UDK: 582.681.81:628.1

Original scientific paper *Izvorni naučni rad*

RECOVERY RESPONSES OF PHOTOSYNTHESIS, TRANSPIRATION, AND WUE IN BLACK POPLAR CLONES FOLLOWING WATER DEFICITS

Topić Mirjana[1], Borišev Milan[1], Župunski Milan[1], Tomičić Marina[2], Nikolić Nataša[1], Pajević Slobodanka[1], Krstić Borivoj[1], Pilipović Andrej[2]

Abstract: Five black poplar (*Populus nigra* L.) genotypes were grown in a semi-controlled environment and were subjected to three different soil water regimes: control (90-70% of maximal field capacity-FC), drought followed by recovery of optimal soil water saturation (90-40% of FC) and permanent drought treatment (50-40% of FC). Variation in leaf gas exchange parameters such as photosynthetic (P) and transpiration intensity (T), as well as water use efficiency (WUE) were investigated among genotypes in their response to water deficit and recovery from drought. Recovery of soil water field capacity from 40% to 90% determined restored values of P, T and WUE. After 16 days of permanent drought (50-40% FC), strong decline of all parameters among examined genotypes was determined. Overall, significant differences among genotypes in leaf gas exchange parameters were found, which can give some indications of superiority of certain genotypes in relation to drought stress.

Key words: *Populus nigra* L., genotypes, drought stress, recovery.

ODGOVORI KLONOVA CRNE TOPOPLE NA OPORAVAK POSLE VODNOG DEFICITA U KONTEKSTU FOTOSINTEZE, TRANSPIRACIJE I EFIKASNOSTI KORIŠČENJA VODE

Izvod: *Pet genotipova crne topole (Populus nigra L.) su u polu-kontrolisanim uslovima izloženi različitim vodnim režimima: optimalnoj zasićenosti vodom (kontrola), suši praćenoj oporavkom optimalnog vodnog kapaciteta i tretmanu konstantne suše. U odgovoru genotipova na vodni deficit i oporavak, ispitivane su promene parametara koji se odnose na razmenu* CO_2 *i vodene pare u listu, kao što su intenzitet fotosinteze (P) i transpiracije (T) i efikasnost koriščenja vode (WUE). Ponovno podizanje vodnog kapaciteta zemljišta na 90%,*

[1] Mirjana Topić, student doktorskih studija, dr Milan Borišev, docent, Milan Župunski, istraživač-pripravnik, Nataša Nikolić, docent, Slobodanka Pajević, redovni profesor, Borivoj Krstić, redovni profesor, Prirodno-matematički fakultet, Departman za biologiju i ekologiju, Univerzitet u Novom Sadu.
[2] Dr Andrej Pilipović, asistent, Institut za nizijsko šumarstvo i životnu sredinu, Univerzitet u Novom Sadu.
[3] Marina Tomičić, istraživač-pripravnik, Institut za ratarstvo i povrtarstvo, Univerzitet u Novom Sadu.

nakon vodnog deficita pri kapacitetu od 40%, uslovilo je oporavak intenziteta fotosinteze, transpiracije i efikasnosti korišćenja vode na nivoe približne vrednostima kontrolnih biljaka. Nakon 16 dana konstantne suše (vodni kapacitet u opsegu 50-40%) utvrđeno je snažno opadanje analiziranih parametara u svim genotipovima. Značajne varijacije analiziranih parametara među genotipovima ukazuju na specifične adaptivne predispozicije određenih gentipova u odnosu na sušni stres.

Ključne reči: *Populus nigra* L., *genotipovi, sušni stres, oporavak.*

INTRODUCTION

Plants have evolved complex systems of defense against, as well as adaption to, the variable and often potentially damaging environmental conditions to which they are exposed during their growth and development (Dobra et al., 2010). Drought is one of the most important factors affecting plant growth, development, survival and crop productivity (Ahuja et al., 2010).

As the key process of primary metabolism, photosynthesis plays a central role in plant performance under drought stress (Chaves et al., 2003; Chaves et al., 2009; Flexas et al., 2004; Flexas et al., 2009; Lawlor and Tezara, 2009; Pinheiro and Chaves, 2011). When plants encounter water deficit, there is a decline in photosynthesis. This may be due to reductions in C fixation per unit leaf area as stomata close or as photo-oxidation damages the photosynthetic mechanisms (Bruce et al., 2002).

Great attention has been drawn to the study of WUE (water use efficiency), in order to detect genotypes that consume less water and are photosynthetically more efficient (Orlović et al., 2002). Genotype adaptive values in terms of gas exchange characteristics should be expressed in high photosynthetic and transpiration potential as well as relatively high and stabile WUE. In this sense, the aim of breeding programs is to select cultivars characterized by sufficient tolerance and resistance to mild and severe drought.

Poplars are among the fastest growing trees in temperate latitudes. However, poplar biomass productivity is closely linked to water availability, and the degree of drought tolerance is an important determinant of productivity in selected clones (Heilman and Stettler, 1986; Tschaplinski and Blake, 1989). Although poplars are among the most susceptible woody plants to drought-induced stress, significant clonal variability in drought tolerance and patterns of response to water deficits has been recorded (Pallardy and Kozlowski, 1981; Gebre and Kuhns, 1991; Chen et al., 1997).

The leaf, being the first organ to show visible signs of drought, may provide a cheap and easy to manipulate trait for selection under water deficit (Anyia and Herzog, 2004). Therefore, the aim of this study was to quantify some leaf gas exchange parameters in five black poplar clones growing on soil which was treated with three different water regimes. The obtained results provide clear evidence for clonal differentiation in their responses to water deficiency.

MATERIAL AND METHODS

Plant Material and Treatments

The experimental material consisted of five black poplar (*Populus nigra* L.) clones (VII/25, IX/30, X/32, XI/36, I/2) obtained by selection of high biomass productivity genotypes at the Institute of Lowland Forestry and Environment, University of Novi Sad, Serbia.

Twenty- centimeter cuttings of five black poplar clones were used. In April 2011, the cuttings were planted in 30 Mitscherlich pots, 4 cuttings per each pot. The plants were grown in a semi-controlled environment (greenhouse) by soil culture method. The temperature was kept under 30°C. The illumination was natural and dependent on the outside light conditions. Until the initiation of treatments, all plants were irrigated to keep the soil moisture in the interval of 70 – 90% of maximal field capacity (FC), during three months.

After growing for three months, all plants had fully developed roots and shoots. Only one shoot per each cutting was selected for further analyses, and all other shoots were removed. Shoots of similar height in all plants were selected. Plants were divided in three treatment groups (10 pots per treatment, 2 pots per clone in each treatment).

At the begging (phase A), all three groups were subjected to 100% of soil water saturation (100% of FC). After that, three groups of plants were assigned to three watering regimes: control (90 − 70% of FC) drought followed by recovery of FC (90 − 40% of FC) and permanent drought (50 − 40% of FC). When plants in second group reached the 40% of FC, they were irrigated to reach 90% of FC, whereas plants in permanent drought group were irrigated only to 50% of FC (phase B – recovery). This was followed by decrease of FC to 40% in both group of plants exposed to mild and severe drought treatment (phase C).

Photosynthesis (P) and transpiration (T) intensity were measured during July of 2011 in all three groups of plants during all three phases: A, B and C. Mesurements in phase A were conducted at the beginning of treatments, in phase B after 10 days and in phase C after 16 days.

Physiological Measurements

Leaf gas exchange parameters (photosynthesis and transpiration) were measured on fully expanded young leaves using the LCpro+ portable photosynthesis system, manufactured by ADC BioScientific Ltd. Light conditions were set using the LCpro+ light unit, which emitted photosynthetically active radiation (PAR) at 1000 μmol • m^{-2} • s^{-1}. The air supply unit provided a flow of ambient air to the leaf chamber at a constant rate of 100 μmol • s^{-1}. Humidity was set at 10 mBar of partial water pressure. Temperature and CO_2 concentration were at ambient levels.

Analyses were conducted at 3 different plants per each treatment and per each clone. At each plant, 3 separated measurements were recorded.

Parameter WUE (water use efficiency) was calculated as the ratio of photosynthesis divided by transpiration: WUE = P/T (μmol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}).

Statistical Analysis

Statistical analyses were conducted by ANOVA two-way factor analyses using Duncan's multiple range test at a significance level of $p<0.05$. The software used was MSTAT C. The average values shown in figures followed by the same letter did not differ significantly. Values are shown as mean ± standard deviation.

RESULTS

At the beginning of treatment, all three groups of plants were irrigated to 100% FC (Phase A). Clone X/32 showed the highest values in photosynthetic intensity (Fig.1). Genotypic behavior in relation to values of T and WUE (Figs. 2 and 3) were quite similar to P under well-watered conditions.

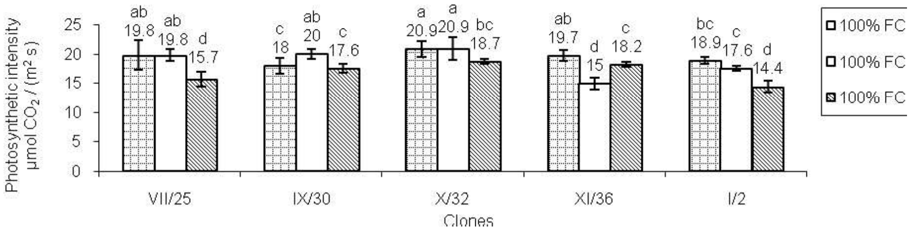

Figure 1. Phase A – photosynthetic intensity (P). All treatments at 100% of field capacity (FC)

Slika 1. Faza A – intenzitet fotosinteze. Svi tretmani na 100% maksimalnog vodnog kapaciteta zemljišta

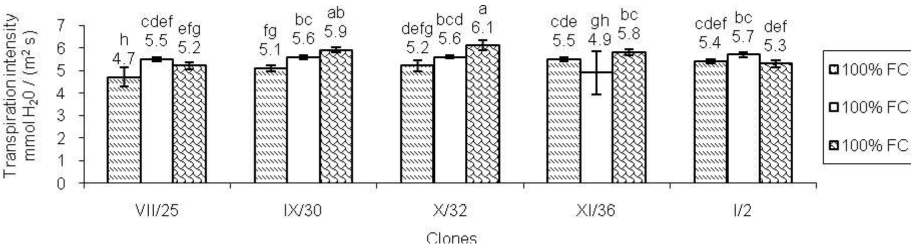

Figure 2. Phase A – transpiration intensity. All treatments at 100% of field capacity (FC)

Slika 2. Faza A – intenzitet transpiracije. Svi tretmani na 100% maksimalnog vodnog kapaciteta zemljišta

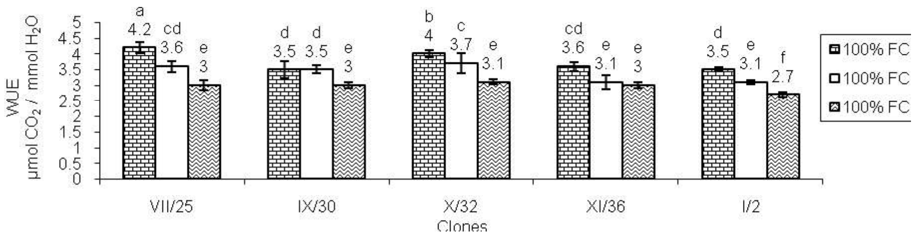

Figure 3. Phase A – Water Use Efficiency. All treatments at 100% of field capacity (FC)

Slika 3. Faza A – Efikasnost korišćenja vode. Svi tretmani na 100% maksimalnog vodnog kapaciteta zemljišta

When FC decreased to 40% in second and third group of plants, second group was irrigated again to recover the water field capacity to 90%, whereas the third group of plant was irrigated only to 50% of FC (permanent drought). During this phase (phase B), photosynthetic intensity (P) (Fig. 4) and transpiration intensity (T) (Fig. 5) in control (90 – 70% FC) and recovered drought treatment (90 – 40% FC) displayed completely restored values, similar to those which were shown in phase A, while permanent drought treatment (50 – 40% FC) values were slightly retarded along with WUE (Fig. 6).

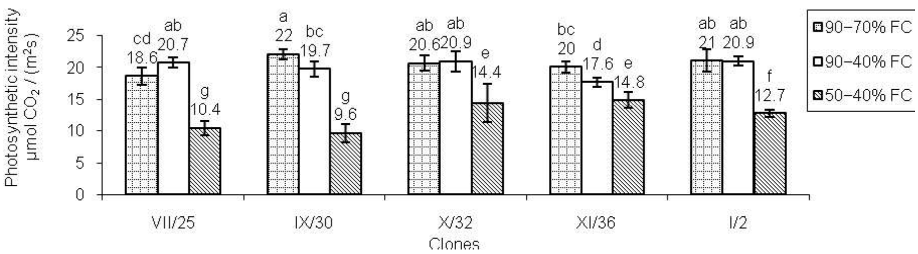

Figure 4. Phase B – recovery, photosynthetic intensity. Control treatment at 90 − 70% FC, drought treatment recovered at 90% FC after 40% FC, permanent drought treatment at 50 – 40% FC

Slika 4. Faza B – oporavak, intenzitet fotosinteze. Kontrola u opsegu 90 − 70% vodnog kapaciteta, tretman suše na 90% vodnog kapaciteta nakon vodnog deficita od 40%, tretman kontinuirane suše u opsegu 50 – 40% vodnog kapaciteta zemljišta

Slight increase of T was determined in clones VII/25, IX/30 and X/32 in plants exposed to 90 - 40% FC. Therefore, values of WUE for these clones, slightly decreased in spite of restored photosynthetic intensity. Permanent drought treatment caused further decrease of WUE (Fig. 6).

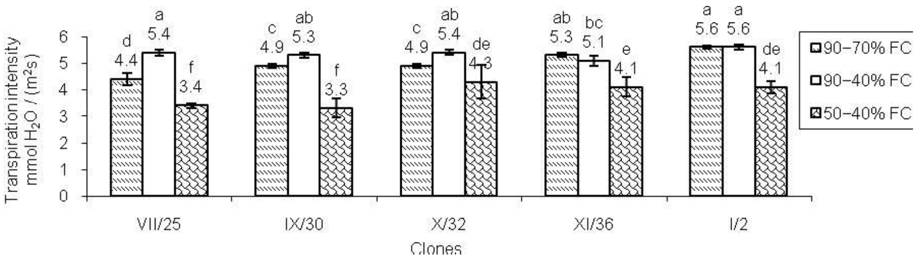

Figure 5. Phase B – recovery, transpiration intensity. Control treatment at 90 – 70% FC, drought treatment recovered at 90% FC after 40% FC, permanent drought treatment at 50 – 40% FC

Slika 5. Faza B – oporavak, intenzitet transpiracije. Kontrola u opsegu 90 – 70% vodnog kapaciteta, tretman suše na 90% vodnog kapaciteta nakon vodnog deficita od 40%, tretman kontinuirane suše u opsegu 50 – 40% vodnog kapacitetazemljišta

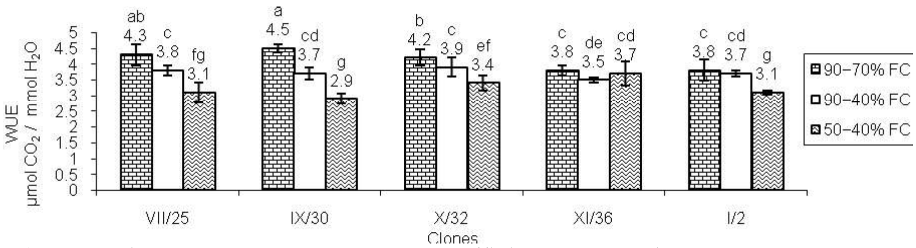

Figure 6. Phase B – recovery, Water Use Efficiency. Control treatment at 90 – 70% FC, drought treatment recovered at 90% FC after 40% FC, permanent drought treatment at 50 – 40% FC

Slika 6. Faza B – oporavak, efikasnost korišćenja vode. Kontrola u opsegu 90 –70% vodnog kapaciteta, tretman suše na 90% vodnog kapaciteta nakon vodnog deficita od 40%, tretman kontinuirane suše u opsegu 50 – 40% vodnog kapaciteta zemljišta

In phase C (Figs. 7, 8, 9) in permanent drought treatment (50 – 40% FC), all examined parameters were almost completely reduced after withholding water for six more days after phase B. However, among the other two treatments, values were similar to each other and were maintained at relatively high levels compared with those in permanent drought treatment.

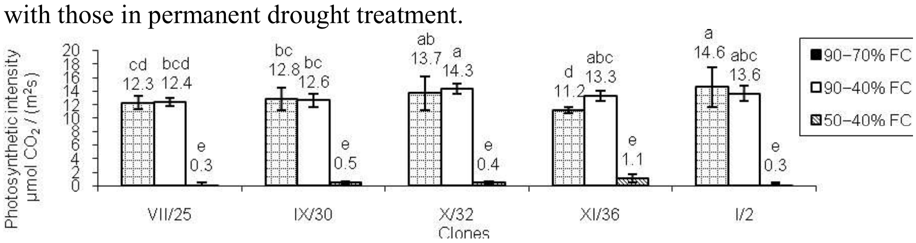

Figure 7. Phase C – photosynthetic intensity. Control treatment at 90 − 70% FC, drought treatment at 40% after recovery at 90% FC, permanent drought treatment at 50 – 40% FC

Slika 7. Faza C − intenzitet fotosinteze. Kontrola u opsegu 90 −70% vodnog kapaciteta, tretman suše na 40% vodnog kapaciteta nakon oporavka na 90%, tretman kontinuirane suše u opsegu 50 – 40% vodnog kapaciteta zemljišta

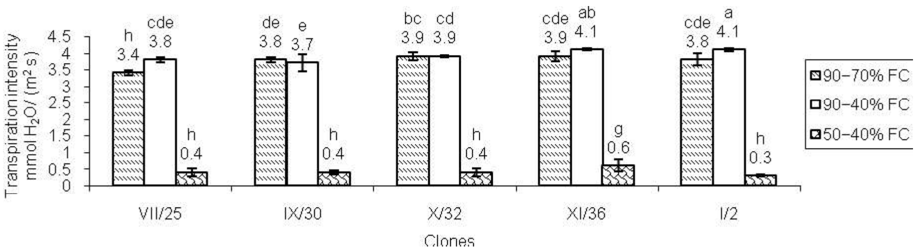

Figure 8. Phase C – transpiration intensity. Control treatment at 90 − 70% FC, drought treatment at 40% after recovery at 90% FC, permanent drought treatment at 50 – 40% FC

Slika 8. Faza C − intenzitet transpiracije. Kontrola u opsegu 90 −70% vodnog kapaciteta, tretman suše na 40% vodnog kapaciteta nakon oporavka na 90%, tretman kontinuirane suše u opsegu 50 – 40% vodnog kapaciteta zemljišta

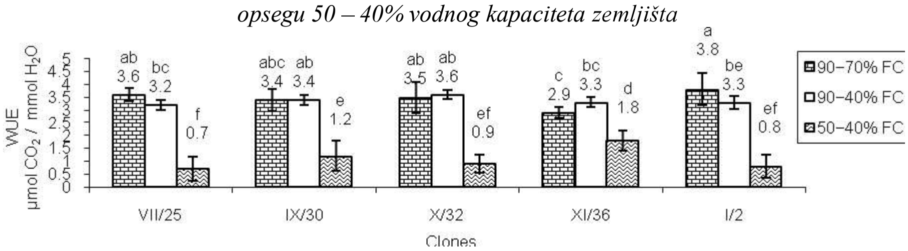

Figure 9. Phase C – Water Use Efficiency. Control treatment at 90 − 70% FC, drought treatment at 40% after recovery at 90% FC, permanent drought treatment at 50 – 40% FC.

Slika 9. Faza C − efikasnost korišćenja vode. Kontrola u opsegu 90 − 70% vodnog kapaciteta, tretman suše na 40% vodnog kapaciteta nakon oporavka na 90%, tretman kontinuirane suše u opsegu 50 – 40% vodnog kapaciteta zemljišta

DISCUSSION

Because poplars grow fast, their demand for water increases as they grow larger. This may induce drought stress under dry conditions (Gebre et al., 1998). In this study five black poplar clones, selected as genotypes with high biomass production were analysed to elucidate genotypic differences in some gas exchange responses to water availability. It has been shown that recovery and prolonged drought significantly affected leaf gas exchange parameters such as: P, T and WUE, under three different soil water regimes.

Variations in P, T and WUE were lower during continuous irrigation at the begging of treatments (Phase A) than in recovery and prolonged drought treatment (Phases B and C). When re-watered after several days without watering, all recovered values were generally similar to the pattern displayed by phase A, except permanent drought treatment (50–40%) in terms of P, where recorded values were the lowest. The recovery values of photosynthesis rate under permanent drought stress are related to the abnormalities in an internal structure of chloroplast. Therefore, the unrecoverable photosynthesis rate after re-watering may be regarded as the rate of internal damage on photosynthesis functions by water stress (Miyashita et al., 2005). Rewatering of investigated poplar plants to 90% of soil water saturation, resulted in full photosynthetic recovery, indicating that disturbances determined by short drought period were not with permanent consequences.

Many reports showed that soil drought could lead to decrease in photosynthetic rate and transpiration (Shao and Chu, 2005; Shao et al., 2005). Because of larger decrease in transpiration (T) than that in photosynthetic rate (P), WUE per leaf would increase (Thomas, 1986; Jie et al., 2001). According to our results, changes of P, T and WUE under prolonged drought (phase C) were not consistent with the above conclusion. During the phase C, there was not larger decrease in T than in P, and WUE per leaf was not increased compared with the phase B. With intensiveness of soil drought (phase C) P, T and WUE of clones decreased, with trend that values for control and recovered drought treatment were similar, while permanent drought treatment values were lower.

Overall, significant genotypic variations in leaf gas exchange parameters were found, which can give some indications of superiority of certain genotypes in relation to drought stress. During prolonged drought (phase C), the fourth (XI/36) clone showed the highest values in P, T and WUE which may suggest genotypic ability to produce a higher level of drought tolerance.

.

Acknowledgement

This paper was part of the project III 043002 financed by the Ministry of Education and Science of the Republic of Serbia.

REFERENCES

Ahuja, I., de Vos, R.C.H., Bones, A.M., Hall, R.D. (2010): Plant molecular stress responses face climate change. Trends in Plant Science Vol. 15, (No.12): 664 — 674.

Anyia, A.O., Herzog, H. (2004): Genotypic variability in drought performance and recovery in cowpea under controlled environment. Journal of Agronomy and Crop Science 190: 151 — 159.

Bruce, V., Ness, H., Hancock, P.J.B., Newman, C., Rarity, J. (2002): Four heads are better than one: Combining face composites yields improvements in face likeness. Journal of Applied Psychology 87: 894 — 902.

Chaves, M.M., Maroco, J.P., Pereira, J.S. (2003): Understanding plant responses to drought – From genes to the whole plant. Functional Plant Bioliology 30: 239 — 264.

Chaves, M. M., Flexas, J., Pinheiro, C. (2009): Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany 103: 551 — 560

Chen, S., Wang, S., Altman, A., Hüttermann, A. (1997): Genotypic variation in drought tolerance of poplar in relation to abscisic acid. Tree Physiology 17: 797 — 803.

Dobra, J., Motyka, V., Dobrev, P., Malbeck, J., Prasil, I.T., Haisel, D., Gaudinova, E., Havlova, M., Gubis, J., Vankova, R. (2010): Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline content. Journal of Plant Physiology 167: 1360 — 1370.

Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T. D. (2004): Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biology 6: 269 — 279.

Flexas, J., Baro´n, M., Bota, J., Ducruet, J.M., Galle´, A., Galme´s, J., Jime´nez, M., Pou, A., Ribas-Carbo´, M., Sajnani, C., Toma`s, M., Medrano, H. (2009): Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (V. berlandieri x V. rupestris). Journal of Experimental Botany 60: 2361 — 2377.

Gebre, G.M., Tschaplinski, T.J., Tuskan, G.A., Todd, D.E. (1998): Clonal and seasonal differences in leaf osmotic potentials and organic solutes of five hybrid poplar clones grown under field conditions. Tree Physiology 18: 645 — 652.

Gebre, G.M., Kuhns, M.R. (1991): Seasonal and clonal variations in drought tolerance of *Populus deltoids*. Canadian Journal of Forest Research 21: 910 — 916.

Heilman, P.E., Stettler., R. F. (1986): Nutritional Concerns in Selection of Black cottonwood and Hybrid Clones for Short Rotation. The Canadian Journal of Botany 16: 860 — 863.

Jie, Y., Yang, H., Cui, M., Luo, X. (2001): Relationship between soil water content and water use efficiency of apple leaves. Chinese Journal of Applied Ecology 12: 387 — 390.

Lawlor, D.W., Tezara, W. (2009): Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany 103:561 — 579.

Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K. (2005): Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environmental and Experimental Botany 53: 205 — 214.

Orlović, S., Pajević, S., Krstić, B. (2002): Selection of Black Poplars for Water Use Efficiency. Proceedings for Natural Sciences, Matica Srpska 102: 45 — 51.

Pallardy, S.G., and Kozlowski, T.T. (1981): Water relations in Populus clones. Ecology 62: 159 — 169.

Pinheiro, C., Chaves, M.M. (2011). Photosynthesis and drought: can we make metabolic connections from available data? Journal of Experimental Botany Vol.62 (No. 3): 869 — 882.

Shao, H.B., Chu, L.Y. (2005): Plant molecular biology in China: challenges and opportunities. Plant Molecular Biology Reporter 23 (No.4): 345 — 348.

Shao, H.B., Liang, Z.S., Shao, M.A.(2005): Molecular mechanisms of higher plant adaptation to environment. Acta Ecologica Sinica 25 (No 7): 1772 — 1781.

Thomas, H. (1986): Characteristics of Dactylis glomerata L., Lolium perenne L. and L. multiflorum Lam. plants. Annals of Botany 57: 211 — 223.

Tschaplinski, T.J., Blake, T.J. (1989): Water relations, photosynthetic capacity, and root/shoot partitioning of photosynthetic as determinants of productivity in hybrid poplar. The Canadian Journal of Botany 67: 1689 — 1697.

Rezime

ODGOVORI KLONOVA CRNE TOPOPLE NA OPORAVAK POSLE VODNOG DEFICITA U KONTEKSTU FOTOSINTEZE, TRANSPIRACIJE I EFIKASNOSTI KORIŠČENJA VODE

Topić Mirjana, Borišev Milan, Župunski Milan, Tomičić Marina, Nikolić Nataša, Pajević Slobodanka, Krstić Borivoj, Pilipović Andrej

Pet genotipova evropske crne topole (Populus nigra L.) su u polu-kontrolisanim uslovima izloženi različitim vodnim režimima: kontrola (90-70% maksimalnog vodnog kapaciteta zemljišta - FC), suši praćenoj oporavkom na optimalnom vodnom kapacitetuzemljišta (90-40% od FC) i tretmanu konstantne suše (50-40% od FC). U odgovoru genotipova na vodni deficit i oporavak, ispitivane su promene parametara koji se odnose na razmenu CO_2 i vodene pare u listu, kao što su intenzitet fotosinteze (P) i transpiracije (T) i efikasnost koriščenja vode (WUE). Ponovno podizanje vodnog kapaciteta zemljišta na 90%, nakon vodnog deficita pri kapacitetu od 40%, uslovilo je oporavak intenziteta fotosinteze, transpiracije i efikasnosti korišćenja vode na nivoe približne vrednostima kontrolnih biljaka. Nakon 16 dana konstantne suše (vodni kapacitet zemljišta u opsegu 50-40%) utvrđeno je snažno opadanje analiziranih parametara u svim genotipovima. Značajne varijacije analiziranih parametara među genotipovima ukazuju na specifične adaptivne predispozicije određenih gentipova u odnosu na sušni stres.