%	90%	87%	92%	87%	89%	86%	100%	87%	91%	91%	90%	88%
9	85%	85%	88%	82%	86%	85%	90%	87%	100%	87%	85%	87%	85%
10	88%	91%	85%	89%	88%	91%	89%	91%	87%	100%	91%	91%	89%
11	86%	90%	86%	90%	86%	93%	87%	91%	85%	91%	100%	95%	90%
12	84%	89%	87%	88%	88%	93%	89%	90%	87%	91%	95%	100%	93%
13	86%	87%	86%	87%	90%	90%	87%	88%	85%	89%	90%	93%	100%

Fig 3. The genetic similarity matrix between wheat genotypes

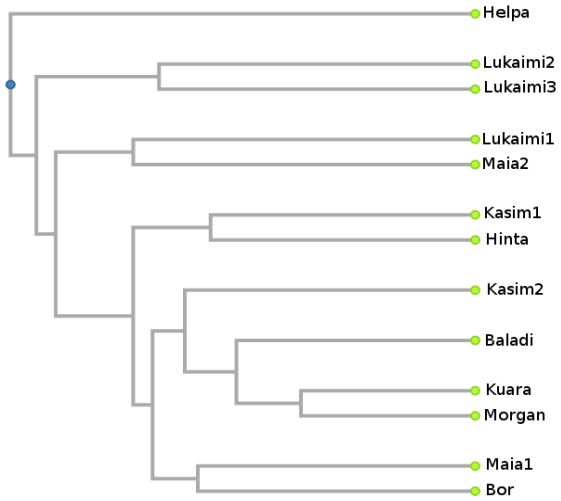


Fig. 4: The phylogenetic tree of wheat genotypes constructed using ISSR and SCoT data

The marker-trait association

Statistical analysis of genetic data with breeding systems helps to develop efficient breeding programs for different crop species [42]. Both ISSR and SCoT assays generated markers were associated with different wheat traits. Eighty-five PCR markers were significantly linked with 32 wheat traits and the SCoT assay were produced 44 markers, while ISSR produced 41 markers. The GW had the highest number of linked markers [17] followed by GR and FLA respectively. Concerning, marker-trait association analysis the p-value ($-\log_{10}$) of markers were ranging from 1.32 to 4.13. The maximum association score was between LL and $SCoT9_{221}$ (4.13) whereas $SCoT9_{221}$ had the highest-scoring marker linked with AC, PH and SCS traits. Also, $SCoT17_{1423}$ had the highest-scoring linked marker with OTSW, GS,and SC, and $SCoT9_{695}$ was linked with SH, CSPL,and SL (**Table 4)**.

Table 4. The highest-scoring and lowest-scoring linked markers associated with agronomic traits; the trait (TR), highest-scoring marker (HL), highest-scoring p-value (-log$_{10}$) (HP), the lowest-scoring marker (LL) and lowest p-value (LP)

Tr	MaxL	MaxP	MinL	MinP
AC	SCoT9-221	1.73	S8-513	1.44
AL	SCoT7-484	1.64	SCoT17-1247	1.31
AS	SCoT17-1639	2.25	SCoT17-1423	1.39
CSPL	SCoT9-695	2.32	S10-976	1.9
DH	S3-109	2.98	S1-641	2.05
FLA	S1-641	2.09	S7-457	1.32
FLB	S8-446	2.78	SCoT7-2437	1.32
FLH	SCoT15-630	1.39	SCoT9-556	1.32
FLL	S1-306	1.92	S1-306	1.92
FLW	SCoT8-553	1.39	SCoT9-556	1.32
GC	S1-467	1.62	S1-467	1.62
GR	S7-457	2.25	S1-641	1.39
GS	SCoT17-1423	2.6	S8-513	1.62
GW	SCoT7-1455	2.84	S7-457	1.32
HDG	S8-513	2.51	S1-306	1.32
LC	SCoT17-330	1.62	S3-109	1.39
LGLP	SCoT17-1011	2.26	SCoT17-330	1.62
LL	SCoT9-221	4.13	S7-457	1.37
OTSW	SCoT17-1423	1.91	S2-278	1.32
PH	SCoT9-221	2.5	SCoT17-370	1.48
SC	SCoT17-1423	2.6	S8-513	1.62
SCS	SCoT9-221	2.6	SCoT9-296	1.31
SG	SCoT7-396	2.05	S10-392	1.79
SGLP	SCoT8-553	2.12	S9-763	1.42
SGS	S11-236	1.45	S11-236	1.45
SH	SCoT9-695	2.25	SCoT9-695	2.25
SL	SCoT9-695	2.84	S9-111	1.62
SS	S8-194	2.78	SCoT10-1093	1.44
WLFL	S1-320	1.62	SCoT8-379	1.32
WLFLB	SCoT17-449	1.75	S3-109	1.39
WLLN	SCoT17-330	1.76	SCoT7-2171	1.39
WLS	SCoT17-449	1.71	SCoT17-449	1.71

For example, Gondo et al. [43] observed that 40 SSR markers were associated with 13 agronomic traits of *Lotus japonicus*. Further, the marker-trait association analysis was used to reveal SSR markers linked with salinity tolerance of chickpea genotypes [44]. Furthermore, 46 markers were detected on 14 associated traits for controlling salt tolerance of barley [45].

The shared markers between different traits could control one or several traits [46]. In this study, The S8$_{446}$ andS2$_{545}$ were linked with 8 wheat agronomic traits, whereas S8$_{446}$ was linked with FLB, PH, SCS, CSPL, LL, WLFLB, AC, and FLA, and S2$_{545}$ was linked with SCS, LL, PH, SC, GS, AC, GW, and FLA. Also, we noticed the S1$_{306}$ and SCoT17$_{1423}$ were linked with 7 agronomic traits, such as SCS, LL, FLL, PH, SS, FLA and HDG for S1$_{306}$ and SC, GS, GW, OTSW, SG, AS and GR for SCoT17$_{1423}$ (**Figure 5**).

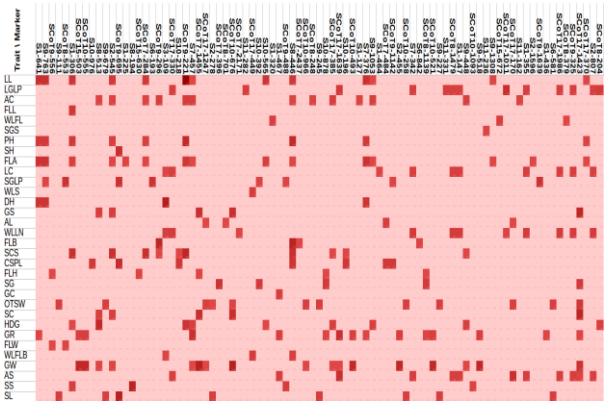


Fig 5. The multiple traits controlling markers, the red color concentration is relative to the p-value score (highest scores have darker red colors)

Association analysis between different agronomic traits was conducted depending on shared molecular markers to measure the genetic relationship and network structure between traits (**Figure 6**). The results showed that three groups of traits had shared linked markers. The traits AS, LGS, LC, and WLLN had shared linked markers ranging from 69% (AS and WLLN and LGS and LC) to 85% (LC and WLLN). Another group contains CSTPL, SCS, PH, FLL, AC, WLLN, HDG and LL the maximum percentage of shared markers was 82% and the minimum was 10%. Finally, SC, GR, GS, and GW shared a quite number of linked markers ranging from 100% (GS and SC) to 10% (GR and GS) respectively.

CONCLUSION

Overall, this study could provide fundamental information for local breeding programs of agronomically important traits in Saudi kinds of wheat. Shared trait-linked molecular markers may provide the opportunity to select several traits in one step and overcome the complex statistical protocols used for avoiding morpho-agronomic traits collateral or untended interactions. Finally, characterizing local Saudi wheat genotypes using molecular markers assays could provide a useful tool for protecting genotypes in seeds GenBank as a fingerprinting tool.

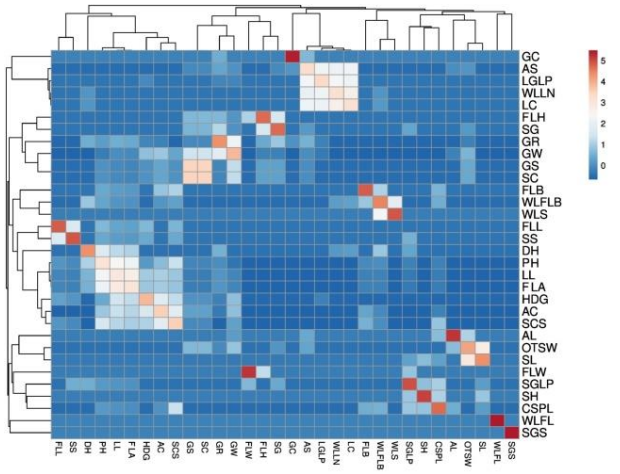


Fig 6. Heatmap for significant shared markers between traits, the blue and red color scale (left side) is relative to the number of shared markers

ACKNOWLEDGEMENTS

I sincerely thank the Employees in Plant Gene bank in National Agriculture & Animal Resources Research Center, Ministry of Environment Water & Agriculture in Saudi Arabia who worked on sample collection and their kind cooperation.

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