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Article

Root Characterization of Myanmar Upland and Lowland Rice in Relation to Agronomic and Physiological Traits under Drought Stress Condition

Moe Myint Sandar [1], Mathurada Ruangsiri [2], Cattleya Chutteang [3], Anuruck Arunyanark [3], Theerayut Toojinda [2] and Jonaliza L. Siangliw [2],*

[1] Master of Science Agricultural Research and Development, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand; lamin9416@gmail.com

[2] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Phahonyothin, Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand; mathurada.rua@ncr.nstda.or.th (M.R.); theerayut@biotec.or.th (T.T.)

[3] Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand; agrcyc@ku.ac.th (C.C.); agrara@ku.ac.th (A.A.)

* Correspondence: jonaliza.sia@biotec.or.th

Abstract: Lowland and upland rice are the two most important rice ecotypes and are grown under different ecosystems of contrasting soil water conditions. The study aimed to characterize root morphological, anatomical, and architectural traits and shoot physiological traits related to agronomic traits in Myanmar lowland and upland rice under drought conditions. Drought experiments were conducted in the field and in the greenhouse. The reduction in root traits under drought was observed in both lowland and upland rice except for the stele and xylem area. Stele area and xylem area were increased under drought in lowland rice while stele area in upland rice was decreased and was not changed in the xylem area. A positive relationship between agronomic traits and root traits of lowland rice was observed including the total number of nodal roots, root number per tiller, and a negative relationship in lateral root density more than anatomical traits. While upland rice illustrated the relationship between root anatomical traits and agronomic traits. In addition, a negative contribution to biomass by photosynthesis rate, stomatal conductance, and transpiration rate was observed in lowland and upland rice. These findings can help improve drought tolerance in rice by selecting the optimal root trait for each rice ecosystem.

Keywords: rice; drought; root morphology; root anatomy

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1. Introduction

Drought is the main important abiotic stress that negatively influences plant growth and development. Drought stress can reduce 30–50% of crop yield. It is especially sensitive to drought stress during reproductive growth and even moderate stress can result in a drastic reduction in grain yield [1–3]. Rice is one of the most important staple food crops and it is grown in 7.2 million hectares representing 34% of the total cultivated area in Myanmar. The drought area is estimated to be 67,700 square kilometers and it constitutes 10 percent of the total area of Myanmar. Farmers in central Myanmar are facing difficulties in rice growing as there is a shortage of water due to drought [4]. Rice is vulnerable to drought stress at all stages of growth, especially reproductive stage leading to a significant reduction in grain yield [5,6]. Improving drought resistance is the one of main targets in the future.

Upland and lowland rice are the two most important ecotypes. Upland rice is mainly cultivated in non-bunded fields without any irrigation facilities. Upland rice varieties are an important genetic resource due to their water-saving ability and drought-resistant characters [7]. Lowland rice has the greatest water requirement of all cereal crops [8].

Moreover, lowland rice plants often experience drought in rainfed environments when rainfall is not sufficient to maintain flooded paddy conditions. Because rice is adapted to saturated soils, the physiology and drought response of lowland rice are different from those of other crops.

Root traits have been claimed to be critical for increasing yield under soil-related stresses [9,10]. The root system of rice is very poor when compared with other cereal crops [11]. The rice root system is divided into three different classes including seminal roots, mesocotyl roots, and nodal roots. Lateral roots emerge from each of these classes. These three classes differ in origin, anatomy, and function [12]. The ability of roots to uptake water and nutrients mainly depends on root morphological and anatomical traits [13]. Extensive studies on rice roots have identified many root traits that provide drought resistance. Rice genotypes that have deep, coarse roots with a high ability of branching and penetration and higher root to shoot ratio are reported as component traits of drought avoidance [14–16]. Increased lateral root formation under drought stress was suggested as a potentially useful adaption to drought in lowland rice [17,18]. Xylem vessels traits (number, diameter, and area) affect axial water conductance while cortical traits and the presence of suberized cell layers may affect radial conductance. Larger xylem vessels and thicker roots are characteristic of upland rice and associated with improved drought tolerance [16]. Moreover, plants with deep rooting can access water from deeper soil layers, which enables the plants to avoid drought stress [19–21]. A well-developed root system will help the plant in maintaining high plant water status [22]. Under drought stress, upland rice develops deep and thick root systems to improve the hydraulic properties of its roots [19,23]. These features greatly enhance the drought resistance of upland rice varieties by allowing the absorption of more water stored in deep soil layers [20,24,25].

The objective of this study was to characterize root morphological, architectural, and anatomical traits of Myanmar upland and lowland rice related to agronomic and physiological traits under drought treatment in lowland environment.

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Drought Treatment

Seven upland and fourteen lowland rice varieties were obtained from Myanmar and one Thai lowland rice variety was used as a drought resistance check (Table S1). In the first experiment, all 21 varieties were grown under well-watered and drought conditions in the field to examine root morphological and anatomical traits. The seeds were germinated on 16 August 2019, in the rice nursery field and seedlings were transplanted 30 days after sowing (DAS) with 25 × 25 cm distance between hills and rows. The experiment was conducted using factorial in RCBD. This experiment was conducted with 3 replications. Water was supplied and maintained at a level of 5 to 10 cm above the soil surface. At 45 DAS, water was drained, and stressing was implemented at maximum tillering stage with soil water potential of -40 to -50 kPa (55–65 DAS) and soil water potential fluctuated from -40 to -70 kPa at booting stage for 9 days (67–75 DAS) (Figure S1A). No re-watering was carried out until harvest for the drought condition. The field soil moisture content was monitored using tensiometers (Daiki soil and moisture, Daiki Rika Kogyo Co., Saitama, Japan) and a weather station was also installed to monitor the air temperature and humidity.

The pot experiment was conducted in the greenhouse to screen root architectural, morphological, and physiological traits. The rice seeds were germinated directly in the plastic pot (20 cm in height and 25 cm in diameter) on 17 August 2020. Each plastic pot was filled with mixed 2.5 kg of dry clay and 2.5 kg of dry sand. Plastic baskets (25 cm diameter and 5 cm height) with 2 mm^2 pore size were buried in the middle of the plastic pot to screen root architectural traits using the basket method [25]. The experimental design was factorial in CRD with 3 replications and 2 conditions including well-watered and drought. Water was maintained in every pot and was poured out at 45 DAS to develop the drought condition. Drought stress was carried out for 15 days, and soil water potential was -40 kPa at day 4 and dropped to -70 kPa at day 8 after the stress (Figure S1B). Then, shoots and roots were harvested at 60 DAS. The two experiments were conducted at Innovative Plant Biotechnology and Precision Agriculture Research Team (APBT), Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand.

2.2. Shoot Measurements

In the field experiment, 50% flowering time (FD) was determined in each plot. Leaf rolling (LR) and leaf drying (LD) were recorded using a standard evaluation system for rice (IRRI, 2002). Plant height (PH) and tiller number (TN) were recorded by measuring the height of the plant from the soil to the tip of the tallest leaf and counting the tillers manually before harvesting shoots and roots at booting stage (65 DAS). Shoot samples were separated from the roots by cutting from base and sun-dried for 14 days before recording the biomass (BIO). Samples for the yield component were harvested from the other plants without root sampling at maturity. One thousand grain weight (1000GW) was obtained by weighing 1000 filled grains and expressing the weight in grams and percent filled grain by weight (FG) was collected by weighing all filled grains in all panicles in a plant and divided by the total number of grains per plant and multiplied by 100.

The physiological traits were performed in a pot experiment. Photosynthesis rate (PR), transpiration rate (TR), and stomatal conductance (SC) were measured using LI-6400 Portable Photosynthesis System (LiCor, Lincoln, NE, USA) at 9:00 a.m.–11:30 a.m. Chlorophyll content was measured by Chlorophyll Meter SPAD-502Plus (Konica Minolta, Inc., Tokyo, Japan). All physiological traits mentioned above were collected at 45, 50, and 55 DAS. Plant height, tiller number, and biomass were collected at 60 DAS with similar protocol as in the field experiment.

2.3. Root Measurements

In the field experiment, root samples were collected at flowering stage (65 DAS) using the same plant collected for biomass. The root systems were extracted from soil using monolith stainless cylinder (20 cm diameter and up to 20 cm soil depth). The roots were washed with water and stored in 70% ethanol for further measurements. The number of nodal roots (NR) was manually counted. Total number of nodal roots was divided by number of tillers to access nodal root number per tiller (NPT). Three 10 cm (from base to tip) nodal roots form each sample were randomly selected and spread on a transparent tray without over lapping. Root images were captured using scanner (EPSON Perfection V700 Photo, Nagano, Japan) at 600 dpi resolution. The lateral root density (LR) was manually counted from 10 cm length nodal root images. For anatomical traits, nodal roots were cut at 5 cm below the base to ensure that the anatomy to view in each sample is at the same positions. Cross sections were generated 20 times with 100 µm thickness using Leica Fully Automatic Vibrating blade microtome (VT 1000S) (Leica Biosystems Division of Leica Microsystems Inc., Buffalo Grove, IL, USA). Three root sections were selected and imaged as representative sub-samples using Leica compound microscopes (4 × magnification). Root thickness (RT), root cortex (RC), stele area (SA), xylem area (XA), and xylem number (XN) was determined using image analysis GIMP software [26].

In the pot experiment, root distribution was evaluated by counting the number of nodal roots that penetrated throughout the basket pores. The angle (in degrees) of each pore of the basket was identified. Percent of nodal roots at 50–90 degrees (%50–90) was classified as vertical root distribution [25,27,28] and calculated by counting the number of nodal roots at 50–90 degrees divided by the total number of nodal roots and multiplying by one hundred. Root samples were washed by water and stored in 70% ethanol after counting the roots from the baskets. The root morphological traits were measured using the same process which was used in the field experiment.

2.4. Statistical Analysis

Genstat 21st Software [29] was used for statistical tests including analysis of variance (ANOVA), mean comparisons by the least significant difference (LSD) test at significant levels of $p < 0.05$, and Pearson's correlation coefficient. The principal component analysis was performed using 'foctoextra' R package [30]. The bulk analysis based on biomass was analyzed using 'tidyr', 'plyr', 'dplyr' R packages to investigate effect of plants with high or low biomass performed in agronomic, physiological, root morphological, and root anatomical traits under drought stress. Path analysis was analyzed using 'lavaan' R package [31].

3. Results

3.1. Field Experiment

The agronomic traits of lowland rice varieties, such as plant height, tiller number, percent filled grain, one thousand grain weight, and biomass showed significant differences among rice varieties in both well-watered and drought conditions. All these shoot traits were reduced under the drought condition (Figure 1A,B). Plant height and tiller number were reduced by 22.5% and 41.7%, respectively. One thousand grain weight was reduced by 13.8% and percent filled grain was reduced by 3.3%. Biomass was reduced by 26.3% (Table 1). Similarly, in upland rice, plant height, tiller number, one thousand grain weight, and biomass were reduced in the drought condition by 29%, 40.9%, 24.8%, and 30.7%, respectively (Figure 1B,C; Table 2). Percent filled grain did not show a significant difference between the two conditions.

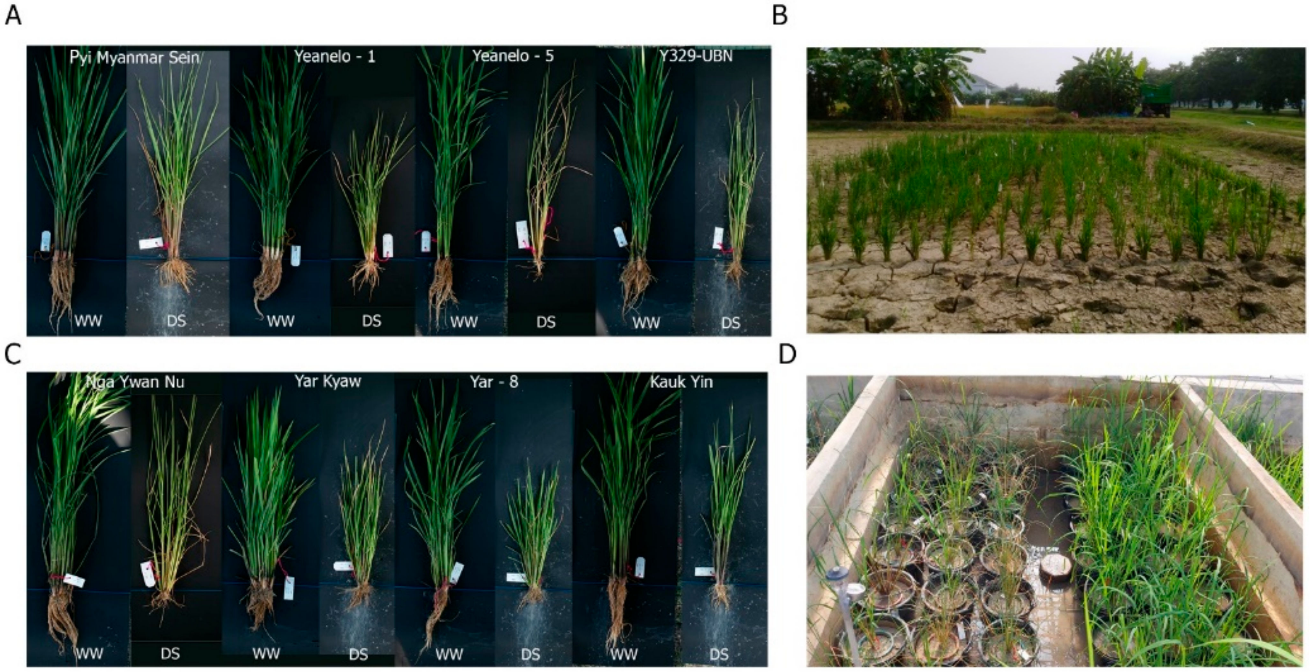


Figure 1. Shoot and root performances, comparing well-watered and drought conditions. (**A**) Lowland rice. (**B**) Drought stress in field experiment. (**C**) Upland rice. (**D**) Plant performances in the pot experiment, comparing drought (**left**) and well-watered conditions (**right**).

Significant genetic variations in root traits of lowland rice varieties were observed in nodal root number per tiller in the well-watered condition. Total number of nodal roots and xylem area were significantly different in both the well-watered and drought conditions (Figure 1A, Table 1). Variation in stele area and percent of aerenchyma were genetically significant in the drought condition. For root morphological traits, nodal roots per tiller, total number of nodal roots, and lateral root density were reduced in the drought condition by 49.4%, 71.5%, and 63.9%, respectively. Additionally, root anatomical traits, such as root thickness, cortex area, and xylem number were decreased under the drought condition by 18.2%, 25.0%, and 8.0%, respectively. However, the stele and xylem area were increased under the drought condition by 25% and 40%, respectively (Table 1).

Table 1. ANOVA, mean, least significant difference (LSD), and range of lowland rice in the field experiment.

Traits	Condition	Mean ± SD			LSD	Min	Max	% Change	G Effect	T Effect	GxT Effect
FD	WW	105	±	12.8	3.7	85	129	−17.1	**	*	**
	DS	87	±	4.3	3.9	82	96		**		
PH	WW	78.5	±	13.0	3.9	42.3	105.3	−22.5	**	*	**
	DS	60.8	±	10.2	6.5	47.7	84.3		**		
TN	WW	24	±	7.6	8.5	15	44	−41.7	**	**	ns
	DS	14	±	3.9	4.2	8	22		**		
FG	WW	90	±	5.2	4.9	77.2	94.4	−3.3	**	*	ns
	DS	87	±	5.5	9.2	78.6	90.6		ns		
1000GW	WW	23.6	±	2.6	1.6	18.9	26.8	−13.8	**	**	ns
	DS	20.3	±	11.4	2.8	16.5	22.8		*		
BIO	WW	30.4	±	3.6	5.4	24.4	38.1	−26.3	*	*	*
	DS	22.4	±	3.9	3.1	16.8	31.5		**		
NPT	WW	17.8	±	4.9	3.7	11.8	29.9	−49.4	**	*	ns
	DS	9.0	±	2.8	4.3	6.7	12.5		ns		
NR	WW	386	±	92.9	63	227	530.3	−71.5	**	**	ns
	DS	110	±	45.5	39.3	60.6	200.5		**		
LR	WW	89.1	±	20.3	40.5	45.5	122.5	−63.9	ns	*	ns
	DS	32.2	±	8.2	18.6	16.8	46.9		ns		
RT	WW	1.35	±	0.14	0.48	1.05	1.56	−11.9	ns	*	ns
	DS	1.19	±	0.25	0.51	0.81	1.6		ns		
CA	WW	1.1	±	0.12	0.49	0.83	1.29	−18.2	ns	*	ns
	DS	0.9	±	0.21	0.47	0.63	1.3		ns		
SA	WW	0.04	±	0.01	0.02	0.03	0.05	25.0	ns	*	ns
	DS	0.05	±	0.01	0.01	0.03	0.07		**		
XN	WW	5.0	±	0.6	1.05	4	5	−8.0	ns	*	ns
	DS	4.6	±	0.9	1.28	3	5		ns		
XA	WW	0.005	±	0.001	0.002	0.003	0.007	40.0	*	*	**
	DS	0.007	±	0.003	0.003	0.002	0.011		**		
PA	WW	32.1	±	8.5	26.7	19.4	45.12	−2.8	ns	ns	ns
	DS	31.2	±	11.7	13.1	11.9	49.64		**		
LRS	DS	5.6	±	0.6	1.5	1.56	4.92		ns		
LDS	DS	3.0	±	1.2	0.7	2.67	4.75		*		

FD, 50% days to flowering (DAS); PH, plant height (cm); TN, tiller number (number); FG, percent of filled grain (%); 1000GW, one thousand grain weight; BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); RT, root thickness (mm); CA, cortex area (cm^2); SA, stele area (cm^2); XN, xylem number (no.); XA, xylem area (cm^2); PA, percent of aerenchyma (%), LRS, leaf rolling score (score), LDS, leaf drying score (score); G effect, genotype effect; T effect, treatment effect; GxT effect, interaction of genotype and treatment effect. *, **, and ns represent the significant differences at 0.05, 0.01 levels and no significant difference, respectively.

Table 2. ANOVA, mean, least significant difference (LSD), and range of upland rice in the field experiment.

Traits	Condition	Mean ± SD			LSD	Min	Max	% Change	G Effect	T Effect	GxT Effect
FD	WW	104	±	11.3	1.61	87	119	−10.6	**	*	ns
	DS	93	±	0.70	2.87	92	93		ns		
PH	WW	82.7	±	13.3	6.75	69.3	102.5	−29.0	**	**	**
	DS	58.7	±	6.74	8.96	51.0	67.4		**		
TN	WW	22	±	10.4	7.00	15	46	−40.9	**	*	**
	DS	13	±	4.23	4.65	9	20		**		
FG	WW	91.8	±	1.37	1.75	89.3	93.7	−11.3	**	ns	ns
	DS	81.4	±	5.37	56.6	77.6	85.2		ns		

Table 2. *Cont.*

Traits	Condition	Mean ± SD	LSD	Min	Max	% Change	G Effect	T Effect	GxT Effect
1000GW	WW	23.8 ± 1.27	1.90	22.5	25.8	−24.8	ns	*	ns
	DS	17.9 ± 0.42	7.86	17.6	18.2		ns		
BIO	WW	30.9 ± 5.00	9.91	25.0	37.3	−30.7	ns	**	ns
	DS	21.4 ± 1.52	4.07	19.5	23.4		ns		
NPT	WW	16.7 ± 6.23	5.30	11.2	29.4	−53.9	**	**	**
	DS	7.7 ± 1.77	5.87	5.1	9.6		ns		
NR	WW	360 ± 100	104	190	505	−74.2	**	**	*
	DS	93 ± 8.68	47.4	80.3	104.7		ns		
LR	WW	94.2 ± 30.5	62.0	53.3	140.4	−56.2	ns	*	**
	DS	41.3 ± 17.96	33.3	27.4	79.5		**		
RT	WW	1.32 ± 0.19	0.68	1.09	1.63	−23.5	ns	*	ns
	DS	1.01 ± 0.29	1.31	0.68	1.44		ns		
CA	WW	1.05 ± 0.16	0.57	0.86	1.34	−30.5	ns	**	ns
	DS	0.73 ± 0.22	0.91	0.47	1.06		ns		
SA	WW	0.043 ± 0.008	0.03	0.030	0.050	−11.6	ns	**	ns
	DS	0.038 ± 0.007	0.05	0.031	0.052		ns		
XN	WW	5 ± 0.18	1.50	5	5	0.00	ns	ns	ns
	DS	5 ± 0.64	1.55	4	5		**		
XA	WW	0.005 ± 0.0003	0.003	0.004	0.005	0.00	ns	ns	ns
	DS	0.005 ± 0.001	0.01	0.004	0.007		ns		
PA	WW	37.1 ± 6.51	36.8	29.6	47.5	−23.7	ns	*	ns
	DS	28.3 ± 8.24	45.5	16.0	39.3		ns		
LRS	DS	2.2 ± 0.8	1.40	1.53	3.5		ns		
LDS	DS	3.1 ± 0.4	0.80	2.25	3.6		ns		

FD, 50% days to flowering (DAS); PH, plant height (cm); TN, tiller number (number); FG, percent of filled grain (%); 1000GW, one thousand grain weight; BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); RT, root thickness (mm); CA, cortex area (cm^2); SA, stele area (cm^2); XN, xylem number (no.); XA, xylem area (cm^2); PA, percent of aerenchyma (%), LRS, leaf rolling score (score), LDS, leaf drying score (score); G effect, genotype effect; T effect, treatment effect; GxT effect, interaction of genotype and treatment effect. *, **, and ns represent the significant differences at 0.05, 0.01 levels and no significant difference, respectively.

There was significant genetic variation in upland rice in nodal root number per tiller and total number of nodal roots under the well-watered condition (Figure 1C). Xylem number showed significant genetic variation in the drought condition. Both root morphological and anatomical traits showed significant reduction in the drought condition except for the xylem number and xylem area which were not affected by the stress (Table 2). In addition, the comparisons of root characters between lowland and upland rice in field experiment were presented in Figure S2 that monitored the nodal root number per tiller and percent of aerenchyma of lowland rice has higher than upland rice, significantly.

3.2. Pot Experiment

Physiological, root morphological, and root architectural traits were investigated in the pot experiments (Figure 1D). For lowland rice, plant height showed significant genetic variations in well-watered and drought conditions while tiller number only showed significant difference in the well-watered condition. No significant genetic variation was found for biomass in both well-watered and drought conditions. Significant differences between conditions were observed in tiller number and biomass, which reduced under the drought condition by 33.3% and 20.0%, respectively. There were significant differences among genotypes for root morphological traits including nodal roots per tiller in the well-watered condition, total number of nodal roots in both conditions and lateral root density in the drought condition. Nodal root number per tiller and total number of nodal roots were decreased under drought by 28.6% and 52.4%, respectively. Percent of nodal roots at 50°–90° differed among genotypes in the drought stress condition but there was no significant treatment effect (Table 3). Moreover, percent of nodal roots at 50°–90° of lowland rice was higher than upland rice (Figure S3).

Table 3. ANOVA, mean, least significant difference (LSD), and range of lowland rice in the pot experiment.

Traits	Condition	Mean ± SD	LSD	Min	Max	% Change	G Effect	T Effect	GxT Effect
PH	WW	84.7 ± 6.48	11.6	72.5	94.4	−2.6	**	ns	ns
	DS	82.5 ± 10.7	21.0	54.9	97.1		**		
TN	WW	15 ± 4.86	10.5	9	24	−33.3	**	**	ns
	DS	10 ± 3.17	5.02	6	15		ns		
BIO	WW	21.5 ± 2.56	7.14	16.5	25.5	−20.0	ns	**	ns
	DS	17.2 ± 1.80	3.66	14.8	20.2		ns		
NPT	WW	18.2 ± 2.65	5.58	13.9	23.0	−28.6	**	**	ns
	DS	13.0 ± 2.06	5.92	10.0	16.5		ns		
NR	WW	269 ± 65.0	152	160	359	−52.4	**	**	ns
	DS	128 ± 47.6	72.1	80.0	213		*		
LR	WW	153.0 ± 36.7	115	92.0	225	5.0	ns	ns	ns
	DS	160.7 ± 48.6	25.0	72.7	230		*		
%50–90	WW	33.3 ± 7.77	15.3	16.7	44.3	−3.6	ns	ns	ns
	DS	32.1 ± 6.72	5.93	11.1	43.3		**		
LRS	DS	5.90 ± 2.32	5.90	3	9		ns		
LDS	DS	3.40 ± 2.66	4.50	1	9		*		

PH, plant height (cm); TN, tiller number (number); BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); %50–90, percent of nodal roots at 50–90 degrees (%); LRS, leaf rolling score (score); LDS, leaf drying score (score); G effect, genotype effect; T effect, treatment effect; GxT effect, interaction of genotype and treatment effect. *, **, and ns represent the significant differences at 0.05, 0.01 levels and no significant difference, respectively.

In upland rice, significant differences among genotypes for shoot traits were observed in plant height in well-watered and drought conditions and tiller number in the well-watered condition. Tiller number was reduced by 29.3% under the drought condition (Table 4). There were no genetic variations in biomass in both conditions, but the conditions were significantly different. Root traits such as nodal root number per tiller was found significantly different in both conditions. Lateral root density and percent of nodal roots at 50°–90° only showed significant genetic variations for the drought stress condition while the total number of nodal roots was only significant in the well-watered condition.

Table 4. ANOVA, mean, least significant difference (LSD), and range of upland rice in the pot experiment.

Traits	Condition	Mean ± SD	LSD	Min	Max	% Change	G Effect	T Effect	GxT Effect
PH	WW	88.3 ± 12.3	13.9	64.0	100.1	−2.9	**	ns	ns
	DS	85.7 ± 13.3	14.6	64.2	107.6		**		
TN	WW	15 ± 4.72	6.63	8	20	−29.3	**	**	ns
	DS	11 ± 2.29	7.05	7	14		ns		
BM	WW	24.7 ± 3.16	13.6	20.7	27.9	−26.0	ns	**	ns
	DS	18.3 ± 1.97	5.35	15.5	20.6		ns		
NPT	WW	18.8 ± 4.63	3.93	12.6	24.3	−25.3	**	**	ns
	DS	14.0 ± 3.17	5.55	10.0	19.5		*		
NR	WW	283 ± 91.5	93.7	95	64	−45.6	**	**	ns
	DS	154 ± 59.1	109	380	218		ns		
LR	WW	148.0 ± 37.30	133.00	81.7	205	6.1	ns	ns	ns
	DS	157.0 ± 46.00	25.00	113.0	251		**		

Table 4. *Cont.*

Traits	Condition	Mean ± SD	LSD	Min	Max	% Change	G Effect	T Effect	GxT Effect
%50–90	WW	26.1 ± 5.44	11.70	20.3	37	−4.6	ns	ns	ns
	DS	24.9 ± 4.07	5.93	19.7	33		*		
LRS	DS	7.3 ± 1.30	5.10	4	9		ns		
LDS	DS	5.4 ± 1.90	5.60	1.7	7		*		

PH, plant height (cm); TN, tiller number (number); BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); %50–90, percent of nodal roots at 50–90 degrees (%); LRS, leaf rolling score (score); LDS, leaf drying score (score); G effect, genotype effect; T effect, treatment effect; GxT effect, interaction of genotype and treatment effect. *, **, and ns represent the significant differences at 0.05, 0.01 levels and no significant difference, respectively.

Physiological traits including chlorophyll content, photosynthesis rate, stomatal conductance, and transpiration rate were measured at 0, 4, and 8 days after stress (or 45, 49, and 53 days after sowing). The photosynthesis rate, stomatal conductance, and transpiration rates were decreased after drought stress occurred in both lowland and upland rice, but the net photosynthesis rate was more reduced in upland rice compared with lowland rice. For the chlorophyll content, lowland and upland rice showed an increase in content 8 days after stress compared to the well-watered treatment, but lowland rice has a more pronounced increase than the upland rice (Figure 2).

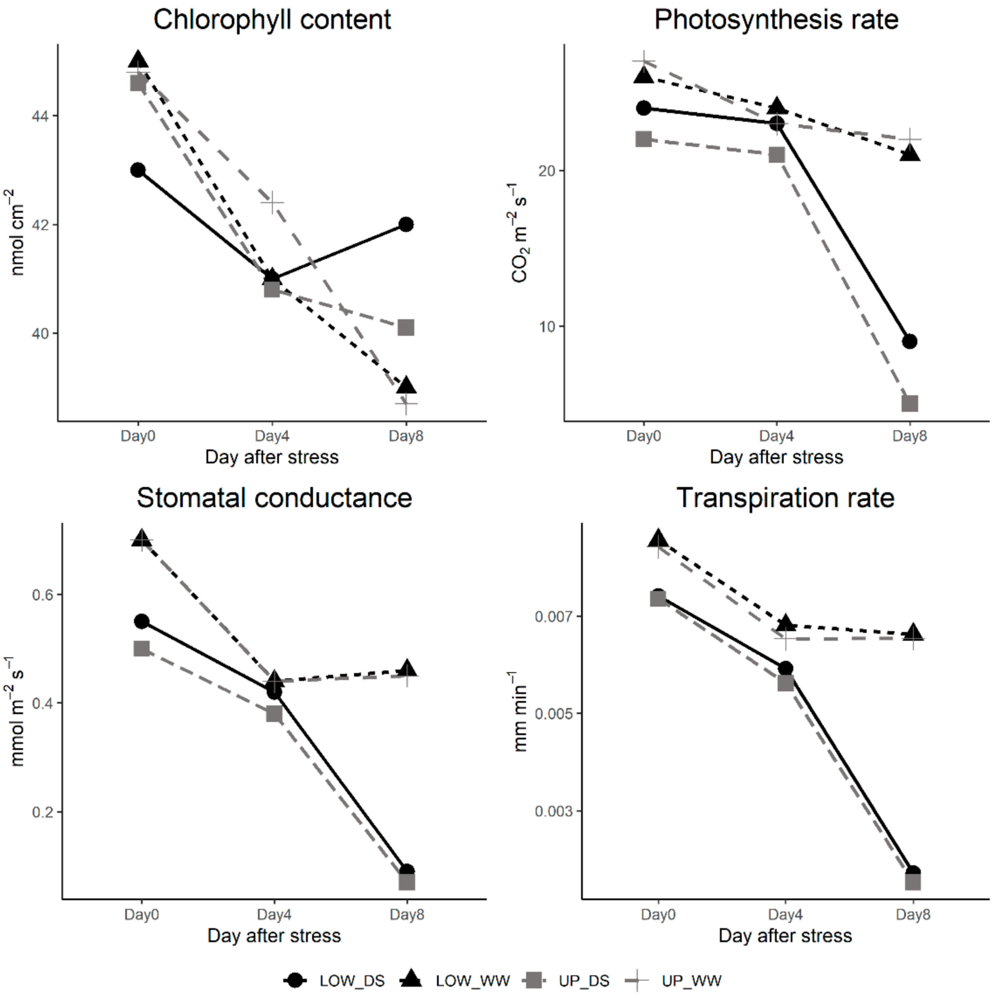


Figure 2. Physiological traits in the pot experiment at day 0, day 4, and day 8 after stress: A comparison between well-watered and drought stress conditions.

3.3. Correlation of Root Traits and Other Traits under Drought

Correlation matrices indicated that the root traits of lowland rice under the drought condition such as nodal root number per tiller (0.85 *) and total number of nodal root (0.97 **) has a significant and positive correlation with biomass. Xylem area (−0.88 **) was negatively correlated with tiller number. Cortical area was positively correlated with root thickness (0.99 **) and stele area (0.94 **). Stele area was also positively correlated with root thickness (0.92 **) (Figure 3A).

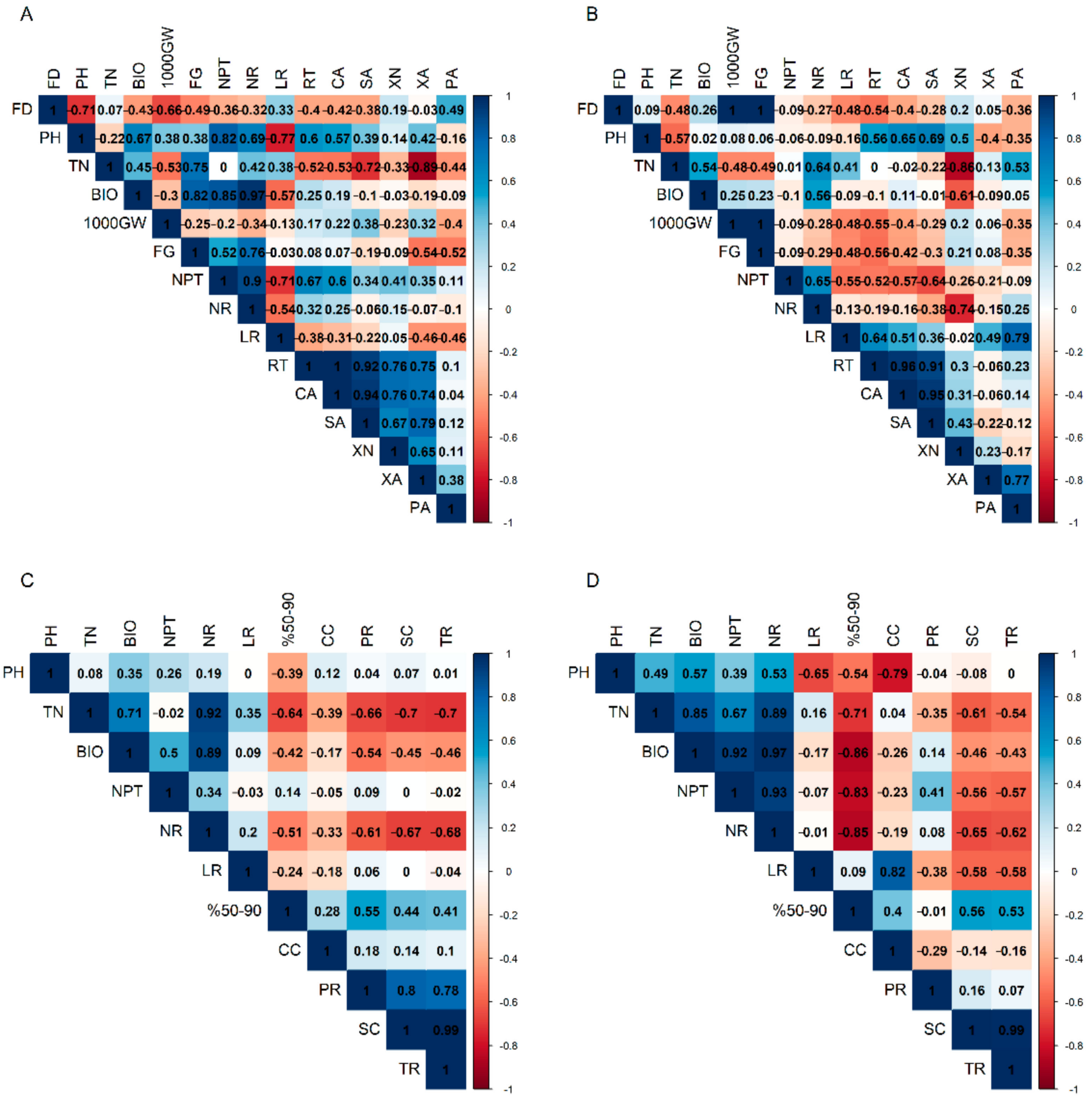


Figure 3. Correlation matrix under drought stress. (**A**) Lowland rice in field experiment, (**B**) upland rice in field experiment, (**C**) lowland rice in pot experiment, and (**D**) upland rice in pot experiment. PH, plant height (cm); TN, tiller number (number); FG, percent of filled grain (%); 1000GW, one thousand grain weight; BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); RT, root thickness (mm); CA, cortex area (cm^2); SA, stele area (cm^2); XN, xylem number (no.); XA, xylem area (cm^2); PA, percent of aerenchyma (%); 50–90, percent of nodal roots at 50–90 degrees (%); CC, chlorophyll content (nmol cm^{-2}); PR, photosynthesis rate (CO$_2$ m^{-2}s^{-1}); SC, stomatal conductance (mmol m^{-2}s^{-1}); TR, transpiration rate (mm min^{-1}).

Negative correlation was observed between xylem number (−0.86 *) and tiller number in upland rice under the drought condition. It was also observed that cortical area was positively correlated with root thickness (0.96 **) and stele area (0.94 **). Stele area was also positively correlated with root thickness (0.90 **) (Figure 3B).

The correlations between shoot, physiological, and root traits were performed in a greenhouse study. Biomass was found to be positively correlated with the total nodal root number (0.89 **) in lowland rice. On the other hand, the total nodal root number was negatively correlated with net photosynthesis rate (0.59 *), stomatal conductance (0.66 *), and transpiration rate (0.68 *). Tiller number was also negatively correlated with net photosynthesis rate (0.63 *), stomatal conductance (0.68 *), transpiration rate (0.69 *), and percent of nodal roots at 50°–90° (0.63 *) (Figure 3C).

Total number of nodal roots was positive correlated with tiller number (0.89 **) and biomass (0.97 **) for upland rice under the drought condition. Moreover, total number of nodal roots (0.97 **) and nodal roots per tiller (0.92 **) were significantly correlated with biomass. Percent of nodal roots at 50°–90° (−0.86 *) was negative correlated with biomass, total number of nodal roots (0.83 **), and nodal roots per tiller (0.85 **). Lateral root density (0.82 *) was positively correlated with chlorophyll content (Figure 3D).

3.4. Principal Component Analysis (PCA)

The principal component analysis was performed on both field and pot experiments under drought stress. In field study, the first four components in PCA analysis with Eigen value >1 contributed 97% of the total variation under drought stress for lowland rice. Six traits contributed in PC1 accounting for 40% of the variation and four characteristics contributed in PC2 accounting for 29.9%. Plant height (0.84), nodal root per tiller (0.83), root thickness (0.93), cortex area (0.90), stele area (0.78), and xylem area (0.72) were positively contributed in PC1 while biomass (0.86) has the same loading directions (negative) with tiller number (0.82), filled grain weight (0.91), and total number of nodal roots (0.80) (Figure 4A, Table S2).

For upland rice, the first four components with Eigen value >1 of upland rice under drought stress accounted for 92% of the total variation. PC1 accounted for 33.9% of the variance. High positive loading was observed in days to 50% flowering days (0.80), one thousand grain weight (0.80), and percent of filled grain (0.80). Lateral root density (−0.77), root thickness (−0.91), cortex area (−0.82), and stele area (−0.71) contributed negatively in PC1. Meanwhile, plant height (0.69) and xylem number (0.84) contributed positively in PC2 accounting for 28.5% and opposite directions were found for tiller number (−0.86), nodal root number per tiller (−0.46), and total number of nodal roots (−0.77) (Figure 4B, Table S3).

In the pot experiment, PCA included shoot, physiological, and root traits in lowland and upland rice under drought stress (Figure 4C,D). The greater Eigen value of more than one was composed by the first four components in lowland rice (83.7%) and the first three in upland rice (90.4%). In lowland rice, PC1 accounted for 46% of the variation and high positive loading score was observed in the percent of nodal roots at 50°–90° (0.67), net photosynthesis rates (0.82), stomatal conductance (0.84), and transpiration rates (0.84). The negative loading score in PC1 was contributed by tiller number (−0.92), biomass (−0.78), and total number of nodal roots (−0.92). PC2 of lowland rice accounted for 15.4% of the variance which was related to two characteristics namely plant height (0.68) and nodal root number per tiller (0.75) (Figure 3C, Table S4). For upland rice under drought stress, PC1 accounted for 50% of the variance which was characterized by tiller number (−0.86), biomass (−0.94), nodal root number per tiller (−0.91), total number of nodal root (−0.98), percent of nodal roots at 50°–90° (0.91), stomatal conductance (0.71), and transpiration rates (0.69). High loading score in PC2 contributing 27.8% of the variation was characterized by plant height (−0.66), lateral root density (0.99), and chlorophyll content (0.85) (Figure 4D, Table S5).

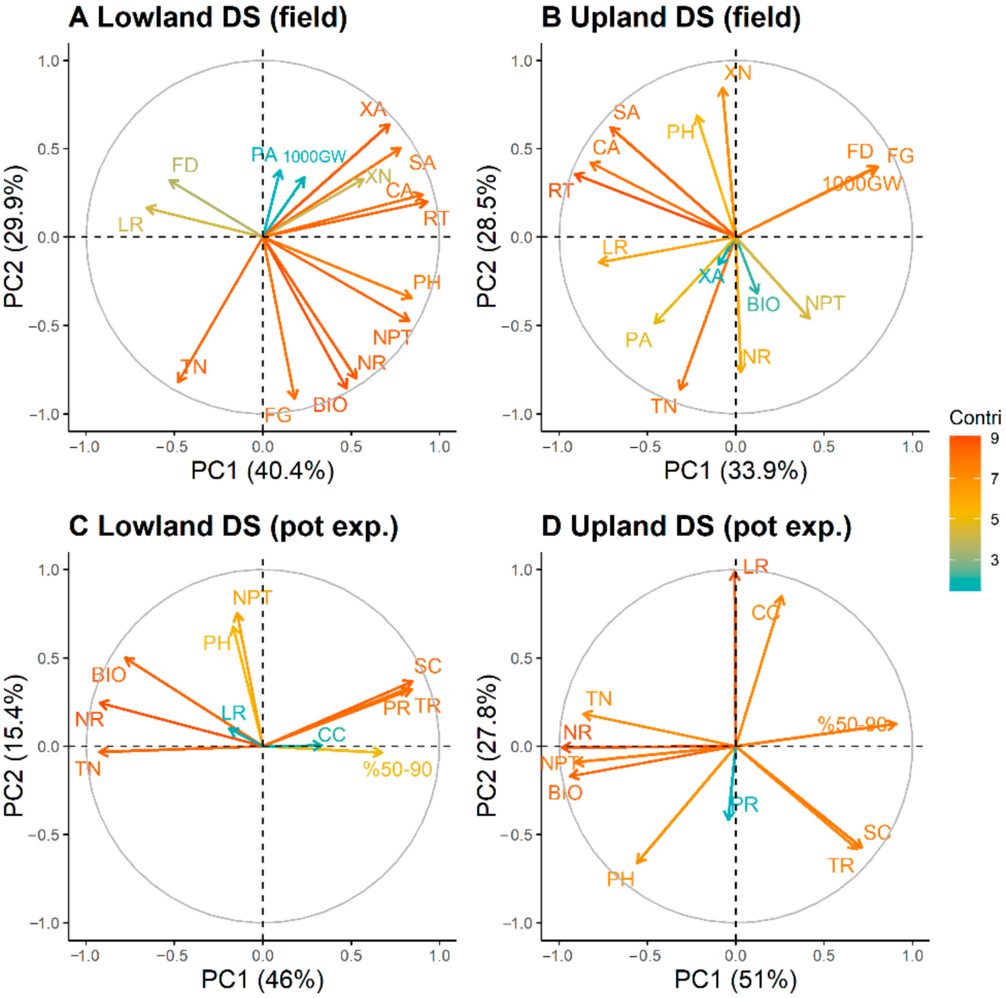


Figure 4. Principal component of phenotypic traits under drought stress. (**A**) Lowland rice in field experiment, (**B**) upland rice in field experiment, (**C**) lowland rice in pot experiment, and (**D**) upland rice in pot experiment. PH, plant height (cm); TN, tiller number (number); FG, percent of filled grain (%); 1000GW, one thousand grain weight; BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); RT, root thickness (mm); CA, cortex area (cm^2); SA, stele area (cm^2); XN, xylem number (no.); XA, xylem area (cm^2); PA, percent of aerenchyma (%); 50–90, percent of nodal roots at 50–90 degrees (%); CC, chlorophyll content (nmol cm^{-2}); PR, photosynthesis rate (CO$_2$ m^{-2}s^{-1}); SC, stomatal conductance (mmol m^{-2}s^{-1}); TR, transpiration rate (mm min^{-1}).

3.5. Bulk Analysis

Bulk segregant analysis was used to identify the traits that affected biomass under the drought condition by selecting the first two individuals with the highest and lowest biomass. The first two top lowland rice varieties, Yeanelo-1 and Pyi Myanmar Sein, had high biomass in the drought condition in the field study. In contrast, Y329-UBN and Yeanelo-5 had the lowest biomass (Table 5). However, no significant difference for the other traits was observed between low and high biomass but the group with high biomass showed a greater tiller number, nodal root number per tiller, and total number of nodal roots (Figure S4). Nga Ywan Nu and Yar Kyaw were grouped into high biomass for upland rice while Yar-8 and Kauk Yin showed the lowest biomass. Significant difference between these two groups was observed for lateral root density. The high biomass group for upland rice showed less lateral root development (Figure S5).

Table 5. Bulk analysis of lowland and upland rice in field experiment.

Ecosystem	Varieties	Group	BIO (DS)	BIO (WW)	PH	TN	1000GW	FG	NPT	NR	LR	CA	SA	XN	XA	PA	RT
Lowland	Y329-UBN	bottom	21.6	38.1	67.8	9	20.9	9.4	8.9	76	22.3	1.07	0.060	5.3	0.011	50	1.37
	Yeanelo-5	bottom	23.9	36.0	70.3	12	22.7	12.8	7.0	101	26.4	0.64	0.037	3.7	0.007	32	0.83
	Yeanelo-1	top	31.5	33.2	84.3	12	19.6	16.9	16.8	201	16.8	1.17	0.053	5.3	0.009	40	1.53
	Pyi Myanmar Sein	top	25.0	30.0	60.0	14	16.5	16.1	8.8	122	31.8	0.79	0.040	5.3	0.006	39	1.03
	p-value (bottom vs. top)		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Upland	Yar-8	bottom	19.5	29.1	55.7	13	0.0	0	7.9	111.8	42.8	0.59	0.037	4.7	0.004	26	1.00
	Kauk Yin	bottom	19.9	28.9	63.5	10	0.0	0	8.5	80.3	40.3	1.06	0.083	5	0.004	14	1.43
	Nga Ywan Nu	top	23.4	35.4	66.5	11	17.6	5.1	8.8	107.5	27.4	0.86	0.060	4.7	0.004	17	1.07
	Yar Kyaw	top	23.1	37.3	51	20	0.0	0	5.4	104.7	29.3	0.66	0.04	4.3	0.006	22	0.87
	p-value (bottom vs. top)		**	*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns

BIO, biomass (g); PH, plant height (cm); TN, tiller number (no.); 1000GW, one thousand grain weight (g); FG, percent of filled grain (%); NPT, nodal root number per tiller (no./tiller); NR, total number of nodal root (no.); LR, lateral root density (no./10 cm); CA, cortex area (cm^2); SA, stele area (cm^2); XN, xylem number (no.); PA, percent of arenchyma (%); RT, root thickness (mm). *, **, and ns represent the significant differences at 0.05, 0.01 levels and no significant difference, respectively.

In the pot experiment, Yeanelo-3 and Pyi Myanmar Sein were the best performing lowland rice varieties and Yeanelo-2 and Sin Thwe Lat were the lowest (Table 6). High biomass varieties showed a higher tiller number and total number of nodal roots, significantly. Higher chlorophyll content was observed in high biomass varieties, but in contrast, a decrease in photosynthesis rate, stomatal conductance, and transpiration rate was also seen (Figure S6). For upland rice, Yar-8 and Ba Ma Htar had the highest biomass and Yar-4 and Nga Ywan Nu had the lowest. Higher nodal root number per tiller and number of nodal roots were found in high biomass varieties. Moreover, high biomass varieties showed a lower percent of nodal roots at 50°–90°, stomatal conductance, and transpiration rate (Figure S7).

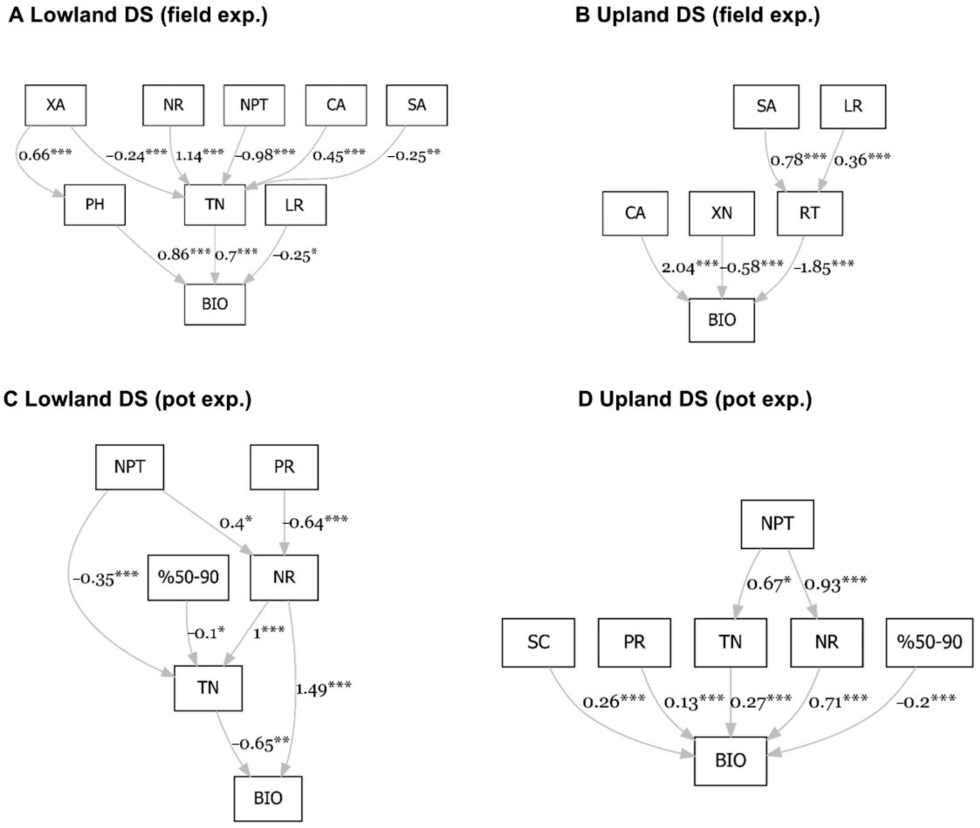
Table 6. Bulk analysis of lowland and upland rice in the pot experiment.

Ecosystem	Varieties	Group	BIO (DS)	BIO (WW)	PH	TN	NPT	NR	LRD	%50–90	CC	PR	SC	TR
Lowland	Yeanelo-2	bottom	15.0	22.6	84.7	7	14.5	87	116	29	40.6	9.7	0.09	0.0018
	Sin Thwe Lat	bottom	15.5	24.6	79.5	9	10.0	91	138	39	40.8	13.6	0.16	0.0029
	Yeanelo-3	top	20.3	23.2	88.7	15	14.1	206	204	21	41.8	1.5	0.04	0.0010
	Pyi Myanmar Sein	top	20.2	22.2	85.8	14	15.3	214	98	32	43.8	5.4	0.02	0.0005
	p-value (bottom vs. top)		**	ns	ns	*	ns	**	ns	ns	ns	ns	ns	ns
Upland	Yar-4	bottom	15.5	27.6	80.2	7	10.0	64	113	33	39.4	8.2	0.11	0.0023
	Nga Ywan Nu	bottom	16.7	22.9	88.7	9	11.3	111	148	24	36	1.2	0.07	0.0015
	Yar-8	top	20.6	28.0	90.6	11	19.5	218	128	20	35.8	11.8	0.06	0.0012
	Ba Ma Htar	top	20.3	25.5	89.7	13	15.4	200	136	23	41.2	2.2	0.08	0.0017
	p-value (bottom vs. top)		*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns

BIO, biomass (g); PH, plant height (cm); TN, tiller number (no.); NPT, nodal root number per tiller (no./tiller); NR, total number of nodal root (no.); LR, lateral root density (no./10 cm); %50–90, percent of nodal roots at 50–90 degrees (%); CC, chlorophyll content (nmol cm^{-2}); PR, photosynthesis rate (CO_2 $m^{-2}s^{-1}$); SC, stomatal conductance (mmol $m^{-2}s^{-1}$); TR, transpiration rate (mm min^{-1}). *, **, and ns represent the significant differences at 0.05, 0.01 levels and no significant difference, respectively.

3.6. Path Analysis

Generally, the valid contribution of traits towards biomass is not represented by a simple correlation. Hence, the path coefficient analysis given by Dewey and Lu [32] provides a view into interrelationships by further separating the correlation coefficients into direct and indirect effects of characters. For lowland rice in the field experiment, plant height (0.86 ***) and tiller number (0.7 **) showed positive direct effects on biomass. In contrast, lateral root density (−0.25 *) showed a negative direct effect on biomass. Xylem area (−0.24 ***), total number of nodal roots (1.14 ***), nodal root number per tiller (−0.98 ***), cortex area (0.45 ***), and stele area (−0.25 *) exhibited indirect effects on biomass via tiller number. Xylem area (0.66 ***) also showed a positive indirect effect on biomass via plant height (Figure 5A). In upland rice under drought stress, the result revealed that the cortex area (2.04 ***) showed a positive direct effect on biomass while xylem number (−0.58 ***) and root thickness (−1.85 ***) showed negative direct effects. Lateral root density (0.36 ***) and stele area (0.78 ***) had positive indirect effects on biomass via root thickness (Figure 5B).



A Lowland DS (field exp.)

B Upland DS (field exp.)

C Lowland DS (pot exp.)

D Upland DS (pot exp.)

Figure 5. Path analysis. (**A**) Lowland rice in field experiment, (**B**) upland rice in field experiment, (**C**) lowland rice in pot experiment, and (**D**) upland rice in pot experiment. PH, plant height (cm); TN, tiller number (number); BIO, biomass (g); NPT, nodal root per tiller (no./tiller); NR, total nodal root number (no.); LR, lateral root density (no./10 cm); RT, root thickness (mm); CA, cortex area (cm^2); SA, stele area (cm^2); XN, xylem number (no.); XA, xylem area (cm^2); %50–90, percent of nodal roots at 50–90 degrees (%); PR, photosynthesis rate (CO_2 $m^{-2}s^{-1}$); SC, stomatal conductance (mmol $m^{-2}s^{-1}$); TR, transpiration rate (mm min^{-1}). *, ** and *** indicate significant levels at 0.05, 0.01 and 0.001, respectively.

In the pot experiment, tiller number (−0.65 **) and total number of nodal roots (1.49 ***) had high direct contributions on the biomass of lowland rice. Percent of nodal root number at 50°–90° and nodal root number per tiller (−0.35 ***) showed negative indirect effects on biomass via tiller number. Photosynthesis rate (−0.64) showed a negative indirect effect on biomass via total number of nodal roots (Figure 5C). For upland rice, the positive direct effects on biomass were contributed by stomatal conductance (0.26 ***), photosynthesis rate (0.13 ***), tiller number (0.27 ***), and total number of nodal roots (0.71 ***). While the percent of nodal root number at 50°–90° showed a negative direct effect on biomass (−0.2 ***). The indirect effect was contributed by nodal root number per tiller via tiller number and total number of nodal roots (0.67 * and 0.93 ***, respectively) (Figure 5D).

4. Discussion

Water deficit is a major constraint to rice production especially in upland and rainfed lowlands. The results in this study illustrated that drought stress affected agronomic traits in both lowland and upland rice such as tiller number, biomass, and plant height. Singh et al. [33] observed the reduction in several traits including plant height, tiller number, spikelet fertility, and grain yield per panicle. Laude [34] observed the reduction in biomass and tiller abortion. The decrease in one thousand grain weight is the most common characteristic of rice under drought stress [35]. Ruangsiri et al. [28] also found the reduction in biomass and 100 grain weight in KDML105-CSSLs population. This indicates that water deficit at the flowering stage is the most serious stress causing yield reduction [36]. Roots are important plant organs. They absorb water and nutrients from the soil and translocate them to plant tops [37–40]. Roots also give mechanical support to plants and supply hormones that affect many physiological and biochemical processes associated with growth and development [41]. In this study, we observed the reduction of root traits such as total number of nodal roots, nodal root number per tiller, lateral root density, cortex area, and root thickness in both lowland and upland rice. Hazman and Brown [42] also found a reduction in nodal roots under drought conditions. Gao and Lynch [43] suggested that a fewer nodal root number can improve deep rooting, stomatal conductance, and photosynthesis under drought stress in maize. In addition, the decrease in nodal root number and lateral root branching was observed in Kato and Okami's [44] experiment under aerobic condition indicating rice roots had responded to aerobic soil as though they were under drought stress. Moreover, reduced lateral root density is important for drought tolerance because this phenotype determines the balance between the mobile and immobile resources [45] and decrease competition for water among roots of the same plant [46]. Drought significantly increased stele area and xylem area in lowland rice. In another study, stele area was observed to increase during drought in lowland rice [17,42]. Conservative stele area under drought could be beneficial for maintaining root penetration ability. Greater stele and xylem area may lead to higher plant water status under water deficit due to higher hydraulic conductivity [47]. Yambao et al. [48] stated that larger xylem diameters were associated with higher axial conductance, thereby enhancing water uptake capacity in rice.

In general, root thickness is considered an important root characteristic contributing to drought resistance [49,50]. In this study, we found that root thickness was decreased under drought stress in both lowland and upland rice. Thick roots have larger xylem vessels, persist longer, produce more and larger branch roots, and thereby increase root length density and water uptake capacity in rice [51]. However, in this study, we observed the reduction under drought stress in both lowland and upland rice. Previously, some research supported that plants with large root thickness or root diameter with large diameter xylem vessels had greater hydraulic conductivity, but less conservative water uses and greater risk of cavitation than those with small diameter vessels [52–56].

In the pot experiment, drought significantly affected the total number of nodal roots and nodal root number per tiller which is similar to the field experiment in both lowland and upland rice. In contrast, drought did not significantly affect lateral root density and the percent of nodal roots at 50°–90° degrees but there is tendency observed that lateral root density increased and the percent of nodal roots at 50°–90° degrees decreased. Lateral roots typically constitute the major portion of root systems, accounting for approximately 90% of the total root length [57,58]. The increased lateral root density in response to drought was observed in several previous studies [42,59–61]. The physiological characters were decreased during drought stress. Drought stress caused a significant reduction in the rate of photosynthesis [62] and the decrease in the transpiration rate as a form of adaptation of plants to drought stress is by closing and reducing the number of stomata so that the transpiration rate can be reduced [63]. Interestingly, chlorophyll content was increased in lowland rice and the same observations were found in potato [64] and sweet potato [65,66]. Delay senescence is the result of a stress-response mechanism characterized by slower chlorophyll degradation over time [67–69].

In lowland rice, we observed that the total number of nodal roots, nodal root per tiller, and xylem area were related to tiller number and biomass under drought conditions. Principal component confirmed the contribution of these traits in lowland rice in both the field and pot experiment (Figure 3A,C). Many studies supported that plants with large diameter xylem vessel resulting in a large xylem area have greater hydraulic conductivity, but less conservative water use and greater risk of cavitation than those with small diameter vessels [52,53,55,56,70]. The relationship among nodal root and tiller number is always connected. Tillering pattern may play a part in genetic variation in assimilating the supply to root growth [21]. Moreover, bulk analysis result showed the groups with a higher biomass always had a higher total number of nodal roots and nodal root number per tiller except in upland rice in the field experiment. Sanhu et al. [71] suggested that a greater number of nodal roots provided yield stability and prevented yield reduction under wet and dry conditions. Path analysis illustrated the large effects contributing to the biomass of lowland rice were lateral root density, nodal root number per tiller, and total number of nodal roots that we found in both field and pot experiments. These finding were observed in Ruangsiri et al. [28]. The higher total number of nodal roots increased the productivity in lowland rice more significantly while the reduction in lateral root density was related to the increase in biomass in lowland rice. Lynch [45] stated that the reduction in lateral roots that was mentioned above is related to the decrease in competition for water among roots.

Root anatomical traits such as cortex area, root thickness, and xylem number had the largest effects on the biomass of upland rice. Reduction in the xylem number and root thickness can improve rice productivity. A decrease in the xylem number could reduce the risk of water losses by xylem leakage [72]. Upland rice varieties which are more drought tolerant, typically have smaller root thickness [16]. Some anatomical traits such as the cortex area also showed the same positive effect in lowland rice. Percent of nodal roots at 50°–90° had a negative effect on biomass in both lowland and upland rice (Figure 3C,D). Additionally, it also showed a negative indirect effect via the tiller number of lowland rice in the pot experiment (Figure 4). Several studies [73–78] indicated that roots are in the topsoil layer and very few roots are found below 30 cm. Vertical deep root penetration would help rice avoid drought stress; however, root penetration is often restricted by the presence of hardpan and our experiment was conducted in pots limiting root penetration. In addition, photosynthesis rate, stomatal conductance, and transpiration rate negatively contributed to the biomass and nodal root number except for the photosynthesis rate in upland rice. This observation was similar to Gao and Lynch's [43] experiment, which found that a low crown root number in maize (nodal root in rice) had a small portion of plant carbon balance devoted to greater water acquisition and better plant water status and greater stomatal conductance and photosynthesis, growth, and yield than high crown root genotype.

5. Conclusions

The study showed that root characters related to agronomic and physiological traits differ in lowland and upland rice. Drought stress experiments were conducted in the field and in a greenhouse and there were different root characters between upland and lowland rice in response to drought stress and the relationship between agronomic and physiological traits. Lowland rice showed a positive relationship between agronomic traits and root traits such as total number of nodal roots and root number per tiller, and a negative relationship with lateral root density more than anatomical traits. While upland rice expressed a relationship between root anatomical traits, such as root thickness, cortex area, xylem number, and anatomical traits. In addition, the photosynthesis rate, stomatal conductance, and transpiration rate negatively contributed to biomass in lowland and upland rice. These finding can help improve drought tolerance in rice by selecting the optimal root trait for each rice ecosystem.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051230/s1, Table S1: Myanmar rice materials. Table S2: Principal component analysis of lowland rice under drought stress in the field experiment. Table S3: Principal component analysis of upland rice under drought stress in the field experiment. Table S4: Principal component analysis of lowland rice under drought stress in the pot experiment. Table S5: Principal component analysis of upland rice under drought stress in the pot experiment. Figure S1:

Soil water potential monitoring by tensiometer. Figure S2: Comparison of upland (U) and lowland (L) rice root characters under drought stress in the field experiment. Figure S3: Comparison of upland and lowland rice root characters under drought stress in the pot experiment. Figure S4: Bulk analysis of lowland rice in the field experiment. Figure S5: Bulk analysis of upland rice in the field experiment. Figure S6: Bulk analysis of upland rice in the pot experiment. Figure S7: Bulk analysis of upland rice in the pot experiment.

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Data Availability Statement: Data is contained within the article or supplementary material. The data presented in this study are available in [Agronomy 2022, 12, 1230].

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