Identification of QTL Controlling Flooding Tolerance in Reducing Soil Conditions in Maize (Zea mays L.) Seedlings

Yoshiro Mano¹, Masanori Muraki² and Tadashi Takamizo¹

¹National Institute of Livestock and Grassland Science, Nasushiobara, Tochigi 329-2793, Japan; ²National Agricultural Research Center for Kyushu Okinawa Region, Miyakonojo, Miyazaki 885-0091, Japan)

Abstract: We investigated the tolerance to flooding in reducing conditions of five maize inbred lines and identified a quantitative trait locus (QTL) for the trait. Flooding treatment with 0.1% to 0.4% starch solution for 14 d reduced soil redox potential to about –200 mV, mimicking reducing conditions in soil. Treatment with 0.2% starch revealed wide varietal differences in dry matter production among the five maize inbred lines. We identified the QTL for flooding tolerance in reducing conditions in a population of 178 F₂ plants derived from a cross of inbred lines F1649 (tolerant) and H84 (sensitive). Flooding tolerance, evaluated as the degree of leaf injury following treatment with 0.2% starch solution, revealed wide variation in the F₂ population. Amplified fragment length polymorphism (AFLP) markers linked to flooding tolerance gene(s) were screened with 64 AFLP primer combinations using 15 of the 178 F₂ plants from each extreme representing the ‘tolerant’ and ‘sensitive’ plants, and found 11 AFLP markers associated with flooding tolerance. Of these, 10 co-segregated and were assigned to chromosome 1. Six SSR primer pairs around these markers were used to construct a linkage map. Composite interval mapping analysis revealed that a single QTL for degree of leaf injury was located on chromosome 1 (bin 1.03-4). Another QTL for flooding tolerance, evaluated as dry matter production under flooding with 0.2% starch, was located at the same position. These results suggest the potential to increase productivity by transferring flooding tolerance genes from F1649 to elite maize inbred lines.

Keywords: Flooding, Quantitative trait locus, Reduction, Starch.

Flooding or waterlogging greatly reduces crop yield in humid temperate and subtropical regions. In Japan, located in the Asian monsoon region, summer crops such as maize, sorghum, and soybean, are required to be grown in upland rice paddy fields to increase productivity and food self-sufficiency. However, during the rainy season, seedlings are damaged by flooding. In addition to excessive water or low oxygen stress, toxic chemical species (e.g., Fe²⁺, H₂S) induced by reducing conditions also pose serious problems in particular a paddy field condition (Yamasaki, 1952). Flooding tolerance is considered to be a quantitative trait in wheat (Boru et al., 2001) and soybean (VanToai et al., 2001). It appears to be quantitatively inherited in maize also, showing relatively low repeatability (Mano et al., 2002). If we separately evaluate genetic variations in several quantitatively inherited flooding-related traits, analyze the mode of inheritance of each trait, and pyramid flooding-tolerance genes, we could breed stable flooding-tolerant lines.

Morphological and anatomical factors such as adventitious root formation (Jackson, 1955; Jat et al., 1975; Tase and Kobayashi, 1992; Lizaso et al., 2001), shallow root system (Oyanagi et al., 1993), and aerenchyma formation in roots (Arikado and Adachi, 1955; Jat et al., 1975; Das and Jat, 1977; Burdick, 1989) affect flooding tolerance, and genetic analyses of these factors have been reported (Ray et al., 1999; Mano et al., 2005a,c). In addition to these approaches, a physiological approach, in particular focusing on tolerance to toxins induced by reduction in waterlogged upland paddies, is required for the breeding of stable flooding-tolerant maize.

Ishihara et al. (1981) used soluble starch treatment to induce reducing conditions (low soil redox potential, Eh) in a study of rice. A. Oyanagi (personal communication) found this same treatment effective in a study of flooding tolerance in wheat. So we evaluated optimal starch concentration for maize and used the treatment in this study.

Little is known of the genetics or mechanisms of flooding tolerance in reducing conditions. Our objectives were: (1) to investigate the response of maize inbred lines to reducing conditions induced by various concentrations of soluble starch; and (2) to identify any QTLs controlling flooding tolerance in reducing conditions by using a segregating F₂ population. The molecular markers we developed may be useful for breeding of flooding-tolerant elite maize lines through molecular marker-assisted selection.
Materials and Methods

1. Variation in flooding tolerance under reducing conditions

(1) Plant materials

We used five maize inbred lines: a yellow flint line, F1649, and four yellow dent lines, B73, Mo17, PA91, and H84. F1649 (accession no. 00002910), PA91 (00094116), and H84 (45099662) were obtained from the Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan; B73 and Mo17 were provided by the Corn and Sorghum Breeding Laboratory, National Institute ofLivestock and Grassland Science, Nasushiobara, Japan. In a previous seedling test, F1649 and B73 showed tolerance and Mo17, PA91, and H84 showed sensitivity to flooding under non-reducing conditions in the soil (Mano et al., 2002; Y. Mano unpublished).

(2) Flooding treatment

The seedlings of the five lines were grown in Wagner pots (1/5000 are; 16 cm diameter, 19 cm depth) filled with granular soil (Kureha Chemical Industry, Tokyo, Japan) fertilized with 1.2 g N, 5.7 g P, and 1.8 g K in each pot, at three plants per pot in a greenhouse (uncontrolled conditions with natural light of 13- to 14-hr daylength and minimum temperature above 20°C). After grown to the four-leaf stage (14 days after sowing), they were divided into the following six groups, six plants per group. Five groups were flooded with 0%, 0.1%, 0.2%, 0.3% or 0.4% soluble starch (Wako, Osaka, Japan) solution to a depth of 1 cm above the soil surface, and the other group was not flooded (control). At 14 days after the start of the above treatment or 28 days after sowing, the shoots of each plant were harvested, dried at 70°C for 5 d, and then weighed.

(3) Measurement of soil redox potential

The effect of starch treatment on soil condition was analyzed by measuring the soil redox potential (Eh). Eh was measured 5 cm below the soil surface every 2 or 3 d with platinum-tipped electrodes and a millivolt meter (Model EHS-120, Fujiwara Scientific Company, Tokyo, Japan). Eh was recorded from two pots per treatment (except control).

2. Mapping QTL for flooding tolerance under reducing conditions

(1) Plant materials

An F2 population of 178 plants derived from a cross between F1649 (tolerant) and H84 (sensitive) was used. The parents were chosen because they differed greatly in flooding tolerance under reducing conditions.

(2) Flooding treatment

At the four-leaf stage, the plant heights were measured and fresh leaves were sampled for DNA isolation. Then the seedlings were flooded with 0.2% starch solution, which caused a wide variation in the reducing condition. At 3, 7, and 14 days after initiation of flooding treatment, leaf injury was scored visually as 0 (chlorosis or necrosis), 1 (chlorosis in some parts of leaves), or 2 (no or slightly leaf injury). Flooding tolerance was evaluated as the sum of the score for each plant, and graded from 0 (sensitive) to 6 (tolerant). As different criteria of flooding tolerance, we also measured dry weights of shoots of plants flooded for 14 days, which was important in the practical breeding.

(3) Screening AFLP markers

In this study, we extracted DNA by a small-scale DNA extraction method just before flooding treatment since sensitive lines became chlorosis after exposure to flooding or could not extract DNA from them. A small amount of DNA (~1-4 µg) was isolated from 50 mg of fresh leaf tissue of each F2 plant of the cross between F1649 × H84 according to the method of Dellaporta et al. (1985) with some modifications (Komatsuda et al., 1998). The quality of DNA extracted by this method is satisfactory for AFLP analysis (Mano et al., 2001). On the contrary, the amount of DNA was not sufficient to construct a linkage map throughout the all 10 chromosomes, thus we screened AFLP markers linked to QTL for flooding tolerance, constructed a partial linkage map at the region and then performed QTL analysis as described below.

Amplified fragment length polymorphism (AFLP) markers linked to flooding tolerance gene(s) were screened using 15 F2 plants from each extreme representing the ‘tolerant’ (score 6) and ‘sensitive’ (scores 0, 1 or 2) classifications out of 178-plant F2 population. To eliminate the effect of initial plant growth on flooding tolerance, we selected plants exhibiting moderate growth just before treatment in the two classes.

The AFLP analysis was done essentially according to the methods described by Vos et al. (1995), with some modifications as described by Mano et al. (2005b). A total of 64 primer combinations of eight EcoRI primers [5’-GACTCGGTACCAATTC-3’ plus AAG (e02), AAA (e03), ACA (e05), ACC (e06), ACG (e07), ACT (e08), AGC (e10), AGG (e11)] and eight MseI primers [5’-GATGAGTCCTGAGTAA-3’ plus CAA (m17), CAC (m18), CAG (m19), CAT (m20), CTA (m29), CTC (m30), CTG (m31) and CTT (m32)] were used to screen AFLP markers that differentiated the 15 flooding-tolerant from the 15 flooding-sensitive F2 plants. The AFLP markers using 64 primer combinations were considered to cover nearly the whole genome since AFLP markers using 32 primer combinations covered about 85% of the total genome (Y. Mano unpublished). Chromosome positions of the selected AFLP markers were estimated using common AFLP marker information in an F2 maize map of B64 × Na4 (Mano et al., 2005b).
(4) Map construction and QTL analysis

On the basis of the data in the MaizeGDB (http://www.maizegdb.org/ssr.php, updated Aug 2004), a total of six simple sequence repeat (SSR) markers locating around the selected AFLP markers were used to construct an SSR-based linkage with the F₂ population (178 plants). SSR analysis was performed as described by Mano et al. (2005b).

An SSR map was constructed by using MAPMAKER/EXP 3.0 (Lander et al., 1987) with six markers. QTL mapping utilizing the F₂ population was performed using the interval mapping (IM) and composite interval mapping (CIM) methods implemented by the software package QTL Cartographer Version 1.14 (Basten et al., 2000). Since the same results were obtained in the IM and CIM analyses, we described the results of only CIM, which was performed using the default setting for model 6 in the program. After 1,000 permutations, we used log-likelihood of odds (LOD) score thresholds of 2.6 for leaf injury, 2.4 for dry weight and 2.4 for plant height, to identify regions containing putative loci associated these traits. The values maintained the chromosome-wide Type-I error rate of 0.05.

Results and Discussion

1. Soil redox potential

Soil Eh values measured 3 hr after the start of flooding treatment were 462 mV in the non-starch flooding treatment (0%) and 333 to 453 mV in the 0.1% to 0.4% starchy treatments (Fig. 1). Two days after the start of the treatment, the Eh in the non-starch treatment was reduced to 152 mV, whereas those in the 0.1% to 0.4% starchy treatments were greatly reduced to –91 to –219 mV. The tendency continued throughout the experiment (Fig. 1). These results agree well with the results of Ishihara et al. (1981). We scored leaf injury at 3, 7, and 14 d after the start of the flooding treatment. During this period, soil Eh was below –100 mV, suggesting the persistence of a low Eh-inducing effect. Chemical changes from Fe₃⁺ to Fe²⁺ and SO₄²⁻ to H₂S in reducing soil conditions were not measured in this study.

2. Variation in flooding tolerance among the five lines

Fig. 2 shows the responses of the five maize inbred lines to 0% to 0.4% starchy solution. In the non-starch flooding treatment (T0), F1649 was highly tolerant (91% of control in dry weight), and H84 was sensitive (34% of control) (Fig. 3). In the 0.1% starchy treatment (T0.1), plant growth was inhibited in all lines except the sensitive line H84. The presence of starch caused leaf injury. In the 0.2% starchy treatment, there were wide varietal differences in the reaction to starch, in particular between tolerant F1649 (45% of control) and sensitive H84 (19% of control, Fig. 3). In the 0.4% starchy treatment, all five lines were severely damaged, and varietal differences were absent (13%–20% of controls). We used F1649 and H84 as the parents of the F₂ mapping population and tested their tolerance to reducing conditions induced by adding 0.2% starchy solution since there were clear differences in plant growth and leaf injury between the two lines.

3. Variation in flooding tolerance in the F1649 × H84 F₂ population

At 3 d after the start of starch treatment, sensitive F₂ plants derived from the cross between F1649 × H84 showed chlorosis. Flooding tolerance, evaluated as the sum of each leaf injury score at 3, 7, and 14 days after the start of flooding treatment, showed wide variations from score 0 (sensitive) to score 6 (tolerant) among the F₂ population (Fig. 4a).
Two weeks after exposure to 0.2% starch solution, the dry weight of the F2 population showed a normal distribution, and transgressive segregation was observed, suggesting the presence of a hybrid vigor effect in the F2 population (Fig. 4b).

In the F2 population, there was a positive correlation between leaf injury score and dry weight ($r = 0.653$, significant at the 0.1%), suggesting that leaf injury reduces dry matter production. There was no significant correlation between plant height just before starch treatment and leaf injury ($r = 0.105$). Therefore, early plant growth did not affect leaf injury caused by reducing conditions.

4. Screening AFLP markers

The comparison of the 15 F2 plants with high leaf injury scores and the 15 F2 plants with low scores selected from the 178-plant F2 population revealed 11 AFLP markers associated with flooding tolerance under reducing conditions by chi-squared test (at the 1% level). Fig. 5 shows an example of an AFLP marker (e07m19-240, arrow) associated with the QTL for flooding tolerance; in the tolerant class, observed segregation ratio of 5 (present) : 1 (absent) was deviated from the expected proportion of 3 (present) : 1 (absent) in dominant marker ($\chi^2 = 15.0$, significant at the 1%). This indicates the relationship between flooding tolerance and AFLP marker e07m19-240. Of the 11, 10 markers (e03m18-210, e05m20-195, e05m30-105, e06m30-325, e06m30-330, e07m19-240, e07m30-220, e08m30-325, e08m30-610, and e10m18-90) co-segregated in the 30 F2 plants. Since two of these AFLP markers (e05m30-105 and e06m30-325) were known to be located on chromosome 1 of an F2 mapping population of B64 × Na4 (Mano et al., 2005b), these ten markers were also located on chromosome 1. The position of the remaining marker (e05m29-125) was unknown because of monomorphism between B64 and Na4 or a lack of marker information in other mapping populations developed by Mano et al. (2005b). Since sensitive line H84 contributed to the QTL associated with this AFLP marker, we did not further analyze the region.

5. Mapping QTL for flooding tolerance under reducing conditions

We constructed a partial linkage map of the 178-plant F2 population for the region of chromosome 1 by using six SSR markers. The map covered 131.5 cM at an average interval of 26.3 cM per marker. The map did not contain 10 AFLP markers found in the marker screening since genotypes of the AFLPs were examined for only 30 F2 plants. By CIM analysis, a QTL for flooding tolerance evaluated by leaf injury was identified between phi001 (bin 1.03) and bnlg1811 (bin 1.04) (LOD = 4.4, additive effect = 0.8, dominance effect = 0.4), which explained 14% of the total variation in flooding tolerance evaluated by leaf injury (a) and dry weight (b) in the F2 population of F1649 × H84. Shaded bars indicate selected F2 plants used for screening of AFLP markers. Arrows indicate the means of the parental lines.

Fig. 3. Comparisons of shoot dry weight among five maize inbred lines. C, Unstressed control; T0, flooding treatment without starch; T0.1-T0.4, flooded with 0.1% to 0.4% starch treatment. The vertical lines on the bars indicate standard deviations. The values in the bars indicate percentages of control.

Fig. 4. Variation in flooding tolerance evaluated by leaf injury (a) and dry weight (b) in the F2 population of F1649 × H84. Shaded bars indicate selected F2 plants used for screening of AFLP markers. Arrows indicate the means of the parental lines.
phenotypic variance (Fig. 6, solid line). Line F1649, with high tolerance to flooding, contributed to the QTL detected in the analysis. The relatively low value of variance explained indicates the presence of other minor factors or classification errors.

By QTL analysis of flooding tolerance evaluated by dry weight, a significant QTL was found at the same position as the QTL evaluated by leaf injury (LOD = 3.4, additive effect = 0.133, dominance effect = 0.024), explaining 10% of the variance (Fig. 6, dotted line). In the QTL analysis of plant height measured just before flooding treatment, no significant QTL was found on chromosome 1 (data not shown). The result confirmed the absence of a significant correlation between plant height just before starch treatment and leaf injury, which suggests that the mechanism of flooding tolerance in reducing conditions differs from the plant growth in unstressed conditions.

Since the effects of the QTLs found in this study are not large, further studies using additional genetic resources are required. Y. Mano (unpublished data) observed in a greenhouse experiment that a new species found on the Pacific coast of Nicaragua, "Zea nicaraguensis" (Bird, 2000; Iltis and Benz, 2000), showed extremely high adaptability to flooding under unstressed conditions. Using that genetic resource, it might be possible to find additional QTLs with larger effects.

Conclusion

The genes controlling flooding tolerance under reducing conditions in maize have not been mapped previously. In this study, we found a QTL for flooding tolerance in reducing conditions on chromosome 1 in a yellow flint inbred line, F1649 (Fig. 6). Since this study relied on a single trial, further studies using an F3 generation are needed to verify the expression of the QTL. By pyramiding QTLs controlling several factors responsible for flooding tolerance such as reduction tolerance (found in this study), adventitious root formation (Mano et al., 2005a,c), and aerenchyma formation (Ray et al., 1999), it should be possible to develop flooding-tolerant elite maize lines.

Acknowledgment

We thank F. Omori for her help in the gel analysis.

References

Arikado, H. and Adachi, Y. 1955. Anatomical and ecological responses of barley and some forage crops to the flooding treatment. Bull. Fac. Agr. Mie Univ. 11 : 1-29.

Basten, C.J., Weir, B.S. and Zeng, Z.B. 2000. QTL Cartographer, Version 1.14. Department of Statistics, North Carolina State University, Raleigh, NC, USA.

Bird, R.McK. 2000. A remarkable new teosinte from Nicaragua: Growth and treatment of progeny. Maize Gen. Coop. News. 74 : 58-59.

Boru, G., van Ginkel, M., Kronstad, W.E. and Boersma L. 2001. Expression and inheritance of tolerance to waterlogging stress in wheat. Euphytica 117 : 91-98.

Burdick, D.M. 1989. Root aerenchyma development in Spartina patens in response to flooding. Am. J. Bot. 76 : 777-780.

Das, D.K. and Jat, R.L. 1977. Influence of three soil-water regimes on root porosity and growth of four rice varieties. Agron. J. 69 : 197-200.

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. Maize DNA miniprep. In R. Malmberg, J. Messing and I. Sussex, eds., Molecular Biology of Plants : A Laboratory Course Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 36-37.

Iltis, H.H. and Benz, B.F. 2000. Zea nicaraguensis (Poaceae), a new teosinte from Pacific coastal Nicaragua. Novon 10 : 382-390.
Ishihara, K., Hirasawa, T., Iida, O. and Kimura, M. 1981. Diurnal course of transpiration rate, stomatal aperture, stomatal conductance, xylem water potential and leaf water potential in the rice plants under the different growth conditions. Jpn. J. Crop Sci. 50 : 25-37**.

Jackson, W.T. 1955. The role of adventitious roots in recovery of shoots following flooding of the original root system. Am. J. Bot. 42 : 816-819.

Jat, R.L., Dravid, M.S., Das, D.K. and Goswami, N.N. 1975. Effect of flooding and high soil water condition on root porosity and growth of maize. J. Ind. Soc. Soil Sci. 23 : 291-297.

Komatsuda, T., Nakamura, I., Takaiwa, F. and Oka, S. 1998. Development of STS markers closely linked to the vrs1 locus in barley, *Hordeum vulgare*. Genome 41 : 680-685.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newburg, L. 1987. MAPMAKER : an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1 : 174-181.

Lizaso, J.I., Melendez, L.M. and Ramirez, R. 2001. Early flooding of two cultivars of tropical maize. 1. Shoot and root growth. J. Plant Nutr. 24 : 979-995.

Mano, Y., Kawasaki, S., Takaiwa, F. and Komatsuda, T. 2001. Construction of a genetic map of barley (*Hordeum vulgare* L.) cross Azumamugi × Kanto Nakate Gold using a simple and efficient amplified fragment-length polymorphism system. Genome 44 : 284-292.

Mano, Y., Muraki, M., Komatsu, T., Fujimori, M., Akiyama, F. and Takamizo, T. 2002. Varietal difference in pre-germination flooding tolerance and waterlogging tolerance at the seedling stage in maize inbred lines. Jpn. J. Crop Sci. 71 : 361-367*.

Mano, Y., Muraki, M., Fujimori, M., Takamizo, T. and Kindiger, B. 2005b. AFLP-SSR maps of maize × teosinte and maize × maize : Comparison of map length and segregation distortion. Plant Breed. 124 : 432-439.

Mano, Y., Omori, F., Muraki, M. and Takamizo, T. 2005c. QTL mapping of adventitious root formation during flooding conditions in tropical maize (*Zea mays* L.) seedlings. Breed. Sci. 55 : 343-347.

Oyanagi, A., Nakamoto, T. and Morita, S. 1993. The gravitropic response of roots and the shaping of the root system in cereal plants. Environ. Exp. Bot. 33 : 141-158.

Ray, J.D., Kindiger, B. and Sinclair, T.R. 1999. Introgenning root aerenchyma into maize. Maydica 44 : 113-117.

Tase, K. and Kobayasi, M. 1992. Studies on flooding tolerance in Italian ryegrass 2. Simple screening technique for flooding tolerance in Italian ryegrass. Hokuriku Sakumotsu Gakkaiho 27 : 76-78***

VanToai, T.T., St Martin, S.K., Chase, K., Boru, G., Schnipke, V., Schmitthenner, A.F. and Lark, K.G. 2001. Identification of a QTL associated with tolerance of soybean to soil waterlogging. Crop Sci. 41 : 1247-1252.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP : a new technique for DNA fingerprinting. Nucleic Acids Res. 23 : 4407-4414.

Yamasaki, T. 1952. Studies on the excess-moisture injury of upland crops in overmoist soil from the view point of soil chemistry and plant physiology. Bull. Nat. Inst. Agr. Sci. B1 : 1-92**.

*In Japanese with English abstract.
**In Japanese with English summary.
***In Japanese.

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Mano, Y., Muraki, M., Fujimori, M., Takamizo, T. and Kindiger, B. 2005a. Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays* ssp. *huazhetangensis*) seedlings. Euphytica 142 : 35-42.