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Floral Polymorfism in Fagopyrum Mill.

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ABSTRACT

Study of genetic regulation of flower development is one of rapidly developing areas of floral development biology. In addition to artificial mutants, defective in the individual genes and controlling the development program of the flower, the taxa with a high level of polymorphism in the flower structure can be used as the models, one of which is the family Polygonaceae Juss. We have studied the flower structure in three different species of the genus *Fagopyrum* Mill, being a part of the above family.We have revealed a high flower structure variability both in number of flower bodies and in their mutual positioning in the studied species *F. esculentum, F. tataricum* and *F. giganteum.* The rate of typical flower structure was 60.6 to 90.1% in different specimens. In addition to typical type, we have also distinguished the most frequent types of flower structure. Changes in the number of flower bodies have most commonly affected an androecium, which sustains the theory of bipolar spatial pattern of flowers of the family *Polygonaceae*.We have revealed a high flower structure variability in both age-old and modern diploid and tetraploid varieties of *F. esculentum*. Short-columnar types prevail among the abnormal flowers.The greatest diversity in the flower structure was observed at the beginning of flowering. During this stage of formation, the abnormal flowers had ones with increased number of bodies prevailing; with decrease in the floral meristem volume during flowering there was observed a prevalence of abnormal flowers with signs of stamens and pistils reduction. *F. giganteum* often had such variants of flower structure with signs common to mutants defective in genes that are responsible for floral meristem formation.In addition to variability by the number of flower bodies, *F. tataricum* had a type of variability determined by underdevelopment of the outer stamens anthers with autogamous pollination, typical of this kind of buckwheat.

Keywords: polymorphism and morphogenesis of a flower, flower formula and diagram, *Fagopyrum.*

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INTRODUCTION

Study of genetic regulation of flower development is one of rapidly developing areas of floral development biology [1]. Based on the results of model objects a model of genetic control of flower body type – the so-called ABC-model [2, 3]. Spatial pattern of floral primordium into separate bodies is highly important during initiation of a flower [4]. In addition to artificial mutants, defective in the individual genes and controlling the development program of the flower, the taxa with a high level of polymorphism in the flower structure can be used as the models, one of which is the family Polygonaceae Juss. [5]. Objective of this study is to investigate floral polymorphism in several species of the gene *Fagopyrum* Mill of the said family.

MATERIALS AND METHODS

Material for study was collected in summer 2012-2014 in competitive variety trial of buckwheat, as well as in the collection nursery of Tatar Scientific and Research Institute of Agriculture (Tatarstan, Russia). Specimens from the collection of N.I. Vavilov All-Russian Scientific and Research Institute of Horticulture and selected varieties of common buckwheat Fagopyrum esculentum Moehch from Tatar Scientific and Research Institute of Agriculture, as well as specimens of Tartary buckwheat Fagopyrum tataricum (L.) Gaertn and and giant buckwheat Fagopyrum giganteum Krotov have served as the subject of research (Table 1). All studied species are annual crops. *F. esculentum* and *F. tataricum* are in the culture.

Table 1: Materials and scope of study

Species	Ploidy	Variety / VIR part No.	Country of origin	Year of material collection	Sample size, number of flowers
F. esculentum	2n	675	Russia	2012	300
	2n	793	Russia	2012	300
	2n	2848	Russia	2012	300
	2n	3646	Russia	2012	300
	2n	Chatyr-Tau	Russia	2012-2013	3900
	4n	Honey	Russia	2013	3000
F. tataricum	2n	K-66	Russia	2014	300
	4n	K-113	Russia	2014	300
	4n	K-62	Canada	2013	300
F. giganteum	4n	K-109	Russia	2014	500

F. esculentum – allogamous heterostylous species. Monoecious flowers with white corollaceous envelope, diameter 5-7 mm.

F. tataricum – autogamous homostylous species. Small, inconspicuous flowers with calyciform envelope, diameter 2-3 mm.

F. giganteum – artificial amphidiploid created by crossing the tetraploid specimen *F. tataricum* and perennial buckwheat *F. cymosum* Meissn. Characterized by entomoppllination, self-fertility, and heterostylism [6]. The flowers are similar in envelope size and color to *F. esculentum.*

The minimum sample size for each variety or varietal sample was 300 flowers. The flowers were fixed in 70% ethanol and further examined in the laboratory through a magnifying glass or a stereoscopic microscope MBS-1. Flower formulas were recorded, diagrams were sketched out.

Buckwheat is characterized by a prolonged flowering period. To find out how the position of the flower affects the plant and, therefore, the time of its blossoming affects its structure, the flower samples of varieties Chatyr Tau and Honey were taken spaced one week apart during the flowering period.

RESULTS AND DISCUSSION

The buckwheat flower is complete, monoecious, and asymmetric. Usually formed of 5 tepals (two outer, two inner, and one intermediate), 8 stamens arranged in 2 whorls (5+3) and ovary formed by fusion of three carpels. Depending on the position of intermediate tepal a distinction is made between levotropic and dexiotropic flowers (Figures 1.6 and 1.7).

Analysis of collected flowers showed a high level of polymorphism in the studied species of flowers. For example, selection varieties *F. Esculentum* may have 3 to 8 tepals, 0 to 12 stamens, and 0-5 ovarian carpels in an individual flower (Table 2). In 2012, the range of variation by the number of tepals and stamens in the flower was higher.

Table 2: Minimum and maximum numbers of flower bodies in specimens *F. esculentum, F. giganteum, F. tataricum*

Species	Specimen / variety	Minimum and maximum characteristic value		
		Number of tepals	Number of stamens	Number of ovarian carpels
F. esculentum	Chatyr-Tau (2012)	3…8	2…12	0…5
	Chatyr-Tau (2013)	4…7	0…10	0…4
	Honey	4…7	2…11	0…5
F. giganteum	К-109	3…6	6…9	2…3
F. tataricum	КК-62	5…6	6…9	3…4
	К-66	4…6	5…10	3…4
	К-113	5…6	6…9	0…3

Reduction in the number of tepals occurs quite seldom and results from either fusion of intermediate and inner tepals (Figure 1.2) or tepals loss (Figure 1.1).

Number of stamens decreases often due to reduction or complete loss of stamens. A reduced stamen has a short filament and immature anther. Reduction and loss of stamens occurs either in outer or inner whorl or both in outer and inner whorls simultaneously (Figures 1.3, 1.4, 1.5).

We have detected some cases of partial to full coalescence of stamen filaments (Figure 1.12). Coalescence occurs in inner stamens more often than in stamens of outer or different whorls.

Number of tepals in the individual flower increases up to 6 typically upon formation of additional tepal resulting from division of intermediate tepal and further formation of three-merous two-whorl envelope (Figure 1.9). It results lesser from division of internal tepal (Figure 1.10). During simultaneous separation of the intermediate and inner tepals a seven-merous envelope was detected (Figure 1.11).

Number of stamens may increase: firstly, due to formation of an additional stamen in the outer whorl in parallel with an additional tepal (Figures 1.9, 1.10); secondly, due to formation of a new stamen in the inner whorl, usually in front of the widest face of the ovary (Figure 1.8); thirdly, at the formation of a new stamen in the inner whorl in parallel with additional ovarian face (Figure 1.1).

Quantitative increase in gynoecium occurs due to formation of additional carpels, which form an ovary (Figure 1.1).

Chatyr-Tau had several cases of coalescence detected (Figure 1.12). Such flowers have consolidated receptacle, envelope and outer whorl of stamens, while inner stamens and pistils are arranged more or less individually. Pistils with atypical number of faces are a common phenomenon. “Double" flowers demonstrate a various degrees of coalescence.

In 2012, Chatyr-Tau had in total 42 variants by the number of flower bodies (flower formula) revealed. In 2013, both Chatyr-Tau and Honey had 36 variants by the number of flower bodies, where 18 of them occurred in both varieties. We have distinguished the most common flower formulas (Table 3). The rate of typical floral structure in varieties was ranged from 88 to 90%.

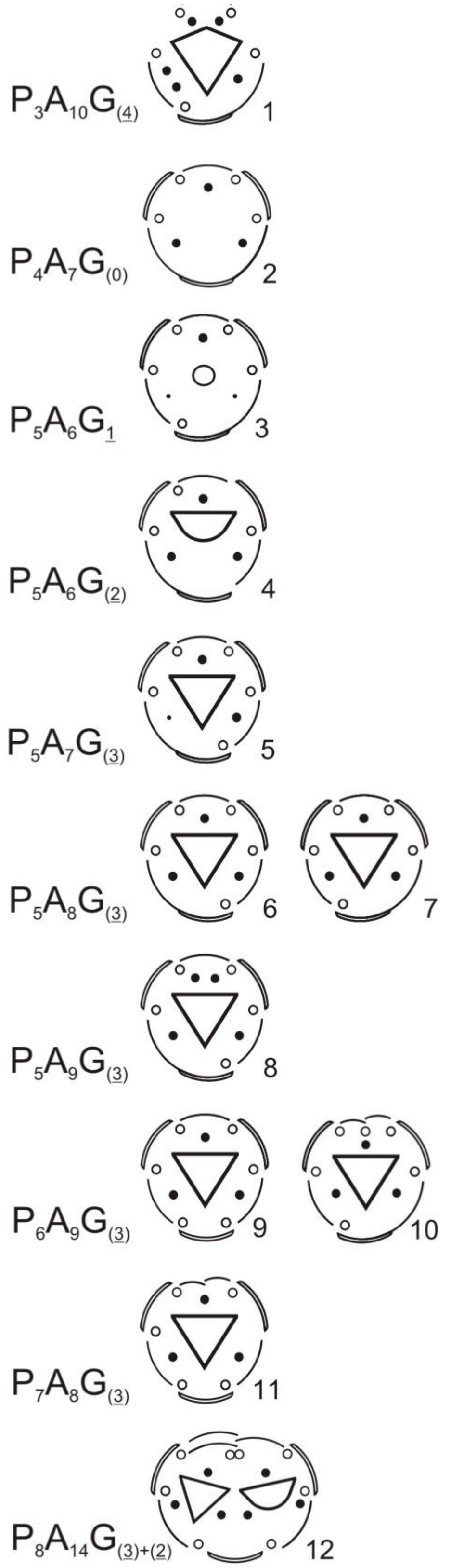

Figure 1: Variants of flower structure of *F. esculentum*

Table 3: The rate of the most common variants in number of flower bodies of *F. esculentum*, %

Flower formula	Chatyr-Tau / 2012	Chatyr-Tau / 2013	Honey / 2013
$P_5A_6G_{(\underline{3})}$	1.2	1.0	1.2
$P_5A_7G_{(\underline{3})}$	3.6	3.1	3.3
$P_5A_8G_{(\underline{3})}$	87.5	90.1	88.9
$P_5A_9G_{(\underline{3})}$	0.9	0.9	1.9
$P_6A_8G_{(\underline{3})}$	0.9	0.9	0.8
$P_6A_9G_{(\underline{3})}$	0.9	1,1	0.8

One variant by the number of flower bodies can match one or more variants of mutual arrangement of flower bodies (the flower diagrams). For example, the variety Honey had flower formula $P_5A_6G_{(\underline{0})}$ matching 3 flower diagrams different in arrangement of the reduced stamens.

Short-columnar types prevailed among the abnormal flowers *F. esculentum*. The ratio of abnormal short-columnar and long-columnar flowers was 64 and 36% in 2012, and 60 and 40% in 2013 for Chatyr-Tau, and 63 and 37% for Honey.

We have suggested, whether such a high variability in the structure of the flower *F. esculentum* results from its breeding. To test the hypothesis, we have studied the flower structure in several age-old varieties *F. esculentum*, created during the national selection. These varieties have been created a long time under the combined action of natural and simple methods of artificial selection [7]. These varieties could be called local, if it were not for the alien crop. However, flower polymorphism in age-old varieties was also significant (Table 4), therefore, it is not the result of selection. The most commonly noted variants are $P_5A_7G_{(\underline{3})}$, $P_5A_8G_{(\underline{0})}$, $P_5A_8G_{(\underline{3})}$, $P_5A_9G_{(\underline{3})}$ and $P_6A_8G_{(\underline{3})}$.

Table 4: Flower structure variability in the age-old samples *F. esculentum*

	Age-old variety cultivated in the territory of			
	Oryol region (No. 675)	Ulyanovsk region (No. 793)	Tatar ASSR (No. 2848)	Kirov region (No. 3646)
Abnormal flower rate in the sample	10.0	13.0	11.3	12.7
Number of floral structure variants by the number of flower bodies	15	9	13	13

The most common deviations from the typical buckwheat flower were due to quantitative changes in the androecium (Table 5): 58% in Chatyr Tau and 68% - in Honey in 2013. 7-10% of changes occurred in the envelope, and 4-9 % - in gynoecium flower. The second most frequent type of changes is those affecting both the flower envelope and androecium, simultaneously. The most rare are the abnormal flowers with changes in both the number of tepals and ovarian carpels: 0-1% only. These findings support a bipolar pattern model suggested for the family *Polygonaceae* [8]. Given the fact that the flowers with changes affecting the number of stamens are the most common among the abnormal buckwheat flowers we may suggest that these floral bodies are the last marked in a flower.

Table 5: Rate of floral structure deviations affecting its certain bodies, %

Variety *F. esculentum* / year	P∗	A∗	G∗	PA	AG	PG	PAG
Chatyr-Tau / 2012	8	58	9	11	7	0	7
Chatyr-Tau / 2013	10	58	4	17	6	1	4
Honey / 2013	7	68	5	10	9	0	1

∗ P – envelope, A – androecium, G – gynoecium.

Study of the flower structure conducted for Chatyr Tau and Honey over time showed that the greatest diversity in the flower structure of both studied varieties was observed in samples taken at the beginning of flowering. This is confirmed both by the frequency of abnormal flowers (Table 6), and by the number of variants by the number of flower parts in different samples. As a result, the number of typical flowers is minimum at the beginning of flowering, then it gradually increases in subsequent samples, and decreases slightly at the end of the growing season. This pattern can be clearly seen in diploid varieties and is not so obvious for variety Honey. This is probably due to a more prolonged formation of reproductive bodies in tetraploid varieties and, as a result, their greater sensitivity to external factors.

Table 6: Rate of abnormal flowers in varieties *F. esculentum*, %

Variety / year	sample No.									
	1	2	3	4	5	6	7	8	9	10
Chatyr-Tau / 2012	27	10	7	7	6	18				
Chatyr-Tau / 2013	24	7	8	7	2	10	11			
Honey / 2013	22	15	8	16	11	15	6	7	8	10

During buckwheat flowering, the abnormal flowers had ones with increased number of bodies prevailing. Further, the ratio of such variants decreases gradually, and at the end of the flowering the abnormal flowers have immature variants with loss of bodies such as stamens and pistils. This phenomenon can be explained by gradual decrease in the floral meristem volume during ontogeny.

Number of flower bodies in the specimens *F. esculentum, F. giganteum, F. tataricum* have been varying within closer range than in the varieties *F. esculentum* (Table 1). Abnormal flower rate of the studied specimen *F. giganteum* was 23.0% in the total sample (Table 7).

All plants *F. giganteum* that we studied were short-columnar, which, however, did not prevent them to form seed harvest. The peculiarity of this species was the discovered variants of the flower structure, where one or rarely two outer tepals were arranged not in a receptacle, but in a peduncle in a manner such that a 5 mm-long internode was formed between the place where a petal should be formed, and the actual place of body formation. Similar signs, when the distance between flower bodies was greater than in normal flowers, were earlier noted in the mutants *Arabidopsis thaliana* defective in the genes responsible for the formation of floral meristem [1]. The rate of these variants was 9.4% in total.

Specimens F. tataricum are characterized by a relatively uniform flower structure (a few variants of flower structure by the number of bodies) and at the same time, a high percentage of abnormal flowers. In addition to the typical variant of structure the variants $P_5A_7G_{(3)}$ and $P_6A_8G_{(3)}$ occurred frequently too. Specimen K-113 is a tetraploid form of K-66. Both specimens have become "champions" in the frequency of abnormal flower occurrence.

Table 7: Flower structure variability in the samples *F. esculentum, F. giganteum, F. tataricum*

Species	specimen	Number of floral structure variants by the number of flower bodies (flower formulas)	Abnormal flower rate	The most frequent types of flower structure (occurrence rate of the relevant variant of structure is given in brackets in %).
F. giganteum	K-109	19	23.0	$P_4A_7G_{(3)}$ (3.0), $P_4A_8G_{(3)}$ (4.4), $P_5A_7G_{(2)}$ (1.0), $P_5A_8G_{(2)}$ (1.4), P5A8G(3) (77.0).
F. tataricum	K-62	8	16.3	$P_5A_6G_{(3)}$ (1.3), $P_5A_7G_{(3)}$ (7.7), $P_5A_8G_{(3)}$ (83.7), $P_6A_8G_{(3)}$ (2.0).
	K-66	13	38.0	$P_5A_6G_{(3)}$ (1.3), $P_5A_7G_{(3)}$ (15.0), $P_5A_8G_{(3)}$ (62.0), $P_6A_8G_{(3)}$ (2.7).
	K-113	10	39.4	$P_5A_6G_{(3)}$ (1.3), $P_5A_7G_{(3)}$ (28.8), $P_5A_8G_{(3)}$ (60.6), $P_6A_8G_{(3)}$ (6.4).

In addition to the variability associated with a decrease and increase in the number of flower bodies, all studied specimens *F. tataricum* had a feature associated with hypoplasia of the anthers. Filaments were fully developed, and the anthers were reduced by half or completely (only one tech was normally developing). In most cases these developmental deviations are common for the stamens of the outer whorl. For example, 1 of 300 studied flowers of specimen K-62 had typical structure and normally developed stamens, 298 flowers had signs of immature stamen of outer whorl, and one had immature stamens in both whorls of androecium. On average, a flower had 3-4 immature anthers in the outer stamens. Obviously, the stamens of the inner whorl are involved in pollination. They, probably, produce the amount of pollen enough for a successful self-pollination, therefore we have lessened control over the stamen development on the part of selection.

F. giganteum and *F. tataricum,* as well as *F. esculentum* had most frequent quantitative changes in flower due to changes in the number of stamens.

SUMMARY

- We have revealed a high level of polymorphism in three species *Fagopyrum* relating to both the number of flower bodies and their mutual arrangement.
- Quantitative changes in flower occur with maximum frequency in the androecium of species *Fagopyrum*.
- High level of flower structure variability in the age-old varieties *F. esculentum* means that the flower polymorphism is not the result of selection.
- The maximum variability of the flower structure in *F. esculentum* was at the beginning of flowering phase. During this stage of formation, the abnormal flowers had ones with increased number of bodies prevailing.
- Feature revealed in the studied specimen *F. giganteum* was the variants of flower structure with one or two outer tepals located on the stem with frequency of 9.4%.
- Most of outer stamens in flowers of the plants *F. tataricum* are immature.

CONCLUSION

We have found a high level of flower polymorphism in three species *Fagopyrum: F. esculentum, F. tataricum* and *F. giganteum.* The rate of typical flower structure was 60.6 to 90.1% in different specimens. In addition to typical type, we have also distinguished the most frequent types of flower structure. Changes in the number of flower bodies have most commonly affected an androecium, which sustains the theory of bipolar spatial pattern of flowers.

We have revealed a high flower structure variability in both age-old and modern diploid and tetraploid varieties of *F. esculentum*. Short-columnar types prevail among the abnormal flowers.The greatest diversity in the flower structure of varieties *F. esculentum* was observed at the beginning of flowering. During this stage of formation, the abnormal flowers had ones with increased number of bodies prevailing; with decrease in the floral meristem volume there was observed a prevalence of abnormal flowers with signs of stamens and pistils reduction.

F. giganteum often had such variants of flower structure with signs common to mutants defective in genes that are responsible for floral meristem formation.

In addition to variability by the number of flower bodies, *F. tataricum* had a type of variability determined by underdevelopment of the outer stamens anthers with autogamous pollination, typical of this kind of buckwheat.

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