

Phenotypic Variation for Root Distribution of IR64, KDML105 and KDML105 Mutant Rice Varieties in Water Deficit and Well-Watered Conditions

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ABSTRACT

Phenotypic variation for root distribution is associated with the ability of plants to acquire water and nutrients available at different soil strata. Rice production is known to be susceptible to water deficit stress (WS) because rice requires much water for cultivation. In this study, we explored natural variation of root distribution among IR64, KDML105 and KDML105 mutant lines (MT1, MT2, MT3) derived from gamma irradiation combined with ethyl methane sulfonate treatment. Plants were grown in a root basket and a root box system. We found that KDML105 had lower root to shoot ratio (27.49%) and less rooting depth (27.25%) compared to IR64. In the root box system, we found that WS decreased an average shoot biomass by 32.9%. The mutant lines maintained their shoot mass under WS while that of KDML105 was significantly reduced. Physiological analysis revealed that MT2 had the lowest evapotranspiration rate of 76.6%. In addition, MT2 and MT3 increased photosynthetic pigments under WS. KDML105 increased its rooting depth while MT1 and MT2 reduced rooting depth by 17.5% and 15.2%, respectively, under WS. Root distribution was associated with crown root number per tiller but not necessary with water use efficiency in our system.

Keywords: Rice, Root, Root distribution, Water deficit, Mutant rice

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Introduction

Rice is one of the most agronomically important crops feeding more than half of the world's population [1]. Presently, the world population is growing at an exponential rate, which substantially increases food demand. Farmers are attempting to raise rice yields in order to keep up with a growing consumer base. It has been reported that 20% of the rice being grown is subjected to water stress [2]. In Asia, most rice are planted in rainfed lowland paddy fields where water-use depends on rainfall. Increasing world temperatures paired with delayed rainfall caused by climate change will have detrimental effects on rice production [3-4].

Khao Dawk Ma Li (KDML105) is a well-known Thai rice cultivar with high commercial value. However, the cultivar requires high fertilizer inputs, and prone to lodging [5]. Moreover, it is sensitive to several abiotic stress particularly drought. Lacking sufficient water reduces growth performances and causes spikelet infertility and eventual loss in grain yield [6-7].

Root distribution associates with the abilities of plants to explore soil domains and the acquisition of and nutrients, which are usually not uniformly distributed in soil strata [8-10]. The advantages of deep root systems are well documented for drought and low nitrogen condition. Plants with greater rooting depth are better able to exploit water and nitrate from deep soil [11]. In rice it was reported that a steep root growth angle, controlled by a locus on chromosome 9 named "DEEPER ROOTING 1" (Dro 1), increases the ability of roots to explore deep soil layers which promote shoot growth and yield under drought [12]. Conversely, root traits allowing for shallow rooting such as high crown root number, increased lateral root branching, and shallow root growth angle can promote the uptake of essential nutrients available near the top soil such as potassium and phosphorus [9]. Therefore, root distribution is an important integrated trait that could be used in plant breeding program for enhanced water and nutrient efficiency.

One of the methods for improving rice characteristics is seed induced mutation by gamma ray irradiation together with EMS (ethyl methane sulfonate) [13]. It has been reported that mutant lines produced by this approach had improved yield and become stress tolerant [14]. In this study, we investigated root distribution of KDML105 in a root basket system compared with IR64, an international rice variety. In addition, we examined root distribution among KDML105 and its selected salt tolerant mutant lines namely MT1, MT2 and MT3 under well-watered and water deficit stress in a root box system.

Materials and Methods

Root basket system

Seeds of IR64 and KDML105 were obtained from the Rice gene discovery unit, Kasetsart University. Seeds were surface-sterilized in 10% NaOCl for 1 min. Then, each seed was germinated at room temperature in a dark chamber for 3 days. After that, each seed was sown at the center of a stainless-steel basket (7.5-cm top diameter, 5.0-cm depth and 2-mm mesh size) that was filled with soil (Figure 1) supplied with Yoshida's solution (pH=5.5) [15]. The ratio of deep rooting and other traits were examined 7 weeks after sowing. Shallow and deep roots were quantified by counting roots emerging through the mesh at 0-50 degree angle with respect to the horizontal as shallow, and those emerging at 51-90 degree angle as deep [16].

Root box experiment

Seeds of gamma irradiation combined with EMS treatment were obtained for molecular marker-screening of salt tolerant mutants [13]. Rice were further screened for salt tolerance by self-pollinated for 5 generations to create M5 seeds. In this study, MT1, MT2, MT3 are the 3 candidate mutant lines for testing their responses to well-watered and water deficit stress. The root box system is a system that allows non-destructive observation of root architectural traits [17]. The root box system (5-mm thick acrylic box, 60-cm height, 30-cm length and 3-cm width) contained sandy loam soil (sand 3:1 soil). A seed was sown in the middle of the box. The plant was watered every two days for two weeks. Then, in water deficit treatment, water was withdrawn for 7 weeks. The root box was weighted weekly for estimating evapotranspiration rate according to Kano-Nakata et al. [18]. Box without the plant used as blank control. On 48 days after water deficit treatment, chlorophyll a, chlorophyll b and total carotenoid contents were analyzed following Cha-um et al. [14]. Leaf rolling score were recorded based on IRRI [19]. At harvest, root box was opened on one side and flipped over to a 60 × 30 cm pin-board, then flushed gently with water to remove soil from the root system. The root was then fixed with the 1 × 1 grid pin-board containing one thousand nails for root architecture observation according to Kano-Nakata et al. [18]. Images of root system were captured and analyzed using ImageJ software [20].

Statistical analysis

R version 3.4.0 (R Development Core Team, 2018) and the R package Agricolae, using analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DMRT) were used to compare differences in root and shoot traits among genotypes and treatments.

Results

Root basket experiment

We found that KDML105 had lower root to shoot ratio compared to IR64 (Figure 1B). On average more than 70% of rice root system penetrated the basket at 0-50 degree angle (Figure 1A and 1C). Among rice varieties, IR64 had 27.2% more deep roots than KDML105 (Figure 1A and 1C).

Root box experiment

We found that water deficit stress (WS) reduced average shoot dry weight and root dry weight by 32.9% and 55.2%, respectively. KDML105 significantly reduced shoot and root dry weight by 40.0% and 55.5% while all mutant lines maintained their biomass under WS. Leaf rolling score was significantly higher in KDML105 (6.3) than its mutant lines (4.0) under WS (Table 1)

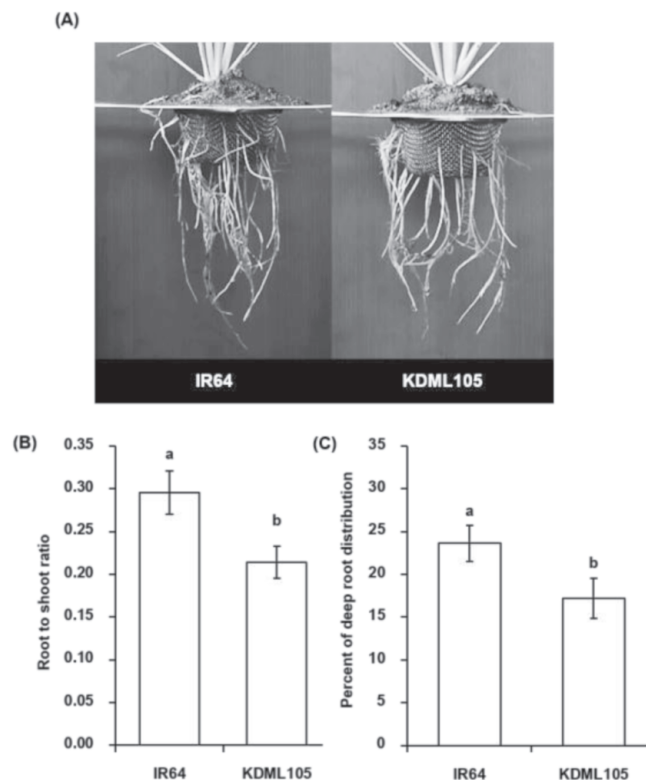


Figure 1 (A) Root distribution compared between IR64 and KDML105. The plants were grown in the baskets for 7 weeks. (B) Root to shoot ratio and (C) Percent of deep root distribution of IR64 and KDML105 at 7 weeks after sowing. Each bar represents \pm SD of each mean value. Difference letters show significant difference at $p \geq 0.05$ analyzed by Duncan multiple range test (DMRT).

Physiological analyses revealed that plant increased evapotranspirational demand under WS. An average evapotranspiration rate was significantly increased in all tested lines by 81.6% under WS (Table 2.). In addition, the WS effects on photosynthetic pigments including chlorophyll a, chlorophyll b and total carotenoid were different among tested lines. While KDML105 and MT1 were not significantly different in chlorophyll a, chlorophyll b and total carotenoid, MT2 and MT3 significantly increased these pigments under WS (Table 2).

Table 1 Shoot dry weight, root dry weight, and leaf rolling score among KDML 105 and KDML105 mutants at 48 days after well-watered (WW) and water deficit stress (WS) treatments.

Genotype	Treatment	Shoot weight (g)	Root weight (g)	Leaf rolling score
KDML105	WW	2.5±1.4 b	0.9±0.3 a	ND
	WS	1.5±0.2 cd	0.4±0.1 b	6.3±1.1 a
MT1	WW	1.8±0.5 bc	0.7±0.2 ab	ND
	WS	1.4±0.5 cd	0.5±0.2 ab	4.0±1.1 b
MT2	WW	0.9±0.3 d	0.2±0.0 b	ND
	WS	0.7±0.4 d	0.3±0.2 b	4.0±1.1 b
MT3	WW	1.4±0.3 cd	0.4±0.1 b	ND
	WS	0.8±0.3 d	0.3±0.0 b	4.0±1.1 b
Significant level		**	*	**

Difference letters within column represent mean±SD with significant differences (*) at $p \geq 0.05$ or highly significant differences (**) at $p \geq 0.01$ analyzed by DMRT. ND stands for “not determined”.

Table 2 Evapotranspiration rate, chlorophyll a content, chlorophyll b content and total carotenoid among KDML 105 and KDML 105 mutants at 48 days after well-watered (WW) and water deficit stress (WS) treatments.

Genotype	Treatment	Evapotranspiration rate (g day ⁻¹)	Chlorophyll a (µg g ⁻¹)	Chlorophyll b (µg g ⁻¹)	Total carotenoid (µg g ⁻¹)
KDML105	WW	2.2 ± 1.0 c	75.5 ± 22.8 a	33.9 ± 11.0 a	23.3 ± 6.2 b
	WS	14.9 ± 1.3 ab	80.2 ± 11.8 b	39.9 ± 6.6 a	23.6 ± 1.2 b
MT1	WW	2.9 ± 3.8 c	86.4 ± 22.2 a	36.4 ± 10.7 a	27.9 ± 5.6 ab
	WS	16.4 ± 4.0 a	68.3 ± 5.8	31.9 ± 2.7 a	24.5 ± 2.4 ab
MT2	WW	2.5 ± 0.8 b	32.7 ± 15.4 b	12.3 ± 10.1 b	13.5 ± 7.7 c
	WS	10.7 ± 4.0 c	77.2 ± 15.8 a	36.7 ± 6.3 a	25.8 ± 5.4 ab
MT3	WW	2.2 ± 0.3 ab	36.4 ± 5.6 b	13.6 ± 2.3 b	15.8 ± 2.5 c
	WS	12.3 ± 2.9 c	76.3 ± 7.0 a	35.1 ± 3.3 a	25.5 ± 1.5 ab
Significant level		**	**	**	**

Difference letters within column represent mean ± SD with highly significant differences (**) at $p \geq 0.01$ analyzed by DMRT.

In general, we found that under WW, the deeper the soils, the lower the root length density (Figure 2A). The highest root length density was located at top soil (0-10 cm). After treated with WS, the location where root length density was highest, was at 30-40 cm in KDML105, meaning that root system became deeper compared to the control. Conversely, the root system of mutant lines remained shallow having the highest root length density at 10-20 cm under WS. These results were consistent with the depth reached by 95% of the root (D_{95}), which is a common parameter to identify plant's rooting depth. Under WW, all rice lines had D_{95} greater than 50 cm. The highest D_{95} was found in MT3 (57.5 cm) while MT1 had the lowest D_{95} (52.5 cm) (Figure 2B). When treated with WS, KDML105 and MT3 showed no significantly changes in D_{95} while MT1 and MT2 significantly reduced in D_{95} by 17.5% and 15.2%, respectively. Further observation of number of crown root indicate that KDML105 had the highest reduction (85.2%) in crown root number per tillers while MT2 had the lowest reduction (67.6%) with the highest number of crown roots under WS (Figure 2C).

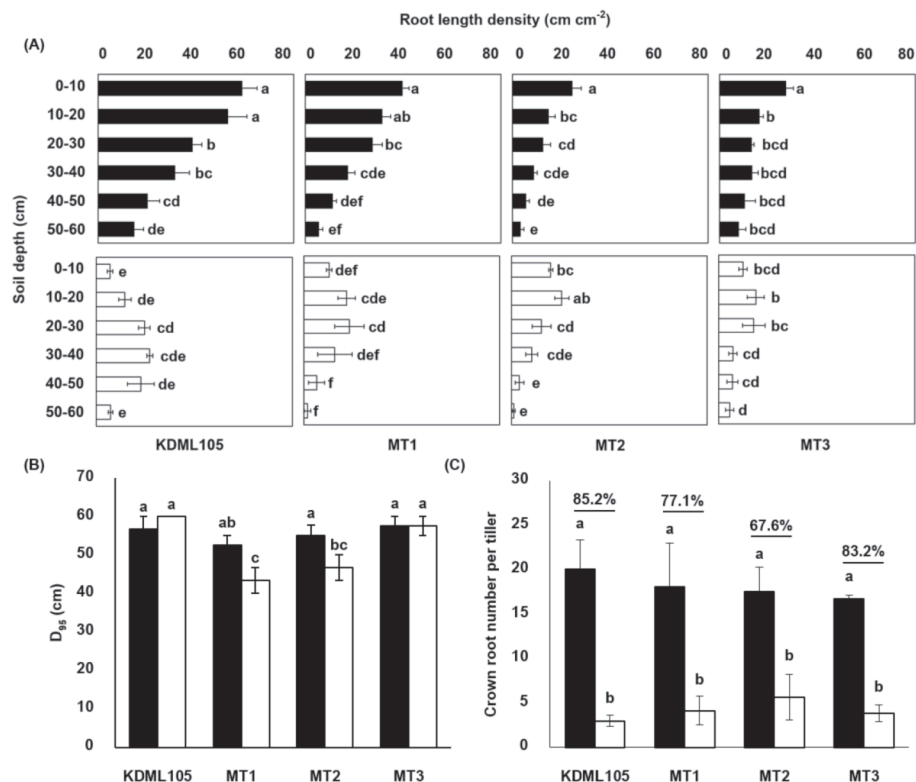


Figure 2 (A) Root length density at each soil depth, (B) Depth reached by 95% of the whole root (D_{95}) and (C) Crown root number per tiller among KDML105 and KDML105 mutants at 48 days after well-watered, WW (■) and water deficit stress, WS (□) treatments. Each bar represents for \pm SD of each mean value. Difference letters show significant difference at $p \geq 0.05$ analyzed by DMRT.

Conclusion and Discussion

Root distribution influences soil exploration and resource acquisition in plants. This present study demonstrates that KDML105 had shallow root system with only 17% penetrating in deep soils. Compared to IR64, KDML105 had lower percentage of deep roots which could reflect its ability to tolerate drought. Our results are consistent with Henry et al. (2012) who reported that IR64 had 20% longer root length, which contribute to greater root depth than KDML105 [21]. While KDML105 suffered a substantial decrease in shoot mass under WS, all mutant lines maintained their shoot growth. We also found that some mutant lines increased chlorophyll content under WS. This is unexpected but not surprising. Under drought, Chlorophyll a & b content could be increased due to leaf rolling. The reduction in leaf expansion results in chlorophyll accumulation and increased a percentage of chlorophyll content. In fact, increased chlorophyll content has been associated with drought tolerant ability. Khayatnezhad

et al. reported that drought tolerant maize genotypes increased chlorophyll content under drought resulting in 50% greater yields than susceptible variety [22].

Interestingly, we found that deep root distribution was not necessarily associated water use efficiency. In our system, the variety that maintained its growth, increased chlorophyll contents, and lowest evapotranspiration appeared to be MT2 which had the shallowest root system among different rice varieties. Drought tolerant ability is then very complex and depends on the integration of physiological, biochemical, and molecular adaptations [23-26].

Root formation is metabolically intensive and requires many resources to be allocated from the shoot. It has been reported that a plant could use more than 50% of the daily photosynthates to maintain a root system [27]. Plants that allocate more carbon from the source (shoot) to the sink (root) increased their vertical root growth [28-29]. For example, Saengwilai et al. demonstrated that maize recombinant inbred lines with fewer crown root numbers could utilize nitrate available in deep soil strata better than lines with many crown roots, resulting in better plant growth and yields in low nitrogen soils [10]. The relationship between rooting depth and root number is also evidenced in our study. Therefore, variation in root distribution is then associated with carbon allocation and resource acquisition efficiency [30].

In summary, we reported here that KDML105, a well-known Thai rice variety [31], had shallower root system than IR64, a high-quality rice variety widely cultivated in many countries originated from Philippines [32]. When compared among KDML105 and KDML105 mutant lines, KDML105 was more WS sensitive despite having a deeper root system. Additionally, we found the association between crown root number and rooting depth. Our results emphasize that water deficit tolerance is a complex trait and plants possess several strategies to cope with the stress. Further experiment is required to identify potential strategies that the mutants used to cope with water deficit.

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