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Naslovnica: Kiku oblika rastlin ajde (foto: C. G. Campbell)
Cover photo: Buckwheat plant habit of Kiku types (Photo: C. G. Campbell)

VSEBINA
CONTENTS

NOTE ON 40 YEARS OF ORGANIZED INTERNATIONAL COOPERATION IN BUCKWHEAT RESEARCH

ZAPIS OB 40 LETNICI ORGANIZIRANEGA MEDNARODNEGA SODELOVANJA PRI RAZISKAVAH AJDE

Ivan KREFT[1]

http://dx.doi.org/10.3986/fbg0061

International Buckwheat Research Association (IBRA) was established on September 3rd, 1980 at Biotechnical Faculty, University of Ljubljana, Slovenia, immediately after the 1st International Symposium on Buckwheat, by participants from Denmark, Poland, Japan and Slovenia. The day of IBRA establishment, September 3rd, was in 2013 by IBRA Assembly proclaimed as the World Day of Buckwheat. International symposia on buckwheat were after 1980 regularly triennially organized in Japan, Poland, Russia, China, Canada, Korea (South), Czech Republic, Slovenia, and in 2019 in India.

The Department of Botany, North-Eastern Hill University (NEHU), Shillong, India in collaboration with ICAR - National Bureau of Plant Genetic Resources (NBPGR), India, and DBT - Institute of Bioresources and Sustainable Development (IBSD), India organized the 14th International Symposium on Buckwheat at North-Eastern Hill University, Shillong from Sept. 3 to 6, 2019 at North Eastern Hill University, Shillong. The Symposium was organized under the ageis of International Buckwheat Research Association on the theme "DIVERSIFYING FOOD SYSTEMS FOR HEALTH AND NUTRITIONAL SECURITY". Prof. S. K. Srivastava, Vice Chancellor, North-Eastern Hill University was the Chief Patron of the Organizing Committee. Dr. Trilochan Mohapatra, Secretary, Department of Agricultural Research and Education, Ministry of Agriculture Govt. of India and Director General, Indian Council for Agricultural Research, Govt. of India was the Chairman of the International Scientific Advisory Committee of the Symposium. Prof. Nikhil Chrungoo

Mednarodno združenje za raziskave ajde (International Buckwheat Research Association, IBRA) je bilo ustanovljeno 3. septembra 1980 na Biotehniški fakulteti Univerze v Ljubljani, Slovenija ob zaključku prvega mednarodnega simpozija o ajdi; ustanovni člani smo bili iz Danske, Poljske, Japonske in Slovenije. Dan ustanovitve organizacije IBRA, 3. september, je bil leta 2013 na skupščini IBRA proglašen za Svetovni dan ajde. Mednarodne simpozije o ajdi smo po letu 1980 redno organizirali vsaka tri leta in sicer na Japonskem, na Poljskem, v Rusiji, na Kitajskem, v Kanadi, Južni Koreji, v Češki republiki in Sloveniji ter leta 2019 v Indiji.

Organizator 14. simpozija o raziskavah ajde leta 2019 je bil Oddelek za botaniko, North-Eastern Hill University (NEHU), Shillong, Indija v sodelovanju z ICAR - National Bureau of Plant Genetic Resources (NBPGR), Indija, in DBT - Institute of Bioresources and Sustainable Development (IBSD), Indija. Simpozij je potekal kot "14th International Symposium on Buckwheat" na North-Eastern Hill University, Shillong od 3. do 6. septembra 2019 v okviru dejavnosti International Buckwheat Research Association na temo „DIVERSIFYING FOOD SYSTEMS FOR HEALTH AND NUTRITIONAL SECURITY". Prof. S. K. Srivastava, Vice Chancellor, North-Eastern Hill University je bil glavni pokrovitelj organizacijskega odbora. Dr. Trilochan Mohapatra, tajnik, Department of Agricultural Research and Education, Ministrstvo za kmetijstvo Vlade Indije in glavni direktor, Indian Council for Agricultural Research, Vlade Indije, je predsedoval mednarodnemu svetovalnemu odboru simpozija. Prof. Nikhil Chrun-

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of NEHU was the Organizing Secretary of the Symposium. Dr. J.C. Rana of Bioversity International and Dr. Aijaz Ahmad Wani of University of Kashmir, Srinagar, India were the Joint Secretaries of the Symposium. Sh. Tathagata Roy, Hon'ble Governor of the Indian province of Meghalaya, graced the Inaugural function of the Symposium as the Chief guest and inaugurated the symposium. Dr. Trilochan Mohapatra, Secretary (DARE), Ministry of Agriculture Govt. of India and DG, ICAR, New Delhi was the Guest of Honour in the inaugural function. Prof. S. K. Srivastava, Vice Chancellor, North-Eastern Hill University, Shillong spoke about importance of Buckwheat as a food crop and also its importance in culture. Dr. T. Mohapatra spoke on the importance of underutilized crops such as Buckwheat in mitigating nutritional insecurity of people. Sh. Tathagata Roy, the Hon›ble Governor of Meghalaya spoke on the importance of buckwheat as a food crop and the need to promote its utilization by masses. Prof. O. Ohnishi, Emeritus Professor, Kyoto University, Japan was conferred «Life time achievement award» for his contribution towards buckwheat research. Sh. Tathagata Roy, the Hon›ble Governor of Meghalaya presented the award to Prof. Ohnishi. Prof. Dr. Meiliang Zhou, of Chinese Academy of Agricultural sciences, Beijing, Dr. Manoj Prasad of NIPGR, New Delhi and Dr. J. C. Rana of Bioversity International, New Delhi were conferred Golden Peacock Awards during the Inaugural function of the Symposium. Dr. Trilochan Mohapatra, Secretary (DARE), Ministry of Agriculture Govt. of India and DG, ICAR, New Delhi presented the awards to the recipients. The organizing committee of the Symposium also included Professors and other scientists from NEHU and elsewhere in India, Research fellows of School of Life Sciences of NEHU and Graduate students of the Department of Botany, NEHU. Prof. Nikhil Chrungoo of NEHU was elected by IBRA Assembly as Chairman of IBRA for the period of the next three years. Poland was by IBRA Assembly decided to be the country for organizing the 15th International Symposium on Buckwheat in 2022.

In 1981 International periodical publication on buckwheat FAGOPYRUM was established in Ljubljana, it moved later to Japan, was edited consecutively by Prof. Toshiko Matano (Shinshu University), Prof. Ohmi Ohnishi (Kyoto University) and Prof. Kiyokazu Ikeda (Kobe Gakuin University). In 2017 it moved again back to Ljubljana, Slovenia, it is published now by the Slovenian Academy of Sciences and Arts.

goo, NEHU, je bil organizacijski tajnik simpozija. Dr. J.C. Rana z Bioversity International in Dr. Aijaz Ahmad Wani z University of Kashmir, Srinagar, Indija sta bila pridružena tajnika simpozija. Sh. Tathagata Roy, guverner indijske province Meghalaye, je kot glavni častni gost otvoril simpozij. Dr. Trilochan Mohapatra, sekretar (DARE), Ministrstvo za kmetijstvo Vlade Indije in DG, ICAR, New Delhi je bil častni gost otvoritvene slovesnosti. Prof. S. K. Srivastava, Vice Chancellor, North-Eastern Hill University, Shillong je predstavil pomen ajde kot rastline za prehrano ljudi in njen kulturni pomen. Dr. T. Mohapatra je predstavil pomen ajde kot ene od ne dovolj uporabljanih poljščin za prehransko varnost ljudi. Sh. Tathagata Roy, guverner Meghalaye je govoril o pomenu ajde kot poljščine za prehrano in nujnosti pospeševanja uporabe ajde v prehrani ljudi. Prof. O. Ohnishi-ju, zasluženemu profesorju, Kyoto University, Japonska je bilo podeljeno priznanje «Life time achievement award» za njegove prispevke na področju raziskav ajde. Sh. Tathagata Roy, guverner Meghalaye je priznanje slovesno izročil prof. Ohnishi-ju. Prof. Dr. Meiliang Zhou, s Chinese Academy of Agricultural sciences, Peking, Dr. Manoj Prasad z NIPGR, New Delhi in Dr. J. C. Rana z Bioversity International, New Delhi so na otvoritveni slovesnosti simpozija prejeli priznanja "Golden Peacock Award". Dr. Trilochan Mohapatra, secretar (DARE), Ministrstvo za kmetijstvo Vlade Indije in DG, ICAR, New Delhi so priznanja izročili prejemnikom. V okviru organizacije simpozija so sodelovali tudi profesorji in drugi znanstveniki univerze NEHU in drugih indijskih inštitucij, zlasti pa sodelavci študijev Life Sciences univerze NEHU ter študenti Oddelka za botaniko NEHU. Prof. Nikhil Chrungoo, NEHU je bil na skupščini IBRA izvoljen za predsednika IBRA za obdobje naslednjih treh let. Poljska je bila na skupščini IBRA izbrana za državo gostiteljico 15. mednarodnega simpozija o ajdi leta 2022.

Leta 1981 je v Ljubljani začela izhajati mednarodna periodična publikacija FAGOPYRUM za objavljanje rezultatov raziskav o ajdi, kasneje se je izhajanje preselilo na Japonsko, kjer so revijo zaporedno urejali prof. Toshiko Matano (Shinshu University), prof. Ohmi Ohnishi (Kyoto University) in prof. Kiyokazu Ikeda (Kobe Gakuin University). Leta 2017 se je izhajanje te recenzirane znanstvene revije vrnilo v Ljubljano, kjer izhaja kot publikacija Slovenske akademije znanosti in umetnosti.

THE ORIGIN OF CULTIVATED BUCKWHEAT IN MANKANG DISTRICT OF THE SANJIANG AREA OF EASTERN TIBET AND ITS DIFFUSION TO INDIA AND THE HIMALAYAN HILLS

IZVOR GOJENE AJDE NA OBMOČJU DISTRIKTA MANKANG OBMOČJA SANJIANG VZHODNEGA TIBETA IN RAZŠIRITEV V INDIJO TER NA OBMOČJE HIMALAJE

Ohmi OHNISHI[1]

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ABSTRACT

The origin of cultivated buckwheat in Mankang district of the Sanjiang area of Eastern Tibet and its diffusion to India and the Himalayan hills

Natural populations of the wild ancestor of cultivated common buckwheat were searched and collected, starting from its discovery in1990 and finishing the collections in 2005. Among the collections, the samples Zhuka, Xihe from Mankang district of Tibet are most closely related to cultivated common buckwheat. On the other hand, cultivated populations of common buckwheat in Zhouba, Zhubalong both from Mankang district are most closely related with the wild ancestor of common buckwheat. This leads to the hypothesis on the origin of cultivated buckwheat in Mankang district in the Sanjiang area. The diffusion route from the original birthplace to India and the Himalayan hills is proposed. Several characteristics of Indian and Himalayan common buckwheat are discussed. A main conclusion of the discussion is that European buckwheat is not of Indian origin nor of the Himalayan origin. It probably came from the northern China through the Silk Road

Key words: buckwheat, wild ancestor, origin, diffusion, India, Himalaya, short day plant

IZVLEČEK

Izvor gojene ajde na območju distrikta Mankang območja Sanjiang Vzhodnega Tibeta in razširitev v Indijo ter na območje Himalaje

Iskali in zbirali so naravne populacije divjega prednika gojene navadne ajde, začeli so leta 1990 in zaključili 2005. Med zbranimi vzorci je bil vzorec Zhuka, Xihe iz Mankanga, Tibet, najbolj soroden gojeni navadni ajdi. Po drugi strani, vzorca gojenih ajd iz Zhouba in Zhubalonga, oboje iz Mankanga, so najbolj sorodni divjemu predniku navadne ajde. Na osnovi tega lahko oblikujemo hipotezo o izvoru gojene ajde v Mankangu na območju Sanjianga. Pot širjenja od prvotnega izvora v Indijo in na območja gorovja Himalaje je predlagana v tej razpravi. Avtor opisuje lastnosti navadne ajde v Indiji in na območju Himalaje. Glavni zaključek je, da ajda v Evropi ne izvira iz Indije ali z območja Himalaje. Verjetno je v Evropo prišla iz Severne Kitajske po Svilni poti.

Ključne besede: ajda, divji prednik, izvor, razširjanje, Indija, Himalaja, rastlina kratkega dne.

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1. INTRODUCTION

Since the time of de Candolle (1883), (a) what is the wild ancestor of cultivated common buckwheat and (b) where is the original birthplace of cultivated buckwheat, these two problems have been the main issues to be solved by buckwheat scientists.

The wild ancestor of cultivated buckwheat was clarified as *F. esculentum* ssp. *ancestrale* Ohnishi, which was first discovered in 1990 in the Wulang river valley in Yongsheng district of Yunnan province, in China by Ohnishi (1991). As for the birthplace of buckwheat, Ohnishi (2004, 2007, 2010, 2016, and 2018) has repeatedly claimed that the Sanjiang area of Yunnan, Sichuang provinces and east Tibet in China is the original birthplace of cultivated buckwheat.

In the Himalayan countries, India, Nepal, Bhutan, and Pakistan, buckwheat is cultivated extensively and buckwheat is consumed well. Buckwheat in India and the Himalayan hills has some characteristics which are not seen in other regions such as China, Japan and European countries.

Today, I discuss the original birthplace of buckwheat in more details, and I consider the diffusion route from the place of origin to India and to the Himalayan hills. Finally, I discuss the characteristics of Indian and the Himalayan buckwheat. As a conclusion, I suggest that the cultivated buckwheat in European countries has never come from India nor from the Himalaya, it probably came from northern China through the Silk Road.

2. THE EXACT ORIGINAL BIRTHPLACE OF COMMON BUCKWHEAT

The wild ancestor of cultivated common buckwheat was first discovered in Yongsheng district in Yunnan province of China in 1990 (Ohnishi, 1991). Ten-years searches for the wild ancestor in Yunnan and Sichuan provinces, and the searches in Mankang district of eastern Tibet in 2002 and 2004, and finally the searches in the Tongyi river valley and the Nyiru river valley in 2004 and 2005 clarified the distribution areas of the wild ancestor of common buckwheat (Ohnishi 2007, see also Ohnishi and Tomiyoshi, 2005).

Photo 1: Cultivated common buckwheat in Sanjian area (Weixi district, Yunnan provice). Flower color in this area near the original birthplace is beautiful pink.

Photo 2: The wild ancestors growing in the Xihe river valley of Mankang district in eastern Tibet. The wild ancestors in this valley are genetically most closely related with cultivated buckwheat. Hence, the Xihe river valley along with the towns in northern Mankang district are considered as the original birthplace of common buckwheat.

Photo 3: The cultivation of common buckwheat in Yanjing town of southern Mankang district. The brown part of the cultivated field is common buckwheat just before harvest. Yanjing town has a good weather condition for buckwheat cultivation and the wild ancestors of commom buckwheat are also growing at the margine of buckwheat fields, although those wild ancestors are not so closely related with cultivated buckwheat.

The wild ancestor is distributed in

1. The Sanjiang area of Yunnan province and eastern Tibet,

2. Sporadic distribution in northwestern Yunnan province and southwest corner of Sichuan province, and

3. The Tongyi river valley in Muli district of Sichuan province and the Nyiru river valley in Shangrila district of Yunnan province. Both the Tongyi river and the Nyiru river are small tributary of the Shuiluo river, a tributary of the Jinshajiang river.

Cultivated common buckwheat and wild ancestors are illustrated with Photos 1 – 4.

Among the collected wild ancestor populations, the wild ancestors from the Sanjiang area were revealed to be the most closely related with cultivated common buckwheat in AFLP variation (Konishi et al., 2005) and allozyme variability (see Figure 1, see Ohnishi and Nishimoto, 1988, for the procedures of the electrophoresis and the names of enzymes analyzed). A part of the data on the frequencies of allozymes can be found in Ohnishi (2007). The N-J tree of Figure 1 was written following by Saitou and Nei (1987) using PAUP* (Phylogenetic analysis using parsimony) version 4.0 (Swofford, 1990, 2002).

The wild ancestors in the Tongyi river valley and the Nyiru river valley are highly variable in AFLP and allozymes, however, the populations from those valleys are most distantly related with cultivated populations of common buckwheat. The sporadically distributed ancestral populations in Yunnan and Sichuan provinces showed intermediate closeness to cultivated buckwheat (Fig. 1).

This may leads to the conclusion that the Sanjiang area is the original birthplace of common buckwheat. Konishi and Ohnishi (2007) showed that close genetic relationship between the wild ancestor in the Sanjiang area and cultivated populations is not due to recent hybridization between them.

Now, by observing Fig. 1 more carefully, you may find that the Zhuka population and the Xihe population from Mankang district are most closely related with cultivated populations. The Adong population (in Yunnan province) and the Yanjing population (close to the border between Yunnan and Tibet, see Fig. 2) are both from the Sanjiang area, but they are slightly far away from the cultivated populations as compared with the Zhuka and the Xihe popultions (Fig. 1).

Furthermore, by observing Fig. 1 from the cultivated population side, you may find that the cultivated populations of Zhubalong and Zhouba (both come from northern Mankang district) are closely related with the wild ancestor of cultivated buckwheat (Figs. 1 and 2).

Now, as a conclusion, we can say that the Yunnan part of the Sanjiang area is not involved in the origin of buckwheat cultivation, rather, Mankang district of the

Photo 4: The landscape of northern Mankang district. Although this area is believed to be the original birthplace of common buckwheat, barley is mainly cultivated in cultivation fields.

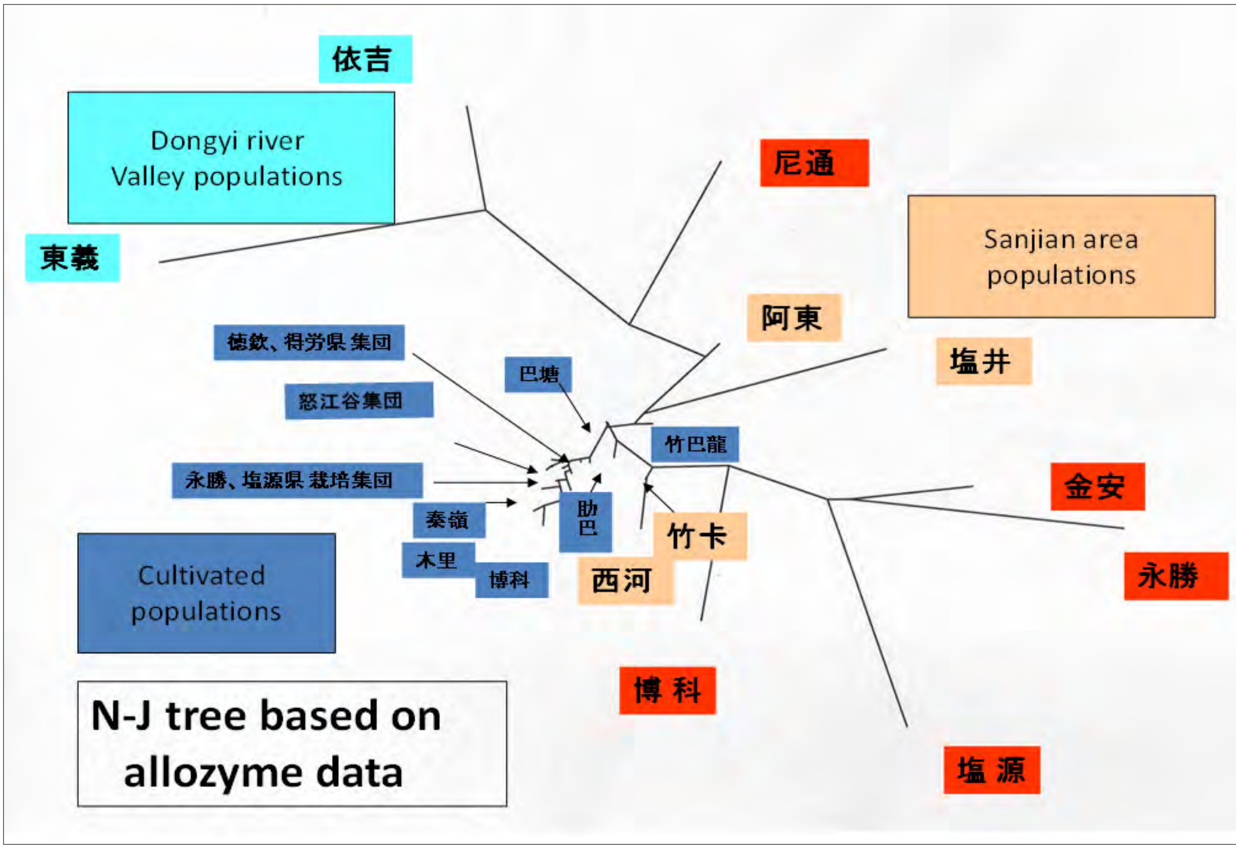

Figure 1: The N-J tree among the populations of wild ancestor and cultivated populations of buckwheat based on allozyme data. The name of locations written in Chinese letters in the figure should be expressed in pinyin as is shown in parentheses. Only the locations appeared in text are listed bellow.
西河*(Xihe)* 竹卡*(Zheke)* 阿東*(Adong)* 塩井*(Yanjing)* 博科*(Boke)* 塩源*(Yanyuang)* 金安*(Jinan)* 永勝*(Yongsheng)*
尼通*(Nidong)* 東義*(Tongyi)* 依吉*(Yiqi)* 竹巴龍*(Zhebalong)* 巴塘*(Batang)* 助巴*(Zhouba)*

Sanjiang area, particularly, north part of Mankang district is the original birthplace of cultivated buckwheat. So, it is reasonable that Wang (1986) reported archaeological remains (buckwheat seed grains) in the archaeological site of Karuo village near Chamdu of east Tibet. This site is close to the original birthplace Mankang district of eastern Tibet (see Fig. 3)..

3. DIFFUSION OF CULTIVATED BUCKWHEAT TO INDIA AND THE HIMALAYAN HILLS

Cultivated common buckwheat migrated from its original birthplace, the Sanjiang area of southwestern China to northern China first, then to the Korean peninsula and Japanese islands (Murai and Ohnishi, 1996). From northern China, cultivated buckwheat migrated west, to the central Asian countries, then to European countries through the Silk Road as I discussed in the previous 13th International Symposium on Buckwheat (Ohnishi, 2016).

From the original birthplace of common buckwheat, Mankang district, cultivated buckwheat first went west (Murai and Ohnishi, 1996), overcoming high mountains, the Hengdan mountains, in the three-river region (the Sanjiang area), and entered to the Yaruzanpu river basin, then finally arrived at Bhutan, Sikkim, Nepal and India. There exists only one route connecting Mankang district in the Sanjian area and the Yaruzampu basin in Tibet as seen in Fig. 3. After

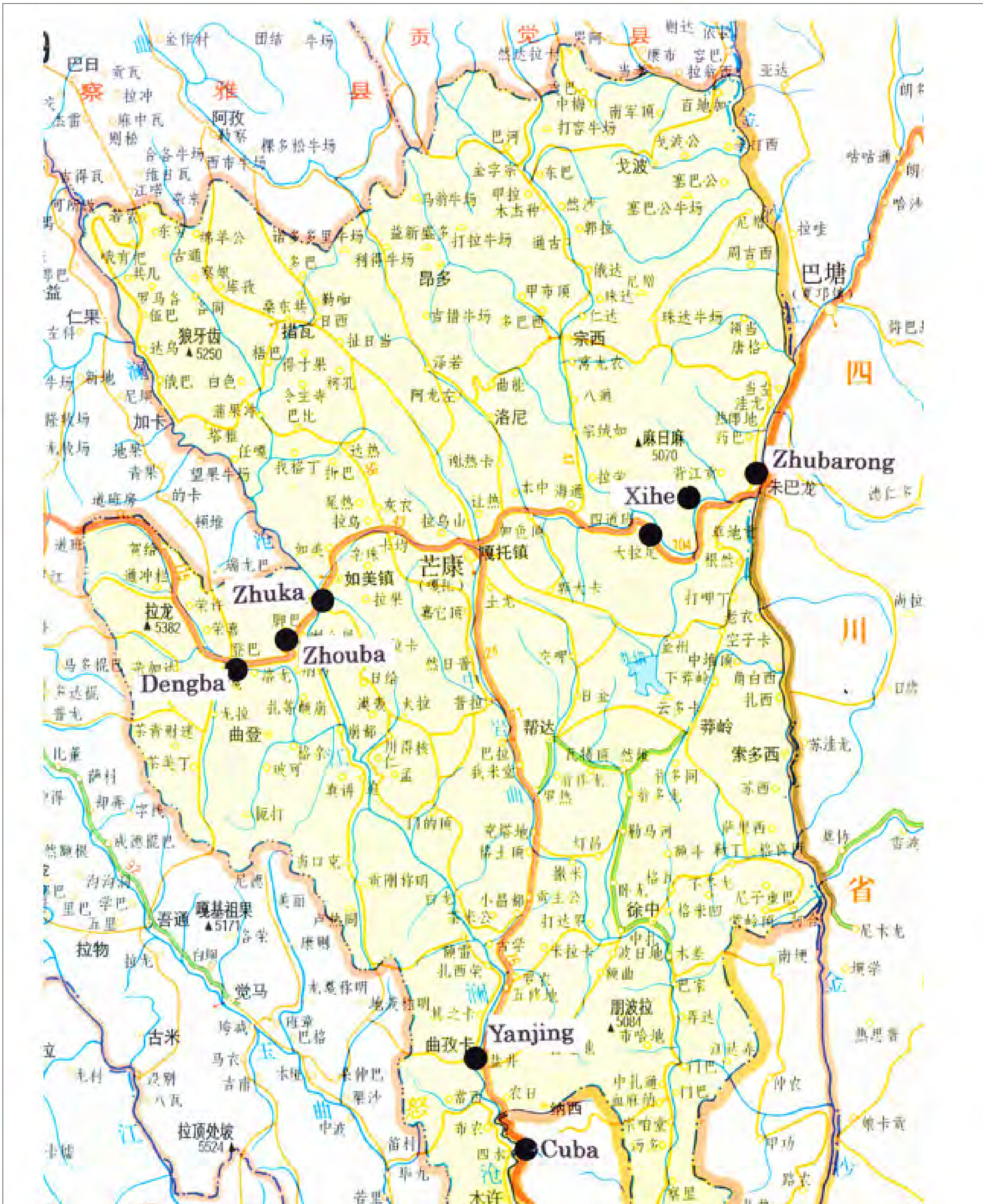

Figure 2. Distribution of the wild ancestor of cultivated common buckwheat in Mankang district of the Sanjiang area. ●: village or town where the wild ancestor was found. Northern population such as Zhouba, Zhuka and Xihe are close to cultivated populations (Fig. 1)

arriving these countries, it is easy for buckwheat to travel further west along the Himalayan hills, because the Himalayan hills may provide a comfortable cultivated conditions for cultivated buckwheat.

Only a few crops diffused through the same route as buckwheat. Tea plant, *Camellia sinensis,* originated in Yunnan province in China, diffused through the same route as common buckwheat, became an important cultivated plant in India and the Himalayan hills. Only the crop diffused opposite direction from the Himalayan hills to the Sanjiang area is the finger millet (*Eleusine coracana*), originated in Africa. It arrived at the Indian subcontinent, India and Pakistan. Then it diffused to southern China, through the same route as of buckwheat, but in the opposite direction (Hoshikawa, 1992).

As shown in Fig. 3, the Karuo archaeological site, from where the oldest buckwheat seed grains were reported, is located not so far away from the original birthplace of cultivated buckwheat. Along the diffusion route, wild perennial buckwheat, *F. cymosum* ssp. *pillus* (syn. *Fagopyrum pillus* Chen, see Chen, 1999) and a weed species *F. gracilipes* are found, near to Dongmai village, Bomi district of Tibet, and Paro of Bhutan, respectively (Fig. 3).

It is well-known that *F. cymosum* growing in the west of the Yaruzampu grand canyon is all tetraploid, and is often called *F. dibotris* in Nepal and India (see Hara, 1972).

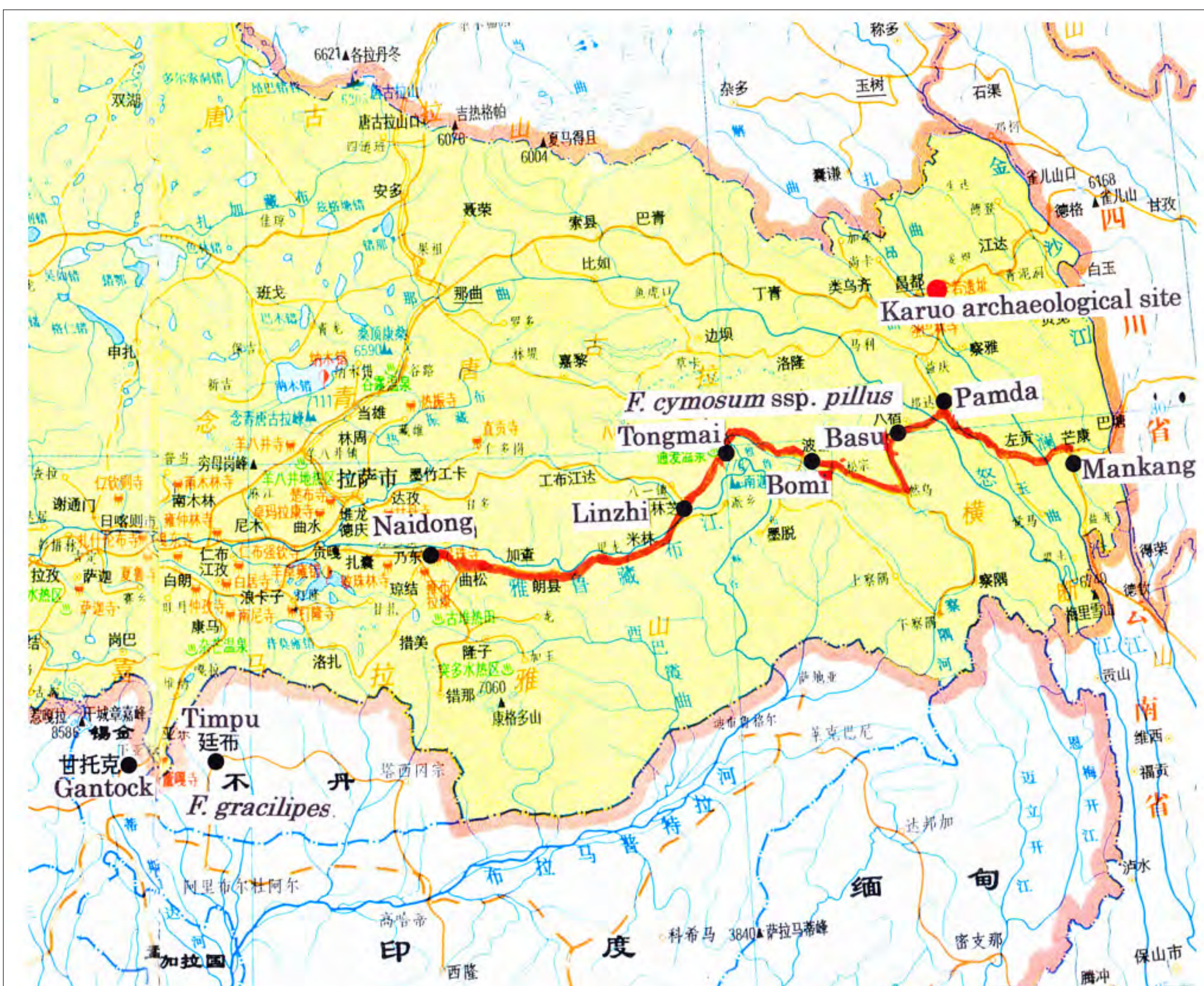

Figure 3. Diffusion route of cultivated buckwheat from Mankang district in the Sanjiang area to India and the Himalayan hills.

4. CHARACTERISTICS OF BUCKWHEAT IN INDIA AND IN THE HIMALAYAN HILLS

Here, I mention several characteristics of buckwheat and buckwheat cultivation in India and the Himalayan hills.

4.1. Buckwheat cultivation as a fresh vegetable in India

Both common buckwheat and Tartary buckwheat are cultivated as a fresh vegetable in India, rather than as a grain crop. As a result of long history of cultivation as a fresh vegetable, local varieties for such purpose have become the varieties with very small grains. I observed such a small grain variety in Bageshwar town, the state of Uttar Pradesh, west India. This custom of buckwheat use as a fresh vegetable is found both in eastern India and western India.

4.2. Common buckwheat in India and in the Himalayan hills is a short day-length plant

Common buckwheat in India and in the Himalayan hills is usually cultivated in fall to early winter, from September to December. As a result of cultivation under the condition of mild temperature and of short day-length, buckwheat in India and the Himalayan hills have become short day-length plant, with characteristics of tall vigorous vegetable parts with relatively longer cultivation period as compared with buckwheat from northern China and Japan.

European common buckwheat has the characteristics of long-day to neutral day-length plant as the descendants of diffused buckwheat through the Silk Road (Ohnishi, 1993, 2016). The characteristics of buckwheat in the Himalayan hills, short-day plant, is opposite to the characteristics of European buckwheat, day-neutral to long day-length plant. This leads to the conclusion that European buckwheat does not have the origin in India nor in the Himalayan hills.

4.3. Making buckwheat noodle by hands in Ladakh of India

Buckwheat noodle was not developed well in Nepal, India, and Pakistan.

Two methods of making buckwheat noodle (Ohnishi, 2016), one using a noodle making wooden equipment, I call this as a Chinese method, one using special cooking knife to cut and make fine noodle of buckwheat dough, I call this as a Japanese method.

Neither methods diffused to Nepal and India. In Bhutan a buckwheat noodle making equipment, called Putta in Bhutan, is used, hence buckwheat noodle is served as a daily food. If peoples know neither methods, what happens for them? In a section of this symposium, Mr. Inazawa and his group will report the method for making buckwheat noodle by hands in Ladakh of India, where peoples make buckwheat noodle by their own hands only without using any special equipments, such as putta in Bhutan or special kitchen knife as in Japan.

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GENETIC CHARACTERIZATION OF BUCKWHEAT ACCESSIONS THROUGH GENOME-WIDE ALLELE FREQUENCY FINGERPRINTS

GENETSKA KARAKTERIZACIJA VZORCEV AJDE Z ODTISI FREKVENCE ALELOV V GENOMU

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ABSTRACT

Genetic characterization of buckwheat accessions through genome-wide allele frequency fingerprints

Genomics-assisted breeding of buckwheat (*Fagopyrum esculentum* Moench) depends on robust genotyping methods. Genotyping by sequencing (GBS) has evolved as a flexible and cost-effective technique frequently used in plant breeding. Several GBS pipelines are available to genetically characterize single genotypes but these are not able to represent the genetic diversity of buckwheat accessions that are maintained as genetically heterogeneous, open-pollinating populations. Here we report the development of a GBS pipeline which, rather than reporting the state of bi-allelic single nucleotide polymorphisms (SNPs), resolves allele frequencies within populations on a genome-wide scale. These genome-wide allele frequency fingerprints (GWAFFs) from 100 pooled individual plants per accession were found to be highly reproducible and revealed the genetic similarity of 20 different buckwheat accessions analysed in our study. The GWAFFs cannot only be used as an efficient tool to precisely describe buckwheat breeding material, they also offer new opportunities to investigate the genetic diversity between different buckwheat accessions and establish variant databases for key material. Furthermore, GWAFFs provide the opportunity to associate allele frequencies to phenotypic traits and quality parameters that are most reliably described on population level. This is the key to practically implement powerful genomics-assisted breeding concepts such as marker-assisted selection and genomic selection in future breeding schemes of allogamous buckwheat.

Key words: Buckwheat (*Fagopyrum esculentum* Moench), genotyping by sequencing (GBS), population genomics, genome-wide allele frequency fingerprints (GWAFFs)

IZVLEČEK

Genetska karakterizacija vzorcev ajde z odtisi frekvence alelov v genomu

Genomsko podprto žlahtnjenje ajde (*Fagopyrum esculentum* Moench) je odvisno od robustnih metod genotipiziranja. Genotipiziranje s spremljanjem sekvenc (genotyping by sequencing, GBS) se je razvilo kot fleksibilna in razmeroma poceni metoda, ki se jo uporablja pri žlahtnjenju rastlin. Uporabnih je več virov GBS za genetsko karakterizacijo posamičnih genotipov, toda te metode niso primerne za predstavitev genetske raznolikosti vzorcev ajde, ki jih vzdržujemo v heterozigotni obliki, kar velja za odprto oplodne populacije. Tu poročamo o razvoju GBS metode, ki, namesto prikazovanja bi-alelnega polimorfizma posameznih nukleotidov (single nucleotide polymorphisms, SNPs), pokaže frekvence alelov v populaciji na nivoju genoma. Ta prikaz frekvence alelov na nivoju genoma (genome-wide allele frequency fingerprints, GWAFFs) z združenimi sto posameznimi rastlinami vsakega vzorca se je pokazal kot visoko ponovljiv in je prikazal genetsko podobnost 20 različnih vzorcev ajde, ki smo jih analizirali v naši raziskavi. Metoda GWAFFs ni uporabna samo kot učinkovito orodje za natančen opis materiala za žlahtnjenje ajde, ponuja tudi možnosti raziskave genetskih razlik med različnimi vzorci ajde in omogoča zbirke podatkov. Nadalje, metoda GWAFFs omogoča povezovanje frekvenc alelov s fenotipskimi lastnostmi in kvalitativnih parametrov, ki so najbolj zanesljivo opisani na nivoju populacij. To je ključ za praktično uporabo z genomiko podprtega žlahtnjenja, kot je z genskimi markerji podprta selekcija in genomska selekcija z GWAFFs.

Ključne besede: ajda (*Fagopyrum esculentum* Moench), genotipizacija s sekvenciranjem (GBS), populacijska genomika, GWAFFs

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1 INTRODUCTION

Plant breeding plays a key role in order to meet the increasing demand for food of the world's growing population. Sophisticated breeding strategies have been developed depending on the reproductive strategy of crop species, but they are all based on one common principle: Through initial crossings, genes and alleles are reshuffled and among the hundreds or thousands of variants produced in the cross, variants outperforming the parents or combining desirable characteristics may be chosen (Fehr 1987; Stoskopf et al. 1999). To select the best variants, plant breeding depended for centuries on the trained breeder's eye. With the establishment of genomics-assisted plant breeding techniques, breeders were given an aid to support the selection process. Genomics-assisted plant breeding incorporates knowledge of the genetic determinant of the trait of interest and allows to select superior variants based on genetic data (Moose & Mumm 2008). This technique allows to select for multiple traits and usually within a fraction of time needed to measure them. Through the steep price drop for next-generation sequencing in the last decades (Wetterstrand 2019), genomics-assisted breeding became not only feasible for major crops but is becoming increasingly popular in orphan crops.

The orphan crop buckwheat (*Fagopyrum esculentum* Moench) is a desired food crop since its gluten-free grains are high in antioxidants and essential amino acids (Li & Zhang 2001). Agronomically, buckwheat has shown beneficial effects in crop rotations and is an attractive bee crop (Falquet et et al. 2015; Teboh & Franzen 2011), but its low seed-set, indeterminate growth and susceptibility to abiotic stresses have hindered wide adoption of buckwheat as a cash-crop in Europe (Lichtenhahn & Dierauer 2000). Buckwheat breeding is conducted in several programs around the world, but is complicated by the heterostylous self-incompatibility system (Ueno et al. 2016). Several attempts have been made to transfer the self-compatibility of its sister species *Fagopyrum homotropicum* Ohnishi (Matsui et al. 2003), while this was successful, the resulting lines often suffered from inbreeding depression (Campbell 1997). Hence, most buckwheat grown today is of outcrossing nature and accessions are maintained as diverse populations. This renders it difficult to fix beneficial alleles in the population, because the 'superior' plants selected at harvest time are already pollinated by the 'inferior' neighbour plants.

Genomics-assisted breeding offers opportunities to select plants containing beneficial alleles based on genetic data and known marker-trait associations. A requirement for this are reliable genotypic data of breeding germplasm. For buckwheat with a haploid genome size of around 1.3 Gb (2n=16), several genomic resources have become available recently (Logacheva et al. 2011; Mizuno & Yasui 2019; Nagano et al. 2000; Yasui et al. 2016). A widely used genotyping method that has evolved as highly flexible is genotyping by sequencing (GBS) (Elshire et al. 2011). In the GBS workflow, genomic DNA is cut by a restriction enzyme and sequencing adaptors are ligated to the cutting sites. After a PCR multiplication step, the fragments are short-read sequenced, which results in repeated coverage of thousands of genetic loci (Elshire et al. 2011). Through alignment of the sequencing reads to a reference genome, a genotyping matrix can be derived that allows for further downstream analysis to compare genotypes or conduct genetic studies. Since buckwheat accessions are populations of genetically distinct individuals, standard genotyping and variant calling pipelines tailored for genotyping single individuals or inbred lines (Li et al. 2009; Mckenna et al. 2010), are of limited use. As an alternative, a large number of single plants can be genotyped and instead of determining the allele present at a certain genetic location, allele frequencies can be calculated. A shortcut and budget-friendly option represents the pooling of multiple individuals before sequencing, which proved to be a highly accurate method in perennial ryegrass (*Lolium perenne*) (Byrne et al. 2013).

The main objective of this study was to find a reliable genotyping method for detailed genetic analyses of buckwheat accessions. Specifically, we adapted the GBS and analysis protocol reported by Byrne et al. (2013) to calculate genome-wide allele frequency fingerprints (GWAFFs) and tested their accuracy in a replicated set of twenty diverse accessions.

2 MATERIAL AND METHODS

2.1 Plant material and DNA extraction

Twenty accessions from Austria (AT), France (FR), Germany (DE), Russia (RU), Slovenia (SI), Ukraine (UA), Czech Republic (CZ) and Switzerland (CH) were grown with a sowing density of 180 seeds/m2 in field plots of 3 x 4m at the ETH Research Station for Plant Sciences in Lindau-Eschikon, Switzerland (47.449N, 8.682E, 520 m a.s.l.). The following accessions were used: Bamby (AT), Billy (AT), Buchsa (CH), Carolin (FR), Carte Noir (FR), Darja (SI), Devyatka (RU), Dialog (RU), Dikul (RU), Drollet (F), Kerntner Hadn (AT), La Harpe (F), Lileja (UA), MinI (DE), Orphe (F), Pyra (CZ), Rosa (CH), Temp (RU), Theophani (DE), Tussi (DE).

DNA was extracted from leaf material cut out with an apple corer to ensure tissues are of approximately the same size. For each accession, pooled samples of 100 randomly selected plants were taken in triplicate. The plant material was flash frozen in liquid nitrogen and milled using mortar and pistil. DNA was extracted using the DNeasy Plant Mini Kit (Quiagen, Hilden, DE) according to the manufacturer recommendation.

2.2 GBS library preparation and DNA sequencing

GBS library was prepared with the restriction enzyme combination PstI and ApeKI at LGC (LGC Ltd, Teddington, UK) according to their in house protocol (Arvidsson et al. 2016). The libraries were 150bp single-end sequenced on an illumina HiSeq (Illumina, Inc., San Diego CA, USA) machine at a depth of approximately 400 million reads for 60 samples.

2.3 Genome-wide allele frequency fingerprints

Demultiplexed fastq files of the GBS data were used for the analysis. The reads were mapped to the available genome assembly FES_r1.0 (Yasui et al. 2016) using BWA (Li & Durbin 2010). A single nucleotide polymorphism (SNP) database was developed by combining read data of the three sample replicates of each accession. At each site where the minimum read depth (RD) of 30 was achieved, the allele frequency of the variant allele was calculated for each cultivar and sites where more than 25 percent of samples had missing data (RD < 30) were removed. The average variant allele frequency at each site was determined [frequency of variant allele / (frequency of reference allele + frequency of variant allele)] and used to filter out sites where the average minor allele frequency was less than 0.01. Allele frequencies were also determined for each sample replicate and a reduced SNP database was generated that included only variant sites with a minimum read depth of at least 30 in all samples. This dataset was used to analyse the similarity of replicates and accessions with R (R CORE TEAM 2008) using the libraries 'psych' and 'pheatmap'. To calculate homozygosity and similarity between accessions, the mean GWAFFs over the three replicates was calculated and loci were considered homozygous if the allele frequency was larger than 0.975 or lower than 0.025.

3 RESULTS

3.1 Allele frequency calling and distribution

Genotyping by sequencing of the 20 buckwheat accessions in triplicate resulted in 3.5-9.5 million reads per pooled sample. Mapping them to the available draft genome (Yasui et al. 2016) resulted in a database containing 40,696 SNPs after filtering. Calculation of the allele frequencies for each sample separately revealed 15,726 high-quality loci after filtering. These loci were distributed on 3363 out of the 387,594 scaffolds reported in the draft genome sequence.

3.2 Reproducibility of GWAFFs

Replicated sampling of the populations resulted in highly comparable GWAFFs within the replicates (Figure 1). The Pearson correlation between replicates of the same accession ranged between 0.971 and 0.999, while between pooled samples of different accessions it ranged between 0.320 and 0.983. All but the accessions Tussi, Theophanu and MinI showed an allele frequency distribution skewed towards the right, indicating that the alternative alleles were present at a low frequency. For Tussi, Theophanu and MinI the allele frequencies were distributed around the extremes (1 or 0, data for Tussi shown in figure 1), implying that the accessions were highly homozygous, which is a consequence of their self-pollinating reproduction system transferred from *F. homotropicum (F.J. Zeller, personal communication).*

3.3 Homozygosity within buckwheat accessions

The buckwheat accessions showed little homozygosity, with the exception of the self-compatible acces-

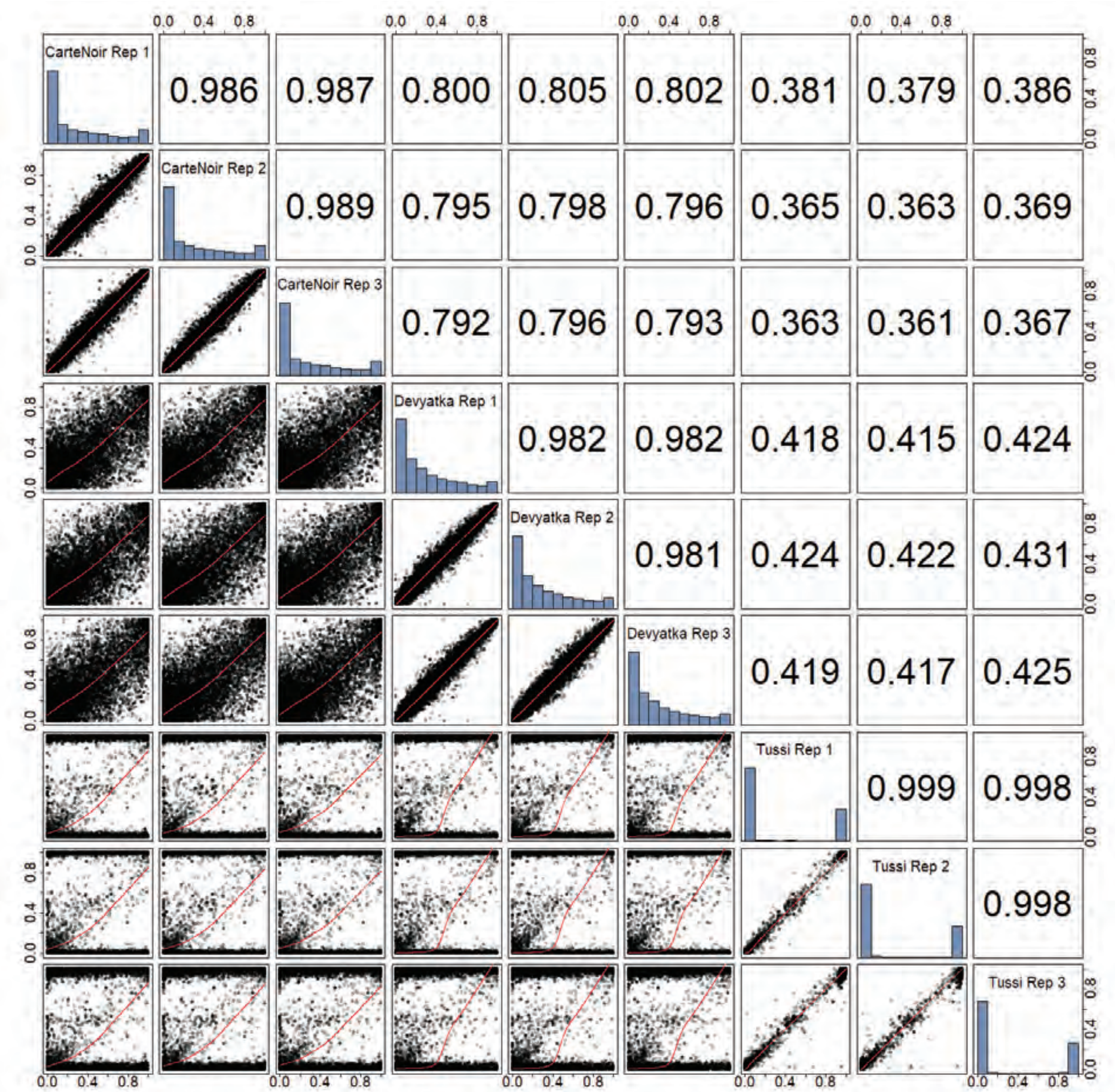

Figure 1: Correlation matrix of genome-wide allele frequency fingerprints within and between the replicated pooled samples of the accessions Carte Noir, Devyatka and Tussi. In the upper diagonal, Pearson correlations between the samples are shown. In the diagonal, histograms of the allele frequency distribution for each sample are shown. In the lower diagonal, the allele frequencies of the two samples are plotted against each other with the red line representing the LOESS (locally estimated scatterplot smoothing) line.
Slika 1: Korelacijska matrica frekvenc odtisov v genomu v in med združenimi vzorci akcesij Carte Noir, Devyatka in Tussi. V zgornji diagonali so prikazane Pearsonove korelacije med vzorci. V diagonali so prikazani histogrami razporeditve frekvenc alelov za vsak vzorec. Pod diagonal so prikazane frekvence alelov dveh vzorcev, rdeča črta označuje LOESS (locally estimated scatterplot smoothing) linijo.

Table 1: Homozygosity rate of 20 buckwheat accessions based on genotyping by sequencing data of 15,726 genetic loci. Genetic loci were regarded as homozygous, if the allele frequency was higher than 97.5% or lower than 2.5%.
Razpredelnica 1: Stopnje homozigotnosti 20 vzorcev ajde, zasnovane na genotipizaciji s pomočjo sekvenciranja 15.726 genetskih lokusov. Lokusi so bili upoštevani kot homozigotni, če je bila pogostnost alela višja od 97,5 % ali nižja od 2,5 %.

Accession	Homozygosity [%]	Accession	Homozygosity [%]
Bamby	22.7	Kerntner Hadn	22.3
Billy	13.4	La Harpe	29.7
Buchsa	17.7	Lileja	15.4
Carolin	17.7	MinI	91.8
Carte Noir	33.3	Orphe	15.5
Darja	12.0	Pyra	15.4
Devyatka	19.4	Rosa	16.6
Dialog	23.4	Temp	25.9
Dikul	18.5	Theophanu	79.0
Drollet	23.5	Tussi	85.7

sions Tussi, Theophanu and Min1 (Table 1). The range of heterozygosity excluding the self-compatible lines ranged from 66.7% (Carte Noir) to 88.0% (Darja) with a mean value of 80.3%.

3.4 Genetic similarity of accessions

The genetic similarity of the accessions was analysed based on a correlation analysis of the mean GWAFFs

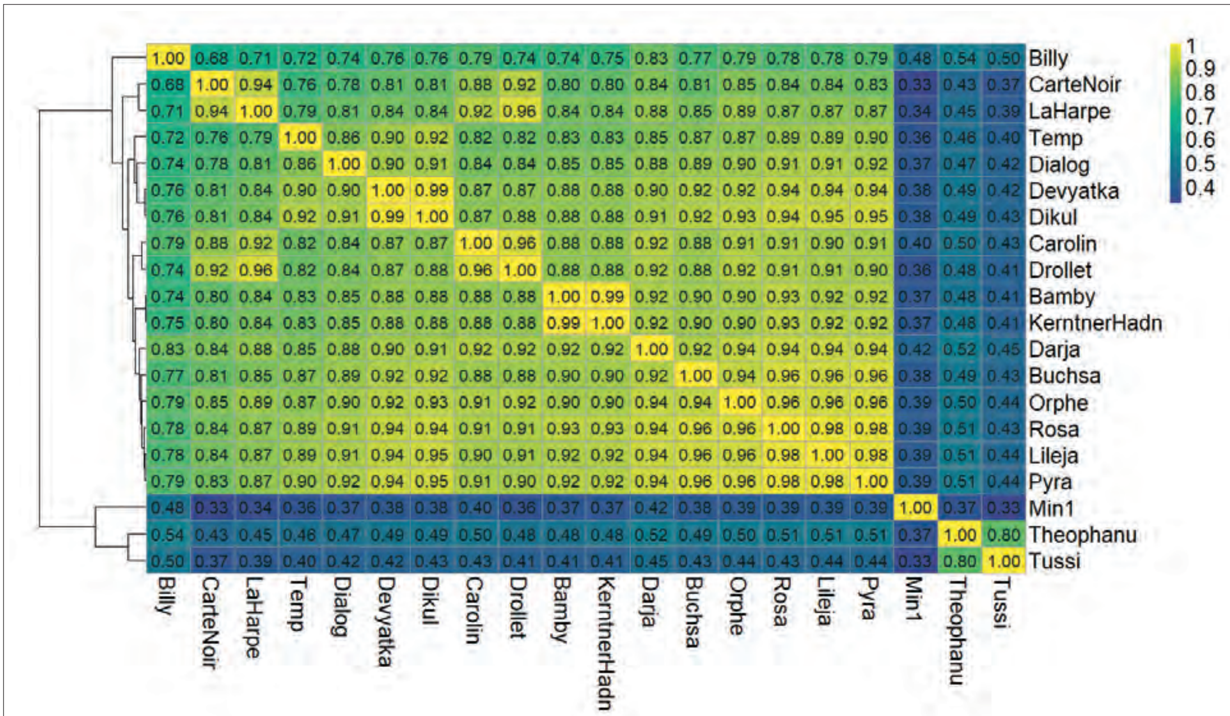

Figure 2. Correlation analysis of genome-wide allele frequency fingerprints of 20 buckwheat accessions. Pairwise Pearson correlations were calculated using the mean allele frequency of the three replicates per accession. On the left side, a hierarchical clustering analysis of the cultivars based on the correlation matrix is shown.
Slika 2. Korelacijska analiza odtisov alelnih frekvenc po celotnem genomu za 20 akcesij ajde. Pearsonove korelacije so izračunane po parih z uporabo srednjih frekvenc alelov treh ponovitev na vsako akcesijo. Na levi strain je prikazano hierarhično združevanje podatkov analiz kultivarjev, zasnovano na korelacijski matrici.

of the 20 accessions (Figure 2). The homozygous, self-compatible accessions Tussi, Theophanu and MinI clustered separately and were distinct from the remaining accessions with Pearson correlations of 0.33-0.54. Within the heterozygous, self-incompatible accessions, several were found to be highly similar, often clustering by the country of origin. A high genetic similarity was revealed between the Central European accessions Bamby, Kerntner Hadn, Darja, Buchsa, Orphe, Rosa, Lileja and Pyra, the Russian accessions Dikul, Devyatka, Dialog and Temp, and the French accessions Carte Noir, La Harpe, Carolin and Drollet (Figure 2).

4. DISCUSSION

4.1 Genome-wide allele frequency fingerprints allow precise genotyping of buckwheat accessions

In this study, we have shown that by pooling 100 individual plants of a buckwheat accession and subjecting them to GBS, representative and highly reproducible GWAFFs can be obtained. This allowed us to genetically characterize 20 buckwheat accessions at unprecedented precision. We identified genetically similar accessions and found that they often cluster by the region of their origin. The genetic similarity of accessions bred in the same country or region may be an indication of the narrow genetic base in each country with limited gene-flow between breeding programs. Analysis of further accessions would yield a better understanding of the buckwheat genetic resources worldwide and may allow to set exchange and conservation priorities.

4.2 Importance of robust genotyping method to implement genomics-assisted breeding in buckwheat

Accurate genotypic data, such as the GWAFFs presented in this work, are crucial to describe buckwheat breeding materials and investigate the genetic diversity between different buckwheat accessions. Furthermore, they enable to associate allele frequencies to plant phenotypic traits and nutrition quality parameters that are most reliably obtained for accessions rather than single plants.

In our study the sequencing reads were aligned to the publicly available reference genome (Yasui et al. 2016), which is still fragmented and does not assign chromosome numbers to the scaffolds. With the upcoming high-quality assembly by NRGene (NRGENE 2018), a better understanding of the genetic distances between polymorphisms and their density on the chromosome will be possible. Assigning genomic locations to the polymorphisms genotyped will increase the efficiency to select for superior germplasm in future crossing-experiments via marker-assisted or genomic selection, and may allow to find candidate genes for certain traits.

4.3 High heterozygosity within buckwheat accessions emphasizes the need to use population genetics approaches

This study was the first to genetically describe buckwheat materials using allele frequencies instead of bi-allelic SNPs. We found that on average 80.3% of the genetic loci covered by GBS were heterzygous. Hence, genotyping a single plant to represent the entire genepool of an accession would result in missing out a large part of the diversity. The accession-specific allele frequencies can, however, be dynamic; for example genetic drift may act if population sizes are small (e.g. seed multiplication from a small batch of seeds) or if certain genotypes within the population cope better with new climatic conditions and therefore contribute more seeds to the next generation (Wright 1931). How these dynamics have affected buckwheat populations in the past is not known, but documenting the changes in allele frequencies in the future may allow to better understand the genetic basis of adaption to new environmental conditions (Günther & Coop 2013).

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BUCKWHEAT BREEDING. PAST, PRESENT AND FUTURE

ŽLAHTNJENJE AJDE V PRETEKLOSTI, SEDANJOSTI IN PRIHODNOSTI

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ABSTRACT
Buckwheat Breeding. Past, Present and Future.

Buckwheat crop improvement by breeding has been taking place over the past 100 years or more. During this time there has been improvements in many desirable agronomic characteristics which has resulted in higher yields in many of the breeding programs. Phenotypic modifications, such as dwarf, semi-dwarf and branching have been reported. There has also been an effort to increase flower number as this has been shown in cross pollinating buckwheat, to increase yields. Flower cluster modifications and their effects on yield have also been studied. Increased reports on the discovery of buckwheat wild species have been reported from several programs with many interspecific crosses having taken place. Several of these crosses were performed with *Fagopyrum esculentum* in efforts to increase variability which can be used to increase yield potential as well as to obtain increased nutritional components. More recent efforts have focused on the development of self-pollinating buckwheat, both from introgression of genes from *Fagopyrum homotropicum* as well as from mutations in cross pollinating buckwheat. The main problem has been in breeding depression which has occurred in many of the reported attempts. However, high yielding homomorphic, self-pollinating varieties have been developed and are now in commercial production. There is now emphasis being placed on many of the nutritional aspects of buckwheat flour as well as value added components. It is expected that this will increase over time.

Key words: Buckwheat breeding, homomorphic, autogamous buckwheat.

IZVLEČEK
Žlahtnjenje ajde v preteklosti, sedanjosti in prihodnosti

Žlahtnjenje ajde poteka že več kot 100 let. V tem času je bila dosežena izboljšava željenih agronomskih lastnosti, kar je pri mnogih programih žlahtnjenja omogočilo večje pridelke. Raziskovalci poročajo o fenotipskih modifikacijah, kot je pritlikava ali pol-pritlikava rast in razvejanje. Za povečanje pridelka so bile raziskane modifikacije socvetij. Število poročil o odkritjih divjih sorodnikov ajde in o mnogih medvrstnih križanjih se je v zadnjem času povečalo. V mnoga od teh križanj je bila vključena navadna ajda (*Fagopyrum esculentum),* da bi povečali variabilnost, kar bi lahko omogočilo povečanje pridelka in izboljšanje prehranskih lastnosti. Novejša prizadevanja so se osredotočila na razvoj samooplodnosti pri ajdi, z vključitvijo genov vrste *Fagopyrum homotropicum,* kot tudi mutacij pri ajdi, ki se je opraševala navzkrižno. Pri tem je bila glavna težava preseči depresijo zaradi samooploditev, depresija se je pojavila pri večih poskusih samooploditve. Ne glede na to je uspelo dobiti visokorodne homomorfne samooplodne sorte za ponudbo na trgu semen. Sedaj se prizadevanja usmerjajo k izboljšanju prehranske vrednosti ajde in pomembnih sestavin v ajdovi moki. Pričakovati je, da se bo pomen prehranske vrednosti ajde sčasoma še povečeval.

Ključne besede: žlahtnjenje ajde, homomorfnost, samooplodna ajda

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INTRODUCTION

Buckwheat (*Fagopyrum esculentum*) crop improvement has been ongoing for a very long time. It is a very nutritious crop and fits well into crop rotations. However, due to its heteromorphic sporophytic crossing system it has been a difficult species for plant breeders to make rapid progress. This has resulted in many other crops obtaining much higher yields and increased nutritional characteristics at a much faster rate that buckwheat. Therefore, there are many reports of countries and areas that have seen decreased production of buckwheat over the past century with marginal production now being the norm in many areas.

Buckwheat crop improvement programs have produced many new varieties with increases in yield or with other increased beneficial nutritional properties. However, when looked at from a commercial outlook there has been little change in yield from these efforts. Perhaps this is due to there being only a few buckwheat breeding programs world-wide focusing only on buckwheat. Many of the present breeding efforts are combined with breeding on other crops and therefore have limited ability to make major improvements. Buckwheat crop improvement must therefore become more coordinated in order that crop improvement objectives can be realized.

PAST BUCKWHEAT BREEDING EFFORTS

Due to *Fagopyrum esculentum* having a heteromorphic, sporophytic incompatibility system initial crop improvement methods were achieved by selection. However, this was maternal selection only as the pollen parent was unknown. Pollinations between individual plants or lines was severely restricted as all crossing parents and the resulting progeny had to have spatial or caged isolation from other buckwheat. Thus the breeding programs could only make limited improvements as compared to those made with rice, wheat or maize.

Early studies have produced homomorphic self-fertile plants (Marshall 1969, Fesenko & Lokhatova 1981) by probable mutations. However, in many cases these also had severe inbreeding depression from possible recessive mutations that could survive in cross-pollinating lines. There have been exceptions to this as the author found one line that was homomorphic with short anthers and pistils and that had no inbreeding depression after several generations.

There has been a lot of recent interest in attempting interspecific crosses, especially after the finding and reporting of new buckwheat species (Ohnishi 1991, 1998a, 1998b, Chen 2016). The finding and identification of these wild species of *Fagopyrum* has triggered, not only a lot of interest in studying the possible interspecific crosses that can be possible, but has also resulted in the development of essential methodology required in the making of the crosses, such as embryo rescue techniques or hot water emasculation. Although reports of successful crosses that have been made (Wang & Campbell 1998, Chen 2016) have created a great deal of interest in this area, many of the interspecific hybrids have been found to be sterile. The major exception to this has been the cross of *Fagopyrum esculentum* by *Fagopyrum homotropicum* (Campbell 1995). This cross has paved the way to successful introgression of the self-compatible character into common buckwheat.

Nutritional aspects of buckwheat has also received interest in the past. Increasing the rutin content has been the focus of some breeding programs and buckwheat germplasm collections have been screened and high rutin accessions have been identified. Certainly buckwheat has long been viewed as having other desirable nutraceutical properties and these have also received attention.

PRESENT BUCKWHEAT BREEDING EFFORTS

Buckwheat breeding programs at the present time are almost all focused on cross-pollinating common buckwheat. Although Tartary buckwheat has received limited interest, especially in easy dehulling types, there has not been a concerted effort on this crop. Much of the common buckwheat in the large area of India, China, Nepal and Bhutan is mainly grown in high, arid regions in the mountains. It is grown as a subsistence crop and as such is an essential part of the diet for these people, however, these areas do not appear to get the notice or support required to better support their needs. Most of the buckwheat grown are local land races that have received selection from the growers although there are some varieties available that have been developed by public institutions.

Buckwheat germplasm efforts have received attention in several countries around the world. Many of these have been characterized and evaluated for qualitative and quantitative traits so that they are now available for utilization in buckwheat improvement programs. However, there is an increasing loss of genetic variability in many areas and this has become a critical issue (Rana, Singh & Yadav 2016).

Efforts in yield improvement in cross-pollinating common buckwheat have been mainly centered on flower number, cluster number, branching habit, plant habit, leaf size and shape, days to maturity and percentage seed set. As the percentage seed set in buckwheat is very low, usually in the range of 11 to 12 percent of flowers produced, this has resulted in plants with more flowers in order to increase yield. The high abortion rate has been the major obstacle of obtaining higher yielding cultivars.

FUTURE BUCKWHEAT BREEDING EFFORTS

Buckwheat breeding efforts, despite the consumption and varied uses of this crop, sadly lacks a major, coordinated effort. It appears that the most interest in past buckwheat breeding programs has been on the commercial aspects of the crop. Although this is a very valid reason for these efforts, the needs of the poor growers that utilize this essential crop for their subsistence has been sadly neglected. Most of the agronomic studies have been conducted to increase yields or nutritional aspects of the crop in high input agricultural management systems and there is a dearth of information on production aspects of this crop under subsistence farming practices which growers in these areas.

As common buckwheat has a very high abortion rate, of almost 88% of flowers produced, this is one of the major concerns which must be addressed in order for higher yielding cultivars to be developed, which can compete better with other crops for grower acceptance. The production of such a high percentage of flowers that do not contribute to yield but require a very large input from the plants will require coordinated efforts to overcome. Unfortunately, the finding of plants/lines that have a much lower flower number but still achieve high yields is extremely difficult. But as a doubling of yield should only require about 25% of the flowers now being produced then the inputs the plant is now putting into the 75% of the flowers which do not contribute to yield could be diverted into seed production. As there is a great deal of variability in cluster shape and number, as well as in flower number, that has been found in self-pollinating buckwheat perhaps this is where the most future efforts should be expended (Figs. 1 – 17).

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Fig. 1: Field of self pollinating buckwheat
Slika 1: Polje s samooplodno ajdo

Fig. 2: Leaf sizes
Slika 2: Velikosti listov

Fig. 3: Plant habit of Kiku types
Slika 3: Kiku oblika rastlin

Fig. 4: Kiku branching habit
Slika 4: Razvejanje pri rastlinah Kiku

Fig. 5: Kiku has very short internodes at first
Slika 5: Na začetku rasti so internodiji zelo kratki

Fig. 6: Early Kiku growth
Slika 6: Začetek rasti pri Kiku

Fig. 7: Early ground cover by Kiku
Slika 7: Rastline Kiku hitro pokrijejo tla

Fig. 8: Plant with early 'weed control' leaves
Slika 8: Rastlina z obliko listov, ki zgodaj zasenčijo rast plevelov

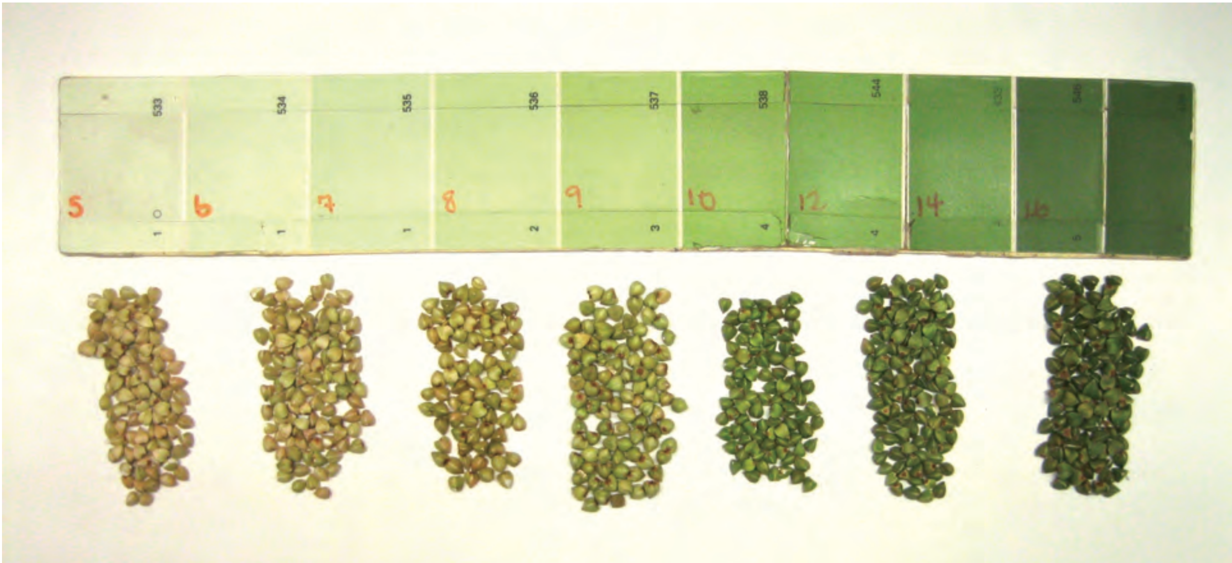

Fig. 9: Enhanced green testa types
Slika 9: Različne stopnje zelenega obarvanja teste

Fig. 10: Flower cluster types that have been found in common buckwheat
Slika 10: Različne oblike socvetij ki smo jih ugotovili pri ajdi

Fig. 11: Enhanced flower clusters
Slika 11: Povečano število socvetij

Fig. 12: Enhanced flower cluster plants
Slika 12: Rastline z obogatenimi socvetji

Fig. 13: Differing auxillary flower cluster types
Slika 13: Različni tipi stranskih socvetij

Fig. 14: Determinant flower clusters
Slika 14: Socvetja ajde s končno rastjo

Fig. 15: Ball cluster
Slika 15: Kroglasto socvetje

Fig. 16: Plant with ballclusters
Slika 16: Rastlina s kroglastimi socvetji

Fig. 17: Red pericarp
Slika 17: Rastlina z rdečim perikarpom

BUCKWHEAT AND AVALOKITEŚVARA

AJDA IN AVALOKITEŚVARA

Yutaka HONDA[1]

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ABSTRACT
Buckwheat and Avalokiteśvara

I investigated the situation of the dedication ceremony of buckwheat noodle in two temples. The buckwheat noodle is dedicated to Avalokiteśvara in both temples. These temples have long history from the foundation, however these ceremonies are never old, if anything new. Buckwheat production increased three times in the past thirty years in Japan. Domestic buckwheat is recognized as good taste and good quality. People visit temple and eat the famous buckwheat noodle in temple town. The interests or merits in the both of restaurants' owners and the temple's priests agree each other. Increasing of production gives birth to new concerned industry or activity of old industry. We can see that the increase of the crop production has the new potential in the human culture.

Key words: buckwheat, hand-made buckwheat noodle, Avalokiteśvara, Buddhism temple

IZVLEČEK
Ajda in Avalokiteśvara

V dveh templjih je avtor raziskoval obred posvečenja ajdovih rezancev. V obeh so ajdovi rezanci posvečeni božanstvu Avalokiteśvara. Templja imata dolgo zgodovino, toda svečanosti posvečene ajdi so se začele šele v novejšem času. V zadnjih tridesetih letih se je obseg pridelovanja ajde na Japonskem potrojil. Na Japonskem pridelana domača ajda je cenjena zaradi dobrega okusa in kakovosti. Ljudje obiskujejo tempelj, da bi v tempeljskem mestu jedli znano ajdo. Lastniki restavracij in svečeniki v templju skupaj skrbijo za kakovost. Povečana izdelava testenin omogoča pojavljanje novih izdelovalcev in razvoj dosedanjih. Povečano pridelovanje ajde je pomembno tudi z vidika kulturnega razvoja.

Ključne besede: ajda, ročno izdelane ajdove testenine, Avalokiteśvara, budistični tempelj

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1 INTRODUCTION

The domestic production of buckwheat increased three times of 1980's in Japan. Present cultivated area increased from about 20,000 ha in 1986 to 63,900 ha in 2018 (Figure 1). According to the increase of buckwheat production the related industries have been activated. Especially the industry of flour millers and buckwheat noodle restaurants increased. Buckwheat noodle restaurants are often located in temple towns which spreads in front of gates of the temples from old times in Japan. The guests of these restaurants are mainly the visitors of the temple to pray or for sightseeing. Therefore owners of the restaurants pray to the temple for thriving business or good harvest of buckwheat. And they execute the ceremony of the dedication of buckwheat noodle for Buddha in a special term, for example buckwheat festival. I am reporting about the ceremony of dedication of buckwheat noodles in two temples.

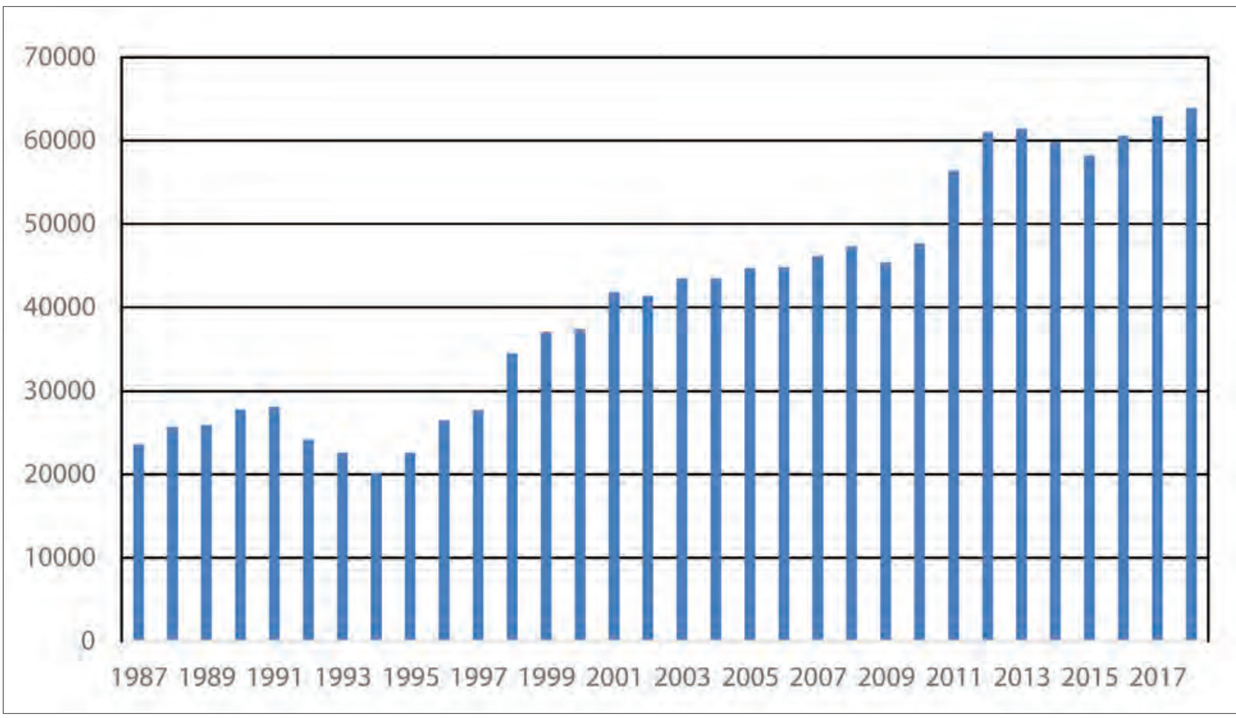

Figure 1: The increase of cultivated area in domestic buckwheat production in Japan
Graf 1: Povečanje obsega domačega pridelovanja ajde na Japonskem

2 METHODS OF RESEARCH

I investigated two famous Buddhist temples near our organization. There are many buckwheat noodle restaurants in front of the gate of these two temples, as if they seem street or town of buckwheat noodle restraunts. These streets are called as the temple town in Japan like cathedral town near church in western world.

One is Jindaiji temple which belongs to Tendai sect, whose general temple is Hieizan Enryakuji temple in Shiga prefecture. The other is Izurusan Manganji temple which generally is called as Idurusan Manganji, which belongs to Chisan school of Shingon sect, whose general temple is Iobusan Negoroji Chishakuin temple, in Kyoto city.

The ceremony about the dedication of buckweat for Avalokiteśvara is performed once a year. I participated in the ceremony, and I went to the temple and heard the details of the ceremony from concerned persons of buckwheat noodle restaurant union, tourism association or the temples.

3 WHAT IS AVALOKITEŚVARA ?

'Avalokiteśvara' is written as 'अवलोकितेश्वर' by devanargary, which is synthesized from avalokita 'अवलोकित' which means 'observe' and 'iśvara' 'ईश्वर' who is a person with supernatural power, translated by Xuanzang who was a famous Buddhist priest in China, AD 7C. And the other word 'Avalokitasvara' 'अवलोकितेश्वर' was the word synthesized from avalokita 'अवलोकित' and svara 'स्वर' which means sound, that is 'observation of sound' in old translation by Kumārajīva who was also translator of the Buddhist priest, arrived from the Western Regions of China, AD 4C to 5C.

These two translations are showed as '观自在/観自在' and '观音/観音' in Chinese letters, respectively. The translation by Kumārajīva was very popular and familiar with East Asian people, threfore Japanese people mainly use the latter word '观音/観音'.

4 JINDAIJI TEMPLE

4.1 Profile of Jindaiji-Temple

Jindaiji-Temple is located at Chofu, Tokyo, which belongs to Tendai Sect whose main believed sutra is lotus of sutra which is very popular one in East Asia, China, Korea and Japan. Avalokiteśvara is written in the 25 chapters of this Sutra, and he rescue people with troubles. And the owners of restaurants dedicated the Avalokiteśvara to wish thriving business or the safety of food for the temple (Photo 1). And they open the ceremony of dedication once a year in a buckwheat festival term of autumn.

Photo 1: Avalokiteśvara in Temple town of Jindaiji-Temple.
Slika 1: Avalokiteśvara v tempeljskem mestu templja Jindaiji

4.2 The order of ceremony

1. Chief priest march with chefs of buckwheat noodles and other priests from the priest's quarter to the front of main temple.
2. Buckwheat chefs dedicates their technics about making buckwheat, making dough from buckwheat flour, stretching the dough and making the belt, cutting the noodle belt for making buckwheat noodles (Photo 2). Priests respond the chanting of Buddhist hymns, kind of religious chorus at the same time in front of main temple.
3. They march from there to the statue of Avalokiteśvara with buckwheat noodles.
4. They dedicate the noodles for Avalokiteśvara in sutra chanting and people offer incense.
5. They march back to the priest's quarter.
6. Visitors eat the dedicated buckwheat noodles in the priest's quarter.

Photo 2: Dedication of making buckwheat noodles in front of the main hall of the Jindaiji -Temple
Slika 2: Posvetitev izdelave ajdovih rezancev pred glavnim poslopjem templja Jindaiji

5 IDURUSAN MANGANJI TEMPLE

5.1 Profile of Idurusan Manganji -Temple

Idurusan Manganji Temple is located at Tochigi whose principal image is the Thousand Armed Avalokiteśvara. Many buckwheat noodle restaurants locate in front of the gate of Manganji - Temple. The Festival of buckwheat exposed to cold are held in January. And the dedication ceremony of buckwheat noodles is held at the same time.

Photo 3: Meditation by sitting under a water fall in Manganji- Temple.
Slika 3: Meditacija pod slapom v templju Manganji

5.2 The order of ceremony

1. Meditation by sitting under a water fall (Photo 3)
2. Dedication ceremony of making buckwheat noodles in front of the main temple (Photo 4).
3. Homa ceremony in main temple (Photo 5)
4. After that people or visitors can eat the new buckwheat noodles made from buckwheat grain exposed to cold

6 DISCUSSION

I have seen buckwheat festival and concerned ceremonies at two temples. These temples have a long history from the foundation. And they have many important and old cultural resources. Especially Jindaiji-Temple is proclaimed as the National Treasure, the Buddha statue is from Hakuho period of 1200 years ago. Compared with these resources the ceremony or festival on buckwheat is not so old. 'Jindaiji Buckwheat Festival' was 37th and 'Idurusan Dedication of Buckwheat Exposed to Cold' was eleventh. Buckwheat was not so important crop in Japanese agriculture about thirty years ago. However after that the domestic production of buckwheat increased for three times. The consumers consider that domestic buckwheat is very important from the viewpoint of quality or taste. The activity of industries concerned with buckwheat become popular in increasing the domestic production. The restaurants utilize the domestic buckwheat naturally, because domestic is recognized as good quality in taste. The good tasting buckwheat noodles make the fun and domestic

Photo 4: Dedication to Avalokiteśvara in front of the main hall of Manganji-Temple.
Slika 4: Čaščenje Avalokiteśvare pred glavno stavbo templja Manganji.

production is increased. The buckwheat noodle restaurants become popular and gather people to temple or restaurant.

Interests concerned in buckwheat matched between temple and restaurant. Here new ceremonies or festivals were born in the temple towns. Increasing of crop production give birth of new industry or increased activity of old industry. We can state that the increase of crop production has new potential in our life and culture.

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Photo 5 : Homa ceremony in the main hall of the Manganji-Temple.
Slika 5: Svečanost v glavni stavbi templja Manganji.

FUNCTIONAL CHARACTERIZATION OF AN ENDOSPERM SPECIFIC PROMOTER *p1062* FROM COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH) FOR DRIVING TISSUE SPECIFIC GENE EXPRESSION

FUNKCIJSKE LASTNOSTI ENDOSPERMSKEGA PROMOTORJA *p1062* NAVADNE AJDE (*FAGOPYRUM ESCULENTUM* MOENCH) ZA OMOGOČANJE TKIVNO SPECIFIČNE EKSPRESIJE GENOV

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ABSTRACT

Functional characterization of an endosperm specific promoter *p1062* from common buckwheat (*Fagopyrum esculentum* Moench) for driving tissue specific gene expression

Seed storage proteins of grain crops meet the major dietary protein requirement of over half of the world population. PCR based genome walking the 5'UTR of the gene coding for a lysine rich legumin type protein amplified a 1.1kb DNA fragment representing the promoter region of the gene. Clustal alignment of this sequence with other sequences in the Genbank database clearly showed 100 percent complementary base match of 282 bases at the 3' end of the sequence, corresponding to nucleotide position 780-1062 with correspondingly similar number of bases on the 5' end of the 1.7kb *Bwleg* gene.We detected one prolamin box and three RY-repeat motifs in the sequence. Seven deletion fragments of the putative promoter were generated by 5' nested PCR and cloned in pCAMBIA1304 upstream of *GUS* gene after excising the CaMV 35S promoter from the vector. *Arabidopsis* plants plants harbouring the deletion construct *pBwlDF1* to *pBwlDF6* clearly showed seed specific expression of the reporter gene. Seeds harbouring the constructs *pBwlDF3*, *pBwlDF4* and *pBwlDF5* showed a nearly threefold decrease in GUS activity than those harbouring the construct with full length promoter.

Key words: buckwheat, DNA, promoter, constructs

IZVLEČEK

Funkcijske lastnosti endospermskega promotorja *p1062* navadne ajde (*Fagopyrum esculentum* Moench) za omogočanje tkivno specifične ekspresije genov

Založne beljakovine semen zrnastih poljščin ustrezajo glavnim potrebam po beljakovinah za več kot polovico svetovnega prebivalstva. S PCR in 5'UTR so za kodiranje kakovostnih beljakovin leguminskega tipa pomnožili odlomek 1,1 kb DNK, ki je promotorsko gensko območje. Vzporejanje te sekvence z drugimi sekvencami podatkovne baze genske banke jasno pokaže popolno komplementarnost 282 baz na 3' koncu sekvence, kar ustreza pozicijam 780-1062 z ustreznim številom baz na 5' koncu gena 1,7 kb *Bwleg*. V sekvenci smo odkrili eno prolaminsko škatljo in tri RY-ponovljene motive. Sedem delecijskih fragmentov putativnega promotorja smo generirali z 5' PCR kloniranjem pCAMBIA1304 navzgor od *GUS* gena po izločitvi promotorja CaMV 35S iz vektorja. Semena s konstrukti *pBwlDF3*, *pBwlDF4* in *pBwlDF5* so izražali skoraj trikratno zmanjšanje GUS aktivnosti v primerjavi s konstrukti, ki so vsebovali polne dolžine promotorjev.

Ključne besede: ajda, DNK, promotor, konstrukti

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INTRODUCTION

Plant genetic resources, representing the entire generative and vegetative reproductive material of species with economic and /or social value for the agriculture of the present and the future, with special emphasis on nutritional plants are the most important gifts of nature to mankind. Of the total available genetic diversity, mankind has utilized only a few plants as major food sources with cereal grains and legume seeds being the major sources of vegetarian dietary proteins for human consumption. However, the nutritional quality of the proteins in both these crops do not match the WHO standards. While the major amino acid deficiency in legume seed proteins is their low content of sulphur containing amino acids cysteine and methionine, cereal proteins have low levels of lysine (Boulter 1981; Shotwell & Larkins 1989).

Strategies developed over the years to improve the level of essential amino acids in seed storage proteins of important crop plants through conventional breeding. However, in most cases these attempts have either led to a severe depletion in seed storage protein levels or abnormalities in seed development. The negative correlation between the seed protein content and the level of essential amino acids per unit protein has, therefore, come as a major handicap in improving the amino acid composition of seed proteins in crops. Due to such limitations in conventional breeding methodologies, molecular approaches have provided alternate strategies to conventional breeding programmes aimed at compensation of amino acid deficiencies in conventional crop plants. With the potentiality of genetic engineering being amply demonstrated a first step towards production of transgenic plants with improved amino acid composition entails the purification and characterization of the specific seed storage protein rich in essential amino acids followed by cloning a full length gene coding for the target protein. Such proteins and their genes have been isolated from soyabean (Hill & Breidenbach 1974), pea (Higgins et al. 1986; Hoffman et al. 1988), *Lupinus albus* (Melo et al. 1994), rice (Takaiwa et al. 1987; Krishnan & Pueppke 1993), oat (Shotwell et al. 1990), *Phaseolus vulgaris* (Goossens et al. 1994), rape seed (Coupe et al. 1993); field bean (Heim et al. 1994); chickpea (Khitha et al. 1995; Mandaokar, & Koundal 1996; Saha & Koundal 1998); *Chenopodium* (Dey et al. 1993); *Brassica* (Dasgupta & Mandal 1991; Utsumi et al. 1993; Dasgupta et al. 1995); grain amaranth (Raina & Datta 1992), buckwheat (Bharali, & Chrungoo 2003). Due to the balanced amino acid composition, high nutrient value and homology with seed storage proteins of leguminous group of plants, the nutritionally rich component of protein of common buckwheat (*Fagopyrum esculentum* Moench) which is a 26kDa basic subunit, having more than 6% lysine and nearly 2% methionine (Bharali, & Chrungoo 2003) could be an important candidate for compensation of limiting amino acid in plants deficient in such amino acids. The present paper describes the functional characterization of an endosperm specific promoter *p1028* from common buckwheat (*Fagopyrum esculentum* Moench) for driving tissue specific gene expression.

MATERIALS AND METHODS

Plant Materials: Grains of common buckwheat (*Fagopyrum esculentum* Moench) [Accession No. IC-188669] were procured from North Eastern Regional Station of National Bureau of Plant Genetic Resources, Shillong. (ii) Seeds of Arabidopsis col-0 (Columbia) ecotype were procured from the University of Nottingham, School of Biosciences through the European Arabidopsis Stock Centre.

DNA Isolation: Total genomic DNA was isolated from 14 days old etiolated seedlings of common buckwheat (acc. no. IC-188669) following a modified CTAB extraction protocol (Murray &Thompson 1980).

RNA Isolation: Grains of common buckwheat were harvested at the mid-maturation stage (16-20DAF) of development and Total RNA was isolated using TRIzol reagent (Invitrogen).

Isolation and *In-silico* analysis of the buckwheat legumin gene: PCR amplification of the legumin gene Bwleg from the cDNA template with primer pair BwlegF (5′GACTAGTATGTCAACTAAACTCATACT3′) and BwlegR (5′ACGCTAGATCTTTAGAAACGCTCCCTC3′). The nucleotide sequences were subjected to in-silico analysis. MOTIF SCAN software was used for motif search on amino acid sequence. The coding regions of the sequences were translated by the EXPASY tool to get the deduced amino acid sequences which were subsequently subjected to BLASTp to determine their homology with other known amino acid sequences in the data bases. Physico-chemical properties like the Molecular weight and isoelectric point (pI) of the deduced amino acid sequences were obtained by ProtParam using EXPASY online tool.

Isolation and characterization of the 5'UTR of legumin gene *Bw*leg of common buckwheat: This study involved construction of genome walking adapter libraries from genomic DNA isolated from common buckwheat and amplification of the buckwheat legumin gene and its 5' upstream region (UTR) from the library. The approach was followed by amplification of the 5' UTR using primers designed from nucleotide sequences obtained during genome walking. After a series of in-silico analysis seven deletions of the putative promoter were created by 5' nested PCR. To further assess the tissue specificity and strength of promoter for driving reporter gene expressions, each deletion fragment was directionally cloned separately in pCAMBIA1304 plant expression vector upstream of GUS and GFP genes for reporter gene expression after excising the CaMV 35S promoter from the vector by digestion with BamHI and SpeI. For verifying the introgression of transgene into the nuclear DNA of transformed Arabidopsis, nuclear DNA was isolated from T1 and T2 generations of transformed Arabidopsis plants. PCR amplification of transgenes was carried out with genomic DNA isolated from transformed Arabidopsis plants as the template and transgene nucleotide sequence specific primers for amplification.

RESULTS AND DISCUSSION

PCR amplification of the legumin gene *Bwleg* from the cDNA template with primer pair BwlegF and BwlegR yielded a single amplicon corresponding to a molecular mass of 1.7kb (Fig.1). BLASTn analysis of the nucleotide sequence showed a maximum homology of 99%, 96% and 90% with a query coverage of 91%, 92% and 85% to common buckwheat legumin gene nucleotide sequences bearing accession numbers D87980, AF152003 and DQ849083, respectively. The sequence has been deposited in GenBank with accession number KM488332. The deduced amino acid sequence of the open reading frame derived from the nucleotide sequence bearing accession no. KM488332 represented a putative 64kDa pre-protein comprised of 565 amino acid residues with a theoretical pI of 5.63. Motif search on the deduced amino acid sequence identified a "Cupin 1" superfamily domain spanning from P'_{49-275} and $P'_{390-539}$. Additionally, domain search on the deduced amino acid sequence using prosite identified an N-terminal signal sequence comprised of 18 amino acid residues represented by the sequence "MSTKLILSFSLCLMVLSC" highlighted in red in figure 2. Sequence analysis of the putative 64kDa pre-protein identified the ASN-GLY proteolytic cleavage site at P'_{377}. While the presence of the ASN-GLY proteolytic cleavage site indicated the presence of an α- and a β-subunit of the protein, the presence of cysteine residues at P´21 and P'381 shows the residues linking the α- and a β- subunit of the protein by a disulphide bond. ExPASy tool identified the α- subunit as a sequence of 376 residues with a predicted molecular mass of 43kDa and a theoretical pI of 5.23. The putative protein showed a lysine content of 1.9%. On the other hand, the β- subunit was identified as a sequence of 181 amino acid residues with a predicted molecular mass of 20kDa and a theoretical pI of 9.51. The subunit showed a lysine content of 5%. Analysis of the number of moles of different amino acids present in the α and β subunits of the putative 64kDa pre-protein revealed the presence of higher levels of lysine and leucine in the β subunit.

The 5' upstream region of buckwheat legumin-type SSP generated was isolated by PCR-based genome walking using Universal Genome Walker Kit from Clontech (USA) which resulted in amplification of a 1.1 kb DNA fragment (Fig.3). The nucleotide sequence of 1062 bases for the amplicon has been deposited in Genbank with accession no. EU595873. It is known that the efficiency of ATG codon recognition is modulated by the context sequence of the codon. The context sequence of ATG at P'_{801} (TCCACCATGTCA) in the nucleotide sequence of p1062*Bwleg* matches the optimal context sequence CCACCATG(G) derived by Kozak (Kozak 1984; Kozak 1986). P**romoter prediction tool (**Neural Network Promoter Prediction**) identified three probable promoter regions** at $P'_{392-442}$, $_{473-523}$ and $_{721-771}$ in the sequence. Out of the three predicted transcription start sites, the TSS at P'_{761} was located closest to the predicted ATG start codon at P'_{801}. The YR rule, i.e., pyrimidine (C/T) at position -1 and purine (A/G) at position +1 of the TSS by Yamamoto (Yamamoto et al. 2007) was suggested to be conserved in *Arabidopsis* and rice genes. The TSS at position 761 also follows the YR rule, having pyrimidine 'C at -1 and purine 'A' at+1 position ($C^{-1}A^{+1}$). Considering 'A' at position 761 (+1) as the predicted TSS and ATG at position 801 (+40) as the initiating codon, the TATA at position $731_{(-62)}$ is therefore considered as the TATA box of the promoter. Apart from TATA box, the sequence revealed several other *cis*-elements, that are involved in the regulation of eukaryotic gene in general and seed-specific expression in particular. Online tool PLACE revealed presence of one prolamin box represented by the -171 core element 5'TGTAAAG3' and three RY-repeat motifs represented by -525, -138, -111 core elements "5'CATGCA3"(Fig 4). The core element 5'CATGCA3' also known as legumin box is considered to be the key element in regulating seed specific expression of genes (Chamberland et al. 1992; Ellerström et al. 1996; Reidt et al. 2000). This box is conserved in all the legumin genes (Shasany & Koundal 2000). The presence of other positive regulatory, enhancer like *cis* elements are only fully functional in conjunction with the core motif 5'CATGCA3' of the legumin box (Bäumlein et al. 1992). While the "P-box" ("TGTAAAG") is a -300 enhancer element present in SSP genes of cereals and several other dicots (Vickers et al. 2006), we detected a "P- box" as a -171 element in the buckwheat legumin gene *Bwleg* promoter p1062*Bwleg*. This element has also been reported to be involved in quantitative regulation of gene expression in seeds (Wu et al. 2000; Chandrasekharan et al. 2003). In many cases the "P-box" and "GCN4" motifs are coupled with each other with only a few nucleotides separating them. This module has been named as "bifactorial endosperm box". Comparative search for regulatory motifs across other seed specific promoters also revealed the highly conserved nature of the 'TGTAAAG',' CANNTG', 'AAAG', 'CACA' and 'CANNTG' (MYC consensus box) motifs in SSP gene promoters of many dicots and monocots (Takaiwa et al. 1996; Sakata et al. 1997).

Seven deletion fragments of the putative promoter were amplified by 5' nested PCR using a common reverse primer *viz*. DLRl and seven forward primers *viz*. DLF1, DLF2, DLF3, DLF4, DLF5, DLF6 and DLF7. This generated a ladder of 7 amplicons with molecular mass of 790kb, 680kb, 560kb, 520kb, 380kb, 250kb and 180kb (Fig.5). Directional cloning of each deletion in pCAMBIA1304 plant expression vector upstream of *GUS* and *GFP* genes for reporter gene expression after excising the CaMV 35S promoter from the vector by digestion with *Bam*HI and *Spe*I generated constructs designated as *pBwlDF7, pBwlDF6, pBwlDF5, pBwlDF4. pBwlDF3, pBwlDF2* and *pBwlDF1*. These constructs were mobilized into *Agrobacterium tumefaciens* (LBA4404) for transformation of Columbia (Col-0) ecotype of *Arabidopsis thaliana*. While PCR amplification with genomic DNA of transformed *Arabidopsis* plants as template revealed amplification of each deletion fragment in conformity with the size of the transgene, no amplification was detected with genomic DNA isolated from untransformed plants of *Arabidopsis thaliana*. Similarly, amplification of GUS reporter gene was observed in the same pattern (Fig.6).

To determine the tissue specific expression and minimum effective promoter length, various plant parts (pod, flower and leaf) of all the seven confirmed homozygous T3 transgenic *Arabidopsis* harbouring buckwheat p1062*Bwleg* deletion construct (*pBwlDF1* to *pBwlDF7*) individually were subjected to GUS and GFP staining (Fig.7). Arabidopsis plants harbouring promoter deletion construct *pBwlDF1* to *pBwlDF6 clearly* showed seed specific expression of the reporter gene. However, wild type and transgenic *Arabidopsis* harbouring deletion construct *pBwlDF7* did not show any reporter gene activity in any tissue. Endosperm specificity of reporter gene expression by previously known seed specific promoters of monocots such as glutelin GluA-2 of rice (Wu et al. 1998), D hordein of barley (Horvath 2000), zein of maize (Russell & Fromm 1997) and dicots like lectin (Phillips et al. 1997), ß-conglycinin ά subunit from soybean (Nishizawa et al. 2003), ARCELIN5 (Goossens et al. 1999) and ß-phaseolin (Burow et al. 1992; van der Geest & Hall 1997) is well known. Reporter gene assay for p1062*Bwleg* clearly revealed the highest activity in plants harbouring the construct *pBwlDF1* followed by a marginally lower activity in plants harbouring the construct *pBwlDF2*. Seeds harbouring the constructs *pBwlDF3*, *pBwlDF4* and *pBwlDF5* showed a nearly threefold decrease in GUS activity than those harbouring the construct with full length promoter. We could not detect any significant GUS activity in plants harbouring the construct *pBwlDF7*. Thus, deletion of 5'CAAT3' $_{(-732)}$ motif, 5'CACA3' $_{(-774)}$ motif, 5'CANNTG3' $_{(-621)}$ motif and 5'AACA3' $_{(-761)}$ motifs had only a marginal effect on promoter activity. On the other hand, deletion of 5'CAAT3' elements at $P'_{-732, -570, -497, -451}$, CACA elements at P'_{-774} and $_{-475}$, 5'CANNTG3' elements at P'_{-621} and $_{-224}$, 5'AACA3' element at P'_{-761} and 5'CATGCA3' element at P'_{-525} caused a marked reduction in GUS expression in the seeds. CATGC, the core motif of legumin box, has been implicated as a key *cis* acting element for seed specific gene expression (Chamberland et al. 1992; Bäumlein et al. 1992). Deletion of this motif within the 2.4 kb *Le*B4 upstream sequence has been reported to lead to a drastic reduction in reporter gene expression, besides driving low level of expression in the leaves (Bäumlein et al. 1992). However, on the basis of their results on progressive deletions, leaving the CATGCATG motif intact in the *Le*B4 promoter, it was concluded that the

CATGC motif was necessary, but not absolutely essential, for SSP for gene expression. *Arabidopsis* plants harbouring the construct *pBwlDF7* did not show any GUS expression in their seeds. This confirms the role of proximal elements like the 5'TGTAAG3' $_{(-171)}$ motif or P-box in regulating the expression of SSP genes. A similar profile of activity was observed for GFP activity in the seeds of transformed *Arabidopsis* plants. This functional analysis revealed the presence of the significant proximal and distal regulatory elements spanned in the biparietal organization of p*1062Bwleg* promoter. Successful introgression of the promoter into a heterologous system for driving spatial and temporal manner of expression of the reporter gene also accentuates its candidature in seed specific gene expression for nutritional enhancement programmes.

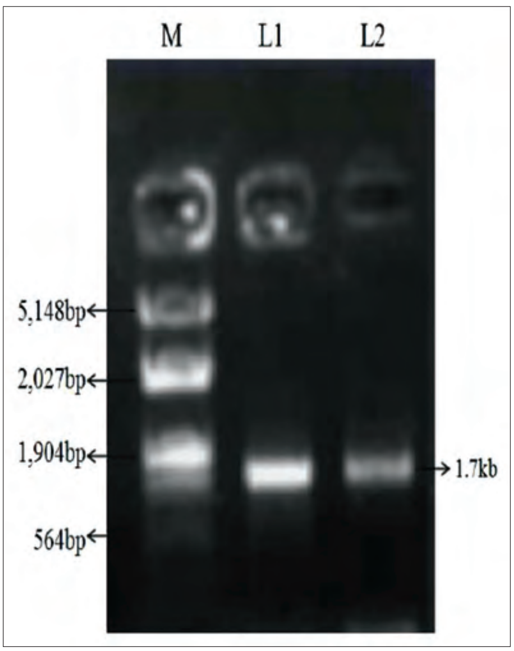

Fig 1: Electrophoresis profile of the legumin gene Bwleg using cDNA as the template with primer pair BlegF and BlegR. M: 500bp ladder and lane 1 shows the 1.7kb profile of the legumin gene.

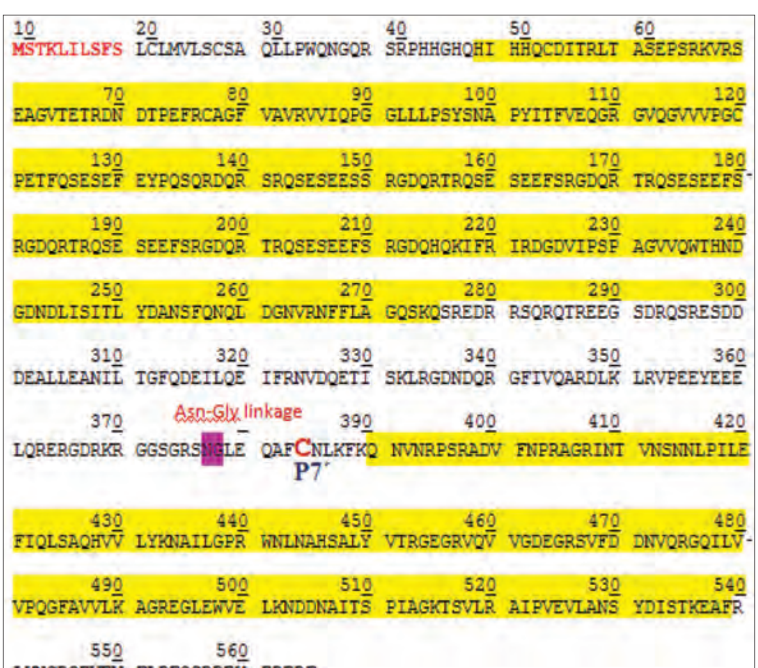

Fig. 2 (a)Sequence analysis of the putative 64kDa pre-protein showing an N-terminal signal sequence comprised of 18 amino acid residues represented by the sequence "MSTKLILSFSLCLMVLSC" highlighted in red and the ASN-GLY proteolytic cleavage site at P'$_{377}$ indicating the presence of an α- and a β-subunit of the protein with the presence of cysteine residues at P´21 and P'381 linking the α- and a β- subunit of the protein by a disulphide bond.

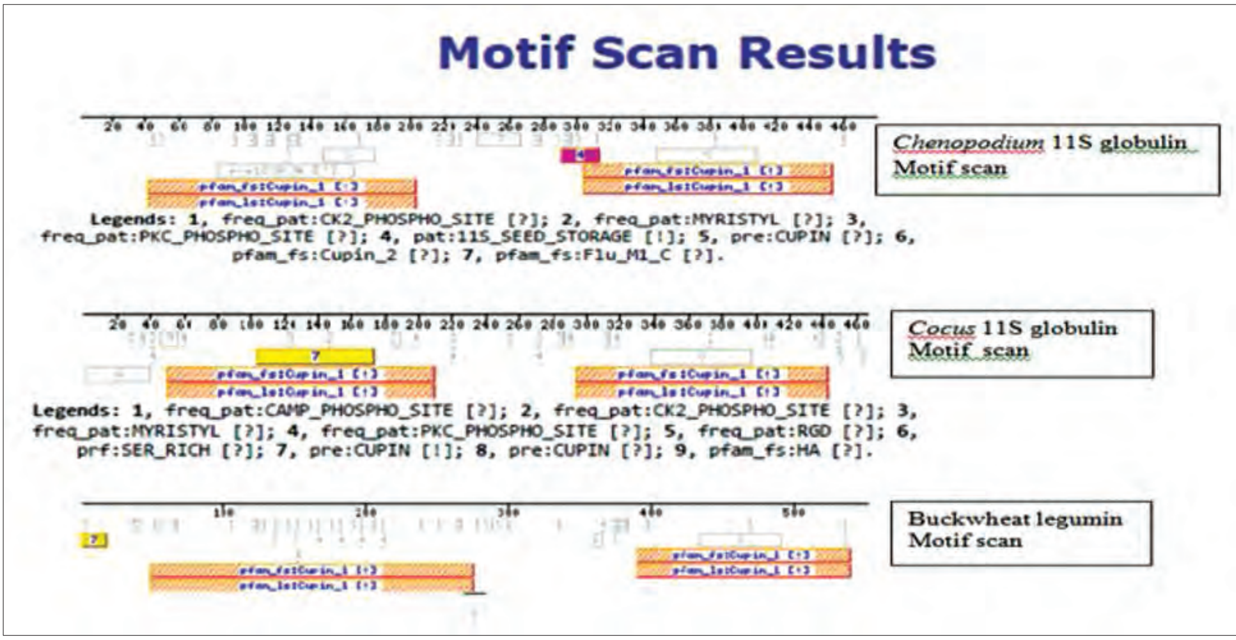

Fig2(b)Motif search on the deduced amino acid sequence showing a "Cupin 1" Superfamily domain spanning from P'$_{49-275}$ and P'$_{390-539}$.

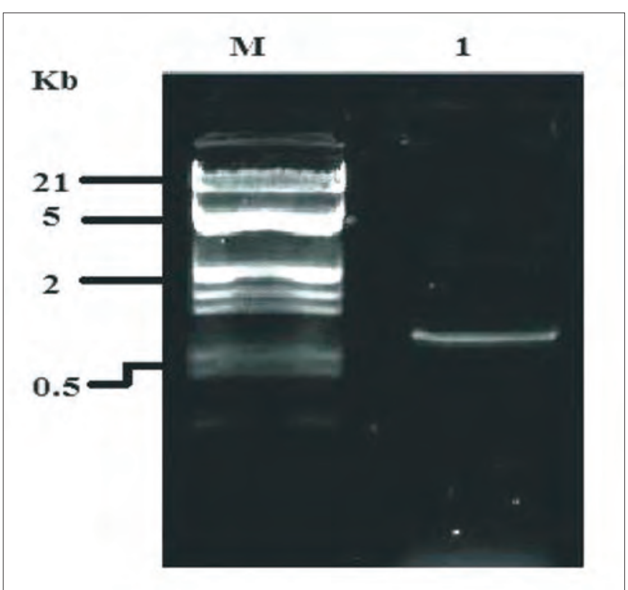

Fig. 3: Electrophoresis profile of the 5'UTR of the legumin like seed storage protein gene. M: EcoRI/HindIII double digest λ DNA, lane 1 shows the profile of the 5'UTR of the legumin like seed storage protein gene.

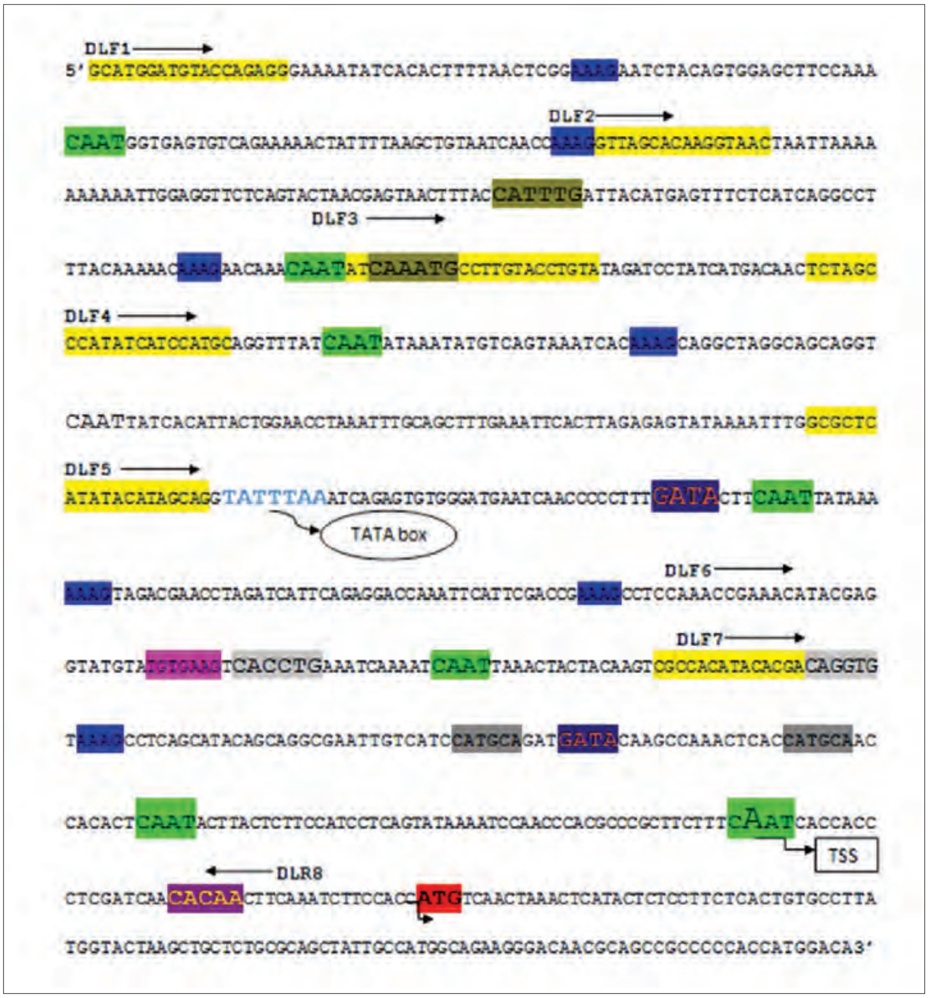

Fig.4: Nucleotide sequence of the the 5' UTR of legumin seed storage protein gene of common buckwheat showing position of primers for deletion analysis along with the position of various regulatory elements.

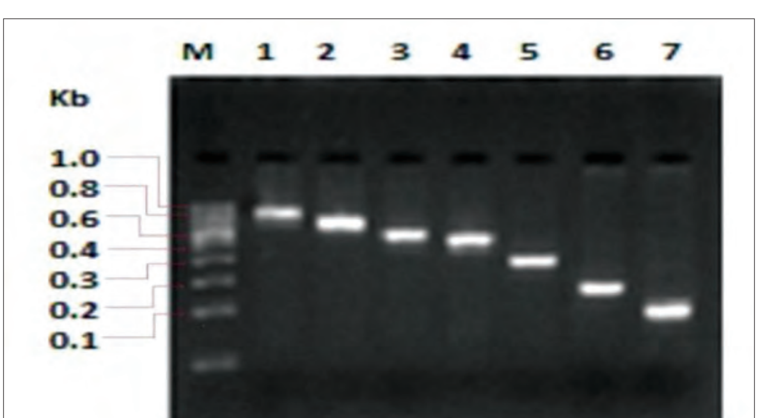

Fig.5: Electrophoresis profile of deletion fragments; Lane M- 100bp DNA ladder,1-DF1(784bp), 2-DF2(668bp), 3-DF3(541bp), 4- DF4(502bp), 5DF5(358bp), 6- DF6(224bp) and 7- DF7(149).

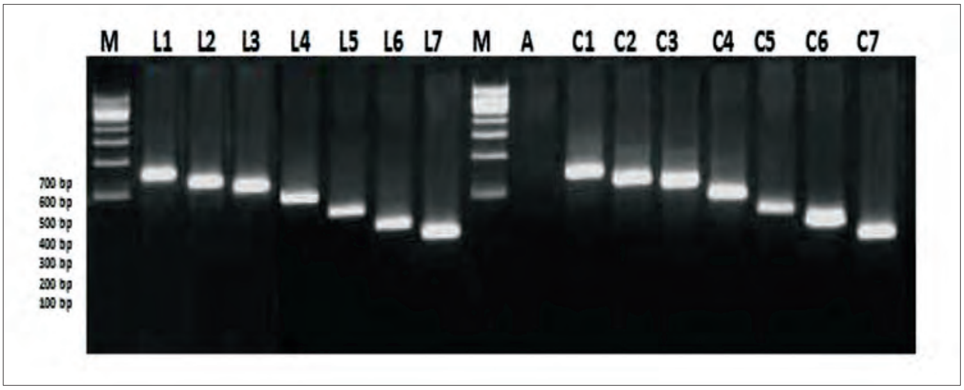

Fig.6: Electrophoresis profile of PCR amplification of (i)Deletion fragments (L1- L7) from transformed plants using genomic DNA isolated from transformed Arabidopsis plants (ii) Deletion fragments (C1- C7) from genomic DNA isolated from common buckwheat (iii) Deletion fragment from Arabidopsis A kept as control using genomic 20DNA isolated M-500bp DNA marker.

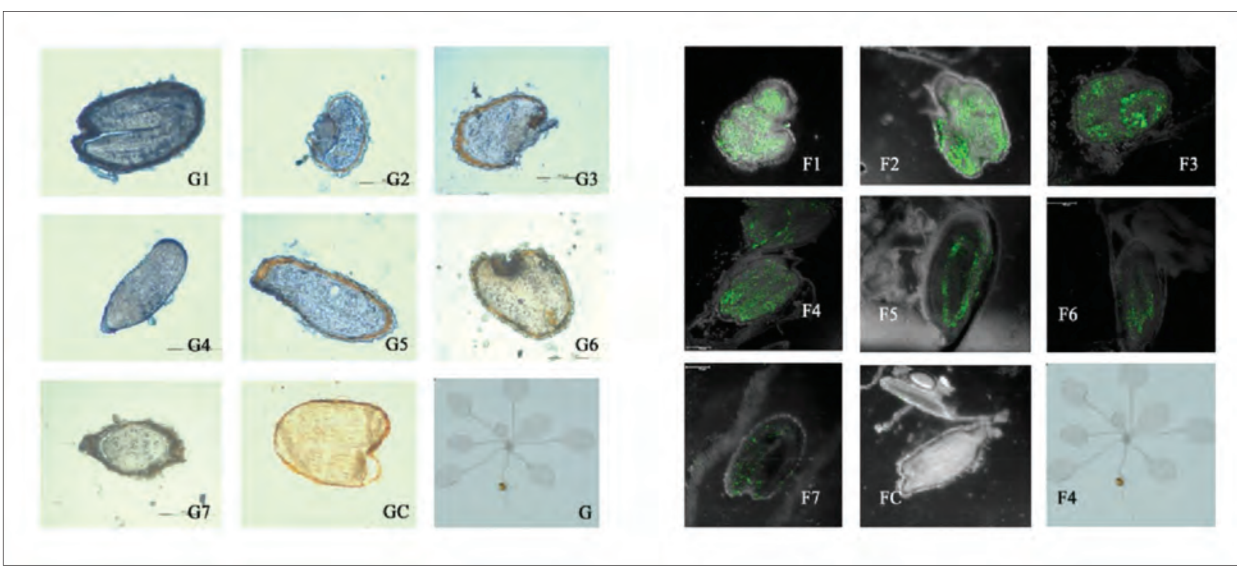

Fig.7: Reporter gene expression profile showing GUS gene (G1-G7) and GFP (F1-F7) activity in transformed Arabidopsis seed sections (transformed with constructs pBwlDF1, pBwlDF2, pBwlDF3, pBwlDF4. pBwlDF5, pBwlDF6 and pBwlDF7)and no activity in GC and FC seed section from control Arabidopsis seed section. leaf and stem of transformed plants (G) also showed no activity.

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BUCKWHEAT IN HUMAN HEALTH - A MEDICAL REVIEW

AJDA IN ZDRAVJE LJUDI – PREGLEDNI ČLANEK

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ABSTRACT

Buckwheat in human health - a medical review

Buckwheat intake has preventive effects at diabetes, obesity, hypertension, high cholesterol levels, stroke, cardiovascular diseases, gallstone formation, cancer, leg oedema as well as level of biomarkers of inflammation in body tissues and remedy in people with gluten sensitivity (celiac disease) as naturally gluten free. A literature review on medical findings is presented.

Key words: buckwheat, health, flavonoids, quercetin, rutin, fiber

IZVLEČEK

Ajda in zdravje ljudi – pregledni članek

Vključevanje ajde v prehrano preventivno vpliva na pojav sladkorne bolezni, visok krvni tlak, visok nivo holesterola, kap, kardiovaskularne bolezni, pojavljanje žolčnih kamnov, novotvorb, kot tudi na nivo bio-označevalcev vnetja telesnih tkiv in je pomebno za ljudi občutljive na gluten zaradi odsotnosti glutena. V delu je predstavljen pregled medicinskih raziskav v zvezi s prehranjevanjem z ajdo.

Ključne besede: ajda, zdravje, flavonoidi, kvercetin, rutin, vlaknine

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INTRODUCTION

Daily intake of different buckwheat food might prevent or reduce many major chronic diseases. Tartary buckwheat contains more rutin (a quercetin-3-rutinoside) than most fruits, vegetables and grain and fagopyritols (especially D-chiro-inositol), with high value in humans with diabetes and obesity. Daily intake of common and Tartary buckwheat cookies in a Slovenian-Swedish-intervention study during 4 weeks in 62 preschool teachers gave interesting effects. The results were a highly significant reduction of total serum cholesterol, less tiredness, improved lung capacity, reduced blood neutrophil biomarkers, all signs of prevention of chronic diseases.

Medical case studies in Sweden with some modern chronic diseases have confirmed similar effects. The addition of 60 gram buckwheat in daily intake reduced the medication at diabetes mellitus, hypercholesterolemia and at hypertension for example.

Buckwheat intake has preventive effects at diabetes, obesity, hypertension, high cholesterol levels, stroke, cardiovascular diseases, gallstone formation, cancer, leg oedema as well as level of biomarkers of inflammation in body tissues and remedy in people with gluten sensitivity (celiac disease) as naturally is gluten free. A literature review on medical findings is presented.

BACKGROUND

Medical effects of buckwheat intake might be difficult to prove, for example there are many different kinds of biomolecular mechanisms suggested and medical research methods for seeking evidence of these effects suggested. An immense coordination of different sciences, and vast diverse disciplines to understand and develop evidence for these mechanisms has been accomplished the last 10 years. Decades ago a study in the Yi people showed effects of buckwheat against hypertension and hypercholesterolemia (He et al.1995). About 2000 years ago the earliest record of the medical function of Tartary buckwheat, TB, came in Chinese history (Lin, 1994). Recently a very interesting study uncovered the relationship and mechanisms of TB and type II diabetes, hypertension and hyperlipidemia. They were using advanced comprehensive systemic approach integrating drug target prediction methods from pharmaceutical research network analysis, so called docking, on biomolecular level pathways between 97 targets and 20 TB composite compounds found in 63 references (Lu et al., 2018). It came as a result of attention as to mechanistic approach in many publications on TB buckwheat health effects in modern chronic diseases. Effects of daily intake of food with healthy effects or functional food indicating prevention or reduction of many major chronic diseases is not at all new modern knowledge as the use in Chinese medicine for thousands of years have been practised. The new thing is that modern food industry is very complicated and concerned with cheap food for consumers than with multidiversity in agriculture for individual diversified needs in different human beings with diverse genetics, some explanation for this will be notified under gluten sensitivity. The importance for minor crops like buckwheat to survive and expand in modern agriculture is major.

COMPONENTS OF HUMAN HEALTH IMPORTANCE

This will be focused in other proceedings, but a few remarks are here noticed. Buckwheat is very rich in trace elements (Zn, Cu, Mn and Se), although different milling fractions have different content of minerals and proteins, starch content (Kreft, 2001). The very low content of prolamins has also been known and is important as it has become a valuable source of dietary protein for gluten-sensitive individuals (Skerrit, 1986). Not to underestimate the comparingly high content of dietary fibers, (3.4-5.2%) out of which 20-30% are soluble. Probably the fiber content is important for the beneficial medical effects in hypertension and hypercholesterolemia. Also the resistant starch, slowly digestable carbohydrates was seen to flatten the glycaemic response curve (Skrabanja and Kreft, 1994). The anti-colon cancer effect might be an effect of the resistant starch in the colon, as it gives fermentation into butyrate in the short chain fatty acids produced by the micro-flora. The molecular composition of buckwheat diets and products has been thoroughly evaluated and studied (Ikeda, 1997).

PHENOLIC COMPOUNDS AND BUCKWHEAT

The importance and content of antioxidant phenolic compounds. Especially Tartary buckwheat (TB) contains more rutin (a quercetin-3-rutinoside) than most fruits, vegetables and grain crops. Epidemiological studies have shown that diets rich in phenolic compounds are connected to a lower risk of diseases associated with oxidative stress, such as cancer and cardiovascular diseases. In a clinical intervention study in 62 teachers 2005 highly significant health effects were found. Before, in between and after eating common or Tartary buckwheat cookies two weeks and then changing (cross-over study) without knowing which type, they were extensively examined. This intervention study during 4 weeks (daily intake), established a highly significant reduction of total serum cholesterol (both types of buckwheat), less tiredness and improved lung capacity (Tartary buckwheat). Buckwheat also may reduce neutrophil inflammation according to blood biomarkers (Wieslander et al., 2011; Wieslander et al., 2012). A major dietary flavonoid, quercetin, abundant in buckwheat, is metabolized after oral intake into its conjugates, such as quercetin–3–*O*–glucuronide and quercetin–3'–*O*–sulfate, whereas no aglycone was found in the human plasma. Therefore, to understand the mechanisms of the biological activity of quercetin focus is on the molecular actions of these conjugates. In the last decade, it has been demonstrated the unique actions of quercetin–3–*O*–glucuronide at sites of inflammation, including specific accumulation in macrophages and the following deconjugation into active aglycone, catalyzed by the macrophage–derived β–glucuronidase.

BUCKWHEAT AND CARDIOVASCULAR DISEASE RISK MARKERS: A SYSTEMATIC REVIEW AND META-ANALYSIS

This study will be described here because of medical importance, as to the vast amount of data gathered. A comprehensive literature search for prospective studies of great interest evaluated the correlation between buckwheat intake and CVD risk between 1960 and 2018 and published in PubMed, Scopus, Ovid, EBSCO, Web of Science, ProQuest databases, Science, JSTOR, Medline and China National Knowledge Infrastructure, were searched using the search terms 'buckwheat' and 'cardiovascular disease' or 'cholesterol' and 'human' or 'animal', and the same terms were applied in each database during the search phase. Thirteen randomized, controlled human studies, two cross-sectional human studies and twenty-one animal studies were identified. In addition manual search was done for all additional potentially relevant papers. CVD was defined to include stroke, aortic disease, peripheral arterial disease and coronary heart disease. The search was restricted to studies on humans and animals and included those that were written in many different languages including English or Chinese. The studies included in this review met the following criteria: (1) a prospective cohort study, (2) normal laboratory animals or free living humans, (3) buckwheat-intake exposure, (4) the results included markers of CVD risk, such as plasma glucose and insulin concentrations and lipid profile. They considered cholesterol was the most common indicator of CVD response to whole-grain foods, cholesterol was used as a primary outcome marker in this review. The result was from using random-effects models, that is the weighted mean difference of post-intervention concentrations of blood glucose, total cholesterol and triglycerides. Significantly decreased values were detected following buckwheat intervention compared with controls [differences in blood glucose: -0.85 mmol/L (95% CI: -1.31, -0.39), total cholesterol: 0.50 mmol/L (95% CI: -0.80, -0.20) and triglycerides: 0.25 mmol/L (95% CI: -0.49, -0.02)]. Responses of a similar magnitude were seen in two cross-sectional studies, which is a remarkable result. For animal studies, nineteen of twenty-one studies showed a significant reduction in total cholesterol of between 12% and 54%, and fourteen of twenty studies showed a significant reduction in triglycerides of between 2% and 74%. There was inconsistency, not surprisingly in HDL cholesterol outcomes in both human and animal studies, the studies were very heterogenous in methodology. The authors hold that it remains unclear whether the outcome on increased buckwheat intake significantly benefits as well other markers of CVD risk, such as weight loss, blood pressure, insulin-, and LDL-cholesterol levels, and they suggest even more studies. This type of studies is of course very recommended to share especially for people in position to be care-giver in outpatient clinics, nutritionists and dietitians in hospitals and in restaurants for the prevention of diseases in the immensely growing elderly populations world-wide, especially in the rich countries to prevent early development of the chronic diseases.

BUCKWHEAT IS NATURALLY GLUTEN-FREE AND HAS NOTHING TO DO WITH WHEAT.

According to the World Gastroenterology Organization data, celiac disease (CD) is a chronic disease, also called gluten sensitivity by diagnosis and the prevalence in healthy adult population varies a lot, between 1 in 100 and 1 in 300 and has a 2:1 female to male ratio (Devaraja, G., Rashmi, BS. Coeliac Disease - A Chronic Enteropathy). In Middle East, North Africa and in India, the prevalence of CD has been found to be same as in western population. In these regions, the prevalence is 3 to 20% at risk population and 3 to 5% in people with type I diabetes. Trigger factors are from ingested gluten and related cereal (wheat, rye, barley) proteins, presence of the individual tissue type (HLA DQ 2/DQ 8 molecules), and generation of circulatory autoantibodies to tissues transglutaminase (tTG) are essential factors for the precipitation of celiac disease. Unless a person has alleles for encoding HLA DQ 2/ DQ 8 molecules, CD generally does not develop. There are clear geographical differences in the prevalence of CD between and within countries. The only remedy, is to avoid gluten totally and in this regard buckwheat food is a good alternative, as long it is not contaminated with other cereals at the milling process. In Sweden buckwheat flour has been used for decades by people with CD and sold in special health stores. The diagnose CD would preferably be done by a medical investigation, to be correct for this specific diagnosis, but there are many individuals that are generally sensitive to the high gluten levels in western food. Buckwheat in a mix with other gluten-free food is then a recommended protein rich substitute in bread, porridge, and cakes and biscuits. The grain **wheat** eaten in at least Europe and America, and gluten is found also in barley and rye, but wheat is the most common food in western countries. Wheat today is **not** the same grain as 100 years ago, and new research in food allergy clinics find more proteins like globulins and other types of wheat related diseases for humans, not even fully understood medically yet. Some of these give type IgE mediated wheat hypersensitization with typical acute severe symptoms and are more easily detected medically (typical antibodies). However, research on IBS or intestinal bowel syndrome shows that 30% are sensitive to substances in wheat. There are also ATS (antitrypsin-inhibiting-substances) related to adverse reactions or sensitivity. New types of mechanisms in modern wheat production can have contributed mostly because of modern agricultural and food technology makes the medical research far behind in time. To get high yields and low prices for consumers have probably been important. It's a pity if people can confuse the buckwheat name with any kind of wheat content which is absolutely misleading. It couldn't be more wrong. There are many reasons to recommend more diversified food items and avoidance of too much wheat consumption in western world, especially as many never get a proper diagnosis of gluten allergy because lack of knowledge of the root cause for many of its various symptoms. Nowadays, it has improved somewhat because of massive knowledge in society and social media and alternatives in the restaurants in some regions like Scandinavia, during the last 20 years. Advice can be very unscientific and misleading, as medical research and knowledge among medical practitioners are lacking it's a difficult situation. Today in social media there are reported many neurological diseases from wheat including anxiety, depression, dementia, migraines, epilepsy, and neuropathy (nerve damage). Medical doctors knowledge is scarce, having one week of studies only altogether in the 70-ties in nutrition science. The eastern saying "food is medicine" is needed in more cultures else than Asian and will be held as an important truth for prevention of some chronic diseases.

BUCKWHEAT ALLERGY

An update on buckwheat allergy can be found in proceedings in The 13th International Symposium on Buckwheat (ISB) (Norbäck, Wieslander, 2016).

CONCLUSIONS

To summarize health effects from food intake of buckwheat (*Fagopyrum* esculentum Moench, *F. tataricum* Gaertner) they are globally used as nutritional foods because of their high levels of minerals, proteins and polyphenols. It has also been used as a functional food in some parts of the world. There are very different kind of studies mentioned here, like epidemiological studies, cost interventional studies, some reports on case studies in sick people, and trials dealing with the effect of buckwheat and its metabolites and hypothesis on the molecular basis for the effect. There are numerous reports of possible potential health benefits with less scientific evidence or control of consuming buckwheat in different kinds of food (groats, biscuits, porridge, supplements or even in the form of pharmaceutical drugs and tea). In this conference security aspects are dealt with and resulting from contamination from pesticides, metals or water contamination or others, and this must be considered. Gluten free buckwheat is important for the coeliac patients (milling must be free of gluten contamination). There are important antioxidative activities of buckwheat, because of high levels of rutin and quercetin. Also anticarcinogenic and preventive effects in coloncancer have been shown. Less hyperlipidaemia, lowering of blood pressure and improved weight regulation have been suggested because of fiber content and resistant starch. The mechanisms behind beneficial effects on diabetes, since lower postprandial blood glucose and insulin response have been reported. Interestingly, buckwheat metabolites, such as rutin, may have beneficial protective effects in preserving insulin signalling. Rutin might have potential therapeutic applications for the treatment of Alzheimer's disease. This is not an overview of the tremendous work to evaluate Buckwheat in Health done over the decades, just some few selection of studies known to me, completed with reviews and meta-analysis of the evidence for health effects in about 30 studies on TB intake in humans (and animals). These are some sign-posts for the new methods coming for new reseachers in "food as medicine" research. However, the literature indicates that buckwheat is safe to consume for most people and may have various beneficial effects on human health.

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RUTIN CONTENT IN BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH, *F. TATARICUM* (L.) GAERTN. AND *F. CYMOSUM* MEISSN.) GROWTH IN THE FAR EAST OF RUSSIA

VSEBNOST RUTINA V AJDI (*FAGOPYRUM ESCULENTUM* MOENCH, *F. TATARICUM* (L.) GAERTN. IN *F. CYMOSUM* MEISSN.) PRIDELANI NA DALJNEM VZHODU RUSIJE

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ABSTRACT

Rutin content in buckwheat (*Fagopyrum esculentum* Moench, *F. tataricum* (L.) Gaertn. and *F. cymosum* Meissn.) growth in the Far East of Russia

The paper presents results of the complex research of different species of *Fagopyrum (F. esculentum* Moench, *F. tataricum* (L.) Gaertn., *F. cymosum* Meissn.) on rutin content and their usage prospects as a resource of flavonoids. Relation between rutin content in the overground mass and the plant colour was found. Biological significance of rutin, prospects of its usage as a diagnostic trait in selection, are also shown in the article. Bio-chemical and technological traits of *F. esculentum* varieties cultivated in the Far East Russia were also studied and reflected in the paper. We therefore examined rutin content in the overground phytomass of the three species of *Fagopyrum* on the phase of mass flowering and discovered that high indices belong to: *F. esculentum* (Izumrud variety) ‒ 3.8 %, *F. tataricum* (sample k-62 from Canada) ‒ 4.4 % and *F. cymosum* (k-4231 from India) ‒ 4.1 %.

Rutin content in the hull of common buckwheat ranged from 0.08 till 0.20 %. Maximum rutin quantity was determined in the hull of Ussurochka (35.7 kg/ha), and minimum (17.8 kg/ha) in the hull of Pri 7. The studies show that the ash content, obtained after burning the hull of *F. esculentum* (600°C) is in average 2 %. The following elements were found in the ash: potassium, sodium, copper, silver, calcium, magnesium, zinc, aluminum, manganese, iron, nickel, chromium, phosphorus, and their concentration depend on variety and type of raw material of *F. esculentum*.

Key words: Fagopyrum esculentum, F. tataricum, F. cymosum, overground mass, hull, rutin.

IZVLEČEK

Vsebnost rutina v ajdi (*Fagopyrum esculentum* Moench, *F. tataricum* (L.) Gaertn. in *F. cymosum* Meissn.) pridelani na Daljnem vzhodu Rusije

V prispevku so predstavljeni rezultati kompleksne raziskave različnih vrst rodu *Fagopyrum* (*F. esculentum* Moench, *F. tataricum* (L.) Gaertn., *F. cymosum* Meissn.) o vsebnosti rutina in možnosti njihove uporabe kot vira flavonoidov. Ugotovljeno je bilo razmerje med vsebnostjo rutina v nadzemni masi in barvo rastlin. V članku je prikazan tudi biološki pomen rutina, možnosti njegove diagnostične uporabe pri selekciji oziroma njegov pomen pri selekciji. V delu so bile proučene tudi biokemijske in tehnološke lastnosti sort *F. esculentum*, ki jih gojijo na Daljnem vzhodu Rusije. Zato smo določali vsebnost rutina v nadzemnih delih treh vrst rodu *Fagopyrum* v fazi cvetenja in ugotovili, da visoki indeksi pripadajo: *F. esculentum* (sorta Izumrud) ‒ 3,8 %, *F. tataricum* (vzorec k-62 iz Kanade) ‒ 4,4 % in *F. cymosum* (k-4231 iz Indije) ‒ 4,1 %. Vsebnost rutina v luščinah navadne ajde je znašala od 0,08 do 0,20 %. Največja količina rutina je bila določena v luščinah Ussurochka (35,7 kg/ha) in najmanjša (17,8 kg/ha) v luščinah Pri 7. Študije kažejo, da je vsebnost pepela, pridobljenega po upopelnjenju luščin *F. esculentum* (600°C), v povprečju 2 %. V pepelu so bili določeni naslednji elementi: kalij, natrij, baker, srebro, kalcij, magnezij, cink, aluminij, mangan, železo, nikelj, krom, fosfor, njihova koncentracija pa je odvisna od sorte in rastlinskega dela vrste *F. esculentum*.

Ključne besede: Fagopyrum esculentum, F. tataricum, F. cymosum, nadzemna masa, luščine, rutin.

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INTRODUCTION

Species of *Fagopyrum* Mill. genus have valuable edible and medicine traits. *Fagopyrum esculentum* Moench is a cereal and melliferous crop which is widely cultivated in many countries of the world. The main producers of buckwheat are China, Russia and Ukraine. In some countries of the South-Eastern Asia (China, India) *F. tataricum* (L.) Gaertn., and *F. cymosum* Meissn. are used as an edible and medicine crop. Plants of species of *F. esculentum* are widely used in popular medicine. As a medicinal raw they use leaves and tops of shoots in blooming stage (Kreft et al. 2006, Hinneburg & Reinhard 2005). Representatives of *Fagopyrum* genus are prospective resources of flavonoids. The main flavonoids is 3-O-rutinozid quercetin (rutin or vitamin P), which has antioxidant, angioprotective, antibacterial and hepatoprotective traits (Odetti et al. 1990, Grinberg et al. 1994, He et al. 1995, Guardia et al. 2001, Holasova et al. 2002, Mashkovsky 2004, Srinivasan et al. 2005).

In some countries (Russia, Canada, Ukraine, Japan) there were developed special buckwheat varieties for rutin production with its high content. As a result of the chemical analysis of buckwheat plants selected for colour, it was found that plants with red coloring of stems contain more rutin in comparison with green plants, green-red and red-green. Buckwheat plants are selected according to the stalk coloring in a fruit formation stage, choosing plants with dark red (anthocyanin) colour (Klykov & Moiseyenko 2005, Anisimova 2011). Our research has shown that intra-variety changes of the plants colour have a wide spectrum (red, red-green, green-red and green) and are affected not only by the variety genotype but largely by variability, which effect depends on various factors: sowing date, mineral fertilizers, seeding rate and method of sowing (Klykov 2000, Klykov & Moiseyenko 2010).

In our view, anthocyanin colour of the stalk is a good diagnostic indicator that can be used for the goal selection of buckwheat plants with high rutin content in the overground part. In connection with this fact, *Fagopyrum esculentum* is a promising domestic source of rutin for the pharmaceutical industry.

MATERIALS AND METHODS

Plant materials: The field experiments were carried out at FSC of agribiotechnology in the Far East named after A.K. Chaika (44.34°N, 131.58°E), Ussuriysk district, Primorsky krai, Russia and in the Pacific Institute of Bioorganic Chemistry named after G.B. Elyakov the Far Eastern Branch of Russian Academy of Sciences. As the research object there were used representatives of the family *Polygonaceae* Juss: cultivated species of the genus *Fagopyrum* Mill. (*Fagopyrum esculentum* Moench, *Fagopyrum tataricum* (L.) Gaertn., *Fagopyrum cymosum* Meissn.).

Soil of the experimental field was meadow-brown, bleached. Power of the arable layer is 24 cm, humus content is 3.8 %, P_2O_5 – 17.1 mg/100 g of soil, K_2O – 14.2 mg/100 g of soil, pH – 5.7.

Representatives of *Fagopyrum* Mill. genus of domestic and foreign origin were received from the Gene Bank of the All-Russia Institute of Plant Growing named after N.I. Vavilov (St. Petersburg, Russia). There wase evaluated collection of 4 Tartary buckwheat (к-6, Germany; к-8, France; к-17, China; к-62, Canada) accessions with different place of origin.

Common buckwheat accessions in tested collection were originated from the states of the former Russia (Primorskaya local, Pri 7, Ussurochka, Pri 10 and Izumrud). The research objects were sample of *F. cymosum* (к-4231, India).

Preparation of aqueous plant extracts: Max. 30 plants of each genotype were used for the analysis. Dry matter content and so called total rutin were determined in whole plants. After drying the plants parts were ground finely. Three analytical methods for rutin determination were tested: high-performance liquid chromatography (HPLC) and spectrophotometric method (Vysochina et al. 1987). The absorption of the extract solution was measured at 360 nm on spectrophotometer Shimadzu UVmini–1240 (Japan) and compared to that of a standard rutin curve.

Rutin determination by HPLC (Kreft et al. 2002). An amount of 0.1-0.5 g of ground plant material was extracted with 10 ml of a solution (methanol-acetic acid-water 100:2:100) for 1 hour on a shaker at laboratory temperature. 2 ml of the extract were centrifuged for 10 min. at 9 000 rot/min. A clear supernatant was filtered through a microfilter with a regenerated cellulose membrane, and it was analysed on a Waters Alliance 2690 liquid chromatograph. The filtrate was applied to a Lichrospher 100RP-18 column and eluted by gradient elution with a mixture of methanol-water: 0-2 min 20 % methanol 2-4 min 60 % methanol. Detection with a

UV detector was carried out at 360 nm. Rutin was eluted at the 6th min. and the peak area was compared with standard solutions of pure rutin. Rutin concentration in samples was determined by a calibration curve.

For rutin identification there were used NMP ^{1}H spectrums, that were registered on spectrometer AC-250 in $CDCl_3$ and d_6. Then they compared the spectrums with the pure rutin ("Chemopol", Czech Republic). Mass-spectrums were produced on the equipment LKB-9000S (Sweden) with straight input under the energy of ionizing electrons equal to 18 and 70 eV(electron-volt).

Polysaccharides were analyzed with the help of X-ray Diffractometer Shimadzu Lab XRD 6000. IR spectroscopy method high-efficient size-exclusion chromatography were used for studying structural pecularities of the found polysaccharides in Institute of Chemistry of Far East Branch of the Russian academy of Sciences (Zemnukhova et al. 2004 a, Zemnukhova et al. 2004 b).

Statistical analysis: The means and standard deviations were calculated using Microsoft Office Excel 2003. Significant differences of these data were calculated using analysis of variance (ANOVA-Duncan's multiple test, SIGMASTAT 9.0). The reliability of the results between the control and experimental samples was evaluated using Student's t-test.

RESULTS AND DISCUSSION

Rutin content depends on genus, species and variety (Klykov et al. 2003, Jiang et al. 2007, Jinfeng Gao et al. 2007, Yan Chai et al. 2007). We investigated rutin content and productivity of overground part of different samples of three *Fagopyrum* species plants. The data show that high rutin content was present in overground part of *F. esculentum* (Izumrud variety) – 3.8 %, *F. tataricum* (sample k-62 from Canada) – 4.4 %, *F. cymosum* (k-4231 from India) – 4.1 % (Table 1).

Table 1: Productivity of the overground part in the mass flowering stage and rutin content in three distinguished samples of *Fagopyrum* species plants

Preglednica 1: Pridelek nadzemnih delov v polnem cvetenju in vsebnost rutina v rastlinah treh vrst *Fagopyrum*

Variety, number in the catalogue ARSRIPG, origin	Green mass, ton /ha		Dry matter, ton /ha		Rutin, %		Rutin, kg /ha	
	lim	$\bar{X} \pm S_{\bar{x}}$	lim	$\bar{X} \pm S_{\bar{x}}$	lim	$\bar{X} \pm S_{\bar{x}}$	lim	$\bar{X} \pm S_{\bar{x}}$
Fagopyrum esculentum								
Izumrud (Russia)	21.1-28.4	25.3±0.2	2.8-3.5	3.2±0.1	3.4-4.2	3.8±0.1	95.1-147.3	121.2±7.1
Primorskaya local (Russia)	18.3-24.5	22.4±0.2	2.5-3.2	2.8±0.1	2.9-3.5	3.2±0.1	72.5-112.3	92.5±4.7
Pri7 (Russia)	19.2-25.3	23.7±0.2	2.7-3.4	3.0±0.1	2.8-3.4	3.1±0.1	76.3-116.2	96.2±3.9
Fagopyrum tataricum								
к-6 (Germany)	17.1-26.2	20.1±0.2	2.5-3.0	2.8±0.1	3.7-4.4	4.1±0.2	92.1-132.3	112.1±6.0
к-8 (France)	20.4-24.3	22.3±0.2	2.2-2.8	2.5±0.1	3.9-4.6	4.1±0.2	86.1-129.6	107.4±5.1
к-17 (China)	25.0-28.5	26.2±0.2	2.8-3.4	3.1±0.1	3.2-4.8	4.2±0.2	89.4-163.3	126.3±7.2
к-62 (Canada)	24.1-32.2	27.1±0.2	2.6-3.3	2.9±0.1	3.6-5.0	4.4±0.1	94.2-165.4	129.1±7.9
Fagopyrum cymosum								
к-4231 (India)	18.4-24.3	20.3±0.2	1.9-2.4	2.2±0.1	3.2-4.4	4.1±0.1	61.3-106.7	83.2±4.4

Productivity of overground mass and dry matter differed between samples of *F. tataricum* k-17 (China), k-62 (Canada), *F. esculentum* – variety Izumrud (Russia, Primorskiy Krai) and *F. cymosum* k-4231 (India). Procurement of raw materials is the most efficient when the maximum rutin concentration coincides with peak growth of the plant phytomass.

Studied species of *Fagopyrum* genus undergo a complete cycle of development: vegetative (shoots-flowering), generative (flowering – seed filling). Total duration of the vegetation period of *Fagopyrum* is 72-90 days. Due to phenological observations it was noted that vegetation period was longer for samples of *F. tataricum* and *F. cymosum* (from 33 to 52 days) than those of *F. esculentum* (24-26 days). The generative period duration for samples of *F. tataricum* ranged from 32 to 46 days, for *F. cymosum* – 38 days, for *F. esculentum* – 48-49 days. The longest vegetation period was observed for *F. cymosum* (k-4231 from India – 90 days), and the shortest period number 63 days for sample of *F. tataricum* (k-6 from Germany). For the varieties of *F. esculentum* it ranged from 72 to 75 days.

Rutin content in the vegetative and generative parts, roots of *F. esculentum* (Izumrud variety, Russia), *F. tataricum* (sample k-62, Canada) and *F. cymosum* (sample k-4231, India) during vegetation varies from the early stages of plant development. The hughest rutin content in all studied samples was observed in flowers (4.7-6.3 %), much less in the stems (0.6-1.4 %) and the lowest rutin content was found in the roots (0.3-0.8 %).

The maximum rutin content was observed in leaves of *F. esculentum* in the budding stage (4.6 %), in *F. tataricum* - at the beginning of flowering (4.8 %), and minimum rutin content in the seed filling phase (2.8 %), as well as at the beginning of vegetation and seed filling phase (*F. esculentum* – 3.0 and *F. tataricum* – 3.1 %, respectively). To determine the optimal harvesting time for the raw materials, when the most rutin from the overground phytomass of the studied species can be got, there was calculated rutin quantity derived from 1 ha of the experimental plot with the density of 120 plants per 1 м^2 (Table 2).

Table 2. Yield of overground part and rutin quantity of three *Fagopyrum* species in different development phases of the plants
Preglednica 2: Pridelek nadzemnih delov in vsebnost rutina pri rastlinah treh vrst *Fagopyrum* glede na faze razvoja rastlin

Species	Development phase	Overground mass yield, ton /ha	Rutin, kg /ha
Fagopyrum esculentum	Vegetation beginning	0.48±0.3	12.0±1.8
	Budding	0.84±0.4	25.2±2.6
	Flowering beginning	1.68±0.5	62.2±5.0
	Mass flowering	2.88±0.7	109.4±7.1
	Seed filling	3.82±0.8	84.6±5.4
Fagopyrum tataricum	Vegetation beginning	0.36±0.2	8.3±0.9
	Budding	1.44±0.4	46.1±4.2
	Flowering beginning	1.80±0.6	73.8±5.4
	Mass flowering	3.00±0.7	117.0±8.3
	Seed filling	4.00±0.8	104.0±8.1
Fagopyrum cymosum	Vegetation beginning	0.24±0.2	4.6±0.6
	Budding	2.28±0.4	66.1±5.8
	Flowering beginning	2.88±0.7	100.8±7.3
	Mass flowering	3.12±0.8	115.4±8.3
	Seed filling	4.92±0.9	108.2±7.9

Maximum rutin quantity (117 kg/ha) is possible to be derived from overground phytomass of *F. tataricum* in the mass flowering stage. Rutin content in dry raw materials in this stage is high enough (4.4 %), and yield of dry phytomass in this period was 3.00 ton/ha. We consider it to be important for gathering rutin per hectar.

Close to them there were rutin output indices of *F. cymosum* (1154.7 g) and *F. esculentum* (109.4 kg) obtained from 1 ha of the experimental plot in the same stage. During the seed filling phase there was also observed high rutin output in the overground phytomass of *F. tataricum* (104 kg) and *F. cymosum* (108.2 kg).

Preparation of raw materials (overground phytomass) of studied *Fagopyrum* species is reasonable to perform in the stage of the plants mass flowering, because during this period, the amount of rutin was the highest. At present time rutin content in the roots of *Fagopyrum* Mill. species is studied scarcely as well as its role in selection on lodging resistance. Therefore, we studied growth dynamics of overground and root mass, the root system maintenance, rutin content in roots of *F. esculentum* and *F. tataricum*.

As a result of the research there were determined significant correlations of *F. tataricum* between rutin content in the roots with aboveground part (r = 0.92) and root mass (r = 0.93), and the root system maintenance (r = 0.89). Intensive growth of *F. esculentum* root mass takes place before the budding stage. Then deceleration in growth is observed and reduction on seed filling phase. It is connected with ageing and decay of the roots. Increase of *F. tataricum* root mass was observed throughout the whole vegetation period.

It was found that *F. esculentum* plants, resistant to lodging, contain 0.68 % of rutin in the roots (bright coloring of the root system), and sensitive plants contain - 0.31 % (dark brown coloring of roots). Apparently, resistance to lodging of plants on a seed filling phase, is connected to physiologically active root system (viable), which directly influences the intense accumulation of rutin, in comparison with sensitive ones. The identified relation between rutin content, the roots coloring and the root mass became the basis for development of selection method of buckwheat plants on lodging resistance (Klykov & Moiseyenko 2003).

This method provides selection of buckwheat forms on three indicators:

1) the root system colouring;
2) maintenance of the root system;
3) rutin content in the roots.

At the starting stage of breeding visual selection was taking into account. These indicators will contribute to increase efficiency in the samples selection with high resistance to lodging.

The study showed that rutin content in seed of buckwheat plant depend on genotype varies from 0.07 to 2.4 % of the dry matter (Table 3). The seed of Tartary buckwheat contains higher amounts of rutin (about 0.8-1.7 % dry weight) than that of common buckwheat (0.01 %) and is rich in vitamins (Raina & Gupta 2015). It was observed that buckwheat groats were coloured in light brown, light green and green. The particular interest presents possibility of rutin content effect upon depend on colouring of buckwheat groats (green color implies increased rutin content). Varieties of *F. esculentum* with green colouring of buckwheat groats had the highest rutin content (0.10-0.15%). Rutin content was significantly lower in *F. esculentum* than that of *F. tataricum* and *F. cymosum*. Wild species *F. tataricum* and *F. cymosum* are valuable in breeding as genetic rutin source. The studied species differed on colouring of buckwheat groats. Thus buckwheat groats of *F. esculentum* variety Pri 7 was light brown, variety Ussurochka - light green, variety Izumrud - green, *F. tataricum* – yellow-green, and *F. cymosum* - bright yellow-green. Colouring of buckwheat groats can serve as a diagnostic sign of visual selection of forms with high rutin content.

Our results show a relationship between rutin content and colouring of the plant different parts (stem, flower, root system, buckwheat groats) of *F. esculentum*, which allows making some adjustments in existing methods of selection of forms with high rutin content, more adapted to abiotic and biotic stresses. There were identified very important diagnostic characteristics that should be used in selection in order to create new varieties with high rutin content in buckwheat groats for production functional foods. The above ground part of *F. esculentum* plant is reasonable to be used as prospective domestic source of rutin for pharmaceutical industry.

Table 3: Rutin content in fruits of three *Fagopyrum* species
Preglednica 3: Vsebnost rutina v plodovih pri treh vrstah *Fagopyrum*

Species	Variety, catalogue number of ARSRIPG, origin	Rutin content in seed, %	Buckwheat groats colouring
Fagopyrum esculentum	Pri 7 (Russia)	0.07±0.01	light-brown
	Ussurochka (Russia)	0.10±0.01	light-green
	Izumrud (Russia)	0.15±0.01	green (salad paint)
Fagopyrum tataricum	к-62 (Canada)	2.4±0.1	yellow-green
Fagopyrum cymosum	к-4231 (India)	1.1±0.1	bright yellow-green

In common buckwheat (*F. esculentum*) production there are formed significant amounts of waste (secondary resources) such as straw and fruit shells (hull), which so far have not been effectively implemented. Proportion of total overground weight of the plant straw depends on the variety and is 40-60 %. As for hull, it is 20-30 % from the grain weight. Straw is usually crushed and remains in the fields or burned.

Rutin content in hull of common buckwheat ranged from 0.08 till 0.20 %. Maximum rutin quantity one can get from the hull of Ussurochka (35.7 kg/ha), minimum (17.8 kg/ha) in hull of Pri 7 (Table 4). The cereal processing plant use hull as carburant. Recently it is used as a filling for pillows. It seems that the most promising would be their use as the secondary resources involvement as an additional source of raw materials for pharmaceutical industry to obtain rutin and micro-fertilizers, as well as to solve problem of environmental pollution.

Table 4: Rutin content in fruit shells (hull) of common buckwheat
Preglednica 4: Vsebnost rutina v luščinah navadne ajde

Variety	Grain yield, ton/ha	Filmness, %	Rutin, %	Number of hull, kg/ha	Rutin, kg/ha
Pri 7	1.12	20.3±0.2	0.08±0.01	223.3±2.3	17.8±0.1
Izumrud	1.34	23.8±0.2	0.10±0.01	309.4±2.9	27.8±0.1
Pri 10	1.63	19.5±0.1	0.15±0.01	312.0±3.0	31.2±0.2
Ussurochka	1.78	21.0±0.2	0.20±0.01	357.0±3.1	35.7±0.2

Buckwheat nowadays is widely used in various industries. The most promising in the near future seems to be involvement in the processing of fruit shells (hull), which accumulates in large volume at cereal processing plants. Chemistry Institute the Far Eastern Branch of Russian Academy of Sciences jointly with FSC of agribiotechnology in the Far East named after A. K. Chaika studied content and composition of polysaccharides of the fruit coating and straw of varieties Izumrud and Pri 7. For this purposes they used sequential extraction with water, solutions of ammonium oxalate and sodium hydroxide (Zemnukhova et al. 2004 a, Zemnukhova et al. 2004 b).

Our study showed that content of polysaccharides depends on the type of raw material (fruit outer coating or straw) and variety. The largest output of polysaccharides derived from the wastes can be received in all the cases using the first (water) extraction. The colour of dry product depends on the source of raw materials and ways of getting the polysaccharides and has white, light brown or almost black coloring (Table 5).

Table 5: Characteristics of polysaccharides from extracts of *F. esculentum* fruit shells (hull)
Preglednica 5: Polisaharidi v ekstraktih luščin *F. esculentum*

Variety	Extragent (concent ration)	Soluble substances in raw materials, %	Characteristics of polysaccharides		
			Output of raw materials, %	Colour	Structure
Pri 7	H_2O	14.7	2.76	light brown	amorphous
	$(NH_4)_2C_2O_4$ (0.5 н)	2.8	1.52	light brown	crystal
	NaOH (0.5 н)	23.7	1.31	black	amorphous
Izumrud	H_2O	14.3	2.63	light brown	amorphous
	$(NH_4)_2C_2O_4$ (0.5 н)	2.6	1.40	light brown	crystal
	NaOH (0.5 н)	19.2	4.40	black	amorphous

Polysaccharides of water and oxalate extractions of all samples are characterized by a high content of glucose.

Polysaccharides of alkaline extraction have a more complex monosaccharide structure and contain residues of rhamnose, arabinose, xylose, mannose, glucose and galactose (Table 6).

Inositol was found in trace amounts. Uronic acids are presented by D-Galakturonic and glucuronic acids.

While extracting raw materials, metals which were in the plant, were extracted into the solution along with organic substances. Obtained data prove that polysaccharides, allocated from the extracts in solid state, absorb metals from the solution. The rest of raw material, which was not dissolved in water, practically doesn't form ashes.

The studies show that the ash content, obtained after burning the hull of *F. esculentum* (600°C) is in average 2 %. The following elements were found in the ash: potassium, sodium, copper, silver, calcium, magnesium, zinc, aluminum, manganese, iron, nickel, chromium, phosphorus, and their concentration depends on variety and type of raw material of *F. esculentum*.

It is important from the point of view of usage of *F. esculentum* fruit shells (hull) as secondary raw material as a source of macro- and micronutrients.

Table 6: Monosaccharide composition of polysaccharides from extracts of F. esculentum fruit shells (hull)
Preglednica 6: Monosaharidna sestava polisaharidov v ekstrakti luščin F. esculentum

Variety	Extraction medium	Monosaccharide composition, molar %							The presence of uronic acids	
		Rha	Ara	Xyl	Man	Glc	Gal	Int	GalA	GlcA
Pri7	H_2O	сл	сл	сл	13	72	15	сл	сл	–
	$(NH_4)_2C_2O_4$	–	18	сл	сл	61	21	сл	+	сл
	NaOH	8	13	17	10	33	19	–	+	+
Izumrud	H_2O	3	4	сл	8	64	21	сл	+	сл
	$(NH_4)_2C_2O_4$	8	21	5	9	39	18	сл	+	–
	NaOH	10	18	25	7	22	18	–	+	+

Notes: Rha – rhamnose, Ara – arabinose, Xyl – xylose, Man – mannose, Glc – glucose, Gal –galactose, Int – inositol; uronic acids: GalA – D-Galacturonic acid, GlcA – glucuronic acid – absent, сл – traces, + presents in small amounts.

Pre-sowing preparation of *Fagopyrum esculentum* seeds is important for productivity increase. We found that treatment of the seeds with the hull ash of *F. esculentum* in quantities of 100 kg/ ton had the greatest harvest increase (0.22 ton/ha).

The literature analysis and our research on testing the possibility of usage of *F. esculentum* waste as raw material in order to obtaining valuable chemical products, shows that the list of the proposed usage goals of the waste is great and, the waste has not yet been fully utilized. The integrated recycling of *F. esculentum* production waste may prove to be economically favorable.

CONCLUSION

The research defined that rutin content in overground part of *F. esculentum* depends on genus, species and variety. As a promising source of rutin we recommend: *F. esculentum* (variety Izumrud, Russia ‑ 3.8 %), *F. tataricum* (sample k-62 from Canada ‑ 4.4 and sample k-17 from China ‑ 4.2 %). There was defined the relation between forms of the plant and amount of rutin, and morphological and economically valuable traits.

Results of *F. esculentum* study as raw material for rutin as well as data on the production maintenance with raw materials can be used in practical application in pharmaceutical industry. There were obtained new data that may become basic for understanding the functional role of rutin in buckwheat, as well as for identifying mechanisms of rutin accumulation in plants depending on various factors, that will allow to define the most promising sources and to develop practical recommendations for their usage in agriculture, food industry and medicine. The above ground part of *F. esculentum* plant can be used as prospective domestic source of rutin for pharmaceutical industry.

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ADDITION OF TRACE ELEMENTS TO COMMON AND TARTARY BUCKWHEAT (*FAGOPYRUM ESCULENTUM* AND *F. TATARICUM*)

DODAJANJE ELEMENTOV V SLEDOVIH NAVADNI IN TATARSKI AJDI (*FAGOPYRUM ESCULENTUM* IN *F. TATARICUM*)

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ABSTRACT

Addition of trace elements to *Fagopyrum esculentum* and *F. tataricum*

Plants need at least 14 elements for normal functioning. Selenium (Se) is on the list of beneficial elements for plants, since it has many positive effects in a propriate concentrations. Iodine (I) is not yet classified on that list since there are not enough studies about the effect of I on plants. Selenium in plants may cause a delay of senescence and promote the growth of the ageing seedlings. Selenium also exhibits protective role in UV treated plants, plants, exposed to water shortage, and in plants, exposed to high or low temperature. High concentration of Se was reported to cause physiological disturbances in plants due to Se binding to cysteine and methionine molecules instead of S, and the inclusion of selenocysteine and selenomethionine in proteins. I might have a positive effect on plants, including its protective role in antioxidant activities in plants, exposed to different stress conditions. Both elements are in deficit in human nutrition in many countries worldwide. I and Se are needed for the optimal function of thyroid gland, thus simultaneous biofortification of crops is feasible for areas deficient in both elements. Selenium and I interfere with each other in pea, common buckwheat plants and in kohlrabi. Sulphur (S) and Se have similar chemical properties, and the assimilation of Se and S follows the S metabolic pathway. S induced the accumulation of Se in Tartary buckwheat in field experiment. Silicon (Si) enhances plant strength, ameliorates the negative effects of salinity, drought, and high or low temperatures, amelio-

IZVLEČEK

Dodajanje elementov v sledovih navadni in tatarski ajdi (*Fagopyrum esculentum* in *F. tataricum*)

Rastline potrebujejo vsaj 14 elementov za normalno rast. Selen (Se) je na seznamu koristnih elementov za rastline, saj ima v ustreznih koncentracijah veliko pozitivnih učinkov na rastline. Jod (I) na ta seznam še ni uvrščen, saj ni dovolj raziskav o vplivu I na rastline. Selen pri rastlinah lahko zakasni proces staranja in pospeši rast sadik. Selen kaže tudi zaščitno vlogo pri rastlinah, izpostavljenih UV žarkom, rastlinah, ki so izpostavljene pomanjkanju vode, in rastlinah, ki so izpostavljene visokim ali nizkim temperaturam. Raziskovalci poročajo, da visoke koncentracije Se povzročajo fiziološke motnje v rastlinah zaradi vezave Se na molekule cisteina in metionina na mesto žvepla in vključitve selenocisteina in selenomethionina v beljakovine. Jod pozitivno vpliva na rastline, vključno s povečanjen njihove antioksidativne aktivnosti pri rastlinah, ki so izpostavljene različnim stresnim razmeram. V mnogih državah po svetu oba elementa primanjkujeta v prehrani ljudi. Jod in Se potrebujemo za optimalno delovanje ščitnice, zato je sočasna biofortifikacija poljščin smiselna na območjih s pomanjkanjem obeh elementov. Dodatek Se in I vplivata na akumulacijo drug drugega pri grahu, navadni ajdi in pri kolerabici. Žveplo (S) in Se imata podobne kemijske lastnosti, asimilacija Se in S pa sledi metabolni poti S. Žveplo je v poljskem poskusu, kjer smo rastlinam foliarno dodajali hkrati oba elementa, povzročilo povečano kopičenje Se v tatarski ajdi. Silicij (Si) povečuje trdnost rastlin, blaži negativne učinke

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rates metal toxicity, and increases plant resistance to different pathogens and herbivores.

Key words: buckwheat, Fagopyrum, selenium, iodine, sulphur, silicon

slanosti, suše in visokih ali nizkih temperatur, blaži strupenost kovin in povečuje odpornost rastlin na patogene in rastlinojede.

Ključne besede: ajda, Fagopyrum, selen, jod, žveplo, silicij

IODINE

In nature, iodine can be found in various forms, such as inorganic sodium salts and potassium salts, iodate and iodide, inorganic and organic iodine (Ahad & Ganie 2010). Plant root cells take up I in the form iodide anion, which follows the chloride transport pathway (White & Broadley 2009). Lack of iodine in the human body is due to insufficient uptake of iodine by food. About two billion people worldwide have iodine deficiency problems. The most effective way of providing enough iodine in the diet is by iodizing salt. Excessive salting of food can cause health problems in humans. An effective solution to iodine deficiency in food for humans is biofortification of plants with iodine (Tonacchera et al. 2013). Iodine is not essential in terrestrial plants. Medrano-Macias et al. (2016) in their review paper reported that there are studies showing beneficial effects of iodine in plants, including better growth, and changes the tolerance to stress and also antioxidant capacity, on the other hand some studies report that the addition of iodine cause no response or even have adverse effects. Uptake of iodine by plants from the soil depend from adsorption–desorption processes in the soil, amount of organic compounds… (Zia et al. 2014). Weng et al. (2013) evidenced that leaf vegetables have higher absorption capacity than fruit vegetable. Smoleń et al. (2011) reported that plants take up iodine through the root system, preferably as iodide. Iodine transport relies more on phloem than on xylem (Blasco et al. 2011). In the recent study of Germ et al. (2019) it was proven that iodine transport exists through phloem, since the amount of iodine increased in seeds in buckwheat plants, previously foliarly treated with iodide and iodate. Golob et al. (2020) shown that similar levels of Se and I in the leaves and tubers in kohlrabi plants showed the translocation of both elements from the leaves to the tubers through the phloem. Transport of iodine by phloem has been previously also evidenced in tomato (Landini et al. 2011). In the study of Dai et al. (2006) it was shown that iodide (I^-) and iodate (IO_3^-) added to the soil, did not significantly affect spinach biomass production. The added iodine can also have a phytotoxic effect, leading to a decrease in biomass and thus reducing crop production (Blasco et al. 2011). Machowiak et al. (2005) found that iodide was more toxic than iodate. Later researchers (Blasco et al. 2012) found that iodide was more available than iodate and at the same time demonstrated that iodine concentration in vegetables was higher when iodide was added than when iodate was added to plants. Blasco et al. (2011) found that the presence of iodide reduced the biomass of the crop of lettuce, while the presence of iodate increased the biomass of the crop.

SELENIUM

Selenium is a trace element that is essential nutrient for humans and animals but also acts environmental toxicant; the boundary between the two is narrow and depends on its concentration, chemical form, and other environmental parameters (Fan et al. 2002). The essentiality of Se to higher plants, however, is still under debate in the scientific world. Selen is harmful for plants in high concentrations, but it has beneficial effects at low concentrations. Health risks for humans and animals can occur in areas where soils are low in bioavailable Se. Although higher plants do not to require Se, in Finland, where the amount of Se in soils is low, the supplementation of fertilizers with sodium selenate affects positively the whole food chain from soil to plants, animals and humans, including the amount of plant yields (Alfthan et al. 2014). Selenium is toxic at high concentrations due to incorporation of Se in place of sulphur in amino acids, with subsequent alteration of protein three-dimensional structure and impairment of enzymatic function (Brown and Shrift 1981). Gorše et al. (2018) measured chlorophyll *a*, anthocyanins, UV absorbing compounds and rutin in Tartary buckwheat sprouts, which become very popular in the food production and nutrition. Sprouts contain significant amount of vitamins and mineral. Amendment of sprouts with iodine and selenium may prevent endemic deficiency of these elements for humans and animals. Tartary buckwheat seeds were soaked in solutions with selenate, iodate or in selenate + iodate. Germination rate of sprouts form seeds, soaked in solution of iodate and combination of selenate and iodate was lower comparing to control group. However, there was no effect of the treatments on the amount of chlorophylls, anthocyanins and UV absorbing compounds. The amount of flavonoid rutin, which is important antioxidant, was the highest in untreated sprouts.

Kreft et al. (2013) studied the impact of Se on foliarly treated Tartary buckwheat. Plants effectively took Se and transported it into the seeds, where its concentration was more than twice as high as in untreated plants. The seeds were then collected and sown to obtain the progeny of Se-treated plants. Three weeks after germination, the Se-treated progeny plants had higher respiratory potential measured via electron transport system (ETS) activity compared to the controls. The potential photochemical efficiency of photosystem (PS) II was also higher in the Se-treated progeny plants than the controls. In adult plants, the leaves dry mass was greater in the Se-treated progeny plants than the control plants. This study demonstrates an impact of Se in Tartary buckwheat on the progeny plants of Se sprayed plants showing an epigenetic effect. Golob et al. (2015) found out that when Se was added to Tartary buckwheat plants, the highest content of Se was found in leaves, followed by seeds and stems. Edible parts of Tartary buckwheat plants were safe for human consumption regarding recommended Se concentration for humans. Authors stated that soil fertilization with 0.5 and 10 mg Se L^{-1} and foliar fertilization with 0.5 mg Se L^{-1} are applicable for cultivation of Tartary buckwheat as a functional food enriched with Se. Golob et al. (2016) foliarly sprayed Tartary buckwheat and hybrid buckwheat with sodium selenate. They found out that in both taxa of buckwheat, Se content was significantly higher in treated plants than in controls. Seeds contained the highest Se concentrations in hybrid and Tartary buckwheat. The main Se species found was selenomethionine. Selenium had positive effect on physiological characteristics like photochemical efficiency of PS II in Tartary buckwheat and hybrid buckwheat. Regarding the concentration of Se in both buckwheat taxa and selenomethionine as the dominant chemical species of Se, Se-enriched buckwheat is a potential source of dietary Se for animals and humans. In addition, Tartary buckwheat sprouts enriched with 30 mg Se/L are a potential source of dietary Se, since concentration of Se does not exceed recommended daily allowance for healthy adults (Štrekelj et al. 2014). Ožbolt et al. (2008) evidenced in their study that soaking seeds of common buckwheat before sowing in a 20 mg SeVI/L solution is a suitable method for obtaining high yield of buckwheat herb with a high, but nutritionally safe, level of Se as well as flavonoids.

To conclude, common buckwheat, Tartary buckwheat and hybrid buckwheat have ability to absorb Se in concentrations, which are safe for human nutrition if Se is added to plants in proper concentrations.

IODINE AND SELENIUM

I and Se are needed for the normal function of thyroid gland, thus simultaneous biofortification of crops is feasible for areas deficient in both elements. Iodine and selenium are not essential elements for plants but both play important roles in human and animal organisms (Smoleń et al. 2014). There are some studies focused on the effect of Se and I on plant physiological and biochemical characteristics (Zhu et al. 2004, Smoleń et al. 2014, 2016, 2019, Jerše et al. 2017, 2018, Germ et al. 2019, Golob et al. 2020). There was no effect of Se (SeO_3^{2-}, SeO_4^{2-}), I (I^-, IO_3^-) and their combination on the germination, amount of chlorophylls, anthocyanins and potential photochemical efficiency of PS II in pea sprouts. On the other hand, all treatments lowered biomass or height of the plants in pea sprouts (Jerše et al. 2017). There was also no effect of soaking of seeds in Se, I or their combinations on common buckwheat microgreens regarding photochemical efficiency of PS II. Germination was unaffected by all combinations with Se and I. Mean seed yield in plants, foliarly treated with both forms of Se and I and their combinations, was similar in treated and control plants (Germ et al. 2019). The simultaneous addition of Se and I has an antagonistic or synergistic effect on accumulation of both elements in common buckwheat plants, thus, biofortification of buckwheat microgreens with Se and I should be performed with care. The biofortification of microgreens with iodate should be delivered at reasonable low concentration, to prevent exceeding the recommended daily intake of this element for humans (Germ et al. 2019).

SULPHUR AND SELENIUM

Due to chemical similarities between selenium and sulphur (S), the uptake, transport and assimilation of selenate follow the sulphate pathway and selenate enters root cells via sulphate transporters. Golob et al. (2016) foliarly treated Tartary buckwheat and common buckwheat plants with solutions of selenate and/or sulphate in order to study the effect of sulphur on selenium accumulation in plants. The concentration of Se in all plant parts was similar in control and S treated plants in both species. However, in Tartary buckwheat the concentration of Se was higher in S and Se treated plants comparing to plants treated only with Se. Sulphur enhanced Se accumulation in all parts of Tartary buckwheat. Selenate exposure competes with sulphate in the growth media, that stimulated the sulphate starvation pathway and activate sulphate transporters, leading to higher accumulation of selenate. Authors also shown that it is possible to produce grain and herb of Tartary and common buckwheat containing appropriate amounts of Se for food without affecting the yield of the plants.

SILICON

Silicon (Si) is important element for plant structure and has very important protective role in plants. It improves their potential to cope with various stresses including drought, extreme temperatures, herbivory and pathogen attacks. Accumulation of silicon differs between different plant taxa and also changes during plant development, resulting to differences in their sensitivity to environmental parameters. Silicon uptake in plants depends on transpiration stream (Grašič et al. 2019). Common and Tartary buckwheat were foliarly treated with different concentration of silicon. Height, number of side branches, number of leaves, dry mass of the grains, leaves and stems and amount of rutin were measured in control and treated groups.

CONCLUSION

In conclusion, addition of selenium led to the accumulation of this element in buckwheat plants. Biofortification with selenium and iodine in different forms simultaneously for human nutrition should be done with care because of synergistic or antagonistic effects of each of these elements.

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UV ABSORBING COMPOUNDS IN BUCKWHEAT PROTECT PLANTS AND PROVIDE HEALTH BENEFIT FOR HUMANS

UV ABSORBIRAJOČE SNOVI V AJDI ŠČITIJO RASTLINE IN PRISPEVAJO K ZDRAVJU LJUDI

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ABSTRACT

UV absorbing compounds in buckwheat protect plants and provide health benefit for humans

Buckwheat became a pan-Eurasian crop, when it expanded via Himalaya to Europe. Common buckwheat is one of the oldest domesticated crops in Asia, while Tartary buckwheat is still thriving as a wild or weedy plant. Buckwheat belongs to dicotyledonous crops that can tolerate poor soils and extreme environment conditions. Buckwheat grows on high elevation, where the intensities of UV radiation are usually high. Buckwheat is a fast-growing plant rich in flavonoids, which absorb UV radiation and have an antioxidant potential. Flavnoids have positive effect also on human health. Besides common buckwheat flour, Tartary buckwheat flour is more and more used in preparing dishes, due to its much higher content of flavonoids rutin and quercetin compared to common buckwheat. Therefore, the studies on how the technological procedures of preparing Tartary buckwheat bread affect the content, availability and efficacy of flavonoids in buckwheat bread have been made. Buckwheat is commonly used in the dishes in Japan (soba noodles), China (buckwheat noodles), Korea (buckwheat noodles), Italy (buckwheat polenta), France (galettes), Slovenia (kasha, žganci). Common buckwheat and Tartary buckwheat are plants suitable for designing foods with good functional value and healthy features. Therefore, it has been determined that different technological procedures, such as hydrothermal treatment of grain, sourdough fermentation, dough preparation and baking influences the availability and changes in the content of flavonoids, rutin and quercetin and antioxidant activity in sour bread and food products, made with buckwheat flour.

Key words: Common buckwheat, Tartary buckwheat, sourdough bread, rutin, quercetin, flavonoids, UV absorbing compounds

IZVLEČEK

UV absorbirajoče snovi v ajdi ščitijo rastline in prispevajo k zdravju ljudi

Ajda je postala vseevrazijska kultura, ko se je preko območja Himalaje razširila v Evropo. Navadna ajda je ena najstarejših gojenih rastlin v Aziji, medtem ko tatarska ajda še vedno uspeva tudi kot divja ali plevelna rastlina. Ajda spada med gojene dvokaličnice, ki lahko prenašajo slaba tla in ekstremne razmere v okolju. Ajda raste na visoki nadmorski višini, kjer je intenziteta ultravijoličnega sevanja običajno visoka. Ajda je hitro rastoča rastlina, bogata z flavonoidi, ki absorbirajo UV sevanje in imajo antioksidativni potencial. Flavonoidi pozitivno vplivajo tudi na zdravje ljudi. Poleg moke iz navadne ajde se moka iz tatarske ajde vse pogosteje uporablja pri pripravi jedi, ker ima v primerjavi z navadno ajdo veliko večjo vsebnost flavonoidov kot sta rutin in kvercetin. Zato so bile narejene študije o tem, kako tehnološki postopki priprave kruha iz tatarske ajde vplivajo na vsebnost, razpoložljivost in učinkovitost flavonoidov v ajdovem kruhu. Ajdo je zelo pogosto uporabljajo v jedeh na Japonskem, na Kitajskem, Koreji, v Italiji, v Franciji, Sloveniji. Navadna ajda in tatarska ajda, sta rastlini primerni za pripravo živil z dobro funkcijsko vrednostjo in lastnostmi ugodnimi za zdravje. Ugotovljeno je bilo, da različni tehnološki postopki, kot so hidrotermična obdelava zrnja, mlečnokislinska fermentacija, priprava testa in peka, vplivajo na dostopnost in spremembe v vsebnosti flavonoidov, rutina in kvercetina in antioksidativno aktivnost kislih kruhov in prehranskih izdelkov pripravljenih iz ajdove moke .

Ključne besede: navadna ajda, tatarska ajda, kruhi s kislim testom, rutin, kvercetin, flavonoidi, UV absorbirajoče snovi

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1 INTRODUCTION

Buckwheat origins in southern China, probably Yunnan province (Ohnishi 1998), from where it gradually spread to the north of China, and further on across Russia and Ukraine (Kreft 1995). It became a pan-Eurasian crop, when it expanded via the Himalaya region to Europe (Hunt et al. 2018). Common buckwheat is one of the oldest domesticated crops in Asia, while Tartary buckwheat is still thriving as a wild or weedy plant (Tsuji & Ohnishi 2009). Buckwheat is an ancient dicotyledonous crop that tolerates poor soils and extreme environments (Bilal Pirzadah et al. 2013). Buckwheat, as a robust and undemanding plant, is becoming an important alternative staple foods crop. The residual nutrients from preceding crops are often sufficient for its adequate growth. Tartary buckwheat is grown in Luxembourg, and as a mixed crop with common buckwheat in Bosnia and Herzegovina. Tartary buckwheat has been introduced also to Slovenia, Italy and Sweden (Gao et al. 2016). Crop production of grains that are botanically not cereals, such as buckwheat (*Fagopyrum* spp.), is increasing (Ačanski et al. 2015). It is a fast-growing plant rich in flavonoids, which contribute efficiently to its biological activity (Horbowicz et al. 2011). Buckwheat grows on high elevation, where the intensities of UV radiation are usually high. Thus, it is feasible to study the effect of UV radiation on the synthesis of UV absorbing compounds. Among grain crops, research on buckwheat is raising attention because of its content of many healthy compounds (Christa & Soral-Śmietana 2008; Chitarrini et al. 2014). The comparative study of Sofic et al. (2010) showed that out of 50 medical plant species, rue (*Ruta graveolens*) plants contained the highest amount of rutin (86.6 mg/g DM) followed by buckwheat flowers (53.5 mg/g DM) (Budzynska et al. 2018). Besides common buckwheat flour, Tartary buckwheat flour is more and more used in preparing dishes, due to its much higher content of rutin and quercetin compared to common buckwheat (Fabjan et al. 2003; Jiang et al. 2007; Kreft 2016). Therefore, the studies on how the technological procedures of preparing Tartary buckwheat bread affect the content, availability and efficacy of flavonoids in buckwheat bread have been made (Vogrinčič et al. 2010; Zhang et al. 2010; Kočevar Glavač et al. 2017; Costantini et al. 2014; Lukšič et al. 2016a, 2016b). Buckwheat is commonly used in the dishes in Japan (soba noodles), China (buckwheat noodles), Korea (buckwheat noodles), Italy (buckwheat polenta), France (galettes), Slovenia (kasha, žganci) (Škrabanja et al. 2018).

2 THE EFFECT OF UV RADIATION ON THE CONTENT OF UV ABSORBING COMPOUNDS

Flavonoids have great potential to scavenge reactive oxygen species (ROS), compounds, which are also produced during water shortage (Hideg & Strid 2017). The production of UV-absorbing compounds in plants like flavonoids and related phenyl-propanoids are primary protective mechanisms protecting plants from potentially damaging solar UV radiation (Zhang et al. 2012; Barnes et al. 2016, Liang et al. 2006). Hideg & Strid (2017) reported that flavonoids can scavenge reactive oxygen species (ROS) by acting as antioxidants. The production is determined by the UV dose, radiation quality and time of exposure. Since UV-B triggers the secondary metabolic pathway, it may serve as a significant stimulator for plant antioxidant activity (Sebastian et al. 2018). Kishore et al. (2010) evidenced the positive correlations between phenolic compounds and the amounts of certain antioxidants and altitude of the growing site of Tartary buckwheat. In buckwheat leaves rutin and rutin-oxidaze enhance the defence against UV radiation, low temperature and water shortage (Suzuki et al. 2015). In the study of Gaberščik et al. (2002) common buckwheat plants (*Fagopyrum esculentum* Moench, variety 'Darja') were grown in outdoor experiments under reduced and ambient UV-B levels, and an UV-B level simulating 17% ozone depletion in Ljubljana (Slovenia). UV-B radiation induced synthesis of UV absorbing compounds. The flavonoid synthesis is significantly enhanced by UV radiation as shown in many studies (Gaberščik et al. 2002; Suzuki et al. 2005; Golob et al. 2018). Regvar et al. (2012) studied the effects of increased UV-B radiation that simulates 17% ozone depletion on fungal colonisation and concentrations of rutin, catechin and quercetin in common buckwheat and Tartary buckwheat. They found out induction of shoot quercetin concentrations in UV-B-treated common buckwheat plants, but no differences in flavonoid concentrations in Tartary buckwheat. Tartary buckwheat had higher concentrations of flavonoids comparing to common buckwheat. Authors presumed that concentrations of these secondary metabolites are the result of genetic pre-adaptation of Tartary buckwheat to higher altitudes, where they protect against UV radiation. Jovanović et al. (2006) studied the behaviour of the enzymatic antioxidant defense system in common buckwheat leaves and seedlings subjected to enhanced UV-B radiation (supplemented UV-B light (radiation 290-320 nm) for 90 min by use of a UV-B lamp (HPQ 100 W Phillips). Plants received 49 kJ m^{-2} biologically effective UV-B radiation. UV-B applied treatment caused enhanced level of methanol-soluble flavonoids, in line with studies from Gaberščik et al. (2002) and Suzuki et al. (2005). The study with fifteen populations of Tartary buckwheat from different elevations exposed to elevated UV-B radiation showed that the sensitivity of plants to UV-B radiation is not only associated with the ambient UV-B level in natural habitats but also with the relative growth rate of genotype (Yao et al. 2007). Thus, future effort to breed for more tolerant cultivars is possible. Yao et al. (2008) studied the effects of enhanced UV-B radiation on crop growth, morphology, reproduction, and physiology in three cultivars of *Fagopyrum esculentum* originating from different altitudes and revealed that enhanced UV-B radiation significantly affected plant growth, development and production, a cultivar originating from Qinghai-Tibet plateau being the most tolerant.

Debski et al. (2016) studied the impact of short-term UV-B treatment on the content of flavonoids and photosynthetic pigments in cotyledons, and the growth of common buckwheat seedlings. Seedlings were subjected to different doses of UV-B, 5 $W{\cdot}m^{-2}$ and 10 $W{\cdot}m^{-2}$. Exposure to UV-B enhanced the amount of anthocyanins in cotyledons while inhibiting hypocotyl elongation, but had no effect on the content of photosynthetic pigments. Exposure to UV-B radiation did not affect rutin levels or cause a decrease in it with respect to different cultivars.

Anthocyanin type and their contents in Tartary buckwheat stems were investigated by Eguchi and Sato (2009). The ratio of each anthocyanin type to total anthocyanins varied with nodal positions in an outdoor experiment. This experiment showed that UV stress influences the ratio of specific anthocyanins to total anthocyanins. This growth chamber experiment showed that the ratio of cyanidin-3-*O*-rutinoside to total anthocyanins was higher under UV conditions in comparison to non-UV conditions. Authors presume that Tartary buckwheat may accumulate cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside systematically to protect plants against UV stress.

In the experiments of Yao et al. (2006), Tartary buckwheat was grown in field plots under near-ambient solar UV-B (approximately 84–88% of solar UV-B), attenuated solar UV-B radiation (43–49% reduction in solar UV-B), and supplemental UV-B radiation (two levels: 5.30 and 8.50 kJ m-2 day-1). The amount of photosynthetic pigments was lowered by the ambient and enhanced UV-B radiation, while the UV-B absorbing compounds and rutin concentration increased, except at the highest level of UV-B irradiance exposure. Authors concluded that Tartary buckwheat is a potentially UV-B sensitive species, and also, that the crop response to UV-B radiation is associated with UV-B intensity, environmental factors and growing season.

Orsak et al. (2001) provided evidence about changes of total polyphenols, phenolcarboxylic acids, and ascorbic acid in three buckwheat samples (seeds, seedlings, and plants of *Fagopyrum esculentum* Moench, cv. Pyra and Emka, and Tartary buckwheat *Fagopyrum tataricum* Gaertner), induced by UV-C irradiation (lambda = 253.7 nm, P = 75 W, 0 - control, 42 and 84 h). Authors found out that *F. tataricum* contained much higher total polyphenol and rutin levels in comparison to *F. esculentum*, and that UV-C irradiation affected seeds causing an increase in the amounts of total polyphenols and rutin.

Tsurunaga et al. (2013) studied the effects of various light compositions on the levels of anthocyanins, rutin, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity in common buckwheat sprouts. Sprouts were irradiated with different sources of visible and ultraviolet light. Authors examined the effect of UV-B at wavelengths of 260-320 nm, 280-320 nm, and 300-320 nm on the synthesis of flavonoid compounds. Their results showed that irradiation with UV-B>300 nm increased the levels of anthocyanins and rutin, as well as the DPPH radical scavenging activity. When sprouts were irradiated with UV-B light at wavelengths of 260-300 nm, yellowing or withering occurred.

The effects of blue and UV-A (365 nm)/UV-C (254 nm), or their combinations, on the levels of total flavonoids, rutin, quercetin, PAL, CHI, rutin degrading enzymes (RDEs), and DPPH radical scavenging activity in Tartary buckwheat sprouts were researched (Ji et al. 2016). Authors found out that blue light in combination with UV-C (BL+UV-C) enhanced the accumulation of total flavonoids, rutin, and quercetin, while this effect was not observed when blue light was combined with UV-A (BL+UV-A).

To conclude, UV radiation stimulates phenylpropanoid biosynthetic pathway leading to the accumulation of compounds that protect plants from the UV caused damage. However, these responses differ regarding the intensity of UV radiation, buckwheat species and on elevation of origin of cultivar. Some studies showed that Tartary buckwheat is more tolerant to UV radiation since it originates in high altitudes.

3 IMPORTANCE OF BUCKWHEAT AS THE STAPLE CROP

Common buckwheat and Tartary buckwheat are used in different parts of the world for making various food products. Bonafaccia et al. (2003) evidenced that the grain of both of these cultivated buckwheat species contains up to 27% fibre. Buckwheat seeds are considered as a prebiotic food because they can increase the lactic acid bacteria in the intestine due to their content of resistant starch (Škrabanja et al. 1998, 2001). Buckwheat has small starch granules and an amylose content of starch higher than cereals, but lower than those of legumes (Škrabanja & Kreft 1998; Schirmer et al. 2013).

Buckwheat (*Fagopyrum esculentum*) herb is used for herbal medicinal products, for preparing green buckwheat tea, for producing buckwheat green leaf flour as an additive to certain food products, and the fresh green plant parts can be used as a vegetable (Kreft et al. 2006).

Buckwheat contains more rutin than the majority of other grain crops, fruits, and vegetables (Li et al. 2011). Many authors reported that seeds of Tartary buckwheat have higher contents of high quality proteins and higher concentrations of flavonoids rutin and quercetin than those of common buckwheat seeds (Fabjan et al. 2003; Gao et al. 2016). Fabjan et al. (2003) reported that Tartary buckwheat contains about 100-fold more rutin than does common buckwheat. Buckwheat is thus an important source of anti-oxidant activity in functional foods (Holasova et al. 2002) due to the presence of the flavonoids rutin and quercetin in buckwheat grain and products, because of their anti-oxidant and anti-inflammatory effects. Rutin and quercetin were also present in baked biscuits made from flour of both species of buckwheat (Wieslander et al. 2011). Buckwheat products decrease cholesterol levels and also improve lung capacity in humans (Wieslander et al. 2011; Yang et al. 2014). Extracts from common buckwheat and Tartary buckwheat can also protect DNA from damage caused by hydroxyl radicals (Vogrinčič et al. 2010). Experiments showed that buckwheat flour can improve diabetes, obesity, hypertension, hypercholesterolemia and constipation (Li & Zhang 2001).

4 RUTIN AND QUERCETIN TRANSFORMATION DURING PREPARATION OF BUCKWHEAT SOURDOUGH BREAD AND THE EFFECT OF HYDROTHERMAL TREATMENT OF TARTARY BUCKWHEAT GRAIN TO THE TRANSFORMATION OF RUTIN TO QUERCETIN

Pseudocereals have received increased interest in recent years due to the growing awareness of the need for healthy diets. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is a pseudocereal rich in dietary beneficial components. It is a popular food source, containing balanced amino-acid composition of its proteins, fiber, retrograded starch, trace elements, vitamins and antioxidants, including flavonoids (Holasova et al. 2002; Bonafaccia et al. 2003; Fabjan et al. 2003; Pongrac et al. 2016). Buckwheat does not contain gluten proteins, so it is safe for people suffering from gluten intolerance (Vogrinčič et al. 2010; Kocjan Ačko 2015). Tartary buckwheat possesses phenolic compounds, such as rutin, quercetin, kaempferol-3-rutinoside and flavanol triglycoside, and has a high antioxidant activity that helps to reduce the risk of major chronic diseases (Tian et al. 2002). Tartary buckwheat contain more rutin (a quercetin-3-rutinoside; 10 and 40 mg/g, respectively) than most vegetables, fruits and grain crops (Li & Zhang 2001), and more rutin (up to about 14.7 mg per g DM) than common buckwheat (up to about 0.1 mg per g DM) (Fabjan et al. 2003; Kreft 2016). Tartary buckwheat was widely grown in the territory of Slovenia since the beginning of 19th century (Fabjan et al. 2003). During the 20th century, the cultivation of Tartary buckwheat gradually decreased, due to cultivation of other crops (Kreft 1995, 2011). However, common buckwheat bread was traditionally made in the past, and its use is reviving in present times. Due to much higher content of rutin and quercetin in Tartary buckwheat flour (Jiang et al. 2007; Qin et al. 2010), the Tartary buckwheat bread has been prepared to investigate the effects of hydrothermal treatment of grain, sourdough fermentation and the baking process on rutin and quercetin content and on the antioxidant activities of common buckwheat and Tartary buckwheat bread (Costantini et al. 2014; Lukšič et al. 2016a, 2016b).

One of the ways to prepare bread involves sourdough fermentation, which can be accompanied by the formation of lactic acid and acetic acid that have an impact on dough processing and the preparation of sourdough bread (Michalska et al. 2008). Many authors have reported that sourdough fermentation can affect the improvement of structural and sensory properties, as well as persistence of sour bread (Gobbetti et al. 2014; Rizzello et al. 2016; Rinaldi et al. 2017; Ua Arak et al. 2017). Experiments showed that sourdough fermentation can improve the availability of proteins and minerals, total content of dietary fiber, total content of phenolic substances and antioxidant activity of sour bread (Boskov Hansen et al. 2002; Gandhi & Dey 2013; Costantini et al. 2014; Rizzello et al. 2016). It has been reported that Tartary buckwheat sour bread had a lower content of carbohydrates, lower glycemic index and a lower energy value than the same amount of Tartary buckwheat flour (Novotni et al. 2012; Costantini et al. 2014). In sour bread, various substances such as alcohols, aldehydes, esters, hydrocarbons, ketones, terpenes, furans and phenols are also produced during the process of lactic acid fermentation (Boskov Hansen et al. 2002). Microorganisms in sourdough starter can also form some new nutritional components, such as peptides and other amino acid derivatives, and some prebiotic polysaccharides (De Vos 2005). It has been found that proteases released by yeast and the enzymes of selected lactobacilli in sourdough starter can metabolise gluten in wheat flour (Weiser et al. 2008).

In the studies, a decrease in the content of rutin and its conversion into quercetin, which is prevented by various types of heat treatment of food products made from Tartary buckwheat and common buckwheat have been reported (Vogrinčič et al. 2010). Changes in the content of other substances with antioxidant activity have also been measured (Vogrinčič et al. 2010; Zhang et al. 2010; Sakač et al. 2011). Cho and Lee (2015) reported that changes in the content of rutin and other antioxidants have not been affected by rapid frying of the noodles, while cooking caused a significant reduction in the content of rutin in the noodles prepared from wheat flour enriched with rutin extract from Tartary buckwheat bran. Jambrec et al. (2015) reported that in full wheat noodles with the addition of pre-autoclaved (120 ° C) flour of common buckwheat, the conversion of rutin into quercetin decreased. While during the cooking of these noodles, the conversion of rutin into quercetin did not occur at all. The decrease of phenolic substances in noodles with the addition of buckwheat flour was comparable to decrease of phenolic substances in control sample (whole grain wheat noodles). In the experiments of Qin et al. (2013) it has been found that soaking of Tartary buckwheat grains (40 °C, 12-14 h) influenced the reduction of the starch and rutin content and influenced the increase in the content of quercetin, kaempferol, isoquercitrin, total flavonoids and phenolic substances. After the pre-soaked grains of Tartary buckwheat were treated with steam (100 , 40-60 min), the content of total flavonoids and total phenolic substances decreased, while the content of rutin in the grain samples increased. It is possible that the process of decomposition of rutin was initiated in the process of soaking grain of Tartary buckwheat, and the steam treatment process triggered a reconnection of rutin. Sensoy et al. (2006) provided evidence that shows that the processing (roasting) of buckwheat flour had no effect on the content of total phenolic substances in buckwheat flour. The DPPH method, used for determination of antioxidant activity, however, showed a slight decrease in antioxidant activity of buckwheat flour while roasting at 200 °C for 10 min, while the roasting at 170 °C had no effect on the reduction of antioxidant activity. The significant decrease in anti-oxidant activity in Tartary buckwheat flour as a result of various thermal treatments such as roasting, steam-pressure heating, and microwaving, has been reported (Zhang et al. 2010). A small decrease in the anti-oxidant activity in common buckwheat flour roasted for 10 min at 200 °C has also been noted (Yasuda & Nakagawa 1994). The results of the experiment suggests that optimization of processing is the key to maintaining healthy substances in buckwheat products.

Bread is a staple food for the majority of the world populations and contributes substantially to the intakes of certain nutrients. Common buckwheat and especially Tartary buckwheat flour possesses some phenolic compounds, such as rutin, quercetin and a high antioxidant activity, that is why this type of flour is suitable to obtain bread with improved nutritional value and healthy features. Therefore, the studies have been made on how the technological procedures of preparing common buckwheat and Tartary buckwheat bread affect the content, availability and efficacy of flavonoids in buckwheat bread. It has been proven that, in bread made with Tartary buckwheat flour, rutin concentration decreased, whereas the quercetin concentration increased and remained stable during processing (Vogrinčič et al. 2010). Lukšič et al. 2016a, 2016b reported that during Tartary buckwheat bread making there was a transformation of a large portion of rutin into quercetin. Breads containing common buckwheat flour, contained several flavonoids, such as rutin and quercetin, and had higher antioxidant activity than wheat bread (Lin et al., 2009). The combined effects of sourdough fermentation and the baking process on the flavonoid concentrations and antioxidant properties of common buckwheat and Tartary buckwheat sourdough starter, bread dough and sourdough bread have also been studied. It has been established that common buckwheat and Tartary buckwheat bread making is feasible without any addition of wheat or gluten, by using the sour bread starter procedure (Costantini et al. 2014; Lukšič et al. 2016a, 2016b). Tartary buckwheat breads made with 100% Tartary buckwheat flour contained the highest phenol (53.3 mg GAE/g) and flavonoid (16.8 mg RE/g) contents, mean-while Tartary buckwheat sour bread, containing 10% of chia (*Salvia hispanica* L.) flour had the highest antioxidant activity (32.0 mmol Fe^{2+} E/g and 128.6 mmol GAE/g, respectively) compared to 100% common buckwheat and wheat sour breads and common buckwheat and wheat sour breads fortified with 10% of chia flour (Costantini et al. 2014). On the contrary, in another experiment a higher antioxidant activity has been measured in common buckwheat bread compared to Tartary buckwheat bread. This might be because of the synthesis of substances with antioxidant properties, including certain Maillard reaction products that occur in bread during thermal treatment (Zhang et al. 2010; Lukšič et al. 2016a). A similar result were established in a study of Vogrinčič et al. (2010) in which Tartary buckwheat bread and breads made of mixtures of Tartary buckwheat and wheat flour were studied. A decrease in polyphenol concentration through baking was observed in all samples. The high DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity in mixed breads (32-56%) and in Tartary buckwheat bread (85-90%) decreased slightly through the bread making process, while an increase of antioxidant activity in bread made of 100% wheat flour during bread making was observed. In the experiments of Lukšič et al. (2016a) sourdough bread was prepared of flour of common buckwheat and of Tartary buckwheat to follow the transformation of rutin and quercetin during sourdough fermentation, bread making procedure and baking of bread. During Tartary buckwheat sourdough fermentation, there was conversion of rutin to quercetin. In the Tartary buckwheat sourdough bread there was no rutin, whereas there was 5.0 mg/g quercetin. In common buckwheat bread, neither rutin nor quercetin were present. Vogrinčič et al. (2010) reported that with the addition of water to mixtures containing Tartary buckwheat during the preparation of the Tartary buckwheat dough made with yeast, rutin concentration decreased, while quercetin concentration increased. The rutin concentration continued to decrease during the bread baking process, while the concentration of quercetin remained stable. After baking, rutin (0.47 mg/g) was present only in bread made of 100% Tartary buckwheat flour along with quercetin (4.83 mg/g). Suzuki et al. (2015) reported that when using a version of Tartary buckwheat with traces of rutinosidase, bread of a flour mixture of Tartary buckwheat and wheat with 0.63 mg/g rutin was prepared, representing approximately 50% of the retained rutin in bread, compared to the source material (flour).

Hydrothermal treatment is a process that involves heating with hot water or steam, followed by cooling and drying of buckwheat groats to produce husked buckwheat or kasha. It is the traditional technology known and still applied in Slovenia, Croatia, Poland, Ukraine and Russia (Kreft 2003). In a study of Lukšič et al. (2016b) the impact of hydrothermal treatment on extractability of flavonoids from starchy matrix was investigated. Tartary buckwheat grain was hydrothermally treated and milled to yield hydrothermally treated flour. In control sample, not hydrothermally treated Tartary buckwheat flour, most of extractable rutin (8 mg of rutin per g DM (dry matter)) was extracted during the first 20 min of extraction. In hydrothermally treated Tartary buckwheat flour only 4 mg of rutin per g DM was extracted in 20 min, and 7 mg of rutin per g DM within 8 h, respectively. This data indicates that, during the hydrothermal treatment, rutin becomes embedded in the flour matrix. Slowly extracted rutin was protected from transformation to quercetin during bread making procedure. From an initial 7 mg of extractable rutin per g DM in hydrothermally treated buckwheat flour, Tartary buckwheat bread contained 2 mg of rutin per g DM, and 6 mg of quercetin per g DM. No other Tartary buckwheat bread making technology which would be able to conserve such an amount of rutin from flour through the process to the final bread product have been reported.

Many studies certificate that both, fermentation process and heat treatment affects changes in content and accessibility of substances with antioxidant properties in buckwheat products. However, these changes differ regarding buckwheat species and plant properties, preparation process, fermentation method and thermal treatment used. This information contributes to a better understanding of the effects of different food preparation methods on substances with antioxidant activity and information on the persistence of rutin and quercetin in sourdough bread and other food products. These findigs are as well important for designing foods with high concentrations of flavonoids and good functional value.

POVZETEK

Ajda izvira iz južne Kitajske, verjetno province Yunnan, od koder se je postopoma razširila na sever Kitajske in naprej po Rusiji in Ukrajini. Postala je vseevropska rastlina ko se je razširila preko Himalaje v Evropo. Navadna ajda je ena najstarejših gojenih rastlin v Aziji, medtem ko tatarska ajda še vedno uspeva tudi kot divja ra-

stlina ali plevel. Ajda je starodavna dvokaličnica, ki prenaša revno prst in ekstremna okolja. Ajda, kot robustna in nezahtevna rastlina, postaja pomemben vir za pridelavo osnovnih živil. Hranilne snovi, ki ostanejo v zemlji iz gojenih rastlin prejšnjih sezon, so pogosto zadostne za njeno ustrezno rast. Tatarsko ajdo sejejo v Luksemburgu in kot mešani pridelek z navadno ajdo v Bosni in Hercegovini. Tatarsko ajdo smo pred kratkim začeli ponovno sejati tudi v Sloveniji, Italiji in na Švedskem. Pridelava ajde (*Fagopyrum* spp.), se povečuje. Je hitro rastoča rastlina, bogata z flavonoidi, ki učinkovito prispevajo k njeni biološki aktivnosti. Ajda raste na visoki nadmorski višini, kjer so intenzitete UV sevanja običajno visoke. Zato je smiselno preučevati učinke UV sevanja na sintezo spojin, ki absorbirajo UV. Število raziskav o ajdi se povečuje ker ima visoko vsebnost snovi, ki pozitivno vplivajo na zdravje ljudi.

Primerjalna študija je pokazala, da je od 50 vrst rastlinskih zdravilnih rastlin vinska rutica vsebovala največjo količino rutina (86,6 mg / g SM), sledijo ajdovi cvetovi (53,5 mg / g SM).

Poleg moke iz navadne ajde, se za pripravo jedi vedno bolj uporablja tatarska ajdova moka, zaradi veliko večje vsebnosti rutina in kvercetina. Zato so izdelane študije o tem, kako tehnološki postopki priprave tatarskega ajdovega kruha ajde vplivajo na vsebino, razpoložljivost in učinkovitost flavonoidov v ajdovem kruhu. Ajda se zelo pogosta uporablja za pripravo jedi na Japonskem (soba rezanci), na Kitajskem (ajdovi rezanci), v Koreji (ajdovi rezanci), Italiji (ajdova polenta), Franciji (galettes), Sloveniji (kaša, žganci).

Zaradi naraščanja zavedanja o pomenu zdrave prehrane, rastline, ki botanično niso žita, med katera uvrščamo tudi tatarsko ajdo (*Fagopyrum tataricum* Gaertn.) ponovno vzbujajo zanimanje med ljudmi. Tatarsko ajdo odlikuje odlična hranilna vrednost, saj ima uravnoteženo aminokislinsko sestavo, vsebuje prehranske vlaknine, retrogradiran škrob, elemente v sledovih, vitamine in antioksidante, vključno s flavonoidi (Holasova et al. 2002; Bonafaccia et al. 2003; Fabjan et al. 2003; Pongrac et al. 2016). Ajda ne vsebuje glutena in je varno živilo tudi za osebe z ugotovljenimi intolerancami na gluten (Vogrinčič et al. 2010; Kocjan Ačko 2015). Tatarska ajda ima visoko antioksidativno aktivnost in vsebuje fenolne spojine, kot so rutin, kvercetin, kaempferol-3-rutinozid in triglikozid flavanol, ki lahko prispevajo k zmanjšanju možnosti za nastanek kroničnih bolezni (Tian et al. 2002). Tatarska ajda vsebuje več rutina (10 in 40 mg/g), kot večina zelenjave, sadja in žit (Li & Zhang 2001) in več rutina (do 14,7 mg/g suhe mase) kot navadna ajda (do 0,1 mg/g suhe mase) (Fabjan et al. 2003; Kreft 2016). Tatarsko ajdo so na območju Slovenije pridelovali že v začetku 19. stoletja (Fabjan et al. 2003). V obdobju 20. stoletja pa se je pridelava tatarske ajde zmanjševala, predvsem zaradi pridelave drugih žit (Kreft 1995, 2011). Kruh iz moke navadne ajde so tradicionalno pekli že v preteklosti, sedaj pa se zanimanje za ajdov kruh ponovno veča. Zaradi večje vsebnosti rutina in kvercetina v moki tatarske ajde (Jiang et al. 2007; Qin et al. 2010), so bili izdelani kruhi iz tatarske ajde, z namenom, da bi preučevali učinke hidrotermične obdelave zrnja, mlečnokislinske frementacije testa in vpliv peke na vsebnot rutina in kvercetina ter na antioksidativno aktivnost kruhov pripravljenih iz moke tatarske ajde in navadne ajde (Costantini et al. 2014; Lukšič et al. 2016a, 2016b).

Eden od načinov priprave kruha vključuje mlečnokislinsko fermentacijo, pri kateri se tvorita mlečna in ocetna kislina, ki vplivata na postopek priprave kislega kruha (Michalska et al. 2008). Raziskovalci so poročali, da mlečnokislinska fermentacija lahko vpliva na izboljšanje strukturnih in senzoričnih lastnosti kislega kruha in lahko podaljša obstojnost takega kruha (Gobbetti et al. 2014; Rizzello et al. 2016; Rinaldi et al. 2017; Ua Arak et al. 2017). V poskusih je bilo ugotovljeno, da mlečnokislinka fermentacija lahko izboljša dostopnost beljakovin, mineralov, skupno vsebnost prehranskih vlaknin, skupno vsebnost fenolnih snovi in antioksidativno ativnost kislih kruhov (Boskov Hansen et al. 2002; Gandhi & Dey 2013; Costantini et al. 2014; Rizzello et al. 2016). Raziskovalci so poročali tudi, da je imel kisel kruh iz tatarske ajde manjšo vsebnost ogljikovih hidratov, nižji glikemični indeks in manjšo energijsko vrednost, kot enaka količina moke tatarske ajde (Novotni et al. 2012; Costantini et al. 2014). V kislih kruhih so med procesom mlečnokislinske fermentacije nastajale tudi snovi, kot so alkoholi, aldehidi, estri, hidrokarboni, ketoni, terpeni, furani, in fenoli (Boskov Hansen et al. 2002). Mikroorganizmi v osnovi za kislo testo prav tako lahko tvorijo nekatere nove prehranske komponente, kot so peptidi in drugi derivati aminokislin, in nekatere prebiotične polisaharide (De Vos 2005). Ugotovljeno je bilo, da lahko proteaze, ki jih v osnovi za kislo testo sproščajo kvasovke in encimi selekcioniranih laktobacilov, presnavljajo gluten v pšenični moki (Weiser et al. 2008).

V raziskavah poročajo, da je, kot posledica različnih načinov toplotne obdelave v živilih, pripravljenih iz tatarske ajde in navadne ajde, prihajalo do zmanjšanja vsebnosti rutina in pretvorb le tega v kvercetin. Izmerili so tudi spremembe v vsebnosti drugih snovi z antioksidativnim učinkom (Vogrinčič et al. 2010; Zhang et al. 2010; Sakač et al. 2011). Cho & Lee (2015) sta poročala o tem, da na spremembe vsebnosti rutina in drugih antioksidantov hitro cvrtje rezancev ni vplivalo, med tem, ko je kuhanje povzročilo znatno zmanjšanje vsebnosti rutina v rezancih, pripravljenih iz pšenične moke in iz z rutinom obogatenega izvečka otrobov tatarske ajde. Jambrec in sod. (2015) so ugotovili, da se je v polnozrnatih pšeničnih rezancih z dodatkom predhodno avtoklavirane (120 °C) moke navadne ajde, pretvorba rutina v kvercetin zmanjšala. Med kuho teh rezancev pa do pretvorbe rutina v kvercetin sploh ni prišlo. Izguba fenolnih snovi v rezancih z dodatkom ajdove moke je bila v območju kontrolnega vzorca (polnozrnatih pšeničnih rezancev). V poskusih, ki so jih opravili Qin in sod. (2013) so ugotovili, da je namakanje zrn tatarske ajde (40 °C, 12-14 h) vplivalo na zmanjšanje v zrnju prisotnega deleža škroba in rutina in vplivalo na povečanje vsebnosti kvercetina, kaempferola, izokvercitrina, skupnih flavonoidov in fenolnih snovi. Po tem, ko so predhodno namočeno zrnje tatarske ajde obdelali še s paro (100 °C, 40-60 min), se je vsebnost skupnih flavonoidov in skupnih fenolnih snovi še naprej zmanjševala, med tem, ko se je vsebnost rutina v vzorcu zrnja povečala. Mogoče je, da je bil proces razgradnje rutina sprožen v procesu namakanja zrnja tatarske ajde, proces obdelave s paro pa je sprožil ponovno spajanje rutina. Sensoy in sod. (2006) so predložili rezultate, ki nakazujejo da procesiranje (praženje) ajdove moke ni imelo vpliva na vsebnost skupnih fenolnih snovi v ajdovi moki. DPPH metoda določanja antioksidativne aktivnosti je sicer pokazala, da je prišlo po praženju ajdove moke (200°C, 10 min) do rahlega zmanjšanja antioksidativne aktivnosti moke ajde, praženje pri 170°C pa ni imelo vpliva na zmanjšanje antioksidativne aktivnosti. Do značilnega zmajšanja antioksidativne aktivnosti v moki tatarske ajde je prišlo, kot posledica različnih načinov toplotne obdelave moke, kot so praženje, segrevanja s paro in mikrovalovno segrevanje (Zhang in sod. 2010). Izmerili so tudi manjše zmanjšanje antioksidativne aktivnosti v moki navadne ajde, ki so jo pražili 10 min na 200 °C (Yasuda & Nakagawa 1994). Rezultati poskusov nakazujejo, da je optimizacija procesiranja ključna za ohranitev zdravju koristnih snovi v ajdovih izdelkih.

Kruh je osnovno živilo za velik del svetovnega prebivalstva in lahko znatno prispeva k vnosu nekaterih hranil. Moka navadne ajde in posebno moka tatarske ajde vsebujeta nekatere fenolne snovi, kot sta rutin in kvercetin ter imata visoko antioksidativno aktivnost, zato sta primerni za pripravo kruhov z izboljšano prehransko vrednostjo in zdravju koristnimi lastnostmi. Poznavanje teh lastnosti moke je privedlo do raziskav v katerih so preučevali, kako tehnološki postopki priprave kruha iz navadne in tatarske ajde vplivajo na vsebnost, dostopnost in učinkovitost flavonoidov v ajdovih kruhih. Preučevali so tudi razmerje med flavonoidoma, rutinom in kvercetinom v ajdi, in do kakšne mere se rutin pretvarja v kvercetin, kot posledica izpostavljenosti različnim tehnološkim postopkom priprave ajdovih kruhov. Ugotovljeno je bilo, da se je v kruhu, pripravljenem iz moke tatarske ajde, vsebnost rutina zmanjševala, vsebnost kvercetina pa je naraščala in ostala stabilna med procesom priprave kruha (Vogrinčič et al. 2010). Kruhi, ki so vsebovali moko navadne ajde so vsebovali flavonoide, kot sta rutin in kvercetin in so imeli večjo antioksidativno aktivnost od pšeničnega kruha (Lin et al. 2009).

Preučevani so bili tudi skupni učinki mlečnokislinske fermentacije in peke na vsebnost flavonoidov in antioksidativne lastnosti testa in kruhov iz moke navadne in tatarske ajde. Ugotovljeno je bilo, da je priprava kruha s postopkom mlečnokislinske fermentacije, iz moke navadne ajde in tatarske ajde možna brez dodatka pšenične moke ali glutena (Costantini et al. 2014; Lukšič et al. 2016a, 2016b). Kruhi pripravljeni iz 100 % moke tatarske ajde so imeli največjo vsebnost fenolov (53,3 mg GAE/g) in flavonoidov (16,8 mg RE/g), v kruhih pripravljenih iz moke tatarske ajde z dodatkom 10% moke oljne kadulje (*Salvia hispanica* L.) pa so izmerili največjo antioksidativno aktivnost (32,0 mmol Fe^{2+} E/g and 128,6 mmol GAE/g) v primerjavi s kruhi izdelanimi iz 100% moke navadne ajde in pšenice in kruhi izdelanimi iz moke navadne ajde in pšenice z dodatkom 10% moke oljne kadulje (Costantini et al. 2014). V nasprotju s temi ugotovitvami, je bila v drugem poskusu, največja antioksidativna aktivnost izmerjena v kruhu iz moke navadne ajde v primerjavi s kruhom tatarske ajde. To je lahko posledica spajanja snovi z antioskidativnim učinkom, vključno snovi, ki lahko nastajajo med Maillardovo reakcijo, pod vplivom toplotne obdelave (Zhang et al. 2010; Lukšič et al. 2016a). O podobnih ugotovitvah so poročali tudi Vogrinčič in sod. (2010), ki so preučevali kruhe iz moke tatarske ajde in kruhe iz mešanice moke tatarske ajde in pšenice. V vseh vzorcih kruhov je med peko prišlo do zmanjšanja vsebnosti polifenolov. V mešanih kruhih iz moke tatarske ajde in pšenice in v kruhih iz moke tatarske ajde je med postopkom peke prišlo do zmanjšanja antioksidativne aktivnosti, ugotovljene z metodo DPPH (2,2-difenil-1-pikrilhidrazil), med tem ko je do povečanja antioksidativne aktivnosti, med postopkom peke, prišlo v kruhih iz 100 % pšenične moke. Lukšič in sod (2016a) so pripravili kisle kruhe iz moke navadne ajde in tatarske ajde, z namenom, da bi spremljali pretvorbe rutina in kvercetina med postopkom mlečnokislinske fermentacije, priprave in peke kruha. Med mlečnokislinsko fermentacijo je prišlo do pretvorbe rutina v kvercetin. V kruhu pripravljenem iz moke tatarske ajde ni bilo rutina, bilo pa je 5,0 mg/g kvercetina. V kruhu iz moke navadne ajde niso izmerili ne vsebnosti rutina in ne kvercetina. Vogrinčič in sod. (2010) so poročali, da se je ob dodatku vode testu iz moke tatarske ajde in kvasa, vsebnost rutina zmanjševala, vsebnost kvercetina pa je naraščala. Vsebnost rutina se je še naprej zmanjševala med postopkom peke kruha, medtem ko je vsebnost kvercetina ostala stabilna. Po peki se je v kruhu iz 100% moke tatarske ajde ohranilo 0,47 mg/g rutina in 4,83 mg/g kvercetina. Suzuki in sod. (2015) so pripravili kruh iz mešanice moke tatarske ajde s sledovi rutinozidaze in pšenice z 0,63 mg/g rutina, kar je približno 50 % delež ohranjenega rutina v kruhu, v primerjavi z izvorno surovino (moko).

Hidrotermična obdelava je postopek, ki vključuje segrevanje z vročo vodo ali paro, ki mu sledi ohlajanje in sušenje ajdovega zrnja z namenom, da bi pripravili oluščeno ajdovo zrnje oziroma kašo. Gre za tradicionalen postopek, ki ga še vedno uporabljamo v Sloveniji, na Hrvaškem, Poljskem, v Ukrajini in Rusiji (Kreft 2003). V raziskavi, ki so jo opravili Lukšič in sod. (2016b) smo preučevali učinek hidrotermične obdelave na dostopnost in izločanje flavonoidov iz škrobnih struktur. Zrnje tatarske ajde je bilo hidrotermično obdelano in zmleto v hidrotermično obdelano moko. V kontrolnem vzorcu, hidrotermično neobdelani moki, je bila večina kvercetina izločena že po 20 minutah ekstrakcije (8 mg/g DM (suhe mase)). V hidrotermično obdelani moki tatarske ajde je bilo po 20 min ekstrakcije, izločenega le 4 mg/g DM rutina, in 7 mg/g DM rutina po 8 h ekstrakcije. Ti podatki nakazujejo, da je med postopkom hidrotermične obdelave, rutin postal vključen v druge strukture v moki in tako postal zaščiten pred pretvorbo v kvercetin med postopkom priprave kruha. Iz začetnih 7 mg/g DM v hidrotermično obdelani moki tatarske ajde, je kruh pripravljen iz te moke vseboval 2 mg/g DM rutina in 6 mg/g kvercetina. Za nobeno drugo metodo priprave kruha iz moke tatarske ajde ni bilo ugotovljeno, da bi se med postopkom priprave kruha ohranilo toliko rutina.

Mnoge raziskave potrjujejo, da postopek mlečnokislinske fermentacije in toplotne obdelave vplivata na spremembe v vsebnosti in dostopnosti snovi z antioksidativnimi lastnostimi v izdelkih iz ajde. Te spremembe se razlikujejo glede na vrsto ajde in lastnosti rastline, postopek priprave, metodo fermentacije in potopek toplotne obdelave. Te informacije prispevajo k boljšemu razumevanju vpliva različnih metod priprave živil z antioksidativno aktivnostjo in obstojnosti rutina in kvercetina v kislih kruhih in drugih prehranskih izdelkih. Te ugotovitve so pomembne tudi za pripravo živil z večjo vsebnostjo flavonoidov in izboljšano funkcijsko vrednostjo.

ZAHVALA

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NAVODILA AVTORJEM

Folia biologica et geologica so znanstvena revija IV. razreda SAZU za naravoslovne vede. Objavljajo naravoslovne znanstvene razprave in pregledne članke, ki se nanašajo predvsem na raziskave v našem etničnem območju Slovenije, pa tudi raziskave na območju Evrope in širše, ki so pomembne, potrebne ali primerljive za naša preučevanja.

1. ZNANSTVENA RAZPRAVA

Znanstvena razprava zajema celovit opis izvirne raziskave, ki vključuje teoretični pregled tematike, podrobno predstavlja rezultate z razpravo in zaključki ali sklepi in pregled citiranih avtorjev. V izjemnih primerih so namesto literaturnega pregleda dovoljeni viri, če to zahteva vsebina razprave.

Razprava naj ima klasično razčlenitev (uvod, material in metode, rezultati, diskusija z zaključki, zahvale, literatura idr.).

Dolžina razprave, vključno s tabelami, grafikoni, tablami, slikami ipd., praviloma ne sme presegati 2 avtorskih pol oziroma 30 strani tipkopisa. Zaželene so razprave v obsegu ene avtorske pole oziroma do dvajset strani tipkopisa.

Razpravo ocenjujeta recenzenta, od katerih je eden praviloma član SAZU, drugi pa ustrezni tuji strokovnjak. Recenzente na predlog uredniškega odbora revije *Folia biologica et geologica* potrdi IV. razred SAZU.

Razprava gre v tisk, ko jo na predlog uredniškega odbora na seji sprejmeta IV. razred in predsedstvo SAZU.

2. PREGLEDNI ČLANEK

Pregledni članek objavljamo po posvetu uredniškega odbora z avtorjem. Na predlog uredniškega odbora ga sprejmeta IV. razred in predsedstvo SAZU. Članek naj praviloma obsega največ 3 avtorske pole (tj. do 50 tipkanih strani).

3. NOVOSTI

Revija objavlja krajše znanstveno zanimive in aktualne prispevke do 7000 znakov.

4. IZVIRNOST PRISPEVKA

Razprava oziroma članek, objavljen v reviji *Folia biologica et geologica*, ne sme biti predhodno objavljen v drugih revijah ali knjigah.

5. JEZIK

Razprava ali članek sta lahko pisana v slovenščini ali katerem od svetovnih jezikov. V slovenščini zlasti tedaj, če je tematika lokalnega značaja.

Prevod iz svetovnih jezikov in jezikovno lektoriranje oskrbi avtor prispevka, če ni v uredniškem odboru dogovorjeno drugače.

6. POVZETEK

Za razprave ali članke, pisane v slovenščini, mora biti povzetek v angleščini, za razprave ali članke v tujem jeziku ustrezen slovenski povzetek. Povzetek mora biti dovolj obširen, da je tematika jasno prikazana in razumljiva domačemu in tujemu bralcu. Dati mora informacijo o namenu, metodi, rezultatu in zaključkih. Okvirno naj povzetek zajema 10 do 20 % obsega razprave oziroma članka.

7. IZVLEČEK

Izvleček mora podati jedrnato informacijo o namenu in zaključkih razprave ali članka. Napisan mora biti v slovenskem in angleškem jeziku.

8. KLJUČNE BESEDE

Število ključnih besed naj ne presega 10 besed. Predstaviti morajo področje raziskave, podane v razpravi ali članku. Napisane morajo biti v slovenskem in angleškem jeziku.

9. NASLOV RAZPRAVE ALI ČLANKA

Naslov razprave ali članka naj bo kratek in razumljiv. Za naslovom sledi ime/imena avtorja/avtorjev (ime in priimek).

10. NASLOV AVTORJA/AVTORJEV

Pod ključnimi besedami spodaj je naslov avtorja/avtorjev, in sicer akademski naslov, ime, priimek, ustanova, mesto z oznako države in poštno številko, država, ali elektronski poštni naslov.

11. UVOD

Uvod se mora nanašati le na vsebino razprave ali članka.

12. ZAKLJUČKI ALI SKLEPI

Zaključki ali sklepi morajo vsebovati sintezo glavnih ugotovitev glede na zastavljena vprašanja in razrešujejo ali nakazujejo problem raziskave.

13. TABELE, TABLE, GRAFIKONI, SLIKE IPD.

Tabele, table, grafikoni, slike ipd. v razpravi ali članku naj bodo jasne, njihovo mesto mora biti nedvoumno označeno, njihovo število naj racionalno ustreza vsebini. Tabele, table, slike, ilustracije, grafikoni ipd. skupaj z naslovi naj bodo priloženi na posebnih listih. Če so slike v digitalni obliki, morajo biti pripravljene u zapisu **.tiff** v barvni skali **CMYK** in resoluciji vsaj **300 DPI/inch.** Risane slike pa v zapisu **.eps.**

Pri fitocenoloških tabelah se tam, kjer ni zastopana rastlinska vrsta, natisne pika.

14. LITERATURA IN VIRI

Uporabljeno literaturo citiramo med besedilom. Citirane avtorje pišemo v kapitelkah. Enega avtorja pišemo » (Priimek leto)« ali »(Priimek leto: strani)« ali »Priimek leto« [npr. (Bukry 1974) ali (Oberdorfer 1979: 218) ali ... Poldini (1991) ...]. Če citiramo več del istega avtorja, objavljenih v istem letu, posamezno delo označimo po abecednem redu »Priimek leto mala črka« [npr. ...Horvatić (1963 a)... ali (Horvatić 1963 b)]. Avtorjem z enakim priimkom dodamo pred priimkom prvo črko imena (npr. R. Tuxen ali J. Tuxen). Več avtorjev istega dela citiramo po naslednjih načelih: delo do treh avtorjev »Priimek, Priimek & Priimek leto: strani« [npr. (Shearer, Papike & Simon 1984) ali Pearce & Cann (1973: 290-300)...]. Če so več kot trije avtorji, citiramo »Priimek prvega avtorja et al. leto: strani« ali »Priimek prvega avtorja s sodelavci leto« [npr. Noll et al. 1996: 590 ali ...Meusel s sodelavci (1965)].

Literaturo uredimo po abecednem redu. Imena avtorjev pišemo v kapitelkah:

– **Razprava ali članek:**

Dakskobler, L, 1997: *Geografske variante asociacije Seslerio autumnalis-Fagetum (Ht.) M. Wraber ex Borhidi 1963.* Razprave IV razreda SAZU (Ljubljana) 38 (8): 165–255.

Kajfež, L. & A. Hočevar, 1984: *Klima. Tlatvorni činitelji.* V D. Stepančič: *Komentar k listu Murska Sobota.* Osnovna pedološka karta SFRJ. Pedološka karta Slovenije 1:50.000 (Ljubljana): 7–9.

Le Loeuff, J., E. Buffeaut, M. Martin & H. Tong, 1993: *Decouverte d'Hadrosauridae (Dinosauria, Ornithischia) dans le Maastrichtien des Corbieres (Aude, France).* C. R. Acad. Sci. Paris, t. 316, Ser. II: 1023–1029.

– **Knjiga:**

GORTANI, L. & M. GORTANI, 1905: *Flora Friuliana.* Udine.

Če sta različna kraja založbe in tiskarne, se navaja kraj založbe.

– **Elaborat ali poročilo:**

PRUS, T., 1999: *Tla severne Istre.* Biotehniška fakulteta. Univerza v Ljubljani. Center za pedologijo in varstvo okolja. Oddelek za agronomijo. Ljubljana. (Elaborat, 10 str.).

– **Atlasi, karte, načrti ipd.:**

KLIMATOGRAFIJA Slovenije 1988: Prvi zvezek: *Temperatura zraka 1951–1980.* Hidrometeorološki zavod SR Slovenije. Ljubljana.

LETNO poročilo meteorološke službe za leto 1957. Hidrometeorološki zavod SR Slovenije. Ljubljana.

Za vire veljajo enaka pravila kot za literaturo.

15. LATINSKA IMENA TAKSONOV

Latinska imena rodov, vrst in infraspecifičnih taksonov se pišejo kurzivno. V fitocenoloških razpravah ali članki se vsi sintaksoni pišejo kurzivno.

16. FORMAT IN OBLIKA RAZPRAVE ALI ČLANKA

Članek naj bo pisan v formatu RTF z medvrstičnim razmikom 1,5 na A4 (DIN) formatu. Uredniku je treba oddati izvirnik in kopijo ter zapis na disketi 3,5 ali na CD-ROM-u. Tabele in slike so posebej priložene tekstu. Slike so lahko priložene kot datoteke na CD-ROM-u, za podrobnosti se vpraša uredništvo.

INSTRUCTIONS FOR AUTHORS

Folia biologica et geologica is a scientific periodical of the Classis IV: Natural history that publishes natural scientific proceedings and review articles referring mainly to researches in ethnic region of ours, and also in Europe and elsewhere being of importance, necessity and comparison to our researches.

1. SCIENTIFIC TREATISE

It is the entire description of novel research including the theoretical review of the subjects, presenting in detail the results, conclusions, and the survey of literature of the authors cited. In exceptional cases the survey of literature may be replaced by sources, if the purport requires it.

It should be composed in classic manner: introduction, material and methods, results, discussion with conclusions, acknowledgments, literature, etc.

The treatise should not be longer than 30 pages, including tables, graphs, figures and others. Much desired are treatises of 20 pages.

The treatises are reviewed by two reviewers, one of them being member of SASA as a rule, the other one a foreign expert.

The reviewers are confirmed by the Classis IV SASA upon the proposal of the editorial board of *Folia biologica et geologica*.

The treatise shall be printed when adopted upon the proposal of the editorial board by Classis IV and the Presidency SASA.

2. REVIEW ARTICLE

On consultation with the editorial board and the author, the review article shall be published. Classis IV and the Presidency SASA upon the proposal of the editorial board adopt it. It should not be longer than 50 pages.

3. NEWS

The periodical publishes short, scientificaly relevant and topical articles up to 7000 characters in lenght.

4. NOVELTY OF THE CONTRIBUTION

The treatise or article ought not to be published previously in other periodicals or books.

5. LANGUAGE

The treatise or article may be written in one of world language and in Slovenian language especially when the subjects are of local character.

The author of the treatise or article provides the translation into slovenian language and corresponding editing, unless otherwise agreed by the editorial board.

6. SUMMARY

When the treatise or article is written in Slovenian, the summary should be in English. When they are in foreign language, the summary should be in Slovenian. It should be so extensive that the subjects are clear and understandable to domestic and foreign reader. It should give the information about the intention, method, result, and conclusions of the treatise or article. It should not be longer than 10 to 20% of the treatise or article itself.

7. ABSTRACT

It should give concise information about the intention and conclusions of the treatise or article. It must be written in English and Slovenian.

8. KEY WORDS

The number of key words should not exceed 10 words. They must present the topic of the research in the treatise or article and written in English and Slovenian.

9. TITLE OF TREATISE OR ARTICLE

It should be short and understandable. It is followed by the name/names of the author/authors (name and surname).

10. ADDRESS OF AUTHOR/AUTHORS

The address of author/authors should be at the bottom of the page: academic title, name, surname, institution, town and state mark, post number, state, or e-mail of the author/authors.

11. INTRODUCTION

Its contents should refer to the purports of the treatise or article only.

12. CONCLUSIONS

Conclusions ought to include the synthesis of the main statements resolving or indicating the problems of the research.

13. TABLES, GRAPHS, FIGURES, ETC.

They should be clear, their place should be marked unambiguously, and the number of them must rationally respond to the purport itself. Tables, figures, illustrations, graphs, etc. should be added within separated sheets. In case that pictures in digital form, **TIFF** format and **CMYK** colour scale with **300 DPI/inch** resolution should be used. For drawn pictures, **EPS** format should be used.

In cases, when certan plant species are not represented, a dot should be always printed in phytocenologic tables.

14. LITERATURE AND SOURCES

The literature used is to be cited within the text. The citation of the authors is to be marked in capitals. One writes the single author as follows: "(Surname year)" or "(Surname year: pages)" or "Surname year" [(Bukry 1974) or (Oberdorfer 1979: 218) or ... Poldini (1991)...]. The works of the same author are to be cited in alphabetical order: "Surname year small letter" [**...**Horvatić (1963 a)... or (Horvatić (1963 b)]. The first letter of the author's name is to be added when the surname of several authors is the same (R. Tuxen or J. Tuxen). When there are two or three authors, the citation is to be as follows: "Surname, Surname & Surname year: pages" [(Shearer, Papike & Simon 1984) or Pearce & Cann (1973: 290-300)...]. When there are more than three authors, the citation is to be as follows: "Surname of the first one et al. year: pages" or "Surname of the first one with collaborators year" [Noll et al. 1996: 590 or Meusel with collaborators (1965)].

The literature is to be cited in alphabetical order. The author's name is written in capitals as follows:

– **Treatise or article:**

Dakskobler, L**,** 1997: *Geografske variante asociacije Seslerio autumnalis-Fagetum (Ht.) M. Wraber ex Borhidi 1963.* Razprave IV. Razreda SAZU (Ljubljana) 38 (8): 165-255.

Kajfež, L**.** & A. Hočevar, 1984: *Klima. Tlatvorni činitelji.* V D**.** Stepančič: *Komentar k listu Murska Sobota.* Osnovna pedološka karta SFRJ. Pedološka karta Slovenije 1:50.000 (Ljubljana): 7–9.

Le Loeuff, J**.,** E. Buffeaut, M. Martin & H. Tong, 1993: *Déecouverte d'Hadrosauridae (Dinosauria, Ornithischia) dans le Maastrichtien des Corbieres (Aude, France).* C. R**.** Acad. Sci. Paris, t. 316, Ser. II: 1023-1029.

– **Book:**

GORTANI, L. & M**.** GORTANI, 1905: *Flora Friuliana.* Udine.

In case that the location of publishing and printing are different, the location of publishing is quoted.

– **Elaborate or report:**

PRUS, T**.,** 1999: *Tla severne Istre.* Biotehniška fakulteta. Univerza v Ljubljani. Center za pedologijo in varstvo okolja. Oddelek za agronomijo. Ljubljana. (Elaborat, 10 str.).

– **Atlases, maps, plans, etc.:**

KLIMATOGRAFIJA Slovenije 1988: Prvi zvezek: *Temperatura zraka 1951-1980.* Hidrometeorološki zavod SR Slovenije. Ljubljana.

LETN0 poročilo meteorološke službe za leto 1957. Hidrometeorološki zavod SR Slovenije. Ljubljana.

The same rules hold for sources.

15. LATIN NAMES OF TAXA

Latin names for order, series, and infraspecific taxa are to be written in italics. All syntaxa written in phytocoenological treatises or articles are to be in italics.

16. SIZE AND FORM OF THE TREATISE OR ARTICLE

The contribution should be written in RTF format, spacing lines 1.5 on A4 (DIN) size. The original and copy ought to be sent to the editor on diskette 3.5 or on CD-Rom. Tables and figures are to be added separately. Figures may be added as files on CD-Rom. The editorial board is to your disposal giving you detailed information.

17. THE TERM OF DELIVERY

The latest term to deliver your contribution is May 31.

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