QTL mapping of root angle in F$_2$ populations from maize 'B73' x teosinte *'Zea luxurians'*

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Abstract: We evaluated variation in nodal root angle in the genus *Zea* and performed quantitative trait locus (QTL) mapping for the trait. Angle (in degrees) of roots emerging from the second (2nd-root angle) and third (3rd-root angle) nodes from the bottom of shoot showed wide variation in nine accessions; relatively high repeatability was obtained. QTL analyses controlling root angle were performed in the two sets of F$_2$ populations (127 individuals in Trial A and 123 in Trial B) developed from different crossings of maize 'B73' (deep-rooting) x teosinte '*Zea luxurians*' (shallow-rooting). In Trial A, we used an SSR-based map with 107 markers, covering 1,329 cM throughout all ten chromosomes. By composite interval mapping analysis, four QTLs were identified, two on chromosome 10 for 2nd-root angle and one each on chromosomes 2 and 7 for 3rd-root angle. In Trial B, using a 1,397 cM SSR-based map with 92 markers, one QTL was located on chromosome 4 and two on chromosome 7 for 2nd-root angle and one each on chromosomes 2, 4 and 7 for 3rd-root angle. The QTL on chromosome 7 (identified as *Qra2nd3rd7.04*) was consistently found across the trials. A potential role of the *Qra2nd3rd7.04* in controlling angles of nodal roots and thus flooding tolerance was discussed.

Keywords: flooding, maize, quantitative trait locus, root angle, shallow root system, teosinte.

Introduction

Flooding of soil occurs over worldwide in crop production areas and results in a reduction of crop productivity (Boyer 1982). In Japan, the Ministry of Agriculture, Forestry and Fisheries has compelled farmers to reduce the number of hectares planted with lowland rice and is encouraging them to plant other crops in upland fields being converted from paddy to increase the level of food self-sufficiency. Maize is required to be grown in such poorly drained upland paddies, even though soil flooding caused by frequent rainfall inhibits growth of maize seedlings due to the poor adaptation of maize to flooding. In order to increase productivity of maize in the temporarily flooded soils, there is a great need to develop of flooding-tolerant lines. However, it is difficult to select reliable tolerant lines in field conditions since flooding tolerance is considered to be a complex trait that relates to multiple factors.

Mano and Omori (2007) summarized factors affecting flooding tolerance in plants as: (1) the ability to grow adventitious roots at the soil surface (surface root) during flooding, (2) the capacity to form root aerenchyma, and (3) tolerance to toxins (e.g., Fe^{2+}, H_2S) under reducing soil conditions. We have already identified quantitative trait loci (QTLs) controlling these traits using wild relatives of maize (various teosinte species) and maize accessions (Mano and Omori 2007). Introgression of these QTLs into elite maize inbred lines is underway (Y. Mano, unpublished). In addition to these factors, the importance of a shallow root system (Oyanagi et al. 2004) and a barrier to radial oxygen loss from aerenchymatous roots (Colmer et al. 1998, Colmer 2003) have also been reported. With further introgression of QTLs controlling traits related to flooding tolerance, it will be possible to develop stable flooding tolerant maize lines. In this study, we focused on shallow root system (vertical root distribution), which was related to not only phosphorus acquisition (Lynch and Brown 2001, Zhu et al. 2005) or root pulling force (Sanguineti et al. 1998) but also flooding tolerance.

Oyanagi et al. (2004) suggested that shallow-rooting double-haploid lines of wheat maintained higher grain yield than deep-rooting double-haploid lines when there was a higher water table. Under

flooding conditions, oxygen in the soil was deficient and shallow-rooting can help plants to obtain dissolved oxygen around the soil surface. The relationship between the root system form in the field and the growth angle of seminal or nodal roots has been clarified in wheat (Oyanagi 1994) and maize (Nakamoto et al. 1991); therefore, root angle may be a useful parameter in vertical root distribution in the field. However, little is known regarding the genetics of the angle of roots in plants.

The objectives of this study were to reveal varietal variation in maize and teosinte accessions and to identify QTLs controlling angle of nodal roots by using F_2 populations derived from the cross between a maize inbred line 'B73' and a teosinte, '*Z. luxurians*'. Furthermore, the relationship between the QTLs controlling root angle and previously reported QTLs controlling root traits will be discussed.

Materials and Methods

Experiment 1: Variation in root angle in species and varieties

Plant materials

The maize (*Zea mays* L.) inbred lines, B73, Na4, Na74, B55 and Mi29, were obtained from the Corn and Sorghum Breeding Laboratory, National Institute of Livestock and Grassland Science, Nasushiobara, Japan, and line B64 (accession No.00094105) was provided by the Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan. Two teosintes, *Z. luxurians* (PI441933) and *Z. mays* ssp. *huehuetenangensis* (PI441934) were provided by the North Central Regional Plant Introduction Station USDA/ARS, NCRPIS, Iowa State University, Ames, Iowa, and one, *Z. nicaraguensis* (CIMMYT13451), came from the International Maize and Wheat Improvement Center (CIMMYT), Mexico.

Root angle

The experiment was conducted in a greenhouse maintained at a temperature of 30°C day/25°C night with natural light at 13-14 hours day length. For measuring root angle, the nine accessions were grown in 16 cm diameter, 19 cm deep pots filled with granular soil (Kureha Chemical Industry, Tokyo, Japan; 1.2 g N, 5.8 g P, 1.8 g K in each pot). In our preliminary experiment, effect of pot size on angle of roots was not so large (data not shown) and our measurement should be applicable for trait evaluation. Twelve plants per accession were evaluated, each accession planted in three pots (two plants per pot), with two replications. The seedlings were irrigated

every second or third day with tap water. The six-leaf-stage seedlings (approximately four weeks old) were excavated from the pots carefully and washed with tap water without root injury. We used nodal roots emerging from the second and third nodes from the bottom of the shoot since these roots are thick and tough for keeping nearly the original angle after washing. On the contrary, the angle of nodal roots emerging from the first node (coleoptilar node) was not evaluated because they are thin or we could not measure the angle accurately. The root angle was determined by measuring the angle between the horizontal (i.e. soil surface) and the line between the root-shoot junction and a point 1 cm from the junction using a protractor in 5° increments. The angle of three to five roots per node were averaged for each plant measured.

Experiment 2: Mapping QTL for root angle

Plant materials

We selected B73 and *Z. luxurians* (Durieu & Asch.) Bird as the parents for the mapping study since we have already constructed a linkage map of the cross between B73 and *Z. luxurians* (Mano et al. 2008), and these two accessions exhibited consistent difference in the degree of root angle based on the results of Experiment 1, B73 exhibiting deep-rooting (large angle) and *Z. luxurians* showing shallow-rooting (small angle). Two populations of 127 (Trial A) and 123 (Trial B) F_2 individuals from a cross between B73 × *Z. luxurians* were used for QTL mapping of root angle. The two F_2 populations were constructed by selfing two different F_1 plants, which were derived from a cross of inbred B73 and one outbred *Z. luxurians* individual. The F_1 individuals, and corresponding F_2 populations, have a 50 % chance of being different for alleles that are heterozygous in the outbred *Z. luxurians* parent.

We used F_2 populations for mapping study due to difficulty of self pollination in F_2 individuals of maize × exotic species teosinte cross (J. Doebley, personal communication, Mano et al. 2007). QTL analyses using an F_2 mapping population of the cross between maize × teosinte have previously been reported (Doebley and Stec 1991, 1993, Bomblies and Doebley, 2006), however, the development of F_3 progeny useful for replication tests has not yet been reported.

Trait evaluation

The angle of three to five roots per node were averaged for each F_2 individual measured as described in Experiment 1 with one exception: seedlings were grown in 11 cm diameter, 30 cm deep pots, one plant

per pot. We also measured plant height, from soil surface to the highest leaf tip, of the six-leaf stage seedlings.

DNA isolation

A small amount of plant DNA (~1–4 μg) was isolated from 50 mg of fresh leaf tissue by the method described by Komatsuda et al. (1998).

SSR analysis

Based on the SSR list available at the MaizeGDB (http://www.maizegdb.org/ssr.php), 107 (Trial A) and 92 (Trial B) SSR primer pairs were selected to construct SSR-based linkage maps. The SSR analysis was performed as described by Mano et al. (2005c). We used the term "bin" which is a traditional method for referring to the location/position of markers within the maize chromosomes.

Map construction and QTL analysis

In Trial A, we used a previously developed linkage map with 107 markers derived from 228 F_2 individuals from the cross between B73 × *Z. luxurians* (Mano et al. 2008); this included 127 F_2 individuals used in Trial A. In Trial B, we constructed a linkage map with 92 SSR markers using MAPMAKER/EXP 3.0 (Lander et al. 1987).

In Trials A and B, composite interval mapping (CIM) in Windows QTL Cartographer Version 2.5 was used to map the QTLs controlling root angle (Wang et al. 2006). CIM was run with the default setting for model 6 in the program (5 background markers and a window size of 10 cM). We reported the LOD threshold (P=0.05) by 1,000 permutations of the

data for each trait using Windows QTL Cartographer. In addition, less significant LOD threshold of 2.5 were used based on Lander and Botstein (1989), since QTLs for root traits generally show a relatively low LOD values due partly to presence of environmental factors.

Results

Experiment 1: Variation in root angle in species and varieties

B73 had the largest root angle (deepest rooting) of the nine accessions. Root angle showed significant and positive correlation between the replications (r=0.827 for 2nd-root angle and r=0.850 for 3rd-root angle). Correlation between 2nd-root angle and 3rd-root angle was high (r=0.910) across the nine accessions (Fig. 1). The degree of root angle in B73 and *Z. luxurians*, used for further mapping analysis, showed great difference (Fig. 2): 2nd and 3rd-root angles were 49° and 51° for B73 and 31° and 28° for *Z. luxurians*, respectively.

Experiment 2: Mapping QTL for root angle

Variation of root angle in the F_2 populations

In trial A, using 127 F_2 individuals, 2nd-root angle, 3rd-root angle and plant height showed continuous distributions. Second-root angle ranged from 18° to 68° (mean=39.8°) while 3rd-root angle ranged from 15° to 63° with the mean of 35.1° (Fig. 3a). Correlation between 2nd-root angle and 3rd-root angle was 0.567, significant at the 0.1 % level (Fig. 4a). Plant height ranged from 49 cm to 90 cm with the mean of 72 cm (data not shown).

In Trial B (123 F_2 individuals), 2nd-root angle ranged 7° to 60° with a mean of 31.1°, while 3rd-root angle ranged -4° to 59° (mean=27.9°), somewhat smaller (shallower rooting) than Trial A (Fig. 3b). There was significant correlation between 2nd and 3rd-root angles (r=0.673, at the 0.1 % level, Fig 4b). Plant height ranged 61 cm to 109 cm (mean 84 cm).
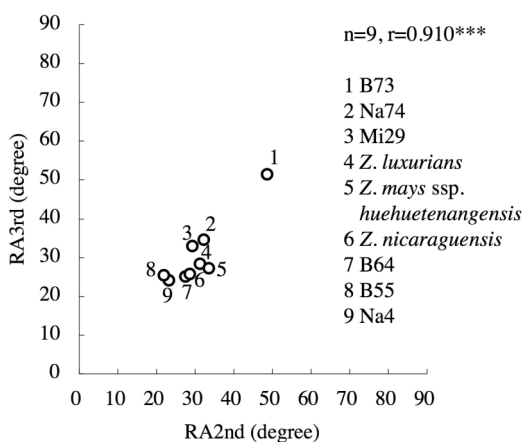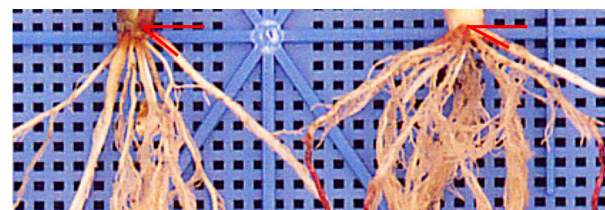

Fig. 1. Relationship between the angles of roots emerging from the second (RA2nd) and third (RA3rd) nodes in nine *Zea* accessions.

Fig. 2. Roots of B73 (left) and *Z. luxurians* (right) at the six-leaf stage. The root angle was determined by measuring the angle between red lines.
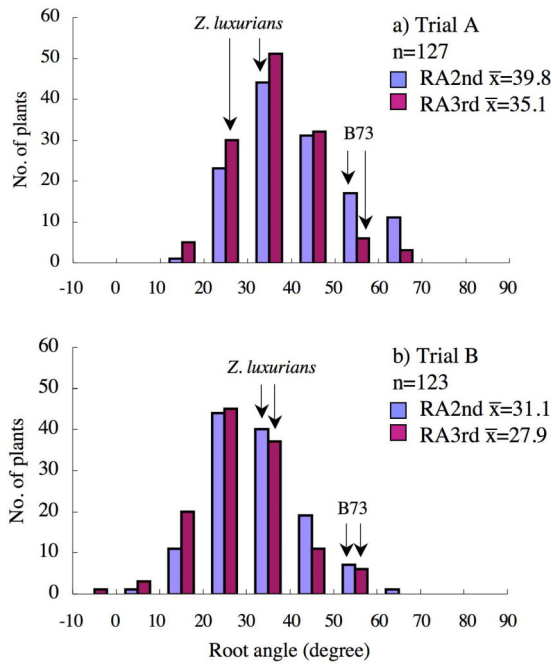
Fig. 3. Frequency distribution for root angles emerging from the second (RA2nd) and third (RA3rd) nodes in the two B73 × *Z. luxurians* F_2 populations. Root angles in B73 evaluated at the same environment were 51°±4° (mean±standard deviation, n=5) for RA2nd and 50°±9° (n=5) for RA3rd in Trial A and, in Trial B, 50°±8° (n=9) for RA2nd and 51°±4° (n=9) for RA3rd, while those of *Z. luxurians* were 35°±9° (n=6) for RA2nd and 22°±3° (n=6) for RA3rd in Trial A and, in Trial B, 33°±6° (n=14) for RA2nd and 31°±5° (n=14) for RA3rd.

QTL mapping for root angle in Trial A

We used an SSR-based map with 107 markers covering 1,329 cM at an average interval of 13.7 cM/locus for the ten chromosomes (Mano et al. 2008, Fig. 5). QTLs were identified in two regions on chromosome 10 for 2nd-root angle and one each on chromosomes 2 and 7 for 3rd-root angle (Table 1). With the exception of one on chromosome 10 (*Qra2nd10.04*), all QTL alleles of *Z. luxurians* decreased root angle (acted towards shallow-rooting).

Four QTLs for plant height were identified on chromosome 1, 4, 5 and 7, that on chromosome 4 being more important (Table 1). Alleles of *Z. luxurians* increased plant height on chromosomes 4 and 7, whereas those of B73 increased plant height on chromosomes 1 and 5.

Map construction and QTL mapping for root angle in Trial B

In Trial B, we first tested the 107 SSR markers that were used in Trial A. Of these, compared to Trial A, 72 SSRs generated the same segregation fragment patterns in B73, *Z. luxurians* and heterozygous after PCR amplification and electrophoresis, whereas 17 SSRs generated the same fragment sizes in B73 but different sizes in *Z. luxurians*. These 89 SSR markers showed clear segregation patterns among the F_2 individuals and could be used for map construction in Trial B.
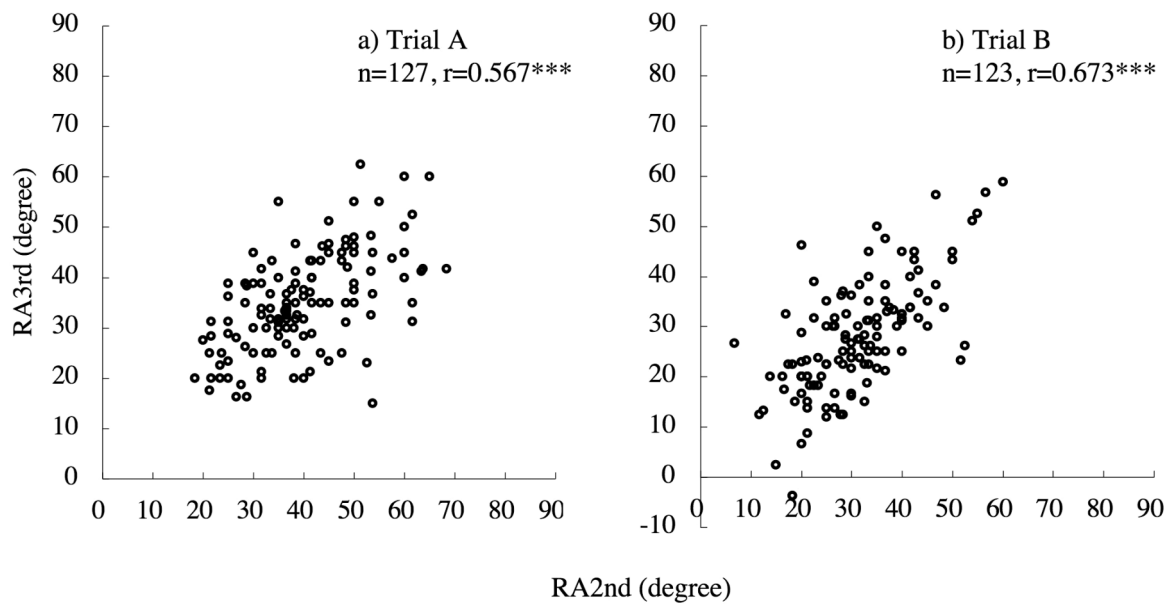

Fig. 4. Relationship between angles of roots emerging from the second (RA2nd) and third (RA3rd) nodes in the two B73 × *Z. luxurians* F_2 populations.
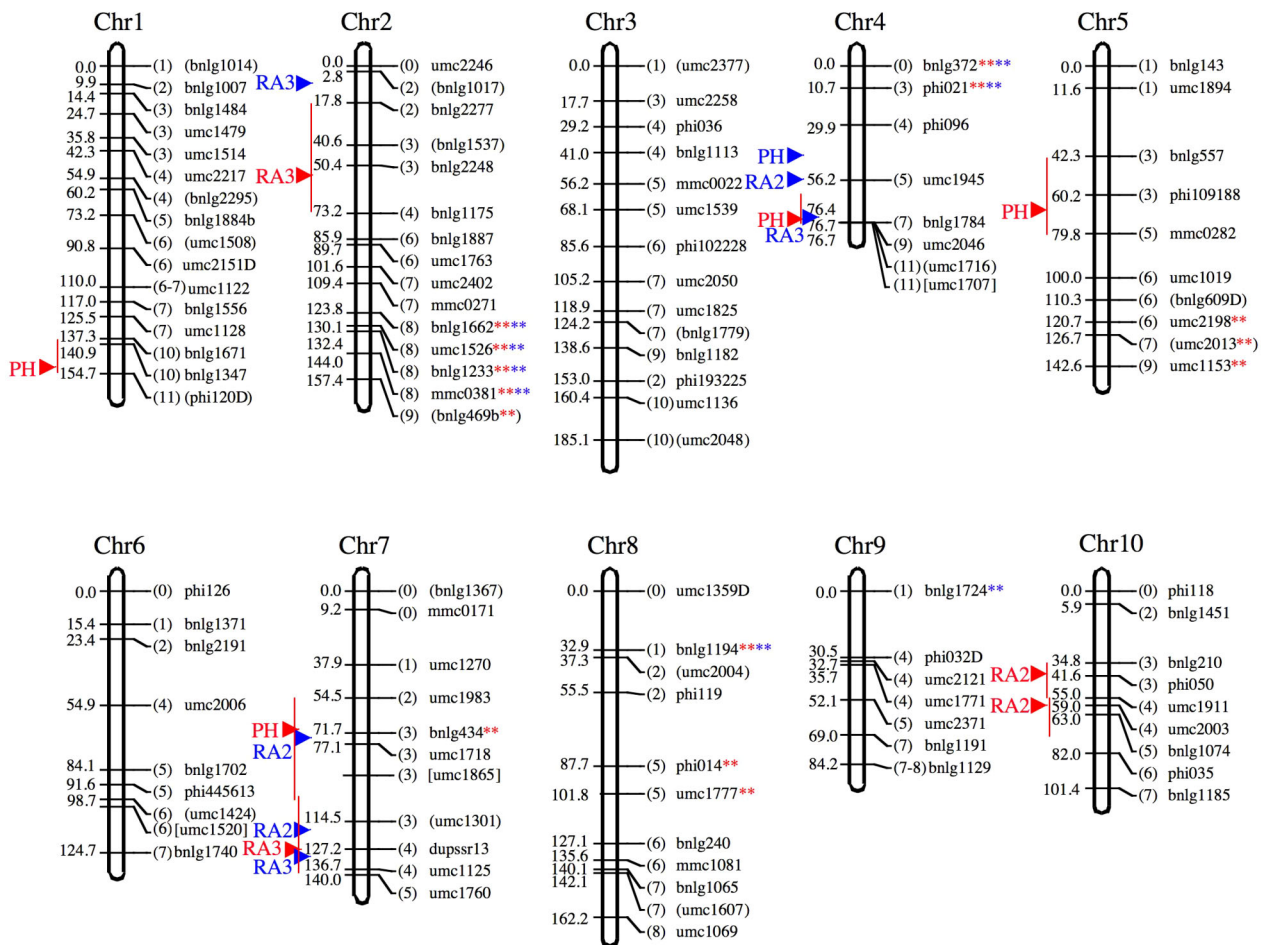
Fig. 5. Linkage map constructed in Trial A and chromosome locations of the QTLs for root angle emerging from the second (RA2) and third (RA3) nodes and plant height (PH) in the B73 × *Z. luxurians* F_2 populations of Trial A and Trial B using composite interval mapping (CIM). QTL positions and segregating markers (significant at the 1 % level; shown **) found in Trial A and B are colored red and blue, respectively. Closed arrowheads indicate the position of the peak LOD in CIM and bars left of the chromosomes indicate a safe support level of 2-LOD likelihood interval. The positions of QTLs found in Trial B are approximate and do not represent the support interval since map distances between Trial A and B are different. The scales are in centimorgans (Haldane units). Markers in parentheses or brackets indicate SSRs used only for Trials A and B, respectively. Bin numbers, a traditional method for referring to the location/position of markers within maize chromosomes, are in parentheses before the marker names.

The remaining 18 SSRs could not be used for Trial B since they did not segregate among the 123 F_2 individuals (monomorphism between the parents) or did not show clear PCR amplification in the genotype of *Z. luxurians*. After preliminary map construction, there were gaps in some chromosome regions due to decreasingly useful markers in Trial B. To fill the gaps, we have used three additional SSR markers (umc1707, umc1520 and umc1865), which are shown with brackets in Fig. 5. Finally, we constructed a 123-F_2 individual map with 92 SSR markers covering 1,397 cM at an average interval of 17.0 cM/locus.

One QTL was located on chromosome 4 and two on chromosome 7 for 2nd-root angle and one each on chromosomes 2, 4 and 7 for 3rd-root angle. *Z. luxurians* possesses both shallow-rooting QTLs and

deep-rooting QTLs (Table 1). For plant height, a single QTL was detected on chromosome 4; the QTL alleles of *Z. luxurians* increased plant height.

Comparisons of QTL positions

We have consistently identified QTL controlling root angle on chromosome 7 at bin 7.04 (named *Qra2nd3rd7.04*), except for the 2nd-root angle in Trial A, and the alleles of *Z. luxurians* in this QTL decreased root angle (acted to shallow-rooting; Table 1, Fig. 5). For 2nd-root angle in Trial A, although the *Qra2nd3rd7.04* was not detected at the criterion of LOD=2.5, a minor QTL with an LOD score of 2.4 was identified at the same region of chromosome 7 (data not shown). An expression of QTLs other than

Qra2nd3rd7.04 may vary according to the trial and the portion of root.

In Trial B, a QTL was found on chromosome 4 for both the 2nd and 3rd-root angles, and alleles of *Z. luxurians* at this QTL acted to increase root angle (deep-rooting). This observation is in contrast to the phenotype of *Z. luxurians* with shallow-rooting. As shown in Figure 5, the position of the *Qra2nd4.05* overlapped that controlling plant height in Trial B (*Qph4.04-5*); the alleles of *Z. luxurians* increased plant height, indicating that the larger plants tend to be deep-rooting. In addition, the position of the *Qra3rd4.07* in Trial B overlapped that of *Qph4.07* (plant height) in Trial A. Also, a correspondence was observed between the *Qra2nd7.03* in Trial B and the *Qph7.03* in Trial A. The correspondence of QTL positions between vertical root distribution and plant height was also reported for rice (Huang et al. 1996,

Yadav et al. 1997). The reason why there is a relationship between root angle (vertical root distribution) and plant height is currently unknown.

Discussion

We have identified QTLs controlling root angle using two F₂ populations of B73 × *Z. luxurians*. Although repeatability of Experiment 1 is relatively high, the observed QTLs found in Experiment 2 do not explain a large portion of the variance of the F₂ populations (from 0.123 to 0.396, Table 1), possibly indicating presence of environmental factors. Also, undetected QTLs are expected. Nevertheless, we have successfully identified a QTL on chromosome 7 (*Qra2nd3rd7.04*) that contributes to shallow-rooting in *Z. luxurians*. Expression of QTLs other than *Qra2nd3rd7.04* changed according to the trial, due

Table 1 Chromosome locations and mode of gene action of QTLs for root angle emerging from the second (RA2nd) and third (RA3rd) nodes and plant height (PH) estimated by composite interval mapping (CIM) in the F₂ populations of the cross between B73 x *Z. luxurians* in two trials

Trait	Trial	QTL	Chr	Position[a]	Marker interval	LOD[c]	a[d]	d[e]	Mode[f]	r²[g]
RA2nd	A	*Qra2nd10.03*	10	41 (35-54)[b]	bnlg210-phi050	2.8	6.31	-3.23	L	0.094
		Qra2nd10.04	10	59 (54-75)	umc2003-bnlg1074	2.8	-5.75	-4.48	B	0.082
									Total[h]	0.123
	B	*Qra2nd4.05*[i]	4	50 (30-74)	phi096-umc1945	4.9*	-6.28	-1.29	B	0.163
		Qra2nd7.03	7	61 (36-75)	bnlg434-umc1718	3.2	-2.95	4.56	B	0.092
		Qra2nd7.03-4	7	105 (75-129)	umc1865-dupssr13	3.2	5.46	2.69	L	0.133
									Total	0.376
RA3rd	A	*Qra3rd2.03-4*	2	54 (18-73)	bnlg2248-bnlg1175	3.8*	4.26	-3.93	L	0.126
		Qra3rd7.04	7	127 (103-140)	dupssr13-umc1125	2.7	4.22	2.74	L	0.077
									Total	0.192
	B	*Qra3rd2.02*	2	8 (0-20)	umc2246-bnlg2277	3.3	4.56	3.39	L	0.113
		Qra3rd4.07	4	69 (52-74)	umc1945-bnlg1784	4.4*	-6.07	-0.16	B	0.131
		Qra3rd7.04	7	115 (93-129)	dupssr13-umc1125	4.7*	5.59	1.28	L	0.125
									Total	0.396
PH	A	*Qph1.11*	1	152 (139-155)	bnlg1347-phi120	4.2*	2.67	2.95	B	0.083
		Qph4.07	4	76 (62-77)	umc1945-bnlg1784	7.9*	-4.77	-1.19	L	0.163
		Qph5.03-5	5	68 (41-80)	phi109188-mmc0282	3.4	2.94	1.86	B	0.086
		Qph7.03	7	69 (54-105)	umc1983-bnlg434	3.8*	-3.20	1.26	L	0.085
									Total	0.418
	B	*Qph4.04-5*	4	38 (23-61)	phi096-umc1945	4.8*	-5.28	-3.41	L	0.210

[a] QTL position in cM from the top of the chromosome. The positions of QTLs between Trial A and Trial B can not be comparable since the map distances between two trials were differ

[b] 2-LOD support interval

[c] An LOD score threshold of 2.5 was presented. Astreisks indicate significant QTL by permutation tests. The LOD thershhold values in permutation tests were 3.6 and 3.6 for RA2nd, 3.5 and 3.4 for RA3rd and 3.5 and 3.6 for PH in Trial A and Trial B, respectively

[d] Additive effect

[e] Dominance effect

[f] Parent contributing lower-value allele (shallow-rooting) for root angle and higher-value allele for plant height, where B=B73, L= *Z. luxurians*

[g] Proportion of phenotypic variance explained

[h] Estimated from multiple interval mapping module in the QTL cartographer

[i] The same background color of QTLs were considered to be the same locus

largely to heterozygosity in *Z. luxurians*.

Using a maize F_2 population of a cross between 'B64' and 'Na4', QTLs controlling adventitious root formation at the soil surface (surface rooting) during flooding were located on chromosomes 3, 7 and 8 (Mano et al. 2005d). When compared, overlap was found between the QTL for root angle (*Qra2nd3rd7.04*) and a QTL for surface root on chromosome 7 (bin7.04-5) of maize inbred line Na4. Furthermore, all of the QTLs controlling root angle and surface rooting in bin7.04 were partially dominant (0.2<|dominance effect/additive effect|<0.8) (data not shown). Therefore, although the parental lines used for the QTL analyses differed between the two reports, it is interesting to find the relationship between root angle (shallow-rooting) and surface rooting under flooded conditions. Mano et al. (2005a) and Bird (2000) observed that *Z. luxurians* and its sister species, *Z. nicaraguensis* showed high ability to form surface roots during flooding, so possibly QTL *Qra2nd3rd7.04* enhances the surface root development. Perhaps shallow-rooting or distributing roots near the soil surface is advantageous to developing surface roots rapidly when flooded. Evaluation regarding the expression of QTL *Qra2nd3rd7.04* under flooded conditions will be implemented to confirm the effect of the marker-assisted introgressed QTL on flooding tolerance. We have previously identified QTLs controlling surface root growth during flooding on several chromosome regions in teosinte and maize (Mano et al. 2005b, Mano et al. 2005d). These QTLs can be classified into morphologically related QTLs (e.g. shallow-rooting *Qra2nd3rd7.04*) and physiological response QTLs like the ethylene-promoted rooting reported by Drew et al. (1979).

Several authors have reported QTLs controlling root traits in maize. Tuberosa et al. (2002) reported that QTLs for primary root length, secondary root weight and root pulling force were located on chromosome 7 (bin 7.04), where QTL *Qra2nd3rd7.04* was identified. Of these, root pulling force was related to vertical root distribution (Sanguineti et al. 1998) and QTL *Qra2nd3rd7.04* might be similar or the same as that controlling root pulling force. The relationship between the remaining QTLs is currently unclear; further study is necessary. For root angle in maize, only a single QTL was identified by Guingo et al. (1998) − using a set of recombinant inbred lines derived from a cross between "F2" × "Io", a QTL controlling the angle of root growth direction at internode 7 was found on chromosome 5 (bin 5.05). However, the relationship was not found in our study.

Yadav et al. (1997) performed QTL mapping controlling vertical root distribution (deep root weight, deep root per shoot ratio and deep root per tiller) in the doubled haploid lines of paddy rice 'IR64' × upland rice 'Azucena' cross and identified QTLs controlling three root traits with highly significant effects on the long arm of chromosome 7. Recently, QTL controlling vertical root distribution of deeper rooting (*Dro1*) was found on the long arm of rice chromosome 9 using upland rice 'Kinandang Patong' × 'IR64' mapping population (Uga et al. 2007). Interestingly, homology between maize chromosome 7 and rice chromosomes 7L and 9L was observed by comparative genome analysis (Wilson et al. 1999), and the QTL *Qra2nd3rd7.04* on maize chromosome 7 may be similar to one of the QTL controlling vertical root distribution located on rice chromosomes 7L and 9L. Also, synteny relationship between maize and rice in several root traits was well characterized (Tuberosa et al. 2003) and rice genome sequence information will help to identify and clone the root trait genes in maize.

Maize B73 used in the mapping study has been widely used for genome analyses and breeding for several agronomically important traits (Troyer 1999, Mikel and Dudley 2006). Also, in our previous study, B73 exhibited a higher degree of flooding tolerance at the seedling stage among the 223 maize accessions (Mano et al. 2002), but this accession has not yet been observed to form surface roots during flooding or root aerenchyma development. By transferring the QTLs controlling root angle into B73 by marker assisted selection, it may be possible to develop a shallow-rooting B73 together with the ability to form surface roots under flood conditions and a higher level of flooding tolerance.

We have separated components related to flooding tolerance and have performed QTL analysis for each of the flooding tolerance related traits (e.g. surface rooting during flooding and root aerenchyma formation, Mano and Omori 2007). These flooding traits were under the control of moderate to minor QTLs (Mano et al. 2005b, Mano et al. 2007), so it may be difficult to select them phenotypically. Also, gene transformation would not be practical due to many desirable target genes. Therefore, our accumulation of basic QTL information and pyramiding those QTLs related to flooding-tolearnt traits, should be a unique and practical approach to developing flooding tolerant maize hybrids that can perform well in upland paddies.

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Fumie Omori's research interest is response of plants to soil flooding such as adventitious rooting at the soil surface and root aerenchyma formation.

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