

Morphometric and biochemical properties of *Cichorium intybus* L. var. *foliosum* as affected by duration of growing period

Tatiana Lavrishcheva¹, Galina Osipova¹, Anton Lavtishchev¹, Zhapparova Aigul², Elmira Saljnikov^{3,4*}

¹ St. Petersburg State Agrarian University, Pererburgskoye sh 2, 196601, St.-Petersburg, Russia

² Department of Soil Science and Agrochemistry, Faculty of Agrobiolgy, Kazakh National Agrarian Research University, Abai Avenue 8, Almaty 050010, Kazakhstan

³ Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia

⁴ Mitscherlich Academy for Soil Fertility (MITAK), GmbH, 14641 Paulinenaue, Prof.-Mitscherlich-Alle 1 Germany

*Corresponding author: E. Saljnikov, soils.saljnikov@gmail.com

Abstract

Cichorium intybus is a valuable crop due to its high nutritional and pharmaceutical value. In this work, the study of the effect of harvesting time on the biometric and biochemical properties of *Cichorium intybus* L. var. *foliosum* (chicory salad witloof) was carried out on five varieties. The period of vegetation affects rosette diameter, number of leaves and root weight. A strong correlation between the weight of roots before laying for forcing and the weight of forcing heads ($r = 0.79$) was revealed. The roots of variety Conus, managed to accumulate a sufficient amount of nutrients for the formation of heads in a 98 days. The accumulation of sugars in forcing heads depended on their initial content in roots with a 75% reliability ($r = 0.75$). The results showed that in the northern latitudes the forcing can be carried out in winter in any room without light at a temperature of 10 to 17°C. In addition, subsurface heating of the substrate or maintaining water in the containers with roots provided a larger yield of heads obtained in a shorter time.

Keywords: *Cichorium intybus*, witloof, forcing, head, root, chlorophyll, sugars, carotenoids

Introduction

The ancient Egyptians cultivated and used chicory (genus *Cichorium* L.) as a medicinal plant, coffee substitute, vegetable crop and as a livestock forage (Aisa et al., 2020). In spite this plant have been used since ancient times it is not well studied yet, in terms of its morphology, genesis, biochemistry, climatic preferences (Street et al 2013; Lavrishcheva and Osipova 2020a,b). The genus *Cichorium* includes six species that generally distributed in Europe and Asia (Bais and Ravishankar (2001). On the <http://www.theplantlist.org/tpl1.1/search?q=cichorium>, 32 plant name records match the search criteria *Cichorium intybus* and 13 plant name records match the search criteria *Cichorium endivia*. While Shevchenko (2000) reported 10-12 species, which are divided into two groups depending on the parts of the plant used: the first group is plants with thick fleshy roots such as *C. intybus* var. *foliosum* (Lavrishcheva and Osipova 2020a) and the second group includes plants with above-ground storage organ (Lavrishcheva et al 2020).

Chicory is known as a frost-resistant plant that can withstand extreme temperatures in all stages of growth (Bais and Ravishankar 2001), but some species showed to be sensitive to duration of sun-light and amount of precipitation (Lavrishcheva et al 2020). *Cichorium intybus* L., is an erect, fairly woody perennial plant, about 1 m in height with a fleshy taproot up to 75 cm in length and large basal leaves (van Wyk 1997; Bais and Ravishankar 2001). *C. intybus* L., possesses a number of valuable properties of both aboveground and belowground parts, such as nutrients, including carbohydrates, proteins, vitamins, minerals, soluble fiber, trace elements and bioactive phenolic compounds, which are responsible for the various nutritional, preventive, and medicinal properties of chicory (Nwafor et al., 2017). According to its cultivation purposes, Cadalen et al. (2010) categorized this chicory four groups: (1) root chicory is cultivated for its taproot as a coffee substitute or for inulin extraction; (2) witloof chicory is cultivated as industrial chicory for etiolated buds (heads) by forcing; (3) leaf chicory is used as fresh or cooked vegetables; and (4) forage chicory, is used to intensify herbage obtainability in pastures. In the second half the the 20th century, it was discovered that the root of *C. intybus* contained up to 40% inulin, which has a negligible impact on blood sugar and thus is suitable for diabetics (Judžentienė and Būdienė, 2008).

Cichorium intybus var. *foliosum* was obtained in the end of XIX century in Brussel as a result of breeding from the Magdeburg root chicory. It was widely used in some European countries as a delicious vegetable crop. Later, the head form of forcing chicory was called witloof, that is, a white leaf (Shevchenko et al., 2016). Witloof is a valuable agricultural crop due to its high nutritional and pharmaceutical value (Shevchenko et al., 2016; Golubkina et al., 2019). It has been revealed that chicory acid isolated from witloof inhibits the aggregation and fibrillation of human hIAPP, which contributes to the treatment of diabetes mellitus (Luo et al, 2020). The seeds of *C. intybus* germinate more tightly than the seeds of the *C. endivia* (Lavrishcheva, 2019a, b), although in the cotyledon phase these plants are indistinguishable. In the first year of vegetation, chicory lettuce plants form a root weighing from 60 -80 g (Gulyaev, 1991) to 100-400 g (Vyutnova et al., 2008), 10 to 45 cm long and 2 to 8 cm and more in diameter, which is used for forcing and processing. As a biennial plant, chicory lettuce produces seeds in its second year of life. The height of the flowering shoot reaches 110-190 cm, significantly exceeding *C. endivia* in this parameter (Lavrishcheva and Osipova 2018). The number of stems on the testes varies from 1 to 10 or more, depending on the variety (Vyutnova et al., 2008).

Currently, the literature has accumulated a certain theoretical material about the peculiarities of growing chicory salad witloof. Methods for obtaining witloof chicory using the heat from fermentation of cow dung in the cold season have been developed (Kumano et al., 2017a). The effect of potassium regime on the growth and development of witloof has been studied (Kumano et al., 2017b, 2017c). Effective methods of chemical and biological protection of chicory plants from pests have been developed (Bengini et al., 2016). Studies have been carried out on the effect of illumination on the accumulation of bitter sesquiterpene lactones and photosynthetic pigments by witloof in leaves

(Wulfkuehler et al., 2014), as well as ways to reduce their content (Wulfkuehler et al., 2013). Van Arkel et al (2012) found that the degree of polymerization of inulin in *C. intybus* highly dependent on the field conditions and harvest time.

Witloof varieties are difficult to distinguish by morphological characteristics, therefore they are distinguished by the timing of the formation of a marketable root and the period of their use for forcing purposes (Shevchenko et al., 2016). Attempts to link the degree of ripeness of roots with the level of any substances have been carried out earlier, but have not yet yielded the desired results (Krug, 2000). Most of the studies are devoted to the study of the features of growing chicory salad in the regions with a warm climate in the open field. However, currently, there is not enough data accumulated on phytochemistry (Street et al 2013) and morphometric characteristics of *C. intybus* grown in northern latitudes. Until now, in Russia, the northernmost latitude where this crop was cultivated in the open field was the Moscow region (Virchenko, 1984; Shevchenko. 2000). Cultivation of *C. intybus* in a greenhouse in the North-West of Russia is of a particular importance to establish the optimal harvesting time for roots, which will later be used for forcing.

The aim of the research was to study the dynamics of changes in biometric and biochemical parameters of various varieties of chicory lettuce (*C. intybus* var. *foliosum*), depending on the duration of cultivation in a protected soil (greenhouse). The tasks included:

- to study the dynamics of changes in the rosette diameter, number of leaves and root weight of plants of various varieties of witloof, depending on the duration of cultivation;
- to study the influence of timing of harvesting the salad chicory on the formation of storage organs: the size and weight of roots, content of sugar, formation of the pigment system in the leaves and forcing heads.

Materials and methods

Experimental design

The plants of chicory lettuce were grown in a film greenhouse on the territory of the Educational and Experimental Garden of the St. Petersburg State Agrarian University (SPbSAU) on the 2x2 m² plots in three random replications in 2014, 2015 and 2016. Sowing scheme was as follows: row spacing - 33 cm, distance between plants - 10 cm. The sowing of witloof was carried out in the greenhouse annually on 23 May. Mass seedlings in all years of research appeared on the eleventh day after sowing. The harvesting of plants was carried out in 2014, on September 28, in 2015 - on September 17, in 2016 - on September 9. Thus, the total duration of the growing season of witloof plants (from the moment of mass germination to harvesting) was: in 2014 - 117 days, in 2015 - 106 days, in 2016 - 98 days.

Studied varieties

Five varieties of witloof were used as objects in the research experiment: Conus, Raketa, Hative, Veneta, Viproda. Two varieties (Conus and Raketa) are included in the State Register of Breeding Achievements of the Russian Federation (2022). Three varieties (Hative, Veneta, Viproda) were obtained from the collection of All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov (VIR) (St. Petersburg, Russia).

Variety Conus (originator is the Federal Scientific Center of Vegetable Growing; selection of the SeDek company) belongs to the group of mid-season varieties. It is a medium early in terms of forcing. The period from mass shoots to technical ripeness is 98-114 days. The period of forcing (from planting roots to mass ripeness of heads) is 17-30 days (State Register 2022). According to the timing of the formation of a commercial root, the Conus variety belongs to the mid-season plant (Shevchenko et al., 2016)

Variety Raketa (selection of the LLC Agrofirmy Poisk) is included in the State Register of the Russian Federation. The period from mass germination to technical ripeness of roots is 130-155 days. The period of forcing (from planting of roots to the economic fitness of heads) is 30 days. (State Register 2022).

Variety Hative is a collection of VIR. The sample number is 48, the introduction number is 351300. The date of inclusion is 1976. The country of origin is France. Biological status is improved variety (Database of Plant and Genetic Resources).

The Veneta variety is a collection of VIR. The sample number is 68, the introduction number is 468051. The date of inclusion is 1984. The country of origin is Netherlands. Biological status is improved variety (Database of Plant and Genetic Resources).

The Viproda variety is a collection of VIR. The sample number is 71, the introduction number is 468053. The date of inclusion is 1984. The country of origin is Netherlands. Biological status is improved variety (Database of Plant and Genetic Resources).

Parameters analyzed

During harvesting, the following biometric indicators were determined: height and diameter of the rosette, number of leaves, weight of the plant, and weight of the root. For these parameters, for each plot, an average value was calculated for one plant per replication. The mass of the above-ground part of the plants was calculated from the difference between the average values of the mass of the plants and the mass of the root for each variant.

At harvest, the plants tops were cut at 2-3 cm from the neck of the root. Roots were stored for 1.5 months in a dark place at a temperature 2°C and a humidity of 90%. For forcing, the roots were placed in peat soil without covering with a soil substrate. Forcing was carried out in a dark room at $t = 12-14^{\circ}\text{C}$ for 30 days.

Analytical methods

The assimilation surface area was calculated by the punching method. The dry matter was determined after drying to constant weight at a temperature of 105°C (GOST 31640-2012).

The amount of sugars was determined quantitatively by the Bertrand method (Ermakov, 1987). The method is based on the ability of reducing sugars possessing a free carbonyl group to reduce copper oxide to ferrous one in an alkaline solution. The amount of the formed copper oxide precipitate strictly corresponds to the amount of sugar in the solution. The resulting ferrous oxide was determined permanganatometrically. The amount of permanganate spent on titration was used to calculate the amount of copper oxide and then the sugar content in the solution.

Quantitatively obtained amount of chlorophyll *a*, *b* and carotenoid, and their ratios in varieties of *C. intybus* var. *foliosum* are important indicators sensitive to changes in ambient conditions (Sonobe et al., 2020). The content of pigments (chlorophylls *a*, *b* and carotenoids) was determined spectrophotometrically (Kachnovich, 2003).

The pigments were extracted from fresh leaves with acetone. The features of the absorption spectra of chlorophylls *a* and *b* allow us to determine their amount in the extract without prior separation. When determining the content of chlorophyll *a* and *b*, the following wavelengths are used: 662 and 644 nm. For solutions of pigments in undiluted acetone, the following equations are used:

$$\text{Chla} = 9,784 \cdot E_{662} - 0,990 \cdot E_{644};$$

$$\text{Chlb} = 21,426 \cdot E_{644} - 4,650 \cdot E_{662},$$

where Chla – content of chlorophyll *a*, (mg/L); Chlb – content of chlorophyll *b*, (mg/L); E_{662} и E_{644} – optical density of the solution.

Statistical processing of the data was carried out using analysis of variance (Dospekhov, 1985).

Results and discussion

The research results showed that almost all *C. intybus* plants formed a root in the first year of vegetation. The exception was plants of the Raketa variety. They had a premature formation of a flowering shoot. The share of such plants was not large and amounted to the years of research: 2014 - 2.2; 2015 - 2.8 and in 2016 - 2.5% of the total number of plants of this variety. Such an anomalous way of development of biennial plants was observed about five decennia ago by Kuperman (1968). In nature, this phenomenon is a biological adaptation of plants to the preservation of species in adverse conditions. Under unfavorable conditions, plants tend to accelerate in development, as a manifestation of the general phylogenetic trend towards a reduction in the period of vegetative development and a faster transition of plant organisms into the generative phases of development.

Wiebe (1989) showed that the effect of low temperature (5-10°C) on the mother plant during the formation of chicory seeds enhanced the subsequent stemming compared to the effect of higher

temperature (15°C). The development of seeds at lower temperatures enhanced the subsequent stemming even at high temperatures of seed germination. This indicates that the seeds were vernalized on the mother plant before they were harvested. In unfavorable years, the number of root shoots in the first year of vegetation can reach 90% (Ludilov et al. 2010). The biometric indicators of studied varieties of chicory salad witloof by the years of research are summarized in Table 1.

Table 1. Dynamics of the morphometric indicators of studied varieties of *Cichorium intybus* by years of research

Duration of vegetation	Variety	Height, cm	Rosette diameter, cm	Number of leaves, pcs	Aboveground biomass, g	Weight of plant, g	Area of assimilation surface, m ²
2014							
115 days	Conus	53.6 b	78.0 b	28.0 b	241.8	396.6 b	1.10
	Raketa	52.4 b	74.5 a	23.5 a	231.0	355.6 a	0.98
	Hative	51.0 a	70.4 a	28.6 b	232.0	416.8 c	1.14
	Veneta	53.2 b	77.6 b	22.8 a	341.8	631.2 d	1.61
	Viproda	51.2 a	87.0 c	24.6 a	387.4	639.4 d	1.69
	LSD ₀₅	1.0	6.1	2.1	–	15.8	–
2015							
104 days	Conus	60.7 b	48.0 a	20.2 c	306.3	409.0 d	1.32
	Raketa	55.0 a	46.0 a	15.9 b	252.4	367.0 c	1.07
	Hative	58.4 b	57.3 c	17.6 c	231.3	340.1 a	0.88
	Veneta	54.0 a	50.8 b	13.3 a	256.8	367.6 c	0.91
	Viproda	58.9 b	57.4 c	14.8 b	241.9	348.0 b	1.37
	LSD ₀₅	2.2	3.6	1.3	–	6.2	–
2016							
98 days	Conus	55.8 b	32.1 c	15.7 d	201.2	241.4 d	1.31
	Raketa	57.4 b	25.7 b	10.9 b	107.1	131.0 b	0.75
	Hative	51.2 a	21.2 a	6.8 a	47.7	68.8 a	0.33
	Veneta	50.5 a	37.0 d	18.0 e	272.7	356.5 e	1.21
	Viproda	58.6 b	57.4 e	14.8 c	180.6	213.9 c	0.93
	LSD ₀₅	4.8	4.3	1.5	–	2.3	–

† different letter denote statistically significant differences between varieties within a column for each year at $p < 0.05$

The data shows that the plant height was predominantly evenly distributed varying within the range from 50.5 to 60.7 cm. Within this deviation the highest plant height was observed in the Conus and Veneta varieties in 2014 and the Conus, Hative and Viproda varieties in 2015, and Raketa and Viproda in 2016, 57.4 and 58.6 cm, respectively.

The formation of a rosette of leaves depended on the duration of the plant vegetation. The smallest rosette diameter in most varieties was recorded in 2016 that varied within 21.2-37.0 cm. The exception was Viproda with 57.4 cm of the rosette diameter, which formed the largest rosette of leaves in all years of study (Table 1).

For all studied varieties, the largest number of leaves over the studied period was formed in 2014. The maximum number of leaves in 2014 and 2015 was found in plants of varieties Conus (28 and 20.2 pieces, respectively) and Hative (28.6 and 17.6 pieces, respectively). In 2016, the largest number of leaves was observed in plants of the Dutch variety Veneta (18 pcs.).

The maximum plant weight in 2014 was found in the Viproda and Veneta varieties (639.4 and 631.2 g, respectively) that significantly exceeded the mass of plants of other studied varieties. In 2015, the differences between varieties in terms of plant weight were less significant, where the highest plant weight showed the varieties Conus (409 g), Raketa (367.0 g) and Veneta (367.6 g). In 2016, the maximum plant weight was recorded for the Veneta variety (356.5 g) that significantly exceeded other studied varieties, which was between 68.8-241.4 g (Table 1). The same patterns were revealed when comparing the weight of the above-ground plants biomass.

The area of the assimilation surface of leaves from a plant strongly correlated with the the above-ground plant biomass ($R = 0.89$). The maximum leaf area in 2014 and 2015 was found in the Viproda variety, which averaged 1.69 and 1.37 m² per plant, respectively. In 2016, the largest area of the assimilation surface of leaves was found in plants of the Conus variety (1.31 m²).

The data on the sizes of roots shows that the average values of the length of roots in most cases do not have significant differences between varieties (Table 2). There were also no clear dependences on the year of research. The largest diameter of the root in 2014 and 2016 was found in the Veneta (5.2 and 3.5 cm, respectively), and the smallest in the Raketa (3.2 and 2.3 cm, respectively). In 2015, the maximum diameter of root was found in the Hative variety (3.8 cm), and the smallest in the Conus variety (2.9 cm).

Table 2. Sizes of the roots of varieties of *Cichorium intybus* by years of research

Variety	Lenth of the root, cm			Diameter of the root, cm			The form index, psc.		
	2014	2015	2016	2014	2015	2016	2014	2015	2016
Conus	16.4 a	13.4 a	16.6 c	3.4	2.9 a	2.7 a	4.82	4.62	6.15
Raketa	15.8 a	14.2 a	15.2 b	3.2	3.0 a	2.3 a	4.94	4.73	6.61
Hative	15.7 a	15.9 b	13.8 a	3.5	3.8 b	2.4 a	4.49	4.18	5.45
Veneta	17.0 b	15.7 b	17.1 c	5.2	3.5 b	3.5 b	3.27	4.49	4.89
Viproda	16.3 b	14.8 b	16.4 c	4.7	3.4 a	2.5 a	3.47	4.35	6.56
LSD ₀₅	1.0	1.1	0.9	1.0	0.5	0.5	–	–	–

† different letter denote statistically significant differences between varieties within a column for each year at $p < 0.05$

According to the classification given by Polyanina (2016), roots in shape and size can be divided into three groups: Group 1 - short conical roots, shape index (SI) -the ratio of the length of the root to its diameter is < 5.0 ; Group 2 - long cylindrical and semi-long roots, SI is from 5.0 to 7.0; and Group 3 - spindle-shaped elongated roots with $SI > 7.0$. In our studies (Table 2), in 2014 and 2015, all studied varieties in terms of the shape index belonged to the first group. In 2016, due to the shortened growing period, the plants did not have time to sufficiently form the thickened shape of the storage organ. With the same length (compared to 2014 and 2015), roots had a small root diameter, which was reflected in their shape index. So, in 2016 varieties Conus, Raketa, Hative and Viproda shifted to the second group. Only the Veneta variety, which formed the largest root, remained in the first group by the morphometric properties of root.

The dynamics of changes in the weight of root of studied varieties of witloof by years of research are shown in Fig. 1. The duration of the growing season had a strong influence on the formation of roots. The largest weight of roots was formed by plants in 2014 (the duration of the growing season was 117 days), with maximum weight found in the varieties Veneta (298.4 g) and

Viproda (252.0 g). In 2015 (the vegetation period was 106 days), the largest weight of root was formed by the Raketa variety (114.6 g). However, it should be emphasized that the lag of other varieties in this indicator in this year was insignificant and fluctuated within 102.7-111.8 g. Reducing the growing season to 98 days had a strong effect on the accumulation of root mass. The weight of the storage organ in 2016 ranged from 21.1-83.8 g, with the highest value recorded in the Veneta variety.

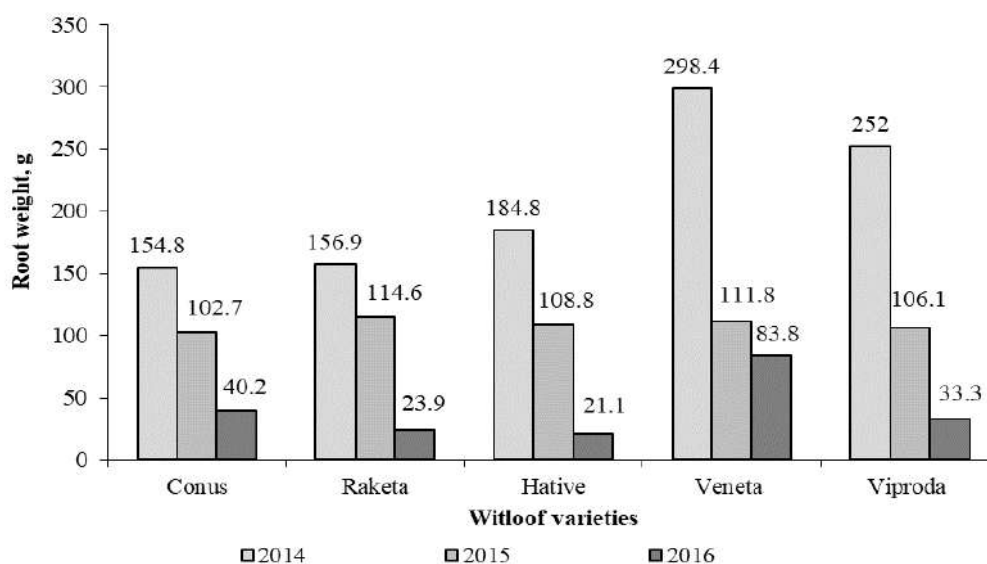


Figure 1. Dynamics of changes in the weight of roots by studied years, g

Table 3 shows the data of the root weight before and after forcing. Before being put into forcing containers, the roots were pruned; therefore, the values of the root weight before forcing were inferior to the values shown in Fig. 1. After forcing, due to the loss of moisture and nutrients, the weight of roots decreased (Table 3). The decrease in the weight of roots in the entire data range ranged from 2 to 17%.

Table 3. Biometric indicators of roots and forcing heads

Variety	Root weight before forcing, g	Root weight after forcing, g	Heads weight, g	Heads height, cm	Heads diameter, cm	Number of head leaves, pcs
2014						
Conus	135.82 b	130.30 b	13.06 c	11.44 c	4.04 a	10.20 b
Raketa	108.42 a	98.72 a	9.74 b	7.89 a	4.34 a	8.72 a
Hative	159.83 c	149.04 c	7.91 a	10.96 c	4.15 a	12.43 c
Veneta	263.33 e	258.08 e	22.65 d	9.67 b	5.04 a	15.17 d
Viproda	186.44 d	172.90 d	28.17 e	19.21 d	5.69 a	14.43 e
LSD ₀₅	9.32	6.91	1.77	1.19	2.22	1.08
2015						
Konus	76.46 a	67.46 a	12.39 e	11.58 b	4.37 c	15.58 d
Raketa	84.96 b	76.66 b	3.81 a	8.10 a	2.53 a	10.53 b
Hative	93.31 c	89.82 c	6.45 b	8.93 a	2.85 a	9.57 a
Veneta	97.58 d	94.98 d	9.72 c	8.00 a	3.88 b	11.49 c
Viproda	94.11 c	90.28 c	10.56 d	11.04 b	4.07 c	11.78 c
LSD ₀₅	2.25	1.23	1.00	1.03	0.32	0.43
2016						
Conus	40.09 d	33.17 d	10.35 c	7.93 b	3.56 c	9.90 b

Variety	Root weight before forcing, g	Root weight after forcing, g	Heads weight, g	Heads height, cm	Heads diameter, cm	Number of head leaves, pcs
Raketa	22.70 b	20.68 b	3.40 a	7.30 a	2.10 a	6.61 a
Hative	20.86 a	19.49 a	3.18 a	8.42 d	2.00 a	6.58 a
Veneta	74.50 e	72.33 e	3.63 a	8.13 c	2.85 b	11.00 c
Viproda	31.28 c	30.56 c	3.68 b	8.75 e	2.82 b	10.75 c
LSD ₀₅	0.78	0.66	0.49	0.26	0.20	0.36

† different letter denote statistically significant differences between varieties within a column for each year at $p < 0.05$

A strong correlation was found between the weight of root before laying for forcing and the weight of forcing heads ($r = 0.79$). In 2014, the maximum weight of heads was found in the varieties Veneta and Viproda, amounting 22.65 and 28.17 g, respectively. The weight of heads in other varieties this year ranged from 7.91-13.06 g. In 2015, the largest weight of heads was observed in the Conus variety (12.39 g), and the smallest in the Raketa variety (3.81 g). The weight of heads in other varieties ranged between 6.45-10.56 g.

In 2016, the smallest root was used for forcing, which affected the formation of heads. In most varieties, their weight varied within 3.18-3.68 g. The exception was the Conus variety with a head weight of 10.35 g. It should be noted that the mass of forcing heads obtained from the root of mid-season Konus variety was stable over the research years and depended little on the duration of growing season. The roots of this variety, apparently, even during the minimum growing period (98 days), managed to accumulate a sufficient amount of nutrients to form full-fledged heads. This is confirmed by the characteristics of the variety given in the state register of breeding achievements, where the period for the formation of a commercial root is indicated from 98 days (Krug, 2000).

In addition to the differences in weight, the heads also differed in shape (Table 3). The highest heights in 2014 and 2015 were distinguished by the varieties Conus and Viproda (11.44 and 19.21 cm in 2014; 11.58 and 11.04 cm in 2015, respectively). In 2016, the highest height was found in the varieties Hative and Viproda (8.42 and 8.75 cm, respectively). The diameter of the heads, depending on the year of research, varied within 4.04-5.69 (in 2014), 2.53-4.37 (in 2015) and 2-3.56 cm (in 2016). The obtained parameters of heads are consistent with the data of other researchers. So, in the work (Fesenko et al., 1984), the authors obtained heads with a height of 15.3-21.2 cm and a diameter of 4.71-5.56 cm. Our results showed that the initial weight of the root (before laying for forcing) influenced the formation of the height and diameter of the heads. The correlation coefficients were $r = 0.52$ and $r = 0.80$, respectively.

Analysis the biochemical composition of plants revealed a clear relationship between the duration of growing the chicory salad and the accumulation of sugars in leaves and roots. The longer the growing season lasted, the more the plants accumulated sugars. This pattern was observed in all treatments when comparing the sugar content as % of the crude substance. The only exception was the early ripening variety Conus and variety Viproda. The sugar content in the leaves of these varieties in 2014 was lower than in 2015 (Fig. 2). This is probably due to the outflow of sugars from leaves into roots at this stage of plant development. This is indirectly confirmed by the fact that the sugar content in the root of the

Conus and Viproda in 2014 was the highest in comparison with the other varieties over the entire observation period (Fig. 3a).

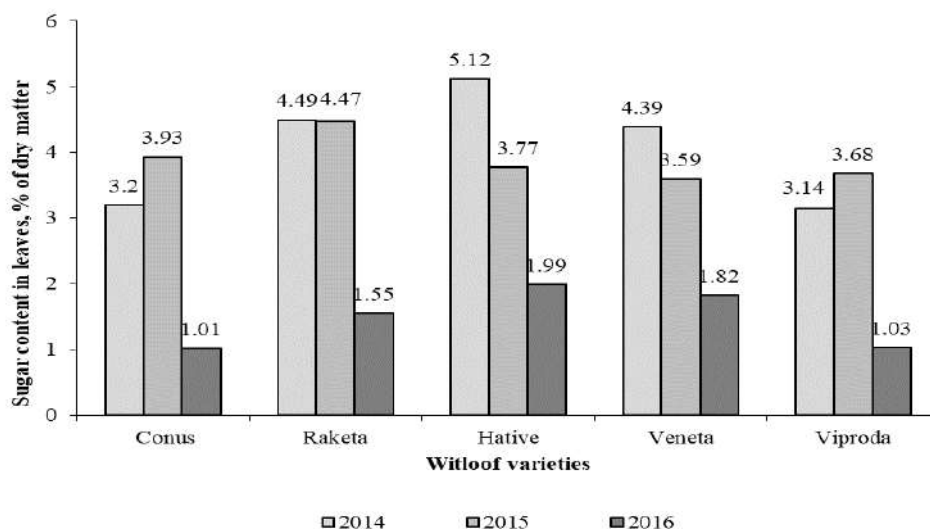


Figure 2. Content of sugars in the leaves of *Cichorium intybus* var. *foliosum*, % of dry matter

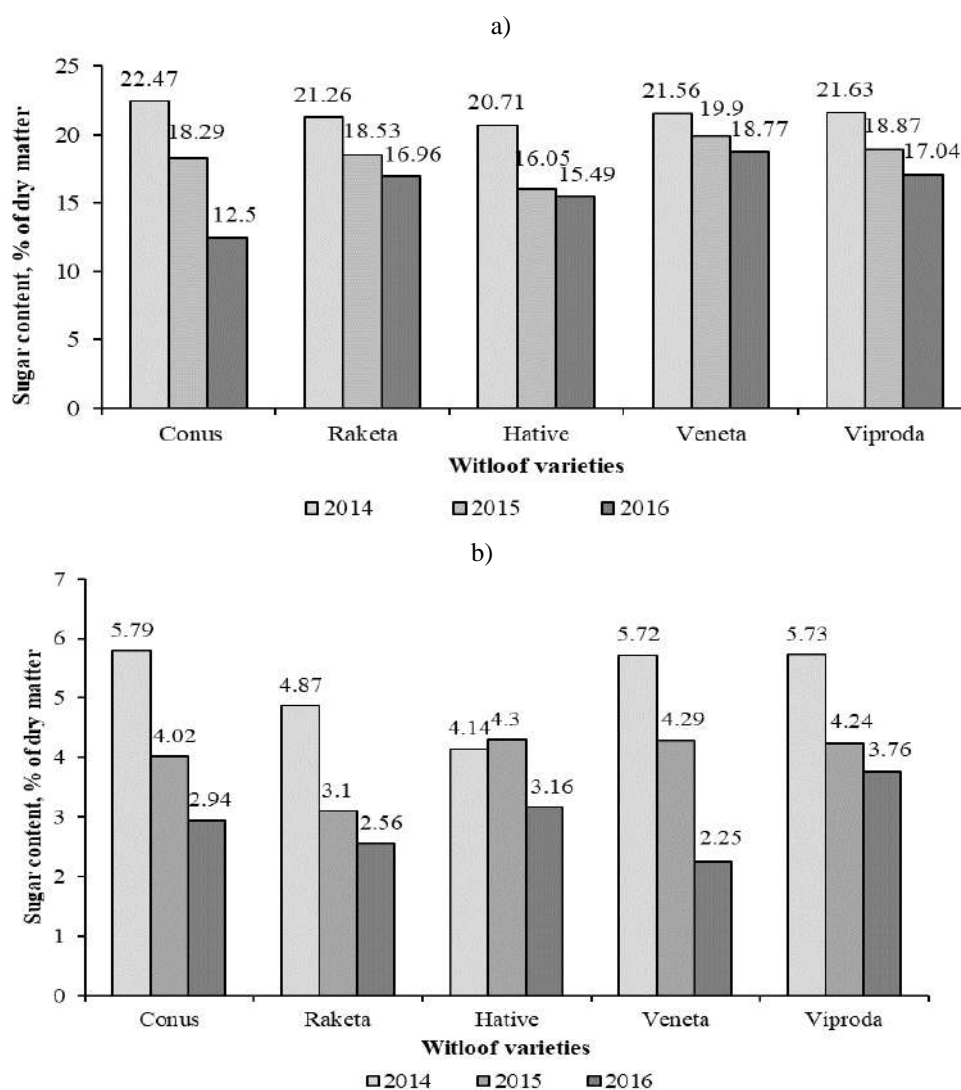


Figure 3. Content of sugars, % of dry matter in a) roots after harvest, b) in heads

The accumulation of sugars in heads depended on their initial content in roots. The higher the initial sugar content in the roots was, the more they accumulated in the heads (Fig. 3b), what is confirmed by strong correlation coefficient ($r = 0.75$). Thus, the sugar content in the heads decreased as the experiment proceeded. In 2014, this indicator ranged from 4.14 to 5.79; in 2015 from 3.1 to 4.3; in 2016 - from 2.25 to 3.76% of dry matter.

The pigment system of plants is the basis for the photosynthetic conversion of solar energy into the energy of chemical bonds (Liu et al., 2020; Sharma et al., 2020). It is represented by chlorophylls and carotenoids (Hermanns et al., 2020). Chlorophylls perform the main photosynthetic function (Agathokleous et al., 2020; Janik-Zabrotowicz et al., 2020). Carotenoids transfer additional energy to chlorophylls, performing a light-harvesting function, and also remove excess energy from chlorophylls, performing a light-protecting function. The efficiency of the pigment system depends on the compliance of its structure and function with climatic and environmental conditions, primarily lighting conditions (Ivanov et al., 2013). Shade-loving plants usually have higher chlorophyll content than light-loving plants, and a higher proportion of chlorophyll *b*, which increases the light-harvesting ability of the leaf in the far red region. Under conditions of high insolation, carotenoids perform the function of protection against photoinhibition (Ivanov et al., 2013; Tselniker, 1978), and therefore, under these conditions, their proportion is often increased. Results of the pigment content in leaves are given in Table 4.

Table 4. Content of pigments in the leaves of *Cichorium intybus* var. *foliosum* after harvest (1) and in the heads after forcing (2).

Variety	Total chlorophyll		Chlorophyll <i>a</i>		Chlorophyll <i>b</i>		Carotenoids		Chlorophyll <i>a</i> /Chlorophyll <i>b</i>		Total chlorophyll/ Carotenoids	
	mg/100 g											
	1*	2	1	2	1	2	1	2	1	2	1	2
2014												
Conus	210.6	9.9	133.8	6.5	76.8	3.4	19.6	2.1	1.7	1.9	10.7	4.7
Raketa	200.0	11.9	121.4	7.7	78.6	4.2	18.3	2.3	1.5	1.8	10.9	5.2
Hative	249.0	13.3	162.5	8.0	86.5	5.3	26.6	7.4	1.9	1.5	9.4	1.8
Veneta	208.0	17.6	137.0	10.9	71.0	6.7	13.7	1.9	1.9	1.6	15.2	9.3
Viproda	413.6	15.4	264.9	9.1	148.7	6.3	27.9	7.6	1.8	1.4	14.8	2.0
2015												
Conus	178.3	12.41	110.6	6.5	67.7	5.9	16.7	6.2	1.6	1.1	10.7	2.0
Raketa	204.7	5.2	118.0	3.3	86.7	1.9	21.0	1.7	1.4	1.7	9.7	3.1
Hative	251.2	23.8	156.9	15.6	94.3	8.2	36.3	2.2	1.7	1.9	6.9	10.8
Veneta	198.8	19.6	121.3	12.8	77.5	6.8	19.7	1.9	1.6	1.9	10.1	10.3
Viproda	263.4	2.9	157.0	2.0	106.4	0.9	29.4	0.7	1.5	2.2	9.0	4.1
2016												
Conus	120.1	2.6	89.2	1.7	30.9	0.9	32.9	1.4	2.9	1.9	3.7	1.9
Raketa	112.3	2.9	83.9	1.9	28.4	1.0	31.3	1.5	3.0	1.9	3.6	1.9
Hative	149.9	10.4	107.2	6.9	42.7	3.5	41.8	1.5	2.5	2.0	3.6	6.9
Veneta	84.8	2.3	64.0	1.6	20.8	0.7	25.5	1.1	3.1	2.3	3.3	2.1
Viproda	85.2	4.1	61.4	3.0	23.8	1.1	24.8	1.7	2.6	2.7	3.4	2.1

*1- content of the pigments in the leaflets at harvest time in September; 2- content of the pigments in forcing heads.

In our studies, the highest content of total chlorophyll in chicory lettuce leaves was observed in 2014 in the Viproda variety (413.6 mg/100 g). In other varieties grown this year, it ranged from 200

to 249 mg/100 g. In 2015, the content of total chlorophyll in the leaves varied in the range of 178.3-263.4 mg/100 g. Moreover, the maximum value of this indicator was also found in the Viproda variety. In 2016, the content of chlorophylls in leaves was the lowest over the entire observation period. Depending on the variety, it ranged from 84.8 to 149.9 mg/100 g

One of the informative indicators characterizing the work of the photosynthetic system is the ratio of chlorophyll *a* to chlorophyll *b* (chl *a*, chl *b*). This ratio is associated with the activity of the “main” chl *a*; the larger it is, the more intense the photosynthesis (Titova et al., 2015; Son et al., 2020). Our results showed that the chl *a* predominated in the composition of chlorophyll over the entire range of presented data. The greatest excess of chlorophyll *a* content over chl *b* was observed in 2016. So, in 2014 and 2015 the chl *a*/chl *b* ratio varied within 1.4-1.9, while in 2016 it was within 2.5-3.1.

The ratio of the chlorophyll to carotenoids (car) (chl *a* + chl *b*/car) plays an equally important role in characterizing the functioning of the photosynthetic system. Normally, this ratio is stable and very sensitive to the changes in the environmental factors (Titova and Rozlomy 2015). In our study, a decrease in the ratio (chl *a* + chl *b*/car) was observed in 2016, indicating a decrease in the light-harvesting function of the pigment complex, due to the shortest growing period (98 days). This is in line with some previous findings reporting that because chl *a* and chl *b* is directly related to the primary production via absorption and conversion of sunlight and water and CO₂, they are sensitive to changes in light intensity (Chen et al 2010). Son et al (2020) confirmed the sensitivity of photophysics of a plant to ambient environmental conditions. This ability may help plant to keep optimal balance between light harvesting and dissipation (Son et al 2020). In our study, forcing the witloof in a dark room influenced the content of the pigment in the heads (Table 4), since lack of light interfered with chlorophyll production. The fact is that cultivation of witloof in the dark was carried out to obtain a white leaf that does not have a bitter taste compared to endive that has a bitter taste.

Conclusion

A clear relationship was revealed between the duration of growing *C. intybus* var. *foliosum* and the biometric parameters (diameter of the rosette and the number of leaves and weight and formation of roots). There was a strong correlation ($r = 0.79$) between the weight of roots before laying for forcing and the weight of forcing heads. The weight of forcing heads obtained from the root of the mid-season Conus variety was stable in all the years studied and did not depend much on the duration of cultivation. Roots of this variety, apparently, even during the minimum growing period (98 days), managed to accumulate a sufficient amount of nutrients for the formation of heads.

The studied biochemical parameters (content of sugars, chlorophyll *a* and *b*, carotenoids and their ratios) were also influenced by the length of growing period. The longer the growing season

lasted, the more the plants accumulated sugars. The higher the initial sugar content was in the roots, the more they accumulated in the heads ($r = 0.75$). The highest content of total chlorophyll in chicory lettuce leaves was observed during the longest growing season. Forcing the witloof in a dark room influenced the pigment content in the heads.

The study allowed to reveal that both the biometric and biochemical parameters of *C. intybus* var. *foliosum* showed a clear relationship with the duration of growing period. The longer the growing season lasted, the more sugar accumulated in the plants. The studied *C. intybus* varieties showed to be promising at 60°C latitude when cultivating in a foil greenhouse with solar heating, which allows extending the growing season. The study results suggest that in the Northwest of Russia the forcing can be carried out in winter in any room without light at a temperature of 10 to 17°C. Moreover, the results imply that with subsurface heating of the substrate or maintaining water in the containers with roots, a larger yield of heads in weight can be obtained in a shorter time.

References

- Agathokleous E, Feng ZhZh, Peñuelas J., 2020. Chlorophyll hormesis: are chlorophylls major components of stress biology in higher plants? *Sci Tot Environ* 726, 138637, <https://doi.org/10.1016/j.scitotenv.2020.138637>.
- Aisa HA, Xin X-L, Tang D. 2020. Chemical constituents and their pharmacological activities of plants from *Cichorium* genus. *Chinese Herbal Medicines*. 12(3):224-236. <https://doi.org/10.1016/j.chmed.2020.05.001>.
- Bais H.P., Ravishankar G.A. 2001. *Cichorium intybus* L.—cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. *Journal of the Science of Food and Agriculture*, 81(5):467–484.
- Benigini M, Cassan L, Leignez S, Durlin L, Oste S. 2016. Control of root aphid (pempighus bursarius L.) in witloof chicory culture (*Cichorium intybus* L. var. *foliosum*). *Crop Prot.* 89:209-15. <https://doi.org/10.1016/j.cropro.2016.07.027>.
- Cadalen T., Mörchen M., Blassiau C. et al. 2010. Development of SSR markers and construction of a consensus genetic map for chicory (*Cichorium intybus* L.). *Molecular Breeding*, 25(4):699–722, <https://doi.org/10.1007/s11032-009-9369-5>.
- Chen W.M., Jin N., Shi Y., Su Y.Q., Fei B.J., Li W., Qiao D.R., Cao Y. 2010. Coordinate expression of light-harvesting chlorophyll a/b gene family of photosystem II and chlorophyll a oxygenase gene regulated by salt-induced phosphorylation in *Dunaliella salina*. *Photosynthetica* 48, 355–360, <https://doi.org/10.1007/s11099-010-0046-z>.
- Database of plant genetic resources VIR [Electronic resource]. Access mode: <http://db.vir.nw.ru/virdb/maindb>.

- Dospikhov B.A. 1985. Field experiment technique (with the basics of statistical processing of research results). 5th ed., Add. and revised, Moscow, Agropromizdat, pp. 351, (in Russ).
- Ermakov A.I., Arasimovich V.V., Yarosh N.P., Peruvian Yu.V., Lukovnikova G.A., Ikonnikov M.I. 1987. Methods of biochemical research of plants. Leningrad, Agropromizdat, pp. 430 (in Russ)
- Fesenko N.V., Khalmirzaev B.Kh. 1984. Economic and biological characteristics of different varieties of lettuce chicory. Use of growth regulators and polymer materials in vegetable growing. Collection of scientific papers, Leingrad, pp. 70-73 (in Russ).
- Golubkina N.A., Shevchenko Yu.P., Kharchenko V.A., Kosheleva O.V., Soldatenko A.V. 2019. Biochemical characteristics and elemental composition of lettuce chicory (*Cichorium intybus* L.) Variety cone. Vegetables of Russia, 3(47):80-86 https://www.elibrary.ru/download/elibrary_38058119_86549661.pdf.
- GOST 24556-89. Products of fruits and vegetables processing. Methods for determination of vitamin C. <http://docs.cntd.ru/document/gost-24556-89>.
- GOST 31640-2012. 2012. Feeds. Methods for determination of dry matter content. Moscow, Standartinform pp. 8, <http://docs.cntd.ru/document/gost-31640-2012>.
- Gulyaev G.V. 1991. Agronomist's Handbook of the Non-Chernozem Zone. Moscow, Agropromizdat, pp. 574 (in Russ).
- Ivanov L.A., Ivanova, L.A., Ronzhina, D.A., and Yudina, P.K. 2013. Changes in the content of chlorophylls and carotenoids in the leaves of steppe plants along the latitudinal gradient in the Southern Urals, Russia. Plant Physiology 60(6):856–864 (in Russ).
- Janik-Zabrotowicz E., Arczewska M, Prochniewicz P, Swietlicka I, Terpiłowski K. 2020. Stability of Chlorophyll a Monomer Incorporated into Cremophor EL Nano-Micelles under Dark and Moderate Light Conditions. Molecules, 25:5059; doi:10.3390/molecules25215059.
- Judžentienė A., Būdienė J. 2008. Volatile constituents from aerial parts and roots of *Cichorium intybus* L. (chicory) grown in Lithuania. *Chemija*, 19:25–28, https://www.researchgate.net/publication/228914575_Volatile_constituents_from_aerial_parts_and_roots_of_Cichorium_intybus_Lchicory_grown_in_Lithuania.
- Kakhnovich L.V. 2003. Methodological recommendations for laboratory classes, tasks for independent work and control of students' knowledge / Minsk.: BSU, 88p (in Russ)
- Krug G. 2000. Vegetable growing. Moscoe, pp. 576 (in Russ).
- Kumano T., Araki H. 2017. Forcing a crop of witloof chicory (*Cichorium intybus* L.) by using heat from the fermentation of cow manure. *Acta Hort.*, 1170:1109-15. <https://doi.org/10.17660/ActaHortic.2017.1170.143>.
- Kumano T., Araki H. 2017. The effect of excessive application of K₂O during root production on plant growth, mineral concentration and yield of edible part in witloof chicory (*Cichorium intybus* L.). *Enviro Cont Biol.*, 55(4):147-54. <https://doi.org/10.2525/ecb.55.147>.

- Kumano T., Araki H. 2017. The potassium absorption capacity of witloof chicory (*Cichorium intybus* L.) in modelled salt accumulated field made by excessive application of methane fermentation digested slurry. *Enviro Cont Biol.*, 55(4):155-64. <https://doi.org/10.2525/ecb.55.155>.
- Kuperman F.M. 1968. Morphophysiology of plants. Novosibirsk: Science, Siberian branch, pp. 71-83, (in Russ).
- Lavrishcheva T.A., Osipova G.S. 2018. Influence of treatments with Epin-extra on biometric indicators and productivity of endive plants. *Bulletin of the St. Petersburg State Agrarian University*, 4(53):21-27, (in Russ) https://www.elibrary.ru/download/elibrary_36817676_34746377.pdf.
- Lavrishcheva T.A. 2019a. Influence of the food area on the productivity of endive lettuce at different planting dates. *Bulletin of the St. Petersburg State Agrarian University*, 3(56):24-31 (in Russ) https://www.elibrary.ru/download/elibrary_41172112_10805795.pdf.
- Lavrishcheva T.A. 2019b. Influence of the food area on the biochemical composition of endive lettuce at different planting dates. *Bulletin of the St. Petersburg State Agrarian University*, 4(57):22-27 (in Russ) https://www.elibrary.ru/download/elibrary_42463195_20800607.pdf.
- Lavrishcheva T.A., Lavrishchev A.V., Litvinovich A.V., 2020. Impact of climatic factors on growth and development of *Cichorium endivia* in greenhouse in Leningrad region, Russia. *Zemljiste i biljka* 69(2):54-67, 2020, http://www.sdpz.rs/images/casopis/2020/zib_69_2_74.pdf.
- Lavrishcheva T.A. 2020. The effect of the duration of cultivation on the growth and development of various varieties of lettuce chicory (*Cichorium intybus* var. *foliosum*) *Bulletin of the St. Petersburg State Agrarian University*, 2(59):14-21 (in Russ) https://www.elibrary.ru/download/elibrary_43116923_82049386.pdf.
- Lavrishcheva T.A., Osipova G.S. 2020a. The accumulation of pigments by leaves of endive lettuce (*Cichorium endivia* L.) depending on the feeding area and planting dates. *Bulletin of the St. Petersburg State Agrarian University* 1(58):20-25. https://www.elibrary.ru/download/elibrary_42816243_49981667.pdf.
- Lavrishcheva T.A. Osipova G.S. 2020b. The effect of harvesting time on the biochemical composition of various varieties of lettuce chicory (*Cichorium intybus* L. var. *leaf-shaped*) *Bulletin of the St. Petersburg State Agrarian University* 3(60):27-35. https://www.elibrary.ru/download/elibrary_44068827_75476815.pdf.
- Liu, J., Friebe, V.M., Frese, R.N. *et al.* 2020. Polychromatic solar energy conversion in pigment-protein chimeras that unite the two kingdoms of (bacterio)chlorophyll-based photosynthesis. *Nat Commun* 11, 1542. <https://doi.org/10.1038/s41467-020-15321-w>.
- Ludilov V.A., Ivanova M.I. 2010. All About Vegetables: A Complete Reference, Moscow, Fiton, pp. 424

- Luo Z, Gao G, Ma Z, Liu Q, Gao X, Tang X, Gao Z, Li C, Sun T. 2020. Cichoric acid from witloof inhibit misfolding aggregation and fibrillation of hIAPP. *Int J Biol Macromol*, 148:1272-9, <https://doi.org/10.1016/j.ijbiomac.2019.10.100>.
- Minocha R, Martinez G., Lyons B., Long S. 2009. Development of a standardized methodology for quantifying total chlorophyll and carotenoids from foliage of hardwood and conifer tree species. *Canadian J Forest Res* 39:849-861, <https://www.fs.usda.gov/treearch/pubs/19820>.
- Polyanina T.Yu. 2016. A diversity of varieties of root chicory in the shape of a root. *Vegetables of Russia*, 2:68-69 (in Russ).
- Sharma, A., Kumar, V., Shahzad, B. *et al.* 2020. Photosynthetic Response of Plants Under Different Abiotic Stresses: A Review. *J Plant Growth Regul* **39**, 509–531. <https://doi.org/10.1007/s00344-019-10018-x>.
- Shevchenko Yu. P. 2000. Breeding of chicory lettuce endive (*Cichorium endivia* var. *crispum*), Escariola (*Cichorium endivia* var. *latifolium* Lam.) and witloof (*Cichorium intybus* L. var. *foliosum* Hegi) for yield, product quality and early maturity: PhD thesis: 06.01.05, Moscow, pp 157.
- Shevchenko Yu.P., Kharchenko V.A., Ushakova I.T., Kurbakov E.L. 2016. Chicory salad – Vitluf. *Vegetables of Russia*, 2 (31):64-67 (in Russ).
- Son, M., Pinnola, A., Gordon, S.C. *et al.* Observation of dissipative chlorophyll-to-carotenoid energy transfer in light-harvesting complex II in membrane nanodiscs. *Nat Commun* **11**, 1295 (2020). <https://doi.org/10.1038/s41467-020-15074-6>.
- Sonobe R, Yamashita H, Mihara H, Morita A, Ikka T. 2020. Estimation of leaf chlorophyll a,b and carotenoid contents and their ratioa using hyperspectral reflectance. *Remote sensing* 12(19), 3265; <https://doi.org/10.3390/rs12193265>.
- State Register of Breeding Achievements 2022 [Electronic resource]. Access mode: <https://reestr.gosortrf.ru/>.
- Street RA., Sidana J., Prinsloo G. 2013. *Cichorium intybus*: Traditional uses, phytochemistry, pharmacology and toxicity. *Evidence-Based Complementary and Alternative Medicine*, Hindawi, Article ID 579319 | <https://doi.org/10.1155/2013/579319>.
- Titova M.S., Rozlomy N.G. 2015. The dynamics of photosynthetic activity of needles of *Picea ajanensis* and *Picea Smithiana* in the green zone of Ussuriysk. *Electronic periodical "Living Bio-Inert Systems"*, No. 12: <http://www.jbks.ru/archive/issue-12/article-4>.
- Trineeva OV., Safonova EF., Slivkin AI., Voropaeva SV. Patent 2531940 (RU). 20.01.2005, https://patents.s3.yandex.net/RU2531940C1_20141027.pdf.
- Tselniker Yu.L. 1978. Physiological bases of shade tolerance of woody plants. Moscow: Nauka, pp. 214 (in Russ).

- van Arkel J, Vergauwen R., Sévenier R., et al. 2012. Sink filling, inulin metabolizing enzymes and carbohydrate status in field grown chicory (*Cichorium intybus* L.). *Journal of Plant Physiology*, 169(15):1520–1529, <https://doi.org/10.1016/j.jplph.2012.06.005>.
- van Wyk B.E., van Oudtshoorn B., Gericke N. 1997. *Medicinal Plants of South Africa*, Briza Publications, Pretoria, South Africa, <https://lib.ugent.be/catalog/rug01:000654435>.
- Virchenko, I.I. Cultivation of planting material for the distillation of lettuce chicory Vitluf : autoref. diss. Candidate of Agricultural Sciences, / Virchenko And I. : 06.01.06. – M., 1984. – 24 p.
- Vyutnova O.M., Polyanina T.Yu. 2008. Root chicory is a valuable crop. *Potatoes and vegetables*, 7: 21-22 (in Russ).
- Wiebe, H.-J. Effects of low temperature during seed development on the mother plant on subsequent bolting of chicory, lettuce and spinach. // *Scientia Horticulturae - SCI Hort-Amsterdam*. 1989. No 38. P. 223-229. <https://doi.org/10.17660/ActaHortic.1989.253.2>.
- Wulfkuehler S, Gras C, Carle R. 2014. Influence of light exposure during storage on the content of sesquiterpene lactones and photosynthetic pigments in witloof chicory (*cichorium intybus* L. var. *foliosum* hegi). *LWT - Food Sci Technol*, 58(2):417-26, <https://doi.org/10.1016/j.lwt.2014.04.017>.
- Wulfkuehler S, Gras C, Carle R. 2013. Sesquiterpene lactone content and overall quality of fresh-cut witloof chicory (*Cichorium intybus* L. var. *foliosum* Hegi) as affected by different washing procedures. *J Agric Food Chem.*, 61(32):7705-14, <https://doi.org/10.1021/jf402189v>.

Morfometrijske i biohemijske osobine *Cichorium intybus* L. var. *foliosum* na koje utiče trajanje perioda rasta

Tatiana Lavrishcheva¹, Galina Osipova¹, Anton Lavtishchev¹, Zhapparova Aigul², Elmira Saljnikov^{3,4*}

¹ St. Petersburg State Agrarian University, Pererburgskoye sh 2, 196601, St.-Petersburg, Russia

² Department of Soil Science and Agrochemistry, Faculty of Agrobiological Sciences, Kazakh National Agrarian Research University, Abai Avenue 8, Almaty 050010, Kazakhstan

³ Institute of Soil Science, Teodora Dradžera 7, 11000 Belgrade, Serbia

⁴ Mitscherlich Academy for Soil Fertility (MITAK), GmbH, 14641 Paulinenaue, Prof.-Mitscherlich-Alle 1 Germany

* Autor za korespondenciju: E. Saljnikov, soils.saljnikov@gmail.com

Izvod

Cichorium intybus je vredna kultura zbog svojih visokih hranljivih i farmaceutskih vrednosti. U ovom radu je proučavan uticaj vremena berbe na biometrijske i biohemijske osobine 5 sorti *Cichorium intybus* L. var. *foliosum*. Period vegetacije utiče na prečnik rozete, broj listova i masu korena. Utvrđena je jaka korelacija između mase korena pre polaganja za forsiranje i mase forsiranih glava ($r=0.79$). Korenje sorte Conus uspelo je da akumulira dovoljnu količinu hranljivih materija za formiranje glavica za 98 dana. Akumulacija šećera u forsiranim glavicama zavisila je od početnog sadržaja u korenu sa pouzdanošću od 75% ($r = 0.75$). Rezultati su pokazali da se u severnim geografskim širinama forsiranje može vršiti zimi u bilo kojoj prostoriji bez svetlosti na temperaturi od 10 do 17°C. Pored toga, podzemno zagrevanje supstrata ili održavanje vode u posudama sa korenjem obezbedilo je veći prinos glavica dobijenih za kraće vreme.

Keywords: *Cichorium intybus*, radič, forsiranje, glavica, koren, hlorofil, šećeri, karotenoidi

Received 25.10.2022

Revised 6.12.2022

Accepted 6.12.2022