ScienceAsia 30 (2004): **411-415**

Relative leaf water content as an efficient method for evaluating rice cultivars for tolerance to salt stress

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Received 16 Apr 2004
Accepted 28 Sep 2004

ABSTRACT: Efficient and reliable screening criteria are a prerequisite for an effective breeding program to incorporate tolerance to abiotic stress in crop plants. In this study we presented preliminary results demonstrating the effective use of one physiological trait (relative water content, RWC) to incorporate salinity tolerance in rice. Sixteen rice genotypes were used in this case study and subjected to salinity stress of 6 dS m^{-1} for 2 weeks. RWC was determined on fully expanded youngest leaves, and shoot tissues were sampled for the measurements of sodium and potassium concentration and hence calculation of the Na^+/K^+ ratio. Rice plants with low salt injury scoring were found to have high RWC and maintain a low Na^+/K^+ ratio, and thus considered as a salt tolerant group. Cultivars with medium RWC also have medium salt injury scores and a moderate Na^+/K^+ ratio and considered as moderately tolerant, while the susceptible group has high salt injury scores and a high Na^+/K^+ ratio. The correlation between salt injury scoring and the Na^+/K^+ ratio was strongly positive, while the relationship between RWC and the Na^+/K^+ ratio was strongly negative. The data of RWC in the tolerant group was clearly distinguishable from the moderately tolerant group and the susceptible group. We therefore conclude that RWC determination could be used as an effective screening technique for salt tolerance in rice. The method is simple and cheap, and allows evaluation of a large number of breeding lines in relatively shorter period of time. The method is also more quantitative compared to visual scoring, and eliminates the need of equipments and expense needed when the Na^+/K^+ ratio is used as screening procedure.

KEYWORDS: salt tolerant rice , salt tolerance screening, Na^+/K^+ ratio, Relative Water Content.

INTRODUCTION

Salt stress is currently one of the major problems facing rice production worldwide. Improving salinity tolerance in rice could enhance productivity in salt affected areas, and help in further expansion of rice production in salt affected areas[1] that are currently not in use. Rice is sensitive to salt stress, however, few landraces were identified with reasonable tolerance. Sensitivity of rice varieties to salt stress varies with the developmental stage of the plants[2]. It is considered very salt-tolerant to during germination, but very sensitive during the early seedling stage and reproduction, and less sensitive during tillering and grain filling stages. Previous studies[3,4] have shown that selection for salt tolerance in rice could be achieved by visual scoring of seedlings grown under salt stress for sufficient time, Na^+ and K^+ uptake analysis and measurements of the Na^+/K^+ ratio. However, these screening procedures are time consuming and expensive, particularly when dealing with a large number of breeding lines. Additionally, replicated tests are often needed to reliably differentiate between tolerant and susceptible lines.

Water deficit associated with high salinity in irrigation water is the major limiting factor in hot dry areas as in the Mediterranean region, where plants are subjected to extreme water deficit during the dry season. Under these conditions, salt accumulated in the soil because of the high evaporative demands and the insufficient leaching of ions because of the scarcity of water resources. A better understanding of physiological responses of crop plants to such stressful conditions may help in developing efficient breeding programs to improve tolerance to drought and/or salt stress. Some physiological mechanisms has been identified that are associated with tolerance to abiotic stresses such as sensitive stomata that regulate water loss and salt uptake, upregulation of the antioxidants system, and active accumulation of compatible solutes such as amino acids, polyamines, and carbohydrates. The later is also associated with maintenance of plant water status and cell turgor necessary for continued growth and functioning under stress.

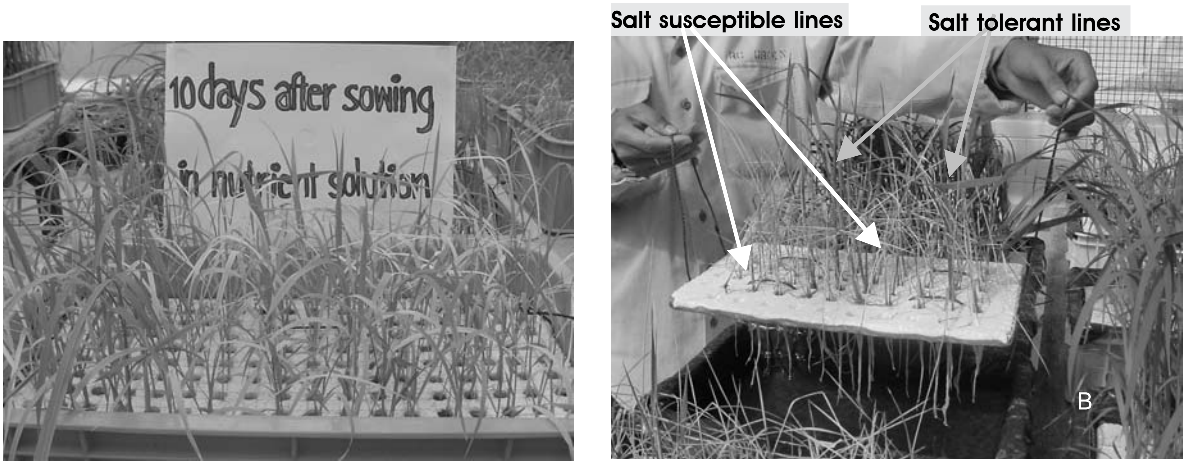
Maintenance of high relative water content (RWC) of rice plant under drought stress could also be associated with salt tolerance. RWC measures the water content of a leaf relative to the maximum amount that the leaf can take under full turgidity and hence is considered as an appropriate measure of plant water status under stress. While leaf water potential has been used as an estimate of plant water status when dealing with water transport in the soil-plant-atmosphere continuum, it does not account for osmotic adjustment (OA) that commonly occurs in plant roots and leaves in response to stress. OA is a powerful mechanism for conserving cellular hydration under drought stress and can be accounted for when measuring leaf RWC. Hence RWC is an appropriate estimate of plant water status because it accounts for both leaf water potential and OA. Plant responses to salt stress during short periods of stress, as experienced at early seedling stage, and responses to water stress have much in common[5] . This is because under saline condition, there are 2 phases of plants responses to salt stress, the first phase is a typical water stress response, due to the high osmotic potential of the soil solution caused by accumulation of soluble salts, and the second phase is the injury caused by ionic stress, mainly seen as ion toxicity and nutritional imbalances. The salt tolerant cultivars, could therefore, be selected based on their ability to maintain high relative water content during the initial phase of salt stress.

This study was conducted to determine the association between RWC and salinity tolerance in rice and to investigate the possibility of using RWC as screening criteria for breeders to incorporate tolerance to salt stress.

Materials and Methods

Test Materials

Sixteen genotypes of rice (*Oryza sativa* L.), namely IR66946-3R-58-1-1 (FL358), IR66946-3R-67-1-1 (FL367), IR66946-3R-111-1-1 (FL411), IR66946-3R-116-1-1 (FL416), IR66946-3R-134-1-1 (FL434), IR66946-3R-143-1-1 (FL443), IR66946-3R-178-1-1 (FL478), IR66946-3R-196-1-1 (FL496), IR66946-3R-223-1-1 (FL523), IR66946-3R-230-1-1 (FL530), IR66946-3R-263-1-1 (FL563) (salt tolerant lines identified by IRRI), Khao Mahk Khaek (KMK), Daeng Dawk Gok (DDG) (last two being Thai landrace cultivars), RD6, Khao Dawk Mali105 (KDML105) (last two being popular aromatic rice genotypes from Thailand), and Pokkali (a traditional salt tolerant genotype from Sri Lanka) were used in this study. Thirty seeds from each cultivar were surface sterilized with 10% clorox (5.25% (w/w) sodium hypochlorite) for 30 minutes, then rinsed with distilled water. Sterilized seeds were incubated in petri dishes at 38-42 °C for 5-7 days to allow germination. Eighteen germinated seeds of each cultivar were placed in small holes on styrofoam plates, one seed in each hole, with a nylon net support at the bottom (Fig. 1, A and B). The plates were floated on a nutrient solution (Yoshida *et al.*, 1976)[7]. After 14 days of growth, the seedlings were subjected to salinization (EC 6 dS m^{-1}) by adding NaCl to the nutrient solution. The nutrient solution was renewed once a week until the experiment was terminated after 16 days from the start of stress treatment (Fig. 1, B). The pH of the solution was adjusted daily at 5.8 by adding either 1N NaOH or 1 N HCl. The seedlings were grown in a



A B

Fig 1. Screening at seedling stage in Yoshida (1976) nutrient solution.
A) 10 days after sowing in nutrient solution.
B) rice palnt under severe stress (21 days after salinization). Susceptible lines were given scores of 7-9, moderately tolerant lines 5-6, and tolerant lines 1-4.

Table 1. Modified standard evaluation score (SES) of visual salt injury.[5]

Score	Observation	Tolerance level
1	Normal growth	Highly tolerant
3	Nearly normal growth; 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 plant dead or dying	Highly susceptible

screenhouse at Ubon Ratchathani Rice Research Center, Ubon Ratchathani, Thailand (day/night temperature = 38/25 °C).

The experimental design used was a 16 x 2 factorial in RCB with 3 replications. The treatments consisted of 16 rice cultivars and 2 salinity levels at 0 and 6 dS m^{-1} (60 mmol NaCl). Each experimental unit consisted of 18 plants. Visual scoring of the stress injury was conducted 16 days after salinization using the Standard Evaluation System of rice (SES, Table 1). Leaf and shoot samples were collected and used to determine RWC and Na^+ and K^+content, respectively. For RWC, the youngest fully expanded leaf was used. One-centimeter long leaf samples were collected from the region at about one-third from the leaf tip. Two sub-samples were weighed to determine fresh weight (FW), soaked in distilled water at 25 °C for 4 h and weighed again to record the turgid weight (TW), then oven dried at 80 °C for 24 h, to determine the dry weight (DW). The Relative Water Content (RWC) was calculated as follow.

$$RWC = \frac{(FW - DW)}{TW - DW} \times 100$$

Shoot samples for Na^+ and K^+ content analysis were oven dried for 3 days at 80 °C. Dried samples were finely ground, and 0.03 g powder from each sample was taken for Na^+ and K^+ analysis using atomic absorption spectrophotometer (PERKIN-ELMER, 1100B s)[8].

Results and Discussion

Screening at seedling stage[3] has been used as an effective method for selection for salt tolerance in rice, because this technique is rapid and effective to use with large number of segregating material commonly evaluated by breeders each year and is reproducible. The technique comprises the use of SES scoring to evaluate seedlings for salt injury within two weeks after imposition of salt stress. Na^+ and K^+ uptake and the Na^+/K^+ ratio have also been used frequently to evaluate material for the extent of tolerance to salt stress. However, these techniques are expensive and require either a good skill (SES) or chemical analysis for sodium

Table 2 Physiological traits contributing to salinity tolerance in 16 rice accessions grown under normal and salt stress conditions.

	At EC 6 dS m^{-1}					At normal condition(0.82 dS m^{-1})			
	Salt tolerant	Salt tolerant	Relative water content(%)*		Na^+/K^+	Salt tolerant	Relative water content(%)*		Na^+/K^+
Lines/cultivars	grouping	scoring*	Midday	Predawn	ratio*	scoring*	Midday	Predawn	ratio*
FL358	MT	5.7 b	85.80 bc	84.28 de	0.346 b	1 a	87.87 b	95.05 a	0.391 a
FL367	MT	5.0 bcd	86.98 abc	87.35 b-e	0.266 bc	1 a	90.39 ab	95.64 a	0.356 a
FL411	MT	5.7 b	87.94 abc	91.61 a-d	0.241 bc	1 a	87.67 b	95.91 a	0.318 a
FL416	MT	3.3 de	91.96 ab	94.11 ab	0.169 bc	1 a	95.13 ab	99.11 a	0.244 a
FL434	MT	5.3 bc	88.50 ab	86.73 b-e	0.382 b	1 a	91.92 ab	96.53 a	0.476 a
FL443	MT	5.3 bc	88.90 ab	85.04 cde	0.232 bc	1 a	92.46 ab	96.73 a	0.303 a
FL478	T	**3.0 e**	**94.16 a**	**97.93 a**	**0.183 bc**	1 a	93.69 ab	97.73 a	0.264 a
FL496	T	**3.0 e**	**94.28 a**	**96.68 a**	**0.158 c**	1 a	96.01 a	98.68 a	0.237 a
FL523	MT	4.7 b-e	91.06 ab	92.51 abc	0.200 bc	1 a	92.05 ab	96.09 a	0.294 a
FL530	T	**3.7 cde**	**93.91 a**	**96.39 a**	**0.126 c**	1 a	92.56 ab	97.84 a	0.271 a
FL563	MT	5.0 bcd	86.04 bc	90.75 a-d	0.318 b	1 a	93.27 ab	97.91 a	0.395 a
KMK	T	4.3 b-e	91.08 ab	90.34 a-d	0.209 bc	1 a	91.49 ab	97.78 a	0.481 a
DDG	T	4.3 b-e	92.00 ab	90.18 a-d	0.233 bc	1 a	94.84 ab	97.66 a	0.364 a
RD6	S	7.7 a	81.35 c	82.06 e	0.729 a	1 a	89.70 ab	97.71 a	0.402 a
KDML105	S	7.7 a	75.00 d	81.80 e	0.668 a	1 a	94.78 ab	96.84 a	0.508 a
Pokkali	T	3.0 e	93.27 a	94.12 ab	0.236 bc	1 a	92.21 ab	95.71 a	0.252 a
CV(%)		32.3	4.2	4.5	71.8	32.3	4.2	4.5	71.8

* The data were collected at 16 days after salinization
T = tolerance
MT = moderately tolerance
S = susceptible
Mean values in any column are not significantly different (p> 0.05), when followed by the same letters

and potassium contents. Hydroponics has also been used to screen seedlings of wheat cultivars for salinity tolerance. The technique involved the use of water-soluble carbohydrate as a marker for selecting salt-tolerant genotypes[9], which accumulated more soluble carbohydrate than the sensitive ones. The accumulation of solutes (such as fructans) has been documented as an adaptive mechanism to maintain osmotic pressure in under salinity stress. The principal role of osmotic adjustment is to maintain cellular water potential and to provide metabolites which act as a surfactant to protect sensitive molecules under stress. As solute concentration increases, water potential decreases and water moves spontaneously from regions of high water potential to regions of low water potential[10]. Therefore, plants with high solute concentration absorbed more water and will therefore, have high water content in their cells. This mechanism is called osmotic adjustment, which is normally observed in plants subjected to water deficit.

So far the relationship between RWC with salinity tolerance and salt uptake has not been established in rice. In this paper, we showed that this relationship does exist in rice (Table 2). We found that the 16 rice lines used in this study can be classified into 3 groups based on the extent of their tolerance to salt stress: 1) salt-tolerant group: Pokkali, FL416, FL478, FL496, FL530, KMK, and DDG, 2) moderately tolerant group: FL358, FL367, FL411, FL434, FL443, FL523, FL563, and 3) susceptible group: RD6 and KDML105. The data were collected when the plants were subjected to 6 dS m^{-1} of salt stress in the nutrient solution, and no significant difference was observed in any of the parameters studied when seedlings were grown in normal nutrient solution (Table 2). Furthermore, a strong positive correlation ($r = 0.84^{**}$) between the Na^+/K^+ ratio and salt tolerance scoring (Table 3 and Fig. 2, A) and a strong negative correlation ($r = -0.71^{**}$) between the Na^+/K^+ ratio and RWC (Table 3 and Fig. 2, B) were observed. Usually, when rice plants are subjected to stress conditions caused by salinity, the tolerant plants will markedly accumulate a number of solute particles in cells[8]. In general, plants that have high affinity K^+ uptake transporters will have low Na^+ uptake. In other words, the lower the Na^+ uptake, the higher the K^+ uptake when rice plants are under stresses[11,12]. This could probably explain the negative association between the Na^+/K^+ ratio with salinity tolerance. This indicated that under stress conditions, plants with low the Na^+/K^+ ratio and high RWC are tolerant to salinity stress. Based on these findings, RCW can effectively be used for screening for salt tolerance in rice. Sinclair and Ludlow (1985) also proposed that leaf relative water content was the better indicator of water status than was water potential[13].

The use of RWC as a screening procedure could have many advantages over the current evaluation techniques. The method is cheap, and eliminates the

Table 3. Correlation between midday RWC, predawn RWC and the Na^+-K^+ ratio in shoots at 16 days after salinization.

	Salt tolerant scoring	RWC_{midday}	$RWC_{predawn}$
RWC midday	-0.89877**		
RWC predawn	-0.87737**	0.82568**	
Shoot Na^+/K^+ ratio	0.88059**	-0.80337**	-0.89950**

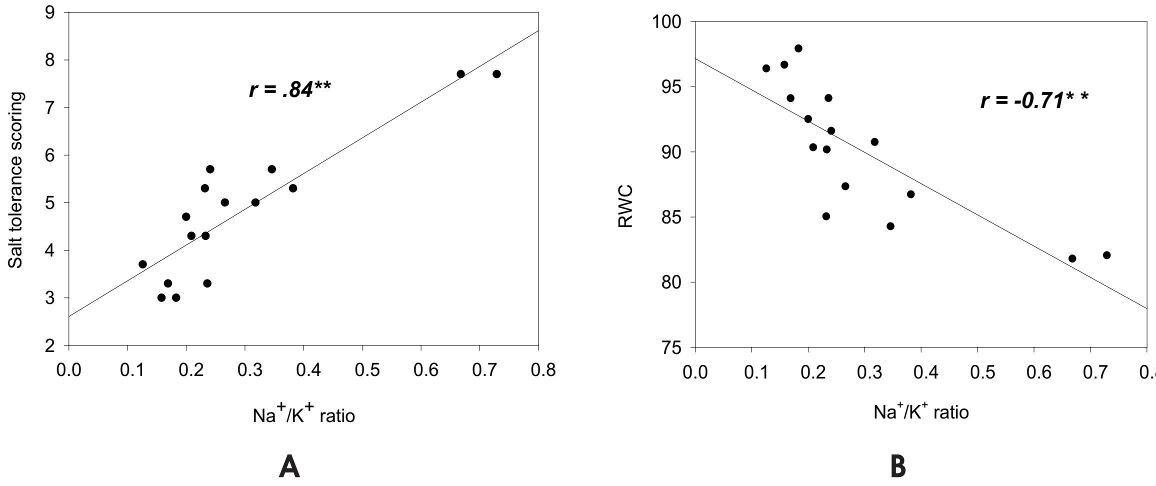


Fig 2. Correlation of parameters studied :
A) correlation between salt tolerant scoring versus the Na^+/K^+ ratio.
B) correlation between % relative water content versus the Na^+/K^+ ratio.

need for the expensive and time consuming mineral analysis commonly used for screening. Tolerant lines could also be selected in a few days after imposition of salt stress, eliminating the need for longer duration required until visual symptoms developed when SES scoring is used. SES scoring is also more subjective and qualitative, and requires a skillful person and sufficient replications to obtain reliable data. RWC values, on the other hand, are easy to use once threshold values are established and few plants are needed for sampling.

ACKNOWLEDGEMENT

We are grateful to the Rockefeller Foundation, for granting a scholarship to the senior author. We acknowledge the International Rice Research Institute, Pathum Thani Rice Research Center for providing the seeds for the experiment. Appreciation was due to Mr. Dome Harnpichitwitaya for helping with the statistical analysis and Dr. Abdelbagi Ismail for editing the manuscript.

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