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## To the methodology for the study of intraspecific variability and selection of wild forms of *Viburnum opulus* L.

The methodological recommendation for the study of intraspecific variability and the selection of wild-growing promising for breeding forms of common *Viburnum* written on the basis of a large amount of factual material obtained from the study of this species in the East Kazakhstan region. It includes an assessment of the current state of *Viburnum opulus* L. populations, with range mapping in ArcMap and ArcGis programs. The geobotanical, phytocenotic description is given. First, the forms are described according to 25 quantitative and qualitative features. Then, on the basis of this, the selection of promising forms, the collection of seeds and cloning with green cuttings is carried out. The distribution of *Viburnum* plants occurred according to the degree of flowering, fruiting, fruit length and diameter. The data were divided into classes with the smallest, average and largest values into five groups, by weight and number of fruits into four groups. The scoring system was applied to seven characteristics: the general condition of plants, winter hardiness, the degree of flowering and fruiting, color, taste, and the nature of the detachment of fruits. The study of endogenous variability of morphological and economic-biological characters is carried out in the crown of one individual. After studying the average indicator of the trait and the amplitude of its variation for different individuals, they proceed to the study of intra-population variability. After mathematical processing, a questionnaire is filled out for each plant according to the selected form, which will make it possible to better represent the studied form to study genotypic and phenotypic variability in the future. The use of this recommendation in research work will allow assessing the genetic potential of the species, the level of its intraspecific variability, and selecting promising forms.

*Keywords:* distribution, quantitative characteristics, degree of flowering, fruits, color, variability.

### Introduction

A comprehensive study of fruit and berry plants in natural habitats is essential for solving issues of ecology, phytocenology, floristics, population biology, and nature conservation.

In the programs for breeding and variety study of fruit and berry crops, the main attention is paid to such leading fruit and berry crops as apple, pear, plum, cherry, apricot, black currant, strawberry (1980). There are several methods for studying rare fruit and berry plants: sea buckthorn [1, 2], blueberries, cranberries [3, 4].

Common viburnum – *Viburnum opulus* L. is the most interesting in terms of studying variability and introducing into cultivation among wild-growing species of fruit and berry plants growing on the territory of the East Kazakhstan region. The advantages of *viburnum vulgare* include high winter hardiness, productivity and usefulness of fruits. Due to its medicinal properties of all parts the plant is widely used in medicine. Its fruits have the richest chemical composition. They contain up to 8% sugars, mainly glucose and fructose, up to 3% tannins. Viburnum bark, which contains up to 4% tannins, has an exceptional therapeutic effect on the human body and most importantly, viburin glycoside, which has a pronounced vaso-constrictive effect [5].

The genus *Viburnum* L., belongs to the *Adoxaceae* family, order *Dipsacales*, includes about 140 species. Common viburnum (*Viburnum opulus* L.) is a multi-stemmed shrub, 1.9 – 4.5 m high with brown-gray bark and smooth light young branches. Buds with two fused outer scales, ovoid, slightly pointed, reddish green. The bushes are multi-stemmed, their number reaches 35 pcs. The leaves are broadly ovate, rounded, 6–13 cm long, 5–11 cm wide, with 3, rarely 5 large-toothed lobes. The middle lobe is larger, with parallel, often elongated lateral margins, shortly pointed at the very apex or with a more or less elongated point. The upper side of the leaves is bare; the lower side is bare or pubescent. *Viburnum vulgare* is a cross-pollinated, anemophilous plant that blooms in late May – early June. White and pink-white flowers are collected in large inflorescences – umbellate corymbs up to 8 cm in diameter, along the edges of the inflorescence there are larger sterile flowers (1.8-2.5 cm) in diameter with white petals. The flowers are heteromorphic, with a double perianth, collected in flat umbellate 6-8- ray panicles, 4.9-7.5 cm in diameter, on a peduncle 2.5-5.0 cm long, marginal flowers on pedicels 1-2 cm long, barren, flat, white, 4-5 times larger than internal, median –

bisexual, sessile or almost sessile, white or pinkish-white, shortly bell-shaped, about 5 mm in diameter. Inflorescences are located at the tops of young branches. The fruit of the viburnum is a bright red spherical drupe 8.3-10.3 mm long and wide, with one large flat stone inside, ripens in September – early October.

*V. opulus* is undemanding to growing conditions, easily tolerates drought and frost, and is most common in the temperate climate of Europe and Asia. It grows in the undergrowth and along the edges of moist deciduous and mixed forests, in tree and shrub thickets along the banks of springs, rivers, lakes, swamps, ravines, gorges and mountain slopes. It grows everywhere in the European part of Russia (except the north and southeast) in Eastern and Western Siberia, the Caucasus, Central Asia, Western Europe, Asia Minor, North Africa.

In Kazakhstan it grows in the following floristic regions: 2. Tob. Ishim. (north), 3. Irtysh, 4. Semipalatininsk forest, 5. Kokshetau, 7. Aktyubinsk, 10a. Ulutau, 11. Eastern Uplands, 12. Zaisan, 22. Altai, 23. Tarbagatai, 24. Dzhungarskiy Alatau (north), 25. Zailiskiy Alatau [6].

It is widely distributed on the territory of the East Kazakhstan region: the Saur ridge, Tarbagatai, in the floodplain of the Kenderlyk river, in the foothills of the Ubinsky ridge, near the village of Cheremshanka, Zimovye, the village of Sharavka, Mount Kozlushka, as well as on the floodplain terraces of the river Bolshaya Talovka, Malaya Talovka, on southeastern slope of Mount Barkhot near the village of Livino, at the foot of the Ivanovsky ridge in the area of the Gornyyak rest house [7].

### *Experimental*

Surveys of natural habitats are carried out by expedition trips in September-October, during the period of harvesting fruit ripeness.

To determine the distribution of common viburnum in one area, we recommend using the Brown-Blanque scale in points:

1 – grows singly, 2 – in groups, 3 – in small spots, 4 – in small colonies or large spots, 5 – in large thickets.

In the description of phytocenoses the concept of layering is used, based on the life forms of the species included in its composition [8]. Latin names of plants are given according to S.K. Cherepanov [9].

At each trial plot, a detailed geobotanical description is carried out, the collection of herbarium, seeds, cloning of selected forms with green cuttings, measurements and description of morphological features in even-aged plants with a continuous count of trees.

### *Results and Discussions*

The selection and description of forms of *viburnum vulgare* in wild populations is carried out in the following order.

Specify the date of the examination.

Assign a number to the plant.

Specify the location through range mapping in ArcMap and ArcGis.

Brief description of the habitat (relief, soil, plant community).

Do a soil analysis.

The description of wild-growing forms of viburnum is carried out according to the phenotype, which takes into account quantitative and qualitative indicators of traits.

Determine the life form: tree, shrub.

Crown shape: round, oval, conical, etc.

Indicate the age state of the described plant (approximate number of years), according to the size of the bush, the diameter of the stems and the general condition.

Shrub height, m.

Stem diameter, cm.

In determining the general condition of the bushes, the main attention should be paid to the ratio of the processes of neogenesis (the number of zero shoots) and the death of old stems in the crown, the diameter of which is higher than 6–8 cm, as well as broken stems. The condition of the plant is assessed as follows: excellent – 3 points, completely healthy, not damaged by pests and diseases, height more than 2.2 m, number of stems from 8 to 16 pieces, no broken and dry stems, growth 18-35 cm, healthy leaves; average – 2 points; not damaged by pests and diseases, height more than 1.8 m, number of stems 6-12 pieces, broken and dry stems up to 10%, growth 12-25 cm, leaf apparatus without damage; bad – 1 point; up to 25% of the leaf apparatus

on the plant is damaged by pests and diseases, the height is more than 1.8 m, the number of stems is 6–12, broken and dry stems are up to 15%, the growth is 8–20 cm.

Winter hardiness: 0 – no damage, 1 – very weak damage, 2 – weak (up to 10% of fruit-bearing branches), 3 – medium (up to 30%), 4 – strong (up to 40%), 5 – very strong (more than 50%).

The color of the bark of skeletal branches (gray, light gray).

The length of annual shoots (with an accuracy of 0.5 cm, the average of 30–40 measurements).

Blade length and width, cm (average of 30 measurements).

The color of the upper side of the leaves is established according to the A.S. Bondartseva (light green, green, dark green), while indicating the intensity of the shade.

The degree of flowering of plants (in points) according to the following scale:

1 – very weak (single brushes, up to 3 pcs. per branch, the number of flowers in a brush up to 35 pcs.); 2 – weak (the number of brushes per branch, up to 6 pcs., The number of flowers in the brush 36–65 pcs.); 3 – medium (number of brushes per branch, up to 9 pcs., number of flowers in a brush 66–95 pcs.); 4 – good (the number of brushes per branch, up to 12 pieces, the number of fruits in the brush is more than 95 pieces); 5 – plentiful (the number of brushes per branch, up to 15 pieces and more, the number of fruits in the brush is more than 95 pieces).

Fruit set: below 30% – low, 40 – 50% – medium, above 60% – high, determined fourteen days after flowering, when an increase in the size of the ovary is noticeable.

The degree of fruiting plants (in points) according to the following scale:

1 – very weak (single brushes, up to 3 pieces per branch, the number of fruits in the brush up to 15 pieces); 2 – weak (the number of brushes on a branch, up to 6 pcs., The number of fruits in a brush 16–30 pcs.); 3 – medium (the number of brushes per branch, up to 9 pieces, the number of fruits in the brush 31–45 pieces); 4 – good (the number of brushes per branch, up to 12 pieces, the number of fruits in the brush is more than 45 pieces); 5 – plentiful (the number of brushes per branch, up to 15 pieces and more, the number of fruits in the brush is more than 45 pieces).

To determine the weight of 100 fruits, with an accuracy of 0.01 g, fruits from different parts of the crown are collected from each plant in triplicate, 25–30 pieces each. Taking into account the population parameters by weight in fruit-bearing plants of *viburnum vulgare* from 0.30 g to 0.79 g, an interval of 0.15 g was adopted in the gradation, four groups: small – below 0.45 g; medium – from 0.46 to 0.60 g; large – from 0.61 to 0.75 g; very large – above 76 g.

In determining the size of fruits with a caliper, with an accuracy of 0.1 mm, measure the length and diameter of 25–30 fruits from different parts of the crown, then determine statistical indicators that will characterize not only this sample (25–30 fruits), but the entire set fruits of a given bush in a particular area of growth.

Describing the shape of the fruit, their index is established – the ratio of the length of the fruit to its diameter, given the index (0.95; 1.01–1.06; 1.07–1.2), the shape of the fruit is described as (round, spherical, oval).

In determining the color of *viburnum* fruits, the colors of the solar spectrum are taken, they are described as (orange, orange-red, reddish-orange, red, cherry). Fruit color: cherry – 15 points, dark red and red – 10 points, orange – 5 points.

The evaluation of fruit taste is determined by the organoleptic method (excellent, good, mediocre, poor). The nature of the taste of fruits (sweet, sour, sour-sweet, sweet-sour, indicating the presence of bitterness. Taste of fruits: dessert – 20 points, pleasant with a slight aftertaste of bitterness – 15 points, with a significant bitter aftertaste – 10 points, bitter – 5 points.

The nature of the detachment of fruits from the hand is dry – the fruit is torn off without juice release (10 points), wet – with juice release (5 points).

Taking into account the population parameters in terms of the number of fruits in the cluster of fruit-bearing plants of *viburnum vulgare*, from 9.7 to 50.9 pieces, an interval of 15 pieces was adopted in the gradation, four groups: few – less than 15 pieces; average – 16–30 pieces; high – 31–45 pieces; very high – more than 45 pcs.

Seeds: a) Color (light brown, brown); b) shape (ovoid, elliptical).

The percentage of seed weight from the weight of fresh fruits is determined within a week.

In terms of ripening, we distinguish early forms, fruit ripening in the third decade of September and late ones in the first decade of October.

Pests and diseases of *viburnum vulgare*, nature of damage.

The study of intraspecific variability of a species (endogenous and individual) is carried out by studying the most common populations within its range, on temporary trial plots.

Variability is one of the basic biological concepts along with the concepts of metabolism, reproduction, and heredity. All changes are divided into two groups: hereditary and non-hereditary [10].

Variability is the main property of all living organisms, therefore in nature there are no individuals absolutely identical in all signs and properties. I.I. Schmalhausen [11] defined variability as the property of living organisms to reproduce similar but not identical. "Variability", he wrote, "is an expression of the continuity of organic forms in the process of reproduction, in which dissimilar structures and functions appear (i.e. develop) in the offspring".

In individual variability, a change characterizes only an individual, and such changes are always caused by the influence of external conditions, i.e. arise as a result of modifications. This kind of variability corresponds more or less to non-hereditary variability.

With group (intrapopulation) variability, the change no longer characterizes an individual, but a whole group of them within the species. Such changes always arise as a result of mutations or combinations, due to which this kind of variability more or less corresponds to hereditary variability.

Baur, on the other hand, suggested that the word "variation" should be given a broader meaning and understood by variations as all three types of possible changes in organisms, i.e. modifications, mutations and combinations. The first group covers a change of a non-hereditary nature, the second and third – hereditary; modifications and mutations arise without any participation of crossing, combinations – as a result of the latter, but all these are different types of variability, as a process or variations.

Thus, the division of variability, in fact, into individual and group is as basic as the division of variations into modifications, mutations and combinations. In one case we are dealing with a known state, in the other with a process.

Crossings are constantly taking place in natural populations, as a result of which new forms appear due to the formation of new combinations from old combinations of hereditary factors. New combinative changes are always of a group nature. Meanwhile, most combinations are split as well as the forms that produced them, due to the fact that each such new phenotype usually includes several different genotypes. In nature, mutational and combinative variability act together, intertwining in the most intimate way with each other. The variability of organisms in a population under the pressure of natural selection leads to the formation of new species and morphogenesis processes.

Studies of the intraspecific variability of common viburnum are carried out on the basis of a population-genetic approach to species assessment, where a species is understood as a system of populations [12-14].

The study of intraspecific variability of common viburnum is carried out according to the method [15] in three stages:

1. The study of endogenous variability of morphological and economic-biological traits in the crown of one individual. In order to determine the endogenous variability, the sample size is 20-50 measurements of each trait, depending on the variability, by crown tiers, exposure sides, etc. (Table).

Table

Accuracy and number of measurements of signs of *Viburnum opulus*

Feature	Measurement accuracy	Number of measurements
Plant height	10 cm	30-40
The number of trunks and the presence of zero shoots, pcs.	1 pcs.	40-45
Length (one-year growth)	0,5 cm	30-40
Number of flowers in inflorescence, pcs.	1 pcs.	40-50
Number of fruits in inflorescence, pcs.	1 pcs.	40-50
Fruit length	0,1 mm	20-25
Fruit diameter	0,1 mm	20-25
Weight 100 pcs. fruits, g	0,01 g	20-25
Weight 100 pcs. seeds, g	0,01 g	30-40

2. After studying the average indicator of the trait and the amplitude of its variation for individuals, they proceed to the study of intrapopulation (organism, individual) variability. The sample size (the number of

model bushes) required to obtain reliable results when characterizing the structure of populations is determined by the level of variability of different traits.

3. Study of interpopulation variability. To assess the degree of variability of traits, a unified scale of variability levels developed [16] is used. According to the scale, the amplitude of variability is estimated by the value of the coefficient of variation: less than 12% – the level of variability is low, 13%-20% – medium, 21%-40% – high, more than 40% – very high.

Phenotypic variability is a set of features and properties of an organism or a group of individuals formed under the combined influence of heredity and environmental factors.

In the study of variability, biometric research methods are used, based in whole or in part on statistical or probabilistic patterns. To find the arithmetic averages –  $M$  and the errors of the averages –  $m$  of the studied parameters, the coefficients of variation  $C\%$ , the standard deviation –  $\sigma$ , the reliability criterion –  $t$ , and the accuracy of the experiment  $P$  for each plant and for a separate population, the obtained digital data are processed using methods of mathematical statistics [17-20].

The calculation of statistical indicators is carried out separately at two levels of variability: endogenous (within the body) and individual within the population (location).

A number of other features: differences in the color of fruits, bark, the nature of taste and separation of fruits are of a qualitative nature. Let us consider an example of comparing the qualitative variability in our case by the color of viburnum fruits. Statistical data show that among the examined plants, the following percentage distribution of the total number of individuals by color was obtained: with red fruits 42.6%, with orange 37.4%, with cherry 20.0%.

With qualitative (alternative variability), no matter how many separate groups (in our case, three), they are divided into two classes: class 1 – having one or another feature and class 0, devoid of it. In cases of qualitative variability, the coefficient of variation completely replaces the quadratic deviation. The calculation of the square deviation is carried out according to the following formula:

$$\sigma = \pm(a)/(a+b)$$

and always expressed as a percentage. The first includes individuals, the second  $b$  individuals. According to the color of the fruits of each of them, it is necessary to contrast with all the others (red – not red, cherry – not cherry, etc.), and for each, the following values of the square deviation are obtained: red 42.6% versus 57.4% not red; orange 37.4% versus 62.6% non-orange; cherry 20.0% versus 80.0% non-cherry.

$$\sigma = \pm(a)/100 = 48,3\%$$

The square deviation is always expressed here as a percentage, and its value does not exceed 50%.

After mathematical processing, it is necessary to fill out a questionnaire for each studied plant in a selected form (data on 25 traits), which will make it possible to better imagine the studied form and study genotypic and phenotypic variability in the future.

### Conclusions

The ecological and biological features of the common viburnum from natural habitats, its comparative characteristics reveal its genetic potential, which is extremely important for the conservation of biological diversity and breeding work.

For each region, the plants of *viburnum vulgare* have their own ecological and biological features. This recommendation will help to study this plant according to the main morphological and economic characteristics, to conduct a comparative characteristic.

In the section objects and methods of research based on actual data, a map has been developed for describing wild-growing forms of viburnum, which is filled in according to 25 quantitative and qualitative characteristics.

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### ***Viburnum opulus* L. жабайы формаларын таңдау және түршілік өзгергіштікті зерттеу әдістемесіне**

Түршілік өзгергіштікті зерттеу және өсіруге перспективалы кәдімгі калинаның жабайы өсетін түрлерін таңдау бойынша әдістемелік ұсыныс Шығыс Қазақстан облысындағы осы түрді зерттеу нәтижесінде алынған үлкен көлемдегі фактілік материалдар негізінде жазылған. Ол ArcMap және ArcGis бағдарламаларында ауқымды карталау арқылы *Viburnum opulus* L. популяцияларының ағымдағы жағдайын бағалауды қамтиды. Геоботаникалық, фитоценоздық сипаттама берілген. 25 сандық және сапалық белгілер бойынша формалардың сипаттамасы жасалған, соның негізінде перспективалық түрлерді іріктеу, тұқым жинау және көк қалемшемен клондау жүргізілген. Калина өсімдіктерін гүлдену, жеміс беру дәрежесі, жемістердің ұзындығы мен диаметрі бойынша бөлу кезінде деректер ең кіші, орташа және ең үлкен мәндері бар (бес топ) кластарға; ал жемістердің массасы мен саны бойынша — төртке бөлінді. Балдық жүйе жеті белгі бойынша қолданылды: өсімдіктердің жалпы жағдайы, қысқа төзімділігі, гүлдену және жеміс беру дәрежесі, түсі, дәмі, жемістердің үзілу сипаты. Морфологиялық және шаруашылық-биологиялық белгілердің эндогендік өзгергіштігін зерттеу бір дарактың жапырағына жүргізілді. Белгінің орташа көрсеткішін және оның өзгеру амплитудасын жеке дарактар үшін зерттегеннен кейін популяция ішілік өзгергіштік зерттелді. Математикалық өңдеуден кейін әрбір өсімдікке таңдап алынған түр бойынша сауалнама толтырылады, бұл зерттелетін түрлерді жақсы білу-

ге және болашақта генотиптік және фенотиптік өзгергіштікті зерттеуге мүмкіндік береді. Бұл ұсынысты ғылыми-зерттеу жұмыстарында қолдану түрдің генетикалық әлеуетін, оның түршілік өзгергіштік деңгейін бағалауға және перспективалы түрлерді таңдауға мүмкіндік береді.

*Кілт сөздер:* таралуы, сандық сипаттамасы, гүлдену дәрежесі, жемістері, түсі, өзгергіштігі.

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## К методике по изучению внутривидовой изменчивости и отбору дикорастущих форм *Viburnum opulus* L.

Методическая рекомендация по изучению внутривидовой изменчивости и отбору дикорастущих форм калины обыкновенной, перспективных для селекции, написана на основе большого фактического материала, полученного при исследовании этого вида на территории Восточно-Казахстанской области. Она включает оценку современного состояния популяций *Viburnum opulus* L., с картированием ареалов в программах ArcMap и ArcGis. Дано геоботаническое, фитоценоотическое описание. Проведено описание форм по 25 количественным и качественным признакам, на основе чего были произведены отбор перспективных форм, сбор семян и клонирование зелеными черенками. При распределении растений калины по степени цветения, плодоношения, длине и диаметру плодов данные были разбиты на классы с наименьшими, средними и наибольшими значениями (пять групп), по массе и количеству плодов — на четыре. Бальная система применена к семи признакам: общее состояние растений, зимостойкость, степень цветения и плодоношения, окраска, вкус, характер отрыва плодов. Изучение эндогенной изменчивости морфологических и хозяйственно-биологических признаков проведено в кроне одной особи. После изучения среднего показателя признака и амплитуды его варьирования для отдельных особей перешли к изучению внутривидовой изменчивости. После математической обработки заполнена анкета на каждое растение по выделенной форме, что даст возможность лучше представить исследуемую форму и в будущем изучать генотипическую и фенотипическую изменчивость. Использование настоящей рекомендации в исследовательской работе позволит дать оценку генетического потенциала вида, уровня его внутривидовой изменчивости, отобрать перспективные формы.

*Ключевые слова:* распространение, количественные признаки, степень цветения, плоды, окраска, изменчивость.

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