POLYSACCHARIDE ACCUMULATION DYNAMICS IN Iris pseudacorus

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It was established by studying carbohydrates of Iris pseudacorus during vegetative periods that the content of water-soluble polysaccharides in the rhizomes and roots is greatest during seed ripening; of pectinic substances, during budding. Glycomannan was isolated from seeds.

Key words: Iris pseudacorus L., water-soluble polysaccharides, pectins, glucomannans, hemicellulose, isolation, accumulation dynamics.

Iris pseudacorus L. or water-flag (Iridaceae) is a perennial rhizomatous herbaceous plant that can propagate by seeds or vegetatively [1].

Roots and rhizomes are found in the M. N. Zdrenko collection for bladder papillomatosis, antacid gastritis, and stomach ulcers. They are used in folk medicine as an astringent and tonic. The decoction is used for respiratory infections, pneumonia, stomach ulcers, and urinary-tract diseases; the extract, for dysmenorrhea [2].

Because iris is cultivated in Uzbekistan as a medicinal plant, its reproductive biology was studied. Herein we present results from an investigation of the polysaccharides in the aerial and subterrean organs of *I. pseudacorus* and their accumulation dynamics as a function of the plant vegetative period.

Plants were collected from a single site in the experimental section of the F. N. Rusanov Botanical Garden of the Scientific-Production Center Botanika of the Academy of Sciences of the Republic of Uzbekistan (Tashkent) during 2004: roots and rhizomes during budding (Apr. 24), flowering (May 15), fruiting (June 30), seed ripening (Sept. 25), and end of vegetation (Nov. 15); leaves and flowers (May 6); and seeds (Sept. 25).

Polysaccharides were isolated from a single batch of raw material successively by water, oxalic-acid and ammoniumoxalate solution, and alkaline solution. Polysaccharides were precipitated from the extracts by alcohol to produce water-soluble polysaccharides (WSPS), pectinic substances (PS), and hemicellulose (HC), respectively.

Figure 1 shows the polysaccharide content in the various iris organs.

The results show that the polysaccharides (PSa) are distributed differently in the various plant organs. WSPS dominate quantitatively in the rhizomes whereas they are distributed almost evenly in the roots, leaves, and subterrean organs.

Iris rhizomes are used for medicinal purposes and contain a significant amount of WSPS and PS. The PSa content can vary depending on the season and plant development stage. Because of the practical interest in PSa and in order to determine the optimal time for collecting the plant raw material, their accumulation dynamics were studied. Table 1 gives the PSa yields.

Table 1 shows that the WSPS content in the rhizomes during all development periods was greater than in the roots, reached a maximum in September, and then decreased. The PS content in the roots during all development periods was greater than in rhizomes except during budding (Apr. 24). The WSPS were a white powder that was very soluble in water and formed nonviscous solutions. The hydrolysate consisted of the neutral sugars: rhamnose, arabinose, mannose, and galactose in a 4.8:1.9:1:10.2 ratio. Total carbohydrates were obtained in 10.5% yield from the air-dried raw material after isolation, precipitation of WSPS by alcohol from the aqueous-alcohol filtrate, and evaporation. Carbohydrates were dialyzed against distilled water. Paper chromatography of the dialysate detected glucose, fructose, and fructooligosaccharides. The undialyzed part was evaporated to a syrup and triturated with acetone to afford a powder, the hydrolysate of which contained mainly fructose and a trace of glucose. Therefore, this PSa was a glucofructan.

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Plant organ and collection time	WSPS, %	PS, %
Rhizome		
24-IV	2.1	9.5
15-V	9.1	4.5
30-VI	12.0	3.9
25-IX	18.5	4.1
15-XI	13.3	5.3
Root		
24-IV	0.44	6.8
15-V	0.9	5.9
30-VI	1.03	6.3
25-IX	1.2	7.0
15-XI	0.2	5.7
%		
30 -	28.7	

TABLE 1. Polysaccharide Accumulation Dynamics in Iris pseudacorus L.

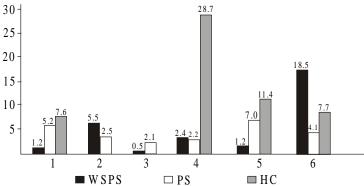


Fig. 1. Polysaccharide content in various organs of *Iris pseudacorus* L.: leaves (1), flowers (2), husk (3), seeds (4), root (5), rhizome (6).

The PS were an amorphous light brown powder that was soluble in water and practically insoluble in most organic solvents. The PS contained 1.9% methoxyls and had $[\alpha]_D^{20}$ +160° (*c* 0.5, H₂O). A molecular weight of 31,000 was determined by viscosimetry [3]. The ash content of the pectin was 2.3%.

The following properties of the pectin were found by titration (%) [4]: free carboxylic acids A_f , 10.8; methoxylated carboxylic acids A_e , 6.3; degree of esterification λ , 36.8. The product of pectin acid hydrolysis contained mainly galacturonic acid in addition to galactose, xylose, and arabinose, the ratio of which was 2.3:1:1.7, respectively.

The HC content was greatest in seeds. The hydrolysate of the HC contained only D-glucose and D-mannose in a 1:1.2 ratio that remained constant during fractionation of the PSa by Fehling solution. Therefore, the PSa was a glucomannan.

The HC was a fibrous white powder that was insoluble in water and soluble in aqueous alkali to form a viscous solution. Neutralization of the alkali solution with acid gave a gelatinous precipitate that was similar to other glucomannans and hemicelluloses.

Thus, the carbohydrates of I. pseudacorus contained WSPS, glucofructans, PS, HC, and glucomannans.

EXPERIMENTAL

Solutions were evaporated in vacuo at $45 \pm 5^{\circ}$ C. Ascending chromatography was performed on FN-1 and -11 paper using butan-1-ol:pyridine:water (6:4:3 v/v). Sugars were developed using anilinium biphthalate for 10 min at 105-110°C and alcoholic urea (5%) and were identified by markers. The ratio of sugar units was determined quantitatively by GC [5].

Seeds were separated from fruit. The main mass consisted of seeds (87.0%) and seed husks (13.0%). Plants were dried in air, ground, and sieved through a 0.5-mm sieve.

Before isolating PSa, ground air-dried specimens of roots (10 g); rhizomes, seeds, and leaves (10 g each); flowers (10 g), and seed husks (3.8 g) were treated at room temperature with $CHCl_3:C_2H_5OH$ (1:1) to extract pigments, lipids, and noncarbohydrate components and again dried in air.

WSPS, PS, and HC were isolated successively from a single batch of raw material by water at room temperature, oxalicacid and ammonium-oxalate solutions (1:1, 1.0%) at 70°C, and alkali (10% aqueous solution). The material was usually extracted twice, the first at a 1:10 ratio; the second, 1:5. PSa were precipitated from the aqueous, acidic, and basic extracts by alcohol (96°, 1:2). The isolated PSa were rinsed with alcohol (80° and then 96°) and dried to afford WSPS, PS, and HC, respectively.

Figure 1 and Table 1 give the yields. The individual sugar compositions of the fractions were determined by hydrolysis with H_2SO_4 (2 N) and analysis of the hydrolysates by paper chromatography and GC [5], using the aldononitrile acetates for the latter.

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