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RESEARCH ARTICLE

Chlorophyll index evaluation and selection of Malaysian salinity tolerant rice (*Oryza sativa* L.)

K. Rahiniza[1], M. M. A. Adam[2], A. R. M. S. Firdaus[3], M. S. Hamidah[4], H. Shahida[4], Z. N. Hidayah[4], S. Elixon[4], R. Asfaliza[5]

1.Rice Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia

2.Technology Commercialisation and Business Centre, MARDI Parit, 32800 Parit, Perak, Malaysia

3.Biotechnology & Nanotechnology Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia

4.Rice Research Centre, MARDI Seberang Perai, 13200 Kepala Batas, Pulau Pinang, Malaysia

5.Director General Office, MARDI Headquaters, 43400 Serdang, Selangor, Malaysia

Corresponding authors email Id: rahiniza@mardi.gov.my

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Abstract

A phenotypic evaluation of 89 potential salinity tolerant rice lines obtained from hybridization of Malaysian elite rice variety (MR 297) and salt tolerant varieties Pokkali and Salinas 2 (NSIC Rc184) was carried out according to chlorophyll index and plant morphological traits. ANOVA test showed a significant difference of chlorophyll index among check varieties when grown under salt-stressed treatment as compared to non-stressed treatment. Further evaluation on chlorophyll index among potential salinity tolerant rice lines found that around 56 lines have higher chlorophyll index compared to tolerant check variety Pokkali ranged from 42.60 to 50.83 SPAD value. Cluster analysis on thirteen selected lines discriminated the lines into two groups according to tolerant and sensitive check varieties. As conclusion, chlorophyll index is one of indicators to determine the inhibitory effect of salinity on photosynthetic efficiency and could be used in identifying potential salt tolerant lines along with other plant morphological traits.

Key words: Rice, chlorophyll index, selection, salt tolerant, morphological traits

Introduction

Changes in sea level and an increase in tidal surges have led to saltwater intrusion into low-lying coastal land as a consequence of climate change. The sea level is projected to rise by 0.5 metres by 2100, causing 20% of rice-growing area to be completely flooded (Ghazali and Awang, 2018). Peninsular Malaysia's northwestern coastline is among the regions that most severely impacted by sea-level rise (Ghazali and Awang, 2018). In 2016, it was reported that seawater had entered the rice fields near Kuala Kedah as a result of high tides (Embun, 2016). This phenomenon affected approximately 30 hectares of rice production areas and yield losses up to 75% (Sah *et al.*, 2021). The seawater inundation on the rice production area poses a risk where the salinity stress interferes with the growth of rice plants. Moreover, high salt level interferes with photosynthesis, cell shrinkage, tissue development and differentiation, nutritional imbalance, and cell membrane damage (Hakim *et al.*, 2014).

There are two major constraints when plant is exposed to high salt; osmotic stress, which compromises a plant's ability to take up water and ionic imbalance that result in excessive accumulation of Na+ and Cl- in intracellular compartments that are metabolically active (Zhao *et al.,* 2020). Salinity stress leads rice plants to experience osmotic shock and the subsequent induction of stomatal closure (Hameed *et al.*, 2021). This event is caused by oxidative stress due to overproduction of reactive oxygen species (ROS) as well as depletion of K+ due to excessive accumulation of Na+ in plant cells (Alscher *et al.,* 1997; Zuo *et al.,* 2021). Furthermore, the changes in structural and functional of chloroplasts reduced photosynthesis efficiency and result in declined photosynthetic rates, decreased biomass production, and lower grain yield (Yang *et al.,* 2020; Zhang *et al.,* 2020). Measurement of chlorophyll content using soil plant analysis development (SPAD) is widely adopted. It is an alternative method based on the reflectance and/or absorbance of radiation by the intact leaf which provides a non-destructive analysis for relative chlorophyll content with rapid and accuracy (Ling *et al.,* 2011). In addition, this equipment is using two light wavelength optical absorbance (650 and 940 nm) to generate relative 'Chlorophyll Index' value but not measuring the absolute chlorophyll content per mass of leaf tissue or per unit leaf area (Cassol *et al.,* 2008; Ling *et al.,* 2011).

There are several indicators to select salinity tolerant rice using morphological and physiological parameters (Krishnamurthy et al., 2016). Salt affected rice plants show symptoms such as white tips of the affected leaves, seedling's growth retardation, low tiller numbers and even plant death in serious cases (Coca et al., 2023). Method for evaluating morphological parameters involves applying salt to rice at various growth stages and observing the damage symptoms such as leaf chlorosis, necrosis and spikelet sterility (Abdullah *et al.,* 2001; Hannachi *et al.,* 2022). Furthermore, physiological parameters examine the metabolite changes and physiological mechanisms involved in plants' ability to deal with excessive salts (Qin et al., 2020). Breeding for local salt tolerance rice varieties is essential not only for improving yield production in saline soil areas, but also for good adaptability and meeting consumer preferences in terms of grain quality. Hence, this study was carried out to evaluate the effect of salt stress on chlorophyll content in breeding lines obtained from hybridization of local elite rice variety (MR 297) and salt tolerant varieties (Pokkali and Salinas 2 (NSIC Rc184)) and selection of tolerant rice lines.

Materials and methods

Early generation of breeding lines consists of 89 progenies (F_3 and BC_1F_3) were obtained from hybridization of local elite rice variety MR 297 with two salt tolerant varieties, Pokkali and NSIC Rc184 (Salinas 2) (Table 1). The breeding lines were evaluated under salt stress condition (electrical conductivity of 6 dS/m) along with sensitive check (IR29) and its parental varieties according to Singh (2006) with slight modifications. The salt stress was given at 21 days after sowing and prolonged until harvest during Main Season 2021/2022 (October 2021 - March 2022) in plant house, MARDI Seberang Perai Research Station, Penang, Malaysia (5.5407°N, 100.4695°E). In order to study the effect of salt stress on rice plant, selected check and parental varieties were grown under non-stressed condition (T1) and salt-stressed condition (T2) using randomized complete block design (RCBD) in three replications. Standard agronomic practices with total fertilizer of 104.30 N:41.70 P_2O5:61.50 K_2O (Kg/ha) were applied.

Table 1: List of genotypes tested

No.	Line/Variety	Types	Crossing	Generation	No. of lines
1	5745	Breeding line	Pokkali x MR 297	F_3	4
2	5746	Breeding line	Pokkali x MR 297	F_3	4
3	5747	Breeding line	Pokkali x MR 297	F_3	4
4	5755	Breeding line	Pokkali x MR 297	F_3	4
5	5757	Breeding line	Pokkali x MR 297	F_3	4
6	SL002	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	1
7	SL006	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	4
8	SL007	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	4
9	SL008	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	3
10	SL010	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	4
11	SL011	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	1
12	SL017	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	2
13	SL021	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	4
14	SL023	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	4
15	SL024	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	1
16	SL026	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	2
17	SL028	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	1
18	SL029	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	2
19	SL030	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	1
20	SL031	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	1
21	SL034	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	3
22	SL036	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	4
23	SL037	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	3
24	SL040	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	F_3	2
25	SL090	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	4
26	SL095	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	4
27	SL100	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	2
28	SL101	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	2
29	SL102	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	3
30	SL104	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	4
31	SL105	Breeding line	MR 297 x NSIC Rc184 (Salinas 2)	BC_1F_3	3
				Total	89
32	IR29 (IRRI)	Sensitive check			
33	Pokkali (India)	Tolerant check (donor parent)			
34	Salinas2 (NSIC Rc184) (Philippines)	Tolerant check (donor parent)			
35	MR 297	Local check variety (recurrent parent)			

Data collection and statictical analysis

Chlorophyll index was measured using Soil Plant Analysis Development (SPAD) (SPAD 502 Plus, Konica-Minolta, Japan) during late vegetative stage. Plant morphological traits such as plant height and panicle length were observed during maturation stage (IRRI, 2013). In addition, survival ratio was calculated according to the number of survival plants until harvest over the treated plants in each line (Singh, 2006). Data of chlorophyll index for the check and parental varieties were subjected to analysis of variance (ANOVA) with the genotypes and treatments as factors which performed using RStudio (version 4.2.1). This analysis was carried out to determine the effect of salinity stress on the chlorophyll index. Further data visualization of chlorophyll index distribution on check and parental varieties was performed using boxplot analysis. Moreover, least significant difference (LSD) test of chlorophyll index was performed on all breeding lines along with the check and parental varieties for mean separation. Additionally, the chlorophyll index value combined with plant morphological traits data of thirteen selected tolerant lines that survived until maturation stage were subjected to cluster analysis to identify the potential tolerant lines for selection purpose and will be evaluated for the next generation.

Results and discussion

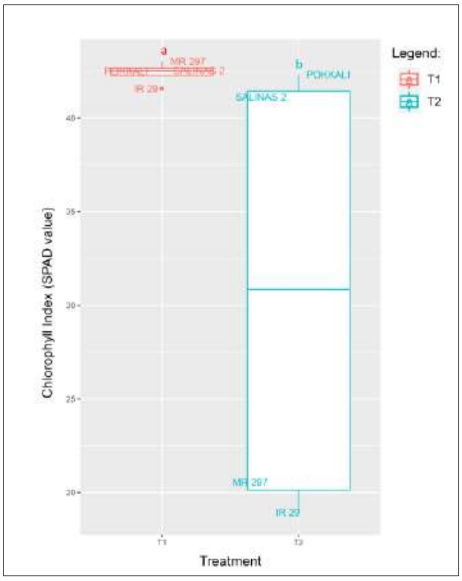
Effect of salt stress on chlorophyll index in check and parental varieties is presented. Analysis of variance showed a significant difference of chlorophyll index among check and parental varieties grown under non-stressed and salt-stressed (6 ds/m) treatments (Table 2). Chlorophyll in plant cells normally degraded when grown under salt stress (Nounjan *et al.,* 2020). According to Islam *et al.,* (2019) and Hameed (2021), salt stress causes structural changes of chloroplast from oval to slightly round in affected rice plant. Besides that, salt stress also causes various chlorophyll content decline, destruction of photosynthetic membrane system (Gill and Tuteja, 2010; Biswas *et al.,* 2021). Generally, the classification of soil salinity based on the electrical conductivity (EC) started with non-saline category (<1 dS/m), followed by very slightly saline (1-2 dS/m), slightly saline (4–8 dS/m), moderately saline (8–16 dS/m), and strongly saline (>16 dS/m) (Schoeneberger *et al.,* 2012). However, rice's EC threshold value only around 3 ds/m and it is considered as very sensitive cereal crop compared to other cereal crops such as wheat and barley with EC threshold value of 5.9 ds/m and 8.0 ds/m, respectively (Munns and Tester, 2008; Grieve *et al.,* 2012). Boxplot and LSD test results showed the significant impact of salt stress on chlorophyll index on tested varieties (Fig. 1). In non-stressed condition, MR 297 showed the highest chlorophyll content with 43.02 SPAD value, followed by Salinas 2 (42.53), Pokkali (42.50) and IR29 (41.57) (Table 3). The chlorophyll index on tested varieties exhibits a significant decreasing trend when the salt stress was imposed except for tolerant check varieties Pokkali and Salinas 2 with SPAD value of 42.31 and 41.14, respectively. Salt-sensitive and salt-tolerant rice plants have different abilities to cope with excessive salt present in plant cells (Munns and Tester, 2008; Zhao *et al.,* 2020). A study conducted by Bissah *et al.,* (2020) used FL478 as salt tolerant check but found that the variety showed a moderately susceptible to salt stress. Other study conducted by Li *et al.,* (2017) reported salt tolerant rice Dongdao-4 exhibits higher leaf chlorophyll content as well as higher photosynthesis rate compared to salt sensitive rice Jigeng-88 under salt stress treatment. According to Kibria *et al.,* (2017), increase in salt stress at 40 and 60 mM NaCl reduced both chlorophyll a and chlorophyll b content. Additionally, chlorophyll content was significantly reduced due to the denaturing of the native structure of membranes or increasing their permeability regardless of the varieties when subjected to salt stress at different durations (Ghosh *et al.,* 2011).

Table 2: ANOVA table of check and parental varieties under normal condition and salt stressed at 6 ds/m

Source	df	Sum Sq	Mean Sq	F value	Pr (>F)
Variety	3	747.32	249.11***	20.3681	2.25e-05***
Treatment	1	816.76	816.76***	66.7828	1.07e-06***
Replication	2	50.60	25.30^{ns}	2.0685	0.16
Variety x Treatment	3	711.05	237.02***	19.3796	2.97e-05***
Residuals	14	171.22	12.23		

*Significance. codes: 0 '***' , 0.001 '**' , 0.01 '*' , 0.05 '^', not significant '^{ns}'.

Fig 1: Boxplot of chlorphyll index distribution of check and parental varieties grown under non-stressed treatment (T1) and salt-stressed treatment (T2). Bars with different alphabets are significantly different from each other (LSD; p < 0.05)



Effect of salt stress on chlorophyll index in breeding rice lines

The Chlorophyll index among breeding lines grown under salt-stressed treatment showed a significant variation and ranked together with check and parental varieties (Table 3). Results showed 56 lines exhibit higher chlorophyll index compared to salt-stressed tolerant check variety Pokkali ranged from 42.60 to 50.83 SPAD value. Line SL095_1 showed the highest chlorophyll index with 50.83 SPAD value (Fig. 2), followed by SL100_1, SL030_1, SL017_4, SL031_1, 5745_2, and 5746_4 with SPAD value of 48.37, 48.23, 48.03, 47.73, 47.67 and 47.53, respectively. In contrast, line SL036_1 showed the lowest chlorophyll index with 1.50 SPAD value, followed by SL021_4, SL040_1, SL023_3 and SL040_2 with SPAD value of 1.6, 2.77, 3.17 and 15, respectively. These low chlorophyll content lines have no significant difference with sensitive check variety and may indicate the salt-sensitive trait. According to Kaur *et al.*, (2016), the study of the chlorophyll content in sensitive and tolerant varieties can gain physiological explanation on how plants adapt to salinity stress. Reduction of chlorophyll content was more exhibited in sensitive genotype as compared to tolerant genotype (Kibria *et al.*, 2017; Riaz *et al.*, 2019).

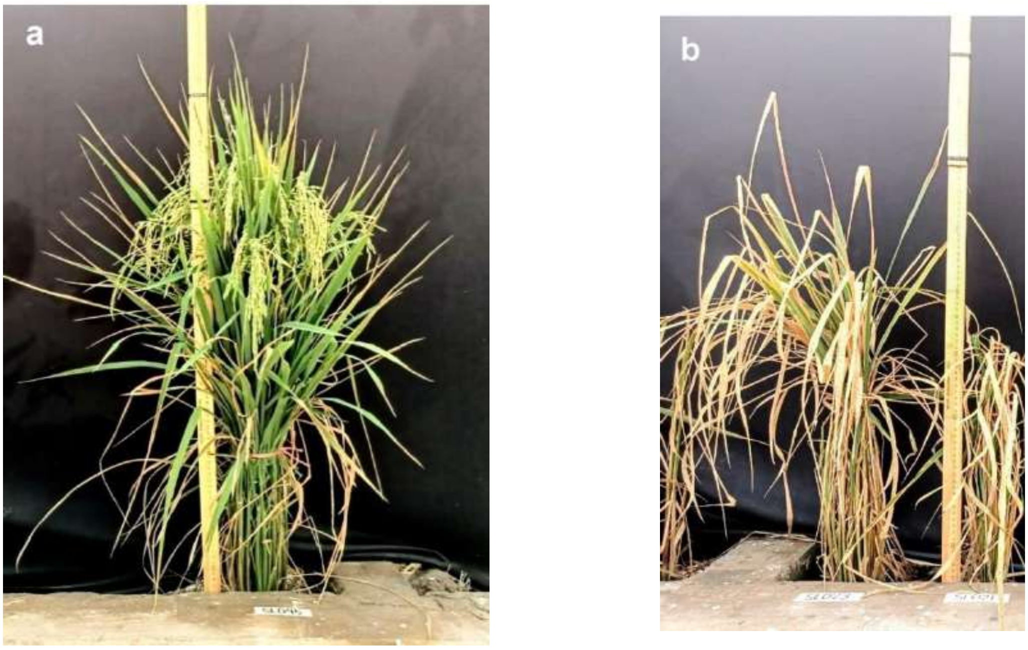
Another study on salt-sensitive rice carrying glutathione peroxidase gene (GPX3) showed significant damage of photosystem II activity and deterioration of chlorophyll content when grown under salt-stressed (Paiva *et al.*, 2019). In addition, high amount of Na+ and Cl- presence in plant cell damage the chlorophyll by impeding the photosystem II main electron transport (PSII) (Hussain et al. 2018). Hence, chlorophyll content is considered as one of the most important trait during breeding selection despite primary traits such as yield components.

Table 3: Chlorophyll index of potential breeding lines evaluated under salt-stressed (T2) and compared with check varieties that were grown under non-stressed condition (T1) and salt-stressed condition (T2)

Line/Variety	SPAD Value	Line/Variety	SPAD Value	Line/Variety	SPAD Value
SL095_1	50.83^{a}	5747_3	44.67^{abc}	SL008_1	41.17^{abc}
SL100_1	48.37^{ab}	5745_3	44.57^{abc}	Salinas 2_T2	41.14^{abcd}
SL030_1	48.23^{ab}	5745_4	44.57^{abc}	5746_2	41.07^{abcd}
SL017_4	48.03^{ab}	5745_1	44.40^{abc}	SL102_1	40.63^{abcd}
SL031_1	47.73^{ab}	SL006_1	44.40^{abc}	SL026_2	40.30^{abcd}
5745_2	47.67^{ab}	5746_3	44.27^{abc}	SL090_3	40.30^{abcd}
5746_4	47.53^{ab}	5757_2	44.27^{abc}	SL010_2	40.23^{abcd}
5757_3	47.20^{abc}	SL101_1	44.03^{abc}	SL036_4	39.60^{abcde}
SL095_3	46.90^{abc}	SL104_2	44.00^{abc}	SL034_1	39.17^{abcde}
SL095_2	46.70^{abc}	5747_2	43.97^{abc}	SL010_4	38.07^{abcde}
SL037_2	46.37^{abc}	SL002_1	43.87^{abc}	SL029_1	38.03^{abcde}
5747_1	46.10^{abc}	SL104_1	43.80^{abc}	SL023_1	36.27^{abcde}
SL010_3	45.83^{abc}	SL006_4	43.77^{abc}	SL037_1	35.37^{abcdef}
5755_4	45.83^{abc}	SL090_2	43.70^{abc}	SL036_3	34.70^{abcdef}
SL100_2	45.80^{abc}	SL007_2	43.70^{abc}	SL036_2	34.00^{abcdef}
SL008_2	45.70^{abc}	SL007_1	43.60^{abc}	SL023_4	33.90^{abcdef}
5746_1	45.60^{abc}	5755_2	43.33^{abc}	SL024_1	32.67^{abcdef}
SL095_4	45.60^{abc}	SL010_1	43.30^{abc}	SL017_2	32.37^{abcdef}
SL101_2	45.60^{abc}	5755_1	43.23^{abc}	SL090_4	32.33^{abcdef}
SL006_2	45.37^{abc}	SL008_4	43.03^{abc}	SL021_2	32.07^{abcdef}
SL104_4	45.33^{abc}	MR 297_T1	43.02^{abc}	SL021_1	29.77^{bcdef}
5747_4	45.23^{abc}	SL034_2	42.80^{abc}	SL037_3	29.37^{bcdef}
SL104_3	45.20^{abc}	5755_3	42.70^{abc}	SL011_2	28.83^{bcdef}
SL105_4	45.17^{abc}	5757_1	42.60^{abc}	SL021_3	26.23^{cdef}
SL102_2	45.17^{abc}	Salinas 2_T1	42.53^{abc}	MR 297_T2	20.54^{defg}
5757_4	45.10^{abc}	Pokkali_T1	42.50^{abc}	IR29_T2	18.95^{efg}
SL026_1	45.10^{abc}	Pokkali_T2	42.31^{abc}	SL040_2	15.00^{fg}
SL102_3	45.03^{abc}	SL007_4	42.20^{abc}	SL023_3	3.17^{g}
SL105_3	45.03^{abc}	SL034_3	41.93^{abc}	SL040_1	2.77^{g}
SL023_2	44.93^{abc}	SL029_2	41.77^{abc}	SL021_4	1.60^{g}
SL105_1	44.87^{abc}	SL028_1	41.73^{abc}	SL036_1	1.50^{g}
SL007_3	44.77^{abc}	SL090_1	41.63^{abc}	Mean	40.01
SL006_3	44.73^{abc}	IR29_T1	41.57^{abc}	CV	14.25

Means with the different letters indicate significant differences at $p \leq 0.05$ level according to LSD. T1=non-stressed treatment, T2= stressed treatment (6 ds/m)

Fig 2: Variation of leaves chlorophyll content among salt-tolerant and salt sensitive lines grown under 6 ds/m salt concentration. a) tolerant line SL095_1 b) sensitive lines SL21_1 and SL23_1

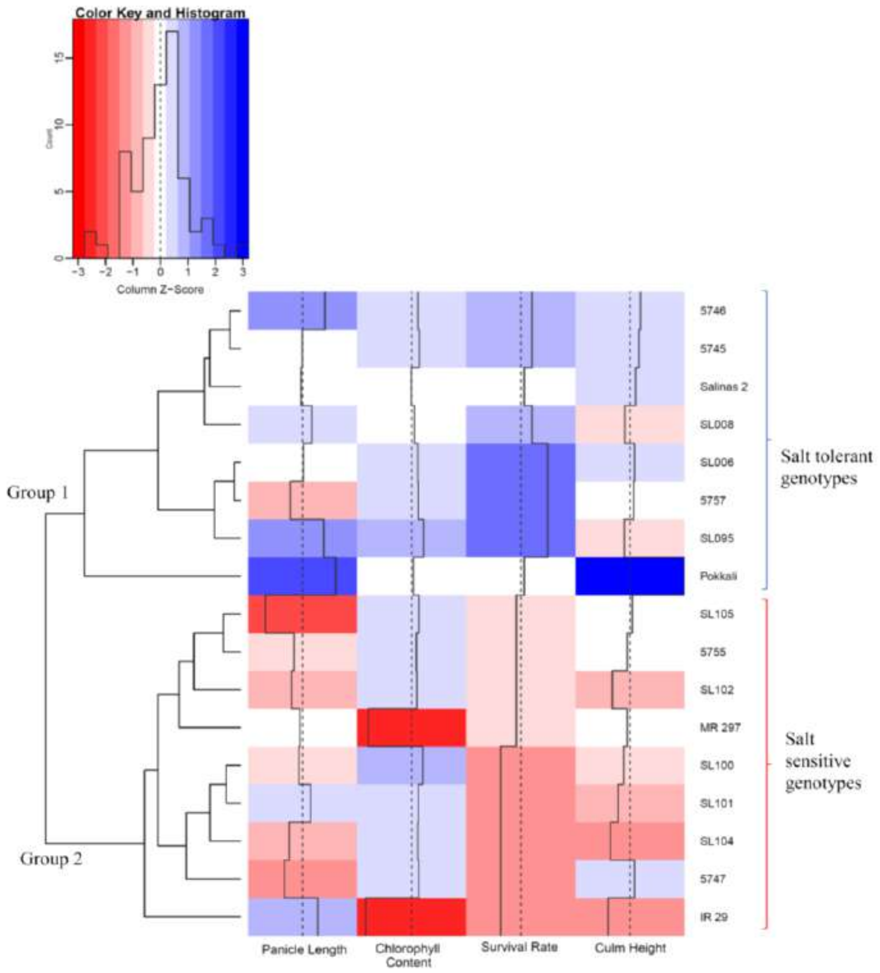


Identified potential tolerant rice lines

Thirteen potential tolerant lines were selected and cluster analysis was then performed based on four traits (panicle length, culm height, survival rate and chlorophyll content) (Fig. 3). The result showed all genotypes tested were clustered into two groups according to check varieties where Group 1 is considered as salt tolerant genotypes while Group 2 is considered as salt sensitive genotypes. Group 1 consists of lines 5746, 5745, SL008, SL006, 5757 and SL095 were categorized in the same group with tolerant varieties Pokkali and Salinas 2. Moreover, Group 2 consist of lines SL105, 5755, SL102, SL100, SL101, SL104 and 5747 were categorized in the same group with sensitive variety IR29 and local check variety MR297. Among the four traits studied, survival rate become the most important parameter to determine the grouping in the cluster analysis. According to Munns (2002), plants treated with high concentration of Na+ and Cl- causes oldest leaf to show symptoms of injury and eventually lead to complete death in some cases if the salinity level is high enough. This event is one of plant's strategy to save young growing meristematic tissues by transporting toxic ions to the older leaves and leaf sheaths since mature leaves have a larger vacuole compared to young leaf cells (Singh, 2006; Reddy *et al.*, 2017).

Various mechanisms are involved in salt-tolerant plant response toward salt stress such as salts transfer into vacuoles which allow accumulation of salt within cytoplasm at fast rate without compromising important metabolic functions (Munns, 1993; Rahman *et al.*, 2000; Munns and Tester, 2008). However, the affected older leaves may enhance premature senescence, and low photosynthesis rate may inhibit the plant from producing flowers and seeds (Munns, 2002). In addition, salt tolerant varieties maintain significantly lower salt concentration in the panicle and flag leaves, hence selection of healthier flag leaf at flowering could be used to screen for salt tolerance at the reproductive stage (Singh, 2006; Hasanuzzaman *et al.*, 2013).

Fig 3: Cluster analysis of 13 potential lines along with the check varieties based on four traits (panicle length, chloropyll content survival rate and culm height



In conclusion, current study investigated the effect of salt stress on the chlorophyll index of selected Malaysian rice varieties and breeding lines. The results demonstrated that salt stress caused a significant decrease in chlorophyll content of sensitive varieties such as MR 297 and IR29, while tolerant varieties such as Pokkali and Salinas 2 maintained relatively higher chlorophyll content. This study also highlighted the development of salt-tolerant rice varieties where several potential lines such as SL095_1, SL100_1, SL030_1, SL017_4, SL031_1, 5745_2, and 5746_4 showed higher chlorophyll index than the tolerant check variety, indicating their potential for salt tolerance. Further cluster analysis based on multiple traits, including chlorophyll content, panicle length, culm height, and survival rate, allowed for the identification of salt tolerant lines such as SL095, 5746, 5745, SL008, SL006 and 5757. These identified potential tolerant lines will be further evaluated in next generation for confirmation of the results. The successful development of local salt tolerant variety will not only contribute to maintaining stable rice production in salt-affected areas but also enhance the resilience of rice crops to changing climatic conditions, particularly in regions experiencing soil salinization.

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References

1. Abdullah, Z. K. M. A., Khan, M. A. and Flowers, T. J. 2001. Causes of sterility in seed set of rice under salinity stress. Journal of Agronomy and Crop Science, 187(1): 25-32.
2. Alscher, R. G., Donahue, J. L. and Cramer, C. L. 1997. Reactive oxygen species and antioxidants: relationships in green cells. Physiol. Plant., 100(2): 224-233.
3. Bissah, M. N., Bimpong, I. K. and Amoah, N. A. K. 2020. Comparative analysis of rice genotypes for tolerance to salt stress at the seedling stage. J. Genet. Genom. Plant Breed., 4(3):147-156.
4. Biswas, S., Chatterjee, P., Biswas, S., Mazumdar, A. and Biswas, A.K. 2021. Salt induced inhibition in photosynthetic parameters and polyamine accumulation in two legume cultivars and its amelioration by pretreatment of seeds with NaCl. Legum. Res., 44(3): 295-301.
5. Cassol, D., De Silva, F. S. P., Falqueto, A. R. and Bacarin, M. A. 2008. An evaluation of non-destructive methods to estimate total chlorophyll content. Photosynthetica, 46: 634-636.
6. Coca, L. I. R., González, M. T. G., Unday, Z. G., Hernández, J. J., Jáuregui, M. M. R. and Cancio, Y. F. 2023. Effects of sodium salinity on rice (*Oryza sativa* L.) cultivation: A review. Sustainability, 15(3): 1804.
7. Embun M. 2016. MADA hopes for RM25 million allocation under 2017 budget. New Straits Times, Malaysia General Busi. New Straits Times Press(M) Bhd.
8. Ghazali, N. H. M., Awang, N. A., Mahmud, M. and Mokhtar, A. 2018. Impact of sea level rise and tsunami on coastal areas of north-West Peninsular Malaysia. Irrig. Drain., 67: 119-129.
9. Ghosh, N., Adak, M. K., Ghosh, P. D., Gupta, S., Sen Gupta, D. N. and Mandal, C. 2011. Differential responses of two rice varieties to salt stress. Plant Biotechnol. Rep., 5: 89-103.
10. Gill, S. S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem, 48(12): 909-930.
11. Grieve, C. M., Grattan, S. R. and Maas, E. V. 2012. Plant salt tolerance. ASCE Manual and Reports on Engineering Practice, 71: 405-459.
12. Hakim, M. A., Juraimi, A. S., Hanafi, M. M., Ismail, M. R., Rafii, M. Y., Islam, M. M. and Selamat, A. J. 2014. The effect of salinity on growth, ion accumulation and yield of rice varieties. J. Anim. Plant Sci., 24(3): 874-885.
13. Hameed, A., Ahmed, M. Z., Hussain, T., Aziz, I., Ahmad, N., Gul, B. and Nielsen, B. L. 2021. Effects of salinity stress on chloroplast structure and function. Cells, 10(8): 2023.
14. Hannachi, S., Steppe, K., Eloudi, M., Mechi, L., Bahrini, I. and Van Labeke, M. C. 2022. Salt stress induced changes in photosynthesis and metabolic profiles of one tolerant (‘Bonica’) and one sensitive (‘Black beauty’) eggplant cultivars (*Solanum melongena L.*). Plants, 11(5): 590.
15. Hasanuzzaman, M., Nahar, K., Fujita, M., Ahmad, P., Chandna, R., Prasad, M. N. V. and Ozturk, M. 2013. Enhancing plant productivity under salt stress: Relevance of poly-omics. Salt stress in plants: Signalling, omics and adaptations, pp 113-156.

16. Hussain, S., Cao, X., Zhong, C., Zhu, L., Khaskheli, M. A., Fiaz, S., Zhang, J. and Jin, Q. 2018. Sodium chloride stress during early growth stages altered physiological and growth characteristics of rice. Chil. J. Agric. Res., 78(2): 183-197.
17. IRRI. 2013. Standard Evaluation System (SES) for Rice 5th Edition. P.O. Box 933. 1099, Los Baños, Manila Philippines, pp 34.
18. Islam, F., Wang, J., Farooq, M. A., Yang, C., Jan, M., Mwamba, T. M., Hannan, F., Xu, L. and Zhou, W. 2019. Rice responses and tolerance to salt stress: deciphering the physiological and molecular mechanisms of salinity adaptation. In advances in rice research for abiotic stress tolerance. Woodhead Publishing, pp 791-819.
19. Kaur, N., Dhawan, M., Sharma, I. and Pati, P. K., 2016. Interdependency of reactive oxygen species generating and scavenging system in salt sensitive and salt tolerant cultivars of rice. BMC Plant Bio., 16(1): 1-13.
20. Kibria, M. G., Hossain, M., Murata, Y. and Hoque, M. A. 2017. Antioxidant defense mechanisms of salinity tolerance in rice genotypes. Rice Sci., 24(3): 155-162.
21. Krishnamurthy, S. L., Sharma, P. C., Sharma, S. K., Batra, V., Kumar, V. and Rao, L. V. S. 2016. Effect of salinity and use of stress indices of morphological and physiological traits at the seedling stage in rice. Indian J. Exp. Biol., 54: 843-850.
22. Li, Q., Yang, A. and Zhang, W. H. 2017. Comparative studies on tolerance of rice genotypes differing in their tolerance to moderate salt stress. BMC Plant Bio., 17: 1-13.
23. Ling, Q., Huang, W. and Jarvis, P. 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. Photosynthesis Res., 107: 209-214.
24. Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59: 651-681.
25. Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant, Cell Environ., 16(1): 15-24.
26. Nounjan, N., Mahakham, W., Siangliw, J. L., Toojinda, T. and Theerakulpisut, P. 2020. Chlorophyll retention and high photosynthetic performance contribute to salinity tolerance in rice carrying drought tolerance quantitative trait loci (QTLs). Agriculture, 10(12): 620.
27. Paiva, A. L. S., Passaia, G., Lobo, A. K. M., Jardim-Messeder, D., Silveira, J. A. and Margis-Pinheiro, M. 2019. Mitochondrial glutathione peroxidase (OsGPX3) has a crucial role in rice protection against salt stress. Environ. Exp. Bot., 158:12-21.
28. Qin, H., Li, Y. and Huang, R. 2020. Advances and challenges in the breeding of salt-tolerant rice. Int. J. Mol. Sci., 21(21): 8385.
29. Rahman, M. S., Matsumuro, T., Miyake, H. and Takeoka, Y. 2000. Salinity induced ultrastructural alterations in leaf cells of rice (*Oryza sativa L*). Plant Prod. Sci. 3: 422429.
30. Reddy, I. N. B. L., Kim, B. K., Yoon, I. S., Kim, K. H. and Kwon, T. R. 2017. Salt tolerance in rice: focus on mechanisms and approaches. Rice Sci., 24(3): 123-144.
31. Riaz, M., Arif, M. S., Ashraf, M. A., Mahmood, R., Yasmeen, T., Shakoor, M. B., Shahzad, S. M., Ali, M., Saleem, I., Arif, M. and Fahad, S. 2019. A comprehensive review on rice responses and tolerance to salt stress. Advances in rice research for abiotic stress tolerance, pp.133-158.
32. Sah, S. S., Maulud, K. N. A., Sharil, S., Karim, O. A. and Nahar, N. F. A. 2021. Impact of saltwater intrusion on paddy growth in Kuala Kedah, Malaysia. J. Sustain. Sci. Manag., 16(6):15-30.

33. Schoeneberger, P. J., Wysocki, D. A. and Benham, E. C. (Eds.) 2012. Field book for describing and sampling soils. Government Printing Office. Lincoln, NE, USA.
34. Singh, R. K. 2006. Breeding for salt tolerance in rice. Plant Breeding, genetics and biotechnologies Division, International Rice Research Institute.
35. Yang, Z., Li, J. L., Liu, L. N., Xie, Q. and Sui, N. 2020. Photosynthetic regulation under salt stress and salt-tolerance mechanism of sweet sorghum. Front. Plant Sci., 10: 1722.
36. Zhang, Y., Ding, J., Wang, H., Su, L. and Zhao, C. 2020. Biochar addition alleviate the negative effects of drought and salinity stress on soybean productivity and water use efficiency. BMC Plant Bio., 20: 1-11.
37. Zhao, C., Zhang, H., Song, C., Zhu, J. K. and Shabala, S. 2020. Mechanisms of plant responses and adaptation to soil salinity. The Innovation, 1(1):1-41.
38. Zuo, Z., Ye, F., Wang, Z., Li, S., Li, H., Guo, J., Mao, H., Zhu, X. and Li, X. 2021. Salt acclimation induced salt tolerance in wild-type and chlorophyl b-deficient mutant wheat. Plant Soil Environ., 67(1): 26-32.