

EVALUATION OF LOW NITROGEN TOLERANCE IN RICE GENOTYPES USING STRESS TOLERANCE INDICES

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ABSTRACT

Nitrogen (N) is one of the most important nutrient for plant growth. Low N condition is usually occur because its character which easily eliminate from the soil. Thirty-eight rice genotypes were evaluated under low N and optimum N environments. The experiment was conducted at Muara Experimental Farm Bogor, Indonesia using augmented design with three replicates under low N (34.5 kg N ha⁻¹) and optimum N (138 kg N ha⁻¹). Seven selection indices for stress tolerance including mean productivity (MP), geometric mean productivity (GMP) tolerance (TOL), yield stability index (YSI), stress tolerance index (STI), and stress susceptible index (SSI) were calculated based on grain yield under low N and optimum N environments. The result of analysis of variance showed significant variations due to genotypes for grain yield in two N environments. PCA analysis showed that te first PCA explained 54.0% of the variation with YSI, SSI, MP, GMP, and STI. Principal component analysis indicated that the first two components accounted for more than 97% of the total variationsfor drought tolerant indices. Positive and

significant correlation of Ys and Yp with MP, GMP, and STI concluded that these indices were the best predictors of yield under low N and optimum N environments.

Keywords: rice, grain yield, low N, stress indices

INTRODUCTION

Grain yield in rice plant commonly affected by less nitrogen (N) nutrient in the soil due to its characters that easy to leaching, Less N conditions is common in all regions due to volatilization (Zhong-cheng *et al.* 2012), denitrification, timing and placement of fertilizer, leaching, run-off, and absorbed by plants (Choudury and Kenedy, 2005) or recovered in crop about 22%–30% of the applied N (Mei-hua *et al.* 2012).that easy to remove by nature or harvest plant. It is unconscious that tolerant rice variety in poor-nitrogen soil or have nitrogen use efficiency is necessary.

Selection under environmental stress condition is required for exploiting genetic variations to improve stress tolerant genotypes (Reynolds and Borlaug 2006; Le Gouis *et al.* 2000; Ortiz *et al.* 2008). However, other researcher stated that in order to develop tolerant cultivars, the selection program should be conducted under stress condition too (Presterl *et al.* 2003; Galais and Coque2005).In this experiment we had selected some population crossed between high yielding varieties with local varieties to obtain new cultivars which using N efficiently. The genotypes resulted from selection then evaluated for low N tolerance using different stress tolerance indices.

Several screening methods have been conducted by scientists to identify any kind of stress condition such as heat stress (Khan and Kabir 2014), drought (Shiferaw *et al.* 2012), and salinity (Singh *et al.* 2014). There were several tolerance indices that usually used to evaluate genotypes under environment stresses. Tolerance index (TOL) is the yield differences under non-stress (Yp) and stress (Ys) conditions. Mean productivity index (MP) as the average of Yp and Ys. Geometric mean productivity (GMP) for separating superior genotypes and stress tolerance index (STI), which can be used to identify genotypes which produce high yields both under stress and non-stress environments. The Stress susceptibility index (SSI) for the assessment of stress tolerant varieties. Yield stability index (YSI) evaluates stress tolerance by calculating the ratio of Ys to Yp. In some research revealed that the Ys and Yp showed highest significant and positive correlations with GMP, MP and STI among indices studied (Golabaldi *et al.* 2006; Ali and El-Sadek 2014; Singh *et al.* 2014). Bahari *et al.* (2013) reported a wheat genotype that tolerant to drought based on MP, GMP, TOL, STI, and SSI. Moosavi *et al.* (2008) introduced three new indices namely: abiotic-stress tolerance index (ATI), stress susceptibility percentage index (SSPI)

and stress non-stress production index (SNPI) to identify relatively tolerant (through ATI and SSPI) and resistant (through SNPI) genotypes under nonirrigated and irrigated conditions. For selection based on a combination of indices, some researchers have used principal component analysis (PCA), a biplot as a better approach than a simple correlation analysis is necessary to identify superior genotypes for both stress and non-stress conditions (Amiri *et al.* 2014). Jalilvandy and Rozrokh (2013) stated that selection of genotypes with low PC1 and high PC2 are suitable for both stress and non-stress environments. Our experiment aimed to evaluate of low nitrogen tolerance in rice genotypes using stress tolerance indices.

MATERIALS AND METHODS

A field experiment was carried out at Muara Experimental Farm Bogor, Indonesia and 200 m above sea level. The soil of the field is latosol. Thirty-eight rice genotypes were evaluated under low N (34.5 kg N ha⁻¹) and optimum N (138 kg N ha⁻¹) environments. This experiment used augmented design with three replications. Grain yield were recorded from 5 m² plot for each rice genotypes and converted as t ha⁻¹. The data were analyzed using Microsoft Excel and Minitab software. A biplot derived from principal component analysis (PCA) based on the two-way data of selection criteria (low N tolerant indices) was conducted using Minitab software. PCA was calculated to analyze relationships among selection criteria, to compare genotypes on the basis of low N tolerance indices. The tolerance indices were calculated as follows:

$$\text{Tolerance (TOL)} = Y_p - Y_s$$

$$\text{Mean productivity (MP)} = \frac{Y_p + Y_s}{2}$$

$$\text{Geometric mean productivity (GMP)} = \sqrt{Y_p \times Y_s}$$

$$\text{Stress susceptible index (SSI)} = \frac{(1 - \frac{Y_s}{\bar{Y}_p})}{(1 - \frac{Y_s}{\bar{Y}_s})}$$

$$\text{Stress tolerance index (STI)} = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$$

$$\text{Yield stability index} = \frac{Y_s}{Y_p}$$

where Y_s is grain yield of each genotype under low N condition, Y_p is grain yield of each genotype under optimum N condition, \bar{Y}_s and \bar{Y}_p are the mean yields of all genotypes under low N and optimum N, respectively.

RESULTS

Analysis of variance and genotypes performace

The result of analysis of variance for grain yield under N suboptimum and N optimum environments are presented in Table 1. Mean squares for combine analysis of variance indicated significant differences among all genotypes for yield ($P < 0.05$). It revealed significant variation due to genotypes for grain yield in two different N environments. Check indicated non-significant differences or expected has a stable grain yield under those two N environments.

Table 1. Analysis of variance showing mean square for yield in rice lines under N suboptimum (N-) dan N optimum (N+) environments.

Source of variation	df	Grain Yield	
		N-	N+
Block	2	0.32	0.49
Genotype	45	1.77*	1.76*
Check	5	1.39	1.00
Error	10	0.55	0.48
Total	62		
CV(%)		15.9	14.8

*=significant at $p < 0.05$

Tolerance indices were calculate on the basis of the genotypes (Table 2). Genotypes showed wide range variations for the estimated indices. The mean yield of genotypes under low N environment varied from 2.05 to 7.82 t ha⁻¹, while mean of yield genotype under optimum N environment varied from 2.20 to 8.39 t ha⁻¹. The genotypes 35, 13, 8, 16, and 5 had the best performace of grain yield in low N condition, while the least were 22, 6, 18, 38, and 12. Genotypes with the best performance under optimum N were 20, 36, 38, 7, and 6 and the least relative tolerant were 1, 2, 8, 12, and 10 genotypes. Based on the tolerant indices, the identification of tolerant genotypes based on a single criterion was contradictory. According to STI, MP, and GMP genotypes 7, 21, and 35 were the most tolerant genotypes whereas genotypes

22, 18, and 12 were the least. For SSI the desirable low N tolerant genotypes were 38, 6, and 36 and the least relative tolerant were 10, 8, and 13. According to TOL the 20, 38, and 6 were the tolerant and 10, 13, and 8 were the least and for YSI the tolerant genotypes were 10, 8, and 13 whereas 36, 6, and 38 were the least relative tolerant.

Correlation between grain yield and indices.

To determine the most desirable tolerance criteria the correlation coefficient between quantitative low N tolerance indices to Ys and Yp were calculated (Table 3). tolerance indices (SSI, MP, TOL, GMP, STI, and YSI), while SSI showed high negative correlation. MP and TOL showed the highest coefficients of correlation. Similar results were observed with Ys against SSI, MP, TOL, GMP, STI, and YSI but TOL were highly negative correlated to Ys, while the most closely correlated to Ys were SSI and YSI. MP was the strongly correlated index to Yp and highly correlated to Ys.

Table 2. The correlation coefficient between Ys and Yp with various N tolerance indices

	Ys	Yp	SSI	MP	TOL	GMP	STI
Yp	-0.274						
SSI	0.806**	-0.698**					
MP	0.631**	0.573**	0.124				
TOL	-0.810**	0.785**	-0.944**	-0.057			
GMP	0.656**	0.524**	0.114	0.981**	-0.103		
STI	0.640**	0.524**	0.093	0.967**	-0.093	0.991**	
YSI	0.806**	-0.698**	1.000	0.124	-0.944**	0.114	0.093

**=significant at p<0.05

Highly significant and positive correlation were observed among each pair of MP, GMP, TOL, and STI (P<0.05). The correlation between Yp and either SSI and YSI was significant and negative. TOL had negatively significant correlated with SSI and YSI. Both Yp and Ys was significantly positive correlation with MP, GMP, and STI. This indicates that these indices were more effective in identifying high yielding lines under low N as well as optimum N environments.

Principal component analysis

In order to assess the relationship between all tolerance indices to identify superior genotypes at once, we used the principal component analysis (Table 3). The first and second components justified 54.00% and 43.90%, respectively and accounted for 97.8% of total variation.

Results demonstrated that there is had positive and high significant correlations with yield in low N conditions (Ys) with some indices such as YSI and SSI, but those YSI and SSI components had negative correlation with TOL (Figure1). Also a high and significant positive correlation was observed between Mp, GMP, and STI.

Table 3. Principal component analysis Ys, Yp and low N tolerance indices of rice lines

Variable	Component 1	Component 2
Ys	0.472	0.097
Yp	-0.208	0.471
SSI	0.436	-0.205
MP	0.234	0.463
TOL	-0.43	0.224
GMP	0.243	0.458
STI	0.235	0.459
YSI	0.436	-0.205
Eigenvalue	4.318	3.509
Percent of variation	54.00	43.90
Cumulative percentage	54.00	97.80

Relationship between grain yield and indices

Linear regression revealed that coefficients of determination between Ys and the tolerance indices were $R^2_{STI/Ys} = 0.4091$, $R^2_{GMP/Ys} = 0.4297$, $R^2_{YSI/Ys} = 0.649$ and $R^2_{MP/Ys} = 0.389$ (Figure 2). These result revealed that YSI index may be considered the best predicate to explain grain yield variations under low N condition. However, relationship between Ys and TOL and SSI were negatively significant with coefficient determination were $R^2_{TOL/Ys} = 0.6569$ and $R^2_{SSI/Ys} = 0.649$, respectively. It showed that those two indices cannot use in grain yield variation under low N condition.

Tabel 4. Mean value of low N tolerance indices for 38 rice genotypes at N suboptimum and N optimum environments

No	Galur	Y _s	Y _p	SSI	MP	TOL	GMP	STI	YSI
1	BPS14250C-100-7-3	4.89	3.21	-18.08	4.05	-1.68	3.96	0.73	1.52
2	BPS14250C-135-1-2	5.27	3.17	-22.95	4.22	-2.10	4.08	0.77	1.66
3	BPS14250C-15-1-1	5.36	4.61	-5.65	4.99	-0.75	4.97	1.15	1.16
4	BPS14250C-16-1-2	4.98	3.29	-17.66	4.13	-1.68	4.05	0.76	1.51
5	BPS14250C-16-2-2	6.24	3.86	-21.21	5.05	-2.37	4.91	1.12	1.61
6	BPS14250C-169-1-1	2.52	6.60	21.37	4.56	4.08	4.08	0.77	0.38
7	BPS14250C-174-2-2	6.05	6.83	3.96	6.44	0.78	6.43	1.92	0.89
8	BPS14250C-176-7-1	6.96	3.01	-45.24	4.99	-3.94	4.58	0.97	2.31
9	BPS14250C-194-2-1	4.57	4.91	2.44	4.74	0.35	4.74	1.04	0.93
10	BPS14262C-105-1-2	5.79	2.20	-56.33	3.99	-3.59	3.57	0.59	2.63
11	BPS14262C-105-2-3	3.53	3.46	-0.65	3.50	-0.06	3.50	0.57	1.02
12	BPS14262C-105-6-1	2.05	2.95	10.59	2.50	0.90	2.46	0.28	0.69
13	BPS14262C-115-1-1	7.22	3.30	-40.97	5.26	-3.91	4.88	1.10	2.18
14	BPS14262C-115-1-2	5.64	4.32	-10.58	4.98	-1.32	4.94	1.13	1.31
15	BPS14262C-128-2-1	5.87	3.66	-20.89	4.76	-2.21	4.63	0.99	1.60
16	BPS14262C-128-2-3	6.24	5.13	-7.50	5.69	-1.11	5.66	1.48	1.22
17	BPS14262C-128-3-1	3.18	3.42	2.36	3.30	0.23	3.30	0.50	0.93
18	BPS14262C-148-1-1	2.42	3.65	11.66	3.03	1.23	2.97	0.41	0.66
19	BPO14250C-104--5-3	3.33	3.83	4.59	3.58	0.51	3.57	0.59	0.87
20	BPO14250C-150-7-2	3.70	8.39	19.35	6.04	4.70	5.57	1.44	0.44
21	BPO14250C-15-2-4	5.42	6.50	5.76	5.96	1.08	5.93	1.63	0.83
22	BPO14250C-15-3-1	2.78	3.48	7.00	3.13	0.71	3.11	0.45	0.80
23	BPO14250C-157-1-1	2.78	5.43	16.86	4.10	2.65	3.89	0.70	0.51
24	BPO14250C-161-2-1	4.09	3.84	-2.32	3.97	-0.26	3.96	0.73	1.07
25	BPO14250C-169-4-2	3.32	6.45	16.77	4.89	3.13	4.63	0.99	0.51
26	BPO14250C-169-4-3	4.66	4.04	-5.30	4.35	-0.62	4.34	0.87	1.15
27	BPO14250C-169-5-3	5.36	4.03	-11.42	4.70	-1.33	4.65	1.00	1.33
28	BPO14250C-172-4-2	3.10	4.97	13.02	4.04	1.87	3.93	0.71	0.62
29	BPO14262C-105-1-3	5.90	3.60	-22.14	4.75	-2.30	4.61	0.98	1.64
30	BPO14262C-114-2-3	5.05	5.38	2.09	5.21	0.32	5.21	1.26	0.94
31	BPO14262C-132-3-3	3.38	5.46	13.14	4.42	2.08	4.30	0.86	0.62
32	BPO14262C-132-6-2	3.39	5.35	12.66	4.37	1.96	4.26	0.84	0.63
33	BPO14262C-155-1-1	4.59	5.62	6.36	5.11	1.03	5.08	1.20	0.82
34	BPO14262C-155-2-1	3.57	4.95	9.64	4.26	1.38	4.20	0.82	0.72
35	BPO14262C-155-2-2	7.82	4.40	-26.94	6.11	-3.43	5.86	1.59	1.78
36	BPO14262C-165--2-2	3.02	7.09	19.85	5.06	4.07	4.63	0.99	0.43
37	BPO14262C-172-1-3	5.11	5.25	0.91	5.18	0.14	5.18	1.24	0.97
38	BPO14262C-173-5-2	2.28	6.86	23.07	4.57	4.58	3.96	0.73	0.33

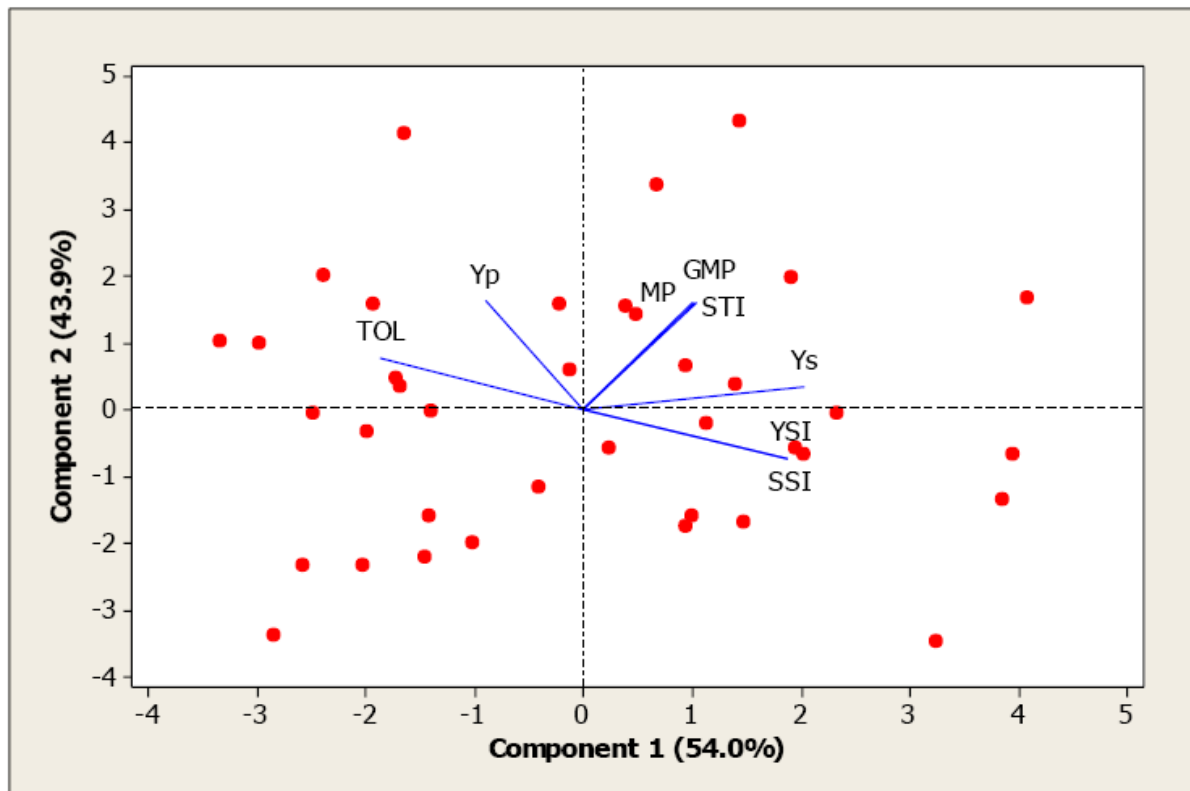


Figure 1. Principal component analysis of stress tolerance indices

DISCUSSION

The rice genotypes had significant variation for grain yield in analysis of variance (Table 1) suggested that the differences in genotypes was sufficient for selecting genotypes with improved low N tolerance genotype. The same results for other stress conditions were finding in wheat (Khan and Khabir 2014; Yasir *et al.* 2014), tef plant (Shiferaw *et al.* 2012), rapeseed (Aliakbari *et al.* 2014), safflower (Majidi *et al.* 2011), and Chickpea (Ganjeali *et al.* 2011). Shiferaw *et al.* (2012) and Yasir *et al.* (2013) also showed that grain yield and yield related traits in tef plant and wheat were significantly affected by genotype under each water stress and non-stress environments. It can conclude that high differences among genotypes indicate the existence of genetic variation and selection possibility for suitable genotypes in both environment conditions (Yasir *et al.* 2013).

The genotype that produce high yield under optimum N environment (Y_p) were 20, 36, and 38 failed to produce high yield under low N environment (Y_s). We found a negative but non-significant correlation between yield in low N and optimum N environments, supported result by Ali and El-Sadek (2016). Khan and Kabir (2014), Yasir *et al.* (2013) found a positive and non-significant association between yield under stress and non-stress conditions. However, other researcher found a significant positive correlation between yield under stress and non-stress conditions (Aliakbari *et al.* 2014; Khakwani *et al.* 2011; Golabadi *et al.* 2006). The positive correlation between Y_p and Y_s indicates that selection under non-stress environment may give high yielding genotypes under stress environment (Shiferaw *et al.* 2012). The all tolerance indices, except TOL, which have significant positive correlation with grain yield in the stress conditions (Y_s)

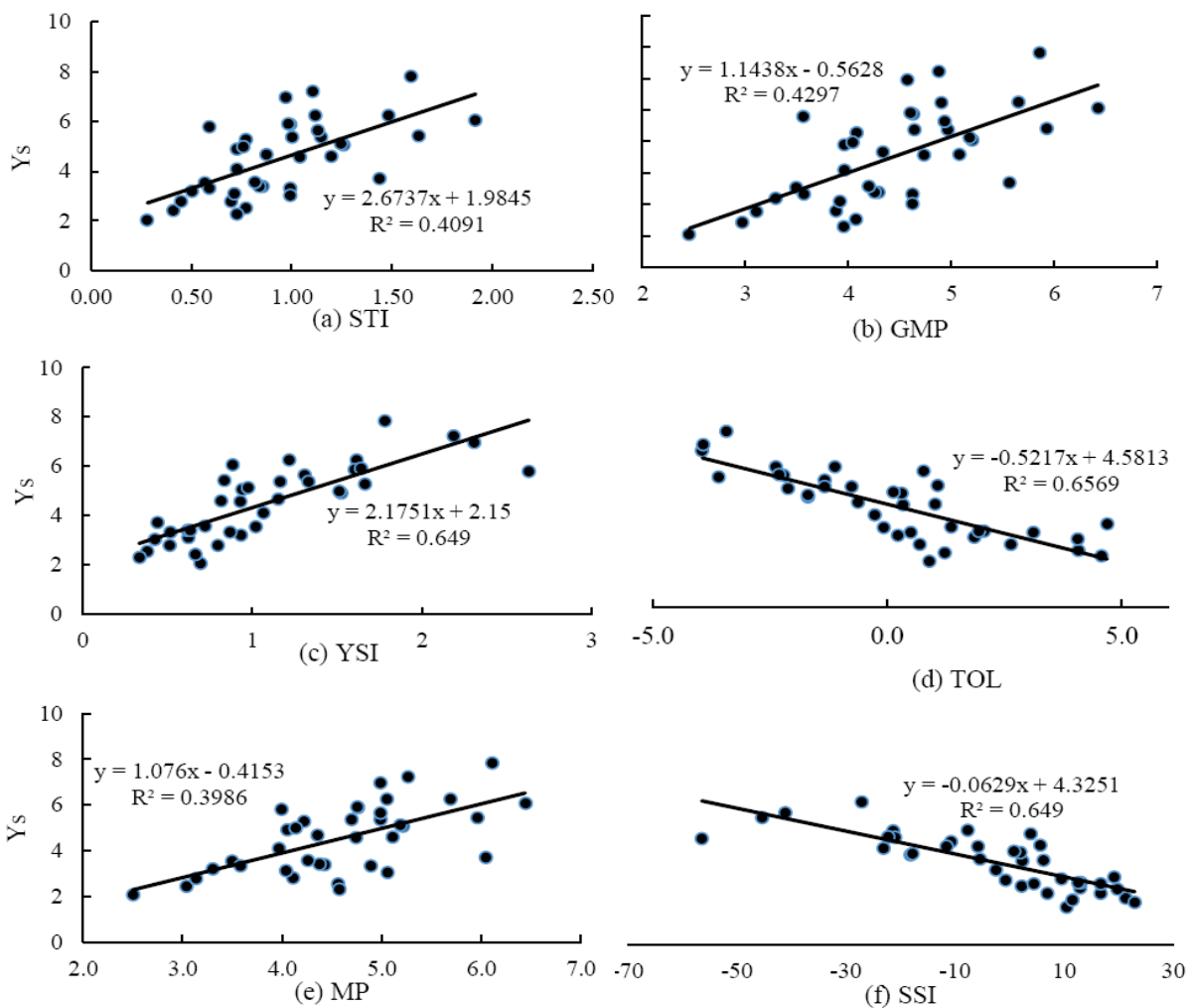


Figure 2. Relationship between grain yield in low N condition (Ys) and (a) stress tolerance index (STI), (b) geometric mean productivity (GMP), (c) yield stability index, and (d) stress tolerance (TOL), (e) mean productivity (MP) dan (f) stress susceptibility index (SSI).

indicating that these indices are suitable criteria for screening tolerant genotypes. In the other hand, if there no significant correlation then they can be discarded as the desirable markers for identifying drought tolerant genotypes (Farshadfar *et al.* 2012). If no significant correlation between yield under non-stress with tolerant indices indicating that those indices were not good indicators to identify the genotype with high yield potential (Aliakbari *et al.* 2014).

Results showed that MP, TOL, GMP, and STI had positive correlation with yield production under optimum N condition, but TOL negatively correlated with yield under low N. This suggested that selection based on low TOL values will reduced grain yield under optimum N environment (Sio-Se Mardeh *et al.* 2006; Talebi *et al.* 2009). Golabadi *et al.* (2006) reported that selection for TOL will be worthwhile only when the target environment is no-drought stressed. Yp and

Ys had positive and significant correlation with MP, STI, and GMP. These results may advise MP, STI and GMP to be the best predicates for both conditions. SSI, TOL and YSI showed disparity against Ys and Yp indicating the population segregated for genes conditioning yield potential and low N tolerance. These result was in line with Ali and El-Sadek (2016), Talebi *et al.* (2009), Mohammadi *et al.* (2012), Ganjeali *et al.* (2011) and Sio-Se Mardeh *et al.* (2006). Majidi *et al.* (2011) reported that GMP and STI indices were similarly able to separate drought sensitive and tolerant genotypes of safflower in both mild and intense water stress environments. Pireivatlou *et al.* (2010) was also noted that STI it self can be a reliable index for selecting high yielding genotypes.

In order to analysis the correlation, a biplot based on principal component analysis was constructed to identify superior genotypes for both low N and optimum N environments. The first component showed high coordination with Ys, SSI, MP, GMP, STI, and YSI. This component had negative correlation with Yp thus, the first dimension can be named as the yield potential and low N tolerance and it separated the tolerant from susceptible genotypes. This component separates low N tolerant genotypes with high yield in both environments. The second component had positive correlation with Yp, TOL, MP, GMP, and STI. This component had negative correlation with SSI and YSI. Hence, the second component can be named the yield potential and susceptible dimension. This finding can be used to discriminate high yielding genotypes which were highly adapted to low N environment. Moosavi *et al.* (2008), Sbei *et al.*

(2014) and Yarnia *et al.* (2011) used biplot analysis to discriminate high yielding wheat, barley, and rapeseed genotypes, respectively, of which were highly adapted to stress conditions.

CONCLUSION

There were significant variations due to genotypes for grain yield in two N environments. PCA analysis showed that the first PCA explained 54.0% of the variation with YSI, SSI, MP, GMP, and STI. Principal component analysis indicated that the first two components accounted for more than 97% of the total variations for drought tolerant indices. Positive and significant correlation of Ys and Yp with MP, GMP, and STI concluded that these indices were the best predictors of yield under low N and optimum N environments.

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