Management of walnut genetic resources to improve the efficiency of the breeding process in the South of Russia

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Abstract. Walnut is one of the main nut crops of world importance. Conservation and efficient use of genetic resources is one of the fundamental principles of breeding work. The most efficient way to work with genetic resources is to create core collections that include most of the genetic diversity with the least number of samples in the collection. There are several different approaches to the creation of core collections, differing in the criteria for the selection of characters and the number of samples in the collection. This article provides an overview of the most effective approaches to creating walnut core collections in southern Russia. Of course, both genetic and phenotypic diversity should be taken into account. Microsatellite markers or SNPs may be the most appropriate tools for assessing genetic diversity, depending on the objectives and sample sizes. The article also provides an overview of the most valuable traits from the point of view of walnut breeding in the North Caucasus.

1 Introduction

Walnut is one of the most widespread crops in the world [1]. The largest walnut-producing countries are the USA and China [2]. The volume of walnuts production in the world is increasing annually. This is due to both the growth of the world's population and the growing needs of the population for nut crops with high nutritional value.

The basis for effective crop cultivation is assortment, the diversity and relevance of which is supported by breeding programs. Of course, artificial selection carried out by breeders can significantly reduce the genetic diversity of a culture, which negatively affects the potential of genetic collections as sources of new traits and their combinations [3].

Breeders in an effort to improve the phenotypic properties of genotypes rely on the genetic diversity of forms in collections. It can be concluded that genetic collections should be as large as possible. However, maintaining large collections is associated with high costs, phenotypic assessment of large collections is a laborious process that requires high labor costs of highly qualified specialists [3]. The most justified way is to create core

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collections for direct breeding work. Such collections include most of the available genetic diversity, the most promising traits from the point of view of breeding. There are different approaches to the formation of core collections, and for each individual case, significant differences are possible [4].

2 Core collection parameters

The core collection should include the greatest variety of alleles with the fewest samples. What kind of alleles these will be and what will be the number of samples after selection is a subject of discussion. The sizes of core collections can vary depending on the size and composition of the overall collection and on the goals of the breeders. According to the literature, the working collection should include 10% of the genotypes of the main collection, reflecting 70% of the available genetic diversity. Estimates for suitable collection sizes range from 10 to 30% of the main collection. Of course, the size of both the main collection and the core collection is limited by the funding and resources available to the curators of the collections. Thus, to create a core collection, it is necessary to assess the genetic diversity of the available forms and their main phenotypic characteristics [5].

3 Assessment of genetic diversity and molecular marker methods

Molecular genetics is a powerful tool in breeding programs. The evolution of methods in this direction has left aside isozyme analysis and concentrated on the analysis of nucleic acids. Analysis of DNA markers. allows you to eliminate the influence of the environment and natural selection, when assessing genetic diversity. From the multilocus markers (RFLP, RAPD, AFLP, ISSR), which are more commonly used in the early stages, researchers have moved to the more popular today microsatellite markers (SSR) and SNP analysis [1,6,7].

SSR markers are easy-to-use, simple and affordable method of analysis, widely used to study the genetic diversity of living organisms. This tool is most widely used in population genetic and phylogenetic studies. Due to the neutral nature of inheritance, a relatively high rate of mutations accumulation, and a large number in the genome, SSR loci make it possible to efficiently assess intraspecific diversity when analyzing large samples or samples of different ecological and geographical origin [1,8–18]. However, when developing core collections, as well as when using methods such as the Genome-Wide Association Study (GWAS), the use of SNP analysis is a more appropriate tool [19].

Axiom ™ J. regia 700K SNP array was developed for walnut SNP analysis [20]. It includes 609658 SNPs, which is quite a sufficient number for this technology. With the use of this tool, the creation and saturation of the adhesion map of the walnut is currently underway [21,22].

The INRAE walnut collection near Bordeaux, including 150 accessions, was genotyped using both SSR markers and the Axiom ™ J. regia 700K SNP array. The authors of this work note that 13 SSR markers reflect 95% of the allelic diversity of SNPs. It is logical to assume that when the task is to compare collections from different sources, in order to effectively replenish collections through exchange, it is enough to use SSR markers. SNP analysis will better reveal its advantages when comparing samples that are closer in their genetic composition. For example, when analyzing local forms, SNP genotyping can be used along with phenotypic assessment. Such applications, in addition to assessing genetic diversity, can provide valuable information for the GWAS. In a joint analysis of genetic and phenotypic data, the search for possible correlations between them can serve as the basis for the creation of genetic markers for QTL (Quantitative Trait Loci).

4 Walnut traits valuable for breeding in the South of Russia

To select genetic resources for core collections, phenotypic data are used along with genetic data. Depending on the region of cultivation, traits valuable for breeding may differ, although there are a number of important parameters that are characteristic of all walnut breeding programs. Here is an overview of the traits most valuable for breeding in the South of Russia.

Lateral fruiting.

One of the most important components affecting the productivity of walnut plants is the ability to set fruits on lateral shoots. With such fruiting, female flowers appear not only on the apical buds, but in almost all axillary buds of young shoots. Apical dominance is reduced in this case. Fruits are located on the tops of lateral growths along the entire length of the branches. Subject to high agricultural technology and good lighting, such shoots bear fruit for a long time. Using data on the laying of fruits in the lateral position, the yield index can be calculated, which significantly correlates with long-term data on the yield of industrial plantings. The presence of pronounced lateral fruiting is also often associated with a short juvenile period, restrained growth, and the ability to stool layering [23].

Late budburst and flowering.

This feature is actualized in countries with a high risk of recurrent frosts. For the South of Russia, frosts are not uncommon in early April and late March, when the walnut begins to emerge from deep dormancy and generative buds open. The most serious losses in productivity are incurred by forms that do not have lateral fruiting, since it is the fruiting apical buds that open first and die. It is enough to reduce the air temperature to zero in order to cause the damage of the apical meristems. It should be noted that the walnut is a thermophilic crop and the growing season lasts about 280 days in a temperate climate. Thus, if the growing season starts too late, trees may suffer from autumn frosts or critical winter frosts [23].

Apomixis.

Apomixis is the ability to set fruit without pollination or fertilization. This symptom is actualized when male buds are damaged by spring frosts or when the timing of flowering of male and female flowers in the garden does not match. Apomictic forms usually bear fruit more stably than those without this trait. The percentage of apomictic fruits is usually small and ranges from 8 to 12%, but in some forms it can be from 23.5 to 81.2%. According to research by Peng-fei et al. [24], under the conditions of China, the experimental cultivar Qinquan1 laid up to 75.7% of apomictic fruits, with an average value of 48.5%.

Homohamy.

Homogamy can be considered an alternative to apomixis in terms of fruiting stability under conditions of a lack of pollinators. Walnut does not have biochemical mechanisms preventing self-pollination, such as gametophytic self-incompatibility of an apple tree or sporophytic self-incompatibility of hazelnuts. Cross-pollination is provided, as a rule, by a mismatch between the timing of flowering of male and female flowers on the same tree. However, the timing can either partially or completely overlap, such plants are called homogamous. These phenological traits can also be of interest for breeding. [25].

Winter hardiness.

One of the most important features of walnut for the South of Russia is its resistance to low temperatures in winter. This trait is due to a high level of dormancy at the beginning of winter, as well as early ripening of fruits and the termination of the growing season. The early transition of plants from active vegetation to deep dormancy allows plants to easily tolerate autumn frosts. Of course, such signs are most often found in plants originating from zones with a continental climate.

Physiological mechanisms of resistance to cold stress are realized due to the presence of cold stress proteins, osmotic pressure of cell sap due to accumulated products of protein and carbohydrate metabolism, as well as the timely transfer of water from cells to the intercellular space.

With a resistance to low temperatures, the walnut is capable of laying stable yields after prolonged winter frosts down to -35 °C. In countries such as the USA, Germany, Belarus, the Netherlands, etc., varieties have been obtained that have similar qualities [23].

As for varieties and forms of walnuts that are not resistant to frost, even a twenty-degree frost, in combination with a strong wind, can damage not only annual growths that form the crop, but even cause the death of whole skeletal branches and the death of a tree. Many varieties that are valuable from the standpoint of yield, zoned in Russia, suffer from winter frosts, which leads to partial or complete loss of yield and significant damage to fruit plantations [26].

Tolerance to bacteriosis and anthracnose.

Bacteriosis, or walnut blight – makes damage to the leaves and fruits of the walnut, caused by bacteria *Xanthomonas arboricola* pv. juglandis. Damage to plants with a strong development of the disease leads to significant crop losses (60% or more). Unfortunately, no immune forms to this disease have been found within the species *Juglans regia* L. However, the disease development degree still depends on the genotype and tolerant forms should be included in the breeding process [27].

Anthracnose, is a widespread disease of walnuts caused by the fungus *Gnomonia leptostyla*. Green parts of the plant suffer from the disease, which leads to a significant decrease in yield, up to a complete loss of yield. Forms from Eastern Europe, where the development of the disease is especially pronounced, can serve as potential sources of anthracnose resistance. In these countries, selection for resistance to this pathogen is underway [28].

Nut and kernel quality.

One of the most important characteristics of the variety is the high fruit quality. The modern walnut market places ever higher demands on products. The nut should be round and smooth. The shell should be moderately thin, within 0.8-1.2 mm, split easily, round and with smooth edges. The last two parameters are important for mechanical procurement and calibration. The inside of the shell must be smooth to allow easy removal of the kernel. The color of the kernel is also important, the lighter it is, the less tannins and cinnamic acids are contained in its shell. These components are often responsible for the tart or bitter taste. The mass of the kernel should be between 48% and 55% of the nut weight. Above these limits, the shell is too thin and can be easily overcome by pathogens and pests, and breaks easily during transport. Kernel yield below 48% usually means low fruit quality [23].

Precocious.

Walnut has a rather long juvenile period, which reaches 12 years. Thus, the rate of entry into fruiting is an important factor affecting crop productivity and growing technology. Breeding for early maturity is carried out in all countries with developed walnut production. According to Germain et al. 1997 [29], in France, within the framework of a breeding program, walnut forms were obtained, bearing fruit in the first year of the growing season. R. Rezaee et al. 2009 [30], notes fruiting in forms in the second year of the growing season, similar results were obtained in the framework of the selection of walnuts in the Krasnodar Territory.

Restrained growth.

The variety of habitus within the J. regia species is quite large. However, it so happened historically that in addition to nutritious and tasty fruits, durable and heat-resistant walnut wood was appreciated. The beautiful texture, combined with mechanical and thermal resistance, has led to the widespread use of walnut wood in the manufacture of furniture and parts of firearms. Accordingly, breeders took into account the high demand for wood when creating walnut varieties. Nevertheless, the general trend towards compacting the crown and intensifying horticulture did not leave aside the breeding programs for walnuts. Dwarf and semi-dwarf walnut forms are found in Central Asia [29]. These forms are also characterized by such a valuable feature as increased rooting ability with vertical layering [31].

Those forms that have received the status of dwarf or semi-dwarf do not exceed 8 meters in height, they are fast-growing, predominantly lay fruit buds and reach the peak of fruiting in a shorter time. A significant disadvantage of these forms is a shorter maturity period of 20-25 years, while more vigorous forms actively bear fruit for 120-150 years or more [29].

In general, dwarf and semi-dwarf forms are of interest for breeding from the point of view of obtaining rootstocks capable of vegetative propagation, as well as varieties adapted for plantings with a high planting density [30].

5 Genotype and phenotype data statistical analysis

Both phenotypic and genetic data are used to select samples for core collections. Both these and other data have both positive and negative sides. There are examples of creating core collections using both approaches [3,18].

According to a study by Kumar et al. 2016 when creating a core collection of saffron, the use of only phenotypic or only genetic data leads to a decrease in the diversity of the obtained collections, therefore, if possible, both approaches should be used [32].

To analyze the data and take into account the effectiveness of the work carried out, one should rely on the indicators of genetic diversity obtained using Bayesian analysis, MANOVA, and PCoA, as well as the geographical origin of the samples and their phenotypic characteristics [32].

The software for creating core collections is based on a maximization strategy (M-strategy), which implies the selection of the largest number of alleles in the smallest number of samples. This task essentially solves the problem, also known as the traveling salesman problem, which implies the optimal route covering the largest number of settlements, with the smallest distance. If there are more than 60 settlements, the problem cannot be solved by a simple selection, since the power of computer technology is insufficient to solve this problem in a reasonable time. To solve such problems, a heuristic algorithm was developed that significantly speeds up the receipt of the correct solution. There are two programs for creating core collections: Powercore and MStrat [3].

6 Conclusions

Thus, to create a core collection of walnuts in southern Russia, both phenotypic and genetic data on diversity should be used. Genetic data can be obtained using both SSR markers and SNPs. When creating a collection, one should take into account traits that are important for breeding, including the characteristics of the cultivation region; for the south of Russia, such traits include resistance to deep winter frosts. The created collection should, if possible, reflect more than 70% of the allelic diversity presented in the original collection.

References

1. F. Bernard, E. Lheureux, Dirlewanger, Tree Genet. Genomes, **14,** 1 (2018)
2. F. Zhao, Y. Liu, D. Gong, Y. Li, Y. Chang, Wang, HortScience **55,** 392 (2020)
3. R. Mahmoodi, M. R. Dadpour, D. Hassani, M. Zeinalabedini, E. Vendramin, S. Micali, F. Z. Nahandi, Sci. Hortic. **249,** 439 (2019)
4. H. Haouane, A. El Bakkali, A. Moukhli, C. Tollon, S. Santoni, A. Oukabli, C. El Modafar, B. Khadari, Genetica **139,** 1083 (2011)
5. W. Liang, L. Dondini, P. De Franceschi, R. Paris, S. Sansavini, S. Tartarini, Plant Mol. Biol. Report, **33,** 458 (2015)
6. U. Erturk and Z. Dalkilic, Romanian Biotechnol. Lett, **16,** 5944 (2011)
7. Q. Ma, J. Zhang, D. Pei, J. Am. Soc. Hortic. Sci, **136**, 422 (2011)
8. M. Aradhya, D. Velasco, Z. Ibrahimov, B. Toktoraliev, D. Maghradze, M. Musayev, Z. Bobokashvili, J. E. Preece, PloS One, 12 (2017)
9. I. Balapanov, I. Suprun, I. Stepanov, S. Tokmakov, A. Lugovskoy, Sci. Hortic, **253**, 322 (2019)
10. L. Chen, Q. Ma, Y. Chen, B. Wang, D. Pei, Sci. Hortic, **168,** 240 (2014)
11. H. Han, K. E. Woeste, Y. Hu, M. Dang, T. Zhang, X.-X. Gao, H. Zhou, X. Feng, G. Zhao, P. Zhao, Tree Genet. Genomes, **12,** 111 (2016)
12. S. Khokhlov, E. Panyushkina, I. Balapanov, I. Suprun, S. Tokmakov, in II Asian Hortic. Congr, 1208, 47 (2016)
13. P. Pollegioni, K. Woeste, F. Chiocchini, S. Del Lungo, M. Ciolfi, I. Olimpieri, V. Tortolano, J. Clark, G. E. Hemery, S. Mapelli, PloS One, 12 (2017)
14. P. Pollegioni, K. Woeste, I. Olimpieri, D. Marandola, F. Cannata, M. E. Malvolti, Tree Genet. Genomes, **7,** 707 (2011)
15. W. Roor, H. Konrad, D. Mamadjanov, and T. Geburek, J. Hered, **108,** 391 (2017)
16. K. Vahdati, S. M. Pourtaklu, R. Karimi, R. Barzehkar, R. Amiri, M. Mozaffari, K. Woeste, Plant Syst. Evol, **301,** 691 (2015)
17. H. Wang, W. Wu, G. Pan, D. Pei, J. Hortic. Sci. Biotechnol, **90,** 563 (2015)
18. Bernard, T. Barreneche, A. Donkpegan, F. Lheureux, E. Dirlewanger, Tree Genet. Genomes, **16,** 76 (2020)
19. Bernard, T. Barreneche, A. Donkpegan, L. Fabrice, E. Dirlewanger, *Management of a Walnut Germplasm Collection: Which of SSR or SNP Markers Are Most Suitable to Preserve Biodiversity?* (Genetics, 2019)
20. Marrano, P. J. Martínez-García, L. Bianco, G. M. Sideli, E. A. Di Pierro, C. A. Leslie, K. A. Stevens, M. W. Crepeau, M. Troggio, C. H. Langley, D. B. Neale, Plant Biotechnol, **17,** 1027 (2019)
21. G. M. Sideli, A. Marrano, S. Montanari, C. A. Leslie, B. J. Allen, H. Cheng, P. J. Brown, D. B. Neale, PLOS ONE, 15(2020)
22. Marrano, G. M. Sideli, C. A. Leslie, H. Cheng, D. B. Neale, Front. Plant Sci, **10,** 1140 (2019)
23. E. Germain, in III Int. Walnut Congr, **442**, 21 (1995)
24. Z. Peng-fei, Y. Yang Jun-qiang, S. Yu-qin, W. Guo-Liang, and C. Yan-hui, in XXVII Int. Hortic. Congr.-IHC2006 II Int. Symp. Plant Genet. Resour. Hortic, 760, 541 (2006)

25. K. Kumar, S. D. Sharma, and R. Sharma, Adv. Hortic. Sci. Riv. Dellortofloroftutticoltura Ital., **19(1),** 1000 2005
26. A. P. Lugovskoy, E. V. Ulyanovskaya, S. N. Artyukh, E. M. Alekhina, N. V. Mozhar, R. Sh. Zaremuk, S. A. Govorushchenko, S. V. Bogatyreva, Horticulture And Viticulture, 21 (2006)
27. M. Kałużna, J. Pulawska, M. Waleron, and P. Sobiczewski, Plant Pathol, **63,** 1404 (2014)
28. M. Hassan, K. Ahmad, Int. J. Environ, **2,** 9 (2017)
29. E. Germain, in Third Int. Walnut Congr., edited by J. A. G. Pereira, J. M. S. Martins, C. P. deAbreu, 21 (1997)
30. R. Rezaee, K. Vahdati, M. Valizadeh, J. Hortic. Sci. Biotechnol, **84,** 228 (2009)
31. K. Vahdati, R. Rezaee, V. Grigoorian, M. Valizadeh, A. Motallebi Azar, J. Hortic. Sci. Biotechnol, **83,** 334 (2008)
32. S. Kumar, H. Ambreen, M. T. Variath, A. R. Rao, M. Agarwal, A. Kumar, S. Goel, A. Jagannath, Front. Plant Sci, **7** (2016)