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Front page photo: Picture of strong PHS line in buckwheat (See Fig. 3 in paper of Suzuki et al. in this issue).

FAGOPYRUM volume 40 (2), September 2023

CONTENTS

REVIEW

Buckwheat Breeding and Seed Production in Poland

Jacek KWIATKOWSKI 29

RESEARCH PAPER

Effects of Rutin and Selenomethionine in Selenium-Enriched Tartary Buckwheat Roasted Grain Tea on Glucose and Lipid Metabolism in db/db Mice

QianWen NIU, Rui DONG, JunJun LI, Pai PENG, Maria Cristina MESSIA, XinZhong HU 41

RESEARCH PAPER

The Quality of Pasta with the Addition of Buckwheat Microgreens or Sprouts

Jana PEXO VÁ KALINOVÁ, Mayowa OLADELE NOJ EEM 55

REVIEW

Breeding of Buckwheat in Kyushu Okinawa Agricultural Research Center, NARO

Tatsuro SUZUKI, Kenjiro KATUS, Nobuo KOCHI, Atsushi HAYASHI 61

RESEARCH PAPER

Evaluation of Buckwheat Genetic Resources in Slovenia within the ECOBREED Project

Aleš KOLMANIČ, Primož TITAN, Pavel SVOBODA, Michal JÁGR, Václav DVOŘÁČEK, Vladimír MEGLIČ, Petra HLÁSNÁ ČEPKOVÁ, Dagmar JANO VSKÁ 67

Review

Buckwheat Breeding and Seed Production in Poland

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ABSTRACT

The history of buckwheat breeding in Poland is almost 80 years old. “Hruszowska” was the first breeding variety, which was in 1956 entered to The Polish National List of Agricultural Plant Varieties (NLI). In the history of buckwheat breeding programs in Poland, 10 varieties have been registered so far. Additionally, 3 breeding lines have been entered into official tests without a decision on entry to the NLI. During the planned economy period in Poland (until 1989), despite the limited number of varieties, the scale of production of certified buckwheat seeds practically covered the entire demand for sowing material. Currently, the small scale of buckwheat cultivation in Poland, and above all, the lack of buckwheat on the EU list of plant species varieties which are subject to national listing and which seed material may be produced, assessed, and controlled limit the scope of breeding work on buckwheat and make it difficult to obtain profitable seed trade.

INTRODUCTION

Probable traces of buckwheat in Poland date back to around 400 BC. (Gibert, 1956). Badura's (1999) reports on the presence of remains of *Fagopyrum esculentum* in two archaeological sites dating back to the 6th-10th century AD and the remains of common buckwheat from the Wolin island dated to the 9th century (Alsleben, 1995) may indicate that its cultivation was known in these areas even before the invasions of the Mongol tribes, which is generally accepted as a buckwheat source in Europe. During the wars with the Tatars (13th century), buckwheat cultivation was popularized and intensified (Badura, 1999; Koszałka and Strzelczyk, 2015).

Materials and Methods

The history of buckwheat breeding and cultivation in Poland, the number of registered varieties and variety movement in the The Polish National List of Agricultural Plant Varieties were presented on the basis of a query of archival materials and old prints as well as legal acts related to the subject and current publications.

Cultivars' characteristics were based on literature sources, source data from Research Centre for Cultivar Testing (COBORU), and accession descriptions from the National Center for Plant Genetic Resources: Polish Genebank (KCRZG). The scale of production was presented on the basis of source data from Statistics Poland (GUS)

and FAOSTAT and a query of historical studies. The characteristics of the seed market were made using source data obtained from Plant Health and Seed Inspection Service (PIORiN).

RESULTS

Buckwheat production in Poland

The first records of buckwheat cultivation in Poland date back to the 15th century (Zemanek et al., 2009). Buckwheat has always been a minor crop. In the agricultural literature of the sixteenth century, it was not recommended to grow it in larger areas but generally in areas after frozen winter crops (Gostomski, 1588). Buckwheat growing was in Poland mostly spread in the 17th century, and since then, it has been gradually replaced by oats (Król, 1986). In the interwar period (1920-1939), its cultivation area was about 150 thousand ha, falling three times in the second half of the twentieth century.

Currently, buckwheat is cultivated in Poland in an area of approx. 114,000 hectares, this is 1.6% in the entire sowing structure and slightly more than 1% in the cereal sowing structure (Fig. 1).

The scale of buckwheat production in Poland corresponds to the size of the sown area, due to the seed yield

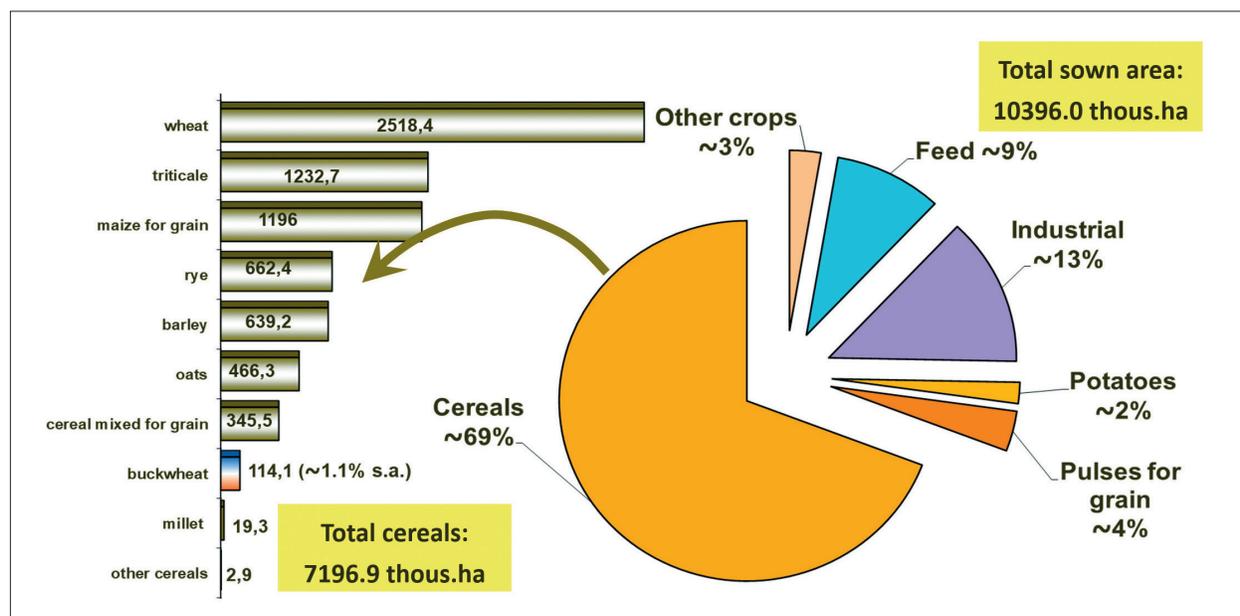


Fig. 1. Sown area in Poland (2022) (Source: own elaboration based on data provided by GUS 2023 (Statistics Poland))

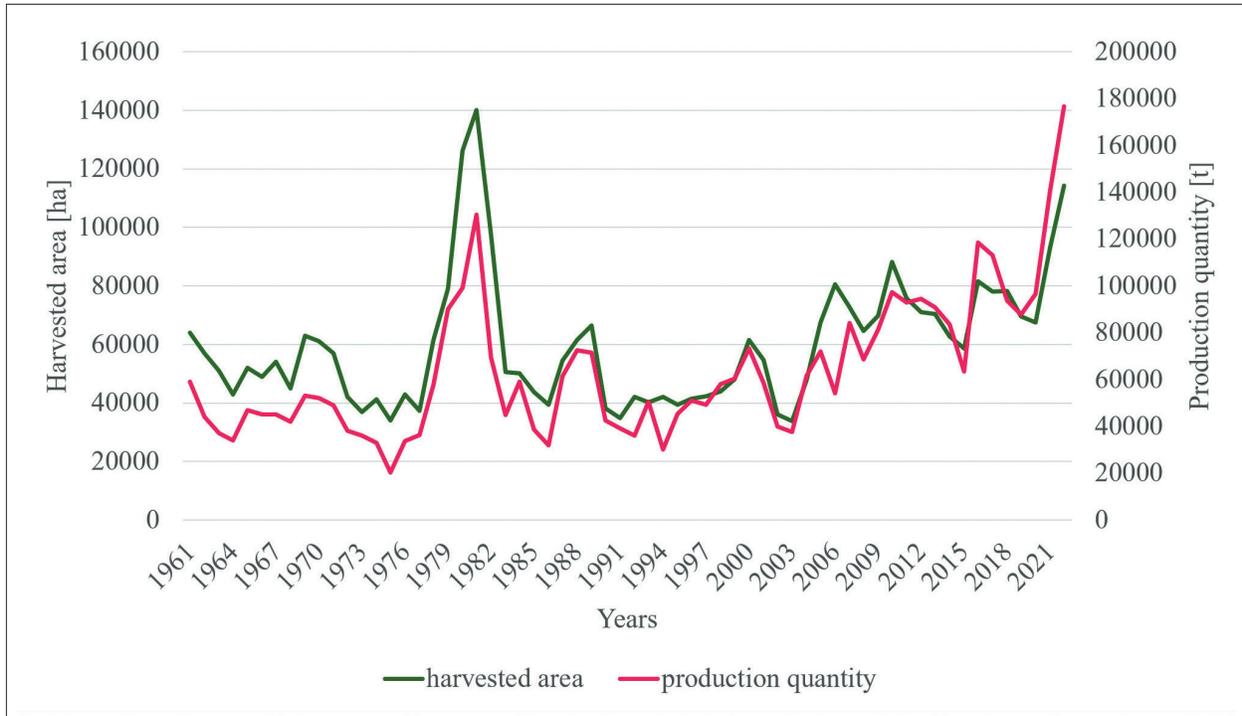


Fig. 2. Harvested area and production quantity of buckwheat in Poland (1961-2022) (Source: own elaboration based on data provided by GUS and FAOSTAT)

oscillating around 1 t per hectare. Until the 1980s, buckwheat production per hectare did not exceed 1 t per ha, which reduced the scale of production. In the last decade, there has been a clear improvement in the yield of this pseudocereal (Fig. 2).

Buckwheat cultivation in Poland is concentrated primarily in the east and north of Poland. The traditional production area remains the Lublin region. The Dolnośląskie Voivodeship also has a relatively large share in buckwheat production (Fig. 3). In addition to historical

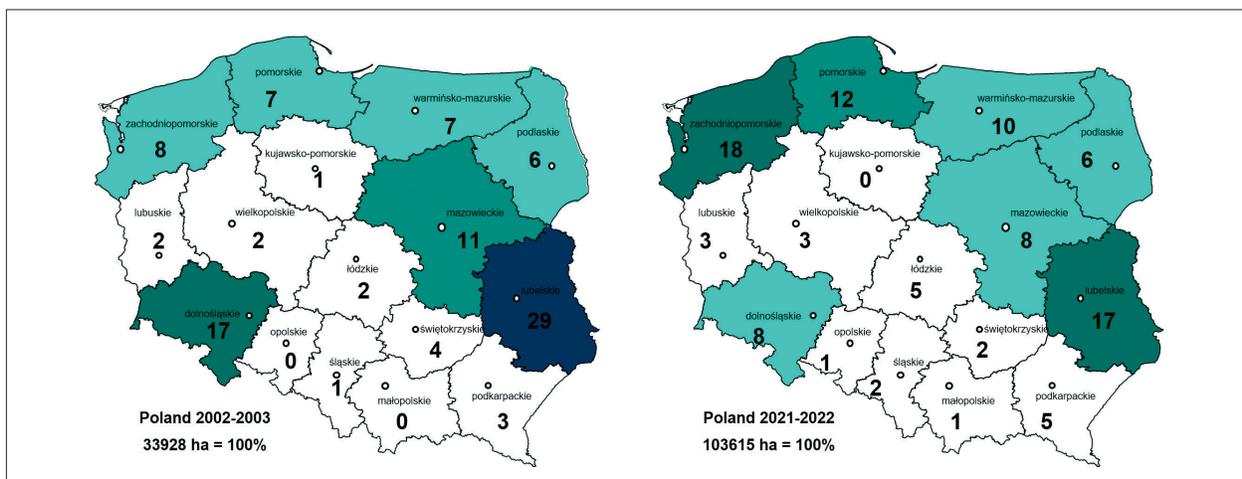


Fig. 3. Distribution of buckwheat growing in Poland (the average for the years 2002-2003 and 2021-2022, compiled based on data from the GUS 2023)

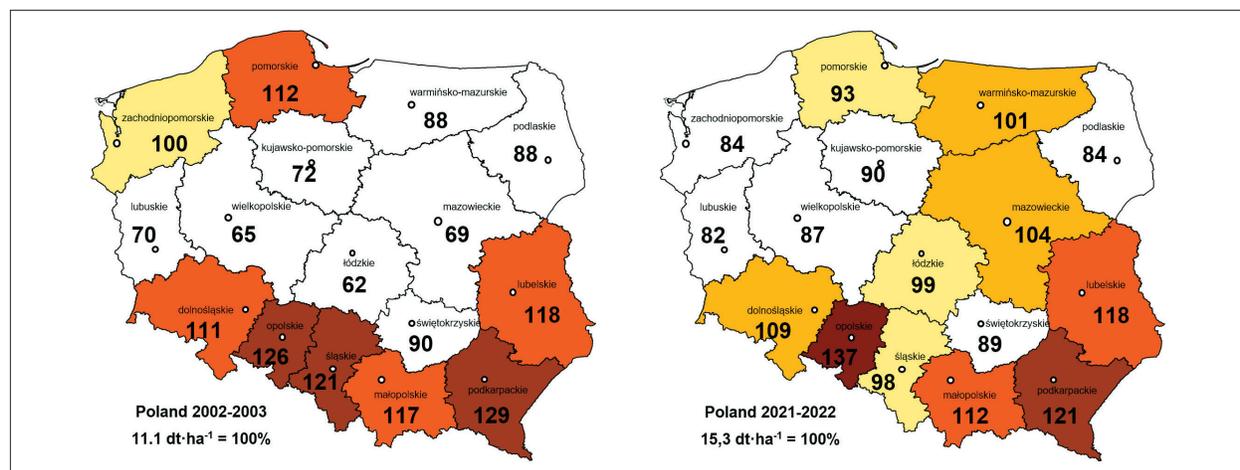


Fig. 4. Relative yields of buckwheat in Poland (the average for the years 2002-2003 and 2021-2022, compiled based on data from the GUS 2023)

traditions, the location of the buckwheat processing industry in Poland still has a significant impact on the distribution of buckwheat cultivation.

The highest yields are obtained in the south-east and south of the country, but considering the scale of production, attention should again be paid to the Lubelskie region and Lower Silesian. In the remaining southern regions, the high productivity reported in statistical reports is due to the extremely small size of crops (single plantations) and not to the mass scale of buckwheat production (Fig. 4).

Buckwheat breeding in Poland

The buckwheat breeding program in Poland began late, compared to other species. In the Polish agricultural literature from the nineteenth century, there are descriptions of two varieties of buckwheat, but this applies to ecotypes and not agricultural cultivars (Tab. 1).

The first form was called black or common buckwheat, with dark brown or black seeds. The second form was called Scottish or Swedish, or silver buckwheat. It was characterized by a light gray color of seeds (less fertile, with higher soil requirements, maturing later, with

Table 1. Species and forms of cultivated buckwheat described in agricultural literature at the turn of the 19th and 20th centuries

Species	Forms	Additional information
Common buckwheat <i>(Fagopyrum esculentum Moench)</i> Tatarka, Hreczka, Gryka	Czarna (Black) or Zwyczajna (Common)	dark brown, almost black small achenes, good yielding form
	Szkocka (Scotch) or Szwedzka (Swedish) or Srebrna (Silver)	the light gray color of achenes, less fertile, with higher soil requirements, later maturing, with larger seeds
Tartary buckwheat <i>(Fagopyrum tataricum (L.) Gaertn.)</i> Tatarka, Gryka syberyjska (Siberian buckwheat) Chińska tatarka (Chinese buckwheat) Rdest tatarski (Tatar knotweed) (<i>Polygonum tataricum</i> or <i>P. emarginatum Rth.</i>)	cultivated and weed	high green biomass, low yield of achenes, late ripening; cultivated to obtain green biomass for fodder; some of its forms as a weed in the cultivation of common buckwheat

finer seeds) (Kowalski, 1885; Jachner 1920). Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn), also known as Siberian buckwheat or Chinese tartare or Tartar knotweed (*Polygonum tataricum* or *P. emarginatum* Rth.) used for green fodder was also cultivated. Even then, attention was paid to the form of Tartary buckwheat, which appeared as a weed in buckwheat fields (Krafft 1876).

In the late thirties of the twentieth century, preliminary breeding studies began at the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy, failing to create expected cultivars (Arseniuk et al., 2009). Breeding work returned at the turn of the 40s and 50s of the twentieth century. In the first period, 9 local buckwheat ecotypes (created by farmers as a result of long-term selection) were collected (Ruszkowski, 1955; Ruebenbauer and Brej, 1957). They were characterized by a relatively short vegetation period, a short stem and little branching, and a low 1,000 seed weight (Ruszkowski, 1983) (Tab. 2).

The first Polish cultivated buckwheat varieties created on the basis of the selection of the best individuals within local ecotypes were: “Hruszowska” (1957), “Puławska” (1958), “Modzurowska” (year not known),

and “Dańkowska” (1963) (Król, 1986). In the 1960s, new breeding directions were set and the new methods used in buckwheat breeding were extended. In 1969, the first tetraploid variety “Emka” was registered. It was created by genomic mutation by doubling the number of chromosomes using colchicine (Komenda-Ronka et al., 1994). Due to the larger size of achenes, requiring changes in the buckwheat processing and a higher share of husks, the variety did not gain the interest of farmers and processors, and work on further polyploids was limited (Tab. 3) (COBORU, 1980; 1983; 1985; 1987; Komenda-Ronka et al., 1994).

Ather Polish varieties (Kora, Luba, Panda) were created by crossing ecologically distant forms. At the turn of the 70s and 80s, mutagenesis developed in Polish breeding of buckwheat (Samborska-Ciania, 1999). The work led to the development of two lines (Gema and Green Corolla), with increased inflorescences and achenes on the plant and thin husk (Komenda-Ronka et al., 1994). In parallel, work was carried out on homostyle self-fertile forms. Despite high hopes placed in this direction of breeding (synthetic, hybrid varieties), it did not bring the desired results (Ruszkowski and Noworolnik, 1983).

Table 2. Local ecotypes of buckwheat grown in Poland until the 1960s (Source: Ruszkowski, 1983)

Ecotype	Vegetation period	Plant height [cm]	1000 seed weight [g]	Soil requirements	Additional information
Borówka	short	25-45	18-24	light soil	Small, dark brown achenes. High yield - 2.0 t·ha ⁻¹ , high yield of groats
Majówka	long	50-70	22-23	from light to heavy soil	Light brown achenes, small yield of achenes and groats
Ruska	long	do 100	15-17		Unstable yield
Podolanka	short	do 40	18-20	light soil	Brown achenes with wings. Stable and good yielding ecotype, also like a cath crop
Holewica	Medium long	do 55	22-23	Wet sandy soil on slope	Very easy infested by Tartary buckwheat
Różowa (Pink)	long	45-55	ok. 20		Pink perianth ecotyp. Dark brown achenes, strongly embedded in the bottom of flower
Biała (White)	short	do 50 cm	20-21		Uniform ripening, brown achenes
Siwa (Gray)	middle	70-80	17-19	light also new soil	Gray-brown achenes with wings, embedded in all inflorescences, without shattering.
Czarna (Black)	short	30-40	13-18	light soil	Uniform ripening, dark brown almost black achenes

Table 3. Polish cultivars of buckwheat (Source: own study based on the : COBORU, 1970; 1976; 1993; 1996; 1998; 2017; 2019; 2023; Arseniuk i in. 2003; Suchecki, 2008; Monitor Polski, 1958; 1959; 1964; 1970; Plant Breeding and Acclimatization Institute – NRI/National Centre for plant genetic resources data base, access: May 2023)

Cultivar	Entry date	Date of deletion	Breeder	Author of cultivar	Description
Hruszowska	1957	2008	Stacja Hodowli Roślin Hruszów	Alina Dziubikowska, A. Bęcik, Bogusław Komenda, Kalina Komenda, J. Got, W. Got	ecotypes from Rejowiec, Hruszów, Radzyń Podlaski and Puławy region
Puławska	1958	1976	IHAR Puławy/ Staszówek/ Chodów	Stefan Lewicki, Marek Ruszkowski	Selection from domestic local population (Lublin region)
Modzurowska	?*	?*	Centralne Zrzeszenie Selekcji Roślin	?*	?*
Dańkowska	1963	1969	Stacja Hodowli Roślin Dańków	Stanisław Janasz, Piotr Kolago, Tadeusz Wolski, Stanisław Ramenda	Selection from domestic local ecotypes (Gościeradowo and Nowe Miasto regions).
Emka (4x)	1969	1996	Stacja Hodowli Roślin Jeleniec	Kalina Komenda, Bogusław Komenda, Mirosław Łapiński	Krupnieplodnaja 4n x form of 4n from Hruszowska cv.
JEC 179	1980	1983	Stacja Hodowli Roślin Jeleniec		Breeding line applied for addition to NLI (The Polish National List of Agricultural Plant Varieties) without acceptance for the official testing
JEC 284	1985	1987	Stacja Hodowli Roślin Jeleniec		Tetraploid (4n) Breeding line applied for addition to NLI without acceptance for the official testing
Kora	1993 2017	2004 (2027)	Stacja Hodowli Roślin Jeleniec / Palikije / Małopolska Hodowla Roślin Spółka z o.o.	Bogusław Komenda, Kalina Komenda, Joanna Komenda-Ronka (Wolińska)	Cultivar's cross
Luba	1998	2004	Stacja Hodowli Roślin Palikije	Bogusław Komenda, Szymon Suchecki	Cross of Jubilejnaja with Hruszowska and own breeding materials
Panda	1998 2017	2004 (2027)	Stacja Hodowli Roślin Palikije / Małopolska Hodowla Roślin Spółka z o.o.	Bogusław Komenda, Szymon Suchecki	Cross within own breeding materials
MHR Korona	2019	(2029)	Małopolska Hodowla Roślin Spółka z o.o.	Szymon Suchecki?*	Cross of Koto x Kora
MHR Smuga	2019	(2029)	Małopolska Hodowla Roślin Spółka z o.o.	Szymon Suchecki?*	Cross of Smugljanka x Kora
GK/2022/1	2022		"Pietrzak" sp. z o.o. sp. k.		Breeding line applied for addition to NLI and accepted in 2023 for the official testing. Origin?

* In the table are question marks where the information is not confirmed

Table 4. Yield of buckwheat seeds in official cultivar studies (Source: own calculations based on COBORU 2015; 2018 data)

Cultivar	Breeder	Seed yield					
		dt·ha ⁻¹					
		2014	2015	2016	2017	2018	Mean
Standard*	MHR, Poland	19.9 (8.1-26.5)	16.2 (9.0-29.0)	21.9 (7.6-28.3)	17.0 (9.2-25.2)	17.3 (10.6-31.4)	18.5
Kora		20.4	16.0	22.4	17.3	17.3	18.7
Panda		18.7	16.2	22.0	16.7	17.3	18.2
PA 13		20.6	16.6				18.6
PA 14				20.2	16.4	18.2	18.3
PA 15			15.8	23.3	17.4	18.7	18.8
		% of standard					
Standard*	MHR, Poland	19.9	16.2	21.9	17.0	17.3	18.5
Kora		103	98	102	102	100	101
Panda		94	99	100	98	100	98
PA 13		103	102				103
PA 14				92	97	105	98
PA 15			98	106	102	108	104
Yield in production areas (dt·ha ⁻¹)		13.3	10.9	14.6	14.5	12.0	13.1
Yield of standard vs. production (%)		150	149	150	117	144	141

*Standard: 2014-2016 – Kora, Panda, PA 13; 2017-2018 – Kora, Panda

After the political and economic transformation in Poland (1989/1990), breeding programs of buckwheat were suspended, and only conservative breeding of owned varieties was carried out, as well as reproduction and evaluation of buckwheat collection from the resources of the Polish gene bank (Suchecki, 2006).

In 2008, the breeding of buckwheat was taken over by a strong, professional breeding company – Małopolska Hodowla Roślin (Małopolska Plant Breeding Company) as a result of consolidation in the Polish breeding and seed sector. It should be mentioned that after Polish accession to the European Union, the situation in buckwheat breeding was difficult because buckwheat was excluded from the varieties registration and seed certification system. Only changes in seed regulations in 2016 allowed for the registration of buckwheat varieties as a species traditionally cultivated in Poland. This change and the new company's strategy resulted in the introduction of new buckwheat varieties MHR Smuga and MHR Korona, to the Polish official catalogue. Cur-

rently, another breeding line (GK/2022/1) from one of the seed companies from Mazovia has appeared in the registry research.

The results of official variety research show high variability in seed yields of buckwheat varieties and new breeding lines between years and places of experiment (Tab. 4).

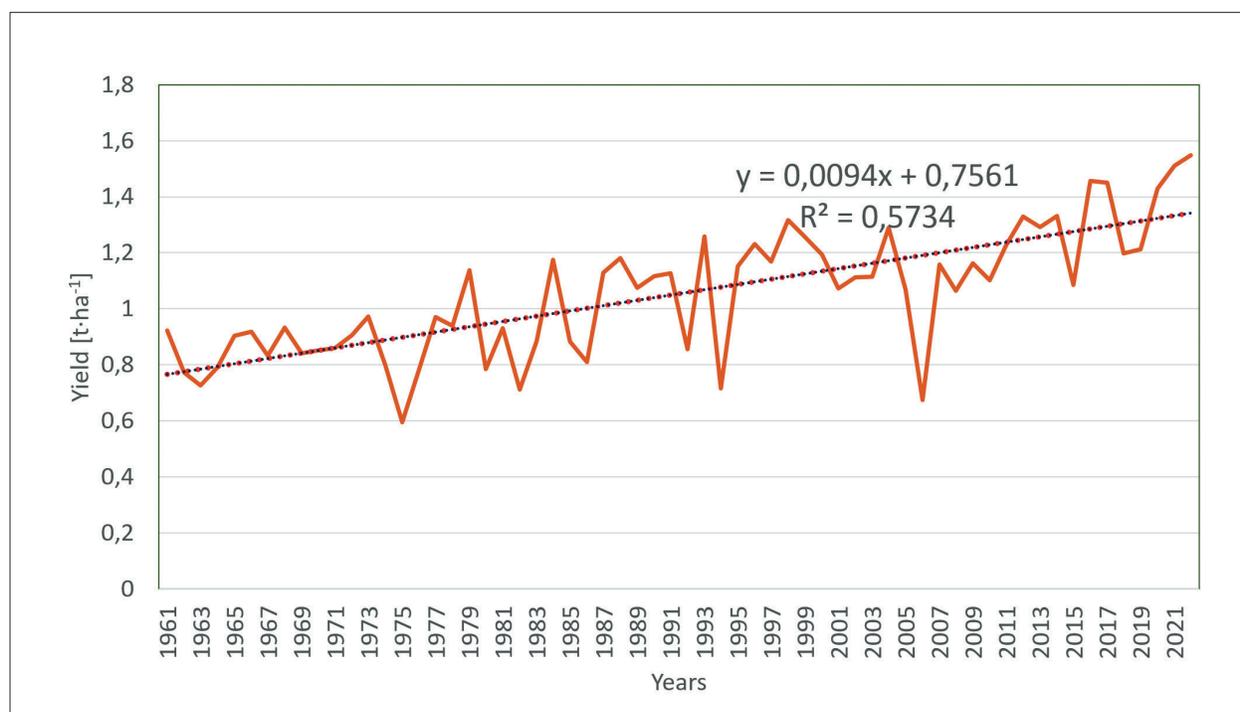
The same is in the results of organic variety testing of buckwheat (Tab. 5).

Although the progress in buckwheat breeding is not big, new varieties are gradually entering into agricultural practice. The introduction of new varieties to production has contributed to a systematic increase in the yield. Although it is very small - an average of about 9.5 kg per hectare per year, the trend is clear (Fig. 5).

For almost 70 years, buckwheat breeding was carried out primarily by the state-owned company Lublin Plant Breeding in the Lublin region (Plant Breeding Station in Hruszów, Jeleniec, Palikije). Over the past 15 years, a breeding program has been conducted at the Malopol-

Table 5. Yield of buckwheat seeds in ecological cultivar experiments in 2022 (Source: COBORU 2023)

Lp.	Cultivar	Białogard		Krzyżewo		Śrem Wójt.		Lućmierz		Cicibór duży		Mean	
		dt·ha ⁻¹	% of standard										
	Standard	14.4		7.5		8.9		25.5		17.3		16.2	
1	Kora	14.0	97	8	108	7.7	87	24.3	95	18.1	104	-0.1	99
2	Panda	15.0	104	7.3	98	8.6	97	24.8	97	17.9	103	0.1	100
3	MHR Korona	14.4	100	8	108	10.8	121	26.5	104	17.4	100	0.4	102
4	MHR Smuga	14.2	99	6.5	87	8.3	94	26.5	104	15.9	92	-0.4	98

**Fig. 5.** Yield of buckwheat achenes in production [t·ha⁻¹] (Source: own elaboration based on data provided by GUS and FAOSTAT)

ska Plant Breeding Company (Plant Breeding Station in Nieznanice).

Buckwheat seed market in Poland

Seed is the carrier of genetic potential for the performance of a crop. Buckwheat seed sector in Poland preceded its breeding by almost a century. From the early periods of buckwheat cultivation to the beginning of

the 19th century, when the foundations of the seed industry began to take shape, farmers used their harvested seeds for sowing. At that time, many farmers believed that only small sized buckwheat seeds should be used for sowing (Sempołowski 1902, Miczyński 1909, Komenda et al. 1994). This view was due to two reasons. The first concerned the volumetric measure of seeds for sowing. The same volume contained more fine seeds, which, un-

der favorable conditions, resulted in a higher density of plants per area unit than large, heavy seeds. The second premise resulted from frequent weed infestation of buckwheat plantations with Tartary buckwheat plants. Tartary buckwheat seeds (with wings) are similar in size to good buckwheat seeds, and therefore when using small common buckwheat seeds for sowing, there was no Tartary buckwheat in the sowing material (Komenda et al. 1994).

With the development of seed testing stations in Europe (including Poland land) in the second half of the nineteenth century, this species' seed samples were submitted for testing. These were few samples because at that time mainly the seeds of small-seeded *Fabaceae* species were tested. However, they show that attention was paid to the quality of the seed material of this cereal. At the end of the 19th century, Sempołowski (1899) based on the testing of many seed samples, proposed the following quality requirements for buckwheat seeds: germination - minimum 80% and purity - minimum 98%. These requirements were slightly stricter (seed purity)

than those in force today in Poland. The system of seed quality standards evolved, and at the beginning of the 20th century, the Polish seed market introduced quality requirements for buckwheat seeds with three quality levels: high (germination – 96%, purity - 98%), medium (90 and 93%) and the lowest (80 and 90%) (Jachner 1920). The results of buckwheat seed quality published in the years 1913-1915 indicate high germination capacity (98-99%) and sufficient purity (83.9-98.4%) of buckwheat seed material (Grabowski 1920).

In 1994-2003 the area allocated to buckwheat seed production ranged from 169 ha in 2002 to 1129 ha in 2000. The leading varieties in reproduction were: Hruszowska, Kora, and later Panda. The weight of certified seed varied from 1996 to 2002, and nearly five-fold differences were observed between the years (Fig. 6).

Following the harmonization of Polish seed laws with EU regulations (2004), buckwheat was removed from the official crop list, and selected varieties were deprived of legal production (no novelty varieties). No official controls were imposed on seed production, supplies were not

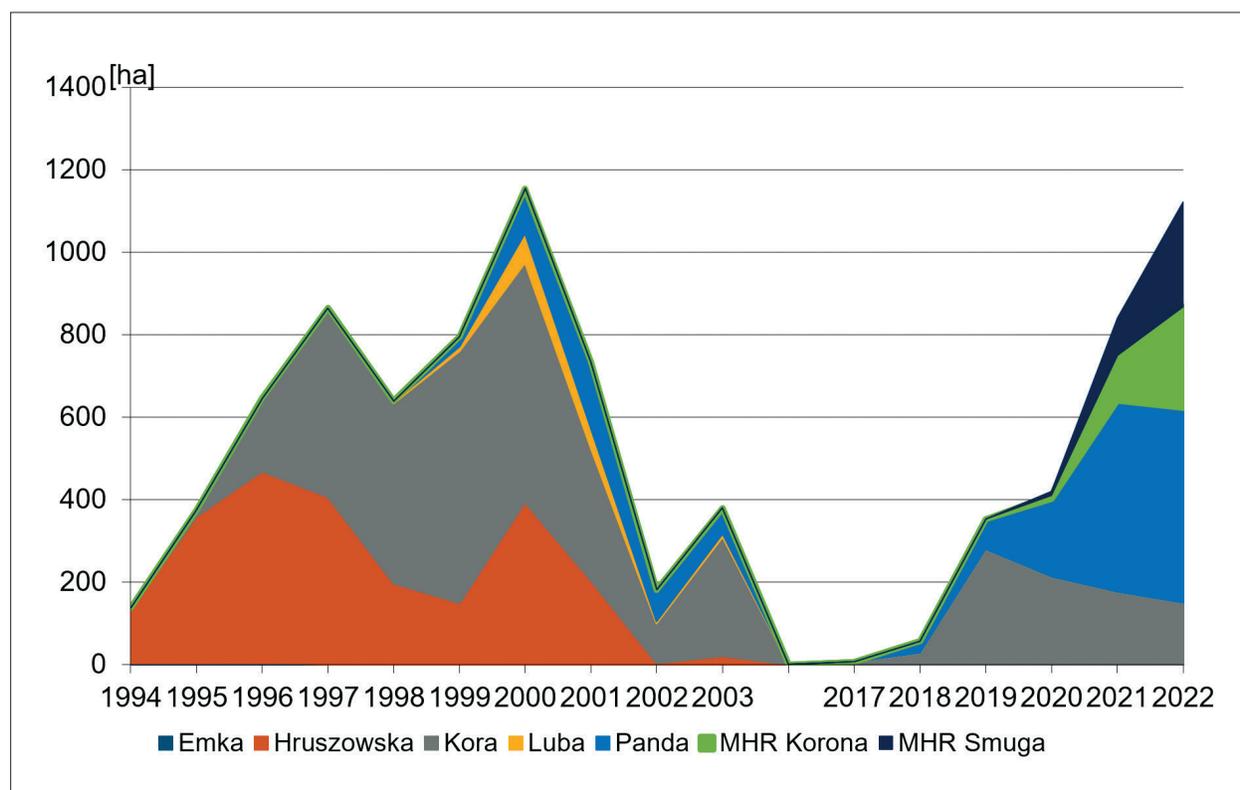


Fig. 6. Seed production area of buckwheat in Poland in 1994-2003 and 2017-2022 [ha] (Source: own elaboration based on data provided by PIORIN (The State Plant Health and Seed Inspection Service))

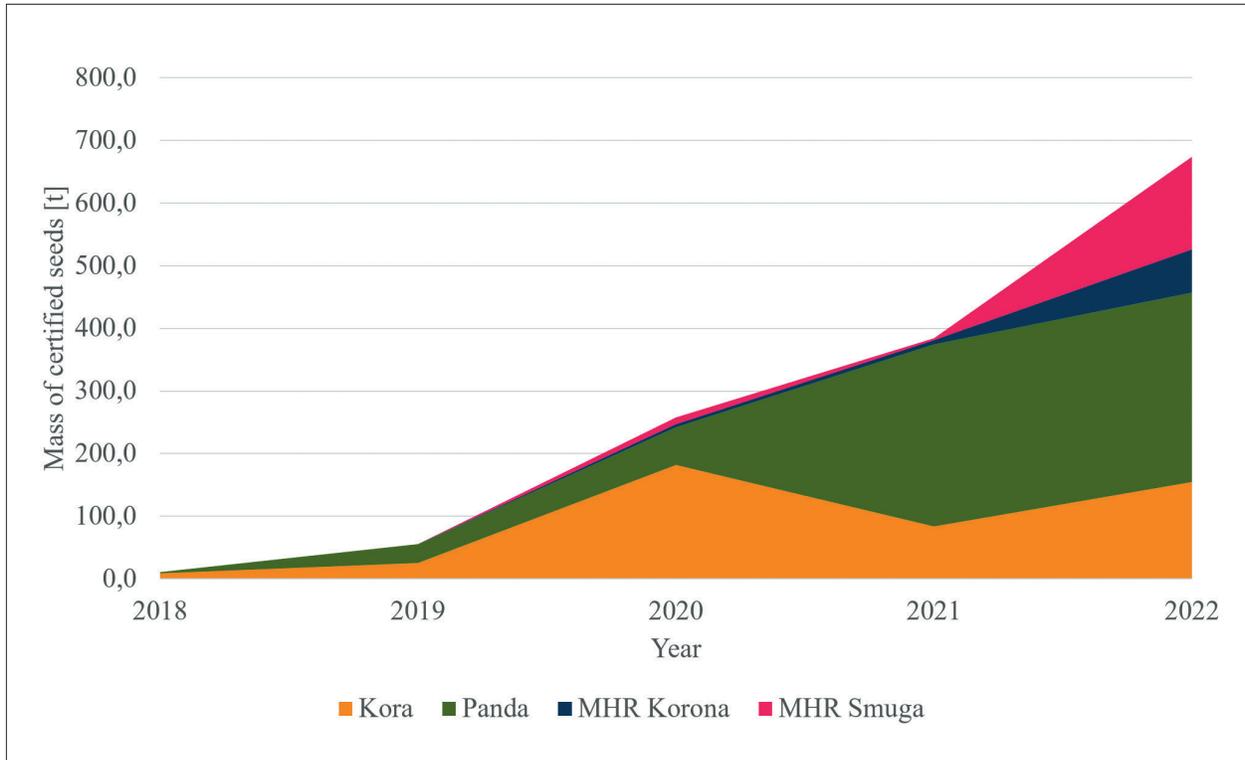


Fig. 7. Mass of certified seed of buckwheat in Poland in 2018-2022 (Source: own elaboration based on data provided by PIORIN)

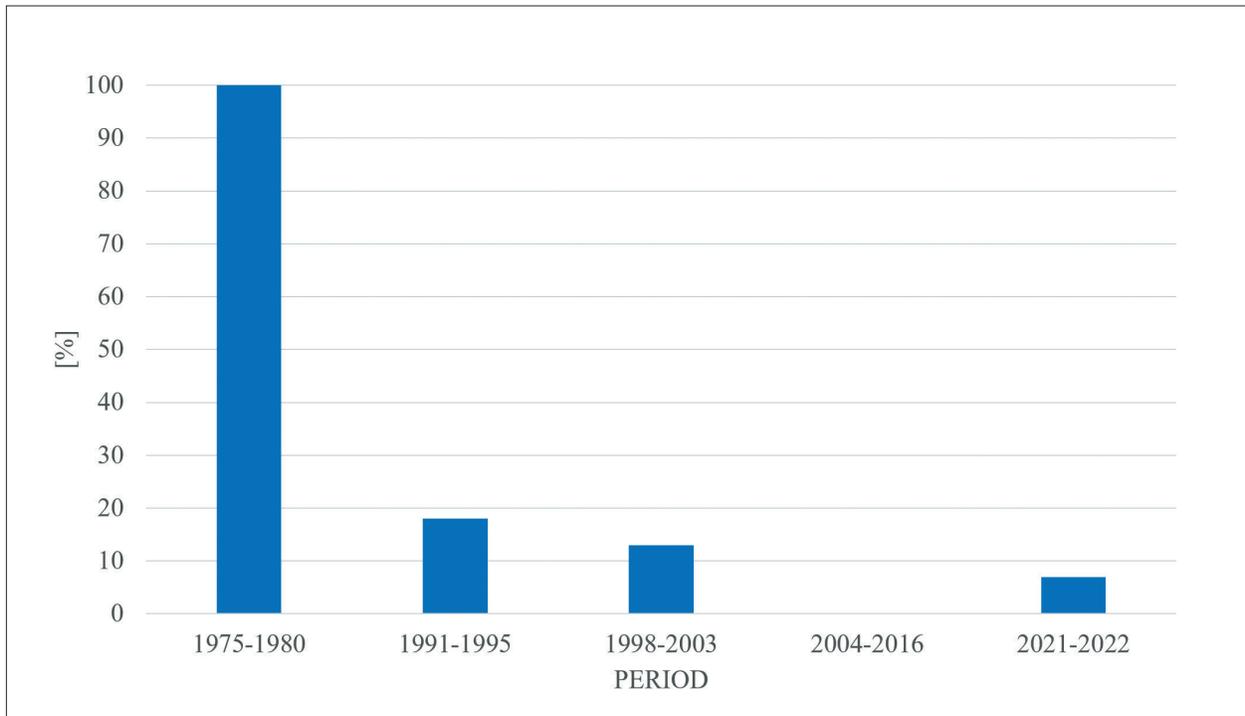


Fig. 8. Percentage of buckwheat production area sown with certified seeds (Source: own calculations based on PIORiN and GUS data)

under obligation to pay license fees, and they were not held accountable for seed quality. The Polish market was flooded with foreign buckwheat varieties produced in the east countries.

With the change of seed regulations in 2016, which enabled the registration of buckwheat varieties, the production of certified seeds of this species was resumed. In the first year after the change in the regulations (2017), two seed material plantations of the Kora and Panda cultivars, with a total area of 6.5 ha, were submitted for evaluation. Since then, there has been a steady increase in both the area of seed plantations and the quantity of certified seed. In 2022, the area of buckwheat seed plantations was nearly 1,090 ha. The Panda cultivar had a dominant share in it (43%). The total share of two new cultivars: Korona and Smuga was similar to Panda. The

quantity of certified seed of buckwheat increased from 10.7t in 2018 to 674t in 2022 (Fig. 7).

Such quantity allows for sowing with certified material on only 7% of the buckwheat's production area. In comparison - in the 1990s, the percentage was twice as high. However, considering current specific status of buckwheat in terms of variety registration and seed certification, non-certified seeds on the seed market can exist, with confirmed or unconfirmed quality in seed laboratories (Fig. 8).

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IZVLEČEK

Žlahtjenje ajde in semenarstvo na Poljskem

Ajdo so na Poljskem začeli žlahtniti pred skoraj 80 leti. 'Hruszowska' je bila prva požlahtnjena sorta, leta 1956 je bila vključena v uradno poljsko sortno listo (The Polish National List of Agricultural Plant Varieties (NLI)). Doslej je bilo uradno potrjenih 10 sort ajde. Dodatno so bile tri žlahtniteljske linije vključene v uradno testiranje, a brez vključitve v uradno listo (NLI). V času planskega gospodarstva (do leta 1989), je bilo ne glede na omejeno število sort dovolj potrebnega semena ajde glede na povpraševanje. Sedaj je zaradi manjšega pridelovanja ajde na Poljskem in glede na to, da ajde ni na uradnem seznamu sort v EU manjše zanimanje za trgovanje s potrjenim semenskim materialom ajde.

Research Paper

Effects of Rutin and Selenomethionine in Selenium-Enriched Tartary Buckwheat Roasted Grain Tea on Glucose and Lipid Metabolism in db/db Mice

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Key words: selenium-enriched Tartary buckwheat tea; selenomethionine; rutin; diabetes

ABSTRACT

Rutin and selenomethionine in selenium-enriched Tartary buckwheat roasted grain tea have certain effects on glucose and lipid metabolism in type II diabetes mellitus, but the mechanism of intervention is still unclear. This study utilized selenium-enriched Tartary buckwheat tea, rutin, and selenomethionine as research materials, with db/db mice serving as the research subjects. Diabetes control (DC), positive control group (PC), rutin group (RC), selenomethionine group (SC), low dose selenium-enriched Tartary buckwheat tea group (TBG-L), medium dose group (TBG-M) and high dose group (TBG-H) were set up. The effects of selenium enriched Tartary buckwheat tea, rutin and selenomethionine on glucose and lipid metabolism in db/db mice were investigated by blood biochemical analysis, liver function and oxidative stress measurement, histopathology and glycogen determination and mRNA expression analysis. Selenium-enriched Tartary buckwheat tea and its active ingredients rutin and selenomethionine can reduce fasting blood glucose and glucose resistance in db/db mice. Dyslipidemia of db/db mice was improved to some extent. The changes of MDA, CAT, T-SOD and GSH-Px enzyme activities in liver also indicate that TBG can improve the oxidative stress of liver and exert liver protection activities. Selenium-enriched Tartary buckwheat tea, rutin and selenomethionine can mediate glycogen storage by increasing glycogen synthesis and inhibiting gluconeogenesis in the liver of diabetic db/db mice, and can effectively mediate the expression of related genes during gluconeogenesis. Among the selenium-enriched Tartary

buckwheat groups, the effect of TBG-H group was the most obvious, which indirectly indicated that rutin and selenomethionine could play synergistic roles in gluconogenesis.

1 INTRODUCTION

Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] is a kind of food and medicine dual-purpose characteristic multigrain crop (Ren et al., 2015). It is rich in protein, lipids, dietary fiber, digestible starch and other nutrients (Qin, 2012), and also contains rutin, quercetin, γ -aminobutyric acid and other active ingredients that are not available in other food crops (Fabjan et al., 2003). The soil in Ankang City of Shaanxi province is rich in selenium, and the planted Tartary buckwheat in Ankang city could enrich a high selenium content (Gao et al., 2014), reaching 118.04 $\mu\text{g}/\text{kg}$ (Niu et al., 2020), which is 20 times higher than that in common Tartary buckwheat (Sun et al., 2005; Park et al., 2002). *Compendium of Materia records* that the Tartary buckwheat has bitter taste, calming cold, beneficial to the strength, the continuation of the spirit, the benefit of the eyes and ears, and the role of widening the intestine and strengthening the stomach. Clinical practice has proved that Tartary buckwheat has beneficial effects on lowering fasting glycemic index and insulin resistance, reducing glycosylated hemoglobin and glycosylated serum protein, as well as lowering blood lipid levels. These effects contribute to inhibiting the development of diabetes and its associated complications (Ren et al., 2015; Qin et al., 2012). Diabetes mellitus is a health problem of common concern in modern society, which brings heavy economic burden for society and family. According to data provided by the International Diabetes Federation (IDF), the global prevalence of type 2 diabetes (T2D) is expected to reach 13.5% in 2040 (Zhou et al., 2018).

T2D is widely recognized as a multifaceted metabolic disease. It is observed that over 90% of individuals with diabetes experience insulin deficiency and insulin resistance in target organs due to malfunctioning pancreatic beta cells (Zhang et al., 2017). Complications caused by T2D also increase the harm of this disease, such as blindness, kidney failure, lower limb amputation, heart disease, kidney disease, and liver disease (Zhang et al., 2017). Diabetes is a pressing issue that requires immediate attention. However, certain drugs used for treating T2D are frequently linked to various side effects, including vitamin B12 deficiency, gastrointestinal problems, liver damage, and lactic acidosis (Zhou et al., 2019; Lee et al., 2016; Hu

et al., 2017). Therefore, it is of great significance to explore the effect and the mechanism of dietary intervention of selenium-enriched Tartary buckwheat and its rutin and selenomethionine in the treatment of T2D.

Selenium has the effect of regulating insulin mediated physiological process to reduce blood glucose (Seale et al., 2013). Selenium mainly exists in the form of selenocysteine in mammals, and is involved in the synthesis of selenoprotein, which is used as the main carrier to play the corresponding biological functions (Niu et al., 2022). Plasma GSH-Px (GPx3) and selenoprotein P are the most common selenoproteins in blood, selenoprotein P is the main and best form of selenium in the body, 60% of selenium is distributed in selenoprotein P, and 30% of selenium is distributed in GPx3 (Tan et al., 2016). GPx3 can remove peroxides produced in the physiological or pathological processes of cells and reduce the damage of reactive oxygen species on the body (Zhou et al., 2019). Selenoprotein P is an extracellular glycoprotein, mainly produced by liver cells and secreted into plasma, which is of great significance for selenium transport and storage, and also has antioxidant stress properties (Thiry et al., 2013).

The close correlation between oxidative stress and T2D suggests that selenium and corresponding selenoproteins have anti-oxidative stress and insulin-like effects. They improve the secretion function of pancreatic β cells, increase the levels of insulin and C-peptide, enhance the content and activity of GSH-Px, and improve the ability to scavenge free radicals. This protection can help preserve insular tissue and insulin from destruction (Zhao et al., 2019; Qin et al., 2013; Rodrigo et al., 2015). The relationship between selenium and T2D has been widely concerned by scholars. Flavonoids can inhibit α -glucosidase, prevent the occurrence of diabetes, reduce blood glucose level, alleviate the development of diabetes and alleviate diabetes complications (Wu et al., 2021). The mechanism of Tartary buckwheat flavonoids to reduce blood lipids is mainly to inhibit the absorption of triacylglycerol and reduce the level of triacylglycerol after meals (Dzah et al., 2020; Hwang et al., 2019; Al-muhayawi et al., 2021). The mechanism of flavonoids to reduce blood glucose is to improve insulin signaling molecules and pathways in the liver, increase glucose

consumption and glycogen production, and assist in insulin regulation to reduce blood glucose content (Chen et al., 2017; Zhu, 2021; Guo et al., 2012; Ruan et al., 2022; Zielińska et al., 2012). The effect mechanism of selenium-enriched Tartary buckwheat tea and its active components selenomethionine and rutin on glucose and lipid metabolism in T2D mellitus is still unclear, and whether there is a synergistic effect needs to be verified.

In this study, a tradition Tartary buckwheat product made from selenium-enriched Tartary buckwheat, was selected as the research material. The feeding effects of different selenium-enriched Tartary buckwheat tea, rutin and selenomethionine on different groups of db/db mice were compared. Blood biochemical analysis, liver function and liver oxidative stress were measured. The effects of selenium-enriched Tartary buckwheat tea, rutin and selenomethionine on glycolipid metabolism in T2D were investigated by histopathology, hepatic glycogen assay and gene expression index, and the study provided theoretical basis and practical reference for selenium-rich Tartary buckwheat products in the adjuvant treatment of T2D mellitus.

2 MATERIALS AND METHODS

2.1 Experimental animals and samples

A total of 84 C57 BL/6 (db/db) species mice were SPF grade healthy males, aged 5 weeks and weighed (30±2) g. They were purchased from Jiangsu Jizhu Pharmaceutical Science and Technology Co., LTD., and kept in the Animal Laboratory of Shaanxi Normal University at an ambient temperature of 23 ± 2 °C and relative humidity of 45%-65%, day and night. After 28 days, the blood, liver, intestines, etc. were slaughtered and frozen for later experiments.

Selenium-enriched Tartary buckwheat tea were planted and processed in Ziyang County, Ankang city, and provided by Lotus Catering Company. Selenium-enriched Tartary buckwheat tea is made from shelled selenium-en-

riched buckwheat seeds, which have been roasted for 10 minutes at 180 °C. The basic ingredients of selenium-enriched Tartary buckwheat tea are shown in Table 1.

Animal db/db mouse models aged 5 weeks were randomly assigned to 7 groups, namely diabetes control (DC), positive control group (PC), rutin group (RC), selenomethionine group (SC), selenium-enriched Tartary buckwheat tea low dose (TBG-L) group, medium dose (TBG-M) group and high dose group (TBG-H), with 12 mice in each group. Each group was fed according to the feed formula table.

2.2 Feed customization

According to the standard feed, the feed is customized according to the principle of conservation of energy intake and the feed formula table is shown in Table 2.

2.3 Measurements of body weight and liver index

From the beginning of the experiment, the body weight (BW) of each mouse was recorded weekly. After killing the mice, the liver index was calculated according to the formula: liver index = liver weight (g)/body weight (100g).

2.4 Methods of fasting blood glucose and oral glucose tolerance test (OGTT)

The mice were fasted for 12 h at a fixed time every week, and drinking water freely. The blood of the mice was collected from the tail vein and the blood glucose level was measured by glucose meter. After 4 weeks of customized feed treatment, the mice were fasted for 12 h at the same time, and were intragastric with glucose solution at a dose of 2 g/kg. Blood from tail vein was collected and blood glucose was measured at 0, 30, 60 and 120 min.

2.5 Blood collection and biochemical analysis

2.5.1 Serum collection

After 4 w of treatment, the mouse eye was removed immediately after wiping with alcohol cotton swab, and

Table 1. Selenium-enriched Tartary buckwheat Tea Component Content (dry base)

	Moisture (%)	Protein (%)	Flavonoids (%)	Se (µg/kg)	Organic selenium (µg/kg)
<i>Tartary buckwheat roasted grain tea</i>	5.50±0.24	16.11±0.15	1.98±0.08	66.63±0.38	49.60±0.14

Table 2. Custom feed formula

AIN-93M (For Maintenance of adult rodents)			DC	PC	SC	RC	TBG-L	TBG-M	TBG-H
Ingredient	Amount	g	g	g	g	g	g	g	g
Casein	14.00%	140	140	140	140	140	108	76	44
Dextrinized Cornstarc	15.50%	155	155	155	155	155	155	155	155
Sucrose	10.00%	100	100	100	100	100	100	100	100
Corn Starch	46.57%	465.7	465.7	465.7	465.7	465.7	365.7	225.7	105.7
Alphacel, Non-Nutritive Bulk	5.00%	50	50	50	50	50	50	50	50
Soybean Oil	4.00%	40	40	40	40	40	36	32	28
Mineral Mix	3.50%	35	35	35	35	35	35	35	35
L-Cystine	0.18%	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Vitamin Mix	1.00%	10	10	10	10	10	10	10	10
Choline bitartrate	0.25%	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Tartary buckwheat roasted grain tea	0.00%	0	0	0	0	0	200	400	600
SeMet	0.00%	0	0	0	8.48µg	0	0	0	0
Rutin	0.00%	0	0	0	0	5.6	0	0	0
Metformin hydrochloride	0.00%	0	0	1.05g	0	0	0	0	0
Total	100.00%	1000.0	1000.00	1000.00	1000.00	1000.00	1064.00	1088.00	1132.00
Mass fraction of Tartary buckwheat	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.37	0.53

the blood was collected. After anticoagulation with sodium citrate, the mouse eye was left for 30 min at room temperature, then after centrifuged at 3 000 r/min. The supernatant was obtained, packaged and labeled, and frozen at -80 °C.

2.5.2 Blood biochemical analysis

The frozen animal liver samples were thawed in the -20°C refrigerator for 8 hours, and then transferred to the 4°C refrigerator before subpackaged. During the dissolution process, it is necessary to gently shake evenly and be careful not to cause bubbles and reduce the formation of precipitation. The serum levels of triglyceride (TG) and total cholesterol (TC) in mice were measured by GPO-PAP method after swirling the samples for 15 s with a scroll instrument. The contents of high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) in serum of mice were directly determined by double reagent method. Serum free fatty acid (FFA) content was quantified using a commercial test kit.

2.6 Measurement of liver function and oxidative stress in the liver

The frozen animal liver samples were thawed in the -20°C refrigerator for 8 hours, and then transferred to the 4°C refrigerator before subpackaged. Serum total protein (TP), albumin (ALB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the protocol of the corresponding kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). Oxidative stress in the liver was assessed by levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The procedure follows the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.7 Histopathological examination and hepatic glycogen assay

The samples were fixed with 4% (w/v) paraformaldehyde and embedded in paraffin. The specimens were successively sliced to 4 mm and dewaxed. To determine

pathological changes and glycogen content in the liver, sections were stained with hematoxylin and eosin (H&E) and periodate Schiff reagent (PAS), respectively. A slide obtained under the ME21 (MSHOT) microscope (Olympus Corporation, Japan).

The content of hepatic glycogen was determined according to the manufacturer's protocol of the commercial kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.8 Quantitative real-time PCR assay

Relative mRNA expression levels of peroxisome proliferator-activated Receptor- γ (PPAR- γ), glucose transporter-4 (GLUT4), phosphoinositol 3-kinase (PI3K), protein kinase B (Akt), glucokinase (GK), and phosphoenolpyruvate carboxykinase in animal liver samples (PEPCK). The primer sequences are listed in Table 3. The evaluation was conducted by quantitative real-time PCR and sequenced by Sevier Gene Technology Co., LTD. (Wuhan, China).

2.9 Statistical analysis

Data were analyzed with a one-way analysis of variance (ANOVA) followed by LSD multiple comparison

Table 3. Primers used in this study

Gene	Primer direction*	Gene sequence
β -actin	F	GTCACGTTGACATCCGTAAAGA
β -actin	R	GCCGGACTCATCGTACTCC
Pparg	F	GGAAGACCACTCGCATTCTT
Pparg	R	GTAATCAGCAACCATTGGGTCA
Slc2a4	F	GGACCGGATTCCATCCCAC
Slc2a4	R	TCCCAACCATTGAGAAATGATGC
Pik3r1	F	CCCCTACTGTAGCCAACAAC
Pik3r1	R	CGTACCAAAAAGGTCCCATCA
Akt	F	ATGAACGACGTAGCCATTGTG
Akt	R	TTGTAGCCAATAAAGGTGCCAT
Gck	F	TGAGCCGGATGCAGAAGGA
Gck	R	GCAACATCTTTACTACTGGCCT
Pck1	F	ATGGGGTGTGGTAGGAGCA
Pck1	R	CCGAAGTTGTAGCCGAAGAA

*F, Forward primer; R, Reverse primer.

tests. All groups were compared with each other for every parameter. The data were shown as the mean \pm SD. Statistical significance was based on $p < 0.05$.

3 RESULT AND DISCUSSION

3.1 Phenotypes on growth of db/db mice during feeding

The changes in body weight and liver index were shown in Fig. 1 (A, B, C).

The final body weight and weight gain of metformin (PC) and selenium-rich Tartary wheat tea groups (TBG-L, TBG-M, TBG-H), selenomethionine groups (SC) and rutin groups (RC) were lower than those of diabetes con-

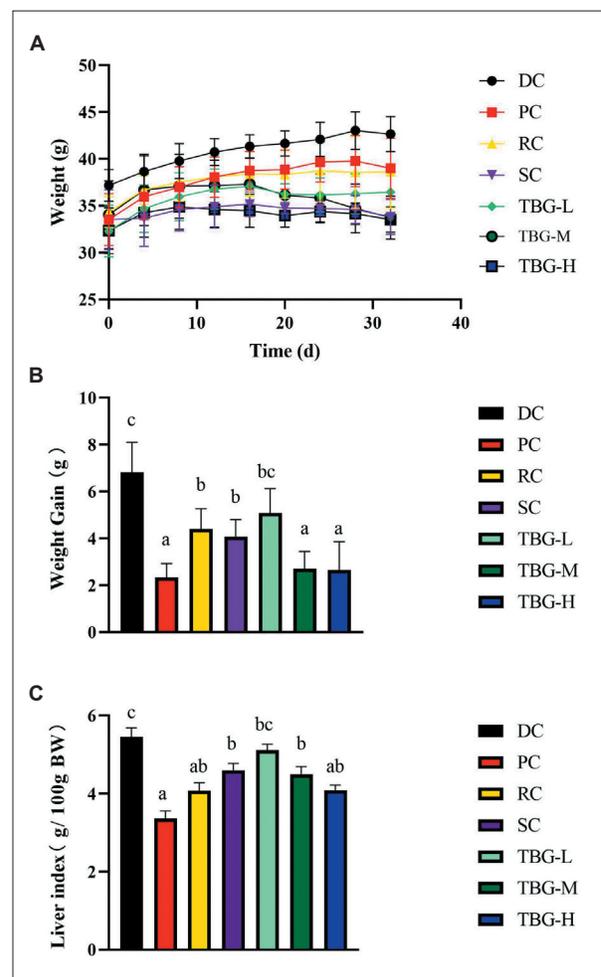


Fig. 1. Changes of body weight and liver index in db/db mice. A weight change of db/db mice; B Final weight; C Changes of liver index in db/db mice ($p < 0.05$)

trol group (DC) ($p < 0.05$). As shown in Figure 1A, during the 4-week experiment, the weight of mice in all groups increased, which showed a continuous rise from 1 to 20 days, and tended to be stable after 20 days. The body weight of the DC group was the highest in the whole experiment, and the final mean value reached 43.5g. There were certain differences in the final mean value of the other groups, which were PC (37.2g), RC (36.4g), TBG-L (35.7g), SC (34.5g) and TBG-M (33.6g), TBG-H (33.0g).

As shown in Fig. 1B, the weight gain of DC group (6.9g) was the largest among all the groups, followed by TBG-L (5.5g), RC (4.8g), SC (4.4g), TBG-M (2.8g), TBG-H (2.7g) and PC (2.3g).

As for the liver index (FIG. 1C), PC showed the smallest value (3.62) among all the groups, and the other groups from small to large were ranked as TBG-H (4.05), RC (4.07), TBG-M (4.25), SC (4.38), TBG-L (5.07), DC (5.36). These results indicated that buckwheat tea, rutin and selenomethionine can effectively intervene in the weight and liver enlargement associated with T2D.

The treatment effect of PC group was the best, and the treatment effect of RC group and SC group was also

more significant. Among the selenium-enriched Tartary buckwheat tea intervention groups, the treatment effect of TBG-H group was the best, followed by TBG-M and TBG-L groups. Both selenoprotein and rutin have obvious therapeutic effect on T2D.

3.2 Changes of glucose homeostasis in db/db mice during feeding

As shown in Fig.2A, db/db mice were treated with diet for four weeks and their fasting blood glucose was measured weekly. Fasting blood glucose in DC group increased with the increase of time and continued to increase throughout the cycle, reaching 7.3 mmol/L on day 7 and 17.6 mmol/L on day 28. The blood glucose value of the other groups showed a decreasing trend after reaching the peak value on day 14, and the peaks were successively RC (14.8 mmol/L), SC (14.1 mmol/L), TBG-L (13.9 mmol/L), TBG-M (13.7 mmol/L), TBG-H (12.7 mmol/L), as a sequence. PC (11.8 mmol/L). The effect of PC group and TBG-H group was more obvious, indicating that high dose of selenium-enriched Tartary buckwheat tea can effectively alleviate the increase of blood glucose.

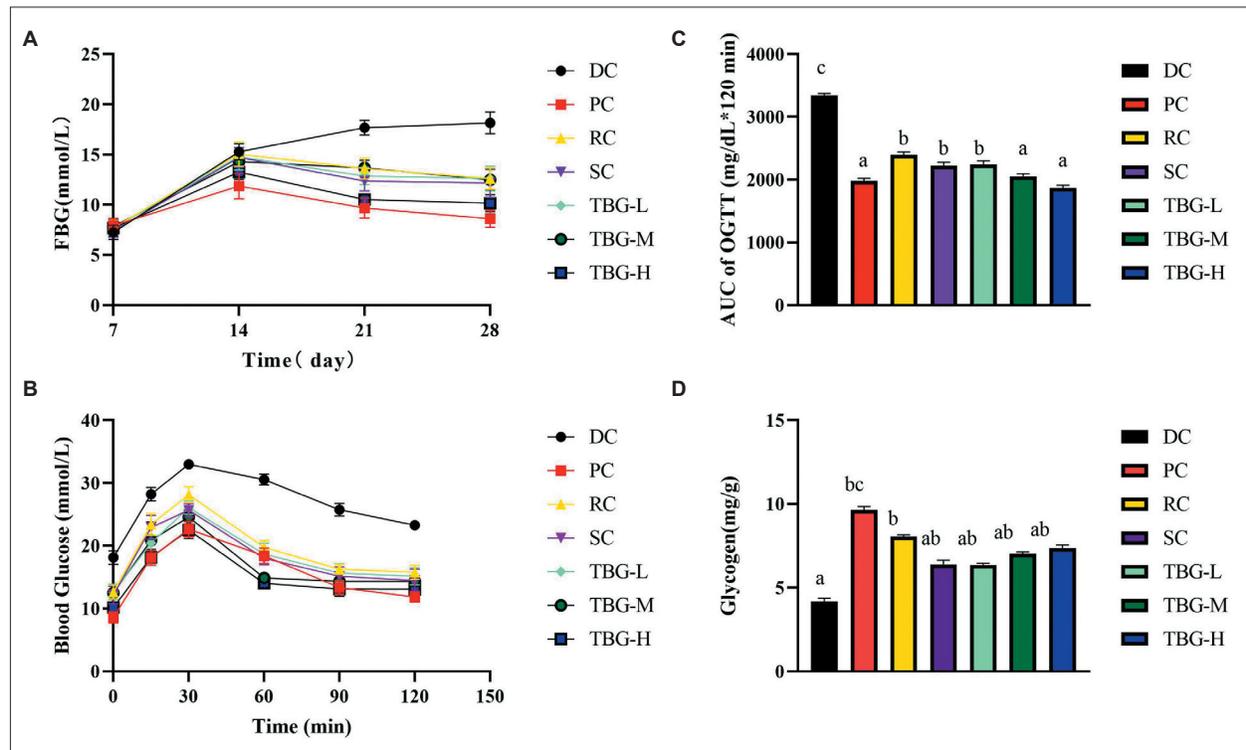


Fig. 2. Changes of glucose-related indexes during feeding of db/db mice
 A Fasting blood glucose (FBG); B Oral glucose tolerance test (OGTT); C Area under the curve of OGTT; D Hepatic glycogen content ($p < 0.05$)

The fasting blood glucose levels and OGTT were carried out through tail vein blood sampling method (Fig.2B). Within 30 min of gastric glucose administration, the blood glucose levels in both the intervention and DC groups increased to a peak value, among which the DC group was higher than all other groups. The peak value of 30 min was lower in PC group and TGB-H group. As shown in Fig.2C, AUC values in PC group and other dietary intervention groups (TBG-L, TBG-M, TBG-H, RC, SC) were significantly lower than those in DC group. These results indicated that selenium-enriched Tartary buckwheat tea, rutin and selenomethionine could alleviate the increase of blood glucose after meals. In selenium-enriched Tartary buckwheat tea group, TBG-H group had the best effect, followed by TBG-M group and TBG-L group, showing a dose-response relationship. Comparing the PC group, RC group and SC group, both SC group and RC group can effectively inhibit glucose tolerance, and the effect of SC group is more significant than that of PC group.

3.3 Changes of blood lipids in db/db mice during feeding

Compared with DC group, the levels of TC, TG and LDL-c in other groups (PC, TBG-L, TBG-M, TBG-H, RC, SC) were significantly increased ($p < 0.05$), and the level of HDL-c was significantly decreased ($p < 0.05$), indicating that the dyslipidemia of db/db mice had been improved. In the intervention groups, TC, TG and LDL-c were lower in PC group, RC group, SC group and TBG-H group, while HDL-c was higher.

Compared with DC group, RC group and SC group were more effective in the treatment of dyslipidemia. In the selenium-enriched Tartary buckwheat group, the treatment effect of TBG-H group was the best, followed by TBG-M group and TBG-L group. These results indicate that rutin and selenomethionine can effectively intervene the dyslipidemia of T2D mellitus, and have a certain synergistic effect.

3.4 Changes of liver indexes in db/db mice during feeding

As shown in Fig.3A and B, the concentrations of TP and ALB in db/db mice were significantly increased after metformin administration compared to the DC group. Similar to the PC group, RC group, SC group, and TBG-M group, the TBG-L group successfully restored the reduction of TP and ALB in db/db mice. There was no significant difference between RC group and SC group.

The metformin group and all dietary interventions exerted a protective effect on the liver by improving the increased activity of ALT and AST. There was no significant difference between RC group and SC group. Among selenium-enriched Tartary buckwheat tea group, TBG-H group had the best effect, followed by TBG-M group and TBG-L group.

The occurrence of T2D is associated with increased lipid peroxidation and oxidative stress (Wu et al., 2016). As a significant biomarker of lipid peroxidation, MDA is a toxic byproduct of lipid oxidation. The level of MDA in the liver indirectly reflects the degree of hepatocyte damage (Si et al., 2017; Hu et al., 2017). CAT, T-SOD and GSH-Px are major antioxidant enzymes that play a central role in the body's antioxidant system (Si et al., 2017).

As shown in Fig.3E, compared with DC group, MDA level in PC group, TBG-L group, TBG-M group, TBG-H group, RC group and SC group decreased by 60.3%, 32.5%, 40.6%, 51.6%, 55.9% and 27.8%, respectively.

After severe oxidative stress occurred in db/db mice, the levels of CAT, T-SOD and GSH-Px were significantly increased by dietary intervention, that is, the dietary intervention of selenium-enriched Tartary buckwheat tea at high, medium and low doses, rutin and selenomethionine (Fig.3F, G, H), indicating that the intervention effectively improved the oxidative stress of db/db mice.

3.5 Pathological observation

In order to evaluate the effects of dietary intervention on liver tissue structure and glycogen storage in diabetic db/db mice, H&E staining was used to observe liver histology, and the contents of hepatic glycogen (Fig. 2D) and free fatty acids (Table 4) were simultaneously determined for combined analysis.

As shown in Fig.5, diabetic db/db mice in the DC group exhibited significant hepatic steatosis and hepatocyte necrosis, characterized by hepatocyte hypertrophy, cytoplasmic vacuolation, and loss of nucleus. The PC group showed effective improvement, and the liver morphology was similar to that of normal mice. After 4 weeks of treatment with selenium-enriched Tartary buckwheat tea, rutin and selenomethionine diet, the liver lesions were improved to varying degrees. In contrast, both hepatocyte hypertrophy and hepatic lipid accumulation were significantly reduced in RC group compared with SC, TBG-L, TBG-M, and TBG-H groups. Excess inflow of free fatty acids (NEFA) is an important cause of triglyceride accumulation in the liver and is associated with insulin re-

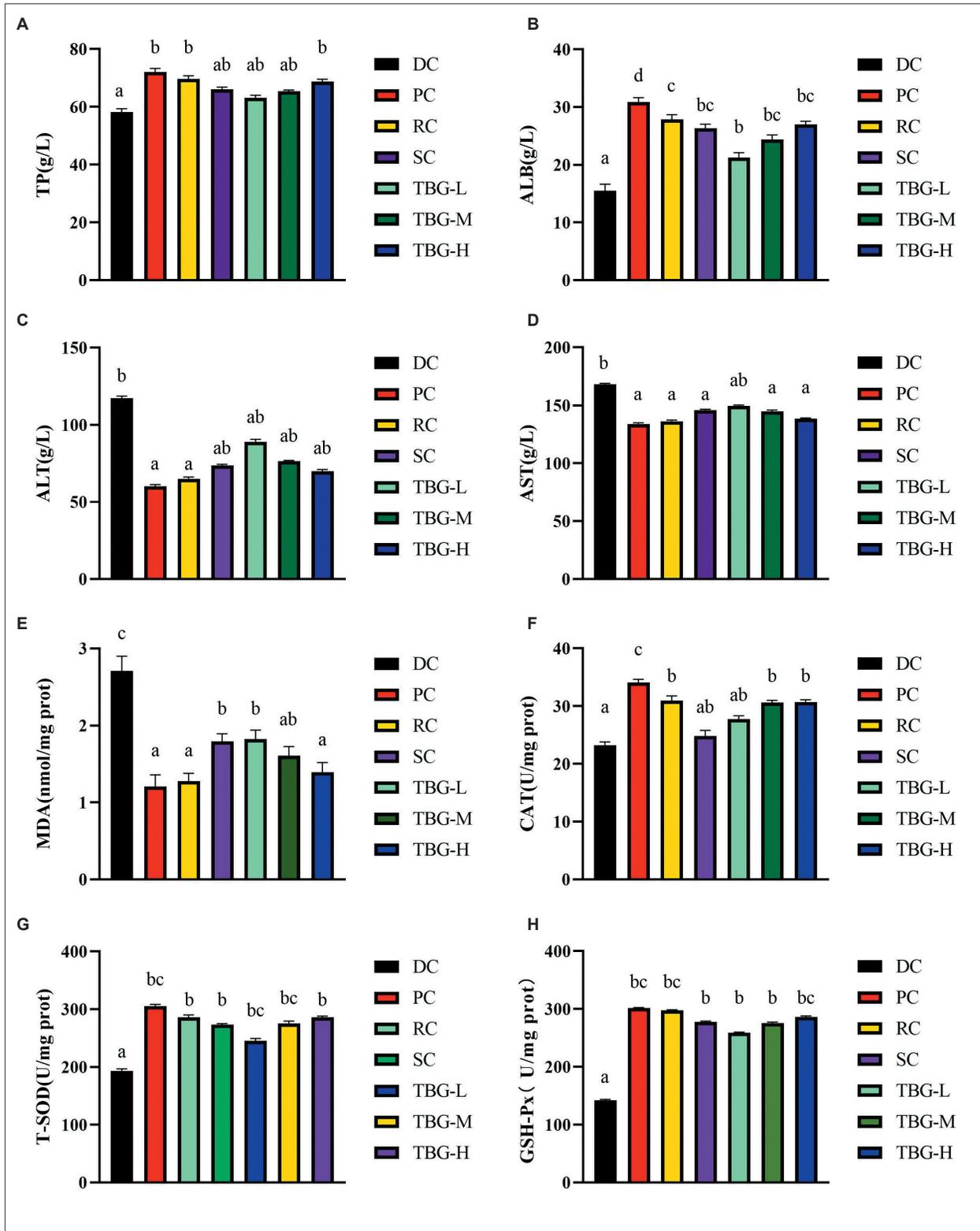


Fig. 3. Effects of feeding on liver function and oxidative stress in *db/db* mice
 A TP level; B ALB level; C the activity of ALT; D AST activity; E MDA level; F CAT; G T-SOD; H GSH-Px ($p < 0.05$)

Table 4. Effects of Tartary buckwheat tea, rutin and selenomethionine on blood lipid in db/db mice

Groups	TC	TG	HDL-c	LDL-c	NEFA
DC	4.94±0.15a	1.95±0.09a	1.24±0.12e	1.17±0.05a	4.67±0.21a
PC	2.01±0.22f	1.34±0.06c	2.58±0.07a	0.48±0.09c	2.89±0.17d
RC	2.78±0.17de	1.41±0.11c	2.47±0.06a	0.59±0.03c	3.02±0.15d
SC	2.91±0.11d	1.55±0.08bc	2.11±0.11b	0.73±0.11b	3.36±0.15c
TBG-L	4.37±0.18b	1.67±0.13b	1.79±0.12d	0.85±0.03b	4.04±0.11b
TBG-M	3.69±0.16c	1.51±0.15bc	1.96±0.09bc	0.71±0.08b	3.83±0.13b
TBG-G	2.81±0.17d	1.42±0.08c	2.06±0.14b	0.66±0.06b	3.67±0.24c

Note: Different letters in the same row indicate the significant difference among groups ($p < 0.05$)

sistance (Hu et al., 2011). As can be seen from the changes of NEFA level in mice from different groups, metformin was significantly reduced after administration, 35.33% in RC group, 28.05% in SC group, 13.49% in TBG-L group and 17.99% in TBG-M group, respectively. TBG-H group decreased the NEFA by 21.41%, compared with DC group.

3.6 Glucose and lipids related gene expression

The liver plays a central role in maintaining blood glucose homeostasis. This function is closely related to two major processes: one is postprandial glycogen conversion and glucose uptake, and the other is fasting glucose

production and glycogen and gluconeogenesis (Postic, 2004). As a key regulator of glucose homeostasis, PPAR- γ has been reported to promote the recovery of insulin sensitivity and has been suggested for the treatment of T2D (Fiévet et al., 2006). Activation of PPAR- γ can increase transcription of transporters such as GLUT4 and increase glucose uptake by the liver (Staels, 2005). The PI3K/Akt signaling pathway is a major insulin effector and has been reported to be involved in hepatic glycogen synthesis (Schultze et al., 2013; Zhu et al., 2016). PI3K is an insulin signaling intermediate, and its activation leads to Akt activation. Activated Akt can mediate glu-

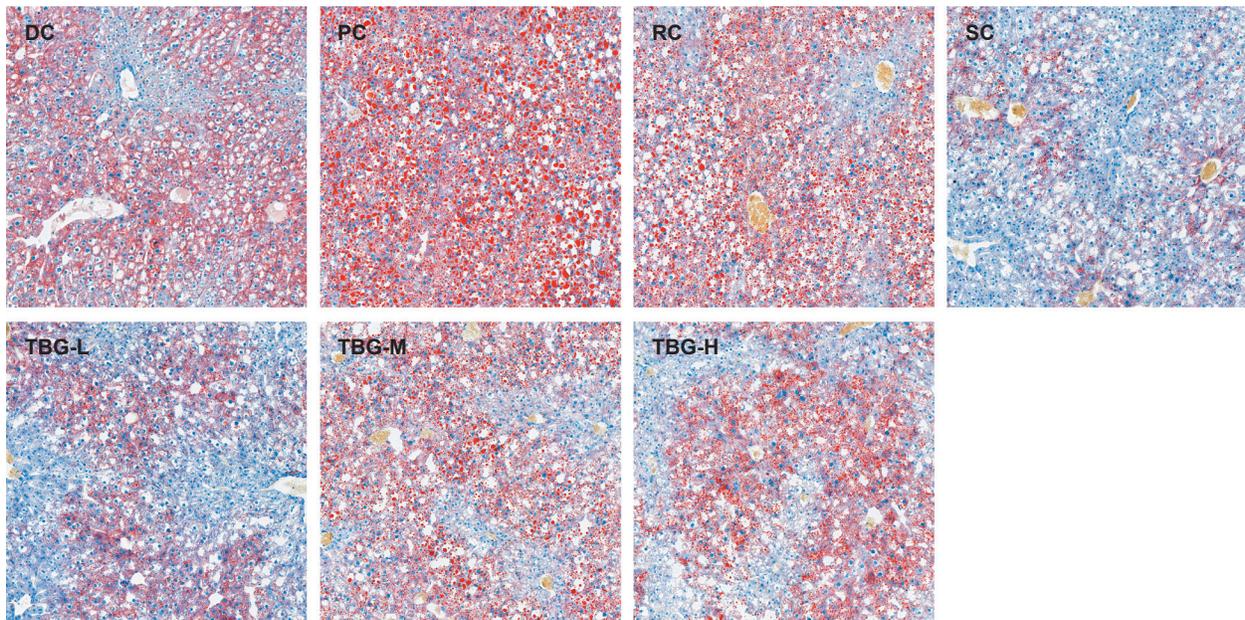


Fig. 4. Changes of liver histological structure in db/db mice during feeding

coarse metabolism in the liver, for example by stimulating glucose uptake and inhibiting gluconeogenesis (Manning et al., 2007). As the first enzyme in the glycogen synthesis pathway, GK promotes glucose phosphorylation and subsequently initiates glycogen synthesis (Agius et al., 2008). PEPCK is a rate-limiting enzyme in gluconeogenesis, which can be indirectly inhibited by activating Akt (Schultze et al., 2012). Down-regulation of PEPCK in db/db mice has been found to increase glycogen content in the liver (Zhou et al., 2015).

The mRNA expressions of Pparg, Slc2a4, Pik3r1, Akt and Gck were the lowest in the liver of DC group, and the expression of Pck1 was the highest. As shown in Figure 5A and B, dietary intervention (selenium enriched Tartary buckwheat tea, rutin, and selenomethionine) significantly increased the expression of Pparg and Slc2a4 (60S except) in the liver of db/db mice ($p < 0.05$), which may have a beneficial effect on increasing glucose uptake. Figure 5C and D showed increased expressions of Pik3r1 and Akt in the dietary intervention group

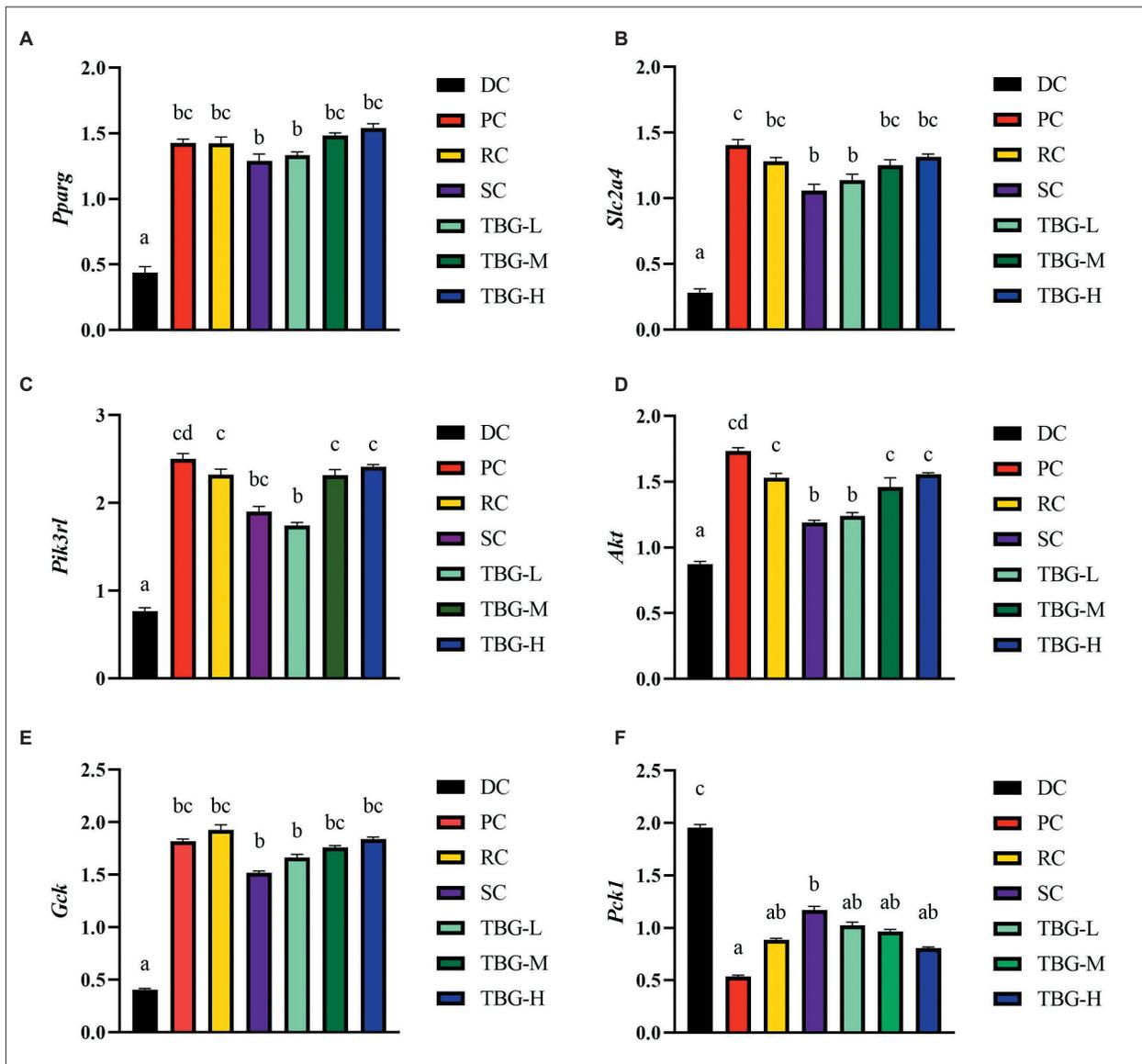


Fig. 3. Effects of feeding on liver function and oxidative stress in db/db mice
 A TP level; B ALB level; C the activity of ALT; D AST activity; E MDA level; F CAT; G T-SOD; H GSH-Px ($p < 0.05$)

(Tartary buckwheat tea, rutin, and selenomethionine), suggesting that glucose metabolism may be mediated through the PI3K/Akt signaling pathway. Compared with the DC group, the relative mRNA expression of Gck in PC, RC, SC, TBG-L, TBG-M and TBG-H groups was increased by 3.21 times, 3.35 times, 2.97 times, 3.01 times, 3.11 times and 3.26 times, respectively (Figure 5E). As shown in Fig. 5F, PC, RC, SC, TBG-L, TBG-M, and TBG-H reversed the increased expression of Pck1 in the DC group. These results suggest that selenium-enriched Tartary buckwheat tea, along with rutin and selenomethionine, can mediate glycogen storage by increasing glycogen synthesis and inhibiting gluconogenesis in the liver of diabetic db/db mice.

Compared with DC group, all indexes in PC group, PC group and SC group effectively mediated the expression of related genes during gluconeogenesis. Among the selenium-enriched Tartary buckwheat groups, the effect of TBG-H group was the most obvious, which indirectly indicated that rutin and selenomethionine could play synergistic roles in gluconogenesis.

4 CONCLUSION

Different doses of selenium-enriched Tartary buckwheat tea and its active components rutin and selenomethionine have certain therapeutic effects on diabetic db/db mice. Feeding selenium-enriched Tartary buckwheat

tea can improve the hyperglycemia, insulin resistance and hyperlipidemia of db/db mice. MDA levels in PC group, TBG-L group, TBG-M group, TBG-H group, RC group and SC group were decreased. The levels of CAT, T-SOD and GSH-Px in db/db mice were significantly increased after dietary intervention of selenium-enriched Tartary buckwheat tea with high, medium and low dose, rutin and selenomethionine, and the oxidative stress of db/db mice was improved. In addition, liver histopathological observation and hepatic glycogen measurement by H&E staining showed that selenium-enriched Tartary buckwheat tea, rutin and selenomethionine increased hepatic glycogen content in db/db mice. Analysis of mRNA expression related to glucose metabolism showed that selenium-enriched Tartary buckwheat tea can increase glycogen synthesis and inhibit gluconeogenesis, thereby mediating glycogen storage in the liver of diabetic db/db mice. TBG has a significant dose-response relationship for DM intervention in db/db mice according to all above parameters.

5 ACKNOWLEDGEMENTS

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IZVLEČEK

Učinek rutina in selenometionina v s selenom obogateni praženi tatarski ajdovi kaši na glukozo in metabolizem lipidov pri db/db miših

Vpliv rutina in selenometionina v s selenom obogatenem praženem čaju iz zrn tatarske ajde na glukozo in lipidni metabolizem pri diabetes II še ni raziskan. Pri tej raziskavi je bil uporabljen s selenom obogaten čaj tatarske ajde kot material za raziskavo in db/db miši kot poskusne živali. Kontrolna skupina z diabetesom (DC), pozitivna kontrolna skupina (PC), skupina z rutinom (RC), skupina s selenometioninom (SC), skupina z nizkim odmerkom selena obogatenim tatarskim ajdovim čajem (TBG-L), skupina s srednjim odmerkom (TBG-M) in skupina z visokim odmerkom (TBG-H) so bile vključene v poskus. Vpliv s selenom obogatenega tatarskega ajdovega čaja, rutina in selenometionina na glukozo in metabolizem lipidov so spremljali v db/db miših z biokemičnimi analizami krvi, analizo funkcije jeter, merjenjem oksidativnega stresa, spremljanjem histopatologije in določanjem glikogena ter ekspresije mRNK. S selenom obogaten čaj tatarske ajde in aktivni sestavini rutin in selenometionin vplivajo na znižanje vsebnosti glukoze in glukozne odpornosti pri db/db miših. Dislipidemija je bila delno izboljšana. Spremembe encimskih aktivnosti MDA, CAT, T-SOD in GSH-Px

v jetrih so nakazovale, da lahko TBG omili oksidativni stres jeter in pokaže aktivnosti zaščite jeter. S selenom obogaten čaj tatarske ajde, rutin in selenometionin lahko posredujejo k povečanju skladiščenega glikogena in zavirajo glukoneogenezo v jetrih diabetičnih db/db miši. Prav tako lahko učinkovito posredujejo k ekspresiji genov med glukoneogenezo. V skupini s selenom obogatene tatarske ajde je pri skupini TBG-H najbolj izrazit posreden vpliv na to, da lahko imata rutin in selenometionin pri glukoneogenezi sinergističen vpliv.

Research Paper

The Quality of Pasta with the Addition of Buckwheat Microgreens or Sprouts

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Keywords: microgreens; pasta; sensory evaluation; sprouts

ABSTRACT

Sprouts and microgreens are important functional foods used as nutrition supplements. The aim of this work was to test the possibility of pasta production with different proportions of buckwheat sprouts or microgreens and evaluate their selected qualitative properties. Semolina pasta containing different proportions of flour from buckwheat sprouts (10-20%) and buckwheat microgreens (10%) were produced. Optimal cooking time, cooking water absorption, swelling, cooking losses, adhesion, color, overall look, surface, elasticity, translucency, flavour, taste, and the overall impression were evaluated. For pasta production, buckwheat sprout flour can be recommended in a portion of up to 10%.

INTRODUCTION

Buckwheat sprouts have a slightly crispy texture and an attractive fragrance (Kim et al. 2004). They are abundant in amino acids (28–38% higher than in seeds), especially lysine, vitamins (especially vitamins C and B, α -tocopherol, and carotenoids), minerals, crude fiber, and flavonoid contents (Sun Lim et al., 2001).

Microgreens are slightly larger and older (usually 5–10 cm tall) than sprouts. They are harvested after the cotyledon leaves have developed, at the first true leaf stage (Treadwell et al. 2020). Microgreens are used as a nutrition supplement, visual enhancement, and food flavour and texture enhancement (Wojdyło et al., 2020). Sprouts and microgreens are considered “functional foods” because they are rich in vitamins, minerals, and antioxidants, and their popularity has been constantly growing (Brentlinger, 2007; Choe and Wang, 2018).

Although sprouts have been a diet staple in Chinese culture for over 5,000 years, they remain relatively unpopular in other countries (Food Source Information 2022). Fortification is the incorporation of bioactive compounds within a food matrix to prevent specific nutrient deficiencies and promote population health (Dwyer et al., 2015). Therefore, a possible way to include sprouts or microgreens in human nutrition is to fortify food such as bread, pasta, noodles, and breakfast flakes with these raw materials.

The global pasta market reached about USD 25.67 billion in 2022 and is expected to reach approximately USD 31.14 billion by 2028 (EMR2023). WHO (the World Health Organization) and FAO (the Food and Agriculture Organization of the United Nations) described pasta as a healthy, sustainable, and quality food model (Bresciani et al., 2022). Clinical data suggest that pasta consumption is not directly associated with overweight or obesity in healthy children and adults (Sanders and Slavin, 2023). Cooked pasta can have a low glycaemic index, is cholesterol-free, and is low in sodium (EMR 2023).

In the last two decades, there has been much research into improving pasta nutritional value by including non-conventional ingredients (dietary fibre, fish ingredients, herbs, inulin, resistant starches, legumes, vegetables, and protein extracts). Buckwheat, amaranth, or quinoa are becoming increasingly popular as ingredients in pasta due to improving nutritional quality too (Kahlon and Chiu, 2015). However, these ingredients can affect the technological properties, such as the texture, taste, and odor of the pasta (Gao et al., 2018). Health effects with

good taste, texture, and an acceptable price are essential for the consumer demand for such foods (Sissons, 2022).

This work aimed to test the possibility of pasta production with different proportions of buckwheat sprouts or microgreens and evaluate their qualitative properties

MATERIAL AND METHODS

Sprouts preparation

The common buckwheat (*Fagopyrum esculentum* Moench) achenes (500g), Jana variety from organic farming were cleaned and freed from foreign seeds and mechanically dehulled. 250 g of seeds surface-sterilized were pre-soaked in sterile water for 4 h. The seeds were directly placed into 40×40 cm plastic trays with two sheets of filter paper (KA 1) saturated with sterile water and incubated in the thermostat at 25±1°C under dark conditions for 96 hours. The seedlings were sprayed with water daily. The plant material was dried in a ventilated oven at 45±1°C for 48 h.

Microgreens preparation

For microgreens, the achenes were directly placed into a double-bottomed container. A hemp non-woven fabric was placed on the upper, perforated bottom of the container with 1x1 cm holes, and buckwheat achenes were placed on the fabric. The container was closed with an opaque lid for five days. Then the lid was removed. Plants were incubated in the thermostat at 25±1°C under dark conditions. Eleven days after the start of the experiments, sprouts were cut 10 mm above the surface for microgreens. The plant material was weighed and dried in a ventilated oven at 45±1°C for 48 h.

Preparation of pasta

At first, sprouts or microgreens were ground into flour. Mixtures of flour were prepared containing 0, 10, 15, and 20% of buckwheat flour from sprouts or 10% of buckwheat flour from microgreens, the remainder up to 100% by weight being durum wheat flour (semolina). Semolina flour was purchased from the firm Granoro Italy (Bari, Italy).

This was followed by kneading the dough of 100 g of a flour mixture with 48 ml of distilled water, warm at 47±1°C and kneaded for 20 minutes. Pasta with the width of 1 cm and the thickness of 2 mm was produced. These were then dried for 6 hours in a hot air oven with a maximum

of 90° C (at 30°C for 30 min, at 50°C for 60 min, at 90°C for 120 min, and at 60°C for 120 min).

Individual dry pasta samples (100g) were boiled for 10 minutes in 1 liter of water at 100° C, adding 10 g of salt.

Evaluated parameters

Optimal cooking time - the time when the white cores of the strands disappeared, according to Approved Method 66-50 of AACC International.

Cooking water absorption - the determination of water content in percentage by weight the pasta was performed during cooking. The drained pasta was left in the sieve for 2 minutes and weighed. Water absorption (WA) was obtained by the following formula: $WA (\%) = (a - b) / b * 100$

Where: a - the weight of the cooked pasta (g), b - the weight of the dry pasta (g).

Cooking yield - the percentage of increased weight of pasta during cooking.

Swelling index - the ratio volume of pasta before (100g) and after cooking, expressed as a multiple of the original volume of solution. The pasta was placed in a graduated cylinder with 700 ml of water, and the water level in ml was read.

Cooking losses - solids (g) from the cooking water were determined by concentrating the cooking water to dryness in an oven at 100 °C.

Stickness - how cooked the pasta sticks to the fork compared to the semolina pasta. Scale: 1 - does not stick to the fork at all; 2 - easily sticks to the fork; 3 - medium glues to the fork, 4 - a lot of sticks to the fork, 5 - strongly sticks to the fork.

Sensory evaluation of pasta was performed by a group of 10 people (5 women and 5 men). The evaluators

were not in contact with each other and were not familiar with the composition of the pasta. The evaluation took place on a scale 1 to 5, with a rating of 1 corresponding to the best and a rating of 5 to the worst impression (1-great, 2- excellent, 3- less excellent, 4- good, 5- unsatisfactory). The overall rating was determined by the arithmetic mean. For raw pasta, the color, overall look (shape, edges), surface properties (1-smooth, 2-less smooth, 3-coarse, 4- slightly rough, 5- rough), elasticity (1-puts up resistance before it breaks, 2- puts up slight resistance before breaking, 3- it breaks easily, 4- it breaks almost immediately, 5-fragile, cannot be bent, breaks immediately), translucency (1-like semolina, 2-almost like semolina, 3-only along the edges, 4-almost none, 5-none). After cooking, it was evaluated: color, look of pasta, flavour, taste, and overall impression.

Statistical analysis

The influence of buckwheat flour portion in cookies and pasta on selected parameters was evaluated by analysis of variance with the post hoc Tukey HSD test in program Statistica 12.0.

RESULTS AND DISCUSSION

Table 1 and 2 summarizes the sensory evaluation of pasta. Most parameters differed significantly ($p < 0.05$) among samples. Higher scores of sensory characteristics of raw pasta were recorded for color and translucency that had pasta with 10% of microgreens and 20% of sprouts. There was no difference among pasta variants with the different proportions of sprouts in elasticity. Similarly, De Marco et al. (2014) found that the elasticity of pasta with different percentages of spirulina also showed no change. The translucency, overall look, and surface prop-

Table 1. Sensory evaluation of raw pasta (mean ± standard deviation)

	Color	Overall look	Surface properties	Elasticity	Translucency	Total mark
0% of sprouts	1 ± 0.5a	1 ± 0.4a	1 ± 0.5a	2 ± 0.5a	1 ± 0.5a	1
10% of sprouts	2 ± 0.4ab	2 ± 0.4b	2 ± 0.0b	3 ± 0.0b	2 ± 0.7a	2
15% of sprouts	3 ± 0.8ab	3 ± 0.5c	3 ± 0.5c	3 ± 0.0b	4 ± 0.0b	3
20% of sprouts	4 ± 0.8b	5 ± 0.5d	3 ± 0.5c	3 ± 0.3b	5 ± 0.0c	4
10% of microgreens	4 ± 0.8b	3 ± 0.4c	3 ± 0.4c	3 ± 0.5b	5 ± 0.4c	4

Different small letters (a–b) indicate significant differences.

Table 2. Sensory evaluation of cooked pasta (mean \pm standard deviation)

Fortification	Color	Overall look	Flavour	Taste	Overall impression	Total mark
0% of sprouts	1 \pm 0a	1 \pm 0a	2 \pm 0.7a	1 \pm 0.5a	1 \pm 1.1a	1
10% of sprouts	3 \pm 0.5b	2 \pm 0.5b	3 \pm 0.5ab	3 \pm 0.8b	3 \pm 0b	3
15% of sprouts	3 \pm 0.5b	3 \pm 0.8b	3 \pm 0.5ab	3 \pm 0.6b	3 \pm 0b	3
20% of sprouts	5 \pm 0.3c	5 \pm 0.5c	5 \pm 0.5b	4 \pm 0.5b	4 \pm 0.5c	5
10% of microgreens	4 \pm 0.7bc	4 \pm 0.5bc	3 \pm 0.7ab	4 \pm 0.3b	4 \pm 0.5c	4

Different small letters (a–b) indicate significant differences.

erties of row pasta were found to be the best in control pasta without buckwheat sprouts and for pasta with 10% of buckwheat sprouts.

During cooking, the starch granules swell and partly solubilize, while the protein becomes insoluble and coagulate (Hager et al., 2012). The presence of gluten positively influences the texture of cooked pasta. Higher scores of sensory properties (color, overall appearance, flavour, taste, overall impression, and also overall marker) for cooked pasta are related to 20% sprouts and 10% microgreens in pasta. Pasta with 15% sprouts was evaluated as less favourable in all characteristics. The higher level of buckwheat sprouts in pasta, the more bitterness was observed. This unpleasant taste was most noticeable in pasta with microgreens. Similarly, Bokić et al. (2022) found that the addition of broccoli sprouts significantly influenced odor and taste of cooked pasta.

The optimal cooking time of pasta with microgreens was significantly higher (18 min) than in pasta from semolina (0% buckwheat sprouts) and pasta with buckwheat sprouts (Table 3). While the lowest (10 min) cooking time was recorded in pasta with 20% of buckwheat sprouts.

The increasing proportion of buckwheat sprout flour in the pasta reduced their cooking time. Bokić et al. (2022) observed that optimum cooking time also decreased with the addition of broccoli sprouts. On the other hand, De Marco et al. (2014) found that increasing amounts of spirulina in pasta decreased optimum cooking time. It was similar in our study when microgreens were added to pasta. According to Fardet et al. (1998), the protein network limits water penetration into the center of the pasta during cooking, so a weaker protein network reduces the cooking time.

The highest swelling (40 ml) and also the highest swelling index (1.59) were observed in pasta with 0% portion of sprouts (the control variant). The lowest swelling (30 and 32 ml) and the lowest swelling index (1.39) was recorded in pasta with 15% of sprouts and 10% of microgreens. There were no significant differences among pasta with different portions of sprouts or microgreens in swelling and swelling index (Table 3).

The cooking water absorption is the amount of water in weight percent that the pasta is taken by cooking. In case of insufficient binding, the cooked pasta is hard

Table 3. Evaluation of cooked pasta (mean \pm standard deviation)

Fortification	Cook. time (min)	Swelling (ml)	Swelling index	Water absorption (%)	Cooking yield (%)	Cooking losses (g)	Stickness
0% sprouts	12 \pm 1ab	40 \pm 0.6b	1.58b	154 \pm 2.4b	254 \pm 2.6b	1.8 \pm 0.2a	1
10% sprouts	15 \pm 2ab	34 \pm 0.5a	1.48a	118 \pm 2.7ab	218 \pm 2.8a	3.2 \pm 0.4c	1
15% sprouts	13 \pm 1ab	30 \pm 4.6a	1.39a	120 \pm 4.7ab	216 \pm 5.6a	3.4 \pm 0.6c	1
20% sprouts	10 \pm 1a	35 \pm 0.8a	1.49a	126 \pm 5.8ab	226 \pm 7.9a	4.5 \pm 0.2d	1
10% microgreens	18 \pm 2b	32 \pm 2.7a	1.39a	108 \pm 5.7a	208 \pm 5.1a	2.3 \pm 0.1b	1

Different small letters (a–b) indicate significant differences.

and rough. On the contrary, with high binding, the pasta is too soft and sticky. The highest cooking water absorption of pasta (154%) was observed in the control variant (0% sprouts), and the lowest cooking water absorption (108%) was recorded in pasta with buckwheat microgreens. The higher cooking water absorption increase was noticed in the pasta with sprouts than in the pasta with microgreens. There was no statistically significant difference between pasta varieties with a different proportion of sprouts in the cooking water absorption (Table 3). Water absorption should be at least 100% of the mass of dry pasta (MSZ 20500/1-1985). All samples met this requirement. Bokić et al. (2022) found that fortification of pasta with broccoli sprouts reduced swelling index but volume (swelling) increased.

The highest cooking yield of pasta (245%) was observed in the control variant (0% of sprouts), and the lowest cooking yield (208%) was recorded in pasta with buckwheat microgreens. The higher cooking yield was noted in the pasta with sprouts than that pasta with microgreens. There was no statistically significant difference between pasta variants with the different proportions of sprouts in the cooking yield.

Cooking losses are defined as the weight of the total solids lost in the cooking water. When pasta is fortified, gluten is diluted by replacing wheat flour with buckwheat sprouts or microgreens, and the protein network is weakened, which facilitates the leakage of amylose into the cooking water (De Marco et al., 2014). The higher portion of the solids lost is, the cooking losses are higher. Replacement of wheat flour by buckwheat sprouts or microgreens significantly ($p < 0.05$) increased the cooking losses. There was a statistically significant difference between pasta variants with different proportions of sprouts. The pasta prepared from flour with 10% of buckwheat microgreens had the most similar cooking losses of the control sample. The highest cooking losses were observed when the pasta was prepared with 20

% of buckwheat sprouts (Table 3). Similarly, Bokić et al. (2022) established that cooking losses increased with the level of broccoli sprouts in pasta. Pasta should exhibit low cooking losses. All pasta samples had acceptable quality because good pasta has cooking losses < 8 g/100 g (Dick and Youngs, 1988).

Stickiness (adhesiveness) is another essential property of pasta. Pasta should exhibit an absence of stickiness. If the pasta sticks, it worsens their consumption. The stickiness of the pasta was tested as adhesion to a fork. Although according to Marchylo et al. (2004), the higher strength and lower.

CONCLUSION

Evaluation of pasta prepared with the addition of buckwheat sprouts flour to wheat flour confirmed that the pasta cooking time, the binding of water during pasta cooking, and the proportion of cooking losses confirmed satisfactory values of these parameters. However, the 10% addition of buckwheat sprouts is acceptable to consumers in terms of look and taste. At the same time, it will positively affect the nutritional quality of the pasta. Based on the obtained results, common buckwheat sprouts can be recommended as an ingredient in pasta. The application of buckwheat to pasta can have an essential role in human nutrition because the consumption of pasta has a long-term upward trend in the population.

As a result of the sensory evaluation, the addition of 20% buckwheat sprouts may adversely affect color and sensory properties. The addition of sprouts than microgreens can be recommended for the fortification of pasta.

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IZVLEČEK

Kakovost testenin z dodatkom ajdovih kalic in mikro zelenja

Kalice in mikro zelenje so pomembna funkcionalna živila, ki jih uporabljajo za prehranska dopolnila. Namen dela je bil preizkusiti možnost izdelave testenin z različnimi deleži ajdovih kalic in ovrednotiti njihove izbrane kvalitativne lastnosti. Pripravljene so bile testenine iz zdroba semoline (durum pšenice) z različnimi deleži moka iz ajdovih kalic (10-20 %) in ajdovega mikro zelenja (10 %). Ocenjevali so optimalen čas kuhanja, vpijanje vode pri kuhanju, nabrekanje, izgubo pri kuhanju, oprijemljivost, barvo, splošni videz, površino, prožnost, prosojnost, aromo, okus in splošni vtis. Za izdelavo testenin se priporoča moka iz ajdovih kalic v deležu do 10 %.

Review

Breeding of Buckwheat in Kyushu Okinawa Agricultural Research Center, NARO

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ABSTRACT

The Kyushu Okinawa Agricultural Research Center is one of the regional agricultural research and development centers of the National Agriculture and Food Research Organization (NARO) in Japan. The center aims to bring NARO's agricultural technologies to the forefront of agriculture.

BREEDING BUCKWHEAT AT NARO

Buckwheat breeding is one of the important programs at the Kyushu Okinawa Agricultural Research Center, as it contributes to the stable production of high-yield and high-quality buckwheat (Fig. 1). In the Kyushu Okinawa Agricultural Research Center, NARO, buckwheat breed-

<p>1) Breeding of common buckwheat</p> <p>Stable production, High Yield</p> <ul style="list-style-type: none"> ① 'Kyushu 9' (DC, SR, PHS) ② 'Kyukei 50' (semidwarf, DC, SR, PHS) <p>High quality</p> <ul style="list-style-type: none"> ③ 'low amylose' <p>2) Breeding of perennial buckwheat</p> <ul style="list-style-type: none"> ④ Rutin and Reduction of bitterness ⑤ Perenniality and non-shattering trait ⑥ 3D plant modeling system 	<p>DC: Double cropping SR: Shattering Resistance PHS: preharvest sprouting resistance</p>
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Figure 1. Main breeding issue of buckwheat in Kyushu Okinawa Agricultural Research Center, NARO.

ing have been conducted twice a year (spring and summer sowing) in test plots of approximately 2.5 hectares each and greenhouses (Fig. 2).

In Japan, shattering and preharvest sprouting (Fig. 3) are significant factors that can decrease buckwheat production. Green flower had leaf like green parts in petals with stronger pedicels than normal buckwheats (Alexeeva et al. 1988). The green-flower lines had higher breaking tensile strengths. In Japan, we identified a green-flower mutant named 'W/SK86GF' (Fig. 4A) in 1999, which was from a progeny of hybridization between Kitawasoba and Skorosperaya 86. Consequently, green-flower appears to be promising as breeding material for shatter resistant varieties. Morishita and Suzuki (2017) performed a large-scale practical test of the green-flower shattering-resistant breeding line by using a seeding machine and combine harvester. In late harvesting with the combine harvester, despite an increased 'head loss' in the green-flower line, the 'threshing and sorting loss' the green-flower line decreased and the 'pre-harvest shatter

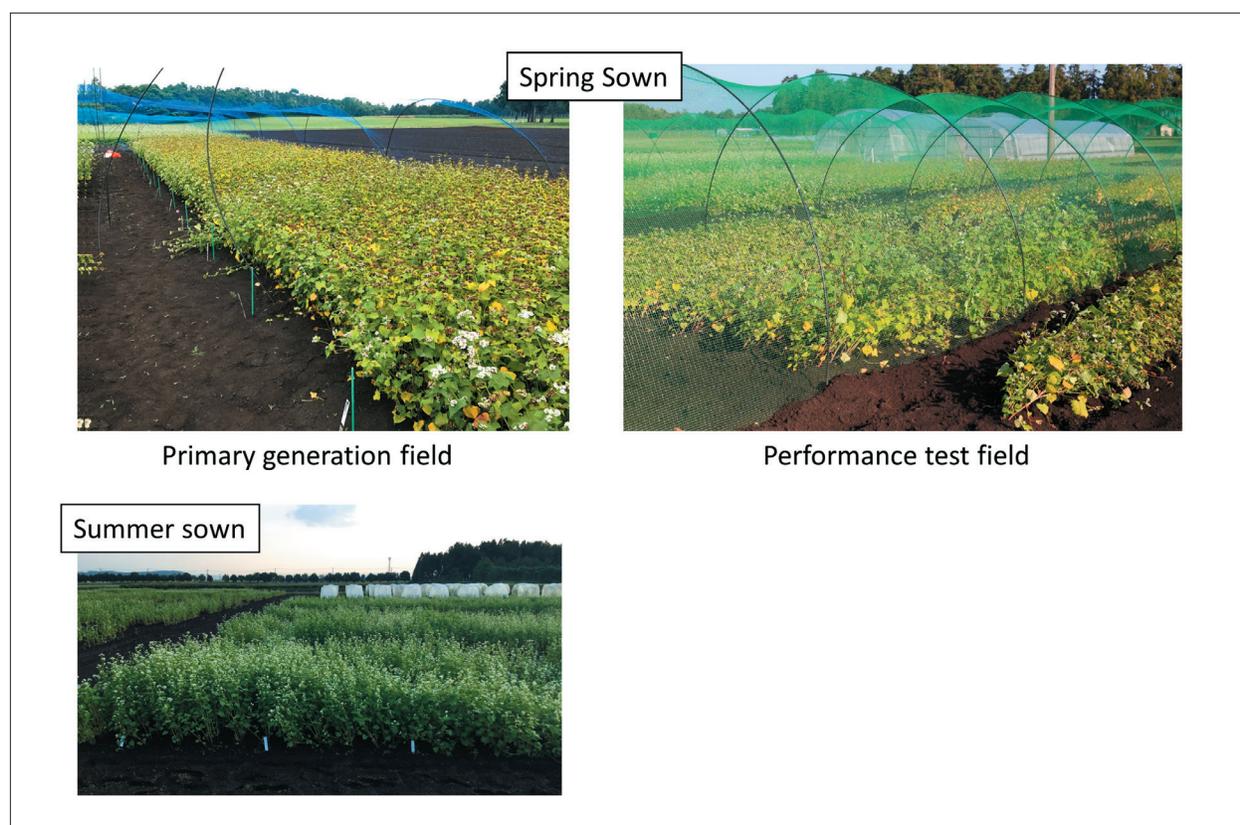


Figure 2. Pictures of buckwheat breeding field. Bird netting is used for spring sowing due to the high bird damage. Summer sowing is not covered because it causes less bird damage.

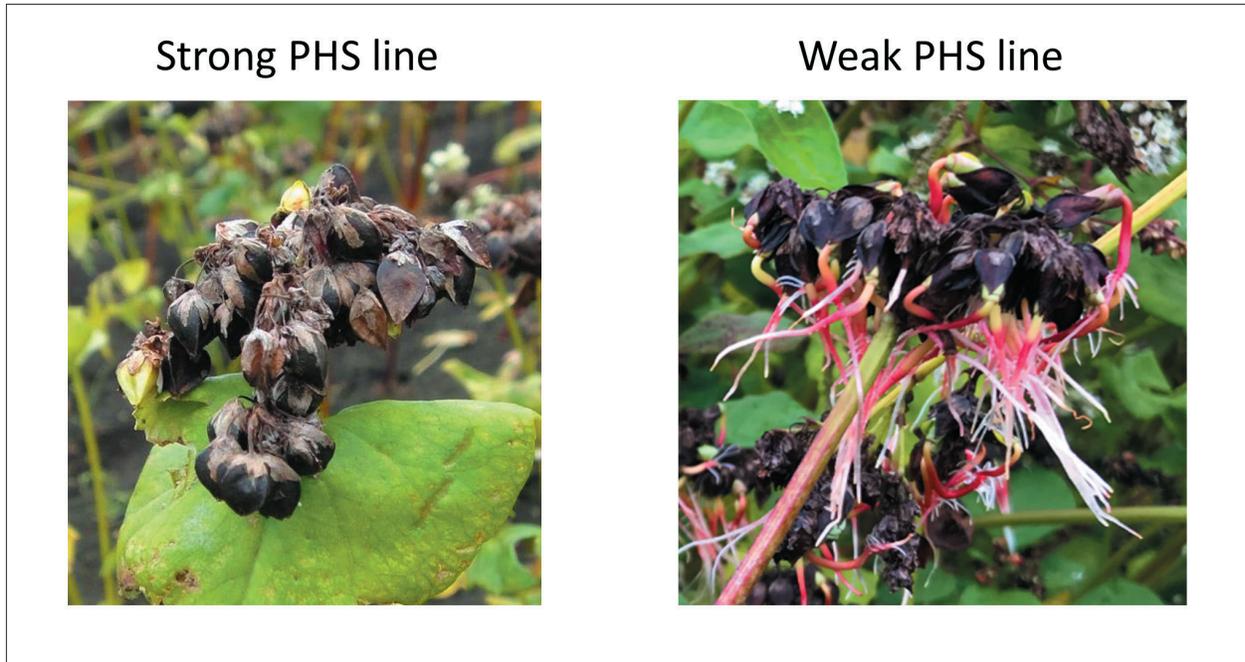


Figure 3. Pictures of PHS in buckwheat.
Pictures were taken in maturing time in spring sown field.

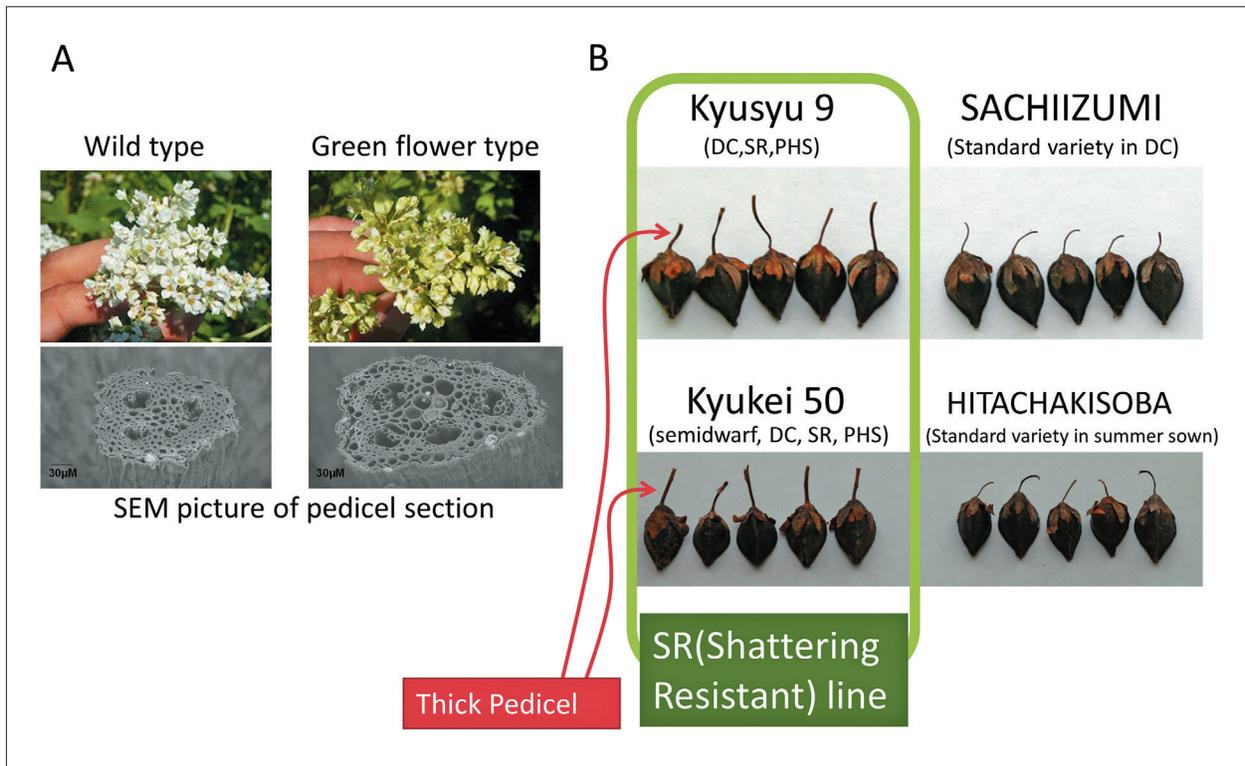


Figure 4. Shattering resistant trait in buckwheat.
A: Pictures of flower and pedicel in greenflower type mutant and wild type. B: Pictures of pedicels in promising breeding line and standard variety.

loss' of 'Kitawasesoba' increased dramatically, so the total loss ratio of the green-flower line was lower than that of 'Kitawasesoba'. Furthermore, the use of green-flower traits was effective for reducing yield loss under conditions when shattering occurs heavily, such as in rain, strong wind, and late to extremely late harvesting. These experiments indicated that it is necessary to reduce 'threshing and sorting loss' by adjustment of the operating conditions of the combine harvester and choosing a suitable harvest time with the combine harvester for practical use with shattering-resistant varieties.

Recently, we developed a promising breeding line named 'Kyushu 9', which exhibits resistance to both shattering and preharvest sprouting. The 'Kyushu 9' possesses the 'green flower type' shattering resistance trait, and as a result, it had a thick pedicel (Fig. 4B) with breaking tensile strength approximately two-fold stronger than that of 'HITACHIAKISOBA', a leading variety of summer-sown buckwheat in Japan (Table 1). The yield of 'Kyushu 9' was also higher than that of 'HITACHIAKISOBA'. Additionally, 'Kyushu 9' has a pre-harvest sprouting resistance class similar to that of 'KANOUZAIRAI', which has the strongest resistance against pre-harvest sprouting. In addition, double cropping (Fig. 5) is essential to increase buckwheat production. However, in buckwheat production areas, contamination caused by shattered

fallen seeds during harvesting is a serious problem. To address this issue, the use of the same variety in both spring and summer is necessary. To achieve this, the use of a variety with an appropriate ecotype, such as an intermediate-autumn type, is required. 'Kyushu 9' has a suitable ecotype for double cropping. Lodging is also an important factor that can reduce buckwheat production. Recently, we also developed 'Kyukei 50' which has a semi-dwarf trait with shattering resistance and practical pre-harvest sprouting resistance (Suzuki et al., 2023a).

Starch is the chief constituent of buckwheat groats, making up to 75% of their dry weight (D.W.). In general, the amylose content of buckwheat starch is 20–30%. The key enzyme for amylose synthesis, GBSS-I, has partly characterized by Chungoo et al., 2012. The varietal difference in amylose content of buckwheat starch is limited compared to that of major cereal starch, such as rice and wheat. In addition, there has been little research into developing buckwheat varieties with waxy-type starch (amylose-free) that would have sticky mechanical characteristics. Gregori and Kreft (2012) reported a low-amylose (3.8–16%) buckwheat mutant. However, the causative genes, starch properties, and physical properties of this food have not yet been characterized. To produce high-quality buckwheat, we identified a promising mutant that lacks the accumulation of granule-bound starch

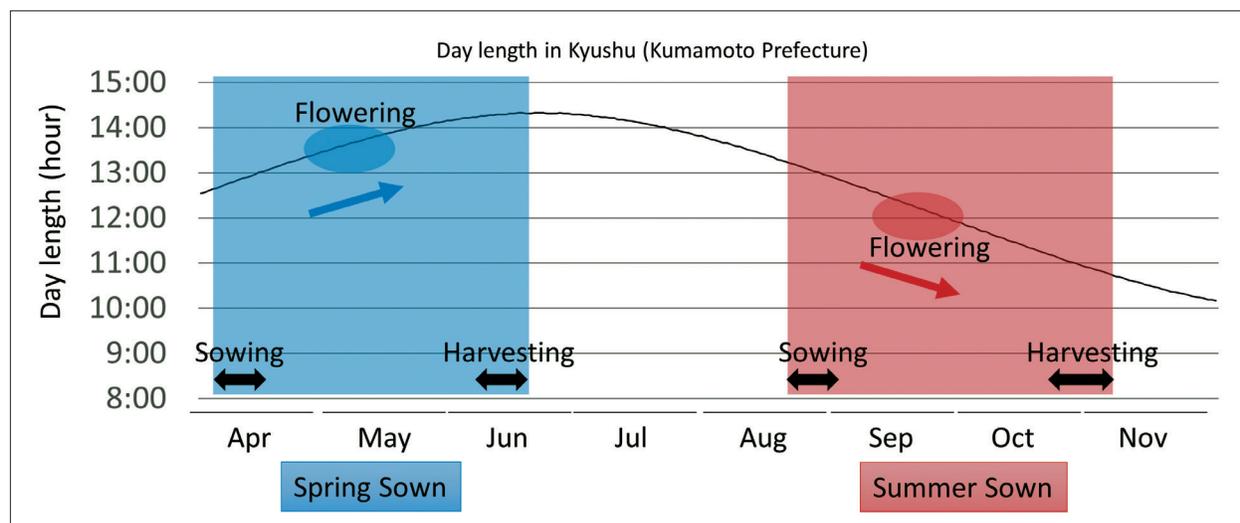


Figure 5. Typical double cropping in buckwheat in Kyushu island.

Spring and summer sowings have significant differences in day length. Therefore, it is important to use varieties suitable for each sowing time (different varieties) in order to achieve the maximum annual yield.

However, due to limited field area in Japan, using different varieties would pose a contamination problem of seed shattering and pollen cross-pollination. As a result, it is necessary to use the same variety for both spring and summer sowings.



Figure 6. Picture of a breeding Field for Perennial Buckwheat.

A: Picture of a breeding Field for Perennial Buckwheat in 2022 summer.

B: Picture of inter-specific hybrids sprouting and growing from last year's plant roots after soil freezing.

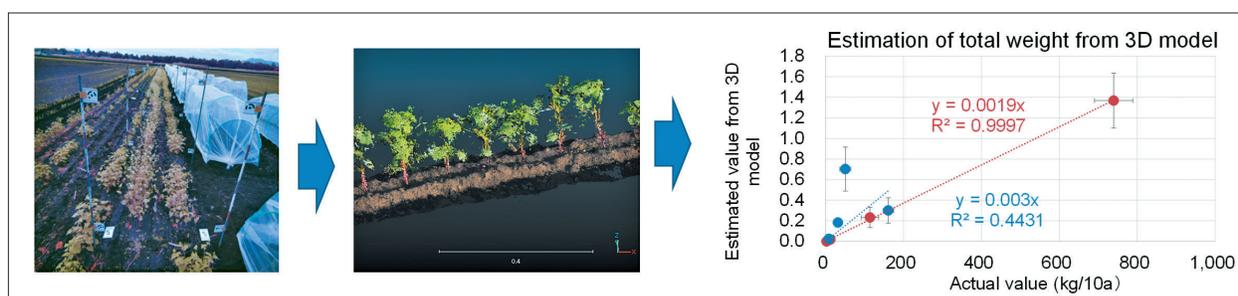


Figure 7. 3D plant modeling system. Brief scheme to make 3D plant modeling and estimation of total weight.

synthase a GBSSa mutant (Suzuki et al., 2021, 2023b). This mutant line has a low amylose concentration compared to the wild type line. The noodles made from the GBSSa mutant were soft and sticky compared to those made from the wild type. Furthermore, the soft texture of the noodle was retained even when stored in chilled conditions.

To develop a practical breeding line of perennial buckwheat, we crossed Tartary buckwheat (*F. tataricum*) and perennial buckwheat (*F. cymosum*) and performed selection suitable for the climate of Japan. We identified the key enzyme that generate strong bitterness in its food (Suzuki et al., 2023c). We have identified some promising breeding lines that exhibit a non-shattering trait with perenniality (Fig. 6). Additionally, we have developed an

3D plant modeling system (Kochi et al., 2021) for buckwheat that operates using images and can measure plants non-destructively without physical contact (Fig. 7). This system is particularly useful for estimating parameters such as total weight during plant growth for individual plants or colonies of plants, especially for perennial buckwheat. It allows for continuous tracking of the same individual plant or plant colony throughout its growth period without the need for any destructive measures.

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Table 1. Result of performance test in 2022.

Variety name↓		sowing time	growth period	Plant height	lodging degree	PHS *1	Green flower trait	breaking tensile Strength	Total weight	Yield		DC Yiled		1000 seeds weight
										rel.	sprintg +August	rel.		
		day	cm	0 : none ~ 4 :	%	g	kg/10a	kg/10a	kg/10a	%	kg/10a	%	g	
NARO-FE-1	Spring sown standard var.	4/6	60	89	0	26	-	39.4	494	206	100			33.1
Spring sown	HITACHAKISOBA Summer sown standard var.	4/6	71	129	0	26	-	44.4	696	120	58			39.2
	Kyushu No. 9	4/6	65	104	0	1	○	103.5	806	196	95			35.8
NARO-FE-1	Spring sown standard var.	8/23	59	54	2.3	68	-	41.7	279	123	100	329	100	27.6
Summer sown	HITACHAKISOBA Summer sown standard var.	8/23	68	82	4.0	79	-	66.0	643	212	172	332	101	36.1
	Kyushu No. 9	8/23	68	61	2.3	21	○	109.6	406	217	176	413	125	31.6

Strong PHS

(The smaller the number, the more PHS.)

Strong SR

(The higher the number, the more SR.)

High DC yield

These results are based on conditions where there was no crop lodging or encounters with typhoons during the harvest season. Therefore, it is believed that the effects of PHS and SR would be more pronounced in the presence of these adverse conditions.

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IZVLEČEK

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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 (Syta, 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 (Martinez-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 at 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 was in Jablje at Ljubljana (GPS 46°08'37.7"N, 14°33'24.5"E at 303 m.a.s.l. Meteorological 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 further laboratory processing.

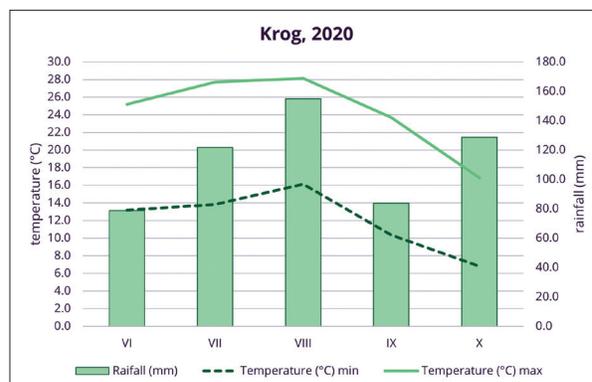
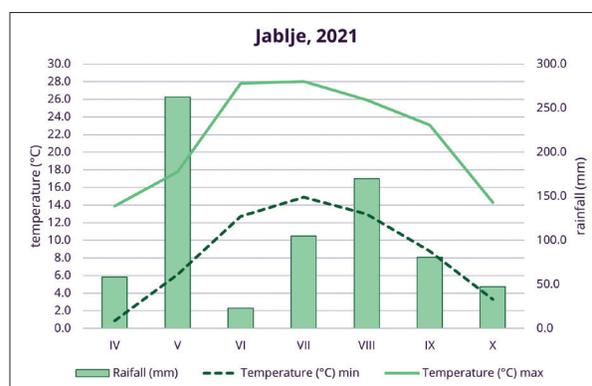
The standards of the phenolic compounds apigenin, caffeic acid, catechin, chlorogenic acid, epicatechin, gallic acid, hesperidin, hyperoside, isoorientin, isoquercetin, iso-vitexin, kaempferol, naringenin, orientin, procyanidins B1 + B3, and procyanidin B2, quercetin, quercitrin, rutin, vitexin, and 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

(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

**Fig. 1.** Weather conditions in Krog, Slovenia in 2020.**Fig. 2.** Weather conditions in Jablje, Slovenia in 2021.

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 $\mu\text{mol TE/g dw}$) was obtained in 2020 while the mean value of AA was slightly lower (11.96 $\mu\text{mol TE/g dw}$) 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

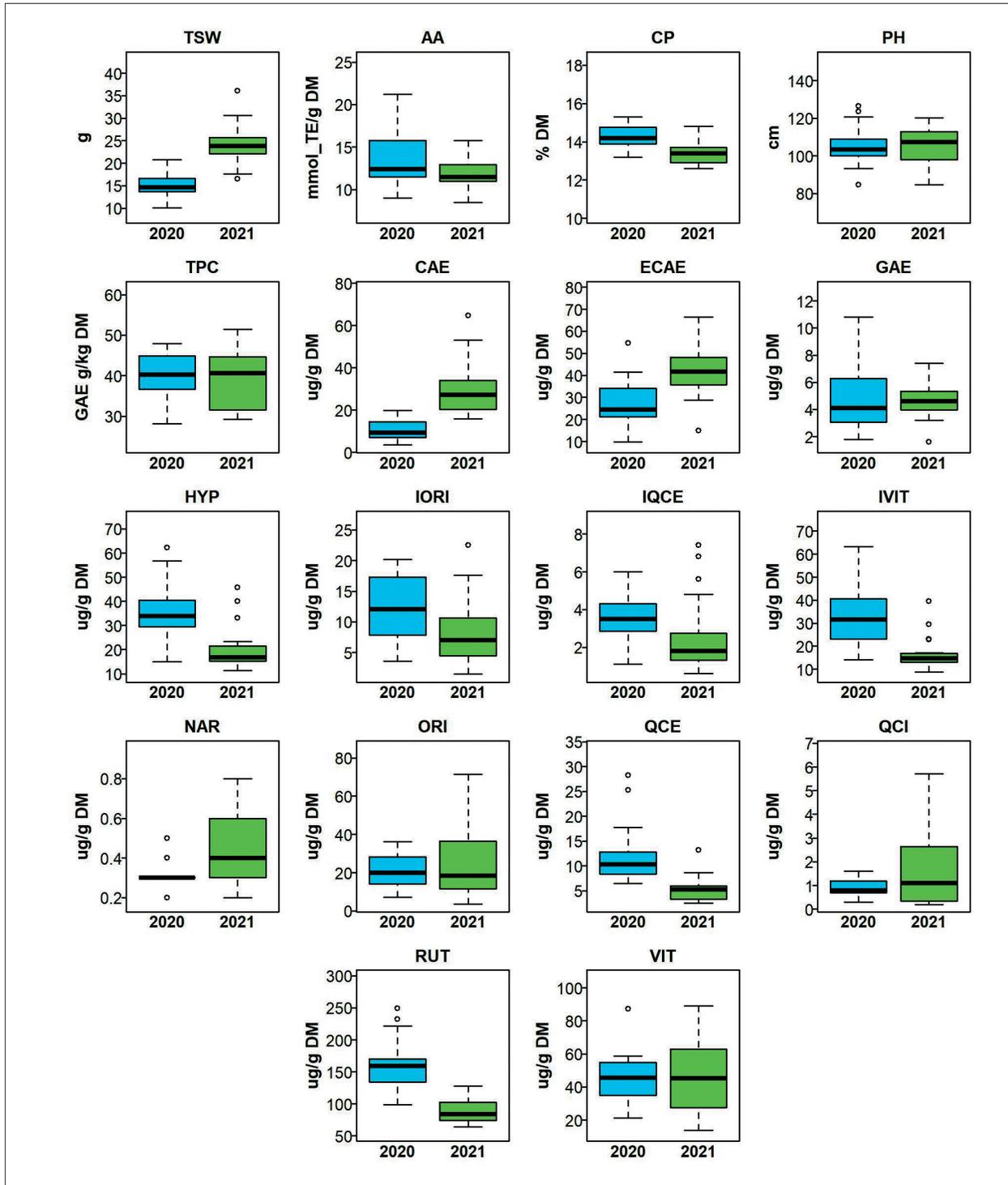


Fig. 3. Distribution of values for set of morpho-phenological parameters and selected chemical compounds measured in 23 common buck-wheat accessions genotypes cultivated in Slovenia, 2020-2021. The abbreviation used in the diagram are as follows: Antioxidant activity (AA), Catechin (CAE), Crude_protein (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)

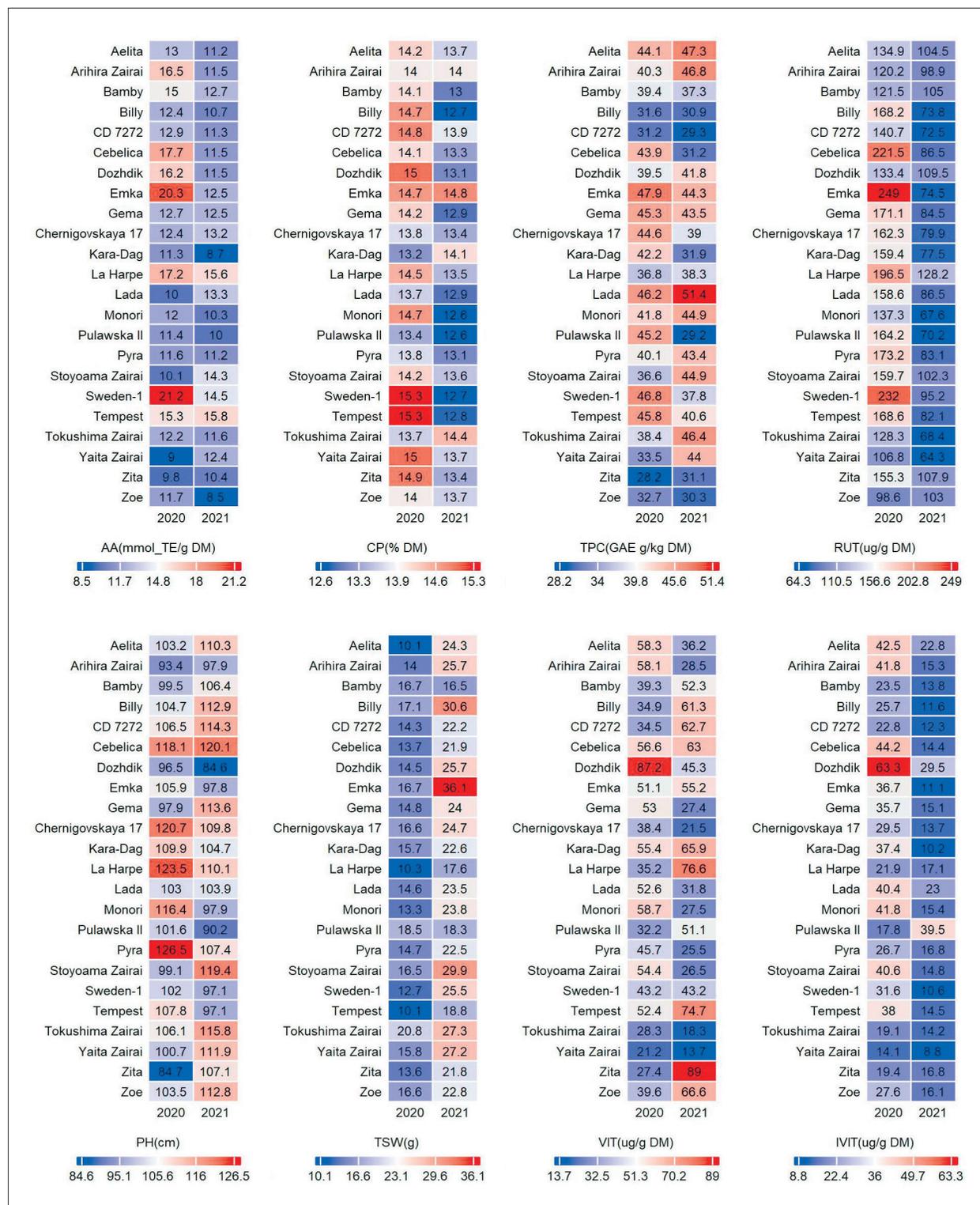


Fig. 4. Heat maps showing the selected morpho-phenological parameters and chemical compounds for the collection of 23 common buckwheat accessions. Values for respective traits from individual years are displayed on a scale from blue (min) to red (max) in accordance with colour key below each heatmap.

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-

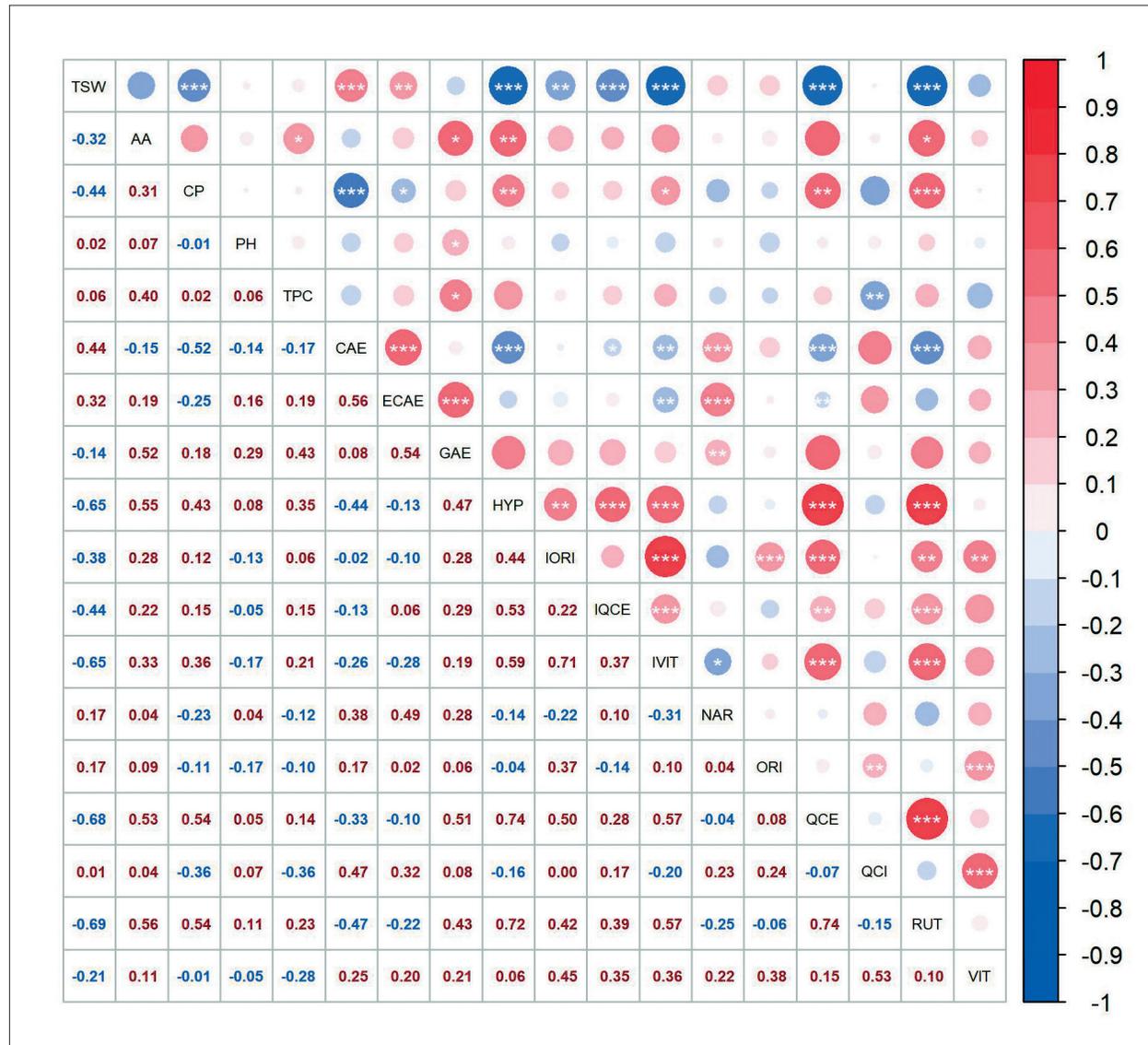


Fig. 5. Spearman's correlation between evaluated descriptors for the collection of common buckwheat accessions. The colour and size of circles above the diagonal determine level of negative (blue) or positive (red) correlation between the pairs of descriptors as illustrated by colour key and correlation 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 odlični 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.