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EMBRYOLOGICAL FEATURES AND SEED PRODUCTIVITY OF TARTARY BUCKWHEAT (Fagopyrum tataricum (L.) Gaertn.)

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ABSTRACT

Tartary buckwheat is a valuable crop and medicinal plant. Nevertheless, its embryological processes remain understudied. Meanwhile, such studies shed light on the reasons for the incomplete realization of the potential of its seed productivity and are a necessary prerequisite for the proper organization of genetic-selection activities. Objective of this research was to study megasporogenesis and gametogenesis, fertilization and embryonic processes in Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn. and their relationship with the seed productivity of plants. The comparative embryological and comparative morphological methods of research were used. It was shown that *F. tataricum* has an orthotrophic, bitegmic, crassinucellar ovule, a *Polygonum*-type embryo sac, porogamic premitotyc fertilization, a nuclear endosperm, a large differentiated embryo with two folded cotyledons, a point of stem growth and an embryonic root. The examination of tetraploid sample of *F. tataricum* revealed abnormalities during embryological processes; as a result, it was behind its diploid analogue in terms of potential and real seed production, fruit setting, and the number of inflorescences formed. The embryological processes in *F. tataricum* and *F. esculentum* Moench in general proceed in a similar way, however, the potential of seed productivity in *F. tataricum* due to self-pollination is realized much more completely than in *F. esculentum*.

Keywords: Tartary buckwheat, common buckwheat, embryological processes, ovule, megasporogenesis, embryo sac, fertilization, seed productivity.

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INTRODUCTION

Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn. is the second most important type of buckwheat after *Fagopyrum esculentum* Moench. Tartary buckwheat is cultivated mainly in the high mountainous regions of Asia [1]. Sprouts and flour obtained from the seeds of Tartary buckwheat, due to their useful properties are used as a basis for functional foods [2, 3]. In connection with the high content of a number of flavonoids in buckwheat plants they are characterized by high antioxidant activity. In addition, buckwheat flour is rich in vitamins, microelements, proteins with a balanced amino acid composition, dietary fiber and polyunsaturated fatty acids [4, 5, 3]. Increasing attention is being paid to the medicinal properties of buckwheat in the treatment of several chronic diseases [6].

Work on the selective improvement of Tartary buckwheat has been carried out since the 90s of the 20th century. The main disadvantage of the culture is that it has a tightly adhering husk that is hard to remove, a low kernel percentage in the seeds, as well as a bitter taste of cereals. The advantages over common buckwheat are its higher productivity, resistance to temperature and other stress, and also the ability to self-pollination [7]. Low yield of Tartary buckwheat in comparison with grain crops and non-uniform maturity of seeds is an obstacle to the spread of this crop [8].

The family *Polygonaceae* Juss., which includes *F. tataricum*, has been well studied from the point of view of embryology. The same can be said for buckwheat *F. esculentum* [9, 10, 11]. In our earlier studies, we described the development of the male reproductive sphere of the flower in *F. tataricum*, the observed developmental disorders and their effect on the amount and fertility of the emerging pollen [12, 13]. Despite the high economic value of Tartary buckwheat its embryological processes remain understudied. Meanwhile, similar studies shed light on the reasons for the incomplete realization of the potential of its seed productivity and are a necessary prerequisite for the proper organization of genetic-selection activities.

Objective of this research was to study megasporogenesis and gametogenesis, fertilization and embryonic processes in Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn. and their relationship with the seed productivity of plants.

RESEARCH METHODS

Samples K-17 (2n), K-108 (4n) *F. tataricum*, obtained from the All-Russian Research Institute of Plant Growing (St. Petersburg, Russia) were investigated. They were sown in the fields of the Tatar Scientific Research Institute of Agriculture (Laishevsky district, Republic of Tatarstan, Russia) in the collection nursery, the area of plant nutrition was 30×10 cm.

In summer 2014-2015, inflorescences, flowers and seeds of Tartary buckwheat were fixed in Chamberlain solution. Subsequently, permanent preparations were prepared from them in the laboratory using the standard cytoembryological technique [14, 15]. The section thickness was 14 μm. The preparations were stained in Heidenhein and Delafield's hematoxylin and hemalaun. The resulted preparations were studied under a microscope.

The following indicators of seed productivity of plants were determined [16]: potential seed productivity (PSP) of samples, percentage of fruit setting (S_{fr}), actual seed productivity (ASP) and productivity index (I_{pr}). All parameters were evaluated for the first-order branch (the sample size was 50 first-order branches for each sample) and for the individual (total 10 plants analyzed). Plants for analysis were randomly selected in the phase of complete ripeness. Statistical data processing was carried out using AGROS selection-oriented software package [17].

RESULTS AND DISCUSSION

The studied embryological processes were generally similar in the diploid and tetraploid samples. The description is given on an example of a diploid sample K-17.

The flower of Tartary buckwheat is characterized by lysicarpous gynoecium, formed by three carpels. The pestle consists of ovary, three styles, ending with head stigmas. Gomostylous.

The ovule of Tartary buckwheat is orthotropic, bitegmic, crassinucellar, located on a short funiculus (Figure 1.1). Nucellus with a massive lateral and halazal zone. The internal integument is two-layer, expanding in the micropyle region to three layers. The outer integument is somewhat shorter than the inner, two-layered over the entire length. Its outer epidermal cells are large, with deposited tannins. Direct micropyle shape is formed by the internal integument. Hypostasis is massive, multi-row, cup-shaped, with cells filled with tannins. Funiculus is straight, short. The conduction bundle approaches the hypostasis.

At the early stages of development, an archesporail cell is formed subepidermally at the ovule apex. This happens when the growing carpels have not yet outgrown the tip of the ovule. The archesporail cell is divided periclinally with the formation of a sporogenic and parietal cell. The latter is divided further anticlinally. At the same time, the internal integument starts its formation.

In the process of further development, the sporogenic cell turns into a megasporocyte. The thickness of parietal tissue reaches more often one, rarely two layers of cells. This stage corresponds to the moment when the outer integument is set.

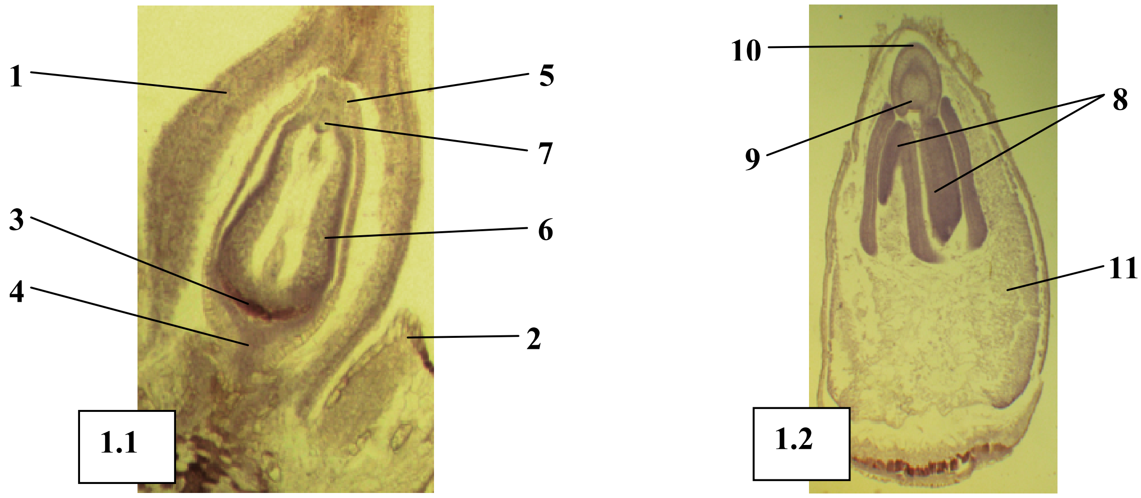

Fig.1. F. tataricum ovule and seed:

1.1 – the structure of the ovule; 1.2 - seed on the longitudinal section: 1 - ovary wall, 2 - nectary, 3 - hypostase, 4 - funiculus, 5 - integuments, 6 - ovule nucellus, 7 - embryo sac, 8 - cotyledons, 9 - stem growth point, 10 - germinal root, 11 - endosperm

As a result of the meiosis of the megasporocyte, a linear tetrad of megaspores is formed. Meiosis proceeded without abnormalities. Halazal megaspore is functional.

Tartary buckwheat has *Polygonum*-type embryo sac. A mature embryo sac is seven-celled, eight-nuclear. An egg cell is large, pear-shaped. Synergids are normally differentiated. Three single-nuclear antipodes, located linearly, are retained for a long time. A central cell is oblong, strongly vacuolated. The polar nuclei merge before fertilization. The secondary nucleus of the central cell in the mature embryonic sac is located near the egg cell. The process of megagametogenesis is accompanied by the closure of the internal integument over the nucellus and the formation of the micropyle.

The diploid sample K-17 had no developmental abnormalities found in the female reproductive sphere. The tetraploid sample K-108, had abnormalities in megagametogenesis recorded at the stage of the binuclear and tetranuclear embryo sac (Figure 2.1), as well as cases of ovule degeneration (Figure 2.2).

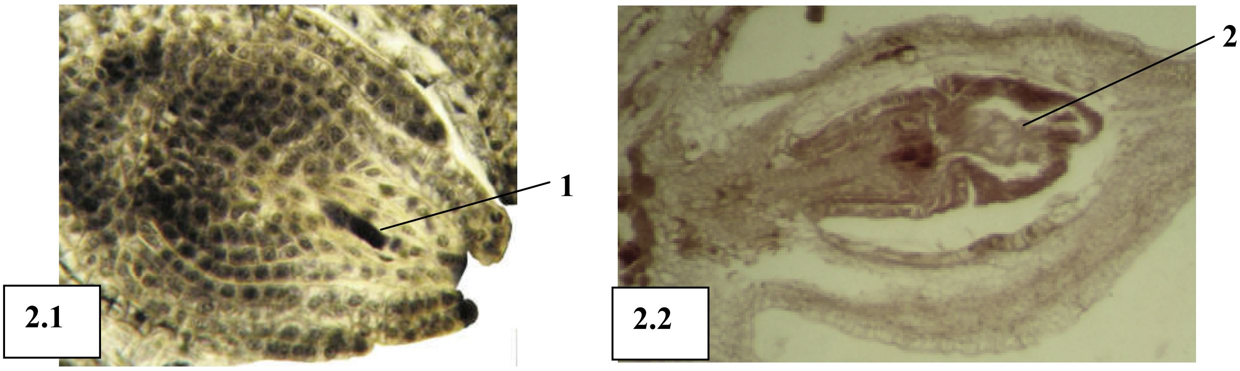

Fig.2. Abnormalities during embryological processes in *F. tataricum*:
2.1 - abnormal megametagenesis at the stage of the binuclear embryo sac, 2.2 - degeneration of the ovule. 1 - collapsing binuclear embryo sac, 2 - collapsing ovule

F. tataricum is characterized by contact autogamy. After the pollen grains fall on the stigma of the pistil, the germination of the pollen tubes begins. Having passed the conductor path of the pistil, the pollen tube penetrates into the ovule through the micropyle (porogamy). Only one pollen tube usually penetrates into the embryo sac, and ends its growth in one of the synergids. In the synergid, which the pollen tube has entered, the disintegrating nucleus of the synergide and the nucleus of the vegetative cell are found.

Double fertilization. As a result of the fusion of an egg cell with sperm, a zygote is formed, from which the embryo subsequently develops, giving rise to a new sporophyte. For *F. tataricum*, a premitotic type of karyogamy is characteristic. After the fusion of the nuclei of the egg cell and sperm the second nucleolus is released in the nucleus of the zygote.

The fusion of the secondary nucleus of the central cell with the second sperm leads to the formation of a triploid primary endosperm nucleus. It begins to divide before the zygote. The endosperm is nuclear; subsequently the process of cytokinesis begins gradually on a part of the micropyle, and the endosperm becomes cellular.

In the center of the filling buckwheat seed a large embryo is localized, surrounded by endosperm cells (Figure 1.2). It is distinguished by the presence of large folded cotyledons. Between the bases of the cotyledons there is a point of growth of the embryo, at the opposite end of the embryo's body a germinal root is laid, oriented toward the micropyle. Around the embryo there is a zone of destroyed endosperm cells.

The conducted study showed that, in general, the course of the investigated embryological processes is similar in the two crop species of buckwheat. Abnormalities in the development of the female reproductive sphere, described for *F. esculentum* [18], were not found in the study object. At the same time, *F. tataricum* had abnormalities found, not typical for common buckwheat. A direct consequence of abnormalities in the female reproductive sphere is a decrease in the seed productivity of the plant.

Synflorescence of Tartary buckwheat consists of florals units of the main shoot and parakladia (branches) of the first, second and subsequent orders. Inflorescences are represented by open-air bracteose thyrsuses. Lateral branches of the thyrsi are multilevel monochasiums.

The study of the morphology and seed productivity of first-order branches showed (Table 1) that the differences between the samples for all the traits studied are reliable [19]. The number of thyrsuses on the branches of sample K-17 is much higher than that of K-108. Each monochasium in diploid plants has on average 4 flowers set, in tetraploid plants – 3 flowers. PSP for the sample K-17 is 3 times higher than for the sample K-108. In the diploid sample, the rest parameters of seed productivity studied were also significantly higher.

The results of the evaluation of the parameters of individuals in the samples studied are presented in Table 2. Analysis of the data obtained showed that the sample K-108 is behind the diploid in terms of the number of thyrsuses on the plant and their average length. Differences in accordance with the last feature and all the parameters of the seed production of the individual studied were significant and confirmed statistically. Thus, a larger number of ovules is set on the plants of the diploid specimen, and they successfully develop in complete seeds.

Table 1. Parameters of first-order branches in the samples of *F. tataricum*

Characteristic	Average ± error of mean	
	K-17	K-108
Number of thyrsuses	8.7±1.0*	4.0±0.4*
Number of monochasiums	34.9±4.8*	16.0±1.6*
PSP	158.5±21.2*	51.5±5.6*
S_{fr}, %	55.6±2.3*	31.4±2.2*
ASP	34.4±4.9*	5.2±0.8*
I_{pr}, %	20.0±1.4*	9.0±1.2*

The sign "*" in this document shall mean average values of the characteristics with reliable differences.

Table 2. Average values of the reproductive characteristics in the samples of *F. tataricum*

Characteristic	Average ± error of mean	
	K-17	K-108
Number of thyrsuses	62.0±16.4	27.4±4.9
Average thyrsus length, cm	3.7±0.4*	2.8±0.2*
PSP	1,174.3±359.2*	369.4±75.7*
S_{fr}, %	59.1±3.3*	33.9±3.2*
ASP	270.3±82.6*	35.0±1.0*
I_{pr}, %	21.5±2.4*	9.0±1.4*

The obtained data allow making interesting comparison of the signs of the reproductive sphere of plants and the indicators of seed productivity between the crop species of buckwheat (Table 3). Thus, the number of thyrsuses on the plant varieties of common buckwheat, according to different data, is 17.5 [23], from 15.5 to 21.8 [24], which is much less than the data obtained for the samples of Tartary buckwheat. Thyrsus in common buckwheat was much denser: each centimeter of the thyrsus has 5.6 to 6.9 monochasiums [23, 24]. The same indicator in Tartary buckwheat for K-17 was 1.1 un/cm, for K-108 – 1.6 un/cm. In addition, the thyrsus in common buckwheat is more branched: the thyrsus of a diploid variety had 7 monochasiums [23], tetraploid – 9 monochasiums [24], the variety of Tartary buckwheat – total 4-5 monochasiums.

Table 3. Morphology of reproductive organs and parameters of seed productivity in *F. esculentum* and *F. tataricum*.

Characteristic	*F. esculentum*		*F. tataricum*	
	2n	4n	2n	4n
Number of thyrsuses in a plant	17.5 [23]	15.5-21.8 [24]	62.0	27.4
Thyrsus density, un/cm	6.9 [23]	5.6-6.1 [24]	1.1	1.6
Number of monochasiums per thyrsus	7 [23]	9 [24]	4-5	4-5
S_{fr}, %	16.4 [22]	8.8 [22] 22.0-24.3 [24]	59.1	33.9
I_{pr}, %	4.4-8.2 [18]	1.5-2.0 [24]	21.5	9.0

Since the index values of seed productivity are less variable, they are more convenient for comparative analysis than the PSP and ASP. Indicator S_{fr} was 59.1% for Tartary buckwheat, and 33.9% for common buckwheat: for a diploid sample – 16.4%, for tetraploid samples – 8.8% [22], 22.0-24.3% [24]. I_{pr} in diploid common buckwheat was 4.4-8.2% [18], in tetraploid – 1.5-2.0% [24]. I_{pr} in diploid Tartary buckwheat was 21.5%, in tetraploid – 9.0%. Thus, both index values were significantly higher in *F. tataricum* as compared with *F. esculentum*. This is due to the different types of pollination in the species being compared: the pollination and fruiting processes in the autogamous species *F. tataricum* are more prosperous and productive than in the allogamous species *F. esculentum.*

SUMMARY

1. *F. tataricum* has an orthotrophic, bitegmic, crassinucellar ovule. Tetrad of megaspores is linear. An embryo sac is of *Polygonum*-type. The tetraploid sample K-108 has abnormalities found at different stages of formation of the female gametophyte, as well as cases of degeneration of the ovules.
2. Fertilization of *F. tataricum* is porogamic. Type of fertilization is premitotic. The endosperm is nuclear. A large differentiated embryo has two folded cotyledons with a stem growing point between them and an embryonic root.
3. The number of developing thyrsuses and monochasiums, the potential and actual seed productivity, the fruit setting and the productivity index are significantly higher in the first-order branches of the diploid sample *F. tataricum* in comparison with the tetraploid sample.
4. Plants of the diploid sample *F. tataricum* significantly exceed the plants of the tetraploid sample in the average length of the thyrsus and the studied parameters of seed productivity.

CONCLUSION

Thus, abnormal development of the female reproductive sphere lead to a decrease in the seed productivity of plants, which was clearly demonstrated by the example of the tetraploid sample *F. tataricum*. The embryological processes in *F. tataricum* and *F. esculentum* generally proceed in a similar way. *F. tataricum* plants produce more friable low-branched thyrsus-inflorescences as compared with *F. esculentum*. The potential of seed productivity in *F. tataricum* due to self-pollination is realized much more completely than in *F. esculentum.*

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