

PAPER • OPEN ACCESS

## Trifolium Repense L. Breeding In The Central Chernozem Region: Main Directions And Methods Of Work

To cite this article: V M Kosolapov *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **901** 012008

View the [article online](#) for updates and enhancements.

You may also like

- [Design and Research of Wind Erosion in Sandy Land and Typical Sand Fixation Project in Small Watershed in Jiangxi Province](#)  
G Zhan Lichao, Chen Meng, Song Yuejun et al.
- [Research progress of active compounds and pharmacological effects in \*Akebia trifoliata\* \(Thunb\) koidz stems](#)  
Y C Liu, H M Wang and X H Zeng
- [The Role Of Perennial Grasses In The Protection Of Soil Resources Of Erosive Ecosystems With Active Development Of Linear Erosion](#)  
V M Kosolapov, V I Cherniavskih, E V Dumacheva et al.



The Electrochemical Society  
Advancing solid state & electrochemical science & technology

### 241st ECS Meeting

May 29 – June 2, 2022 Vancouver • BC • Canada

Abstract submission deadline: Dec 3, 2021

Connect. Engage. Champion. Empower. Accelerate.  
**We move science forward**



**Submit your abstract**



# TRIFOLIUM REPENSE L. BREEDING IN THE CENTRAL CHERNOZEM REGION: MAIN DIRECTIONS AND METHODS OF WORK

V M Kosolapov<sup>1</sup>, V I Cherniavskih<sup>1,2,3</sup>, E V Dumacheva<sup>1,2,3</sup>, N A Sopina<sup>3</sup>, V I Tseiko<sup>1,2</sup>, E I Markova<sup>3</sup>

<sup>1</sup> Federal Williams Research Center of Forage Production & Agroecology, 1 building, Scientific town, Lobnya, Moscow region, 141055, Russia

<sup>2</sup> All-Russian Research Institute of Phytopathology, 5 Ownership, Institute St., r.p. Big Vyazemy, Odintsovo district, Moscow region, 143050, Russia

<sup>3</sup> Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia

E-mail: cherniavskih@mail.ru

**Abstract.** Varieties and breeding samples of creeping clover *Trifolium repense* L. of lawn direction were studied in the nursery of competitive varietal trials. Using the methods of recurrent breeding involving local populations of creeping clover growing on chalk outcrops as initial forms, two varieties 'Krasnoyarskiy' and 'Ilyok' were obtained, as well as a new breeding sample PO 17/07. Varieties 'Krasnoyarskiy', 'Ilyok' and selection sample PO 17/7 are characterized by high seed productivity, the possibility of cultivation on soils with high carbonate content (burial of construction waste in an urban environment, reclamation of man-made-disturbed landscapes, etc.); high resistance to trampling. Seed yield over three years of tests in varieties 'Krasnoyarskiy', 'Ilyok' and breeding sample PO 17/7 significantly exceed the standard by an average of 24,6-26,8%; have uniform foliage at the level of 48-53%. Breeding sample PO 17/7 is planned to be submitted to the State variety testing.

**Keywords:** seed productivity, aboveground productivity, morphological characters, genetic resources, variety 'Krasnoyarskiy', variety 'Ilek'.

## 1. Introduction

Creeping clover (*Trifolium repense* L.) is a perennial leguminous plant that has a creeping, easily rooted stem and white, loose flower heads. Wild clover is widespread in nature, grows in meadows, pastures and is found in abundance on roadsides. In culture it is widely used in the U.S., Canada and New Zealand. In these countries it is valued for its good bribery and high quality honey. From 1 hectare of its crops can be collected on 100-125 kg of honey. But the take from white clover is not constant. Their nectar contains from 0.050 to 0.102 mg of sugar. Honey production varies depending on the abundance of flowers on 1 ha from 2 to 5 kg at thinned crops to 50-100 kg per 1 ha under favorable conditions [1].

*T. repense* is considered as a raw material for pharmaceutical industry and creation of new medicines [2-4].

However, *T. repense* has become widely known as a lawn grass. A complex and least studied issue is the selection of components (grass species and varieties) used for lawn grasses depending on their



purpose or specific growing conditions. There is no consensus on the optimal species composition of the components of lawn grass mixtures. In this regard, lawn grass selection is actively carried out. New varieties are created, and collections of varieties and breeding samples are formed [5-8].

The researchers widely apply in their work the classical methods of mass, individual, ecotypic and negative selection, and for the selection of synthetic varieties - polycross method. They study the resistance of *T. repense* varieties and breeding samples to unfavorable climatic factors (winter hardiness and frost resistance), their ability to improve the soil as a result of nitrogen fixation, etc. [9-13].

In Russia, creeping clover, more commonly called white clover, occupies more and more areas. *T. repense* is used in green construction for the reclamation of disturbed lands, for creating lawns, and also as a honey crop. Breeding work with creeping clover has been carried out in the Belgorod region since 2006. Using the concept of formation of a secondary anthropogenic microgenetic center of formation of synanthropic plant species in the south of the Central Russian Upland as a methodological basis for research, the genetic resources of local flora are actively involved in the breeding work [14-17]. A genetic collection of *T. repense* including more than 150 numbers has been formed [18].

The aim of breeding work with *T. repense* species in the region is to create low-growing varieties with high ornamental qualities for lawns, with high seed productivity and manufacturability when cultivated for seed.

## 2. Methods and materials

We studied *T. repense* cenopopulations in natural phytocenoses of the Cretaceous south of the Srednerusskaya Upland since 2006. [19,20].

During 2006-2010, elite plants were isolated, which became the basis for further breeding work and obtaining new varieties. Breeding work was carried out on the basis of the breeding plot of ZAO Krasnoyarskaya Grain Company and in the botanical garden of Belgorod State University.

Scientists from the federal research centers of the Russian Academy of Sciences participated in the research as part of joint scientific programs: Williams Reserch Center for Forage Production and Agroecology, All-Russian Research Institute of Phytopathology.

Small station, competitive station and inter-station competitive variety trials were conducted at the breeding plot of JSC "Krasnoyarskaya Grain Company" during the period from 2007 to 2018. Breeding nurseries were sown by the standard method in double repetition in one-row plots. The standard variety 'Volat' was sown every four numbers. The method of "halves" was used in the work: a reserve of seeds for further use was left from each population.

The selected breeding numbers of *T. repense* were multiplied in isolated plots (from the reserve seeds) and studied under field experiment conditions according to standard methods [21]. Objects of the study: the standard variety 'Volat', new local selection varieties 'Krasnoyarsky' and 'Ilek', as well as a new breeding sample PO 17/07.

The soil of the experimental plot is typical carbonate medium eroded chernozem. Humus content is 2.4 %. On average, the sum of average monthly temperatures varied from 5.9 7.8 oC to 7.8 oC during the years of research. The amount of precipitation was close to the norm - 2.6 mm. Field experiments were made by split plot method. Repeatability of the experiments was six times. The area of registration plots was 10 m<sup>2</sup>. Sowing method - row, with a row spacing of 45 cm. Yield accounting was carried out by plot-by-plot method. Evaluation of breeding samples was carried out in accordance with the table of features of the test methodology for distinctiveness, uniformity and stability.

## 3. Results and discussion

The basis of successful ecological breeding is the use of source material obtained by attracting local and wild forms that have undergone natural selection under ecotopic conditions and are adapted to a particular zone of growth.

In this connection, breeding work with *T. repense* was started with the study of local forms growing in phytocenoses of ravine and gully complexes, near settlements, on farm plots, along roads, etc.

Populations of *T. repense* in chalk outcrops and intensively grazed pastures in the floodplains of the Korocha and Oskol rivers of the Belgorod region were identified and studied. These populations served as starting material for the cultivar 'Krasnoyaruhsky'. The variety was obtained by recurrent selection (periodic selection) from the variety 'Volat' and local populations.

The variety 'Ilyok' was obtained by individual-family selection from the variety Volat, selection number PO 12/06 and local populations of *T. repense*, growing on chalk outcrops in the Belgorod region and intensively grazed pastures in the floodplain of the rivers Korocha and Oskol, with re-pollination of selected forms in polycross nursery and subsequent cultivation in an isolated area.

The selection number PO 17/07 was obtained by recurrent selection (periodic selection) using local source material - clover price populations growing in the floodplain and on the slopes of the river Manjokha of the Belgorod region.

The results of comparative tests of varieties and breeding sample on average for 3 years are shown in the table 1.

**Table 1.** Evaluation of varietal and biological properties and breeding model *T. repense* (2016-2018)

characteristics	Variety, breeding sample			
	'Volat'	'Krasnoyaruhs ky'	'Ilek'	PO 17/07
Leaf size, cm	1.5-2.6	0.9-1.5	0.7-1.8	0.5-1.7
Stem height, cm	12-15 to 35	10-12 to 20	10-14 to 20	9-12 to 18
Frequency of plants with white marks	medium	high	low	low
Flowering time	late	medium	late	medium
Coloration of knots	green	green	anthocyanin	anthocyanin
Middle tier leaflets: shape	rounded	rounded	elongated	rounded
Middle tier leaflets: color	green	green	anthocyanin	anthocyanin
Total decorative value of lawns	high	high	high	high
Seed yield, kg*(m <sup>2</sup> ) <sup>-1</sup>	0.029±0.001	0.037±0.004	0.038±0.002	0.037±0.003
The appearance of mass shoots (in days after sowing)	9-10	8-9	8-9	7-8
Beginning of spring growth (date)	10-12 April	08-09 April	05-07 April	05-07 April
End of vegetation (date)	15-18 October	20-22 October	23-26 October	23-26 October
Projective coverage, %	60-100	80-100	85-100	85-100

All varieties and breeding sample tested in the experiment were resistant to frequent mowing, weeding, disease and pest damage.

Variety 'Krasnoyaruhsky' varieties differ from 'Volat' varieties by smaller leaves, low stature (32,0 % lower on average), high shoot-forming ability, white-pink flowers, high seed production - 26,8 % higher than standard; higher occurrence of leaves with a white spot. The abundance of individuals in the variety is uniform and amounts to 48%, while in the variety 'Volat' it was 42%. The stems are of medium

coarseness and lack pubescence. Nodes are violet in color, branching is medium to high. The average number of internodes varies from 9 to 16. Bushiness is average The number of stems per bush averaged 14 to 20 (figure 1).



**Figure 1.** General view of the sowing of the creeping clover 'Krasnoyarskiy' variety (photo by V.I. Cherniavskiy)

The inflorescence head is 1.5-2.0 cm long, medium friability, white-pink in color. The beans are small, about 0.5-0.8 cm; straight, brownish-brown in color. The seeds are small, rounded, yellow (most) to brown, with a hardness of 8 to 26%.

Variety 'Ilyok' from individuals of variety 'Volat' differs more small leaves, low stature - lower on average 28.6%, high shootability, high seed production - higher than standard by 29.3%. The stem, on which the head is located, has anthocyanin coloration, which increases as the seeds mature; the white spot on the leaves is absent or faintly pronounced.

The foliage of individuals of the variety is uniform and amounts to 51-53%. Nodes have anthocyanin coloring, branching varies from medium to high. The average number of internodes varies from 7 to 15. Average number of stems per bush is 15 to 20.

Inflorescence is a head of 1.5-2.0 to 1.8-2.5 cm in length, with a medium degree of friability. Beans small, 0.5-0.8 cm; straight, brownish-brown in color.

The seeds are small, rounded, yellow (most) to brown in color, with a hardness of 8 to 26%.

In general, the variety 'Ilek' is distinguished by white flowers, which after pollination acquire a pink hue; a longer head; midstory leaflets elongated, have the anthocyanin coloration, leaf coloration from green to dark green; node coloration anthocyanin.

Breeding specimen PO 17/07 differs from the standard by smaller leaves, low stature (36,9 % lower on average), high shoot-forming ability, white-pink flowers retaining color after pollination; high seed production, 24,6 % higher than the standard; high incidence of leaves with white spot, at the level of the standard. Widening of individuals in the variety is uniform, higher than the standard and is 51-53%.

Stem knots have weak anthocyanin coloring, medium to high branching. The average number of internodes varies from 6 to 14. The average number of stems per bush is 13 to 17.

Inflorescence is 1.5 to 2.0 cm long, with a medium degree of friability. Pods small, 0.5-0.7 cm; erect, brownish-brown in color.

The seeds are small, rounded, brown in color (most of them), with a hard seed size of 10 to 28%.

#### 4. Conclusion

1. Varieties and breeding samples of local selection 'Krasnoyarskiy', 'Ilek', PO 17/07 have a longer growing season, low growth rate, high drought tolerance and winter-hardiness. The high seed yield of seed crops in the crop ensures guaranteed seed production of local *T. repense* varieties.

2. Seed yield over three years of tests in the varieties 'Krasnoyarskiy', 'Ilyok' and breeding sample PO 17/7 significantly exceed the standard on average 24.6-26.8 %; have uniform foliage at 48-53 %. Breeding sample PO 17/7 is planned to be submitted to the State variety trial.

3. Varieties and breeding samples of local selection 'Krasnoyarskiy', 'Ilek', PO 17/07 are suitable for industrial technology of cultivation, mechanized harvesting and processing. In lawns can be sown both pure and in mixtures with grasses of local selection: the varieties 'Streletsky' and 'Stepniak' (*Lolium perenne*), 'Gostenka' and 'Vezelka' (*Festuca rubra*). A higher and more guaranteed seed productivity makes it possible to reduce the cost of lawn grasses using the new *T. repense* varieties by 20-22%.

### Acknowledgments

The research was carried out within the framework of the project of the Belgorod Scientific and Educational Center of the World Level «Innovative Solutions in Agro-Industrial Complex».

### References

- [1] Bong, J., Loomes, K.M., Schlothauer, R.C., Stephens, J.M. 2015. Fluorescence Markers in Some New Zealand Honeys. *Food Chemistry*. 192: 1006-1014.
- [2] Malca-Garcia, G.R., Zagal, D., Graham, Ja., Nikolić, D., Friesen, J.B., Lankin, D.C., Chen, Sh.N., Pauli, G.F. 2019. Dynamics Of The Isoflavone Metabolome Of Traditional Preparations Of *Trifolium Pratense* L. *Journal of Ethnopharmacology*. 238: 111865.
- [3] Mikhailov, A.L., Timofeeva, O.A., Ogorodnova, U.A., Stepanov, N.S. 2019. Comparative Analysis Of Biologically Active Substances In *Trifolium Pratense* And *Trifolium Repens* Depending On The Growing Conditions. *Journal of Environmental Treatment Techniques*. 7 (Special Issue): 874–877
- [4] Ahmad, S., Zeb, A. 2020. Phytochemical Profile and Pharmacological Properties of *Trifolium Repens*. *Journal of Basic and Clinical Physiology and Pharmacology*: 20200015.
- [5] Kosolapov, V. M., Kostenko, S. I., Tyurin, Yu. S., Shamsutdinova, E. Z., Piskovskii, Yu. M. 2021. Perennial forage grasses – the basis for greening agricultural production. *IOP Conference Series: Earth and Environmental Science*. DOI: 10.1088/1755-1315/663/1/012022.
- [6] Mammadyarova, K., Asgarov, A., Akparov, Z. 2019. Studies On A Clover (*Trifolium* L.) Collection From Lenkaran-Lerik Region Of Azerbaijan. *Genetic Resources and Crop Evolution*. 66 (3): 611–618.
- [7] Cherniavskih, V.I. 2018. Breeding of Leguminous Grass in Central Black Soil Region. *Plodovodstvo i Yagodovodstvo Rossii*. 54: 81–87. (in Russia)
- [8] Jeremy, M., Masonb, M., Ambrusa, A. 2018. Urban greenspace is associated with reduced psychological stress among adolescents. *A Geographic Ecological Momentary Assessment (GEMA) analysis of activity space Landscape and Urban Planning Volume*. 174: 1–9.
- [9] Olivo, C.J., Meinerz, G.R., Agnolin, C.A., Ziech, M.F., Skonieski, F.R., Steinwandter E. 2010. Forage Production And Stocking Rate Of Coastcross Pastures Overseeded With Winter Grasses. *Revista Brasileira de Zootecnia*. 39 (1): 68–73.
- [10] Zhou R.-l., Zhao M., Zhang P., Wang Y.-j., Zhao Y.-h. 2012. Physiological Differences of *Trifolium Repens* and *Trifolium Pratense* in Response to Freezing-Thawing Stress. *Chinese Journal of Ecology*. 31 (6): 1334–1340.
- [11] Dahlin, A.S., Campbell, C.D., Hillier, S., Ramezani, A., Öborn, I. 2015. Waste Recovered By-Products Can Increase Growth of Grass-Clover Mixtures in Low Fertility Soils and Alter Botanical and Mineral Nutrient Composition. *Annals of Applied Biology*. 166 (1): 105–117.
- [12] Lin, H., Liu, C., Li, B., Dong, Y. 2021. *Trifolium Repens* L. Regulated Phytoremediation Of Heavy Metal Contaminated Soil By Promoting Soil Enzyme Activities And Beneficial Rhizosphere Associated Microorganisms. *Journal of Hazardous Materials*. 402: 123829
- [13] Matse, D.T., Huang, C.-H., Huang, Y.-M., Yen, M.-Y. 2020. Effects Of Coinoculation Of *Rhizobium* With Plant Growth Promoting *Rhizobacteria* On The Nitrogen Fixation And

- Nutrient Uptake Of *Trifolium Repens* In Low Phosphorus Soil. *Journal of Plant Nutrition*. 43 (5): 739-752.
- [14] Cherniavskih, V. I., Sidelnikov, N. I., Dumacheva, E. V., Borodaeva, Z. A., Glubsheva, T. N., Gorbacheva, A. A., Vorobyova, O. V., Korolkova, S. 2019. Biological Resources of Natural Forage Grassland of the Cretaceous South of the European Russia. *EurAsian Journal of BioSciences*. 13 (2): 845–849.
- [15] Chernyavskikh, V. I., Dumacheva, E. V., Lisetsky, F. N., Tsugkiev, B. G., Gagieva, L. Ch. 2019. Floral Variety of Fabaceae Lindl. family in gully ecosystems in the South-West of the Central Russian Upland // *Bioscience Biotechnology Research Communications*. 12 (2): 203–210.
- [16] Dumacheva, E.V., Cherniavskih, V.I., Tokhtar, V.K., Tokhtar, L.A., Pogrebnyak, T.A., Horolskaya, E.N., Gorbacheva, A.A., Vorobyova, O.V., Glubsheva, T.N., Markova, E.I., Filatov, S.V. 2017. Biological Resources of the *Hyssopus L.* on the South Of European Russia and Prospects of its Intro-duction. *International Journal of Green Pharmacy*, 11 (3): 476–480.
- [17] Chernyavskikh, V.I., Dumacheva, E.V., Sidelnikov, N.I., Lisetsky, F.N., Gagieva L.Ch. 2019. Use of *Hissopus officinalis L.* culture for phytoamelioration of carbonate outcrops of anthropogenic origin the South of European Russia. *Indian Journal of Ecology*, 46 (2): 221–226.