Evaluation of genetic variability of buckwheat varieties (*Fagopyrum esculentum*) using ISSR-analysis

A.G. Klykov*, P.V. Fisenko, A.S. Chibizova, E.N. Barsukova

FSC of Agribiotechnology in the Far East named after A.K. Chaika, Ussurijsk, Russia

DOI 10.18699/ICG-PlantGen2019-14

* e-mail: alex.klykov@mail.ru

Abstract: The article presents analysis of the genetic polymorphisim on ISSR primers of five varieties of buckwheat (*Fagopyrum esculentum* Moench) (Izumrud, Kitavase soba 1, Kitavase soba 2, Cheremshanka, Bashkirskaya krasnostebelnaya). The obtained results demonstrated the high level of polymorphism and the prevalence of intra- over intervarietal variability. There were defined genetic distances between the studied varieties and a dendrogram reflecting the genetic differences between vatieties was constructed. The most largest Nei's genetic distance (D_N = 0.2430) was found between 'Izumrud' and 'Bashkirskaya krasnostebelnaya'. The results of analysis of phylogenetic differences can be useful in buckwheat selection for developing new forms with high flavonoids contents.
Key words: *Fagopyrum esculentum* Moench; selection; variety; ISSR analysis; flavonoids.

1. Introduction

Fagopyrum esculentum Moench is an important cereal and nectar-bearing crop widely cultivated in many countries of the world. The genus *Fagopyrum* Mill. possesses valuable nutrient and medical qualities. Representatives of the genus *Fagopyrum* are promising sources of flavonoids, among which the main is 3-O-rutinoside quercetin (rutin or vitamin P), which has antioxidant, angioprotective, antibacterial, and hepatoprotective properties (Klykov et al., 2018). The most important task of both foreign and Russian science is improvement of the existing varieties of *F. esculentum* and creation of new ones adapted to the growing conditions, diseases and pests and with high flavonoid content. A successful solution of the problem of this scale is possible on the basis of interdisciplinary research in the field of genetics, breeding, and biochemistry.

Buckwheat, despite its popularity, is not a mass object of genetic research. At the same time, the study of the intraspecific genetic diversity of *F. esculentum* is extremely relevant, due to the presence of a large number of varietal samples differing in the content of flavonoids. Inter-simple sequence (ISSR) repeat markers are widely used to detect intraspecific polymorphism, as well as variability in closely related genotypes of cultivated plants (Joshi et al., 2000; Sica et al., 2005).

The aim of this study was to evaluate the genetic polymorphism of *Fagopyrum esculentum* varieties of different origin with high flavonoid content using molecular marking.

2. Materials and methods

The plant material includes five varieties of *F. esculentum* – Izumrud (Russia, Primorsky Krai), Kitavase soba 1 (Japan), Kitavase soba 2 (Japan), Cheremshanka (Russia, Republic of Tatarstan), and Bashkirskaya krasnostebelnaya (Russia, Republic of Bashkortostan) – isolated in the field conditions of Primorsky Krai for the flavonoid content in the aboveground mass, fruits and other agronomically valuable traits (Klykov et al., 2018).

Ten seeds of each buckwheat variety were germinated in the conditions of the culture room. DNA was isolated from the leaves by the method of Aljanabi et al. (1997) with additional purification using a chloroform/isoamyl alcohol mixture (24/1). The amount of DNA in the sample was determined using a BioSpec-Nano spectrophotometer (Shimadzu). PCR was carried out 2–3 times using four ISSR primers. We used the ready-to-use reaction mix BioMaster HS-Taq PCR-Color (Biolabmics) with the addition of magnesium chloride to a final concentration of 2.5–3.0 mM, depending on the primer. To control contamination and nonspecific hybridization of primers, there was used a blank sample containing a complete reaction mixture without adding DNA.

Amplification was performed in the MJ Mini amplifier (BioRad) with preliminary denaturation for 5 min at 94 °C followed by 40 cycles: 30 sec at 94 °C; 30 sec at 49–60 °C (depending on the primer, the annealing temperature was selected individually); 1 min at 72 °C. The reaction products were separated by electrophoresis in 2 % agarose gel, colored with ethidium bromide, in 0.5 X TBE buffer. Visualization was performed by irradiation of the gel with ultraviolet light using a Gel Doc XR+ (BioRad).

For each primer, binary matrices were constructed where the presence or absence of a fragment was marked as 1 or 0, respectively. Genetic distances and construction of dendrograms was carried out using the software package POPGENE and TFPGA.

3. Results and discussion

Studies have noted that the quantitative and qualitative yield of DNA from the tissues of buckwheat (freeze-dried leaves) is much lower than from the same number of tissues of other crops (soybeans, rice). When using green leaves, the situation improves, but the final concentration of total DNA is still lower. Perhaps this is due to the fact that buckwheat, compared with other crops, has the fewest chromosomes in the karyotype.

As a result, the PCR revealed 106 amplicons, 105 of which were polymorphic. Intravarietal polymorphism varied considerably, from a minimum of P = 50 % and 50.94 % in 'Izumrud' and 'Bashkirskaya krasnostebelnaya', respectively, and to a maximum of P = 75.47 % in 'Cheremshanka'. The varieties Kitawase soba 1 and Kitawase soba 2 have similar level of polymorphism, P = 65.09 and 64.15 %, respectively.

Table 1
Genetic distances (D_N) between the of *F. esculentum* varieties

Variety	Izumrud	Kitawase soba 1	Kitawase soba 2	Cheremshanka
Izumrud	***			
Kitawase soba 1	0.1046	***		
Kitawase soba 2	0.1192	0.0372	***	
Cheremshanka	0.1196	0.0543	0.0577	***
Bashkirskaya krasnostebelnaya	0.2430	0.1833	0.1791	0.1872

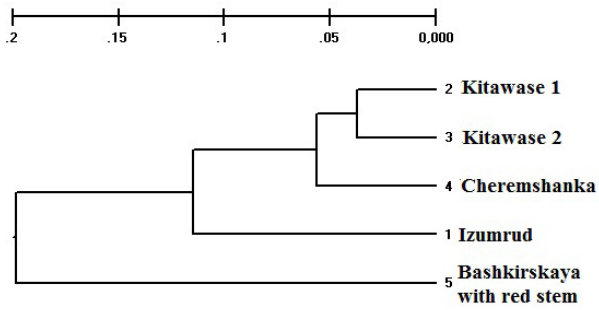

Figure 1. UPGMA dendrogram of phylogenetic relationships of buckwheat varieties constructed on the base of ISSR analysis.

Nei's genetic distances (D_N) (Nei M. 1972) were calculated on the basis of ISSR analysis. The highest value of $D_N = 0.2430$ was found between 'Izumrud' and 'Bashkirskaya krasnostebelnaya', the lowest $D_N = 0.0372$ was between 'Kitawase soba 1' and 'Kitawase soba 2' (Table 1).

To visualize genetic differences using the TFPGA software package, a phylogenetic relationship dendrogram was constructed using the pairwise intragroup unweighted (UPGMA) algorithm. The length of the branches reflects the level of genetic differences (Figure 1). 'Kitawase soba 1' and 'Kitawase soba 2' formed a cluster with the shortest branches, and 'Bashkirskaya krasnostebelnaya', having the highest value of genetic distances in relation to the other cultivars, formed a separate cluster with the longest branch.

We calculated the population differentiation index (G_{st}), which shows the distribution of intra- and inter-population variability. The value of $G_{st} = 0.3125$ indicates the predominance of intra-population variability over inter-population variability, which may be a consequence of the peculiarities of the biological features of the crop under study, namely, cross-pollination, a large number of different biotypes in the variety population, leading to an increase in population variability compared to self-pollinating crops (Klykov, 2011). In our early studies, there was defined a diagnostic trait (anthocyanin color of stem, flowers, root system, nuclei) which is efficient for use in breeding in order to create new varieties of *Fagopyrum esculentum* with high rutine contents (Klykov et al., 2018).

4. Conclusion

Thus, according to the results of ISSR analysis, the varieties Izumrud, Krasnoznamennaya Bashkirskaya and Kitawase soba 1, which have the greatest genetic differences were recommended for breeding process in order to create new genotypes with high flavonoid contents. In breeding, as the initial forms of selection, the choice of high-grade varieties with the highest level of differences, can lead to heterosis in hybrids. The data from ISSR analysis can be used to select the best plants within the hybrid buckwheat population.

The work was done in the framework of the complex program of fundamental research of the FEB RAS "Far East" in 2018-2020 project No. 18-5-025.

References

Aljanabi S.M., Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acid Research*. 1997;25(22):4692–4693.

Joshi S.P., Gupta V.S., Aggarwal R.K., Ranjekar P.K., Brar D.S. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. *Theor. Appl. Genet*. 2000;100:1311–1320.

Nei M. Genetic distance between populations. *American Naturalist*. 1972;106(949):283–292.

Klykov A.G., Moiseenko L.M., Gorovoy P.G. Biological resources of species of the genus of buckwheat (*Fagopyrum* Miil.) in the Russian Far East. Monograph. Vladivostok, 2018;304.

Klykov A.G. Study of intravarietal variability and the possibility of its use in the selection of buckwheat biotypes with certain qualitative indicators. *Reports of the Russian Academy of Agricultural Sciences*. 2011;2:12–14.

Sica M., Gamba G., Montieri S., Gaudio L., Aceto S. SSR markers show differentiation among Italian population Asparagus acutifolius L. *BMC Genetics*. 2005;6:6–17.

Conflict of interest. The authors declare no conflict of interest.