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SURVIVAL AND JUVENILE-AGE PERFORMANCE OF *SALICACEAE* CLONES FOR BIOMASS

PREŽIVLJAVANJE I ODLIKE KLONOVA IZ FAMILIJE *SALICACEAE* ZA PROIZVODNJU BIOMASE U JUVENILNOJ FAZI

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Abstract

The adaptability of three *Salicaceae* clones selected for biomass (*Salix* x 'Terra Nova', *Populus* x 'AF-18' and *Populus* x 'AF-8') was tested in the first year of growth at nursery in comparison with a poplar cultivar for wood production (*Populus* x 'I-214').

An experimental plantation was established in April 2020 with standard hardwood cuttings on 0.2 ha of nursery land along the Danube River in Central Northern Bulgaria. Principal leaf gas exchange parameters were measured. Diameter increment, height growth and increment, and normalized difference vegetation index were monitored on representative samples of plants. The influence of the genotype was analyzed, and correlations with main climate parameters were sought.

The willow clone showed early increment culmination and a sharp decline in growth and viability with the suspension of watering. It had the lowest net photosynthetic rate (8.312 ± 0.107 µmol $(CO_2)\cdot m^{-2}\cdot s^{-1}$), low biomass growth (0.098 ± 0.01 kg DM) and a high survival rate.

Unlike the other poplar genotypes, 'I-214' showed rapid growth at the beginning of the summer, a high transpiration rate (1.222 ± 0.034 mmol $(H_2O)\cdot m^{-2}\cdot s^{-1}$) and a stronger dependence on moisture. Clones 'AF-8' and 'AF-18' had high photosynthetic (10.238 ± 0.231 and 11.480 ± 0.193 µmol $(CO_2)\cdot m^{-2}\cdot s^{-1}$, respectively) and low transpiration (0.672 ± 0.024 and 0.682 ± 0.015 mmol $(H_2O)\cdot m^{-2}\cdot s^{-1}$, respectively) rates, and their growth was positively affected by the temperatures. The water use efficiency of the poplar biomass clones was the highest. 'AF-8' genotype showed the best diameter and biomass (0.194 ± 0.036 kg DM per plant) growth, while the 'AF-18' genotype grew best in height.

Key words: adaptability, growth, *Populus* 'AF-8', *Populus* x 'AF-18', *Salix* x 'Terra Nova'

1. INTRODUCTION / UVOD

The increase in carbon dioxide concentration in the atmosphere is of particular concern because of its role in global climate change. Efforts to reduce its amount are being made, as there is increasing evidence of climate warming, particularly over the past 50 years. One of the main carbon mitigation strategies is the maximum wood usage strategy to substitute for other high-energy materials or fuels (Kohlmaier et al., 1998) and the short-rotation crops from fast-growing tree species are the principal sources of woody biomass. Energy crops have been defined as short-rotation, high-density systems of selected genotypes grown under specific cultural regimes (Ceulemans & Deraedt, 1999). They are usually managed by repeated coppicing and therefore poplars and willows are among the most appropriate species to be used due to their fast growth and ability to sprout. Back in 1985, the International Poplar Commission (according to Sixto et al., 2007) defined the main characteristics of the ideal clone appropriate for short-rotation crops: rapid juvenile growth, high ability to sprout and re-sprout, ability to grow in high densities, ample utilization of the vegetation period and positive response to tendings. With the advancement of knowledge and experience on establishment, growth and utilization of the lignocellulosic plantations, new priorities have also been added to the list (Paris et al., 2011): i) high rooting percentage (>80-90%) of hardwood cuttings under field conditions; ii) high survival rates of sprouts (>80%) after frequent coppicing; iii) sprout density not lower than 5000 per hectare; iv) continuous high dendromass yield, never lower than 10 Mg of dry matter ha^{-1} year^{-1}, over frequent and repeated coppicing; and v) balanced stem dimensions (diameter and height) suitable for mechanical harvesting.

Several *Salicaceae* genotypes have been selected over time for use in short-rotation crops (Caslin et al., 2012; FAO - International Poplar Commission, 2016). However, the selected plant material is adapted to the climatic and photoperiodic conditions of the breeding site and genetic tests in the deployment area before commercial utilization are needed to examine their adaptability and growth potential (Stener & Westin, 2017). Poplars and willows are diverse pioneer species that have the inherent ability to grow rapidly as a result of their capability to capture light efficiently through the process of photosynthesis and take up large quantities of water and nutrients from the soil (Richardson et al., 2014). While photosynthetic rate correlates directly and positively with productivity and transpiration favours the good plant status through the thermoregulation and the synthetic processes through the water transport, the water use efficiency (WUE) describes the rate of dry matter production for a given rate of water loss (Fischer et al., 2015). Fischer et al. (2015) pointed out that detailed knowledge of the relationship between water use and biomass productivity for the particular poplar and willow species and their hybrids under realistic field conditions is critically needed to match appropriate crops to prevailing agroclimatic conditions and the importance of WUE evaluation for short-rotation crop species has been emphasized (Fischer et al., 2015; Kalaydzhiev et al., 2015; Rodzkin et al., 2015). The normalized difference vegetation index (NDVI), a physiological parameter related to the photosynthetic activity of the vegetation canopies (Myneni et al., 1995; Myneni et al., 1997), also can serve in the assessment of the adaptive potential of the tested genotypes. It is considered sufficiently stable to permit evaluation of the inter-annual changes in vegetation growth and activity (Huete et al., 2002) and therefore can be viewed as an indirect indicator of the dynamics in plant condition.

Several studies investigating the biomass production potential of fast-growing broadleaves, traditionally grown for timber in Bulgaria, were carried out in 2013–2020 within the framework of two research projects. As a result, generalized and clone-specific allometric relationships for estimation of the aboveground biomass of *Populus x euramericana* (Dode) Guinier cultivars from easily measured tree and stand variables were developed (Stankova et al., 2016, 2017, 2021). The effect of spacing, genotype, root age and harvesting cycle on the aboveground woody biomass production of the black poplar hybrids was investigated in a set of spacing trials (Stankova et al., 2019, 2021). Research on the total chlorophyll content and NDVI (Anev et al., 2018) and on the root systems (Gyuleva et al., 2018) of the poplar clones at different levels of spacing was undertaken. A study exploring and comparing the growth and adaptability of two basket willow (*Salix viminalis* L.) clonal varieties - *rubra* and *purpurea* - at specific edaphic and climate conditions in North Central Bulgaria was also conducted (Stankova et al., 2022). However, results from investigations on the adaptivity and productivity of poplar and willow genotypes bred specifically for biomass and grown in Bulgaria, have not been published so far. A clonal collection with *Salicaceae* clones for biomass was established in the spring of 2019 on the territory of Vardim nursery along the Danube River, Central Northern Bulgaria. The main objective of this study was to test the adaptability of three selected biomass genotypes from the *Salicaceae* family (*Salix* x 'Terra Nova', *Populus* x 'AF-18' and *Populus* x 'AF-8') during the first year of growth in the nursery along the Danube River in Northern Bulgaria, and in comparison with a poplar cultivar for timber production (*Populus* x 'I-214').

2. MATERIAL AND METHODS / MATERIJAL I METOD RADA

The study was carried out in an experimental plantation with 3 poplar clones and 1 willow clone established in a forest nursery in the village of Vardim. The nursery belongs to Svishtov State Forest Enterprise, Central Northern Bulgaria and is located at 25m a. s. l. along the Danube River (43°36'39.66" N, 25°29'2.53" E). The region is characterized by *Calcic Chernozems* (Teoharov et. al, 2014). It is poorly stocked with humus, total nitrogen and phosphorus. The supply of potassium oxide is good (21.7mg·100g^{-1}) and the soil reaction at the experimental plot is highly alkaline: pH= 8.78. The decomposition degree of the organic substances is optimal /Mull/ (Mihalska & Angacheva, 2012). Average annual temperatures are in the range of 10.6–11.7 °C, and the average number of days with temperatures above 10 °C is between 194 and 210. The annual amount of precipitations is between 493 and 661 mm with a maximum in June and a minimum in February-March. The snow cover lasts between 41 and 63 days. The duration of the growth period is 6–6.5 months. Extreme climatic anomalies that have an adverse effect in the region are late spring frosts, summer-autumn droughts and strong winds ("Silva 2003", 2016).

The experimental plantation was established in mid-April 2020 on an area of 0.2 ha. Soil preparation consisted of autumn deep plowing (down to 60 cm), followed by disking and milling. Pre-sowing fertilization with triple superphosphate (30 kg·dka^{-1}) and potassium nitrate (10 kg·dka^{-1}) was carried out. The poplar clones *Populus* x 'AF8', *Populus* x 'AF18' and *Populus* x 'I-214' and the willow clone *Salix* x 'Terra Nova' were studied. *Populus* x 'AF8' is a female hybrid whose mother clone *P.* x *generosa* '103-86' is a hybrid between the American black poplar clone *P. deltoides* '583' (Iowa - USA) and the balsam poplar clone *P. trichocarpa* '196' (Oregon - USA) and the paternal genotype is the balsam poplar clone *P. trichocarpa* 'PEE' (Washington - USA) (Marco Giletta, personal communication 21 July 2021). It is known to tolerate some exposure to adverse weather conditions, high altitude and drought. It is susceptible to windbreaks and moderately susceptible to leaf rust (Lindegaard, 2020). *Populus* x 'AF18' is a female interspecific hybrid obtained by a controlled cross between the paternal genotype *P. nigra* '365-94' (Italia) and the maternal clone *P. x deltoides* '62-98' (Iowa-USA). It was created in 2004 (Marco Giletta, personal communication 21 July 2021). *Populus* x ‘I-214’ is a female hybrid clone imported to Bulgaria in the 1950s. It grows equally well both on light and airy soils and on heavy and compact soils. It does not tolerate excess moisture and grows poorly on dry soils. It is one of the fastest-growing poplar clones in Bulgaria (Tsanov & Mikov, 1997). *Salix* x ‘Terra Nova’ is a female willow interspecific hybrid. The maternal genotype ‘LA940140’ is a hybrid between *Salix viminalis* L. ‘Bowles Hybrid’ and *Salix triandra* L. ‘Dark Newkind’, and the paternal genotype belongs to the bushy willow species *Salix miyabeana* Seemen (Caslin et al., 2012).

The propagules used were hardwood cuttings, 20–25 cm in length. One-hundred cuttings of ‘AF-8’ were delivered from Italy that spring, while the remaining poplar cuttings were obtained in late winter from the one-year-old shoots of the saplings grown in the clonal collection. The willow cuttings were collected from one- and two-year-old shoots of saplings grown in Gulyantsi forest nursery (Central Northern Bulgaria). The cuttings were placed vertically, with their uppercuts at 1 cm below the soil surface, in single rows at 290 cm between-row and 40 cm within-row distances. The non-standard (all willow) cuttings were treated with Aminosol (9.4% total nitrogen (N) 115 $g \cdot l^{-1}$, 1.1% total potassium oxide (K_2O) 15 $g \cdot l^{-1}$). They were dipped in a 1% water solution for 15 min to promote rooting formation.

Mechanized inter-row weeding was carried out twice a month and manual hoeing in the row once a month, from May to October. Fertilization was done twice: in the last week of May and in the middle of June with ammonium nitrate (25 $kg \cdot dka^{-1}$). Drip irrigation for 72 hours was carried out 4 times during the growing season - in the middle of May, June, July and August (64.8 $m^3 \cdot dka^{-1}$). The standard for poplar nursery pest control was carried out: one treatment against rust with Sistan Ecozom (active substance myclobutanil, 165 $ml \cdot dka^{-1}$); one treatment against poplar leaf beetle (*Chrysomela populi* L.) with Mospilan (active substance acetamiprid, 13 $g \cdot dka^{-1}$) and one treatment against poplar shoot-borer (*Gypsonoma aceriana* Duponchel) with Tepeki (active substance flonicamid, 14 $g \cdot dka^{-1}$). In mid-July, infestation by mites (*Tetranychus urticae* C. L. Koch) was found on 30% of the willows. Treatment with Akarzin (active substance paraffin oil 85%, 817 $ml \cdot dka^{-1}$) was applied twice with good results.

Measurements were taken on saplings - one row per clone - from the innermost representative part of the plantation. The rooting was reported on 15 July 2020, and the percentage of the rooted plants that survived was recorded on 22 October 2020. The tree heights were measured once a month during the period July 15 - October 22 (a total of 4 times). Ten plants per clone, between the 25th and 75th percentile in height, were sub-sampled for additional data collection. Measurements of height, diameter at 2 cm above the root collar (total of 7 times) and normalized difference vegetation index - NDVI (total of 4 times) were taken on these test plants every two weeks to assess the growth increment and the changes in the plant vitality.

Thirty saplings from each clone were destructively sampled in April 2021, following the clone-specific pooled diameter distributions of all survived saplings. Length, basal and breast-height diameter and fresh weight of each sampled plant were measured. Samples of lignified biomass (100–200 g), combining parts of stems and branches proportionally to their share in the whole, were obtained by diameter classes. The samples were oven-dried in a laboratory at 105 °C to constant mass. The duration of drying was determined by periodic control measurements. The heights of the standing trees were measured with a measuring pole, and of the harvested saplings - with a tape measure, with 1 cm accuracy. The diameters were measured with a digital caliper with 0.1 cm accuracy, and the fresh weight of the total above-ground biomass (stem and branches) – with a scale of 5 g accuracy. The weight of the lignified samples, before and after drying, was measured with a laboratory scale with an accuracy of 0.001 g. The normalized difference vegetation index was measured on leaf petioles with the PlantPen NDVI-300 portable device (PSI Corporation, Czech Republic).

Measurement of the physiological parameters net photosynthetic rate (μmol(CO_2)·m^{-2}·s^{-1}), transpiration rate (mmol(H_2O)·m^{-2}·s^{-1}), stomatal conductance (mol(H_2O)·m^{-2}·s^{-1}), chlorophyll content (mg·cm^{-2}) and leaf vapor pressure deficit (kPa) was done once, on 14 July 2020. The measurement was taken on fully developed leaves of 5 plants per clone using a Li-6400 portable apparatus. For the willow clone 'Terra Nova', 5 saplings were selected from each of the two plant groups, originating from one- and two-year-old shoots, respectively. Water use efficiency (WUE, μmol(CO_2)·mmol(H_2O)$^{-1}$) was also determined as a ratio of the net photosynthetic rate and transpiration rate.

Soil samples were collected with an auger with a volume of cylinder 502.4 cm^3. The samples were collected in 5 replicates from depths of 0–20 and 20–40 cm. In the laboratory the following parameters: pH in distilled H_2O, coarse fragment's content by weight method, soil structure, relative density and porosity trough calculation, organic carbon (OC) content by Tyurin method, total N content by Kjeldahl method and the relation C/N - trough calculation (Donov et al., 1974), were determined. Organic carbon stock was calculated following the GPG-LULUCF by IPCC (IPCC, 2006). For the study area, climatic data were collected daily during the growing season (March - October) from World Weather Online. This information was used to calculate the total precipitation, average minimum and maximum temperatures, and average wind speed, monthly and biweekly.

The influence of the clone on the physiological indicators measured once with a Li-6400 or repeatedly during the summer period (NDVI), as well as on the height growth (15.07 - 22.10.2020, 4 measurements) was investigated by one-way analysis of variance (ANOVA). When significant, Tukey's post-hoc test was applied to distinguish the statistically significant groups of means by variables. Homogeneity and normality of the residual distributions were examined by graphical (plot of residuals against predicted values and Quantile-Quintile plot) and analytical (Shapiro-Wilk normality test) tests. If any of the requirements for the residuals were violated, a non-parametric Kruskall-Wallis rank sum test was applied instead of ANOVA.

The course of the height and diameter increments for the two-week periods from 15 July to the end of September was analyzed graphically. Mean minimum and mean maximum temperatures, total precipitation and mean wind speed were calculated for the five two-week growing periods from the collected climate information. For a more accurate assessment of the moisture, available to plants, the water quantities supplied by irrigation were added to the precipitation amounts. The relationships between the climate indicators and the increments were investigated.

The relative performance of the four *Salicaceae* clones on the study site in terms of survival, height, diameter and biomass growth was evaluated and compared using a standardized index (Filat et al., 2010):

$$SI = (x-\bar{x})/s \qquad (1)$$

where *SI* is the standardized index, *x* is the mean value of the variable for a specific clone, $\bar{x}$ is the mean and *s* is the standard deviation of the variable for all clones. A complex evaluation of the adaptability and growth, as an arithmetic mean of the standardized indices, was made both for all clones and only for poplars. In the second case, the indices of the four variables were used, and due to the differences in the plant form of the willow and poplar clones, the assessment for all four genotypes was based only on survival and biomass growth.

3. RESULTS / REZULTATI

During the growing season of 2020, the maximum amount of precipitation was in June, when it reached 205.4 mm·m^{-2}, followed by May (121.6 mm·m^{-2}). In July, August and September, the precipitations were minimal, below 30 mm·m^{-2}. In April, when the rooting experiment was set up, a rainfall amount as low as 24 mm·m^{-2} was recorded (Figure 1). The highest maximum temperature average was 33.8°C and was reached in August, and the lowest minimum temperature average during the growing season was 6°C and was found in March.

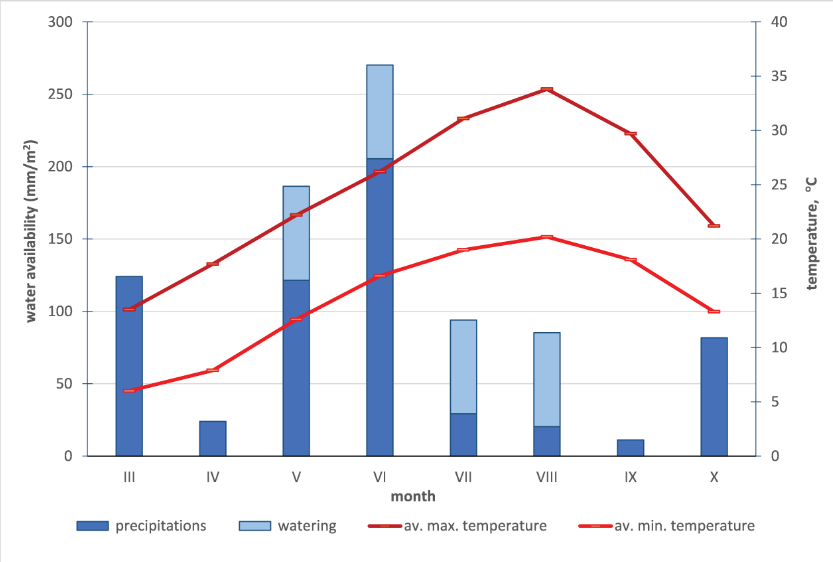

Figure 1. Climatic variables of the study region during the growth period of 2020 / **Slika 1**. Klimatske karateristike područja u kojem se nalazi ogled tokom vegetacionog perioda u 2020. godini

The determined pH of the studied soil varies slightly - between 7.43 and 7.66 for all soil layers. Therefore, the soils of the study site are characterized by a neutral to very slightly alkaline reaction, over the entire investigated depth of the soil profile, according to the classification of Atanasov et al. (2009). The bulk density of the soil varies in depth between 1.13 and 1.73 g·cm^{-3}. A tendency of slight compaction in-depth and the related reduction of porosity in the deeper 20–40 cm soil layer is established. The relative density of the soil is between 1.9 and 3.2. The calculated total porosity ranges from 28 to 55%, which defines the soils as slightly porous to porous and correlates with the established soil texture.

The results for the soil texture of the studied samples show a higher total content of the sandy fractions (between 52 and 64%) compared to the clay fractions (from 36 to 48%). No differentiation of the profile in terms of soil texture and movement of silt from the upper horizons to the lower ones are noticeable. According to the Kaczynski scale (Donov et al., 1974), soils are classified as medium sandy-clay to heavy sandy-clay, which correlates positively with the established bulk density.

The humus content of the studied soils is on average 2.55%. The average carbon content is 1.48%, ranging from 0.67 to 2.99%, and decreases with depth. The degree of enrichment of soils with nitrogen "follows" the distribution of organic carbon, is defined as low and varies from 0.11% to 0.06%. The studied soils are characterized by an average enrichment of humus with nitrogen - C/N is on average 16 and varies between 10 and 26. The carbon stock of the soil in the studied area is on average 41 t·ha^{-1}, varying widely between 17 and 86 t· ha^{-1}. The surface soil layer 0–20 cm has a carbon stock between 31 and 86 t·ha^{-1}, and the deeper (20–40 cm) soil layer between 17 and 47 t·ha^{-1}.

A low percentage of rooting was reported for the poplar clones: 36–54%. Due to the limited quantity and quality of the willow cuttings, they were treated with a stimulating substance and a high rooting rate (87%) was achieved. At the end of the growing season, relatively high survival rates of the rooted saplings were reported, varying from 88 to 97% among the clones ('AF-18' - 88%, 'AF-8' - 96%, 'I-214' - 93%, ‘Terra Nova’ - 97%).

Due to the violation of the requirement for normal distribution of the residuals, the influence of the clone on the sapling heights on each of the 4 measurement dates was analyzed by a non-parametric test (Kruskall-Wallis rank sum test). Height differences within the clone were large throughout the growing season, and the clone was not proven to be a significant source of variation of this variable for any of the four measurement dates (P>0.05). However, a marginally significant genotype effect (P<0.1) was reported at the first and last measurements and therefore the clones were compared pairwise. The comparison showed that significant differences at the first and the last date were found only between the clones 'AF-8' and 'I-214' (P<0.03). While at the beginning of the measurements (mid-July) (Figure 2a) clone 'I-214' surpassed 'AF-8' in height, after the end of growth 'AF-8' had the advantage (Figure 2b).

The physiological parameters net photosynthetic rate, transpiration rate, stomatal conductance, leaf vapor pressure deficit, chlorophyll and water use efficiency were analyzed by one-way Analysis of variance, as the graphical and analytical tests did not show violation of the re-

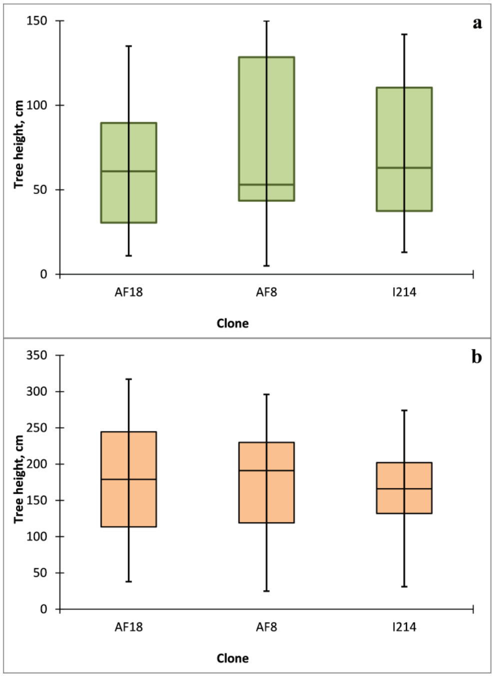

Figure 2. Heights of poplar saplings at the beginning and at the end of the measurements. a) 15 July 2020; b) 22 October 2020. Medians are shown with lines, 25% and 75% quartiles - with boxes, minimum and maximum - with whiskers. / **Slika 2**. Visine sadnica topole na početku i na kraju mjerenja. a) 15 juli 2020; b) 22 oktobar 2020. Medijane su prikazane linijama, kvartil 25% i kvartil 75% prikazani su pravougaonicima, a minimumi i maksimumi sa horizontalnim linijama (eng. whiskers)

quirement for normal distribution of residuals. The genotype was proven as a significant source of variation in all physiological parameters studied (Table 1). The results show that the poplar clones photosynthesize more intensively than the willow one. The highest photosynthetic intensity was reported for clone 'AF-18', significantly lower was the photosynthetic intensity of clone 'AF-8', and the cultivar 'I-214' had an intermediate value (Figure 3a). Significantly highest transpiration rate and stomatal conductance were recorded for clone 'I-214'. Plants originating from large-sized willow cuttings (from biennial shoots) showed higher stomatal conductance and transpiration rate compared to willow saplings from smaller-sized cuttings (from one-year-old shoots). Transpiration rate and stomatal conductance were low for both poplar clones for biomass (Figures 3b, 3c). In contrast to the transpiration rate and stomatal conductance results, the highest water use efficiency was found for the two poplar genotypes, selected for biomass, followed by the willow saplings from small-sized cuttings. Cultivar 'I-214', which showed the highest transpiration rate, had the lowest value of water use efficiency of all poplar clones (Figure 3d). The highest leaf vapor pressure deficit was reported for the saplings from the willow clone. Clone 'AF-8' also showed a relatively high leaf vapor pressure deficit, while the other two poplar clones were at the lower end of the scale (Figure 3e). The chlorophyll content of the leaves of the willow saplings was twice as high as that of the poplars (Figure 3f).

The measurements of the normalized difference vegetation index in the middle and at the end of July did not show significant differences between the studied clones (Figures 4a, 4b). A distinct inter-clone variation for this indicator (P<0.05) was manifested in August when the genotype 'AF-8' showed significantly the highest values, and the clone 'Terra Nova' lagged significantly (Figures 4c, 4d).

Table 1. Analysis of variance results about the effect of the clone on principal physiological parameters / **Tabela 1.** Rezultati analize varijanse efekta klona na glavne fiziološke parametre

Parameter	Factor	*DF*	*SS*	*MS*	*F-test*	*P*
Photosynthesis	Clone	4	56.58	14.15	66.81	<0.001
	Residuals	20	4.234	0.212		
Transpiration	Clone	4	1.353	0.338	119.3	<0.001
	Residuals	20	0.057	0.003		
Conductivity of the stomata	Clone	4	$6.11x10^{-3}$	$1.53x10^{-3}$	158.0	<0.001
	Residuals	20	$1.93x10^{-4}$	$9.67x10^{-6}$		
Water use efficiency	Clone	4	310.8	77.70	68.67	<0.001
	Residuals	20	22.63	1.131		
Leaf vapour pressure deficit	Clone	4	$4.22x10^{-2}$	$1.05x10^{-2}$	146.4	<0.001
	Residuals	20	$1.44x10^{-3}$	$7.20x10^{-5}$		
Chlorophyll	Clone	4	$2.39x10^{-3}$	$5.96x10^{-4}$	17.22	<0.001
	Residuals	20	$6.93x10^{-4}$	$3.46x10^{-5}$		

Note. Abbreviations: DF - degrees of freedom; SS - sum of squares; MS - mean square; F-test - Fisher's criterion; P - parameter of statistical significance / **Napomena.** Skraćenice: DF - stepeni slobode; SS - suma kvadrata; MS - sredina kvadrata; F-test - Fišerov kriterijum; p - parametar statističke značajnosti

Three of the clones ('Terra Nova', 'AF-18' and 'I-214') reached their maximum height increment in the second half of July, except for clone 'AF-8', whose growth in height was most intense in the first half of August. As expected, the height increment of the willow clone was more than twice smaller than the height increment of the poplar clones. It showed a sharp decline already in the first half of August. The course of height increment of cultivar 'I-214' and clone 'AF-18' were similar, but the increment of the cultivar 'I-214' was less than that of the two clones for biomass throughout the observed growth period. After the end of August, clones 'AF-18' and 'AF-8' had similar values of height increment. The height increment decline of all clones decreased after the last irrigation on 15 August and increased again at the end of August with the cessation of watering (Figure 5a).

The willow clone first reached its maximum diameter increment in the second half of July, which also coincided with the period of its maximum height increment. The diameter in-

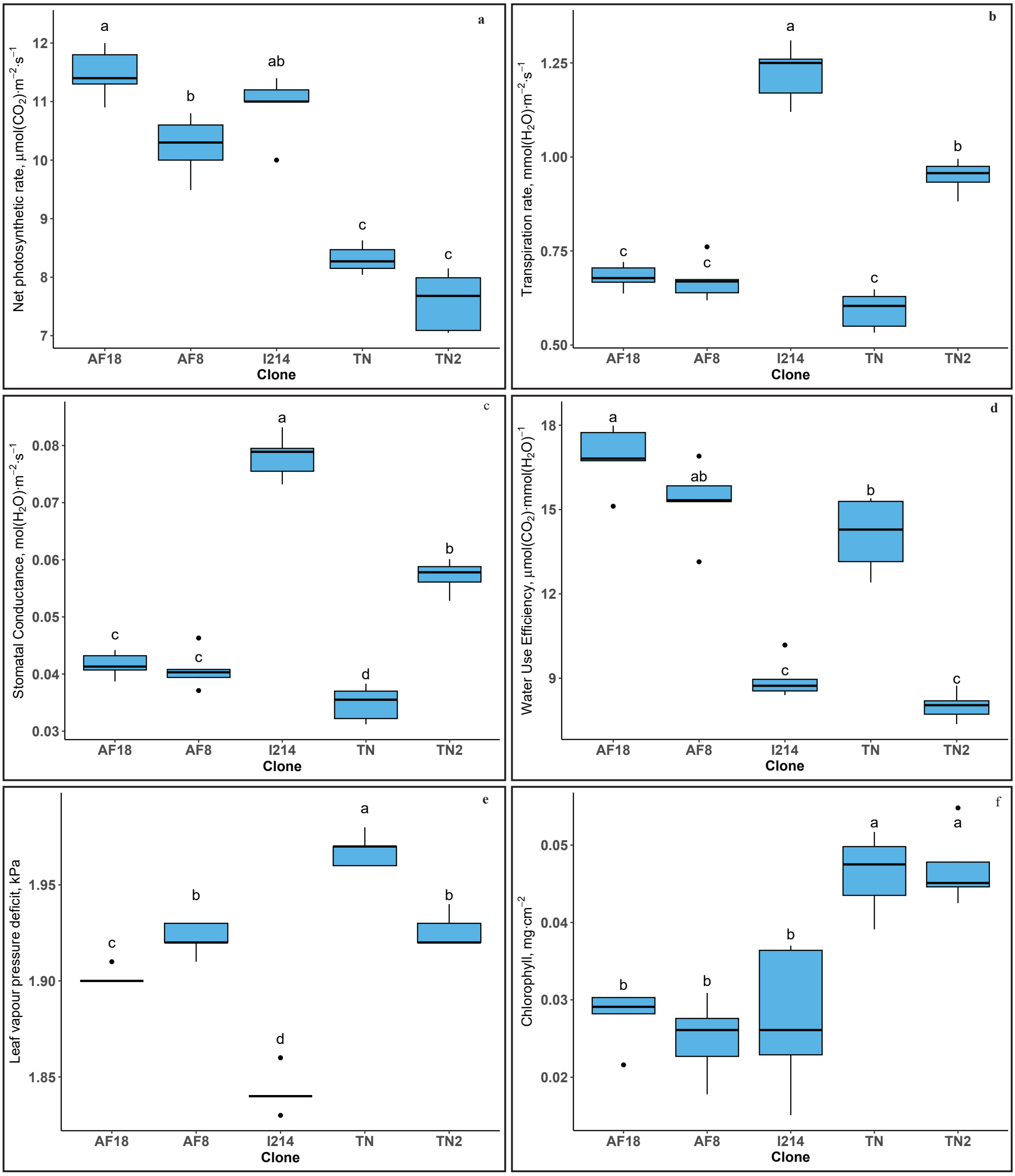

Figure 3. Physiological parameters of the studied genotypes, measured on 15 July 2020 with Li- 6400. a) net photosynthetic rate (µmol(CO_2)·m^{-2}·s^{-1}), b) transpiration rate (mmol(H_2O)·m^{-2}·s^{-1}), c) stomatal conductance (mol(H_2O)·m^{-2}·s^{-1}), d) water use efficiency - WUE (µmol(CO_2)·mmol(H_2O)$^{-1}$), e) leaf vapor pressure deficit (kPa), f) chlorophyll content (mg·cm^{-2}). Medians are shown with with lines, 25% and 75% quartiles - with boxes, minimum and maximum - with whiskers. / **Slika 3**. Fiziološki parametri proučavanih genotipova mjerenih 15 jula 2020. sa uređajem Li- 6400. a) neto fotosinteza (µmol(CO_2)·m^{-2}·s^{-1}), b) transpiracija (mmol(H_2O)·m^{-2}·s^{-1}), c) stomatalna provodljivost (mol(H_2O)·m^{-2}·s^{-1}), d) efikasnost upotrebe vode - WUE (µmol(CO_2)·mmol(H_2O)$^{-1}$), e) deficit pritiska vodene pare (kPa); f) sadržaj hlorofila (mg·cm^{-2}). Medijane su prikazane linijama, kvartil 25% i 75% pravougaonicima, minimumi i maksimumi sa horizontalnim linijama (eng. *whiskers*)

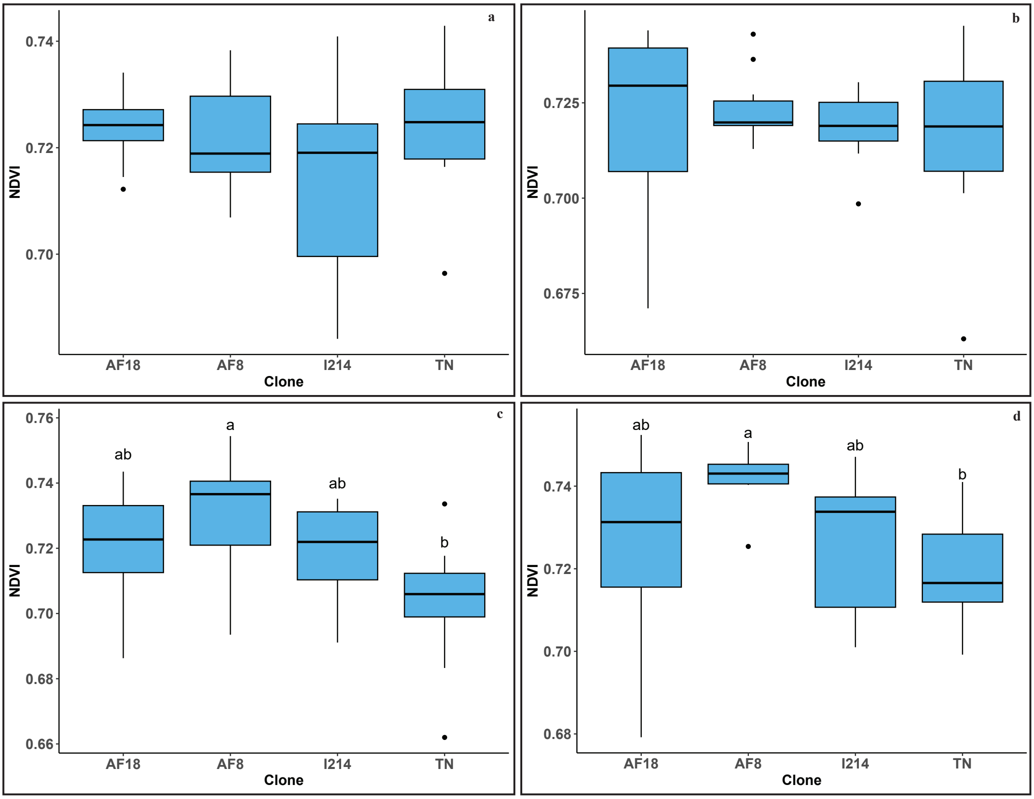

Figure 4. Normalized difference vegetation index (NDVI) of the studied clones during the summer of 2020. a) 15 July; b) 30 July; c) 14 August; d) 30 August. Medians are shown with lines, 25% and 75% quartiles - with boxes, minimum and maximum - with whiskers. / **Slika 4**. Normalizovani diferencijalni vegetacioni indeks (NDVI) proučavanih klonova tokom ljeta 2020. a) 15. juli; b) 30. juli; c) 14. avgust; d) 30 avgust. Medijane su prikazane linijama, kvartil 25% i 75% pravougaonicima, minimumi i maksimumi sa horizontalnim linijama (eng. *whiskers*)

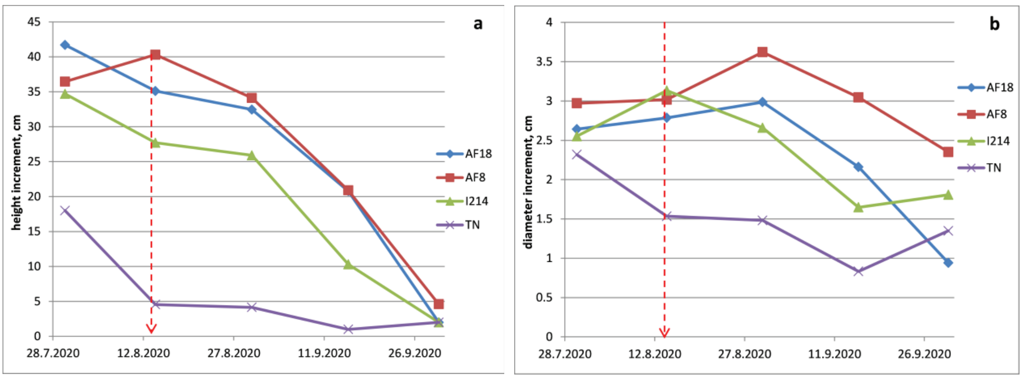

Figure 5. The course of increments during the growth period by clones. a) height increment; b) diameter increment / **Slika 5**. Tendecije prirasta klonova tokom perioda rasta. a) visinski prirast; b) prirast prečnika

crement of cultivar 'I-214' peaked in the first half of August, earlier than the other two poplar clones. Clones 'AF-8' and 'AF-18' reached the maximum diameter increment in the second half of August, with clone ‘AF-8’ surpassing all other genotypes in this variable (Figure 5b).

Height increment showed a strong correlation with the water supply. The correlations with temperature and wind speed were weaker (about 0.3) and the direction of correlation with the wind speed was negative (Table 2). The correlations between the diameter increments and the climate variables were similar but less strong.

Despite the general trends established for all clones, the studied genotypes showed also individual specificities concerning the meteorological factors. Clone 'Terra Nova' was characterized by the strongest positive form of dependence on moisture retention (correlation coefficients 0.708 and 0.553 for height and diameter increments, respectively, Table 2). Its correlations with the temperature were low and not statistically significant. Cultivar 'I-214' also showed a high positive correlation between the height increment and the moisture supply (correlation coefficient 0.695), exceeding the average for all clones. In contrast to the other clones, the poplars, selected for biomass ('AF-8' and 'AF-18') exhibited a stronger positive correlation with the temperature, both in terms of height and diameter increments (Table 2).

Table 2. Correlations between the increments of the studied clones and the climate parameters during the vegetation period [a] / **Tabela 2.** Korelacije između prirasta proučavanih klonova i klimatskih parametara tokom vegetacionog perioda [a]

Clone	Parameter	Water supply (rain+irrigation)	Average min. temperature	Average max. temperature	Average wind speed
Height increment	All clones	0.531 ***	0.265 ***	0.373 ***	-0.330 ***
	AF 8	0.562 ***	0.469 **	0.459 **	-0.448 **
	AF 18	0.529 ***	0.334 *	0.453 **	-0.297 *
	I 214	0.695 ***	0.268 '	0.426 **	-0.406 **
	Terra Nova	0.708 ***	-0.185 NS	0.066 NS	-0.368 *
Diameter increment	All clones	0.326 ***	0.176 *	0.265 ***	-0.167 *
	AF 8	0.191 NS	0.349 *	0.296 *	-0.198 NS
	AF 18	0.385 **	0.301 *	0.415 **	-0.162 NS
	I 214	0.286 *	0.162 NS	0.319 *	-0.069 NS
	Terra Nova	0.553 ***	-0.102 NS	0.077 NS	-0.252 '

Note. [a] Levels of the significance of the established correlations are indicated as follows: *** - P < 0.001, ** - P < 0.01, * - P < 0.05, ‘ - P < 0.1, NS - P > 0.1 / **Napomena.** [a] Nivoi značajnosti utvrđenih korelacija su prikazani kako slijedi: *** - P < 0.001, ** - P < 0.01, * - P < 0.05, ‘ - P < 0.1, NS - P > 0.1

The results showed that in terms of survival, 'Terra Nova' performed best among all the clones and 'AF-8' among the poplars (Table 3). The worst survival was observed in genotype 'AF-18', but on the other hand, its height growth was the best. Cultivar 'I-214' had the lowest and negative growth score for height and diameter, while clone 'AF-8' had the highest score for diameter. When evaluating the biomass of all tested clones, 'AF-8' performed the best, and the least productive was the willow clone 'Terra Nova'. The least amount of biomass from the poplar clones was estimated in 'AF-18'. Of the 3 poplar clones tested, the highest adaptability and growth potential in the first year of development under nursery conditions in North Central Bulgaria was shown by clone 'AF-8', while the other poplar clone for biomass ('AF-18') was with the lowest complex index compared to all tested clones. Thanks mainly to its good survival, the willow clone 'Terra Nova' performed above average compared to the other clones in terms of its adaptive capacity (Table 3).

Table 3. Standardized indices for growth and adaptivity evaluation [a] / **Tabela 3.** Standardizovani indeksi za rast i evaluacija adaptibilnosti [a]

Clone	Plant survival		Height	Basal diameter	Biomass		[b] **Complex score - poplar clones**	**Complex score - all clones**
	All clones	Poplar clones			All clones	Poplar clones		
AF-18	-1.30 **0.882**	-1.01	0.24 **1.87**	-0.18 **1.60**	-0.19 **0.103**	-0.24	***-0.30***	***-0.75***
AF-8	0.72 **0.965**	0.99	0.08 **1.78**	0.37 **1.95**	0.52 **0.194**	0.40	***0.46***	***0.62***
I-214	-0.26 **0.925**	0.02	-0.32 **1.56**	-0.19 **1.59**	-0.10 **0.115**	-0.16	***-0.16***	***-0.18***
Terra Nova	0.84 0.970		1.35	0.91	-0.23 **0.098**			***0.31***
Mean variable value	*0.936*	*0.924*	*1.737*	*1.714*	*0.127*	*0.137*		
Standard deviation	*0.041*	*0.041*	*0.541*	*0.634*	*0.128*	*0.143*		

Note. [a] Survival, height, basal diameter and dry biomass are presented below the standardized index values in bold. Plant survival is expressed as a proportion of the rooted cuttings, height is measured in meters, basal diameter is measured in centimeters, and biomass is measured in kilograms of dry weight. [b] The complex scores of growth and adaptability based on the standardized indices are shown in bold, italics / **Napomena.** [a] Preživljavanje, visina, prečnik i suva masa su prikazane ispod standardizovanog indeksa koji je prikazan podebljanim fontom. Preživljavanje biljaka je mjereno kao odnos ukorijenjenih reznica, visina je mjerena umetrima, prečnik osnove u centimetrima, a biomasa u kilogramima suve mase. [b] Složeni rezultati rasta i prilagodljivosti bazirani su na standardizovanim indeksima i prikazani su podebljanim slovima i u kurzivu

4. DISCUSSION / DISKUSIJA

Ceulemans & Deraedt (1999) generalized that the poplar clones are characterized by a high rate of rooting and survival during the year of establishment. Average rooting rates reported are around 80–100% (Bergante & Facciotto, 2011; Paris et al., 2011). Rooting problems were found only for *P. deltoides* clones and hybrids (Bergante & Facciotto, 2011; Ceulemans & Deraedt, 1999; Paris et al., 2011). In the present study, a high survival rate of the rooted plants was reported (88–97%), but the rooting rate of the poplar clones was low (36–54%), including 'I-214' cultivar, which shows traditionally high rooting percentages (over 80%) under the specific conditions. A possible explanation for the observed result is the unfavorable moisture regime of the soil during the establishment stage, with the precipitation amount in April being only 24 mm/m^2, and the lack of pre-treatment of the cuttings to stimulate the rooting process. In contrast to results reported for Slovakia (Demo et al., 2013), where 'Terra Nova' clone showed a low rooting rate (56%), compared to the other 7 tested willow clones, the rooting percentage of this clone in our experiment was high (87%).

Ayik (1989) reported that poplar grows best in soils having 35% or less clay content and well-aerated, at pH values of 6.5–8. The poplar needs for organic matter are higher than 1.15% (Tufekcioglu et. al., 2005). Our results describe the soils with a mean acid reaction of 7.56, medium sandy-clay with an average clay content of 41.76%, slightly porous to the porous structure, and moderately rich in organic carbon content (Artinova, 2014), which was 1.48% on average (or 14.83 $g \cdot kg^{-1}$). Considering the referenced values of the soil characteristics recommended for growing poplar clones, we can assume that the soil conditions in our experimental area, were not completely satisfying the needs of the poplar saplings, of clay content higher than 35%, which leads to sub-optimal soil aeration. The acid reaction of the soil and the organic carbon content were in the optimum zone according to Ayik (1989) and Tufekcioglu et. al (2005).

Apart from the exceptions (for clones 'AF-8' and 'I-214' on the first and the last date), we did not find significant differences between the heights of the tested clones, due to the large variation of the variable within the clones. This is probably related to the fact that the growth in the first year is highly dependent on the process of formation of the root system of the new plants and is therefore limited by it. In some studies, (Orlandi et al., 2017), data from the first year are not even used when comparing the growth potential of different clones. In a study conducted in western Slovakia (Heilig et al., 2021) collating the growth and survival of 21 poplar clones on 4 sites, even after 3 years of growth, significant differences between clones were found in only one of the sites. On the other hand, when comparing four poplar clones in an experiment conducted in Latvia, interclone differences were found after the second but not after the third year of growth (Lazdiņa et al., 2014). In this case, the variation was probably obscured by the different fertilizer treatments.

In a comparative study conducted in Slovakia, the willow clone 'Terra Nova' showed the lowest growth rate in the first year compared to seven other clones (Demo et al., 2013). Its average shoot length value is comparable to that measured in our nursery experiment (1.36 vs 1.35 m).

Clone 'AF-8' has been tested and compared with other clones in several experimental plantations across Europe. Sabatti et al. (2014) found that 'AF-8' outperformed five other poplar genotypes grown in northern Italy after 2 years of growth. In the same experiment, cultivar 'I-214' showed the lowest parameter values. In another experiment in Latvia, 'AF-8' was not the best-performing clone, but showed increasing with age height increment (Lazdiņa et al., 2014). On the other hand, 'AF-8' showed the lowest height and diameter increments by the sixth year compared to 8 other clones grown in an experiment in Poland, where the average annual temperature was 7 °C and the annual rainfall was over 620 mm (Niemczyk et al., 2016). Our study showed that of the three tested poplar clones, the highest adaptability and growth potential in the first year in the nursery of Vardim was shown by clone 'AF-8', and the lowest by the other biomass poplar clone 'AF-18' '. The superiority of the poplar clone 'AF-8', compared to 5 other tested clones after one year of growth, was also confirmed on 5 sites in Romania, two of which are located along the Danube River (Filat et al., 2010). However, the saplings of 'AF-8' in these experiments were superior in height and diameter to those grown in our nursery plantation, with tree height below 2 m and basal diameter less than 2 cm only on the two worst sites. On the other hand, the one-year-stem heights were in the range of 51–124 cm for 33 poplar clones, 'AF-8' and 'AF-18' among them, when grown under cooler conditions in Latvia (Šēnhofa et al., 2021). Similar to our results, in the Latvian experiment 'AF-18' was superior to 'AF-8' in height (Table 3), but in both clones, the average height did not exceed 1 m.

Filat et al. (2010) reported the most intensive height growth of the one-year-old poplar saplings in July and August and its decline thereafter, which agrees with our findings. The course of growth during the growing season is closely related to the climatic variables, which in our study was proved by the established positive correlations with the temperature and especially with the precipitations. Our results are also comparable to the reported positive relationships of the heights of 12 poplar clones with the temperature and precipitations during the growing season in Latvia (Šēnhofa et al., 2018). In an experiment in central Italy, the relationship between the growth of 'AF-8' and 'Monviso' clones with the climatic variables was analyzed separately for the first and the second half of the growing season (Orlandi et al., 2017). In the first half of the growing season (till mid-July), a strong positive correlation of height increment with temperature and solar radiation was established. In the second half of the growing season, the direction of this correlation changed to negative, and the positive influence of the precipitations increased. In contrast to our experiment, which covered only the second half of the growing season, the growth of the poplar saplings in the study by Orlandi et al. (2017) was analyzed at 2–5 years of age, when they were grown without irrigation and with average monthly rainfall amounts below 50 mm·m^{-2}.

Orlandi et al. (2017) generalized that in terms of drought tolerance, poplar clones can be classified into two categories: isohydric and anisohydric. Isohydric clones have a tight control of stomatal conductance, while the opposite is true for anisohydric ones. It is therefore hypothesized that isohydric clones will have higher water use efficiency and consequently better drought tolerance. Conversely, anisohydric poplar clones will maintain high stomatal conductance, which will decrease their vitality and survival in a prolonged drought. The once-measured physiological parameters of net photosynthesis rate, transpiration rate and stomatal conductance of the studied clones in mid-July suggest that the two poplar biomass clones are more isohydric than the cultivar 'I-214' and the willow clone 'Terra Nova'. Their values show both lower stomatal conductance and transpiration rate and higher water use efficiency of 'AF-8' and 'AF-18' clones. Rodzkin et al. (2015), in a study on four willow clones, also found that higher water use efficiency was associated with lower transpiration rates. Our assumption of an anisohydric strategy of the willow clone 'Terra Nova' and the poplar cultivar 'I-214' is also confirmed by the higher correlations with the moisture supply found for these 2 clones. There was also a sharper decline in their height and diameter increments during the drier period in late summer after the irrigation was stopped. The significantly lower values of the normalized difference vegetation index of the willow clone 'Terra Nova' at the end of August, on the other hand, suggests that its anisohydric behavior was not successful at the experimental site, as it led to reduced plant vigor. In contrast, the naturalized poplar cultivar 'I-214' shows a vitality comparable to the isohydric clones 'AF-8' and 'AF-18' under unfavorable moisture regime.

5. CONCLUSION / ZAKLJUČAK

The willow clone showed the highest dependence on moisture availability, early culmination of increment, a sharp decline in growth with the suspension of watering and a decrease in the viability indicator NDVI. It had the lowest net photosynthetic rate and low biomass growth, but a high survival rate.

Unlike the other poplar genotypes, the cultivar ‘I-214’, showed rapid growth at the beginning of the summer, had a high transpiration rate and a stronger dependence on moisture storage. Clones ‘AF-8’ and ‘AF-18’ showed high net photosynthetic and low transpiration rates and their growth was positively affected by temperatures during the growing season. The water use efficiency of the poplar biomass clones was the highest. 'AF-8' genotype showed the best diameter and biomass growth, while the 'AF-18' genotype grew best in height. The production of propagules from the clones for biomass in nurseries along the Danube River can be recommended.

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Sažetak

Adaptibilnost tri klona iz familije *Salicaceae* selekcionisanih za proizvodnju biomase (*Salix* x 'Terra Nova', *Populus* x 'AF-18' and *Populus* x 'AF-8') testirani su u prvoj godini rasta u rasadniku i upoređeni sa klonom koji je namijenjen za proizvodnju drveta (*Populus* x 'I-214').

Eksperimentalni zasad uspostavljen je u aprilu 2020. godine sa reznicama na površini od 0,2 hektara na zemljištu koje se nalazi pored rijeke Dunav centralnom dijelu sjevernog dijela Bugarske. Izmjereni su glavni parametri razmjene gasova lišća, prirast prečnika, visina i visinski prirast, a na reprezentativnim induviduama praćen je normalizovani diferencijalni vegetacijski indeks. Analiziran je uticaj genotipa te su tražene korelacije sa glavnim klimatskim parametrima.

Klon vrbe pokazao je ranu kulminaciju prirasta i oštar pad rasta i vitalnosti pri zaustavljanju navodnjavanja. Navedeni klon vrbe imao je neto fotosintezu (8.312 ± 0.107 µmol $(CO_2)\cdot m^{-2}\cdot s^{-1}$), nizak prirast biomase (0.098 ± 0.01 kg DM) i visoku stopu preživljavanja.

Za razliku od drugih genotipova topola, klon I-214 pokzao je brz rast na početku ljeta, visoku stopu transpiracije (1.222 ± 0.034 mmol (H_2O)·m^{-2}·s^{-1}) i jaču zavisnost od količine vlage. Klonovi 'AF-8' i 'AF-18' imali su visoke stope fotosinteze (10.238 ± 0.231 i 11.480 ± 0.193 µmol (CO_2)·m^{-2}·s^{-1}) i nisku transpiraciju (0.672 ± 0.024 i 0.682 ± 0.015 mmol (H_2O)·m^{-2}·s^{-1}) a na njihov rast temperatura je pozitivno djelovala. Najveća efikasnost upotrebe vode registrovana je kod klonova topola. 'AF-8' genotip postigao je najveći prečnik i proizvedenu količinu biomase (0.194 ± 0.036 kg DM po biljci), dok je genotip 'AF-18' pokazao najbolji rast u visinu.

Ključne riječi: adaptibilnost, *Populus* 'AF-8', *Populus* x 'AF-18', rast, *Salix* x 'Terra Nova'