

Breeding for flooding tolerant maize using “teosinte” as a germplasm resource

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Abstract: Flooding or waterlogging is a major factor in reducing crop yields. In order to increase crop productivity in temporarily flooded soils, development of flooding-tolerant lines is required. Three primary factors affecting flooding tolerance in plants have been reported: (1) the ability to grow adventitious roots at the soil surface during flooding; (2) the capacity to form root aerenchyma; and (3) tolerance to toxins (e.g., Fe^{2+} , H_2S) under reducing soil conditions. By analyzing these components separately, it could be possible to perform selections for genotypes exhibiting varying degrees of flooding tolerance. In quantitative trait locus (QTL) analyses for flooding tolerance, using teosinte as a germplasm resource, we have identified several QTLs associated to flooding traits. Based on the DNA marker information linked to flooding tolerance-related QTLs, the potential transfer of QTLs conferring flooding tolerance from teosinte to maize and pyramiding these QTLs into selected maize lines are now possible.

Keywords: adventitious roots at the soil surface, aerenchyma, flooding, maize, quantitative trait loci, teosinte.

Introduction

Yield reduction due to flooding or waterlogging is a major component in reduced crop yields (Boyer 1982). In Japan, promoting the cultivation of summer crops such as maize, sorghum and soybean in upland fields converted from paddy is one of the most important agricultural policies to redress the over production of rice. Moreover, the self-sufficiency regarding the production of major upland crops in Japan is very low. For example, grain maize production in Japan is negligible in statistics, soiling maize production was 4.7 million ton and soybean produc-

tion was only 0.16 million ton, while 16.5 million ton grain maize and 4.4 million ton soybean were imported in 2004 (Abstract of Statistics on Agriculture Forestry and Fisheries in Japan, <http://www.maff.go.jp/toukei/abstract/index.htm>).

Therefore, genetic improvements of summer crop varieties implementation of superior cultivation and management practices may be useful approaches for generating and identifying cultivars that can be utilized in rice paddy. During the late spring and early summer, the Asian monsoon season provides a constant threat of flooding. Maize hybrid seedlings growing this period exhibit poor adaptation to flooding conditions (Fig. 1). In such temporarily flooded soils, the development of improved flooding-tolerant maize lines is required.

In a study of flooding tolerance, selection tests have been reported using a large number of accessions in barley (Takeda and Fukuyama 1987, Takeda 1989), wheat (Ueno and Takahashi 1997) and soybean (Hou and Thseng 1991). In maize, studies have identified cultivar variability for pre-germination flooding tolerance (Martin et al. 1988, Lemke-Keyes and Sachs 1989) and flooding tolerance at the seedling stage (Fausey et al. 1985, Mano et al. 2002). In addition, molecular and cellular



Fig. 1. Maize seedlings damaged by flooding stress.

responses during flooding in maize have been reported (Sachs et al. 1996, Subbaiah and Sachs 2003). However, superior genetic resource useful for the development of a flooding tolerant maize has not yet been identified.

Wild species have often provided good genetic resources for introducing superior levels of biotic or abiotic stress tolerance (Harlan 1976). Wild barley *Hordeum marinum*, for example, exhibits superior flooding tolerance when compared to cultivated barley (McDonald et al. 2001). The distant relative of maize “teosinte” consists of various subspecies of *Zea mays* such as *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis* and *Z. mays* ssp. *huehuetenangensis* and the separate species of *Z. diploperennis*, *Z. perennis*, *Z. luxurians* and *Z. nicaraguensis* (Doebley and Iltis 1980, Iltis and Doebley 1980, Iltis and Benz 2000). Teosintes obtained from regions that are known to receive frequent rainfall in Mexico, Guatemala, Honduras and Nicaragua may provide a superior genetic resource for the development of flooding-tolerant maize. For example, an accession of *Z. nicaraguensis* has shown adaptation to the northwest coastal plain of Nicaragua and is known to tolerate frequent flooding during a 6-month rainy season (Bird 2000, Iltis and Benz 2000). Superior features of teosinte as a potential germplasm resource for the breeding of maize will be discussed in the review of flooding tolerance.

Factors affecting flooding tolerance

One of the most difficult problems for the breeding of flooding tolerant crops is that the varietal ranking for the trait is constantly changing with the growth stages. In addition, when the comparison is made at a similar growth stage, it is difficult to select reliable tolerant lines since multiple factors related to flooding under field conditions reduce repeatability of the experiments. In quantitatively inherited traits such as flooding tolerance, marker-assisted selection may be useful for varietal improvement. Studies utilizing soybean cultivars under field conditions have identified that multiple factors related to flooding tolerance result from various environmental interactions and as such, it was difficult to develop reliable DNA marker(s) for marker-assisted selection (Reyna et al. 2003). Therefore, it is important to differentiate the factors affecting flooding tolerance and to analyze each component separately.

Three primary factors affecting flooding tolerance in plants have been reported: (1) the ability to grow adventitious roots at the soil surface during flooding conditions (Bird 2000, Lizaso et al. 2001, Mano et al. 2005a,b,c); (2) the capacity to form root aerenchyma (Arikado and Adachi 1955, Jat et al. 1975, Drew et al.

1979, Burdick 1989, Armstrong et al. 1991, McDonald et al. 2001, Shimamura et al. 2003, Shimamura and Mochizuki 2005); and (3) tolerance to toxins (e.g., Fe^{2+} , H_2S) under reducing soil conditions (Yamasaki 1952, Ponnampereuma 1984). We will describe the three factors in this review.

In addition to these three components, the importance of a shallow root system and the barrier to radial oxygen loss (ROL) from aerenchymatous root has also been reported. Oyanagi et al. (2004) suggested that shallow-rooting double-haploid lines of wheat maintained higher grain yield than deep-rooting double-haploid lines in conditions with a high level of ground water. The barrier to ROL was suggested to occur in the aerenchymatous plant root. In plants that form aerenchyma in the root cortex under flooded conditions, oxygen is diffused through the aerenchyma from the basal part to the apex of roots. The oxygen, however, may be consumed by respiration or be lost by radial loss from the root during the diffusion through aerenchyma. As the result of this phenomenon, the amount of oxygen available for root elongation at the apical meristem zone is decreased. The development of a barrier to ROL in the peripheral portion of the root acts to enhance oxygen diffusion to the root apex (Colmer et al. 1998, Colmer 2003). By analyzing these components separately, it may be possible to perform reliable selections for such a complex trait.

Superior features of teosinte for flooding tolerance

Adventitious root formation at the soil surface during flooding

Adventitious (i.e., shoot-borne crown) root formation at the soil surface is one of the most important adaptations to flooding conditions. Dicotyledonous plants (e.g., soybean and tomato) generally form taproot system but develop adventitious roots under flooding conditions (McNamara and Mitchell 1990, Bacanamwo and Purcell 1999). This characteristic allows the root system to obtain oxygen directly from the air because the adventitious roots develop in shallow layer of the soil and even at the soil surface. Cereal plants have fibrous root system that consists of a primary seminal root (originating from radicle) and numerous adventitious roots (i.e., other seminal roots, mesocotyl roots and nodal roots). During flooding, some adventitious roots of cereal plants may show similar eco-physiological behavior to those of dicotyledonous plants. The importance of adventitious root formation during flooding has been previously reported for barley (Stanca et al. 2003) and Italian ryegrass (Tase and Kobayashi 1992).

In the genus *Zea*, some flooding tolerant maize lines formed adventitious roots at the soil surface

during experimental flooding conditions (Mano et al. 2005a). In addition, teosinte *Z. luxurians* and *Z. mays* ssp. *huehuetenangensis* have been observed to exhibit a higher capacity for adventitious root formation than some maize inbreds (Mano et al. 2005a). In an extremely severe flooding condition (with 12cm of standing water), *Z. mays* ssp. *huehuetenangensis* seedlings were observed to exhibit a high adaptability to flooding by developing adventitious roots above the soil surface (Mano unpublished). As a consequence, the adventitious roots of this teosinte can obtain oxygen and this characteristic may play an important role in its adaptation to flooding conditions.

In an analysis using a 94 F₂ individual population by crossing maize B64 × teosinte *Z. mays* ssp. *huehuetenangensis* (Fig. 2), quantitative trait loci (QTLs) controlling adventitious root formation at the soil surface under flooding condition (not reducing soil conditions) were identified on chromosomes 4, 5 and 8 (Mano et al. 2005b). From work in progress (Mano et al. unpublished), success is believed to have achieved in transferring a QTL for adventitious root formation with larger effect on chromosome 8 from teosinte to maize. In addition, evaluation of flooding tolerance in a near-isogenic line possessing adventitious root forming QTL is now in progress. Recently, Mano (unpublished) found that *Z. mays* ssp. *huehuetenangensis* formed adventitious roots at the soil surface in the field condition during the rainy season (Fig. 3). In this instance, the water level is below at the soil surface, suggesting the capacity of adventitious root formation in teosinte could be important to obtain oxygen in not only experimental flooded condition but in the field condition with frequently rainfalls.

Root aerenchyma

Flooding tolerant wetland species of rice (Jackson et al. 1985, Colmer 2003) and *Sagittaria lancifolia* (Schussler and Longstreth 1996) are known to grow well under flooded conditions, at least in part, by supplying oxygen through root aerenchyma. Also, they have been observed to form aerenchyma in well-aerated hydroponic solution. Maize does not typically form aerenchyma in non-flooding or non-stressed conditions but can form lysigenous aerenchyma in aerated hydroponic cultivation of seedlings if nitrogen levels are low (Konings and Verschuren 1980). Aerenchyma formation in maize roots has been investigated when not flooded (Jat et al. 1975); however an evaluation of the capacity of various genotypes to form aerenchyma at a relatively early growth stage, when severe damage can be caused by flooding, has not been reported. Also, little is known about varietal variation for aerenchyma formation in maize during non-flooding conditions.

This character may be relevant to increase flooding tolerance since a plant that possesses aerenchyma channels when not flooded, may be able to adapt more rapidly to flooding conditions when they occur. In an earlier study, under well-aerated and drained conditions, *Z. luxurians* was reported to develop well-formed aerenchyma in adult plants (Ray et al. 1999). At the seedling stage, Mano et al. (2006c) investigated the capacity of aerenchyma development in drained soil and found that some accessions of *Z. nicaraguensis* and *Z. luxurians* formed clear aerenchyma (Fig. 4).

In an F₂ population of maize B64 × teosinte *Z. nicaraguensis*, QTLs controlling aerenchyma formation in roots when not flooded were identified on chromosomes 1, 5 and 8 (Mano et al. 2006b), and additional QTLs were found in a different maize B73 × teosinte *Z. luxurians* mapping population (Omori et al. unpublished). The development of near-isogenic lines for aerenchyma forming QTL is now in progress. By comparing flooding tolerance between



Fig. 2. Two-week old flooded lines. Left: Teosinte *Z. mays* ssp. *huehuetenangensis* (with adventitious roots), right: Maize B64 (without adventitious roots). Shoots were removed from the plants in order to clearly observe the adventitious roots at the soil surface. The picture was reproduced from Mano (2006) Livestock Technology September p. 18.



Fig. 3. Teosinte *Z. mays* ssp. *huehuetenangensis* seedling with adventitious roots at the soil surface in the field condition during the rainy season.

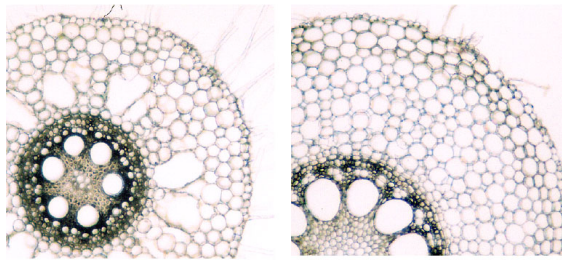


Fig. 4. Cross sections of adventitious roots that emerged from the second node in four-week old seedlings when not flooded (15-20 cm from the root base) showing aerenchyma in teosinte *Z. luxurians* (left) and showing the lack of aerenchyma of maize B73 (right).

maize and near-isogenic lines possessing an aerenchyma forming gene, it will be possible to elucidate the effects of aerenchyma on flooding tolerance as well as the possible mechanisms of aerenchyma formation by molecular and cellular analyses.

Tolerance at reducing soil condition

During the rainy season in upland field converted from paddy, toxic chemicals (e.g., Fe^{2+} , H_2S), induced by reducing soil conditions, also pose serious problems in addition to excessive water or low oxygen stress conditions (Yamasaki 1952). Mano et al. (2006a) found a QTL for flooding tolerance in reducing conditions on chromosome 1 in the yellow flint maize inbred line, F1649. Since the effect of the QTL found in F1649 was not large, further studies using additional genetic resources are required. Mano (unpublished) has observed in a greenhouse experiment that teosinte *Z. nicaraguensis* exhibited an extremely high adaptability to flooding under reducing soil conditions. Using this genetic resource as a donor parent, it might be possible to find additional QTLs with larger effects.

Perspective

We have reported several studies regarding experiments focused on understanding flooding tolerance in *Zea*. Based on the DNA marker information linked to flooding-related QTLs, transferring QTLs related to flooding tolerance from teosinte to maize and pyramiding these QTLs is now in progress and the evaluation of flooding tolerance in developed lines at the field condition will soon be investigated. We hope our flooding study will shed some light on flooding tolerance characteristics and contribute to the practical and applied breeding for flooding tolerance in maize.

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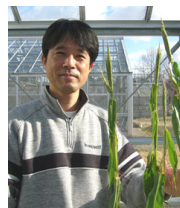
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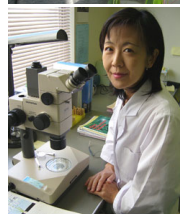
References

- Arikado H, Adachi Y 1955 Anatomical and ecological responses of barley and some forage crops to the flooding treatment. *Bull. Fac. Agric. Mie Univ.* 11: 1-29.
- Armstrong W, Justin SHFW, Beckett PM, Lythe S 1991 Root adaptation to soil waterlogging. *Aquat. Bot.* 39: 57-73.
- Bacanamwo M, Purcell LC 1999 Soybean root morphological and anatomical traits associated with acclimation to flooding. *Crop Sci.* 39: 143-149.
- Bird RMCK 2000 A remarkable new teosinte from Nicaragua: Growth and treatment of progeny. *Maize Gen. Coop. Newsl.* 74: 58-59.
- Boyer JS 1982 Plant productivity and environment. *Science* 218: 443-448.
- Burdick DM 1989 Root aerenchyma development in *Spartina patens* in response to flooding. *Am. J. Bot.* 76: 777-780.
- Colmer TD, Gibberd MR, Wiengweera A, Tinh TK 1998 The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. *J. Exp. Bot.* 49: 1431-1436.
- 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.
- Doebley JF, Iltis HH 1980 Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. *Am. J. Bot.* 67: 982-993.
- 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.
- Fausey NR, VanToai TT, McDonald Jr MB 1985 Response of ten corn cultivars to flooding. *Trans. ASAE* 28: 1794-1797.
- Harlan JR 1976 Genetic resources in wild relatives of crops. *Crop Sci.* 16: 329-333.
- Hou FF, Thseng FS 1991 Studies on the flooding tolerance of soybean seed: varietal differences. *Euphytica* 57: 169-173.
- 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.
- Jackson MB, Fenning TM, Drew MC, Saker LR 1985 Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressure of oxygen. *Planta* 165: 486-492.
- Jat RL, Dravid MS, Das DK, Goswami NN 1975 Effect of flooding and high soil water condition on root porosity and growth of maize. *J. Indian Soc. Soil Sci.* 23: 291-297.
- Konings H, Verschuren G 1980 Formation of aerenchyma in roots of *Zea mays* in aerated solutions, and its relation to nutrient supply. *Physiol. Plant.* 49: 265-270.
- Lemke-Keyes CA, Sachs MM 1989 Genetic variation for

- seedling tolerance to anaerobic stress in maize germplasm. *Maydica* 34: 329-337.
- Lizaso JL, Melendez LM, Ramirez R 2001 Early flooding of two cultivars of tropical maize. I. shoot and root growth. *J. Plant Nutr.* 24: 979-995.
- Mano Y, Muraki M, Komatsu T, Fujimori M, Akiyama F, 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. (in Japanese with English abstract)
- 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. (in Japanese with English abstract)
- 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, Omori F, Muraki M, Takamizo T 2005c QTL mapping of adventitious root formation under flooding conditions in tropical maize (*Zea mays* L.) seedlings. *Breed. Sci.* 55: 343-347.
- Mano Y, Muraki M, Takamizo T 2006a Identification of QTL controlling waterlogging tolerance in reducing soil conditions in maize (*Zea mays* L.) seedlings. *Plant Prod. Sci.* 9: 176-181.
- Mano Y, Omori F, Takamizo T, Kindiger B, Bird RMcK 2006b Identification of QTL controlling root aerenchyma formation in teosinte seedlings. *Breed. Res.* 8 (Suppl. 1): 192. (in Japanese)
- Mano Y, Omori F, Takamizo T, Kindiger B, Bird RMcK, Loaisiga CH 2006c Variation for root aerenchyma formation in flooded and non-flooded maize and teosinte seedlings. *Plant Soil* 281: 269-279.
- Martin BA, Smith OS, O'Neil M 1988 Relationships between laboratory germination tests and field emergence of maize inbreds. *Crop Sci.* 28: 801-805.
- McDonald MP, Galwey NW, Colmer TD 2001 Waterlogging tolerance in the tribe Triticeae: the adventitious roots of *Critesion maritimum* have a relatively high porosity and a barrier to radial oxygen loss. *Plant Cell Environ.* 24: 585-596.
- McNamara ST, Mitchell CA 1990 Adaptive stem and adventitious root responses of two tomato genotypes to flooding. *HortScience* 25: 100-103.
- Oyanagi A, Kiribuchi-Otobe C, Yanagisawa T, Miura S, Kobayashi H, Muranaka S 2004 Growth and grain yield of wheat experimental lines with deep and shallow root system in wet paddy fields. *Jpn. J. Crop Sci.* 73: 300-308. (in Japanese with English abstract)
- Ponnamperuma FN 1984 Effects of flooding on soils. In: Kozlowski TT, Ed., *Flooding and plant growth*. Academic Press, Orlando, Florida, USA, pp.9-45.
- Ray JD, Kindiger B, Sinclair TR 1999 Introgressing root aerenchyma into maize. *Maydica* 44: 113-117.
- Reyna N, Cornelious B, Shannon JG, Sneller CH 2003 Evaluation of a QTL for waterlogging tolerance in southern soybean germplasm. *Crop Sci.* 43: 2077-2082.
- Sachs M, Sabbaiah C, Saab I 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.
- Shimamura S, Mochizuki T, Nada Y, Fukuyama M 2003 Formation and function of secondary aerenchyma in hypocotyls, roots and nodules of soybean (*Glycine max*) under flooded conditions. *Plant Soil* 251: 351-359.
- Shimamura S, Mochizuki T 2005 Secondary aerenchyma formation and its relation to flooding tolerance of plants. *Root Res.* 14: 149-155. (in Japanese with English abstract)
- Stanca AM, Romagosa I, Takeda K, Lundborg T, Terzi V, Cattivelli L 2003 Diversity in abiotic stress tolerances. In: von Bothmer R, van Hintum Th, Knüpfper H, Sato K, Eds., *Diversity in Barley (Hordeum vulgare)*. Elsevier Science BV, Amsterdam, pp. 1-280.
- Subbaiah CC, Sachs MM 2003 Molecular and cellular adaptations of maize to flooding stress. *Ann. Bot.* 91: 119-127.
- Takeda K 1989 Varietal variation of flooding tolerance in barley seedlings, and its diallel analysis. *Jpn. J. Breed.* 39 (Suppl. 1): 174-175. (in Japanese)
- Takeda K, Fukuyama T 1987 Tolerance to pre-germination flooding in the world collection of barley varieties. *Barley Genet.* V: 735-740.
- Tase K, Kobayasi M 1992 Studies on flooding tolerance in Italian ryegrass 2. Simple screening technique for flooding tolerance in Italian ryegrass. *Hokuriku Sakumotsu Gakkaikaiho* 27: 76-78. (in Japanese)
- Ueno K, Takahashi H 1997 Varietal variation and physiological basis for inhibition of wheat seed germination after excessive water treatment. *Euphytica* 94: 169-173.
- Yamasaki T 1952 Studies on the excess-moisture injury of upland crops in overmoist soil from the viewpoint of soil chemistry and plant physiology. *Bull. Natl. Inst. Agric. Sci.* B1: 1-92. (in Japanese with English summary)



Dr. Yoshiro Mano's research interest is genetic improvement for soil flooding tolerance in maize using "teosinte" as a germplasm resource.



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