ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Pollen Productivity and Fertility of Pollen in the Genus *Fagopyrum* Mill.

Luisa R. Kadyrova *, and Regina R. Mukhametshina.

Kazan Federal University, Kazan, Kremliovskaya St., 18.

ABSTRACT

In recent years, a lot of hopes to improve buckwheat breeding are set on interspecific hybridization. To improve the efficiency of hybridization and in further successful seed reproduction of plants the quantity and quality of pollen released are of great importance. The objective of this paper was a comparative evaluation of pollen productivity and fertility of *Fagopyrum esculentum Moench, Fagopyrum tataricum (L.) Gaertn.* and *Fagopyrum giganteum Krotov* pollen. In this paper was used a comparative-embryological method and iodine method for pollen fertility determination. The development of anther in all three species happened in a similar manner, the difference was in the number of developing mother cells of microspores in the anther nest. Here were calculated average and potential pollen productivity of a flower, and p/o ratio of three representatives of *Fagopyrum Mill.* Realization of potential pollen productivity prevents violations during embryological processes. All three representatives of the genus revealed pauses in the development of sporogenous tissue and abnormalities in meiosis. Cytomixis was found only in *Fagopyrum esculentum*. The number of normally developed stamens in *Fagopyrum esculentum* and *Fagopyrum giganteum* was close to eight and in *Fagopyrum tataricum* samples was 4.9 and 6.0. The weakening of control over the development of *Fagopyrum tataricum* outer circle stamens is due to the fact that pollen from autogamous species produces more than it is necessary for its self-pollination. In all three species was discovered significant percentage of sterile pollen. Even in favorable for the plant's growth and development conditions the pollen fertility of *Fagopyrum tataricum* and *Fagopyrum giganteum* tetraploid sample did not exceed 56 %. On the example of *Fagopyrum esculentum* there was proved a significant impact of drought on pollen fertility.

Keywords: pollen productivity, embryological processes, p/o ratio, pollen fertility, *Fagopyrum*.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In recent years, in connection with the discovery of buckwheat *Fagopyrum homotropicum Ohnishi* new species there are great expectations to improve the common buckwheat breeding by interspecific hybridization with the big-seeded *cymosum*-group species [1]. Apart from the common buckwheat *Fagopyrum esculentum Moench* into this group are included *F. homotropicum Ohnishi, F. tataricum (L.) Gaertn., F. cymosum Meisn.* [2]. Promising to be introduced into the culture after appropriate breeding species refinement is also considered a form of *F. giganteum Krotov* [1] which is an artificial amphidiploid from *tetraploid F. tataricum* and *F. cymosum* crossbreeding[3].

To improve the efficiency of crossbreeding and in further successful plants' seed reproduction the quantity and quality of pollen formed is of great importance. The object of this paper was a comparative assessment of pollen productivity and pollen fertility in common buckwheat *F. esculentum Moench*, the Tatar buckwheat *F. tataricum (L.) Gaertn.* and buckwheat giant *F. giganteum Krotov*. If considered from this point of view the first type is fairly well studied, but *F. tataricum* and *F. giganteum* are not studied at all.

The development of the male reproductive system of *F. esculentum* flower is described in the works of some researchers [4, 5, 6]. The development of the pollen nest wall is carried out according to the type of monocots. The wall of the formed pollen nest consists of epidermis, fibrous, intermediate layers and tapetum. The cells of sporogenous tissue are located in the nest anther in a row. Microspores are formed by simultaneous type. Later from microspores there are formed pollen grains, in which is occurring microgametogenesis, culminating in the formation of sperm cells. Mature pollen grain – is three-cellular. The maximum pollen size and fertility are specific to the lower inflorescence flowers of main stem and branches [7, 8].

Pollen productivity of *F. esculentum* [9, 10] flower was calculated, and it amounted to 1784 pollen grains in average for long-columnar flowers and 1096 for short-columnar. The phase of plants' blossoming can affect amount of the pollen produced in a flower, besides genotypes with high pollen efficiency can be allocated [11].

The percentage of abortive pollen in diploid buckwheat rarely exceeds 1.0%, the increased sterility of pollen and embryo sacs in tetraploid forms is explained by disturbances in meiosis [12, 13, 14]. At the same time in the experiment water deficit resulted in a significant decrease in the percentage of fertile pollen [15] in the period preceding the diploid *F. esculentum* pollen formation.

In experiments on *F. esculentum* pollen germination was revealed its high (over 90 %) viability [16, 17, 18, 19]. In our studies of diploid common buckwheat its pollen fertility averaged 80 %, the reduction in the viability of pollen could be explained by a high sensitivity of embryological processes to abiotic stresses, particularly to drought [6].

MATERIALS AND METHODS

Materials for research were collected in the fields of the Tatar Research Institute of Agriculture (Laishevsky region, Tatarstan, Russia) in the competitive strain testing of buckwheat and collector's nursery in the summer of 2013-2015. There were examined varieties of Chatyr Tau (2n) and Medovaya (4n) *F. esculentum* breeding of Tatar Research Institute of Agriculture, samples K-17 (2n), K-108 (4n) *F. tataricum* and K-109 (4n*) F. giganteum* obtained from N.I. Vavilov Russian Scientific Research Institute of Plant Production (Saint-Petersburg, Russia).

For studying the anthers' structure and the processes of pollen formation in the Chamberlain fixator there were registered inflorescences in the phase of plants budding. There were made permanent preparations out of them according to standard methods [20, 21].

In the process of studying flower morphology in buckwheat species there was estimated the number of stamens in the flowers. For this in the phase of initial flowering from each of the samples were randomly selected 300 flowers, fixed in 70 % ethanol, and then studied in the laboratory with a magnifying glass or stereoscopic microscope MBS-1.

ISSN: 0975-8585

In the summer of 2015 was conducted a field study of buckwheat pollen fertility. Was used iodine method of assessment [20]. For each sample in the phase of beginning of flowering was estimated pollen fertility of 50 flowers. To do this, anthers of large buds were removed and opened with a dissecting needle, were treated with iodine solution and studied under a microscope MBD-1. For each flower were taken into account the ratio of fertile and sterile pollen grains in 5 visual fields of the microscope. In *F. tataricum,* due to a small flower size and complexity of anthers extraction from buds, in a drop of iodine solution on a glass slide there were dipped whole fresh-blown flowers with anthers opened.

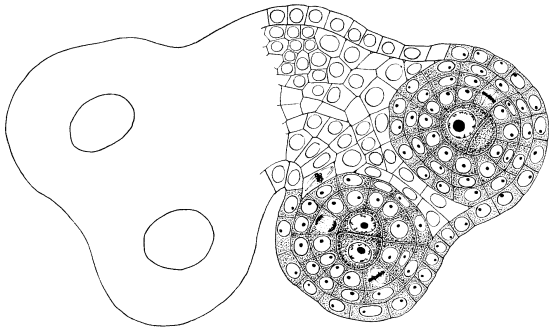
Data on the main meteorological parameters were provided by the meteorological station of Tatar Research Institute of Agriculture (vil. Bolshiye Kabany, Laishevsky district, Tatarstan, Russia). The results of all studies were processed using the software package of statistical and biometrics-genetic analysis in crop production and breeding AGROS [22].

RESULTS AND DISCUSSION

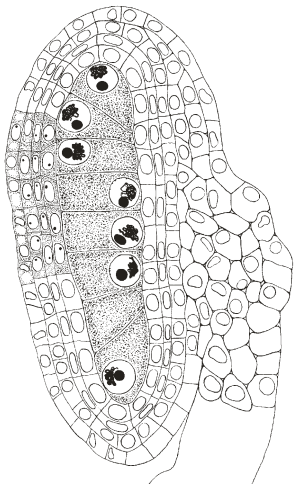
In all studied representatives of the genus *Fagopyrum Mill.* the anther was four-nested (Figure 1.1). Sporogenic tissue is located in a single layer in the anther's nest. The formation of anther wall in *F. tataricum* and *F. giganteum* is occurring as in *F. esculentum*, as in monocots. In mature anther the wall consists of four layers of cells. On the longitudinal section through an anther you can count the number of sporogenous tissue cells (Figure 1.2). This index in the family of *Polygonaceae Juss*., where belongs *Fagopyrum* genus, is considered systematically important at the species level [23, 24].

As can be seen from Table 1, the number of microspore mother cells, into which transform the cells of sporogenous tissue, for buckwheat averaged 10.9, which is considerably higher than of other species. For *F. tataricum* and *F. giganteum* this index was close, which makes sense, given that *F. tataricum* is one of the parental forms for *F. giganteum*. Calculation of Student's *t*-test [25] confirmed the significance of differences between the mean values of the parent cells number of microspores in the studied species.

Currently, there are several weight methods for assessing "pollen productivity" of the flower [26]. At the same time, knowing the amount of microsporocytes, the productivity of flower pollen can also be determined by calculation. Each microspore parent cell as a result of meiosis gives a tetrad (four) microspores, each of the latter in the further development process becomes a male gametophyte – pollen grain.

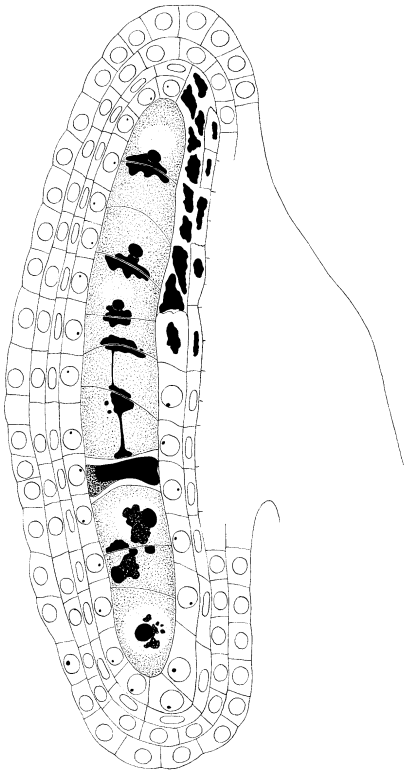


1.1



1.2

ISSN: 0975-8585



1.3 1.4

Fig. 1.1 - Cross-section through the anther of *F. esculentum.* The increase of 338 times.

Fig. 1.2 - Longitudinal section through the anther of *F. tataricum.* Microspore mother cells. Prophase I. The increase of 338 times.

Fig. 1.3 - Photo of a longitudinal section through 2 anther nests of *F. giganteum*. Among normal pollen grains there are visible smaller pollen grains. The increase of 420 times.

Fig. 1.4 - Longitudinal section through the anther of *F. esculentum*. Cytomixis. The increase of 338 times.

Knowing that in the anther of the studied species have 4 pollen nests, and in average the flower has 8 stamens, it is possible to calculate the efficiency of pollen as

$$N = n \times 4 \times 4 \times 8 = 128\ n,$$

where N - is a number of pollen grains formed in the flower, n - is the number of microspores mother cells in the anther nest.

Table 1: Number of microspores mother cells in the anther nest and pollen productivity of the species *Fagopyrum* flower

Index	*F. esculentum*	*F. tataricum*	*F. giganteum*
The number of microspore mother cells in the anther nest			
Mean ± error of the mean	10.9 ± 0.55	6.1 ± 0.18	5.9 ± 0.15
The minimum and maximum feature value	8…16	4…8	4…8
Pollen productivity of the flower			
Potential pollen productivity	2048	1024	1024
Average pollen productivity	1396.5	778.2	750.1

Using this formula, was calculated the average and maximum (potential) pollen productivity of the studied species (Table 1). The results of calculations for *F. esculentum*, fixed without taking into account the type of columnar flowers, were quite consistent with those of our predecessors [9, 10].

All three considered representatives of the genus *Fagopyrum* contain only one single ovule in the flower, therefore, p/o ratio [27] for *F. esculentum* is 1397 on average, for *F. tataricum* – 778, *F. giganteum* – 750. A high value of p/o ratio for *F. esculentum* confirms its tendency to xenogamy, relatively low values of p/o ratio for *F. tataricum* and *F. giganteum* indicates their greater tendency to autogamy. Buckwheat giant, though inherited the structure of heterostylous flowers from *F. cymosum*, is, however, self-fertile [28].

Our research has shown that the calculated potential of pollen productivity cannot be implemented in the Republic of Tatarstan. In the process of pollen formation from the time of the young anther primordium laying to ripening of pollen grains there are possible various deviations from the normal development. Previously detected irregularities in microspore and microgametogenesis in diploid varieties of *F. esculentum* [6] were also mostly detected in the other two species.

Table 2 shows the possible deviations from the normal process of pollen grains formation and their alleged effects. In all three members of the genus *Fagopyrum* were found pauses in the development of sporogenous tissues and violations in meiosis. The latter appear in the form of polyads with a different number of microspores and incomplete tetrads of microspores and micronuclei. As a result, the formed pollen grains have different sizes (Figure 1, 3). Very often violations of meiosis were observed in tetraploid sample K-108 *F. tataricum* and *F. giganteum*.

Cytomixis was found in the sporogenous tissue cells in seven of the nine previously studied diploid varieties of *F. esculentum* [6] (Figure 1.4). Cytomixis is proposed to be determined as phenomenon of cytoplasm part replacement with the nucleus or part of the nucleus from one cell to a neighboring [29]. It is believed that cytomixis is most typical for plants with disturbed homeostasis and, consequently, sensitive to various external influences. As the most significant cytomixis consequences is indicated the partial pollen sterility [29].

Another factor affecting the flowers pollen productivity is the variability in the number of stamens in the flower. Its value in the studied species of buckwheat can be seen from the data in Table 3.

Table 2: Violations during microsporogenesis and microgametogenesis and their implications

Description of developmental disorders	*F. esculentum*	*F. tataricum*	*F. giganteum*	The consequences of developmental disorders
The destruction of the sporogenous tissue cells before entry into meiosis and / or destruction of tapetum cells	+	+	+	Reduction of part or all of the anther
Cytomixis	+			Partial or complete pollen sterility
Violations during meiosis	+	+	+	
Tetrads with fully or partially destroyed microspheres		+	+	Reducing in the amount of pollen produced
Violation of the tapetum functions	+	+	-	

With the sign "+" are marked irregularities identified in the specific type repeatedly.

ISSN: 0975-8585

Table 3: Number of normally developed anthers (stamens) in flower and pollen fertility in the representatives of *Fagopyrum* genus

Index	*F. esculentum*		*F. tataricum*		*F. giganteum*
	Chatyr Tau (2n)	Medovaya (4n)	K-17 (2n)	K-108 (4n)	K-109 (4n)
Number of normally developed anthers (stamens) in flower					
Mean ± error of the mean	7.9 ± 0.03	8.0 ± 0.04	4.9 ± 0.05	6.0 ± 0.07	7.9 ± 0.02
The minimum and maximum feature value	4…10	3…10	3…7	3…8	6…9
Pollen fertility, %					
Mean ± error of the mean	67.1 ± 1.9	81.0 ± 1.3	81.5 ± 1.7	56.1 ± 2.0	55.2 ± 1.7
The minimum and maximum feature value	18.3…93.3	26.3…100	20.0…100	4.7…100	10.1…79.1

In addition to the increase and decrease in the number of stamens in all studied species there is often observed stamens reduction. We believe that this is an external demonstration of the sporogenous tissue development suspension. Such stamens when counting the number of stamens in the flower due to their sterility was not taken into account. Despite the identified variation in number of stamens the average value of the trait in *F. esculentum* and *F. giganteum* was very close to 8. In *F. tataricum* was also recorded only one flower with 8 normally developed stamens at K-108 sample. In all other cases there was observed partial (reduction of one theca) or complete reduction of stamens in the flowers.

The vast majority of cases, abnormalities touched stamens of the outer circle and very rarely the stamens of the inner circle, which provide contact pollination of flower stigmas. Thus, we observe a weakening of control over the development of five outer circle stamens in *F. tataricum*. Perhaps in the course of further evolution it will lead to reduction of stamens outer circle in this species. *F. tataricum* plants are self – pollinating, and calculated index of the average p/o ratio is clearly very high for autogamous species.

In all the species was discovered some percentage of sterile pollen (Table 3). The latter is usually different from the normal one by a small size, and at the tetraploid samples the differences in the sizes of fertile and sterile pollen were more obvious. The determination of pollen fertility was carried out in the phase of beginning of flowering. Despite the fact that all the samples were sown simultaneously, the passing rate of phenological phases was very different. For the varieties of *F. esculentum* the determination of pollen fertility was carried out on the 26th of June, for *K-17 F. tataricum* – on the 18th July, for K-108, K-109*,* and again for Chatyr Tau on the 25th of July. Since weather conditions in June and July were radically different from each other, it would be incorrect to compare the results of pollen fertility studies, obtained at different times. To assess the plants water availability at the moment of pollen formation process was determined Selyaninov hydrothermal coefficient (HTC) [30] for the two-week period preceding the date of pollen fertility evaluation. In 2 weeks preceding the date of pollen fertility evaluation in the cultivars Chatyr Tau and Medovaya, the HTC was 0.16, which corresponds to severe drought conditions. The HTC during the pollen formation in July in samples of *F. tataricum* and *F. giganteum* was, respectively, 2.77 and 1.82, which indicates the absence of drought and even excessive moisture.

Low values of pollen fertility in the cultivars of *F. esculentum* are explained by the drought in the time of the most important stages of microspore- and micro gametogenesis. Contrary to expectations, the fertility of diploid pollen varieties amounted to 67.1 %, while for tetraploid species it was significantly higher (Table 3). Some time ago there was the possibility [14] to obtain tetraploid forms with low-frequency violations in meiosis by repeated selection for productivity.

Obviously, Medovaya is one of these forms, which together with the relative drought tolerance allowed it to maintain the pollen fertility at a relatively high level in unfavorable conditions. Re-assessment of Chatyr Tau variety in the phase of early fruit formation (25 July), when conditions for plant growth were favorable, demonstrated pollen fertility at the level of 85.8 ± 1.29 %, which is significantly higher than in the phase of early flowering. Thus, weather conditions have a significant impact on *F. esculentum* pollen fertility.

F. tataricum and *F. giganteum* pollen formation took place under favorable conditions. In spite of this pollen fertility in the studied samples appeared to be low, especially in tetraploid samples (55-56%), which we explain by the frequent disturbances in chromosome segregation in meiosis.

SUMMARY

- Anther development in all three species was held in a similar manner. The studied species significantly differ in the number of microspores maternal cells in the anther nest. This indicator was the highest in *F. esculentum* allogamous species.
- By calculation there were determined the average and potential pollen productivity in the species studied, and the p/o ratio.
- There were found numerous violations in the formation of pollen, resulting in poor pollen productivity. In all three species was detected a pause in the development of sporogenous tissue and abnormalities in meiosis. Cytomixis was found only in *F. esculentum*.
- The number of normally developed stamens in *F. esculentum* and *F. giganteum* was close to eight, and for the samples of *F. tataricum* it was 4.9 and 6.0. In *F. tataricum*, there was noticed a weakening of control over the flower's outer circle stamens development due to the fact that in autogamous species there is produced more pollen than it is necessary for self-pollination.
- In all the three species was revealed a significant percentage of sterile pollen. Even in favorable for the growth and development of plants conditions, *F. tataricum* and *F. giganteum* tetraploid sample's pollen fertility did not exceed 56 %. For example, for *F. esculentum* was proved a significant impact of drought on pollen fertility.

CONCLUSION

Thus, in *F. esculentum*, *F. tataricum* and *F. giganteum* in the weather conditions of the Republic of Tatarstan due to the numerous violations in the process of microspore and microgametogenesis was observed a decrease in the number of emerging pollen and its fertility. As a result, the potential productivity of pollen is not realized to the full.

ACKNOWLEDGEMENTS

The work was performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

REFERENCES

[1] Fesenko I.N. Genetics of reproductive barriers and morphological differences between species of *Fagopyrum Mill* large-seeded groups. // Author. Diss. Dr. Sci. Biol. – SPb., 2013. 50 P.

[2] 2.Yamane K., Yasui Y., Ohnishi O. Intraspecific cpDNA variations of diploid and tetraploid perennial buckwheat, *Fagopyrum cymosum (Polygonaceae*) // American Journal of Botany. 2003. Vol. 90. N 3. P. 339–346.

[3] Krotov A.S., Dranenko E.T. Amphidiploid of buckwheat *Fagopyrum giganteum* Krotov sp.Nova // Bull. VIR. 1973. No.30. P. 41-44.

[4] Maryahina I.Y., Mikulovich T.P., Baleva S.V. Cytoembryological description of organogenesis stages of buckwheat in connection with heterostyly. // Morphogenesis of plants. – Moscow: MSU publishing house, 1961. - Vol. 1. - P. 301-304.

[5] Pausheva Z.P. Cytological and embryological features of buckwheat // Genetics of flower and the problem of compatibility in buckwheat. - M.: Nauka. 1988. P. 53-66.

[6] Kadirova L., Sitnykov A. Reproductive biology of buckwheat // Advances in buckwheat research: Proceedings of the 11th International Symposium on Buckwheat. – Orel, 2010. P. 331-340.

[7] Pausheva Z.P. Biological heterogeneity of pollen grains of buckwheat // Izvestiya TAA. 1960. Vol. 2. P. 207-213.

[8] Gorina E.D., Anokhina T.A. To breeding of buckwheat for precocity // Selection and seed production. 1980. No.2. P. 16-17.

[9] Doida, Y. Heterostyly and pollen grain number in buckwheat // Ann. Rep. Nat. Inst. Genet. 1958. № 9. P. 57-58.
[10] Fesenko I.N. The change of the index "number of pollen grains on the flower" during the transition to self-fertilization in buckwheat // Briefing of 2nd Congress of VOGiS. – SPb., 2000. - Vol. 1. - P. 162-163.
[11] Alekseyeva E.S., Bureyko A.L. Bee visitation, nectar and pollen efficiency of common buckwheat // *Fagopyrum.* 2000. Vol. 17. P. 77-80.
[12] Frolova S.L., Sakharov V.V., Mansurova V.V. Microsporogenesis in autotetraploid buckwheat (*Fagopyrum esculentum Mnch.)* and its fertility // bull. MOIP. The Department of biology. - 1946. - Vol. 51. - Ed. 4-5. - P. 114-125.
[13] Poddubnaya-Arnoldi V.A. Comparative-embryological study on diploid and tetraploid forms of buckwheat // Botanical journal. 1948. Vol. 33. No.2. P. 181-194.
[14] Tkachev A.T. The problem of fertility in autotetraploid buckwheat. Report 1. Meiosis and fertility of experimental autotetraploids in *Fagopyrum esculentum Moench* // *Fagopyrum*. 1990. Vol. 10. P. 95-122.
[15] Nizovtseva R.V. Microsporogenesis in buckwheat with a lack of water in the soil // XXII Gertsenovskiye chteniya. Natural Sciences (Mat. of Interuniversity conference). – L., 1970. - P. 32-35.
[16] Hirose T., Ujihara A., Kitabayashi H., Minami M. Interspecific cross-compatibility in *Fagopyrum* according to pollen tube growth // Breeding Science. 1994. V. 44. P. 307-314.
[17] Adhikari K.N., Campbell C.G. In vitro germination and viability of buckwheat (*Fagopyrum esculentum Moench*) pollen // Euphytica. 1998. V. 102. P. 87-92.
[18] Cawoy V., Lutts S., Kinet J-M. Osmotic stress at seedling stage impairs reproductive development in buckwheat (*Fagopyrum esculentum*) // Physiologia Plantarum. 2006. V. 128. P. 689-700.
[19] Chen Q.F., Hsam SLK, Zeller F.J. Cytogenetic studies on diploid and autotetraploid common buckwheat and their autotriploid and trisomics // Crop Science. 2007. V. 47. P. 2340-2345.
[20] Pausheva Z.P. practical work on Cytology of plants. – 4th ed., rev. and extra – M.: Agropromizdat, 1988. 271 p.
[21] Barykina R.P. Handbook of Botanical micro-technology. Foundations and methods. – M.: Publishing House of MGU, 2004. 312 p.
[22] The software package of statistical and biometrics-genetic analysis in plant breeding and AGROS. The version-2.08. Tver, 1999.
[23] Solntseva M.P. The family of *Polygonaceae* // Comparative embryology of flowering plants. *Phytolaccaceae – Thymelaeaceae.* – L.: Nauka, 1983. P. 53-58.
[24] Kamelina O.P. Systematic embryology of flowering plants. Bipartite. – Barnaul: publishing house of "ARTIK", 2009. 501 p.
[25] Lakin G.F. Biometrics. Guide for boil. spec. universities. – 4th ed., rev. and add. – M.: Visshaya Sch., 1990. 352 p.
[26] Antecology: program and instructional materials / Ed.: E.S. Ivanov, E.P. Pribylova; Yessenin Ryaz. state Univ. – Ryazan, 2008. – 28 p.
[27] Cruden R.W. Pollen-ovule rations: a conservative indicator of breeding systems in flowering plants // Evolution. 1977. Vol. 31. P. 32-46.
[28] Fesenko N.N., Fesenko I.N. New species of buckwheat – *Fagopyrum hybridum* // Vestnik of Orel state agrarian University. 2010. No.4. P. 78-81.
[29] Orlova I.N. Cytomixis // Embryology of flowering plants. Terminology and concept. Editor – T.B. Batygina. Vol. 1. – SPb.: «World and family», 1994. P. 115-117.
[30] Agricultural encyclopedic dictionary. – M.: Soviet encyclopedia, 1989. – 656 p.