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THE EFFECT OF WATER STRESS INDUCED WITH PEG SOLUTION ON MAIZE SEEDLINGS

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ABSTRACT

Developing maize genotypes that can perform well under drought is an important goal throughout the world and in Romania, too. It is known that drought tolerance is a complex trait making the search for efficient selection traits, breeding and screening methods difficult. In winter wheat, seedling tests under controlled conditions have been successful in predicting drought or heat tolerance, but similar results have not been enough reported in maize. In this respect, we tried to develop a simple screening method to evaluate drought tolerance of maize seedlings.

The experiments were conducted in growth chamber. Eighteen maize genotypes were grown under drought stress induced by polyethylene glycol 10.000 (10% PEG 10.000, inducing an osmotic potential of - 0.30 MPa). Decreasing of leaf area, root length, root volume, and dry matter accumulation showed important metabolism disturbances of maize plants grown under water stress. The possibility of using certain physiological traits as screening criteria in breeding maize programs for drought tolerance is discussed.

Key words: limited water supply, polyethylene glycol, genetic diversity, maize.

INTRODUCTION

Expanding world's human population with greater food and energy needs has led to increased demand for greater global maize (*Zea mays* L.) production. Unfortunately, environmental limitations such as temperature and drought continued to decrease maize production levels in latest decades and in many areas, and this decrease is predicted to worsen with changing climates Meeks et al., 2013). Developing cultivars of maize that can perform well under heat and drought is an important goal of breeding programs throughout the world, as well as in Romania. It is known that drought tolerance is a complex trait making the search for efficient selection, breeding and screening methods difficult. An initial focus only on yield stability under water stress has resulted in relatively good progresses, but this approach is limited due to cost and testing volume. Identification and utilization of correlated simpler agronomic and physiological traits, easier to assess in field or laboratory, would be desirable for providing better progress in the future. These traits include, but are not limited to shortened anthesis-silking interval (ASI), delayed leaf senescence, increased rooting depth and density, osmotic adjustment, high leaf number and short plant height, performance with limited available nitrogen, seedling vigour and epicuticular wax (Betran et al., 2003; Ludlow et al., 1990; Wan et al., 2000; Meeks et al., 2013).

Additional traits and methods that can help identify drought tolerant genotypes at different stages of growth would be extremely useful for plant breeders, physiologists and agronomists to use in the development of new cultivars. While it is widely recognized that flowering is the most critical time for drought stress to impact maize yield, drought can damage a field anytime throughout the season and the seedling stage has received little attention (Bänziger and Araus, 2007). Assessment of drought tolerance at seedling stage is necessary to predict a good crop stand at maturity (Qayyum et al., 2012). So, one possibility is to use seedlings, and water stress

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to be induced by different osmotic substances, such as polyethylene glycol. Such a method has been successfully used in discriminating cultivars of alfalfa or wheat (Bănică et al., 2008; David, 2012). This is an attractive approach because it is a rapid, low costing and low space method, with fast turnaround time; however, to our knowledge, a such methods has not reported yet in maize.

For the development of elite lines having drought tolerance, the existence of variability in the available germplasm of maize is a key to success for the maize breeders.

The aim of this research was to explore the variation and to determine the target traits conferring drought tolerance in maize.

MATERIAL AND METHODS

Seeds of the eighteen maize hybrids, released by corn breeding department from National Research and Development Institute Fundulea (NARDI) were sterilized with 2% sodium hypochlorite and germinated on filter paper rolls (eight seeds per roll) in water under controlled conditions (25±1°C temperature and 14 h day length), in a 2 factors split plot with 2 replications. After seven/eight days from sowing, for half of rolls, water stress was induced with 10% polyethylene glycol 10.000 solutions (inducing osmotic potential of - 0.30 Mpa) for ten days. The other half of rolls was grown in tap water (control plants). One of the genotypes studied (F 376) is known as resistant to drought.

Root volume (in cm^3, measured by water displacement), the main root length (mm), height of plant (mm), root fresh weight (g) and shoot fresh weight (g) were determined.

P field superiority indexes (PFSI) for grain yield, proposed by Lin and Binns (1988), were computed using the following formula:

$$Pi = (\sum (Xij - Mj)^2)/(2n)$$

where:

n = number of locations (7); xij = yield (from 2015) for genotype i in location j; Mj = yield of genotype with the largest yield in location j. Yield data have been provided by Corn Breeding Department from NARDI.

ANOVA for 2 factor split plot where main plot was the water stress level and hybrids were splitted within the main plots and correlation coefficients among studied traits as well as between studied traits and grain yield were computed (Ceapoiu, 1968).

RESULTS AND DISCUSSION

Effect of water levels showed significant difference at $p<0.01$ for all measured traits. The genotypes were also significant at the level of $p<0.01$ for main root length and root volume and at $p<0.1$ for height of plant and leaf area, while the interaction between treatments and genotypes influenced significantly the studied traits ($p<0.5$ and $p<0.1$) (Table 1).

Table 1. Mean squares and F values for analysis of variance for four seedlings traits in maize genotypes

Source of variance	DF	F value and probability			
		Height of plant	Leaf area	Main root length	Root volume
Water level (control, drought)	1	328.25***	170.90***	78.16***	154.02***
Genotype	17	5.72**	6.20**	34.43***	53.53***
Treatment x genotype	17	2.25*	4.39*	6.1**	6.54**
Error	102	MS = 559	MS = 153314	MS = 780	MS = 0.28

*, **, *** - significant at p=0.05, 0.01 and 0.001, respectively.

Drought tolerance of crop plants is a complex genetically controlled trait, but expression of the plant traits are determined by genotype and environment interaction (Moaveni, 2011).

Positive and significant correlations were observed between height of plants with leaf area, fresh weight of shoots with leaf area and root volume, fresh weight of root with fresh weight of shoots, and volume of root, at both levels of water stress; the coefficients of correlation between root volume and main root length and between fresh weight of root and main root length were significant only in drought conditions while correlation coefficients between fresh weight of shoots and height of plant, root volume and leaf area and between fresh weight of root and leaf area were significant only in control (no water stress). These results were somehow expected; fresh weight of shoot and fresh weight of root were correlated practically with all other traits and could be considered as candidates for large volume screenings being relatively easy to be measured (Table 2).

Table 2. Phenotypic correlation coefficients among studied traits

Trait	Growing conditions	Height of plant	Leaf area	Main root length	Root volume	Fresh weight of shoots
Leaf area	Control	0.68***				
	Drought	0.63***				
Main root length	Control	0.35	0.16			
	Drought	0.20	0.24			
Root volume	Control	0.36	0.75***	0.31		
	Drought	0.10	0.23	0.51**		
Fresh weight of shoot	Control	0.74***	0.87***	0.17	0.68***	
	Drought	0.40	0.63***	0.19	0.58**	
Fresh weight of root	Control	0.26	0.66***	0.23	0.83***	0.73***
	Drought	0.13	0.31	0.49*	0.94***	0.68***

*, **, *** - significant at p=0.05, 0.01 and 0.001, respectively.

Genotypes with greater plant height are often larger in overall plant size, intercept more light and use water faster by transpiration, leading to lower plant water status, higher leaf death scores and more spikelet sterility. Thus, height of plant or respectively stem biomass is negatively related to harvest index (Edmeades et al., 1999). In our case, the correlation between height of plant and yield was lower and not significant for unstressed plants but under drought conditions a positive correlation (r = 0.53**) was registered (Table 3).

Table 3. The correlation coefficients between studied traits and maize yield

Trait	Growing conditions	Yield	
		Control	Drought
Height of plant	Control	0.063	
	Drought		0.53**
Leaf area	Control	0.18	
	Drought		0.40
Main root length	Control	0.43*	
	Drought		0.53**
Root volume	Control	0.33	
	Drought		0.43*
Fresh weight of shoot	Control	-0.05	
	Drought		0.20
Fresh weight of root	Control	-0.008	
	Drought		0.39

The performance of hybrids under water stress is correlated with main root length and root volume. This means that a greater depth and extent of soil water extraction could increase the amount of water absorbed, and avoid of water deficits at critical growth stages (r = 0.53**, r = 0.43*) (Table 3). Main root length and root volume, easy to measure, are highly positively correlated with yield in both stress levels and consequently could constitute root bioassay candidates for correlated traits with grain yield potential in both stress levels. Data are still limited, new and more extensive study being necessary to confirm the validity of these correlated simple traits, such as root length and root volume.

Comparative evaluation of maize genotypes studied

Height of plant, leaf area and fresh weight of shoot

Under optimum conditions, the height of plants varied between 222 and 313 mm (genotypes F 445 M and HSF 344-12), while plant height under drought treatment decreased drastically, maximum height being of 202 mm (genotype HSF 474-11). We can appreciate performance of hybrids Milcov, Oituz and other seven genotypes tested, in terms of plant growth (as measured by height) under optimum moisture (very significant from the overall average) and under drought stress two of the analysed genotypes (HSF 474-11, HSF 128-09) had a satisfactory behaviour (Table 4).

Table 4. The effect of water stress on height, leaf area and fresh weight of shoot for maize genotypes

Genotype	Height of plants (mm)			Leaf area (cm^2)			Fresh weight of shoot (g)			PFSI
	Ct	Dt	% reduction	Ct	Dt	% reduction	Ct	Dt	% reduction	Rank
Milcov	277	180	35	3200	1086	66	4.88	2.29	53	5
Oituz	287	187	34	2761	1048	62	4.16	2.1	49	11
F 475 M	222	172	22	2361	977	58	3.85	1.98	35	4
F 376	251	146	41	2368	634	73	4.49	1.98	55	10
HSF 128-09	236	196	16	1978	733	62	3.16	1.73	45	12
HSF 160-11	261	178	31	2796	885	68	4.6	1.81	60	13
HSF 2809-11	234	173	34	2124	745	64	3.3	2.08	36	18
HSF 474-11	303	202	33	2796	1461	47	4.44	2.84	36	8
HSF 31-11	304	189	37	2694	868	67	4.64	2.26	51	9
HSF 465-11	286	197	31	1509	978	39	3.18	1.42	44	14
HSF 21-11	244	183	25	1509	978	35	2.89	1.93	33	2
HSF 170-11	255	170	33	2330	841	63	3.55	2.04	42	1
HSF 265-11	236	189	19	2216	1049	52	3.65	2.26^0	38	6
HSF 334-12	293	188	35	2613	1077	58	4	2.21	44	7
HSF 344 -12	313	173	44	2985	796	73	5.88	2.15	63	17
HSF 417-12	265	167	36	2030	749	63	3.24	1.64	49	3
HSF 418-12	287	155	45	2420	875	63	4.24	1.61	52	15
HSF 495-12	253	146	58	1187	654	44	2.16	1.4	35	16
Average	***267***	***177***	***32***	***2348***	***909***	***60***	***4.88***	***2.29***	***47***	
LSD5%	***20.94***			***346***			***0.23***			

Ct = control; Dt = drought.

The reduction of plant height to an optimal range could increase yield potential and simultaneously reduce the risk of lodging (Araus et al., 2008). The reduction of plant height could also lead to enhanced partitioning towards the growing ear, which in turn might result in increasing number of kernels per ear. This explains why hybrid F 376, considered resistant to water stress was among genotypes that showed a clear reduction in height due to the action of water stress.

Our results are consistent with those of El Neomani et al. (1990) who showed that water stress occurred during early stage of growth decreases dramatically height of the maize although dry matter production depends on the levels of photo-assimilated products. Hogenboom et al. (1987) also noted the negative effect of drought on plant height by reducing photosynthesis and, consequently, reduce the length of internodes.

Genotypes Milcov, Oituz, HSF 474-11 could be highlighted with a large leaf area (above average) in both conditions. These genotypes were superior in terms of biomass accumulation (Table 4).

The fraction of radiation intercepted by the canopy averaged across crop cycle can be increased by selecting genotypes that reach full soil cover early in the growing season (Araus, 2002). Leaf area maintenance would improve yield stability during drought stress due to better radiation interception when water is available.

Water stress significantly reduced the accumulation of biomass from aerial part in all maize genotypes studied (Table 4)

Short-term water stress effects at the beginning of intensive vegetative growth stage on biomass have also been reported by other authors (Salvador and Pearce, 1995), and could be explained by a decline in plant extension growth, delayed leaf tip emergence and limited leaf size.

Length, volume and fresh weight of roots

Most root traits are controlled by multiple genes, each governing small effects and often with a degree of epistasis or interaction effects that can change with environmental conditions (De Dorlodot et al., 2007; Cooper et al., 2009). Reducing the length of root due to exposure to water stress may be due to cessation of cell division and roots elongation.

Our results are consistent with studies showing that most often root growth is less inhibited in the water stress conditions compared with the aerial part (Sharp et al., 1988, Kramer and Boyer, 1995).

In our case aerial part was inhibited by an average of 32%, while the root was by only 20% (Table 5). The explanation is the fact that maize root continues to grow at levels of water potential which completely inhibits growth of the aerial part. It was suggested that root growth in maize can be maintained under low water potential conditions by the increased elasticity of the cell wall (Wu et al. 1994).

Studies have shown that the capacity of elongation was maintained only on cells located within millimetres from the apex, then gradually as the distance from the tip of the root, was inhibited, resulting however a decrease in area pieces (Sharp et al., 1988).

The average length of the main root of maize plants under water stress was smaller by 63 mm compared to the length of the roots of the plants grown carried out under optimum conditions (Table 5).

Oituz, F 376 and HSF 495-12 genotypes performed better than HSF 2809-11, HSF 21-11, HSF 265-11 and HSF 344-12, whereas F 475 M and HSF 170-11 genotypes were associated with the highest primary root length in water stress conditions (Table 5).

Highest root volume was shown by F 376, both under normal conditions (8.5 cm^3) and drought condition – induced with 10% PEG solution (7.5 cm^3) (Table 5), indicating its ability to explore a greater soil volume (density of roots) and that roots are thicker as shown in figure 1. By comparison HSF 465-11 genotype had the lowest volume of the root system (Figure 2).

Previous experiments concerning drought tolerance under field conditions displayed a net superiority of F 376 as compared with other hybrids (Mitu, 2003).

Under normal conditions, highest fresh root weight was shown by F 376 followed by other seven genotypes. The best performance regarding fresh root weight under water stress was achieved by Milcov, F 376, F 475 M, HSF 31-11, HSF 474-11 and HSF 265-11 (>5.5 g/plant). The lowest fresh root weight was registered by HSF 495-12, HSF 21-11, HSF 465-11 and HSF 2809-11 under both conditions. Similarly, Avramova et al. (2016) showed that analysis of root growth in rhizotrons under drought conditions revealed a strong reduction in total root length, as well as rooting depth and width. This was reflected by corresponding decreases in fresh and dry weight of the root system.

Moreover the authors showed that phenotypic differences between lines with different geographic origin (African vs. European) and in drought tolerance under field conditions could already be identified at the seedling stage by measurements of total root length and shoot dry weight of the plants.

Table 5. The effect of water stress on length, volume and fresh weight of roots

Genotype	Length of main root (mm)			Root volume (cm^3)			Fresh weight of root (g)			PFSI
	Ct*	Dt*	± (% to ct)	Ct	Dt	± (% to ct)	Ct	Dt	± (% to ct)	Rank
Milcov	337	261	- 23	6.5	6	- 8	5.52	6.03	+ 9	5
Oituz	398	289	- 27	6.0	4.5	- 25	4.6	4.2	- 9	11
F 475 M	391	380	- 3	6	6	0	5.47	5.18	- 5	4
F 376	327	298	- 9	8.5	7.5	- 12	6.09	5.68	- 7	10
HSF 128-09	316	273	- 14	6	5	- 17	4.25	4.12	- 3	12
HSF 160-11	459	296	- 36	7.8	5	- 36	6	4.58	- 24	13
HSF 2809-11	288	195	- 32	3	2.5	- 17	3.77	2.92	- 23	18
HSF 474-11	289	265	- 8	7	7	0	*5.07*	*5.42*	+ 7	8
HSF 31-11	342	270	- 21	5.5	7	+ 27	5.98	5.82	- 3	9
HSF 465-11	385	271	- 30	3	2	- 33	2.87	2.22	- 23	14
HSF 21-11	234	170	- 27	4	2.9	- 28	3.56	3.45	- 3	2
HSF 170-11	404	382	- 5	6	5.5	- 8	5.26	4.78	- 9	1
HSF 265-11	336	244	- 27	5.5	6.6	+20	4.49	5.66	+ 26	6
HSF 334-12	306	296	- 3	5	2.5	- 50	4.12	3.6	- 13	7
HSF 344 -12	324	240	- 26	6.5	5	- 23	5.45	4.5	- 17	17
HSF 417-12	359	329	- 8	4.5	4.5	0	3.88	4.05	+ 4	3
HSF 418-12	349	266	- 24	4.5	4.1	- 9	4.92	3.25	- 34	15
HSF 495-12	355	334	- 6	2.9	2	- 31	3.98	2.69	- 32	16
Average	344	281	- 18	5.5	4.7	- 15	4.73	4.34	- 9	
LSD5%	24.74			0.84			0.22			

Ct = control, Dt = drought.

Figure 1. F 376 genotype
Highest volume root under both conditions

Figure 2. HSF 465–11 genotype
Lowest volume root (adventitious roots few and thin)

Differences in rooting patterns change the amount and timing of water availability to the crop. Greater depth and extent of soil water extraction could increase the amount of water transpired; if this results in the avoidance of water deficits at critical growth stages, it would increase harvest index.

CONCLUSIONS

The results reported in this paper underlined the genetic variability of the investigated traits, especially in drought treatment. Differences in physiological and morphological characteristics of the seedlings seemed to be related to drought tolerance and could be used for large scale screening of maize breeding material, or as an additional screening measure for drought tolerant hybrid selection.

The accumulation of biomass, plant height, root system volume and length of main root would be recommended as physiological traits for a preliminary characterization of breeding material.

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