EVALUATION AND CHARACTERIZATION OF ADVANCED RICE MUTANT LINE OF RICE (*ORYZA SATIVA*), MR219-4 AND MR219-9 UNDER DROUGHT CONDITION

PENILAIAN DAN PENCIRIAN WARISAN MUTAN PADI (*ORYZA SATIVA*), MR219-4 DAN MR219-9 DI BAWAH KEADAAN KEMARAU

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Abstract

Two advance rice mutant lines, MR219-4 and MR219-9 derived from mutagenesis of Oryza sativa cv. MR219 with gamma radiation at 300Gy were evaluated in simulated drought condition in the greenhouse at Malaysian Nuclear Agency. The mutants were evaluated simultaneously with ARN1, a drought resistant variety and MR211 a susceptible cultivar as a check. Randomized complete block design with three replicates was used in the experiment. The evaluation and selection were done based on leaf rolling and leaf drying as well as other agronomic traits, such as, number of tillers per plant, plant height, flag leaf area, grain weight per plant, grain yield per plant, 100-grain weight, harvest index, panicle length and plant biomass. The mutants MR219-4 showed moderate tolerance and MR219-9 showed tolerance to drought respectively as compare to the check variety (ARN1, MR211) and control MR219. Leaf rolling, leaf drying, days to flowering and days to maturity are valuable secondary traits that may provide additional information for selection because of associating with the plant survival under water stress. Further research on expression of drought-tolerant lines under different drought conditions is essential in order to identify particular traits that are associated with drought tolerance and high yield potential. Similarly the importance of secondary traits, relative to other putative traits for drought tolerance, needs to be tested in various environments.

Abstrak

Dua warisan mutan padi, MR219-4 dan MR219-9 terhasil dari mutagenesis Oryza sativa cv. MR219 dengan sinaran gama pada 300Gy telah dinilai dalam simulasi keadaan kemarau di rumah hijau, Agensi Nuklear Malaysia. Warisan mutan tersebut dinilai serentak dengan ARN1, varieti tahan kemarau dan MR211 kultivar yang rentan sebagai kultivar semakan. Rekabentuk penyelidikan RCBD telah digunakan yang diulang sebanyak 3 replikasi. Penilaian dan penyaringan telah dijalankan berdasarkan ke atas daun yang bergulung dan kering dan ciri-ciri agronomi yang lain. Ciri-ciri seperti bilangan tiller, tinggi pokok, luas daun pengasuh, berat biji per pokok, hasil biji per pokok, berat 100-biji, berat kering pokok, panjang panikel, biomas dan indeks tuaian direkodkan. Keputusan menunjukkan MR219-4 didapati sederhana rintang dan MR219-9 didapati rintang kepada kemarau berbanding dengan varieti semakan (ARN1, MR211) dan kawalan, MR219. Skala daun bergulung, daun kering, masa untuk berbunga dan masa untuk matang adalah ciri-ciri sekunder bernilai yang boleh memberi maklumat tambahan untuk pemilihan kerana ciri-ciri ini didapati berkait rapat terhadap kemandirian pokok kepada tekanan air. Kajian seterusnya ke atas eksperesi warisan padi yang rintang kemarau di kawasan kemarau yang lain adalah sangat perlu bagi mengenalpasti ciri-ciri yang berkaitan dengan rintang kepada kemarau dan potensi hasil yang tinggi. . Begitu juga kepentingan ciri-ciri kedua yang berkait rapat dengan ciri-ciri yang tahan kepada kemarau perlu diuji di beberapa lokasi yang berbeza.

Keywords/Kata kunci: mutant line, drought-tolerant, gamma radiation

INTRODUCTION

Rice is susceptible to drought as compared to other crop species. This sensitivity normally severe at reproductive stage and it can lead to various degrees of sterility (Tuong & Bhuiyan 1994). The efforts have been taken in identifying and mapping quantitative trait loci (QTLs) affecting traits related to drought tolerant in order to facilitate marker assisted selection (MAS) for genetic improvement of drought tolerance in rice (Sariam et al. 2010). Despite a large number of drought tolerance QTLs identified, progress in breeding for drought tolerance by MAS has been slow.

Drought tolerance refers to the extent to which plants maintain their metabolic function when leaf water potential is markedly low (Athar & Ashraf 2009). Although mechanism of drought tolerance is poorly understood, osmotic adjustment is considered to be associated with dehydration tolerance. According to Neumann (2008), osmotic adjustment is the accumulation of organic or inorganic solutes in response to water stress thereby maintaining tissue turgor potential. However, in view of earlier studies it is believed that plant tolerance to drought is an adaptive feature involving plant responses at cellular and at whole plant level such as synthesis and accumulation of organic compatible solutes, synthesis of stress proteins, up-regulation of antioxidant enzymes, development of deep and dense root system, epicuticular wax, leaf rolling and leaf relative water content (Neumann 2008).

The development of drought-resistant cultivars and lines of rice through selection and breeding is of considerable economic value for increasing rice production in areas with low precipitation or without any proper irrigation system (Subbarao et al. 2005). Despite this fact the issue of food security will become more serious due to the forecasted global climatic changes in combination with the increasing world population (FAO 2006).

There were many successful varieties of drought tolerance that have been developed and improved since it has been an important aim of plant breeders for a long time (Khush & Virk 2002). However, availability of genetic variation at inter-specific, intra-specific and intra-varietal levels is of prime importance for selection and breeding for enhanced resistance to any stress (Serraj et al. 2005a). In order to develop drought-tolerant cultivars, it is imperative to develop efficient screening method and suitable selection criteria. Various agronomic, physiological and biochemical selection criteria for drought tolerance are being employed to select drought tolerant plants, such as grain yield, harvest index, dry weight, leaf water potential, osmotic adjustment, water use efficiency and stomatal conductance (Neumann 2008). In addition, Subbarao et al. (2005) stated that there is no single trait that breeders can use to improve productivity in a water deficit environment since many adaptative traits are effective only for certain aspects of drought tolerance and over a limited range of drought stress. Hence, physiological or morphological traits that contribute to check water loss through transpiration and enhance water use efficiency and yield are suggested as traits of interest.

Classical studies have generated significant amounts of information regarding the morphological traits related to drought tolerance in rice (Subbarao et al. 2005). Fischer et al. (2003) stated that grain yield is the primary trait for selection in breeding programs for drought-prone environments. However, as grain yield is a quantitative trait, selection directly on grain yield is ineffective. Heritability value for grain yield is usually low. Therefore, indirect selection on secondary traits such as morphological traits that are related to as being important to drought tolerance can be useful to develop drought tolerance rice varieties. According to Fischer et al. (2003), secondary traits are morphological characteristics associated with yield. Nine secondary traits i.e. plant height, flag leaf length and width, panicle length, total number of tillers, days to 50% flowering, days to maturity, leaf rolling and leaf drying, suggested as putative traits for drought tolerance by Fischer et al. (2003) would be evaluated.

In 2003 radiation mutagenesis study on developing new rice cultivar adaptable to low water requirement was carried out in collaboration between Malaysian Nuclear Agency, Malaysian Agriculture Research Development Institute and Universiti Putra Malaysia. Two promising mutant lines namely MR219-4 and MR219-9 has been generated from the studies which has prone to law water input as well as grow in aerobic condition (Abdullah et al. 2010). Further studies to verify the advance mutant lines is essential in order to enhance more information on its traits as compare to the variety tolerant and susceptible to drought.

Two mutant lines (MR219-4 and MR219-9) and two local rice varieties (MR211 and MR219) together with Aeron 1 (ARN 1) were used in this study. Mutant lines MR219-4 and MR219-9 derived from mutagenesis of MR219 with gamma radiation, showed high yield potential under aerobic condition. These two lines were used in this experiment for further evaluation to determine the yield potential under drought condition. The aims of the investigation are i) to determine the morphological traits associated with drought tolerance in rice ii) to determine the yield potential of drought tolerance lines.

MATERIALS AND METHODS

Two mutant lines MR219-4 and MR219-9, varieties MR211, MR219 and ARN1 were grown in a greenhouse at the Malaysian Nuclear Agency (MNA), Bangi. Two troughs of 35 cm depth filled with clay loam soil were designed for water stress and control which were considered as treatments. Each trough had an area of 16 m^2. The MR219-4 and MR219-9 and check varieties MR211, MR219 and ARN1 were assigned in a randomized complete block design (RCBD) with three replications. The pre-germinated seeds were sown in trays containing wet soil. Healthy seedlings were transplanting 26 days after germination. The seedlings were planted in a row consisting of nine plants each genotype were replication three represented 15 rows, all together. The planting distance was 23 cm within and 23 cm between rows. The space between two adjacent troughs was 1 m. The water were drained at 30 days after transplanting (DAT) and was re-irrigated periodically when soil water tension fell below -50kPa. The control was continued with standing water until maturity.

Evaluation on Morphological Traits. Morphological traits evaluated on single plant basis were plant height, days to flowering, number of tillers, flag leaf area, panicle length and days to maturity. The plants were scored for leaf rolling and leaf drying by observing visually using 0-9 scale (Table 1 & Table 2) based on Standard Evaluation System adopted for rice (IRRI 1996).

Harvesting. The process of harvesting the grains was done manually when the plants reached maturity. Grains from each plant were packed in an envelope. The culms and leaves were cut at ground level and wrapped with newspaper. The grains and plant parts were dried in an oven at 37°C for 48 hours.

Table 1 Leaf rolling score description

Scale	Description
0	Leaves healthy
1	Leaves starts to fold
3	Leaves folding (deep V-shaped)
5	Leaves fully cupped (U-shaped)
7	Leaves margins touching (O-shaped)
9	Leaves tightly rolled

Table 2 Leaf drying score description

Scale	Description	Rate
0	No symptoms	Highly resistant
1	Slight tip drying	Resistant
3	Tip drying extended to ¼ length in most leaves	Moderately resistant
5	¼ to ½ of the leaves fully dried	Moderately susceptible
7	More than 2/3 of all leaves fully dried	Susceptible
9	All plants apparently dead	Highly susceptible

Measurement on Agronomic Traits. The measured agronomic traits for each plants were grain weight, grain yield, 100-grain weight, dried plant weight, biomass and harvest index.

Analysis of Variance **(ANOVA).** Data were analysed using the statistical analysis system (SAS 9.1.3) for windows software. All the data obtained were subjected to a two-way analysis of variance (ANOVA) and the mean differences were compared by least significant differences (LSD).

RESULTS AND DISCUSSION

Leaf Responses to Drought **Tolerance.** Leaf rolling and leaf drying are used as an indication of tolerance in drought studies. Table 3 shows the results of ANOVA for leaf rolling and leaf drying for 25 rice lines. The results showed that there were significant differences among the evaluated lines for leaf rolling and leaf drying.

Table 3 Analysis of variance for leaf rolling and leaf drying traits

Source	Df	Mean squares	
		Leaf rolling	Leaf drying
Lines (L)	24	0.5494*	0.4691*
Replications/L (R/L)	50	0.1821	0.2914
Plants/Plot	150	0.0001	0.0004

*significant at level $p<0.05$

Leaf rolling and drying. The early sign of soil water declining is leaf rolling which is a simple expression of leaf wilting. Fischer et al. (2003) have suggested leaf rolling as a criterion for scoring drought tolerance in rice cultivars. Therefore, leaf rolling is useful for quick screening hundred of lines (Lafitte et al. 2004a). The method of screening is by scoring the plants on a scale 0 to 9 (Table 1) according to Standard Evaluation System adopted for rice (IRRI 1996).

Table 4 shows that ARN 1, a drought resistant check variety had the best score of 0 for leaf rolling. Meanwhile, MR211, the susceptible check variety had a score of 7 indicating susceptibility to drought. MR219 had a score of 5 which was considered as moderately susceptible. For the mutant lines, MR219-4 scored 3 and MR219-9 scored 1. Based on Revandy et al. (2010), MR219-4 and MR219-9 are known to be tolerant to submergence condition. Kadioglu and Terzi (2007) stated that leaf rolling is a hydronastic mechanism that reduces light interception, transpiration and leaf dehydration. Fischer et al. (2003) mentioned that leaf rolling is correlated with internal water status of the leaf tissue and it is also related with the stomatal closure and decreases transpiration from rice leaves. Leaf rolling might play a similar role in osmotic adjustment to maintain internal plant water status (Subahsri et al. 2005). The internal water status of the leaf tissue is affected by drought since the results showed that most of the evaluated lines of DTY 2.2 and DTY 3.1 populations had high leaves wilting scores 5 to 7. According to Kadioglu and Terzi (2007), high level of leaf rolling can be advantageous in terms of prevention of water loss.

According to Fischer et al. (2003), typically leaf drying begins at the tip of the leaf, which is usually under greater water deficit than the basal part that closer to the stem. Leaf drying was observed visually by scoring the plants on a scale 0 to 9 (Table 2) based on Standard Evaluation System adopted for rice (IRRI 1996).

Table 4 shows that resistant check variety, ARN 1 had the best score of 1 indicating highly resistant. MR211 which is the susceptible check variety scored 7, indicating susceptibility. MR219 scored 3 thus it could be considered as moderately resistant. Revandy et al. (2010) mentioned that MR219-4 and MR219-9 are known to be tolerant to submergence condition. From the results, both of the mutant lines MR219-4 and MR219-9 scored 1, and were considered as being resistant to drought.

According to Fischer et al. (2003), leaf water deficiency can be further reduced beyond the point of turgor loss in which reaching the point of tissue death. According to Kadioglu and Terzi (2007), low score of leaf drying can be advantageous in terms of less damage under water stress. In this study, lower score in MR219-9 and MR219-4 which had scored 1 indicating that they were less damage from water stress.

Table 4 Mean value of leaf rolling and leaf drying traits of evaluated lines

Line	Leaf rolling mean score	Leaf drying mean score
MR219-4	3	1
MR219-9	1	1
MR211	7	7
MR219	5	3
ARN 1	0	1
$LSD_{(0.05)}$	0.29	1.70

Morphological Traits. Table 5 shows that there were significant differences between treatments for number of tillers, days to flowering, plant height, flag leaf area and days to maturity. Lines showed significant differences ($p<0.05$) for number of tillers, days to flowering, flag leaf area, panicle length and days to maturity (Table 5). There were significant interaction between treatments and lines for number of tillers, days to flowering, flag leaf area, panicle length and days to maturity.

Number of Tillers. The number of tillers was significantly different between treatments (Table 5). Table 6 shows the mean values of tiller number for each of the evaluated line under non-stress and stress conditions. ARN 1 showed significant difference for number of tillers per plant between normal water supply and water stress with the mean of 3.79 tillers and 6.34 tillers respectively. It shows that ARN 1 had 67% higher number of tillers under water stress compared to non-stress. On the other hand, varieties MR211 and MR219 had significantly lower tiller number per plant under water stress than under non-stress. MR211 had the mean of 8.06 tillers under non-stress and 6.89 tillers under water stress, and MR219 had the mean of 7.04 under non-stress and 5.89 tillers under water stress. The mutant lines MR219-4 and MR219-9 also showed significantly decrease in number of tillers under stress, with each line decreased by 24% and 41% respectively.

According to Teng et al. (2004), water stress causes the plants to experience less water uptake thus inhibiting sufficient food preparation and inhibiting cell division of the meristematic tissue. Tripathy et al. (2000) also reported that water stress had affected tiller number per plant by reducing the number of tillers production. Ichwantoari et al. (1989) stated that cultivars that produced less number of tillers under normal water supply condition were less susceptible to drought than cultivars producing a higher number of tillers. Thus, the lines S4* and S15* that have less tillers when grown under normal water supply can be considered as less susceptible to drought than other of the evaluated lines.

Days to Flowering. Flowering time is an important determinant of grain yield under prolong or severe drought conditions. According to Pantuwan et al. (2002), earlier flowering genotypes would escape the severe water stress and had higher grain yields. Table 5 shows that the days to flowering were significantly different between treatments. Water stress delayed flowering as shown in Table 6. Hence, it shows that days to flowering were affected by water stress.

ARN 1 reached the flowering stage significantly earlier under normal water supply than under water stress with the means of 50 days and 55 days respectively. Similarly, MR219 reached the flowering stage significantly earlier under normal water supply than under water stress. On the other hand, MR211 was flowered earlier under water stress than under non-stress. Meanwhile, water stress had effect on days to flowering for MR219-4 and

MR219-9 as the means showed significantly different between non-stress and stress. Both of the mutant lines were flowered earlier under non-stress than under water stress.

Plant Height. Table 5 shows that there was significant difference between treatments for plant height but there was no significant difference among the lines for plant height. The means of plants for the lines were in the range of 82.89 cm to 199.40 cm under no-stress and 83.78 cm to 119.11 cm under water stress. Water stress had no effect on plant height for ARN 1 as the mean between non-stress and stress was not significantly different, with the means of 110.72 cm and 112.33 cm respectively. MR211 showed similar result with no significant difference in plant height mean between non-stress (95.56 cm) and water stress (92.33 cm). For MR219, water stress had caused significant reduction in plant height with the mean of 83.11 cm compared to 199.40 cm under normal water supply. Meanwhile, mutant lines MR219-4 and MR219-9 were not affected by water stress for plant height as there were no significant differences in their plant height means between non-stress and water stress (Table 6). According to Halim & Yazid (2010), water deficiency reduced plant height. However, the results in this study contradicted with their results as the plant height for most of the lines were not affected by water stress. This may be due to the water stress that was given after the vegetative stage. When the stress occurred after vegetative stage, there was no effect on plant height as the plants had already achieved their normal height.

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Table 5 Analysis of variance for morphological traits

Source	Df	Mean squares					
		Number of tillers	Days to flowering	Plant height (cm)	Flag leaf area (cm^2)	Panicle length (cm)	Days to maturity
Treatments (T)	1	0.07	0.0034*	17391	532.12*	30.63	0.0111*
Replications/T (R/T)	4	0.06	0.0001	9296	93.56	11.69	0.0001
Lines (L)	4	0.18*	0.1440*	6385	168.40*	29.25*	0.0820*
T × L	4	0.13*	0.0016*	1113	169.13*	7.96	0.0004*
(R/T) × L	16	0.03	0.0003	9496	45.01	19.85	0.0002
Plants/Plot	60	0.02	0.0001	9061	24.81	7.38	0.0001

*significant at level $p<0.05$

Table 6 Mean value of morphological traits of evaluated lines for non-stress and stress

Line	Morphological traits											
	Number of tiller		Days to flowering		Plant height (cm)		Flag leaf area (cm^2)		Panicle length (cm)		Days to maturity	
	*NS	*S	NS	S	NS	S	NS	S	NS	S	NS	S
MR219-4	8.87	6.72	82.99	83.67	110.33	98.44	28.83	32.23	26.62	25.24	103.99	109.67
MR219-9	11.79	6.98	83.37	84.00	102.33	93.11	28.95	17.70	25.54	25.19	105.33	112.00
MR211	8.06	6.89	75.51	74.33	95.56	92.33	31.75	29.86	26.87	27.23	97.61	99.33
MR219	7.04	5.89	83.95	87.99	199.40	83.11	30.10	26.02	27.53	24.37	108.00	112.99
ARN 1	3.79	6.34	50.00	55.00	110.72	112.33	34.75	24.26	24.22	22.92	73.00	79.00
$^{a}LSD_{(0.05)}$	0.21	0.13	0.09	0.07	31.75	14.72	7.98	10.26	3.75	3.19	0.05	0.04
$^{b}LSD_{(0.05)}$	0.20		0.11		25.23		10.62		7.50		0.19	

[a]LSD value for comparing the line within the treatment at $p<0.05$
[b]LSD value for comparing line mean of the treatment at $p<0.05$
*NS for non-stress treatment
*S for stress treatment

Flag Leaf Area. ARN 1 showed no significant difference in flag leaf area between non-stress and water stress with the mean values of 34.75 cm^2 and 24.26 cm^2 respectively. The MR211 and MR219 also did not show significant difference in flag leaf area between non-stress and water stress treatments. For mutant line MR219-4, water stress did not affect the flag leaf area. However, for MR219-9 the water stress caused significant reduction in flag leaf area with the mean value 17.70 cm^2 compared to 28.95 cm^2 under normal water supply. Therefore, MR219-9 was affected by water stress for flag leaf area.

Panicle Length. Table 5 shows that there were no significant differences between treatments, significantly different among the lines and significant interaction between treatments and lines for panicle length. The means of evaluated lines for panicle length in non-stress and stress are shown in Table 6. The means of panicle length for ARN 1, MR211, MR219, and mutant lines (MR219-4 and MR219-9) were not significantly different between non-stress and water stress. Hence, it shows water stress had no effect on panicle length for these check varieties and mutant lines. Therefore, the water stress did not affect the panicle length which corroborate with report by Champoux et al. (1995) that suggested the water stress imposed no effect on assimilate translocation from leaf to vegetative growth of panicles.

Days to Maturity. The water stress had delayed the plants to reach maturity in all of the lines (Table 6). For ARN 1 the water stress delayed the maturity stage by 6 days compared to the non-stress with the mean of 73 days and 79 days respectively. Table 6 also shows that the means of days to maturity for MR211, MR219, MR219-4 and MR219-9 in the non-stress were significantly delayed than water stress. Therefore, water stress had affected the days to maturity for MR211, MR219, MR219-4 and MR219-9. The results corroborate with Levitt (1980), who stated that plants that were under water stress took longer time to flower and to mature as compared to plants that were well watered.

Agronomic Traits. Table 7 shows that there were significant differences ($p<0.05$) between treatments for grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight and harvest index. The lines were significantly different ($p<0.05$) for grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight, biomass and harvest index (Table 7). There were significant interaction between treatments and lines for grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight, biomass and harvest index.

Table 7 Analysis of variance for agronomic traits

Source	Df	Mean squares					
		Grain weight per plant (g)	Grain yield per plant (g)	100-grain weight (g)	Dried plant weight (g)	Biomass (g)	Harvest index
Treatments (T)	1	267.67*	79.88*	0.41*	596.86*	65.13	0.040*
Replications/T (R/T)	4	20.42	9.80	0.10	173.80	186.63	0.010
Lines (L)	4	104.95*	58.33*	2.16*	636.93*	1214.72*	0.009*
T × L	4	124.87*	55.91*	0.21	350.76*	864.66*	0.005
(R/T) × L	16	21.29	7.65	0.71	170.03	173.51	0.006
Plants/Plot	60	20.59	11.94	2.25	110.17	167.49	0.003

*significant at level p<0.05

Table 8 Mean value of agronomic traits of evaluated lines for non-stress and stress

Line	Agronomic traits											
	Grain weight per plant (g)		Grain yield per plant (g)		100-grain weight (g)		Dried plant weight (g)		Biomass (g)		Harvest index	
	*NS	*S	NS	S	NS	S	NS	S	NS	S	NS	S
MR219-4	14.54	9.68	9.18	8.46	3.82	3.71	31.05	36.06	45.59	45.74	0.21	0.20
MR219-9	21.17	9.49	16.17	8.60	4.06	3.78	43.19	35.69	64.37	45.19	0.25	0.20
MR211	11.26	9.66	9.48	7.79	3.46	3.45	31.54	33.91	42.80	43.58	0.21	0.18
MR219	9.80	12.18	7.98	10.07	3.70	3.63	24.20	40.51	34.00	52.70	0.23	0.21
ARN 1	9.61	8.12	8.43	6.89	3.73	3.52	18.10	27.65	27.72	35.77	0.30	0.21
[a]$LSD_{(0.05)}$	10.41	4.46	6.96	3.54	0.40	0.26	11.11	12.72	18.03	14.90	0.08	0.06
[b]$LSD_{(0.05)}$	9.68		6.64		0.38		13.96		27.25		0.09	

[a]LSD value for comparing the line within the treatment at p<0.05
[b]LSD value for comparing line mean of the treatment at p<0.05
*NS for non-stress treatment
*S for stress treatment

Grain Weight per Plant. Grain weight is the weight of dried seeds per plant. The highest value of grain weight per plant is not necessarily considered as high yielding because there is a possibility of the plant containing a lot of empty seeds and thus affecting the weight of grain per plant. The grain weight per plant is shown to be significantly different between treatments (Table 7). Table 8 shows the mean values of grain weight for each of evaluated lines under non-stress and water stress.

Table 8 shows that the mean values of grain weight per plant for ARN 1, MR211 and MR219 were not significantly different between normal water supply and water stress.Therefore, the water stress had no effect on grain weight for these varieties. For mutant line MR219-9, the water stress greatly affected the grain weight with the mean of 9.49 g compared to the mean of grain weight under non-stress was 21.17 g. However, water stress had no effect on grain weight per plant for MR219-4 as the mean was not significantly different.According to Ding et al. (2005), the reduction of grain weight under stress condition might be due to the fact that under water stress, plants are not able to absorb sufficient nitrogen. Hence, it influences the grain weight of rice plants.

Grain Yield per Plant. Grain yield per plant is an important agronomic trait (Richards 2000). Venuprasad et al. (2002) reported that water deficiency reduced yield in *Oryza sativa*. Adam et al. (2002) reported that rice crops are susceptible to drought which causes large yield loss in many countries. In this study, the water treatment had significant effect on grain yield per plant (Table 7). Table 8 shows the means of grain yield for each of the evaluated lines under non-stress and stress conditions. For ARN 1, MR211 and MR219, the water stress had no effect on grain yield as their mean values were not significantly different between non-stress and stress treatments. For the mutant lines, water stress treatment had significant reduction on grain yield for MR219-9 with the mean of 16.17 g under non-stress and 8.60 g under water stress. However, the water stress had no effect on grain yield for MR219-4 as the mean values were not significantly different between non-stress (9.18 g) and stress (8.46 g) treatments.

100-Grain Weight. The 100-grain weight was significantly different between treatments (Table 7). Table 8 shows the mean value of 100-grain weight for each of the evaluated lines under non-stress and stress conditions. The means of the 100-grain weight for ARN 1, MR211, MR219 and the mutant lines (MR219-4 and MR219-9) were not significantly different between non-stress and water stress treatments. Hence, these check varieties and the mutant lines showed that the water stress had no effect on 100-grain weight. The high value of 100-grain weight suggests the large grain size and higher density. Water stress had inhibited the effectiveness of the distribution of photosynthetic products to the reproductive parts which can affect the size of grains formed into seeds (Koh 2005). However, in this study most of the lines were not affected by water stress for 100-grain weight indicating that the lines were tolerant to drought.

Dried Plant Weight. Table 8 shows that the means of the dried plant weight for ARN 1, MR211, MR219-4 and MR219-9 were not significantly different between non-stress and water stress. Therefore, the water stress had no effect on dried plant weight for these check varieties and the mutant lines. However, MR219 showed significantly higher dried plant weight under water stress with the mean 40.51 g compared to non-stress with the mean 24.40 g. Surajit (1981) reported that water stress reduced dried plant weight as number of tillers reduced. In this study, not all of the evaluated lines are affected by water stress for dried plant weight. This may be due to the dried processing of plants parts which are not totally dried in an oven. It might be also due to the occuring of technical problem to control the temperature of the oven at 37°C. Therefore, accurate results for dried plant weight cannot be obtained as the plants are not totally dried.

Biomass. Water stress had no effect on biomass for ARN 1, MR211 and MR219 as the means between non-stress and stress were not significantly different. For mutant lines, MR219-4 and MR219-9 were also not affected by water stress for biomass as there were no significant differences in their biomass means between non-stress and water stress (Table 8). Biomass is highly dependent on grain weight per plant and dried plant weight. According to Peng et al. (2000), grain crops will increase the grain weight per plant as biomass increase. In this study, water stress had no effect on grain weight for most of the evaluated lines as the means between non-stress and stress were not significantly different. It was also showed that water stress had no effect on dried plant weight for most of the evaluated lines as no significant differences between non-stress and water stress. Therefore, the results had proved that biomass is highly related to grain weight and dried plant weight as water stress also had no effect on biomass for most of the evaluated lines.

Harvest Index. The harvest index value was significantly different between treatments (Table 7). Table 8 shows the means of harvest index for each of the evaluated lines under non-stress and water stress. The results showed that ARN 1 had significantly different between non-stress and water stress as it had the mean of 0.30 and 0.21 respectively. Water stress had no effect on harvest index for MR211, MR219, MR219-4 and MR219-9 as the means between non-stress and stress were not significantly different (Table 8). The harvest index is the fraction of total dry matter that is in the grain; for cereals in general and rice in particular; when the grain fills only at the end of the crop's life it is expected that late stress will decrease the harvest index more than early stress. But the effect of stress on grain filling has been suggested to be not great (Peng 1994). Moderate drought stress did not result in a change of the harvest index of crops but severe drought stress does, where it had causing harvest index to decrease (Setter et al. 1996). Genotypes that are adapted to areas of late-season drought should also have high harvest index. In this study, water stress had no effect on harvest index for most of the evaluated lines as the means between non-stress and stress were not significantly different. Hence, it might be due to the level of stress that is moderate stress which did not result in a change of the harvest index.

According to Mohamed et al. (1994), harvest index is a feature that measures the ratio of photosynthesis to the distribution of reproductive (seed) compared to the vegetative parts (stem, young leaves, and roots). Low harvest index is due to inefficient distribution of photosynthesis (Donald 1962). Hay (1995) also stated that the harvest index can be used as a criterion for the selection of high yielding plants. Plants with high harvest index value have good efficiency in the distribution of photosynthesis to the plant that has economic value, such as rice grains (Khanna 1991). According to Chandler (1969), modern high yielding rice cultivars have harvest index values in the range of 0.47 to 0.57. Harvest index for all of the evaluated lines in this study appears to be lower than the suggested range. This may be due to the inefficiency factor distribution of photosynthesis and water deficiency, thus reducing yield.

CONCLUSIONS

Generally, leaf rolling and leaf drying are the results from water loss in the plants under drought condition. Based on leaf rolling and leaf drying, none of the evaluated lines was better than the drought resistant check variety, ARN 1. Water stress caused delay in flowering and maturity. Besides, water stress had effect on number of tillers, plant height and flag leaf area. Water stress also caused reduction in grain weight per plant, grain yield per plant, 100-grain weight, dried plant weight and harvest index. However, water stress had no effect on panicle length and biomass. Overall in this study, leaf rolling, leaf drying, days to flowering and days to maturity can be suggested as valuable secondary traits that may provide additional information for selection because these traits appear to be associated with plant survival under water stress.Further research on expression of drought-tolerant lines under different drought conditions will be needed to identify particular traits that are associated with drought tolerance and high yield potential. Similarly the importance of secondary traits, relative to other putative traits for drought tolerance, needs to be tested in various environments.

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