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Influence of *Rheum* taxa and harvesting date on the content of L-ascorbic acid and oxalic acid in the climatic conditions of South Moravia (Czech Republic)

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ABSTRACT

Rhubarb (*Rheum* L.) is a well-known medicinal and culinary plant. Apart from its rich nutritional value, rhubarb contains a higher concentration of oxalates. In this study, the content of L-ascorbic acid (AA) and oxalic acid (OA) within three rhubarb species (*Rheum rhabarbarum*, *R. rhaponticum* and *R. palmatum* × *wittrockii*) differentiated to 16 accessions in a gene bank rhubarb collection (Lednice, Czech Republic) in the condition of conventional production in the South Moravia region during the harvesting period was evaluated. While L-ascorbic acid is essential in human nutrition, oxalic acid is considered toxic, and high doses may cause serious health issues. AA and OA content, the morphology evaluation and ISSR (inter simple sequence repeats)-based genetic analysis were performed. The results of this study confirm the significant influence of taxonomy and harvesting time on the content of AA in Rheum accessions. The content of AA was determined from 6 mg · 100 g^{-1} to 10 g · 100 g^{-1} fresh weight (FW) at the beginning of the harvesting season (May) up to 25 mg · 100 g^{-1} FW at the end of the harvesting period. The content of OA strongly varied from 300 mg · 100 g^{-1} to 1800 mg · 100 g^{-1} FW. Regarding the antinutrient character of oxalate, the optimal harvest period of this region was estimated to be from May to early June, when the OA content was the lowest. The role of AA as a precursor of oxalate formation in rhubarb was not affirmed by the results of this study.

Keywords: Chinese rhubarb, gene bank collection, genotypes, ISSR analysis, oxalate, rhubarb, vitamin C

INTRODUCTION

Rhubarb (*Rheum* L.) is a perennial plant of the Polygonaceae family that is extensively grown for its rich nutritional value. Although the leaves are referred to as toxic, the petioles are widely processed in the food industry in both raw and cooked forms (Neuss, 2021). The main bioactive compounds described for rhubarb petioles include flavan-3-ols, anthraquinones, anthocyanin, high-quality fibre, and vitamins such as retinol, thiamin, riboflavin, niacin or L-ascorbic acid (Nguyen and Savage, 2020; Bhat, 2021). According to the United States Department of Agriculture (USDA) database, the content of L-ascorbic acid in raw rhubarb is 8 mg · 100 g^{-1} (USDA, 2019). However, rhubarb plants are known especially for their high concentration of oxalates.

Oxalic acid generally occurs as oxalate salts in plants and humans (Peck et al., 2016). Oxalic acid is considered as the end product in mammalian metabolism, for example, metabolism of some amino acid, glycolate or ascorbic acid (EMEA, 2004). Oxalic acid forms

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water-soluble salts with Na^+, K^+ and NH_4^+ ions or water-insoluble forms with Ca^{2+}, Fe^{2+} and Mg^{2+} and from which the calcium oxalate is the most important constituent of kidney stones (Noonan and Savage, 1999). In plants, oxalates exist in partly insoluble forms such as oxalates, free oxalic acid and calcium oxalate (Silberhorn, 2005). Generally, they can be found in relatively small amounts in many plants and their content varies within species and also among cultivars. Nevertheless, genera such as *Beta*, *Portulaca, Rheum* or *Spinacia* are referred to as 'oxalate-rich foods' and the consumption of these vegetables poses health risks (Noonan and Savage, 1999). Reported lethal oral doses of pure oxalic acid for humans range from 5 g to 30 g (Silberhorn, 2005; Olson, 2018). However, poisoning by oxalic acid is mostly referred to in the industry where it is used in dyes, metal cleaners, bleaches, detergents and rust removers. The toxicological effects reported in humans are quite rare. However, they include gastrointestinal effects, hypocalcemia secondary to calcium oxalate crystal formation and renal toxicity (Gawarammana et al., 2009; Dassanayake and Gnanathasan, 2012). The ingestion of even 5–15 g of oxalic acid has caused death (Olson and Hung, 2012). For *Rheum* species, the reported total oxalate content in *Rheum rhabarbarum* varied from 275 mg · 100 g^{-1} up to 1336 mg · 100 g^{-1} of fresh weight (FW) in raw petioles and from 260 mg · 100 g^{-1} to 455 mg · 100 g^{-1} in stewed petioles (Noonan et Savage, 1999; Siener et al., 2006; Nguyen and Savage, 2020). Many environmental effects associated with seasonal change (e.g. precipitation, day length, hours of sunlight) might alter oxalate levels (Chairiyah et al., 2021). However, it is still not known how season or temperature affects oxalate content in plants. Extensive research is required to understand the role of season or temperature on oxalate accumulation in plants (Rahman and Kawamura, 2011).

Although the major functional role usually attributed to L-ascorbic acid is its function as a water-soluble antioxidant, it also plays a role as a precursor of oxalate in many plants (Yang and Loewus, 1975; Williams et al., 1979). Oxalates might be a final product of L-ascorbic acid degradation in food. On the other hand, it was proven that L-ascorbic acid is also metabolized by pathways other than those resulting in oxalate formation (Knight et al., 2016). Oxalate-accumulating plants (e.g. spinach, wood sorrel, shamrock) produce oxalic acid from dehydro-L-ascorbic acid, an oxidation product of L-ascorbic acid-1-(14)C (Yang and Loewus, 1975). The results of a study on *Pistia stratiotes* showed that idioblasts have metabolic machinery to synthesize oxalic acid from L-ascorbic acid using carbons 1 and 2 of L-ascorbic acid. Further, this oxalic acid is used for crystal formation (Kostman et al., 2001). Such oxalate crystals are typical in *Rheum* species as well.

Abnormal intake of L-ascorbic acid could also be a significant risk factor for the development of kidney stones as well as the oxalates themselves (Knight et al., 2016).

According to the TRIDGE database, the highest export values reached 959.7 million USD in China, followed by Belgium, Spain and Mexico (approximately from 523 million to 430 million USD). Next is Poland and the Netherlands at export values up to 300 million USD. Egypt, France, Turkey and the United States reached export values of around 100 million USD in 2023 (TRIDGE database, 2023).

The present study is focused on the evaluation of L-ascorbic and oxalic acid content during the harvesting period of three rhubarb species (*R. rhabarbarum*, *R. rhaponticum* and *R. palmatum* × *wittrockii*) in the condition of conventional production in the South Moravian region. The effects of weather conditions and taxonomy were assessed. The possibility of L-ascorbic acid's role as the oxalate precursor for *R. rhabarbarum* was also examined.

MATERIALS AND METHODS

Plant material and field trial conditions

The influence of *Rheum taxa* and harvesting time on the L-ascorbic and oxalic acid content was evaluated in 16 accessions of the *Rheum* genetic resources, containing 13 accessions of *R. rhabarbarum*, representing the most cultivated species of culinary rhubarb in the Czech Republic, two accessions of *R. rhaponticum* and one hybrid of *R. palmatum* × *wittrockii* (Table 1). The input taxonomic classification of the studied accessions was stated according to the plant providers. The rhubarb plants were cultivated on experimental fields at Mendel University in Brno, the Faculty of Horticulture in Lednice, Czech Republic (48°47′36″ N, 16°47′48″ E). Plants have been grown on this plot since 2015 and the 42H7500047 accession since 2018.

Meteorological data were recorded with automatic sensors located near the experimental field. Recorded data comprise the amount of precipitation (mm), air temperature (°C) and sunshine duration, defined as the number of sunny hours. The long-term average values (2015–2021) of selected climate characteristics and the values of the studied period (2022) are shown in Table 2 (CHMI, 2022).

According to the agrochemical analyses of the soil from the experimental field in 2022, the nitrogen content was 0.146%, the content of phosphorus was 37.794 mg · kg^{-1} and the potassium content was 198 mg · kg^{-1}. The calcium and magnesium contents were 4961 mg · kg^{-1} and 198 mg · kg^{-1}, respectively. The pH (H_2O) of the soil at a depth of 0.3 m was 7.71. The area of cultivation of each accession was 2.9 m^2. Plants were grown without additional irrigation.

Evaluation of morphological parameters and selected substance content

Sixteen accessions of *Rheum* genetic resources were evaluated during the years 2015, 2019, 2020, 2021 and 2022 using the morphological characteristics of petioles and the content of L-ascorbic acid, oxalic acid and

Table 1. Description of *Rheum* accessions analysed in the study.

No.	Plant ID[1]	Species	Cultivar	Propagation[2]	Origin
1	42H7500001	*Rhabarbarum*	Jara	V	CZ
2	42H7500003	*Rhabarbarum*	Victoria	V	GB
3	42H7500004	*Rhabarbarum*	Dawes Chalenge	V	USA
4	42H7500005	*Rhabarbarum*	The Sutton	V	GB
5	42H7500006	*Rhabarbarum*	Holsteiner Blut	V	D
6	42H7500008	*Rhabarbarum*	–	G	GB
7	42H7500011	*Rhabarbarum*	–	G	GB
8	42H7500012	*palmatum* × *wittrockii*	–	G	UA
9	42H7500014	*rhaponticum*	–	G	BG
10	42H7500016	*Rhabarbarum*	Timperley Early	V	GB
11	42H7500020	*rhaponticum*	–	G	PL
12	42H7500023	*Rhabarbarum*	–	G	GB
13	42H7500025	*Rhabarbarum*	Glaskins Perpetual	G	GB
14	42H7500030	*Rhabarbarum*	Krupnochereshkovyj	V	RUS
15	42H7500044	*Rhabarbarum*	–	V	CZ
16	42H7500047	*Rhabarbarum*	–	V	CZ

[1]Plant ID according to GRIN CZECH database (GRIN Czech, 2023).
[2]Propagation V—vegetative, G—generative.

Table 2. Selected climate characteristics for Lednice location.

Period	Average day temperature (°C)	The sum of precipitation (mm)	Sum of sunshine (hr)
May 2015–2021	15.1	56.3	208.0
June 2015–2021	20.5	46.8	269.9
July 2015–2021	21.6	64.8	260.9
May 2022	16.8	26.4	270.9
June 2022	21.1	76.9	296.4
July 2022	21.6	46.3	290.6

soluble solids. All accessions were evaluated in full maturity (May till July) and marketable quality. Ten petioles from several plants of the same accessions were hand-harvested by breaking out. Petioles with removed leaf blades were washed and evaluated in the laboratory. For the evaluation, the descriptor list of Turečková et al. (2001) was used. The classification consists of six morphological parameters (petiole skin colour above the base, skin colour at the base, shape of the petiole base in cross-section, surface of the petiole abaxial side, flesh colour at the petiole base and petiole thickness diameter) and three plant content analyses (L-ascorbic acid, oxalic acid and total soluble solids) (Table 3).

The concentration of L-ascorbic acid was determined by the modified high-performance liquid chromatography (HPLC) method according to Arya et al. (2000). Three parts were taken from each petiole – the basal, central and upper parts – and pooled and then homogenized. For analyses, 10 g of the mixture was mixed in the blender with 20 mL of 0.1 M oxalic acid. The homogenate was filtered and adjusted with 0.1 M oxalic acid to a volume of 100 mL. Then 20 mL of the mixture solution was centrifuged at 3500 rpm for 10 min. The supernatant was filtered through a microfilter (PVDF (polyvinylidene difluoride membrane) 0.45 µm) and used for the determination (ECOM, 1999). The analyses were performed by RP-HPLC (reversed-phase high-performance liquid chromatography) (ECOM, Czech Republic) at 254 nm using a UV-VIS (ultraviolet visible) detector on column (C18). All samples were evaluated in three technical replicates. The amount of L-ascorbic acid was expressed as mg · 100 g^{-1} FW.

In a 4-year evaluation, the standard titration with the potassium permanganate method as described by Karamad et al. (2019) was used. In 2022, a more precise method to quantify oxalic acid content was applied. The content was determined by a modified HPLC method according to Rahman et al. (2007). The petioles were cut, dried at 70°C in a forced-air dryer for 48 hr and ground into a powder. The sample (0.5 g) was extracted by 15 mL of 1 M hydrochloric acid solution. The sample suspension was heated in a bath of boiling water for 18 min. After cooling, the mixture was filtered, rinsed with distilled water and adjusted to 50 mL. The filtrate of the acid extract was adjusted to pH 3.0 using a 5 M sodium hydroxide solution. The filtrates were further filtered through a syringe filter with a 0.45 µm hydrophilic membrane before analysis by RP-HPLC (ECOM, Czech Republic). A 10 µL sample was injected at a column (C18) using a mobile phase of 15 mM NaH_2PO_4 (pH 2.7), a flow rate of 0.5 mL · min^{-1} and a wavelength of 210 nm. Measurement of each accession was performed in three technical replicates. The amount of oxalic acid was expressed as mg · 100 g^{-1} FW.

The content of total soluble solids was evaluated according to Zbíral (2005). The samples were sliced and pressed, and the extracted juice was dropped on the measuring glass plate of the refractometer. Measurements were then conducted on the digital optical instrument HI 96801 (Hanna Instruments Ltd., USA) measuring refractive index, which was converted to the sucrose

Table 3. Classification of leaf petiole morphological data and selected substances content (Turečková et al., 2001).

Trait	Description
Leaf – petiole skin colour above base	1 = green, 2 = rose, 3 = red stripped
Leaf – petiole skin colour at the base	1 = green, 2 = rose, 3 = red stripped, 4 = red, 5 = dark red
Leaf – shape of petiole base in cross-section	1 = oval, 2 = reniform, 3 = semi-circular
Leaf – surface of petiole lower (abaxial) side	1 = smooth, 2 = ribbed
Leaf – flesh colour at the petiole base	1 = green, 2 = green to white, 3 = green to rose, 4 = red to dark red
Leaf – petiole thickness (diameter) (mm)	1 = very small (<20), 3 = small (20–25), 5 = intermediate (26–30)
Leaf – petiole L-ascorbic acid content (mg · kg^{-1} FW)	1 = very low (<50), 3 = low (50–150), 5 = intermediate (151–250), 7 = high (251–350), 9 = very high (>350)
Leaf – petiole oxalic acid content (mg · kg^{-1} FW)	1 = very low (<1500), 3 = low (1500–2500), 5 = intermediate (2501–3500), 7 = high (3501–4500), 9 = very high (>4500)
Leaf – petiole total soluble solids content (%)	1 = very low (<2.0), 3 = low (2.0–3.5), 5 = intermediate (3.6–5.0), 7 = high (5.1–6.5), 9 = very high (>6.5)

FW, fresh weight.

Table 4. Characteristics of 15 ISSR primers used in the study.

Primer name	Primer sequence (5′→3′)	Ta[1]	No. of bands	Product size range (bp)
UBC-807	AGA GAG AGA GAG AGA GT	52	16	250–1100
UBC-808	AGA GAG AGA GAG AGA GC	51	13	300–1300
UBC-809	AGA GAG AGA GAG AGA GG	51	13	180–1000
UBC-810	GAG AGA GAG AGA GAG AT	46	4	220–510
UBC-815	CTC TCT CTC TCT CTC TG	49	3	700–1400
UBC-817	CAC ACA CAC ACA CAC AA	51	10	650–1300
UBC-836	AGA GAG AGA GAG AGA GYA	51	4	200–430
UBC-840	GAG AGA GAG AGA GAG AYT	48	5	650–1300
UBC-842	GAG AGA GAG AGA GAG AYG	52.5	5	220–680
UBC-844	CTC TCT CTC TCT CTC TRC	51	4	580–850
UBC-845	CTC TCT CTC TCT CTC TRG	53	13	700–2100
UBC-848	CAC ACA CAC ACA CAC ARG	52	18	280–1200
UBC-849	GTG TGT GTG TGT GTG TYA	49	3	750–1300
UBC-850	GTG TGT GTG TGT GTG TYC	52.5	4	450–1400
UBC-884	HBH AGA GAG AGA GAG AG	45	3	490–1000

[1]Ta: annealing temperature, B = (C, G, T), H = (A, G, T), R = (A, G), Y = (C, T).

sugar concentration (%) in water suspensions. For each sample, three technical replicates (measurements) were performed.

In 2022, a detailed observation of the development of L-ascorbic and oxalic acid content and soluble solids content in rhubarb petioles was done. The evaluation was performed at six-time points: May 3rd, May 17th, May 31st, June 14th, June 28th and July 12th, 2022.

DNA extraction and ISSR (inter simple sequence repeats) PCR (polymerase chain reaction) reaction

Based on the morphological diversity of the studied *R. rhabarbarum* accessions, a genetic analysis based on ISSR (inter simple sequence repeats) reaction was performed. Total genomic DNA was extracted from young frozen leaves (0.1 g) using the DNeasy Plant Mini Kit (Qiagen, Germany). The DNA quality was estimated using electrophoresis on a 0.8% agarose gel compared with lambda DNA standards containing 200 ng · µL^{-1} and 400 ng · µL^{-1} DNA. The concentration was measured by the Modulus Single Tube Multimode Reader (Turner BioSystems, USA) and adjusted to the final concentration of 12.5 ng · µL^{-1}.

Initially, 17 ISSR primers (Tabin et al., 2016) were screened for polymorphism and finally 15 polymorphic primers (Table 4) were selected for generating ISSR profiles of the *Rheum* samples. The reaction mixture consisted of 1.5 µL DNA, 4 µL 5 × GoTaq Flexi Buffer, 0.5 µL dNTPs (10 mM), 1.2 µL $MgCl_2$ (25 mM), 1.5 µL primer (10 µM), 0.7 µL 5 × Green GoTaq Flexi Buffer, 0.2 µL GoTaq G2 Flexi DNA polymerase (5 U · µL^{-1}, Promega, USA) and nuclease-free water to make a final volume of 25 µL. The PCR (polymerase chain reaction) cycles were: one activation cycle at 94°C for 5 min, followed by 45 cycles at 94°C for 1 min, annealing temperature for each primer given in Table 4 for 1 min and extension at 72°C for 2 min. The final extension cycle was carried out at 72°C for 7 min. PCR products were separated on a 1.5% agarose gel stained with Midori green Advance dye (Nippon Genetics, Japan) and the size of each fragment was estimated by

comparison with a 100 bp DNA ladder (New England BioLabs, United Kingdom) and a TrackIt 1 Kb Plus DNA Ladder (Invitrogen, USA).

Data analysis

Morphological data analysis was performed based on the six leaf-petiole quality characters obtained for the same input material as for the L-ascorbic and oxalic acid content. The representative values of individual descriptors for each accession were calculated using the 4-year dataset. These data were subjected to distance-based clustering analysis using the unweighted pair-group method with arithmetic averages (UPGMA) and Jaccard's similarity coefficient by the FreeTree v.0.9.1.50 software (Hampl et al., 2001). The phylogenetic tree was visualized by FigTree v1.4.4 (Rambaut, 2010) software.

Data of L-ascorbic and oxalic acid content were transformed by the decimal logarithm and analysed by repeated measures analysis of variance (ANOVA) and Tukey's multiple comparison tests with an α level of 0.05 by the Statistica v.12.0.0 software (StatSoft, USA). The obtained results were examined by residual analysis using the same software. The putative role of L-ascorbic acid as the oxalate precursor in *R. rhabarbarum* accessions was examined by the correlation of L-ascorbic and oxalic acid measured in individual sampling terms using the regression analysis of Statistica v.12.0.0 software with an α level of 0.05. For ordination analysis to evaluate the relationship between accessions and sampling terms and the correlation of L-ascorbic acid and oxalic acid content, the program Canoco 5 (Biometris, Wageningen University and Research Centre, Wageningen, The Netherlands; University of South Bohemia in České Budějovice, České Budějovice, Czech Republic) was used.

For genetic analysis, each amplified fragment of the ISSR reaction was scored manually and converted into a binary matrix based on the presence (1) and absence (0) of the respective band. Subsequently, the data were used to construct a difference/similarity matrix based on UPGMA and Jaccard's similarity coefficient and to construct a phylogenetic tree using FreeTree v. 0.9.1.50 (Hampl et al., 2001) and FigTree v. 1.4.4 (Rambaut, 2010) software. Based on the taxonomical relationships, the accession 42H7500012 (*R. palmatum* × *wittrockii*) was used as the outgroup.

The gradient for the response data in the ordination analysis was 0.6 units long, and redundancy analysis (RDA) was chosen as a statistical method. The statistical significance of the results was calculated with the Monte-Carlo permutation test (499 permutations). Results are significant at a significance level of $p = 0.002$.

RESULTS AND DISCUSSION

Long-term observation of selected Rheum accessions

Four-year observation of six morphological characters of petioles and the content of L-ascorbic acid, oxalic acid and soluble solids in 16 accessions of *Rheum* genetic resources brought the following results. The *R. palmatum* × *wittrockii* petioles (42H7500012) were characterized by red skin on the base to green skin above the base, a semi-circular shape in the cross-section, a ribbed abaxial surface, green flesh and a petiole diameter up to 20 mm (Table 5). *R. rhaponticum* petioles (42H7500014) are distinguished from the *Rheum* hybrid accession by the green to rose flesh and green colour of the skin on both evaluated parts (at and above the base). The rest of the petiole traits were identical. For accession 42H7500020 (*R. rhaponticum*), the affiliation with the *R. rhabarbarum* species was presumed based on the morphological analysis.

The majority of the evaluated accessions of *R. rhabarbarum* showed red or dark red petiole colour at the base and from green (42H7500011, 42H7500016, 42H7500025, 42H7500044) to rose (42H7500001, 42H7500003, 42H7500005, 42H7500006, 42H7500008) and red stripped (42H7500004, 42H7500023, 42H7500030, 42H7500047) colouring above the base. The cross-sections were mostly reinformed, with both smooth and ribbed shapes. The predominant colour of the flesh was green. Except for accessions 42H750001, 42H750004, 42H750006 and 42H750044, whose petioles showed a diameter from 20 mm to 25 mm, the *R. rhabarbarum* plants had values of petiole diameter similar to those of *R. rhaponticum* and *R. palmatum* × *wittrockii* species (<20 mm).

According to the average content of L-ascorbic acid, oxalic acid and soluble solids, evaluated accessions created groups independently of the groups based on taxonomy or morphological characteristics. In the case of L-ascorbic acid, eight accessions (42H7500001, 42H7500003, 42H7500004, 42H7500006, 42H7500012, 42H7500014, 42H7500016 and 42H7500020) showed values of 5–15 mg · 100 g^{-1} FW, representing a low content of L-ascorbic acid. The value of L-ascorbic acid content reported for *R. rhabarbarum* cultivated in Latvia was 15.62–68.80 mg · 100 g^{-1} FW, representing rhubarb growing areas further north of the Czech Republic (Duma et al., 2016). The rest of the accessions showed intermediate values (15–25 mg · 100 g^{-1}). Compared to Romania, placed further south, where L-ascorbic acid gained a content of 339–438 mg · 100 g^{-1} FW (Stoleru et al., 2019); all the evaluated accessions had lower L-ascorbic acid content. In Table 5, the descriptor value representing the given content range of L-ascorbic acid presents a 4-year average (2015, 2019, 2020 and 2021).

Similarly, in Table 5, the 4-year average of oxalic acid content measured in the harvest season is expressed by a descriptor value according to Table 3. For all accessions, a very high content of oxalic acid (>450 mg · 100 g^{-1} FW) was found. Significantly lower content was presented by Stoleru et al. (2019) ranging from 256 mg · 100 g^{-1} to 377 mg · 100 g^{-1} FW in rhubarb grown in Romania. The oxalic acid content in our study was comparable to experiments from Latvia (Duma et al., 2016) or New Zealand (Nguyen and Savage, 2020). The content of

Table 5. Data of 4-year observation of *Rheum* petiole descriptors.

Plant ID	Petiole skin colour above base	Petiole skin colour at the base	Shape of petiole base in cross-section	The surface of the petiole abaxial side	Flesh colour at the petiole base	Petiole thickness diameter (mm)	Petiole L-ascorbic acid content (mg · kg^{-1} FW)	Petiole oxalic acid content (mg · kg^{-1} FW)	Petiole soluble solids content (%)
42H7500001	2	2	2	1	1	3	3	9	5
42H7500003	2	4	1	2	3	1	3	9	5
42H7500004	3	3	3	1	2	3	3	9	5
42H7500005	2	5	3	1	3	1	5	9	5
42H7500006	2	5	1	2	4	3	3	9	5
42H7500008	2	4	3	2	1	1	5	9	5
42H7500011	1	5	2	1	1	1	5	9	5
42H7500012	1	4	3	2	1	1	3	9	3
42H7500014	1	1	3	2	3	1	3	9	5
42H7500016	1	5	2	2	3	1	3	9	5
42H7500020	3	2	3	1	4	1	3	9	5
42H7500023	3	4	3	1	2	1	5	9	5
42H7500025	1	5	2	2	1	1	5	9	3
42H7500030	3	5	2	2	1	1	5	9	5
42H7500044	1	4	2	1	3	3	5	9	5
42H7500047	3	4	2	2	1	1	5	9	5

FW, fresh weight.

oxalic acid is strongly variable, probably depending on local climate and soil conditions, and taxonomy. Moreover, the variability in content may be affected by the selected analysis type (titration, HPLC, catalytic kinetic spectrophotometry).

For the content of soluble solids, the intermediate content (3.6%–5%) was the most prevalent. The results found in Slovakia (3.54–4.37°Bx) (Mezeyová et al., 2021) were close to our values.

The UPGMA analysis based on morphological data created a phylogenetic tree with four main clades, gathering 12 of 13 *R. rhabarbarum* accessions into Group I, II and IV (Figure 1). Interestingly, the species *R. palmatum* × *wittrockii* (42H7500012) and *R. rhaponticum* (42H7500014) created Group III together with *R. rhabarbarum* 'Victoria' (42H7500003) and *R. rhabarbarum* (42H7500008). However, the cultivar 'Victoria' is referred to as the *R. rhaponticum* species by several authors (Walkey and Matthews, 1979; Noonan and Savage, 1999; Zhao et al., 2009) and its categorization into Group III based on visual similarities was possible to expect.

The first clade (Group I) linked *R. rhabarbarum* accessions 42H7500004 ('Dawes Chalenge'), 42H7500005 ('The Sutton') and 42H7500023 to *R. rhaponticum* 42H7500020 and *R. rhabarbarum* 42H7500006 ('Holsteiner Blut') as the least similar ones. Accessions with the closest relationship shared the same values for four of the six evaluated traits. The second and fourth clade (Group II, Group IV) consisted of the other seven *R. rhabarbarum* accessions, 42H7500001 ('Jara') and 42H7500044 (both Group II) and 42H7500011, 42H7500016 ('Timperley Early'), 42H7500025 ('Glaskin Perpetual'), 42H7500030 ('Krupnochereshkovyj') and 42H7500047 (Group IV). Group IV included the most homogenous subclades, where five of the six characteristics were identical. Nevertheless, the common characteristics of the whole Group IV were only the shape of the petiole cross-section and the petiole diameter.

Development of L-ascorbic and oxalic acid content through the Rheum harvesting period

To evaluate the development of L-ascorbic and oxalic acid, the content of those acids was evaluated in six sampling terms during the year 2022: May 3rd, May 17th, May 31st, June 14th, June 28th and July 12th, covering the common rhubarb harvesting period in South Moravia (Czech Republic). Regarding the climate conditions, values of the average daily temperature and the sum of sunshine did not differ from average values recorded in the last 6 years (2015–2021, Table 2). A different value was determined for the precipitation, where the long-term precipitation in May (56.3 mm) differed from 2022 by almost 30 mm (26.4 mm in 2022). Contrary to the June long-term value of 46.8 mm, the precipitation in 2022 was markedly higher (76.9 mm) (CHMI, 2022). The lowest sum of precipitation was measured in the 2 weeks before the third sampling term (9.5 mm) and the highest before the fourth term (49.7 mm). Some accessions showed a significant increase in L-ascorbic acid content between those two terms. The content of

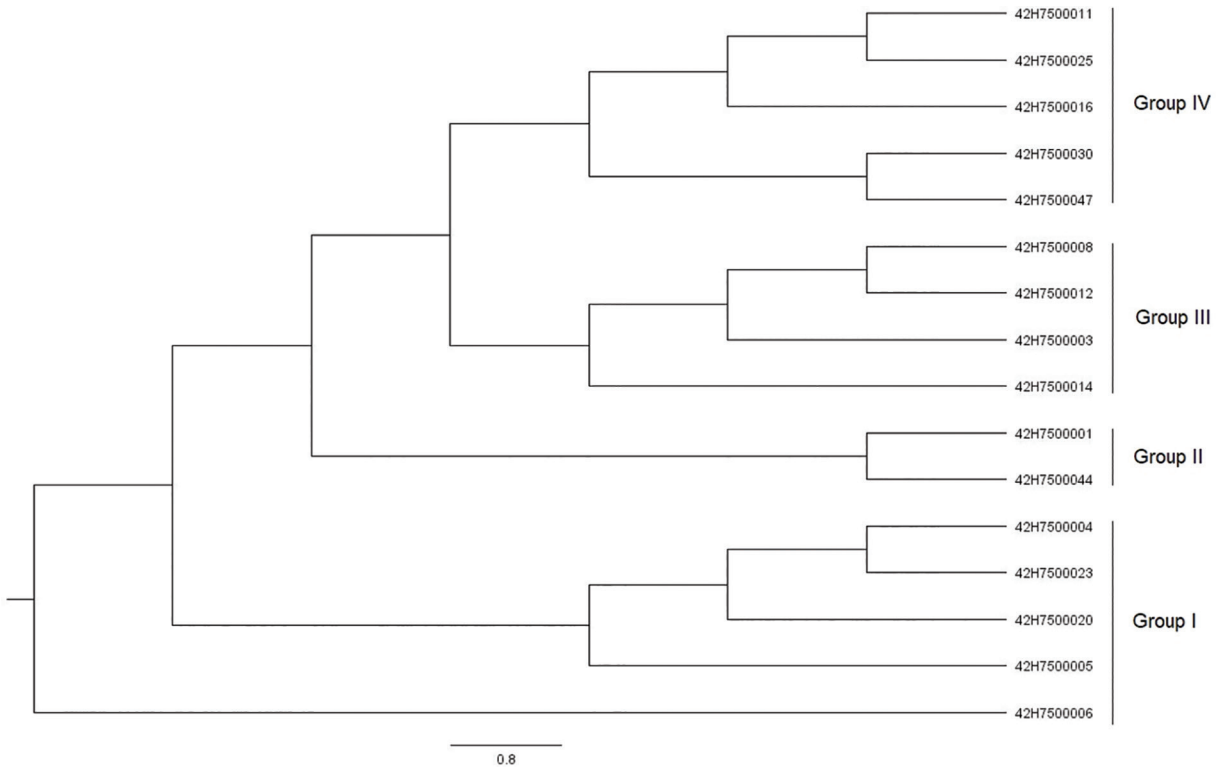

Figure 1. Phylogenetic tree based on UPGMA analysis and Jaccard's similarity coefficient of *Rheum* morphological data. The distance-based clustering analysis was performed using FreeTree v.0.9.1.50 software (Hampl et al., 2001). UPGMA, unweighted pair-group method with arithmetic averages.

L-ascorbic acid increased by around 50% or more in *R. rhabarbarum* accessions 42H7500003, 42H7500005, 42H7500006 and 42H7500030. The highest daily average temperature and maximum daily temperature were recorded in the 2 weeks before the fourth sampling term (21.16°C and 28.24°C, respectively) (Supplementary Table 1), which could contribute to higher L-ascorbic acid content. However, most of the accessions showed only a slight increase and, in some cases, a significant decrease was recorded (42H7500020). The results of Duma et al. (2016) also suggest a low influence of the sudden precipitation, as the presented value of L-ascorbic acid obtained for 'Victoria' in May was 15.62 mg · 100 g^{-1} FW. The same cultivar in our study (42H7500003) showed the value of 11.98 mg · 100 g^{-1} FW. Generally, studies assessing temperature effects on L-ascorbic acid content in other agricultural crops (e.g. potatoes or tomatoes) suggest that higher temperature growth conditions might increase the L-ascorbic content in plants (Hamouz et al., 2018; Hernández et al., 2018). Nevertheless, not many studies investigating the effects of climate conditions on rhubarb have been conducted yet.

The content of L-ascorbic acid developed as follows: in most (10 of 16 accessions), the increase of L-ascorbic acid between the first and second sampling terms was recorded, with the most significant increase in the *R. rhabarbarum* accessions 42H7500004, 42H7500006 and 42H7500023. Next, the decreasing tendency of L-ascorbic acid in the third term, followed by an increase in the fourth term, was detected. Similarly, the content of L-ascorbic acid decreased in the fifth sampling term in most accessions. *R. rhabarbarum* accessions 42H7500016, 42H7500020 and 42H7500047 and *R. rhaponticum* 42H7500014 did not show such a decrease in the fifth sampling term. During the sixth sampling term, the content of L-ascorbic acid increased in all accessions except *R. rhabarbarum* accession 42H7500047 (Table 6 and Supplementary Graph 1). The development of L-ascorbic acid in *Rheum* species was monitored only by Duma et al. (2016) where the increase of L-ascorbic acid from May to June, followed by a little decrease in July, was reported.

The statistical analysis of the influence of taxonomy and harvesting time on the content of L-ascorbic acid showed significant differences for both factors. In the *Rheum* taxonomy, *R. palmatum* × *wittrockii* and *R. rhaponticum* significantly differ from the tested *R. rhabarbarum* accessions, except for accessions 42H7500004 and 42H7500044 in the case of *R. palmatum* × *wittrockii* and accessions 42H7500016 and 42H7500023 for *R. rhaponticum*. On the other hand, four accessions of *R. rhabarbarum* (42H7500005, 42H7500008, 42H7500020 and 42H7500047) significantly differed from all other accessions. The other *R. rhabarbarum* accessions were similar to the maximum of two other accessions, indicating individual

Table 6. Average L-ascorbic acid content in *Rheum* species (mg · 100 g^{-1} FW) during the studied period.

Taxa	03/05/2022	17/05/2022	31/05/2022	14/06/2022	28/06/2022	12/07/2022
R. palmatum × *wittrockii*						
42H7500012	8.76 ± 0.18	11.03 ± 0.08	10.11 ± 0.30	13.36 ± 0.12	6.74 ± 0.09	25.62 ± 0.37
R. rhabarbarum						
42H7500001	15.08 ± 0.34	9.28 ± 0.21	10.63 ± 0.56	15.16 ± 0.24	9.59 ± 0.25	16.72 ± 0.75
42H7500003	12.37 ± 0.11	13.84 ± 0.10	7.69 ± 0.57	15.97 ± 0.32	8.31 ± 0.39	18.72 ± 0.48
42H7500004	15.15 ± 0.36	21.06 ± 0.28	18.88 ± 0.46	15.24 ± 0.49	14.65 ± 0.23	18.69 ± 0.20
42H7500005	16.07 ± 0.12	16.12 ± 0.55	16.44 ± 0.44	29.62 ± 0.38	28.08 ± 1.15	33.49 ± 0.64
42H7500006	7.07 ± 0.17	17.36 ± 0.36	8.75 ± 0.09	20.36 ± 0.35	13.21 ± 0.03	28.24 ± 0.50
42F7500008	20.98 ± 0.21	25.21 ± 0.30	27.02 ± 0.06	33.53 ± 1.03	21.42 ± 0.28	32.39 ± 0.22
42H7500011	12.27 ± 0.23	13.88 ± 0.03	13.14 ± 0.02	18.43 ± 0.21	13.15 ± 0.36	19.55 ± 0.34
42H7500016	9.52 ± 0.08	11.10 ± 0.26	8.90 ± 0.46	11.44 ± 0.21	11.50 ± 0.21	23.20 ± 0.29
42H7500020[1]	9.50 ± 0.14	6.37 ± 0.27	15.38 ± 0.11	7.06 ± 0.17	9.88 ± 0.48	13.42 ± 0.09
42H7500023	6.77 ± 0.21	11.76 ± 0.32	13.07 ± 0.52	12.56 ± 0.16	9.05 ± 0.09	14.88 ± 0.11
42H7500025	12.80 ± 0.05	14.05 ± 0.32	10.16 ± 0.52	18.10 ± 0.32	11.39 ± 0.30	14.79 ± 0.12
42H7500030	13.48 ± 0.54	12.28 ± 0.03	6.66 ± 0.09	18.88 ± 0.13	10.16 ± 0.14	23.54 ± 0.31
42H7500044	13.39 ± 0.60	12.54 ± 0.19	15.26 ± 0.12	21.27 ± 0.13	20.27 ± 0.08	24.81 ± 0.65
42H7500047	14.67 ± 0.05	10.39 ± 0.14	18.59 ± 0.02	16.90 ± 0.17	18.69 ± 0.07	16.84 ± 0.11
R. rhaponticum						
42H7500014	15.05 ± 0.24	16.96 ± 0.18	12.10 ± 0.06	18.04 ± 0.18	19.10 ± 0.08	22.19 ± 0.24

[1]Originally described as *R. rhaponticum*.
FW, fresh weight.

differences within the *R. rhabarbarum* species in the case of L-ascorbic acid content (Supplementary Table 2). The sampling term was a significant factor in most of the evaluated accessions. The highest values of L-ascorbic acid were obtained for *R. rhabarbarum* 42H7500008, where the content of L-ascorbic acid was >20 mg · 100 g^{-1} FW during the whole evaluated period. These results suggest that the content of L-ascorbic acid in *Rheum* plants depends on both taxonomy and harvesting date. Nevertheless, the development of L-ascorbic acid during the evaluated period showed similar trends.

The content of oxalic acid in the *Rheum* accessions was significantly influenced by the taxonomy and harvesting time. The accessions of *R. rhaponticum* (42H7500014) and three *R. rhabarbarum* (42H7500003, 42H7500016 and 42H7500044) significantly differed from all of the other accessions. Accessions 42H7500014 and 42H7500044 represented *Rheum* with a high content of oxalic acid during the evaluated period, whereas accessions 42H7500003 (*R. rhabarbarum* 'Victoria') and 42H7500016 (*R. rhabarbarum* 'Timperley Early') showed low values. The content of oxalic acid in the first sampling ranged from 296.99 mg · 100 g^{-1} ± 0.008 mg · 100 g^{-1} FW (*R. rhabarbarum* accession 42H7500016) to 942.77 mg · 100 g^{-1} ± 0.031 mg · 100 g^{-1} FW *(R. rhabarbarum* accession 42H7500044). For most of the accessions, the content of oxalic acid was slightly increasing in time, with the highest values on June 14th (the fourth sampling, Supplementary Graph 2). However, for 12 out of 16 (total) accessions, the decrease during the fifth sampling term (June 28th) was recorded. In four accessions; *R. rhabarbarum* 42H7500003, 42H7500004, *R. rhaponticum* 42H7500014 and *R. palmatum* × *wittrockii* 42H7500012; the content of oxalic acid increased in the fifth sampling term. The first three listed herein above belong to the same group (Group III) according to molecular data from 15 ISSR markers, suggesting possible similarities based on genetics. The content of oxalic acid in *R. rhabarbarum* from organic production was observed by Stoleru et al. (2019). The presented values in this study ranged from 256 mg · 100 g^{-1} to 377 mg · 100 g^{-1} FW, approximately two to three times lower than in our study. The possible differences may be caused by different methodological approaches when catalytic kinetic spectrophotometry was used by Stoleru et al. (2019), while titration and HPLC (high-performance liquid chromatography) were in our study. Another factor causing this difference may be climatic and weather conditions affecting the content of oxalic acid. Several studies reported a higher content of oxalic acid as crystals of calcium oxalate (druse crystals) in plant tissues (e.g. taro, sweet potatoes) under stress conditions such as drought (Woolfe, 1992; Oscarsson and Savage, 2007; Gouveia et al., 2018).

The ordination diagram (Figure 2) shows relationships between accessions, sampling terms and the content of oxalic acid and L-ascorbic acid. The content of L-ascorbic acid was the highest at the last, sixth, sampling term. The diagram also indicates an obvious negative correlation between oxalic acid content and the first sampling term, representing the lowest content of oxalic acid at the beginning of the harvesting season. Accessions *R. rhabarbarum* 42H7500008 and 42H7500005 were among those with the highest content

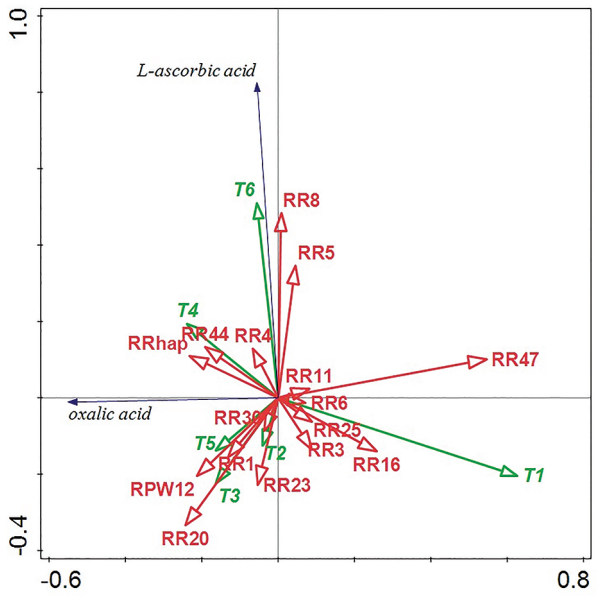

Figure 2. RDA ordination diagram showing the relationships between accessions, sampling terms and content of oxalic and L-ascorbic acid, pseudo –F = 7.1, $p = 0.002$. The position of accessions RR8 and RR5 in the diagram close to T6 (Term 6) and L-ascorbic acid content implicate a positive correlation between these variables and factors. Distinctive negative correlations are found between accession RR47 and oxalic acid content, RR20, RR12, and RR23, and L-ascorbic acid content. Regarding the content of acids in terms, the position of T1 (first term) expresses a negative correlation to oxalic acid content, and the contrary T6 (last term) indicates a notable positive correlation to L-ascorbic content. RDA, redundancy analysis.

of L-ascorbic acid, and *R. palmatum* × *wittrockii* 42H7500012, *R. rhabarbarum* 42H7500020, 42H7500023 had the lowest ones. Regarding oxalic acid, a notable negative correlation between accession *R. rhabarbarum* 42H7500047 and oxalic acid content was found. In the fourth sampling term, both oxalic acid and L-ascorbic acid content increased. No correlation between oxalic acid and L-ascorbic acid was found in this analysis.

The measured values of both acids obtained from May to July were evaluated from the perspective of a possible role of L-ascorbic acid as a precursor of oxalate formation in *R. rhabarbarum* accessions. The regression analysis performed for individual sampling terms suggested the presence of a negative correlation for accession 42H7500001 on 3rd May and a positive correlation for accessions 42H7500006, 42H7500025 and 42H7500044 on 17th May and for 42H7500016 on 28th June (Supplementary Table 3). These results do not support the conversion of L-ascorbic acid to the oxalate form as was described for other oxalate-accumulating plants such as spinach, wood sorrel shamrock and begonia by Yang and Loewus (1975) or in yucca Horner et al. (2000). On the other hand, the increase of L-ascorbic acid with unchanged content of oxalic acid in low-temperature stressed spinach plants was also published (Proietti et al., 2009). However, a more comprehensive dataset would be required for the confirmation of these findings in the case of *Rheum* species.

Characterization of Rheum accessions by ISSR analysis

The analysis based on the molecular ISSR markers clustered 15 *Rheum* accessions into 3 main clusters (Figure 3), where the first cluster consisted of 3 closely related groups (Groups Ia, Ib and Ic). The only groups that remained from the morphological analysis were *R. rhaponticum* (42H7500014) and *R. rhabarbarum* 'Victoria' (42H7500003). These accessions were clustered into Group III of the molecular-based tree with a small genetic distance from *R. rhabarbarum* 42H750004 ('Dawes Chalange') and 42H7500023. Nevertheless, the visually similar *R. rhabarbarum* 42H7500008 occurred in close Group II, and the hybrid species *R. palmatum* × *wittrockii* was used as the outgroup of ISSR data analysis. The rest of the studied accessions did not show similarities between morphological and genetic results. The differences in phylogenetic results based on morphology and molecular characteristics are generally presented, as well as the close similarities (Persson et al., 2000). ISSR primers from our study were used for the diversity assessment in the cases of *R. emodi*, *R. spiciforme* and *R. webbianum* by Tabin et al. (2016). The analysis not only clustered individual species into groups under their geographical location but also indicated differences in the genetic background of the included populations.

Explanatory notes			
RR1	42H7500001 *R. rhabarbarum* Jara	RRhap	42H7500014 *R. rhaponticum*
RR3	42H7500003 *R. rhabarbarum* Victoria	RR16	42H7500016 *R. rhabarbarum* Timperley Early
RR4	42H7500004 *R. rhabarbarum* Dawes Chalenge	RR20	42H7500020 *R. rhabarbarum*
RR5	42H7500005 *R. rhabarbarum* The Sutton	RR23	42H7500023 *R. rhabarbarum*
RR6	42H7500006 *R. rhabarbarum* Holsteiner Blut	RR25	42H7500025 *R. rhabarbarum* Glaskins Perpetual
RR8	42F7500008 *R. rhabarbarum*	RR30	42H7500030 *R. rhabarbarum* Krupnochereshkovyj
RR11	42H7500011 *R. rhabarbarum*	RR44	42H7500044 *R. rhabarbarum*
RPW12	42H7500012 *R. palmatum* × *wittrockii*	RR47	42H7500047 *R. rhabarbarum*
T1–T6	Sampling terms 1 to 6		

Table 7. Oxalic acid content for *Rheum* species (mg · 100 g^{-1} FW) in the studied period.

Taxa	03/05/2022	17/05/2022	31/05/2022	14/06/2022	28/06/2022	12/07/2022
R. palmatum × *wittrockii*						
42H7500012	670.19 ± 0.02	1036.48 ± 0.04	1413.99 ± 0.03	1636.8 ± 0.02	2315.14 ± 0.12	1454.68 ± 0.04
R. rhabarbarum						
42H7500001	453.79 ± 0.01	1375.85 ± 0.02	1991.31 ± 0.03	1752.99 ± 0.03	1504.59 ± 0.02	1463.98 ± 0.05
42H7500003	540.69 ± 0.03	616.96 ± 0.02	723.53 ± 0.04	620.69 ± 0.01	1111.35 ± 0.02	761.63 ± 0.02
42H7500004	792.38 ± 0.00	1005.73 ± 0.02	889.51 ± 0.05	981.64 ± 0.05	2287.14 ± 0.08	711.01 ± 0.04
42H7500005	598.80 ± 0.00	987.47 ± 0.05	793.82 ± 0.01	843.32 ± 0.06	707.70 ± 0.02	1317.66 ± 0.11
42H7500006	354.81 ± 0.02	585.79 ± 0.00	926.35 ± 0.04	1686.64 ± 0.14	1050.84 ± 0.01	1194.20 ± 0.01
42F7500008	407.26 ± 0.01	448.45 ± 0.01	1743.34 ± 0.03	1927.36 ± 0.14	1281.33 ± 0.08	1546.97 ± 0.03
42H7500011	451.24 ± 0.01	456.33 ± 0.03	833.45 ± 0.00	817.21 ± 0.02	700.95 ± 0.01	936.17 ± 0.00
42H7500016	296.99 ± 0.01	432.20 ± 0.01	402.99 ± 0.00	807.39 ± 0.05	486.89 ± 0.00	699.58 ± 0.01
42H7500020	460.39 ± 0.01	1523.02 ± 0.05	1442.98 ± 0.01	2034.83 ± 0.08	1229.00 ± 0.01	1138.73 ± 0.04
42H7500023	921.14 ± 0.01	869.45 ± 0.01	861.78 ± 0.01	1527.04 ± 0.03	539.44 ± 0.04	836.12 ± 0.00
42H7500025	570.38 ± 0.02	561.60 ± 0.01	656.61 ± 0.01	906.80 ± 0.05	679.91 ± 0.00	689.21 ± 0.00
42H7500030	436.23 ± 0.01	671.23 ± 0.00	985.26 ± 0.01	1839.81 ± 0.03	835.38 ± 0.02	881.95 ± 0.01
42H7500044	942.77 ± 0.03	1345.46 ± 0.07	1573.44 ± 0.03	1691.24 ± 0.04	1276.30 ± 0.02	1835.46 ± 0.03
42H7500047	756.72 ± 0.03	1473.44 ± 0.05	1330.53 ± 0.02	1739.93 ± 0.04	981.73 ± 0.00	972.80 ± 0.04
R. rhaponticum						
42H7500014	811.57 ± 0.03	1628.48 ± 0.01	1983.39 ± 0.01	1974.29 ± 0.07	2266.68 ± 0.02	1479.83 ± 0.05

[1]originally described as *R. rhaponticum.*
FW, fresh weight.

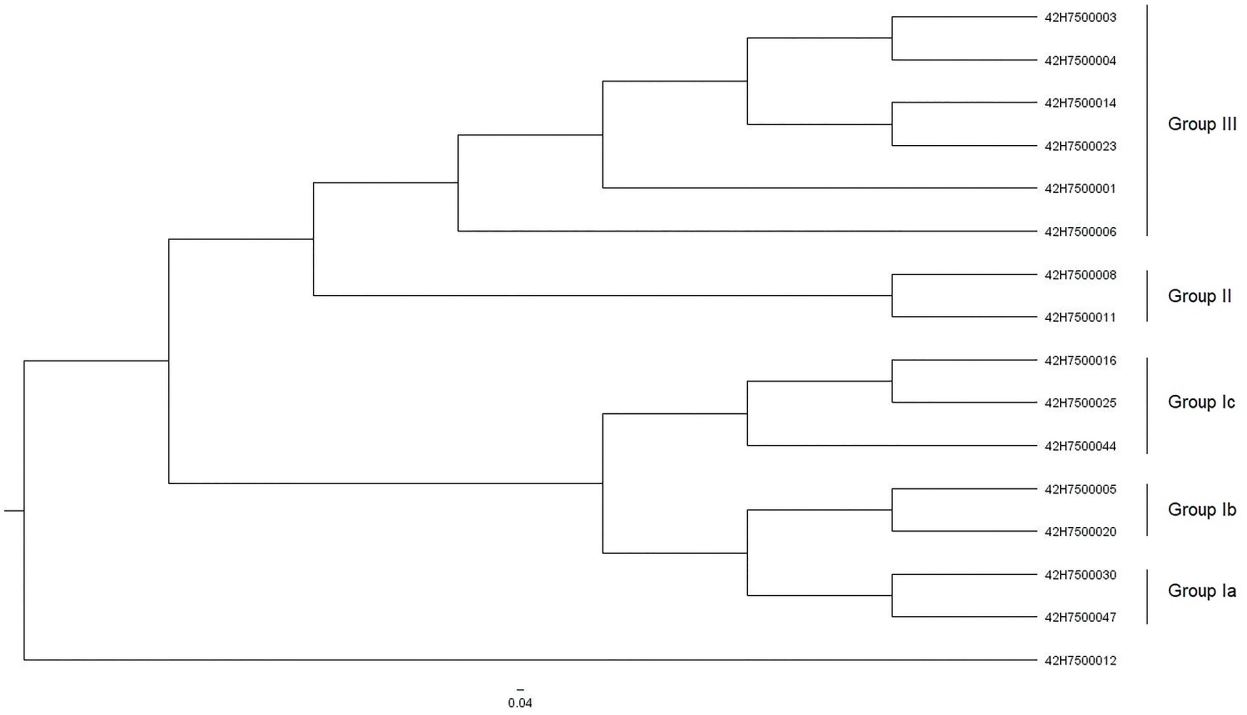

Figure 3. UPGMA tree based on *Rheum* molecular data from 15 ISSR markers. The difference/similarity matrix based on UPGMA and Jaccard's similarity coefficient was used to construct the tree by the FreeTree v.0.9.1.50 software (Hampl et al., 2001). UPGMA, unweighted pair-group method with arithmetic averages.

Results of RDA ordination analysis evaluating relationships between the content of oxalic acid and L-ascorbic acid and groups based on molecular data (Figure 3) and sampling terms indicate several correlations (Figure 4). Accessions from Group II had a higher content of L-ascorbic acid at the sixth sampling term. A negative correlation between oxalic acid content and Group Ia suggests a lower content of oxalic acid in this group. Conversely, some positive correlation indicating a higher content of oxalic acid was observed

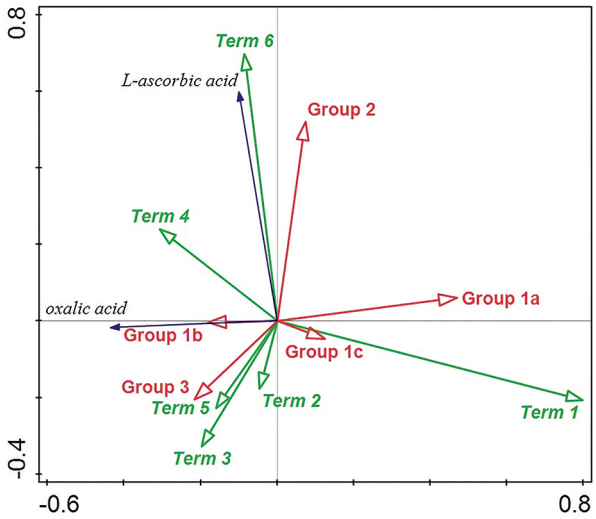

Figure 4. RDA ordination diagram evaluating the relationships between groups (based on molecular data Figure 3), sampling terms and content of oxalic and L-ascorbic acid, pseudo –F = 7.4, $p = 0.002$. The position of Group 2 in the diagram close to L-ascorbic content and Term 6 indicates a strong positive correlation between these factors and variables. The position of Group 1a in the diagram shows a notable negative correlation to oxalic acid content. A weaker positive correlation with oxalic acid content occurs for Group 1b or 3 according to this ordination diagram. RDA, redundancy analysis.

in Group Ib and Group III. The results of this analysis can explain the possible effect of taxonomy in a few groups (Group 2, Group 1a) on L-ascorbic and oxalic acid content development during the harvesting season. In other groups, the correlation was weaker. The content of L-ascorbic and oxalic acid in accessions from Group 1c was probably affected by other factors not included in this analysis.

CONCLUSIONS

The estimation of phenotypic and genotypic variance plays an important role in plant breeding, the preservation of genetic resources and the food industry, leading to the targeted use of species with a beneficial effect on human health. According to our observaton, the content of L-ascorbic acid, one of the most well-known antioxidant agents, is dependent on taxonomical membership at the level of species but may vary between individual cultivars. A significant impact was also confirmed for the harvesting time, showing an increasing trend of L-ascorbic content during the cultivation season. Based on the level of oxalic acid determined in rhubarb petioles during the harvesting period, the recommended time for the harvest and use of rhubarb petioles in the conditions of the South Moravia region in the Czech Republic is from May to early June. At this time, the content of oxalic acid has decreased and the possible risk of calcium oxalate formation is predicted to be minimalized. The best results were observed for *R. rhabarbarum* 'Timperley Early'. The assumption of L-ascorbic acid as a precursor of oxalate formation in the *R. rhabarbarum* species was not confirmed in this study. Results also suggest that L-ascorbic and oxalic acid content might be affected not only by weather conditions but also by genotype. Ordination analysis in this study confirmed several differences in the content of L-ascorbic and oxalic acid between groups sorted by molecular data. However, more complex research in control conditions is necessary to precisely determine and identify the effects of various factors such as precipitation, drought, temperatures or taxonomy.

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AUTHOR CONTRIBUTIONS

J.N., E.H. and J.Č. designed the research study. J.N., E.H., J.Č., J.R. and D.T. performed the research. R.P. provided help and advice on supervising the research study. E.H. and L.N.R. analysed the data. J.N., E.H., L.N.R. wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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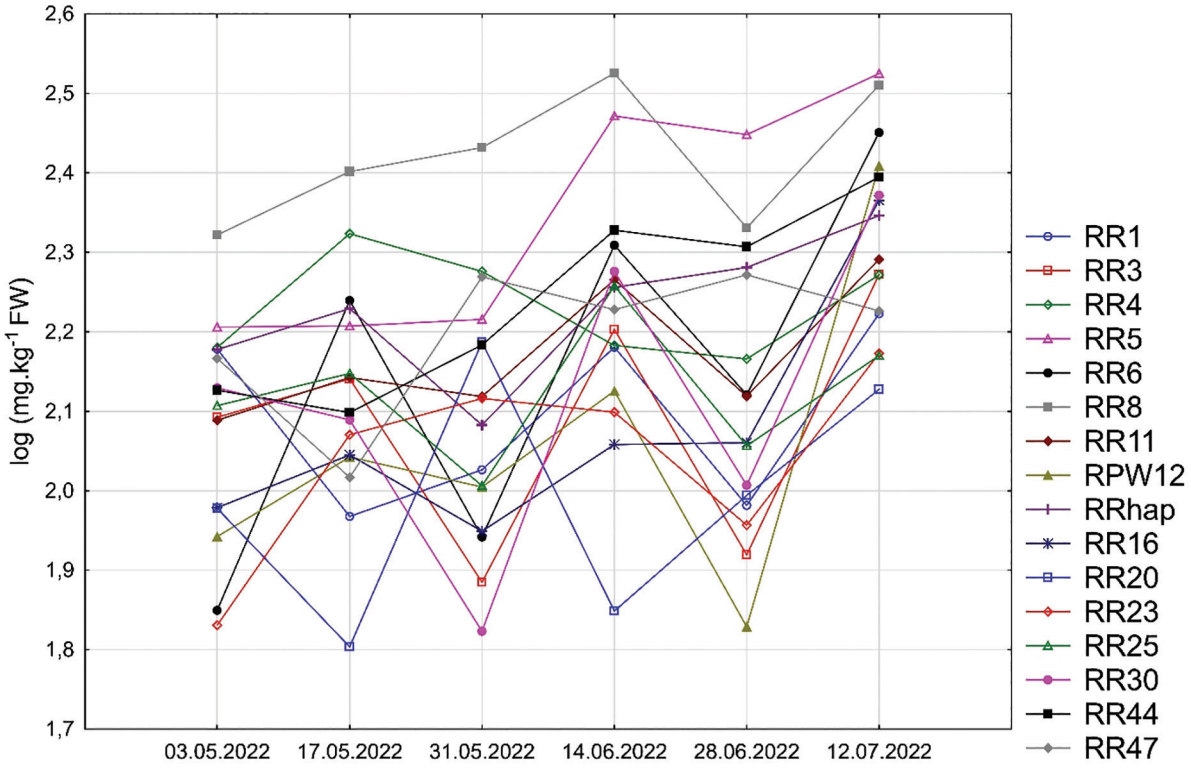
SUPPLEMENTARY MATERIALS

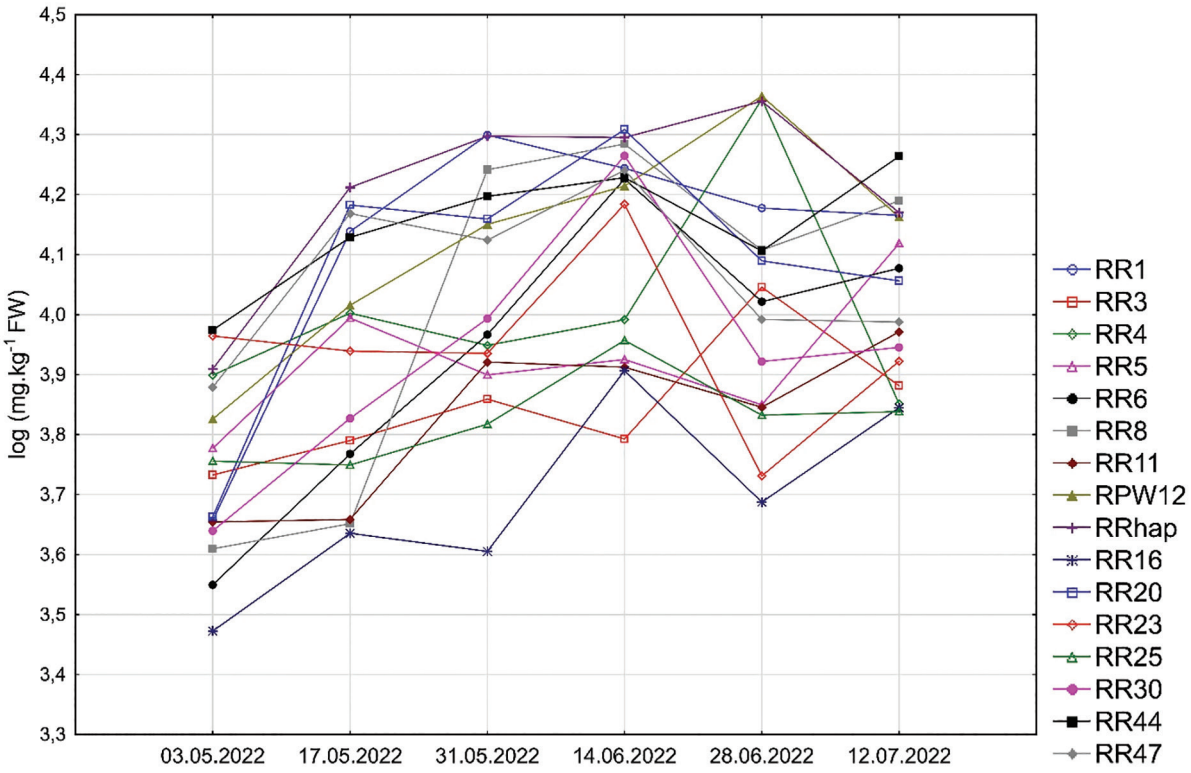
Supplementary Table 1. Weather conditions during sampling terms.

Sampling term	3.5.2022	17.5.2022	31.5.2022	14.6.2022	28.6.2022	12.7.2022
Period of meteorological data record	19.4.–2.5. 2022	3.5.–16.5. 2022	17.5.–30.5. 2022	31.5.–13.6. 2022	14.6.–27.6. 2022	28.6.–11.7. 2022
Day temperature average (°C)	11.09285714	17.28571429	16.57142857	19.85	21.15714	21.3
Maximum day temperature (°C)	17.1	24.09285714	22.86428571	26.1	28.24286	27.54286
Precipitation (mm)	11.8	16	9.5	49.7	28.1	21.2



Supplementary Graph 1. Content of L-ascorbic acid (mean values) in rhubarb accession during six sampling terms.

Explanatory notes		
42H7500001 *R. rhabarbarum* Jara	RRhap	42H7500014 *R. rhaponticum*
42H7500003 *R. rhabarbarum* Victoria	RR16	42H7500016 *R. rhabarbarum* Timperley Early
42H7500004 *R. rhabarbarum* Dawes Chalenge	RR20	42H7500020 *R. rhabarbarum*
42H7500005 *R. rhabarbarum* The Sutton	RR23	42H7500023 *R. rhabarbarum*
42H7500006 *R. rhabarbarum* Holsteiner Blut	RR25	42H7500025 *R. rhabarbarum* Glaskins Perpetual
42F7500008 *R. rhabarbarum*	RR30	42H7500030 *R. rhabarbarum* Krupnochereshkovyj
42H7500011 *R. rhabarbarum*	RR44	42H7500044 *R. rhabarbarum*
42H7500012 *R. palmatum* × *wittrockii*	RR47	42H7500047 *R. rhabarbarum*



Supplementary Graph 2. Content of oxalic acid (mean values) in rhubarb accession during six sampling terms (explanatory notes – see hereabove after Supplementary Graph 1).

Supplementary Table 2. Statistical differences in the content of L-ascorbic acid in *Rheum* accessions (red values are significant at α level 0.05).

Accession	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
42H7500001		0.998556	0.000000	0.000000	0.000000	0.000000	0.000000	0.000129	0.000000	0.384580	0.000000	0.000000	0.000557	0.034173	0.000000	0.000000
42H7500003	0.998556		0.000000	0.000000	0.000000	0.000000	0.000000	0.006179	0.000000	0.983678	0.000000	0.000000	0.00001	0.000861	0.000000	0.000000
42H7500004	0.000000	0.000000		0.000000	0.000000	0.000000	0.000000	0.000000	0.999997	0.000000	0.000000	0.000000	0.000000	0.000000	0.999775	0.000028
42H7500005	0.000000	0.000000	0.000000		0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
42H7500006	0.000000	0.000000	0.000000	0.000000		0.000000	0.182537	0.000000	0.000000	0.000000	0.000000	0.000000	0.004003	0.000052	0.000000	0.000001
42F7500008	0.000000	0.000000	0.000000	0.000000	0.000000		0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
42H7500011	0.000000	0.000000	0.000000	0.000000	0.182537	0.000000		0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
42H7500012	0.000129	0.006179	0.000000	0.000000	0.000000	0.000000	0.000000		0.000000	0.000000	0.000000	0.271620	0.000000	0.000000	0.000000	0.000000
42H7500014	0.000000	0.000000	0.999997	0.000000	0.000000	0.000000	0.000000	0.000000		0.000000	0.000000	0.000000	0.000000	0.000000	0.942159	0.000307
42H7500016	0.384580	0.983678	0.000000	0.000000	0.000000	0.000000	0.000000	0.311562	0.000000		0.000000	0.000000	0.000000	0.000006	0.000000	0.000000
42H7500020	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000		0.000000	0.000000	0.000000	0.000000	0.000000
42H7500023	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.271620	0.000000	0.000068	0.000000		0.000000	0.000000	0.000000	0.000000
42H7500025	0.000557	0.000000	0.000000	0.000000	0.004003	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000		0.995451	0.000000	0.000000
42H7500030	0.034173	0.000861	0.000000	0.000000	0.000052	0.000000	0.000000	0.000000	0.000000	0.000006	0.000000	0.000000	0.995451		0.000000	0.000000
42H7500044	0.000000	0.000000	0.999775	0.000000	0.000000	0.000000	0.000000	0.000000	0.942159	0.000000	0.000000	0.000000	0.000000	0.000000		0.000001
42H7500047	0.000000	0.000000	0.000028	0.000000	0.000001	0.000000	0.011563	0.000000	0.000307	0.000000	0.000000	0.000000	0.000000	0.000000	0.000001	

Supplementary Table 3. Correlation coefficient of L-ascorbic acid and oxalic acid in *Rheum rhubarbarum* accessions for individual harvesting terms (red values are significant at α level 0.05).

Accession	03.05.2022	17.05.2022	31.05.2022	14.06.2022	28.06.2022	12.07.2022
42H7500001	−0.999992	0.900138	0.722004	0.693218	0.462415	0.953726
42H7500003	−0.551535	−0.764839	−0.795861	−0.275743	−0.464849	0.268219
42H7500004	0.995162	−0.011417	−0.980462	0.010893	−0.918308	−0.259346
42H7500005	0.693380	0.721893	−0.840521	0.306089	0.418912	0.033346
42H7500006	−0.495380	0.999667	−0.972016	−0.170747	−0.111774	−0.181601
42F7500008	0.963472	−0.850060	0.519917	−0.255376	0.242785	−0.190471
42H7500011	0.992796	0.878552	−0.336647	0.878170	−0.757353	−0.978263
42H7500016	0.925750	−0.986940	−0.760368	0.630009	0.997758	−0.785389
42H7500020	−0.564960	−0.278109	0.000230	0.175259	0.988205	0.941517
42H7500023	0.822302	0.570501	0.699590	−0.974260	−0.875252	0.668926
42H7500025	0.291533	0.999393	−0.969792	0.992495	−0.888897	−0.005509
42H7500030	0.200544	0.729887	−0.707420	0.325131	0.744921	0.995225
42H7500044	−0.931785	0.999230	−0.097143	−0.026990	−0.694324	0.879198
42H7500047	0.787395	−0.138509	−0.524755	−0.340026	0.514848	−0.619189