IN VITRO SELECTION OF TRUE POTATO SEED GENOTYPES TOLERANT TO DROUGHT STRESS

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Abstract

The biological material used in this study was produced from true potato seed (TPS). Nine genotypes (MIL19-01-08, MIL19-01-22, MIL19-01-37, ZIL19-02-01 , ZIL19-02-11, ZIL19-02-43, GIL19-03-07, GIL19-03-29 and GIL19-03-38) were tested *in vitro* for drought tolerance. Four treatments were used to induce *in vitro* water stress: MS medium with three different concentrations of PEG (1%, 1.5%, 2%) and one variant of MS medium without PEG as control. On culture medium variant with highest concentration of PEG (2%) GIL19-03-29 obtained best results for plantlet height (11.08 cm), leaf number (9.50), root number (5.33), fresh plant weight (0.167 g). In stress conditions GIL19-03-07 recorded best results for plant fresh weight (0.173 g), root length (7.17 cm), plantlet height (12.28 cm) on PEG 1%. Also, ZIL19-02-43 obtained higher values on the culture medium variants with the highest level of water stress for parameters such as root fresh weight (0.146 g), plant fresh weight (0.163 g), root length (7.08 cm) on PEG 1.5%. The potato genotypes GIL19-03-29, GIL19-03-07 and ZIL19-02-43 showed the best tolerance to the water deficit and were selected for further assessments both in protected area (greenhouse) and open-field conditions.

Key words: potato, plant tissue culture, TPS, polyethylene glycol, water stress tolerance

In the scenario of global climate change, drought is considered to be one of the abiotic stress that affects plant growth and development, food security and causes the highest crop losses. Developing climate change adaptation strategies will be a real challenge for agriculture and new tools should be used to ensure that plant genetic resources support food security in the world's poorest regions. It is absolutely essential that agricultural biodiversity to cope with the anticipated impacts of climate change not only as a source of characteristics (genetic, traditional, scientific, socio-economic), but especially as a basis for development of agro-ecosystems farm (Frison E.A. *et al*, 2011).

Abiotic stress refers to the negative impact of environmental factors on plant growth and development. Drought is the environmental factor with a major impact on plant growth, productivity and distribution in various areas (Rukundo P. *et al*, 2012). It is the factor of abiotic stress that causes the greatest damage in agriculture worldwide (Ober E., 2008). Drought affects over 10% of arable land (Bray E.A. *et al*, 2000; Zidenga T., 2006) and the negative effects of these conditions are exacerbated by population growth, continuous soil deterioration, lack of water and climate change. As it is known that drought severely affects crop survival and production, while increasing costs, finding drought-tolerant potato genotypes will increase farmers profitability through the efficient use of soil water resources. Identification of germplasm tolerant to water stress is a very important aspect, which is the subject of applied research in breeding programs in most crops (Romero P. *et al*, 2004, Akbarpour E., 2017).

In the current global context, when we face the climate shock, energy and food crisis, the potato remains one of the most important crops, which will play a key role in solving food security problems for the next decades. However, the effects of climate change will have a major influence both on the areas cultivated with potatoes and on the yields.

Efforts to identify stress-tolerant species are of great importance in increasing crop productivity. In recent years, plant tissue cultures based on *in vitro* selection have been considered a feasible and effective tool for obtaining stress-tolerant plants (Manoj K.R. *et al*, 2011). *In vitro* cultures can be used as a tool to obtain drought tolerant plants, assuming that there is a correlation between plant response at the cellular level and in *in vivo* conditions (Mohamed M.A.H. *et al*, 2000).

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The main purpose of this study was the *in vitro* selection of drought tolerant individuals within populations derived from true potato seeds. The assessment of crops drought stress tolerance can be done in field conditions, but the factors of the external environment (attack of diseases and pests, land uniformity, rain, strong wind, air temperature etc.) are difficult to control. An effectivealternative that allows the minimization of these inconveniences is the plant tissue culture.

In the literature there are numerous studies on the use of *in vitro* cultivation methods in order to induce water stress by adding various chemical agents that reduce the water potential in the culture medium. Polyethylene glycol (PEG) is the most recommended inducer because it does not penetrate plant cells and at the same time reduces the water potential of the culture medium in which plants grow (Manoj K.R. *et al*, 2011).

MATERIAL AND METHOD

In this study nine potato genotypes derived from true potato seed were tested for tolerance to water stress induced *in vitro*: MIL19-01-08, MIL19-01-22, MIL19-01-37, ZIL19-02-01, ZIL19-02-11, ZIL19-02-43, GIL19-03-07, GIL19-03-29 and GIL19-03-38. Potato microplants obtained after seeds germination were multiplied *in vitro* using stem cuttings containing an axillary bud with the afferent leaf and half of the neighboring internodes. Different concentrations of polyethylene glycol (PEG) was used to induce *in vitro* drought stress (*table 1*). After obtaining a healthy (virus free) stock material for all nine potato genotypes, uninodal microcuttings were inoculated on four culture medium variants. MS nutrient medium specific to the *in vitro* cultivation of potato (Murashige T., Skoog F., 1962) was used as control. Different amounts (10, 15 and 20 g/l) of PEG were added to the other three growth medium variants. All four medium variants were supplemented with 20 g/l sucrose and 9 g/l agar. The pH of the medium was adjusted to 5.7.

Table 1

Variants of the culture media used for inducing *in vitro* water stress

Culture media variants
V1 - MS (Murashige-Skoog) - control
V2 - MS + 10 g/l PEG
V3 - MS + 15 g/l PEG
V4 - MS + 20 g/l PEG
Sucrose (20 g/l) Agar (9 g/l) pH: 5.7

After explants inoculation, cultures were transferred to a growth chamber and exposed daily to 16 hours light, for 4 weeks. The growth chamber temperature was maintained at 20±2 °C.

After 4 weeks of *in vitro* cultivation potato plantlets were evaluated for following parameters: plantlet height, leaf number, root length, root number, fresh plant and root weight.

The study was carried out at the National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Brașov, Research Laboratory for Plant Tissue Culture. The experiment was bifactorial with nine genotypes and four culture medium variants. The results were subjected to statistical analysis according to the completely randomised experiment design with three replications. As a control, was established for each analyzed parameter the average values. Statistical interpretation of the obtained results was made using analysis of variance method (Săulescu N.A., Săulescu N.N., 1967).

RESULTS AND DISCUSSIONS

Statistical interpretation of the combined influence between culture media and genotype on the plantlet height (*table 2*) revealed that the genotype GIL19-03-07 recorded a distinctly significant positive difference (4.66 cm) on the media variant added with 1% PEG and significantly positive difference (3.34 cm) for the media variant added with 2% PEG, compared with the control. Also, the genotype GIL19-03-29 obtained distinctly significant positive differences for the media added with 1% PEG and 1.5% PEG (4.71 cm and 4.12 cm, respectively) and for the media in which the concentration of PEG was 2% the difference is very significant positive (6.25 cm). The last genotype mentioned, even under in vitro conditions of water stress tends to form tall and vigorous plantlets.

By comparing the V2 (PEG 1%) medium variant with control medium (V1 – MS) we observed that genotype GIL19-03-29 is remarkable for plantlet height with a positive difference (0.75 cm), but not significant. Also, for this genotype no significant differences were identified between the culture media with a concentration of 1.5% PEG (-1.50 cm) and 2% PEG (-0.50 cm) respectively, compared to the control medium (*table 2*).

As shown in *table 3*, the *in vitro* behavior of the genotypes on the all culture media variants was different in terms of plantlet height. Thus, the best results were obtained for the genotypes GIL19-03-29 (11.271 cm) and GIL19-03-07 (10.583 cm), with distinctly significant positive differences (3.622 cm and 2.935 cm, respectively). The most affected genotypes to induced water stress were MIL19-01-22 and ZIL19-02-01, which recorded the lowest values of plantlet height (5.296 cm and 5.413 cm, respectively) and significant negative differences (-2.353 cm and -2.236 cm).

Another element studied was the number of leaves (*table 4*). Analyzing the results obtained regarding this parameter, the genotype GIL19-03-

29 exceeded by the large number of leaves obtained under conditions of water stress, which recorded a significant positive difference (2.96) on the media variant added with 1% PEG and distinctly significant positive difference (3.39) for the media variant added with 2% PEG.

Table 2

Combined effect of genotypes and in vitro water stress treatments on the plantlet height

Genotypes	V1	Diff. (cm)	Sign.	V2	Diff. (cm)	Sign.	V3	Diff. (cm)	Sign.	V4	Diff. (cm)	Sign.
MIL19-01-08	16.70	4.52	**	7.60	-0.03	ns	7.63	1.67	ns	2.50	-2.33	ns
MIL19-01-22	10.75	-1.43	ns	6.17	-1.46	ns	1.60	-4.36	oo	2.67	-2.16	ns
MIL19-01-37	13.08	0.91	ns	4.00	-3.63	o	7.92	1.95	ns	2.67	-2.16	ns
ZIL19-02-01	9.08	-3.09	o	8.17	0.54	ns	1.65	-4.31	oo	2.75	-2.08	ns
ZIL19-02-11	12.00	-0.18	ns	8.17	0.54	ns	5.50	-0.46	ns	4.25	-0.58	ns
ZIL19-02-43	11.92	-0.26	ns	2.75	-4.88	ooo	8.00	2.04	ns	5.92	1.09	ns
GIL19-03-07	13.63	1.46	ns	12.28	4.66	**	8.25	2.29	ns	8.17	3.34	*
GIL19-03-29	11.58	-0.59	ns	12.33	4.71	**	10.08	4.12	**	11.08	6.25	***
GIL19-03-38	10.83	-1.34	ns	7.17	-0.46	ns	3.03	-2.93	o	3.47	-1.36	ns
Mean (Ct)	12.18	-	-	7.63	-	-	5.96	-	-	4.83	-	-

LSD 5% = 2.76 cm; 1% = 3.68 cm; 0.1% = 4.80 cm

Genotypes	Diff. v_2-v_1 (cm)	Sign.	Diff. v_3-v_1 (cm)	Sign.	Diff. v_4-v_1 (cm)	Sign.
MIL19-01-08	-9.10	ooo	-9.07	ooo	-14.20	ooo
MIL19-01-22	-4.58	oo	-9.15	ooo	-8.08	ooo
MIL19-01-37	-9.08	ooo	-5.17	oo	-10.42	ooo
ZIL19-02-01	-0.92	ns	-7.43	ooo	-6.33	ooo
ZIL19-02-11	-3.83	o	-6.50	ooo	-7.75	ooo
ZIL19-02-43	-9.17	ooo	-3.92	oo	-6.00	ooo
GIL19-03-07	-1.35	ns	-5.38	oo	-5.47	ooo
GIL19-03-29	0.75	ns	-1.50	ns	-0.50	ns
GIL19-03-38	-3.67	o	-7.80	ooo	-7.37	ooo

LSD 5% = 2.83 cm; 1% = 3.91 cm; 0.1% = 5.40 cm

Table 3

Effect of genotype on plantlet height

Genotypes	Plantlet height (cm)	Diff. (cm)	Sign.
MIL19-01-08	8.608	0.960	ns
MIL19-01-22	5.296	-2.353	o
MIL19-01-37	6.917	-0.732	ns
ZIL19-02-01	5.413	-2.236	o
ZIL19-02-11	7.479	-0.169	ns
ZIL19-02-43	7.146	-0.503	ns
GIL19-03-07	10.583	2.935	**
GIL19-03-29	11.271	3.622	**
GIL19-03-38	6.125	-1.524	ns
Mean (Ct)	7.649	-	-

LSD 5% = 2.015 cm; 1% = 2.932 cm; 0.1% = 4.397 cm

Table 4

Combined effect of genotypes and *in vitro* water stress treatments on the number of leaves

Genotypes	V1	Diff.	Sign.	V2	Diff.	Sign.	V3	Diff.	Sign.	V4	Diff.	Sign.
MIL19-01-08	8.67	0.72	ns	5.17	-1.54	ns	6.67	0.39	ns	3.67	-2.44	o
MIL19-01-22	7.00	-0.94	ns	6.00	-0.70	ns	4.33	-1.94	ns	4.33	-1.78	ns
MIL19-01-37	7.67	-0.28	ns	7.00	0.30	ns	7.33	1.06	ns	7.50	1.39	ns
ZIL19-02-01	6.17	-1.78	ns	6.83	0.13	ns	4.00	-2.28	ns	5.33	-0.78	ns
ZIL19-02-11	6.50	-1.44	ns	6.83	0.13	ns	6.67	0.39	ns	6.67	0.56	ns
ZIL19-02-43	7.83	-0.11	ns	5.17	-1.54	ns	7.00	0.72	ns	7.00	0.89	ns
GIL19-03-07	10.83	2.89	*	9.00	2.30	ns	7.00	0.72	ns	6.50	0.39	ns
GIL19-03-29	9.83	1.89	ns	9.67	2.96	*	7.83	1.56	ns	9.50	3.39	**
GIL19-03-38	7.00	-0.94	ns	4.67	-2.04	ns	5.67	-0.61	ns	4.50	-1.61	ns
Mean (Ct)	7.94	-	-	6.70	-	-	6.28	-	-	6.11	-	-

LSD 5% = 2.40; 1% = 3.20; 0.1% = 4.17

Genotypes	Diff. v_2-v_1	Sign.	Diff. v_3-v_1	Sign.	Diff. v_4-v_1	Sign.
MIL19-01-08	-3.50	oo	-2.00	ns	-5.00	ooo
MIL19-01-22	-1.00	ns	-2.67	o	-2.67	o
MIL19-01-37	-0.67	ns	-0.33	ns	-0.17	ns
ZIL19-02-01	0.67	ns	-2.17	ns	-0.83	ns
ZIL19-02-11	0.33	ns	0.17	ns	0.17	ns
ZIL19-02-43	-2.67	o	-0.83	ns	-0.83	ns
GIL19-03-07	-1.83	ns	-3.83	oo	-4.33	ooo
GIL19-03-29	-0.17	ns	-2.00	ns	-0.33	ns
GIL19-03-38	-2.33	o	-1.33	ns	-2.50	o

LSD 5% = 2.29; 1% = 3.12; 0.1% = 4.22

Table 5

Effect of genotype on the number of leaves

Genotypes	Number of leaves	Diff.	Sign.
MIL19-01-08	6.04	-0.72	ns
MIL19-01-22	5.42	-1.34	o
MIL19-01-37	7.38	0.62	ns
ZIL19-02-01	5.58	-1.18	ns
ZIL19-02-11	6.67	-0.09	ns
ZIL19-02-43	6.75	-0.01	ns
GIL19-03-07	8.33	1.57	*
GIL19-03-29	9.21	2.45	**
GIL19-03-38	5.46	-1.30	o
Mean (Ct)	6.76	-	-

LSD 5% = 1.25; 1% = 1.83; 0.1% = 2.74

In terms of the leaf number, the potato genotypes showed a different behavior for all variants of culture media (*table 5*). Water deficiency reduces the number of leaves, their size and their lifetime by decreasing the water potential of the culture medium. However, the best results were obtained by GIL19-03-29 (9.21) followed by GIL19-03-07 (8.33) with distinctly significant positive (2.45) and significant positive (1.57) differences, respectively comparative to control (mean of all values for studied genotypes). MIL19-01-22 and GIL19-03-38 genotypes registered the lowest number of leaves on ”in vitro” treatments applied, with significantly negative differences (-1.34 and -1.30) compared to control (mean of all values). Plants experience water stress when the water supply to the roots becomes difficult. A vigorous root system helps plants survive under water stress. Both the number of roots and their length are an important aspect in adapting plants to water deficit.

According to the results presented in *table 6*, of all the 9 genotypes studied, GIL19-03-29 stands out, which obtained the highest number of roots (5.00), registering a significantly positive difference (1.19) compared to control.

Following the interpretation of the combined influence of the culture media and the genotype on the number of roots (*table 7*) it can be seen that the genotype GIL19-03-29 registered a distinctly positive difference (2.22) on the culture medium variant with the highest concentration of polyethylene glycol (2%).

Table 6

Effect of genotype on the number of roots

Genotypes	Number of roots	Diff.	Sign.
MIL19-01-08	3.83	0.02	ns
MIL19-01-22	3.29	-0.52	ns
MIL19-01-37	3.33	-0.48	ns
ZIL19-02-01	2.83	-0.98	ns
ZIL19-02-11	3.88	0.06	ns
ZIL19-02-43	4.54	0.73	ns
GIL19-03-07	4.67	0.85	ns
GIL19-03-29	5.00	1.19	*
GIL19-03-38	2.94	-0.88	ns
Mean (Ct)	3.81	-	-

LSD 5% = 1.17; 1% = 1.71; 0.1% = 2.56

Table 7

Combined effect of genotypes and *in vitro* water stress treatments on the number of roots

Genotypes	V1	Diff.	Sign.	V2	Diff.	Sign.	V3	Diff.	Sign.	V4	Diff.	Sign.
MIL19-01-08	5.83	0.89	ns	3.17	-0.38	ns	4.50	0.85	ns	1.83	-1.28	ns
MIL19-01-22	4.17	-0.78	ns	3.83	0.29	ns	2.67	-0.98	ns	2.50	-0.61	ns
MIL19-01-37	4.67	-0.28	ns	3.17	-0.38	ns	3.67	0.02	ns	1.83	-1.28	ns
ZIL19-02-01	3.00	-1.94	o	4.00	0.45	ns	2.33	-1.31	ns	2.00	-1.11	ns
ZIL19-02-11	4.00	-0.94	ns	4.17	0.62	ns	3.67	0.02	ns	3.67	0.56	ns
ZIL19-02-43	6.17	1.22	ns	3.00	-0.55	ns	5.00	1.35	ns	4.00	0.89	ns
GIL19-03-07	5.67	0.72	ns	4.33	0.79	ns	4.33	0.69	ns	4.33	1.22	ns
GIL19-03-29	6.67	1.72	*	4.00	0.45	ns	4.00	0.35	ns	5.33	2.22	**
GIL19-03-38	4.33	-0.61	ns	2.25	-1.30	ns	2.67	-0.98	ns	2.50	-0.61	ns
Mean (Ct)	4.94	-	-	3.55	-	-	3.65	-	-	3.11	-	-

LSD 5% = 1.66; 1% = 2.22; 0.1% =2.89

Table 8

Combined effect of genotypes and *in vitro* water stress treatments on the root length

Genotypes	V1	Diff. (cm)	Sign.	V2	Diff. (cm)	Sign.	V3	Diff. (cm)	Sign.	V4	Diff. (cm)	Sign.
MIL19-01-08	4.58	-0.66	ns	3.80	-0.64	ns	4.50	0.98	ns	1.67	-1.24	ns
MIL19-01-22	4.83	-0.41	ns	3.83	-0.60	ns	1.17	-2.35	o	1.92	-0.99	ns
MIL19-01-37	5.75	0.51	ns	3.08	-1.35	ns	3.83	0.32	ns	3.00	0.09	ns
ZIL19-02-01	5.92	0.68	ns	4.92	0.48	ns	0.80	-2.72	oo	1.83	-1.07	ns
ZIL19-02-11	4.92	-0.32	ns	6.00	1.56	ns	2.25	-1.27	ns	2.90	-0.01	ns
ZIL19-02-43	5.75	0.51	ns	3.00	-1.44	ns	7.08	3.57	***	4.67	1.76	ns
GIL19-03-07	5.67	0.43	ns	7.17	2.73	**	3.75	0.23	ns	3.33	0.43	ns
GIL19-03-29	5.25	0.01	ns	5.08	0.65	ns	4.50	0.98	ns	3.75	0.84	ns
GIL19-03-38	4.50	-0.74	ns	3.03	-1.40	ns	3.77	0.25	ns	3.08	0.18	ns
Mean (Ct)	5.24	-	-	4.44	-	-	3.52	-	-	2.91	-	-

LSD 5% = 1.82 cm; 1% = 2.42 cm; 0.1% = 3.15 cm

Genotypes	Diff. v_2-v_1 (cm)	Sign.	Diff. v_3-v_1 (cm)	Sign.	Diff. v_4-v_1 (cm)	Sign.
MIL19-01-08	-0.78	ns	-0.08	ns	-2.92	oo
MIL19-01-22	-1.00	ns	-3.67	ooo	-2.92	oo
MIL19-01-37	-2.67	oo	-1.92	o	-2.75	oo
ZIL19-02-01	-1.00	ns	-5.12	ooo	-4.08	ooo
ZIL19-02-11	1.08	ns	-2.67	oo	-2.02	o
ZIL19-02-43	-2.75	oo	1.33	ns	-1.08	ns
GIL19-03-07	1.50	ns	-1.92	o	-2.33	o
GIL19-03-29	0.17	ns	-0.75	ns	-1.50	ns
GIL19-03-38	-1.47	ns	-0.73	ns	-1.42	ns

LSD 5% = 1.72 cm; 1% = 2.35 cm; 0.1% = 3.17 cm

Root length also plays an important role in plant tolerance to stress caused by reduced water reserves. The longer the root, the better it manages to penetrate into the deeper layers of the soil, which are richer in water. The values presented in *table 8* show that the best results in terms of root length were recorded in the genotypes GIL19-03-07 (7.17 cm) on the medium added with 1% PEG and ZIL19-02-43 (7.08 cm) on the medium added with 1.5% PEG, obtaining a distinctly significant positive difference (2.73 cm) and very significantly positive difference (3.57 cm), respectively.

Regarding the behavior of potato genotypes on media that induce water stress compared to the control (MS), in terms of root length, the genotype GIL19-03-29 was highlighted with a positive difference (0.17 cm) for the medium added by 1% PEG. Also, no significant differences were identified in this genotype between the culture medium with a PEG concentration of 1.5% (-0.75 cm) and 2% (-1.50 cm), respectively, compared to the control medium.

The lowest values of root length were recorded for the genotypes ZIL19-02-01 (0.80 cm) and MIL19-01-22 (1.17 cm) on the culture medium variant in which the concentration of polyethylene glycol was 1.5%. They obtained a distinctly significant negative difference (-2.72 cm) and a significantly negative difference (-2.35 cm), respectively, compared to mean of values for all genotypes on medium with 1.5% PEG.

The influence of genotype on root length highlighted two of the nine genotypes studied, namely ZIL19-02-43 and GIL19-03-07 (*table 9*). They obtained significantly positive differences (1.10 cm and 0.96 cm, respectively) from the control. The smallest values of root length were recorded in the MIL19-01-22 genotype (2.94 cm),

with a significantly negative difference (-1.09 cm) compared to the control.

Analyzing the data presented in *table 10* which refers to plant fresh weight, the genotypes ZIL19-02-43, GIL19-03-07 and GIL19-03-29 are noted. Thus, GIL19-03-29 obtained the best results (0.167 g) on the culture medium with the highest concentration of polyethylene glycol (2%), registering a very significant positive difference (0.098 g) compared with the control. Also, the genotype GIL19-03-07 obtained a distinctly significant positive difference (0.076 g) for the culture medium variant with the addition of 1% PEG, and for the genotype ZIL19-02-43 the difference was distinctly significant positive (0.081 g) on the culture medium variant added with 1.5% PEG.

Regarding the behavior of genotypes on the four culture medium variants (*table 11*), the highest values of plant fresh weight were obtained at GIL19-03-07, ZIL19-02-43 and GIL19-03-29 with distinctly significant positive differences (0.048 g and 0.043 g) and significantly positive difference (0.032 g), respectively. At the opposite pole were the genotypes MIL19-01-22 and ZIL19-02-01, with significant negative differences (-0.038 g and -0.039 g, respectively).

Table 9

Effect of genotype on root length

Genotypes	Root length (cm)	Diff. (cm)	Sign.
MIL19-01-08	3.64	-0.39	ns
MIL19-01-22	2.94	-1.09	o
MIL19-01-37	3.92	-0.11	ns
ZIL19-02-01	3.37	-0.66	ns
ZIL19-02-11	4.02	-0.01	ns
ZIL19-02-43	5.13	1.10	*
GIL19-03-07	4.98	0.96	*
GIL19-03-29	4.65	0.62	ns
GIL19-03-38	3.60	-0.43	ns
Mean (Ct)	4.03	-	-

LSD 5% = 0.93 cm; 1% = 1.35 cm; 0.1% = 2.02 cm

Table 10

Combined effect of genotypes and *in vitro* water stress treatments on the plant fresh weight

Genotypes	V1	Diff. (g)	Sign.	V2	Diff. (g)	Sign.	V3	Diff. (g)	Sign.	V4	Diff. (g)	Sign.
MIL19-01-08	0.207	0.051	*	0.090	-0.007	ns	0.090	0.007	ns	0.010	-0.059	o
MIL19-01-22	0.110	-0.046	ns	0.083	-0.014	ns	0.023	-0.059	o	0.037	-0.032	ns
MIL19-01-37	0.150	-0.006	ns	0.053	-0.044	ns	0.093	0.011	ns	0.033	-0.035	ns
ZIL19-02-01	0.100	-0.056	o	0.100	0.003	ns	0.017	-0.066	oo	0.030	-0.039	ns
ZIL19-02-11	0.093	-0.063	o	0.103	0.006	ns	0.070	-0.013	ns	0.063	-0.005	ns
ZIL19-02-43	0.263	0.107	***	0.037	-0.060	o	0.163	0.081	**	0.113	0.045	ns
GIL19-03-07	0.213	0.057	*	0.173	0.076	**	0.107	0.024	ns	0.103	0.035	ns
GIL19-03-29	0.123	-0.033	ns	0.133	0.036	ns	0.110	0.027	ns	0.167	0.098	***
GIL19-03-38	0.143	-0.013	ns	0.100	0.003	ns	0.070	-0.013	ns	0.060	-0.009	ns
Mean (Ct)	0.156	-	-	0.097	-	-	0.083	-	-	0.069	-	-

LSD 5% = 0.048 g; 1% = 0.065 g; 0.1% =0.084 g

Table 11

Effect of genotype on plant fresh weight

Genotypes	Plant fresh weight (g)	Diff. (g)	Sign.
MIL19-01-08	0.099	-0.002	ns
MIL19-01-22	0.063	-0.038	o
MIL19-01-37	0.083	-0.019	ns
ZIL19-02-01	0.062	-0.039	o
ZIL19-02-11	0.083	-0.019	ns
ZIL19-02-43	0.144	0.043	**
GIL19-03-07	0.149	0.048	**
GIL19-03-29	0.133	0.032	*
GIL19-03-38	0.093	-0.008	ns
Mean (Mt)	0.101	-	-

LSD 5% = 0.029 g; 1% = 0.043 g; 0.1% = 0.064 g

Table 12

Combined effect of genotypes and *in vitro* water stress treatments on the root fresh weight

Genotypes	V1	Diff. (g)	Sign.	V2	Diff. (g)	Sign.	V3	Diff. (g)	Sign.	V4	Diff. (g)	Sign.
MIL19-01-08	0.023	-0.016	ns	0.013	-0.004	ns	0.012	-0.015	ns	0.003	-0.007	ns
MIL19-01-22	0.022	-0.017	ns	0.017	0.000	ns	0.003	-0.024	ns	0.004	-0.005	ns
MIL19-01-37	0.042	0.003	ns	0.007	-0.009	ns	0.008	-0.019	ns	0.003	-0.006	ns
ZIL19-02-01	0.039	0.000	ns	0.015	-0.001	ns	0.008	-0.019	ns	0.003	-0.006	ns
ZIL19-02-11	0.006	-0.033	ns	0.013	-0.004	ns	0.005	-0.022	ns	0.013	0.004	ns
ZIL19-02-43	0.102	0.063	**	0.012	-0.005	ns	0.146	0.119	***	0.026	0.016	ns
GIL19-03-07	0.078	0.039	ns	0.046	0.029	ns	0.014	-0.013	ns	0.008	-0.001	ns
GIL19-03-29	0.016	-0.023	ns	0.010	-0.007	ns	0.010	-0.017	ns	0.017	0.007	ns
GIL19-03-38	0.024	-0.015	ns	0.018	0.001	ns	0.038	0.011	ns	0.006	-0.003	ns
Mean (Ct)	0.039	-	-	0.017	-	-	0.027	-	-	0.009	-	-

LSD 5% = 0.043 g; 1% = 0.057 g; 0.1% =0.074

Regarding the roots fresh weight, among the nine studied genotypes, ZIL19-02-43 was highlighted, in which the roots grown on the medium with the addition of 1.5% PEG had an average weight of 0.146 g, registering a very significant positive difference (0.119 g) compared to the control. For the other genotypes the difference from the control was insignificant *(table 12*).

CONCLUSIONS

In general, drought slows growth, closes the stomata and thus reduces photosynthesis (Nemeth M. *et al*, 2002). The main effects of water stress on the potato plant are the reduction of leaf area and number of leaves, decrease in plant weight, number of tubers and their weight, decrease in quality and production, reduction of biomass and number of roots (Tourneux C. *et al*, 2003; Schittenhelma S. *et al*, 2006; Arvin M.J., Donnelly D.J, 2008; Hassanpanah D., 2009).

The intensification of drought stress threatens agricultural production and global food security. Taking into account these aspects, the sustainability of agricultural production will depend on the identification and use of new drought-tolerant genotypes (Cochard H. *et al*, 2008).

The results obtained in this study suggest that the genotypes GIL19-03-29, GIL19-03-07 and ZIL19-02-43 can be used as possible potato genotypes tolerant to water stress, but further research is needed to evaluate them both in protected areas as well as in field conditions.

On culture medium variant with highest concentration of PEG (2%) GIL19-03-29 obtained best results for plantlet height (11.08 cm), leaf number (9.50), root number (5.33), fresh plant weight (0.167 g). In stress conditions GIL19-03-07 recorded best results for plant fresh weight (0.173 g), root length (7.17 cm), plantlet height (12.28 cm) on PEG 1%.

ZIL19-02-43 obtained higher values on the culture medium variants with the highest level of water stress for parameters such as root fresh weight (0.146 g), plant fresh weight (0.163 g), root length (7.08 cm), on PEG 1.5%.

Based on the results obtained in this work and in other scientific research carried out in this field worldwide, it is possible to use in vitro culture as an useful method for selection of drought tolerant potato genotypes.

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