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ACCUMULATION OF PHOTOSYNTHETIC PIGMENTS AND SECONDARY METABOLITES IN LEAVES OF GALEGA (*Galega orientalis* Lam.) cv. GALE DEPENDING ON STAND AGE AND AGROTECHNOLOGIES DURING INTRODUCTION IN THE MIDDLE TAIGA OF WESTERN SIBERIA

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Abstract

Plant biomass production and accumulation of bioactive substances are determined by a complex of physiological and biochemical mechanisms, environmental factors and agrotechnologies. The use of *Galega orientalis* as a forage crop throughout the world is largely due to its unique environmental adaptability and a large yield potential. Despite the widespread use of forage *G. orientalis* around the world, research data on photosynthetic pigments, vitamin C and flavonoids in green mass of the plants under a new environment are scarce, and for the north of Russia, it is completely absent. Earlier, we were the first to describe the phenological, eco-morphological features and photosynthetic potential, the productivity of green mass and seeds of *G. orientalis* for the zone of the Middle taiga of Western Siberia. This paper systematizes our first data on the accumulation of photosynthetic pigments, vitamin C, and flavonoids in *G. orientalis* plants at the site of introduction. The study aimed to characterize the content of these compounds during adaptation to new environment, depending on cropping practices and the age of the herbage. Introductory studies were carried out on the cv. Gale (an experimental plot, the village of Barsovo, Khanty-Mansi Autonomous Okrug — Yugra, Surgut district, 61°15′00″ N, 73°25′00″ E. 2013-2015). Plants were grown using peas as a cover crop, in monoculture with pre-sowing treatment of seeds with the Baikal-EM1 microbiological preparation (OOO NPO EM-Center, Russia), and in monoculture without treatment. The effects of the cropping practices on the total chlorophylls (Chl a + Chl b) in the leaves appeared in the 2nd year plants. Upon seed pre-sowing treatment with the Baikal-EM1 preparation, in the 2nd and 3rd year plants, the level of total chlorophylls by plant development phases was 19-22 % and 16-18 % higher than in the control). In mixed sowing total chlorophylls decreased at the end of the 2nd year but exceeded the control (by 33 %) by the end of the 3rd year. In the control, the Chl a level in the leaves of the 1st, 2nd and 3rd year plants averaged 1.23±0.10, 1.29±0.12 and 1.32±0.14 mg/g dry weight over the growing season. Over the 2nd year of growth, the content of Chl a in the leaves increased by 15 % on average upon the Baikal-EM1 application compared to the control and remained within the control values (1.20±0.23 mg/g) ($p \leq 0.05$) in the mixed stands with pea plants. For the microbiological preparation, the average Chl a/Chl b ratio significantly ($p \leq 0.05$) decreased over 3 years, which may indicate an increase in the adaptive potential of plants, and for the mixed crops, it remained within the control values. The proportion of chlorophylls (Chl a + Chl b) localized in the light-harvesting complexes (LHC) varied from 20 to 90 % depending on the plant phenophase, stand age, and the agrotechnology. In the control and two treatments, the correlation coefficients between Chl a/Chl b and the proportion of chlorophylls (Chl a + Chl b) localized in the LHC were $r = -0.83$, $r = -0.93$, and $r = -0.65$, respectively. Treatments did not lead to a statistically significant change in the Chl/Car index. Nevertheless, after inoculation with the Baikal-EM1 biological and in mixed sowing with peas, the accumulation of carotenoids exceeded the control. For all treatments over the years, the accumulation of all pigments in the leaves directly correlated with the hydrothermal coefficient (HTC). The content of Chl b and carotenoids turned out to be weaker associated with the temperature regime, while the first parameter directly correlated with precipitation during the season, and a negative correlation occurred for the second parameter. When inoculated with Baikal-EM1, the leaf level of vitamin C in the 1st and 2nd year plants increased compared to the control and was almost equal to the control in the 3rd year plants. In the 3rd year mixed sowing, the vitamin C content decreased compared to control. After application of the microbiological preparation and in the control, the content of flavonoids in the 3rd year plants switched to generative development sharply decreased, while in the sowing with the cover crop, where the virginal stage continued, it sharply increased (1.6 times compared to previous years). In general, our findings indicate that the biological Baikal-EM1 largely contributed to the adaptation of the 2nd and 3rd year plants of *G. orientalis* cv. Gale to a new environment.

Keywords: photosynthesis pigments, vitamin C, flavonoids, *Galega orientalis* Lam., cv. Gale, introduction, Baikal-EM1

In the countries of the European Union, especially in Central and Northern Europe, there has been a shortage of feed protein for many years, which is primarily associated with unfavorable climatic conditions, the short growing season and frequent droughts. The potential of perennial leguminous crops, a significant part of which are resistant to drought, is still not realized, although their protein yield is often 2 times higher than that of annual crops [1]. This is due to the attention to alternative perennial legumes that can provide more stable yields of green mass with high feed value.

As such, the eastern galega (*Galega orientalis Lam.*), a perennial forage legume plant (family *Fabaceae*) which has a complex of valuable properties, is increasingly being used. The eastern galega possesses winter hardiness, drought resistance, and high efficiency of using spring moisture reserves. Plants show early regrowth in spring and rapid growth, significant foliage (60-70%), and stability of seed production (up to 6 kg/ha or more). Longevity of crop use makes 10-15 years or more with high productivity (for 2 mowing, up to 60-70 t/ha of green mass, up to 10-15 t/ha of hay) and nutritional value (1 feed unit contains 150-270 g of digestible protein) [2, 3].

The possibilities of widespread use of eastern galega are largely due to its biological features, and in particular, its high yield potential and exceptional adaptability to various environmental conditions [4]. The natural territory of habitat of the eastern galega is the North Caucasus and the Transcaucasia (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:495682-1) [5]. The Eurasian Caucasian region is considered the geographical center of origin of this species [6]. However, at present it has significantly expanded its range, including due to introduction [5, 7]. As a forage plant, eastern galega is cultivated in Ukraine, Belarus, Estonia, China, in Western Europe, e.g., in Austria, France, where the naturalization of the species is also noted [7], Baltic countries, the Czech Republic and Slovakia, Kazakhstan [5], Moldova [8], Canada where productivity was compared within the geographical coordinates of 45-56 N 52-120 W [9], and in Japan [10].

In Russia, studies on the introduction of eastern galega have been carried out in many regions, in the Central Chernozem Zone [11], the Volga region [12, 13], the Middle Urals [14], and Siberia [15, 16]. Based on the data obtained, regional technologies for growing eastern galega are being developed and optimized, considering the timing and norms of seeding, the effectiveness of cover crops, the use of microbiological preparations, the number of mowing, the impact on the soil, the duration of economic use of crops, seed productivity. The authors of these works mainly evaluated the photosynthetic potential, crop productivity, the content of dry matter, protein, and amino acids in the green mass during the growing season and depending on the cultivation method. However, there are other components in the green mass of galega that characterize its feed advantages and quality, i.e., fiber, pectins, chlorophylls, secondary metabolites (vitamin C, flavonoids, carotenoids), as well as anti-nutritional substances, for example, tannins [17-19] which worsen protein digestibility and give plants a bitter taste, undesirable in feed cultures. The number and ratio of such components can also change in plant ontogenesis and under the influence of environmental conditions and cultivation technologies.

The physiological role of chlorophylls, carotenoids, vitamin C and flavonoids is diverse. These are strong natural antioxidants [20-22], whose protective properties are due to the ability to prevent or slow down oxidative damage to cells caused by physiological oxidants, including reactive oxygen species, nitrogen, and free radicals [23]. In addition, carotenoids play an important role in metabolism (vitamin A is a derivative of beta-carotene) [24]. Along with the antioxidant effect, the anti-inflammatory, hepatoprotective, antibacterial, antiviral, and anticancer activity of flavonoids is well known [22]. Chlorophylls and carotenoids are pigments involved in photosynthesis. They are part of the main pigment-protein complexes of the photosynthetic apparatus [25]. The photosynthetic apparatus is capable of restructuring, which ensures the successful growth and development of plants in continuously changing lighting conditions. The key components of the photosynthetic apparatus, the pigment-protein complexes are characterized by constancy of composition and structure, and adaptive transformations are carried out by changing their number and correlation in thylakoid membranes [25]. Ascorbic acid (AA) is a low-molecular-weight antioxidant, most common in plants where it is involved in a variety of metabolic processes, including reactions that determine resistance to stress and adaptive response to environmental influences [26]. The role of ascorbic acid in maintaining photosynthesis and protecting the photosynthetic apparatus from reactive oxygen species and photoinhibition is known [26, 27]. Ascorbic acid can be an electron donor that ensures the full functioning of the photosynthetic electron transport chain [28-30]. Flavonoids are secondary metabolites of plants with high biological activity they can directly or indirectly weaken or prevent cellular damage caused by free radicals [22, 31]. Flavonoids play an extremely important functional role in the interactions of plants and the environment. They participate in the regulation of auxin transport, creating its gradients. This leads to the formation of phenotypes with various morphoanatomical features, which can be of great importance in stress-induced morphogenic response of plants [22].

Despite the rather long history of the introduction of *G. orientalis* as a forage crop in different regions of the world and in Russia, there is little information about the accumulation of photosynthetic pigments, vitamin C and flavonoids in galega plants when adapting to new growing conditions [4, 17-19, 32, 33], and for the north of Russia there are none.

Earlier, we described for the first time the phenological, eco-morphological features and assessed the photosynthetic potential, productivity of green mass and seeds [34-36] in eastern galega and prospects for growing in the Middle taiga zone of Western Siberia [37-39]. In this paper, we systematize the data we obtained for the first time on the accumulation of photosynthetic pigments, vitamin C and flavonoids in the plants of the eastern galega at the site of introduction.

The aim of the study was to characterize the content of chlorophyls, carotenoids, vitamin C and flavonoids in the Eastern galega (*Galega orientalis* Lam.) plants when adapting to new environmental conditions of cultivation with different agrotechnical techniques (pre-sowing seed preparation, the use of peas as a cover crop) and depending on the age of the herbage.

Materials and methods. Introduction studies of *G. orientalis* were carried out in 2013-2015 at an experimental site in the village of Barsovo (Khanty-Mansi

Autonomous Okrug — Yugra, Surgut district, 61°15'00" N., 73°25'00" E.) on the variety Gala (in 1988, the variety was included in the State Register of Breeding achievements admitted to use). The seeds were purchased in 2013 (OOO AF Seeds of the Ob region, Novosibirsk, category RS1 — first reproduction).

The soil of the experimental site is sandy podzolic, cultivated, the mass fraction of organic matter is 5.63%, pH 5.21, the soil is 4.7 mmol/100 g absorbed bases, 3.85 mg/kg N-NH^4, 129 mg/kg N-NO$_3$, 396.1 mg/kg P_2O_5, and 66.5 mg/kg K_2O [36]. The growing season of 2013 was arid, the sum of the average daily temperatures was 1751 °C, precipitation was 252.7 mm, HTC (hydrothermal coefficient) = 1.4 (with an average annual value of HTC = 1.7). In the warm periods of 2014 and 2015, the sum of average daily temperatures was equal to 1546 and 1579 °C, respectively, with excessive accumulation of moisture — 356 and 458 mm, respectively (with a norm of 1648.6 °C and 287 mm), HTC = 2.3 in 2014, HTC = 2.9 in 2015 [36]. During the growing seasons, there were sharp fluctuations in the main meteorological parameters, generally unfavorable for the growth and development of the eastern galega. Monitoring of weather conditions at the site of introduction was carried out based on data from the Surgut weather station.

The influence of meteorological factors of the growing seasons were assessed in micro plot field tests. Sowings were performed in 2013 (for herbages of 1-3 years of life in 2013-2015), in 2014 (for herbages of 1-2 years of life in 2014-2015), and in 2015, taking into account in 2015 (for an herbage of the 1st year of life in 2015). The plants were grown in three variants. Control was a single-species sowing without seed treatment. The second option was a single-species sowing with seed pretreatment with a microbiopreparation Baikal-EM1 based on a complex of lactic acid, photosynthetic, nitrogen-fixing bacteria, saccharomycetes (NPO EM-Center LLC, Moscow Ulan-Ude, Russia). A 1:1000 dilution of the preparation was used for seed soaking during 30-60 min. The third treatment included a mixed sowing with peas as a cover culture without pre-sowing bacterial inoculation of galega seeds. The seeding rate was 2.8 million seeds per 1 ha for galega and 1 million seeds per 1 ha for peas. Weeding was not carried out. At the end of the growing season, the herbage of the eastern galega was not mowed. The biological repeatability in each variant was 4-fold, the placement of plots is randomized [40], 1.5 m^2 per plot, the total test area for each year is 18 m^2. Phenological observations were carried out as reported [41], the phases of ontogenesis were recorded, the formation of morphological structures was considered [42-46]. At each phenophase of development and at the end of vegetation [41], functionally mature leaves from 20 plants were selected for analysis and a combined sample was formed (the total number of samples was 372). The samples were air-dried and crushed.

To determine the amount of photosynthetic pigments — chlorophylls a and b (Chl a, Chl b) and carotenoids (Car), a 0.05-0.08 g portion of biomass was extracted with 96% ethyl alcohol with the addition of $CaCO_3$ and filtered to a colorless state. The optical density of the extract was determined at $\lambda = 665$ nm (chlorophyll a), $\lambda = 649$ nm (chlorophyll b) and $\lambda = 470$ nm (carotenoids) (SF-56, Lumex LLC, Russia), control was 96% ethyl alcohol [47]. The proportion of chlorophylls in light-collecting complexes (CCCs) was calculated as (Chl b + Chl 1.2 b)/(Chl a + Chl b), assuming that all Chl b is in the CC of photosystem II (PSII), and the ratio of Chl a/Chl b in total is approximately 1.2 [48, 49]. The ratios of Chl a/Chl b and (Chl a + Chl b)/Car were determined.

The amount of amino acids (AA) was determined by E.J. Hewitt and G.J. Dickes [50] in the modification of G.N. Chupakhina [51]. A sample of vegetable raw materials (0.3 g) was poured with 5% metaphosphoric acid, ground and extracted for 10 minutes at 4 °C and 20 minutes in a thermostat at 100 °C. The extracts were transferred to an ice bath followed in 1 hour by a photometric measurement at λ = 520 nm (SF-56, Lumex LLC, Russia); control was 5% metaphosphoric acid.

The concetration of flavonoids was determined in accordance with recommendation [52] in a color reaction with aluminum chloride. A 0.25 g sample was extracted with 70% ethyl alcohol for 30 minutes with heating in a water bath. The optical density was determined at λ = 410 nm vs. the standard rutin solution (SF-56, Lumex LLC, Russia).

Statistical data processing (Microsoft Office Excel 2016 software package and the Statistica 6.0 program, StatSoft, Inc., USA) included calculation of arithmetic means (*M*) and standard errors of means (±SEM). The significance of the differences was assessed by the Student's *t*-test at $p = 0.05$. Pearson pair correlation analysis was used to assess the interrelationships of the studied parameters.

Results. Plant productivity and accumulation of biologically active substances are determined by complex physiological and biochemical interactions, environmental factors and agricultural technologies. The introduction of plants in northern latitudes is limited by unfavorable soil and weather conditions. In the Middle taiga of Western Siberia, this is a cold climate, sharp daily temperature fluctuations, frosts, an increase in daylight hours in the first half of the growing season, a short growing season, low fertility, and high soil acidity. Earlier, we showed that the yield of green mass in eastern galega of the Gale variety in the Surgut District (the Khanty-Mansi Autonomous Okrug, 61°15'00" N, 73°25'00" E) on average for 3 years was 243.0 c/ha in the control, 280.0 c/ha when using Baikal fertilizer-EM1, and 66.7 c/ha in mixed sowing with peas. The dry matter was 68.8, 76.4 and 19.9 c/ha, respectively {35].

Photosynthetic pigments. When growing eastern galega, the effect of the compared methods with respect to the number of chlorophylls in the leaves by the phases of plant development was statistically significant ($p \leq 0.05$) from the 2nd year of life (Table 1). Thus, when inoculated with a microbiological preparation for the 2nd and 3rd years of life at germination phase, the number of green pigments was higherby 22 and 16%, , respectively, at the tillering phase by 26 and 19%, at stem branching by 19 and 18% vs. control. In mixed sowing with peas in plants of the 2nd and 3rd years of life, the amount of chlorophylls in the leaves increased statistically significantly ($p \leq 0.05$) by 19.4% at germination phase, by 24.0% at tillering, by 18.7% at stem branching, but decreased at the end of the 2nd year of life by 53.0% vs. control. On the 3rd year, with the cover crop at tillering and stem branching, the total content of chlorophylls, on the contrary, first decreased (by 46 and 21%), and at the end of the growing season was 33% higher vs. control.

1. **The content of photosynthetic pigments** (mg/g of dry matter) **in the leaves of *Galega orientalis* Lam. cv. Gale depending on the age of herbage and agrotechnology during introduction** (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District)

PD	Chl a		Chl b		Chl a + Chl b		Car		Chl a + Chl b + Car	
	M±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %
The 1st year of life										
Monoculture (not treated seeds, control)										
Sown in 2013										
1	1.28±0.02	17.0	0.33±0.04	37.0	1.61±0.05	14.0	1.28±0.12	16.8	2.89±0.02	18.3
2	1.55±0.02	14.3	0.42±0.04	54.0	1.97±0.03	16.2	1.12±0.10	14.5	3.04±0.02	15.0
3	1.76±0.07	15.6	0.54±0.08	24.7	2.30±0.05	15.0	0.92±0.14	18.0	3.22±0.08	17.4
7	0.21±0.05	18.2	0.10±0.06	22.0	0.31±0.09	16.0	0.49±0.09	15.3	0.80±0.05	20.3
Sown in 2014										
1	1.30±0.03	24.1	0.18±0.12	32.4	1.48±0.04	22.3	1.18±0.10	17.4	2.66±0.09	19.4
2	1.62±0.09	19.0	0.42±0.10	50.1	2.04±0.03	18.7	1.09±0.14	19.2	3.13±0.07	17.0
3	1.88±0.02	17.3	0.46±0.05	48.0	2.34±0.10	15.6	0.83±0.10	16.4	3.17±0.05	15.8

Continued Table 1

7	0.20±0.03	15.0	0.55±0.07	32.0	0.73±0.07	27.3	0.42±0.09	15.0	1.15±0.06	16.4
Sown in 2015										
1	1.34±0.05	16.3	1.29±0.05	27.6	2.63±0.06	19.2	1.22±0.08	14.8	3.85±0.07	15.0
2	1.53±0.07	21.0	1.47±0.03	25.3	3.00±0.04	20.0	1.18±0.10	19.2	4.18±0.08	14.8
3	1.75±0.05	12.0	1.57±0.09	52.0	3.32±0.06	24.5	0.85±0.13	19.0	4.17±0.10	22.3
7	0.20±0.08	12.7	0.11±0.07	47.3	0.30±0.07	19.3	0.33±0.09	14.3	0.63±0.09	17.5
Phenophase average (2013-2015)										
1	1.30±0.02	12.0	0.61±0.35	54.8	1.91±0.36	33.0	1.23±0.03	18.0	3.29±0.29	15.2
2	1.53±0.06	16.0	0.77±0.35	41.0	2.34±0.33	24.6	1.13±0.03	14.0	3.47±0.36	17.8
3	1.80±0.04	14.3	0.86±0.36	39.2	2.65±0.33	21.8	0.87±0.03	15.0	3.52±0.33	16.0
7	0.20±0.03	13.7	0.25±0.15	40.0	0.86±0.15	31.0	0.41±0.05	19.0	0.86±0.15	30.8
Average for the growing season (2013-2015)										
	1.22±0.18	52.5	0.62±0.15	84.0	1.84±0.29	54.0	0.91±0.09	37.0	2.75±0.36	25.3
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)										
Sown in 2013										
1	1.37±0.07	15.4	0.21±0.03	41.3	1.58±0.10	22.1	1.20±0.02	17.3	2.78±0.07	15.3
2	1.42±0.12	14.0	0.38±0.05	52.0	1.80±.005	15.4	1.13±0.01	11.7	2.93±0.05	17.8
3	1.64±0.09	19.2	0.43±0.05	37.8	2.07±0.09	15.0	1.00±0.03	15.6	3.07±0.09	21.0
7	0.21±0.10	23.0	0.12±0.09	50.3	0.33±0.07	15.8	0.51±0.03	22.0	0.84±0.05	14.0
Sown in 2014										
1	1.37±0.05	20.2	0.48±0.12	48.6	1.85±0.08	16.7	1.17±0.03	24.2	3.02±0.12	19.1
2	1.72±0.05	17.1	0.51±0.10	470	2.23±0.10	21.0	1.08±0.05	19.4	3.31±0.10	18.0
3	2.00±0.03	18.3	0.58±0.08	34.2	2.58±0.13	19.3	0.84±0.04	17.6	3.42±0.09	23.0
7	0.16±0.10	16.0	0.07±0.03	51.0	0.23±0.15	27.4	0.42±0.02	19.0	0.65±0.07	20.0
Sown in 2015										
1	1.37±0.07	14.3	1.21±0.08	36.8	2.58±0.15	19.5	1.21±0.02	20.0	3.79±0.05	15.7
2	1.42±0.10	24.1	1.34±0.05	45.9	2.76±0.09	18.0	0.97±0.05	18.3	3.73±0.07	14.2
3	1.72±0.12	18.0	1.75±0.05	37.4	3.47±0.06	20.3	0.80±0.09	24.5	4.27±0.21	20.0
7	0.19±0.05	16.0	0.10±0.09	28.4	0.29±0.10	17.8	0.42±0.01	30.0	0.67±0.08	23.7
Phenophase average (2013-2015)										
1	1.37±0.09	13.2	0.63±0.29	31.0	1.99±0.30	26.1	1.19±0.01	33.0	3.20±0.30	16.5
2	1.50±0.10	12.7	0.74±0.30	25.8	2.26±0.35	24.6	1.06±0.05	24.6	3.32±0.23	12.0
3	1.79±0.11	11.3	0.92±0.42	35.0	2.71±0.41	26.2	0.88±0.06	21.8	3.59±0.36	17.2
7	0.19±0.02	15.0	0.09±0.01	43.0	0.72±0.06	14.5	0.45±0.03	21.0	0.72±0.60	14.5
Average for the growing season (2013-2015)										
	1.22±0.19	54.0	0.60±0.12	31.0	1.84±0.30	57.9	0.89±0.09	33.0	2.71±0.37	27.0
Mixed culture with pea										
Sown in 2013										
1	1.25±0.03	16.2	0.48±0.08	42.4	1.73±0.12	20.0	1.25±0.05	17.8	2.98±0.09	20.0
2	1.70±0.01	15.0	0.57±0.13	38.0	2.27±0.09	19.2	1.17±0.08	14.5	3.44±0.15	31.3
3	1.70±0.05	17.3	0.64±0.19	27.4	1.77±0.08	15.6	0.98±0.08	16.0	2.75±0.10	27.8
7	0.71±0.03	14.5	0.36±0.05	30.0	1.07±0.12	17.3	0.61±0.07	21.8	1.68±0.08	21.0
Sown in 2014										
1	1.25±0.04	23.0	0.53±0.07	49.0	1.78±0.10	18.0	1.22±0.05	13.0	3.0±0.16	15.3
2	1.53±0.09	20.0	0.18±0.05	41.3	1.71±0.09	21.3	0.93±0.04	15.0	2.64±0.21	18.9
3	1.92±0.12	16.7	0.34±0.07	36.5	2.26±0.19	18.3	0.83±.09	18.4	3.09±0.09	20.0
7	0.21±0.06	15.0	0.14±.12	31.3	0.35±0.23	20.0	0.36±.03	20.0	0.71±0.10	16.8
Sown in 2015										
1	1.28±0.14	22.0	1.32±0.06	28.4	2.60±0.07	14.6	1.24±0.13	18.7	3.84±0.07	22.0
2	1.64±0.09	14.8	1.64±0.05	28.0	3.28±0.09	18.0	1.17±0.05	14.8	4.45±0.06	19.3
3	1.83±0.15	18.2	1.98±0.09	37.6	3.81±0.10	17.5	0.97±0.05	15.2	4.78±0.03	24.8
7	0.21±0.08*	19.0	0.14±.10	37.0	0.35±0.16	20.1	0.28±0.09	16.0	0.63±.05	16.0
Phenophase average (2013-2015)										
1	1.26±0.01	14.0	0.78±0.27	28.4	2.04±0.28	23.9	1.24±0.01	12.0	3.27±0.28	14.9
2	1.62±0.05	15.7	0.80±0.43	42.0	2.42±0.46	32.8	1.09±0.08	13.0	3.51±0.53	25.8
3	1.82±0.06	16.3	0.99±0.50	37.6	2.61±0.61	40.8	0.93±0.05	19.0	3.54±0.63	30.7
7	0.38±0.17	16.0	0.21±0.07	26.0	1.00±0.34	58.1	0.42±0.1	41.0	1.01±0.34	38.0
Average for the growing season (2013-2015)										
	1.27±0.17	46.7	0.69±0.18	39.0	1.92±0.3	54.3	0.92±0.09	37.0	2.83±0.38	46.0
The 2nd year of life										
Monoculture (not treated seeds, control)										
Sown in 2013										
1	1.32±0.06	14.0	0.27±0.04	17.0	1.59±0.16	18.0	1.12±0.18	12.5	2.71±0.05	20.0
2	1.43±0.06	16.3	0.31±0.09	21.0	1.74±0.22	16.3	1.10±0.05	14.8	1.93±0.03	23.7
3	1.57±0.03	15.2	0.35±0.12	14.5	1.92±0.19	14.7	0.83±0.05	25.0	2.75±0.07	28.1
7	0.62±0.08	14.8	0.34±0.09	16.0	0.96±0.07	16.0	0.66±0.09	19.1	1.62±0.07	28.0
Sown in 2014										
1	1.42±0.04	24.0	0.30±0.07	22.0	1.72±0.07	25.7	0.19±0.07	10.0	1.91±0.05	14.6
2	1.52±0.05	16.3	0.34±0.05	16.3	1.86±0.10	22.1	0.79±0.12	24.6	2.65±0.09	23.0
3	1.74±0.07	14.0	0.34±0.05	20.0	1.92±0.11	20.8	0.68±0.15	27.5	2.60±0.08	18.4
7	0.25±0.01	16.0	0.15±0.08	19.7	0.40±0.08	23.5	0.30±0.10	13.0	0.70±0.05	29.0

Continued Table 1

Phenophase average (2014-2015)										
1	1.37±0.04	15.0	0.29±0.02	10.0	2.25±0.19	21.3	0.94±0.27	18.0	2.31±0.40	24.5
2	1.48±0.03	14.0	0.33±0.11	17.0	2.30±0.16	17.2	0.64±0.07	15.0	2.29±0.36	22.0
3	1.64±0.05	16.0	0.36±0.01	15.6	2.69±0.05	14.2	0.75±0.06	16.0	2.68±0.08	13.9
7	0.42±0.11	13.0	0.27±0.05	30.0	1.17±0.21	43.0	0.49±0.07	30.0	1.16±0.46	56.0
Average for the growing season (2014-2015)										
	1.23±0.18	42.0	0.30±0.02	23.0	1.50±0.19	36.2	0.71±0.12	47.0	2.10±0.25	34.0
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)										
Sown in 2013										
1	1.40±0.12	17.3	0.43±0.02	24.0	1.83±0.07	14.0	1.00±0.05	20.0	2.83±0.03	12.3
2	1.63±0.10	19.2	0.47±0.03	22.0	2.10±0.07	16.0	0.87±0.08	17.8	2.97±0.03	17.8
3	1.90±0.10	23.4	0.52±0.05	18.4	2.42±0.09	16.3	0.82±0.13	14.5	3.24±0.09	16.0
7	0.57±0.09	14.5	0.29±0.08	15.3	0.86±0.12	22.0	0.73±0.10	18.0	1.59±0.05	19.0
Sown in 2014										
1	1.38±0.50	18.0	0.43±0.02	17.0	1.84±0.05	15.4	1.14±0.09	16.4	2.95±0.03	25.4
2	1.85±0.13	12.8	0.48±0.07	21.0	2.33±0.07	14.0	0.97±0.14	22.0	3.30±0.02	15.6
3	2.30±0.08	14.0	0.52±0.05	23.2	2.82±0.05	27.0	0.72±0.10	13.8	3.54±0.07	16.0
7	0.48±0.02	16.3	0.29±0.03	15.7	0.77±.010	18.4	0.50±0.08	14.0	1.27±0.05	20.0
Phenophase average (2014-2015)										
1	1.39±0.02	19.0	0.43±0.01	18.3	2.89±0.03*	17.0	1.04±0.05	11.0	2.89±0.06	12.8
2	1.80±0.07*	18.0	0.48±0.01	16.1	3.10±0.08*	16.2	0.92±0.02	14.0	3.14±0.17	17.4
3	2.14±0.12*	12.0	0.53±0.02	17.4	3.12±0.07*	14.9	0.80±0.05	13.0	3.29±0.15	16.2
7	0.53±0.02	18.0	0.29±0.01	20.3	1.42±0.08*	12.6	0.66±0.06	19.0	1.43±0.16	15.8
Average for the growing season (2014-2015)										
	1.44±0.23*	44,3	0,43±0,03	22,0	1,90±0,26	38.9	0.84±0.07	24.0	2.71±0.29	34.0
Mixed culture with pea										
Sown in 2013										
1	1.27±0.05	15.0	0.32±0.10	17.0	1.59±0.05	16.8	1.20±0.12	19.2	2.79±0.02	11.0
2	1.78±0.07	17.0	0.10±0.05	16.2	1.88±0.07	17.2	1.15±0.08	20.0	3.03±0.02	16.0
3	1.53±0.05	14.5	0.08±0.03	15.4	1.61±0.03	21.3	0.81±0.06	27.3	2.42±0.02	13.7
7	0.19±0.03	12.3	0.09±0.03	14.5	0.28±0.03	17.0	0.35±0.04	15.0	0.63±0.05	14.2
Sown in 2014										
1	1.33±0.04	14.0	0.62±0.08	14.0	1.95±0.03	14.3	0.93±0.05	19.0	2.88±0.08	19.2
2	1.78±0.05	13.5	1.28±0.12	17.3	2.76±0.07	15.0	0.76±0.05	18.2	3.52±0.07	18.0
3	1.53±0.08	15.0	1.35±0.07	15.2	3.08±0.05	19.0	0.52±0.08	16.3	3.60±0.05	20.0
7	0.19±0.12	15.7	0.11±0.05	20.3	0.28±0.04	16.4	0.18±0.06	14.0	0.46±0.03	13.7
Phenophase average (2014-2015)										
1	1.30±0.03	14.0	0.51±0.07*	19.0	2.79±0.05*	18.2	1.10±0.08	14.0	2.84±0.06	12.4
2	1.78±0.01*	19.0	0.77±0.30*	16.8	3.04±0.12*	15.2	0.96±0.09	20.0	3.28±0.25	10.6
3	1.51±0.01	14.0	0.93±0.29*	15.0	3.31±0.26*	20.8	0.70±0.07	19.0	3.01±0.59	27.7
7	0.19±0.01*	19.0	0.11±0.01*	12.0	0.55±0.05*	19.5	0.28±0.04	26.0	0.55±0.09	22.0
Average for the growing season (2014-2015)										
	1.20±0.23	52,0	0,53±0,18*	44,0	1,70±0,36	60,3	0,74±0,13	50,0	2,41±0,43	50,1
The 3d year of life										
Monoculture (not treated seeds, control)										
Sown in 2013										
1	1.40±0.04	18.2	0.40±0.05	17.3	1.78±0.08	15.8	1.40±0.05	17.3	2.93±0.07	25.0
2	1.60±0.05	17.4	0.43±0.08	15.0	1.99±0.12	18.1	0.83±0.08	15.6	2.92±0.04	30.0
3	1.70±0.03	15.3	0.45±0.08	18.0	2.13±0.09	12.9	0.93±0.06	20.0	2.99±0.07	24.8
4	2.50±0.08	12.8	0.32±0.03	22.0	2.82±0.17	12.3	1.35±0.10	14.3	4.17±0.03	29.0
5	1.90±0.05	14.3	0.23±0.09	27.0	2.10±0.10	14.3	0.92±0.12	12.8	3.02±0.05	25.0
6	0.52±0.07	19.0	0.16±0.05	19.8	0.68±0.09	15.4	0.73±0.08	14.0	1.41±0.02	30.4
7	0.50±0.12	19.2	0.27±0.07	23.0	0.73±0.14	18.2	0.63±0.08	16.0	1.36±0.07	27.8
Average for the growing season (2015)										
	1.45±0.27	50.0	0.32±0.04	34.0	1.75±0.29	44.6	0.94±0.09	26.0	2.68±0.38	37.0
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)										
Sown in 2013										
1	1.43±0.04	16.0	0.69±0.03	14.5	2.13±0.08*	19.8	1.56±0.07	16.0	3.68±0.07	29.0
2	1.57±0.07	15.4	0.72±0.05	16.1	2.45±0.03*	12.9	1.10±0.05	22.3	3.39±0.09	24.5
3	1.91±0.05	20.0	0.75±0.04	17.0	2.63±0.02*	12.4	1.25±0.09	17.4	3.91±0.05	28.3
4	2.30±0.03	17.2	0.48±0.10	17.3	2.74±0.03*	12.9	1.42±0.07	16.5	4.20±0.06	27.1
5	1.42±0.05*	14.9	0.35±0.04	14.6	1.69±0.10*	18.7	0.83±0.05	14.3	2.60±0.08	30.0
6	0.70±0.02*	16.1	0.27±0.08	18.2	0.92±0.01*	13.0	0.74±0.08	16.0	1.67±0.09	32.1
7	0.60±0.05	17.0	0.55±0.04	18.0	0.74±0.03*	18.4	0.54±0.10	18.2	1.32±0.05	18.6
Average for the growing season (2015)										
	1.42±0.23	43.0	0.54±0.07*	35.0	1.90±0.30	41.6	1.06±0.14	35.0	2.97±0.43	38.0
Mixed culture with pea										
Sown in 2013										
1	1.40±0.07	20.0	0.55±0.05	18.0	1.92±0.02	12.8	1.35±0.09	15.7	3.29±0.09	19.0
2	0.80±0.10*	14.7	0.34±0.05	16.1	1.07±0.02*	13.9	0.56±0.05	16.8	1.65±0.10	25.7
3	0.50±0.08*	16.2	0.18±0.08*	15.8	0.63±0.03*	12.9	0.81±0.13	18.0	1.48±0.09	29.0
7	0.42±0.04	16.0	0.35±0.09	16.0	1.05±0.02*	13.5	0.86±0.10	17.2	1.93±0.15	32.4

Continued Table 1

Average for the growing season (2015)									
0.86±0.19*	45.0	0.36±0.06	43.0	1.20±0.27	44.8	0.89±0.16	37.0	2.09±0.24	39.0
Average over the years of study									
Monoculture (not treated seeds, control)									
1.28±0.12	47.9	045±0.07	36.5	1.78±0.16	47.2	0.86±0.06	37.0	2.54±0.2	41.2
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)									
1.33±0.12	46.5	0.53±0.07	32.0	1.9±0.29	46.9	0.92±0.06	32.0	2.78±0.2	38.9
Mixed culture with pea									
1.18±0.12	49.0	0.58±0.10	45.0	1.72±0.19	56.6	0.84±0.07	40.0	2.57±0.25	46.6
Average for the 1st year of life									
1.23±0.10	49.5	0.64±0.09	52.3	1.88±0.18	55.9	0.91±0.05	35.0	2.76±0.21	44.6
Average for the 2nd year of life									
1.29±0.12	45.5	0.42±0.07**	48.0	1.68±0.15	44.7	0.76±0.06	39.0	2.41±0.19	38.9
Average for the 3d year of life									
1.32±0.14	48.4	0.40±0.04	43.0	1.69±0.17	43.0	0.98±0.07	32.0	2.66±0.19	38.2

Note. PD — phase of devlopment; 1 — seedlings (regrowth for the 2nd and the 3d years of life), 2 — tillering, 3 — stem branching, 4 — budding, 5 — flowering, 6 — fruiting, 7 —the end of vegetation; Chl a, Chl b, Car — chlorophylls and carotenoids.

* Differences vs. control are statistically significant at $p \le 0.05$.

** Differences vs. the value in the previous year are statistically significant at $p \le 0.05$.

Quantitative and qualitative changes in the pigment complex reflect the state of the photosynthetic apparatus and physiological status of plants [53, 54]. With quantitative changes in the pigment apparatus of leaves (the content of Chl a, Chl b, Chl a + Chl b, Chl a/Chl b, the content of carotenoids and the Chl/Car ratio) in response to environmental conditions, light is the main factor, but other conditions, the temperature and humidity have a certain influence [55]. When adapting to new environmental conditions, quantitative changes may occur in the pigment complex [56] and LHC [57]. If the light flux collected by the plant does not limit photosynthesis, the amount of LHC decreases and the ratio Chl a/Chl b increases [7]. At high latitudes, the percentage of blue-violet rays absorbed by carotenoids increases in the spectrum of scattered radiation, and a proportion of carotenoids increase in the profile of photosynthetic pigments. This indicates an increase in their protective role with the advance to the north [53].

The content of Chl a. In our tests, the leaf content of Chl a (see Table. 1) in the galega plants of the year of sowing, for the 2nd and 3rd years of life, averaged 1.23±0.10; 1.29±0.12 and 1.32±0.14 mg/g of dry weight (control values). With the use of microbiological fertilizer, the values for the 2nd year of life significantly ($p \le 0.05$) increased vs. control (by 18% at tillering and by 24% at stem branching). In mixed sowing, for the 2nd year of life, the content of Chl a was maximum at tillering (1.78±0.01 mg/g) with a significant ($p \le 0.05$) excess over the control value by 17% and a decrease to 1.51 mg/g at stem branching and to 0.19 mg/g by the end of the growing season. On average, in the 2nd year, when using Baikal-EM1 fertilizer, the content of Chl a in leaves ($p \le 0.05$) increased by 15% compared to the control, while in mixed sowing with peas, it remained within the control values (1.20±0.23 mg/g).

In the 3rd year of vegetation, a gradual increase in the Chl a content in the leaves occurred in control and bacterial inoculation, starting from the regrowth phase to budding (from 1.40 to 2.50 mg/g of dry matter). At the end of the growing season, the Chl a content decreased to 0.50-0.60 mg/g of dry matter. Under inoculation, the Chl a content was significantly lower than in the control (by 25%) during the flowering period and higher than the control (by 26%) at fruiting phase. In the mixed sowing, the content of Chl a in leaves reached its maximum during the regrowth period and at the end of the growing season.

The Chl a/Chl b ratio. The Chl a/Chl b values (Table. 2) in the galega leaves ranged from 2.78 to 4.41 depending on the age of the plants. The analyzed parameter for the 2nd year of life significantly increased (by 37%) vs. that of plants in the 1st year of life. Upon reaching the generative age of plants (for the 3rd year of life), it significantly decreased to 3.44.

2. The ratio of photosynthetic pigments and the proportion of chlorophylls in light-harvesting complexes (LHC) **in the leaves of *Galega orientalis* Lam. cv. Gale depending on the age of herbage and agrotechnology during introduction** (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District)

PD	Chl a/Chl b		(Chl a + Chl b)/Car		Pproportion of Chl a + Chl b in LHC, %	
	M±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %
The 1st year of life						
Monoculture (*not treated seeds, control*)						
Sown in 2013						
1	3.88±0.02	17.0	1.28±0.07	22.4	52.0±4.12	45.0
2	3.69±0.02	12.8	1.78±0.05	18.3	57.3±5.00	37.4
3	3.26±0.05	19.4	2.50±0.05	18.0	60.2±6.18	40.3
7	2.10±0.03	21.0	0.63±0.02	17.4	71.7±3.48	42.0
Sown in 2014						
1	7.22±0.08	12.6	1.25±0.03	14.0	27.8±6.52	58.2
2	3.86±0.03	14.0	1.87±0.10	19.0	60.0±5.30	32.4
3	4.09±0.03	13.0	2.82±0.04	18.2	53.6±5.00	29.5
7	2.00±0.06	12.7	0.71±0.02	22.0	96.4±7.24	40.3
Sown in 2015						
1	1.04±0.07	15.0	2.16±0.06	19.3	75.3±6.32	48.2
2	1.04±0.03	13.4	2.54±0.04	13.4	60.0±8.00	36.0
3	1.11±0.04	19.5	3.91±0.07	12.4	70.2±5.41	34.3
7	1.82±0.02	20.0	0.94±0.12	13.7	81.3±3.87	52.0
Phenophase average (2013-2015)						
1	4.05±1.79	12.7	1.56±0.29	33.0	50.2±6.00	47.2
2	2.86±0.91	19.0	2.06±0.24	20.0	60.7±5.09	32.4
3	2.82±0.89	19.3	3.08±0.43	24.0	60.3±4.70	36.0
7	1.97±0.08	18.0	0.76±0.09	21.0	70.2±7.12	38.1
Average for the growing season (2013-2015)						
	2,96±0,52	61,0	1,87±0,28	51,0	74,8±11,6	28,7
Monoculture (*seeds pre-treated with microbiological agent* Baikal-EM1)						
Sown in 2013						
1	6.52±0.04	21.0	1.32±0.09	14.0	29.4±7.10	40.0
2	3.74±0.07	11.4	1.59±0.08	19.3	48.3±5.00	48.0
3	3.85±0.08	18.3	2.07±0.06	22.4	50.4±6.41	35.7
7	1.75±0.03	22.0	0.65±0.04	20.0	80.1±7.00	29.3
Sown in 2014						
1	2.85±0.21	14.7	1.58±0.12	24.1	57.2±6.42	50.0
2	3.37±0.19	21.2	2.06±0.15	20.6	60.0±6.00	34.1
3	3.45±0.24	24.0	3.07±0.22	15.7	62.7±8.34	29.0
7	2.28±0.18	19.3	0.55±0.09	17.0	67.0±5.42	35.7
Sown in 2015						
1	1.13±0.14	20.5	2.13±0.04	14.3	62.0±6.70	32.4
2	1.06±0.22	15.7	2.84±0.07	12.8	70.0±9.10	35.0
3	0.98±0.15	11.8	4.34±0.09	18.0	69.7±4.35	31.0
7	1.97±0.20	28.0	0.76±0.13	21.4	76.0±7.12	28.7
Phenophase average (2013-2015)						
1	3.50±1.59	49.0	1.68±0.29	25.0	50.3±5.43	36.4
2	2.72±0.84	53.0	2.16±0.36	29.0	60.8±9.12	30.5
3	2.76±0.89	56.2	3.16±0.66	36.0	60.0±8.00	29.0
7	2.03±0.18	16.7	0.65±0.06	16.0	70.4±5.21	32.7
Average for the growing season (2013-2015)						
	2.76±0.46	57.0	1.91±0.32	58.0	68.4±7.83	24.6
Mixed culture with pea						
Sown in 2013						
1	2.60±0.18	14.3	1.38±0.09	14.1	61.0±8.00	21.5
2	2.98±0.24	18.2	1.94±0.09	10.8	75.4±4.68	29.4
3	1.77±0.21	12.4	1.81±0.05	14.0	80.0±7.39	32.3
7	1.97±0.17	19.0	1.75±0.14	12.3	74.4±8.22	24.5
Sown in 2014						
1	2.36±0.08	19.4	1.46±0.08	14.5	66.3±6.00	21.3
2	8.50±0.08	15.6	1.84±0.15	16.0	63.0±8.00	27.8
3	5.65±0.12	23.0	2.73±0.18	13.5	55.0±7.84	30.0
7	1.50±0.15	21.0	0.97±0.12	20.0	88.0±9.37	29.5
Sown in 2015						
1	0.97±0.09	16.4	2.10±0.24	22.1	45.2±5.46	19.7
2	1.00±0.12	17.3	2.80±0.09	18.4	70.4±7.00	32.4
3	0.92±0.12	15.2	3.93±0.02	15.3	73.2±6.21	25.3

Continued Table 2

7	1.50±0.14	16.3	1.25±0.28	27.0	88.8±4.78	29.2
Phenophase average (2013-2015)						
1	1.98±0.51	45.2	1.65±0.23	24.0	60.2±3.42	30.0
2	4.16±2.24	52.0	2.19±0.34	21.0	70.6±7.55	24.8
3	2.78±1.46	34.0	2.82±0.61	38.0	70.7±5.00	26.0
7	1.66±0.16	16.4	1.32±0.23*	30.0	80.50±.31	21.3
Average for the growing season (2013-2015)						
	2.64±0.65	48.2	2.00±0.24	41.2	75.30±8.5	28.7
The 2nd year of life						
Monoculture (not treated seeds, control)						
Sown in 2013						
1	4.8±0.07	18.8	1.42±0.04	21.0	37.1±4.45	32.0
2	4.6±0.06	12.6	1.58±0.04	14.5	39.6±7.00	30.0
3	4.5±0.12	14.0	2.31±0.08	20.0	40.8±5.60	24.8
7	1.8±0.09	12.3	1.45±0.05	15.8	78.2±4.89	26.2
Sown in 2014						
1	4.7±0.03	12.0	1.05±0.13	17.3	66.4±8.00	27.3
2	4.4±0.05	18.8	2.35±0.09	22.3	75.8±9.17	32.0
3	4.7±0.03	16.7	3.1±0.10	18.3	74.6±5.00	19.8
7	1.7±0.08	19.3	1.33±0.18	15.6	33.2±4.04	25.3
Phenophase average (2014-2015)						
1	4.73±0.09	13.0	1.29±0.08	13.0	50.1±8.15	27.0
2	4.5±0.1	13.0	1.93±0.22	23.0	50.6±9.03	25.0
3	4.6±0.1	13.0	2.74±0.19	14.0	60.4±7.14	30.0
7	1.75±0.05	14.0	1.4±0.03	14.0	70.5±5.08	28.3
Average for the growing season (2014-2015)						
	3.9±0.47	34.0	1.82±0.07	38.0	56.1±7.3	30.0
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)						
Sown in 2013						
1	3.26±0.09	14.4	1.83±0.07	19.3	52.2±6.31	29.0
2	3.16±0.08	18.0	2.44±0.12	13.7	49.3±8.00	27.6
3	3.65±0.10	22.3	2.95±0.08	22.0	47.4±5.78	28.4
7	1.97±0.12	15.7	1.18±0.15	18.0	74.6±9.10	39.5
Sown in 2014						
1	3.21±0.07	17.0	1.59±0.07	16.4	52.7±10.2	32.4
2	3.85±0.07	14.6	2.40±0.06	21.0	45.9±7.06	24.3
3	4.42±004	18.0	3.92±0.08	14.7	41.4±5.00	28.5
7	1.66±0.03	15.4	1.54±0.04	17.0	83.0±6.33	34.2
Phenophase average (2014-2015)						
1	3.25±0.03	15.0	1.68±0.08*	19.0	50.0±4.89	24.6
2	3.66±0.19	17.0	2.42±0.02*	16.0	60.5±8.00	28.0
3	4.04±0.38	14.0	3.44±0.29*	17.0	70.6±5.30	38.4
7	1.82±0.16	12.0	1.37±0.12	17.0	80.40±4.01	36.2
Average for the growing season (2014-2015)						
	3.18±0.33	25.0	2.23±0.32	40.0	55.40±5.27	42.0
Mixed culture with pea						
Sown in 2013						
1	3.97±0.08	19.3	1.33±0.12	18.2	44.50±6.00	30.0
2	17.80±0.10	12.4	1.63±0.21	15.7	37.30±7.42	32.7
3	19.13±0.07	18.5	1.99±0.09	18.0	11.80±5.65	30.0
7	2.10±0.05	14.0	0.80±0.25	22.0	71.30±4.85	28.4
Sown in 2014						
1	2.15±0.08	18.3	2.10±0.30	19.3	70.60±9.36	25.6
2	1.16±0.09	20.0	3.63±0.15	14.8	56.20±8.00	32.4
3	1.28±0.08	22.0	5.92±0.24	41.0	96.10±7.15	30.1
7	1.55±0.05	30.4	1.56±0.17	21.0	89.00±5.10	36.3
Phenophase average (2014-2015)						
1	3.05±0.48	32.0	1.74±0.19*	24.0	40.70±8.04	28.7
2	9.48±0.60	28.7	2.59±0.56*	37.0	50.20±11.02	38.0
3	10.21±0.54*	25.6	3.65±1.03*	40.0	40.40±7.30	29.0
7	1.83±0.28	22.0	1.18±0.24*	66.0	60.30±5.80	35.4
C Average for the growing season (2014-2015)						
	6.14±2.71*	45.0	2.73±0.58*	22.7	61.88±12.70	27.4
The 3d year of life						
Monoculture (not treated seeds, control)						
Sown in 2013						
1	3.50±0.03	17.4	1.59±0.04	22.0	50.20±8.34	35.1
2	3.72±0.05	17.4	2.18±0.09	19.3	50.40±6.00	28.4
3	3.78±0.03	21.0	2.50±0.03	19.8	50.30±4.78	26.3
4	7.82±0.18	15.2	2.09±0.04	18.3	30.40±9.01	38.4
5	8.26±0.09	14.5	2.32±0.04	14.0	20.40±5.06	40.2
6	2.60±0.12	18.0	0.98±0.05	15.6	50.00±7.12	21.8
7	2.50±0.09	13.3	1.11±0.04	18.4	60.20±5.00	33.5

Continued Table 2

Average for the growing season (2015)						
	4,60±0,91	52,0	1,83±0,23	19,0	44,30±5,28	22,0
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)						
Sown in 2013						
1	2.07±0.06*	15.0	1.36±0.09	23.2	70.04±8.36	27.8
2	2.18±0.07*	25.0	2.08±0.09	25.0	70.12±7.01	40.3
3	2.55±0.12*	22.3	2.30±0.05	22.1	60.1±9.12	30.3
4	4.79±0.05*	16.8	1.96±0.03	19.0	40.4±8.00	40.2
5	4.06±0.05*	15.7	2.13±0.04	19.0	40.0±6.32	39.0
6	2.59±0.08*	18.0	1.31±0.07*	19.2	60.0±5.00	35.4
7	1.50±0.13*	12.3	1.85±0.05*	13.4	90.3±7.31	28.6
Average for the growing season (2015)						
	2.82±0.44*	42.0	1.82±0.13	19.0	61.40±4.78	30.1
Mixed culture with pea						
Sown in 2013						
1	2.55±0.09*	20.0	1.44±0.13	23.0	60.4±5.10	32.5
2	2.35±0.07	23.0	2.04±0.07	18.7	60.2±6.23	40.0
3	2.78±0.09*	18.1	0.84±0.08*	23.4	70.0±4.57	42.5
7	2.40±0.10*	21.0	1.19±0.05	25.0	80.3±6.00	30.7
Average for the growing season (2015)						
	2.52±0.09*	38.0	1.34±0.26*	37.0	67.5±4.79	26.7
Average over the years of study						
Monoculture (not treated seeds, control)						
	3.65±0.37	53.2	1.85±0.16	37.0	61.3±6.13	38.4
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)						
	2.90±0.25*	44.0	1.99±0.17	45.0	62.7±4.20	40.0
Mixed culture with pea						
	3.79±0.98*	47.0	2.02±0.23	56.0	69.50±6.00	29.8
Average for the 1st year of life						
	2.78±0.31	66.0	1.93±0.15	49.0	73.00±5.30	37.5
Average for the 2nd year of life						
	4.41±0.92**	58.0	2.14±0.23	53.0	57.8±5.00**	49.2
Average for the 3d year of life						
	3.44±0.44**	54.0	1.73±0.12*	29.0	56.1±4.05	41.3

Note. PD — phase of devlopment; 1 — seedlings (regrowth for the 2nd and the 3d years of life), 2 — tillering, 3 — stem branching, 4 — budding, 5 — flowering, 6 — fruiting, 7 —the end of vegetation; Chl a, Chl b, Car — chlorophylls and carotenoids.

* Differences vs. control are statistically significant at $p \leq 0.05$.

** Differences vs. the value in the previous year are statistically significant at $p \leq 0.05$.

In the 2nd and 3rd years of life, in mixed culture with peas, statistically significant differences in the value of Chl a/Chl b in leaves depend on the phases of galega plant development (see Table 2). In the 2nd year, the Chl a/Chl b value significantly decreased (by 36%) during regrowth and increased 2-fold at tillering and stem branching vs. control (4.73±0.09, 4.50±0.10, and 4.60±0.10, respectively). In the 3rd year of life, a statistically significant decrease in the Chl a/Chl b value occurred during the regrowth phase (by 27%), at tillering (by 37%), and at stem branching (by 26%) vs. 3.50±0.07, 3.72±0.09, and 3.78±0.08 in the control, respectively. With pre-sowing seed inoculation with Baikal-EM1, the Chl a/Chl b values changed statistically significantly only in the 3rd year of life (for all phases of development, on average, the values y were 33-51% lower compared to control).

On average over 3 years, the Chl a/Chl b values in mixed crops remained within the control range and amounted to 3.79±0.98. When using a microbiological preparation, the Chl a/Chl b significantly decreased by 21%, to 2.90±0.25 vs. 3.65±0.37 in control. A decrease in the Chl a/Chl b values may indicate an increase in the adaptive potential of plants under stress and their stability [57-59].

In plants of the Russian European north-east taiga, antenna (light-collecting) chlorophylls were reported to account for 55-65% of the total green pigments [53]. In our tests, the proportion of the leaf LHC chlorophylls varied from 20 to 90% depending on the phenological phase, the age of the herbage and the agrotechnology (control, inoculation, mixed sowing) (see Table. 2). There was a strong negative correlation between the value of Chl a/Chl b and the proportion of chlorophylls (Chl a + Chl b) in the LHC. In general, the lower the Chl a/Chl b (x) value, the higher the proportion of the LHC chlorophylls (*r* = –0.83; R^2 = 0.666, y = –7,698x + 84,994). The correlation in the control (*r* = –0.80; R^2 = 0.694, y = –6.2859x + 79.81) was lower than when using Baikal-EM1 (*r* = –0.93; R2 = 0.856, y = –12.971x + 98.602), but higher than in binary crops (*r* = –0.65; R^2 = 0.429, y = –2.3476x + 76.206). Correlations between the sum of leaf green pigments and the Chl b content were the same. For (Chl a + Chl b) to Chl b proportion in control, pre-sowing treatment with a microbiological preparation, and binary sowing), accounted for *r* = 0.57, *r* = 0.55, and *r* = 0.89 ($p \le 0.05$).

The content of carotenoids. A sufficiently high accumulation of carotenoids in the galega leaves is quite expected (see Table 1). It is known that in the spectrum of scattered radiation at high latitudes, the percentage of blue-violet rays absorbed by carotenoids increases. Carotenoids can additionally perform a light-harvesting function during white nights [53]. Thanks to carotenoids, plants can use light energy in the blue region of the spectrum [54]. In addition, they protect chlorophyll and other components of photosystems from light overexcitation [54]. We consider the accumulation of carotenoids noted in our experiments as an adaptive response of the photosynthetic apparatus to the conditions of high geographical latitudes [60, 61].

On average, in our tests, the Chl/Car value in the year of sowing was 1.93±0.16, in the 2nd year it increased to 2.44±0.36, but statistically significantly increased (by 19%) only in the 3rd year of plant life (see Table 2). Chl/Car values in the range of 2.0-3.9 correspond to a high content of carotenoids vs. the content of green pigments [53]. The Chl/Car = 3 was reported for plants of the Circumpolar Urals, among which the proportion of Arctic and Arctic-Alpine species is high [53]. This indicates a raising role of carotenoids with the advance to the north.

In all years of observations, in the control, inoculated and mixed sowing, the Chl/Car ratio decreased to minimum values in the autumn period (0.76-1.85) compared to the spring-summer time (2.30-3.65), when intensive linear growth occurs (see Table 2).

The agrotechnologies we compared did not lead to a statistically significant change in the Chl/Car ratio. Nevertheless, when inoculated with Baikal-EM1 and grown together with peas, there was an excess in accumulation of carotenoids in the galega leaves vs. control (see Table 2).

In general, in our tests, there was a wide variation in the Chl/Car ratio, which, in our opinion, can be used in the selection of crops based on productivity and adaptability to the conditions of the Middle taiga of Western Siberia.

Hydrothermal conditions and pigment content. On average, over the years of the study, the accumulation of pigments in the leaves of eastern galega directly correlated with LHC (x) (*r* = 0.90, R^2 = 0.839, y = 0.804x + 0.5586) for all treatments. The pigment content decreased with an increase in the sum of active temperatures during the growing season (Fig. 1). The Chl a content in the leaves inversely depended on the average daily air temperature. The content of Chl b and carotenoids was less associated with the temperature regime of the region (see Fig. 1).

Eastern galega, like all legumes, is demanding of the amount of moisture, which is consistent with a high correlation between the content of all photosynthetic pigments in plant leaves and the amount of precipitation during the growing season (*r* = 0.80, $p \le 0.05$) (see Fig. 1). The content of Chl b directly correlated with the amount of precipitation during the growing season (*r* = 0.71), whereas for carotenoids, there was an inverse relationship (*r* = –0.72) ($p \le 0.05$ for all correlation coefficients obtained).

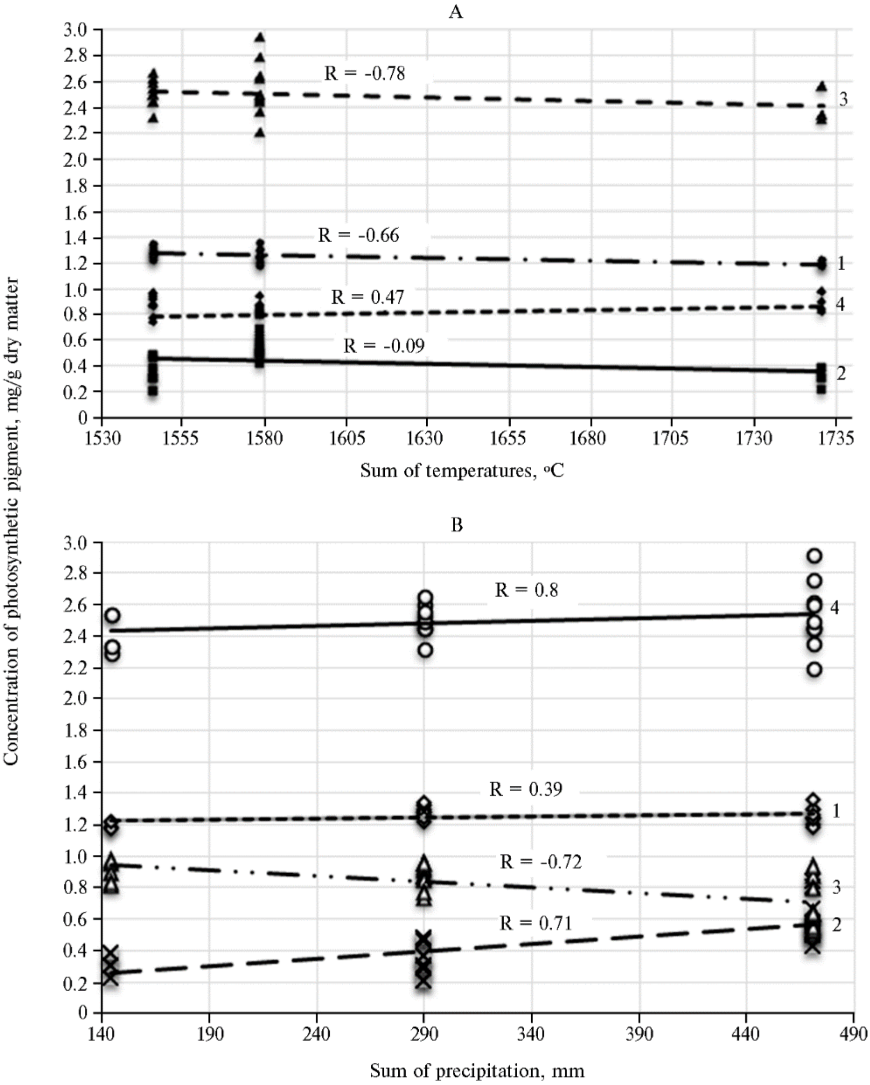

Fig. 1. Accumulation of photosynthetic pigments in the leaves of *Galega orientalis* Lam. cv. Gale depending on the sum of active temperatures ≥ 10 °C (A) **and the sum of precititation** (B)**:** 1 — Chl a, 2 — CHl b, 3 — Car, 4 — total pigments (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District, 61°15′00″ N, 73°25′00″ E, 2013-2015).

The content of vitamin C. Although most mammals are able to synthesize ascorbic acid (AA), its amount may not be sufficient for full growth and ensuring high productivity of animals or under stress, and therefore additives containing AA are used to enrich feed [62-65]. According to reports, the feed mass of the eastern galega contains from 136.2 to 522.1 mg of AA per 100 g of dry matter, at the beginning of the growing season this value may be 800-900 mg% [66]. Earlier we showed that the plant mass of *Galega orientalis* Lam. is a source of ascorbic acid after plants enter the generative phase of development with a predominant (96%) localization of vitamin in leaves [67], which is expected given the role of ascorbic acid in photosynthesis [27]. In our tests, the concentration of AA during the observation period increased from 37 mg% in plants of the 1st year of life to 60 mg% in the 3rd year of life [67]. In the leaf mass of the 3-year-old plants, the content of ascorbic acid (60 mg%) exceeded 1.6 times the same parameter for the 1st and 2nd years of vegetation (37 and 39 mg%, respectively).

When inoculated with the Baikal-EM1 preparation, in the year of sowing, the accumulation of AA in the plant mass was 20% higher (41 mg%, $p \leq 0.05$), in the 2nd year 26% lower (31.0 mg%, $p \leq 0.05$) than the control, in the 3rd year, it was at the control level (61-62 mg%). In mixed sowing with peas, in the 3rd year of herbage life, a significant ($p \leq 0.05$) decrease to 56.0 mg% was noted, which is 6 mg% less than in the control.

We have not revealed a relationship between the AA accumulation and water availability (data are not shown). With a decrease in the average daily air temperature (x), the vitamin C content in the green mass increased ($r = -0{,}69$; $R^2 = 0.47$, $y = -8{,}0838x + 133{,}73$). A strong negative correlation occurred between the AA content in the leaves and the the specific leaf surface (r from –0.83 to –0.88) [67].

The content of flavonoids. According to V.I. Filatov et al. [68], during the introduction of eastern galega in Eastern Siberia, the amount of flavonoids was 0.40% at branching, 0.35% at budding, 0.27% at flowering, and 0.25% to dry matter at fruiting. In our tests, the average content of flavonoids in the aboveground biomass of galega varied from 0.7 to 3.2% over the years of research for all treatments. When inoculated with Baikal-EM1, the maximum amount of flavonoids in aboveground biomass was degected in the 1st year of vegetation (2.4% with 1.9% in the control). In the 2nd year of life, both during inoculation with a microbiological preparation and in the control, the content of flavonoids increased by another 0.3%, in the 3rd year it decreased sharply (to 0.7%), but it did not differ significantly from the control values. In plants under the cover of peas in the 1st and 2nd year of life, the content of flavonoids in the aboveground biomass was 2.1-2.4%, in the 3rd year it increased sharply (to 3.2%), significantly exceeding the indicators in the other two variants of the experiment.

In general, in our tests, the content of flavonoids in the leaves of galega plants was higher than in the stems, and varied from 0.3 to 2.8% (0.2-0.5%in the stems).

In the 1st year of vegetation, we did not detect significant differences in the content of flavonoids in the leaves according to the experimental variants (the values were 1.6-1.9%). In the 2nd year, in control and inoculation with Baikal-EM1, the analyzed parameter increased by 0.5%, in mixed sowing it remained the same as in the 1st year of life (1.7%). In the 3rd year, the content of flavonoids in the leaves decreased sharply in the control (up to 0.05%, i.e., 3-fold compared to the 1st year and 5-fold compared to the 2nd year) and when using a microbiological preparation (6- and 8-fold, respectively). In crops with peas, it sharply increased and exceeded the value for the previous years by 1.6 times (2.8% vs. 1.8 and 1.7%, respectively). We associate a sharp decrease in the content of flavonoids in the 3rd year of life in the control and when using a microbiological preparation with the transition of plants to generative development and entry into the phases of flowering and fruiting (unlike binary sowing, where the virginal stage continued). The use of a microbiopreparation contributed to a more intensive growth of vegetative organs in the pregenerative period, the formation of a larger number of peduncles and fruit formation. It should be noted that studies on different plant species have described both similar [69, 70] and inverse [71] patterns.

One of the factors that was associated with the content of flavonoids during intensive vegetative growth is the amount of precipitation (x) ($r = 0.79$, $R^2 = 0.63$; $y = 0.0046x + 0.5037$).

In eastern galega, we also found a close inverse correlation between the content of vitamin C, on the one hand, and the accumulation of flavonoids and carotenoids, on the other (for all ages of the herbage and experience variants) (Fig. 2).

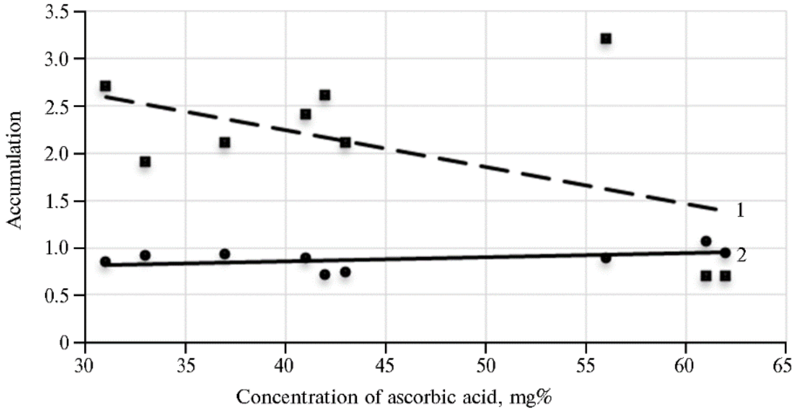

Fig. 2. Accumulation of flawonoids (%, 1) **and carotenoids** (mg/g dray mattem, 2) **in the leaves of *Galega orientalis* Lam. cv. Gale depending on the ascorbic acid concentration** (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District, 61°15′00″ N, 73°25′00″ E, 2013-2015).

The obtained results allow us to conclude that the eastern galega of the Gale variety successfully adapts to the natural and climatic factors of the Middle taiga zone of Western Siberia and is promising as a fodder crop. The temperature and moisture availability at the point of introduction were sufficient for the operation of the photosynthetic apparatus formed by the plants of the eastern galega in the light conditions of the region (intensity and spectral composition of solar radiation, daylight duration) during the growing season. As a result, the productivity of the herbage was 23-35 t/ha. To ensure high and stable yields, the highest protein content and high nutritional value of feed, it is advisable to improve the elements of crop cultivation technologies, including through the selection of microbiopreparations, growth regulators [72], effective cover crops [4]. As an additional reserve, optimization of harvesting techniques through fractionation of its elements (leaves and stems) [1].

A detailed study of biochemical composition of eastern galega which also contains substances classified as anti-nutritional, e.g., trypsin inhibitors, lectins [2], coumarins, saponins, tannins, alkaloids [19], and of physiological and biochemical mechanisms of their accumulation in the plant is important both in matters of feeding and in view of future breeding of the crop. For example, coumarin-based preparations are already used in clinical practice, and many coumarins and their derivatives are considered as potential medicines [73], but sweet clover contains coumarin which in hay, under the action of mold fungi, turns into dicumarol (3,3-methylene-bis-4-oxycoumarin), preventing blood clotting, as a result of which painful bleeding may occur in cattle [74]. Tannins and saponins in high concentrations are considered anti-nutritive substances, but tannins serve as a preservative in feed, and saponins have an immunomodulatory effect [75]. Saponins can promote intestinal health in chickens (76). Alkaloids, tannins and saponins was reported to influence the nutritional behavior of cattle and sheep [77].

The influence of fertilizers and the accumulation of micro- and macroelements, heavy metals in the biomass of galega is also subject to assessment [78, 79]. Other promising areas are the study of the root system, allowing galega plants to use nutrients better, the elucidation of the influence of galega as a precursor on the yield of agricultural plants, and the determination of its suitability in the system of extensive organic farming [3, 4].

Finally, the ecological aspect of the galega introduction is extremely important. Legumes are one of the leaders in the harmful effects of plant invasion [7, 80]. In Central Russia, legumes occupy the fifth place among alien species. The aggressiveness of legumes is associated with their mass use as fodder grasses and “green fertilizers”. *G. orientalis* is one of the most aggressive invasive species of legumes [80]. During invasions, changes occur at the ecosystem level, so even the complete removal of insiders does not return the community to its original status [80].

Thus, during the introduction of eastern galega cv. Gale in the North of Russia (61 15'00" N, 73 25'00" E), the effect of three studied agrotechniques (t.e., monoculture, monoculture with pre-sowing treatment of seeds with microbial preparation Baikal-EM1, and mixed culture with peas) on the Chl a + Chl b in the leaves appeared since the 2nd year of plant life. For the 2nd and 3rd years of life, this value, as influenced by a microbiological preparation, was higher than in the control (by 19-22% and 16-18% over the development phases). In mixed sowing it decreased at the end of the 2nd year, but by the end of the 3rd year it exceeded the control values by 33%. In the control, the content of Chl a in the leaves in the year of sowing, for the 2nd and 3rd years of life was 1.23±0.10, 1.29±0.12 and 1.32±0.14 mg/g of dry weight. On average, in the 2nd year, when using Baikal-EM1 fertilizer, the content of Chl a in the leaves increased by 15% compared to the control. In mixed sowing with peas, it remained within the control values (1.20±0.23 mg/g). Over 3 years, when using a microbiological preparation, the value of Chl a/Chl b in leaves significantly decreased ($p \leq 0.05$), which may indicate an increase in the adaptive potential of plants. In mixed crops it remained within the control values. The proportion of Chl a + Chl b localized in the light-harvesing complexes (LHC) varied from 20 to 90% depending on the phenological phase, the age of the herbage and the treatment. In the control, under inoculation with a microbial preparation and in mixed spwings, the correlation between Chl a/Chl b and the proportion of chlorophylls Chl a + Chl b localized in the LHC was characterized by $r = 0.83$, $r = 0.93$ and $r = 0.65$ ($p \leq 0.05$), respectively. The used agrotechniques did not significantly change the Chl/Car values. Nevertheless, during inoculation with Baikal-EM1 and in mixed sowing with peas, the accumulation of carotenoids in the leaves of eastern galega exceeded that in the control. On average, over the years of the study, for all variants of the experiment, the accumulation of all pigments in the leaves directly correlated with the LHC. The content of Chl b and carotenoids was less associated with the temperature regime of the region, while the first parameter directly correlated with the amount of precipitation for the season and the second parameter showed a negative correlation. When inoculated with Baikal-EM1, the content of ascorbic acid in the leaves in the 1st and 2nd year of plant life increased cpared to control, by the 3rd year, it was almost equal to the control values, in mixed sowing for the 3rd year it decreased vs. the control. The content of flavonoids in the leaves of 3-year-old plents with the microbiological preparation and in the control (when the plants switched to generative development) decreased sharply, while the mixed sowing, where the virginal stage continued, it sharply increased (1.6 times compared to previous years). In general, the data obtained indicate that the use of the microbiological preparation Baikal-EM1 largely contributed to the galega plant adaptation to new environmental conditions during the 2nd and 3rd years of life.

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