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# EVALUATION OF KHAO DAWK MALI 105 CHROMOSOME SEGMENT SUBSTITUTION LINES POSSESSING VARIOUS DROUGHT TOLERANCE QTLS UNDER DROUGHT STRESS

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## SUMMARY

Rice crops are mostly grown under the rainfed lowland ecosystem and often experiencing drought stress throughout its growth stages. The appropriate approach in sustaining the rice yield under this constraint is to utilize drought-resistant varieties. However, drought tolerance is a complex trait, and it is difficult to develop because numerous QTLs distributed throughout the rice genome control this trait. The ability of the roots to uptake water from the deeper soil layers may increase grain yield under drought stress. The objective of this study was to evaluate root responses and grain yield of chromosome segment substitution lines (CSSLs) in the genetic background of Khao Dawk Mali 105 (KDML105) to drought stress. Lines containing single quantitative trait loci (QTLs) for drought tolerance on chromosomes 1, 3, 4, and 9 that contained chromosome segments from DH212 and also chromosome 8 that contained segments from DH103 were identified. Root length, root length density, root surface area, root dry weight, and root to shoot ratio increased under drought stress while root diameter was reduced. Moreover, drought stress increased root length density at deeper soil layers in CSSLs rather than in KDML105. The drought tolerance index (DTI) of drought tolerant lines sharply increased under drought stress. There traits related to root adaptation under both mild and severe drought stress are useful as selection criteria. Moreover, the CSSL #6, #9, #10, and #15 were the genotypes that had greater root adaptation such as root length, root surface area, root diameter, and lower yield reduction than KDML105 under drought stress. These results suggested that the elite CSSLs were improved and can be used as genetic materials in breeding programs.

**Key words:** Water deficit, quantitative trait loci, root length density, drought tolerance, grain yield, dry weight

**Key findings:** Root traits respond to late season drought for rooting depth associate with grain yield under severe stress**.** The CSSLs #6, #9, #10, and #15 were the genotypes that have good root adaption such as root length, root surface area, root diameter, and low yield reduction under different drought stress.

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## INTRODUCTION

Rice (*Oryza sativa* L.) is a semiaquatic species (physio-morpho and anatomically adapted to flooded conditions) which can be grown in a wide range of environments including upland and lowland areas (Javahar, 2012). Drought is major constraint for rice production. Rice grown in rainfed ecosystems are always exposed to drought stress resulting in yield loss. Pandey *et al.* (2008) reported that an average of 40% yield losses were caused by severe drought in Eastern India. In Northeast Thailand, over 50% yield loss were also due to drought (Polthanee *et al.*, 2014). Breeding for drought tolerance is thus a requirement. However, the response of the rice plant to drought stress has not been well understood since those traits are complex and often confounded by genetic × environment interactions which lead to difficulties in crop improvement.

Numerous studies have been subjected to identify traits that adapt to drought stress such as functional roles of the roots (Niones *et al.*, 2012), drought yield index (Raman *et al.*, 2012), and agro-morphological traits (Bocco *et al.*, 2012). The root system is one of the most important parts of the rice plant that functions in soil, water, and nutrients uptake. Comas *et al.* (2013) reported that root traits associated with maintaining plant productivity under drought included small and fine root diameters, specific root length and root length density, especially the available soil water level. Roots with higher dry weight, root length density, especially at 30 cm of depth has an advantage for water uptake from deeper soil layers (Siopongco *et al*., 2005). Although, several studies aimed to identify new traits associated with drought tolerance (Li *et al*., 2011; Jie *et al*., 2006), this information has not been clearly understood for the rainfed lowland areas of Thailand. Khao Dawk Mali 105 (KDML105) is a Thai jasmine rice with good cooking and eating qualities. It's mostly grown in Northeast Thailand, the region that often has water deficit. The improvement of KDML105 and/or derived lines using appropriate selection criterions will be a beneficial approach for the breeding program.

In the previous research, "KDML105" was used as a recurrent parent which was crossed with DH103 (drought tolerant) and DH212 (drought tolerant), to develop chromosome segment substitution lines (CSSLs). This population is carrying the QTL for drought tolerance located on chromosomes 1, 3, 4, 8, and 9 (Kanjoo *et al*., 2012) and the candidate materials for identifying the genetic regions for drought tolerance as well as the CSSLs were evaluated for agronomic traits. This study is interested in root traits which are important to improve agricultural productivity in low input systems. Limited information has been available in the response of CSSLs populations' root systems to drought. A better understanding on how the CSSLs' root traits respond to drought stress is important for the development of drought resistance cultivars. Therefore, the objective of this study was to evaluate the root responses of CSSLs of KDML105 under drought stress. The outputs were elite CSSLs which can be used as genetic materials in breeding programs.

## MATERIALS AND METHODS

### Genetic materials

The parental lines of DH103 (IR68586-F2-CA-31) and DH212 (IR68586-F2-CA-143) were derived from a cross between CT9993 × IR62266, which had a good root system and high osmotic adjustment under drought environment. CSSLs were derived from a cross between KDML105 × DH103 (cross 1) and KDML105 × DH212 (cross 2). The DH212 was assigned as a drought tolerant due to QTL (DT-QTL) on chromosomes 1, 3, 4, and 9 while the DH103 DT-QTL was on chromosome 8. The information of KDML105 CSSLs and their parents were presented in Supplementary Table 1. Details of CSSLs development were described previously (Kanjoo *et al*., 2012).

### Cultivation and experimental design

The field experiment was carried out at Chum Phae Rice Research Center, Khon Kaen, Thailand (latitude 16$^{o}$ 32' 2.6'' N, longitude 102$^{o}$ 7' 6.2'' E, altitude 215 m) in 2012 and 2013. The rice plants were assigned in 2 × 23

**Table 1.** Shoot dry weight (g plant$^{-1}$), root dry weight (g plant$^{-1}$), root to shoot ratio and drought tolerant index (DTI) of 20 KDML105 CSSLs and parental lines (KDML105, DH103 and DH212) responses to drought stress in 2012.

| Genotypes | Shoot dry weight | | | Root dry weight | | | Root to shoot ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | WW | DS | DTI | WW | DS | DTI | WW | DS | DTI |
| CSSL#1 | 15.8 | 7.0 | 0.50 | 0.55 | 0.63 | 1.21 | 0.035 | 0.120 | 3.55 |
| CSSL#2 | 18.0 | 5.8 | 0.32 | 0.45 | 0.58 | 1.29 | 0.025 | 0.100 | 4.03 |
| CSSL#3 | 14.0 | 6.1 | 0.45 | 0.49 | 0.60 | 1.28 | 0.035 | 0.105 | 3.05 |
| CSSL#4 | 27.2 | 6.7 | 0.33 | 0.57 | 0.66 | 1.21 | 0.025 | 0.110 | 5.34 |
| CSSL#5 | 20.7 | 7.6 | 0.36 | 0.62 | 0.61 | 1.00 | 0.030 | 0.085 | 2.73 |
| CSSL#6 | 10.9 | 8.1 | 0.75 | 0.46 | 0.81 | 1.80 | 0.045 | 0.110 | 2.38 |
| CSSL#7 | 12.8 | 5.7 | 0.53 | 0.53 | 0.61 | 1.17 | 0.045 | 0.115 | 2.93 |
| CSSL#8 | 19.5 | 7.0 | 0.36 | 0.53 | 0.68 | 1.28 | 0.025 | 0.095 | 3.57 |
| CSSL#9 | 21.4 | 13.8 | 0.64 | 0.51 | 0.94 | 1.91 | 0.025 | 0.070 | 3.07 |
| CSSL#10 | 9.6 | 6.8 | 0.70 | 0.39 | 0.64 | 1.65 | 0.045 | 0.105 | 2.36 |
| CSSL#11 | 16.3 | 8.1 | 0.93 | 0.48 | 0.72 | 1.69 | 0.040 | 0.090 | 2.82 |
| CSSL#12 | 24.8 | 9.8 | 0.40 | 0.82 | 0.69 | 0.89 | 0.035 | 0.070 | 2.18 |
| CSSL#13 | 6.9 | 9.7 | 1.41 | 0.45 | 0.58 | 1.31 | 0.065 | 0.090 | 0.97 |
| CSSL#14 | 20.7 | 7.8 | 0.37 | 0.64 | 0.65 | 1.03 | 0.030 | 0.075 | 2.93 |
| CSSL#15 | 12.2 | 10.0 | 0.80 | 0.52 | 0.65 | 1.32 | 0.050 | 0.090 | 1.62 |
| CSSL#16 | 13.9 | 6.9 | 0.55 | 0.66 | 0.59 | 0.89 | 0.050 | 0.090 | 1.69 |
| CSSL#17 | 17.0 | 6.2 | 0.50 | 0.62 | 0.56 | 0.93 | 0.050 | 0.135 | 2.21 |
| CSSL#18 | 25.1 | 5.7 | 0.28 | 0.86 | 0.65 | 0.76 | 0.050 | 0.100 | 2.90 |
| CSSL#19 | 17. 6 | 8.5 | 0.48 | 0.54 | 0.79 | 1.65 | 0.030 | 0.080 | 3.28 |
| CSSL#20 | 20.6 | 7.7 | 0.37 | 0.61 | 0.56 | 0.96 | 0.025 | 0.070 | 2.66 |
| KDML105 | 12.6 | 12.5 | 0.99 | 0.57 | 0.81 | 1.44 | 0.065 | 0.125 | 1.32 |
| DH103 | 18.6 | 4.1 | 0.24 | 0.81 | 0.52 | 0.67 | 0.040 | 0.095 | 2.89 |
| DH212 | 19.3 | 5.9 | 0.30 | 0.73 | 0.58 | 0.80 | 0.040 | 0.060 | 2.61 |
| Max | 27.2 | 13.8 | 1.41 | 0.86 | 0.94 | 1.91 | 0.77 | 0.14 | 5.34 |
| Min | 6.9 | 4.1 | 0.24 | 0.39 | 0.52 | 0.67 | 0.03 | 0.06 | 0.97 |
| Mean | 17.2 | 7.7 | 0.56 | 0.54 | 0.66 | 1.22 | 0.04 | 0.10 | 2.74 |
| F-test | ns | ns | | ns | ns | | ns | ns | |
| CV(%) | 44.75 | 34.05 | | 21.94 | 21.85 | | 44.64 | 30.41 | |
| $P \leq 0.05$ | | | | | | | | | |
| W | 2071** | | | 0.125* | | | 0.071** | | |
| G | 28ns | | | 0.019ns | | | 0.00044ns | | |
| W*G | 33ns | | | 0.032* | | | 0.00059ns | | |
| CV (%) | 45.81 | | | 21.68 | | | 36.51 | | |

* and ** significant different at the level of 0.05 and 0.01 respectively

factorial in a randomized complete block design with two replications. Factor A was the two water regimes including well-watered and drought stress treatments. Factor B was the twenty-three rice genotypes including twenty KDML105 CSSLs and three parents. Seeds were sown on August 2012 and 2013. Thirty day-old seedlings were transplanted using 1 seedling per hill at a spacing of 20 cm × 20 cm between rows in a plot size of 1.0 m × 2.0 m. The water level was maintained at 5-10 cm above soil surface until 50 days after transplanting (DAT), and the water under drought stress condition was drained out. Under irrigated conditions, water was distributed by sprinkler until 90 DAT. The water application rate in well-watered was 4 cm day$^{-1}$

and 0 cm day$^{-1}$ in drought stress. The fertilizer rate of 29.1-21.6-21.6 kg N-$P_2O_5$-$K_2O$ ha$^{-1}$ was split applied at tillering stage and panicle initiation stage. Chemical pest control was done as needed.

**Data collection**

Relative humidity, rainfall, maximum, and minimum temperatures were recorded daily from the date of transplanting until the final harvest by the weather station at Chum Phae Rice Research Center. Soil samples from the two water regimes were examined at 50 DAT and final harvest taken at the depth of 0-5, 10-15, 25-30, 40-45, and 55-60 cm using the gravimetric method.

The shoot of each plot was cut at soil surface and oven dried at 80 $^oC$ for 48 hours or until constant weight. Root/shoot ratio was calculated as root dry weight to shoot dry weight. Plants within the harvest area of the plot (4 rows x 10 hills) were harvested to examine the grain yield.

Root samples of individual plants were collected by auger method using the core tube (Welbank *et al*., 1974) at final harvest. A core tube of 7.16 cm diameter and 1 m long was used for sampling. Root samples were taken at 0-15, 15-30, 30-45, and 45-60 cm of depth. The roots were washed to separate of plant debris and maintained in tap water and stored at 10 $^oC$ for root measurements. The root measurements included root length (cm), root surface area (cm$^2$ plant$^{-1}$), root diameter (mm), and root volume (cm$^3$ plant$^{-1}$). Root data were analyzed using the WINRHIZO Pro2004a software (REGENT instruments Inc., QC, Canada). Root length density (RLD) was calculated as the ratio between root lengths (cm) to soil volume (cm$^3$). Percent RLD was also calculated.

Drought tolerant index (DTI) was determined after harvesting. This method was used to determine the DTI of shoot dry weight, root dry weight, root to shoot ratio, root length, root surface area, average root diameter, and root volume in rice. These traits in both experiments were used for estimating the DTI as follows: DTI = stress treatment/well water treatment.

**Data analysis**

Analysis of variance (ANOVA) was performed in all traits for each year according to randomize complete block design (RCBD) with two replications using MSTAT-C package (Bricker, 1989). Mean comparison was done by Duncan's Multiple-Range Test (DMRT) at a 0.05 probability. Error variance for 2 years was tested for homogeneity by Bartlett's method and combined analysis of variance for each characteristic was performed over the two years (Gomes and Gomez, 1984). Data of all traits for each year were analyzed separately as the G × E interaction variance was detected. Correlations among traits were also examined.

## RESULTS

**Weather and soil conditions**

The meteorological data was shown in Figure 1. Maximum and minimum air temperature during 2012 ranged from 26.3 $^oC$ to 36.4 $^oC$ and 15.9 $^oC$ to 25.5 $^oC$ (Figure 1a). In 2013, the maximum and minimum air temperature ranged from 21.7 $^oC$ to 35.2 $^oC$ and 11.6 $^oC$ to 26.3 $^oC$, respectively (Figure 1c). Range of average air temperature during crop growth in 2012 was 21.1 $^oC$ to 30.4 $^oC$ (Figure 1a) whereas it was 17.8 $^oC$ to 30.8 $^oC$ in the year 2013 (Figure 1c), respectively. Average relative humidity values were 80.5% and 81.1% in 2012 (Figure 1b) and 2013 (Figure 1d), respectively. The total rainfall in 2012 was 224.9 mm (Figure 1b) while it was 359.5 mm in 2013 (Figure 1d). These indicated that in 2013, the rainfall was higher resulting to a higher relative humidity and a lower temperature. Soil moisture was observed at final harvest under well-watered and drought stress in 2012 and 2013 (Figure 2). The results showed difference between well-watered and drought stress at all soil depth in both years. The soil moisture content at soil depth 0-5, 10-15, 25-30, 40-45, and 55-60 cm were similar to all soil depth under well-watered. In contrast, the soil moisture content in 2013 was higher than 2012 because in second year, rainfall occurred after the water was drained.

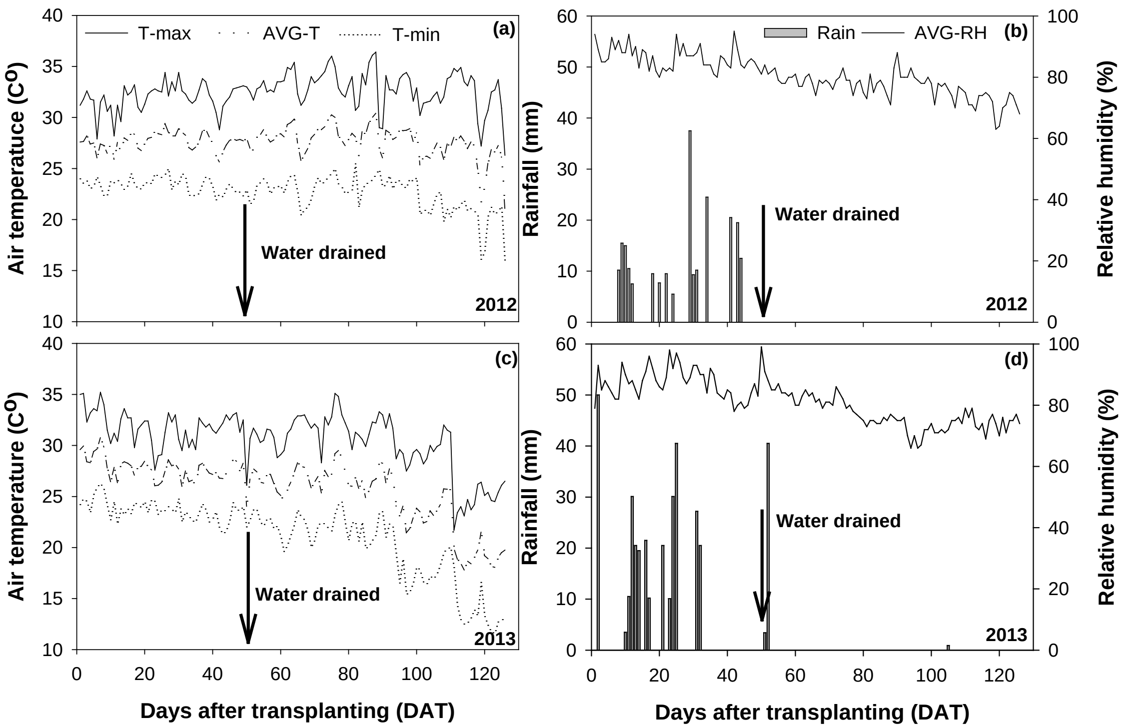


**Figure 1.** Climatological data at Chum Phae Rice Research Center from (a) September 2012 to December 2012 and (b) September 2013 to December 2013.

## Effect of drought stress on yield and yield reduction

Grain yield of CSSLs and their parents was presented in Figure 3. The CSSLs #8, #9, #13, #14, and #16 gave higher yield under drought stress than KDML105 in 2012. The CSSLs #2, #4, #8, #9, #14, and #15 gave higher yield under drought stress as compared to KDML105 in 2013. Severe drought stress caused yield reduction in all genotypes whereas CSSLs #2, #4, #8, #11, #12, #14, and #18 had lower yield reduction than KDML105 (Figure 4). Especially, CSSLs #8 and #14 had consistently higher grain yield and lower yield reduction than KDML105 under drought stress in both years. These indicated that yield reduction depicted severe drought in 2012 and mild drought in 2013.

## Effect of drought stress on root system

In the first year, the differences among shoot dry weight, root dry weight, and root to shoot ratio were observed due to differences in water regimes (Table 1). Severe drought stress reduced shoot dry weight (DTI < 1) whereas, root dry weight and root to shoot ratio increased (DTI > 1). Shoot dry weight, root dry weight, and root to shoot ratio were not significantly different among genotypes under both well-watered and drought conditions. For example, CSSL #13 was higher in DTI for shoot dry weight than others and increased under drought stress (DTI > 1). This indicated that this genotype could maintain shoot dry weight when exposed to drought stress. Moreover, CSSLs #6, #9, #10, #11, and #19 have higher DTI than KDML105 in shoot dry weight. In addition, root to shoot ratio was thus increased in most genotypes, especially in CSSLs #1, #2, #3, and #4 which carried the DT-QTL on chromosome 1, they have higher DTI for root to shoot ratio under drought stress. Thus, it was revealed that the CSSL on chromosome 1 had high performance in root adaptation under drought stress.

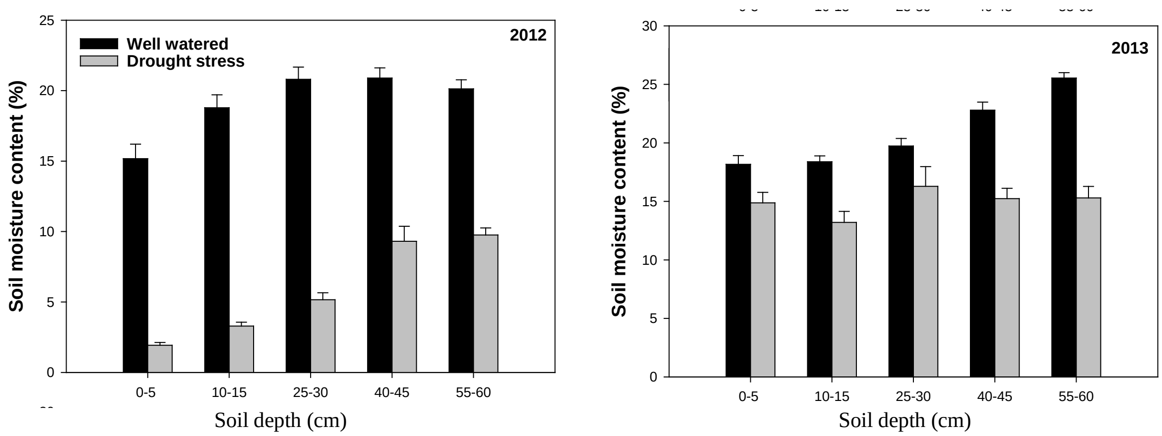


**Figure 2.** Soil moisture content at final harvest under well watered and drought stress conducted at Chum Phae Rice Research Center, Thailand during July–December 2012 (a) and in 2013 (b) at the depth of 0-5, 10-15, 25-30, 40-45 and 55-60 cm.

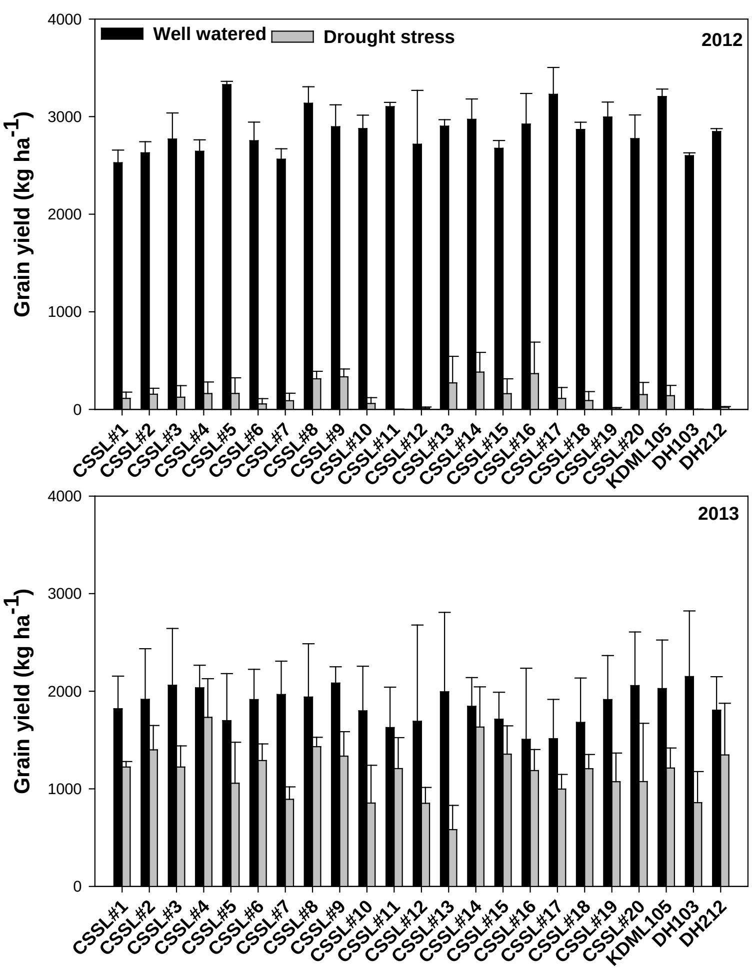


**Figure 3.** Grain yield of CSSLs KDML105 and their parents grown under non stress and drought stress at final harvest in 2012 and 2013.

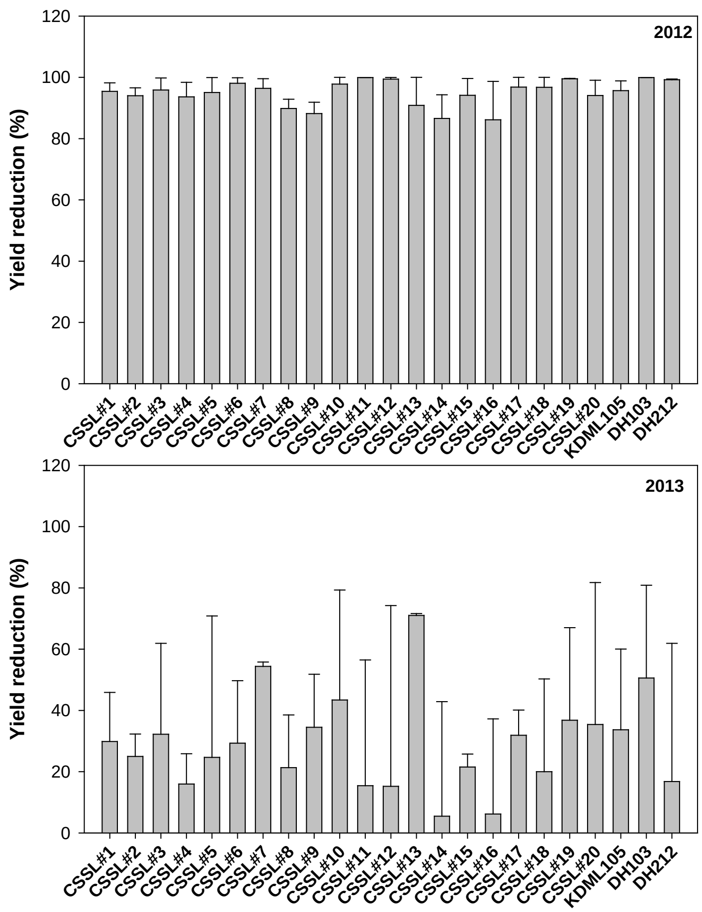


**Figure 4.** Yield reduction of CSSLs KDML105 and their parents grown under non stress and drought stress at final harvest in 2012 and 2013

Although, root surface area and root volume were not significantly different between water regimes (Table 2), root length was increased by severe drought stress while average root diameter was reduced. CSSLs #1, #6, and #9 have higher root length under drought stress compared to well-watered; their DTI was also high for root length (2.85, 3.31, and 3.72, respectively). Adaptation in total root length when plants are imposed to drought indicates the potential of the genotype to extract water in deeper soil layers. While the root diameter was associated with maintaining plant productivity under drought stress.

Root length density was highest in top soil (0-15 cm), and they were slightly reduced with increasing soil depth (Figure 5). Among parental lines (KDML105, DH103, and DH212), root length density at 15-60 cm of soil depth were 4.5%, 30.2%, and 20.7%, respectively. Most CSSLs had higher percentage of root length density at 15-60 cm soil depth than KDML105, especially CSSLs #8, #9, #18, and #20. The depth of CSSLs root system might be from DH103 which affected the population by obtaining the drought tolerance segment from chromosome 8 as showed in CSSLs #13, #14, #15, and #16 (Figure 5).

In second year, there was no significant difference in shoot dry weight and root dry weight between water regimes while root to shoot ratio was significantly increased by drought stress (Table 3). CSSL #6 tended to be higher in root to shoot ratio than KDML105 and it was higher in DTI (2.31) than other genotype. This indicated that late season with mild drought

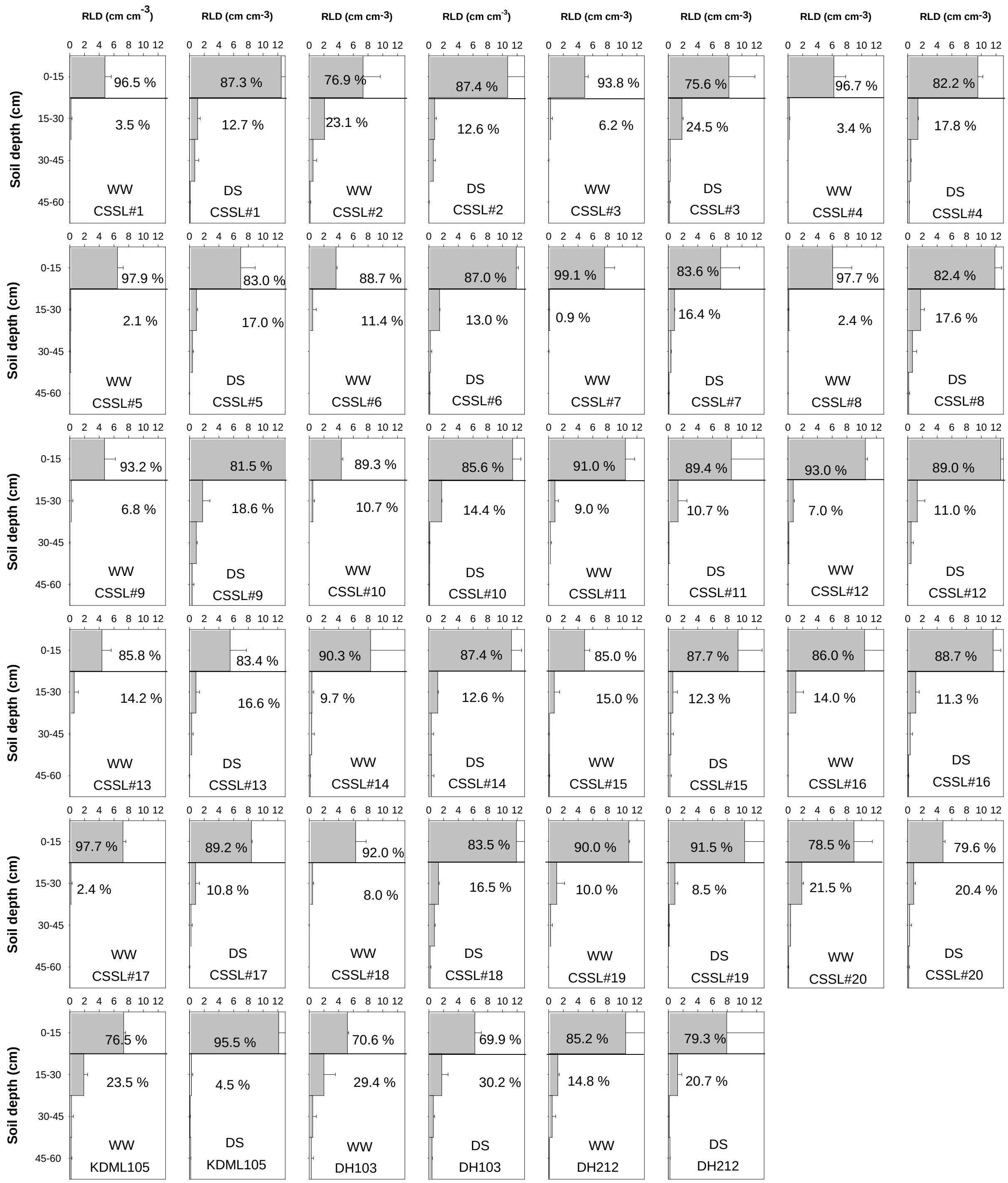


**Figure 5.** Root length density with four soil depth for evaluation of KDML105 CSSLs grown under non stress and drought stress at final harvest in 2012. Numeric values in above and below line exhibited percentage of root length density of 0-15 and 15-60 cm soil layers.

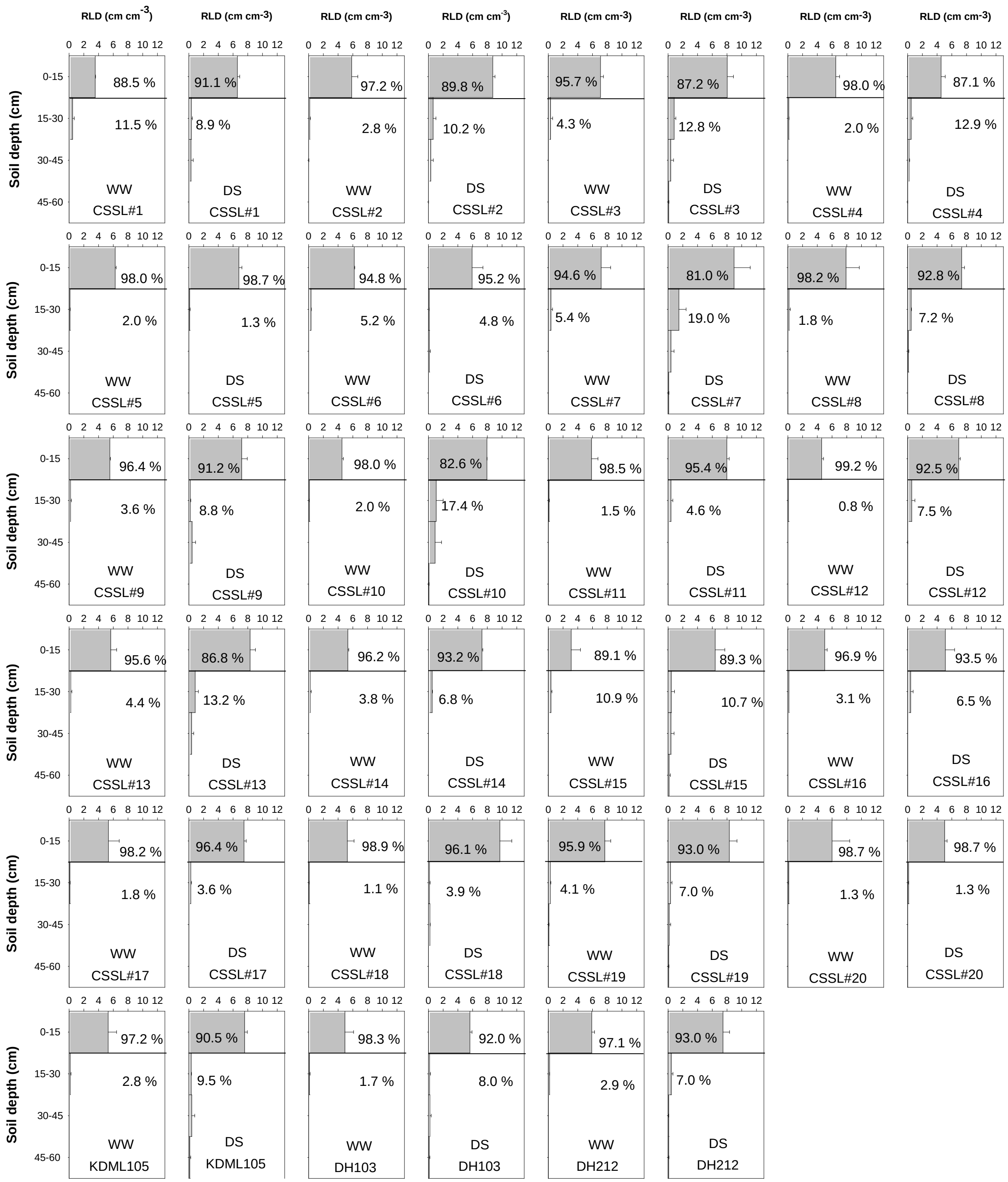


**Figure 6.** Root length density with four soil depth for evaluation of KDML105 CSSLs grown under non stress and drought stress at final harvest in 2013 Numeric values in above and below line exhibited percentage of root length density of 0-15 and 15-60 cm soil layers.

**Table 2.** Root length (cm $plant^{-1}$), root surface area ($cm^2 plant^{-1}$), average root diameter (mm), root volume ($cm^3 plant^{-1}$) and drought tolerant index (DTI) of all traits of 20 KDML105 CSSLs and parental lines (KDML105, DH103 and DH212) responses to drought stress in 2012.

| Genotype | Root length | | | Root surface area | | | Average root diameter | | | Root volume | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WW | DS | DTI | WW | DS | DTI | WW | DS | DTI | WW | DS | DTI |
| CSSL#1 | 3001 | 8693 | 2.85 | 338 | 398 | 1.17 | 0.57 | 0.60 | 1.22 | 3.75 | 3.55 | 1.03 |
| CSSL#2 | 6074 | 7345 | 1.34 | 514 | 500 | 1.05 | 0.56 | 1.04 | 1.80 | 3.83 | 4.23 | 1.13 |
| CSSL#3 | 3153 | 6338 | 2.05 | 364 | 521 | 1.54 | 0.54 | 0.48 | 0.91 | 3.52 | 3.76 | 1.19 |
| CSSL#4 | 3892 | 7006 | 1.89 | 482 | 426 | 0.88 | 0.81 | 0.47 | 0.58 | 5.73 | 3.29 | 0.57 |
| CSSL#5 | 3997 | 5044 | 1.31 | 450 | 449 | 1.07 | 0.83 | 0.65 | 0.81 | 4.21 | 3.59 | 0.92 |
| CSSL#6 | 2520 | 8257 | 3.31 | 302 | 668 | 2.21 | 0.58 | 0.61 | 1.05 | 3.27 | 4.97 | 1.53 |
| CSSL#7 | 4636 | 5067 | 1.06 | 417 | 415 | 1.00 | 0.63 | 0.55 | 0.90 | 3.58 | 3.04 | 0.91 |
| CSSL#8 | 3729 | 8683 | 2.91 | 397 | 617 | 1.74 | 0.64 | 0.53 | 0.85 | 3.84 | 3.66 | 1.01 |
| CSSL#9 | 3002 | 9933 | 3.72 | 297 | 786 | 3.04 | 0.65 | 0.81 | 1.27 | 2.62 | 6.02 | 2.69 |
| CSSL#10 | 2960 | 8019 | 2.71 | 334 | 595 | 1.86 | 0.61 | 0.59 | 0.97 | 3.56 | 4.03 | 1.28 |
| CSSL#11 | 6979 | 5995 | 0.98 | 597 | 478 | 0.83 | 0.68 | 0.62 | 0.91 | 4.85 | 3.59 | 0.74 |
| CSSL#12 | 6833 | 8713 | 1.29 | 658 | 574 | 0.89 | 0.83 | 0.63 | 0.77 | 5.62 | 4.22 | 0.78 |
| CSSL#13 | 3017 | 4075 | 1.30 | 312 | 428 | 1.36 | 0.54 | 0.70 | 1.40 | 2.80 | 4.34 | 1.56 |
| CSSL#14 | 5573 | 7931 | 2.15 | 515 | 626 | 1.45 | 0.60 | 0.54 | 0.91 | 4.19 | 4.50 | 1.09 |
| CSSL#15 | 3474 | 6443 | 2.10 | 403 | 523 | 1.38 | 0.59 | 0.54 | 0.93 | 4.09 | 3.86 | 0.97 |
| CSSL#16 | 6947 | 7953 | 1.21 | 582 | 580 | 1.02 | 0.65 | 0.54 | 0.88 | 4.25 | 3.63 | 0.85 |
| CSSL#17 | 4471 | 5722 | 1.29 | 475 | 468 | 1.00 | 0.79 | 0.51 | 0.80 | 4.35 | 3.30 | 0.86 |
| CSSL#18 | 4115 | 8547 | 2.21 | 559 | 616 | 1.10 | 1.21 | 0.47 | 0.44 | 8.12 | 3.85 | 0.55 |
| CSSL#19 | 7393 | 6851 | 0.99 | 657 | 514 | 0.82 | 0.74 | 0.52 | 0.71 | 5.40 | 3.45 | 0.67 |
| CSSL#20 | 6776 | 3640 | 0.56 | 609 | 434 | 0.75 | 0.62 | 0.64 | 1.05 | 5.02 | 3.96 | 0.82 |
| KDML105 | 5829 | 7631 | 1.31 | 501 | 680 | 1.37 | 0.50 | 0.69 | 1.37 | 3.81 | 5.38 | 1.43 |
| DH103 | 4823 | 5477 | 1.16 | 541 | 405 | 0.79 | 1.00 | 0.41 | 0.48 | 8.30 | 2.59 | 0.43 |
| DH212 | 7384 | 5763 | 0.94 | 631 | 459 | 0.80 | 0.68 | 0.60 | 0.89 | 4.52 | 3.21 | 0.73 |
| Max | 7393 | 9933 | 3.72 | 658 | 786 | 3.04 | 1.21 | 1.04 | 1.80 | 8.30 | 6.02 | 2.69 |
| Min | 2520 | 3640 | 0.56 | 297 | 398 | 0.75 | 0.50 | 0.41 | 0.44 | 2.62 | 2.59 | 0.43 |
| Mean | 4808 | 6919 | 1.77 | 475 | 529 | 1.27 | 0.69 | 0.59 | 0.95 | 4.48 | 3.91 | 1.03 |
| F-test | ns | ns | | ns | ns | | ns | ns | | ns | ns | |
| CV(%) | 39.02 | 25.04 | | 27.13 | 22.9 | | 32.15 | 35.58 | | 42.19 | 23.09 | |
| P≤.05 | | | | | | | | | | | | |
| W | 102500000** | | | 65043ns | | | 0.19* | | | 7.49ns | | |
| G | 4350775ns | | | 19541ns | | | 0.02ns | | | 1.51ns | | |
| W*G | 6472638ns | | | 29593ns | | | 0.06ns | | | 3.70ns | | |
| CV (%) | 34.08 | | | 25.83 | | | 34.1 | | | 34.91 | | |

* and ** significant different at the level of 0.05 and 0.01 respectively.

**Table 3.** Shoot dry weight (g plant$^{-1}$), root dry weight (g plant$^{-1}$), root to shoot ratio and drought tolerant index (DTI) of 20 KDML105 CSSLs and parental lines (KDML105, DH103 and DH212) responses to drought stress in 2013.

| Genotypes | Shoot dry weight | | | Root dry weight | | | Root to shoot ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | WW | DS | DTI | WW | DS | DTI | WW | DS | DTI |
| CSSL#1 | 20.6 | 24.2 | 1.23 | 0.40 | 0.85 | 2.08 | 0.020 | 0.035 | 1.84 |
| CSSL#2 | 22.7 | 33.5 | 1.48 | 0.70 | 0.85 | 1.16 | 0.032 | 0.027 | 0.82 |
| CSSL#3 | 31.3 | 22.3 | 0.68 | 0.80 | 0.90 | 1.10 | 0.026 | 0.044 | 1.67 |
| CSSL#4 | 24.4 | 17.3 | 0.71 | 0.70 | 0.55 | 0.79 | 0.029 | 0.032 | 1.11 |
| CSSL#5 | 26.0 | 32.1 | 1.21 | 0.65 | 0.75 | 1.18 | 0.025 | 0.025 | 1.00 |
| CSSL#6 | 26.5 | 16.4 | 0.60 | 0.70 | 0.95 | 1.51 | 0.027 | 0.057 | 2.31 |
| CSSL#7 | 23.8 | 30.2 | 1.26 | 0.85 | 1.05 | 1.14 | 0.037 | 0.034 | 0.92 |
| CSSL#8 | 22.3 | 19.4 | 0.91 | 0.75 | 0.65 | 0.85 | 0.037 | 0.034 | 0.95 |
| CSSL#9 | 29.6 | 18.5 | 0.70 | 0.70 | 0.55 | 0.83 | 0.024 | 0.029 | 1.27 |
| CSSL#10 | 34.5 | 37.9 | 1.65 | 0.60 | 1.00 | 1.97 | 0.020 | 0.027 | 1.43 |
| CSSL#11 | 23.4 | 24.5 | 1.06 | 0.70 | 0.85 | 1.14 | 0.032 | 0.034 | 1.17 |
| CSSL#12 | 27.8 | 22.0 | 0.79 | 0.75 | 0.70 | 1.00 | 0.026 | 0.033 | 1.27 |
| CSSL#13 | 19.1 | 17.5 | 0.91 | 0.55 | 0.85 | 1.86 | 0.030 | 0.052 | 2.02 |
| CSSL#14 | 12.4 | 24.5 | 2.09 | 0.55 | 0.65 | 1.33 | 0.043 | 0.030 | 0.67 |
| CSSL#15 | 15.6 | 23.5 | 1.56 | 0.45 | 0.70 | 2.29 | 0.030 | 0.031 | 1.29 |
| CSSL#16 | 34.9 | 21.8 | 0.67 | 0.90 | 0.60 | 0.70 | 0.026 | 0.040 | 1.56 |
| CSSL#17 | 19.6 | 14.1 | 0.92 | 0.75 | 0.65 | 0.89 | 0.042 | 0.048 | 1.45 |
| CSSL#18 | 19.6 | 30.2 | 1.62 | 0.65 | 1.05 | 1.49 | 0.037 | 0.033 | 0.90 |
| CSSL#19 | 36.7 | 17.8 | 0.53 | 0.95 | 0.70 | 0.94 | 0.025 | 0.041 | 1.69 |
| CSSL#20 | 27.4 | 30.1 | 1.12 | 0.85 | 0.75 | 0.96 | 0.030 | 0.024 | 0.82 |
| KDML105 | 25.1 | 33.4 | 1.44 | 0.70 | 1.05 | 1.59 | 0.028 | 0.031 | 1.10 |
| DH103 | 26.0 | 19.8 | 0.74 | 0.75 | 0.60 | 0.85 | 0.028 | 0.033 | 1.24 |
| DH212 | 23.2 | 27.8 | 1.27 | 0.55 | 0.90 | 1.74 | 0.025 | 0.034 | 1.37 |
| Max | 36.7 | 37.9 | 2.09 | 0.95 | 1.05 | 2.29 | 0.04 | 0.06 | 2.31 |
| Min | 12.4 | 14.1 | 0.53 | 0.40 | 0.55 | 0.70 | 0.02 | 0.02 | 0.67 |
| Mean | 24.9 | 24.3 | 1.09 | 0.69 | 0.79 | 1.28 | 0.03 | 0.04 | 1.30 |
| F-test | ns | ns | | ns | ns | | ns | ns | |
| CV(%) | 37.74 | 32.74 | | 33.43 | 26.56 | | 31.07 | 27.41 | |
| P<.05 | | | | | | | | | |
| W | 8.3ns | | | 0.218 ns | | | 0.00074** | | |
| G | 81.3ns | | | 0.037 ns | | | 0.00012ns | | |
| W*G | 74.0ns | | | 0.051 ns | | | 0.00010ns | | |
| CV (%) | 35.93 | | | 29.48 | | | 30.21 | | |

* and ** significant different at the level of 0.05 and 0.01 respectively.

stress had different effects on shoot and root growth with some genotypes have increased in shoot or root growth after imposed to mild drought. Most of the genotypes' root and shoot dry weight were increased under mild drought.

The root length, root surface area, root diameter, and root volume were significant under drought stress (Table 4), and the root length and root surface area were significantly increased. Root length values under well-watered conditions ranged from 2325 to 4878 cm while in drought stress ranged from 3076 to 6586 cm. Root surface area values under well-watered ranged from 330 to 675 $cm^2$ $plant^{-1}$ while under drought stress ranged from 397 to 773 $cm^2$ $plant^{-1}$. Similarly, the CSSL #7 had high root surface area under well-watered and drought stress. Average root diameter was decreased from 0.33 mm to 0.47 mm as affected by drought stress in the first year. The reduction of root diameter affected the root volume. Most of genotypes were reduced under drought condition and even under mild stress. CSSL #15 was higher in average root diameter than other genotypes. This indicated that the plant adapted to drought by reducing root diameter to increase root surface area as well as penetration for water uptake ability. Root length density was highest in top soil (0-15 cm), and slightly reduced with increasing soil depth similar to first year (Figure 6). Among parental lines, KDML105 was higher in RLD and %RLD in sub soil (15-60 cm) than other parental lines. Mild drought stress affected to increase RLD and %RLD of DH103 and 212 especially depth of soil layer (15-60 cm). CSSLs #7, #10, and #13 showed the highest RLD and %RLD in deeper soil layer (19.0%, 17.4%, and 13.2%, respectively), which was higher than KDML105, DH103, and DH212 (Figure 6).

## Combined analysis

Combined analysis of variance showed that differences among water regimes (W) were significant for all root traits (Table 5). Year (Y) contributed to a large portion of the total variation for root diameter, root volume, shoot dry weight, and root to shoot ratio (32.3, 75.0, 35.8, and 27.9%, respectively). Genotype (G) had smaller effect to root length (7.3%), root diameter (4.1%), root volume (1.9%), shoot dry weight (5.7%), root dry weight (8.3%), and root to shoot ratio (3.7 %). The interactions between year and water (Y × W) regimes was not significant for most traits except for shoot dry weight and root to shoot ratio. Genotypes x water regime interaction and the interactions Y×W×G have more effect to root volume due to the difference of water stress in two year. The severity of stress would be the main effect to the interaction. The result of this study showed that drought stress had more effect on root characters.

## Correlation between root traits with grain yield and dry weight

Correlation among traits under drought stress was presented in Table 6. In 2012, positive and significant correlations were found between shoot dry weight and most root traits such as root surface area ($r$ = 0.61**), root diameter ($r$ = 0.69**), and root volume ($r$ = 0.87**). Moreover, relationship between grain yield with root surface area (0.41*) and root volume (0.46*) were positively significant. The results indicated that plant above ground dry weight should be affected to underground character. The great root system contributed to good plant stands and yield production under drought condition. Similar with 2013, shoot dry weight was found correlated with root surface area ($r$ = 0.56**) and root volume ($r$ = 0.58**). Moreover, grain yield showed negative correlation with root length ($r$ = -0.50*) in 2013.

# DISCUSSION

## The effect of weather to drought severity

Soil moisture content between well-watered and drought stress of both years is clearly different at each soil depths (Figure 2). However, the soil moisture contents under drought stress are lower in first year than in second year. It is due to higher rainfall after the water is drained out (Figure 1). The soil moisture sharply increases after rewatering especially in dry soil (Xu and Zhou, 2011). Forty millimeters of rainfall after water is drained out in 2013 are the major effect to low yield reduction (Figure 4) and great root

**Table 4.** Root length (cm plant$^{-1}$), root surface area (cm$^{2}$plant$^{-1}$), average root diameter (mm), root volume (cm$^{3}$plant$^{-1}$) and drought tolerant index (DTI) of all traits of 20 KDML105 CSSLs and parental lines (KDML105, DH103 and DH212) responses to drought stress in 2013.

| Genotype | Root length | | | Root surface area | | | Average root diameter | | | Root volume | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WW | DS | DTI | WW | DS | DTI | WW | DS | DTI | WW | DS | DTI |
| CSSL#1 | 2436 | 4375 b-g | 1.80 | 303.0 | 577.5 a-h | 1.91 | 0.43 | 0.36 d-f | 0.82 | 4.00 | 4.10 a-f | 1.06 |
| CSSL#2 | 3651 | 5880 a-c | 1.64 | 490.2 | 672.4 a-f | 1.38 | 0.35 | 0.40 a-f | 1.16 | 4.00 | 3.75 c-g | 0.93 |
| CSSL#3 | 4471 | 5615 a-d | 1.25 | 602.6 | 699.4 a-d | 1.16 | 0.42 | 0.39 b-f | 1.00 | 4.50 | 3.90 b-g | 0.92 |
| CSSL#4 | 4010 | 3152 g | 0.78 | 506.8 | 397.5 h | 0.79 | 0.46 | 0.43 a-d | 1.00 | 4.00 | 3.75 c-g | 0.92 |
| CSSL#5 | 3859 | 4160 c-g | 1.08 | 468.3 | 527.1 c-h | 1.12 | 0.52 | 0.36 d-f | 0.69 | 4.00 | 4.10 a-f | 1.01 |
| CSSL#6 | 3945 | 3739 d-g | 0.95 | 518.4 | 544.0 b-h | 1.07 | 0.49 | 0.44 ab | 0.91 | 4.50 | 4.55 ab | 1.02 |
| CSSL#7 | 4555 | 6586 a | 1.44 | 618.7 | 773.4 a | 1.25 | 0.51 | 0.39 a-f | 0.76 | 4.50 | 3.80 b-g | 0.88 |
| CSSL#8 | 4879 | 4777 a-g | 1.06 | 609.1 | 547.9 a-h | 0.94 | 0.41 | 0.37 c-f | 0.91 | 4.00 | 3.80 b-g | 0.86 |
| CSSL#9 | 3472 | 4735 a-g | 1.36 | 520.8 | 459.5 e-h | 0.89 | 0.48 | 0.39 b-f | 0.81 | 5.00 | 3.50 d-g | 0.70 |
| CSSL#10 | 2793 | 6022 a-c | 2.14 | 425.4 | 767.2 ab | 1.90 | 0.43 | 0.33 f | 0.81 | 5.00 | 4.35 a-c | 0.90 |
| CSSL#11 | 3607 | 5066 a-f | 1.45 | 489.0 | 613.8 a-h | 1.29 | 0.46 | 0.45 ab | 0.99 | 4.00 | 3.80 b-g | 0.88 |
| CSSL#12 | 2800 | 4549 b-g | 1.64 | 454.5 | 522.3 c-h | 1.16 | 0.50 | 0.40 a-f | 0.82 | 5.00 | 3.65 c-g | 0.69 |
| CSSL#13 | 3563 | 5778 a-c | 1.63 | 394.6 | 636.7 a-g | 1.67 | 0.47 | 0.36 c-f | 0.79 | 3.50 | 3.50 d-g | 1.00 |
| CSSL#14 | 3343 | 4713 a-g | 1.41 | 392.8 | 513.9 c-h | 1.35 | 0.49 | 0.39 b-f | 0.80 | 3.50 | 3.45 e-g | 0.94 |
| CSSL#15 | 2325 | 4480 b-g | 2.78 | 364.4 | 481.8 d-h | 2.16 | 0.51 | 0.47 a | 0.90 | 4.50 | 3.55 d-g | 0.78 |
| CSSL#16 | 3120 | 3351 fg | 1.10 | 545.3 | 417.4 gh | 0.83 | 0.49 | 0.38 b-f | 0.78 | 5.50 | 4.25 a-d | 0.76 |
| CSSL#17 | 3292 | 4686 b-g | 1.53 | 463.7 | 473.7 d-h | 1.05 | 0.37 | 0.38 b-f | 1.04 | 4.50 | 3.35 fg | 0.77 |
| CSSL#18 | 3209 | 6080 ab | 2.00 | 504.3 | 731.1 a-c | 1.55 | 0.44 | 0.37 c-f | 0.83 | 5.00 | 3.75 c-g | 0.76 |
| CSSL#19 | 4829 | 5374 a-e | 1.12 | 673.0 | 590.6 a-h | 0.97 | 0.47 | 0.33 f | 0.70 | 4.00 | 3.25 g | 0.81 |
| CSSL#20 | 3665 | 3076 g | 0.96 | 534.7 | 445.8 f-h | 0.91 | 0.46 | 0.43 a-e | 0.95 | 5.00 | 4.85 a | 0.96 |
| KDML105 | 3307 | 5088 a-f | 1.66 | 499.6 | 680.7 a-e | 1.40 | 0.41 | 0.33 f | 0.81 | 5.00 | 4.20 a-e | 0.85 |
| DH103 | 3041 | 3697 e-g | 1.29 | 475.2 | 446.1 f-h | 1.03 | 0.39 | 0.35 ef | 0.90 | 4.50 | 3.65 c-g | 0.76 |
| DH212 | 3669 | 4847 a-g | 1.32 | 458.2 | 624.8 a-h | 1.44 | 0.44 | 0.43 a-e | 0.97 | 4.00 | 3.80 b-g | 0.99 |
| Max | 4879 | 6586 | 2.78 | 673.0 | 773.4 | 2.16 | 0.52 | 0.47 | 1.16 | 5.50 | 4.85 | 1.06 |
| Min | 2325 | 3076 | 0.78 | 330 | 397 | 0.79 | 0.35 | 0.33 | 0.69 | 3.50 | 3.25 | 0.69 |
| Mean | 3558 | 4793 | 1.45 | 491.8 | 571.2 | 1.27 | 0.45 | 0.39 | 0.88 | 4.41 | 3.85 | 0.87 |
| F-test | ns | * | | ns | * | | ns | * | | ns | * | |
| CV(%) | 24.93 | 19.18 | | 30 | 19.31 | | 17.44 | 9.9 | | 17.99 | 9.62 | |
| $P < 0.05$ | | | | | | | | | | | | |
| W | | 34050000** | | | 145987** | | | 0.096** | | | 7.1** | |
| G | | 1646567* | | | 22236ns | | | 0.004ns | | | 0.6ns | |
| W*G | | 1159087ns | | | 17616ns | | | 0.002ns | | | 0.2ns | |
| CV (%) | | 21.41 | | | 24.24 | | | 14.73 | | | 15.13 | |

* and ** significant different at the level of 0.05 and 0.01 respectively.

**Table 5.** Sum of squares in combined analysis of variance for root length (cm plant$^{-1}$), root surface area (cm$^2$ plant$^{-1}$), root diameter (mm) root volume (cm$^3$ plant$^{-1}$), shoot dry weight (g plant$^{-1}$), root dry weight (g plant$^{-1}$) and root: shoot ratio of twenty CSSLs of rice CV. KDML105 and their parents under well watered and drought stress in 2012 and 2013.

| Source of variance | DF | Root length (cm plant$^{-1}$) | | Root surface area (cm$^2$ plant$^{-1}$) | | Root diameter (mm) | | Root volume (cm$^3$ plant$^{-1}$) | | Shoot dry weight (g plant$^{-1}$) | | Root dry weight (g plant$^{-1}$) | | Root: shoot ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year (Y) | 1 | 132400000 | (16.01)$^{ns}$ | 40462 | (1.08)$^{ns}$ | 2.27 | (32.31)$^{**}$ | 659.35 | (75.01)$^{**}$ | 6747.1 | (35.88)$^{**}$ | 0.70 | (9.70)$^{ns}$ | 0.056 | (27.92)$^{ns}$ |
| Rep / year | 2 | 42780000 | (5.17) | 50140 | (1.33) | 0.04 | (0.56) | 0.02 | (0.00) | 202.4 | (1.08) | 0.11 | (1.57) | 0.014 | (6.93) |
| Water (W) | 1 | 127300000 | (15.39)$^{**}$ | 202960 | (5.39)$^{**}$ | 0.28 | (4.02)$^{**}$ | 4.52 | (0.51)$^{**}$ | 1171.6 | (6.23)$^{**}$ | 0.33 | (4.59)$^{**}$ | 0.043 | (21.53)$^{**}$ |
| Genotype (G) | 22 | 60510000 | (7.32)$^{ns}$ | 500675 | (13.30)$^{ns}$ | 0.29 | (4.16)$^{ns}$ | 17.15 | (1.95)$^{ns}$ | 1079.3 | (5.74)$^{ns}$ | 0.60 | (8.30)$^{ns}$ | 0.008 | (3.73)$^{ns}$ |
| Y*W | 1 | 9191446 | (1.11)$^{ns}$ | 8070 | (0.21)$^{ns}$ | 0.01 | (0.12)$^{ns}$ | 3.05 | (0.35)$^{ns}$ | 908.1 | (4.83)$^{**}$ | 0.01 | (0.08)$^{ns}$ | 0.029 | (14.28)$^{**}$ |
| Y*G | 22 | 71440000 | (8.64)$^{ns}$ | 418434 | (11.12)$^{ns}$ | 0.37 | (5.33)$^{ns}$ | 16.40 | (1.87)$^{ns}$ | 1346.0 | (7.16)$^{ns}$ | 0.65 | (9.08)$^{ns}$ | 0.005 | (2.42)$^{ns}$ |
| W*G | 22 | 96130000 | (11.62)$^{*}$ | 594483 | (15.80)$^{ns}$ | 0.77 | (10.90)$^{ns}$ | 41.10 | (4.68)$^{*}$ | 1132.9 | (6.03)$^{ns}$ | 1.02 | (14.11)$^{ns}$ | 0.007 | (3.33)$^{ns}$ |
| Y*W*G | 22 | 71770000 | (8.68)$^{ns}$ | 444127 | (11.80)$^{ns}$ | 0.68 | (9.71)$^{ns}$ | 40.48 | (4.60)$^{*}$ | 1242.9 | (6.61)$^{ns}$ | 0.83 | (11.55)$^{ns}$ | 0.009 | (4.26)$^{ns}$ |
| Error | 90 | 215400000 | (26.05) | 1504254 | (39.97) | 2.31 | (32.90) | 96.90 | (11.02) | 4971.7 | (26.44) | 2.96 | (41.02) | 0.031 | (15.59) |
| Total | 183 | 827000000 | | 3763607 | | 7.03 | | 878.97 | | 18802.1 | | 7.21 | | 0.201 | |

Numbers in the parentheses are percent (%) of sum squares to total sum of squares.
ns, *,** Non significant, significant and highly significant at $P \leq 0.05$ and $\leq 0.01$ probability levels, respectively.

**Table 6.** Correlation analyses among traits of CSSLs and their parents under drought stress in 2012 and 2013.

| Parameters | Shoot dry weight | Root dry weight | Root shoot ratio | Grain yield |
|---|---|---|---|---|
| 2012 | | | | |
| Root length | 0.31$^{ns}$ | 0.62** | 0.10$^{ns}$ | 0.24$^{ns}$ |
| Root surface area | 0.61** | 0.76** | -0.20$^{ns}$ | 0.41* |
| Root diameter | 0.69** | 0.44* | -0.71** | 0.26$^{ns}$ |
| Root volume | 0.87** | 0.72** | -0.60** | 0.46* |
| 2013 | | | | |
| Root length | 0.23$^{ns}$ | 0.65** | 0.22$^{ns}$ | -0.50* |
| Root surface area | 0.56** | 0.90** | 0.05$^{ns}$ | -0.14$^{ns}$ |
| Root diameter | -0.04$^{ns}$ | -0.09$^{ns}$ | 0.07$^{ns}$ | -0.11$^{ns}$ |
| Root volume | 0.58** | 0.24$^{ns}$ | -0.38$^{ns}$ | 0.40$^{ns}$ |

ns, *,** Non significant, significant and highly significant at $P \leq 0.05$ and $\leq 0.01$ probability levels, respectively.

traits. The rewatering or rainfall after water deficit mainly improve root growth, grain quality, and grain yield (Yang *et al*., 2017). The grain yield of rice is mainly caused by soil moisture content especially under drought conditions. The low yield reduction in 2013 is mainly due to 40 mm. of rainfall after water stress treatment and high soil moisture content (Figures 1 and 2). Sometimes, the aerobic soil condition contributes to high grain yield than anaerobic condition in rice production system (Zainudin *et al*., 2014) as well as the intermittent drought condition showed lower grain yield reduction than terminal drought condition (Monkham *et al*., 2015). Previous study of (Kanjoo *et al*., 2012) showed CSSLs with the DT-QTL on chromosome 4 and 8 had higher performance in yield under drought and well-watered than KDML105 (check cultivar). In this study, it was confirmed that CSSLs #14 and #16 have consistently higher grain yield and lower yield reduction than KDML105 under drought stress in both years (Figure 4), and these lines carried the DT-QTL on chromosome 3 and 8, respectively. The result suggests that both lines could be well adapted under both severe and mild stress in 2012 and 2013 based on grain yield. Moreover, the stress condition affected the root trait such as the pattern of RLD (Figures 5 and 6). The severity of water stress influenced the high RLD in deeper soil layer (15.24%) compared to mild stress in 2013 (8.38%). Water deficit determined RLD distributions accurately, especially in the field, because the distributions change with different soils moisture content (Zuo *et al*., 2006). More than 90% of roots are embedded in shallow soil layer under mild stress condition (2013) because of the moisture zone (0-15 cm depth). Therefore, the rewatering after a short period of water deficit could affect grain yield and root length density especially the high ratio of root at 0-15 cm depth.

**The role of root to shoot of CSSLs**

Our results show significant variation among the CSSLs for shoot and root dry weight. Drought stress reduced the shoot dry weight (Tables 1 and 3) similar to previous reports (Kano *et al*., 2011) and (Champoux *et al*., 1995). Moreover, the performance of root to shoot ratio was higher in flooded than in aerobic conditions (Kato *et al*., 2010). The results suggest that shoot dry weight is correlated with root traits such as root surface area and root volume under drought stress (Table 6). Root dry weight could be contributed to shoot growth of this CSSLs population. Similar result was observed that deep roots contributed to biomass (Comas *et al*., 2013). Root characteristics are believed to play a significant role in the drought resistance mechanism by absorbing greater amount of water from deeper soil layers. Moreover, drought has an effect to P-deficiency, the ability of root extraction is efficient to biomass accumulation and grain yield (Kato *et al*., 2016). This could be due to root traits did not change when drought occurred at panicle initiation (Lilley and Fukai, 1994). CSSLs chro.4 show higher ability than KDML105 in increasing the total root length under severe stress and mild stress (Tables 2 and 4). Some researchers reported that primary root traits such as deep and thick roots contributed to drought avoidance to maintain plant water status and often found to be more important for higher yield under stress than tolerance mechanism (Kamoshita *et al*., 2008).

Root surface area is an important parameter for nodule development and absorption of water and nutrients (Ansari *et al*., 1995). Most of CSSLs tended to increase root surface area under drought stress because of increased absorption area by root than KDML105 (Tables 2 and 4). Moreover, the root surface area is highly correlated with shoot dry weight. The CSSLs #6 and #9 showed great root surface area than check varieties in 2012 and #1 and #15 in 2013. With thick and deep root system, plants can gain better access to water and show higher drought tolerance (Jeon *et al*., 2011). The rice varieties with thicker roots are more tolerant to drought than those with thin roots (O'Toole and Chang, 1979). The results of this study showed that the root diameter of the CSSLs had a high correlation with shoot dry weight under severe drought stress (2012). Small xylem diameters in targeted seminal roots could save water in deep profile for crop maturation and improved yields (Comas *et al*., 2013). Upland accessions have thick root and high deep root dry matter, which are beneficial to breeding for drought resistance in rice (Thanh *et al*., 1999). Moreover, deep rooting and root branching are useful for drought selection (Kato *et al*., 2013). Drought stress increased the root length density in deeper soil layers compared to well-watered (Figures 5 and 6). The response in root length density is better in most CSSLs which is similar to KDML105 under severe stress while average root diameter is reduced. This is probably caused by lateral root branching and root elongation. KDML105 is more plastic in root growth under drought condition as it has good root branching in the upper soil layer (Kameoka *et al.*, 2016). Similar observation has also been reported in other crops such as wheat (Wang *et al*., 2014), chickpea (Kashiwagi *et al*., 2006), and peanut (Jongrungklang *et al*., 2011).

The increase in root volume under severe drought stress affected the grain yield and high root length is less useful under mild stress (Table 6). Morphological traits seem to influence total root length, surface area, root diameter, and root tissue density (Comas *et al*., 2013). Thus, root traits could be used as criterions in breeding for drought resistance in rice. Plants have the ability to survive from limited water conditions if high root length and root length density in deeper soil layers and good adaptation for root diameter, volume, and surface area are integrated through rice breeding for drought tolerance program.

**DTI and root response of different drought types in the field**

DTI were the effective of genotypes under each stress but high value of each traits was not showed the ability of genotype. Therefore, the high effective genotypes should be selected. The good DTI compared with KDML105 should be suitable for root increasing trait under drought condition. In this study, there were no significant differences among genotypes for almost all root traits. However, the DTI > 1 in most of root traits indicated that roots would be activated by stress condition. Since selection for root traits are rather difficult and with high variability, DTI would be a good alternative selection criteria for drought tolerance. In 2012, CSSLs #6 and #9 had better total root dry weights than checks and contributed to high DTI in root length and root surface area. Even though, the severe drought stress had no effect to the root traits but the DTI was quite different between lines. Under mild stress in 2013, most of the root traits such as root length, root surface area, root diameter, and root volume were significantly different under stress condition. The difference in the performance of DTI in each trait showed different line rankings but CSSLs #10 and #15 have good DTI for root length and root surface area. However, high root length was negatively correlated with grain yield ($r$ = -0.50*) under mild stress in which DTI (root length) was closely related with root length under stress ($r$ = 0.46*). Therefore, the low grain yield under stress due to sink strength under water deficit condition slowed down the carbohydrate synthesis (Davatgar *et al*., 2009), which translocated to the root rather than the shoot and affected the grain yield. However, the good DTI could show the potential of traits under drought stress condition and its usefulness as selection criteria.

In conclusion, drought stress induced high root length density at top soil layer and affected grain yield and yield reduction than root dry weight. Moreover, difference in level of drought stress caused different responses. Root traits respond to late season drought for rooting depth associated with grain yield under severe stress. Moreover, most root traits showed relationship with shoot dry weight. Some CSSLs having a large root system could maintain grain yield under drought condition and DTI was used as the selection criteria in terms of genotype response. The CSSLs #6, #9, #10, and #15 are the genotypes that have good root adaption such as root length, root surface area, root diameter, and low yield reduction under different drought stress which could be used as germplasm for further breeding program.

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## REFERENCES

Ansari SA, Kumar A, Gupta BN (1995). Root surface area measurements based on adsorption and desorption of nitrite. *Plant Soil* 175: 133-137.

Bocco R, Lorieux M, Seck PA, Futakuchi K, Manneh B, Baiey H, Ndjiondjop MN (2012). Agro-morphological characterization of a population of introgression lines derived from crosses between IR64 (*Oryza sativa* indica) and TOG5681 (*Oryza glaberrima*) for drought tolerance. *Plant Sci*. 183: 65-76.

Bricker AA (1989). MSTAT-C user's guide. Michigan State University.

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC , Huang N, McCouch SR (1995). Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theor. Appl. Genet.* 90(7): 969-981.

Comas LH, Becker SR, Cruz VMV, Byrne PF, Dierig DA (2013). Root traits contributing to plant productivity under drought. *Front Plant Sci*. 4: 1-16.

Davatgar N, Neishabouri MR, Sepaskhah AR, Soltani A (2009). Physiological and morphological responses of rice (*Oryza sativa* L.) to varying water stress management strategies. *Int. J. Plant Prod*. 3(4): 19-32.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research 2nd ed., John Wiley and Son, New York.

Javahar RP (2012). Overview of literature on waterlogging and salinity interaction. In: R.P. Javahar, ed., *Physiological and anatomical implications of salinity on rice as a semi-aquatic species*. Cambridge Scholars Publishing, Newcastle upon Tyne, pp. 5-60.

Jeon JS, Jung KH, Kim HB, Suh JP, Khush GS (2011). Genetic and molecular insights into the enhancement of rice yield potential. *Plant Biol*. 54(1): 1-9.

Jie C, Da-zhou C, Guang-jie L, Kang-le Z, Jie-yun Z (2006). Development of chromosome segment substitution lines from a backcross recombinant inbred lines population of interspecific rice cross. *Rice Sci*. 13(1): 15-21.

Jongrungklang N, Toomsan B, Vorasoot N, Jogloy S, Boote KJ, Hoogenboom G, Patanothai A (2011). Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress. *Field Crops Res*. 120: 262-270.

Kameoka E, Suralta RR, Mitsuya S, Yamauchi A (2016). Developmental plasticity of rice root system grown under mild drought stress condition with shallow soil depth; comparison between nodal and lateral roots. *Plant Prod. Sci.* 19(3): 411–419.

Kamoshita A, Babu RC, Boopathi NM, Fukai S (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Res*. 109: 1-23.

Kanjoo V, Punyawaew K, Siangliw JL, Jearakongman S, Vanavichit A, Toojinda T (2012). Evaluation of agronomic traits in chromosome segment substitution lines of KDML105 containing drought tolerance QTL under drought stress. *Rice Sci*. 19(2): 117-124.

Kano M, Inukai Y, Kitano H, Yamauchi A (2011). Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. *Plant Soil* 342: 117-128.

Kashiwagi J, Krishnamurthy L, Crouch JH, Serraj R (2006). Variability of root length and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. *Field Crops Res*. 95: 171-181.

Kato Y, Ryosuke T, Homma K, Toriumi A, Yamagishi J, Shiraiwa T, Mekwatanakarn P, Jongdee B (2013) Root growth response of rainfed lowland rice to aerobic conditions in northeastern Thailand. *Plant Soil.* 368: 557–567.

Kato Y, Tajima R, Toriumi A, Homma K, Moritsuka N, Shiraiwa T, Yamagishi J, Mekwatanakern P, Chamarerke V, Jongdee B (2016). Grain yield and phosphorus uptake of rainfed lowland rice underunsubmerged soil stress. *Field Crops Res*. 190: 54–59.

Kato Y. Okami M (2010). Root growth dynamics and stomatal behavior of rice (*Oryza sativa* L.) grown under aerobic and flooded conditions. *Field Crops Res*. 117: 9-17.

Li J, Wang D, Xie Y, Zhang H, Hu G, Li J, Dai A, Liu L, Li Z (2011). Development of upland rice introgression lines and identification of QTLs for basal root thickness under different water regimes. *J Genet Genom.* 38: 547-556.

Lilley JM, Fukai S (1994). Effect of limiting and severity of water deficit on four diverse rice cultivars. I. Rooting pattern and soil water extraction. *Field Crop Res*. 37: 205-213.

Monkham T, Jongdee B, Pantuwan G, Sanitchon J, Mitchell JH, Fukai S (2015). Genotypic variation in grain yield and flowering pattern in terminaland intermittent drought screening methods in rainfed lowland rice. *Field Crops Res*. 175: 26-36.

Niones JM, Suralta RR, Inukai Y, Yamauchi A (2012). Field evaluation on functional roles of root plastic responses on dry matter production and grain yield of rice under cycles of transient soil moisture stresses using chromosome segment substitution lines. *Plant Soil*. 359: 107-120.

O'Toole JC, Chang TT (1979). Drought resistance in cereals-rice: a case study. In: H. Mussell, and R.C. Staples, eds., *Physiology of Crop Plants*. John Wiley and Sons, New York, pp. 374-405.

Pandey S, Bhandari H (2008). Drought: economic costs and research implication. In: R. Serraj. J. Bennett and B. Hardy, eds., *Drought frontiers in rice: crop improvement for increased rainfed production*. World Scientific: International Rice Research Institute, Philippines, pp. 3-18.

Polthanee A, Promkhumbut A, Bamrungrai J (2014). Drought impact on rice production and farmer's adaptation strategies in Northeast Thailand. *IJERD*. 45-52.

Raman A, Satish VB, Nimai MP, Mukund VV, Shukla DJ, Dwivedi LB, Singh NO, Singh N, Padmini S, Ashutosh MK, Robin S, Chandrababu R, Jain A, Ram T, Hittamani S, Haefele S, Piepho HP, Kumar A (2012). Drought yield index to select high yielding rice lines under different drought stress severities. *Rice*. 5(31): 1-12.

Siopongco JD, Yamauchi A, Salekdeh H, Wade LJ (2005). Root growth and water extraction response of doubled-haploid rice lines to drought and rewatering during the vegetative stage. *Plant Prod Sci*. 8(5): 497-508.

Thanh ND, Zheng HG, Dong NV, Trinh LN, Ali ML, Nguyen HT (1999). Genetic variation in root morphology and microsatellite DNA loci in upland rice (*Oryza sativa* L.) from Vietnam. *Euphytica*. 105: 43-51.

Wang C, Liu W, Li Q, Ma D, Lu H, Feng W, Xie Y, Zhu Y, Guo T (2014). Effects of different irrigation and nitrogen regimes on root growth and its correlation with above-ground plant parts in high-yielding wheat under field conditions. *Field Crops Res*. 165: 138-149.

Welbank PJ, Gibb MJ, Taylor PJ, Williams ED (1974). Root growth of cereal crops. In: Rothamsted Experimental Station Annual Report 1973, Part 2. Rothamsted Experimental Station, Harpenden, UK, pp. 26–66.

Xu Z, Zhou G (2011). Responses of photosynthetic capacity to soil moisture gradient in perennial rhizome grass and perennial bunchgrass. *BMC Plant Biol*. 11:21.

Yang J, Zhou Q, Zhang J (2017). Moderate wetting and drying increases rice yield and reduces water use grain arsenic level and methane emission. *Crop J*. 5: 151-158.

Zainudin H, Sariam O, Chan CS, Azmi M, Saad A, Alias I, Marzukhi H (2014). Performance of selected aerobic rice varieties cultivated under local condition. *J. Trop. Agric. and Fd. Sc*. 42(2): 175-182.

Zuo Q, Shi J, Li Y, Zhang R (2006). Root length density and water uptake distributions of winter wheat under sub-irrigation. *Plant Soil*. 285: 45–55.

## APPENDIX

**Supplementary Table 1.** The genotypes and pedigree of all lines which cross between KDML105 and IR68586-F2-CA-143 (DH212) and KDML105 and IR68586-F2-CA-31 (DH103) and there trait carrying.

| Genotypes | Pedigree | Substitution segment | Chrom. | Donor | Size of DT segment | Traits |
|---|---|---|---|---|---|---|
| CSSL#1 | RGD05164-11-MAS39 | RM212 - RM3362 | 1 | DH212 | | |
| CSSL#2 | RGD05164-11-MAS25 | RM212 - RM3362 | 1 | DH212 | 49 cM | PN, PH, LWP, |
| CSSL#3 | RGD05164-11-MAS10 | RM212 - RM3362 | 1 | DH212 | | DS, CT |
| CSSL#4 | RGD05164-11-MAS18 | RM212 - RM3362 | 1 | DH212 | | |
| CSSL#5 | RGD05169-2-MAS12 | RM3413 - RM3807 | 3 | DH212 | | |
| CSSL#6 | RGD05162-3-MAS56 | RM3413 - RM3807 | 3 | DH212 | 14.8 cM | GY, HI, |
| CSSL#7 | RGD05162-3-MAS44 | RM3413 - RM3807 | 3 | DH212 | | DFAIG |
| CSSL#8 | RGD05162-8-MAS41 | RM3413 - RM3807 | 3 | DH212 | | |
| CSSL#9 | RGD05131-4-MAS39 | RM142 - RM559 | 4 | DH212 | | |
| CSSL#10 | RGD05131-6-MAS5 | RM142 - RM559 | 4 | DH212 | 53 cM | GY, TSN |
| CSSL#11 | RGD05128-10-MAS12 | RM142 - RM559 | 4 | DH212 | | PSS, PN |
| CSSL#12 | RGD05128-4-MAS40-MAS11 | RM142 - RM559 | 4 | DH212 | | |
| CSSL#13 | RGD06063-69-MAS24 | RM5353 - RM3480 | 8 | DH103 | | |
| CSSL#14 | RGD06064-6-MAS52 | RM5353 - RM3480 | 8 | DH103 | 60 cM | BY, PSS, |
| CSSL#15 | RGD06064-6-MAS16-MAS2 | RM5353 - RM3480 | 8 | DH103 | | PN, PH |
| CSSL#16 | RGD06064-26-MAS45-MAS8 | RM5353 - RM3480 | 8 | DH103 | | |
| CSSL#17 | RGD05160-6-MAS29 | RM242 - RM205 | 9 | DH212 | | |
| CSSL#18 | RGD05157-5-MAS8 | RM242 - RM205 | 9 | DH212 | 30 cM | BY, HI, DS |
| CSSL#19 | RGD05159-4-MAS56 | RM242 - RM205 | 9 | DH212 | | |
| CSSL#20 | RGD05159-4-MAS52-MAS4 | RM242 - RM205 | 9 | DH212 | | |
| | KDML105 | | Recurrent parent | | | |
| | DH103 | | Donor parent | | | |
| | DH212 | | Donor parent | | | |

PN; panicle number, PH; plant height, LWP; leaf water potential, DS; drought score, CT; canopy temperature, GY; grain yield, HI; harvest index, DFAIG; days to flowering after initiation of irrigation gradient, TSN; total spikelet number, PSS; percent spikelet sterility, PN; panicle number, BY; biological yield (Kanjoo et al., 2012).