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Seed productivity of Rosa oxyodon Boiss

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Abstract. This article presents the results of the study examining the pollination process and seed reproduction in the sharp-toothed wild rose (Rosa oxyodon Boiss.) endemic to the Caucasus. The objects of the study are natural populations of the studied species. To study the processes of pollination, loose buds were isolated with flesilin bags using the method of I.A. Shantser. When studying the germination of R. oxyodon seeds, various sowing options were carried out on the Gunib Plateau: 1) sowing with seeds collected from fruits of different degrees of maturity in August, September, and October in open ground; 2) spring sowing with seeds collected from mature fruits after stratification, scarification and without them. In each variant, 100 seeds were sown. The aim of the study is to examine the reproductive characteristics of R. oxyodon endemic to the Caucasus. Our studies demonstrate that zero percent of R. oxyodon fruits form through self-pollination and geitonogamy. In its natural environment, the species yields enough seeds for potential seed reproduction of a population. Our findings revealed that R. oxyodon is an obligate self-incompatible entomophilous species with low productivity coefficient but high fecundity potential, which guarantees stable seed reproduction of the species. R. oxyodon seeds are characterized by mixed and long dormancy period.

1 Introduction

The genus Rosa L., according to various taxonomists, includes from 190 to 500 species distributed exclusively in the North. There are many reasons that make it difficult to distinguish between species and create a stable system of the above-named genus. This is due to weak morphological and genetic differences between species, intraspecific polymorphism, the ability of many species to hybridize, the prevalence of polyploidy, and the presence of balanced heterogamy. The foregoing fully applies to the species Rosa oxyodon Boiss. Rosa oxyodon is a morphologically variable species [1, 2] endemic to the Caucasus. To date, in the flora of Dagestan, there are 46 species of wild rose. According to the influence on the formation of the general phytogeographic spectrum of the wild rose flora, the dominant geoelements form the following descending series: caucasian - boreal - xerophilic - steppe. The wild rose flora is a conglomeration of species of different origin, the main core of which is Caucasian and Dagestan species [2]. An important problem remains the conservation of the biodiversity of the flora of the territories with the participation of populations of endemic plants adapted to certain conditions of existence and constituting a vulnerable part of regional

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floras. R. oxyodon is a 1,50-1,80 m tall shrub that grows and forms clumps in the mountainous regions of Dagestan, on forest edges, mountain slopes, along roads and in abandoned terraced areas across the middle and upper elevation zones [3, 1, 4]. This species is attributed to the mesophilous phytocenoses on the thick and moderately developed soils. Rosa oxyodon differs in complex biosystematics and strong variability in all morphological characters. This species is included in the ancient group of related species R. oxyodon Boiss. sl, including R. oxyodon, R. prokhanovii Galushko, and R. sosnovskyana Tamamsch., is characterized by ecological plasticity and vitality, which makes it an interesting object for population and bioecological studies [1, 2]. Like other wild rose shrubs, R. oxyodon shrubs form a peculiar system of aerial (vegetative, generative and old) shoots and xylopodia – a basal part of a stem attached to a lignified hypocotyl [5]. In the general structure of the R. oxyodon clump, it is rather difficult to distinguish individual bushes, and the boundaries of such individuals have not been methodically determined. The vegetative mobility of bushes and high fertility potential with a low seed production coefficient are the basis of the spatiotemporal dynamics of R. oxyodon populations. Thus, conducting comprehensive studies of the reproductive potential of R. oxyodon in the conditions of Dagestan is relevant.

Modern botany pays much attention to research on reproduction of flowering plants, especially seed reproduction, which is a multistage, multifactorial and self-regulated process [6, 7, 8, 9]. Seed reproduction can occur through both sexual reproduction and apomixis [10], (xenogamy) is characteristic of the vast majority of Rosa species; however, self-pollination and apomixis occur in a number of species of this genus as well [11, 12]. Studies of reproductive systems of various Rosa species show that cross-pollination results in the highest number of nutlets, whereas self-pollination and apomixis yield a considerably lower number of nutlets [13].

The aim of the present study was to examine the reproductive characteristics of R. oxyodon. In this regard, determining the type of pollination and productivity coefficient of R. oxyodon are the objectives of the study.

2 Methods

The objects of the study were natural populations of R. oxyodon that grow on the Gunib Plateau (1620 m.a.s.l.); 10 quadrats measuring 1m2 were placed along transect lines over each sampling area.

To study self-fertility and self-sterility, loose buds were isolated by means of pouches made out of interfacing fabric (non-woven fabric made from cellulose fibers) using Shancer's method [12]. The experiment was carried out in three ways:

1) in order to study the ability to self-pollinate, R. oxyodon buds were isolated with the use of pouches made out of interfacing fabric. Three plants were used for this purpose, with 10 buds being isolated on each one of them;
2) in order to study geitonogamy (pollen is transferred from one flower to a stigma of another flower on the same plant), sepals, petals and stamens were removed from the buds, while stigmas were pollinated with pollen from the same R. oxyodon plant; next, the fertilized buds were isolated using the pouches. The total of 30 buds was isolated;
3) in order to study the efficiency of cross-pollination, 30 buds were stripped of their sepals, petals and stamens, while stigmas were pollinated with pollen from another plant, after which the buds were isolated;
4) the control sample included another 30 buds, which were marked beforehand.

In the process of studying germination capacity of R. oxyodon, we used different methods of sowing on the Gunib Plateau: 1) sowing seeds from rose hips of various degrees of maturity in open ground in August, September and October; 2) using scarified, stratified and normal seeds from mature rose hips to sow them in spring. Each method involved planting 100 seeds.

The weight and size measurements were obtained through “Ohaus” digital scale accurate to within 1 mg and the calipers accurate to within 0,1 mm respectively. The program STATISTICA 5.5 was used for statistical data analysis.

3 Results

To study the processes of pollination, experiments were carried out at the end of May on the Gunib plateau. During self-pollination, flowers were isolated on three plants, 10 buds each. Self-pollination did not result in fruit set. When studying the processes of geitonogamy, 30 buds were pollinated and isolated on one bush. In this variant, the formation of fruits was also not observed. While experiments on artificial cross-pollination and free pollination in nature have led to R. oxyodon fruit set. In each of these variants, 30 buds were initially isolated and marked. In the case of artificial cross-pollination, 26 fruits were formed, with free pollination - 28. Our isolation experiments on R. oxyodon buds resulted in zero percent of fruits forming through self-pollination and geitonogamy. Hips in the control sample developed normally. These results demonstrate that R. oxyodon is an obligate self-incompatible and cross-pollinating species. I. A. Shantser obtained same results with R. majalis Herrm. and R. donetzica Dubovik, which are closely related to R. oxyodon.

One of the conditions needed for seed reproduction is the ability of a plant to produce viable seeds. Potential seed productivity (PSP) is the maximum amount of seeds that a plant can produce in a given time period, provided that all ovules develop into mature seeds [14, 15, 16]. Being one of the main characteristics of seeds reproduction [17], PSP depends on the following factors: the number of generative shoots of a plant, the number of flowers per shoot, the number of ovaries of a flower and the number of ovules in an ovary.

The number of ovules per rose hip (an accessory fruit) in R. oxyodon amounted to 38+1, while the number of rose hips per shoot amounted to 18+3. Potential seed productivity does not directly affect seed reproduction, since only fully developed seeds are able to germinate. The number of fully developed seeds determines real seed productivity (RSP), which amounted to 7+1 seeds per fruit in relation to R. oxyodon. In our calculation, RSP is characterized by a very high coefficient of variation — 67,3%, whereas PSP varies at 19,9% (Table 1).

Table 1. Seed productivity of R. oxyodon.

	Potential seed productivity	**Real seed productivity**	**Productivity coefficient, %**
$\bar{x} \pm s_{\bar{x}}$	38,0±1,03	7,0±1,05	18,0+1,02
CV,%	19,9	67,3	58,0

PSP is a parameter genetically determined quite clearly. RSP is an ecological parameter that represents a combination of stochastic and energy processes, which co-occur with anthesis and fruit development [17, 18, 19]. Thus, high variability level of real seed productivity is likely a result of a large number of factors that control seed development: sufficiency of pollination, the amount of resources used for seed development, vitality of a specimen, the amount of time it takes for a seed to mature, herbivore activity etc. [10, 17]. The high level of variability of the trait allows the assumption that the trait is under strong selection pressure. The number of carpels and ovules, on the other hand, is strictly determined by genotype [20, 21]. A number of researchers [22, 23] found that one of the reasons of lower seed productivity is abnormalities in embryonic development. In some cases, externally normally developed nuts may not be viable due to underdevelopment or premature degeneration of the embryo. Such nuts contain an air-filled cavity instead of an embryo. The variation of the studied signs of R. oxyodon is a property of the species, reflecting a certain range of responses to external influences. These reactions are considered as adaptive, allowing to ensure the successful reproduction of an individual and, consequently, a high degree of reliability of the preservation of the species. To give a more complete characterization of R. oxyodon, we used productivity coefficient, which shows the ratio of real seed productivity to potential seed productivity expressed as a percentage [10, 24]. The productivity coefficient of the studied population of R. oxyodon is equal to 18%. In woody plants and herbaceous plants the productivity coefficient does not exceed 32,7% and 57,2% respectively [25]. Low productivity coefficient is caused by the following factors: pollination deficit, resource scarcity, damage caused by herbivores and diseases [10]; they depend on abiotic and biotic factors in a complex way.

One generative shoot of R. oxyodon yields 18+3 fruits on average (Table 2), RSP is 7 seeds per fruit.

Table 2. Yield and real seed productivity on the Gunib Plateau.

Density of generative shoots per m^2	The average number of fruits per shoot, pcs.	Real seed productivity per m2
4,2$\pm$0,44	18,0$\pm$3,11	538,02

It is possible that the cause of such low RSP value is the hybrid nature of R. oxyodon. In previous works was made to study the genetic relationships of R. oxyodon with related species R. pendulina, R. majalis, and R. donetzica by sequencing the sequence of the trnL-trnF chloroplast spacer [2]. The sample size for chloroplast DNA analysis was 48 samples. DNA isolation was carried out by the STAB method from dried leaves. In all studied samples, the sequence of the chloroplast intergenic spacer trnL-trnF was sequenced. The sequenced sequences of the trnL-trnF chloroplast spacer were manually aligned using the BioEdit v7.0.5 program. Data analysis was carried out using the statistical parsimony method implemented in the TCS v. 1.21. The analysis included 83 sequences. The alignment length was 293 positions. As a result of the work done, 18 haplotypes were identified, 7 of which were absent among the samples and were hypothetical extinct. The remaining haplotypes were distributed as follows:

1) R. pendulina: A, B, C, D, E;
2) R. majalis: F, L, M;
3) R. oxyodon: A, F, G, H, I;
4) R. donetzica: E, F.

R. oxyodon is characterized by haplotypes A and F, genealogically distant from each other and occurring in R. pendulina, R. majalis, and R. donetzica, respectively. Such a distribution of haplotypes in the three species under consideration, including disjunctions in the distribution of haplotype A, suggests the hybrid origin of R.oxyodon as a result of multiple reciprocal (at least two) hybridizations between R. pendulina and R. majalis [2].

The density of generative shoots per square meter in Gunib population averaged 4,2, with RSP from this area amounting to 538,02 seeds (4,2*18,0*7,0). In its natural environment, R. oxyodon yields enough seeds for potential seed reproduction of a population.

Real seed productivity is an integral parameter that includes, among other factors, seed quality, which is determined by germination capacity of seeds and their viability [17]. R. oxyodon seeds, as seeds of other Rosa species, have a form of single-seeded triquetrous indehiscent nutlets surrounded by a thick lignified layer with tightly sealed pores containing a large embryo and no endosperm [26]. The seeds are characterized by long dormancy period, which can last for as long as two or more years [27, 28].

The timing of harvest is known to be one of the main conditions that determine germination speed of seeds. Hrzhanovskij V.G. [3] believes that rose seeds germinate the fastest if harvested during the period when rose hips turn brown. It is during this period that seeds reach full maturity and seed coats are still not hard as in the later stages of maturity. To evaluate the quality of seeds taken from fruits of varying maturity degrees, we conducted experiments applying various methods of seed pre-treatment (Table 3).

Table 3. Germination of seeds under different methods of pre-sowing treatment.

Stratification and heat treatment	**Germination capacity %**	**Acid treatment without stratification**	**Germination capacity %**	**No treatment and stratification**	**Germination capacity %**
Freezing seeds for 9 days at -15^{0}C	15	5% solution of H_2SO_4 for 30 minutes	1	Seeds from half-mature fruits	4
Treating seeds with boiling hot water after freezing them for 9 days at -15^{0}C	4	10% solution of H_2SO_4 for 30 minutes	12	Seeds from mature fruits	0
Boiling hot water treatment	15	5% solution of HCl for 30 minutes	33	Half-mature fruits	0
Stratification without heat treatment (control)	0	10% solution HCl for 30 minutes	0	Mature fruits	0

When R. oxyodon seeds extracted from half-mature fruits were sown in summer without pre-treatment, first scattered seedlings emerged in 18 months, while the germination capacity amounted to 4%. Half-mature whole fruits planted in summer developed no seedlings. When planting seeds extracted from mature fruits, we applied various methods of pre-sowing treatment, as R. oxyodon seed coats significantly affect embryo activity [3]. Moreover, the condition of an embryo is also determined by physiological factors [26]. First of all, we had to get rid of the physical barrier created by a seed coat, which prevented seeds from germinating. In order to achieve that, we used heat and acid treatments (Table 3). The presented data show that R. oxyodon germination capacity is relatively low and ranges from 0% to 33%.

Before stratification, R. oxyodon seeds were subjected to various treatments, which increase germination capacity. One of the reasons of low germination capacity in our case is a strong inhibitory effect of a pericarp, which can be eliminated through varying temperature and acid treatments. The longest R. oxyodon seeds were stratified was 16 months: 4 months in a refrigerator at +50C and 12 months in sand, in natural conditions. Such long period of stratification, according to Nikolaeva M.G. [26], indicates a mixed dormancy, which combines physical and endogenous (physiological) types of dormancy. The obtained result is consistent with literature data [24, 28].

The obtained results demonstrate that the most effective method of pre-sowing treatment of seeds is scarification with 5% solution of HCl for 30 minutes.

4 Conclusion

The results of studying the pollination process of R. oxyodon allow us to define it as an obligate self-incompatible entomophilous species. It was found that the studied species is characterized by low productivity coefficient but high fecundity potential, which guarantees stable seed reproduction of the species. Seeds of R. oxyodon are characterized by mixed and long dormancy period. The most effective method of pre-sowing treatment is scarification with 5% solution of HCl for 30 minutes (33%).

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