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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 37: November 30, 2019.

**Front page photo:** Buckwheat pasta with 1.7% addition of seaweed, see Asami et al., 5-11.

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Research paper

# Mechanical characterization of buckwheat noodles mixed with seaweed (fu-nori)

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**Keywords:** common buckwheat, mechanical characteristics, noodles, seaweed

## ABSTRACT

The present study was conducted to clarify the effect of incorporation of seaweed, i.e. *funori* (*Gloiopeltis tenax* (Turner) J. Agardh) into buckwheat noodles on their mechanical characteristics. Mechanical analysis of buckwheat noodles with *funori* showed that incorporation of *funori* into buckwheat noodles enhanced breaking stress and energy. On the other hand, incorporation of *funori* into buckwheat noodles enhanced decreased solubility of the albumin plus globulin fraction. The present study findings suggest that the endogenous protein may be an important factor responsible for the mechanical characteristics of buckwheat noodles with seaweed.

## INTRODUCTION

Buckwheat (*Fagopyrum spp.*) is an important crop in some regions of the world (Kreft et al., 2003; Ikeda, 2002). Buckwheat flour contains various beneficial components for human health such as protein, polyphenolics, rutin and minerals at high levels (Ikeda 2002; Ikeda and Yamashita 1994). Thus, buckwheat can contribute as an important dietary source of such beneficial components.

There is a large variety of buckwheat products produced on a global basis (Ikeda, 2002). Attention has been currently paid to the palatability and acceptability of buckwheat products from the perspective of their cooking and processing. However, there are still unanswered questions on the palatability and acceptability of buckwheat products. As buckwheat flour has low cohesiveness, dough-binders, such as wheat flour, egg, seaweed, Japanese yam flour, are often added in preparing buckwheat noodles (ZMCS, 2004). A variety of buckwheat noodles with various dough-binders has been traditionally available in Japan. We reported mechanical effects by addition of various dough-binders to common and Tartary buckwheat noodles in view of two analysis, i.e., tensile analysis and breaking analysis (Ikeda, et al., 2005). However, further systematic analysis is needed to understand the exact mechanical effects of various dough-binders to buckwheat products.

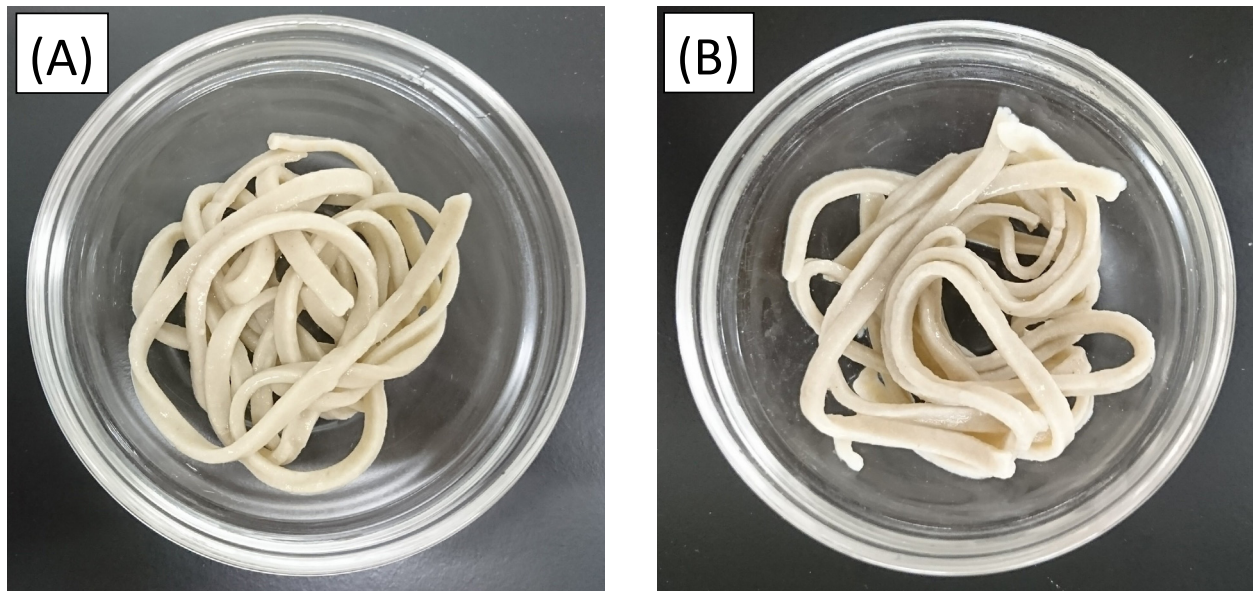
In Niigata district, located in the middle region of Japan, there is a famous buckwheat dish. This dish is

called “*hegi-soba*”. These buckwheat noodles are prepared by incorporating into buckwheat dough a kind of seaweed, i.e. *funori*, (*Gloiopeltis tenax* (Turner) J. Agardh) as a dough-binder (Zen-men-kyo, 2014). Before ingestion, the noodles prepared with *funori* are usually put on a unique wooden-tray which is called “*hegi*”; so these buckwheat noodles are called “*hegi-soba*”. Although this buckwheat dish is traditionally utilized only in Niigata region, many Japanese people currently often enjoy these local buckwheat noodles. *Hegi-soba* noodles have a unique masticatory sense with refreshing sense on ingestion. Mechanical characterization of “*hegi-soba*” noodles is an interesting subject in view of buckwheat research. In this background, the present study was conducted to characterize noodles made from buckwheat flour with seaweed.

## MATERIALS AND METHODS

### Materials

Buckwheat flour (*Fagopyrum esculentum* Moench, var. Kitawase-soba), which was harvested in Hokkaido (in 2017), was used in this research. Buckwheat flour was kindly provided prepared from Terao Milling Co. (Hyogo, Japan) and stored at -80°C until use. Ground seaweed, i.e. *fu-nori* in Japanese, *Gloiopeltis tenax* J. Agardh) used in this study was a commercial product (Oowaki-man-zou-shoten Co., Fukui, Japan).



**Fig. 1.** Buckwheat noodles. (A), non added seaweed; and (B) added seaweed (1.7% addition).

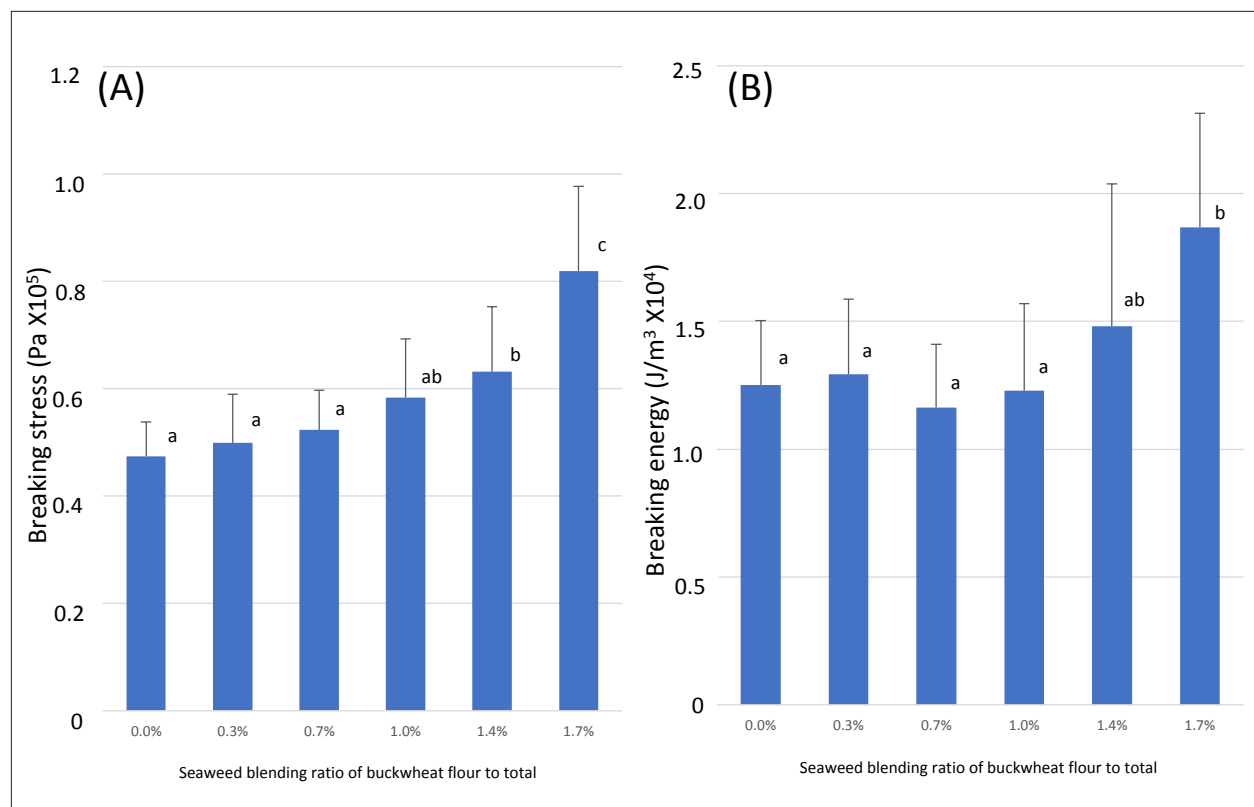
### Mechanical measurements

For the study of the effects of the seaweed on the mechanical characteristics of buckwheat noodles, buckwheat noodles were prepared by hand. The mechanical characteristics of buckwheat noodles were evaluated by breaking analysis. Prior to the mechanical analysis, the buckwheat flour which had been stored at  $-80^{\circ}\text{C}$  was placed in a desiccator at room temperature until the flour exhibited a constant moisture content. The moisture of the flour was measured with a moisture analyzer (ML-50, A&D Co. Ltd., Japan). Seaweed was boiled, and sticky seaweed was added to buckwheat flour. The buckwheat dough was prepared just prior to mechanical analysis to have a moisture content of 42% by adding the appropriate amount of distilled water. Then the buckwheat noodles were made from the buckwheat dough using a hand-made pasta machine (SP-150, Imperta Co., Torino, Italy). Figure 1 shows buckwheat noodles prepared in this study. The buckwheat noodles obtained were subjected to mechanical analysis. Before the mechanical analysis, buck-

wheat noodles prepared were heated in boiling water for 150 seconds and subsequently were cooled for 150 seconds at  $4^{\circ}\text{C}$ . Immediately after cooling, mechanical measurements of the noodles were performed. The breaking analysis of the buckwheat noodles was performed with Rheoner RE2-3305C (Yamaden Co. Ltd., Japan). Measurements of breaking analysis were performed with a load cell of 200N and 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 RE2-3305C. Mechanical measurements were replicated twenty times for each sample.

### Protein determination

For chemical analysis of the combined fractions of buckwheat albumin plus globulin (AG) in the heated noodle samples which had been subjected to the mechanical measurements, the noodle samples were lyophilized and then ground into flour. The flours obtained were extracted with a ten-fold (v/w) volume of 0.2M NaCl for 1hr at



**Fig. 2.** Breaking characteristics of buckwheat noodles made with seaweed. (A), breaking stress; and (B), breaking energy. Vertical bars in the figure show the standard deviations. Values that within the same row that are not followed by the same letter are significantly different at  $P < 0.05$ .

40°C. After extraction, the suspensions were centrifuged at 17,000 Xg for 20 min. Protein concentration was determined using the Bradford method with bovine serum albumin as a standard protein.

**Statistical analysis**

Statistical analysis was conducted using a personal computer with the program Excel (Microsoft Co., USA), Ekuseru-Toukei 2015 (Social Survey Research Information Co., Japan) and SPSS Ver.23.0 (IBM, USA).

**RESULTS AND DISCUSSION**

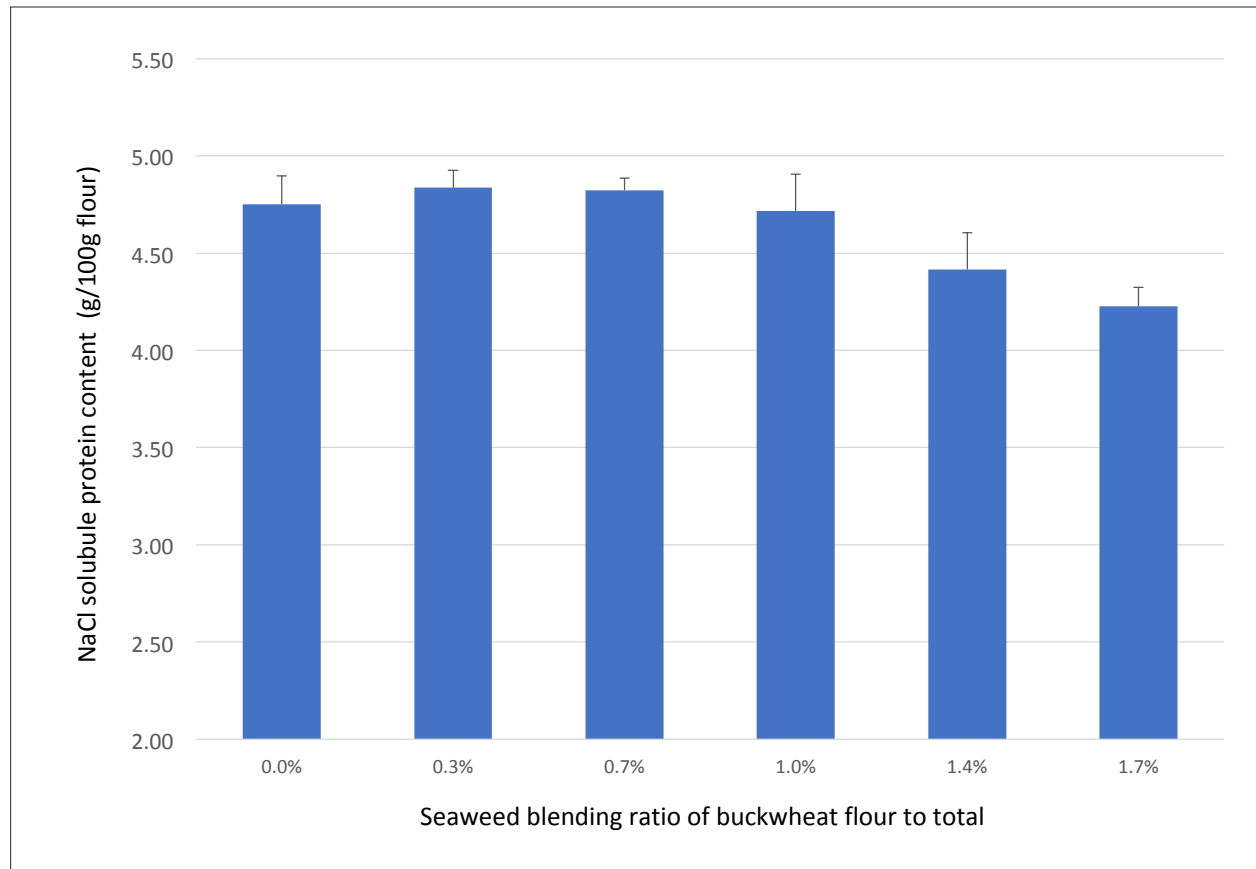
**Mechanical characteristics of buckwheat noodles made with seaweed**

Figure 2 shows the breaking characteristics of *hegi-soba* buckwheat noodles prepared with *funori*-seaweed. The breaking stress and energy of the *hegi-soba* noodles gradually increased as the added concentration of *funori*

seaweed increased (Fig. 2 (A and B)). A significant high breaking stress (Fig. 2 (A)) was found with *hegi-soba* buckwheat noodles with a concentration of *funori* seaweed with 1.4% or over as compared the buckwheat without *funori* seaweed ( $P < 0.05$ ). Similarly, a significant high breaking energy (Fig. 2 (B)) was found with buckwheat noodles with a concentration of *funori* seaweed with 1.7% as compared the buckwheat without *funori* seaweed ( $P < 0.05$ ). These findings characterize showed the unique mastication characteristics of *hegi-soba* noodles.

**Protein compositions of buckwheat noodles made with seaweed**

Figure 3 shows the NaCl-soluble protein content of buckwheat noodles made with seaweed. The NaCl-soluble protein exhibits the combined fraction of the major buckwheat proteins, i.e., albumin plus globulin (Ikeda, 2002), designated as the AG fraction below. Changes by the addition of the seaweed in solubility of the AG fraction were found (Fig. 3). Incorporation of seaweed



**Fig. 3.** NaCl-soluble protein content of buckwheat noodles made with seaweed. Vertical bars in the figure show the standard deviations.



into buckwheat dough was found to reduce the solubility of the AG fraction in buckwheat dough as the *funori* seaweed added increased (Fig. 3). The seaweed contains dietary fiber at high levels (Ooishi, 1993). Judged from our previous findings (Ikeda and Kusano 1983), this phenomenon may be due to in-solubilization of proteins arisen by dietary fiber in seaweed. Interest in the nutritional function of dietary fiber for humans is currently increasing. Dietary fiber has many beneficial effects on human such as blood glucose increase suppression and antihypertensive (Mori and Tsuji, 1997). Considering in view of current nutritional science concerning the beneficial effects of dietary fiber, the intake of buckwheat noodles with seaweed with high level of dietary fiber, should be recommended as a key source of dietary fiber.

Relationships of the observed breaking characteristics (Fig. 2) to the protein components (Fig. 3) was analyzed. The AG fraction content (Fig. 3) negatively correlated to their observed breaking stress (Fig. 2 (A)) with

$r = -0.934$  ( $P < 0.01$ ), breaking energy (Fig. 2 (B)) with  $r = -0.942$  ( $P < 0.01$ ). These findings suggest that proteins in the AG fraction may be an important factor involved in the observed changes in mechanical characteristics arisen by the addition of *funori* seaweed.

Finally, the present study shows changes in mechanical characteristics of buckwheat noodles made with seaweed. The present study suggests that changes in the protein of AG fraction in buckwheat noodles with seaweed may be an important factor affecting the mechanical characteristics of buckwheat noodles, although the exact mechanism remains uncertain. The present findings provide a scientific basis in the understanding of palatability and acceptability of buckwheat noodles.

#### ACKNOWLEDGMENT

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#### IZVLEČEK

##### Mehanska karakterizacija ajdovih rezancev z dodatkom morskih alg *funori*.

Namen raziskave je bil ugotoviti kako vpliva dodatek morskih alg *funori* (*Gloiopeltis tenax* (Turner) J. Agardh) na mehanske lastnosti ajdovih rezancev. Ugotovljeno je, da dodatek alg poveča občutljivost na lomljenje in energijo. Dodatek alg *funori* pospeši zmanjšanje topnosti albuminske in globulinske frakcije beljakovin. Rezultati raziskave kažejo, da so proteini testenin pomemben dejavnik, ki vpliva na mehanske lastnosti rezancev z dodatkom alg.



Research paper

# Non-destructive methodology in comparative physiology of buckwheat genotypes within the different origin

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**Keywords:** chlorophyll fluorescence, phenolic acid, non-destructive measurement, flavonoids, anthocyanins

## ABSTRACT

In the presented study has been used non-destructive method for prescreening of flavonoids, anthocyanins and pigments from early stage of growth till flowering period of buckwheat genotypes of different origin. The similar increasing tendency in the changes of FLAV, ANTH and MFI indexes of Chinese genotypes compared to the tendency of Ukrainian genotypes has been observed. Genotypes of *F. tataricum* compared to the genotypes of *F. esculentum* have been shown to lower ANTH index during seedling growth. *SFR index* which relates to chlorophyll concentration shown different dynamic between Ukrainian and Chinese genotypes. In the middle phase 30 days after sowing (DAS) of growth has been discovered significant increase of SFR index almost in all experimental genotypes. On 36 DAS just two Ukrainian genotypes which characterized also high ANTH and MFI indexes has been kept tendency to increase SFR index. HPLC analysis of experimental samples found that presence of p-anisic acid was typical for *F. tataricum* species compared to the experimental cultivars of *F. esculentum* of both origin. Buckwheat genotypes of different origin can vary in flavonoid, anthocyanins and pigments content during stages of growth, but changes in their contents can be similar for representatives of the same origin.

## INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) supposes that the world's population in 2050 will be 34% greater than it is today. Currently, 49% of the world's population lives in urban areas, while in 2050 this will be closer to 70% (Alexandratos and Bruinsma 2012). During this time period, climate change and the development of biofuel production will present major risks to long-term food security. Population growth, urban civilization and climate change can effectively support high crop concurrence as possible sources of food, bioenergy, fiber and other industrial needs. Going forward, these problems will require innovative approaches to the genetic and agronomic components of systems of crop production and developing concurrent food market.

While we are entering a period of increasingly rapid climate change, the overall objective of the project initiative is to design new strategies to maintain high yield and qualitative parameters of crop plants produced under changed environmental conditions, using novel crop plants which is promising for development in food market. Crop yield is a complex trait depending on the successful completion of different steps of vegetation (phase of reproductive organ development), which can be sensitive to environmental factors.

Among innovative approaches is high interest in plant biotechnology and non-destructive methods of prescreening agriculture plants regarding content of specific secondary metabolites, regarding development of some diseases, stress reaction for the missing of nitrogen supply or to discover plant species biodiversity. In targeted plant metabolites, the main goal is to achieve a high throughput. Therefore, there is often an initial desire for a rapid pre-screening of the samples. This is especially the case when dealing with many sample numbers, where only a limited number of individuals might be expected to be different (Verhoeven et al. 2006). This is the case when searching for valuable genetic resources (e.g. those having a high content of desired compounds) within natural populations or population obtained by crosses or mutagenesis (Sytar et al. 2015; 2017).

Nowadays many scientific groups in the functional food area are looking for plants with high content of phytochemicals as an important source of active pharmaceuticals or with valuable nutritional properties (Abuajah et al. 2015; Varzakas et al. 2016). Buckwheat among these plants got a prime position as potential source of gluten free food products as well as rich source of antioxidant

compounds (Saturni et al. 2010). The various types of bioactive compounds presented in different buckwheat varieties provide basic background needed for the efficient production of buckwheat foods with added value (Sytar et al. 2016). Two main buckwheat species have been commonly produced and consumed: common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* Gaertn). The biodiversity of buckwheat plant species which can be used farther are wide. Therefore, its is important to develop study difference between buckwheat species of different origin.

The non-invasive fluorescence-based phenomics method for determination of plant phenolics based on the strictly UV-absorbing properties, the effects of phenolic compounds on visible light-induced chlorophyll fluorescence is negligible, whereas their presences strongly suppress the chlorophyll fluorescence emission under UV excitation (Cerovic et al. 2002). This phenomenon has been successfully applied for estimation of transmittance of UV radiation by chlorophyll fluorescence (Burchard et al. 2000; Ounis et al. 2001). In the previous decades, the numerous studies examined and confirmed possibility to use the chlorophyll fluorescence signal in the estimation of phenolics and anthocyanins. In addition to self-constructed devices or standard fluorometers combined with external light sources and filters, which were used in the majority of studies, the factory-made special devices for this purpose were also introduced (Sytar et al. 2015).

In this scientific work we would like to combine the non-invasive fluorescence-based, and traditional biochemical methods (HPLC, spectrophotometry etc.), typically used for plant phenomics studies to discover growth characteristics of buckwheat species of different origin.

## MATERIAL AND METHODS

### Plant material

Plants of 7 buckwheat samples from Ukraina (*Fagopyrum tataricum himalaicum*, *Fagopyrum tataricum rotundatum* – red, *Fagopyrum esculentum* cv. Rubra - red, *Fagopyrum esculentum* cv. Karadag) and Chinese cultivars (*Fagopyrum esculentum* cv. SuQiao 1, *Fagopyrum esculentum* cv. YuQiao 4, *Fagopyrum esculentum* cv. NingQiao 1) were exposed immediately from sowing to direct sunlight in open field conditions for 52 days. The non-destructive measurements were started from early growth stage (stage of vegetation). First measurements were done at

stage of two leaves (22 days after sowing (DAS)), other measurements were carried out at stage of 4-5 leaves (30 DAS), at the beginning of flowering stage when buckwheat plants had 5-6 leaves (36 DAS) and last measurements were done in flowering stage (51 DAS). The leaves for biochemical analysis were collected in the flowering phase.

Cultivar Rubra with high anthocyanins content (3.87–4.41 mg/100 g DW) in the vegetative organs has been received by family selection method from chemo mutants. Cultivar Karadag is received from the Scientific Research Institute of Groat Crops in Ukraine. *F. tataricum* G. is a one-year plant which, among the species researched, has a better pollination of flowers and a higher grain production. *F. giganteum* Krot. is amphidiploid obtained after crossing *F. tataricum* G. with the perennial plant *F. cymosum* Meissn. (Krotov and Dranenko 1973). *F. cymosum* Meissn. is a polyploid with 32 chromosomes. The collection of buckwheat germplasm, which is maintained at the Scientific Research Institute of Groat Crops in Ukraine comprises nearly 1000 samples which are readily available for breeding research.

### Chlorophyll fluorescence records and analyses using fluorescence excitation ratio method

The chlorophyll fluorescence analysis was done using the portable optical fluorescence sensor Multiplex-3® (Force-A, Paris, France). Multiplex-3® is a hand-operated, multi-parametric sensor based on light-emitting-diode excitation and filtered photodiode determination that is arranged to work in the field, greenhouse and laboratory conditions. The sensor of Multiplex-3® has three, red-blue-green LED-matrices emitting light at 470 nm (blue), 516 nm (green) 153 and 635 nm (red). There are three integrated photodiode detectors for fluorescence recording: far-red, red and yellow (Ghozlen et al. 2010). We used values of fluorescence measured at UV (375 nm), green light (516 nm), red light (635 nm) and 735 nm (FRF).

The evaluation of phenolic compounds contents in plants was done via calculation of fluorescence values detected after excitation by light of the defined wavelengths (details are below). In analogy to the spectrophotometric method for assessing leaf absorbance, the parameters were based on the Beer-Lambert's law and calculated as logarithm of the fluorescence ratio values.

UV absorbing compounds (mostly flavonols) content described by flavonoid (FLAV) index (Cerovic et al. 2002; Agati et al. 2011) was estimated using the modified for-

mula of Zivcak et al. (2017), as the logarithm of the ratio of the red-light induced far-red fluorescence ( $FRF_R$ ) and the UV-induced far-red fluorescence ( $FRF_{UV}$ ):

$$FLAV = \log[FRF_R / (k_{UV} * FRF_{UV})]$$

Similarly, the ANTH Index that provides estimates of green-light absorbing compounds ( $\log FER_{R/G}$ ), mostly red-colored flavonoids and anthocyanins, was calculated as the logarithm of the ratio of the red-light induced fluorescence ( $FRF_R$ ) and the green light-induced fluorescence ( $FRF_G$ ):

$$ANTH = \log[FRF_R / (k_G * FRF_G)]$$

The correction coefficients  $k_{UV}$  or  $k_G$  was applied to measurements of fluorescence to avoid negative values (Zivcak et al. 2017). The constant values of the coefficients were used as the minimum values of the  $FRF_{UV}/FRF_R$  and  $FRF_G/FRF_R$  ratios found in the database that contains several thousand records from over three hundred plant species grown in diverse environments (Zivcak et al. unpublished results). The same constants have been used when processing data across all experiment and cultivars. We also calculated the modified Flavonoid Index (MFI) that provides a better estimate of total flavonoid content when plants with different colors are compared (Zivcak et al. 2017). The MFI was calculated as the logarithm of the ratio of the red-light induced fluorescence ( $FRF_R$ ) and the green light-induced fluorescence ( $FRF_G$ ).

$$MFI = \log[2 * FRF_R / (k_G * FRF_G + k_{UV} * FRF_{UV})]$$

The values of correction coefficients ( $k_G$ ,  $k_{UV}$ ) for MFI were the same as for ANTH and FLAV.

Chlorophyll content was estimated from values of fluorescence measured at 735 nm (FRF) and at 685 nm (RF) after excitation by red light (635 nm). The Simple fluorescence ratio (SFR) was calculated as:

$$SFR = FRF_R / RF_R$$

Because the diameter of the measuring area was only 50 mm, 6-7 measurements were taken on each plant in different position to account for heterogeneity in leaf color and structure. This number of measurements from the top view provides sufficient data to characterize the entire plant.

### Anthocyanins estimation

0.1 - 0.5 g plant material was homogenized on ice with 3 ml of acidified methanol (1% HCl) and then incubated at 4 °C for 12 h with moderate shaking. The mixture was centrifuged for 10 min at 14 000 rpm at 4 °C. Absorption of the extracts at 530 and 657 nm wavelengths was determined spectrophotometrically. The blank was acid-

ified methanol. The concentration of the anthocyanins was expressed as  $\text{mg}\cdot\text{g}^{-1}$  dry weight and was calculated by formula:

$$\text{anthocyanins} = [A530 - (0.25 \cdot A657)] \cdot V / (W \cdot 1000),$$

where A is absorbance; V is total volume of the extract (ml) and W is weight of the dry leaf tissue (g).

### Total phenolics estimation

Total phenolic content in the buckwheat leaves extracts was determined by standard spectrophotometric method of Lachman et al. (2003) by using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). 0.25 g powdered samples (freeze-dried) was extracted for 16-18 hours with 20 ml of 80% ethanol. After the time of extraction a volume of 100  $\mu\text{l}$  of the plant extract was pipetted into 50 ml volumetric flask. 2.5 ml of Folin-Ciocalteu reagent was added to the extract. Then after 3 minutes (agitation) 5 ml 20%  $\text{Na}_2\text{CO}_3$  solution was mixed. After two hours at 25 °C the absorbance was measured on the spectrophotometer Jenway UV/Vis 6405 (Jenway, UK) at wavelength  $\lambda = 765$  nm against blank. Gallic acid was used as a reference standard for plotting calibration curve. Total phenolic content was expressed as  $\text{mg}\cdot\text{kg}^{-1}$  gallic acid equivalent of dry matter.

### Analysis of hydroxycinnamic acid derivatives

Analysis of hydroxycinnamic acid derivatives has been previously developed (Mewis et al. 2010). Samples were taken after finishing the freeze-drying process where the material was ground by a flint mill (20 000 g for 2 min). A total of 20 mg ground samples from leaf suspension were extracted for 15 min using 0.75 mL 70% methanol (v/v, pH 4.0, phosphoric acid) in an ultrasonic water bath on ice. Then samples were centrifuged for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice more with 0.5 mL of 70% methanol (HPLC-Gradient grade, VWR chemicals). Coumaric acid or cinnamic acid (Sigma-Aldrich Chemie GmbH) (40  $\mu\text{L}$  of 3 mM solution) was added as internal standard to the first extraction. The combined supernatants from each sample were reduced to near dryness in a centrifugation evaporator (Speed Vac., SC 110) at 25 °C.

Samples were added up to 1 mL with 40% acetonitrile (HPLC Ultra Gradient Grade, Roth). The samples were filtrated using 0.22-mm filters and then analyzed with HPLC. The chromatography was performed using a DionexUltiMate 3000 HPLC System with a diode array detector (DAD-3000) with a WPS-3000 SL auto sampler,

LPG-3400SD pump and a TCC-3000RS Column Compartment (Dionex Corp., Sunnyvale, CA, USA).

Extracts (1 mL) were analyzed at a flow rate of 0.4 mL/min and a column temperature of 35 °C. The column used is Narrow-Bore Acclaim PA C16-column (3 mm, 120A, 2.1  $\times$  150 mm, Dionex). A 49-min gradient program was used with 0.1% v/v phosphoric acid in ultrapure water (eluent A) and 40% v/v acetonitrile in ultra-pure water (eluent B) as follows: 0–5 min: 0.5% B, 5–9 min: 0–40% B, 9–12 min: 40% B, 12–17 min: 40–80% B, 17–20 min: 80% B, 20–24 min: 80–99% B, 24–32 min: 99–100% B, 32–36 min: 100–40% B, 36–49 min: 40–1% B. The gradient program was followed by a 4-min period to return to 0.5% B and a 5-min equilibration period resulting in a total duration of 39 min. Peaks were monitored at 290, 330 and 254 nm respectively. The phenolic acid quantity was calculated from HPLC peak areas at 290 nm. The retention times in the HPLC for the experiments were 12.13 min for vanillic acid, 12.72 min for chlorogenic acid, 13.29 min for caffeic acid, 15.98 min for the internal standard p-coumaric acid and 21.59 min for cinnamic acid. For the identification of unknown phenolic compounds, a semiquantitative analysis was performed using HPLC coupled with mass spectrometric detection (LC/MS) and NMR (Mewis et al., 2010).

### Statistical analysis

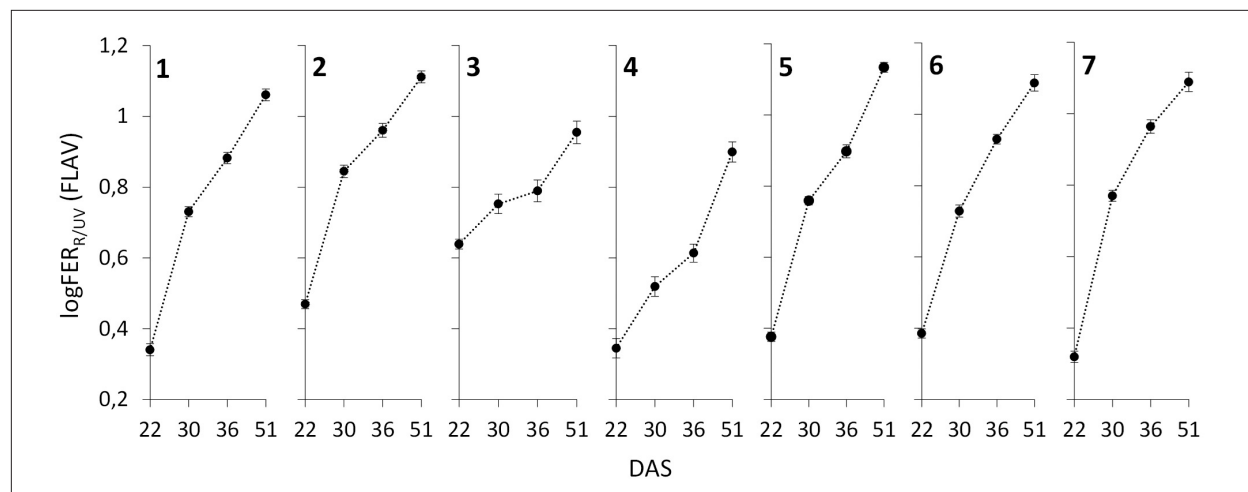
Means and standard deviations were calculated by the Microsoft Office Excel 2013. Significant differences of these data were calculated using analysis of variance ANOVA Duncan's multiple test (STATISTICA 10, StatSoft, Tulsa, USA). All results were expressed as mean + standard deviations from replications  $n = 50$ .

## RESULTS AND DISCUSSION

### Chlorophyll fluorescence records and analyses of FLAV, ANTH, MFI and SFR indices using fluorescence excitation ratio method

Chlorophyll fluorescence records and analyses using fluorescence excitation ratio method of Ukrainian and Chinese genotypes during growth periods of 51 DAS shown significant increasing of FLAV index from beginning of seedlings stage to the of flowering stage (Fig.1).

The highest increase of FLAV index which is connected with flavonols content has been observed in the Chinese genotypes. The high FLAV index at the flowering stage was not depended from flavonols content on

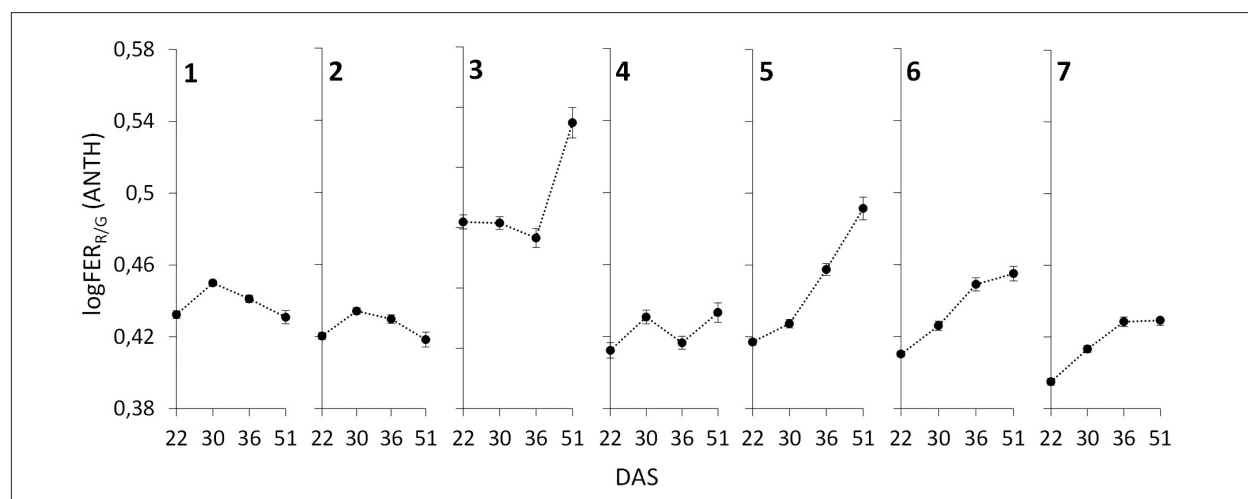


**Figure 1.** Process of flavonols accumulation ( $\log\text{FER}_{R/UV} - \text{FLAV}$ ) in the leaves of investigated buckwheat plant species exposed to direct sunlight during 51 days after seedlings (numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum cv. Rubra*, 4 – *F. esculentum cv. Karadag*, 5 – *F. esculentum cv. SuQiao 1*, 6 – *F. esculentum cv. YuQiao 4*, 7 – *F. esculentum cv. NingQiao 1*).

the beginning of seedlings stage (22 DAS). For example, Ukrainian genotype *F. esculentum cv. Rubra* on 22 DAS has a highest FLAV index (0.64 RU) compared to other experimental genotypes. At the flowering stage on 51 DAS FLAV index in this experimental genotype was 0.95 RU which was lower compared to the other experimental genotypes. Plus other Ukrainian genotype *F. esculentum cv. Karadag* on the beginning of seedlings stage (22 DAS)

got FLAV index 0.35 RU which was similar to the level of FLAV index of Ukrainian *F. tataricum rotundatum* and Chinese *F. esculentum cv. SuQiao 1*, 6 – *F. esculentum cv. YuQiao 4*, 7 – *F. esculentum cv. NingQiao 1*. At the flowering stage on 51 DAS FLAV index in these experimental genotypes was in range 1.06-1.13 RU.

The different tendency of ANTH index increasing from beginning of seedlings stage to the flowering stage



**Figure 2.** Process of anthocyanins accumulation ( $\log\text{FER}_{R/G} - \text{ANTH}$ ) in the leaves of investigated buckwheat plant species exposed to direct sunlight during 51 days after seedlings (numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum cv. Rubra*, 4 – *F. esculentum cv. Karadag*, 5 – *F. esculentum cv. SuQiao 1*, 6 – *F. esculentum cv. YuQiao 4*, 7 – *F. esculentum cv. NingQiao 1*)

has been observed for Ukrainian genotype *F. esculentum* cv. Rubra compared to other investigated buckwheat plant genotypes (Fig. 2). At the beginning of seedlings stage this genotype has highest ANTH index (0.50 RU) and at the flowering stage on 51 DAS (0.57 RU) too. At the flowering stage came to significant increase of ANTH index in *F. esculentum* cv. Rubra, on the contrary to other Ukrainian genotypes *F. tataricum rotundatum* and *F. tataricum himalaicum*, in which has ANTH index decreasing character from beginning of flowering stage. On the 51 DAS at the flowering stage in *F. tataricum* genotypes ANTH index was almost on the same level as at the beginning of seedlings growth after significant increasing at the 30 and 36 DAS. ANTH index for other investigated genotypes (was) ranged from 0.39 to 0.43 RU at the beginning of seedlings stage. Genotypes of *F. tataricum* compared to the genotypes of *F. esculentum* of both origin shown decreasing of ANTH index during seedling growth. All *F. esculentum* genotypes of both origin has increasing on ANTH index on 51 DAS (Fig.2).

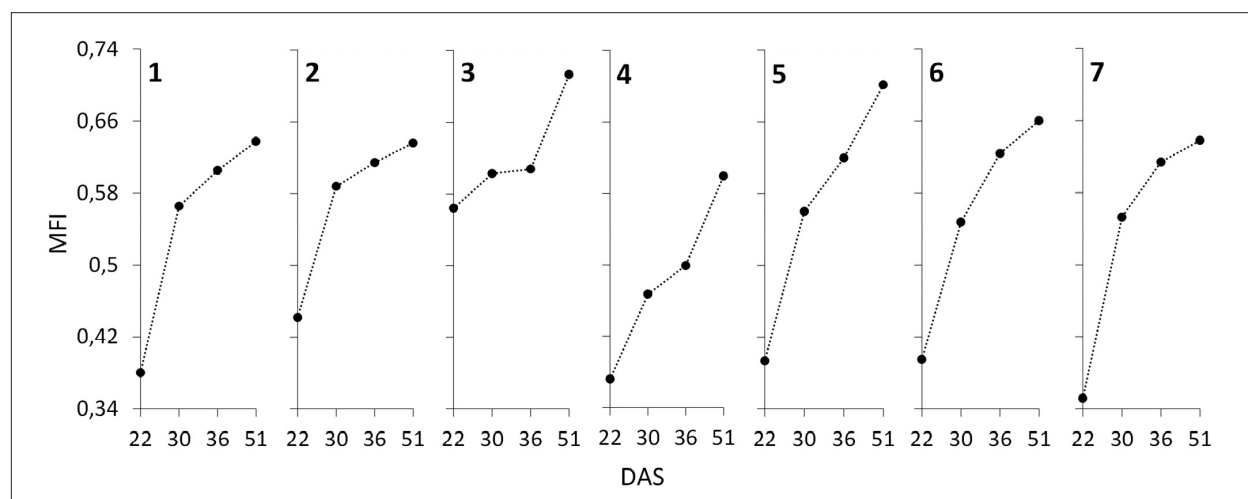
MFI, the parameter that takes into consideration the accumulation of both flavonols and anthocyanins, is a better estimate of flavonoids than FLAV (Zivcak et al., 2017). Results of statistical analyses using MFI are highly similar to those that use data of biochemical analysis (Zivcak et al., 2017). MFI index did not shown significant difference between *F. tataricum* and *F. esculentum* genotypes of both origin but highest values has been found

for *F. esculentum* cv. Rubra during all growth stages. Based on the obtained prescreening results during 51 DAS of growing period is possible to conclude that non-destructive methodology can be used to choose genotypes with high antioxidant content.

The majority of published vegetation indices for non-invasive remote sensing techniques are not sensitive to rapid changes in plant photosynthetic status brought on by common environmental stressors. The SFR index is connected with chlorophyll concentration in the leaves. In presented experiment with buckwheat genotypes of different origin the tendency of SFR index changes was different between genotypes of Ukrainian and Chinese origin (Fig.4). In the middle phase (30 DAS) of growth has been observed significant increase of SFR index almost in all experimental genotypes. On 36 DAS just two Ukrainian genotypes which characterized also high ANTH and MFI indexes has been kept tendency to increase SFR index - *F. esculentum* cv. Rubra and *F. esculentum* cv. Karadag. The Chinese genotypes shown decreasing of SFR index on 36 DAS with farther decreasing at 51 DAS of flowering stage with parallel increasing of ANTH, FLAV and MFI indexes.

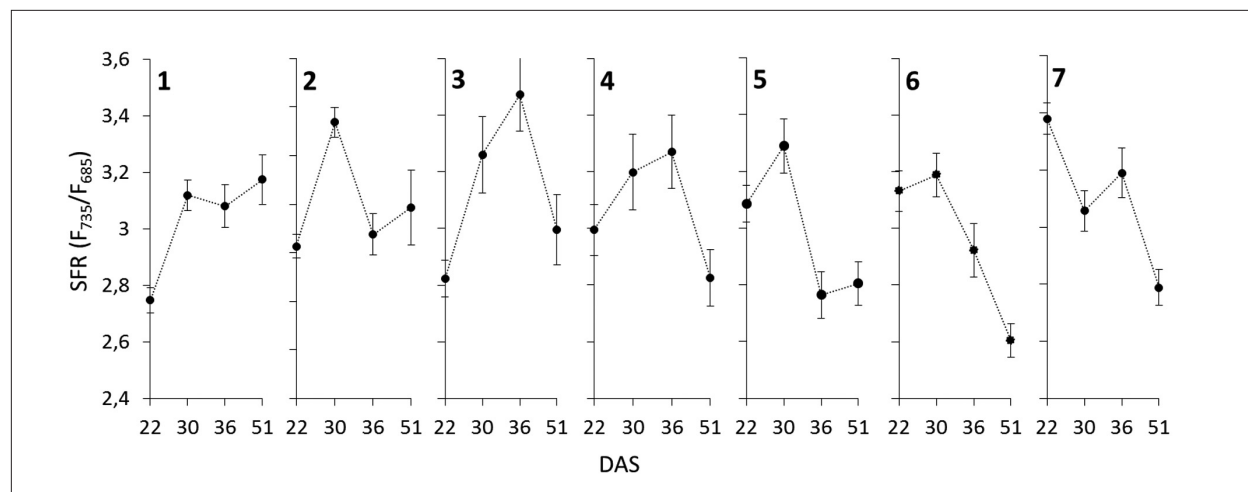
### Total phenolics and anthocyanins estimation

The biochemical analysis among experimental buckwheat genotypes of different origin has been shown that highest total phenolic contents was found in Ukrainian



**Figure 3.** Values of MFI index in the leaves of investigated buckwheat plant species exposed to direct sunlight during 51 days after seedlings (numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. Rubra, 4 – *F. esculentum* cv. Karadag, 5 – *F. esculentum* cv. SuQiao 1, 6 – *F. esculentum* cv. YuQiao 4, 7 – *F. esculentum* cv. NingQiao 1).



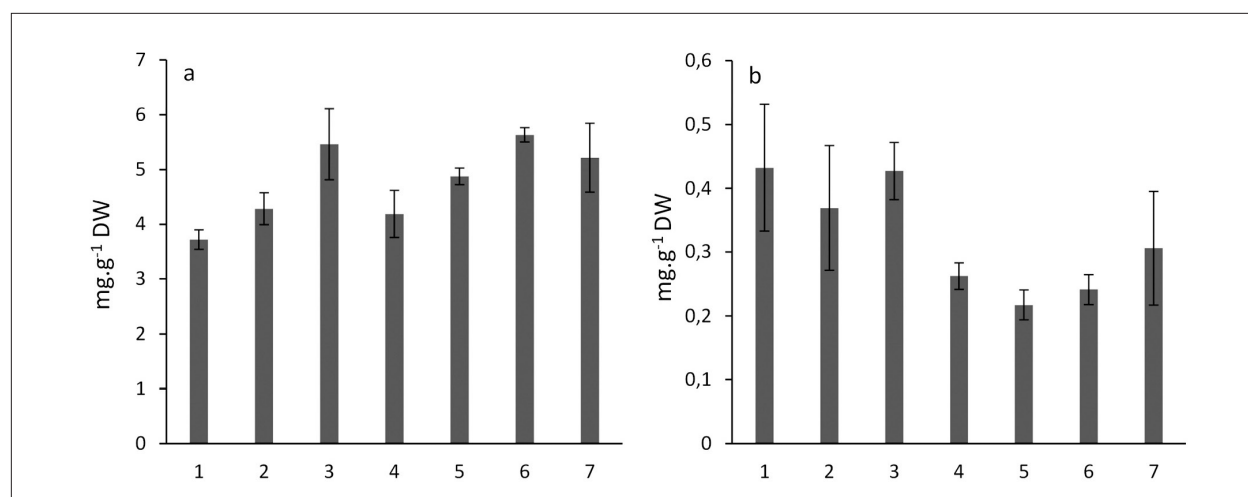


**Figure 4.** Values of SFR (simple fluorescence ratio) in the leaves of investigated buckwheat plant species exposed to direct sunlight during 51 days after seedlings (numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. *Rubra*, 4 – *F. esculentum* cv. *Karadag*, 5 – *F. esculentum* cv. *SuQiao 1*, 6 – *F. esculentum* cv. *YuQiao 4*, 7 – *F. esculentum* cv. *NingQiao 1*).

buckwheat *Fagopyrum esculentum* cv. *Rubra* and Chinese genotypes (Figure 5). At the same time leaves of Ukrainian *Fagopyrum esculentum* cv. *Rubra* has been characterized by highest anthocyanins content compared to the other experimental buckwheat genotypes of different origin. This data is connected with previous research where was studied role of anthocyanins as marker for selection of buckwheat plants with high rutin content in *Fagopyrum esculentum* cv. *Rubra* (Sytar et al. 2014).

#### Analysis of hydroxycinnamic acid derivatives

HPLC prescreening buckwheat genotypes of different origin on (51 DAS) has been identified chlorogenic, *p*-coumaric, *p*-anisic, cinnamic, methoxycinnamic, ferulic and vanilic acids. It was found highest content of chlorogenic and *p*-coumaric acids in the Chinese genotypes *F. esculentum* cv. *NingQiao 1* and *F. esculentum* cv. *YuQiao 4*. The highest *p*-anisic acid content was found for buckwheat genotypes of *F. tataricum himalaicum*



**Figure 5.** Content of total phenolics (5a) and anthocyanins (5b) in the buckwheat leaves determined by HPLC method (numbers indicate individual cultivars of buckwheat as follow: 1 – *F. tataricum rotundatum*, 2 – *F. tataricum himalaicum*, 3 – *F. esculentum* cv. *Rubra*, 4 – *F. esculentum* cv. *Karadag*, 5 – *F. esculentum* cv. *SuQiao 1*, 6 – *F. esculentum* cv. *YuQiao 4*, 7 – *F. esculentum* cv. *NingQiao 1*)

| Leaves of buckwheat                 | chl. acid | p-coum. acid | p-anis. acid | cinn. acid | Methox. acid | fer. acid | van. acid |
|-------------------------------------|-----------|--------------|--------------|------------|--------------|-----------|-----------|
| <i>F. tataricum rotundatum</i>      | 0.4±0.1   | 8.4±0.2      | 0.6±0.0      | 0.0±0.0    | 0.8±0.2      | 0.2±0.0   | 0.1±0.0   |
| <i>F. tataricum himalaicum</i>      | 0.3±0.0   | 0.2±0.1      | 0.8±0.1      | 0.0±0.0    | 2.6±0.1      | 0.2±0.0   | 0.1±0.0   |
| <i>F. esculentum</i> cv. Rubra      | 0.1±0.0   | 9.4±0.3      | 0.0±0.0      | 0.4±0.1    | 11.2±2.8     | 0.5±0.1   | 0.5±0.0   |
| <i>F. esculentum</i> cv. Karadag    | 0.2±0.0   | 9.4±0.8      | 0.0±0.0      | 0.1±0.1    | 4.8±0.6      | 2.2±0.3   | 0.2±0.0   |
| <i>F. esculentum</i> cv. SuQiao 1   | 0.3±0.1   | 20.1±8.3     | 0.1±0.0      | 0.0±0.0    | 3.0±0.3      | 0.2±0.1   | 0.3±0.0   |
| <i>F. esculentum</i> cv. YuQiao 4   | 0.4±0.1   | 21.2±5.0     | 0.0±0.0      | 0.0±0.0    | 2.4±0.1      | 0.2±0.0   | 0.2±0.0   |
| <i>F. esculentum</i> cv. NingQiao 1 | 0.5±0.0   | 25.1±0.6     | 0.1±0.1      | 0.0±0.0    | 4.0±0.3      | 0.1±0.0   | 0.3±0.0   |

**Table 1.** Content of phenolic acids identified via HPLC analysis in the experimental buckwheat samples

(Table 1). It is important to admit that genotypes of *F. tataricum* characterized by higher p-anisic acid content compared to the experimental genotypes of *F. esculentum* of both origins.

The nondestructive technique of infrared spectroscopy is recommended as alternative technique for routine analysis of main flavonoids like rutin, quercetin and quercitrin in aerial parts of buckwheat (Ladan et al. 2017). It can be pointed out that individual bioactive compounds compositions are suitable indicators of the physiological stage of crop plants.

The phenotype of a plant is the result of a complex interaction between morphological, ontogenetical, physiological, and biochemical factors (Gratani 2014). The highest increasing of FLAV index has been observed on 51 DAS of flowering stage which is connected with flavonoids content in the Chinese genotypes. Rutin content of the grain of 22 buckwheat genotypes (*F. esculentum* and *F. tataricum*) grown in same region of origin had variation (Bai et al. 2009), so its important to use non-destructive methods for prescreening of flavonoids content for different genotypes. The high FLAV index at the flowering stage was not depended on flavonoids content on the beginning of seedlings stage (22 DAS).

Other investigated genotypes for ANTH index in range from 0.39 to 0.43 RU at the beginning of seedlings stage. Genotypes of *F. tataricum* compared to the genotypes of *F. esculentum* of both origin shown decreasing of ANTH index during seedling growth. Liu et al. (2008) have shown that ethanol extracts of Tartary buckwheat sprouts had higher free radical scavenging activity and

superoxide anion scavenging activity than those of common buckwheat sprouts (Liu et al. 2008). Total phenolics and rutin in tested samples were related to the antioxidant activities (Holasova et al. 2002).

The SFR index is linked to the Chl concentration of leaves (Diago et al. 2016). Leaf Chl and FLAV concentration on a surface basis depends on leaf age and the amount of light radiation received during their development. Both increase with leaf expansion and light exposure until veraison, while afterwards, leaf Chl usually decreases (Louis et al. 2009) while FLAV remain unvaryingly high (Downey et al. 2003). Such tendency has been confirmed in the our experiments with buckwheat genotypes of different origin just development of SFR index changes was different between Ukrainian and Chinese genotypes origin.

The antioxidant capacity can be connected with total phenolic and anthocyanin contents and variety of plant species plus maturity (Prior et al. 1998; Kim et al. 2003). Flavonoids and phenolic acids have relevant antioxidant properties (Barriada-Bernal et al. 2014). The concentration is affected by environmental conditions, age, and phenological stage (Almaraz-Abarca et al. 2013), while the qualitative phenolic profiles are more stable and vary among different groups of plants with a species-specific tendency (Emerenciano et al. 2001).

It was observed that presence of p-anisic acid was typical for *F. tataricum* genotypes compared to the experimental genotypes of *F. esculentum* of both origins – Ukrainian and Chinese. p-anisic acid is one of the isomers of anisic acid which has antiseptic properties (Bhimba et

al. 2010). It is also used as an intermediate in the preparation of more complex organic compounds.

Cinnamic acid has low toxicity and in the search for novel pharmacologically active compounds, cinnamic acid derivatives are important and promising compounds with high potential for development into drugs (Sova 2012). The high content of cinnamic acid at 52 d of flowering period found in Ukrainian *F. esculentum* cv. Rubra, which is characterized by high anthocyanins content. In plants, flavanone biosynthesis begins with the hydroxylation of cinnamic acid to *p*-coumaric acid by a membrane-bound P450 monooxygenase, cinnamate 4-hydroxylase (C4H) (Yan et al. 2005). At 52 day of flowering period the *p*-coumaric acid content was higher more than 2 times in Chinese genotypes compared to the Ukrainian genotypes of *F. esculentum*.

## CONCLUSION:

The screening of biological active compounds of phenolic nature in the early stages of growth with non-destructive chlorophyll fluorescence techniques can be used for qualitative traits analysis of plant sprouts, especial-

ly buckwheat. The high flavonoids level at the flowering stage is not dependent on flavonoids content on the begging of seedlings stage. Plant genotypes of different origin can vary in flavonoid, anthocyanins and pigments content during stages of growth but changes in their contents can be similar for representatives of the same origin. The presence of some phenolic acid can be typical for genotypes of *F. tataricum* compared to the genotypes of *F. esculentum*. To support natural plant biodiversity research it would be good to develop use of fast pre-screening methods of plants during all stages of development what can be helpful in applied food plant research.

**Abbreviations:** FLAV- flavonoids, ANTH – anthocyanins, SFR - simple fluorescence ratio, Chl – chlorophyll, DAS – days after sowing

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## IZVLEČEK

Predstavljeni so nedestruktivni načini ugotavljanja flavonoidov, antocianinov in pigmentov tekom osebkovega razvoja pri genotipih ajde različnega izvora, do faze cvetenja. Podobno naraščanje indeksov FLAV, ANTH in MFI je bilo ugotovljeno pri kitajskih in ukrajinskih genotipih. Genotipi *Fagopyrum tataricum* so v primerjavi z genotipi *F. esculentum* imeli tekom rasti kalic nižji indeks ANTH. Indeks SFR, povezan s koncentracijo klorofila je kazal različno dinamiko v primerjavi genotipov iz Ukrajine in Kitajske. V srednji fazi (30 dni po setvi) je bil pri večini genotipov ugotovljen povečan indeks SFR. Na 36 dan po setvi sta dva ukrajinska genotipa z visokima indeksoma ANTH in MFI imela naraščajoč indeks SFR. HPLC analize raziskanih vzorcev so pokazale vsebnost *p*-anisinske kisline, značilne za *F. tataricum* v primerjavi s kultivarji *F. esculentum*. Rastline genotipov ajde lahko imajo različno vsebnost flavonoidov, antocianinov in pigmentov tekom faz rasti in razvoja, vendar podobne vsebnosti pri vzorcih enakega porekla.



Research paper

# Research results of local buckwheat varieties and forms of Ukrainian origin

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## ABSTRACT

The national collection of buckwheat in Ukraine consists of 578 local samples and forms of Ukrainian origin added into the collection during 1929 to 2012. In 1994-2000 and 2014-2018, the material has been studied and described, the common characteristics of samples peculiar of a certain area of origin, feature manifestation peculiarities, material flexibility and stability of the quantitative and qualitative characteristics depending on the weather in growing years have been identified. The range of studies included sample performance indicators, architectonic values, grain characteristics, and so on. A common characteristic of local buckwheat varieties is a significant sensitivity to changes in growing conditions, which is reflected in the change of growth processes (increase in the plant height by lengthening of internodes, the number of branches of the second and higher orders, the number of leaves per plant) and adjustment of the growth duration (lengthening the duration of the growing season after the onset of more favorable conditions for growth and development). The performance indicators related to the grain characteristics are quite stable. However, the general trends characteristic of plants from a certain origin of the collection material remained unchanged.

## INTRODUCTION

Buckwheat has not become one of the most demanded crops by the international community yet. The main reason is its limited distribution around the world and low yields. However, the conclusions made in recent years concerning the maintenance of full-fledged human life, health and outstanding buckwheat plant properties indicate a significant need in buckwheat products (Kreft, 2010, Alekseeva et al., 2005). Gradually, the buckwheat products are distributed from the major regions of its traditional consumption to the new areas, where it gains the status of the core component of a healthy diet and becomes the foundation for a healthy lifestyle.

As a result of in-depth study of biochemical composition of buckwheat grain and the whole plant, involving new types of crops into the research, the scientists around the world have greatly expanded the application areas of buckwheat products from traditional to exotic. To carry out this work, the genetic material with extensive properties and characteristics, most of which is concentrated in banks of plant genetic resources, is used (Alekseeva et al., 2004).

Genetic diversity preservation has long been an imperative of global importance. The work to create the banks of genetic plant resources, launched more than a hundred years ago, not only validated the feasibility of this direction development, but also confirmed an unvalued contribution to ensuring food security worldwide (Alexanian, 2003).

To solve scientific problems, the collection material of various eco-geographical origin and biological status is critical. However, given the importance of addressing the issue of the plant productive potential realization in contrasting environmental conditions, the study of local varieties and forms, the research of adaptive mechanisms and biochemical plant components became of paramount importance.

## MATERIALS AND METHODS

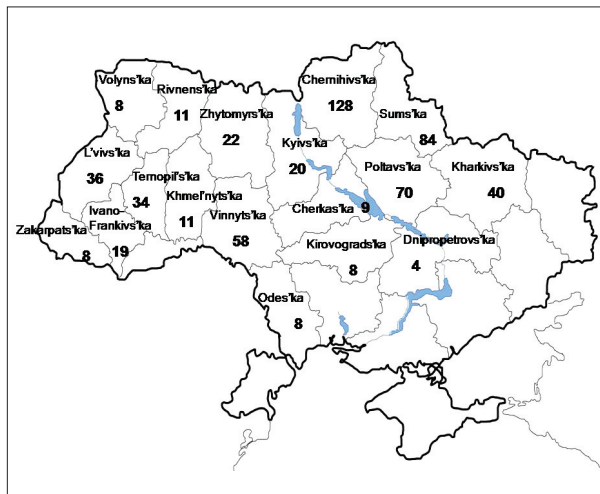
A part of the National Buckwheat Collection in Ukraine, which is stored in Ustymivska Experimental Station of Plant Production (Poltavs'ka region), includes over 1,600 authentic samples, of which 1.1 thousand samples are the local varieties and forms of the common buckwheat (*Fagopyrum esculentum* Moench.) (Tryhub et al., 2015). 578 samples in the collection have Ukrainian origin. The material included in the collection is the result

of its collection by employees of the N. I. Vavilov All-Union Institute of Plant Genetic Resources (VIR, Leningrad) during the missions (Fesenko et al., 2006), by researchers of Ternopil Breeding and Research Station, the Research Institute of Agriculture and Livestock of the Western Regions of Ukraine led by Olena Alekseeva (Alekseeva, 1967), as well as employees of the National Center for Plant Genetic Resources of Ukraine, assisted by the Ustymivska Experimental Station of Plant Production (Kirjan et al. 2014) in the territory of Ukraine from 1929 to 2012. As regards the ecological and geographical origin, there are samples from 18 regions. These are the representatives of the Carpathian region, Woodlands, Forest Steppe and Steppe zones. The overwhelming majority is the samples from the North, Central and Western parts of Ukraine, and a small number comes from the Eastern and Southern regions (see Table 1 and Pic. 1).

| Natural-climatic zones and regions of Ukraine | Number of accesses |
|---|--------------------|
| <b>Forest Steppe</b>                          | <b>294</b>         |
| Kyiv's'ka (part)                              | 12                 |
| Sums'ka (part)                                | 60                 |
| Khmel'nyts'ka                                 | 11                 |
| Ternopil's'ka                                 | 34                 |
| Cherkas'ka                                    | 9                  |
| Poltavs'ka                                    | 70                 |
| Vinnyts'ka                                    | 58                 |
| Kharkiv's'ka                                  | 40                 |
| <b>Steppe</b>                                 | <b>20</b>          |
| Kirovohrads'ka                                | 8                  |
| Dnipropetrovs'ka                              | 4                  |
| Odes'ka                                       | 8                  |
| <b>Polissya and Carpathian region</b>         | <b>264</b>         |
| Zakarpats'ka                                  | 8                  |
| Ivano-Frankiv's'ka                            | 19                 |
| L'viv's'ka                                    | 36                 |
| Rivnens'ka                                    | 11                 |
| Volyn's'ka                                    | 8                  |
| Chernihiv's'ka                                | 128                |
| Kyiv's'ka (part)                              | 8                  |
| Sums'ka (part)                                | 24                 |
| Zhytomyr's'ka                                 | 22                 |
| <b>Total</b>                                  | <b>578</b>         |

**Table 1.** Distribution of origin of the local sample collection by climatic zone of Ukraine





**Fig. 1.** Territorial distribution of origin of the local buckwheat samples (name of district and number of samples).

The research of the collection material of buckwheat varieties and forms was carried out as required by the “Complete unified classifier of the genus Buckwheat (*Fagopyrum* Mill.)” (Tryhub et al., 2013), “Descriptors for buckwheat (*Fagopyrum* spp.)” (Descriptors..., 1994), “Guidelines for the study of collection samples of corn, sorghum and groats” (Krotov, 1968), “Analysis of the structure of buckwheat plants (Methodical recommendations)” (Bochkareva, 1994).

The material study and description was carried out in several stages during 1994-2000 and 2014-2018, and the results obtained were compared to the standard variety of Ukrainka. The common characteristics of samples peculiar of a certain area of origin, feature manifestation peculiarities, material flexibility and stability of the quantitative and qualitative characteristics depending on the weather in growing years have been identified. The range of studies included the sample performance indicators (the number of seeds and buds per plant, inflorescence yield), architectonic parameters (plant height, number of branches and inflorescences, height of attachment of the inflorescences and branches, the length of the branching and grain production zone), grain characteristics (grain size, evenness and husk content), the length of the growing season and its components, and so on.

## RESULTS AND DISCUSSION

To obtain high and stable buckwheat yields, it is required to continue creating new varieties combining high

performance, friendly maturation, resistance to drought, temperatures below zero, lodging, grain shattering, pests and diseases, as well as a high grain quality. For this purpose, in selection, as noted by N.I. Vavilov, we need to use the local material subjected to prolonged exposure to natural selection and adapted to specific conditions. This material has great value, and is widely used in selection (Korynyak et al., 2017).

It should be noted that in terms of soil and climatic conditions, the research region, namely the south of Poltav's'ka region (central part of Ukraine, Forest Steppe zone) is one of the most favorable for growing buckwheat. Still, it has recently been affected by the ongoing significant climate change towards higher temperatures during flowering and insufficient precipitation during initial growth, which is typical for most areas of buckwheat cultivation in Ukraine. These trends are typical for most regions of the world where the buckwheat is sown. The features of sign manifestation were identified based on a set of researches conducted in two stages. The samples were studied following a three-year cycle during 1994-2000 and 2014-2018. The first cycle of study (1994-2000) was remarkable for more favorable weather conditions with precipitation and temperature regime of the growing season close to the average long-term data. The second cycle of study (2014-2018) was characterized by a high temperature in summer and a significant lack of moisture in the spring.

The data obtained in the course of study indicate a considerable variety of feature manifestations by the local plant varieties and forms. There is a considerable dependence in the levels of feature manifestation on climatic zones of the sample origin. A significant impact on the plant characteristics was exercised by the climatic factors, precipitation and temperature regime during the years of cultivation. However, a set of the studied traits showed the characteristic, genetically determined features of samples and allowed describing the quantitative and qualitative material characteristics.

Given the considerable heterogeneity of the climatic zones of Ukraine in terms of soil conditions, heat and precipitation, the entire buckwheat gene pool of these areas has been further divided into smaller, but more similar regions. To characterize the samples from the western regions of Ukraine, the classification proposed of Olena Alekseeva (Alekseeva, 1999) was applied. For other areas, the author's classification was applied.

### **Samples from Forest Steppe of Ukraine**

In terms of soil and climatic conditions, the Forest Steppe Zone was subdivided into western, central and eastern parts, each with its own characteristics, significantly differing among themselves, but with rather similar buckwheat growing conditions within each part. The western part includes the samples from Khmel'nyts'ka, Ternopil's'ka and Vinnyts'ka regions, having more precipitation as compared to the other parts of the Forest Steppe Zone, and their more even distribution during the vegetation period, lower average daily temperatures and fertile soils. The central part of the Forest Steppe (Cherkas'ka, Poltavs'ka, Sums'ka and Kyivs'ka regions) is characterized by harsher weather conditions in terms of the temperature and humidity. Here is sufficient precipitation during the growing season, but its distribution is very uneven. The cover in most of the area is presented with fertile soil with excellent quality characteristics. The eastern part of the Forest Steppe zone includes Kharkivs'ka region and the south of Sums'ka region. This part shows the most extreme weather conditions with little precipitation and high temperatures, especially during the buckwheat flowering and graining.

In general, the gene pool of this region is represented by 294 samples.

The samples of this group are the most malleable genetic material grown in the area with conditions, which are the most suitable for the buckwheat cultivation. Such a genepool was the source material for most of the modern high-tech varieties, and today the scientists are searching for the forms remarkable for their performance indicators combined with resistance to abiotic environmental factors, grain quality, and so on primarily among these samples.

The samples from the western part of the Forest Steppe Zone of Ukraine show an extensive diversity in terms of the key parameters of the plant organism structure, which requires their subdivision into two subgroups by the growing season duration, i.e. mid-late-ripening (78-85 days) and early-ripening (70-77 days). Mid-late-ripening samples form tall plants (1.5 m high) with a large number of internodes (9-13 pcs.), a large number of leaves and branches (9-16 pcs.). Lower interstitials are thickened, forming resistance to lodging. The samples' yield is 180-220 g/m<sup>2</sup>, and the productivity is 1.8-2.6 g/plant. They have medium-sized grain, up to 25.6 g/1000 grain with uniformity up to 82% and husk content 22.7-23.5%. The early-ripening samples have the

plants 85-110 cm high with 10 internodes and the ratio of grain formation zone vs. branching zone as 1.0-1.3. The plants form a large number of inflorescences, up to 55 pieces per plant. Samples have large dark brown grain as for the local forms; the weight of 1,000 grains is 25.8-26.9 g, the husk content increased to 23.8%, and a good inflorescences uniformity, i.e. 85%. The yields of these samples is within 165-200 g/m<sup>2</sup>, and the productivity is 1.6-2.2 g/plant. Plants have a significant number of leaves and the medium number of branches (5-8 pcs.), are resistant to lodging and moderately resistant to grain shattering after ripening.

The central part of the Forest Steppe Zone has a more form genepool in terms of manifestation of quantitative and qualitative traits of the buckwheat plant organism. Local varieties from this part feature the medium-term ripening (70-80 days), medium and large plant height (100-150 cm), 7 to 16 internodes on the main stem and the ratio of the graining zone vs the branching zone as 1.1-1.3. The plants from this group are resistant to lodging, have many leaves and 8-14 branches (including 5-8 first-order branches) and 85 buds per plant. The grain of these samples is gray and dark brown with an average weight of 1,000 grains as 24.2-27.3 g, grain husk content as 22.1-23.5%, and 90% uniformity. The average grain yield varies between 185-236 g/m<sup>2</sup>, and the productivity – within 1.8-2.8 g/plant.

The plants from the local samples of eastern part of the Forest Steppe of Ukraine are potential sources of the variety resistant to abiotic environmental factors (high temperatures and drought). Samples from this part form the medium-term ripening (75 days) plant up to 120 cm high with 5-9 internodes, 5-12 branches and 65 buds per plant. These samples are characterized by high resistance to lodging and medium resistance to the grain shattering. Grain has a dark brown color with a clear or blurry pattern in the form of dots or dashes, the weight of 1,000 grains is 23.5-26.7 g, the medium husk content (up to 23.1%) and evenness (75%). The yield of samples from this group is 168-218 g/m<sup>2</sup> with the plant performance at 1.45-2.05 g/plant.

### **Samples from Polissya and Carpathian region**

The material from this zone also features extensive diversity due to a significant difference between the weather, climate and soil conditions within the region. The samples from this area were subdivided into 3 groups: the north-western, western, northern and Carpathian.

The north-western part includes the samples originating from Volyns'ka and Rivnens'ka regions, the climate in which is characterized by excessive precipitation, moderate temperature conditions and poor nutritional composition of their soils. The western part (Ivano-Frankivs'ka, L'vivs'ka and Zhytomyrs'ka region) is characterized by a moderate precipitation and temperature conditions favorable for plant growth and development, with soils of varying fertility. The northern part of the Polissia zone (Chernihivs'ka and a part of Kyivs'ka and Sums'ka regions) has the most fertile soils in this region, sufficient precipitation and heat. The gene pool of the Carpathian region (Zakarpats'ka and a part of Ivano-Frankivs'ka region) includes representatives of foothill and mountain areas, featuring moderate and sometimes low temperature during the growing season, often excessive precipitation and poor soils. The collection of local varieties and forms from this region includes 264 samples.

The samples of the north-western part of the region are characterized by a short growing season, low (1.2 m) stem with 4-5 internodes; slight branching (3-7 branches, of which 2-3 are the first-order branches) with a ratio of the graining zone vs the branching zone as 0.8, the medium number of leaves, the sample yield at 120-180 g/m<sup>2</sup> per plant and the productivity as 0.8-1.0 g/plant; the medium grain particle size (up to 23.5 g/1000 grains), uniformity (80%) and husk content (up 23.5%), brown grain with a clear patten; resistant to lodging and having a medium degree of grain shattering after ripening.

The samples from the western part feature a significant heterogeneity by the growing season duration; the samples vegetation varies from early-ripening (70 days) to late-ripening (90 days), and respectively, the plant height and the number of internodes ranged from 80-100 cm and 5-7 pieces. to 125-130 cm and 9-12 pieces. In general, all samples were dark brown and gray, with medium and large grain size (23.7-26.8 g/1000 seeds), medium evenness (85%) and high husk content (24%); the plants have many leaves with a medium degree of grain subsidence and lodging. The samples' yield was 175-210 g/m<sup>2</sup>, and the plant productivity – 1.6-2.2 g/plant.

The samples from the northern part feature a high yield (up to 250 g/m<sup>2</sup>) and plant productivity (up to 3.0 g/plant); large grain (24.5-26.4 g/1000 grain), good evenness (up to 85%), and medium husk content (22.6-23.1%), brown and dark brown color. The plants of these samples are tall (1.2-1.5 m) with a large number of branches (13-18 pcs.) and buds (70 pcs.) per plant,

as well as many leaves. The stem is thick and resistant to lodging.

The samples from the Carpathian region (foothill areas of Zakarpats'ka and Ivano-Frankivs'ka regions) are characterized by a considerable length of the growing period (80-90 days) and tall plants (150 cm) with a significant number of branches (15 pcs.) and internodes (10-13 pcs.); the ratio of the graining zone vs the branching zone is 0.9-1.1; the plants have a large number of leaves and buds (80 pcs.); yield at 180-200 g/m<sup>2</sup> and productivity up to 2.2 g/plant; the medium grain size (up to 25.1 g/plant), medium husk content (23%) and evenness (85%). The plants are remarkable for a low resistance to plant lodging and grain shattering. Samples from the Carpathian mountain areas have a medium length of the growing period (75 days), the plants are lodging heavily due to thin stems and lots of leaves, the medium number of branches per plant (7-10 pcs.) and internodes per stem (6-9 pcs.); the ratio of the graining zone vs the branching zone is 0.6-0.9. The sample yield is medium, 160 g/m<sup>2</sup> per plant, and the productivity is 0.9-1.5 g/plant, while the number of buds is - 50 pcs. The grain features a medium size 21.3-24.6 g/1000, low evenness (70%) and high husk content (23.4-24.6%); gray or brown color with a distinct pattern.

### Samples from Steppe of Ukraine

This is the least numerous group of samples available in the National Collection of Ukraine. The total gene pool of the region comprises 20 samples. However, in terms of its climatic conditions, each of the regions included in this group has the contrasting features and requires subdivision into two parts, namely the gene pool of Odes'ka region, which was formed in conditions of unstable humidity, relatively poor soils and high temperature during the vegetation period, as well as samples originating from Kirovohrads'ka and Dnipropetrovs'ka regions, where the sample forming conditions are remarkable for unstable, but more moderate precipitation, more favorable temperature conditions and availability of fertile soils.

The samples originating from Odes'ka region feature the early ripening (68-75 days), low height (110 cm), a small number of internodes per stem (48 pcs.), low resistance to lodging due to a thin stem, a small number of branches (5-9 pcs.) and a moderate number of leaves per plant. The graining zone vs the branching zone ratio is 1.1-1.3. The sample productivity level varies considerably, several times, depending on the weather conditions.

The average yield in this group of samples ranged from 68.5 to 174 g/m<sup>2</sup>, and the plant productivity was 0.4-2.3 g. Samples form a quite fine grain, 21.6-23.2 g/1,000 grain, with a medium husk content – 22.8-23.4%, and low evenness (75%).

The samples originating from Kirovohrads'ka and Dnipropetrovs'ka regions are more grainful (179-236 g/m<sup>2</sup>) with the productivity of 1.9-2.6 g/plant. They also form larger (24.7-26.5 g/1,000 grain), even (85%) grain with thinner husk (up 23.1%). The samples of these plants have more leaves, branches (7-13 pcs.) and buds (80 pcs.) per plant. The plant height is medium, 125 cm, with 9-13 internodes. The plants are more resistant to the grain shattering after ripening and resistant to lodging due to the thickened lower internodes.

A common characteristic of all local buckwheat varieties of Ukrainian origin is a significant sensitivity to changes in growing conditions, which is reflected in the change of the growth processes (increase in the plant height by lengthening of internodes, the number of branches of the second and higher orders, as well as the number of leaves per plant) and adjustment of the growth

process duration (extension of the growing season length after the onset of more favorable conditions for growth and development). A quite stable performance is related to grain characteristics (size, husk content and uniformity, color, grain, etc.). However, the general trends, characteristic of the collection material origin, remained unchanged.

Extensive variety options are important for selection, because enables finding a material with specific characteristics among varieties and forms, identifying and examining the same according to a set of indicators. While working to study and describe the sample collection, some contrasting forms in comparison with the original populations were discovered (in terms of the shape of plants and buds, flower color and size, availability and varying degrees of anthocyanin color, initial growth rate, a lack of branching, ability to counter or avoid the extreme environmental factors, etc.). This is the most valuable genepool, because it allows expanding the variety polymorphism, extending the traditional and starting the new areas of buckwheat selection or use of products obtained from its cultivation.

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## IZVLEČEK

Zbirko ajde Ukrajine tvori 578 lokalnih vzorcev, ki so bili nabrani in vključeni v zbirko v letih 1929 do 2012. V letih 1994-2000 in 2014-2018 so potekale raziskave in opisovanje vzorcev, zlasti običajnih lastnosti, značilnih za posamezna območja izvora, posebnosti vzorcev, variabilnost in stalnost kvantitativnih in kvalitativnih lastnosti v odvisnosti od vremena in leta pridelovanja. Raziskave so med drugim vključevale kazalce rodnosti, opis zgradbe rastlin in lastnosti zrn. Običajne lastnosti lokalnih varietet ajde so odvisne od sprememb v razmerah pridelovanja, kar se kaže v spremembah poteka rasti (večanje višine rastlin z daljšanjem internodijev, števila stranskih vej drugega in višjega reda, števila listov na rastlino). Kazalci povezani z lastnostmi zrn so precej stabilni. Vseeno pa ostajajo lastnosti rastlin glede na izvor v znatni meri nespremenjene.

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**Manuscripts** should be typed double-spaced on DIN A4 format (21x29cm or 8.5x11 inch) with sufficiently wide margins (2.5-3cm), in one column (we will transfer later the text to two parallel columns). All pages, including the tables, legends and references, should be numbered consecutively. The manuscript should be arranged in the following order, or other suitable similar order:

1. *Title page (page 1)*

- Title (the title should be as short as possible, but should contain adequate information to indicate the contents)
- Author's full name(s)
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2. *Key words/Running head (not to exceed 50 letters including spaces) (page 2)*

- Key words (maximum of 8, in alphabetical order, suitable for indexing)

3. *Abstract (brief and informative, not to exceed 250 words).*

4. *Main text*

- Introduction, Materials and Methods, Results, Discussion
- The relative importance of headings and subheadings should be clear.

5. *The approximate location of figures and tables could be indicated in the margin or in the text.*

- The use of footnotes is to be avoided.

6. *After the main text*

- Acknowledgements (also grants, support etc., if any) should follow the text and precede the references.

7. *References*

Abstract in Slovenian will be for foreign authors made by the editors.

Review papers are welcome, main text has to be organised according to authors' suggestion.

**The literature references** should be arranged alphabetically, typed double spaced and in the text referred to as: author and year of publication, e.g., Budagovskaya (1998), (Inoue et al. 1998). Citation of personal communications and unpublished data should be avoided, unless absolutely necessary. Such citations should be in text, appear only as (R. Brown, personal communication), and not in the reference list. To abbreviate titles of periodicals, refer to recent issues of FAGOPYRUM.

Each literature source listed in the list of references should contain at the end the doi number, if such number exists, like:

**If the cited paper has a doi number, follow the case:**

Chetry, U., L. Dohtdong and N.K. Chrungoo, 2018. Analysing structural diversity of seed storage protein gene promoters: Buckwheat a case study. *Fagopyrum* 35: 5-17. <https://doi.org/10.3986/fag0004>

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Budagoskaya, N., 1998. Changes in the state of photoautotrophic and heterotrophic organs of buckwheat plants at iron deficiency and low pH. *Fagopyrum* 15: 1-7.

Inoue, N., M. Hagiwara, H. Y. Kim and T. Matano, 1998. A preliminary study for modeling seed production in common buckwheat. *Fagopyrum* 15: 35-41.

**Books (edited by someone other than author of article)**

Hattermer, H. and H. G. Gregorius, 1990. Is gene conservation under global climate meaningful? In: Jackson, M. T., B. V. Ford-Lloyd and M. L. Parry (Eds.), *Climatic Change and Plant Genetic Resources*, pp. 158-166, Bellhaven Press, London.

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Campbell, C.G., 1997. *Buckwheat*. IPGRI, Rome.

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Authors will be contacted by the Editor-in-Chief when the initial review process is completed. Correspondence with authors will be by e-mail. When preparing final versions of revised manuscript, the revised manuscripts including figures should be prepared and forwarded to the Editor-in-Chief.

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