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Variation in Root Development Response of Napier Grass to Drought Stress

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ABSTRACT

Global climate change and increasing agricultural activity are the main causes of biotic and abiotic stresses, which negatively affect the plant growth and crop yields. The plant root system is the first organ for sensing the soil moisture limitation; therefore root growth under elevated water deficit is an important indicator for plant's drought tolerance. Although the previous studies focused on the morphological traits of Napier grasses under water stresses, the root growth changes due to drought levels remain largely unclear. In order to evaluate variation in root performance to respond to drought stress, four cultivars named "Cỏ voi thuần" (CVT), King grass, Packchong, and VA06 were grown for 10 days under drought conditions under polyethylene glycol 6000 (PEG6000): 0% PEG6000 as control, 5% PEG6000, 10% PEG6000, 15% PEG6000 and 20% PEG6000. As compared to control, the root growth of all cultivars was reduced under drought treatments; however, significant variation in the root development response to drought levels was found. Among Napier cultivars, "Cỏ voi thuần" expressed drought-tolerant genotypes. The information on the root length, diameter, surface area and volume of the cultivars reveals interesting guidelines for further studies to explore the mechanisms behind root adaptation of Napier grasses to drought.

Keywords: Drought levels, Napier grasses, root response, root length.

INTRODUCTION

Global climate change and increasing agricultural activity are the main causes of biotic and abiotic stresses, which negatively affect the plant growth and crop yields [Raza et al., 2019]. Abiotic stresses such as salinity, cold, waterlogging and drought have adverse impacts on crop yield and about 50–70% of crop yield reduction is attributed to such abiotic stresses [Francini & Sebastiani, 2019]. Drought stress is one of the most severe abiotic stresses that directly affect the growth and development of crop thus affecting its productivity [Yavad et al., 2019]. For instance, a recently published report indicates that between 1983– to 2005, 75% of global cultivated land (454 million hectares) experience drought-induced yield losses, which account to about 166 billion United States dollars [Kim et al., 2019].

The impact of drought on major crops has been reported in several studies, including meta-analyses and summary studies. Severe drought-induced crop yield losses of 14.0% and 21.8% were reported for maize and soybean, respectively [Wang et al., 2020]. Under drought, the wheat and rice yields decreased by 27.5% and 25.4%, respectively [Zhang et al., 2018]. Water stress reduced the yield attributes and grain yield in sorghum [Jabereldar et al., 2017]. Farooq et al. [2009] documented the economic yield reduction and critical stages of growth affected by drought stress in barley, maize, rice chickpea, and pigeon pea common beans, sunflower, canola, soybean and potato. Besides field crops, drought stress also affect grasses and fodder crop growth, yield and productivity. Severe drought reduces the nutritive quality of forage legumes [Kuchenmeister et al., 2013, Liu et al., 2018]. Drought decreases the shoot and root biomass, plant height, tiller number and leaf growth of rhizomatous grasses (*Pascopyrum smithii* and *Elymus lanceolatus*) [Zhang et al., 2017]. Guinea (*Panicum maximum*) and Napier (*Pennisetum purpureum* Schumach.) grasses exhibit decreased plant height and herbage mass under drought stress [Purbajanti et al., 2012].

Napier grass (*Pennisetum purpureum* Schumach.), also known as elephant grass, is a major C_4 perennial forage crop grown in many tropical and subtropical regions of the world [Negawo et al., 2017]. Despite being native to East and Central Africa, Napier grass is currently distributed in Central and South America, Asia, Australia, Middle East and Pacific islands [Singh et al., 2013]. The factors behind the wide adoption of Napier grass as a fodder crop include; high forage productivity, rapid regeneration and fast growing characteristics, drought tolerance and high water use efficiency [Purbajanti et al., 2012, Kabirizi et al., 2015]. Napier grass, like many other C_4 plants, has numerous drought-coping adaptation mechanisms [Lopes et al., 2011]. During limited water availability, Napier grass exhibits a larger root system in combination with a less restrictive stomata regulation to maximize carbon assimilation [Cardoso et al., 2015].

Plant root systems play a crucial role in detecting the changes in soil moisture; thus, they develop appropriate drought survival mechanisms [Zhang et al., 2017]. It has been indicated that altering the root structure of crops grown under water stress can increase their yield [Lynch et al., 2014]. In different crops, root traits such as small fine root diameters, specific root length, root length density, specific root area and root angle have been suggested as desirable traits for improving plant productivity under drought stress [Comas et al., 2013, Wasaya et al., 2018]. Many studies on crops such as wheat, cow pea and rice have focused on the variation in root responses of cultivars to drought [Nguyen et al., 2015, Santos et al., 2020, Figueroa-Bustos et al., 2020, Kim et al., 2020, Fang et al., 2021]. In Napier grass, a few studies have focused on germplasm screening for water use efficiency under drought stress [Mwendia et al., 2016, Habte et al., 2019]. However, little is known about the root development response to drought stress in Napier grass. Therefore, in this study, four Napier grass varieties were assessed for their genetic variation in root development responses under drought stress.

MATERIALS AND METHODS

Materials

Four Napier cultivars named "Cỏ voi thuần" (CVT), King grass, Packchong and VA06 were used in this study. For each Napier grass, uniform stem cuttings were used, having 2 nodes that were 25 cm in length, 35 g in weight and 1.5 cm in diameter.

Experimental design

In order to induce root and bud development, the cuttings of each cultivar were inserted in humid sandy soil for 3 days. Then they were transplanted and fixed into position in the polystyrene board with soft silicone rubber. The board with cuttings was then placed and floated on a modified Kimura B nutrient solution (composed of 0.36 mMCa $(NO_3)_2{\cdot}4H_2O$, 0.36 mM $(NH_4)_2SO_4$, 0.18 mM KH_2PO_4, 0.18 mM KNO_3, 0.54 mM $MgSO_4{\cdot}7H_2O$, 40 μM,Fe(III)-EDTA, 18.8 μM H_3BO_3, 13.4 μM $MnCl_2{\cdot}4H_2O$, 0.32 μM $CuSO_4{\cdot}5H_2O$, 0.3 μM $ZnSO_4{\cdot}4H_2O$, and 0.03 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$)) in a plastic container (61 cm × 42 cm × 20 cm). The root system in container was continuously aerated by two air pumps 1.0 L min^{-1} at opposite ends of the container. Seven days after transplanting (DAT), the plants were treated for 10 days under drought levels. In this study, polyethylene glycol 6000 was used to simulate drought tress. Five drought stress levels were 0% PEG6000 (control), 5% PEG6000, 10% PEG6000, 15% PEG6000 and 20% PEG6000 in the Kimura B solution (Fig. 1). The solutions were changed every 3 days to avoid nutrient depletion. The experiments were conducted in a greenhouse and designed with the randomized complete block design with 6 replications (6 boxes) per PEG6000 level treatment. A plant for each genotype in a box was used as a replication for data analysis.

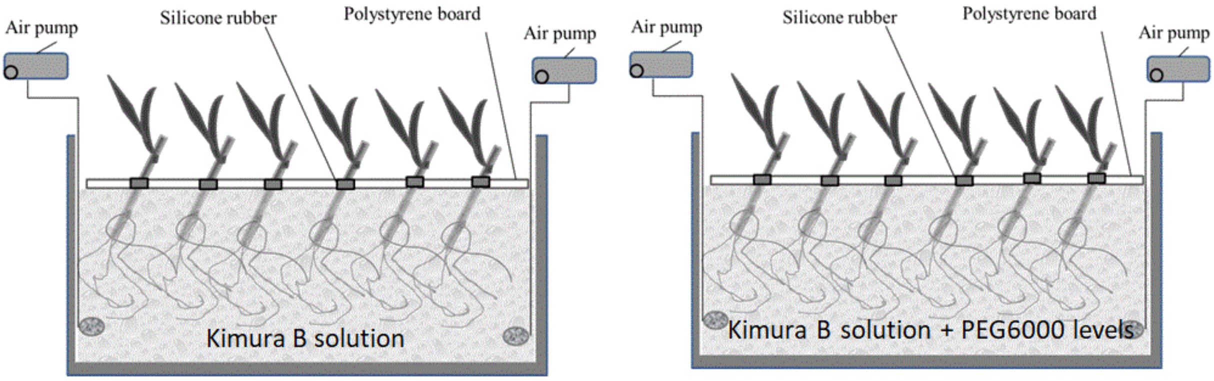

Figure 1. Drought treatments in hydroponic culture method

Data collection

The root growth traits including root length (RL), diameter (RD), surface area (RSA) and volume (RV) were determined after 10 days under drought treatments, according to the method of previous studies [Nguyen et al., 2017; Nguyen et al., 2020]. By using an image scanner (V700/V750 2.08A-Epson), the root systems for each individual plant were imaged. The images were then analyzed by WinRhizo system (Regent Instruments, Inc., Quebec City, Canada) (Fig. 2) to investigate RL, RD, RSA and RV. RL was calculated by skeleton-based root analysis. Roots were classified into ten groups from k_1, k_3 …, k_{10} (mm) with unequal width ($k_1 > 4.5$; $4.0 > k_2 \leqq 4.5$; $3.5 > k_3 \leqq 4.0$; $3.0 > k_4 \leqq 3.5$; $2.5 > k_5 \leqq 3.0$; $2.0 > k_6 \leqq 2.5$; $1.5 > k_7 \leqq 2.0$; $1.0 > k_8 \leqq 1.5$; $0.5 > k_9 \leqq 1.0$; $k_{10} \leqq 0.5$ for RD measurements according to WinRhizo instruction. RSA and RV were determined by the following formulas: $RSA = \pi \times RL \times RD$ and $RV = \pi \times RL \times (\frac{RD}{2})^2$. The shoot dry weight (SDW) and root dry weight (RDW) values were evaluated after drying samples at 80 °C for 72 hours until constant weight.

Statistical analysis

The data were analyzed using R software. The effects of cultivar, drought, cultivar by drought interaction on the measured traits were analyzed by two-way ANOVA; separating mean values by Tukeys's honest significant difference test at P<0.05.

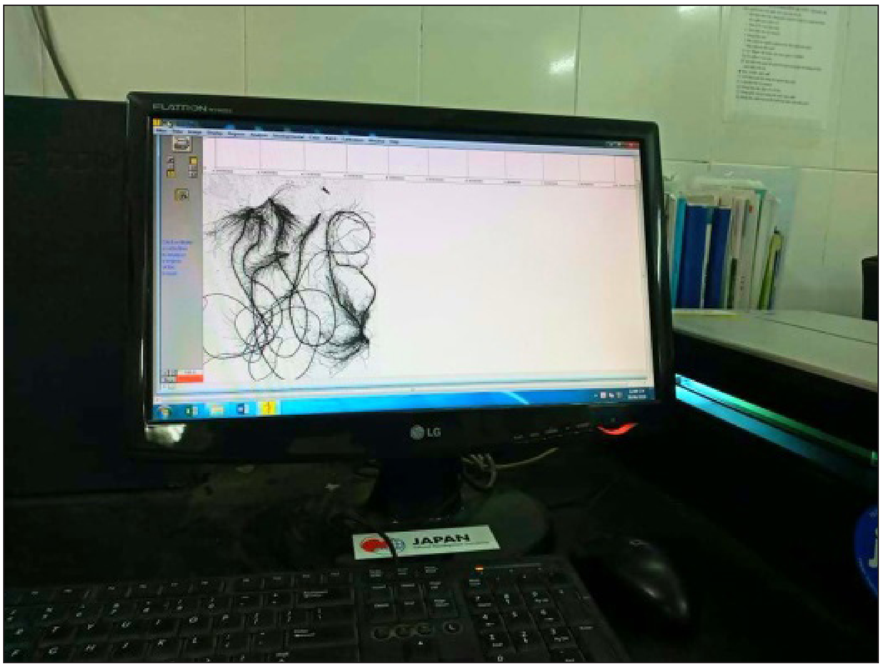

Figure 2. WinRhizo system used to measure root morphological traits

RESULTS

ANOVA analysis

Table 1 shows the data on the effects of cultivar (C), drought treatments (T), and their interaction (T×C) on the measured traits. It was found that C, T, and T×C had significant effects on all measured traits except RD.

Effects of cultivar, drought level and their interaction on root dry weight (RDW, g plant^{-1}) and shoot dry weight (SDW, g plant^{-1})

RDW and SDW of almost cultivars were not significant different at the lower drought levels 5% PEG6000 compared with plants at 0% PEG6000, except for RDW in King grass (Fig. 3 and Fig. 4). Significant reductions were observed in RDW

Table 1. Data of two-way ANOVA analysis on the root and shoot growth traits of the Napier grasses under drought conditions

Traits	F-value		
	Treatments (T)	Cultivar (C)	T x C
Root length (RL, cm)	195.65***	24.27***	18.66***
Root surface area (RSA, cm^2)	74.75***	9.86***	5.90***
Root volume(RV, cm^3)	29.25***	5.02**	2.18*
Root diameter (RD, mm)	1.03	1.01	0.99
Root dry weight (RDW, g)	73.60***	5.68**	6.48***
Shoot dry weight (SDW, g)	38.18***	32.01***	3.82***
Total dry weight (TDW, g)	74.63***	30.59***	6.00***

Significant at *P<0.05, ** P<0.01, and *** P<0.001

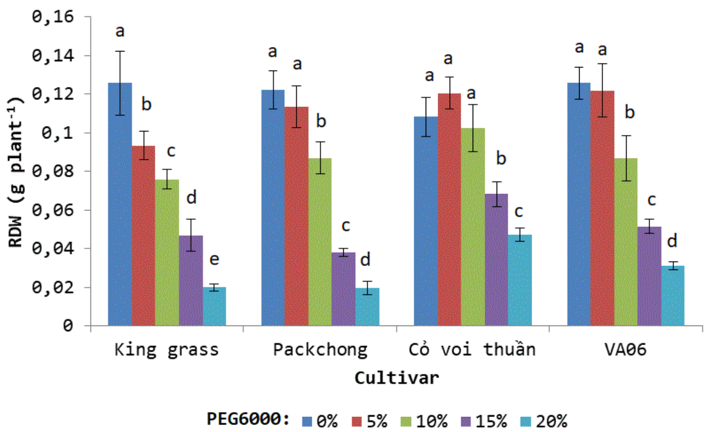

Figure 3. Root dry weight (RDW, g plant^{-1}) of four cultivars under drought conditions. The treatments with different letters in each genotype are significantly different according to LSD-test at p< 0.05

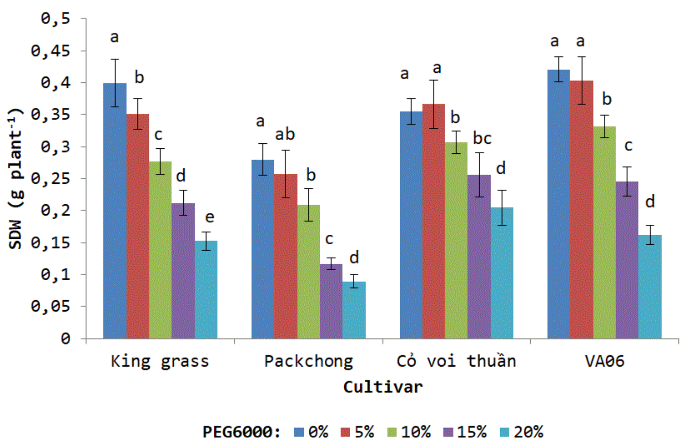

Figure 4. Shoot dry weight (SDW, g plant^{-1}) of four cultivars under drought conditions. The treatments with different letters in each genotype are significantly different according to LSD-test at p< 0.05

and SDW of all cultivars at 10% PEG6000. In the comparison among cultivars, "Cỏ voi thuần" showed higher drought tolerance; while Packchong and King grass were drought-sensitive (Fig. 3 and Fig. 4).

Effects of cultivar, drought level and their interaction on root traits

Significant variations in root trait responses (RL, RSA, and RV) to drought level were found among cultivars (Fig. 5–7, Fig. 9, Table 2–4). Significance difference was not found in RD of cultivars under drought (Fig. 8, Table 5). Similar to RDW and SDW, "Cỏ voi thuần" showed higher drought tolerance; while Packchong and King grass were drought-sensitive (Table 2–4).

DISCUSSION

Napier grass distribution and accessions in various gene banks around the world has been reported [Negawo et al., 2017]. Genetic variation and Napier grass germplasm characterization using various kinds of DNA markers has been reported in several previous studies [Lowe et al., 2003, Kawube et al., 2015]. Similarly, morphological characterization of Napier grass has also been studied. Budiman et al. [2012] evaluated three Napier grass cultivars in regards to their above ground morphological characteristics, such as plant height, stem diameter and leaf stem ratio. However, there are fewer studies on variation on root performance under drought stress in Napier grass. In this study, four cultivars of Napier grass were evaluated for their root response to drought stress using polyethylene glycol 6000 (PEG6000) at 0% (control), 5%, 10%, 15% and 20% drought levels.

The obtained results indicated significant difference in root and shoot growth response to drought among the Napier grass cultivars (Table 1). The four Napier grass cultivars exhibited reduced root length growth as compared to control (0% PEG6000). Among the four cultivars; CVT exhibited the highest root length (RL), RSA and RV development at all drought levels, thus indicating drought-tolerance traits, while King grass and Packchong were drought sensitive (Tables 2, 3 and 4, Figure 3–5). The ability of Napier grass

Table 2. Results of the two-way ANOVA for the effects of drought by cultivar interaction on root length (RL, cm) of Napier grasses

PEG6000	Cultivar	Lsmean	SE	Df	Lower.CL	Upper.CL	.group
20%	Packchong	55.2	38.9	100	-21.89	132	a
20%	King grass	71.6	38.9	100	-5.54	149	ab
20%	VA06	146.6	38.9	100	69.50	224	abc
15%	King grass	243.1	38.9	100	165.94	320	abcd
15%	Packchong	265.8	38.9	100	188.70	343	bcd
20%	CVT	271.9	38.9	100	194.81	349	cd
15%	VA06	391.0	38.9	100	313.87	468	d
15%	CVT	630.5	38.9	100	553.42	708	e
10%	Packchong	705.4	38.9	100	628.24	782	ef
10%	King grass	864.7	38.9	100	787.60	942	fg
10%	VA06	1009.0	38.9	100	931.89	1086	gh
5%	King grass	1077.5	38.9	100	1000.42	1155	hi
0%	VA06	1093.6	38.9	100	1016.50	1171	hi
10%	CVT	1219.7	38.9	100	1142.56	1297	ij
5%	VA06	1246.6	38.9	100	1169.47	1324	ij
0%	CVT	1246.8	38.9	100	1169.66	1324	ij
5%	Packchong	1379.7	38.9	100	1302.62	1457	jk
0%	Packchong	1469.6	38.9	100	1392.43	1547	kl
0%	King grass	1496.7	38.9	100	1419.61	1574	kl
5%	CVT	1637.8	38.9	100	1560.69	1715	l

PEG6000 and Cultivar with different letters are significantly different according to Tukeys's honest significant difference test at P<0.05.

Table 3. Results of the two-way ANOVA for the effects of drought by cultivar interaction on root surface area (RSA, cm^2) of Napier grasses

PEG6000	Cultivar	Lsmean	SE	Df	Lower.CL	Upper.CL	.group
20%	Packchong	4.19	4.92	100	-5.58	14.0	a
20%	King grass	5.41	4.92	100	-4.36	15.2	a
20%	VA06	11.48	4.92	100	2.07	21.6	a
15%	King grass	18.36	4.92	100	8.60	28.1	a
15%	Packchong	21.86	4.92	100	12.09	31.6	a
20%	CVT	22.51	4.92	100	12.74	32.3	a
15%	VA06	29.06	4.92	100	19.29	38.8	ab
15%	CVT	49.94	4.92	100	40.17	59.7	bc
10%	Packchong	58.06	4.92	100	48.29	67.8	cd
10%	King grass	68.94	4.92	100	59.17	78.7	cde
10%	VA06	79.74	4.92	100	69.97	89.5	def
0%	VA06	88.89	4.92	100	79.12	98.7	efg
5%	King grass	90.50	4.92	100	80.73	100.3	efgh
0.1	CVT	92.15	4.92	100	82.38	101.9	efgh
5%	VA06	104.45	4.92	100	94.68	114.2	fghj
0%	CVT	105.49	4.92	100	95.72	115.3	ghj
5%	Packchong	112.38	4.92	100	102.61	122.2	ghj
0%	King grass	115.71	4.92	100	105.94	125.5	hj
0%	Packchong	124.99	4.92	100	115.22	134.8	j
5%	CVT	127.38	4.92	100	117.61	137.2	j

PEG6000 and Cultivar with different letters are significantly different according to Tukeys's honest significant difference test at $P<0.05$.

Table 4. Results of the two-way ANOVA for the effects of drought by cultivar interaction on root volume (RV, cm^3) of Napier grasses

PEG6000	Cultivar	Lsmean	SE	Df	Lower.CL	Upper.CL	.group
20%	Packchong	0.0256	0.05	100	-0.0737	0.125	a
20%	King grass	0.0329	0.05	100	-0.0663	0.132	a
20%	VA06	0.0766	0.05	100	-0.0226	0.176	ab
15%	King grass	0.1114	0.05	100	0.0122	0.211	ab
15%	Packchong	0.1435	0.05	100	0.0443	0.243	abc
20%	CVT	0.1507	0.05	100	0.0515	0.250	abc
15%	VA06	0.1755	0.05	100	0.0762	0.275	abc
15%	CVT	0.3183	0.05	100	0.2190	0.418	bcd
10%	Packchong	0.3842	0.05	100	0.2850	0.483	cde
10%	King grass	0.4448	0.05	100	0.3456	0.544	def
10%	VA06	0.5171	0.05	100	0.4178	0.616	defg
10%	CVT	0.5559	0.05	100	0.4567	0.655	defgh
0%	VA06	0.5805	0.05	100	0.4813	0.680	efgh
5%	King grass	0.6078	0.05	100	0.5086	0.707	efghi
5%	VA06	0.7020	0.05	100	0.6028	0.801	fghi
0%	CVT	0.7164	0.05	100	0.6171	0.816	ghi
0%	King grass	0.7180	0.05	100	0.6188	0.817	ghi
5%	Packchong	0.7325	0.05	100	0.6332	0.832	ghi
5%	CVT	0.7966	0.05	100	0.6973	0.896	hi
0%	Packchong	0.8550	0.05	100	0.7558	0.954	i

PEG6000 and Cultivar with different letters are significantly different according to Tukeys's honest significant difference test at $P<0.05$.

to produce deep roots systems with large amounts of biomass has been previously reported [Sekiya et al., 2013]. These root morphological characters make Napier more tolerant to drought stress than other grass species, in addition to being C4 and perennial in nature [Purbajanti et al., 2012]. Thus, several researchers across the world have been involved in evaluating different Napier grass accessions for water use efficiency and productivity, and this has been mainly crucial in arid and semi-arid regions that are greatly affected by long-term droughts [Nyambati et al., 2010,

Table 5. Results of the two-way ANOVA for the effects of drought by cultivar interaction on root diameter (RD, mm) of Napier grasses

PEG6000	Cultivar	Lsmean	SE	Df	Lower.CL	Upper.CL	.group
15%	VA06	0.237	0.0115	100	0.214	0.260	a
10%	CVT	0.240	0.0115	100	0.217	0.263	a
15%	King grass	0.240	0.0115	100	0.217	0.263	a
20%	King grass	0.242	0.0115	100	0.219	0.265	a
20%	Packchong	0.242	0.0115	100	0.219	0.265	a
0%	King grass	0.245	0.0115	100	0.222	0.268	a
5%	CVT	0.248	0.0115	100	0.225	0.271	a
10%	VA06	0.252	0.0115	100	0.229	0.275	a
15%	CVT	0.253	0.0115	100	0.230	0.276	a
10%	King grass	0.257	0.0115	100	0.234	0.280	a
0%	VA06	0.257	0.0115	100	0.234	0.280	a
20%	VA06	0.258	0.0115	100	0.235	0.281	a
5%	Packchong	0.259	0.0115	100	0.236	0.282	a
15%	Packchong	0.263	0.0115	100	0.240	0.286	a
10%	Packchong	0.263	0.0115	100	0.240	0.286	a
20%	CVT	0.265	0.0115	100	0.242	0.288	a
5%	VA06	0.267	0.0115	100	0.244	0.290	a
5%	King grass	0.268	0.0115	100	0.245	0.291	a
0%	CVT	0.268	0.0115	100	0.245	0.291	a
0%	Packchong	0.270	0.0115	100	0.247	0.293	a

PEG6000 and Cultivar with different letters are significantly different according to Tukeys's honest significant difference test at $P<0.05$.

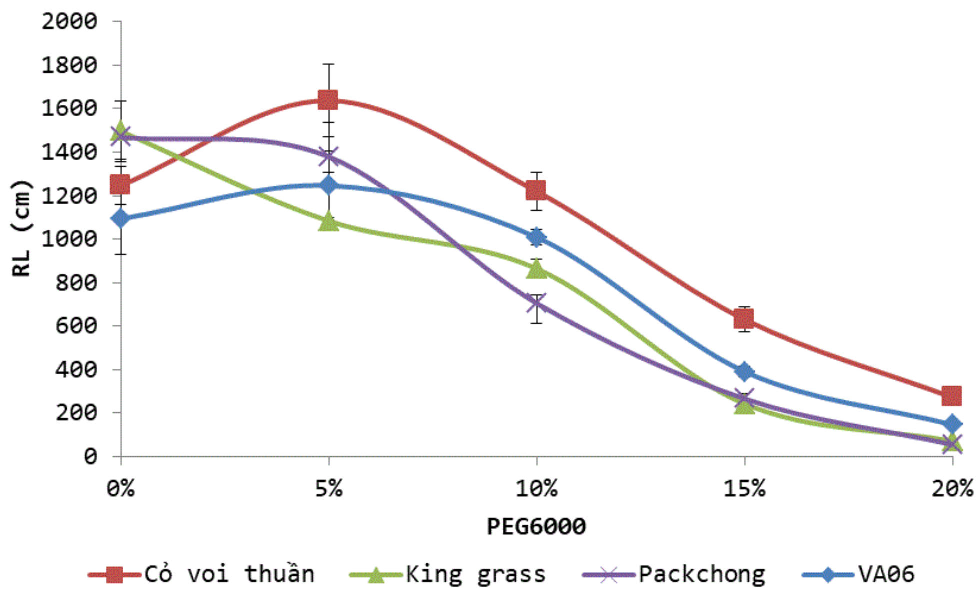

Figure 5. Root length (RL, cm) of four cultivars under drought conditions

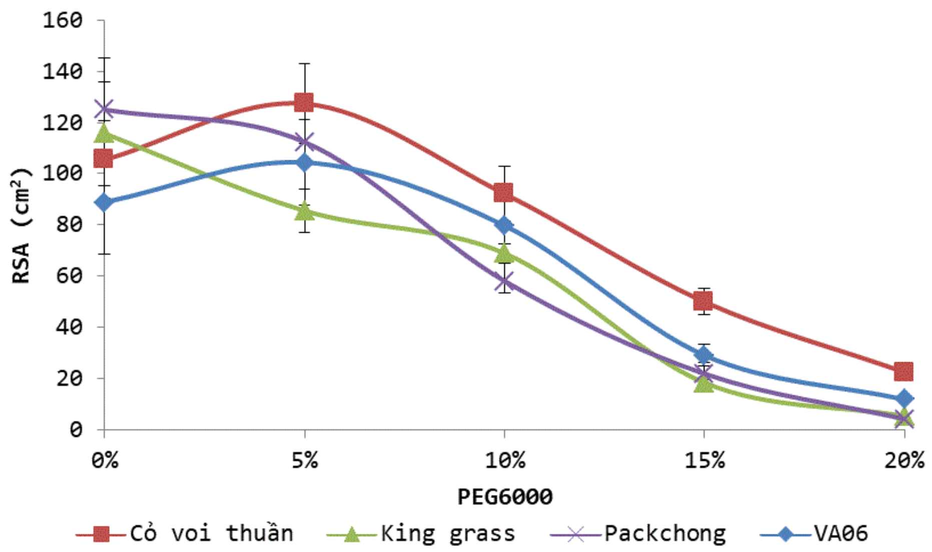

Figure 6. Root surface area (RSA, cm^2) of four cultivars under drought conditions

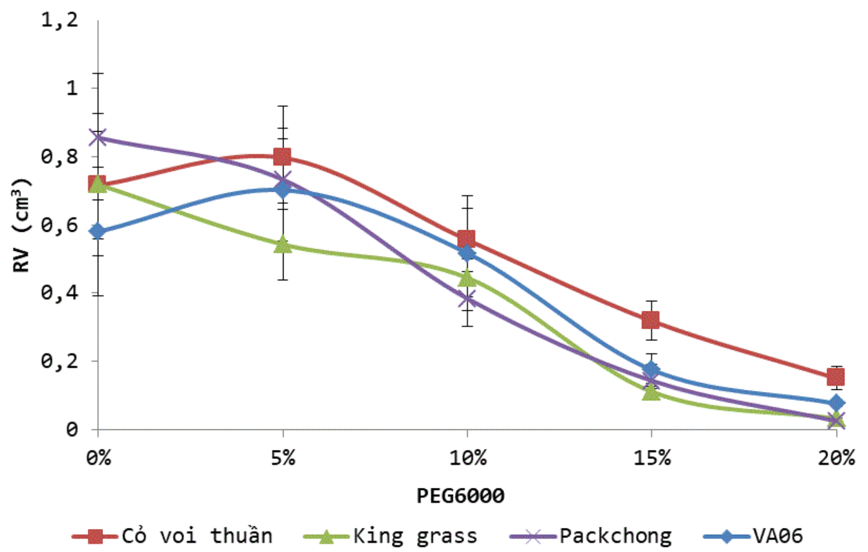

Figure 7. Root volume (RV, cm^3) of four cultivars under drought conditions

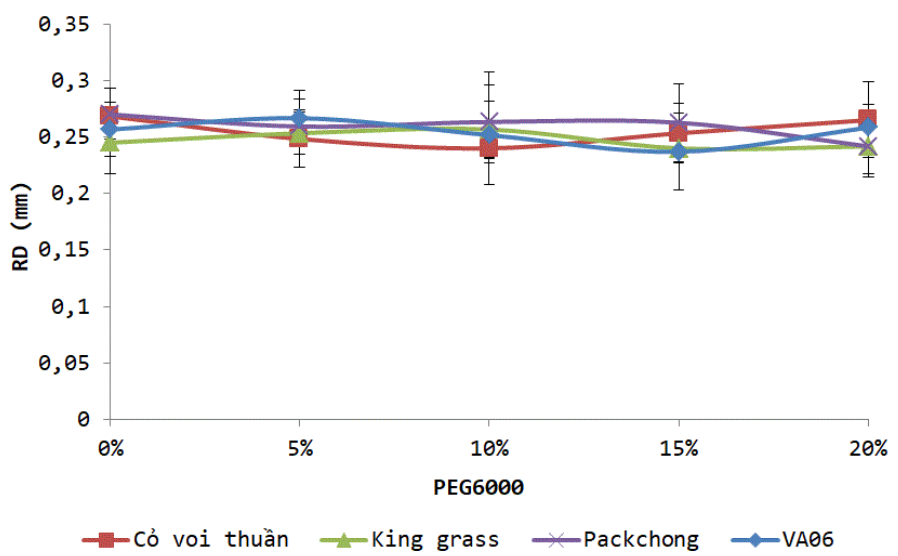

Figure 8. Root diameter (RD, cm^3) of four cultivars under drought conditions

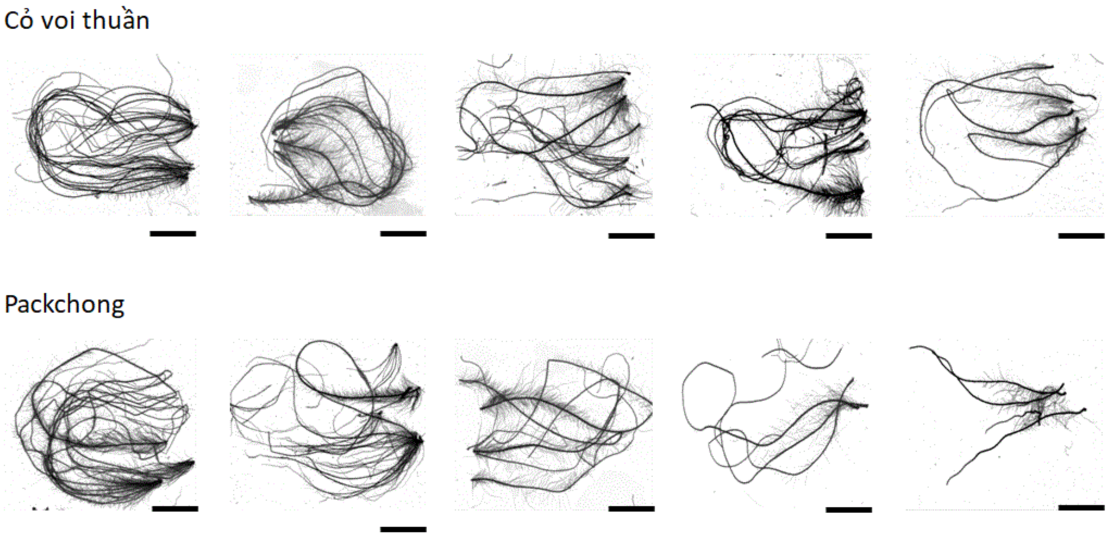

Figure 9. Root system of "Cỏ voi thuần" and Packchong under drought conditions. Horizontal bars = 4 cm

Budiman et al., 2012, Mwendia et al., 2016]. This study, therefore, indicates a Napier grass "Cỏ voi thuần (CVT)" could be an important genetic plant material resource for breeding of better Napier grass cultivars that are tolerant to drought and high yielding.

Higher development (as root branching and elongating) in root responses to water stresses is more advantageous to plant acquiring water but also nutrient uptake [Palta et al., 2011]. Packchong and King grass presented drought-sensitive cultivars with rapid decrease of RL and RSA. In addition, it was found that "Cỏ voi thuần (CVT)" was better adapted to drought as compared to the other cultivars. These results suggest that by a combined analysis of root plasticity and its association with water uptake and water use efficiency a more mechanistic understanding of factors involved in Napier root responses to drought will be reached and this should be the next research step.

Acknowledgments

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