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Content of Some Groups of Compounds in the Vegetative Organs of *Colchicum autumnale* (Melanthiaceae)

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Abstract

The quantitative composition of storage compounds for *Colchicum autumnale* was determined. It was established for the first time that under the conditions of the forest-steppe zone of the West Siberia in the vegetation period in leaves and bulbotubers of the introduced species *Colchicum autumnale*, sugars, starch, saponins, ascorbic acid, pectins, protopectins, catechins, and flavonols are accumulated.

Key words: bulbotuber, leaf, storage compounds, *Colchicum autumnale*, Siberia

INTRODUCTION

The development of pathways and possibilities of the adaptation under conditions of the introduction is relevant and associated with biochemical processes, in particular, with the accumulation of storage compounds in vegetative organs of plants. Among them, bulbotuberiferous plants wintering in the forest-steppe zone of the West Siberia take a special place. The nature of the plant raw material of modified shoots of ornamental plants has been studied insufficiently. One of them is *Colchicum autumnale* L., of the family Melanthiaceae from the genus *Colchicum* L., popular in the ornamental floriculture of our region [1]. In the nature, this taxon grows on wet meadows and clearings in the warm-temperate zone of the Mediterranean, in Europe, Caucasus, Crimea and Krasnodar Territory [2], mesophyte. According to [3, 4], plants of the genus *Colchicum* L. contain a number of flavonoids: luteolin-7-laminariobioside, apigenin-7-sambubioside, apigenin, luteolin, and apigenin-7-diglucoside.In bulbotubers, alkaloids were found, *viz*., colhamine and colchicine. Colhamine is used when treating leucosis and oncological skin diseases [5]. Colchicine, as a major alkaloid of *Colchicum autumnale* (the content is about 0.25 % [6]), is used in small doses when treating gout [7]. Its biological properties influence specifically the spindle apparatus of cells [8, 9] and are often used for selection purposes, for increasing the ploidy of chromosomes in plants [10]. The species have the medicinal and ornamental value. However, the data about the content of metabolites of the major groups of substances in bulbotubers and leaves of *Colchicum autumnale* were not found by us, what laid the foundation for this research.

The goal of the work is a comparative study of the composition of some groups of compounds in bulbotubers and leaves of *Colchicum autumnale* under conditions of the forest-steppe zone of the West Siberia.

EXPERIMENTAL

Research was carried out at the Central Siberian Botanical Garden (CSBG) of the SB RAS in 2009–2011. Test plants were grown up

in the introduction plot located in the south-eastern region of Ob′ River, Novosibirsk. Bulbotubers of the generative age state *Colchicum autumnale* L. introduced starting with 1989 were used in the work [1]. The material was received from the department of ornamental plants of the experimental station of the Vavilov Research Institute of Plant Industry (Pushkino City). Under conditions of Novosibirsk, the specie vegetates in the second-third decades of May–June, rests relatively in July–August, and blooms in autumn (September). In 2009–2010 the vegetation of aboveground organs (leaves) was continued and in September, because, by hydrothermal conditions, 2009 was chilly, excessively moist, while 2010 was warm, moderately wet. The year of 2011 was slightly dry, hence the vegetation of leaves ended in August.

Freshly harvested raw materials were used for the quantitative definition of substances (pectins, protopectins, catechins, sugars, starch, saponins, tannins, and ascorbic acid). Samples for analysis (sample mass of 5–10 g) were selected, according to the development phenophases during the vegetation period (May, June, September) until the start of sustainable frosts.

Pectin substances were determined by the carbazole method based on getting specific purple-pink colouring uronic acids with carbazole in the sulphuric acid medium [11]. The presence in samples of sugars hampers the determination of pectin substances. To remove them, the crushed sample weight of the freshly harvested raw materials was filled in with hot ethanol (from the calculation to obtain the final alcohol concentration of about 80 %) and extracted on a boiling hot bath during 20–30 min. The extraction was repeated three times. The residue was dried at a slight temperature until no smell of the alcohol, extracted with water for the isolation of water-soluble pectins, and then protopectins remaining in the sample mass were hydrolysed.

The alcoholic extract obtained in the previous experiment was used for the determination of the quantitative content of sugars. It contains the simple sugars only and there are no proteins that complicate the identification. The alcohol was removed in a vacuum evaporator; the amount of sugars in the aqueous residue was determined by the method described in [11]. The amount of catechins (flavan-3-ols) was determined by the spectrophotometric method using 70 % ethanol extract [12]. Catechins with 1 % solution of vanillin in concentrated hydrochloric acid give crimson staining. Starch was determined by the acidic hydrolysis method [13] using hydrochloric acid. The amount of sugars formed earlier was subtracted from the amount of glucose obtained after hydrolysis; the remainder was multiplied by 0.9, since one mass part (m. p.) of glucose corresponds to 0.89996 m. p. of starch.

When determining saponins crushed samples were extracted by chloroform in a Soxhlet apparatus to extract lipids, resins, *etc.* The samples were dried and extracted on a water bath at 70 °C for 30 min. consistently with 50, 60 and 96 % solutions of ethanol. The amount of saponins was determined by the weight method [14, 15].

The quantitative identification of flavonols was carried out by the method [16] using the reaction of complexation of flavonols with aluminum chloride. The extraction of 1 g of raw materials was carried out three times on a boiling water bath with 70 % ethanol with a return refrigerator over a period of 30 min. The optical density of the combined extract was measured on a spectrophotometer at the wavelength of 415 nm. The concentration of flavonols was calculated by the schedule constructed on rutin.

To determine the content of ascorbic acid, the average sample was selected (no less than 10 plants), in which all tissues of each species in appropriate proportions were present. Samples were grinded in a mortar up to the homogeneous state in the presence of 1 % solution of hydrochloric acid solution, and then 1 % solution of oxalic acid was added to give the stability in the extract to ascorbic acid. The quantitative determination of ascorbic acid was conducted by the titration with Tillmans paint. To exclude substances reacting with 2,6-dichlorophenolindophenol, a parallel test was conducted. When heated with a solution of copper sulphate, ascorbic acid decomposes. The resulting correction was subtracted from the data of the titration of test extracts [10]. The acidity (free acids) was determined by the way of titration with an alkali of the aqueous extract of the crushed freshly collected material [17]. All biochemical indicators except of ascorbic acid were calculated on the weight of absolutely dry raw materials. The definitions were carried out the triplicate.

RESULTS AND DISCUSSION

The analysis of results of the biochemical study of the composition of bulbotuber and leaves in the period of the spring-autumn vegetation of colchicum autumn allowed to reveal individual and general regularities. Eight compounds were found in the composition of bulbotubers and leaves. Seven from them were present in all organs studied.

Sugars

The analysis showed that the highest content of sugars (30.07–33.18 %) was marked in all observation years in the aboveground organs for *C. autumnale* in the first decade of June *i. e.* in the period of the mass regrowth of leaves. Accordingly, it reduced by 2–4 times by autumn. It was established that the greatest content of sugars in leaves was discov-

TABLE 1

Content of spare substances in aboveground and underground organs of *Colchicum autumnale*

Months	Years					
	2009		2010		2011	
	1	2	1	2	1	2
			Sugars, %			
May	22.06	13.44	22.27	11.00	22.91	12.48
June	33.18	3.56	30.07	1.93	30.65	7.24
September	8.70	8.70	30.82	5.01	–	2.27
			Ascorbic acid, mg/%			
May	114.69	25.91	128.94	19.13	137.14	22.34
June	56.14	35.71	60.39	17.29	177.80	19.03
September	15.07	15.07	21.68	8.67	–	11.03
			Pectins, %			
May	0.22	0.09	1.05	0.49	1.05	0.66
June	0.29	0.15	0.11	0.59	–	0.80
September	0.65	0.65	0.73	0.76	–	0.69
			Protopectins, %			
May	6.52	4.87	3.97	6.37	4.60	7.10
June	3.33	3.31	5.24	4.25	5.83	5.15
September	3.45	3.45	9.41	0.02	–	3.18
			Catechins, %			
May	0.19	0.22	0.06	0.12	0.13	0.10
June	0.16	0.04	0.33	0.22	0.29	0.29
September	0.03	0.03	0.14	–	–	–
			Saponins, %			
May	–	0.58	5.07	5.30	15.87	8.74
June	17.37	2.71	6.87	2.48	15.56	6.48
September	1.84	1.84	30.98	8.05	–	6.10
			Humidity, %			
May	84.50	77.24	84.60	75.10	87.65	84.38
June	86.59	72.65	85.17	75.10	86.59	73.61
September	68.49	68.49	87.93	67.86	–	66.58

Note. 1 – aboveground organs (leaf), 2 – underground (bulbotuber).

ered in 2010 (22.37–30.82 %). Obviously, this is associated with weather conditions of vegetation, since by hydrothermal conditions, this vegetation period was warm and moderately moist. A high content of sugars in the bulbotuber was registered in May (11.0–13.44); however, it was two times lower, in comparison with the data for leaves (Table 1). By autumn, the amount of sugars in the bulbotuber reduced by 2.5–6 times.

Ascorbic acid

It was established that the content of this substance in all organs decreased from May till September in all observation years. However, in May and June, it is 2–6 times higher in leaves (114.6–137.1 %), in comparison with the bulbotuber.

Pectins

Pectin substances are present in plants in the form of pectin water-soluble, calcium and magnesium salts of pectin acid and protopectin, *i. e.* pectin substances derivative, insoluble in water. The analysis of the data obtained showed that the content of pectins in aboveground and underground organs of *C. autumnale* was insignificant and varied by years. Thus, in the period of the mass spring regrowth the amount of pectin in leaves is 23 times greater, than in bulbotubers. By autumn, pectins get accumulated in underground organs and on the quantity differ by 8 times (2009), by 1.5 times (2010) and insignificantly (2011) from the data for May (see Table 1). Such a tendency for aboveground organs was marked in 2009 only (the increase by 3 times), in comparison with 2010–2011.

Protopectins

During all years of observation, the content of protopectins in bulbotubers was reduced by 2–10 times to the autumnal pre-winter. The maximal data were received for leaves (4.6–6.52 %) in the spring regrowth period.

Catechins

It is the most reductive group of flavonoids showing a wide spectrum of the biological effect. Overwintered (mother) bulbotubers of the autumn colchicum in May contain the largest number (0.10–0.22 %) of catechins. By September, their content reduces and was insignificant (only 0.03 %) in the young bulbotuber in 2009, and catechins were not discovered in 2010–2011. A high content of these components in leaves was observed in May (0.19 %).

Saponins

They belong to poisonous glycosides of the steroidal and nonsteroidal type. Their quantitative composition for this taxon varied greatly by years of research and had individual indicators. Thus, a gradual growth of the content of saponins in bulbotubers by 3 (2009) and 1.5 times (2010) was marked from May to September. In 2011, it decreased in the bulbotuber of from 8.74 to 6.10 %. The highest content of saponins in leaves was marked in May–June, *viz*, 15.87–17.37 % (see Table 1).

Starch

As a polymer of glucose consisting of two polysaccharides (linear polysaccharide of amylase and "branchy" amylopectin), starch was detected in bulbotubers. In May, its content was

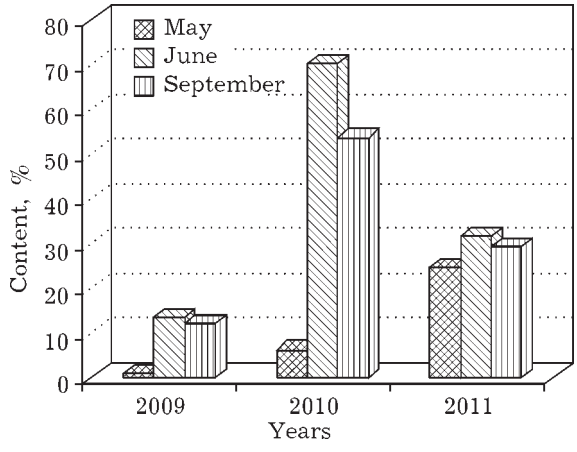

Fig. 1. Content of starch in the bulbotubers of *Colchicum autumnale* in 2009–2011.

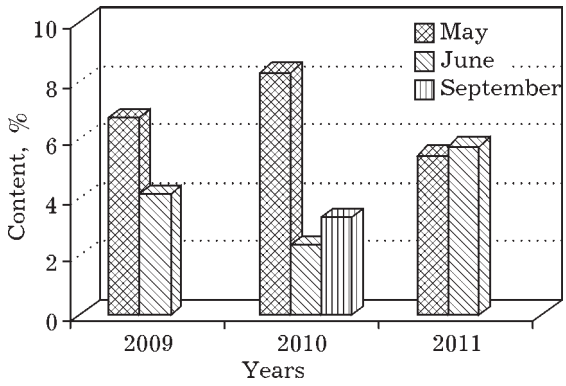

Fig. 2. Content of flavonols in the leaves of *Colchicum autumnale* in 2009–2011.

minimal. By September, the accumulation of starch was 12.08 % (2009), 53.65 % (2010) and 29.40 % (2011), what is 12, 9, 1.5 times greater, respectively, in comparison with the period of the spring vegetation of the genus (Fig. 1).

Flavonols

They were found in aboveground organs (leaves). Their maximum was marked in May–June (5.76–8.31 %). By autumn, their content was reduced (Fig. 2) in all years of observations.

CONCLUSIONS

Thus, under conditions of Novosibirsk (Priobye forest-steppe climatic province) for *C. autumnale*, we established for the first time that the quantitative content of starch in bulbotubers was increased from spring to the pre-winter, and that of sugar was reduced. The increase of the content of starch as insoluble polysaccharide promotes the acceleration of metabolic processes in storage tissues of bulbotubers, which determines their significant frost resistance and strengthens adaptation possibilities during the winter dormancy in Siberia. *C. autumnale* is perennial polycarpic, that is its bulbotubers get replaced (get renewed) annually. These processes pass on intensively in spring-summer-autumn periods. By autumn (pre-winter), for this taxon, a young (daughterly) bulbotuber that spends winters at low positive temperatures to the start of the spring vegetation was formed. Thus, we established that for *C. autumnale* that spends winters in the open ground of the forest-steppe zone of West Siberia, the content of sugar in bulbotubers to pre-winter is decreased by two times, in comparison with spring, and the content of starch increases. Obviously, the accumulation and distribution of starch in storage organs, that is a bulbotuber, plays a significant part in the winter hardiness. Consequently, a high content of starch in pre-winter indicates a good frost resistance of bulbotubers of C. *autumnale* and the possibility of their wintering in the open ground under harsh conditions of Siberia.

The presence of saponins, ascorbic acid and catechins in the composition of overwintered bulbotubers promotes the steadiness of this species to adverse environmental factors, microflora in the period of the intense growth and development. A small amount of pectins and protopectins during the whole vegetation period is caused by their low content in underground organs, in comparison with aboveground at the period of formatting of the daughterly bulbotuber. The quantitative content of starch in bulbotubers of *C. Autumnale*s pending winters in the open ground in the pre-winter is higher by 2–9 times while that of sugar is lower by 2–6 times. The content of ascorbic acid in the period of spring regrowth of leaves is by 5–6 times higher, than for overwintered bulbotubers. The dynamics of the accumulation of saponins, catechins, pectins, and protopectins during the seasonal development is changed in aboveground and underground organs, which is associated with the growth rhythm and development of *C. autumnale*.

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