



Identification of quantitative trait loci for seedling habit, grain color and cuticular wax from Tibetan semi-wild wheat (*Triticum aestivum* ssp. *tibetanum*)

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Abstract

Aiming at detecting possible potentials of Tibetan semi-wild wheat in breeding programs, we have identified several Quantitative Trait Loci (QTLs) for seedling habit, grain color and cuticular wax that are related to stress resistance such as cold resistance, pre-harvest sprouting and drought resistance using recombinant inbred line population derived from a cross between Tibetan semi-wild wheat (Q1028) and Zhengmai 9023 (ZM9023). A total of 17 QTLs were detected. Four QTLs were detected for seedling habits on chromosomes 2A, 2B, 2D, 6B, explaining 4.64%-26.40% of phenotypic variance. Of these, QTLs on chromosomes, 2A, 2B and 2D were stable across two growing years. Two stable QTLs were detected for grain color on chromosomes, 3A and 3B explaining 7.59%-12.81% of phenotypic variance. Five QTLs for spike wax, detected on chromosomes, 1B (2 QTLs), 2B, 2D and 5A explained 6.53%-13.20% of phenotypic variance, while six QTLs for stem wax were detected on chromosomes, 1A, 2B, 2D, 4A, 6A and 6B explaining 5.59%-21.03% of phenotypic variance. The QTLs identified in this study broadened our understanding on Tibetan semi-wild wheat and laid the foundation for their further utilization in breeding program in order to improve the cold resistance, pre-harvest sprouting and drought tolerance.

Key words: Tibetan semi-wild wheat, seedling habits, grain color, cuticular wax, stress resistance

Introduction

Common wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD) is one of the three major food crops in the world. A good stress tolerance/resistance in genotypes plays positive effects on improvement in yield and quality of wheat. Thus, attempts to enhance stress tolerance of wheat have always been an important task in breeding programs. In China, winter freezing

injury is one of the major natural disasters in wheat production. Breeding and development of new wheat varieties with cold hardiness is a most important strategy. The wheat seedling habits can be used as an index for identification of cold resistance (Liu 2005). Seedling habits can be divided into spreading (with high cold resistance), semi-spreading (with moderate cold resistance), semi-upright (weak cold resistance), and upright (weaker cold resistance). Thus, identification of seedling habits could be a simple method for screening genotypes with cold resistance.

Pre-harvest sprouting (PHS) or a tendency of grain to germinate in the spike prior to harvest is a worldwide natural disaster in wheat (Mares and Mrva 2014). It is reported that grain color could influence PHS/dormancy in wheat (Gerjets et al. 2009). Red kernel wheat appears to be more resistant to PHS than white kernel wheat. Thus, red-grain color has been used in wheat breeding programs as a morphological marker screening for resistance to PHS. Jiang et al. (2015) have earlier identified several quantitative trait loci (QTL) controlling seed dormancy. One of the objectives in the present study is to validate the genetic relationship between PHS and grain color by QTL analyses.

Cuticular wax is a common character in plants. It is likely to provide protection against water loss as well as abiotic and biotic stresses including drought (Fischer and Wood 1979), pests, pathogens and natural radiation (Jenks and Ashworth 1999; Misra and Ghosh 1991). In wheat, the surfaces of leaf, stem and spike

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are often covered by epicuticular waxes, thus showing a glaucousness characteristic (Jensen and Driscoll 1962). The expression of glaucousness depends on the arrangement of wax deposits rather than amount of wax (Johnson et al. 1983). The variation in presence of wax on leaf surface is scattered and is characterized by the presence of different homologues of carbon atoms ranging from C₂₀ to C₄₄ (Das et al. 1999). Genetics for each carbon homologue is expected to be differently governed. In one such study, Durgesh et al. (2009) reported that carbon homologue C₃₂ is controlled by a recessive allele and that the presence of wax was not scorable in F₁ but F₂ individuals showed quantitative difference in C-compounds, which corresponds to phenotypic score under field conditions. Several researchers have reported the wax producing genes and inhibitors for waxiness. Earlier work has also indicated that the wax producing genes and the inhibitors are allelic (Tsunewaki 1966). A number of researchers have identified quite a few of loci controlling the glaucous and non-glaucous phenotypes of wheat on chromosomes 1A, 1B, 1D, 2B, 2D, 3A, 4A, and 5A (Gadaleta et al. 2009; Li et al. 2016; Mason et al. 2010; Mondal et al. 2015; Wu et al. 2013; Zhang et al. 2015)..

Tibetan semi-wild wheat (*T. aestivum* ssp. *tibetanum*) is a hexaploid wheat collected from Tibet (Shao et al. 1980) which has been characterized by not only unique and primitive traits such as hulled glumes and brittle rachis (Jiang et al. 2015; Luo et al. 2016a) but also numerous desirable traits including tolerance to nutrient deficiency and strong seed dormancy, useful for improving common wheat (Jiang et al. 2014; Sun et al. 1998). Further efforts have been made to tap the advantages of Tibetan semi-wild wheat which led to identification of quite a few novel QTLs governing agronomic traits as well (Zhou et al. 2016; Luo et al. 2016b). A systematic work towards exploiting breeding potentialities of Tibetan semi-wild wheat in cultivar development, a study was undertaken to dissect several QTLs for wheat seedling habit, grain color, and cuticular wax that are related to stress resistance such as cold resistance, PHS, and drought resistance.

Materials and methods

Plant materials

A total of 186 RILs (generations varying between F₁₀ and F₁₂) derived from a cross between Tibetan semi-wild wheat accession Q1028 (Dong and Zheng, 2000)

and a common wheat cultivar Zhengmai 9023 (ZM9023, spring wheat) were used in this study. The Tibetan semi-wild wheat shows semi-upright seedling, produces red grain and the spike and stem are glaucous, whereas the seedlings of ZM9023 are semi-upright, the grain is white and the spike and stem are non-glaucous (Fig. 1). The RILs and parents were planted in the experimental farm of the Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China (average annual temperature, ~15.2 to 16.6°C; average annual rainfall, ~ 873 to 1265 mm and average relative humidity is ~70 to 80%). Each line was two 2m long with 30 cm distance between rows and 10cm spacing within rows. Field management followed common practices for wheat production without irrigation.

Phenotype evaluation

The seedling habits were assessed using 0 (spreading), 1 (semi-spreading), 2 (semi-upright), and 3 (upright) scale as described by Liu (2005). The color of the grain was determined after harvest and air drying. White grain was scored as 0 and the red as 1. Visual glaucousness on spike (spike wax) and stem (stem wax) was scored using a 0 (non-glaucousness) and 2 (glaucousness) scale after heading was completed. For each investigated trait, three to five plants were measured. The mean of the tested plants was used for further analysis. The data for these traits were collected from two wheat growing seasons (2014-2015 and 2015-2016). Frequency distribution was calculated using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL). The estimated broad-sense heritability of the investigated traits was calculated as follows: $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_e/r)$, where σ^2_G is the genetic variance, σ^2_e is the residual variance, and r is the number of replicates per genotype.

QTL mapping

A whole-genome genetic map constructed previously (Jiang et al. 2014) was adopted for QTL mapping. The genetic map was constructed using a population of 186 RILs. Five hundred and sixty-five of 2, 000 DArT and 117 of 1, 089 SSR polymorphic markers were screened for map construction. These markers were distributed in 22 linkage groups and covered a total genetic distance of 2, 727 cM (Jiang et al. 2014). The relative positions of contiguous markers were tested through maximum likelihood (ML) mapping. QTL analysis was initially conducted using QTL IciMapping version 3.2 which was followed by inclusive composite interval mapping (ICIM) (Wang et al. 2012). Thereafter,

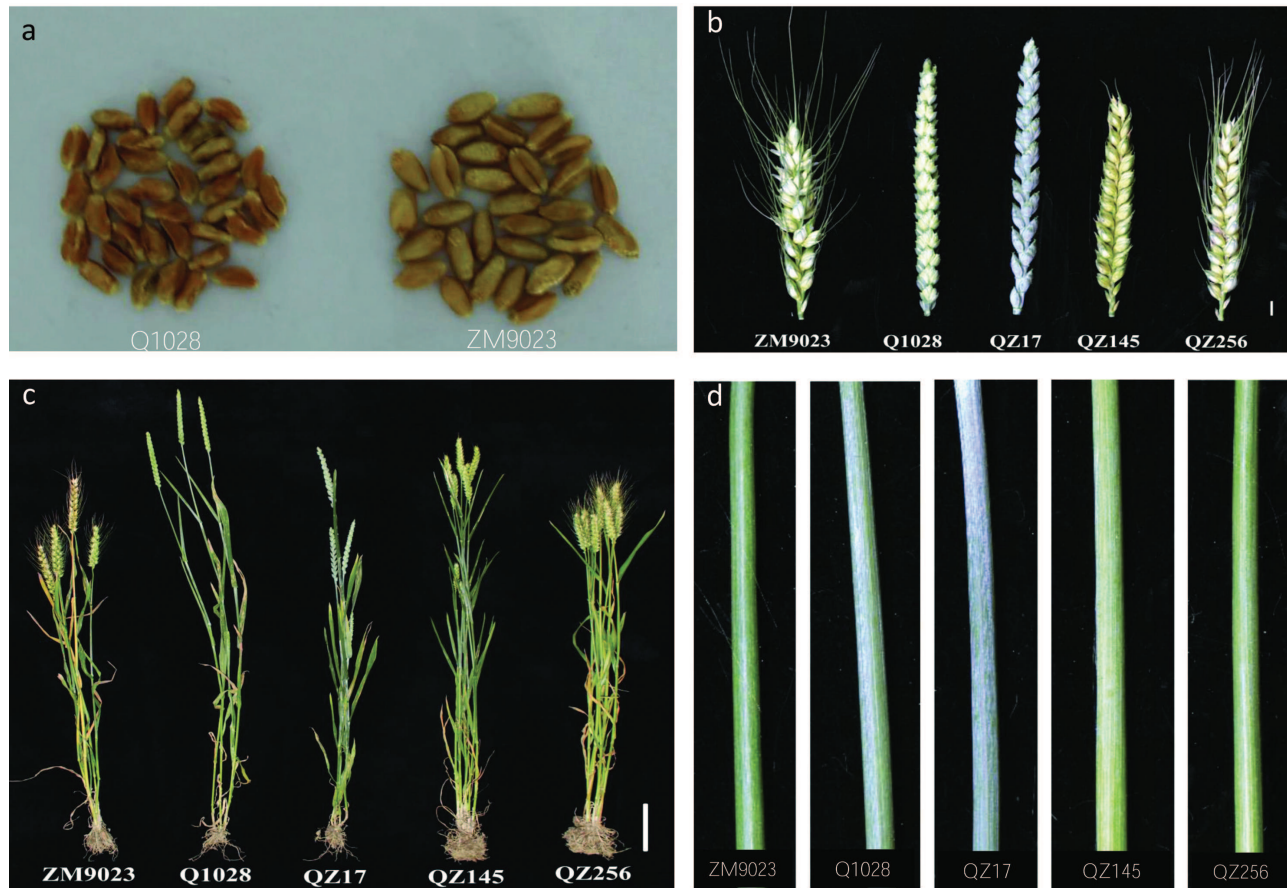


Fig. 1. Phenotypes of the parents and several lines a. grain color; b. spike wax, the scale bar represents 1 cm ; c. whole plant, the scale bar represents 10 cm; d. stem wax

single marker analysis (SMA) was applied to ascertain the QTLs' authenticity. LOD of 2.5 was used to determine the presence of a QTL (Lin et al. 1996). WinQTLCart 2.5 (North Carolina State University, Raleigh, NC, USA) was further used to detect QTL with CIMat a LOD threshold of 2.5.

Results and discussion

Efforts were made to tap possible potentials of Tibetan semi-wild wheat in breeding programs and we identified several QTLs for seeding habit, grain color and cuticular wax that are related to stress resistance such as cold resistance, PHS, and drought resistance. The frequency distributions of the investigated traits showed continuous variation with approximately normal distribution indicating that the traits are quantitative in nature and are suitable for QTL analysis (Fig. 2). The heritability values were 92% for seeding habits, 45% for grain color, 65% for spike wax and 58% for stem wax indicating that they are stable traits and mainly

controlled by genetic factors. As anticipated, the QTLs detected in the present study contain those that have been reported previously and some novel ones (Table 1). A majority of these QTLs were also detected at surrounding positions of the same chromosomes using WinQTLCart 2.5 (Supplementary Table 1 available online at <http://www.isgpb.co.in>), further indicating the reliability of the obtained results. The identification of these QTLs further increased our knowledge on Tibetan semi-wild wheat.

Three stable QTLs for seeding habits could be used for screening wheat for cold resistance

Four QTLs for seeding habits were detected. The QTLs on chromosomes 2A, 2B, and 2D were stably detected across two growing seasons and a QTL on 6B was detected in one growing season only. The phenotypic variation explained for these QTLs ranged from 4.61 to 26.4%. The positive alleles of the QTLs on 2D and 6B were contributed by ZM9023 and the remaining

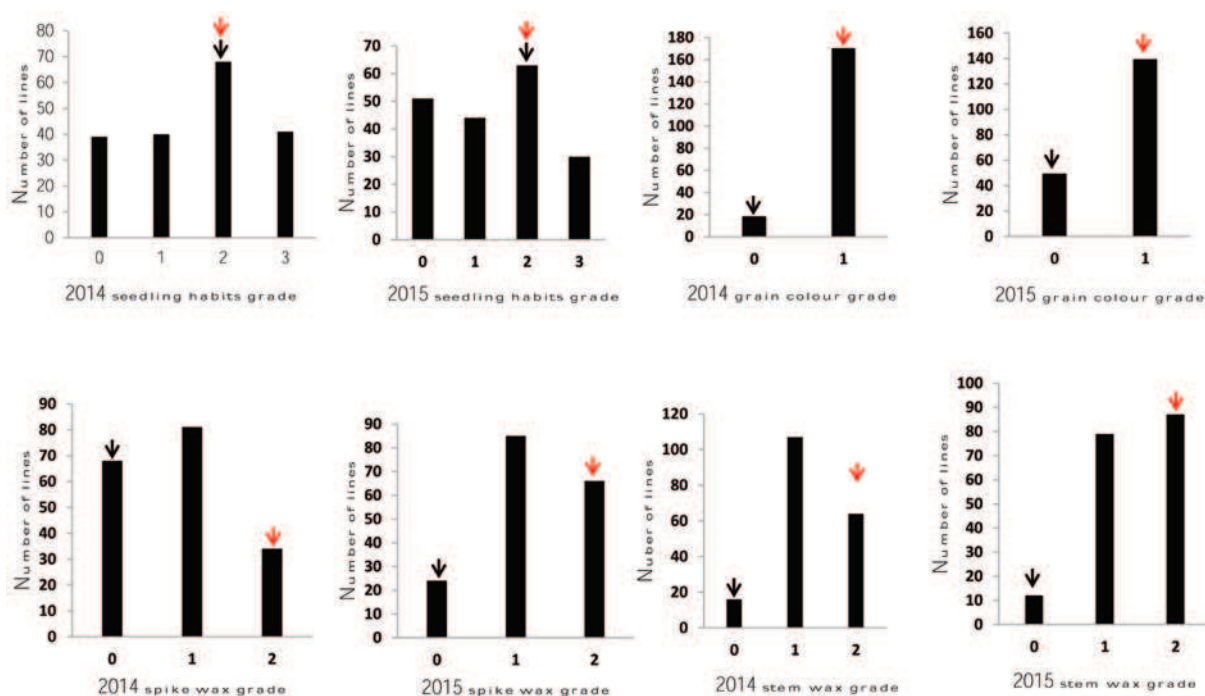


Fig. 2. Frequency distributions of the investigated traits from the RIL population Data was based on mean of the investigated experiments. The black arrow indicates the value of Q1028 and the red arrow indicates ZM9023

Table 1. Estimated additive of QTLs for the investigated traits

Trait	Year	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add
SH	2014	2A	24	gwm636	wPt-664128	4.51	7.49	0.29
	2014	2B	1	wPt-3561	wPt-6932	12.59	23.07	0.5
	2014	2D	193	wPt-3692	wPt-666518	4.62	7.83	-0.29
	2014	6B	75	wPt-2564	wPt-8183	2.88	4.64	-0.23
	2015	2A	24	gwm636	wPt-664128	2.81	4.81	0.23
	2015	2B	1	wPt-3561	wPt-6932	13.27	26.40	0.54
	2015	2D	193	wPt-3692	wPt-666518	4.1	7.28	-0.29
GC	2014	3A	187	wmc153	gwm155	6.22	12.81	0.11
	2014	3B	120	wPt-4222	wPt-10179	5.55	12.55	0.1
	2015	3A	189	wmc153	gwm155	3.16	7.59	0.12
	2015	3B	118	wPt-4222	wPt-10179	4	11.31	0.15
SPW	2014	1B	36	wPt-2075	wPt-3477	2.89	7.07	-0.19
	2014	2B	98	gwm210	wmc314	2.8	6.53	0.18
	2014	5A	66	wPt-7185	gpw4457	3.58	8.55	0.21
	2015	1B	8	wPt-1782	wPt-4574	2.9	10.91	-0.22
	2015	2D	197	wPt-666518	wPt-730613	3.11	7.83	0.19
	2015	5A	67	gpw4457	wPt-9094	5.17	13.20	0.25
STW	2014	1A	49	wPt-667634	wPt-1709	3.46	6.55	-0.16
	2014	2B	96	gwm210	wmc314	6.4	11.62	0.21
	2014	4A	14	gpw4182	gpw4448	3.4	6.27	0.15
	2014	6A	53	wPt-667618	gpw4257	4.13	8.05	0.17
	2014	6B	2	wPt-669607	wPt-5480	3.17	5.59	-0.14
	2015	2D	198	wPt-666518	wPt-730613	8.13	21.03	0.29
	2015	6B	1	wPt-1325	wPt-669607	3.54	8.04	-0.18

SH: seedling habit; GC: grain color; SPW: spike wax; STW: stem wax

QTLs were contributed by Q1028 (Table 1 and Fig. 3). The QTL on 2B was co-mapped with those for both heading date (HD) (*QHd.sau-2B*) and anthesis date (AN) (*QAn.sau-2B*) identified earlier by Luo et al. (2016b). In our earlier study, we also concluded that the two QTLs (*QHd.sau-2B* and *QAn.sau-2B*) were possibly controlled by photoperiod gene-B1 (*Ppd-B1*)

displaying their genetically co-locations on chromosome 2B (Luo et al. 2016b; Mohler et al. 2004). Taken together, it is most likely that the *Ppd-B1* was as well responsible for affecting the seedling habit controlled by the locus on 2B. The QTL on 2D and those for HD (*QHd.sau-2D2*) and AN(*QAn.sau-2D2*) were genetically co-mapped between markers wPt-

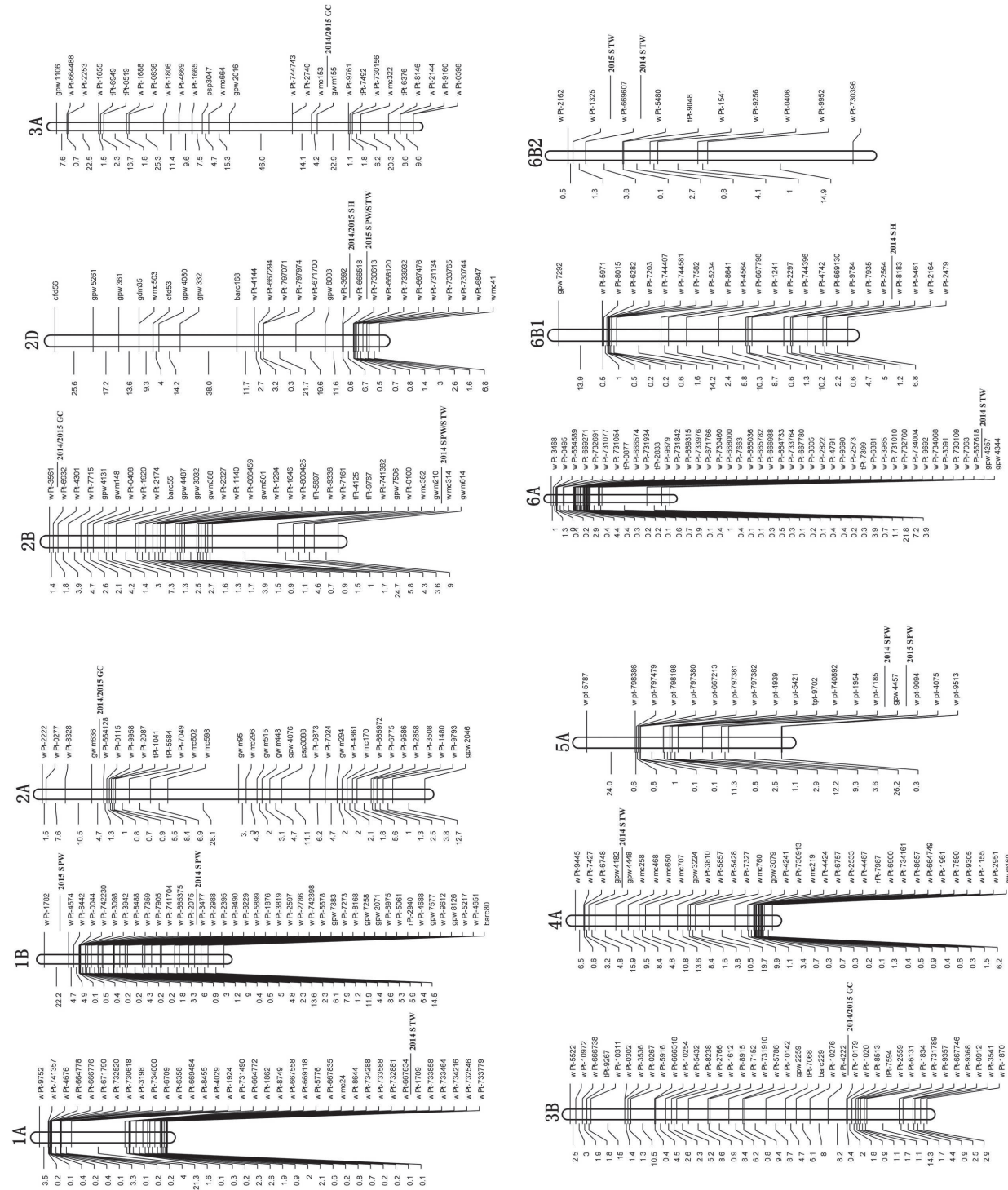


Fig. 3. Distribution of the identified QTLs SH: seedling habit; GC: grain color; STW: stem wax; SPW: spike wax; STW: stem wax

3692 and wPt-666518, indicating that they are likely controlled by a single locus. The other stable QTL on 2A was not co-mapped with any others for HD and AN. These results combined with those reported previously suggest that seedling habits were likely to be controlled genetically by complex genes. Liu (2005) observed that seedling habit was positively and significantly related with a good degree of cold injury: the more decumbent wheat seedling, higher will be the cold resistance. They further concluded that seedling habit could be used as an intuitionistic index for evaluating cold resistance. Thus the stable QTLs for seedling habits detected in this study could be useful for screening wheat genotypes with high cold resistance.

Genetic validation of the relationship between grain color and PHS

Two stable QTLs on chromosomes 3A and 3B were identified across two growing seasons. Each of these loci explained between 7.59 and 12.81% of the phenotypic variation. The positive alleles of these QTLs were contributed by Q1028 (Table 1 and Fig. 3). Previously, we employed tamyb10-genome-specific primers determined to be efficient for detecting *R-1* genes controlling grain color (Himi et al. 2011) to genotype the present Q1028 x ZM9023 RILs population (Jiang et al. 2015). The genetic locations of the *R-A1*(1.3 cM away from wmc153) and *R-B1*(4.2 cM away from wPt-4222) markers are consistent with those for the QTLs for grain color on 3A and 3B (sharing same flanking markers of wmc153 and wPt-4222), respectively, confirming the accuracy of the identified QTLs in the present study. Interestingly, we observed that two QTLs for PHS on 3A and 3B identified previously and those for grain color are genetically co-mapped between wmc153 and gwm155 (4.2 cM) and wPt-4222 and wPt-10179 (10.4 cM) (Jiang et al. 2015). The present finding validated earlier results that grain color could influence PHS in wheat (Gerjets et al. 2009). Jiang et al. (2015) have earlier mapped *R* homeologous gene *R-D1* on 3D. It is noteworthy that we failed to detect the locus for grain color gene on 3D in the present study. It is likely that the expression of *RD-1* was repressed and thus the QTL on 3D could not be detected.

Identification of possible novel QTLs for wax

Five QTLs for spike wax were identified explaining phenotypic variation ranging from 6.53 to 13.2%. Except the QTL on chromosome 5A being detected in two growing seasons, the others QTLs were detected on

1B, 2B and 2D in one growing season only (Table 1 and Fig. 3). The positive alleles of the QTLs on 1B were contributed by ZM9023 and the remaining QTLs were contributed by Q1028. Six QTLs mapped on chromosomes, 1A, 2B, 2D, 4A, 6A and 6B for stem wax were detected with phenotypic variation ranging from 6.27 to 21.03 %. The QTL on 6B was stably detected across two growing seasons and the others were identified in one season only (Table 1 and Fig. 3). The positive alleles of the QTLs on 1A and 6B were contributed by ZM9023 and those of the remaining QTLs were contributed by Q1028. To our knowledge, none of the QTLs for wax was identified on 6B before (Li et al. 2016) and the stable QTL on 6B in this study could be a novel locus. Given the importance of cuticular wax (Jenks and Ashworth 1999; Misra and Ghosh 1991), the next step of fine mapping and cloning this novel QTL is underway.

Authors' contribution

Conceptualization of research (JM, XJL); Designing of the experiments (JM); Contribution of experimental materials (XJL); Execution of field/lab experiments and data collection (WL, NNQ, XHZ, YMW, PFQ, QTJ); Analysis of data and interpretation (WL, NNQ, XHZ); Preparation of manuscript (JM, XHZ).

Declaration

The authors declare no conflict of interest.

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Supplementary Table 1. Estimated additive of QTLs for the investigated traits detected by WinQTLCart 2.5

Trait	Year	Chromosome	Position	Left Marker	Right Marker	LOD	Add
SH	2014	2A	23.8	wpt-8328	wpt-7094	4.0	0.3
	2014	2B	5.2	wpt-3561	gpw4131	10.0	0.5
	2014	2D	194.9	gpw8003	wpt-730744	4.3	-0.3
	2015	2D	194.9	gpw8003	wpt-730744	4.3	-0.3
GC	2014	2A	133.2	wpt-3508	wpt-1480	2.7	0.1
	2014	2A	146.2	wpt-9793	gpw2046	2.6	0.1
	2014	3A	187.0	wpt-744743	wpt-8761	7.6	0.1
	2014	3B	120.6	wpt-10276	wpt-10179	5.5	0.1
	2015	3B	116.1	wpt-10276	wpt-10179	3.4	0.1
	2015	5B	25.1	wpt-7101	wpt-428	2.8	-0.2
SPW	2014	1B	35.0	wpt-4574	wpt-9490	4.0	-0.2
	2014	2D	9.4	cf56	gpw5261	3.1	0.2
	2014	5A	66.5	wpt-7185	wpt-9094	4.9	0.2
	2015	1B	7.6	wpt-1782	wpt-4574	3.8	-0.2
	2015	2B	4.4	wpt-3561	gpw4131	12.1	0.5
	2015	2D	197.7	gpw8003	wpt-730744	3.8	0.2
	2015	2D	6.0	cf56	gpw5261	2.6	0.2
	2015	5A	67.4	wpt-7185	wpt-9094	4.9	0.2
STW	2014	2B	49.1	wpt-1294	wpt-1646	3.0	0.1
	2014	2B	57.1	wpt-9336	tpt-9767	2.8	0.1
	2014	2B	86.0	gpw7506	gwr614	6.7	0.2
	2014	5B	10.8	wpt-3298	wpt-8163	2.8	-0.2
	2014	6B1	102.5	wpt-2479	wpt-5069	2.9	0.1
	2014	7B	104.0	wpt-5069	wpt-6276	3.4	0.1
	2015	2D	198.4	gpw8003	wpt-730744	9.0	-0.3
	2015	6B1	84.7	wpt-8183	wpt-2479	4.0	0.0

SH: seedling habit; GC: grain color; SPW: spike wax; STW: stem wax