QTL mapping of above-ground adventitious roots during flooding in maize x teosinte "*Zea nicaraguensis*" backcross population

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Abstract: Quantitative trait locus (QTL) analyses were performed to map the genes controlling adventitious root formation on the soil surface (ARF-SS) under flooding conditions in seedlings of 317 BC_3F_1 individuals derived from a cross between elite maize Mi29 x teosinte *Zea nicaraguensis*. An SSR-based linkage map was developed using 94 markers, covering 896.3 cM of the ten chromosomes. The ability of ARF-SS under flooding conditions showed continuous variation in the BC_3F_1 population. By single point regression and interval mapping analyses, the QTLs for ARF-SS were located on chromosomes 3 (bin 3.04), 7 (bin 7.04) and 8 (bin 8.03). Alleles of *Z. nicaraguensis*, which has a high ability of ARF-SS, increased the level of ARF-SS for all the QTLs. By comparing chromosome positions of ARF-SS loci to previously reported loci, the region on chromosome 3 was shown to be unique to this teosinte. A possible application of the new QTL to breed flooding tolerant maize is discussed.

Keywords: adventitious root, flooding, quantitative trait loci, teosinte, waterlogging

Introduction

In Japan, soil waterlogging during late spring to early summer may result in substantial yield losses in summer upland crops, including maize. Several morphological, anatomical and physiological responses are known to adapt many species to flooding stresses (for review see: Yamasaki 1952, Vartapetian and Jackson 1997, Setter and Waters 2003, Mano and Omori 2007). Adventitious (shoot-borne crown) root formation at the soil surface (ARF-SS) or surface rooting is considered to be a key to adaptation to flooding because the adventitious roots can obtain oxygen directly from the air. The ability of ARF-SS during flooding has been observed in upland crops such as maize (Lizaso et al. 2001, Mano et al. 2005a), teosinte (a wild relative of maize) (Mano et al. 2005b) and barley (Stanca et al. 2003), and in particular, wide variation within genus *Zea* was reported (Mano et al. 2005a).

In 1989, a new species of teosinte, *Zea nicaraguensis*, was discovered in far northwest Nicaragua in an area frequently flooded for many weeks. It has several unique traits that make it a most interesting experimental material. When not flooded, the plants have 15 to over 30 primary, secondary and tertiary tillers, many of them could be cut and rooted, which allows clonal plants to be tested in many ways.

In *Z. nicaraguensis*, there is a striking difference between clones grown unflooded and those flooded. Plants in Nicaragua were observed to grow up to 5.3 m tall (Bird 2000, Iltis and Benz 2000), however, progeny grown in Mexico grew about 2 m tall at tasseling. In experiments (Bird 2000), roots and lower stems of young experimental plants in Raleigh, NC were constantly flooded both indoors and outside, winter and summer. These plants changed in root form: (1) masses of adventitious roots grew in the water above the soil; (2) small roots 0.2-0.4 mm in diameter emerged on those roots near the air, extending upward 0.5-2.5 cm towards the water surface in dense clumps, about 40 per 3 cm of adventitious root.

To date, we have identified QTLs controlling ARF-SS during flooding in F_2 populations of maize inbred line 'B64' × teosinte '*Zea mays* ssp. *huehuetenangensis*' (Mano et al. 2005b) and maize 'B64' × maize 'Na4' (Mano et al. 2005d). However a QTL controlling ARF-SS during flooding had not yet

been identified in the unique teosinte '*Z. nicaraguensis*'. The objectives of this study were to identify any new QTL controlling ARF-SS by using segregating BC_3F_1 population derived from a cross between maize inbred line 'Mi29' and '*Z. nicaraguensis*' and to compare chromosome position of ARF-SS loci to the known QTLs for ARF-SS. The possibility of using QTLs controlling ARF-SS to breed flooding tolerant maize is discussed.

Materials and Methods

Plant materials

An elite dent maize inbred line, Mi29, developed at the National Agricultural Research Center for Kyushu Okinawa Region (Ikegaya et al. 1999), was obtained from the Forage Crop Breeding Research Team, National Institute of Livestock and Grassland Science, Nasushiobara, Japan, and the teosinte, *Z. nicaraguensis* (CIMMYT 13451), was obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico. During flooding, seedlings of Mi29 exhibit a low capacity for ARF-SS (Y. Mano, unpublished), while those of *Z. nicaraguensis* exhibit a high tendency for ARF-SS (Bird 2000). A single F_1 plant derived from a cross between Mi29 × *Z. nicaraguensis* was crossed to Mi29 (as the female) and 36 BC_1F_1 plants were obtained. These 36 BC_1F_1 plants were backcrossed using Mi29 as the pollen parent to produce BC_2F_1 seeds. Fifteen BC_2F_1 seeds from each of 36 BC_1F_1 spikes (a total of 540 seeds) were sown in the field at the National Institute of Livestock and Grassland Science, Japan and 262 randomly selected BC_2F_1 plants were backcrossed a third time using Mi29 as the pollen parent. For this analysis, a total of 350 BC_3F_1 individuals from 1 or 2 BC_3F_1 seeds from each of the 262 BC_2F_1 spikes were used.

SSR analysis

Approximately 1–4 µg of DNA was isolated from 50 mg of fresh leaf tissue using the method described by Komatsuda et al. (1998). The SSR analysis was performed as described by Mano et al. (2005c).

Flooding treatment

For evaluating ARF-SS under flooding conditions, the 350 BC_3F_1 individuals and their parents (n=20, for each) were grown in pots of 11 cm in diameter and 15 cm in depth (single plant/pot) in a greenhouse. Of these, a total of 317 plants grew well and were used for trait evaluation. Seedlings at the three-leaf-stage were immersed in water until the

water level reached 3 cm above the soil surface. Detailed description of the experimental methods can be found in the report of Mano et al. (2005b) with an exception of trait evaluation. In this study, the amount of ARF-SS of each individual seedling was evaluated in four indices; total root length visually observed above the ground at one week and at two weeks after flooding (TRL1 and TRL2) and root number likewise (RN1 and RN2). Lateral roots generated from adventitious roots were not counted. We also measured plant height just before flooding treatment (PH0), and one week (PH1) and two weeks after flooding (PH2).

Map construction and QTL analysis

We have previously constructed a SSR-based map using 94 markers for the 214 plants of the BC_2F_1 population derived from the Mi29 × *Z. nicaraguensis* cross (Mano and Omori 2008). In this study, we used the previous BC_2F_1 map information for the 350 BC_3F_1 population. For the linkage map construction, the order of markers was the same as that presented in the study of Mano and Omori (2008). Recombination was computed in Haldane's mapping function using the QGene program (Nelson 1997). The frequency of donor chromosome segments (heterozygotes) in the recurrent parent (Mi29) genome was calculated using QGene.

QTL analysis

Single-point regression analysis (SPA) and interval mapping (IM) were used to map the QTLs controlling ARF-SS in the BC_3F_1 individuals using QGene. The experiment-wise significance threshold in SPA and IM were established at P<0.05 levels by permutation tests (Churchill and Doerge 1994). Based on 10,000 permutations, the threshold for SPA corresponded to F values of 13.8 for TRL1, 12.3 for TRL2, 12.8 for RN1 and 12.3 for RN2, and that for IM corresponded to LOD score values of 3.1 for TRL1, 2.7 for TRL2, 2.9 for RN1 and 2.7 for RN2.

Results

Trait evaluation

The degree of ARF-SS in the parents and 317 BC_3F_1 individuals was evaluated one and two weeks following flooding treatment. The teosinte parent differed greatly from Mi29 in amount of ARF-SS (Fig. 1). The BC_3F_1 population showed continuous distributions in the four traits (Fig. 2), suggesting the ARF-SS related traits are under multiple-gene control. Positive significant correlations were found among

Fig. 1. Two-week old flooded maize and teosinte seedlings. Left: Mi29 (without adventitious roots), right: *Z. nicaraguensis* (with adventitious roots).

the four ARF-SS related traits (TRL1, TRL2, RN1 and RN2), from 0.600 to 0.849 (Table 1).

We also measured plant height (PH0, PH1 and PH2) under flooding conditions and the traits showed continuous distributions (data not shown). Correlation coefficients between the ARF-SS related traits and plant height measured just before flooding treatment (PH0) were generally low (0.208 to 0.262), suggesting that the effect of original plant size on the traits related to ARF-SS is low. Relatively low but

positive correlations were found between PH2 and ARF-SS related traits two weeks after flooding treatment (TRL2 for 0.258 and RN2 for 0.310; Table 1).

Proportion of donor chromosome segments

The frequency of heterozygotes in the BC_3F_1 individuals ranged from 0.00 to 0.33 with a mean being 0.12 (n=350, standard deviation=0.06). This overall mean is quite close to the expected value of 0.125 for a BC_3F_1 generation.

Linkage map

We constructed a linkage map derived from 350 BC_3F_1 individuals from the cross between Mi29 × *Z. nicaraguensis*. This SSR-based map covered 896.3 cM at an average interval of 10.7 cM/locus for the ten chromosomes. This is close to the 852.7 cM reported in the BC_2F_1 map of the same cross (Mano and Omori 2008). By comparing published maize SSR maps (Sharopova et al. 2002) based on bin information, the coverage of the map was satisfactory for QTL mapping. Segregation distortion regions (at the 0.01 level) in the BC_3F_1 map were found on chromosome 4 (bin 4.07; associated with a higher frequency of the heterozygous genotype) and chromosome 5 (5.07; associated with a higher frequency of Mi29) (data not shown). Their positions corresponded well to those in the BC_2F_1 map (Mano and Omori 2008).
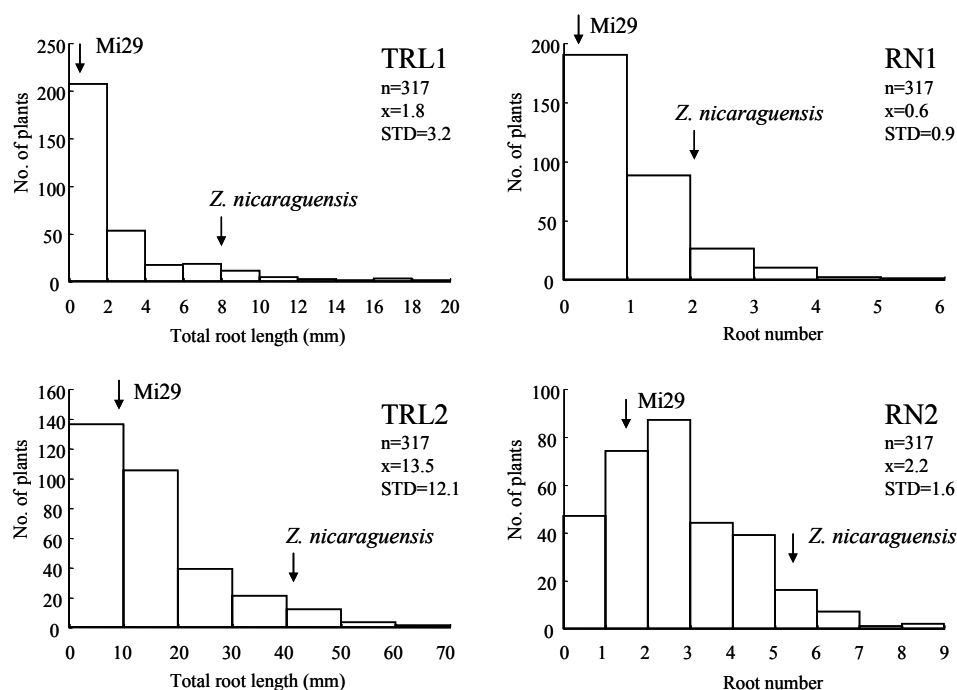

Fig. 2. Frequency distributions of traits related to ARF-SS in the Mi29 × *Z. nicaraguensis* BC_3F_1 population.

Mapping ARF-SS QTLs in the BC₃F₁ population

Using SPA and IM analyses, QTLs were identified for trait TRL2 on chromosome 3 (bin 3.04) and 7 (bin 7.04), for RN1 on chromosome 8 (bin 8.03) (signifi-cant only for IM analysis), and for RN2 on chromosome 3 (bin 3.04). No significant QTL was detected for the trait TRL1 (Table 2). Figure 3 shows the LOD curves of the ARF-SS related QTLs. The positions of the QTLs for TRL2 and RN2 on chromosome 3, with

Table 1. Correlation coefficients among seven traits in the BC₃F₁ individuals of the cross between Mi29 × *Z. nicaraguensis*

	TRL1	TRL2	RN1	RN2	PH0	PH1
TRL2	0.677**					
RN1	0.849**	0.626**				
RN2	0.600**	0.837**	0.621**			
PH0	0.232**	0.220**	0.262**	0.208**		
PH1	0.169**	0.151**	0.162**	0.196**	0.629**	
PH2	0.225**	0.258**	0.229**	0.310**	0.479**	0.734**

TRL1, total adventitious root length on the soil surface at one week after flooding.
TRL2, total adventitious root length on the soil surface at two weeks after flooding.
RN1, adventitious root number on the soil surface at one week after flooding.
RN2, adventitious root number on the soil surface at two weeks after flooding.
PH0, plant height just before flooding treatment.
PH1, plant height one week after flooding treatment.
PH2, plant height two weeks after flooding treatment.
**Significant at the 1 % level.

Table 2. Chromosome locations and mode of gene action of QTLs for ARF-SS during flooding estimated by single-point analysis (SPA) and interval mapping (IM) in the BC₃F₁ individuals of the cross between Mi29 × *Z. nicaraguensis*

Traits	QTL	Chr	Bin	Nearest marker	Phenotypic mean		SPA				IM	Increased effect
					AA[a]	AB[b]	F-value[c]	p-value	LOD	Var[d]	LOD[e]	
TRL1[f]	-	-	-	-	-	-	-	-	-	-	-	-
TRL2	*Qarf3.04*	3	3.04	bnlg1113	12.4	21.3	18.4	< 0.0001	3.9	0.06	3.9	*Z. nicaraguensis*
	Qarf7.04	7	7.04	dupssr13	12.6	20.8	13.9	0.0002	3.0	0.04	3.4	*Z. nicaraguensis*
RN1[g]	*Qarf8.03*	8	8.03	umc2075	0.5	1.1	11.9	0.0006	2.6	0.04	2.9	*Z. nicaraguensis*
RN2	*Qarf3.04*	3	3.04	bnlg1113	2.0	3.2	20.0	< 0.0001	4.2	0.06	4.2	*Z. nicaraguensis*

[a] Homozygous for Mi29 alleles at nearest marker.
[b] Heterozygous (Mi29/*Z. nicaraguensis*) at nearest marker.
[c] Significant thresholds levels (13.8 for TRL1, 12.3 for TRL2, 12.8 for RN1 and 12.3 for RN2) were detremined by permutation tests.
[d] Proportion of phenotyphic variance explained.
[e] Significant thresholds levels (3.1 for TRL1, 2.7 for TRL2, 2.9 for RN1 and 2.7 for RN2) were detremined by permutation tests.
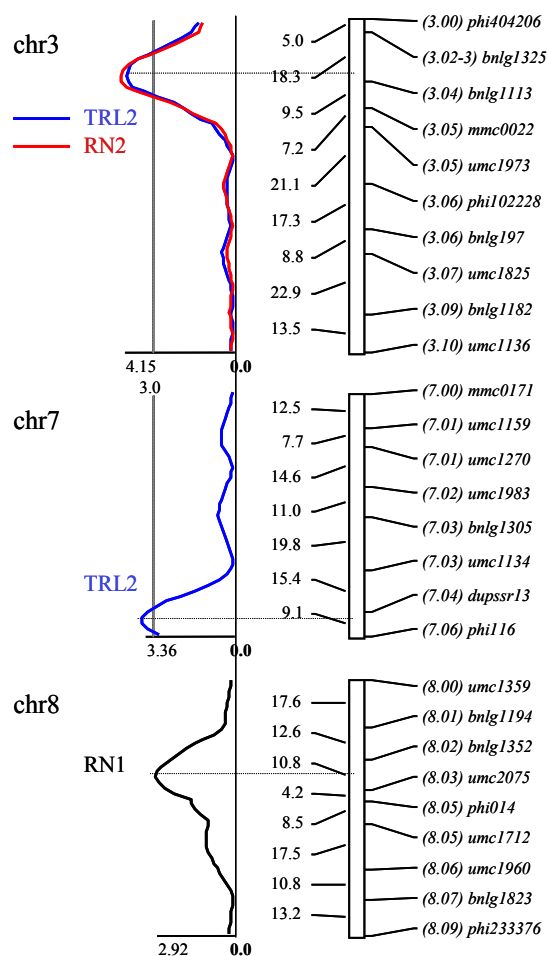[f] No significant QTL was identified.
[g] Significant only in IM analysis.

Fig. 3. Chromosome locations of the QTLs for ARF-SS related traits of TRL2, RN1 and RN2 on chromosomes 3, 7 and 8 in the Mi29 × *Z. nicaraguensis* BC$_3$F$_1$ population determined by IM. Only significant chromosomes are presented. Short arms of the chromosomes are located at the top. The scale is expressed in centimorgans (Haldane units).

LOD scores of 3.9 and 4.2 respectively, corresponded well. For all the QTLs, alleles of *Z. nicaraguensis* in heterozygous genotypes increased the degree of ARF-SS. Chromosome positions of QTLs controlling ARF-SS did not overlap those of the QTLs for plant height (PH0, PH1 and PH2) (data not shown).

Comparison of QTL positions with those in different mapping population

Figure 4 summarizes chromosome positions of the QTLs controlling ARF-SS due to flooding found in four mapping populations. The region here conditioning the TRL2 trait of ARF-SS in this study, on chromosome 7 (bin 7.04), overlapped that found in the B64 × Na4 mapping population (Mano et al. 2005d). In addition, the position of the QTL for RN1

on chromosome 8 (bin 8.03) was close to that found in the B64 × *Z. mays* ssp. *huehuetenangensis* population (Mano et al. 2005b). The position of the QTL controlling TRL2 and RN2 on chromosome 3 (bin 3.04) in this study did not overlap any previously identified ARF-SS loci, suggesting the QTL is a newly identified locus. This indicates the composition of ARF-SS QTLs can be changed according to the genotype.

Discussion

The ability of ARF-SS under flooding or waterlogging conditions noted in the rare teosinte *Z. nicaraguensis* and *Z. mays* ssp. *huehuetenangensis* (Bird 2000, Mano et al. 2005b) was shown to be heritable. At least some of the *Z. nicaraguensis* alleles were expressed in the heterozygotic BC$_3$F$_1$ population.

We have previously mapped QTLs controlling flooding-induced ARF-SS to several chromosome regions in teosinte (Mano et al. 2005b) and maize (Mano et al. 2005d). In this study, a new QTL has been successfully identified on chromosome 3 using a BC$_3$F$_1$ population of Mi29 × *Z. nicaraguensis* and this QTL did not overlap known QTLs controlling several other root traits (summarized by Tuberosa et al. 2002, Tuberosa et al. 2003). We have not identified a single major QTL controlling ARF-SS in our studies involving two teosintes, *Z. mays* ssp. *huehuetenangensis* (Mano et al. 2005b, Y. Mano unpublished) and *Z. nicaraguensis* (this study). Instead, the results strongly indicate multiple gene control. Although the effect of each QTL on ARF-SS detected in our studies is minor, it may be possible to develop maize lines with extremely high levels of ARF-SS during flooding by pyramiding ARF-SS QTLs from both *Z. mays* ssp. *huehuetenangensis* and *Z. nicaraguensis* (and perhaps also *Z. luxurians*) by using a marker-assisted selection approach.

Omori and Mano (2007) reported that, in tropical maize Na4, the QTL for ARF-SS during flooding and the QTL for angle of adventitious root in teosinte '*Z. luxurians*' were both located in the same part of chromosome 7 (bin 7.04). In this study, we have confirmed the correspondence between the QTL for ARF-SS on chromosome 7 in *Z. nicaraguensis* (Fig. 3) and the QTL for angle of roots in its sister species, *Z. luxurians* (Omori and Mano 2007), a very useful verification of the importance of this chromosome segment (or perhaps gene) as observed in the earlier experiments involving similar materials.

The importance of ARF-SS or surface rooting during flooding has been reported for barley (Stanca et al. 2003), Italian ryegrass (Tase and Kobayashi 1992) and riverside species *Rumex* (Blom et al. 1994).
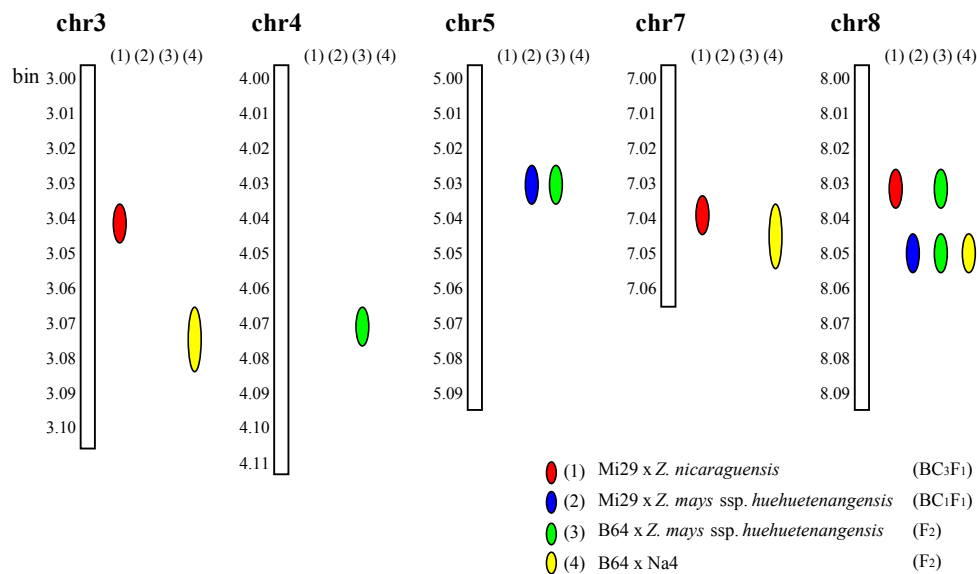
Fig. 4. Comparison of chromosome positions of QTLs controlling ARF-SS during flooding in four mapping populations. (1) Mi29 × *Z. nicaraguensis* BC$_3$F$_1$ (this study), (2) Mi29 × *Z. mays* ssp. *huehuetenangensis* BC$_1$F$_1$ (Y. Mano, unpublished), (3) B64 × *Z. mays* ssp. *huehuetenangensis* F$_2$ (Mano et al. 2005b). (4) B64 × Na4 F$_2$ (Mano et al. 2005d).

In this study, among the 317 BC$_3$F$_1$ individuals, relatively low but positive correlations were found between ARF-SS related traits and flooding tolerance evaluated by plant height in particular at two weeks after flooding (Table 1). This tendency was also reported for 27 *Zea* accessions in a greenhouse experiment (Mano et al. 2005a), further suggesting a positive relationship between ARF-SS and flooding tolerance. In field experiments using 25 tropical and subtropical maize accessions, no relationship between surface rooting (related to ARF-SS) and flooding tolerance was observed based on grain yield (Zaidi et al. 2007). This disagreement between the latter study and ours may be due to differences in materials, amount of flooding and/or the stage of growth when flooded. This situation may soon be clarified by comparing flooding tolerance between maize and its near-isogenic lines possessing QTLs controlling ARF-SS from *Z. mays* ssp. *huehuetenangensis* that we have recently developed (Mano et al. 2007).

Also, we have been developing a series of chromosome segment substitution lines (CSSLs) each possessing a chromosome segment from *Z. nicaraguensis* in the genetic background of the recurrent line (Mi29). The CSSLs will be useful in verifying the QTL results reported in this study, in search for recessive QTLs, which could not be detected in this study of heterozygous BC$_3$F$_1$ individuals and in comparing the root traits between flooding and normal conditions.

In conclusion, a new ARF-SS QTL was identified on chromosome 3 in *Z. nicaraguensis*. However, it has yet to be determined whether this and the other ARF-SS QTLs of *Z. nicaraguensis* can aid in withstanding temporary flooding. *Z. mays* ssp. *huehuetenangensis* has been observed to develop above-surface adventitious roots in the field and greenhouse conditions without flooding (Mano and Omori 2007, Y. Mano, unpublished), so by combining ARF-SS QTLs from *Z. mays* ssp. *huehuetenangensis* and *Z. nicaraguensis*, it may be possible to develop an elite maize line that possesses the ability of ARF-SS in both flooded and non-flooded conditions.

Acknowledgments

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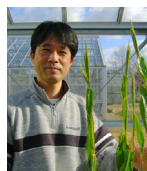
Dr. Yoshiro Mano's research interest is genetic improvement for soil flooding tolerance in maize using "teosinte" as a germplasm resource.

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