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# Genetic Divergence of Rice on Some Morphological and Physiochemical Responses to Drought Stress

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## ABSTRACT

Twelve Malaysian rice genotypes were evaluated for drought-related morphological and physiochemical responses to determine the degree of genetic divergence. Preliminary investigation showed a considerable reduction in plant growth, total chlorophyll content, chlorophyll stability index (CSI %), and total soluble protein in all the genotypes studied. On the other hand, a sharp increase in the accumulation of proline was also noticed. An analysis of variance revealed significant variations for those traits among the genotypes, which was adequate for the estimation of genetic diversity. Hierarchical cluster analysis of multivariate approach was performed for the genotypes exposed to water deficit stress as well as for the control conditions. Meanwhile, the genotypes were classified into groups based on the deferential responses. However, the analysis was unable to reveal how sensitive or tolerant the genotypes to drought condition, hence a discriminant functional analysis was carried out. The result obtained from canonical discriminant function clearly distinguished the genotypes based on sensitivity to drought stress. Furthermore, the study demonstrated the relevance of morphological and physiochemical responses in screening drought tolerance in rice. Hence, it is suggested that discriminant functional analysis can be used as a potential screening tool to identify drought tolerance genotypes at early stages in rice.

**Keywords: Free proline, total soluble protein, genetic divergence, multivariate analysis, drought responses in Malaysian rice**

## INTRODUCTION

Drought is one of the major factors limiting rice production worldwide. Uneven distribution of rainfall makes rice growers to depend heavily on irrigation. However, increasing the frequency of irrigational input is not possible due to water shortage and inadequate management of infrastructures (Wardlaw, 2000; Llorens *et al*., 2004; Ober *et al*., 2005; Flexas *et al.*, 2006). Therefore, in order to sustaining crop production, it is essential to have improved rice varieties with less sensitivity to water deficit condition.

Improving drought tolerance and productivity is the most difficult task for cereal breeders because of the diverse strategies adopted by plants at various stages of development among the species and cultivars to cope with water stress (Chaves *et al*., 2003). It has been reported (Mansfield and Atkinson, 1990; Nayyar and Gupta, 2006; Yang *et al*., 2006) that the first and foremost response of plants to acute water deficit is the Stomatal closure to prevent transpiration loss, and it has primarily resulted in a reduction in the photosynthesis rate. Fisher

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*et al.* (1998) found that stomatal conductance and rate of photosynthesis were positively correlated with the increase in yield in wheat cultivars. Meanwhile, Sibournheuang *et al.* (2006) observed genotypic variation among the rice cultivars for leaf water potential (LWP) and suggested that it might be due to the differences in stomatal conductance or differences in the root water uptake.

Several reports (Morgan, 1984; Hoekstra *et al.*, 2001; Ramanjulu and Bartels, 2002; Mahajan and Tuteja, 2005) showed that water deficit condition has resulted in the loss of structural integrity of membrane, disruption in cellular compartmentalization and reduction in enzyme activity. Therefore, to combat the adverse effect, plant synthesis accumulates various compounds such as sugars, amino acids, inorganic ions, and organic acids. These compounds help to maintain their hydrated state in the cell and provide resistance against drought and cellular dehydration.

In general, responses to drought are numerous and interconnected. It is well-established that drought stress impairs numerous metabolic and physiological processes in plant which ultimately resulted in reduce plant growth, loss of chlorophyll pigments, accumulation of osmolytes, etc. (Lima *et al*., 2002; Colom and Vazzana, 2003; Souza *et al*., 2004; Ekmekci *et al.*, 2005; Li *et al*., 2006; Nayyar and Gupta, 2006; Yang *et al*., 2006; Efeoglu *et al*., 2009).

Despite the great deal of research in the physiology of drought, only limited information is available on genetic background. Many scientists (e.g. Rush and Epstein, 1976; Greenway and Munns, 1980; Wyn Jones, 1981; Epistein and Rains, 1987; Cheeseman, 1988; Jacoby, 1999; Shannon and Greve, 1999; Ashraf, 2002; Munns, 2002) have suggested that the physiology of stress would offer valuable information to design efficient and accurate screening techniques for improving drought tolerant traits. Difficulty in breeding complex traits could thus be resolved by identifying reliable morphological and physiological characters that are closely linked to yield in water limiting environment and by integrating the approach of stress physiology with molecular genetics (Tuberosa *et al*., 2002; Ober *et al*., 2005). However, the effectiveness of selection primarily depends on the magnitude of genetic variability present in the breeding material. Pradhan and Ray (1990), as well as Roy and Panwar (1993) emphasized the importance of genetic divergence for the selection of suitable genotypes. Knowledge on genetic diversity maximizes the exploitation of the germplasm resources (Belaj *et al*., 2002; Rasul and Okubo, 2002) and it can be estimated through multivariate approach. Multivariate analysis is a useful tool to quantify the extent of divergence at genetic level. This approach visualizes the interaction between the genotype and traits involved in the study, and it thus provides information about the superior and inferior genotypes (Ober *et al*., 2005). The present study was undertaken to determine the degree of genotypic diversity for drought-related morphological and physiochemical traits such as plant growth, chlorophyll content, chlorophyll stability index, proline and protein using multivariate analysis, and to determine whether these traits can be used to select drought tolerance genotypes at early stage in rice.

## MATERIALS AND METHODS

Twelve rice genotypes, namely MR167, MR211, MR219, MR220, MR232, Mahawi, Bahagia, Makmur, Seberang, Ria, Masqia, Gaya, grown in 30 cm x 30 cm plastic pots filled with clay loam soil, were obtained from the Malaysian Agricultural Research and Development Institute (MARDI). The seeds of the rice varieties were planted in pots of 30 cm diameter and 30 cm height, filled with clay loam soil and arranged in randomized block design with three replications. Each replication consisted of five pots. About ten seeds were planted in each pot at 2-3 cm depth. Two weeks after sowing, the seedlings were thinned to five plants per pot. In each pot, about 5 cm of the standing water was maintained and 10 g of slow release commercial fertilizer (15%N, 15%P and 15%K) was then added to maintain a healthy crop stand. Two sets of the experimental materials were maintained; one

was kept as a control and the other for water stress treatment. Drought was initiated 45 days after sowing (45 DAS) by withholding water for a period of 7-10 days. The physiological parameters were measured for both the control and water-stressed plants at the onset of drought initiation. Plants were uprooted carefully after the start of the drought treatment and then separated into root and shoot. Root and shoot lengths were measured (cm), while the number of leaves was counted for each rice genotypes, and the data were also recoded.

## DETERMINATION OF THE TOTAL CHLOROPHYLL CONTENT

Chlorophyll content was determined by following the methods of Harbone (1984). Leaf tissue (500 mg) was homogenized in 80% chilled acetone. After appropriate dilution, the chlorophyll levels in the supernatant were determined spectrophotometrically using the following formula:

Total chlorophyll content (mg $ml^{-1}$) = 17.3 $A_{646}$ + 7.18 $A_{663}$

Chlorophyll *a* (mg $ml^{-1}$) = 12.21 $A_{663}$ – 2.81 A.646

## ESTIMATION OF CHLOROPHYLL STABILITY INDEX (CSI)

CSI in the leaf was estimated using a spectrometer, following the method of Koleyoreas (1958). Two leaf samples of 250 mg each were put in two test tubes containing 10 ml of distilled water. One of the test tubes was placed in a water bath and heated to 65°C for 30 minutes while the other was kept as a control. Then, the total chlorophyll content was estimated using a spectrophotometer at 652 nm (Koleyoreas, 1958). CSI was calculated using the following formula:

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll content (heated)}}{\text{Total chlorophyll content (control)}} \times 100$$

## ESTIMATION OF FREE PROLINE CONTENT

Proline was determined following the procedure by Bates *et al*. (1973). A fresh leaf sample (0.5 g) was homogenized in 5 ml of 3% sulphosalicylic acid and the homogenate was centrifuged at 9000 xg. The reaction mixture consisted of 2 ml of the supernatant, 2 ml of acid ninhydrin (1.25 g ninhydrin dissolved in 30 ml of glacial acetic acid, and 20 ml of 6M orthoposphoric acid) and 2 ml of glacial acetic acid which was boiled at 100°C for 1 h. After termination of the reaction on ice, the reaction mixture was extracted with 4 ml of toluene, and the absorbance was read at 520 nm.

## ESTIMATION OF THE TOTAL PROTEIN

About 1.0 g of leaf tissue was ground in cold mortar. The grinding medium (4-6 ml/g fresh mass) consisted of 50 mM Tris-HCL buffer (pH 8.0), 1 mM PMSF, 10% (v/v) glycerol and homogenizing beads. The homogenate was filtered through four layers of cheesecloth and centrifuged at 12000 rpm for 20 minutes at 4°C and the supernatant was taken. An aliquot of the extract was used for protein concentration, following the method of Bradford (1976) with bovine serum albumin (BSA) as a standard.

## STATISTICAL ANALYSIS

The experiment was performed using a randomized block design with three replications. The multivariate analysis was carried out for control as well as drought stress induced genotypes to assess the differences between the stress induced and control. Meanwhile, the statistical variance analysis was performed using ANOVA and compared with the least significant differences (LSD) at 5% level. Grouping of genotypes was done using the SPSS (Version. 11) statistical programme.

TABLE 1A
Mean (± SE) of the morphological traits of twelve rice genotypes recoded for the control and water stressed conditions

| S. no | Rice varieties | Shoot length (cm) | | | Root length (cm) | | | Ratio (root/shoot) | | | No. of leaves/ plant | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | control | stress | C-S | control | stress | C-S | control | stress | C-S | control | stress | C-S |
| 1 | MR 167 | 20.6 ± 2.19 | 17.8 ± 4.21 | 2.8 | 10.4 ±1.14 | 10.0 ± 1.87 | 0.4 | 0.505 | 0.562 | -0.055 | 5.4 ± 1.14 | 5.0 ±1.00 | 0.4 |
| 2 | MR 211 | 21.8 ± 2.77 | 21.2 ± 1.92 | 0.6 | 6.6 ± 1.14 | 7.8 ± 1.92 | -1.2 | 0.303 | 0.368 | -0.065 | 4.8 ± 0.84 | 4.6 ± 1.14 | 0.2 |
| 3 | MR 232 | 13.6 ± 1.67 | 16.6 ± 2.07 | -3.0 | 8.0 ± 1.58 | 9.4 ± 1.14 | -1.4 | 0.588 | 0.566 | 0.022 | 9.4 ± 2.30 | 6.6 ± 2.41 | 2.8 |
| 4 | MR 219 | 21.2 ± 1.30 | 7.6 ± 1.52 | 13.6 | 6.2 ± 0.84 | 8.8 ± 1.30 | -2.6 | 0.292 | 1.158 | -0.865 | 5.4 ± 1.52 | 7.8 ± 0.84 | -2.4 |
| 5 | MR 220 | 25.4 ± 3.85 | 24.0 ± 1.58 | 1.4 | 11.0 ± 0.79 | 11.9 ± 0.61 | -0.86 | 0.433 | 0.494 | -0.061 | 6.2 ± 2.49 | 6.4 ± 2.07 | -0.2 |
| 6 | Mahawi | 20.8 ± 1.48 | 19.0 ± 1.58 | 1.8 | 10.9 ± 0.89 | 9.6 ± 1.52 | 1.3 | 0.524 | 0.505 | 0.019 | 3.2 ± 0.84 | 3.4 ± 0.55 | -0.2 |
| 7 | Bahagia | 21.0 ± 3.54 | 17.2 ± 1.92 | 3.8 | 9.6 ± 1.14 | 9.4 ± 2.07 | 0.2 | 0.457 | 0.546 | -0.089 | 5.2 ± 1.92 | 4.4 ± 1.67 | 0.8 |
| 8 | Makmur | 16.2 ± 4.15 | 16.4 ± 2.07 | -0.2 | 10.0 ± 1.58 | 10.2 ± 1.92 | -0.2 | 0.617 | 0.622 | -0.005 | 5.0 ± 1.58 | 4.8 ± 1.79 | 0.2 |
| 9 | Seberang | 15.0 ± 3.39 | 14.0 ± 2.55 | 1.0 | 7.2 ± 1.92 | 7.6 ± 1.50 | -0.44 | 0.480 | 0.546 | -0.06 | 8.0 ± 2.55 | 7.6 ± 1.14 | 0.4 |
| 10 | Ria | 33.6 ± 3.05 | 9.8 ± 1.48 | 23.8 | 7.2 ± 0.84 | 9.0 ± 1.58 | -1.8 | 0.214 | 0.918 | -0.704 | 7.2 ± 0.84 | 6.4 ± 1.14 | 0.8 |
| 11 | Masqia | 37.2 ± 1.92 | 9.4 ± 1.52 | 27.8 | 6.6 ± 0.55 | 10.4 ± 1.14 | -3.8 | 0.177 | 1.106 | -0.929 | 6.8 ± 0.84 | 6.2 ± 0.84 | 0.6 |
| 12 | Gaya | 45.2 ± 2.39 | 41.2 ± 2.17 | 4.0 | 7.8 ± 2.28 | 11.0 ± 2.00 | -3.2 | 0.173 | 0.267 | -0.094 | 6.8 ± 0.84 | 5.2 ± 1.64 | 1.6 |

TABLE 1B
Mean (± SE) of chlorophyll pigment content of twelve rice genotypes for the control and water stressed conditions

| S. no | Rice varieties | Chlorophyll a | | | Chlorophyll b | | | Ratio between Chlorophyll a/b | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | control | stress | C-S | control | stress | C-S | control | stress | C-S |
| 1 | MR 167 | 7.86 ± 0.05 | 6.04 ± 0.11 | 1.82 | 5.90 ± 0.37 | 2.16 ± 0.14 | 3.74 | 1.33 | 2.79 | -1.46 |
| 2 | MR 211 | 3.88 ± 0.04 | 2.38 ± 0.08 | 1.50 | 1.53 ± 0.06 | 0.99 ± 0.12 | 0.54 | 2.54 | 2.40 | 0.14 |
| 3 | MR 232 | 8.01 ± 0.04 | 0.13 ± 0.03 | 7.87 | 12.35 ± 0.09 | 11.88 ± 0.13 | 0.47 | 0.65 | 0.01 | 0.64 |
| 4 | MR 219 | 6.94 ± 0.35 | 5.39 ± 0.09 | 1.54 | 2.48 ± 0.17 | 1.20 ± 0.05 | 1.28 | 2.80 | 4.49 | -1.69 |
| 5 | MR 220 | 8.81 ± 0.32 | 6.80 ± 0.03 | 2.01 | 6.38 ± 0.15 | 3.34 ± 0.08 | 3.04 | 1.38 | 2.03 | -0.65 |
| 6 | Mahawi | 9.72 ± 0.14 | 8.40 ± 0.02 | 1.32 | 3.07 ± 0.19 | 0.60 ± 0.04 | 2.48 | 3.17 | 14.0 | -10.83 |
| 7 | Bahagia | 5.09 ± 0.24 | 3.83 ± 0.04 | 1.25 | 1.33 ± 0.22 | 0.36 ± 0.17 | 0.97 | 3.82 | 10.64 | -6.82 |
| 8 | Makmur | 7.58 ± 0.30 | 5.80 ± 0.08 | 1.77 | 3.02 ± 0.14 | 2.50 ± 0.25 | 0.51 | 2.51 | 2.32 | 0.19 |
| 9 | Seberang | 6.59 ± 0.80 | 3.16 ± 0.02 | 3.43 | 2.44 ± 0.33 | 0.25 ± 0.07 | 2.19 | 2.70 | 12.64 | -9.94 |
| 10 | Ria | 0.72 ± 0.16 | 0.60 ± 0.01 | 0.12 | 10.11 ± 0.02 | 9.65 ± 0.15 | 0.46 | 0.07 | 0.06 | 0.01 |
| 11 | Masqia | 9.55 ± 0.33 | 5.20 ± 0.02 | 4.35 | 4.99 ± 0.02 | 3.75 ± 0.16 | 1.24 | 1.91 | 1.39 | 0.52 |
| 12 | Gaya | 4.48 ± 0.11 | 3.64 ± 0.04 | 0.84 | 1.41 ± 0.09 | 1.41 ± 0.05 | 0.00 | 3.18 | 2.58 | 0.6 |

## RESULTS AND DISCUSSION

The results obtained in the study revealed that drought had caused considerable morphological and physiochemical changes in plant growth, chlorophyll content, chlorophyll stability index, proline, and protein content (Tables 1a and 1b; *Fig. 1a, b, c* and *d*). Meanwhile, the severity of the drought affects the plant growth and it was measured by reduction in root and shoot length. All the genotypes had registered significant differences ($p<0.05$) for shoot length, root length and root to shoot ratio, but the extent of variation was strongly cultivar dependent (Table 1a). Among the genotypes, Masqia, Ria and MR 291 had shown greater reductions in shoot length under drought stress condition. However, the same genotypes had recorded increases in the root length and root to shoot ratio due to water deficit condition. As for Mukmur, the plant growth was not affected by drought stress.

The study showed that the length of seedling was significantly shorter than the length of the control. In their study, Nayyar and Gupta (2006) reported that leaf growth was inhibited relatively

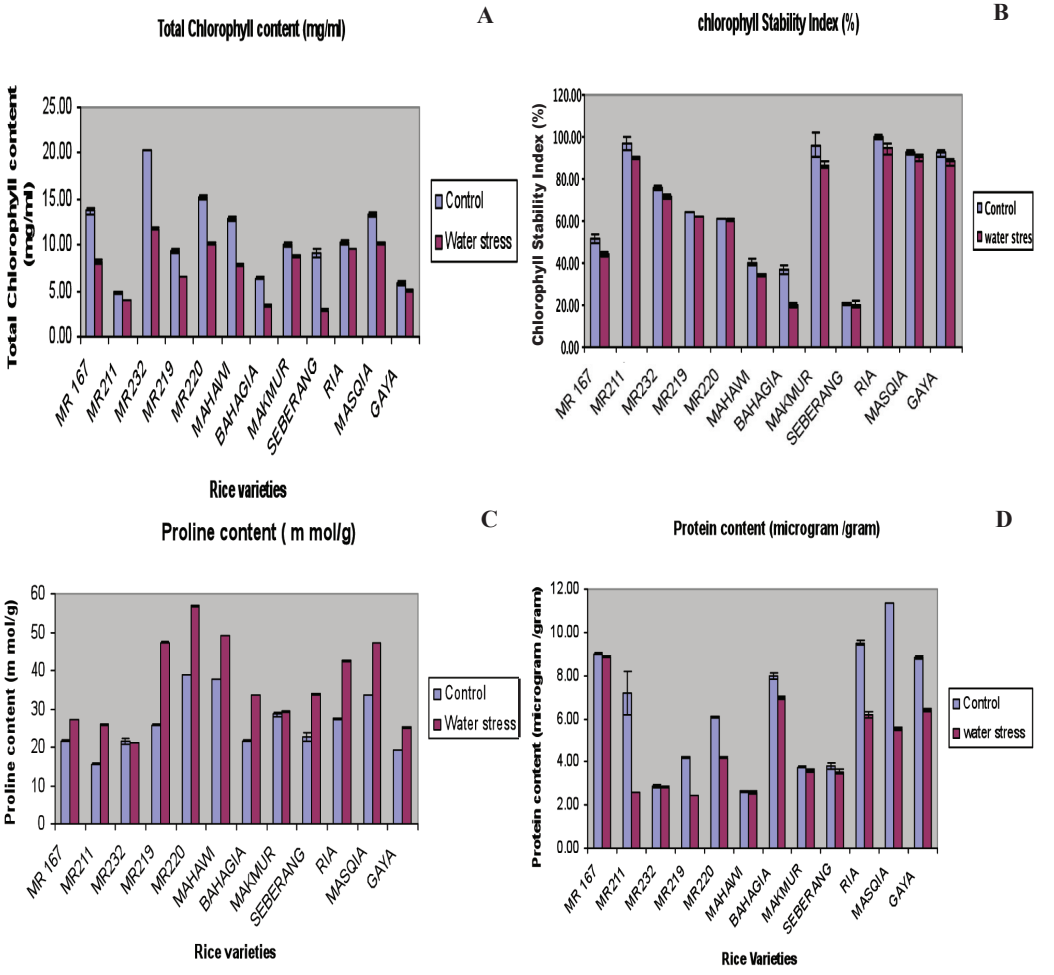


*Fig. 1: Effects of drought stress on certain morphological and physiochemical parameters of 12 rice genotypes; A) Total chlorophyll content (mg/g), B) Chlorophyll Stability Index (%), C) Proline concentration (µg/ g) and D) Protein content (µg/ g). All the treatments differed significantly from the control ($p < 0.05$)*

more than root growth in a stressed environment. Changes in plant growth were also recorded for Masqia, Ria, and MR219 in response to drought exhibited primary signal for drought adaptation. Plant growth is one of the most drought sensitive physiological processes due to the reduction of turgor pressure. In particular, water stress greatly suppresses cell expansion and growth due to the low turgor pressure (Kartikeyan *et al*., 2007; Jaleel *et al*., 2007; Manivannan *et al.*, 2007).

Therefore, to understand the photosynthetic ability of the genotypes studied under water deficit condition, chlorophyll a, chlorophyll b and total chlorophyll content were determined. A significant reduction in the chlorophyll content (a, b and a + b) was noticed in all the rice genotypes under stressed condition (Table 1b). Meanwhile, the total chlorophyll content showed differences which ranged from 8.60 mg $g^{-1}$ FW in MR 232 to 0.8460 mg $g^{-1}$ FW in Gaya (Table 1b) for both the control and stressed plants. A strong decline in chlorophyll a content was noticed for MR 232, Masqia, and Seberang. Depletion in chlorophyll a indicates that the drought stress impairs photosynthetic reaction centres but the ill effect was compensated by the increase in chlorophyll b for all these genotypes, except for Seberang. Furthermore, the ratio of chlorophyll a/b was also found to be less for these genotypes. On the contrary, a few genotypes, namely Mahawi, Seberang, and Bahagia had very high ratio for this particular trait under water-stressed condition. Some previous studies indicated that drought tolerant genotypes were able to maintain a higher chlorophyll content than the susceptible genotypes. The changes in the chlorophyll a/b ratio were less for these genotypes. Cicek and Cakirlar (2008) reported that the soybean salt stressed cultivars seemed to adapt to the stress by reducing their chl a/b ratio. Accordingly, the genotypes MR 232 and Masqia were found to be tolerant under water stress, whereas Mahawi, Seberang and Bahagia recorded very high chl a/b ratios and they might be sensitive to drought stress.

In addition, the heat stability of chlorophyll pigments has been described as an index for drought tolerance in plants. There was a general decreasing trend observed for CSI (%) in all the genotypes due to drought stress (*Fig. 1b*). A greater reduction was noticed for Bahagia, Makmur, MR 167 and MR 211, whereas slight decreases in CSI (%) were found in Seberang, MR 220, and MR 219.

The high CSI value obtained in the result indicated a better availability of chlorophyll in the plant that helps to withstand stress. On the contrary, Ali *et al*. (2008) reported that low CSI value and high sink strength were found to directly correlate with the productivity of pearl millet cultivars.

Proline is one of the amino acid which appears more commonly in response to stress. There was a steep increase in the proline content in all the genotypes (*Fig.1c*). The differences in accumulation of proline ranged from 0.25 m mol/ FW to 21.49 m mol/ FW. A slight increase in the accumulation of proline was also noticed in MR 232, while the maximum was recoded in MR 219. The synthesis of osmolyte, including proline, is widely used by plants to stabilize membranes and maintain the conformation of proteins at low leaf water potentials. Proline is known to be involved in reducing photo damage in thalakoid membranes by scavenging and/or reducing the production of $O_2$ (Reddy, 2004). Furthermore, proline plays a role as enzyme stabilizing agent and has the ability to mediate osmotic adjustment and stabilize sub-cellular structure (Hassanein, 2004; Yokota *et al*., 2006). The values of free proline content appear to be related to tolerance, however, the synthesis and accumulation of proline have been found to vary among the cultivars. Zhu (2001) suggested that lower accumulation of osmolyte function in protecting macromolecules either by protecting the tertiary structure of protein or by scavenging ROS (reactive oxygen species) produced in response to drought. The accumulation of proline was invariably observed in all the genotypes under stress and it was

TABLE 2
Analysis of variance for some morphological and physiochemical traits of 12 rice genotypes subjected to water deficit condition and controlled condition

| Traits | Df | Mean sum of square | | Error sum of square | | F ratio | | Sig (P<0.05) |
|---|---|---|---|---|---|---|---|---|
| | | Control | Stress | Control | Stress | Control | Stress | 0.00 |
| Root length (cm) | 11 | 16.22 | 7.45 | 1.744 | 2.577 | 9.30* | 2.89* | 0.00 |
| Shoot length (cm) | 11 | 459.18 | 388.99 | 7.825 | 4.725 | 58.68* | 82.33* | 0.00 |
| No. of leaves/ plant | 11 | 13.54 | 9.000 | 2.60 | 2.117 | 5.19* | 4.25* | 0.00 |
| Chlorophyll A (mg/g ) | 11 | 34.88 | 32.19 | 0.099 | 1.923 | 353.00* | 16.74* | 0.00 |
| Chlorophyll B (mg /g) | 11 | 63.50 | 70.22 | 0.035 | 1.923 | 1806.38* | 4109.20* | 0.00 |
| Total chlorophyll (mg / g) | 11 | 98.89 | 43.30 | 0.061 | 0 .009 | 1593.93* | 4561.09* | 0.00 |
| CSI (%) | 11 | 3729.66 | 3894.84 | 4.593 | 1.518 | 812.06* | 2565.69* | 0.00 |
| Proline (m mol /g) | 11 | 261.1 | 652.74 | 0.708 | 0 .221 | 368.87** | 2949.67* | 0.00 |
| Protein (µg/ g) | 11 | 43.17 | 21.96 | 0.092 | 0.005 | 469.47 | 4632.08* | 0.00 |

*Significance at p= 0.05 level

found to be higher in stress-sensitive genotype. This result is in accordance with an earlier observation reported for other species such as cassava (Sundaresan and Sudhakaran,1995), Mediterranean scrub (Ain-Lhout *et al*., 2001), European beech (Peuke *et al*., 2002), and wheat (Rampino *et al*., 2006).

There was a general decreasing trend for the total soluble protein content in all the genotypes due to water deficit stress (*Fig. 1d*). A greater reduction was noticed in Masqia followed by MR 211. Sarhan and Perras (1987) suggested that the quantitative changes in polypeptides might be responsible for the adjustments in metabolic pathways under stressed condition. This feature can be used as an indicator for improving stress tolerance (Pareek *et al*., 1997), depending on the nature of cultivar.

The result presented in Table 2 revealed an adequate significant genetic variation ($p<0.05$) for the morphological and physiochemical responses of drought stress. Genetic diversity was then estimated for these responses using the multivariate analysis. Multivariate approach helps to visualize the relationship between the genotypes with traits and presents a picture of superior and inferior genotypes. Meanwhile, clustering of genotypes was established based on the Euclidean distance matrix derived from standardized data and the results obtained are presented in *Figs. 2a* and *2b*. The grouping pattern indicated in *Figs. 2a* and *2b* has revealed the response of the genotypes towards water deficit condition. All the traits tested using the Wilk's criteria have shown pronounced differences among the genotypes. The principal component analysis yielded eight functional eigenvalue for the control and six values for the stress induced genotypes (Tables 3a and 3b). The first two principal axes accounted for 82.1% of the total variation in control, while 68.6% in the stress-induced genotypes. From the data presented in Tables 3a and 3b, it is evident that the characters shoot length, chlorophyll b, total chlorophyll content, CSI (%), proline and protein content recorded greater eigenvalue. In

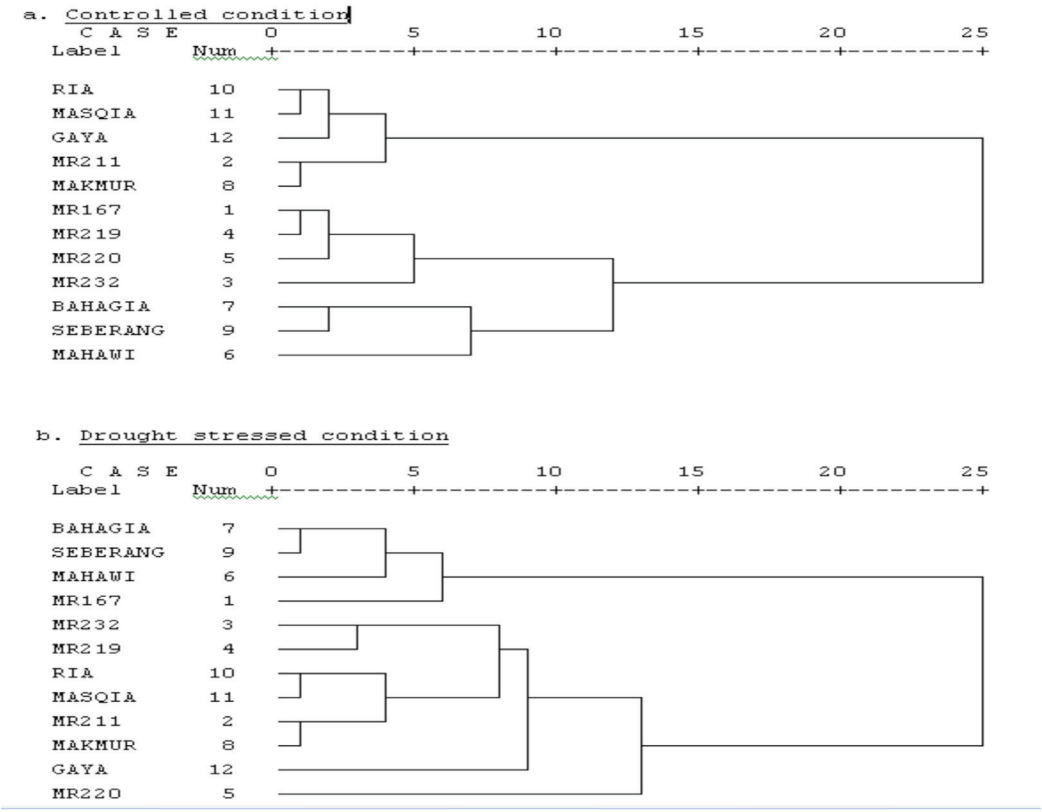


*Fig. 2: Dendrogram of hierarchical cluster analysis depicting grouping in rice genotypes based on the morphological and physiochemical responses in the controlled and stress induced conditions. 2a) Grouping of genotypes under controlled condition. 2b) Grouping of genotypes under drought stressed condition*

more specific, the large eigenvalue obtained in the study explained that the proportion of variance is associated with a strong function, and it indicates the proportion between the group and within group sum of squares. These values are related to the canonical correlations and they also describe how much discriminating ability a function possesses. Meanwhile, a large eigenvalue is associated with strong function, and thus, the traits involved in the study have contributed more towards genetic divergence. The investigation had further accounted for 53.5% of the first canonical root in controlled condition and 41.9% in stressed condition. Based on the values of the principal component scores, grouping was performed using the hierarchical cluster analysis, as depicted in *Figs. 2a* and *2b.* The position of genotypes in the dendrogram was apparently distributed into three groups in the control and six groups in the drought induced. The result obtained from the cluster analysis clearly revealed the differential responses of the genotypes under stress. However, the hierarchical cluster analysis was unable to state the nature of the genotype responses, i.e. how sensitive or tolerant to drought stresses. The data were further subjected to the discriminant function analysis to determine the magnitude of discriminating abilities of the rice genotypes based on the morphological and physiochemical parameters under drought stress. Hence, a two dimensional scatter diagram was constructed using the values of the first two canonical vectors (functions 1 and function 2) as the coordinates

TABLE 3a
Eigen values and percentage of variation for the morphological and physiochemical characters in 12 rice genotypes in controlled condition

| Function | Eigen value | % of Variance | Cumulative % | Canonical correlation |
|---|---|---|---|---|
| Shoot length | 735.225 | 53.5 | 53.5 | .999 |
| No. of leaves | 393.376 | 28.6 | 82.1 | .999 |
| Chlorophyll A | 107.248 | 7.8 | 89.9 | .995 |
| Chlorophyll B | 89.610 | 6.5 | 96.4 | .994 |
| Total Chlorophyll | 40.473 | 2.9 | 99.4 | .988 |
| CSI (%) | 5.896 | 0.4 | 99.8 | .925 |
| Proline | 2.436 | 0.2 | 100.0 | .842 |
| Protein | .467 | 0.0 | 100.0 | .564 |

TABLE 3b
Eigen values and percentage of variation for the morphological and physiochemical characters in 12 rice genotypes in water stressed condition

| Function | Eigen value | % of Variance | Cumulative % | Canonical correlation |
|---|---|---|---|---|
| Shoot length | 1913.800 | 41.9 | 41.9 | 1.000 |
| Chlorophyll B | 1223.488 | 26.8 | 68.6 | 1.000 |
| Total chlorophyll | 817.711 | 17.9 | 86.5 | .999 |
| CSI (%) | 427.251 | 9.3 | 95.9 | .999 |
| Proline | 173.596 | 3.8 | 99.7 | .997 |
| Protein | 15.297 | .3 | 100.0 | .969 |

for the graphical presentation (*Figs. 3a* and *3b*), where $F_1$ served as x axis and $F_2$ as y axis. The grouping which was obtained by hierarchical clusters was compared with two dimensional representation of the canonical discriminant function analysis. It is interesting to note that the results obtained matched with the magnitude of divergence measured by dendrogram. Moreover, it is evident from the scatter plot diagram that the response of the genotypes varies widely as indicated by the change in the localization of genotypes in both the control and drought stressed conditions. The positions of Seberang and Mahawi in the graph have shifted far below the central axis, indicating a drastic reduction in their function and revealing that they are considered as sensitive to stress. Meanwhile, MR 232 and Masqia were found close to the axis, and this indicated stabile in their performance. Meanwhile, the positions of Ria, Gaya, and MR 167 were shown to be above the central axis and they might be tolerant to water deficit stress. Furthermore, the scatter plot of canonical discriminant function reflected the relative importance of character contributing towards divergence.

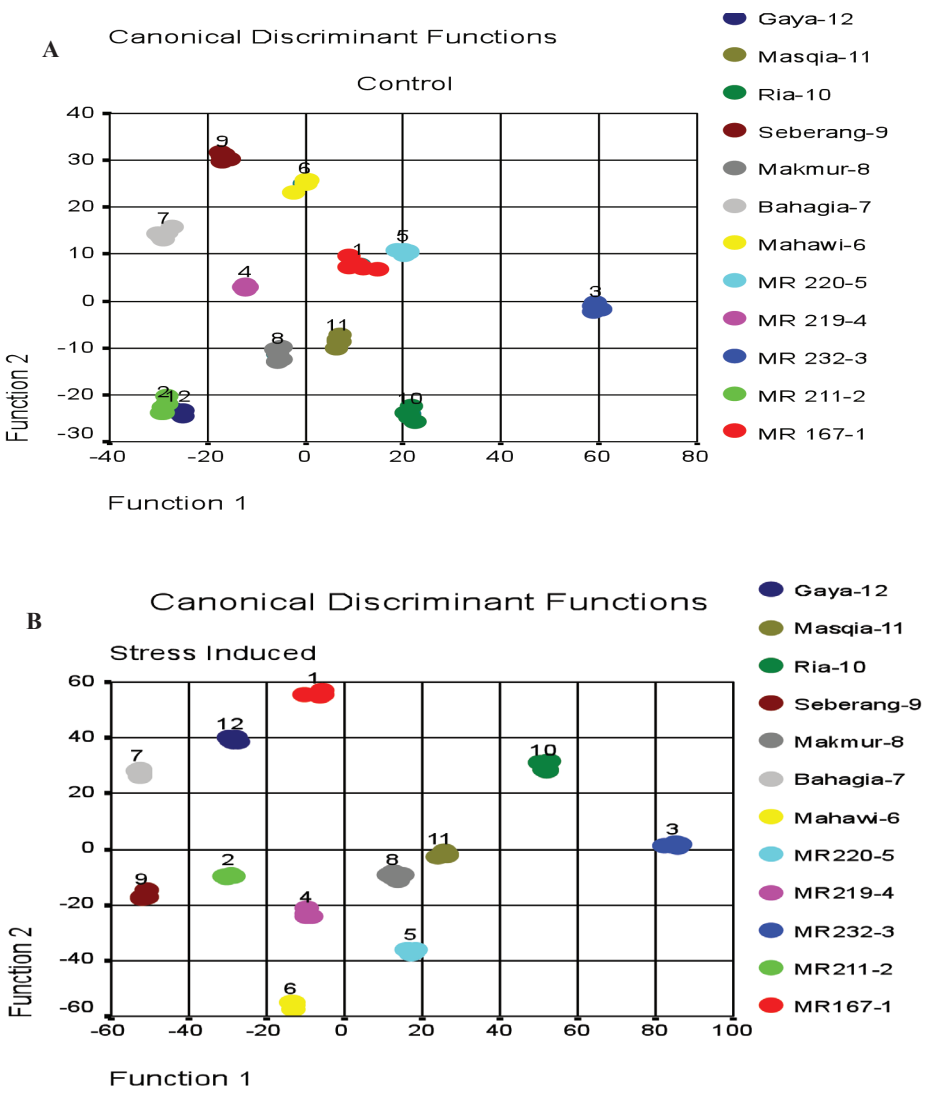


*Fig. 3: Two dimensional representation of drought stress response of 12 rice genotypes obtained from the canonical discriminant functions; a) Genotypes response obtained under controlled environment, b) Genotype response obtained under water stressed condition*

## CONCLUSIONS

The study has demonstrated that the morphological and physiochemical traits investigated have greater relevance to future breeding programme, particularly for screening drought tolerance at early stage. The traits total chlorophyll content, chlorophyll stability index, proline and protein have contributed the maximum towards genetic divergence under stressed condition and classified the genotypes as tolerance and sensitive to drought stress. Furthermore, the correlation coefficient presented in Tables 3a and 3b shows a strong association with those physiochemical traits. Therefore, it is suggested that these traits can be employed as potential indicators for screening

drought tolerance at early stage. Therefore, the present investigation has demonstrated that laboratory-based measurements on growth rate, total chlorophyll content, CSI %, proline and protein enable large number of genotypes to be screened in a shorter period time. The traits examined are promising and can be used as potential selection criteria for improving drought tolerance in rice. More importantly, the techniques used to screen the genotype at the laboratory level are economical and effective alternatives to select drought and stress tolerant genotypes at early stage.

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