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THE STEM GROWTH MEASURED IN SEEDLINGS AFTER 20% PEG TREATMENT 15 DAYS AFTER SOWING IS SIGNIFICANTLY CORRELED WITH FIELD RESPONSE TO DROUGHT IN THE FIELD

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ABSTRACT

The extreme variability of climatic conditions, which is a particularly important characteristic of Romania's climate, poses difficult problems not only to agricultural production, but also to breeding programs, including wheat breeding. The alternation of dry years with rainy years, as well as the different moments in which rains occur, make it impossible to carry out a continuous selection pressure for a superior behaviour of the genetic material under water stress. Therefore, identifying indirect methods that would allow characterization of breeding material in all years, regardless of weather, could significantly contribute to accelerating genetic advancement towards future varieties with superior performance under water stress. At Simnic, an experiment was performed in the laboratory using the PEG 6000 solution at 15% and 20%. We determined: stem length at three different moments. For 13 varieties that were field tested for the period (2002-2015), the correlation between the YI-specific drought tolerance index calculated from yield data from the field and determinations under PEG-induced drought revealed a significant positive correlation between drought tolerance index and the ratio of the stem measured after treatment with 20% PEG and that measured in the control (treated with water), at the first moment of determination (15 days after sowing) and in average over all moments.

Key words: wheat, drought, stem length, yield index YI, polyethylene glycol (PEG) treatment.

INTRODUCTION

Drought is probably the most important abiotic stress factor limiting crop growth and crop productivity globally, but also in Romania, especially in the south, east and southeast. Drought, heat, low temperatures, insects and diseases are considered to be the consequences of climate change most relevant to agriculture, and breeding can reduce their impact by improving water efficiency and high temperature tolerance, adapting the vegetation period, disease and insects resistance.

Among the factors which affect wheat crop, drought, especially lack of adequate humidity for yield formation, has a significant limiting effect can overcome the negative impact of all other cumulative abiotic factors (Barnabas et al., 2008). In a reference study on drought in Romania, Șișești quantified its size by specifying that it include time intervals of at least 10 days without rains in summer and at least 14 days without precipitation in winter (Sin and Popescu, 2015). Drought accompanied by high temperatures represents a major danger for the yields formation, Romania registering changes in the thermal regime in consensus with the global context of climate change (Sandu and Mateescu, 2014). The same authors specify that, without having a very rigorous cyclical character, in the previous century the drought phenomenon occurred generally at intervals of 10-15 years.

Plant breeding can contribute to reducing the impact of drought, by focusing its efforts on obtaining genetic material characterized by increased water and nutrient utilization Among the proposed indirect methods for examining differences in water stress response among genotypes, the exposure of plantlets to modified osmotic potential by the addition of polyethylene glycol (PEG) was widely used (Andersen et al., 1987; Petcu et al., 2007; Munns et al., 2010; Guo et al., 2013; Chachar et al., 2016). PEG may be used to alter the osmotic potential of the nutritive solution and can induce in plant a

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water deficit under relatively controlled conditions.

Our research aimed to contribute to the acceleration of genetic advancement in the creation of more drought-resistant wheat varieties by identifying indirect methods that would allow the characterization of the breeding material in all years, regardless of the weather and the identification of possible sources of genes for drought tolerance. Among the ways to reduce drought impact on agricultural production in general and wheat yield in particular, the creation of new varieties capable of delivering less water stress-affected productions is a considered among the most important.

MATERIALS AND METHODS

At Simnic, the experiment was performed in the laboratory using the PEG 6000 solution at 15% and 20%. We determined: stem length at three different moments (T1 = 15 days from sowing date, T2 = 24 days from sowing date and T3 = 35 days from sowing date.

Thirteen wheat varieties of various origins were tested in the laboratory to detect differences for the ratio of stem growth under stress and growth in control in terms of their length.

Sowing was done in pots containing the same amount of soil. Eighteen seedlings of each wheat genotype of control (water) were transferred to plastic pots each with 6 plants and introduced into the Sanyo Growth Chamber, previously adjusted to the temperature, light and atmospheric humidity parameters for the proper growth of wheat plants.

The following experimental variants were established:

- control: the plants were maintained in optimal conditions all during the experiment;
- treatment 1: plants were treated during 35 days with polyethylene glycol (PEG) at a concentration of 15%;
- treatment 2: plants were treated during 35 days with polyethylene glycol (PEG) at a concentration of 20%.

Stem length was measured at 3 different times: 25 november (15 days after sowing), 4 december (24 days after sowing) and 15 december (35 days after sowing).

The 13 varieties were also field tested for the period 2002-2015, and the YI-specific drought tolerance index was calculated from yield data from the field, taking into account the average yield of years with the most severe drought 2002 and 2003 as Ys.

Yield index (Gavuzzi et al., 1997)

$YI = \frac{Ys}{\overline{Ys}}$ best reflects the behavior under stress conditions, compared to the average of all varieties. It is not influenced by other conditions and therefore seems the most adequate to characterize the ability of indirect methods to describe the drought resistance.

The correlations between the YI-specific drought tolerance index and determinations under PEG-induced drought were calculated.

Weather conditions during 2002-2015 at ARDS Simnic

At Simnic, during the period 1957-2015, annual rainfall varied greatly from year to year, showing first a decreasing and then a spectacular increase in recent years. In order to correlate the results of laboratory tests with behaviour in water stress conditions in the field, we analyzed the climatic conditions during the period 2002-2015.

The presentation of the pluviometric regime for these years was based on the Angot Index, which shows the more or less rainy feature of a month in relation to the overall precipitations in one year. Coefficient can be higher or lower than 1. The index is calculated as follows:

For months with 31 days (31/365 = 0.085)
$q = 11.76\ p/P$;
For months with 30 days (30/365 = 0.082)
$q = 12.19\ p/P$;
For months with 28 days (28/365 = 0.077)
$q = 12.99\ p/P$;
where p = monthly precipitations and P = the precipitations of one year.

The major advantage of this coefficient is that it shows how the month is by point of view pluviometric in relation to the annual average of that location**.** A month when 60 mm rainfalls were registered in a location 2000 mm per year is considered arid, but it is wet in a location with an average of 400 mm.

In our case, the Angot index was modified in the sense that P was considered to be the 57-year multiannual average (fixed amount of 565.1 mm) and not the rainfall in that particular year, because we wanted to highlight how was the month studied in relation to the general climate characterization of the area, with a base period of 14 years.

In summary, the situation is (Table 1):

Table 1. The frequency of the months according to the drought susceptibility classes

Susceptibily clasees	Angot index	Absolute frequency (months number)	Relative frequency (%)
Very dry months	under 0,99	72	57,2
Dry months	1-1,49	23	18,2
Moderate months	1,5-1,99	11	8,7
Wet months	2-2,49	9	7,2
Very wet months	> 2,5	11	8,7
Total		126	100

Based on the Angot Index, the dry and very dry months in this study accounted for 75.4% of the total number of analyzed months.

RESULTS AND DISCUSSION

I. Variation of wheat yields during the period 2002-2015 at ARDS Simnic

Climate conditions are integrated by wheat plants all growing season, so it can be said that grain yields best describe the favorability of these conditions.

Yield was determined by harvesting 3 m^2 of each experimental plot and then calculating at standard humidity of 14%.

In period 2002-2015 the yield varied very much, the average for the varieties tested in all years ranging from 381 kg / ha in 2002 to 5992 kg / ha in 2005 (Table 2).

Lower yields were associated with drought conditions in years like 2002 and 2003, but also with excessive rainfall, for example in 2014.

Also interesting is the analysis of the correlations between the yields obtained in different years by the varieties tested during the whole period 2002-2015 (Table 3).

Correlations varied, from very significant positive (for example between yields in the dry years 2002 and 2003, but also between those years and years that were on average more favorable) to even significant negative correlations (for example between the yields obtained in 2002 or 2003 with those from 2008). This illustrates the extreme variability of the climate conditions in Şimnic during the study period.

It is interesting to note that the average yield of 2002 and 2003 was closely correlated with the average yield over the whole period 2002-2015. This was another argument to consider the average of 2002 and 2003 yield for characterization of field behaviour in drought conditions.

Table 2. The average yields obtained during the period 2002-2015

Variety/ year	2002	2003	2002+ 2003 average	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2002-2015 average
Glosa	427	3460	1944	6080	6820	5223	3329	3185	5698	5190	4648	4400	2043	1557	5155	4087
Alex	455	2510	1483	5620	6960	4533	3507	3970	5554	5815	4590	4091	2710	1839	4149	4022
Izvor	524	3260	1892	6730	5840	4400	2868	3178	5621	4948	4523	4471	2931	995	4668	3926
Gruia	537	3250	1894	7670	5670	4632	3100	2964	5214	6027	4048	4417	2812	764	2748	3847
Crina	391	2700	1546	6430	6130	5043	3141	3598	5069	5835	3835	3628	3393	1216	2998	3815
Faur	700	2880	1790	6160	6000	5051	2448	2424	5535	4439	4263	4215	2820	1040	4748	3766
Delabrad	385	3080	1733	5410	5730	5375	2712	4306	5785	4394	4227	3605	2441	1159	3966	3755
Boema	358	2270	1314	5160	5500	4885	3086	4241	4989	4796	4112	4041	2516	1988	4365	3736
Simnic 30	309	2470	1390	5650	6560	4378	2489	4224	5235	4345	3779	4025	2897	1756	3298	3673
Dropia	391	2530	1461	5440	5440	4271	2905	3579	5231	4857	3962	4021	3349	1229	3626	3631
Romulus	113	2370	1242	5270	6690	4579	3036	3938	4878	5040	3479	3589	2280	924	2874	3504
Lovrin 34	237	2100	1168	5440	5550	4463	2239	3955	5400	5484	3587	3995	2432	724	3108	3480
Bezostaia 1	130	2520	1325	4860	5000	4552	2503	3553	5551	4340	3726	3795	2760	1434	3787	3465
13 varieties average	381	2723	1552	5840	5992	4722	2874	3624	5366	5039	4060	4022	2722	1279	3807	3747

Table 3. Analysis of the relationships between the yields obtained in different years in the varieties tested during the period 2002-2015

Year average yield	2002	2003	2002+ 2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Average
	381	2723	1552	5840	5992	4722	2874	3624	5366	5039	4060	4022	2722	1279	3807	3747
2002	1															
2003	0.59	1														
2002+2003	0.77	0.97	1													
2004	0.67	0.68	0.74	1												
2005	0.11	0.15	0.16	0.12	1											
2006	0.28	0.45	0.44	0.07	0.13	1										
2007	0,18	0.31	0.3	0.26	0.52	0.18	1									
2008	-0.65	-0.58	-0.65	-0.63	0.06	-0.1	0.01	1								
2009	0.34	0.54	0.53	0.04	-0.02	0.31	-0.15	-0.24	1							
2010	0.16	0.09	0.12	0.55	0.27	-0.08	0.57	-0.13	-0.24	1						
2011	0.7	0.67	0.74	0.34	0.31	0.35	0.51	-0.33	0.63	0.1	1					
2012	0.64	0.52	0.61	0.6	0.08	-0.18	0.16	-0.59	0.32	0.2	0.62	1				
2013	0.26	-0.09	0.01	0.26	-0.3	-0.36	-0.06	-0.18	-0.23	0.1	-0.13	-0.05	1			
2014	-0.07	-0.2	-0.18	-0.43	0.27	0.08	0.36	0.42	-0.01	-0.23	0.33	-0.01	-0.06	1		
2015	0.47	0.43	0.49	-0.08	0.13	0.38	0.16	-0.31	0.62	-0.36	0.8	0.48	-0.29	0.41	1	
2002-2015 average yield	0.68	0.7	0.76	0.55	0.53	0.37	0.69	-0.31	0.4	0.38	0.9	0.58	-0.06	0.3	0.56	1

II. Rainfall – yield relationship during the period 2002-2015 at ARDS Șimnic

A strong correlation between the average yield of varieties tested throughout the period and the average Angot Index (r = 0.8885) was found for the period 2002-2009, which justifies its relevance and represents a good way to form a referance basis for the performance in the field (Figure 1).

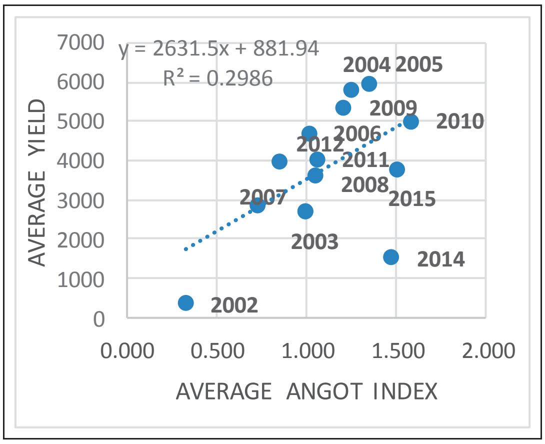

Figure 1. The relationship between average yield of the varieties tested in the period 2002-2015 and the average Angot index

The correlation is much more pronounced for the period 2002-2009 when the variability of the average Angot index explains 88% of the variability of average yield (Figure 2).

When the excess rainfall occurred (2010-2015) the correlation did not exist (Figure 3).

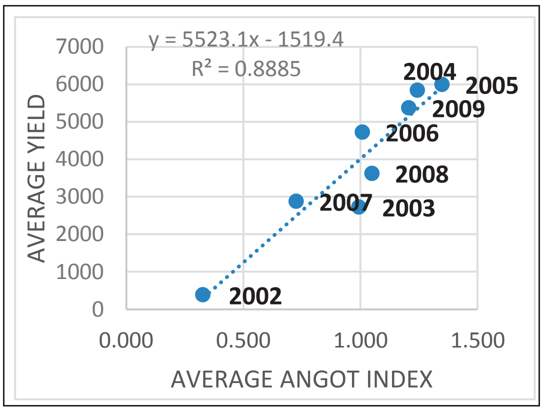

Figure 2. The relationship between average Angot index and the average yield of the varieties tested in the period 2002-2009

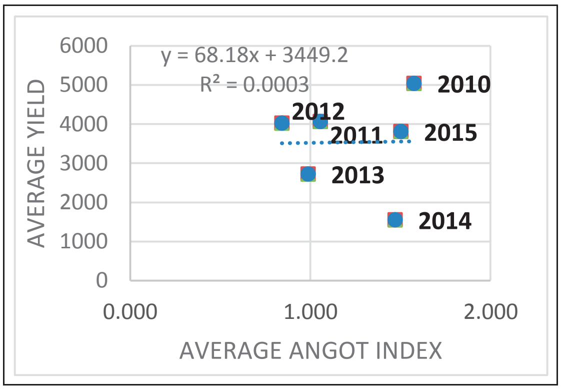

Figure 3. The relationship between average Angot index and the average yield of the varieties tested in the period 2010-2015

Taking into account the average Angot index only for the months of March - June, very important months in the development of wheat crops, there was a strong correlation with yield, but only for the period 2002-2009 (r = 0.746) (Figure 4)

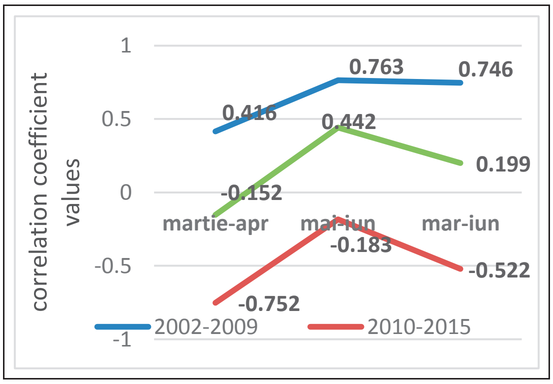

Figure 4. The value of the correlation coefficients between the average Angot index and average yield for different vegetation and testing periods

The same Angot index, for March-April month interval is significantly negatively correlated with yield for the period 2010-2015 (r = -0.752).

The correlation is more pronounced for May-June month interval, corresponding to the ear emergence and filling grain phases, for the period 2002-2009 when the variability of the Angot index explained 58% of the variability of average yield. For the period 2002-2015, for the same months, the correlation coefficient was 0.442 not significant.

When the excess rainfall occurred (May and June 2010-2015), the correlation diminished considerably, becoming not significant, with a negative trend, with a coefficient of determination of only 3.3%.

On the basis of these data, the conclusion was that the average of the years 2002 + 2003 may best represent the drought conditions. Studies previously conducted at Simnic, based on the climatic data recorded during 1957-1993, showed that the yield increases with the increase of rainfall up to 582 mm during the vegetation period, after which the yield decreases (Păunescu et al., 1994). This aspect was also evidenced by polynomial relationship calculated based on our present data. In the last years (2010-2015), as the amount of precipitation reached 600 mm, the precipitation-yield relationship was not as conclusive as the one for the period 2002-2009 (Figure 5).

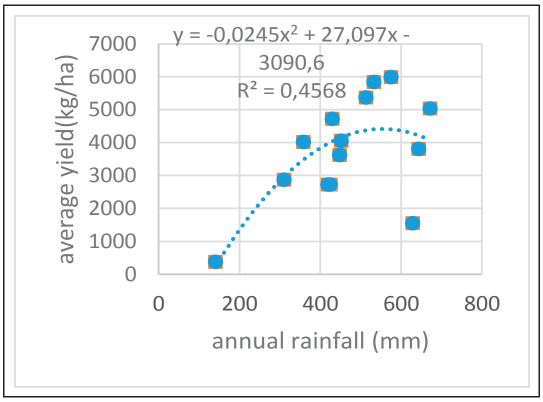

Figure 5. The rainfall - yield relationship for the cultivars tested during 2002-2015

III. Analisys of stress susceptibility index

Based on the analysis of several drought sensitivity indices proposed in scientific literature (data not shown), taking into account our objective to establish a classification of cultivars in terms of the water stress response in the field, on the basis of which to appreciate the utility laboratory measurements, we focused on the Yield Index suggested by Gavuzzi et al. (1997).

We considered this index, which reflects the relative behaviour under stress conditions, reported to the average behaviour of all varieties, without reference to the optimal yield potential, as the most appropriate as a reference point for analysing the utility of indirect methods for drought resistance. According to this index, the ranking of the varieties tested during the whole period 2002-2015 is presented in Table 4.

Table 4. Classification of cultivars according to the yield index (YI) in drought conditions

Cultivars	YI
Glosa	1.252
Gruia	1.220
Izvor	1.219
Faur	1.153
Delabrad	1.116
Crina	0.996
Alex	0.955
Dropia	0.941
Simnic 30	0.895
Bezostaia 1	0.854
Boema	0.847
Romulus	0.800
Lovrin 34	0.753

Our researches focused on the identification of indirect methods, which could be appliede in the absence of water stress in the field, and would allow a ranking of varieties similar to that obtained in yield trials under conditions of severe drought.

IV. Variability of stem length and relationships with yield index YI

For the 13 varieties tested during 2002-2015, the correlation between the YI specific drought tolerance index calculated from field behavior and PEG-induced laboratory determinations in different concentrations and at different times of application revealed that there was a significant positive correlation of the tolerance index with the ratio between the stem measured on treatment with 20% PEG and that measured on the control (water treatment) at the first application time and on average (Table 5). Thus, the high value of the specific drought tolerance index YI (a variety with relatively higher yields under drought conditions) corresponds to a high value of the ratio (so the stem growth is not inhibited by PEG treatment which induce drought).

Relatively high values, but not-significant, of the correlation between YI and the ratio of the stem measured on 15% PEG treatment to that measured at the control (water treatment) at the first application time and on average, were also recorded.

Table 5. The correlation of the YI index with the ratio of stem length to treatment with PEG 15% and 20% and the stem length on water treatment

Cultivar	YI	Ratio stem length PEG 15%/ stem length control				Ratio stem length PEG 20%/ stem length control			
		T1 25 Nov.	T2 04 Dec.	T3 15 Dec.	Average	T1 25 Nov.	T2 04 Dec.	T3 15 Dec.	Average
Glosa	1.252	1.251	1.031	0.888	1.057	1.08	0.987	0.856	0.974
Gruia	1.220	1.523	1.184	0.95	1.219	1.506	1.055	0.794	1.118
Izvor	1.219	1.151	1.167	0.985	1.1	1.135	1.228	0,971	1.111
Faur	1.153	0.988	0.972	0.921	0.96	1.005	0.984	0.929	0.973
Delabrad	1.116	0.795	0.836	0.843	0.825	0.931	0.862	0.895	0.896
Crina	0.996	0.877	1.07	1.063	1.003	1.075	0.937	0,794	0.935
Alex	0.955	1.105	0.941	0.824	0.956	1.02	0.849	0.76	0.876
Dropia	0.941	0.852	0.848	0.798	0.833	0.927	0.957	0.789	0.891
Şimnic 30	0.895	0.911	0.803	1.037	0.917	0.918	0.95	0.96	0.943
Bezostaia 1	0.854	0.838	0.986	0.787	0.87	0.992	0.913	0.884	0.93
Boema	0.847	1.006	0.879	0.785	0.89	0.954	0.756	0.722	0.811
Romulus	0.800	0.802	1.15	1.139	1.03	0.846	1.049	1.05	0.981
Lovrin 34	0.753	1.022	0.924	0.763	0.903	0.958	0.853	0.621	0.81
Correlations with YI		0.592	0.384	0.150	0.562	0.632*	0.538	0.250	0.693*

CONCLUSIONS

The ratio between the stem measured after 20% PEG treatment and that measured in the control (water treatment) 15 days after sowing, as well as on average on three determinations (15, 24 and 35 days from sowing) was identified as the best indicator for drought tolerance selection.

These ratios showed the best correlation with YI, calculated on the basis of field behavior of the 13 varieties tested during the period 2002-2015.

The cultivars Izvor, Gruia and Glosa showed high values of the ratio between the stem measured after 20% PEG treatment and that measured at the control (water treatment) 15 days after sowing.

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