

Initial material of hybrid clover (*Trifolium hybridum* L.) of various ecogeographical origins in the Carpathian region

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Hybrid clover is a valuable component of onions and pastures. Twenty-six hybrid clover cultivars of various ecological and geographical origins, obtained by different methods, served as the material for the research. The standard is the Pridnestrovskaya variety. The initial material was evaluated according to the "Methodology for the selection of perennial legumes and crops in the Carpathian Region" and "The methodology for forming a collection of field crops resistant to the biotic factors". Statistical processing of the data was carried out using correlation and variance methods and assessing the significance of the difference in the mean of the sample using the t-criterion using the TIBCO Statistica 13.5.0.17 software package (1984-2018 Tibco Software inc.). The coefficients of variation were 1.4-29.7, depending on the biological and ecological characteristics of the hybrid clover.

Keywords: Relationships, Source material, Economic and biological characteristics, Cluster analysis, Productivity.

Introduction

Hybrid clover, pink, or swedish (*Trifolium hybridum* L.) ($2n=16-32$) is a perennial plant, a valuable legume component for the creation and improvement of cultivated onions and pastures. They were used for hay, green food, and grazing. In its pure form, through a bitter taste determined by the content of cyanogenic glycosides, it is eaten worse than red clover, and therefore it is more often used in a mixture with it and cereal grasses. It is nutritionally as good as red clover and has a lower fiber content (Hai-xia 2013 et al.; Scot & Sutherland 1984). Hybrid clover grows on acidic, peat-boggy, clayey soils. It is moisture-loving, does not tolerate drought well, withstands flooding well (up to 15-20 days), but does not tolerate stagnant water. Under cultural conditions, it lasts 2 years, liquefies strongly after harvesting seeds. Mainly yields a high yield of hay for 2 years in a row (two mows per year) and seeds-once. The seed yields are more stable and higher than that of the meadow clover.

There are two ecological forms of hybrid clover:

1. *Trifolium fistulosum* Gilib.-a plant with hollow stems. It grows in northern regions and areas with high humidity.
2. *Trifolium elegans* Savi is a full stemmed plant. Mostly low-productive, wild-growing forms are found in southern regions in dry places.

Its highest yield is obtained in the second year after sowing. On moist soils, where red clover is less productive, it is advisable to sow it in mixes, which winters better and does not freeze out as quickly as red clover (Lugic et al., 2009; Ford & Barrett, 2011). It can be plowed in like green manure. *T. hybridum* is common throughout Europe and frequently occurs in waterlogged meadows. As the amount of liming decreases, the soils quickly become acidified, which reduces the chances of growing acid-sensitive plant species and increases the likelihood of increased hybrid clover seed production of hybrid clover (Townsend, 1964; Scott et al., 1974; Lang & Vejrka, 2012). According to Paplauskiene V. and Dabkeviciene G., the agromorphological characteristics of hybrid clover varied within wide limits (V 0.6-71.9%). Seed yield was the most variable indicator (V 38.7-71.9%). They found nine ISSR markers for the ratio of agromorphological traits to quality indicators (Paplauskiene & Dabkeviciene, 2012).

Currently, farmers are using the harvest of harvested seeds and researching varieties imported from other countries. In search of hybrid clover varieties with improved performance for New Zealand conditions, some accessions and local varieties introduced from Russia were evaluated for the 3 leading economically valuable indicators (Widdup & Ryan, 1994). Naydenova G. assessed the phenotypic variability in dry matter and seeds and their components in hybrid clover populations originating from different regions of Europe. According to the results of the study, significant genotypic variation ($P < 0.05$) genotypic variation was observed between plants for all studied traits (Naydenova, 2017). Determining plant genetic diversity is the first step in breeding (Heathcliff & Krohn, 2010). The study of forage grasses is laborious and time-consuming since most of them are perennial and perennial plants and the traits analyzed are quantitative. Usually, breeding programs for hybrid clover are based on mass phenotypes or periodic breeding, and therefore heterogeneous varieties with highly heterozygous individuals have been created (Willmore et al., 2007).

Forming collections to create the initial material of perennial leguminous grasses provides for a preliminary study of the breeding value of varieties and their effective use in the breeding process in terms of yield and other morphobiological characteristics (Karagic et al., 2010). The ecological adaptability of varieties includes the reaction of hybrid clover to changes in growing conditions and the realization of their genetic potential against the background of these changes. Also, it characterizes the level of significance

of varieties as possible sources of valuable traits. To date, the features of the manifestation of quantitative and qualitative traits of culture, depending on genotype, growing conditions, and the like, have not been sufficiently studied.

Materials and Methods

The studies were carried out in 2016-2018, on the experimental base of the Peredkarpatsky department of scientific research of the Institute of Agriculture of the Carpathian region of the NAAS (Lishnya village, Drohobych district, Lviv region).

The soil of the experimental field is typical for the indicated region, drained by pottery drainage of soddy-middle-podzolic leaves, superficially gleyed middle oxides, loamy, formed on diluvial deposits. Weather conditions 2016-2018 They had several characteristics. Over the years of research, significant differences were observed from the long-term average data of precipitation and temperatures during the summer months, which made it possible to more thoroughly assess the growth and development of hybrid clover and the effect of unfavorable environmental conditions on productivity. Twenty-six hybrid clover cultivars of various ecological and geographical origins, obtained by different methods, served as the material for the research. The standard is the Pridnestrovskaya variety.

The initial material was assessed according to the "Methodology for the selection of perennial legumes and grasses in the Carpathian region" (Konyk et al., 2015) and the "Methodology for the formation of a collection of field crops resistant to the biotic factors" (Petrenkova et al., 2015). Statistical processing of the data was carried out using correlation and variance methods and assessing the significance of the difference in the mean sample by the t criterion (Dospekhov, 1985) using the TIBCO Statistica 13.5.0.17 software package (1984-2018 Tibco Software Inc).

Results and Discussion

For grass pasture and hay use, the height of the stand is an essential indicator, directly affecting the yield of plants and the suitability of varieties for mechanized harvesting. The height of hybrid clover plants varied depending on the variety, meteorological parameters, and nutritional conditions and was 68.00-79.00 cm; the coefficient of variation for this trait is 3.79% (Table 1). The number of stems per plant was 6-22 (CV=29.7%). The plant leaves are a valuable component in the biomass of forage crops, as they contain 2-3 times more protein than the stems. Hybrid clover is characterized by good leafiness. The plants are most leafy in the early phases of the growing season. As they grow and develop, the number of leaves decreases. The plants had a large leafing in areas with imitation of pasture, 54.9-61.8%. With the use of hay, this figure was significantly lower, 43.10-47.90%.

The formation of the hybrid clover seed yield of hybrid clover is the result of the genotype of the interaction of the plant genotype and environmental conditions, that is, a genetically determined process. So, for example, in the Carpathian region, overwintering conditions, air temperature, and the amount of precipitation from flowering to full ripeness have the most significant influence on the yield of clover seeds. In this regard, one of the tasks of the study was to study and select the varieties of the collection that are most productive in the specific agroclimatic conditions of the Carpathian region. According to the methodological approach to the analysis, which reveals the reason for the different seed productivity, the structure of the yield was taken as a basis in connection with the morphological structure of the plant: the number of stems per plant and the number of inflorescences on the stem, diameter of the inflorescence, the number of flowers in the inflorescence, the weight of 1000 seeds.

Table 1. Morphobiological characteristics of hybrid clover cultivars (average for 2016-2018).

Feature	M ± SE	min	max	SD	CV, %	t-value
Plant height, cm	73.81 ± 0.55	68.00	79.00	2.80	3.79	134.40
Number of stems per plant	10.34 ± 0.60	6.00	22.00	3.07	29.70	17.12
Number of inflorescences per stem	7.33 ± 0.08	6.50	8.00	0.40	5.45	93.05
Inflorescence diameter, cm	2.40 ± 0.03	2.00	2.70	0.17	7.08	71.13
Number of flowers per inflorescence	61.12 ± 0.87	53.00	69.00	4.44	7.26	70.20
Number of seeds per inflorescence	50.77 ± 1.17	40.00	60.00	5.98	11.78	433.24
1000 seeds weight, g	0.71 ± 0.01	0.68	0.73	0.01	1.40	250.81

We determined that hybrid clover blooms evenly, 20-28 days. It is characterized by the axillary lateral placement of the heads. The beginning of flowering in the Carpathian region occurs 5-10 days earlier than in meadow clover. The lowest head blooms first; internodes grow above it; their number increases and new heads appear. The same happens on lateral shoots.

The uniformity and duration of flowering depend on the meteorological conditions: the unfavorable the weather, the longer the hybrid clover blooms. The number of inflorescences on the stem was 6.5-8.0 pcs, and the variation coefficient was 89.0%. The diameter of the inflorescence on the plant ranged from 2.00 to 2.70 cm, with a variation of 7.08%.

Analysis of inflorescences showed that the number of flowers in the inflorescence of hybrid clover depends on the origin of the population and the growing conditions. In wild populations, the number of flowers is less than in cultivated ones. On average, the number of flowers per inflorescence ranged from 53 to 69 pcs. The coefficient of variation was 7.26%, the mass of 1000 seeds was 0.68-0.73 g, and the coefficient of variation for this trait was 1.4%. We revealed that the structure of the seed yield depends on the biological and ecological characteristics of the varieties. The coefficient of variation ranged from 1.4% for the weight of 1000 seeds to 29.7% for the number of stems per plant.

To establish the similarity of the samples for individual groups of traits andir totality and the degree of genetic divergence, cluster analysis was carried out, which allowed the classification of the hybrid clover breeding material. When constructing the dendrogram, the Euclidean metric and the single-link method were used. This method combines two samples that are most similar to each other. In the next iteration, a sample with the maximum similarity is attached to them, which leads to the formation of a cluster. Euclidean

distances are used as a measure of genetic divergence to distinguish genetically close groups of varieties. When the samples were clustered by seed yield, three clusters were formed. The largest cluster was uniting 12 varieties (No. 01716, No. 00707, No. 00546, No. 00973, No. 01719, No. 00708, No. 01810, No. 01812, No. 01758, No. 00710, No. 01820, No. 01811) with a yield of 2, 04-2,12 c ha⁻¹ (Fig. 1).

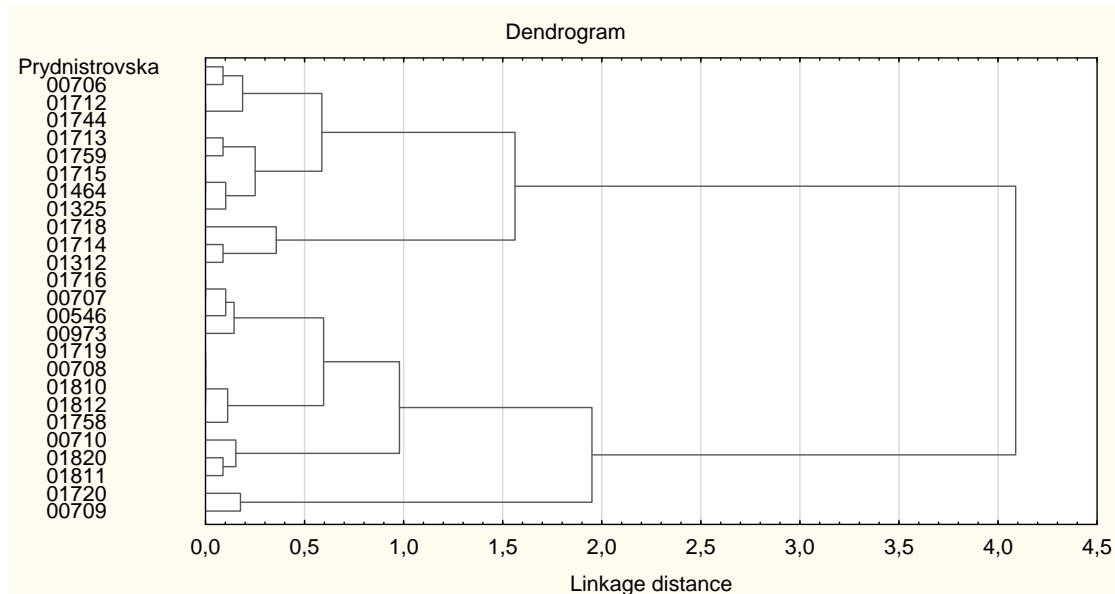


Fig. 1. Clustering of hybrid clover samples by seed yield (horizontally, Euclidean distances, vertically, sample numbers).

When divided into three groups according to the average value of the seven analyzed characteristics, the best was the group in which 12 cultivars were concentrated (Pridnestrovskaya, No. 01716, No. 00973, No. 01744, No. 01718, No. 01713, No. 01719, No. 01812, No. 01720, No. 01759, No. 01325, No. 01712, No. 00708, No. 00709, No. 00706, No. 00710) with signs that exceeded the average in other groups (Fig. 2).

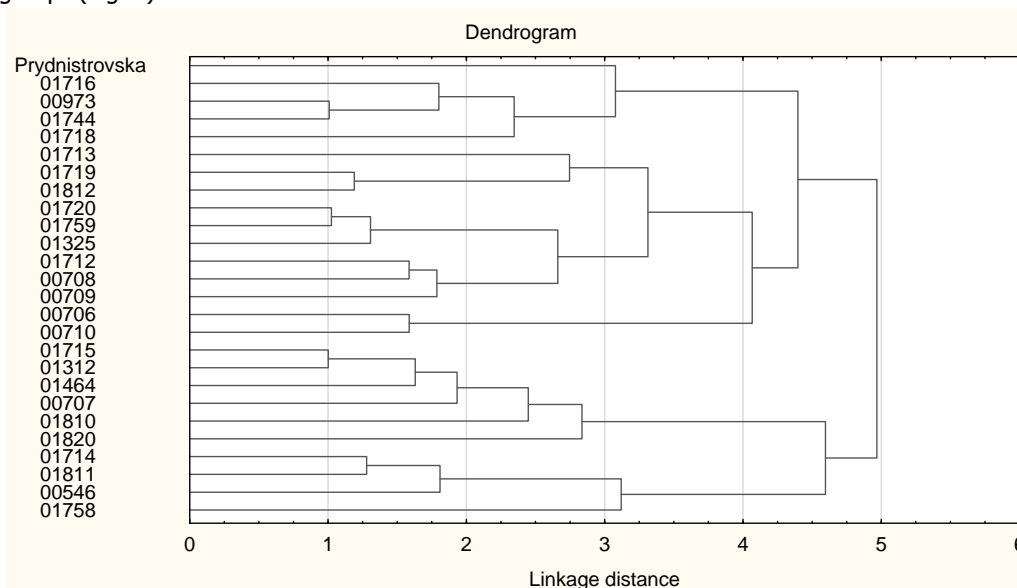


Fig. 2. Clustering hybrid clover samples according to the main economic and biological characteristics (horizontally-Euclidean distances, vertically-sample numbers).

We found that reliable correlation coefficients were obtained between the height of the plants and the number of stems per plant ($r=-0.41$), between the number of seeds in the inflorescence and the diameter of the Inflorescence ($r=0.44$), between the yield seeds and the number of inflorescences on the stem ($r=0.80$), between the yield of seeds and the number of seeds in the inflorescence ($r=0.46$), between the weight of 1000 seeds and the yield of seeds ($r=0.94$) (Table 2). Therefore, these traits should be taken into account when selecting high-performance biotypes.

Table 2. The relationship between yield and economic and biological characteristics of hybrid clover.

Feature	Plant height, cm	Number of stems per plant	Number of inflorescences per stem	Inflorescence diameter, cm	Number of flowers per inflorescence	Number of seeds per inflorescence	Number of 1000 seeds per seeds weight, g
Number of stems per plant	-0.11						
Number of	-0.07	0.11					

inflorescences per stem							
Inflorescence diameter, cm	-0.17	0.0001	-0.191				
Number of flowers per inflorescence	0.25	-0.23	-0.37	0.16			
Number of seeds per inflorescence	-0.14	-0.02	0.15	0.44*	0.01		
1000 seeds weight, g	-0.14	-0.14	-0.13	0.07	-0.13	0.17	
Seed yield, c ha ⁻¹	0.48*	0.11	0.80*	-0.17	-0.092	0.46*	0.94*

Significant at p <0.05.

Conclusion

The basis for creating new varieties of hybrid clover is the corresponding source material and knowledge of its morpho-biological characteristics. The coefficient of variation ranged 1.4-29.7, depending on the biological and ecological characteristics of the varieties. In terms of the average value of all seven traits analyzed, the best was the group, in which 13 variety specimens were concentrated, which exceeded the average in other groups.


We revealed reliable correlation coefficients between the height of plants and the number of stems per plant, between the number of seeds in the inflorescence and the diameter of the inflorescence, between the yield of seeds and the number of inflorescences on the stem between the yield of seeds and the number of seeds in the inflorescence, and between the mass of 1000 seeds and the yield of seeds.

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