QTL Mapping of Adventitious Root Formation under Flooding Conditions in Tropical Maize (*Zea mays* L.) Seedlings

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Quantitative trait loci (QTLs) controlling adventitious root formation (ARF) on the soil surface were evaluated under flooding conditions in 110 individuals of an F₂ population derived from a cross between a dent inbred line 'B64' and a tropical Caribbean flint inbred line 'Na4'. The ARF capacity of seedlings suggested the existence of continuous variation in the F₂ population. The QTLs for ARF were located on chromosomes 3 (bin 3.07-8), 7 (bin 7.04-5) and 8 (bin 8.05). Alleles of line Na4, with a high capacity for ARF, increased ARF in the case of all the QTLs. By comparing chromosome positions of ARF loci in the B64 × Na4 population with those in a B64 × teosinte (*Zea mays* ssp. *huehuetenangensis*) population, the region conditioning ARF on chromosome 8 was consistent across the two populations. In the present study, overlap between the QTLs for ARF in the B64 × Na4 cross and QTLs for root traits measured in aerated hydroponic culture was also observed as reported in other mapping populations.

Key Words: adventitious root, flooding, quantitative trait loci, tropical maize, waterlogging.

Introduction

Waterlogging or flooding often occurs from late spring to early summer in the Asian monsoon region. In Japan, maize is required to be grown in upland rice paddy fields in order to maximize land productivity, in spite of its poor adaptation to waterlogging. In maize, varietal variations in the pre-germination flooding tolerance (Martin *et al.* 1988, Lemke-Keyes and Sachs 1989) and waterlogging tolerance at the seedling stage (Fausey *et al.* 1985, Mano *et al.* 2002) or molecular and cellular responses during flooding (Sachs *et al.* 1996, Subbaiah and Sachs 2003) have been investigated. However, stable tolerant lines for use as breeding materials have not yet been established. To develop waterlogging-tolerant lines, it is necessary to enhance the understanding of the genetics related to waterlogging tolerance.

Waterlogging tolerance appears to be quantitatively inherited in crops such as rice (Xu and Mackill 1996), wheat (Boru *et al.* 2001) and soybean (VanToai *et al.* 2001). Also in maize, waterlogging tolerance at the seedling stage showed continuous variation in an F₂ population and the trait is considered to be a quantitative trait (Mano *et al.* 2005d). In quantitatively inherited traits, marker-assisted selection (MAS) may be useful. Under field conditions in soybean, however, since multiple factors related to waterlogging result in environmental interactions, it is difficult to develop reliable marker(s) for MAS (Reyna *et al.* 2003). Therefore,

it is important to differentiate the factors affecting waterlogging tolerance (e.g. adventitious rooting, capacity of root aerenchyma formation and tolerance to toxins under reduction conditions) and to analyze each component separately. Of several morphological and cellular responses during flooding in plants (Krizek 1982, Subbaiah and Sachs 2003), adventitious (shoot-borne crown) root formation (ARF) on the soil surface is one of the most important adaptations to flooding conditions. This characteristic allows the root system to obtain oxygen directly from the air. Importance of ARF during flooding has been reported for maize (Mano *et al.* 2005a), wild relatives of maize, teosinte (Bird 2000), barley (Stanca *et al.* 2003) and Italian ryegrass (Tase and Kobayashi 1992). Based on an evaluation of over 4,000 barley accessions under paddy field conditions, it was concluded that germplasm accessions with superior flooding tolerance developed a large number of adventitious roots (Takeda 1989, Stanca *et al.* 2003).

In a recent study on QTLs related to ARF in teosinte (*Zea mays* ssp. *huehuetenangensis*), the QTLs were found to be located on chromosomes 4 and 8 (Mano *et al.* 2005b). In addition to *Zea mays* ssp. *huehuetenangensis*, a tropical maize inbred line 'Na4', exhibiting tolerance to waterlogging, also formed a large number of adventitious roots above the soil surface under flooding conditions (Mano *et al.* 2005a). Therefore, a QTL analysis of ARF using Na4 should enable to further identify and classify QTLs. In the present study, we identified loci controlling ARF by using an F₂ population derived from a cross between the maize inbred lines B64 and Na4. The objectives were to compare the chromosome positions of ARF loci in the B64 × Na4 population

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with those in a B64 $\times$ *Zea mays* ssp. *huehuetenangensis* population, which had been previously reported (Mano *et al.* 2005b). We also examined the relationships between the QTLs for ARF in the B64 $\times$ Na4 cross and the reported QTLs for root traits using aerated hydroponics in other mapping populations (Tuberosa *et al.* 2002).

Materials and Methods

Plant materials

Evaluation of ARF was performed in an F₂ population consisting of 110 individuals of maize (*Zea mays* ssp. *mays*) derived from a cross between a dent inbred line 'B64' and a tropical Caribbean flint inbred line 'Na4'. B64 exhibited a low capacity for ARF on the soil surface under flooding conditions, while Na4 exhibited a high tendency for ARF (Mano *et al.* 2005a).

Flooding treatment

The capacity of the 110 F₂ plants and their parents for ARF had previously been evaluated in order to estimate heritabilities based on correlation and regression coefficients between the F₂ and F₃ individuals (Mano *et al.* 2005a). Detailed description of the experimental methods adopted to obtain flooding conditions can be found in the report of Mano *et al.* (2005b). Briefly, seedlings at the four-leaf-stage were immersed in water until the water level reached 3 cm above the soil surface. At two weeks after the initiation of the flooding treatment, the degree of ARF above the soil surface for each individual seedling was scored visually as follows: 0 (none), 1 (low), 2 (medium) and 3 (high). Under the experimental conditions, the effect of Fe²⁺ and/or H₂S toxicity was negligible.

Map construction and QTL analysis

An SSR-AFLP-based map with 437-point markers for the 94 plants of the F₂ population derived from the B64 $\times$ Na4 cross had been previously constructed (Mano *et al.* 2005c). In addition, we conducted a marker analysis for additional 16 F₂ plants. We selected a subset of 161 markers (81 SSRs and 80 AFLPs) and reconstructed a map of 110 F₂ plants using MAPMAKER/EXP 3.0 (Lander *et al.* 1987).

QTL mapping for ARF in the F₂ population was performed by composite interval mapping (CIM) using QTL Cartographer Version 2.0 (Basten *et al.* 2000). CIM was performed with default setting for model 6 (five background markers and a window size of 10 cM). A log-likelihood (LOD) score threshold of 3.0 was used to identify a significant LOD peak.

Results and Discussion

Phenotypic variations

ARF in the parental germplasm indicated the presence of a high level of variation. B64 exhibited a low capacity for ARF whereas Na4 exhibited a high capacity for ARF

(Fig. 1). In the F₂ population, ARF showed a continuous normal distribution (Fig. 2, the histogram had already been presented in the report of Mano *et al.* 2005a), suggesting that ARF is controlled by multiple genes.

Mapping of QTLs for ARF in the F₂ population

In the present study, an SSR-AFLP-based map was constructed using 161 markers, covering 1,715 cM at an average interval of 11.4 cM/marker for each of the ten maize chromosomes. A region with segregation distortion was found on the long arm of chromosome 9 (significant at the 1% level) and was associated with the genotype of Na4. CIM enabled to detect three QTLs for ARF on chromosomes 3 (bin 3.07-8), 7 (bin 7.04-5) and 8 (bin 8.05), and they explained 44% of the total phenotypic variance. Alleles of line Na4, with a high capacity for ARF, increased ARF in the case of all the QTLs. (Fig. 3a and Table 1).

In the *Zea* genus, genetic analyses of waterlogging or flooding-related traits have been reported. Anoxia tolerance in maize seedlings has been found to be dominant and

Fig. 1. Two-week old flooded maize inbred lines. Left: Na4 (with adventitious roots), right: B64 (without adventitious roots). Shoots were removed from the plants in order to clearly observe the adventitious roots.

Fig. 2. Frequency distribution of ARF in the B64 $\times$ Na4 F₂ population. ARF scores in the parental lines were 2.7 (n=7) for Na4 and 0.0 (n=7) for B64 in identical environments. The histogram had already been presented in the report of Mano *et al.* (2005a).

Fig. 3. (a) Chromosome location of the QTLs for ARF in the B64 × Na4 population obtained by CIM. (b) Comparison of the location of QTL on chromosome 8 with that of the QTL in the B64 × *Z. mays* ssp. *huehuetenangensis* mapping population (Mano *et al.* 2005b). In the B64 × Na4 population, only significant chromosomes are presented. Short arm of the chromosomes is located at the top. The scale is expressed in centimorgan (Haldane units). Bars to the left of the chromosomes indicate 1-LOD likelihood intervals, and arrowheads indicate the position of the peak LOD in the interval. The markers that are common between the two populations are connected by solid lines. The QTLs for ARF found in the B64 × *Z. mays* ssp. *huehuetenangensis* population are separated by about 30 cM between the two experiments but are likely to represent two QTLs at the same locus (Mano *et al.* 2005b). The position of the QTL with a larger effect is presented in the figure.

Table 1. Chromosome location and mode of gene action of QTLs for ARF in the F₂ population of B64 × Na4

Chromosome	Interval	LOD ¹⁾	a ²⁾	d ³⁾	PVE ⁴⁾	Pa ⁵⁾
3 (bin 3.07-8 ⁶⁾)	bnlg1605-phi046	3.2	−0.33	−0.33	0.10	Na4
7 (bin 7.04-5)	dupssr13-phi069	5.1	−0.52	0.26	0.21	Na4
8 (bin 8.05)	bnlg162-bnlg240	4.9	−0.47	0.22	0.15	Na4
	Total ⁷⁾				0.44	

¹⁾ LOD score.

²⁾ Additive effect.

³⁾ Dominance effect.

⁴⁾ Proportion of phenotypic variance explained.

⁵⁾ Parent contributing higher-value allele.

⁶⁾ The concept of using the terminology “bin” is a traditional method for referring to the location/position of markers within the maize chromosomes.

⁷⁾ Estimated from multiple interval mapping module in the QTL Cartographer.

indicated a simple segregation (Sachs *et al.* 1996), while it was suggested that aerenchyma formation in teosinte (*Z. luxurians*) was controlled by a dominant gene (Ray *et al.* 1999). However, in these studies, the chromosome location of the genes for waterlogging-related traits had not been determined. In the present study, since we identified the chromosome location of QTLs for ARF, reliable MAS of waterlogging tolerance may be possible by using molecular markers linked to QTLs related to ARF.

Comparison of QTLs with those in different mapping populations

QTLs for ARF in *Z. mays* ssp. *huehuetenangensis* have recently been mapped on chromosomes 4 (bin 4.07) and 8 (bin 8.03 and/or 8.05) using an F₂ population (Mano *et al.* 2005b). Figure 3b illustrates a comparison of the QTL for ARF on chromosome 8 between the B64 × Na4 and B64 × *Z. mays* ssp. *huehuetenangensis* mapping populations. The position of the QTL for ARF in the B64 × Na4 cross (nearest marker is bnlg162) was very close to that of the B64 ×

Z. mays ssp. *huehuetenangensis* cross. In future studies, utilizing different teosinte species (e.g. *Z. luxurians* and *Z. nicaraguensis*) exhibiting a high capacity for ARF (Bird 2000, Mano *et al.* 2005a), should provide additional information of QTLs for ARF along with enabling to identify common QTLs and species-specific QTLs in a number of *Zea* species.

Relationships between QTLs for ARF and QTLs for root traits

In maize, Tuberosa *et al.* (2002) identified 11 QTLs controlling primary root length in plants grown in an aerated hydroponic system using an Lo964 × Lo1016 mapping population, and co-location between the QTLs for primary root length and QTLs for ARF in a B64 × *Z. mays* ssp. *huehuetenangensis* population was reported (Mano *et al.* 2005b). In the present study, overlap between the QTLs for ARF with the QTLs for root traits in hydroponic culture was observed; QTLs for ARF were mapped on chromosomes 3 (bin 3.07-8), 7 (bin 7.04-5) and 8 (bin 8.05) in the B64 × Na4 population (Table 1), whereas those for primary root weight or primary root length were mapped on chromosomes 3 (bin 3.09), 7 (bin 7.04-5) and 8 (bin 8.05) (Tuberosa *et al.* 2003). On chromosome 8, a noticeable correspondence was observed that the nearest marker for the QTLs for ARF and primary root length was SSR bnlg162.

The existence of maize plants with a high tendency for ARF indicated that all the adventitious roots, including the underground roots, tended to elongate more than those of the plants with a low capacity for ARF (Mano *et al.* 2005a). This observation suggests that ARF, on the soil surface during flooding, could be controlled by a root elongation gene expressed in both the primary and adventitious roots. Further studies should be carried out to confirm this assumption.

Conclusion and perspective

In the present study, regions that are involved in the control of ARF were identified using a B64 × Na4 mapping population. Na4 harbors a QTL on chromosome 8 common to *Z. mays* ssp. *huehuetenangensis*'s QTL which shows the largest effect in the *Z. mays* ssp. *huehuetenangensis* genome (Mano *et al.* 2005b). In addition, Na4 harbors line-specific alleles for high ARF on chromosomes 3 and 7. As a result, Na4 may be a source of germplasm for combining additional QTLs for waterlogging-related traits (e.g. aerenchyma formation and tolerance to toxins under reduction conditions such as Fe²⁺, H₂S and NO₂⁻) through a MAS breeding process.

Comparative analyses revealed the co-linearity of the genomes between maize and Gramineae species such as rice (e.g. Wilson *et al.* 1999), wheat (Ahn *et al.* 1993) and sorghum (Pereira *et al.* 1994). Using the co-linearity information about the genome, it may be possible to identify homologous QTLs for ARF throughout not only the *Zea* genus but also Gramineae species. In this process, functional analysis of ARF genes and wide use of the ARF gene in a MAS breeding program in Gramineae have become possible.

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