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FAGOPYRUM is open to everyone who is interested in buckwheat and will cover all aspects of buckwheat research: genetics, cytology, breeding, agronomy, nutrition, utilization, biochemistry, ethnobotany and others. FAGOPYRUM will accept manuscripts in English only, which meet the scientific requirements set by the Editorial Board and which have not been published or submitted for publication elsewhere. Announcements concerning the promotion of research on buckwheat (workshops, symposium and so on), bibliographies and other information related to buckwheat will also be published. Deadline for receiving manuscripts for volume 36: June 30, 2018.

Front page photo: Forming buckwheat dough into a chrysanthemum flower-form, see Asami et al., 19-27.

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ORIGINAL PAPERS

Analysing Structural diversity of Seed storage protein gene promoters: Buckwheat a case study

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ABSTRACT

Multiple sequence alignment of 5'UTR of SSP genes from accessions of *Fagopyrum esculentum* revealed the invariant nature of sequences with the transcription start site at P_{761} and TATA box located -30bp upstream the TSS. Other *cis*-elements identified in the sequences included the legumin box (-581, -524, -184, -135, -91), the -131 prolamin box, DOF element (-718, -649, -540,-432, -272,-225, -128) and CAAT box (-692, -530, -475, -411, -282, -168, -54). Other elements identified included those involved in abscisic acid signalling *viz*., ABI3 at $P_{-470,-95,-68,}$ RAV1 at P_{-694} and $_{-543}$ and AGL15 at P_{-671}. A comparative analysis of regulatory elements of SSP gene promoters of distantly related species the presence of five *cis*-regulatory elements *viz*. TATA BOX, E-BOX, RY- element, CAAT box and the Endosperm box, which interplay in seed specific SSP gene expression. Other modulators influencing seed specific gene expression detected in the sequences included the ABA-responsive elements ABI3, RAV1 and AGL15 which play an integral role in seed maturation. Identification of potential nucleosome binding sites in SSP gene promoters of *Cicer arietinum, Brassica napus, B. campestris, Vicia faba, and Pisum sativum* at positions 78, 635, 195, 112 and 152 respectively surmises the spatial fine tuning of SSP gene transcriptional regulation in these species. On the other hand, absence of nucleosome binding sites in the promoters of *Fagopyrum esculentum, Zea mays, Avena sativa, Triticum aestivum and Oryza sativa* may indicate relatively easier access of transcription factors to the proximal promoter, thereby providing higher level of gene expression.

INTRODUCTION

Seed storage proteins are a major class of proteins that not only serve as a source of nutrition to germinating seedlings but also as an important source of dietary proteins for human consumption. Genes encoding such proteins are under tight spatial and temporal transcriptional control. The basic building blocks for promoters of such genes are regions of *cis*-regulatory DNA, which in eukaryotes often comprises clusters of *cis*-regulatory elements (CREs) that modulate gene expression through their interaction with *trans*-acting factors. Plant *cis*-regulatory motifs are often reported as consensus sequences which are commonly delineated by reporter gene expression assays (Guilfoyle, 1997). Neverthless, PLACE database, a collection of experimentally characterized plant *cis*-regulatory elements sequences, remains an invaluable resource for annotating motifs discovered in sequences that have not been characterized experimentally **(**Higo et al.,1998). Majority of contemporary computational approaches for the discovery of *cis*-regulatory elements use the position weight matrix (PWM) motif model, based on the frequencies of nucleotides at each position in a collection of regulatory elements **(**GuhaThakurta, 2006). The exponential growth in development of bioinformatics tools to discover specific motifs in DNA or protein sequences and creation of genomic resources representing specialized databases of plant *cis*-acting elements have greatly facilitated *in silico* analysis of promoters. However, discovery of such elements is hindered by the variability within their sequences, which typically tolerate nucleotide substitutions without loss of functionality. Further, in majority of the cases, and especially so in higher eukaryotes, TFs often regulate gene expression by binding to specific elements in the promoter regions of different genes independenty or in synergy with other regulatory proteins. Different *cis*-elements of a given promoter are also known to interact with different parts of *cis*-regulatory module (CRM), where the relative positions of *cis*-elements and the distances between them are crucial.an overall regulatory complex (Arnone and Davidson, 1997).

Detailed analysis of expression of genes coding for seed storage proteins has revealed that the expression of SSP genes and accumulation of the proteins is limited to the endosperm/ embryos or cotyledons of the seeds (Perez-Grau and Goldberg, 1989; Fujino et al., 2001; Milisavljevic et al*,* 2004; Jain, 2004). Seed-specific expression has been shown to be conferred by the promoter regions of various storage protein genes (Devic et al, 1996; Lee et al, 2007; Moreno-Risueno et al, 2008). Signature *cis*-elements identified in the promoters of specific class of plant genes include the "legumin box" comprising of the core “RY motif " having the sequence 5’CATGCA3’ (Baumlein et al., 1986, Dickinson et al*,* 1988, Forde et al, 1985) and the "vicilin box" having the core sequence 5’GCCACCTCAT3’ in legumes (Vincente et al.,1997; Weschke et al., 1988) and the “prolamin box”(5’TGTAAAG3’) or endosperm motif (E-motif) in cereals (Vicente et al., 1997; Shewry and Halford, 2003.). The promoter region of prolamin genes comprises of three CREs including the

GCN4-like (GLM) element (5'GRTGAGTCAT3'), the prolamin-box (5'TGTAAAGT3') and the AACA (5'AACAAACTCTATC3') element that respectively interact with bZIP, DOF and MYB family transcription factors (Fauteux and Strömvik, 2009). A comprehensive analysis of the *napA* gene promoter in rapeseed (*Brassica napus* L.) has revealed the presence of two regulatory complexes which include the B-box, that contains the distB element (5'GCCACTTGTC3') together with the proxB element (5'TCAAACACC3'), and the RY/G complex which contains two RY repeats (5'CATGCA3') and one G-box (5'CACGTG3') **(**Ezcurra et al.,1999; Chandrasekharan et al., 2003)**.** G-box, CCAAAT box, E-box (5'CACCGT3') and RY elements have been demonstrated to have a strong role in mediating gene expression in embryos (Lindstrom et al.,1990). Motifs conferring seed-specific expression are known to lie in the proximal region of the promoter, often within 500 bp upstream of the transcriptional start (Wu et al., 2000; Fujimori S et al., 2005).

Although the availability of *cis*-acting regulatory element database and tools of bioinformatics help to predict the transcriptional properties of new entry sequences with considerable accuracy, understanding the structural features of DNA, such as GC skew, bendability, topography, free energy, curvature and nucleosome positioning gives a better understanding of the regulatory landscape of such genes (Florquin et al., 2005; Kanhere and Bansal 2005b). The present study decribes the profiling of the 5'UTR of legumin-like seed storage protein gene in ten accessions of common buckwheat *vis. a vis*. seed storage protein gene promoters from distantly related species. This uncovered the presence of specific conserved motifs in SSP gene promoters across plant species and moderate nuclosome biding potential in 5'UTR of buckwheat legumin genes.

MATERIALS AND METHODS

Nucleotide sequences of the 5'UTR of legumin like seed storage proteins of ten accessions of common buckwheat viz. IC-107090, IC-107285, IC-107265, IC-108517, IC-79192, IC-16550, IC-188669, IC-324313, IC-18864 and IC-363973 were generated by nucleotide sequencing of the relevant amplicons. Nucleotide sequences of the promoter regions of seed storage protein genes of other distantly related species were retrieved from Genbank database of NCBI for comparative analyses. The accession numbers of the sequences retrieved from Genbank data bases included EU595873 of *Fagopyrum esculentum*, AF420598 of *Brassica napus*, X67833.1 of *B. junceae*, Y13108 of *B. campestris*, Y13166 of *Cicer arietinum*, S60289.1 of *Vicia faba*, X02983.1 of *Pisum sativum*, X65064.1 of *Hordeum vulgare,* X65064.1 of *Oryza sativa*, EU189096.1 of *Triticum aestivum* and JQ241267 of *Zea mays.*

Sequence analysis

BLASTn analysis of the nucleotide sequences of the 5'UTR of legumin like seed storage proteins of ten accessions of common buckwheat was carried out using the BLAST tool of NCBI. The sequences were aligned using the multiple alignment too MULTALIN http://multalin.toulouse.inra.fr/multalin/). Distribution of *cis*-elements within the sequences was identified out by PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html) and AtPAN (http://atpan.itps.ncku.edu.tw). Neural Network Promoter Prediction tool (http://www.fruitfly.org/seq_tools/promoter.html) was used to identify the transcriptional start site in the target sequences.

Nucleosome formation potential

Comparative analysis on the nucleosome formation potential of the representative sequences from each species was performed with Strong Nucleosome tool (http://strn-nuc.haifa.ac.il:8080/ mapping/home.jsf). Sequences with statistical value of scoring peaks between 50-65 were used to determine the potential position of nucleosome along the DNA sequence.

Result and discussion

Profiling of the 5'UTR of buckwheat legumin gene

BLAST analysis of nucleotide sequences from all the accessions revealed more than 98% homology with 5'UTR of sequence bearing accession no. EU595873, the gene coding for legumin like seed storage protein gene of common buckwheat. Alignment of the sequences using MULTALIN clearly showed a highly conserved nature of the sequences (Fig. 1). Promoter prediction tool (Neural Network Promoter Prediction) identified three probable promoter regions between $P'_{392\text{-}442}$, $_{473\text{-}523}$ and $_{721\text{-}771}$ in the sequences. 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 TSS at P'_{761} also followed the YR rule ($C^{-1}A^{+1}$), having the pyrimidine 'C' at -1 and the purine 'A' at +1 position (Yamamoto et al. 2007). Considering 'A' at position 761 (+1) as the predicted TSS and ATG at position 801 (+40) as the initiating codon, the TATA at position $731_{(-62)}$ was identified as the TATA box of the promoter. Apart from TATA box, the sequences revealed several other *cis*-elements, that are involved in the regulation of eukaryotic gene expression in general and seed-specific expression in particular. The transcription start site predicted for the sequences of all the accessions followed the YR rule with the TATA box motif being localized at P'_{-30} relative to the TSS. Alignment of the context sequences around TATA, TSS and ATG-start codon of buckwheat seed storage protein gene with the corresponding regions of seed storage protein genes from other accessions clearly

established the high degree of conservation in spacing between these elements. Sequence analysis identified 3 legumin boxes comprised of the core sequence 5'CATGCA3' at P'$_{-470}$, $_{-95}$, and $_{-68}$, a single prolamin box, comprising of the sequence 5'TGTAAAG3' at P$_{-131}$ and 7 DOF motifs with the core sequence 5'AAAG3' at P $_{-718, -649, -540, -432, -272, -225,}$ and $_{-128}$ with respect to TSS. Legumin box is considered to be the key element in regulating seed specific expression of genes coding for legumin type proteins (Bäumlein et al., 1992; Ellerström et al., 1996; Reidt et al., 2000). Destruction of the legumin box by a 6 bp deletion in an otherwise intact 2.4 kb 5'-noncoding upstream sequence of *Vicia faba* legumin gene LeB4 was shown to drastically reduce LeB4 expression in seeds (Baumlein et al., 1992). Similar observations were made by Ezcurra et al. (1999) for RY elements in the promoter region of napin gene. Baumlein et al. (1992) has shown that the enhancer-like *cis*-elements in 5'UTR were fully functional only in conjunction with the core motif 5'CATGCATG3' of the legumin box, thereby indicating a possible role of legumin box in modulating enhancer activity in promoter of SSP genes. RY motif has been shown to interact with the conservative B3-domain of the transcriptional activators *VP*1 of maize (McCarty, 1995) and *fus*3 and *abi*3 proteins of *Arabidopsis* (Ezcurra et al., 1999; Reidt et al., 2000). Analysis of several other seed specific promoters has confirmed the importance of RY elements for quantitative expression of seed specific genes as well as the potential of this motif in repression of gene expression in non-seed tissues (Mönke et al., 2004; Singh, 1998)

While the "P-box" (5'TGTAAAG3') is a -300 enhancer element present in SSP genes of cereals and several other dicots (Vickers et al., 2006), we detected the "P- box" as a -131 element in the buckwheat legumin gene promoter. 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". The CAAT box, noted as an enhancer element involved in quantitative regulation of gene expression, was located at positions -692,-530,-475,-411,-282,-168, and -54. Sequence analysis also revealed the presence of SEF1 binding motif, having the core motif 5'ATATTTATA3' at P'$_{-307.}$ Lessard et al. (1990) have demonstrated strong interaction between SEF1 and A-T rich sequences presnet far upstream in genes coding for α′ and β subunits of β-conglycinin. They suggested SEF1 recognizes its binding site with greater affinity than the other SEF factors and it may be involved in directing nucleosome phasing within the promoter region, analogous to mammalian high mobility group chromosomal proteins (HMG-1). Zhou et al. (2014) have reported that while deletion of SEF3 and SEF4 binding motifs from promoter of seed-specific allergen gene of *Arachis hypogaea* did not affect promoter activity, deletion of three E-boxes and one SEF1 motif caused a marked decrease in promoter activity. Their results suggest the possibility of a role for E-box and SEF1 binding motifs in regulating seed-specific expression of genes.

Seed storage protein gene promoters exhibit signature motifs

Comparative analysis using AtPan sofware generated the co-occurrence of *cis*-motifs in the promoters of *Cicer arietinum, Brassica.napus, B.campestris, Vicia faba, Pisum sativum*, *Fagopyrum esculentum, Zea mays, Avena sativa, Triticum aestivum and Oryza sativa* (Table 1). The overall consensus generated from the SSP gene promoters investigated in the present study can be broadly divided into five conserved composite motifs (CREs). These included CRE1, which is 20 to 30 bases upstream of the TSS. This element has been reported universally from all promoters (Joshi, 1987)**.** CRE2 included a G-box-like and a CAAT motif, nested into an E-box (5'CANNTG3'). CRE3 comprised of the RY element (5'CATGCA3') with core motif CATG. This motif is known to be highly conserved in seed specific promoters of both dicots and monocots **(**Dickinson et al., 1988). CRE4 comprised of the CAAT box which has been suggested to act as an an enhancer element involved in quantitative regulation of gene expression (Schirm et al, 1987; Wu et al., 2000). CRE5 included P-box or the endosperm box "5'TGTAAAG3' that interacts with the transcription factor DOF which plays a key role in activating the expression of prolamin genes in cereals. Forde et al. (1985) has suggested the presence of atleast two types of controls operating on prolamin gene expression. While one was responsible for coordinating induction of genes during endosperm development, the other regulated subsequent rates of prolamin accumulation. It was suggested that these two controls have the ability to act differentially on subsets of prolamin genes. The two control systems were together named as the endosperm box which is a bipartite motif consisting of the prolamin box and the GCN4 like motif. The GCN4 motif has been reported to be a target of basic leucine zipper transcription factor that belongs to maize Opaque-2 (O2)-like protein family which is also known as *RISBZ* in rice. Yamamoto et al. (2006) have demonstrated that the prolamin binding factor transactivated several storage protein genes via an AAAG target sequence located within the promoters of such genes. They observed a synergism between RPBF and *RISBZ1* in recognizing the GCN4 motif (TGA(G/C)TCA) for inducing expression of SSP genes. It was suggested that RPBF gene, which predominatly expressed in maturing endosperm and coordinately expressed with seed storage protein genes, was involved in quantative regulation of genes expressed in the endosperm in cooperation with *RISBZ1*.

Nucleosome Mapping determines potential nucleosome binding site for SSP promoters

Variations in position of different *cis*-elements in the promoter sequences are expected to affect gene expression either through their interaction with transcription factors or through differences in nucleosome favouring and/or nucleosome excluding sequences (Tirosh et al.,2008). Besides the knowledge of *cis*-acting regulatory elements for prediction of transcriptional control of genes, information about structural features of DNA, such as GC skew, bendability, topography, free energy, curvature and nucleosome positioning would give a better understanding of the regulatory landscape. Therefore mapping of promoters for potential nucleosome binding sites would generate a deeper insight into long range interactions that may not be evident from sequence variations alone. Nucleosome positioning demarcates the promoter region and transcription start site. While promoters which confer ubiquitous gene expression are essentially free of nucleosomes, Levitsky et.al (2001) have suggested that promoters conferring tissue specific expression of genes display higher nucleosome formation potential**.** Nucleosome positioning map of each seed storage gene promoter, generated using Strong Nucleosome Mapping tool, revealed the highest scoring peak between 45-51. While a a scoring peak > 65 is considered to be statistally significant in determining potential position of the nucleosome along the DNA sequence, values between 50-60 indicate a moderate affinity towards accommodating a nucleosome. This is due to involvement of determinants such as CpG islands or epigenetic regulation in modulating nucleosome positioning. With a scoring peak of >50, the promoters of seed storage proteins of *C. arietinum, B. napus, B. campestris, V. faba, and P. sativum* showed potential nucleosome binding sites at positions P'$_{78}$, $_{635}$, $_{195}$, $_{112}$ and $_{152}$ respectively. The sites were located at positions -100 to -300 with respect to the TSS. On the other hand, analysis of nucleotide sequences of 5'UTR of genes coding for seed storage proteins in *Fagopyrum esculentum, Zea mays, Avena sativa, Triticum aestivum and Oryza sativa* by Strong Nucleosome tool revealed a score of <45, thereby indicating absence of nucleosome binding sites in the promoters of SSP genes of these crops. Jiang and Pugh (2009) has suggested that in comparison to the transcribed region of DNA, the UTR was essentially free of nucleosome biding potential. Our results indicate that compared to the promoters of genes coding for SSPs in *C. arietinum, B. napus, B. campestris, V. faba, and P. sativum*, those of *Fagopyrum esculentum, Zea mays, Avena sativa, Triticum aestivum* and *Oryza sativa* have lower accessibility to nucleosome, therby ensuring easy access to transcription factors and a consequent higher level of gene expression.

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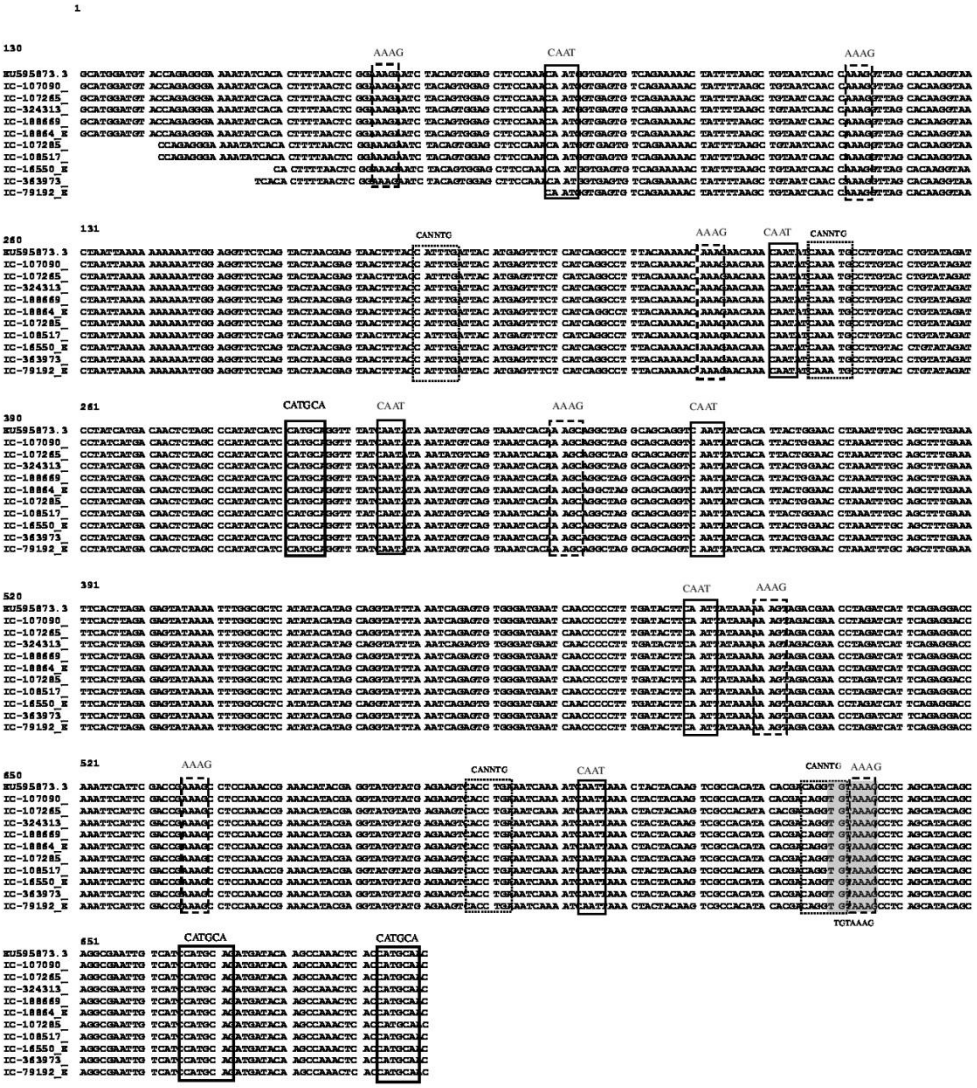

Fig 1: Multiple sequence alignment of 5'UTR of seed storage protein gene from ten accessions of Fagopyrum esculentum (Moench)

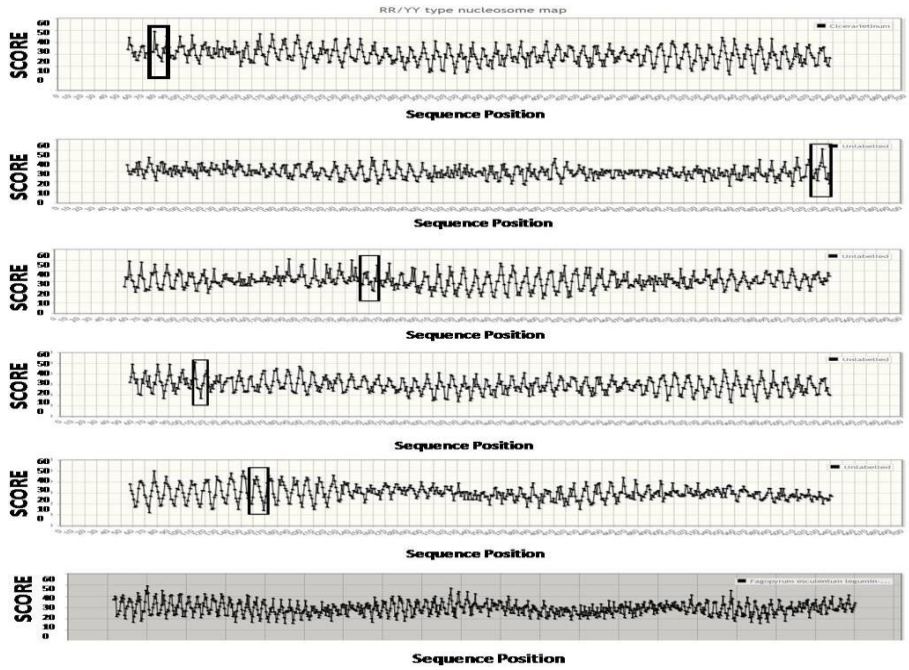

Fig. 2: Nucleosome positioning map highlighting the scoring peak at the probable nucleosome binding site in nucleotide sequences of 5'UTR of legumin genes of (A) *C.arietinum* [acc. no. Y13166] ; (B) *B.napus* [acc. no. X67833.1], (C) *B.campestris* [acc. no. Y13108], (D) *V. faba* [acc. no. X02983.1], and (E) *P. sativum* [acc. no. X02983.1]. A representative image of non existence of nucleosome binding site in *Fagopyrum esculentum, Zea mays* [acc. no. JQ241267], *Avena sativa* [acc. no. EU595873], *Triticum aestivum* [acc. no. (EU189096.1] *and Oryza sativa* [acc. no. (X65064.1] is given in (F). The nuclosome binding site for the respective accessions is boxed.

Table1 :Tabular representation of the significant regulatory elements present in SSP promoter across different species.

SITE	MOTIF	Oryza sativa	Zea mays	Hordeum vulgare	Triticum aestivum	Piscum sativum	Vicia Faba	Cicer arrientum	Brassica napus	Brassica campestris	Brassica juncaea	Fagopyrum esculentum	SIGNIFICANCE
PBF	**TGTAAAG**	-34,-156,-161,-224,-320,-345,-454,-466,-476	-66,-136,-158,-173,-315,-540,-571	-45,-53,-65,-164,-273,-374	-50,-58,-69,-145,-169,-271,-332,-336	-124,-236,-260,-294,-308,-430,-480	--157,-259,-285,-438,-452,-576,-911,-927,-993,-1135,-1143,-1159	-155,-289,-369,-429,-786,-903,-1052,-1203,-1224,-1539,-2050,-2117,-2192	-1064,-225	--161,-259,-327,-611,-659,-726,-827,-1056,-1139,-1319,-1386	-225,-1068	-131	Core site required for binding of Dof proteins in maize
E-BOX	**CANNTG**	--391,-409	-804,-503,-263,-517	-355,-379	-182	-407,-824,-1156,-1124,-1251,-1285	-58,-126,-567,-849,-901	-517,-635,-747,1060,-1344,-2226	-912,-887,-594,-523	-107,-360,-560,631,-920,-1143,-1299,-1336	-58,-80,-138,-598,-651,-841,-917	-581,-524,-184,-135,-91	E-box of napA storage-protein gene of Brassica napus
CAATBOX1	**CAAT**	-10,-247,-402,-411	-196,-329,-415,-425,-504,-547,-870,-880,-940	-72,-109,-147	-77,-152	-62,-83,-493,-649,-715,-798,-904,-979,-1109	-370,-549,-676,-757,-938,-1104,-1130	-198,-473,-541,-951,-1010,-1100,-1613,-2023,-2122	-926,-829,-715,-345,-86	-46,-371,-902,-913,-1095,-1258,-1409	-86,-347,-405,-720,-827,-897,-924	-692,-530,-457,-411,-282,-168,54	"CAAT promoter consensus sequence" found in legA gene of pea;
DOF	**AAAG**	-34,-156,-161,-224,-320,-345,-454,-466,-476	-66,-135,-158,-174,-258,-315,-540,-572	-45,-53,-65,-164,-273,-374	169,-271,-332,-336	-124,-236,-260,-294,-308,-430,-480	--157,-259,-285,-438,-452,-576,-911,-927,-993,-1135,-1143,-1159	-155,-289,-369,-429,-786,-903,-1052,-1203,-1224,-1539,-2050,-2117,-2192	-225,-1064	--161,-259,-327,-611,-659,-726,-827,-1056,-1139,-1319,-1386	-225,-1068	-131	Core site required for binding of Dof proteins in maize
TATABOX	**TTATTT**	-23	-23	-20	-24	23	-25	-23	-24	-21	-24	-30	TATA box elements are critical for accurate initiation
OPAQUE-2	**TGAGTCA**	-210			--------	----------	---------	----------	----------	----------	-------	------	GNC4 motif is the recognition site forOpaque-2 (O2)-like proteins
RISBZI	**TGAGTCA**	-210			---------				----------	----------	------	------	Required for the exp GNC4 motif ression of
SEF1						-1136	-803					-307	SEF1binding motif; sequence found-upstream region;ofsoybean βconglicinin gene

14th international Symposium on Buckwheat in India 2019, first communication

The organizers are announcing to have the 14th international Symposium on Buckwheat from Sept. 3- 6, 2019 at Shillong, followed by a conducted excursion on 7th & 8th Sept. We shall be having the IBRA General Assembly on evening of Sept. 2, 2019. The general assembly to decide on the venue for 15th International symposium on buckwheat shall be held on evening of Sept 6,2019.

Expected sessions:

Session I: Germplasm resources, evolution and phylogeny

Session II: Genetics and breeding

Session III: Physiology and cultivation practices

Session IV: Biotechnology and value addition

Session V: Bioactive molecules in buckwheat

Session VI: Processing technology and buckwheat as a functional food

Session VII: Buckwheat in medicine

Each session would have at least one key note speaker and 3 invited talks followed by paper presentations.

Symposium organizers are finalizing the website for 14th international Symposium on Buckwheat. The site shall have information on the theme, composition of the International scientific advisory committee, national organizing committee, technical sessions, link for registration and submission of abstracts, Names of Key note speakers, Symposium tour, post symposium brain storming session, details about local weather, links to hotels, and how to reach Shillong.

We would expect the delegates to arrive at Guwahati airport (GAU) on September 1 or morning of Sept. 2. The organizers shall be making arrangements for travel of participants from Guwahati to Shillong by chartered cars/mini vans. It takes 2 hours to reach Shillong from Guwahati by road. The delegates can book their return journey from Guwahati to Delhi by the afternoon /evening flights of Sept. 8. The return tickets from Delhi can be booked for Sept. 10. Those who would not be participating in the brainstorming meeting in Delhi may book their return travel from Delhi on September 9.

For participants from Europe the tickets could be booked from their respective places to Delhi (DEL) via either Vienna or Istanbul or Frankfurt and then from Delhi to Guwahati. We are appointing a travel desk for facilitating the bookings of delegates. Delhi has direct air connections with most European cities. Even Beijing or Guangzhou, Narita (Japan) or Incheon (Korea) has direct flights to Delhi.

The arrangements for stay would be facilitated in different resorts/hotels in Shillong. The travel desk would be facilitating this too. This would be announced on the Symposium website.

The email 14thisb@gmail.com is fully functional.

E-mail address of organizers: Prof. Nikhil Chrungoo <14thisb@gmail.com>

www.14isb.in

Fagopyrum 35: 19-27 (2018)

Research paper

Analysis of traditional preparation methods of buckwheat noodles in Japan

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Keywords:
common buckwheat, mechanical characteristics, noodles, traditional buckwheat noodle method

ABSTRACT

The present study was undertaken to clarify two subjects, i.e., one subject is to clarify the mechanical characteristics on the *kukuri* (kneading) and *kiku-neri* (forming into a chrysanthemum flower-like shape) processes on the preparation of buckwheat noodles; and another problem, to compare mechanical characteristics with buckwheat flours with different particle size. The present study shows some factors which lie behind traditional methods preparing buckwheat noodles, i.e., some factors behind the *kukuri* and *kiku-neri* processes. Furthermore, this present study shows that the particle size of buckwheat flour may be an important factor affecting the mechanical characteristics of buckwheat noodles.

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) is an important crop in many countries of the world (Ikeda, 2002; Kreft et al., 2003). Buckwheat flour is processed into various products such as noodles, pasta etc. There is a large variety of buckwheat products globally. In view of their processing, increasing attention has been paid to clarifying scientific basis for the palatability and acceptability of various buckwheat products.

Noodles made from buckwheat flour-water dough are popular in some regions including Japan (Ikeda, 2002). In Japan, buckwheat noodles are a popular, traditional food. Traditional methods preparing buckwheat noodles have been cultivated in the Japan for about four hundred years or over (Zen-men-kyo, 2014; Asami et al., 2016). The traditional methods preparing buckwheat noodles generally consist of six successive processes. The first process is mixing buckwheat flour by hand in a wooden bowl with water in the presence of some additives such as wheat flour to make dough. This process is called *mizu-mawashi* which means mixing buckwheat flour with water. The second process is kneading buckwheat dough and foaming it into a circle form. This process is called *kukuri* which means kneading and forming. The third process, is binding the dough into a chrysanthemum flower-like shape (Fig. 1). This process is called *kiku-neri*: *Kiku* means chrysanthemum flower; and *neri*, binding. It is generally thought that both the *kukuri* and *kiku-neri* processes may be the most important ones in the preparation of buckwheat noodles. In addition, the fourth process is extending the ball-shaped dough into a thin, rectangular shape using a long wooden-bar. This process is called *nobashi* which means expanding. The fifth process is softly-folding the thin, rectangle-shaped dough. This process is called *tatami* which means folding. The last process is cutting the folded dough using a big knife to prepare long resultant buckwheat noodles. This process is called *houchou* which means cutting with the knife. In general, buckwheat noodles with high palatability are prepared by the traditional method in Japan. Many Japanese people enjoy palatable buckwheat noodles. It appears that scientifically-excellent techniques may lie behind each process of such traditional methods. In viewpoint of food science, there are various questions concerning traditional methods for buckwheat noodles.

Mechanical characteristics on human mastication may be important on various foods, especially buckwheat noodles. On the other hand, there are various kinds of buckwheat flour with different particle size of the flour. Such different buckwheat flours

are widely utilized for the preparation of various buckwheat noodles in Japan. It is also interesting to clarify what mechanical sense on human mastication may be arisen with each of various, different buckwheat flours, respectively. We have recently undertaken a series of studies to clarify the scientific basis responsible for traditional processing techniques for buckwheat noodles with special regards to mechanical analyses (Ikeda et al., 1997; Ikeda et al., 1999; Asami et al., 2008; Asami et al., 2009; Asami et al., 2010; Asami et al., 2012; and Asami et al., 2016). However, there are still many, unanswered questions for traditional, excellent techniques in the preparation of buckwheat noodles. Especially, the scientific reason for the *ukuri* and *kinu-neri* processes is the subject of much interest. In addition, comparison study on analysis of noodle preparing methods using various buckwheat flour with different particle sizes is also an interesting subject. The present study was undertaken to clarify two subjects, i.e., one subject is to clarify mechanical characteristics of the *kukuri* (kneading) and *kiku-neri* (forming buckwheat noodles into a chrysanthemum flower-like shape) processes on the preparation of buckwheat noodles; and another problem, to compare mechanical characteristics with various, different buckwheat flours.

MATERIALS AND METHODS

Materials

Buckwheat flour (*Fagopyrum esculentum* Moench, var. Kitawase-soba), which was harvested in Hokkaido (in 2016), was used in this research. Different buckwheat flours with different particle sizes and wheat medium flour were kindly provided prepared by Terao Milling Co. (Hyogo, Japan) and stored at -80℃until use.

Preparation of buckwheat noodles

Buckwheat noodles were hand-made by Y. Yamashita and T. Oka, who are the present authors, and buckwheat noodle-preparation experts. Y. Yamashita has the fifth grade, the highest grade, of preparing buckwheat noodles, which was awarded by the Japan Buckwheat Noodles Association (Zen-men-kyo). T. Oka has the fourth grade awarded by the Zen-men-kyo. Buckwheat noodles were prepared by Y. Yamashita and T. Oka according to the traditional method described previously (Zen-men-kyo, 2014). Two experiments, I and II, were conducted in the present study. Experiment I was performed to analyze the mechanical role of *kiku-neri* process of preparing buckwheat noodles, i.e., mechanical characteristics of buckwheat noodles prepared under three different conditions in the *kiku-neri* process were compared. Experiment II was performed to compare mechanical characteristics of three buckwheat noodles prepared with three different types of flour particle size.

Mechanical measurements

Before mechanical measurements, prepared buckwheat noodles were cooked in boiling water for 40 sec and were subsequently cooled for 40 sec at 4℃. Immediately after cooling, mechanical measurements of the noodles were performed. Breaking characteristics of buckwheat noodles were evaluated using a Rheoner RE2-3305C (Yamaden Co. Ltd., Japan). Measurements of breaking analysis were performed with a load cell of 2000N and a measurement speed of 0.50 mm/sec. A wedge-style plunger (No.49: W 13mm, D 30mm, H 25mm) was used in measurements with the Rheoner RE-3305. Mechanical measurements of the buckwheat noodles were repeated twenty times for each sample.

Measurements of particle size

Measurements of the particle size of buckwheat flours in experiment II were performed by Shimadzu Techno-Research, Inc., Japan. Measurements of the particle size were performed using a SALD-2300 (Shimadzu, Japan). The easurements were performed with a cyclonic type dry measuring unit DS5, a dispersion pressure of 0.4MPa, a table lifting speed of 4mm/sec, a refractive index using 1.50-0.01i.

Measurements of water absorption

Measurements of maximum water absorption capacity (MaxWAC) of buckwheat flours in experiment II were assayed using the method of Hashimoto (1991).

Statistical analysis

Statistical analysis was conducted using a personal computer with the program Excel (Microsoft Co., USA) and Ekuseru-Toukei 2015 (Social Survey Research Information Co., Japan).

RESULTS AND DISCUSSION

Experiment I: Analyze the role of dough-kneading and dough-forming in to a chrysanthemum-flower shape

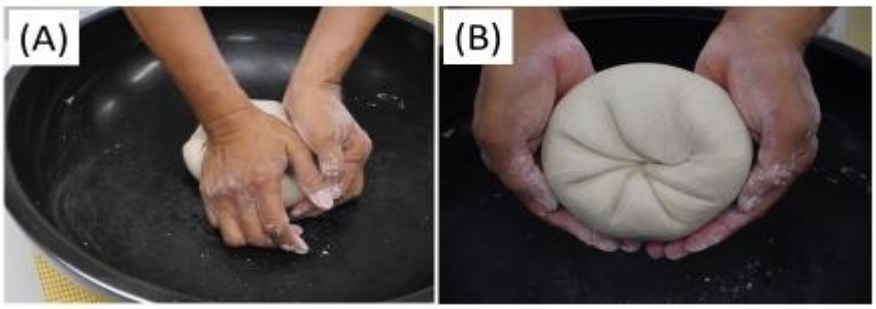

Fig. 1 Forming buckwheat dough into a chrysanthemum flour-form. This figure is cited from Asami *et al.,* (2016) the reference in the present paper. (A, left) shows *kukuri* (kneading) process; (B, right), *kiku-neri* (forming into a chrysanthemum-flower shape) process.

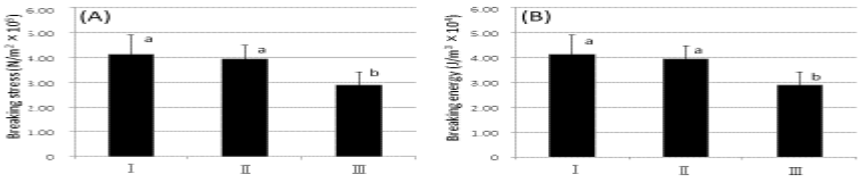

Fig. 2 Mechanical comparison of buckwheat noodles prepared by three different buckwheat noodles prepared by three different preparing methods in view both of kneading (*kukuri* process) and of forming a chrysanthemum flower-like dough (*kiku-neri* process). (A), breaking stress and (B), breaking energy. I, noodles prepared both with kneading and with forming into a chrysanthemum flower-like dough; II, noodles prepared with kneading but without forming into a chrysanthemum flower-like dough; and III, noodle prepared both without kneading and without forming into a chrysanthemum flower-like dough. Vertical bars show standard deviations. Values that within the same row that are not followed by the same letter are significantly different at $p<0.05$.

Figure 2 shows mechanical comparison of buckwheat noodles prepared by three different buckwheat noodles prepared by three different preparing methods in view both of kneading, abbreviated as *kukuri* process, and of forming a chrysanthemum flower-like shape (abbr. as *kiku-neri* process).

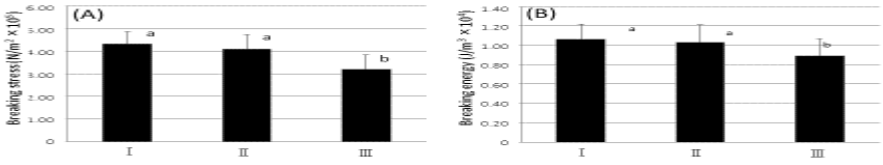

Fig. 3 Mechanical characteristics of three buckwheat noodles prepared with buckwheat flours with three different flour particle size. (A), Breaking stress and (B), Breaking energy. Noodles I was prepared with fine flour; noodles II, middle size flour; and noodles III, large flour. Vertical bars in the figure show standard deviations. Values that within the same row that are not followed by the same letter are significantly different at $P<0.05$

Buckwheat noodles I were prepared both with *kukuri* process and with *kiku-neri* process; noodles II, prepared with *kukuri* process but without *kiku-neri* process; and noodles III, prepared without *kukuri* process and without *kiku-neri* process. The mechanical values of noodles III (III in Fig. 2 A and B) was significantly lower than those of noodles I and II (I and II in Fig. 2 A and B). This finding suggests that *kukuri* process, i.e., kneading process may be important in mechanical characteristics in preparation of buckwheat noodles. On the other hand, there was no significant difference in the mechanical values between noodles I and II shown in I and II in Fig. 1 (A) and (B)（$P>0.05$）. Noodles I were prepared both with *kukuri* process and *kiku-neri* process, whereas noodles II were prepared with *kukuri* process but without *kiku-neri* process. This finding shown in Fig. 2 (A) and (B) suggests that *kiku-neri* process may exhibit little or substantially no effect on mechanical characteristics of buckwheat noodles. On the other hand, some cracks in buckwheat dough were often found in buckwheat dough prepared without *kiku-neri* process, whereas no cracks were found in buckwheat dough prepared with *kiku-neri* process (data not shown). Finally, the present study concludes that *kiku-neri*

process, i.e., forming buckwheat dough into a chrysanthemum-like form maybe smoothly promotes subsequent procedures after *kiku-neri* process. Although there may be a possibility that the *kikuneri* process may remove air from buckwheat dough which may bring about some oxidation reaction in buckwheat dough. Further research is needed.

Experiment II: Comparison of three buckwheat noodle samples prepared from three different particle size flours

Figure 3 shows mechanical characteristics of three buckwheat noodles prepared with buckwheat flours with three different flour particle size. Noodles I were prepared with fine flour; noodles II, middle size flour; and noodles III, large flour (Table 3). Table 1 shows the content of water (%) added to buckwheat flour prepared, shown in Fig. 3. Table 2 shows percentage of water absorption to buckwheat noodles presented in Fig. 3. Table 3 shows average diameter of buckwheat flour particles used in Fig. 3. The mechanical values of noodles III (III in Fig. 3A and B) was significantly lower than those of noodles I and II (I and II in Fig. 3 A and B). Relationships were analyzed on various mechanical characteristics (Fig. 3 I to III) of buckwheat flour (Tables 1 to 3). There was a significant positive correlation between the average diameter of buckwheat flour particles (Table 3) and maximum water absorption capacity (MaxWAC) (Table 2) of buckwheat flour ($r= 0.999$, $p<0.01$). This is finding that buckwheat flour with larger particle size can exhibit higher MaxWAC than buckwheat flour with smaller particle size. On the other hand, there is a significant positive correlation between WAC of buckwheat flour (Table 2) and the particle size (Table 3) ($r= 0.998$, $P<0.05$). This finding well agrees with the above observed finding. Furthermore, a relationship of the obtained mechanical characteristics (Fig. 3) to the water addition rate at noodle preparing (Table 1) was analyzed. Interestingly, the MinWC (Table 1) correlated negatively to breaking stress with $r= -0.998$ ($P<0.05$), to breaking energy with $r= -0.998$ ($p<0.05$). These findings show that the particle size of buckwheat flour may be an important factor affecting the mechanical characteristics of buckwheat noodles.

Table 1 Percent of water added to buckwheat doughs prepared in Fig. 3.

Buckwheat flour	Percent of added Water addition rate (%)
I	40.76
II	42.00
III	50.00

Table 2 Percent of water absorption to buckwheat flour used in Fig. 3

Buckwheat flour	Water absorption rate (%)*
I	139.2 ± 2.8
II	143.2 ± 1.4
III	192.9 ± 9.4

*: Amount of water absorbed per 100 g of buckwheat flour.

Table 3 Average diameter of buckwheat flour used in Fig. 3

Buckwheat flour	Average diameter of flour (μm)
I	108.9 ± 0.4
II	160.6 ± 0.4
III	687.3 ± 0.1

Finally, the present study shows some factors which lie behind traditional methods preparing buckwheat noodles, i.e., some factors behind the *kukuri* (kneading) process and *kiku-neri* (forming into a chrysanthemum flower-like shape) process. Furthermore, the present study shows that the particle size of buckwheat flour may be an important factor affecting the mechanical characteristics of buckwheat noodles.

The exact mechanisms involved in traditional preparation methods for buckwheat noodles will be an interesting subject in the future.

ACKNOWLEDGMENT

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Research paper

Formation of buckwheat genepool collection in Ukraine and directions of its usage

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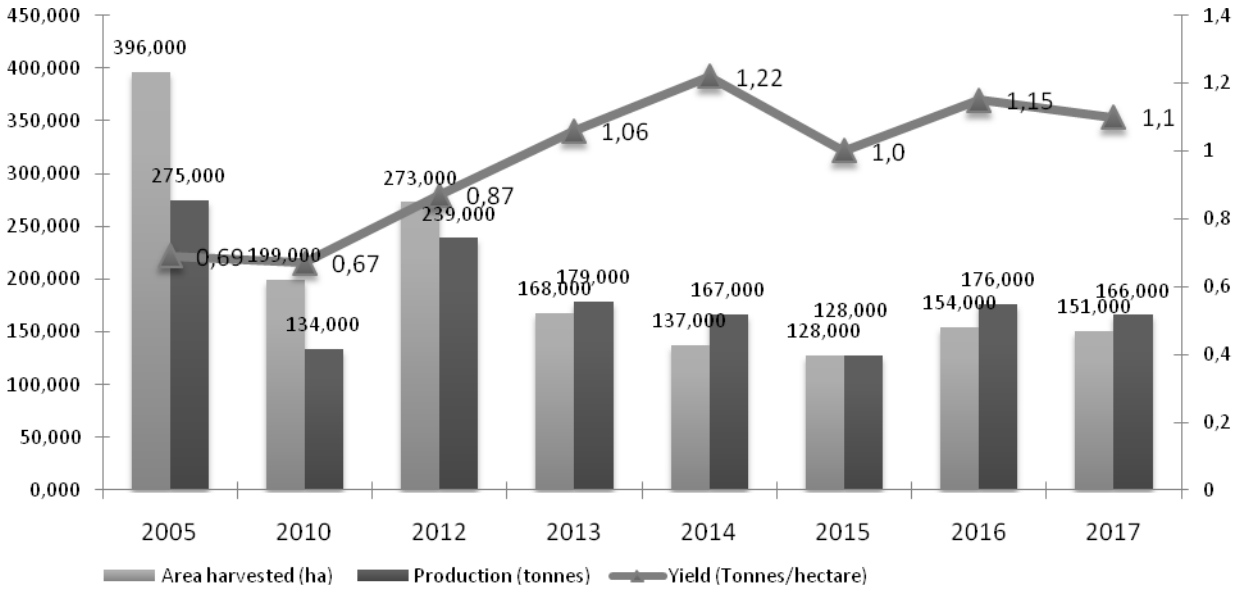
Keywords: buckwheat, genepool, collection, accesses, agronomic traits

ABSTRACT

The national collection of buckwheat in Ukraine consists of more than 2,000 samples. The material is studied, reproduced and preserved in special storage facilities with controlled environmental conditions and temperature, humidity of grain in the hermetic containers. The research work, conducted over the genepool, has allowed separating the source of valuable for selection treats: high yield and productivity, large grain, low-growing plant, high seedling vigor, resistance towards abscission and impact of abiotic and biotic environmental factors. As a result of the fulfilling research program "Plant genetic resources" following actions are conducted annually: allocation of 10-15 sources of selection and agronomic traits of plant productivity, grain quality, adaptability of the material, etc .; software research and breeding facilities standards, sources and donors of valuable traits for breeding and other research - about 100 collection samples; transmission of 1-2 educational collections (30-50 samples) to educational institutions.

INTRODUCTION

Ukraine is one of the TOP 5 producers of buckwheat in the world. Anually 150,000 sq. hectares are used to grow buckwheat, and its yield is about 1.2-1.5 tons per hectare (Fig.1) (Maslak, 2017). Only one species - buckwheat common Fagopyrum esculentum Moench -. is grown industrially. Environmental and climatic conditions on most territory of Ukraine are very favorable for buckwheat cultivation (summer temperatures, humidity during spring and summer periods). In order to gain the highest yield possible, the main factor is the cultivation of varieties with highest production capabilities (Alekseeva et al., 2005). 26 varieties of buckwheat have been added into the State register of plant varieties suitable for dissemination in Ukraine in 2018, and only 2 of them were originating from abroad (Russian Federation) (State register, 2018).



The potential yield level of modern Ukrainian varieties ranges within 3.5 – 4.2 tons per hectare. Currently this level is implemented for 30-40% of the potential in field conditions. This situation is mainly caused by the sensitivity of buckwheat plants to environmental conditions and non-compliance with cultivation technologies etc. (Alekseeva et al., 2005). Current production volume covers 90-95 % of the national needs for buckwheat grain. Yet it

is very obvious that under the appropriate conditions Ukraine can become an exporter of this product. Quality of Ukrainian buckwheat grain and functional buckwheat products fully complies with all world standards.

In 2017 International association of research institutions, producers, processors and functional buckwheat products was founded in Ukraine. The main purpose of this Institution is the coordination of the activities of its participants and assistance in resolving questions related to research activities, grain production, growing,

processing and producing groats and other functional buckwheat products. The Association's task is to unite the producers of buckwheat products, strengthen the cooperation between producers and selection institutions, develop modern technologies, including the organic ones. The Association founders are the leading state and private research institutions, processors with significant production volumes.

It is necessary to resolve a number of factors to increase the production volumes, such as:

- Yield level stabilization;
- Reduction of dependencies on environmental factors (temperature fluctuations during inflorescence period and yield formation);
- Decrease of plant sensitivity towards soil humidity level.

Selection allows resolving these issues by using ecologically diverse initial material. This material can be obtained from plant genetic resources collections.

Description of National buckwheat collection of Ukraine

The National buckwheat collection of Ukraine is located in 2 institutions (Tryhub, 2015):

- Research Institute cereals them. Olena Alekseeva Podolsky State Agricultural and Technical University;
- Ustymivska experimental station of Plant Production of Plant Production Institute nd. a. V. Ya. Yuryev of NAAS.

The total volume of the collections located in these institutions is over 2000 authentic samples.

Scientific-Research Institute of Groat Crops nd. a. O. Alekseeva is located in western Forest-steppe area of Ukraine. The genepool of the institute has all categories of plant genetic resources belonging to 13 species of Fagopyrum: local populations, hybrid populations, selection varieties of common and intensive types, wild species, botanic forms, polyploids, genetic markers and mutants. The collection formation took place in 1950-1971. The first samples were from western region of Ukraine. Over 500 samples in total were collected (Alekseeva, 1967). These samples had diverse morphological treats and different technological quality and biochemical content of grain. Starting from 1960 the experimental mutagenesis usage has started for selection purposes. A large number of original forms have been created: with significant branching capabilities, dwarfs, short-stem, green flower, with salad and anthocyan coloring of plant, different forms of inflorescences, large size of grain etc. Thanks to the significant input of academic Olena Alekseeva and her successors the basic collection of the institute has increased to over 1600 samples collected from all over the territory of former Soviet Union and 14 other countries (Alekseeva et al., 2005b). 115

collection samples are *Fagopyrum tataricum* Gaertn. (Nikitchuk, 2001). The collection of this institute is the short-term storage collection. A few years ago the institute has reinstated its active research work and started gathering and reinstating the collection; studying the industrial and biologic traits of both common and tartary buckwheat, cultivation technologies and selection of new varieties (Alekseeva et al., 2002).

Ustymivska Experimental Station of Plant Production is located in the central part of Ukrainian Forest-steppe territory with extremely favorable environmental conditions for buckwheat cultivation, soil quality and human expertise availability. The work with the collection in the Experimental Station started in 1954. The prolific weather conditions, huge diversity of genetic material of this region, significant planting acreage and stable grain yield volumes were the main factors of collection formation here (Tryhub, 2016). The collection formation supervisors were scientists from All-Union institute of plant production (Leningrad) – Krotov A. and Avezdzhanov R.

Nowadays the collection of Experimental Station consists of 1629 samples, 991 of which originate from Ukraine. The collection contains 6 samples of *Fagopyrum tataricum* Gaertn. and one - *Fagopyrum giganteum* Krotov. 23 samples are tetraploid. The collection has a wide diversity of varieties and species that allow in full and in a short time to assess the gene pool of buckwheat on a range of agronomic traits of adaptability to abiotic and biotic factors. Collection has a broad representation of global diversity and contains materials from 23 countries of the world. This includes 20 regions of Ukraine, 28 - the Russian Federation and 5 - the Republic of Belarus. The collection contains buckwheat samples gathered during the expeditions performed by Vavilov N. during the mid-20th of the XX century.

As a result of years long research in the Ustymivska Experimental Station Krotov A. and Dranenko E., created a new species - *Fagopyrum giganteum* Krotov.

Fig. 2. *Fagopyrum giganteum* Krotov

The introduction of new material, its studying, reproduction and preservation is conducted during the work with the collection.

As a result of the fulfilling research program "Plant genetic resources" following actions are conducted annually:

– the introduction of new valuable varieties into the gene bank of Ukraine in the amount of 5-10 samples;

– the gene pool study based on economically valuable indicators (cold-, drought- and heat resistance, diseases- and pests-resistance and the productivity of its elements, the quality of the crop, etc.) - more than 100 samples;

– allocation of 10-15 sources of selection and agronomic traits of plant productivity, grain quality, adaptability of the material, etc .;

– cultivation and transfer to storage in the storage doublet repository of Research Station (about 200 samples - to collection and 100 samples - to storage in a National repository of Ukraine);

– software research and breeding facilities standards, sources and donors of valuable traits for breeding and other research - about 100 collection samples;

– transmission of 1-2 educational collections (30-50 samples) to educational institutions;

– the formation and transmission of the attributive and special collections for the registration to the National Center GRPU;

–unique samples of collection of the gene pool are sent to the registration;

– supplying with the information on the gene pool of plant breeding and research institutions of Ukraine and other countries (publication of 2-3 articles).

The storage of seed collection is conducted in the special repository that was provided in the mid-1990th as part of the cooperation with IPGRI. The controlled temperature (+2–+4^{0}C) and humidity of environment (not more than 30%) are kept in the storage. Seed is stored for preservation with humidity of 6-7%.

All collections samples are entered into passport database, information about most of them is available in the Internet.

In the National Center PGRU seven buckwheat gene pool collections are registered by the station: base collection; educational collection of buckwheat gene pool; four indicative collections of buckwheat gene pool by yield and largest grain, productivity and drought resistance and heat resistance, adaptation to mechanized cultivation and productivity of the material and core collection.

In addition to the mandatory set of traits to determine the yield characteristics of the collection material, research has been done to determine:

– plant resistance to shattering and breakage of the stem (as lodging resistance characteristics) using special devices;

– drought resistance in growing material under controlled conditions (special containers and other), as well as the study of physiological parameters (water shortages, water-holding capacity, changes in germination of seeds after heating, etc.);

– heat resistance by using special methods;

– flower size in different samples and species; protein and starch contents in the grain, etc.

In addition, the most early-maturing samples (60-70 days) were cultivated, with stem fasciation, determinant type of growth, two or three carpal and long lateral inflorescences, two leaves at the node near the inflorescence, with lateral inflorescences in the form of branches, with narrow leaflets, high attachment of the lower inflorescence (more than 30 cm), with a small number of nodes on the main stem (6 pcs.); pest-resistant; low intensity of transpiration and water shortages, high water-holding capacity.

The method of isolation with the help of tetraploid buckwheat is used for reproduction of diploid samples. The distance between diploid samples is 8 – 10 m.

Annually in order to fulfill the requests made by different institutions and on the own initiative, consumers are being given more than 100 unique samples. Over the past 10 years, we sent more than 500 samples after examination and evaluation to the main selection institutions.

Based on the latest tendencies of buckwheat usage directions expansion we have started researching in the new vectors: selection of the forms which could be used as green manure fertilizer, studying antioxidant traits of common buckwheat from different ecologic and geographic origin.

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