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Utilization of Selection Indexes for Adaptive Responses of Black Poplar (*Populus nigra* L.) to Drought Stress

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ABSTRACT: Drought tolerance in plants refers to the degree to which a plant is adapted to arid or drought conditions. Therefore identification of these adaptive responses, known as drought escape, avoidance and tolerance, with several mathematical indices is highly important for development of resistant tree species. In the current study, black poplar clone collection including 300 individuals collected from all around Turkey was tested in a field trial. The growth and biomass production capacities of these individuals with several physiological traits were measured and compared in rainy and drought periods. Mean Relative Performance (MRP) and Stress tolerance Index (STI) were used to identify adaptive responses of black poplar to drought stress. In the study, MRP favored drought tolerant genotypes having the highest biomass production. Genotypes with higher STI values exhibited drought avoidance mechanism which characterized with low productivity but much higher drought resistance. On the other hand, lower STI was associated to drought escape genotypes which revealed the lowest growth performance and the highest defoliation rates under drought. In the current study, relationship between antioxidant enzyme activities and drought adaptations were also investigated on the leaves of selected clones. Higher enzyme activities attributed to drought escape adaptation in the study.

Keywords-*Populus nigra, drought, adaptation, selection index*

1. Introduction

Drought stress is the most important environmental limitation for plant growth, productivity and survival. Destructive effects of drought stress have been reported to become more severe and frequent especially on forest and fruit tree species with global warming in recent years (Basu et al., 2016). Trees are long lived sessile organisms that grow across all climatic regions in the world and are always compelled to withstand all types of environments throughout their lifespans. Therefore, they evolved many morphological, physiological and biochemical adaptation strategies against several stress factors, drought stress in particular (Wilkins et al., 2009; Hamanishi and Campbell, 2011). Identification of these adaptation strategies and selection of the most drought resistant genotypes are an urgent need to develop trees that can cope with more prolonged drought conditions.

Drought resistance is a general term applied to all plant species that could be divided in to three adaptive features; drought escape, avoidance, or tolerance (Lei 2006; Ryan 2011). ‘Drought escape’ is attained through a shortened growing season, allowing plants to complete their lifetime before dry season. This adaptation strategy involves rapid vegetative growth and seed reproduction stages before soil water depletion and little or no vegetative growth during dry season. These plants are considered not to have any special morphological, physiological, or biochemical adaptations to drought. Therefore, they tend

to shed their leaves to reduce water loss and standby a dormant state during drought stress. On the other hand, 'Drought avoidance' is characterized with higher tissue water content despite strong reduction in soil water content. This is achieved through a variety of adaptive traits involving water uptake maintenance through increased rooting and hydraulic conductance and minimization of water loss by having small transpiration area (small leaves and small plant body) or shedding of older leaves to keep the limited water in meristematic tissues (Blum, 2005). 'Drought tolerance' (DT) is defined as the ability of plants to withstand low tissue water content under drought through adaptive traits such as cell turgor maintenance through osmotic adjustment, cellular elasticity and protoplasmic resistance (McDowell et al., 2008, Lei 2008).

Improvement of yield and maintaining yield stability of trees, under normal as well as drought stress conditions is essential for the food and wood security of the world. This security could be achieved by identification of these adaptation strategies in trees and selection of the best individuals for breeding programs. These adaptation strategies have been well characterized for agricultural species with several mathematical selection indices. Some of these indices such as Mean productivity index (Hossain et al., 1990) and geometric mean of productivity (Ramirez-Vallejo and Kelly., 1998) simply depended on average yield ratio of a genotype under stress and non-stress conditions. On the other hand, stress related indices like Stress tolerance index (Fernandez., 1992) and Stress susceptibility index (Fischer and Maurer., 1978) assessed the reduction in yield caused by stress condition compared to non-stress environments. These indices have been used for identification of adaptation strategies in crop plants and selection of the best drought resistant individual (Raman et al. 2012). However, such types of indices have not been applied for tree species due to lack of knowledge on adaptive responses of trees to drought stress. Poplar species could be one of the most suitable plant species to investigate genetic architecture of trees and their responses to drought stress. After sequencing the whole genome of *Populus trichocarpa*, poplar was accepted as a model organism for many fruit trees and deciduous plants (Wilkins et al., 2009).

Therefore, we selected black poplar to test their adaptive responses to drought stress in the current study. Some mathematical indices used for identification of drought tolerant crop plants were also used to identify these adaptation responses. Furthermore, relationship between antioxidant enzyme activities and drought adaptation strategies were also assessed to find out the potential usage of selection indices and antioxidant enzyme activities for identification of the best trees possessing high productivity and enhanced drought tolerance.

2. Material and Methods

2.1. Field trial and selection of clones exhibiting different drought response and yield

Breeding and conservation programs of black poplar were initiated several decades ago in Turkey and preceded with selection of best growing black poplar individuals from their natural populations and old plantations throughout the country. The effects of drought stress on this collection were firstly assessed in a field trial established with 300 black poplar clones. In the field trial, four sites were firstly established in the area and drainage pits were excavated around them to withhold the external water from the trial. Five cuttings (fifteen- centimeter) per clone were randomly planted into each site as a one row plot in

March 2011. With this randomized block design, each clone in the experiment was represented with 20 (4 site x 5 trees/replication in a row plot) ramets and totally 6000 trees were tested in the field trial (300 clones x 5 ramets/clone x 4 site = 6000).

The trial was located into an agricultural area found in Behiçbey nursery/Ankara/Turkey. The location of the field trial was found in Central Anatolia, which receives the least annual rainfall of Turkey. The weather in this region characterized with extreme heat, with almost no rainfall between 15th of July and 15th of September in summers. Therefore, to estimate the growth of the clones during non-stressed (rainy) period, the shoot and diameter growth (from ground level) of the seedlings were measured in 1th of June and 15th of July 2013. The same measurements were also taken in 1th September 2013 to record the growth of the clones during drought period. Average rainfall in the rainy and drought periods were recorded as 43.4 kg/m³ and 10.2 kg/m³, respectively. No rain was recorded at around field trial during the drought season. Decrease in soil moisture was followed by 20 tensiometer (92-38 Field Use of Tensiometers,USA) distributed to field trial.

The biomass production of the clones in the field trial was expressed as decimeter cubic stem volume, which was calculated by the following formula (Gertner, G.Z. 1991):

$$\text{Stem volume (SV)} = 0.42 * B * H \quad (1)$$

,where B is tree basal area at ground level and H is tree shoot height.

In addition to growth performances, leaf abscission rates (LA) of clones were also observed during drought periods in the field trial. Clones were scaled from 0 (non-defoliated) to 4 (all defoliated) in terms of drought dependent leaf abscission rates. Further morphological characters such as bud flash at spring and branch angel of the first branch of the seedlings were also measured during the field trial.

2.2. Selection indices used to identify drought adaptation strategies in black poplar

As mentioned in introduction part, several selection criteria have been proposed for crop species to evaluate genetic differences in drought tolerance based on their performance in stress and non-stress environments (Raman et al 2012). The objective of black poplar breeding program was to select the clones that combine both productivity and resistance to drought stress. Therefore, we applied Mean relative performance (MRP) and Stress Tolerance Index (STI) to select the clones having the most productive and drought resistant clones. MRP and STI indexes were applied as described in Hossain et al. (1990) and Fernandez (1992), respectively.

$$\text{MRP} = \frac{(Y_i)_S}{(Y)_S} + \frac{(Y_i)_{NS}}{(Y)_{NS}} \quad (2)$$

$$\text{STI} = \frac{(Y_i)_S * (Y_i)_{NS}}{(Y)_{NS}^2} \quad (3)$$

In these indices, $(Y_i)_S$ denotes the yield of the i^{th} genotype under drought stress, $(Y_i)_{NS}$ the yield of the i^{th} genotype under non-stress (i.e., rainy) conditions and Y_S and Y_{NS} the mean yields of all genotypes evaluated under stress and non-stress conditions, respectively.

2.3. Selection of black poplar clones having different response to drought

In the current study, MRP and STI values of each individuals were distributed in a line plot (Figure 1) to select clones that have the highest and the lowest values. Then, twelve black poplar clones represented in the Figure 1 were selected to carry out further physiological and biochemical analysis and to test their drought response. In the field experiment, to understand relationship between drought adaptations and water content of the clones, leaf water potential (LWP) of the selected black poplar genotypes was measured in rainy and drought periods weekly. The measurements of LWP were carried out at mid-nights with 15 fully expanded leaves with Sholander pressure chamber (PMS Instrument Co., Corvallis, OR, USA).

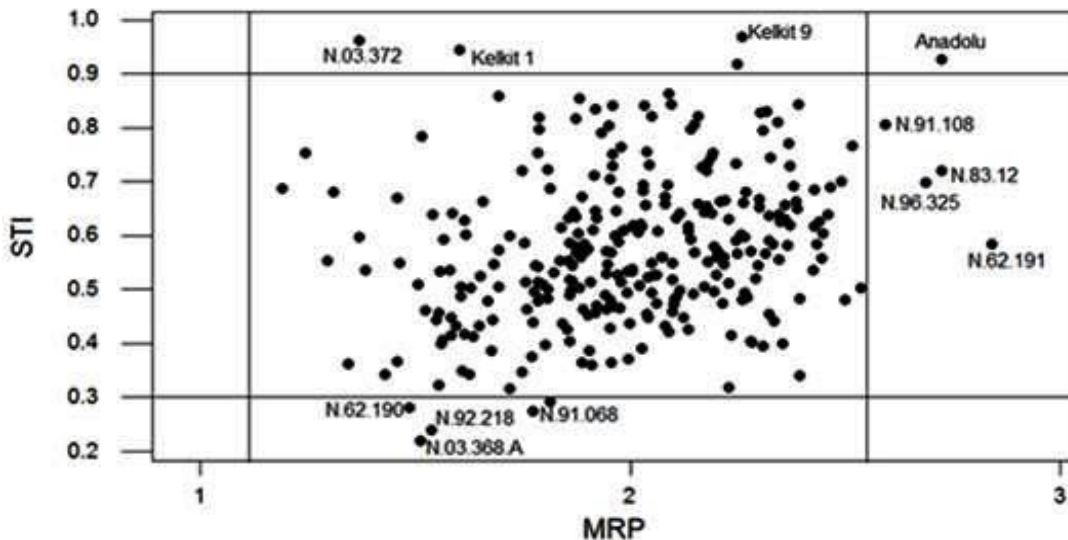


Figure 1. According to the averages of stem volume increments of the tested clones in the field trial under rainy and drought periods, mean relative productivity (MRP) and stress tolerance index (STI) values of each clone was calculated and distributed in a line plot. The clones having the highest and the least STI and MRP values were selected to test their drought adaptation strategies and associate to indices.

2.4. Antioxidant Enzyme Activity Measurements

To measure the changes in antioxidant enzyme activities and H_2O_2 content, the fully expanded leaves were harvested in the noon in rainy and drought periods from the seedlings of selected clones. All the collected leaf samples were immediately frozen in liquid nitrogen and kept at $-80\text{ }^{\circ}\text{C}$ until use. All the leaf samples were grounded into powder with liquid nitrogen by using mortar and pestle. Protein content of the leaves were isolated the quantity was determined by Bradford method (Bradford, 1976) using a commercial protein assay kit (Thermo Fisher Scientific, Germany). Ascorbate peroxidase (APX) activity procedures used in this study were taken from Murshed et al., (2008) and superoxide dismutase (SOD) activity was measured according to Giannopolitis and Ries (1977). All enzyme assays were performed with the 96 well UV- micro-plate at $25\text{ }^{\circ}\text{C}$ by using Epoch Microplate Spectrophotometer (BioTek,France) equipped with an internal temperature incubator for kinetic analysis. Samples were analyzed in triplicate. Blank corrections for non-enzymatic reduction of the enzymes were carried out in the absence of

the enzyme sample. In addition to antioxidant enzyme activities, hydrogen peroxide (H₂O₂) content of the clones was also estimated according to Brent and Bergmeyer(1974).

Statistical Analysis

The results of all measurements, collected during field trial, were statistically analyzed with SAS by taking the average of seedling for each genotype. All variables recorded in the measurements were analyzed with two way analyses of variance (ANOVA) by Proc Mixed Procedure of SAS (SAS INST, 2009) to test the effects of replication on clone performance in rainy and drought periods. Correlations between the measurements and regression analysis were also carried out by SAS to understand relationship between measured traits.

3. Results

3.1. Changes in soil moisture content in the field trial

The effects of drought on the black poplar collection including 300 clones were firstly assessed with the current study in a field trial. Shoot and diameter growth of the three years old seedlings was measured under different water availabilities (WA) to evaluate the effects on drought over the clones. These measurements were taken in rainy (1 June-15 July) and drought (15 July- 1 September) seasons in the summer of 2013. Soil moisture levels in field trial was also evaluated with 20 tensiometer dipped into a depth of 40 cm. The results of soil moisture did not significantly changed for three weeks between 15-30 Julys, which is coded as non-stressed (NS) condition. The first statistically significant ($p < 0.05$) decrease in soil moisture content was recorded in the first week of August 2013 and recorded as pre-drought (PD). Soil moisture content was then continued to decrease in each subsequent week and coded as mild-drought (MiD), moderate drought (MD) and severe drought (SD) in the experiment.

3.2. Yield and growth performances of clones in the field trial

Results of two-way ANOVA on the growth under rainy and drought periods indicated highly significant Clone x WA interaction ($p < 0.0001$). Site effect on the stem growth of the clones found to be insignificant ($p > 0.072$). Year x Clone interaction was also found to be insignificant ($p > 0.098$) for the stem volume increments in rainy and drought periods. Average shoot and diameter (ground level) growth increment of the clones during the rainy period (non-stress) was measured as 91.4 ± 15.2 cm (range: 129-40 cm) and 11.5 ± 1.9 (range: 18-6 mm), respectively (Table 1). Drought period strongly constrained the productivity of the clones that the shoot growth increment decreased %57 (range: 88-17 cm) in that period. The reduction in diameter increment was estimated as %51 (range: 12.6-2.4 mm) in the same period. Final stem volume, shoot and radial growth of the clones at the end of three years reached to 1.76 ± 0.93 dm³ (ranged; 0.64 – 4.2), 455 ± 55 cm (ranged; 296-630) and 33 ± 6 mm (ranged; 19 – 56.6), respectively. To determine the most useful criteria for selection of drought resistant and high yielding genotypes, the correlation coefficients between selection indices and morphological traits were calculated and expressed in Table 2. MRP has an upward bias when the differences between non-stress and stress conditions are large and it favors genotypes with higher yield potential and higher rate of productivity. Therefore, the correlations between MRP and stem volume increment in rainy ($r=0.52$) and drought periods ($r=0.47$) indicated a strong positive

relationships. On the other hand, higher STI values indicate lower differences in yield across stress and non-stress conditions, in other words, more resistance to drought. As it was expected, stem volume increment in drought period was positively correlated with STI ($r=0.45$), whereas biomass increment in rainy period ($r= -0.25$) was negatively associated with STI. Both index indicated a strong positive association with each other ($r = 0.28$) which means that selection of drought resistance clones with high yielding performance is quite possible. Another important negative correlation was recorded between STI and drought depended leaf abscission (-0.35). This result indicated that drought tolerance highly related with leaf abscission and this trait could be used for elimination of especially drought sensitive genotypes. According the distribution of the clones in a regression line plot (Figure 1), clones that have the highest and the lowest MRP and STI values were selected and evaluated according to their response to rainy and drought conditions in the field (Table 1).

Table 1. Morphological and growth performances traits of the selected clones transferred to greenhouse. The clones were selected according to their mean relative productivity (MRP) and stress tolerance (STI) performances in the field trial. In addition to growth parameters such as stem volume, shoot elongation and diameter increment in rainy and drought (Dry) periods, morphological traits such as leaf abscission (LA) rate of the clones under drought periods.

Selection indices	Clone Name	STI	MRP	L.A	Final Height (m)	Final Diameter (cm)	Final Stem Volume (dm ³)	Shoot Elongation in Rainy Period (cm)	Diameter Increment in Rainy Period (mm)	Shoot Elongation in Dry Period (cm)	Diameter Increment in Dry Period (mm)
Lowest STI	N.03.368.A	0.22	1.51	3.60	4.35	6.1	2.2	100.3	10.9	22.0	6.5
	N.92.218	0.24	1.54	2.50	5.32	6.2	2.7	99.7	13.4	23.8	5.3
	N.91.068	0.27	1.77	1.20	4.77	5.3	1.8	110.1	11.7	30.2	6.4
	N.92.160	0.34	2.39	1.63	5.42	5.6	2.3	138	13.1	46.9	6.3
					4.9±0.4	5.8±0.4	2.3±0.3	112±15.5	12.3±1.1	37±9.8	6.1±0.9
Highest STI	KELKİT 9	0.97	2.26	0.33	5.56	5.6	2.3	77.2	12.9	74.9	6.5
	N.03.372	0.96	1.37	0.5	2.95	3.7	0.6	47.1	8.70	45.3	5.0
	KELKİT 1	0.95	1.60	0.13	3.85	4.6	1.2	55.6	8.90	52.6	7.5
	ANADOLU	0.93	2.72	0.67	5.27	5.5	2.1	95.7	12.3	88.8	8.9
					4.4±1.1	4.8±0.8	1.5±0.7	69±19	10.7±1.9	65.4±17.3	7.2±1.4
Highest MRP	N.62.191	0.58	2.84	0.14	6.27	6.4	3.4	129.3	15.2	75.6	6.5
	N.83.12	0.72	2.72	0.38	5.59	5.6	2.3	111.1	13.3	79.9	7.3
	N.96.325	0.7	2.69	1.13	5.43	5.5	2.2	111.3	13.0	77.8	7.3
	N.91.108	0.81	2.59	0.83	5.34	5.4	2.1	123.1	11.6	79.9	7.9
					5.6±0.6	5.75±0.4	2.5±0.5	118.7±10.7	13.2±1.3	78.3±1.8	7.2±0.5

Drought Sensitive (S) Genotypes (Lowest STI): A lower STI values implies lower tolerance of a clone to drought stress. Therefore, the clones (N.03.368.A, N.92.218, N.92.160, and N.91.068) found in this group defined as drought sensitive group. The average shoot and

diameter growth of these clones reached 112 ± 15.5 cm and 12.2 ± 1 mm in rainy period, respectively. However, the growth of these clones was severely affected by water deficit that shoot growth and diameter increments decreased more than 73% and 70% under drought period, respectively. In addition to severe reduction in growth parameters, drought dependent leaf abscission rate was also higher in these clones compared to other selected clones. Moreover, terminal shoots of the some ramets of N.03.368.A and N.92.218 desiccated under drought period in the field trial (Figure 2).

Table 2. Correlation coefficients between mean relative productivity(MRP), stress tolerance index (STI), final stem volume (SV_Final), under rainy (SV_RP) and drought (SV_DP) periods, , leaf abscission (LA) rate under drought in the clones tested in the field trial.

	MRP	STI	SV_Final	SV_RP	SV_DP
STI	0.268**				
SV_Final	0.489**	-0.022 ^{ns}			
SV_RP	0.520**	-0.248**	0.783**		
SV_DP	0.477**	0.454**	0.558**	0.133*	
L.A	-0.187*	-0.348**	-0.290**	-0.149*	-0.300**

Drought Resistant Genotypes (R) (Highest STI): The clones (Anadolu, Kelkit 1, N.03.372, and Kelkit 9) that have the highest STI values were defined as the most resistant clones to drought stress in the study. Although, their shoot growth and diameter increment was smaller (69 ± 18.9 cm and 10.7 ± 1.9 mm) than the sensitive genotypes under rainy period, growth of them was slightly reduced (shoot growth; 65 ± 17.3 cm and diameter increment; 7 ± 1.4 mm) during drought stress period. Among these resistant clones commercial black poplar clone named as Anadolu indicated highest MRP and STI values meaning that it combined both drought resistance and high productivity in the field trial (Figure 2).

Moderate Drought Resistant (MR) Genotypes (Highest MRP): Among all tested clones in the field trial the highest shoot (118.7 ± 10.7 cm) and diameter (13.2 ± 1.3 mm) growth increments under rainy periods were exhibited by the clones (N.62.191, N.83.12, N.96.325 and N.91.108) found in this group. The drought period caused 31% and 46% reduction for shoot and diameter growth increments, respectively. Despite this significant reduction in growth under drought, shoot (78.3 ± 1.3) and diameter growth (7.2 ± 0.5 mm) of these clones under drought stress were found to be still higher than all selected genotypes. The group members also exhibited the highest shoot growth (565 cm) at the end of three years and N.62.191 was recorded as the tallest tree (627 cm) among all the clones tested in the field trial. Moreover, the least leaf abscission rate was also recorded for these clones under drought period (Figure 2).

3.3. Predawn leaf water potential (LWP) of selected clones under drought

The most important discriminative indicator of drought response among black poplar genotypes was pre-dawn leaf water potential (LWP). For the sensitive genotypes, LWP started to decrease at PD (Figure 2/LWP) and reached its maximum value at SD (-11.6 ± 0.9 bars). On the other hand, the same parameter was not significantly decreased until MD for the other three genotypes. At SD, LWP of the moderate resistant genotypes was also significantly decreased to -9.7 ± 0.7 bars. The highest LWP values were recorded in the resistant genotypes (-4.5 ± 0.2 bars) at SD, indicating highest leaf water content under drought compared to other studied genotypes.

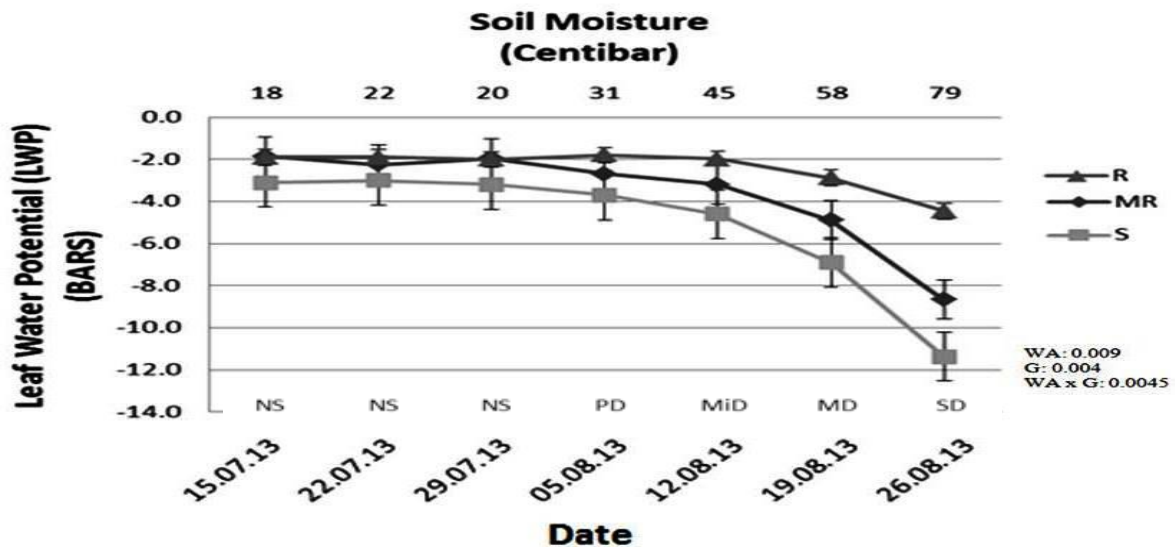


Figure 2. The leaf water potential and shoot growth increment/ week in drought period (15 July-30 August) for the four types of genotypes. Tensiometers were used in the experiment to measure soil moisture content which is expressed as centibar. The results indicated that soil moisture did not significantly change for three weeks which is coded as non-stressed (NS) condition. The first statistically significant ($p < 0.05$) decrease in soil moisture was recorded the first week of August 2013 and recorded as pre-drought (PD). Soil moisture content was decreased in each subsequent week and coded as mild-drought (MiD), moderate drought (MD) and severe drought (SD) in the experiment. Genotypes characterized as moderate resistant (MR), sensitive (S), and resistant (R) included four clones each of which represented with 20 individuals in field trial. WA, the effect of the water availability; G, the effect of the genotypes; WA \times G, the water availability \times genotype interaction effect, as determined by ANOVA

3.4. Leaf Area and Drought Dependent Defoliation Rates of selected genotypes

Among the studied genotypes, the lower leaf area was recorded for resistant genotypes. The same trait was the largest in sensitive genotypes (Figure 3). The selected black poplar clones also manifested contrasting leaf abscission responses to drought stress. The older leaves of the sensitive genotypes desiccated suddenly when the soil moisture decreased to MD, indicating earlier response to drought stress than the other genotypes. The leaves of these sensitive genotypes were almost completely defoliated at SD.

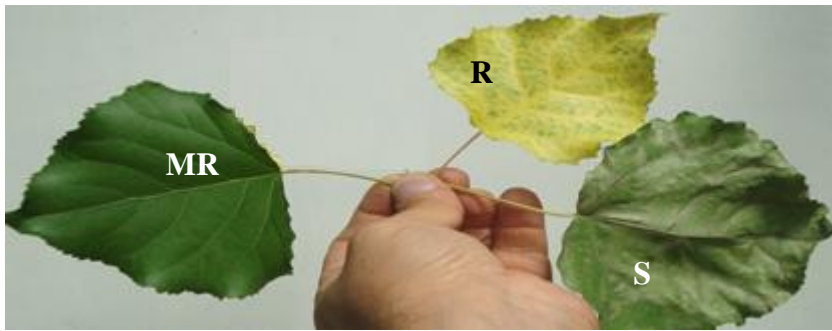


Figure 3. Leaf sizes of the Moderate resistant (MR), Resistant (R) and Sensitive (S) genotypes and leaf senescence symptoms at moderate drought level.

Until the end of the drought period, just a few leaves remained on the stems of the sensitive clones. Contrary to sensitive clones, leaves of the moderate resistant clones formed only senescence symptoms such as yellowing and necrotic lesions. However, the moderate resistant clones did not exhibit severe leaf shedding even after exposure to SD. Drought dependent leaf abscission rate of the moderate resistant genotypes could be considered as non-senescent compared to sensitive clones. Resistant clones exhibited a different drought response in terms of leaf abscission. Interestingly, these genotypes selectively shed their older leaves as they grow under non-stressed condition. Drought dependent defoliation was not observed until SD in resistant clones and only flavescent leaf formation was observed in older leaves located on the lower parts of the stems. Limited number of the leaves defoliated in the seedlings of resistant clones after exposure to SD.

3.5. Effects of Drought Stress on H_2O_2 content and Antioxidant Defense System

Superoxide dismutase (SOD), ascorbate peroxidase (APX), activities gradually increased under progressive drought stress (Figure 4). Although the activities of these antioxidant enzymes were not much significantly differentiated between the genotypes in the non-stressed condition, sensitive genotype possessed significantly higher increments in especially SOD and APX activities than did other genotypes especially under SD. Compared to non-stressed condition, significant increments in SOD activity appeared much earlier and higher in sensitive genotypes than the others. The SOD activity increment ratios from NS to MD and SD in sensitive genotype were 74% and 90%, respectively. The same ratios were only around 30 % at MD and 50 % at SD in other three genotypes. The most significant difference among the genotypes was measured in APX activity, which increased more than nine fold in sensitive genotypes from NS to SD. On the other hand, increase in APX activity between the same conditions was not bigger than four folds in other three genotypes (Figure 4/APX). The hydrogen peroxide (H_2O_2) contents were significantly increased with the severity of the drought. H_2O_2 levels had parallel increments in the all genotypes when they were exposed to progressive drought stress (Figure 4/ H_2O_2). Compared to NS, significant increments in H_2O_2 levels occurred at MD in all genotypes. Interestingly, as drought increased further, the H_2O_2 level reduced in SD in all genotypes indicating effective activity of antioxidant enzymes in that drought level. Although there were no significant differences between the genotypes in H_2O_2 level during NS, H_2O_2 accumulations were more significant in sensitive genotype at MD than in other three genotypes. The increments of H_2O_2 concentration at MD was 98.6 % for sensitive genotypes compared to NS, whereas the same increments were only 31%, and 54% in moderate resistant, and resistant genotypes, respectively.

4. Discussion

4.1. Usage of selection indices to identify drought adaptation strategies in black poplar

In the current study, we investigated drought related physio-biochemical processes at different stages of water availability to understand the adaptation mechanisms of black poplar to drought stress. Drought resistance is defined by Hall (1993) as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988). Since drought resistance is a yield based trait, selection could vary depending on which index is chosen by the breeder. Therefore we selected stress tolerance index (STI) and mean relative productivity (MRP) indices to test their success in the selection of black poplar clones combining both high productivity and enhanced drought tolerance.

Strong correlation between MRP and overall shoot and diameter increment in field trial indicated that MRP is suitable when the breeding objective is directed toward testing performance under favorable and stress conditions. MRP favored the clones with the highest biomass production at the end of three years, whereas the above ground growth of these clones reduced almost %50 during drought period in the field trial. Therefore, the selected clones with the highest MRP values were defined as moderate resistant clones (N.62.191, N.83.12, N.96.325 and N.91.108) in the study. Physiological, morphological and biochemical analysis in the experiment indicated that these moderate resistant clones had a **dehydration tolerance mechanism** to cope with drought stress. Drought dependent leaf abscission was very limited in these clones that it can be accepted as 'non-senescent' compared to sensitive genotypes. Leaves of the moderate resistant clones formed only senescence symptoms such as yellowing and small necrotic lesions. Another important property of dehydration tolerant genotypes was higher survival rates under lower leaf water potentials. In the current study, leaf water potential values of moderate resistant genotypes decreased (-9.7 ± 0.7 bars) more at SD as compared to resistant genotypes. Despite high drought tolerance, lower leaf water potential was generally associated with osmotic adjustment in many reports (Kramer and Boyer, 1995; Babu et al, 1999; Blum, 2005). Osmotic adjustment comprises accumulation of solutes (sugars and proteins) in the plant cell vacuoles in response to drought. This accumulation results in reduction of osmotic potential of the plant cells, which initiate attraction of water into the cells and maintain turgor pressure. This mechanism sustains higher leaf water content in the cells at low LWP. Although we did not measure the accumulation of one of these types of solutes, high drought tolerance with lower LWP may depend on osmotic adjustment in moderate resistant genotypes. From these results it is highly possible to conclude that MRP could be used to select the dehydration tolerant genotypes within a collection. By this way we can combine both drought resistance and high productivity in the breeding program.

STI assesses the reduction in yield caused by drought stress compared with non-stressed condition. Lower STI values indicated lower differences in yield across stress levels, in other words, more resistance to drought. Therefore, in the current study the clones that have the highest STI values were defined as the resistant clones which were characterized with almost no reduction in above-ground growth during drought period as compared to non-stress environment in the field test. The results of the experiment indicated that these resistant clones had physiological properties of **dehydration avoidance strategy**, which was defined as a plants' ability to maintain high leaf water status under the effect of

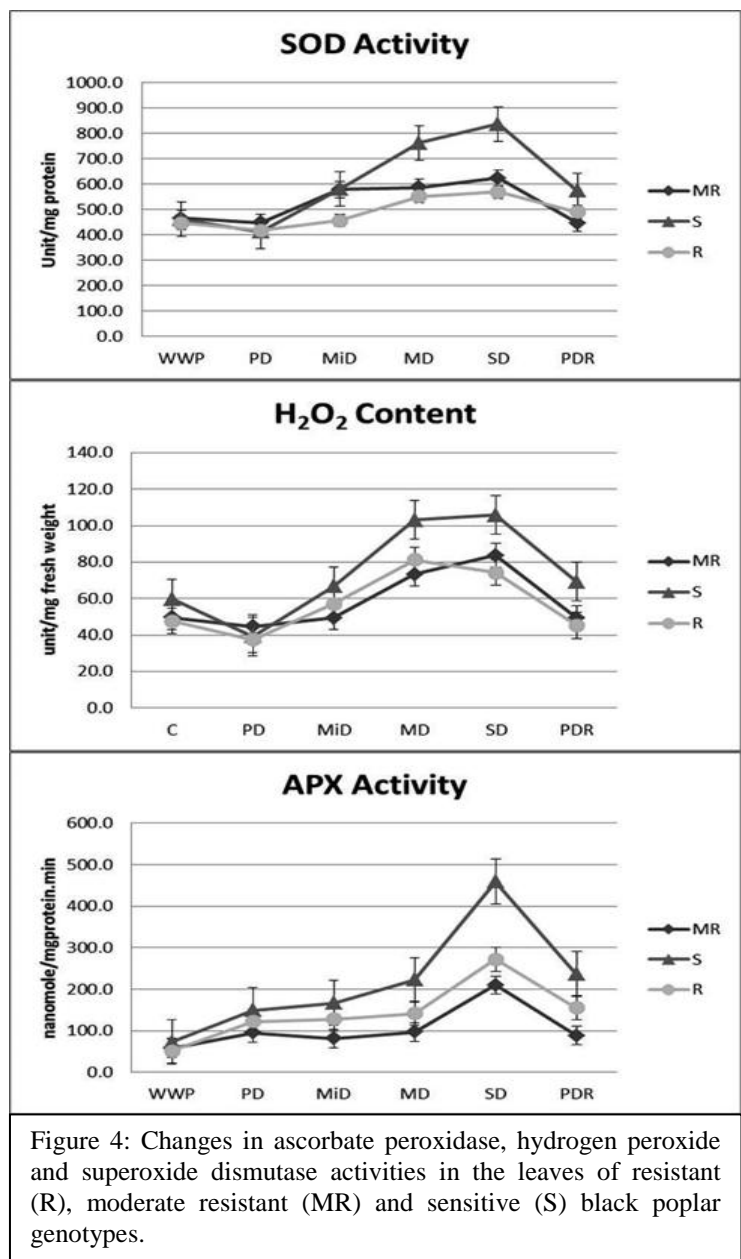
drought conditions (Kozłowski and Pallardy, 2002; Touchette et al., 2007). The strategy mostly relies on limiting the water usage and transpiration under drought stress (Blum, 2005; Klein et al., 2011). The plants that have this strategy reduce stomatal conductance as a first response to limit water transpiration during drought stress. This response enables constant leaf water potential regardless of drought intensity (Rood et al., 2000; Blum, 2005; McDowell et al., 2008). Although we did not measure the stomatal conductance of the clones in the experiment, the highest leaf water potential (-4 ± 0.6 bars) during the severe drought stress indicated that resistant clones could keep the limited water in the leaves especially found on the upper parts of stem. The high leaf water content in the leaves of a plant under stress conditions generally associated with promotion in water homeostasis. It has been reported that to maintain this homeostasis in dehydration avoided plants, older leaves are selectively killed under stress while the remaining young leaves retain high turgor (high LWP) (Blum and Arkin, 1984). In the present study, the older leaves located at the lower parts of stem of these resistant clones started to defoliate during non-stressed conditions. This could be considered as an adaptation and preparation to drought period. As the older leaves were selectively killed in the seedling, the upper part of the stems such as newly formed younger leaves and apical shoot of the seedlings could keep water effectively during drought period. By this way, the clones having this strategy could resist more prolonged drought stress without excess reduction in leaf water potential and complete leaf defoliation. Blum (2005) reported that plants designed for this type of constitutive moderation of water use could not attain high yield potential. Therefore, these types of plants are characterized with reduced plant size and leaf area, which are also useful in reduction of transpiration under drought stress (Kozłowski and Pallardy, 2002; Blum, 2005). The results of our experiment were also corresponded well with these suggestions that the overall biomass production of resistant clones ($517 \pm 86 \text{ cm}^3$) was much smaller than the moderate resistant clones ($668 \pm 108 \text{ cm}^3$) at the end of three years in the field test. Resistant clones were also had smaller leaf area as compared to sensitive and moderate resistant clones (Figure 3). Therefore, usage of STI index could be beneficial to select clones that can resist more prolonged drought conditions, whereas the utilization of this index as selection criteria could result in loss of biomass production in the breeding program.

Among the investigated clones, the sensitive genotypes in the current study were suggested to evolve a **drought escape adaptation strategy**. In this strategy, active portion of plant life generally takes place mostly during water abundant periods. Therefore, the most important biomass production takes place in well-watered period. The results of the current study indicated that sensitive black poplar clone had the high shoot elongation rate under non-stressed conditions. Fast growth of drought sensitive poplar genotypes under non stressed periods were also reported in previous studies (Tschaplinski et al., 1998; Yin et al., 2005; Regier et al., 2009; Yang et al., 2009). Despite high growth performances under non-stressed condition, sensitive clones indicated lowest yield productivity under drought period. Leaf shedding is known as a drought escape strategy to reduce leaf surface area and consequently the overall transpiration rate of a tree. (Kozłowski and Pallardy, 2002; Jansson et al., 2010; Zapater et al., 2012). In this study, the highest and the earliest leaf defoliations were observed at MD in only sensitive clones (Figure 3). At the severe drought conditions, sensitive genotypes shed the leaves almost completely. Only a few leaves remained on the stems until the end of the experiment, whereas these leaves could not recover themselves and shed in the re-watering period. Among the investigated clones the highest reduction in leaf water potential was recorded in sensitive genotype (-12 ± 0.9 bars) clone under severe drought conditions. Among the sensitive genotypes N.03.368.A and

N.92.218 exhibited an extreme form of stress sensitivity that terminal shoots of ramets of these clones desiccated at the SD. Prolonged reductions in leaf water potentials have been previously reported to result in xylem cavitation and embolism which cause subsequent hydraulic failure and branch sacrifice in many poplar species (Guo et al 2010; Rood et al., 2000; Jansson et al., 2010; Klein et al., 2011). These results indicated that STI may not be recommended for selection of the clones that combine both drought resistance and productivity, whereas STI could be used to eliminate the sensitive genotypes in a breeding program.

4.2. Association between antioxidant enzyme activities and drought adaptation strategies

The most important effect of drought stress is known to inhibit photosynthetic activity in leaf tissues due to an imbalance between light capture and its utilization (Foyer and Noctor, 2000). Down regulation of especially photosystem II (PSII) activity results in an imbalance between the generation and utilization of electrons. These changes in the photochemistry of chloroplasts in the leaves of drought stressed plants result in the dissipation of excess light energy in the PSII core, thus generating reactive active oxygen (ROS) species (O_2^- , O_2 , H_2O_2 , OH), which are potentially dangerous under drought stress conditions (Reddy et al., 2004; Sofu et al., 2005; Anjum et al., 2010). It has also been reported that increase in ROS, such as H_2O_2 level, generate signals in the cell to trigger the defense response during the drought stress. The highest values of H_2O_2 content were recorded for the drought sensitive clones during the drought treatment (Figure 4). As stated in the result section, sensitive clones were the most adversely affected clones under the drought stress in terms of growth and leaf abscission which are highly related with increased level of ROS in the plants. H_2O_2 level mostly depends on existence of ROS under drought stress



and conversion of these ROS into H_2O_2 by SOD (Asada, 1999; Sharma et al., 2012). Investigation of SOD activity along the drought treatment demonstrated this clear linkage between H_2O_2 and SOD enzyme activity that the highest increase of SOD activity was also

observed in sensitive clones throughout the drought treatments (Figure 4). In fact, increased activity of SOD is often correlated with increased tolerance of the plant against environmental stresses. Even, it was suggested that SOD could be used as a selection criterion for screening of drought-resistant plants (Sharma et al., 2012; Regier et al., 2009; Yang et al., 2010). However, a reverse situation was recorded in the current study, which could be explained by the increased need of ROS removal from the leaves of sensitive clones. Under drought conditions, activity of APX also increased much greater extent in sensitive clones than other genotypes. The APX has a central function in ascorbate-glutathione cycle and a crucial role in the management of ROS during many stressful conditions. The basic activity of the APX is to reduce H_2O_2 into water (Sharma et al., 2012). In the current study, APX activities exhibited very compatible variations with SOD and H_2O_2 changes throughout the stress treatment. The rate of APX increase for the sensitive black poplar genotypes was more than four folds higher than it was in the other clones. This seemed to be matched with the suggestion of increased need of ROS removal in leaves of the drought sensitive clones.

Relatively lower antioxidant enzyme activities in the resistant and moderate resistant genotypes could be associated with the high plant water status or osmotic adjustment during drought periods. Drought tolerance is generally defined as the plant capacity to maintain high plant water status or cellular hydration under the effect of drought. By this mechanism plants avoid being stressed because tissues are relatively unexposed to dehydration (Kramer and Boyer 1995; Kozlowski and Pallardy, 2002; Blum, 2005). In the current study, LWP values of the resistant clones were much higher than moderate resistant and sensitive genotypes at SD. This situation indicated that resistant kept the limited water in their leaves under drought conditions and might not need to increase their antioxidant enzyme activities due to the lower level of ROS in the leaf tissues. Despite the significant decrease of LWP in moderate resistant clones at SD, antioxidant enzyme activities were not increased in these clones as compared to sensitive ones. This situation could be explained by osmotic adjustments of the moderate resistant clones under drought stress to maintain turgor pressure. By this adjustment drought might not cause increase in ROS and antioxidant enzyme activities in drought treated leaves of moderate resistant clones. On the other hand, increased accumulation of ROS in the leaves of sensitive genotypes could be the main reason for earlier and severe leaf abscission as well as higher antioxidant enzyme activities. ROS attack the most sensitive biological macromolecules in cells impairing their functions. Deleterious effects of ROS molecules on biological structures include DNA nicking, amino acid and protein oxidation, and lipid peroxidation (Asada, 1999; Johnson et al., 2003). Another function of ROS molecules in the plant cells are initiation of a catastrophic cascade including expression of leaf abscission related hormones such as abscisic acid or ethylene and activation of the genes functional antioxidative systems and antioxidants (Mano, 2002). Although this series of ROS scavenging regulatory mechanisms have activated within the plant cells, oxidative damage remains a potential problem, since it causes perturbations in metabolism, such as a loss of coordination between energy production (source) and energy utilization (sink) processes during photosynthesis in green leaves which could be resulted with cell death (Anjum et al., 2010). Therefore, increase in antioxidant enzyme activities in the sensitive clones could not be enough to protect the leaf cells under prolonged drought conditions.

4.3. Usage of clonal variation in drought response for wood production

In Turkey, about 99 % of forest lands and wood resources are under the direct control of the governmental agencies. Roughly, 7.5-8 million m³ of industrial round wood and 5.0 million m³ fuelwood are produced from the state forests annually. Despite the small area of private forests, which are mostly poplar plantations (200,000 ha), industrial round wood production from this plantations is relatively high. Private sector fast-growing plantations and agro-forestry sites produce about 3.2 million m³ industrial round wood and 1.5 million m³ fuel-wood annually. However, wood production from this state and private forest is not enough to compensate the rising demand of forest products in Turkey (Yıldırım et al., 2011). Poplar plantations seem to be the most efficient way to meet this wood demand. According to a survey, if 5% of the irrigated land in Turkey is reserved for poplar plantations, about 2.5 million m³ wood could be produced annually (Işık and Toplu, 2004). However, most of these irrigated lands were generally utilized for agricultural practices. Therefore, development of drought tolerant poplar cultivars, which enable establishment of poplar plantations in arid or semi-arid zones, is crucial to increase wood production in Turkey. The results of field test in the current study indicated that black poplar clones gathered from all around Turkey evolved three contrasting adaptation strategies for survival and growth under limited water availability. Therefore, identification of black poplar adaptation strategies to different watering regimes in this study could be used for selection of clones for wood production plantations established in different regions.

In the black poplar collection, a few clones were found to have drought escape adaptations. In the both field and greenhouse experiment it was found that this strategy contributed to fast growth rates if there was available water resources. However, when they encountered with water limitations, these drought evaded clones were found to withstand only mild drought conditions. Therefore, these types of clones could be very suitable for gallery plantations which are established in river sites. When the total length of the river basin (100.000 km) of Turkey is considered, gallery plantations that are established with these types of clones would be beneficial in terms of wood production.

The clones with dehydration tolerance strategy combine high productivity with drought tolerance. Our observations in the field trial indicated that clones that have this strategy comprised 10% of the black poplar collection. Despite the reduction in growth parameter under drought stress, in all different water availabilities these clones exhibited highest growth performances in both greenhouse and field trial. Therefore, the clones that have this adaptation strategy could be used in industrial plantations for wood and biomass production in especially arid and semi-arid zones of Turkey. Among the five commercial black poplar clones of Turkey, four of them (Gazi, Kocabey, Geyve, Behiçbey) included in this dehydration tolerant group. However, there were many clones in the collection (e.g. N.62.191) that the productivity and drought tolerance properties were much better than these commercial ones.

The last adaptation strategy in black poplar collection was dehydration avoidance mechanism, which has high capacity for drought tolerance but lower growth rates under both stressed and non-stressed condition. The great majority of the black poplar collection (70%) fell into this group. Due to their slow growth rates, the usage of these types of clones in the wood production plantations is not recommended. However, breeding studies require

high genetic diversity to select the best individuals against other types of biotic and abiotic environmental stresses. Therefore, these drought avoided clones should be kept in black poplar collection to increase genetic diversity for future breeding studies.

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