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SELECTION INDEX BASED ON MULTIVARIATE ANALYSIS FOR SELECTING DOUBLED-HAPLOID RICE LINES IN LOWLAND SALINE PRONE AREA

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SUMMARY

Salinity is one of major abiotic stresses in rice crop. It affects rice growth and yield, especially those planted in the coastal areas. It needs a solution, among them is to breed adaptive variety to saline environment through doubled-haploid rice lines. The doubled-haploid plant derived from anther culture can accelerate plant breeding program. Meanwhile, interaction of genotype and environment of doubled-haploid rice can be a problem in selecting the adaptive genotype. The objective of the study was to develop selection index based on multivariate analysis and to select doubled-haploid rice lines adaptive to saline prone environment. The research was carried out at the Pusakanagara Experimental Station (normal area) and farmer field in Truntum, Subang (saline prone area) from March until July 2018. The experimental design used was nested randomized complete block design with two-factors (genotypes and locations). The genotypes consisted of 36 doubled-haploid lines and four varieties as control and repeated three times. The analysis used was genetic and multivariate analysis. Based on the phenotypic and genetic correlation and genetic path analysis, it showed that productive tiller was the best-supporting character to the yield. The stress tolerance index (STI) was the suitable tolerance index as a basis to develop the selection index to determine adaptability of genotypes to salinity. The selection index involved principal component analysis and corrected by its direct genetic influence (zAI) was 0.441 productivity STI + 0.145 productive tillers STI. The selection index had repeatability of 102.4%. Based on the positive index selection value, 21 doubled-haploid lines were adaptive to salinity stress and nine of the doubled-haploid lines had better adaptability to salinity stress than Ciherang (mega variety). The present investigation indicates that the selection index based on multivariate analysis increased the effectiveness in selecting doubled-haploid rice lines adaptive to saline environment.

Key words: Climate change, effective selection, high homozygosity, productive tiller, saline environment

Key findings: The multivariate analysis approach increased effectiveness of selection index in selecting doubled-haploid rice lines adaptive to saline environment. Combined productivity and its supporting yield character, i.e. productive tiller, can be used in developing the selection index. Based on the selection index, 21 doubled-haploid lines were adaptive to salinity stress.

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INTRODUCTION

Rice is a main staple food for Asian countries so that its production needs to be increased from year to year. However, drastic climate change has led to an increase in suboptimal environment for plants which threatens the increase of rice production (Aydinalp and Cresser, 2008). One impact of climate change is an increase of saline areas, especially in archipelago countries or countries with long coastlines such as Indonesia.

Salinity has a negative effect on growth and development of rice which has an impact on decreasing the yield. According to Linh *et al*. (2012) an increase in soil salinity of up to 6 dS/m can reduce rice productivity to > 50% and when it reached 12 dS/m the paddy will fail to produce. According to Ismail *et al*. (2013), the magnitude of the salinity effect is shown by several stresses such as osmotic stress, imbalance of ion homeostatic, the toxicity of Na^+ and Cl^- ions and oxidative stress. It makes salinity a major abiotic stress in rice field of coastal areas. Therefore, the problem needs to be resolved, one of which is through the development of varieties adaptive to salinity stress.

Previous research has found several doubled-haploid rice lines adaptive to salinity stress through an indirect selection approach based on selection indices combination of the agronomic selection criteria in potential field condition and the salinity tolerance in hydroponic screening (manuscript in preparation). The lines need to be evaluated further for their adaptability directly in the saline prone environment around the coastal area. Adaptability of breeding lines to abiotic stress can be assessed by using tolerance indices that have been developed by several researchers (Singh *et al*., 2015). Fernandez (1992) has developed the formulas for stress tolerance index (STI) and geometric mean productivity (GMP). Fischer and Maurer (1978) developed the stress susceptibility index (SSI) formula. Rosielle and Hamblin (1981) developed the formulas for mean productivity (MP) and tolerance index (TOL) and Gavuzzi *et al*. (1997) developed the yield index (YI). However, the use of these indices only focused on one character (Kamyab-Talesh *et al*., 2014), in this case productivity. Productivity has a complex mechanism and is strongly influenced by its yield supporting characters (Kassahun *et al*., 2013), thus it is very risky to select only based on productivity. This principle can be applied to doubled-haploid rice lines which have high homozygosity but their productivity are not free from the influence of environment and interactions between genotypes and the environment. Therefore, the use of yield supporting characters is important in the selection.

The index selection is an effective selection method which uses some characters. However, the important things in developing selection index are the determination of selection characters and weighting coefficient of those selection characters, thus the selection can be more representative and accurate (Singh and Chaudhary, 2007). One method that can be used to determine the selection characters and character's weighting coefficient is multivariate analysis (Sabouri *et al*., 2008; Hasan *et al*., 2016; Kose *et al*., 2018). Multivariate analysis can simplify and process data with large variables so that it is easier to interpret (Mattjik and Sumertajaya, 2011). The use of multivariate analysis in the development of selection index has been carried out by several researchers including Hasan *et al*. (2016) with discriminant analysis, Sabouri *et al*. (2008) with path analysis and Peternelli *et al*. (2017) with discriminant analysis and artificial neuron networks. The objective of the study was to develop selection index based on multivariate analysis and to select doubled-haploid rice lines adaptive to saline prone environment.

MATERIALS AND METHODS

Experimental materials

The genotypes used were 40 genotypes consisting of 36 doubled-haploid lines derived from anther culture of F1s and four control varieties. The control varieties were obtained from Indonesian Center for Rice Research (ICRR), namely Ciherang, Inpara 5, Inpari 29, and Inpari 34 Salin Agritan. Ciherang is a mega rice variety in Indonesia (Toledo *et al*., 2015) and based on electrophysiology measurement is tolerant to salinity at around 100 mM NaCl levels or EC 9.8 dS/m (Hariadi *et al*., 2015). Inpara 5 and Inpari 29 are high yielding and salt tolerant rice varieties (Safitri *et al*., 2017) which were used as parents in hybridization programme to obtain F1s for anther donor plant. Inpari 34 Salin Agritan is tolerant to salinity at seedling phase.

Experimental procedures

Planting and harvesting

Experiments were conducted from March to July 2018 in two sites representing normal and saline conditions. The experiment in normal condition was carried out in the Pusakanagara Experimental Station, Indonesian Center for Rice Research, Subang with coordinates point of 6°16′54.7″ S and 107°52′0.08″ E. The experiment in saline condition was done in the farmer field at Truntum, Subang with coordinate's point of 6°15′12.3″ S, and 107°44′0.71″ E. The site used for the experimental site in Truntum was located 100 m from the beach and previously the soil EC has been measured to gave a range of 6-15 dS/m, while water EC could reach 8-18 dS/m depending on the level of water. However, when the seedling was planted the level of salinity decrease to 2.0 dS/m due to irrigation water used for land preparation. One month after planting, the level of water EC slightly increase to 2.5–3 dS/m. Other observation showed that the water EC fluctuated until harvest in the range of 3-8 dS/m.

Each genotype was sown as much as 35 g in a seedbed of 0.25 m^2. After 21 days, the seedlings from the nursery were transplanted to the prepared paddy fields. Maintenance included fertilizing with a dose of 200 kg ha^{-1} Urea, 100 kg ha^{-1} SP-36, and 100 kg ha^{-1} KCl, control of plant pests and diseases, and irrigation. Urea fertilizer was applied in 3 stages with a proportion of 1/3 dose at planting time, 28 days after planting (DAP) and at 49 DAP, respectively. The KCl fertilizer was given in two stages with ½ dose at planting time and the remaining half at 49 DAP. Meanwhile, the SP-36 was given all doses at the planting time. Harvest was marked by 80% of rice grains turned yellow.

Experimental design

The experiment used a nested randomized complete block design (RCBD) with two factors namely genotype and environment. The replications were three times and nested in the environment. Based on the design, the total experimental units were 240. The plot area of the experimental unit was 8 m^2 with plant spacing of 25 cm x 25 cm.

Data collection

Observations of agronomic characters were carried out on 14 quantitative characters, namely vegetative plant height (60 days after sowing), reproductive plant height, number of total tillers, number of productive tiller, days to flower, flag leaf length, panicle length, 1000 grains weight, number of filled grain, number of unfilled grain, number of total grains, filled grain percentage, unfilled grain percentage, and productivity.

Data analysis

Statistical analysis used included analysis of variance, repeatability, multivariate analysis, and tolerance indices. Phenotypic and genetic correlation and genetic path analysis were carried out to determine yield supporting character. The principal component analysis was used for weighting the selection index. Then the selection index was evaluated by the index repeatability. The software's used in the analyses were META-R from CIMMYT 2016 for phenotypic and genetic correlation, STAR 2.0.1 from IRRI for analysis of variance, and Excel 2013 for genetic path analysis and repeatability.

Phenotypic and genetic correlation analysis

Correlation analysis aims at predicting the closeness of the relationship between a character to other characters. Correlation in breeding was divided into two, namely the phenotypic correlation which was the same as the general correlation and genetic correlation which eliminate environmental influences in correlation (Manjunatha *et al*., 2017). The phenotypic and genetic correlation coefficients were estimated by the following formula:

$$r_{gxy} = \frac{GCOV_{xy}}{\sqrt{\sigma_{gx}^{2} . \sigma_{gy}^{2}}} \qquad r_{pxy} = \frac{PCOV_{xy}}{\sqrt{\sigma_{px}^{2} . \sigma_{py}^{2}}}$$

Notes:

r_{pxy} = Phenotypic correlation coefficient between traits x and y;

r_{gxy} = Genotypic correlation coefficient between traits x and y;

σ_p^{2} = Phenotypic variance

σ_g^{2} = Genetic variance

PCOVxy = Phenotypic covariance between variables x and y; and

GCOVxy = Genotypic covariance between variables x and y (Kassahun *et al*., 2013).

Estimated salinity tolerance index

Salinity tolerance index for each genotype was calculated using the tolerant indices developed by several researchers as follow: Yield stability index (YSI) from Bouslama and Schapaugh (1984); Stress susceptibility index (SSI) from Fischer and Maurer (1978); Yield index (YI) from Gavuzzi *et al*. (1997); Mean productivity (MP) from Rosielle and Hamblin (1981); Tolerance index (TOL) from Rosielle and Hamblin (1981); Stress tolerance index (STI) and Geometric mean productivity (GMP) from Fernandez (1992).

Path analysis

The path analysis was used to identify components that have a direct or indirect impact on the rice yield character. It was based on equations as suggested by Singh and Chaudhary (2007) according to the following formula:

$$C = R_y R_x^{-1}$$

$$\begin{bmatrix} r_{1y} \\ r_{2y} \\ \vdots \\ r_{py} \end{bmatrix} = \begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1p} \\ r_{21} & r_{22} & \cdots & r_{2p} \\ \vdots & \vdots & \cdots & \vdots \\ r_{p1} & r_{p2} & \cdots & r_{pp} \end{bmatrix} \begin{bmatrix} C_1 \\ C_2 \\ \vdots \\ C_p \end{bmatrix}$$

Notes:

C = path coefficient

R_x^{-1} = inverse correlation matrix between independent characters

R_y = vector correlation coefficient between independent and dependent characters

Principal component analysis

The principal component analysis (PCA) was an analysis involved Eigen values as compressing complex dimensions into simple dimensions. The PCA algorithm was generally as follows (Jolliffe, 2002):

$$\alpha_k' x = \alpha_{k1}x_1 + \alpha_{k2}x_2 + \alpha_{k3}x_3 + \cdots + \alpha_{kp}x_p = \sum_{j=1}^{p} \alpha_{1p}x_p$$

Notes:

$\alpha_k' x$ = linear functions of the maximum variant elements x of *k*th principal components

α_{kp} = Constant vector *k* to *p*-variables,

(‵) = transpose from vector matrix

There were three ways which could be used to determine the number of principal components (PC). First, it was based on the total variance that could be explained more than 80%. The second way was to identify Eigen value PC > 1. The third way was to observe the scree plot by looking at the elbow fault from the scree plot (Mattjik and Sumertajaya, 2011). In this study, determination on the number of suitable PC in the PCA analysis was based on the cumulative proportion of more than 80%.

Repeatability of selection index

The repeatability of the selection index followed the heritability formula proposed by Nordskog (1978) as follows:

$$h_i^2 = \frac{b'Gb}{b'Pb}$$

Notes :

h_i^2 designated as R =character repeatability

b = regression coefficient vectors, (b') transpose of regression coefficient vector

G = genetic variance and covariance matrix

P = phenotypic variance and covariance matrix

RESULTS AND DISCUSSION

The ANOVA results showed that all characters were highly significant different toward their variance source with high repeatability value more than 50%, except for the number of filled grains which have repeatability value 45.22% (Table 1). It indicated that there were characters which genetically have different response in normal and saline condition (vegetative plant height, generative plant height, number of total tillers, days to flowering, flag leaf length, number of unfilled grain, 1000 grain weight, and yield) and other characters have stable response in both conditions (number of productive tillers, panicle length, number of filled grain, number of total grain, percentage of filled grain, and percentage of unfilled grain). The characters which have different response in normal and saline condition can be explained by the significant interaction between genotype and environment and vice versa (Akçura and Çeri, 2011). Based on Table 1, productivity as the main character has significant interaction. Therefore, it needs further analysis to select the adaptive genotype by minimizing environmental effects and their interactions so that the selected genotypes could maintain their adaptability in other sites.

The phenotypic and genetic correlations can be used to identify the influence of the yield components to productivity (Krishnamurthy *et al*., 2013). Both of these correlations were crucial in this study as productivity was polygenic (Kassahun *et al*., 2014) and its variance was significantly influenced by genotype and interactions of genotype and environment (Table 1). Based on both phenotype and genetic correlations (Table 2), number of total tillers and productive tillers were significantly and positively correlated to productivity, while generative plant height and days to flower showed the opposite correlation. Although the two correlations had almost the same results, there were two differences between the two. First, the genetic correlation had one added character (number of filled grains) which was significantly and negatively correlated with productivity. Second, the genetic correlation value was relatively higher than the phenotype correlation toward the significantly correlated characters. The higher value of genetic correlation

Table 1. Mean squares and genetic parameters of agronomic characters of doubled-haploid lines grown in normal and saline environments.

Characters	G	E	GxE	CV	Vg	Vp	R
VPH	105.71**	14193**	20.05*	3.73	14.28	17.62	81.03
GPH	120.59**	6826.67**	27.26**	3.35	15.56	20.10	77.39
NTT	24.41**	38.96tn	4.78*	11.25	3.27	4.07	80.42
NPT	5.45**	110.43tn	2.26tn	9.89	0.53	0.91	58.53
DF	76.80**	437.4**	8.18**	2.09	11.44	12.80	89.35
FLL	61.10**	362.60*	2.48**	7.22	9.77	10.18	95.94
PL	7.205**	320.17**	2.43tn	4.69	0.80	1.20	66.27
NFG	450.47*	4627.94tn	246.77tn	15.10	33.95	75.08	45.22
NUG	441.73**	24660**	108.31*	28.35	55.57	73.62	75.48
NTG	1053.97**	7922.36*	334.06tn	12.77	119.99	175.66	68.30
PFG	95.92**	8709.14**	18.52tn	6.09	12.90	15.99	80.69
PUG	95.92**	8709.14**	18.52tn	24.33	12.90	15.99	80.69
GW	10.08**	285.56*	3.58**	5.37	1.08	1.68	64.48
Productivity	1.81**	1.14tn	0.648*	12.13	0.19	0.30	64.20

Notes: ** significant at p(ɑ) 0.01, * significant at p(ɑ) 0.05, G = genotype, E= environment, CV= coefficient of variance, Vg= genetics variance, Vp = phenotypic variance, R = repeatability, VPH = vegetative plant height, GPH = generative plant height, NTT = number of total tiller, NPT = Number of productive tiller, DF = days to flowering, FLL= flag leaf length, PL = panicle length, NFG = number of filled grain, NUG = number of unfilled grain, NTG = number of total grain, PFG = percentage of filled grain, PUG = percentage of unfilled grain, GW = 1000 grains weight. tn = not significant

compared to the phenotype correlation was also reported by Fotokian and Agahi (2014) who worked on rice. According to Krishnamurthy *et al*. (2014) and Manjunatha *et al*. (2017), the low phenotypic correlation was caused by the high environmental influence on the total of variance between the two characters. Therefore, genetic correlation values were preferred as a basis for selection rather than the phenotypic correlation, especially in doubled-haploid rice lines. However, according to Krishnamurthy *et al*. (2014), the identification of the yield supporting characters based solely on correlation was still considered inaccurate because the correlation value was influenced by covariance of other characters. Therefore, the use of advanced analysis was important in determining the best supporting character, one of which through path analysis (Fotokian and Agahi, 2014).

Path analysis separates correlation values into direct and indirect effects to the main character (Manjunatha *et al*., 2017; Kose *et al*., 2018). Direct influence could be defined as the standard deviation given by a character to the total standard deviation of the main intended characters (Singh and Chaudhary, 2007). Path analysis was based on characters which were significantly correlated to phenotypic and genetic correlations, but the correlation value used as a basis of path analysis was only its genetic correlation. The genetic correlation was free from environmental effects, thus the determination of supporting characters would be more effective and efficient. The use of genetic correlation as the basis of path analysis in the identification of yield supporting characters was also reported by Krishnamurthy *et al*. (2014) in rice. Based on Table 2, the best character showing the greatest direct influence was number of productive tiller with a value of 0.43. This result was also in accordance with

Table 2. Phenotype (below diagonal) coefficient correlation, genetic coefficient correlation(above diagonal) among agronomic characters of rice , direct influence and indirect influence of important rice characters on doubled-haploid rice yield grown in normal and saline environments.

	VPH	GPH	NTT	NPT	DF	FLL	PL	NFG	NUG	NTG	PFG	PUG	GW	Pr
VPH		0.27	-0.63	-0.80	-0.26	0.73	0.84	-0.38	0.30	0.06	-0.37	0.37	0.71	-0.18
GPH	0.28		-0.04	-0.36	0.49	0.07	-0.17	0.83	0.05	0.40	0.20	-0.16	-0.48	-0.89**
NTT	-0.47	-0.06		0.73	0.06	-0.17	-0.37	0.29	-0.43	-0.16	0.45	-0.41	-0.64	0.50**
NPT	-0.57	-0.26	0.50		-0.48	-0.12	-0.28	-0.61	-0.64	-0.73	0.51	-0.51	-0.40	0.82**
DF	-0.28	0.33	-0.01	-0.28		-0.34	-0.42	1.00	0.68	1.00	-0.44	0.47	-0.47	-0.58**
FLL	0.60	0.00	-0.01	-0.10	-0.30		0.93	-0.34	0.33	0.04	-0.40	0.44	0.01	0.13
PL	0.66	-0.04	-0.23	-0.14	-0.42	0.75		-0.44	0.36	0.00	-0.41	0.45	0.13	0.17
NFG	-0.24	0.37	0.10	-0.17	0.60	-0.35	-0.21		0.50	0.76	-0.19	0.19	-0.82	-0.46**
NUG	0.27	-0.03	-0.36	-0.43	0.52	0.20	0.21	0.16		0.89	-0.98	1.00	0.19	-0.11
NTG	0.02	0.23	-0.18	-0.39	0.74	-0.10	0.01	0.76	0.77		-0.79	0.77	-0.29	-0.32
PFG	-0.32	0.17	0.34	0.36	-0.34	-0.35	-0.29	0.12	-0.95	-0.55		-1.00	-0.23	0.06
PUG	0.32	-0.17	-0.34	-0.36	0.34	0.35	0.29	-0.12	0.95	0.55	-1.00		-0.23	0.06
GW	0.56	-0.13	-0.46	-0.44	-0.32	0.02	0.27	-0.34	0.11	-0.15	-0.17	0.17		-0.26
Pr	-0.21	-0.62**	0.36**	0.49**	-0.45**	0.10	0.21	-0.14	-0.20	-0.22	0.13	-0.13	-0.01	

Characters	Direct effect	Indirect effect				Residual
		GPH	NTT	NPT	DF	
GPH	-0.71		-0.01	-0.15	-0.02	0.03
NTT	0.16	0.03		0.31	0.00	0.03
NPT	0.43	0.25	0.12		0.02	0.03
DF	-0.04	-0.34	0.01	-0.21		0.03

Notes: The significance was focused on the productivity character (Pr), ** significantly correlated at p(α) 0.01, VPH = vegetative plant height, GPH = generative plant height, NTT = number of total tiller, NPT = Number of productive tiller, DF = days to flowering, FLL= flag leaf length, PL = panicle length, NFG = number of filled grain, NUG = number of unfilled grain, NTG = number of total grain, PFG = percentage of filled grain, PUG = percentage of unfilled grain, GW = 1000 grains weight.

Table 3. Pearson coefficient correlation of several tolerance indices for productivity (Y) of doubled-haploid rice lines under normal and saline conditions.

Tolerance indices	Yp	Ys	YSI	YI	STI	GMP	SSI	MP	TOL
Yp	1.00								
Ys	0.47	1.00							
YSI	-0.52	0.50	1.00						
YI	0.48	1.00	0.49	1.00					
STI	0.86	0.85	-0.03	0.85	1.00				
GMP	0.86	0.85	-0.02	0.86	1.00	1.00			
SSI	0.52	-0.50	-1.00	-0.49	0.03	0.02	1.00		
MP	0.86	0.86	-0.02	0.86	1.00	1.00	0.02	1.00	
TOL	0.52	-0.50	-0.99	-0.50	0.02	0.02	0.99	0.02	1.00

Notes:Yp = productivity under normal condition, Ys = productivity under saline condition,YSI = yield stability index, YI = yield index, STI = stress tolerance index, GMP = geometric mean productivity, SSI = susceptibility index, MP = mean productivity (MP), TOL = tolerance index.

previous studies by Safitri *et al*. (2016) and Anshori *et al*. (2018) who reported that the number of tiller was a character that directly influenced yield and could predict productivity. Therefore, number of productive tiller can be used as supporting character in the selection index to obtain genotype adaptive to salinity stress.

Salinity tolerance indices were very important in the assessment of doubled-haploid lines adapted to salinity stress. The index can combine or become a midpoint in selecting tolerant doubled-haploid lines with high yield potential in saline environment (Singh *et al*., 2015). However, the determination of the best tolerance index was the key to select adaptive lines under salinity stress (Kamyab-Talesh *et al*., 2014). The best salinity tolerance index was able to correlate with productivity response in normal and saline environments so that the index could characterize the adaptability of a genotype (Ali and El-Sadek, 2016). Based on Pearson coefficient correlation of different tolerance indices for productivity (Table 3), the tolerance indices which have a good correlation to normal and saline condition with the value above 0.8 were stress tolerance index (STI), geometric mean productivity (GMP), and mean productivity (MP). Among the three tolerance indices, STI was more likely to be selected as the tolerance index in this study. STI has a dynamic concept because the tolerance index considered the average response of all genotype under normal condition to select the tolerant genotype, whereas GMP and MP did not include average response of all genotypes. The STI concept is similar to the dynamic stability concept which considered more adaptive than the static stability concept, thus the selected genotypes would maintain their adaptability when they were planted in other saline sites (Lin *et al*., 1986). The use of STI as a basis for the tolerance index in salinity stress was also reported by Anshori *et al*. (2018) when determining tolerant indices which can be used in selection of salinity tolerant rice genotypes using pot experiment in the green house.

The selection index in this study was developed based on the character of productivity and productive tiller according to the genetic correlation and genetic path analysis. Multivariate analysis used in determining character’s weight was the principal

Table 4. Eigen vectors, eigen values and variance of 10 principles components of the STI for agronomic character of doubled-haploid rice lines.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
VPH	0.224	-0.365	0.270	0.226	-0.089	0.171	0.160	0.190	-0.018	0.420
GPH	0.116	0.186	0.424	0.439	0.216	0.002	0.452	0.407	0.015	-0.158
NTT	-0.217	0.196	-0.306	0.273	0.151	0.761	0.168	-0.208	0.242	-0.043
NPT	-0.312	0.047	-0.337	-0.034	0.199	-0.417	0.675	-0.155	-0.166	0.163
DF	0.261	0.409	-0.025	-0.030	0.114	0.096	-0.059	0.026	-0.628	-0.414
FLL	0.113	-0.334	-0.208	0.457	0.261	0.019	-0.217	-0.229	-0.515	0.266
PL	0.137	-0.366	-0.117	0.419	-0.243	-0.228	0.078	-0.274	0.213	-0.628
NFG	0.115	0.417	0.049	0.254	-0.479	-0.076	0.026	-0.315	-0.031	0.283
NUG	0.435	0.041	-0.186	-0.114	0.008	0.013	0.129	0.064	0.059	0.043
NTG	0.361	0.270	-0.123	0.076	-0.302	-0.081	0.113	-0.116	0.096	0.185
PFG	-0.380	0.116	0.314	0.162	-0.158	-0.017	-0.096	-0.133	-0.182	0.020
PUG	0.408	-0.059	-0.261	-0.140	0.169	0.071	0.085	0.052	0.092	0.040
GW	0.060	-0.320	0.264	-0.398	-0.296	0.355	0.426	-0.303	-0.310	-0.117
Productivity	-0.214	-0.105	-0.441	0.088	-0.531	0.110	0.038	0.612	-0.240	-0.044
CP	0.337	0.574	0.711	0.824	0.891	0.928	0.955	0.971	0.983	0.992
EigenValues	4.720	3.320	1.915	1.584	0.940	0.520	0.371	0.219	0.166	0.131

Notes: STI= stress tolerance index, VPH = vegetative plant height, GPH = generative plant height, NTT = number of total tiller, NPT = Number of productive tiller, DF = days to flowering, FLL= flag leaf length, PL = panicle length, NFG = number of filled grain, NUG = number of unfilled grain, NTG = number of total grain, PFG = percentage of filled grain, PUG = percentage of unfilled grain, GW = 1000 grains weight. PC = principal component, CP = cumulative proportion.

components analysis (PCA). The PCA has been used by Godshalk and Timothy (1988) and Akbar *et al*. (2018) as weighting characters on the selection index. The principal component analysis can be used to compress a large dimension into a simpler dimension by retaining most of variance of the initial data. Each principal component produced was a variant eigenvector combination of all variables that are free from multi collinearity, thus PC results are not over estimated (Jolliffe, 2002). Akbar *et al*. (2018) reported that the eigenvector of the supporting characteristics of the selected PC had the same direction as the productivity eigenvector itself, so it was relevant to be used as the weighting base.

The PCA analysis, based on the cumulative proportion of more than 80% of total variance and by including all STI characters, indicated that there were four principal components (PC) which can be used as the references in weighting the selection index (Table 4). Then, the selection of the best weighting PC can be based on the dominance of productivity eigenvector which determines the variant direction in grouping genotypes on a particular PC (Akbar *et al*., 2018). Based on these principles, PC3 was the best PC as the weighting base. The negative value on the eigenvector only showed the absolute position of characters in the grouping quadrant (Jolliffe, 2002), thus the eigenvector value can be used as a weighting character. Based on PC 3, the resulting selection index formula was 0.441 productivity STI + 0.337 number of productive tiller STI. However, based on the results of path analysis (Table 2), number of productive tiller only have a direct effect around 0.43, thus the weight coefficient of number of productive tiller must be corrected by its direct influence to become 0.43 x 0.337 = 0.145. The use of path analysis results was also reported by Sabouri *et al*. (2008) in the development of the selection index. After correcting the weight coefficient of number of productive tiller, the selection index formula for adaptability of genotype to salinity was formulated as follow:

Selection index = 0.441 productivity STI + 0.145 number of productive tiller STI

The selection results based on the selection index showed 22 genotypes had an adaptability response to salinity above average or had a standardization index value (zAI) > 0 (Table 5). Standardizing index values was an objective way of determining the best line boundaries based on rank (Peternelli *et al*., 2017). Among the control variety, Ciherang, was classified as having better adaptability response. If the selection was based on the best control variety, 9 doubled-haploid lines showed better adaptability to salinity than Ciherang. Therefore, those nine doubled-haploid lines have very good adaptability in this study and can be continued to be used in the next evaluation.

The effectiveness and efficiency of the selection index in the doubled-haploid lines can be measured by the selection index repeatability, due to high homozygosity of doubled haploid lines. DH plants show high homozygosity for every locus in the genome, thus they do not have dominance gene action to affect their traits (Seymour *et al*., 2011), and that make all traits highly heritable. The repeatability of selection index was measured by combining variance and covariance to initial repeatability from all selection characters in index (Nordskog 1978). The repeatability based on Nordskog (1978) formula was 102.4%, which value exceeds the maximum limit of repeatability (100%), and then the selection index repeatability was considered 100%. Compared to the direct selection, which consider only productivity, the repeatability reached 64.2% (Table 1), thus selection index was more stable in genetic approach than only focused to yield or productivity. The repeatability value indicated that selection index was able to increase the genetic role of productivity so that index selection becomes more stable than direct selection. Therefore, the use of a selection index with a multivariate analysis approach was considered more effective than a single selection based on productivity such as in direct selection approach.

CONCLUSION

The number of productive tiller is the best-supporting character to be used as selection character along with productivity under salinity stress. Stress tolerance index (STI) is a dynamic tolerance index which can be used in determining tolerance index for salinity stress. The selection index formula produced related to adaptability of doubled-haploid rice line under salinity stress is 0.441 productivity + 0.145 productive tillers. It is considered effective and efficient based on the repeatability of the selection index. Based on the positive index selection value, 22 genotypes including Ciherang were considered adaptive to salinity stress and 9 of them had better adaptability responses than Ciherang.

Table 5. Mean, salinity tolerance index and standardized selection index for number of productive tiller and productivity of DH rice lines grown in normal and saline environments.

R	Genotype	NPT-P	Pr- P	NPT-T	Pr-T	NPT - Av	Pr - Av	NPT- STI	Pr - STI	AI	zAI
1	FU24	16.5	6.50	18.9	5.93	17.7	6.22	1.27	1.27	0.75	1.72
2	FU35	16.5	6.50	17.1	6.03	16.8	6.27	1.15	1.30	0.74	1.61
3	FU19	15.3	5.56	18.4	6.70	16.9	6.13	1.15	1.23	0.71	1.29
4	FU34	16.1	6.14	17.6	6.00	16.9	6.07	1.16	1.22	0.70	1.18
5	FU12	16.4	5.62	17.1	6.38	16.8	6.00	1.14	1.18	0.69	1.07
6	FU29	15.7	6.10	17.0	5.85	16.4	5.98	1.09	1.18	0.68	0.96
7	FU22	15.1	5.96	17.3	6.03	16.2	6.00	1.06	1.19	0.68	0.96
8	FU11	15.8	5.81	17.5	6.06	16.7	5.94	1.13	1.16	0.68	0.96
9	FU17	17.0	5.75	19.1	5.67	18.1	5.71	1.33	1.08	0.67	0.86
10	Ciherang	17.3	6.20	19.1	5.13	18.2	5.67	1.36	1.05	0.66	0.75
11	FU20	15.9	6.04	17.9	5.46	16.9	5.75	1.17	1.09	0.65	0.64
12	FU14	14.3	5.31	17.5	6.35	15.9	5.83	1.03	1.11	0.64	0.53
13	FU25	16.8	5.60	17.1	5.73	17.0	5.67	1.17	1.06	0.64	0.53
14	FU4	15.7	6.11	17.3	5.34	16.5	5.73	1.11	1.08	0.64	0.53
15	FU32	16.6	6.26	16.7	5.19	16.7	5.73	1.13	1.07	0.64	0.53
16	FU23	14.5	5.79	17.3	5.58	15.9	5.69	1.03	1.07	0.62	0.31
17	FU30	15.3	5.74	16.2	5.62	15.8	5.68	1.01	1.07	0.62	0.31
18	FU28	16.1	5.58	17.7	5.24	16.9	5.41	1.17	0.97	0.60	0.10
19	FU10	14.3	5.61	16.7	5.63	15.5	5.62	0.97	1.04	0.60	0.10
20	FU26	15.7	5.64	16.2	5.42	16.0	5.53	1.04	1.01	0.60	0.10
21	FU15	15.4	5.42	14.7	5.84	15.1	5.63	0.93	1.05	0.60	0.10
22	FU31	16.5	5.52	17.9	5.33	17.2	5.43	1.21	0.97	0.60	0.10
23	FU21	15.1	5.30	17.2	5.60	16.2	5.45	1.06	0.98	0.59	-0.01
24	FU9	16.2	5.75	17.1	5.10	16.7	5.43	1.13	0.97	0.59	-0.01
25	FU16	17.7	5.55	16.3	5.12	17.0	5.34	1.18	0.94	0.58	-0.12
26	Inpara 5	16.9	5.62	18.9	4.72	17.9	5.17	1.30	0.88	0.58	-0.12
27	FU33	14.7	5.82	16.9	5.03	15.8	5.43	1.01	0.97	0.57	-0.23
28	FU18	16.8	5.99	16.3	4.69	16.6	5.34	1.12	0.93	0.57	-0.23
29	FU1	15.8	5.48	19.5	4.74	17.7	5.11	1.26	0.86	0.56	-0.34
30	FU13	14.9	5.30	16.7	5.20	15.8	5.25	1.02	0.91	0.55	-0.44
31	FU27	14.5	5.68	17.4	4.74	16.0	5.21	1.03	0.89	0.54	-0.55
32	FU2	15.8	5.07	16.0	5.12	15.9	5.10	1.03	0.86	0.53	-0.66
33	Inpari 29	15.2	5.30	17.7	4.86	16.5	5.08	1.10	0.85	0.53	-0.66
34	FU3	16.9	5.19	15.7	4.82	16.3	5.01	1.08	0.83	0.52	-0.77
35	FU8	13.3	4.48	15.8	5.24	14.6	4.86	0.86	0.78	0.47	-1.31
36	FU5	14.8	3.98	15.0	5.65	14.9	4.82	0.91	0.74	0.46	-1.42
37	FU7	14.5	4.34	14.3	5.00	14.4	4.67	0.84	0.72	0.44	-1.64
38	FU6	14.1	4.37	14.1	5.11	14.1	4.74	0.81	0.74	0.44	-1.64
39	Inpari 34 Salin Agritan	15.5	4.05	16.7	3.58	16.1	3.82	1.06	0.48	0.36	-2.50
40	FU36	14.5	4.02	16.1	3.68	15.3	3.85	0.95	0.49	0.35	-2.61

Notes: R= rank, NPT = number of productive tiller, Pr = productivity, P = Pusakanagara, T = Truntum, Av = average, STI = stress tolerance index, AI = adaptability index, zAI = standardized adaptability index.

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REFERENCES

Akçura M, Ceri S(2011). Evaluation of drought tolerance indices for selection of Turkish oat (*Avena sativa* L.) landraces under various environmental conditions. *Žemdirbystė Agric.* 98: 157-166.

Ali MB, El-Sadek A (2016). Evaluation of drought tolerance indices for wheat (*Triticum aestivum* L.) under irrigated and rainfed conditions. *Commun. in Biometry and Crop Sci.* 11: 77-89.

Akbar MR, Purwoko BS, Dewi IS, Suwarno WB, Sugiyanta (2018). Agronomic and drought tolerance evaluation of doubled haploid rice breeding lines derived from anther culture. *SABRAO J. Breed. Genet*. 50: 115-128.

Anshori MF, Purwoko BS, Dewi IS, Ardie SW, Suwarno WB, Safitri H (2018). Determination of selection criteria for screening of rice genotypes for salinity tolerance. *SABRAO J. Breed. Genet.* 50: 279-294.

Aydinalp C, Cresser MS (2008). The effects of global climate change on agriculture. *American-Eurasian J. Agric. Environ. Sci.* 3: 672-676.

Bouslama M, Schapaugh WT (1984). Stress tolerance in soybean. Part. 1: Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci*. 24: 933-937.

Fernandez GCJ (1992). Effective selection criteria for assessing stress tolerance. In: C.G. Kuo, eds., *Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress*. AVRDC Publication, Tainan, pp. 257-270.

Fischer RA, Maurer R(1978). Drought resistance in spring wheat cultivars, 1. Grain yield response. *Aust. J. Agric. Res.*29: 897-907.

Fotokian MH, Agahi K (2014). Genetic worth and stability of selection indices in rice (*Oryza sativa* L.). *Progress in Biol. Sci.* 4: 153-166.

Gavuzzi P, Rizza F, Palumbo M, Campaline RG, Ricciardi GL, Borghi B (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Can. J. Plant Sci.* 77: 523-531.

Godshalk EB, Timothy EB (1988). Factor and principal component analyses as alternatives to index selection. *Theor. Appl. Genet.*76 : 352-360.

Hariadi YC, Nurhayati AY, Soeparjono S, Arif I (2015). Screening six varieties of rice (*Oryza sativa*) for salinity tolerance. *Procedia Environ. Sci.* 28 : 78 – 87.

Hasan R, Akand M, Alam N, Bashar A, Huque AKMM (2016). Genetic association analysis and selection indices for yield attributing traits in available chilli (*Capsicum annum* L.) genotypes. *Mol. Plant Breed.*7: 1-9.

Ismail AM, Platten JD, Miro B (2013). Physiological bases of tolerance of abiotic stresses in rice and mechanisms of adaptation. *Oryza*. 50: 91-99.

Jolliffe IT (2002). Principal Component Analysis, Second Ed., Springer-Verlag, New York, USA, pp. 167-169.

Kamyab-Talesh F, Mausavi SF, Asadi R, Rezaei M, Khaledian MR (2014). Evaluation of some rice cultivars response to salinity stress using resistance indices. *Arch. Agron. Soil Sci.* 2014: 1-12.

Kassahun BM, Alemaw G, Tesfaye B (2013). Correlation studies and path coefficient analysis for seed yield and yield components in Ethiopian coriander accessions. *Afr. Crop Sci. J*. 21: 51-59.

Kose A, Onder O, Bilir O, Kosar F (2018). Application of multivariate statistical analysis for breeding strategies of spring safflower (*Carthamus tinctorius* L.). *Turk. J. Field Crops*. 23:12-19.

Krishnamurthy SL, Sharma SK, Gautama RK, Kumar V (2014). Path and association analysis and stress indices for salinity tolerance traits in promising rice (*Oryza sativa* L.) genotypes. *Cereal Res. Commun*. 42 : 474-483.

Lin CS, Binns MR, Lefkovirch LP (1986). Stability analysis: where do we stand? *Crop Sc*i. 26: 894-900.

Linh LH, Linh TH, Xuan TD, Ham LH, Ismail AM, Khanh TD (2012). Molecular breeding to improve salt tolerance of rice (*Oryza sativa* L.)

in the Red River Delta of Vietnam. *Int. J. Plant Genomics.* ID 949038 : 1-9.

Manjunatha GA, Kumar MS, Jayashree M (2017). Character association and path analysis in rice (*Oryaza sativa* L.) genotypes evaluated under organic management. *J. Pharmacogn. Phytochem*. 6: 1053-1058.

Mattjik AA, Sumertajaya IM (2011). Sidik Peubah Ganda dengan Menggunakan SAS. Statistika F-MIPA IPB, Bogor, Indonesia, pp. 119-128.

Nordskog AW (1978). Some statistical properties of an index of multiple traits.*Theor. Appl. Genet.* 52: 91–94.

Peternelli LA, Moreira EFA, Nascimento M, Cruz CD(2017). Artificial neural networks and linear discriminant analysis in early selection among sugarcane families. *Crop Breed. Appl. Biotechnol.*17: 299-305.

Rosielle AA, Hamblin J (1981). Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci*. 21: 943-946.

Sabouri H, Rabiei B, Fazlalipour M (2008). Use of selection indices based on multivariate analysis for improving grain yield in rice. *Rice Sci*. 15:303-310.

Safitri H, Purwoko BS, DewiIS, Ardie SW (2016). Morpho-physiological response of rice genotypes grown under saline conditions. *ISSAAS J.*22: 52-63.

Safitri H, Purwoko BS, Dewi IS, Ardie SW (2017). Salinity tolerance of several rice genotypes at seedling stage. *Indonesian J. Agric. Sci*. 18: 63-68.

Seymour DK, Filiault DL, Henry IM, Monson-Miller J, Ravi M, Pang A, Comai L, Chan SWL, Maloof JN (2011). Rapid creation of Arabidopsis doubled haploid lines for quantitative trait locus mapping. *PNAS* 109: 4227-4232.

Singh RK, Chaudhary BD(2007). Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publisher, New Delhi, India, pp. 69-78.

Singh S, Sengar RS, Kulshreshtha N, Datta D, Tomar RS, Rao VP, Garg D, Ojha A (2015). Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). *J. Agric. Sci*. 7: 49-57.

Toledo AMU, Ignacio JCI, Casal Jr. C, Gonzaga ZJ, Mendioro MS, Septiningsih E.M (2015). Development of improved Ciherang-*sub*1 having tolerance to anaerobic germination conditions. *Plant Breed. Biotech*. 3:77-87.