# Selection in Recombinant Inbred Lines of rice (*Oryza sativa* L.) by Drought Tolerant Indices

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The development of drought tolerant cultivars is paramount to attain stability of production in the rainfed lowlands. Inefficiencies of screening techniques for drought flagged the use drought index, which is a mathematical association between yield under stress and non-stress conditions. This study aimed to determine the effectiveness of indices in classifying and identifying drought tolerant rice genotypes. An experiment was conducted in the Philippine Rice Research Institute Central Experiment Station on ten recombinant inbred lines developed through single cross of popular local varieties in replicated RCBD. Each population was subjected to two cycles of seasonal selection, under non-stress ( $E_{ns-1}$ ) and reproductive stage drought ( $E_{s-1}$ ) on the 1<sup>st</sup> cycle at severe stress intensity (SI) of 0.94 and during the 2<sup>nd</sup> cycle (SI = 0.27, moderate stress) under non-stress (Ens-2) and favorable rainfed (Es-2) conditions. Eleven drought tolerance indices viz., relative drought index (RDI), stress tolerance (TOL), mean productivity (MP), yield stability index (YSI), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean (HAM), drought resistance index (DI), sensitivity drought index (SDI), stress susceptibility index (SSI) and yield index (YI) were calculated. High heritability  $(h^2)$  were computed for yield in  $E_{ns-1}$  (h<sup>2</sup> = 0.91),  $E_{s-1}$  (h<sup>2</sup> = 0.63) and  $E_{ns-2}$ ,  $E_{s-2}$  (h<sup>2</sup> = 0.93). Significantly positive correlation of GMP, STI and HAM to yield under  $E_{s-1}$ ,  $E_{s-2}$ , and  $E_{ns-2}$  showed that these indices were effective in identifying stable and high yielding genotypes across three environments. Screening genotypes through drought indices, correlation, principal component and genotype x environment analyses delineated, PR39269-B-3-B-1-3 derived from cross combination of PSB Rc10 and NSIC Rc138 as high yielding and stable under reproductive stage drought, favorable rainfed and non-stress environment.

Keywords: selection, drought tolerant index, correlation, genotype x environment analysis, rice

# Introduction

Drought stress occurs frequently in rice ecosystems that are either rainfed or rely on impounded surface water, affecting about 20 - 25 million hectares (ha)

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worldwide (Atlin et al., 2008). In Asia, about 50% of all rice land is rainfed and although rice yields in irrigated systems have doubled and tripled over the past 30 years, only modest gains have occurred in the rainfed systems (Fischer et al., 2003). The main share of total rice production will continue to come from irrigated systems but there are indications that irrigated systems alone will not be able to supply the additional amount needed in the near future (Pingali *et al.*, 1997). In the Philippines, 1.48 million ha or about 46% of the 4.73 million ha of areas designated for rice are rainfed, but only 32% of rice produced is sourced through this ecosystem. These areas are geographically distributed in the archipelago's three major islands, 340,000 ha in Luzon, 730, 000 ha in Visayas and 420,000 ha in Mindanao (BAS, 2015), wherein more distinct areas are located in the Cagayan Valley, Ilocos, IloIlo and on the coastal plains of Visayas (Maclean, 2002). Since these areas fully rely on rainfall as its only source of irrigation, yield continually remains low and inconsistent due to intermittent rainfall patterns, occurrence of drought and/or submergence with varied intensity across seasons and years. In addition, most irrigated areas rely on surface irrigation from run-off river systems and reservoirs can suffer from conditions similar to rainfed conditions during drought years.

In the context of current and predicted water scarcity scenarios, irrigation is generally not a viable option to alleviate drought problems in rainfed ricegrowing systems (Serraj *et al.*, 2008), therefore there is an imperative need for a different strategy such as varietal development for drought-prone environments. Although, varietal development for drought tolerance is complicated by the lack of fast, reproducible screening techniques and the ability to routinely create defined repeatable water stress conditions where large populations can be efficiently evaluated (Ramirez and Kelly, 1998). The development of rice cultivars that combine improved yield under stress with high yield potential can be obtained by screening breeding lines for both yield potential in favorable environments and yield under managed stress (Atlin *et al.*, 2008).

The preliminary point in the selection of desirable genotypes is distinguishing genotypes expressing comparative superiority in both stress and non-stress environments due to unpredictable rainfed conditions (Mohammadi *et al.*, 2010). Although some researchers believe in selection under favorable condition (Betran *et al.*, 2003), in target stress condition (Mohammadi *et al.*, 2011) and selection under both favorable and stress conditions (Nouri *et al.*, 2011). Selection of suitable genotypes based on relative yield performance has been considered a reliable technique for evaluating a large number of genotypes in drought stressed conditions (Panthuwan *et al.*, 2002).

Several selection criteria have been proposed for selecting genotypes based on their performance in stress and non-stress environments (Fischer and Maurer, 1978, Rosielle and Hamblin, 1981, Fernandez, 1992). Drought indices, which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions, have been used for screening genotypes for drought tolerance (Mitra, 2001).

Although there are several studies on the use of drought indices for selection in barley (Amini *et al.*, 2012; Eizavi *et al.*, 2013), bread wheat (Abdi *et al.*, 2012; Dehbalaei *et al.*, 2013; Drikvand *et al.*, 2012; Nouraein *et al.*, 2013), corn (Kiani, 2013; Moradi *et al.*, 2012; Naghavi *et al.*, 2013), oat (Akcura and Ceri, 2011; Rabiei *et al.*, 2012), rapeseed (Rad and Abassian, 2011; Zebarjadi *et al.*, 2011), soybean (Bahari and Nasirifard, 2014), sorghum (Menezes *et al.*, 2013) and sunflower (Safavi *et al.*, 2005), studies in rice are uncommon. Selection of drought tolerant genotypes among advanced breeding lines through drought tolerant indices may prove to be a good selection criterion in hastening and improving selection process for cultivar development programs.

## **Materials and Methods**

#### Plant Genetic Materials and Field Experiment

The study was conducted in an experimental field in the Philippine Rice Research Institute Central Experiment Station, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines, which is, located at latitude 15° 40' N, longitude 120° 53' E and an elevation of 60.4 m above sea level during the 2014 dry and wet cropping seasons. Selection program was initiated by screening ten  $F_7$  to  $F_8$  generation recombinant inbred lines (RIL) from cross combination of popular high yielding local varieties (Table 1). Rice seedlings were planted (21 days old) in 20 cm x 20 cm spacing. Fertilizers were applied prior to sowing and side dressing preceding panicle initiation following local fertilizer recommendations at the rate of 120-60-60 on the dry season and 90-60-60 of N-P-K during the wet season.

Genotype	Genotype	Parentage	
G1	PR39955-B-2-1-3-2	PSB Rc14/PSB Rc82	
G2	PR39269-B-3-B-1-3	PSB Rc10/NSIC Rc138	
G3	PR39172-B-19-B-B-2	PSB Rc62/NSIC Rc138	
G4	PR39949-B-5-2-2-2	PR25769-B-9-1/PSB Rc14	
G5	PR39955-B-4-2-4-2	PSB Rc14/PSB Rc82	
G6	PR39954-B-15-2-4-1-3	PSB Rc14/PSB Rc68	
G7	PR40029-B-20-1-7-2-1	PSB Rc14/NSIC Rc152	
G8	PR40029-B-14-B-2-2-2	PSB Rc14/NSIC Rc152	
G9	PR40029-B-16-1-1-1	PSB Rc14/NSIC Rc152	
G10	PR40028-B-6-B-5-1-3	PSB Rc14/NSIC Rc158	

**Table 1.** List of recombinant inbred lines (RIL), its corresponding pedigree and cross combinations during the 2014 dry and wet season divergent trials

## **Divergent Field Trials**

The 1<sup>st</sup> cycle of screening was in a 5.6 m<sup>2</sup> plot under non-stress and managed reproductive stage drought conditions. Second cycle screening was in an  $8m^2$  plot under non-stress and rainfed conditions. During the 1<sup>st</sup> cycle reproductive drought, two series of drought were imposed in the trial until crop maturity. Initiation of differential irrigation started at panicle initiation or at 36 days after transplanting (DAT) by withholding water for 27 days, and then the field was surface flooded water for 3 days. The 2<sup>nd</sup> series of drought followed and continued for 32 days. Water below soil surface reached 104 - 114 cm and 15-20% soil moisture at 30 cm soil depth prior re-irrigation. Moreover, the 1<sup>st</sup> cycle screening received 26 mm of rainfall. The 2<sup>nd</sup> cycle of rainfed plots were not provided with any supplemental irrigation after transplanting other than precipitation amounting to 635 mm in the course of the season. The non-stress experiment maintained 2 - 3 cm standing water from transplanting to 10 days before maturity by providing water by supplementary irrigation through a water pump as required.

#### **Drought Tolerant Indices Computation**

After physiological maturity stage, non-stress yield  $(Y_{ns1-2})$  and stress yield  $(Y_{s1-2})$  were harvested, threshed, sundried and measured for weight and moisture content. The yield was adjusted based on 14% moisture content and computed as tons per hectare  $(t.ha^{-1})$ . Narrow sense heritability  $(h^2)$  was computed based on the ratio of genotypic to phenotypic variance. In order to ascertain the best drought tolerance indices as well as the drought tolerant lines computations were done with 11 different indices: TOL, MP, HAM, SSI, GMP, STI, YI, YSI, DI, RDI and SDI using yield under non-stress and stress conditions.

Drought Tolerant Index (DTI)	Equation	References		
Stress susceptibility index (SSI)	$\frac{1 - Y_s / Y_{ns} / SI, \text{ where;}}{\text{stress intensity SI= } 1 - (\bar{Y}_s / \bar{Y}_{ns})}$	Fischer and Maurer, 1978		
Relative drought index (RDI)	$\mathbf{Y}_{\mathrm{s}}$ / $\mathbf{Y}_{\mathrm{ns}}$ / $\mathbf{\bar{Y}}_{\mathrm{s}}$ / $\mathbf{\bar{Y}}_{\mathrm{ns}}$			
Tolerance Index (TOL)	$Y_{ns} - Y_s$	Rosielle and Hamblin, 1981		
Mean productivity (MP)	$Y_{ns} + Y_s / 2$	Rostene and Hamolin, 1981		
Yield stability index (YSI)	$Y_s / Y_{ns}$	Bouslama and Schapaugh, 1984		
Geometric mean productivity (GMP)	$\sqrt{(Y_s * Y_{ns})}$	Fernandez, 1992		
Stress tolerance index (STI)	$Y_s * Y_{ns} / \bar{Y}_{ns}^2$			
Harmonic mean (HAM)	$2(Y_{s}*Y_{ns}) / Y_{s} + Y_{ns}$	Kristin et al., 1997		
Yield index (YI)	$Y_s/\bar{Y}_s$	Gavuzzi <i>et al.</i> , 1997		
Drought resistance index (DI)	$Y_s * (Y_s / Y_{ns}) / \bar{Y}_s$	Lan, 1998		
Sensitivity drought index (SDI)	$Y_{ns}$ - $Y_s$ / $Y_{ns}$	Farshadfar and Javadinia, 2011		

 Table 2. Drought tolerant indices and correponding formula and references

 $Y_s$  (stress yield),  $Y_{ns}$  (non-stress yield),  $\bar{Y}_s$  (stress mean yield) and  $\bar{Y}_{ns}$  (non-stress mean yield)

#### Statistical Analysis

Experiments were planted in Randomized Complete Block Design (RCBD) in three replicates under two divergent trials. Analysis of variance (ANOVA), Pearson's correlation, genotype x environment and principal component analyses (PCA) were generated using CRAN packages: agricolae, GGEBiplotGUI, FactoMineR and factoextra of R: A language and environment for statistical computing.

# **Results and Discussion**

#### Stress Intensity and Heritability

Association of drought tolerant indices through yield in contrasting target population environments (TPE) were used to assess drought tolerance in RILs. During the 1<sup>st</sup> Cycle, results showed that water stress reduced the grain yield of all genotypes during the 1<sup>st</sup> cycle. The mean yield in non-stressed and stressed conditions were 6.93 t.ha<sup>-1</sup> and 0.16 t.ha<sup>-1,</sup> which indicated that the stress intensity, (SI) was extremely severe (0.94). In the 2<sup>nd</sup> cycle of selection, mean yield under non-stress resulted to 4.73 t.ha<sup>-1</sup> and 3.46 t.ha<sup>-1</sup> under rainfed condition and stress intensity was (0.27) or moderately stress. At the reproductive stage, yield reduction in rice is significant even with moderate stress (Verulkar *et al.*, 2010). High heritability (h<sup>2</sup>) were computed for yield

 $Y_{ns-1}$  (h<sup>2</sup> = 0.91),  $Y_{s-1}$  (h<sup>2</sup> = 0.63) and  $Y_{ns-2}$ ,  $Y_{s-2}$  (h<sup>2</sup> = 0.93). Thus, seletion for yield across two cycles of selection is appropriate since it is highly heritable.

# Correlation Analysis

Correlation is useful in finding out the overall linear association between two variables or traits. Significant associations present in yield under stress to non-stress conditions and drought indices present a suitable criterion for selecting drought tolerant genotypes. Correlation analysis didn't reveal any significant association between stress and non-stress yield in the 1<sup>st</sup> cycle of selection (Table 3), comparable results were obtained by (Amini *et al.*, 2012, Drikvand *et al.*, 2012, Farshadfar *et al.*, 2013 and Kiani, 2013). The lack of correlation between an optimum and stress environment ascertains that selection for high yield under stress does not fully guarantee high yield in non stress environment. The absence of correlation deduced only selection for either stress or non-stress environment since selection for both environments will not surely create directly proportional yield gains.

Significantly positive correlation were observed for  $Y_{ns-1}$  to TOL (r=0.96) and MP (r = 0.95). Selection from high values of these indices would provide high yield if selected TPE is under non-stress condition. Whereas, perfect correlation was recorded for YI (r=1.00) to  $Y_{s-1}$ . Moreover, highly significant and positive correlation of  $Y_{s-1}$  were identified for RDI, YSI, HAM (r=0.99); STI (r=0.98); GMP, DI (r=0.97), while negative correlation were observed for SDI, SSI (r=-0.99). Thus, genotypes with high values of YI, RDI, YSI, HAM, STI, GMP and DI and low values of SDI and SSI will yield higher under stress conditions.

In the 2<sup>nd</sup> cycle of selection, correlation was observed for  $Y_{ns-2}$  to  $Y_{s-2}$  (r=0.717), similar connections were detailed by Akcura and Ceri, 2011, Eizavi *et al.*, 2013 and Menezes *et al.*, 2014 Positive correlation of  $Y_{ns-2}$  were noted for MP (r=0.93), STI (r=0.92), GMP (r=0.91), HAM (r=0.88) and YI (r=0.72). Yield under rainfed condition ( $Y_{s-2}$ ) was seamlessly correlated to YI (r=1.00), and positively interrelated with HAM (r=0.96), GMP (r=0.94); STI, DI, MP (r=0.92); RDI, YSI (r=0.67) while negative relationship were observed for SDI and SSI (r=-0.67) (Table 3). MP, GMP, STI, HM and YI are simultaneously related to  $Y_{ns-2}$  and  $Y_{s-2}$ . Highly correlated indices to both  $Y_s$  and  $Y_{ns}$ , are most appropriate in identifying stress tolerant cultivars (Farshadfar and Javadinia, 2011).

Indices	Y <sub>ns-1</sub>	$Y_{s-1}$	RDI	TOL	MP	YSI	GMP	STI	HM	DI	SDI	SSI	YI
Y <sub>ns-2</sub>	1	-0.17	-0.32	0.96	0.95	-0.32	0.07	0.01	-0.15	-0.3	0.32	0.32	-0.17
Y <sub>s-2</sub>	0.72	1	0.99	-0.45	0.16	0.99	0.97	0.98	1	0.97	-0.99	-0.99	1
RDI	-0.03	0.67	1	-0.59	0	1	0.92	0.94	0.98	0.98	-1	-1	0.99
TOL	0.42	-0.33	-0.92	1	0.81	-0.59	-0.23	-0.28	-0.43	-0.56	0.59	0.59	-0.45
MP	0.93	0.92	0.34	0.06	1	0	0.39	0.34	0.18	0.02	0	0	0.16
YSI	-0.03	0.67	1	-0.92	0.34	1	0.92	0.94	0.98	0.98	-1	-1	0.99
GMP	0.91	0.94	0.39	0	1	0.39	1	0.99	0.97	0.89	-0.92	-0.92	0.97
STI	0.92	0.92	0.34	0.05	1	0.34	1	1	0.99	0.93	-0.94	-0.94	0.98
HM	0.88	0.96	0.44	-0.06	0.99	0.44	1	0.99	1	0.97	-0.98	-0.98	1
DI	0.39	0.92	0.9	-0.67	0.7	0.9	0.74	0.71	0.78	1	-0.98	-0.98	0.97
SDI	0.03	-0.67	-1	0.92	-0.34	-1	-0.39	-0.34	-0.44	-0.9	1	1	-0.99
SSI	0.03	-0.67	-1	0.92	-0.34	-1	-0.39	-0.34	-0.44	-0.9	1	1	-0.99
YI	0.72	1	0.67	-0.33	0.92	0.67	0.94	0.92	0.96	0.92	-0.67	-0.67	1

**Table 3.** Correlation matrix  $Y_s$ ,  $Y_{ns}$  and 11 DTI in the 1<sup>st</sup> cycle (above diagonal) and 2<sup>nd</sup> cycle (below diagonal) selection

Values in bold are different from 0 with a significance level alpha=0.05

Many studies indicated that MP, GMP and STI were the most appropriate indices in identifying drought tolerant genotypes (Abdi *et al.*, 2012; Bahari and Nasirifard. 2014; Drikvand *et al.*, 2012), there also studies which include HAM along with STI, MP and GMP (Farshadfar and Elyasi, 2012; Menezes *et al.*, 2014). Ultimately considering the two cycles of divergent selection, indices GMP, STI and HAM, which were similarly present as positively significant correlation for the 1<sup>st</sup> cycle ( $E_{s-1}$ ) and 2<sup>nd</sup> cycle ( $E_{s-2}$  and  $E_{ns-2}$ ), were categorized as the most suitable indices for selecting drought tolerant genotypes.

#### **Principal Component Analysis**

PCA is one way to compress data sets of high dimensional vectors into lower dimensions (Abdi *et al.*, 2013). PCA revealed that the 1<sup>st</sup> component explained 77.5 % and 68.9 % of the variation with Y<sub>s</sub>, Y<sub>ns</sub> and the selected indices in the 1<sup>st</sup> and 2<sup>nd</sup> cycle, respectively. Thus, the first PCA can be named as the yield potential and drought tolerance dimension (Ali and El-Sadek, 2016). High and positive values of PC<sub>1</sub> will be equivalent to high yielding genotypes under a stress environment. The second PCA amounted to 22.1 % and 31.0 % of the total variability, thus it is the non-stress dimension. The correlation coefficient among any two indices is approximately the cosine of the angle between their vectors. Thus, r= cos180  $\cong$  -1, cos 0  $\cong$  1 and cos 90  $\cong$  0 (Yan and Rajcan, 2002).

In the 1<sup>st</sup> cycle, the most prominent relations present in the biplot are: (1) Strong positive correlation between  $Yn_{s-1}$  to TOL and MP.  $Y_{s-1}$  is positively associated to GMP, STI, HM, YI, YSI, RDI and DI as indicated by acute angles formed between their segment vectors; (2) Near zero association between  $Y_{ns-1}$  to GMP, STI as shown by the near perpendicular vectors and (3) Negative association between  $Y_{s-1}$  to SDI and SSI as indicated by the obtuse angles formed through their vectors (Figure 1A). First cycle selection was able to delineate two groups, wherein the 1<sup>st</sup> group contains G3, G5, G7 and G9, with high values of TOL and MP with negative PC<sub>1</sub> and positive PC<sub>2</sub> values. Additionally, the 2<sup>nd</sup> group have positive PC<sub>1</sub> scores which contains G2 and G4, which will perform best in stress environment. G2 and G4 have high values of GMP, STI, HAM, YI, DI, YSI and RDI. During the 2<sup>nd</sup> cycle, both  $Y_{s-2}$  and  $Y_{ns-2}$  are positively correlated with STI, GMP, MP, HAM and YI, while  $Y_{ns-2}$  has almost no observed correlation with RDI, YSI, SDI and SSI while  $Y_{s-2}$  also has no correlation to TOL.



**Figure 1.** PCA biplot of (A)  $1^{st}$  cycle (B)  $2^{nd}$  cycle of selection among 10 genotypes,  $Y_{ns}$ ,  $Y_s$  and 11 drought tolerant indices

Significant negative associations of  $Y_{S-2}$  were recorded for SDI and SSI. Second cycle selection was able to identify three groups: (1) Indices such as SDI, SSI was correlated to G8, G9 and G10 and are also negatively associated with  $Y_{ns-2}$  and with positive PC<sub>1</sub> and negative PC<sub>2</sub>; (2) Both  $Y_{ns-2}$  and  $Y_{s-2}$  have positive association to STI, MP, GMP, HM and YI containing G1, G2 and G3; (3) Negative association of DI, YSI and RDI for  $Y_{s-2}$  were detailed for G5 and G6 which will perform best only in stress environment.

### Genotype x environment (GGE) biplot analysis.

The combined ANOVA for  $Y_{ns}$  and  $Y_s$  during two divergent trials indicated highly significant variation (P < 0.001) (Table 4). The environment (E) effect was a predominant source of variation and accounted for 95.66 % of the total sum of squares, while G and GE interaction sources of variation accounted for 1.48 % and 2.33 % of the total variation. The first two principal components explained 84.4 % of the total GGE variation. First principal component accounted for 62.99 % whereas the second principal component is equal to 24.99 %.

**Table 4**. Combined analysis of variance of yield data in two divergent trials, 2014 dry and wet seasons

Source	Df	Sum of Squares	Mean Square	Pr(>F)
Genotype(G)	9	10.24	1.14	< 2.2e-16 ***
Environment (E)	3	663	220.99	6.081e-12 ***
GxE	27	16.14	0.6	< 2.2e-16 ***
Residuals	72	3.71	0.05	

Significance codes: 0.0001 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



**Figure 2.** Biplot showing which won where among 10 genotypes in two divergent trials, 2014 dry and wet seasons.

Visualization of the "which-won-where" pattern of multi-environment data is important for studying the possible existence of different environments in a region (Yan *et al.*, 2001). The polygon view of a biplot is the best way to visualize the interaction pattern between genotypes and environments and to effect vely interpret a biplot (Yan and Kang, 2003). The polygon view showing "which-won-where" of the GGE biplot revealed that there were two mega environments (Figure 2). The 1<sup>st</sup> environment contains  $E_{ns-1}$  (1<sup>st</sup> cycle non-stress environment), indicating G7 as the best genotype in the environment along with G5 and G9. The 2<sup>nd</sup> mega-environment consisted of  $E_{S-1}$  (1<sup>st</sup> cycle stress environment),  $E_{ns-2}$  (2<sup>nd</sup> cycle non-stress environment) and  $E_{S-2}$  (2<sup>nd</sup> cycle stress environment) which was succeeded by G2 and followed by G1.

### Conclusion

Several studies have reported that selection of drought tolerant genotypes can be based on the relative performance of a combination of drought indices in contrasting environments. The use of drought indices with apposite interpretations of statistical tools including correlation, principal component and genotype x environment analyses can be predictors of drought tolerant genotypes. Selection for reproductive stage drought, rainfed and irrigated environments based on the combination of HAM, GMP and STI resulted in the identification of probable drought tolerant genotype, PR39269-B-3-B-1-3 with high yield and stability across three environments.

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2690

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