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Original Paper

Physiological Indicators of Drought and Heat Resistance of Plants of the Moraceae Family

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The observed increase in global average air temperature has a profound effect on the climate, and the effects of this phenomenon are expected to be more significant in the next 30–50 years. As a result, their physiological condition deteriorates, and their defenses and productivity decrease. This fact emphasizes the relevance and feasibility of the study, which is aimed at determining the drought and heat tolerance of plants of the Moraceae family. The work uses generally accepted methods for determining the resistance of breeding samples to heat and drought. The research established that mulberry plants of Victoria, Halyna, Merefyanska, Slobozhanska, Maclura varieties showed increased adaptive resistance to high temperatures (heat and drought). In particular, damage to leaf plates in the 40–80 °C temperature range did not exceed 50%. Scoring scales were developed for evaluating plants of the Moraceae family for drought and heat resistance, which increased the accuracy and informativeness of their determination. It is emphasized that the general level of physiological processes in germinated seeds under conditions of drought indicates the high resistance of adult plants, which ensures the possibility of preserving and growing valuable species for further breeding. From a practical point of view, the complex diagnostic assessment of Moraceae plants for adaptive resistance by various methods provided a comprehensive assessment and selection of breeding material with high adaptive potential to stressful growing conditions.

Keywords: Moraceae family, drought resistance, varieties, water deficit, moisture retention capacity

1 Introduction

The global climate is undergoing significant changes, with notable impacts on the global and Ukrainian conditions due to global warming. Droughts are becoming more frequent, significantly affecting the biological cycle of insect development and plant germination, survival, and growth. The combined impact of heat and drought on plant productivity and leaf mass is more pronounced than the effect of a single stressor (Dreesen et al., 2012; Rollins et al., 2013). Drought is the most significant environmental stress for plants. Some scientists have highlighted that by the end of the 21st century, rising air temperatures will lead to an increased risk of drought. This is particularly relevant in the context of intensive anthropogenic transformation of natural landscapes, which disrupts general metabolic processes and the hormonal balance of plants, causing changes in subcellular structures. However, drought tolerance varies from plant species to plant species, so ultra-fast and objective assessment is critical for implementing appropriate irrigation strategies (Seleiman et al., 2021; González, 2023).

A typical representative of the Moraceae family is mulberry, which is successfully grown not only in temperate and subtropical zones (Biasiolo et al., 2004) but also in Ukraine. It is considered the only culture that is economically important in silk production and occupies one of the leading places among the members of the Mulberry family, whose leaves are used as the main food for the mulberry silkworm (Khamenei-Tabrizi et al., 2020; Jiang et al., 2022). Wild mulberry plantations are

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found in Africa, Asia, and North America (Awasthi et al., 2004; Mei et al., 2012). Mulberry leaves contain seven types of carbohydrates: fiber, araban, oligosaccharides, sugars, galactans, dextrins, and starch, the amount of which varies during the growing season (Mei et al., 2012; Thaipitakwong et al., 2018). However, the fodder qualities of the leaves must meet the needs of the caterpillars and ensure their normal development (Zhang et al., 2019). The presence of carbohydrates in the mulberry leaves in a certain ratio to proteins is of great importance for the development rate and viability of mulberry silkworm caterpillars. However, the negative effects of global warming and heat stress affect the biochemical composition (reduction of cell volume) and yield of mulberry leaves and fruits (Sharma et al., 2020; Ackah et al., 2022). Mulberry demonstrates a high tolerance to environmental stresses, including salinity, drought, and heavy metals. However, current research on determining the adaptive capacity of mulberry to unfavorable environmental factors is mainly focused on the selection of sustainable resources and the determination of physiological parameters (Gan et al., 2021).

In sericulture, as in other crop production sectors, breeding aimed at increasing plant tolerance to adverse environmental conditions is important (Cao et al., 2020) and creating drought- and heat-resistant varieties (Sun et al., 2023). At the same time, not all varieties of the Moraceae family are suitable for this purpose. In this context, only those identified and isolated from the genera *Morus* and Maclura play a key role, and their representatives are also in the unique collection of the Laboratory of Sericulture and Technical Entomology of the NSC "IECVM" (National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine") (Kharkiv). The collection serves as the primary source material for breeders, facilitating the development of novel, high-yielding, environmentally resilient fruit varieties with enhanced fruit taste, and elevated levels of biologically active compounds, vitamins, and microelements. The collection currently comprises 113 varieties and breeding forms from 12 countries, including 8 from russia, 4 from Bulgaria, 2 from Romania, 1 from Italy, 1 from Georgia, 11 from Uzbekistan, 1 from Azerbaijan, 2 from India, 1 from China, 2 from Korea, and 6 from Japan. Additionally, 71 varieties are from different regions of Ukraine, and the collection is unique in Ukraine.

Nevertheless, the collection, as a National Heritage of Ukraine, must be preserved in the form of species-specific plantations of mulberry trees of varying ages, restored, replenished, and cultivated as valuable planting material. The primary challenge in replenishing the genetic resources of drought-resistant and heat-resistant mulberry varieties is the inadequacy of the system for assessing the parameters of vegetative reproduction of fruiting mulberry (*Maclura rhomifera* (Rafin.) Schneid). In this regard, a comprehensive investigation of the influence of elevated temperatures on diverse biological entities not only anticipates long-term implications for their capacity to adapt and persist (Bogach et al., 2020; Paliy et al., 2021; Korkh et al., 2023), but also facilitates the expeditious development of resistant animal breeds and plant varieties by breeders (Zhang et al., 2021; Worku et al., 2023).

Given the above, it is crucial to determine the biological basis of resistance of different varieties of the Moraceae family to dehydration and create drought-resistant, heat-resistant, highly productive mulberry varieties. The solution to this urgent task will, first of all, reduce the cost of growing mulberry saplings with high leaf and berry yields, and, as a result, further replenish the mulberry gene pool collection of the NSC "IECVM" with valuable genetic material.

The objective of the research is to ascertain the drought and heat resistance of plants in the Moraceae family.

2 Material and Methods

2.1 Characteristics of Weather Conditions and the Research Object

The study was conducted on plants of the Moraceae family from the gene pool of the NSC "IECVM" using accepted methods for determining the resistance of breeding samples to heat and drought throughout 2023. According to the meteorological data, the average annual air temperature in the area where the sericulture laboratory is located is +6.9 °C. The coldest month is January (the average monthly temperature according to long-term data is -7.3 °C), and the warmest is July, with an average monthly temperature of +20.8 °C. The minimum temperature in January reaches -22 °C and the maximum temperature in August is 38 °C. The average annual precipitation is 538.0 mm, with average monthly fluctuations from 31 mm (February) to 69 mm (June). The peculiarities of atmospheric circulation in the region cause uneven precipitation in winter and summer. The lowest precipitation (96 mm) falls in winter (December–February), and the highest (109 mm) in summer (June–August). High-yielding, zoned varieties were selected from the gene pool of mulberry varieties and breeding forms of four species of the genus *Morus* from the Moraceae family. The following varieties were selected for testing: Nadiia, Belisma, Chance, Halyna, Surprise, Ukrainska 107, Kharkivska 14, Merefianska, Slobozhanska, Viktoriia, and Maclura. Kharkivska 3 was used as a control variety. Agricultural techniques for growing different species of mulberry on experimental plantations are mostly favorable, while one of the negative abiotic environmental factors that affects the development of planting material is a long period of drought with insufficient and uneven rhythm of natural moisture in the soil and trees during the growing season. This primarily leads to the cessation of their growth, budding, drying, cracking, and premature aging. For this reason, the Institute's scientists are conducting ongoing research aimed at preventing the occurrence of undesirable signs and the mandatory assessment of plants for drought and heat resistance.

The varieties were evaluated visually during drought (air temperature <30 °C) in the field, according to the methodology of state testing and the methodology for the examination of plant varieties of fruit, berry, nut, subtropical, and grape for suitability for distribution in Ukraine (Tkachyk et al., 2016). Observations of drought tolerance of the varieties began with the onset of soil moisture deficit (>50%) during the growing season, high temperatures (<30 °C), and dry winds. During daytime hours, the condition of the plants was assessed visually based on external signs of damage, including leaf curl, premature yellowing, wilting and drying of leaves, shoots, and inflorescences, loss of turgor in plant organs, and the ability to restore it at night. Furthermore, we monitored the recovery of drought damage. Based on a set of traits that characterize the reaction of varieties to dry conditions, the varieties were classified into resistance groups with a corresponding score. During the growing season, a logbook was maintained to record air temperature and precipitation.

2.2 Water Regime Assessment Methodology

The water regime of Moraceae plants was determined using the weight method (Verslues et al., 2006; Li et al., 2022). The study was conducted during the second decade of each month from May to September, under sunny weather conditions. The samples were collected at the same time of the day at 8 a.m. To obtain measurements, 20 shortened shoots were cut from the middle part of the crown of the selected model trees. The total water content, deficit, relative turgorescence, and water-holding capacity were then determined. The total water content was calculated as a percentage by weight, based on the plants' dry weight after being dried to a constant weight. The water-holding capacity was studied by monitoring the rate of water loss by the plants to the initial wet weight throughout 2, 4, 6, 12, and 24 hours. To achieve this, the collected shoots were immersed in water for 12 hours to ensure they were fully saturated. The water loss was then determined with an accuracy of ±0.01 g. Between weightings, the shoots were placed on filter paper under conditions of constant temperature and humidity in the laboratory.

Water deficit was considered as the amount of water deficit in the leaves, expressed as a percentage of its total content in the state of full saturation; relative turgorescence was the amount of water content in the leaves at the time of determination, expressed as a percentage of its content in the state of full saturation with water.

Based on the fact that the adaptation of plants to stressful environmental conditions is considered a complex physiological reaction of the organism to the action of the listed factors, the methods used were based on the determination of the effect of temperature limits on the germination of seeds and maintenance of the functional state of vegetative organs of plants. The basic difference between the actual research and the simple diagnostics of breeding material for stress resistance is that the selected samples were planted directly in the soil (field, vegetation containers). Seeds obtained in the current year were used in subsequent cycles of evaluation and selection by traits, as well as to determine physiological indicators of resistance in the final stage of selection.

2.3 Methodology for Assessing Heat and Drought Resistance of Varieties

Heat resistance was determined by the percentage of germinated seeds at the limit temperatures (41.0–43.0 ±0.5°C), adjusted for laboratory germination. Under these conditions, the percentage of seed purity (%), its laboratory germination (%), and suitability for economic use (%) were calculated.

To assess the purity of the seeds after thorough mixing, three 0.5 g weights were taken, poured onto clean, glossy paper, and carefully examined. All clean and complete seeds were separated from impurities using a dissecting needle. The cleaned seeds obtained after this sorting were reweighed to the nearest ±1 mg. The percentage of seed purity, as an average between three weights, was determined using the formula 1:

$$p = \frac{P_2 \cdot 100}{P_1} \quad (1)$$

where: p – the percentage of seed purity, %; P_2 – the final weight of the sample (after cleaning), mg; 100 – conversion factor to percentage; P_1 – the initial weight of the sample (before cleaning), mg

Laboratory germination of seeds of the experimental varieties was determined on three samples of pure seed fraction, 100 pieces each. Seeds were germinated in Petri dishes on filter paper. The Petri dishes were sealed and placed in a thermostat at 28.0 ±0.5 °C. The number of germinated seeds was continuously evaluated during the 20 days of the experiment. Germination was determined as a percentage of the total number of seeds taken for germination by the formula 2:

$$X = \frac{a \cdot 100}{b} \tag{2}$$

where: *X* – laboratory germination of seeds, %; *a* – number of germinated seeds, pcs; 100 – conversion factor to percentage; *b* – a total number of seeds taken for germination, pcs

The overall indicator of the economic suitability of seeds was calculated based on their germination and purity by the formula 3:

$$ES = \frac{p \cdot X}{100} \tag{3}$$

where: *ES* – economic suitability of seeds, %; *p* – percentage of seed purity, %; *X* – laboratory germination of seeds, %; 100 – conversion factor to percentage

Drought resistance was evaluated by germinating seeds in osmotic sucrose solutions at different pressures (18–22 atm), increasing its value with each selective cycle. This method allows for the assessment of the general physiological and biochemical processes in germinated seeds under drought conditions. It also evaluates the resistance of mature plants to drought, highlighting the potential for conserving and cultivating valuable genotypes for future selection.

For a more reliable and objective evaluation and selection of lines, the values of heat and drought resistance were also determined on vegetative plants in the dynamics of their development using the heat resistance index (Bao et al., 2024). In the course of the study, plants (5 samples of each variety) were immersed in a water bath at 40.0 ±0.5 °C for 30 min. The first leaf sample from the water bath was transferred to a desiccator with water at room temperature. Then the temperature of the water bath was gradually increased by 10.0 ±0.5 °C and the next samples were taken after 10 min. Then, the water in the desiccator was replaced with 0.2 N hydrochloric acid solution and the results were obtained after 20 minutes. The leaf membranes destroyed by the high temperature lost their selective properties and allowed acid to pass through, causing the leaves to turn brown due to chlorophyll pheophytinization. At a temperature of 90.0 ±0.5 °C (lethal level), the experiment was stopped. The degree of heat tolerance of the samples was determined by the Matskov method, which consists of determining the degree of browning of leaves caused by pheophytin formed as a result of penetration of 0.2 N hydrochloric acid into the leaf tissue damaged by overheating (55–57 °C) on a three-point scale. Plants with minimal damage to the leaf surface (up to 50%) were considered as 1 point (+), 2 points (++) – 50%, and 3 points (+++) – 100% damage.

The selection of heat-resistant forms was carried out by a method based on the preservation of pollen viability after exposure to high temperatures (39.0–42.0 ±0.5 °C) compared to the control (25.0 ±0.5 °C). The collected pollen was applied to the stamens of the fruits after heating in a thermostat at a temperature of 39.0–42.0 ±0.5 °C, with an exposure time of 30 min. The evaluation and selection of heat-tolerant plants was done by the number of seeds formed on the fruits compared to the control. Pollen-pollinated fruits kept at room temperature were used as a control. In addition, heat and drought tolerance were determined using a modified method that takes into account changes in leaf tissue electrical resistance (LTER) under overheating and dehydration (Sarakhan, 2006).

2.4 Statistical Analysis

The obtained results were processed using methods of variation statistics using the software package for analysis of variance (ANOVA) Stat Plus 5 (6.7.0.3) (Analyst Soft Inc., USA). The results are presented as mean ± standard error (x ±SE). Tukey's test was used within a row to compare the difference in mean values between samples, where differences were considered statistically significant at p <0.05 for all data.

3 Results and Discussion

Based on visual observations of the aboveground part of plants of the Moraceae family, it was found that the most drought-resistant plants are Maclura, Slobozhanska, Merefianska, Surprise, and Halyna. Plants of varieties Nadiia, Belisma, Viktoriia, and Kharkivska 14 were found to be moderately resistant to drought. Low resistance to drought was observed in plants of varieties Kharkivska 3, Ukrainska 107, and Chance (Table 1).

The data obtained were supplemented with studies measuring the total water content in the leaves and their water-holding capacity values. It should (Table 2) be noted that the total water content in the leaves of Moraceae plants was unstable and gradually decreased by the end of the growing season, which is consistent with the results obtained by (Reddy et al., 2017).

Table 1 Scoring of drought tolerance of Moraceae plants in the field (on a 10-point scale)

Variety name	Points
Kharkivska 3 (control)	6
Nadiia	7
Belisma	8
Viktoriia	8
Halyna	9
Surprise	9
Ukrainska 107	5
Kharkivska 14	8
Merefianska	9
Slobozhanska	9
Chance	4
Maclura	10

Some foreign scientists (Huang et al., 2013) emphasize that mulberry responds to heat stress by increasing the area of moisture absorption and increasing the ability to retain water. This thesis was also reflected in their studies. The average water content during the experiment ranged from 60.6% to 68.1%, including the values of total water content in leaves of plants from June to July decreased by 2.8%, from July to August – by 2.7%, from August to September – by 2.0%, and in general during the experimental period – by 7.5%. The maximum content of total water in leaves was recorded in plants of varieties Maclura – 66.2%, Surprise – 65.6%, Slobozhanska – 65.5%, Merefianska – 65.1%, Halyna – 65.1%, in comparison with the control Kharkivska 3 and the average data for the observation period, where this indicator was 62.7 and 64.2%, respectively. The minimum level of total water in leaves was determined in plants of Chance and Ukrainska 107 varieties, which reached the values of 62.1 and 62.3%, respectively. The results of determining the values of the leaf water regime (water deficit and relative turgorescence) of Moraceae plants are presented in Table 3.

The lowest rate of water deficit was observed in the leaves of Maclura – 3.7%, Surprise – 4.2%, Slobozhanska – 4.7%, Merefyanska – 4.9%, the highest – in Chance and Ukrainska 107, respectively 35.5 and 26.2%. The average indicators of leaf water regime were determined in varieties Halyna – 13.7%, Victoria – 15.5%, Belisma – 16.2%, Kharkivska 14 – 17.5%, Nadiya – 18.7% and Kharkivska 3 – 19.5%. The values of relative turgorescence ranged from 64.5% to 97.3%, with an average of 85.5%. The highest relative turgorescence was recorded in varieties Maclura, Surprise, Slobozhanska, Merefyanska – 97.3%, 95.8%, 95.3%, and 95.1%, respectively. The lowest value of turgorescence (64.5%) was found in plants of the variety Chance. Thus, there was a significant difference between the plants of the Moraceae family in terms of water deficit and relative turgorescence. The results are consistent with the material published by (Ding et al., 2018).

The determination of the water holding capacity during wilting also shows variations in this indicator. The wilting process in the laboratory lasted 24 hours for all selected samples, but only variety Chance lost more than 30% of water in 12 hours. The highest water loss during the first two hours of wilting was observed in varieties Chance (12.1%) and Ukrainska 107 (10.2%), the lowest – in varieties Maclura (3.5%) and Surprise (4.2%). The average water loss during the first two hours

Table 2 Total water content in plant leaves in % (x ±SE, n = 20)

Variety name	Observation period				The average by variety
	June	July	August	September	
Kharkivska 3 (control)	66.2 ±1.3[a]	63.3 ±1.2[ab]	61.6 ±1.1[b]	59.6 ±1.6[b]	62.7 ±1.2
Nadiia	67.9 ±1.0[a]	64.2 ±1.3[b]	62.7 ±1.3[b]	60.5 ±1.4[b]	63.8 ±1.3
Belisma	68.3 ±1.0[a]	65.8 ±1.3[a]	62.6 ±1.5[b]	60.8 ±1.1[b]	64.4 ±1.2
Viktoriia	68.9 ±1.0[a]	65.2 ±1.2[b]	63.5 ±1.2[bc]	61.4 ±1.1[c]	64.8 ±1.1
Halyna	68.7 ±0.9[a]	66.4 ±1.4[a]	63.3 ±1.2[b]	61.8 ±1.3[b]	65.1 ±1.1
Surprise	69.3 ±1.0[a]	67.1 ±1.5[ac]	63.9 ±1.3[bc]	61.9 ±1.1[b]	65.6 ±1.3
Ukrainska 107	66.7 ±1.3[a]	63.2 ±1.4[ac]	60.8 ±1.7[bc]	58.3 ±1.8[b]	62.3 ±1.5
Kharkivska 14	67.0 ±1.4[a]	64.5 ±1.3[ac]	61.7 ±1.1[bc]	58.8 ±1.7[b]	63.0 ±1.3
Merefianska	68.5 ±1.3[a]	66.6 ±1.2[ac]	63.2 ±1.2[bc]	61.9 ±1.2[b]	65.1 ±1.1
Slobozhanska	69.4 ±1.2[a]	66.8 ±1.3[ac]	63.9 ±1.3[bc]	61.7 ±1.2[b]	65.5 ±1.2
Chance	66.7 ±1.1[a]	63.4 ±1.4[ac]	60.2 ±1.7[bc]	58.1 ±1.0[b]	62.1 ±1.4
Maclura	69.8 ±0.9[a]	67.3 ±1.1[ac]	65.3 ±1.2[bc]	62.3 ±1.5[b]	66.2 ±1.3

different letters within a row indicate significant differences between observation terms, according to the Tukey test

Table 3 Dynamics of changes in the water regime of Moraceae plants during wilting (x ± SE, n = 20)

Variety name	Water deficit (%)	Water lost during wilting (%, in hours)					Relative turgorescence (%)
		2	4	6	12	24	
Kharkivska 3 (control)	19.5	9.6 ±0.7^{a}	13.7 ±0.7^{b}	19.8 ±2.2^{c}	24.2 ±1.3^{c}	29.6 ±1.1^{d}	80.5
Nadiia	18.7	8.8 ±0.7^{a}	11.6 ±1.4^{b}	19.1 ±1.9^{b}	23.9 ±0.9^{b}	29.5 ±1.8^{c}	81.3
Belisma	16.2	6.7 ±0.1^{a}	12.7 ±1.5^{b}	18.5 ±1.8^{c}	23.7 ±2.0^{c}	29.1 ±1.5^{d}	83.8
Viktoriia	15.5	6.4 ±0.8^{a}	7.6 ±1.1^{b}	11.8 ±0.9^{c}	19.5 ±1.3^{d}	22.0 ±2.7^{d}	84.5
Halyna	13.7	4.9 ±0.4^{a}	7.7 ±0.3^{b}	11.9 ±0.7^{c}	18.3 ±0.5^{d}	22.1 ±1.4^{c}	86.3
Surprise	4.2	4.2 ±0.3^{a}	7.9 ±0.7^{b}	10.5 ±1.8^{b}	16.5 ±2.0^{c}	18.9 ±2.1^{c}	95.8
Ukrainska 107	26.2	10.2 ±1.8^{a}	16.1 ±1.7^{b}	20.8 ±2.7^{b}	29.1 ±2.5^{c}	34.5 ±1.2^{c}	73.8
Kharkivska 14	17.5	9.5 ±1.3^{a}	13.3 ±1.5^{a}	19.6 ±0.8^{b}	26.1 ±1.9^{c}	28.5 ±0.1^{c}	82.5
Merefianska	4.9	5.5 ±0.1^{a}	8.3 ±0.7^{b}	13.4 ±0.9^{c}	17.3 ±1.4^{d}	19.2 ±1.1^{d}	95.1
Slobozhanska	4.7	4.9 ±0.7^{a}	7.1 ±1.2^{a}	12.7 ±1.2^{b}	15.1 ±0.9^{b}	18.3 ±0.5^{c}	95.3
Chance	35.5	12.1 ±0.9^{a}	17.6 ±0.5^{b}	21.8 ±0.7^{c}	32.1 ±0.7^{d}	37.5 ±1.1^{c}	64.5
Maclura	3.7	3.5 ±0.1^{a}	5.6 ±1.3^{ab}	8.5 ±1.8^{bc}	11.6 ±2.0^{c}	15.8 ±2.1^{c}	97.3

different letters within a row indicate significant differences between water losses during observation, according to Tukey's test

of the experiment was 7.2%. During the next 2 hours after the beginning of wilting, they ranged from 5.6% to 17.6%, while the average was 10.8%. Compared to the first two hours, water loss was 3.6% less. After 6 hours, the water loss in the leaves was 15.7%, compared to the previous period (after 4 hours) and amounted to 4.9%, which is 1.3% less. With each subsequent wilting period, an increase in water loss was observed. Within 12 hours, there was a significant difference in water loss between Maclura and Chance plants, 11.6% and 32.1%, respectively, but in one day it averaged 25.4%. During the day, the least amount of water was lost by the Maclura variety, which amounted to 15.8%. The largest amount of moisture was lost by the mulberry variety Chance, 37.5%. The results obtained indicate that the unequal potential drought resistance of the studied plants of the Moraceae family is due to their ability to retain water differently during wilting. Similar changes to those found in the experiment were observed (Secchi et al., 2017) in other varieties of this family.

The ability of leaves to regain turgor and green color after wilting was directly related to the degree of dehydration. A similar ability of leaves to restore turgor and green color after wilting was found in grapes (Galmés et al., 2007; Vandeleur et al., 2009). The longer it took to lose 30–35% of the moisture from the initial mass, the more deeply bound water was contained. This indicates the potential ability of leaves to tolerate deep wilting, and the ability to restore leaf turgor after such wilting indicates the ability to resume physiological processes in their tissues without significant changes.

Based on the research, we developed a scoring scale for assessing physiological parameters characterizing the level of drought tolerance and plants of the Moraceae family, according to which the varieties were divided into three groups: high, medium, and low drought tolerance (Table 4).

The scale for evaluation of plants of the Moraceae family on the level of total water content, water deficit, relative turgorescence and water retention capacity in leaves made it possible to increase the accuracy and informative value of determination of the drought tolerance group. When determining the effectiveness of the implementation of the evaluation scale, it was found that none of the experimental plants was included

Table 4 Scale for assessing physiological parameters of a group of plants of the Moraceae family by drought resistance Total water content in plant leaves in % (x ±SE, n = 20)

Physiological indicator	Drought resistance group (%)		
	I (high)	II (medium)	III (low)
Total water content	65–75	60–65	55–60
Water deficit	0–10	10–20	20–40
Relative turgorescence	90–100	75–90	60–75
Water retention capacity	10–20	20–30	30–40

in the group with low drought tolerance by the total water content. The most drought-resistant plants were found to be Maclura, Surprise, Slobozhanska and Merefyanska. This is confirmed by the fact that they belonged to the first group (high drought tolerance) in terms of total water content, water deficit, relative turgorescence and water holding capacity.

Plants of varieties Kharkivska 3, Nadiia, Belisma, Viktoria, Halyna, and Kharkivska 14 were assigned to the second group (medium drought tolerance) based on similar indicators. The varieties Chance and Ukrainska 107 were placed in the third group (low drought tolerance) in terms of water deficit, relative turgorescence, and water holding capacity, and only in terms of total water content in leaves – in the second group.

The general observation of the samples made it possible to prove that Maclura plants, compared to other plants of the Moraceae family, are the most drought-resistant (Zhu, 2016). During the dry period observed, Maclura leaves were characterized by a higher water content, but its release was less intense.

The level of heat tolerance, studied by the percentage of seeds germinated at the limiting temperatures with adjustment for laboratory germination, is shown in Table 5.

It was found that almost all plant varieties had significantly lower values of biological traits of seeds in the experimental varieties than in the control. In particular, the level of laboratory germination of seeds ranged from 88.3% in the variety Chance to 99.2% in the Maclura variety, with average values of 94.9%, which was 1.3% lower than the control values. A more pronounced difference (6.3%) was found in the economic suitability of the seed. Plants of varieties Victoria, Halyna, Merefianska, Slobozhanska, and Maclura had higher laboratory germination rates ranging from 96.2% to 99.2%, while the average data for all varieties in the experimental variants was 94.9%. The same plant varieties also had higher rates of economic viability of seeds in the experimental variants from 88.7% to 97.5%, while the average data of the total sample were at the level of 82.2%, which indicates the stability of heat resistance in them. This is confirmed by the findings of other experts (Liu et al., 2015).

As part of the next stage of the work, we determined the effect of limiting temperatures on seed germination and the preservation of the functional state of plant vegetative organs (Table 6).

It should be noted that, on average, 50% of the seeds germinated in a sucrose solution with an osmotic pressure of 14 atm, so a solution with a pressure of 18 atm (20–30% higher) was also used for selection. In each subsequent stage of the work, the selected resistant forms were studied using more concentrated solutions: 20 and 22 atm. To determine the adaptive potential of the selected plants of the Moraceae family, a constant selection background of 18 atm was also maintained. The third cycle of selection was carried out on a solution with an osmotic pressure of 22 atm. Plants of varieties Victoria, Halyna, Merefianska, Slobozhanska, and Maclura had higher indicators of adaptive potential, which ranged from 30% to 50%, while the average values within the studied varieties were 29.3%.

Table 5 Heat resistance level by the percentage of germinated seeds at limiting temperatures adjusted for laboratory germination (x ±SE, n = 20)

Variety name	Seed purity (%)		Laboratory germination of seeds (%)		Economic suitability of seeds (%)	
	control	experiment	control	experiment	control	experiment
Kharkivska 3	94.2 ±2.6[a]	93.8 ±0.2[a]	93.1 ±3.2[a]	92.3 ±5.4[a]	78.1 ±1.6[a]	66.0 ±4.0[b]
Nadiia	98.0 ±2.0[a]	97.2 ±0.5[a]	97.5 ±2.5[a]	95.0 ±10.3[a]	85.0 ±1.7[a]	78.6 ±12.5[a]
Belisma	98.4 ±6.4[a]	94.4 ±5.6[a]	97.8 ±2.4[a]	95.5 ±7.9[a]	90.1 ±1.7[a]	80.7 ±7.6[a]
Viktoriia	98.0 ±1.6[a]	97.3 ±0.5[a]	96.7 ±1.5[a]	96.2 ±7.3[a]	93.4 ±1.9[a]	88.7 ±6.0[a]
Halyna	98.6 ±0.1[a]	98.1 ±15.1[a]	96.5 ±1.8[a]	96.4 ±4.5[a]	88.9 ±1.9[a]	89.2 ±8.0[a]
Surprise	81.0 ±3.8[a]	77.8 ±7.4[a]	90.2 ±0.9[a]	89.9 ±0.9[a]	79.6 ±1.7[a]	70.1 ±1.2[b]
Ukrainska 107	97.3 ±1.0[a]	92.0 ±3.4[a]	97.2 ±1.0[a]	95.0 ±4.9[a]	95.0 ±2.2[a]	83.2 ±2.9[b]
Kharkivska 14	98.1 ±1.4[a]	96.3 ±2.6[a]	96.9 ±2.9[a]	95.0 ±10.2[a]	94.7 ±4.1[a]	82.9 ±1.6[b]
Merefianska	99.3 ±0.7[a]	96.7 ±1.5[a]	99.6 ±0.4[a]	98.0 ±9.8[a]	94.7 ±2.0[a]	92.6 ±9.2[a]
Slobozhanska	98.9 ±0.6[a]	97.0 ±0.5[b]	99.4 ±1.8[a]	98.2 ±4.9[a]	93.3 ±1.9[a]	92.9 ±3.7[b]
Chance	91.4 ±1.8[a]	81.5 ±5.6[a]	89.8 ±3.0[a]	88.3 ±4.7[a]	70.5 ±1.6[a]	64.4 ±2.2[b]
Maclura	99.9 ±0.6[a]	97.9 ±6.1[a]	99.8 ±1.5[a]	99.2 ±9.3[a]	98.4 ±1.9[a]	97.5 ±11.4[a]

different letters within a row indicate significant differences between experimental and control plant samples, according to Tukey's test

Table 6 Determining the effect of limiting temperatures on seed germination of plants belonging to the family Moraceae (x ±SE, n = 20)

Variety name	Selection background (atm)				
	control	14	18	20	22
Kharkivska 3	48 ±1.3[a]	42 ±0.9[b]	34 ±0.9[c]	26 ±1.3[d]	18 ±1.6[e]
Nadiia	56 ±09[a]	48 ±1.3[b]	36 ±1.8[c]	28 ±1.1[d]	19 ±1.0[e]
Belisma	60 ±1.8[a]	49 ±1.8[b]	41 ±1.5[c]	36 ±1.6[d]	28 ±1.2[e]
Viktoriia	61 ±1.5[a]	51 ±0.8[b]	36 ±0.8[c]	32 ±1.4[d]	30 ±1.5[d]
Halyna	65 ±0.6[a]	52 ±0.9[b]	38 ±1.4[c]	36 ±1.9[d]	32 ±1.6[d]
Surprise	41 ±1.3[a]	39 ±0.6[a]	20 ±0.9[b]	16 ±0.3[c]	14 ±0.9[d]
Ukrainska 107	65 ±0.9[a]	50 ±0.8[b]	32 ±1.6[c]	29 ±1.6[c]	18 ±1.2[d]
Kharkivska 14	48 ±1.9[a]	40 ±1.1[b]	24 ±0.9[c]	20 ±1.8[cd]	18 ±1.4[d]
Merefianska	72 ±1.8[a]	62 ±1.6[b]	52 ±0.9[c]	50 ±0.8[cd]	49 ±0.6[d]
Slobozhanska	78 ±1.3[a]	65 ±1.3[b]	58 ±0.8[c]	56 ±1.3[cd]	55 ±0.8[d]
Chance	42 ±0.1[a]	38 ±1.6[b]	23 ±1.3[c]	19 ±0.9[d]	16 ±0.8[e]
Maclura	80 ±0.8[a]	65 ±0.9[b]	58 ±0.8[c]	56 ±1.3[cd]	55 ±0.8[d]

different letters within a row indicate significant differences between plant selection backgrounds, according to Tukey's test

It should be noted that, on average, 50% of the seeds germinated in a sucrose solution with an osmotic pressure of 14 atm, so a solution with a pressure of 18 atm (20–30% higher) was also used for selection. In each subsequent stage of the work, the selected resistant forms were studied using more concentrated solutions: 20 and 22 atm. To determine the adaptive potential of the selected plants of the Moraceae family, a constant selection background of 18 atm was also maintained. The third selection cycle was carried out on a solution with an osmotic pressure of 22 atm. Plants of varieties Victoria, Halyna, Merefianska, Slobozhanska, and Maclura had higher indicators of adaptive potential, which ranged from 30% to 50%, while the average values within the studied varieties were 29.3%.

The results of observations of the aboveground part of plants of the Moraceae family show that the most drought-resistant were plants of the varieties Maclura, Slobozhanska, Merefianska, Viktoriia, and Halyna, and medium drought-resistant were plants of the varieties Nadiia, Belisma, Ukrainska 107 and Kharkivska 14. Low drought resistance was observed in the varieties Kharkivska 3, Surprise, and Chance plants.

Table 7 Comparative evaluation of the degree of heat resistance of leaves of Moraceae plants

Variety name	Degree of leaf damage by high temperature (°C)					
	40.0 ±0.5	50.0 ±0.5	60.0 ±0.5	70.0 ±0.5	80.0 ±0.5	90.0 ±0.5
Kharkivska 3	+	+	+	++	+++	+++
Nadiia	+	+	+	++	+++	+++
Belisma	+	+	+	++	+++	+++
Viktoriia	+	+	+	+	++	++
Halyna	+	+	+	+	++	++
Surprise	+	+	++	+++	+++	+++
Ukrainska 107	+	+	+	++	+++	+++
Kharkivska 14	+	+	+	++	+++	+++
Merefianska	–	+	+	+	++	++
Slobozhanska	–	+	+	+	++	++
Chance	+	+	++	+++	+++	+++
Maclura	–	+	+	+	++	++

"–" – no damage, "+" – minimal damage, "++" – 50% damage, "+++" – 100% damage

Since the signs of heat and drought tolerance are formed throughout ontogeny, the evaluation and selection of Moraceae plants for stress resistance was carried out not only at the stages of seed germination but also during the growing season. The results obtained convincingly show that the leaves of Moraceae plants are able to withstand an increase in water temperature in the range of 40.0 ±0.5 °C to 50.0 ±0.5 °C for 20 min without significant damage (Table 7).

Similar damage to mulberry leaf blades under the influence of high air temperatures was also reported by Chaitanya et al., 2001. On the other hand, damage to the leaf blades of plants of the Moraceae family began with their veins. At a temperature of 60.0 ±0.5 °C, the first signs of significant damage to the leaf blade of the Surprise and Chance varieties were recorded, which in percentage was 55.6% and corresponded to 2 points on the evaluation scale. Damage to leaf blades of varieties Kharkivska 3, Nadiia, Belisma, Halyna, Viktoria, Ukrainska 107, Kharkivska 14, Merefianska, Slobozhanska and Maclura at 60.0 ±0.5 °C did not exceed 50% of the total area. At 70.0 ±0.5 °C the varieties Kharkivska 3, Nadiia, Belisma, Ukrainska 107, and Kharkivska 14 received 2 points based on browning of the leaf blade tissues.

According to the maintenance of pollen viability after exposure to high temperatures (39.0–42.0 ±0.5 °C) compared to the control (25.0 ±0.5 °C), plants of varieties Victoria, Halyna, Merefianska, Slobozhanska, and Maclura were determined to be heat-resistant. The percentage of seeds in the fruits of these varieties at a pollen heating temperature of 40.0 ±0.5 °C ranged from 81.4% to 89.8%, while at a temperature of 42.0 ±0.5 °C – from 80.2% to 88.4%, indicating the effectiveness of the selection of plants of the Moraceae family for the trait under study (Table 8).

As the results demonstrated in the graph show, the varieties Victoria, Halyna, Merefianska, Slobozhanska, and Maclura are characterized by a rather high adaptive resistance to high temperatures (heat and drought). At the same time, under the influence of desiccation, the water content in the leaves of Moraceae plants decreased. Evaluation of vegetative plants of the family Moraceae for drought tolerance by determining the change in electrical resistance of leaf tissues after drought (compared to the control), which had an inverse relationship with water loss, proved that they are not equally adapted to dehydration. Rapid water loss during the period of active growth and seed formation in the fruits indicates that plants are less adaptable to drought, as emphasized in the study (Sutka et al., 2016).

The change in ERLT is inversely related to water loss. Under the influence of drought, ERLT parameters changed significantly. In particular, in the phase of 4–5 leaves, the percentage of ERLT in varieties Merefianska, Slobozhanska Maclura ranged from 70% to 60%, in the flowering phase – from 90% to 80%, in the phase of fruit ripeness – from 110% to 90%, compared to the control Kharkivska 3, in which these values were 80, 100 and 120%, respectively. This is due to the adaptive capacity of plants to moisture loss. Drought-tolerant plants of Merefianska, Slobozhanska, and Maclura varieties are characterized by low values of

Table 8 Efficiency of selection of plants of the Moraceae family for heat resistance while maintaining pollen viability during overheating

Variety name	Control	39.0 ±0.5 °C		40.0 ±0.5 °C		42.0 ±0.5 °C	
	seeds in sub-fruits	seeds in sub-fruits	% to control	seeds in sub-fruits	% to control	seeds in sub-fruits	% to control
Kharkivska 3	65	52	80.0	50	76.9	48	73.8
Nadiia	86	72	83.7	68	79.1	67	77.9
Belisma	89	78	87.6	70	78.7	69	80.2
Viktoriia	96	85	88.5	80	83.3	79	82.3
Halyna	98	88	89.8	82	83.7	81	82.7
Surprise	64	36	56.3	32	50.0	29	45.3
Ukrainska 107	72	45	62.5	43	59.7	40	55.6
Kharkivska 14	66	40	60.6	39	59.1	35	53.0
Merefianska	106	95	89.6	93	87.7	91	85.8
Slobozhanska	112	102	91.1	99	88.4	98	87.5
Chance	68	40	58.8	34	50.0	32	47.1
Maclura	147	134	91.2	132	89.8	130	88.4

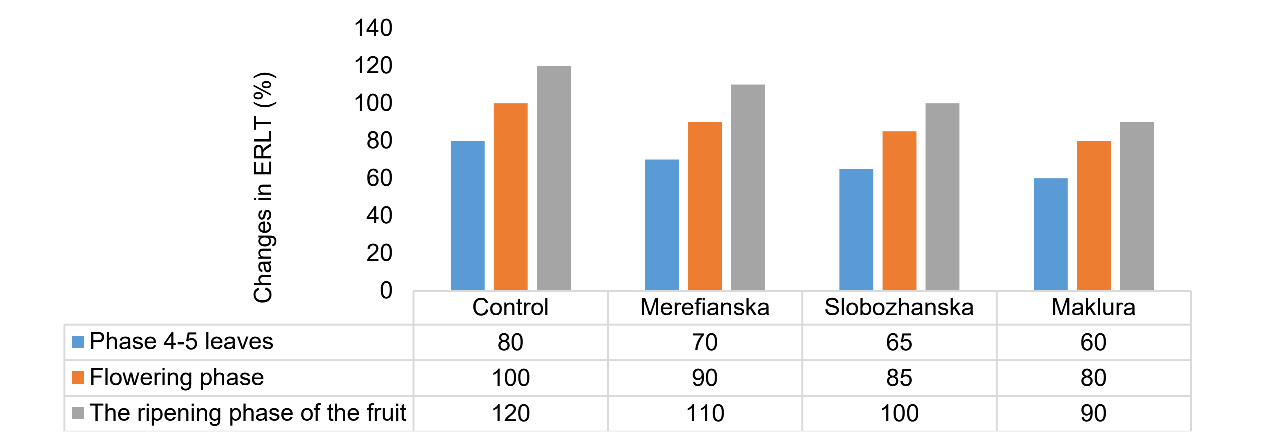

Figure 1 Changes in electrical resistance of leaf tissues in %

Table 9 Comprehensive scoring scale for the assessment of plants of the Moraceae family by adaptive resistance

Physiological indicator	Adaptive resistance group (%)		
	I (high)	II (medium)	III (low)
Germination of seeds in osmotic sucrose solutions with different pressures (drought tolerance)	50–30	29–18	16–10
Preservation of pollen viability (heat resistance)	80–90	70–80	40–60
Change in electrical resistance of the leaf tissue (drought resistance)	60–70	71–80	81–90

EOTL changes after drought exposure. Plants selected in osmotic solutions had greater adaptability to drought conditions due to lower changes in ERLT at different stages of development (Figure 1).

Summarizing the results obtained, we developed a comprehensive scale for assessing the physiological parameters of drought and heat tolerance of Moraceae plants with the identification of three groups: high, medium, and low adaptive capacity (Table 9).

Plants of varieties Victoria and Halyna were assigned to the first group (high adaptive capacity) according to the index of seed germination in osmotic sucrose solutions with different pressures of 18–22 atm (drought resistance), evaluation by Matskov method (heat resistance), preservation of pollen viability (heat resistance), and to the second group (medium adaptive capacity) only by the change in electrical resistance of leaf tissues (drought resistance). The varieties Chance and Surprise were assigned to the third group (low adaptive capacity) according to the index of seed germination in osmotic sucrose solutions with different pressures of 18–22 atm (drought resistance), evaluation by the method of Matskov (heat resistance), preservation of pollen viability (heat resistance) and changes in electrical resistance of leaf tissues (drought resistance).

4 Conclusions

The substantiated physiological parameters of drought and heat tolerance of plants of the Moraceae family should be used as indicators for their objective assessment in the context of climate change. It is emphasized that the varieties Victoria, Halyna, Merefianska, Slobozhanska, and Maclura have higher adaptive resistance to high temperatures (heat and drought), i.e. they are most suitable for cultivation in the forest-steppe of Ukraine. Two scoring scales for the evaluation of plants of the family Moraceae in terms of drought and heat tolerance have been developed, based on which they are divided into three groups: high, medium, and low adaptive resistance. It is argued that the overall level of physiological processes in germinated seeds under drought conditions indicates the stability of adult plants, and thus the possibility of preserving and growing valuable varieties for further breeding.

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