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Effect of mist, irrigation and plant density levels on rutin content of common buckwheat (*Fagopyrum esculentum* Moench.)

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Abstract
To determine the effects of some environmental factors on rutin content of common buckwheat, an experiment was conducted in split-split-plot, based on RCBD design with three replications. The relative humidity (mist and non-mist treatments), irrigation (20 and 40 mm/m^2 treatments) and plant density (33.3 and 100 plants/m^2 series) were performed. Results showed that the rutin content was significantly affected by single and interaction effects of the factors studied at the 0.01 level of probability, so that, in the case of rutin content, mist and irrigation of 20 mm/m^2 and 100 plant/m^2 treatments showed high concentration. The greatest content of rutin was obtained from treatments comprising non-mist, 20 mm/m^2 water irrigation and 100 plants/m^2.

Key words: Buckwheat, rutin, mist, plant density.

Introduction
Common buckwheat (*Fagopyrum esculentum* Moench.) is an agronomic species of the Polygonaceae family and is ranked as the second crop for many countries. Also, it has yet persisted through centuries of civilization and enters into the agriculture of nearly each country where cereals are cultivated. Main producer countries of it are China, Russian Federation, Ukraine and Kazakhstan. Although, the crop is not a cereal, its seeds (strictly achenes) are usually classified among the cereal grains because of their similar usage. The small leaves and shoots are used as leafy vegetables. In addition, the flowers and green leaves are used for rutin extraction in medicinal usage [1, 2].

Buckwheat leaves usually contain 3–8% rutin [3] and it has a potential source for industrial extraction of this compound. Rutin and its hemisynthetic derivatives cause different pharmacologically demonstrated effects like normalization of increased vascular permeability and fragility, oedema protection, hyaluronidase inhibition [4], antioxidant [5, 6], hypotensive and anti-inflammatory effects [7]. It is possible that leaf rutin content could fluctuate because of environmental conditions. A researcher indicated the effect of light intensity on rutin content [8]. The aim of the present research was to study the influence of different levels of irrigation, relative humidity and plant population density on rutin content of common buckwheat.

Materials and Methods
The experiment was established in the experimental field of the Dept. of Horticulture, University of Tehran. The climate is classified as a semi-arid type with average annual rainfall of 224.4 mm. The texture of soil was classified as clay loam (clay 32%, sand 25.48% and silt 42.52%). The experimental design consisted of three treatments such as relative humidity (M), irrigation (I) and plant density (D) which were performed to determine rutin content from dry matter. The experimental layout was a randomized complete block design (RCBD) in a split-split-plot arrangement with two levels of relative humidity as a whole-plot treatment [mist (m_1) and non-mist (m_2)], two levels of irrigation as a split-plot arrangement [20 mm/m^2 (i_1) and 40 mm/m^2 (i_2)] and split-split-plot was two series of plant densities [33.3 plants/m^2 (d_1) and 100 plants/m^2 (d_2)] which were replicated three times. The seeds of common buckwheat were planted in experimental plots (1 m × 1 m) with inter-plot space of 50 cm. The plots were watered with flood irrigation until seedlings emergence. Thinning was carried out in 2-3 leaves stage. Afterwards, they were watered with 3-day intervals until 4-5 leaves stage. Plant densities were the mean number of plants per m^2 after emergence. Thereafter, irrigation and relative humidity treatments were performed. To determine the time of irrigation, a tensiometer was placed at the depth of 15 cm in the plot with irrigation treatment of 40 mm/m^2. Time of mist application was 4 a.m. to sun-up (that is similar to climate with latent rainfall). All plants (in two series of densities) were cut and collected separately (leaf and stem segments) at full-bloom stage and dried at 30-50ºC. Samples were grinded, 30 ml of methanol-

formamid solution (80/20 v/v respectively) was added to 3 g of grinded samples, stirred for 20 min and filtered after it. Obtained solutions were removed to new tube. This action was repeated and the final volume of solution in tube was recorded. The rutin content in each processed product was measured by high performance liquid chromatography (HPLC) system (Table 1) based on a method of Kim *et al.* [9] and expressed on dry weight basis.

Table 1. HPLC condition for rutin analysis.

Apparatus	Character
Detector	Absorbance Detector Waters 48
Wave length	280 nm
Column	Partisil 10 ODS_2 250x4/6mm
Mobile phase	0.1 M methanol-phosphoric acid
Flow rate	1 ml/min.
Column temp.	25°C
Injection volume	10 μl of standard sample

Results and Discussion

All treatments had significant influence on rutin content ($p<0.01$) as seen in Table 2. In addition, interaction effects significantly affected all studied factors. In the case of simple effects, high rutin content was obtained from mist (m_1), irrigation of 20 mm/m^2 (i_1) and 100 plant/m^2 (d_2) treatments. The interaction effects of $m_1 \times i_1$, $d_2 \times i_1$ and $m_1 \times d_2$ showed high rutin content separately. In addition, the highest rutin content was recorded for $m_1 \times i_1 \times d_2$ (Table 3), $m_2 \times i_1$ (Table 4), $m_2 \times d_2$ (Table 4), $d_2 \times i_1$ (Table 4) and $m_2 \times i_1 \times d_2$ (Table 3) obtained from leaf segments.

Rutin (quercetin-3-rutinoside) is a flavonoid compound and glucose molecules possess in its skeleton as a simple sugar. Due to this reason, any condition which results to last stomata opening in buckwheat leaves causes increase of the carbohydrate content and, consecutively, increase of the rutin content. Air humidity is also a decisive factor to govern water loss. These considerations illustrate the important function of stomata for the gas exchange of the leaves. The regulation of the stomatal aperture determines how high the rate of CO_2 assimilation may be without the plant losing too much essential water [10]. Furthermore, relative humidity by affecting on dew creation on plant leaves, especially in drought stressed plants, retardates the new stress period and helps to prepare water requirements from leaves. These results are in line with the finding [11] that rutin content and dry matter were higher under greenhouse conditions compared to field cultivation. About irrigation treatment, it can be concluded that a majority of phenolic compounds are formed from phenylalanine (a product from shikimate pathway) by phenylalanine ammonia lyase (PAL) that is one of the most

Table 3. Interaction effects of air humidity, irrigation and plant density on rutin content (%) in different parts of plant.

Treatment			Rutin content (%)		
Relative humidity	Irrigation (mm/m²)	Plant density (plants/m²)	Leaf	Stem	Total
Mist	20	33.3	2.516 e	2.106 i	2.311 c
		100	3.352 b	2.349 g	2.851 a
	40	33.3	2.336 h	1.110 n	1.723 g
		100	1.448 l	1.617 j	1.533 h
No mist	20	33.3	2.942 c	1.125 m	2.033 d
		100	3.695 a	0.940 o	2.317 b
	40	33.3	2.822 d	1.118 m	1.970 f
		100	2.416 f	1.543 k	1.980 e

intensively studied enzymes of plant secondary metabolism. Therefore, every brief drought stress results to yield mRNA (which codes the PAL). So, formation of PAL and other secondary metabolites will be increased by such conditions. No significant differences in rutin percentage could be detected in 17 buckwheat strains tested for a 3-year period [12]. Differences between years, however, were significant.

It is possible that leaf rutin content could fluctuate because of environmental conditions. Furthermore, the content of rutin fluctuates with light intensity [8]. In addition, it can be inferred that, low irrigation rate resulted in decreasing of dry matter and rutin content increased in weight unit of dry matter of vegetative parts, accordingly. Furthermore, the higher net assimilation rate and larger leaf area, which accounted for the higher crop growth rate of the low moisture plot, were due both to avoiding suppression of the photosynthetic rate and leaf expansion owing to water stress and to maintaining high rates of leaf photosynthesis and a large leaf area during leaf senescence[13]. The results of the present study are in contrast with the findings[14] that water stress decreased stem height and leaf expansion and successively decreased photosynthetic rate in buckwheat plants which resulted to reduced rutin content. Plant density stress takes place in high population density, and reduction in dry matter from each plant is attributed to the high plant density stress. Increasing of yield with alternate or narrow rows, particularly at high populations, can be explained by greater solar energy interception, shading the soil surface more completely during the early part of season [15, 16]. Contrary to our results, it has been demonstrated [17] that total phenolic concentration of birch seedlings declined by increasing plant density. In addition, it can be noted that light quality and quantity change under different plant density. The sweet buckwheat cultivars had a great number of stomata in the middle and bottom of leaves under blue light but a great number of stomata in the upper part under red light [18].

Table 2. Results of two-way ANOVAs showing the effects of different factors on rutin content (%) of common buckwheat.

	Source of variation						
	M	I	M x I	D	D x M	Dx I	Dx I x M
df	1	1	1	1	1	1	1
MS of rutin content	0.01**	3.992**	1.699**	0.31**	0.002**	0.757**	0.156**

M Relative humidity, I Irrigation, D Plant density, MS Mean square, ** significant at p ≤ 0.01.

Table 4. Interaction effects of air humidity and irrigation, air humidity and plant density, and irrigation and plant density treatments on rutin content (%).

		Treatment							
		Irrigation (mm/m²)				Plant density (plants/m²)			
		20		40		33.3		100	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Relative	Mist	2.934b	2.227d	1.892e	1.364f	2.426c	1.608f	2.400d	1.983e
humidity	No mist	3.318a	1.032h	2.619c	1.331g	2.882b	1.121h	3.055a	1.242g
Irrigation	20	...	...	...	...	2.729b	1.615f	2.579c	1.114h
(mm/m²)	40	...	...	...	...	3.523a	1.644e	1.932d	1.580g

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