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Research review paper

Strategic enhancement of genetic gain for nutraceutical development in buckwheat: A genomics-driven perspective

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ABSTRACT

Buckwheat (*Fagopyrum* spp.) under the family Polygonaceae is an ancient pseudocereal with stupendous but less studied nutraceutical properties. The gluten free nature of protein, balanced amino acid profile and health promoting bioactive flavonoids make it a golden crop of future. Besides a scanty basic research, not much attention has been paid to the improvement of plant type and breeding of nutraceutical traits. Scanning of scientific literature indicates that adequate genetic variation exists for agronomic and nutritional traits in mainstream and wild gene pool of buckwheat. However, the currently employed conventional approaches together with poorly understood genetic mechanisms restrict effective utilization of the existing genetic variation in nutraceutical breeding of buckwheat. The latest trends in buckwheat genomics, particularly availability of draft genome sequences for both the cultivated species (*F. esculentum* and *F. tataricum*) hold immense potential to overcome these limitations. Utilizing the transgenic hairy rot cultures, role of various transcription factors and gene families have been deduced in production and biosynthesis of bioactive flavonoids. Further, the acquisition of high-density genomics data coupled with the next-generation phenotyping will certainly improve our understanding of underlying genetic regulation of nutraceutical traits. The present paper highlights the application of multilayered omics interventions for tailoring a nutrient rich buckwheat cultivar and nutraceutical product development.

1. Introduction

Diversifying food resources through incorporation of ancient pseudocereals possessing unparalleled nutritive value and nutraceutical properties in modern cropping systems is one potential strategy to combat the hidden hunger (Boukid et al., 2018). Buckwheat (BW, *Fagopyrum* spp.) is one such pseudocereal valued for its centuries-old historical cultivation and immense nutraceutical potential. By virtue of its wide adaptability to fragile hilly, tribal and marginal ecosystems, it has been treated as a subsistence and security crop for a long time in

these areas. Among the 26 known species of genus *Fagopyrum* (Table 1), *F. esculentum* (common buckwheat) and *F. tataricum* (tartary buckwheat) are the two cultivated species (Fig. 1) predominantly diploid with 16 chromosome pairs (Joshi et al., 2019). Tartary buckwheat (TB) is mainly cultivated in the highlands of China and Himalayas, whereas common buckwheat (CB) is widely distributed throughout the world (Ohsako and Ohnishi, 2000). Subsequently, BW has been rekindled with its potential as a nutraceutical crop for various food formulations in recent decades (Fig. 2). Additionally, bioactive compounds with therapeutic properties of diverse pharmaceutical uses also make it a

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Table 1
List of various cultivated and wild species of genus *Fagopyrum*.

No.	Latin name	Ploidy	Notes	Reference
1	<i>F. esculentum</i>	2n = 2 × = 16	Cultivated species	Li and Suk-pyo (2003)
	<i>F. esculentum</i> ssp. <i>ancestralis</i>		Subspecies	Ohsako and Ohnishi (2000)
2	<i>F. tataricum</i>	2n = 2 × = 16	Cultivated species	Li and Suk-pyo (2003)
	<i>F. tataricum</i> ssp. <i>potanini</i>		Subspecies	Ohsako and Ohnishi (2000)
3	<i>F. statice</i>	2n = 2 × = 16	Species	Li and Suk-pyo (2003)
4	<i>F. urophyllum</i>	2n = 2 × = 16	Species	Li and Suk-pyo (2003)
5	<i>F. caudatum</i>	2n = 2 × = 16	Species	Li and Suk-pyo (2003)
6	<i>F. gilesii</i>	2n = 2 × = 16	Species	Li and Suk-pyo (2003)
7	<i>F. lineare</i>	2n = 2 × = 16	Species	Li and Suk-pyo (2003)
8	<i>F. gracilipes</i>	2n = 4 × = 32	Species	Li and Suk-pyo (2003)
	<i>F. gracilipes</i> var. <i>odontopterum</i>		Variety	
9	<i>F. leptopodium</i>	2n = 2 × = 16	Species	Li and Suk-pyo (2003)
	<i>F. leptopodium</i> var. <i>grossii</i>		Variety	
10	<i>F. cymosum</i>	2n = 2 × = 16	Synonyms: <i>F. dibotrys</i>	Li and Suk-pyo (2003)
	<i>F. pilus</i>	2n = 4 × = 32	<i>F. cymosum</i> complex	Chen (1999)
	<i>F. megaspartanum</i>	2n = 2 × = 16	<i>F. cymosum</i> complex	Chen (1999)
		2n = 2 × = 16		
11	<i>F. zuogongense</i>	2n = 4 × = 32	Species	Chen (1999)
12	<i>F. homotropicum</i>	2n = 2 × = 16	Species	Ohsako and Ohnishi (2000)
		2n = 4 × = 32		
13	<i>F. pleioramosum</i>	2n = 2 × = 16	Species	Ohsako and Ohnishi (1998)
14	<i>F. callianthum</i>	2n = 2 × = 16	Species	Ohsako and Ohnishi (1998)
15	<i>F. capillatum</i>	2n = 2 × = 16	Species	Ohsako and Ohnishi (2000)
16	<i>F. jinshaense</i>	2n = 2 × = 16	Species	Ohsako et al. (2002)
17	<i>F. gracilipedoides</i>	2n = 2 × = 16	Species	Ohsako et al. (2002)
18	<i>F. rubrifolium</i>	2n = 2 × = 16; 2n = 4 × = 32	Species	Ohsako and Ohnishi (1998)
19	<i>F. macrocarpum</i>	2n = 2 × = 16	Species	Ohsako and Ohnishi (1998)
20	<i>F. crispatifolium</i>	2n = 4 × = 32	Species	Liu et al. (2008)
21	<i>F. pugense</i>	2n = 2 × = 16	Synonyms: <i>F. densovillosum</i>	Tang et al. (2010)
22	<i>F. qiangcai</i>	2n = 2 × = 16	Synonyms: <i>F. polychromofolium</i>	Shao et al. (2011)
23	<i>F. wenchuanense</i>	2n = 2 × = 16	Species	Shao et al. (2011)
24	<i>F. luojishanense</i>	2n = 2 × = 16	Species	Hou et al. (2015)
25	<i>F. hailuogouense</i>	2n = 2 × = 16	Species	Zhou et al. (2015a)
26	<i>F. longzhoushanense</i>	2n = 2 × = 16	Species	Wang et al. (2017a)

valuable crop. Based on the background, BW research has attracted more and more attention in the last decades, and the major advances have been made to project it as an economically viable crop of modern agroecosystems.

In the present work, we attempted to emphasize the unparalleled nutritive value and importance of BW biofortification in the context of emerging global nutritional and health scenario. We have also explored the nutraceutical properties and provided a brief account of various environmental factors, agrotechniques and processing methods affecting the nutritive value of BW. In the end, we have specifically highlighted the role of emerging omics and biotechnological interventions for enriching nutraceutical developments in BW. We believe this review could provide a reference for the study of metabolic mechanism and molecular breeding of BW in the future.

2. The fundamentals of buckwheat

Generally, the BW plant is a tall, annual herb that can grow up to 0.5–2.5 m height. Of the two cultivated species, common BW is well adapted to lower elevations (< 1000 m), while TB performs well in the higher hills (> 2500 m) (Rana et al., 2016). Additionally, the two species exhibit variation for inflorescence characteristics and seed shape, and can be distinguished by floral characteristics alone. CB is an auto-incompatible species having dimorphic flower, either a pin type (long pistil and short stamen) or thrum type (short pistil and long stamen) flowers, whereas TB is a self-compatible species bearing homomorphic flowers. CB seeds are generally triangular in shape, whereas TB is characterized by ovate or broadly ovate and surfaces grooved seeds.

Although nutraceutical potential of BW has gained global prominence recently, the historical evidence of its cultivation could be traced

back to first and second century BCE in China (Tang et al., 2016). Most of the BW species have both the centre of origin and diversification in southwest China (Ohsako and Ohnishi, 2000). The centre of origin of *F. esculentum* is Eastern Tibetan plateau and Yunnan province in China, while the Eastern Tibet or North-western Yunnan is the centre of origin of *F. tataricum* (Konishi et al., 2005). The rest of the BW species are believed to originate from North-western Sichuan province in China (Ohsako and Ohnishi, 2000). Largest BW producers are Russia, China, and Ukraine respectively (FAOSTAT, 2018). The production and area under BW cultivation are continues to decline compared to major food crops globally (Fig. 3). Lack of crop improvement efforts is a plausible reason for the decline of BW production, compared to the major cereals.

Taxonomically, genus *Fagopyrum* is placed under the order Caryophyllales of family Polygonaceae. It includes both diploid and tetraploid species (Table 1) with a basic chromosome number of 8 (Ohsako and Ohnishi, 2000). The genome size of two cultivated species *F. esculentum* and *F. tataricum* is ~1.2 Gbp (Yasui et al., 2016) and ~0.48 Gbp (Zhang et al., 2017a) respectively, which is very small as compared to maize (~2.4 Gbp) and wheat (~16.8 Gbp). The taxonomic position and interspecific relationships of the genus *Fagopyrum* is poorly understood due to the high phenotypic variation. The widely accepted classification separates the genus into two groups, *Cymosum* group with big achene and *Urophyllum* group with small achene (Ohsako and Ohnishi, 2000). This taxonomic classification was further validated through molecular phylogenetic studies (Zhou et al., 2014). To date, the ancestors of cultivated BW species are unknown, although morphological and molecular studies suggested *F. megaspartanum* and *F. pilus* as probable progenitors of *F. esculentum* and *F. tataricum*, respectively (Ohsako and Ohnishi, 2000). Recently, a new perennial species, *F. hailuogouense*, identified from Sichuan province of China and characterized by succulent and ligneous rhizomes with numerous

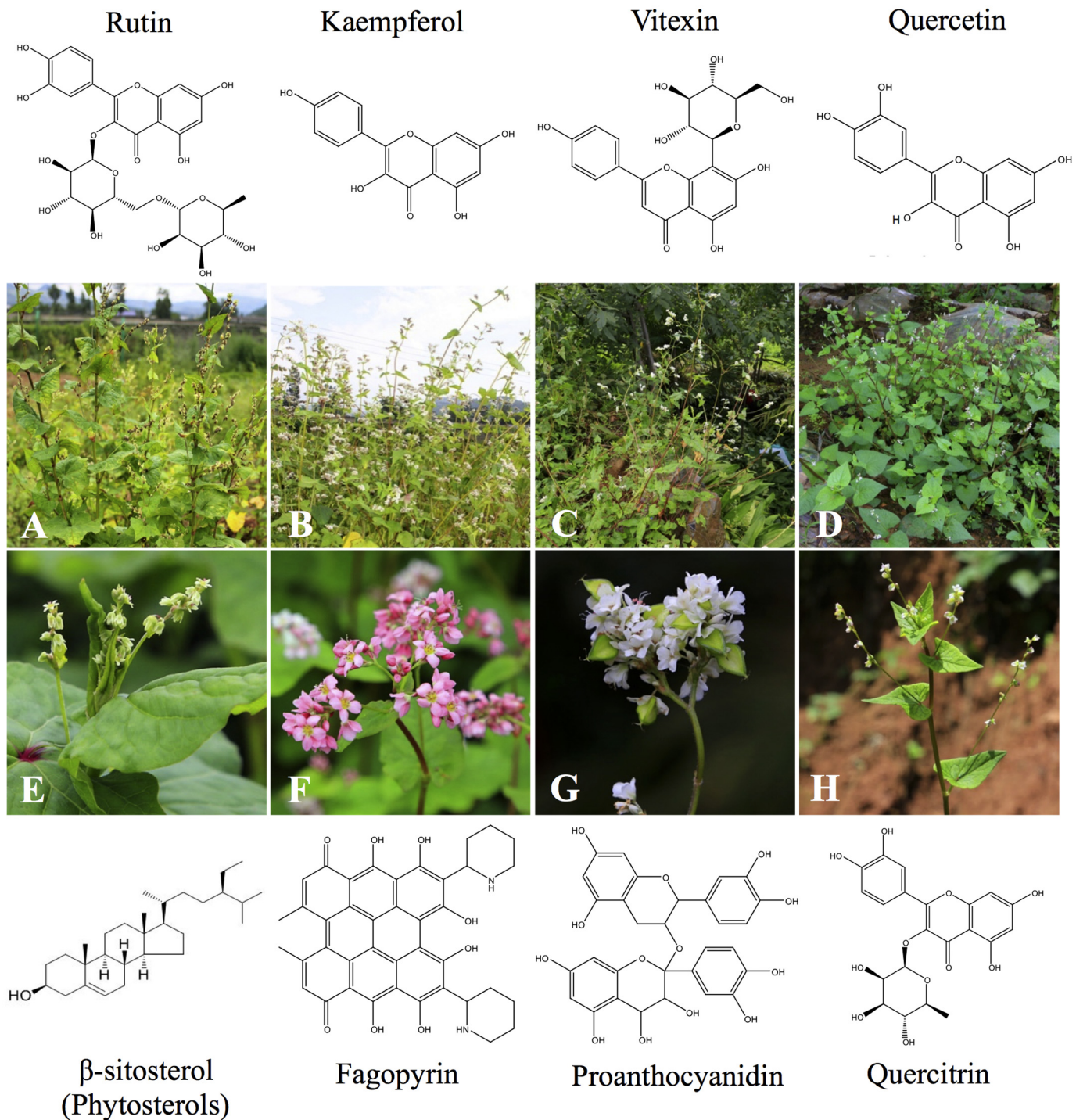


Fig. 1. Buckwheat is a promising pseudocereal for future agroecosystems.

From A to D: phenotypes of four buckwheat species (*F. tataricum*, *F. esculentum*, *F. cymosum* and *F. gracilipes* respectively); E to H: Inflorescence of four buckwheat species (*F. tataricum*, *F. esculentum*, *F. cymosum* and *F. gracilipes* respectively).

adventitious roots and smooth epicarp surface of the achene is expected to be the probable ancestor of BW and becomes another subdivision of the genus *Fagopyrum* (Zhou et al., 2015b).

3. Buckwheat is a nutritional powerhouse

With its unparalleled nutritive value and versatile adaptability, BW is emerging as a golden crop of modern agroecosystems, which have already been perceived the most nutritious compared with all major cereals in the world.

3.1. Proximate composition, minerals and vitamins

Similar to the protein quantity of most cereals, the protein content of BW ranged from 12.0 to 18.9%. However, BW contains well-balanced protein with 12 amino acids including lysine, arginine, cysteine, and methionine, especially the lysine (5.9 g/100 g of protein), which is considered as the limiting amino acid in majority of the cereal protein (Guo and Xiong, 2013; Ahmed et al., 2014). Among sulphur containing amino acids, BW grains are a good source of methionine (3.7 g/100 g of protein) and cysteine (2.6 g/100 g of protein) compared to all cereals

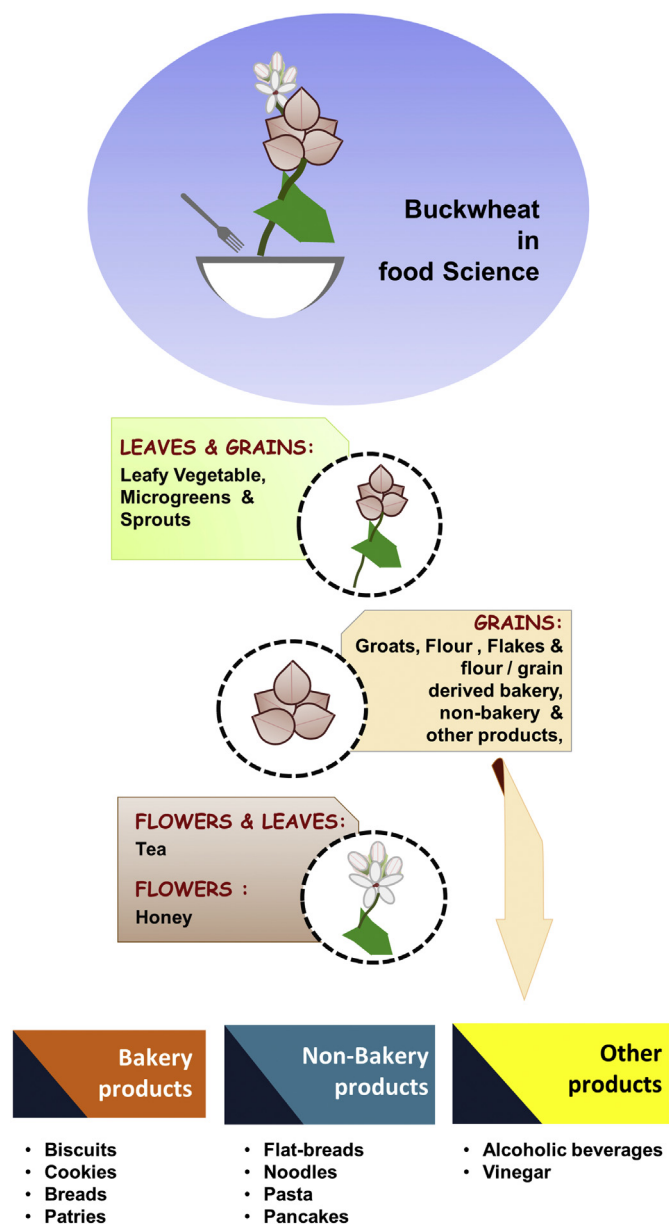


Fig. 2. Versatile applications of buckwheat in food science for addressing global health and nutrition scenario.

and pulses (Bonafaccia et al., 2003). Further, on the quality characteristics, ratios of lysine to arginine and methionine to glycine are significantly lower in BW protein than that of other plant proteins (Krkošková and Mrázová, 2005). Such rare plant proteins are widely recognized due to their cholesterol-lowering effects in blood and serve as a healthy food for patients suffering from hypercholesterolemia. BW proteins contain on an average 50–60% albumins and globulins, 11–20% glutelins and 1–7% of prolamins (Wei et al., 2003). The low proportion of prolamins and lack of α -gliadin protein contribute to the gluten-free nature of BW protein, which is the key specialty in comparison to the protein from wheat, barley, and oats (Gimenez-Bastida et al., 2015). In the gluten-free market, BW products such as bread, cookies, noodles, pasta, and beer with its uncompromised nutritional qualities, are popular among celiac patients across the world.

Compared with other cereals, BW seeds are stuffed with proportionately more starch and less fat. The starch of BW flour, which mainly concentrates from the central endosperm, is accounting 70–91% depending on different milling methods (Zhu, 2016). The amylose

content of TB is close to 39.0%, whereas the total amylose in the starch of CB could be up to 43% (Gao et al., 2016). Polyphenols and lipids in the BW flour can make non-covalent complexes with starch. These complexes inhibit the alpha-amylase activity resulting in the formation of amylase-resistant BW starch produced by heating (Skrabanja et al., 2001). Because of this unique characteristic of starch, BW has become a befitting diet part of hyperglycemic patients (Kawa et al., 2003). Among minerals, BW grains and sprouts are exceptionally rich in calcium (Ca), sulphur (S), and molybdenum (Mo) compared to other cereals and also contain potassium, iron and other trace elements in good amount (Pongrac et al., 2016). BW is a rich source of vitamins, particularly vitamin B1, B2, B3, B6, vitamin C and vitamin E. The BW flour is the better sources of B-group vitamins compared to the corn and rice flour. Generally, TB contains more vitamin B1, B2 and B3 than the CB, but holds comparably less vitamin E than the CB (Ikeda et al., 2006). Among the eleven different gluten-free flours, the highest concentration of vitamin B2 (0.22 mg/100 g of flour) is observed in BW (Rybicka and Gliszczynska-Świąto, 2017).

BW holds high dietary fiber, low calories and practically no fat. The dietary fiber of BW helps digestion improvement in humans. The soluble dietary fiber especially reduces the absorption of certain nutrients and at the same time slow down the glucose absorption. Normally, a serving of 100 g BW groats supplies 2.7 g of dietary fiber (USDA <https://ndb.nal.usda.gov/ndb/search/list>), which could ease the food transport through the digestive tract and regulate the bowel movement. The lipids in dehulled seeds of BW are neutral lipids (810–850 mg/g), phospholipids (80–110 mg/g) and glycolipids (30–550 mg/g). Maximum lipid contents are observed in embryo, while, the lowest concentration (4–9 mg/g) is in the BW hull (Joshi et al., 2019).

In nutshell, BW could be treated as a nutritional powerhouse because of its gluten-free protein, well-balanced amino acids, starch, vitamins, and mineral elements. The abundant genetic variations observed in crop germplasm provide ample scope for the selection of trait specific (nutrient-rich) genotypes for their inclusion in breeding programs. Hybridization of these nutritionally superior genotypes with agronomically well-adapted cultivars will help in BW biofortification through the development of nutrient-rich cultivars to combat hidden hunger, particularly in low-income food deficient countries. However, some aspects should be noticed and highlighted. An antinutritional factor, like protease inhibitors and tannins, present in BW seed coat is resistant to thermal processing and inhibits the digestibility of BW protein thereby hindering its efficient utilization and bioavailability. The excellent bioavailability of BW minerals is attributed to the formation of tightly bound complexes within phytate salts, flavonoids, and polyphenols compounds.

3.2. Bioactive flavonoids of buckwheat

The flavonoid compounds of BW contain a phenylbenzopyrone structure and possess a variety of biological activities. These flavonoids make BW a valuable dietary supplement.

3.2.1. Rutin and quercetin

BW contains six major flavonoids, rutin, quercetin, orientin, homoorientin, vitexin, and isovitexin, in its leaves, flowers, stem, and sprouts. Among all the six flavonoids, rutin has numerous bioactive properties, including anti-oxidant, anti-inflammatory, anti-hypertension, as well as gastric lesions protectant activities (Kreft et al., 2002). It also has an ability to suppress microglia activation thereby endowed it with neuroprotective effect exhibited on different animal models. Rutin is also known to prevent cognitive impairments like Alzheimer's disease by ameliorating oxidative stress (Javed et al., 2012). Unlike the major cereals and other pseudocereals, almost all parts of BW are good sources of dietary rutin, including seeds, leaves, and stems. Among the two cultivated species, rutin contents are five-fold higher in TB than CB. Additionally, compared with CB seeds, the TB contain comparatively

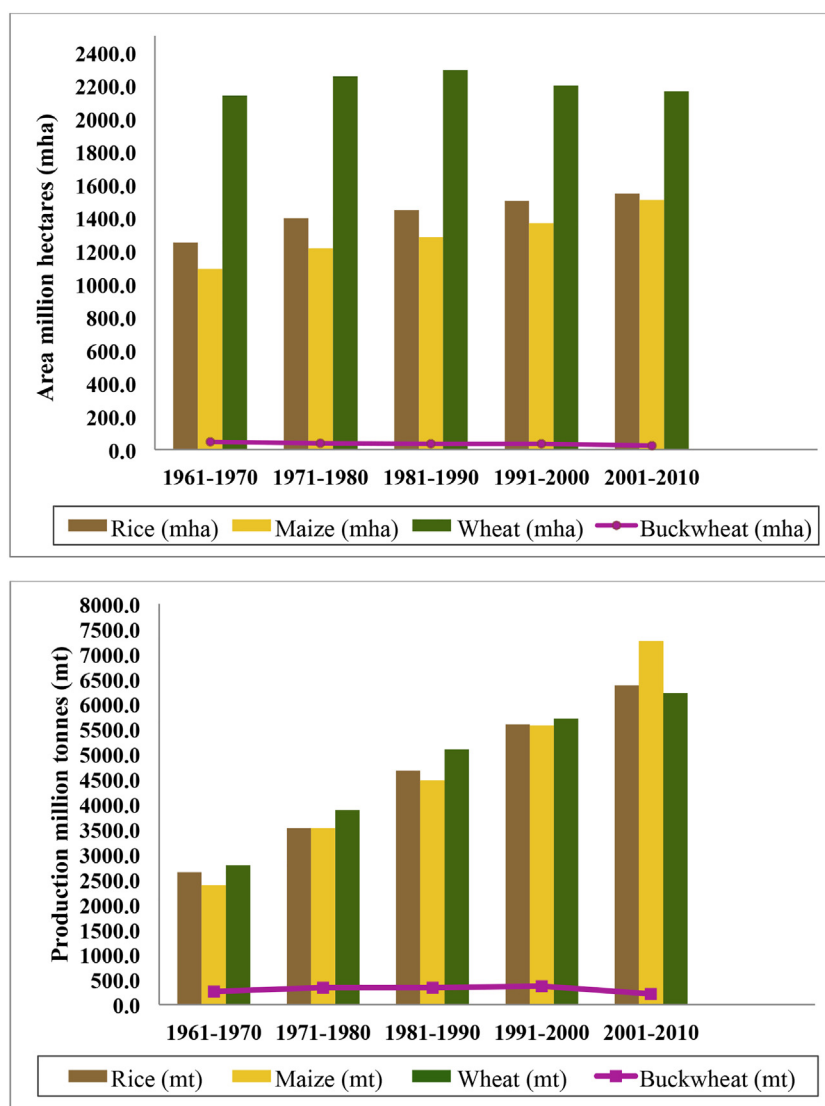


Fig. 3. Global trends in area and production of buckwheat compared to three major cereals. (FAOSTAT, 2018.)

more quercetin along with high levels of rutinoidase activity (Suzuki and Morishita, 2016). The extremely high concentration of rutin in TB provide strong bitterness to its flour and provide tolerance to UV radiation, chilling injury and insect pests (Suzuki et al., 2015). The maximum rutin content in TB sprouts could reach 109 mg/100 g fresh weight (Koyama et al., 2013). In contrast, the C-glycosylflavones such as orientin, vitexin, isoorientin, and isovitexin in CB are reported to be in higher concentration than those in TB (Seo et al., 2013). In general, BW grain contains a higher concentration of rutin than quercetin, however, the concentration of these two flavonoids could reverse during processing and heat treatment, due to the activity of rutin degrading enzymes. During processing, the conversion of rutin to quercetin is acceptable, because the quercetin is widely distributed in animal tissues and also possesses similar bioavailability and health effects like rutin (Sikder et al., 2014). Interestingly, quercetin becomes undetectable in BW sprouts on day seventh after germination, which should be noticed during the BW healthy micro-greens and sprouts processing (Ren and Sun, 2014).

3.2.2. Fagopyrin and fagomine

Fagopyrin is a phototoxic flavonoid, which accumulates in BW grains with the low concentration and hard to isolate. Characterization of BW fagopyrin through different spectrometric techniques like NMR,

UV-Vis absorption, and mass spectroscopy revealed presence of at least six fagopyrin derivatives, fagopyrin A to fagopyrin F (Tavčar et al., 2014). Among the three different species (*F. esculentum*, *F. tataricum*, and *F. cymosum*), the maximum fagopyrin content was observed in the flowers of *F. cymosum* (20,700 µg/100 g; Stojilkovski et al., 2013). The highest concentration of fagopyrin is observed during seed germination and the increment of fagopyrin is intense under the light condition because light is necessary to convert protofagopyrins into fagopyrins (Kreft et al., 2013).

D-fagomine in BW is a strong inhibitor of α -glucosidase and β -galactosidase in mammals and exhibits potential anti-hyperglycaemic effect for the diabetic treatment. It can also reduce the risk of pathogenic bacterial infection after dietary supplement (Amezqueta et al., 2012). CB contains more D-fagomine than the TB, and the highest concentration of D-fagomine was determined in BW grouts that could reach 44 mg/kg (Amezqueta et al., 2012).

3.2.3. Tannin and phenolic acids

Tannin has protection potential against a variety of biotic and abiotic stresses, which is the secret behind the consumption of large amounts of BW bran for nutritional or medicinal purposes (Steadman et al., 2001). Generally, the concentration of tannin rises up during the development of young BW seedlings. BW tannin functions by affecting

the metabolic pathway of bacterial enzymes, disturbing nutrient availability, and functionality of biological membranes. Tannins also have a range of pharmacological effects such as free radical scavenging activity, anti-cancer, anti-microbial as well as cardio-protective properties. For instance, the tannin isolated from rhizomes of *F. cymosum* is reported to have excellent anti-tumour and anti-oxidant effects (Barbehenn and Constabel, 2011). Apart from tannin, BW also contains natural polyphenolic antioxidants including gallic, gentisic, vanillic, caffeic, coumaric, ferulic, salicylic acid, which plays an important role in different metabolic diseases (Oniszczuk, 2016).

The above described numerous bioactive flavonoids of BW label it as a super food due to their profound beneficial effects on human health. However, flavonoid content of BW varies between different genotypes, tissues, developmental phases, growing conditions, and it also changes in response to the region of its cultivation (Zhou et al., 2017). For instance, *F. esculentum* hulls contain six flavonoids, while only rutin and isovitexin were isolated from its seeds (Dorota and Wieslaw, 1999). The rutin content in flowers were much higher than that in the leaves and stem (Dorota and Wieslaw, 1999). Therefore, it is necessary to detect the characteristics and distribution of these flavonoids and explore the efficient processing methods to enhance their content in BW based functional foods. Furthermore, key regulatory genes of flavonoids biosynthesis pathway still need to be identified from not only from the cultivated species but also in the wild relatives. The interaction between different regulatory factors and the links between different metabolic biosynthesis pathways still need to be deciphered.

4. Factors influencing the nutritional value of buckwheat

The nutritional profile and the accumulation of bioactive compounds of BW are influenced by various environmental factors and agricultural techniques. Therefore, critical emphasis has been placed on evaluating the effect of these factors on nutritional value of BW. It would be of great interest to have more detailed investigations to understand more clearly the interaction of agronomic practices with climate conditions, which along with genetic background, are the critical determinants of nutritive value and nutraceutical potential of BW. Likewise, for the development of food products with potential nutrient qualities, it is crucial to consider the effects of processing methods on the product quality itself and on the bioactive compounds of BW as well (Sytar et al., 2016; Table 2).

5. Buckwheat is the nature's basket

Studies have confirmed that diets based on pseudocereal grains such as amaranth, BW, and quinoa are preventive against several degenerative diseases such as myocardial infarction, diabetes and few types of cancers (Li and Zhang, 2001). In the case of BW, the anti-hemorrhagic properties of *F. esculentum* seeds have been widely recognized and documented in the British Herbal Pharmacopoeia (Zhou et al., 2015a). Similarly, the potent phytochemicals of *F. cymosum* and *F. tataricum* with immense medicinal importance are being utilized in Chinese medicine since time immortal (Joshi et al., 2019). The numerous bioactive compounds with potential therapeutic applications (Fig. 4) provide new horizons for nutraceutical developments from BW, although the efficacy of specific phytochemicals requires rigorous scientific studies before medical application. Nutraceutical properties of BW are discussed in details in the following sections.

5.1. Anti-oxidant and anti-aging properties

In the last couple of years, plant based dietary antioxidants has gained tremendous attention because of their proven role in the prevention of cellular oxidative stress to maintain a proper homeostatic balance (Kumar et al., 2016). BW grains, particularly the bran aleurone layer, contain large amount of various phenolic compounds and

Table 2

Composition of different nutritional compounds and their concentration in common and tartary buckwheat grains (Bonafaccia et al., 2003; Pongrac et al., 2016).

Nutrients	Common buckwheat	Tartary buckwheat
Proximate composition of flour (g/100 g)		
Protein	10.6	10.3
Ash	1.82	1.80
Fat	2.34	2.45
Starch	78.4	79.4
Diatery Fiber (Total)	6.77	6.29
Diatery Fiber (Soluble)	0.88	0.52
Diatery Fiber (Insoluble)	5.89	5.77
Amino acid composition of flour (g/100 g basis)		
Arginine	0.991	0.963
Cysteine	0.273	0.266
Glycine	0.609	0.592
Leucine	0.692	0.711
Lysine	0.584	0.618
Methionine	0.141	0.142
Fatty acid composition of flour (g/100 g basis)		
Palmitic	15.6	19.7
Linoleic	39.0	36.6
Linolenic	1.0	0.7
Saturated	20.5	25.3
Unsaturated	79.3	74.5
Unsaturated/Saturated	3.87	2.94
Vitamin B composition of flour (mg/g basis)		
Vit. B1	0.28	0.40
Vit. B2	0.14	0.28
Vit.B6	0.15	0.18
Mineral composition of flour (mg/100 g basis)		
Magnesium	213.0	280.0
Phosphorus	391.0	386.0
Sulphur	124.0	149.0
Potassium	507.0	628.0
Calcium	56.2	79.1
Manganese	1.1	2.57
Copper	0.45	0.64
Molybdenum	0.08	0.13

catechins, having strong antioxidant activity and protect the DNA damage and its pleiotropic symptoms caused by reactive oxidative stress (Quettierdeleu et al., 2000). Additionally, the linoleic acid's peroxidation inhibiting ability has also been reported from BW bran and hull (Heř et al., 2012). Among the three cultivated species, *F. tataricum* is known to possess the highest antioxidant properties due to the abundance of rutin and total flavonoids. Together with dietary fibers in the colonic lumen and other molecules in the gut epithelium, the phenolic compounds of BW provide protection against oxidative stress to animal body. A diet comprised of BW enhanced the activity of antioxidant enzymes like glutathione peroxidase and glutathione S-transferase in rats suggesting its protective role against cellular oxidative stress (Kim et al., 2012). BW based food products and extracts are reported to delay aging and related chronic disorders by strongly inhibiting the *in vitro* DNA damage and double strand breaks induced by reactive oxygen species (Zhou et al., 2012a). Thermal and other processing techniques are known to suppress the free radical scavenging activities of BW flavonoids except for rutin, which is known to exert strong antioxidant effect even after digestion (Bao et al., 2016).

5.2. Anti-neoplastic properties

Owing to rich in polyphenols, including hydrobenzoic acids, vanillic acid, syngiric acid, p-hydroxy-benzoic acid, and p-coumaric acids, BW grains have potential anticarcinogenic properties. Apart from flavonoids, lectin protein purified from TB seeds is also reported to induce apoptosis of human leukaemia cells (U937) in a dose dependent manner indicating its role in reducing the rate of spontaneous and induced

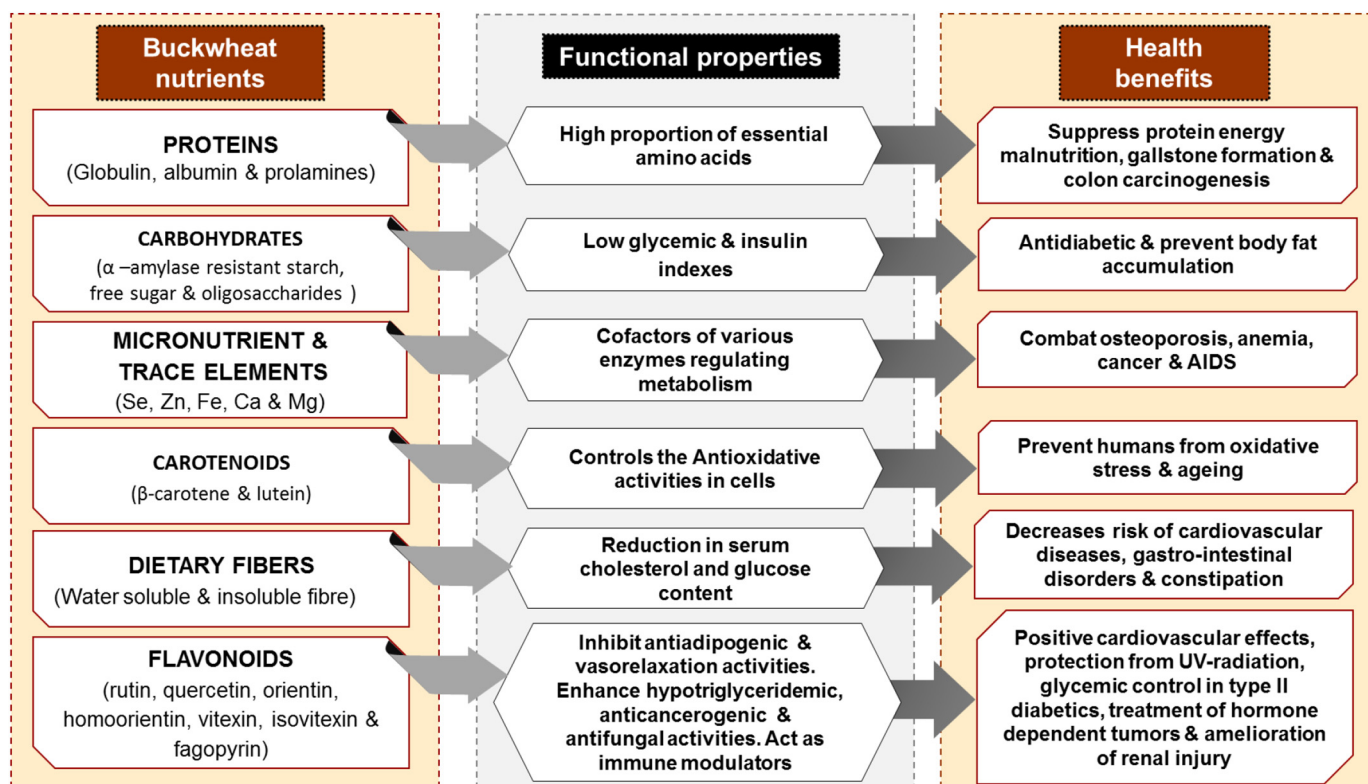


Fig. 4. Functional and nutraceutical properties of various bioactive compounds of buckwheat.

tumour proliferation (Bai et al., 2015). The MTT and flow cytometry assays confirmed that ethanolic extracts of *F. cymosum* rhizomes exert synergistic anti-carcinogenic effects on three carcinoma cell lines (CaEs-17, SGC-7901, and A549), when combined with extracts of *Rosa roxburghii* (Liu et al., 2012). Likewise, seven novel phenylpropanoid glycosides isolated from the extracts of *F. tataricum* roots has been found to have a potential cyto-toxic effect on cancer cell lines such as A-549, HCT116, ZR-75-30 and HL-60 implicating that these major constituents of BW roots may have anti-cancer properties (Zheng et al., 2012). Out of the seven potent glycosides, tatariside C (3) was found to be the most effective compound against the four tested cell lines (Zheng et al., 2012). Additionally, ethanolic extracts of BW sprouts that are specifically poisonous to cancer cell lines A549, AGS, MCF-7, Hep3B and Colo205 can prove an ideal natural bioactive chemotherapeutic agent for the treatment of different type of cancers without any negative pleiotropic effect (Sun et al., 2012). Likewise, chlorogenic acid (16 mg/100 g DW) and *p*-anisic acid (872 mg/100 g DW) isolated from the inflorescence of *F. esculentum* (green flowers) have been found to have strong blocking effect on tumour growth and proliferation (Sytar, 2015). Being rich in selenium (up to 0.1208 mg/g), an essential trace element that is specifically known for its anticarcinogenic and anti-HIV function, BW grains are ideal nutraceutical food for curing these life-threatening diseases (Zheng et al., 2011).

5.3. Anti-diabetic and anti-adipogenic properties

Diabetes and obesity are the chronic disorders of complex etiology, affecting a large proportion of human population worldwide. Plant based dietary interventions, enriched with bioactive compounds have emerged as a promising therapeutic tool against these diseases with negligible deleterious effects on human health. Of these, BW-based food formulations especially prepared from BW flour are known to have lower glycemic index and stabilize blood sugar level (Skrabanja et al., 2001; Stokić et al., 2015). The consequent elevation of glucose in the blood after diet could be effectively managed by inhibiting the activity

of two key enzymes (α -glucosidase and pancreatic amylase) regulating carbohydrate hydrolysis mechanism in the human body. Interestingly, the natural antioxidants of BW seed coat were reported to be the effective inhibitor of these enzymes (Bao et al., 2016). For instance, the antioxidant properties of CB-based diet were shown to improve the glycemic index of type II diabetes mellitus rats by decreasing the blood glucose and serum insulin level (Han et al., 2008). While the anti-nutritional factors such as tannin may also contribute toward anti-diabetic property of BW, the most widely known hyperglycaemic response is attributed to the presence of six different types of fagopyritols, which constitute the 40% soluble carbohydrate in its seeds (Steadman et al., 2000; Wu et al., 2018). Fagopyritols are galactosyl derivatives of D-chiro-inositol (DCI), which are known to act as insulin mediators, in the treatment of non-insulin dependent diabetes mellitus (NIDDM) (Wu et al., 2018). Of the six types of fagopyritols isolated from BW seeds, fagopyritol A1 is the most prominent fagopyritol, which is mainly present in bran section (Steadman et al., 2000) and known to lower the blood glucose level in rats (Obendorf et al., 2012). Thus, BW fagopyritols have the tremendous potential to emerge as a dietary treatment for reducing the symptoms of NIDDM, which is known to affect over 50% of the people in certain populations.

Obesity is often related to metabolic disorders including cardiovascular diseases (CVDs) and type II diabetes (Hill and Peters, 1998). TB extracts are known to inhibit the adipogenesis and exert hypolipidemic response, therefore, seems to be a prospective ingredient to obviate obesity-associated disorders in humans. For instance, the 70% ethanolic extracts of TB at the concentration of 100 μ g/100 ml are reported to reduce the intracellular triglycerides in 3T3-L1 adipocyte cells by 22.3% (Lee et al., 2017). Further studies revealed that TB extracts act against hyperlipidemia by reducing triglyceride content and affecting glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity and regulate transcription of key genes of fatty acid biosynthesis pathway (PPAR- γ , CEBP- α , aP2, ACC, FAS, and SCD-1) during adipocyte differentiation of 3T3-L1 cells (Lee et al., 2017).

5.4. Cardio-protective properties

Cardio vascular disorders such as hypertension, and imbalance of cholesterol (LDL and HDL) are the leading CVDs across the globe. Epidemiological evidence showed communities consuming traditional whole grains have lower risks of cardiovascular diseases and associated abnormalities (Mellen et al., 2008). In fact, the epidemiological studies conducted in southwest China (He et al., 1995) and inner Mongolia (Zhang et al., 2007) revealed that low density lipoproteins (LDL) and serum total cholesterol were considerably low in participants consuming BW. BW-based dietary interventions are known to significantly reduce the total cholesterol in rats compared to controls, providing valuable protection against coronary heart diseases (Li et al., 2018). Owing to its remarkable slow digestibility, a strong hypocholesterolemic effect of BW protein was observed in the blood plasma of rats fed with cholesterol-enriched diets (Kayashita et al., 1997). Recently, in a study conducted on rodents, TB protein is reported to be a major cardio protective agent by reducing the total cholesterol in blood plasma, with the four times efficiency than rice and wheat proteins (Zhang et al., 2017b). The hypocholesterolemic effect of TB protein is mediated by regulating the excretion of hepatic cholesterol-7 α -hydroxylase and also by interfering cholesterol absorption by repressing intestinal Niemann-Pick C1-like protein 1 (NPC1L1), acyl CoA:cholesterol acyltransferase 2 (ACAT2), and ATP binding cassette transporters. In human, BW-based food products such as BW-enriched wheat bread, TB tea, and BW flour were able to effectively control the LDL, HDL, total cholesterol and triglycerides in serum thus providing a better cardiovascular health (Stokić et al., 2015; Yu et al., 2016; Dinu et al., 2017). The various studies conducted on humans have shown that BW based dietary supplement significantly affect the determinants of CVD.

However, thorough studies are needed for the identification of bioactive compounds responsible for the cardio-protective effects. Therefore, exhaustive biochemical and human based clinical studies are required for promoting BW as a potential dietary intervention for cardiovascular health.

5.5. Hepato-protective properties

The BW flavonoids particularly rutin and quercetin are the major bioactive compounds providing protection to hepatic injury by preventing oxidative liver damage, regulating vasoactive substances, improving lipid metabolism and stabilizing cell membranes (Hu et al., 2016). The oral administration of 70% ethanol extracts from germinated CB seeds at the rate of 100 or 200 mg/kg body weight for eight weeks were found to effectively inhibit fatty acid deposition in rat models fed on high fat diet (Choi et al., 2007). The HPLC analysis revealed that the germination process for 48 h enhanced the rutin content in seeds from 8 to 145 mg/g, which eventually resulted in strong anti-fatty liver activity by reducing the triglyceride and total cholesterol levels in rat liver. In addition to this, the high rutin and quercetin content in ethanolic extracts suppressed the expression of key adipogenic transcriptional factors in hepatocytes. Likewise, hepatoprotective effect of BW phenolic extracts in rabbits fed on high fat diet was also observed through reduction of total cholesterol, triglyceride, and ascorbate free radicals in liver cells (Wojcicki et al., 1996). These findings in animal models indicated that BW phenolic extracts provide opportunities for the development of BW-based nutraceutical product with potent hepato-protective properties. For instance, BW honey, a rich source of rutin, hesperetin, and *p*-coumaric acid is known to control carbon tetrachloride induced liver and DNA damage in mice (Cheng et al., 2015). The administration of BW honey for 10 weeks in mice exerted hepato-protective effect by decreasing hepatic malondialdehyde and increasing hepatic antioxidant enzymes (superoxide dismutase and glutathione peroxidase) (Cheng et al., 2015).

5.6. Anti-bacterial properties

The phytochemicals and antioxidants of BW have broad spectrum anti-microbial properties. BW honey, widely consumed nutraceutical food in China is known to have strong antibacterial activity (Zhou et al., 2012b). This observation is also supported by a study showing BW honey possess higher cellular antioxidant activity than manuka honey (popular honey of New Zealand) and exhibit antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Deng et al., 2018). The higher antioxidant and antibacterial property of BW honey was attributed to the presence of phenolic compounds (hydroxybenzoic acid, chlorogenic acid, and *p*-coumaric acid).

Owing to its versatile applications, *F. cymosum* (also named *F. dibotrys*) is one of the well-recognized plants in Chinese medicine (Joshi et al., 2019). The ethanol extract of *F. cymosum* may exert bacteriostatic activity providing protection from *Haemolytic streptococcus*, *Pneumococcus* and *Streptococcus pneumoniae* by *in vitro* and *in vivo* studies (Jing et al., 2016). *F. cymosum* is known to control lung injury caused by *Klebsiella pneumoniae* through down regulation of mRNA and the protein expression in lung tissues of rat models (Dong et al., 2012). The 75% ethanolic extract of BW is known to regulate the immune response of T helper-2 cell (Th2), which is a key response of food allergy. The rutin exerted inhibitory effect by reducing the expression of Th2 markers, such as GATA3 and *p*-STAT6 in PMA/ionomycin, induced EL4 T cells in mice (Shen et al., 2012). These findings indicated utility of BW-based food in improving anti-cellular allergic responses.

6. Genomics-driven gene discovery for nutritional trait

The genus *Fagopyrum* includes a large variation in species having different phenotypes. At present, > 10,000 germplasm accessions of wild and cultivated species are being conserved in different gene banks across the world (Li et al., 2019). However, due to their large number, adequate phenotyping records pertaining to traits of nutraceutical importance are not available. Consequently, these collections remain unexploited for the improvement of nutraceutical traits in BW. One potential strategy to enhance the utilization of these collections is sampling 'core collections' with a drastically reduced size representing genetic variation in genus *Fagopyrum* (Upadhyaya et al., 2014). Due to small size, core collections can be extensively evaluated for in-depth characterization of expensive and laboratory-based nutraceutical traits. Further, these diverse collections can be used for identifying novel variation and genome wide association studies (GWAS) to characterize the genomic regions (QTLs)/candidate genes responsible for the traits of economic importance in BW.

Though the wild relatives of BW remain untapped, but lately efforts have been made to characterize variation for the traits of nutritional importance. The wild relatives *F. cymosum*, *F. gracilipes*, and *F. urphyllum* possess much higher protein content, total amount of essential amino acids and bioactive flavonoids of medicinal importance than the cultivated species (*F. esculentum* and *F. tataricum*) and this could be used to improve these traits in BW (Joshi et al., 2019). Latest advancements in BW genome sequencing (Yasui et al., 2016; Wang et al., 2017a; Zhang et al., 2017b) will allow decoding of the whole genome of potential wild species, thus shedding new light on novel allelic variation pertaining to traits of nutritional importance and therapeutic uses.

6.1. Deciphering complex nutrient-gene and nutrient-protein interactions

Recently, omics-based approaches have emerged as a robust tool for deciphering the role of candidate genes in synthesis and regulation of nutritional traits and bioactive compounds across the crop plants (Atanasov et al., 2015). An array of functional genomics resources governing transcriptional and translational machinery underlying nutrient accumulation and bioactive compounds has been developed in BW (Table 3). Subsequently, whole transcriptome shotgun sequencing

Table 3
List of differentially expressed genes and proteins identified from different functional genomics studies in buckwheat.

Trait/Process	Species	Genotypes	Plant part/stage utilized	Methodology	DEGs/protein	References
Rutin content	<i>F. esculentum</i>	Tanno-original and Tanno-ruttin Mi 11	Stem, root and seed	Hig-throughput RNA sequencing	<i>FeCH1, FeFLS2, FePAL, FeC4H, FeCHS, FeF3H, FeFSL</i> and <i>FeDFR</i> 11, 676 DEGs	Li et al. (2014)
Nutrient and flavinoids accumulation	<i>F. tataricum</i>	Xide, No.2 Pingqiao	Pre-filling stage, filling stage, and mature stage of grain Seed filling	Hig-throughput RNA sequencing	180,568 transcripts and 109,952 unigenes	Huang et al. (2017) Gao et al. (2017)
Flavonoid, storage protein and starch biosynthesis	<i>F. esculentum</i> <i>F. tataricum</i> <i>F. esculentum</i> ssp. <i>ancestrale</i>	-	Flowers	Hig-throughput RNA sequencing	DEGs corresponding to sugar biosynthesis and metabolism	Logacheva et al. (2011)
Sugar biosynthesis and metabolism	<i>F. esculentum</i> <i>F. tataricum</i>	-	Seed maturity	1D-PAGE and LC-ESI-Q/TOF mass spectrometry	67 unique proteins	Kamal et al. (2011)
Embryo and endosperm development	<i>F. esculentum</i>	-	Leaf and stem	1D-PAGE and MALDI-TOF mass spectrometry	79 and 81 unique proteins belonging to <i>F. esculentum</i> and <i>F. tataricum</i> respectively	Shin et al. (2010)
Light dependent protein network of buckwheat sprouts	<i>F. esculentum</i> <i>F. tataricum</i>	-				

(WTSS) based on next generation sequencing (NGS) has improved global expression profiling of several differentially expressed genes (DEGs) that respond to the accumulation of nutrients and bioactive compounds in different tissues. For instance, RNA-sequencing has been performed to study the transcription machinery and regulation mechanism underlying nutrient biosynthesis and accumulation during seed development in *F. tataricum* (Huang et al., 2017). This effort has generated a tissue and stage specific expression atlas for TB genes and identified a total of 11,676 DEGs. This remarkable data can be used in the development of functional markers for the traits of nutritional significance in a less explored crop like BW with a poorly characterized genome.

Recent advances in proteome mapping, protein-protein interactions, and comparative proteomics is a valuable source of functional characterization of proteins regulating various biological processes (Altelaar et al., 2013). Many important proteins involved in seed maturation, endosperm development, and carbohydrate metabolism have been identified by employing different proteomic approaches in BW (Table 4). For instance, mass spectrometric analysis of BW sprouts identified 166 unique cytosolic proteins involved in cellular metabolism during light stress. A total of 79 proteins were unique to CB, while 81 proteins showed exclusive expression in TB and 6 proteins were commonly expressed in both the species (Lee et al., 2016). Gluten-free nature of BW makes it a healthy alternative food for the celiac patients. However, BW allergy is well recognized in parts of Europe, Asia and Britain (Heffler et al., 2011). Five different proteins of molecular weight 4, 19, 16, 10, and 9 kDa were reported to be the major allergen candidates of BW (Matsumoto et al., 2015). The tools of proteomics could potentially be utilized for absolute quantification of these allergen proteins, deciphering the underlying gene network and their post-translational modifications in CB seed proteome to make it more appealing for food industry.

6.2. Metabolomics assisted interventions for nutraceutical development

Metabolomics and ionomics allow a thorough insight in nutraceutical value of plants by determination of metabolic profile and identification of ionic contents, and phytochemicals (Sumner et al., 2003; Kumar et al., 2017). Considering that, major contributors toward buckwheat's nutraceutical properties are flavonoids and polyphenols, it requires complete metabolic profiling for elucidation of the nutraceutical potential of different varieties and germplasm lines. Further, metabolic phenotyping of different cultivated and wild species of genus *Fagopyrum* will generate metabolites as potential markers for nutraceutical trait development. This approach has effectively been utilized to harness the metabolic diversity as a complementary tool to conventional phenotyping for trait identification in rice (Chen et al., 2014). In BW, Olschlager et al. (2010) demonstrated potential of flavonoid phenotypes improvement by selecting the appropriate parents in a cross between *F. esculentum* and *F. homotropicum*. Metabolic profiling of TB uncovered a series of changes in seed development and flavonoids distribution during five different stages of seed development (Song et al., 2016). Likewise, metabolic profiling of BW sprouts treated with methyl jasmonate revealed enhancement in isoorientin, orientin, rutin, and vitexin content (Kim et al., 2011). Dehulling is a major bottleneck in BW breeding, responsible for drudgery prone postharvest processing of TB. Recently Song et al. (2019) unravelled key metabolic pathway alterations capable of changing lignin and cellulose contents of seed hull and their relationship with dehulling capacity in TB. The investigation observed the highest concentration of lignin and lowest concentration of cellulose in dehulling friendly genotype compared to dehulling recalcitrant genotype. Taken together, metabolic profiling suggests the interaction between auxin, lignin, and cellulose in the hull during seed development, and provides a direction for future research in the dehulling trait of TB. In nutshell, high throughput metabolic platforms can generate biomarkers (metabolite markers) for the

Table 4
Update on metabolic engineering attempts by hairy root cultures in buckwheat.

Species	Strain used	Explant used	Metabolites	Reference
<i>F. esculentum</i>	<i>A. rhizogenes</i> R1000	Leaf	Rutin	Lee et al. (2007)
<i>F. esculentum</i>	<i>A. rhizogenes</i> 15,834	Excised cotyledons and hypocotyls	Rutin	Park and Park (2018)
<i>F. tataricum</i>	<i>A. rhizogenes</i> R1000	Stem	Rutin, quercetin, epicatechin, catechin hydrate, gallic acid, ferulic acid, chlorogenic acid and caffeic acid	Kim et al. (2010)
<i>F. esculentum</i>	<i>A. rhizogenes</i> 15,834	Stem	Rutin	Kim et al. (2010)
<i>F. tataricum</i>	<i>A. rhizogenes</i> R1000	Sterile young stems	Epigallocatechin, caffeic acid, chlorogenic acid and rutin	Park et al. (2011)
<i>F. esculentum</i>	<i>A. rhizogenes</i> R1000	Young stems	Rutin	Park et al. (2012)
<i>F. tataricum</i>	<i>A. rhizogenes</i> R1000	Hypocotyl	Rutin, quercetin, gallic acid, caffeic acid, ferulic acid, 4-hydroxybenzoic acid and anthocyanin	Thwe et al. (2013)
<i>F. tataricum</i>	<i>A. rhizogenes</i> R1000, R1200, R13333, R15834, R1601, LBA9402 and A4	Germinated seeds	Rutin, anthocyanins (cyanidin3-O-glucoside and cyanidin3-O-rutinoside)	Thwe et al. (2016)

biofortification breeding of BW.

In fact, integration of BW seed metabolome with emerging genome sequences, cost-effective genotyping assays, transcriptomics, and proteomic information will provide a cutting-edge data set for use in research as well as therapy.

6.3. Next generation phenotyping and genomic selection for nutraceutical development

Notwithstanding the remarkable advancement in crop genomics, the unavailability of cost-effective high throughput phenotyping assays impedes the progress of understanding the genetic mechanism underlying the traits of nutraceutical importance and implementation of these traits in breeding programs. The high operational cost and low throughput of the currently employed plant phenotyping assays for extraction and quantification of dietary flavonoids and polyphenols limits their large-scale utilization in crop improvement programs. Recently, the use of high throughput phenotyping platforms based on near infrared spectral assays (Belalcazar et al., 2016), hyperspectral reflectance (Yendrek et al., 2017), micro plate assays (Herald et al., 2012), ultrasound assisted assays (Vardanega et al., 2014; Wang et al., 2018) and mass spectrophotometry (Cocuron et al., 2019) supported with robust statistical and mathematical models have paved the way for phenotyping of these health promoting compounds with high throughput, precision and accuracy. Unlike the conventional assays, these automated phenotyping platforms have the potential to characterize the micronutrient and flavonoid diversity in large breeding populations in a rapid yet precise manner. For instance, the near infrared (NIR) based phenotyping assay screened a considerably larger number of cassava genotypes (≥ 100 genotypes) for root carotenoids compared with the low number of genotypes (10 samples) phenotyped through conventional HPLC each day (Belalcazar et al., 2016). In a crop like BW, automated high throughput phenotyping platforms would be important because classical methods are time consuming, labour intensive and not precise screening large genetic populations.

Since the advent of single nucleotide polymorphism (SNP), genomic selection (GS) or genomic prediction has emerged as the potent molecular breeding approach to enhance the genetic gain for complex traits per unit time and cost (Crosa et al., 2017). The GS combines genotypic and phenotypic data of a breeding population to calculate the genomic estimated breeding values (GEBVs) of individuals in the test population based on their genotyping only. Although use of GS in minor crops like BW is intricate but promising results in a pilot study suggested its use in enhancing genetic gain for complex traits with low heritability (Yabe et al., 2018). Traits targeted for nutraceutical breeding program in BW, e.g. flavonoid content, micronutrient density and rutinase activity, are mostly controlled by multiple genes that are highly influenced by G \times E interaction. Therefore, relying alone on phenotypic selection thereby neglecting the underlying genetic mechanism and the

interaction between multiple genes and environments cannot make appreciable genetic gain for nutraceutical traits in a short span of time. Considering the low heritability and complex genetic architecture of these traits, traditional marker assisted selection (MAS) is also not the best choice. Therefore, acquisition of genomic prediction models based on high throughput genotypic and phenotypic data will be important to unlock the BW germplasm repository of unexplored diversity for nutraceutical traits. As a foremost step for implementing GS in BW breeding, a training population (TP) is needed to be constituted comprising elite cultivars, advanced breeding lines and genetic stocks for which multiple season phenotypic data for nutraceutical traits across the environments is available. Utilizing the abundant genome wide distributed marker (e.g. SNPs) profiling data, GEBVs can be estimated for each genotype of training population. Based on this information GS can help either to remove the low performing genotypes for nutraceutical traits (low or moderate heritability) or even to select superior performing genotype (high heritability) having potential to express high genetic advance in next generation. Genotypes with superior GEBVs are selected as parents from the training population (TP) and crosses are made to develop candidate population (CP). Higher efficiency in GS for nutraceutical trait breeding in BW can be achieved by (i) enhancing marker density across the genome by SNP array, developing SNP bead chips at different marker densities or whole genome resequencing and (ii) designing the robust training populations from advanced breeding lines and elite cultivars assembled from different BW breeding programs across the globe for which phenotyping data for their performance have already been documented.

6.4. Speed breeding: Accelerating the genetic gain for complex traits

The conventional plant breeding is time and labour-intensive process that requires 10–12 years for the release of new genetically stable cultivars. Recently, a novel plant breeding technique for rapid cultivar development named ‘speed breeding’ has been deployed in major crops to achieve six crop cycles per year compared to one or two cycles under normal field and glasshouse conditions (Watson et al., 2018). Speed breeding is carried out in environment controlled growth chambers with extended photoperiods and controlled temperature regimes. Under optimum field conditions, the growing period for buckwheat lasts three to four months (Joshi et al., 2019). However, speed breeding under fully controlled conditions using high temperature and induced short photoperiods could potentially be applied to reduce the generation time of BW to achieve six crop cycles in a year. The sharp reduction in generation time through speed breeding could greatly accelerate the development of mapping populations such as recombinant inbred lines (RILs) in BW for genomics assisted breeding of nutraceutical traits. Furthermore, genomic selection together with speed breeding under controlled conditions could be used to screen breeding population for nutraceutical traits development during off seasons. On the whole,

rapid generation advance to homozygosity following crossing with emerging genomics and phenomics tools will improve nutritional traits and allow quick development of nutrient-rich BW cultivars.

6.5. Genes knockdown through reverse genetics and genome editing

Despite of its nutritional benefits, BW has certain amount of anti-nutritional factors (ANF) limiting its utilization as major food resource. Of these, α -amylase inhibitor, trypsin inhibitor and certain allergen proteins are the major anti-nutritional factors (Wang and Zhu, 2015). Of the two main cultivated species of BW, ANFs are reported to be in higher amount in TB, sometimes leading to discomfort symptoms such as nausea and vomiting after consumption (Wang and Zhu, 2015). In addition to this, the rutin hydrolysis activity mediated by rutinoidase enzyme in TB flour is a major hindrance in formulating its functional food products (Suzuki and Morishita, 2016).

RNA interference (RNAi) technology based on the principle of reverse genetics is a popular functional genomics approach, widely utilized for deducing the gene function. The RNAi-induced gene silencing has been well exploited in silencing the genes leading to poor digestibility of millets protein and stable transformants producing grains with improved digestibility were developed (Da Silva et al., 2011). This approach could potentially be utilized in BW nutri-genomics for knockdown of genes governing antinutritional factors and allergen proteins to make it more suitable for food industry.

The RNA guided CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat)-Cas9 based genome editing is a groundbreaking technology in the field of functional genomics (Bortesi and Fischer, 2014). It has huge potential to revolutionize the trait modification in crop plants by gain-of-function and loss-of-function through inserting single guide RNA (sgRNA) in plant genomes in a rapid yet precise manner. To date, no RNAi and gene-editing tool has been developed in BW nutri-genomics. However, rapid advancements in BW transformation provide the opportunity for the development of high-quality sustainable nutraceutical products through these technologies in the near future. The CRISPR-edited BW products will be expected to have reduced ANFs, enhanced protein, essential amino acids, and bioactive compounds compared to the commercial cultivars.

6.6. Metabolic engineering through hairy roots culture models

In the last few decades, genetically engineered hairy roots have emerged as a sustainable biological model for metabolic engineering of bioactive compounds across the crop plants. Hairy roots develop as a consequence of the infection by a soil borne gram-negative bacterium, *Agrobacterium rhizogenes*. During infection, the *rol* genes from the root inducing plasmid of *A. Rhizogenes* integrate into the host genome and results the formation of hairy roots (Sharma et al., 2013). BW is highly sensitive to both, *A. tumefaciens* and *A. rhizogenes* infection (Park and Park, 2018). Therefore, *in vitro* hairy root cultures yielding higher amount of flavonoids have been well studied (Fig. 5; Table 4). Studies have been conducted to investigate the effect of hairy root cultures on biosynthesis of rutin and many other bioactive molecules in *F. esculentum* (Lee et al., 2007; Kim et al., 2010; Park et al., 2012; Park and Park, 2018) and *F. tataricum* (Kim et al., 2010; Park et al., 2011; Thwe et al., 2013; Thwe et al., 2016). Among the various factors, growth media is known to strongly influence the root phenotype and overall pattern by which different root components contribute to flavinoid production. For instance, Lee et al. (2007) reported that, of the four tested culture media (half strength B5, B5, half-strength MS, and MS medium), half-strength MS medium induced the highest levels of growth and rutin production in hairy root cultures of CB. Interestingly, supplementation of growth media with auxins increased the growth rate of roots with no effect on rutin biosynthesis. Contrasting root morphological phenotypes of TB were observed when cultured on MS media and vitamins (Park et al., 2011). The thin roots produce higher

total dry weight and almost double the amount of secondary metabolites like (-)-epigallocatechin, caffeic acid, chlorogenic acid, and rutin) juxtaposed with thick phenotype. Transgenic hairy root cultures of both CB and TB have been developed to facilitate in depth molecular and physiological studies pertaining to flavonoid biosynthesis. For instance, over expression of AtMYB12 in hairy root cultures of CB induced flavonoid biosynthesis genes (Park et al., 2012). The expression of these genes induced rutin biosynthesis up to 0.9 mg/g dry wt. Collectively, these findings suggest that BW hairy root cultures can be used as an alternative approach for secondary metabolites production. The establishment of hairy roots model is helpful for the characterization of the key genes and promoting the molecular breeding in BW (Zhou et al., 2017; Zhang et al., 2018; Li et al., 2019). The R2R3-MYB transcription factor FtMYB11 represses rutin accumulation via interacting with the importin protein FtsAD2 (Sensitive to ABA and Drought 2), and this interaction depends on two conserved Asp residues of its SID-like (SID2 Interacting Domain) motif. FtsAD2 could mediate the transport of FtMYB11 into the nucleus ((Zhou et al., 2017). Later, Zhang et al. (2018) reported 4 JA-responsive MYB transcription factors, FtMYB13/14/15/16. FtMYB13/14/15 mainly expressed in stem and inflorescence while FtMYB16 in root, repress rutin biosynthesis. FtMYB13/14/15 can interact with FtsAD2 via conserved SID motif, while FtMYB16 lack SID motif and showed no interaction with FtsAD2 (Zhou et al., 2017; Zhang et al., 2018). Recently, Li et al. (2019) demonstrated that Ftimportin- α 1 directly regulates the nucleocytoplasmic trafficking signaling pathway of its cargo protein FtMYB16 lacking of a SID motif. Additionally, by virtue of their high genetic stability, hairy roots are the ideal biological model for deciphering the metabolic pathway involved in the accumulation of nutraceutically important bioactive compounds of BW.

7. Summary and perspectives

The growing demand for healthier food and increased awareness of public has promoted nutraceutical market of BW and renaissance of the crop. Additionally, the wide adaptation makes BW a well-suited alternative to diversify the modern agroecosystems and mitigate the hostile weather extremes. Large-scale deployment of genetic resources is imperative for nutraceutical trait development and nutri-rich BW commercial cultivars. This will require: (i) reliable and high throughput screening of genetic resources for nutritional traits and bioactive compounds, (ii) multi-location testing quantifying the genotype and environment interaction for traits of nutraceutical importance and (iii) enhanced utilization of untapped wild BW species for nutraceutical development. The fast emerging 'multilayered genetic and omics development' could play a pivotal role in complementing the above-mentioned approaches for nutraceutical trait development in BW. However, breeding programs for nutraceutical development utilizing various omics interventions in BW is lagging behind, compared to major crops. The latest developments in plant molecular breeding, such as 'genomic selection', 'genome editing via CRISPR-Cas9' and 'speed breeding', will reduce the time and allow simultaneous improvement of nutraceutical traits and yield. Full exploitation of multilayered omics tools in BW breeding can bridge the gap to major crops that have been bred intensively for over a century. In nutshell, improving nutraceutical potential of BW can play an important role in altering modern cropping systems according to the future consumer needs.

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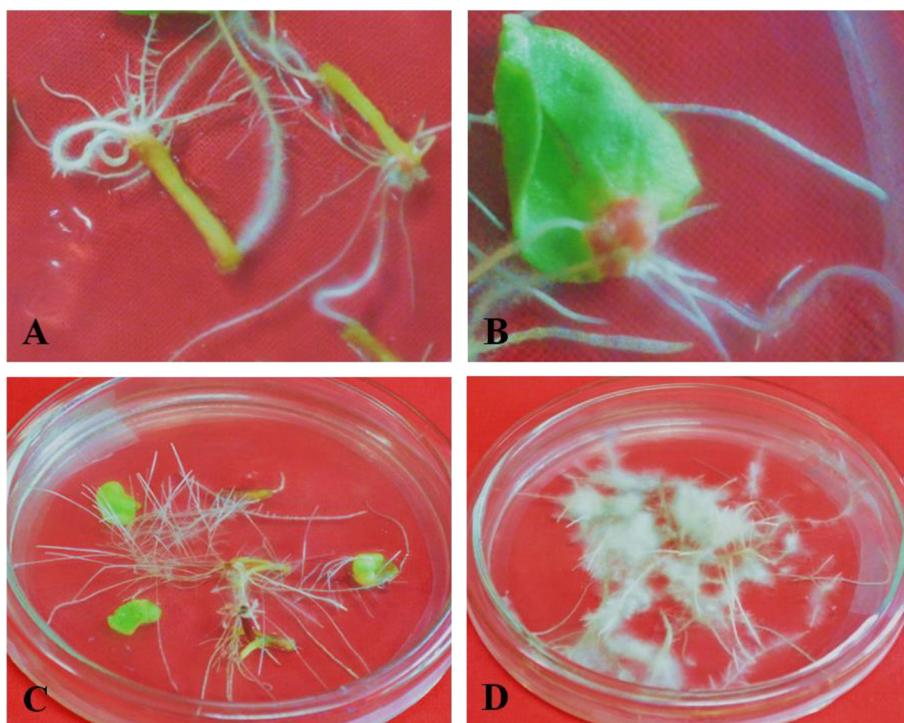


Fig. 5. Buckwheat hairy roots induced by *Agrobacterium rhizogenes*.

A. Buckwheat hairy roots induced from hypocotyls mediated by *Agrobacterium rhizogenes* A4 strain after 10 days culture; B. Buckwheat hairy roots induced from cotyledons mediated by A4 strain after 10 days culture; C. Buckwheat hairy roots induced by A4 strain after two weeks culture; D. Individual hair root line cutting from explants growing on MS solid medium.

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