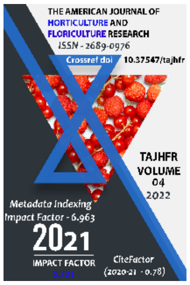
The American Journal of Horticulture and Floriculture Research
(ISSN – 2689-0976)
VOLUME 04 ISSUE 03 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 251) (2021: 5. 731) (2022: 6. 19)
OCLC – 1121086298 METADATA IF – 6.963
Publisher: The USA Journals



Journal Website: https://theamericanjournals.com/index.php/tajhfr

Research Article

SEASONAL DYNAMICS OF THE MAIN MARKERS OF STRESS IN PLATANUS ORIENTALIS LEAVES IN THE CONDITIONS OF THE URBAN ENVIRONMENT SEMIARIDE ZONE

Submission Date: February 25, 2022, **Accepted Date:** March 15, 2022,
Published Date: March 26, 2022 |
Crossref doi: https://doi.org/10.37547/tajhfr/Volume04Issue03-01

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ABSTRACT

Today, the resistance of plants to stress is one of the most basic and important problems. Plants are sufficiently resistant to oxidative disorders that occur with sudden changes in the physiological state of the organism. This suggests that plant cells have an antioxidant system that can protect against oxygen radicals and singlet oxygen. They are different from animals that have an immune system. The article describes the state of the antioxidant system of the Platanus orientalis tree in the urban semiarid zone from June to September. In addition to the impact of climatic conditions, the impact of an additional man-made stress factor in the form of a highway was also assessed. It is known that all living organisms have the ability to adapt to biotic and abiotic environmental factors and protect themselves from adverse conditions. The article describes the state of the antioxidant system of the plane tree plant growing in Uzbekistan in the summer. During the season, the amount of malondialdehyde and proline was studied.

KEYWORDS

Proline, malondialdehyde, antioxidant system, stress-resistant plants, an active form of oxygen.

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INTRODUCTION

An important part of this task is to study the resistance of ornamental plants to adverse conditions of the city by the Decree of the President of the Republic of Uzbekistan "On measures to improve the system of landscaping and architectural landscaping of roads" (PP-3262 No. 09/11/2017). It should also be noted that the level of air pollution along city highways is very high. The selection of plants resistant to these contaminants, as well as to the harsh continental climate, requires great effort. The study of the developmental dynamics of oxidative stress in the leaves of ornamental plants growing in such conditions will serve as a basis for the selection of plants in the subsequent greening of the city and the propagation of greenery.

THE MAIN RESULTS AND FINDINGS

The study of cellular and molecular mechanisms in the adaptation of plants to unfavorable conditions of nature is now one of the fundamental problems. The formation of an adaptive response to abiotic stressors in the plant organism occurs as a result of many metabolic changes [5,7]. Several benefits of proline in plant resistance to abiotic stressors, including inhibiting the accumulation of the active form of oxygen in the cell, its osmoprotective role in the protection of macromolecules, and maintaining homeostasis in the plant organism, are essential processes in plant life. The role of proline in osmoregulation is that it increases the osmotic pressure of plant sap and ensures plant resistance in a decrease in the water potential of the soil solution [8]. In addition to its osmoprotective function, proline stabilizes "chemical" chaperone function, membrane structures, and proteins under stress, and is a scanner of CFS as an antioxidant. It also plays an important role in regulating the expression of stress-controlling genes and maintaining the cell pH environment. In the recovery phase, it serves as a source of energy, excess nitrogen, and carbon [1]. During the stress relief phase, proline controls cell proliferation and death. In short, proline is more multifunctional than other osmolytes, and it performs several important functions in stressful and moisture-deficient conditions - regulatory, metabolic, and protective, according to the "chemical" chaperone model [12]. The antioxidant properties of proline also depend on its molecular structure. The molecule, which contains a hydrated pyrrole heterocyclic ring, can participate in redox reactions. Other heterocyclic compounds that retain many functional groups, such as nucleotide bases, can form hydrogen bonds between proline molecules to form a polymer chain. Proline retains a tertiary carbon atom. The formation of such a stable radical can "shut down" or stop the cascade of free radical reactions initiated by superoxide, peroxide, or hydroxyl radical [10,11].

In an urban environment, plants maintain their decorative state from the outside but undergo significant physiological and biochemical changes under the influence of various factors. As a result of various extreme factors, primarily the membrane structure is damaged, the activation of free radicals in plant cells leads to an increase in the end product of peroxidation of lipids – malondialdehyde (MDA). MDA is a widely used marker of oxidative lipid injury caused by environmental stress. A number of studies have investigated MDA of plants under different stress conditions. Zhou et al. studied forest trees grown in soil which was exposed to Pb with different levels of water stress, and the results indicated that water stress significantly increased superoxide dismutase (SOD) and peroxidase (POD) activities and MDA content under different Pb concentrations [13]. The

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data from Jbir-Koubaa suggested that salinity stress might cause a shock and photo-oxidative stress, which caused MDA accumulation in leaves [3]. Jin et al. studied physiological responses of oilseed rape under herbicide ZJ0273 stress, and the results indicated that MDA contents showed a linear trend with the increasing of ZJ0273 [4]. The above studies indicated that lipid peroxidation was a common phenomenon in plants under stress, and MDA could be used as an important indicator of physiological status during plant growth.

Therefore, it is important to determine the amount of MDA and proline in the leaves of plants, which shows the importance of using experimental results in determining the resistance of ornamental plants to adverse environmental conditions.

The object of the study was the leaves of Maple or Eastern Platan (Platanus orientalis) growing in the green plantations of the Botanical Garden and the National University of Uzbekistan named after Mirzo Ulugbek ("Botanical Garden" group) and the leaves of plants growing at the intersection of Central Street and Amir Temur Square in Tashkent. The leaves are collected in the morning (at 7–9 a.m.). Trees about one-year-old were used for the study.

Determination of free proline. The amount of free proline was determined by the Bates method [9]. To do this, 500 mg of fresh or 200 mg of dried plant material was homogenized in 10 ml of an aqueous solution of 3% sulfosalicylic acid, then filtered to the homogenate. 1,5 ml of extract was obtained to carry out the chromogenic reaction. 1,5 ml of glacial acetic acid and 1,5 ml of ninhydrin reagent (1,25 g of ninhydrin, 20 ml of 6M H_3PO_4, 30 ml of glacial acetic acid) were added to the extract. Then 3 ml of toluene was added to the mixture and shaken vigorously twice for 30 seconds with a 1-minute break (to vigorously mix the aqueous and organic phases and convert the chromophore to toluene). To estimate the chromophore concentration, the optical density of the nidrin-proline solution in toluene was measured at 520 nm. The proline content was determined using a calibration curve constructed using a set of standard proline solutions in 3% sulfosalicylic acid.

Determination of malondialdehyde. This method is based on the reaction between malondialdehyde and thiobarbituric acid, resulting in the formation of a trimethyl complex in an acidic environment at high temperatures. The maximum absorption rate of this complex is 532 nm. To determine MDA, freshly cut plant leaves are homogenized with 20% trichloroacetic acid. It is then centrifuged for 15 minutes (10000g). To 0.5 ml of supernatant is added 1.5 ml of 0.5% TBK (dissolved in 20% trichloroacetic acid) and incubated at 95 ° C for 30 minutes. After incubation, the samples are cooled in an ice bath and the amount of MDA at 532 nm and 600 nm wavelengths is measured using Synergy HT microplates (BioTekInstruments, USA) [2].

Various abiotic factors, in particular drought, the high temperatures that occur in the summer months lead to the activation of adaptive and protective reactions in plants. The adaptation of plants to abiotic stresses is accompanied by the accumulation of free proline in the tissues. In the response of plants to various influences, proline can regulate the oxidation-reduction potentials, neutralize oxygen radicals, act as an osmoprotectant, in addition, this amino acid stabilizes the tertiary structure of proteins. An increase in proline content can serve as a quantitative measure of dehydration and stress. The more unfavorable the growth conditions of the plant, the greater the amount of proline in its tissues [6]. At the beginning of the season, the amount of proline in the leaves of plants growing in the botanical garden was not more than

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3,69 µmol/g dry mass (DM). By August, the amount of proline had increased significantly by 1,6 times compared to the initial figure. The amount of proline in Platanus orientalis leaves growing near the highway was 5,4 µmol/g (DM) at the beginning of the season, which is 1,5 times higher than that of botanical garden plants. This difference suggests that there is additional anthropogenic stress in the form of atmospheric air pollution near busy highways, which in turn has an impact on plant growth. Later, in July, when the highest temperature was observed (average daily temperature did not fall below 39-41°C), this figure was 5,9 µmol/g (DM). This is a significant increase of 1,1 compared to the beginning of the season. At the same time, the amount of proline in maple leaves growing in botanical garden conditions was also higher than at the beginning of the season, reaching 5,7 µmol/g (DM). This amount is 4% less than the existing conditions of additional anthropogenic stress.

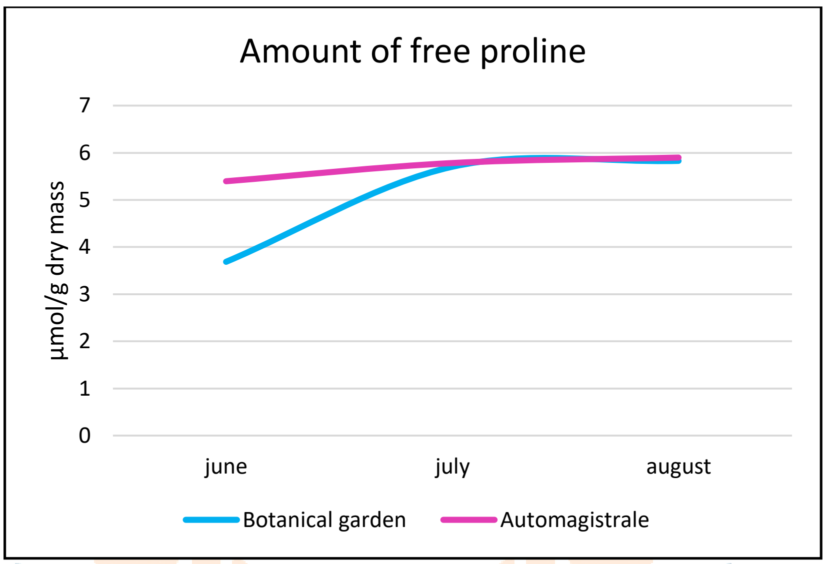

Figure 1. Seasonal variation of proline accumulation in *Platanus orientalis* leaves depending on the place of growth (n = 20, p <0.05).

By August, proline levels had increased 1,1 times, 5,9 µmol/g (DM). However, it should be noted that compared to the figures of trees growing in the botanical garden, this figure was still 1,02 times higher.

For an overall assessment of oxidative stress, we used to measure the accumulation of malondialdehyde (MDA), the end product of the peroxidation process of lipids. We found that in the early stages of the growing season (April-May) its amount is minimal. Therefore, during our study, the MDA level in April-May was taken as basic or "zero". By June, the amount of MDA in maple leaves growing in the botanical garden was 14 µmol/g relative to dry mass, while in July this figure decreased by 3,5 times (4 µmol/g dry mass). By the end of the season, the amount of MDA was almost unchanged, i.e. 4,5 µmol / g relative to the dry mass.

It was observed that the amount of MDA in maple leaves growing near the highway was significantly different from that in botanical garden plants. At the beginning of the season, the amount of MDA was 10 µmol/g relative to the dry mass. In July, the figure rose to 50%. By August, the amount of MDA was observed to increase 2,7 times compared to the beginning of the season (27 µmol/g dry mass).

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That is, the difference between the amount of MDA in maple leaves growing in the trunk conditions of the botanical garden was 11%. To evaluate the activity of the antioxidant system (AOS), the ratio of MDA numbers in the summer months was calculated, which showed that the figure was different in both cases, but in the botanical garden this figure was much lower and the antioxidant system activity of maple under additional anthropogenic stress was almost 6 times higher (Figure 2).

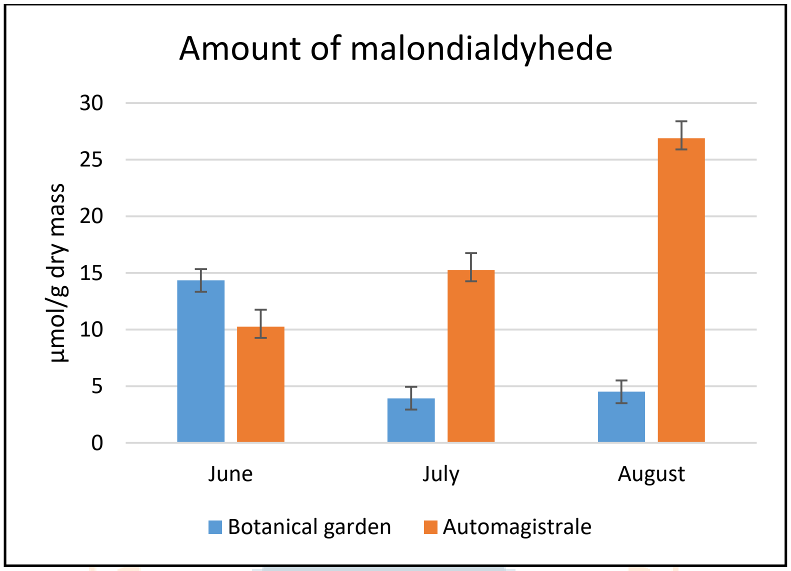

Figure 2. Dynamics of accumulation of MDA in *Platanus orientalis* leaves in the Botanical Garden and on the automagistrale (at the beginning of the season, the amount of MDA was taken as 0; n = 20; p <0.05)

CONCLUSION

Thus, the data obtained show that the resistance of the Platanus orientalis to high temperatures and anthropogenic impacts is associated with various physiological, biochemical, and molecular genetic mechanisms, among which the activation of AOS activity plays an important role. Based on our data, it can be assumed that the implementation of the protective effect of the antioxidant system under the influence of stress factors on ornamental plant species requires the activation of several or more antioxidant reactions. In this case, the selectivity in activating specific biochemical reactions that protect from oxidative stress is determined not only by the constitutional characteristics of the plant species but also by the nature of the stressor acting.

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