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The effects of a QTL for root development under hypoxia on yield of soybean exposed to 7-day waterlogging at seedling stage

Dang Thi Thu Hien[1], Nguyen Van Loc[2], Tomomi Abiko[3], and Toshihiro Mochizuki[3]

[1]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 744, Motooka, Nishi-ku, Fukuoka, 819-0395, Japan
[2]Faculty of Agronomy, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, 131000, Vietnam
[3]Faculty of Agriculture, Kyushu University, 111 Harumachi, Kasuya-cho, Kasuya-gun, Fukuoka, 811-2307, Japan
Dang Thi Thu Hien and Nguyen Van Loc are co-first authors

***Corresponding author: mochizuki@farm.kyushu-u.ac.jp**

Abstracts

In humid parts of the world, waterlogging is a major environmental stress limiting the yield of soybean and its root development is an important indicator of tolerance. Recently, quantitative trait loci (QTLs) for this trait were detected on the chromosome 12 of soybean through the analyses of recombinant inbred lines (RILs) developed from a cross between a hypoxia-sensitive cultivar, Tachinagaha, and a tolerant landrace, Iyodaizu. In this study, in order to get a detailed understanding of these QTLs, NIL-9-4-5, a near-isogenic line (NIL), was selected from Tachinagaha/Iyodaizu BC_6F_2 population through marker assisted selection (MAS). NIL-9-4-5 was then tested for yield performance under waterlogging conditions exposed at the seedling stage. The test was conducted in a greenhouse in 2015 and 2018 at the Experimental Farm of Kyushu University, Fukuoka, Japan. Soybean plants were grown in pots containing loam soil. From six days after germination, seedlings were grown for 7 days under two treatments: drained (control) and waterlogging conditions. Pots were kept in containers where the water level was maintained at 2.0 cm depth from bottom in the control and at 2.0 cm above the soil surface in waterlogged condition. Experimental results consistently showed that seed yields of both Iyodaizu and NIL-9-4-5 were not reduced under waterlogging. Compared to Tachinagaha, NIL-9-4-5 showed higher root development and increased seed yield under waterlogging. These indicate that the tested QTLs are able to increase yield by stimulating root development under waterlogging.

Keywords: Soybean, waterlogging tolerance, seed yield, root development, near-isogenic lines.

Abbreviations: CARD_Change in average root diameter; Chr._Chromosome; DAS_days after sowing; NIL_Near-isogenic line; QTL_Quantitative trait loci; RD_Root diameter; RIL_Recombinant inbred lines; RL_Root length; RLD_Root length development; RSA_Root surface area; RSAD_Root surface area development; WTI_Waterlogging tolerance index; Y_Yield.

Introduction

In humid parts of the world, waterlogging is a major environmental stress limiting soybean production (Kokubun et al., 2013). It inhibits growth and seed yield, leading to decreased root growth, nitrogen fixation, leaf chlorophyll content, and photosynthesis activity (Scott et al., 1989; Oosterhuis et al., 1990; Sung, 1993; Linkemer et al., 1998; Bacanamwo and Purcell, 1999; Pedó et al., 2015; Suematsu et al., 2017). Therefore, breeding varieties with strong waterlogging-tolerance is a desirable trait in countries with much rainfall (Van Nguyen et al., 2017).

Hypoxia is one of main effects of waterlogging due to rapid decrease of soil's available oxygen (Armstrong, 1979). Under short-term waterlogging, roots and micro-organisms' respiration depletes the remnant oxygen and the environment becomes hypoxic. The soil environment becomes anoxic if waterlogging is prolonged (Blom and Voesenek, 1996; Bailey-Serres and Voesenek, 2008). These conditions strongly inhibit root growth (Suematsu et al., 2017). To contribute for plant growth under waterlogging stress, a common morphological response of plants to low oxygen condition is the production of adventitious roots (Colmer and Voesenek, 2009; Rich et al., 2012). Under waterlogging, adventitious roots are formed/initiated in upland crops such as maize (Zaidi et al., 2003), tomato (McNamara and Mitchell, 1990), wheat (Mano and Omori, 2007) and soybean (Thomas et al., 2005; Bacanamwo and Purcell, 1999; Shimamura et al., 2003; Henshaw et al., 2007) under waterlogging. Another response to waterlogging is the ability to maintain root elongation and branching under hypoxia (Sakazono et al., 2014; Suematsu et al., 2017; Van Nguyen et al., 2017). Therefore, maintaining root development plays an important role in waterlogging tolerance of soybean plant.

Near-isogenic lines (NILs) are useful and valuable materials for gene mapping. Backcrossing and maker assisted selection methods are common used to create NILs. Backcrossing is a well-known and long-established breeding strategy where a characteristic is introgressed from a donor parent into the genomic background of a recurrent parent (Babu et al., 2004). Marker assisted selection (MAS) is simply understood as the use of genetic markers to follow regions of the genome that encode specific characteristics of a plant (Hospital and Charcosset, 1997). Therefore, NILs have been shown to be more suitable for efficient the identification of specific genes. We found QTLs conferring root development including root length development (RLD) and root surface area development (RSAD) on soybean chromosome (Chr.)12 and developed a NIL population carrying targeted QTLs at BC_6F_2. QTLs for RLD and RSAD (*Qrld-12*, *Qrsad-12*) on Chr.12 are stable across years. NIL-9-4-5, carrying the targeted QTLs, performed well under both hypoxia and waterlogging conditions, confirming the effect of these QTLs. In this study, we tested whether detected QTLs involve in seed yield improvement of soybean plant under waterlogging.

Results

Effects of genotype and waterlogging treatments

Table 1 shows the ANOVA results of the effects of genotypes (G), waterlogging treatments (T), and their interaction (G×T) on the root traits. We found that G had non-significant effects on all measured traits except RD. T had a significant effect on all traits except Y. G ×T interaction had a significant effect on all traits except RD and CARD.

Root development and seed yield of parents and NIL-9-4-5 under waterlogging

Under condition treated with waterlogging, RL, RLD, RSA and RSAD of Tachinagaha were reduced significantly than those of Iyodaizu. Mean of WTI values for these traits was 0.4 in Tachinagaha and 0.8 in Iyodaizu (Table 2). NIL-9-4-5 maintained root development under waterlogging (Table 2). RD of all genotypes tended to increase under waterlogging (WTI >1) but no significantly deference in both of Iyodaizu and NIL-9-4-5 (Table 2).

Seed yield of Tachinagaha was greatly decreased, but those of Iyodaizu and NIL-9-4-5 showed no significant decrease (Figure 2). Compared to Tachinagaha, NIL-9-4-5 showed higher root development and seed yield under condition treated with waterlogging in both year experiments (Figure 2).

Trait correlations

Pearson correlation coefficients were calculated to analyze the relationship between the studied traits under control and waterlogging conditions (Table 3). Positive correlations among root traits such as RL, RLD, RSA, and RSAD were observed under both control and waterlogging treatments. RD and CARD showed positive correlation with other root traits under control while they tended to negatively correlated under waterlogging. The results obtained a strong and highly significant correlation between seed yield (Y) and each of the root traits. However, contrasting responses were found for the correlation between Y and root diameter traits (RD and CARD). RD and CARD showed a positive correlation with Y under controls, while they presented a negative correlation under waterlogging.

Discussion

QTL analysis for waterlogging tolerance is usually challenging. However, several researches have been conducted to detect QTLs for waterlogging tolerance in soybean. QTLs for flooding tolerance of soybean plant at germination stage which were named *Sft1, Sft2, Sft3* and *Sft4* (Sayama et al., 2009). QTLs for waterlogging injury score and tolerance index at vegetative stages were found and mapped on Chrs. 2, 6, 7, 10, 11, 14, and 19 (Githiri et al., 2006), Chrs. 3, 5, 12, 13, and 16 (Cornelious et al., 2005), Chrs. 11 and 13 (Nguyen et al., 2012), and Chr. 12 (Van Nguyen et al., 2017). QTL associated with better soybean growth and grain yield under waterlogging was identified on Chr. 18 near marker Sat_064 (VanToai et al., 2001). Although many QTL studies of waterlogging tolerance in soybean have been reported, their effects on overall seed yield of soybean have not yet been validated.

The detected QTLs for a trait are important, but just the first step in molecular breeding program for crop genetic improvement. They should be confirmed in genetic background and different environments. Currently, approaches utilizing near iso-genic lines (NILs) have been effectively used to validate the identified QTLs (Collard and Mackill, 2008). Genes or QTL detection studies in relation to root related abiotic stress using introgression lines or near isogenic lines were conducted in rice (Steele et al., 2006; Uga et al., 2013), and barley (Naz et al., 2012, 2014). Uga et al. (2013) reported that positional cloning of QTL underlying DEEPROOTING1 (Dro1) gene in rice. They developed NIL containing Dro1 in IR64 background via marker selection. Dro1-NIL demonstrated significant increase in shoot biomass, yield and drought stress avoidance as compared to control genotype IR64 suggesting that the alteration of root system architecture improves yield and drought avoidance in rice plant. In this study, NILs was used to confirm the contribution of the region in LG H (Chr. 12) carrying the root development QTLs under waterlogging. The resultant increase in root development of NIL-9-4-5 was most likely inherited from the waterlogging-tolerant parent, Iyodaizu (Table 2). These finding indicates that genes from Iyodaizu could reduce cultivars' waterlogging susceptibility, and that QTLs for root development are strongly involved in it.

The relationship between RL and RSA in soybean plants is consistent with findings of Sallam and Scott (1987). This consistency supports the hypothesis that the maintenance of root extension may serve as an indicator of soybean waterlogging tolerance (Sakazono et al., 2014). Pearson correlation analysis between traits indicated strong positive relationships among root traits such as RL, RSA, and root development traits included RLD and RSAD under both water treatments. RD and CARD tended to increase under waterlogging as compared to controls due to a reduction in root elongation and branching (Sakazono et al., 2014; Suematsu et al., 2017). Our results show that RD tended to be negatively correlated with other root traits (Table 3).

Table 1. Analysis of variance results for the growth and root traits of studied cultivars under control and waterlogging treatment in 2015

Traits	Analysis of Variance (*F*-Ratio)		
	Genotype (G)	Waterlogging treatment (T)	GxT
RL (cm)	2.29	29.85**	14.29**
RLD (cm)	2.00	26.06**	13.03**
RSA (cm^2)	3.44	21.47**	13.11**
RSAD (cm^2)	0.95	19.39**	12.06**
RD (mm)	18.79**	12.73*	2.90
CARD (mm)	3.47	0.91	0.75
Y (g plant^{-1})	1.32	2.64	11.69**

Significant at*$P < 0.05$ and **$P < 0.01$; RL = root length, RLD = root length development, RSA = root surface area, RSAD = root surface area development, RD = root diameter, CARD = change in average root diameter, Y = seed yield.

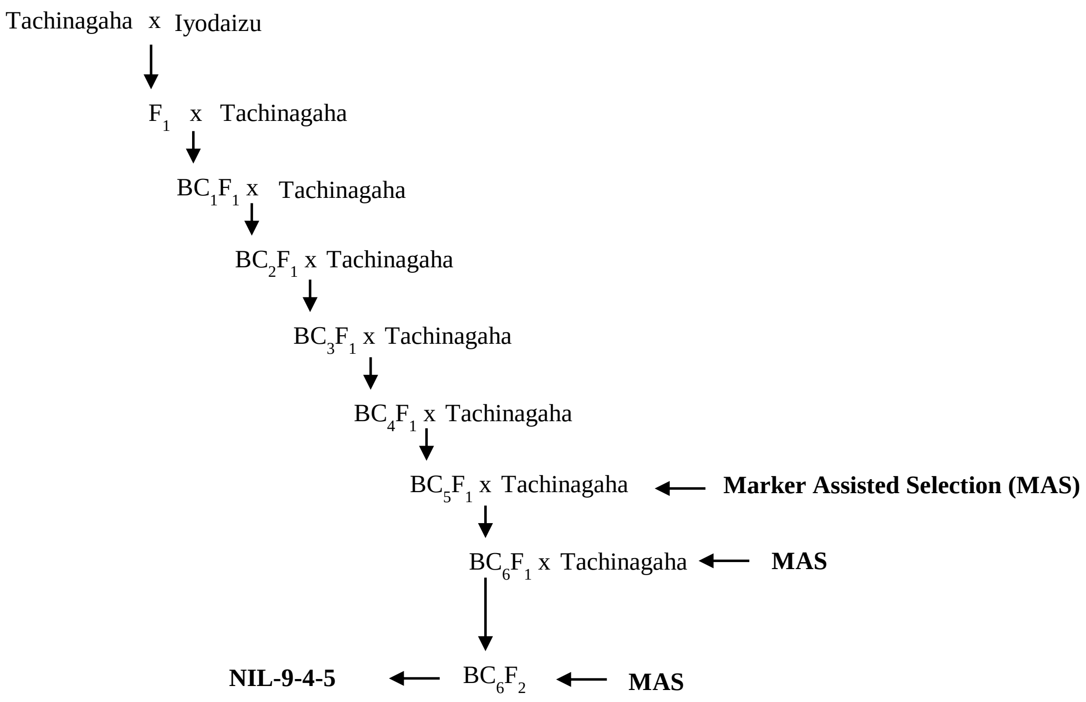

Fig 1. Diagram showing the material development of NILs at BC$_6$F$_2$

Table 2. Root traits and waterlogging-tolerance index (WTI) of Tachinagaha, Iyodaizu and NIL-9-4-5 in 2015

Traits	Tachinagaha			Iyodaizu			NIL-9-4-5		
	Control	Waterlogging	WTI	Control	Waterlogging	WTI	Control	Waterlogging	WTI
RL (cm)	441±54	181*±17	0.4	386±12	297*±11	0.8	282±30	291^{NS}±21	1.0
RLD (cm)	367±57	107*±15	0.3	334±18	243*±17	0.7	241±28	254^{NS}±18	1.1
RSA (cm^2)	89±13	43*±4	0.5	70±3	56*±3	0.8	51±7	55^{NS}±3	1.1
RSAD (cm^2)	71±14	25*±3	0.3	58±3	43*±3	0.7	41±7	46^{NS}±4	1.1
RD (mm)	0.64±0.24	0.75*±0.02	1.2	0.57±0.01	0.61^{NS}±0.01	1.1	0.58±0.02	0.61^{NS}±0.04	1.1
CARD (mm)	-0.13±0.26	-0.03*±0.07	0.2	-0.15±0.09	-0.15^{NS}±0.09	1.0	-0.21±0.04	-0.20^{NS}±0.07	1.0

RL = root length, RLD = root length development, RSA = root surface area, RSAD = root surface area development, RD = root diameter, CARD = change in average root diameter, WTI = waterlogging-tolerance index; Means with * within a genotype are significantly different by *t*-test at $p< 0.05$ (n=5).

Table 3. Phenotypic correlation coefficients among traits under control condition (above diagonal) and waterlogging (below diagonal) in 2015

Traits	RL	RLD	RSA	RSAD	RD	CARD	Y
RL	-	0.987**	0.979**	0.979**	0.682**	0.515**	0.683**
RLD	0.991**	-	0.958**	0.98**	0.635**	0.448**	0.603**
RSA	0.96**	0.95**	-	0.988**	0.809**	0.488**	0.681**
RSAD	0.947**	0.965**	0.974**	-	0.759**	0.481**	0.644**
RD	-0.92**	-0.929**	-0.793**	-0.826**	-	0.28*	0.44**
CARD	-0.768**	-0.793**	-0.614**	-0.639**	0.863**	-	0.807**
Y	0.832**	0.842**	0.805**	0.844**	-0.866**	-0.581**	-

Significant at *$P < 0.05$ and **$P < 0.01$; RL = root length, RLD = root length development, RSA = root surface area, RSAD = root surface area development, RD = root diameter, CARD = change in average root diameter, Y = seed yield.

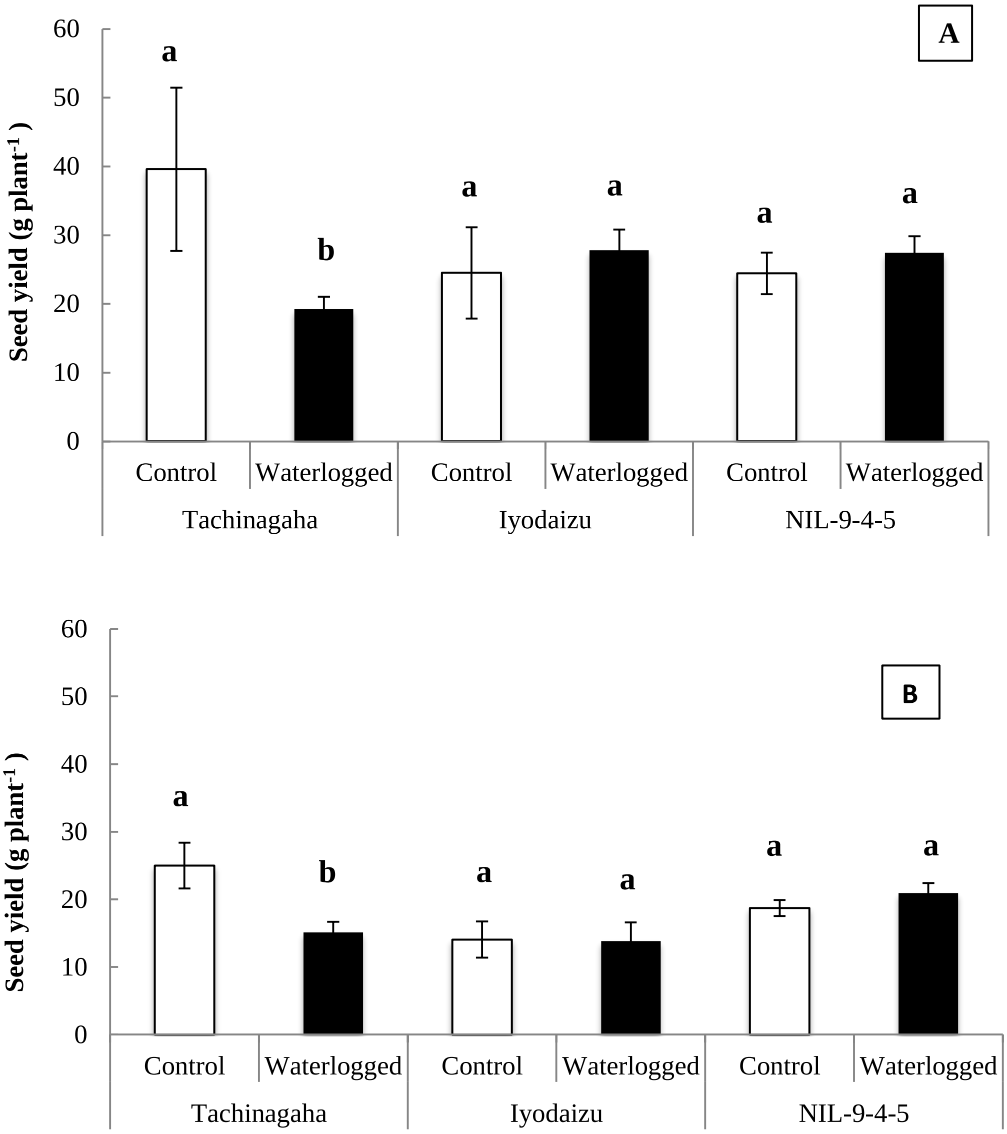

Fig 2. Seed yield of parents and NIL-9-4-5 under control and waterlogging in 2015 (A) and 2018 (B). Bars with the same letter within a genotype are not significantly different at $P < 0.05$ by *t*-test (*n*=5).

Waterlogging hampers both shoot and root growth (Sillam and Scott, 1987; Araki et al., 2012; Sauter, 2013). Normally, waterlogging reduced root growth before it reduces shoot growth (Shimamura et al., 2003). Bacanamwo and Purcell (1999) also concluded that the shoot biomass of soybean plant was not altered during the first 7 days of waterlogging. However, roots are vital for plant function, which play an important role in the absorption, translocation of water and nutrients (Garcia, 2015). High and strong correlation was observed between root development trait and seed yield in this study (Table 3). In this study, root development of Tachinagaha was strongly decreased and as a consequence the seed yield was reduced under waterlogging. Interestingly, experimental results consistently showed that seed yields of both Iyodaizu and NIL-9-4-5 were not reduced under waterlogging (Figure 2), these results are most probably related to their ability to maintain root development.

In conclusions, the obtained results showed that RLD and RSAD of Tachinagaha were greatly decreased under condition treated with waterlogging, but those of Iyodaizu and NIL-9-4-5 showed a slight decrease and no decrease, respectively. Interestingly, NIL-9-4-5 also had increased seed yield compared to Tachinagaha, indicating that the tested QTLs are able to enhance increase yield under waterlogging.

Materials and methods

Plant materials

Parents Iyodaizu and Tachinagaha plus NIL-9-4-5 were used in this study. Iyodaizu is hypoxia-tolerant, and Tachinagaha is hypoxia-susceptible (Sakazono et al., 2014). NIL-9-4-5 carrying the QTL region derived from Iyodaizu in the genetic background of Tachinagaha was selected at BC_6F_2 through recurrent backcrossing (Figure 1) and maker assisted selection (Supplementary Table 1).

Experimental design

The waterlogging experiment was conducted in a greenhouse at the Experimental Farm of Kyushu University, Fukuoka, Japan (33°37′N and 130°27′E) in two years of experiments.

In 2015, the experiment was conducted to evaluate the effects of QTLs for root development under hypoxia on root traits and seed yield of soybean exposed to 7days waterlogging at seedling stage. Eight seeds on Petri dish were incubated at 23°C and 70% RH for 3 days to uniform rate of germination. To select uniform seedlings for next steps, treated seeds were sown in moist vermiculite (Midori Sangyo, Fukuoka, Japan) at 23°C for 2 days. Selected seedling was transplanted into a pot (16 cm diameter and 18 cm height) containing 3 kg of dried paddy soil (56.1% sand, 23.2% silt, and 20.7% clay). Then all pots were putted into ten big trays kept 2 cm water level on its surface bottom. The factorial experiments were arranged in a Randomized Complete Bock Design with five replications. Three plants of each genotype in a big tray were used as a replication. From six days after germination, seedlings were grown for 7 days under two treatments; drained (control) and waterlogging conditions. Under control condition, water level was maintained at 2.0 cm from bottom of container. Under waterlogging, water level was maintained at 2.0 cm above soil surface. At before and after treatments, a plant in each replication was sampled to measure root traits. All the remaining pots treated with waterlogging were transferred to new containers to keep water level at 2.0 cm from the bottom like control conditions. Then, all plants were grown under the drained (control) conditions until pod harvesting.

In 2018, the yield experiments were conducted again to confirm parents and NIL-9-4-5 yield performance under waterloging conditions.

Measurements

The root systems of plants at before and after the treatments were scanned with an image scanner using the WinRhizo software to measure root morphological traits including root length (RL), root surface area (RSA), and root diameter (RD). RL was calculated on the so called skeleton images of root system. Roots were classified into ten groups (from x_1 to $x_{10,}$ mm) with unequal width ($x_1 \leqq 0.5$, $0.5 > x_2 \leqq 1.0$, $1.0 > x_3 \leqq 1.5$, $1.5 > x_4 \leqq 2.0$, $2.0 > x_5 \leqq 2.5$, $2.5 > x_6 \leqq 3.0$, $3.0 > x_7 \leqq 3.5$, $3.5 > x_8 \leqq 4.0$, $4.0 > x_9 \leqq 4.5$, $x_{10} > 4.5$) for evaluation of root diameter (RD). RSA was automatically calculated by software based on RD and RL of root in each class. Root development including root length development (RLD), root surface area development (RSAD) and change in root diameter (CARD) were calculated to measure their changes between before and after treatments in each replication. WTI, waterlogging-tolerant index was calculated for each root trait as the ratio of the value under waterlogging to that in control.

To measure seed yield (Y), three components of seed yield of each genotype were assessed: number of pods per plant, number of seeds per pod, and average of seed weight.

Statistical analysis of phenotypic data

All statically analyses were done with SYSTAT 13 (SYSTAT software, Inc., Washington, USA). Simple *Pearson* correlation coefficients between traits were calculated using value means. The genotype and treatment effects on the level of each trait were assessed by two-way ANOVA. The *t*-test was used to compare the average values among treatments.

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