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Effect of environment of the rutin content in leaves of *Fagopyrum esculentum* Moench.

S. Dražić[1], Đ. Glamočlija[1], M. Ristić[2], Ž. Dolijanović[3], M. Dražić[3], S. Pavlović[2], M. Jaramaz[4], D. Jaramaz[4]

[1]Serbian Association of Plant Breeders and Seed Production, Zemun, Serbia
[2]Institute for Medicinal Plant Research Dr Josif Pančić, Belgrade, Serbia
[3]University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia
[4]City of Zagreb, Zagreb, Croatia

ABSTRACT

The experiments were conducted in four locations in Serbia: Valjevo, Kucevo, Nova Pazova and Surduk in 2012 and 2013. A working hypothesis that growing conditions would not affect the rutin content in buckwheat leaves was set up. The content of rutin in leaves of buckwheat was determined by the HPLC/DAD technique, using the external standard calibration method. Out of the basic biometric parameters, average value, variance, and the variation coefficient were estimated. Results were processed by the analysis of variance for the factorial experiment. The analysis of variance showed existence of significant differences in the rutin content, over locations, but only in the first year of testing. The average content of rutin in the first and the second year was 3.30% and 2.61%, respectively, and in both years of testing it amounted on average to 2.82%. The variation in the rutin content was larger in the second year of testing. On the other hand, this variation was lower in the first year (5.3–29.0%) in comparison to the second year (16.2–28.6%). Relatively lower rutin contents were recorded in samples collected at lower-altitude locations.

Keywords: quercetin rutinoside; bioflavonoid content; natural sources of rutin; cultivar Golubica; environmental conditions

Rutin was discovered in 1842. The use of rutin as a medicinal agent for the treatment of vascular disorders characterized by abnormally fragile or permeable capillaries has stimulated interest in this compound (Campbell 1977).

The genus *Fagopyrum* consists of 15 annual and perennial species, of which only three are important for cultivation: common buckwheat (*Fagopyrum esculentum* Moench.), Tatar buckwheat [*Fagopyrum tataricum* (L) Gaertn.] and perennial buckwheat (*Fagopyrum cymosum* Meisn.) (Popovic et al. 2013a). Rutin was identified in *Fagopyrum esculentum*, *F. tataricum* and *F. cymosum*. It occurs in concentrations of 3–6% of the dry weight. Now, there is a trend back to natural sources and a higher concentration of rutin would make the processing of buckwheat more economically feasible.

Investigations of the chemical composition and active principles of buckwheat revealed that plant was rich in constituents, which could be used in contemporary phytotherapy. The most important constituents in buckwheat herb are flavonoids, while rutin, quercetin and hyperoside are the most abundant (Popovic et al. 2013a). Buckwheat has a significant content of rutin (quercetin-3-rutinosid) and other polyphenols. The content of rutin is listed among one of the most important characteristics from the viewpoint of nutrition (Campbell 1977). Anti-oxidative potential of buckwheat, in comparison to those recorded in the most used

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grains, is significantly higher, because of a high content of polyphenolic constituents, first of all rutin (Kreft et al. 2006).

Rutin is mainly located in flowers and green parts of buckwheat plants. Buckwheat samples may differ according to the rutin content in regard to the genotype, growing conditions and technology (Park et al. 2000).

Buckwheat is a plant species that can be cultivated under different growing conditions. It can be successfully cultivated especially in areas with chilly continental climate, and poor soils, the conditions prevailing in hilly-mountainous regions of Serbia. Due to these reasons, experiments were conducted during two years in lowland as well as mountainous regions of Serbia. A working hypothesis that growing conditions would not affect the rutin content in buckwheat leaves was set up.

Buckwheat ecology. Buckwheat is generally considered a short-day plant, thus flowering of buckwheat was stimulated by a short-day treatment. Furthermore, buckwheat is a short-duration crop (3–4 months) and requires a moist and cool temperate climate to grow. Buckwheat has little tolerance to frost and thus is usually grown at lower altitudes than is Tartary buckwheat (Popovic et al. 2013b). Buckwheat thrives well on sandy, well-drained soils. When moisture is limited, the plant is very sensitive to high temperatures and hot dry winds. This usually results in the loss of flowers, a condition called 'blasting'. Krotov (1963) reported that flowering at temperatures above 30°C was accompanied by desiccation of the fruit and lowering of yield. According to Gubbels (1978) the yield increased with high soil moisture although seed set remained essentially the same. This indicated that seed size increased with increased soil moisture content. Adequate soil moisture appears to be essential for high yields. If buckwheat is grown under fertile soil conditions it can lodge badly, especially if subjected to high winds and heavy rains (Campbell 1977).

The aim of the present study is an attempt to determine the effect of environment on the rutin content in leaves of buckwheat, especially growing conditions in different locations.

MATERIAL AND METHODS

Setting of buckwheat cultivation experiments. During 2012 and 2013, the experiments were conducted in four locations in Serbia: Valjevo, Kucevo (Kučevo), Nova Pazova and Surduk. Valjevo is situated around 100 km SW of Belgrade, surrounded with hills and mountains. The experiment was set up on the grey forest soil with the shallow arable layer of poor fertility in the hilly-mountainous location at the altitude of over 300 m a.s.l. Kučevo is situated about 140 km NE from Belgrade, where altitudes of 250–940 m a.s.l. prevail. There, the experimental field was located on the brown acidic soil on lower slopes of the Homolje Mts. (480 m a.s.l.). Nova Pazova is situated in the northern part of Serbia, and the southern part of the Pannonian Plain, 30 km far from Belgrade. The experiment there was set up on chernozem, 87 m a.s.l. Surduk is a village situated also in the county of Srem (as Nova Pazova), but on the bank of the Danube, around 40 km far from Belgrade. Agrochemical analyses showed that the experimental plot was set up on chernozem placed over the loess plateau type locations in which the cultivation experiments were set up, climate was moderately continental, with certain specificities, which were manifested through elements of sub-humid and micro-thermal climate. Average annual temperatures were balanced (11°C), while July was the warmest month (with average temperatures of around 21°C). The average annual precipitation sum was the highest in Valjevo (780 mm), while the corresponding levels in other locations were lower (500–600 mm).

Experiments were set up in four replications with the elementary plot size amounting to 16 m^2. Continuous sowing was done in the interrow distance of 50 cm with a manual single-breasted planter in the first decade of May in both years. Cropping practices were regularly applied. Unlike other cereals, the number of blossoms irregularly blooming on the stem can be found, thus the flowering period could be extended even over 30 days. Flowering started 25–30 days from sprouting. In this phase of ontogenesis, the rutin content increases, and the leaf samples collected in this phase-were subsequently air dried.

Statistical analysis. Out of the basic biometric parameters, average value, variance, and the variation coefficient were estimated. Results were processed by the analysis of variance for the factorial experiment (StatSoft 2010)

Determination of the rutin content in buckwheat leaves. The content of rutin in the aboveground part of buckwheat was determined by the

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HPLC/DAD technique, using external standard calibration method (ESD).

Extraction of the samples and preparation of test solutions. At the analytical balances 1 g of pulverized drug was weighed into 50 mL flat bottom flask, covered with 15 mL methanol (p.a.), equipped with reflux condenser, and kept boiling on the water bath for 30 min. After cooling, extract was filtrated into 25 mL volumetric flask, plant material and filter paper used were quantitatively returned to extraction vessel, and after addition of fresh 10 mL of methanol extraction was repeated for further 30 min. After cooling the second extract was filtrated into the 25 mL volumetric flask (already containing the first one) and volumetric flask was filled with methanol up to the mark.

High performance liquid chromatography (HPLC). The analysis was carried out on Agilent Technologies A-1200 HPLC (Waldbronn, Germany), equipped with ALS, autoinjector and Diode Array Detector (DAD), on the Lichrospher 100 RP-18e column (250 mm·× 4 mm; 5 μm) Agilent Technologies, in 3 repetitions. Working solutions (20 μL) were chromatographed in gradient mode, at the flow rate of 1 mL/min, starting from the water-methanol mixture 70:30, whose composition has linearly changed to the opposite ratio in 10 min, and kept unchanged in additional 5 min. Chromatograms were recorded at the wavelength of 361 nm (by bandwidth of 4 nm). In described chromatographic conditions retention time of rutin was around 8.7 min. Processing of the raw chromatographic data was accomplished by the usage of instrument's ChemStation software (Rev. A.09.03, Santa Clara, USA).

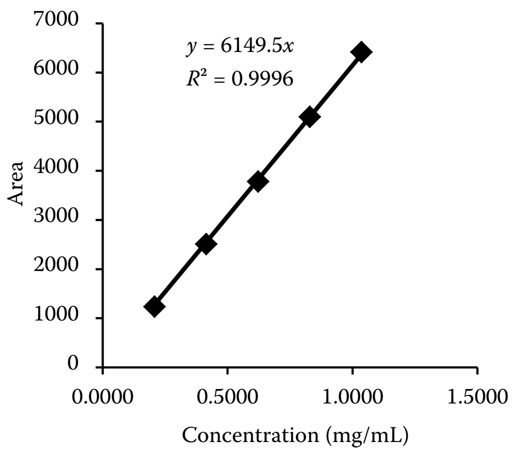

Figure 1. Calibration curve for the determination of rutin (external standard calibration method)

Calibration (ESD). Five-point calibration in 3 repetitions was accomplished with reference solutions containing 0.21, 0.41, 0.62, 0.82, and 1.04 mg/mL of rutin, whose stock solution was prepared by dissolving rutin analytical standard (Serva, Heidelberg, Germany) in methanol. Working calibration curve for determination of rutin by the ESD method is presented in Figure 1.

Quantification of rutin in tested samples. Measuring solution of tested samples was chromatographed in 3 repetitions in the sequence, as described earlier, and the rutin content was determined from the previously prepared calibration curve by ESD.

RESULTS AND DISCUSSION

The comparison of calculated and tabular *F*-values shows that variances in locations and years were both significant and insignificant, respectively. If the latter was found to be significant, it would be justified to point at possible combined influence of the year and the location on the rutin content. As it was not found in our experiment, separate conclusions can be derived for treatments of each of the tested factors. On the basis of the *F*-test results, having in mind significant differences among locations, the set hypothesis in general was rejected (Table 1).

The determination of the presence and absence of statistically significant differences among average values of the rutin content revealed very reliable differences among locations in 2012. The rutin content varied from 2.17% in the location of Surduk to 3.43%** in the location of Valjevo. Reliably higher average values were recorded in two remaining locations. Simultaneously, the co-

Table 1. Analysis of variance of factorial experiment

Source of variation	*df*	MS	*F*	F_{tab}	
				0.05	0.01
A (year)	1	1.410	3.53	4.3	7.8
B (location)	3	1.392	3.48*	3.0	4.7
AB (interaction)	3	0.442	1.09	3.0	4.7
Error	24	0.404	–	–	–
Total	31	–	–	–	–

*P = 0.05

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Table 2. Average values and variability of the rutin content

Location	2012						2013					
	average	min.	max.	width	variance	*CV*	average	min.	max.	width	variance	*CV*
Valjevo	3.43**	2.78	3.95	1.17	0.181	12.4	2.86	1.95	3.95	2.00	0.215	16.2
Kucevo	3.29**	2.82	3.72	0.90	0.155	12.0	2.96	2.24	4.30	2.06	0.474	23.3
Nova Pazova	3.24*	2.96	3.41	0.45	0.030	5.3	2.29	1.77	3.41	1.64	0.423	28.6
Surduk	2.17	1.60	2.55	0.95	0.397	29.3	2.34	1.75	3.01	1.26	0.270	22.2

*$P < 0.05$; **$P < 0.01$; *CV* – coefficient of variation

efficient of variation in tested locations ranged from 5.3–29.0% (Table 2).

Average values of the rutin content in 2013 were relatively lower in comparison to the values obtained in the previous year, but more balanced (2.29–2.96%). The range of variation of average values varied from 16.2–28.6% and, in general, exceeded those recorded in the first year of testing (Table 2).

On the whole, the rutin content was relatively higher in buckwheat leaf samples from plants cultivated in hilly-mountainous regions (Valjevo, Kucevo). It was already established in previous studies, aimed at defining environmental and related factors which could affect the rutin content in the buckwheat leaves (Krotov 1963, Campbell 1977, Gubbels 1978).

It should be pointed out that variations in the rutin content are not appreciated if raw material of standard and homogeneous quality is needed. The range of variations presented in Tables 1 and 2 points to possible differences in the tested buckwheat population.

Minimal and maximal values of the rutin content in buckwheat were recorded in different locations and different years. In the second year of testing broad variability was registered (Table 2), pointing to the presence of plants with expressed values of that characteristic (Max) in comparison to the average population, which depends upon the influence of different factors (genetic and non-genetic ones).

Buckwheat is considered as the best source of rutin in human nutrition. However, the rutin content varies considerably, depending on cultivar, as well as agro-ecological conditions in cultivation (Jiang et al. 2007). Although the rutin content in buckwheat leaves has been studied rather long, information/data about varietal differences are modest. McGregor and McKillican (1952) suggested that no significant differences in rutin percentages could be detected in 17 strains tested for a 3-year period. Differences between years, however, were highly significant indicating the possibility that the rutin content in leaves could fluctuate because of environmental conditions.

Hurusawa and Harada (1961), who determined the rutin content in seeds of strains collected from various areas of Japan, did not observe any differences among the strains. A study conducted by Suzuki et al. (1987) indicated that the rutin content fluctuated with light intensity. The grain, however, contains one or more dyes which, as a result of fluorescence are photodynamically active. Ohsawa and Tsutsumi (1993) reported that the varietal differences in the rutin content in seeds were significant in 12 strains and varieties mainly from Japan.

Kitabayashi et al. (1995) evaluated the varietal differences and heritability of the rutin content in the seed and leaf in buckwheat. The results showed that the rutin content in seeds was one of the traits with a relatively high heritability among the main traits in common buckwheat, and that the rutin content in leaves was a trait comparatively affected by environmental conditions.

In conclusion, the analysis of variance shows the existence of significant differences in the rutin content in locations, but only in the first year of testing. The average content of rutin in the first and the second year was 3.30% (2.17–3.43%) and 2.60 (2.29–2.96%), respectively, while it amounted to 2.82% in both years of testing.

The variations for the rutin content were larger in the second year of testing (1.26–2.06). According to variability, variances and factors of variances, variation in the rutin content was lower in the first year (5.3–29.0%) in comparison to the second year (16.2–28.6%). Relatively lower rutin contents

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were recorded in samples collected in locations situated in lower altitudes.

In the first year the analysis of variance showed significant differences in the rutin content between locations with higher and lower-altitude, but also in the second year such tendency could be seen with higher levels of rutin in the buckwheat leaves cultivated in higher-altitude locations. This could be caused by higher sun irradiation.

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Correspoding author:

Mr. sc. Miroslava Jaramaz, univ.spec.oecol., mag.ing.agr, City of Zagreb, Public Procurement Office, Trg Stjepana Radića 1, 10 000 Zagreb, Croatia; e-mail: mima22@net.hr