



Field Report

# Evaluation of a Newly Identified Mutation Gene that Promotes Root Elongation for Improvement of Drought Avoidance in Rice

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**Abstract.** Drought is one of the most serious abiotic stresses for rice cultivation, even under a water saving production system such as the Alternate Wetting and Drying (AWD) system and upland irrigated fields. In this study, we evaluated the performance of larger root systems in a KM07 mutant under such water management conditions. This mutant line was utilized for breeding and development of new rice germplasm adaptable to drought stress conditions in Mwea, Kenya, by crossing it with the recurrent parent of New Rice for Africa (NERICA), WAB56-104. Three mutant-type F<sub>6</sub> lines grown under AWD and upland irrigated conditions showed different responses to drought stress. The mutant type's greater root growth was observed in lines 2 and 3 under AWD and upland irrigation conditions, respectively. These results suggest that the highly developed root system derived from the KM07 mutant can avoid drought stress under field conditions in Kenya, and it should be utilized as breeding material for drought stress avoidance, along with water management technology.

**Key words:** Kenya, rice, root system, drought, water saving technology

## Introduction

Rice is the staple food for more than half of the world's population. Rice is one of the most important crops, not only for its calories, but also as an economic commodity, particularly in many developing countries<sup>1)</sup>. Rice consumption among those countries is growing rapidly, especially in sub-Saharan Africa (SSA) where it is the fastest growing staple food<sup>2)</sup>. However, the productivity of rice in SSA is less than half that of the world's average, and more than 60% of the total rice consumed there is imported, mainly from Asia<sup>3)</sup>.

Drought caused by limited water resource is considered a major constraint to both upland and rainfed lowland rice production in SSA<sup>4)</sup>. Therefore, in order to address this challenge and use the limited water resource more efficiently, water management practices are necessary. A water saving technology, known as 'Alternate Wetting and Drying' (AWD), has been developed. In the AWD system, rice is grown without continuously waterlogged (CWL) in the field, and irrigation water is applied to obtain flooded conditions after a certain number of days have passed since the disappearance of ponded water<sup>5)</sup>. AWD was implemented after several studies showed that

it reduced irrigation water input by up to 38%<sup>6, 7)</sup>. Another newly developed method is the aerobic or upland irrigated method, where adapted rice varieties are grown as normal field crops with or without supplementary irrigation in well-drained, non-puddled, and non-saturated soils. Water-saving techniques, however, risk yield reduction because plants may experience drought stress. By analyzing the data from in total 31 field experiments, it is reported that 92% of the AWD treatments resulted in yield reductions varying from just above 0% to 70% compared with flooded control plots<sup>5)</sup>. Therefore, in order to minimize drought stress without decreasing yield under water saving condition, water input needs to be optimized and rice varieties possessing drought tolerance or drought avoidance mechanisms should be selected. Adaptability of root systems to drought stress have been studied intensively under various water management systems, including AWD, aerobic conditions, different soil moisture conditions, and fluctuating soil moisture conditions<sup>8-16)</sup>. For adaptation to these stress environments, one of the most important root attributes is to maintain total root length under water deficient and rewatering conditions<sup>9, 17)</sup>. These adaptations resulted in increased water uptake and improved shoot dry matter production<sup>13, 18)</sup>.

In this study, we identified a rice mutant line, KM07, that has a highly-developed root system under hydroponic conditions at the seedling stage. In order to evaluate the possibility of using these mutant root traits for avoiding drought stress under field conditions in Mwea, Kenya, we prepared F<sub>6</sub> lines derived from the cross between KM07 mutant and WAB56-104, an upland improved variety and parent of NERICA 1 to NERICA 11, for improving rice productivity in Africa.

## Materials and Methods

### Plant materials

Rice root mutant line KM07 and its parental variety, Kimmaze (*Oryza sativa* L.) were utilized for pot experiments. Three F<sub>6</sub> lines derived from the cross between the KM07 mutant and WAB56-104 were evaluated in the field. Each F<sub>6</sub> line was classified into normal root phenotype (Normal-type) and mutant root phenotype (Mutant-type).

### Pot experiment

#### Growth conditions

Kimmaze and the KM07 mutant were evaluated in pot experiments conducted in a glasshouse at Nagoya University, Nagoya, Japan (35°6'42"N, 137°4'57"E) in 2017. Kimmaze and KM07 mutant seeds were sterilized by soaking in water containing benomyl fungicide (2.5 g L<sup>-1</sup>) in a growth chamber at 28°C under continuous light for 4

d. Three pre-germinated seeds each of Kimmaze and the KM07 mutant were sown in a pot (20 cm in height and 16 cm in diameter) filled with 4.0 kg of air-dried sandy loam soil analyzed in our previous paper<sup>19)</sup>. The soil was mixed with fertilizer containing 120 mg nitrogen, 120 mg phosphorus, and 120 mg potassium. The seedlings in each pot were thinned to one at 10 days after sowing (DAS). Kimmaze and the KM07 mutant were grown under CWL conditions.

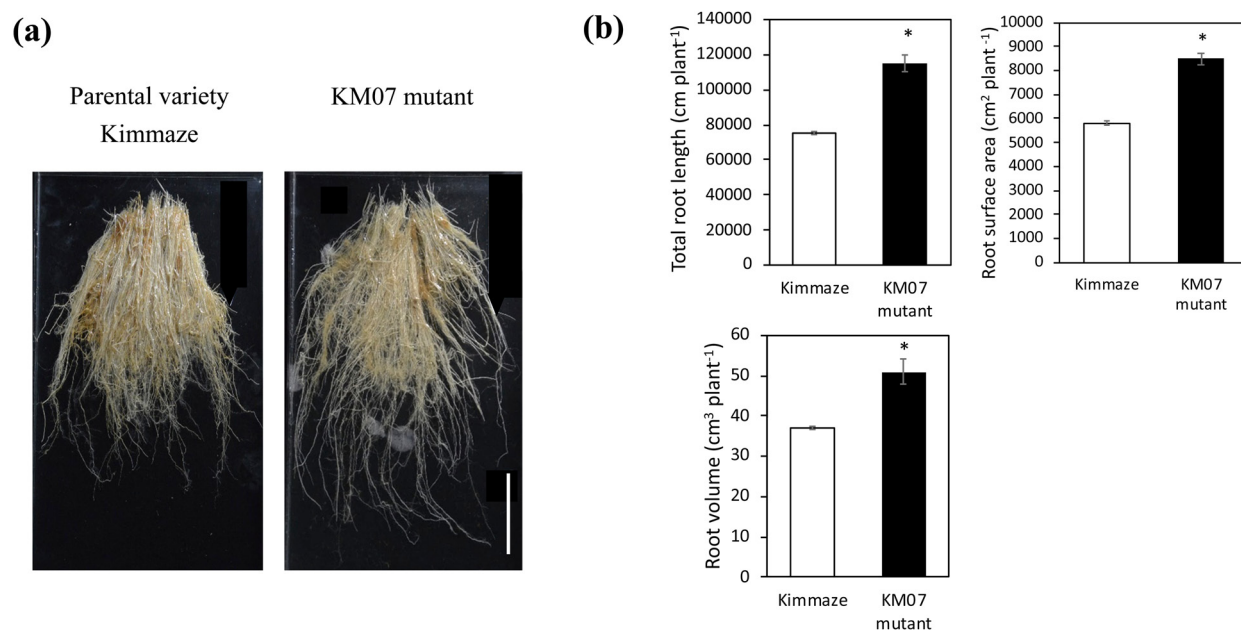
### Root sampling and measurement

Roots were collected from three plants of each genotype at 60 DAS. Roots were washed under running water to remove soil. Cleaned roots were spread on the acrylic tray filled with water without overlapping. Digital images were taken using an Epson scanner (ES-10000G) and then saved as tiff files (6600 × 4879 pixels). Each root trait was analyzed using WinRHIZO software v. 2013 (Regent Instruments, Quebec, Canada).

### Field experiment

#### Growth condition

Three F<sub>6</sub> lines were evaluated in a field experiment conducted at Kenya Agriculture and Livestock Research Organization-Mwea Centre, Kenya (KALRO-Mwea Research Farm (0°40'35"S, 37°18'06"E) from April to September 2017. The soil of the experimental field was Nitisol analyzed in our previous paper<sup>20)</sup>. Plant materials were grown under CWL, AWD, and upland irrigated field conditions (Fig. 3b, c). For the upland irrigated field condition, three seeds were directly sown per hill on April 12, 2017 and then the seedlings in each hill were thinned to one at 10 DAS. Pre-germinated seeds were sown in seedling trays on April 12, 2017 for CWL and AWD treatment, and then seedlings were transplanted in the field on May 3, 2017. As shown in Fig. 3a, seeds or seedlings were grown in seven rows, seven plants per row at a spacing of 20 cm × 20 cm between plants. Each line and genotype had three replicates. The AWD field was maintained in a flooded condition for 21 days after transplanting (DAT), and then re-irrigated up to 5 cm depth from the soil surface whenever the water depth reached 20 cm from the soil surface. Water depth in the AWD field was measured using a water tube buried into the soil. The upland irrigated field was kept wet but not flooded by applying water using a sprinkler until harvest. Basal fertilizer was applied at a per-hectare rate of 25 kg N, 25 kg P<sub>2</sub>O<sub>5</sub>, and 25 kg K<sub>2</sub>O at the time of transplanting. Top-dressing fertilizer was applied at a per-hectare rate of 25 kg N at 21, 45, and 75 DAT (42, 66, and 96 DAS in the upland irrigated field, respectively).



**Fig. 1.** Root system profiles (a) and root traits (b) of parental variety (Kimmaze) and KM07 mutant plants at 60 days after transplanting under continuously waterlogged (CWL) conditions. Bar = 10 cm.

\*, significant at  $P < 0.05$  between the two genotypes.

#### Root sampling and measurement

Roots were collected from four plants of each line and genotype at 125 DAT. Before root sampling, shoot parts were cut at 5 cm above the ground surface. A metal tube (30 cm in height and 20 cm in diameter) was inserted into the soil up to a depth of 20 cm and then pulled out to sample a core of soil and roots. The root samples from the core were washed with running water to remove soil. Each root trait was measured by the same methods used in the pot experiment.

## Results

#### Root development of the KM07 mutant

The differences in root development between the KM07 mutant and Kimmaze were evaluated in the pot experiment at the vegetative stage. The roots of the KM07 mutant were found to be distributed wider and deeper than those of Kimmaze (Fig. 1a). As shown in Fig. 1b, under CWL conditions, total root length (TRL), root surface area (SA) and root volume (RV) in the mutant were 53%, 47% and 38% higher than those in the Kimmaze, respectively.

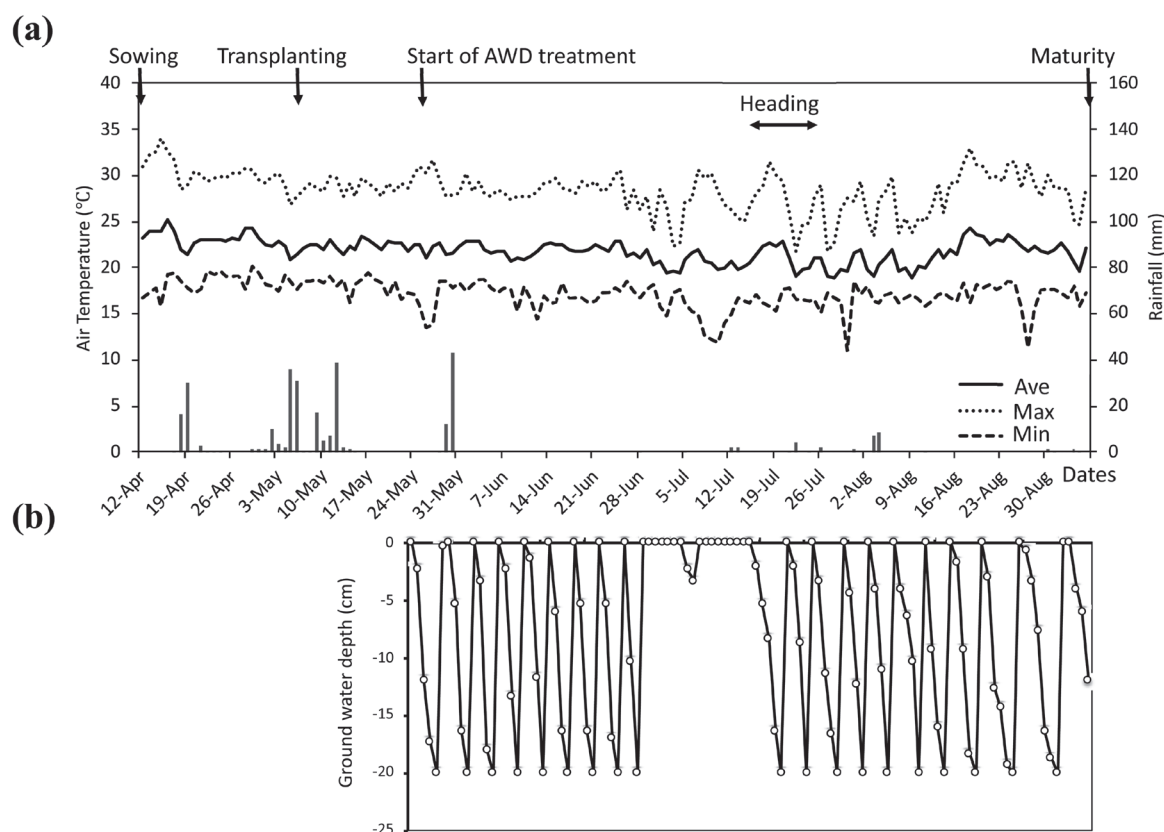
#### Water depth in AWD field and weather conditions at the experimental site

In the AWD field, the cycles of wetting and drying were repeated 19 times until harvesting (Fig. 2b). The air temperature reached below 15°C three times from the booting stage to the maturity stage (Fig. 2a), and the cold damage

resulted in severe sterility in both genotypes (Fig. 3d).

#### Root phenotypic evaluation

Each root trait was analyzed using digital images in WinRHIZO (Table 1). The rice root system typically consists of two types of roots, nodal roots (NR) formed at lower stem nodes and lateral roots (LR) derived from the nodal roots. In this study, the root components, NR and LR, were classified based on their diameter (D.). NR and LR were included when their diameters were  $D. > 0.3$  mm and  $D. \leq 0.3$  mm, respectively. LR was divided into three ranges, thin LR, middle LR and thick LR when diameters were  $0 \text{ mm} < D. \leq 0.080$  mm,  $0.080 \text{ mm} < D. \leq 0.15$  mm and  $0.15 \text{ mm} < D. \leq 0.30$  mm, respectively<sup>21</sup>). Under CWL condition, there was a tendency that Mutant-type plants showed slightly higher root development than that of Normal-type plants among all three F<sub>6</sub> lines, however, in all root traits there were no significant differences between Normal-type and Mutant-type plants. Meanwhile, the effects of water treatment in AWD and upland irrigated on root development were significant between the two genotypes. Under AWD conditions, only Mutant-type plants of line 2 maintained significantly higher TRL, SA, RV, total nodal root length (TNRL), total lateral root length (TLRL) and thin TLRL compared with Normal-type plants. Under upland irrigated conditions, only Mutant-type plants of line 3 maintained significantly higher TRL, TLRL, and lateral root length (LRL) at each diameter compared with Normal-type plants. As a result, Mutant-type plants of line



**Fig. 2.** Air temperature and rainfall in Mwea, Kenya (a) and ground water levels under alternate wetting and drying (AWD) field (b) in 2017. Max, Ave, and Min represent maximum, average, and minimum temperature, respectively.

2 in AWD conditions and Mutant-type plants of line 3 in upland irrigated conditions had significantly higher developed root systems, but Mutant-type plants of line 1 did not have significant higher root growth under either AWD or upland irrigated conditions compared with Normal-type plants of line 1 grown under both conditions.

## Discussion

In this study, we evaluated the performance of root systems observed from the KM07 mutant under different water management conditions, including CWL, AWD, and upland irrigated conditions in Mwea, Kenya. Under AWD and upland irrigated conditions, Mutant-type plants developed larger root system with increased root length compared with those in Normal-type plants, suggesting that Mutant-type plants responded to the water deficit and rewatering more strongly, thereby maintaining a highly developed root system under drought stress conditions. Mutant-type plants exhibited greater root growth in lines 2 and 3 under AWD and upland irrigate condition, respectively.

East Africa including Mwea, Kenya is located in cold-prone highlands. In these location, cold stress, one

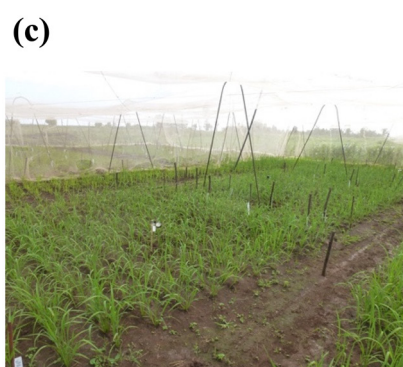
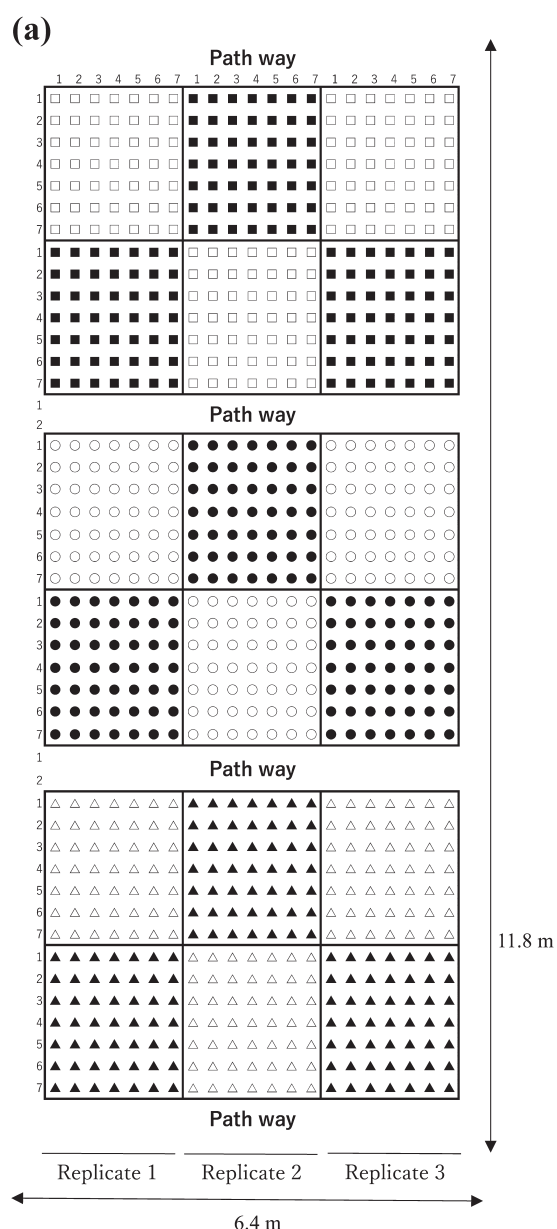
of the most important abiotic stresses for rice cultivation, caused yield losses of up to 100%<sup>16</sup>. In this study, the air temperature reached below 15°C several times from the booting stage to the maturity stage, resulting in severe sterility in both Normal-type plants and Mutant-type plants. Importantly, Mutant-type plants of lines 2 and 3 maintained highly developed root systems even under a severe cold stress environment. We previously reported a cold tolerant gene locus that functions well in the cold-prone highlands in Kenya<sup>22</sup>. In order to achieve stable yield under water deficit as well as cold-prone conditions, we are now trying to produce breeding lines that have both the cold tolerant gene and the KM07 mutant gene.

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**Fig. 3.** Field map in continuously waterlogged (CWL), alternate wetting and drying (AWD), and upland irrigated field (a), growth conditions in CWL (b, left), in AWD (b, right) and in upland irrigated (c) and panicle damaged by cold stress (d). □, Normal-type of line 1; ■, Mutant-type of line 1; ○, Normal-type of line 2; ●, Mutant-type of line 2; △, Normal-type of line 3; ▲, Mutant-type of line 3. Each symbol represents one plant per hill.

## References

- Hegde S, Hegde V. (2013) Assessment of global rice production and export opportunity for economic development in Ethiopia. *Int J Sci Res* 2: 257–260.
- Nwanze KF, Mohapatra S, Kormawa P, Keya SO, Bruce-Oliver S. (2006) Perspective. Rice development in sub - Saharan Africa. *J Sci Food Agric* 86: 675–677.
- Seck PA, Tollens E, Wopereis MCS, Diagne A, Bamba I. (2010) Corrigendum to: Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. *Food Policy* 36: 325–327.
- Balasubramanian V, Sie M, Hijmans RJ, Otsuka K. (2007) Increasing rice production in Sub-Saharan Africa: Challenges and Opportunities. *Advan Agron* 94: 55–133.
- Bouman, BAM, Tuong TP. (2001) Field water management to save water and increase its productivity in irrigated lowland rice. *Agric Water Manag* 49: 11–30.
- Vries ME. (2010) Rice production with less irrigation water is possible in a Sahelian environment. *F Crop Res* 116: 154–164.
- Lampayan RM, Rejesus RM, Singleton GR, Bouman BAM. (2015) Adoption and economics of alternate wetting and drying water management for irrigated lowland rice. *F Crop Res* 170: 95–108.
- Kamoshita A, Wade LJ, Yamauchi A. (2000) Genotypic Variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction during the drought period. *Plant Prod Sci* 3: 189–196.
- Bañoc DM, Yamauchi A, Kamoshita A, Wade LJ, Pardales JR. (2000) Genotypic variations in response

**Table 1.** Root growth of Normal-type and Mutant-type F<sub>6</sub> plants under CWL, AWD, and upland irrigated conditions at maturity stage

Water treatment	Traits	Diameter (mm)	F <sub>6</sub> Line 1		F <sub>6</sub> Line 2		F <sub>6</sub> Line 3	
			Normal-type	Mutant-type	Normal-type	Mutant-type	Normal-type	Mutant-type
CWL	TRL (cm)		36248.3	42213.2 <sup>ns</sup>	30831.1	35078.9 <sup>ns</sup>	27535.7	29078.7 <sup>ns</sup>
	SA (cm <sup>2</sup> )		3333.3	4013.2 <sup>ns</sup>	35078.9	3681.2 <sup>ns</sup>	2925.5	2952.8 <sup>ns</sup>
	RV (cm <sup>3</sup> )		24.5	30.9 <sup>ns</sup>	26.3	30.8 <sup>ns</sup>	25.0	24.0 <sup>ns</sup>
	TNRL (cm)	0.3 ≤ D.	8769.5	10223.6 <sup>ns</sup>	8376.7	9887.8 <sup>ns</sup>	7936.6	10366.5 <sup>ns</sup>
	TLRL (cm)	0 < D. ≤ 0.30	27437.2	31938.9 <sup>ns</sup>	22422.3	25154.9 <sup>ns</sup>	19570.6	17864.2 <sup>ns</sup>
	LRL (cm)	0 < D. ≤ 0.080	16189.2	19034.9 <sup>ns</sup>	13820.1	14160.8 <sup>ns</sup>	10878.9	11820.6 <sup>ns</sup>
	LRL (cm)	0.080 < D. ≤ 0.15	6519.2	7481.0 <sup>ns</sup>	5035.7	5979.0 <sup>ns</sup>	4859.7	4880.6 <sup>ns</sup>
	LRL (cm)	0.15 < D. ≤ 0.30	4728.8	5423.1 <sup>ns</sup>	3566.5	5015.0 <sup>ns</sup>	3712.1	4241.7 <sup>ns</sup>
AWD	TRL (cm)		20492.1	19532.9 <sup>ns</sup>	16553.8	24092.7 *	18010.9	15829.3 *
	SA (cm <sup>2</sup> )		2075.9	2245.9 <sup>ns</sup>	1738.5	2546.0 *	1972.3	1758.0 <sup>ns</sup>
	RV (cm <sup>3</sup> )		16.8	20.8 *	14.7	21.5 *	17.3	15.6 <sup>ns</sup>
	TNRL (cm)	0.3 ≤ D.	5359.0	5785.2 <sup>ns</sup>	4563.3	6421.3 *	5172.7	4640.2 <sup>ns</sup>
	TLRL (cm)	0 < D. ≤ 0.30	15110.7	13727.8 <sup>ns</sup>	11972.5	17643.5 *	12819.7	11171.3 <sup>ns</sup>
	LRL (cm)	0 < D. ≤ 0.080	8444.0	7942.9 <sup>ns</sup>	6772.1	10196.0 *	6974.8	6224.3 <sup>ns</sup>
	LRL (cm)	0.080 < D. ≤ 0.15	3790.7	3348.9 <sup>ns</sup>	2984.3	4285.7 <sup>ns</sup>	3233.7	2790.7 <sup>ns</sup>
	LRL (cm)	0.15 < D. ≤ 0.30	2876.0	2436.0 *	2216.0	3161.7 <sup>ns</sup>	2611.2	2156.4 <sup>ns</sup>
Upland irrigated	TRL (cm)		10357.4	13102.4 <sup>ns</sup>	13344.1	11297.5 <sup>ns</sup>	11898.6	16881.6 *
	SA (cm <sup>2</sup> )		1493.3	1381.8 <sup>ns</sup>	1230.4	1533.0 <sup>ns</sup>	1370.7	1703.8 <sup>ns</sup>
	RV (cm <sup>3</sup> )		13.3	13.6 <sup>ns</sup>	11.7	14.4 <sup>ns</sup>	12.6	13.8 <sup>ns</sup>
	TNRL (cm)	0.3 ≤ D.	3956.0	4589.0 <sup>ns</sup>	5921.2	3737.8 <sup>ns</sup>	3574.0	6347.7 <sup>ns</sup>
	TLRL (cm)	0 < D. ≤ 0.30	6948.7	8187.6 <sup>ns</sup>	9902.9	6747.0 <sup>ns</sup>	8312.2	10651.5 *
	LRL (cm)	0 < D. ≤ 0.080	3711.6	4829.6 <sup>ns</sup>	4860.5	4125.1 <sup>ns</sup>	4459.8	6807.1 *
	LRL (cm)	0.080 < D. ≤ 0.15	1679.0	2457.8 <sup>ns</sup>	2136.4	1753.7 <sup>ns</sup>	1894.3	2924.1 *
	LRL (cm)	0.15 < D. ≤ 0.30	1652.1	2210.1 <sup>ns</sup>	2253.7	1669.2 *	1958.1	2676.8 *

CWL, continuously waterlogged; AWD, alternate wetting and drying; TRL, total root length; SA, root surface area; RV, root volume; TNRL, total nodal root length; TLRL, total lateral root length; LRL, lateral root length at each indicated diameter (D.). ns, not significant; \*, significant at  $P < 0.05$  between the two genotypes within each water treatment.

of lateral root development to fluctuating soil moisture in rice. *Plant Prod Sci* 3: 335–343.

10. Suralta RR, Inukai Y, Yamauchi A. (2008a) Genotypic variations in responses of lateral root development to transient moisture stresses in rice cultivars. *Plant Prod Sci* 11: 324–335.
11. Suralta RR, Inukai Y, Yamauchi A. (2008b) Utilizing Chromosome Segment Substitution Lines (CSSLs) for evaluation of root responses to transient moisture stresses in rice. *Plant Prod Sci* 11: 457–465.
12. Suralta RR, Inukai Y, Yamauchi A. (2010) Dry matter production in relation to root plastic development, oxygen transport, and water uptake of rice under transient soil moisture stresses. *Plant Soil* 332: 87–104.
13. Kano-Nakata M, Inukai Y, Wade LJ, Siopongco JDL, Yamauchi A. (2011) Root development, water uptake, and shoot dry matter production under water deficit conditions in two CSSLs of rice: Functional Roles of Root Plasticity. *Plant Prod Sci* 14: 307–317.
14. Kano-Nakata M, Gowdab VRP, Henry A, Serraj R, Inukai Y, Fujita D, Kobayashi N, Suralta RR, Yamauchi A. (2013) Functional roles of the plasticity of root system development in biomass production and water uptake under rainfed lowland conditions. *F Crop Res* 144: 288–296.
15. Sandhu N, Jain S, Battan KR, Jain RK. (2012) Aerobic rice genotypes displayed greater adaptation to water-limited cultivation and tolerance to polyethyleneglycol-6000 induced stress. *Physiol Mol Biol Plants* 18: 33–43.
16. Wainaina CM, Makihara D, Samejima H, Kikuta M, Menge DM, Kimani JM, Inukai Y. (2017) Development of a new cultivation technology for cold stress escape through flowering time manipulation by water management in the highlands of East Africa. *J Intl Cooper Agric Dev* 15: 32–41.
17. Wade LJ, Kamoshita A, Yamauchi A, Azhiri-Sigari T. (2000) Genotypic variation in response of rainfed lowland rice to drought and rewetting. I. Growth and water use. *Plant Prod Sci* 3: 173–179.
18. Kameoka E, Suralta RR, Mitsuya S, Yamauchi A. (2015) Matching the expression of root plasticity with soil moisture availability maximizes production of rice plants grown in an experimental sloping bed

- having soil moisture gradients. *Plant Prod Sci* 18: 267-276.
19. Menge DM, Kameoka E, Kano-Nakata M, Yamauchi A, Asanuma S, Asai H, Kikuta M, Suralta RR, Koyama T, Tran TT, Siopongco JDLC, Mitsuya S, Inukai Y, Makihara D. (2016) Drought-induced root plasticity of two upland NERICA varieties under conditions with contrasting soil depth characteristics. *Plant Prod Sci* 19: 389–400.
  20. Njinju SM, Samejima H, Katsura K, Kikuta M, Gweyi-Onyango JP, Kimani JM, Yamauchi A, Makihara D. (2018) Grain yield responses of lowland rice varieties to increased amount of nitrogen fertilizer under tropical highland conditions in central Kenya. *Plant Prod Sci* 21: 59–70.
  21. Yamauchi A, Kono Y, Tatsumi J. (1987) Quantitative analysis on root system structures of upland rice and maize. *Japan Jour Crop Sci* 56: 608–617.
  22. Wainaina CM, Makihara D, Nakamura M, Ikeda A, Suzuki T, Mizukami Y, Nonoyama T, Doi K, Kikuta M, Samejima H, Menge DM, Yamauchi A, Kitano H, Kimani JM, Inukai Y. (2018) Identification and validation of QTLs for cold tolerance at the booting stage and other agronomic traits in a rice cross of a Japanese tolerant variety, Hananomai, and a NERICA parent, WAB56-104. *Plant Prod Sci* 21: 132–143.