

## Evaluation for Salt Tolerance in Rice Using Multiple Screening Methods

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### ABSTRACT

Screening methods that are effective in the early stage of growth will potentially provide the largest quantity of breeding material. Although various screening methods under greenhouse have been proposed, potential effective approaches must be described a good correlation with results under field conditions. This study was aimed to assess the correlations between traits obtained from four salt screening methods, hydroponic culture, soil culture, pot and field methods. Salt injury scores from the soil culture and hydroponic methods at the seedling stage under salinity of 12 dS m<sup>-1</sup>, were not correlated, but were able to identify the variety Pokkali as being a tolerant variety, and IR29 as a susceptible variety. Traits in the pot and field experiments were significantly related to the rice varieties and salt salinity levels. The correlations at the seedling stage were found between salt injury score in the soil culture and plant height, proline content of leaves and panicle length in the field experiment ( $r = -0.886$ ,  $r = 0.992$  and  $r = -0.933$ , respectively). Also, traits from the pot experiment showed significant correlations with those from the field experiment. Salt injury scores obtained from soil culture method provide a simple and efficient method for indirect selection for salt tolerance in rice.

**Keywords:** Hydroponic culture, Na<sup>+</sup>/K<sup>+</sup> ratio, Rice, Salt tolerance, Soil culture.

### INTRODUCTION

Rice is one of the most important cereal crops in the world. In many countries in Africa and Asia, rice cultivation is considered the principle agricultural activity and source of income. More than 90% of the world's rice is grown and consumed in Asia (Khush and Virk, 2000), where arid or semi-arid environments are often associated with soil salinity problems, because the rainfall is insufficient to leach soluble salts from the soil. Soil salinity, one of the most destructive abiotic stresses, is a global problem that threatens crop production in many areas. Soils with an Electrical Conductivity (ECe) of saturation extracts

above 4 dS m<sup>-1</sup> are considered saline soils (Marschner, 1995). Over the past several decades, many studies have indicated that rice is sensitive to salinity, especially in the seedling and reproductive stages, with the vegetative stage appearing to be more tolerant with increase in the age of the rice plant (Aref and Rad, 2012; Gregorio *et al.*, 1997; Abdullah *et al.*, 2001; Mohammadi-Nejad *et al.*, 2010).

The effects of salinity stress on the morphological, physiological and biochemical traits have been investigated in rice. Salinity reduced tillering, spikelet filling, florets per panicle, 1,000 grain weight, grain yield, harvest index, shoot and root dry matter and K<sup>+</sup> uptake and increased leaf and root Na<sup>+</sup> and Cl<sup>-</sup> (Mohammadi-

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Nejad *et al.* 2008; 2010; De Leon *et al.*, 2015; Morales *et al.*, 2012). Although the genetics of salt tolerance is useful for breeders in helping with the development of tolerant varieties, progress in selecting tolerant genotypes in large scale breeding programs is still limited. This is mainly due to the fact that salt stress conditions are not uniformly distributed or stable throughout an area, thereby making it difficult to identify tolerant genotypes. The variation in salt tolerance occurs not only among species, but also among cultivars within species (Arzani, 2008; Ashraf and Foolad, 2013). In addition, stages of growth, soil salt composition and environmental conditions are additional factors affecting response of plant to salinity (Arzani, 2008). Therefore, salinity tolerance in rice is a complex trait that makes the evaluation of the phenotypes under field conditions very difficult. To enhance salinity tolerance in rice, it is very important to find sufficient variation and to devise appropriate screening techniques which are reliable and able to identify salt tolerant genotypes. The objective of this study was the evaluation of parental lines for salt tolerance in rice using multiple screening methods.

## MATERIALS AND METHODS

### Plant Materials

The study used six rice varieties: RD6, which is the most popular variety in Thailand, with high grain quality and moderate salt tolerance (Rice Thailand, 1977); SRN1, the photoperiod insensitive variety with moderate salt tolerance (Rice Thailand, 1977); IR62266-KKU and IR62266-RGD, which are good performing varieties under saline conditions, originating from different sources [KKU and RGD (Rice Gene Discovery Unit)]; IR29 and POKKALI, which are known susceptible and tolerant checks, respectively (Pathan *et al.*, 2004).

### Experimental Design and Cultural Practices

#### Experiment 1: Screening at the Seedling Stage

The hydroponic conditions used followed Gregorio *et al.* (1997), while the nutrient solution was prepared following Yoshida (1976). The experiment was in the form of a split-plot design with 3 replications. The main plot treatments comprised 3 salinity levels; 0 (control), 8 and 12 dS m<sup>-1</sup>; the sub-plots comprised 6 rice varieties. Seeds were sowed individually in small holes in styrofoam plates, with a nylon net providing support at the bottom (8 seedlings/variety). The styrofoam plates were floated in rectangular plastic trays half filled with nutrient solution until 21 Days-After-Sowing (DAS) the salinization treatments were subjected at 0, 8 dS m<sup>-1</sup> and 23 DAS were added up to 12 dS m<sup>-1</sup> Electrical Conductivity values (ECe) by adding NaCl. The nutrient solution was renewed every 4 days and the pH was maintained at 5.0 (being adjusted daily by adding either 1N NaOH or HCl). The ECe measurements were taken daily. Data on Salt Injury Score (SIS) were collected following the standards of IRRI (1996) at 31, 37 and 41 DAS.

The soil culture conditions based on modified Gregorio *et al.* (1997) and the experimental design, were the same for the hydroponic growing conditions. Seven days old seedlings were transferred into 4.7×4.7×5.0 cm<sup>3</sup> plastic pots filled with soil and soaked in the water tank, with one seedling per pot (4 pots/variety). The pots were fertilized with 25 kg ha<sup>-1</sup> N, 25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 13 kg ha<sup>-1</sup> K<sub>2</sub>O at 14 DAS. The salinization treatments were adjusted by adding NaCl until ECe reached 8 dS m<sup>-1</sup> at 21 DAS and 12 dS m<sup>-1</sup> at 23 DAS. The salt solution level was maintained at 2 cm above the soil surface throughout the growing period. The solution was renewed once a week and the pH was maintained daily at 5.0 (adjusted by adding either 1N NaOH or HCl). The recorded Salt Injury

Score (SIS) followed the standards of IRRI (1996) at 31, 36 and 40 DAS.

Experiment 2: Screening at the Reproductive Stage

A pot experiment was conducted at Khon Kaen University, Thailand, in the wet season of 2012. The study focused on individual plant responses under constant stress. The layout of the 6×4 factorial experiment was according to a CRD, with 3 replications. Factor A was 6 rice varieties, while factor B was 4 levels of NaCl in the soil [0.0, 0.1, 0.2 and 0.4% (w/w of soil)]. Plastic pots with a diameter of 30 cm and a height of 30 cm were filled with 10 kg of regular soil. The soil was sieved through a 1 cm sieve and mixed with NaCl at the treatment rates, then flooded with water 5 cm above the soil surface for 7 days before transplanting. Sowing took place on 14 June 2012 and transplanting on 14 July 2012. Fertilizer was applied at a rate of 25 kg ha<sup>-1</sup> N, 25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 13 kg ha<sup>-1</sup> K<sub>2</sub>O at 37 DAS, with a further 72 kg ha<sup>-1</sup> N being applied at 67 DAS. The electric conductivity (dS m<sup>-1</sup>) and pH of the solution above the soil surface were recorded at 37, 44, 58, 72, 86 and 100 DAS, using an EC500 meter.

The field experiments were conducted in farmer's fields at Udon Thani province in Northeast Thailand in wet season 2013. The physical and chemical properties of the soil are summarized in supplementary Table 1. The experimental design was a RCBD with four replications. Individual treatment plots were 1×1 m<sup>2</sup>, with a 0.25×0.25 m<sup>2</sup> between plants and rows. Seeds were sown on July 24<sup>th</sup> 2013 and transplanted on August 30<sup>th</sup> 2013. The electric conductivity (dS m<sup>-1</sup>) and pH of the solution above the soil surface, and the *EC<sub>e</sub>* of the soil, were recorded at 51, 65, 79, 93 and 107 DAS (3.01, 1.40, 4.35, 8.66 and 9.13 dS m<sup>-1</sup>, respectively) using an EC500 meter. Fertilizer applied to the plots comprised 25 kg ha<sup>-1</sup> N, 25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 13 kg

ha<sup>-1</sup> K<sub>2</sub>O at 52 DAS and 72 kg ha<sup>-1</sup> N at 63 DAS.

Two reproductive experiments, SIS were collected using the standard evaluation system of IRRI (1996). Data collected included plant height, Days-To-Flowering (DTF), panicle length, Shoot Dry Weight (SDW), Grain Yield (GY), panicle number/hill, number of seeds/panicle, filled and unfilled grain weight. Moreover, sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) contents were measured by Flame Photometer follow the procedure described by Yoshida (1976) and the measured proline content follows Bunnag and Pongthai (2013). The physical and chemical properties of the soil were summarized in supplementary Table 1.

### Data Analysis

Analysis of variance was performed and treatment means were compared by Least Significant Difference (LSD) at the 5% probability level using Crop Stat 7.2. Correlation between traits within the experiment of hydroponic and soil culture at the salinity of 12 dS m<sup>-1</sup> were characterized as well as correlation of same traits between those two screening methods. Under field and pot experiment 8 dS m<sup>-1</sup> and 0.4 % (w/w soil) were used with approximately 8 dS m<sup>-1</sup> (Elhag and Abdalla, 2014). On the other hand, the correlation between seedling stage (hydroponic and soil culture) and reproductive phase (pot and field experiments) stage were using 8 dS m<sup>-1</sup> (the highest salinity level could be achieved).

## RESULTS

Experiment 1: Screening at the Seedling Stage

The salt injury scores for all 6 varieties were recorded at 41 and 40 DAS under hydroponic and soil culture conditions, respectively. Under hydroponic conditions, the score showed a significant variation at 8 dS m<sup>-1</sup> of NaCl, which POKKALI showed



the highest tolerance to salinity with a score of 4.38: SRN1 was classified as moderate tolerant, while IR62266-RDG was susceptible. The salt injury score at 8 and 12 dS m<sup>-1</sup> showed identical responses for the POKKALI variety, indicating that the variety is moderate salt tolerance, while other varieties were classified as susceptible, especially IR29 which showed the highest susceptibility with a score of 9.00 (Table 1). Under the soil culture conditions, the injury scores showed significant variation at 8 and 12 dS m<sup>-1</sup>. Similar to hydroponic POKKALI showed a tolerance in both conditions. For other varieties, except for SRN1, they showed a stable response of being tolerant and moderately tolerant in both growing environments (Table 1). The results indicated that the injury scores were significantly influenced by genotype and salt conditions. The high concentration of salt in the hydroponic method was better in classification tolerance varieties than soil culture method.

Experiment 2: Screening at the Reproductive Stage

### Pot Experiment

The traits related to salt tolerance of all 6 varieties, including SPAD, Na<sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup>/K<sup>+</sup> ratio, SFW, SDW, plant height, DTF, tillers/hill, panicles/hill, panicle length, seeds/panicle and GY, were recorded at 0.1 to 0.4% (w/w) NaCl in the pot experiment. The responses to salinity showed a high level of trait variability being reflected in the distribution of the traits representing the different rice genotypes and salt concentrations (Supplementary Figure 2).

For all the rice varieties, the mean values of K<sup>+</sup>, SFW, SDW, plant height, panicle length, seed/panicle, and GY, tended to decrease from 0 to 0.1% to 0.2 and 0.4%, while the mean values for SPAD, Na<sup>+</sup>, Na<sup>+</sup>/K<sup>+</sup> and DTF tended to increase in response to increasing NaCl levels. Surprisingly, SPAD showed more stability than other traits in all salt concentrations.

**Table 1.** Mean comparison of salt injury score under hydroponic and soil culture with 8 and 12 dS m<sup>-1</sup> at 41 and 40 DAS, respectively, under greenhouse condition.<sup>a</sup>

| Varieties   | Hydroponic |                      |          |                       |          |         | Soil culture         |          |                       |          |         |                      |          |
|-------------|------------|----------------------|----------|-----------------------|----------|---------|----------------------|----------|-----------------------|----------|---------|----------------------|----------|
|             | 41 DAS     |                      |          | 40 DAS                |          |         | 41 DAS               |          |                       | 40 DAS   |         |                      |          |
|             | Control    | 8 dS m <sup>-1</sup> | Reaction | 12 dS m <sup>-1</sup> | Reaction | Control | 8 dS m <sup>-1</sup> | Reaction | 12 dS m <sup>-1</sup> | Reaction | Control | 8 dS m <sup>-1</sup> | Reaction |
| RD6         | 1.00       | 7.03 c               | S        | 8.10 bc               | S        | 1.00    | 5.00 ab              | T        | 5.50 a                | MT       | 1.00    | 5.00 ab              | T        |
| IR62266-KKU | 1.00       | 8.22 d               | S        | 8.70 c                | S        | 1.00    | 5.00 ab              | T        | 5.00 a                | T        | 1.00    | 5.00 ab              | T        |
| IR29        | 1.00       | 8.88 d               | S        | 9.00 c                | S        | 1.00    | 6.13 c               | MT       | 7.00 b                | MT       | 1.00    | 6.13 c               | MT       |
| POKKALI     | 1.00       | 4.38 a               | T        | 5.75 a                | MT       | 1.00    | 4.25 a               | T        | 5.00 a                | T        | 1.00    | 4.25 a               | T        |
| SRN1        | 1.00       | 6.69 c               | MT       | 8.14 bc               | S        | 1.00    | 5.13 b               | MT       | 7.13 b                | S        | 1.00    | 5.13 b               | MT       |
| IR62266-RGD | 1.00       | 5.52 b               | MT       | 7.22 b                | S        | 1.00    | 5.00 ab              | T        | 5.63 a                | MT       | 1.00    | 5.00 ab              | T        |
| CV (b) (%)  |            |                      |          | 13.76                 |          |         |                      |          | 14.08                 |          |         |                      |          |

<sup>a</sup> DAS: Day After Sowing; S= Susceptible; MT= Moderate Tolerance, and T= Tolerance. Means within columns followed by the same letters are not significantly different at 0.05 probability level using LSD test.

This means that rice would be able to stay green under salt stress. The mean values of tillers/hill and panicles/hill tended to decrease with increasing NaCl concentration in all varieties, except IR62266-RGD, which showed an increase in response to the higher salt conditions in the soil (Supplementary Figure 2). The results indicated that the response of IR62266-RGD to salinity was significantly different from other varieties.

Pearson correlations were calculated among the 15 traits (Supplementary Table 2), and there are 7 correlation pairs that reached significant levels under the pot experiment conditions. Late flowering genotypes had a higher shoot fresh weight and dry weight ( $r=0.935^{**}$  and  $r=0.941^{**}$ , respectively). High tiller number affected the high panicle numbers ( $r=0.925^{**}$ ). Genotypes with a higher concentration of  $K^+$  were associated with a shorter plant height ( $r=-0.913^{**}$ ) (Supplementary Table 2).

### Field Experiment

The traits that responded to salt levels in all rice varieties included plant height, panicles per hill, GY, DTF, proline,  $Na^+$ ,  $K^+$ ,  $Na^+/K^+$ , seeds/panicle, panicle length, filled grain weight, unfilled grain weight and SDW, were recorded in the field experiment. All the traits except proline, unfilled grain weight and SDW, showed a significant response among POKKALI and IR29, which are defined as tolerant and susceptible varieties, respectively (Table 2). The results indicated that proline, unfilled grain weight and SDW could not be used as selection criteria for salt tolerance screening under field condition. Pearson correlations among 15 traits were also calculated (Supplementary Table 3). There were 19 correlation pairs with significance in the field experiment, such as grain yield which were correlated with lower concentration of  $Na^+$  ( $r=-0.826^*$ ). A higher ratio of  $Na^+/K^+$  was reflected in a lower filled grain ( $r=-0.938^{**}$ ), especially for the late maturity varieties (Supplementary Table 3).

**Table 2.** Mean comparison of traits under field condition of 6 rice varieties in wet season 2013.

| Variety            | Plant height (cm) | Panicle /Hill | Panicle length (cm) | Seed /Panicle | FG <sup>a</sup> (g m <sup>-2</sup> ) | UFG <sup>b</sup> (g m <sup>-2</sup> ) | GY <sup>c</sup> (g m <sup>-2</sup> ) | SDW <sup>d</sup> (g m <sup>-2</sup> ) | DTF <sup>e</sup> (Days) | Proline | Na     | K      | Na/K   |
|--------------------|-------------------|---------------|---------------------|---------------|--------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|-------------------------|---------|--------|--------|--------|
| RD6                | 104.2 c           | 13.0 a        | 22.3 b              | 134.4 bc      | 112.1 b                              | 22.4 bc                               | 289.3 c                              | 583.2 ab                              | 98.3 bc                 | 139.9a  | 1.02 b | 2.04 b | 0.50 b |
| RD6 qB1, 2, 11, 12 | 106.6 c           | 11.9 a        | 22.2 b              | 134.7 bc      | 112.1 b                              | 22.6 c                                | 277.0 bc                             | 526.3 ab                              | 97.8 b                  | 197.6a  | 0.94 b | 2.10 b | 0.49 b |
| IR62266-KKU        | 117.7 d           | 10.6 a        | 27.0 d              | 142.9 c       | 111.8 b                              | 31.1 d                                | 214.7 bc                             | 637.6 b                               | 100.3 c                 | 122.2a  | 0.89 b | 2.03 b | 0.45 b |
| IR62266-RGD        | 73.1 b            | 22.8 b        | 22.7 b              | 95.7 a        | 62.5 a                               | 33.2 d                                | 163.3 ab                             | 649.8 b                               | 113.0 d                 | 152.0a  | 1.42 c | 1.23 a | 1.16 d |
| POKKALI            | 124.8 d           | 9.4 a         | 25.5 c              | 119.7 b       | 108.9 b                              | 10.8 a                                | 318.4 c                              | 542.8 a                               | 94.8 a                  | 124.9a  | 0.66 a | 2.97 c | 0.23 a |
| IR29               | 60.4 a            | 19.0 b        | 19.0 a              | 94.2 a        | 79.2 a                               | 15.0 a                                | 80.2 a                               | 461.2 a                               | 98.6 bc                 | 239.6a  | 1.33 c | 1.53 a | 0.87 c |
| CV (%)             | 5.22              | 19.08         | 2.58                | 9.01          | 13.23                                | 21.60                                 | 33.96                                | 16.71                                 | 1.26                    | 65.63   | 13.72  | 12.01  | 20.30  |

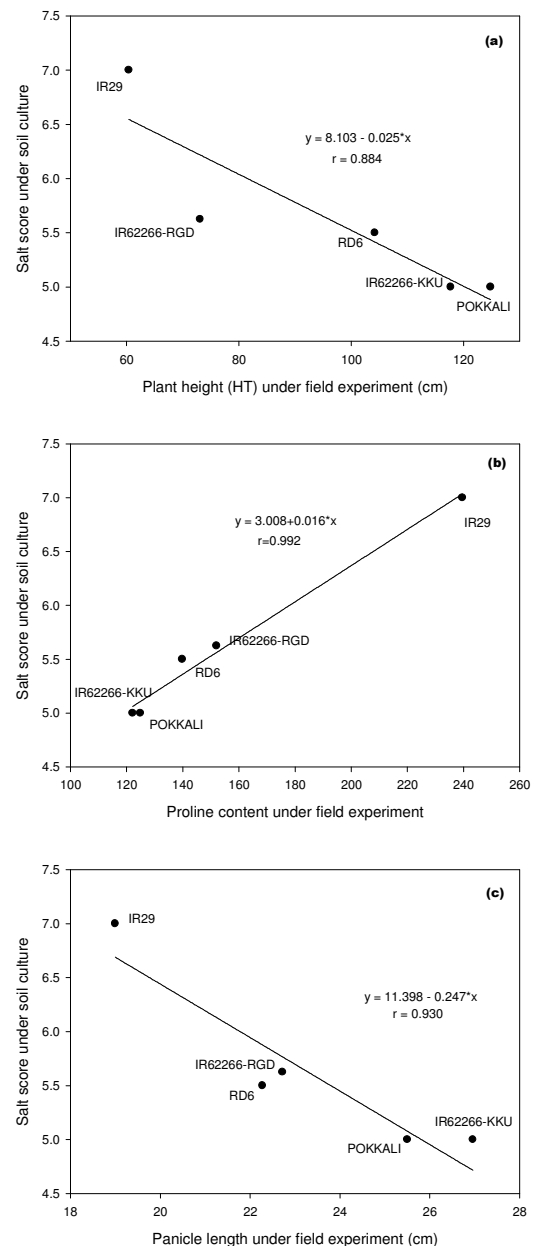
<sup>a</sup> Fill Grain; <sup>b</sup> Unfill Grain; <sup>c</sup> Grain Yield; <sup>d</sup> Shoot Dry Weight; and <sup>e</sup> Day To Flowering. Means within columns followed by the same letters are not significantly different at 0.05 probability level using LSD test.



### Relationship of Traits under Salt Condition Using Multiple Screening Methods

Pearson correlations between hydroponic and soil culture methods at  $12 \text{ dS m}^{-1}$  (Supplementary Figure 1) showed positive trends, although the correlations were not pronounced. The varieties with a high score under hydroponic culture conditions seemed to have a high score in the soil culture.

The correlations between the field and pot experimental methods are shown in Table 3. There were 16 correlation pairs with statistical significance under field condition.  $\text{K}^+$  in the pot experiment was negatively correlated with  $\text{K}^+$  ( $r = -0.968$ ), but was positively correlated with  $\text{Na}^+$ ,  $\text{Na}^+/\text{K}^+$  and panicles/hill ( $r = 0.989$ ,  $r = 0.968$  and  $r = 0.956$ , respectively). Other traits, including panicles/hill, panicle length and seeds/panicle in the pot experiment were correlated to DTF, panicle length, seeds/panicle and GY, in the field experiment (Table 3). These results indicate that the responses to salinity of the tested varieties were similar in the pot and field experiment methods. Based on the test times, the methods can be divided into 2 stages of rice growth, seedling stage and reproductive phase. Early growth stage tests comprised the hydroponic and soil culture methods, while late growth stage tests comprised the pot and field experiments. The relationships were defined among the two groups, based on the classification of test times. In the soil culture method, injury score was related to plant height, proline content and panicle length in the field experiment ( $R^2 = 0.783$ ,  $0.985$  and  $0.871$ , respectively). POKKALI and tolerant varieties with a low salt injury score were associated with high plant height and panicle length, but negatively correlated with proline content (Figure 1). The results indicated that the injury scores in seedling



**Figure 1.** The relationship between, (a) Plant height under field condition and salt score under soil culture experiment; (b) Proline content under field condition and salt score under soil experiment, and (c) Panicle length under field condition and salt score under soil culture experiment of 6 rice varieties.

stage were related to traits in the reproductive phase.

**Table 3.** Correlation coefficients calculated for traits between pot experiment (0.4% NaCl) and field condition in wet season 2013.

| Field | Pot experiment                  |                 |                |                                 |                  |              |                  |              |                |              |                 |
|-------|---------------------------------|-----------------|----------------|---------------------------------|------------------|--------------|------------------|--------------|----------------|--------------|-----------------|
|       | Traits                          | Na <sup>+</sup> | K <sup>+</sup> | Na <sup>+</sup> /K <sup>+</sup> | SDW <sup>a</sup> | Plant height | DTF <sup>b</sup> | Panicle/Hill | Panicle length | Seed/Panicle | GY <sup>c</sup> |
|       | Na <sup>+</sup>                 | 0.276           | 0.989**        | -0.302                          | -0.529           | -0.942**     | -0.546           | 0.610        | -0.601         | -0.730       | -0.791          |
|       | K <sup>+</sup>                  | -0.051          | -0.969**       | 0.504                           | 0.520            | 0.838        | 0.500            | -0.591       | 0.382          | 0.639        | 0.742           |
|       | Na <sup>+</sup> /K <sup>+</sup> | 0.383           | 0.968**        | -0.187                          | -0.553           | -0.867*      | -0.575           | 0.720        | -0.511         | -0.847       | -0.678          |
|       | SDW                             | -0.343          | 0.100          | -0.379                          | 0.287            | 0.299        | 0.353            | 0.580        | 0.740          | -0.053       | 0.069           |
|       | Plant height                    | -0.417          | -0.895*        | 0.116                           | 0.633            | 0.971**      | 0.672            | -0.400       | 0.796          | 0.719        | 0.725           |
|       | DTF                             | 0.249           | 0.782          | -0.206                          | -0.295           | -0.499       | -0.293           | 0.906*       | -0.033         | -0.766       | -0.432          |
|       | Panicle/Hill                    | 0.483           | 0.956**        | -0.086                          | -0.526           | -0.905*      | -0.568           | 0.711        | -0.619         | -0.859       | -0.702          |
|       | Panicle length                  | -0.361          | -0.677         | 0.047                           | 0.322            | 0.916*       | 0.387            | -0.163       | 0.947**        | 0.378        | 0.784           |
|       | Seed/Panicle                    | -0.844          | -0.649         | -0.431                          | 0.616            | 0.773        | 0.708            | -0.419       | 0.820          | 0.875*       | 0.395           |
|       | GY                              | -0.654          | -0.846         | -0.140                          | 0.634            | 0.800        | 0.688            | -0.670       | 0.592          | 0.963**      | 0.492           |

<sup>a</sup> Shoot Dry Weight; <sup>b</sup> Day To Flowering; and <sup>c</sup> Grain Yield; 100 GW= 100 Grain Weight; \*, \*\*, Are significant at P< 0.05 and P< 0.01, respectively.

## DISCUSSION

An appropriate screening method that is effective in early stages of growth would potentially provide a rapid method for primary screening of large quantities of plant material. Although various screening methods under greenhouse, as well as field conditions, have been proposed, these methods are very complex and even under controlled conditions, are expensive and time-consuming when screening large quantities of germplasm. So, effective alternative screening approaches must be proven to have correlation of results in the early phase of growth, in both pot and field conditions. The visual symptoms of salt stress may still be the most appropriate for mass screening.

In this study, 6 rice varieties were used for a comparison of screening methods in two stages of growth, the seedling and reproductive stages. The seedling stage assessment included hydroponic and soil cultures, while reproductive stage included pot and field experiments.

### Seedling Stage Test

Salt injury score due to salinity is reflected in leaf symptoms, such as leaf rolling and burning when rice is grown under saline conditions. Rolling of the leaf tends to minimize water loss by respiration affected by water deficit. Under severe salinity, leaves showed symptoms of burning (Amirjani, 2010). In this study, injury score was depicted as criteria for salt tolerance evaluation under hydroponic and soil cultures. This approach can be used for the classification of rice varieties (Table 1). The score for injury obtained from soil culture was higher (more severe) than those screened under hydroponic. Similar response was found in the Na<sup>+</sup> content. This indicates that the salt stress induced more severe oxidative damage in the hydroponic culture than the soil culture (Bayram *et al.*, 2014). When comparing these two methods,



hydroponic seems a more effective technique for salt tolerance screening in seedling stage.

### Reproductive Stage Test

At the reproductive stage, several morphological and physiological parameters, such as shoot fresh weight, shoot dry weight, plant height and N uptake, are well correlated with crop salt tolerance and thus can be used as indicators for salt tolerance (Ashraf *et al.*, 1999; Noreen and Ashraf, 2008). Under salt stress, reductions in yield and yield components are a common response in crop plants.

### Pot Experiment

In all of the rice varieties tested, salinity caused a significant reduction in  $K^+$ , Shoot Fresh Weight (SFW), Shoot Dry Weight (SDW), plant height, tillers/hill, panicles/hill, panicle length and seeds/panicle, when compared with control (Supplementary Figure 1). The reductions in sensitive genotypes under salt stress for tillers/plant, SFW, SDW, seedling growth and biomass, were also reported in numerous experiments (Hakim *et al.*, 2014; Ali *et al.*, 2004; Hussain *et al.*, 2013; Haq *et al.*, 2009).

Plants have two processes that are affected by salinity, water relations and ionic relations. Water stress will occur during initial exposure to salinity. This influences the reduction of leaf expansion and/or leaf rolling to minimize water loss. On the other hand, ionic stress will be experienced as a result of long-term exposure to salinity, and can lead to premature senescence of mature leaves (Amirjani, 2010). The water stress response under salinity is due to reduction of water potential related to osmotic adjustment (Munns *et al.*, 1995) with increasing the concentration of  $Na^+$  in their tissue. Therefore, some genotypes which accumulate high levels of  $Na^+$  may

experience toxic effects and cell damage (Amirjani, 2010). In the present study, the injury score at the seedling stage (Table 1) may reflect the accumulation of  $Na^+$ , which tends to increase with increasing salt stress (Supplementary Figure 1).

The number of tillers/hill is also an important yield parameter under saline conditions, as it is correlated with panicle grain yield (Supplementary Table 2). Salinity caused a significant reduction in the number of tillers/hill when compared to the control treatment (Supplementary Figure 1). On a relative basis, all varieties except RD6 (stable) and IR62266-RGD (increased) experienced a reduction in tiller number in response to salt stress. The greatest reductions in tillers/hill and panicles/hill were recorded in the susceptible variety, IR29. Interestingly, RD6 showed stability in tillers/hill and panicles/hill under the salt stress conditions. This probably reflected the long adaptation to the growing environment in Northeast Thailand, which often experiences salinity due to regular drought condition which accompany with soil salinity in late growing season. The development of more tillers may be a salt tolerance adaptation mechanism in plants, resulting in salt dilution in plants (Aslam *et al.*, 1989).

### Field Experiment

All traits except proline, Unfilled Grain Weight (UFG) and Shoot Dry Weight (SDW) showed significance among the tested varieties (Table 2). Proline has been used as a drought tolerance indicator in many plant species (Igarashi *et al.*, 1997). Tolerant varieties or species exhibited a high proline accumulation (Demiral and Türkan, 2005). In this study, proline was not an appropriate selection criterion for salt tolerance under field conditions. The field experiment was able to distinguish the tolerant and susceptible varieties in the same way as the use of seedling stage tests, especially the soil culture method. This



finding was confirmed by correlations of the field condition with the culture method (Figure 1). Correlations between seedling tests and field experiments for salt tolerance have also been reported in muskmelon (Franco *et al.*, 1997) and wheat (Chhipa and Lal, 1995; Salam *et al.*, 1999; Ahmad *et al.*, 2005; Turki *et al.*, 2014).

The correlations between the field and pot experiment are shown in Table 3. There were 16 paired traits which showed significant correlation in the field condition. All traits, except for SDW from the field condition, correlated with traits in the pot experiment. This result indicates that the responses to salinity of tested varieties were similar under the pot and field condition.

Under the field condition, data of the photoperiod sensitive variety, SRN1, was not included, as data for some traits related to flowering could affect yield of rice. However RD6 *qBl* 1, 2, 11, 12 (Suwannual, unpublished data), an improved line through QTL pyramiding for blast resistance, was used to replace SRN1 for this testing. The results showed that RD6 *qBl* 1, 2, 11, 12 responded to salt in the same way as RD6, the recurrent parent, in all parameters under field conditions (Table 2). This infers that the RD6 *qBl* 1, 2, 11, 12 has a similar genetic background to RD6. In addition, POKKALI showed greater salinity tolerance than other tested varieties. The contrast response between RD6 *qBl* 1, 2, 11, 12 and POKKALI to salt stress depicts availability of the segregating population constructed from RD6 *qBl* 1, 2, 11, 12 and POKKALI, for mapping of QTLs associated with salinity tolerance.

### CONCLUSIONS

Rice varieties are highly variable in performance under salinity stress. Among the salt tolerance traits, injury score in the soil culture method was correlated with plant height and panicle length in the field condition, suggesting the potential to be a high performance method for a large scale of

salt tolerance screening. In addition, the rice varieties POKKALI and RD6 *qBl* 1, 2, 11, 12 are promising variety lines for using as parental lines to breed for salt tolerance and blast resistance in terms of gene pyramiding (POKKALI) and recipient (RD6 *qBl* 1, 2, 11, 12) parents for the improvement providing resistance to the rice blast and the salt tolerant disease .

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## ارزیابی میزان تحمل نمک در برنج با استفاده از روش های چندگانه غربالگری

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### چکیده

روش های غربالگری موثر در مراحل اولیه رشد به طور بلقوه، بیشترین مقدار مواد پرورش را فراهم میکنند. اگرچه روش های غربالگری زیادی در گلخانه پیشنهاد شده اند اما روش هایی عملاً موثر خواهند بود که ارتباط خوبی با نتایج مزرعه داشته باشند. این مطالعه با هدف دستیابی به ارتباط بین ویژگی های بدست آمده از چهار روش غربالگری کشت هیدروپونیک، کشت خاکی، گلدان و مزرعه انجام شد. میزان آسیب های ناشی از نمک در کشت خاکی و هیدروپونیک در مرحله ی جوانه تحت شوری 12ds/m ارتباطی با هم نداشتند اما قادر به شناسایی رقم Pokkali به عنوان رقم مقاوم و رقم IR29 به عنوان رقم حساس بودند. ویژگی های مربوط به کشت گلدانی و مزرعه بطور قابل ملاحظه ای به رقم های برنج و مقادیر شوری مرتبط بودند. در مرحله ی جوانه مقادیر آسیب ناشی از نمک در کشت خاکی با ارتفاع گیاه، میزان پرولین برگ ها و طول خوشه در محیط مزرعه مرتبط بودند (به ترتیب  $r=0.992$ ،  $r=0.886$  و  $r=-0.933$ ). همچنین مشخصات آزمایشهای کشت گلدانی ارتباط قابل ملاحظه ای با نتایج مزرعه نشان دادند. مقادیر آسیب ناشی از نمک در روش کشت خاکی، روش ساده و موثری برای تعیین غیر مستقیم مقادیر تحمل شوری در برنج ایجاد کرد.