Genome-wide association study for colour traits of steamed wheat flour bread using single nucleotide polymorphism markers

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Summary
In this study, a genome-wide association study (GWAS) was conducted to locate the genetic determinants that monitor colour quality of steamed wheat bread. Two hundred and five wheat varieties were firstly genotyped with the high-density Illumina 90K (Davis Genome Center, Davis, California, USA) single nucleotide polymorphism (SNP) array. GWAS results revealed a total of 276 marker-trait associations (MTAs) for colour-related traits (mapped onto 21 chromosomes of wheat) with phenotypic variance ranging from 5.8 % to 14.0 %, of which, 23 MTAs were highly significant markers, 9 MTAs were stable markers and 17 MTAs were multi-trait markers. These MTAs can be used to track the effects of gene loci on steamed bread colour traits, to assist the selection of desirable wheat breeding lines.

Keywords
bread colour; marker-trait association; single nucleotide polymorphism; model; wheat

Steamed bread is a traditional fermented food of China, accounting for about two-thirds of total flour consumption in northern China and about a half throughout the country [1]. Among the characteristics of steamed bread, colour is one of the most important quality indices, which influences both the product sensory properties and commercial value [2–4]. Usually, consumers prefer steamed bread with white colour, so steamed bread with high commercial value also requires a white colour. In the past, much attention was placed on effects of processing-related variables such as ingredients, additives and processing conditions on the properties of steamed wheat flour bread [5–8]. Limited studies were conducted from a genetic perspective on the end-use quality traits of such steamed bread, although genetic inheritance of a wheat material may likely be an important determinant of the sensory and quality characteristics of derived bread. Among the few publications in this field, the recent work accomplished by our research group achieved significant progress, in which loci were found that affect the specific volume of steamed wheat bread from a single gene level, and seven main quantitative trait loci (QTL) were also found useful for molecular marker-assisted selection in wheat breeding programmes.

Recent development in the emerging area of complex trait genetics further stimulate the improvement of approaches to mapping genes affecting quantitative traits. Genome-wide association studies (GWAS) capable of identifying very small chromosomal regions that code for an important trait in crops, were able to estimate the size and direction of the effects of alleles in known loci [9]. Compared with QTL mapping, GWAS have notable merits including high degree of confidence, resolution of QTL and potential for molecular marker-assisted selection [10]. To obtain accurate

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location of genes, GWAS demand a lot of markers distributed across the whole genome of crops, to minimize the downside risk associated with the effects of population structure [11, 12]. As one type of the molecular markers, single nucleotide polymorphism (SNP) markers, are advantageous regarding genetic stability, abundance, wide distribution and ease of detection, thereby suitable for high-throughput analysis such as GWAS with improved statistical validity [13]. In particular, SNPs enable the analysis of the evolution of domesticated wheat species on a genomic scale, thus, can improve the success rate of positional cloning, association analysis, QTL mapping, kinship evaluation and construction of genetic linkage maps [14–17]. Accordingly, QTL detection through GWAS using SNP markers can improve the purpose and accuracy of marker-assisted selection, and then improve the efficiency of breeding [18, 19]. At present, GWAS is the main approach to dissect the genetic bases of complex traits, and GWAS using SNP markers for colour traits of steamed bread can identify the gene loci that control colour traits and help to understand colour traits from the molecular level.

This study aimed to identify the genomic regions and effective markers that capture genetic determinants of the colour traits of steamed wheat flour bread, using a panel of 205 diverse Chinese winter wheat varieties and 24 355 SNP markers covering the whole genome of wheat. The results obtained are expected to support molecular marker-assisted selection in wheat breeding programmes.

MATERIALS AND METHODS

Plant materials

The association panel was composed of 205 wheat varieties, of which 203 varieties were from 10 winter wheat-producing provinces in China, and 2 from Mexico and France, respectively. Among these 205 varieties, 132 were founder parents and 73 were breeding lines, which were all chosen from the varieties grown in Shandong province of China (Tab. 1).

Experimental design and phenotypic trait evaluation

The wheat seeds were grown in Dezhou city (116°29'E, 37°45'N) and Tai'an city (116°36'E,

Tab. 1. Wheat varieties used in this study.

Origin	Number of varieties	Variety name
Shandong, China	138	Zhaoshan15, Shannong17, Shannong19, Shannong20, Shannong10-2, Shannong11, Shannong12, Shannong06-278, Shannongyoumai2, Shannongyoumai3, Shannong0919, Shannong55843, Shannong22, Shannong23, Shannong055849, New shannong11, Taishan1, Taishan21, Tainong18, Tainong9236, Taiong19, Lumai6, Lumai9, Lumai14, Lumai23, Luyuan502, Luyuan205, Jining3, Jining16, Jining6058, Jimai19, Jimai21, Jimai22, Jinan17, Yannong21, Yannong19, Yannong999, Yan99102, Weiyin84137, Wei60182, Weimai8, Zimai12, Lainong8621, Qifeng2, Wenhang1, Wennong17, Bo8, Bonong6, Liangxing66, Lingxing99, Xingmai18, Ningmaizi22, Ningmaizi28, Hemai13, Hemai17, He9946, Laizhou9361, Lin4, Linmai2, Xinmai296, Yuanjin97-28, Lunzao3, Xizhi8222, Yuejin5, Hemai0302; Breedering lines (73)
Henan, China	24	He0927, Yumai34, Yunong416, Yunong949, Yu70-36, Zhengmai7698, Zhengmai0856, Zhengzi8780-2, Zhoumai16, Zhoumai22, Zhoumai24, Zhoumai26, Zhoumai18, Luo86036, Luo88079, Lianfeng85, Zhu0263-541, Fan7030, Luo22, Mengxian201, Aikang58, Fanmai5, Huapei3, Yumai57
Hebei, China	14	ShiB07-4056, Shi08-534, Gan5092, Gan05-093, Heng5229, Heng5364, Heng4371, Hengguan76, Hengguan35, Jifu8512, Shiluan02, Xingmai11, Gaocheng8901, Shi4185
Anhui, China	8	Jinghe91-P39, Wan38, Wan50, Wanmai52, Wanmai53, Fu84111, Huancheng3366, Wo85
Jiangsu, China	6	Lianmai2, Xuzhou24, Lian0809, Lian0756, Zhen8906, Zhenmai18
Beijing, China	5	Kenong199, Kenong2009, Kenong3106, Zhongyu01089, Zhongyu01095
Shaanxi, China	4	Xinong157, Xinong979, Shannong33, Xiaoyan22
Gansu, China	2	Aifeng3, Bima6
Guizhou, China	1	Fengyou04
Ningxia, China	1	Ningdong11
France	1	Soissons
Mexico	1	Ci-5

36°57'N), Shandong province in China, for two consecutive years (2013 and 2014). Land with similar conditions was used for growing each wheat genotype to avoid experimental errors, and a randomized block design with replications per species was applied to all the wheat varieties. The trial was laid out with blocks containing rows (2 m long, with a row-to-row distance of 25 cm and 3 rows per seed variety). Conventional standard field management and agronomic practices were applied to wheat growth, no serious diseases or water stress and significant insect pests were found during the growing seasons.

After grown to maturity, the wheat plants were harvested and ground into flour. The obtained flour was labelled according to its original wheat variety, and used for making steamed bread following the same standard steamed bread production procedure [20].

As soon as the steamed bread was cooked, its external surface colour and internal colour were measured at room temperature (20 ± 2 °C) using a Minolta CR-400 colorimeter (Konica Minolta, Tokyo, Japan), and expressed as L^*, a^* and b^* values: L^* value defines the lightness ($L^* = 0$ for black and $L^* = 100$ for white), and a^* and b^* values define the red-greenness (green [–] to red [+]) and yellow-blueness (blue [–] to yellow [+]), respectively. The instrument was calibrated using a white reference tile ($L^* = 97.09$, $a^* = –4.86$, $b^* = 7.02$) before measurements. Bread external colour was determined based on triplicate measurements for the same spot located in the top of per outside surface. After that, steamed bread was cut into three parallel slices, and bread internal colour was determined through measuring the spot located inside the middle slice of steamed bread. The normal correlation analysis of steamed bread's colour-related traits was carried out using SPSS 18.0 software (IBM, Armonk, New York, USA).

DNA extraction

DNA was extracted from the fresh young leaf tissues of each of the selected wheat plant following the previously published protocol [21]. The obtained DNA samples were subjected to quality check via electrophoresis using 0.8% agarose gel, and their concentrations were determined using a NanoDropND-1000 UV-Vis spectrophotometer (Nano Drop Technologies, Wilmington, North Carolina, USA).

Single nucleotide polymorphism markers and genotyping

SNP genotyping was carried out at the University of California, Davis Genome Center (Davis, California, USA) using the new 90K iSelect SNP chip [22], consisting of 81 587 SNP markers mapped on all 21 chromosomes of wheat. By using the Illumina Beadstudio genotyping software (Illumina, San Diego, California, USA) for SNP calling, the SNP data were gathered and called minor allele frequency (0.05) and detection/call (0.8) to avoid false positive associations [23].

Marker-trait association analysis

Marker-trait associations were determined using the general linear model and the mixed linear model as implemented in TASSEL 3.0 software (NCSU, Raleigh, North Carolina, USA), along with the Q method (population structure results generated from Structure software (Stanford, Palo Alto, California, USA), the kinship (K) method (the level of genetic covariance between pairs of genotypes as a random effect generated from TASSEL software) and the Q+K method (family relatedness correction) were also applied for GWAS. The analysis results were compared and the best fitting statistical model was chosen. SNPs with $p < 0.001$ were considered to be in association with target traits. SNPs with $p < 0.0001$ were highly associated with the target traits. Consensus maps were constructed for molecular markers using intergrated map based on six doubled haploid genetic populations [22].

RESULTS AND DISCUSSION

Phenotypic data

The phenotypic data of colour-related traits in four different environments are summarized in Tab. 2. Most of the evaluated indicators for the colour-related traits had a large coefficient of variation (*CV*) in each environment, with the internal a^* values in Environment 1 having the highest *CV* (71.7 %) and the external L^* values in Environment 4 having the lowest *CV* (3.7 %). The *CV* values of both internal a^* and external a^* were over 20 %, suggesting high levels of genetic variation in internal and external a^* values. The *CV* values of internal and external b^* were between 10 % and 20 %. These four a^* and b^* traits all had significant *CV* values, indicating great potential for improvement, i.e. allow further studies on the present wheat varieties using different approaches to locate and characterize the genomic regions that control the colour attribute of steamed bread. Further, most of the absolute values of skewness and kurtosis were less than 1.0, suggesting that the phenotypic data followed

Tab. 2. Phenotypic values for colour-related traits of the nature population in four cultivation environments in wheat.

Trait	E	Minimum	Maximum	Range	Mean ± *SD*	*CV* [%]	Skewness	Kurtosis	h_B^2 [%]
External *L**	E1	12.97	87.65	74.68	60.45 ± 12.59	20.8	–0.58	0.733	30.8
	E2	42.05	87.65	45.60	65.58 ± 10.72	16.3	–0.115	–0.762	
	E3	25.44	89.88	64.44	81.31 ± 6.37	7.8	–0.786	1.325	
	E4	68.93	87.10	18.17	82.32 ± 3.04	3.7	–1.472	1.542	
External *a**	E1	0.11	1.98	1.87	0.63 ± 0.51	40.2	0.765	–0.426	67.5
	E2	0.01	2.19	2.18	0.94 ± 0.63	66.7	0.133	–0.339	
	E3	0.15	2.65	2.5	1.33 ± 0.48	36.2	–0.024	–0.041	
	E4	0.12	2.76	2.64	1.25 ± 0.51	41.0	0.225	0.125	
External *b**	E1	6.01	19.89	13.88	11.14 ± 2.51	22.4	0.628	0.758	64.1
	E2	5.56	18.42	12.86	11.60 ± 2.38	20.5	0.266	0.35	
	E3	14.44	28.09	13.65	18.87 ± 2.30	12.1	0.696	1.022	
	E4	13.13	23.56	10.43	17.92 ± 2.27	12.6	0.353	–0.358	
Internal *L**	E1	31.00	87.08	56.08	57.32 ± 8.99	15.6	–0.152	0.778	26.0
	E2	33.33	87.08	53.75	61.73 ± 8.10	13.1	0.044	0.567	
	E3	18.31	94.96	76.65	74.06 ± 9.03	12.1	–0.892	1.861	
	E4	29.96	85.35	55.39	77.53 ± 4.43	5.7	–0.913	1.279	
Internal *a**	E1	0.01	2.03	2.02	0.67 ± 0.48	71.7	0.704	–0.294	27.8
	E2	0.06	2.53	2.47	0.89 ± 0.58	65.7	0.525	–0.769	
	E3	0.01	1.74	1.73	0.74 ± 0.43	58.4	0.175	–0.913	
	E4	0.02	2.46	2.44	0.95 ± 0.53	55.3	0.379	–0.454	
Internal *b**	E1	0.81	17.64	16.83	12.63 ± 2.45	19.4	–0.823	0.954	84.3
	E2	7.68	17.89	10.21	13.13 ± 1.88	14.3	0.19	–0.141	
	E3	12.62	23.98	11.36	17.69 ± 2.16	12.2	0.042	0.005	
	E4	13.41	22.69	9.28	17.62 ± 2.06	11.7	0.344	–0.381	

E – environment (E1 – grown in Tai'an, China in 2013, E2 – grown in Dezhou, China in 2013, E3 – grown in Tai'an, China in 2014, E4 – grown in Dezhou, China in 2014).
SD – standard deviation, *CV* – coefficient of variation, h_B^2 – broad-sense heritability.

a normal distribution and were suitable for GWAS.

The results of ANOVA showed that the variances of genotype for all the investigated colour-related traits were significant at $p < 0.01$. Significant differences were also found in environment for all traits except for internal b^*. Broad-sense heritability of the investigated colour-related traits ranged from 26.0 % (internal L^*) to 84.3 % (internal b^*), indicating that both genetic and environmental factors played roles in the formation of these measured colour-related traits (Tab. 2 and Tab. 3). All the presented colour-related traits were found to be correlated with each other across all of the four environments except for external b^* and external L^* (Tab. 4).

Consensus genetic map for molecular markers

After SNP genotyping with the 90K iSelect wheat chip, 38381 out of 81587 markers were identified as polymorphic markers in the selected material with a polymorphic percentage of 47.0 %. SNP markers with a genotype detection rate over 80 % and/or minor allele frequency less than 5 % were removed and, as a result, 32432 SNP markers were retained. Among the retained markers, only 24355 SNPs had the corresponding chromosome location and were genetically suitable for the construction of consensus linkage map and further trait-marker analysis.

The locus of each chromosome and the length of integrated linkage group are listed in Tab. 5. The average genetic distance between markers was 0.15 cM and the length of the whole linkage group (LG) map was 3674.16 cM. Among the 21 chromosomes, chromosome 1B and 4D had the largest ($n = 2390$) and smallest number of significant marker-trait associations (MTAs).

Marker-trait associations

In total, 276 MTAs were identified ($p < 0.001$) for colour-related traits in the four analysed environments, accounting for 5.8–14.0 % of the phenotypic variance (Tab. 6 and Tab. 7). The greatest and smallest MTA values were with external L^*

(74), and internal *b** (24), respectively. Medium MTA values were with external *a** (61), internal *a** (47), external *b** (40) and internal *L** (30). Among these MTAs, 23 MTAs had highly significant association with colour-related traits ($p < 0.0001$), 9 MTAs were detected in two or more environments and 31 MTAs being high genetic contributors (Tab. 8 and Tab. 9).

For internal *L**, thirty MTAs were identified on 11 of the 21 chromosomes, accounting for 6.4–14.0 % of the phenotypic variance. Among these MTAs, highly significant ($p < 0.0001$) MTAs were found on chromosomes 1D, 2A, 7A and 7D, responsible for 9.0–14.1 % of the trait variation. One MTA (BS00023200_51) on chromosome 7A was detected in two environments, with 7.1% and 9.3% of trait variation, respectively. Three MTAs had a trait variation over 10 %, suggesting their high genetic contribution. The strong association region containing 7 MTAs for internal *L** was located on chromosome 3A (86–87 cM).

For internal *a**, 47 MTAs were located on 14 of the 21 chromosomes, explaining 6.2–12.1 % of the phenotypic variance. Two MTAs on chromosomes 7A and 7D were highly significant markers ($p < 0.0001$), accounting for 9.7–10.7 % of the trait variation. The markers BS00023037_51 on chromosome 3B and wsnp_ku_c7102_12271493 on chromosome 4A were detected in two environments as stable markers. Four MTAs were high genetic contributors with a trait variation over 10 %. The strong association region containing 5 MTAs for internal *a** were found on chromosome 1A (116 cM).

For internal *b**, 24 MTAs were distributed on 7 of the 21 chromosomes, accounting for 5.8–9.6 % of the phenotypic variance. Among them, two stable markers (Tdurum_contig63834_340 and Excalibur_c22201_907) on chromosomes 1B and 2B were detected in two environments, with 6.3–8.7 % of the phenotypic variance. A strongly associated locus containing 6 MTAs for internal *b** was mapped onto chromosome 1B (89–90 cM).

For external *L**, 74 MTAs were detected on 13 of the 21 chromosomes, with 6.4–14.0 % of the phenotypic variance. Among them, 15 MTAs on chromosomes 2A, 2B, 5B, 7A and 7D showed highly significant associations with external *L** ($p < 0.0001$) and explained 9.0–12.0 % of the trait variation. One MTA (wsnp_Ex_c14654_22713386) on chromosome 7A was noted in two different environments with 7.7% and 8.3% of trait variation, respectively. Fifteen MTAs were high genetic contributors with a trait variation over 10 %. Two strong association regions harboring 19 and 10 MTAs were detected on chromosome 5B (143–144 cM) and 7A (89 cM), respectively.

For external *a**, 61 MTAs were distributed across 10 of the 21 chromosomes, with 6.3–13.8 %

Tab. 3. Analysis of variance for bread colour-related traits of the nature population in four cultivation environments in wheat.

Trait	Source of variation	Mean square	*F* value
External *L**	Genotype	60.307	3.053[a]
	Environment	135.481	6.860[a]
	Error	19.751	
External *a**	Genotype	0.433	9.278[a]
	Environment	0.208	4.449[b]
	Error	0.047	
External *b**	Genotype	12.313	6.315[a]
	Environment	6.893	3.535[b]
	Error	1.95	
Internal *L**	Genotype	84.384	2.123[a]
	Environment	239.92	6.035[a]
	Error	39.752	
Internal *a**	Genotype	0.587	6.266[a]
	Environment	1.521	16.242[a]
	Error	0.094	
Internal *b**	Genotype	10.498	5.488[a]
	Environment	1.955	1.043
	Error	1.913	

Lowercase letter in superscript indicates the statistical significance (a – correlation significant at $p < 0.01$, b – correlation significant at $p < 0.05$).

Tab. 4. Pairwise correlation coefficients among bread colour-related traits of the nature population.

Bead colour-related traits	External *L**	External *a**	External *b**	Internal *L**	Internal *a**
External *a**	0.163[b]				
External *b**	0.153	0.442[a]			
Internal *L**	0.384[a]	0.187[b]	0.177[b]		
Internal *a**	0.232[a]	0.734[a]	0.415[a]	0.307[a]	
Internal *b**	0.187[b]	0.608[a]	0.555[a]	0.391[a]	0.685[a]

Lowercase letter in superscript indicates the statistical significance (a – correlation significant at $p < 0.01$, b – correlation significant at $p < 0.05$).

Tab. 5. Single nucleotide polymorphism markers in the integrated linkage group map.

Locus	Number of markers	Length of linkage group [cM]
1A	1506	161.35
2A	1462	185.46
3A	1154	184.56
4A	1145	164.12
5A	1243	144.15
6A	1463	180.74
7A	1550	232.13
1B	2390	174.10
2B	1977	180.33
3B	1628	150.97
4B	882	118.91
5B	2187	219.77
6B	1786	127.54
7B	1471	178.85
1D	629	196.97
2D	769	151.92
3D	331	156.06
4D	78	161.10
5D	240	207.32
6D	234	156.53
7D	230	241.28
Total	24355	3674.16

of the phenotypic variance. One MTA on chromosomes 6D was a highly significant marker ($p < 0.0001$), with 13.8 % of trait variation. Four MTAs were high genetic contributors with the trait variation over 10 %. The markers BS00110512_51 on chromosome 6A and wsnp_Ku_c7102_12271493 on chromosome 4A were stable markers, explaining 7.0–9.1 % of the phenotypic variance. Two strong association regions containing 20 and 8 MTAs for external *a** were located on chromosomes 6A (141 cM) and 7A (113 cM), respectively.

Tab. 6. Summary of marker–trait associations for colour-related traits in four cultivation environments.

Trait	Environment				Total MTA	R^2 [%]
	E1	E2	E3	E4		
Internal *L**	14	1	0	15	30	6.4–14.0
Internal *a**	12	9	10	16	47	6.2–12.1
Internal *b**	6	3	2	13	24	5.8–9.6
External *L**	2	3	55	14	74	6.4–14.0
External *a**	28	5	10	18	61	6.3–13.8
External *b**	7	6	24	3	40	6.7–10.2
Total	69	27	101	79	276	5.8–14.0

E1 – grown in Tai'an, China in 2013, E2 – grown in Dezhou, China in 2013, E3 – grown in Tai'an, China in 2014, E4 – grown in Dezhou, China in 2014.
MTA – marker–trait association ($p < 0.001$), R^2 – phenotypic variation contribution.

For external *b**, 40 MTAs were detected on 9 of the 21 chromosomes, with 6.7–10.2 % of the phenotypic variance. Among them, one MTA (RFL_Contig4953_469) on chromosome 1A was detected in two environments with a phenotypic variance of 9.9–10.6 %, indicating that this MTA could be considered a stable marker for the trait. Five MTAs were high genetic contributors with a trait variation over 10 %. A strong association region harboring 12 MTAs was detected on chromosome 1B (71 cM).

Multi-trait association

A few markers co-associated with two or more traits (termed as "multi-trait MTAs"). Seventeen multi-trait MTAs for colour-related traits were detected with their chromosome positions and effect values listed in Tab. 10. Seven MTAs on chromosomes 1B, 2A, 2D, 4A, 7A and 7B exhibited a pleiotropic effect on internal *L** and external *L**. Four MTAs mapped onto chromosomes 4A, 4B, 6A and 6B were pleiotropic loci for internal *a** and external *a**. Three MTAs on chromosomes 4B, 7A and 7D showed a pleiotropic effect on internal *L**, internal *a** and external *L** traits. Moreover, internal *a** and external *L** shared a common MTA on chromosome 4A, internal *b** and external *b** had MTA in common on chromosome 5B, and both internal *b** and external *a** had MTA on chromosome 5B.

Analysis method

SNPs are particularly important molecular markers to reflect both natural genetic variability and genetic drifts created by breeders in crops [24]. Hexaploid wheat is an allopolyploid species and contains three genomes (A, B and D). Therefore, a large number of repetitive DNA sequences exist in the wheat genome, which make wheat genome sequencing very difficult and have led to much slower development and utilization of SNP markers in wheat compared with those in maize [25], rice [26] and barley [27]. Bread wheat has a relatively low level of useful SNP-derived markers due to low SNP frequencies within each of the diploid genomes of wheat (A, B or D) revealed in genome-wide studies [28]. With the recent advancement of sequencing technology, great progress has been made in engaging SNP markers with GWAS and/or high-throughput technologies, which allows efficient genotyping of wheat [29–31]. In this study, 24355 SNP loci were found

Tab. 7. A list of 276 chromosomal loci significantly associated with colour-related traits.

Trait	Locus	Site	Marker	*p* value	R^2 [%]	Environment
Internal *L**	2A	156	Kukri_rep_c76782_289	2.23×10^{-5}	10.6	E4
	3A	86	wsnp_Ex_c5929_10402147	7.68×10^{-4}	6.9	E1
	3A	86	BS00095423_51	6.42×10^{-4}	7.1	E1
	3A	86	IAAV6474	6.96×10^{-4}	7.0	E1
	3A	86	BS00010531_51	6.96×10^{-4}	7.0	E1
	3A	86	RAC875_rep_c70746_582	4.30×10^{-4}	7.6	E1
	3A	87	Ku_c17569_905	6.96×10^{-4}	7.0	E1
	3A	87	BS00041121_51	6.96×10^{-4}	7.0	E1
	3A	88	wsnp_Ex_c11085_17973016	7.51×10^{-4}	6.9	E1
	3A	89	RAC875_c5310_1729	6.19×10^{-4}	7.1	E1
	3A	91	Kukri_c34195_357	6.19×10^{-4}	7.1	E1
	3A	91	BobWhite_rep_c49102_169	6.19×10^{-4}	7.1	E1
	3A	91	Kukri_c80104_809	6.19×10^{-4}	7.1	E1
	4A	49	wsnp_Ex_c13091_20706489	5.39×10^{-4}	6.9	E4
	4A	49	RAC875_c37611_302	3.84×10^{-4}	7.3	E4
	4A	146	RAC875_c1126_1769	9.76×10^{-4}	6.8	E4
	7A	42	wsnp_Ex_c14654_22713386	2.92×10^{-4}	7.7	E4
	7A	45	Kukri_c18677_823	1.34×10^{-6}	14.0	E4
	7A	136	Kukri_c7579_614	6.85×10^{-4}	6.7	E4
	7A	228	BS00023200_51	6.89×10^{-4}	7.1	E1
	1B	57	Tdurum_contig60037_441	5.90×10^{-4}	6.8	E4
	1B	123	BS00064052_51	7.45×10^{-4}	7.3	E4
	4B	61	Tdurum_contig4974_355	1.46×10^{-4}	8.6	E4
	5B	69	wsnp_Ex_c5594_9848626	8.89×10^{-4}	6.7	E1
	7B	82	BobWhite_c3541_152	6.35×10^{-4}	9.7	E4
	7B	116	Tdurum_contig63207_82	9.02×10^{-4}	6.4	E4
	1D	68	tplb0044p22_2257	7.39×10^{-5}	13.0	E2
	2D	37	Kukri_c13329_800	4.04×10^{-4}	7.8	E4
	7D	56	D_contig06359_118	8.65×10^{-5}	9.0	E4
Internal *a**	1A	70	Excalibur_c32608_64	9.11×10^{-4}	6.6	E1
	1A	81	BS00089894_51	7.26×10^{-4}	6.9	E1
	3A	130	wsnp_BG262734A_Ta_2_3	5.80×10^{-4}	9.8	E1
	3A	173	wsnp_Ex_rep_c104125_88923836	5.84×10^{-4}	9.4	E2
	3A	173	Kukri_c2273_365	4.99×10^{-4}	9.7	E2
	4A	144	Kukri_c50736_53	2.27×10^{-4}	10.9	E2
	4A	144	Tdurum_contig15260_591	1.09×10^{-4}	12.1	E2
	4A	146	BS00104640_51	8.92×10^{-4}	9.5	E2
	4A	147	wsnp_Ku_c7102_12271493	2.07×10^{-4}	8.4	E1
	4A	147	wsnp_Ku_c7102_12271493	6.29×10^{-4}	9.3	E2
	4A	147	BobWhite_c3259_96	1.19×10^{-4}	12.0	E2
	5A	93	Excalibur_c24051_1028	6.67×10^{-4}	7.1	E3
	5A	93	wsnp_Ex_rep_c101994_87256479	6.67×10^{-4}	7.1	E3
	5A	93	RAC875_rep_c109969_119	6.67×10^{-4}	7.1	E3
	5A	94	Excalibur_c49550_97	5.67×10^{-4}	7.3	E3
	5A	125	Excalibur_c49297_159	5.63×10^{-4}	6.8	E4
	6A	41	RAC875_c29753_800	8.71×10^{-4}	6.5	E4
	6A	141	Ra_c2235_3340	3.64×10^{-4}	9.5	E1
	7A	42	wsnp_Ex_c14654_22713386	4.92×10^{-5}	9.7	E4
	7A	127	BS00110424_51	9.43×10^{-4}	6.9	E3
	1B	116	wsnp_Ku_c18881_28259811	2.71×10^{-4}	8.1	E1
	1B	116	Excalibur_c25597_508	2.33×10^{-4}	8.2	E1
	1B	116	IACX6230	4.20×10^{-4}	7.7	E1
	1B	116	TA004668-0687	2.71×10^{-4}	8.1	E1
	1B	116	BS00021876_51	3.50×10^{-4}	7.7	E1
	2B	102	RAC875_c15396_90	5.44×10^{-4}	6.9	E4
	2B	104	Kukri_c50842_573	3.43×10^{-4}	7.3	E4

Tab. 7. *continued*

Trait	Locus	Site	Marker	*p* value	R^2 [%]	Environment
	2B	104	Tdurum_contig13653_255	6.26×10^{-4}	7.3	E4
	3B	61	BS00023037_51	7.25×10^{-4}	8.4	E3
	3B	61	BS00023037_51	8.60×10^{-4}	7.3	E4
	3B	73	GENE-1617_131	4.42×10^{-4}	7.6	E3
	3B	73	GENE-1617_188	4.42×10^{-4}	7.6	E3
	3B	80	Kukri_c64989_168	6.67×10^{-4}	7.1	E3
	4B	61	Tdurum_contig4974_355	2.57×10^{-4}	8.6	E4
	4B	68	Excalibur_c23433_474	9.71×10^{-4}	6.2	E4
	6B	63	IACX9279	7.58×10^{-4}	6.5	E4
	6B	65	CAP11_c2683_177	8.78×10^{-4}	6.4	E4
	6B	65	BobWhite_c10140_297	5.68×10^{-4}	7.0	E4
	6B	65	Tdurum_contig70639_196	5.68×10^{-4}	7.0	E4
	6B	72	GENE-4183_1109	3.27×10^{-4}	7.4	E4
	6B	72	RAC875_c96675_51	8.31×10^{-4}	6.4	E4
	7B	93	RAC875_c21537_368	3.30×10^{-4}	8.0	E1
	7B	95	BS00029287_51	4.05×10^{-4}	7.6	E1
	2D	80	Excalibur_c61922_195	5.68×10^{-4}	7.3	E3
	2D	137	Ku_c19185_1569	6.06×10^{-4}	9.5	E2
	7D	24	BS00059457_51	9.89×10^{-4}	9.1	E2
	7D	56	D_contig06359_118	1.81×10^{-5}	10.7	E4
Internal *b**	2A	108	wsnp_BF475068A_Ta_2_1	3.32×10^{-4}	6.9	E4
	2A	108	BobWhite_c4743_63	2.69×10^{-4}	7.1	E4
	4A	53	RAC875_c37840_704	6.83×10^{-4}	7.2	E1
	7A	163	IAAV5268	8.23×10^{-4}	6.0	E4
	7A	166	Excalibur_c25630_537	5.77×10^{-4}	9.6	E2
	1B	64	GENE-0411_656	9.60×10^{-4}	5.8	E4
	1B	71	RFL_Contig1601_750	3.95×10^{-4}	7.6	E1
	1B	72	BS00081127_51	3.95×10^{-4}	7.6	E1
	1B	72	GENE-0165_389	4.58×10^{-4}	7.5	E1
	1B	72	RFL_Contig3343_2115	4.58×10^{-4}	7.5	E1
	1B	74	Tdurum_contig63834_340	4.09×10^{-4}	7.6	E1
	2B	120	Tdurum_contig47_185	6.14×10^{-4}	9.5	E2
	2B	173	Excalibur_c22201_907	6.82×10^{-5}	6.3	E2
	2B	173	Excalibur_c22201_907	5.76×10^{-4}	6.3	E4
	4B	78	Kukri_c6388_1030	6.89×10^{-4}	7.1	E3
	5B	53	IACX5818	4.51×10^{-4}	6.6	E4
	5B	161	D_GDEEGVY01AU4CW_149	5.06×10^{-4}	7.5	E3
	5B	74	Tdurum_contig63834_340	3.05×10^{-4}	8.7	E4
	5B	89	Kukri_c1932_300	7.57×10^{-4}	6.0	E4
	5B		Kukri_c1932_624	8.13×10^{-4}	6.0	E4
	5B	90	Ex_c69066_186	1.70×10^{-4}	7.6	E4
	5B	90	Excalibur_rep_c69066_270	1.70×10^{-4}	7.6	E4
	5B	90	RAC875_c32848_578	1.70×10^{-4}	7.6	E4
	5B	90	RAC875_c62171_386	1.70×10^{-4}	7.6	E4
External *L**	2A	143	Tdurum_contig86243_288	1.50×10^{-4}	9.3	E3
	2A	156	Kukri_rep_c76782_289	2.23×10^{-5}	10.6	E4
	3A	24	Kukri_c53085_574	2.31×10^{-4}	10.9	E2
	4A	49	wsnp_Ex_c13091_20706489	5.39×10^{-4}	6.9	E4
	4A	49	RAC875_c37611_302	3.84×10^{-4}	7.3	E4
	4A	146	RAC875_c1126_1769	9.76×10^{-4}	6.8	E4
	4A	127	wsnp_Ex_c6094_10663424	2.10×10^{-4}	8.8	E3
	4A	127	BS00032472_51	5.27×10^{-4}	8.4	E3
	4A	133	wsnp_Ex_c1246_2393978	3.88×10^{-4}	8.1	E3
	4A	133	Ex_c1246_1162	5.39×10^{-4}	7.7	E3
	4A	133	BobWhite_c914_465	4.70×10^{-4}	7.9	E3

Tab. 7. *continued*

Trait	Locus	Site	Marker	*p* value	R^2 [%]	Environment
	4A	146	BS00104640_51	3.27×10^{-4}	8.6	E3
	4A	151	Tdurum_contig31852_251	4.05×10^{-4}	8.7	E3
	6A	141	GENE-1795_81	4.98×10^{-4}	7.8	E3
	7A	42	wsnp_Ex_c14654_22713386	1.45×10^{-4}	8.3	E3
	7A	42	wsnp_Ex_c14654_22713386	2.92×10^{-4}	7.7	E4
	7A	45	Kukri_c18677_823	1.34×10^{-6}	14.0	E4
	7A	65	Ra_c16330_1160	7.48×10^{-4}	7.4	E3
	7A	89	IAAV1940	3.56×10^{-5}	11.4	E3
	7A	89	Kukri_c106476_350	3.41×10^{-5}	11.2	E3
	7A	89	BS00007429_51	3.16×10^{-5}	11.3	E3
	7A	89	RFL_Contig3271_810	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_328	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_522	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_630	3.41×10^{-5}	11.2	E3
	7A	89	Tdurum_contig20214_181	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig20214_279	3.16×10^{-5}	11.3	E3
	7A	89	BS00062706_51	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_522	3.16×10^{-5}	11.3	E3
	7A	136	Kukri_c7579_614	6.85×10^{-4}	6.7	E4
	1B	57	Tdurum_contig60037_441	5.90×10^{-4}	6.8	E4
	1B	123	BS00064052_51	7.45×10^{-4}	7.3	E4
	2B	48	GENE-1676_57	6.74×10^{-4}	7.1	E1
	2B	49	wsnp_Ra_rep_c106119_89961852	8.55×10^{-4}	6.8	E1
	2B	116	BS00102614_51	1.79×10^{-5}	12.0	E3
	2B	134	wsnp_Ex_rep_c101342_86720058	4.30×10^{-4}	7.9	E3
	2B	134	Tdurum_contig49532_368	4.30×10^{-4}	7.9	E3
	4B	61	Tdurum_contig4974_355	1.46×10^{-4}	8.6	E4
	5B	52	wsnp_Ku_c3869_7094615	8.12×10^{-4}	7.2	E3
	5B	93	wsnp_Ra_c9155_15344108	8.26×10^{-4}	9.0	E2
	5B	95	wsnp_Ra_c2105_4092507	9.33×10^{-4}	8.7	E2
	5B	104	Kukri_c49101_731	1.57×10^{-4}	9.2	E3
	5B	104	Tdurum_contig27797_1114	1.91×10^{-4}	9.0	E3
	5B	105	wsnp_Ku_c21770_31551190	1.91×10^{-4}	9.0	E3
	5B	107	Ra_c20970_500	9.99×10^{-4}	6.9	E3
	5B	143	Excalibur_c22518_1683	5.13×10^{-4}	7.7	E3
	5B	143	Kukri_c55317_179	5.06×10^{-4}	7.7	E3
	5B	144	Excalibur_c4623_876	5.13×10^{-4}	7.7	E3
	5B	144	BobWhite_c36154_81	5.13×10^{-4}	7.7	E3
	5B	144	GENE-2932_119	8.95×10^{-4}	7.3	E3
	5B	144	Kukri_c41_858	4.06×10^{-4}	8.7	E3
	5B	144	Kukri_c5739_276	5.13×10^{-4}	7.7	E3
	5B	144	RAC875_rep_c108860_477	5.13×10^{-4}	7.7	E3
	5B	144	BS00088733_51	9.95×10^{-4}	6.9	E3
	5B	144	BS00095157_51	9.95×10^{-4}	6.9	E3
	5B	144	Kukri_c3576_1212	9.95×10^{-4}	6.9	E3
	5B	144	RAC875_c62400_267	9.95×10^{-4}	6.9	E3
	5B	144	RAC875_c62400_639	9.95×10^{-4}	6.9	E3
	5B	144	RAC875_c62400_840	9.95×10^{-4}	6.9	E3
	5B	144	RAC875_c62400_924	9.95×10^{-4}	6.9	E3
	5B	144	Tdurum_contig44115_132	9.95×10^{-4}	6.9	E3
	5B	144	Tdurum_contig44115_561	9.95×10^{-4}	6.9	E3
	5B	144	Tdurum_contig44115_720	9.95×10^{-4}	6.9	E3
	5B	144	Tdurum_contig92938_632	9.95×10^{-4}	6.9	E3
	5B	151	wsnp_Ex_c7196_12357989	5.98×10^{-4}	7.5	E3
	5B	151	wsnp_Ex_rep_c67783_66469848	5.98×10^{-4}	7.5	E3
	5B	152	BS00062618_51	7.43×10^{-4}	7.3	E3

Tab. 7. *continued*

Trait	Locus	Site	Marker	*p* value	R^2 [%]	Environment
	5B	153	wsnp_Ex_c7911_13433794	5.98×10^{-4}	7.5	E3
	5B	155	BobWhite_c6685_1922	3.15×10^{-5}	11.3	E3
	7B	82	BobWhite_c3541_152	6.35×10^{-4}	9.7	E4
	7B	116	Tdurum_contig63207_82	9.02×10^{-4}	6.4	E4
	2D	37	Kukri_c13329_800	4.04×10^{-4}	7.8	E4
	3D	107	Excalibur_rep_c93332_58	4.02×10^{-4}	8.0	E3
	3D	113	BobWhite_c9622_723	7.61×10^{-4}	7.3	E3
	7D	56	D_contig06359_118	8.65×10^{-5}	9.0	E4
External *a**	1A	111	Tdurum_contig75762_377	8.58×10^{-4}	10.7	E2
	4A	133	RAC875_c12455_353	8.17×10^{-4}	6.4	E4
	4A	147	wsnp_Ku_c7102_12271493	6.75×10^{-4}	7.4	E4
	4A	147	wsnp_Ku_c7102_12271493	6.75×10^{-4}	7.0	E1
	4A	147	wsnp_Ku_c7102_12271493	3.87×10^{-4}	9.1	E2
	6A	79	wsnp_Ku_c38451_47086066	8.40×10^{-4}	6.7	E1
	6A	79	Excalibur_c4152_1031	8.40×10^{-4}	6.7	E1
	6A	134	BS00110512_51	4.72×10^{-4}	7.4	E1
	6A	134	BS00110512_51	4.72×10^{-4}	7.4	E4
	6A	138	wsnp_CAP7_c5823_2616381	3.31×10^{-4}	7.4	E4
	6A	138	wsnp_JD_c7795_8868122	3.31×10^{-4}	7.4	E4
	6A	141	wsnp_Ex_c29648_38653281	3.03×10^{-4}	7.9	E1
	6A	141	BS00109913_51	2.44×10^{-4}	8.4	E1
	6A	141	Ex_c28973_935	2.73×10^{-4}	8.0	E1
	6A	141	Excalibur_c17487_51	4.30×10^{-4}	7.5	E1
	6A	141	IAAV5620	1.69×10^{-4}	8.7	E1
	6A	141	RAC875_c41438_223	3.54×10^{-4}	8.7	E1
	6A	141	wsnp_Ex_c28973_38050204	2.73×10^{-4}	8.0	E1
	6A	141	wsnp_Ex_c28973_38050756	2.73×10^{-4}	8.0	E1
	6A	141	wsnp_Ex_c29648_38653339	4.33×10^{-4}	7.5	E1
	6A	141	BS00075803_51	1.59×10^{-4}	10.5	E1
	6A	141	BobWhite_c10832_1131	3.22×10^{-4}	7.8	E1
	6A	141	BS00083914_51	3.30×10^{-4}	7.8	E1
	6A	141	Ex_c28973_947	3.24×10^{-4}	7.9	E1
	6A	141	Kukri_rep_c70558_246	2.73×10^{-4}	8.0	E1
	6A	141	Ra_c2235_3340	1.16×10^{-4}	11.7	E1
	6A	141	RAC875_c16731_1548	2.57×10^{-4}	8.1	E1
	6A	141	TA005098-0959	1.29×10^{-4}	8.9	E1
	6A	141	wsnp_Ex_c2325_4355706	3.25×10^{-4}	7.8	E1
	6A	141	Excalibur_c18265_399	1.43×10^{-4}	8.8	E1
	6A	141	RAC875_c12821_466	3.25×10^{-4}	7.8	E1
	7A	113	wsnp_Ex_c13337_21022241	6.12×10^{-4}	7.2	E3
	7A	113	wsnp_Ex_c13337_21022658	5.56×10^{-4}	7.3	E3
	7A	113	wsnp_Ku_c57674_60718050	5.56×10^{-4}	7.3	E3
	7A	113	BS00067746_51	7.60×10^{-4}	6.9	E3
	7A	113	BS00110010_51	5.56×10^{-4}	7.3	E3
	7A	113	Excalibur_c13337_219	5.56×10^{-4}	7.3	E3
	7A	113	Excalibur_s114755_67	5.87×10^{-4}	7.2	E3
	7A	113	IAAV4945	5.08×10^{-4}	7.4	E3
	1B	82	Excalibur_c14911_976	4.18×10^{-4}	7.5	E1
	2B	160	BS00026037_51	9.13×10^{-4}	7.3	E4
	4B	68	Excalibur_c23433_474	1.47×10^{-4}	8.4	E4
	4B	68	Ra_c27465_564	4.12×10^{-4}	7.2	E4
	4B	72	wsnp_Ku_c13052_20918857	2.62×10^{-4}	7.7	E4
	4B	72	BS00020575_51	2.62×10^{-4}	7.7	E4
	4B	76	wsnp_CAP12_c1101_569783	6.74×10^{-4}	6.6	E4
	4B	76	BS00068540_51	6.46×10^{-4}	6.7	E4
	4B	76	BS00076033_51	6.74×10^{-4}	6.6	E4

Tab. 7. *continued*

Trait	Locus	Site	Marker	*p* value	R^2 [%]	Environment
	4B	76	Excalibur_c106884_135	6.46×10^{-4}	6.7	E4
	5B	178	Excalibur_c23452_401	9.86×10^{-4}	8.7	E2
	5B	178	Kukri_c1214_2316	9.86×10^{-4}	8.7	E2
	5B	178	Kukri_c1214_825	9.86×10^{-4}	8.7	E2
	5B	6	Kukri_rep_c109397_59	6.70×10^{-4}	7.0	E3
	5B	161	D_GDEEGVY01AU4CW_149	5.95×10^{-4}	7.2	E3
	6B	28	BS00033641_51	8.55×10^{-4}	7.0	E1
	6B	39	Excalibur_c46399_307	8.77×10^{-4}	6.3	E4
	6B	63	wsnp_Ex_c18669_27544717	8.74×10^{-4}	6.3	E4
	6B	63	IACX9279	7.67×10^{-4}	6.5	E4
	6B	63	BobWhite_c686_387	8.74×10^{-4}	6.3	E4
	6B	119	BS00064478_51	5.81×10^{-4}	7.1	E1
	6D	158	Excalibur_c43912_389	2.54×10^{-5}	13.8	E1
External *b**	1A	35	wsnp_Ex_c4876_8692849	2.27×10^{-4}	8.5	E1
	1A	70	RFL_Contig4953_469	8.32×10^{-5}	10.6	E1
	1A	70	RFL_Contig4953_469	9.15×10^{-5}	9.9	E3
	1A	138	BS00022824_51	3.85×10^{-4}	7.8	E1
	4A	137	RFL_Contig3841_1986	9.65×10^{-4}	6.7	E1
	4A	138	BS00093255_51	5.73×10^{-4}	9.5	E1
	4A	138	Tdurum_contig75819_559	7.93×10^{-4}	7.6	E1
	6A	3	RAC875_c16649_322	9.06×10^{-4}	7.0	E3
	6A	32	wsnp_Ex_rep_c115803_95396724	3.84×10^{-4}	10.1	E2
	6A	32	RAC875_c26860_648	3.84×10^{-4}	10.1	E2
	1B	71	wsnp_Ex_c31567_40338517	3.40×10^{-4}	8.2	E3
	1B	71	wsnp_Ex_rep_c66883_65286958	3.40×10^{-4}	8.2	E3
	1B	71	Ku_c70461_480	3.40×10^{-4}	8.2	E3
	1B	71	Kukri_c27114_294	3.40×10^{-4}	8.2	E3
	1B	71	Kukri_c38105_143	3.40×10^{-4}	8.2	E3
	1B	71	Kukri_c38105_452	3.40×10^{-4}	8.2	E3
	1B	71	Kukri_c49015_1037	3.40×10^{-4}	8.2	E3
	1B	71	Kukri_c49015_1606	3.40×10^{-4}	8.2	E3
	1B	71	BobWhite_rep_c51642_1057	3.40×10^{-4}	8.2	E3
	1B	71	Ra_c45721_2630	3.40×10^{-4}	8.2	E3
	1B	71	BobWhite_c28635_785	3.40×10^{-4}	8.2	E3
	1B	71	BobWhite_c28635_896	3.40×10^{-4}	8.2	E3
	1B	76	Kukri_c49015_1700	3.40×10^{-4}	8.2	E3
	3B	67	BobWhite_c11540_60	3.38×10^{-4}	8.2	E3
	3B	68	BobWhite_c5968_868	4.18×10^{-4}	8.0	E3
	3B	69	RAC875_s115213_106	7.80×10^{-4}	7.4	E3
	3B	71	Excalibur_c5182_93	6.16×10^{-4}	7.5	E3
	3B	71	TA004228-0191	6.16×10^{-4}	7.5	E3
	3B	71	BS00024783_51	6.16×10^{-4}	7.5	E3
	3B	73	BS00066060_51	5.84×10^{-4}	7.6	E3
	5B	53	IACX5818	3.81×10^{-4}	7.2	E4
	5B	72	Tdurum_contig42301_1583	3.59×10^{-4}	8.6	E3
	5B	161	BS00065864_51	6.10×10^{-4}	6.7	E4
	6B	28	Excalibur_c39830_862	4.46×10^{-4}	9.9	E2
	6B	57	RAC875_c2730_620	9.38×10^{-4}	7.0	E3
	6B	114	RAC875_c49043_93	7.37×10^{-4}	8.1	E4
	5D	70	Kukri_c444_833	4.79×10^{-4}	7.6	E1
	6D	9	wsnp_Ku_c19587_29102203	5.73×10^{-4}	9.5	E2
	6D	9	RFL_Contig2815_1135	3.58×10^{-4}	10.2	E2
	6D	15	Excalibur_c10358_1800	3.62×10^{-4}	10.1	E2

E1 – grown in Tai'an, China in 2013, E2 – grown in Dezhou, China in 2013, E3 – grown in Tai'an, China in 2014, E4 – grown in Dezhou, China in 2014; R^2 – phenotypic variation contribution.

Tab. 8. Highly significant or of high genetic contribution and stable association marker-trait associations for colour-related traits in four cultivation environments.

Trait	Locus	Site	Marker	*p* value	R^2 [%]	Environment
Internal *L**	1D	68	tplb0044p22_2257	7.39×10^{-5}	13.0	E2
	2A	156	Kukri_rep_c76782_289	2.23×10^{-5}	10.6	E4
	7A	45	Kukri_c18677_823	1.34×10^{-6}	14.0	E4
	7A	228	BS00023200_51	6.89×10^{-4}	7.1	E1
				3.57×10^{-4}	9.3	E4
	7D	56	D_contig06359_118	8.65×10^{-5}	9.0	E4
Internal *a**	3B	61	BS00023037_51	7.25×10^{-4}	8.4	E3
				8.60×10^{-4}	7.3	E4
	4A	144	Tdurum_contig15260_591	1.09×10^{-4}	12.1	E2
	4A	144	Kukri_c50736_53	2.27×10^{-4}	10.9	E2
	4A	147	wsnp_Ku_c7102_12271493	2.07×10^{-4}	8.4	E1
				6.29×10^{-4}	9.3	E2
	4A	147	BobWhite_c3259_96	1.19×10^{-4}	12.0	E2
	7A	42	wsnp_Ex_c14654_22713386	4.92×10^{-5}	9.7	E4
	7D	56	D_contig06359_118	1.81×10^{-5}	10.7	E4
Internal *b**	1B	74	Tdurum_contig63834_340	3.05×10^{-4}	8.7	E1
				4.09×10^{-4}	7.6	E4
	2B	173	Excalibur_c22201_907	6.82×10^{-4}	6.3	E2
				5.76×10^{-4}	6.3	E4
External *L**	2A	156	Kukri_rep_c76782_289	2.23×10^{-5}	10.6	E4
	2B	116	BS00102614_51	1.79×10^{-5}	12.0	E3
	3A	24	Kukri_c53085_574	2.31×10^{-4}	10.9	E2
	5B	155	BobWhite_c6685_1922	3.15×10^{-5}	11.3	E3
	7A	42	wsnp_Ex_c14654_22713386	1.45×10^{-4}	8.3	E3
				2.92×10^{-4}	7.7	E4
	7A	45	Kukri_c18677_823	1.34×10^{-6}	14.0	E4
	7A	89	IAAV1940	3.56×10^{-5}	11.4	E3
	7A	89	BS00007429_51	3.16×10^{-5}	11.3	E3
	7A	89	RFL_Contig3271_810	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_328	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_522	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig20214_181	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig20214_279	3.16×10^{-5}	11.3	E3
	7A	89	BS00062706_51	3.16×10^{-5}	11.3	E3
	7A	89	Tdurum_contig14075_630	3.41×10^{-5}	11.2	E3
	7A	89	Kukri_c106476_350	3.41×10^{-5}	11.2	E3
	7D	56	D_contig06359_118	8.65×10^{-5}	9.0	E4
External *a**	1A	111	Tdurum_contig75762_377	8.58×10^{-4}	10.7	E2
	4A	147	wsnp_Ku_c7102_12271493	6.75×10^{-4}	7.0	E1
				3.87×10^{-4}	9.1	E2
				6.75×10^{-4}	7.4	E4
	6A	134	BS00110512_51	4.72×10^{-4}	7.4	E1
				4.72×10^{-4}	7.4	E4
	6A	141	Ra_c2235_3340	1.16×10^{-4}	11.7	E1
	6A	141	BS00075803_51	1.59×10^{-4}	10.5	E1
	6D	158	Excalibur_c43912_389	2.54×10^{-5}	13.8	E1
External *b**	1A	70	RFL_Contig4953_469	8.32×10^{-5}	10.6	E1
				9.15×10^{-5}	9.9	E3
	6A	32	wsnp_Ex_rep_c115803_95396724	3.84×10^{-4}	10.1	E2
	6A	32	RAC875_c26860_648	3.84×10^{-4}	10.1	E2
	6D	9	RFL_Contig2815_1135	3.58×10^{-4}	10.2	E2
	6D	15	Excalibur_c10358_1800	3.62×10^{-4}	10.1	E2

E1 – grown in Tai'an, China in 2013, E2 – grown in Dezhou, China in 2013, E3 – grown in Tai'an, China in 2014, E4 – grown in Dezhou, China in 2014; R^2 - phenotypic variation contribution.

Tab. 9. Site cluster for colour-related traits in four cultivation environments.

Trait	Locus	Site	Number of markers	*p* value	R^2 [%]	Environment
Internal *L**	3A	86–87	7	10^{-4}	6.9–7.6	E1
Internal *a**	1B	116	5	10^{-4}	7.7–8.2	E1
Internal *b**	1B	89–90	6	10^{-4}	6.0–7.6	E4
External *L**	5B	143–144	19	10^{-4}	6.9–8.7	E3
	7A	89	10	10^{-5}	11.2–11.4	E3
External *a**	6A	141	20	10^{-4}	7.5–11.7	E1
	7A	113	8	10^{-4}	6.9–7.4	E3
External *b**	1B	71	12	10^{-4}	8.2	E3

E1 – grown in Tai'an, China in 2013, E2 – grown in Dezhou, China in 2013, E3 – grown in Tai'an, China in 2014, E4 – grown in Dezhou, China in 2014; R^2 - phenotypic variation contribution.

to be polymorphic using the 90K iSelect chip, which were greater than the number of loci detected using traditional molecular markers targeted by amplified fragment length polymorphisms (AFLPs) or simple sequence repeats (SSRs) [32]. Therefore, SNP play a particularly important role in genotyping and genetic analysis.

In this study, we detected 23 MTAs for colour-related traits ($p < 0.0001$) on chromosomes 1A, 1D, 2A, 2B, 5B, 6D, 7A, 7D, which accounted for 9.0 % to 14.1 % of the phenotypic variation. The highly associated marker RFL_Contig4953_469 on chromosome 1A was a stable marker for external *b**. Liu et al. [33] detected 37 MTAs for stem rupture strength-related traits ($p < 0.0001$) on chromosomes 1A, 1B, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 5B, 5D, 6B, 7A, 7B, 7D, which accounted for 7.7 % to 26.3 % of the phenotypic variation. The highly associated marker Tdurum_contig4974_355 on chromosome 4B had a trait variation over 10 %. Among SNP markers detected in the current study, the highly significant or stable MTAs and multi-trait MTAs, such as the four highly significant markers for internal *a** and the stable MTA for external *b**, can be used to develop cleaved amplified polymorphic sequence markers for

Tab. 10. Multi-trait marker for colour-related traits in four cultivation environments.

Marker	Locus	Site	Marker R^2 [%]					
			Internal *L**	Internal *a**	Internal *b**	External *L**	External *a**	External *b**
Tdurum_contig60037_441	1B	57	6.8 (E4)			6.8 (E4)		
BS00064052_51	1B	123	7.3 (E4)			7.3 (E4)		
Kukri_rep_c76782_289	2A	156	10.6(E4)			10.6 (E4)		
Kukri_c13329_800	2D	37	7.8 (E4)			7.8 (E4)		
BS00104640_51	4A	146		9.5 (E2)		8.6 (E3)		
RAC875_c1126_1769	4A	146	6.8 (E4)			6.8 (E4)		
wsnp_Ku_c7102_12271493	4A	147		9.3 (E2)			7.0 (E1)	
Tdurum_contig4974_355	4B	61	8.6 (E4)	8.6 (E4)		8.6 (E4)		
Excalibur_c23433_474	4B	68		6.2 (E4)			8.4 (E4)	
IACX5818	5B	53			6.6 (E4)			7.2 (E4)
D_GDEEGVY01AU4CW_149	5B	161			7.5 (E3)		7.2 (E3)	
Ra_c2235_3340	6A	141		9.5 (E1)			11.7 (E1)	
IACX9279	6B	63		6.5 (E4)			6.5 (E4)	
wsnp_Ex_c14654_22713386	7A	42	7.7 (E4)	9.7 (E4)		7.7 (E4)		
Kukri_c7579_614	7A	136	6.7 (E4)			6.7 (E4)		
BobWhite_c3541_152	7B	82	9.7 (E4)			9.7 (E4)		
D_contig06359_118	7D	56	9.0 (E4)	10.7 (E4)		9.0 (E4)		

E1 – grown in Tai'an, China in 2013, E2 – grown in Dezhou, China in 2013, E3 – grown in Tai'an, China in 2014, E4 – grown in Dezhou, China in 2014; R^2 - phenotypic variation contribution.

marker-assisted selection and to identify colour-related candidate genes via bioinformatics analysis, such as multiple sequence alignments and gene annotations.

Genetic structure analysis

In this study, GWAS was applied using SNP markers for colour-related traits. GWAS was characterized by its high mapping resolution, which can help narrow down the chromosomal region of putative QTLs and predict causal genes [34]. False positive results caused by population stratification and kinship could be effectively eliminated in this study, through the use of mixed linear statistical model, the incorporation of Q matrix and K matrix of each individual (identified by the population structure analysis) into the regression analysis as covariate, and the use of a tight constraint on high threshold value, the threshold of statistical significance for the associations between loci and traits being set at $p < 0.0001$). As a result, the credibility of associated results has been greatly increased. It was also anticipated that the markers detected in different environments would not be completely consistent with each other, which is a prominent feature of quantitative traits controlled by multi-genes. Some SNPs within a gene could directly affect the structure of protein or the level of gene expression, while these SNPs might also become the functional sites of candidate genes. Compared with SSRs, SNPs may make closer contact with the functional genes [35].

CONCLUSIONS

Based on a GWAS, we identified 276 MTAs for colour-related traits in four environments. Among them, 23 MTAs were highly significant markers ($p < 0.0001$), 9 MTAs were stable markers and 17 MTAs were multi-trait markers. The highly significant MTAs, stable MTAs and multi-trait MTAs can be of practical use for the investigation on gene loci associated with steamed bread colour traits with those explaining a high percent trait variation having potential of increased genetic gain per selection cycle. This study also indicates that the combined use of GWAS, SNP markers and high-throughput technologies, such as the 90K iSelect chip, could be an efficient approach to gain insight into the genetic determinants that monitor the colour quality of consumer wheat-based foods. All these findings can be used in molecular marker-assisted breeding programmes.

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