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Review

A Crop of High Nutritional Quality and Health Maintenance Value: The Importance of Tartary Buckwheat Breeding

Ivan Kreft [1,2], Aleksandra Golob [1] and Mateja Germ [1,*]

[1] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; ivan.kreft@guest.arnes.si (I.K.); aleksandra.golob@bf.uni-lj.si (A.G.)
[2] Nutrition Institute, Tržaška 40, SI-1000 Ljubljana, Slovenia
* Correspondence: mateja.germ@bf.uni-lj.si

Abstract: Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.), originating in the Himalayan area, is cultivated in central Asia and northern, central, and eastern Europe. Tartary buckwheat grain and sprouts are rich in flavonoid metabolites rutin and quercetin. The synthesis of flavonoids in plants is accelerated by UV-B solar radiation to protect the plants against radiation damage. During Tartary buckwheat food processing, a part of rutin is enzymatically converted to quercetin. Rutin and quercetin are able to pass the blood–brain barrier. Studies have investigated the effects of rutin and quercetin on blood flow to the brain, consequently bringing more nutrients and oxygen to the brain, and causing improved brain function. In addition to the impact on blood flow, rutin and quercetin have been shown to have antioxidative properties. The goals of breeding Tartary buckwheat are mainly to maintain and enhance the high nutritional quality. The goals could be reached via the breeding of Tartray buckwheat for larger cotyledons. Other main breeding efforts should be concentrated on the easy husking of the grain, the prevention of seed shattering, and the improvement in growth habits to obtain uniformity in grain ripening and a stable and high yield.

Keywords: *Fagopyrum tataricum*; flavonoids; rutin; quercetin; food; nutrition; breeding

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1. Introduction

The place of origin of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is the mountain area of the Himalayas. Tartary buckwheat cultivation takes place at high altitudes around the Himalayas, in China, Nepal, Bhutan, India, and Pakistan, as well in some other countries: Korea, Japan, Kazakhstan, Russia, Ukraine, Belarus, Sweden, Poland, Italy, Luxemburg, and Slovenia [1,2] (Figure 1a–d). In Bosnia and Herzegovina, Tartary buckwheat is grown mainly as a mixed crop with common buckwheat (*Fagopyrum esculentum* Moench) (Figure 1d) [1].

Tartary buckwheat seeds are protected by a thick husk and phenolic substances, and may remain alive but dormant in the soil for many years. Under suitable environmental conditions, they can grow again. Tartary buckwheat survives under strong ultraviolet radiation, which takes place at high altitudes. During their evolution, the plants survived while gradually accumulating genes for substances that allow the plants to reproduce and survive in the stony areas of the high Himalayas, exposed to intense UV-B radiation. The main protective substances that will be discussed in the present paper are flavonoids and other phenolic substances. There are many steps in the synthesis of flavonoid substances and phenolic acids based on phenylalanine; synthesis is enabled by genes and enzymes, active under the impact of UV-B radiation (Figure 2) [2]. The important function of Tartary buckwheat phenolic substances is thus the protection of plants against UV-B radiation.

Many genes involved in the biosynthesis of phenolic substances and their regulation were established in buckwheat [3]. The feasibility of Tartary buckwheat to be able to survive in conditions of high levels of abiotic stress is attributed to the development of

several complexes of genes influencing the transduction of signals and the regulation of genes [3]. Such changes in the genetic layout cause the adaptation of plants to harsh ecological conditions. The significance of the genes for the biosynthetic pathway of rutin and the relevant MYB transcription factors (Figure 2) [3–5] is well-known.

Figure 1. (**a**) Flowering and ripening plant of Tartary buckwheat. (**b**) Inflorescence with ripening Tartary buckwheat grain. (**c**) Field of Tartary buckwheat in Wermland, Sweden. (**d**) Fields growing a mixture of Tartary and common buckwheat in the central part of Bosnia and Herzegovina.

Buckwheat metabolites with benzene rings included protect buckwheat plants in several ways. The protection of buckwheat plants from fungal attacks is important for fungi that form mycotoxins in the grain [6–8]. In food, however, the plant-protecting substances of Tartary buckwheat are important for protecting human health.

The flavonoid rutin is present in the grain of Tartary buckwheat, mainly in the cotyledons [9–11]. In addition to rutin, there are also enzymes in the grain which transform rutin into quercetin (Figure 3). Rutin and quercetin molecules are similar, with the difference that rutin has two sugar molecules attached to the aglycone part of the molecule. The enzyme rutinasidase enables the splitting apart of the sugar part of the aglycone and the transformation of rutin into quercetin. In the intact grain, rutin is separated from the enzyme and thus is protected from the transformation. After crushing Tartary buckwheat seeds, the rutin-degrading enzyme is mixed with rutin and during the preparation of dough, after moistening, the conditions are suitable for the decomposition of rutin molecules and the appearance of quercetin. As the consequence, the bitter substance quercetin appears [12–14].

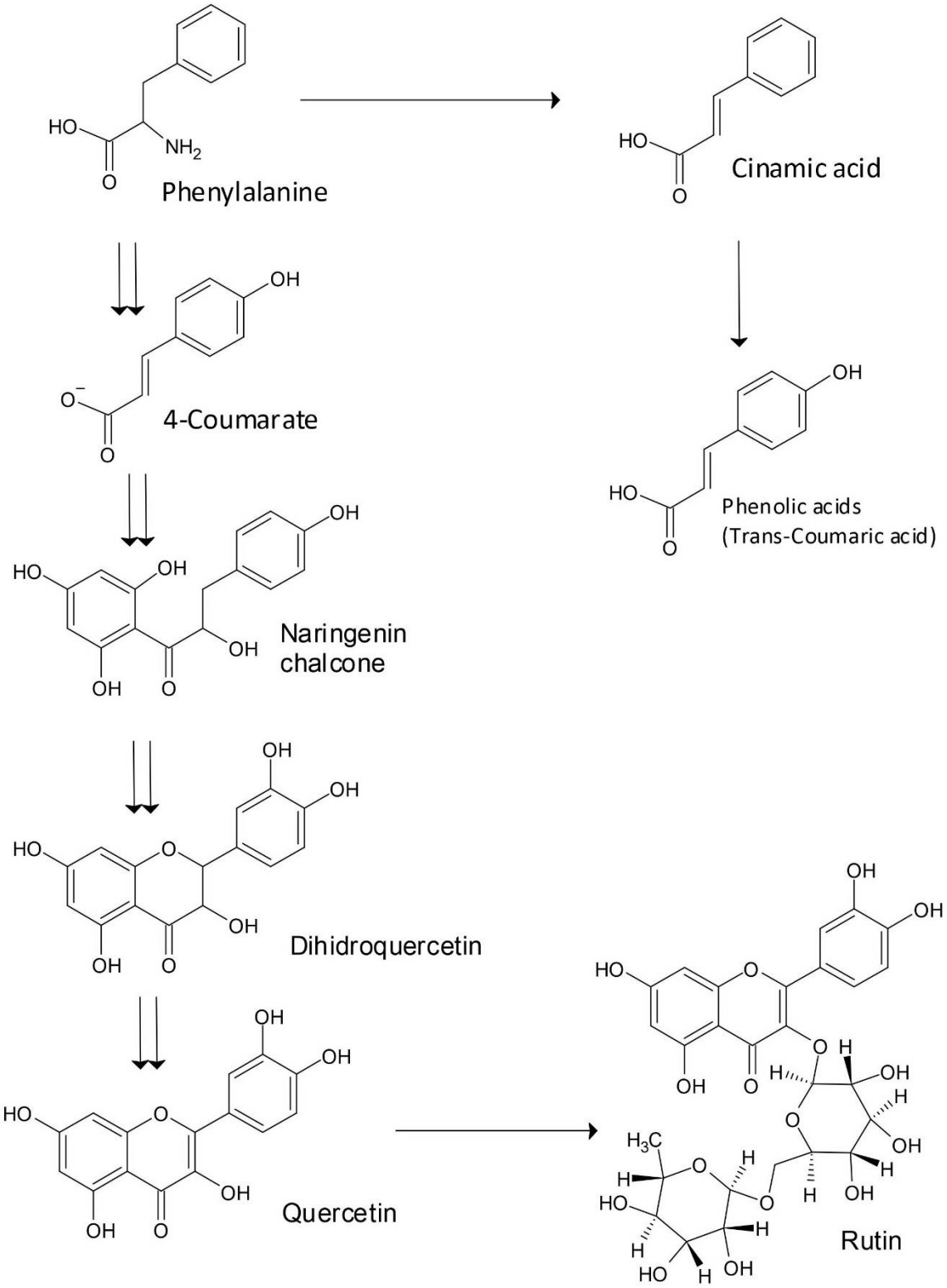

Figure 2. Main steps in the biosynthesis of rutin from phenylalanine in buckwheat plants, each supported by respective genes. A side branch of the synthetic pathway leads to phenolic acids.

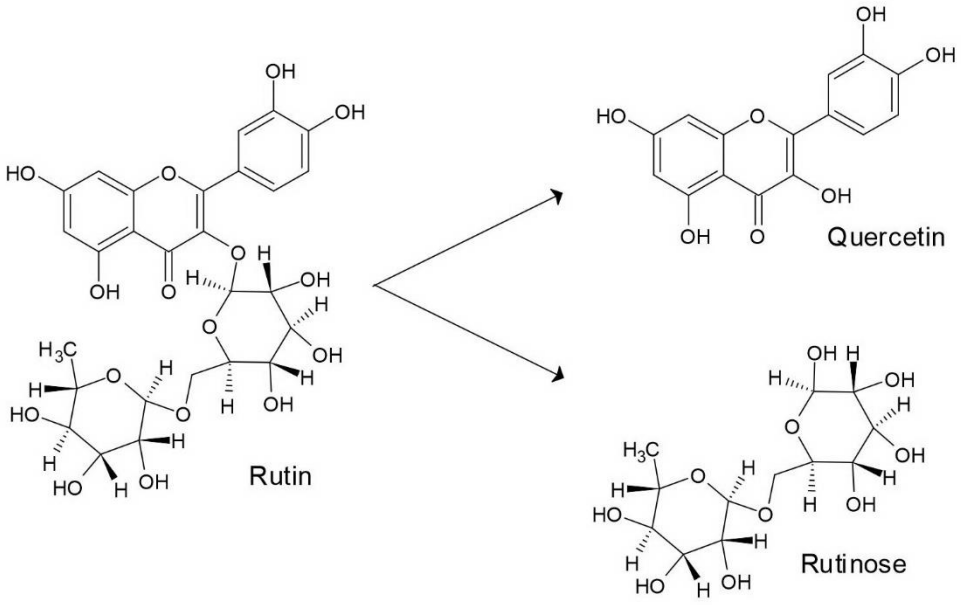

Figure 3. The enzymatic degradation of rutin to flavonoid quercetin and sugar rutinose.

Tartary buckwheat has, in comparison to cereals, a high nutritional value. It has good fatty acid composition and excellently balanced amino acid composition, with favorable vitamin B content [15–17], and a very high rutin level [18–20]. Rutin is known for its ability to strengthen blood vessels, supporting the impact of vitamin C and aiding in reducing blood clots and cholesterol levels [21–23]. Consuming dishes with Tartary buckwheat is important for the prevention of gallstone formation, hypertension, cardiovascular problems, and obesity [24–26]. The effects are mainly due to slowly digestible proteins, digestion-resistant starch, phenolic substances, and their interactions with other grain constituents [27]. From a comparison of the amounts of flavonoids rutin and quercetin in the collection of Tartary buckwheat grain and sprout samples from Europe and Asia, it was reported that the Nepal grain samples had the highest rutin content (13.3 g/kg in dry matter). The concentration of rutin in the sprouts from the same Himalayan area was up to 54.4 g/kg in dry matter. The concentration of quercetin in sprouts was 10–90-fold higher in comparison to that established in the grain [27,28]. Tartary buckwheat grain and sprouts have important potential for providing flavonoids in the human diet and for the production of functional dishes, rich in flavonoids.

When comparing common buckwheat and Tartary buckwheat, there is a considerable difference in the metabolic layout of the two species. It was found that 61 flavonoids and 94 non-flavonoid substances had significantly higher content (at least double) in Tartary buckwheat in comparison to common buckwheat. Tartary buckwheat flour has a yellow color, compared to the light gray color of common buckwheat flour (Figure 4a). The dough and bread made from Tartary buckwheat flour also express a yellow-green hue (Figure 4b–d). Tartary and common buckwheat grains are rich in secondary metabolites beneficial to human health and among them, non-flavonoid metabolites are important. They contribute high health-supporting effects in Tartary buckwheat compared to common buckwheat [29,30]. Zhang et al. [31] reported the sequencing and assembly of Tartary buckwheat [31–33]. Milling the grain of Tartary buckwheat and mixing the flour with water results in the formation of quercetin, which is a degradation product of rutin after rutinosidase activity [14,32,33].

Figure 4. (**a**) Tartary buckwheat flour (**left**) compared to common buckwheat flour (**right**) at the farmers' market in Xichang, Liangshan Yi Autonomous Prefecture, Sichuan, China. (**b**) Dough made from Tartary buckwheat flour, expressing a yellow-green hue due to Tartary buckwheat's secondary metabolites. (**c**) Pretzels made from a mixture of Tartary buckwheat (40%) and wheat flour, expressing a green hue due to the Tartary buckwheat's secondary metabolites content. (**d**) Slovenian olive-oil-seasoned vegan soup, thickened with boiled husked common buckwheat grain (to the left) and husked Tartary buckwheat grain.

Many novel dishes have recently been developed based on traditional Tartary and common buckwheat grain materials, rich in rutin and quercetin (Figure 4c,d) [11,34,35]. Due to the protein content, with suitable amino acid composition, innovative foods based on Tartary buckwheat are a source of high quality proteins in the diet of vegans and other people who do not consume animal proteins. In Korea and China, Tartary buckwheat sprouts are developed as a food material, rich in flavonoids rutin and quercetin [36].

2. Bioactivity of Flavonoids Rutin and Quercetin

In Tartary buckwheat, the complexation of quercetin with starch has been reported [37]. Enzyme molecules hinder the in vitro digestion of starch complexed with quercetin [37,38]. This causes the transformation of native starch to the resistant one and changes the physical and chemical characteristics of the Tartary buckwheat starch [39]. The effects of such a quercetin–starch complexation are that foods made from Tartary buckwheat will have limited digestibility. Quercetin in Tartary buckwheat can reduce the concentration of low-density lipoprotein, serum triacylglycerols, and body weight. In experimental animals, a diet with 0.1% quercetin was shown to significantly impact the lowering of low-density lipoprotein concentrations in serum, without any effect on high-density lipoprotein. Tartary buckwheat has also prevented an increase in body weight and fat deposition during high-

fat intake in rats [40]. A buckwheat diet can improve glucose intolerance in patients by reducing the insulin response [41]. The reduced postprandial glycemic responses in common buckwheat were shown [41].

Phenolic substances are often transformed in the gut before their absorption. The microbiota in the colon enable this process [42]. Small-sized phenolic molecules, which result from the colon's microbial conversion, are more easily absorbed than large-sized phenolic metabolites [43–45].

Radiation-induced brain injury is a serious adverse effect of brain radiotherapy in oncology. Flavonoid quercetin has a wide range of biological activities, including the ability to regulate gut microbiota. After the oral submission of quercetin preparations in experimental mice, the spontaneous activity behavior, short-term memory ability, and anxiety level were improved [46]. The long-term administration of buckwheat whole meal flour suppresses cognitive decline by increasing hippocampal brain-derived neurotrophic factor production in experimental mice [47].

Important bioactivities have been found for quercetin and the related molecules, not just in the gastrointestinal system but also in blood vessels, muscles, and the brain [48]. The blood–brain barrier is a highly selective semipermeable border, consisting of endothelial cells. It prevents solutes from circulating blood from non-selectively crossing into the extracellular space of the central nervous system [49–51]. Ingested rutin and quercetin can accumulate in the brain tissue after crossing the blood–brain barrier [52]. The challenge in treating Alzheimer's disease and some other neurological conditions is the inability of medical substances to enter the brain due to poor solubility and the blood–brain barrier [53]. Quercetin could cross the blood–brain barrier and exerts neuroprotective effects in many neurological disease situations [54]. In vitro and in vivo experiments have shown that molecules, including quercetin, and quercetin derivatives, can penetrate the blood–brain barrier without toxicity; quercetin could be delivered to the ischemic area of the brain, ensuring the targeted delivery and antioxidative impact. The mitochondrial targeting of damaged neurons is also achieved in such a way. Substances with quercetin as part of the molecules may have better neuroprotective ability than quercetin itself [55].

Taile et al. [56] established that polyphenols are important dietary antioxidants with anti-inflammatory action, able to improve the effects of stroke and other cerebrovascular problems. The protective impact of quercetin and some other polyphenolic substances is connected with their bioavailability. It is important to apply polyphenol-based strategies for the improvement of the clinical picture of stroke.

Blood–brain barriers hinder not only the endogenous or exogenous toxicants, but also compounds with therapeutic properties. A group of chemo-sensing receptors was identified in the blood–cerebrospinal fluid barrier. Chemosensing bitter taste receptors are promising as potential targets of drugs. Quercetin, resveratrol and other metabolites with neuroprotective activity as ligands for transporting molecules can potentially counteract drug resistance in the delivery to the brain for the treatment of central nervous system disorders [57,58]. Extracts from buckwheat leaves and flowers have an impact on the antioxidant situation of the liver and brain of mice. Such extracts were given orally to mice for 21 days. The effects in the mouse brain were the following: the amount of glutathione and malondialdehyde was reduced, superoxide dismutase activity was significantly decreased, and catalase activity significantly increased [59].

Choi et al. [60] studied the impact of rutin and the n-butanol fraction extracted from Tartary buckwheat on learning and memory deficits in a mouse model of amyloid beta (A beta)-induced Alzheimer's disease. The impaired cognition and memory of experimental animals were attenuated by the oral submission of an n-butanol fraction and rutin extracted from plants of Tartary buckwheat. According to the results from Choi et al. [60], the n-butanol fraction and rutin extracted from Tartary buckwheat had protective effects, and therapeutic applications for the treatment of Alzheimer's disease are thus suggested.

3. Precautions for Possible Adverse Effects of Tartary Buckwheat Metabolites

One of buckwheat's secondary metabolites, fagopyrin, has a health threat when the green parts of common or Tartary buckwheat are eaten [61–63]. But the ingestion of buckwheat grain products seems to be safe due to the low concentration of fagopyrin in the grain [63–65]. A buckwheat allergy is a very rare event [66].

Suzuki et al. [12] investigated the potential toxicity of rutin-rich dough made from Tartary buckwheat through acute and subacute toxicity studies in experiments with laboratory rats. The concentration of rutin in Tartary buckwheat material was 1570 mg/100 g. In the experiment, no toxic or other non-regular symptoms were detected. The resulting body weight was not significantly different among the groups of experimental animals. The conclusion of the experiment was that Tartary buckwheat at a given dose was without noticeable adverse effects. The results of Vogrinčič et al. [67] and Suzuki et al. [68] also established that no genotoxic effects were expressed in Tartary buckwheat grain materials.

Quercetin is genotoxic to salmonella, but it was approved to be safe for human application [69]. The study of Cunningham et al. [70] confirmed the safety of quercetin for use in mice when applied daily at 12.5, 25, or 50 mg/kg of body weight for 98 days. In any case, more studies have to be performed to determine any possible quercetin toxicity effects after the chronic ingestion of Tartary buckwheat or other quercetin-rich food [70].

4. Main Goals and Methods for Breeding Tartary Buckwheat

Tartary buckwheat is a semi-wild plant [1]. It was not long ago when people started to cultivate it. Even now, wild forms of Tartary buckwheat grow outside the fields on the rocky slopes of the Himalayas. Tartary buckwheat is found as a weed in common buckwheat crops or other crops. The shattered seeds may return from the fields to less friendly areas, as wild plants, occupying poorly fertile soil. For the propagation of wild plants, the shattering is a very important trait. In newly domesticated crop-like Tartary buckwheat, seed shattering is a detrimental characteristic for growing in fields and consistent efforts must be made to eliminate this trait during breeding. When Tartary buckwheat is cultivated, an important part of the yield could be lost by the shattering of seeds. Therefore, breeding against shattering and obtaining the uniform ripening of Tartary buckwheat seeds are important goals in Tartary buckwheat breeding. In practical terms, the selection against shattering could be performed by selecting thicker peduncles supporting individual seeds. Other selection methods against seed shattering could be through the vibrating of plants and scoring which portion of seeds remain on the plants after the vibration impact. This selection method is not very effective because of the non-uniform ripening of the seeds on Tartary buckwheat plants. An important issue in obtaining less shattering is a uniform time for ripening of all seeds on the plant. In common buckwheat, the gene for the determinate growth habit is known [71–73]. Determinate buckwheat plants have more uniform ripening and stronger side branches, which supports the prevention of seed shattering. But until now, no report on determinate growth habits in Tartary buckwheat is known. It should be desirable to find Tartary buckwheat with determinate growth habits. One way to reach this goal could be a mass screening of the Tartary buckwheat population for this trait, or mutation breeding. Determinate common buckwheat plants are lower, so they could be hidden in the canopy. Determinate plants in the tight canopy condition have a lower possibility of surviving and reproducing seeds. Therefore, in canopies, selection pressure is working against plants with determinate growth habits, unless plants grow in conditions with less competition from neighboring plants [71,74,75]. Why determinate Tartary buckwheat plants have not yet been found among the wildly growing populations is unknown. Determinate Tartary buckwheat would be interesting because of its expected resistance to lodging, the simultaneous and uniform seed set and the resistance to seed shattering. One of the main problems with the production of Tartary buckwheat is its indeterminant growth. As a result, the seeds ripen very unevenly and the first ripe seeds can fall off before the last formed and filled seeds mature.

Mutation breeding in Tartary buckwheat should not be too complicated as it is a self-fertile plant and homozygotes for the mutated gene could already be expressed and scored in early generations after treatment, with mutagenic impact.

The lodging of plants does not seem to be a serious problem in Tartary buckwheat. If lodged, plants are very soon recovered. If plants can be created by breeding the determinate Tartary buckwheat, they are expected to be more resistant to lodging than non-determinate plants. For example, in common buckwheat, determinate plants are much better resistant to lodging in comparison to non-determinate plants [71].

As Tartary buckwheat grains have coarse and hard husks, it is very difficult to dehusk them to obtain groats. Only a few producers can make husked Tartary buckwheat [1]. Several easily husked or "Rice-Tartary" buckwheat forms are known. Rice-Tartary is a special type of Tartary buckwheat, with seeds with a loose husk that make dehusking easier [76,77]. It was established that by reciprocally crossing Tartary and rice-Tartary buckwheats, and backcrossing rice-Tartary-type progeny with Tartary buckwheat, the non-adhering husk trait depends on a single recessive gene [78]. However, according to the new results from Duan et al. [79], three genes regulate the trait of easily husked Tartary buckwheat.

In crossing, hot water emasculation was used. The hybrid rice-Tartary buckwheat cultivar Mikuqiao18 was obtained in China through the pedigree selection of crossbreeding 'Miqiao' with 'Jingqiaomai2' [77]. The hybrid had a lower yield than parent varieties because the grain mass was lower due to the thinner husk.

In Tartary buckwheat grain, the most valuable substances for good nutritional value and the maintenance of human health are cotyledons. The increased size of cotyledons is important for a high content of proteins and polyphenols. It is not yet clear how to select plants to achieve this goal. One possibility would be to evaluate the size of cotyledons through the transverse sections of grain. Another possibility would be to estimate the size of cotyledons through the evaluation of milling fractions. Contrary to the situation in common buckwheat, in Tartary buckwheat all the grain on a given Tartary buckwheat plant is genetically uniform because of self-fertilization. So, a certain grain from a Tartary buckwheat plant would be, genetically, a representative sample for other seeds from the same plant.

It has been established that the expression levels of genes for phenylalanine ammonia lyase (PAL) and 4-coumaric acid coenzyme A ligase (4CL) were positively correlated with the content of flavonoids in Tartary buckwheat [80]. This finding suggests the possibility for breeding Tartary buckwheat with stable or enhanced flavonoid content, which is very important for the utilization value of Tartary buckwheat.

Other important quality traits are plant height, leaf blade width, stem color (green or red), number of primary branches, inflorescence length, flower color (greenish-yellow, white, pink, red), seed anthocyanin color (green, brown, black), seed shape (ovate, with sharp edges or with growths) and seed weight. These accessions can be of vital significance for future buckwheat breeding programs.

Traditional breeding and selection methods are chiefly concentrated on visible properties, including plant morphology and yield. The changes in metabolic profile cannot be observed by the methods usually used. Research on changes in the metabolite profile during domestication and breeding efforts is very important. Metabolome profiling in Tartary buckwheat will make genetic improvements of traits possible in Tartary buckwheat, which is important for medical use and resistance against diseases [81].

Tartary buckwheat is mainly tolerant to pests and plant diseases. In any case, strains of *Rhizoctonia solani*, a soil-born pathogen, may damage the plants [82]. Using a multiomic approach, it is feasible to identify genes related to resistance against this pathogen in Tartary buckwheat. This finding could accelerate the molecular breeding of *Rhizoctonia*-resistant cultivars in Tartary buckwheat [82]. Regarding climatic changes, obtaining Tartary buckwheat cultivars that are tolerant to drought is essential. Relevant genes and regulatory systems connected to drought tolerance have been reported in Tartary buckwheat [83].

The transformation and genome editing of common and Tartary buckwheat have some restrictions in their application [84]. In several European countries (for example, Austria, Czech Republic, and Italy), common buckwheat and Tartary buckwheat are grown and used in nutrition as ecological crops. According to the official demands for ecological crops, the use of transgenic cultivars is not allowed. Moreover, transgenic cultivars should not be cultivated close to ecological crops, to prevent the pollination of ecologically grown plants with the pollen of transgenic plants [84].

5. Future Perspectives

Due to its excellent nutritional value and its adaptability to adverse climatic and soil conditions, Tartary buckwheat undoubtedly has a future. Above all, it is necessary to increase the yield. Care must be taken to ensure that entire yield is harvested on the fields, i.e., it is necessary to improve the resistance of Tartary buckwheat to weather incidents, to prevent lodging and losing seeds through shattering. The starting point for the successful breeding of Tartary buckwheat is genetic material that must be collected and used in breeding.

The genetic material of Tartary buckwheat is available from diverse sources. The starting material for breeding could be the domestic populations of Tartary buckwheat grown by farmers, especially in areas of the Himalayas. The starting genotypes for successful Tartary buckwheat breeding could also be wild plants, and weedy Tartary buckwheat plants growing in the fields among the plants of common buckwheat. Weedy Tartary buckwheat plants are already adapted to grow in cultivated fields. Within Tartary buckwheat populations there is a great diversity and polymorphism, so selection could be very effective.

To increase the diversity and accelerate breeding, induced mutations are feasible, as well as obtaining tetraploids or crossing Tatary buckwheat plants with plants of other, related species.

When breeding for increased yield, care must be taken to maintain and improve the resistance of plants against diseases, pests and weather disasters. Care should also be taken to preserve the excellent nutritional value of Tatary buckwheat, especially its high content of flavonoids, other polyphenolic substances, proteins and fiber.

6. Conclusions

Tartary buckwheat grain and sprouts are rich in flavonoids rutin and quercetin. After crushing Tartary buckwheat grain and mixing the obtained material with water, the rutin-degrading enzyme of the grain starts its activity of decomposing the rutin molecules, as the result the concentration of rutin becomes lower and the concentration of quercetin becomes higher.

Studies have shown the effects of flavonoids rutin and quercetin on the human body, including the brain, producing promising results. Rutin and quercetin have been shown to have anti-inflammatory effects, which are believed to play a role in various neurological conditions, including depression, anxiety, and Alzheimer's disease. Despite the promising results, further research is needed to completely understand the effects of rutin and quercetin and to determine the optimal dose for long-term use. Tartary buckwheat is a valuable food source of flavonoids rutin and quercetin and other phenolic metabolites. However, maintaining a balanced diet is most important, and relying on a single food as the main source of nutrients should be avoided.

Tartary buckwheat is a self-fertile plant, so mutation breeding is feasible. Few generations after mutagenic treatment, homozygotes for mutated genes could appear and mutated traits would be visible for selection. Another breeding method is screening Tartary buckwheat varieties and wild populations for desirable traits, and using hybridization to introduce the desirable traits to the target cultivars.

The main goal of Tartary buckwheat breeding is suggested to be maintaining and enhancing the excellent properties of flavonoid content and the high nutritional value of proteins.

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