

## Detection of consensus genomic regions associated with root architecture of bread wheat on groups 2 and 3 chromosomes using QTL meta-analysis

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### Abstract

Root architecture is an important bread wheat phenomenon that highly influences its production and adaptation to environmental stresses, in particular drought stress. Several QTL studies have been conducted to ascertain chromosomal regions associated with root morphology resulting in identification of various loci depending on evaluated population types and experimental conditions. In order to identify the most consistent and reliable QTLs involved in various root morphological traits in bread wheat, a meta-QTL (MQTL) analysis was performed using 106 QTLs derived from 12 different populations under both normal and drought stress conditions. Among them, 125 QTLs related to root traits were successfully projected onto the reference map and further meta-analysis was focused on chromosomes of homeologous groups 2 and 3 with most assigned QTLs. Consequently, a total of seven MQTLs were identified on chromosomes 2A, 2B, 3A and 3B originated from 2 to 17 initial QTLs with a confidence interval (CI) of 5.3–6.6 to 39.5–55.0 cM. Three MQTLs located on 2A, 3A and 3B derived from 7 to 17 QTLs related to different root morphological traits pointed out the most important chromosomal regions. A reduction in the average 95% confidence interval from 20.8 cM to 6.4 cM was observed when comparing the individual QTL to the MQTL. Further analysis on investigation of candidate genes located in these genomic regions resulted in identification of some genes mainly associated with lignin catabolic process, potassium transporters and leucine-rich repeats receptor-like kinases (LRR-RLKs). These results provide fundamental information on most important genomic regions and candidate genes related to root morphology in bread wheat.

**Keywords:** Bread wheat; Root traits; Quantitative trait loci; Meta-QTL analysis.

**Abbreviations:** QTL\_quantitative trait loci; MQTL\_meta-QTL; CI\_confidence interval; R<sup>2</sup>\_phenotypic variance explained by the QTL; LOD\_logarithm of odds; AIC\_akaike information criterion; RL\_root length; RSA\_root surface area; RV\_root volume; RN\_root number; RDW\_root dry weight; BC\_backcrossed lines; DH\_doubled haploid lines; RIL\_recombinant inbred lines.

### Introduction

Root morphological traits are fundamental for multiple functions that are at the basis of plant productivity, which including efficiency of water and inorganic nutrients absorption, root development directly affects the growth and biomass production, and is important factor of the high and stable yield in plants (Sharma et al., 2011). The effect of root architecture on yield and related traits and its role in increasing drought tolerance have been widely reported for all major crops (Tuberosa et al., 2002a, b; de Dorlodot et al., 2007, Christopher et al., 2013). Growth and development of root are not only affected by climate and cultivations condition but also controlled by numerous genes. Caradus (1995) suggested that the traits associated with root size such as root length, weight, volume, number, surface area, and the ratio of root to shoot dry weight have the highest heritability in compare to other root morphological traits such as root diameter, density, length of hairs. Several studies revealing the extent and nature of root morphological genetic variation

have profound implications for improving water- and nutrient-use efficiency of crops or for enhancing their productivity under abiotic stresses or suboptimal soil conditions.

Genotypes with the greatest root vigor under drought conditions showed the lowest yield reductions under severe water stress (Motzo et al., 2013). The previous studies indicated that root architecture determined yield potential under drought conditions; for example, high yielding upland rice varieties with longer root length. (Steele et al. 2007). Although root traits have vital effects on plant yield under water-limited conditions, they are rarely considered as selection parameter for improvement of wheat and other plants because they are difficult to measure. In general it can be said that the inherent difficulties in root system evaluation (because of the quantitative nature of root characteristics, their complex genetic control, and the strong environmental effects) has so far slowed the pace of mutants, genes and

QTL discovery for roots and lead to a paucity of root traits-associated molecular markers to be used in MAS. This has largely precluded breeding programs to exploit root traits in breeding. The genetic variation observed for root morphology characters has been used to study the inheritance of various root traits under control and drought stress conditions (Richard et al., 2105; Chapagain et al., 2014; Comas et al., 2013; Whalley et al., 2013). However, only limited information available for the genetic control of root characters in wheat. As per the Monyo and Whittington (1970) reports the single genes, as well as polygenic systems controlling growth period, had an effect on both root and shoot. MacKey (1973) demonstrated that genetic control of root traits was spread over the whole genome by using a set of monosomics lines in bread wheat. Richards and Passioura (1981) observed that control of 3 traits i.e., maximum xylem vessel diameter, number of seminal root axes, and number of metaxylem vessels in bread wheat was multigenetic. Ehdai and Waines (1997) had used a set of ditelosomic and dimonotelosomic lines of Chinese Spring, and demonstrated that the expression of root biomass in bread wheat had been affected by at least 13 chromosome arms belonging to the A, B, and D genomes.

Genomic loci controlling such traits are called quantitative trait loci (QTL). With the advent and development of molecular markers, this capability is established that estimate the gene position and size of QTL, including those for root morphology traits (Sharma et al. 2011). The applications of molecular marker techniques and outcomes of quantitative trait locus (QTL) mapping have facilitated a better understanding of the genetic basis of root traits and grain yield. Identification of QTLs associated with wheat root morphology provides useful information for avoiding drought stress and maintaining yield production under the irrigation condition. In some cases, these studies have suggested possible roles for these QTL in determining plant yield, due to the overlap of QTL for root features with those for traits related to productivity (Tuberosa et al., 2002a, b; Steele et al., 2007). A number of QTLs for root traits in rice (Horii et al. 2005; Steele et al., 2007), barley (Arifuzzaman et al., 2014), maize (Tuberosa et al., 2002b; Burton et al., 2015), durum wheat (Maccaferri et al., 2016) have been mapped in many studies. The more and fresh amount of data for specific QTL, enhances our physiological and evolutionary understanding and reveals links between root morphology and root functions that will be essential in designing root surface area for target environments. For instance, a major QTL for root length of chromosome 9 in rice, which has been deployed in smart breeding and marker-assisted selection (Steele et al., 2006), has shown a degree of QTL by environment interaction under drought that suggests its greater effectiveness in limited water conditions. The same QTL is also associated with the size and volume of individual adventitious root axes, rather than branching of seminal roots (Horii et al., 2005). Overlap of QTLs for root system morphology in maize with those for grain yield suggests the possible role of these QTL in determining the grain yield (Tuberosa et al., 2002b).

Since different populations (Austin et al., 2000), generations (Li et al., 2007), and environmental conditions (Li et al., 2003; Lan et al., 2005) were commonly found to have great influence on the results of QTL detection experiments, direct comparisons of QTLs data across different studies were difficult. The integration of QTL data from diverse resources is informative for the eventual QTL cloning and breeding application. Meta-analysis is a method of combining data from different sources in a single study to

determine co-locations between genes and QTLs (Goffinet and Gerber 2000; Arcade et al., 2004). Pooling of results from several studies allows greater statistical power for QTL detection and more precise estimation of their genetic effects. Hence, a meta-analysis can yield conclusions that are stronger than those of individual studies and can give greater insight into the genetic architecture of complex traits (Wu and Hu, 2012). This approach has been used to study different quantitative traits in various cereals such as abiotic stresses tolerance in barley (Zhang et al., 2016), yield in rice (Wu et al., 2016), and disease resistance in maize (Zhao et al., 2015). Meta-analysis of QTLs has been also employed for root architecture in rice (Courtois et al., 2009), *Brassica napus* (Zhang et al., 2016) and adaptation to drought and heat stress in bread wheat (Acuña-Galindo et al., 2014). Despite of different studies, research is limited in using meta-analyses for studying root system morphology in wheat. Therefore, the objectives of this study were to identify, using meta-QTL analysis, chromosomal regions where 'real' QTL involved in traits related to root morphology are located that will aid breeders in targeted genetic improvement.

## Results

### *Distribution of initial QTLs associated with traits of root morphology*

The QTLs used in this study derived from various population types included backcrossed lines (BC), recombinant inbred lines (RIL), doubled haploid lines (DH) and F<sub>2</sub> plants and the population sizes ranged from 85 to 206 plants (Table 1). A total of 243 QTLs related to root morphology were identified in the 12 experiments subjected to the current meta-analysis. The number of QTLs on each chromosome varied between 1 (chromosome 5B, 6B and 6D) to 31 (chromosome 1A) (Supplementary Table 1). Among them, 125 QTLs with sufficient information and common markers with the reference map including 105 QTLs in normal conditions and 20 QTLs under drought stress conditions were selected for further analysis (Fig 1).

Of the 105 QTLs in normal conditions 61 QTLs of 10 populations were associated with root length and root number, 28 QTLs of six populations with root surface area, root volume and 16 QTLs of five populations with root dry weight, roots angle and root diameter (Fig 1). Of the seven traits associated with root morphology, root length (13.4%) and roots angle (2.1%) had the highest and the lowest average R<sup>2</sup> value, respectively (Fig 2). LOD scores were ranged from 2.05 to 29.9, for QTLs associated with root volume and root length, respectively. Average LOD for all traits was estimated as 4.6 (Table 2). Most of the QTLs for root traits were located on homoeologous group 2 and 3 chromosomes with 35 and 18, respectively (Table 3). QTLs for root length (12 QTLs) and root surface area (7 QTLs) were mainly on homoeologous group 2 and 3 chromosomes and QTLs for root number (10 QTLs) and root volume (7 QTLs) were mainly located on homoeologous groups 2 and 3 chromosomes. Due to presence of most root related QTLs on homoeologous group 2 and 3 chromosomes, they were subjected to further analysis of metaQTL.

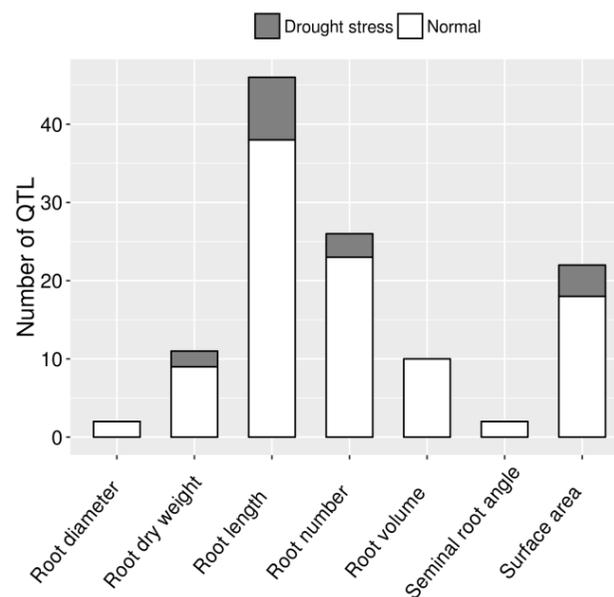
### *Meta-analysis of QTLs for root morphology*

A total of 53 QTLs related to root morphology on homoeologous groups 2 and 3 chromosomes were anchored

**Table 1.** The origin of QTLs related to root morphological traits included in the meta-analysis.

Parents of Population	Population size	Population type	Number of marker	Map density	Marker type	Experiment environments	Reference
Weimai8 × Luohan 2	179	RILs	496	2.64	SSR	N and DS	Zhang et al., 2014
Weimai8 × Yannong19	175	RILs	496	2.64	SSR	N and DS	Zhang et al., 2014
Weimai 8 × Jimai 20	172	RILs	496	2.64	SSR	N and DS	Zhang et al., 2014
Chinese Spring × SQ1	90	DH	338	8.45	SSR	N and DS	Czyczyło-Mysza et al., 2013
Xiaoyan54 × Jing411	142	RILs	470	7.3	SSR	N	Ren et al., 2012
Creso × Pedroso	123	F <sub>7</sub>	600	3	SSR & DArT	N	Petraruloet al., 2014
WL711 × C306	206	RIL (F <sub>9</sub> /F <sub>10</sub> )	173	21.2	SSR & STS	N & DS	Kadam et al., 2012
Mironovskaya808 × Chinese Spring	100	F <sub>2</sub>	122	19.5	SSR & SNP	N and ABA treatment	Iehisaet al., 2014
Hanxuan10 × Lumai14	150	DH	395	9.9	SSR & RFLP	N and DS	Liu et al., 2013
Opata85 × W7984	114	RILs	800	4.4	SSR	N and PEG <sup>b</sup>	Landjeva et al., 2013
Chinese Spring × Synthetic6	85	DILs <sup>a</sup>	80	6.87	SSR	N and PEG	Landjeva et al., 2008
Huapei3 × Yumai57	168	DH	323	7.67	SSR & EST	N	Zhao et al., 2014

N= Normal condition, DS= Drought stress, a. D genome introgression lines, b. Polyethylene glycol



**Fig 1.** Number of QTLs associated with root morphological traits under normal and drought stress conditions in 12 different populations.

**Table 2.** Number of QTLs related to root morphology identified on each chromosome.

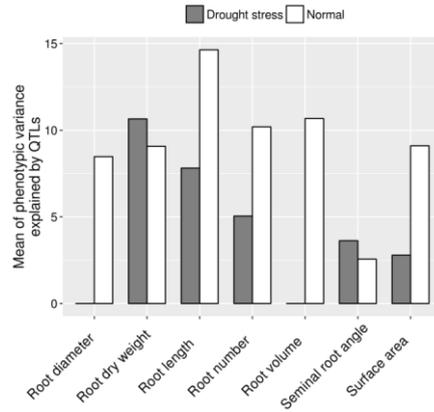
Wheat genome	Chromosome number							Total
	1	2	3	4	5	6	7	
A	30/13	19/17	14/9	5/4	4/2	15/8	6/2	55/93
B	16/2	23/14	25/6	11/7	14/6	11/6	11/4	45/111
D	1/1	4/4	4/4	4/4	9/4	8/4	11/4	25/41
Total	47/16	46/35	43/19	20/15	27/12	34/18	28/10	125/245

Numbers after and before slash indicate number of QTLs identified in evaluated studies and used in meta-QTL analysis, respectively.

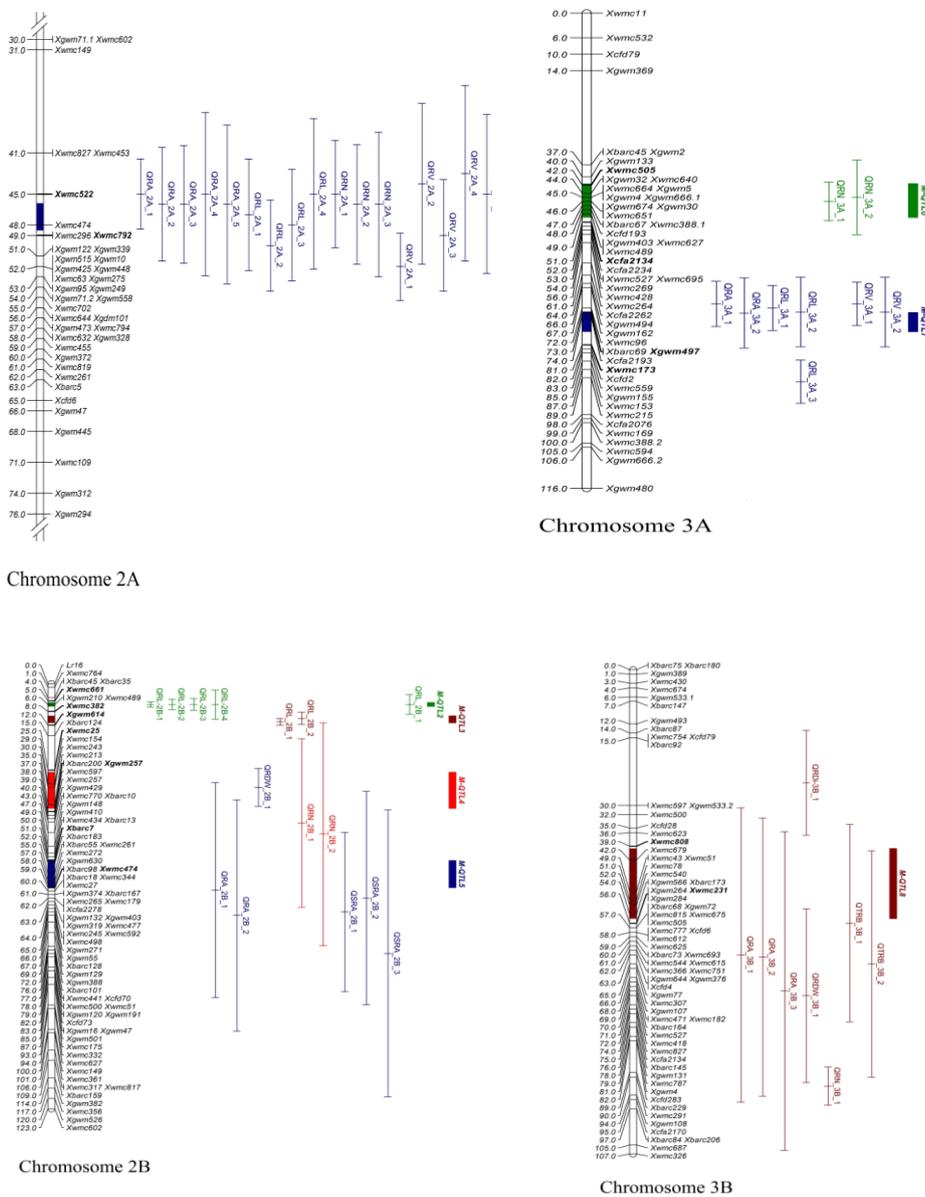
**Table 3.** List of QTLs associated with root morphological traits on 2A, 2B, 3A and 3B chromosomes used for the QTL meta-analysis.

Num.	QTL name	Trait	Chr.	Conditions	LOD	R <sup>2</sup>	Position on consensus map	Confidence interval of QTL
1	QRA_2A_1	Root area	2A	Normal	3.81	19.6	45	41.62 - 48.38
2	QRA_2A_2	Root area	2A	Normal	2.85	12.1	46	40.52-51.48
3	QRA_2A_3	Root area	2A	Normal	2.53	11.7	46	40.34-51.66
4	QRA_2A_4	Root area	2A	Normal	2.58	8.4	45	37.11-52.89
5	QRA_2A_5	Root area	2A	Normal	2.05	8.6	46	38.30-53.70
6	QRA_2B_1	Root area	2B	Drought	2.86	2.86	59.7	28.60-90.80
7	QRA_2B_2	Root area	2B	Drought	2.86	2.86	67	33.55-100.45
8	QRA_3A_1	Root area	3A	Normal	3.73	12	71	65.48-76.52
9	QRA_3A_2	Root area	3A	Normal	2.13	7.7	73.21	64.6-81.82
10	QRA_3B_1	Root area	3B	Drought	2.72	2.72	63	30.50-95.50
11	QRA_3B_2	Root area	3B	Normal	3.12	3.12	63.5	32.84-94.16
12	QRA_3B_3	Root area	3B	Drought	2.72	2.72	71	35.83-106.17
13	QRDi-3B_1	Root diameter	3B	Normal	3.15	7.36	25	13.39-36.61
14	QRDW_2B_1	Root dry weight	2B	Normal	3.82	6.65	30	24.50-35.50
15	QRDW_2D_1	Root dry weight	2D	Normal	3.13	8.61	90	85.76-94.24
16	QRDW_2D_2	Root dry weight	2D	Normal	3.5	9.45	41	36.81-45.19
17	QRDW_3B_1	Root dry weight	3B	Normal	2.4	8.3	72	52.79-91.21
18	QRL_2A_1	Root length	2A	Normal	2.86	12.2	47	41.57-52.43
19	QRL_2A_2	Root length	2A	Normal	3.22	15.2	50	45.64-54.36
20	QRL_2A_3	Root length	2A	Normal	2.71	12.2	48	42.57-53.43
21	QRL_2A_4	Root length	2A	Normal	2.55	9.1	45	37.72-52.28
22	QRL-2B-1	Root length	2B	Normal	29.89	68	6	5.160-6.840
23	QRL-2B-2	Root length	2B	Normal	16.42	38.8	6	4.52-7.480
24	QRL-2B-3	Root length	2B	Normal	12.64	34	6	4.31-7.69
25	QRL-2B-4	Root length	2B	Normal	2.91	8.2	6	1.75-10.25
26	QRL-2B-5	Root length	2B	Normal	26.27	59	11	10.03-11.97
27	QRL-2B-6	Root length	2B	Normal	11.98	32	10	8.21-11.79
28	QRL-2B-7	Root length	2B	Normal	7.48	20.3	6	3.17-8.83
29	QRL-2D-1	Root length	2D	Drought	4.54	4.54	48	26.93-69.07
30	QRL_3A_1	Root length	3A	Normal	3.74	12	72	66.48-77.52
31	QRL_3A_2	Root length	3A	Normal	2.13	7.8	73	64.51-81.49
32	QRL_3A_3	Root length	3A	Normal	2.92	10.9	90	84.73-95.27
33	QRL_3B_1	Root length	3B	Normal	4.39	4.39	56	34.21-77.79
34	QRL_3B_2	Root length	3B	Normal	3.83	3.83	65	40.02-89.98
35	QRN_2A_1	Root number	2A	Normal	2.44	12.8	45	39.82-50.18
36	QRN_2A_2	Root number	2A	Normal	2.14	11.5	46	40.24-51.76
37	QRN_2A_3	Root number	2A	Normal	2.14	9.5	46	39.03-52.97
38	QRN_2B_1	Root number	2B	Drought	3.04	3.04	40.3	15.90-64.70
39	QRN_2B_2	Root number	2B	Normal	2.15	2.15	43.5	11.25-75.75
40	QRN_3A_1	Root number	3A	Normal	3.92	14.2	46	41.33-50.67
41	QRN_3A_2	Root number	3A	Normal	2.73	6.3	45	35.89-54.11
42	QRN_3B_1	Root number	3B	Drought	3.13	6.72	92	87.83-96.17
43	QRN_3D_1	Root number	3D	Normal	2.46	8.8	36	29.48-42.52
44	QRV_2A_1	Root volume	2A	Normal	4.01	20.4	52	48.75-55.25
45	QRV_2A_2	Root volume	2A	Normal	2.69	8.5	44	36.20-51.80
46	QRV_2A_3	Root volume	2A	Normal	2.52	12.2	49	43.57-54.43
47	QRV_2A_4	Root volume	2A	Normal	2.45	7.8	43	34.51-51.49
48	QRV_2A_5	Root volume	2A	Normal	2.05	8.6	45	37.30-52.70
49	QRV_3A_1	Root volume	3A	Normal	3.55	12.5	71	65.70-76.30
50	QRV_3A_2	Root volume	3A	Normal	2.13	7.8	73	64.51-81.49
51	QSRA_2B_1	Root angle	2B	Drought	4.15	4.15	66	42.95-89.05
52	QSRA_2B_2	Root angle	2B	Drought	3.1	3.1	62	31.14-92.86
53	QSRA_2B_3	Root angle	2B	Normal	2.14	2.14	78	36.50-119.5
Average					4.9	12.25	-	20.8

LOD= logarithm (base 10) of odds, R<sup>2</sup>= phenotypic variance explained by individual QTL (%).



**Fig 2.** The average variance explained by the QTLs ( $R^2$ ) associated with root morphological traits under normal and drought stress conditions.



**Fig. 3.** Chromosomal locations of QTLs with 95% confidence intervals associated with root morphological traits on chromosomes 2A, 2B, 3A and 3B in bread wheat. Markers name are on the right; the distances in centiMorgan are on the left. Chromosomes were referenced from the wheat consensus map of Somers et al. (2004). The BioMercator program used to integration genetic maps and QTLs towards discovery of MQTLs.

**Table 4.** Consensus QTLs of six root morphological traits identified by meta-analysis on 2A, 2B, 3A and 3B chromosomes.

Chr.	MQTL	AIC <sub>a</sub>	Position on consensus map (cM)	Confidence interval M-QTL	Flanking markers of the position	Number of initial QTLs	Trait
2A	M-QTL1	86.5	47.2	45.9–48.5	<i>Xwmc522–Xwmc792</i>	17	RV, RSA, RL, RN
2B	M-QTL2	112.1	6.0	5.3–6.6	<i>Xwmc661–Xwmc382</i>	5	RL
	M-QTL3	112.1	10.8	9.9–11.6	<i>Xwmc382–Xgwm614</i>	2	RL
	M-QTL4	112.1	30.8	25.5–36.1	<i>Xwmc25–Xgwm257</i>	3	RN
	M-QTL5	112.1	59.0	51.9–59.0	<i>Xbarc7–Xwmc474</i>	5	RSA
	M-QTL6	58.7	45.8	41.6–49.9	<i>Xwmc505–Xcfa2134</i>	2	RN
3A	M-QTL7	58.7	75.5	73.1–77.8	<i>Xgwm497–Xwmc173</i>	8	RV, RSA, RL
3B	M-QTL8	86.8	47.3	39.5–55.0	<i>Xwmc808–Xwmc231</i>	7	RV, RSA, RL, RDW

RL= root length; RSA= root surface area; RV= root volume; RN= root number; RDW= root dry weight.

a. The Akaike information criterion is a criterion for model selection used to regularized maximum likelihood (ML) estimators.

onto the consensus genetic map (Somer et al., 2004) and utilized in meta-QTL analysis. Over the past decade, the hexaploid wheat microsatellite-based consensus linkage map by Somers et al. (2004) has been widely used as a reference for bread wheat genomics studies. As a result eight significant MQTLs were identified according to the lowest Akaike information criterion (AIC) values (Fig 3). Isolated MQTLs were distributed unevenly on group 2 and 3 wheat homoeologous chromosomes: one MQTL on chromosome 2A; four on chromosome 2B, and two MQTLs on chromosomes 3A and one MQTL on chromosome 3B named as MQTL1 to MQTL8 according to their chromosomal locations (Table 4; Fig. 3). Of these identified MQTLs, five were derived from at least five initial QTLs, five with confidence interval no more than 5 cM, and seven with  $R^2$  value of more than 20.0%. Among them MQTL1, MQTL2, MQTL3 and MQTL7 derived from more than five initial QTLs with confidence interval (CIs) of less than 5 cM on chromosome 2A, 2B, 2B and 3A, respectively. MQTL1 located on the long arm of chromosome 2A was detected for various root morphological traits including root number, root length, root surface area and root volume that explains 11.8% of phenotypic variation with a confidence interval of 2.61 cM. This MQTL was consistently detected in the same marker interval *Xwmc522–Xwmc792* (bin 45.93–48.54) at Meta-QTL analysis. Moreover, four MQTLs (MQTL2, MQTL3, MQTL4 and MQTL5) were identified by an integrated meta-analysis of 7 root length, 3 root number, and 5 root surface area QTLs. MQTL2 and MQTL3 were associated with root length. MQTL2 on short arm of chromosome 2B merged five QTLs from four populations, with flanking markers *Xwmc661–Xwmc382* at 5.3 cM and 6.6 cM of the consensus map, a CI of 1.3 cM and a  $R^2$  value of 15.8%. MQTL3 on short arm of chromosome 2B merged two QTLs, predicted from five populations, into a single consensus QTL. The flanking markers for this MQTL lay at 9.9 cM and 11.6 cM (*Xwmc382–Xgwm614*), the CI of this MQTL was 1.7 cM, and its  $R^2$  value was estimated as 9.4%. MQTL4 on short arm of chromosome 2B merged three QTLs, predicted from two populations, into a single consensus QTL. The flanking markers (*Xwmc25–Xgwm257*) for this site lay at 25.5 and 36.1 cM, the CI of the QTL was 4.6 cM, and its  $R^2$  value was 8.7%. MQTL5 on short arm of chromosome 2B combined five QTLs, predicted from three populations, into a single consensus QTL. The flanking

markers for this site lay at 51.9 and 59.0 cM, the CI of the QTL was 7.1 cM, and its  $R^2$  value was 14.8%.

Meta-analysis was carried out on root morphology traits on chromosome 3A to explaining the number of meta-QTLs for the trait and gets an accurate estimate of their position. A total of 10 individual QTLs on chromosome 3A were used to explore MQTLs and their accurate position of root morphological traits. As results, two MQTLs (MQL6 and MQL7) were identified within an interval of 36 cM (Table 4; Fig. 3), at 45.8 cM (bin *Xwmc505–Xcfa2134*) and 75.5 cM (bin *Xgwm497–Xwmc173*) distance according to the reference consensus map. MQTL6 was associated with number of roots. MQTL6 and MQTL7 comprised two and eight initial QTLs, each with a large CI, resulting in broad MQTL CI>25 cM, respectively. On chromosome 3B, eight QTLs were projected (Table 4; Fig 3), resulted in identification of MQTL8 comprising six and two initial QTLs with positions at 47.3 and 109.1 cM, respectively. They were associated with root length, root surface area, root volume and root dry weight (Fig. 3). The marker interval for this MQTL lay at 9.9 cM and 11.6 cM (between SSR markers *Xwmc808* and *Xwmc231*), the CI of this QTL was 1.7 cM.

## Discussion

In the near future, cereal crop production will face two major challenges, (i) the sharp increase in cereal production required by the growing world population, and (ii) the ongoing climate change and global warming that result in increased extreme drought and heat episodes. In this scenario, enhancing the genetic capacity of the plant to acquire soil resources (water and nutrients) is a primary target and can be accomplished by including the crop root system in the list of traits of interest for plant breeders (Lobet et al., 2013). From a methodological standpoint, phenotyping roots of crops is highly cost effective for evaluating hundreds of genotypes as required in QTL discovery studies (Maccaferri et al., 2016). The role of root architecture in plant performance in the field is indicated by the coincidence of root QTL and other agronomic importance traits. A meta-analysis combines results from multiple QTL studies, allows confirmation of QTL locations across genetic backgrounds and environments and providing more insight into genomic region associated with traits. To date, there are few reports about QTL meta-analysis for different traits in wheat (Mao et al., 2010), rice (Khowaja et al., 2009), barley (Li et al., 2013), soybean (Qi

et al., 2011) and maize (Wang et al., 2015; Hao et al., 2010). In this study, we performed meta-QTL analysis on root morphological traits as an important part of wheat architecture with high impact on the plant yield and adaptation, but less studied due to some practical difficulties. Finding MQTL that explains several root traits at the sometime can be useful to combine, or to pyramid, a number of root characteristics by marker assisted selection (MAS). Consequently, we combined the results of 53 root QTLs studies under both normal and drought stress conditions in wheat that resulted in identification of eight MQTLs on groups 2 and 3 chromosomes. The total length of wheat linkage map used in this study was 1086.9 cM. Nine QTLs with  $R^2$  value higher than 15% were integrated in to these MQTLs. The confidence interval at all MQTLs ranged from 1.4 to 15.5 cM (Table 4). Three meta-QTLs, MQTL1, MQTL2 and MQTL7, were much more important. Each of these included 7–17 initial QTLs reflecting clusters of multiple QTLs for 3–4 root morphological traits.

The integrated map used to conduct the MQTL analysis is prosperous in molecular markers that correspond to gene sequences, and that can give indications on feasible candidate genes for more studies (Marone et al., 2013). Marker-assisted selection (MAS) is an approach of selecting desirable individuals in a breeding program based on DNA molecular markers patterns instead of, or in addition to, their traits values. The functional markers in wheat have been predominantly generated from individual QTL studies. The candidate genes, especially those co-located with the ranked meta-QTL could be strong candidates for developing suitable markers for root morphological trait (Li et al. 2013). Information was recovered for the putative functions of sequences relevant to the molecular markers underneath the individual QTL and MQTL identified for these root traits. Further analysis revealed that under MQTL1 on chromosome 2A, SSR marker *Xwmc522* is broadly identical to a member of potassium transporter gene family in Arabidopsis. In plants, individual genes are involved in soil potassium availability and uptake and possibly auxin distribution in roots (Gierth and Mäser, 2007). The characterization of Arabidopsis mutants defective in *AtKUP/HAK/KT* transporter disclosed that these genes affect developmental processes mainly involved in cell elongation and development of root-hairs. Rigas et al. (2001) and Vicente-Agullo et al. (2004) also reported prevention of *Tiny Root Hair 1 (TRH1)* leads root development and stops root-hair elongation due to damaged auxin transport agravitropic. The microsatellite marker *Xwmc661*, under MQTL2 on chromosome 2B, corresponds to a putative LRR receptor-like serine/threonine-protein kinase. Receptor-like kinases including leucine-rich repeats (LRR-RLKs) form a large part of the RLK family in the plant genome, and comprise of three domains: an extracellular LRR domain, a single transmembrane domain, and a cytoplasmic serine/threonine kinase domain (Shiu and Bleecker, 2001). It is shown that LRR-RLKs have a key role in diverse signal transduction pathways involved in plant growth and development. Three CLAVATA1-related receptor kinases, *BARELY ANY MERISTEM 1 (BAM1)*, *BAM2* and *BAM3*, act in a positive way in the development of shoot and root apical meristems (DeYoung et al., 2006). *HYPERNODULATIONABERRANT ROOT FORMATION1 (HARI)* is another gene from this family that is taken part in root development and nodulation process. The *HARI* locus in *Lotus japonicus* plants changes the phenotype of roots and a hyper-nodulation. Root structure alteration is due to inhibition in root length elongation, radial expansion and

increase in lateral and seminal root initiation (Wopereis et al., 2000).

The *Xgwm497* marker located in MQTL7 on chromosome 3A is relevant to lignin catabolic process and oxygen oxidoreductase activity. Lignification, which is the metabolic process of sealing a plant cell wall by lignin deposition, happens within the course of typical tissue development and is a significant step during root growth. Lignin is one of the final products of phenylpropanoid metabolism and it plays an important role in resistance to biotic and abiotic stresses tolerance (Chen et al., 2013). Some enzymes of this metabolic pathway, such as phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), and peroxidase (POD), come with the synthesis and polymerization of monolignols that result, in premature lignification (Boerjan et al., 2003; Vanholme et al., 2010). Reduction in root length has been linked to the cell wall lignification induced by allelochemicals. In general, lignification makes the cell wall firm with concomitant increases in PAL and POD activities. In fact, rise of PAL activity is closely connected to decline of root growth and lignin production in maize, cucumber, and soybean vulnerable to the action of phenylpropanoid allelochemicals (Rama Devi and Prasad 1996; Herrig et al., 2002; dos Santos et al., 2004).

Interestingly by most of the identified chromosomal regions resulted were from MQTL analysis involved in the control of a number of different root traits. The meta-analysis reported here will help the selection of targeted root QTLs by marker assisted selection, and provides fundamental information that obtaining QTL-related genes in wheat. These MQTLs associated genomic regions can be further verified by performing fine mapping on large-effect QTLs to narrow down the genomic region responsible for a specific root trait or by developing chromosome segment substitution.

## Materials and methods

### QTLs related to root morphology of bread wheat

Here we analyzed QTLs related to root morphology in wheat under both normal and drought stress conditions resulted from 12 independent experiments published within 2005–2014 (Table 1). The evaluated root characters included length, surface area, average diameter, volume, number of tips and dry weight. The information on traits, the position of QTLs, chromosome number, confidence interval (CI), phenotypic variance explained by the QTL ( $R^2$ ) and LOD values are presented in table 2. In cases the confidence interval was not available, it was calculated using the following formula with 95% confidence interval:

$$CI = 530 / (N \times R^2) \text{ for } F_2 \text{ lines} \quad (1)$$

$$CI = 287 / (N \times R^2) \text{ for DH lines} \quad (2)$$

$$CI = 163 / (N \times R^2) \text{ for RILs} \quad (3)$$

Where N is the number of lines in the mapping population and  $R^2$  is the percentage of phenotypic variation explained by the related QTL.

### Analysis of meta-QTL

The analysis of meta-QTL involved two stages: first the integration of the different genetic maps and then the meta-QTL analysis itself. A consensus map contained 1,238 SSR markers spanning 2,569 centiMorgan (cM) with an average of 2.06 cM distance between two adjacent loci (Somers et al., 2004) were used as a reference map for Meta-QTL analysis. The projections of QTLs on the consensus map were performed using BioMercator 4.2 software based on common

markers among different maps (Arcade et al., 2004) BioMercator is based on the algorithm developed by Goffinet and Gerber (2000) in which a maximum likelihood function is produced to determine (i) the number of MQTLs given a set of input QTLs on a common genetic map, (ii) the consensus position of detected MQTLs based on the variance of input QTLs positions and were estimated as the mean QTLs distribution maximum likelihood, and (iii) a 95% CI for each MQTLs based on the variance of input QTLs intervals and QTLs is deduced by the algorithm (Arcade et al., 2004): The 95% confidence interval of the consensus:

$$C.I. = 3.92' \sqrt{1/\hat{\mathbf{a}} \sum_{i=1}^n (1/\sigma_i^2)}$$

where  $\sigma_i^2$  is the variance of position of the *i*th QTLs of the distribution. The BioMercator computes the consensus QTLs by models 1, 2, 3, 4 and *n*. Model 1 represents that all the input QTLs are in a single location on the linkage group. Model *n* means all the input QTLs are in *n* different locations. The model with the lowest Akaike criterion (AIC value) is the most probable model. In each model, a confidence interval is calculated for each detected meta-QTLs. The drawing bar chart and chi square test ( $\chi^2$ ) were performed using Rstudio software. The molecular linkage map was drawn by the Mapchart 2.2 software (Voorrips 2002) ([www.wageningenur.nl/en/show/Mapchart.htm](http://www.wageningenur.nl/en/show/Mapchart.htm)).

### Exploring functional candidate genes

The confidence intervals (cM) of identified microsatellite markers linked to MQTL regions on the bread wheat physical map were used to search for the candidate genes in *Triticum aestivum* on the “Ensembl Plants” website ([http://plants.ensembl.org/Triticum\\_aestivum/Info/Index](http://plants.ensembl.org/Triticum_aestivum/Info/Index)). Moreover, the sequences were functionally annotated by BLAST analysis, and classified according to the “Uniprot” database (<http://www.uniprot.org/>).

### Conclusion

Analysis of meta-QTL allowed us to use collected QTLs from different published studies to obtain consensus QTLs across different genetic backgrounds and conditions, and thus provide a better definition on genomic regions involved in the control of root morphological traits and finally chances are there to facilitate the identification of positional candidate genes. Moreover, identified MQTLs can be targeted for future studies on root architecture, breeding and genetic improvement of stress tolerance in bread wheat.

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