



# Natural Hybridization of the Beans (*Phaseolus vulgaris* L.) as a Method of Initial Material for Selection

Almas Asadova<sup>1</sup>

10.18805/ag.DF-695

## ABSTRACT

**Background:** This review deals with natural hybridization, an important subject in conservation biology. This process is uncommon in terms of the total number of individuals involved, but is much less unusual if we consider the number of species that hybridize. Thus, natural hybridization may be an important process in the shaping of the evolutionary trajectories of many plant and animal species. The possible consequences of natural hybridization, which can either promote or prevent evolutionary divergence between taxa and will involve many ecological factors, are analysed here.

**Methods:** Local AzePHA-16 samples (flowers light pink, beans striped on a green background, stems green, seeds purple) participated in the hybridization as a high-quality mother form. The introduced AzePHA-t/15 (with purple flowers, dark purple pods, anthocyanin-rich stem and light-brown seeds) samples were used as a high-quality father form. To confirm that natural hybridization had occurred and to determine the genetic characteristics of the hybrids, individual characteristics such as flower, fruit and seed color and shape, growth rate and yield were compared.

**Result:** Natural hybrids have been obtained by cross-pollination between different genotypes of beans without human intervention. The observation of seeds and beans of different shapes and colors indicates that the parents have different genetic characteristics and that these characteristics are expressed in different combinations. The fact that hybrid plants have different phenotypes proves genetic variation. Our findings highlight the often underestimated conservation value of hybrid populations.

**Key words:** Common bean, Cross-pollination, Floral structure, Natural hybridization, Seed's color.

## INTRODUCTION

The importance of legumes, as an alternative source of nutrition, is associated with the content of essential amino acids, as well as vitamins, minerals and microelements (Neethu *et al.*, 2022). *P. vulgaris* is one of the most economically important legumes in the world. In sub-Saharan Africa, more than 200 million people depend on beans as their most important pulse. Beans are grown for their green leaves (often fed to livestock) and immature pods and seeds, but it is the dry seeds (which are nutritious and store well) that are the primary end product. Dry beans (*Phaseolus vulgaris* L.) are an extremely important aliment, not only representing the main source of dietary protein for humans in several world regions but also contributing greatly to diet with starch, fiber, vitamins and minerals. Annual global production is presently approximately 26.5 million tons, most of which is used for human consumption (FAOSTAT, 2018). Although in the most developed countries, the importance of dried bean consumption has diminished with the increase of meat consumption, producing animal protein is far more expensive and is generally considered unsustainable (Gonzalez *et al.*, 2011). Red meat in particular also has negative effects on health, for example being associated with the development of cardiovascular disease and cancer (Gonzales *et al.*, 2014). International organizations and specialists therefore recommend increasing the consumption of beans and other legumes to fulfill our nutritional needs and decrease inputs of food production.

Of the major food crops, beans and especially common beans, are among the most variable. Growth habit, seed

<sup>1</sup>Institute of Genetic Resources, National Academy of Sciences (NAS) of Azerbaijan.

**Corresponding Author:** Almas Asadova, Institute of Genetic Resources, National Academy of Sciences (NAS) of Azerbaijan. Email: almas.i.asadova@gmail.com

**How to cite this article:** Asadova, A. (2025). Natural Hybridization of the Beans (*Phaseolus vulgaris* L.) as a Method of Initial Material for Selection. Agricultural Science Digest. 1-7. doi: 10.18805/ag.DF-695.

**Submitted:** 07-11-2024 **Accepted:** 12-05-2025 **Online:** 28-06-2025

size, shape and color, days to maturity and many other factors vary across varieties. There is hidden diversity too, not only in resistance to pests and diseases but also in the nutritional value of the seeds. CGIAR research centers, notably CIAT, have been instrumental in breeding beans that are more productive and more nutritious, benefitting millions of households.

The main goal of the research is to monitor and study natural hybridization in common bean (*P. vulgaris* L.) in Absheron conditions and its use in breeding. Thus, cross-pollination in beans is of theoretical and practical interest for breeding. Using natural hybridization, it is possible to develop methodological aspects of creating new genotypes of beans.

## MATERIALS AND METHODS

The studies were conducted in 2021-2023 at the Institute of Genetic Resources (IGR) of the National Academy of Sciences (NAS) of Azerbaijan. The IGR is located on the

Absheron peninsula (80 m above sea level), in a dry subtropical climate with very sunny and dry summers, warm and sunny falls and mild almost snowless winters. The average temperature is 13.5-14.5°C. Frost in winter is rare. In summer, the temperature climbs up to 38-40°C and since 2010 this can reach to 40-45°C. The driest months are July and August. Most of the rainfalls occur in winter-spring period. Average yearly rainfall is mediocre and constitutes 120-150 mm and relative humidity is 70.6%. Summer is almost always dry. The soil is sandy and very poor. Caspian Sea and semi-arid plains surrounding the peninsula has big impact to the climate. The following method was used during the research: Methodology for the definition of a key set of characterization and evaluation descriptors for bean (*Phaseolus vulgaris* L.) (Alercia, 2011).

To confirm that natural hybridization had occurred and to determine the genetic characteristics of the hybrids, individual traits such as flower, fruit and seed color and shape, growth rate and yield were compared. A wide range of phenotypic variation was observed in the F1 and F2 generations.

Sowing of collection samples was carried out in duplicate with an area of food of one plant 10 × 60 cm at the optimum time, in the spring at the end of April. A standard sample was sown after every 10 samples, with the Method of systematic placement of experimental plots. In the process of growing, the ranks made phenological observations, determined the time of onset of phenological phases. The onset of the phase was noted when there were signs in 10% of the plants and complete-in the presence of signs in 75% of the plants. The dates of the onset of the main phases and interphase periods were noted: seedlings, flowering, fruiting and ripening of beans. Harvesting and threshing of grain was done individually.

110 samples were used as research material: 87 of them were local forms and 23 were samples obtained from VİR.

Natural cross-pollination between bean varieties occurred in 2014 and was observed by chance. In the following year, the hybrids found in the mother plants were planted separately in a 5 × 5 m plot. Each year, the next generation hybrid plants were planted separately and monitored. Local AzePHA-16 samples (flowers light pink, beans striped on a green background, stems green, seeds purple) participated in the hybridization as a high-quality mother form. The introduced AzePHA-t/15 (with purple flowers, dark purple pods, anthocyanin-rich stem and light-brown seeds) samples were used as a high-quality father form.

15 forms were selected to enrich the collection and to be used in future selection works.

All samples identified based on morphology and stored at the Institute of Genetic Resources (IGR) of the National Academy of Sciences (NAS) of Azerbaijan.

## RESULTS AND DISCUSSION

### Natural hybridization in common bean

The common bean is mainly self-pollinated; however, many authors have reported outcrossing or natural hybridization

in both wild and cultivated populations. Ibarra-Perez *et al.* (1997) reported cross-pollination rates between 0 and 85%. Low occurrence of cross-pollination depends on the biological characteristics of the flowers of beans. The floral structure of *P. vulgaris* contributes to the high rate of self-pollination: Anther dehiscence and stigma receptivity occur at the same time, before the flower is fully open and the anthers and stigma are positioned near one another at the time of anther dehiscence and stigma receptivity (Webster, Tucker and Lynch, 1977).

According to the researchers, random cross-pollination in beans is very rare compared to self-pollination, which is up to 3% in black soil regions, 0.5-1% in non-black soil regions and up to 5% in the Caucasus (Ivanov, 1961).

Although *P. vulgaris* is self-fertilized, recent evidence indicates that the high nectar production level in this species is completely out of character with its pollination mechanism.

The degree of cross-pollination of common beans is also highly dependent on the environment and the presence of certain pollinators. In close plantings, hybridization between neighboring ones can be from 0.5% to about 5%. In our study, it was 3.43% (Omer Sozen *et al.*, 2018).

### Study of hybrid forms obtained as a result of natural hybridization and selection of hybrid forms with complex traits

Natural hybridization in beans has occurred through cross-pollination of self-pollinated flowers that opened with pollen from unrelated, closely growing genotypes. Pollen transfer between bean flowers may be due to the influence of wind, insects and other pollination factors. It is thought that it is carried out more often by representatives of the bee family (Apidae). Their highest activity was observed during the period of mass flowering of the bean plant. Hybrid plants differed from their parents in the shape and color of the bean and seed. The diversity obtained in the color and shape of the seed and bean is an indicator of genetic variation. In F2, deviations from normal splitting were detected in individual quality traits, which is expressed in an increase in the frequency of alleles in the population. It was also determined that natural hybridization occurred between the parent form AzePHA-16 (light pink flowers, striped beans on a green background, green stem, purple seeds) and the parent form AzePHA-t/15 (purple flowers, dark purple pods, anthocyanin-rich stem and light brown seeds). This was determined based on the plant from which the hybrid seeds were obtained.

In natural hybridization, the splitting of the F2 generation occurred according to Mendel's laws. Thus, the F2 generation is obtained as a result of self-pollination or crossbreeding of the F1 generation hybrids. The splitting in these figures (Fig 1-7) also corresponds to the F2 generation. Because in the F2 generation, phenotypic diversity and splitting of characteristics were observed according to Mendel's law. Beans and seeds with different shapes and colors indicate genetic variation between

hybrids. On the other hand, if the resulting plants were hybrids obtained from two different parental lines, then different seed shapes and colors should appear in the F2 due to the splitting of dominant and recessive traits. This was also encountered in our study.

During this study, a rich source material for selection on a broad genetic basis was created.

Bean hybrids were not observed in the first generation of harvested seeds. The hybrid seed looked identical to the parent seed F1 (Fig 1-3).



Fig 1: Mother type.



Fig 2: Father type.



Fig 3: Hybrid seed F1.

The flowers of the hybrid plants obtained from hybrid seeds were purple, the pods were dark purple, the stem was anthocyanin and the grains were ala on a light-brown background (Fig 4-5).

In the second generation, the division became more complex. In F2, the division by phenotype occurred according to the law of free (independent) distribution of traits (genes), as in polyhybrid crosses. Since self-pollination occurred, various forms were obtained from them for the next generations. Thus, in F2, 28 genetic classes were obtained by phenotype.

In the study, more complex combinations were determined as a result of the epistatic and complementary effect of genes in subsequent generations. As a result of hybridization, it was possible to obtain not only forms that include the signs and characteristics of the parent form, but also organisms that can develop completely new qualities. As can be seen, common bean has shown extremely high variability of variation due to recombination, genotypic and somatic variation during natural hybridization. Among the natural variations, the color and shape of the seed has a wide place. Although the color of the beans is the same in the hybrid plants, there is a considerable diversity in terms of the seed coat (Fig 7).

The upper generation hybrid forms obtained from natural hybridization differed from the parental forms and other specimens of the collection in terms of productivity, resistance to stress factors, mainly drought.

It should be noted that grains with such colored seed coat were not found in our collection.

Success in improving yield, quality, resistance to biotic and abiotic resistance of derived forms mainly depends on the natural variability present in the population. Selection for grain yield was continued until the material was homozygous.

#### The inheritance of the traits color of seeds

In our experience, the most variability was found in the colors of the seeds. A single factor was identified as responsible for pigmentation, while two linked factors were identified to control mottling; this appears to be the first report of linkage in beans. Furthermore, Sax (1923) was the first to report linkage between a Mendelian character (seed coat pigmentation) and a QTL (for seed size).



Fig 4: Plants grown from hybrid seeds (F2).



Later, Lamprecht showed the existence of 2 main color genes for shaping the color of a number of organs in beans: The P gene, which is necessary for determining the color of the seed coat, flower and stem and the T gene, which determines one color in all seeds and flowers. In addition to these basic color genes, Lamprecht distinguished 6 color genes (additional Prakken factors).

The C gene determines the sulphur-white color of the seed coat (geschwefeltes weisse).

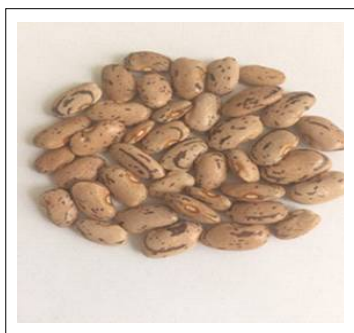
The J-gene determines the pink-yellow color (rohseidengelb) and strong luster of the seed coat, as well as the yellow-brown to ochre-yellow color of the scar margin. The G-gene determines the pale yellow color of the seed coat and the yellow-brown color of the scar margin.

The B-gene determines the purple-white color and brightness of the seed coat, *i.e.* fine purple spots on a white background, as well as the yellow-brown color of the edge of the scar.

The V gene has a pleiotropic effect on the flower, stem and seed coat, coloring the corolla deep purple, the stem red and the seed coat greenish-white (glaucescens) and causing the seed coat to shine.

The R-gene determines the manifestation of light pink color and generally red color in combination with other genes, as well as the brightness of the seed coat.

Research is ongoing to determine the heritability of seed color characteristics in common bean. So, in recent studies, researchers have come to the following conclusion (Dittmer, 1937).



**Fig 5:** Seed obtained from a hybrid plant (F2).

Alleles of the genes: Gy, C, R, J, G, B, Rk interact with the gene V, as well as among each other and as a result of that, produce different shades of the common bean seed coat color. Shaw and Norton subdivided the group of yellow-black colored common bean seeds on the following subgroups: seeds with yellow seed coat (control of the gene C), brown (gene F) and black (gene G). They define the following dominance between genes:  $G \rightarrow F \rightarrow C$ . These authors suggest that the light-brown color of the seeds was determined by the additional gene H. Kooiman proposes a new scheme for determination of the seed's color, which is completely different from the previous ones. According to this scheme, three genes interact with the essential gene A, to display the corresponding seed coat color. These three genes are B, C and D. The genotype A-B- defines a lemon-yellow color, while the A-C- determines the yellow or orange color. A-D- control gray-yellowish seed coat color. All of these genes, in different genotypic combinations, control the diversity of common bean seed coat colors and patterns. (Basset *et al.*, 2002).

In our experiment, the formation of different shades of color of the seed coat has once again confirmed the interaction of genes.

#### Economically significant signs

In Azerbaijan, common beans are the most widely used legume. They are used in almost all seasons of the year in the preparation of various dishes, from cold snacks (salads) to hot dishes. Despite such widespread use, the variety of beans in our country is quite small (Asadova *et al.*, 2016). To meet the need for beans, they are necessarily imported from abroad. This also means high costs. Our goal in the research conducted is to enrich the collection using the existing opportunities and various methods and to create new varieties suitable for local conditions. Each breeder must be able to correctly assess the opportunities provided to him and direct them in the direction of his interests. When appropriate methods are used efficiently and in a timely manner in the creation of a new form or variety, the result is high. One of these methods is natural hybridization. We studied these hybrids discovered by chance and evaluated them in the direction of our interests. High heritability accompanied with high genetic advance as per cent of mean was recorded for plant height (cm),



**Fig 6:** Phenotypic diversity of seeds and beans in F2.

bean size (length-width) (cm), the number of grains in a bean number of 100 pieces (g), yield 1 km (g) indicating the preponderance of additive gene action which may be exploited through simple selection procedures (G. Bhargavi *et al.*, 2017). Of the obtained hybrid forms, H-15, H-2, H-7, H-8, H-9, H-12, H-17, H-20, H-22, H-22, H-26, H-27, H-28 forms were selected for their high productivity and drought resistance. In the selected hybrid plants, the plant height was 65-180 cm, the bean size was 10.0-18.0 cm, the

number of grains in the bean was 5-8, the mass of 100 grains was 20.5-39.5 g and the yield was 198-280 g (Table 1).

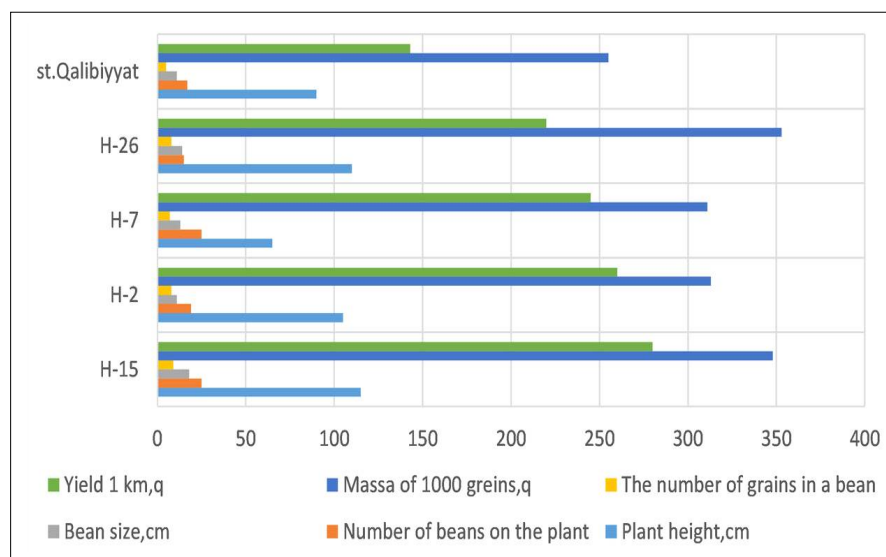
In the course of this research, a rich source material was created for selection on a broad genetic basis. Currently, lines derived from plants isolated from spontaneous hybrid populations are being studied in the hybrid nursery, as well as in preliminary and competitive variety trials. 15 forms were selected to enrich the collection and to be used in future selection works.



**Fig 7:** Diversity of grains in the same bean according to seed coat.

**Table 1:** Main economic indicators of selected samples of hybrid forms.

| Hybrid forms | Plant height, cm | Number of beans on the plant | Bean size (length-width), cm | The number of grains in a bean | Number of 100 pieces, g | Yield 1 km, q |
|--------------|------------------|------------------------------|------------------------------|--------------------------------|-------------------------|---------------|
| H-15         | 115              | 25                           | 18,0-1,2                     | 9                              | 34,8                    | 280           |
| H-2          | 105              | 19                           | 11,5-1,3                     | 8                              | 31,3                    | 260           |
| H-7          | 65               | 25                           | 11,0-1,0                     | 7                              | 31,1                    | 245           |
| H-8          | 180              | 18                           | 13,5-1,5                     | 6                              | 27,0                    | 235           |
| H-9          | 80               | 22                           | 10,0-1,0                     | 6                              | 33,2                    | 233           |
| H-12         | 120              | 24                           | 11,5-1,2                     | 5                              | 30,0                    | 230           |
| H-17         | 110              | 21                           | 13,5-1,2                     | 6                              | 36,5                    | 227           |
| H-20         | 155              | 21                           | 13,5-1,2                     | 6                              | 20,5                    | 225           |
| H-22         | 120              | 18                           | 13,5-1,2                     | 6                              | 32,0                    | 222           |
| H-23         | 125              | 17                           | 14,0-1,0                     | 7                              | 39,5                    | 221           |
| H-26         | 110              | 15                           | 14,0-1,0                     | 8                              | 35,3                    | 220           |
| H-27         | 125              | 1                            | 13,5-1,0                     | 6                              | 28,7                    | 198           |
| H-28         | 120              | 15                           | 13,0-1,0                     | 8                              | 33,3                    | 198           |

**Fig 8:** Evaluation of samples according to their quantitative and qualitative characteristics, the relationship between their characteristics.

We proceeded to the evaluation of samples for quantitative and qualitative characteristics, identifying the relationship between their characteristics. As a result of studies, the most high-yielding and high-quality samples H-15, H-2, H-7, H-26 were found in the studied beans samples (Fig 8).

## CONCLUSION

Natural hybrids have been obtained by cross-pollination between different genotypes of beans without human intervention. The observation of seeds and beans with different shapes and colors indicates that the parents have different genetic characteristics and that these characteristics are expressed in different combinations. The fact that hybrid plants have different phenotypes proves genetic variation. The genetic fragmentation that occurred in F2 indicates that this fragmentation is caused by the

combination of dominant and recessive traits in accordance with Mendel's laws. Thus, the emergence of different phenotypes in F2 from a homogeneous (having the same characteristic) phenotype in the F1 generation indicates that this is fragmentation in accordance with Mendel's laws. Our findings highlight the often underestimated conservation value of hybrid populations and indicate that they can help save species through evolution.

Natural hybrids obtained as a result of cross-pollination and considered valuable starting material have been evaluated as new forms in our collection. Of the obtained hybrid forms, H-15, H-2, H-7, H-8, H-9, H-12, H-17, H-20, H-22, H-22, H-26, H-27, H-28 forms were selected for their high productivity and drought resistance. In the selected hybrid plants, the plant height was 65-180 cm, the size of the bean was 10.0-18.0 cm, the number of grains in the bean was 5-8, the mass of 100 grains was 20.5-39.5 g and the yield was 198-280 g.

H-15, H-2, H-7 and H-26 hybrids were included in the collection as a new line because they have complex economic characteristics.

On the other hand, these forms, which differ from the collection samples in terms of their biomorphological characteristics, are also new for the Republic of Azerbaijan.

### Disclaimers

The views and conclusions expressed in this article are solely those of the author and do not necessarily represent the views of their affiliated institutions. The author is responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

### Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

### REFERENCES

- Alercia, A. (2011). Key characterization and evaluation descriptors: Methodologies for the assessment of 22 crops. Bioersity International Rome, Italy, 602 p. Methodology for the definition of a key set of characterization and evaluation descriptors for bean (*Phaseolus vulgaris* L.). p. 42-72.
- Asadova, A.I., Amirov, L.A., Abbasov, M.A. (2016). Some Types of Leguminous Plants of Azerbaijan. Baku. 183 p.
- Bassett, M. J., Lee, R, Otto, C. and McClean, P. E. (2002). Classical and molecular genetic studies of the strong greenish yellow seedcoat color in Wagenaar' and Enola' common bean. J Am Soc Hort Sci. 127: 50-55.
- Bhargavi, G., Satyanarayana, V. Rao, Ratna Babu, D., Narasimha Rao, K.L. (2017). Genetic variability studies in virginia bunch groundnut (*Arachis hypogaea* L.). Agricultural Science Digest. 37(4): 310-313. doi: 10.18805/ag.D 4524.
- Dittmer, E.E. (1937). Cultural Flora of the USSR. Edited by prof. E.W. Wulf. IV. Cereal legumes. Leningrad. pp. 478-479.
- FAOSTAT (2018). Food and Agriculture Data. Rome: FAOSTAT. [Google Scholar].
- Gonzales, J. F., Barnard, N. D., Jenkins, D. J. A., Lanou, A. J., Davis, B., Saxe, G. *et al.* (2014). Applying the precautionary principle to nutrition and cancer. J. Am. Coll. Nutr. 33 239-246. 10.1080/07315724.2013.866527 [PubMed] [CrossRef] [Google Scholar].
- Gonzalez, A.D., Frostell, B., Karlsson-Kanyama, A. (2011). Protein efficiency per unit energy and per unit greenhouse gas emissions: Potential contribution of diet choices to climate change mitigation. Food Policy. 36: 562-570. 10.1016/j.foodpol.2011.07.003 [CrossRef] [Google Scholar].
- Ibarra-Perez, F., Ehdai B, Waines, J.G. (1997). Estimation of outcrossing rate in common bean. Crop Science 37: 60-65.
- Ivanov N.R. (1961). Beans.
- Neethu, T.M., Nagarajappa Adivappar., Srinivasa, V., Girijes, G.K. (2022). Effect of spacing and fertigation on growth and yield parameters of french bean (*Phaseolus vulgaris* L.) under protected cultivation. Legume Research. 45(6): 742-748. doi: 10.18805/LR-4288.
- Omer Sozen, Ufuk Karadavut, Huseyin Ozcelik, Hatice Bozoglu, Mevlut Akcura (2017). Genotype × environment interaction of some dry bean (*Phaseolus vulgaris* L.) genotypes. Legume Research. 41(2): 189-195. doi: 10.18805/LR-354
- SAX, K. (1923). The association of size differences with seed-coat pattern and pigmentation in phaseolus vulgaris. Genetics 8: 552-560.
- Webster, B.C. Tucker, Y.S. Lynch. (1977). A morphological study of the development of reproductive structures of *Phaseolus vulgaris* L. J. Am. Soc. Hortic. Sci. 102:640-643.