Breeding buckwheat for nutritional quality

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Common buckwheat (Fagopyrum esculentum Moench, CB) and Tartary buckwheat (Fagopyrum tataricum (L.) Gaertn., TB) are used in human nutrition. The idea to screen in the haploid phase for genes affecting low amylase concentration opens the possibility for the effective search of low amylase (waxy) genotypes in CB populations. Self-pollinated homozygous plants of TB might allow us to use a part of endosperm for screening of amylase content. Phenolic substances have a significant inhibitory effect on the digestion of CB and TB proteins, thus metabolites may have impact on protein digestibility. Digestion-resistant peptides are largely responsible for the bile acid elimination. Breeding to diminish polyphenols and anti-nutritional substances might have negative effects on the resistance of plants against pests, diseases and UV-radiation. Bread and pasta are popular CB and TB dishes. During dough making most of CB or TB rutin is degraded to quercetin by rutin-degrading enzymes. The new trace-rutinosidase TB variety makes possible making TB bread with considerable amount of rutin, preserving the initial rutin from flour. Breeding CB and TB for larger embryos would make it possible to increase protein, rutin, and essential minerals concentration in CB and TB grain.

Key Words: flavonoids, low-amylose, quercetin, recessive genes, rutin, waxy starch.

Introduction

Two buckwheat species, common buckwheat (Fagopyrum esculentum Moench, CB) and Tartary buckwheat (Fagopyrum tataricum (L.) Gaertn., TB) are used to make various foods and dishes. In Japan, China and Korea, CB and TB are used mostly to prepare noodles and other pasta products. In Italy, CB flour is used to prepare pasta, and in Slovenia and Austria, traditional dishes are CB and TB porridge (žganci) (Fig. 1A, 1B), and bread (Bonafaccia et al. 2003a, Costantini et al. 2014, Kreft et al. 2007, Lukšič et al. 2016, Vogrinčič et al. 2010). French CB pancakes (galettes) and Russian blini are popular the world over (Fig. 1C, 1D). In South Korea, TB sprouts are a new vegetable, used for salads and smoothies (Kim et al. 2004, 2008, Park et al. 2000). In Korea, TB is recently used for a non-alcoholic drink, in Japan, Korea and China, CB is used for strong drinks (in Japan soba shochu), and in Luxembourg, Germany, Slovenia and Italy, attempts are underway to make a beer-like CB or TB drink. CB groats dishes are very important in Slovenia, Croatia, Poland, Ukraine, Belarus and Russia, in Slovenia groats are made also from TB.

Japan, China, Korea, Poland, Ukraine, Belarus, Russia, and Slovenia have a long-standing tradition of breeding CB, and many new varieties were released, chiefly those of CB, but also some new varieties of TB. The utmost attention in CB and TB breeding was given to secure high and stable yields of grain in specific environmental and agricultural conditions. Some attention was focused, especially in the case of TB, on the breeding which would facilitate husking and provide pleasant taste. Fewer efforts have been devoted to improve the nutritional quality of CB and TB by breeding.

Nutritional importance of polyphenol-protein interactions

CB and TB proteins have a very well-balanced amino acid composition (Eggum et al. 1981, Javornik et al. 1981). Differences between amino acid compositions of CB samples,
and between CB and TB, are not considerable (Bonafaccia et al. 2003b). Javornik and Kreft (1984) established some differences between solubility fractions in the amino acid composition, but these differences are not great. As lysine content is higher in albumins and globulins, these fractions contribute to the well balanced amino acid composition of CB and TB, breeding to change the proportion of solubility fractions could thus somewhat improve the overall amino acid composition of proteins (Javornik and Kreft 1984). CB and TB breeding aimed to produce bigger embryos could be a promising possibility to increase the concentration of proteins and to improve their quality.

CB and TB proteins have a low digestibility (Eggum et al. 1981). Polyphenols, naturally present in CB and TB, lower the true digestibility of proteins, but do not adversely affect the biological value of proteins (Eggum et al. 1981, Skrabanja et al. 1998, 2000). As reported by Ikeda et al. (1986), phenolic substances have a significant inhibitory effect on the in vitro peptic and pancreatic digestion of globulin, thus CB and TB secondary metabolites may have impact on protein digestibility. Considerable interaction between polyphenols and proteins was observed after hydrothermal treatment (Skrabanja et al. 2000).

It was stated by Annor et al. (2017) that protein digestibility in millet is slower comparing to other cereals, may be because of binding of polyphenolic substances to proteins. Similar explanation can be suggested for CB and especially TB, having higher concentration of low molecular mass phenolic substances (for example rutin and quercetin) in comparison to cereals. The interaction between phenolic substances and proteins reduces the digestion of proteins through the small and large intestine. However, microbial processes in the colon enhance the digestibility of protein otherwise blocked by polyphenols in hydrothermally processed CB (Skrabanja et al. 1998, 2000).

CB and TB proteins can reduce the concentration of cholesterol in the serum by increasing the fecal excretion of steroids, which is induced by the binding of steroids to undigested proteins (Wieslander et al. 2011, 2012). Digestion-resistant peptides are largely responsible for the bile acid elimination. CB proteins have been reported to prevent gallstone formation more strongly than soy protein isolates, and they may slow mammary carcinogenesis by lowering serum estradiol, as well as suppress colon carcinogenesis by reducing cell proliferation (Tomotake et al. 2000). These effects are most probably connected with the limited digestibility of CB proteins.

CB and TB proteins are mainly connected in the embryo, this is indicated by the location of sulphur in the embryo and by the distribution of nutrients among milling fractions (Chetry et al. 2018, Pongrac et al. 2013, 2016, Skrabanja et al. 2004, Vombergar and Luthar 2018). Breeding CB and TB for larger embryos would make it possible to increase protein concentration in grain. As rutin and minerals are collocated with proteins (Skrabanja et al. 2004), breeding CB and TB for larger embryos would have an impact on the increase of rutin, protein and minerals.

It could be expected that breeding CB and TB for lower content of polyphenols could enhance the nutritional quality of proteins, this, on the other hand, could be unfavorable for the desired effects of polyphenol-protein complexes (Skrabanja et al. 2000, Wieslander et al. 2012). Here we should bear in mind that polyphenols are responsible for the protection of CB and TB plants against diseases, pests and UV radiation (Germ et al. 2013). Breeding to diminish polyphenols might have negative effects on the resistance of plants.

**Starch, amylose and amylpectin**

CB flour contains about 70–90% of starch, depending on the sample and milling method (Skrabanja et al. 2004). The amylose part of starch is the basis for the formation of retrograded starch during the hydrothermal processing of CB materials (Skrabanja et al. 2001). CB groats were found to be a prebiotic food because they can affect the increase of lactic acid bacteria in the intestine due to the presence of resistant starch (Skrabanja et al. 1998, 2001).

CB and TB are known to have relatively small starch granules, and the amylose content in starch granules is higher than that of cereals (Gao et al. 2016, Gregori and Kreft 2012, Skrabanja and Kreft 1998). Starch in TB has amylose content close to 39%, in addition to a high flavonoid content, indicating that TB has the potential to be exploited for the production of functional foods with a low glycemic index (Gao et al. 2016). In regard to amylose content, there are differences among varieties (Gao et al. 2016), so it is possible to alter the amylose concentration by breeding for the desired size of starch granules. CB and TB have, in comparison to cereals, much smaller starch granules, this could be a starting point for breeding aimed at even smaller size of starch granules and for the production of fat replacers (Gao et al. 2016). However, obtaining a
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low amyllose and high amylopectin content in CB or TB could be a challenge, as it would require obtaining varieties suitable for making cakes and other products with desired eating qualities. Waxy (amylose-free) mutants are known to lack an effective starch granule-bound protein, known as waxy (Wx) protein, connected with amylose biosynthesis (Chrungoo et al. 2016). According to Hung et al. (2006), the development of waxy and high-amylose wheats with varying amylose contents is contributing to produce diverse noodle types with desired eating qualities. Similar variation in noodle qualities could be expected in the case of waxy CB, if the characteristic could be stable through generations. As waxy mutants are expected to be recessive in comparison to non-waxy alleles, a search for a waxy form was performed by investigating starch in CB pollen grains. The haploid phase of pollen grain is expected to have a full expression of recessive genes (Gregori and Kreft 2012). This type of mutants is known in rice and in several other cereals, but not yet in CB or TB. The search for the mutant endosperm has so far produced no result, as the impact of the recessive low amylose mutated gene could be in diploid CB covered by a dominant normal allele. The search for this type of mutation by screening a haploid pollen grain has resulted in finding a low amylose type in a population of Slovenian CB (Gregori and Kreft 2012). In the studied waxy material, the plants had many defective pollen grains (some slightly smaller than normal) and defective endosperm, so there were problems in propagation and maintenance of this genetic material. Crossing within the progeny of plants with the mutated gene resulted in grain with very low amyllose content, but endosperm was poorly developed and in most cases not able to support the germinating embryo with enough amount of assimilates (Gregori and Kreft 2012).

The idea to screen in the haploid phase for genes affecting low amyllose concentration opens the possibility for the effective search of low amyllose (waxy) CB genotypes in CB populations. If so, it would be suitable to use one of the best local varieties as a starting material, to avoid too many backcrossing generations afterwards, for getting the desired gene in a suitable high yield locally adapted variety. On the other hand in self-pollinated homozgyous plants of TB, we would utilize a part of endosperm for screening of amyllose content, and directly use the rest of the seed for further propagation in vitro, or by embryo rescue method. This would be a suitable method of breeding for waxy TB.

Rutin, rutinosidase and quercetin

CB and TB are important sources of antioxidant activity in functional foods (Gaberščik et al. 2002, Holasova et al. 2002, Kreft 2016, Matsui et al. 2018, Pexová Kalínová et al. 2019, Zhang et al. 2018). CB and TB products decrease the level of cholesterol, reduce fatigue symptoms and improve lung capacity in humans (Sidker et al. 2014, Wieslander et al. 2011, 2012, Yang et al. 2014). CB and TB extracts can also protect DNA from damage caused by hydroxyl radicals (Vogrinčič et al. 2010). DNA protecting effects of CB and TB extracts are not attributed solely to rutin or quercetin, but also to a spectrum of CB and TB grain constituents; flavonoids, however, are one of the main factors (Vogrinčič et al. 2013).

Flavonoids (rutin, quercetin) are thus of special interest when it comes to CB and TB grain and products. Rutin tends to prevent flour deterioration in CB and TB (Suzuki et al. 2005a). According to Suzuki (2016), rutin is involved in the reactions, important in generating volatile compounds of boiled CB noodles (soba). Some volatile compounds found in his experiment are important contributors to the unique flavor of CB noodles (soba). Enzymatic activity in flour is important for flavor generation of boiled CB noodles whereas rutin does not have important function. Suzuki (2016) suggests that it is a need to develop the variety with enhanced flavor, but not easily exposed to the deterioration.

Rutin is important for protecting CB and TB plants from solar UV radiation, cold, desiccation and pests (Gaberščik et al. 2002, Kreft et al. 2002, Suzuki et al. 2005b). The combination of high levels of rutin content and the activity of the rutin-degrading enzyme rutinosidase produces a strong bitterness after grazing, which protects CB and especially TB, from being eaten by animals (Suzuki et al. 2015a). Chittarrini et al. (2014) suggest that rutin-derived quercetin is more efficient in inhibiting aflatoxin biosynthesis by Aspergillus flavus than rutin.

During bread making most of rutin is degraded to quercetin by rutin-degrading enzymes (Yasuda and Nakagawa 1994), thus no rutin passes untransformed during the procedure and none remains in TB bread (Fig. 2) (Germ et al. 2019, Vogrinčič et al. 2010). By scalding TB flour with hot water, a considerable amount of rutin is conserved from flour through the process to the final bread product (Lukšič et al. 2016). Suzuki et al. (2002, 2014, 2015a, 2015b, 2015c) used new trace-rutinosidase TB variety to produce wheat-TB combined bread with considerable amount of rutin, preserving about 50% of the initial rutin from TB flour in the bread. Trace-rutinosidase TB may become more popular in Japan. Among Japanese consumers, TB dishes are generally less popular since they expect “sweet” taste of

![Rutin transformation to quercetin and rutinose.](image)
soba noodles. For the markets where customers prefer gentle taste of CB and TB dishes, TB could be transferred to improve the taste of TB varieties by backcrossing the new trace-rutinosidase TB.

In breeding CB and TB it is possible the use in vitro tissue culture methods to regenerate for example from one cotyledon several genetically identical regenerated plants (Luthar and Marchetti 1994). However, the possibility of regenerating plants from tissue cultures is in CB and TB limited by a high concentration of phenolic substances.

The possibility for breeding for high content of micro-elements

Differences in micro-elements distribution between CB and TB were studied by Bonafaccia et al. (2003a) and Ikeda et al. (2006). Research by Breznik et al. (2005) and Golob et al. (2015) suggested CB and TB, enriched with Se, as an important source of nutritionally Se. Pongrac et al. (2013, 2016) established that valuable essential elements, like Mg, P, S, K, Mn, Fe and Zn are located mainly in the embryo. To enhance their concentration by CB and TB breeding efforts, it could be feasible to breed for larger embryos. Cross sections of CB and TB grain are suitable to estimate the size of embryos (Kreft and Kreft 2000, Pongrac et al. 2013). Ca is concentrated in husk, allocation of Cu, which could be toxic in high concentrations due to polluted soil, is more evenly dispersed among tissues of TB grain (Pongrac et al. 2013).

Anti-nutritional factors concerning CB and TB breeding

CB and TB plants contain some fagopyrin, a phototoxic substance. It is mainly concentrated in green parts (sprouts, leaves) and less in grain (Kočevar Glavač et al. 2017, Kreft et al. 2013, Stojilkovski et al. 2013). The possibilities to avoid fagopyrin by breeding have not been studied yet. Fagopyrin could play a role in protection of plants against UV radiation, pests or diseases, so breeding for low concentration of fagopyrin may bring lateral, undesired effects.

CB and TB, like their relatives from the Polygonaceae family (like rhubarb, Rheum rhabarbarum L.), contains Ca oxalate druses and oxalic acid involved in the protection against solar radiation and against aluminum toxicity respectively (Golob et al. 2018, Klug and Horst 2010). According to Peng et al. (2002), there is a genotypic difference in aluminum resistance and oxalate exudation in CB. Genotypic differences could be the starting points for breeding for low content of oxalates in CB and TB. However, breeding aimed to reduce the concentration of oxalic acid may result in sensitivity of CB and TB plants to solar radiation or aluminum toxicity. Among anti-nutritional factors are also some metabolites with inhibitory potency against enzymes, like dietary fiber, tannins, phytates, and a protein protease inhibitor (Ikeda et al. 1986). In a study of impact on the in vitro pepsin-pancreatin digestibility of proteins, the protein inhibitor exhibited the highest inhibitory capacity among the substances examined, while that of phytate was the lowest (Ikeda et al. 1986).

Standard TB variety flour exhibits a high level of α-glucosidase inhibitory activity, whereas the newly developed cultivar ‘Manten-Kirari’ with a low level of a rutin-degrading enzyme exhibits no α-glucosidase inhibitory activity. Quercetin, present in TB grain, may be a factor, responsible for α-glucosidase inhibitory activity (Ikeda et al. 2017). These effects are interesting in regard to diabetes mellitus prevention (Ikeda et al. 2017). Quercetin may be a factor responsible for the ability of TB to lower blood sugar in patients suffering from diabetes mellitus. This research is important in the development of more powerful antidiabetes drugs and wider utilization of TB, already used in the diet of diabetic patients’ prevention (Ikeda et al. 2017, Li et al. 2009, Ren et al. 2018). TB sprouts, leaves and even TB roots extracts have a potential to be utilized as a functional food for preventing diabetes due to α-glucosidase inhibitory activity. Glucosidase inhibitors are used as drugs to treat diabetes (Li et al. 2009, Ren et al. 2018, Sharma et al. 2012).

Enzyme inhibitors lower digestibility of proteins or carbohydrates. Removal of these “anti-nutrients” by breeding would improve digestibility of metabolites in CB and TB grain, on the other hand, it could harm other, desired nutritional effects of CB and TB flour (like suppressing gall-stone formation, lowering plasma cholesterol, preventing diabetes mellitus).

Conclusions, challenges and prospects for the future

It is very important to collect CB and TB samples from around the world, in countries and places where domestic varieties and wild relatives still exist. Cross-fertilized populations, like CB, are genetically and phenotypically very heterogenous. In screening CB genetic resources for valuable recessive genes, it is important to take into account that recessive genes could be hidden in the population as a part of heterozygotes. CB and TB breeding was in many places not very intense, so there could still exist in populations the genes involved in synthesis of valuable nutrients. It is necessary to evaluate populations and find valuable genes for breeding CB and TB, including breeding for improved nutritional value of CB and TB. This holds true for TB, though it is expected that plants are homozygous because of selfing and that valuable properties are expressed. However, due to less rigorous breeding of TB, many varieties are a group of self-pollinating lines. In such a mixture, it is difficult to find nutritionally valuable properties with bulk analyses, it is better to isolate lines from the populations and screen them for valuable genes individually.

It is expected that in future more attention will be focused on breeding for nutritional quality of CB and TB.
Nutritional quality of CB and TB is a result of complex interactions between genes, and as well between primary and secondary plant metabolites.

**Author Contribution Statement**

I.K. initiated and coordinated the writing, wrote part on polyphenol-protein interactions, and conclusions, prepared the final version of the manuscript. M.Z. wrote the parts on micro-elements, anti-nutritional factors, breeding, and contributed remarks to other parts. A.G., M.G., M.L and K.D. wrote the abstract, introduction, part on rutin, rutinosidase, quercetin, and coordinated cited literature. Z.L. was leading the writing, wrote the part on starch, amylose, amyllopectin, recessive genes, and contributed the figures.

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