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REGULAR ARTICLE

QTL analysis for stomatal density and size in wheat spike organ

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ABSTRACT

Plant changes its own photosynthetic rate and transpiration rate through regulating stomatal aperture, stomatal density and stomatal distribution. In this study, stomatal density, length and width of wheat spike organs, including palea, lemma and glume, at the third day after flowering were investigated, using a wheat doubled haploid population from a cross of Hanxuan10 and Lumai 14 in 2012 and 2013. And quantitative trait loci (QTL) of the above three traits were analyzed. There were stomata in the abaxial surface of palea, lemma and glume, but not in the adaxial surface for DH lines and their parents. A total of fourteen additive QTLs for those traits were identified. On the marker interval Xgwm291-Xgwm410-WMC340 on chromosome 5A, Q_{MLsd}-*5A* for stomatal density at middle of lemma and Q_{DGsd}-*5A* for stomatal density at down of glume, and Q_{AGsl}-*5A* for stomatal length at apex of glume were detected in 2012 and 2013, but with opposite direction of additive effect. In the previous study, *Qsd-5A.3* and *Qsd-5A.4* for stomatal density of wheat leaf, and *Qsl-5A.1* for stomatal length of wheat leaf were also detected at the same marker region, and also with opposite direction of additive effect. These findings provided genetic basis for significantly negative correlation between stomatal density and length for wheat leaf and spike organs, but also implied stomatal density and length for wheat leaf and spike organs may be governed by the same or pleiotropic genes.

Keywords: Stomatal density; Stomatal length; Stomatal width; Spike organ; QTL

INTRODUCTION

Non-foliar green organs in wheat are another major source of photosynthesis, except for leaf organ. Specially, spike organs at the top of the canopy are conducive to the interception of photosynthetic CO_2 (Zhang et al., 2006). The photosynthetic product from spike organs is transported directly to the grain without waste (Zhang et al., 2008). Changes of physiological indexes reflecting the level of photosynthetic capacity, such as stomatal conductance, net photosynthetic rate, and transpiration rate, are mainly implemented through the stomata. Plant can change its own photosynthetic rate and transpiration rate through regulating stomatal aperture, stomatal density and stomatal distribution, when it is stressed by biotic or abiotic factors (Meidner 1986).

It was showed that stomata existed on all green parts of spike organs. Teare et al. (1971) found that there were stomata in the abaxial surface of glume, lemma, palea and awn, but not in the adaxial surface. Ziegler-Jons et al. (1989) recognized that there were stomata only in the adaxial surface of glume, in the abaxial and adaxial base of lemma in wheat. Zhang et al. (2006) thought there were stomata on different parts of spike organs. Obviously, there are different views about stomatal distribution of spike organs in wheat. So far, there were many reports of genetic analysis of traits related to stomata in leaf, for example, in rice, two QTLs for adaxial stomatal frequencies and two QTLs for abaxial stomatal frequencies in the middle part of the top fully expanded leaves were identified (Ishimaru et al., 2001a). Ten QTLs for stomatal density and four QTLs for size were detected across growth stages and leaf surfaces (adaxial and abaxial) (Laza et al., 2010). In wheat, twenty additive QTLs and 19 pairs of epistatic QTLs for stomatal density, stomatal length and width of leaf were identified under drought stress, the other twenty QTLs and 25 pairs epistatic QTLs were obtained under well-water at the heading, flowering, and mid- and late grain filling stages (Wang et al., 2016). However, there have not been about genetic analysis of traits related to stomata in spike organs. In this study, stomatal density, stomatal length and

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width in palea, apex of lemma, middle of lemma, apex of glume, middle of glume, down of glume were measured and QTLs for those traits were analyzed. The purpose was to gain insights into the changing pattern and molecular basis of stomatal density and size in spike organs.

MATERIALS AND METHODS

Plant material and field design

The wheat DH population, derived from a Hanxuan 10 (H10) ×Lumai 14 (L14) cross, comprised of 150 lines (Jing et al., 1999). All 150 lines and parents were grown at the experimental farm (37°25'N, 112°35'E, 799.6 m.a.s.l.) of Shanxi Agricultural University in 2012 and 2013. The experimental soil was sandy with, soil organic matter of 16.7 g kg-1, total nitrogen of 2.9 g kg-1, available phosphorus of 11.2 mg kg-1, available potassium of 110.7 mg kg-1.The field design consisted of randomized complete blocks with three replications. Each block was a length of 2 m and a width of 40 m. 150 lines and two parents with 0.25 m between rows and 40 seeds per row were sown. Irrigations with $650m^3/hm^2$ were applied at Zadoks growth stages (GS)22, 30, 40 and 65, respectively.

Measurement and calculation of stomatal density and size in spike organs

Three flowering plants in each line and parents were tagged. The three plants looked like the same. Middle spikes of the three plants were quickly placed into 2 mL centrifuge tube with FAA solution (alcohol 70%: acetic acid: formalin=18:1:1) 3 days after anthesis, respectively. Every tube was gently shaken, then placed in a refrigerator at 4C°.

After a spike was taken out, the first flower on the base was selected. The surfaces of all parts were cleaned using a degreased cotton ball dipped in alcohol, and then carefully smeared with a thin film of nail varnish on the under epidermis. When dry, the film was peeled from the leaf surface, mounted on a glass slide, and immediately covered with a cover slip.

Five views were selected in palea, apex of lemma, middle of lemma, apex of glume, middle of glume, down of glume, respectively. The numbers of stomata per view were scored, and stomatal lengths and widths were measured under a 40X objective lens of a photomicroscope fitted with objective and eyepiece micrometers. Stomatal averages of five view areas ($S=\pi r^2$, r=view radius) were calculated, and stomatal density was defined as N/S (number of stomata mm-2). Three random stomata per view were randomly selected for measuring lengths and widths which were then meant as the value for each plant.

Statistical analyses and QTL detection

Analyses of variance (ANOVA) of the data were conducted by the SPSS v.17.0 statistical package to assess the variances among DHLs and to calculate the mean, variance, standard deviation, coefficient of variation, kurtosis and skewness of DHLs for stomatal density and size and differences of the same traits between two years.

QTLs for stomatal density and size were detected using QTL Network 2.0 for composite interval mapping (CIM) of a mixed linear model. Significance levels of $P<0.001$ and $P<0.005$ were adopted for identifying additive effects of QTLs. QTLs were named according to the rule of "QTL + trait + chromosome + gene number".

RESULTS

Stomatal distribution of spike organs in wheat

There were stomata in the abaxial surface of palea, lemma and glume, but not in the adaxial surface for DH lines and their parents. For palea, there were two to three rows of stomata at the crease part of the edge of the abaxial surface and were covered by trichome. From the apex to the middle to the base of lemma, the row number of stomata changed from more to one to zero, and the density of stomata also became smaller. More rows of stomata existed at the edge of glume, and from the apex to the base, the row number of stomata became littler, the density of stomata gradually became smaller (Fig. 1).

Phenotypic variation in stomatal density and size in the DHLs and their parents

Except for apex, middle and down of glume of Lumai14 and down of glume of Hanxuan10 (Fig. 2), differences of stomatal densities for the rest parts of spike organs between

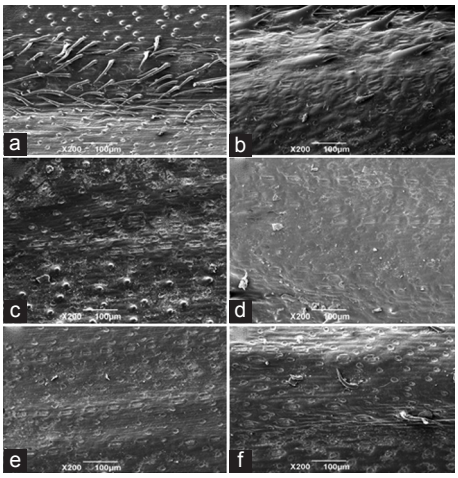

Fig 1. The distribution of stomata on spike organs of wheat. a: Palea, b: Apex of Lemma, c: Middle of Lemma, d: Apex of Glume, e: Middle of Glume, f: Down of Glume.

Table 1: The phenotype of stomatal density, length and width of spike organs in DH population and their parents

Trait	Environment	H10	L14	t	Min.	Mix.	Mean	SD	Skewness	Kurtosis	CV (%)
P_{SD}	2012	92.785a/A	81.395a/A	1.1837	71.167	111.507	88.722a/A	9.208	0.203	–0.473	10.31
	2013	82.556a/A	85.458a/A	1.8865	55.932	105.989	81.375b/B	7.885	0.029	0.507	9.79
AL_{SD}	2012	87.852a/A	76.607a/A	4.4745*	66.488	114.368	86.11a/A	8.331	0.231	0.334	9.56
	2013	86.981a/A	84.66a/A	7.6414*	69.788	101.781	84.270a/A	6.749	0.105	–0.392	8.01
ML_{SD}	2012	79.328a/A	59.088a/A	0.0642	48.75	113.098	71.62a/A	10.618	0.654	1.101	14.78
	2013	84.679a/A	61.754a/A	0.4724	48.968	99.242	67.252b/B	7.535	0.59	2.107	11.15
AG_{SD}	2012	88.686a/A	80.525a/A	0.8505	43.636	110.341	85.044a/A	10.488	–0.374	0.832	12.27
	2013	87.054a/A	73.016b/B	0.3065	62.026	105.771	79.438b/B	7.857	0.464	0.507	9.89
MG_{SD}	2012	76.172a/A	76.063a/A	23.372*	50.927	111.103	77.893a/A	10.461	0.297	0.243	13.36
	2013	81.069a/A	60.393b/B	2.6286	51.144	93.366	73.473b/B	8.259	0.04	–0.08	11.18
DG_{SD}	2012	72.254b/A	72.907a/A	82.2725**	42.439	104.356	70.367a/A	10.954	0.152	0.387	15.1
	2013	80.946a/A	62.026b/A	3.544	39.174	104.9	68.269a/A	10.5	0.107	0.768	15.10
P_{SL}	2012	47.083a/A	44.166a/A	1.089	36.25	49.625	42.933b/A	2.463	0.126	0.18	5.75
	2013	44.479a/A	43.125a/A	0.7559	37.727	47.875	43.178a/A	1.849	0.316	0.231	4.28
AL_{SL}	2012	48.541a/A	46.77a/A	0.072	41.354	54.327	47.092a/A	2.306	–0.065	0.166	4.89
	2013	48.333a/A	46.77a/A	0.1677	42.125	51.5	46.567a/A	1.882	0.128	–0.439	4.04
ML_{SL}	2012	47.708a/A	47.083a/A	1.4245	40.781	51.458	46.581a/A	2.008	–0.294	0.276	4.31
	2013	47.604a/A	45.52a/A	3.355	40.5	51.5	45.806b/B	1.9	0.114	–0.072	4.15
AG_{SL}	2012	45.104a/A	47.083a/A	0.8505	39.546	50.938	45.719a/A	2.304	–0.125	–0.464	5.03
	2013	44.583a/A	44.687a/A	0.3065	40.625	49.75	45.664a/A	2.066	–0.163	–0.486	4.52
MG_{SL}	2012	47.916a/A	46.354a/A	23.3720*	39.375	52.188	46.014a/A	2.227	–0.07	–0.203	4.85
	2013	45a/A	46.25a/A	2.6286	40.5	51	46.082a/A	2.206	–0.316	–0.193	4.78
DG_{SL}	2012	48.229a/A	46.458a/A	82.2725**	38.281	50.833	45.22a/A	2.56	–0.106	-0.32	5.67%
	2013	43.645b/A	46.562a/A	3.544	38.125	50	44.708a/A	2.38	–0.006	–-0.502	5.32
P_{SW}	2012	26.25a/A	24.687a/A	0.661	20.833	29.25	25.64a/A	1.303	–0.41	1.752	5.08
	2013	25.208a/A	24.791a/A	3.2167	21.477	29.75	24.603b/B	1.06	1.055	4.471	4.30
AL_{SW}	2012	29.479a/A	28.125a/A	1	23.75	33.462	27.239a/A	1.775	0.843	1.038	6.49
	2013	25.416b/A	25.208a/A	1.8715	23.854	30.5	25.921b/B	1.013	1.095	2.356	3.91
ML_{SW}	2012	26.562a/A	26.25a/A	0.9116	24.063	34.583	27.062a/A	1.623	1.212	2.975	5.99
	2013	26.77a/A	25.52a/A	3.6056	23.75	29.625	25.967b/B	1.067	0.748	0.663	4.11
AG_{SW}	2012	26.666a/A	33.75a/A	0.8505	24.375	37.789	29.133a/A	2.327	0.915	1.608	7.97
	2013	29.375a/A	28.125b/A	0.3065	25.125	33.5	27.662b/B	1.29	1.237	2.861	4.66
MG_{SW}	2012	29.652a/A	30a/A	23.372*	25.625	38.125	29.499a/A	2.123	0.703	1.218	7.17
	2013	28.229a/A	28.75a/A	2.6286	24.5	32.969	27.883b/B	1.565	0.482	0.518	5.61
DG_{SW}	2012	30a/A	30.729a/A	82.2725**	24.531	37.604	29.271a/A	2.384	0.625	0.39	8.11
	2013	28.645a/A	31.562a/A	3.544	23.75	34.375	27.484b/B	1.868	0.97	1.437	6.77

SD, stomatal density; $_{SL}$, stomatal length; $_{SW}$, stomatal width; P, palea; AL, apex of lemma; , ML, middle of lemma; , AG, apex of glume ; MG, middle of glume; DG, down of glume; SD, standard deviation; CV, coefficient of variation; * and ** indicate significance at P<0.05 and P<0.01, respectively

the two years were not significant. For DH lines, stomatal densities of the rest parts in 2012 were significantly more than those in 2013, except for apex of lemma and down of glume (Table 1).

Except for down of glume of Hanxuan10, differences of stomatal length for the rest parts of spike organs between the two years were not significant for the two parents. Differences of stomatal length for the rest parts between the two years were also not significant for DH lines, except for palea and middle of lemma (Table 1).

Except for apex of lemma of Hanxuan10 and apex of glume of Lumai14, differences of stomatal width for the rest parts of spike organs between the two years were not significant for the two parents and DH lines (Table 1).

Correlation for stomatal density and size

For all parts of spike organ**,** stomatal density showed a highly significant negative correlation with stomatal length in 2012 and 2013. But correlation between stomatal density and stomatal width was not significant. Stomatal length was significant and highly significant positive correlation with stomatal width in 2012, and except for middle of lemma, apex and down of glume in 2013 (Table 2).

Additive QTL for traits related to stomata in wheat spike organ

Stomatal density and size of spike organs for DHLs showed continuous transgressive segregation with smaller skewed value and kurtosis values (Table 1), suggesting normal distribution. All target traits were thus quantitatively controlled by multiple genes and were suitable for QTL mapping.

Table 2: Correlation analysis of stomatal density, stomatal length and stomatal width of various parts of spikelet in different years

Trait	Environment	P_{SD}	AL_{SD}	ML_{SD}	AG_{SD}	MG_{SD}	DG_{SD}	P_{SL}	AL_{SL}	ML_{SL}	AG_{SL}	MG_{SL}	DG_{SL}
P_{SW}	2012	0.09[2])	–0.08	–0.04	–0.13	0.06	0	0.18*	0.19*	0.27**	0.15	0.19*	0.18*
	2013	0	–0.17*	–0.04	–0.16	–0.14	–0.05	0.41**	0.21**	0.17*	0.07	0.23**	0.24**
AL_{SW}	2012	0.05	–0.01	0.13	0.1	0.08	0	0.14	0.20*	0.12	0.19*	0.14	0.18*
	2013	–0.06	–0.13	–0.04	–0.03	0.03	0	0.16	0.22**	0.11	0.14	0.16	0.19*
ML_{SW}	2012	0.03	–0.04	0.06	–0.09	0.01	–0.02	0.13	0.27**	0.20*	0.27**	0.20*	0.16
	2013	–0.02	–0.11	0.06	–0.03	0.06	0.06	0.27**	0.18*	0.16	0.12	0.12	0.29**
AG_{SW}	2012	0.15	–0.02	0.11	–0.09	0.1	0.09	0.13	0.26**	0.27**	0.17*	0.21*	0.17*
	2013	0.07	–0.13	0	–0.1	–0.09	0.12	0.1	0.15	0.13	0.04	0.11	0.18*
MG_{SW}	2012	0.13	–0.05	0.08	0.01	0.08	0.08	0.11	0.30**	0.32**	0.21**	0.21*	0.26**
	2013	–0.01	–0.19*	–0.08	–0.04	–0.04	0.04	0.11	0.25**	0.18*	0.12	0.24**	0.13
DG_{SW}	2012	0.19*	–0.12	0.07	–0.01	0.02	–0.01	0.09	0.26**	0.22**	0.20*	0.20*	0.26**
	2013	0.09	–0.06	0.13	–0.09	0.03	0.01	–0.06	0.08	0.04	0.03	0.13	0.05
P_{SL}	2012	–0.20*	–0.41**	–0.35**	–0.46**	–0.33**	–0.27**						
	2013	–0.30**	–0.25**	–0.22**	–0.29**	–0.24**	–0.16						
AL_{SL}	2012	–0.11	–0.48**	–0.29**	–0.38**	–0.33**	–0.22**						
	2013	–0.17*	–0.51**	–0.18*	–0.29**	–0.27**	–0.15						
ML_{SL}	2012	0.19*	–0.34**	–0.35**	–0.29**	–0.20*	–0.21**						
	2013	–0.16*	–0.41**	–0.43**	–0.30**	–0.32**	–0.31**						
AG_{SL}	2012	0	–0.44**	–0.33**	–0.46**	–0.37**	–0.34**						
	2013	–0.20*	–0.53**	–0.31**	–0.45 **	–0.43**	–0.27**						
MG_{SL}	2012	–0.02	–0.50**	–0.43**	–0.50**	–0.51**	–0.47**						
	2013	–0.14	–0.39**	–0.28**	–0.33**	–0.43**	–0.41**						
DG_{SL}	2012	0	–0.37**	–0.34**	–0.40**	–0.41**	–0.38**						
	2013	–0.12	–0.33**	–0.24**	–0.23**	–0.26**	–0.31**						

SD, stomatal density; $_{SL}$, stomatal length; $_{SW}$, stomatal width; P, palea; AL, apex of lemma; , ML, middle of lemma; , AG, apex of glume ; MG, middle of glume; DG, down of glume; SD, standard deviation; * and ** indicate significance at P<0.05 and P<0.01, respectively

Table 3: Additive effect QTL for stomata-related traits of spike organs in DH population

Traits	QTL	Marker interval	2012				2013			
			Position [a]	A[b]	P-value	H^2 [c]	Position [a]	A[b]	P-value	H^2 [c]
P_{SD}	*QP_{sd}-5B*	Xgwm371-Xgwm335	46.5	-2.61	0.000018	11.05%				
AL_{SD}	*QAL_{sd}-5A-1*	Xgwm410-WMC340	109.5	4.99	0.000017	11.10%				
	QAL_{sd}-5A-2	WMC410-WMC74					21.2	2.3037	0.000574	2.30%
	QAL_{sd}-5D	Xgdm68-Xgdm3					11.5	-2.336	0.000463	8.35%
ML_{SD}	*QML_{sd}-5A*	Xgwm291-Xgwm410	79.1	4.537	0.000047	10.04%	79.1	3.9435	0.0000	15.06%
AG_{SD}	*QAG_{sd}-5A*	Xgwm410-WMC340	109.5	5.921	0.000058	9.86%				
MG_{SD}	*QMG_{sd}-5A-1*	WMC410-WMC74	37.2	4.211	0.000027	10.48%				
	QMG_{sd}-5A-2	Xgwm410-WMC340					109.5	6.2509	0.0000	17.72%
DG_{SD}	*QDG_{sd}-5A*	Xgwm291-Xgwm410	72.1	4.57	0.000021	10.73%	74.1	6.1264	0.0000	19.85%
ML_{SL}	*QML_{sl}-5A*	P3616.5-P3616.6					54.4	0.5265	0.001745	10.12%
AG_{SL}	*QAG_{sl}-5A*	Xgwm410-WMC340	108.5	-1.61	0.0000	15.33%	105.5	-1.121	0.000054	9.93%
DG_{SL}	*QDG_{sl}-3B*	P3156.1-P5138					163.4	1.1792	0.000817	6.98%
ML_{SW}	*QML_{sw}-2A*	Xpsp3088-WMC296					57.4	-0.58	0.000134	8.97%
AG_{SW}	*QAG_{sw}-4B*	WMC47-3459.1	121	-0.96	0.000098	9.30%				

[a] Positions (cM) represents the distance to the first marker in the linkage group; [b]A represents the additive effect. Positive value indicates the Hanxuan 10 allele having positive effect on the trait, and negative value represents Lumai 14 allele having positive effect; [c] H2(A) indicates the phenotypic variance explained by additive QTL.$_{SD}$, stomatal density; $_{SL}$, stomatal length; $_{SW}$, stomatal width; P, palea; AL, apex of lemma; , ML, middle of lemma; , AG, apex of glume ; MG, middle of glume; DG, down of glume; SD, standard deviation.

A total of nine additive QTLs for stomatal density of spike organ were detected in two years. Phenotypic variation of these QTLs ranged from 2.30 to 19.85%. Among these QTLs, *$Q_{ML.sd}$-5A* and *$Q_{DG.sd}$-5A* were detected in 2012 and 2013, with additive effects from favorable alleles of H10. *QP_{sd}-5B*, *QAL_{sd}-5A-1*, *QAG_{sd}-5A* and *QMG_{sd}-5A-1* were detected in 2012, the rest three QTLs were mapped in 2013(Table 3).

Three additive QTLs for stomatal length of spike organ were detected in two years with phenotypic variation range from 6.98% to 15.33%. *QAG_{sl}-5A* detected in 2012 and 2013 were at the same marker region with *$Q_{ML.sd}$-5A* and *$Q_{DG.sd}$-5A*, but with opposite direction of additive effect. The rest two QTLs were mapped in 2013 (Table 3).

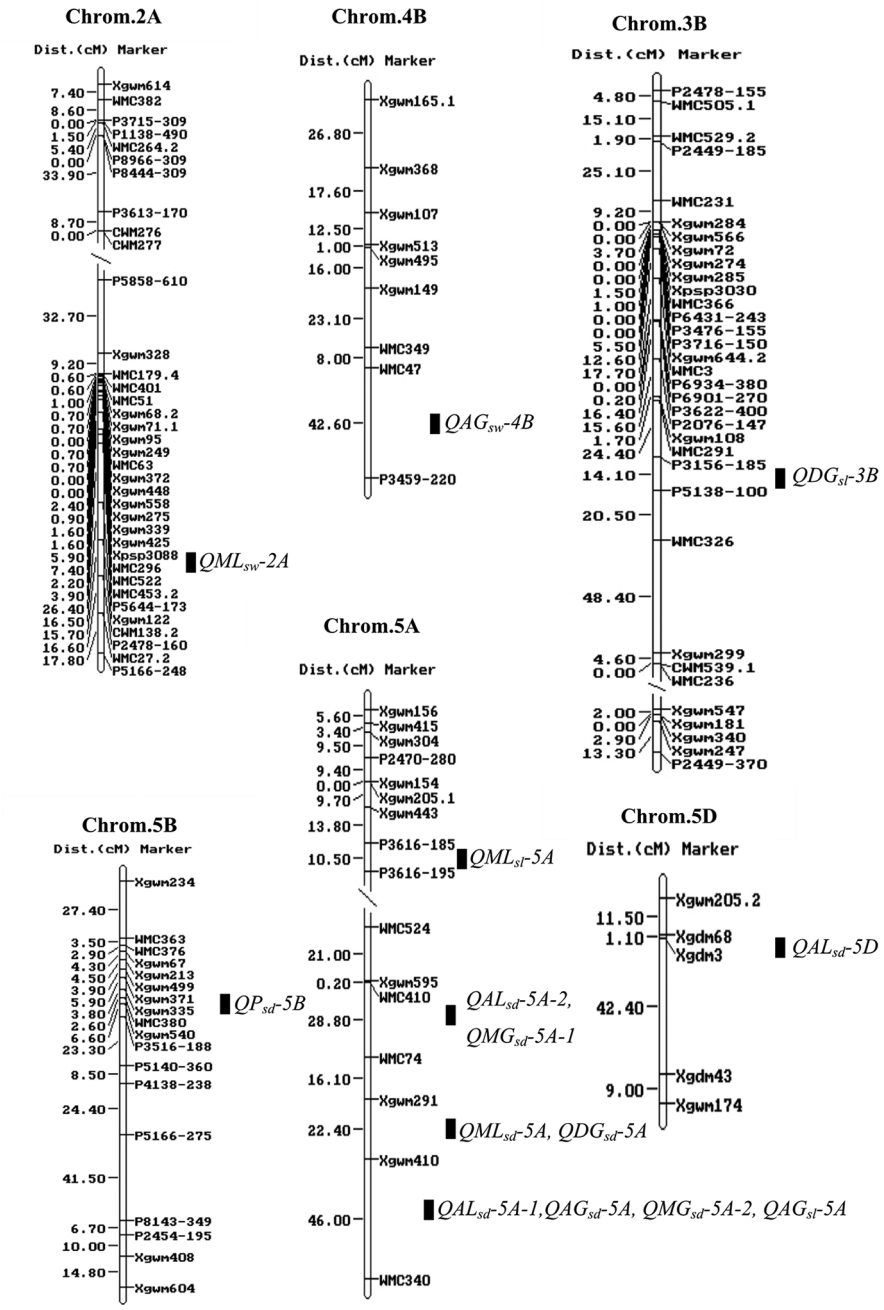

Fig 2. The distribution of QTLs for traits related to the stomata of wheat spike organs on genetic linkage groups constructed on the DH population (produced from the Hanxuan 10×Lumai 14 cross).

One additive QTLs for stomatal width of spike organ were detected in 2012 and 2013, respectively (Table 3).

DISCUSSION

Stomatal density and size in rice leaves are strongly negatively correlated (Ishihara et al., 1979; Ishimaru et al., 2001a; Ohsumi et al. 2007). Stomatal density was also negatively correlated with stomatal length under different water conditions in jujube (Liu et al., 2006) and *Platanus acerifolia* leaves (Zhang et al., 2004). The present study found stomatal density showed a highly significantly negative correlation with stomatal length for all parts of spike organ in 2012 and 2013. But correlation between stomatal density and stomatal width was not significant. Stomatal length was significantly or highly significantly positive correlation with stomatal width in 2012, and except for middle of lemma, apex and down of glume in 2013. In the previous study, we found that stomatal density of wheat leaf was always significantly and negatively correlated with stomatal

length at the heading, flowering, and mid- and late grain filling stage, but a negative relationship between stomatal density and width was significant only at heading under well-watered condition and at flowering and late grain filling under drought stress. Stomatal length and width of wheat leaf showed positive and significant correlations, except at heading under drought stress and flowering under well-watered (Wang et al., 2016). Therefore, all green organs of plant can improve their adaptability to different environment conditions through co-ordinated or compensatory variation in stomatal density and length, stomatal density and width, or stomatal length and width. On the other hands, stomatal density and width of wheat spike organs in 2012 were more than those in 2013, but the difference of stomatal length between the two years was not significant. These results indicated that stomatal density and width may have higher plasticity than stomatal length in response to different environments.

Tanksley et al. (1996) thoughtthat main effect QTL can explain more than 10% of the phenotypic variation. In this study, a total of fourteen additive QTLs were detected for traits related to stomata of spike organs. Eight of these QTLs explained more than 10% of the phenotypic variation, suggesting that all target traits were quantitative and co-controlled by major and minor genes. In the present study, among the nine additive QTLs for stomatal density, seven increasing stomatal density were alleles from H10. Except for QAG_{sl}-*5A,* the rest two additive QTLs increasing stomatal length were also from H10 alleles. But two additive QTLs increasing stomatal width were from L14 alleles (Table 3). The findings confirmed large genetic differences existed between the two parents.

Various studies have found that QTLs for closely correlated traits may be located at, or near, the same chromosomal positions (Hervé et al., 2001; Fracheboud et al., 2002; Tuberosa et al., 2002). Phenotypic correlations between stomatal density and length were found in our work and in previous studies (Zhang et al., 2004; Wang et al., 2016). In the present study, there were Q_{MLsd}-*5A* for stomatal density at middle of lemma and Q_{DGsd}-*5A* for stomatal density at down of glume, and Q_{AGsl}-*5A* for stomatal length at apex of glume were detected on the marker interval Xgwm291-Xgwm410-WMC340 on chromosome 5A in 2012 and 2013, but with opposite direction of additive effect. Previously, *Qsd-5A.3* and *Qsd-5A.4* for stomatal density of wheat leaf, and *Qsl-5A.1* for stomatal length of wheat leaf were also mapped at the same marker region, similarly with opposite direction of additive effect (Wang et al., 2016). These findings implied stomatal density and length for wheat leaf and spike organs may be governed by the same or pleiotropic genes, and once again provided the molecular basis for close correlation between stomatal density and length.

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Author's contributions

Shuguang Wang and Fanfan Dong conducted the experiment, analyzed the results, discussed the results, and drafted the manuscript. Daizhen Sun supervised and designed the research. Yaoyu Chen and Xue Yan analyzed the results and made figures. Ruilian Jing provided materials and reviewed the manuscript.

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SUPPLEMENTARY

Supplementary Table 1: SSR marker sequences linked with QTLs detected in the study

Marker	Forward primer	Reverse primer
Xgwm371	GACCAAGATATTCAAACTGGCC	AGCTCAGCTTGCTTGGTACC
Xgwm335	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC
Xgwm410	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA
WMC410	GGACTTGAAAGGAAGCTTGTGA	CATGGATGGCATGCAGTGT
WMC74	AACGGCATTGAGCTCACCTTGG	TGCGTGAAGGCAGCTCAATCGG
Xgdm68	GCCTGACCACTCCCATAAAA	TCGGAAGGGGGACTATACAA
Xgdm3	GTATCTCGGTGATGCAGCAA	GTGTGATGTTTGAATACGCA
Xgwm291	CATCCCTACGCCACTCTGC	AATGGTATCTATTCCGACCCG
Xpsp3088	GTGGTGTTACTTTGTAGGTTTCTCC	GGACCATTGGTATGTTTTCTAGTC
WMC296	GAATCTCATCTTCCCTTGCCAC	ATGGAGGGGTATAAAGACAGCG
WMC47	GAAACAGGGTTAACCATGCCAA	ATGGTGCTGCCAACAACATACA