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Drought tolerance indices for selection of drought tolerant, high yielding upland rice genotypes

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Abstract

The purposes of this study were (1) to assess the effectiveness of drought tolerance indices for selection of drought tolerance in upland rice, and (2) to identify the most suitable drought tolerance indices to select for drought tolerant, high yielding upland rice genotypes. This study employed a Split Plot design consisting of irrigation levels as the main plots, and rice genotype as the sub-plot treatments. There were three main plots: 100% field capacity (FC); 75% FC; and 50% FC level. The subplot treatments consisted of 40 upland rice genotypes. Grain yields under no-stress and stressed conditions were used to calculate drought tolerance indices. There were significant variations in grain yields and drought tolerance indices between different rice genotype treatments. The indices from the literature found to be most suitable for the selection of drought tolerant upland rice cultivars were STI, GMP, MRP, HARM, REI, ATI, YI, SNPI. Ten genotypes from among the 40 tested – namely HK-07, ADN-04, PMK-01, ADN-05, NGR-022, ALR-02, HK-06, and KMD-01 – were selected as combining drought tolerance with high yield potential.

Keywords: upland rice, genotypes, drought tolerance index, grain yield.

Abbreviations: SSI_stress susceptibility index; RDI_relative drought index; STI_stress tolerance index; MP_mean productivity; DRI_drought response index; GMP_geometric mean productivity**;** REI_relative efficiency index; MRP_mean relative performance; MISTIk1_modified STI1; MISTIk2_modified STI2; ATI_abiotic tolerance index; SSPI_stress susceptibility percentage index; SNPI_stress/non-stress production index; HRM_harmonic mean yield; RDY_relative decrease in yield.

Introduction

One of the major abiotic stresses influencing crop productivity is water deficit. This effect is more pronounced in crops such as rice that mainly completes its life cycle under water saturated condition. Water stress is, therefore, a major abiotic constraint for rice productivity worldwide, most notably in Asia (Bray et al., 2000; Kumbhar et al., 2015). Diminishing water supplies for agriculture worldwide is an increasing trend. This necessitates the search for drought adaptation in crops like rice. Screening for rice varieties that are tolerant of water stress is seen as an important step in sustaining future development of rice production (Pandey and Shukla, 2015).

Selection of drought tolerance is more urgent for upland rice than for lowland, fully irrigated rice. Upland rice is generally cultivated under water-unsaturated conditions, especially during the recent phenomena of prolonged droughts due probably to global climate change (Halliwell, 2006). Improvement of rice adaptation to drought, via selection of drought-tolerant varieties, relies on the availability of a gene pool for the desired traits. Upland rice germplasm is an invaluable genetic resource that can be employed for improvement of drought tolerance.

Improvement of drought tolerance requires an understanding of plant diversity in relation to adaptation to drought (Alonso-Blanco et al., 2009**),** followed by design of the most effective selection strategy for plant survival and productivity under conditions of moisture-stress (Sarkar et al., 2013). Evaluation of genotypic variability under different moisture stress conditions is an essential step for a successful breeding program focused on drought tolerance (Abenavoli et al., 2016; Anower et al., 2015).

A collection of pigmented upland rice germplasm from East Nusa Tenggara, Indonesia has previously been evaluated for genetic diversity (Mau et al., 2017) and for resistance to blast (Mau et al., 2018). Pigmented rice with red and black/purple pericarp is rich in carbohydrate, protein, vitamins, mineral content, and also anthocyanins, which confer various health-promoting properties (Tsuda et al., 2002**;** Hyun et al., 2004**;** Nam et al., 2006; Shao et al., 2011). This germplasm collection represents a gene pool that can be explored for selection and improvement of drought tolerance in upland rice, as well as for the production of superior pigmented upland rice cultivars that are currently of limited availability in Indonesia (Santika and Rozakurniati, 2010; IAARD, 2012) and perhaps in other parts of the world as well.

Some studies have investigated the use of osmo-priming compounds such NaCl, KCl or polyethylene glycol (PEG) to assess the effect of soil moisture stress on seed germination and on seedling growth (Islam et al., 2012; Singh et al., 2017a; Swapna and Shylaraj 2017; Mishra and Panda 2017). These priming methods, require specific standard methods to validate the moisture stress generated under field conditions (Singh et al., 2017b). Other studies on drought resistance in rice have frequently used indirect selection indicators such as morphological and physiological responses (Fukai and Cooper 1995; Rahman et al., 2002; Yue et al., 2006; Farooq et al., 2010; Kumar et al., 2015; Singh et al., 2017b; Purbajanti et al., 2017). Nevertheless, such indirect selection methods are more labor and resource intensive, as many characters need to be evaluated and the selected tolerant genotypes may not necessarily be high yielding. For this reason, selection for drought tolerance based on indices developed from grain yield is considered a more rapid and effective approach to selecting genotypes that combine drought tolerance with general high yield potential.

Many indices of drought tolerance have previously been proposed for use in selection of drought tolerant genotypes in various crops. These indices include stress susceptibility index (SSI) (Fischer and Maurer, 1978); relative drought index (RDI) (Fischer and Wood, 1979); mean productivity (MP) (Rosielle and Hamblin, 1981); drought response index (DRI) (Bidinger et al., 1987); stress tolerance index (STI) and geometric mean productivity (GMP) (Fernandez 1992); relative efficiency index (REI) and mean relative performance (MRP) (Hossain et al., 1999; modified STI1 (MISTIk1) and modified STI2 (MSTIK2) (Farshadfar and Sutka, 2002). More recently, proposals for indices of drought tolerance have included abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI), and stress/non-stress production index (SNPI) (Moosavi et al., 2008); harmonic mean yield (HARM) (Dadbakhsh et al., 2011); and relative decrease in yield (RDY) (Farshadfar and Elyasi, 2012).

In summary, to date, more than 20 indices have been employed for selection of drought-tolerant varieties, with varying degree of effectiveness depending on the particular crop species. Among these, STI, SSI, MP, MRP, HARM, REI, ATI, GMP, MSTIK1, MSTIK2, SNPI, SSPI, YI, RDI, and RDY have previously been reported to be the most suitable indices for various crops (Naghavi et al., 2003; Sio-Se et al., 2006; Moosavi et al., 2008; Bahar and Yildirim, 2010**;** Golbashy et al., 2010; Dadbakhsh et al., 2011; Farshadfar and Elyasi, 2012; Mau et al., 2014; Ali and El-Sadek, 2016; Bennani et al., 2017) but these have not been assessed in upland rice. Thus, the purposes of the study reported in this paper were (1) to assess the effectiveness of drought tolerance indices for selection of drought tolerance in upland rice, and (2) to determine the most suitable index for identifying drought tolerant, high-yielding upland rice genotypes.

Results

Grain yield and drought tolerance indices

The grain yield was recorded from rice plots grown under non-stressed (100% field capacity (FC) and drought-stressed conditions (75% FC/Stress Level 1 and 50% FC/Stress Level 2). Two-way ANOVA (Table 1) showed that there were highly significant differences in grain yield between genotypes (P<0.001) under both non-stressed and stressed conditions. The mean grain yield under no stress conditions was the highest (3.43 t/ha) (Table 2), which can be considered as the potential yield (YP). The mean yield at 75% FC (2.66 t/ha) was 22% lower than the potential yield while that at 50% FC (2.28 t/ha) was 34% lower than the potential yield. The stress intensity at 75% FC (SL 1) was, therefore, considered mild with a stress intensity value of 0.22 while that at 50% FC (SL 2) was considered moderate with a stress intensity level of 0.34.

Interaction between drought stress level (SL) and genotype (G) affected all drought indices either significantly or highly significantly. Five drought tolerance indices, i.e. REI, ATI, SNPI and YSI differed between the two stress levels (SL1 0.22 and SL2 0.34), whereas the drought indices STI, GMP, MRP, HARM, MP, YI, MSTIK1, RDI, SSI and MSTIK2 were stable across the two stress levels. As there was no real difference in grain yield (YS) between SL1 and SL2, and because most of the drought indices were stable across the two stress levels, only the data from the higher stress level (SL2 with 0.34 /moderate stress intensity) was used for the evaluation described below in the rest of this paper.

Drought tolerance indices and grain yield under non-stressed and moderately stressed conditions (SL2 0.34) are presented in Table 2. Table 2 shows that the grain yield for genotypes under no-stress conditions ranged from 1.73 to 7.39 t ha^{-1} (mean of 3.43 t ha^{-1}) while that at 0.34 stress intensity ranged from 1.33to 3.88 t ha^{-1} (mean of 2.28 t ha^{-1}). Based on a ranking method and a 25% selection intensity, ten best genotypes were selected: i.e. ADN-04, HK-07, PMK-01, SBD-02, ADN-05, ISN-03, SBD-04, MGR-04, NGR-022, and HK-06. Interestingly, nine of these ten genotypes, i.e. all except SBD-02, ranked among the best ten within each of the drought indices STI, GMP, MRP, HARM, and ATI and in grain yield (YP and YS). Of the remaining indices, seven genotypes were selected by REI, four genotypes by YI and MSTIK1, and two genotypes by SNPI.

Correlation among yield and drought tolerance indices

Table 3 shows the coefficients of correlation among grain yield and drought tolerance indices under 0.34 stress levels. Yield (YP) under non-stressed conditions was highly correlated with yield (YS) under 0.34 stress level. YP and YS were also highly significantly and positively associated with drought indices STI, GMP, MRP, HARM, MP, REI, and ATI at 0.34 stress level (Table 3). At the moderate drought stress level (0.34), correlation between each of the drought indices YI, MISTIK1, SSPI, and SSI was significant only with YP. A similar situation was found for the indices SNPI and MISTIK2 but the direction of the correlation was negative. YS and RDI were negatively correlated with YS but their correlations with YP were absent at 0.34 stress level.

Under the 0.34 stress level, a perfect correlation was found between drought indices STI, GMP, MRP, HARM, and MP. A similar tight correlation was found between another cluster of drought indices: YI, MSTIK1, SSPI, and RDI. SSPI was significantly and negatively correlated with almost all indices. As with SSPI, MISTK2 also showed significant and negative correlation with other indices, except with HARM. SSI had a significant and positive correlation with other indicators but its correlation with SNPI was negative.

Principal component analysis

Results of Principal Component Analysis (PCA) applied to the variables in the correlation coefficient matrix revealed that two principal components explained 96.79% variations for drought tolerance indices. The first component explained about 62.99% and the second explained 33.80% of the observed variations.

The results also showed that PC1 was positively correlated with almost all drought indices, except SNPI and MSTIK2 which were negative (Fig 1). PC2 had a positive relationship with drought indices STI, GMP, MP, HARM, MRP, ATI, SNPI and MSTIK2 and a negative relationship with YI, YSI, MSTIK1, RDI, and SSI, while its relationship with REI and SSPI was negligible.

The biplot diagram (Fig 1) shows that drought indices STI, GMP, GM, HARM, ATI, and MRP were the best indices to select for drought tolerant genotypes with high and stable grain yield, i.e. genotypes 29 (HK-07), 31 (KMD-01), 38 (ADN-05), 22 (NGR-22), 4 (ALR-02) and 6 (ADN-04).

Discussion

This study revealed a decrease in mean grain yield of 34% in the drought-stressed main plot in which soil moisture declined to 50% field capacity level in the post flowering stage of the rice life cycle, relative to the unstressed main plot treatment in which irrigation ensured field capacity through until harvest. Decrease of grain yield due to drought stress in cereal crops has been reported previously by many workers (Rahman et al., 2002; Yue et al., 2006; Golabadi et al., 2006; Moosavi et al., 2008; Dadbakhsh et al., 2011; Farshadfar and Elyasi, 2012; Dixit et al., 2014; Bennani et al., 2017). The drought stress-induced grain yield reduction reported here was of moderate level. Moderate drought stress is reported to be suitable for selecting drought-tolerant genotypes in wheat (Ali and El-Sadek, 2016).

Under the level of moderate stress (SL2: 0.34 stress level), ten best genotypes (HK-06, HK-07, ADN-04, KMD-01, ALR-01, PMK-01, NGR-022, ADN-05, MGP-01, and SBD-02) were selected based on mean rank of grain yield. Seven of these genotypes were among the best ten genotypes in the drought indices STI, GMP, MRP, HARM, REI, ATI, YI, SNPI and in grain yields YP and YS. These findings imply that selecting drought tolerant genotypes based on mean rank of multiple drought indices is effective but additional methods of selection based on correlation analysis, principal component analysis, and bi-plot diagram may need to be included to make the selection more effective. A previous study by Farshadfar and Elyasi (2012) successfully used a mean-rank method in combination with correlation analysis, and Principal Component Analysis to select for drought tolerant genotypes of bread wheat landraces. Drought indices such as STI, MP, GMP, MRP, HARM, RDI, ATI, REI, and MISTIK1 have also been reported to be effectively used to select drought tolerant, high yielding genotypes in various crops (Dadbakhsh et al., 2011; Farshadfar and Elyasi, 2012; Naghavi et al., 2013; Ali and El-Sadek, 2016; Bennani et al., 2017).

Results in this study indicated a positive and highly significant correlation between YP and YS under moderate/0.34 stress level, suggesting that genotypes that showed high grain yield under non-stressed condition also demonstrated high yield under stressed condition. A positive correlation between YP and YS has also been reported in earlier studies (Moosavi et al., 2008; Dadbakhsh et al., 2011; Naghavi et al., 2013; Bennani et al., 2017). The drought indices STI, GMP, MRP, HARM, MP, REI and ATI also showed highly significant correlations with YP and YS, which suggests that the drought indices are able to identify high yielding genotypes under non-stressed and stressed conditions. The same drought indices were also previously reported by Bennani et al. (2017) to have a positive and high correlation with YP and YS in bread wheat. Farshadfar and Elyasi (2012) and Moosavi et al. (2008) also observed a positive and significant correlation between YP and YS with STI, GMP, MP, ATI, and other indices in bread wheat. Positive and significant correlation between STI, GMP and MP with YP and YS have also been previously reported by other workers (Golabadi et al., 2006; Jafari et al., 2009; İlker et al., 2011*;* Toorchi et al., 2012). The high correlations between YP, YS and drought indices may serve as good indicators for selection of the best indices and the best genotypes. Drought indices having a significant correlation with grain yield in both non-stressed and stressed conditions are reported to be suitable for selecting drought tolerant genotypes (Mitra, 2001).

A perfect correlation was observed between drought indices STI, GMP, MRP, HARM, and MP; near perfect correlations occurred also for another cluster of drought indices YI, MSTIK1, SSPI, and RDI. The results suggest that drought indices that show perfect correlation can perhaps be used interchangeably. Interchangeable drought indices have also been reported in wheat by Ali and El-Sadek (2016) and Bennani et al. (2017).

Drought indices showing positive and significant correlation with YP and YS, i.e., STI, GMP, MRP, HARM, MP, REI, and ATI were also the indices that selected the ten best genotypes based on mean-rank of all employed drought indices and YP and YS. Thus STI, GMP, MRP, HARM, MP, REI, and ATI are suitable indices to select for drought tolerant and high yielding upland rice genotypes. As with ranking method, correlation analysis, PC analysis, and bi-plot diagram showed that the drought indices STI, GMP, GM, HARM, ATI, MRP, and REI are the most suitable indices to select for drought tolerant genotypes.

Upland rice genotypes number 29 (HK-07), 6 (ADN-04), 37 (PMK-01), 38 (ADN-05), 22 (NGR-22), and 4 (ALR-02) were selected as the best genotypes based on drought indices STI, GMP, GM, HARM, ATI, and MRP. Interestingly, six (HK-07, ADN-04, PMK-01, ADN-05, NGR-022, and ALR-02) among the seven selected genotypes were also selected based on the ranking method using drought indices STI, GMP, MRP, HARM, REI, ATI, YI, SNPI. The present study results were in line with the results of previous workers (Dadbakhsh et al., 2011; Farshadfar and Elyasi, 2012; Naghavi et al., 2013; Ali and El-Sadek, 2016; Bennani et al., 2017) where the drought indices STI, GMP, MRP, HARM, REI, ATI, YI, SNPI have also been reported to be the most suitable for selecting for drought tolerant genotypes in various crops.

Overall, the present study results revealed that drought indices STI, GMP, GM, HARM, ATI, MRP, REI, YI, and SNPI are the most suitable drought indices to select for drought tolerant upland rice genotypes. By considering various methods of selection employing these multiple drought indices, eight upland rice genotypes were selected, i.e., HK-07, ADN-04, PMK-01, ADN-05, NGR-022, ALR-02, HK-06, and KMD-01. These genotypes are drought tolerant and also high yielding in both non-stressed and moderate drought-stress conditions. All these are pigmented (red and black/purple pericarp) upland rice genotypes (Mau et al., 2017; Mau et al., 2018), while four of them (ADN-04, KMD-01, HK-06,

Table 1. Results from analyses of variance of grain yield and of estimated indices of drought tolerance for 40 upland rice genotypes grown under three different soil moisture regimes (non-stressed; mildy stressful SL1 0.22; and moderately stressful SL2 0.34); mean squares for soil moisture regimes for genotypes, and for their interaction.

Source of Variation	Two-way ANOVA			ANOVA SL0: No stress	ANOVA SL1: 0.22	ANOVA SL2: 0.34
	Stress level (SL)	Genotype (G)	SL x G	Genotype Effect	Genotype Effect	Genotype Effect
Grain Yield (YP and YS)	27.5239***	4.0110***	0.4336**	2.6518***	-	-
Grain Yield (YS)	5.9287	1.9124**	0.3140**		1.5532**	0.6773**
STI	0.8744	1.0391**	1.0391**		0.7604**	0.3438**
GMP	2.2328	2.9556***	0.1207**		1.9200**	1.1594**
MRP	0.0001	1.4040***	0.0427**		0.8434**	0.6021**
HARM	3.0562	2.7560***	0.1686**		1.8841**	1.0397**
MP	1.4822	3.1931***	0.0785**		1.9573**	1.3139**
REI	24.2597*	0.9331**	0.4088*		1.2646**	0.0786**
ATI	46.9413**	5.7792**	2.3337**		7.4649**	0.6507**
YI	0.0001	0.4527**	0.2010**		0.2196**	0.4340**
MSTIK1	1.8474	0.2767**	0.1155**		0.0939**	0.2977**
SNPI	1139.5062*	13.9499**	13.5995**		26.3757**	1.1737**
SSPI	46.9413***	5.7702**	2.3337**		7.469**	0.6507**
YSI	8.7473*	0.0209**	0.0506**		0.0283**	0.0437**
RDI	11.2912	0.0437**	01016**		0.0465**	0.0990**
SSI	0.1781	0.6057**	0.2347**		0.5267**	0.3153**
MSTIK2	2.9241	0.1992**	0.0833*		0.0774**	0.2053**

$^{*, **, ***}$: significance at $p < 0.05$, 0.01, and 0.001 respectively.

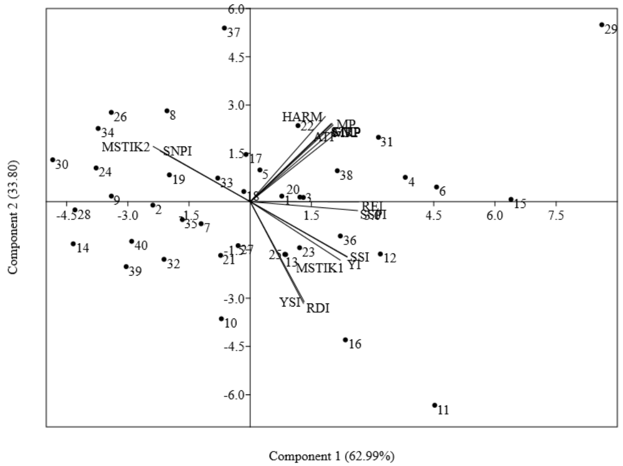

Fig 1. Biplot diagram for drought tolerance indices in 0.34 stress level. (Genotypes – 1:ADN-03, 2:TLB-04, 3:SBD-02, 4: ALR-01, 5:NGR-01, 6:ADN-04, 7:P.WANGI, 8:SBD-04, 9:SBR-01,10:CBL-01, 11:PJ-01, 12:MANU-04, 13:SLR-07, 14:TLB-04, 15:HK-06, 16:TLB-02, 17:SBD-03, 18:MGR-04, 19:NGR-21, 20:WTN-21, 21:WTN-22, 22:NGR-22: 23:PAU-01, 24:SBD-05, 25:ISN-03, 26:ISN-02, 27:PM-01, 28:BLU-01, 29:HK-07, 30:SLT-01, 31:KMD-01, 32:SBD-01, 33:G.MUNGKUR, 34:AEK SIBUND., 35:ALR-02, 36:MGP-01, 37:PMK-01,38: ADN-05, 39:SBD-12, 40.IR-20)

Table 2. Mean grain yield and drought tolerance indices for 40 upland rice genotypes grown under non-stressed conditions (i.e. on soils at field capacity throughout) and under moderately stressed conditions (on soils at 50% field capacity during the post flowering phase of plant development).

Genotype	YP	YS	STI	GMP	MRP	HARM	MP	REI	ATI	YI	MSTIK1	SNPI	SSPI	YSI	RDI	SSI	MSTIK2
ADN-03	3.66	2.4	0.75	2.96	2.12	2.9	3.03	0.37	1.05	1.12	1.52	4.41	18.3	0.32	0.49	0.96	1.49
TLB-05	2.55	2.01	0.44	2.27	1.63	2.25	2.28	0.17	0.6	0.7	1.27	5.33	7.89	0.26	0.39	0.59	1.8
SBD-02	3.84	2.44	0.8	3.06	2.19	2.98	3.14	0.4	1.1	1.2	1.58	4.3	20.5	0.34	0.51	1.03	1.44
ALR-01	4.93	2.67	1.11	3.61	2.61	3.44	3.8	0.59	1.54	1.45	1.86	3.99	32.9	0.35	0.53	1.26	1.24
NGR-01	3.66	2.58	0.8	3.07	2.2	3.02	3.12	0.32	1.07	0.94	1.43	4.79	15.7	0.27	0.41	0.82	1.6
ADN-04	5.16	2.61	1.15	3.67	2.65	3.47	3.88	0.65	1.59	1.62	1.97	3.79	37.1	0.38	0.57	1.38	1.16
P. WANGI	2.81	2.03	0.48	2.39	1.71	2.35	2.42	0.24	0.68	0.92	1.39	4.78	11.5	0.33	0.5	0.78	1.64
SBD-04	3.34	2.88	0.82	3.1	2.24	3.09	3.11	0.15	0.92	0.45	1.16	6.25	6.64	0.12	0.19	0.38	1.96
SBR-01	2.3	1.94	0.38	2.11	1.52	2.1	2.12	0.12	0.5	0.52	1.19	5.9	5.35	0.21	0.32	0.45	1.91
CBL-01	2.36	1.47	0.29	1.86	1.33	1.81	1.92	0.25	0.34	1.22	1.62	4.27	13.1	0.57	0.86	1.06	1.41
PJ-01	3.97	1.33	0.45	2.29	1.74	1.98	2.65	0.55	0.28	2.19	3	3.29	38.6	0.81	1.22	1.86	0.77
MANU-04	4.24	2.12	0.77	2.99	2.17	2.82	3.18	0.54	1.03	1.65	2.01	3.76	30.9	0.47	0.71	1.41	1.14
SLR-07	3.34	2	0.57	2.58	1.86	2.5	2.67	0.37	0.77	1.33	1.68	4.14	19.6	0.44	0.66	1.12	1.37
TLB-04	1.73	1.42	0.21	1.56	1.13	1.55	1.57	0.09	0.23	0.52	1.23	6.9	4.38	0.31	0.47	0.47	1.88
HK-06	5.92	2.54	1.27	3.86	2.84	3.53	4.23	0.79	1.71	1.85	2.38	3.56	49.4	0.42	0.63	1.6	0.97
TLB-02	3.39	1.58	0.46	2.31	1.68	2.15	2.48	0.45	0.47	1.76	2.15	3.65	26.5	0.65	0.98	1.5	1.06
SBD-03	3.65	2.69	0.83	3.13	2.24	3.08	3.17	0.29	1.08	0.84	1.37	5.04	14	0.24	0.36	0.74	1.67
MGR-04	3.37	2.39	0.7	2.84	2.03	2.8	2.88	0.3	0.92	0.95	1.43	4.74	14.3	0.3	0.46	0.83	1.6
NGR-21	2.89	2.31	0.57	2.59	1.86	2.57	2.6	0.19	0.76	0.65	1.25	5.44	8.41	0.22	0.33	0.56	1.82
WTN-21	3.83	2.42	0.79	3.04	2.18	2.96	3.13	0.4	1.08	1.17	1.59	4.37	20.4	0.33	0.51	1.02	1.44
WTN-22	2.77	1.86	0.44	2.27	1.62	2.22	2.31	0.27	0.59	1.07	1.49	4.5	13.2	0.41	0.62	0.92	1.52
NGR-22	4.21	3.01	1.08	3.56	2.55	3.51	3.61	0.37	1.44	0.94	1.4	4.74	17.5	0.23	0.34	0.8	1.63
PAU-01	3.51	2.07	0.62	2.69	1.93	2.6	2.79	0.4	0.84	1.35	1.69	4.1	21	0.43	0.65	1.15	1.34
SBD-05	2.41	2.11	0.43	2.25	1.63	2.25	2.26	0.1	0.52	0.4	1.14	6.62	4.39	0.15	0.23	0.35	1.99
ISN-03	3.33	2	0.57	2.58	1.85	2.5	2.67	0.37	0.77	1.32	1.67	4.14	19.4	0.44	0.66	1.12	1.37
ISN-02	2.96	2.69	0.68	2.82	2.05	2.82	2.83	0.09	0.66	0.3	1.1	7.51	4.02	0.09	0.14	0.26	2.06
PM-01	2.98	1.96	0.5	2.41	1.73	2.36	2.47	0.3	0.68	1.13	1.53	4.45	14.9	0.4	0.59	0.95	1.51
BLU-01	1.92	1.67	0.27	1.79	1.29	1.79	1.8	0.09	0.36	0.44	1.15	6.28	3.74	0.21	0.32	0.37	1.97
HK-07	7.39	3.88	2.44	5.35	3.86	5.09	5.64	0.92	3.34	1.56	1.91	3.84	51.3	0.25	0.38	1.34	1.19
SLT-01	2.17	2	0.37	2.08	1.51	2.08	2.08	0.06	0.39	0.25	1.08	7.62	2.47	0.11	0.16	0.22	2.1
KMD-01	4.84	2.98	1.23	3.8	2.72	3.68	3.91	0.52	1.69	1.25	1.63	4.23	27.1	0.28	0.43	1.08	1.4
SBD-01	2.26	1.66	0.32	1.94	1.39	1.92	1.96	0.19	0.44	0.88	1.36	4.87	8.81	0.39	0.58	0.75	1.67
G. MUNG.	3.25	2.42	0.67	2.8	2.01	2.77	2.83	0.26	0.91	0.84	1.34	4.96	12.1	0.26	0.38	0.72	1.7
A. SIBUN.	2.76	2.48	0.58	2.62	1.9	2.61	2.62	0.1	0.58	0.31	1.12	7.82	4.11	0.11	0.16	0.28	2.04
ALR-02	2.69	1.99	0.46	2.32	1.66	2.29	2.34	0.21	0.63	0.85	1.35	4.96	10.2	0.31	0.46	0.72	1.7
MGP-01	3.96	2.71	0.91	3.28	2.35	3.22	3.34	0.37	1.26	1.03	1.46	4.57	18.2	0.27	0.41	0.88	1.56
PMK-01	4.33	3.85	1.42	4.08	2.96	4.07	4.09	0.16	1.31	0.36	1.12	6.73	6.95	0.08	0.11	0.31	2.02
ADN-05	4.29	2.67	0.98	3.39	2.43	3.29	3.48	0.46	1.36	1.24	1.6	4.25	23.6	0.31	0.47	1.05	1.42
SBD-12	1.93	1.49	0.24	1.69	1.22	1.68	1.71	0.14	0.31	0.73	1.31	5.36	6.5	0.39	0.59	0.64	1.74

IR-20	2.19	1.68	0.31	1.91	1.38	1.89	1.93	0.15	0.37	0.7	1.33	5.82	7.35	0.33	0.51	0.62	1.75
Average	3.43	2.28	0.70	2.77	2.00	2.70	2.85	0.32	0.90	1.00	1.52	5.00	16.8	0.32	0.48	0.86	1.58

Note: YP and YS are grain yields (t/ha) under non-stressed, and under moderately stressed conditions (SL2 0.34) respectively.

Table 3. Spearmen's correlation coefficients between YP, YS and drought tolerance indices determined across 40 rice genotypes at the SL2 0.34 stress level.

	YP	YS	STI	GMP	MRP	HARM	MP	REI	ATI	YI	MSTIK1	SNPI	SSPI	YSI	RDI	SSI	MSTIK2
YS	0.71																
STI	0.92	0.91															
GMP	0.92	0.91	1.00														
MRP	0.93	0.91	1.00	1.00													
HARM	0.88	0.95	0.99	0.99	0.99												
MP	0.96	0.87	0.99	0.99	0.99	0.97											
REI	0.86	0.32	0.64	0.64	0.65	0.58	0.71										
ATI	0.88	0.87	0.96	0.96	0.95	0.97	0.95	0.67									
YI	0.66	0.02	0.38	0.37	0.38	0.30	0.46	0.94	0.41								
MSTIK1	0.66	0.02	0.38	0.37	0.38	0.30	0.46	0.94	0.41	1.00							
SNPI	-0.68	-0.04	-0.40	-0.39	-0.40	-0.32	-0.48	-0.94	-0.44	-1.00	-0.99						
SSPI	0.83	0.27	0.60	0.60	0.61	0.53	0.68	1.00	0.63	0.96	0.96	-0.96					
YSI	0.16	-0.50	-0.16	-0.16	-0.16	-0.23	-0.07	0.57	-0.10	0.80	0.80	-0.78	0.62				
RDI	0.16	-0.50	-0.16	-0.16	-0.16	-0.24	-0.07	0.57	-0.10	0.80	0.80	-0.77	0.62	1.00			
SSI	0.66	0.02	0.38	0.37	0.38	0.30	0.46	0.94	0.41	1.00	1.00	-0.99	0.95	0.80	0.80		
MSTIK2	-0.66	-0.02	-0.38	-0.37	-0.38	-0.30	-0.47	-0.94	-0.42	-1.00	-1.00	0.99	-0.96	-0.80	-0.80	-1.00	

Notes: YP and YS are grain yields (t/ha) under non-stressed, and under moderately stressed conditions (SL2 0.34) respectively.

Bold numbers are significant at 5% significance level.

PMK-01) were reported to be moderately resistant or resistant/highly resistant to one or multiple races of the blast disease fungus *Pyricularia grisea* (Mau et al., 2018). Thus, these genotypes could be used as parents for the development of high yielding, blast resistant, drought tolerant varieties of pigmented upland rice or could be recommended for direct release as new varieties.

Materials and methods

Research location

This research was carried out in the Field Agriculture Laboratory of Universitas Nusa Cendana, Kupang, East Nusa Tenggara, Indonesia during the dry season (June to October) 2017. The research site was located at 10.15432 S Latitude and 123.66997 East Longitude, about 110 m above sea level (asl). The soil type of the research site was an Entisol (Vertisol: USDA).

Experimental design

Evaluation of drought tolerance was carried out during the dry season to allow an appropriate setting for imposition of drought stress. The experiment employed a Split Plot design consisting of irrigation level (I) as the main plots and upland rice genotypes (G) as the subplot treatments. The main plots consisted of three irrigation levels, i.e. I_0: irrigation at an optimum level (100% Field Capacity), I_1: irrigation at 75% field capacity level and I_2: irrigation at 50% field capacity. Subplot treatments consisted of 40 rice genotypes.

In the normal/non-stressed treatment (100% FC) (I_0), the plants were watered daily to maintain the soil at field capacity from the time of planting until harvest. In the moisture-stressed treatments, the plants were irrigated on a daily basis to maintain soil at 100% field capacity level from the time of planting until the initial flowering stage, followed by reduced irrigation to either a 75% FC level (I_1) or a 50% FC level (I_2) from the early flowering stage until harvest. Irrigations were based on calculated water loss through evapotranspiration based on assumed mean Et_0 values in the research area estimated over the previous five years (5.24 - 5.28 mm day^{-1} during July to October) and the assumed values for the Kc coefficient of rice plants during the flowering and reproductive stage (1.1).

Plant materials

The research materials used in the study were 40 upland rice genotypes consisting of 37 local cultivars of red and black rice from East Nusa Tenggara Province in Indonesia along with three check cultivars kindly provided by the Indonesian Rice Research Institute, i.e. Aek Sibundong (Indonesian released superior red rice variety), Gajah Mungkur (drought tolerant variety) and IR20 (drought susceptible variety). All treatments consisted of two replicates, and in total, 80 experimental units were observed per irrigation main-plot and 240 units in all combined main-plot and sub-plots.

Plant cultivation

The planting field was first cleared, and a total of 240 plots of 2 m x 2 m size were prepared for growing the rice plants. These 240 plots were arranged in two replicates of the main plot treatment, each consisted of 120 plots (80 plots for each of three irrigation level). All the treatments were placed randomly, starting from the main-plot treatment followed by placement of sub plots treatment within each of the assigned main-plot treatments. Between sub-plot spacing was 50 cm, and between main-block spacing was 1.0 m.

The plots were irrigated until field capacity level before planting the rice seeds. Rice seeds were planted manually in planting holes of 20 cm x 25 cm plant spacing. Three seeds were planted in each planting hole, and only one plant per hole was retained for the evaluation. Irrigation was done according to the assigned treatments.

The rice plants were provided with Urea, SP36, and KCl as basal fertilizers at a rate of, respectively, 80 g/4m^2 sub-plot (equal to 200 kg ha^{-1}), 60 g/4m^2 sub-plot (equal to 150 kg ha^{-1}), and 40 g/4m^2 sub-plot (equivalent to100 kg ha^{-1}). SP36 and KCL were applied at planting time while Urea was applied twice at planting time and 45 days after planting.

Observation and data analysis

The main observed variable was dry grain weight per 4 m^2 sub-plot which then was converted into grain yield/ha (t ha^{-1}). The grain yields in non-stressed and stressed treatments included YS (potential yield of given genotype under stress condition), YP (potential yield of a given genotype under non-stressed condition), Y.S (average yield of all genotypes under stress condition) and Y.P (average yield of all genotypes under non-stressed condition). The grain yield data were then used to calculate drought tolerance indices SSI (Fischer and Maurer, 1978); RDI (Fischer and Wood, 1979); MP (Rosielle and Hamblin, 1981), DRI (Bidinger et al., 1987); STI and GMP (Fernandez, 1992**);** REI and MRP (Hossain et al., 1999); MISTIK1 and MSTIK2 (Farshadfar and Sutka, 2002); ATI, SSPI, and SNPI (Moosavi et al., 2008); HARM (Dadbakhsh et al., 2011); and RDY (Farshadfar and Elyasi, 2012).

Calculated grain yield and drought tolerance index data were then subjected to analysis of variance to determine the treatment effects. The accumulated data were then subject to correlation analysis and Principal Component Analysis (Farshadfar and Elyasi, 2012, Ali and El-Sadek, 2016; Bennani et al., 2017). ANOVA was performed using GenStat version 12 software (VSNi, 2009) while correlation analysis and Principal Component Analysis were performed using PAST (Hammer et al., 2001).

Conclusion

Among fifteen drought-tolerance indices evaluated, STI, GMP, MRP, HARM, REI, ATI, YI, SNPI were considered to be the most effective for selection of drought tolerant, high yielding upland rice genotypes. The genotypes HK-07, ADN-04, PMK-01, ADN-05, NGR-022, ALR-02, HK-06, and KMD-01 were selected as superior upland rice genotypes based on the chosen indices. The selected genotypes are recommended to be used as parents in a breeding program for improvement of drought tolerance and grain yield of upland rice varieties, in particular pigmented upland rice.

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