CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Evaluation of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) genetic resources

MASTER'S THESIS

Prague 2023

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Declaration

I hereby declare that I have done this thesis entitled “Evaluation of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) genetic resources” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 22 April 2023

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Nitkamon Iamprasertkun

Acknowledgments

I gratefully appreciate my supervisor specialist, Ing. Petra Hlásná Čepková, Ph.D. (Gene Bank, Crop Research Institute, Prague) for supporting and guiding me in the research as well as my living in Prague which helped me to finish my master's degree journey successfully and having a great valuable time during my studying.

I am so thankful to my supervisor Ing. Iva Viehmannová, Ph.D. (Czech University of Life Sciences Prague) for her fast-responding, guidance, and recommendation about my thesis that made it as perfect as we can.

Many thanks to the teams, especially Daniela Pátková, Olga Uhlířová, Tomáš Čermák, and Michal Jágr, at the Crop Research Institute Prague for taking care of the plant in the field, providing the Tartary buckwheat samples, and allowing me to do the research in their laboratories. Moreover, I would like to thank Lucie Dostalíková, Ph.D. student, who taught and trained me in laboratory work.

Last but not least, I am grateful for the heart-warming support from my family as well as the financial support from the Anadamahidol Foundation for my study and living aboard. This thesis and degree could not succeed without them.

This work was financially supported by the Ministry of Agriculture of the Czech Republic (no. MZE-RO0423), the Internal Grant Agency of the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague IGA (Project No. 20223105), and the ECOBREED project, European Union's Horizon 2020 research and innovation program, grant agreement No. 771367.

I gratefully acknowledge the Subsidy Program-the National Program for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity (no. MZE-62216/2022-13113) for information about Tartary buckwheat genetic resources in the Czech Republic.

Abstract

Because of its beneficial nutrients, particularly its protein content and suitability for a gluten-free diet, Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.), a pseudo-cereal, is gaining great attention. The objective of this thesis was to evaluate the yield, morphological traits, and nutrition component from selected genotypes of Tartary buckwheat under the climatic condition of the Czech Republic. Morphological traits of the samples in 2022, including plant height, branching, inflorescence characteristics, yields, etc., were evaluated, while the data from 2019 – 2021 were provided by the Crop Research Institute (CRI) to compare them with the results obtained in 2022. The nutrition components of the samples in 2019 – 2022, including protein content, total phenolic content, and antioxidant activity were analyzed. The morphological traits of samples from different origins did not show significant differences. Although the yield and weight of thousand seeds (WTS) were less in 2022 compared to the previous years, the average protein content, total phenolic content, and antioxidant activity were 11.30% of dw, 144.58 mg_{GAE}/g_{dw}, and 39.76 $\mu mol_{TE}/g_{dw}$, respectively. The total phenolic content, antioxidant activity, and protein content of each production year (from 2019 – 2021) were significantly different, while the results from samples belonging to each origin were not different. Some genotypes showed great potential. The genotype from Slovenia represented the highest yield under unflavored weather conditions, whereas the American genotypes showed a high number of seeds per cymes. Hence, these were proposed for further investigation into improving the Tartary buckwheat for the conditions in the Czech Republic.

Keywords: genetic resources, nutritive compound, phenotypic traits, plant breeding, Tartary buckwheat

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1. Introduction

Pseudo-cereal crops are playing the main role as the superfood in the 21st century with high and well-balanced nutrition. Among pseudo-cereals, buckwheat especially Tartary buckwheat (*Fagopyrum tataricum*) is the best source of phenolic compound (Martinez-Villaluenga et al. 2020) which is 10 – 60 folds of the total phenolic in amaranth (Mir et al. 2018) as well as the balanced source of the essential amino acids (Luthar et al. 2021b). Moreover, Tartary buckwheat is confirmed for various beneficial effects on human health (Zhu 2016). However, the cultivation of pseudo-cereal crops is complicated due to the poor agronomical traits which affect the yield and decision of the farmers (Pirzadah & Malik 2020); for example, the self-incompatibility in common buckwheat (Ueno et al. 2016), seed shattering, flower abortion, or difficult hulling (Dar et al. 2018). Although Tartary buckwheat contains various downsides in cultivation, it also has profitable agronomical traits; for instance, drought and cold resistance (Song et al. 2020; Aubert et al. 2021).

After Tartary buckwheat was in the spotlight, the breeding attempts for better in both quantity and quality were more studied. Negative traits as bitter taste, seed dormancy, and allergic protein, are targeted to be removed, while the positive traits, such as high phenolic, are desired (Woo et al. 2016; Luthar et al. 2021b). The resistance to the various environmental conditions can maintain the yields, whereas the dehulling ability, increasing of nutritive compounds, and the satisfying of taste is considered as the improvement of quality (Wang & Campbell 2007; Suzuki et al. 2014; Kreft et al. 2020; Wang et al. 2022b).

The main objective of the study was to evaluate the morphological traits together with the nutritive compounds, including protein content, total phenolic content, and antioxidant activity of Tartary buckwheat genotypes accessed from the gene banks under the conditions of the Czech Republic. The good performance genotypes can be used as information for the other breeders for further breeding programs in Central Europe.

2. Literature Review of Tartary Buckwheat

2.1. Taxonomic classification

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) belongs to the Polygonaceae family, Caryophyllales order, Magnoliopsida class, and Tracheophyta division. *Fagopyrum* genus presently contains 25 species distributed in South Asia, regularly on the southern edge of the Qinghai-Tibetan Plateau (Ohsako & Li 2020).

Only two species from this genus, common buckwheat and Tartary buckwheat, are cultivated (Ohsako & Li 2020). In addition, the cultivated and wild forms are dissected and classified as subspecies. Wild and cultivated common buckwheat is *F. esculentum* ssp. *ancestrale* and ssp. *esculentum* while wild and cultivated Tartary buckwheat is *F. tataricum* ssp. *potanini* and ssp. *tataricum* (Ohnishi 1998).

Although Tartary buckwheat has a long history, over a thousand years, cultivated and domesticated in the Chinese ethnic culture, the Yi people in Liangshan, China (Song et al. 2019), the first taxonomy study of *Fagopyrum* was reported in 1742 by Tourn, then it was grouped into *Polygonum* by Linnaeus in 1753. After that, the taxonomic classification of *Polygonum* and *Fagopyrum* was a tangled systematized in the 19th century (Zhou et al. 2018d).

In the early 19th century, *Fagopyrum* and *Tiniaria*, a genus in the Polygonaceae family, genera were combined into a *Fagopyrum* genus with a subsection of *Tiniaria* and *Eufagopyrum* which were classified by the morphology of the inflorescences and the persistent of the perianth. In the late 19th century, the *Fagopyrum* genus was assorted into two groups by achene size consisting of *F. esculentum sensu lato* and *F. gilesii sensu lato* which is close to the classification in the present. However, nowadays, *Fagopyrum* has been accepted as a genus and discriminated from other genera by the position and morphology of the embryo (Ohsako & Li 2020).

In addition, *Fagopyrum* has been distinguished into two groups consisting of the *cymosum* group and the *urophyllum* group which is firmly supported by both morphological and molecular studies. Both groups represent monophyletic characters in the phylogenetic tree analyzed from *accD 5'* and *rbcL* sequencing (Yasui & Ohnishi 1998). By the morphological characteristics, the species in *cymosum* group have long horizontal cotyledons, large lusterless achene, and are partially covered by persistent perianths. On the contrary the species in *urophyllum* group have laterally long or round cotyledons, small lustrous achenes, and are fully covered by persistent perianths (Ohnishi 2016).

2.2. Origin, geographical distribution, and production

F. tataricum is believed to originated in the southwest of China, Yunnan same as *F. esculentum*, and domesticated in eastern Tibet, southwest of China (Yunnan and Sichuan) because of the high genetic diversity and genetic similarity of the landraces in this area (Zhou et al. 2018c). The amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) analyses support China as the origin of the relationship between wild species and cultivated landraces (Tsuji & Ohnishi 2000, 2001). Although, there is no evidence such as the pollen fossils and macrofossils of *F. tataricum* records that can confirm its presence in China. The researchers predicted that the lack of fossil evidence might be caused by the less production of pollen and the morphological character of the flower, cleistogamous (Hunt et al. 2018).

Naturally, the distribution of Tartary buckwheat is in the subtropical mountain area, in southwest China, which has low temperatures and highland regions with an altitude of 400 – 4,400 m above sea level (Zhou et al. 2018a). From the archaeology studies, Tartary buckwheat and common buckwheat were well spread and domesticated to the other parts of China in the mid of 6th millennium cal BP (approximately 4th millennium cal BC). Two millenniums after, they were distributed to the Tibetan plateau/Himalayan region and went west entering and ubiquitous in the temperate zone in Europe from the 3,000 – 4,000 cal BP (1,050 – 2,050 cal BC) via the southerly route. At the same time, they were distributed to Japan via the Korean peninsula from northern China at 4,000 cal BP (2,050 cal BC) (Campbell 1997; Hunt et al. 2018). The research estimated that buckwheat was stepped into Russia between the 9 – 12th centuries, but the route is unclear (Suvorova & Zhou 2018). However, nowadays, Tartary buckwheat is widespread on three continents around the world, including Asia, Europe, and North America. Tartary buckwheat presently distributes to several countries; for example, Afghanistan, Bangladesh, Belgium, China, the Czech Republic, Slovakia, Denmark, France, Germany, Great Britain, Japan, Kazakhstan, Mongolia, Myanmar, Nepal, Netherlands, Poland, Romania, Sweden, Ukraine, etc. (Figure 1).

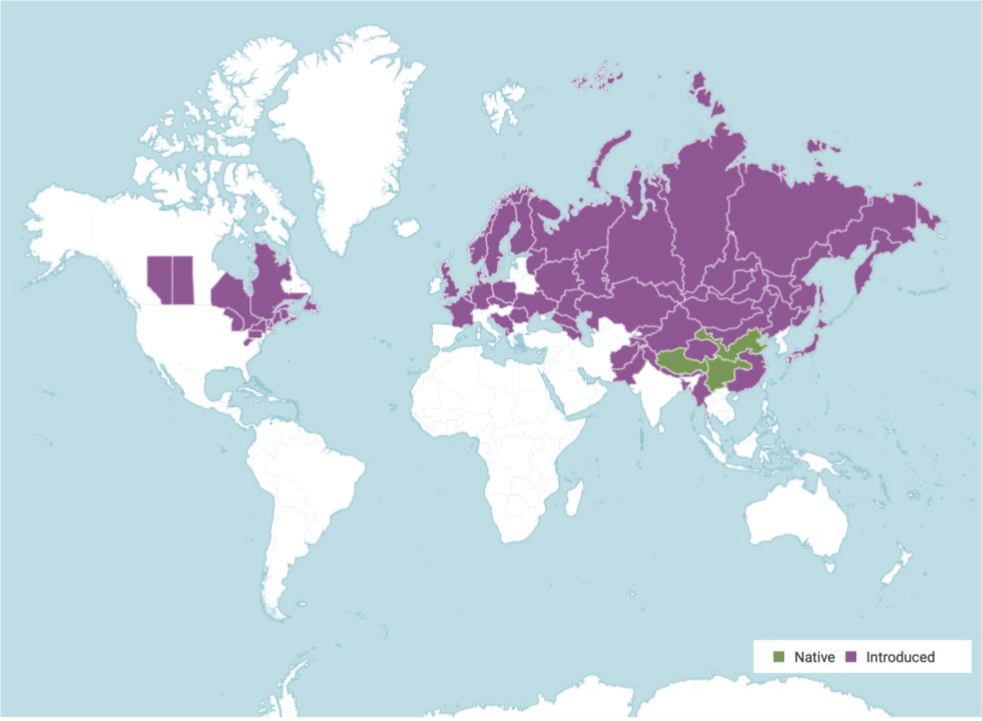

Figure 1 The world distribution of Tartary buckwheat (*Fagopyrum tataricum*)

(Source: Plant of the World Online by the Royal Botanical Garden, Kew, 2022)

Tartary buckwheat is cultivated in a few countries, for example, China, Nepal, Bhutan, Pakistan, and India (Chandra et al. 2021). China was reported as the main source of Tartary buckwheat in 2018, with an approximate production of 300,000 – 400,000 tons/year and the greatest area of cultivation around 200,000 – 300,000 km^2 (Zhou et al. 2018a), which is approximately 30% of the buckwheat planting areas (Suvorova & Zhou 2018). The world production reports of Tartary buckwheat are not available because they were mixed between Tartary buckwheat and common buckwheat. However, the Tartary buckwheat production is a minor proportion due to the less distribution compared to common buckwheat.

From 2001 – 2010, China had the greatest production of total buckwheat grains in the world with a production of 800,000 tons/year, followed by 635,000 tons/year (Russian Federation) and 248,000 tons/year (Ukraine), while the other countries represented significantly different production with less than 100,000 tons/year. On the contrary, from 2011 to 2020, the highest production of buckwheat was from Russian Federation with 927,000 tons/year, followed by China with 563,000 tons/year, which illustrated the bulky gap with other European countries (Figure 2). This data showed that the main production of buckwheat in the past 2 decades was in China and Russian Federation (FAOSTAT 2022).

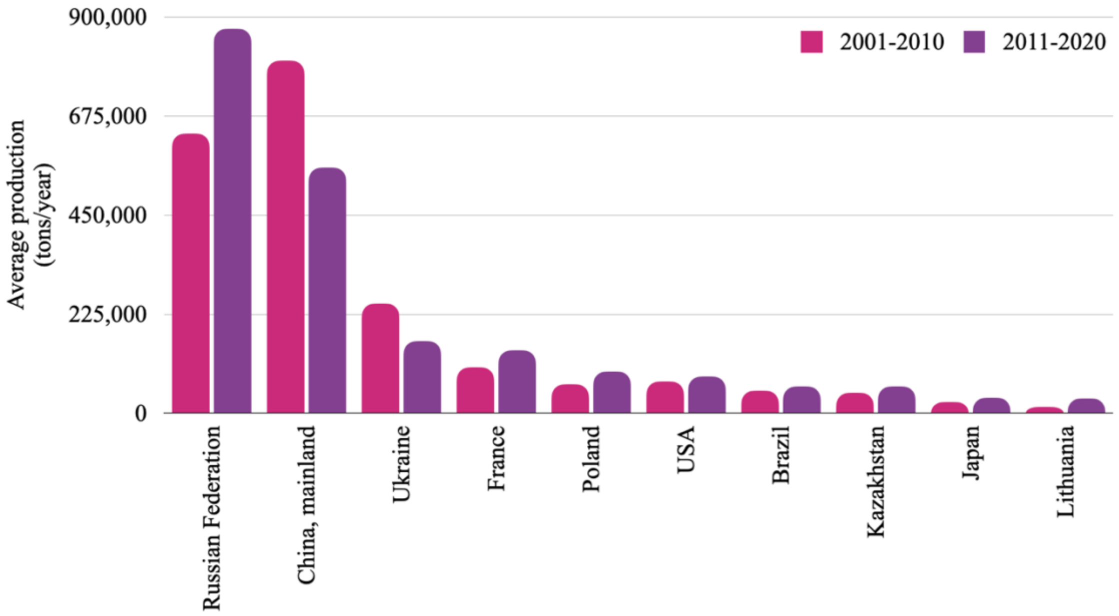

Figure 2 The average production of buckwheat (common and Tartary buckwheat) in the years 2001 – 2010 and 2011 – 2020 of the first-tenth greatest production countries in the world according to FAOSTAT (2022)

2.3. Botanical description

Tartary buckwheat is an annual herb ascending or erect with a green, yellowish green to red colour, branched stem. The height of the stem is between 10 – 100 cm tall but usually 30 – 80 cm (Freeman & Reveal 2005).

Petioles are 1 – 7 cm long but can be short as 0.5 cm (Freeman & Reveal 2005). Leaf blades are broadly triangular to broadly hastate. Normally, the width and length are equal to 2 – 8 cm (Campbell 1997). Leaf bases are truncate or cordate, margins are entire or ciliate, and apexes are acute to acuminate. Leaf veins are palmate with 7 – 9 primary basal and papillate along the veins on both sides of the leaf (Figure 3) (eFloras 2008).

Inflorescences are generated at both positions of the terminal and axillary buds. Inflorescences are racemelike with paniculate with 2 – 10 cm long. Bracts are ovate with 2 – 3 mm, and acute apex. Peduncles are 1 – 6 cm long and pedicels are 1 – 4 mm long. Flowers are often cleistogamous or close flowers which are automatic self-pollination (Freeman & Reveal 2005). Perianths are white to greenish with approximately 2 mm long tepal. Tepals are triangular to ovate with the entire margin and obtuse to acute apex. 8 yellow nectary glands are presented between the stamens (Campbell 1997). Stamens are as long as perianths around 2 mm long. Stigmas are capitate (Figure 3) (eFloras 2008).

Fruits are dry indehiscent achene. They are 5 – 6 mm long, 3 – 5 mm in width, and often observe with the persistent perianth. Achenes are grey, black-brown, and rarely mottled with blackish spots medially. Achene shapes are narrowly ovoid or trigonous with grooved surfaces. Angles are round at the bottom and sharply acute above, possibly observe with sinuate-dentate (Zhou et al. 2018a).

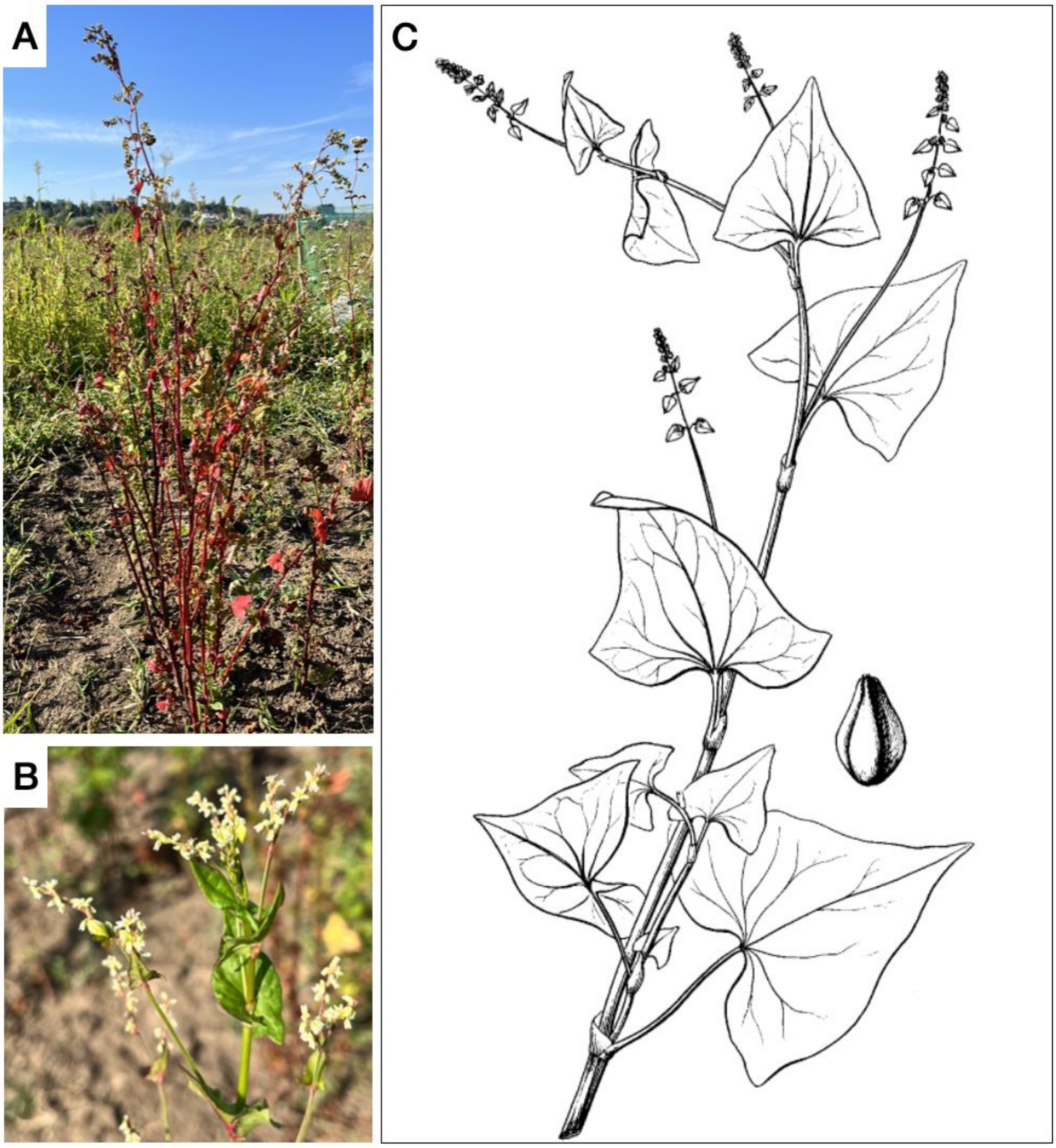

Figure 3 The botanical characters of Tartary buckwheat (*Fagopyrum tataricum*): habit (A), inflorescences (B), and botanical drawing of leaves, inflorescences, and seed (C)

(Source: Flora of China, vol 5, 2008)

2.4. Nutritive properties

2.4.1. Carbohydrates

The principal components in Tartary buckwheat seed are carbohydrates which can be assorted into 3 groups, starch, dietary fiber, and D-chiro-inositol. Starch is the major component in the seeds, with over 70% of the dry weight (dw), followed by dietary fiber and D-chiro-inositol, respectively. The starch granules are usually polygonal, approximately 2 – 15 µm in size (Zhu 2016). The dietary fiber content is 8.4 – 26% depending on the genotype, while the resistant starch is 13.1 – 22.5%. The D-chiro-inositol, a nutritive carbohydrate that lowers blood pressure and glucose levels in the blood, is 0.23 – 18% and 0.37 – 0.39% in dry seed and leaf, respectively (Zhu 2016; Luthar et al. 2021b). However, the varying carbohydrate structures depend on the varieties, the planting environment, and the food processing (Luthar et al. 2021b).

2.4.2. Proteins

Tartary buckwheat grain shows a lower protein content and amino acids composition than the common buckwheat and other wild varieties, *F. cymosum*, *F. gracilipes*, and *F. caulophyllum* (Zhou et al. 2018b); however, it contains a well-balanced level of protein and essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and tryptophan), with 10.30% of protein content, 10.21% of amino acids, and 3.37% of essential amino acids (Zhou et al. 2018b; Luthar et al. 2021b).

The protein classification system in cereal is based on the solubility of the proteins. There are four classes of protein fractions, including albumins, globulins, prolamins, and glutelins. Albumins are a fraction that dissolves in a hypotonic solution or water. Globulins are soluble in an isotonic or salty solution. Prolamins are dissolved in ethanol or other alcohol. Glutelins are a fraction that is soluble in diluted acids or bases. The last two fractions, prolamins and glutelins, can form gluten which is the reason for celiac disease (Škrabanja & Kreft 2016).

By the reason of the non-cereal family, Tartary buckwheat contains fewer prolamins or gluten content than other cereals (Wijingaar & Arendt 2006). The majority fraction in Tartary buckwheat is albumins, followed by globulin and gliadin (one of the prolamins) with 51.52%, 13.69%, and 8.83%, respectively (Ruan et al. 2020). Thus, it is recommended for celiac disease patients (Luthar et al. 2021b).

The consumption of hydrothermally processed buckwheat is suggested because the hydrothermal processes can reduce the effect of the polyphenols in the husk. The digestibility of buckwheat protein is interfered with the polyphenols, especially tannic acid and catechin, which represent a strong inhibitory effect on in vitro digestion enzymes, peptic and pancreatic (Ahmed et al. 2013; Luthar et al. 2021b). In addition, the allergen protein, Fag t2, is found in Tartary buckwheat seed but has no clinical report yet. The researchers expected that the allergic symptoms would be like the allergy in common buckwheat, urticaria, asthma, angioedema, and allergic rhinitis (Zhu 2016).

2.4.3. Vitamins and minerals

The buckwheat seeds are identified as a good source of vitamins including vitamins B, C, and E, which are able to alter the concentration via the germination process (Zhu 2016). Vitamin E is the significant vitamin in seed, with about 127.2 µg/g, followed by 28.1 µg/g of vitamin B (Zhu 2016; Zhou et al. 2018b). In addition, the Tartary buckwheat grains, grain products, leaves, and leaves infusion contained the mineral elements, for instance, Se, Zn, Fe, Co, Ni, Sb, Cr, and Sn. Nevertheless, Tartary buckwheat indicated the Cr absorption ability and the represented Cr content in all plant parts. Hence, the cultivation area and the soil contamination should be concerned (Luthar et al. 2021b).

2.4.4. Phenolic compounds

Phenolic compounds are the most abundant secondary metabolites found in the plant kingdom. Over 8,000 phenolic compounds were identified from several plant species (Pandey & Rizvi 2009). In addition, they perform board range of bioactivities and medical therapeutic effects. Hence, polyphenols were used in traditional medicine and were interested in drug development processing. Phenolic compounds are aromatic compounds attached to one or more hydroxyl groups (Ghani 2020).

Phenolic compounds are a bulky group of compounds, so the basic classification of this group is achieved by the chemical structure. From the chemical skeleton, phenolic compounds can be divided into three classes, including phenolic acid, flavonoids, and non-flavonoids. Each class can be separated into several groups (Figure 4) (Rambaran 2020). However, the studies in Tartary buckwheat show the presence of some groups of phenolic compounds, including phenolic acids, flavonoids, anthraquinones, phenylpropanoid glycosides, stilbenes, and fagopyrin (Zhu 2016).

Similar to common buckwheat, Tartary buckwheat represents a high phenolic compound content. The total phenolic content in Tartary buckwheat grain is around 50 – 100 µmol of gallic acid equivalent/g of dw which is varying due to the genotypes and locations which refer to the environment (Guo et al. 2011). In addition, the total phenolic content is equal to approximately 23 – 50 folds of the total phenolic in corn and wheat (Adom & Liu 2002) and high than 2 – 13 folds in cranberry and apple (Sun et al. 2002); hence, Tartary buckwheat is suggested as the excellent dietary source of phenolic.

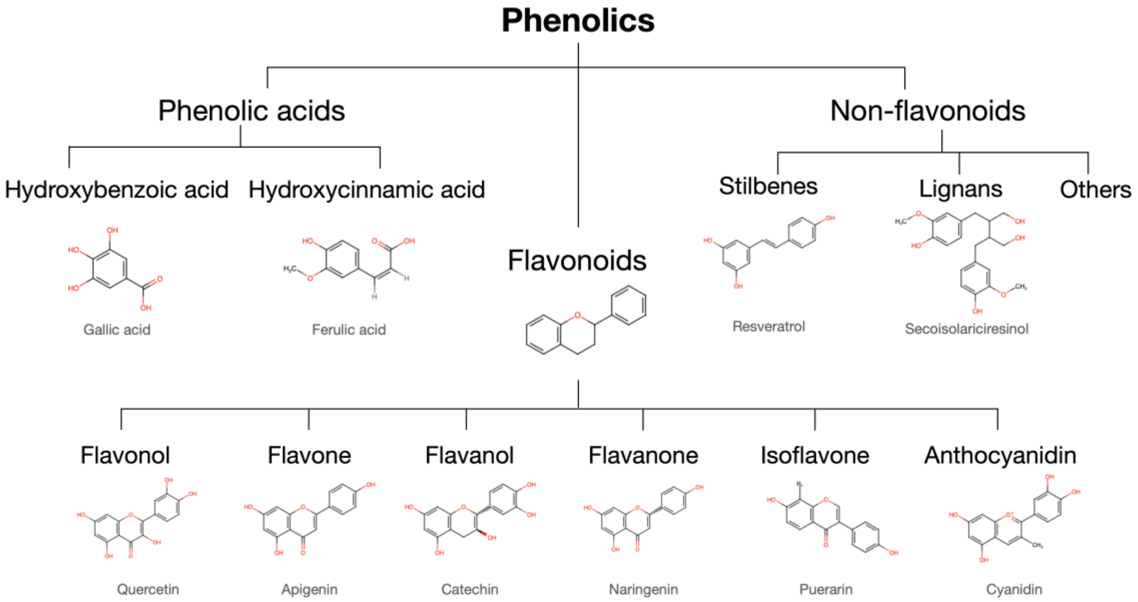

Figure 4 The classification of polyphenols according to Rambaran (2020)

2.4.4.1. Phenolic acids

By various techniques such as High-Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), and Nuclear Magnetic Resonance Spectrometer (NMR), the Tartary buckwheat phenolic acids were identified. The studies show that phenolic acids in Tartary buckwheat consist of caffeic acid, 6-coumaric acid, *p*-coumaric acid, ferulic acid, tannic acid, *p*-hydroxybenzoic acid, syringic acid, gallic acid, protocatechuic acid, chlorogenic acid, neochlorogenic acid, and vanillic acids (Zhu 2016; Ruan et al. 2020; Kreft et al. 2022b). Usually, the phenolic acids in Tartary buckwheat are more present in the free form than bound form and the environmental factors play an essential role in the total phenolic acid content (Guo et al. 2011).

In addition, hull and bran represent different main acids. The major phenolic acid in the hull is protocatechuic acid, accounting for 65.54% of all phenolic acid, while 58.61% of all phenolic acid in bran is *p*-hydroxybenzoic acid (Ruan et al. 2020), followed by caffeic acid, chlorogenic acid, and protocatechuic acid (Zhu 2016). Furthermore, the phenolic acid contents in Tartary buckwheat, especially chlorogenic acid, grow during the sprouting period (Kim et al. 2008). On the contrary, the total phenolic content tends to decrease after the dough-making because of the activation of enzymes by mixing the flour with water at moderated temperature, and then degradation of neochlorogenic acid occurs. However, a high temperature, more than 80 °C, can nullify this effect (Kreft et al. 2022b).

2.4.4.2. Flavonoids

The most important flavonoid found in this species is rutin, followed by the other flavonoids, including quercetin, quercitrin, vitexin, catechin, epicatechin, and epicatechin gallate (Kreft et al. 2022b). The greatest number of flavonoids was present in the leaves (81.2 mg/g), followed by 12.9 mg/g from the fruit. Compared to the other buckwheat species, the Tartary buckwheat illustrated the highest content of flavonoids in the leaves but the second rank in the flavonoid content in the fruit. The greatest fruit flavonoid content was found in *F. cymosum* with 17.5 mg/g (Zhou et al. 2018b).

However, flavonoid content in Tartary buckwheat was highly variable. From the research, the flavonoid content varies between 6.65 – 22.74 mg/g. The flavonoid's richest genotype is the variety "Mei-Hua-Shan" from China (Zhu 2016). While the highest rutin content variety is from Nepal (Kreft et al. 2022a). Normally, the rutin content varies between 0.8 and 2.9% in grains and approximately between 0.1 and 3.4% in leaves depending on leafage. The rutin content is increased by aging in leaves as well as the sprouts. Young sprouts contain approximately 0.3% of rutin and it grows up to 2.5% in developed sprouts (Kreft et al. 2022b).

The flavonoid content depends on both environmental factors, such as high temperature and radiation, and genetic factors. Tartary buckwheat varieties originating in the mountain regions display higher flavonoid content than others because of the high light intensity in the mountain areas (Kreft et al. 2022b). In addition, it tends to increase during the germination or food process; for example, the rutin content can be raised up 4-folds after 7 days of germination, and quercetin content in sprouts is higher than in seeds 10-90 times (Zhu 2016). Rutin can be changed to quercetin and rutinose by the rutinosidase during food processing which is the reason for the rocket increasing in quercetin content. Moreover, quercetin is able to form quercetin-polyphenol complexation with the starch in the grain causing low digestibility that is recommended for weight loss (Luthar et al. 2021b).

2.4.4.3. Anthraquinones and fagopyrin

There are a few anthraquinones identified from Tartary buckwheat including aurantio-obtusin, aloe-emodin, emodin, rhein, physcion, chrysophanol, questinol, ω-hydroxyemodin, and emodin-8-O-glucoside (Wu et al. 2014; Yang et al. 2020). However, there is one anthraquinone derivative, fagopyrin, which is the signature compound found in *Fagopyrum* ssp.

Fagopyrin is the plant's secondary metabolite related to hypericin and causes fagopyrism, a phototoxic effect on livestock and human involving serious exudate and skin irritation (Luthar et al. 2021b). Originally, *Fagopyrum* species store the protofagopyrins which are able to transform into fagopyrin after exposure to sunlight for at least an hour. The fagopyrin content is variable by the plant parts and time exposed to sunlight. In Tartary buckwheat, the highest content is found in the flowers and inflorescences which are over 8 times more than other plant parts (Kim & Hwang 2020).

Although the Tartary buckwheat leaves have a high content of rutin, their consumption should be aware due to the plentiful fagopyrins. On the contrary, the Tartary buckwheat grains are more recommended because of their high rutin and low fagopyrin content (Stojilkovski et al. 2013). Nevertheless, fagopyrin shows an anti-proliferative activity that might be beneficial for medical treatment in the future (Zhu 2016).

2.4.5. Bioactivities

Tartary buckwheat represents plenty of bioactivities, for example, antioxidant, anti-bacterial, anti-genotoxicity, anti-cancer, anti-hypertension, anti-inflammatory, digestive enzyme inhibition, improving blood glucose profile, and prevention of several chronic diseases such as obesity, cardiovascular diseases, gallstone formation, and hypertension (Zhu 2016; Cheng et al. 2022; Kreft et al. 2022a; Kreft et al. 2022c).

2.4.5.1. Antioxidative activity

Due to the high content of phenolic in all parts of Tartary buckwheat, the extraction from both this plant and its products shows antioxidant activity by various chemical assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric ion reducing antioxidant power (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Tsai et al. 2012; Ma et al. 2013; Wang et al. 2013). Tartary buckwheat displays 3 – 5 times more antioxidative activity than common buckwheat. The groat of Tartary buckwheat presents higher antioxidative activity than the hull of Tartary buckwheat. The antioxidant activity of groat Tartary buckwheat is between 78 – 147 mmol_{TE}/g, while hull Tartary buckwheat is around 30 – 80 mmol_{TE}/g depending on the analysis methods (Lee et al. 2016).

The antioxidant activity can be decreased by food thermal processing such as roasting, pressured-steam heating, and microwave heating. This processing affects the total phenolic content which is related to the antioxidant activity (Zhang et al. 2010). To maintain better nutrition, the processing method should be concerned before preparing Tartary buckwheat.

2.4.5.2. Antimicrobial activity

Tartary buckwheat shows antimicrobial activity due to the high content of phenolic. The barn extract can inhibit the growth of bacteria, including *Staphylococcus aureus*, *S. epidermis*, and *Propionibacterium acnes*, with MIC value 260 – 2050 µg/mL (Wang et al. 2013). The sprout extract shows both antimicrobial and antifungal activity. The sprout extract act against the growth of two gram-negative bacteria (*P. lachrymans* and *S. typhimurium*) and three gram-positive bacteria (*B. subtili*, *S. albus*, and *S. aureus*) with the MIC value 0.8 – 3.2 mg/mL but it shows low inhibition of fungi spore germination, lower than 20% of inhibition (Zhong et al. 2022). However, the antimicrobial efficiency depends on the extraction method and solution.

Quercetin is a compound that plays an essential role in antimicrobial activity. From the previous paragraph, the quercetin extracted from both barn and sprout is the main active compound against the microbes. The pure extraction of quercetin shows the highest inhibition in both bacteria and fungi. Quercetin represents an almost similar spore inhibition result to a commercial antibiotic (Wang et al. 2013; Zhong et al. 2022). Moreover, the other chemicals presented in Tartary buckwheat also have an alterability to quercetin, for example, the digestion of rutin by rutinosidase enzyme (Luthar et al. 2021b).

2.4.5.3. Digestive enzyme inhibition

Three major phenolics found in Tartary buckwheat are rutin, quercetin, and isoquercetin. These three compounds show inhibitory activities on the digestion of starch and lipid by inhibiting of enzymes and binding with the substrates to prevent the activity of enzymes, for example, the forming of hydrogen bonds between the glycoside in rutin and starch (Wang et al. 2022a). The inhibition of digestive enzymes by these compounds are found in three enzymes, including α-glucosidase, α-amylase, and pancreatic lipase (Zhu 2016).

Although these phenolics can inhibit three digestive enzymes, they use different mechanisms to inhibit the enzymes and efficiency. The inhibition of α-amylase is competitive inhibition by the binding of rutin, quercetin, and isoquercetin with the enzymes directly. The best inhibitor is isoquercetin, followed by quercetin and rutin, respectively (Li et al. 2009a). The inhibition of α-glucosidase is a mixed type of noncompetitive and anticompetitive whereas the best inhibitor is quercetin, sequently followed by isoquercetin and rutin (Li et al. 2009b). The inhibition of pancreatic lipase is noncompetitive as well as the best inhibitor is rutin, followed by isoquercetin and quercetin, respectively (Li et al. 2011). However, all of these inhibitory behaviours show a similar trend of a dose-dependent manner (Li et al. 2009a; Li et al. 2009b; Li et al. 2011).

2.5. Tartary buckwheat breeding and breeding objectives

The major purpose of the world breeding program is the improvement of seed yields in both quality and quantity. Obtaining a better yield is depended on various factors. Not only the increase in seed size, groat percentage, and shattering resistance, which is the direct effects on the yield, but also the resistance of the plant to the fluctuated environment, the earlier maturity, dehulling abilities, and seed coat colour, which are related to the plant adaptabilities, post-harvest processing, and nutrition of seed, should be considered (Campbell 1997).

Moreover, some negative traits are targeted. The bitter taste, sensitivity to photoperiod, strong seed dormancy, indeterminate flower, and allergenic protein should be removed. On the contrary, high nutritive compounds cultivars for instance high rutin and protein are desired (Woo et al. 2016; Luthar et al. 2021a).

Likewise, the Tartary buckwheat breeding programs emphasize the resistant characteristics and improving the quality of the grains, particularly the dehulling ability, increasing of nutritive compounds, and improvement of the taste (Wang & Campbell 2007; Suzuki et al. 2014; Kreft et al. 2020; Wang et al. 2022b).

2.5.1. Improvement of dehulling properties

Normally, the Tartary buckwheat hull is hard to remove because it is attached to the testa layer of the groat tightly. Fortunately, rice-Tartary buckwheat germplasms were found in China, Nepal, and Tibet. The rice-Tartary buckwheat is a specific type of buckwheat with smaller grain and lower yield but a thinner hull and easier dehulling, called ‘Mi-Qiao’ in Chinese. It distributes in the Himalayan Mountain areas (Wang & Campbell 2007).

From its easily dehulling property, rice-Tartary buckwheat was used as the parent for the breeding program with various methods such as hybridization and pedigree selection (Wang & Campbell 2007; Wang et al. 2022b). In 2022, the researchers successfully cross-bred normal Tartary buckwheat (‘Jing-Qiao-Mai2’) with rice-Tartary buckwheat as ‘Mi-Ku-Qiao18’ by a pedigree selection of crossbreeding. ‘Mi-Ku-Qiao18’ has a better level of flavonoids, taste quality, and thinner hull; however, it still shows a significantly lower yield and lower content of amino acids than usual (Wang et al. 2022b).

2.5.2. Improvement of taste and nutritive property

Tartary buckwheat is known as bitter buckwheat because of the presence of a high content of quercetin which is the bitter flavonoid in many fruits (Suzuki et al. 2021). However, the highest flavonoid in Tartary buckwheat is not quercetin but rutin. Tartary buckwheat also contains an enzyme named rutinosidase which can finally alter rutin to quercetin (Luthar et al. 2021b). Hence, Japanese plant breeders were trying to breed the non-bitter Tartary buckwheat by limiting the activity and amount of rutinosidase. Finally, the Tartary buckwheat cultivar ‘Manten-Kirari’ was obtained in 2012 and officially registered as a new variety in 2014 by an artificial cross of a trace-rutinosidase variety in Nepal and a normal-rutinosidase variety ‘Hokkai T8’ (Suzuki et al. 2014).

The ‘Manten-Kirari’ cultivar successfully shows no bitter taste, higher yields, and rutin content compared to the parental line. The rutinosidase isozyme is not detectable in this cultivar; hence, this cultivar represents higher rutin and lower quercetin content after cooking (Suzuki et al. 2014). However, the improvement of tasting causes less nutritive properties due to decreased quercetin. Quercetin is the main inhibitory flavonoid to α-glucosidase which is beneficial for diabetes patients (Kreft et al. 2020) and reduces the forming of quercetin-polyphenol complexation with the starch which is beneficial for weight loss (Luthar et al. 2021b).

2.6. Genetic resources of Tartary buckwheat

Due to the substitute of better new varieties on the old landraces, the genetic diversity of Tartary buckwheat is disturbed and becomes endangered. In 1945, the Food and Agriculture Organization (FAO) was the first who picks up the topic of the danger of genetic erosion caused by the development of agriculture. Three years after, the

International Board for Plant Genetic Resources (IBPGR) was established with the purpose of conservation and sustainable use of genetic resources (Singh et al. 2020).

2.6.1. World genetic resources

Unfortunately, the Tartary buckwheat samples are collected under the name of buckwheat (Suvorova & Zhou 2018). Hence, it is not possible to estimate the world genetic resources of Tartary buckwheat separately. In terms of buckwheat, both common buckwheat and Tartary buckwheat, the Crop Genetic Resources Institute of Chinese Academy of Agricultural Science (CAAS) has the highest record of the germplasm numbers with 2,804 collections, followed by N.I. Vavilov Research Institute of Plant Industry (VIR) in Russia with 2,230 collections (Singh et al. 2020).

Because of the enlargement of buckwheat market and the emerge of new varieties in Europe, five countries had an agreement to preserve the existing population of buckwheat in their research organizations, including Station d'Amélioration des Plantes, Institut National de la Recherche Agronomique (INRA) in France, Plant Breeding and Acclimatization Institute in Poland, Crop Research Institute, Department of Gene Bank in the Czech Republic, Biotechnical Faculty, University of Ljubljana in Slovenia, Institute of Crop Science, Federal Research Centre for Agriculture and Institute for Plant Genetics and Crop Plant Research Genebank in Germany. The exact number of Tartary buckwheat collections in 2018 is presented (Table 1) (Luthar 2018).

In terms of on-farm conservation, over 300 varieties are cultivated in their local origin, for example, the mountain region in the south of the Yantze River in China. (Suvorova & Zhou 2018). Thirteen landraces were found in the Yi people's community. Due to the culture of this community which is closely connected to Tartary buckwheat, this community continues doing the on-farm conservation (Song et al. 2019).

Nepal also has an excellent genetic diversity of Tartary buckwheat varieties. The Nepalese Tartary buckwheat represents unique characteristics such as rice-Tartary buckwheat which is used as the parent in the improvement of dehulling breeding. In addition, some varieties are cultivated only in a specific region, for instance, 'Kalo Kishe' and 'Seto Kishe' in Dolpa, Nepal (Suvorova & Zhou 2018). However, on-farm conservation is precarious because of the changing of lifestyle and allocation of local people.

Table 1 The genetic resources of Tartary buckwheat in Europe in 2018

Country	Organization	Number of samples in collection
France	Station d'Amélioration des Plantes, Institut National de la Recherche Agronomique (INRA)	80
Poland	Plant Breeding and Acclimatization Institute	-
Czech Republic	Crop Research Institute, Department of Gene Bank	25
Slovenia	Biotechnical Faculty, University of Ljubljana	26
Germany	Institute of Crop Science, Federal Research Centre for Agriculture	8
Germany	Institute for Plant Genetics and Crop Plant Research Genebank	84

(Sources: Hlásná Čepková et al. 2009; Luthar 2018)

2.6.2. The Czech Republic genetic resources

The conservation of genetic resources of Tartary buckwheat in the Czech Republic was started in 1993. The samples are collected in the Gene Bank of the Crop Research Institute (CRI) in Prague Ruzyně (Hlásná Čepková et al. 2009). Presently, there are 25 Tartary buckwheat accessions in the working collection. The collections are mainly supported by foreign gene banks. Eleven samples are originating in Bhutan, four samples are originating from China, and two samples are originating in the USA. One sample is provided by Germany, Pakistan, Mexico, and Nepal. There is only one sample originating in the Czech Republic (VURV 2022). Other details are presented in Table 2.

Table 2 The genetic resources of Tartary buckwheat in the Gene Bank of the Czech Republic

Accession	Name	Origin
01Z5100001	PI 481644	Bhutan
01Z5100002	PI 481645	Bhutan
01Z5100003	PI 481646	Bhutan
01Z5100004	PI 481652	Bhutan
01Z5100005	PI 481656	Bhutan
01Z5100006	PI 481658	Bhutan
01Z5100007	PI 481659	Bhutan
01Z5100008	PI 481670	Bhutan
01Z5100009	PI 481671	Bhutan
01Z5100010	Lifago	Germany
01Z5100011	903016	Pakistan
01Z5100012	-	Czech Republic
01Z5100013	PI 451723	Mexico
01Z5100014	PI 476852	United States

Table 2 The genetic resources of Tartary buckwheat in the Gene Bank of the Czech Republic (continue)

Accession	Name	Origin
01Z5100017	Weswod Ican	unknown
01Z5100019	RCAT 068749	unknown
01Z5100025	290	Bhutan
01Z5100028	PA 160	unknown
01Z5100030	PI 427239	Nepal
01Z5100037	PI 481661	Bhutan
01Z5100041	Jianzui	China
01Z5100042	Liuqiao-3	China
01Z5100044	Zhaoqiao-1	China
01Z5100046	Jinqiao-2	China
01Z5100050	Sarasin a Ployes	United States

3. Aims of the Thesis

This diploma thesis's objective was to evaluate the selected genotypes of Tartary buckwheat (*Fagopyrum tataricum*) for a further breeding program.

The specific aims were:

1. To evaluate the phenotypic traits in selected genotypes
2. To evaluate the protein contents in selected genotypes
3. To evaluate the phenolic compound in selected genotypes
4. To evaluate the antioxidant activity in selected genotypes
5. To evaluate the effect of environmental conditions on the traits by comparing four years (2019 – 2022) of the result

The thesis aims based on the hypotheses that

1. The phenotypic traits and nutritive compounds are related to the place of origin.
2. The phenotypic traits and nutritive compounds are related to the year of production.
3. The sample originating in Central Europe, like the Czech Republic, will have better quantity production than the others.

4. Methods

4.1. Weather conditions

The weather condition was recorded by the weather station in the Crop Research Institute (CRI), Prague Ruzyně, during the vegetation period of Tartary buckwheat in 2022.

4.2. Plant materials

One hundred and ten Tartary buckwheat genotypes (Table 3) were cultivated under field conditions in 2022 in CRI, Prague Ruzyně. The seeds were provided by the Gene Bank of Crop Research Institute of the Czech Republic, the Ustymivka Experimental Station of Plant Production of Ukraine, the Gene Bank of Leibniz Institute of Plant Genetics and Crop Plant Research of Germany, and the Gene Bank of the U.S. Department of Agriculture. All samples were sown in two rows with 1 m in length, 25 cm apart, and 50 seeds per row. The protection of cross-pollination is not needed due to its cleistogamous or close flowers, which are automatically self-pollinated.

The leaf samples were taken during the flowering stage (27 July 2022) from 36 different genotypes. Because most of the genotype's seeds were first time received by other gene banks, the list of genotypes in 2022 was different from the list in 2019 – 2021, which was provided by CRI for nutrition analyses. The list of genotypes is in Table 3.

Table 3 The plant material details

No.	Name	Origin	2019	2020	2021	2022
1	01Z5100014	USA	X	X	X	X
2	01Z5100019	-	X	X	X	X
3	Kails Farm Germany	DEU	X	X	X	X
4	UC0100180	CHN	X	X	X	X
5	UC0100291	IND		X	X	X
6	UC0101037	HUN	X	X	X	X
7	UC0101690 (Lira)	UKR		X	X	X
8	UC0101691 (Peremoga)	UKR	X	X	X	X
9	SRGB02223 (Sloveni Gradec)	SVN	X	X	X	X
10	SRGB02224 (Osrednje Goričko)	SVN	X	X	X	X
11	SRGB02257 (Radohova vas)	SVN	X	X	X	X
12	SRGB02258 (Dolina Krme na Gorenjskem)	SVN	X	X	X	X
13	SRGB02304 (Rut nad Tolminom)	SVN		X	X	X
14	SRGB02316 (Sveti Miklavž nad Litijo)	SVN	X	X	X	X
15	SRGB02321 (Novo Mesto)	SVN	X	X	X	X
16	SRGB02337 (Sevnica)	SVN	X	X	X	X
17	SRGB02356 (Koroška)	SVN		X	X	X
18	SRGB02409 (Straža)	SVN	X	X	X	X
19	Reitz Farm	DEU		X	X	X
20	FAG 21	-				X
21	FAG 26	-				X
22	FAG 27	-				X
23	FAG 34	-				X
24	FAG 40	-				X
25	FAG 48	BLR				X

Table 3 The plant material details

No.	Name	Origin	2019	2020	2021	2022
26	FAG 49	-				X
27	FAG 50	CHN				X
28	FAG 98	SVK				X
29	FAG 99	CHN				X
30	FAG 100	BLR				X
31	FAG 111	SVK				X
32	FAG 112	SVK				X
33	FAG 113	SVK				X
34	FAG 149	-				X
35	PI 199769 (PA 160)	USA				X
36	PI 427237	NPL				X
37	PI 427238	NPL				X
38	PI 427239	NPL				X
39	PI 427240	NPL				X
40	PI 476852 (Madawaska)	USA				X
41	PI 481644	BTN				X
42	PI 481645	BTN				X
43	PI 481646	BTN				X
44	PI 481647	BTN				X
45	PI 481648	BTN				X
46	PI 481649	BTN				X
47	PI 481650	BTN				X
48	PI 481651	BTN				X
49	PI 481652	BTN				X
50	PI 481653	BTN				X

Table 3 The plant material details

No.	Name	Origin	2019	2020	2021	2022
51	PI 481654	BTN				X
52	PI 481655	BTN				X
53	PI 481656	BTN				X
54	PI 481658	BTN				X
55	PI 481659	BTN				X
56	PI 481660	BTN				X
57	PI 481661	BTN				X
58	PI 481662	BTN				X
59	PI 481663	BTN				X
60	PI 481664	BTN				X
61	PI 481665	BTN				X
62	PI 481666	BTN				X
63	PI 481667	BTN				X
64	PI 481668	BTN				X
65	PI 481669	BTN				X
66	PI 481671	BTN				X
67	PI 481672	BTN				X
68	PI 481673	BTN				X
69	PI 481674	BTN				X
70	PI 503879 (Sarasin a Ployes)	USA				X
71	PI 647612 (Clfa 38)	USA				X
72	PI 647613 (Clfa 39)	USA				X
73	PI 658425 (2049)	NPL				X
74	PI 658429 (G 32048)	USA				X
75	PI 658430 (G 32049)	USA				X

Table 3 The plant material details

No.	Name	Origin	2019	2020	2021	2022
76	PI 658431 (G 32050)	USA				X
77	PI 658438 (Martin’s Tarbary)	USA				X
78	PI 658439 (Madawaska Native)	USA				X
79	PI 673845 (Zhong Dian Ku Qiao)	CHN				X
80	PI 673846 (Yuan Ke Qiao)	CHN				X
81	PI 673847 (Ying Pan Ku Qiao)	CHN				X
82	PI 673848 (Xing Hou Dou Ma Ku Qiao)	CHN				X
83	PI 673849 (Xing Qiao, Ku Qiao)	CHN				X
84	PI 673850 (Xiao Jin Qiao)	CHN				X
85	PI 673851 (Xiao Hei Yuan Zi Ku Qiao)	CHN				X
86	PI 673852 (Xiao Cun Ku Qiao)	CHN				X
87	PI 673853 (Qiao)	CHN				X
88	PI 673854 (Pu Xian Ku Qiao)	CHN				X
89	PI 673855 (Ni Xi Ku Qiao)	CHN				X
90	PI 673856 (Ma Jie Ku Qiao)	CHN				X
91	PI 673857 (Ma Ji Ku Qiao)	CHN				X
92	PI 673858 (Ma Hei Qiao)	CHN				X
93	PI 673859 (Luo He Ku Qiao)	CHN				X
94	PI 673860 (Lu Qiao)	CHN				X
95	PI 673861 (Long Tan Ku Qiao)	CHN				X
96	PI 673862 (Lin Xian Qiao Mai)	CHN				X
97	PI 673863 (Jin Qiao Mai)	CHN				X
98	PI 673864 (Hui Cha Ku Qiao)	CHN				X
99	PI 673865 (Huang Hua Ku Qiao)	CHN				X
100	PI 673866 (Hua Ning Ku Qiao)	CHN				X

Table 3 The plant material details

No.	Name	Origin	2019	2020	2021	2022
101	PI 673867 (Hei Xiao Qiao)	CHN				X
102	PI 673868 (He Ka Ku Qiao)	CHN				X
103	PI 673869 (Ha Ba Ku Qiao)	CHN				X
104	PI 673870 (Fu Gong Ku Qiao)	CHN				X
105	PI 673871 (Da Xing Ku Qiao)	CHN				X
106	PI 673872 (Da Bian Zi Ku Qiao)	CHN				X
107	PI 673873 (Da Bai Yuan Ku Qiao)	CHN				X
108	PI 673874 (Ci Qiao)	CHN				X
109	PI 673875 (Bai Lu Qiao)	CHN				X
110	PI 673876 (Bai Gan Qiao)	CHN				X
111	Madawaska Canada	-	X			
112	Madawaska USA	-	X			
113	UC0101692 (Kalyna)	UKR		X	X	

Belarus (BLR), Bhutan (BTN), China (CHN), Germany (DUE), Hungary (HUN), India (IND), Nepal (NPL), Slovakia (SVK), Slovenia (SVN), Ukraine (UKR), and United States of America (USA)

4.3. Phenotypic evaluation

The morphological and phenotypical traits of the genotypes in 2022 were evaluated during the growing season, whereas the traits of 19 genotypes in 2019 – 2021 were provided by CRI. The selected traits were evaluated according to the descriptor for buckwheat IPGRI (1994). The number of days to emerge, flowering, and physiological maturity were recorded. In the flowering stage, the growth and branch shoot habit, plant length, number of branching of the primary stem, leaf blade size, and compactness of the inflorescence were recorded. After fruit set and seed maturity, the yield, and weight of thousand seeds (WTS) were recorded. Then, the representative sample was collected and stored in the dark and cool for further evaluation.

4.4. Chemical analyses

4.4.1. Sample preparing

Approximately 10 g of the dry seed and leaf sample were grounded in a grinding mill (IKA A11 basic, IKA® Werke, Staufen, Germany). Five grams of the ground sample was dried in an electric hot-air drier at 105 °C, for 4 hours to obtain the dry matter content according to the standard method CSN EN ISO 662 (CSN 2001). The remaining sample was placed in a plastic bag and stored in the dark and cool.

4.4.2. Protein contents

The protein contents were determined by the Kjeldahl mineralization method and calculated with a conversion factor of 6.25 (CSN 2012). 0.5 g of samples were used in the measuring and glycine was used as the control. The analyses were done twice for the repetitions of each sample.

4.4.3. Total phenolic contents

The total phenolic content (TPC) was determined by the Folin assay modified according to previous literature (Holasova et al. 2002). Two grams of sample were extracted in 20 mL of 80% methanol for 60 min in dark. 0.5 mL of the extract was diluted with distilled water to 50 mL. Then, 2.5 mL of Folin-Ciocalteau reagent (PENTA, Prague, the Czech Republic) and 7.5 mL of 20% sodium carbonate were added and incubated for 2 hours at dark and room temperature. The absorbance at wavelength $\lambda = 765$ nm was measured by the spectrophotometer Thermo GENESYS™ 10UV UV-Vis (Thermo Scientific, Waltham, MA, USA). The standard curve was quantified by the gallic acid standard (Merck, Germany). The results were presented in the unit of gallic acid equivalents (GAE) and repeated twice for the repetitions of each sample.

4.4.4. Antioxidant activity

An antioxidant activity was evaluated by DPPH assay (Şensoy et al. 2006). One gram of the sample was extracted with 20 mL of 80% methanol in the dark and shaken for 90 min. Twenty µL of the extract were mixed with 150 µL of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution with the initial absorbance at 550 nm (A = 0.6) and incubated in the dark for 10 min. Then, the absorbance at wavelength λ = 550 nm was measured by the spectrophotometer (Sunrise absorbance reader, Tecan, Switzerland). Trolox (Sigma-Aldrich, Germany) was used as the standard with a concentration between 0.0 – 0.2 µmol/L; the results were expressed in the term of Trolox equivalent (TE). The analyses were done twice for the repetitions of each sample.

4.4.5. UHPLC-ESI-MS/MS Analysis

The extraction procedure was followed from the previous literature (Janovska et al. 2021). Grounded samples (0.1 g) were extracted twice with 1 mL of extraction solvent (0.1 µg/mL of probenecid in 80% methanol) for 60 min at 45 °C in an ultrasonic bath. The samples were centrifuged at 25 °C and 13,500 rpm for 15 min, and then the supernatants were filtrated through 0.2 µm nylon syringe filters (Thermo Scientific, Rockwood, TN, USA). The extracted samples were sent to the Quality and Plant Products, Crop Research Institute, for further analysis.

4.5. Statistical analyses

The data analyses were performed by the R program and Microsoft Office Excel. The normality distribution and the equality of variance were tested by the “rstatix” package. The analysis of variance (ANOVA) and Kruskal Wallis H test were suitability applied to the traits, followed by Tukey’s, and Dunn’s tests (if significant). Heatmaps were provided by the “heatmaply” package.

5. Results

5.1. Weather conditions analysis

In Prague Ruzyně, 2022, warmer and highly variable rainfall compared to the average from 1953 to 2020 was recorded. The temperature generally tended to rise to approximately 1 – 3 ºC in each month (except for September). The average precipitation from late spring to the summer (May to August) was constantly distributed from 61 – 72 mm per month. Then, the rainfall was half drop to around 30 mm per month in autumn. While in 2022, the precipitation indeed fluctuated in each month. The rainfall was 15.7 mm in May. After that it was ten-time increased to 146.9 mm in June, then fell to 68.6 mm in July. Afterward, the precipitation raised up to 85.1 mm and reduced by half in each month to 15.4 mm in October (Figure 5).

During 2019 – 2021, the precipitation in the years 2019 and 2020 was mostly similar except in October when the year 2020 showed double higher precipitation. Compared to the average from 1953 – 2020, the precipitation of both years was analogous content with the average, excluding the precipitation in July that was lower than the average. The precipitation in 2021 fluctuated. It, 104 mm, was twice higher than 2019, 2020, and the average in May. Then, it dropped slightly to 39.7 mm in August which was lower than the others. Afterward, it peaked at 92.1 mm in September and fell to 12.9 mm in October. The monthly average temperature in 2019 – 2021 was generally higher than the average (1953 – 2020), except in May and August (Figure 5).

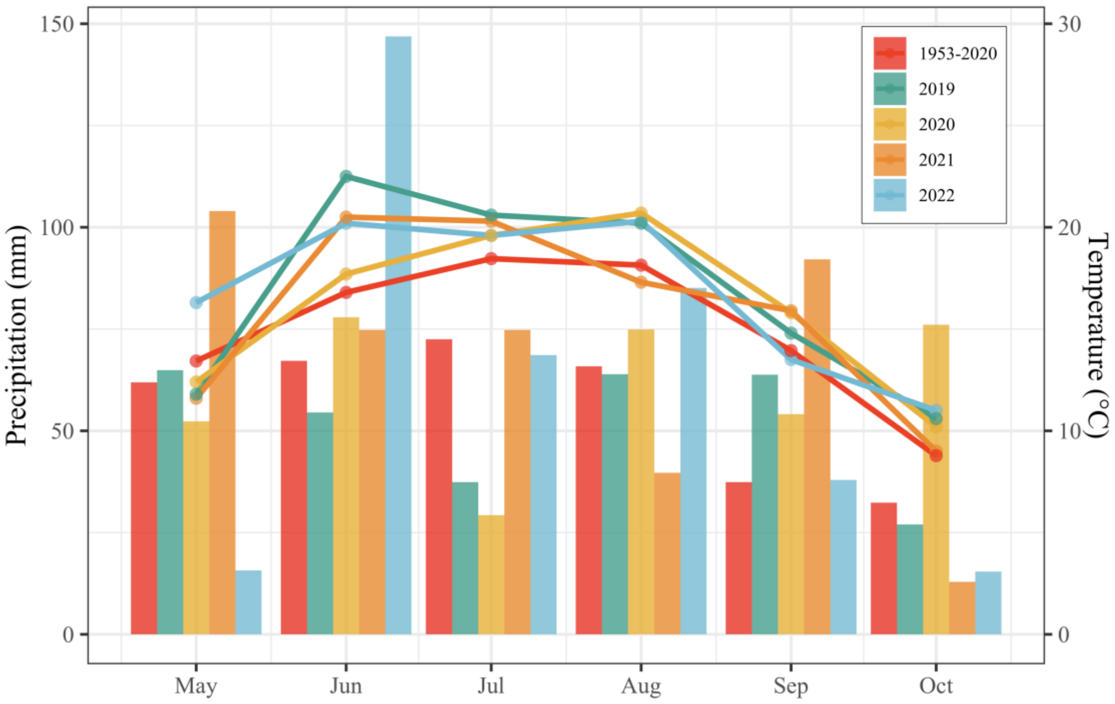

Figure 5 **The precipitations (bar) and temperature (line) in 2019 – 2022 and the average (1953 - 2022) during the vegetation period of Tartary buckwheat from May to October in the Crop Research Institute, Prague Ruzyně**

5.2. Morphological evaluation

One hundred and ten genotypes of Tartary buckwheat were sown on 16 May 2022. Seeds germinated at varying times from 14 – 23 days after sowing, then started flowering from 52 – 91 days after sowing and were ready for harvesting after 119 – 149 days. Although all genotypes were germinating, only 70 genotypes were flowering, and just 30 genotypes could complete the growth cycle with the seed production.

The genotype status was considered by the flowering and harvesting ability. Non-flowering represented the genotypes which unable to flower or die after germination. Flowering without yield showed the plants which were able to be flowering but unable to produce the grains. Flowering with yield indicated the group that can finish the life cycle with the production. The ratio of genotype status in each country is shown in Figure 6. The number of genotypes in each country fluctuated and was represented after the country name.

The highest ratio of flowering with yield genotypes was found in the genotypes from Hungary, India, and Ukraine, with 100%. Then, half of the genotypes from Germany and unknown origin were able to be flowering and harvested, followed by the USA, China, Slovakia, and Bhutan, respectively. The genotypes originating in Nepal could not flower and survive, while the samples from Belarus could flower but could not be harvested. However, the numbers of genotypes in each country were too low to be used as the representative of the country (Figure 6).

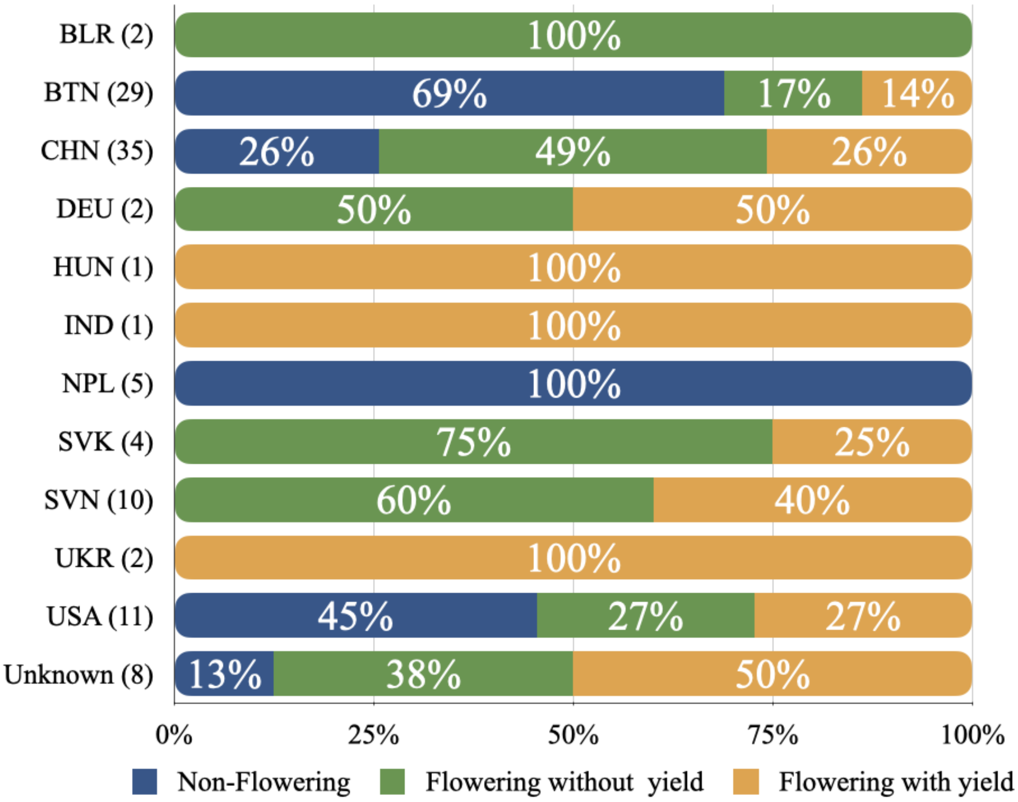

Figure 6 The percentage of non-flowering, flowering without yield, and with yield of Tartary buckwheat genotypes classified by the origin

Belarus (BLR), Bhutan (BTN), China (CHN), Germany (DEU), Hungary (HUN), India (IND), Nepal (NPL), Slovakia (SVK), Ukraine (UKR), and the United States of America (USA). The number after the origin represents the number of genotypes in each origin.

The highest mean yield was recorded in the varieties originating in Ukraine with 11.42 ± 3.07 g/m^2, followed by Slovenia and China with 11.07 ± 9.83 g/m^2 and 10.40 ± 3.15 g/m^2, respectively. On the other hand, the genotypes originating in Belarus and Nepal displayed no yield in 2022 (Table 4). However, the yields, WTS, and seed number in each origin (BTN, CHN, SVN, USA, and Unknown) indicate no significant difference in the Kruskal-Wallis H test ($p \geq 0.5$).

Table 4 The mean yield, WTS, and seed number observed from the different originating genotypes in 2022

Origin	**Yield (g/m^2)**	**WTS (g)**	**Seed number (seeds)**
Belarus	-	-	-
Bhutan	2.42 ± 1.97	8.64 ± 1.32	118.50 ± 85.65
China	10.40 ± 3.15	10.23 ± 0.85	480.22 ± 145.82
Germany	0.47 ± NA	12.32 ± NA	19.00 ± NA
Hungary	7.66 ± NA	10.33 ± NA	371.00 ± NA
India	0.92 ± NA	18.36 ± NA	25.00 ± NA
Nepal	-	-	-
Slovakia	0.97 ± NA	10.12 ± NA	48.00 ± NA
Slovenia	11.07 ± 9.83	9.73 ± 0.82	617.50 ± 545.95
Ukraine	11.42 ± 3.07	8.96 ± 0.80	631.50 ± 110.50
United States of America	2.29 ± 0.56	11.08 ± 1.65	103.00 ± 22.65
Unknown	8.88 ± 3.78	11.29 ± 0.62	412.00 ± 175.63
Overall	**7.43 ± 1.73**	**10.44 ± 0.47**	**364.97 ± 89.08**

The table represents mean ± standard error, and NA shows a non-replication sample.

Individually, the highest yield was found in the variety named SRGB02258 (Dolina Krme na Gorenjskem) from Slovenia with 40.50 g/m^2. This variety was provided by the Gene Bank of Crop Research Institute of the Czech Republic. The second and third originated in China, named PI673861 (Long Tan Ku Qiao) and PI673871 (Da Xing Ku Qiao), with 26.78 and 20.74 g/m^2, respectively (Figure 7).

The mean value of the WTS was 10.44 ± 0.47 g. The highest WTS was recorded in UC0100291, with 18.36 g from India, followed by PI673871 (Da Xing Ku Qiao) and PI647612 (Clfa_38) from China and USA, with 16.21 g and 14.06 g, respectively. Regarding the origins and excluding the non-replication sample, the unknown region showed the highest mean WTS with 11.29 ± 0.62 g, followed by the USA and China-originated genotypes with 11.08 ± 1.65 g and 10.23 ± 0.85 g, respectively.

The genotypes from Ukraine showed the highest mean seed number per cyme with 631.50 ± 110.50 seeds, followed by Slovenia and China (Table 4). The greatest seed number was found in the SRGB02258 genotype, with 2,251 seeds from Slovenia, followed by PI673861 (Long Tan Ku Qiao) and 01Z5100019 (Control) with 1,346 and 845 seeds from China and unknown origin. While the three lowest productions were only 6, 7, and 8 seeds from unknown origin (FAG34), Slovenia (SRGB02409), and Bhutan (PI481647), respectively.

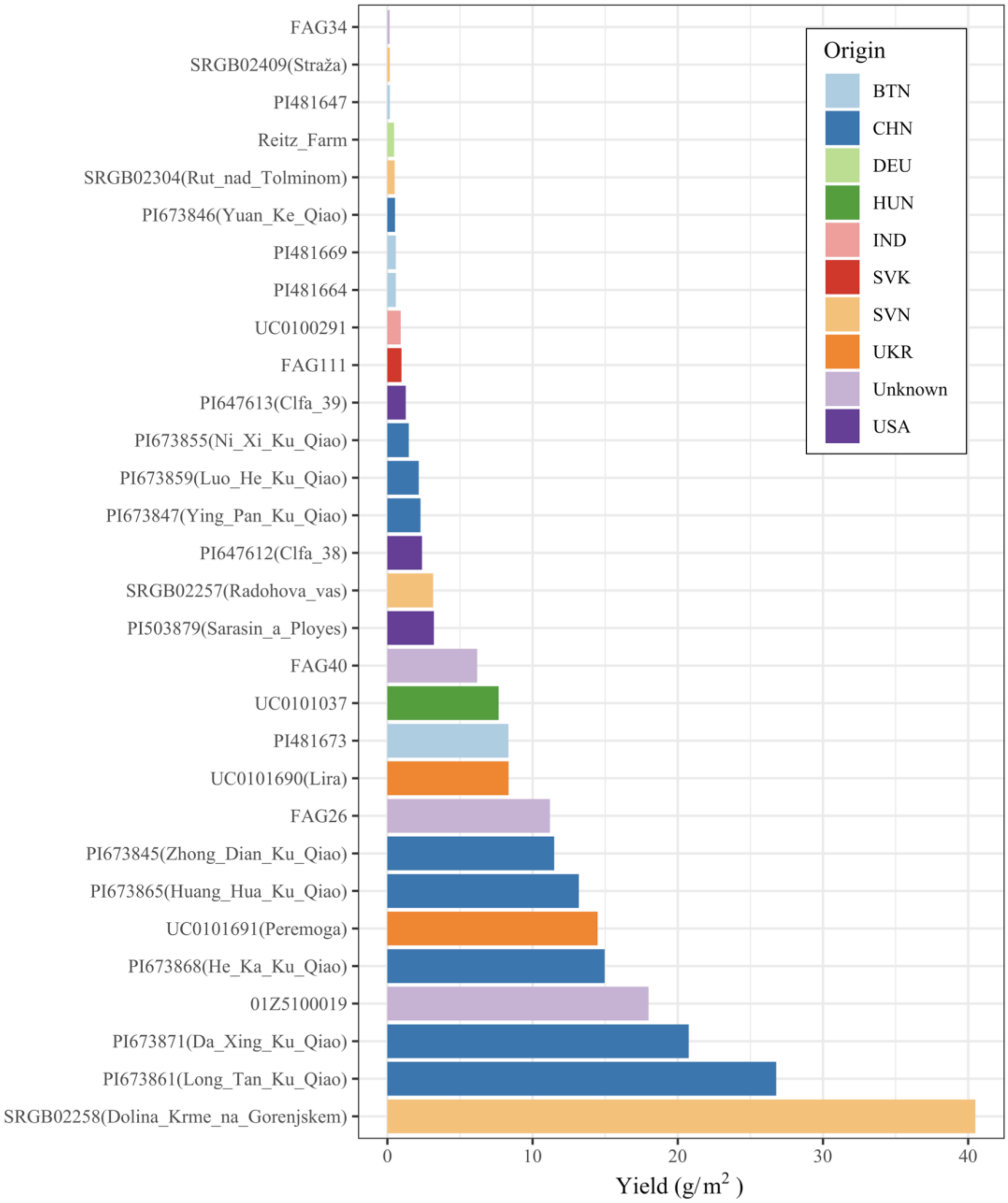

Figure 7 Yield (g/m^2) from 30 genotypes of Tartary buckwheat cultivated in 2022

Belarus (BLR), Bhutan (BTN), China (CHN), Germany (DEU), Hungary (HUN), India (IND), Nepal (NPL), Slovakia (SVK), Ukraine (UKR), and the United States of America (USA). The number of the origin represents the number of genotypes in each origin.

The correlation between days to emerge, days to flowering, days to maturity, seed developing days (from flowering to the mature stage), seed number, WTS, and yield were calculated using Spearman's correlation coefficient. The yield proved to be significantly related to the number of seeds (coefficient value = 0.98, $p < 0.001$), while it did not have any relation with the grain weight, represented by the WTS (Figure 8). The greatest yield genotype, SRGB02258, showed the greatest seed numbers, whereas the WTS was lower than the average, with only 8.99 g. The highest WTS was found in UC0100291; however, the yield of this genotype was only 0.92 g/m^2 due to the low seed number.

In addition, the number of days to flowering and days to maturity (coefficient value = 0.51, $p < 0.05$) as well as the number of days to maturity and the seed developing days had a significant relation (coefficient value = 0.80, $p < 0.001$). Although the other parameters showed differences in correlation coefficient values, they had no significant relations due to the low *p-value* (Figure 8).

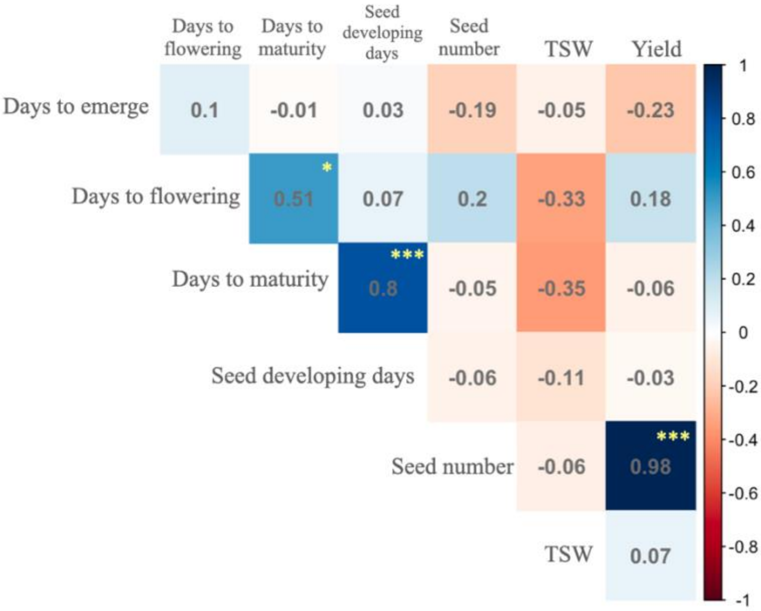

Figure 8 Spearman's correlation between selected evaluated descriptors of the Tartary buckwheat genotypes

The colour and number indicate the correlation between each pair of descriptors. Red represents negative values, whereas blue shows positive values. Significant correlations are marked by *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$).

The plant height of Tartary buckwheat was 76.12 ± 2.51 cm. The highest plant height was found in the PI673865 (Huang Hua Ku Qiao) genotype from China, 109 cm tall, whereas the shortest was PI647612 (Clfa 38) genotype from the USA, with only 45 cm tall. Considering the origins, the Chinese genotypes, with 88.45 ± 3.91 cm height on average, were the highest, whereas the USA was the shortest with 54.33 ± 13.74 cm height (Figure 9).

Tartary buckwheat leaf size was 4.35 ± 0.12 cm in length and 5.88 ± 0.18 cm in width in general. The Slovakian varieties tended to have the smallest leaves with 3.10 ± 0.64 and 3.90 ± 0.68 cm in length and width, while the largest leaves were found in the German genotype with 5.10 ± 0.51 cm in length and 7.30 ± 0.60 cm width as the average.

The number of flower clusters per cyme was distributed between 2 to 5 clusters per cyme. The number of flower clusters was approximately 3 clusters on average, except for the USA genotypes, which have only 2 clusters per inflorescence. However, the seeds per cyme in the USA varieties showed the highest value with 41 ± 10 seeds per cyme, followed by the Hungarian and Ukrainian genotypes with mean of 29.33 ± 6.65 and 26.08 ± 4.32 seeds per cyme, respectively, while the average of seed per cyme was 21.25 ± 1.26 seeds (Figure 9).

According to the descriptor for buckwheat according to IPGRI (1994), some traits, consisting of growth and branch shoot habit, lodging, branching level, and compactness of inflorescence, were evaluated as the levels. Two levels of plant growth and branch shoot habit were found equally, including semi-erect shorter (3) and semi-erect longer (5). The same origins seem to have similar growth and branch shoot habit except for Slovenian and Ukrainian genotypes (Figure 10).

Four levels of branching were observed, including weak (level 3; 2 – 3 branches), intermediate (level 5; 4 – 5 branches), strong (level 7; 6 – 7 branches), and very strong (level 9; over 8 branches). The majority of genotypes showed a strong branching level, with 61.76%, followed by the intermediate (20.59%), very strong (11.76%), and weak (5.88%), respectively. The very strong branching was found in four genotypes from Bhutan, China, Slovakia, and Ukraine, whereas two weak genotypes were from China and the USA.

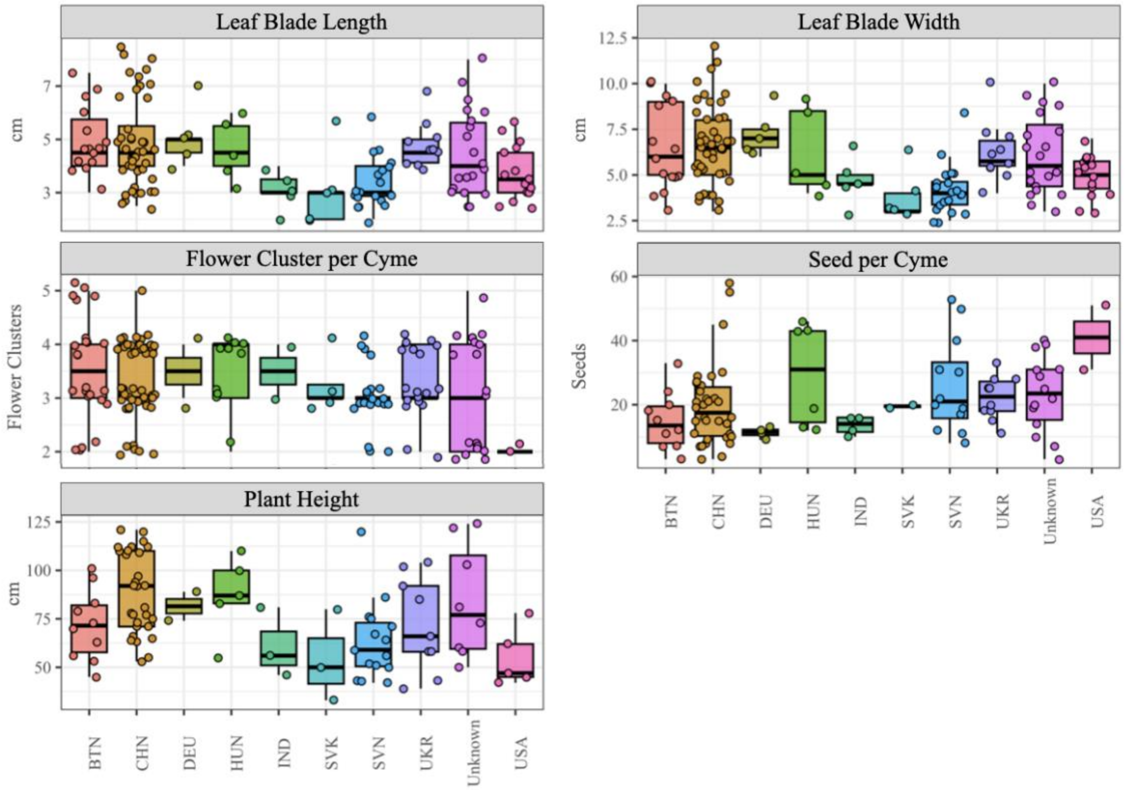

Figure 9 The box plot illustrates the distribution of the morphological traits, including plant height, leaf blade length, leaf blade width, number of flower clusters per cyme, and number of seeds per cyme, of the Tartary buckwheat genotypes classified by the origin

Bhutan (BTN), China (CHN), German (DEU), Hungary (HUN), India (IND), Slovakia (SVK), Slovenia (SVN), Ukraine (UKR), and the United States of America (USA).

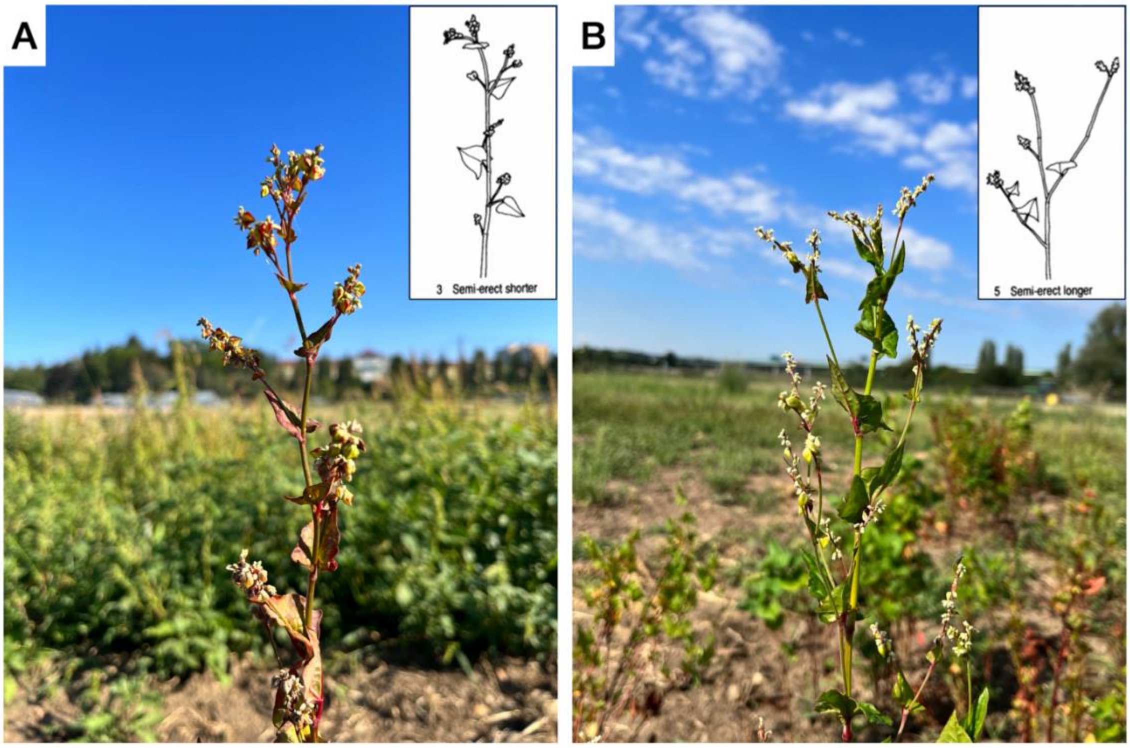

Figure 10 The growth and branch shoot habit of Tartary buckwheat: semi-erect shorter (A) and semi-erect longer (B)

In each place of origin, they contained both genotypes with loose cyme (3) and semi-compact cyme (5) character in compactness of inflorescence except for the origin with only one sample, including German, Hungarian, Indian, and Slovakia varieties. The genotypes were generally the semi-compact cyme around 70% of the genotypes (infructescence in Figure 11). The lodging levels of Tartary buckwheat were mainly very low (1), 81.82% of samples, followed by low and intermediate lodging, 13.64% and 4.55%, respectively.

The seed characteristics, colour and shape, were evaluated (Figure 12). Four seed colours were shown in this evaluation. However, seeds were generally brown, 60%, or brown-black, 33%, in colour. There were only two genotypes which were brown-grey and grey colour. The seeds were primarily oval, 83.33%, whereas the others were in a triangle shape, with 16.67%. The triangle shape was found in only five genotypes, one from Bhutan, two from China and another two from Slovenia and India.

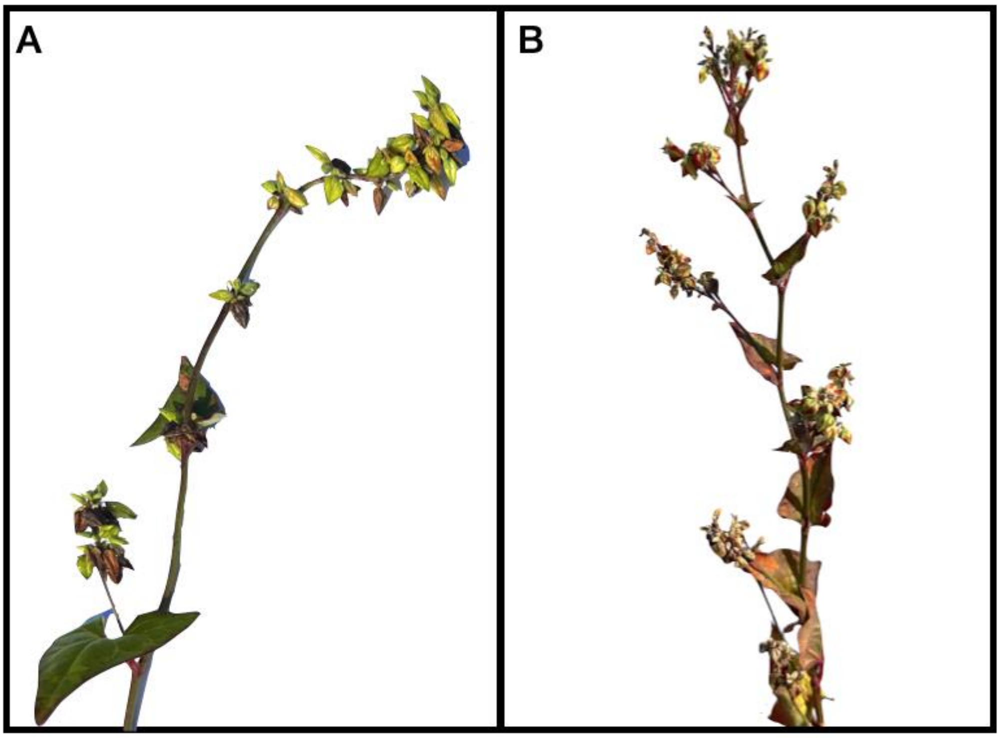

Figure 11 The compactness of infructescence of Tartary buckwheat: cyme loose (A) and cyme semi-compact (B)

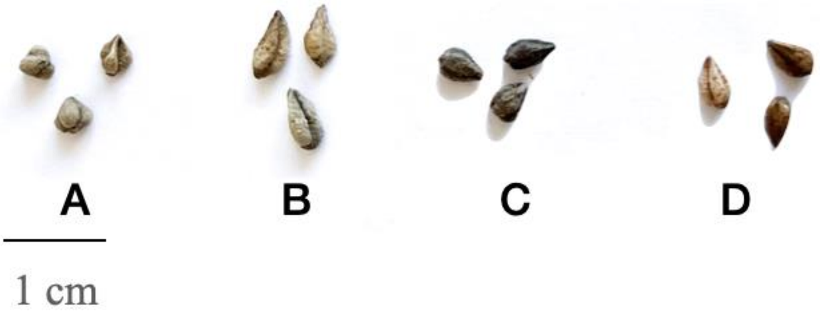

Figure 12 The seed characters of Tartary buckwheat: triangle gray seed (A), oval brown-gray seed (B), oval black-brown seed (C), and oval brown seed (D)

5.3. Nutrition components

Even though 30 genotypes gave production in 2022, only nine had enough grains for further nutrition analyses, while the leaf samples were taken from 36 different genotypes.

5.3.1. Leaf samples

Thirty-six leaf samples were taken from genotypes of different origins, consisting of Bhutan (2), China (20), Germany (1), Hungary (1), India (1), Slovenia (4), Ukraine (2), the USA (3), and unknown region (2). Tartary buckwheat leaf mostly had 12.05 ± 0.39% of dw of protein content, 598.01 ± 19.16 mg_{GAE}/g_{dw} of phenolic content, and 473.36 ± 16.06 $\mu mol_{TE}/g_{dw}$ of antioxidant activity (Table 5).

Considering the places of origin, the genotypes from unknown origin represented the highest mean protein content with 13.74 ± 3.13% of dw, followed by Bhutan (13.54 ± 0.61% of dw) and China (12.99 ± 0.48% of dw) while Hungarian genotype showed the least protein content with 6.87 ± 0.16%. However, the Chinese mean protein content was significantly different from the Hungarian, Ukrainian, and American genotypes, tested by Tukey’s test.

The highest mean phenolic content was found in the German variety with 730.51 ± 29.22 mg_{GAE}/g_{dw}, followed by Hungarian (701.53 ± 25.98 mg_{GAE}/g_{dw}) and the USA genotypes (681.91 ± 47.40 mg_{GAE}/g_{dw}), whereas the highest antioxidant activity was found in Chinese genotypes with 546.46 ± 17.88 $\mu mol_{TE}/g_{dw}$, followed by the USA (479.82 ± 37.17 $\mu mol_{TE}/g_{dw}$) and German variety (462.19 ± 14.66 $\mu mol_{TE}/g_{dw}$). In addition, the statistical analyses of phenolic content and antioxidant activity represented a similar trend. The total phenolic content of the sample from China and the USA was significantly different from the Indian genotype, while the antioxidant activity of Chinese genotypes was notably different from Hungarian, Indian, and Ukrainian genotypes.

Table 5 The mean content of protein content, phenolic content, and antioxidant activity in leaves measured from the samples of different countries (results from 2022)

Origin	**Protein content (% of dw)**	**Total phenolic content (mg_{GAE}/g_{dw})**	**Antioxidant activity (μmol_{TE}/g of dw)**
Belarus	-	-	-
Bhutan	13.54 ± 0.61^{abc}	565.14 ± 57.35^{ab}	419.04 ± 42.71^{ab}
China	12.99 ± 0.48^{ab}	617.28 ± 26.16^{a}	546.46 ± 17.88^{a}
Germany	10.99 ± 0.01^{bcde}	730.51 ± 29.22^{ab}	462.19 ± 14.66^{ab}
Hungary	6.87 ± 0.16^{cde}	701.53 ± 25.98^{ab}	277.26 ± 20.48^{b}
India	9.74 ± 0.02^{bcde}	259.02 ± 4.25^{b}	214.91 ± 4.58^{b}
Nepal	-	-	-
Slovakia	-	-	-
Slovenia	12.26 ± 1.51^{abcde}	533.44 ± 44.88^{ab}	423.89 ± 50.60^{ab}
Ukraine	8.37 ± 0.52^{cde}	504.37 ± 102.42^{ab}	306.48 ± 16.68^{b}
United States of America	9.64 ± 0.18^{e}	681.91 ± 47.40^{a}	479.82 ± 37.17^{ab}
Unknown	13.74 ± 3.13^{abcde}	586.55 ± 59.80^{ab}	363.60 ± 49.54^{ab}
Overall	**12.05 ± 0.39**	**598.01 ± 19.16**	**473.36 ± 16.02**

The table represents mean ± standard error. The different letters show the significant differences tested by Tukey's test.

5.3.2. Seed samples

Nine seed samples from 4 places of origin, including China, Slovenia, Ukraine, and unknown origin, were harvested and applied to nutrition analysis in 2022. The grains were mainly obtained from Chinese varieties with 5 varieties, followed by 2 samples from unknown origins and 2 samples from each Slovenia and Ukraine. Tartary buckwheat grain showed approximately 11.30 ± 0.20% of dw of protein content, 144.58 ± 1.44 mg_{GAE}/g_{dw} of phenolic content, and 39.76 ± 5.85 $\mu mol_{TE}/g_{dw}$ of antioxidant activity (Table 6).

Table 6 The mean value of protein content, phenolic content, and antioxidative activity in seeds measured from the different genotypes in 2022

	Origin	**Protein (%)**	**Phenolic (mg_{GAE}/g_{dw})**	**Antioxidant activity ($\mu mol_{TE}/g_{dw}$)**
01Z51000019	Unknown	13.03 ± 0.22^{abc}	139.26 ± 7.14	33.77 ± 0.18^{a}
FAG26	Unknown	10.54 ± 0.29^{defg}	134.37 ± 0.57	42.98 ± 1.40^{b}
PI673845	China	12.28 ± 0.05^{abcd}	137.56 ± 2.55	36.24 ± 0.20^{ab}
PI673861	China	11.27 ± 0.18^{bcdef}	152.36 ± 0.30	36.87 ± 1.23^{ab}
PI673865	China	9.98 ± 0.29^{efg}	138.96 ± 0.57	40.11 ± 1.96^{ab}
PI673868	China	10.17 ± 0.34^{defg}	152.96 ± 4.27	41.38 ± 0.29^{b}
PI673871	China	9.67 ± 0.01^{defg}	139.34 ± 3.19	42.42 ± 1.74^{b}
SRGB02258	Slovenia	12.45 ± 0.29^{abcd}	154.91 ± 2.32	10.10 ± 1.51^{ab}
UC0101691	Ukraine	12.30 ± 0.21^{abcd}	151.52 ± 5.70	43.66 ± 1.58^{b}
Overall	**-**	**11.30 ± 0.20**	**144.58 ± 1.44**	**39.76 ± 5.85**

The table represents mean ± standard error. The different letters show the significant differences tested by Tukey's test.

Considering the individual genotype, the highest protein content was observed from the unknown origin, 01Z5100019, with 13.03 ± 0.13%, while the greatest phenolic content was found in the Slovenia genotype, SRGB02258, with 154.91 ± 1.34 mg_{GAE}/g_{dw} and the Ukrainian variety, UC0101691, represented the highest antioxidant activity with 43.66 ± 0.91 $mmol_{TE}/g_{dw}$. On the other hand, the lowest protein content, phenolic content, and antioxidative activity were found in PI673871 (China), FAG26 (Unknown), and 01Z51000019 (Unknown), respectively (Table 6).

The Chinese genotypes showed a low protein content (10.67 ± 0.23%), phenolic content (144.23 ± 1.70 mg_{GAE}/g_{dw}), and antioxidant activity (39.40 ± 0.64 $\mu mol_{TE}/g_{dw}$) compared with the Slovenian and Ukrainian genotype. However, the comparison between the origin was not performed by reason of not enough and unequal sample size.

5.4. The comparison between each year of production

5.4.1. Morphological evaluation

Nineteen samples from the year 2019 to 2022 were evaluated for yield and morphology traits. The yield was strongly influenced by the planting year (Figure 13). The highest average yield was found in 2021, with 94.59 ± 12.11 g/m^2, followed by 2020 (22.41 ± 7.54 g/m^2) and 2019 (19.90 ± 6.31 g/m^2), while the lowest average yield was found in 2022, with 9.42 ± 3.98 g/m^2. After the Kruskal Wallis H test and Dunn test were done, the yield in 2021 was significantly higher than the others. The highest yield was SRGB02316 in 2021, with 223.35 g/m^2, while the lowest yield was SRGB02409 in 2022, with 0.17 g/m^2.

In contrast, WTS was influenced by both year of cultivation and genotype (Figure 13). Considering by the year, the lowest WTS was found in 2022, with 10.85 ± 0.94 g, followed by 2019 and 2020, with 11.24 ± 0.61 g and 12.32 ± 0.46 g. While the highest WTS was found in 2021 with 14.06 ± 0.45 g. WTS in 2022 was significantly higher than WTS in 2019 and 2022 but was not significantly different from the production in 2020. However, significant differences were not found in genotype and place of origin in both yield and WTS.

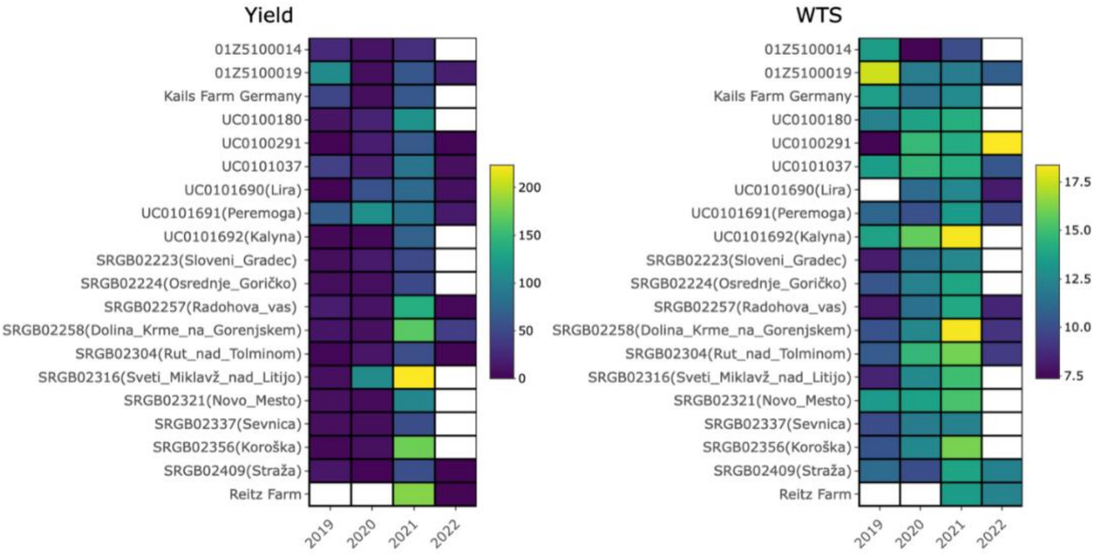

Figure 13 Diversity of 19 Tartary buckwheat genotypes in terms of yield (g/m^2, left) and weight of thousand seed (WTS, right) value, illustrated using a heatmap

White rectangles indicate the missing values for giving traits for a given genotype. Values for the respective traits are displayed on a scale from blue (min) to red (max), according to the colour key below each heatmap.

5.4.2. Nutrition component

Seeds of nineteen samples from the year 2019 to 2021 were evaluated for the stability of the nutrition, including protein content, total phenolic content, antioxidant activity, as well as the content of each phenolic compound by UHPLC-ESI-MS/MS analysis. The highest protein content was detected in 2019, with 13.37 ± 0.14%, followed by 2020 and 2021, with 13.24 ± 0.12% and 10.69 ± 0.11%, respectively (Table 7). Moreover, the protein content of the seeds in 2021 was significantly less than in 2019 and 2020 ($p < 0.001$).

On the other hand, the grain in 2021 represented significantly higher total phenolic content and antioxidant activity, with 158.80 ± 3.42 $\mathrm{mg_{GAE}/g_{dw}}$ and 25.32 ± 0.90 $\mathrm{mmol_{TE}/g_{dw}}$, than the grain from 2019 and 2020 ($p < 0.001$). The lowest phenolic content was found in the year 2020 with 128.04 ± 2.49 $\mathrm{mg_{GAE}/g_{dw}}$, while the grain in 2019 showed the lowest antioxidative activity with 18.58 ± 0.79 90 $\mu mol_{TE}/g_{dw}$. However, statistical significance was not found in 2019 and 2020 (Table 7).

Considering the place of origin, Tartary buckwheat grains did not show a significant difference in the content of protein, total phenolic, and antioxidative activity. The average protein content, total phenolic, and antioxidative activity of each origin in 2019 – 2021 are shown in Appendix 1.

Table 7 The mean protein content, phenolic content, and antioxidative activity in seeds in 2019 - 2021

Year	Protein (%)	Phenolic (mg_{GAE}/g_{dw})	Antioxidant activity ($\mu mol_{TE}/g_{dw}$)
2019	13.37 ± 0.14^{a}	140.01 ± 2.81^{b}	18.58 ± 0.79^{b}
2020	13.24 ± 0.12^{a}	128.04 ± 2.49^{b}	20.92 ± 1.36^{b}
2021	10.69 ± 0.11^{b}	158.80 ± 3.42^{a}	25.32 ± 0.90^{a}

The table represents mean ± standard error. The letters show a significantly different, tested by Dunn's test.

Twenty-two phenolic compounds, including gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, orientin, isoorientin, vitexin, isovitexin, hyperoside, isoquercetin, rutin, hesperidin, quercitrin, quercetin, naringenin, luteolin, kaempferol, apigenin, isorhamnetin, rhamnetin, and emodin were evaluated from the grain in 2019 – 2021.

The phenolic compound found in Tartary buckwheat were mainly quercetin (492.50 ± 6.84 $\mu g/g_{dw}$) and rutin (3,521.91 ± 24.18 $\mu g/g_{dw}$), while 18 compounds, consisting of gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, orientin, isoorientin, vitexin, isovitexin, hyperoside, isoquercetin, quercitrin, naringenin, luteolin, kaempferol, apigenin, isorhamnetin, and emodin presented in low concentration (less than 100 µg/g) in some genotypes. However, hesperidin and rhamnetin were not present in any samples.

The significant differences of each phenolic compound content in different years and origins were found after test by the Kruskal Wallis H test, except hesperidin and rhamnetin. Considering by harvested years, the rutin content of 2019 seeds, 3,141.32 ± 26.66 µg/g, showed a significantly lower than the seeds in 2020 and 2021, with 3,609.64 ± 36.17 µg/g and 3,723.84 ± 41.50 µg/g. Likewise, the quercetin content in 2019, 392 ± 7.36 µg/g, was notably lowest compared to 2020 (486.30 ± 11.29 µg/g) and 2021 (571.58 ± 10.65 µg/g) (Figure 14).

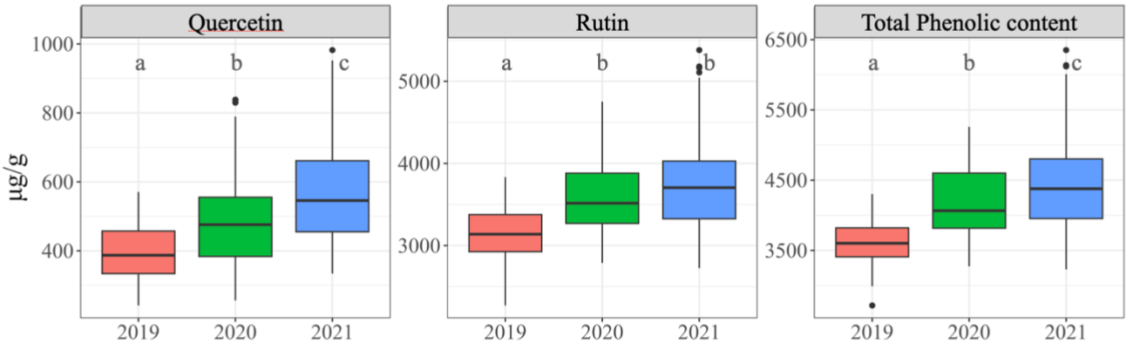

Figure 14 The box plot illustrates quercetin, rutin, and total phenolic content (TPC) of Tartary buckwheat in all samples from different years (2019 to 2021)

The letters show a significantly different, tested by Dunn’s test. Points represent the outlier of the data.

On the other hand, the phenolic compounds of the samples from difference places of origin showed significantly different after test by Kruskal Wallis H test with $p < 0.001$, except kaempferol, isoorientin, hesperidin, and rhamnetin. The lowest rutin content was found in Slovenian grain with 3,331.48 ± 21.59 µg/g which was significantly different from the other origins excluding Germany and the USA origin. In contrast, the quercitin content from Slovenian grain, 459.15 ± 7.29 µg/g, was notably lower than Ukrainian genotypes, 585.80 ± 28.09 µg/g, but not significantly different from the other origins (Figure 15).

The other phenolic compounds varied differently in each year of production, presented in Appendix 2. However, the total content of twenty-two phenolic compounds in the grain in 2019, approximately 3,604.97 ± 26.18 µg/g, was notably lower than in 2020 (4,191.28 ± 39.78 µg/g) and 2021 (4,406.63 ± 50.23 µg/g) affected by the lower content of rutin and quercetin (Figure 14). Considering the place of origin, the sum of twenty-two phenolic compounds content in the grain from Slovenia, 3,883.47 ± 26.47 µg/g, showed significantly lower content than all other origins except Germany and the USA origin as same as the content of rutin. The highest average sum of twenty-two compounds was found in Chinese genotypes with 4,485.13 ± 167.01 µg/g; however, it was not significantly higher than other origins except Slovenia (Figure 15).

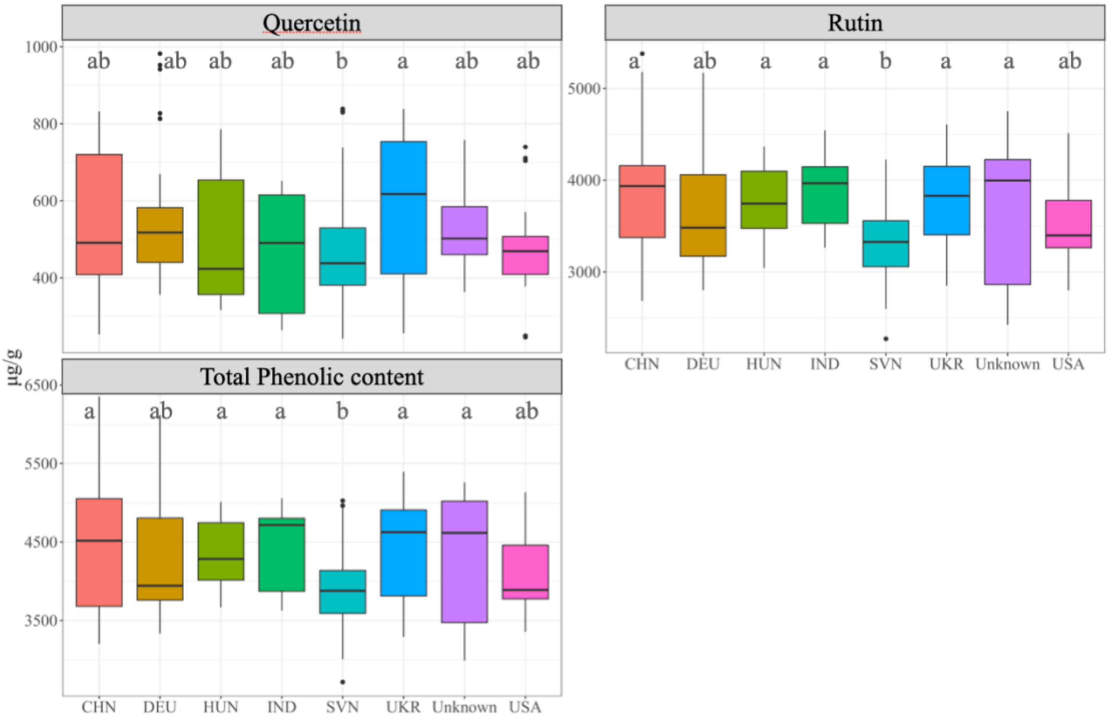

Figure 15 The box plot illustrates quercetin, rutin, and total phenolic content (TPC) of Tartary buckwheat samples of different origins

China (CHN), German (DEU), Hungary (HUN), India (IND), Slovenia (SVN), Ukraine (UKR), and United States of America (USA). The letters show a significantly different, tested by Dunn's test. Points represent the outlier of the data.

6. Discussion

6.1. Yield and morphological characters

The growth and development stages of Tartary buckwheat were separated into three stages, consisting of emerging, flowering, and maturing of the plant. Tartary buckwheat generally germinates within 1 – 3 days on moist paper in a Petri dish, but it takes approximately 8 – 15 days to emerge depending on the planting depth in the field conditions (Born & Corns 1958), while the seed emerging in this research was approximately a week longer with 14 – 23 days to emerge. However, previous research in Prague Ruzyně, 2006 – 2009, showed a similar time to emerge, with 9 – 25 days from sowing (Hlásná Čepková et al. 2009). Hence, the delay in seed emergence was probably related to the weather conditions. The rainfall affects soil moisture content which causes changes in the water absorbance and imbibition duration. The germination of Tartary buckwheat usually takes 30 – 88 hours for the seed imbibition before germinating (Bai et al. 2022). Whereas the temperature decline effect the delay of germination in both Petri dish and pot plantation (Born & Corns 1958).

Considering the flowering and maturing period, the plant started to flower around 52 – 91 days after sowing (47 – 73 days after emergence) and the seed became mature approximately 119 – 149 days after sowing (102 – 135 days after emergence) which was a longer period than other reports. The study performed in Kathmandu, Nepal showed a shorter time before flowering and ripening with an average of 30 days and 88 days after sowing to flower and mature (Joshi et al. 2011). As well as, the range for flowering in the previous studies was 22 – 37 days after sowing while the maturing was 60 – 118 days after sowing (Joshi et al. 2011), and 74 – 106 days after sowing (Joshi 2008). In addition, the study in Prague Ruzyně also showed a shorter time before flowering with the range of 40 – 53 days to flowering (after the emergence) but the ripeness period, 101 – 148 days to ripeness (after the emergence), is still in the similar range to this study (Hlásná Čepková et al. 2009).

The delay in flowering and maturing was possibly caused by the heavy rainfall in June 2022, two times higher than the average of the previous years. The excess soil moisture or flooding in the early seedling stage reduced the growth rate, leaf size, and leaf numbers in Tartary buckwheat cv. Nepal as reported by Matsuura et al. (2005). Likewise, all Nepal-originated genotypes died after a short period of time after emerging in this study. Furthermore, the delay in vegetation growth can also be the reason for the incomplete growth cycle due to the limited cultivation time before winter came. Eleven genotypes, eight from China and three from Belarus, Slovenia, and an unknown region, could set the fruit but did not have enough time for ripening.

The average WTS in this research, 10.44 g, is lower than the results in the previous reports, with 15.24 g in Nepal (Joshi et al. 2011), 14.07 g in the Czech Republic (Hlásná Čepková et al. 2009), and 16.5 g (in 2018) and 17.9 g (in 2019) in China (Hou et al. 2022). The number of seeds per cyme of each genotype varied between 5 – 41 seeds and 22.25 seeds per cyme on average which showed a similar range but marginally higher compared to the study, with 5 – 37 seeds per cyme and 18 seeds on average (Joshi et al. 2011). The plant branching from the main stem showed a similar range with the previous study, 2 – 8 branches (Joshi et al. 2011). The plant height in this study, 76.12 cm on average, demonstrated a middle length compared to the others, 41.8 cm (Joshi et al. 2011), 142.24 cm, and 130.92 cm (Hou et al. 2022). The varying in plant height was assumed that depends more on environmental conditions than on genetic variation (Hlásná Čepková et al. 2009). The plant height was affected by both temperature and water stress. The water deficit declined the plant height, whereas the high temperature stimulated the plant growth and increased height; however, the response to the stress was also affected by the varieties (Aubert & Quinet 2022).

Due to the fluctuation of weather conditions in 2022, fewer genotypes survived causing fewer samples as well as non-normal distributions of the data. Hence, the statistical analysis of morphological traits could not be performed. The places of origin did not affect the yield quantity; likewise, the report under greenhouse conditions in the Netherlands (Aubert & Quinet 2022). However, the genotypes from neighboring countries in Central and Eastern Europe, Ukraine (11.42 g/m^2) and Slovenia (11.07 g/m^2) showed slightly higher yield on average compared to the average of all genotypes, 7.43 g/m^2. Considering individually, the top tenth highest yield genotypes were from only four regions, including Slovenia, China, Ukraine, and unknown origin.

The cultivation in 2021 showed a high yield indeed, with 94.59 g/m^2 on average. Compared to the observed years that the yield was in the range of 9.42 – 22.41 g/m^2 on average. Hence, the production in 2021 was in a similar range of yield compared to the previous research, with 75.62 g/m^2 as the average (Joshi et al. 2011) and in the range of 30.0 – 358.4 g/m^2 (Joshi 2008), whereas the other years of production represented a low in quantity. The differences in yield are probably caused by the fluctuation and variation in the weather condition each year, especially temperature and soil water content. The great yield in 2021 might be related to the high rainfall in September which is the seed development period. Although the temperature showed a stronger effect on the yield in Tartary buckwheat, the water stress also declined the yield by interfering with the plant growth (Aubert & Quinet 2022), lower the number of inflorescence (Aubert et al. 2021), as well as changing the physiological traits in the photosynthesis process, including the chlorophyll content, transpiration rate, and stomatal conductance (Xiang et al. 2013). Not only the water deficit but the low temperature (4 °C) also decreased the yield by affecting the carbon-nitrogen metabolism and the growth of the plant (Jeon et al. 2018). While the high temperature (28/26 °C during day and night) had a strong effect on the yield by interfering with seed development and causing an abortion (Aubert & Quinet 2022).

6.2. Nutritive compounds

Tartary buckwheat is considered a nutritive pseudo-cereal due to the good balance of protein content and total phenolic which refer to the antioxidant activity. The grain protein content, 11.30%, in this study showed similar content compared to the other research on this species, which is in the range of 6.82 – 15.02% of protein content (Qin et al. 2010; Luthar et al. 2021b). Compared to other pseudo-cereals, quinoa and amaranth, contain around 9.1 – 16.7% and 13.1 – 21.5% of protein content, respectively (Martinez-Villaluenga et al. 2020).

Due to the fewer production varieties in 2022, the comparison between each origin could not be performed. The protein content, total phenolic content, and antioxidant activity from the grain in 2019 – 2021 did not show a statistical difference in each origin, but it significantly differed in the years of production. The nutrition analyses of the grains in 2021 showed a significantly lower protein content than the other two years. Hence, the differences in the protein component of Tartary buckwheat might be affected more by the fluctuation of the weather conditions in each year than the place of origin or the genetical properties.

Although the previous research about the environmental and genetic effects on the protein content in Tartary buckwheat was not found, they can be confirmed in common buckwheat that the protein content varies by the environment of planting cite and the places of origin (Barta et al. 2004; Sytar et al. 2016). In addition, in the year 2021, the increase of the yield probably was the reason of protein content declining due to the limiting of nitrogen in the soil; likewise, the increase of the yield in maize can result in lower protein concentration, except when the N-fertilizer was applied (Radulov et al. 2010).

On the other hand, the total phenolic compound content in Tartary buckwheat grain was notably high in concentration, with 144.58 $\text{mg}_{\text{GAE}}/\text{g}_{\text{dw}}$ on average, compared to the report in the Chinese cultivars, which have approximately 50 – 100 $\mu\text{mol}_{\text{GAE}}/\text{g}_{\text{dw}}$ (8.51 – 17.01 $\text{mg}_{\text{GAE}}/\text{g}_{\text{dw}}$) (Guo et al. 2011). In addition, Tartary buckwheat is also classified as an excellent phenolic source due to its high phenolic content compared with other pseudo-cereals; for example, amaranth with 12.4 $\text{mg}_{\text{GAE}}/100\ \text{g}_{\text{dw}}$ and quinoa with 90 – 200 $\text{mg}_{\text{GAE}}/100\ \text{g}_{\text{dw}}$ depended on the grain types (Škrovánková et al. 2020).

In accordance with the high content of total phenolic compound, Tartary buckwheat also represented high antioxidant activity. The average antioxidant activity in this study, 39.76 $\mu\text{mol}_{\text{TE}}/\text{g}_{\text{dw}}$, was slightly lower than the previous report, approximately 52.9 – 57.4 $\mu\text{mol}_{\text{TE}}/\text{g}_{\text{dw}}$ in dehulled grains (Morishita et al. 2015). The differences might be caused by the dehulling process because phenolic compounds are mainly located in the outer layer of the seed (Hung & Morita 2008) then the antioxidant activity was lower in the hull than whole seed (Lee et al. 2016). Compared with other pseudo-cereals, the Tartary buckwheat indicated approximately ten times higher than the other pseudo-cereals (1.05 $\mu\text{mol}_{\text{TE}}/\text{g}_{\text{dw}}$ in amaranth and 3.12 – 4.02 $\mu\text{mol}_{\text{TE}}/\text{g}_{\text{dw}}$ in quinoa) (Škrovánková et al. 2020).

As a result, significant differences in total phenolic content and antioxidant activity were not found when compared to the place of origin, but the grain in 2021 showed significantly higher in both total phenolic content and antioxidant activity than the production in 2019 and 2020.

As the defense mechanism of the plant, the total phenolic content usually increases under stress conditions; for example, the water deficit buckwheat showed a higher concentration of polyphenol than the well-watering (Siracusa et al. 2017), which contrasts with the result in 2019 – 2021. However, the phenolic content is possibly affected by other parameters. Consequently, total phenolic content and antioxidative activity are probably affected more by the environmental effect as same as the protein content in the grain. From the previous studies, the total phenolic compound and antioxidant activity proved that they were affected by the cultivar, place of origin, and plantation area (Guo et al. 2011; Luthar et al. 2021b).

Although the concentration of the phenolic compounds varied, rutin was the highest phenolic compound found in Tartary buckwheat grain, followed by quercetin as same as the previous report (Kreft et al. 2022b). Considering rutin, quercitin, and the sum of phenolic compounds, the production in 2021 showed the highest content same as the total phenolic content tested by Folin’s assay. However, other significances were found in the concentration of rutin, quercitin, and the sum of phenolic compounds, which might be caused by the development and size of the embryo. These phenolics are generally in the embryo; hence, the increase in embryo size can also improve the rutin profile in the grain (Luthar et al. 2020). In addition, the lower content of rutin is possibly caused by the high concentration and activity of rutinosidase, which can transform rutin to quercetin (Luthar et al. 2020; Kreft et al. 2022a).

7. Conclusions

According to the results, Tartary buckwheat showed both semi-erect shorter and semi-erect longer in growth and branch shoot habit. Generally, the plant had 2 – 9 branches from the main stem, with 76.12 cm height on average. Leaves had more width, 5.88 cm on average, than length, 4.35 cm on average. The inflorescences were loose cyme and semi-compact cyme. The number of flower cymes per inflorescence was usually 3 cymes, but it can vary from 2 – 5 cymes. Each cyme contains 21.25 seeds on average. The major color and shape of the grain was brown and oval.

The yield and WTS in 2022 was lower than the other years. Tartary buckwheat showed slightly low protein content, 11.30% of dw, but was high in total phenolic content, 144.58 $\text{mgGAE/g}_{\text{dw}}$, and antioxidant activity, 39.76 $\mu\text{molTE/g}_{\text{dw}}$.

This study did not confirm the tendencies of the samples originating in the same geographical area to display similar morphological or chemical traits. Despite this fact, the average yields from Ukraine and Slovenia were higher in comparison to other countries.

The results also showed that both phenotypic and nutritive compounds are strongly dependent on the year of production and weather conditions. The production in 2019 and 2020 demonstrated a significantly higher protein content than in 2021 and 2022; however, the total phenolic content and antioxidant activity varied between each year of production.

In this study, some genotypes showed great potential. The genotype from Slovenia (SRGB02258) represented the highest yield under unflavored weather conditions, whereas the American genotypes (PI 476852 and PI 199769) showed a high number of seeds per cymes. Hence, these were proposed for further investigation into improving the Tartary buckwheat for the conditions in the Czech Republic.

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Appendices

List of the Appendices

Appendix 1 The mean protein content, phenolic content, and antioxidative activity in grain classified according to the country of origin in 2019 – 2021

Year	Origin	Average protein (%)	Average phenolic ($\mathrm{mg_{GAE}/g_{dw}}$)	Average antioxidant ($\mathrm{mmol_{TE}/g_{dw}}$)
2019	China	13.96 ± 0.28	122.53 ± 0.57	14.28 ± 0.19
	Germany	12.58 ± 0.34	128.46 ± 2.28	18.31 ± 0.33
	Hungary	13.81 ± 0.05	132.82 ± 1.13	15.61 ± 0.13
	India	-	-	-
	Slovenia	13.38 ± 0.26	141.03 ± 4.16	17.61 ± 1.00
	Ukraine	13.86 ± 0.20	128.62 ± 2.83	18.17 ± 0.41
	USA	13.37 ± 0.24	147.41 ± 7.03	18.70 ± 0.34
	Unknown	12.95 ± 0.03	128.62 ± 2.83	25.87 ± 2.59
		13.37 ± 0.14^{a}	**140.01 ± 2.81**^{b}	**18.58 ± 0.79**^{b}
2020	China	12.48 ± 0.67	133.63 ± 0.79	17.15 ± 0.04
	Germany	12.71 ± 0.17	126.64 ± 1.67	40.84 ± 0.38
	Hungary	13.26 ± 0.03	114.67 ± 0.65	15.70 ± 0.21
	India	13.10 ± 0.78	122.80 ± 0.75	16.32 ± 0.06
	Slovenia	13.38 ± 0.18	121.11 ± 2.34	20.29 ± 1.63
	Ukraine	12.84 ± 0.26	147.53 ± 4.92	23.29 ± 4.48
	USA	12.97 ± 0.26	159.99 ± 0.24	21.38 ± 0.17
	Unknown	14.04 ± 0.46	133.90 ± 0.51	15.04 ± 0.15
		13.24 ± 0.12^{a}	**128.04 ± 2.49**^{b}	**20.92 ± 1.36**^{b}
2021	China	9.99 ± 0.58	134.13 ± 0.69	21.23 ± 0.13
	Germany	10.43 ± 0.24	176.30 ± 7.44	29.91 ± 0.86
	Hungary	10.28 ± 0.15	151.37 ± 3.69	27.93 ± 0.72
	India	11.30 ± 0.13	144.81 ± 0.35	20.42 ± 0.39
	Slovenia	10.73 ± 0.17	150.48 ± 2.48	22.22 ± 0.97
	Ukraine	11.16 ± 0.17	157.55 ± 2.22	29.91 ± 0.85
	USA	10.56 ± 0.24	205.07 ± 3.21	35.36 ± 0.86
	Unknown	10.57 ± 0.23	201.00 ± 2.98	31.27 ± 0.56
		10.69 ± 0.11^{b}	**158.80 ± 3.42**^{a}	**25.32 ± 0.90**^{a}
Overall		**12.42 ± 0.14**	**142.28 ± 2.03**	**21.45 ± 0.65**

The table represents mean ± standard error.

Appendix 2 The mean phenolic content in grain classified according to the country of origin in 2019 – 2021

Phenolic compounds	**Year**	**Phenolic content (μg/g)**								
		Origin								
		CHN	**DEU**	**HUN**	**IND**	**SVN**	**UKR**	**USA**	**Unknown**	**Average**
Apigenin	2019	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	NA	0.03 ± 0.00	0.26 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.01^{a}
	2020	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.10 ± 0.04	0.03 ± 0.00	0.03 ± 0.00	0.10 ± 0.01	0.07 ± 0.00	0.04 ± 0.00^{b}
	2021	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00^{c}
Caffeic acid	2019	0.13 ± 0.00	0.10 ± 0.00	0.14 ± 0.00	NA	0.20 ± 0.00	0.16 ± 0.00	0.15 ± 0.00	0.09 ± 0.00	0.17 ± 0.00^{a}
	2020	0.22 ± 0.01	0.18 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.20 ± 0.00	0.19 ± 0.00	0.44 ± 0.01	0.23 ± 0.01	0.21 ± 0.01^{b}
	2021	0.19 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.18 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.30 ± 0.01	0.21 ± 0.00^{b}
Catechin	2019	0.79 ± 0.03	0.85 ± 0.03	0.98 ± 0.01	NA	1.58 ± 0.04	1.73 ± 0.07	1.07 ± 0.01	2.60 ± 0.07	1.48 ± 0.05^{a}
	2020	1.40 ± 0.03	3.32 ± 0.05	0.70 ± 0.01	0.93 ± 0.02	2.23 ± 0.15	4.87 ± 0.13	3.50 ± 0.10	1.86 ± 0.07	2.44 ± 0.12^{b}
	2021	1.36 ± 0.04	3.69 ± 0.14	2.51 ± 0.05	2.00 ± 0.07	3.47 ± 0.07	4.19 ± 0.09	2.93 ± 0.05	6.74 ± 0.13	3.47 ± 0.09^{c}
Chlorogenic acid	2019	0.78 ± 0.07	0.39 ± 0.04	3.50 ± 0.04	NA	0.89 ± 0.04	1.66 ± 0.25	0.58 ± 0.04	4.43 ± 0.30	1.32 ± 0.11^{a}
	2020	4.07 ± 0.29	10.57 ± 0.83	0.40 ± 0.04	2.67 ± 0.22	1.95 ± 0.37	9.06 ± 0.85	15.61 ± 0.46	2.80 ± 0.36	4.22 ± 0.40^{b}
	2021	0.73 ± 0.02	1.28 ± 0.13	0.90 ± 0.09	1.99 ± 0.32	1.08 ± 0.05	0.90 ± 0.04	1.76 ± 0.04	15.46 ± 0.46	1.89 ± 0.25^{c}
Emodin	2019	6.26 ± 0.27	6.20 ± 0.14	10.62 ± 0.23	NA	7.41 ± 0.19	7.45 ± 0.19	5.88 ± 0.08	4.81 ± 0.11	7.18 ± 0.16^{a}
	2020	7.87 ± 0.18	8.02 ± 0.23	9.37 ± 0.62	5.25 ± 0.11	9.4 ± 0.26	7.43 ± 0.11	13.63 ± 0.69	9.72 ± 0.23	9.02 ± 0.20^{b}
	2021	5.68 ± 0.65	7.65 ± 0.56	7.22 ± 0.09	4.06 ± 0.07	5.12 ± 0.24	6.28 ± 0.21	9.66 ± 0.21	10.41 ± 0.17	6.11 ± 0.19^{c}

The table represents mean ± standard error, - shows 0 μg/g, and NA presents no sample. The letters show a significantly different, tested by Dunn’s test.

China (CHN), German (DEU), Hungary (HUN), India (IND), Slovenia (SVN), Ukraine (UKR), and the United States of America (USA).

Appendix 2 The mean phenolic content in grain classified according to the country of origin in 2019 – 2021 (Continue)

Phenolic compounds	Year	Phenolic content (µg/g)								
		Origin								
		CHN	**DEU**	**HUN**	**IND**	**SVN**	**UKR**	**USA**	**Unknown**	**Average**
Epicatechin	2019	7.88 ± 0.33	11.28 ± 0.47	13.01 ± 0.96	NA	9.89 ± 0.27	9.25 ± 0.39	11.20 ± 0.12	26.07 ± 1.19	11.27 ± 0.43^{a}
	2020	14.50 ± 0.66	25.69 ± 0.93	7.64 ± 0.37	9.97 ± 0.12	17.58 ± 0.80	21.10 ± 0.47	22.63 ± 0.27	27.08 ± 0.39	18.11 ± 0.57^{b}
	2021	11.35 ± 0.35	23.73 ± 1.02	15.03 ± 0.29	12.92 ± 0.51	19.83 ± 0.52	21.43 ± 0.70	21.55 ± 0.84	67.77 ± 2.36	21.96 ± 0.92^{c}
Gallic acid	2019	0.51 ± 0.01	0.42 ± 0.03	0.56 ± 0.03	NA	0.96 ± 0.03	0.65 ± 0.02	0.48 ± 0.01	0.48 ± 0.01	0.77 ± 0.03^{a}
	2020	0.79 ± 0.01	0.96 ± 0.02	0.63 ± 0.02	0.50 ± 0.01	0.90 ± 0.03	0.90 ± 0.04	1.92 ± 0.04	1.01 ± 0.05	0.93 ± 0.03^{b}
	2021	0.52 ± 0.01	0.81 ± 0.03	0.67 ± 0.01	0.67 ± 0.01	0.93 ± 0.03	0.82 ± 0.04	1.20 ± 0.08	2.06 ± 0.06	0.93 ± 0.03^{b}
Hesperidin	2019	-	-	-	NA	-	-	-	-	-
	2020	-	-	-	-	-	-	-	-	-
	2021	-	-	-	-	-	-	-	-	-
Hyperoside	2019	0.11 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	NA	0.10 ± 0.00	0.89 ± 0.11	0.10 ± 0.00	0.07 ± 0.00	0.16 ± 0.02^{a}
	2020	0.21 ± 0.01	0.10 ± 0.01	0.16 ± 0.01	0.18 ± 0.00	0.26 ± 0.01	0.13 ± 0.01	0.38 ± 0.03	0.33 ± 0.02	0.23 ± 0.01^{b}
	2021	0.20 ± 0.01	0.17 ± 0.01	0.18 ± 0.00	0.21 ± 0.00	0.18 ± 0.02	0.15 ± 0.01	0.21 ± 0.02	0.19 ± 0.00	0.18 ± 0.01^{c}
Isoorientin	2019	0.21 ± 0.07	0.03 ± 0.01	0.01 ± 0.00	NA	0.06 ± 0.02	0.04 ± 0.01	-	-	0.06 ± 0.01^{a}
	2020	-	-	-	-	0.03 ± 0.01	-	0.05 ± 0.01	0.12 ± 0.02	0.03 ± 0.00^{a}
	2021	-	-	-	-	0.03 ± 0.01	-	0.02 ± 0.01	-	0.02 ± 0.00^{b}

The table represents mean ± standard error, - shows 0 µg/g, and NA presents no sample. The letters show a significantly different, tested by Dunn's test.

China (CHN), German (DEU), Hungary (HUN), India (IND), Slovenia (SVN), Ukraine (UKR), and the United States of America (USA).

Appendix 2 The mean phenolic content in grain classified according to the country of origin in 2019 – 2021 (Continue)

Phenolic compounds	Year	Phenolic content (μg/g)								
		Origin								
		CHN	DEU	HUN	IND	SVN	UKR	USA	Unknown	Average
Isoquercetin	2019	5.45 ± 0.37	4.60 ± 0.07	4.53 ± 0.07	NA	6.21 ± 0.18	5.45 ± 0.16	5.50 ± 0.21	7.41 ± 0.36	5.90 ± 0.13^{a}
	2020	11.70 ± 0.18	7.96 ± 0.11	7.71 ± 0.31	10.07 ± 0.56	9.16 ± 0.16	7.62 ± 0.42	10.11 ± 0.36	9.31 ± 0.60	9.09 ± 0.14^{b}
	2021	11.87 ± 0.47	10.03 ± 0.23	5.89 ± 0.30	11.24 ± 0.11	9.48 ± 0.20	8.96 ± 0.34	9.40 ± 0.08	15.98 ± 0.48	9.85 ± 0.18^{c}
Isorhamnetin	2019	0.3 ± 0.01	0.27 ± 0.02	0.40 ± 0.01	NA	0.51 ± 0.01	0.57 ± 0.01	0.44 ± 0.02	0.56 ± 0.03	0.47 ± 0.01^{a}
	2020	1.30 ± 0.06	0.53 ± 0.02	0.67 ± 0.03	0.73 ± 0.02	1.54 ± 0.04	0.67 ± 0.01	1.23 ± 0.06	0.76 ± 0.03	1.20 ± 0.04^{b}
	2021	0.52 ± 0.01	0.61 ± 0.01	0.66 ± 0.01	0.76 ± 0.02	0.96 ± 0.03	0.79 ± 0.03	0.59 ± 0.02	1.29 ± 0.03	0.86 ± 0.02^{c}
Isovitexin	2019	0.74 ± 0.16	0.13 ± 0.02	0.09 ± 0.00	NA	0.36 ± 0.07	0.17 ± 0.02	-	-	0.29 ± 0.05^{a}
	2020	0.07 ± 0.03	0.02 ± 0.00	0.04 ± 0.00	-	0.07 ± 0.01	0.01 ± 0.00	0.26 ± 0.11	0.30 ± 0.05	0.08 ± 0.01^{b}
	2021	0.12 ± 0.02	-	0.06 ± 0.01	0.03 ± 0.01	0.10 ± 0.02	0.20 ± 0.07	0.14 ± 0.04	0.04 ± 0.00	0.10 ± 0.01^{b}
Kaempferol	2019	44.46 ± 6.01	43.93 ± 1.88	35.46 ± 1.10	NA	41.96 ± 1.44	26.43 ± 0.44	39.83 ± 4.74	46.71 ± 1.25	40.89 ± 1.07^{a}
	2020	48.53 ± 3.50	37.30 ± 3.77	45.96 ± 4.10	35.43 ± 2.17	55.99 ± 2.17	50.70 ± 5.13	62.17 ± 6.56	49.06 ± 6.13	51.99 ± 1.54^{b}
	2021	98.18 ± 2.48	64.64 ± 4.79	86.71 ± 2.37	69.36±1.75	59.61 ± 1.18	68.47 ± 1.80	41.79 ± 1.54	46.44 ± 4.04	63.41 ± 1.26^{c}
Luteolin	2019	0.05 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	NA	0.11 ± 0.00	1.56 ± 0.18	0.06 ± 0.00	0.08 ± 0.00	0.20 ± 0.04^{a}
	2020	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.00	0.07 ± 0.00	0.13 ± 0.01	0.15 ± 0.00	0.38 ± 0.01	0.18 ± 0.01	0.15 ± 0.01^{b}
	2021	0.10 ± 0.00	0.13 ± 0.01	0.13 ± 0.00	0.11 ± 0.00	0.12 ± 0.00	0.14 ± 0.00	0.13 ± 0.01	0.18 ± 0.01	0.12 ± 0.00^{b}

The table represents mean ± standard error, - shows 0 μg/g, and NA presents no sample. The letters show a significantly different, tested by Dunn’s test.

China (CHN), German (DEU), Hungary (HUN), India (IND), Slovenia (SVN), Ukraine (UKR), and the United States of America (USA).

Appendix 2 The mean phenolic content in grain classified according to the country of origin in 2019 – 2021 (Continue)

Phenolic compounds	Year	Phenolic content (µg/g)								
		Origin								
		CHN	DEU	HUN	IND	SVN	UKR	USA	Unknown	Average
Naringenin	2019	0.02 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	NA	0.11 ± 0.00	0.08 ± 0.00	0.07 ± 0.01	0.12 ± 0.00	0.09 ± 0.00^{a}
	2020	0.08 ± 0.00	0.08±0.01	0.06 ± 0.00	0.04 ± 0.00	0.12 ± 0.00	0.08 ± 0.00	0.23 ± 0.02	0.12 ± 0.00	0.11 ± 0.00^{a}
	2021	0.06 ± 0.01	0.10 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	0.10 ± 0.00	0.10 ± 0.01	0.17 ± 0.00	0.15 ± 0.00	0.1 ± 0.00^{a}
Orientin	2019	0.09 ± 0.02	-	-	NA	0.04 ± 0.01	-	-	-	0.03 ± 0.01^{a}
	2020	-	-	-	-	0.01 ± 0.00	-	0.04 ± 0.01	0.10 ± 0.02	0.02 ± 0.00^{b}
	2021	-	-	-	-	0.01 ± 0.01	-	0.01 ± 0.01	-	0.01 ± 0.00^{a}
Quercetin	2019	399.60 ± 34.47	478.37 ± 10.26	364.56 ± 11.10	NA	372.4 ± 8.59	379.79 ± 11.96	399.80 ± 45.60	496.60 ± 12.70	392.71 ± 7.36^{a}
	2020	433.92 ± 28.29	435.06 ± 29.57	440.19 ± 33.76	328.81 ± 22.05	486.81 ± 13.45	562.60 ± 48.28	555.11 ± 41.22	567.59 ± 55.53	486.30 ± 11.29^{b}
	2021	785.46 ± 18.12	653.63 ± 44.56	692.09 ± 19.48	603.25 ± 12.96	503.67 ± 9.95	712.00 ± 22.86	461.29 ± 13.92	549.97 ± 38.30	571.58 ± 10.65^{c}
Quercitrin	2019	0.43 ± 0.01	0.34 ± 0.01	0.38 ± 0.01	NA	0.31 ± 0.01	0.44 ± 0.05	0.33 ± 0.01	0.17 ± 0.00	0.33 ± 0.01^{a}
	2020	0.38 ± 0.01	0.37 ± 0.01	0.39 ± 0.01	0.47 ± 0.05	0.39 ± 0.01	0.28 ± 0.01	0.48 ± 0.02	0.37 ± 0.01	0.38 ± 0.01^{b}
	2021	2.00 ± 0.03	1.41 ± 0.19	0.59 ± 0.01	2.30 ± 0.06	2.33 ± 0.23	0.54 ± 0.01	1.20 ± 0.03	1.84 ± 0.03	1.85 ± 0.13^{c}
Rhamnetin	2019	-	-	-	NA	-	-	-	-	-
	2020	-	-	-	-	-	-	-	-	-
	2021	-	-	-	-	-	-	-	-	-

The table represents mean ± standard error, - shows 0 µg/g, and NA presents no sample. The letters show a significantly different, tested by Dunn's test.

China (CHN), German (DEU), Hungary (HUN), India (IND), Slovenia (SVN), Ukraine (UKR), and the United States of America (USA).

Appendix 2 The mean phenolic content in grain classified according to the country of origin in 2019 – 2021 (Continue)

Phenolic compounds	Year	Phenolic content (µg/g)								
		Origin								
		CHN	DEU	HUN	IND	SVN	UKR	USA	Unknown	Average
Rutin	2019	3077.40 ± 111.42	2973.12 ± 36.97	3563.20 ± 61.13	NA	3143.51 ± 32.16	3097.04 ± 56.98	3392.09 ± 28.67	2727.55 ± 68.55	3141.32 ± 26.67[a]
	2020	3935.21 ± 22.21	3342.61 ± 39.15	4166.95 ± 81.83	3766.21 ± 181.94	3339.9 ± 30.25	3901.07 ± 76.22	4027.46 ± 105.54	4264.27 ± 141.39	3609.64 ± 36.17[b]
	2021	4530.39 ± 180.53	4320.00 ± 149.57	3611.03 ± 135.54	4032.55 ± 34.95	3474.28 ± 38.4	3991.48 ± 84.66	3098.13 ± 72.21	4115.04 ± 32.25	3723.84 ± 41.50[b]
Vitexin	2019	0.77 ± 0.14	0.14 ± 0.03	0.18 ± 0.06	NA	0.37 ± 0.07	0.20 ± 0.02	-	-	0.30 ± 0.04[a]
	2020	0.10 ± 0.04	0.02 ± 0.00	0.05 ± 0.00	0.03 ± 0.01	0.07 ± 0.01	0.01 ± 0.00	0.28 ± 0.11	0.42 ± 0.06	0.09 ± 0.01[b]
	2021	0.12 ± 0.02	0.01 ± 0.00	0.07 ± 0.01	0.04 ± 0.01	0.13 ± 0.03	0.21 ± 0.07	0.15 ± 0.05	0.05 ± 0.01	0.12 ± 0.02[b]
Total	2019	3546.00 ± 80.08	3520.39 ± 47.78	3997.84 ± 69.20	NA	3587.02 ± 34.88	3533.81 ± 61.25	3857.62 ± 31.64	3317.78 ± 73.75	3604.97 ± 26.18[a]
	2020	4460.49 ± 41.52	3872.96 ± 24.14	4681.22 ± 95.35	4161.61 ± 205.55	3926.77 ± 36.94	4566.90 ± 106.75	4716.02 ± 94.59	4935.70 ± 88.17	4194.28 ± 39.78[b]
	2021	5448.90 ± 193.37	5088.13 ± 187.55	4424.06 ± 149.99	4741.81 ± 28.96	4081.66 ± 44.88	4816.92 ± 96.95	3650.61 ± 69.53	4833.93 ± 72.16	4406.63 ± 50.23[c]

The table represents mean ± standard error, - shows 0 µg/g, and NA presents no sample. The letters show a significantly different, tested by Dunn’s test.

China (CHN), German (DEU), Hungary (HUN), India (IND), Slovenia (SVN), Ukraine (UKR), and the United States of America (USA).