

AZ-97 (*Oryza sativa* ssp. *Indica*) exhibits superior biomass production by maintaining the tiller numbers, leaf width, and leaf elongation rate under phosphorus deficiency

Yasuhiro Tsujimoto , Mitsukazu Sakata , Viviane Raharinivo , Juan Pariasca Tanaka & Toshiyuki Takai

To cite this article: Yasuhiro Tsujimoto , Mitsukazu Sakata , Viviane Raharinivo , Juan Pariasca Tanaka & Toshiyuki Takai (2020): AZ-97 (*Oryza sativa* ssp. *Indica*) exhibits superior biomass production by maintaining the tiller numbers, leaf width, and leaf elongation rate under phosphorus deficiency, Plant Production Science, DOI: [10.1080/1343943X.2020.1808026](https://doi.org/10.1080/1343943X.2020.1808026)

To link to this article: <https://doi.org/10.1080/1343943X.2020.1808026>



© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



[View supplementary material](#)



Published online: 31 Aug 2020.



[Submit your article to this journal](#)



Article views: 172



[View related articles](#)



[View Crossmark data](#)

AZ-97 (*Oryza sativa* ssp. *Indica*) exhibits superior biomass production by maintaining the tiller numbers, leaf width, and leaf elongation rate under phosphorus deficiency

Yasuhiro Tsujimoto^a, Mitsukazu Sakata^b, Viviane Raharinivo^c, Juan Pariasca Tanaka^a and Toshiyuki Takai^a

^aJapan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan; ^bFaculty of Agriculture and Marine Science, Kochi University, Nankoku, Japan; ^cDepartment of Rice Research, Centre National de Recherche Appliquée au Développement Rural (FOFIFA), Antananarivo, Madagascar

ABSTRACT

Shoot morphology in rice plants changes in response to P deficiency. However, how genotypic variations in these morphological changes affect the canopy development and biomass production have hardly been explored. The study aimed to identify specific shoot morphological traits that confer biomass production under P deficiency. Four rice genotypes, including AZ-97 (WAS 63-22-5-9-10-1), which exhibits high performance under highly P-deficient fields in Madagascar, were grown for 56 days in flooded pots over several P application rates to simulate P-sufficient and moderately, severely, and extremely P-deficient conditions. AZ-97 had superior shoot P contents and biomass than Takanari, a high-yielding cultivar, and X265, a common high-yielding cultivar in Madagascar at severely to moderately P-deficient conditions. Shoot biomass was highly correlated with projected leaf area (PLA) from the early growth stage, and tiller number, leaf width, and leaf elongation rate explained the variations in PLA. These morphological traits reduced significantly with decreased P application rates, while reduction in AZ-97 was small relative to the other genotypes, even for equivalent shoot P contents. As the result, AZ-97 had greater PLA per unit of shoot P content at equivalent shoot P contents. The result indicates that lower sensitivity and degrees of change in shoot morphology when exposed to P deficiency stress could be a key trait facilitating the maintenance of captured radiation and subsequently influencing genotypic differences in external P uptakes and biomass production. AZ-97 is a potential donor with such traits that can offer an additional avenue for genetic improvement toward P-efficient rice production.

Abbreviations DAT: days after transplanting; LN: leaf number in the main stem; PLA: projected green leaf area; PUE: phosphorus use efficiency; SSA: Sub-Saharan Africa.

ARTICLE HISTORY

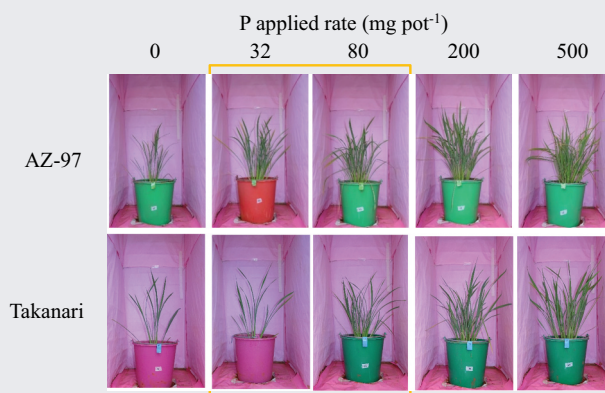
Received 20 March 2020

Revised 27 May 2020

Accepted 28 July 2020

KEYWORDS

Leaf elongation rate; leaf width; *Oryza Sativa*; P deficiency; projected leaf area; shoot morphology; tillering



Introduction

Existing rock phosphate reserves could be exhausted over the next 50–100 years and the quality of reserves

is declining with the increasing costs of extraction, processing, and shipping (Cordell et al., 2009). Considering the finite nature of P fertilizer resources, it is vital to investigate potential sustainable crop production

CONTACT Yasuhiro Tsujimoto ✉ tsjmt@affrc.go.jp

Supplemental data for this article can be accessed [here](#).

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

strategies that involve the efficient utilization of the supplied P and available P in soils. Such strategies are obviously required in high-input systems in developed countries where excess P fertilizer amounts applied in agricultural systems cause environmental challenges such as the eutrophication of lakes and marine estuaries (Vance et al., 2003). Moreover, efficient use of P is critical in low-input systems such as in rice production by small-holder farmers in Sub-Saharan Africa (SSA), where rice yield – despite the increasing demand of rice for food security – is restricted largely by P-deficient soils and inadequate P fertilizer inputs (Nziguheba et al., 2015; Saito et al., 2019; Tsujimoto et al., 2019).

Genetic improvement is one of the major strategies for the efficient utilization of the supplied P and available P in soils. For instance, the identification of a P starvation tolerance gene (*PSTOL1*), which enhances early root growth and promotes P uptake (Gamuyao et al., 2012), and the identification of certain genotypes with high root efficiency (P uptake per root surface area) (Mori et al., 2016) could facilitate the development of rice cultivars that produce high yields even under highly P-deficient soils with low rates of P fertilizer application. Wissuwa and Ae (2001) demonstrated that genotypic variations in P uptake in rice were largely explained by their root sizes when cultivated under P-deficient conditions. In addition, understanding root architectural traits, such as steep root angle (Uga et al., 2013), deep rooting (Obara et al., 2010), and shallow rooting (Uga et al., 2012), and the use of such quantitative trait loci (QTL) in breeding activities could also offer avenues for improving P-use efficiencies by enabling the cultivation of rice with root systems that are adapted to the P availability conditions in local soil profiles or localized P applications. Lynch and Brown (2001) reported the development of more surface roots as a typical plant response to P deficiency because most of the available P are accumulated in the topsoil. As such, the root system plays a primary role under P deficiency and has been studied extensively for rice genetic improvement to enhance P deficiency stress tolerance (Campos et al., 2018; Wissuwa, 2005).

In contrast, hardly any studies have explored above-ground morphological variations and adaptations of rice genotypes in response to P deficiency. Rice plants exhibit unique above-ground morphological changes under P deficiency stress, including reduced tillering, narrow leaves, erect leaves, and retarded leaf elongation rates (Dobermann & Fairhurst, 2000; Luquet et al., 2005). Such morphological changes are potentially linked to leaf area expansion and captured radiation, and, therefore, influence photosynthetic assimilation and biomass production under P-deficient conditions. The most well-

understood physiological mechanism in shoot morphological change in rice is an increase in strigolactones in root exudates, which plays a dual role under P deficiency; (i) inducing hyphal branching of arbuscular mycorrhizal (AM) fungi for exploiting available P in soils and (ii) inhibiting tiller bud outgrowth in rice seedlings (Umehara et al., 2010). Then, it is considered that assimilates that are no longer required for new tiller growth are partitioned to maintain root growth, which results in high root to shoot mass ratio as an initial plant response to P deficiency (e.g., Mollier & Pellerin, 1999; Wissuwa et al. 2005). However, none of the studies above explored genotypic variations and how the inhibition of tillering under P deficiency influence canopy development and biomass production. Therefore, understanding genotypic variations in the above-ground morphological characteristics and their correlation with biomass production under P-deficient conditions could offer an additional avenue for genetic improvement that could facilitate P-efficient rice production. We hypothesized that canopy development is an important trait to maintain the positive chain cycle among photosynthetic carbon assimilation, allocation of assimilates to the root system, and external P uptakes, which in turn lead to high biomass under P deficiency.

In the current study, we targeted two rice accessions that demonstrated the highest grain yields among 300 accessions tested under severely P-deficient fields in the central highlands of Madagascar in our preliminary on-farm trials. The two accessions were AZ-78 (FACAGRO 64::IRGC 82,059, *indica* type originating from India) and AZ-97 (WAS 63–22-5-9-10-1, *indica* type originating from Senegal). The set of 300 accessions were selected from 3000 rice accessions that cover a wide range of genotypes and are available publicly at the Rice SNP-Seek Database (<http://snp-seek.irri.org>) (Mansueto et al., 2017). We conducted pot experiments to 1) verify the superior performance, and 2) identify specific above-ground morphological changes/adaptations of the two varieties and their relationship with biomass production under various P-deficient conditions.

Materials and methods

Experiment design

Pot experiments were conducted in a screenhouse at the National Center for Applied Research on Rural Development (FOFIFA) at Antananarivo, Madagascar (18° 52'S, 47°33'E, 1310 m alt.). The screenhouse was approximately 4 m high and all sides were covered with mesh nets that ensured more or less ambient temperature and natural sunlight conditions. The daily mean temperature

inside the screenhouse was recorded by Thermo Recorder (TR-72 U, T&D Corporation, Nagano, Japan) and ranged from 20.0°C to 25.3°C throughout the experimental period.

The experiment soil was collected from a farmer's field (0–20 cm). The major physico-chemical properties are summarized in Table 1. Briefly, the experimental soil was clay loam with a pH of 5.4 and with extremely low amounts of P and cations. The amount of oxalate-extractable P – a suitable indicator of P availability for lowland rice production in the region (Rabeharisoa et al., 2012) – is merely 40.7 mg kg⁻¹, which is the lowest level among many lowland and upland rice fields in Madagascar (Kawamura et al., 2019).

After being air-dried and sieved, this extremely P-deficient soil was put into 5-L plastic pots (20 cm in height, 20 cm in diameter) with 4 kg of soil per pot. Afterward, five P treatments were established by applying NaH₂PO₄ · 2H₂O at rates of 0, 32, 80, 200, and 500 mg P pot⁻¹ (0P, 32P, 80P, 200P, and 500P, respectively, hereafter). The P application rates were determined based on the result of preliminary pot experiments that revealed a wide range of morphological changes in tiller number and leaf width by growing X265, a popular high-yielding variety in the region. The rates were approximately equivalent to 0, 10, 25, 64, and 159 kg P ha⁻¹, as estimated based on pot size. Nitrogen (N) and Potassium (K) were supplied adequately to all the pots using NH₄NO₃ and KCl, respectively, at the rates of 608 mg pot⁻¹ for both N and K, to avoid any potential influence of N and K deficiency on rice growth. All the nutrients were mixed uniformly with soils and puddled a day prior to transplantation.

Four genotypes were used – the aforementioned two *indica* varieties (AZ-78 and AZ-97), X265, and Takanari, a high-yield *indica* variety developed in Japan. Twenty-day-old seedlings of the four varieties grown in P-free

sand were transplanted one plant per hill and one hill per pot. Five levels of P treatments factorially combined with the four varieties were allocated in a randomized complete block design with six replicates and grown under continuously flooded conditions.

Measurements

Tiller number, leaf age or the number of leaves on the main stem (LN), leaf width of the open-top leaf, and projected green leaf area (PLA) in each pot were observed weekly from 19 days after transplanting (DAT). PLA was estimated using digital imagery analysis according to the method of Tsujimoto et al. (2016). Briefly, each pot was sequentially put into a 1.6 m-height carton box with a small lens hole on the top and one side open (the opposite side of the side illuminated by sun's rays) to avoid reflectance from direct sunlight, and then a digital photograph was taken virtually from the lens hole. The PLAs on individual images were determined using image processing software (Image J, NIH, USA). LN was determined by counting the number of leaves in the main stem starting from the incomplete (prophyll) leaf. If the top leaf had not expanded fully, LN was estimated based on the ratio of the length of the elongating top leaf to that of the preceding leaf.

At 56 DAT, shoot biomass was determined by sampling the whole plants at the soil surface and dried at 70°C for 3 days in a ventilated oven. Each shoot sample was ground to fine powder using a high-speed vibrating sample mill (T1–100, CMT Co. Ltd., Fukushima, Japan). Afterward, shoot P concentrations were determined using the molybdate blue method (Murphy & Riley, 1962) after dry-ashing at 550°C for 2 h and digesting with 0.5 M HCl. Shoot P content and internal PUE were determined as follows:

$$\text{Shoot P content (mg P pot}^{-1}\text{)} = \text{shoot biomass} \\ \times \text{shoot P} \\ \text{concentration}$$

$$\text{PUE (g biomass per mg P)} = \text{shoot biomass/shoot} \\ \text{P content}$$

Based on the synchronous leaf and tiller development theory – rice has the potential to produce one tiller on the *n*th node when the new leaf on *n* + 3th node emerges (Katayama, 1951) –, potential tiller number as a function of LN was calculated at 41 DAT using the following equation:

$$\text{Number of potential tiller} = 0.5 \times LN^2 - 4.5 \times LN \\ + 13 \quad LN > 4 \quad (1)$$

based on the assumptions that 1) the first tiller emerged from the 1st node on the main stem and 2) only primary and secondary tillers emerged.

Table 1. Soil properties of the pot experiments.

Parameter	Unit	
Clay ^a	%	30.7
Silt ^a	%	35.9
Sand ^a	%	33.5
pH (1:5 H ₂ O)	-	5.4
Total N ^b	g kg ⁻¹	1.5
Total C ^b	g kg ⁻¹	18.5
Oxalate-P ^c	mg kg ⁻¹	40.7
Available P ^d	mg kg ⁻¹	8.0
CEC ^e	c mol kg ⁻¹	7.4
Exchangeable Ca ^e	c mol kg ⁻¹	0.33
Exchangeable K ^e	c mol kg ⁻¹	0.17
Exchangeable Mg ^e	c mol kg ⁻¹	0.34
Exchangeable Na ^e	c mol kg ⁻¹	0.05

a: Sieving and pipetting method.

b: NC analyzer, Sumigraph NC-220F (SCAS, Tokyo, Japan).

c: Inductively coupled plasma mass spectrometer (ICPE-9000, Shimadzu, Japan) after oxalate extraction.

d: Bray-I extraction method.

e: Ammonium acetate extract method at pH 7.0.

Table 2. F value and level of significance in the GLM test.

	Shoot Biomass	P uptake	PUE	PLA at 41 DAT	Tiller No. at 41 DAT	LN at 41 DAT	Leaf width at 41 DAT
Genotype (G)	7.1***	14.4***	5.6**	13.6***	63.8***	83.9***	5.6**
P level (P)	437.6***	1792.9***	442.8***	534.7***	668.0***	196.5***	442.8***
G×P	3.6*	13.3***	3.1*	3.8*	3.7*	ns.	3.1*

***P<0.001, **P<0.01, *P<0.05, ns. not significant.

Further, we assessed the efficiency in canopy development under P deficiency by dividing PLA by shoot P content. We used the PLA at 41 DAT before the start of shoot elongation and leaf senescence.

Statistical analysis

JMP v14 software (SAS Institute Inc., Tokyo, Japan) was used to perform the statistical analyses. A generalized linear model (GLM) was used to determine the single and interaction effects of genotype (G) and P application rate (P) on the measured variables. The replicate (n = 6) was treated as a random factor. F-values from the GLM tests for each variable are summarized in Table 2. Afterward, mean values were compared separately for each P application rate using Tukey's honestly significant difference test at a 5% significance level. The linear regression coefficients of shoot P content on biomass (Δ Shoot biomass/ Δ shoot P content) or the P-response slope parameters with the intercept at 0 were compared among genotypes by the analysis of covariance. A stepwise regression analysis was performed to identify significant explanatory factors of PLA using LN, tiller number, leaf width, and total number of leaves as candidate factors. In the stepwise process, the 'selection' and 'removal' of factors were controlled with an F-value of P < 0.05. Thereafter, a multiple regression model was developed using the selected parameters.

Results

Genotype differences in shoot biomass production, shoot phosphorus content, and phosphorus-use efficiency under various phosphorus deficiency status

Shoot biomass ranged from 5.0 to 36.4 g pot⁻¹ at 56 DAT, with significant effects of and interaction between genotype and P application rates (Figure 1, S1 Fig). The various

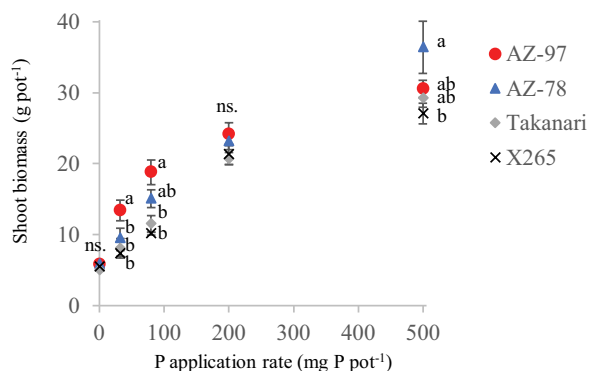


Figure 1. Genotype comparison in shoot biomass at 56 DAT at difference levels of P fertilizer applied.

P application rates in the present study produced diverse P-deficiency statuses. Shoot biomass in the P-sufficient condition (500P) more or less plateaued against increased shoot P content (Figure 2). In the moderately P-deficient condition (200P), biomass production was 63–79% of the rate of production under 500P and curved against increased shoot P content. Under the severely P-deficient conditions at 80P and 32P, the biomass production was 26–43% (61% for AZ-97 at 80P) of the production at 500P, and responded linearly to an increase in shoot P content. In the extremely P-deficient condition at 0P, biomass production was <20% of the production under 500P. No significant genotypic differences were observed at 0P. However, AZ-97 produced significantly greater biomass than the other three genotypes, by 41–83%, at 32P, and, significantly greater biomass than X265 and Takanari, by 63–85%, at 80P. Genotypic differences in biomass became less apparent at 200P, while AZ-97 retained slightly higher biomass than Takanari and X265. At the P-sufficient condition of 500P, AZ-78 produced the greatest biomass, which was significantly greater than that of X265 by 35%.

The linear regression coefficients of shoot P content on biomass (intercept = 0) at the low P application rates from

Table 3. Genotype comparison in shoot Phosphorus content at 56 DAT at different levels of P fertilizer applied.

Genotype	P application rate									
	0P		32P		80P		200P		500P	
AZ-97	5.4	a	14.0	a	20.8	a†	31.1	a	60.9	b
AZ-78	5.5	a	9.5	b	16.9	a†	35.5	a	76.9	a
Takanari	4.1	a	7.7	b	12.0	b	27.8	a	54.6	b
X265	4.4	a	5.9	b	10.5	b	31.1	a	56.2	b

Within each column, the same alphabets indicate no significant mean differences at P<0.05.

†Significant at P=0.10.

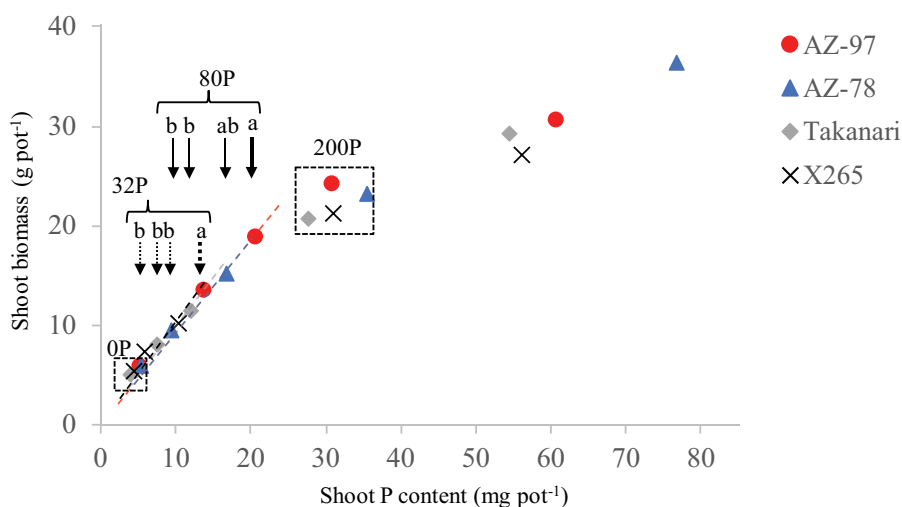


Figure 2. Relationship between shoot P content and shoot biomass at 56 DAT.

0P to 80P were 0.93 g mg^{-1} for AZ-97, 0.93 g mg^{-1} for AZ-97, 0.99 g mg^{-1} for Takanari, and 1.04 g mg^{-1} for AZ-97, showing no significant genotypic differences (Figure 2). The result indicates that greater biomass production of AZ-97 at 32P and 80P treatments was attributable primarily to the greater shoot P contents. The shoot P content of AZ-97 was significantly greater than the other three genotypes by 47–136% at 32P and by 73–99% than X265 and Takanari at 80P (Table 3).

It should be noted, however, that AZ-97 had equivalent PUE with the other genotypes despite its greater shoot P content at 80P, and it retained relatively high PUE at 200P (tended to be greater than AZ-78 and greater than X265 at $P = 0.09$), when shoot P content was not

significantly different among the genotypes (Figure 3(a), Table 3). Therefore, AZ-97 tended to have higher PUE than the other varieties when PUE was compared at similar shoot P contents, approximately 20–40 mg plant^{-1} , as estimated from the PUE reduction curves plotted against shoot P content (Figure 3(b)).

Genotype variations in morphological responses to different phosphorus deficiency status

Projected green leaf area (PLA) and PLA per unit of shoot P content

PLA was closely correlated with shoot biomass from the early growth days at 19 DAT irrespective of genotype

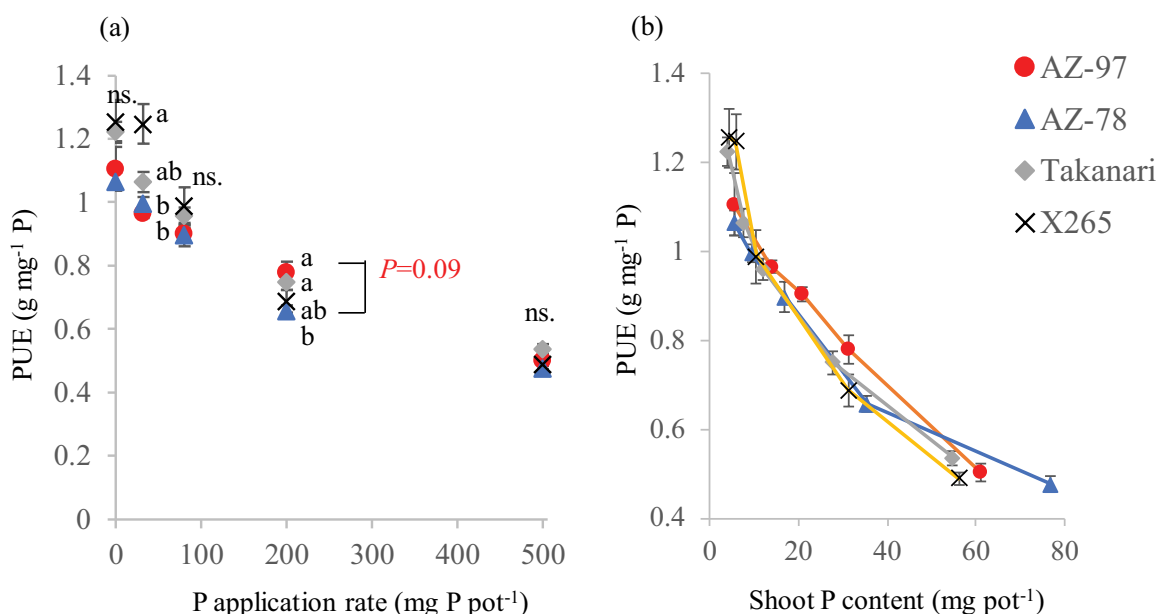


Figure 3. A comparison of PUE changes among genotypes against (a) P application rate (b) shoot P content.

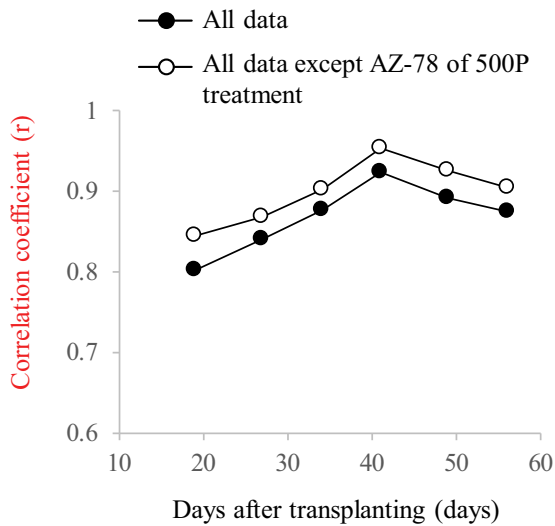


Figure 4. Changes in coefficient of determinations between PLA (projected green leaf area) and shoot biomass at different growing days after transplanting.

(Figure 4). AZ-78 at 500P tended to have relatively low PLA despite its high biomass production rate (potentially because AZ-78 had erect leaves as illustrated in S1 Fig.). When AZ-78 data at 500P were excluded, the correlation coefficient (r) in the simple linear regression between the PLA and shoot biomass ranged from 0.85 to 0.95 at different DATs, with the peak value at 41 DAT.

AZ-97 had significantly greater PLA, by 57–169% at 32P, and 46–102% at 80P, than the other three genotypes at 41 DAT (Figure 5). AZ-97 retained slightly greater PLA than X265 and AZ-78, by 8–11%, at 200P. In addition, AZ-97 had significantly greater PLA per unit of shoot P content at $13.3 \text{ cm}^2 \text{ mg}^{-1}$ than Takanari

($10.0 \text{ cm}^2 \text{ mg}^{-1}$) and AZ-97 ($10.8 \text{ cm}^2 \text{ mg}^{-1}$), and than X265 ($11.9 \text{ cm}^2 \text{ mg}^{-1}$) at the significance level of $P = 0.10$ when compared at equivalent shoot P contents at 200P. The greater PLA of AZ-97 under low P application rates from 32P and 200P were consistently observed from the early stages of plant growth (S2 Fig). Genotype differences were less apparent at 0P and 500P, excluding in the case of Takanari, which had consistently lower PLA than the other three genotypes.

Tiller number and leaf elongation rate

AZ-97 consistently produced higher tiller numbers than the other three genotypes under all the P application rates (Figure 6(a)). The maximum number of tillers in AZ-97 was 32.5 at 500P, which was 46–60% higher than the other genotypes. The number of tillers decreased gradually in all genotypes under lower P application rates while this reduction rate was relatively small for AZ-97. The proportion in the number of tillers relative to 500P for AZ-97 vs. other genotypes were 75% vs. 64–72% at 200P, 61% vs. 35–46% at 80P, and 46% vs. 27–34% at 32P (Figure 6(b)). Consequently, the differences in the number of tillers between AZ-97 and the other three genotypes were particularly large at 200P (24.2 vs. 14.2–16.2), at 80P (19.7 vs. 8.5–9.5), and at 32P (15.0 vs. 6.0–7.0). Higher tiller numbers in AZ-97, particularly from 32P to 200P, were observed consistently from the early plant growth stages (data not shown). Tiller number was highly restricted even in AZ-97 at 0P, and the differences among genotypes became less significant.

AZ-97 also demonstrated significantly more rapid leaf elongation rate or shorter phyllochrons than in the other three genotypes under all the P application rates (Figure 7

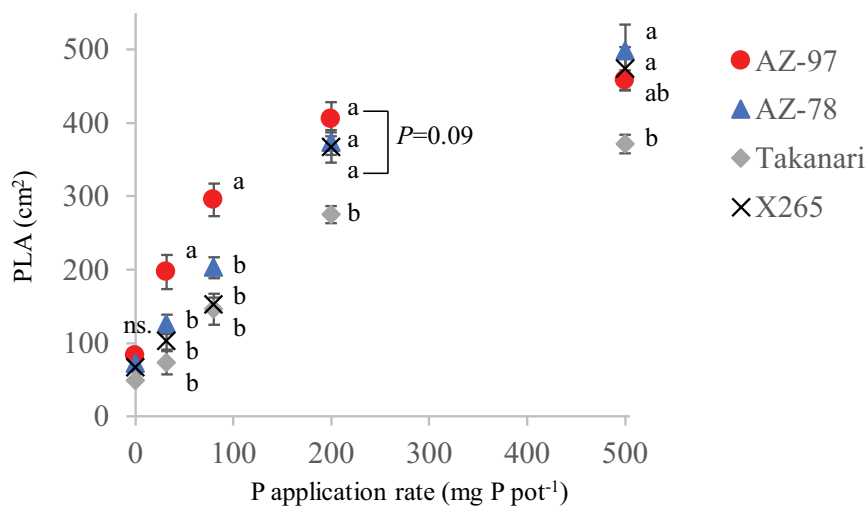


Figure 5. Genotype comparison in PLA (projected green leaf area) at 41 DAT at difference levels of P fertilizer applied.

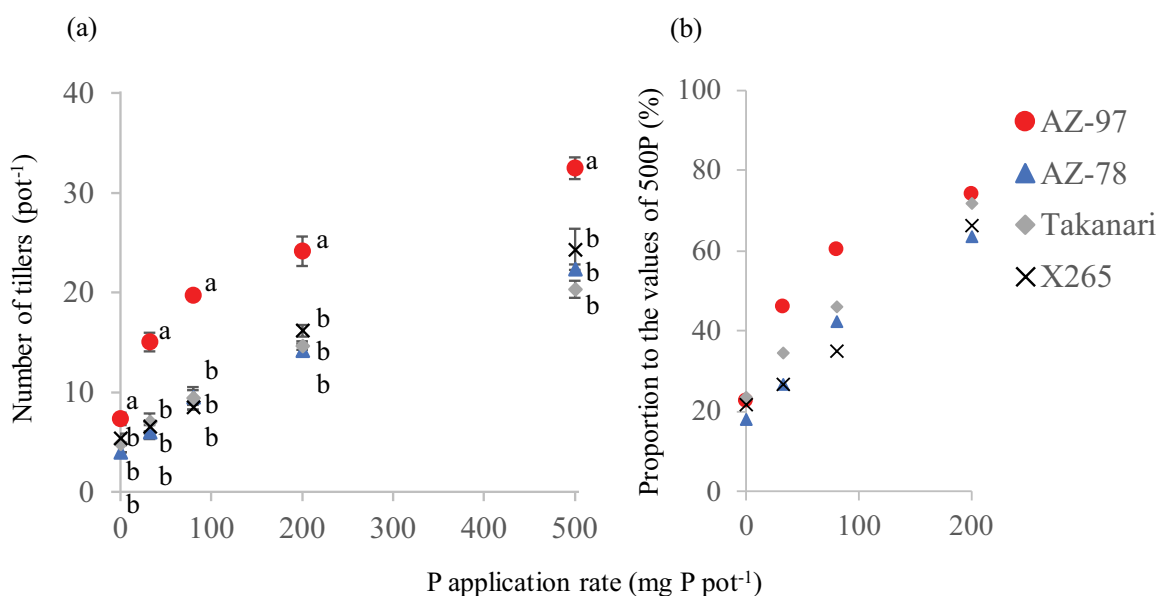


Figure 6. Genotype comparison in (a) the number of tillers at 41 DAT at difference levels of P fertilizer applied and (b) proportion in the number of tillers to the values of 500P treatment.

(a). The superior leaf elongation rate of AZ-97 was consistently observed from the early stages of plant growth (data not shown). More importantly, change in LN was low in AZ-97 relative to the other genotypes, while leaf elongation rate was gradually retarded with a decrease in P application rates in all the genotypes. The reductions in LN when compared with the case in the 500P treatment were 0.06 leaves for AZ-97 vs. 0.13–0.55 leaves for the other three genotypes at 200P, 0.37 vs. 0.93–1.00 leaves at 80P, 0.87 vs. 1.27–1.44 leaves at 32P, and 1.37 vs. 1.67–1.93 leaves at 0P (Figure 7(b)). Therefore, the differences

in LN between AZ-97 and the other genotypes were apparently large under P deficient conditions.

Figure 8 illustrates the relationship between LN and number of tillers for each genotype based on different P application rates, demonstrating that low P supply suppressed the number of tillers through (1) reduced potential tiller numbers as a result of slower leaf elongation rates (Equation 1) and (2) reduced number of developed tillers in comparison to the potential tiller number. For instance, the tiller number decreased from 24.3 at 500P to 5.3 at 0P, in the case of X265, which was related to the reduction in

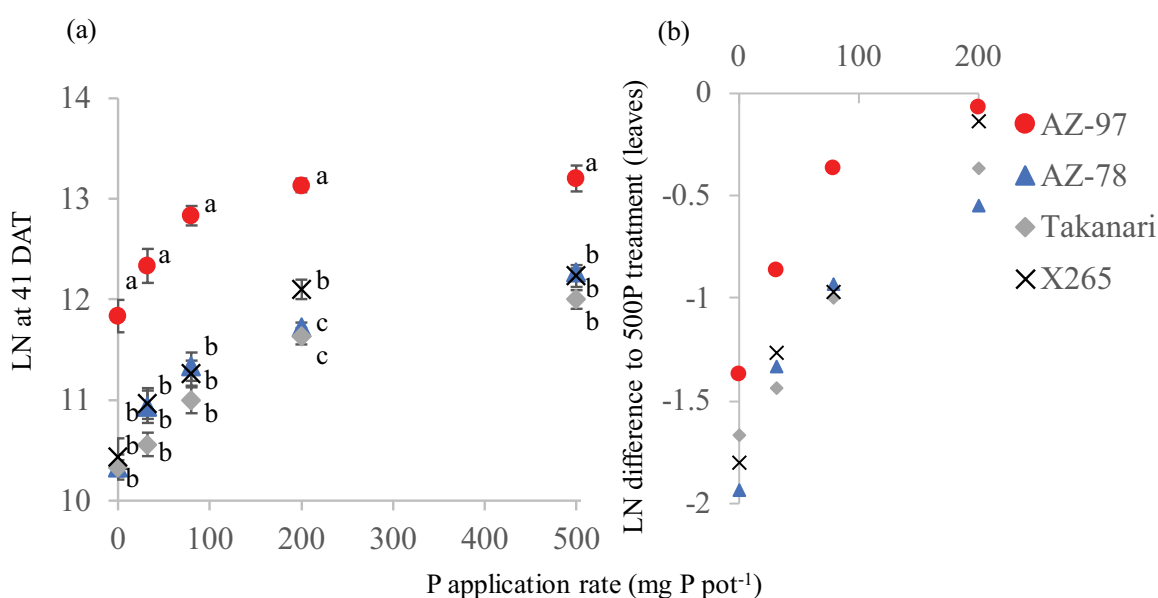


Figure 7. Genotype comparison in (a) LN (the number of leaves on the main stem) at 41 DAT at difference levels of P fertilizer applied and (b) the LN reduction against the 500P treatment.

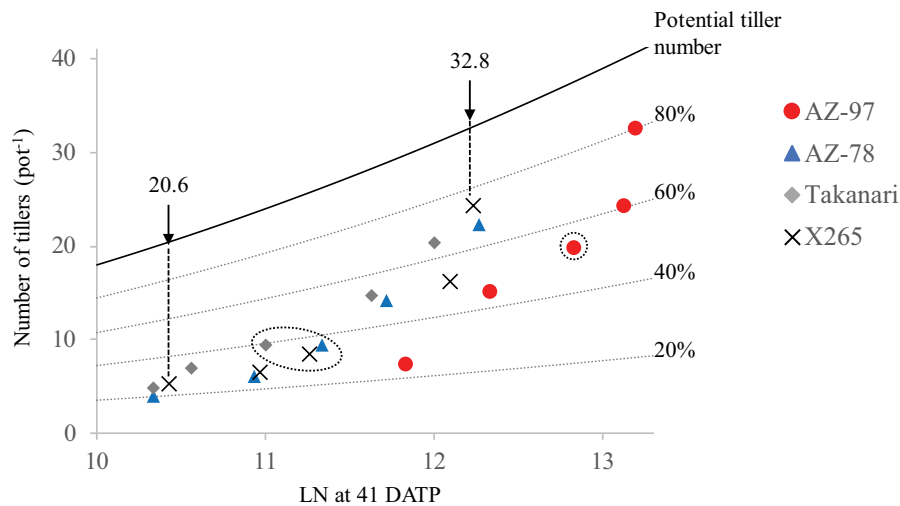


Figure 8. Relationship between the LN (number of leaves on the main stem) and the number of tillers at 41 DAT for each genotype by P application rate.

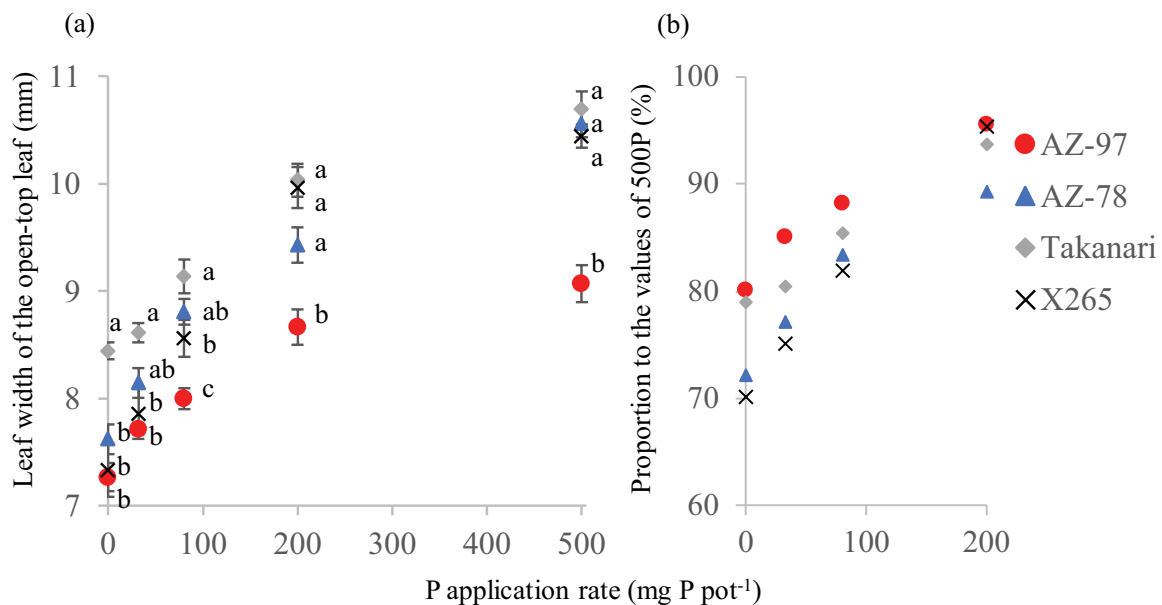


Figure 9. Genotype comparison in (a) the leaf width of the open-top leaf at the main stem at 41 DAT at difference levels of P fertilizer applied and (b) proportion in the leaf width to the values of 500P treatment.

potential tiller number from 32.8 to 20.6 due to lower LN and the concomitant reduction in the proportion of developed tillers against the potential numbers from 74% to 26%. The proportion of developed tillers against the potential tiller numbers reduced at lower P supply similarly for the other genotypes. However, this proportion remained relatively high for AZ-97 vs. the other genotypes for all the P application rates excluding 0P; 80% vs. 66–74% at 500P, 60% vs. 49–52% at 200P, 52% vs. 33–39% at 80P, and 45% vs. 25–33% at 32P. At 0P, the proportion was similarly restricted to 20% across all the genotypes while the AZ-97

still had slightly higher tiller number due to the greater LN, by 1.4–1.5 leaves, than the other three genotypes.

Leaf width

Contrary to the observed tiller number, AZ-97 had narrower leaves as measured from the open-top leaf in the main stem, while there was a significant interaction between genotype and P application rate (Table 2). The leaf width of AZ-97 was narrower than those of the other three genotypes at 500P by 1.4–1.6 mm (Figure 9(a)). The difference in leaf width became less considerable with

a decrease in P application rates. In particular, the leaf width in AZ-78 and X265 decreased sharply at the lower P application rates. The leaf widths at 0P and 32P were 70–72% and 75–77% relative to the leaf widths at 500P, respectively, for both AZ-78 and X265, whereas these values were retained at 86% and 88%, respectively, for AZ-97 (Figure 9(b)). As a result, there were no significant differences in leaf width among AZ-97, AZ-78, and X265 in the 0P and 32P treatments. Takanari had wide leaves consistently relative to the other genotypes under all the P rates.

A multiple regression model after a stepwise selection confirmed that the number of tillers ($P < 0.001$), leaf width ($P < 0.001$), and LN ($P < 0.05$) were closely and positively correlated with PLA and, in turn, with shoot biomass, irrespective of genotype or P application rate. The standardized partial regression coefficients indicated that the number of tillers, leaf width, and LN at 41 DAT explained 55%, 32%, and 13% of the variations in PLA, respectively, with the determinant coefficient (R^2 after being df adjusted) of the regression model at 0.91.

Discussion

Superior growth of AZ-97 on severe to moderate P-deficiency stresses

AZ-97 had superior shoot biomass production relative to the common high-yielding *indica* cultivars, i.e., Takanari and X265, under severely to moderately P-stressed conditions (32P, 80P, 200P). The varieties had equivalent productivity when P was fully supplied (500P) (Figure 1), implying that the genotype variations in treatments with lower P supply rates originated from their different responses to P deficiency stress.

With regard to P uptake and PUE, the superior shoot biomass production in AZ-97 was attributed primarily to greater shoot P contents in response to the low amounts of P applied (Figure 2) and secondly to the maintenance of relatively high PUE even when the shoot P contents increased (Figure 3). Such AZ-97 traits with both high P acquisition capacity and high efficiency in converting acquired P into shoot biomass are highly relevant to rice breeding under P deficiency stress (Wang et al., 2010). Rose et al. (2015) highlighted the importance of comparing PUE among genotypes at equal shoot P contents to avoid the risk of screening false genotypes (or related QTLs) whose PUE are apparently high but only because the genotypes have low shoot P contents – see X265 at 0P and 32P in Figure 3(a). Similarly, excess allocation of assimilates to root systems for P acquisition can be a cost and a trade-off against efficient shoot biomass

production (Wissuwa et al., 2009). AZ-97 may have prospective traits to maintain a balance between external P acquisition and shoot biomass production following exposure to P deficiency stress. Notably, there were significant genotypic differences neither in shoot P contents nor in shoot biomass at 0P, which could be because the genetic traits related to P acquisition capacity or PUE are hardly exerted not only when available P is abundant but also when available P is extremely low in soils.

Key morphological traits of AZ-97 facilitating superior growth under phosphorus deficiency stress

PLA or canopy coverage are key parameters that influence the amount of radiation intercepted by a canopy, and, in turn, biomass production (Monteith, 1977). The present study confirmed a close correlation between PLA from early growth stages and subsequent shoot biomass production, irrespective of genotype or P application rate (Figure 4). The result indicated that rapid canopy development or the maintenance of high leaf area following exposure to P deficiency conferred an advantage to biomass production. In this regard, AZ-97 had a trait to retain the leaf expansion even when the plants were exposed to the same level of P deficiency status (superior PLA per unit of shoot P content at 200P). In addition, shoot morphological observations revealed that the variation in PLA can be mostly explained by the tiller number, leaf width, and LN, which is anticipated because tiller number and LN influence the number of leaves per plant, and single leaf size is largely dependent on the changes in leaf width while P deficiency does not much influence leaf length of rice (Luquet et al., 2005).

At the P-sufficient condition (500P), no genotypic differences in PLA were observed. This is probably because an advantage of AZ-97 having a large number of tillers and LN could be counteracted by its significantly narrower leaves. However, AZ-97 gradually achieved a major advantage in PLA relative to the other genotypes under lower P application rates as AZ-97 had less significant changes in all of the parameters. The trends in morphological changes across the genotypes under P deficiency – AZ-97 exhibited lower degrees of change – were already observed at 200P, at which the final shoot P contents were more or less equivalent among genotypes. The result implied that AZ-97 tended to retain shoot morphology, and in turn, canopy coverage relative to the other genotypes even when exposed to the same level of P deficiency status.

According to Umehara et al. (2010), the inhibition of the production of new tillers is a potential adaptive strategy in rice for saving the limited P resources for

existing tillers. Conversely, our results implied that ‘too sensitive shoot morphological changes’ may not be advantageous for biomass production, at least under moderate to severe P-deficient conditions.

Interrelationship among canopy development, phosphorus uptake and biomass production

Less significant changes in shoot morphology of AZ-97 might be an advantageous trait to eventually produce large biomass under P deficiency by retaining captured radiation, consistent supply of carbon assimilates to the root system, and external P uptake. It is generally understood there is a close interaction among PLA or leaf area expansion, root growth, and external P uptakes via photosynthetic assimilation and allocation of assimilates into the root system. Such interactions could gradually amplify genotypic differences in P uptakes and biomass production. For instance, Mollier and Pellerin (1999) reported that P deficiency first reduces leaf expansion to maintain root growth, and the morphological response subsequently inhibits root production due to smaller leaf area and inadequate carbohydrate supply. This study indicates that excess allocation of assimilates to roots at a cost of leaf expansion may eventually cause a negative effect on biomass production under P deficiency. Wissuwa (2005) demonstrated that P deficiency-tolerant genotypes in rice maintained root to shoot ratios even under P stress conditions, while intolerant genotypes disproportionately increased root to shoot ratio. Less sensitive shoot morphological responses to P deficiency as observed in AZ-97 could be partly linked to such genotypic variations in P-deficiency tolerance.

In addition, Luquet et al. (2005) noted that rapid canopy development should be advantageous in fields by facilitating the acquisition of limited nutrient resources in the presence of weed competitors. Saito et al. (2015) also pointed out that rapid initial growth is an important trait for rice breeding in P-deficient and low-input production systems in SSA. Such aspects could also be related to the results of our preliminary field trial in which AZ-97 had the highest yield among 300 accessions under low-input and poor-nutrient soils in Madagascar. In summary, since AZ-97 exhibited superior shoot biomass production, it is a potential donor for traits such as high P acquisition capacity and high leaf expansion capacity from early growth stages based on less significant morphological changes in the number of tillers, leaf width, and leaf elongation rate, even under limited P fertilizer input amounts under severely P-deficient soils.

Acknowledgments

The authors are grateful to Dr Tomohiro Nishigaki and Ms Mayumi Yonemura for chemical analysis of soils and plants, and Ms Mihoko Enami for imagery analysis. The authors thank Dr Matthias Wissuwa, Japan International Research Center for Agricultural Sciences, for providing us with valuable comments and suggestions to improve the draft of this manuscript.

Disclosure statement

The authors declare no conflict of interest in this paper.

Funding

This research was funded by the JIRCAS research program ‘Development of sustainable agricultural productivity and improve food security in Africa’ and by the Science and Technology Research Partnership for Sustainable Development (SATREPS), Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA) (Grant No. JPMJSA1608).

ORCID

Yasuhiro Tsujimoto  <http://orcid.org/0000-0001-7738-9913>
 Juan Pariasca Tanaka  <http://orcid.org/0000-0002-9488-4729>
 Toshiyuki Takai  <http://orcid.org/0000-0002-6498-610X>

References

- Campos, P., Borie, F., Cornejo, P., Lopez-Raez, J. A., Lopez-Garcia, A., & Seguel, A. (2018). Phosphorus acquisition efficiency related to root traits: is mycorrhizal symbiosis a key factor to wheat and barley cropping? *Frontiers in Plant Science*, 9, 752. <https://doi.org/10.3389/fpls.2018.00752>
- Cordell, D., Drangert, J.-O., & White, S. (2009). The story of phosphorus: Global food security and food for thought. *Global Environmental Change*, 19(2), 292–305. <https://doi.org/10.1016/j.gloenvcha.2008.10.009>
- Dobermann, A., & Fairhurst, T. H. (2000). *Rice: Nutrient disorders & nutrient management*. Potash & Phosphate Institute (PPI), Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute (IRRI).
- Gamuyao, R., Chin, J. H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., Slamet-Loedin, I., Tecson-Mendoza, E. M., Wissuwa, M., & Heuer, S. (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature*, 488, 535–539. <https://doi.org/10.1038/nature11346>
- Katayama, T. 1951. *A study on tillering systems in rice and wheat*. Yokendo Ltd. (in Japanese).
- Kawamura, K., Tsujimoto, Y., Nishigaki, T., Andriamananjara, A., Rabenarivo, M., Asai, H., Rakotoson, T., & Razafimbelo, T. (2019). Laboratory visible and near-infrared spectroscopy with genetic algorithm-based partial least squares regression for assessing the soil phosphorus content of upland and lowland rice fields in madagascar. *Remote Sensing*, 11(5), 506. <https://doi.org/10.3390/rs11050506>

- Luquet, D., Zhang, B. G., Dingkuhn, M., Dexet, A., & Clément-Vidal, A. (2005). Phenotypic plasticity of rice seedlings: Case of phosphorus deficiency. *Plant Production Science*, 8(2), 145–151. <https://doi.org/10.1626/pps.8.145>
- Lynch, J. P., & Brown, K. M. (2001). Topsoil foraging – An architectural adaptation of plants to low phosphorus availability. *Plant and Soil*, 237, 225–237. <https://doi.org/10.1023/A:1013324727040>
- Mansueto, L., Fuentes, R. R., Borja, F. N., Detras, J., Abriol-Santos, J. M., Chebotarov, D., Sanciango, M., Palis, K., Copetti, D., Poliakov, A., Dubchak, I., Solovyev, V., Wing, R. A., Hamilton, R. S., Mauleon, R., McNally, K. L., & Alexandrov, N. (2017). Rice SNP-seek database update: New SNPs, indels, and queries. *Nucleic Acids Research*, 45(D1), 1075–1081. <https://doi.org/10.1093/nar/gkw1135>
- Mollier, A., & Pellerin, S. (1999). Maize root system growth and development as influenced by phosphorus deficiency. *Journal of Experimental Botany*, 50(333), 487–497. <https://doi.org/10.1093/jxb/50.333.487>
- Monteith, J. L. (1977). Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 281, 277–294. <https://doi.org/10.1098/rstb.1977.0140>
- Mori, A., Fukuda, T., Vejchasarn, P., Nestler, J., Pariasca-Tanaka, J., & Wissuwa, M. (2016). The role of root size versus root efficiency in phosphorus acquisition in rice. *Journal of Experimental Botany*, 67(4), 1179–1189. <https://doi.org/10.1093/jxb/erv557>
- Murphy, J., & Riley, J. P. (1962). A modified single method for the determination of phosphates in natural waters. *Analytica Chimica Acta*, 27, 31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- Nziguheba, G., Zingore, S., Kihara, J., Merckx, R., Njoroge, S., Otinga, A., Vandamme, E., & Vanlauwe, B. (2015). Phosphorus in smallholder farming systems of sub-Saharan Africa: Implications for agricultural intensification. *Nutrient Cycling in Agroecosystems*, 104, 321–340. <https://doi.org/10.1007/s10705-015-9729-y>
- Obara, M., Tamura, W., Ebitani, T., Yano, M., Sato, T., & Yamaya, T. (2010). Fine-mapping of qRL6.1, a major QTL for root length of rice seedlings grown under a wide range of NH₄(+) concentrations in hydroponic conditions. *Theoretical and Applied Genetics*, 121(3), 535–547. <https://doi.org/10.1007/s00122-010-1328-3>
- Rabeharisoa, L., Razanakoto, O. R., Razafimanantsoa, M. P., Rakotoson, T., Amery, F., & Smolders, E. (2012). Larger bio-availability of soil phosphorus for irrigated rice compared with rainfed rice in Madagascar: Results from a soil and plant survey. *Soil Use and Management*, 28, 448–456. <https://doi.org/10.1111/j.1475-2743.2012.00444.x>
- Rose, T. J., Mori, A., Julia, C. C., & Wissuwa, M. (2015). Screening for internal phosphorus utilisation efficiency: Comparison of genotypes at equal shoot P content is critical. *Plant and Soil*, 401, 79–91. <https://doi.org/10.1007/s11104-015-2565-7>
- Saito, K., Vandamme, E., Johnson, J.-M., Tanaka, A., Senthilkumar, K., Dieng, I., Akakpo, C., Gbaguidi, F., Segda, Z., Bassoro, I., Lamare, D., Gbakatchetche, H., Abera, B. B., Jaiteh, F., Bam, R. K., Dogbe, W., Sékou, K., Rabeson, R., Kamissoko, N., & Wopereis, M. C. S. (2019). Yield-limiting macronutrients for rice in sub-Saharan Africa. *Geoderma*, 338, 546–554. <https://doi.org/10.1016/j.geoderma.2018.11.036>
- Saito, K., Vandamme, E., Segda, Z., Fofana, M., & Ahouanton, K. (2015). A screening protocol for vegetative-stage tolerance to phosphorus deficiency in upland rice. *Crop Science*, 55, 1223–1229. <https://doi.org/10.2135/cropsci2014.07.0521>
- Tsujimoto, Y., Pedro, J. A., Boina, G., Murracama, M. V., Tobita, S., Oya, T., Nakamura, S., Cuambe, C. E., & Martinho, C. (2016). An application of digital imagery analysis to understand the effect of N application on light interception, radiation use efficiency, and grain yield of maize under various agro-environments in Northern Mozambique. *Plant Production Science*, 20(1), 12–23. <https://doi.org/10.1080/1343943X.2016.1240013>
- Tsujimoto, Y., Rakotoson, T., Tanaka, A., & Saito, K. (2019). Challenges and opportunities for improving N use efficiency for rice production in sub-Saharan Africa. *Plant Production Science*, 22(4), 413–427. <https://doi.org/10.1080/1343943X.2019.1617638>
- Uga, Y., Hanzawa, E., Nagai, S., Sasaki, K., Yano, M., & Sato, T. (2012). Identification of qSOR1, a major rice QTL involved in soil-surface rooting in paddy fields. *Theoretical and Applied Genetics*, 124(1), 75–86. <https://doi.org/10.1007/s00122-011-1688-3>
- Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y., Wu, J., Matsumoto, T., Takai, T., Okuno, K., & Yano, M. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*, 45(9), 1097–1102. <https://doi.org/10.1038/ng.2725>
- Umehara, M., Hanada, A., Magome, H., Takeda-Kamiya, N., & Yamaguchi, S. (2010). Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. *Plant and Cell Physiology*, 51(7), 1118–1126. <https://doi.org/10.1093/pcp/pcq084>
- Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157, 423–447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>
- Wang, X., Shen, J., & Liao, H. (2010). Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? *Plant Science*, 179(4), 302–306. <https://doi.org/10.1016/j.plantsci.2010.06.007>
- Wissuwa, M. (2005). Combining a modelling with a genetic approach in establishing associations between genetic and physiological effects in relation to phosphorus uptake. *Plant and Soil*, 269, 57–68. <https://doi.org/10.1007/s11104-004-2026-1>
- Wissuwa, M., & Ae, N. (2001). Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding*, 120(1), 43–48. <https://doi.org/10.1046/j.1439-0523.2001.00561.x>
- Wissuwa, M., Gamat, G., & Ismail, A. M. (2005). Is root growth under phosphorus deficiency affected by source or sink limitations? *Journal of Experimental Botany*, 56(417), 1943–1950. <https://doi.org/10.1093/jxb/eri189>
- Wissuwa, M., Mazzola, M., & Picard, C. (2009). Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil*, 321, 409–430. <https://doi.org/10.1007/s11104-008-9693-2>