Clustering analysis of salinity responses in indigenous rice by sorting algorithm

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ABSTRACT: The aims of this study were to screen thirty indigenous rice at the seedling stage grown under salinity. And the possibility of clustering analysis with our data pattern was studied by matrix operation. Sodium chloride at the concentration of 0 and 103 mM added into the nutrient solution was used for control and salt stress treatments, respectively. Physiological responses including shoot dry weight, root dry weight, survival rate and visual-symptom score were recorded. Results showed that the grouping based on plant responses under salt stress can be performed from the pattern of nine criteria of four parameters via the software MATLAB, and it is firstly evident. All examined lines were classified into 10 related subgroups, which were then detailed analyzed into three main groups including tolerant, moderately tolerant and susceptible regarding to tolerant responses. Eight of 30 lines of indigenous rice were placed to categories of tolerant group. Based on these data, accession no. KKU.ULR076 (named, How-klang), KKU. ULR198 (Brown sticky rice) as well as the tolerant-ecotype-Pokkali exhibited a similar ability in salinity responses. Additionally, 10 of 30 lines of indigenous rice were identified for susceptible group.

Keywords: salt stress, rice, clustering analysis, sorting algorithm

Introduction

Rice (*Oryza sativa* L.) is the major agriculture crops grown in South-East Asia. It provides a staple food and supports people all over the world. The demand of carbohydrates crop will gradually increase because of the increasing of the human population. It was proposed that Asian population in the year 2000 will increase from 3.7 billion to 4.8 billion by 2030 (Lem et al., 2014). For rice production, typical practice is to cultivate improved varieties to get better grains milling quality than traditional rice. The improved varieties are identified as moderated sensitive to water stress such as drought and salinity at the seedling and reproductive stages (Moradi and Ismail, 2007). They are planted in flooded paddy fields using more fertilizer whereas indigenous rice, especially upland rice is commonly grown in the mountainous regions with low-fertility production systems (Saito and Futakuchi, 2009). Saito et al. (2006) studied the responses of traditional and improved upland rice cultivars to major nutrients such as nitrogen and potassium fertilizer in the rice of northern Laos in order to investigate how fertilizers effect on grain yields at natural farm location. The authors concluded that upland rice can grow well under resource-poor conditions and respond well to applied N fertilizer. In Thailand, the yield evaluation of 252 upland rice lines was investigated for rice breeding purposes. It has been shown that upland rice produced a two

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times higher yield than the control lines (Srihanoo and Sanitchon, 2011) .

For many years, rice cultivation is exposed to abiotic stresses affecting growth and development from seedling, flowering throughout seed maturation (Cha-Um et al., 2009; He and Serraj, 2012). Drought stress affecting crop productivity is an important stress that can enhance the accumulation of salt ions such as Na^{+} and Cl^{-} on soil surface in particular areas. Moreover, some mineral deficiency may be more regulated during salt stress period (Gregorio et al., 2002). Understanding plant responses to stress and characterizing of indigenous rice may be contributed for breeding purposes. And, the conservation of indigenous rice might be responsible for rice genetic diversity. It has been showed that there was genetic variation in physio-morphological traits for drought resistance in rice. Thus, physiological-morphological characterization as well as physiological-genetics research of traditional varieties under multiple stresses is basically needed to provide the background knowledge of stress physiology and increase the efficiency of plant breeding (Fukai and Cooper, 1995).

The aims of this study were to screen thirty indigenous rice at the seedling stage grown under salinity. And the possibility of clustering analysis with our data series was studied according to (Li and Parkin, 1997). Li and Parkin (1997) method via the software MATLAB. It was reported that a simple and efficient sorting algorithm can be used to perform the principle function of group technology. Group technology is the method designed for grouping machines and parts to minimize interactions in factory layout. In the present work, application of sorting algorithm for plant grouping based on the responses to NaCl stress was firstly reported. Previous work by Ninsuwan et al. (2012) demonstrated that some traditional rice would grow under salt stress during seedling stage. It was hypothesized in this work that plant responses to NaCl stress can be indicating the tolerance level of the rice. The indigenous rice was received from the collection of Rice Germplasm Unit, Khon-Kaen University, Thailand. This unit has been collecting traditional rice grains specimens from regions of Thailand for specific reasons such as propagating, conserving and breeding purposes

Methodology

Salinity treatment

Germplasm with thirty indigenous rice lines was kindly provided from Dr. Jirawat Sanitchon, Khon Kaen University, Thailand, and the control rice lines were received from Rice Gene Discovery Unit, BIOTEC, Kasetsart University, Thailand (Table 1). Thirty varieties of indigenous rice and the control lines were grown in the Yoshida's Nutrient solution (Gregorio et al., 1997) for two weeks. For salt stress treatment, the rice was grown in solution containing 103 mM NaCl compared to control solution and maintained for a week. Shoot and root dry weight (DW), survival rate and visual-symptom score were recorded after exposed to salt stress

Sorting algorithm analysis

Physiological responses were listed for four parameters including salt-visual-symptom score 1 to 9, survival rate, shoot DW and root DW.

Clustering analysis was applied by matrix operation and sorting algorithm using the software MATLAB (Li and Parkin, 1997). The analysis started with the incidence A matrix that provides a yes or no answer to the question as described in the following steps.

1) Matrix (0 and 1) format (A matrix) was arranged according to the relationship between rice lines (row) and criteria (column). Individual rice was identified for only single specified criterion (Table 2); if it operated on criteria, then score was equal to 1, otherwise it was equal to zero.

2) Closeness matrix, called B matrix, was calculated from multiplying A matrix and A^t matrix.

3) The closeness values from B matrix were then rearranged in order to generate D matrix which exhibited the relationship between number of rice (row) and maximum value of closeness (column). Members of D matrix were counted from closeness number for each row. Further, D matrix was sorted by column.

4) Next, P matrix was generated from D matrix. The members of P was a_{ij}, a was equal to 1 when i was number of rice, j was the sorted order otherwise it was equal to zero.

Finally, matrix multiplication between P and A was calculated. Grouping matrix A_{sort} was finished.

Table 1 Accession number* with name /or origin of thirty indigenous rice (no. 1-30), and the control (no. 31-34)

Accession number	Name	Accession number	Name
1. ULR078	How-Dang	18. KKU.ULR200	a brown sticky rice
2. ULR152	unidentified from Phu-Ruea	19. KKU.ULR229	unidentified
3. ULR159	unidentified from Dan-Chan	20. KKU.ULR303	Phare-Rice
4. ULR192	unidentified from Keg-Noi	21. KKU.ULR-T4	Chaing-Mai
5. ULR198	a brown sticky rice	22. KKU.ULR-T33	Ban-Wang-Wah
6. ULR234	unidentified from Coke-Khun	23. KKU.ULR-T5	Nai-Niphun
7. ULR238	unidentified	24. KKU.ULR-T30	Jow-Mong 3
8. ULR239	a brown sticky rice	25. KKU.ULR-T15	Sil-kleng
9. ULR254	a purple rice	26. KKU.ULR-T18	Sil-Thong 1
10. ULR284	a brown sticky rice	27. KKU.ULR-T20	Mae-Win 1
11. KKU.ULR016	Khun-Wang K.	28. KKU.ULR-T35	Muan-Ran
12. KKU.ULR031	japanese sticky rice	29. KKU.ULR-T36	Leum-Na
13. KKU.ULR038	unidentified from Keg-Noi	30. KKU.ULR-T13	Mong purple-sticky-rice
14. KKU.ULR064	Kow-Jow-Mong	31. KDML105	
15. KKU.ULR076	How-Klang	32. IR28	
16. KKU.ULR121	unidentified	33. IR29	
17. KKU.ULR198	a brown sticky rice	34. POKKALI	

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Results and Discussion

Data of the responses including shoot dry weight, root dry weight, survival rate and visual-symptom score were recorded from all examined rice subjected to 0 and 103 mM NaCl for ten days. The salt-tolerant (Pokkali) and salt-sensitive (IR28 and IR29) were grown as the control. All data were then listed to parameters with theirs specified criteria. As shown in Table 2, criteria number 1, 4, 6 and 8 represent tolerant characteristics whereas 2, 3, 5, 7 and 9 show non-tolerant aspects. Matrix (0 and 1) format was identified for only single specified criterion, if it operated on criteria, then score was equal to 1, otherwise it was equal to zero. The A matrix with 9 rows of criteria and 34 columns of rice lines was constructed (Table 3). The result from table 3 was then calculated using sorting algorithm, and ten related subgroups were obtained as the output grouping (Figure 1).

Table 2 The specific criteria of the parameters for data classificatio

Parameters	Criteria*
A. visual-symptom score	1. score1-3 (normal or nearly normal growth and tillering)
	2. score 4-5 (growth retarded, most leaf rolled, a few leaves elongated)
	3. score 6-9 (almost all plants dead or drying)
B. survival rate	4. more than or equal to 75%
	5. less than 75%
C. shoot dry weight	6. no-difference between stress and control
	7. significant difference between stress and control
D. root dry weight	8. no-difference between stress and control
	9. significant difference between stress and control

*Each line was placed into only single criterion of each parameter.

Table 3 The incidence A matrix showing 9 rows of criteria and 34 columns of rice lines

	rice lines																																	
criteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1
2	1	1	1	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	0	1	1	0	1	0	0	0
3	0	0	0	0	0	1	0	0	1	0	1	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0
4	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1
5	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	0
6	1	0	0	1	0	0	0	1	1	1	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	1
7	0	1	1	0	1	1	1	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	1	0	0	1	1	0	1	1	0	1	0	0
8	0	0	1	1	0	1	0	1	1	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	0	1	0	1	1	0	1	1
9	1	1	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	1	0	0	1	0	0

To carry out the grouping, detailed analysis was performed. Tolerant rating of 1 to 4 was counted from criteria 1, 4, 6 and 8, and the main groups were reported. The results revealed that all examined lines were classified on the basis of the rating into 3 main groups including tolerant, moderately tolerant, and susceptible groups (Table 4). There were 4 lines, KKU.ULR076, KKU. ULR198, IR29 and Pokkali, in the tolerant groups with rating 4. The appearances of indigenous tolerant rice seedlings, KKU.ULR076 and KKU. ULR198, and the salt-tolerant Pokkali under control and salinity treatments were shown in Figure 2. It is consistent with the report that Pokkali is classified as highly salt tolerant ecotype; however, IR29 and KDML105 are known as moderate to sensitive lines (Moons et al., 1995). It seems that IR29 and KDML105 might develop the tolerance ability to 103 mM NaCl under this experiment condition.

This grouping methodology is an efficient sorting algorithm with simple steps for analyzing data, compared to the other algorithm techniques for example iteration calculation. Although, sorting algorithm was designed for grouping a great number of parts and machines in order to minimize interactions in factory layout (Li and Parkin, 1997; Ninsuwan and Hassamontr, 2014). In the present work, it was evident that sorting algorithm was possibly analyzed with nine criteria of four parameters. Specified criteria representing tolerant or non-tolerant characteristics were identified for the last steps. Consequently, the related subgroups as well as the main groups would be clearly observed for the output results with the specified criteria. However, sorting algorithm must be developed by person having experiences of the software MATLAB, especially matrix operation. Regarding to the pattern of 9 criteria from this study, it was also shown that some rice such as number 1 (ULR078), 3 (ULR159) and 9 (ULR254) could be classified to more than one group. The findings suggested that the grouping may depend on more parameters of respond data and preparation for a number of inputs is required for precisely output. However, this three rice would be possibly identified for moderately tolerant. The results demonstrated that the tolerant group revealed all of four tolerant characteristics such as salt tolerant score one to three, survival rate up to 75%, no difference of shoot and root dry weight between stress and control treatment, in contrast to characteristics of moderately tolerant and susceptible members.

It had been proposed that salt tolerant ability of rice was related to vegetative growth of both shoots and roots. Additionally, relative water content, accumulation of proline, polyamines and Na^+/K^+ ratio may play an important role in plant during salt stress treatment (Summart et al., 2010; Nounjan et al., 2012; Sripinyowanich et al., 2013). It was also confirmed that transgenic rice plants, salt and drought tolerance, had higher survival rate, fresh weight and accumulated higher soluble sugar content than what found in the control (Zhou et al., 2009) .

Conclusion

The clustering application by sorting algorithm was possibly analysed with this data pattern of nine criteria of four parameters. The examined rice lines were classified into three main groups. Based on these data, it was suggested that indigenous tolerant rice including KKU. ULR076, named How-Klang, and KKU.ULR198, a brown sticky rice, were identified as tolerant rice, and were similar to what found in the salt-tolerant Pokkali. There were about 10 rice number were identified for susceptible among 30 indigenous rice used in this study. In addition, it is firstly evident that the grouping based on plant responses under salt stress can be performed by matrix operation via the software MATLAB. A greater number of parameters may be required for the precisely classification. These findings on rice grouping *may* provide a basis for stress physiology research and breeding programmes.

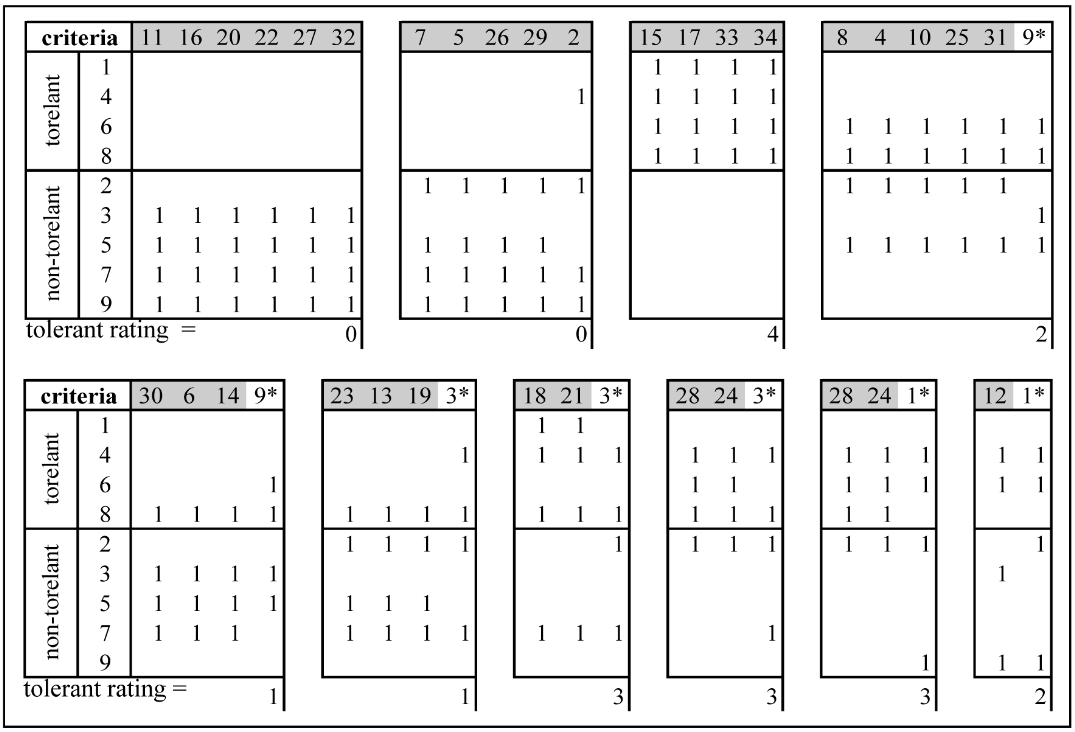

Figure 1 Ten related subgroups from sorting algorithm analysis

* Lines including no. 1, 3 and 9 could be classified to more than one group

Table 4 Three main groups of the examined lines according rating of the tolerant criteria

Tolerance	rating	Rice number*
1. Tolerant	4	15, 17, 33, 34
	3	18, 21, 28, 24, 3, 1
2. Moderately tolerant	2	8, 4, 10, 25, 31, 9, 12, 1
	1	30, 6, 14, 9, 23, 13, 19, 3
3. Susceptible	0	11, 16, 20, 22, 27, 32, 7, 5, 26, 29, 2

*31 to 34 were control.

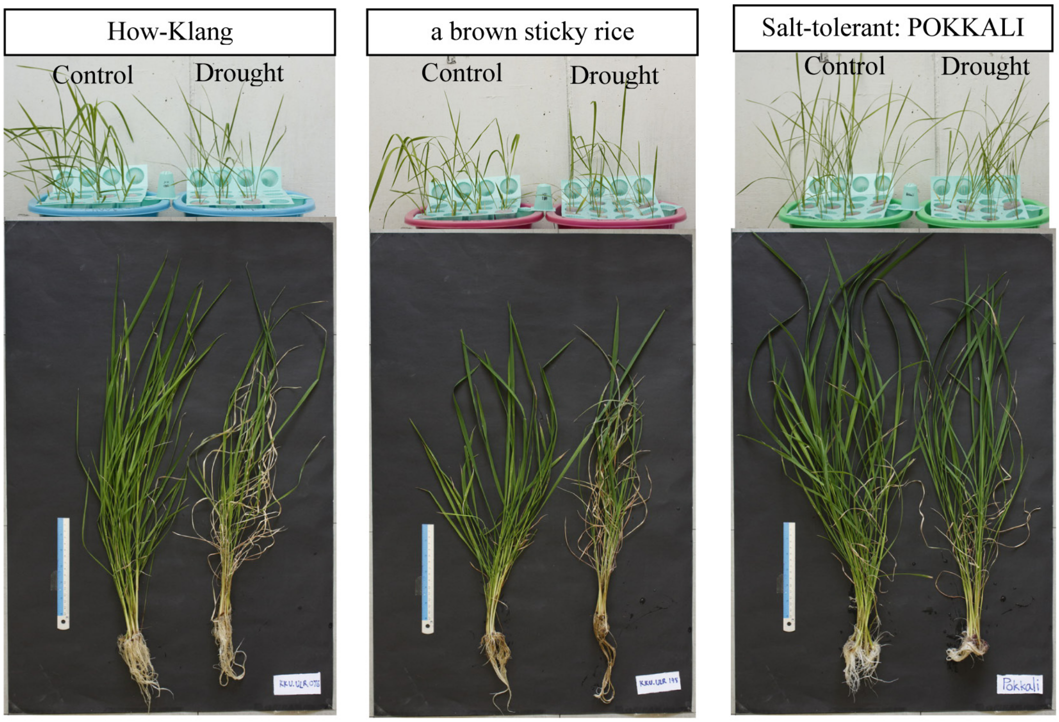

Figure 2 The characteristics of indigenous tolerant rice such as KKU.ULR076 named How-Klang) and KKU. ULR198 (brown sticky rice) and the salt-tolerant Pokkali under salt stress compared to control condition, scale bar = 15 cm

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