Current method in the selection of legume grasses

S A Bekuzarova1,2, S G Kozyrev1, A H Kozyrev1, G V Lushchenko2, L S Gishkaeva3 and A T Doeva1

1 Gorsky State Agrarian University, Vladikavkaz, Russia
2 Vladikavkaz Scientific Center of the Russian Academy of Sciences, Vladikavkaz, Russia
3 Chechen State University, Grozny, Russia

E-mail: bekos37@mail.ru

Abstract. The paper presents the results of research on the formation of complex hybrid populations, and evaluates collection samples of clover, alfalfa, and sainfoin based on a set of economically valuable traits for creating hay-pasture varieties. The research was based on promising plants and their biomechanical mixture, which was sown in a wide row and individually in a row. Weak and underdeveloped plants were mowed down by negative selection. During the period of budding - the beginning of flowering, economic and valuable traits were taken into account, cutting plants at a height of 10-12 cm. The direct dependence of seed formation of the studied crops on weather conditions is noted. In the first year of life, alfalfa plants developed unevenly, reaching a maximum height of 58 cm and a minimum height of 26 cm. Evaluation of the productivity of the studied legume grasses (clover, alfalfa, esparcet), which was determined by the calculation method based on the mass of seeds from one plant, showed that the maximum level of seed yield was formed by samples that exceeded the standard variety by 15-20%. Polycross nurseries were created for three crops (clover, alfalfa, and sainfoin). Samples were selected for a complex of economically valuable features. Correlation dependences of selected plants on the number of internodes and seed productivity were revealed. Plants were selected based on their productivity and adaptability to form new varieties. A data bank of clover, alfalfa and sainfoin was created to form varieties with high seed productivity.

1. Introduction

To solve the problem of providing animal feed in the conditions of the North Caucasus, an important element is the cultivation of perennial legumes. Due to their ability to fix air nitrogen in symbiosis with nodule bacteria, they enrich the soil and improve its hydrophysical properties [1, 2].

Among legume grasses in the Republic of North Ossetia-Alania, the leading place is occupied by clover, alfalfa and sainfoin. Existing breeding methods allow us to create intensive varieties with potentially high but unstable productivity over the years, with reduced tolerance to bio- and abiotic stress factors [3, 4].

The issue of creating highly productive varieties can be solved by developing principles and methods for the synthesis of productive, adaptive, balanced varietal populations with different genetic structures. Such populations should consist of morphologically similar, biologically compatible, genetically different and complementary individuals that represent a balanced heterotic organism [5, 6, 7, 8].
The method of creating complex hybrid populations or synthetic varieties is most widely used. Their components can be existing varieties, clones isolated by selecting several genotypes within inbred progeny [2, 9, 10].

For cultures with hard-controlled pollination (clover, alfalfa, sainfoin) is the most appropriate polycross. It includes growing plants in a polycross nursery, where plants are freely re-pollinated among themselves or crossed with further testing of progeny [2, 4, 6].

Using the method of selection of samples in polycross nurseries with further evaluation and testing, a number of valuable varieties were created [6, 11].

In the works of herb breeders, the combination ability of the original varieties selected for synthetic populations is an important selection and genetic characteristic.

Due to the presence of open flowering, cross-pollination and selective fertilization of plants with different genotypic properties and features, a mass formation of hybrid organisms occurs with the most favorable gene interaction, which increases their viability and, as a result, productivity. In such mixtures, with their annual reproduction, the effect of multiple heterosis may appear. The meaning of this phenomenon is that the value of multiple heterosis consists in the number of generations in which the usual heterosis effect is manifested and, consequently, used [1, 4].

In order to obtain a promising source material for the formation of new varieties, polycross nurseries were created for three crops (clover, alfalfa, and sainfoin). Samples were selected for a complex of economically valuable features. Correlation dependences of selected plants on the number of internodes and seed productivity were revealed, followed by the formation of varieties with high seed productivity and adaptability in specific growing conditions.

2. Methods and objects of research
Research was conducted in breeding nurseries of complex hybrid populations (CHP) during 2014-2020. on the experimental basis of the North Caucasus research Institute of mountain and foothill agriculture of the Vladikavkaz Scientific Center of the Russian Academy of Sciences in nurseries of complex hybrid populations. The area of each plant species was 500m², sown in a wide row (row spacing 45 cm), where phenological observations of plant growth and development were made. During the growing season, poorly developed individuals were removed, leaving 235-365 plants in each nursery for further observation. Before the flowering phase, plants with low leafiness and a long period of generative stem formation were rejected, leaving plants well developed for flowering and seed formation. In the period of budding - the beginning of flowering, we took into account the height of plants, leafiness and biomass of plants, which were determined in the dynamics of development.

All the records and observations were carried out according to the method of the V. R. Williams Institute of feed [5], according to which rejected plants were removed in polycross nurseries with individual plant placement. As a result of the negative selection, insufficiently developed plants were mowed down. When selecting the best plants, attention was paid to such indicators as the number of generative stems (at least 6-8 pcs.), leafiness within 50-60%, plant height, resistance to diseases and pests, the number of internodes (as an indicator of longevity), and inflorescence semination. According to the complex of such characteristics, promising plants are left for cross-pollination, with further obtaining a biomechanical mixture and subsequent testing of progeny.

The object of research was clover varieties of domestic and foreign selection, wild samples of mountain phytocenoses. As a control, the following varieties were used: for clover - Daryal, alfalfa - Ossetian, and esparcet – double-crop North Caucasian. In statistical data processing, the best samples were compared with the standard of each culture.

On the basis of the best tolerant samples, complex hybrid populations were formed for re-pollination and formation of new promising varieties.
3. Discussion of experimental data and results of scientific research

The nursery of complex hybrid populations involves 26 varieties of clover obtained from different regions of Russia, wild forms of mountain phytocenoses, and formed synthetic populations. Out of 26 samples, 7 were selected that exceeded the standard - the zoned variety Daryal (table 1).

**Table 1.** Evaluation of promising samples of meadow clover settlement 2019 (accounting for the 2nd year of life) 1st mowing.

| The name of the sample | Plant height, cm | Leafiness, % | Green mass From the 1st m², kg | In % to standard |
|------------------------|-----------------|--------------|-------------------------------|-----------------|
| Daryal – standard      | 81.0            | 48.7         | 3.4                           | -               |
| Alan                   | 125             | 52.4         | 4.0                           | 117.6           |
| Vladikavkaz            | 120             | 50.8         | 3.2                           | 94.1            |
| Nart                   | 98              | 51.6         | 3.6                           | 105.9           |
| Farn                   | 100             | 52.0         | 3.5                           | 102.9           |
| Syn 305-03             | 115             | 54.0         | 3.7                           | 108.8           |
| Syn 300-99             | 115             | 53.5         | 3.8                           | 111.8           |
| T-100 (VIC)            | 105             | 50.2         | 3.7                           | 108.8           |

From the data given in table 1, it follows that the maximum excess of the green mass yield was achieved by the Alan variety, which exceeded the Daryal standard by 17.6%.

Local varieties Vladikavkazsky and Alan (110-120 cm) reached the maximum height. Synthetic populations were highly leafy: Syn 305-03 and SYN 300-99 (53-54%). After mowing, these samples are left for free re-pollination and obtaining seeds of complex hybrid populations.

Based on the account of seed productivity, samples of T-46 (selection of the Institute of feed), a new synthetic population of Syn 274-94 and the zoned variety Daryal were selected.

Counting the number of flowers of the studied varieties showed that this indicator varies between 75-90 pieces in one head, and they formed seeds from 17 to 70 pieces, which fully depends on the quality of nectar and pollinating activity of insects - pollinators. (table 2).

**Table 2.** Seeding of meadow clover inflorescences (2nd year of life).

| The name of the sample | Quantity of flowers, pcs. | Quantity of seeds, pcs. | Seediness of inflorescences, % |
|------------------------|---------------------------|-------------------------|-------------------------------|
| Daryal – standard      | 90.6                      | 48.8                    | 53.8                          |
| Alan                   | 89.4                      | 49.1                    | 54.9                          |
| Vladikavkaz            | 81.1                      | 35.4                    | 43.6                          |
| Nart                   | 88.3                      | 38.2                    | 43.3                          |
| Farn                   | 71.3                      | 35.7                    | 50.0                          |
| Syn 274-94             | 81.1                      | 26.6                    | 32.7                          |
| Syn 295-97             | 75.4                      | 17.7                    | 23.4                          |
| Syn 300-99             | 94.0                      | 58.8                    | 62.5                          |
| Syn 305-03             | 75.4                      | 17.7                    | 21.2                          |
| T-46                   | 90.8                      | 60.5                    | 66.7                          |
| T-70                   | 75.2                      | 31.0                    | 41.2                          |

The flowering plants left for re-pollination had different seminations, ranging from 23-66%.
In the crops, in different years of testing, seminations were observed in individual plants, the seeding rate of which did not exceed 15-20%, which is explained by unstable weather conditions and, as a result, the absence of pollinator insects.

In the alfalfa breeding nursery, plants from the new variety Osetinskaya were selected. The basis for the study was promising plants and their biomechanical mixture, which was sown in a wide row. Weak and underdeveloped plants were mowed down by negative selection. During the period of budding - the beginning of flowering, economically valuable traits were taken into account, discarding unproductive and underdeveloped plants. The total number of plants selected for further study was 235.

One of the indicators of alfalfa that correlate with high winter hardiness is the number of internodes. The number of internodes also affected the survival of plants during the drought period (the third ten days of July). 36 items were selected out of 235 studied. The correlation coefficient was from 0.68 to 0.72.

Accounting for green mass showed that from the selected samples (28 pcs.), the yield of green mass in the first year reached about 100g. per plant.

The selected plants were resistant to the diseases ascochitosis and blue mould (table 3).

| № of breeding samples | Ascochitosis | Blue mould |
|-----------------------|--------------|------------|
|                       | % of defeat  | % of development | % of defeat | % of development |
| 18                    | -            | -           | 3.0        | 0.6           |
| 23                    | 4.0          | 1.0         | -          | -             |
| 27                    | 1.3          | 0.5         | -          | -             |
| 31                    | 15.0         | 1.9         | -          | -             |
| 35                    | 5.0          | 0.6         | 4.0        | 0.3           |
| 38                    | 15.0         | 1.9         | 8.0        | 1.0           |
| 42                    | 12.0         | 1.5         | 5.0        | 0.6           |
| 49                    | 22.0         | 0.8         | 6.0        | 0.7           |

The noted plants with morbidity had a low number of leaves and offsets. Such undeveloped plants were removed.

In the first year of life, alfalfa plants developed unevenly, reaching a maximum height of 58 cm and a minimum height of 26 cm. The characteristics of plants in the nursery are shown in table 4.

| Values               | Plants best | medium | worst |
|----------------------|-------------|--------|-------|
| Plant height, cm     | 36          | 56     | 143   | 60.8  |
| Bushiness, pcs/m²    | 23          | 148    | 64    | 27.2  |
| Green mass, c/ha     | 32          | 68     | 135   | 57.4  |

From the data shown in table 4, it follows that plants of the average level for green mass are 28.9 %, which is more than 2 times higher than the best. The minimum number of selected plants for tilling capacity in the best plants is 9.8%. The largest number of plants with low indicators is rejected at the beginning of budding.
In the nursery of CHP (complex hybrid populations), 50 plants were selected from 365 esparcets based on economically valuable traits, of which 5 groups were formed based on phenotypic traits. These groups were compared in research with the standard zoned variety double-crop North - Caucasian.

The studied varieties differed slightly in the duration of the growing season, which was 95-97 days (table 5).

**Table 5. Duration of the phases of development of sainfoin.**

| Examples                        | Duration of interphase periods, days | Length of the growing season, days |
|---------------------------------|-------------------------------------|-----------------------------------|
|                                 | budding-flowering | flowering-fruit formation | fruit formation-maturation |                                 |
| Double-crop North Caucasus (standard) | 13 | 25 | 21 | 95 |
| 1-10                           | 14 | 27 | 22 | 97 |
| 11-20                          | 13 | 27 | 21 | 95 |
| 21-30                          | 12 | 25 | 20 | 96 |
| 31-40                          | 12 | 26 | 20 | 95 |
| 41-50                          | 13 | 25 | 21 | 95 |

The duration of the budding -flowering period in most samples was 12-14 days, and the flowering -fruiting period was 25-27 days. An increase in the flowering period of the sainfoin is important in achieving the optimal multiplicity of pollination transfer by insects and increasing the probability of fertilization of the flower. Varieties 1-10 and 11-20 were characterized by a longer duration of this period.

When studying the biology of esparcet flowering, it was found that the largest number of flowers form samples №11-20 and №41-50, exceeding the standard variety by 3 and 2 pcs./inflorescence. However, cultivars №41-50 are significantly concede to the standard in the number of ovaries per inflorescence (table 6).

**Table 6. Indicators of flowering and setting of esparcet fruits.**

| Examples                        | Number flowers, pcs./inflorescence | Number open flowers, pcs./inflorescence | Number ovaries, pcs./inflorescence | Fruit set, % |
|---------------------------------|------------------------------------|----------------------------------------|-------------------------------------|-------------|
| Double-crop North Caucasus (standard) | 40 | 37 | 12 | 32.4 |
| 1-10                           | 41 | 39 | 11 | 28.2 |
| 11-20                          | 43 | 40 | 14 | 35.0 |
| 21-30                          | 40 | 38 | 12 | 31.6 |
| 31-40                          | 40 | 39 | 13 | 33.3 |
| 41-50                          | 42 | 39 | 11 | 28.2 |

It was found that up to 43 flowers are formed in the inflorescences of the studied varieties of esparcet. The fruit setting rate was 28.2-35.0%.

Important technological and morphological characteristic of a plant variety is the height of the plants. For haymaking grass, increasing its height increases the coefficient of alienation of its ground mass in the absence of lodging (table 7).
Table 7. Main indicators of esparcet productivity.

| Examples                        | Heigh, cm | Leafiness, % | Quantity of beans, pcs./escape | The number of seeds in a bean, pcs. | Weight of 1000 seeds, g. |
|---------------------------------|-----------|--------------|---------------------------------|-------------------------------------|-------------------------|
| Double-crop North Caucasus (standard) | 92.4      | 41.5         | 13.7                            | 4.8                                 | 17.3                    |
| 1-10                            | 93.1      | 43.0         | 13.9                            | 4.5                                 | 17.5                    |
| 11-20                           | 97.7      | 45.2         | 14.5                            | 5.0                                 | 18.7                    |
| 21-30                           | 94.5      | 43.7         | 14.0                            | 4.7                                 | 18.3                    |
| 31-40                           | 95.0      | 42.6         | 13.5                            | 4.5                                 | 18.0                    |
| 41-50                           | 93.8      | 43.3         | 14.1                            | 5.2                                 | 17.9                    |

At the same time, the very high height of the stems is one of the reasons for the lodging of the grass, which leads to a deterioration in the quality of feed, a decrease in yield, and difficulties in harvesting.

In our experiments, the tallest samples were №11-20 - 97.7 cm, significantly exceeding the standard height by 5.3 cm.

For the formation of high yields of forage crops, both the height of plants and their leafiness are of great importance.

Up to 95% of the total dry matter mass accumulated by forage crops during the growing season is created during photosynthesis. In this regard, the study of the leafiness of esparcet plants, the selection of varieties with optimal leaf surface sizes and photosynthetic potential indicators are of significant theoretical and practical importance.

Thus, the efforts of breeders should be aimed at increasing the area of the assimilation surface, complete preservation of the leaf apparatus and increasing the duration of its life.

Of the studied varieties, №11-20 and 21-30 were characterized by the highest leafiness. The plants of the two selected groups of cultivars exceeded the standard in leafiness by 2.2 and 3.7%, respectively.

Performance evaluation as determined by the calculation method, based on the weight of seeds per plant and plant population, and the analysis of its structure found that the maximum yields of seed formed samples №11-20. The yield of this group of varieties exceeded the standard double-crop North Caucasian variety by 0.07 t/ha.

4. Conclusions
The difficult-hybrid populations were obtained. The assessment of collection samples of clover, alfalfa, and esparcet is given based on a complex of economically valuable traits for creating hay-pasture-type varieties.

The best samples were selected for the yield of green mass, bushiness, and plant height.

The source material was obtained based on the characteristics of adaptability, green mass yield, leafiness and seed productivity.

Selected promising clover samples for high seed productivity, reaching more than 60%.

Based on the cross-pollination of the best genotypes complex hybrid populations were formed for further breeding and evaluation.

Reference
[1] Bekuzarova S A and Sokolova L B 2015 Evolution and introduction of clover plants Izvestiya of Gorskiy GAU (Vladikavkaz) 52(1) 219-23
[2] Polyudina R I 2017 Clover in Siberia (Novosibirsk, Russia: Monograph) p 168-77
[3] Kozlov N N and Banadysev S A 1987 Physiological aspects of selection of meadow clover for winter hardiness and productivity Sb. n. Tr. ARSRI of feeds 137-41
[4] Kuleshov G F and Kramnik A N 1986 The use of heterosis to improve productivity Bulletin of agricultural science 11 86-91
[5] 2012 Methodological Guidelines for the Selection of Perennial Herbs (Moscow, Russia: VIC)
p 116

[6] Novoselova A S 1989 Results and prospects of selection of meadow clover Selection of forage crops 14-5

[7] Taylor N L 2008 A century of clover breeding developments in the United States Crop Science 1 1-13

[8] Afsharmanesh G 2009 Study of some morphological traits and selection of-drought-resistant alfalfa cultivars (Medicago sativa L.) Plant Ecophysiology 3 109-18

[9] Bekuzarova S A, Samova M T, Kotaeva M A and Gelashvili K TS published on 27.02.2012 Method of Selection of Initial Samples of Meadow Clover in Natural Phytocenosis (Russia) Patent №2464778

[10] Bell M L, Goldberg R, Hogrefe C, Kinney P L, Knowlton K, Lynn B, Rosenthal J, Rosenzweig C and Patz J A 2007 Climate change, ambient ozone, and health in 50 US cities Climatic Change 82 61-76

[11] Sakanoue S 2005 Demography of red clover seeds in mixed-sown meadows The Journal of Agricultural Science 2 193-7

[12] Naidovich V and Popov T N 2014 And meteorological effects on seed productivity of alfalfa in the arid Volga River basin Reports of Russian Academy agricultural Sciences