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# Correlation among Agro-Morphological Variation and Genetic Diversity of Rice (*Oryza sativa* L.) under Drought Stress

Pham Thi Thu Ha [1,2, a], Do Tan Khang [1, b], Phung Thi Tuyen [1, c], Luong The Minh[1, d], Truong Ngoc Minh [1, e], Nguyen Thi Lang [2, f], Bui Chi Buu[3, g], Tran Dang Xuan[1, f, *]

[1]Graduate School for International Development and Cooperation (IDEC), Hiroshima University, 739-8529, Japan.

[2]Cuu Long Delta Rice Research Institute, Thoi Lai, Can Tho, Viet Nam.

[3]Institute of Agricultural Sciences for Southern Vietnam (IAS).

[a]phamthithuhabt@gmail.com, [b]dtkhang@ctu.edu.vn, [c]phungtuyen@gmail.com, [d]ltminh87@gmail.com,[e] minhtn689@gmail.com, [f]tdxuan@hiroshima-u.ac.jp, [g]ntlang@hcm.vnn.vn, [h]buichibuu@hcm.vnn.vn.

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**Abstract.** In this study, the correlation coefficients among agro-morphological variation, genetic diversity, and drought tolerance in 44 rice cultivars were analyzed. The drought tolerance at seeding stage (DTS) was significantly proportional to drought tolerance at vegetative stage (DTV) (r = 0.60). In addition, DTS and DTV had strong significant positive correlation to leaf roll (r = 0.87 and 0.54, respectively). Means of unfilled grains and tilling per panicle were proportionally correlated to DTS (r = 0.22 and 0.25, respectively), and DTV (r = 0.20 and 0.36, respectively). However, weight of 1000 grains and filled grains were recorded no correlation to DTS and DTV. At a homologous coefficient of 16.85 integrated from cluster analysis of agro-morphological, quantitative characteristics and drought tolerant scores, the rice cultivars were divided into five groups. Maximum polymorphic information content (PIC) values were detected in three markers including RM11125, RM21, and RM5629, which were from 0.78 to 0.79. Cluster analysis of microsatellite markers revealed that by a genetic distance of 0.63, the rice varieties were separated into three clusters. The results provide valuable information for rice breeders to select donors in breeding rice integrated with drought tolerance and good agronomic characteristics.

## 1. Introduction

Rice (*Oryza sativa* L.) is the staple food of more than three billion people in the world. Recent estimates on climate change predict that because of water deficiency, the intensity and frequency of drought are becoming a serious problem for crop production, especially in rice cultivation [1,2]. Rice is highly sensitive to water stress [3,4]. Due to irregular rain, rice plants can be damaged by drought during seedling, flowering, and maturity stages. The injuries normally occur on leaves at the seedling stage and on sterile spikelets at the reproductive stage. Particularly, at the reproductive stage, floral fertility in rice is extremely sensitive to water deficiency.

Conventional plant breeding approaches for yield improvement under drought conditions are time-consuming and laborious because the field conditions are required to be carefully managed [5]. Recent studies at the IRRI (International Rice Research Institute) found that there is moderate to high heritability of grain yield under drought stress [4,6,7]. Basically, the main objectives of a breeding program for drought tolerant rice are to determine standard tolerant varieties, to identify important traits, and to evaluate the tolerant levels at seedling and reproductive stages. Therefore, information about agro-morphological and genetic diversity among the drought tolerant rice varieties to select potential parental donors is essential for breeding programs.

Recent advances in molecular biology, principally the development of the polymerase chain reaction (PCR) for amplifying DNA or DNA sequencing has resulted in powerful techniques which can be exploited for screening, characterizing and evaluating genetic diversity of rice. Several types

of molecular markers have been extensively applied for evaluating the genetic variation in rice [8]. These include restriction fragment length polymorphism (RFLP) [9], random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites or simple sequence repeats (SSRs) [10]-[13]. Commonly, SSR markers have been extensively used in genetic diversity in rice because of their high level of polymorphism to establish relationships among individuals even with fewer number markers [14].

This study was carried out to assess drought tolerant levels, agro-morphological and quantitative variations, and genetic diversity of 44 selected rice varieties collected from the Genebank of Cuu Long Rice Research Institute (CLRRI), Vietnam and the International Rice Research Institute (IRRI). The correlation coefficients among them were also analyzed to provide information to breed drought tolerant rice integrated with agro-morphological traits.

## 2. Materials and Methods

### 2.1 Plant materials

Forty-four rice varieties were obtained from the Gene Bank of CDRRI and IRRI. They are either drought tolerant or high yield commercial rice, and selected from preliminary experiments.

### 2.2 Screening of drought tolerance

The experiment of evaluation drought tolerance was laid out in a randomized complete block design with three replications at the reproductive stage under drought stress. These seeds were soaked, germinated in plastic trays in an incubator. Each experimental plot included 30 $m^2$/variety. After 15 days, they were transplanted into cement basins. The row- to- row and plant-to-plant spacing of 20 x 15 cm was maintained. Ten days after transplanting, the drainage through drain taps was set up, without provided water until flowering. Fertilizer was applied at the rate of 100-40-30 kg N-$P_2O_5$-$K_2O$ $ha^{-1}$.

The drought tolerance of rice was evaluated following the standard evaluation system IRRI [15] with scores 0-3 (tolerant), score 5-9 (susceptible).

### 2.3 Agro-morphological character evaluation

The agronomic characters and quantitative traits, including panicle length (cm), number of panicles per plant, 1000-grain weight (g), days to maturity, filled grains, unfilled grains, and yields were recorded from all treatments. The yields were determined by the following formula:

Yield = weight of harvest grain (g)/no. of hills x no. of possible hills x MF,

where:

$$MF = \frac{100-MC}{86}.$$

MC (Moisture content of the harvest grains) per plot was determined immediately after weighing using a moisture meter.

**Table 1**. Leaf rolling level.

| Score | 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 |

### 2.4 Evaluation genetic diversity using SSR markers

DNA extraction was prepared according to a method described by McCouch et al. [14]. A piece of young rice leaf (2 cm) was collected and placed in a 1.5 ml centrifuge tube sitting on ice. The leaf

was ground using a polished glass rod in a well of a Porcelain Spot Test Plate (Thomas Scientific) after adding 400 µl of extraction buffer. Grinding was done until the buffer turned into green, an indication of cell breakage and releasing of chloroplasts and other cell contents. Another quantity of 400 µl of extraction buffer was added into the well. An aliquot of 400 µl of the lysate was replaced to a new tube. The lysate was deproteinized using 400 µl chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and the DNA was then precipitated using absolute ethanol. Afterward, it was air-dried and re-suspended in 50 µl of TE buffer.

Microsatellite primers were used to analyze the genetic polymorphism among the samples. A total of 165 primers were randomly selected from the available microsatellite markers currently for rice [13]. The PCR reactions were conducted with mineral oils and they were processed in a Programmable Thermal Controller programmed for 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 2 min 72 °C, with a final extension at 75 °C for 5 min. After amplification, 10 µl of stop solution was added to the PCR products, which were then denatured at 94 °C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel for observing the amplified DNA bands.

DNA band detection and scoring were conducted as follows: plates were separated using a plastic wedge and removed from the tank. The polyacrylamide gel was soaked in ethidium bromide staining solution for 15 to 20 min. Bands in the stained gels were detected and photographed under UV light. Allelic bands were scored as 1 or 0 for presence or absence, respectively. Pair-wise comparisons of the lines based on the presence or absence of unique and shared polymorphic products were used to calculate genetic similarity coefficients. Similarity of coefficients was calculated by Nei and Li [16] distance measurement in the NTSYS–PC (Numerical Taxonomy and Multivariate Analysis System [17]. The lines were clustered using the unweighted pair group method using an arithmetic averages (UPGMA) clustering algorithm.

### 2.5 Data analysis

Correlation analysis: Correlation coefficient (r) is a measure of the association between two or more variables. It is a measure of symmetrical association between variables and does not measure the dependence of one variable over other. Correlation among agro-morphological traits was calculated by using an SAS program.

In this research, all trials were conducted three times in a completely randomized complete block design. Cluster analysis was carried out for an agro-morphology–based genetic distance matrix using an UPGMA clustering method in the NTSYS program. The distance matrix was calculated by means of Euclidean Distance Coefficient [18]

$$Eij = [\sum_k (X_{ki} - X_{kj})^2]^{1/2}$$

where: Eij = 0 to ∞, the larger the value, the more distant degrees of relationship; Xi and Xj are the standardized values for the $i^{th}$ and $j^{th}$ characters in $k^{th}$ varieties.

Polymorphic information content (PIC) value that provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles expressed, but also relative frequencies of those alleles, was estimated using the formula suggested by Nei [19]

$$PIC = 1-\sum x^2_k$$

where, $x^2_k$ represents the frequency of the $k^{th}$ allele.

## 3. Results

### 3.1 Evaluation of drought tolerance

The drought tolerant levels are presented in Table 2. One of the most important characters of drought tolerance evaluation is leaf roll (LR), which was scored from 0-9. There were 38 varieties, which obtained LR scores of 0-3, whereas 6 varieties had LR scores of 5-9. Regarding drought-tolerance at the seedling stage, 36 varieties were in the 0-3 score range, 14 varieties scored 5, whereas 4 varieties were in the 7-9 score range. At the vegetative stage, the number of susceptible varieties slightly increased.

**Table 2.** Results of drought-tolerant evaluations of 44 rice lines/varieties.

| No. | Name of line/variety | LR score | DTS score | DTV score | Origin |
|---|---|---|---|---|---|
| 1 | OM4900 | 3 | 1 | 3 | CLLRI[1] |
| 2 | OM1490 | 9 | 7 | 7 | CLLRI |
| 3 | AS996 | 1 | 3 | 5 | CLLRI |
| 4 | M362 | 0 | 0 | 5 | IRRI[2] |
| 5 | Basmati | 1 | 3 | 7 | IRRI |
| 6 | Basmati DB | 3 | 5 | 5 | IRRI |
| 7 | OM6162 | 3 | 5 | 3 | CLLRI |
| 8 | SwarnaSub1 | 7 | 9 | 9 | IRRI |
| 9 | IR 64Sub1 | 5 | 9 | 7 | IRRI |
| 10 | IRGA318-11-6-9-2B | 3 | 5 | 7 | IRRI |
| 11 | IR78966-B-10-B-B-B-2 | 1 | 1 | 3 | IRRI |
| 12 | IR78913-B-10-B-B-B | 3 | 5 | 5 | IRRI |
| 13 | IR75499-73-1-B | 0 | 0 | 0 | IRRI |
| 14 | IR78913-B-19-B-B-B | 3 | 5 | 5 | IRRI |
| 15 | Azucena | 3 | 1 | 5 | IRRI |
| 16 | IR78933-B-24-B-B-2 | 0 | 1 | 5 | IRRI |
| 17 | IR78933-B-24-B-B-3 | 1 | 1 | 5 | IRRI |
| 18 | IR78933-B-24-B-B-4 | 0 | 0 | 3 | IRRI |
| 19 | IR79008-B-11-B-B-1 | 5 | 5 | 3 | IRRI |
| 20 | IR75499-38-1-B | 0 | 0 | 5 | IRRI |
| 21 | V3M-92-1 | 0 | 0 | 1 | IRRI |
| 22 | IR75499-21-1-B | 0 | 0 | 1 | IRRI |
| 23 | V3M-109-2 | 0 | 0 | 1 | IRRI |
| 24 | WAB272-B-B-8-H1 | 0 | 0 | 3 | IRRI |
| 25 | WAB340-B-B-2-H2 | 1 | 1 | 3 | IRRI |
| 26 | WAB176-42-HB | 1 | 1 | 3 | IRRI |
| 27 | IR78937-B-20-B-B-1 | 5 | 5 | 7 | IRRI |
| 28 | WAB880-1-38-18-20-P1-HB | 1 | 5 | 0 | IRRI |
| 29 | WAB881SG9 | 1 | 1 | 3 | IRRI |
| 30 | IR78997-B-16-B-B-B-SB2 | 0 | 0 | 1 | IRRI |
| 31 | IR78966-B-10-B-B-B-SB1 | 0 | 1 | 3 | IRRI |
| 32 | IR78944-B-8-B-B-B | 3 | 5 | 5 | IRRI |
| 33 | IR78941-B-16-B-B-B | 3 | 3 | 3 | IRRI |
| 34 | IR78948-B-21-B-B-B | 1 | 1 | 1 | IRRI |
| 35 | IR78942-B-2-B-B-2 | 1 | 3 | 5 | IRRI |
| 36 | IR78937-B-20-B-B-3 | 1 | 3 | 7 | IRRI |
| 37 | IR78985-B-13-B-B-B | 1 | 3 | 7 | IRRI |
| 38 | IR78933-B-24-B-B-1 | 3 | 5 | 7 | IRRI |
| 39 | WABC165 | 3 | 5 | 5 | IRRI |
| 40 | IR80315-49-B-B-4-B-B-B | 3 | 5 | 7 | IRRI |
| 41 | IR78966-B-16-B-B-B | 0 | 1 | 3 | IRRI |
| 42 | IR78913-B-22-B-B-B | 1 | 5 | 3 | IRRI |
| 43 | OMCS2000 | 9 | 7 | 5 | CLRRI |
| 44 | IR78939-B-9-B-B-B | 3 | 5 | 5 | IRRI |

CLRRI: Cuu Long Rice Research Institute; IRRI: International Rice Research Institute

The record plant recovery for each entry following 0-9 score of the standard evaluation system (IRRI,1996) ( 0- 3: tolerance; 5- 9: susceptible )

### 3.2 Variance of agro-morphological characters

The agro-morphological characteristics were evaluated as shown in Table 3, 4. The growth duration (GD) was from 85 to 140 days. Seven rice varieties had a short GD < 90 days. The plant height ranged from 96.7 to 142.0 cm. Three varieties had the low plant heights < 100 cm including OM1490, OMCS2000 and AS996.

**Table 3.** Descriptive statistics of quantitative traits among 44 lines/varieties.

| Traits | Max | Min | Mean | CV | P |
|---|---|---|---|---|---|
| Growth duration (day) | 140.0 | 85.0 | 103.0 | | <0.05 |
| Plant height (cm) | 142.0 | 96.7 | 117.0 | 1.4 | <0.05 |
| No. of panicles | 17.6 | 4.3 | 11.6 | 12.0 | <0.05 |
| Filled grains/panicle | 212.5 | 99.4 | 150.2 | 9.5 | <0.05 |
| Unfilled grains/panicle | 62.3 | 4.6 | 20.4 | 8.7 | <0.05 |
| Grain weight 1000 (g) | 28.6 | 23.4 | 26.6 | 2.2 | <0.05 |
| Yield (ton/ha) | 8.6 | 3.5 | 5.6 | 9.6 | <0.05 |

Significantly different at p< 0.05

The number of panicles per hill ranged from 4.3 to 17.6. There were 4 varieties with maximum numbers of panicles per hill, from 15 to 17.6. The number of filled grains per panicle varied highly among varieties. Seven cultivars had filled grains per panicle < 115, whereas there were 20 varieties showed filled grains per panicle > 150. The rate of unfilled grains per panicle ranged from 4.6 to 62.3%. The low rate of unfilled grain/panicle (from 5-10%) comprised of 2 varieties V3M-109-2, and WAB272-B-B-8-H. There were 22 varieties with the average rate of unfilled grain/panicle (from 10-20%). The rest of varieties with the rate of unfilled grain/panicle were very high (more than 30%) including Basmati, M362, Basmati DB, IR64Sub1, OM1490, and OMCS2000. The weight of 1000 grains varied among cultivars ranged from 23.4 to 28.6 g. There were 17 cultivars with a weight of 1000 grains > 27 g. The productivity ranged from 3.5 to 8.6 tons/ha (Table 3). Specifically, there were 16 varieties with the yield from 3 to 5 tons/ha, 21 varieties with the yield from 5 to 7 tons/ha. Seven lines/ varieties had high yield (more than 7 tons/ha) including OM4900, V3M-92-1, IR78985-B-13-B-B-B, WAB881SG9, IR78913-B-22-B-B-B, IR78997-B-16-B-B-B-SB2, and IR75499-73-1-B.

**Table 4.** Grain yield characters of rice lines/varieties evaluated under drought stress at reproductive

| No. | Lines/varieties | GD (day) | PH (cm) | No. of P | FG/P | UG (%) | GW (g) | Yield (ton/ha) |
|---|---|---|---|---|---|---|---|---|
| 1 | OM4900 | 100 | 109.5 lm | 14.1 b-e | 209.8 ab | 14.2 jk | 26.6 e-l | 7.2 bcd |
| 2 | OM1490 | 85 | 96.7 n | 14.7 bc | 122.3 m-q | 51.2 b | 26.3 h-l | 4.1 mno |
| 3 | AS996 | 85 | 97.5 n | 13.9 b-f | 146.9 g-m | 28.5 ef | 27.5 a-g | 5.2 h-l |
| 4 | M362 | 106 | 113.0 ijk | 5.2 mn | 141.8 i-n | 32.1 de | 26.6 e-l | 5.6 g-k |
| 5 | Basmati | 120 | 133.9 b | 9.4 i-l | 155.3 e-k | 31.2 de | 28.2 abc | 4.8 j-n |
| 6 | Basmati DB | 100 | 111.5 jkl | 11.2 f-i | 146.6 g-m | 33.4 cd | 27.3 b-i | 5.2 h-l |
| 7 | OM6162 | 95 | 108.0 m | 9.2 i-l | 127.1 l-q | 10.4 lm | 28.6 a | 6.7 c-f |
| 8 | Swarna Sub1 | 114 | 116.5 e-h | 13.8 b-g | 155.5 e-k | 12.6 jkl | 26.4 f-l | 4.2 l-o |
| 9 | IR64Sub1 | 105 | 109.7 lm | 11.3 e-i | 147.1 g-m | 36.5 c | 26.5 f-l | 6.2 d-h |
| 10 | IRGA318-11-6-9-2B | 110 | 125.9 c | 10.1 ijk | 186.3 a-d | 29.7 ef | 28.4 ab | 5.9 e-i |
| 11 | IR78966-B-10-B-B-B-2 | 105 | 124 cd | 13.7 b-g | 145.6 g-m | 24.5 ghi | 26.7 d-k | 6.3 d-g |
| 12 | IR78913-B-10-B-B-B | 105 | 117.0 ef | 11.7 d-i | 174.6 c-f | 23.1 hi | 27.4 a-h | 5.9 e-i |
| 13 | IR75499-73-1-B | 100 | 114.5 f-i | 17.6 a | 200.5 abc | 24.6 ghi | 27.2 b-i | 8.6 a |
| 14 | IR78913-B-19-B-B-B | 120 | 116.0 e-i | 7.4 klm | 144.4 h-m | 21.3 i | 28.4 ab | 4.1 mno |
| 15 | Azucena | 102 | 115.7 e-i | 10.6 hij | 187.0 a-d | 26.5 fgh | 26.3 h-l | 6.8 cde |
| 16 | IR78933-B-24-B-B-2 | 140 | 111.7 jkl | 11.5 d-i | 165.7 d-h | 24.5 ghi | 25.9 jkl | 4.0 no |
| 17 | IR78933-B-24-B-B-3 | 100 | 131.2 b | 15.9 abc | 174.6 c-f | 27.3 fg | 26.7 d-k | 4.2 l-o |
| 18 | IR78933-B-24-B-B-4 | 105 | 123.7 cd | 9.2 i-l | 183.1 b-e | 22.3 i | 26.5 f-l | 4.2 l-o |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 19 | WAB326-B-B-7-H1 | 100 | 116.7 efg | 10.1 ijk | 148 g-m | 24.1 ghi | 26.7 d-k | 5.2 h-l |
| 20 | IR79008-B-11-B-B-1 | 85 | 115.5 e-i | 9.2 i-l | 159.9 d-j | 21.3 i | 25.4 lm | 6.7 c-f |
| 21 | IR75499-38-1-B | 90 | 116.9 ef | 10.7 hij | 141.3 i-n | 12.1 j-m | 26.8 d-k | 6.8 cde |
| 22 | V3M-92-1 | 90 | 125.1 cd | 9.1 i-l | 135.3 i-o | 10.3 lm | 27.4 a-h | 7.2 bcd |
| 23 | IR75499-21-1-B | 86 | 141.5 a | 10.1 ijk | 158.7 d-j | 12.3 jkl | 27.9 a-d | 6.5 c-g |
| 24 | V3M-109-2 | 104 | 109.1 lm | 10.7 hij | 110.3 opq | 4.6 n | 26.5 f-l | 5.2 h-l |
| 25 | WAB272-B-B-8-H1 | 140 | 122.1 d | 16.4 ab | 173.4 c-g | 8.6 m | 28.4 ab | 4.1 mno |
| 26 | WAB340-B-B-2-H2 | 140 | 142.0 a | 11.0 g-j | 122.4 m-q | 13.5 jkl | 26.9 d-k | 4.2 l-o |
| 27 | WAB176-42-HB | 140 | 117.9 e | 11.3 e-i | 119.6 l-q | 15.6 j | 27.8 a-e | 4.6 k-n |
| 28 | IR78937-B-20-B-B-1 | 100 | 116.9 ef | 8.3 jkl | 113.2 n-q | 14.6 jk | 24.3 lm | 3.5 o |
| 29 | WAB880-1-38-18-20-P1-HB | 85 | 115.9 e-i | 7.1 lmn | 131.2 k-p | 12.3 jkl | 27.9 a-d | 6.6 c-g |
| 30 | WAB881SG9 | 99 | 116.7 efg | 4.3 n | 168.3 d-h | 13.5 jkl | 24.3 lm | 7.4 bc |
| 31 | IR78997-B-16-B-B-B-SB2 | 112 | 115.8 e-i | 9.4 i-l | 103.8 pq | 14.6 jk | 27.6 a-f | 8.2 ab |
| 32 | IR78966-B-10-B-B-B-SB1 | 90 | 123.5 cd | 11.1 f-j | 111.1 opq | 12.6 jkl | 25.8 kl | 4.9 i-n |
| 33 | IR78944-B-8-B-B-B | 105 | 115.8 e-i | 14.9 abc | 113.9 n-q | 11.5 klm | 27.1 c-j | 4.2 l-o |
| 34 | IR78941-B-16-B-B-B | 110 | 113.5 g-k | 13.7 b-g | 99.4 q | 13.2 jkl | 23.4 m | 5.1 i-m |
| 35 | IR78948-B-21-B-B-B | 105 | 115.9 e-i | 11.6 d-i | 103.6 pq | 14.2 jk | 25.4 lm | 4.1 mno |
| 36 | IR78942-B-2-B-B-2 | 105 | 116.9 ef | 14.3 bcd | 122.2 m-q | 23.5 hi | 26.2 i-l | 4.0 no |
| 37 | IR78937-B-20-B-B-3 | 90 | 111.1 klm | 11.7 d-i | 144.9 h-m | 22.1 i | 26.4 g-l | 4.2 l-o |
| 38 | IR78985-B-13-B-B-B | 85 | 113.9 f-k | 14.9 abc | 161.3 d-i | 12.3 jkl | 27.8 a-e | 7.2 bcd |
| 39 | IR78933-B-24-B-B-1 | 95 | 122.8 cd | 13.9 b-f | 173.5 c-g | 14.4 jk | 23.5 m | 5.2 h-l |
| 40 | WABC165 | 100 | 123.3 cd | 15.0 abc | 155.0 e-k | 12.6 jkl | 28.4 ab | 6.2 d-h |
| 41 | IR80315-49-B-B-4-B-B-B | 95 | 109.0 lm | 14.8 abc | 173.6 c-g | 24.1 ghi | 23.4 m | 5.7 f-j |
| 42 | IR78966-B-16-B-B-B | 100 | 122.2 d | 6.7 lmn | 150.0 f-l | 12.3 jkl | 23.5 m | 6.2 d-h |
| 43 | IR78913-B-22-B-B-B | 95 | 113.3 h-k | 14.7 bc | 212.5 a | 11.5 klm | 26.5 f-l | 7.5 bc |
| 44 | OMCS 2000 | 90 | 97.0 n | 13.4 c-h | 186.0 a-d | 62.3 a | 26.5 f-l | 6.2 d-h |
| | CV (%) | | 1.4 | 12 | 9.5 | 8.7 | 2.2 | 9.6 |

Note: GD: Growth duration day; Plant height: PH; Tilling/P: Tilling/Panicle; FG/P: Filled grain; UG: Unfilled grain

### 3.3 Correlation among agro-morphological traits and drought tolerance

The correlation coefficients among agro-morphological traits, drought tolerance at seedling stage (DTS), and drought tolerance at vegetative stage (DTV) are shown in Table 5.

**Table 5**. Correlation coefficients for among agro-morphological traits.

| Traits | GD | PH | Tilling/P | FG/P | UG | 100w | Yield | LR | DTS | DTV |
|---|---|---|---|---|---|---|---|---|---|---|
| GD (day) | 1 | | | | | | | | | |
| PH (cm) | 0.32ns | 1 | | | | | | | | |
| Tilling/P | 0.02ns | -0.16ns | 1 | | | | | | | |
| FG/P | -0.08ns | 0.01ns | 0.25ns | 1 | | | | | | |
| UG (%) | -0.11ns | -0.41* | 0.10ns | 0.23ns | 1 | | | | | |
| 1000 w | 0.14ns | 0.14ns | 0.06ns | 0.06ns | 0.03ns | 1 | | | | |
| Yield (t/ha) | -0.42* | -0.08ns | -0.08ns | 0.37ns | -0.10ns | 0.12ns | 1 | | | |
| LR (score) | -0.04ns | -0.29ns | 0.29ns | 0.13ns | 0.22ns | 0.13ns | -0.10ns | 1 | | |
| DTS (score) | -0.02ns | -0.28ns | 0.22ns | 0.02ns | 0.25ns | 0.02ns | -0.17ns | 0.87** | 1 | |
| DTV (score) | 0.13ns | -0.16ns | 0.20ns | 0.07ns | 0.36ns | 0.24ns | -0.32ns | 0.54* | 0.60** | 1 |

Note: DT: drought-tolerance; ns: not significant; DTV: DT at vegetative stage; DTS: DT at seedling stage; GD: Growth duration day; Plant height: PH; Tilling/P: Tilling/Panicle; FG/P: Filled grain; UG: Unfilled grain; 1000 w: 1000 weight; LR: leaf roll; Means with * and ** are significantly different at $p< 0.05$ and 0.01, respectively.

It was found that both DTS and DTV did not correlate with filled grains and weight of 1000 grains. Leaf roll values strongly correlated with DTS and DTV ($r = 0.87$ and 0.54, $p < 0.01$, 0.05 respectively), indicating that it should be used as an index for drought tolerance of rice. A negative significant correlation was observed in growth duration x yield ($r = 0.42$). DTS also found to correlate

significantly with DTV (r = 0.60). Despite the correlation coefficients not being markedly different, DTS and DTV were proportional to unfilled grains and tilling per panicle, while they were negatively proportional to rice yield and plant height.

### 3.4 Cluster analysis among 44 varieties based on phenotypic

The value of ten agro-morphological characters including seven yield component traits and three parameter of drought tolerance evaluation were used to carry out analysis. The detail result showed in Fig. 9. The similarity coefficient of the group was from 2.75 to 21.55. At homologous coefficient of 16.85 was the lines/varieties were divided into five main clusters marked A, B, C, D, and E.

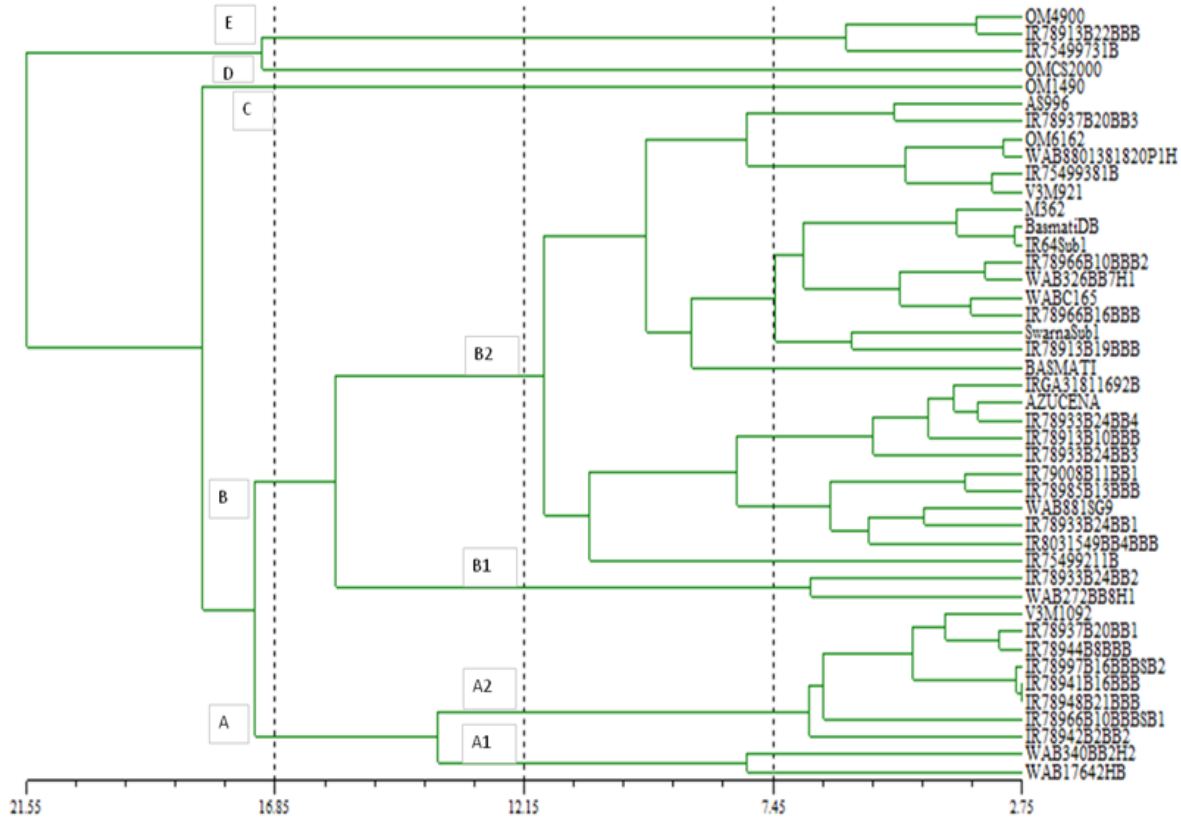


**Fig. 1.** Dendrogram of cluster analysis of 44 rice lines/varieties using UPGMA method based on both agro-morphiological characters and drought tolerant scores.

At similarity coefficient of 12.15, cluster A was separated into two sub-clusters, A1 and A2. Sub-cluster A1 included two varieties WAB176-42-HB and WAB 340-B-B-2-H2. These varieties had a long GD of 140 days, number of panicles/hill 11 to 12, the number of filled grains/panicle from 122 to 120, rate of unfilled grains/panicle from 13 to 16%, the weight of 1000 grains from 27 to 28 g, yield from 4.2 to 4.6 tons/ha, level of leaf roll from score 1 to score 5, level of drought tolerance at seedling stage from score 1 to score 3, and level of drought tolerance at vegetative stage from score 3 to score 7 (Tables 2, 4; Fig. 1). Sub-cluster A2 comprised of eight cultivars. This cluster had GD from 90 to 110 days, plant height from 109 to 123 cm, yield from 4 to 8 tons/ha, level of leaf roll from score 1 to score 3, level of drought tolerance at seedling stage from score 1 to score 3, and level of drought tolerance at vegetative stage (from score 0 to 7) (Tables 2, 4; Fig. 1).

Cluster B was divided into two sub-clusters as cluster B1 and B2. Sub-cluster B1 consists of two varieties, this group had GD 140 days, plant height from 111 to 122 cm, number of panicles/hill from 11 to 16, number of filled grains/panicle from 165 to 173, rate of unfilled grains/panicle from 8 to 25%, weight of 1000 grains from 26 to 28 g, yield of 4 tons/ha, level of leaf roll from score 0 to score 1, level of drought tolerance at seedling score 1, and the level of drought tolerance at vegetative stage from score 3 to score 5 (Tables 2, 4; Fig. 1). As well cluster B2 had yield 4 to 7 tons/ha, GD from 85 to 90 days, level of leaf roll from score 0 to score 3, level of drought tolerance at seedling stage from score 0 to 3, and level drought tolerance at vegetative stage from score 1 to 7 (Tables 2, 4; Fig. 1).

Cluster C was with only one variety (OM1490). So that this variety had low growth time (85 days) low yield (4.1 tons/ha), level of leaf roll (score 9), drought tolerance at seedling (score 7), level of drought tolerance at vegetative (score 7). Similarly, cluster D had one variety (OMCS2000) with low growth time (90 days), the average yield (6.2 tons/ha), level of drought tolerance at seedling (score 3), level of dried leaf (score 3), level of drought tolerance at vegetative (score 5) (Fig. 1).

Cluster E included 3 varieties (OM4900, IR78913-B-22-B-B-B, and IR75499-73-1-B) and in this group had GD from 95 to 100 days, the plant height from 110 to 115 cm, number of panicles/hill from 14 to 18, number of filled grains from 200 to 215, rate of unfilled grains/panicle from 12 to 25%, weight of 1000 grains from 26 to 27 g, yield from 7.2 to 8.6 tons/ha), level of leaf roll from score 0 to 9, level of drought tolerance at seedling stage from score 0 to 7, and level of drought tolerance at vegetative stage from score 0 to 5 (Tables 2, 4; Fig. 1).

### 3.5 Polymorphism of microsatellite markers

The result of microsatellite analysis showed that there were 73 out of 165 SSR markers found to be polymorphic. The number of amplified fragments ranged from 2 to 9 alleles, therefore the average of alleles was 5.12 per locus. Three primers RM10890, RM21539 (chromosome 7), and RM222 (chromosome 10) produced the highest number of alleles (9). The lowest PIC values were noted in the primer RM5908 (0.23), followed by RM252 (0.30). Whereas, the highest values were observed in the primer RM11125 (0.79), followed by the RM21 and RM5629 (0.78) markers (Table 6).

**Table 6.** Results of polymorphic analysis based on SSR markers

| No. | Primer | Chr | No.of alleles | Size (bp) | PIC value | No. | Primer | Chr | No. of alleles | Size (bp) | PIC value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RM105 | 9 | 5 | 210-215 | 0.46 | 38 | RM154 | 2 | 9 | 160-180 | 0.71 |
| 2 | RM10115 | 1 | 4 | 240-250 | 0.49 | 39 | RM231 | 3 | 6 | 200-210 | 0.67 |
| 3 | RM243 | 1 | 3 | 190-210 | 0.45 | 40 | RM21539 | 7 | 9 | 205-210 | 0.45 |
| 4 | RM10649 | 1 | 6 | 180-210 | 0.45 | 41 | RM122 | 5 | 4 | 205-230 | 0.64 |
| 5 | RM24 | 1 | 4 | 200-205 | 0.63 | 42 | RM510 | 6 | 4 | 220-230 | 0.42 |
| 6 | RM7643 | 1 | 4 | 205-220 | 0.66 | 43 | RM547 | 8 | 5 | 200-210 | 0.49 |
| 7 | RM472 | 1 | 3 | 210-242 | 0.64 | 44 | RM23662 | 9 | 8 | 210-220 | 0.64 |
| 8 | RM11125 | 1 | 5 | 160-200 | 0.79 | 45 | RM219 | 9 | 7 | 200-215 | 0.65 |
| 9 | RM10843 | 1 | 5 | 180-200 | 0.73 | 46 | RM24013 | 9 | 8 | 215-220 | 0.42 |
| 10 | RM3412b | 1 | 6 | 190-200 | 0.64 | 47 | RM3 | 6 | 7 | 220-225 | 0.50 |
| 11 | RM10793 | 1 | 6 | 210-220 | 0.63 | 48 | RM223 | 8 | 8 | 200-210 | 0.46 |
| 12 | Salt 1 | 1 | 4 | 200-220 | 0.74 | 49 | RM315 | 1 | 5 | 210-230 | 0.49 |
| 13 | Salt 2 | 1 | 2 | 210-220 | 0.45 | 50 | RM13 | 5 | 5 | 190-210 | 0.63 |
| 14 | RM 152 | 8 | 3 | 175-200 | 0.63 | 51 | RM166 | 2 | 6 | 190-200 | 0.65 |
| 15 | RM5806 | 10 | 6 | 210-230 | 0.66 | 52 | RM140 | 1 | 4 | 200-210 | 0.63 |
| 16 | RM5806 | 10 | 6 | 230-250 | 0.64 | 53 | RM220 | 1 | 5 | 210-220 | 0.64 |
| 17 | RM211 | 2 | 4 | 200-215 | 0.65 | 54 | RM227 | 3 | 4 | 200-220 | 0.65 |
| 18 | RM17 | 12 | 4 | 160-190 | 0.79 | 55 | RM148 | 3 | 6 | 190-210 | 0.43 |
| 19 | RM310 | 8 | 5 | 200-210 | 0.72 | 56 | RM471 | 4 | 5 | 213-250 | 0.60 |
| 20 | RM27877 | 12 | 6 | 215-240 | 0.63 | 57 | RM252 | 4 | 5 | 200-215 | 0.50 |
| 21 | RM221 | 2 | 5 | 220-230 | 0.66 | 58 | RM1155 | 4 | 3 | 200-245 | 0.40 |
| 22 | RM28746 | 12 | 2 | 200-210 | 0.63 | 59 | RM279 | 2 | 4 | 200-263 | 0.50 |
| 23 | RM5436 | 7 | 3 | 200-210 | 0.73 | 60 | RM555 | 2 | 2 | 190-240 | 0.30 |
| 24 | RM3867 | 3 | 7 | 210-230 | 0.74 | 61 | RM71 | 2 | 3 | 210-230 | 0.56 |
| 25 | RM6329 | 3 | 5 | 220-230 | 0.64 | 62 | RM324 | 2 | 6 | 210-235 | 0.54 |
| 26 | RM249 | 5 | 5 | 210-230 | 0.64 | 63 | RM418 | 7 | 4 | 200-215 | 0.56 |
| 27 | RM5626 | 3 | 5 | 200-210 | 0.78 | 64 | RM455 | 7 | 5 | 200-245 | 0.58 |
| 28 | RM18 | 7 | 4 | 190-200 | 0.64 | 65 | RM125 | 7 | 6 | 200-215 | 0.56 |
| 29 | RM21 | 11 | 4 | 210-220 | 0.78 | 66 | RM8300 | 9 | 7 | 200-417 | 0.54 |

| No. | Primer | Chr | No.of alleles | Size (bp) | PIC value | No. | Primer | Chr | No. of alleles | Size (bp) | PIC value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | RM163 | 5 | 3 | 255-260 | 0.45 | 67 | RM24712 | 9 | 4 | 200-332 | 0.75 |
| 31 | S11049 | 11 | 6 | 200-210 | 0.74 | 68 | RM222 | 10 | 9 | 180-245 | 0.79 |
| 32 | RM140 | 1 | 8 | 190-200 | 0.61 | 69 | RM590 | 10 | 5 | 185-236 | 0.23 |
| 33 | RM169 | 5 | 8 | 240-250 | 0.73 | 70 | RM2010 | 2 | 2 | 180-245 | 0.56 |
| 34 | RM9 | 1 | 6 | 230-240 | 0.49 | 71 | RM1024 | 2 | 3 | 190-255 | 0.45 |
| 35 | RM10852 | 1 | 3 | 220-230 | 0.64 | 72 | RM7396 | 4 | 6 | 200-265 | 0.63 |
| 36 | RM10890 | 1 | 9 | 205-510 | 0.66 | 73 | RM463 | 12 | 4 | 210-225 | 0.52 |
| 37 | RM10927 | 1 | 7 | 240-245 | 0.40 | Average | | | 5.12 | | |

Chr: Chromosome PIC: Polymorphic Information Content

A dendrogram based on cluster analysis using (UPGMA) method with the module of SAHN in the NTSYS-pc package was generated and showed in Fig 2. The genetic variation among rice varieties ranged from 0 to 0.84. At genetic distance of 0.63 there were three major clusters namely, A, B, and C. At genetic distance of 0.42, group A was divided 5 sub-clusters with group A1 (IR78944-B-8-B-B), group A2 (OM6162), group A3 (Swana Sub1, Basmati DB, and Basmati), group A4 (19 varieties), and group A5 (14 varieties).

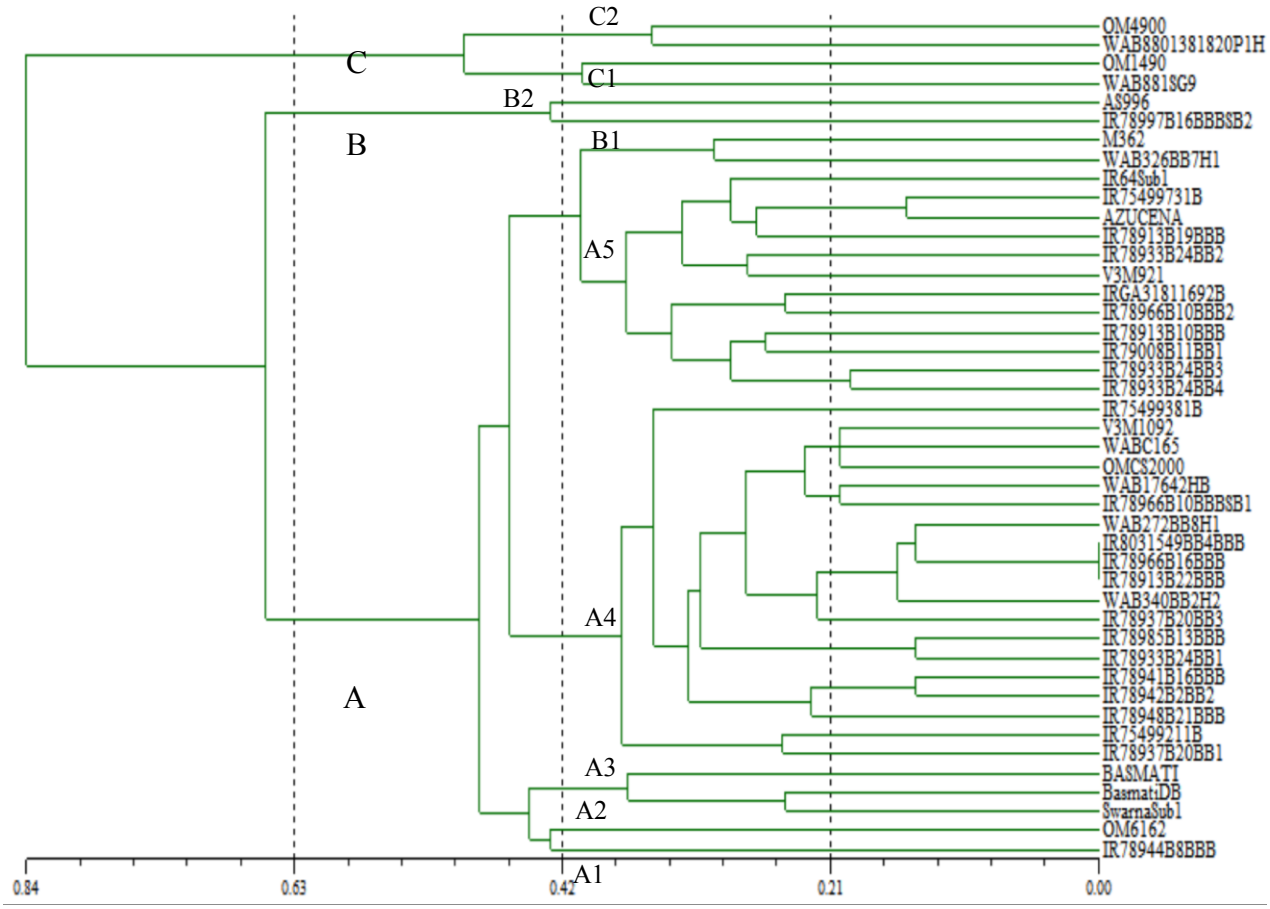


**Fig. 2.** Cluster dendrogram of 44 rice lines/varieties by the genetic distance of using UPGMA method with the SAHN based on SSR markers.

At genetic distance of 0.42, group B was separated into two sub-clusters with Group B1 (IR78997-B-16-B-B-B-SB2), and group B2 (AS996). Similarly, group C included two sub-clusters with group C1 (OM1490, and WAB881-SG9), and group C2 (OM4900 and WAB8801381820P1H).

## 4. Discussions

The standard evaluation system of IRRI was applied to evaluate the drought tolerant level of rice for nearly 20 years with high reliability. The lower of the evaluated scores indicates the higher tolerant levels of rice. Among 44 rice lines/varieties shown in this study, many varieties had the scores between 0 and 3 obtained from IRRI. There were six lines including IR75499-73-1-B, V3M-92-1, IR75499-21-1-B, V3M-109-2, IR78997-B-16-B-B-B-SB2, and IR78948-B-21-B-B-B achieving high drought-tolerant levels (score from 0 to 1). They are potential parents for breeding of drought-tolerant rice varieties.

Rice is vulnerable to abiotic stress such as drought caused by shortage of water and irregular rain, especially at the productive stage. If the duration of the reproductive stage is shorter, the levels of drought stress can be increased [20]. In several drought stress conditions, the early flowering feature was a very important mechanism to escape from drought stress [21]. Therefore, selection of drought-tolerant parental varieties for breeding is highly related to agro-morphological characters including pre-mature duration. In this study, maximum values were obtained in IR75499-73-1-B (8.6 t/ha).

Frequency distribution of rice varieties with respect to maturity, plant height, panicles per plant, number of filled grains, number of unfilled grains, 1000 grain weight, yield, level for drought tolerance, showed a great diversity among 44 varieties studied. Phenotypic measurement is very important for identifying quantitative trait loci (QTLs) because quantitative traits are much affected by environment, especially for measuring drought-tolerance [11]. The variation in agro-morphological characters of rice in this study can provide important information for plant breeders to breed drought-tolerant rice integrated with good agronomic traits.

The genetic basis of drought traits is governed by one recessive allele located on 12 chromosomes. Therefore, application of molecular markers linked tightly to target drought genes is considered as a powerful tool to select drought rice varieties faster and more effective. Studies by Garris et al. [22] and Ram et al. [23] showed that the number of alleles per locus, polymorphic information content and gene diversity were 4.8-14.0, 0.6-0.7, and 6.2-6.8, respectively. The average alleles per locus were 3.9 in 416 rice accessions collected from China [24].

The PIC values obtained from the markers RM17616 and RM316 were 0.146 and 0.756, respectively, in *Indica* accessions [25]. The lowest PIC values were noted in the RM5908 primer (0.23), followed by RM252 (0.30). The maximum values were at primers RM11125 (0.79), followed by RM21 (0.78) and RM5629 (0.78). The maximum genetic diversity is the most important criterion to select parental varieties for breeding because of increase the choice of desirable genotypes [26]. Findings of this study were similar to previous studies, in which the PIC values were from 0.16 to 0.78 for European Chinese rice collection of 416 accessions [24,27]. In addition, Chen et al. [28] screened 300 rice accessions with 372 SNP markers for 0.358 of diversity and found that polymorphic was 0.285. Gene diversity < 0.68 was reported [29]. However, most rice diversity worldwide has the gene diversity of 0.5 to 0.7 [8,22,29]

## Conclusions

Most of rice varieties showed high drought-tolerant levels. They could serve as important parental donors in breeding drought-tolerant rice. In addition, there was a high variation of agro-morphological character and genetic diversity in these 44 cultivars. The detected three clusters of rice in this study are evidence that can form a basic for effort to improve productivity of drought-tolerant rice. In the integration of drought tolerance, agro-morphological traits, and genetic diversity, four lines/cultivars (OM4900, IR78913-B-22-B-B-B, OM6162 and IR75499-73-1-B) appear as the most promising parental donors for developing agronomic and drought-tolerant rice.

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