Evaluating the rice germplasm for salinity tolerance based on phenotypic traits

Site Noorzuraini A R1*, Mohd Ramdzan O1, Nur Idayu A R1, Muhammad Hafiz M S1

1MARDI Seberang Perai, Jalan Paya Keladi, 13200 Kepala Batas, Pulau Pinang, Malaysia.

* Corresponding author: zuraini@mardi.gov.my

Abstract. Salinity is one of the major problems which hampers yield production. Salinity stress can be perceived either in the soil or irrigation water. Forty rice germplasms were selected based on their origin country. Salinity level at 12dSm-1 were imposed at seedling stage (10 Days after sowing). The findings observed shoot length and root length were decreased under salt stress. Although, the rice germplasms responded to salt stress differently, however, leaf injury score and percent of survival seedlings successfully differentiated the salt tolerant genotypes. BULASTOG, AZUCENA and ZHEN-LONG 13 were performed well under salt stress with exhibited lowest leaf injury score compared to the tolerant control varieties. BULASTOG also showed the highest percent of survival seedlings under salt stress. The second highest on percent of survival seedlings observed in KHAO TAH HAENG, and followed with CITANDANG. Cluster analysis was constructed based on leaf injury score, shoot length, root length, and percent of survival seedlings grouped the rice germplasms into three major groups. The tolerant control varieties, POKKALI I and POKKALI II were grouped in Group 2. BULASTONG and KHAO TAH HAENG were obviously closed to tolerant control varieties in the group. In conclusion, BULASTONG and KHAO TAH HAENG are the most promising rice germplasms under salinity condition, the germplasms can be recommended for further improvement program for salt tolerant in rice. In addition, shoot length, leaf injury score, and percent of survival seedlings should be emphasized during selection of salt tolerant genotypes of rice at seedling stage.

1. Introduction
Salinity is one of the major problems in rice growing areas and considered as a serious limitation to increase rice production worldwide [1]. Based on report of FAO [2], there are over 800 million ha of worldwide land areas are affected by salt, which 20% of that are irrigated areas suffered with various degrees of salinity problems. Salt accumulation in irrigation water caused by high trace amount of sodium chloride (NaCl) [3], while, in rainfed agriculture, salinity problems occurred because the areas are situated adjacent to coastal areas [4]. In Malaysia, rice mainly planted in granary areas which many of the areas are situated adjacent to the coastal saline land such as Muda, Seberang Perai, Krian, and Barat Laut Selangor [5]. The areas are prone to salt water intrusion, thus can caused serious damage to the rice plants [5].

In Pakistan, about 64% reduction in crop yield reported due to salinity [6]. In Vietnam, about 45 million USD which equivalent to 1.5% of annual rice production in the Mekong Delta loss due to salt intrusion [7]. Salinity conditions with high salt concentration (NaCl) in soils may decreased the water uptake abilities in plants [4]. High concentration of Na+ and Cl- can negatively affect plant growth by...
impairing metabolic processes and decreasing photosynthetic efficiency through the occurrence of osmotic stress and accumulating of Na\(^+\) stress [8]. Stomatal closure in order to reduce transpiration process also appears to be one of the main caused for the decreasing of the photosynthetic rate [9].

Rice plant is reported sensitive to salinity particularly at early seedling stage and flowering stage. Although rice is a salt sensitive crop [10], however, some of rice landraces possess remarkable tolerance to salt stress. One example of a rice landrace and tolerant genotype to high salinity is Pokkali, the Indian landrace and used widely as a donor of salt-tolerance traits in breeding programs. Therefore, it is prerequisite to screen the existing rice germplasm for a good source of salt tolerance genotypes.

Screening of rice germplasm at seedling stage is readily acceptance as it is based on a simple criterion of selection, provides rapid screening method [11] and saving in time [12]. However, the salt tolerance at early growth stages does not exactly correlate with the subsequent growth stages [13, 14]. In this study, screening the potential of salinity tolerant among the rice germplasms was done at early growth stage i.e. seedling stage. The study aims to assess the performance of selected rice germplasm in term of seedling growth parameter at 12dSm\(^{-1}\) salinity level. The identified potential rice germplasm is useful in improvement programs for development salinity tolerant rice cultivars.

2. Material and Method

2.1. Plant materials

The screening for salinity tolerance at the seedling stage of the landrace genotypes of rice germplasms (Table 1) was carried out on the basis of morphological characterization. The rice germplasms were selected based on their origin country, in which each country had suffered serious flooding as a result of the intrusion of seawater. The idea to select the rice germplasms based on their origin country because these genotypes have been cultivated for several decades in their home country and these may genetically allow them to develop a complex set of mechanisms for salt tolerant.

There are two accessions of POKKALI were selected as tolerant control varieties and IR64 the advance line from IRRI, Philippines was selected as sensitive control variety.

Table 1. List of landraces of rice germplasms

| No. | Accession No. | Variety Name     | Country of Origin          |
|-----|---------------|------------------|-----------------------------|
| 1   | MRGB02491     | POKKALI I        | Tolerant Control Variety from India |
| 2   | MRGB11679     | POKKALI II       | Tolerant Control Variety from India |
| 3   | MRGB07854     | IR64             | Sensitive Control variety from IRRI |
| 4   | MRGB00292     | CHANDINA         | Bangladesh                  |
| 5   | MRGB01192     | PANBIRA          | Bangladesh                  |
| 6   | MRGB01296     | PURBACHI         | Bangladesh                  |
| 7   | MRGB02073     | TILOCKACHARI     | Bangladesh                  |
| 8   | MRGB02426     | BIPLAB           | Bangladesh                  |
| 9   | MRGB02770     | SHA TIAO TSAO    | China                       |
| 10  | MRGB10753     | SI-FENG 43       | China                       |
| 11  | MRGB10754     | ZHEN-LONG 13     | China                       |
| 12  | MRGB10817     | QI-CAI-ZHAN      | China                       |
| 13  | MRGB10894     | ZHE 733          | China                       |
| 14  | MRGB00023     | ALURSANNA 199    | India                       |
| 15  | MRGB01732     | SOORYA           | India                       |
| 16  | MRGB06962     | TRIVENI          | India                       |
Table 1. List of landraces of rice germplasms (continued)

| No. | Accession No. | Variety Name      | Country of Origin |
|-----|---------------|-------------------|-------------------|
| 17  | MRGB04538     | THAVALU           | India             |
| 18  | MRGB06269     | MUTHU MANIKAM     | India             |
| 19  | MRGB00200     | BANGAWAN          | Indonesia         |
| 20  | MRGB00235     | BRONDOL PUTEH     | Indonesia         |
| 21  | MRGB01803     | TJAHAYA           | Indonesia         |
| 22  | MRGB01804     | TJERE MAS         | Indonesia         |
| 23  | MRGB07493     | CITANDANG         | Indonesia         |
| 24  | MRGB00382     | DAWK MALI 109     | Thailand          |
| 25  | MRGB00640     | KAO SAARD 108     | Thailand          |
| 26  | MRGB00699     | KHAO RAJA         | Thailand          |
| 27  | MRGB00700     | KHAO TAH HAENG    | Thailand          |
| 28  | MRGB01088     | NAM DOCK MAI 2028 | Thailand         |
| 29  | MRGB07903     | PADI MASIN        | Malaysia          |
| 30  | MRGB12981     | PADI BARIO        | Malaysia          |
| 31  | MRGB08109     | MAYANG KELUBI     | Malaysia          |
| 32  | MRGB12421     | PADI BIDOR        | Malaysia          |
| 33  | MRGB11892     | PADI LUMPUR       | Malaysia          |
| 34  | MRGB00242     | BULASTOG          | Philippines       |
| 35  | MRGB05145     | AZUCENA           | Philippines       |
| 36  | MRGB10829     | SALUMPIKIT        | Philippines       |
| 37  | MRGB10837     | TANDUKAN          | Philippines       |
| 38  | MRGB05233     | DINORADO          | Philippines       |
| 39  | MRGB01790     | TEP SAIGON        | Vietnam           |
| 40  | MRGB00276     | CADUNG KET        | Vietnam           |
| 41  | MRGB00525     | HIN TRANG         | Vietnam           |
| 42  | MRGB01093     | NANG QUOT         | Vietnam           |
| 43  | MRGB01728     | SOC NAM           | Vietnam           |

2.2. Salt stress imposition

The rice germplasms were screened for salt tolerance at seedling stage based on Gregorio et al. [11] with some modifications. The viable seeds of each rice germplasm were germinated in the Petri dish and filled with distilled water. For a period for 5 days, after germination, the seedlings were transferred into plastic tray. Seedling of each rice accessions were arranged separately according to the rows on the plastic tray. The seedlings were kept in non-saline distilled water for about 5 days to allow the seedlings recovered from injuries during transferring process.

The salinity condition at desired EC level (12dSm\(^{-1}\)) was prepared using seawater and diluted to reduce the salt concentration with added non-saline distilled water. The salinity level of the solution was confirmed using EC meter (Model Hanna HI 9300).

2.3. Phenotypic study of salinity tolerance at seedling stage

Salinized and non-salinized conditions were setup with 4 replicates. The modified standard evaluation system (SES) as proposed by Gregorio et al. [11] was used in rating the visual symptoms of salt
toxicity on leaf (Table 2) of each rice germplasm. The scoring is important to discriminate the susceptible from the tolerant and the moderately tolerant genotypes [15]. The scoring was done at day 7 after imposed to salinized condition. The phenotypic data i.e. shoot length and root length were recorded for salinized and non-salinized conditions. The percent of survival seedlings was also recorded based on number of surviving seedlings after seven days under salinized condition.

Table 2. Modified standard evaluation score (SES) of visual salt injury score at seedling stage

| Score | Observation                                           | Tolerance       |
|-------|-------------------------------------------------------|-----------------|
| 1     | Normal growth on leaf symptoms                        | Highly tolerant |
| 3     | Nearly normal growth, but leaf tips or few leaves whitish and rolled | Tolerant        |
| 5     | Growth severely retarded, most leaves rolled; only a few are elongating | Moderately tolerant |
| 7     | Complete cessation of growth; most leaves dry; some plants dying | Susceptible     |
| 9     | Almost all plants dead and dying                      | Highly Susceptible |

2.4. Statistical analysis
The experiments were carried out in split plot design with four replicates which each replication consisted of 12 seedlings. The shoot length, root length, percent of survival seedlings, and leaf injury score were analysed using SAS statistical software. Cluster of the rice germplasm based on the data recorded were constructed using Paleontological Statistical Software Package (PAST 4.0). Euclidean distance and Unweighted Pair Group Method Arithmetic Average (UPGMA) was considered for clustering.

3. Result and Discussion

3.1 Visual leaf injury score
Leaf injury score showed highly significant difference among the replications (Table 3). The rice germplasm showed wide variation on leaf injury score with the lowest is score 3 (tolerant) and highest is score 7 (susceptible). POKKALI I and POKKALI II as salt tolerant control varieties showed the average score is 3.3. BULASTOG, AZUCENA and ZHEN-LONG 13 showed the most salinity tolerant with average score is 3.0. Whereas, KHAO TAH HAENG, NAM DOCK MAI 2028, SOORYA, QI-CAI-ZHAN, and ZHE 733 showed average score is 3.5 (Figure 1). The leaf injury score for POKKALI I, POKKALI II, BULASTOG, AZUCENA, and ZHEN-LONG 13 are significantly different with other rice germplasms under EC level at 12dSm⁻¹ (Figure 1).

Salt tolerant seedlings can be distinguished from the sensitive seedlings at 12dSm⁻¹. The symptoms on the sensitive seedlings were obvious on the first and second leaves and visualized by leaf rolling, whitish of leaf tip, drying of leaves, stunted growth, and leading to dying of seedlings [11]. According to Lin et al. [16], damage of leaves was attributed by the accumulation of Na⁺ in the shoot.

Table 3. Statistical analysis for leaf injury score

| Source          | DF | Anova SS | Mean Square | F Value | Pr > F  |
|-----------------|----|----------|-------------|---------|---------|
| Variety         | 40 | 215.16   | 5.38        | 1.87    | 0.1766  |
| Replication     | 3  | 284.85   | 94.95       | 33.03   | <.0001  |
| Variety*Replication | 120| 218.40   | 1.82        | 0.63    | 0.8622  |
Visual Leaf Injury Score among the rice germplasm

Figure 1.

3.2 Seedlings growth performance based on phenotypic traits

The distribution for shoot length and root length showed wide fluctuation among the varieties under EC level at 12dSm$^{-1}$. The shoot length ranged from 26.34 to 43.11 which the highest shoot length observed in POKKALI II and the lowest in SI-FENG 43 (Figure 2). Whereas, the root length ranged from 11.94 to 18.56 which the highest root length found in TANDUKAN and the lowest observed in QI-CAI-ZHAN (Figure 3).

This study observed several rice germplasms exhibited higher shoot length under salinity condition namely POKKALI II, KHAO RAJA, KHAO TAH HAENG, THAVALU, POKKALI I, DAWK MALU 109, and TILOCKACHARI. Bhowmik et al. [15] found highly significant and positive correlations between plant height and dry matter at seedling stage under salinized condition. Peng at al. [17] stated that increasing in plant height would allow to greater biomass production. Increased in biomass production is important as it increase the yield potential [18]. Reduction in seedling growth under salinized condition may be due to accumulation of salt in older leaves thus caused premature senescence, and eventually reduced photosynthetic rate of a plant to a level that cannot maintain growth [19]. In this study, those rice germplasms that exhibited higher shoot length also performed well under the salinity condition.

Root length at seedling stage also decreased with increasing salinity [12]. POKKALI II showed root length is 16.37, whereas POKKALI I is 14.83. There are four rice germplasms that have lowest leaf injury score (resistant to salinity) and also showed root length more than 16.00 which are BULASTOG, ZHEN-LONG 13, KHAO TAH HAENG, and NAM DOCK MAI 2028. However, the findings look as root length and leaf injury score showed no relevance relation in other salt tolerant rice germplasms. Similar to Nasim Ali et al. [12], that observed insignificant relation between root length and tolerance index for leaf injury score at higher salinity levels and concluded that root length may not be a good descriptor of salinity tolerance [12].
3.3 Seedling survival rate under salinity condition
The seedling survival rate is based on the number of survival seedling at the end of the screening. According to Lutts et al. [20], seed germination, growth, and survival of seedlings decreased due to effect of salinity in rice. The highest survival rate observed in POKKALI II (100%) and the lowest survival rate in IR64 (65.62%). POKKALI I and POKKALI II showed remarkable tolerance level compared to the sensitive control variety, IR64. BULASTOG also showed highest survival rate which is 100%. Whereas, sixteen rice germplasms showed more than 90% survival rate namely KHAO TAH HAENG, CITANDANG, SOORYA, MAYANG KELUBI, BIPLAB, ZHEN-LONG 13, AZUCENA, KAO SAARD 108, NAM DOCK MAI 2028, NANG QUOT, PADI MASIN, TJERE MAS, TANDUKAN, KHAO RAJA, TEP SAIGON, and TRIVENI.

The study observed the salt symptoms appeared as whitish on older leaf tips in tolerant rice germplasms after one week of stress. In moderately tolerant rice germplasms, most leaves are still elongating but some leaves started to dry after one week. Whereas, in susceptible rice germplasms, the leaves completely dried and dying within a week. The rice germplasms that have more than 90% of survival seedlings were most durable and showed remarkable growth ability under salinity condition.
3.4 Identification of salt tolerant among the rice germplasm
Hierarchical clustering using Euclidean Distance as distance measure was used for grouping the rice germplasms (Figure 5). The dendrogram was constructed based on four data collected namely leaf injury score, shoot length, root length, and percent of survival seedlings. There are three groups generated from the clustering analysis. The tolerant control varieties, POKKALI I and POKKALI II were grouped in Group 2 with another 15 rice germplasms. Whereas, the susceptible control variety, IR64 was grouped in Group 1 with PANBIRA, MUTHU MANIKAM, and DINORADO.

In Group 2, two rice germplasms namely KHAO TAH HAENG and BULASTOG are obviously closed to tolerant control varieties. BULASTOG showed highest percent of survival seedlings (100%) and lowest leaf injury score (3.00) compared to the tolerant control varieties. KHAO TAH HAENG also produce high shoot length (41.22) and high percent of survival seedlings (96.88%). These indicate the rice germplasms presenting the higher limit of tolerance under salinity condition. Thus, this can be concluded that BULASTOG and KHAO TAH HAENG have potential and can be utilized for further breeding program against salinity in rice.
Figure 5. Dendrogram for 40 rice germplasms under salinity condition

4. Conclusion
Highly significant differences among the rice genotypes under salinity condition were observed in this study. The differential responses of rice germplasms based on leaf injury score, shoot length, root length, and % of survival seedlings were successfully distinguished the promising rice germplasms for salinity tolerant. The findings will be helpful for both breeders and farmers to face and address salinity issues in their areas. The identified rice germplasms that have been identified can be recommended and utilized suitably for further improvement program for salt tolerant in rice. Shoot length, leaf injury score, and % of survival seedlings should be emphasized during selection of salt tolerant genotypes of rice at seedling stage.

Acknowledgements
Financial support through an R&D grant under Development Fund of Ministry of Agriculture and Agro-based Industry, Malaysia is gratefully acknowledged. Thanks also due to the support given by the contract staff of Rice Genebank, MARDI Seberang Perai.

References
[1] Gregorio G B 1997 Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). PhD thesis, University of the Philippines, Los Baños 118
[2] Food and Agriculture Organization (FAO) 2010 Report of salt affected agriculture. http://www.fao.org/ag/agl/agll/spush/
[3] Tester M and Davenport R 2003 Na+ tolerance and Na+ transport in higher plants Ann Bot 91 503-527
[4] Bararathkumar S, Jena PP, Kumar J, Yasin Baksh SK, Samal R, Gouda G, Murkherjee M, Donde R, Vijayan J, Parida M, Reddy JN 2016 Identification of new alleles ins alt tolerant rice germplasm lines through phenotypic and genotypic screening Int J Agric Biol. 18 441-448
[5] Hashim G M 2003 Salt affected soils of Malaysia ftp://ftp.fao.org/agl/agll/ladados/malaysia.doc
[6] Afzal I, Basra SMA, Ahmad N, Farooq M 2005 Optimization of hormonal priming techniques
for alleviation of salinity stress in wheat (*Triticum aestivum* L.). Caderno de Pesquisa serie Biologia 17 95-109

[7] Ministry of Agriculture and Rural Development 2005 Vietnam News Agency

[8] Maser P, Eckelman B, Vaidyanathan R, Horie T, Fairbairn DJ, Kubo M, Yamagami M, Yamaguchi K, Nishimura M, Uozumi N, Robertson W, Sussman MR, Schroeder JL. 2002. Altered shoot/root Na⁺ distribution and bifurcating salt sensitivity in Arabidopsis by genetic disruption of the Na⁺ transporters AtHKTI *FEBS Lett* 531 157-161

[9] Kaiser W M 1987 Effects of Water Deficit on Photosynthetic capacity *Physiol. Plant.* 71 142-149

[10] Mass E V 1990 Crop salt tolerance. In: agricultural salinity assessment and management (Eds.): K. K. Tanji. ASCE Manuals and Reports on Engineering American Society of Civil Engineers, New York 71 262-304

[11] Gregorio G B, Senadhira D, Mendoza R D 1997 Screening rice for salinity tolerance *IRRI Discussion Paper Series. International Rice Research Institute, Laguna Philippines* 22 1-30

[12] Nasim Ali Md, Yeasmin L, Gantait S, Goswami R, and Chakraborty S. 2014. Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. *Physiol Mol Biol Plants.* 20 (4): 411-423 (12)

[13] Zeng L, Shannon MC, Grieve CM 2002 Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters *Euphytica* 127 235-245 13

[14] Ferdose J, Kawasaki M, Taniguchi M, Miyake H 2009 Differential sensitivity of rice cultivars to salinity and its relation to ion accumulation and root tip structure *Plant Prod Sci* 12 453-461

[15] Bhowmik S K, Titov S, Islam M M, Siddika A, Sultana S, Shahidul Haque M D 2009 Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *African Journal of Biotechnology* 8(23) 6490-6494

[16] Lin HX, Zhu MZ, Ysno M, Gao JP, Liang ZW, Su WA, Hu XH, Ren ZH, Chao DY 2004 QTLs for Na⁺ and K⁺ uptake of the shoots and roots controlling rice salt tolerance *Theor Appl Genet* 108 253-260

[17] Peng S, Cassman KG, Virmani SS, Sheehy J, Khush GS 1999 Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential *Crop Sci.* 39 1552-1559

[18] Zhang ZH, Li P, Wang LX, Hu ZL, Zhu LH, Zhu YG 2004 Genetic dessection of the relationships of biomass production and partitioning with yield and yield related traits in rice. *Plant Sci.* 167 1-8

[19] Munns R 2002 Comparative physiology of salt and water stress *Plant Cell Environ.* 25 239-250

[20] Lutts S, Kinet JM, Bouharmont J 1995 Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance *J Exp Bot.* 46 1843-1852