Thesis of doctoral (PhD) dissertation

LABORATORY AND FIELD TEST OF OSMOTIC STRESS TOLERANCE OF POTATO GENOTYPES

Hanász Alexandra

PhD candidate

Supervisor:

Dr. László Zsombik

Scientific advisor

UNIVERSITY OF DEBRECEN

Kerpely Kálmány Doctoral School of Crop Production and Horticultural Sciences

Debrecen, 2025

1. THE ANTECEDENTS AND THE OBJECTIVES OF THE DOCTORAL DISSERTATION

The vulnerability of the crop production sector to climatic factors indirectly threatens the security of the food supply, and we cannot even make approximate estimates regarding the development of the expected yield. The goal of modern plant breeding is to support effective adaptation to difficult conditions by breeding and using plant species and varieties that adapt to the given microclimate and have excellent stress tolerance. Stress is one of the most important concepts, which has a decisive and influencing role in the life, development and growth of the plant. One of these abiotic stressors is drought. The drought tolerance and osmotic stress tolerance of the plant depends on its specific morphological, physiological and biochemical properties. The potato (*Solanum tuberosum* L.) is cultivated almost all over the world, and based on the amount of crops produced worldwide, the potato is the fourth most important food crop after corn, wheat and rice. In our country only about 8000 hectares are cultivated, and we need significant imports. The decrease in area is likely to be the result of the difficult growing conditions, such as unpredictable weather and meeting consumer requirements, as well as the combined effect of potatoes coming in from abroad. Although irrigation has a better chance of reducing the risk of crop loss, the costs also increase significantly. Therefore, even if it is possible to irrigate, as far as possible, it is advisable to choose plant varieties with outstanding stress-resistant properties, with which the difficulties of cultivation can be minimized. Due to its biologically active compounds, it also plays an important role as a functional food in preventing cardiovascular diseases. The primary goal of the breeding program carried out during the research is to examine the developmental stages and productivity of potato genotypes under optimal water supply and drought conditions, as well as to promote the selection and cultivation of breeding lines resistant to extreme weather conditions. In addition, we were looking for a simple but effective way to validate the results of our *in vitro* experiments under *in vivo* conditions.

Summarized in points, the following examination objectives were realized:

1. Testing the osmotic stress tolerance of potato breeding lines in vitro

- Based on stress index (SI) calculated from simple morphological parameters, comparison of 27 breeding lines with drought-tolerant referent lines;
- Application of different osmotic agents, selecting the most effective method for modeling osmotic stress *in vitro* and for selecting genotypes based on their stress tolerance. The targeted trait-specific selection, initiated under *in vitro* conditions, can significantly reduce the time required to develop a new variety. Thus, the results of laboratory studies conducted prior to field trials can provide a solid foundation for the subsequent stages of plant breeding.

2. Testing of potato breeding lines under isolated and greenhouse conditions

- The effect of plant density and planting according to tuber size as influencing factors on the physiological state of breeding lines.

3. Further in vivo testing of selected potato breeding lines

- Testing the drought tolerance of potato genotypes *in vivo* by applying different amounts of water and using different sized tubers;
- Comparison of developmental, morphological and yield characteristics (number of tubers, weight and size distribution) under drought stress;
- Detection of the after effects of drought in the following growing season;
- Comparison of developmental, morphological and yield characteristics of the tested potato breeding lines with known drought-tolerant control varieties ('Desiree', 'Boglárka').

2. MATERIAL AND METHODS

Location of laboratory (*in vitro*) osmotic stress tolerance tests were (*figure 1 a*) the Centre for Agricultural Genomics and Biotechnology of the Faculty of the Agricultural and Food Science and Environmental Management of the University of Debrecen and the Research Institute of Nyíregyháza Institutes for Agricultural Research and Educational Farm of the University of Debrecen. In the experiment, 29 potato genotypes, including 27 breeding lines and 2 referent lines, were examined in 2019. These referent genotypes were selected during previous *in vivo* experiments, where their drought tolerance was also tested. The *in vitro* shoot cultures were started from seeds, after surface sterilization and placement on Murashige–Skoog medium (hereafter MS), which contained salts and vitamins, 3% sucrose and 0.7% agar-agar, they were incubated in the dark at 24 °C for 5 days. After this time, they were placed in a plant breeding room at 22/15 ± 2 °C day/night temperature and 16 hours of daily light (65 µmol m^{-2} s^{-1} PPFD) and continued to develop. After 4 weeks, the plant materials of the referent and breeding lines were replanted on MS medium.

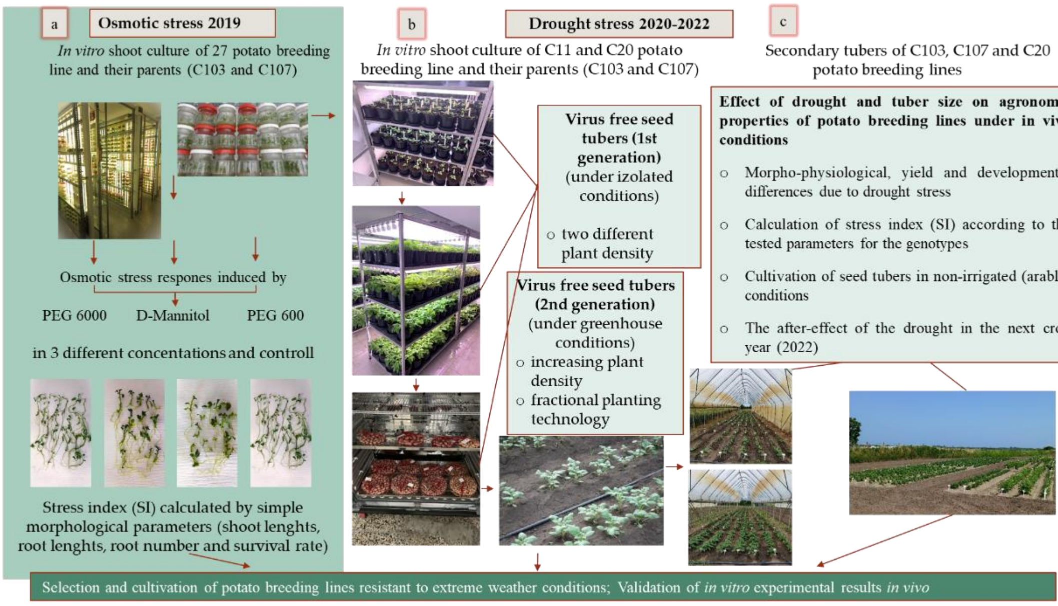

figure 1: The experiments that form the basis of the dissertation (Nyíregyháza, 2019-2022)

Nodal segments containing one axillary bud were used as explants from the *in vitro* 4-week-old shoot cultures. The explants were placed on general MS culture medium, which contained 0.3 % sucrose, 0.7 % agar and polyethylene glycol of different molecular weights (PEG 600 and PEG 6000) in concentrations of 2.5, 5.0, 7.5 and 5.0, 7.5, 10.0 %, and D-mannitol 0.1, 0.2 and in a concentration of 0.3 M, which were used to induce osmotic stress, while the control medium did not contain any osmotic. I measured the osmolality of the osmotics to be included in the experiment (D-mannitol, PEG 600 and PEG 6000) in different concentrations together with the nutrient medium constituents. The type of culture medium is MS basic culture medium, except for the solidifying agent. The concentrations used in the experiment were selected on the basis of previous establishing experiments (*table 1.*).

table 1: Osmotics, their concentrations and osmolality used in the experiment (Source: HANÁSZ et al., 2023, revised)

PEG 6000 Molecular weight: 5400–6600 MS +3% sucrose		**PEG 600** Molecular weight: 570–630 MS +3% sucrose		**D–mannitol** Molecular weight: 182.17 MS +3% s sucrose		**Control** (osmotic free) MS +3% sucrose
% w/v	mOsm kg^{-1}	% w/v	mOsm kg^{-1}	M	mOsm kg^{-1}	mOsm kg^{-1}
5.0	228.8	2.5	240.0	0.1	350.0	191.3
7.5	251.3	5.0	309.7	0.2	454.0	
10.0	274.0	7.5	417.3	0.3	539.5	

In the first step of the test, a calibration line was prepared. The calibration was done with a 500 mOsmol NaCl solution. The calibration was performed in at least four repetitions to obtain the baseline.

20 explants and 50 mL of nutrient medium were placed in a glass jar in 5 repetitions, a total of 100 plants were observed per treatment. The entire experiment was repeated twice. The shoot cultures were grown under controlled conditions, with a day/night temperature of 22/15 ± 2 °C and 16 hours of daily illumination at a light intensity of 65 μmol m^{-2} s^{-1} PPFD for another 4 weeks. At the end of the experiment, we observed the explant survival rate (SR), shoot length (SL), and the number (RN) and length (RL) of the surviving individuals. Before the evaluation, the results were expressed as a percentage, compared with the results of the individuals measured on the control medium (*table 2*).

table 2: Stress index equations for the various parameters (*in vitro*) (Source: KPOGHOMOU et al., 1990, revised)

Metodology of SI calculation
SI_SL = Shoot lengths of treated plants (mm)/shoot lengths of untreated (control) plants (mm) × 100
SI_RL = Longest root of treated plants (mm)/Longest root of untreated (control) plants (mm) × 100
SI_RN = Number of roots of treated plants/Number of roots of untreated (control) plants × 100
SI_SR = Survival rate of treated plants (per jar)/Survival rate of untreated (control) plants (per jar) × 100

In the next stage of the experiment (*Figure 1 B*), 1st and 2nd generation tubers of breeding lines with different osmotic stress tolerance properties were examined under isolated and greenhouse conditions. The *in vitro* and acclimatization phase in the Centre for Agricultural Genomics and Biotechnology of the Faculty of the Agricultural and Food Science and Environmental Management of the University of Debrecen, the greenhouse experiment in the Research Institute of Nyíregyháza Institutes for Agricultural Research and Educational Farm of the University of Debrecen were carried out. The starting subjects of the experiment are *in vitro* shoot cultures of potato parent lines (C103, C107) and their breeding lines (C11, C20), which were selected based on the previous *in vitro* osmotic stress tolerance test (HANÁSZ et al., 2022). The breeding lines included in the study (C11, C20) are crosses from the parent lines. The first step of introducing the breeding lines into *in vitro* culture was the surface sterilization of the shoot buds taken from the tuber, during which they were first treated in a 12% $Ca(OCl)_2$ solution for 5-8 minutes, then, after rinsing with sterile distilled water, the shoots were soaked in 70 % ethanol for 30 seconds and it was repeatedly rinsed with distilled water three times. After that, a meristem with a size of approximately 0.1-0.3 mm was prepared and then placed on a starter culture medium (DOBRÁNSZKI, 2006). The MS-based media (MURASHIGE és SKOOG, 1962) used during the *in vitro* cultivation of potatoes contain macro- and microelements as well as vitamins, 3 % sucrose and 0.7 % agar. The pH of the culture medium was set to 5.7 before autoclaving. Sterilization was performed at 121°C, 105 Pa pressure in an ST-124/2 type autoclave for 30 minutes. After starting from the meristem, plants with 7-8 leaves developed within 1-2 months, from which the rooted shoot cultures for the experiments were produced by micropropagation from the lines that proved to be virus-free. During micropropagation, single-bud shoot pieces (nodal segments) were placed on the culture medium (20 explants/glass jar). For breeding, we used 400 ml cylindrical glass jars containing 50 ml of culture medium. The plants were grown under 16 hours of light, 22/15+2°C day/night temperature range, 65 µmol $m^{-2}s^{-1}$ PPFD light intensity for four weeks. For the experiment, we produced 10 culture pots per genotype, i.e. 200 *in vitro* plants. The 4-week-old shoot cultures were grown in an acclimatization room for the next 3 months. The nutrient medium was a general potting soil. In the first month, the plants grew under 16 hours of illumination, and in the second two months under 12 hours of illumination. The set temperature is 21 °C, the relative humidity is 55 %. The required amount of water was given to the plants twice a week, which meant 500 ml per pot (3 l capacity). Each genotype was planted in 10 pots, singly, and in another 97 pots, we planted the plants in pairs, with a total of 428 pots. One week before harvesting, we removed the leaves of the plants. At harvest, the individually planted plants (10 pieces/genotype) were harvested separately. The number of tubers was recorded, both in the case of plants planted singly and in pairs. After harvesting, the tubers were placed in a climate chamber at a temperature of 12 °C for the rest period, for 60 days. To break dormancy, gibberellic acid treatment (5 mg l^{-1}) was applied to the minitubers (COLEMAN ÉS COLEMAN, 2000; SASANI et al., 2009).

The 1st generation tubers were planted in the greenhouse conditions on July 27, 2020. Genotypes in 18 replicates, with 2 different plant distances (25 cm (T1) and 15 cm (T2)), divided into two different tuber size ranges (d1 (20-45 mm) and d2 (-19 mm)), d1 planted with T1, d2 planted with T2 plant distances, in 3 m x 0.75 m plots, in a randomized block layout, with sprinkler irrigation (DAP (Day After Planting) 79 until 5 l m^{-2} day^{-1}, between DAP 79 and 93 with an intensity of 2.5 m^{-2} day^{-1}).

We observed the dynamics of germination according to genotype and tuber size range, recorded plant height and leaf weight, before harvesting, we performed fluorescence measurements on parental lines C103 and C107, examined total chlorophyll, chlorophyll-a and chlorophyll-b content by spectrophotometric measurement, and the tuber fractions distribution and the weight of the harvested tubers were assessed per plot according to genotype and planting density.

Chlorophyll-a, -b, and total chlorophyll content were determined using spectrophotometric method (FELFÖLDY, 1987). According to genotype and T1, T2 plant density, 500 mg of fresh shredded leaf were taken in 4 replicates. The isolates were stored at -80 °C in an ultra-deep freezer until use. The fresh shredded leaf were mechanically crushed using MgO and quartz sand, then the absorbance of the solutions was examined with a spectrophotometer using a solvent at 653, 666, and 750 nanometers (A653; 666; 750) light wavelengths. During the calculation, the value measured at 750 nanometers was subtracted from the absorbance measured at both 653 and 666 nanometers, which represents the turbidity in the solution. The values were calculated according to the following method:

- chl a = 17.12 A_{666} – 8.68 A_{653}
- chl b = 32.23 A_{653} – 14.55 A_{666}
- chl a + chl b = 2.57 A_{666} + 23.6 A_{653}
- The unit of measure μg g^{-1} fresh weight of leaf (FW)

The measurements were carried out with an OS5p modulated fluorimeter instrument, on C103 and C107 genotypes at T1 and T2 plant distances, in four repetitions. One fully developed leaf was sampled per measurement and per plant. With the help of cover clips, after keeping in the dark for 30 minutes, we measured the Fv/Fm ratio (potential quantum efficiency of the PSII reaction center), which shows the utilization efficiency of the absorbed energy, according to the protocol of the fluorimeter software. It has no unit of measure, like other efficiency parameters.

The *in vivo* drought tolerance experiment (*figure 1 c*), during which the effect of drought and tuber size on the agronomic properties of the selected potato breeding lines was tested, in the Research Institute of Nyíregyháza Institutes for Agricultural Research and Educational Farm of the University of Debrecen were carried out. The *in vivo* experiments were performed in two consecutive years, 2021 and 2022. Information on local meteorological data was provided by Pessl Instruments µMetos meteorological station. The soil type was humic sandy (1.52% humus; pH 6.32; P_2O_5: 231 mg kg^{-1}, K_2O 131 mg kg^{-1}, NO_3+NO_2 8.1 mg kg^{-1}).

During the *in vivo* experiment conducted in 2021, we used the 2nd generation of virus-free seed tubers (minitubers) with a size range of 10-35 mm, which were derived from *in vitro* shoot cultures. Three potato breeding lines (C103, C107 and C20) were included in greenhouse experiment, which were selected based on the experimental phases described above. Larger tuber sizes (≥25 mm) of the same breeding lines were propagated and observed under field conditions. The breeding line C20, which is a crossing of C103 and C107, was selected for its high survival ability to osmotic stress. Before planting, the tubers were classified according to their size into the following categories: 25-35, 20-24, 15-19, 10-14 mm. The tubers were planted in between May 5-6, 2021, and the harvest was carried out on September 27, 2021. In addition to the breeding lines (C103, C107 and C20) tested during the *in vivo* experiment conducted in 2022, the varieties 'Boglárka' and 'Desiree' were included as control varieties in the experiment.

In the greenhouse experiment conducted in 2021, minitubers of potato breeding lines were planted and the effect of tuber size and three water treatments (including optimal water supply) were examined in three repetitions.

In the *in vivo* experiment conducted in 2022, the potato minitubers (C103, C107 and C20) harvested in the greenhouse experiment of the previous year (in 2021) were grouped according to the previous water treatment. Only tubers in the 25-35 mm size category were used for planting, and two water treatments were applied in four repetitions. The tubers were planted in 3.0 m × 0.75 m plots with a spacing of 0.25 m, in a randomized block layout. We planted twelve mini-tubers in each plot. Cultivation was carried out according to the protocol used for potatoes, with the application of appropriate crop protection.

In the experiment carried out in 2021, in the early stages of development, in order to achieve a homogeneous plant culture, the plants were given an optimal amount of water (5 mm day^{-1}). The water was applied through a drip water supply system (drip irrigation tape, flow rate 1.6 l h^{-1}). From the 38th day after planting (DAP), we started to reduce the water doses. With a unique water measuring device: 5 mm per day (W1, 100 %: control), 3 mm per day (W2, 60 %) and 1 mm per day (W3, 20 %) were dosed until DAP 62. From DAP 63, we further reduced the amount of water by 50 % (2.5, 1.5, 0.5 mm day^{-1}) to maintain the drought stress induction. This amount was given to the plants for another 25 days. The tubers were propagated in the field plots without irrigation, with a plot size of 10 m × 0.75 m and a spacing of 0.25 m (40 tubers $plot^{-1}$).

In the experiment conducted in 2022, the potato tubers received the optimal amount of water (5 mm day^{-1}) until the desired initial development level was reached (DAP 37). After that we started applying reduced water doses, 5 mm (W1: control) and 1 mm (W2) per day. During the tuber ripening stage, we further reduced the amount of water by 50% (2.5 and 0.5 mm day^{-1}) - using the previous year's method.

The same observations were made during the experiments conducted in 2021 and 2022. The emergence rate (in % form) was observed on DAP 20, DAP 23, DAP 28 and DAP 30 days, and the flowering rate (in % form) on DAP 48, DAP 51 and DAP 54 days. The physiological state of the plants was evaluated by measuring the NDVI value (4 measurements per plot) with a Trimble® GreenSeeker hand-held sensor. The measurements were made at a distance of 50 cm from the plant's foliage, at the same time. We examined the effect of water deficit on the photosynthetic activity of plants at a given developmental stage (DAP 35, DAP 45, DAP 75 days).

Plant height (SL, cm) was measured at 76 days DAP (4 measurements per plot), and samples were collected to determine fresh and dry mass of above-ground biomass (FAB and DAB, g $plant^{-1}$). The remaining foliage was removed by cutting, and the fruit was harvested on DAP 102. We recorded the number of tubers per plant (TN) and the fresh tuber yield (TY, g per plant). Then, we calculated the stress indices (SI) as percentages of the results obtained from the control plots (*Table 3*), in order to compare the breeding lines responses to different levels of drought stress. According to the formula, the stress index (SI) is generally a maximum of 100, and the higher the value, the better the variety's stress tolerance. We also examined the changes in size of the harvested tubers due to water deficit (based on counting and weight measurements). The statistical evaluation of the data collected during certain stages of the experiments in the research program was performed as summarized in *Table 4.*

Table 3: Determination of stress indices (Source: KPOGHOMOU et al., 1990, revised)

The methodology for calculating the SI (Stress Index)
SI_ FAB = Fresh above-ground biomass weight of treated plants (g $parcel^{-1}$; W2, W3)/ Fresh above-ground biomass weight of untreated (control) plants (g $parcel^{-1}$; W1) × 100
SI_DAB = Dry above-ground biomass weight of treated plants (g $parcel^{-1}$; W2, W3)/ Dry above-ground biomass weight of untreated (control) plants (g $parcel^{-1}$; W1) × 100
SI_PH = Height of treated plants (cm; W2, W3)/ Height of untreated (control) plants (cm; W1) × 100
SI_TN = Number of tubers in treated plants (pieces; W2, W3)/ Number of tubers in untreated (control) plants (pieces; W1) × 100
SI_TY = Yield of treated plants (g; W2, W3)/ Yield of untreated (control) plants (g; W1) × 100

Table 4: Statistical methods used in the different phases of the research

Experimental phase	*In vitro* osmotic stress tolerance experiment	*In vivo* experiment (acclimatized and field-grown)	*In vivo* drought tolerance experiment
Data management	SPSS Statistics 27.0 (IBM, USA, New York)		
Applied tests	ANOVA LSD Tukey-B ($p < 0.05$)	LSD Tukey-B ($p < 0.05$)	ANOVA Tukey-B ($p < 0.05$) Spearman's rank correlation

3. RESULTS

This chapter summarizes the most significant results of the parameters examined in the previously discussed experimental phases, in terms of the dissertation. The other experimental results are included in the publications by HANÁSZ et al. (2022), HANÁSZ et al. (2023), and HANÁSZ et al. (2024).

3.1 *In vitro* osmotic stress tolerance experiments on seedling cultures of potato breeding and referent lines

The interpretation in the multi-parameter stress index (SI), which is based on the percentage comparison to the control (untreated plantlets), enables a clearer understanding of the differences between genotypes according to their unique stress tolerance. Accordingly, we determined a stress index from our results for each examined treatment and evaluated the examined genotypes based on their SI values (*table 5*). The research basis of the dissertation is the morpho-physiological responses to osmotic stress of these parameters (shoot and root length (SL, RL), number of roots (RN) and survival rate (SR), which summarize adaptation and damage reactions. After calculating the SI values, we compared the osmotic stress tolerance and response of 27 breeding lines and 2 drought-tolerant referent potato genotypes. From the calculated stress index values, which are based on the averages of all treatments and measured breeding and reference lines, we concluded that the inhibition of shoot growth (40.1) was higher than that of the root system (60.8, 82.6). During the experiment, we also established that the treatments affected all morpho-physiological parameters, and we found differences between the genotypes.

table 5: The effect of different osmotic agents and stress levels on morpho-physiological parameters based on stress index (SI) values (Source: own data, Nyíregyháza, 2019)

Parameter (SI, %)	**PEG 6000**			**PEG 600**			**D-mannit**			Total μ
	5 %	**7,5 %**	**10 %**	**2.5 %**	**5.0 %**	**7.5 %**	**0.1M**	**0.2M**	**0.3M**	
Survival rate	87.8	87.8	78.4	85.9	56.1	21.7	98.5	92.2	78.5	
μ	83.7			54.6			89.8			76.0
Shoot length	45.1	39.3	38.2	55.7	32.3	18.3	68.9	39.1	23.7	
μ	40.9			35.4			43.9			40.1
Root length	61.5	64.9	58.0	76.4	47.5	30.1	90.5	70.0	48.3	
μ	61.5			51.3			69.6			60.8
Root number	96.6	99.4	105.2	80.8	62.2	41.5	93.9	88.3	75.8	
μ	100.4			61.5			86.0			82.6
Total μ	71.6			50.7			72.3			64.9

Note: μ: averages

We also detected significant interactions between the genotypes and the type and concentration of the osmotic agent.

The survival rate (SR) stress index (SI) values (*Table 6*) were significantly reduced or most often not affected by 5 % PEG 6000. A significantly lower SR SI value was obtained for some breeding lines than in the case of the referent lines. The increase of PEG 6000 (7.5 %) significantly reduced SR SI values in few cases, while other breeding lines reacted similarly to those observed at 5 % PEG. The 10% concentration of PEG 6000 resulted in a significant decrease in the SR SI values of seven potato breeding lines (C2, C17, C20, C28, C41, C42 and C57) compared to the 7.5% values.

Although the best SR results were obtained for the referent line C103 and breeding lines C8 and C30, similar SI values were also observed for eight other breeding lines (C2, C10, C11, C12, C14, C20, C21, C22). However, the SR SI value of the C107 referent line was significantly reduced.

All potato breeding lines and both referent lines responded with reduced shoot length (SL) to the presence of PEG 6000 at the tested concentrations. In general, inhibition of shoot growth increased with increasing PEG 6000 concentration to 7.5 %, but higher PEG doses did not result in further significant reduction. The best result (76.8 %) was obtained in breeding line C9, although the SL SI values of some lines were very similar (C2, C22 (73.7 %), C30 and C41 (68.0 %)). At elevated PEG 6000 concentration (7.5 %), five breeding lines (C2, C9 (64.1 %), C12, C30 and C32 (54.2 %)) showed significantly higher SI values compared to both referent lines. At the highest level of PEG 6000 (10 %), only one breeding line (C2) achieved a significantly higher SI value compared to both reference lines, but we measured a higher stress index value for most of the breeding lines, than in the case of the C107 referent line.

The use of 5% PEG 6000 on medium reduced the root length (RL) in all breeding lines and in the referent lines. 10 breeding lines (C5, C8 (83.8 %), C9 (87.7 %), C10 (88.2 %), C14 (88.1 %), C19 (67.5 %), C22 (74.7 %), C30, C35 (89.0 %) and C63 (71.2 %)) RL SI values were significantly higher than those of the referent lines. When 7.5 % PEG 6000 was applied, increased SI values were obtained in some breeding lines, and the values of four breeding lines (C10 (87.9 %), C14 (111.8 %), C22 (90.0 %), and C30) were significantly higher than those of the two referent lines. When 10 % PEG 6000 was added to the medium, only the C22 (95.9 %) and C5 breeding lines had a higher RL SI value than the C103 referent line, and the C107 genotype had a lower RL SI value than most breeding lines. With few exceptions (C14, C30), the application of

Table 6: Stress index values belonging to the highlighted 9 breeding and 2 referent lines (cells marked in blue: outstanding SI values according to the selection criteria) lines (Source: HANÁSZ et al., 2022, revised)

Parameter	Breeding line	PEG 6000			PEG 600			D-Mannitol		
		5%	7.5%	10%	2.5%	5%	7.5%	0.1M	0.2M	0.3M
SR (survival rate)	C2	100	99	92	100	63	24	100	100	100
	C5	93	92	83	92	58	36	100	95	89
	C8	100	100	100	100	77	34	100	100	100
	C11	100	99	96	100	47	27	100	95	92
	C12	99	100	96	100	57	34	100	97	74
	C14	87	100	96	89	57	34	100	94	77
	C30	100	100	100	100	64	13	100	100	100
	C58	79	83	89	93	95	40	87	73	70
	C63	98	54	80	84	78	34	100	100	100
	C103	95	100	100	99	87	53	100	100	68
	C107	93	96	74	74	69	45	90	85	44
SL (shoot length)	C2	65.9	62.4	56.7	57.9	48.1	17.4	54.4	34.6	23.0
	C5	24.9	26.9	30.3	61.4	28.6	16.0	65.4	29.2	24.5
	C8	62.2	22.8	35.4	68.7	21.9	5.0	93.2	46.8	24.2
	C11	41.4	36.5	41.8	61.4	22.7	14.0	68.3	36.1	19.0
	C12	52.3	49.2	57.8	24.2	29.3	20.4	119.7	51.8	36.1
	C14	34.7	40.3	34.2	45.3	28.7	19.3	89.0	52.6	40.9
	C30	67.8	62.5	41.5	42.0	9.5	2.8	67.8	35.0	17.7
	C58	38.1	34.8	37.0	67.6	44.1	23.5	81.2	46.5	24.7
	C63	52.3	26.0	37.0	54.9	33.6	12.5	89.3	42.5	25.1
	C103	37.5	42.6	52.3	70.4	49.3	43.5	60.6	41.1	23.3
	C107	26.1	31.5	24.4	62.7	35.6	25.6	51.0	34.9	13.2
RL (root length)	C2	50.7	69.5	46.8	57.7	31.1	8.5	97.9	61.0	37.6
	C5	69.4	84.2	85.1	99.5	92.3	46.7	86.5	73.3	78.5
	C8	83.8	58.4	72.3	73.9	26.0	3.7	101.9	84.6	70.0
	C11	47.4	33.7	48.0	93.4	25.5	14.5	90.0	79.3	60.3
	C12	47.2	76.7	58.8	51.8	66.1	27.4	94.5	69.3	56.5
	C14	88.1	111.8	57.0	92.4	25.4	9.3	93.7	50.6	21.7
	C30	91.0	106.3	81.5	49.0	8.9	2.5	74.7	68.9	39.8
	C58	63.2	79.5	82.4	81.7	59.0	44.1	88.6	78.9	52.0
	C63	71.2	43.0	53.7	131.1	107.8	73.4	119.9	96.5	53.0
	C103	56.5	73.6	84.7	98.6	87.1	64.2	108.8	103.7	44.2
	C107	55.4	67.1	46.3	84.9	59.7	54.2	85.4	77.9	68.4
RN (root number)	C2	108.1	111.9	135.1	109.3	103.0	58.9	91.4	74.4	67.6
	C5	77.6	76.9	80.3	74.2	72.2	56.7	113.2	93.1	97.5
	C8	98.2	154.9	121.2	70.7	47.4	22.5	108.0	100.9	77.0
	C11	110.2	89.7	113.9	96.7	48.9	35.3	80.5	121.2	121.7
	C12	101.9	117.7	110.8	50.7	43.2	28.4	80.5	67.7	45.0
	C14	90.2	87.9	80.6	81.5	59.8	35.7	108.5	94.7	70.6
	C30	70.7	67.2	110.2	91.9	32.4	25.4	105.3	119.4	96.5
	C58	84.8	96.8	112.4	80.2	73.3	41.6	105.1	104.8	87.7
	C63	78.8	66.4	71.3	84.8	58.6	28.8	84.2	83.3	91.4
	C103	149.7	173.0	180.3	94.3	73.0	72.2	100.9	82.4	68.8
	C107	138.2	161.0	220.0	83.6	61.4	57.7	90.5	76.6	67.0

PEG 6000 to the root lengths (RL) of the tested breeding lines showed a decreasing trend in parallel with the increase in concentration. Significantly higher stress index values for the root lengths of the shoot cultures were calculated for genotypes C22, C30, as well as C5 and C58 breeding lines, although not significantly in all cases, but higher values.

PEG 6000 treatments caused a significant growth in the number of roots (RN) in the referent lines and some breeding lines, however, the best RN SI values were obtained in the referent genotypes.

5 % PEG 6000 added to the medium resulted in reduced root number in several breeding lines, however, some lines responded to the elevated (7.5 %) PEG concentration with increased SI values. Increasing the osmotic concentration level to 10 % in case of four breeding lines, C8, C26, C41 and C57 resulted in a significant decrease in SI values, in contrast in C2, C3 (82.7 %), C11, C30, C35 (111.8 %), C37 (129.0 %) and C58 breeding lines showed significantly elevated values. The PEG 6000 osmotic agent generally resulted in reduced root number (RN) in 1/3 of the genotypes, however, high stress index values were calculated in the remaining cases. In addition to the referent lines C103 and C107, high SI values were obtained in the C2, C4, C8, C11, C12, C19, C30, C32, C37, C42, C57 and C58 breeding lines.

The survival rate (SR) SI values decreased with increasing PEG 600 levels. At the lowest (2.5 %) PEG 600 concentration, the SI values of the survival rates were generally higher than 70 %, and some breeding lines reached 100 % as well. C9 and C32 breeding lines explants survived at a significantly lower rate than the reference lines. The next (5 %) level of PEG 600 was associated with a considerable decrease in the survival rate of several tested breeding lines and referent lines. When comparing the referent lines C103 and C107 with C57 (94 %), C58 and C19 (88 %) breeding lines, higher SI values were obtained, but the results only showed a significant difference compared to the referent line C107. In the case of breeding lines C19 (88 %) and C20 (87 %), we measured outstanding survival ability based on their high SI values. Similarly, the survival ability of the breeding line C20 proved to be high compared to both referent lines, although the highest level of PEG 600 (7.5 %) caused a significant decrease in the TA SI values for all genotypes without exception. Most of the breeding lines generally showed a significantly lower survival rate (SR) stress index value than the referent lines. The breeding and referent lines responded with significantly reduced shoot length (SL) SI to the presence of PEG 600 at 2.5 % concentration, and reduced shoot growth was observed with increasing PEG 600 concentration. Based on the SL stress index value, the reference genotypes showed outstanding stress tolerance compared to the breeding lines. In the case of the referent genotypes, higher stress index values were measured for the

C103 line compared to the C107 line, as well as the increase in PEG 600 levels resulted in a greater difference between the SI values of the two referent lines. At the 2.5 % level of PEG 600, only the SI values of C6 (75.7 %) and C42 (79.5 %) significantly exceeded the stress index values of the two referent lines. An excellent SI values were also measured for the breeding line C19 at two stress levels (2.5 % and 5.0 % PEG; 75 and 53.9 %). At the highest concentration level (7.5 %), the referent lines achieved the highest SI values among the genotypes.

The growth inhibitory effect of PEG 600 on root lengths (RL) of *in vitro* potato shoot cultures increased with increasing levels of osmotic agent. The 2.5 % concentration of PEG 600 caused a growth-stimulating effect on the length of their roots in 4 breeding lines based on their SI values (C5, C20 (105.6 %), C57 (105.6 %) and C63) to a significantly different extent from the reference lines. The C63 genotype showed outstanding RL stress index values at all levels of the osmotic. Based on stress index values, the C5 breeding line also tolerated the osmotic stress induced by PEG 600 treatments well.

The increase in PEG 600 levels caused a decrease in SI values for root number in most of the tested breeding lines. At the lowest osmotic level of PEG 600 (2.5 %) high stress index values were calculated for genotypes C2 and C3 (108.0 %) and at the next osmotic level (5.0 %) they also showed significantly higher values compared to the reference and breeding lines. The highest level of PEG 600 (7.5 %) reduced the stress index values less in the referent lines than in the other breeding lines, however, there was a significant difference between them.

D-Mannitol had no effect on the stress index for survival (SR) values in almost half (41 %) of the breeding lines, moreover, the SI value was 100 for the C2, C8, C20, C30 and C63 genotypes at all applied osmotic levels. At the lowest (0.1 M) osmotic stress level, lower survival values were obtained for the C9, C21 and C58 breeding lines and the C107 referent line, compared to the SI value of the C103 referent line. Compared to the stress index value of the C107 referent line, the breeding lines generally had higher stress index values. As a result of the higher (0.2 M) osmotic level, significantly lower stress index values were obtained for the C6, C22 and C10 genotypes compared to the SI values of the plants grown at the 0.1 M osmotic level. The highest applied osmotic concentration (0.3 M) also reduced the SI value of 12 breeding lines and the C103 reference line, and we calculated a higher SI value for all of the tested genotypes than for the C107 reference line.

The lowest osmotic level of D-mannitol (0.1 M) caused a decrease in shoot length (SL) growth in all genotypes and a general decrease as the concentration of the osmotic agent increased. Among the tested genotypes, C12 had a stress index value higher than 100 %, and we calculated significantly higher SI values for breeding lines C3 (67.4 %), C5, C8, C11, C14, C17 (74.1 %), C19 (87.4 %), C22 (71.4 %), C26 (70.8 %), C30, C32 (75.1 %), C58 and C63 compared to the referent lines. The results were similar at higher concentrations of D-mannitol (0.2 and 0.3 M), and in both cases several of the breeding lines had significantly higher SI values compared to the referent lines. The increase in osmotic levels caused a decrease in stress index values and the highest SL SI values were calculated for breeding lines C12, C14 and C17.

In the root length (RL) SI values, the 0.1 M concentration of D-mannitol caused a significant decrease in the breeding lines C4, C6, C21, C22 and C30 compared to the reference lines, but on the contrary, we experienced an increase in the SI values of the C41 (112.7 %), C57 (104.8 %) and C63 (with significantly higher SI value, then C103 referent line) genotypes. Among the referent lines, C103 showed increased SI values in response to osmotic stress. We generally observed a significant decrease in stress index values with increasing osmotic levels. Increasing the concentration (0.2 M) did not cause a decrease in the C103 referent- and the C17 and C63 breeding lines, or the decrease did not differ significantly. Almost all tested breeding lines showed a decrease in SI values at the 0.2 M osmotic level. Significantly higher SI values were calculated for the C17 (97.7 %) and C63 breeding lines compared to the C107 referent line. The highest level of D-mannitol (0.3 M) had a decreasing effect on the SI values of the genotypes tested and the C5, C8, C11, C12, C20 (55.9 %), C32 (57.9 %), C57 (64.3 %), C58, C63 breeding lines significantly exceeded the SI value of the C103 referent line. To a lower dose of D-mannitol (0.1 M), the C8, C10, C41, C57, C63 and C103 breeding- and referent lines responded with increased root length compared to the control shoot cultures (i.e. with a stress index value higher than 100 %), however, the increase in the osmotic concentration level resulted in a decrease in SI values and reached a maximum of 78.5 % (C5 breeding line).

The SI values for the number of roots (RN) in breeding lines C4, C6, C9, C11, C12, C20, C21, C35, C37, C41, C42, C57 and C63 showed a decreasing tendency for a mild (0.1 M) concentration of D-mannitol in compared to referent lines. The breeding lines C5, C17, C19 and C32 showed significantly higher SI values (between 100.9 and 134.0 %) compared to both referent lines. The increase in osmotic level (0.2 M) was generally associated with a decrease in SI values of the breeding lines, but in the breeding lines C8, C11, C14, C17 (104.9 %), C22 (94.6 %), C26 (95.3 %), C28 (93.2 %), C30, C32 (173.2 % highest SI value) C35 (96.8 %) and C58, the opposite was true, with an increase in SI values. At a concentration of 0.3 M D-Mannitol, significantly higher values were calculated for breeding lines C5, C11, C17 (98.4 %), C19 (83.8 %), C30, C32 (135.2 %), C57 (85.9 %), C58 and C63 compared to the values of the referent lines. Based on the root number (RN) SI values of the tested breeding lines, higher doses of 0.2 and 0.3 M D-mannitol added to the culture medium were suitable for effective selection.

3.2 *In vivo* experiments on 1st and 2nd generation tubers of potato breeding lines with different osmotic stress tolerance

The yield of both harvested 1st and 2nd generation tubers decreased significantly for those planted at smaller plant distance (*table 7*). Significantly different results were measured in the quantity of harvested 1st generation tubers for three breeding lines (C103, C107 and C20), while for 2nd generation tubers two breeding lines (C103 and C20) were highlighted.

table 7: Effect of plant distance on tuber yield from 1st and 2nd generation seed tubers; (Source: HANÁSZ et al., 2023, revised)

1st generation tuber		**Breeding line**	**2nd generation tuber**	
***No** (tuber plant^{-1})	****Na** (tuber plant^{-1})		*******T_1/d_1 (tuber plant^{-1})	********T_2/d_2 (tuber plant^{-1})
5.2 bc	3.5 d	**C103**	7.2 A	3.4 B
6.9 a	5 bc	**C107**	2.1 D	1.1 D
3.9 c	4 c	**C11**	0.7 EF	0.2 F
6.3 ab	5.2 bc	**C20**	5.9 AB	2.1 C

Note: No (Normal)*: 1 tuber pot^{-1}; Na (Narrow)**: 2 1 tuber pot^{-1}; T1 (plant distance)/d1 (tuber diameter)***: 25 cm/20–45 mm; T2 (plant distance)/d2 (tuber diameter)**** 15 cm/–19 mm); Lowercase letters in the table indicate statistically different values of yield (number of tuber) harvested from 1st and uppercase letters from 2nd generation tubers between genotypes and treatments ($p<0.05$)

On the size distribution of the number of tubers, narrowing had a positive effect in the case of breeding lines C103 (20-24 mm) and C20 (15-19 mm), while it had a slight decreasing effect of C107 and C11 breeding lines (15-19 mm).

Considering the size distribution calculated based on the number of tubers ($\%_{TN}$) (Table 8), higher values were measured in the C103 (20–24 mm: +14 %) and C20 (15–19 mm: +44 %) genotypes in the increased plant density, while for the C107 (15–19 mm: -10 %) and C11 (15–19 mm: -3 %) potato lines, a slight decrease was observed.

Looking at the distribution calculated according to the tuber yield ($\%_{TY}$) (*table 8*), the C107 and C11 genotypes (15–19 mm: -21 %; -17 %) showed a decreasing trend in the most common tuber size with the increase in plant density. However, the proportions changed, and the share of larger tubers (20-24 mm) increased. This trend was also observed in the C20 potato breeding line, although here the most common tuber size remained the same in the increased plant density, with a higher proportion (15–19 mm: +17 %).

table 8: Size distribution of primary tubers from normal (1 tuber per culture vessel) and narrowed (2 tubers per culture vessel) laboratory (*in vitro*) shoot cultures (Source: HANÁSZ et al., 2023, revised)

Tuber size (mm)	**Normal**							
	C103		**C107**		**C11**		**C20**	
	$\%_{TN}$	**$\%_{TY}$**	**$\%_{TN}$**	**$\%_{TY}$**	**$\%_{TN}$**	**$\%_{TY}$**	**$\%_{TN}$**	**$\%_{TY}$**
36–45	0	0	0	0	0	0	0	0
25–35	16	43	0	0	0	0	2	8
20–24	28	32	14	26	11	18	0	0
15–19	34	20	65	67	62	71	29	50
10–14	20	4	17	7	24	11	52	38
–10	2	1	3	2	3	0	2	4
Tuber size (mm)	**Narrowed**							
	C103		**C107**		**C11**		**C20**	
	$\%_{TN}$	**$\%_{TY}$**	**$\%_{TN}$**	**$\%_{TY}$**	**$\%_{TN}$**	**$\%_{TY}$**	**$\%_{TN}$**	**$\%_{TY}$**
36–45	1	0	0	0	0	0	0	0
25–35	8	43	1	2	1	2	1	3
20–24	42	32	26	47	23	39	11	26
15–19	23	20	55	46	59	54	73	67
10–14	18	4	14	4	14	5	11	3
–10	8	0	5	1	3	0	3	0

Note: $\%_{TN}$: size distribution based on the number of tubers; $\%_{TY}$: size distribution based on the yield of tubers; The cell color indicates the increase in proportion: the darker the shade, the higher the proportion of the given tuber size.

Table 9 shows the size distribution of harvested secondary tubers from different potato genotypes. In the C103 parent line, the order of decreasing proportions was as follows: tubers between 25 and 35 mm (a-c), under 10 mm (a–d, -3.9 %), between 10 and 14 mm (a–d, -4.7 %), and between 20 and 24 mm (a–e, -8.8 %) were the most common.

table 9: Size distribution of potato breeding lines tubers according to T1 (25 cm) and T2 (15 cm) plant spacing (Source: HANÁSZ et al., 2023, revised)

TS (mm)	**C103**		**C107**		**C11**		**C20**	
	T1	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
45–	3.29 b–e	1.07 c–e	0.00	0.00	0.00	0.00	0.00	0.00
36–45	5.06 b–e	3.38 b–e	1.64 c–e	0.58 e	0.00	0.00	2.98 b–e	0.00
25–35	6.62 a–c	5.82 a–e	2.02 c–e	0.98 de	0.00	0.00	6.4 a–d*	1.6 cde*
20–24	6.04 a–e	5.91 a–e	2.24 b–e	1.38 c–e	1.91 c–e	0.00	7.91 ab*	3.64 a–e*
15–19	4.36 a–e	4.58 a–e	1.96 c–e	1.87 c–e	1.69 c–e	0.00	4.98 a–e	3.56 a–e
10–14	6.31 a–d *	8.93 a*	1.96 c–e	2.48 b–e	2.53 b–e	1.33 c–e	5.96 a–e	5.78 a–e
–10	6.36 a–d	4.93 a–e	3.2 b–e	3.24 b–e	1.6 c–e	0.76 de	4.22 a–e	4.18 a–e

Note: GM: tuber size; lowercase letters indicate statistically significant differences between genotypes for T1 and T2 spacings. The "*" symbol shows significant differences between the same genotype at different spacings ($p<0.05$). Darker cell colors indicate higher proportions of the given tuber size. Unit: pieces m^{2-1}.

Less frequent were the tubers between 36 and 45 mm (a–e, -23.6 %), between 15 and 19 mm (a–e, -34.1 %), and larger (consumption size) tubers above 46 mm (b–e, -50.3 %) in plants grown at the normal (T1) plant density. In general, smaller tubers were predominant, but in terms of tuber numbers, more were harvested compared to other breeding lines. The change in plant density (T2) led to an increase in the proportion of tubers between 10 and 14 mm (a, +41.5 %), while there were differences in the proportions of tubers under 10 mm, between 36 and 45 mm, and above 46 mm, though these were not statistically significant. In the C107 parent line, the order of decreasing proportions started with tubers under 10 mm (b–e) and between 20–24 mm (b–e, -30 %). Fewer tubers were found in the ranges between 25–35 mm (c–e), 15–19 mm, 10–14 mm (c–e), and 36–45 mm (c–e), with differences of 30 %, 36.9 %, 38.8 %, and 48.8 %, respectively. Increasing plant density (T2) did not have a significant effect on the tuber size distribution, though a slight increase was observed in the proportion of tubers between 10 and 14 mm, while a decrease was noted for tubers under 10 mm, between 25–35 mm, between 36–45 mm, and above 46 mm. Among the breeding lines, fewer tubers were harvested from the C11 genotype compared to the C103 and C107 genotypes. Regarding the size distribution, the decreasing order of proportions was as follows: 10–14 mm (b–e), 20–24 mm (c–e), 15–19 mm (c–e), and under 10 mm (c–e), with differences of 24.5 %, 33.2 %, and 36.8 %. Increasing the plant density (T2) had a reducing effect on both the number and size range of harvested tubers, with 10–14 mm and under 10 mm tubers being more predominant. In the case of the C20 potato genotype, the decreasing order of tuber size distribution was as follows: 20–24 mm (ab), 25–35 mm (a–d), 10–14 mm (a–e), 15–19 mm (a–e), under 10 mm (a–e), and 36–45 mm (b–e), with decreases of 19.1 %, 24.7 %, 37 %, 46.7 %, and 62.3 %. Increasing plant density (T2) led to a reduction in the different tuber sizes, with a significant decrease in the amount of 20–35 mm tubers harvested per unit area. When comparing all the tuber sizes and plant densities (T1 and T2) for the parent lines (C103 and C107), statistically significant differences were measured. The C103 genotype consistently showed higher yields per unit area compared to the C107 parent line. In the analysis of the C11 genotype, significant differences in tuber sizes were found, with higher values in favor of the C103 parent line. Compared to the C20 genotype, significant differences were also found in terms of yield between the C103 and C107 parent lines as well as the C11 breeding lines.

3.3 Effect of drought and tuber size on agronomic properties of potato breeding lines under *in vivo* conditions

In the 2021 crop year, the different seed tuber sizes of the C103, C107 and C20 potato breeding lines were subjected to further testing with three types of water applied, which we started to administer from DAP 38 with doses of W1, W2 and W3. The aim of the experiment was to find the most suitable tuber size category for analyzing drought tolerance characteristics and the amount of water withdrawal required for interpreting drought stress on genotypes in a given climate environment.

The tuber size was a determining factor in the emergence rate of the genotypes. In tubers between 20 and 35 mm, emergence rates were generally higher (C103: 86.1–98.2 %; C107: 95.4–100 %; C20: 98.2 %) than in tuber sizes between 10 and 19 mm. During the experiment, tubers between 10 and 19 mm of breeding line C20 emerged in the lowest rate (67.6–75.0 %), and most plants were measured in lines C103 and C107, as well as between tuber size and genotype, tuber size dominated. Under field conditions, where tubers larger than 25 mm were planted, rapid germination was observed in all genotypes at a high percentage (95.3 %, 99.7 %, and 98.8 %) for the C103, C107, and C20 breeding lines, respectively.

The NDVI values increased in parallel with the time since planting (DAP: 35, 45 and 75) and the development of the plants. The effect of seed tuber size on NDVI values was detectable during DAP 35 and DAP 45 observations for the tested genotypes. The size of the seed tuber was also influential in the development of NDVI values, the NDVI values of tubers larger than 20 mm were significantly higher than those below 20 mm. We sought correlations between NDVI results and yield parameters (tuber number (TN) and tuber yield (TY)) for the examined breeding lines. Significant correlations were mainly found for the C103 and C107 potato breeding lines. However, the measurement time, genotype, and water treatments also had a significant influence on tuber number (TN) and yield (TY). While NDVI measurements showed promising potential for yield prediction, the results in our study were not consistent across all breeding lines and water treatments.

Water deprivation caused a decrease in tuber number, and the SI values showed variability, but no clear trend was observed. Based on the SI values for tuber number (TN), the C20 breeding line was the most sensitive genotype to drought. Plants receiving the least water (W3 treatment) had SI values for tubers that did not exceed 40.0 %, and no tubers could be harvested from plants that developed from the smallest tuber size (10–14 mm). At the same water level, the harvested yield SI (TY) values were lower compared to the tuber count. In the experiment, water deprivation was applied once a developmentally homogeneous plant population was established (including the early phase of tuber initiation), which plays a crucial role in the final yield formation. During the experiment, we observed that even low levels of drought stress significantly reduced both tuber number (TN) and tuber yield (TY).

Considering the average of all planted tuber sizes, the stress index (SI) for tuber count was as follows: 74 %, 86 %, and 64.7 % for the C103, C107, and C20 breeding lines, respectively. The yield-related indices followed a similar pattern. With increasing drought stress (W3), the TN SI values decreased for the C107 and C20 breeding lines, while the C103 line showed an increase in tuber count (84.7 % SI, compared to 74 % SI for the W2 treatment plots). However, examining the tuber yield (TY) SI values, the C103 breeding line under W3 treatment showed an SI of 67.9 %, which was lower than the SI calculated from the W2 plots. This suggests that although the number of tubers increased, their size likely decreased due to drought stress, as previously reported by others (NASIR and TÓTH, 2022).

In the analysis of yield parameters (*table 10*), it was found that the C20 breeding line was the most sensitive to drought stress, as it showed very low SI values (TN: 22.6% and TY: 23.6%). The causes of yield loss could be smaller tubers, fewer harvested tubers, or a combined effect of both (SCHAFLEITNER et al., 2007). Under moderate drought (W2), the TN decreased at the same rate as the TY for all breeding lines. However, under more severe drought (W3), the decrease in TY was significantly greater than the decrease in TN for the C103 and C107 breeding lines, while for the C20 line, the reduction was the same for both.

table 10: SI values of the number (TN) and weight (TY) of tubers in the average of all seed tuber sizes (Source: HANÁSZ et al., 2024, revised)

Breeding line	**W2**		**W3**	
	TN (SI, %)	**TY (SI, %)**	**TN (SI, %)**	**TY (SI, %)**
C103	73.98 ± 6.13	73.03 ±3.32	84.7 ± 8.16	67.93 ± 6.84 a
C107	86.0 ± 6.64	83.33 ± 7.08	78.2 ± 5.99	65.58 ± 7.37 a
C20	64.7 ± 10.74	65.88 ± 16.11	22.6 ± 3.73	23.6 ± 9.41 b

Note: Different lowercase letters indicate significant differences between the means of SI values of different genotypes (Tukey post hoc test; $p < 0.05$)

Examining the yield (TY) SI values (*table 10*), the C103 breeding line under W3 treatment showed an SI of 67.9, which was lower than the SI calculated from the W2 plots. This indicates that although the number of tubers increased, their size likely decreased due to drought stress. In the analysis of yield parameters, it was found that the C20 breeding line was the most sensitive to drought-induced stress, as it showed very low SI values (TN: 22.6 % and TY: 23.6 %). Under moderate drought (W2), the TN decreased at the same rate as the TY for all breeding lines. However, under more severe water shortage (W3), the decrease in TY was significantly greater than the decrease in TN for the C103 and C107 breeding lines, while for the C20, the reduction rate was the same for both.

The size distribution of the harvested tubers was significantly influenced by genotype, drought stress, and tuber size. For the C103 and C107 breeding lines, increased water shortage led to a decrease in the number (TN) and yield (TY) of larger tubers (*tables 11 and 12*). In the case of the C20 breeding line, no tubers larger than 35 mm were harvested. Regardless of the planted size fraction (i.e., when the planted tuber size categories were combined), the tuber size distribution showed that with decreasing water levels, the number of large tubers slightly decreased, and the harvested tuber size distribution heavily depended on the genotype. The greater reduction in the proportion of large tubers can be attributed to the effects of water shortage, especially when considering both tuber count and yield.

In the 2022 growing year, there was no significant difference in the height of the plants between the control potato varieties and the breeding lines that received a reduced amount of water. In 2021, there was no significant difference in the height of the plants grown from tubers that received a reduced and optimal amount of water (PW2, PW3 and PW1) either (62.5–83.3 cm). We measured a significant difference between the results regarding the breeding lines in both years. The stress index values for the breeding lines were above 80.0 %, lower values were obtained for the control varieties 'Boglárka' and 'Desiree'. In the SI values of the C103 breeding line, we observed an increase parallel to the application of the previous year's water deficit, a decrease in the C107 breeding line, and stagnation in the case of the C20 breeding line, and the values varied between 87.0 % and 90.6 %.

There was no significant difference in the SI values of fresh and dry aboveground biomass (FAB and DAB) samples taken from control plots (W1, i.e. plots that received an optimal water dose) between tubers that received a lower water amount (PW2 and PW3) and an optimal water dose (PW1) in the 2021 growing year, but the FAB stress index values were higher in the C103 and C107 breeding lines and only the lower water dose (PW3, 1 mm day^{-1}) resulted in increased SI values.

The after-effect of priming was not significantly detectable on yield parameters (TN and TY) during the experiment, although in the control plots (which received optimal water dose, W1), more tubers were harvested per plant in the case of breeding lines C107 and C20, 18.9 and 16.8 tubers respectively. PW3 (1 mm day^{-1}) water priming also resulted in an increase in yield parameters (TY) compared to PW1 and PW2 water priming (3 and 5 mm day^{-1}).

table 11: The effect of genotype, planted tuber size and different water doses (W1,W2,W3) on the ratio of the yield harvested from tubers planted according to size ranges based on tuber number (TN %) (Source: HANÁSZ et al., 2024, revised)

Genotype	Harvested tuber size (mm)	Planted tuber size (mm)											
		25–35			20–24			15–19			10–14		
		W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
C103	>45	21.52	17.77	10.37	14.27	20.26	9.06	21.07	20.33	9.77	15.35	10.88	7.01
C103	36–45	25.82	25.73	30.02	24.86	17.62	26.63	26.82	26.45	23.14	23.65	27.2	27.1
C103	25–35	24.81	28.39	29.47	26.47	33.93	35.13	26.44	28.39	32.55	33.2	24.26	28.98
C103	20–24	11.39	15.12	12.28	15.42	12.33	11.34	12.64	11.93	11.39	12.86	16.32	15.88
C103	15–19	7.85	7.42	6.55	9.2	7.49	10.2	6.52	8.06	9.04	4.98	9.2	8.41
C107	>45	2.6	1.79	1.94	3.38	0	3.81	3.38	5.54	4.39	0	0	4.17
C107	36–45	15.84	17.47	9.84	15.09	11.01	11.42	16.1	22.63	17.4	17.27	13.5	11.57
C107	25–35	33.54	31.64	34.49	31.6	37.98	33.41	41.71	35.1	32.04	31.59	35.54	29.01
C107	20–24	19.93	21.71	18.7	23.93	20	18.82	18.84	19.17	17.22	22.04	23.97	20.06
C107	15–19	12.62	11.52	15.51	10.14	11.63	12.05	9.82	6.93	11.02	10.23	10.74	13.27
C20	25–35	19.18	9.22	25.25	28.71	21.79	0	0	0	20	10.73	3.81	0
C20	20–24	27.9	21.67	38.38	43.07	34.64	0	38.33	16.26	35.83	26.84	52.46	0
C20	15–19	19.78	40.11	18.18	17.7	27.94	0	38.33	55.83	29.17	42.95	26.62	0

Note: The color of the cell indicates the degree of distribution: the darker the shade of green, the higher the distribution ratio (%).

table 12: The effect of genotype, planted tuber size and different water doses (W1,W2,W3) on the ratio of the yield harvested from tubers planted according to size ranges based on tuber yield (TY %) (Source: HANÁSZ et al., 2024, revised)

Genotype	Harvested tuber size (mm)	Planted tuber size (mm)											
		25–35			20–24			15–19			10–14		
		W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
C103	>45	58.17	49.71	32.79	42.66	53.54	31.48	54.02	44.75	34.11	46.79	32.31	26.73
C103	36–45	28.59	31.93	46.78	35.61	23.94	41.65	30.47	27.65	38.06	30.97	41.48	45.54
C103	25–35	10.3	14.91	17.05	16.7	18.96	22.76	12.47	13.64	22.98	19.11	18.78	21.78
C103	20–24	1.99	2.68	2.4	3.62	2.66	2.91	2.22	13.1	3.23	2.31	5.24	4.46
C103	15–19	0.66	0.57	0.66	1.01	0.67	0.97	0.61	0.73	1.08	0.43	1.31	0.99
C107	>45	58.58	7.89	9.87	42.66	0	17.82	54.02	16.48	17.8	46.79	0	19.32
C107	36–45	28.3	42.72	26.99	35.61	30.98	27.15	30.47	40.89	38.04	30.97	35.69	26.4
C107	25–35	10.2	35.05	46.72	16.7	50.5	40.31	12.47	33.26	33.72	19.11	46.06	37.35
C107	20–24	1.97	10.51	11.03	3.62	13.94	9.76	2.22	7.32	7.01	2.31	13.82	11.59
C107	15–19	0.66	2.63	3.77	1.01	3.1	2.97	0.61	1.37	2.16	0.43	2.88	3.86
C20	25–35	49.79	26.53	49.69	53.83	49.21	0	0	0	44.98	30.61	11.11	0
C20	20–24	33.2	31.51	39.34	38.28	35.15	0	64.61	39.02	36.33	20.41	69.44	0
C20	15–19	11.62	36.48	8.28	6.34	12.83	0	28.09	52.44	15.57	43.37	16.67	0

Note: The color of the cell indicates the degree of distribution: the darker the shade of green, the higher the distribution ratio (%).

The breeding lines did not show increased drought tolerance even at the reduced water dose (W2, 1 mm day^{-1}) based on their stress index values (*table 13*). There was no significant difference between the yield results of the plots receiving either optimal (W1, 5 mm day^{-1}) or reduced water dose (W2, 1 mm day^{-1}).

table 13: SI values calculated from the number of tubers (TN) and yield (TY) of plants developed from tubers exposed to drought stress after being primed (2021) for the breeding lines and the control varieties ('Boglárka' and 'Desiree') (Source: HANÁSZ et al., 2024, revised)

Parameter	Water dose(2021)	C103	C107	C20	'Boglárka'	'Desiree'
TN (SI)	PW1 (5 mm m^{-2} nap^{-1})	100,2 ± 8,71 ab	120,8 ± 2,34 a	78,2 ± 3,85 bc	80,2 ± 11,51 bc	54,6 ± 5,31 c
	PW2 (3 mm m^{-2} nap^{-1})	96,7 ± 11,2 ab	110,9 ± 5,84 ab	98,7 ± 3,96 ab	–	–
	PW3 (1 mm m^{-2} nap^{-1})	110,1 ± 10,53 ab	73,1 ± 9,76 bc	73,5 ±8,85 bc	–	–
	Average	102,8 ± 5,58 a	101,6 ± 7,12 a	82,5 ± 4,56 a	80,2 ± 11,51 ab	54,6 ± 5,31 b
TY (SI)	PW1 (5 mm m^{-2} nap^{-1})	61,7 ± 7,35	73,1 ± 4,03	41,9 ± 0,82	57,7 ± 11,63	41,2 ± 8,75
	PW2 (3 mm m^{-2} nap^{-1})	52,8 ± 12,48	74,0 ± 4,92	62,2 ± 5,8	–	–
	PW3 (1 mm m^{-2} nap^{-1})	56,5 ± 3,91	43,1 ± 4	51,1 ± 10,88	–	–
	Average	57,0 ± 4,65	63,4 ± 4,88	51,7 ± 4,49	57,7 ± 11,63	41,2 ± 8,75

Note: Water doses of the previous year (2021): The lowercase letters indicate statistically significant differences in means between tuber sizes within each breeding line (Tukey's post-hoc test, where $p < 0.05$).

In the average of all priming treatments (PW1, PW2 and PW3) in 2021, the effect of drought stress was statistically detectable in the size distribution (TN) calculated based on the number of harvested tubers of different sizes, and as a result of water shortage, the proportion of the majority tuber size decreased and the smaller size categories expanded. A similar trend was observed when examining the size distribution calculated based on the harvested crop (TY). In general, the largest size category of tubers harvested from control (W1, 5 mm day^{-1}) areas showed a greater or lesser decrease under drought stress (W2, 1 mm day^{-1}), and the amount of smaller sized tubers increased. Priming treatments (PW1, PW2 and PW3) generally had no significant effect on the number of tubers (TN) of genotypes in either the control (W1) or water-deprived (W2) stands, however, water deprivation caused differences, generally resulting in a decrease in the proportion of larger tuber sizes. The highest size range of tubers was measured for the C103 breeding line (over 25 mm), followed by C107 (25-45 mm) and C20 (20-45 mm). Based on the harvested yield (TY), there was no clearly measurable significant difference in the proportion of primed tubers among genotypes, however, drought stress (W2) caused a general decrease, and the size range of the majority of tubers expanded with smaller sizes. In the case of the C103 breeding line, the highest size (over 45 mm) was typical, while in the C107 and C20 breeding lines (over 36 mm and between 25-45 mm) sizes were typical.

4. NEW CONTRIBUTIONS TO ACADEMIC KNOWLEDGE

In accordance with the experimental objectives, the following conclusions can be interpreted as new scientific findings:

1. The selection based on the osmotic stress-inhibiting effect on the studied genotypes was made possible by the PEG 6000 at 7.5 % and 10 %, and D-mannitol at concentrations of 0.2 M and 0.3 M.

2. During the *in vitro* stress index selection of 27 potato genotypes, the C2 (80 %), C5 (76 %), C8 (88 %), C11 (77 %), C12 (84 %), C14 (81 %), C30 (84 %), C58 (80 %), and C63 (72 %) breeding lines were identified as outstanding in terms of osmotic stress tolerance and are promising lines for breeding purposes.

3. In field trials, tuber size and genotype influenced every examined growth and development parameter. Significant differences were observed in the early developmental phases (germination), with considerable variation in the parameters of genotypes and tuber sizes. The most significant developmental differences were measured between tubers larger and smaller than 20 mm, with a germination rate of 87 % for tubers below 20 mm and 69 % for those above 20 mm.

4. Water withdrawal had no detectable effect on flowering rate and NDVI values but had a significant impact on yield development (tuber number, yield, and size distribution). The performance and drought stress index (SI) of the breeding lines included in the study were similar to or higher than those of the control potato varieties 'Desiree' and 'Boglárka' in most of the examined parameters. Based on both *in vitro* and *in vivo* results, stable drought tolerance was confirmed for the C103 and C107 breeding lines.

5. Water withdrawal applied to secondary tubers in 2021 did not cause statistically significant differences in either morpho-physiological or yield parameters in the examined genotypes in the following production year.

5. PRACTICAL USE OF THE RESULTS

1. The selection process carried out under laboratory conditions, with the help of osmotic agents, can significantly speed up the work of breeders, which helps to create the primary goal, drought-tolerant potato varieties.

2. In experiments conducted in isolated environments and in field conditions, tuber size was found to be an important factor in terms of development and drought tolerance. In the tested breeding lines, tuber sizes above 20 mm were found to be suitable for crop production even under less than optimal water supply conditions.

3. During drought tolerance studies, it is advisable to conduct the correlation between NDVI and yield parameters according to genotype-specific phenophases, and to specifically analyze yield differences, which makes the process more time- and cost-effective.

4. In the case of seed tuber multiplication, based on our studies, the value of seed tubers from water-deficient years does not change compared to tubers from optimal water supply conditions.

6. REFERENCES

1. *Coleman, W. K. – Coleman, S. E.* 2000. Modification of potato microtuber dormancy during induction and growth in vitro or ex vitro. *Am. J. Pot. Res.* 77, 103–110. DOI: 10.1007/BF02853737
2. *Dobránszki, J.*: 2006. A biotechnológia alkalmazási lehetőségei a modern mezőgazdaságban. [In: Varga (szerk.) Versenyképes növénytermesztés I., Tantárgyi segédlet.] NYF MMFK. ROP–3.3.1.–05/1.–2005–08–005/37 P. 123–155
3. *Felföldy, L.*: 1987. Biological water qualification. Water Management Institute. 190.
4. *Hanász, A. – Dobránszki, J. – Mendler–Drienyovszki, N. – Zsombik, L. – Magyar–Tábori, K.* 2022. Responses of Potato *(Solanum tuberosum* L.) Breeding Lines to Osmotic Stress Induced in in vitro Shoot Culture. Hortic. 8, 591. DOI: 10.3390/horticulturae8070591
5. *Hanász, A. – Dobránszki, J. – Mendler–Drienyovszki, N. – Zsombik, L. – Magyar–Tábori, K.* 2022. Responses of Potato *(Solanum tuberosum* L.) Breeding Lines to Osmotic Stress Induced in in vitro Shoot Culture. Hortic. 8, 591. DOI: 10.3390/horticulturae8070591
6. *Hanász, A. – Dobránszki, J. – Zsombik, L.* 2023. Eltérő ozmotikus stressztoleranciájú burgonya szülő és nemesítési vonalak primer és szekunder gumóinak tesztelése izolált és fóliasátras termesztési közegben. *Növénytermelés.* 72 (4), 37-61, 2023.
7. *Hanász, A. – Zsombik, L. – Magyarné Tábori, K. – Mendlerné Drienyovszki, N.* 2024. Effect of Drought and Seed Tuber Size on Agronomical Traits of Potato (Solanum tuberosum L.) under In Vivo Conditions. *Agron.* Basel. 14 (6), 1-41, 2024.
8. *Kpoghomou, B. K. – Sapra, V.T. – Beyl, C. A.* 1990. Sensitivity to drought stress of three soybean cultivars during different growth stages. *J. Agron. Crop Sci.*164, 104–109.
9. *Murashige, T. – Skoog, F.* 1962. A revised medium for rapid growth and bioassays with tobacco tisssue cultures. Phys. Plant. 15, 473–497.
10. *Nasir, M. W. – Tóth, Z.* 2022. Effect of Drought Stress on Potato Production: A Review. *Agronomy.* 12, 635. https://doi.org/10.3390/agronomy12030635.
11. *Sasani, R. – Khazaee, H. R. – Nezami, A.* 2009. Effects of gibberellin, benzyl adenine, zeatine hormones and temperature on dormancy breaking of potato minituber *(Solanum tuberosum*). *J. Hortic. Sci.* 23, 2, 61–67.
12. *Schafleitner, R. – Gutierrez, R. – Legay, S. – Evers, D. – Bonierbale, M.* 2009. Drought Stress Tolerance Traits of Potato [In Proceedings of the 15th International Symposium of the International Society for Tropical Root Crops (ISTRC)]. Lima, Peru, 2009. November 2–7.

7. LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/385/2024.PL
Subject: PhD Publication List

Candidate: Alexandra Hanász
Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10062963

List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (1)

1. **Hanász, A.**, Dobránszki, J., Zsombik, L.: Eltérő ozmotikus stressztoleranciájú burgonya szülő és nemesítési vonalak primer és szekunder gumóinak tesztelése izolált és fóliasátras termesztési közegben.
   Növénytermelés. 72 (4), 37-61, 2023. ISSN: 0546-8191.

Foreign language scientific articles in international journals (3)

2. **Hanász, A.**, Zsombik, L., Magyarné Tábori, K., Mendlerné Drienyovszki, N.: Effect of Drought and Seed Tuber Size on Agronomical Traits of Potato (Solanum tuberosum L.) under In Vivo Conditions.
   Agronomy-Basel. 14 (6), 1-41, 2024. EISSN: 2073-4395.
   DOI: http://dx.doi.org/10.3390/agronomy14061131
   IF: 3.3 (2023)

3. **Hanász, A.**, Dobránszki, J., Mendlerné Drienyovszki, N., Zsombik, L., Magyarné Tábori, K.: Responses of Potato (Solanum tuberosum L.) Breeding Lines to Osmotic Stress Induced in In Vitro Shoot Culture.
   Horticulturae. 8 (7), 1-24, 2022. EISSN: 2311-7524.
   DOI: http://dx.doi.org/10.3390/horticulturae8070591
   IF: 3.1

4. Magyarné Tábori, K., Mendlerné Drienyovszki, N., **Hanász, A.**, Zsombik, L., Dobránszki, J.: Phytotoxicity and Other Adverse Effects on the In Vitro Shoot Cultures Caused by Virus Elimination Treatments: Reasons and Solutions.
   Plants-Basel. 10 (4), 1-62, 2021. ISSN: 2223-7747.
   DOI: http://dx.doi.org/10.3390/plants10040670
   IF: 4.658

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Foreign language abstracts (2)

5. Magyarné Tábori, K., **Hanász, A.**, Mendlerné Drienyovszki, N., Zsombik, L., Dobránszki, J.: Study of osmotic stress tolerance of potato breeding clones in vitro shoot cultures.
   In: 20th EAPR Triennial Conference : "Potato facing global challenges" : Keynote lectures and abstracts, European Association for Potato Research, Paris, [278] (P098), 2017.

6. **Hanász, A.**, Magyarné Tábori, K., Zsombik, L., Dobránszki, J.: Effect of antiviral chemicals on the morphological traits of in vitro potato shoots.
   In: International Conference "Climatic changes, a permanent challenge for agricultural research on potato, sugar beet, cereals and medicinal plants" Abstract of Papers and Posters. Ed.: S. C. Chiru, Minister of National Education and Scientifix Research, Brasov, Románia, 17, 2016.

Patents (1)

7. Magyarné Tábori, K., Dobránszki, J., **Hanász, A.**, Zsombik, L.: Burgonya vírusmentesítése kemoterápiás eljárással. 2021
   Hatáskör: Magyarország
   Bejelentés ideje: -
   Ügyiratszám: P1700238/10 ()
   Szabadalmi szám: 320
   Szabadalom státusza: Oltalom fennáll - Végleges oltalom alatt áll

List of other publications

Foreign language scientific articles in Hungarian journals (1)

8. Zsombik, L., **Hanász, A.**, Sipos, T., Basal, O., Magyarné Tábori, K.: Seedling morphology of different wheat genotypes at early stages under hydrocultural conditions.
   Agrártud. közl. 1, 249-254, 2021. ISSN: 1587-1282.
   DOI: http://dx.doi.org/10.34101/actaagrar/1/8301

Hungarian abstracts (4)

9. **Hanász, A.**, Zsombik, L., Dobránszki, J.: Burgonya (Solanum tuberosum L.) kallusz kultúrák ozmotikus stressztűrésének vizsgálata különböző ozmotikumokkal.
   In: XXIII. Tavaszi Szél Konferencia 2020: Nemzetközi Multidiszciplináris Konferencia. Absztrakt kötet I.. Szerk.: Barna Boglárka Johanna, Kovács Petra, Molnár Dóra, Pató Viktória Lilla, Doktoranduszok Országos Szövetsége, Budapest, 36, 2020. ISBN: 9786155586705

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

10. Magyarné Tábori, K., **Hanász, A.**, Zsombik, L., Dobránszki, J.: Exogén citokininek hatása őszi búza in vitro hajtástenyészeteinek növekedési paramétereire.
In: Maróti Mihály emlékülés Budapest 2017. április 21, [s.n.], Budapest, 13, 2017.

11. **Hanász, A.**, Magyarné Tábori, K., Zsombik, L., Dobránszki, J.: Őszi búza tájfajták in vitro hajtástenyészeteinek ozmotikus vizsgálatai.
In: Maróti Mihály emlékülés Budapest 2017. április 21, [s.n.], Budapest, 14, 2017.

12. **Hanász, A.**, Magyarné Tábori, K., Dobránszki, J.: Burgonya nemesítési klónok ozmotikus stressztűrésének vizsgálata in vitro hajtástenyészeteken.
In: XXII. Növénynemesítési Tudományos Nap : Összefoglalók. Szerk.: Veisz Ottó, Polgár Zsolt, Magyar Tudományos Akadémia, Budapest, 85, 2016. ISBN: 9789633960851

Foreign language abstracts (5)

13. Mendlerné Drienyovszki, N., Magyarné Tábori, K., **Hanász, A.**, Dobránszki, J.: In vitro osmotic stress tolerance experiments with buckwheat shoot cultures.
In: Abstract Book of 12th International Congress on Plant Biotechnology and Agriculture, [s.n.], [s.l.], 43, 2019. ISBN: 9789591642608

14. **Hanász, A.**, Magyarné Tábori, K., Zsombik, L., Dobránszki, J.: Osmotic stress tolerance experiments on in vitro shoot cultures.
In: Fiatal Biotechnológusok Országos Konferenciája. Szerk.: Tamás László, Zelenyánszki Helga, JATEPress, Szeged, 121, 2018. ISBN: 9789633153703

15. Magyarné Tábori, K., **Hanász, A.**, Mendlerné Drienyovszki, N., Zsombik, L., Dobránszki, J.: Evaluating of phytotoxicity and antiviral activity of two nucleoside analogues in potato.
In: 20th EAPR Triennial Conference : "Potato facing global challenges" : Keynote lectures and abstracts, European Association for Potato Research, Paris, [230] (P055), 2017.

16. Magyarné Tábori, K., **Hanász, A.**, Mendlerné Drienyovszki, N., Zsombik, L., Dobránszki, J.: Combined application of Virasole and Zidovudine for elimination of PVM and PVS viruses from in vitro potato plantlets.
In: "XII Simposio Internacional de Biotecnología Vegetal" : Cayo Santa María, Cuba, del 5 al 8 de abril del 2016 : Libro Resumen, Instituto de Biotecnología d las Planta, Santa Clara, 6-7, 2016.

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

17. Seres, E., Erdős, Z., **Hanász, A.**, Magyarné Tábori, K., Zsombik, L.: Evaluation of osmotic stress tolerance of winter wheat landraces.
In: International Conference "Climatic changes, a permanent challenge for agricultural research on potato, sugar beet, cereals and medicinal plants" Abstract of Papers and Posters. Ed.: S. C. Chiru, Minister of National Education and Scientifix Research, Brasov, Románia, 30, 2016.

Total IF of journals (all publications): 11,058
Total IF of journals (publications related to the dissertation): 11,058

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

25 June, 2024