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Research Paper

Evaluation of Buckwheat Genetic Resources in Slovenia within the ECOBREED Project

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ABSTRACT

Understanding the diversity in morpho-phenological characteristics, as well as variations in nutrients and nutraceutical compounds among the different buckwheat genetic resources, plays an important role in their effective use and breeding purposes. In 2020 and 2021, a total of 23 buckwheat accessions were grown in the Slovenian environment. During this period, selected morpho-phenological traits were evaluated together with the analysis of protein content, total content, antioxidant activity, and 17 different phenolic compounds. A remarkable variation in the thousand seed weight was observed between the years 2020 and 2021, while the protein content remained constant in both years and locations. Regarding the content of phenolic compounds, it was confirmed that buckwheat grains are an excellent source of these compounds. Among these compounds, rutin, vitexin, epicatechin, and orientin were identified as the predominant components in the grains. The evaluation of common buckwheat accessions revealed considerable diversity in the studied characteristics within the Slovenian environment. The varieties Sweden-1, ´Tempest, and Dozhdik performed well under the Slovenian conditions.

INTRODUCTION

Often overlooked or less acknowledged, certain crops that were significant components of ancient civilizations' diets are making a resurgence in modern times. Among these are pseudocereals, a classification that encompasses various lesser-known grains. One such pseudocereal is buckwheat (*Fagopyrum* Mill. Polygonaceae), a genus comprising fewer than 30 species, primarily distributed in China (Ohsako and Li, 2020). Within this genus, only two species, common buckwheat and Tartary buckwheat, hold culinary importance (Zhou et al., 2018). Common buckwheat (*Fagopyrum esculentum* Moench) is an annual dicotyledonous plant native to Yunnan Province, China (Ohnishi, 1990). It has considerable agricultural importance in regions such as China, Korea, Japan, Russia, the USA, Ukraine, and various parts of Europe (Ohnishi, 2016). Traditionally, buckwheat is cultivated for its nutritional value, as the hulled seeds contain several essential nutrients (Sytar, 2015; Singh et al., 2020). The protein content varies between 8.51% and 18.87% depending on the variety (Krkoskova and Mrazova, 2005). Buckwheat flour obtained by various milling methods consists of 70-91% (w/w) starch (Skrabanja et al., 2004). Research has identified rutin, vitexin, isovitexin, and hyperoside as the major phenolic compounds in four different buckwheat varieties (Kalinova et al., 2019).

Furthermore, numerous research publications have confirmed the extensive diversity of buckwheat, encompassing morphological and agronomic characteristics (Ghiselli et al., 2016; Tang et al., 2016; Rauf et al., 2020;), as well as nutrient composition (Bai et al., 2015) and content of health-promoting compounds (Kiprovski et al., 2015; Kalinova et al., 2019; Li et al., 2019). Notably, buckwheat exhibits mechanisms for drought avoidance, with its physiological parameters remaining largely unaffected even under extreme drought conditions (Martínez-Goñi et al., 2023). Buckwheat has been cultivated as a traditional crop in numerous countries, resulting in the development of numerous new varieties since the 1920s (Singh et al., 2020). Over the past three decades, extensive exploration of buckwheat's genetic resources has occurred, with a focus on identifying valuable traits for enhancing quality. Presently, more than 10,000 samples of buckwheat genetic resources are conserved and stored within various gene banks worldwide (Zhou et al., 2018). The genetic resources housed in these gene banks require thorough evaluation and proper characterization to ascertain the extent of their useful and valuable genetic diversity. This characterization and assessment process is vital prior to selecting suitable materials for specific purposes such as breeding (Hawkes et al., 2002).

One of the main objectives of the ECOBREED project was to assess buckwheat accessions from gene banks, with a specific focus on agro-morphological characteristics and the presence of particular compounds under Slovenian conditions. The recognition of the most promising accessions and the subsequent findings will assist buckwheat breeders in choosing the most suitable material customized for the Slovenian environment.

MATERIAL AND METHODS

A total of 23 common buckwheat accessions were utilized in this study (Table 1). Seventeen accessions were sourced from the Gene Bank of the Crop Research Institute, Czech Republic, while six accessions were obtained from commercial varieties purchased from seed companies.

At two different sites, which differ mainly in the course of weather during the growing season, 23 buckwheat accessions were selected for evaluation. In 2020, the evaluation site was in Krog ati Murska Sobota (GPS 46°38'04.7"N 16°08'13.6"E) at 189 m.a.s.l. and in 2021, the evaluation site wasin Jablje at Ljubljana (GPS 46°08'37.7"N, 14°33'24.5"E at 303 m.a.s.l. Meteo data were provided from the nearest meteorological station Brnik (for location Jablje) and from the meteorological station Rakičan (Krog). All samples were grown in two rows 1 m long, 25 cm apart, with 50 seeds per row. During the growing season, selected morphological and phenological traits were evaluated according to the List of Descriptors (IPGRI, 1994). The weather conditions of Krog and Jablje are described in Fig. 1 and Fig. 2, respectively. A representative sample of 10 g was taken from the harvest. The samples were then kept in cool, dark conditions at a temperature of -18 °C until to further laboratory processing.

The standards of the phenolic compounds apigenin, caffeic acid, catechin, chlorogenic acid, epicatechin, gallic acid, hesperidin, hyperoside, isoorientin, isoquercetin, iso-vitexin, kaempferol, naringenin, orientin, procyanidins B1 + B3, and procyanidin B2, quercetin, quercitrin, rutin, vitexin, and the internal standard probenecid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol (LC-MS grade, ≥99.9%) was obtained from Riedel de Haën (Seelze, Germany). Formic acid

Table 1. List of evaluated common buckwheat accessions.

No.	Accession Number	Accession Name	Country of origin
1	01Z5000001	AELITA	SUN
2	01Z5000007	Lada	SUN
3	01Z5000017	Monori	UKN
4	01Z5000047	Tokushima Zairai	JPN
5	01Z5000049	Yaita Zairai	JPN
6	01Z5000050	Stoyoama Zairai	JPN
7	01Z5000055	Arihira Zairai	JPN
8	01Z5000063	Pyra	CSK
9	01Z5000067	Chernigovskaya 17	UKR
10	01Z5000071	Dozhdik	RUS
11	01Z5000111	Emka	POL
12	01Z5000112	Gema	POL
13	01Z5000123	Kara-Dag	UKR
14	01Z5000137	Pulawska II	POL
15	01Z5000140	Tempest	CAN
16	01Z5000141	Sweden-1	SWE
17	01Z5000143	CD 7272	CSK
18	commercial variety	Bamby	AUT
19	commercial variety	Billy	AUT
20	commercial variety	Čebelica	SVN
21	commercial variety	La Harpe	FRA
22	commercial variety	Zita	CZE
23	commercial variety	Zoe	CZE

AUT-Austria, CAN-Canada, CSK-Czechoslovakia, CZE-Czech Republic, FRA-France, POL-Poland, JPN-Japan, RUS – Russian Federation, SUN-Former Soviet Union, SVN-Slovenia, SWE-Sweden, UKN-unknown, UKR-Ukraine

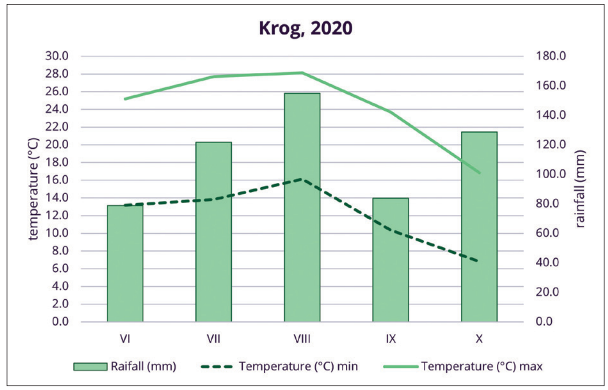

Fig. 1. *Weather conditions in Krog, Slovenia in 2020.*

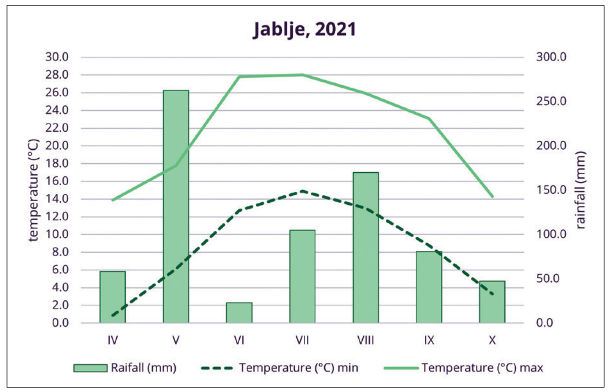

Fig. 2. *Weather conditions in Jablje, Slovenia in 2021.*

(LC-MS grade, 99%) was purchased from VWR (Leuven, Belgium). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Standard preparation, sample isolation, and UHPLC-ESI-MS/MS analysis were performed according to Janovská et al. 2021. The identification of the phenolic compounds in the buckwheat samples was based on their retention times in relation to the genuine standards and on the mass spectrometric data obtained through LC-MS. These data included a precise mass determination that lead to the derivation of the elemental composition and fragmentation patterns for the molecular ion. These LC-MS results were then cross-referenced with findings from earlier research involving Orbitrap analysis of phenolic compounds (Kiprovski et al., 2015; Huda, 2021. Calibration curves were constructed by plotting the peak area (normalized with probenecid as internal standard) against the concentration of the corresponding reference standard. Each sample was measured in at least three replicates. Data analysis was performed using Statistica 12 Software (TIBCO software, Palo Alto, CA, USA).

For the chemical analyses, the dry weight (dw) of the seed samples (5g) was dried in an electric hot-air dryer at 105 °C for 4h, according to the standard method CSN EN ISO 662. The content of protein (CP) was determined using the Kjeldahl mineralization method and calculated using the conversion factor 6.25 (EN ISO 5983-2 (467035). For determination of total phenolic content (TPC) Folin assay with slight modification was used (Holašová et al., 2002). Antioxidant activity (AA) was evaluated by DPPH assay (Şensoy et al., 2006). Analyses were performed in two replicates for each sample. Statistical analysis was mainly performed using the R programming language (R Development Core Team 2021) and Microsoft Office Excel v. 2016.

RESULTS AND DISCUSSION

Weather conditions in both Slovenian localities in 2020 and 2021 were characterized by constant temperature conditions with the highest values during summer months of July and August. More than 250 mm of precipitation fell in May 2021, in contrast to June 2021 when there was practically no rain. Rainfall in 2020 was evenly distributed over the months.

Buckwheat varieties reached higher plant height (PH) in 2021, but the highest value was observed in the Czech variety Pyra (126 cm) in 2020. There was only a slight difference observed between the mean values of PH in 2021 and 2020, measuring 106.22 cm and 105.70 cm, respectively. In 2020, the range of values of PH in Krog was not as wide as in Jablje in 2021. The height of buckwheat plants was significantly higher as compared to the local Himalayan buckwheat, as indicated by Sharma et al. (2023). However, the recorded values were close to those documented for 964 common buckwheat genotypes in China (Zhou et al., 2018) and buckwheat genotypes grown in the Czech Republic (Janovská et al., 2021) (Fig.3 and Fig.4).

The weight of thousand seeds (WTS) exhibited a remarkable increase in 2021 in Jablje, with the mean TSW of 24.05 g, in contrast to the previous year's mean of 14.85 g in 2020 in Krog, a difference was almost 10 g. It is possible, that the increased rainfall in May 2021, combined with slightly lower temperatures during August compared to 2020, contributed to improved seed development.The research of Janovská et al. (2021) also confirmed the significant differences observed between years and underlined the significant impact of weather conditions. In 2021, the Polish variety Emka exhibited the highest WTS of 36.1 g, while in 2020, the Japanese variety Tokushima Zairai had the highest value with a WTS of 20.8 g. Lower values of WTS were observed in local buckwheat varieties in the Eastern Himalayas in India (Sharma et al. 2023).

Further, protein content (CP), antioxidant activity (AA), total phenolic content (TPC), and 17 phenolic compounds were evaluated (Fig. 3 and Fig. 4). In general buckwheat grains are considered as high quality protein source (Eggum et al., 1980; Ikeda et al., 2002) with a balanced amino acid composition and with special bioactivities such as cholesterol lowering effect and improving constipation, antihypertensive effect together with influence on obesity by acting similar to dietary fiber (Rao and Poonia, 2023). Different research studies have reported diverse values for protein content, which according to Qin et al. (2010) showed a wide range from 8.06 to 12.44%, and an even wider range from 8.51 to 18.87% depending on the variety (Krkošková a Mrázová, 2005). Here the range was between 12.55 and 14.75% dw in 2021 and between 13.17 and 15.32% dw in 2020. The evaluated samples had higher content of protein in 2020 in Krog with a mean value 13.36% dw where the maximum value being reached in the varieties Sweden-1 and Tempest (15.32% and 15.28 % dw, respectively). In Jablje in 2021, the highest mean values reached Emka and Tokushima Zairai (14.75% and 14.4% dw, respectively).

The mean values obtained agrees well with the results of a study conducted under conditions in the Czech Republic (Janovská et al., 2021). The protein content seems to remain consistent across different years and environments.

The consumption of buckwheat grains and their positive impact on human health have been extensively documented in numerous studies, focusing on the favorable assessment of phenolic compound content and antioxidant activities (Sedej et al., 2012; Kalinová et al., 2019; Knez et al., 2023).

The mean TPC value of buckwheat grains was slightly higher in 2020 in Krog (40.12 mg GAE/g dw) than in 2021 in Jablje (39.37 mg GAE/g dw). The variety Emka had the highest content of TPC (48 mg GAE/g dw) in 2020, while the variety Lada (51.40 mg GAE/g dw) in 2021 in Jablje. In the case of AA the higher mean value (13.55 µmol TE/g dw) was obtained in 2020 while the mean value of AA was slightly lower (11.96 µmol TE/g dw) in 2021. These values are in agreement with the results of other studies (Li et al. 2013; Janovská et al., 2021). These findings from buckwheat samples cultivated in the Slovenian environment further support the notion that buckwheat grains serve as a valuable antioxidant source. In the overall evaluation of 17 phenolic compounds in the samples collected across both years and sites, rutin emerged as the predominant compound, followed by vitexin, epicatechin, and orientin. Hyperoside, catechin, and isovitexin

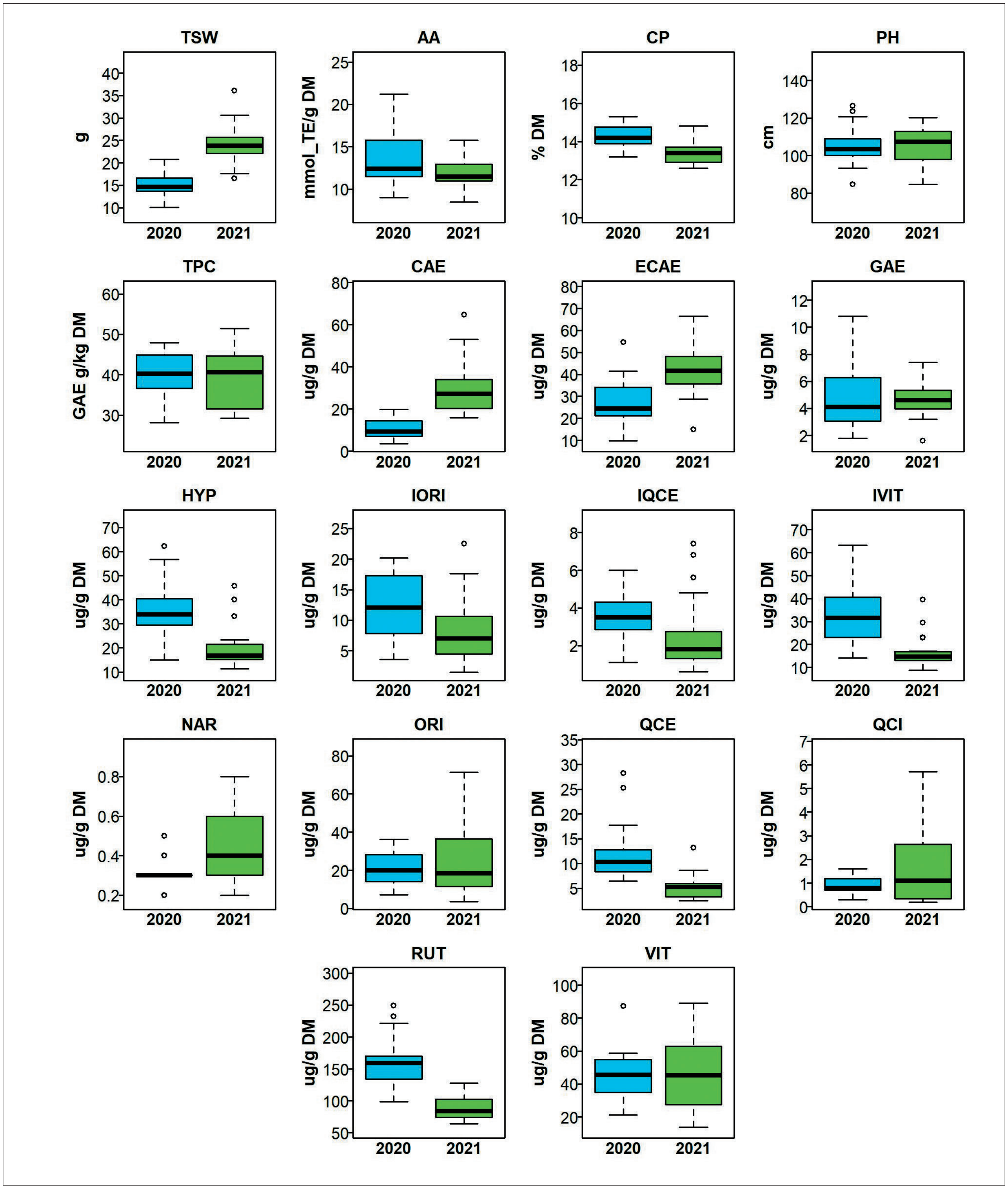

Fig. 3. Distribution of values for set of morpho-phenological parameters and selected chemical compounds measured in 23 common buckwheat accessions genotypes cultivated in Slovenia, 2020-2021. The abbreviation used in the diagram are as follows: Antioxidant activity (AA), Catechin (CAE), Crude_protein (CP), Epicatechin (ECAE), Gallic acid (GAE), Hyperoside (HYP), Isoorientin (IORI), Isoquercetin (IQCE), Isovitexin (IVIT), Naringenin (NAR), Orientin (ORI), Plant height (PH), Quercetin (QCE), Quercitrin (QCI), Rutin (RUT), Total phenolic content (TPC), Thousand seeds weight (TSW), Vitexin (VIT)

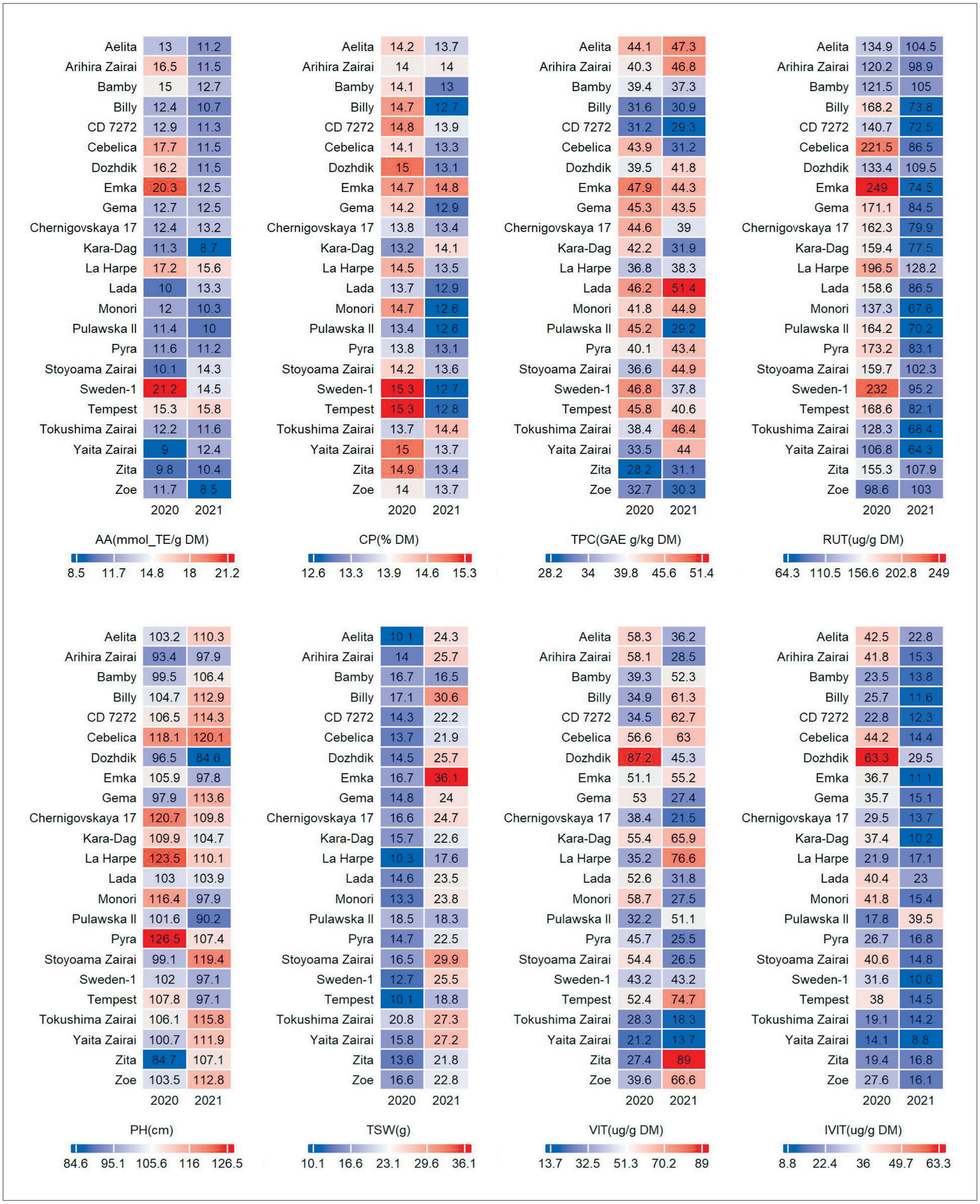

Fig. 4. *Heat maps showing the selected morpho-phenological parameters and chemical compounds for the collection of 23 common buckwheat accessions. Values for respective traits from individual years are displayed on a scale from blue (min) to red (max) in accordance with colour key below each heatmap.*

were present in relatively lower concentrations within the grains. Notably, buckwheat grains displayed minimal or trace amounts of quercetin, quercitrin, isoquercetin, isoorientin, gallic and caffeic acid, as well as apigenin. Based on the evaluation of all parameters for each accession, it is interesting to note that at each site, different accessions were among the best. While in Jablje in 2021 it was La Harpe, Dozhdik and Pulawska II, in Krog in 2020 it was Emka, Tempest and Sweden-1. However, all the varieties mentioned came in the top 10 of the ranking within the Slovenian conditions (Fig. 3).

The mean content of rutin was higher in 2020 (159.18 μg/g dw) than in 2021 (88.08 μg/g dw). The buckwheat plant probably faced more extreme weather conditions, with heavy rainfall in May 2021 contrary to drought in June 2021 while weather patterns were more balanced in 2020. The variety Emka had the highest content of rutin (249.00 μg/g dw in 2020) and, in 2021 the varie-

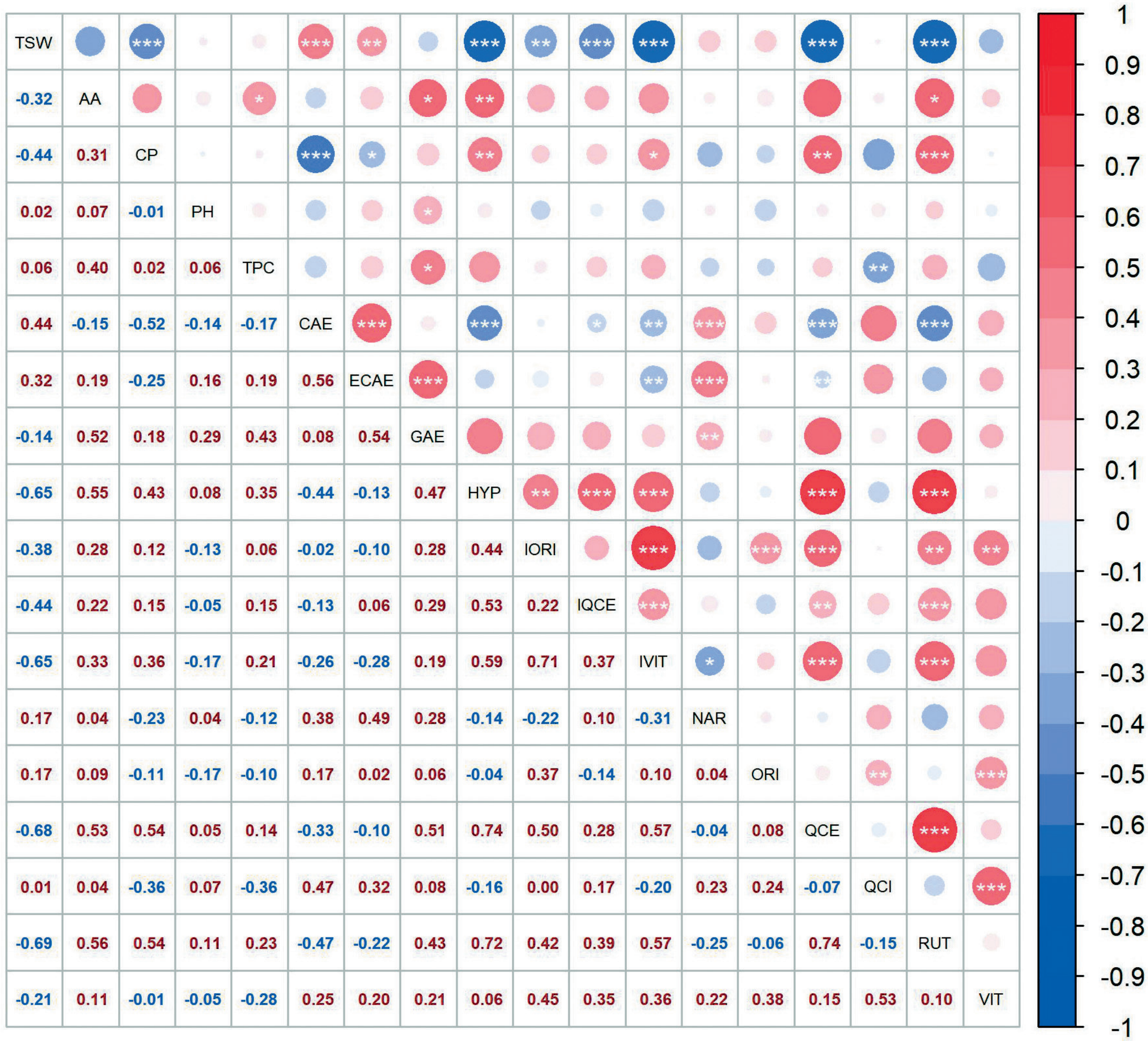

Fig. 5. *Spearman´s correlation between evaluated descriptors for the collection of common buckwheat accessions. The colour and size of circles above the diagonal determine level of negative (blue) or positive (red) correlation between the pairs of descriptors as illustrated by colour key and correlation values below diagonal. Significant correlations are represented in the circles by * (p < 0.05), ** (p < 0.01), and *** (p < 0.001), respectively.*

ty La Harpe exhibited the highest rutin content (128.00 μg/g dw). Similarly, buckwheat for grains cultivated in the regions of Central and South Italy exhibited closely comparable rutin levels in both the Emka and La Harpe varieties (Brunori and Vegvari, 2007).

A positive correlation was found between the content of rutin and hyperoside, hyperoside and quercetin, quercetin and isovitexin, and isovitexin and isorientin. Conversely, a negative correlation was found between the thousand seed weight (TSW) and the contents of rutin, quercetin, isovitexin, and hyperoside (Fig. 5).

The obtained results reveal a significant range of variation among the evaluated buckwheat varieties in the assessed traits. This diversity can serve as a basis for future efforts in breeding and the selection of appropriate varieties well-suited to varied environments. Substantial evidence exists to demonstrate that buckwheat is capable of thriving in extreme weather conditions, encompassing abundant rainfall on the one hand and drought on the other. The need of conserving germplasm is to safeguard genetic resources encompassing numerous important traits, which can be strategically integrated into existing varieties when the need arises (Yu et al., 2023).

CONCLUSION

Essentially, the assessment of plant genetic resources within the framework of the ECOBREED project extends beyond a mere scientific pursuit. By uncovering the hidden potential of buckwheat accessions and matching them with Slovenian conditions, this research serves as a valuable basis for sustainable and targeted breeding efforts that promote the development of resilient crops that can thrive and contribute to the agricultural landscape of Slovenia.

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ABBREVIATIONS

PH—plant height, CFA—Caffeic acid, GAE—Gallic acid, CAE—Catechin, HYP—Hyperoside, CGA—Chlorogenic acid, ECAE—Epicatechin, IORI—Isoorientin, ORI—Orientin, IQ— Isoquercetin, VIT—Vitexin, IVIT—Isovitexin, RUT—Rutin, QCE—Quercetin, QCI—Quercitrin, NAR—naringenin, TPC—Total polyphenols, WTS—weight of thousand seeds, AA—Antioxidant activity, CP— Crude protein, DPPH—2,2-diphenyl-1-picrylhydrazyl.

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IZVLEČEK

Vrednotenje genskih virov ajde v Sloveniji v okviru projekta ECOBREED

Razumevanje raznolikosti morfofenoloških značilnosti ter razlik v vsebnosti hranil oziroma nutrientov med različnimi genskimi viri ajde je pomembno za njihovo učinkovito uporabo in žlahtnjenje. V letih 2020 in 2021 je bilo v slovenskem okolju poskusno pridelovanih skupno 23 akcesij ajde. Pri tem so bile ovrednotene izbrane morfofenološke lastnosti skupaj z analizo vsebnosti beljakovin, antioksidativnih aktivnosti in vsebnosti 17 različnih fenolnih spojin. Med letoma 2020 in 2021 in lokacijama je bila opažena izjemna razlika v masi tisoč semen, vsebnost beljakovin pa je ostala konstantna v letih oziroma na lokacijah. Glede vsebnosti fenolnih spojin je bilo potrjeno, da so ajdova zrna odličen vir teh snovi. Med temi spojinami so rutin, vitexin, epikatehin in orientin prevladujoče fenolne snovi v zrnih. Vrednotenje akcesij ajde je pokazalo precejšnjo raznolikost preučevanih lastnosti v slovenskem okolju. Sorte Sweden-1, ´Tempest in Dozhdik so v slovenskih razmerah pokazale dobre rezultate.