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Correlation between endogenous elements and development of hollowing in the root of radish (*Raphanus sativus* L.) cultivars

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Abstract

The present investigation was carried out to discover the relationship between endogenous element content determined by Wavelength-Dispersive X-Ray Fluorescence (WDXRF) spectrometer and development of hollowing in the radish root by varying cultivars. Four radish (*Raphanus sativus* L.) cultivars ('Siyah', 'Beyaz', 'Antep' and 'Iri Kirmizi') were used as plant material in this work. Cultivars varied significantly in their resistance to development of hollowing. 'Siyah' was found to be more resistant than 'Antep', 'Beyaz' and 'Iri Kirmizi'. Significant correlations between development of hollowing and endogenous element content of the radish cultivars used in this study: N ($r = 0.701$, $P < 0.01$), Na ($r = 0.703$, $P < 0.01$), Cl ($r = 0.672$, $P < 0.01$), Ca ($r = 0.544$, $P < 0.01$), Mn ($r = 0.414$, $P < 0.05$), Zn ($r = 0.567$, $P < 0.01$), Br ($r = 0.393$, $P < 0.05$) and Ba ($r = 0.528$, $P < 0.01$). In addition to simple correlation coefficients of hollowing and endogenous elements; similar results were obtained in stepwise multiple regression analysis. Regression equation [Y = 35.104 + (49.94 x Na) – (2935.93 x Ba) – (4.29 x N) – (20978.88 x Sn)] and R square (0.823, $P < 0.01$) were determined. According to results of this study, the providing of extra or deficient plant nutritional elements in soil or growing media such as especially N, Cl, Ca, Mn, Zn, Br, Sn and Ba may affect development of hollowing in the case of cvs. 'Beyaz', 'Antep' and 'Iri Kirmizi'. However, cv. 'Siyah' may not be affected by the endogenous elements because of its resistance and thus hollowing does not develop.

Key words: *Raphanus sativus* L., radish, hollowing, element content, WDXRF.

Introduction

Radish, *Raphanus sativus* L., is a member of the *Brassicaceae* family. They are cool season, fast maturing, easy to grow annual or biennial herbaceous plants grown for their roots and they do not grow well in hot and dry weather (Decoteau, 2000). Although, it is generally considered hardy in cold temperatures, its optimum growth temperature is 14–16ºC (Günay, 2005). In addition, they are cultivated almost all year round throughout Turkey with approximately 170.000 tons of annual production. However, it is known that hollowing is one of the biggest physiological disorders and inhibits their production for fresh marketing in Turkey and other producer countries.

This physiological disorder, hollowing, most frequently occurs when radishes are sown during midsummer, and it causes serious problems for growers (Kano, Fukuoka, 1995). Development of hollowing originates from the intercellular spaces formed in the central region of the pith (Kano, 1989); spaces that are normally filled with large cellular elements as some of the protruding cells formed within the space continue cell division and enlargement (Fukuoka, Kano, 1992).

Several investigations have reported about occurring or suppressing of development of hollowing on radish roots. Kano (1989), Kano and Fukuoka (1995) reported that anatomical observations of the development of hollowing revealed that heat treatment, in which the soil temperature was kept around 35°C during the middle of the growth period caused the intercellular spaces to coalesce into longitudinal cavities. Other than this, the frequency of hollow root is lowered by spraying plants with a high concentration of the sodium salt of alpha-naphthaleneacetic acid (NAA), mainly due to the severe suppression of the rate of root growth (Kano, 1987). Kano and Fukuoka (1996 a) to see the effects of 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) on the development of hollowing in the roots of Japanese radishes, solutions of 5 mg l^{-l} of CPPU were sprayed on the leaves of plants in different times after sowing. The size of the hollow cavity in the roots of Japanese radishes became significantly smaller with CPPU treatment, in contrast to that of the control. Similarly, Kano and Fukuoka (1996 b) reported that the production of endogenous cytokinin in the roots of radish cultivars, prone to hollowness, was reduced at higher soil temperatures; while in cultivars that were resistant to hollowness cytokinin production was higher.

Physiological disorders in vegetable production can be associated with nutritional imbalances within the plant or non-optimal environmental conditions during the growing season (Decoteau, 2000). For example, on soils deficient in boron, symptoms appear as curling and rolling of the leaves, deformed foliage, brown curds or flower buds, and hollow stem centres in broccoli and cauliflower (Swiader et al., 1992). Examples of relations with elements and physiological disorders can be increased such as magnesium deficiency and Chlorosis, calcium deficiency and Black Heart in celery; extra nitrogen and Catfacing in tomato or boron and magnesium deficiency and poorly flavored in muskmelons (Swiader et al., 1992).

Although many researchers have shown relations between development of hollowing and different factors, such as soil temperature (Kano, 1989; Kano, Fukuoka, 1995), plant density (Kano, Fukuoka, 1991), endogenous cytokinin activity, suppressive effect of CPPU or NAA (Kano, 1987; Kano, Fukuoka, 1996 a, b) have been reported and most researches do not cover the relationship between endogenous element content and hollowing in radish.

Therefore, the present investigation was carried out to discover the relationship between endogenous element content determined by Wavelength-Dispersive X-Ray Fluorescence (WDXRF) spectrometer and development of hollowing in the radish root by varying cultivars.

Material and methods

The study was conducted in field conditions at Atatürk University, College of Agriculture, Erzurum, Turkey, in 2004 and 2005. In this work, four radish (*Raphanus sativus* L.) cultivars ('Siyah', 'Beyaz', 'Antep' and 'Iri Kirmizi') were used as plant material.

The soil type of the experimental area was a loam, Lithosol (according to FAO) with neutral pH. It had 1.80% organic matter, 3.69 kg ha^{-1} P_2O_5 and 278 kg ha^{-1} K_2O.

Seeds were sown on plots of 6 m^2 in field on 12 July in both 2004 and 2005, in rows 280 cm long, at a separation between rows of 40 cm and between plants of 20 cm (Güvenç, 1995; Ara et al., 1999). The plants were thinned after the emergence when they formed 4–5 leaves. The plants were irrigated with furrow irrigation. During the development phases, all of the plant care practices were applied in each plot.

All plots received 100 kg ha^{-1} N and 80 kg ha^{-1} P_2O_5 as calcium ammonium nitrate and triple super phosphate, respectively (Thapa et al., 2003). All of the P_2O_5 and half of the nitrogen fertilizer were applied uniformly prior to planting onto soil surface by hand and incorporated. The remaining half of the nitrogen was given 20 days after emergence (Srinivas, Naik, 1990).

Twenty plants were sampled in 45 plants and hollowing was measured. To measure the degree of hollowing, roots were cut lengthwise along the central axis 80 days after sowing and the maximum cross and length-wise dimensions of any cavities were measured. Any root with a cavity larger than 1.5–2 cm^2 was classed as a hollow root and the ratio of hollow roots was calculated for each cultivar (Fukuoka, Kano, 1997). After this process, hollow roots and other roots were dried separately for all cultivars in an oven at 67.5ºC and this material was used for all XRF analyses.

A WDXRF spectrometer (Rigaku ZSX-100e with Rhodium target X-ray) was used for the analysis of the roots element content (Figure). This instrument was controlled by a computer using ZSX software ("Rigaku", Japan). The ZSX-100e WDXRF spectrometer characteristics included; analysis of elements from B to U, 4 kW 70 kV end-window X-ray tube, micro area mapping down to 0.5 mm, up to five primary beam filters, 4 analyzing crystals [LiF, Ge (111), PET (002) and TAP (100)], and eight limiting area diaphragms, optional secondary collimators, automatic sample changer, compact design and multi window, multi-function fundamental parameters software.

Dry roots were ground in a porcelain mortar. The ground material was used for all XRF analyses. In the XRF laboratory, 2 g weight, 2.5 mm thickness and 35 mm diameter pellets were produced by applying 15 t of pressure with a spex press (Cat. B25). After the preparation of the pellets, they were incubated at 80ºC for 20 min to remove all moisture and were then used for element determination. LiF, Ge (111), PET (002) and TAP (100) crystals were used to separate the X rays coming from the pellets in WDXRF spectrometer. At the end of the WDXRF analysis, the major, minor and trace element contents of hollow and non-hollow radish roots were determined as mass percentage (wt%) (wt% = element mass x 100 / total mass).

The laboratory and field experiments were conducted as randomized complete block designs, with four replicates. Arcsine transformation was made in percentage data before statistical analysis. Data obtained were subjected to *Anova* and differences between means compared using Duncan's multiple range test. Additionally, stepwise multiple regression and the correlation coefficient (r) between hollowing and endogenous elements were determined.

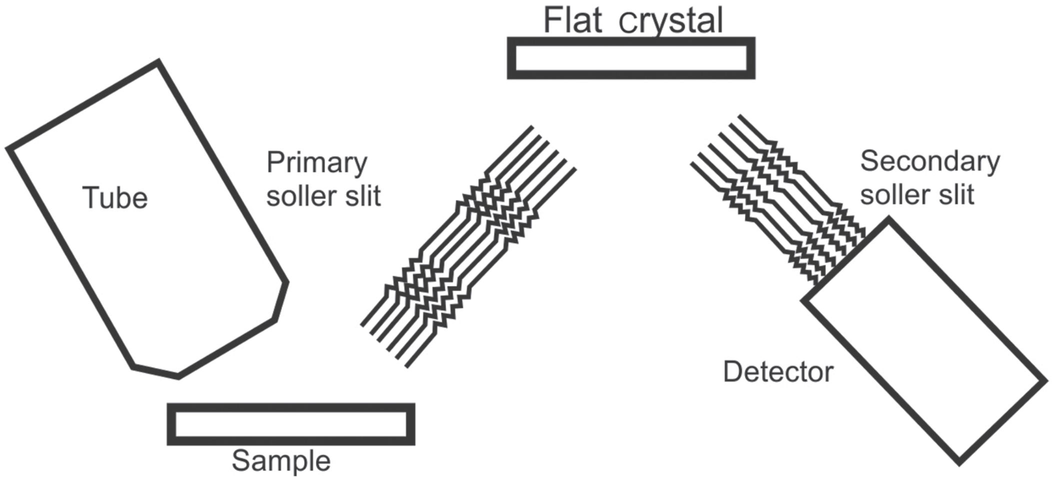

Figure. The detection geometry of the spectrometer

Results and discussion

Cultivars varied significantly ($P < 0.01$) in their resistance to development of hollowing (Table 1). 'Siyah' was found to be more resistant than 'Antep', 'Beyaz' and 'Iri Kirmizi'. In other words, it can be clearly said that 'Siyah' was resistant cultivar to hollowing in view of the fact that it did not develop in 'Siyah' both 2004 and 2005 although hollowing for 'Antep', 'Beyaz' and 'Iri Kirmizi' roots were determined in different ratios (Table 1).

Table 1. Development of hollowing in the roots of different radish cultivars (%)

Cultivars	2004	2005	Mean
'Antep'	26.92 a**	35.35 a**	31.13 A**
'Beyaz'	32.26 a	34.73 a	33.49 A
'Iri Kirmizi'	22.33 a	24.94 a	23.63 A
'Siyah'	1.28 b	1.28 b	1.28 B

Note. ** – indicated that 0.01 probability level ($P < 0.01$).

Alan et al. (1995) and Kaymak (2006) reported that although hollowness did not occur in 'Siyah', development of hollowing in other cultivars varied with sowing time both spring and summer sowings. At the same time, Özzambak et al. (1996) obtained that both sowing time and late harvest affected the development of hollowing and hollowing increased as the harvest was delayed. Besides, in this study, sowings were made in mid July and the maximum air and soil temperature (5 cm depth) of experimental area were on average 28–30ºC and 25–26ºC respectively, in experimental years. These temperatures might have affected the development of hollowing as it was reported that hollowing often occurs when radishes are sown during midsummer (Kano, Fukuoka, 1995). Results of the study were similar to previous studies conducted by Alan et al. (1995), Kano, Fukuoka (1995), Özzambak et al. (1996), Kaymak (2006).

As it was shown in Table 2, major and minor elements were varied according to the cultivars and differences among cultivars were statistically significant at 0.01 probability level in both years of experiment. Data about the element content (major, minor and trace) could not be collected for 'Siyah' due to the fact that development of hollowing was not seen in all experimental years (Table 1). The major elements with contents of ≥ 0.1 wt% in hollow and non-hollow roots were N and K. The amounts of N in non-hollow roots ranged from 2.79 wt% ('Antep') to 4.00 wt% ('Iri Kirmizi') in experimental years. The amounts of K were 3.12 wt%, 4.72 wt%, 5.74 wt% and 7.23 wt% in 'Siyah', 'Antep', 'Beyaz' and 'Iri Kirmizi', respectively. Nitrogen contents were less in hollow roots than non-hollow roots in 2004 and 2005. However, K contents were higher in hollow roots than non-hollow roots except for 'Beyaz' and 'Iri Kirmizi' in 2005.

Minor elements with content values of less than 0.1 wt% in all samples in both experimental years were: Na, Mg, P, S, Cl and Ca (Table 2). We found similar results in minor elements as found in major ones. Minor element content of roots was higher in hollow roots than non-hollow roots in all experimental years, except P and S in 'Beyaz' and 'Iri Kirmizi'.

Table 2. The amounts of major and minor elements in different radish cultivars (wt%)

			Cultivars				
			'Antep'	'Beyaz'	'Iri Kirmizi'	'Siyah'	
			means of 2004 and 2005				*P* values
Non-hollow roots	major elements	N	2.79	3.29	4.00	3.25	0.387
		K	4.72	5.74	7.23	3.12	0.000
	minor elements	Na	0.25	0.31	0.21	0.13	0.000
		Mg	0.27	0.32	0.40	0.21	0.010
		P	0.45	0.57	0.55	0.37	0.080
		S	0.62	0.69	0.80	0.62	0.006
		Cl	0.26	0.35	0.42	0.18	0.001
		Ca	0.51	0.63	0.81	0.48	0.000
Hollow roots	major elements	N	2.16	1.36	1.98	–^{z}	0.258
		K	6.52	6.20	7.75	–	0.330
	minor elements	Na	0.34	0.62	0.38	–^{z}	0.006
		Mg	0.38	0.34	0.38	–	0.534
		P	0.57	0.50	0.43	–	0.346
		S	0.73	0.58	0.72	–	0.200
		Cl	0.46	0.54	0.44	–	0.178
		Ca	0.77	0.84	0.90	–	0.399

Note. '–'^{z} – indicated that development of hollowing was not seen, i.e. data could not be collected.

Trace elements were varied according to the cultivars for both experiments. The differences between cultivars were statistically significant at 0.05 and 0.01 probability levels for both years (Table 3). Trace elements among all samples were: Mn, Fe, Cu, Zn, Al, Ti, Ni, Br, Rb, Sr, Sn and Ba. The values of trace elements were ranging between 0.0003 wt% and 0.026 wt% in all samples (Table 3).

The WDXRF analyses allowed the determination of major, minor and trace elements which changed depending on cultivars (Table 2 and 3) in hollow and non-hollow roots. Decoteau (2000) reported that radishes had 30 mg 100 g^{-1} Ca, 31 mg 100 g^{-1} P, 1 mg 100 g^{-1} Fe and 322 mg 100 g^{-1} K. Additionally, Peirce's (1987) results certify Decoteau (2000) findings. Similarly, Salerno et al. (2005) reported that radish cultivars had 7252 mg kg^{-1} Ca, 80140 mg kg^{-1} K, 9300 mg kg^{-1} Mg, 6982 mg kg^{-1} P, 202 mg kg^{-1} Fe, 9 mg kg^{-1} Cu, 31 mg kg^{-1} Mn, 70 mg kg^{-1} Zn and 39700 mg kg^{-1} N in 1 kg dry matter and elements. Our present results on elements were obtained from non-hollow roots in accordance with previous studies (Peirce, 1987; Decoteau, 2000; Salerno et al., 2005). However, we did not find any detailed reports in other researches about the element content in hollow roots of radishes.

Table 3. The amounts of trace elements in different radish cultivars (wt%)

	Trace elements	Cultivars				
		'Antep'	'Beyaz'	'Iri Kirmizi'	'Siyah'	
		means of 2004 and 2005				*P* values
Non-hollow roots	Mn	0.002	0.002	0.003	0.002	0.001
	Fe	0.018	0.020	0.019	0.034	0.000
	Cu	0.0010	0.0007	0.0008	0.0008	0.596
	Zn	0.0020	0.0023	0.0027	0.0023	0.040
	Al	0.024	0.019	0.025	0.045	0.000
	Ti	0.0016	0.0018	0.0015	0.0034	0.003
	Ni	0.0013	0.0010	0.0009	0.0008	0.120
	Br	0.0030	0.0034	0.0036	0.0015	0.000
	Rb	0.0028	0.0033	0.0038	0.0013	0.001
	Sr	0.0037	0.0043	0.0048	0.0030	0.045
	Sn	0.0008	0.0007	0.0005	0.0005	0.035
	Ba	0.0058	0.0058	0.0054	0.0049	0.418
Hollow roots	Mn	0.003	0.003	0.003	–^{z}	0.975
	Fe	0.024	0.034	0.019	–	0.105
	Cu	0.0008	0.0007	0.0006	–	0.274
	Zn	0.0040	0.0040	0.0084	–	0.014
	Al	0.025	0.027	0.017	–	0.180
	Ti	0.0022	0.0030	0.0013	–	0.045
	Ni	0.0010	0.0009	0.0009	–	0.767
	Br	0.0037	0.0042	0.0036	–	0.718
	Rb	0.0029	0.0025	0.0025	–	0.851
	Sr	0.0048	0.0044	0.0047	–	0.910
	Sn	0.0003	0.0007	0.0006	–	0.000
	Ba	0.0045	0.0042	0.0036	–	0.689

Note. '–'^{z} – indicated that development of hollowing was not seen, i.e. data could not be collected.

It was mentioned that physiological disorders in vegetable production can be associated with nutritional imbalances within the plant. Similarly, as shown in Table 4, significant correlations between development of hollowing and endogenous element content of the radish cultivars were evident. N (r = 0.701, P < 0.01), Na (r = 0.703, P < 0.01), Cl (r = 0.672, P < 0.01), Ca (r = 0.544, P < 0.01), Mn (r = 0.414, P < 0.05), Zn (r = 0.567, P < 0.01), Br (r = 0.393, P < 0.05) and Ba (r = 0.528, P < 0.01) were significantly and positively correlated with hollowing (Table 4).

In the present study, high N content promoted hollowness concomitant with Na, Cl, Ca, Mn, Zn, Br and Ba. Thus we can conclude that higher N contents in radish roots may cause cellular enlargement. Because, although it is known that the hollow cavity in the root is formed by intercellular spaces (Fukuoka, Kano, 1992), these spaces are usually filled with large and globular cellular elements (Kano, Fukuoka, 1992).

In addition to simple correlation coefficients of hollowing and endogenous elements; similar results were obtained in stepwise multiple regression analysis (Table 5). In other words, near direct effect of endogenous elements, indirect effect of endogenous elements was determined. Regression equation and R square (0.823, P < 0.01) were shown in Table 5. According to stepwise multiple regression analysis Na, Ba, N and Sn clearly and significantly affected the development of hollowing in radishes.

Researchers have shown relations between hollowing and soil temperature (Kano, 1989; Kano, Fukuoka, 1995), plant density (Kano, Fukuoka, 1991), endogenous cytokinin activity, suppressive effect of CPPU or NAA (Kano, 1987; Kano, Fukuoka, 1996 a, b). Similarly, the statistical results clearly revealed that endogenous elements had a significant effect on the development of hollowing in the root of radish and that the development of hollowing was closely related to extra or deficient of elements (Table 4 and 5).

Table 4. Simple correlation coefficients of hollowing and endogenous elements in radish cultivars (average of 2004–2005)

	HR[1]	N	K	Na	Mg	P	S	Cl	Ca	Mn	Fe	Cu	Zn	Al	Ti	Ni	Br	Rb	Sr	Sn
N	–0.701**																			
K	0.354	0.025																		
Na	0.703**	–0.493**	0.550**																	
Mg	0.300	0.079	0.760**	0.357																
P	0.034	0.339	0.549**	0.323	0.743**															
S	−0.128	0.430*	0.696**	0.042	0.660**	0.691**														
Cl	0.672**	−0.363	0.571**	0.632**	0.788**	0.438*	0.150													
Ca	0.544**	−0.175	0.903**	0.661**	0.792**	0.453*	0.534**	0.749**												
Mn	0.414*	0.003	0.859**	0.601**	0.700**	0.581**	0.676**	0.513**	0.873**											
Fe	0.191	−0.095	−0.085	0.373	−0.133	0.184	0.124	−0.095	0.060	0.282										
Cu	−0.226	0.460*	−0.001	−0.067	−0.100	0.179	0.046	−0.291	−0.098	0.123	0.058									
Zn	0.567**	−0.456*	0.446*	0.320	0.279	−0.177	0.084	0.385*	0.529**	0.335	−0.173	−0.270								
Al	−0.216	0.225	−0.399*	−0.177	−0.340	−0.007	0.095	−0.470*	−0.302	0.001	0.814**	0.235	−0.400*							
Ti	0.108	−0.155	−0.314	0.185	−0.221	0.070	−0.008	−0.160	−0.110	0.071	0.905**	0.031	−0.261	0.831**						
Ni	−0.141	0.352	0.160	0.091	0.051	0.243	0.045	−0.106	−0.002	0.117	−0.188	0.786**	−0.141	−0.169	−0.292					
Br	0.393*	−0.111	0.848**	0.787**	0.609**	0.590**	0.533**	0.546**	0.792**	0.810**	0.175	0.112	0.217	−0.232	−0.023	0.266				
Rb	−0.121	0.403*	0.712**	0.270	0.595**	0.758**	0.716**	0.267	0.487**	0.626**	−0.084	0.275	−0.119	−0.231	−0.239	0.371	0.701**			
Sr	0.194	0.137	0.817**	0.500**	0.826**	0.808**	0.814**	0.518**	0.775**	0.846**	0.194	0.056	0.163	−0.078	0.060	0.143	0.825**	0.760**		
Sn	−0.267	0.036	−0.051	0.151	−0.118	0.005	−0.191	−0.077	−0.179	−0.180	−0.149	0.098	−0.179	−0.232	−0.224	0.363	0.095	0.186	−0.026	
Ba	–0.528**	0.362	−0.005	−0.155	−0.351	0.057	0.190	–0.562**	−0.283	−0.043	0.076	0.209	–0.574**	0.179	0.034	0.162	0.153	0.369	0.050	0.234

Note. [1] – hollowing; *, ** – significant differences among cultivars at 0.05 and 0.01 probability levels, respectively.

Table 5. Stepwise multiple regression analysis of hollow root ratio and endogenous elements in radish cultivars

	Unstandardized coefficients		Standardized coefficients		
	B	Std. error	Beta	*t*	*P* values
Constant	35.104	7.207		4.871	0.000
Na	49.942	9.477	0.541	5.270	0.000
Ba	−2935.928	1092.151	−0.260	−2.688	0.013
N	−4.287	1.388	−0.330	−3.089	0.005
Sn	−20978.884	6981.742	−0.277	−3.005	0.006
R square = 0.823					
Y = 35.104 + (49.94 x Na) – (2935.93 x Ba) – (4.29 x N) – (20978.88 x Sn)					

Conclusion

Consequently, it can be concluded that endogenous elements, most likely connected with soil, influence development of hollowing in the roots and this effect in hollowness may result from deficient or extra plant nutritional elements in soil. In addition, according to results of this study, simple correlation coefficients and stepwise multiple regression analysis show that the providing of extra or deficient plant nutritional elements in soil or growing media especially such as N, Na, Cl, Ca, Mn, Zn, Br, Sn and Ba may affect development of hollowing in the case of cvs. 'Beyaz', 'Antep' and 'Iri Kirmizi'. However, our resistant cultivar 'Siyah' did not develop hollowing because it might not be affected by the endogenous elements.

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Ryšys tarp endogeninių elementų kiekio ir valgomojo ridiko (*Raphanus sativus* L.) šaknų tuščiaviduriškumo formavimosi

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Santrauka

Tyrimai atlikti siekiant nustatyti ryšį tarp bangas sklaidančios rentgeno spinduliuotės fluorescencijos (BSRSF) spektrometru nustatyto endogeninių elementų kiekio ir įvairių veislių ridikų šaknų tuščiaviduriškumo formavimosi. Tirtos keturios valgomojo ridiko (*Raphanus sativus* L.) veislės: ʽSiyah', ʽBeyaz', ʽAntep' ir ʽIri Kirmizi'. Tirtos veislės iš esmės skyrėsi atsparumu šaknų tuščiaviduriškumo formavimuisi. Veislė ʽSiyah' pasirodė atsparesnė už veisles ʽAntep', ʽBeyaz' ir ʽIri Kirmizi'. Nustatyta esminė koreliacija tarp tuščiaviduriškumo formavimosi ir valgomojo ridiko veislių endogeninių elementų kiekio: N (r = 0.701, P < 0.01), Na (r = 0.703, P < 0.01), Cl (r = 0.672, P < 0.01), Ca (r = 0.544, P < 0.01), Mn (r = 0.414, P < 0.05), Zn (r = 0.567, P < 0.01), Br (r = 0.393, P < 0.05) ir Ba (r = 0.528, P < 0.01). Be nustatytų ridikų šaknų tuščiaviduriškumo ir endogeninių elementų koreliacijos koeficientų, panašūs rezultatai gauti atlikus regresijos daugialypę analizę. Nustatyta regresijos lygtis [Y = 35.104 + (49.94 x Na) – (2935.93 x Ba) – (4.29 x N) – (20978.88 x Sn)] ir R kvadratas (0.823, P < 0.01). Tyrimų rezultatai parodė, kad dirvos arba auginimo terpės papildymas trūkstamomis maisto medžiagomis, ypač N, Cl, Ca, Mn, Zn, Br, Sn ir Ba, turėjo įtakos tuščiaviduriškumo formavimuisi valgomojo ridiko veislių ʽBeyaz', ʽAntep' ir ʽIri Kirmizi' šaknyse. Tačiau endogeniniai elementai neturėjo įtakos veislei ʽSiyah' dėl jos atsparumo, todėl šios veislės ridikų šaknyse tuščiaviduriškumas nesiformavo.

Reikšminiai žodžiai: *Raphanus sativus* L., valgomasis ridikas, tuščiaviduriškumas, elementų kiekis, BSRSF.