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ASSESMENT OF SPRING BARLEY GENOTYPES RESISTANCE TO DROUGHT AT TWO DEVELOPMENTAL STAGES UNDER DIFFERENT SIMULATED CONDITIONS

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Abstract

Barley is a plant that can be grown in various conditions, from a pedoclimatic point of view, because it has a good drought tolerance. This study tested the drought tolerance of 25 spring barley genotypes using two drought induction methods, the polyethylene glycol method (PEG 10000) at NARDI Fundulea in a controlled climate (M1), and the sodium chlorate method ($NaClO_3$) in the experimental field from ARDS Turda (M2). Drought stress is one of the most important issue for the two-row spring barley yield, since it simultaneously affects many properties at the morphological and physiological level, mainly the production elements and, implicitly, the final yield. Following this study, the Daciana and Jubileu varieties and spring barley perspective genotypes (4 lines from advanced generations To 2168-01, To 2115-10, To 2054-97 and To 2027-10) were shown to be tolerant to both types of induced drought (both in the seedling and adult plant developmental stages).

Key words: *drought tolerance, spring barley, polyethylene glycol, sodium chlorate.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated plants that has played a significant role in the development of human civilization, agronomic, physiological and genetic sciences in plant breeding. It was probably first used in human food in raw form or in the form of bread, porridge or soups. Barley will later be used mainly as fodder, in the manufacture of malt and beer and in the distillation processes (Boanta et al., 2019).

Barley is a plant that can be grown in various conditions, from a pedoclimatic point of view, because it has a good drought tolerance (Mishra & Shivakumar, 2000; Valenzuela & Smith, 2002 cited by Porumb, 2018) and is more resistant to high temperatures in the vegetation period than wheat, rye and oats (Muntean, 1993).

The demand and production of barley is growing for various reasons, namely: wide adaptability, the genetic diversity, being a model crop for molecular research and having a wide range of uses, such as food, in the beer industry and animal feed. This is the reason why special importance is given to this plant through various studies at the molecular, cellular, physiological and morphological levels, barley having the ability to adapt more easily to the recent climate change (Kebede et al., 2019).

Drought is a major abiotic stress factor, which severely affects the crop production worldwide. The agronomic and physiological features associated with drought tolerance are adequate indicators in the breeding program (Rong et al., 2006) the identification of drought tolerant genotypes, in order to reduce the impact of water deficit on the crop yields.

Agricultural drought refers to a very low level of rainfall that cannot ensure the proper growth and development of crops during the growth season. Drought occurs for various reasons: quantitatively reduced rainfall, the timing of water availability depending on the requirements of each plant or the reduction of the soil water supply (Kebede et al., 2019).

Therefore, drought stress is an important aspect in the formation of barley production elements, as it simultaneously affects many traits through morphological, physiological and metabolic changes that occur in all plant organs, eventually leading to a decreased production (Sacks et al., 1997; Cellier et al., 1998; Cochard et al., 2002 cited by Rong et al., 2006).

MATERIALS AND METHODS

Within the theme of the ADER 2.1.2 research project, two studies were performed using two methods on drought tolerance of 25 genotypes of spring barley tested in two localities (Turda and Fundulea). At National Agricultural Research and Development Institute (NARDI) Fundulea, the spring barley genotypes were tested in the seedling phase by inducing stress with polyethylene glycol (PEG 10000), determining the root length, seedling height and leaf area. The seeds were sterilized with 1% sodium hypochlorite solution for 10 minutes, washed thoroughly with distilled water and germinated on rolls of filter paper in Berzelius beakers in tap water. They were kept in the growth chamber for five days at a temperature of 24°C and 16 hours of illumination. After five days, half of the rolls were further kept in tap water (control) and the remainder were transferred in to 15% polyethylene glycol solution (method M1). Both variants were kept in the growth chamber at the same parameters for two weeks (Petcu et al., 2020).

Using a ruler, biometric measurements were performed on the length of the stem, root and leaves (the length and width of the leaf were measured) and the leaf area was calculated using the following formula:

$LA = (L * w * 0.66)$

where:

LA - leaf area (mm^2);

L - leaf length (mm);

w - leaf width (mm);

0.66 - the coefficient used to calculate the leaf area specific to barley.

At Agricultural Research and Development Station Turda (ARDS Turda), drought tolerance was tested according to the method of Blum et al., 1998 (method M2), by spraying the plants with sodium chlorate ($NaClO_3$) 14 days after flowering. During the experiment, 4 rows with a length of 1 linear meter (two rows for check and two for sodium chlorate treatment) were delimited for each experimental variant, where the treatment was applied by spraying this desiccant on the entire plant. Each variant was harvested individually and processed in the laboratory. Through this method, determinations were made on the yield elements, namely: ear length (EL), ear weight (EW), grains weight per ear (GW/E) and one thousand kernel weight (TKW). To determine these traits, 15 plants were analyzed in three repetitions.

The experiment was conceived as a two-factor type A × B, where: factor A is the treatment (with two graduations, check and treated); factor B is the genotype (with 25 graduations).

The POLIFACT statistical analysis program was used to process the obtained data.

RESULTS AND DISCUSSIONS

Assessing drought tolerance in the seedling stage is very important because it affects all subsequent stages and, ultimately, the yield per unit area. Such a study was also conducted by Sallam et al., in 2019. At this stage, the studies of genetic variation in drought tolerance were focused mainly on the characteristics of leaves and roots as in our case. Analysis of the variance revealed the significant effect of PEG-induced drought on the seedling height of spring barley genotypes (Table 1).

Table 1. Analysis of the variance for the seedling height at the spring barley genotypes studied

Source of variation	DF	MS	F
Factor A (Treatment)	1	106027.60	59.524*
Error A	2	1781.25	-
Factor B (Genotype)	24	1783.97	4.280***
Interaction (AxB)	24	963.09	2.310***
Error B	96	416.85	-

DF - Degrees of freedom; MS - Mean squares.

Along with the treatment factor (A), both genotypes (B) and the double interaction between treatment and genotype (A x B) have a more important contribution in the variance of seedling height, the values of sample F corresponding to the hereditary factor being very significant, suggesting differences between genotypes in terms of drought stress behavior. Similar results were obtained by Petcu et al. (2020) in a study conducted on 10 genotypes of winter barley.

The height of the seedlings in optimal humidity conditions was between 133 and 232 mm, and in drought conditions the recorded values were between 98 and 154 mm. The most affected variants were: the lines To 2172-01, To 2149-99, To 2013-99, To 2011-92, To 2198-13 and the Adina variety with the biggest differences being distinctly negative (Table 2).

Table 2. The effect of induced drought in seedling height in a series of spring barley genotypes tested at NARDI Fundulea

No.	Genotype	Seedling height (mm)			
		Check	Treatment (drought)	Diff.	Sign.
1	Daciana	174	140	-34	-
2	Turdeana	191	128	-53	-
3	Romanița	197	131	-66	0
4	**Adina**	**210**	**130**	**-80**	**00**
5	To 2270-94	195	145	-50	0
6	**To 2198-13**	**215**	**141**	**-74**	**00**
7	To 2096-10	187	151	-36	-
8	**To 2172-01**	**232**	**118**	**-114**	**00**
9	To 2168-01	187	125	-62	0
10	To 2115-94	173	123	-50	0
11	To 2036-02	189	136	-53	0
12	To 2054-97	195	138	-57	0
13	**To 2013-99**	**195**	**126**	**-70**	**00**
14	To 2095-01	208	154	-54	0
15	**To 2149-99**	**206**	**110**	**-96**	**00**
16	To 2017-93	174	127	-47	0
17	To 2014-99	207	157	-51	0
18	To 2247-01	150	126	-24	-
19	To 2167-01	133	126	-7	-
20	To 2051-10	188	131	-56	0
21	To 2123-01	197	145	-52	0
22	To 2027-10	123	111	-12	-
23	To 2170-01	161	98	-63	0
24	**To 2011-92**	**192**	**122**	**-70**	**00**
25	Jubileu	185	137	-49	0
LSD (p 5%)		41 mm			

Diff. - difference; Sign. - significance.

If in the case of seedling height (Table 1) the factor F registered significant values for the treatment as source of variation, in the case of root length, the analysis of variance showed the participation of both factors A (treatment) and B (genotype) and also their interaction in expressing this traits (Table 3).

Table 3. Analysis of the variance for root length of studied spring barley genotypes

Source of variation	DF	MS	F
Factor A (Treatment)	1	163152	194.099***
Error A	2	840.56	-
Factor B (Genotype)	24	1583.22	3.163***
Interaction (AxB)	24	1284.75	2.567***
Error B	96	500.50	-

DF - Degrees of freedom; MS - Mean squares.

It is obvious that the induced water stress affected the root system of the seedlings more than their aerial part. In terms of a well-developed root system in both environmental conditions, the genotypes Daciana, Turdeana, Romanița, Adina, To 2270-94 and To 2095-01 can be noticed.

On the other hand, the most affected genotypes under conditions of induced water stress were the varieties Turdeana, Romanița, Jubileu and the To 2036-02 line (Table 4). This is reflected in the differences between the check and the treatment as well as in their (very significant negative) meanings and it seems that these genotypes are not drought tolerant regarding the root system (Table 4). In this case it have to analyze them in relation to the length of the root under normal condition where these have the most developed root system. After exposure to induced drought condition, however, they have the most developed root system compared to other genotypes.

Table 4. The effect of induced drought on the root length in a series of spring barley genotypes tested at NARDI Fundulea

No.	Genotype	Root length (mm)			
		Check	Treatment (drought)	Diff.	Sign.
1	**Daciana**	**194**	**158**	-36	-
2	**Turdeana**	**249**	**128**	**-121**	**000**
3	**Romanița**	**251**	**146**	**-105**	**000**
4	**Adina**	**242**	**153**	-89	00
5	**To 2270-94**	**215**	**149**	**-66**	00
6	To 2198-13	232	141	-92	00
7	To 2096-10	204	131	-73	00
8	To 2172-01	181	147	-34	-
9	To 2168-01	209	135	-74	00
10	To 2115-94	180	121	-60	00
11	**To 2036-02**	**221**	**104**	**-117**	**000**
12	To 2054-97	221	153	-68	00
13	To 2013-99	195	143	-52	0
14	**To 2095-01**	**228**	**132**	-96	00
15	To 2149-99	208	123	-85	00
16	To 2017-93	205	155	-50	0
17	To 2014-99	192	137	-54	0
18	To 2247-01	133	132	-1	-
19	To 2167-01	196	142	-54	0
20	To 2051-10	186	150	-36	-
21	To 2123-01	179	149	-30	-
22	To 2027-10	194	137	-57	0
23	To 2170-01	159	104	-55	0
24	To 2011-92	189	144	-45	0
25	**Jubileu**	**221**	**121**	**-101**	**000**
LSD (p 5%)		40 mm			

Diff. - difference; Sign. - significance.

For the leaf area, the analysis of variance showed that the highest variation is due to factor A (treatments) (Table 5). A similar situation is presented by Petcu et al. (2020) in a study conducted on 10 winter barley genotypes (varieties and lines).

Kang (1998) states that the genotype-environment interaction is a significant challenge in the genotype development. Table 5 shows the influence of the double interaction in expressing the variance of the leaf area. Water stress inhibits the growth of leaf area by disrupting photosynthesis and the metabolic processes due to stomatal closure, and tissue dehydratation, respectively (Petcu et al., 2020).

Table 5. Analysis of the variance for the leaf area of the studied spring barley genotypes

Source of variation	DF	MS	F
Factor A (Treatment)	1	3408081	338.40***
Error A	2	10071.23	-
Factor B (Genotype)	24	20902.40	3.81***
Interaction (AxB)	24	15450.38	2.82***
Error B	96	5488.07	-

DF - Degrees of freedom; MS - Mean squares.

Among the studied traits, it seems that the leaf area is the most affected trait in conditions of induced water stress, fact highlighted in Table 6 from the very low values in treatment conditions compared to the check, as well as from the very significantly negative differences. The most affected genotypes in this respect were the spring barley varieties Turdeana, Romanița, Adina and the lines To 2172-01, To 2013-99, To 2149-99, To 2011-92.

The reduction in plant height may be due to the shortening of the internodes or the reduction of the leaf area (Jafarzadeh and Poostini, 2004). This connection between the leaf area and the height of the plants is reflected by the behavior of the above mentioned lines (To 2172-01, To 2149-99, To 2013-99 and To 2011-92), which were among the most affected by the water stress induced, both in the case of plant height and of the leaf area. Elements of yield that breeders use in assessing drought tolerance for barley and wheat include the seedling vigor, plant height, the number of days to maturity, ear length, the number of ear spikes, root length, the number of grain ears, grain weight, etc. (Sallam et al., 2019). Drought tolerance as a property can be assessed by any of these traits or by using drought indices that accurately assess the response of genotypes to drought stress.

Using the method proposed by Blum (1998), to test drought tolerance, we applied desiccant 14 days after anthesis, making determinations on ear length, ear weight, grains weight/ear and TKW. Observing the significance of the F factor for the treatments factor we can say that the variance of the length of the ear is not that much influenced by environmental conditions. An important contribution in expressing this feature seems to be the genetic factor (genotype), which records very significant values (Table 7).

Table 6. The effect of induced drought on the leaf area in a series of spring barley genotypes tested at NARDI Fundulea

No.	Genotype	Leaf area (mm^2)			
		Check	Treatment (drought)	Diff.	Sign.
1	Daciana	510	215	-296	00
2	**Turdeana**	**552**	**172**	**-380**	**000**
3	**Romanița**	**563**	**179**	**-384**	**000**
4	**Adina**	**578**	**182**	**-394**	**000**
5	To 2270-94	540	208	-332	000
6	To 2198-13	661	220	-441	000
7	To 2096-10	532	235	-297	000
8	**To 2172-01**	**603**	**129**	**-474**	000
9	To 2168-01	507	230	-277	00
10	To 2115-94	403	164	-240	00
11	To 2036-02	565	176	-389	000
12	To 2054-97	587	205	-381	000
13	**To 2013-99**	**548**	**148**	**-400**	**000**
14	To 2095-01	667	325	-309	00
15	**To 2149-99**	**662**	**166**	**-463**	**000**
16	To 2017-93	540	310	-229	00
17	To 2014-99	603	355	-247	00
18	To 2247-01	383	227	-156	0
19	To 2167-01	287	197	-90	-
20	To 2051-10	525	311	-214	00
21	To 2123-01	472	245	-227	00
22	To 2027-10	342	197	-145	0
23	To 2170-01	411	162	-249	00
24	**To 2011-92**	**543**	**240**	**-302**	**00**
25	Jubileu	515	293	-222	00
LSD (p 5%)		132 mm^2			

Diff. - difference; Sign. - significance.

Table 7. Analysis of variance for ear length (cm) in spring barley genotypes tested at ARDS Turda

Source of variation	DF	MS	F
Factor A (Treatment)	1	6.78407	59.579*
Error A	2	0.11387	-
Factor B (Genotype)	24	1.13417	2.709***
Interaction (AxB)	24	0.29684	0.709
Error B	96	0.41871	-

DF - Degrees of freedom; MS - Mean square.

The length of the ear for the plants tested in normal humidity conditions was on average

9.4 cm, with the highest values recorded by the genotypes Turdeana, Romanița, lines To 2096-10, To 2115-94, To 2017-93 and To 2247-01.

Under induced drought condition by the application of desiccant, average values of 9.0 cm were recorded, the most affected being the lines To 2149-99 and To 2115-94.

The least affected by the drought were the varieties Daciana, Jubileu and To 2198-13, To 2168-01, To 2167-01 and To 2011-99 lines (Figure 1).

Another important quantitative trait is the weight of the ear and the grains weight/ear. The genetic variation in grain size and seed germination rate allows for a high flexibility of plants in response to genotypes in varying environmental conditions (Giles, 1990, quoted by Ellis & Marshal, 1998). Variability is the basic phenomenon for plant improvement and consists of the appearance of different individuals at the genetic level, differences that are also due to the interaction of the genotype with the environmental conditions (Jalata et al., 2011).

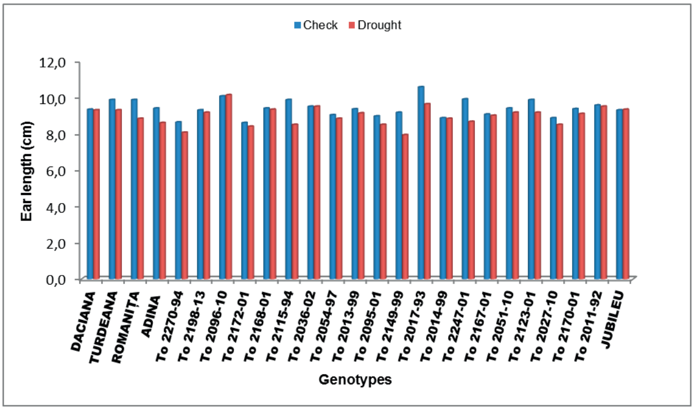

Figure 1. The effect of induced drought on ear length in spring barley genotypes, ARDS Turda

The analysis of the variances for ear weight and grain weight/ear is presented in Table 8. The treatment factor has a very significant involvement in the variability of the two traits (EW and GW/E), so the effects of induced drought and extreme heat in the post anthesis period can be observed. Even in the conditions of chemical desiccation, the values of the variance corresponding to the treatment for the two properties (s^2 = 2.39 and s^2 = 1.80) suggest that the environment has the most significant contribution in their variation. Also, the genetic factor (genotype) is of particular importance in expressing these traits, presented very significant values (Table 8).

Table 8. Analysis of variance for ear weight (g) and grains weight/ear (g) in spring barley genotypes tested at Turda

Source of variation	DF	EW (g)		GW/E (g)	
		MS	F	MS	F
Factor A (Treatment)	1	2.39402	156.978***	1.80841	379.438***
Error A	2	0.01525	-	0.00477	
Factor B (Genotype)	24	0.15275	3.019***	0.12697	4.075***
Interaction (AxB)	24	0.06485	1.282	0.06354	2.039***
Error B	96	0.05060	-	0.03116	

DF - Degrees of freedom; MS - Mean square.

Drought can occur during flowering and can extend until the grain filling, thus affecting the number of grains in the ear and the grains weight, two important components of yield. Because yield is a complex polygenic-controlled trait, breeders often use indirect selection and utilize traits well correlated with this trait to improve yield potential under normal environmental conditions (Sallam et al., 2014).

The average weight of the ear under normal environmental conditions is between 1.36 and 1.88 g, while under stress conditions caused by drought induced during the post anthesis spring barley period, this trait decrease significantly, registering values between 1.65 and 0.75 g (Figure 2).

The average grains weight/ear under normal conditions is between 1.09 and 1.54 g and in the conditions of chemical desiccation it decreases very significantly registering oscillating values, between 0.93-1.32 g/ear, the amplitude of variation between the maximum value under normal conditions and the minimum under stress conditions being 8.98 g (Figure 3).

The most affected genotypes from the application of the desiccant for both properties were the Turdeana variety, but also the To 2172-01 and To 2017-93 lines for both traits. The least affected genotypes by drought were the Daciana and Jubileu varieties, but also the lines To 2168-01, To 2115-10, To 2054-97 and To 2027-10, these being considered tolerant genotypes (Figure 2 and 3).

These statements are also supported by Sallam in a study conducted in 2019, saying that when wheat and barley plants are exposed to drought or heat stress during grain filling, photosynthesis decreases rapidly which reduces the assimilates available in the grain. Consequently, there is a dramatic reduction in grain weight (Sallam et al., 2019).

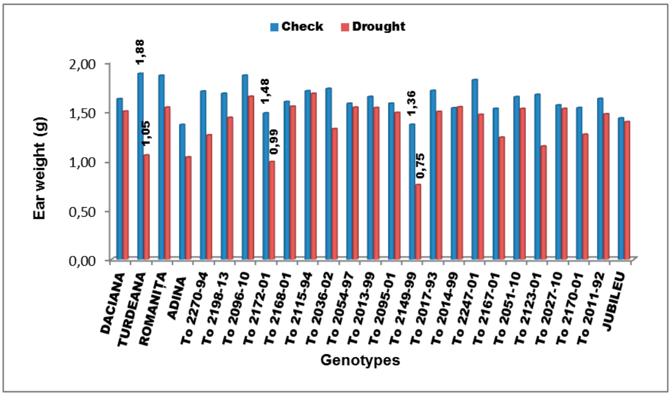

Figure 2. The effect of induced drought on ear weight (g) in spring barley genotypes tested in Turda

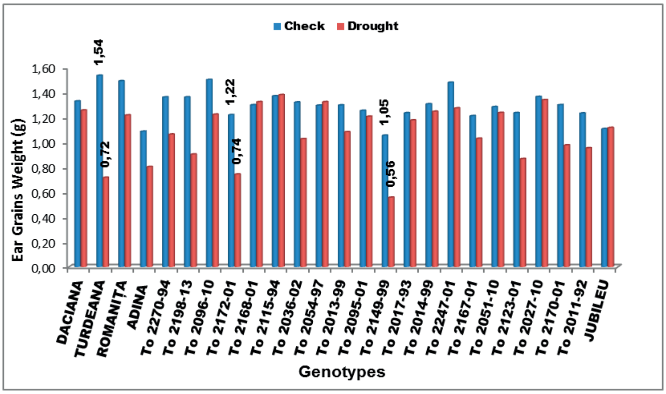

Figure 3. The effect of induced drought on grains weight/ear (g) in spring barley genotypes tested at Turda

Many papers in the speciality literature mention that, among the elements of yield, TKW is the least affected by environmental conditions (Porumb et al., 2018), which can also be observed in our case, wich implies that for TKW there are no meanings for factor F

(Table 9). However, looking at the values of the variance corresponding to the treatment for this property (s^2 = 144367), it can be suggested that the environment in this case had a rather important contribution.

Table 9. Analysis of variance for TKW (g) in spring barley genotypes tested in Turda

Source of variation	DF	MS	F
Factor A (Treatment)	1	144367	1.334^{ns}
Error A	2	108252.40	-
Factor B (Genotype)	24	104966	0.976
Interaction (AxB)	24	108990.30	1.014
Error B	96	107515.30	-

DF - Degrees of freedom; MS - Mean square.

An important element of yield with indisputable implications on quality is TKW. The size of the embryo and implicitly the amount of reserve substances accumulated in the grain, necessary to ensure a good germination and at the same time a higher germination energy (Porumb, 2018) is closely related to this feature.

By directly reflecting the weight of the grains and indirectly their size, the genotypes analyzed in the present study under normal environmental conditions could be divided according to TKW values: medium grain genotypes, with TKW between 43-50 g and large grain genotypes, with TKW over 51 g. The most important values of this property in normal drought conditions were recorded by the Romanița variety (53.74 g). TKW values under stress conditions (Figure 4) ranged from 25.97 g (spring barley line To 2149-99) to 48.57 g (Daciana variety).

The wide range of values obtained from the application of the desiccant suggests the different impact of climatic conditions on this property and especially from the period from flowering to the time of treatment.

In addition to the Turdeana variety and the To 2172-01 line, which were noted and presented previously as the most affected, the To 2198-13 and To 2149-99 lines are also noted for TKW.

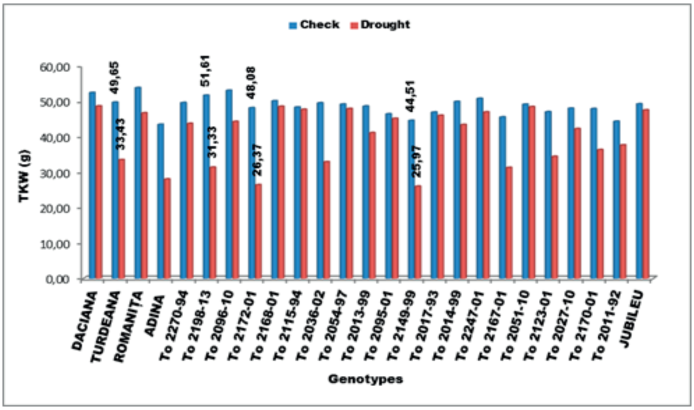

Figure 4. The effect of induced drought on TKW (g) in spring barley genotypes tested at Turda

CONCLUSIONS

In terms of a well-developed root system under both normal and stress conditions, the spring varieties Daciana, Turdeana, Romanița, Adina, To 2270-94 and To 2095-01 lines can be noticed.

The lines To 2172-01, To 2149-99, To 2013-99 and the barley varieties Adina and Turdeana can be considered as very sensitive to both water stress and atmospheric stress, since they are most affected in both drought induced conditions (M1 and M2).

The genotypes affected to an insignificant extent by the drought induced by both methods (M1-PEG and M2-$NAClO_3$) are varieties Daciana, Romanița, Jubileu and To 2198-13, To 2168-01, To 2011-92 and To 2095-01 lines. These can be considered drought tolerant as they do not show significant decreases in the values of the studied traits under normal conditions and in conditions of induced drought.

The Daciana variety stands out is distinguished by the most important values in conditions of water stress or heat for most of the analyzed traits.

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