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To cite this article: W. A. E. Abido *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1262** 052055

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## Polyethylene Glycol (PEG) Induced Changes in Germination, Seedling Growth, Water Relation and $\alpha$ - $\beta$ Amylase of some Hungarian Wheat Landraces Varieties

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**Abstract.** Wheat productivity has been steadily declining over the past few years, and abiotic stresses are responsible for more than half of all yield losses. Stress from drought is one of the most significant factors that can impede plant growth and production wherever in the world. The main goal of this study was to examine how low water potential, which was caused by polyethylene glycol (PEG 6000), on the germination and biochemical parameters of Hungarian wheat landraces. Seven Hungarian wheat landraces varieties (Szentesi; Nyirádi; Kiszombori; Háromfai; Tapiószelei; Nagykállói, and Szajlai) were tested for germination characteristics; seedling parameters; water relative content; tolerance index; and enzyme activities in the presence of different five concentrations of water stress (0; 5; 10; 15 and 20%) of polyethylene glycol (PEG 6000). At the Research Institute of Nyiregyhaza in Hungary, a laboratory experiment using Factorial Experiment in Randomized Completely Design (RCD) was carried out in four replicates. According to the data, the Háromfai landrace outperformed all other landraces under research and recorded the highest values of all analyzed features, followed by Tapiószelei; Kiszombori; Szajlai; Nyirádi; Szentesi and Nagykállói landraces varieties. All characteristics under study were significantly decreased as water stress concentrations (PEG 6000) were increased from 0 up to 20%. In comparison to other wheat landraces, Háromfai and Tapiószelei landraces varieties had the highest water relative content (WRC), tolerance index (TI), and  $\alpha$  as well  $\beta$ -amylase activities, and were able to induce better drought tolerance. These results suggest that these landraces could be a good resource for breeding programs and cultivation under drought stress conditions. Furthermore, the results of correlation analysis did not neither positive nor negative correlation between the Hungarian wheat landraces varieties and all of analyzed traits. On contrary, the correlation analysis results show negative correlation between the drought stress levels and studied traits.

**Keywords.** Wheat, PEG, Germination, Enzyme activity.



## 1. Introduction

The global phenomenon of climate change and the limited availability of water resources in the natural environment exert a substantial influence on the productivity of wheat crops [1]. In several regions of the world, wheat (*Triticum spp* L.) is regarded as the primary cereal crop [2]. Hungary produced around 5.12 million tonnes resulted from 936620 hectares (ha) of wheat cultivated area, while the total cultivated area in the world reached 219.01 million ha with total production 760.93 million tonnes during 2020 growing season, according to the [3]. According to recent estimations, over 25% of the world's agricultural lands are now affected by and suffering from water stress, which has a significant impact on the growth of wheat from the germination phase to yield production [4]. The current global of weather variation, the increase in temperature, and changing precipitation, have made this situation more serious, especially for the wheat crop production [5,6]. In most agricultural fields around the world, drought is one of the environmental factors that puts the most strain on the environment and significantly affects morphological, physiological, molecular, and biochemical processes to inhibit its growth as well reduces crop production [7,8, 9]. One of the most important stages in the plant's life cycle is germination. Furthermore, seed germination and the early phase of seedling development are essential stages for plant establishment under stress circumstances [10]. The increases of water stress levels decreased germination percentage, decreased enzyme activity, which had a detrimental impact on glucose metabolism, prevented water absorption, decreased calcium and potassium requirements and cell elongation, membrane structure and function as well altered hormones that are released by seeds during the germination period [11,12,13,14]. The process of enzymatic reactions, development of roots and shoots, transit of metabolites, hydrolysis of proteins, lipids, and carbohydrates in the storage tissues of germinating seeds all depend on the availability of water [15,16]. According to the results detected by [17]. Hungarian wheat landraces had the best behavior and were able to improve drought resistance under water stress in most of the studied characteristics.

Multiple techniques were developed and evaluated within a controlled laboratory setting to create water stress in plants. The objective was to assess the drought tolerance of wheat plants by employing different chemicals, including polyethylene glycol (PEG) [18]. PEG 6000 is commonly employed as a means to simulate and induce drought stress in plants. This compound is a non-ionic water-soluble polymer that lacks the ability to penetrate seeds or cells. Its utility lies in its capacity to effectively impose, experimentally investigate, and evaluate the drought resistance traits of plants during the initial stages of seedling development, both in controlled laboratory settings and in natural field conditions [19,20]. The germination rate, vigor index, seedling characteristics, water relative content, tolerance index, and  $\alpha$  &  $\beta$ -amylase activities of wheat cultivars were considerably reduced by 20 to 25% water stress levels induced by PEG 6000 (references 21 and 22).

Concerning to the interaction amongst factors under study, earlier studies on identifying drought-tolerant wheat cultivars using varied levels of PEG 6000 revealed substantial changes in germination and seedling characteristics [23,24]. Water stress levels (PEG 6000) has a favorable impact on the germination parameters, seedling characteristics, water relative content, tolerance index, and  $\alpha$  &  $\beta$ -amylase activities of wheat landraces. Lewucei and Mateteleki landraces had the best behavior and were able to improve drought resistance under the highest concentrations of water stress (24% of PEG 6000) in most of the studied characteristics [25,26]. In these circumstances, the main goal of the current inquiry was to evaluate the reaction of seven Hungarian wheat landraces under drought stress generated by PEG 6000 in order to identify drought resistant wheat landraces that may be employed in a breeding program for maximize germination, seedlings and biochemical parameters of Hungarian wheat landraces under drought stress conditions. This research might be used to demonstrate that applied sciences are extremely important in life due to their numerous applications in the present and past [27,28,29,30,31,32].

## 2. Materials and Methods

### 2.1. Site Description and Experimental Design and Treatments

A controlled laboratory experiment was conducted in October 2022 at Research Institute of Nyiregyhaza, Hungary, in a phytotron at a temperature of  $20 \pm 1$  °C and relative humidity 70% for 18/6

h day/night lighting for germination. Treatments were assigned in a Factorial Experiment with four replicates based on a Randomized Completely Design (RCD). The 1<sup>st</sup> factor comprised seven Hungarian wheat landraces (Szentesi; Nyirádi; Kiszombori; Háromfai; Tapiószelei; Nagykállói, and Szajlai). Hungarian wheat landraces were developed by using simple selection methods for local adaptation and a wide variety of quality traits. The 2<sup>nd</sup> factor consisted of five imitation drought stress concentrations *i.e.*, 0; 5; 10; 15 and 20%. Drought stress was induced by polyethylene glycol (PEG 6000) according to [33]. 25<sup>th</sup> of healthy uniformed seeds of each Hungarian landrace were allowed to germinate on a filter paper into plastic Tarson Aseptic Petri dishes. Before beginning the experiment, the seeds of Hungarian wheat landraces under study were sterilized with 0.1% of HgCl<sub>2</sub> for 1 minute, washed 3 times with distilled water to prevent fungal infection, and air dried at room temperature according to, [34]. All plastic Tarson Aseptic Petri dishes were labeled, moistened with 8 mL distilled water (control) and by PEG-6000 solution at the different artificial water stress concentrations and incubated in a in a phytotron at a temperature of (20 ± 1 °C) and relative humidity 70% for 18-hour day and 6-hour night for germination for fifteen day. The seeds were deemed germinated when the emerging radical protrusion reached about 2 mm in length. According to the International Rules of Seed Testing Association [35]. germination of seeds was documented daily, with the first and final accounts completed after 4 and 8 days, respectively.

### 2.2. Measurements Observations on the Morphological and Biochemical Parameters

The subsequent germination characteristics were assessed: The germination percentage (GP%) was determined by dividing the number of germinated seeds after 8 days by the total number of germinated seeds. The germination speed (GS) was calculated by dividing the number of germinated seeds after 8 days by the total number of days required for germination, as outlined in reference [36].

After 12 days from sowing, 10 seedlings from each treatment were randomly choice to estimate: Shoot and root length (cm); Seedling vigor: was calculated by multiplying (shoot length + root length) in GP% according to, [37].; Shoot fresh and dry weights (g); Root fresh and dry weights (g); Relative water content (RWC) was calculated by using the following equation:  $RWC = (FW - DW) / (TW - DW) \times 100$  as described by [38], Where: FW is the fresh weight of shoots; TW is the weight at all full turgor measured after immersed in double dist. water for 4 hours in the light at room temperature and DW is the weight estimated after drying the shoots at 80 ± 1°C in a hot air oven for 6 hours until a constant weight; Tolerance Index (TI): calculated by dividing the dry weight of seedlings under water stress on the dry weight of seedlings under control treatment [39]. and  $\alpha$  and  $\beta$ -amylase activities: According to the method given by [40].,  $\alpha$  and  $\beta$ -amylase activities were determined by incubating a combination of 3 ml of soluble starch (2% v/v) and 3 ml of extract at 30 C° for about 60 minutes. The alkaline color reagent was made by dissolving 1g of 3,5-dinitrosalicylic acid in a solution of 40 ml 1 N NaOH solution and 30 ml H<sub>2</sub>O after the incubation period. A 1ml incubation mixture was added, then solid potassium sodium tartrate was added and liquefied. The mixture was then finished to a final volume of 100 ml and heated for approximately 5 minutes over a boiling water bath. After preparing the standard curve with various maltose concentrations between 0 and 1.5  $\mu\text{mol L}^{-1}$ , the absorbance at 546 nm was measured in comparison to a blank (1 ml H<sub>2</sub>O plus 1 ml alkaline reagent). However, the prior technique was used to measure  $\beta$ -amylase using amylopectin instead of soluble starch.

### 2.3. Statistical Analysis

All of the presented data are the averages of four separate replicates with standard error (SE). IBM SPSS Statistics v19 was used to do analysis of variance (ANOVA) and averages comparisons using the Duncan's Multiple Range test, (New York, NY, USA) [16]. Pearson's correlation coefficients were also determined using SPSS statistical software (17.0 version; SPSS Inc., Chicago, IL) to assess the link between observations on the morphological and biochemical characteristics of wheat Hungarian landraces.

### 3. Results and Discussion

Analysis of variance (ANOVA) found significant variations for all investigated variables between the Hungarian wheat landrace varieties, PEG levels, and their interaction. Mean square, F. value, and significant \*, \*\*, at  $p=0.05$  and  $0.01$  level, based on an F-test are given in Table 1.

**Table 1.** Analysis of variance for different traits under study.

Source of variance (SOV) Characters	Mean Square, F. value and significant				CV%
	Hungarian wheat landraces varieties "Factor A"	Drought stress "Factor B"	Interaction "A * B"	Error	
	Df	6	4	24	
Germination percentage	761.484	14097.215	65.899	3.626	2.6
	210.001**	3887.712**	18.173**		
Germination rate	0.650	13.998	0.055	0.002	2.2
	266.908**	5749.473**	22.471**		
Shoot length	59.154	935.506	1.311	0.492	4.5
	120.277**	1902.172**	2.665**		
Root length	17.580	159.497	0.369	0.102	3.5
	172.029**	1560.783**	3.607**		
Seedling vigor	216.237	3414.171	2.626	0.910	4.7
	237.580**	3751.146**	2.885**		
Shoot fresh weight	0.009	0.082	0.001	0.001	2.1
	204.404**	1840.516**	12.064**		
Shoot dry weight	0.001	0.017	0.000	0.001	1.9
	274.636**	3492.550**	10.214**		
Root fresh weight	0.001	0.008	0.000	0.001	2.1
	102.540**	869.637**	2.492**		
Root dry weight	0.001	0.011	0.001	0.001	3.4
	338.309**	3528.341**	7.322**		
Relative water content	844.993	3379.745	15.198	7.729	4.4
	109.324**	437.268**	1.966**		
Tolerance index	0.713	0.392	0.007	0.001	3
	751.756**	413.073**	7.271**		
$\alpha$ -amylase activity	264.890	55.392	0.702	0.370	1.7
	716.348**	149.797**	1.898**		
$\beta$ -amylase activity	538.305	15751.615	53.542	2.462	2.2
	218.681**	6398.936**	21.751**		

\*\*, Significant different ( $p < 0.05$ ) using Duncan New Multiple Range Test (DMRT's test).

#### 3.1. Performance of Hungarian Wheat Landraces Varieties

As showed in Tables 2 and 3 there are significant differences among Hungarian wheat landraces varieties for germination, seedling properties, water relation and enzymes activity, except for (GP) between Szentesi and Nagykállói landraces, (root length) between Szentesi and Nyirádi landraces, for (relative water content and  $\beta$ -amylase activity) between Kiszombori and Tapiószelei wheat landraces. Our results indicated that the Háromfai wheat landrace outperformed all other landraces under research and recorded the highest values of all germination, seedling parameters as well as relative water content, followed by Tapiószelei; Kiszombori; Szajlai; Nyirádi; Szentesi and Nagykállói wheat landraces. It can be noticed that Háromfai wheat landrace significantly surpassed other wheat landraces Tapiószelei; Kiszombori; Szajlai; Nyirádi; Szentesi and Nagykállói by (2.79, 10.09, 11.89, 17.18, 21.21 and 21.33%); (5.89, 12.44, 15.21, 20.56, 31.13 and 24.23%); (8.03, 15.81, 20.15, 22.26, 27.87 and 24.01%); (7.86, 19.35, 23.25, 30.41, 39.58 and 35.41%); (4.80, 9.32, 12.71, 15.25, 19.49 and 16.95%), (2.29, 7.63, 11.45, 14.50, 19.85 and 16.03%); (2.29, 7.63, 11.45, 14.50, 19.85 and 16.03%); (7.58, 16.67, 22.73, 27.27, 34.85 and 31.82%); (8.40, 10.06, 13.22, 19.23, 30.53 and 22.95%); (3.18, 8.83, 13.60, 16.16, 19.76 and 17.75%); (4.77, 12.52, 19.56, 25.18, 45.16 and 29.80%)

and (5.34, 9.11, 22.89, 36.99, 38.79 and 36.14%) respectively for GP %, shoot length, root length, seedling vigor, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, relative water content, tolerance index,  $\alpha$  and  $\beta$ -amylase activities. On the other hand, germination rate was decreased by (3.92, 9.40, 13.12, 18.75, 23.73 and 28.81%), respectively for Tapiószelei; Kiszombori; Szajlai; Nyirádi; Szentesi and Nagykállói wheat landraces varieties as compared with Háromfai landrace. The variations between Hungarian wheat landrace types may be attributed to changes in genetic variables and genetic structure. Furthermore, the Hungarian wheat landraces varieties that were significantly tolerant to water stress had major changes in their root system,  $\alpha$  and  $\beta$ -amylase activities and photosynthetic rate as well as efficient utilization of available water.

### 3.2. Effect of Water Stress

Regarding to the influence of water stress (PEG-6000) on germination, seedlings characters, relative water content, tolerance index and activity of  $\alpha$  and  $\beta$ -amylase as showed in Tables 2 and 3. In the present study, all characters under studied significantly affected due to drought stress levels. Most of studied traits were decreased due to increasing water stress up to 20% (PEG-6000), with except mean germination rate increased. The gradual decreases were (5.30, 16.80, 42.55, and 61.81%); (15.21, 34.73, 56.57 and 66.99%); (8.21, 20.11, 39.19, 55.31%); (17.17, 41.06, 71.14 and 85.05%); (6.96, 19.07, 30.15 and 38.92%); (6.76, 16.22, 32.43 and 47.97%); (8.33, 15.48, 21.43 and 29.17%); (10.00, 36.25, 56.25 and 68.75%); (7.31, 18.79, 34.19 and 37.10%); (7.78, 26.62, 48.01 and 65.66%); (4.98, 10.34, 18.27 and 26.67%) and (5.27, 10.70, 15.11 and 18.04%) for germination, shoot length, root length, seedling vigor, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, relative water content, tolerance index,  $\alpha$  and  $\beta$ -amylase activities, respectively as compared with control treatment "0 without drought stress". The reduction in the shoot and root lengths due to the high levels of PEG up to 20% might be due to some disturbance posed by the osmotic stress conditions in cell division, elongation and on membrane structure and function led to inhibits the process of water uptake [14,17]. Furthermore, water availability is crucial for the process of enzymatic reactions, hydrolysis of endosperm starch into metabolizable sugars by amylase enzymes during seed germination is crucial for the formation of roots and shoots [15,25]. Additionally, increased water stress decreased the activity of enzymes like  $\alpha$  and  $\beta$ -amylase, which had detrimental effects on carbohydrate metabolism, prevented water uptake or reduced water potential, decreased calcium and potassium requirements, and altered the activation of enzymes and seed hormones during the germination phases, [41].

#### 3.2.1. Effect of the Interactions

Concerning to the interaction between Hungarian wheat landraces and drought stress levels. As presented in Table 1 of analysis of variance for different traits under study. There is significant interaction showed for germination characters, seedlings characters, water relative content and  $\alpha$  and  $\beta$ -amylase activities. The two Hungarian wheat landraces (Kiszombori and Tapiószelei) performed better and showed maximum average of germination percentage (Fig. 1); germination rate (Fig. 2); shoot length (Fig. 3); root length (Fig. 4); seedlings vigor (Fig. 5); relative water content (Fig. 6); tolerance index (Fig. 7);  $\alpha$ -amylase (Fig. 8) and  $\beta$ -amylase (Fig. 9) under drought stress, followed by Kiszombori; Szajlai; Nyirádi; Szentesi and Nagykállói. Under high levels of drought stress conditions, the relative water content, tolerance index,  $\alpha$ -amylase as well as  $\beta$ -amylase activities are most important indicator of the water status in wheat.

**Table 2.** Germination percentage (GP%); germination rate (GR); shoot and root lengths (cm); seedling vigor (SV); shoot fresh and dry weight (g) averages as impacted by wheat landraces and drought stress as well as their interactions.

Characters Treatments	Germination percentage (GP %)	Germination Rate (GR)	Shoot length (cm)	Root length (cm)	Seedling vigor (SV)	Shoot fresh weight (g)	Shoot dry weight (g)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<i>A. Hungarian wheat landraces performance:</i>							
Szentesi	66.140 f ±27.947	2.562 a ±0.564	14.050 f ±5.677	8.397 e ±2.657	16.979 f ±11.244	0.294 f ±0.065	0.110 f ±0.029
Nyirádi	69.627 e ±26.374	2.362 c ±0.720	14.730 e ±5.887	8.590 e ±2.593	18.294 e ±11.548	0.300 e ±0.066	0.112 e ±0.028
Kiszombori	75.587 c ±23.030	2.176 e ±0.824	16.237 c ±6.462	9.303 c ±2.692	21.202 c ±12.279	0.321 c ±0.059	0.121 c ±0.027
Háromfai	84.069 a ±17.526	1.989 g ±0.895	18.543 a ±6.912	11.050 a ±2.314	26.288 a ±12.313	0.354 a ±0.045	0.131 a ±0.023
Tapiószelei	81.720 b ±20.744	2.067 f ±0.873	17.450 b ±6.535	10.163 b ±2.508	24.222 b ±12.437	0.337 b ±0.050	0.128 b ±0.023
Nagykállói	66.240 f ±28.631	2.461 b ±0.647	12.770 g ±5.431	7.970 f ±2.793	15.884 g ±11.051	0.285 g ±0.064	0.105 g ±0.030
Szajlai	74.073 d ±24.386	2.250 d ±0.780	15.723 d ±6.585	8.823 d ±2.497	20.176 d ±12.064	0.309 d ±0.062	0.116 d ±0.028
<i>B. Influence of drought stress (polyethylene glycol "PEG-6000"):</i>							
Control "0"	98.948 a ±1.396	1.135 e ±0.365	23.956 ±2.605	12.176 a ±0.795	35.779 a ±3.614	0.388 a ±0.013	0.148 a ±0.004
PEG 5%	93.703 b ±4.307	1.730 d ±0.290	20.313 ±2.270	11.176 b ±1.126	29.634 b ±4.445	0.361 b ±0.015	0.138 b ±0.007
PEG 10%	82.322 c ±7.462	2.508 c ±0.184	15.637 ±2.259	9.728 c ±1.157	21.088 c ±4.610	0.314 c ±0.026	0.124 c ±0.011
PEG 15%	56.850 d ±9.747	2.880 b ±0.117	10.403 ±1.277	7.404 d ±1.052	10.325 d ±3.027	0.271 d ±0.042	0.100 d ±0.013
PEG 20%	37.789 e ±12.208	3.080 a ±0.056	7.908 ±1.540	5.442 e ±1.314	5.348 e ±2.824	0.237 e ±0.026	0.077 e ±0.011
<i>C. Interaction effect:</i>							
AB "F. test"	**	**	**	**	**	**	**

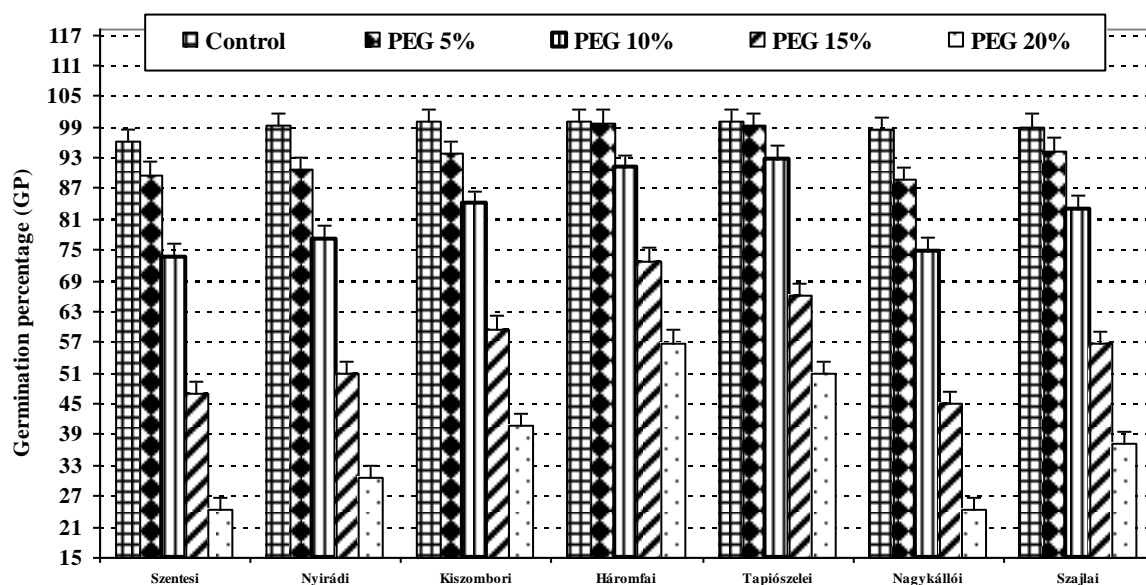
Values are mean of four replicates ± standard deviation. Mean values followed by the same letter are not significantly different at the 5% probability level according to are not significantly different ( $p < 0.05$ ) using Duncan New Multiple Range Test (DMRT's test).

**Table 3.** Root fresh and dry weights (g); relative water content (RWC); tolerance index (TI);  $\alpha$ -amylase and  $\beta$ -amylase activities averages as impacted by wheat landraces and drought stress as well as their interactions.

Characters Treatments	Root fresh weight (g)	Root dry weight (g)	Relative water content (RWC)	Tolerance Index (TI)	$\alpha$ -amylase activity	$\beta$ -amylase activity
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<i>A. Hungarian wheat landraces performance:</i>						
Szentesi	0.110 f ±0.029	0.045 f ±0.022	56.967 e ±11.530	65.288 f ±27.589	0.987 f ±0.121	16.274 f ±1.226
Nyirádi	0.112 e ±0.028	0.048 e ±0.023	59.713 d ±11.495	66.549 e ±27.377	1.052 e ±0.087	16.056 e ±1.393
Kiszombori	0.121 c ±0.027	0.055 c ±0.022	66.493 b ±12.324	72.370 c ±24.769	1.230 c ±0.118	23.161 c ±1.584

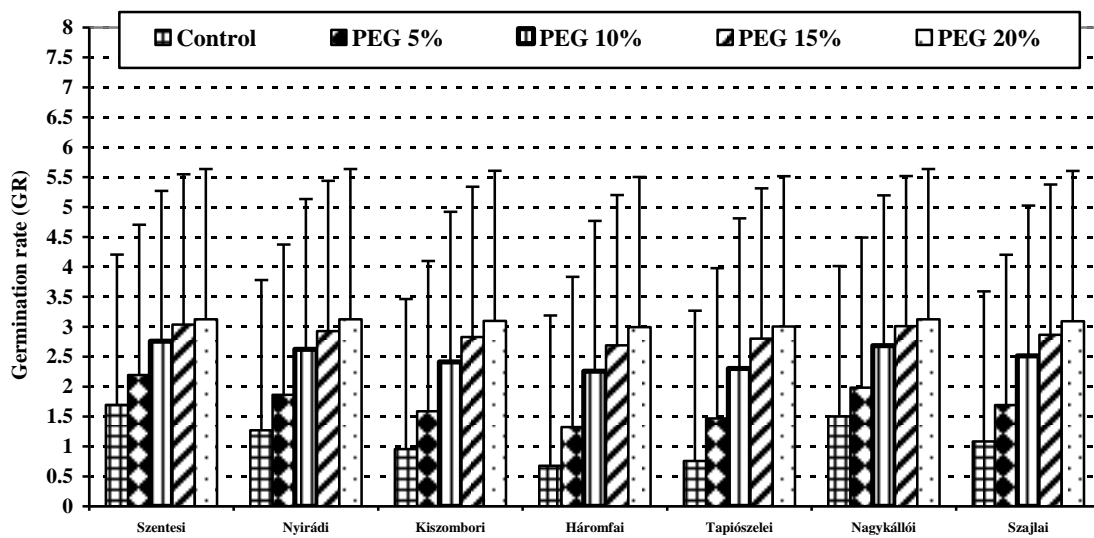
Characters Treatments	Root fresh weight (g)	Root dry weight (g)	Relative water content (RWC)	Tolerance Index (TI)	$\alpha$ - amylase activity	$\beta$ - amylase activity
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Háromfai	0.131 a $\pm$ 0.023	0.066 a $\pm$ 0.020	73.933 a $\pm$ 13.496	79.376 a $\pm$ 20.522	1.406 a $\pm$ 0.172	25.482 a $\pm$ 2.465
Tapiószelei	0.128 b $\pm$ 0.023	0.061 b $\pm$ 0.021	67.720 b $\pm$ 14.105	76.853 b $\pm$ 22.066	1.339 b $\pm$ 0.111	24.122 b $\pm$ 1.460
Nagykállói	0.105 g $\pm$ 0.030	0.043 g $\pm$ 0.022	51.360 f $\pm$ 9.202	63.695 g $\pm$ 29.353	0.771 g $\pm$ 0.198	15.598 g $\pm$ 1.413
Szajlai	0.116 d $\pm$ 0.028	0.051 d $\pm$ 0.023	64.160 c $\pm$ 12.149	68.581 d $\pm$ 26.660	1.131 d $\pm$ 0.106	19.648 d $\pm$ 1.649
<i>B. Influence of drought stress (polyethylene glycol "PEG-6000"):</i>						
Control "0"	0.168 a $\pm$ 0.008	0.080 a $\pm$ 0.006	78.123 a $\pm$ 8.974	100.000 a $\pm$ 0.000	1.286 a $\pm$ 0.191	22.233 a $\pm$ 4.198
PEG 5%	0.154 b $\pm$ 0.010	0.072 b $\pm$ 0.008	72.413 b $\pm$ 8.167	92.221 b $\pm$ 3.815	1.222 b $\pm$ 0.199	21.061 b $\pm$ 4.361
PEG 10%	0.142 c $\pm$ 0.010	0.051 c $\pm$ 0.011	63.442 c $\pm$ 8.200	73.380 c $\pm$ 8.216	1.153 c $\pm$ 0.212	19.853 c $\pm$ 4.064
PEG 15%	0.132 d $\pm$ 0.007	0.035 d $\pm$ 0.009	51.413 d $\pm$ 6.532	51.995 d $\pm$ 9.236	1.051 d $\pm$ 0.232	18.873 d $\pm$ 3.892
PEG 20%	0.119 e $\pm$ 0.006	0.025 e $\pm$ 0.008	49.142 e $\pm$ 6.453	34.340 e $\pm$ 8.185	0.943 e $\pm$ 0.225	18.223 e $\pm$ 3.648
<i>C. Interaction effect:</i>						
AB " F. test"	**	**	**	**	**	**

Values are mean of four replicates  $\pm$  standard deviation. Mean values followed by the same letter are not significantly different at the 5% probability level according to are not significantly different ( $p < 0.05$ ) using Duncan New Multiple Range Test (DMRT's test).

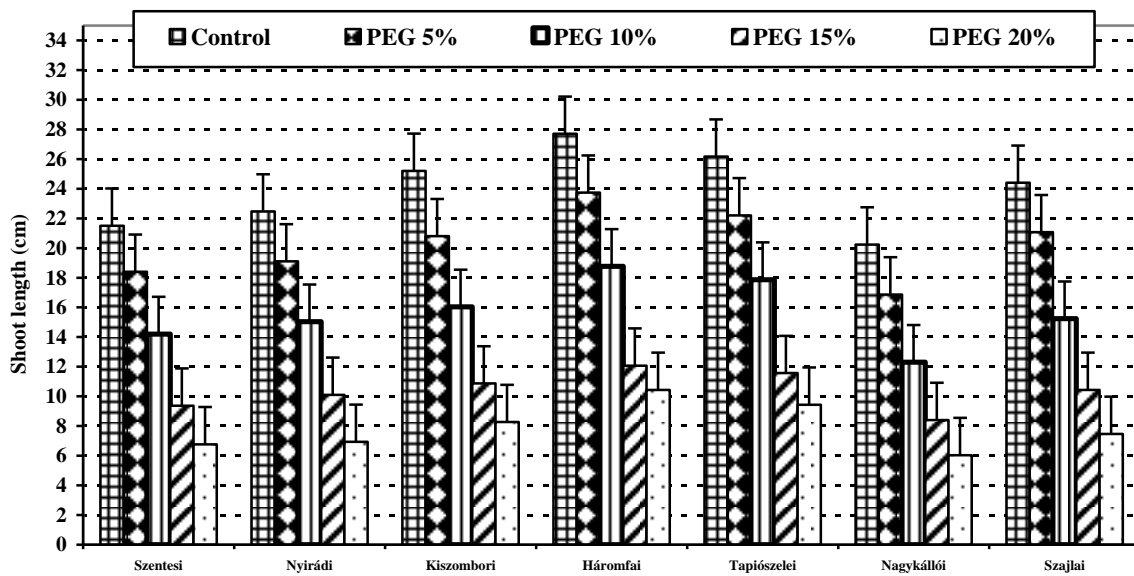


**Figure 1.** Means of germination percentage (GP) as affected by the interaction between wheat landraces varieties and drought stress.

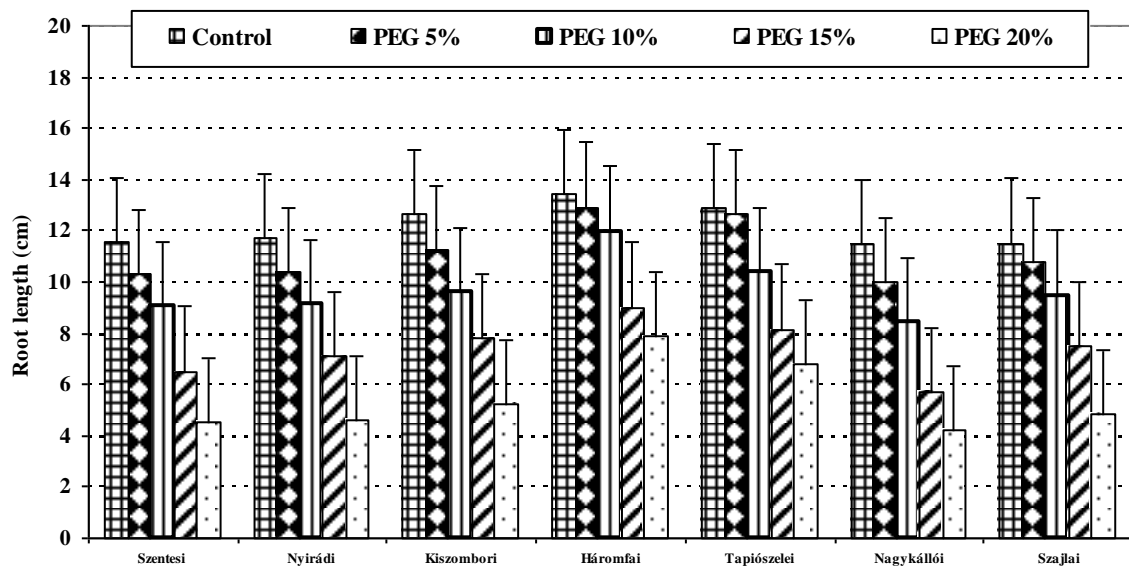




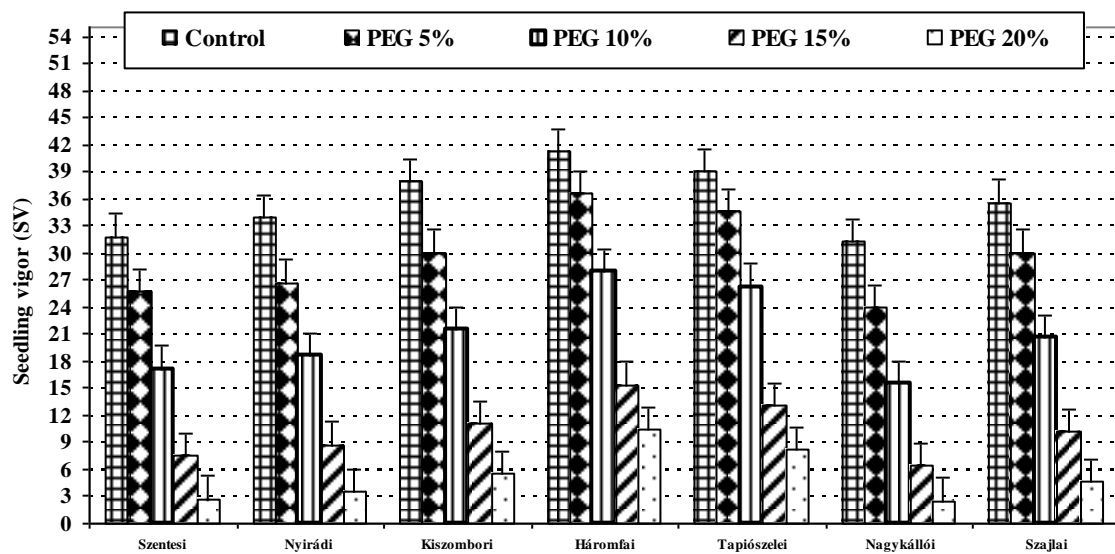
**Figure 2.** Means of germination rate (GR) as affected by the interaction between wheat landraces varieties and drought stress.



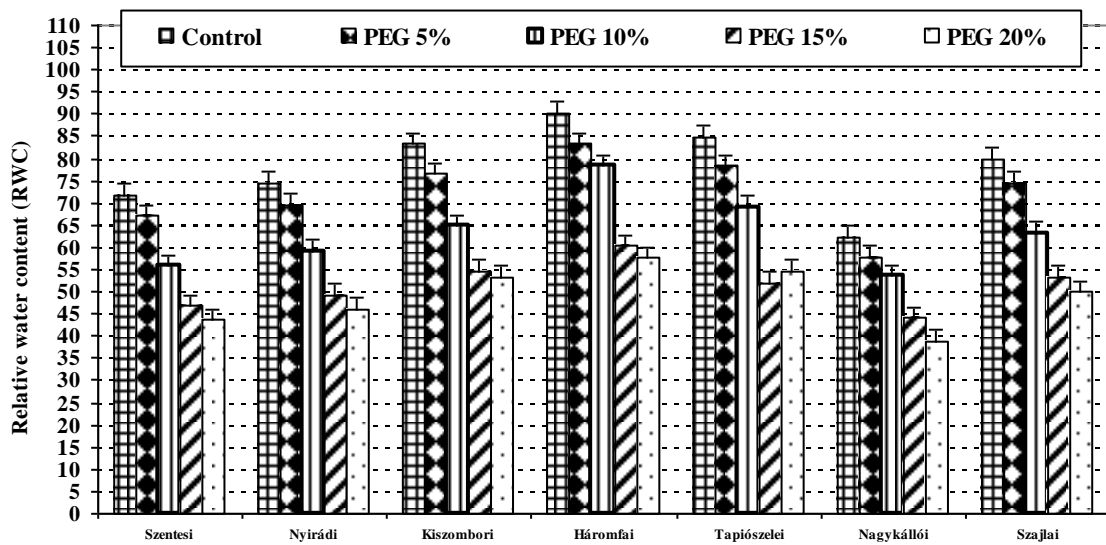
**Figure 3.** Means of shoot length (cm) as affected by the interaction between wheat landraces variety and drought stress.



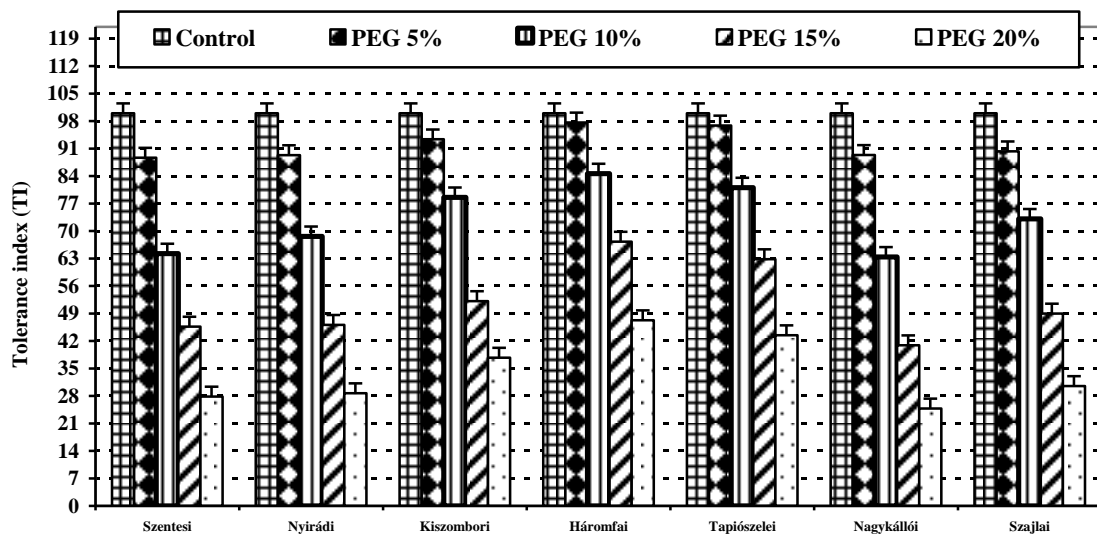
**Figure 4.** Means of root length (cm) as affected by the interaction between wheat landraces varieties and drought stress.



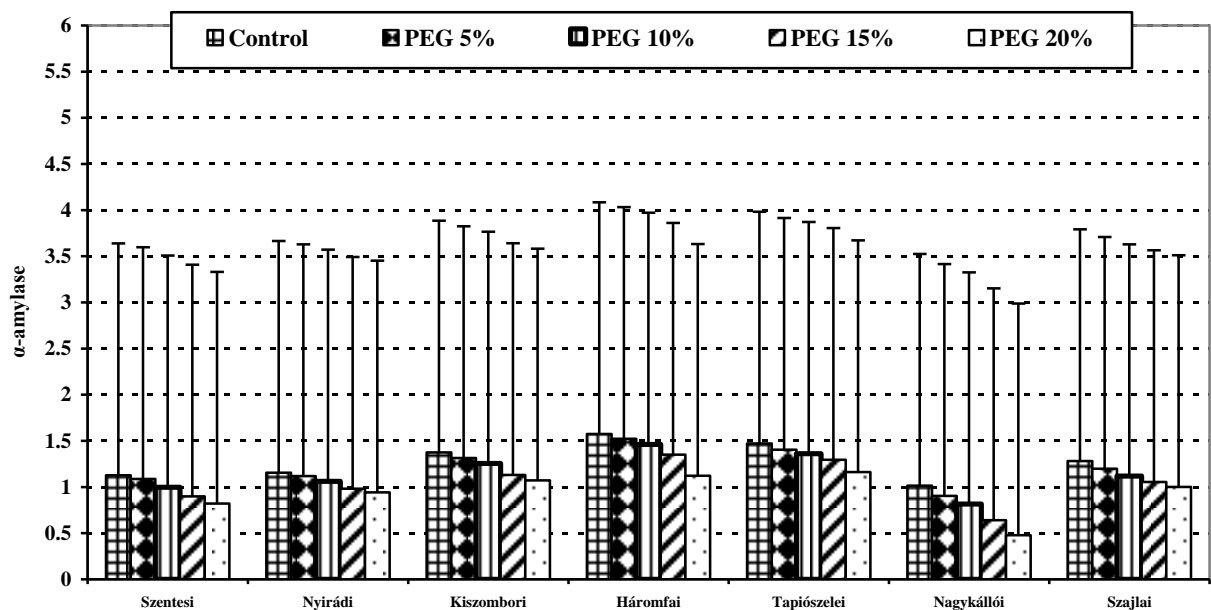
**Figure 5.** Means of seedling vigor (SV) as affected by the interaction between wheat landraces varieties and drought stress.



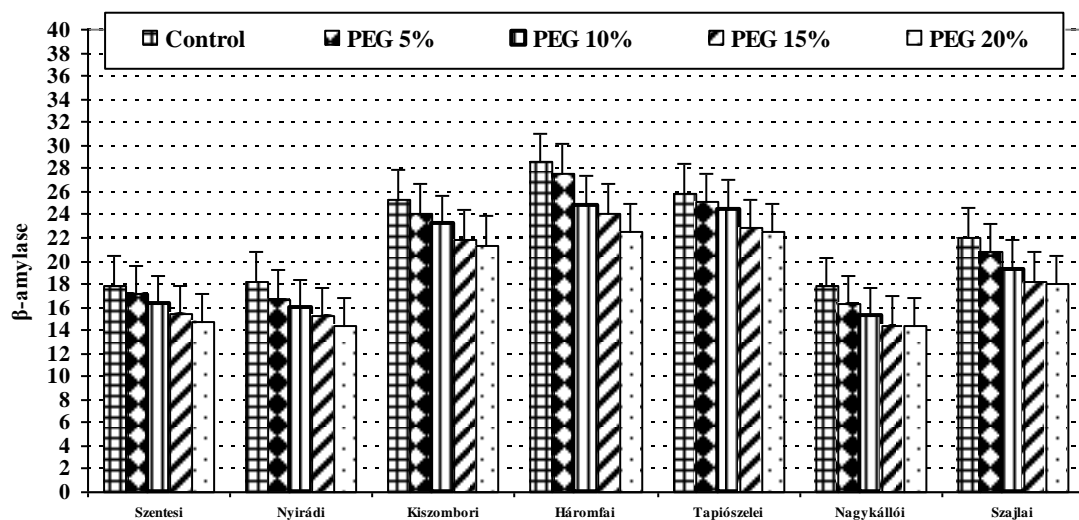
**Figure 6.** Means of relative water content (RWC) as affected by the interaction between wheat landraces varieties and drought stress.



**Figure 7.** Means of tolerance index (TI) as affected by the interaction between wheat landraces varieties and drought stress.



**Figure 8.** Means of  $\alpha$ -amylase activity as affected by the interaction between wheat landraces varieties and drought stress.



**Figure 9.** Means of  $\beta$ -amylase activity as affected by the interaction between wheat landraces varieties and drought stress.

### 3.3. Correlation of Traits

A Pearson's correlation analysis was performed to analyze the connection between the germination, seedlings, and biochemical characteristics of Hungarian wheat landraces under water stress condition. The data presented in Table 4 showed that result of correlation analysis did not show any connection between the Hungarian wheat landraces varieties and all of analyzed germination, seedlings and biochemical characters. The correlation analysis results shows tight and negative correlation between the drought stress and germination percentage (-0.920\*\*), germination rate (-0.933\*\*), shoot length (-0.943\*\*), root length (-0.906\*\*), seedling vigor (-0.946\*\*), shoot fresh weight (-0.904\*\*), shoot dry weight (-0.923\*\*), root fresh weight (-0.906\*\*), root dry weight (-0.920\*\*), relative water content (-0.820\*\*) and tolerance index (-0.955\*\*); furthermore, low or medium connection with the  $\alpha$ -amylase enzyme activities (-0.504\*\*) and  $\beta$ -amylase enzyme activities (-0.344\*\*). The connection was tight

and positive between the germination percentage and germination rate (0.997\*\*), shoot length (0.937\*\*), root length (0.971\*\*), seedling vigor (0.957\*\*), shoot fresh weight (0.958\*\*), shoot dry weight (0.986\*\*), root fresh weight (0.920\*\*), root dry weight (0.950\*\*), relative water content (0.880\*\*), and tolerance index (0.983\*\*); furthermore, medium connection with the  $\alpha$ -amylase enzyme activities (0.693\*\*) and  $\beta$ -amylase enzyme activities (0.541\*\*). The correlation was positive, close or medium between the germination rate and shoot length (0.944\*\*), root length (0.971\*\*), seedling vigor (0.959\*\*), shoot fresh weight (0.961\*\*), shoot dry weight (0.988\*\*), root fresh weight (0.924\*\*), root dry weight (0.953\*\*), relative water content (0.884\*\*),  $\alpha$ -amylase enzyme activities (0.692\*\*),  $\beta$ -amylase enzyme activities (0.531\*\*) and tolerance index (0.987\*\*). The correlation indicated positive, tight or medium between the shoot length and root length (0.962\*\*), seedling vigor (0.995\*\*), shoot fresh weight (0.957\*\*), shoot dry weight (0.956\*\*), root fresh weight (0.967\*\*), root dry weight (0.977\*\*), relative water content (0.945\*\*),  $\alpha$ -amylase enzyme activities (0.713\*\*),  $\beta$ -amylase enzyme activities (0.583\*\*) and tolerance index (0.960\*\*). The correlation was positive, tight or medium between the root length and seedling vigor (0.975\*\*), shoot fresh weight (0.968\*\*), shoot dry weight (0.981\*\*), root fresh weight (0.956\*\*), root dry weight (0.967\*\*), relative water content (0.925\*\*),  $\alpha$ -amylase enzyme activities (0.757\*\*),  $\beta$ -amylase enzyme activities (0.623\*\*) and tolerance index (0.969\*\*). There was positive, close or medium correlation between the seedling vigor and shoot fresh weight (0.968\*\*), shoot dry weight (0.969\*\*), root fresh weight (0.971\*\*), root dry weight (0.985\*\*), relative water content (0.941\*\*),  $\alpha$ -amylase enzyme activities (0.713\*\*),  $\beta$ -amylase enzyme activities (0.587\*\*) and tolerance index (0.973\*\*). The correlation was positive, close and medium between the shoot fresh weight and shoot dry weight (0.975\*\*), root fresh weight (0.954\*\*), root dry weight (0.976\*\*), relative water content (0.915\*\*),  $\alpha$ -amylase enzyme activities (0.754\*\*),  $\beta$ -amylase enzyme activities (0.633\*\*) and tolerance index (0.970\*\*). There was positive, tight or medium correlation between the shoot dry weight and root fresh weight (0.949\*\*), root dry weight (0.969\*\*), relative water content (0.904\*\*),  $\alpha$ -amylase enzyme activities (0.741\*\*),  $\beta$ -amylase enzyme activities (0.595\*\*) and tolerance index (0.989\*\*). The correlation analysis indicated positive, close or medium connection between the root fresh weight and root dry weight (0.967\*\*), relative water content (0.935\*\*),  $\alpha$ -amylase enzyme activities (0.765\*\*),  $\beta$ -amylase enzyme activities (0.654\*\*) and tolerance index (0.940\*\*). There was positive tight or medium correlation between the root dry weight and relative water content (0.936\*\*),  $\alpha$ -amylase enzyme activities (0.741\*\*),  $\beta$ -amylase enzyme activities (0.629\*\*) and tolerance index (0.976\*\*). The correlation was positive and close between the relative water content and  $\alpha$ -amylase enzyme activities (0.832\*\*),  $\beta$ -amylase enzyme activities (0.720\*\*) and tolerance index (0.885\*\*). The correlation was positive, close or medium between the  $\alpha$ -amylase enzyme activities and  $\beta$ -amylase enzyme activities (0.893\*\*) and tolerance index (0.666\*\*). The correlation was positive and medium between the  $\beta$ -amylase enzyme activities and tolerance index (0.515\*\*). These results are in harmony with resulted by [42,43].

**Table 4.** Pearson's correlation coefficients (r) describing the association between the germination, seedlings, and biochemical characteristics of Hungarian wheat landraces under water stress levels.

Person's correlation	A	B	GP	GR	SL	RL	SV	SFW	SDW	RFW	RDW	WRC	$\alpha$ -amylase	$\beta$ -amylase	T I
A	1														
B	0.00	1													
GP	0.06	0.920*	1												
GR	0.02	0.933*	0.997*	1											
SL	0.02	0.943*	0.937*	0.944*	1										
RL	0.02	0.906*	0.971*	0.971*	0.962*	1									
SV	0.04	0.946*	0.957*	0.959*	0.995*	0.975*	1								
SFW	0.03	0.904*	0.958*	0.961*	0.957*	0.968*	0.968*	1							
SDW	0.02	0.923*	0.986*	0.988*	0.956*	0.981*	0.969*	0.975*	1						

Person's correlation	A	B	GP	GR	SL	RL	SV	SFW	SDW	RFW	RDW	WRC	$\alpha$ -amylase	$\beta$ -amylase	TI
RFW	0.031	- 0.906*	0.920*	0.924*	0.967*	0.956*	0.971*	0.954*	0.949*	1					
RDW	0.043	- 0.920*	0.950*	0.953*	0.977*	0.967*	0.985*	0.976*	0.969*	0.967*	1				
WRC	0.032	- 0.820*	0.880*	0.884*	0.945*	0.925*	0.941*	0.915*	0.904*	0.935*	0.936*	1			
Alpha	0.006	- 0.504*	0.693*	0.692*	0.713*	0.757*	0.713*	0.754*	0.741*	0.765*	0.741*	0.832*	1		
Beta	0.173	- 0.344*	0.541*	0.531*	0.583*	0.623*	0.587*	0.633*	0.595*	0.654*	0.629*	0.720*	0.893**	1	
TI	0.024	- 0.955*	0.983*	0.987*	0.960*	0.969*	0.973*	0.970*	0.989*	0.940*	0.976*	0.885*	0.666**	0.515**	1

## Conclusion

According to the results of the current study, there is a wide range of genotypic variation, and this confers a wide response to PEG triggered drought stress in wheat genotypes. The evaluation of tolerance index (TI), water relative content (WRC),  $\alpha$  and  $\beta$ -amylase enzyme activities, as well as other factors, may be viewed as a tool for the effective selection of drought-resistant Hungarian wheat landrace varieties in further studies. Between the Hungarian wheat landraces under studied, Háromfai and Tapiószelei landraces had favorable results in all of the drought stress treatments investigated, and they would be a good resource for breeding programs and cultivation under drought stress conditions. Furthermore, the results of correlation analysis did not neither positive nor negative correlation between the Hungarian wheat landraces varieties and all of analyzed traits. On contrary, the correlation analysis results show negative correlation between the drought stress levels and studied traits.

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