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CLASSIFICATION OF JERUSALEM ARTICHOKE ACCESSIONS BY LINEAR DISCRIMINANT ANALYSIS OF MINERAL CONCENTRATION IN TUBERS AND LEAVES

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SUMMARY

Linear discriminant analysis (LDA) was used to classify 138 accessions of Jerusalem artichoke (*Helianthus tuberosus* L.). The analysis was performed using mineral element concentrations of tubers and leaves for N, P, Ca, Mg, K, Fe, Mn, Zn and Cu. Higher classification accuracy was obtained using tuber (92.8%) than leaf (78.3%) mineral concentrations. Elements that contributed most to discrimination were Zn, Mg, and Cu for tubers and P, Mg, Zn and Cu for leaves. Accession separation in LDA figures was acceptable. Three distinctive groups that matched accession origins from the USA, Montenegro and cultivars were found according to tuber mineral concentrations. Leaf mineral concentrations provided a LDA graph where accessions from Montenegro and cultivars were grouped in two distinctive groups while accessions from the USA overlapped mostly with cultivars and to a certain degree with Montenegrin accessions. The obtained results indicate that LDA of tuber mineral composition can be useful as an additional tool for classification of Jerusalem artichoke accessions according to their origin using data of five elements (Na, Zn, Mg, Cu, Fe and K).

Key words: ***Helianthus tuberosus*, genetic variability, mineral concentration, tubers, leaves, linear discriminant analysis**

INTRODUCTION

As a cultivated plant, Jerusalem artichoke has always been behind traditional species, such as potato. It was grown on larger acreage only in France after the Sec-

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ond World War, on about 300.000 ha. Because of relatively low investments in Jerusalem artichoke breeding, complex multiplication in comparison to cultivars that multiply only by seed, and significant influence of photoperiod on its vegetative phases, selection and creation of new cultivars proved to be rather difficult. Genetic resources of Jerusalem artichoke exist in many centers, with some of them (Canada, France, Serbia) having more than 100 accessions (Kays and Nottingham, 2007).

Plant differentiation has been practiced even before the first cultivated plants and it was also the first step in selection and breeding. Differentiation was made based on the phenotype, mostly according to the plant architecture and fruit/seed yield. Development of science and technology allowed an increase in number of analyzed traits and enabled plant comparison based purely on genotype differences excluding environmental effect.

One of the available statistical methods that can be used to help accession differentiation and the breeding process is the discriminant analysis, which has been used with success for various species or byproducts like tomatoes, olives, onions *etc*. (Bontempo *et al*., 2011; Lopez *et al*., 2008; Rodrıguez Galdon *et al*., 2008). In this paper, we determined the concentrations of mineral elements in leaves and tubers of Jerusalem artichoke accessions to investigate how they differ. A linear discriminant analysis was carried out to investigate the relationship between the analyzed minerals and to evaluate their usefulness as origin markers for accession differentiation.

MATERIALS AND METHODS

The trial was conducted at Rimski Šančevi experimental field near Novi Sad (45°19'40"N, 19°49'41"E) in 2007. Tested germplasm included 138 accessions of Jerusalem artichoke (Table 1) supplied by the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS).

Table 1: Jerusalem artichoke accessions examined for mineral element concentrations

Type	No	Accession	Origin
Wild and land races	1-73	TUB-CG 3-63, 65-67, 69, 70, 72, 75-80	Montenegro
Wild	74-111	TUB 6-8, 15, 16, 20, 26 675, 1540, 1625, 1628, 1698-1705, 1945, 1959, 2024, 2045-2047, 2050-2052, 2061, 2062, 2066, 2067, 2069-2071, 2080, 2089, 2189,	USA
Cultivars	112-138	TUB BP 1-27	Serbia, France, Hungary, Slovenia, Austria, Germany

Planting was done in the second half of March. The field was given conventional cultivation practices used for cultivated sunflower. Tubers were planted in 7.5 m long rows each with 15 plants spaced 0.5 m between. Single rows were spaced 1 m apart resulting in plant density of 20 000 plants/ha. Plants within a plot served as replications.

Leaf samples were collected in July during the period of flowering from the middle part of the stem. At the end of the growing season, the parts above the ground were cut and tubers were dug. Samples of leaves and tubers were each a mixture of five different plants. Tubers were thoroughly washed with tap water. Sliced tubers were oven dried to constant mass at +50ºC. Leaves were dried to constant mass at room temperature, on sheets of clean paper. Dry samples were then ground in a mill and stored in paper bags prior to chemical analysis. A total of 9 minerals were determined in tubers and leaves (N, P, Ca, Mg, K, Fe, Mn, Zn, Cu). Analysis of all samples was done in two independent replications. Total nitrogen concentrations were determined using Kjeldahl procedure (Burns 1989) on a Gerhardt Vapodest VAP 50 distillation unit. P was determined spectrophotometrically (Spectrophotometer DU Series 60, Beckman), by ammonium-molybdate-vanadate method (Faithfull, 2002). Total concentrations of macro- and microelements (Ca, Mg, K, Fe, Mn, Zn, Cu) in plant tissues were determined by inductively coupled plasma optical emission spectrometry (ICP-OES Varian, Vista-Pro) after plant material was ashed at t=450°C and digested in 1 ml of H_2O_2 and 10 ml of HCl (25%). The detailed data on the results of the chemical analysis and mineral element concentrations are not shown in this paper although are referred to in order to support the main presented results.

The non-parametric Mann-Whitney test was used to test hypothesis whether the accessions with different origin were drawn from the same group. The differences among the groups were visualized by box-plots based on Tukey's five number summary. Linear discriminant analysis - LDA as a multivariate statistical tool was used to classify the accessions using the *a priori* information about their origin. This information was used to develop the discrimination function that resulted in an optimal discrimination of the groups of accessions. In addition, variables with the highest discrimination power were identified by partial Wilks lambda statistics. Variables with 0.0 Wilks lambda statistics had ideal discriminatory power. The increase of this value leads to the lower discriminatory power of variable. The inclusion of variables into model was made by forward selection procedure. Prior to conducting the LDA all variables were normalized by Box-Cox transformation which brings the data as close as possible to normality. The results of first two discriminant functions were visualized by means of two-dimensional graph.

RESULTS AND DISCUSSION

Mann-Whitney test showed that elements with high discrimination power exist for each of the three used origin groups. Element ratios important in ruminant nutrition like Ca/P and K/(Ca+Mg) were also found to be useful for discrimination (Table 2). The lowest number of useful variables was found for differentiation between US and cultivar origin when comparing leaf sample mineral composition. In this trial US accessions had the highest concentration for most of the analyzed elements except Zn in tubers, while in leaves they had relatively similar concentrations with the cultivar group and that resulted in small number of discriminating variables (Table 2).

Table 2: Mann-Whitney test showing variables significant for origin group differentiation

Group	Leaves		Tubers	
	Montenegrin	USA	Montenegrin	USA
USA	N, P, Ca, Mg, K, Cu[a], Fe, Mn, Zn, Ca/P, K/(Ca+Mg)		Cu, Fe, Mn, Zn, K/(Ca+Mg)	
Cultivars	P, Ca, Mg, K, Fe[a], Zn, Ca/P, K/(Ca+Mg)	Zn, Ca/P	N, P, Ca, Mg, Mn, Zn, K/(Ca+Mg)	N, P, Ca, Mg, Cu, Fe, Mn, Zn, K/(Ca+Mg)

[a] Significantly different at $P < 0.05$

Table 3: Percentage of correctly classified Jerusalem artichoke accessions using LDA of tuber and leaf mineral composition

Group	Tubers			Leaves		
	Monte negrin	Cultivars	USA	Monte negrin	Cultivars	USA
Montenegrin	69	2	1	62	4	5
Cultivars	2	24	2	2	19	6
USA	2	1	35	9	4	27
Group size	73	27	38	73	27	38
n (correct)	69	24	35	62	19	27
proportion	0.945	0.889	0.921	0.849	0.704	0.711
n / *n* (correct)		0.928			0.783	

Correct classification concerning Jerusalem artichoke accessions according to their mineral composition was 92,8% for tubers and 78.3% for leaf. Proportion of correct accession classifications according to tuber mineral composition was the highest for Montenegrin accessions (95%) followed by US (92%) and cultivars (89%). In comparison to tubers, leaves provided less accurate classification but with the same group accuracy. Montenegrin were classified with highest accuracy (85%), followed by US (71%) and cultivars (70%) (Table 3).

In Figures 1 and 2 the groupings of accessions by different mineral composition are shown. Accession separation in LDA figures was acceptable. Three distinctive groups were found according to tuber mineral concentrations that matched accession origins from the USA, Montenegro (MN) and cultivars (Figure 1). The existing variability within the group was one of the reasons for misclassification of cultivated accession TUB BP16, found among US accessions and US accessions TUB 2070 and TUB 2089 found among cultivars (Figure 1). TUB BP 18 of the cultivar group and TUB CG 7 of the MN group were positioned furthest from their supposed origin groups which implies that for the analyzed traits they may be closer to the groups they were positioned in (Figure 1).

Leaf mineral concentrations provided a LDA graph where accessions from Montenegro and cultivars were grouped in two distinctive groups while accessions from the USA overlapped mostly with cultivars and to a certain degree with Montenegrin accessions. This was a result of relatively similar leaf mineral concentrations of US and cultivar group accessions that resulted in small number of discriminating variables (Figure 2).

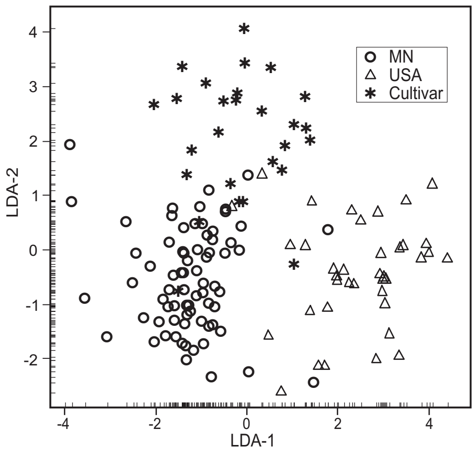

Figure 1: Discriminant plot for Jerusalem artichoke accession classification using tuber mineral composition

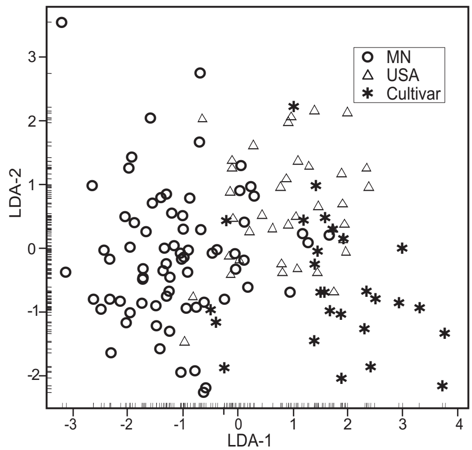

Figure 2: Discriminant plot for Jerusalem artichoke accession classification using leaf mineral composition

Increased overlapping in the leaf LDA graph may also be due to differences in mineral element composition between leaves and tubers. Higher Ca and lower P content in leaves in comparison to tubers combined with their lower classification contribution may have led to lower percentage of correctly classified accessions (Tables 4, 5). One other specification of Jerusalem artichoke is the accumulation of dry matter in tubers, when mineral element concentration is also changing between aerial parts and tubers. Even though in this work tubers proved to be more convenient for accession classification, aerial plant parts were found useful for chemometric characterization of some species using as low as four variables of the total 16 analyzed (Cantarelli *et al.*, 2010).

When nine mineral elements analyzed in this work were compared for contribution to accession classification, Zn, Mg and Cu were found to be most informative for tubers and P, Mg, Zn and Cu for leaves (Tables 4, 5). Significantly lower partial Wilks were found for Zn and Mg in tubers when element ratios Ca/P and K/(Ca+Mg) were included in the analysis which shows how important it is to adequately select variables for the model (Table 4).

Table 4: Contribution of variables to accessions classification based on tuber mineral composition. A analyzed with included element ratios, B analyzed without element ratios.

A		B	
Element	Partial Wilk's λ	Element	Partial Wilk's λ
Zn	0.471†	Zn	0.464
Mg	0.534	Mg	0.743
Cu	0.793	Cu	0.801
Fe	0.821		
K	0.872		

†all variables in model are highly significant

If single concentrations of the analyzed elements are compared to their contribution in accession classification it is evident that they are not related and that the complete element pattern affects discrimination. One of the papers that describe genetic variability for leaf element concentration (Seiler and Campbell, 2004) mentions that substantial proportion of the total variation for N, P, Ca, Mg, K, and the Ca/P was due to genotype with genotype to phenotype variance ratio greater than 0.93. Based on those results it could be expected that all of the mentioned elements could contribute to classification. In this trial, at least for leaf mineral composition, concentrations of K and Ca did not significantly contribute to accessions classification using LDA, which also confirms the effect of the complete element pattern.

Table 5: Contribution of variables to accessions classification based on leaf mineral composition. A analyzed with included element ratios, B analyzed without element ratios.

A		B	
Element	Partial Wilk's λ	Element	Partial Wilk's λ
Zn	0.852†	P	0.717
Mg	0.892	Mg	0.837
Ca/P	0.897	Zn	0.843
P	0.903	Cu	0.892
N	0.924		

†all variables in model are significant

The most influential factor for the creation of an element pattern is found to be the specific production area. As Ariyama *et al.* (2007) stated, the fingerprint of an element pattern is not not easily changed by the variations of fertilization, year, variety and soil type if appropriate elements were chosen. Even though the production area is the most significant factor affecting element pattern, varieties can also be discriminated using the same method when cultivated in the same conditions (Di Giacomo *et al.*, 2007).

CONCLUSION

Correct classification concerning Jerusalem artichoke accessions according to their mineral composition was higher when using tuber than leaf mineral composition. Classification was more accurate among Montenegrin accessions than US and cultivated, regardless of the material analyzed, tubers or leaves. The obtained results indicate that linear discriminant analysis of tuber and to a lesser extent of leaf mineral composition can be useful as an additional tool for classification of Jerusalem artichoke accessions according to their origin using data of five elements (Na, Zn, Mg, Cu, Fe and K) obtained by ICPOES.

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