EVALUATION OF BREEDING INDICES FOR DROUGHT TOLERANCE IN ALFALFA (*Medicago*) GENOTYPES

Raisa VOZHEHOVA, Andrii TYSHCHENKO, Olena TYSHCHENKO, Oleksandr DYMOV, Olena PILIARSKA, Pavlo LYKHOVYD

Institute of Irrigated Agriculture, Naddniprianske, Kherson, Ukraine, 73483

Corresponding author email: pavel.likhovid@gmail.com

Abstract

The goal of the study is to find out the best indices for drought tolerance evaluation in alfalfa genotypes to distinguish the best ones for further use in the plant breeding process for drought resistance. The study was carried out during 2017-2020 at the experimental field of the Institute of Irrigated Agriculture of NAAS (Kherson, Ukraine) with accordance to modern standards of scientific work in agronomy. We studied 24 varieties and populations of alfalfa in the conditions of optimal (irrigation) and stress (rainfed) humidification in the South of Ukraine, which is characterized as a semi-arid climatic zone. To evaluate the resistance of the studied genotypes of the crop to drought, 14 different indices were applied. Based on the results of the study, new index for drought tolerance evaluation in alfalfa, named stress resistance index (ISR), was introduced. We selected five genotypes: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr/C., which had the highest yield of 8.30-8.47 kg/m² under the moisture stress as the most prospective for their further use in the plant breeding process. Four indices, namely, yield index (YI), mean geometric productivity (GMP), harmonic mean productivity (HMP), stress sensitivity index (SSI) and the developed stress resistance index (ISR) were selected as the best ones for characterization of alfalfa varieties by drought resistance as they do not only characterize the genotype in terms of its drought resistance, but also in terms of productivity under the stress conditions.

Key words: alfalfa, drought resistance indices, productivity, stress conditions, yield.

INTRODUCTION

Alfalfa is a perennial forage crop, which is cultivated all over the world, and it is characterized, in comparison to ther forage legumes, by high biomass productivity, nutritional value with high protein content. Alfalfa helps to increase soil fertility (Latrach et al., 2014), protects soils from wind and water erosion (Abdelguerfi & Abdelguerfi-Laouar, 2002), and increases the resilience of crop livestock production and systems (Annicchiarico et al., 2011). Besides, the fixation of atmospheric nitrogen makes it the best fore crop for other crops.

Alfalfa grows in a wide range of climatic conditions, from the equator to almost the Arctic polar circles (Annicchiarico et al., 2011). According to numerous forecasts, global climate change will lead to higher temperatures, changes in the geographical structure of precipitation and in the future to an increase in the frequency of extreme climatic events (Aleksandrov, 2002; Harrison et al., 2014), which is already observed in southern Ukraine. Abiotic stresses are the main factors that reduce crop productivity. Drought is the most significant, as it limits the capabilities of agricultural plants, reducing their productivity in arid and semi-arid areas (Hussain et al., 2012; Mollasadeghi et al., 2011). The intensity and severity of the drought can affect a sensitive and strategic sector, such as agriculture, which can threat food security. The detrimental effects of abiotic stress are a serious limitation for cultivation of this crop (Vasconcelos et al., 2008; Wang et al., 2015). But due to its strong and branched root system, it is considered to be a crop with high drought resistance and wide adaptability to arid conditions (Lemaire, 2006; Li et al., 2020). However, like any other crop, it reacts negatively to drought and, in order to adapt and survive under stress, it undergoes morphological, physiological, biochemical or molecular changes, which must be taken into account when creating drought-resistant varieties while increasing yields and product quality.

During the dry season, alfalfa plants (*Medicago*) reduce the aboveground vegetative

mass (Bellague et al., 2016; Durand, 2007), which limits the leaf area index, and consequently reduces the productivity of biomass. Therefore, to stabilize and increase the productivity of alfalfa, it is necessary to increase the drought resistance of alfalfa plants, and the study of this trait is an important step in plant breeding programs (Yu, 2017).

The amount of moisture loss through the evapotranspiration is steadily increasing, and this trend will only worsen in the future (Aleksandrov, 2002), so declining yields are a major problem and at the same time a basis for plant breeders to strengthen crops to adapt to climate change and increase their productivity under the stressful conditions (Cattivelli et al., 2008). Identification and development of drought-resistant genotypes is one of the main tasks of plant breeding programs, but the creation of high-yielding varieties and the realization of their yield potential in arid conditions is an extremely difficult task for plant breeders (Mustatea et al., 2003: Richards, 2006; Richards et al., 2002). The development of drought-resistant varieties is hindered by low heredity of traits and lack of effective selection strategies (Kirigwi et al., 2004). The selection of drought-resistant populations is difficult due to the strong interaction between genotypes and the environment and the limited knowledge of the functions and role of resistance mechanisms. Different researchers have used different methods to assess genetic differences in drought resistance. Some researchers believe that it is reasonable to conduct the selection of genotypes only in favorable conditions (Betran et al., 2003), and others - in arid conditions (Ceccarelli, 1987; Ceccarelli & Grando, 1991). However, there are many researchers who have chosen both paths and use the selection of genotypes in both favorable and stressful conditions (Clarke et al., 1992; Fernandez, 1992).

Plant susceptibility to drought is defined as a function of reduced yields under water stress (Koleva & Dimitrova, 2018), compared to potential yields (Ramirez & Kelly, 1998). Therefore, different breeding indices are used to differentiate genotypes from drought resistance, which are based on plant productivity in optimal and stressful conditions (Fisher & Maurer, 1978; Lin & Binns, 1988), and are used to select drought-resistant

genotypes (Boussen et al., 2010; Mitra, 2001; Zou et al., 2007).

Rosielle et al. (1981) proposed to use the Tolerance Index (TOL) as the difference between yield under irrigation and yield under natural humidification, and the mean productivity (MP), as the arithmetic mean of yield under stress and optimal conditions. Blum (1988:2005) determined the Drought Resistance Index (DI), which was generally accepted to determine the genotypes that provide high yields, both under stress and in better conditions. Fisher & Maurer (1978) recommend the use of a Stress Sensitivity index (SSI) to determine the stability of plant productivity, which records the value of yield in optimal and stressful conditions. The Stress Sensitivity Index (SSI) is a good one for identifying high-yielding genotypes that are also highly resistant to stress. As a rule, a lower level of SSI indicates less variation in crop vield under stress and under optimal conditions. Fernandez (1992) recommends using the Stress Tolerance Index (STI), and Saba et al. (2001) recommended its use in plant breeding programs for screening high-yielding genotypes in conditions of stress and its absence. Stable varieties have higher values of this index. Studying the yield of genotypes of mung beans (Vigna radiata L.) in stressful and optimal environments, Fernandez (1992) classified them into four groups:

group A - varieties that have equally high productivity in both environments;

group B - varieties that have high productivity only in optimal conditions;

group C - varieties with high yields under stress;

group D - varieties with low yields in both environments.

To determine the sensitivity of varieties to stress due to different drought intensities in different years Fernandez (1992) and Schneider et al. (1997) proposed the use of Geometric Mean Productivity (GMP) of the varieties in both environments. Besides, Gavuzzi et al. (1997), Bouslama & Schapaugh (1984) and Choukan et al. (2006) proposed to use the Yield Index (YI), the Yield Stability Index (YSI) and the Yield Reduction Index (YRI), respectively.

During the study of the drought resistance indices of corn, Moghaddam & Hadizadeh

(2002) stated that a low Tolerance Index (TOL) does not necessarily mean a high yield of a variety under stressful conditions, because the yield of a certain variety may be low under optimal conditions and show less reduction under stress, which leads to a decrease in TOL and this variety can be defined as drought resistant. However, Fernandez (1992) believed that the TOL and SSI indices reflect the drought resistance of the variety better. The use of the SSI index to identify drought-resistant varieties is a false direction. They believe that because the formula for the index calculation used the share of vield of a particular variety under stress to optimal conditions, as well as the ratio of productivity in stress to non-stress conditions in all varieties, the two varieties with high or low yields in both environments may have the same SSI value. With regard to MPI, the authors found that the use of a Mean Productivity Index often leads to the selection of varieties with high yields under optimal conditions that are less tolerant to stress. Malek-Shahi et al. (2009) presented MPI as an appropriate index for determining droughtresistant varieties. Shirani Rad & Abbasian (2011) while studying the sensitivity to stress in six varieties of winter oilseed rape found out that the indices of GMP, STI and MPI are the most appropriate indices for the determination of drought-resistant varieties. The same opinion is held by Sio-Se-Mardeh et al. (2006), which point out the importance of GMP, STI and MPI indices as the most effective ones for the exclusion of the varieties with high yields in both dry and optimal conditions (Yarnia et al., 2011).

In order to increase the effectiveness of the STI index, Farshadfar & Sutka (2002) proposed modified stress resistance indices (M1STI, M2STI), which adjust the STI. For screening drought-resistant genotypes in different environmental conditions, Moosavi et al. (2008) developed the percentage of sensitivity to stress index (SSPI).

Hao et al. (2011) recommend the index as an integrated selection criterion (SI) because it provides an assessment of drought stress resistance based on yield and related agronomic characteristics and will thus be useful for determining drought-resistant genotypes in plant breeding programs (Khalili et al., 2016).

Therefore, there are 14 indices for determining the drought resistance of genotypes, which we used in our studies. The goal of the study is to evaluate the response of varieties and populations of alfalfa in different environments and determine the best one not only by drought resistance but also by productivity under stress for their further use in the plant breeding process, and to select indices that allow distinguishing the genotypes with such traits.

MATERIALS AND METHODS

The study was conducted at the Institute of Irrigated Agriculture of NAAS (Ukraine, Kherson, vil. Naddnipryanske, 46°44'50.1"N 32°42'30.0"E), located on the Ingulets irrigated array, in 2017-2020 in the field conditions. The object of the study were varieties and populations of alfalfa: Unitro, Elehiya, Prymorka, M.g./P.p., Sin(s)/Prymorka, LR/H, Prymorka/Sin(s), A.-N. d. № 114, A.-N. d. № 15, A.-N. d. № 38, D. k.s. Ram. d., (Emeraude/T.)2, T./Emeraude, M.g. CP-11, M.agr./C., A.r. d., M.g./M.agr., M.g. d., FHNV², B.11/P.d., Zh./TsP-11 at forage use under two conditions of humidification: irrigation (drip irrigation) and natural humidification on the herbage of the first and second years of use. Productivity and drought resistance were determined using different indices:

$$MPI = \frac{Yp + Ys}{2} \quad \begin{array}{c} Rosielle \&\\ Hamblin, (1981) \end{array}$$
(1)

Where: MPI - mean productivity index, Yp - yields at the optimal conditions, Ys - yields at the stress conditions.

$$SSI = \frac{1 - \frac{Ys}{Yp}}{1 - \frac{\overline{Ys}}{\overline{Yp}}}$$
 Fisher &
Maurer, (2)
(1978)

Where: SSI - stress sensitivity index, \overline{Yp} - mean productivity of all the varieties in the optimal conditions, \overline{Ys} - mean productivity of all the varieties in the stress conditions.

$$TOL = Yp - Ys$$

$$Hamblin (3)$$

$$(1981)$$

Where: TOL - index of drought toleramce.

$$YSI = \frac{Y_s}{Yp} \qquad \begin{array}{c} Bouslama \&\\ Schapaugh\\ (1984) \end{array} (4)$$

~

Where: YSI - yield stability yindex.

$$YI = 100 \times \frac{Y_{s}}{\overline{Y_{s}}} \qquad \begin{array}{c} \text{Gavuzzi et} \\ \text{al. (1997);} \\ \text{Lin et al.} \\ (1986) \end{array}$$
(5)

Where: YI - yield index.

$$STI = \frac{YS \times Yp}{\overline{Yp}^2}$$
 Fernandez (6)

Where: STI - stress tolerance index.

-- --

$$GMP = \sqrt{Ys \times Yp}$$
 Fernandez
(1992);
Schneider (7)
et al.
(1997)

Where: GMP - mean geometric (proportion) productivity.

* 7

$$RDI = \frac{\frac{Ys}{Yp}}{\frac{\overline{Ys}}{\overline{Yp}}} \qquad \begin{array}{c} Fischer \& \\ Maurer \\ (1978) \end{array}$$
(8)

Where: RDI - relative drought tolerance index.

$$DI = \frac{Y_{S} \times \left(\frac{Y_{S}}{\overline{Yp}}\right)}{\overline{Ys}} \quad Lan (1998) \quad (9)$$

Where: DI - drought tolerance index.

$$SSPI = 100 \times \frac{Yp - Ys}{2 \times \overline{Yp}} \quad \begin{array}{c} Moosa \\ \text{vi et al.} \\ (2007) \end{array}$$

Where: SSPI - stress sensitivity predisposition index.

$$M_1 STI = STI \times \left(\frac{Yp}{Yp}\right)^2$$
 Farshadfar (11)
& Sutka

$$M_2 STI = STI \times \left(\frac{Ys}{\overline{Ys}}\right)^2$$
 (2002) (12)

Where: M₁STI, M₂STI - modified indices of the stress tolerance.

$$ATI = \frac{Yp - Ys}{\frac{\overline{Yp}}{\overline{Ys}}} \times \sqrt{Yp \times Ys} \quad Moosavi et al. (13) (2007)$$

Where: ATI - abiotic tolerance index.

$$HMP = 2 \times \frac{Yp \times Ys}{Yp + Ys}$$
Kristin et al.
(1997);
Chakherchaman
et al. (2009);
Jafari et al.
(2009) (14)

Where: HMP - harmonic mean productivity.

Statistical processing of the experimental data was performed using AgroSTAT, XLSTAT, Statistica (v. 13) software at p<0.05.

RESULTS AND DISCUSSIONS

Weather conditions over the years of the study differed both in temperature and in the amount and characteristics of precipitation, which made it possible to analyze varieties and populations of alfalfa for resistance to stress (drought) conditions. The hydrothermal coefficient (HTC) in 2017, 2018 and 2020 fluctuated between 0.51-0.55, which indicates very arid climatic conditions, while in 2019 it was 0.88, which belongs to arid conditions. Analysis of resistance of the alfalfa varieties and populations to stress was performed using 14 different drought tolerance indices: MPI, SSI, TOL, YSI, YI, STI, GMP, RDI, DI, SSPI, M1STI, M2STI, ATI, HMR and ISR stress resistance index, which was developed by our scientific group.

In the plant breeding for drought resistance, an important aspect is not only the resistance of plants to drought, i.e., the ability of plants to tolerate significant dehydration and overheating and survive drought with the lowest yield reduction, but also to show the maximum productivity under the stress. For example, genotypes may show a slight decrease, i.e., the difference in the yield obtained under optimal and stress conditions, but also low productivity under the stress. On the contrary, the population has high productivity during droughts, but a greater difference between yields in the optimal and stress conditions. For convenience, alfalfa populations are divided into three groups according to yielding Under irrigation (Yp) the capacities. populations with yields above 20.00 kg/m² were classified as high, 19.00-20.00 with medium and below 19.00 kg/m² - with low yields, under the stress conditions (Ys) - with a yield of more than 8.00 kg/m2 - with a high, 7.00-8.00 - medium and below 7.00 kg/m^2 to the group with a low yield.

The obtained experimental data allowed us to identify 5 populations of alfalfa by the yields in the stress conditions: M.g./P.p., LR/H, Ram.d., M. g./CP-11 and M.agr./C. with a herbage yield of 8.30-8.47 kg/m² with a high MPI (13.82-14.37), which shows the potential yield of the genotype in different cultivation conditions. In the variety Elehiya and population B11/P.d. there is also observed a high MPI (14.07; 13.94), which was formed because of high yields under the optimal conditions (irrigation), but low or medium - under the stress, therefore, the MPI cannot be considered as a reliable index for the determination of the resistance of genotypes to stress.

The drought sensitivity index (SSI) ranged from 0.91 to 1.12. It characterizes how sensitive the genotype is to the effects of drought and the lower the rate is, the greater the drought resistance of the genotype is. Nine populations were identified according to this index: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C., Prymorka/Sin(s), Zymostiyka/MK, A.r. d., M.g./M.agr. with its value of 0.91-0.96. But the last four genotypes entered this group due to low yields under the optimal conditions and mean ones under the stress. Therefore, we believe that this index can be used to determine drought resistance of genotypes, but in combination with other indices, to identify stress-resistance and high drought productivity more accurately.

The Drought Tolerance Index (TOL) and the Stress Sensitivity Predisposition Index (SSPI) are similar in nature and show the loss of yield under the influence of drought, the first in absolute units, the second in percent.

The population M.g./M.agr. is characterized with the lowest TOL - 10.35 and predisposition to the stress SSPI - 26.61. Its low yield of 17.95 kg/m² in the optimal conditions and a mean of 7.60 kg/m² under the stress does not mean that it is more resistant to drought and has greater productivity under the stress than the population M.g./P.p. and M.g./CP-11 with TOL indices 11.52 and 12.14, and SSPI 29.62 and 31.22, which according to the indices do not belong to drought-resistant but formed a high yield under the stress - 8.44 and 8.33 kg/m². Therefore, low TOL and SSPI will mean stress resistance, but there is a very high probability that the population will be more productive under the stress, although with the best TOL and SSPI indices will not be allocated as drought resistant.

According to the yield stability index (YSI), i.e., the ratio of the yield under the stress to the yield under the optimal conditions, with fluctuations from 0.31 to 0.44, ten populations significantly distinguished: FHNV², were M.g./CP-11, A.p.d., M.g./M.agr., M.agr./C., Zymostiyka/M.K., Ram.d., Prymorka/Sin(s), M.g./P.p., LR/H, in which this index varied within 0.40-0.44, but, as in the previous index. the mean vield under the stress and low under the optimal conditions led to high index rates in some populations. This means that the YSI index should be used only in comparison with others, because more productive populations, both under the optimal conditions and drought, may not be included into the group of drought resistant.

Yield index (YI), geometric mean productivity (GMP) and harmonic mean productivity (HMP) express the yield of a particular genotype under the stress conditions to the mean yield of the studied genotypes in these conditions, but the indices YI, GMP, HMP are calculated using different formulas. It is believed that they are less sensitive to large differences between the values of potential yields and yields under stressful conditions.

According to these indices, there were five populations distinguished: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C. with the indices 111.25-113.53, 12.72-13.03 and 11.67-11.86, respectively. We believe that these indices most fully characterize the resistance of the populations to drought and their high productivity under the stress (8.30-8.47 kg/m²). The Stress Tolerance Index (STI), with a range of 0.31 to 0.45, characterizes the ability of the genotype to form a stable yield regardless of stress factors. According to this index, there were eight populations that significantly surpassed the mean population, but they can be divided into two groups:

M.g./P.p., LR/H, Ram.d., M. g./CP-11 and M.agr./C. - STI index varies from 0.43 to 0.45 and they have a high yield under the stress of 8.30-8.47 kg/m² and mean under the irrigation - 19.22-20.44 kg/m²;

FHNV², B11/P.d., Sin(s)/Prymorka, in which the STI index is 0.40, but high or moderate yield under the irrigation $(19.5-20.65 \text{ kg/m}^2)$ and mean under the stress $(7.26-7.76 \text{ kg/m}^2)$.

According to the relative drought resistance index (RDI), nine populations were identified: M.g./CP-11, Zymostiyka/M.K., A.r. d., M.g./M.agr., M.agr./C., Prymorka/Sin(s), LR/H, M.g./P.p., Ram.d. with a variation of the index from 1.06 to 1.14.

According to the drought resistance index (DI), there were eight populations that significantly exceeded the mean population, but they can be divided into two groups:

M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C. - DI index varies in the range of 0.45-0.49 and they have a high yield under the stress of 8.30-8.47 kg/m²;

Prymorka/Sin(s), B.11/P.d, Sin(s)/Prymorka, Zymostiyka/M.K. and M.g./M.agr., in which the DI index is 0.43, but low yields under irrigation (17.95-18.65 kg/m²) and average under the stress (7.60-7.76 kg/m²).

Studying the Modified Stress Tolerance Indices (M1STI, M2STI), eight (Ram.d., M.agr./C., B11/P.d., LR/H, Sin(s)/Prymorka, M.g./P.p., M.g./CP-11, Elehiya), with an M1STI index of 0.42-0.47 and five populations of alfalfa (M.agr./C., LR/H, Ram.d., M.g./P.p., Elehiya) with an index of M2STI - 0.54-0.57 were distinguished. The M1STI index is not always suitable for the selection of populations for drought resistance, as the selected populations are Sin(s)/Prymorka and B11/P.d. had high rates of 0.44-0.46 due to high yields under the irrigation (20.65; 20.36 kg/m²), but low or moderate under the stress (7.26; 7.52 kg/m²). more accurately The M2STI index characterizes drought-resistant genotypes that have high performance under the stress.

The Abiotic Tolerance Index (ATI) ranges from 57.77 to 67.95, in our case, it is not suitable for characterizing the resistance of alfalfa populations to stress, as, using this index, five populations were identified. (A.N.d. №15, M.g./CP-11. B11/P.d., Sin(s)/Prvmorka. Elehiya), of which the last three had a high yield in the optimal conditions (20.36-20.65 kg/m²), but medium or low under the stress (6.66-7.52) and only one population M.g./CP-11 can be classified as drought-resistant with an ATI index of 60.67.

Based on the results of the study and their analysis, we proposed the stress resistance index ISR, which in our opinion characterizes the genotypes by the stress resistance not only by a smaller difference in the yield in the optimal and limiting conditions, but also considers high stress productivity.

Stress Resistance Index ISR is determined by the formula:

$$ISR = \frac{Yp \times Ys}{(Yp - Ys) \times (1 - \frac{Ys}{Yp})}$$
(15)

According to the Stress Resistance Index (ISR), nine populations were identified that significantly surpassed than the mean population, but they can be divided into two groups:

M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C. - ISR index varies between 23.53-26.67 and they have a high yield under the stress of 8.30-8.47 kg/m²;

Prymorka/Sin(s), Zymostiyka/MK, A.r.d. and M.g./M.agr., in which the ISR index is 22.29-22.86, but low yields under the irrigation $(17.95-18.65 \text{ kg/m}^2)$ and average under the stress $(7.60-7.76 \text{ kg/m}^2)$.

Yields of alfalfa populations under the stress (Ys) have a high positive correlation (r = 0.901-1.000) with indices YSI, YI, GMP, HMP, STI, DI, RDI, ISR and negative with SSI (r = -0.901); with the indices MPI, TOL, SSPI - the average correlation (r = 0.627; r = -0.609; r = -0.609), and with the ATI index there is no connection (r = -0.092).

CONCLUSIONS

Analyzing alfalfa populations by forage productivity by the largest number of indices (11-13), we selected five genotypes: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C., which had the highest yield of 8.30-8.47 kg/m² under the stress. Four indices YI, GMP, HMP, SSI and the proposed ISR stress resistance index were selected, which not only characterize the population in terms of drought resistance, but also in terms of productivity under the stress conditions.

| ISR | 18.26 | 14.00 | 15.31 | 25.34 | 17.27 | 26.06 | 22.76 | 12.64 | 15.85 | 18.56 | 16.03 | 26.67 | 20.63 | 18.04 | 23.53 | 22.48 | 25.43 | 22.29 | 22.86 | 19.02 | 21.41 | 18.91 | 18.30 | 19.44 | 20.04 | 19.23 | 1.818 | 19.41 | 0.794 |
|--------------------|-------------|---------|----------|------------|-------------------|--------|-------------------|--------------|------------|--------------|-----------|---------|------------------------------|-------------|------------|-----------------|----------|---------|--------------|---------|-------------------|------------|------------|-------------|------------------------|---------|-------------------|-------|-------|
| HMP | 10.60 | 10.17 | 9.71 | 11.86 | 10.74 | 11.85 | 10.96 | 9.25 | 10.15 | 10.60 | 9.59 | 11.71 | 11.00 | 10.54 | 11.81 | 10.92 | 11.67 | 10.79 | 10.68 | 10.36 | 11.10 | 10.98 | 10.65 | 10.65 | 10.76 | 10.71 | 0.327 | 6.51 | 0.143 |
| ATI | 56.78 | 67.95 | 52.61 | 57.37 | 62.90 | 55.66 | 50.27 | 56.84 | 57.99 | 55.74 | 47.99 | 52.71 | 55.90 | 56.51 | 60.67 | 50.32 | 54.50 | 48.90 | 46.38 | 50.75 | 55.41 | 60.96 | 57.42 | 53.87 | 55.27 | 55.70 | 2.312 | 8.95 | 1.010 |
| M_2STI | 0.36 | 0.30 | 0.25 | 0.57 | 0.38 | 0.57 | 0.41 | 0.21 | 0.30 | 0.36 | 0.24 | 0.55 | 0.42 | 0.35 | 0.56 | 0.41 | 0.54 | 0.39 | 0.37 | 0.33 | 0.43 | 0.41 | 0.36 | 0.36 | 0.39 | 0.38 | 0.048 | 26.28 | 0.021 |
| M ₁ STI | 0.39 | 0.46 | 0.31 | 0.47 | 0.45 | 0.45 | 0.35 | 0.32 | 0.37 | 0.38 | 0.27 | 0.42 | 0.40 | 0.38 | 0.50 | 0.35 | 0.43 | 0.33 | 0.31 | 0.32 | 0.40 | 0.44 | 0.39 | 0.36 | 0.39 | 0.39 | 0.028 | 15.36 | 0.012 |
| IdSS | 31.86 | 38.08 | 31.73 | 29.62 | 34.43 | 28.88 | 28.00 | 34.95 | 33.43 | 31.37 | 29.62 | 27.77 | 30.63 | 31.86 | 31.22 | 28.11 | 28.67 | 27.67 | 26.61 | 29.42 | 30.19 | 33.02 | 32.07 | 30.37 | 30.82 | 30.50 | 1.254 | 8.71 | 0.548 |
| DI | 0.36 | 0.28 | 0.30 | 0.48 | 0.34 | 0.49 | 0.43 | 0.25 | 0.31 | 0.36 | 0.32 | 0.49 | 0.40 | 0.36 | 0.45 | 0.43 | 0.48 | 0.42 | 0.43 | 0.37 | 0.41 | 0.37 | 0.36 | 0.38 | 0.39 | 0.38 | 0.031 | 17.39 | 0.014 |
| RDI | 0.96 | 0.81 | 0.90 | 1.10 | 0.92 | 1.12 | 1.08 | 0.80 | 0.90 | 0.97 | 0.94 | 1.14 | 1.02 | 0.96 | 1.06 | 1.08 | 1.11 | 1.08 | 1.10 | 1.01 | 1.04 | 0.96 | 0.96 | 1.00 | 1.00 | 1.01 | 0.044 | 9.42 | 0.019 |
| GMP | 11.94 | 11.96 | 11.11 | 12.98 | 12.24 | 12.92 | 12.03 | 10.90 | 11.63 | 11.91 | 10.86 | 12.72 | 12.23 | 11.89 | 13.03 | 12.00 | 12.74 | 11.85 | 11.68 | 11.56 | 12.30 | 12.37 | 12.00 | 11.89 | 12.03 | 11.98 | 0.276 | 4.92 | 0.121 |
| STI | 0.38 | 0.38 | 0.33 | 0.45 | 0.40 | 0.44 | 0.38 | 0.31 | 0.36 | 0.38 | 0.31 | 0.43 | 0.40 | 0.37 | 0.45 | 0.38 | 0.43 | 0.37 | 0.36 | 0.35 | 0.40 | 0.40 | 0.38 | 0.37 | 0.38 | 0.38 | 0.018 | 10.01 | 0.008 |
| Ιλ | 97.31 | 89.27 | 87.66 | 113.13 | 97.31 | 113.53 | 104.02 | 81.09 | 91.42 | 97.58 | 87.53 | 112.86 | 102.54 | 96.64 | 111.25 | 103.48 | 111.66 | 102.27 | 101.87 | 96.24 | 104.02 | 100.80 | 97.72 | 98.79 | 100.00 | 99.79 | 4.078 | 8.72 | 1.781 |
| ΥSI | 0.37 | 0.31 | 0.35 | 0.42 | 0.35 | 0.43 | 0.42 | 0.31 | 0.34 | 0.37 | 0.36 | 0.44 | 0.39 | 0.37 | 0.41 | 0.41 | 0.43 | 0.41 | 0.42 | 0.39 | 0.40 | 0.37 | 0.37 | 0.38 | 0.38 | 0.39 | 0.017 | 9.46 | 0.007 |
| TOL | 12.39 | 14.81 | 12.34 | 11.52 | 13.39 | 11.23 | 10.89 | 13.59 | 13.00 | 12.20 | 11.52 | 10.80 | 11.91 | 12.39 | 12.14 | 10.93 | 11.15 | 10.76 | 10.35 | 11.44 | 11.74 | 12.84 | 12.47 | 11.81 | 11.98 | 11.86 | 0.488 | 8.71 | 0.213 |
| ISS | 1.02 | 1.12 | 1.06 | 0.94 | 1.05 | 0.92 | 0.95 | 1.12 | 1.06 | 1.02 | 1.04 | 0.91 | 0.99 | 1.03 | 0.96 | 0.95 | 0.93 | 0.95 | 0.94 | 1.00 | 96.0 | 1.02 | 1.02 | 1.00 | 1.00 | 1.00 | 0.027 | 5.86 | 0.012 |
| MP | 13.46 | 14.07 | 12.71 | 14.20 | 13.96 | 14.09 | 13.21 | 12.85 | 13.32 | 13.38 | 12.29 | 13.82 | 13.61 | 13.41 | 14.37 | 13.19 | 13.91 | 13.01 | 12.78 | 12.90 | 13.63 | 13.94 | 13.53 | 13.28 | 13.45 | 13.43 | 0.248 | 3.95 | 0.108 |
| $\gamma_{\rm S}$ | 7.26 | 6.66 | 6.54 | 8.44 | 7.26 | 8.47 | 7.76 | 6.05 | 6.82 | 7.28 | 6.53 | 8.42 | 7.65 | 7.21 | 8.30 | 7.72 | 8.33 | 7.63 | 7.60 | 7.18 | 7.76 | 7.52 | 7.29 | 7.37 | 7.46 | 7.44 | 0.304 | 8.72 | 0.133 |
| Yp | 19.65 | 21.47 | 18.88 | 19.96 | 20.65 | 19.70 | 18.65 | 19.64 | 19.82 | 19.48 | 18.05 | 19.22 | 19.56 | 19.60 | 20.44 | 18.65 | 19.48 | 18.39 | 17.95 | 18.62 | 19.50 | 20.36 | 19.76 | 19.18 | 19.44 | 19.53 | 0.386 | 4.26 | 0.169 |
| Abbreviation | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 | G13 | G14 | G15 | G16 | G17 | G18 | G19 | G20 | G21 | G22 | G23 | G24 | | | | | |
| Name | Unitro, St. | Elehiya | Prymorka | M.g./ P.p. | Sin (s)./Prymorka | LR/H | Prymorka / Sin(s) | AN. d. № 114 | AN.d. № 15 | AN. d. Nº 38 | Dobir k.s | Ram. d. | (Emeraude /T.) ² | T./Emeraude | M.g. CP-11 | Zymostiyka/M.K. | M.agr/C. | A.r. d. | M.g./ M.agr. | M.g. d. | FHNV ² | V.11/P. d. | Zh./ CP-11 | Sybir. 8, d | Mean by the population | Mediana | LSD ₀₅ | V, % | Sx |

| ISR | | | | | | | | | | | | | | | | | ı |
|----------------------|----------------------|------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------|----------|--------|--------|--------|
| HMP | | | | | | | ı | ı | | ı | | | | | | ı | 0.910 |
| ATI | | | ı | | ı | | - | ı | | | | | | | | 0.078 | -0.323 |
| M_2STI | | | - | | - | - | ı | - | - | - | - | | ı | | 0.065 | 0.991 | 0.917 |
| M_1STI | - | - | | - | ı | - | - | ı | - | · | - | - | - | 0.704 | 0.753 | 0.711 | 0.375 |
| IdSS | | | | | | | | ı | | | | | 0.288 | -0.470 | 0.845 | -0.466 | -0.772 |
| DI | | | | | ı | | ı | ı | | | | -0.768 | 0.388 | 0.923 | -0.311 | 0.920 | 0.998 |
| RDI | | | | | | | 1 | · | - | | 0.973 | -0.891 | 0.173 | 0.811 | -0.513 | 0.814 | 0.973 |
| GMP | | | - | | - | - | - | - | - | 0.633 | 0.788 | -0.217 | 0.870 | 0.955 | 0.337 | 0.965 | 0.776 |
| STI | | | ı | | ı | | | ı | 1.000 | 0.633 | 0.789 | -0.219 | 0.871 | 0.958 | 0.335 | 0.965 | 0.778 |
| ΥI | | | | | ı | | | 0.907 | 0.906 | 0.901 | 0.974 | -0.609 | 0.583 | 0.981 | -0.092 | 0.985 | 0.967 |
| ΥSI | | | ı | | ı | | 0.901 | 0.633 | 0.633 | 1.000 | 0.973 | -0.891 | 0.173 | 0.811 | -0.513 | 0.814 | 0.973 |
| TOL | | | | | | -0.891 | -0.609 | -0.219 | -0.217 | -0.891 | -0.768 | 1.000 | 0.288 | -0.470 | 0.845 | -0.466 | -0.772 |
| ISS | | | | | 0.891 | -1.000 | -0.901 | -0.633 | -0.633 | -1.000 | -0.973 | 0.891 | -0.173 | -0.811 | 0.513 | -0.814 | -0.973 |
| MP | | | | -0.228 | 0.236 | 0.228 | 0.627 | 0.896 | 0.897 | 0.228 | 0.439 | 0.236 | 0.996 | 0.740 | 0.717 | 0.749 | 0.427 |
| $\gamma_{\rm S}$ | | | 0.627 | -0.901 | -0.609 | 0.901 | 1.000 | 0.907 | 0.906 | 0.901 | 0.974 | -0.609 | 0.583 | 0.981 | -0.092 | 0.985 | 0.967 |
| \boldsymbol{Y}_{P} | | 0.018 | 0.790 | 0.416 | 0.782 | -0.416 | 0.018 | 0.437 | 0.439 | -0.416 | -0.202 | 0.782 | 0.821 | 0.178 | 0.993 | 0.187 | -0.213 |
| | \boldsymbol{Y}_{P} | $\gamma_{\rm S}$ | MP | ISS | TOL | ISY | Ιλ | ITZ | GMP | RDI | DI | IdSS | M_1STI | M_2STI | ATI | HMP | ISR |

Table 2. Drought tolerance - sown in 2017-2019 (total for two years)

REFERENCES

- Abdelguerfi, A., & Abdelguerfi-Laouar, M. (2002). Forage and pasture species: The uses in Maghreb (Algeria, Morocco, and Tunisia). FAO, Rome, Italy.
- Aleksandrov, V. (2002). Climate change on the Balkan Peninsula. Ecology and Future. Vol. 1(2-4), 26-30.
- Annicchiarico, P., Pecetti, L., Abdelguerfi, A., Bouizgaren, A., Carroni, A. M., Hayek, T., ... & Mezni, M. (2011). Adaptation of landrace and variety germplasm and selection strategies for lucerne in the Mediterranean basin. Field Crops Research, 120(2), 283-291.
- Annicchiarico, P., Pecetti, L., & Tava, A. (2013). Physiological and morphological traits associated with adaptation of lucerne (Medicago sativa) to severely drought-stressed and to irrigated environments. Annals of Applied Biology, 162(1), 27-40.
- Betran, F. J., Beck, D., Bänziger, M., & Edmeades, G. O. (2003). Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop Science, 43(3), 807-817.
- Blum, A. (2018). Plant breeding for stress environments. CRC press.
- Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive?. Australian Journal of Agricultural Research, 56(11), 1159-1168.
- Bouslama M., & Schapaugh W.T. (1984). Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. Crop Science, 24(5), 933–937.
- Boussen, H., Ben Salem, M., Slama, A., Mallek-Maalej, E., & Rezgui, S. (2010, March). Evaluation of drought tolerance indices in durum wheat recombinant inbred lines. In Proceeding of Second International Conference on Drought Management FAO-CIHEAM, Istanbul, Turkey (pp. 4-6).
- Cattivelli, L., Rizza, F., Badeck, F. W., Mazzucotelli, E., Mastrangelo, A. M., Francia, E., ... & Stanca, A. M. (2008). Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field crops research, 105(1-2), 1-14.
- Ceccarelli, S. (1987). Yield potential and drought tolerance of segregating populations of barley in contrasting environments. Euphytica, 36(1), 265-273.
- Ceccarelli, S., & Grando, S. (1991). Selection environment and environmental sensitivity in barley. Euphytica, 57(2), 157-167.
- Choukan, R., Taherkhani, T., Ghanadha, M. R., & Khodarahmi, M. (2006). Evaluation of drought tolerance in grain maize inbred lines using drought tolerance indices. Iranian Journal of Agricultural Sciences, 8(1), 79-89.
- Clarke, J. M., DePauw, R. M., & Townley-Smith, T. F. (1992). Evaluation of methods for quantification of drought tolerance in wheat. Crop Science, 32(3), 723-728.
- Bellague, D., M'Hammedi-Bouzina, M., & Abdelguerfi, A. (2016). Measuring the performance of perennial

alfalfa with drought tolerance indices. Chilean journal of agricultural research, 76(3), 273-284.

- Durand, J. L. (2007). Les effets du stress hydrique sur la plante: The effects of water stress on the plant: Physiological aspects. Fourrages, 190, 181-195.
- Farshadfar, E., & Sutka, J. (2003). Multivariate analysis of drought tolerance in wheat substitution lines. Cereal Research Communications, 31(1), 33-40.
- Fernandez, G. C. (1992). Effective selection criteria for assessing plant stress tolerance. In Proceeding of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, Aug. 13-16, Shanhua, Taiwan, 1992 (pp. 257-270).
- Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. Australian Journal of Agricultural Research, 29(5), 897-912.
- Gavuzzi, P., Rizza, F., Palumbo, M., Campanile, R. G., Ricciardi, G. L., & Borghi, B. (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Canadian Journal of plant science, 77(4), 523-531.
- Hao, Z. F., Li, X. H., Su, Z. J., Xie, C. X., Li, M. S., Liang, X. L., ... & Zhang, S. H. (2011). A proposed selection criterion for drought resistance across multiple environments in maize. Breeding science, 61(2), 101-108.
- Harrison, M. T., Tardieu, F., Dong, Z., Messina, C. D., & Hammer, G. L. (2014). Characterizing drought stress and trait influence on maize yield under current and future conditions. Global change biology, 20(3), 867-878.
- Hussain, S. S., Raza, H., Afzal, I., & Kayani, M. A. (2012). Transgenic plants for abiotic stress tolerance: current status. Archives of Agronomy and Soil Science, 58(7), 693-721.
- Khalili, M., Pour-Aboughadareh, A., & Naghavi, M. R. (2016). Assessment of drought tolerance in barley: integrated selection criterion and drought tolerance indices. Environmental and Experimental Biology, 14(1), 33-41.
- Kirigwi, F. M., Van Ginkel, M., Trethowan, R., Sears, R. G., Rajaram, S., & Paulsen, G. M. (2004). Evaluation of selection strategies for wheat adaptation across water regimes. Euphytica, 135(3), 361-371.
- Koleva, M., & Dimitrova, V. (2018). Evaluation of drought tolerance in new cotton cultivars using stress tolerance indices. AGROFOR, 3(1), 11-17.
- Schneider, K. A., Rosales-Serna, R., Ibarra-Perez, F., Cazares-Enriquez, B., Acosta-Gallegos, J. A., Ramirez-Vallejo, P., ... & Kelly, J. D. (1997). Improving common bean performance under drought stress. Crop Science, 37(1), 43-50.
- Latrach, L., Farissi, M., Mouradi, M., Makoudi, B., Bouizgaren, A., & Ghoulam, C. (2014). Growth and nodulation of alfalfa-rhizobia symbiosis under salinity: electrolyte leakage, stomatal conductance, and chlorophyll fluorescence. Turkish Journal of Agriculture and Forestry, 38(3), 320-326.
- Lemaire, G. (2006). La luzerne: Alfalfa. Productivity and quality. In: Forage Fabaceae diversity and their symbionts: biotechnological, agronomic and

environmental applications. International Workshop, Algiers, Algeria, pp. 174-182.

- Lin, C. S., & Binns, M. R. (1988). A superiority measure of cultivar performance for cultivar× location data. Canadian journal of plant science, 68(1), 193-198.
- Malekshahi, F., Dehghani, H. A. M. I. D., & Alizadeh, B. A. H. R. A. M. (2009). A study of drought tolerance indices in canola (Brassica napus L.) genotypes. Journal of Science and technology of agriculture and natural resources, 13(48 (B)), 77-90.
- Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. Current Science, 80, 758-763.
- Moghaddam, A., & Hadizadeh, M. H. (2002). Response of corn (Zea mays L.) hybrids and their parental lines to drought using different stress tolerance indices. Journal of Seed and Plant Improvement, 18(3), 255-275.
- Mollasadeghi, V., Valizadeh, M., Shahryari, R., & Imani, A. A. (2011). Evaluation of end drought tolerance of 12 wheat genotypes by stress indices. World Applied Sciences Journal, 13(3), 545-551.
- Mousavi, S. S., YAZDI, S. B., Naghavi, M. R., Zali, A. A., Dashti, H., & Pourshahbazi, A. (2008). Introduction of new indices to identify relative drought tolerance and resistance in wheat genotypes. Desert, 12, 165-178.
- Mustāþea, P., Sãulescu, N. N., Ittu, G., Pãunescu, G., Stere, I., Tanislav, N., ... & Voinea, I. (2003). Genotypical differences in wheat response to drought under conditions of the Year 2002. Romanian Agricultural Research, 19-20, 39-48.
- Bagavathiannan, M. V., & Van Acker, R. C. (2009). The biology and ecology of feral alfalfa (Medicago sativa L.) and its implications for novel trait confinement in North America. Critical Reviews in Plant Sciences, 28(1-2), 69-87.
- Ramirez-Vallejo, P., & Kelly, J. D. (1998). Traits related to drought resistance in common bean. Euphytica, 99(2), 127-136.
- Richards, R. A. (2006). Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural water management, 80(1-3), 197-211.
- Richards, R. A., Rebetzke, G. J., Condon, A. G., & Van Herwaarden, A. F. (2002). Breeding opportunities for

increasing the efficiency of water use and crop yield in temperate cereals. Crop science, 42(1), 111-121.

- Rosielle, A. A., & Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environment. Crop science, 21(6), 943-946.
- Saba, J., Moghadam, M., Ghasemi, K., & Nishabouri, M. R. (2001). Genetic properties of drought resistance indices. Journal of Agricultural Science technology, 3, 43-49.
- Rad, A. H. S., & Abbasian, A. (2011). Evaluation of drought tolerance in winter rapeseed cultivars based on tolerance and sensitivity indices. Žemdirbystė (Agriculture), 98(1), 41-48.
- Li, S., Wan, L., Nie, Z., & Li, X. (2020). Fractal and topological analyses and antioxidant defense systems of alfalfa (Medicago sativa L.) root system under drought and rehydration regimes. Agronomy, 10(6), 805.
- Mardeh, A. S. S., Ahmadi, A., Poustini, K., & Mohammadi, V. (2006). Evaluation of drought resistance indices under various environmental conditions. Field Crops Research, 98(2-3), 222-229.
- Vasconcelos, E. S. D., Barioni Júnior, W., Cruz, C. D., Ferreira, R. D. P., Rassini, J. B., & Vilela, D. (2008). Alfalfa genotype selection for adaptability and stability of dry matter production. Acta Scientiarum. Agronomy, 30(3), 339-343.
- Wang, Z., Ke, Q., Kim, M. D., Kim, S. H., Ji, C. Y., Jeong, J. C., ... & Kwak, S. S. (2015). Transgenic alfalfa plants expressing the sweetpotato Orange gene exhibit enhanced abiotic stress tolerance. PLoS One, 10(5), e0126050.
- Yarnia, M., Arabifard, N., Khoei, F. R., & Zandi, P. (2011). Evaluation of drought tolerance indices among some winter rapeseed cultivars. African Journal of Biotechnology, 10(53), 10914-10922.
- Yu, L. X. (2017). Identification of single-nucleotide polymorphic loci associated with biomass yield under water deficit in alfalfa (Medicago sativa L.) using genome-wide sequencing and association mapping. Frontiers in Plant Science, 8, 1152.
- Zou, G. H., Liu, H. Y., Mei, H. W., Liu, G. L., Yu, X. Q., Li, M. S., ... & Luo, L. J. (2007). Screening for drought resistance of rice recombinant inbred populations in the field. Journal of Integrative Plant Biology, 49(10), 1508-1516.