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CLONAL DIFFERENCES OF BLACK POPLAR CUTTINGS FOR MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES TO SOIL WATER DEFICITS

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ABSTRACT

Five black poplar clones were subjected to three different soil water regimes (well-watered and two water-stressed treatments) to evaluate their morpho-physiological and biochemical responses to water deficits. Plants were grown in a semi controlled environment (greenhouse) by soil culture method. The three-month-old plants were exposed to 90-70% of maximum soil water saturation - control, mild drought followed by recovery of optimal soil water saturation (90-40%) and severe drought (50-40% of soil water saturation) for 21 days. Prolonged drought caused reduction in photosynthetic (A) and transpiration (E) intensity in all clones, but after recovery these parameters were enlarged considerably. Instantaneous water use efficiency (WUEi) was significantly increased under conditions unfavorable for A and E, where genotypes IX/30 and I/2 showed the highest values. The best recovery of A and WUEi exhibited genotype I/2. Proline accumulation in leaves was increased with the increasing intensity of drought, whereas VII/25 and I/2 showed better osmotic adjustments and higher drought tolerance than the other examined clones. No direct correlation was found between water deficit level and malondialdehyde (MDA) content in genotypes IX/30 and X/32, while the other clones showed significant MDA accumulation in one or both drought treatments. Water deficits significantly slowed down apical growth and shoot height growth in all clones except in X/32, while VII/32 and X/32 showed similar number of leaves during all treatments with no significantly differences among values per clone. The obtained results provide clear evidence for clonal differentiation in their responses to water deficiency in all examined parameters.

Key words: Black poplar genotypes, soil water deficits, recovery, morpho-physiological parameters, biochemical parameters.

INTRODUCTION

The global warming expected at the end of the 21st century will produce an increased probability of drought episodes, larger vapour pressure deficits and in general more frequent and more severe extreme climatic events (Saxe *et al.*, 2001). The permanent or temporary water deficit severely hampers the plant growth and development more than any other environmental factor (Anjum *et al*., 2011).

Plants have developed genotype specific strategies to cope with drought (Chaves *et al.*, 2002), and they involve either the stress avoidance or the tolerance mechanisms. Under drought stress, plants are capable to reduce water use (WU) (Blum, 2005). Understanding how plants respond to drought, can play a major role in breeding programs which aim is to produce clones characterized by superior growth and resistance to mild and severe drought.

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). Therefore, the ability to maintain photosynthetic machinery functional under water stress is of major importance for drought tolerance (Zlatev and Yordanov, 2004). In addition, great attention has been drawn to the study of WUE, in order to detect genotypes that consume less water and are photosynthetically more efficient (Orlović *et al.*, 2002).

Drought also reduces vegetative growth of plants, in particular shoot growth, and leaves growth is generally more sensitive than the roots growth (Mahajan and Tuteja, 2005).

The generation of reactive oxygen species (ROS) is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. During drought, ROS levels increased dramatically resulting in oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). The ROS such as O_2^-, H_2O_2 and •OH radicals, can directly attack membrane lipids and increase lipid peroxidation (Mittler, 2002). To evaluate such damages caused by ROS, measurement of malondialdehyde (MDA) content, one of the end product of lipid peroxidation (LP), can be used as indicative parameter (Munne´-Bosch and Alegre 2003; Molinari *et al.*, 2007). The capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance of plants (Tsugane *et al.*, 1999).

On the other hand, osmolytes play a major role in osmotic adjustment and protect the cell by scavenging ROS (Miller *et al.,* 2010). One of the most widely studied osmolite is proline because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration is generally higher in stress-tolerant than in stress-sensitive plants (Anjum *et al.,* 2011).

To contend with water limitation, trees must make appropriate physiological and developmental adjustments (Wilkins *et al.,* 2009). Poplars are usually known as one of the most drought-sensitive woody plant groups, but their drought tolerance varies greatly among species, populations and clones due to their great genetic diversity (Zhang *et al.,* 2004; Monclus *et al.,* 2006). Thus, it was hypothesized that responses of black poplar clones to soil water deficits could differ among each other. Therefore, the aim of this study was to quantify some morpho-physiological and biochemical parameters in five black poplar clones growing on soil which was treated with three different watering regimes.

MATERIALS AND METHODS

Plant material and experimental design: The experimental material consisted of five black poplar (*Populus nigra* L.) genotypes (VII/25, IX/30, X/32, XI/36, I/2) obtained from the Institute of Lowland Forestry and Environmental Protection, Novi Sad, Serbia.

The experiment was set when homogeneous 20 cm long, woody-stem cuttings of *P. nigra*, with one shoot per cutting, were pricked in 5-l Mitscherlich pots and filled with homogenized soil of the same weight, at the beginning of April 2011, when the growing season started. The number of cuttings was eighty-three and they were selected randomly in 30 Mitscherlich pots (from one to five cuttings per pot).

The plants were grown in a semi-controlled environment (greenhouse) by soil culture method. The temperature range was 18–35°C and illumination was natural and dependent on the outside light conditions.

After growing for three months, the cuttings were subjected to three different soil water regimes: well watered, mild drought followed by recovery and severe drought treatment. The first treatment was the control group of plants with the lowest limit of soil water saturation set at 70%. The other two treatments had the lowest limit of soil water saturation at 30–40%. The soil water content was controlled by weighing method every day and supplemented with some water to reach upper level of soil water saturation. For the first and second treatment this level was 90%, and for the third one, it was 50%. The water amount for adding to each treatment was recorded by flask. Every treatment consisted of 10 Mitscherlich pots, arranged in three rows, one behind the other. Each treatment included 5 clones and two replications per clone.

The experimental layout was completely randomized with two factors (species and watering regime).

Leaf gas exchange parameters (A, E and WUEi) were measured during July, five times (phases from 1 to 5) in mild drought treatment followed by rewatering in order to determine how clones respond to drought and recovery. Soil water saturation was determined in every phase of the measurement during this treatment. The recovery of A, E and WUEi was measured 2 days after re-watering (phase 4, 90% of soil water saturation).

Instantaneous WUE (WUEi = A/E) was calculated by dividing photosynthetic rate by transpiration and was expressed in unit μmol CO_2/mmol H_2O.

Measurements related to free prolin content (Pro) and lipid peroxidation (LP) activity were applied on all treatments at the end of the experiment. A fully expanded fifth leaf from the apex of each plant was sampled for mentioned measurements.

Morphological parameters were measured twice in July: one day before treatments application – when all cuttings were approximately of equal height and were on their maximum of soil water saturation (100%), and at the end of the experiment, three weeks after the treatments started, when all three treatments were on their lowest limit of soil water saturation.

Leaf gas exchange parameters: Photosynthesis and transpiration were measured 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. Measurement was conducted in 9 replications on 3 plants per one clone (45 measurements per treatment in total).

Free proline content: Free proline content was determined using the Bates method (Bates *et al.,* 1973). Plant material (1g), which consisted of young leaves, was ground with 10 ml of 3% sulfosalicylic acid. The homogenate was filtered and 2 ml of glacial acetic acid and 2 ml acid ninhydrin reagent were added to 2 ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 15 minutes. After that, it was transferred to ice bath and warmed to room temperature. Four ml toluene was added to the mixture and the upper toluene layer was measured at 520 nm using UV spectrophotometer. Measurement was made at

the end of the experiment on fully expanded leaves and was conducted in 3 replications on 3 plants per one clone (45 measurements per treatment in total). Contents of proline were expressed as μg g^{-1} fresh weight.

Assessments of Malondialdehyde: Malondialdehyde (MDA) content was estimated by the following manner: leaf tissue (0.5 g) was homogenized using 4.5 ml of the extraction solution, containing 10 ml 10% $HClO_4$ saturated with thiobarbituric acid (TBA) and 30 ml 20% trichloracetic acid. The mixture was heated in boiling water for 20 min, and then quickly cooled in an ice bath. The absorbance of the supernatant at 532 nm was determined with a DU-65 Beckman spectrophotometer after centrifugation at 3500 × g for 10 min. The MDA production was expressed as nmol MDA g^{-1} fresh weight. (Placer *et al.,* 1966).

Growth traits: Apical growth was measured with a ruler from the point which was 3 cm below the top of the stem. The increase of shoot height was measured also with a ruler, but for the whole length of the stem. The increase of leaves number expressed as the number of all leaves per cutting, both young and mature leaves. Measurements were made in 4 replications on 4 plants per clone (20 measurements per treatment in total.

Statistical analysis: Statistical analyses were conducted by ANOVA two-way factor analyses. The Duncan's multiple range test was used to compare mean values of studied parameters between treatments and genotypes at a significance level of $p<0.05$ (Duncan, 1955). The average values shown in figures followed by the same letter did not differ significantly. Values are shown as mean ± standard deviation.

RESULTS AND DISCUSSION

Leaf gas exchange parametres: Prolonged drought decreased the rate of gas exchange in all clones (Figs 1-3). The maximum decrease in photosynthetic and transpiration rates were observed in the third phase of the experiment (40% of soil water saturation). Previous studies noticed the reduction in the photosynthetic rate due to several coordinated events, such as stomatal closure, decreased ATP synthesis and RuBP supply, and the reduced activity of photosynthetic enzymes (Lawlor and Cornic 2001; Chaves *et al.,* 2003).

WUEi (Fig. 3) was significantly increased under conditions unfavorable for photosynthesis and transpiration (40% of soil water saturation), where genotypes IX/30 and I/2 showed the highest values, while XI/36 had the lowest. Thus, this data suggested that examined clones may develop two contrasting WUE strategies for survival under limited water availability. Genotypes IX/30 and I/2 had conservative WUE strategy, while XI/36 had prodigal strategy. Zang *et al.,* (2004) suggested that the first strategy may be beneficial for drought tolerance, when water is limited, while the second strategy may increase growth when water is available. WUE increased in drought, primarily because stomatal conductance, and thus water loss, declined more than carbon fixation (Edwards *et al.,* 2012). Monclus *et al.* (2009) and Guo *et al.* (2010) observed that high WUE under drought conditions has often been correlated with high drought resistance and productivity in hybrid poplars.

When re-watered after 10 days without watering (phase 4, 90% of soil water saturation), the plants reached the levels of A, E and WUEi which were similar to those from the first phase. These results suggest rapid recovery of these parameters since our measurements were conducted two days after re-watering. Arango-Velez *et al.* (2011) considered that rapid recovery from drought may be among the highly desirable traits alleviating drought effects on growth of poplar trees. The best recovery of A and WUEi, compared with the first phase, displayed I/2 while in terms of E, it were clones XI/36, I/2 and VII/25. Remarkably rapid recovery was mainly due to the restoration from low photosynthesis rate by stomata closure and accumulation of ABA, except for cell destruction or the damage on metabolism (Miyashita *et al.,* 2005).

Free proline content: Data presented in Fig. 4 showed that proline accumulation in leaves increased with the increasing intensity of drought. However, clones VII/25 and I/2 showed a higher increase in free proline content during severe drought (50-40%) than the rest, which suggested that mentioned clones possessed better osmotic adjustments and higher drought tolerance than the other examined clones. Ssignificantly increased levels of free proline in plants exposed to severe drought treatment indicated its important role in the osmotic adjustment of poplar plants to drought stress. Osmotic adjustment has been considered as one of the crucial mechanisms in plant adaptation to various stresses, but it varies greatly among genotypes (Zhang *et al.,* 2004, Gunes *et al.,* 2008). The observed correlation between proline concentration and water deficit is consistent with those reported by Gunes *et al.* (2008) for chickpea, Xiao *et al.* (2009) for poplar, Din *et al.* (2011) for canola cultivars and Geravandi *et al.* (2011) for bread wheat.

Assessments of Malondialdehyde: The effects of water deficits on the levels of MDA content in leaves of examined clones are shown in Fig. 5. There was no statistically significant difference in MDA content between watering regimes in genotypes IX/30 and X/32. These results showed that mentioned clones controlled the overproduction of ROS more efficiently than did the other clones. It is consistent with the results obtained in some other studies (Xiao *et al.,* 2008). Other clones were more sensitive to water deficits and more liable to

oxidative stress due to the fact that they showed significant MDA accumulation in one or both drought treatments. This study confirms the fact that MDA level is a suitable marker for membrane lipid peroxidation and its content has been considered an indicator of oxidative damage (Anjum *et al.,* 2011).

Growth traits: Growth inhibition is one of the earliest responses of plants to water deficiency (Zhang *et al.,* 2004; Lei *et al.,* 2006). Water deficits significantly slowed down apical growth and shoot height growth in all clones except in X/32, which showed similar values in mentioned parameters during tree watering regimes (Figs. 6, 7). Manivannan *et al.* (2007) found that reduction in plant height could be due to decline in the cell enlargement and more leaf senescence in the plant under water stress. Effect of drought on plant growth per day (cm day^{-1}) was shown in Table 1. The lowest values were recorded in clone I/2 at both treatments. Clone X/32 had the lowest growth per day irrespective of the soil water saturation, and remained 100% and 72% of the control values at 90-40 and 50-40% treatments, respectively.

Genotypes VII/25 and X/32 showed similar number of leaves (Fig. 8) during all treatments with no significantly differences among values per clone, in spite of the fact that water deficits reduce the number of leaves per plant, as was observed in study of Anjum *et al.* (2011). The rest clones presented disordered values in mentioned parameter.

Overall, Yang and Miao (2010) observed that plant growth is responsive to drought stress and the reactions depend on the adaptation to the rapidity, severity and duration of the drought event.

Table 1. Effect of drought on plant growth per day (cm day^{-1}). Values are means of five measurements ±S.D. Values in parenthesis represent percent of control.

	Treatments		
Clones	90-70% soil water saturation	90-40% soil water saturation	50-40% soil water saturation
VII/25	0.6 d ± 0.02	0.40 fg ± 0.04 (67%)	0.18 kl ± 0.03 (30%)
IX/30	1.02 a ± 0.04	0.90 b ± 0.07 (88%)	0.24 jk ± 0.08 (23%)
X/32	0.46 ef ± 0.03	0.46 ef ± 0.02 (100%)	0.33 gh ± 0.01 (72%)
XI/36	0.75 c ± 0.04	0.31 hi ± 0.03 (42%)	0.28 hij ± 0.04 (37%)
I/2	0.53 de ± 0.05	0.25 ijk ± 0.04 (47%)	0.10 l ± 0.03 (19%)

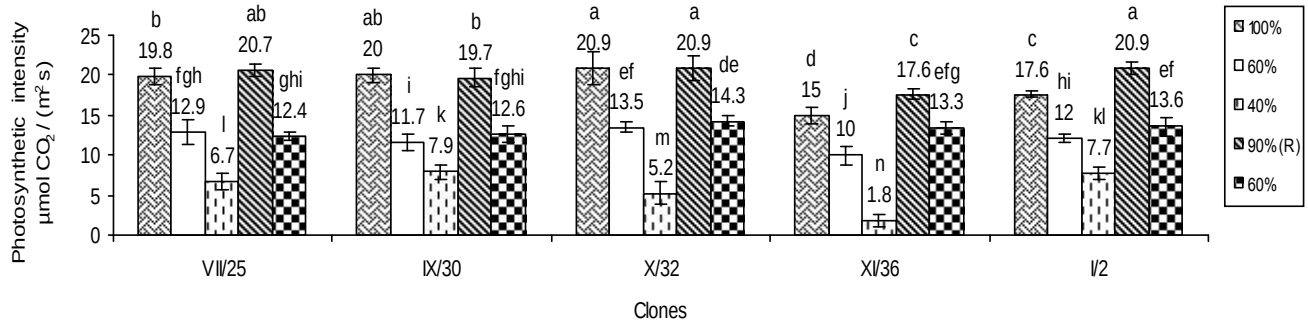

Fig. 1. ***Photosynthetic intensity in mild drought treatment during five phases***

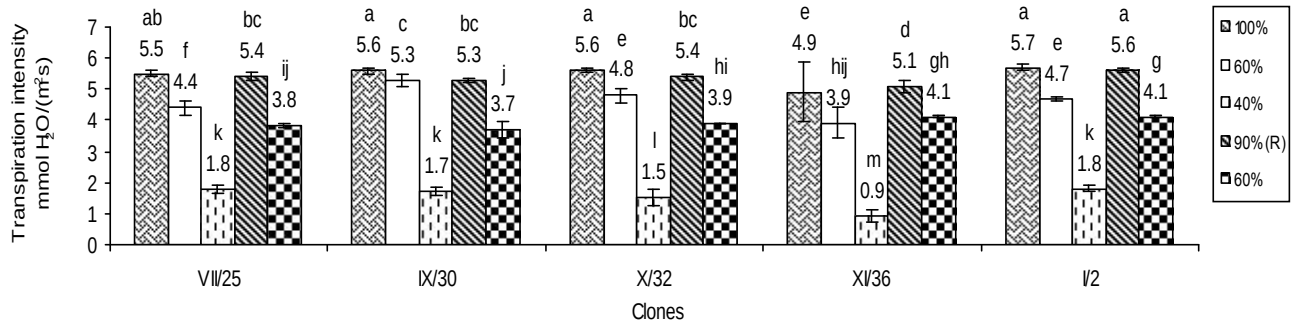

Fig. 2. ***Transpiration intensity in mild drought treatment during five phases***

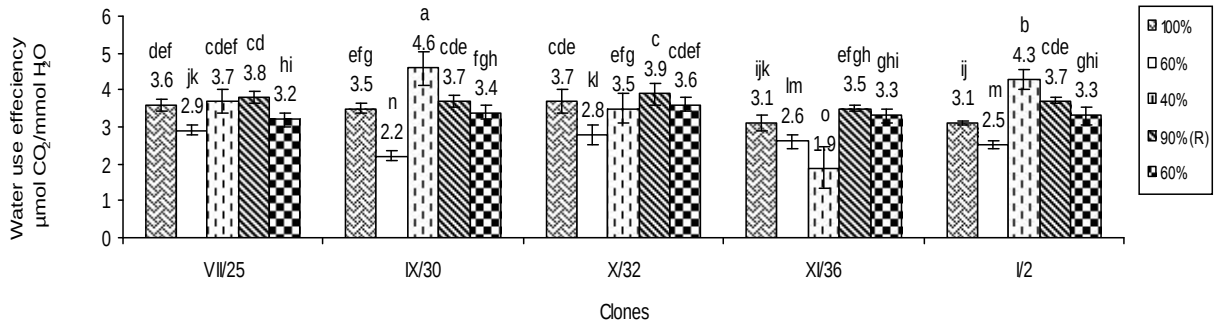

Fig. 3. ***Water use efficiency in mild drought treatment during five phases***

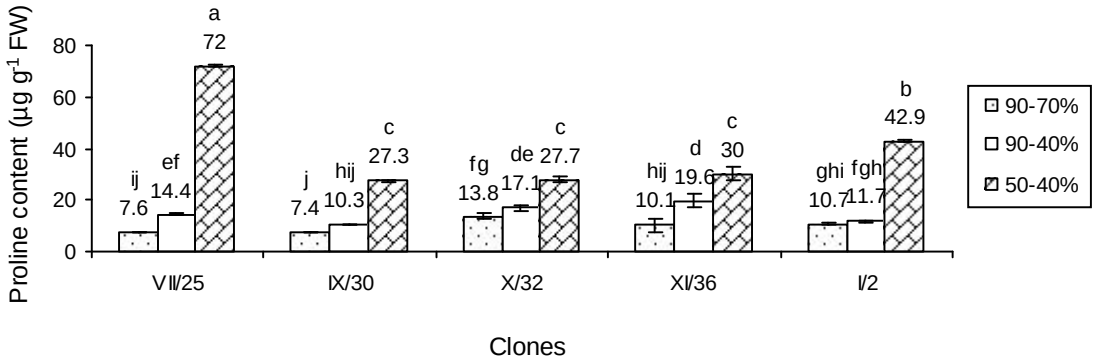

Fig. 4. ***Free proline content in control and drought stressed black poplar clones***

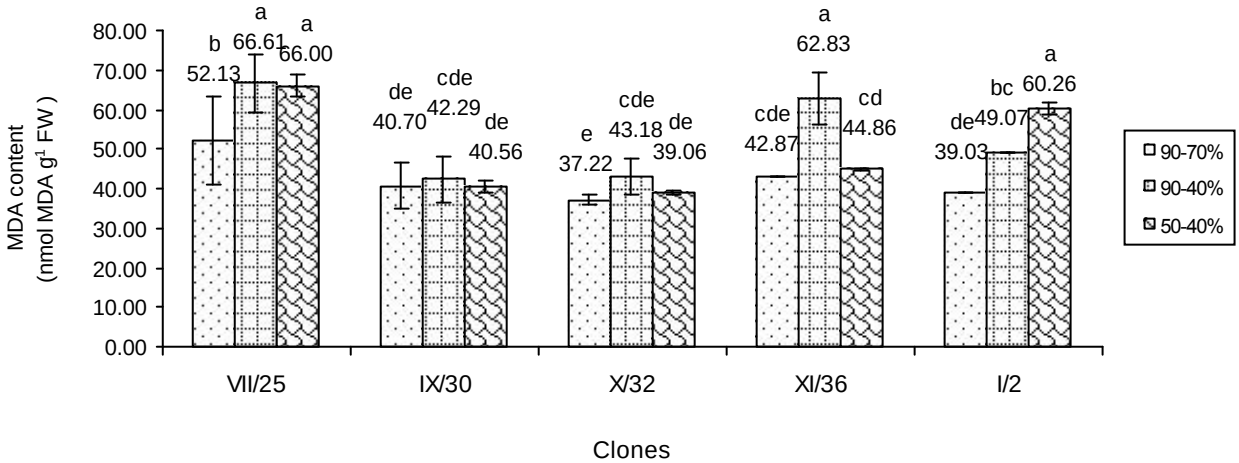

Fig. 5. ***Change in Malondialdehyde (MDA) content in control and drought stressed black poplar clones***

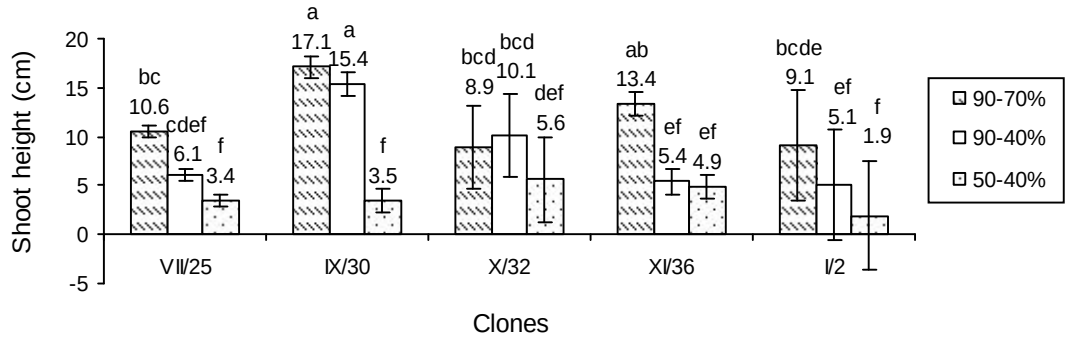

Fig. 6. ***Apical growth of control and drought stressed black poplar clones***

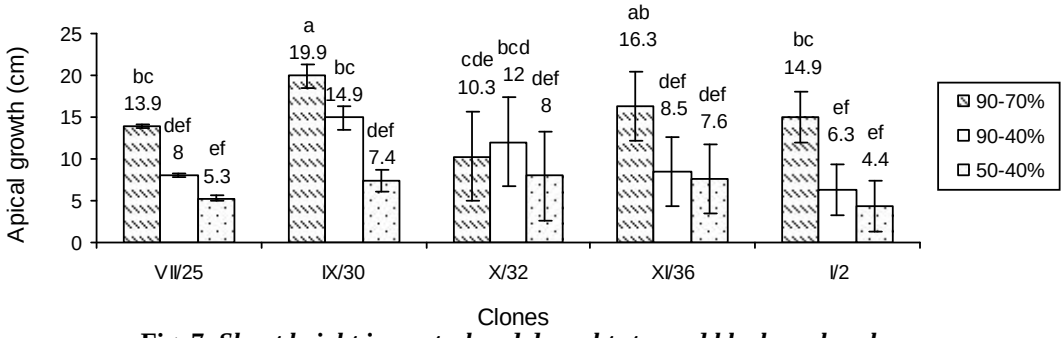

Fig. 7. ***Shoot height in control and drought stressed black poplar clones***

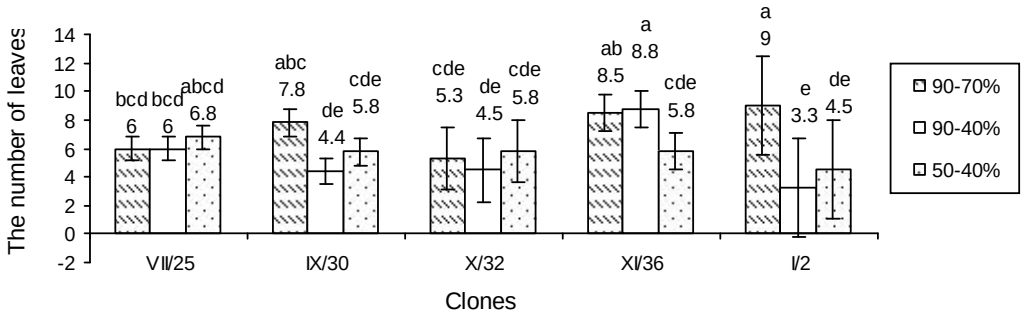

Fig 8. ***Number of leaves in control and drought stressed black poplar clones***

Conclusion: The study presented significant morpho-physiological and biochemical differences between five black poplar clones in their responses to soli water deficits. Clone I/2 was evaluated as drought tolerant since it had high values in free proline content and iWUE under drought conditions as well as the best recovery of A and iWUE. Clones IX/30 and X/32 were superior in controlling the overproduction of ROS while morphological adaptations allowed X/32 clone to maintain growth when water availability was decreased.

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