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Suitability of drought tolerance indices for selecting alfalfa (*Medicago sativa* L.) genotypes under organic farming in Austria

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ABSTRACT

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In eastern Austria, alfalfa is usually grown as a rainfed crop in crop rotations in organic farming systems, where year-to-year rainfall fluctuations cause different levels of drought stress. To identify the suitability of different alfalfa genotypes and drought tolerance indices, 18 contrasting alfalfa genotypes were evaluated under irrigated and rainfed conditions at the research station of the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria, during 2006-08. The first study year (2006) was considered as the establishment year. Five drought tolerance selection indices were estimated based on shoot dry matter, total biomass yield and biological nitrogen fixation (BNF) data. The correlation between irrigated and rainfed performances increased (from r=-0.17 to 0.56) with decreasing stress intensity from the first to the second year. Genotypes Sitel, Plato ZS, Vlasta and NS-Banat were the best genotypes based on their performance under both conditions. Drought tolerance selection indices TOL and SSI showed high correlations (r = 0.32 to 0.81) only with rainfed performance, and SSI was the index that best identified genotypes with high yield potential under rainfed conditions. Indices STI and GMP were the ones that best identified genotypes with high performance under both conditions.

Keywords: biological nitrogen fixation, Iranian ecotypes, shoot dry matter, stress intensity, total biomass yield

INTRODUCTION

Legume fodder crops such as alfalfa (*Medicago sativa* L.) are an essential component of organic farming systems, especially under arid and semiarid conditions. Stockless organic farming is predominant in the dry Pannonian region of eastern Austria, and alfalfa-with its high biological nitrogen fixation (BNF) ability and drought tolerance-is the best known fodder crop in that region. Alfalfa is usually grown as a rainfed crop in crop rotations where the amount, frequency and duration of rainfall fluctuate from year-to-year. Annual rainfall fluctuations may cause different levels of water availability, from optimum conditions to high intensity drought stress. The effect of drought stress is intensified when accompanied by high temperatures, biotic stresses and undesirable soil characteristics such as low water-holding capacity.

Selection solely under favorable or solely under stress conditions may lead to specifically adapted genotypes, i.e., genotypes with a suitable response to specific conditions. Ceccarelli and Grando (1991) stated that breeding programs can produce cultivars with contrasting adaptation patterns by adopting distinct genetic bases (each including materials with the desired adaptive response), distinct selection environments (each representative of the target population in a particular environment), or both. Thus, there are three approaches for choosing a breeding strategy for stress environments. Some researchers believe in selecting under non-stress conditions and subsequent yield testing in stress environments (Roy and Murty, 1970; Mederksi and Jeffers, 1973; Richards, 1996; Betran *et al.*, 2003). They assume that genotypes that are superior under favorable conditions will also produce relatively good yields under stress conditions, and that genotypes selected under stress conditions will show low yield potential in more favorable environments (Ceccarelli, 1987). Followers of the second approach rely on direct selection under the target stress conditions (Boyer and McPherson, 1975; Johnson, 1980; Buddenhagen, 1983; Ceccarelli, 1987; Ceccarelli and Grando, 1991), which they believe is most efficient for increasing yield in those conditions. Accordingly, direct selection in stress environments will decrease yield in non-stress environments unless genetic variances in stress environments are considerably greater than those in non-stress environments, or genetic correlations between stress and non-stress environments are positive and close to 1 (Rosielle and Hamblin, 1981).

Based on the specific strengths and weaknesses of the two above-mentioned approaches, simultaneous selection under stress and non-stress conditions seems more likely to produce results. Based on this, some researchers believe in a third approach: parallel selection under favorable and unfavorable conditions (Fischer and Maurer, 1978; Clarke *et al*., 1992; Fernandez, 1992).

Based on genotypic performance in stress and non-stress environments, Fernandez (1992) proposed a system for classifying genotypes into four groups: genotypes with uniform superiority in both stress and non-stress environments in Group A; genotypes that perform favorably only in non-stress environments in Group B; genotypes that have relatively high yields only in stress environments in Group C; and genotypes with poor performance in both stress and non-stress environments in Group D. Several selection criteria have been proposed for selecting genotypes based on the mathematical relationship between their performance under stress and non-stress conditions (Rosielle and Hamblin, 1981; Fischer and Maurer, 1987; Fernandez, 1992). The ideal selection index and criterion would distinguish Group A (widely adapted genotypes) from the other three groups quantitatively.

In the context of fodder crop breeding, forage yield trials under two contrasting conditions, non-stress and stress, are widely used to select genotypes suitable for and adapted to both environments. In addition to shoot yield (harvestable biomass), the yield of non-harvestable biomass (stubble and root) can play an important role in total biological nitrogen fixation (BNF) and in supplying nitrogen for the following crop, especially in management systems where forage shoot biomass is harvested and removed (for example, to use as organic dairy feed). In such systems, most of the fixed N_2 is removed when the forage legumes are harvested, reducing the benefit to the subsequent crops (Pietsch *et al*., 2007). However, 30-60% of the legume's total plant N may be below ground, associated with roots and nodules (Peoples *et al*., 2009). Consequently, crop legume residues may still contain considerable fixed N even after a large amount of N is removed at harvest. Therefore, considering total biomass production and BNF along with shoot dry matter can help to select genotypes that are superior in crop rotations.

The objectives of this study were: (1) to identify the most widely adapted genotypes and the most tolerant to moisture stress among 18 alfalfa genotypes based on shoot dry matter, total biomass yield and biological nitrogen fixation; and (2) to evaluate five drought tolerance indices and identify the one that works best under different stress levels.

MATERIALS AND METHODS

Site and experiment description

To identify the suitability of different alfalfa genotypes and drought tolerance indices, this study included two separate trials, namely irrigated (no water stress) and rainfed (water stress), at two different organically managed fields, Gross-Enzersdorf (48º12' N, 16º33' E) and Raasdorf (48º15' N, 16º37' E), respectively. Both fields are located at the research station of the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. Farm management was organic, stockless and no organic manures were applied. The soils are Calcaric Phaeozems (WRB) from loess with a silty loam texture. Site classification was mainly related to the level of summer drought stress (irrigated and rainfed cropping) and the differing water-holding capacity of the locations (depth of A horizon and soil organic matter content) (Table 1).

The average annual precipitation (1971-2000)

Table 1. Selected properties of the soil in two test fields.

	Gross-Enzersdorf (Irrigated)	Raasdorf (Rainfed)
Texture	**Silty loam**	**Silty loam**
Organic carbon content (%)		
0-30 cm	**1.5**	**2.0**
30-60 cm	**1.4**	**0.7**
Depth of A horizon	**45-50 cm**	**25-35 cm**
Bulk density (g cm^{-3})	**1.4-1.6**	**1.3-1.4 (Pietsch *et al*., 2007)**

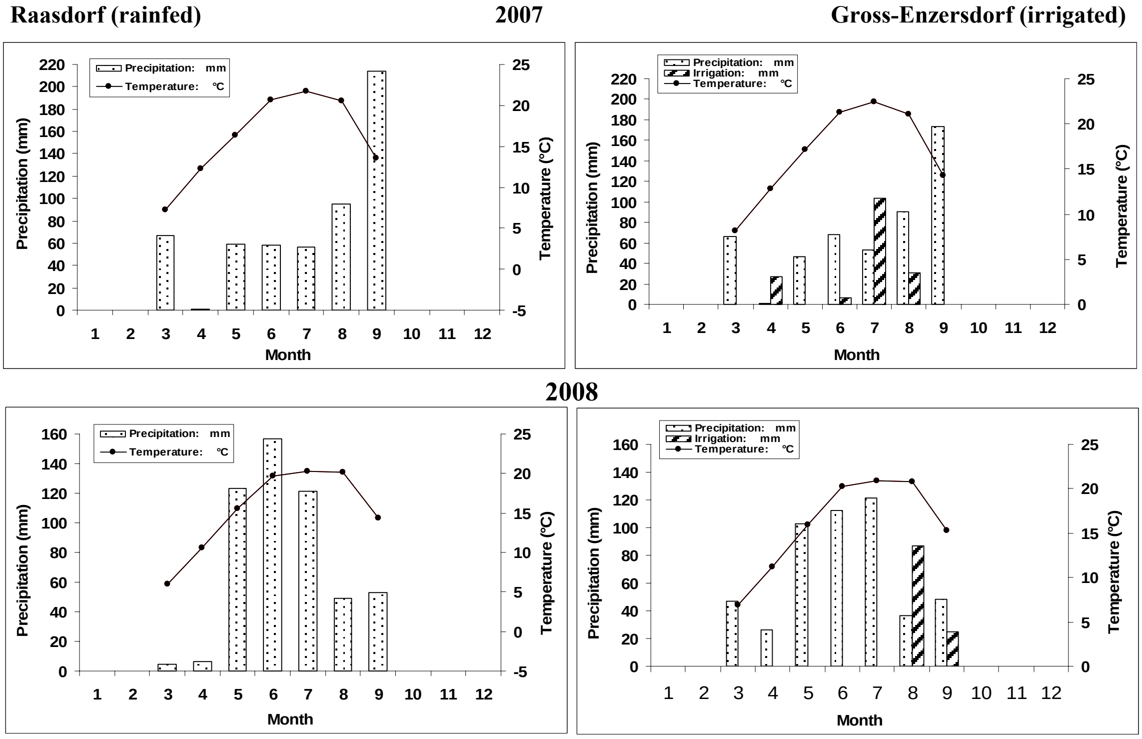

Fig. 1. Monthly precipitation, average temperature and applied irrigation water from March to September 2007 and 2008.

was 520 mm. The amount of precipitation and applied irrigation water and the average temperature from March to September in 2007-08 are shown in Fig. 1. Meteorological data were assessed by two weather stations in Raasdorf and Gross-Enzersdorf.

Experimental treatment and design

Eighteen alfalfa cultivars and ecotypes, including eight Iranian ecotypes, Mohajeran (1), Khorvande (2), Famenin (3), Ghargholog h (4), Ordobad (5), Shorakat (6), Ghara-aghaj (7) and Hokmabad (8), and ten European varieties, Sitel (9), Verko (10), Vlasta (11), Monz 42 (12), Fix 232 (13), NS-Banat (14), Sanditi (15), Alpha (16), Plato (17) and Niva (18), were evaluated during 2006-08. Both trials were hand-seeded in May, 2006. The first test year was considered as the establishment year. Field plots in both trials were laid out in an α-lattice design with two replications. To estimate BNF, nine field plots in each trial were hand-seeded with a mixture of four grasses as a reference crop in the first and the last plot of each incomplete block. The grass mixture consisted of tall oat-grass (*Arrhenatherum elatius* var**.** *arane*), red fescue (*Festuca rubra* var. *gondolin*), cocksfoot grass (*Dactylis glomerata* var. *amba*) and perennial ryegrass (*Lolium perenne* var. *pimpernell*). Seeding density was 25 kg ha^{-1} in all cases, adjusted by the cultivars' germination rate. Each genotype was seeded in 12 rows, 2 m in length, in the rainfed trial at Raasdorf and 8 rows, 1.5 m in length, in the irrigated trial at Gross-Enzersdorf. Spacing between rows in both trials was 12.5 cm.

In the irrigated trial, soil moisture content was monitored weekly by four FDR (Frequency Domain Reflectometry, ThetaProbe ML2x, UMS GmbH, München, Germany) probes at 15, 40, 80 and 120 cm soil depths; these devices were installed in one plot in each incomplete block including cultivars 1, 9 and 18 in each replication. Irrigation was started at 50% depletion of soil available water (SAW) content (SAW = Water content difference between field capacity and permanent wilting point) based on the FDR probe at 15 cm soil depth. The amount of applied irrigation water was calculated for 0-30 cm depth based on soil moisture content up to field capacity. Plots were irrigated by a drip irrigation system with 28 drippers per plot and an outflow of 2 liters of water per hour and dripper.

Data collection

Plots were hand-clipped three times to a 5-cm stubble height using garden scissors at 30-40% of flowering. Root dry matter, stubble dry matter and inorganic nitrogen at 30-60 cm and 60-90 cm soil depths were recorded only at the third harvest in each year. Shoot (SHDM) and stubble (STDM) yield data (t ha^{-1}) were adjusted on a dry matter basis by sub-sampling approximately 200 g and 50 g of fresh shoot and stubble, respectively, from 0.5 m^2 of the plots at each harvest, and drying the samples at 60 °C for 72 h. Annual shoot dry matter production was determined by summing the yield data over the harvests in each year. Root dry matter (RODM) (t ha^{-1}) was determined using a soil corer with a 9 cm diameter. Two samples, one in the row and one between the rows, were taken from each plot down to a depth of 30 cm, and fresh root materials were washed and dried at 60 °C for 72 h.

Biological Nitrogen Fixation (BNF) was estimated for each plot by the "extended difference method" (Giller, 2001). Based on this method, the BNF of the legume crop was taken as the difference between the legume's total N uptake and that of the non-nodulating plant (reference crop), both of which were grown at the same time in the same field; differences in soil inorganic N content between plots were also accounted for. Inorganic nitrogen was extracted from soil samples taken after each harvest using a CaCl2 (0.01 M) solution at a soil-solution ratio of 1 to 4. Nitrate-N in the extracts was measured according to standard ÖNORML1091 (1991) using a UV–VIS-Photometer. Plant samples were taken on each harvest date. Nitrogen content in dry plant organs was determined using an isotope ratio mass spectrometer (IRMS-ThermoQuest Finnigan DELTA plus, Bremen, Germany) in the laboratory of the Department of Chemical Ecology, University of Vienna.

Drought tolerance indices

Five suggested drought tolerance selection indices-tolerance (TOL) (Rosielle and Hamblin 1981); mean productivity (MP) (Rosielle and Hamblin, 1981); stress susceptibility index (SSI) (Fischer and Maurer, 1978); geometric mean productivity (GMP) (Fernandez, 1992); and stress tolerance index (STI) (Fernandez, 1992), along with superiority statistic (P_i) (Lin and Binns, 1988)—were calculated using the Excel spread sheet program. Stress intensity (SI) ranged from 0 to 1, and larger SI values indicate higher stress intensity:

$$\left[\text{SI (Stress intensity)} = 1 - \frac{\overline{Y}_s}{\overline{Y}_p} \right]$$

where $\overline{Y}_s$ and $\overline{Y}_p$ are the mean yields of all genotypes evaluated under stress and non-stress conditions, respectively. Larger MP, GMP and STI values and smaller P_i, TOL and SSI values are desirable. When ranking indices, a low rank number is desirable for all indices.

Statistical analysis

The data were analyzed based on repeated measure analysis of variance based on an alpha-lattice design by PROC MIXED in SAS software (SAS Institute, 2004). Adjusted least square (LS) means were estimated for each year-location combination (environment), and also over two years for each location (condition) separately; they were used to calculate the different indices mentioned above. Based on genotypic means, simple correlations were calculated among drought tolerance indices, SHDM, TBY and BNF performance of genotypes under rainfed and irrigated conditions (n=18).

RESULTS

Analyses of variance for SHDM, TBY and BNF showed significant differences among locations (L) ($P< 0.001$), years (Y) ($P< 0.001$), and genotypes (G) ($P< 0.001$), and significant interaction effects for GL ($P< 0.001$), GY ($P<0.05$ to $P<0.001$) and GYL ($P<0.05$ to $P<0.001$) (table not shown). The mean genotypic values for these three traits under rainfed (RF) and irrigated (IR) conditions in the first and second years and the average of both years are given in Table 2. Irrigation scheduling in the irrigated trial was aimed at preventing moisture stress among genotypes, whereas the rainfed trial was not irrigated, similar to organic farmers' management in eastern Austria. Temperature patterns were similar across the two years and locations, while precipitation distributions were different between the two years but similar for the two locations (Fig. 1). The amount of precipitation was nearly twice as much from June to August 2008 compared to 2007. So the genotypes in the rainfed trial were exposed to mid-season water stress during 2007 and late-season stress during 2008. The second and third harvests were affected by water stress in 2007, but only the third harvest was affected in 2008. Stress intensities (SI) for SHDM, TBY and BNF were 0.48, 0.38 and 0.35, respectively, in the first year, and 0.25, 0.20 and 0.14, respectively, in the second year. Based on the results of SI calculations, the first and second years of the study were considered to represent high and low stress pressure, respectively.

To identify the index that best distinguishes the superior genotypes under both conditions (irrigated and rainfed), genotypes were classified into four groups (A to D) (Fernandez, 1992) in scatter plots by drawing reference lines through the x (irrigated) and y (rainfed) axes using the grand mean of all genotypes in each environment (irrigated and rainfed) (Figs. 2 to 4). For all three characters, the number of selected superior genotypes using different indices was derived from the number of genotypes classified in Group A (Figs. 2 to 4) under each condition.

Table 2. Shoot DM, total biomass and BNF yield of genotypes under irrigated and rainfed conditions during the first and sec

		Shoot DM (t ha^{-1})						Total biomass (t ha^{-1})					
		Irrigated			Rainfed			Irrigated			Rainfed		
Code	Genotype	1st	2nd	Ave.	1st	2nd	Ave.	1st	2nd	Ave.	1st	2nd	Ave.
1	Mohajeran	19.6	17.3	18.4	7.8	11.0	9.4	28.9	26.8	27.8	14.3	21.3	17.8
2	Khorvande	10.6	12.2	11.4	6.2	9.4	7.8	20.9	25.6	23.3	12.0	19.3	15.7
3	Famenin	14.4	16.5	15.5	9.7	10.9	10.3	23.1	26.9	25.0	15.5	20.2	17.8
4	Gharghologh	12.6	14.7	13.6	7.0	12.6	9.8	24.3	26.9	25.6	16.0	22.6	19.3
5	Ordobad	15.7	14.6	15.1	5.5	11.0	8.3	27.0	25.2	26.1	12.2	20.3	16.2
6	Shorakat	17.6	15.1	16.3	6.4	13.3	9.8	28.8	23.6	26.2	16.1	23.6	19.9
7	Ghara-aghaj	13.9	17.2	15.5	9.8	13.2	11.5	22.3	25.3	23.8	17.8	22.0	19.9
8	Hokmabad	14.4	14.3	14.3	6.3	10.5	8.4	24.6	25.3	25.0	14.9	19.4	17.1
9	Sitel	17.1	17.7	17.4	10.9	13.7	12.3	24.0	30.1	27.1	16.6	23.6	20.1
10	Verko	17.2	17.6	17.4	7.5	12.0	9.7	27.3	26.2	26.7	12.9	20.9	16.9
11	Vlasta	15.8	17.0	16.4	10.8	12.6	11.7	23.8	25.8	24.8	21.8	22.1	21.9
12	Monz 42)	15.5	15.5	15.5	8.0	10.1	9.1	23.1	26.9	25.0	15.7	18.3	17.0
13	Fix 232	17.5	17.0	17.2	7.9	13.3	10.6	28.4	30.0	29.2	13.1	21.4	17.2
14	NS-Banat	16.2	16.0	16.1	7.7	14.0	10.9	28.5	28.2	28.4	14.9	22.4	18.6
15	Sanditi	14.7	15.8	15.3	10.6	12.7	11.6	21.0	24.9	23.0	17.4	21.8	19.6
16	Alpha	16.0	17.6	16.8	7.4	11.5	9.5	21.5	26.6	24.0	12.5	20.5	16.5
17	Plato ZS	17.6	16.4	17.0	9.0	12.7	10.9	25.1	25.4	25.3	17.3	20.6	18.9
18	Niva	15.0	18.1	16.5	8.5	13.7	11.1	20.4	29.6	25.0	14.6	23.0	18.8
	Mean	15.6	16.1	15.9	8.2	12.1	10.1	24.6	26.6	25.6	15.3	21.3	18.3
	Std error	0.78$^{\zeta}$	16.1	0.62				1.19 $^{\zeta}$		0.85			

1st, 2nd and Ave. are first year, second year and average values of both years of study, respectively.

ζ = Standard error for year-location combinations.

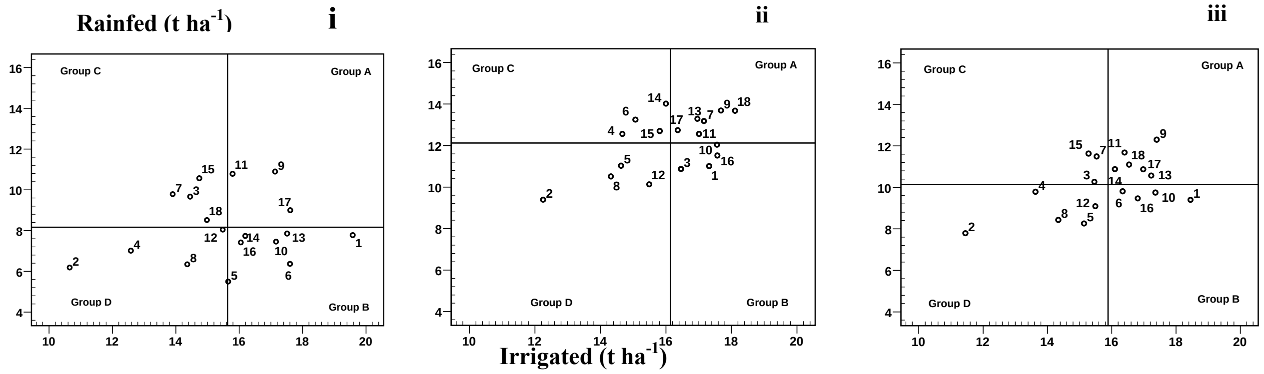

Fig. 2. Scatter plots of shoot DM yield (t ha^{-1}) of genotypes under rainfed and irrigated conditions during the first (i) and second years (ii), and the two-year average (iii). (Cross lines show average points).

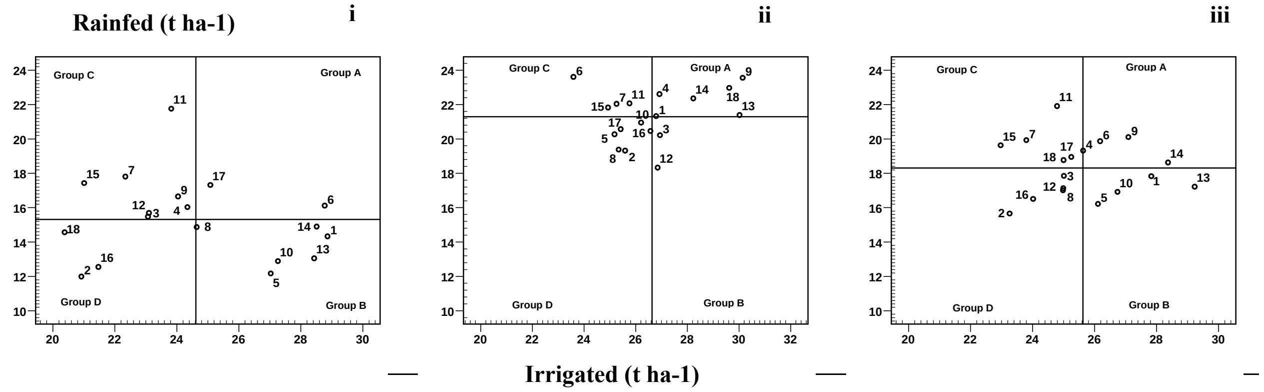

Fig. 3. Scatter plots of total biomass yield (t ha^{-1}) of genotypes under rainfed and irrigated conditions in the first (i) and second years (ii) and the two-year average (iii).

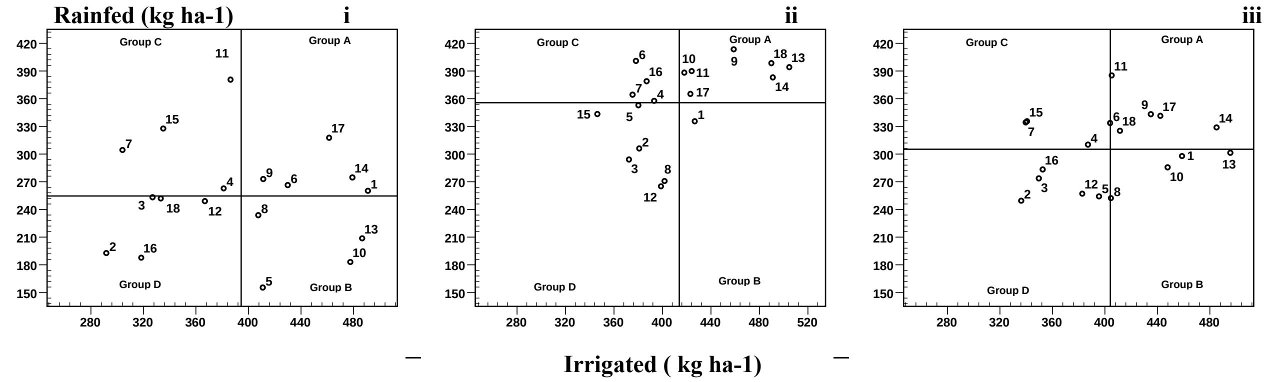

Fig. 4. Scatter plots of BNF estimation (kg ha^{-1}) of genotypes under rainfed and irrigated conditions in the first (i) and second (ii) years and the two-year average (iii).

Shoot Dry Matter (SHDM)

The highest yield under IR conditions was produced in the first year by ecotype Mohajeran, which also had the best two-year average, and by Niva in the second year. Under RF conditions, Sitel produced the highest yield in the first year and the best two-year average, and NS-Banat produced the highest yield in the second year (Table 2). The correlation coefficients between SHDM under IR and RF conditions for high SI (first year), the two-year average and low SI (second year) were r = 0.13, r = 0.49* and r = 0.56*, respectively (Table 4). In high and low SI, genotypes 3 and 6 were classified in Group A. Genotypes 9 (Sitel), 17 (Plato) and 11 (Vlasta) in the first year (high SI) (Fig 2i); genotypes 18 (Niva), 9 (Sitel), 7 (Ghara-aghaj), 13 (Fix232), 11 (Vlasta) and 17 (Plato) in the second year (low SI) (Fig. 2ii); and genotypes 9 (Sitel), 11 (Vlasta), 13 (Fix232), 17 (Plato), 18 (Niva) and 14 (NS-Banat) based on the two-year average (Fig. 2iii) were categorized in Group A. The ranking of genotypes based on different indices is presented in Table 3. Based on TOL, none of the Group A genotypes was selected in high and low SI, whereas based on the two-year average, only Sitel and Vlasta were identified. Based on MP, most Group A genotypes-Sitel and Plato ZS in the first year (high SI), Niva, Sitel, Ghara-aghaj, Fix232 and NS-Banat in

Table 3. Ranking of genotypes using different stress tolerance indices based on shoot DM yield (SHDM), total biomass yield (TB

Index		TOL			MP			GMP			SSI			
Genotype		**1st**	**2nd**	**Ave.**	**1st**	**2nd**	**Ave.**	**1st**	**2nd**	**Ave.**	**1st**	**2nd**	**Ave.**	**1st**
Mohajeran	SHDM	18	18	18	2	11	4	5	12	9	16	18	18	5
	TBY	16	9	17	4	6	6	5	6	6	15	9	15	5
	BNF	15	13	16	4	10	6	4	10	6	15	14	14	4
Khorvande	SHDM	3	4	2	18	18	18	18	18	18	6	9	6	18
	TBY	11	13	11	18	17	18	18	16	18	12	15	13	18
	BNF	6	11	9	18	15	18	18	15	18	10	12	11	18
Famenin	SHDM	4	16	7	8	13	13	6	14	13	4	15	9	6
	TBY	7	16	9	14	11	12	13	11	13	8	16	9	13
	BNF	4	12	6	15	17	17	15	16	17	4	13	9	15
Ghargologh	SHDM	6	3	3	17	14	15	16	13	15	8	3	3	16
	TBY	9	5	6	8	5	7	8	5	7	9	5	5	8
	BNF	8	8	7	10	11	10	9	11	10	6	8	6	9
Ordobad	SHDM	16	6	15	15	15	16	17	15	16	18	11	17	17
	TBY	17	7	16	12	15	14	15	15	16	18	7	17	15
	BNF	16	5	13	16	13	14	16	13	14	18	6	15	16
Shorakat	SHDM	17	1	13	9	12	12	14	11	11	17	1	12	14
	TBY	13	1	7	2	9	5	2	8	4	13	1	4	2
	BNF	12	1	5	5	8	7	5	8	7	11	1	4	5
Ghara-aghaj	SHDM	1	10	4	11	3	8	8	3	6	2	8	2	8
	TBY	3	3	3	10	8	10	7	9	9	3	3	3	7
	BNF	1	4	2	13	12	12	11	12	12	1	4	2	11
Hokmabad	SHDM	10	9	10	16	17	17	15	17	17	14	13	13	15
	TBY	12	11	12	11	18	15	10	18	14	10	14	11	10
	BNF	13	17	14	11	16	13	10	17	13	13	17	17	10
Sitel	SHDM	7	11	6	1	2	1	1	2	1	5	7	5	1
	TBY	5	14	8	7	1	1	6	1	1	5	11	8	6
	BNF	10	9	10	7	4	5	6	3	5	9	9	8	6
Verko	SHDM	15	15	17	7	6	7	9	7	10	15	14	16	9
	TBY	15	8	15	9	10	11	14	10	11	16	8	16	14
	BNF	18	6	17	9	6	9	13	6	9	17	5	16	13
Vlasta	SHDM	5	13	5	4	7	2	2	6	2	3	12	4	2
	TBY	1	4	1	1	7	3	1	7	2	1	4	1	1
	BNF	2	7	3	2	5	3	1	5	2	2	7	3	1
Monz 42	SHDM	9	14	12	12	16	14	12	16	14	9	17	14	12
	TBY	6	17	13	13	16	16	12	17	15	7	18	12	12
	BNF	7	18	12	12	18	15	12	18	16	8	18	13	12
Fix 232	SHDM	14	8	14	5	4	5	7	4	5	13	5	11	7
	TBY	18	18	18	6	3	4	9	3	5	17	17	18	9
	BNF	17	16	18	6	1	2	8	1	4	16	15	18	8
NS-Banat	SHDM	11	2	8	10	5	9	11	5	8	11	2	7	11
	TBY	14	10	14	3	4	2	4	4	3	14	10	14	4
	BNF	14	15	15	3	3	1	3	4	1	14	16	12	3
Sanditi	SHDM	2	5	1	6	10	10	4	10	7	1	4	1	4
	TBY	2	2	2	15	13	13	11	12	12	2	2	2	11
	BNF	3	2	1	8	14	11	7	14	11	3	2	1	7
Alpha	SHDM	13	17	16	14	9	11	13	9	12	12	16	15	13
	TBY	10	12	10	17	12	17	17	13	17	11	13	10	17
	BNF	9	3	4	17	9	16	17	9	15	12	3	5	17
Plato ZS	SHDM	12	7	11	3	8	3	3	8	3	10	6	10	3
	TBY	8	6	5	5	14	8	3	14	8	6	6	7	3
	BNF	11	10	11	1	7	4	2	7	3	7	10	10	2
Niva	SHDM	8	12	9	13	1	6	10	1	4	7	10	8	10
	TBY	4	15	4	16	2	9	16	2	10	4	12	6	16
	BNF	5	14	8	14	2	8	14	2	8	5	11	7	14

See Table 2 for abbreviations.

the second year (low SI), and Sitel, Vlasta, Plato ZS, Fix232 and Niva based on the two-year average—were differentiated from the other groups. The correlations between MP and SHDM were positive and significant under both IR and RF conditions (Table 4). Based on SSI, only Vlasta in high SI, Fix232 and Plato ZS in low SI, and Sitel and Vlasta for the two-year average, were distinguished as Group A genotypes.

Correlations between SSI and SHDM were positive under IR conditions, while correlations were significant and negative under RF conditions in high SI, the two-year average and low SI (Table 4). The results of GMP and STI were identical in ranking. Based on STI, all Group A genotypes were distinguished from other groups, except for Plato ZS (17) in low SI and NS-Banat (14) based on the two-year average (Table 3). In high SI, STI was able to distinguish all Group A genotypes. Based on the two-year average, NS-Banat (14), as a Group A genotype, was ranked after Ghara-aghaj (7) and Sanditi (15) from Group C with high rainfed yields, and in low SI, Plato ZS (17) was ranked after NS-Banat (14) from Group C and Verko (10) from Group B. The correlations between STI and SHDM in both conditions were positive and significant (Table 4). However, the association of STI with SHDM under RF conditions was greater than that under IR conditions. Using P_i, most Group A genotypes were discriminated, except for Vlasta (11) in high SI, Plato ZS (17) in low SI, and NS-Banat (14) and Niva (18) based on the two-year average. There were negative and significant correlations between P_i and SHDM (Table 4), showing that selecting by P_i will increase yield under both conditions.

Total Biomass Yield (TBY)

Mohajeran, Sitel and Fix232 under IR conditions, and Vlasta, Shorakat and Vlasta under RF conditions had produced the greatest total biomass in the first and second years and the two-year average, respectively (Table 2). Correlations between TBY of genotypes under IR and RF conditions were -0.17, -0.04 and 0.26 for high SI, the two-year average and low SI, respectively (Table 4). Two and six genotypes were selected as Group A in high and low SI, respectively (Fig. 3). The ranking of genotypes using the different indices is presented in Table 3. In high SI, TOL and SSI were not able to identify Group A genotypes, while other indices distinguished only Shorakat from Group A (Table 3 and Fig. 3i). In low SI, TOL and SSI distinguished only one genotype—Ghargholgh (4)-out of six genotypes in Group A, while other indices identified all Group A genotypes (Table 3 and Fig. 3i). Fix 232, a Group A genotype, was ranked as unsuitable by TOL and SSI because of the large yield difference between the two conditions (Tables 2 and 3). As for the two-year average, TOL could not distinguish Group A genotypes, while GMP, STI and Pi identified most of them (Table 3 and Fig. 3iii). All indices ranked Vlasta first because of its high total biomass yield under rainfed conditions and high SI, and its two-year average. Correlations between indices and TBY under IR and RF conditions were similar to those of SHDM (Table 4).

Biological Nitrogen Fixation (BNF)

With regard to BNF, the highest estimates were as follows: under IR conditions, Mohajeran in the first year and Fix232 in the second year and the two-year average; under RF conditions, Vlasta in the first year and the two-year average, and Sitel in the second year of the study (Table 2). Correlations between BNF of genotypes under IR and RF conditions were -0.05, 0.22 and 0.48* for high SI, the two-year average and low SI, respectively (Table 4). Five and seven genotypes were classified as Group A under high and low SI, respectively (Fig. 4). The ranking of genotypes using the different indices is presented in Table 3.

Similar to SHDM and TBY, TOL and SSI were not able to identify Group A genotypes under high SI; only Verko and Vlasta under low SI, and Vlasta and Shorakat for the two-year average were distinguished from the other groups. With minor changes in ranking, MP, STI and P_i showed the same efficiency in separating Group A genotypes from the other groups (Table 3 and Fig. 4). They were able to identify most Group A genotypes as being separate from the other groups.

On average for these traits, STI, GMP, MP, Pi, SSI and TOL differentiated about 77, 77, 66, 66, 11 and 0% of genotypes in Group A under high SI; 75, 75, 67, 69, 30 and 22% for the two-year average; and 94, 94, 89, 94, 26 and 15% under low SI, respectively. The correlation between TOL and SSI was positive, large and significant for all three studied traits. Indices MP, GMP, STI and Pi were highly correlated to each other (data not shown). There were no significant correlations between TOL or SSI and other indices for the three traits.

DISCUSSION

Year-to-year fluctuations in the amount, frequency, time and duration of rainfall occurred under rainfed conditions. Variation in precipitation patterns caused varying levels of stress in one site in different years, such as low to high stress intensity in

Table 4. Simple correlations between drought tolerance indices, irrigated and rainfed SHDM, TBY and BNF across 18 genotypes.

		SHDM		TBY		BNF	
		Irrigated	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**
Rainfed	1st	0.13		-0.17		-0.05	
	2nd	0.56*		0.26		0.48*	
	Ave.	0.49*		-0.04		0.22	
TOL	1st	0.75**	-0.56*	0.81**	-0.72**	0.78**	-0.67**
	2nd	0.55*	-0.39	0.71**	-0.50*	0.52*	-0.51*
	Ave.	0.66**	-0.33	0.74**	-0.71**	0.73**	-0.50*
MP	1st	0.81**	0.68**	0.72**	0.56*	0.75**	0.63**
	2nd	0.89**	0.87**	0.84**	0.74**	0.86**	0.86**
	Ave.	0.90**	0.83**	0.71**	0.67**	0.84**	0.72**
GMP	1st	0.62**	0.86**	0.51*	0.76**	0.57*	0.79**
	2nd	0.86**	0.90**	0.79**	0.79**	0.84**	0.88**
	Ave.	0.83**	0.89**	0.58*	0.79**	0.78**	0.79**
SSI	1st	0.46	-0.81**	0.65**	-0.85**	0.62**	-0.80**
	2nd	0.32	-0.61**	0.59*	-0.63**	0.38	-0.63**
	Ave.	0.36	-0.63**	0.60**	-0.83**	0.59*	-0.65**
STI	1st	0.59*	0.87**	0.48*	0.78**	0.56*	0.79**
	2nd	0.85**	0.91**	0.80**	0.79**	0.85**	0.86**
	Ave.	0.82**	0.90**	0.58*	0.78**	0.77**	0.79**
P_i	1st	-0.86**	-0.54*	-0.53*	-0.70**	-0.63**	-0.70**
	2nd	-0.88**	-0.81**	-0.87**	-0.58*	-0.82**	-0.86**
	Ave.	-0.92**	-0.72**	-0.54*	-0.73**	-0.77**	-0.75**

SHDM: shoot dry matter; TBY: total biomass yield; BNF: biological nitrogen fixation; see Table 2 for abbreviations.

*** and ** Significant at 5 and 1 % probability level, respectively.**

the two successive years of this study under rainfed conditions as the target environment. Stress intensity variability in a given environment affects and determines a breeding program's selection strategy (different approaches mentioned in the introduction) and also the choice of a suitable index for identifying superior genotypes. Although the amount of precipitation did not differ considerably, the test genotypes were exposed to mid-term (high intensity) and late season (low intensity) stress during the first and second years of the study, respectively, under rainfed conditions (Fig. 1). The SI values showed that, in addition to different stress intensity caused by rainfall fluctuations in the two study years, drought stress had different effects on different plant parts. SHDM was reduced the most (compared with TBY and BNF) in both years due to drought conditions. This parallels the results of Showemimo and Olarewaju (2007), who reported different effects of drought stress on various traits of sweet pepper (*Capsicum annuum* L.).

The relationship between the performance of genotypes under IR and RF conditions (Table 4) was affected by the level of SI and by the traits. The strength of the correlation between the two conditions increased as stress intensity (SI) decreased from the first to the second year of the study, suggesting that selection of drought tolerant genotypes based on yield under non-stress conditions is unreliable, especially when stress intensity is high in target environment. Moreover, the degree of association between the two conditions differed among the studied traits. SHDM, as a constituent of TBY, showed a stronger correlation between performance under the two conditions than TBY and BNF. This indicates the role of non-harvestable plant parts, especially root DM, in the selection process for tolerant or Group A genotypes. On the other hand, the degree of association between the two conditions influenced the number of genotypes in Group A. The number of genotypes in Group A decreased with increasing SI (Figs. 2 to 4). This shows the weak positive and occasionally negative correlations between IR and RF conditions (Table 4). Also, we observed the same trend of fewer Group A genotypes for TBY and BNF compared to SHDM. This reduction suggests there is a lower possibility of finding genotypes that express uniform superiority under both stress and non-stress conditions. Considering the variable relation between performances under stress and non-stress conditions, the simultaneous assessment of genotypes under both conditions (the third approach mentioned in the introduction) can be a reliable way of identifying suitable genotypes for rainfed cropping systems. In other words, to be suitable for selecting superior genotypes, selection approaches and drought tolerance indices have to take into account the variable stress levels (from normal to high stress intensity) under rainfed conditions as the target environment. With regard to the correlation between IR or RF performance and drought tolerance indices, genotype selection based on TOL and SSI will increase overall yield only under RF conditions, while MP, GMP, STI and P_i will increase it under both conditions.

In the current study, MP and STI (or GMP) were the best mean predictors of IR and RF performance, respectively. Akhtar *et al*. (2000) reported that MP would best identify genotypes with high performance in non-stress environments, and GMP would best identify genotypes with high performance in stress environments. Sio-Se Mardeh *et al*. (2006) found a negative and significant correlation between P_i and the yield of wheat genotypes under non-stress conditions, and a positive and non-significant correlation under stress conditions. Fernandez (1992), however, stated that identifying Group A genotypes is a better approach than correlation analysis because the effectiveness of genetic gain based on the observed correlation may not reflect the genetic gain of individual genotypes. Indices TOL and SSI were not suitable for identifying Group A genotypes, but did select genotypes such as Vlasta for their high yields under rainfed conditions. In general, SSI was a suitable index for identifying genotypes (mostly in Group C) with high performance under stress conditions, such as Sanditi, Vlasta and Ghara-aghaj. The correlation between SSI and rainfed performance was stronger under high SI than medium and low SI, confirming the suitability of SSI for selecting tolerant and high yielding genotypes under stress conditions.

Although most Group A genotypes were identified by MP, GMP, STI and P_i (Table 3 and Figs. 2 to 4), STI and GMP were the indices that best separated Group A genotypes with regard to correlations among these criteria (Table 4) because they were more efficient in ranking the genotypes. Farshadfar and Sutka (2003) suggested MP, GMP and STI as the most suitable drought-tolerance criteria for screening wheat substitution lines. Fernandez (1992) and Akhtar *et al*. (2000) concluded that STI is the best index for identifying Group A genotypes in mungbean (*Vigna radiata* L. Wilczek). However, the suitability of indices decreased with increasing SI (regarding the number of identified Group A genotypes), which indicates that their relevance depends on the severity of stress (Table 3 and Figs. 2 to 4).

In conclusion, the utility of different approaches for breeding for stress environments depends on stress intensity. In low SI, when the correlation between yield under stress and non-stress conditions is moderate to high and yield reduction due to stress is low, the first approach (selection under non-stress conditions and subsequent yield testing in stress environments) apparently works better than others. In high SI, when the correlation between yields under stress and non-stress conditions is low and sometimes negative, and yield losses due to stress are high, the second approach (direct selection under the target stress conditions) apparently works better than others. Based on the present and other studies, however, the third approach-simultaneous evaluation of genotypes under both conditions-ensures the selection of superior genotypes for both conditions, especially under moderate stress intensity. In the present study, Sitel, Plato ZS, Vlasta and NS-Banat were the best genotypes based on their performances under both conditions. As specific adaptations, Vlasta and Sitel can be selected for stress and non-stress conditions, respectively. Among Iranian ecotypes, the best performance was achieved by Mohajeran under irrigated conditions and by Ghara-aghaj and Gharghologh under rainfed conditions. STI and GMP were the best indices to distinguish Group A genotypes from other groups, while SSI was the one that best identified genotypes with high yield potential under stress conditions.

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