UDK 536.463:661.74:633.88
DOI: 10.7562/SE2016.6.01.02
Original article
www.safety.ni.ac.rs

STAMENKOVIĆ SLAVIŠA[1]
MARKOVIĆ MARIJA[2]
STANKOV JOVANOVIĆ VESNA[3]
MITIĆ VIOLETA[4]
ILIĆ MARIJA[5]

[1,2]University of Niš,
Faculty of Science and Mathematics,
Department of biology and ecology
[3,4,5]University of Niš,
Faculty of Science and Mathematics,
Department of Chemistry

sjvesna@yahoo.co.uk

TOTAL CONTENT OF ORGANIC ACIDS IN PLANTS COLLECTED SECOND YEAR AFTER THE WILD FIRE

Abstract: *The wild fire, which occurred in the summer of 2007 in the mountain Vidlič (Southeast Serbia) destroyed large areas of beech and oak forests, dry meadows and rocky terrains. The aim of the present study was to investigate the effect of wild fire on the organic acids content in underground and above-ground parts of plants Geranium macrorhizum, Doronicum columnae, Glechoma hirsuta, Chelidonium majus and Primula veris, second year after the accident. As a control material were used samples of plants from areas that were not exposed to fire.*

Key words: organic acids, wild fire, Geraniaceae, Asteraceae, Lamiaceae, Papaveraceae, Primulaceae.

INTRODUCTION

Wild fires dramatic consequences on ecosystems are well known and documented. Wild fire leads to the destruction of flora, vegetation, fauna, their habitats as well as plant and animal species in the affected areas. The restoration of the original situation demands a considerable period of time, which is measured by decades or centuries.

On the mountain Vidlič in southeastern Serbia, in the summer 2007 an enormous wild fire occurred, which burned down more than 2500 hectares of oak forests, scrubs of hornbeam, thermophilic meadows, rocky terrains and beech forests.

Forest fires create conditions that are favorable for certain plant species that were not present in this ecosystem before the fire. These plants are known as pioneer plants. Pioneer plants are specific, because they face many adverse environmental factors, such as high intensity lighting, high temperatures, low moisture content, increased evaporation as well as significant changes in the soil composition [1]. Pioneer and native plants become competitive. Some plants on the wild fire affected areas very quickly adapt, while others are absent, and they appear only after the establishment of the conditions that suit them.

The fire might affect phytochemical characteristics of the plants, which was the subject of several studies [2,3,4,5,6,7]. After the wild fire on the Vidlič mountain, it's impact on the phytochemical characteristics of plants was assessed in terms of: heavy metal content [8,9,10,11,12,13] content of chloroplast pigments [1,14], content of organic acids first year after the fire [15,16], the quantity and chemical composition of essential oils [17], antioxidant characteristics of plants [18,19,20,21] and activity of the enzyme catalase [22].

Organic acids are secondary metabolites that are formed during the decomposition process of carbohydrates, fats and proteins. The most common are: oxalic, citric, formic, fumaric, acetic, phosphoric, and others. The accumulation of some specific organic acids in the plant tissues is usually in connection with the enzymatic reactions. Biochemical role of organic acids in plants depends on the environmental conditions. They act as proton donors, play a role in some oxidation-reduction reactions, for example in the conversion of malic to oxalic acid. Some previous studies have shown that the content of organic acids in plants depends on a variety of biochemical processes in plants (transpiration), and growth stages of the plants [23]. According to the results obtained by Vergano & Gabrielli [24], Trajkovic [25], Trajkovic et al. [26] higher concentrations of heavy metals cause an increase in the content of organic acids in plants.

Organic acids in plants have an important role in detoxification that is caused by heavy metals [27,28,29]. They form chelate complexes with heavy metals and thus allow the circulation of heavy metals through the plant. These complexes of organic acids and heavy metals, which accumulate in the plant organs, can later be removed from the plant [27,30,31]. On the other hand, it has been shown that iron deficiency causes substantial accumulation of organic acids in root tissues, as well as a significant increase in hydrogen ions and excretion of organic acids [32,33,34]. In addition, the lack of phosphorus [35,36,37] or increased concentration of Al^{3+} ions leads to increased exudation of organic acids anions in some plant species [38,39].

MATERIALS AND METHODS

The organic acids in the plant material were determined volumetrically [32]. Due to the interferences caused by plant material, procedure was slightly modified, to the extent that such a change does not affect the validity of the results. Actually, instead of the classical volumetric titration potentiometric titration was applied in order to obtain sharp titration ending point.

Plant material (10 g) was extracted in a mortar and pestle with 100 ml of distilled water. The extract was transferred without any loss to the Erlenmeyer flask of 100 ml and left in the water bath at a temperature of 75ºC for an hour. After this, Erlenmeyer flask was rapidly cooled under cold running water, the content was filtered to a volumetric vessel of 100 ml and supplemented with distilled water to the mark. Probe of 50 ml of the filtrate was transferred into a beaker and titration was carried out by 0.1000 mol/dm^3 NaOH standard solution, applying glass electrode and pH-meter, with constant stirring until pH of 8 was achieved.

Calculation of the total acidity was done using the formula:

X = a x T x b x 10 /m x c

Where:

X - the amount of acid in the examined sample in meq per 1 g of fresh weight

a - the volume of spent 0.1000 mol/dm^3 standard NaOH solution (cm^3)

T - correction factor of 0.1000 mol/dm^3 standard NaOH solution

b - general volume of the extract (cm^3)

c- volume of filtrate used for titration

m - amount of plant material used for the extraction (g)

10 - factor of calculating to acid miliequivalents (1 cm^3 of 0.1000 mol/dm^3 standard NaOH solution corresponds to 0.1 meq acid).

RESULTS AND DISCUSION

Examination of the total organic acids content in plants from the wild fire affected areas, second year after the fire was performed, along with a comparison with the plants from the areas not affected by wild fire, taking into account their importance as an indicator of the physiological state of the plants and the effect of environmental factors on them. The total content of organic acids in plants from areas affected and not affected by wild fire was determined one year after the occurrence and the results for whole plants indicated that total organic acids content vary for different plants, but in most cases was greater for the plants form the wild fire affected areas than those from areas not affected by wild fire. The exceptions are *Geranium macrorhizum* and *Aegopodium podagraria*, and for them the opposite is true [15]. Since the level of organic acids in plants *Geranium macrorhizum* and *Aegopodium podagraria* were higher in plants at not affected compared to the fire-affected areas, it is probable that they have developed different defense mechanisms to survive under stressful conditions such as wild fire. The lowest level of organic acids right after the fire was recorded in plant *Aegopodium podagraria* [15].

Trajkovic et al. [26] noticed that the content of organic acids varies in underground and above-ground organs of the same plant. Underground and above-ground organs of plants have different morphology, anatomy, and developed various defense mechanisms in response to stress conditions, which are genetically predetermined [25]. It is related to the different roles of the mentioned organs, their different needs and different active processes in which they participate. Therefore, the second year after the fire, unlike the first, the main task was to analyze separately content of acids in underground and above-ground parts of plants (Tab. 1).

Table 1. *Comparison of the total organic acids content (mEq / g fresh weight) in plants from not affected (NA) and fire affected areas (FA) of beech forests in the Vidlič mountain second year after wild fire.*

Plant species	Plant part	TAC in plants on NA (mEq/g fresh weight)	TAC in plants on FA (mEq/g fresh weight)	Ratio of TAC in plants from FA and NA (%)
Geranium macrorrhizum	AG	0.214	0.373	174.30
	UG	0.191	0.244	127.75
Doronicum columnae	AG	0.154	0.111	72.08
	UG	0.115	0.046	40.00
Glechoma hirsuta	AG	0.065	0.042	64.62
	UG	0.026	0.014	53.85
Chelidonium majus	AG	0.052	0.070	134.62
	UG	0.051	0.067	121.57
Primula veris	AG	0.061	0.072	118.03
	UG	0.037	0.041	110.81

NA - fire non affected areas, FA - fire affected areas, TAC - total acid content, AG - above-ground plant part, UG - underground plant part.

As expected, in a significant number of studied plants (*Geranium macrorhizum, Chelidonium majus* and *Primula veris*) content of organic acids is higher at affected than in wild fire not affected areas, both in underground and aboveground parts. For plant species *Doronicum columnae* and *Glechoma hirsuta* the

situation is reversed, i.e. both in their underground and above-ground organs, acids content were higher at not affected versus affected area.

In all tested plants, total content of organic acids is higher in aboveground parts in than in underground parts, disregarding if area was or wasn't affected by wild fire. Overall, in the second year (2009) compared with the first year (2008) after the fire [15], lower levels of organic acids was detected, which is associated with the fact that in second year after the accident stress impact is less pronounced compared in the first year after the fire.

CONCLUSION

Plants that grow at the areas affected by wild fire are highly adaptive plants, characterized with a distinctive metabolism and mechanisms for survival. The changed conditions after the wild fire require from plants anatomical, physiological and biochemical adaptations. The ability of plants to adapt to stressful conditions is crucial for their survival. Different plants exhibited different responses to stress, depending on the different genetic background, various phenophase, morphological and anatomic characteristics of the tested plants.

The fact that mainly increased heavy metals content in the areas affected by wild fire entails enhancement of the total organic acids content. Synthesis of organic acids is one of the defense mechanisms of plants in response to stress conditions that occur at the fire affected areas.

ACKNOWLEDGEMENT

This work was performed under the project of the Ministry of Education, Science and Development of Republic of Serbia OI 171025.

REFERENCES

[1] Marković M., Pavlović D., Tošić S., Stankov-Jovanović V., Krstić N., Stamenković S., Mitrović T., Marković V. (2012): Chloroplast pigments in post-fire grown cryptophytes on Vidlič Mountain (Southeastern Serbia), Archives of Biological Sciences, Belgrade, 64 (2): 531-538.

[2] Knapp A.K., Giliam F.S. (1985): Responce of Andropogon gerardii (Poaceae) to Fire Induced High vs. Low Irradience Environments in Tallgrass Praire: Leaf Structure and Photosynthetic Pigments. American Journal of Botany, Vol. 72, No. 11, 1668-1671.

[3] Frazer J.M., Davis S.D. (1988): Differential survivalof chaparral seedlings during first summer drought after wildfire, Oecologia 76: 215-221.

[4] Fleck I., Grau D., Sanjosé M., Vindal D. (1996): Carbon isotope discimination in *Quercus ilex* resprouts after fire and tree-feel, Oecologia105: 286-292.

[5] Alonso M., Rosados M.J., Vega J.A., Perez-Gorostiaga P., Cuinas P., Fonturbel M.T., Fernandez C. (2002): Biochemical responces of *Pinus pinaster* trees to fire induced trunk girdling and crown scorch: secondary metabolites and pigments as needle chemical indicators, J. Chem. Ecol. 28(4): 687-700.

[6] El Omari B., Fleck I., Aranda X., Abadia A., Cano A., Arnao M. (2003): Total antioxidant activity in *Quercus ilex* resprouts after fire, Plant Physiology and Biochimistry 41: 41-47.

[7] Antiléan M., Araya N., Briceno M., Escudey M. (2006): Changes of chemical fraction of heavy metals in Chilean soils amended with sewerage sludge affected by a thermal impact, Soil Research 44(6): 619-625.

[8] Marković M., Pavlović-Muratspahić D., Stankov-Jovanović V., Mandić S., Mitić V. (2008): Heavy metals distribution in plant and soil samples from post-fire area on the Vidlic Mountain, 6th Aegean Analytical Chemystry Days (AACD), Denizli, Turkey, 9-12 october 2008, Book of Abstracts: 266.

[9] Mitić V., Marković M., Pavlović-Muratspahić D., Stankov-Jovanović V., Nikolić-Mandić S. (2008): Heavy metals distribution in two plant species growing in burnt and unburnt soils from the Vidlic Mountain, 20th Congress of Chemists and Technologysts of Macedonia, Book of Abstracts.

[10] Ilić M., Marković M., Mitić V., Mandić S., Stankov-Jovanović V. (2010): The content of heavy metals in soil and plants of Lamiaceae family on fire affected and nonaffected areas of the Vidlič mountain, 10th Symposium on the Flora of Southeastern Serbia and Neighbouring regions, Vlasina, 17 to 20 june 2010, Book of abstracts: 67. (In Serbian)

[11] Ilić M., Mitić V., Marković M., Stankov-Jovanović V., Nikolić-Mandić V. (2011a): Heavy metals determination in species of family Geraniaceae and coresponding soils after wild fire, International conference "Medicinal and aromatic plants in generating of new values in 21 st century" Sarajevo, 9-12 November, 2011, Book of Abstracts, 227.

[12] Stankov-Jovanović P.V., Ilić D.M., Marković S.M., Mitić D.V., Nikolić-Mandić D.S., Stojanović S.G. (2011): Wild fire impact on copper, zinc, lead and cadmium distribution in soil and relation with abundance in selected plants of Lamiaceae family from Vidlic Mountain (Serbia). Chemosphere 84: 1584-1591.

[13] Jovanović S., Marković M., Simonović S., Ilić M., Stankov-Jovanović V., Mitić V., Nikolić-Mandić S.. (2012): Distribution of iron, zinc copper, cadmium and lead in *Aegopodium podagraria, Chelidonium majus* and *Hypericum perforatum* from Vidlič mountain (Serbia), Society of Chemist and Technologists of Macedonia, 22 nd Congress with international participation, Ohrid, September, 5-9, 2012, Abstract book BFP-43.

[14] Krstić N., Marković, M., Stamenković S, Stankov-Jovanović V., D. Pavlović D., Marković V. (2011): Chloroplast pigments of *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. from the place on a fire of Vidlič Mountain, EUROanalysis, 16th European Conference of Analytical Chemystry, Belgrade, EN 45 Session B.

[15] Nešić M., Marković M., Trajković R., Pavlović D., Ilić M., Mitić V., Stankov-Jovanović V. (2010a): Total content of organic acids in plants from fire affected forest, Biologica nyssana 1 (1-2): 65-69.

[16] Nešić M., Marković M., Trajković R., Pavlović D., Stankov-Jovanović V., Mitić V., Ilić M. (2010b): Content of totally organic acids in plants from fire affected forest, 10th Symposium on the Flora of

Southeastern Serbia and Neighbouring regions, Vlasina, 17 to 20 june 2010, Book of abstracts: 101.

[17] Mitić D.V., Stankov-Jovanović P.V., Jovanović P.O., Palić I., Đorđević S.A., Stojanović S.G. (2011): Composition ond Antioxidant Activity of Hydrodistilled Assential Oil of Serbian *Ajuga chamaepytis* (L.) Schreber. ssp. *chia* (Schreber) Arcangeli, Journal of Essential Oil Research Vol 23., Novembar/Decembar, 70-74.

[18] Dimitrijević M., Cvetković J., Mitić V., Marković M., Ilić M., Stankov-Jovanović V. (2010): Antioxidant properties of some plant species in the fire affected areas at the Vidlič Mountain, 10th Symposium on the Flora of Southeastern Serbia and Neighbouring regions, Vlasina, 17 to 20 june 2010, Book of abstracts: 93. (In Serbian)

[19] Ilić M., Marković M., Mitić V., Stankov-Jovanović V., Stojanović G. (2011b): Antioxidant properties of Seseli rigidum Waldst. et Kit. extracts of different polarity, International conference "Medicinal and aromatic plants in generating of new values in 21 st century" Sarajevo, 9-12 November, 2011, Book of Abstracts, 41.

[20] Stojanović G., Mitić V., Stankov-Jovanović V., Ilić M. (2011): Antioxidant activity of selected plants species growing under normal and post fire conditions on Vidlič mountain (Serbia), 10th Indo-Iitalian workshop on chemistry and biology of antioxidants, Sapienza University, Rome, Italy, Book of Abstracts, 2011, 63.

[21] Stojanović S.G., Mitić D.V., Stankov-Jovanović P.V., Ilić D.M., Jovanović P.O., Petrović M.G. (2013): Antioxidant characteristics of selected plants growing post-fire environmental conitions, Oxidation Communications 36, No 1, 26-32.

[22] Marković S.M., Ilić S.B. Miladinović L.D., Stamenković M.S., Trajković R., Stankov-Jovanović P.V., Djelić T.G. (2015): Activity of a catalase enzyme in plants from the burned areas of the Vidlič mountain's beech forest, Oxidation Communications, No 2, 860-868.

[23] Gašić O. (1992): Biochemistry of plants, Scientific book, Belgrade. (In Serbian).

[24] Vergano O., Gabrielli R. (1987): The response of plants to heavy metals, organic acids production. G. bot. "Ital." 121, N 3-4: 209-212.

[25] Trajković R. (1995): The impact of sir pollution on some biochemical and physiological parameters in plants from the industrial area of Kosovska Mitrovica and Obilić, PhD thesis, Faculty of Science and Mathematics, Priština. (In Serbian).

[26] Trajković R., Nešić M, Blagojević N. (2007): Content of totally organic acids in plant from industrial area of Bor town, 9th Symposium on the Flora and Vegetation of Southeastern Serbia and Neighbouring regions, Niš, Book of abstracts: 145-151.

[27] Jones D.L. (1998): Organic acids in rhizosphere – a critical review, Plant Soil 242, 235-243.

[28] Ma J.F. (2000): Role of organic acids in detoxification of aluminium in higher plants. Plant cel physiology, 41(4): 383-390.

[29] Ma J.F., Ryan P., Delhaize E. (2001): Aluminium tolerance in plants and the complexing role of organic acids. Trends in Plant Science, 6(6), 273-278.

[30] Ma J.F., Furukawa J. (2003): Recent progress in the research of external Al detoxification in higher plants: a mini review. Journal of Inorganic Biochemistry 97, 46-51.

[31] Arnetoli M., Montegrossi G., Buccianti A., Gonnelli C. (2008): Determination of organic acids in plants of *Silene paradoxa* L. by HPLC. Journal of Agricultural and Food Chemistry 56: 789-795.

[32] De Vos C.R., Lubb erding H.J., Bienfait H.F. (1986): Rhisosphere acidification as a response to iron-deficiency in bean plants. Plant Physiology 103: 695-702.

[33] Guerinot M.L., Yi Y. (1994): Iron: nutritious, noxious and not readily available. Plant Physiology 104, 815-820.

[34] Ohwaki Y., Sugahara K. (1997): Active extrusion of protons and exudation of carboxylic acids in response to iron deficiency by roots of chickpea (*Cicer arietinum* L.), Plant Soil 189: 49-55.

[35] Hoffland E., Van Den Boogaard R., Nelemans J., Findenegg G. (1992): Biosynthesis and root exudation of citric and alic acids in phopphatestarved rape plants. New Phytology 122: 675-680.

[36] Johnson J.F., Allan D.L., Vance C.P., Weiblen G. (1996): Root carbon dioxide fixations by phosphorous-deficient Lupus albus. Contribution to organic acid exudation by proteoid roots. Plant Physiology 112, 19-30.

[37] Dakora F.D., Philips D.A. (2002): Root exudates as mediators of mineral acquisition in law-nutrient environments. Plant Soil 245: 35-47.

[38] Li X.F., Ma J.F., Matsumoto H. (2002): Aluminium-induced secretion of both citrate and malate in rye. Plant Soil 242, 235-243.

[39] Pirenos M.A., Magalhaes J.V., Alves V.M.C., Kochian L.V. (2002): The physiology and biophysics of an aluminium tolerance mechanism based on root citrate exudation in maze. Plant Physiology 129: 1194-1206.

[40] Плешков Б.П. (1985): Прктикум по биохемиирастений, Издателство "Колос" Москва. (in Russian).

BIOGRAPHY

Slaviša Stamenković was born in Niš. He graduated in Biological sciences at Faculty of Sciences and Mathematics in Belgrade, completed M.S and Ph.D at Faculty of Biology, University of Belgrade. His main areas of interest are Ecology, Environmental protection and Lichenology.

He is currently working as an associate professor at the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš.

SADRŽAJ ORGANSKIH KISELINA U BILJKAMA DRUGE GODINE POSLE POŽARA

Stamenković Slaviša, Marković Marija, Stankov Jovanović Vesna, Mitić Violeta, Ilić Marija

Apstrakt: *Požar koji se dogodio u leto 2007. godine na planini Vidlič u Jugoistočnoj Srbiji uništio je velike površine bukovih i hrastovih šuma, suvih pašnjaka i kamenjara. Cilj našeg istraživanja je bio da ispitamo uticaj požara na sadržaj organskih kiselina u podzemnim i nadzemnim delovima biljnih vrsta Geranium macrorrhizum, Doronicum columnae, Glechoma hirsuta, Chelidonium majus i Primula veris druge godine posle požara. Kao kontrolni materijal korišćeni su uzorci biljaka sa površina koje nisu bile izložene dejstvu požara.*

Ključne reči: organske kiseline, požar, Geraniaceae, Asteraceae, Lamiaceae, Papaveraceae, Primulaceae.