

# A linkage map of maize × teosinte *Zea luxurians* and identification of QTLs controlling root aerenchyma formation

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**Abstract** The objectives of this study were to construct a linkage map and identify quantitative trait loci (QTLs) controlling root aerenchyma formation in drained soil conditions using 195 F<sub>2</sub> individuals derived from a cross between maize inbred line B73 × teosinte *Zea luxurians*. A 107 SSR marker based map covering 1,331 cM across all ten chromosomes was developed. One significant difference between the parents utilized in the study was that under non-flooding conditions, B73 exhibits a minor capacity to develop root aerenchyma, whereas *Z. luxurians* exhibits a high tendency to form aerenchyma. Linkage analysis indicated segregation distortion regions on chromosomes 2, 4 and 8, and severe recombination suppression on the long arm of chromosome 4. Multiple interval mapping analysis suggests that five QTLs for aerenchyma formation in non-flooding conditions are located on chromosomes 2, 3, 5, 9 and 10, and these explained 36.3% of the

total phenotypic variance. The *Z. luxurians* alleles in all five QTLs increased the capacity to form aerenchyma and the locations of these QTLs did not overlap those previously identified in the teosinte *Z. nicaraguensis*. By transferring aerenchyma-forming QTLs from both *Z. luxurians* and *Z. nicaraguensis*, it may be possible to pyramid these genes and develop a maize line with exceptional aerenchyma formation and a high level of tolerance to flooding conditions.

**Keywords** Aerenchyma · Flooding · Quantitative trait locus · Recombination suppression · Root-deficient · Teosinte

## Introduction

The teosintes, wild relatives of cultivated maize (*Zea mays* L. ssp. *mays*), consist of three subspecies of *Zea mays* L. in section *Zea*: *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis* and *Z. mays* ssp. *huehuetenangensis*, and three species of section *Luxuriantes*: *Z. luxurians*, *Z. diploperennis*, *Z. perennis* (Doebley and Iltis 1980; Iltis and Doebley 1980). Recently, Iltis and Benz (2000) classified populations of teosinte discovered near the northern Pacific Coast of Nicaragua as a new form that was given the name *Z. nicaraguensis*. With the exception of tetraploid *Z. perennis* (2n = 4x = 40), all other species of teosinte possess the same chromosome number as

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maize ( $2n = 2x = 20$ ) and fertile  $F_1$  hybrids can be generated at the diploid level.

Various molecular linkage maps covering all the genome have been constructed for some maize  $\times$  diploid teosinte of *Zea mays* ssp. *mexicana* (Doebley and Stec 1991), *Z. mays* ssp. *parviglumis* (Doebley and Stec 1993), *Z. mays* ssp. *huehuetenangensis* (Mano et al. 2005c) and *Z. nicaraguensis* (Mano et al. 2007b), and for a *Z. diploperennis*  $\times$  *Z. mays* ssp. *parviglumis* cross (Westerbergh and Doebley 2002), with *Z. luxurians* being the single exception. In those prior studies, segregation distortion and severe recombination reduction were observed across several chromosome regions (Doebley and Stec 1991, 1993). The construction of a maize  $\times$  *Z. luxurians* linkage map will provide additional useful information with regard to marker order, segregation distortion and recombination suppression in a distinctive teosinte and also QTL information for exploiting the unique characteristics of *Z. luxurians* for maize improvement.

Teosintes from the regions that receive frequent rainfall within the tropical and subtropical zones of Mexico and Central America have recently been examined as a germplasm resource for the breeding of flooding tolerant maize (Mano and Omori 2007). *Z. luxurians* and *Z. nicaraguensis* possess unique flooding-related traits such as the capacity to form root aerenchyma under non-flooding conditions (Ray et al. 1999; Mano et al. 2006) and the ability to form adventitious roots at the soil surface under flooding conditions (Bird 2000; Mano et al. 2005a). In this paper, we focus on the capacity of *Z. luxurians* to form root aerenchyma under non-flooding conditions and acquiring the fundamental data to transfer this trait to maize for the development of flooding tolerant maize hybrids.

Two types of root aerenchyma formation have been described; lysigenous, in which cortical cells die creating air spaces in the root system and schizogenous, in which aerenchyma cell development is the result of cell separation (Evans 2004). Flooding-induced lysigenous aerenchyma formation in maize has been reported (e.g., Drew et al. 1979; Konings 1982) and aerenchyma formation mediated by ethylene has been well documented from prior physiological and cellular analyses (Jackson et al. 1985a; He et al. 1996; Saab and Sachs 1996; Gunawardena et al. 2001; Subbaiah and Sachs 2003). Physiological roles determining maize aerenchyma formation under conditions of sulfur or phosphorus starvation have also been

investigated (Fan et al. 2003; Bouranis et al. 2006). In various upland cereals, it has been observed that under non-flooded and aerated conditions, lysigenous aerenchyma typically does not develop or the degree of aerenchyma formation is relatively low in species such as wheat (Thomson et al. 1992; McDonald et al. 2001), barley (Garthwaite et al. 2003; Pang et al. 2004) and maize (Drew et al. 1979; Zaidi et al. 2007). In contrast, flooding tolerance in rice (Jackson et al. 1985b; Colmer 2003) and several wetland species (Smirnov and Crawford 1983; Justin and Armstrong 1987; Schussler and Longstreth 1996) has been observed to involve formation of lysigenous or schizogenous aerenchyma in well-aerated or drained soil conditions. The capacity to form aerenchyma in non-flooded soil conditions or the tendency to possess aerenchyma channels in root systems prior to flooding may pose an agronomic advantage to those plants in that they may be able to adapt more rapidly to sudden and temporary soil flooding conditions.

Ray et al. (1999) analyzing  $F_1$  hybrid of *Z. luxurians*  $\times$  maize, suggested that a dominant gene(s) control the capacity to form lysigenous aerenchyma at the adult plant stage in aerated conditions; however, this preliminary study did not associate the trait to any teosinte chromosome. In a recent study of QTLs related to lysigenous aerenchyma in unflooded *Z. nicaraguensis*, QTLs were found to be located on two regions of chromosome 1 (*Qaer1.02-3* and *Qaer1.07*), chromosome 5 (*Qaer5.09*) and chromosome 8 (*Qaer8.06-7*) (Mano et al. 2007b). A QTL analysis of aerenchyma formation using *Z. luxurians* should also be able to identify QTLs associated with aerenchyma formation in this species. The objectives of this study were to construct a molecular linkage map; to compare the linkage map to the known maize  $\times$  teosinte maps; to evaluate segregation distortion and recombination suppression regions; and to identify new loci controlling lysigenous aerenchyma formation by utilizing an  $F_2$  population derived from a cross between the maize inbred lines B73 and *Z. luxurians*.

## Materials and methods

### Plant materials

The maize (*Zea mays* L.) inbred line, B73, was obtained from the Corn and Sorghum Breeding

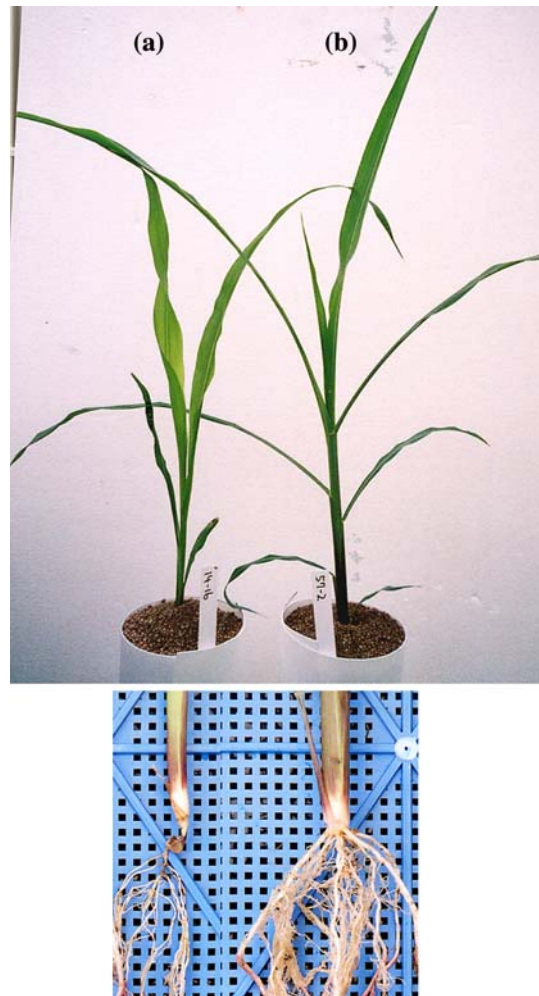
Laboratory, National Institute of Livestock and Grassland Science, Nasushiobara, Japan and *Z. luxurians* (Durieu & Asch.) Bird (PI441933) from the North Central Regional Plant Introduction Station (NCRPIS), USDA-ARS/Iowa State University, Ames, Iowa. Previous analysis of these materials has indicated that B73 exhibits a low capacity for developing aerenchyma in roots under non-flooding conditions; while *Z. luxurians* accession exhibits a high capacity (Mano et al. 2006).

We used 228 F<sub>2</sub> plants (sowing 10–15 seeds per day) derived from a single aerenchyma-forming F<sub>1</sub> plant of the cross between B73 × *Z. luxurians* in a greenhouse maintained at a temperature of 30°C day/25°C night exposed to natural light at 13–14 h day length. Two hundred and twenty-two plants grew well and the remaining 6 plants did not generate adventitious (shoot-born crown) roots (Fig. 1). Twenty-seven of the 222 F<sub>2</sub> plants could not be evaluated for their degree of aerenchyma development at the preferred six-leaf growth stage due to time limitations. A total of 195 F<sub>2</sub> plants were utilized for the evaluation of root aerenchyma formation under non-flooding conditions.

In this study, evaluation of aerenchyma formation was conducted as a single trial using an F<sub>2</sub> mapping population because the development of F<sub>3</sub> progeny or recombinant inbred lines for replication tests has not been reported due to the difficulty of self pollination in some F<sub>2</sub> plants of maize × exotic teosinte crosses (J. Doebley, personal communication). In addition, single study evaluations have successfully identified QTLs for root aerenchyma formation in an F<sub>2</sub> mapping population of a cross between B64 × *Z. nicaraguensis* (Mano et al. 2007b). Therefore, the utilization of an F<sub>2</sub> mapping strategy for a B73 × *Z. luxurians* F<sub>2</sub> population should be equally applicable.

### Root anatomy

Aerenchyma channels were observed in the adventitious roots emerged at the second node of six-leaf stage seedlings (approximately four weeks old) under non-flooded conditions as described in Mano et al. (2006). Briefly, fresh-root cross sections of 80–100 μm thick were made using a microtome. The amount of aerenchyma in the root cortex was visually scored: 0 (no aerenchyma), 0.5 (partial formation), 1 (radial



**Fig. 1** Root and shoot morphology of (a) adventitious root-deficient and (b) normal four week-old plants

formation) and 2 (radial formation extended toward epidermis) and these values were reflected to determine an approximate percentage of aerenchyma in the root cortex, corresponding to 0, ~10, ~20 and ~35%, respectively. For the QTL analysis, we averaged scores at 10 and 15 cm from the root tips of two adventitious roots (a total of 4 positions), where a higher degree of aerenchyma formation is observed when compared to other cm length/positions (Mano et al. 2006).

### SSR analysis

Small amounts of DNA were isolated using the method described by Komatsuda et al. (1998). Based

on the SSR list available at the MaizeGDB (<http://www.maizegdb.org/ssr.php>), a total of 107 SSR primer pairs for the B73 × *Z. luxurians* F<sub>2</sub> population were used to construct a linkage map as described by Mano et al. (2005c).

### Map construction and QTL analysis

A linkage map was constructed with 107 markers using an F<sub>2</sub> model of MAPMAKER/EXP 3.0 (Lander et al. 1987). Markers were grouped according to a two-point analysis at LOD > 3.0 with a recombination fraction of 0.3. A framework map was constructed according to the “three-point” and “order” command first at the LOD > 3.0 and then at LOD > 2.0. The “ripple” command was used to verify the marker order in the linkage group. Haldane’s mapping function (Haldane 1919) was used to calculate map distances. Segregation ratios at each marker were tested for goodness of fit to the expected 1:2:1 or 3:1 ratios using the Chi-square method. To obtain a genome-wide significance criterion of  $\alpha \leq 0.05$  for this number (107) of 3- or 2-class Chi-square tests, an approximate sequential Bonferroni-Holm correction (Rice 1989) was applied to ensure an appropriate test-wise significance criterion (i.e.,  $\alpha' = 0.05/107 = 0.0005$ ). Composite interval mapping (CIM) using Windows QTL Cartographer Version 2.5 (Wang et al. 2006) was used to map the QTLs controlling aerenchyma formation in the F<sub>2</sub> population. CIM was run with the default program setting for model 6 (5 background markers and a window size of 10 cM). A permutation test was performed to estimate average LOD thresholds. After 1,000 permutations, an average LOD threshold of 3.6 was chosen to determine if a putative QTL was significant and maintained a chromosome-wide Type-I error rate of 0.05. The multiple interval mapping (MIM) was performed with QTL Cartographer to refine the QTL positions, to search for more QTLs and to investigate epistatic effects among the detected QTL (Kao et al. 1999). For the MIM analysis, model selection began with an initial genetic model suggested by the CIM results and continued in a search for additional QTLs through several cycles of a forward search method.

## Results

### Segregation of root-deficient plants

We found six of 228 F<sub>2</sub> plants had not grown nodal adventitious roots at the six-leaf stage (Fig. 1). The segregation ratio of 222 (normal):6 (root-deficient) indicate that the trait is controlled by multiple genes. The subterranean morphology of adventitious root-deficient F<sub>2</sub> plants resembles that of an *rtcs* maize mutant (Hetz et al. 1996; Hochholdinginger et al. 2004).

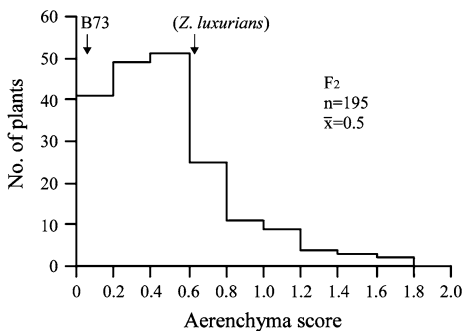
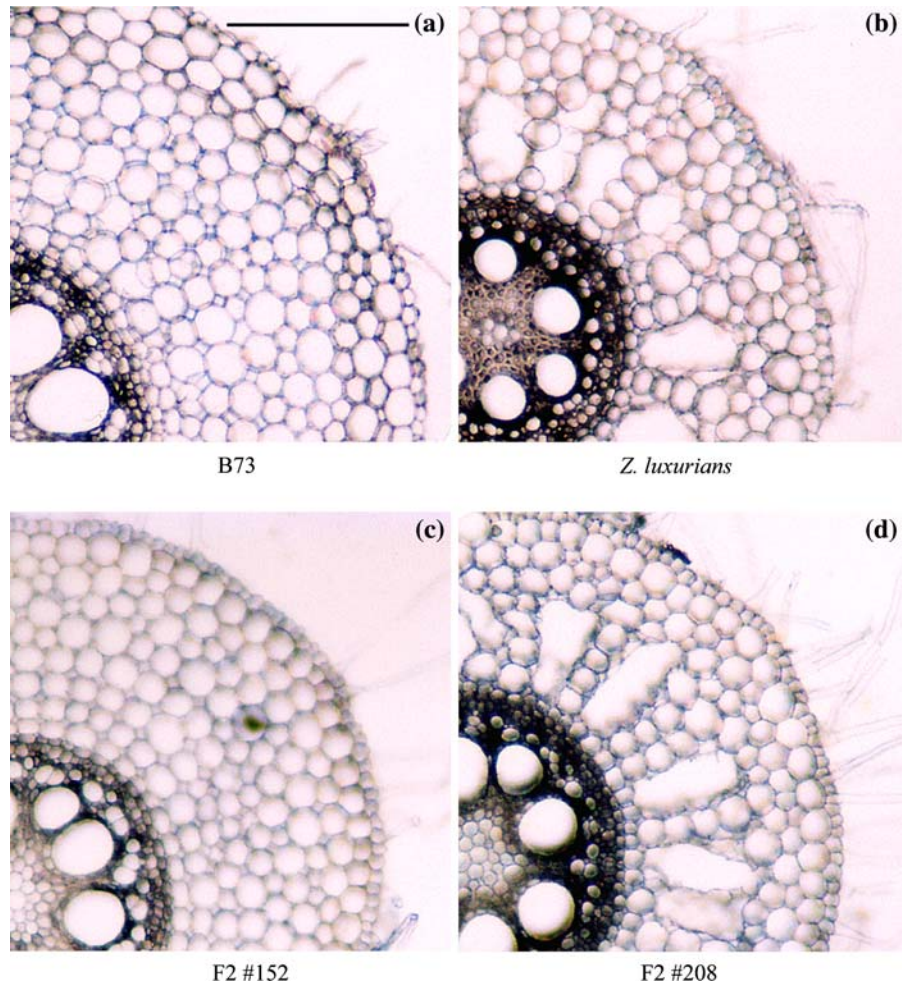
### Aerenchyma formation

The average aerenchyma scores at 10 and 15 cm from the root tips was  $0.1 \pm 0.1$  (mean  $\pm$  standard deviation) for B73 ( $n = 10$ ) and  $0.6 \pm 0.5$  for *Z. luxurians* ( $n = 10$ ) (Fig. 2). The frequency distribution of the aerenchyma score in the F<sub>2</sub> population is provided in Fig. 3. *Z. luxurians* scores were observed to range considerably (0.3–2.0). Since the *Z. luxurians* accession used in this study is not a pure line and as a consequence would segregated many genes, this variable score is anticipated to be caused by the segregation of genes having effects on aerenchyma formation. For this study, we selected a single aerenchyma-forming F<sub>1</sub> plant for the development of the F<sub>2</sub> mapping population. As a consequence, the average score of 0.6 in *Z. luxurians* may not correspond well to the single *Z. luxurians* parent in this mapping study. In essence, only a single *Z. luxurians* gamete provided for the variability of aerenchyma formation in this study. As a consequence, we present the score of *Z. luxurians* in parentheses in the histogram of the F<sub>2</sub> population (Fig. 3) to reflect this point.

### Map construction

We constructed an SSR-based map using 107 markers, which covered 1,331 cM for an average marker interval of 13.7 cM for the ten chromosomes (Fig. 4). Of these, five (4.7%) were dominant markers. The map location of the SSR markers in our B73 × *Z. luxurians* map are in good agreement with the published map of the MaizeGDB (<http://www.maizegdb.org/ssr.php>).

**Fig. 2** Cross sections of adventitious roots at 10–15 cm from the root tip that emerged from the second node in six-leaf stage seedlings when not flooded. The lack of aerenchyma is shown in (a) B73 (score 0) and (c) F<sub>2</sub> plant #152 (score 0), and aerenchyma-forming in (b) *Z. luxurians* (score 1) and (d) F<sub>2</sub> plant #208 (score 2). Bar = 0.25 mm for all



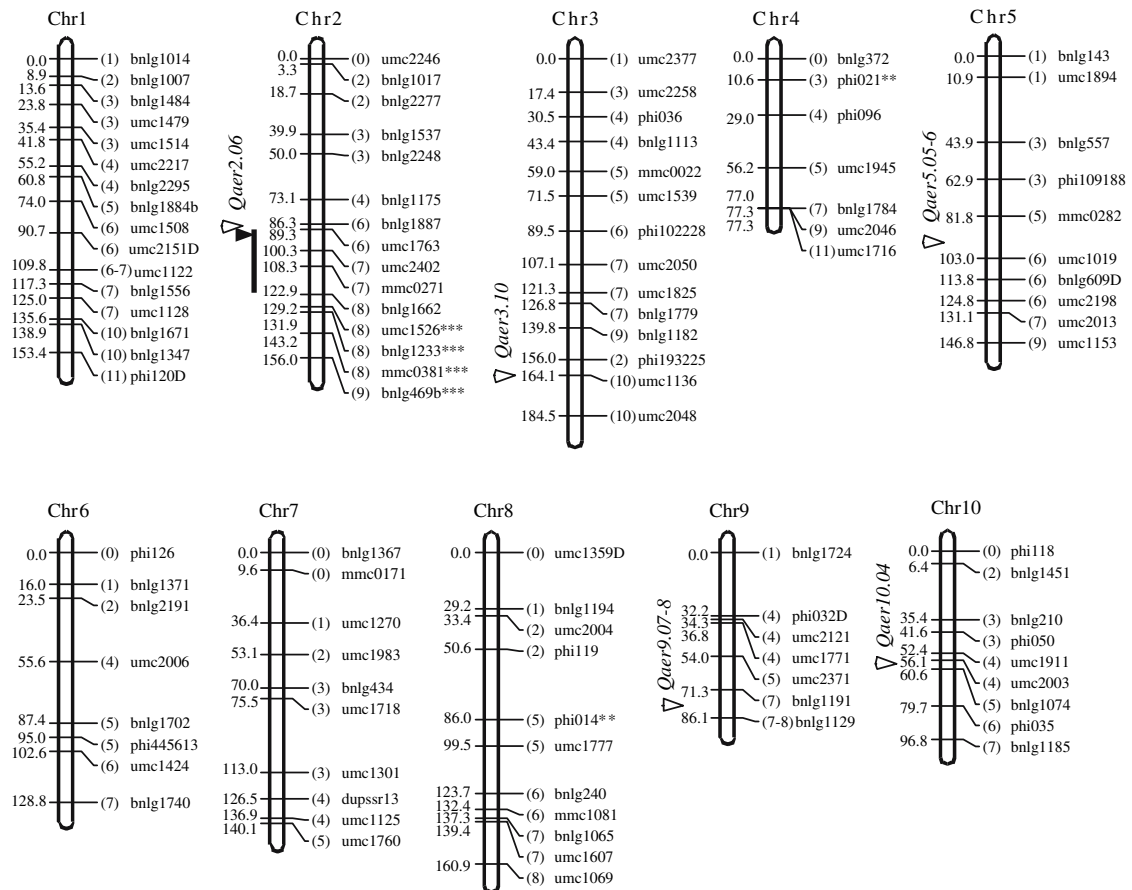
**Fig. 3** Frequency distribution for aerenchyma formation of the B73 × *Z. luxurians* F<sub>2</sub> population. Average score of two adventitious roots at 10 and 15 cm from the root tip (four positions in total) was used as the value for each F<sub>2</sub> individual

One exception was the SSR ‘phi193225’ locus: which maps to bin 3.02 in the MaizeGDB; however, in this study, maps to bin 3.09–10. The ‘phi193225’ generated

a single fragment in B73 or *Z. luxurians*, suggesting that this disagreement may not be due to map the different fragment located on the different locus between this study and published map.

#### Segregation distortion

For the 107 mapped SSR markers, six (5.6%) indicated a distorted segregation ( $P < 0.05$ ) in a total of three regions: chromosome 2 (the most extreme segregating region being at bin 2.09), chromosome 4 (4.03) and chromosome 8 (8.05) (Fig. 4). The distorted segregation region on chromosome 2 was associated with the lower frequency of the *Z. luxurians* genotype (or the higher frequency of B73 genotype), whereas that on chromosome 4 was associated with lower frequency of the genotype of



**Fig. 4** Chromosome locations of the QTLs controlling aerenchyma formation in the B73 × *Z. luxurians* F<sub>2</sub> populations identified using composite interval mapping (CIM) and multiple interval mapping (MIM). Short arms of the chromosome are on the top. The scale represents centimorgan (Haldane units). Bars to the left of the chromosomes indicate a safe support level of 2-LOD likelihood intervals, and closed

arrowheads indicate the position of the peak LOD in CIM. Opened arrowheads indicate the position of the peak LOD in MIM. Bin numbers are in parentheses before marker names. \*\* and \*\*\* indicate markers having distorted segregation of  $P < 0.01$  and  $P < 0.001$ , respectively and the significance levels are corrected according to the Bonferroni-Holm sequential methods (Rice 1989)

B73 (or higher frequency of *Z. luxurians*). The region on chromosome 8 favored a heterozygous genotype together with the lower frequency of *Z. luxurians* genotype (data not shown).

between bnlg1784 and bnlg1890 (closely linked to umc1716) are estimated to be 57.9 cM (SSR Consensus 1998, J Romero-Severson unpublished) and 92.8 cM between bnlg2291 (closely linked to bnlg1784) and umc1716 (T218 × GT119 2004.4, Sharopova et al. 2002).

### Recombination suppression

A high degree of recombination suppression was identified at bin 4.07-11 on the long arm of chromosome 4. The distance between the SSR “bnlg1784” and “umc1716” in the B73 × *Z. luxurians* population was 0.3 cM (Fig. 4) whereas it was estimated to be over 50 cM in the maize × maize maps as shown in the MaizeGDB. For example, the standard distances

### Mapping QTL for aerenchyma formation

With an LOD threshold of 3.6, the CIM analysis detected a single QTL for aerenchyma formation on chromosome 2 (bin 2.06) with an LOD score of 4.1, designated as *Qaer2.06* (Fig. 4, Table 1). This locus explains 9.1% of the total phenotypic variance. Using

MIM analysis, four additional QTLs exhibiting minor effects were found on chromosome 3 (*Qaer3.10*), chromosome 5 (*Qaer5.05-6*), chromosome 9 (*Qaer9.07-8*) and chromosome 10 (*Qaer10.04*) (Table 1). The additive effect values are positive for all QTLs, suggesting alleles of *Z. luxurians* increased the capacity to form aerenchyma for all five QTLs. The  $r^2$  value in the MIM model fitted to the five QTL and their epistatic interaction was 0.363.

There was no clear trend for dominance relationships within the five root aerenchyma formation loci (Table 1). Three of the detected loci *Qaer2.06*, *Qaer5.05-6* and *Qaer9.07-8* exhibited the same direction for their additive (positive) and dominant (positive) effects, which suggested that these *Z. luxurians* alleles were dominant to the corresponding B73 alleles. Another detected locus *Qaer10.04* exhibited a negative value of dominance effect, indicating recessive relationships in the *Z. luxurians* alleles. The remaining *Qaer3.10* locus indicated additive gene action.

## Discussion

We have constructed an SSR-based molecular linkage map covering the genome of an  $F_2$  population

derived from a cross between the maize inbred line B73 and the annual teosinte *Z. luxurians*. This data represents the first linkage map covering the ten chromosomes of *Z. luxurians*. The study also characterized several unique phenotypic and genotypic features of this material: (1) root-deficient segregants; (2) a strong segregation distortion region on the long arm of chromosome 2; and (3) a severe level of recombination suppression region on the long arm of chromosome 4. All these observations were also found in another B73  $\times$  *Z. luxurians*  $F_2$  population derived from a different  $F_1$  plant (F. Omori and Y. Mano, unpublished). In addition, QTLs have been successfully identified that control root aerenchyma formation during unflooded conditions. This QTL information will be used to develop flooding tolerant maize lines.

## Unique phenotypic and genotypic features

Several mutants affecting shoot-borne root development traits have been reported for maize and rice (Inukai et al. 2001; Hochholdinger et al. 2004). In maize, the root-deficient mutant “*rtcs*” completely inhibits the formation of crown and lateral seminal

**Table 1** Chromosome locations and mode of gene action of QTLs for root aerenchyma formation estimated by composite interval mapping (CIM) and multiple interval mapping (MIM) in the  $F_2$  population of the cross between B73  $\times$  *Z. luxurians*

QTL	Chr	Position <sup>a</sup>	Marker interval	LOD	a <sup>c</sup>	d <sup>d</sup>	Mode <sup>e</sup>	r <sup>2</sup> <sup>f</sup>
CIM								
<i>Qaer2.06</i>	2	93 (89–121) <sup>b</sup>	umc1763-umc2402	4.1	0.140	0.075	L	0.091
MIM								
<i>Qaer2.06</i>	2	88	bnlg1887-umc1763	3.4	0.155	0.058	L	0.091
<i>Qaer3.10</i>	3	164	umc1136-umc2048	1.8	0.127	−0.002	L	0.063
<i>Qaer5.05-6</i>	5	96	mmc0282-umc1019	2.6	0.060	0.112	L	0.042
<i>Qaer9.07-8</i>	9	79	bnlg1191-bnlg1129	1.5	0.092	0.102	L	0.045
<i>Qaer10.04</i>	10	57	umc2003-bnlg1074	1.8	0.083	−0.072	L	0.037
Interaction between <i>Qaer2.06</i> and <i>Qaer5.05-6</i>				0.9		−0.285		0.042
Interaction between <i>Qaer5.05-6</i> and <i>Qaer10.04</i>				0.9		0.218		0.044
							Total	0.363

<sup>a</sup> QTL position in cM from the top of the chromosome

<sup>b</sup> 2-LOD support interval

<sup>c</sup> Additive effect

<sup>d</sup> Dominance effect

<sup>e</sup> Parent contributing higher-value allele, where L = *Z. luxurians*

<sup>f</sup> Proportion of phenotypic variance explained

roots and has been reported in a flint inbred line DK105 × dent *c-m68613* cross, possessing the *En* transposable element at the *C1* locus (Hetz et al. 1996). The *rtcs* was recently cloned by Taramino et al. (2007). This mutant is characterized as being controlled by a single recessive gene located on the short arm of chromosome 1 and no pleiotropic effects on other parts of the plants have been observed. In this study, the frequency of the root-deficient plants was 2.6%, suggesting multigene control. Contrary to the maize *rtcs* mutant without pleiotropic effects, our root-deficient F<sub>2</sub> plants exhibited erect leaf types and poor growth compared to their normal plants (Fig. 1). These observations suggest that root-deficient F<sub>2</sub> plants identified in this study could differ from the *rtcs* mutant. We have been investigating the chromosome locations of the root-deficient genes using the F<sub>2</sub> plants of B73 × *Z. luxurians* and currently identified three candidate chromosomal regions related to root-deficient (Y. Mano, unpublished).

A total of three regions were associated with segregation distortion. The presence of ovule abortion in the F<sub>2</sub> generation of maize × *Z. luxurians* hybrids has been previously identified (B. Kindiger, unpublished) and as a consequence, some chromosome regions exhibiting segregation distortion could be associated with ovule abortion. We compared the segregation distortion regions to the known segregation distortion regions found in other maize × teosinte (*Zea mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, *Z. mays* ssp. *huehuetenangensis* and *Z. nicaraguensis*) mapping populations (Doebley and Stec 1993; Mano et al. 2005c, 2007b). The positions of the segregation distortion regions on chromosomes 4 and 8 in our B73 × *Z. luxurians* map were identical or closed to the at least one of the previously known segregation distortion regions and the directions of the favorite alleles corresponded. The region on chromosome 4 contains a well characterized gametophytic factor gene, *Gal*, reported to be located to bin 4.01-02 in several teosintes (Doebley and Stec 1993; Westerbergh and Doebley 2002; Mano et al. 2005c). Here the region on chromosome 2, showing the strongest segregation distortion (Chi-squared value of 50.7,  $P < 0.001$ ), was identified to be within the known segregation distortion region of SDR2.3 reported in the maize LH200 × LH216 mapping population (Lu et al. 2002), although our region may differ from the SDR2.3 region due to the

difference in the direction of skewness. The lower frequency of the *Z. luxurians* genotype contrasts with the higher frequency of a heterozygous genotype in the LH200 × LH216 map.

Severe recombination suppression was identified to bin 4.07-11 on the long arm of chromosome 4, suggests the presence of structural differentiation between B73 and *Z. luxurians* in this region. Recombination suppression at the same region has been reported in the cross between maize B64 × *Z. nicaraguensis* (Mano et al. 2007b), which is the closest relative of *Z. luxurians* (Iltis and Benz 2000). In contrast, although severe recombination reduction was observed in several chromosome regions of previous maize × teosinte maps (e.g., Doebley and Stec 1991), severe suppression on the long arm of chromosome 4 was not found in other maize × teosinte crosses (Doebley and Stec 1991, 1993; Mano et al. 2005c) or in a *Z. diploperennis* × *Z. mays* ssp. *parviglumis* cross (Westerbergh and Doebley 2002). In addition, remarkable recombination suppression on the long arm of chromosome 4 has not yet been identified in maize × maize linkage maps (e.g., Senior et al. 1996; Sharopova et al. 2002; Falque et al. 2005). Recombination suppression is widely recognized in interspecific or wide-cross hybridization of plants (Causse et al. 1994; Copenhaver et al. 1999; Choi et al. 2003). In tomato, recombination was completely suppressed on the long arm of chromosome 10 in the cross between cultivated tomato *Lycopersicon esculentum* × Peruvian wild nightshade *Solanum lycopersicoides* (Chetelat et al. 2000). Their study suggested that the recombination suppression may indicate a chromosome rearrangement such as a paracentric inversion. By constructing a linkage map on the long arm of chromosome 4 in a cross between *Z. luxurians* × *Z. nicaraguensis*, each including strong recombination suppression in this region when crossed to maize, it may be possible to compare marker order to the maize × maize maps and determine if an inversion is present or not in *Z. luxurians* and *Z. nicaraguensis*. In addition to the linkage analysis, cytological observation may also clarify this situation.

#### QTLs controlling root aerenchyma formation

We have identified five QTLs controlling root aerenchyma formation and the  $r^2$  value in the MIM



model fitted to the five QTLs and their epistatic interaction was 0.363 (Table 1), suggesting that the observed QTLs do not explain a large portion of the variance in the B73  $\times$  *Z. luxurians* F<sub>2</sub> population. The value of 0.363 is much lower than in our previous study of QTL mapping for aerenchyma formation of 0.621 using a B64  $\times$  *Z. nicaraguensis* F<sub>2</sub> mapping population (Mano et al. 2007b). Since we performed these two experiments in the identical greenhouse, under the identical conditions and the same researcher conducted the trait evaluation with the same experimental procedure, the lower value of the total variance explained in this study may be due to the presence of undetected QTLs, not to experimental errors. In addition, because Cheng and Ukai (1995) found by a computer simulation study that a lethal factor, causing strong segregation distortion, decreased the sensitivity of QTL detection, segregation distortion found here in several chromosome regions may affect QTL detection.

The positions of five QTLs controlling root aerenchyma formation found in this study do not overlap the known aerenchyma loci on chromosome 1 (*Qaer1.02-3* and *Qaer1.07*), chromosome 5 (*Qaer5.09*) and chromosome 8 (*Qaer8.06-7*) identified in a previous B64  $\times$  *Z. nicaraguensis* mapping study (Mano et al. 2007b). These data suggest that those QTLs identified in *Z. luxurians* are newly identified loci. Therefore, it may be possible to develop maize lines with extremely high levels of aerenchyma-formation, equivalent to rice or wetland species, by transferring aerenchyma-forming QTLs from both *Z. luxurians* and *Z. nicaraguensis* into maize using a marker-assisted selection approach.

We have begun the process of transferring QTLs for aerenchyma formation from *Z. luxurians* to B73 using a marker assisted selection approach and are developing near-isogenic lines of B73. Since B73 provides high yielding hybrids and has been widely used for genome analyses and breeding for several agronomically important traits (Troyer 1999, Mikel and Dudley 2006), an aerenchyma-forming B73 line should be useful for practical breeding of flooding tolerant maize lines. In addition, the relationship between the QTLs for aerenchyma formation and genes induced by ethylene, hypoxia or anoxia (Sachs et al. 1996) is currently unknown, so near-isogenic lines possessing QTLs for aerenchyma formation may also prove useful for molecular, cellular and

physiological analyses of the trait not only related to hypoxia but also suboptimal nutrient availability of S or P-starvation (Fan et al. 2003; Bouranis et al. 2006).

The capacity to form aerenchyma is controlled by multiple QTLs with small to moderate effects in *Z. luxurians* (Table 1) and in *Z. nicaraguensis* (Mano et al. 2007b). Another flooding-related trait, the ability of adventitious root formation at the soil surface during flooding (ARF-SS), is also controlled by multiple QTLs (Mano et al. 2005b, 2005d). As a consequence, the potential transfer of these possible flooding tolerance traits to maize should be reliably and efficiently performed by the application of a molecular marker assisted phenotypic selection technique. A near-isogenic line of maize B64 possessing the QTLs controlling the ability of ARF-SS will soon be completed (Mano et al. 2007a) and a similar process is being undertaken for elite inbred lines B73 and Mi29 (Y. Mano, unpublished). Further introgression of aerenchyma-forming QTLs from both *Z. luxurians* and *Z. nicaraguensis* is underway for developing superior flooding-tolerant maize lines.

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## References

- Bird RMcK (2000) A remarkable new teosinte from Nicaragua: growth and treatment of progeny. *Maize Gen Coop Newsl* 74:58–59
- Bouranis DL, Chorianoopoulou SN, Kollias C, Maniou P, Protonotarios VE, Siyiannis VF, Hawkesford MJ (2006) Dynamics of aerenchyma distribution in the cortex of sulfate-deprived adventitious roots of maize. *Ann Bot* 97:695–704
- Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu KS, Xiao JH, Yu ZH, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138:1251–1274
- Cheng R, Ukai Y (1995) Effect of a lethal factor on QTL mapping based on a DNA polymorphism linkage map. *Jpn J Breed* 45(Suppl. 1):125
- Chetelat RT, Meglic V, Cisneros P (2000) A genetic map of tomato based on BC<sub>1</sub> *Lycopersicon esculentum*  $\times$  *Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homeologous genomes. *Genetics* 154:857–867

- Choi P, Mano Y, Ishikawa A, Odashima M, Umezawa T, Fujimura T, Takahata Y, Komatsuda T (2003) Construction of a high-density AFLP and SSR map using recombinant inbred lines of cultivated  $\times$  weedy soybean. *Breed Sci* 53:335–344
- Colmer TD (2003) Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). *Ann Bot* 91:301–309
- Copenhaver GP, Nickel K, Kuromori T, Benito MI, Kaul S, Lin XY, Bevan M, Murphy G, Harris B, Parnell LD, McCombie WR, Martienssen RA, Marra M, Preuss D (1999) Genetic definition and sequence analysis of *Arabidopsis* centromeres. *Science* 286:2468–2474
- Doebley JF, Iltis HH (1980) Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. *Am J Bot* 67:982–993
- Doebley J, Stec A (1991) Genetic-analysis of the morphological differences between maize and teosinte. *Genetics* 129:285–295
- Doebley J, Stec A (1993) Inheritance of the morphological differences between maize and teosinte: comparison of results for two F<sub>2</sub> populations. *Genetics* 134:559–570
- Drew MC, Jackson MB, Giffard S (1979) Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in root may be adaptive responses to flooding in *Zea mays* L. *Planta* 147:83–88
- Evans DE (2004) Aerenchyma formation. *New Phytol* 161:35–49
- Falque M, Décousset L, Dervins D, Jacob A-M, Joets J, Martinant J-P, Raffoux X, Ribière N, Ridet C, Samson D, Charcosset A, Murigneux A (2005) Linkage mapping of 1454 new maize candidate gene loci. *Genetics* 170:1957–1966
- Fan M, Zhu J, Richards C, Brown KM, Lynch JP (2003) Physiological roles for aerenchyma in phosphorus-stressed roots. *Func Plant Biol* 30:493–506
- Garthwaite AJ, von Bothmer R, Colmer TD (2003) Diversity in root aeration traits associated with waterlogging tolerance in the genus *Hordeum*. *Func Plant Biol* 30:875–889
- Gunawardena AH, Pearce DM, Jackson MB, Hawes CR, Evans DE (2001) Characterisation of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (*Zea mays* L.). *Planta* 212:205–214
- Haldane JBS (1919) The combination of linkage values and the calculation of distance between the loci of linked factors. *J Genetics* 8:299–309
- He CJ, Finlayson SA, Drew MC, Jordan WR, Morgan PW (1996) Ethylene biosynthesis during aerenchyma formation in roots of maize subjected to mechanical impedance and hypoxia. *Plant Physiol* 112:1679–1685
- Hetz W, Hochholdinger F, Schwall M, Feix G (1996) Isolation and characterization of *rtcs*, a maize mutant deficient in the formation of nodal roots. *Plant J* 10:845–857
- Hochholdinger F, Park WJ, Sauer M, Woll K (2004) From weeds to crops: genetic analysis of root development in cereals. *Trends Plant Sci* 9:42–48
- Iltis HH, Benz BF (2000) *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. *Novon* 10:382–390
- Iltis HH, Doebley JF (1980) Taxonomy of *Zea* (Gramineae). II Subspecific categories in the *Zea mays* complex and a generic synopsis. *Am J Bot* 67:994–1004
- Inukai Y, Miwa M, Nagato Y, Kitano H, Yamauchi A (2001) Characterization of rice mutants deficient in the formation of crown roots. *Breed Sci* 51:123–129
- Jackson MB, Fenning TM, Drew MC, Saker LR (1985a) Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen. *Planta* 165:486–492
- Jackson MB, Fenning TM, Jenkins W (1985b) Aerenchyma (gas-space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. *J Exp Bot* 36:1566–1572
- Justin SHFW, Armstrong W (1987) The anatomical characteristics of roots and plant response to soil flooding. *New Phytol* 106:465–495
- Kao C-H, Zeng Z-B, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. *Genetics* 152:1203–1216
- Komatsuda T, Nakamura I, Takaiwa F, Oka S (1998) Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*. *Genome* 41:680–685
- Konings H (1982) Ethylene-promoted formation of aerenchyma in seedling roots of *Z mays* L. under aerated and non-aerated conditions. *Physiol Plant* 54:119–124
- Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181
- Lu H, Romero-Severson J, Bernardo R (2002) Chromosomal regions associated with segregation distortion in maize. *Theor Appl Genet* 105:622–628
- Mano Y, Muraki M, Fujimori M, Takamizo T (2005a) Varietal difference and genetic analysis of adventitious root formation at the soil surface during flooding in maize and teosinte seedlings. *Jpn J Crop Sci* 74:41–46
- Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B (2005b) Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays* ssp. *huehuetenangensis*) seedlings. *Euphytica* 142:33–42
- Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B (2005c) AFLP-SSR maps of maize  $\times$  teosinte and maize  $\times$  maize: comparison of map length and segregation distortion. *Plant Breed* 124:432–439
- Mano Y, Omori F (2007) Breeding for flooding tolerant maize using “teosinte” as a germplasm resource. *Plant Root* 1:17–21
- Mano Y, Omori F, Kikawada T (2007a) Development of maize near-isogenic lines possessing the teosinte’s QTL controlling the ability to form adventitious root at the soil surface during flooding. *Root Res* 16:63
- Mano Y, Omori F, Muraki M, Takamizo T (2005d) QTL mapping of adventitious root formation during flooding conditions in tropical maize (*Zea mays* L.) seedlings. *Breed Sci* 55:343–347
- Mano Y, Omori F, Takamizo T, Kindiger B, Bird RMCK, Loisiga CH (2006) Variation for root aerenchyma

- formation in flooded and non-flooded maize and teosinte seedlings. *Plant Soil* 281:269–279
- Mano Y, Omori F, Takamizo T, Kindiger B, Bird RMcK, Loaisiga CH, Takahashi H (2007b) QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte “*Zea nicaraguensis*” cross. *Plant Soil* 295:103–113
- McDonald MP, Galwey NW, Ellneskog-Staam P, Colmer TD (2001) Evaluation of *Lophopyrum elongatum* as a source of genetic diversity to increase the waterlogging tolerance of hexaploid wheat (*Triticum aestivum*) *New Phytol* 151:369–380
- Mikel MA, Dudley JW (2006) Evolution of North American dent corn from public to proprietary germplasm. *Crop Sci* 46:1193–1205
- Pang JY, Zhou MX, Mendham N, Shabala S (2004) Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. *Aust J Agric Res* 55:895–906
- Ray JD, Kindiger B, Sinclair TR (1999) Introgressing root aerenchyma into maize. *Maydica* 44:113–117
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Saab IN, Sachs MM (1996) A flooding-induced xyloglucan endo-transglycosylase homolog in maize is responsive to ethylene and associated with aerenchyma. *Plant Physiol* 112:385–391
- Sachs MM, Sabbaiah CC, Saab IN (1996) Anaerobic gene expression and flooding tolerance in maize. *J Exp Bot* 47:1–15
- Schussler EE, Longstreth DJ (1996) Aerenchyma develops by cell lysis in roots and cell separation in leaf petioles in *Sagittaria lancifolia* (Alismataceae). *Am J Bot* 83:1266–1273
- Senior ML, Chin ECL, Lee M, Smith JSC, Stuber CW (1996) Simple sequence repeat markers developed from maize sequences found in the GENBANK database: map construction. *Crop Sci* 36:1676–1683
- Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez-Villeda H, Gardiner J, Bergstrom D, Houchins K, Melia-Hancock S, Musket T, Duru N, Polacco M, Edwards K, Ruff T, Register JC, Brouwer C, Thompson R, Velasco R, Chin E, Lee M, Woodman-Clikeman W, Long MJ, Liscum E, Cone K, Davis G, Coe EH (2002) Development and mapping of SSR markers for maize. *Plant Mol Biol* 48:463–481
- Smirnov N, Crawford RMM (1983) Variation in the structure and response to flooding of root aerenchyma in some wetland plants. *Ann Bot* 51:237–249
- Subbaiah CC, Sachs MM (2003) Molecular and cellular adaptations of maize to flooding stress. *Ann Bot* 91:119–127
- Taramino G, Sauer M, Stauffer Jr JL, Multani D, Niu X, Sakai H, Hochholdinger F (2007) The maize (*Zea mays* L.) *RTCS* gene encodes a LOB domain protein that is a key regulator of embryonic seminal and post-embryonic shoot-borne root initiation. *Plant J* 50:649–659
- Thomson CJ, Colmer TD, Watkin ELJ, Greenway H (1992) Tolerance of wheat (*Triticum aestivum* cvs. Gamenya and Kite) and triticale (*Triticosecale* cv. Muir) to waterlogging. *New Phytol* 120:335–344
- Troyer AF (1999) Background of U.S. hybrid corn. *Crop Sci* 39:601–626
- Wang S, Basten CJ, Zeng Z-B (2006) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, USA. <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>
- Westerbergh A, Doebley J (2002) Morphological traits defining species differences in wild relatives of maize are controlled by multiple quantitative trait loci. *Evolution* 56:273–283
- Zaidi PH, Selvan PM, Sultana R, Srivastava A, Singh AK, Srinivasan G, Singh PR, Singh PP (2007) Association between line *per se* and hybrid performance under excessive soil moisture stress in tropical maize (*Zea mays* L.). *Field Crop Res* 101:117–126