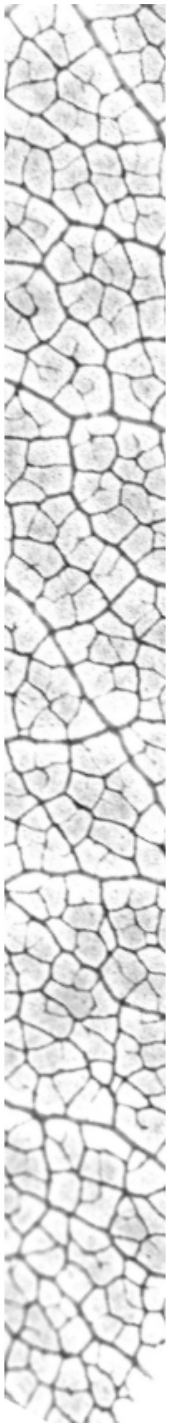

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 733-737 (2019)

Features of intra population variability of *Medicago varia* Mart. with the expressed *mf*-mutation on a complex qualitative characteristics

Vladimir I. Cherniavskih [1*], Elena V. Dumacheva [1], Zhanna A. Borodaeva [1], Anzhelika A. Gorbacheva [1], Elena N. Horolskaya [1], Larisa Ch. Gagieva [2], Nadezhda V. Kotsareva [1,3], Svetlana V. Korolkova [1]

[1] Department of Biology, Belgorod State University, Belgorod, 308015, RUSSIA
[2] Department of Biological and Chemical Technology, Gorsky State Agrarian University, Vladikavkaz, the Republic of North Ossetia-Alania, RUSSIA
[3] Department of Plant Growing Breeding and Vegetable Growing, Belgorod State Agricultural University Named after V. Gorin, Belgorod, RUSSIA
*Corresponding author: chernyavskih@bsu.edu.ru

Abstract
Lucerne has multifunctional value: it is a source of a high-protein forage for farm animals and valuable raw materials for pharmaceutical industry. The purpose of this work was studying of features of intra population variability of a lucerne with a mf-mutation on a complex of the qualitative signs, valuable as for the forage production as well as for the pharmaceutical industry. In the years 2016-2018, researchers of the 30 breed population, received as a result of an individual selections from a breed lucerne Krasnoyaruzhskaya 1 on the basis of a high expression of a mutation of multifoliolation (mf-mutation). The high variability of a population of the lucerne in the posterity of the second generation on the basis of expressiveness of the mf-mutation was established. The breed population was distributed by the index of an expression of the mf-mutation as follows: the group with low expressiveness of the sign included 52.8% of numbers, 9.9% of the numbers had the index of an expression up to the standard, 36.3% of the breed population exceeded the standard for 9.3-62.7%. As a result of researches the direct correlation between degree of an expression of the mf-mutation and the feeding efficiency of a lucerne is not revealed. There was established the breed population SP-11 at which the high expression of the mf-mutation and high efficiency of the elevated phytoweight against the background of the low maintenance of saponin and antotcyanin. The population of SP-11 will be included in the selection program for the creation of the lucerne breeds with a high fodder quality. The breed population SP-23, SP-24, SP-28 has a high expression of the mf-mutation, high efficiency of the elevated phytoweight against the background of the high content of the biologically active agents (saponins and antocyanins). These forms will be included in the selection program for creation of breeds of a lucerne with the high content of biologically active agents for the pharmaceutical industry.

Keywords: *Medicago varia* Mart., multifoliolation, *mf*-mutation, saponins, antocyanins, feeding efficiency, raw materials for the pharmaceutical industry

Cherniavskih VI, Dumacheva EV, Borodaeva ZA, Gorbacheva AA, Horolskaya EN, Gagieva LCh, Kotsareva NV, Korolkova SV (2019) Features of intra population variability of *Medicago varia* Mart. with the expressed *mf*-mutation on a complex qualitative characteristics. Eurasia J Biosci 13: 733-737.

INTRODUCTION

Lucerne is one of the leading world agricultural cultures which has multifunctional value. On the one hand the lucerne is a source of a high-protein forage for farm animals, and with another it gives the valuable raw materials for the pharmaceutical industry (Bian et al. 2017, Chen et al. 2017, El-Dabae et al. 2018, Ford and Claydon 2011, Hassan et al. 2010, Ivanova et al. 2015, Kayce et al. 2017, Lamb et al. 2007, Vogel et al. 2013).

The important direction of researches of a lucerne is a studying of a recessive genetic mutation of a multifoliolation (mf-mutation). The mutation is controlled by a recessive factor (mj) and two more factors influencing its manifestation (Petkova 2003, Petkova and Djukic 2007, Petkova and Panayotova 2007, Popescu et al. 2016, Vyšniauskienė et al. 2015, Yancheva et al. 2012).

The genotypes with a high expression of a mutation show high quality of a forage (decrease in a content of

Received: December 2018
Accepted: May 2019
Printed: July 2019

an anti-nutrients and increase in efficiency of elevated weight) (Bingham and Murphy 1965, Petkova 2003, 2010, Petkova and Djukic 2007, Petkova and Panayotova 2007, Rumbaugh 1971, Sheaffer et al. 1995, Tashiro 2009).

The mutation demonstrates most obviously in difficult ecological conditions (Dumacheva and Cheriavskih 2013, Dumacheva et al. 2015, 2018).

Studying of biological resources of a lucerne and issues of its selection are directed to receive forms with high phytoefficiency (Jones et al. 2003, Juan et al. 1993, Popovic et al. 2001, Lamb et al. 2006, Veronesi et al. 2006, 2010).

The only shortcoming at cultivation of a lucerne for a forage is contents in its green material of high amount of biologically active agents causing diseases of ruminants, in particular saponin. Saponins are complex organic compounds with a high biological activity of glycosidic character. They belong to group of substances the water solutions of wich forms the resistant foam, similar to soap (Bissinger et al. 2014, Mugford and Osbourn 2013, Rumbaugh 1971, Sadowska et al. 2014).

In this regard, at the selection on the fodder purposes there is conducted selection of forms with the low maintenance of saponin (Dumacheva et al. 2015, 2018, Dumacheva and Cheriavskih 2013).

However, vegetable saponins are used in pharmaceutical industry as initial raw materials for synthesis of steroid hormonal medicines, anti-diabetic, all-strengthening, positively influencing the cardiovascular system of the person (Kovalev et al. 2017, Mugford and Osbourn 2013, Sadowska et al. 2014).

The work purpose — features studying of the intra population variability of a lucerne with a mf-mutation on a complex of the qualitative signs valuable to forage production and the pharmaceutical industry.

MATERIALS AND METHODS

Lucerne was explored in 2016-2018 on the selection site of CJSC “Krasnoyaruzhsky Grain Company». The soil: chernozem typical carbonate of medium power and of low-humus heavy-loamy on a chalk residual soil. The humus content before starting the experiment if 3.12-3.56%, pHKCl – 7.35-7.42. The content of the easily hydrolyzed nitrogen is 77-78 mg/kg, P_2O_5 according to Machigin is 11.8-12.0 mg/kg, K_2O – 208-212 mg/kg. Weather conditions on the sums of temperatures and quantities draft were close to average long-term values (Dumacheva and Cheriavskih 2013, Dumacheva et al. 2018, Lisetskii et al. 2010).

As the object were served 30 type population received as a result of the individual selections from the breed lucerne Krasnoyaruzhskaya 1. The sign of a mf-mutation was marker sign for carrying out selection. As the uterine plants for selections were individuals with the index of an expression mf 3. The efficiency of the elevated phytoweight was the second marker sign for selections.

The index of an expression was calculated as the sum of works of plants number (escapes) which are in each category mf on total number of plants (stalks) in population. Categories mf: 0 – lack of a mutation, 1 – 1 mf-leaf on 1 stalk, 2 – 2-3 mf-leaves on 1 stalk, 3 – 4-5 mf-leaves on 1 stalk, 4 – 6-7 mf-leaves on 1 stalk, 5 – more than 8 mf-leaves on 1 stalk (Sheaffer et al. 1995).

The descendants of uterine plants (F2) were sowed on separate allotments (length – 1 m, a row-spacing – 0.4 m). As a standard was the sort Krasnoyaruzhskaya 1. Researches were conducted in fivefold biological frequency, with plants of the 1st hay crop. A harvest of an elevated phytoweight was defined (kg/sq.m of a.s.c.).

The level of the anthocyanin pigments was determined by the scale: 0 – absence, 1 – coloring on the basis of scapes, 2 – on the basis of a stalk, 3 – coloring on a stalk except for scapes, 4 – coloring on all stalk, 5 – bright coloring of a stalk and scapes.

The content of saponins was calculated on foaming coefficient with use of a physical method. The foam-forming activity was defined on the 3rd to a ball scale: 1 point – weak foaming, no more than 15 sec.; 2 points – average foaming, no more than 15-30 sec.; 3 points – strong foaming, reaction keep more than 1 min. (Rumbaugh 1971).

Statistical data processing was carried out with the use of the common software Microsoft Excel (2010).

RESULTS AND DISCUSSION

The high population variability of a lucerne in descendants of the second generation on the basis of expressiveness of a mf-mutation was set. The breed population was distributed by the index of an expression of a mf-mutation as follows: the group with low expressiveness of sign in comparison with the standard included 52.8% of numbers, 9.9% of numbers had the index of an expression up to the standard, 36.3% of the breed population exceeded the standard on expressiveness of sign for 9.3-62.7% (tab. 1). SP-23 breed population had the maximum index – 2.62.

Table 1. Intra population variability of *M. varia* Mart. According to expression sign of an *mf*-mutation in the generation F2 (on an average 2016-2018)

Index of the mf-mutation expression	Breed population
1.61±0.04	St
< ST	SP-1, SP-2, SP-3, SP-4, SP-5, SP-7, SP-9, SP-10 ,SP-13, SP-14, SP-17, SP-19, SP-22, SP-25, SP-29, SP-30
On the stage ST	SP-6, SP-8, SP-26,
> ST	SP-11, SP-12, SP-15, SP-16, SP-18, SP-20, SP-21, SP-23, SP-24, SP-25, SP-27, SP-28

Table 2. Intrapopulation variability of M. varia Mart. on collecting of solid material in the generation F2 (on an average 2016-2018)

Collecting elevated phytoweight, kg/sq.m	Breed population
0.87±0.02	St
< ST	SP- 2, SP-3, SP-5, SP-8, SP-12, SP-13, SP-14, SP-16, SP-17, SP-18, SP-19, SP-20, SP-21
On the stage ST	SP-4, SP-6, SP-9, SP-15, SP-22
> ST	SP-1, SP-7, SP-10, SP-11, SP-23, SP-24, SP-25 SP-26, SP-27, SP-28, SP-29, SP-30

Table 3. Intrapopulation variability of *M. varia Mart.* on degree of expressiveness of antacyan coloring in the generation F2 (on an average 2016-2018)

Antacyans contents, rating	Breed population
1.94±0.06	St
< ST	SP-8, SP-9, SP-11, SP-16, SP-17,
On the stage ST	SP-1 – SP-4, SP-6, SP-7, SP-10, SP-12, SP-13, SP-14, SP-18, SP-19, SP-20, SP-21, SP-22, SP-23, SP-25, SP-26, SP-28, SP-29, SP-30
> ST	SP-5, SP-15, SP-24, SP-27

Table 4. Intrapopulation variability of *M. varia Mart*. on the basis of foaming in the generation F2 (on an average 2016-2018)

Foaming coefficient	Breed population
1.0	St
< ST	SP-7, SP-11, SP-20
On the stage ST	SP -1, SP-4, SP-6, SP-8, SP-14, SP-17, SP-18, SP-19, SP-21, SP-26, SP-27
> ST	SP-3, SP-5, SP-7, SP-9, SP-10, SP-12, SP-13, SP-15, SP-16, SP-22, SP-23, SP-24, SP-25, SP-28, SP-29, SP-30

According to a number of authors, the high degree of an expression of a mf-mutation directly correlates with efficiency of elevated phytoweight. The productivity of the elevated phytoweight the studied breed population were distributed as shown in **Table 2**.

The studied varieties showed rather high population variability on an indicator of collecting dry basis of the elevated phytoweight. At the same time, 42.9% of varieties of the elevated efficiency changed ranging from 0:29 up to 0.77 kg/sq.m, 16.5% of variety had a collecting elevated phytoweight up to the standard, at 39.6% – exceeded the standard on average for 12.2-72.5%. The maximum efficiency was shown by numbers SP-24 and SP-28 – 1.48 and 1.50 kg/sq.m respectively.

Antocyans are important biologically active agents increasing resistance to stress of plants. In separate researches the part of marker signs of ecological fitness is assigned to them (Dumacheva and Cheriavskih 2013, Dumacheva et al. 2015, 2018).

The intrapopulation variability of the individuals in F2 posterity on the basis contents of the antacyans pigments is presented in the **Table 3**. Those data confirm low variation of this sign at the studied variety.

Only 16.5% of breed population of an individual had low expressiveness of this sign, and 16.5% had high (at the level of 3-4 points). 77.0% of the breed population had the level of antocyans on a ball scale conformed to the standard.

The evaluation of saponin contents by determination of coefficient of foaming allowed to establish high intrapopulation variability on this sign (**Table 4**). The breed population were distributed in a next way: 36.3% had foaming coefficient up to the standard; 9.9% – below the standard – at the level of 0.4-0.8; 36.3% of the breed population coefficient of foaming was an average and did not exceed coefficient 2. 16.5% of breed population (SP-3, SP-23, SP-24, SP-28, SP-30) had a high coefficient of foaming and reached level 3.

Between an indicator of foaming and the saponin content at a lucerne the high correlation dependence at the level of 0.77-0.81 is established (Streltsina et al. 2001).

It gives us the reason to believe that at lucerne breed samples with the high coefficient of foaming in our researches the content of saponins are also high. On the basis of the maintenance of saponins it is possible to conduct the further selection on high fodder efficiency with the low content of saponin for forage production and also with the high content of saponin for use as raw materials for pharmaceutical industry.

CONCLUSION

In the F2 lucerne posterity received as a result of the individual selections, the direct correlation between expressiveness of a mf-mutation and fodder efficiency is not established.

The breed population SP-11 is separated and its individuals have a high expression of a mf-mutation and high efficiency of elevated phytoweight against the background of the low content of biologically active agents potentially dangerous to ruminants – saponin and antocyan is allocated. The population SP-11 will be included in the selection program for creation of a lucerne breeds with high fodder qualities.

There are allocated the breed populations SP-23, SP-24, SP-28 which, against the background of a high expression of a mf-mutation and high efficiency of elevated phytoweight, has the high content of biologically active agents (saponin and antotsian).

These breed populations is planned to include in the selection program for creation of the lucerne breeds with a high content of biologically active agents for use in the pharmaceutical industry.

REFERENCES

Bian X, Zhao Y, Guo X, Zhang L, Fu T, Wang W, Yin Y, Chen G, Li P, Liu J (2017) Chiisanoside, a triterpenoid saponin, exhibits anti-tumor activity by promoting apoptosis and inhibiting angiogenesis. RSC Advances, 7(66): 41640-41650. https://doi.org/10.1039/C7RA08041G

Bingham ET, Murphy RP (1965) Breeding and morphological studies on Multifoliolate selections of alfalfa, Medicago sativa L., Crop. Sci., 5: 233-35. https://doi.org/10.2135/cropsci1965.0011183X000500030010x

Bissinger R, Modicano P, Alzoubi K, Honisch S, Abed M, Lang F, Faggio C (2014) Effect of saponin on erythrocytes. International Journal of Hematology, 100(1): 51-59. https://doi.org/10.1007/s12185-014-1605-z

Chen Y, Liu Y, Xu J, Xie Y, Zheng Q, Yue P, Yang M (2017) A natural triterpenoid saponin as multifunctional stabilizer for drug nanosuspension powder. AAPS PharmSciTech, 18(7): 2744-2753. https://doi.org/10.1208/s12249-017-0756-7

Dumacheva EV, Cheriavskih VI (2013) Particular qualities of micro evolutionary adaptation processes in cenopopulations Medicago L. On carbonate forest-steppe soils in European Russia. Middle East Journal of Scientific Research, 17(10): 1438-1442.

Dumacheva EV, Cherniavskih VI, Gorbacheva AA, Vorobyova OV, Borodaeva ZA, Bespalova EN, Ermakova LR (2018) Biological resources of the Fabaceae family in the cretaceous south of Russia as a source of starting material for drought-resistance selection. International Journal of Green Pharmacy, 12(2): 354-358.

Dumacheva EV, Cherniavskih VI, Markova EI, Klimova TB, Vishnevskaya EV (2015) Spatial pattern and age range of cenopopulations medicago l. In the conditions of gullying of the southern part of the Central Russian upland. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(6): 1425-1429.

El-Dabae WH, Ata NS, Hussein HA, Rohaim MA, Reda IM, El-Safty MM (2018) Saponin-adjuvanted vaccine protects chickens against velogenic newcastle disease virus. Archives of Virology, 163 (9): 2423-2432. https://doi.org/10.1007/s00705-018-3917-4

Ford JL, Claydon RB (2011) Inheritance of multifoliolate leaves in white clover Agronomy Society of New Zealand Special Publication. Grassland Research and Practice Series, 6: 167-170.

Hassan SM, Haq AU, Cartwright AL, Bailey CA, Byrd JA, Berhow MA (2010) Haemolytic and antimicrobial activities of saponin-rich extracts from guar meal. Food Chemistry, 119(2): 600-605. https://doi.org/10.1016/j.foodchem.2009.06.066

Ivanova N, Pavlyuchik E, Ambrosimova N, Panteleeva T, Epifanova N (2015) Formation of productivity of pasture agrophytocenosis designed based on perennial ryegrass and festulolium under drained soils of upper Volga. Nauka i studia, 7: 48-58. (In Russian).

Jones ES, Hughes LJ, Drayton MC, Abberton MT, Michaelson-Yeates TPT, Bowen C, Forster JW (2003) An SSR and AFLP molecular marker-based genetic map of white clover (Trifolium repens L.). Plant Science, 165: 531-539. https://doi.org/10.1016/S0168-9452(03)00212-7

Juan NA, Sheaffer CC, Barnes DK (1993) Temperature and photoperiod effects on multifoliolate expression and morphology of alfalfa. Crop Sci. 33: 573-578. https://doi.org/10.2135/cropsci1993.0011183X003300030030x

Kayce P, Böke SN, Kirmizigül S, Pekmez M, Arda N (2017) The structure and cytotoxic activity of a new saponin: cephoside a from cephalaria elazigensis var. Purpurea. Turkish Journal of Chemistry, 41(3): 345-353. https://doi.org/10.3906/kim-1607-68

Kovalev S, Demeshko O, Kocherga V, Kovalev V (2017) The research of the organic acids of medicago varia herb. Ukrainian biopharmaceutical journal, 3: 52-55. https://doi.org/10.24959/ubphj.17.118

Lamb JFS, Jung H-JG, Sheaffer CC, Samac DA (2007) Alfalfa leaf protein and stem cell wall polysaccharide yields under hay and biomass management systems. Crop Science Society of America, 47: 1407-1415. https://doi.org/10.2135/cropsci2006.10.0665

Lamb JFS, Sheaffer CC, Rhodes LH, Sulc RM, Undersander DJ, Brummer EC (2006) Five decades of alfalfa cultivar improvement: Impact on forage yield, persistence and nutritive value. Crop Science, 46: 902–909. https://doi.org/10.2135/cropsci2005.08-0236

Lisetskii FN, Chernyavskikh VI, Degtyar OV (2010) Pastures in the zone of temperate climate: trends for development, dynamics, ecological fundamentals of rational use. Pastures: Dynamics, Economics and Management: 51-84.

Mugford ST, Osbourn A (2013) Saponin synthesis and function. Isoprenoid Synthesis in Plants and Microorganisms. New Concepts and Experimental Approaches: 405-424. https://doi.org/10.1007/978-1-4614-4063-5_28

Petkova D (2003) Morphological and economical characteristics of alfalfa multifo-liolate variety. Plant Science, 40: 190-192.

Petkova D (2010) Multifoliate Alfalfa line with 23-24 leaves on a leaf stalk. Journal of Crop and Weed, 6(1): 1-5.

Petkova D, Djukic D (2007) Perfomance and stability of some agronomical traits of trifoliate and multifoliate alfalfa gern plasms. A Periodical of scientific research on field and vegetable crops, 44: 35-38.

Petkova D, Panayotova G (2007) Comparative Study of Trifoliolate and Multifoliolate Alfalfa (Medicago sativa L.). Synthetic Populations. Bulgarian Journal of Agricultural Science, 13: 221-224.

Popescu S, Boldura O-M, Ciulca S (2016) Evaluation of the geneticvariability correlated with multileaflet trait in alfalfa. AgroLife Scientific Journal, 5(2): 125-130.

Popovic S, Grljusic S, Cupic T, Tucak M, Stjepanovic M (2001) Protein and fiber contents in alfalfa leaves and stems. Quality in lucerne and medics for animal production. Zaragoza: CIHEAM, 45: 215-218.

Rumbaugh MD (1971) Inderitance of foaming properties of plant extracts of alfalfa. Croh. Sci., 9: 321-632.

Sadowska B, Budzyńska A, Wieckowska-Szakiel M, Paszkiewicz M, Rózalska B, Stochmal A, Moniuszko-Szajwaj B, Kowalczyk M (2014) New pharmacological properties of medicago sativa and saponaria officinalis saponin-rich fractions addressed to Candida Albicans. Journal of Medical Microbiology, 63(8): 1076-1086. https://doi.org/10.1099/jmm.0.075291-0

Sheaffer CC, McCaslin M, Volenec JJ, Cherney JH, Johnson KD, Woodward WT, Viands DR (1995) Multifoliolate Leaf Expression (Leaves With Greater Than 3 Leaflets. Leaf): 2.

Streltsina SA, Zhukova MA, Chachko EV, Dzyubenko NI, Konarev AV (2001) Comparative analysis of intra-population variability of alfalfa (Medicago sativa L.) and Eastern goat (Galega orientalis L.) by biochemical quality traits. Agricultural biology, 5: 37-47.

Tashiro RM (2009) From the field to the flowerbed to the lab: ornamental white clover breeding and leaf trait mapping. Master of Science. Athens, Georgia: 64.

Veronesi F, Brumers EC, Huyghe C, Boller B, Posselt UK, Veronesi F (2010) Alfalfa. Fodder Crops Amenity Grasses. Series: Handbook of Plant Breeding Springer, New York, USA, 5: 395-437. https://doi.org/10.1007/978-1-4419-0760-8_17

Veronesi F, Huyghe G, Delgado I (2006) Lucerne breeding in Europe: results and research strategies for future developments. Proceedings of the 21th general meeting of the European Grassland federation Badajos, Spain. Grassland Scienece in Europe, 11: 232-242.

Vogel A, Eisenhauer N, Weigelt A, Fester T, Scherer-Lorenzen M, Schmid B, Weisser WW (2013) Separating drought effects from roof artifacts on ecosystem processes in a grassland drought experiment, 8(8): 979-997. https://doi.org/10.1371/journal.pone.0070997

Vyšniauskienė R, Rančelienė V, Patamsytė J, Čėsnienė T, Žvingila D, Naugžemys D (2015) ISSR and chloroplast DNA analyses indicate frequent hybridization of alien medicago sativa subsp. Sativa and native m. Sativa subsp. Falcata. Plant Systematics and Evolution, 301(10): 2341-2350. https://doi.org/10.1007/s00606-015-1232-z

Yancheva C, Petkova D, Sevov A (2012) Studies onquality of multifoliolate alfalfa. Scientific Papers. Series A. Agronomy: 261-264.