Creation of a new breeding material for creeping clover (*Trifolium repens* L.) hay-pasture type using the hybridization method

A A Ivanova

Federal State Budgetary Institution "Federal Scientific Center for Forage Production and Agroecology named after V.R. Williams ", 141055, Russia, Moscow region, Lobnya, st. Scientific town, bldg. 1

Corresponding author e-mail: alinkaiv85@mail.ru

**Abstract.** The studies were carried out in the Moscow region in 2019-2021. In order to create a new breeding material for creeping hay-pasture clover, samples of the Lodian type (giganteum variety), characterized by high peduncles and longer leaf petioles, and a low content of hydrocyanic acid (0.3 mg%), were selected as parental forms. Reciprocal crossing was carried out. Hybrids F₁ and F₂ in the first year of life were assessed by the main economic and biological characteristics: the length of the peduncles, the number of inflorescences on the plant, fodder and seed productivity, early maturity, winter hardiness and other indicators. Based on the assessment results, the best hybrids and individual biotypes were identified.

**Keywords:** creeping clover, hybrid, selection.

1. Introduction

Solving the problem of feed protein both in the Russian Federation and throughout the world remains the most important priority of agricultural science and practice. Creeping clover (*Trifolium repens* L.) is a valuable legume component for the creation of cultivated meadow and pasture agrophytocenoses in regions with a temperate climate. The crop is characterized by extremely high feeding properties. It contains from 17.5 to 26.0% crude protein, depending on the cut. High palatability ensures excellent eatability of creeping clover, and its digestibility is about 80%. Pastures that include creeping clover in the mixture do not require additional nitrogen fertilization and do not adversely affect animal health due to the accumulation of non-protein nitrogen and nitrates in the feed. The productivity of herbage containing creeping clover is equivalent to cereal, under which 120-180 kg / ha of nitrogen fertilizers are applied (Piskovatskaya, Shmatkova, Makaeva, 2019). In the soil and climatic conditions of Russia, in different regions, pasture binary mixtures of creeping clover with perennial ryegrass or festulolium are of economic importance and are highly effective forage agrophytocenoses, which make it possible to receive from 6.3 to 7.1 thousand fodder in three to four grazing cycles annually. unit / ha with low cost and content of 10.5-10.6 MJ of exchange energy in 1 kg of dry matter; 1.12-1.24 t / ha of crude protein, to ensure the production of 77-80 GJ / ha of exchangeable energy (Kutuzova, Provornaya, Sedova, 2007).

Currently, with an increase in the number of cattle and the intensification of processes in dairy and beef cattle breeding, the need for highly productive grassland agricultural systems and an increase in...
their areas is significantly increasing. Constant improvement of natural hayfields and grazing for livestock, reanimation of degraded cultivated pastures in various ways on an area of at least 0.5 million hectares annually require more than 1.0 thousand tons of creeping clover seeds in the coming years, and in the future - about 2.0 thousand t of high-quality seed material of this culture (Zolotarev, Pereravo, 2019).

Despite the many valuable properties of this crop, seed production has a number of difficulties, including due to the imperfection of the existing harvesting technique for harvesting stunted grass stands.

The source material, as N.I. Vavilov, predetermines the success of breeding work. Therefore, the correct choice and its use in breeding are of great importance. Wild populations, local and breeding varieties of both domestic and foreign origin, as well as forms created using hybridization, mutagenesis, cell selection, polyploidy, etc., can serve as a source material for breeding.

The aim of the research was to create a new hybrid material with increased seed and fodder productivity for hay-pasture use.

2. Methods of the research
This work was carried out under the conditions of a selection greenhouse complex and on growing plots. Initial samples in 2019 were studied in conditions of a selection greenhouse complex in boxes. The cultivation was carried out in vessels with a capacity of 3 liters, in each variant 20 genotypes were studied. During the research, manual cross-pollination was carried out. Study and evaluation of hybrids \( F_1 \) and \( F_2 \) was carried out on the growing plots of the selection greenhouse complex in 2020 (\( F_1 \)) and in 2021 (\( F_2 \)). In the experiment, 4 hybrid combinations of 50 genotypes of each hybrid were evaluated \( F_1 \) and \( F_2 \). The VIK 70 variety was used as a standard. The experiment was laid by nesting seedlings, one plant per hole. Planting scheme 50x50 cm. Sowing of meadow timothy served as an isolate between the variants. The experiments, observations and counts were carried out according to the methodology (Guidelines for selection and primary seed production of clover, 2002).

Agrochemical soil characteristics: pH salt extract 7.05; \( \text{P}_2\text{O}_5 \) – 30 mg per 100 g of soil (according to Kirsanov); \( \text{K}_2\text{O} \) – 26 mg per 100 g of soil (according to Maslova); humus - 4.6% (according to Tyurin); hydrolytic acidity - 0.6 mg equivalent per 100 g of soil.

3. Results of the research
The creation of a variety using hybridization methods implies the selection of parental pairs for crossing based on knowledge of the characteristics of the parents who want to combine in new varieties. In this breeding work with creeping clover, the method of intraspecific hybridization was used based on artificial crosses and free-limited cross-pollination between selected cultivars and individual biotypes. In order to create a new breeding material for creeping hay-pasture clover, samples of the Lodian type were selected as parental forms (variety giganteum): Atholay (Latvia), Gigant (Ukraine) and Trefle blancs (Netherlands). The samples were characterized by large leaf blades, long petioles and peduncles, as well as a low content of cyanogenic glycosides (0.3 mg%). The protein content of the Atolay, Gigant and Trefle blancs varieties is at the standard level. The parental form was planted in the vessels in March 2019. The reciprocal crosses were carried out in June. As a result, hybrid seeds Atolay x Trefle blanc, Trefle blanc x Atolay, Gigant x Trefle blanc and Trefle blanc x Gigant were obtained.

Evaluation of hybrids \( F_1 \) and \( F_2 \) according to the intensity of the onset of plant development phases in the first years of life, showed that the number of early maturing biotypes in the Trefle blanc x Atolay and Atolay x Trefle blanc hybrids is 4.50% and 3.25% higher than the standard, respectively.

Based on the analysis of the main morpho-biological characters, the Trefle blanc x Atolay hybrid is of particular interest. This hybrid combination exceeds the standard: in the length of the peduncle by 47%, the size of the leaf blade by 26-33%, the diameter of the bush by 34%, the number of peduncles per plant by 68% and the number of flowers in one head by 36%. Data on the morpho-biological characteristics of the hybrids \( F_1 \) and \( F_2 \) creeping clover in the first years of life were identical, in connection with which the data were combined and the average value was calculated (table 1).
The crude protein content in absolutely dry matter varies from 16.50% (F1 Atolay x Trefle blanc) to 18.81% (F1 Giant x Trefle blanc) and is at the standard level (VIC 70 17.31%).

**Table 1.** Morpho-biological characteristics of F1 and F2 hybrids of creeping clover on average over the first years of life (2020-2021).

| Variant                  | Sheet length plates, cm | % to the standard | Leaf blade width, cm | % to the standard | Leaf petiole length, cm | % to the standard | Petiole diameter sheet, mm | % to the standard | Inflorescence length, cm | % to the standard | Inflorescence diameter, cm | % to the standard |
|--------------------------|-------------------------|-------------------|----------------------|-------------------|------------------------|-------------------|-----------------------------|-------------------|--------------------------|-------------------|-----------------------------|-------------------|
| VIK 70 standard          | 2.40                    | 100,00            | 1.90                 | 100,00            | 12.32                  | 100,00            |
| F1₂ Atolay x Trefle blanc| 3.10*                   | 106,25            | 2.00                 | 105,26            | 12.46                  | 101,14            |
| F1₂ Trefle blanc x Atolay| 3.20*                   | 133,33            | 2.40*                | 115,79            | 15,32*                 | 124,35            |
| F1₂ Giant x Trefle blanc | 2.90*                   | 120,83            | 2.20                 | 111,50            | 13,07                  | 106,09            |
| F1₂ Trefle blanc x Giant | 2.50                    | 104,17            | 2.30*                | 121,05            | 13,60*                 | 110,39            |
| LSDₙ₀₅                   | 0.40                    | 0.35              | 1.04                 |                   |                         |                   |

| Variant                  | Peduncle length, cm | % to the standard | Diameter peduncle, mm | % to the standard | Stolon diameter, mm | % to the standard |
|--------------------------|---------------------|-------------------|-----------------------|-------------------|---------------------|-------------------|
| VIK 70 standard          | 18.00               | 100,00            | 0.23                  | 86.96             | 0.25                | 100,00            |
| F1₂ Atolay x Trefle blanc| 21.60*              | 120,00            | 0.20                  | 96.00             | 0.26                | 104,00            |
| F1₂ Trefle blanc x Atolay| 26.36*              | 146,45            | 0.24                  | 104,35            | 0.24                | 96.00             |
| F1₂ Giant x Trefle blanc | 20.10*              | 111,67            | 0.22                  | 95.65             | 0.27*               | 108,00            |
| F1₂ Trefle blanc x Giant | 22.00*              | 122,22            | 0.22                  | 95.65             | 0.26                | 104,00            |
| LSDₙ₀₅                   | 1.80                | 0.01              | 1.01                  |                   |                     |                   |

| Variant                  | Bush diameter, cm | % to the standard | Number of peduncles per plant, pcs. | % to the standard | Number of flowers in one head, pcs. | % to the standard |
|--------------------------|------------------|-------------------|-------------------------------|-----------------|-----------------------------------|------------------|
| VIK 70 standard          | 41.00            | 100,00            | 21.83                         | 100,00          | 77.80                             | 100,00           |
| F1₂ Atolay x Trefle blanc| 50.00*           | 121,95            | 30.95*                        | 141,78          | 106.20*                           | 136,50           |
| F1₂ Trefle blanc x Atolay| 55.00*           | 134,15            | 36.59*                        | 167,61          | 105.80*                           | 135,99           |
| F1₂ Giant x Trefle blanc | 42.00            | 102,44            | 19.80                         | 90.70           | 114.20*                           | 146,79           |
| F1₂ Trefle blanc x Giant | 47.00*           | 114,63            | 19.45                         | 89.10           | 109.80*                           | 141,13           |
| LSDₙ₀₅                   | 4.50              | 3.46              | 11.6                          |                 |                                   |                   |

Note: * – significant difference from the standard at a 95% probability level.
The studied hybrids are characterized by an erect bush shape, which is characteristic of the variety *giganteum*. In the genotypes of the studied hybrids, samples with distinctive features are distinguished. In the Atolay x Trefle blanc hybrid, plants with pink inflorescences stand out. Among the genotypes of the Trefle blanc x Gigant hybrid, there are individual plants with large inflorescences with up to 300 flowers per head.

The characteristic of creeping clover hybrids in terms of seed productivity was determined by the mass of seeds from one bush. The Trefle blanc x Altayskiy hybrid was distinguished by the maximum seed productivity - 6.50 g per plant, which exceeded the standard by 20%. By the weight of 1000 seeds, the studied samples do not exceed the indicator of the standard (0.54 g).

Winter hardiness was determined by the number of plants before winter and the number of plants after regrowth. At this stage of research, the winter hardiness of hybrids is at the standard level, which is 95-100%.

4. Conclusion
The study of the phenotypic variability of the main economically important traits of hybrids of the first and second generations of creeping clover confirmed the presence of a reserve for the selection of valuable plant forms and the possibility of creating a new genetically stable creeping clover material with specified traits and properties for hay-pasture use. The analysis of the F1 and F2 hybrids showed that the F1 Trefle blanc x Atolay hybrid turned out to be the most promising material for further breeding work.

References
[1] Zolotarev V.N. Actual problems of selection and varietal seed production of creeping clover (Trifolium repens L.) in Russia and directions of their solution in the context of import substitution / V.N. Zolotarev, N.I. Perepravo // Feed production.- 2019. - № 12. – p. 26-34).
[2] Kutuzova A.A. Clover-ryegrass mixtures for pastures in the Non-Black Earth Zone / A.A. Kutuzova, E.E. Provornaya, E.G. Sedova // Feed production.- 2007. - №4. – p. 6-10.).
[3] Guidelines for selection and primary seed production of clover. - M., 2002. - 71p.
[4] Piskovatskaya R.G. The main directions of selection of creeping grassland clover for use in irrigated conditions / R.G. Piskovatskaya, A.A. Shmatkova, A.M. Makaeva // Irrigated agriculture.- 2019.- № 1.- p. 22-23.