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Seasonal Accumulation Dynamics for Reserve Substances in *Scilla sibirica* and *Ornithogalum ponticum* Bulbs

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Abstract

Accumulation dynamics under the conditions of the forest-steppe zone of the West Siberia has been for the first time established for sugars, starch, saponines, ascorbic acid, pectines, protopectines, catechines, tannins in bulbs of introduced species such as *Scilla sibirica* Haw. and *Ornithogalum ponticum* Zahar. Bulb componential composition has been described for these taxons.

Key words: bulb, reserve substances, bluebell, starflower, Siberia

INTRODUCTION

Reserve substances exert a considerable influence upon the features of the formation of generative organs as well as upon the winter hardiness of bulbous plants. The distribution dynamics of reserve substances in vegetative organs of ornamental plants is still not adequately investigated, which is especially urgent for their cultivation in West Siberia, the region with a pronounced continental climate. Among ornamental plants, *Scilla sibirica* Haw. (the Siberian bluebell) and *Ornithogalum ponticum* Zahar. (syn. *O. hyrenacum* auct. non L.) are early-spring bulbous geophytes and being of practical use [1]. The Siberian bluebell (mesophyte) naturally grows within the forest zone of the European part of the former USSR, Crimea, Caucasus, Asia Minor and Forward Asia. The natural habitat of the *Ornithogalum ponticum* (xeromesophyte) is confined to the bottom and middle mountain zone of Caucasus and Crimea. Studies concerning the biochemical structure of these species were almost not carried out, merely the authors of [2–4] noted that plants belonging to the genus *Scilla* L. contain apigenine, homoisoflavanones such as scyllasciline and 3,9-dihydroeucomnaline. The multifunctional adaptation of vegetative organs at the morphological, anatomic, physiological and biochemical levels promotes active or passive vital functions of plants. Data concerning the content of the metabolites of the main groups of substances in the bulbs of these species would allow differential approaching to the preparation of raw material during the springtime or prewinter period.

The present work is aimed at the studies of seasonal accumulation dynamics for reserve substances in the bulbs of the Siberian bluebell (*Scilla sibirica* Haw.) and the *Ornithogalum ponticum* Zahar. under the conditions of the forest-steppe zone of West Siberia.

EXPERIMENTAL

The studies were carried out at the Central Siberian Botanical Garden (CSBG), SB RAS, during 2007. Experimental plants were grown on the introduction plot located in the southeast Priob'ye area of the Novosibirsk. For the experiments we used regenerative age condition bulbs of *Scilla sibirica* (Siberian bluebell) and *Ornithogalum ponticum* introduced under the conditions of Novosibirsk since 1989 [1]. For the quantitative determination of substances (pectines, protopectines, catechines, sugar, starch, saponines, tannins, ascorbic acid) in bulbs of the species under investigation we used fresh-harvested plant raw material. Samples for the analysis (weighed portions of 5–10 g in mass) were chosen according to the phenological stages of development in the course of vegetative period (May, June, July, October) even to steady frosts.

Pectin-like substances were determined employing a carbazole method based on obtaining specific violet-red colouring of uronic acids with carbazole in sulphuric acid medium [5]. The determination of pectin-like substances is complicated by the presence of sugars in samples. In order to remove them, we grinded a weighed portion of fresh harvested raw material then covered it with hot ethyl alcohol (in such a way as to obtain of the final concentration of alcohol about 80 %), extracted it with the use of a boiling water bath during 20–30 min. The extraction was repeated three times. The residue obtained was dried at a moderate temperature up to reaching the absence of alcohol smell, then it was extracted with water for the isolation of water-soluble pectines, then protopectines remained in the weighed portion were exposed to hydrolysis.

For determining the quantitative content of sugars we used the alcoholic extract obtained wherein only simple sugars are contained and there are no proteins those complicate the determination of sugars. Alcohol was removed employing a vacuum evaporator, whereas the amount of sugars in an aqueous residue was determined according to the method described in [5]. Catechines were determined by means of a spectrophotometric method using the alcoholic extract obtained in the course pectin determination. Catechines (flavanols) interacting with 1 % vanillin in concentrated HCl result in a crimson colouring [6]. Starch was determined employing the method of acid hydrolysis [7] performed with the use of HCl, in the following manner. We subtracted the amount of sugars obtained earlier from the amount of glucose obtained after the hydrolysis and then the result was multiplied by 0.9, since 1 mass part of glucose corresponds to 0.89996 mass parts of starch. In order to determine saponines, the grinded samples were exposed to the extraction with chloroform using a Soxhlet apparatus for the extraction of lipids, resins, *etc*. The samples were dried and extracted successively with 50, 60 and 96 % ethanol using a water bath at 70 °C, during 30 min. The amount of saponines was determined employing a gravimetric method [8]. The amount of tannins was determined by permanganatometry. The extraction of fresh harvested grinded material was carried out with water employing a boiling water bath for 50 min [9].

For the determination of ascorbic acid, we took an average sample (not less than 10 plants) wherein there were all the tissues of each plant in corresponding proportions, triturated employing a mortar to obtain homogeneous condition in the presence of 1 % HCl solution. Then we added a 1 % solution of oxalic acid for stabilizing ascorbic acid in the extract. The quantitative determination of ascorbic acid was carried out employing Tillman's dye titration. In order to exclude the substances reacting with 2,6-dichlorophenolindophenol we executed parallel experiments. On heating with copper sulphate solution, ascorbic acid decomposes. The correction obtained was subtracted from the data of titration of experimental extracts [5]. The acidity (free acids) was determined *via* alkali titration of an aqueous extract of grinded fresh harvested material [10]. All the biochemical parameters except for the data for ascorbic acid were calculated with respect to the mass of absolutely dry raw material. The determinations were carried out in triple replication.

RESULTS AND DISCUSSION

The analysis of the results of the studies concerning reserve substances in the bulbs of *Scilla sibirica* and *Ornithogalum ponticum* within the period of their growth and development, summer dormancy and pre-winter time has allowed us to establish individual and general regularities of reserve substances accumulation dynamics. We have determined that the bulbs of the taxons under investigation contain eight components (Table 1).

Within the period of spring vegetation and flowering (May) the content of sugar in the

TABLE 1

Data concerning the content of reserve substances in the bulbs of *Scilla sibirica* and *Ornithogalum ponticum* growing under the Novosibirsk conditions, %

Phenological data	Pectines	Proto-pectines	Catechines	Sugars	Starch	Saponines	Tannins	Ascorbic acid
			Ornithogalum ponticum					
14.05	1.8	3.5	16.2	10.3	20.8	8.5	–	26.4
20.06	3.2	4.1	–	6.2	15.5	4.1	0.9	15.4
17.07	3.0	7.3	–	5.3	5.9	2.9	0.3	18.1
04.10	1.0	2.2	–	15.6	24.6	3.8	0.5	8.1
			Scilla sibirica					
14.05	1.3	2.5	10.8	8.4	18.7	14.3	–	24.2
20.06	3.0	5.6	–	4.1	6.5	1.4	0.8	34.2
17.07	1.8	3.4	6.1	5.7	2.9	3.1	0.4	14.5
04.10	–	–	19.0	9.4	28.1	12.1	0.6	12.8

Notes. 1. The content of catechines and ascorbic acid is presented in mg%. 2. Dash – not revealed.

bulbs of *Scilla sibirica* and *Ornithogalum ponticum* is equal to 8.4 and 10.3 %, respectively. However, already in June, *i. e.* during the fructification period and in the beginning of summer relative dormancy of bulbs, its content exhibits a two-fold decrease. In the pre-winter time (October) the content of sugar in the bulbs of *Scilla sibirica* exhibited a two-fold increase (9.4 %), whereas in the bulbs of *Ornithogalum ponticum* this value exhibited a three-fold increase (up to 15.6 %).

The quantitative content of starch in the bulbs of both species in May exceeds the content of sugar therein amounting to 20.8 % for *Ornithogalum ponticum* and 18.6 % for *Scilla sibirica* (Fig. 1). By the summer dormancy period (June, July) the content in the bulbs of *Ornithogalum ponticum* decreased from 15.5 to 5.8 % and by October this value increased again up to 24.5 %. The same regularity is noted and for *Scilla sibirica*: the content of starch is reduced in June and July from 6.5 to 2.9 %, whereas by the autumn period this value increases by 7–9 times.

As far as the quantitative ratio of saponines is concerned, we have established that a high content of saponines (14.3 %) in *Scilla sibirica* is observed within the flowering period in May (see Fig. 1, *a*). However, in the course of summer dormancy in June, an abrupt reduction of saponine content (1.4 %) and then its gradual increase up to 3.1 % in July were observed. By the period of the beginning of winter dormancy

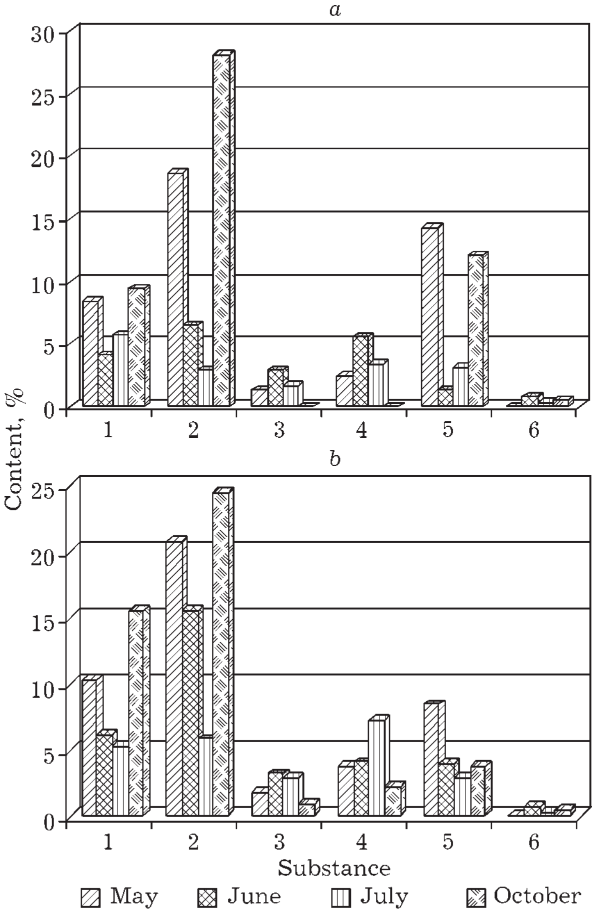

Fig. 1. Distribution dynamics of reserve substances in the bulbs of *Scilla sibirica* (*a*) and *Ornithogalum ponticum* (*b*) from May to October: 1 – sugar, 2 – starch, 3 – pectine, 4 – protopectine, 5 – saponine, 6 – tannins.

(October), the content of saponines in the bulbs of *Scilla sibirica* have amounted to 12.1 %. As far as *Ornithogalum ponticum* is concerned, a 2.5-fold decrease has been noted in the content of saponines within the period from May to October (from 8.5 to 3.8 %).

For the plant species under investigation, single type regularity has been observed concerning the distribution and accumulation of protopectines and pectines in the course of seasonal development. The analysis of the data obtained has demonstrated that the content of pectines and protopectines in the bulbs of both species is insignificant. From May to July the content exhibits an increase, abruptly reducing by the beginning of pre-winter time, whereas immediately before the winter dormancy period the pectines and protopectines in bulbs are absent. So, in May the bulbs of *Ornithogalum ponticum* contain an insignificant amount of protopectines (3.5 %), in July this value reaching a maximum (7.3 %), in October the content of protopectines being low (2.2 %). As far as the bulbs of *Scilla sibirica* is concerned, a low content of these substances is also observed therein in May (2.8 %), in June this value exhibits an increase up to 5.6 %, in July this value is reduced down to 3.4 %, whereas in October the substances were not revealed. The content of pectines in May in the bulbs of *Ornithogalum ponticum* and *Scilla sibirica* is equal to 1.8 and 1.3 %, respectively. In June this value is maximal for both species (3 and 3.2 %, respectively). In October the content of pectines decreases down to 1 % for *Ornithogalum ponticum*, whereas in bulbs of *Scilla sibirica* these substances were not revealed.

The maximal content of ascorbic acid (26.4 mg%) in the bulbs of *Ornithogalum ponticum* has been observed in May. During the summer dormancy period (June–July) and the pre-winter time the content of ascorbic acid in the bulbs of this species decreased by three times amounting to 8.1 mg%. Its maximal content in the bulbs of *Scilla sibirica* has been dated for June being equal to 34.2 mg%.

As a whole, within the vegetative development period from June to October the content of tannins in the bulbs of *Ornithogalum ponticum* decreases from 0.8 to 0.5 %, whereas this value for *Scilla sibirica* decreases from 0.8 to 0.6 % (see Fig. 1).

As the bulbs of species *Ornithogalum ponticum* are concerned, catechines were revealed only in May, the content of these substances amounted to 16.2 mg%. In the bulbs of another species under investigation – *Scilla sibirica* – these substances were not revealed in June; their content is equal to 10.8 mg% in May, to 6.4 mg% in July, to 19 mg% in October.

The humidity of bulbs from May to October increased from 56.8 to 76.5 % for *Ornithogalum ponticum* and from 53.3 to 73.9 % for *Scilla sibirica*. Their acidity amounted to 0.2–1.4 and 0.5–1.3 %, respectively, whereas the minimal value has been registered in July, and the maximal one was observed in October.

CONCLUSIONS

Thus, we have first established for ornamental plants of the species such as *Scilla sibirica* (Siberian bluebell) and *Ornithogalum ponticum* that under the conditions of the Novosibirsk (Priob′ye forest-steppe climatic province) the quantitative content of starch and sugar in bulbs exhibits an increase from the springtime to the pre-winter time. In this case, in the springtime the content of starch is twice higher than the content of sugar. Within the summer seasonal period, the content of starch in the bulbs of the *Ornithogalum ponticum* is twice higher, than its content in the bulbs of the Siberian bluebell. The increase in the content of starch as an insoluble polysaccharide promotes the acceleration of metabolic processes in the tissues of the reserving bulb scales, which causes their considerable frost resistance and strengthens the adaptabilities of these taxons in the course of winter dormancy in Siberia. The accumulation of sugar and starch in bulbs before their winter dormancy provides the stability of generative organs wintering in the bulb renewal buds of these ephemeroids. In this case the frost resistance level of Siberian bluebell bulbs is higher comparing to those for the *Ornithogalum ponticum* bulbs. Certainly, this fact is connected with a genotypic origin of these species and their norm of reaction with respect to external factors. It has been established that during the period of vegetation and flowering, the highest content of saponines, protopectines, pectines in the bulbs of *Scilla sibirica* and *Ornithogalum ponticum* could be observed, whereas the amount of tannins, catechines and ascorbic acid is insignificant. In this case the content of saponines in the springtime and pre-winter period observed for *Scilla sibirica* is twice higher comparing to *Ornithogalum ponticum*. The quantitative content of catechines exhibits individual variability; for *Ornithogalum ponticum* these features are noted only in May (16.2 mg%), whereas for *Scilla sibirica* their increase was observed from May to October (from 10.8 up to 19 mg%).

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