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Invited Review

Genetic regulation of root traits for soil flooding tolerance in genus *Zea*

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Flooding stress caused by excessive precipitation and poor drainage threatens upland crop production and food sustainability, so new upland crop cultivars are needed with greater tolerance to soil flooding (waterlogging). So far, however, there have been no reports of highly flooding-tolerant upland crop cultivars, including maize, because of the lack of flooding-tolerant germplasm and the presence of a large number of traits affecting flooding tolerance. To achieve the goal of breeding flooding-tolerant maize cultivars by overcoming these difficulties, we chose highly flooding-tolerant teosinte germplasm. These flooding-tolerance-related traits were separately assessed by establishing a method for the accurate evaluation of each one, followed by performing quantitative trait locus (QTL) analyses for each trait using maize × teosinte mapping populations, developing introgression lines (ILs) or near-isogenic lines (NILs) containing QTLs and pyramiding useful traits. We have identified QTLs for flooding-tolerance-related root traits, including the capacity to form aerenchyma, formation of radial oxygen loss barriers, tolerance to flooded reducing soil conditions, flooding-induced adventitious root formation and shallow root angle. In addition, we have developed several ILs and NILs with flooding-tolerance-related QTLs and are currently developing pyramided lines. These lines should be valuable for practical maize breeding programs focused on flooding tolerance.

Key Words: adventitious roots at the soil surface, aerenchyma, reducing soil conditions, radial oxygen loss (ROL) barrier, root angle, teosinte, waterlogging.

Introduction

Many regions of the world, including Southeast Asia, peninsular India, eastern Africa and the northern half of the Andes, have experienced a dramatic increase in flooding due to an increase in precipitation events associated with climate change (Hirabayashi *et al.* 2013, Sasidharan *et al.* 2017, Voesenek and Bailey-Serres 2015). In South and Southeast Asia, for example, over 18% of the maize (*Zea mays* subsp. *mays*) production area is affected by soil flooding (waterlogging) (Cairns *et al.* 2012, Zaidi *et al.* 2010). Flooding stress threatens upland crop production and food sustainability (Pedersen *et al.* 2017), so upland crop cultivars with greater tolerance to soil flooding (waterlogging) are needed for these areas. Apart from flooding related to climate change, Japan is faced with a unique situation: in order to counteract the decreases in land productivity and food self-sufficiency caused by overproduction of rice for human consumption, maize is grown in uncultivated upland fields converted from rice paddies, which are frequently flooded or waterlogged during the rainy season (**Fig. 1**). To develop flooding-tolerant maize, an understanding of genetic and physiological aspects of tolerance is essential.

Despite the importance of developing flooding-tolerant upland crops, there are no reports of highly flooding-tolerant maize cultivars for two main reasons: (1) the lack of flooding-tolerant germplasm useful for practical breeding approaches and (2) the presence of multiple and complex genetic factors related to flooding tolerance, which reduces the repeatability of experiments and increases environmental interactions (Mano and Oyanagi 2009). To overcome these difficulties, we and our colleagues at the Institute of Livestock and Grassland Science, NARO, and at Nagoya University followed a unique approach using highly flooding-tolerant teosinte germplasm in the following strategy: (1) separate flooding-tolerance-related traits and establish methods for the accurate evaluation of each one, (2) perform quantitative trait locus (QTL) analyses for each trait using maize × teosinte mapping populations, (3) develop introgression lines (ILs) or near-isogenic lines (NILs) of maize containing QTL alleles for flooding

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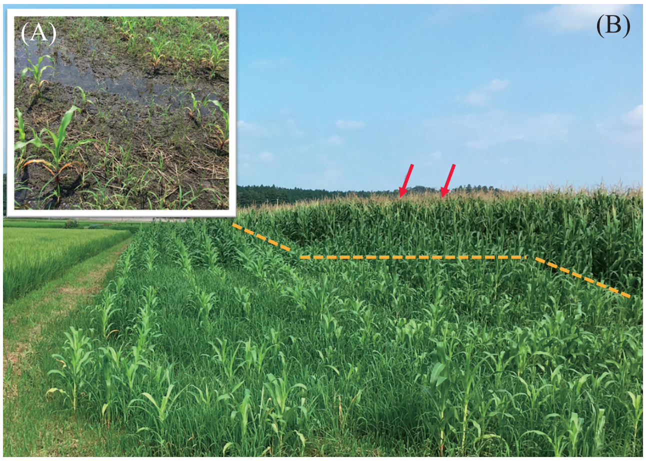

Fig. 1. Flooding-affected maize field converted from rice paddy. (A) Maize plants damaged by natural flooding stress, 8 July 2017. (B) Loss and delayed growth of maize plants in low-lying flooded area (below dotted line) while maize plants in a higher part of the field were tasseling (arrows), 31 July 2017.

tolerance from teosinte and (4) pyramid useful traits. In this review, we present the current status of investigations regarding flooding tolerance in genus *Zea*, which are focused on root traits.

Teosinte germplasm

Wild relatives of crops offer a diversity of traits useful for increasing tolerances to multiple stresses (Harlan 1976, Warburton *et al.* 2017). In the genus *Zea*, for example, teosinte (a set of species related to maize) is tolerant to insects, diseases (de Lange *et al.* 2014, Mammadov *et al.* 2018) and flooding (Mammadov *et al.* 2018, Mano and Omori 2007). Teosinte consists of three polytypic annual subspecies of *Zea mays* (*Z. mays* subsp. *parviglumis*, *Z. mays* subsp. *mexicana* and *Z. mays* subsp. *huehuetenangensis*) and four additional species: a diploid perennial, *Z. diploperennis*; a tetraploid perennial, *Z. perennis*; and two diploid annual species, *Z. luxurians* and *Z. nicaraguensis* (Doebley and Iltis 1980, Iltis and Doebley 1980, Iltis and Benz 2000). Of these, *Z. nicaraguensis* is found in flooded lowlands in the northwest coastal plain of Nicaragua (Bird 2000, Iltis and Benz 2000) and *Z. luxurians* accession JSG-593 is adapted to high-precipitation regions in Oaxaca, Mexico (e.g., receiving 3669 mm during the growing season; Sánchez *et al.* 2011, 2018). These teosintes adapted to flooded or wet environments are considered to possess unique and favorable genes that would be useful for breeding flooding-tolerant maize. Because it is easier to obtain, we have been using mainly *Z. nicaraguensis* for flooding studies. An advantage to the use of teosinte in the development of flooding-tolerant maize lines is that all of the teosinte species, with the exception of *Z. perennis*, can be easily crossed to maize, followed by selfing or backcrossing.

Root traits related to flooding tolerance

Flooding tolerance is a complex quantitative trait controlled by multiple genes. Flooding-tolerance-related traits have widely been reported in several wetland and upland plants (e.g., Mustroph 2018, Pedersen *et al.* 2021a, Vartapetian and Jackson 1997), and many of them are root traits. Root traits related to flooding tolerance include (1) the capacity to form aerenchyma, (2) formation of a barrier to radial oxygen loss (ROL), (3) tolerance to toxic soil constituents under reducing soil conditions, (4) flooding-induced adventitious root formation at the soil surface (ARF-SS) and (5) shallow root angle. In addition, a higher cortex-to-stele ratio (CSR; Yamauchi *et al.* 2021) was recently found to promote oxygen transport from the shoot base to root tips of plants (Pedersen *et al.* 2021a), and comparison of upland and wetland crops suggested a positive relationship between CSR and flooding tolerance (Yamauchi *et al.* 2019).

Flooding-tolerant *Z. nicaraguensis* has all of the flooding-tolerance-related root traits listed above (Mano and Omori 2007, Mano *et al.* 2016), with the exception that its CSR has not been well investigated. These different root traits are considered to be controlled by different genetic mechanisms, so we analyzed these components separately. For genetic analyses using segregants of a cross between maize and *Z. nicaraguensis*, we used maize inbred line Mi29 as a female parent, because this line is widely used in Japanese breeding programs (Ikegaya *et al.* 1999, Ito *et al.* 2004). In addition to performing QTL analyses for root traits, we have developed a series of ILs each possessing a chromosome segment of *Z. nicaraguensis* in the genetic background of Mi29 (Mano and Omori 2013a). The ILs, and subsequently developed NILs possessing QTLs introduced by backcrossing, can be used as breeding materials directly as well as for genetic, physiological and anatomical analyses.

Constitutive aerenchyma formation

Root aerenchyma in cortical tissue contains air spaces that provide plant roots with oxygen under flooded or low-oxygen conditions. In upland crops, lower capacity for oxygen transport from shoot to root tip under flooded conditions inhibits plant growth and induces yield loss because of incomplete or partial aeration through root aerenchyma. Therefore, higher capacity for oxygen transport from shoot to root tip via aerenchyma is essential for improving flooding tolerance (summarized in Takahashi *et al.* 2014 and Yamauchi *et al.* 2013).

In some wetland crops such as rice (*Oryza sativa*), lysigenous aerenchyma, which is produced by cortical cell death, is constitutively formed even in drained soil, and its formation is further induced by waterlogged or low-oxygen conditions (Shiono *et al.* 2011). These mechanisms are respectively described as constitutive and inducible aerenchyma formation (Yamauchi *et al.* 2018). On the other hand, flooding-sensitive upland crops such as maize, wheat and barley form inducible aerenchyma under flooded or low-oxygen conditions but lack constitutive aerenchyma (Drew *et al.* 1979, Yamauchi *et al.* 2016). Because the induction of aerenchyma after exposure to flooding or low-oxygen conditions takes several hours or days, roots of upland crops without constitutive aerenchyma are damaged by flooding until they form sufficient amounts of inducible aerenchyma (Herzog *et al.* 2016, Rajhi *et al.* 2011, Yamauchi *et al.* 2014). The importance of constitutive aerenchyma, which enables the plant to adapt more rapidly to flooding conditions, is widely known (Pedersen *et al.* 2021a); however, accurate comparisons between materials with and without aerenchyma were not made in earlier studies because these investigations compared different genera or species.

In an earlier study, Ray *et al.* (1999) revealed that 2- to 3-month-old plants of *Z. luxurians* and F_1 hybrids of *Z. luxurians* × maize formed clear constitutive aerenchyma in well-aerated environments. Mano *et al.* (2006b) evaluated constitutive aerenchyma formation in the seedlings of *Zea* species grown under drained soil conditions and found that accessions of *Z. nicaraguensis* and *Z. luxurians* formed clear aerenchyma. Because diploid teosinte can be crossed to maize, development of NILs for constitutive aerenchyma formation and precise comparisons between aerenchyma-forming and -non-forming maize became possible. QTLs for constitutive aerenchyma formation have been mapped in several chromosome regions by using F_2 and backcross populations of maize × teosinte crosses and self-pollinated *Z. nicaraguensis* (**Table 1**). Among these, QTL for aerenchyma formation on the long arm of chromosome 1 at bin 1.06–.07 (*Qaer1.06-1.07*) showed the largest effect (Mano *et al.* 2007, Mano and Omori 2008).

Recently, we developed a pyramided line, IL-AE91, possessing four QTLs (*Qaer1.06-1.07*, *Qaer1.11*, *Qaer5.09n* and *Qaer8.05*) in the genetic background of Mi29 (**Fig. 2A**, **Table 2**) and found greater flooding tolerance than in Mi29 at the seedling stage under both oxygen-deficient stagnant hydroponic growth and flooded potting-mix soil conditions (Gong *et al.* 2019). By measurement of ROL and cell viability in IL-AE91 and Mi29, effective O_2 transport and cell survival in root tips under stagnant conditions were verified in IL-AE91. Although Mano and Omori (2013b) revealed that a greater capacity to form constitutive aerenchyma enhanced flooding tolerance by comparing *Z. nicaraguensis* accessions with high and low levels of its formation, a study by Gong *et al.* (2019) was the first to reveal the advantage of constitutive aerenchyma formation to flooding tolerance in materials with a similar genetic background. Because IL-AE91 still contains ~14% of the *Z. nicaraguensis* genome, deletion of undesirable *Z. nicaraguensis* genome regions is necessary to use IL-AE91 for practical breeding. For this purpose, detailed mapping of *Qaer1.06-1.07*, which has the highest genetic effect among the four aerenchyma QTLs, is now in progress.

Formation of barrier to radial oxygen loss (ROL)

The presence of a ROL barrier in adventitious roots, which facilitates oxygen diffusion to root apices by preventing radial loss of oxygen from the roots, is an important adaptation under low-oxygen conditions in plants (Colmer 2003, Watanabe *et al.* 2013). Flooding-tolerant rice and some wetland species induce a ROL barrier under stagnant or flooded conditions (referred to as inducible ROL barrier formation; Colmer *et al.* 1998, Colmer 2003, Shiono *et al.* 2011), whereas a few wetland species form ROL barriers under aerated or well drained conditions (constitutive ROL barrier formation; McDonald *et al.* 2002, Visser *et al.* 2000). In recent investigations, wild species of *Echinochloa* and Amazonian wild rice (*Oryza glumaepatula*) were found to constitutively form ROL barriers under aerated conditions (Ejiri and Shiono 2019, Ejiri *et al.* 2020). In this review, we focus on genetic mechanisms for inducible ROL barrier formation in *Z. nicaraguensis*.

By screening of a series of ILs derived from a cross between Mi29 × *Z. nicaraguensis* for ROL barrier formation, we found that only IL#11, which contains segments of chromosomes 1, 3 and 4 from *Z. nicaraguensis*, formed ROL barriers. In further genetic analyses using backcross progeny of the same cross combination, we found that the capacity to produce an inducible ROL barrier is controlled by a single major locus on the short arm of chromosome 3 (bin 3.04), and the mode of gene action was complete dominance (Watanabe *et al.* 2017). This locus was tentatively named *RBF1* (ROL Barrier Formation 1; **Table 1**), and we developed a NIL, designated NIL468-3, possessing *RBF1* in the genetic background of maize Mi29 (**Table 2**). By detailed mapping, the chromosome region containing *RBF1* was narrowed down to 232 kb, and candidate genes were selected (Ide *et al.* 2017). In the most recent investigation, *RBF1* induced a ROL barrier both in the main axis of adventitious roots and in the lateral roots when grown in stagnant, deoxygenated solution (Pedersen *et al.* 2021b). Isolation of *RBF1* and investigations of the mechanism of ROL formation (e.g., the signal for ROL formation and the composition of the barrier) are now in progress using *Agrobacterium*-transformed Mi29 containing the *RBF1* allele from *Z. nicaraguensis*.

Tolerance to toxic soil constituents under flooded reducing soil conditions

During the rainy season in Japan (late spring to early summer) in upland paddies, toxic soil constituents (e.g., Fe^{2+}, Mn^{2+} and H_2S) induced by reducing soil conditions (low redox potential) are a severe problem in addition to the stress caused by excessive water or low oxygen

Table 1. QTL and genes for flooding-tolerance-related traits in *Zea* species

Trait	Name[a]	Chr.	Bin	PVE[b]	Population[c]	References
Constitutive aerenchyma formation	*Qaer1.02-1.03*	1	1.02–1.03	0.11	B64 × ***Z. nicaraguensis***	Mano *et al.* (2007)
	Qaer1.05-1.06	1	1.05	0.25	Mi29 × ***Z. nicaraguensis***	Mano and Omori (2009)
			1.06	0.17	Mi29 × ***Z. nicaraguensis***	Mano and Omori (2008)
	Qaer1.06-1.07	1	1.06–1.07	0.08–0.17	***Z. nicaraguensis*** (S1)	Mano *et al.* (2012)
			1.07	0.12	B64 × ***Z. nicaraguensis***	Mano *et al.* (2007)
	Qaer1.11	1	1.11	0.12	Mi29 × ***Z. nicaraguensis***	Mano and Omori (2008)
	Qaer2.06	2	2.06	0.09	B73 × ***Z. luxurians***	Mano *et al.* (2008)
	Qaer5.09	5	5.09	0.11	**B64** × *Z. nicaraguensis*	Mano *et al.* (2007)
	Qaer5.09n			0.06	Mi29 × ***Z. nicaraguensis***	Mano and Omori (2008)
	Qaer7.01	7	7.01	0.12	***Z. nicaraguensis*** (S1)	Mano *et al.* (2012)
	Qaer8.05	8	8.05	0.07	Mi29 × ***Z. nicaraguensis***	Mano and Omori (2009)
ROL barrier formation	(*RBF1*)	3	3.04	Major locus	Mi29 × ***Z. nicaraguensis***	Watanabe *et al.* (2017)
Flooding tolerance under reducing soil conditions	(*Qft-rd1.03-1.04*)	1	1.03–1.04	0.14	**F1649** × H84	Mano *et al.* (2006a)
	Qft-rd4.07-4.11	4	4.07–4.11	0.13–0.42	Mi29 × ***Z. nicaraguensis***	Mano and Omori (2013a)
Adventitious root formation at the soil surface (ARF-SS)	*Qarf3.04*	3	3.04	0.06	Mi29 × ***Z. nicaraguensis***	Mano *et al.* (2009)
	(*Qarf3.07-3.08*)	3	3.07–3.08	0.10	B64 × **Na4**	Mano *et al.* (2005c)
	(*Qarf4.07*)	4	4.07	0.09	B64 × ***Z. mays* subsp. *huehuetenangensis***	Mano *et al.* (2005b)
	(*Qarf5.03*)	5	5.03	0.14	B64 × ***Z. mays* subsp. *huehuetenangensis***	Mano *et al.* (2005b)
	Qarf7.04	7	7.04	0.04	Mi29 × ***Z. nicaraguensis***	Mano *et al.* (2009)
	(*Qarf7.04-7.05*)		7.04–7.05	0.21	B64 × **Na4**	Mano *et al.* (2005c)
	Qarf8.03	8	8.03	0.04	Mi29 × ***Z. nicaraguensis***	Mano *et al.* (2009)
				0.10	B64 × ***Z. mays* subsp. *huehuetenangensis***	Mano *et al.* (2005b)
	(*Qarf8.05*)	8	8.05	0.25	B64 × ***Z. mays* subsp. *huehuetenangensis***	Mano *et al.* (2005b)
				0.15	B64 × **Na4**	Mano *et al.* (2005c)
Root angle	*CRA1*	1	–[d]	0.04	**DH1M** × T877	Li *et al.* (2018)
	CRA2	1	–	0.03–0.04	**DH1M × T877**[e]	Li *et al.* (2018)
	Qra3rd2.03-2.04	2	2.03–2.04	0.13	B73 × ***Z. luxurians***	Omori and Mano (2007)
	Root-ABA1	2	2.04	0.32	Os420 × **IABO78**	Giuliani *et al.* (2005)
	Qra2nd4.05	4	4.05	0.16	**B73** × *Z. luxurians*	Omori and Mano (2007)
	Qra3rd4.07	4	4.07	0.13	**B73** × *Z. luxurians*	Omori and Mano (2007)
	CRA3	6	–	0.03	**DH1M** × T877	Li *et al.* (2018)
	Qra3rd7.04	7	7.04	0.13	B73 × ***Z. luxurians***	Omori and Mano (2007)
	CRA4	9	–	0.03–0.04	**DH1M** × T877	Li *et al.* (2018)
Root relative dry weight	*rdw1*	1	1.04	0.04	HZ32 × **K12**	Qiu *et al.* (2007)
	rdw3	3	3.04	0.05	HZ32 × **K12**	Qiu *et al.* (2007)
	qWT5.04	5	5.04	0.10–0.15	**A3237** × A3239	Yu *et al.* (2019)
Root dry weight (treatment)	*rdw4-1*	4	4.05	0.07	HZ32 × **K12**	Osman *et al.* (2013)

[a] QTL or gene names in parentheses are tentative and are based on our previous nomenclature.
[b] Proportion of phenotypic variance explained.
[c] Mapping populations; boldface indicates parent contributing higher-value allele.
[d] Unknown.
[e] The parent contributing the allele with a positive effect differed depending on the environment.

(Ponnamperuma 1984). Under artificial reducing soil conditions produced by adding 0.1% or 0.2% soluble starch solution to the soil, wide variations were observed among maize inbred lines (Mano *et al.* 2006a) and between maize Mi29 and *Z. nicaraguensis* for the amount of leaf injury (Mano and Omori 2013a). Adventitious roots of sensitive maize Mi29 seedlings became black, whereas those of *Z. nicaraguensis* showed only a slight visible change, if any (**Fig. 2C**). We evaluated tolerance to toxic soil constituents as leaf injury (possibly correlated to root damage) under artificial reducing soil conditions.

Mano *et al.* (2006a) found a QTL for flooding tolerance in reducing soil conditions on chromosome 1 by using a maize mapping population from a cross between F1649 (tolerant) × H84 (sensitive) (**Table 1**). Because the effect of the QTL found in F1649 was not very large, we used *Z. nicaraguensis* as a donor for further genetic analyses. By screening ILs derived from a cross between Mi29 × *Z. nicaraguensis* under reducing soil conditions, we identified a tolerant line, IL#18a (**Fig. 2C**; Mano and Omori 2013a). IL#18a contains most of the long arm of chromosome 4 of *Z. nicaraguensis* in the genetic background of Mi29, and we named the QTL as *Qft-rd4.07-4.11* (QTL for flooding tolerance under reducing soil conditions at bin 4.07–4.11).

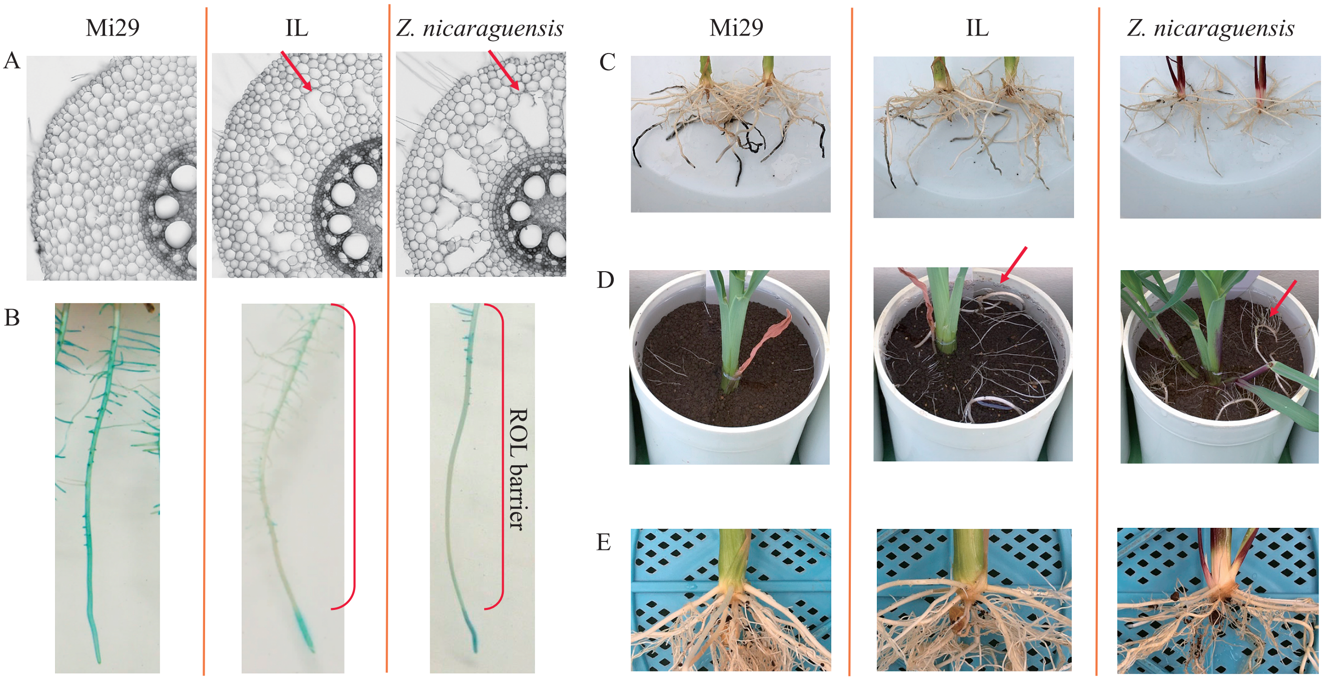

Fig. 2. Flooding-tolerance-related traits in *Z. nicaraguensis* and various introgression lines. (A) Capacity to form constitutive aerenchyma (arrows). (B) Barrier to radial oxygen loss (ROL), illustrated by methylene blue staining. Blue: O_2 loss through root; colorless: no oxygen loss through root. The figure is adapted from Mano *et al.* (2016) with permission. (C) Tolerance to toxic soil constituents under flooded reducing soil conditions (primary and seminal roots were removed to clearly reveal the adventitious roots). Black indicates root damage. (D) Ability to form adventitious roots at the soil surface during flooding (arrow). (E) Shallow root angle (grown under flooded conditions). IL: introgression line [A: IL-AE91 (possessing *Qaer1.06-1.07*, *Qaer1.11*, *Qaer5.09n* and *Qaer8.05*; Gong *et al.* 2019); B: IL#11 (*RBF1*: Watanabe *et al.* 2017); C: IL#18a (*Qft-rd4.07-4.11*; Mano and Omori 2013a); D: IL#468-4 (*Qarf3.04*; Y. Mano and F. Omori, unpublished); E: IL#32 (*Qra7.04*; Y. Mano and F. Omori, unpublished)].

We introgressed *Qft-rd4.07-4.11* into maize inbred lines Mi47 and Na50 by backcrossing eight times and developed two ILs, Mi47*Qft-rd4.07-4.11* and Na50*Qft-rd4.07-4.11* (**Table 2**; Y. Mano and F. Omori, unpublished). The effectiveness of *Qft-rd4.07-4.11* for flooding tolerance in these two different genetic backgrounds of maize (Y. Mano and F. Omori, unpublished) and in F_1 hybrids (Mano and Omori 2015) was confirmed in our greenhouse experiments. However, no recombination was observed in the *Qft-rd4.07-4.11* region. This recombination suppression is caused by a large chromosome inversion, which was verified by linkage analyses (Mano and Omori 2013a) and modifications of the FISH signal patterns (Braz *et al.* 2020).

We have been targeting QTLs in addition to *Qft-rd4.07-4.11* for tolerance to toxic soil constituents under reducing soil conditions because the region of *Qft-rd4.07-4.11* on chromosome 4 may contain genes for undesirable traits (linkage drag) such as lower kernel row number, late flowering and low pollen amount, but they cannot be eliminated because of recombination suppression. We identified another IL (IL#39) containing mainly the short arm of chromosome 9 of *Z. nicaraguensis* (**Table 2**), which showed equivalent tolerance under reducing soil conditions (Mano and Omori 2013a; **Supplemental Fig. 1**). Because IL#39 contains teosinte chromosome fragment(s) other than the short arm of chromosome 9 and the precise position of the QTL was not determined, detailed mapping is now in progress. Unlike *Qft-rd4.07-4.11*, the QTL for tolerance to toxic soil constituents in IL#39, possibly located on the short arm of chromosome 9, may be useful for practical breeding after the elimination of undesirable chromosome segments of *Z. nicaraguensis* by backcrossing.

Flooding-induced adventitious root formation at the soil surface (ARF-SS)

Adventitious (i.e., stem-base crown) root formation at the soil surface (ARF-SS) is one of the most important adaptations to soil flooding, because adventitious roots that develop close to or above the soil surface can obtain oxygen directly from the air. Similar to many flooding-tolerant wetland species (Justin and Armstrong 1987, Visser *et al.* 1996), some maize lines induce ARF-SS under flooded conditions (Lizaso *et al.* 2001, Mano *et al.* 2005a). Furthermore, *Z. nicaraguensis*, *Z. luxurians* and *Z. mays* subsp. *huehuetenangensis* formed larger numbers of adventitious soil-surface roots than did maize (Bird 2000, Mano *et al.* 2005a, 2009). This trait was observed in both greenhouse and field experiments (Mano and Omori 2007).

QTL analyses using parents with contrasting degrees of

Table 2. Introgression lines possessing flooding-tolerance-related traits developed at Institute of Livestock and Grassland Science, NARO and Nagoya University

Trait	Name	QTL[a]	Chr.	Bin	Genetic background	Donor	Fig. showing line	References
Constitutive aerenchyma formation	IL-AE91	*Qaer1.06-1.07* *Qaer1.11* *Qaer5.09n* *Qaer8.05*	1 1 5 8	1.06–1.07[b] 1.11 5.09 8.05	Mi29	*Z. nicaraguensis*	**Fig. 2A**	Gong *et al.* (2019)
ROL barrier formation	IL#11	(*RBF1*)	3	3.04	Mi29	*Z. nicaraguensis*	**Fig. 2B**	Watanabe *et al.* (2017)
	NIL#468-3[c]	(*RBF1*)	3	3.04	Mi29	*Z. nicaraguensis*	–	Ide *et al.* (2017)
Flooding tolerance under reducing soil conditions	IL#18a, IL#18b	*Qft-rd4.07-4.11*	4	4.07–4.11	Mi29	*Z. nicaraguensis*	**Fig. 2C**	Mano and Omori (2013a)
	Mi47-*Qft-rd4.07-4.11*	*Qft-rd4.07-4.11*[d]	4	4.07–4.11	Mi47	*Z. nicaraguensis*	–	Mano and Omori (2015)[e]
	Na50-*Qft-rd4.07-4.11*	*Qft-rd4.07-4.11*[d]	4	4.07–4.11	Na50	*Z. nicaraguensis*	–	Y. Mano and F. Omori (unpublished)
	IL#39	–[f]	–	–	Mi29	*Z. nicaraguensis*	**Supplemental Fig. 1**	Mano and Omori (2013a)
Adventitious root formation at the soil surface (ARF-SS)	IL#468-4	*Qarf3.04*	3	3.04	Mi29	*Z. nicaraguensis*	**Fig. 2D**	Y. Mano and F. Omori (unpublished)
	IL#32	*Qarf7.04*	7	7.04	Mi29	*Z. nicaraguensis*	**Supplemental Fig. 2**	Y. Mano and F. Omori (unpublished)
	Na110	*Qarf5.03* *Qarf8.05*	5 8	5.03 8.05	Mi29	*Z. mays* subsp. *huehuetenangensis*	–	H. Tamaki *et al.* (unpublished)
Shallow root angle	IL#32	*Qra7.04*	7	7.04	Mi29	*Z. nicaraguensis*	**Fig. 2E**	Y. Mano and F. Omori (unpublished)

[a] QTL or gene names in parentheses are tentative and based on our previous nomenclature.

[b] Detailed mapping is now in progress.

[c] Near isogenic line possessing *RBF1* (undesirable segments of *Z. nicaraguensis* from IL#11 have been eliminated).

[d] BC_8F_4 generation.

[e] Developed to BC_3 generation in this study; subsequent backcrosses were made by Y. Mano and F. Omori (unpublished).

[f] Different from *Qft-rd4.07-4.11*, but precise position was not determined.

ARF-SS revealed QTLs controlling ARF-SS on chromosomes 3, 7 and 8 in a mapping population from maize B64 × maize Na4 (Mano *et al.* 2005c), on chromosomes 4 and 8 in a population from B64 × *Z. mays* subsp. *huehuetenangensis* (Mano *et al.* 2005b), and on chromosomes 3, 7 and 8 in a population from Mi29 × *Z. nicaraguensis* (Mano *et al.* 2009) (**Table 1**). Three of the identified QTL regions could be detected in two different populations: bin 7.04–7.05 (donor parents were *Z. nicaraguensis* and maize Na4), bin 8.03 (*Z. nicaraguensis* and *Z. mays* subsp. *huehuetenangensis*) and bin 8.05 (*Z. mays* subsp. *huehuetenangensis* and maize Na4) (**Table 1**).

By selecting the series of ILs described above and backcrossing, we have developed two ILs for ARF-SS: IL#468-4, possessing QTL for adventitious root formation at the soil surface at bin 3.04 (*Qarf3.04*; **Fig. 2D**), and IL#32 (*Qarf7.04*; **Supplemental Fig. 2**). These lines should be useful for verifying the effect of ARF-SS on flooding tolerance and revealing its genetic and physiological mechanisms. In addition, we have generated pyramided line Na110, which contains both *Qarf5.03* and *Qarf8.05* (derived from *Z. mays* subsp. *huehuetenangensis*) in the genetic background of Mi29 (**Table 2**). Since 2013, yield-related data for F_1 hybrids (Na110 × Mi47 and Mi29 × Mi47 [control]) have been obtained under both flooded and unflooded field conditions (Mano *et al.* 2016).

Shallow rooting angle

Root angle is related to tolerance to several abiotic stresses such as flooding, drought and low soil P conditions (Lynch and Wojciechowski 2015, Oyanagi *et al.* 2004, Wasson *et al.* 2012). Research on root angle is focused mainly on drought tolerance, and deep rooting is an important means of reaching water stored deep in the soil. Genetic factors controlling deep rooting include *DRO1–DRO5* in rice (e.g., Uga *et al.* 2013, 2015), nine root-growth-angle QTL clusters in wheat (Maccaferri *et al.* 2016) and *Root-ABA1* and *CRA1–CRA4* in maize (**Table 1**). Regarding shallow rooting, Kitomi *et al.* (2020) recently identified a gene responsible for a QTL associated with root growth angle in rice, *qSOR1*, and demonstrated that a shallower root growth angle could enhance rice yields in saline paddies.

Shallow-rooting wheat lines had higher tolerance to flooding under paddy field conditions than deep-rooting lines (Oyanagi *et al.* 2004). In *Zea* species, Omori and Mano (2007) reported four QTLs controlling the angle of root emergence from the 2nd and 3rd nodes from the bottom at the seedling stage: QTL for root angle emerged from 3rd node from the bottom at bin 2.03–4 (*Qra3rd2.03-4*), *Qra2nd4.05*, *Qra3rd4.07* and *Qra3rd7.04* (**Table 1**). Some QTLs for root angle overlap with those for ARF-SS. For example, both *Qra3rd4.07* and *Qarf4.07* were located at bin 4.07 on chromosome 4, and *Qra3rd7.04* and *Qarf7.04* were located at bin 7.04 on chromosome 7 (**Table 1**). The overlap in QTL regions for these two root traits suggests that shallow roots can easily emerge from the soil surface under flooding. IL#32, which contains only a chromosome segment corresponding to bin 7.03–7.06 of *Z. nicaraguensis* in the genetic background of Mi29 (**Fig. 2E**, **Table 2**), should be useful for understanding the relationship between root angle and ARF-SS by backcrossing and subsequent dissection of *Qra3rd7.04* (root angle QTL) and *Qarf7.04* (ARF-SS).

Although QTLs for flooding tolerance in *Zea* have been widely investigated (Mano *et al.* 2006a, Zaidi *et al.* 2015, Zhang *et al.* 2013), QTLs associated with root traits other than those in our investigations are limited. Some examples of QTLs controlling root relative dry weight (Qiu *et al.* 2007, Yu *et al.* 2019) and root dry weight under waterlogged conditions (Osman *et al.* 2013) are listed at the bottom of **Table 1**.

Breeding strategy and perspective

Table 2 summarizes ILs possessing flooding-tolerance-related traits developed at the Institute of Livestock and Grassland Science, NARO, and at Nagoya University. Because combinations of root traits (e.g., constitutive aerenchyma formation plus a ROL barrier) should increase flooding tolerance by increasing oxygen transport to the root tip via aerenchyma under flooded conditions, pyramiding of these root traits would be valuable for maize breeding. The genetic background of most of these lines is Mi29, so it should be relatively easy to pyramid multiple genes in Mi29 by crossing and selfing together with marker-assisted selection. Indeed, we have developed pyramided lines possessing several flooding-tolerance-related traits (Y. Mano and F. Omori, unpublished).

In addition to generating ILs and pyramided lines, understanding the physiological mechanisms for flooding-tolerance-related traits is essential to applying QTLs for efficient breeding of maize and other flooding-sensitive upland crops. The ILs and NILs listed in **Table 2** can be useful for physiological and functional analyses. Indeed, we used IL-AE91 for physiological analysis of constitutive aerenchyma (Gong *et al.* 2019) and NIL#468-3 or IL#11 for physiological and functional analyses of ROL barrier formation (Ide *et al.* 2017, Pedersen *et al.* 2021b, Watanabe *et al.* 2017). Through these investigations, the relationships between QTLs for flooding-tolerance-related traits and physiological mechanisms such as ethylene-induced aerenchyma formation (reviewed by Drew *et al.* 2000), suberin-related ROL barrier formation (reviewed by Watanabe *et al.* 2013) and auxin-induced adventitious root development (reviewed by Steffens and Rasmussen 2016) are being revealed.

As mentioned in this review, we have been applying a unique approach using a highly flooding-tolerant teosinte germplasm to develop flooding-tolerant maize. During the development of NILs or ILs possessing flooding-tolerance-related traits, recombination suppression caused by a large chromosome inversion (a problem often seen in crosses involving wild relatives) was observed in the *Qft-rd4.07-4.11* region on the long arm of chromosome 4 (Mano and Omori 2013a). The recombination suppression prevented us from fine-mapping of *Qft-rd4.07-4.11* by linkage analysis, so we could not generate a NIL for the *Qft-rd4.07-4.11* locus. To overcome this difficulty, the following approach is now in progress: we have constructed a high-density F_2 linkage map of the long arm of chromosome 4 derived from a cross between *Z. nicaraguensis* × *Z. luxurians*, both of which possess an inverted chromosome segment in this region (Mano and Omori 2013a). Because these parents showed a clear difference in flooding tolerance under reducing soil conditions (Y. Mano, unpublished), it may be possible to perform fine-mapping of *Qft-rd4.07-4.11* by linkage analysis and subsequent isolation of the responsible gene. By using genome editing targeting the orthologous gene in maize, it may be possible to improve flooding tolerance in maize without introducing teosinte's undesirable traits. These efforts can be used to prevent the flooding damage promoted by global climate change and other causes.

Author Contribution Statement

YM wrote the main text and generated figures and tables; MN wrote the main text and generated a figure.

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