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QTL MAPPING OF PLANT HEIGHT, SPIKE LENGTH, PEDUNCLE LENGTH AND NUMBER OF GRAINS PER PLANT IN BARLEY (*HORDEUM VULGARE* L.) USING 'STEPTOE/MOREX' DH POPULATION GROWN IN NORTHWEST OF CHINA

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Abstract

In the present study, 120 out of 150 barley double haploid lines, obtained by crossing 'Steptoe' x 'Morex', were screened for the 4 traits including plant height, peduncle length, spike length and grains plant^{-1}, during three crop years in the area of Northwestern China. Quantitative trait loci (QTL) mapping was performed from the available genotype datasets using the 4.1Version of QTL IciMapping. In total, 15 QTLs were mapped on six linkage groups (1H, 2H, 3H, 4H, 5H and 7H) which explained 5.42% to 47.27% phenotypic variation. Four QTLs were recognized in three years for plant height including two QTLs of major impact, one on chromosome 2H in 2014-15 and 2015-16, and the other was detected only in 2016-17 on chromosome 4. While for spike length, four QTLs were found. Six QTLs were identified underlying the trait peduncle length with three major QTLs, two on chromosome 2H in 2015-16 and 2016-17, and one on 3H in 2014-15 and 2015-16. One major QTL for the trait grains plant^{-1} was identified in 2015-16. Our study could lay the foundation for the fine mapping of agronomic traits studied and could increase the efficiency of marker assisted selection (MAS) in barley breeding programs aiming at improvement of these traits.

Key words: Double haploid, Plant height, Spike length, Peduncle length, No. of grain plant^{-1}, QTL; Genetic mapping, barley.

Introduction

Barley is one of the oldest domesticated cereal crops and ranks the fourth in production after maize, rice and wheat (Horsley *et al.,* 2009; Yangcheng *et al.,* 2016). It is well-adapted to drought conditions and can be grown normally in moisture stress and poor soil conditions, where performance of other cereals is not good (Shakhatreh *et al.,* 2010). Besides its economic importance, barley has remained a model species in cereal genetic and physiological studies due to the diploid nature and rich genetic resource (Koorneef *et al.,* 1997). Many important traits are quantitatively inherited in nature (Teng *et al.,* 2002). It is quite pivotal to clone the functional genes which control the agronomic traits of importance in barley that not only contribute to barley genetic improvement, but also provide the potential reference for other cereal crops. Quantitative trait loci (QTL) mapping is an efficient method for genetic studies of these traits. In this method, segregation of polygenic characters and molecular markers is simultaneously investigated and the QTL location in the genome is detected along with number of genes, their gene action and the rate of gene, which could be transferred into desired cultivars or lines (Hayes *et al.,* 1993; Yin *et al.,* 2003). Molecular markers are particular components of DNA, which are efficient and useful tools in the improvement of major and minor crop species (Rao, 2014). Marker-assisted selection is thus of importance in the exploitation of markers linked to various QTLs of importance in the evolution of improved cultivars. QTL analysis is widely applied to many crops including barley (Sharma *et al.,* 2011; Wang *et al.,* 2014, Liu *et al.,* 2015; Zhang *et al.,* 2017). These investigations have resulted in the identification of many QTLs underlying complex agronomical and morphological traits, yield components, disease resistance, tolerance to abiotic stresses and malting quality. Rao *et al*., (2007), Siahsar *et al*., (2009), Siahsar & Aminfar (2010), Mehravaran *et al*., (2014), Shahraki & Fakheri (2016) worked on QTL analysis in Double Haploid population derived from 'Steptoe' x 'Morex' crosses. However, the potential application of QTLs mapping based on different types of molecular markers or morphological traits in determining DH population in barley is yet inconclusive. Hence in the present study, QTLs mapping of some important barley traits has been performed in a "Steptoe x Morex" DH population for its importance in marker assisted (MAS) breeding programs.

Materials and Methods

Plant materials and genotyping data: In this study 120 out of 150 DH Lines were used along with their parents 'Steptoe' and 'Morex', which were kindly provided by NABGMP (the North American Barley Genome Mapping Project). Parent 'Steptoe' had smaller values of studied traits than the parent Morex. (http://www.css.orst.edu/barley/nabgmp/qtlsum.htm). The "Steptoe × Morex" DH genetic population is a most widely used barley mapping population on the world level, which has been greatly genotyped with Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) markers. Additionally, "Steptoe × Morex" genetic map is the reference map of the NABGMP in which many QTLs controlling agronomic and malting traits are mapped. The genotyping data with 437 SSR and RFLP markers (Kleinhofs *et al.,* 1993) was downloaded from Grain gene database (http://wheat.pw.usda.gov/ggpages/SxM/) and used in constructing the genetic map and the genotyping data of the selected 120 lines extracted for QTL mapping.

Phenotypic data collection of morphological traits: All the lines and parents were sown in the trial field of NWSUAF (N34.28, E108.06) under rain-fed conditions in three crop seasons 2014-15, 2015-16 and 2016-17, respectively. The DH lines were arranged in Randomized Complete Block Design with three replications and a plot size of $1m^2$. The plant height, peduncle length and spike length of each line was measured manually with the engineering rule and the grains plant^{-1} were counted from 10 plants per replication. QTL mapping was done using the Version 4.1 of QTL IciMapping (http://www.isbreeding.net/ software/, access by 2016-3-8) software with the inclusive composite interval mapping of additive (ICIM-ADD) and epistatic QTL (ICIM-EPI) methods. 1.0cM steps were employed in the detection of additive QTLs. The stepwise regression was adopted with the significance probability set at 0.001. 1000 permutations were used to determine significant LOD thresholds. Type I error was set at $p<0.05$. Epistatic QTLs were recognized by using a scanning step of 5.0cM with a probability of 0.0001 in stepwise regression with a significant LOD threshold of 3.0.

Results

The results of statistical analysis of the double haploid population and their parents for the studied traits are presented in Table 1. The height of genotype 'Morex' was taller than 'Steptoe' and height of DH lines ranged from 71.00 cm to 133.00 cm. The evaluation of spike length showed that 'Morex' had longer spikes than 'Steptoe' in all the three years. In DH lines it varied between 2.66 cm and 12.00 cm in three years. These lines varied extensively for this trait. The data showed that 'Morex' had longer peduncles than 'Steptoe' in two years. The peduncle length of DH lines varied from 17.16 cm to 48.60 cm in three years. There was sufficient variation in the peduncle length of DH lines. The value for the trait number of grains plant^{-1} was higher in 'Morex' than that of 'Steptoe', whereas those for DH lines varied from 16.00 to 84.33 in three years. There was sufficient phenotypic variability in this trait which ranged from 10% in 2014-15 to 22% in 2016-17. The high per cent of variation in this trait and their smaller values in year 2016-17 was due to comparatively dry crop season in this year. The DH populations showed considerable variation and also transgressive segregation as DH progeny had extreme values in both the directions. All the traits exhibited normal distribution with skewness and kurtosis values between -1 and +1 (Table 1) except grains plant^{-1} which showed higher skewness towards positive side in the year 2014-15. Smaller skewness values with negative sign indicated left-skewed distribution and with positive sign exhibited right-skewed distribution.

QTL analysis: In total 15 QTLs were found on chromosomes 1H, 2H, 3H, 4H, 5H and 7H for the four morphological traits in three consecutive years and were mapped (Table 2; Figs. 1, 2). Eight, seven and three QTLs were found in 2014-15, 2015-16 and 2016-17 years, respectively. These QTLs accounted for 6.29%-19.18%, 5.61%-46.46% and 9.22%–13.90% phenotypic variation in the three years.

QTLs for plant height: Four QTLs underlying plant height were found in three consecutive years, whose LOD value varied from 2.73 to 5.79 and individually caused 9.22–16.58% phenotypic variation in this trait (Table 2). One major QTL was identified on chromosome 2H, localized in the *ABR338 - *ABG356 marker interval at 72cM, in 2014-15 as well in 2015-16 which could be regarded as a stable marker. It accounted for 16.58% (2014-15), 12.73% (2015-16) and 14.66% (mean value of two years) phenotypic variation and was responsible for a decrease in plant height. The dwarfing allele came from parent 'Steptoe'. Two QTLs on chromosome 3H were detected, one QTL, flanking with markers *ABG398 - *ABR334 at position 56cM was detected in 2014-15, with LOD score of 3.53 and explained 9.74% phenotypic variation and the other QTL flanked with markers *ABG3198 - *ABC172 and localized at position 188cM was detected in 2016-17, with LOD score of 2.73 and individually explained 9.22% phenotypic variation. Both QTLs had a positive impact in increasing plant height. One more major QTL on linkage group 4H was identified in the year 2016-17 at *WG622-*MWG634 marker interval and 174cM position, with LOD score of 3.99, which accounted for 13.9% phenotypic variation and positively influenced plant height. The allele with increasing effect came from parent 'Morex'.

Table 1. The statistics of the 120 lines of DH population and parents for morphological traits based on data from three consecutive years (2014-15, 2015- 16 and 2016-17).

Trait	Year	Steptoe		Morex		DH Lines						
		Mean	SD	Mean	SD	Min.	Max.	Mean	SD	Skewness	Kurtosis	CV%
PH	2014-15	103	11.31	106.5	2.12	84.66	132.6	113.2	9.5	-0.09	-0.1	0.08
	2015-16	107.5	14.84	109	2.82	73.33	129	100.8	9.85	0.09	0.53	0.1
	2016-17	75.8	11	83.6	4.93	71	133	101.7	12.43	-0.47	-0.02	0.12
SL	2014-15	7.5	0.7	9.5	0.7	5	12	7	1.39	0.46	-0.26	0.18
	2015-16	8	1.41	10.5	0.7	4.66	11.6	7.66	1.43	0.29	-0.35	0.19
	2016-17	5.8	1.52	6.83	0.76	2.66	9.66	6.28	1.4	0.22	-0.32	0.22
PL	2014-15	27.5	4.94	36	4.24	23.3	48.6	34.9	5.58	0.13	-0.42	0.16
	2015-16	31	14.14	36.5	2.12	20.33	45.66	32	5.11	0.04	-0.25	0.16
	2016-17	29	2.59	20.8	3.54	17.16	47.66	32.73	6	-0.06	-0.29	0.18
SLS	2014-15	72	8.48	72	8.48	42	83.3	65.1	6.3	-0.03	1.18	0.1
	2015-16	72	8.48	81	12.7	47.66	84.33	61.7	7.1	0.31	0.2	0.11
	2016-17	48	10.19	58	9.16	16	74	49	10.8	-0.32	-0.38	0.22

Abbreviations= (PH), Plant height, (SL) Spike length, (PL) Peduncle length and (SLS) number of grain plant^{-1}

Table 2. Chromosome mapping of various QTLs with nearest linked molecular markers for four morphological traits.

Traits	Year	Chromosome	Position (cM)	Left marker	Right marker	LOD	PVE%	Add	Left Cl	Right Cl
Plant height	2014-15	2H	72	*ABR338	*ABG356	5.79	16.58	-4.25	71.5	72.5
		3H	56	*ABG398	*ABR334	3.55	9.74	3.26	54.5	56.5
	2015-16	2H	72	*ABR338	*ABG356	3.36	12.73	-3.5	71.5	72.5
	2016-17	3H	188	*ABG319B	*ABC172	2.73	9.22	3.78	182.5	188
		4H	174	*WG622	*MWG622	3.99	13.9	4.72	173.5	174
Spike length	2014-15	1H	40	*ABG380	*ABC158	3.4	6.29	0.35	36.5	44.5
		3H	56	*ABG398	*ABR334	19.18	19.18	0.97	54.5	56.5
	2015-16	2H	72	*ABG338	*ABG356	3.75	5.61	-0.38	71.5	72.5
		3H	56	*ABG398	*ABR334	21.28	46.46	1.09	55.5	56.5
		5H	135	*iPgd2	*Cmwg733	3.4	5.42	0.37	132.5	140.5
Peduncle length	2014-15	2H	49	*ABG459	*MWG520A	3.32	9.22	1.7	48.5	50.5
		3H	55	*ABG399	*ABG398	4.52	12.37	1.96	54.5	56.5
		7H	61	*CDO504	*MWG514B	3.05	8.35	-1.59	58.5	66.5
		7H	141	*WG530	*WG541	3.16	8.44	1.61	139.5	144.5
	2015-16	2H	50	*MWG520A	*ABG005	4.58	15.05	1.9	48.5	50.5
		3H	55	*ABG399	*ABG398	5.09	16.87	2.01	54.5	56.5
	2016-17	2H	54	*Pox	*ABC454	3.04	11.94	2.11	50.5	54.5
Number of grains plant^{-1}	2015-16	2H	71	*Bmy2	*ABC306	2.77	11.35	-2.36	69.5	71.5

QTLs for spike length: Four QTLs controlling the length of spike were identified in three years with LOD score ranging between 3.40 and 21.28 and individually explained 5.42% to 46.46% phenotypic variation. On chromosome 3H, one major QTL was identified flanking with markers *ABG398 - *ABR334 in 2014-15 and 2015-16 at position 56cM, which explained an average of 32.82% phenotypic variation in the two years and had increasing effect on spike length. The other three QTLs detected on 1H (2014-15), 2H (2015-16) and 5H (2015-16) linkage groups, were minor QTLs which collectively explained 17.32% phenotypic variation. The QTLs located on 1H and 5H had increasing, while that on 2H had decreasing effect.

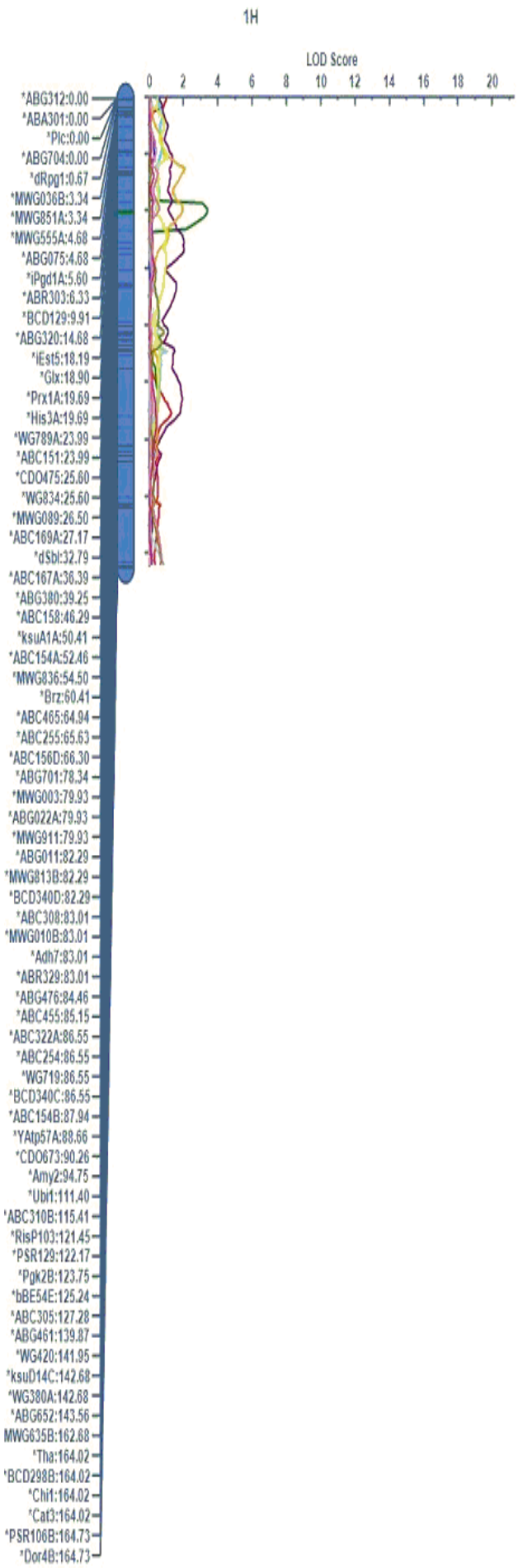
QTLs for peduncle length: In total six QTLs were recognized underlying peduncle length in three years with LOD score ranging from 3.04 to 5.09, and individually explained 8.35% to 16.87% phenotypic variability in peduncle length. A major QTL on 3H linkage group, near markers *ABG399 - *ABG398, was identified in 2014-15 and 2015-16 at position 55cM which explained 12.37% and 16.87% phenotypic variability, respectively, and had a positive impact of increasing peduncle length. On chromosome 2H, three more QTLs were found, each in year 2014-15, 2015-16 and 2016-17 which individually explained 9.22%, 15.05% and 11.94% phenotypic variability, respectively, with positive effect for increasing peduncle length. Two minor QTLs were also detected on 7H linkage group in the year 2014-15, localized in *CDO504-*MWG514B and *WG530-*WG541 marker intervals at 61cM and 141cM positions, respectively. Both separately explained 8.4% average phenotypic variability. Former QTL showed a negative and the latter had a positive increasing effect on the length of peduncle.

QTLs for number of grains plant^{-1}: Only one QTL for this trait was identified on 2H in the year 2015-16 in *BMY2 – *ABC306 marker interval and was localized at 71cM position, with 2.77 LOD score which explained 11.35% phenotypic variation. This QTL had decreasing effect and the allele for decreasing effect came from parent 'Steptoe'.

Discussion

The breeding programs of barley involve selection for many yield influencing traits. In these programs, marker assisted selection (MAS) approaches need a thorough knowledge of the genetic architecture underlying complex agronomic traits. Many traits of importance are polygenic in nature (Wang *et al.,* 2014). For determining the genetic structure of polygenic characters, different important genetic and genomic resources are developed in many species (Mora *et al.,* 2015) including barley (Close *et al.,* 2009; Zhou *et al.,* 2015). QTL studies point out the chromosome regions and specific alleles responsible for variation in a complex trait. The present study examined a double haploid barley population extracted from a cross 'Steptoe' x 'Morex' for QTL mapping.

Plant height is a most significant agronomic trait influencing the yield potential (Ogrodowicz *et al.,* 2017). Reduced height and the culm stiffness are the two most important plant characters which determine lodging resistance in cereals (Keller *et al.,* 1999). The introgression of these genes in elite lines has greatly improved crop yields by reducing yield losses due to lodging and has increased the harvest index (Wang *et al.,* 2014). Plant height is under polygenic control (Zhou *et al.,* 2015) and is influenced by so many qualitative genes and QTLs with minor effects (Tang *et al.,* 2007; Arriagada *et al.,* 2017). In the present study, four QTLs underlying plant height were identified on 2H, 3H and 4H linkage groups. Two out of four QTLs were localized on 2H and 3H linkage groups. These results were similar to those of Pasam *et al*., (2012) who detected majority of QTLs on 2H and 3H linkage groups. One major QTL recognized on 2H at position 72cM in 2014-15 and 2015-16, explained 12.73% and 16.58% phenotypic variability, respectively, suggesting that this QTL was comparatively less influenced by the environment. One more major QTL was also found on 4H only in the year 2016-17 which was responsible for 13.9% phenotypic variability in plant height.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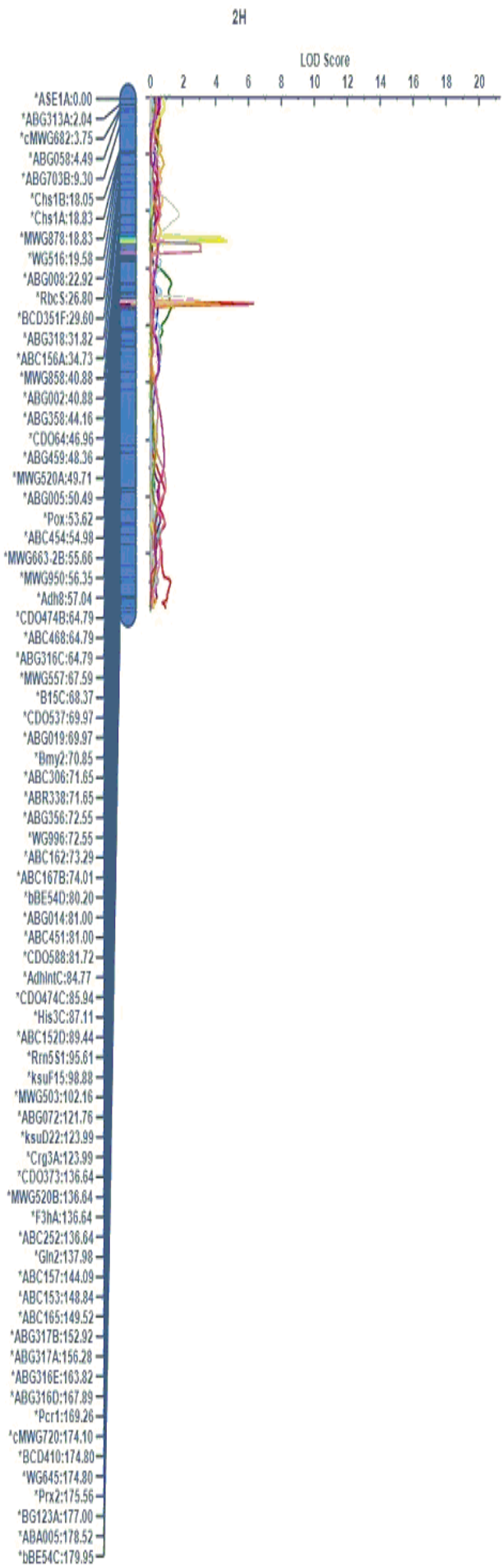
*Plc:0.00
*ABG704:0.00
*dRpg1:0.67
*MWG036B:3.34
*MWG851A:3.34
*MWG555A:4.68
*ABG075:4.68
*iPgd1A:5.60
*ABR303:6.33
*BCD129:9.91
*ABG320:14.68
*iEst5:18.19
*Glx:18.90
*Prx1A:19.69
*His3A:19.69
*WG789A:23.99
*ABC151:23.99
*CDO475:25.60
*WG834:25.60
*MWG089:26.50
*ABC169A:27.17
*dSbl:32.79
*ABC167A:36.39
*ABG380:39.25
*ABC158:46.29
*ksuA1A:50.41
*ABC154A:52.46
*MWG836:54.50
*Brz:60.41
*ABC465:64.94
*ABC255:65.63
*ABC156D:66.30
*ABG701:78.34
*MWG003:79.93
*ABG022A:79.93
*MWG911:79.93
*ABG011:82.29
*MWG813B:82.29
*BCD340D:82.29
*ABC308:83.01
*MWG010B:83.01
*Adh7:83.01
*ABR329:83.01
*ABG476:84.46
*ABC455:85.15
*ABC322A:86.55
*ABC254:86.55
*WG719:86.55
*BCD340C:86.55
*ABC154B:87.94
*YAtp57A:88.66
*CDO673:90.26
*Amy2:94.75
*Ubi1:111.40
*ABC310B:115.41
*RisP103:121.45
*PSR129:122.17
*Pgk2B:123.75
*bBE54E:125.24
*ABC305:127.28
*ABG461:139.87
*WG420:141.95
*ksuD14C:142.68
*WG380A:142.68
*ABG652:143.56
MWG635B:162.68
*Tha:164.02
*BCD298B:164.02
*Chi1:164.02
*Cat3:164.02
*PSR106B:164.73
*Dor4B:164.73


2H
LOD Score
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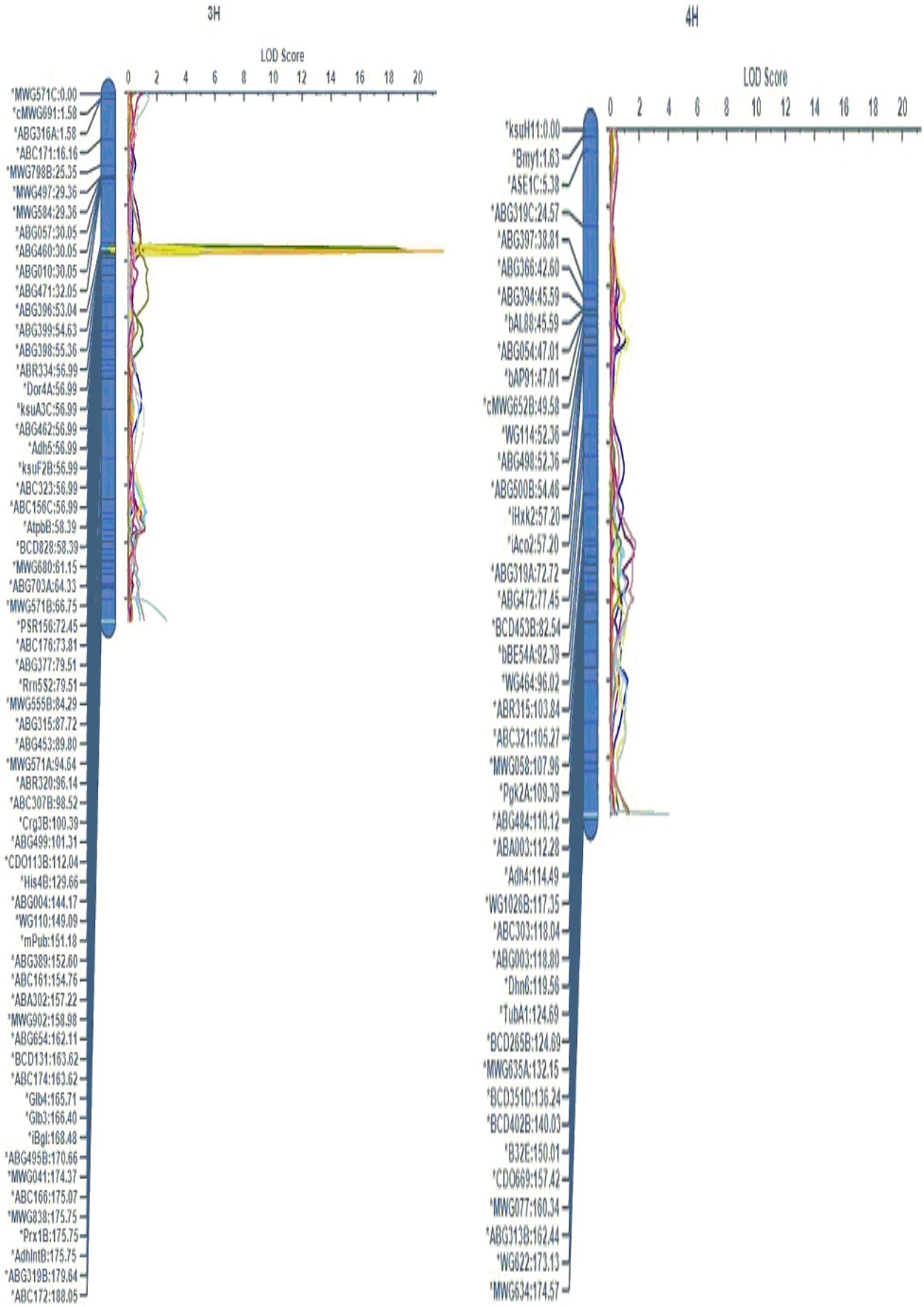
*cMWG682:3.75
*ABG058:4.49
*ABG703B:9.30
*Chs1B:18.05
*Chs1A:18.83
*MWG878:18.83
*WG516:19.58
*ABG008:22.92
*RbcS:26.80
*BCD351F:29.60
*ABG318:31.82
*ABC156A:34.73
*MWG858:40.88
*ABG002:40.88
*ABG358:44.16
*CDO64:46.96
*ABG459:48.36
*MWG520A:49.71
*ABG005:50.49
*Pox:53.62
*ABC454:54.98
*MWG663-2B:55.66
*MWG950:56.35
*Adh8:57.04
*CDO474B:64.79
*ABC468:64.79
*ABG316C:64.79
*MWG557:67.59
*B15C:68.37
*CDO537:69.97
*ABG019:69.97
*Bmy2:70.85
*ABC306:71.65
*ABR338:71.65
*ABG356:72.55
*WG996:72.55
*ABC162:73.29
*ABC167B:74.01
*bBE54D:80.20
*ABG014:81.00
*ABC451:81.00
*CDO588:81.72
*AdhIntC:84.77
*CDO474C:85.94
*His3C:87.11
*ABC152D:89.44
*Rrn5S1:95.61
*ksuF15:98.88
*MWG503:102.16
*ABG072:121.76
*ksuD22:123.99
*Crg3A:123.99
*CDO373:136.64
*MWG520B:136.64
*F3hA:136.64
*ABC252:136.64
*Gln2:137.98
*ABC157:144.09
*ABC153:148.84
*ABC165:149.52
*ABG317B:152.92
*ABG317A:156.28
*ABG316E:163.82
*ABG316D:167.89
*Pcr1:169.26
*cMWG720:174.10
*BCD410:174.80
*WG645:174.80
*Prx2:175.56
*BG123A:177.00
*ABA005:178.52
*bBE54C:179.95

Fig. 1. (Cont'd.).


3H
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*ABG057:30.05
*ABG460:30.05
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*ABR334:56.99
*Dor4A:56.99
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*ABG462:56.99
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*ABC323:56.99
*ABC156C:56.99
*AtpbB:58.39
*BCD828:58.39
*MWG680:61.15
*ABG703A:64.33
*MWG571B:66.75
*PSR156:72.45
*ABC176:73.81
*ABG377:79.51
*Rrn5S2:79.51
*MWG555B:84.29
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*ABR320:96.14
*ABC307B:98.52
*Crg3B:100.39
*ABG499:101.31
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*His4B:129.66
*ABG004:144.17
*WG110:149.09
*mPub:151.18
*ABG389:152.60
*ABC161:154.76
*ABA302:157.22
*MWG902:158.98
*ABG654:162.11
*BCD131:163.62
*ABC174:163.62
*Glb4:165.71
*Glb3:166.40
*iBgl:168.48
*ABG495B:170.66
*MWG041:174.37
*ABC166:175.07
*MWG838:175.75
*Prx1B:175.75
*AdhIntB:175.75
*ABG319B:179.64
*ABC172:188.05
4H
LOD Score
0 2 4 6 8 10 12 14 16 18 20
*ksuH11:0.00
*Bmy1:1.63
*ASE1C:5.38
*ABG319C:24.57
*ABG397:38.81
*ABG366:42.60
*ABG394:45.59
*bAL88:45.59
*ABG054:47.01
*bAP91:47.01
*cMWG652B:49.58
*WG114:52.36
*ABG498:52.36
*ABG500B:54.46
*iHxk2:57.20
*iAco2:57.20
*ABG319A:72.72
*ABG472:77.45
*BCD453B:82.54
*bBE54A:92.39
*WG464:96.02
*ABR315:103.84
*ABC321:105.27
*MWG058:107.96
*Pgk2A:109.39
*ABG484:110.12
*ABA003:112.28
*Adh4:114.49
*WG1026B:117.35
*ABC303:118.04
*ABG003:118.80
*Dhn6:119.56
*TubA1:124.69
*BCD265B:124.69
*MWG635A:132.15
*BCD351D:136.24
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*B32E:150.01
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*MWG077:160.34
*ABG313B:162.44
*WG622:173.13
*MWG634:174.57

Fig. 1. (Cont'd.).

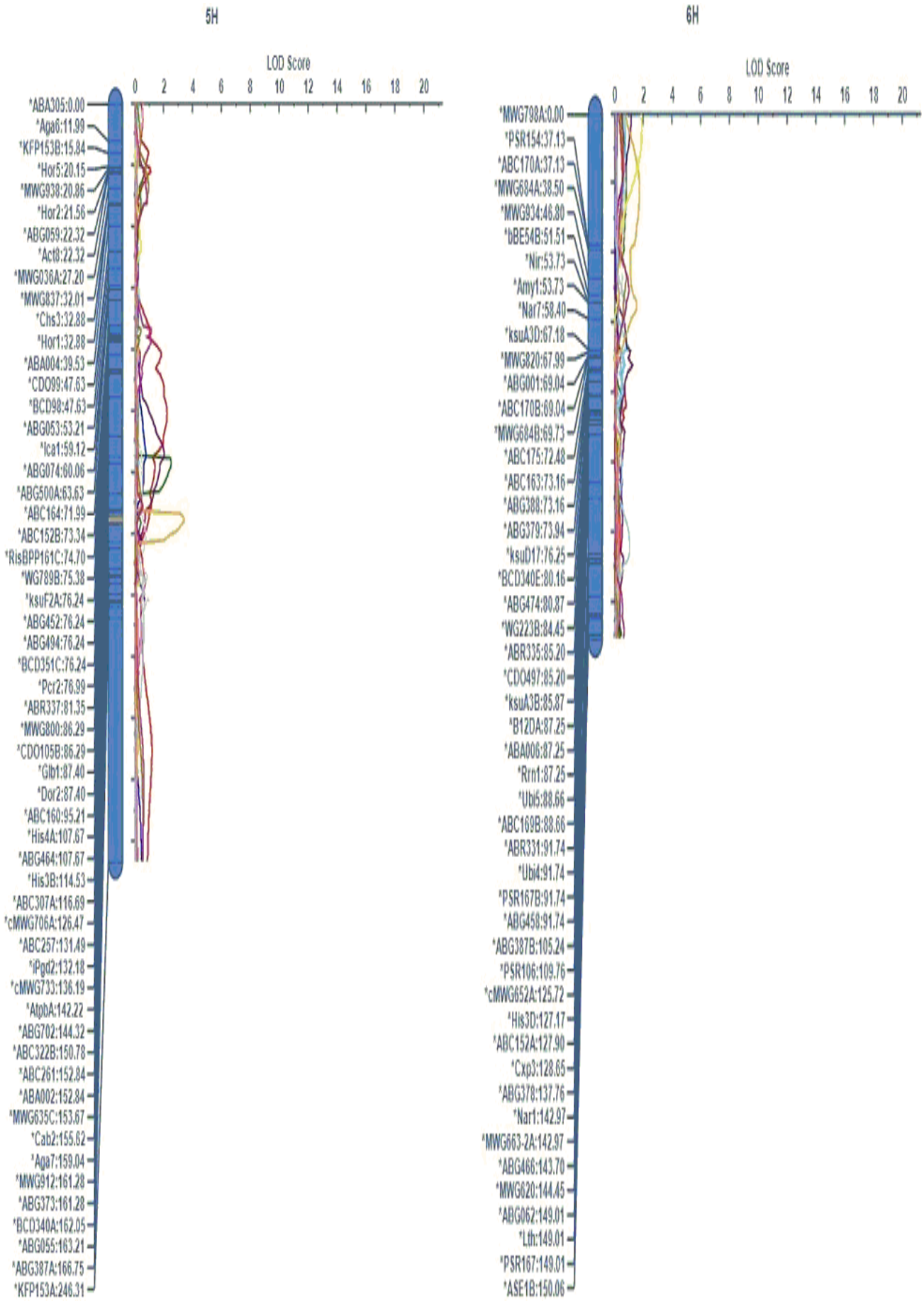
5H
LOD Score
0 2 4 6 8 10 12 14 16 18 20
*ABA305:0.00
*Aga6:11.99
*KFP153B:15.84
*Hor5:20.15
*MWG938:20.86
*Hor2:21.56
*ABG059:22.32
*Act8:22.32
*MWG036A:27.20
*MWG837:32.01
*Chs3:32.88
*Hor1:32.88
*ABA004:39.53
*CDO99:47.63
*BCD98:47.63
*ABG053:53.21
*Ica1:59.12
*ABG074:60.06
*ABG500A:63.63
*ABC164:71.99
*ABC152B:73.34
*RisBPP161C:74.70
*WG789B:75.38
*ksuF2A:76.24
*ABG452:76.24
*ABG494:76.24
*BCD351C:76.24
*Pcr2:76.99
*ABR337:81.35
*MWG800:86.29
*CDO105B:86.29
*Glb1:87.40
*Dor2:87.40
*ABC160:95.21
*His4A:107.67
*ABG464:107.67
*His3B:114.53
*ABC307A:116.69
*cMWG706A:126.47
*ABC257:131.49
*iPgd2:132.18
*cMWG733:136.19
*AtpbA:142.22
*ABG702:144.32
*ABC322B:150.78
*ABC261:152.84
*ABA002:152.84
*MWG635C:153.67
*Cab2:155.62
*Aga7:159.04
*MWG912:161.28
*ABG373:161.28
*BCD340A:162.05
*ABG055:163.21
*ABG387A:166.75
*KFP153A:246.31
6H
LOD Score
0 2 4 6 8 10 12 14 16 18 20
*MWG798A:0.00
*PSR154:37.13
*ABC170A:37.13
*MWG684A:38.50
*MWG934:46.80
*bBE54B:51.51
*Nir:53.73
*Amy1:53.73
*Nar7:58.40
*ksuA3D:67.18
*MWG820:67.99
*ABG001:69.04
*ABC170B:69.04
*MWG684B:69.73
*ABC175:72.48
*ABC163:73.16
*ABG388:73.16
*ABG379:73.94
*ksuD17:76.25
*BCD340E:80.16
*ABG474:80.87
*WG223B:84.45
*ABR335:85.20
*CDO497:85.20
*ksuA3B:85.87
*B12DA:87.25
*ABA008:87.25
*Rrn1:87.25
*Ubi5:88.66
*ABC169B:88.66
*ABR331:91.74
*Ubi4:91.74
*PSR167B:91.74
*ABG458:91.74
*ABG387B:105.24
*PSR106:109.76
*cMWG652A:125.72
*His3D:127.17
*ABC152A:127.90
*Cxp3:128.65
*ABG378:137.76
*Nar1:142.97
*MWG663-2A:142.97
*ABG466:143.70
*MWG820:144.45
*ABG062:149.01
*Lth:149.01
*PSR167:149.01
*ASE1B:150.06

Fig. 1. (Cont'd.).

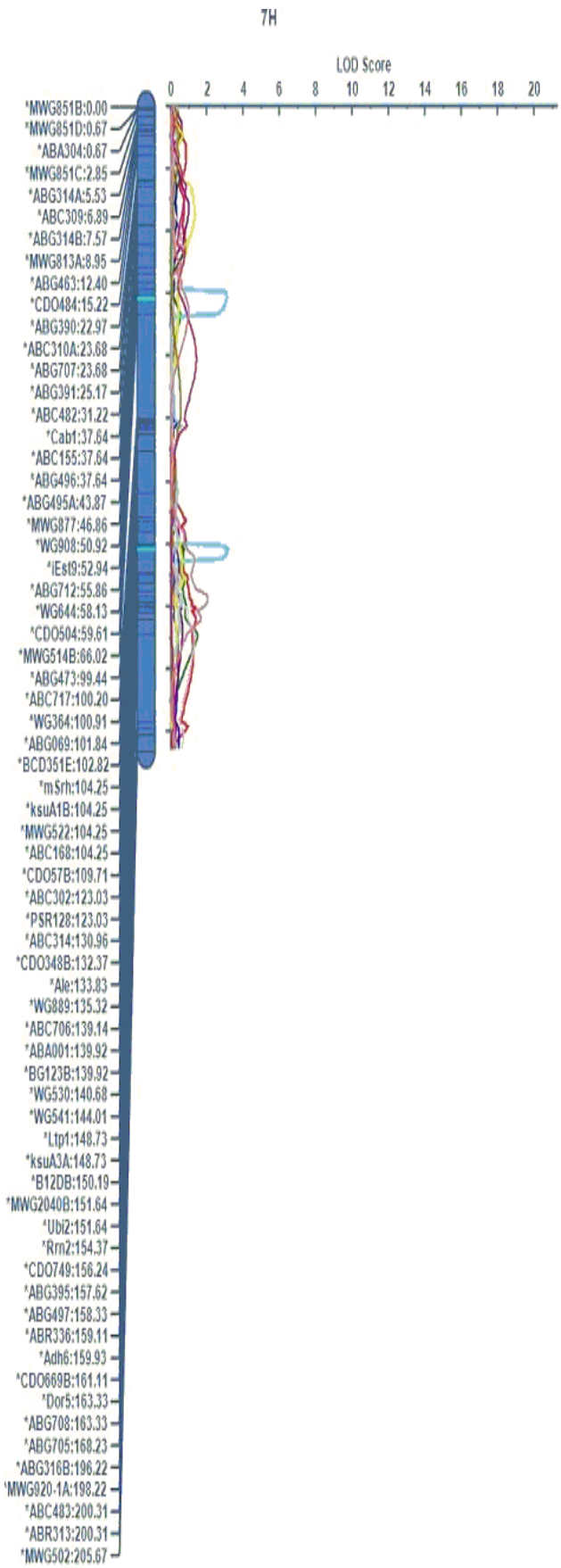

Fig. 1. Genetic map of DH barley population for the seven linkage groups.

The other two QTLs, with minor effects, were detected on 3H linkage group in one year only, which expressed only 9% phenotypic variability in plant height. These QTLs showed greater interaction with the environment. The alleles inducing decrease or increase in height came from both the parents, thus resulted in transgressive segregation observed. Previous studies show genome wide QTLs distribution for plant height. Chutimanitsakun *et al.,* (2011) reported QTLs underlying plant height on 1H, 2H, 3H and 4H. Mora *et al*., (2016) detected plant height QTLs on 2H, 3H, 4H and 6H. Malosetti *et al.,* (2011) detected significant plant height-SNP associations on 2H, 3H, 5H and 7H, the important QTL located on 3H carried semi-dwarfing genes (Wang *et al.,* 2014). Semi-dwarfing genes uzu 1, sdw 1 and denso have been detected on 3H (Barua *et al*., 1993). Zhou *et al.,* (2015) identified major QTLs located on 3H and 7H which explained 44.5% and 23.2% phenotypic variation respectively. Ogrodowicz *et al.,* (2017) detected a QTL on a region of short arm of 2H showing significant interaction with environment.

Spike length is also an important morphological trait affecting yield potential and the malt extract yield (Hori *et al.,* 2003, Wang *et al.,* 2010). In the present study, four QTLs were found on 1H, 2H, 3H and 5H linkage groups underlying this trait. One major QTL was recognized on 3H, in *ABG398-*ABR334 markers interval at 56cM position in two consecutive years, 2014-15 and 2015-16, which induced 47.27% and 46.46% phenotypic variability in spike length, respectively. Its repeated occurrence suggested that this QTL showed less interaction with environment. Three minor QTLs were found on 1H, 2H and 5H which explained 6.29%, 5.61% and 5.42% phenotypic variation respectively. QTLs influencing spike length are reported to be present on all of the seven linkage groups. Chutimanitsakun *et al*., (2011) reported QTLs controlling spike length on 1H, 2H, 3H, 5H and 6H. Abbaszadeh *et al*., (2014) detected two QTLs on 2H and 3H, localized in 3.5cM and 8cM which explained 14.9% and 2.7% phenotypic variability in spike length. Peighambari *et al*., (2005) also reported two QTLs on 2H and 3H. Wang *et al*., (2014) identified QTLs underlying this trait on 1H, 2H, 5H and 7H, in a double haploid population. Wang *et al*., (2016) in a DH population extracted from cross Huaai 11 x Huadamai 6 used CIM mapping and detected 26 QTLs for spike length in five years, which individually explained 1.16% to 52.72% of phenotypic variation. Among them a major QTL was detected on 2H, accounting for 7.27% to 18.7% phenotypic variation. Wang *et al.,* (2016) also reported 3 main cluster regions linked with 10 traits on 2H, 4H and 7H. Ren *et al.,* (2013) also detected one significant QTL underlying spike length on 2H that induced decrease of main spike length.

Peduncle length is also one of the most important traits in cereals, which decided the ideal architecture and dry matter remobilization towards seed setting and seed development (Rao *et al.,* 2007). In green revolution era, plant height was decreased by inducing genes for

dwarfing trait that shortened internodes and this also shortened the peduncle length. In the varieties with shortened peduncles the spike emergence from flag leaf is sometimes incomplete resulting in low seed-setting in the florets covered by flag leaf and also caused a high thrip infestation in the spike. According to Borner *et al*., (2002), the short peduncled plants are susceptible to diseases as the peduncle length is of more importance in disease escape and breeding for spike disease resistance. However, at present there is a little information as for as the genetics of peduncle length in barley is concerned. In the present study, six QTLs were identified on 2H, 3H and 7H linkage groups, which explained 8.35% -16.87% phenotypic variations. A QTL with major impact was identified on 3H in *ABG399-*ABG398 marker interval at position 55cM in 2014-15 and 2015-16 growing seasons, explaining 12.37% and 16.87% variation in the peduncle length, respectively. This QTL contributed positively to the peduncle length and was comparatively less influenced by the environment. Two other major QTLs were found on 2H in the years 2015-16 and 2016-17 which explained 15.05% and 11.94% phenotypic variability in the peduncle length, respectively. Three more minor QTLs with positive effect were recognized on 2H and 7H. Rao *et al.*, (2007) reported two major impact QTLs for this trait, each on 2H and 3H and another putative QTL was found on 1H with smaller effect on the peduncle length. Abbaszadeh *et al.*, (2014) found one QTL underlying peduncle length on linkage map 3, localized at 5.70cM position. Mora *et al.*, (2016) reported 20 QTLs on 2H, 3H, 4H and 6H.

Number of grains plant^{-1} is also a trait of importance and is directly related to yield potential. Only one significant QTL was detected on chromosome 2H, only in the year 2015-16, accounting for 11.35% phenotypic change. Wang *et al.*, (2016) also detected one QTL underlying this trait on 2H. Chutimanitsakun *et al*., (2011) detected one minor QTL on 1H and two major QTLs on 2H. Ren *et al.*, (2013) observed two QTLs on 5H and 7H, with 8.07% and 26.00% phenotypic variability. The QTLs controlling grains plant^{-1} detected in other studies varied according to the populations and environmental conditions. The differences may be due to different habits of growth of studied lines and various climatic conditions. Many of the QTLs found in the present study were associated with 2H and 3H. The linkage groups with the lowest QTL number were 4H, 5H and 7H. Similar results were reported by Mora *et al.*, (2016). The present study coincided with the previous QTLs detected by Chutimanitsakun *et al.*, (2011) on 3H for plant height and on 5H for spike length. In this study marker ABG398 on 3H was concomitantly linked with plant height, spike length and peduncle length. The chromosome 2H carried QTLs for plant height, spike length and grains plant^{-1} and the 3H had those for plant height, spike length and peduncle length on the same chromosome region. Presence of many QTLs on the same chromosomal position is indicative of pleiotropy of the genes or of a tight linkage among genes of the quantitative traits (Kuczynska *et al.*, 2014; Wang *et al.*, 2016). Co-localization of many QTLs underlying yield components indicate that major loci for development are linked with many associations in barley (Comadran *et al.*, 2011). Several major QTLs recognized on 2H and 3H could be the candidates for map-based pleiotropism studies, recombination hot spots, gene-rich regions and QTL clustering. 2H is of special importance because it had QTLs for all the four traits analyzed in this study. The markers recognized could contribute to further fine mapping these traits and also provide the useful tool for marker assisted selection of lines in breeding programs of barley.

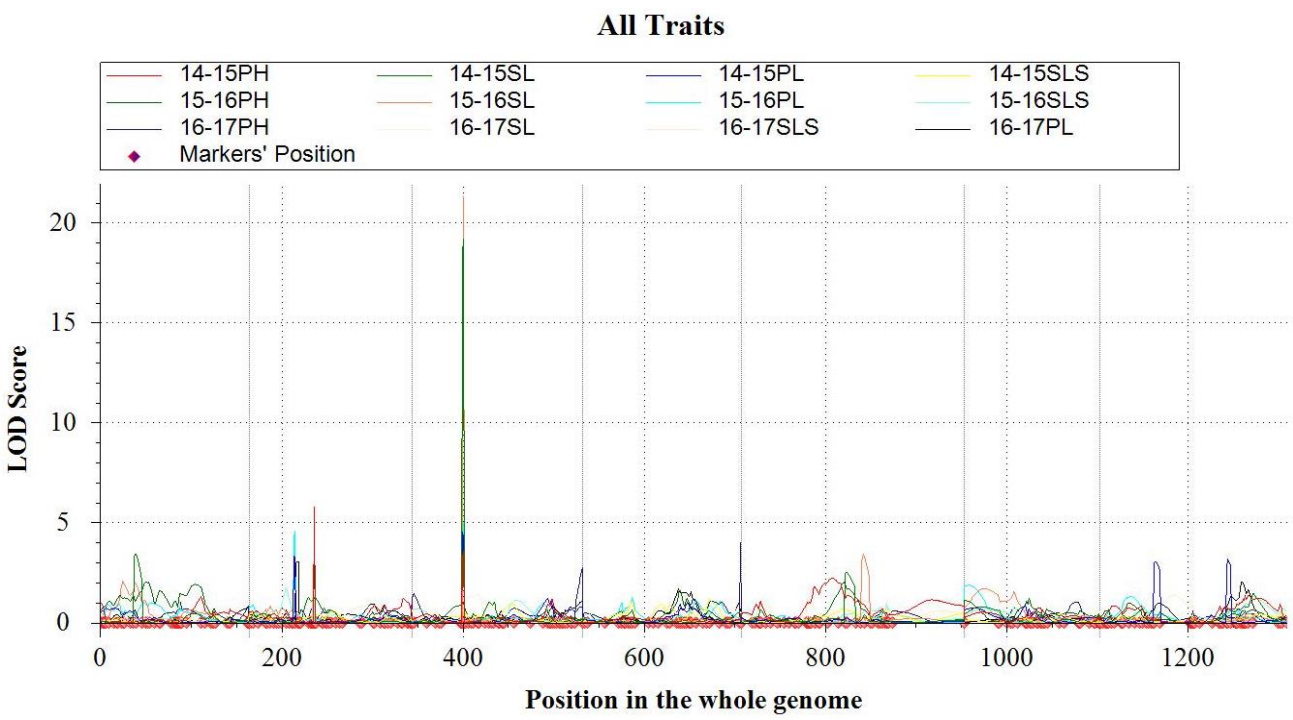

Fig. 2. Chromosome mapping of various QTLs with nearest linked molecular markers for four morphological traits.

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