Features of Flavonoids Accumulation in Wild-Growing and Strange Plants *Panzerina lanata* subsp. *argyracea* (Kuprian.) Krestovsk. Growing in Khakassia

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Abstract

Panzerina lanata subsp. *argyracea* (Kuprian.) Krestovsk. is a valuable drug plant that grows in Khakassia. It contains a complex of biologically active compounds including flavonoids. Resources of panzerina raw material steadily go down owing to anthropogenic load. Its domestication solves the problem of its preservation. A wild-growing cenopopulation with the greatest content of flavonoids in the aboveground part has been revealed that is of interest as a starting material for plant introduction. The content of flavonoids in particular members of plants *Panzerina lanata* subsp. *argyracea* that grows in various cenopopulations of Khakassia and under conditions of plant introduction has been examined. Dynamics of accumulation of flavonoids for strange silvery panzerina has been determined in various vegetation phases. It was found that plants in the crop exhibit a high level of variability in their content of flavonoids.

INTRODUCTION

Natural vegetative resources, as they are a source of raw material for various industries, are also of invaluable importance in environment formation. Sustainable utilization of natural resources of any region depends on the development of scientific bases of nature management and prospective programs of preservation and reproduction of flora. Two ways of particular mobilization of vegetative resources are possible: adoption of a useful plant as a natural raw material or its domestication [1].

Biochemical research occupies one of the main places in comprehensive study of useful plants of natural flora. The research includes not only biochemical inventory of fodder, medicinal, technical, and alternative groups of plants, but also forecasting the rational ways to use vegetative resources. The important stage of this research is recognizing the plants that hold promise for further in-depth study.

The possibility and character of using drug plants in medicine depends on the active substances available in them and the diversity of compounds may play this part. Flavonoid-containing plants attract the attention of explorers as raw material to produce antiphlogistic, cholagogue, radioprotective, antitumorigenic, capillary protection and alternative preparations [2]. Practically universal distribution of these materials in flora is also indicative of their great importance in life of plants, too [3].

A stem *Panzerina* (Sojak) (previously *Panzeria* Moench. – panzeria) that is eurysynusic in Central Asia is represented in Khakassia by panzerina silvery, *P. lanata* subsp. *argyracea* (Kuprian.) Krestovsk. [4]. According to data [5], *P. lanata* represents a peculiar edificator plant of desertificated steppes of Khakassia and it

grows also in genuine steppes, with a smaller abundance though. In steppe areas, the plant grows on rocky slopes along river valleys [6]. It is low productive under natural conditions. The numbers of this species in natural habitals steadily taper off in relation to an increasing anthropogenic load, namely, cattle pasturage near to settlements, and heavy use of reserve stocks. In addition, large-scale harvesting of aerial materials by the population leads to an almost complete extermination of its populations.

Panzerina was at all times applied in folk medicine as a remedy for edema and rheumatism, for heart pain, as diuretic and sedative. Accordingly, it was registered in the Hungarian pharmacopoeia as diuretic agent and remedy to cure edema [7]. Sedative and vasodilating action of panzerina has been found that is highly competitive with valerian and motherwort (*Leonurus*) in its efficiency [8]. Panzerina is in general use by local population when treating thyroid gland diseases.

Plants of this stem contain significant amount of flavonoid compounds, derivatives of hydroxycinnamic acids, tannins (derivatives of pyrocatechol), *etc*. Pharmacological studies of the sum of flavonoids have revealed their spasmolytic and hypotensive action. A combined preparation that is made up of caffeic, neochlorogenic, and chlorogenic acids shows also the antihypertensive effect, moderate negative chronotropic action and it tangibly raises amplitude of cardiac contractions [9]. According to authors of [10, 11], aerial material of *Panzeria lanata* (L.) Bunge contains 0.03–0.06 % of alkaloids, together with saponins, tanning agents, organic acids, iridoids, triterpenoids. The aerial material of panzeria silvery – *Panzeria argyracea* Kuprian. – contains alkaloids (0.03 %), bitter matter of undetermined nature and up to 0.02 % of essential oil. In addition, hydroxybutanedioic and tannic acids together with vitamin C are revealed in grass of panzeria [12]. Diversity of chemical composition defines a biological potency of plants of this stem and may be of great practical interest.

One of the ways to preserve panzerina silvery is its domestication. Creating agropopulations with high productivity indexes and content of biologically active compounds in the crop will allow us to preserve this valuable species in the nature.

The purpose of this work is to study the content of flavonoids of panzerina silvery that is cultivated in botanical garden of the Research Institute of Agricultural Problems of Khakassia and that growing in natural conditions of Khakassia.

EXPERIMENTAL

Cenopopulations of panzerina silvery in Shira and Ust-Abakan districts (Khakassia) have been studied. In Shira district, plants of panzerina were collected in halophytic mixed grass assemblage of bunchgrass steppe in vicinities of Kiprino Lake on a rocky flank. In Ust-Abakan district, two cenopopulations have been studied: on a motley grass wormwood long fallow field in vicinities of Il'ich settlement and in motley grass wormwood assemblage of gentle rolling disturbed steppe in vicinities of the 2nd branch of Chernogorskoye experimental farm.

Climate of the research areas is sharply continental, with cold winter with little snow and with hot and dry summer. Rainfall comprises 298 mm/year, and their basic part (57.8 %) precipitates during summer months. Vernal period is typified by low relative humidity of air, by soil moisture deficiency, and by strong dehumidifying winds. Climatic conditions of the plant introduction site (Zelenoye settlement, Ust-Abakan district) practically do not differ from the conditions of the research areas [13].

The plant seeds of natural cenopopulations were sowed at an experimental field of the Botanical Garden of Research Institute of Agrocultural Problems of Khakassia by broad-row method with 60 cm row widths. The seeding rate was 50 seeds per a running metre. Plants were grown without showering and fertilization. Samples of strange plants were collected during the following development phases: that of the formation of racemes (the visible phase), that of budding, that of blooming, and that of beginning of seeds ripening. Sampling in natural localities was conducted during the period of plants blooming. Total content of flavonoids was determined in an average sample that was composed of 15–20 plants of panzerina silvery.

The procedure of quantitative determination of flavonoids has its origins in a method [14] that makes use of a reaction of complex formation of flavonoids with aluminium chloride.

Spectrophotometric determination of the combined content of flavonoids in raw material of panzerina silvery was conducted as follows. An accurately weighted sample of air-dried raw material (~0.5 g) that was grinded and riddled through a 1 mm diameter holed sieve was put in a flask with the capacity of 100 mL and exhaustive extraction was performed with 70 % ethanol, with the control of the completeness of the extraction by reaction with 5 % NaOH solution (to reach the disappearance of yellow colouring); then the volume of the filtered aggregated extract was measured. Next, 0.1 mL of the extract was put in a measuring tube, 0.2 mL of 2 % $AlCl_3$ solution in 96 % ethanol was added, and the volume was brought to reach 5 mL by adding ethanol of the same concentration. When doing the reference run, 1–2 drops of 30 % acetic acid were poured into 0.1 mL of the extract and next, the volume was brought to reach 5 mL. The solutions were agitated and 40 min later, the optical density of the solution with aluminium chloride was measured in a SF-26 spectrophotometer at 415 nm in a sample cell with the layer 1 cm thick, using solution with the acid as the reference.

Combined content of flavonoids (as % from mass of the air-dried raw material) was determined as $X = YV_1V_2100/(MV_3 \cdot 10^6)$, where Y is the content of flavonoids in 1 mL of the tested solution that was found from the calibration curve plotted in terms of rutin, μg; V_1 is the extract volume, mL; V_2 is the dilution volume, mL; V_3 is the extract volume that was taken for the analysis, mL; M is the mass of air-dried raw material, g.

To make an estimate of the variability of the flavonoid content, a scale of variability levels was used [15]. According to this scale, the variability rate is considered: very low at $V < 7$ %, low at $V = 8–12$ %, moderate at $V = 13–20$ %, high at $V = 21–40$ %, and very high at $V > 40$ %.

RESULTS AND DISCUSSION

Plants of natural cenopopulations exhibit a certain tendency in accumulation of flavonoids during their blooming. Based on the flavonoid content of racemes, leaves, and caules, populations can be arranged in the following order: Il'ich > Chernogorskoye > Shira. The content of flavonoids comprises 2.31–2.37 % in racemes of plants from Chernogorskoye and Il'ich cenopopulations, and 1.32 % in racemes of plants from Shira cenopopulation. The flavonoid content of leaves is almost twice less by comparison to the content of racemes. Plants of Chernogorskoye cenopopulation are typified by the greatest flavonoid content of their aerial material (1.34 %), while those of Il'ich cenopopulation, by the minimal one (0.37 %) (Fig. 1).

In wild growing samples in the blooming phase, the interpopulation variability of their content of flavonoids in different members was characterized by a high variability V (%): racemes 29.4, leaves 28.1, caules 29.5, above-ground parts 68.6, roots 44.7 (Fig. 2).

Transfer of plants from the conditions of natural habitation into culture conditions is inevitably related to a change in its usual environment. Efficiency of the plant introduction depends for the most part on that how much the ecological conditions of culture are close to the conditions where the given species was shaped [13].

On the average, the following regularity is evidenced in accumulation of flavonoids in strange plants of panzerina silvery during the course of two years. The quantity of flavonoids in racemes in the blooming phase is more by comparison to that in the budding stage. The level of the flavonoid content drops down during seeds ripening. The flavonoid content of leaves, caules, and aerial material during the

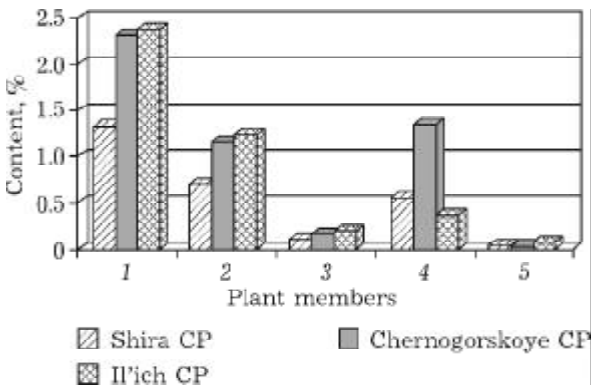

Fig. 1. Flavonoid content of various members for middle-aged generative plants of panzerina silvery in natural cenopopulations (CP) of Khakassia: *1* – racemes, *2* – leaves, *3* – caules, *4* – aboveground part, *5* – roots.

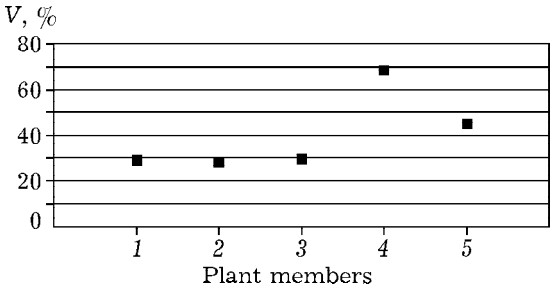

Fig. 2. Interpopulation variability of the flavonoid content (*V*) in various members of *Panzerina lanata* subsp. *argyracea*. For designations see Fig. 1.

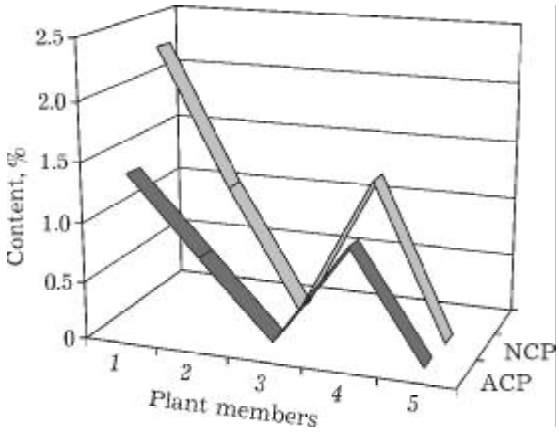

Fig. 3. Content of flavonoids during the blooming phase of *Panzerina lanata* subsp. *argyracea* in natural cenopopulation (NCP) and in agrocenopopulation (ACP). For designations see Fig. 1.

budding stage is lower as compared with the phase of formation of racemes, and then it increases again in the blooming phase. In the samples that were taken in 2002, flavonoids in the leaves accumulated gradually from the moment when germs of racemes appeared until blooming; their content was unaffected in the budding stage. Roots contain very small quantity of flavonoids (Table 1).

Strange plants exhibited very high variability of the flavonoid content of racemes, leaves, caules, and roots for the growing season of 2001, and anomalously high variability (V = 140.5 %) for that of the aerial material. In 2002, a moderate level of variability of the flavonoid content during the vegetation (17.3 %) is typical for caules of the plants only; it is very high (45.4–64.2 %) for the remaining parts of plants.

Under culture conditions, the average (for two years) content of flavonoids during the blooming phase in racemes (1.38 %), leaves (0.74 %), caules (0.11 %), and above ground part (0.96 %) of panzerina silvery from Chernogorskoye agropopulation drops off when compared to natural conditions: 2.31, 1.16, 0.16, 1.34 %, respectively (Fig. 3).

CONCLUSION

Hence, the study of regularities of flavonoids accumulation in panzerina silvery has demonstrated that the maximum content of flavonoids (2.31–2.37 %) has been observed in the blooming phase in racemes of the plants of Chernogorskoye and Il'ich cenopopulations.

Based on the content of flavonoids of the aerial material (1.34 %), wild plants of Chernogorskoye cenopopulation are most promising for plant introduction. Under conditions of culture, the content of flavonoids tapers off when compared to natural conditions.

TABLE 1

Content of flavonoids in various members of panzerina silvery from Chernogorskoye agrocenopopulation, % from air-dried mass

Phases of vegetation	Racemes	Leaves	Caules	Aboveground part	Roots
Shaping of racemes	–/–	0.48/0.23	0.05/0.10	0.11/0.20	0.02/0.04
Budding	0.35/0.84	0.10/0.47	0.01/0.07	0.07/–	0.03/0.09
Blooming	1.60/1.17	0.67/0.80	0.13/0.10	1.23/0.68	0.07/0.02
Maturing	–/1.06	–/0.27	–/0.10	–/0.31	–/0.04
M	0.82/0.84	0.42/0.44	0.07/0.09	0.47/0.40	0.04/0.05
V, %	83.2/45.4	69.0/59.4	91.8/17.3	140.5/62.5	61.6/64.2

Notes. 1. The first value is the content of the samples that were taken in 2001, the second – in 2002.
2. *M* is average content of flavonoids, %; *V* is variation factor, %. 3. The dash implies that data are unavailable.

The presented materials may form a basis for a sustainable utilization of this species as raw material to produce flavonoid-containing preparations.

REFERENCES

1 K. A. Sobolevskaya, Uspekhi introduktsii rasteniy, Nauka, Moscow, 1973, pp. 43–65.
2 V. G Minaeva, Introduktsii rasteniy v Sibiri, Nauka, Novosibirsk, 1977, pp. 165–175.
3 M. N. Zaprometov, Osnovy biokhimii fenolnykh soyedineniy, Vyssh. Shk., Moscow, 1974.
4 Flora Sibiri: *Pyrolaceae – Lamiacea (Labiatae)*, Nauka, Novosibirsk, 1997, p. 11.
5 V. V. Reverdatto, *Tr. Tom. Un-ta*, 147 (1957) 203.
6 Poleznye rasteniya Khakassi. Resursy i introduktsiya, Nauka, Novosibirsk, 1989.
7 V. G Minaeva, Lekarstvennye rasteniya Sibiri, Nauka, Novosibirsk, 1970.
8 N. V. Vershinin, D. D. Yablokov, Novye lekarstvennye rasteniya Sibiri i ikh lechebnye preparaty, Izd-vo Tom. un-ta, Tomsk, 1944, issue 1, pp. 3–9.
9 V. I. Vavilov, Khimichesskoye issledovaniye otechestvennykh predstaviteley roda pantserii (Author's abstract of Candidate's Dissertation in Pharmacology), Kharkov, 1973.
10 I. A. Gubanov, I. L. Krylova, V. L. Tikhonova, Dikorastushchiye poleznye rasteniya SSSR, Mysl', Moscow, 1976.
11 L. P. Markova, L. M. Belenovskaya, T. P. Nadezhina *et al.*, Dikorastushchiye poleznye rasteniya flory Mongolskoy Narodnoy Respubliki, Nauka, Leningrad, 1985.
12 V. I. Vavilov, E. V. Gella, *Rast. Res.*, 6, 2 (1970) 235.
13 A. V. Kuminova (Ed.), Rastitelny pokrov Khakassii, Nauka, Novosibirsk, 1976.
14 V. V. Belikov, *Rast. Res.* 21, 3 (1985) 350.
15 S. A. Mamaev, Zakonomernosti vnutrividovoy izmenchivosti listvennykh drevesnikh porod, Sverdlovsk, 1975.