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VARIATION IN MORPHO-PHYSIO TRAITS OF SELECTED RICE (*ORYZA SATIVA* L.) GERMPLASM IN RESPONSE TO WATER STRESS DURING THE VEGETATIVE STAGE

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ABSTRACT

Achieving drought resistance in rice requires a deeper understanding of the mechanisms and traits that could facilitate the process. An experiment was conducted to identifying the morpho-physio traits of rice in response to water stress during the vegetative stage at Rice Research and Development Institute, Batalagoda during *maha* 2012/13 season. Eleven genotypes; CNI 9024, DSN 22, DSN 37, DSN 56, Bg 300, At 307, Bg 352, Bg 357, Moroberecan, Black gora and N 22 were tested in well watered (WW) and water stress (WS) conditions in Complete Randomized Design with 3 replicates as a pot experiment. Two cycles of water stresses were given 25 days after sowing (DAS) at 10 days intervals. The water levels in WW bags were kept at saturated throughout the growing period and data were recorded at 45 DAS. DSN 22, DSN 37, DSN 56 and Black gora had high densities of leaf trichomes. Black Gora, Moroberecan and Bg 352 had deep root systems. Bg 357 did not produce roots below 30 cm of soil depth in WS condition. Total root distribution of Black gora, DSN 56 and Moroberecan was significantly high in WS condition. The thickest roots below 30 cm of soil depth were found in Moroberecan. Accordingly, Black gora, Moroberecan, DSN 37, DSN 56 and Bg 352 have combinations of many traits of drought avoidance and were identified as the most promising drought resistant genotypes. At 307 and DSN 22 and DSN 37 were also performed better while Bg 357 was identified as the least resistant variety.

KEYWORDS: Morpho-physio traits, Drought avoidance, Rice, Water stress.

INTRODUCTION

Drought is becoming a particularly important production constraint for rice cultivation in Sri Lanka. Almost all agro ecological regions of the Dry Zone and Low Country Intermediate Zone (where rice is the major crop cultivated) are highly vulnerable to drought (Chithranayana and Punyawardana, 2008). It is alarming that by the year 2050s, the Dry Zone begin to experience below normal annual rainfall while by the year 2080s, rainfall decreases up to 22% compared to the baseline period of 1961-1990 (Punyawardana *et al.*, 2013). Droughts occur once in every 3 to 4 years and severe droughts of national significance occur within 10 years or so in the country (Anon, 2011). In this context, almost all rice varieties which will be released in future should have high yield potential, short to medium duration and both biotic and abiotic stress tolerance. Thus, screening of genotypes, identification of donor parents and creating drought resistant rice varieties with high yield potential are utmost important for the sustainability of rice sector.

Drought sensitive periods of rice is categorized as early (from vegetative to mid tillering), intermittent (during mid tillering to panicle initiation) and late (panicle initiation to grain filling) according to their growth stages (Piyasiri *et al.,* 2012). Water stress during vegetative stage could be recovered but can reduce plant height, tiller number, and yield due to reduction in leaf expansion and photosynthesis. Late drought has severe effect on rice and becomes a main factor contributing to yield loss. Plants use various mechanisms to cope with drought and are classified as drought escape, drought avoidance and drought tolerance (Manavalan *et al.*, 2009).

Achieving drought resistance in rice requires a deeper understanding of the traits and mechanisms that could facilitate the process. In rice, trichomes presence on the leaf surface are important because they appear as grey or white and reflect light and reduce water loss (Biaolin *et al,*. 2013). In lowland rice, the penetration of hard layer is achieved through the possession of large root diameters (Cook *et al.,* 1997). Gowda *et al.* (2011) reported that thick roots can produce more branches and have greater xylem vessels and reduce the resistance to water flux. Root to shoot ratio (the proportion of root dry matter to shoot dry matter) is a good indicator to measure the dry matter partitioning to roots from shoots. The genotypes that have deep, coarse roots with a greater ability of branching and penetration, higher root to shoot ratio, elasticity of leaf rolling, early stomatal closure and high cuticular resistance are reported as components traits in drought avoidance (Gowda *et al.*, 2011).

Grain yield of rice is the primary trait used in selection for drought resistance but the heritability of yield under stress is much low. Therefore use of secondary traits (morphological and physiological) which are highly heritable and correlated with yield can improve the precision of screening (Verulkar and Verma, 2014). In addition, they can be measured easily and rapidly. These traits can be also used to focus specific type of drought where final yield is a summation of many other biotic and abiotic stresses not directly associated with water. Therefore, the present experiment was conducted to identify the variability in some morpo-physio traits of drought avoidance in response to water stress during the vegetative stage of rice.

MATERIAL AND METHODS

The experiment was carried out in Rice Research and Development Institute (RRDI), Batalagoda during *maha* 2012/13 season. Eleven genotypes namely CNI 9024, DSN 22, DSN 37, DSN 56 (lines selected as drought tolerant with high yield potentials at RRDI) (Piyasiri *et al.*, 2012), Bg 352, Bg 357, Bg 300, At 307 (popular high yielding varieties), Moroberecan, Blackgora and N 22 (drought tolerant exotic varieties) were used. The experiment was conducted as two separate pot trials for well watered (WW) and water stressed (WS) conditions under a rain shelter in Complete Randomized Designs with 3 replicates.

Soils collected from rice field were uniformly mixed and filled into black polythene bags (250G, 20 cm diameter and 50 cm height) up to the height of 40 cm. Each bag was ensured to fill equal volume of soil and allowed to settle for two weeks with standing water. Pre-germinated seeds (soaked in clean water for 24 hours and incubated for 48 hours) were sown as five seeds/bags/genotype and were kept at field capacity for 7 days. Five grams of basal fertilizer mixture (N:P:K 5:30:20 kg/ha) was applied to each bag at the time of sowing. The water levels of the bags were maintained at 3 cm of height from 7 days after sowing (DAS) until the WS was induced. Seedlings were thinned out leaving two healthy seedlings per bag 10 DAS. Each bag was fertilized by 3 g of urea and 5 g of 55N:20K kg/ha mixture at 14 and 25 DAS, respectively. The bags subjected to WS were kept without watering for 10 days from the 25th DAS. The water levels of the bags in WW were always kept at saturated throughout the growing period. Ten days after the first WS, the bags were again re-watered by adding 5 l of water/bag and kept for another 10 days without watering.

Tiller numbers and plant height of the genotypes both in WW and WS conditions were recorded 10 days after the 2nd WS (45 DAS). Leaf trichomes were observed under the optical microscope. Degree of leaf pubescence and leaf rolling was recorded according to the method described by IRRI (1996). Soil moisture contents of bags subjected to WS was determined at 30 cm depth in dry weight basis. Each polythene bag was then cut horizontally into 10 cm segments. The roots were carefully harvested by washing and removing soils and they were collected into paper bags separately. Length, volume, surface area and average diameter of roots were recorded in each 10 cm segment by using Epsom Perfection Photo 700 root scanning machine. Dry weights of plant and root were also recorded.

Analysis of Variance was performed for the quantitative traits and the count data (normally distributed) using SAS computer package. Mean separation was done by using Duncan's Multiple Range Test at p=0.05. Kruskal-Wallis test was performed to analyze the percentage data. Hierarchical cluster analysis for shoot and root traits in water stress condition were performed separately using Ward method based on principle component analysis using MINITAB.

RESULTS AND DISCUSSION

Average soil moisture contents 10 days after the second water stress inducement in dry weight basis at 30 cm soil depth varied from 8.4% to 9.8% among the genotypes but they were not significant at p=0.05 level.

Variation in shoot traits

Variations observed in leaf pubescence, roughness and leaf rolling among the genotypes in WS condition are given in Table 1. The highest density of leaf trichome was observed in DSN 56 (272/cm^2) followed by DSN 37, DSN 22 and Black Gora (201, 187 and 145/cm^2). Trichomes present in CNI 9024, Bg 357 and N 22 were comparatively low. Leaf blade pubescence score of DSN 37, DSN 56, DSN 22 and Black gora was '3' (pubescence) while the score was '2' (intermediate) in Bg 300, At 307, Bg 352 , Bg 357 and Moroberecan.

Table 1. **Variations in leaf trichomes, leaf blade pubescence and leaf rolling score in water stress condition.**

Genotype	Leaf trichome density (number /cm^2)	Leaf blade pubescence score *	Leaf rolling score**
CNI 9024	24	1	9
DSN 22	187	3	5
DSN 37	201	3	1
DSN 56	272	3	7
Bg 300	68	2	9
At 307	90	2	9
Bg 352	76	2	7
Bg 357	23	1	7
Black gora	145	3	1
Moroberekan	50	1	1
N 22	28	1	1

Notes: *Leaf blade pubescence scores; 1= glabrous, 2= intermediate, 3=pubescent.**leaf rolling scores; 0=leaves are healthy, 1=leaves start to fold (V shape), 3=leaves folding (deep V shape), 5=leaves fully cupped (U shape), 7=leaf margin touching, 9= leaves tightly rolled.

Du *et al.* (2009) reported that, soybean cultivars with high density of pubescence had restricted loss of water via transpiration during drought, thus enhancing photosynthesis in comparison to the cultivars with low density of pubescence. Different degrees of leaf rolling were observed in response to WS and DSN 37, Black gora, Moroberecan and N 22 delayed in leaf rolling. Leaf rolling reduces rate of transpiration and begins at a relatively high water potential in rice. It is used to make a decision on time of irrigation in WS condition before water content reduced to a lethal level (John and Rolando, 1980).

Significant variations in plant height, and shoot dry weight were observed among the genotypes (Table 2). Maximum plant height was observed in Moroberecan both in WW and WS conditions. The minimum reduction in WS was given by Black gora (5%) and Moroberecan (6%). Tiller numbers produced in WS (mean 6.6) were lower than in WW (mean 8.6) condition. At 307 did not reduce its tiller number at WS while Moroberecan produced the lowest tiller numbers both in WW and WS conditions. Shoot biomass was drastically reduced at WS in majority of genotypes (Table 2). The highest reduction was observed in Bg 357 (76%) followed by N 22 (69%). Bimpong *et al.* (2011) stated that, water deficit reduces carbon assimilation due to reduction of stomatal conductance and leads to low biomass production and dry matter partitioning. In contrast, DSN 22 and Black gora increased their dry weights by 13% and 12% at WS.

The dendrogram produced for quantitative shoot traits using hierarchical cluster analysis illustrates two distinct clusters at a Euclidian distance of -27.72 and four clusters at a Euclidian distance between 14.86 and 57.43 (Figure 1). The cluster comprised with CNI 9024, N22, Bg 300 and Bg 357 was characterized by both low trichome densities and shoot dry weights. DSN 22, DSN 37 and DSN 56 consisted with high leaf trichomes were grouped into a separate cluster. At 307 and Black gora which grouped together had significantly high shoot dry weights.

Variation in root traits

The vertical distribution of roots as given by root lengths differed among the genotypes in response to soil depth and water availability (Figure 2). The root distributions in different soil depths at WS were considerably lower than those found in WW condition in all genotypes except in DSN 37, Black gora and Moroberecan. These varieties showed comparatively higher lengths of roots in some of the soil layers in WS than in WW condition. Black Gora, Moroberecan and Bg 352 had deep root systems at WS and have ability to use water stored at deeper soil layers during the water stress. In contrast, Bg 357 did not produce roots below 30 cm of depth at WS. It also had poor root distribution below 30 cm of depth even in WW condition and identified as a variety having a shallow root system and therefore may not suitable for cultivating in drought prone areas. Gowda *et al.* (2011) reported that the length and mass of rice roots decrease exponentially with the depth.

Table 2. **Variations in mean plant height, tiller number and shoot dry weight among genotypes in WW and WS conditions.**

Genotype	Plant height (cm)			Tiller number /plant (g)			Shoot dry weight/plant (g)		
	WW	WS	Reduction (%)	WW	WS	Reduction (%)	WW	WS	Reduction (%)
CNI 9024	72.3 c	58.8 de	18.6	8.0 a	5.7 bc	28.8	9.8 bc	4.1 ef	58.2
DSN 22	95.8 ab	79.0 bc	17.5	8.7 a	6.0 b	31.0	5.4 c	6.1 cde	(13.0)
DSN 37	95.8 ab	76.5 bc	20.1	10.0 a	6.3 b	37.0	13.1 ab	9.3 ab	29.0
DSN 56	99.8 ab	82.8 b	17.0	8.3 a	6.3 b	24.1	16.0 a	8.3 abc	48.2
Bg 300	89.0 abc	53.8 e	39.5	9.3 a	7.0 b	24.7	13.0 ab	8.0 bcd	38.5
At 307	90.3 abc	67.8 cd	24.9	9.0 a	9.7 a	(7.8)	16.9 a	9.4 ab	44.3
Bg 352	81.5 bc	66.5 cde	16.6	8.7 a	6.3 b	27.6	12.6 ab	5.0 def	60.3
Bg 357	72.3 c	59.5 de	17.7	9.7 a	5.7 bc	41.2	13.2 ab	3.2 f	75.8
Black gora	101.0 a	95.8 a	5.1	8.7 a	8.3 ab	46.0	10.0 bc	11.2 a	(12.0)
Moroberekan	107.0 a	101.3 a	6.3	4.0 b	3.3 c	17.5	16.3 a	8.5 abc	47.8
N 22	99.8 ab	75.0 bc	24.8	10.0 a	7.7 ab	23.0	16.7 a	5.2 def	68.9
Mean	91.3	74.3	18.9	8.6	6.6	26.6	13.0	7.1	34.6
CV%	8.2	7.3		14.1	21.5		16 .2	18.2	

Notes: WW = Well Watered, WS = Water Stress. Within each column, the means followed by the same letter are not significantly different at p=0.05.Values within Parenthesis are percentage increment.

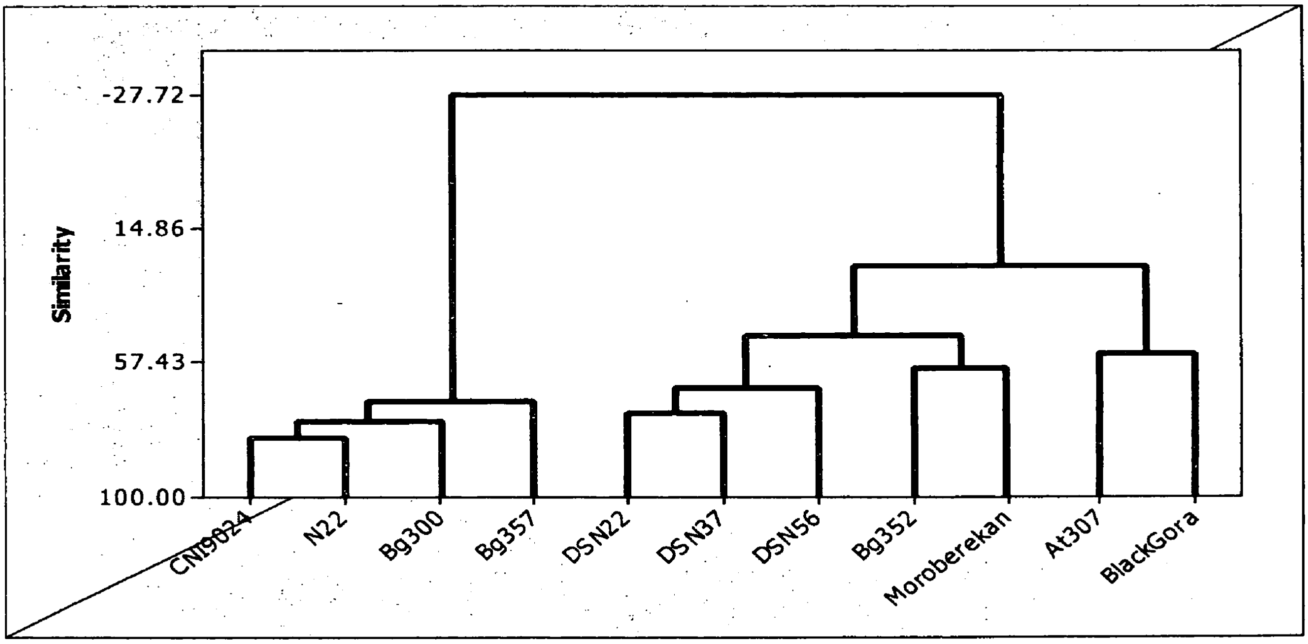

Figure 1. **Dendrogram of eleven rice genotypes based on shoot traits for water stress condition.**

Significant variations in total root distribution (total root system) were observed among the genotypes (Table 3). At 307, Bg 300, DSN 56, Bg 352 and Bg 357 had high total root length in WW condition implying that they are efficient in extracting soil nutrients when the soil is saturated or irrigated with sufficient water. Black gora, DSN 56 and Moroberecan had significantly higher total root distributions in WS condition while the lowest was observed in Bg 357. Abd *et. al.* (2010) reported that drought resistant genotypes of rice have high proportion of roots being present in lower soil layers below 20 cm. In this study, the proportion of roots distributed below 30 cm varied from 0.08-0.33 (WW) and 0-0.4 (WS). Bg 352, Black gora, N 22, Moroberecan and CNI 9024 had greater portions of deep roots (more than 0.2) in WW condition and may have ability to withstand late drought if water is adequately available during their vegetative stages. Comparatively higher deep root proportions were observed in Bg 352, Bg 300, Black gora, Moroberecan and N 22 at WS indicating that they have ability to penetrate into deeper soil layers searching their water requirements.

Significant differences were observed in total root surface area, root volume and average root diameter among the genotypes in WW and WS conditions (Table 4). Root surface area ranged between 266.9 cm^2 (Bg 352) to 905.2 cm^2 (At 307) in WW condition. Conversely, the highest root surface area in WS condition was observed in Black gora (758.6 cm^2) followed by Bg 352 (621.7 cm^2). The lowest were found in Bg 357 followed by Bg 300, N 22 and CNI 9024. At 307 had comparatively higher root surface areas in both conditions. Root volume which is a function of total root length and the average root diameter was significantly high in Moroberecan, At 307, DSN 37, Bg 300 and Bg 357 in WW condition (Table 4). The highest root volume at WS was observed in Black Gora (5.7 cm^3) and it was higher than in WW condition (3.1 cm^3). In WS condition, large diameter roots were found in Moroberecan, Black gora, and DSN lines, At 307, Bg 352 and N 22. The thickest roots below 30 cm of soil depth were found in Moroberecan at both WW and WS (0.44 and 0.57 mm, respectively). Abd *et al.* (2010) stated that in lowland rice cultivation, the ability to penetrate the hardpan is achieved due to large root diameters. Thick roots also have greater capacity to uptake water because they can persist longer and produce more and larger root branches with greater xylem vessels.

Variations in root dry weight and root to shoot ratio

High root dry weights in WW condition was observed in Moroberican and At 307 (Table 5). In WS condition, the highest root dry weight was found in DSN 37 followed by Black gora, Moroberecan and At 307 while the lowest were found in Bg 357. Root to shoot ratio is a measure of the allocation of resources between different plant components (Gowda *et al.*, 2011). Asch *et al.*, (2004) reported that the proportion of root to shoot allocation is an average of 0.05-0.1 at flowering; however genetic variations among the genotypes existing. In this study, root to shoot ratio ranged in between 0.03-0.1 in WW condition while it was 0.02-0.09 in WS condition. The highest dry matter allocation in WW condition was found in DSN 22. The dry matter partition in WS condition was high in DSN 37, Black gora and Moroberecan. It has been proven that more dry mater is partitioned to roots than shoots at drought stress, but this relationship was observed only in DSN 37, At 307, Bg 352 and in Black gora where root to shoot ratio increased from WW to WS. The lowest root to shoot ratio was found in DSN 56 and Bg 300 at WS.

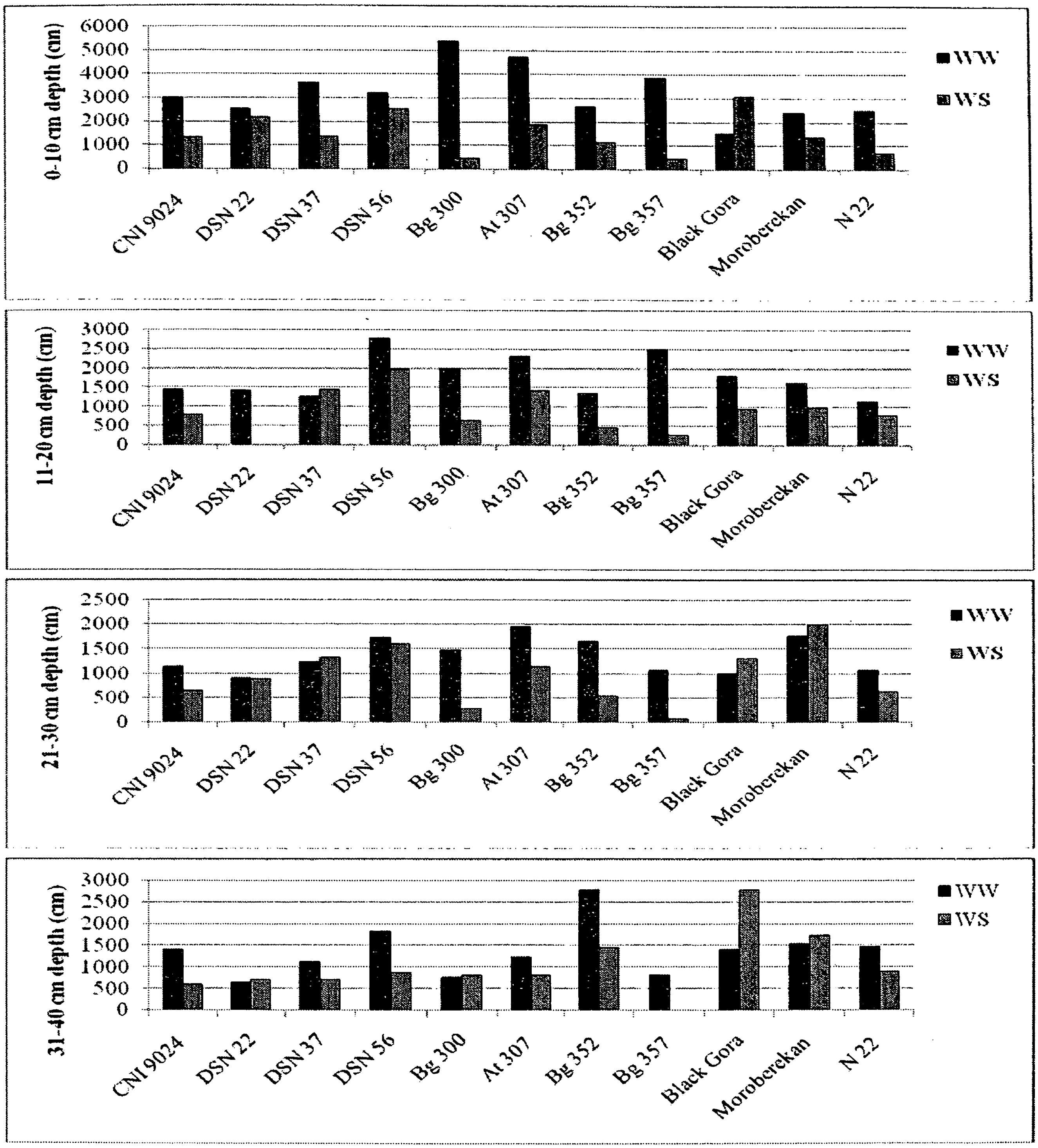

Figure 2. **Mean values of root distribution (expressed in lengths) at different soil layers.**

Table 3. **Variations in total root distribution and proportion of deep root distribution below 30 cm depth in WW and WS conditions.**

Genotype	Total root length (cm)		Proportion of deep root distribution below 30 cm depth	
	WW	WS	WW	WS
CNI 9024	6,994.5 bc	3,314.8 cde	0.20	0.18
DSN 22	5,466.6 cd	3,767 cde	0.11	0.14
DSN 37	7,237.6 bc	4,854.3 bcde	0.16	0.14
DSN 56	9,529.1 ab	6,953.7 ab	0.19	0.13
Bg 300	9,609.7 ab	2,180.9 cd	0.08	0.37
At 307	10,176.7 a	5,238.6 bcd	0.12	0.17
Bg 352	8,424.4 abc	3,570.7 cde	0.33	0.40
Bg 357	8,237.1 abc	806.9 f	0.10	0.00
Black gora	5,718.9 cd	8,092.3 a	0.25	0.34
Moroberekan	7,362.5 bc	6,081.5 abc	0.21	0.28
N 22	6,152.1 cd	3,005.9 cde	0.24	0.30
Mean	7,719.0	4,351.5	0.18	0.22
CV %	15.7	25.7		

Note: Within each column, the means followed by the same letter are not significantly different at p=0.05

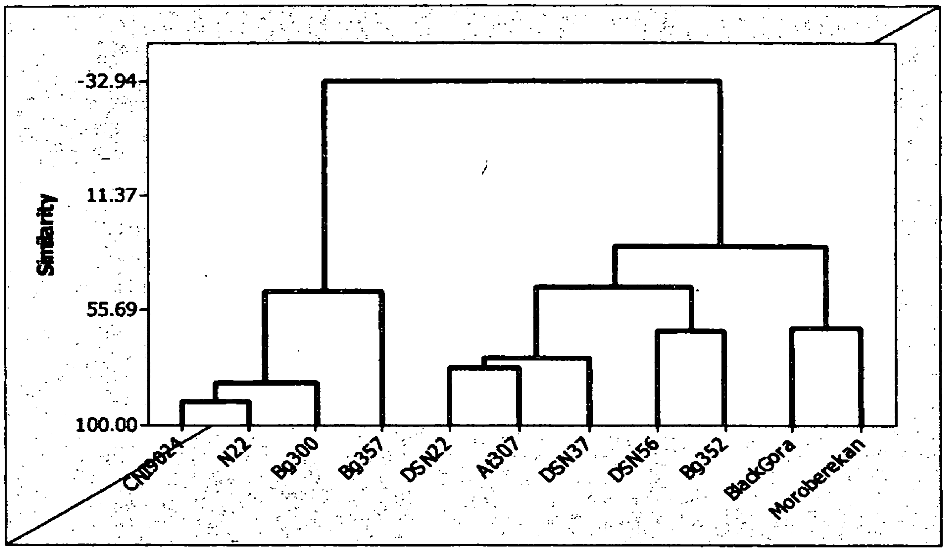

Figure 3. **Dendrogram of eleven rice genotypes based on root traits for water stress condition.**

The dendrogram produced for quantitative root traits under WS condition illustrates that there were five distinct clusters of genotypes at a Euclidian distance between 11.37 and 55.69 (Figure 3). Bg 357, which is characterized by the lowest root length, surface area, volume and dry weight under WS condition, produced a single cluster. In contrast, Black Gora and Moroberecan which had higher values for the above traits were grouped together. Bg 352 and DSN 56 formed a single cluster and separated from DSN 22, At 307 and DSN 37 due to high root surface area and diameter.

Table 4. Variations in total root surface area, total root volume and average diameter of roots.

Variety	Total root surface area (cm^2)		Total root volume (cm^3)		Root volume below 30 cm (cm^3)		Average diameter of roots (mm)		Average diameter of roots below 30 cm (mm)	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
CNI 9024	554.8 c	236.8 cd	3.5 c	1.7 cdef	0.42 cd	0.20 bc	0.40 abcde	0.23 bc	0.21 bc	0.22 bc
DSN 22	507.8 c	409.0 bc	3.9b c	2.8 bcd	1.54 ab	0.19 bc	0.38 abcde	0.29 abc	0.21 bc	0.28 b
DSN 37	809.4 a	471.9 b	7.6 a	3.3 bc	0.61 bcd	0.27 b	0.49 bcde	0.31 abc	0.27 bc	0.19 bc
DSN 56	513.6 c	624.8 ab	2.8 c	2.5 bcde	0.56 bcd	0.19 bc	0.30 de	0.36 ab	0.21 bc	0.15 c
Bg 300	838.6 a	147.9 d	6.1 ab	0.8 ef	0.29 d	0.19 bc	0.52 abc	0.22 bc	0.25 bc	0.21 bc
At 307	905.2 a	552.9 ab	7.9 a	3.9 b	1.39 abc	0.12cd	0.55 a	0.36 ab	0.31 b	0.23 bc
Bg 352	266.9 d	621.7 ab	3.7 c	1.6 cdef	0.42 cd	0.37 a	0.33 cde	0.30 abc	0.21 bc	0.20 bc
Bg 357	800.8 a	61.7 d	6.0ab	0.4 f	0.27 d	0.00 d	0.35 bcde	0.17 c	0.18 c	0.0 d
Black gora	439.2 cd	758.6 a	3.1 c	5.7 a	0.59 bcd	0.23 b	0.28 d	0.36 ab	0.24 bc	0.30 b
Moroberekan	782.5 ab	517.6 b	8.0 a	3.6 b	1.65 a	0.21 bc	0.54 ab	0.4 a	0.44 a	0.57a
N 22	587.9 bc	225.4 cd	4.8 bc	1.4 def	1.00 abcd	0.19 bc	0.41 abcde	0.27 abc	0.25 bc	0.22 bc
Mean	637.0	420.8	5.4	2.5	0.81	0.2	0.41	0.30	0.25	0.23
CV%	14.4	21.7	19.3	29.9	41.4	20.4	19.0	22.3	20.4	21.4

Note: Within each column, the means followed by the same letter are not significantly different at $p=0.05$

Table 5. **Variations in total root dry weights and root: shoot ratio (dry weight basis) in well water and water stress conditions.**

Genotype	Total root dry weight/plant (g)		Root: Shoot ratio	
	WW	WS	WW	WS
CNI 9024	0.52 bcd	0.18 cd	0.05	0.04
DSN 22	0.55 bcd	0.45 bc	0.10	0.07
DSN 37	0.74 bcd	0.88 a	0.06	0.09
DSN 56	0.49 cd	0.13 d	0.03	0.02
Bg 300	0.78 bc	0.19 cd	0.06	0.02
At 307	1.08 a	0.65 ab	0.06	0.07
Bg 352	0.48 d	0.24 cd	0.04	0.05
Bg 357	0.77 bcd	0.10 d	0.06	0.03
Black Gora	0.54 bcd	0.84 a	0.05	0.08
Moroberekan	1.26 a	0.66 ab	0.08	0.08
N 22	0.79 b	0.21 cd	0.05	0.04
Mean	0.72	0.41	0.06	0.05
CV%	16.4	29.0		

Note: Within each column, the means followed by the same letter are not significantly different at $p=0.05$

CONCLUSIONS

This study revealed that significant variations exist among the genotypes for morpho-physio traits studied in response to water stress during the vegetative stage. DSN 22, DSN 37, DSN 56 and Blackgora were characterized as genotypes having high densities of leaf trichomes. Plant height and tiller number of all genotypes reduced when the moisture content of the soil decreased during the vegetative stage. Shoot dry weight drastically declined at WS in almost all genotypes, but DSN 22 and Black gora had 13% and 12% increment respectivly. Black gora, Moroberecan and Bg 352 had deep root systems while Bg 357 did not produce roots below 30cm of depth at WS. Total root distribution of Black gora, DSN 56 and Moroberecan was significantly high in WS condition. The thickest roots below 30 cm of soil depth were found in Moroberecan at both WW and WS. Dry matter partition to roots at WS was high in DSN 37, At 307, and Bg 352 and in Black gora. Accordingly, Black gora, Moroberecan, DSN 37, DSN 56 and Bg 352 have combinations of many morpho-physio traits in drought avoidance and were identified as the most promising drought resistant genotypes. At 307 and DSN 22 were also performed better while Bg 357 was identified as the least resistant variety. These traits should be studied further to identify heritable variations linked to the yield.

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