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Efficiency of drought tolerance indices under different stress severities for bread wheat selection

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Abstract

Drought is a world-wide spread problem adversely affecting bread wheat production in rainfed agro-ecosystems. Development and identification of efficient selection criteria for developing drought tolerant wheat varieties with stable and high yield potential is of paramount importance. This study was carried out to evaluate 24 indices for selecting the best high yielding and drought tolerant cultivars, among 40 bread wheat genotypes, under four levels of stress intensities: no stress, mild (0.25, 0.35) and severe (0.57). The mean productivity (MP), modified stress tolerance index (MSTIk), superiority index (Pi), mean relative performance (MRP), relative efficiency index (REI), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean (HARM) and relative decrease in yield (RDY) showed a high power of discrimination among genotypes, and expressed significant correlations with yields under both stress and non-stressed conditions at all stress intensities. This group of indices was capable to select the highest mean yield associated with the least mean variance at 20 % selection pressure. However, as the stress intensity became greater (>35 %), the efficiency of these indices decreased, especially at high stress intensity (57%), where only Pi and MP were still able to target the highest performances. MRP, REI, GMP, RDY and STI can be used interchangeably. Based on GGE analysis, the best performing genotypes were AUS30355, followed by Gladius, Amir-2 and AUS30354 that showed high yield and stability across all the environments. These genotypes are recommended for direct release and/or for use as parents in the breeding programs.

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Keywords: Drought stress, grain yield, stress intensity, tolerance indices, *Triticum aestivum*.
Abbreviations: ATI_Abiotic Tolerance Index, DI_Drought Resistance Index, DRI_Drought Response Index, DTE_Drought Tolerance Efficiency, GM_Golden Mean, GMP_Geometric Mean Productivity, HARM_Harmonic Mean, MP_Mean Productivity, MRP_Mean Relative Performance, MSTIk1_Modified Stress Tolerance Index 1, MSTIk2_Modified Stress Tolerance Index 2, Pi_Superiority Index, RDI_Relative Drought Index, RDY_Relative Decrease in Yield, REI_Relative Efficiency Index, Red_Reduction, SSI_Stress Susceptibility Index, SSPI_Stress Susceptibility Production Index, STI_Stress Tolerance Index, TOL_Tolerance Index, YI_Yield Index, b_Coefficient of regression, bN_Relative adaptability to drought, SNPI_Stress/Non-stress Production Index.

Introduction

Bread wheat is one of the main crops for food security worldwide, representing about 95 % of the wheat grown (Rajaram, 2000). In Morocco, bread wheat is mainly cultivated in rainfed agroecosystems (91 %), characterized by highly variable and unpredictable precipitation pattern and large inter- annual fluctuations (Jlibene, 2009).

The Mediterranean region is identified as one of the most prominent drought hotspots in future climate change projections; especially in North Africa and Middle East (IPCC, 2007). In this context, the adoption of appropriate technological package, principally drought tolerant varieties and other cropping techniques such as fertilization and adequate mechanisation, may reduce the negative impact of the climate change (Gommes et al., 2009).

Drought tolerance is a complex trait, involving several morphological and physiological characters. Thus, efficient screening techniques are a pre-requisite for success in selecting desirable genotypes through any breeding program (Mitra, 2001). Until now, however, no efficient standard selection criteria have been proposed (Golabadi et al., 2006; Sio-Se Mardeh et al., 2006). Selection for yield automatically integrates all the known and unknown factors that contribute to drought tolerance (Richards, 1996). Nevertheless, the heritability of a quantitative trait such as grain yield is very low (Saba et al., 2001). In this perspective, several drought tolerance indices (Table 1) have been suggested to quantify tolerance and select the genotypes tolerant to stress on the basis of a mathematical relationship between yield under drought stress and non-stress conditions (Talebi et al., 2009; Pireivatlou et al., 2010).

A good drought tolerance index should have the power to discriminate between genotypes and must be able to identify superior ones in both drought prone and favorable environments (Saba et al., 2001; Farshadfar et al., 2012). However, the effectiveness of selection indices in differentiating resistant cultivars depends on the stress intensity of target environment varying over years and locations (Panthuwan et al., 2002; Farshadfar et al., 2012). The lines with outstanding performances over different stress intensities should be selected. Many studies (e.g. Moosavi et al., 2008; Talebi et al., 2009; Mohammadi et al., 2010; Mohammadi et al., 2011; Farshadfar and Elyasi, 2012; Farshadfar and al., 2012; Raman et al., 2012; Farshadfar et al., 2013; Golinezhad et al., 2014) have highlighted the efficiency of the indices for drought tolerance selection. However, none of these studies had treated all the major indices listed in the literature to establish the relationships between them and avoid the redundancy of their use. Moreover, the comparison of the effectiveness of these indices was based on simple statistics, mainly analysis of variance, correlation with yield combined with principal component analysis. Finally, the effect of target environment and stress intensity on the indices effectiveness was often mentioned but without further explanations.

In this perspective, the present study aimed to i) investigate the effectiveness of 24 drought indices in screening tolerant cultivars using more statistical proofs (heritability, repeatability, comparison of genotypes selection of different indices) under different drought stress intensities ii) evaluate the relationships among these indices; and iii) identify the highest yielding and the most stable genotypes.

Results and Discussion

Comparative analysis of cropping seasons pattern

The 2014 cropping season was characterized by a drier climate compared to the 2015 season for both experimental sites. The rainfall amount was 30 % less in the stressed site (Sidi El Aidi; 185 and 258 mm during 2014 and 2015 seasons, respectively), and about 33 % in the non-stressed site (Taoujdate; 278 mm versus 413 mm during 2014 and 2015, respectively) (Supplementary Table 1). According to growth stage, drought occurred for all environments at reproductive stage (heading and flowering stages) which is considered as drought of mid-season.

Grain yield

The Pearson’s correlation coefficient showed highly significant association between crop yield and season rainfall ($r = 0.914$; $p<0.001$), indicating that the rainfall is the main source of variation among environments (Site x Year). The combined ANOVA indicated highly significant variability over years and sites; and among genotypes ($p<0.001$) for grain yield (Supplementary Table 2). Moreover, the combined ANOVA over the 4 environments showed highly significant differences among genotypes and environments ($p<0.001$) (Table 2). The Bonferroni test also showed significant differences among the 4 environments confirming our initial assumption (Supplementary Table 3). Accordingly, the stress intensity was used to compare among the four environments and generate the drought stress levels. Over the four environments, the highest mean grain yield (4.49 t/ha) was achieved at the favorable site (Taoujdate, 2015) during the 2015 season. Thus, it can be considered as the potential yield (Yp). During the 2014 season, the mean grain yield in the favorable site (3.35 t/ha) was 25 % lower than the potential yield. Accordingly, Taoujdate in 2014 can be considered as the low moisture stress level (S1) at a stress intensity value of 0.25. During 2015, the stressed site (Sidi El Aidi, 2015) recorded 2.91 t /ha yield level; with 35 % reduction compared to its yield potential. This environment represents the mild stress level (S2), with stress intensity level of 0.35. These two stress levels indicated that the genotypes experienced a mild drought stress (< 50 %). The last stress condition (S3) was based on the grain yield of stressed site during 2014 season (1.93 t/ha). In this case, the stress intensity was stronger (0.57) with 57 % of yield reduction compared to yield potential. This stress level can be considered as severe (more than 50 %) and occurred at Sidi El Aidi, 2014.

Correlation between yield potential and yield under different stress intensities was positive but not significant ($r = 0.302$, $p= 0.059$; $r = 0.280$, $p= 0.08$; $r = 0.128$, $p= 0.432$, respectively for the 3 stressed levels). Thus, the improvement of yield potential may not automatically improve the yield under stressed conditions even under low to moderate stress intensity (Fernandez, 1992; Talebi et al., 2009; Mohammadi et al., 2010; Nouri et al., 2011; Farshadfar et al., 2013).

Heritability estimate measures the standing genetic variation of a population. Considering the grain yield over the four environments, the heritability was only 6 %. In literature, selection mainly for grain yield under drought stress conditions is difficult due to its low heritability resulting from variations in the intensity of the stress through the field (Blum, 1988; Saba et al., 2001). Thus, the improvement of yield under stress must combine a reasonably high yield potential with specific factors which would buffer against a severe yield reduction under stress (Chandler and Singh, 2008).

Drought indices

The results of combined ANOVA (Table 2) indicated that the drought tolerance indices DI, DTE, GMP, HARM, MP, MSTIk1, MSTIk2, Pi, RDY, Reduction, SNPI, SSPI, STI and TOL had significant differences among the three stress levels. This indicates that these indices were influenced by stress conditions, unlike indices ATI, DRI, GM, MRP, RDI, REI, SSI and YI which demonstrated their stability.

Based on one-way ANOVA (within each particular stress intensity) (Table 2), the drought indices DRI, GMP, HARM, MP, MRP, MSTIk1, RDY, REI, STI and YI showed significant differences among genotypes for all stress levels. These indices discriminate among genotypes performances in relation to water stress regardless of stress intensity. The indices RDI, DI, DTE, Reduction, SSI, SSPI, and TOL were significant only at 0.35 and 0.57 stress intensities, showing that they were not able to discriminate between genotypes under slight stress severity. On the other hand, SNPI, Pi, MSTIk2 and GM exhibited significant genotypic differences at moderate stresses (0.25 and 0.35) and lost their efficiency at severe stress (0.57). Therefore, these indices are not useful in discriminating genotypes under severe stress. Finally, significant differences were noted between genotypes for ATI only at 0.25 and 0.57 but not at 0.35 stress intensity (Table 2). The heritability of calculated drought indices is the estimates of the average repeatability of the genetic expressions over the three moisture stress levels (Table 2). Almost all the drought indices showed an important heritability.

Table 1. List of the 24 drought tolerance indices and formula.

Index	Abbr.	Formula	References
Mean productivity	MP	$(Ypi + Ysi)/2$	Rosielle and Hamblin (1981)
Mean relative Performance	MRP	$\left(\frac{Ysi}{Ys}\right) + (\frac{Ypi}{Yp})$	Hossain et al. (1999)
Stress susceptibility index	SSI	$(1 - (\frac{Ysi}{Ypi}))/SI$ Where $SI = 1 - (\frac{Ys}{Yp})$	Fischer and Maurer (1978)
Stress tolerance index	TOL	$Ypi - Ysi$	Rosielle and Hamblin (1981)
Geometric Mean Productivity	GMP	$\sqrt{Ypi \ \times Ysi}$	Fernandez (1992)
Relative efficiency index	REI	$\left(\frac{Ysi}{Ys}\right) \times (\frac{Ypi}{Yp})$	Hossain et al. (1999)
Stress Tolerance Index	STI	$(Ysi \ \times Ypi)/(Yp^2)$	Fernandez (1992)
Modified Stress Tolerance Index 1	MSTIk1	$(\frac{Ypi^2}{Yp^2}) \times STI$	Farshadfar and Sutka (2002)
Modified Stress Tolerance Index 2	MSTIk2	$\left(\frac{Ysi^2}{Ys^2}\right) \times STI$	Farshadfar and Sutka (2002)
Harmonic mean of yield	HARM	$2 \ \times \frac{Ypi \ x \, Ysi}{Ypi + Ysi}$	Dadbakhsh et al. (2011)
Coefficient of regression	b	$\sum Yij \ Yj \ / \sum Y^2$ where i refers to genotypes, j environments, Y overall mean of all genotypes in all environments	Bansal and Sinha (1991)
Relative adaptability to drought	bN	b/a where a is the intercept of regression model	Karamanos and Papatheohari (1999)
Yield Index	YI	${}^{Ysi}/_{Ys}$	Gavuzzi et al. (1997); Lin et al. (1986)
Superiority Index	Pi	$\sum_{i=1}^{n}(Yij - Mj)^2/4$ where I is the genotype, j the environment, M the highest yielding genotype in the environment j	Clarke et al. (1992); Lin et al. (1986)
Reduction	Red	$\left(\frac{Ypi - Ysi}{Ypi}\right) \times 100$	Farshadfar and Javadinia (2011)
Relative drought index	RDI	$\left(\frac{Ysi}{Ypi}\right) \div (\frac{Ys}{Yp})$	Fischer and Wood (1979)
Drought Resistance Index	DI	$Ysi \ \times \left(\frac{Ysi}{Ypi}\right) / \ Ys$	Lan (1998)
Golden Mean	GM	$(Ypi + Ysi)/(Ypi - Ysi)$	Moradi et al. (2012)
Abiotic Tolerance Index	ATI	$\left(\frac{Ypi - Ysi}{\frac{Yp}{Ys}}\right) \times (\sqrt{Ypi \ \times Ysi}$	Moosavi et al. (2008)
Stress Susceptibility Percentage Index	SSPI	$\left(\frac{Ypi - Ysi}{2 \times Yp}\right) \times 100$	Moosavi et al. (2008)
Stress/non-stress Production Index	SNPI	$\sqrt[3]{\frac{Ypi + Ysi}{Ypi - Ysi}} \times \sqrt[3]{Ypi \ \times Ysi \ \times Ysi}$	Moosavi et al. (2008)
Drought Response Index	DRI	$(YA - Yes)/Ses$ where Y_A is yield estimate by regression in stress conditions; Y_{es} Real yield in stress conditions; S_{es} =Standard error of estimated grain yield of all genotypes	Bidinger et al. (1987)
Relative decrease in yield	RDY	$100 - (\left(\frac{Ysi}{100}\right) \times Ypi)$	Farshadfar and Elyasi (2012)
Drought tolerance efficiency	DTE	$\left(\frac{Ysi}{Ypi}\right) \times 100$	Fischer and Wood (1981)

Ysi: Yield under stress for genotype "i; Ypi: Yield under non-stress for genotype "i"; Ys: Mean of grain yield under stressed; Yp: Mean of grain yield under non-stress conditions.

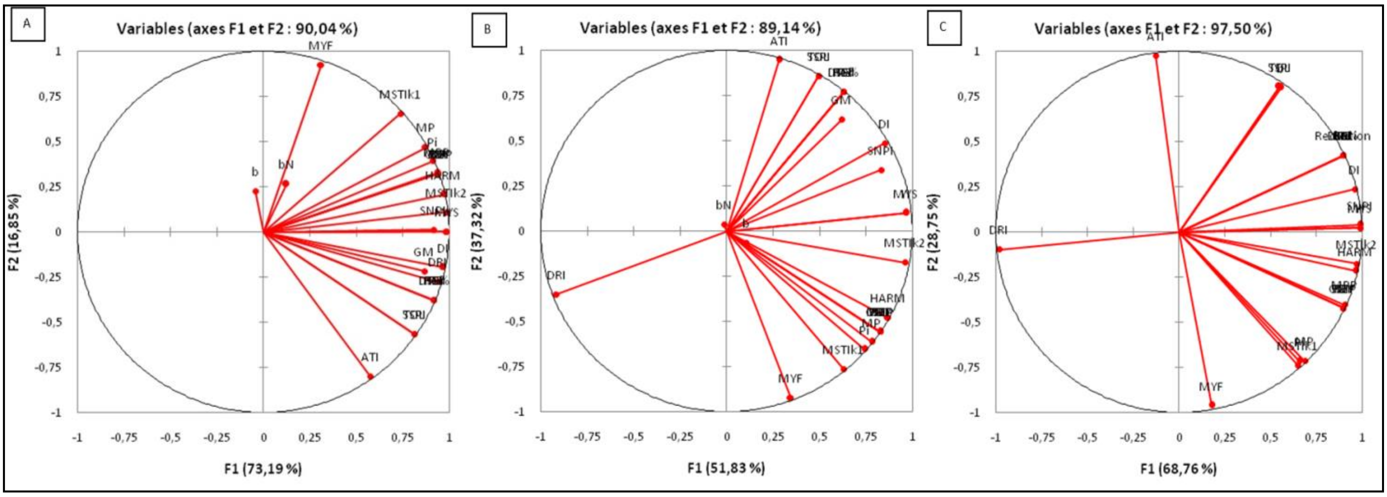

Fig 1. Biplot of drought indices based on Principal Component analysis at 0.25 (A), 0.35 (B) and 0.57 (C) stress severities, respectively.

Table 2. Mean Square of analysis of variance of drought tolerance indices and grain yield for the 40 genotypes across stress levels, genotypes and their interactions

Source of variation	Two way ANOVA						ANOVA S1 25 %	ANOVA S2 35 %	ANOVA S3 57%	h^2 across mean stress levels
	Stress Level	VE (%)	Genotype (G)	VE (%)	SL x G	VE (%)	Genotype Effect	Genotype effect	Genotype effect	
ATI	3.36	5.67	12.46***	21.08	2.26	3.82	7.74*	6.66	1.89*	82
DI	2.6***	60.84	0.43 ***	10.04	0.14	3.37	0.15	0.32 **	0.18*	66
DRI	0.015	0.004	0.83	0.24	1.53***	0.44	1.54***	1.38**	0.99**	0
DTE	36054.2 ***	85.84	2038.2***	0.48	448.9	0.11	737.8	1311.5*	605.3*	78
GM	972.4	19.28	1695.8 ***	33.63	1624.7***	32.2	4433***	419.5 *	83.65	4
GMP	31.69 ***	79.55	1.39***	3.51	0.34	0.86	0.48***	1.005***	0.58**	75
HARM	46.81***	83.51	1.53***	2.74	0.49	0.87	0.52***	1.19***	0.75**	68
MP	16.17***	70.77	1.28 ***	5.59	0.19	0.84	0.45***	0.83***	0.39**	85
MRP	0.028	1.09	0.42***	16.64	0.099	3.9	0.12***	0.27***	0.23**	77
MSTIk1	3.89***	53.96	0.63***	8.68	0.063	0.87	0.29***	0.36***	0.12**	90
MSTIk2	1.87**	32.23	1.01***	17.38	0.33	5.6	0.35***	1.002***	0.29	68
Pi	11.89***	51.05	2.76 ***	11.87	0.55	2.37	0.81**	2.32***	0.73	80
RDI	0.007	0.31	0.57***	23.16	0.12	5.07	0.13	0.28*	0.33*	78
RDY	0.134 ***	80.26	0.0066***	3.91	0.0015	0.9	0.003 ***	0.005 ***	0.002**	77
REI	0.011	0.45	0.43 ***	17.19	0.095	3.78	0.12***	0.27***	0.22**	78
Red	36054.2 ***	85.84	2038.2***	4.85	448.9	1.07	737.8	1311.5 *	605.3*	78
SNPI	174.63***	52.62	74.51***	22.45	38.36***	11.6	86.7***	59.73***	5.78	49
SSI	0.27	6.03	1.86***	41.5	0.502	11.2	1.16	1.28*	0.19 *	73
SSPI	8454.2***	84.71	345.2***	3.46	80.5	0.81	120.1	188.4*	151.8*	77
STI	3.31***	80.26	0.16***	3.91	0.037	0.9	0.07***	0.13 ***	0.041**	77
TOL	68.24***	84.7	2.79***	3.46	0.65	0.81	0.97	1.52*	1.23*	77
YI	0.014	0.89	0.22***	14.29	0.093	6.1	0.05***	0.16***	0.17**	57
Yield	134.17***	94.25	1.62***	1.14	0.67**	0.47	0.86***	1.48***	0.78**	

VE: Percentage of Variation explained (%), *, **, *** Significant at 0.05, 0.01 and 0.001 levels respectively

Table 3. Spearman's rank correlation between grain yields (Ys and Yp) and drought indices over the three stress levels.

Variables	SL1 25 %		SL2 35 %		SL3 57 %	
	YS	YP	YS	YP	YS	YP
YS	1	0.239	1	0.309	1	0.152
YP	0.239	1	0.309	1	0.152	1
MP	0.683	0.837	0.862	0.714	0.658	0.820
MRP	0.738	0.797	0.933	0.594	0.891	0.536
REI	0.731	0.798	0.933	0.584	0.878	0.551
SSI	0.688	-0.494	0.913	-0.066	0.893	-0.229
TOL	0.554	-0.625	0.805	-0.266	0.546	-0.677
GMP	0.731	0.798	0.933	0.584	0.878	0.551
STI	0.731	0.798	0.933	0.584	0.878	0.551
MSTIk1	0.522	0.932	0.718	0.852	0.613	0.836
MSTIk2	0.952	0.483	0.989	0.404	0.969	0.327
HARM	0.789	0.736	0.964	0.488	0.961	0.365
YI	1.000	0.239	1.000	0.309	1.000	0.152
Pi	0.632	0.862	0.894	0.636	0.621	0.811
Red	0.688	-0.494	0.913	-0.066	0.893	-0.229
RDI	0.688	-0.494	0.913	-0.066	0.893	-0.229
DI	0.901	-0.146	0.975	0.120	0.967	-0.047
SSPI	0.554	-0.625	0.805	-0.266	0.546	-0.677
GM	0.579	-0.348	0.804	0.080	0.893	-0.229
ATI	0.360	-0.789	0.560	-0.554	-0.099	-0.986
SNPI	0.782	-0.009	0.871	0.306	0.998	0.139
RDY	0.731	0.798	0.933	0.584	0.878	0.551
DTE%	0.688	-0.494	0.913	-0.066	0.893	-0.229
DRI	-0.957	0.001	0.947	0.038	-0.990	-0.082
b	0.075	0.032	-0.024	0.148	0.555	-0.670
bN	0.018	0.005	0.135	0.233	0.893	-0.229

Bold values are significant at 5% level of probability.

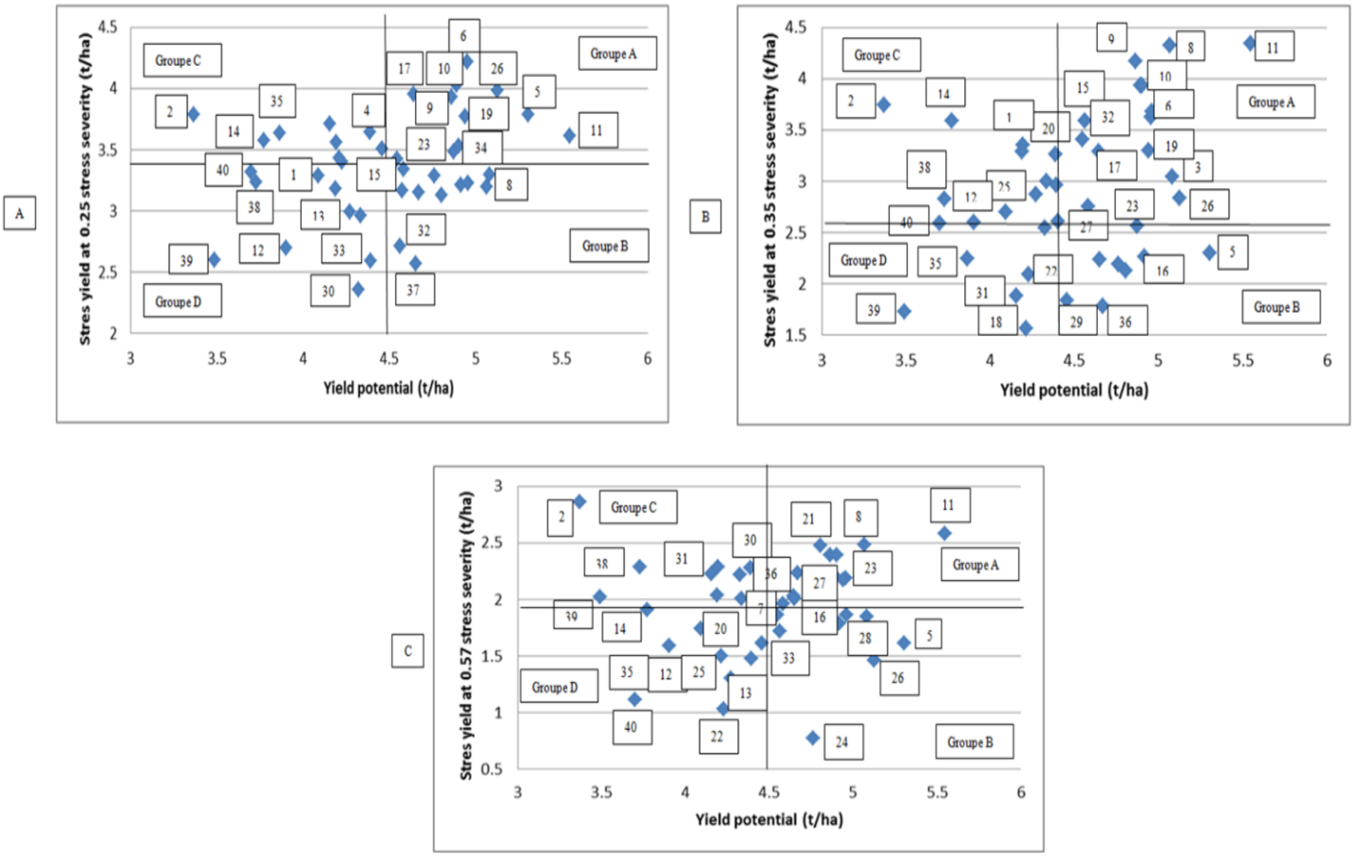

Fig 2. Relationships between Yield potential and Stress yield at 0.25 (A), 0.35 (B) and 0.57 stress severities (C).

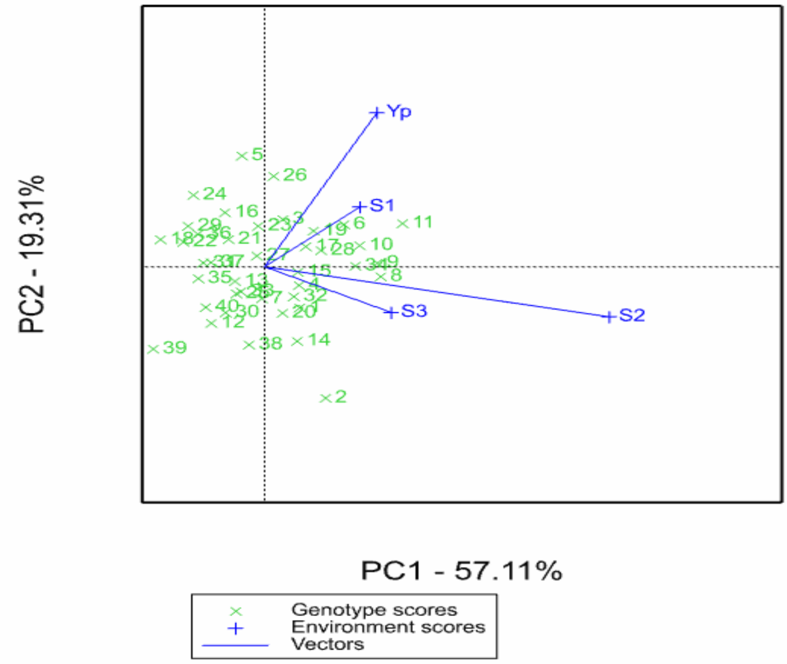

Fig 3. GGE analysis based on grain yield at four environments.

Table 4. Mean yield and variance of the selected top 20% genotypes selection for the 24 drought indices at 0.25, 0.35 and 0.57 stress intensities.

	Stress Level 25 %			Stress Level 35 %			Stress Level 57 %		
Index	Mean yield	Mean variance	Top 20% Genotypes selected	Mean yield	Mean variance	Top 20% Genotypes selected	Mean yield	Mean variance	Top 20% Genotypes selected
MP	4.47	0.36	6, 10, 26, 17, 9, 2, 5, 19	4.47	0.33	11, 8, 9, 10, 34, 2, 28, 6	3.68	1.75	2, 11, 8, 21, 9, 34, 38, 1
MRP	4.47	0.36	11, 5, 26, 3, 8, 28, 6, 19	4.46	0.27	11, 8, 9, 34, 10, 28, 6,19	3.62	1.51	11, 8, 21, 34, 9, 6, 10, 19
REI	4.47	0.36	6, 11, 26, 5, 10, 9, 19, 17	4.46	0.27	11, 8, 9, 34, 10, 28, 6, 32	3.68	1.75	11, 5, 26, 3, 8, 28, 6, 19
GMP	4.47	0.36	6, 26, 11, 5, 10, 9, 19, 17	4.46	0.27	11, 8, 9, 34, 10, 28, 6, 32	3.68	1.75	11, 8, 34, 21, 9, 6, 19, 10
STI	4.47	0.36	6, 26, 5, 11, 10, 9, 19, 17	4.46	0.27	2, 14, 9, 8, 10, 34, 1, 32	3.68	1.75	11, 8, 21, 34, 9, 6, 10, 2
MSTIk2	4.47	0.36	2, 14, 35, 40, 31, 38, 17, 6	4.46	0.27	2, 14, 9, 8, 1, 20, 38, 10	3.60	1.46	11, 8, 34, 9, 21, 6, 19, 10
HARM	4.47	0.60	2, 14, 35, 40, 31, 38, 1, 17	4.46	0.27	11, 8, 9, 34, 10, 28, 6, 32	3.62	1.51	11, 8, 21, 34, 9, 6, 10, 19
RDY	4.47	0.36	6, 26, 5, 11, 10, 9, 19, 17	4.46	0.27	11, 8, 9, 34, 10, 28, 6, 32	3.68	1.75	11, 8, 21, 34, 9, 6, 10, 19
Pi	4.46	0.40	6, 26, 5, 11, 10, 9, 19, 17	4.47	0.33	11, 5, 26, 3, 8, 28, 6, 19	3.68	1.75	2, 38, 39, 14, 1, 31, 30, 4
MSTIk1	4.46	0.44	11, 5, 26, 6, 10, 19, 9, 3	4.46	0.37	11, 8, 9, 34, 10, 28, 6, 3	3.68	1.75	2, 38, 39, 1, 31, 4, 21, 30
YI	4.35	0.24	6, 10, 26, 17, 9, 2, 5, 19	4.39	0.25	11, 8, 9, 34, 10, 28, 6, 32	3.51	1.23	2, 11, 8, 21, 9, 34, 38, 1
DI	4.08	0.09	26, 5, 6, 11, 10, 9, 19, 34	4.29	0.17	2, 14, 9, 8, 10, 34, 1, 32	3.42	1.09	2, 38, 39, 1, 31, 4, 21, 30
SNPI	4.07	0.09	2, 14, 35, 40, 31, 38, 17, 6	4.38	0.22	11, 8, 9, 10, 34, 2, 28, 6	3.51	1.23	11, 8, 34, 9, 21, 6, 19, 10
b	4.00	0.52	2, 14, 35, 40, 31, 38, 17, 6	3.66	0.74	26, 2, 38, 40, 23, 16, 28, 33	3.09	0.76	24, 22, 40, 13, 35, 26, 5, 33
bN	3.96	0.57	2, 6, 35, 14, 17, 31, 10, 9	3.66	0.74	26, 2, 40, 38, 23, 33, 28, 16	3.19	0.82	2, 11, 21, 8, 9, 38, 34, 1
GM	3.89	0.06	2, 14, 35, 40, 31, 38, 1, 17	4.16	0.17	2, 8, 9, 14, 11, 10, 34, 32	3.19	0.82	2, 38, 39, 1, 31, 4, 21, 30
SSI	3.85	0.06	14, 35, 40, 31, 38, 17, 6, 1	4.14	0.15	2, 14, 9, 8, 1, 20, 38, 10	3.19	0.82	2, 38, 39, 1, 31, 4, 21, 30
Red	3.85	0.06	2, 14, 35, 40, 38, 31, 1, 39	4.14	0.15	14, 9, 8, 10, 34, 1, 32, 20	3.19	0.82	2, 38, 21, 1, 8, 11, 31, 4
RDI	3.85	0.06	14, 35, 6, 31, 17, 10, 40, 9	4.14	0.15	2, 14, 38, 9, 1, 20, 40, 8	3.19	0.82	2, 38, 39, 14, 1, 31, 30, 4
DTE	3.85	0.06	6, 26, 5, 11, 10, 9, 19, 17	4.14	0.15	14, 8, 9, 11, 10, 34, 32, 28	3.19	0.82	2, 39, 38, 14, 40, 35, 12, 31
TOL	3.76	0.05	2, 14, 35, 40, 31, 38, 17, 6	3.96	0.14	11, 8, 9, 34, 10, 28, 6, 32	3.09	0.76	11, 8, 21, 34, 9, 6, 10, 19
SSPI	3.76	0.05	30, 37, 33, 32, 39, 12, 7, 13	3.96	0.14	2, 14, 9, 8, 10, 34, 1, 32	3.09	0.76	2, 38, 39, 1, 31, 4, 21, 30
ATI	3.60	0.06	8, 21, 32, 3, 9, 14, 24, 4	3.79	0.15	2, 8, 14, 9, 11, 6, 10, 34	2.83	0.94	2, 38, 39, 1, 31, 4, 21, 30
DRI	3.46	0.64	11, 35, 16, 6, 27, 7, 33, 37	4.31	0.19	11, 8, 9, 34, 10, 28, 6, 19	2.86	2.61	2, 38, 39, 14, 1, 31, 30, 4

Table 5. List of the 40 bread wheat genotypes used for this study.

Entry Code	Name	Origin	Entry Code	Name	Origin
1	NEJMAH-11	ICARDA	21	SB062	CIMMYT
2	NEJMAH-14	ICARDA	22	SB109	CIMMYT
3	SHIHAB-12	ICARDA	23	SB169	CIMMYT
4	AL-ZEHRAA-2	ICARDA	24	SsrT02	CIMMYT
5	BAASHA-21	ICARDA	25	SsrT09	CIMMYT
6	AMIR-2	ICARDA	26	SsrT14	CIMMYT
7	ATTILA	CIMMYT	27	SsrT16	CIMMYT
8	SOKOLL	CIMMYT	28	SsrT17	CIMMYT
9	GLADIUS	AUSTRALIA	29	SsrW35	CIMMYT
10	AUS30354	CIMMYT	30	SsrW47	CIMMYT
11	AUS30355	CIMMYT	31	ARREHANE	Morocco
12	AUS30518	CIMMYT	32	ACHTAR	Morocco
13	AUS30523	CIMMYT	33	MARCHOUCH	Morocco
14	QG-170-4.1	CIMMYT	34	KANZ	Morocco
15	QG-58-5.1	CIMMYT	35	AMAL	Morocco
16	HARTOG	AUSTRALIA	36	MASSIRA	Morocco
17	DRYSDALE	AUSTRALIA	37	AGUILAL	Morocco
18	SB003	CIMMYT	38	BT05A104	Morocco
19	SB165	CIMMYT	39	BT05A106	Morocco
20	SB069	CIMMYT	40	RAJAE	Morocco

The highest heritability was expressed by MSTIk1 (90 %), MP (85 %); ATI (82 %); Pi (80 %); DTE, RDI, REI, Reduction (78 %); SSPI, STI, TOL, RDY, MRP (77 %) and GMP (75 %). These indices are the most interesting as they show high repeatability across samples of environments and stress levels; indicating that selection based on them will be more fruitful. However, the lowest heritability was observed for GM (4 %), bN (16 %) and the coefficient of regression b (28 %).

Spearman correlations among grain yield (Yp and Ys) and drought indices

The index MP kept high correlation with both Yp and Ys at the three drought stress intensities (Table 3). Several studies stated the effectiveness of this widely used drought index under moderate stress (Golabadi et al., 2006; Sio-Se Mardeh et al., 2006; Mohammadi et al., 2010; Farshadfar and Elyasi, 2012; Farshadfar et al., 2012; Farshadfar et al., 2013) and severe stress (Moosavi et al., 2008; Talebi et al., 2009; Mohammadi et al., 2011; Moradi et al., 2012). MP represents the average yield under stress and non-stress conditions; allowing to describe yield variation under various moisture regimes (Rosielle and Hamblin, 1981). However, this index is maximized even when yield in either normal or stressed environment is too high (Najafian, 2009). The same findings were observed for (MSTIk1 and Pi) (Table 3) which is in agreement with numerous studies for MSTIk1 under moderate (Farshadfar and Elyasi, 2012; Farshadfar et al., 2012) and severe stress (Naghavi et al., 2013; Gholinezahed et al., 2014); and for Pi under moderate stress (Saba et al., 2001; Mohammadi et al., 2011) and severe stress (Mohammadi et al., 2010; Akçura and Ceri, 2011). However, their correlation with Ys became moderate at severe stress (0.57). This result can be explained by the fact that Pi formula is based on the distance mean square between the cultivar's response and the maximum response over environments (Lin and Binns, 1988). Hence, Pi instantly relates to the agronomic target of identifying genotypes with relatively high yield potential. Regarding MSTIk1, the equation is favoring mainly the yield potential power rather than the stress yield. MSTIk2 showed high positive correlation with Ys for all the stress intensities; while its relationship with Yp seemed to be always moderate (Table 3). This is in concordance with Farshadfar and Elyasi (2012); Farshadfar et al. (2012) and Gholinezhad et al. (2014) studies. This result can be explained by the formula which favors the yield under stress conditions (correction coefficient) rather than the yield potential. For the indices MRP, REI, GMP, STI, HARM and RDY, the correlation is strong with both yields; however, their correlation with Yp was high only at 0.25 stress intensity, but became moderate at 0.35 and 0.57 (Table 3). The results concerning HARM, GMP and STI are in agreement with Talebi et al. (2009); Mohammadi et al. (2011); Moradi et al. (2012) and Farshadfar et al. (2013). However, Rahmani et al. (2013) found an absence of correlation of Yp with HARM, GMP and STI under severe stress (0.6). Moreover, the association between yield and RDY was observed at slight moderate stress (Farshadfar and Elyasi, 2012); moderate (Farshadfar et al., 2013; Gholinezhad et al., 2014) and severe stress level (Gholinezhad et al., 2014). Similar to our findings, REI and MRP were useful in identifying genotypes with high yield potential in Bennani et al. (2016). All these indices have in common the yields product (Ys and Yp) in their equation and consider the effect of both yields in balance. Thus, the selected genotypes based on these indices are characterized by drought tolerance and will improve yield under stress conditions. Ys was positively and significantly correlated with SSI, RED, RDI, GM and DTE. However, the correlation of Yp with the same group was significant at 0.25 stress intensity; and non-significant at 0.35 and 0.57 (Table 3). These findings are in concordance with Mohammadi et al. (2010); Farshadfar et al. (2012); Moradi et al. (2012) and Rahmani et al. (2013). These indices are influenced by the variation between yields under stress and favorable conditions and permit to select drought tolerant genotypes. This can be explained by their formula which is favoring the stability more than the high yielding. Tolerance index (TOL) is computed as a tolerance degree. The positive correlation between TOL and Ys was strong at moderate stress levels (0.25 and 0.35); but became moderate at 0.57 stress intensity (Table 3). These results are in agreement with Talebi et al. (2009) and Moradi et al. (2012) but in contradiction with the findings of Farshadfar et al. (2012) and Farshadfar et al. (2013) where there is no association even at moderate stress;

and with Naghavi et al. (2013) and Rahmani et al. (2013) where high correlations were noted between the two components. However, strong negative correlation was found between TOL and Yp at all stress levels (Table 3) (Talebi et al., 2009; Akçura and Ceri, 2011; Moradi et al., 2012; Farshadfar et al., 2012; Rahmani et al., 2013). Sio-Se Mardeh et al. (2006) suggested that selection based on TOL could result in reduced yield under well-watered conditions (low Yp and Ys). TOL index only assess the plasticity of the genotypes under study, whereas a variety may rank first in both environments but still have higher TOL than the other varieties (Saba et al., 2001).

The indices YI, DI, SNPI and SSPI showed strong positive correlation only with Ys except the moderate correlation for SSPI at 0.57 stress intensity (Table 3). The same findings were found in Moosavi et al. (2008) and Gholinezhad et al. (2014). On the other hand, negative correlation characterized the relationship between Ys and DRI at 0.25 and 0.57 as stated by Farshadfar et al. (2012) and Ys with b and bN at severe stress level only (0.57) as indicated in Mohammadi et al. (2010) and Akçura and Ceri (2011) studies. The two former indices (YI and DI) formula are mainly focusing on yield under stress; while SNPI and SSPI rely on crop survivals in stress conditions revealing the relative yield stability of genotypes with changing conditions. The result of selection was appropriate for cultivars with potential stress tolerance, but may not be for cultivars with high yield in both conditions (Moosavi et al., 2008).

The index ATI showed a high significant negative correlation with Yp at 0.25 and 0.57 stress intensities (-0.79 and -0.99 respectively); while moderate correlation was observed between Yp and ATI at 0.35 (-0.55) (Table 3). These findings are in concordance with the results of Moosavi et al. (2008), Farshadfar et al. (2012), Rahmani et al. (2013) and Gholinezhad et al. (2014). However, significant positive correlation of 0.36 and 0.56 were observed between ATI and Ys at stress intensities of 0.25 and 0.35 respectively, which, however disappeared at 0.57 (Table 3). The same results were observed by Farshadfar and Elyasi (2012); Farshadfar et al. (2012); Rahmani et al. (2013). For this index, the yield stability is also more important than the high yield under non-stressed conditions. However, it has more emphasis on Yp than SSPI, SSI and TOL (Moosavi et al., 2008).

Relationships between drought tolerance indices

The principal component analysis was used to describe the interrelationships among all traits on the basis of overall pattern of the data (Fig 1). The bi-plot presents a whole picture about the interrelationships among the drought indices through the cosine of the angle between the vectors. The more the cosine between two indices is high, the more the traits (indices) are different, and the reverse is true (Yan et al., 2000). Low correlation between various indices suggests that each index may be a potential indicator of differential biological response to drought.

The two first principal components (PC) explained 89, 90 and 97.5 % at 0.25, 0.35 and 0.57 stress intensities, respectively. The PC1 regrouped at the three stress levels Ys, MRP, REI, GMP, STI, MSTIk2, HARM, YI, DI, SNPI and RDY. This component can be called “Stress tolerance component”. On the other hand, the PC2 showed consistent positive correlation with Yp and MSTIk1. This component can be called “Yield potential component” (Fig 1).

Over all the drought intensities, the indices MRP, REI, GMP, RDY and STI consistently showed (at all stress levels) an overlapping of their vectors. The indices HARM followed by MSTIk2 had strong correlation with this group (vector angle below 90°). The same observations were found for Pi, MP and MSTIk1. These relationships became less strong at 0.57 compared to 0.25 and 0.35 stress intensities. These results were reported by Mohammadi et al. (2011); Farshadfar et al. (2012); Raman et al. (2012) and Rahmani et al. (2013). SNPI had also strong correlation with Ys at 0.25 stress intensity (below 90°); however, the overlap of vectors was observed at 0.35 and 0.57 stress levels. Furthermore, an overlapping of vectors was found between Ys and YI at all stress levels with strong correlation with SNPI (Fig 1). The observed relationships between YI and SNPI are consistent with those reported by Farshadfar and Elyasi (2012).

Moreover, the indices SSI, RED, RDI, and DTE also showed the overlapping of vectors (Fig 2). The same results were observed by Moosavi et al. (2008), Yarnia et al. (2011) and Nouraein et al. (2013). Strong correlations were observed between this group and GM and DI (below 90 °C) especially at severe stress intensity (0.57) where we have an overlapping of vectors. SSPI and TOL had also collapsing of vectors as reported by Moosavi et al. (2008); Farshadfar and Elyasi (2012); Farshadfar et al. (2012); Naghavi et al. (2013); Rahmani et al. (2013).

The indices b and bN contributed consistently in the genetic variation observed only at 0.57. The coefficient of regression (b) always had high correlation with bN as stated by Mohammadi et al. (2010). Overall, one of the indices MRP, REI, GMP, RDY and STI can be used interchangeably as an alternative for the others in genotypes selection. The same observation can be made for the group (SSI, RED, RDI, and DTE) and for the two indices b and bN.

Comparison of genotypes selection based on yield performances and drought indices

For the three stress intensities, the genotypes were classified in the four Fernandez groups (A, B, C and D) based on the relationship between Yp and Ys (Fig 2). The group A includes high yield in both conditions. The group B contains high yield under non-stress conditions. The group C incorporates good yield under stressed conditions, while the group D integrates low yield in both conditions (Fernandez, 1992).

Overall, the genotypes 6, 9, 10, 11, 17, 19 and 34 belonged to the group A at the three stress levels and can be considered as high yielding and stable genotypes across all the environments studied. Based on GGE outputs, the GGE biplot explained about 76 % of the total variation (Fig 3). The environment played the most important part in yield variation (97%), while, based on total sum of squares (Data not Shown), only 1.2 % and 0.47 % of variation were attributed for both genotypes and interaction effects. The genotype G11 followed by G9, G6 and G10 had the best performances of high yield and stability across all the environments.

Each index provides proper genotypes ranking according to its appropriate formula. At 0.25 stress intensity, in comparison with Fernandez groups outputs (Fig 2), the indices MP, MRP, REI, GMP, STI, MSTIk2, HARM, Pi and RDY selected efficiently the group A (6, 11, 26, 5, 10, 9, 19, 17) when considering 20 % of selection pressure (Table 4). The indices MISTk1 and Yi selected the group A; but MSTIk1 integrated genotype 3 which belongs to group B instead of genotype 17; while Yi selected genotype 2 belonging to the group C instead of genotype 11. At 0.35 stress intensity (Table 4), the indices MP, REI, GMP, STI, MSTIk1, Pi, and RDY were composed at 87.5 % from group

A, and one genotype from group B (G21) (Fig 2). MRP and HARM were formed mainly by group A (75 %) and one genotype from group B (G21) and group C (G2). At 0.57 stress intensity (Table 4), the indices MP and Pi selection was based mainly on group A (87.5 %) and one genotype from group B (G28) (Fig 2). The indices MRP, REI, GMP, STI, MSTIk1, MSTIk2, HARM, RDY, YI, DRI selection was composed by 75 % of group A. The index SNPI selection was formed by 62.5 % of group A, 25 % of group B and one genotype from group D (G14); while DI selection was constituted by 62.5 % of group A, and the rest of genotypes belonged equally to the remaining groups (B, C and D). The remaining indices were not able to target the group A and didn't select more than 50% of the 20% genotypes selection pressure (Table 4).

Regarding the mean yield and mean variance of the genotypes selected (Table 4), the indices MP, MRP, REI, GMP, STI, MSTIk2, HARM, RDY, Pi and MSTIk1 showed the highest mean yield (4.47 t/ha) associated with mean variance (0.37) at 0.25 of stress level. At 0.35, the same indices exhibited the best mean yield that didn't differ from the one at 0.25 (4.46 t/ha) but the variance was reduced (0.29). However, at severe stress (0.57), the reduction of mean yield by 18 % was obvious and reached 3.66 t/ha for the same indices and showed an increase of variance (1.67).

Selection of the best drought tolerance indices

The objective of this study was to identify the best drought yield indices able to identify breeding lines with superior performances over various stress severities (non-stress, slight stress, moderate stress, severe stress). These indices must better express genetic differences, high heritability and repeatability across samples of the environments, high correlation with yields under stressed and non-stress conditions and must target the highest performances (group A) (Fernandez, 1992; Mitra, 2001; Bennani et al., 2016). Our study aims to study all the known drought indices cited in the literature, under a contrasting inter annual and inter site Moroccan climate, to elucidate their efficiency under three drought scenarios based on more statistical proofs.

The drought indices MP, MSTIk1, Pi, MSTIk2, MRP, REI, GMP, STI, HARM and RDY showed high significant differences among genotypes at each stress level, showing a high degree of discrimination. They expressed significant correlation with both yields (potential and stressed yields) at all stress intensities. The index MP kept high correlation at the three stress intensities. However, the correlation of MSTIk1 and Pi with Ys was moderate at severe stress (0.57); while the indices (MRP, REI, GMP, STI, HARM and RDY) showed moderate association with Yp at 0.35 and 0.57. This selection of indices also exhibited the highest mean yield for all drought intensities associated with the lowest mean variance of genotypes selected at 20 % of pressure. Regarding Fernandez groups selection (1992) at 20 % selection pressure, the same indices (MP, MSTIk1, Pi, MSTIk2, MRP, REI, GMP, STI, HARM and RDY) selected efficiently the group A at 0.25 except MSTIk1 which integrated one genotype from group B to its list of selection. Many studies have reported the effectiveness of these indices in selecting high yielding and drought tolerant genotypes (Rosielle and Hamblin, 1981; Fernandez, 1992; Ramirez and Kelly, 1998; Hohls, 2001; Golabadi et al., 2006; Jafari et al., 2009; Talebi et al., 2009; Mohammadi et al., 2010; Farshadfar et al., 2012; Raman et al., 2012). However, the drought indices are influenced by stress intensity and difference in drought patterns among locations and years. Therefore, when the stress became more intense, these indices became less efficient, as stated also by Mohammadi et al. (2010). In fact, At 0.35 stress severity, MP, REI, GMP, STI, MSTIk1, Pi, RDY remained the best and selected 7 genotypes from group A out of 8, and one genotype from group B; while MRP and HARM selected 6 genotypes from group A and incorporated 2 genotypes respectively from group B and group C. At severe stress (0.57), only MP and Pi kept high performances by selecting 7 genotypes from group A out of 8; while MRP, REI, GMP, STI, MSTIk1, MSTIk2, HARM and RDY selected 6 genotypes from group A and 2 genotypes from group B and C respectively.

Overall, based on the statistical analysis, the indices MP, REI, GMP, STI, MSTIk1, Pi and RDY represent the appropriate selection criteria for drought tolerance; especially MP and Pi. As REI, GMP, STI and RDY can be used interchangeably, a cross selection based on MP REI, MSTIk1 and Pi should be the best combination for an efficient selection of the best performances under drought stress.

Materials and Methods

Plant materials and experimental design

Forty spring bread wheat genotypes, from diverse origins (Australia, Morocco, ICARDA, CIMMYT) (Table 1), were chosen based on their broad range of response to drought stress and yield performance and were planted in Randomized Complete Block design (RCBD) with three replications in two contrasting experimental fields (stressed and non-stressed) during 2013-14 and 2014-15 cropping seasons. Each combination "Site x Year" was considered as an environment.

Each plot (9 m²) was composed of 6 rows of 5 m length, with inter-row distance of 0.25 m. The sowing was performed in late November and harvesting was carried out on mid-May for stressed fields and mid-June in non-stressed experimental site. The fertilizers (N18 - P46 - K00) and Urea (33.5 %) were applied at a rate of 1 quintal/ha before planting and tillering stage respectively. The plants were protected against foliar diseases by applying fungicides (Impact) at a rate of l/ha twice (at booting and heading stages), and weeds were controlled manually and by herbicides using Cossak (1l/ha) at the beginning of the season and Mustang (1l/ha) at reproductive stages.

Experimental Sites

The study considered two contrasting experimental sites in terms of long term average rainfall, namely "Taoujdate" and "Sidi El Aidi", belonging to the National Institute of Agricultural Research of Morocco. The favorable site (Taoujdate) is located at 33°55'49''N latitude, 5°16'33''W longitude, at an elevation of 550 m above sea level. The soil is deep clay. The yearly average maximum and minimum temperatures are 19.9 °C and 2.8°C respectively, and average annual rainfall is 470 mm. The "Sidi El Aidi" station represents the stressed semi-arid site with 300 mm as mean rainfall. It is located at 33°07'16''N latitude, 7°37'44'' longitude, at an elevation of 240 m. The soil is deep clay. The yearly average maximum and minimum temperatures are 19.5 °C and -4°C respectively.

Statistical analysis

The grain yield of each plot was evaluated based on 9 m², and converted to the standard unit at metric ton per hectare (t/ha).

The stress intensity calculation was based on the formula: $SI = 1 - (\frac{Ys}{Yp})$ considering all the combinations between the yields across years and sites. The Yp refers to potential yield at the favorable season in Taoujdate, while Ys is the stressed yield. We assume that rainfall is the main driving force of yield in these environments. Then, based on the mean grain yield across trials under non-stress, moderate and severe stress conditions, conventional drought tolerance indices were calculated (Table 1).

The combined analysis of variance (ANOVA) was carried out for grain yield considering the effects of three factors (years, sites and genotypes) according to the model:

$$Y = Year + Site + Bloc\ (site) + Genotypes + Genotype \times Year + Genotype \times Site + Genotype \times Year \times Site + Error$$

The second model of two-ways ANOVA was used for single environments for grain yield and drought indices using the model:

$$Y = Stress\ Level\ or\ environment + Genotypes + Genotype \times Stress\ Level\ or\ environment + Error$$

Finally, the third model was used for each stress level separately to detect the genotypic effect per stress level using the model:

$$Y = Genotype + Bloc + Error$$

For each combined ANOVA, the magnitude of variation attributable to each factor was estimated as percentage of variance explained (VE %) of total sum of squares.

The broad sense heritability of grain yield was computed based on mean square variations according to the formula developed by Lush (1940) and Robinson et al. (1949) as follows:

$$h^2(\%) = \left(\frac{Vg}{Vp}\right) * 100$$

Where, Vg is the genotypic variance

Vp is the phenotypic variance

For ranking the genotype that had the least of SSI, TOL, Pi, SSPI, ATI, RDY, Reduction, bN and b indices value and the most of HARM, MP, MRP, REI, GMP, STI, MSTIk1, MSTIk2, YI, RDI, DI, GM, SNPI, DTE and DRI earned the first position (rank 1).

The ANOVA was performed using GENSTAT software (Discovery edition 3, VSN International, UK). The correlation and PC analysis were carried out using XLSTAT (Free trial version 2015, Addinsoft, Inc., Brooklyn, NY, USA); while the GGE analysis was performed using BMS software.

Conclusion

Significant differences among genotypes in grain yield were observed across the four environments (non-stress, 0.25, 0.35 and 0.57 stress intensities). Over all the stress intensities, a cross selection based on the indices REI, MSTIk1, GMP, STI, RDY, MP and Pi (especially the 2 last ones) can enable breeders to select efficiently advanced bread wheat lines. The indices REI, GMP, STI and RDY can be used interchangeably. Based on indices selection and GGE analysis, AUS30355, Gladius, Amir-2 and AUS 30354 were the best high yielding and drought tolerant genotypes among the 40 lines evaluated. These genotypes are recommended for direct release and/or parentage purposes in the breeding programs.

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