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DETERMINATION OF SELECTION CRITERIA FOR SCREENING OF RICE GENOTYPES FOR SALINITY TOLERANCE

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SUMMARY

Identification of tolerant rice genotypes to salinity is strongly associated with the environment, criteria, and methods of selection. The aim of this study was to determine a salinity tolerant index, an agronomic character as a criterion of selection and electrical conductivity (EC) as an optimal selection to increase the effectiveness of tolerance screening in saline soil. This study was conducted in a greenhouse using soil media. The experimental design used was a randomized complete block factorial design (RCBD) consisting of two factors: varieties and NaCl concentrations, and replicated three times. The varieties used were Pokkali, as the salinity tolerant variety, and IR29 as the salinity sensitive variety. The NaCl concentrations used consisted of 0,10, 20, 30, and 40 mM measured at EC 2.14, 4.78, 6.57, 7.44, and 8.43 dS m^{-1}, respectively. Various calculations of salinity tolerance indices, such as stress tolerance index (STI), geometric mean productivity (GMP), stress susceptibility index (SSI), tolerance index (TOL), mean productivity (MP), yield index (YI), and yield stability index (YSI), were applied to the yield per plant in each combination. The results of this study indicated that STI, GMP, and YSI were suitable tolerant indices which can be used in selection of salinity tolerant rice genotypes. The agronomic character that could be used as salinity selection criterion was the number of total tillers. The critical EC which could be used to select salinity tolerant rice was about 5.62 dS m^{-1}.

Key words: Rice, salinity, critical EC, salinity tolerant index, total tiller number

Key findings: Total tiller number can be used as a selection criterion based on path analyses of salinity tolerance indices.

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INTRODUCTION

Among abiotic stresses, salinity is one of the major constraints reducing rice productivity in Indonesia, an archipelago affected by the rise in sea level, which is due to global warming that can induce seawater abrasion and increase in soil salinity around the coast (Aydinalp and Cresser, 2008). According to Rachman *et al*. (2007), the total area of saline land in Indonesia of 0.44 M ha is divided into moderate saline (0.304 M ha) and saline (0.140 M ha). Asian Development Bank (2009) reported that 15% of the total rice production in Indonesia is contributed by rice fields located in the coastal areas, therefore, increased salinity can reduce rice production in Indonesia.

Salinity affects both plant growth and yield through three kinds of stresses. The first way is through the osmotic stress that causes the plants to experience physiological drought, in which water cannot be absorbed by plants (Yamamoto *et al*., 2011). Second, is through the toxic stresses of sodium (Na^+) which can disrupt metabolism (Chandna *et al*., 2013) and photosynthesis (Sultana *et al*., 1999) in plants. The last is the ionic stress that causes homeostatic imbalances of ions in plant cells, especially the essential elements like potassium (K^+) (Hossain *et al*., 2015). Therefore, the salinity of soil negatively affects the growth and yield of rice crops.

The easiest way to address the problem of salinity is through the development of a salt tolerant variety. The success of this development depends on the determination of selection method, selection environment, and selection criteria used. An effective selection method should be repeatable and easy to apply (Titov *et al*., 2009). There are several selection methods developed for selection of salinity tolerant rice, such as germination (Subasingle *et al*., 2007; Pradheeban *et al*., 2014), hydroponics (Ali *et al*., 2004; Titov *et al*., 2009; Ali *et al*., 2014; Mondal and Borromeo, 2016), and saline soils (Egdane *et al*., 2003; Hariadi *et al*., 2014, Safitri *et al*., 2016). The most widely used method is the hydroponics method which is an effective rapid screening method (Titov *et al*., 2009). However, the disadvantage of this method is that it highlights only the seedling or vegetative phase and it cannot predict the effects on the next growth stage (Ali *et al*., 2014). Saline soil method reflects the actual environment so that the plant growth response to salinity can be depicted in the reproductive phase. However, in its application, much saline induction is given at the critical point of plant reproduction (Egdane *et al*., 2003; Hariadi *et al*., 2014). Safitri *et al*. (2016) induced salinity in the early phase of saline selection in greenhouses, but the selection process is still based on the yield characters. Therefore, additional information based on other secondary characters is needed and which should be related to the response of rice crop to saline soil from vegetative to reproductive phase.

Artificial selection environments have attempted to reflect the real stress environment in the field so that genes related to salinity tolerance can be expressed. Determination of the salinity selection environment should bring out the diversity of tolerance trait among the genotypes of population used (De Costa *et al*., 2012). Therefore, it is necessary to approach the plant characters as selection criteria. The salinity selection environment reflects the electrical conductivity (EC) value and is affected by the concentration and composition of dissolved salts (Munns and Tester, 2008).

Criteria for selection can be agronomic characters that have high heritability and correlate with yield under stress conditions. The direct selection of yield under stress conditions has low heritability (Fritsche-Neto and DoVale, 2012). According to Acquaah (2007), the agronomic characters can be used as selection criteria when a direct selection of yield has barriers or low heritability. Thus, the agronomic characters can be considered for the development of a tolerance index. The tolerance index is one way to improve the selection accuracy of tolerant lines (Kamyab-Talesh *et al*., 2014) and Singh *et al*. (2015) suggested many approaches to the tolerance index. Therefore, the objective of this study was to obtain the suitable tolerance index and agronomic character as selection criteria and to obtain critical EC that can be used to screen salinity tolerance in a saline soil method.

MATERIALS AND METHODS

This study was conducted at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) Greenhouse, Bogor from September 2016 to January 2017. The experimental design was a randomized complete block factorial design (RCBD), consisting of varieties and NaCl concentrations (measured to EC values). Varieties used consisted of two genotypes i.e. Pokkali as a salinity tolerant variety and IR29 as a salinity sensitive variety. NaCl concentration consisted of five levels, namely 0, 10, 20, 30, and 40 mM which then measured to EC 2.14, 4.78, 6.57, 7.44, and 8.43 dS m^{-1}, respectively. The experiment consisted of three replications, resulting in 30 experimental units and each experimental unit consisted of one plant per pot.

Procedures

Rice seeds were germinated in containers until 25 days. The seedlings were transferred into pots (one seedling per pot) containing soil, manure, and water in a ratio of 7:1:3. NaCl was added to medium according to treatment and 900 ml water was added and then stirred slowly. Electrical conductivity (EC) of the standing water in each treatment was measured by 470 model Jenway EC meter. Maintenance included watering, fertilizing, and weeding. Watering was done at least once a day to maintain water conditions relatively at the same level. Fertilization of NPK 15:15:15 with a dose of 6 g/pot at 1 week after planting (WAP) and urea at the 3 and 9 WAP with a dose of 1.5 g/pot were applied. Harvesting was done when 80% of panicles turned yellow.

The variables included plant height, flag leaf, panicle length, number of total tillers, number of productive tillers, number of grains (filled, unfilled, and total) per panicle, 1000 grain weight, and grain yield per hill.

Estimation of salinity tolerance index

To calculate the salinity tolerance index of each genotype, we used the following formulae:

- Yield index (YI) = Ys / Ȳs (Gavuzzi *et al*., 1997);
- Stress tolerance index (STI) = Yp × Ys / Ȳp2 (Fernandez, 1992);
- Yield stability index (YSI) = Ys / Yp (Bouslama and Schapaugh, 1984);
- Geometric mean productivity (GMP) = $\sqrt{Yp \times Ys}$ (Fernandez, 1992);
- Stress susceptibility index (SSI) = (1 - Ys / Yp) / SI; SI = 1 - Ȳ s / Ȳ p (Fischer & Maurer, 1978);
- Mean productivity (MP) = (Yp + Ys) / 2 (Rosielle and Hamblin, 1981);
- Tolerance Index (TOL) = Yp - Ys (Rosielle and Hamblin, 1981).

Yp refers to yield of each genotype in normal condition (EC 2.14 dS m^{-1}). Ys refers to yield of each genotype in stress condition (EC 4.78, 6.57, 7.44, and 8.43 dS m^{-1}). Meanwhile, Ȳp and Ȳs refer to the average yield of all genotypes in the normal and saline condition, respectively.

Data analysis

The data were processed to obtain mean and standard deviation. *F*-test followed by DMRT real difference test, *t*-Student test, correlation test, principal component analysis (PCA), path analyses, multivariate regression test, and linear regression test were used for analyses. The error level used in this study was 5%. The statistical software programs used in the analyses were SAS 9, STAR, R package Agricolae (De Mendiburu, 2014), and Curve expert.

RESULTS AND DISCUSSION

General response of tolerant and sensitive rice varieties to salinity

Analysis of variance related to several vegetative and reproductive characters was shown in Tables 1 and 2. The observations in both group of characters indicated significant differences as a response between Pokkali and IR29 so that both varieties can be differentiated in normal and stress conditions. In addition, the NaCl concentration factor, which in this case was elaborated in EC, showed very different results for all characters. This explained that the concentration differences affected all the characters observed in both varieties. Similar results were reported by Razzaque *et al*. (2009), Sultana *et al*. (2014), and Safitri *et al*. (2016) which showed a decrease in response to vegetative and reproductive characters due to increased NaCl concentration. In this study, the interaction of varietal treatment and NaCl concentrations were only significantly different for the

Table 1. Mean squares of variance analyses of vegetative and reproductive characters.

Variance Sources	d.f	VH	TT	PT	FL
Replication	2	189.06	0.33	0.11	4.69
Genotype (G)	1	29296.86 **	2.35 *	2.58 **	3754.02 **
NaCl concentrations (S)	4	3113.41 **	12.99 **	13.5 **	1083.97 **
G * S	4	227.46	0.58	0.90 *	86.75 **
Error	18	100.41	0.29	0.3	12606
Coefficient of variance		9.39	17.07 t1	18.23 t1	10.74

Notes: (t1): the result of transformation $\sqrt{(0.5 + x)}$; ** Highly significant different , *significantly different; Vegetative character: the vegetative height (VH), and the number of total tiller (TT); Reproductive characters: the productive tiller (PT), and the flag leaf length (FL).

Table 2. Mean squares of variance analyses of reproductive characters.

Variance Sources	d.f	PL	NFG	NUG	Yh	NTG	W1000
Replication	2	13	3.89	0.03	0.85	1023	6.13
Genotype (G)	1	347.54 **	37.16 **	2.68 **	7.68 **	21549 **	447.34 **
NaCl concentrations (S)	4	275.83 **	91.20 **	0.31 **	54.93 **	19270 **	249.22 **
G * S	4	53.11 **	3.44	0.70 **	2.99 *	1588 *	44.64 **
Error	18	4.42	1.93	0.059	0.84	407	7.23
Coefficient of variance		11.3	20.69 t1	19.36 t2	25.97 t1	23.52	13.33

Notes: (t1) the first result of the transformation $\sqrt{(0.5 + x)}$, (t2) the second results of a transformation log(1 + x);** Highly significant different , *significantly different; the panicle length (PL), the number of filled grains (NFG), the number of unfilled grains (NUG), the yield per hill (Yh), the number of total grains (NTG), Weight of 1000 grains (W1000).

reproductive characters, i.e. the productive tillers, the length of flag leaf, the length of panicle, the number of unfilled grains, yield per hill, total grain number, and 1000 grain weight. It is clear that the impact of salinity stress was different for tolerant and sensitive varieties, especially in the reproductive phase (Bhowmik *et al*., 2007). Therefore, a combination of NaCl concentration treatment and genotypes can detect tolerance index, selection criteria, and critical EC to select the salinity tolerant rice genotypes.

The average data for each vegetative and reproductive character were shown in Tables 3 and 4. Table 3 shows that the characters of plant height and the number of total tillers in IR29 decreased drastically when given salinity stress of 4.78 dS m^{-1}. However, the decline begins to stabilize when an EC increase exceeds 4.78 dS m^{-1} with a quadratic and cubic response. Meanwhile, Pokkali showed a gradual decline in these characters at severe level of salinity with a linear and quadratic pattern. This illustrated that IR29 retained plant height and total tiller to a certain EC (4.78 dS m^{-1}) as a threshold. In contrast, Pokkali still have not reached a critical point while at EC 4.78 dS m^{-1}. The EC critical point of Pokkali occured at EC 7.44 dS m^{-1}.

Flag leaf length (Table 3), productive tillers, panicle length, number of filled grains, yield per hill, and number of total grains showed a similar pattern with vegetative characters to salinity stress in both varieties (Table 4). However, the stagnation of decline in IR29 begins at EC 6.57 dS m^{-1}, whereas Pokkali showed varied responses among characters toward their decline stagnation. The decrease of 1000 grain weight (W1000) in IR29 was more drastic than Pokkali. These results were consistent with the study of Safitri *et al*. (2016), who stated that the reduction in 1000 grain weight of IR29 was higher than that of Pokkali. In addition, the number of unfilled grain (NUG) for IR29 does not have a specific response pattern, while Pokkali was quadratic. This character was much related to the total amount of grain produced per panicle and the ability of the plant to translocate the photosynthate into the grains (Amrullah *et al*., 2014) so that this character had a different pattern in comparison with the other characters.

The observations of all characters explained the difference in responses between Pokkali versus IR29 when stressed with salinity. It was stated by Bhowmik *et al*. (2007) that Pokkali was tolerant under salinity at all plant growth phases illustrating that salinity affects all plant characters during all growth stages. However, tolerant varieties have a mechanism to defend themselves from the effects of stress. Therefore, they can normally grow and produce under saline conditions.

Determination of rice salinity tolerant index in saline soil

Assessing the yield potential of a genotype in a harsh environment requires a certain criterion known as a tolerant index. Some researchers have used several approaches to tolerant index with consideration to the plant yield at the optimum and the stress environment (Singh *et al*., 2015). The calculation results of some tolerant indices are shown in Table 5. Table 5 indicated the presence of different ranking patterns of some reported tolerant indices. This difference was related to the approach used in each tolerant index. The tolerant indices i.e. STI and GMP have a pattern of ranks similar to yield per hill under stress condition (Ys). In contrast, the TOL and SSI indices have an inverse pattern with other indices. The highest value in both of the indices indicated the sensitivity combination. The results of tolerant index calculations were further analyzed by using student's *t*-test, correlation, and principal component analysis (PCA). The *t*-student test was conducted to obtain a tolerant index that can differentiate yield between Pokkali and IR29 at each stress level. The H1 hypothesis used in this test was the IR29 has a lower mean value than Pokkali, except in the tolerant TOL and SSI indices with the opposite hypothesis.

The results of the analysis showed that STI, YSI, YI, GMP, and SSI were the tolerant indices which significantly differ for yield between Pokkali and IR29 at each EC level (Table 6). The YI and GMP indices showed a very significant difference at 1% error and thus YI and GMP were particularly relevant to differentiate tolerant and sensitive varieties in salinity stress. However, the *t*-test results have not been able to explain the linear relationship between the five indices to the yield per hill. Therefore, the correlation analysis was performed.

Table 3. Average and Duncan Multiple Range Test of vegetative and reproductive character.

Varieties	EC (dS m^{-1})	VH (cm)	TT	FL (cm)
IR29				
	2.14	113.00	32.3	38.98b
	4.78	73.00	8.7	28.55c
	6.57	62.67	5.0	22.23d
	7.44	67.00	3.7	19.57d
	8.43	61.17	2.0	0.00e
Response	quadratic	quadratic	cubic	cubic
Pokkali				
	2.14	174.33	28.0	60.45a
	4.78	151.33	17.7	56.48a
	6.57	134.00	9.0	40.93b
	7.44	117.33	6.0	31.82c
	8.43	112.33	3.7	31.52c
Response	quadratic	Linear	quadratic	cubic

Notes: letters in one column show the differences and similarities between combinations of treatments; Vegetative characters: vegetative height (VH), and number of total tiller (TT); Reproductive character: flag leaf length (FL).

Table 4. Average and Duncan Multiple Range Test of reproductive characters.

Varieties	EC (dS m^{-1})	PT	PL (cm)	NFG	NEG	W1000 (g)	Yh (g)	NTG
IR29								
	2.14	30.0a	25.33a	123.8	24.9bcd	24.00ab	70.43a	148.7b
	4.78	9.7cd	19.54bc	54.1	11.9d	23.50abc	12.87c	65.1dc
	6.57	5.7de	16.34dc	22.6	14.1cd	18.67c	2.00d	36.7d
	7.44	3.3e	14.81d	22.2	22.6bcd	13.00d	1.15d	44.4dc
	8.43	0.0f	0.00e	0.0	0.0e	0.00e	0.00d	0.0e
Response	quadratic	quadratic	cubic	quadratic	-	quadratic	cubic	cubic
Pokkali								
	2.14	20.3b	27.29a	145.3	47.4ab	28.50a	65.03a	192.7a
	4.78	14.7bc	26.21a	131.8	34.7abcd	27.00a	40.07b	166.5ab
	6.57	9.7cd	21.17b	62.4	15.9bcd	24.83ab	11.57c	79.5c
	7.44	3.3e	18.38bcd	30.2	44.7abc	20.83c	1.97d	58.2dc
	8.43	2.3e	16.99dc	5.1	61.0a	18.50c	0.21d	66.1dc
Response	quadratic	cubic	linear	linear	quadratic	linear	quadratic	quadratic

Notes: the letters in one column show the differences and similarities between the combination of treatments; productive tiller (PT), panicle length (PL), number of filled grains (NFG), number of empty grains (NEG), Weight of 1000 grains (W1000), yield per hill (Yh), the number of total grains (NTG).

Table 5. Average tolerant indices from combination of varieties and electrical conductivity levels.

Combination	Ys (g)	STI	YSI	YI	GMP	TOL	MP	SSI
IR29 (4.78)	12.87 (2)	0.224 (2)	0.163 (3)	0.49 (6)	28.37 (2)	57.57 (6)	41.65 (2)	1.37 (1)
IR29 (6.57)	2.00 (4)	0.035 (4)	0.027 (5)	0.30 (7)	11.26 (4)	68.43 (3)	36.22(4)	1.08 (2)
IR29 (7.44)	1.15 (6)	0.020 (6)	0.016 (6)	0.74 (5)	8.10 (6)	69.29 (2)	35.79 (5)	1.01 (3)
IR29 (8.43)	0.00 (8)	0.000 (8)	0.000 (8)	0.00 (8)	0.00 (8)	70.43 (1)	35.22 (6)	1.00 (4)
Pokkali (4.78)	40.07 (1)	0.539 (1)	0.709 (1)	1.51 (2)	49.54 (1)	24.97 (8)	52.55 (1)	0.48 (8)
Pokkali (6.57)	11.57 (3)	0.165 (3)	0.195 (2)	1.71 (1)	27.14 (3)	53.47 (7)	38.30 (3)	0.90 (7)
Pokkali (7.44)	1.97 (5)	0.026 (5)	0.035 (4)	1.26 (3)	10.73 (5)	63.07 (5)	33.50 (7)	0.99 (6)
Pokkali (8.43)	0.21 (7)	0.003 (7)	0.004 (7)	1.99 (4)	3.37 (7)	64.82 (4)	32.63 (8)	1.00 (5)

Notes: yield under stress condition (Ys), yield stability index (YSI), Yield index (YI), stress tolerance index (STI), geometric mean productivity (GMP), stress susceptibility index (SSI), mean productivity (MP), tolerance Index (TOL), number in the bracets indicate the rank of the treatments in each variable.

Table 6. *t*-Student pairwise test results in some tolerant indices. H0: μ1 = μ2; H1: μ1 < μ2

μ1	μ2	DF	*t* value	Pr (<t)
Ys 1	Ys 2	11	-2.86	0.0078**
STI1	STI2	11	-2.29	0.0215*
YSI1	YSI2	11	-2.42	0.0171*
YI1	YI2	11	-4.33	0.0006**
GMP1	GMP2	11	-2.76	0.0093**
TOL2	TOL1	11	-1.31	0.1077
MP1	MP2	11	-0.35	0.3661
SSI2	SSI1	11	-2.19	0.0254*

Notes: ** Highly significant different , *significantly different, yield under stress condition (Ys), yield stability index (YSI), Yield index (YI), stress tolerance index (STI), geometric mean productivity (GMP), stress susceptibility index (SSI), mean productivity (MP), tolerance Index (TOL).

Table 7 showed that the tolerant indices of STI, YSI, GMP, TOL, and MP were highly correlated with yield under stress conditions. However, only TOL had a significant negative correlation to yield, whereas other indices were positive. This result explains that tolerant index which was significant in the *t*-test does not necessarily correlates to yield of each genotype in saline condition (Ys). This was proven by the tolerant index of YI and SSI which can distinguish tolerant and sensitive varieties but does not significantly correlate with the yield. This also applies in reverse as shown by tolerant indices i.e. TOL and MP. The tolerant indices that have a significant response in both *t*-student and Pearson correlation tests are STI, YSI, and GMP, which were relevant to be used as tolerant indices.

Some researchers (Akçura and Çeri, 2011; Hosseini *et al*., 2012; Singh *et al*., 2015; Abdi *et al*., 2013; Ali and El-Sadek, 2016) also conducted PCA analysis as the basis for determining tolerant indices used under stress conditions. PCA analysis is a data processing technique that can compact data sets from high dimension into the lower dimension, so it is easy to be represented and visualized (Ilin and Raiko, 2010). In addition, PCA analysis results can be visualized by biplot analysis to provide an overview of the direction and variance of the tolerant index used. The PCA results showed the presence of two PCs describing the variance of the yield character and the tolerance index under saline conditions (Table 8). Yield in saline environment (Ys) was in the same direction with STI, YSI, GMP, and MP in PC1, while in PC2, the yield is in line with YSI, GMP, STI, and TOL. This indicated that the three indices, i.e. STI, GMP, and YSI, which have the same direction as the yield under saline environment (Ys) in both components. Furthermore, they also have the same direction with the best combination, which was marked as number 5 (Figure 1). It was the combination of Pokkali and EC 4.76 dS m^{-1}, as the tolerant combination. Based on the three analytical results, the suitable tolerant indices to be used as a salinity tolerant index in this study were STI, GMP, and YSI. According to Singh *et al*. (2015), the correct tolerant indices for wheat in saline and normal conditions were MP, GMP, and STI. According to Hosseini *et al*. (2012), the tolerant indices of STI, GMP, and MP were also relevant in selecting salinity tolerant rice genotypes. Krishnamurthy *et al*. (2013) also used STI in determining the tolerant genotype of rice against salinity stress. Meanwhile, Kamyab-Talesh *et al*. (2014) also suggested that the YSI index was good for selection of saline tolerant rice genotypes.

Determination of selection criteria based on rice agronomic characters under salinity stress

The determination of agronomic characters as selection criteria for salinity can be obtained through correlation between agronomic characters and tolerance index under saline condition. The correlation between them is expected to produce an agronomic character that has the same ability as the tolerance index in differentiating tolerant and sensitive genotypes and correlates with yield under salinity. This will improve the effectiveness and efficiency of selection in predicting the tolerant genotypes.

Table 7. Pearson correlation index of salinity tolerance to yield per hill.

	Ys	STI	YSI	YI	GMP	TOL	MP	SSI
Ys	1							
STI	0.996 **	1						
YSI	0.996 **	0.983	1					
YI	0.308 ns	0.2758	0.337	1				
GMP	0.954 **	0.968	0.9328	0.314	1			
TOL	-0.984 **	-0.9714	-0.9876	-0.4578	-0.9376	1		
MP	0.978 **	0.9833	0.9647	0.1207	0.9343	-0.9249	1	
SSI	-0.663 ns	-0.5916	-0.7287	-0.4826	-0.4976	0.7209	-0.5694	1

Notes: The correlation focus on Ys character, ** highly correlated at the level of 1%, ns=not significantly correlated, yield under stress condition (Ys), yield stability index (YSI), yield index (YI), stress tolerance index (STI), geometric mean productivity (GMP), stress susceptibility index (SSI), mean productivity (MP), tolerance Index (TOL).

Table 8. The PCA results of some tolerant indices and the yield.

Variables	Ys	STI	YSI	YI	GMP	TOL	MP	SSI	SD	CP	E
PC1	-0.39	-0.388	-0.394	-0.155	-0.37	0.39	-0.38	0.28	2.53	0.80	6.42
PC2	-0.08	-0.153	-0.04	0.831	-0.14	-0.06	-0.28	-0.42	1.04	0.94	1.08

Notes: yield under stress condition (Ys), stress tolerance index (STI), yield stability index (YSI), yield index (YI), geometric mean productivity (GMP), tolerance Index (TOL), mean productivity (MP), stress susceptibility index (SSI), standard deviation (SD), cumulative proportion (CP), EigenValue (E). PC1= principle component 1, PC2= principle component 2.

Table 9. Correlation between all observation characters and tolerant index.

	VH	TT	PT	FL	PL	NFG	NUG	W1000	Ys	NTG	STI	GMP
TT	0.72 *											
PT	0.59	0.95 **										
FL	0.88 **	0.87 *	0.83 *									
PL	0.7	0.77 *	0.81 *	0.94 **								
NFG	0.69	0.99 **	0.95 **	0.84 *	0.75 *							
NUG	0.56	0.11	-0.03	0.49	0.46	0.05						
W1000	0.66	0.75 *	0.84 *	0.91 **	0.99 **	0.74 *	0.60					
Ys	0.65	0.98 **	0.91 **	0.78 *	0.65	0.98 **	0.05	0.59				
NTG	0.82 *	0.94 **	0.86 *	0.95 **	0.85 *	0.92 **	0.41	0.78 *	0.91 **			
STI	0.61	0.98 **	0.93 **	0.77 *	0.66	0.97 **	0.01	0.62	1 **	0.9 **		
GMP	0.63	0.98 **	0.92 **	0.78 *	0.65	0.98 **	0.03	0.61	1 **	0.91 **	1 **	
YSI	0.68	0.97 **	0.88 **	0.78 *	0.64	0.97 **	0.08	0.57	1 **	0.92 **	0.98 **	0.99**

Notes : ** Highly significant correlated , *significantly correlated, vegetative height (VH), number of total tiller (TT), productive tiller (PT), flag leaf length (FL), panicle length (PL), number of filled grains (NFG), number of unfilled grains (NUG), Weight of 1000 grains (W1000), yield under stress condition (Ys), the number of total grains (NTG), stress tolerance index (STI), geometric mean productivity (GMP), yield stability index (YSI).

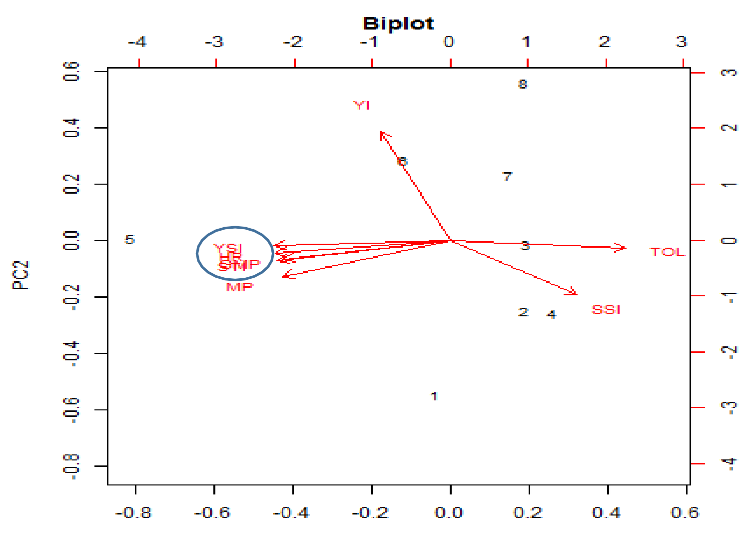


Figure 1. Biplot PC1 and PC2 in the all selection indices and the yield to the combination of treatments.

Table 9 shows the correlation between the agronomic characters in saline conditions and the tolerant index obtained in the previous analysis. All tolerant indices have high significant correlations to the number of total tillers (TT), productive tillers (PT), number of filled grains (NFG), and the number of total grains (NTG) at 1% level. This explained that TT, PT, NFG, and NTG were agronomic characters which have effect in the yield under salinity. Similar results were reported by Krishnamurthy *et al*. (2013), Gopikannan and Ganesh (2013), and Fiyaz *et al*. (2011). However, the correlation results have not been able to explain the magnitude of the direct influence of each character in any tolerant index. Therefore, it is necessary to do a partition of each correlation to determine the direct and indirect effects of each character in the tolerant index partitioning the correlations using path analysis (Singh and Chaudhary, 2007). Path analysis is used by breeders to assist in determining the character that can be used as selection criterion (Miligan *et al*., 1990). The results of the path analysis are shown in Table 10. The result of path analysis in STI showed that number of total tiller had the most influential effect on STI index (Table 10). In addition, the total tillers also have a high indirect effect in other characters. The results of the path analysis of GMP showed that the total tiller and number of filled grain have a direct influence in GMP (Table 10). These characters also gave an indirect effect with a proportion that was almost equal to its direct effect. However, the total tiller proportion was higher than the number of filled grains. The YSI tolerant index showed the same result as the GMP index (Table 10). However, the proportion of both characters were almost equal to both the direct and indirect influences. The results of correlation analysis can also be a reference to create the formulation through multivariate regression analysis. The results of the multivariate regression analysis also

Table 10. Results of path analysis and multivariate regression analysis of STI, GMP and YSI.

Character	The Direct Effect	TT	PT	NFG	NTG	Residual
Stress tolerance index (STI) = -0.128401 + 0.036633 TT (R 2 adj = 0.948)						
TT	1.33	-	-0.07	-0.09	-0.20	0.03504
PT	-0.07	1.27	-	-0.08	-0.18	0.03504
NFG	-0.09	1.32	-0.07	-	-0.19	0.03504
NTG	-0.21	1.25	-0.06	-0.08	-	0.03504
Geometric mean productivity (GMP) = -75 834 + 21.02 TT (R 2 adj = 0.951)						
TT	0.74	-	-0.19	0.53	-0.10	0.031013
PT	-0.20	0.70	-	0.51	-0.09	0.031013
NFG	0.53	0.73	-0.19	-	-0.10	0.031013
NTG	-0.10	0.70	-0.17	0.49	-	0.031013
Yield stability index (YSI) = -0.183423 + 0.046977 TT (R 2 adj = 0.933)						
TT	0.73	-	-0.47	0.72	0.00	0.031479
PT	-0.50	0.70	-	0.69	0.00	0.031479
NFG	0.72	0.72	-0.47	-	0.00	0.031479
NTG	- 0.005	0.69	-0.43	0.66	-	0.031479

Notes: number of total tiller (TT), productive tiller (PT), number of filled grains (NFG), the number of total grains (NTG).

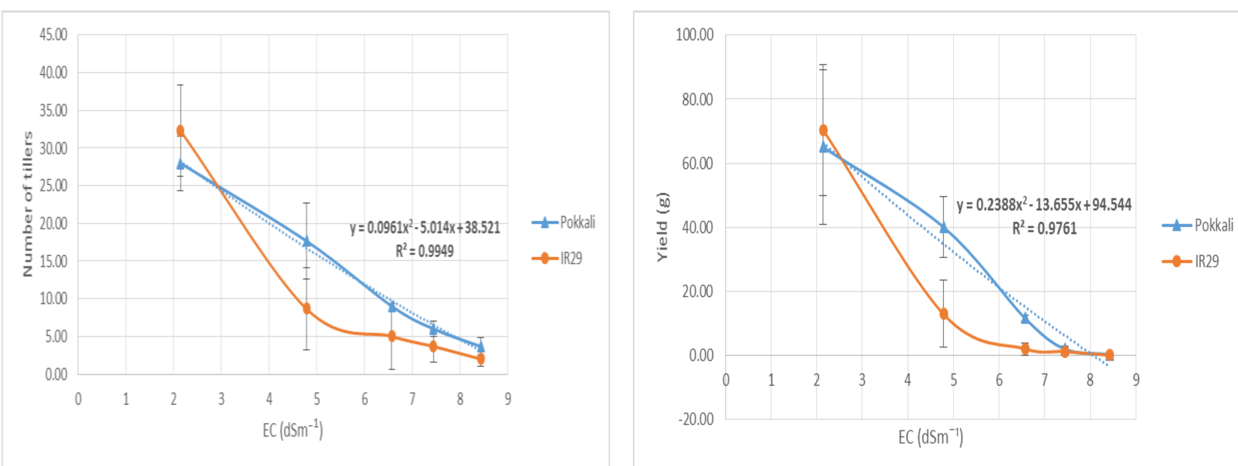
Table 11. Critical EC and NaCl concentration on the important characters of rice to salinity stress in pots.

Parameter	TT	Yh
EC (dS m^{-1})	5.62	5.42
The concentration of NaCl (mM)	14.91	13.85
Responses	quadratic spline	quadratic spline

Notes: number of total tiller (TT), Yield per hill (Yh).

showed that the three tolerant indices were highly determined by the total tillers (TT) (Table 10). It can be seen from the formulae of each tolerant index that the total tiller number was the only character fit in determining the value of each index.

The overall analysis that had been carried out showed that the number of total tillers was the relevant character to be used as a selection criterion in the salinity stress screening for rice. According to Hasanuzzaman *et al*. (2009) and Safitri *et al*. (2016), the total and productive tillers were drastically decreased in rice under saline condition. In addition, Mansuri *et al*. (2012) and Munns and Tester (2008) stated that the total tillers can be one of the indicator character in predicting the yield and selecting tolerant genotypes under salinity stress.



A **B**

Figure 2. Graphics and regression of the number of total tiller (A) and the yield per hill (B) towards EC levels of salinity in the pot.

Determination of critical EC for selection of rice genotype in stress environment

The number of total tillers was an effective and efficient selection criteria because it can be observed before reproductive stage. The total number of tillers under the saline environment is almost the same as the number of productive tillers. This explains that the tillers produced in the saline environment were only the productive tillers, so that the total tillers produced in the vegetative phase reflected the plant's adaptability to salinity stress.

The critical EC describes an effective and efficient selection environment for determining salinity tolerance in rice genotypes. Critical EC determination is expected to produce high diversity, so that selection can be optimally performed in salinity stress. In this study, the number of total tillers can be used in determining the critical EC, in addition to the yield per plant. In aluminum stress tolerant study, Bakhtiar *et al*. (2007), used the relative root length (RRL) criterion as a determinant of the Al tolerant genotype by using 50% RRL. Therefore in this study, the same approach was applied to the total tillers and yield in finding critical EC points which caused a 50% decrease on the character of Pokkali. The decline can be obtained through regression analysis.

Figure 2 showed the number of total tillers and yield per hill, respectively. Both graphs showed that the tested EC had not reached the expected critical EC. Therefore, determination of the critical EC of both characters were obtained through interpolation of regression. The critical EC determination used the software Curve expert. The results were shown in Table 11.

The results showed that critical EC of number of total tillers (TT) was 5.62 dS m^{-1} or equivalent to 14.91 mM of NaCl concentration (Table 11). Meanwhile, the character of the yield per hill (Yh) indicated that the critical EC was at 5.42 dS m^{-1}, equivalent to 13.85 mM of NaCl in the saline soil media. Both characters have similar critical EC. The critical EC of total tillers was correlated and directly affected all tolerant indices, therefore, it can be used to select salt tolerant rice genotypes in pots.

The critical EC obtained in this study slightly differed from previous findings. EC of about 6 dS m^{-1} was used by Bhowmik *et al.* (2007) and Egdane *et al*. (2003) at the reproductive phase in soil media under controlled fertilization in the water tank. In addition, Safitri *et al*. (2016) suggested that EC of about 6.2 dS m^{-1} was the recommended EC to select salt tolerant genotypes. However, present study have set slightly lower critical EC, due to different approach in determining the selection criteria. The present study used the number of total tillers as the selection criteria instead of yield characters.

CONCLUSION

Salt tolerance index i.e STI, GMP, and YSI can be used to determine either sensitive or tolerant rice varieties and were positively correlated with the yield under salinity in pots. The number of total tillers can be used as selection criterion. The critical EC to select salt tolerant rice genotypes was about 5.62 dS m^{-1}.

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