Sugars in *Iris pseudoacorus* L. seeds

W. MACIEJEWSKA-POTAPCZYK, M. SANIEWSKI, M. PELCZYŃSKA

Plant Physiology Department, Institute of Physiology and Cytology Łódź University, Poland

(Received: September 27, 1973.)

Abstract

Qualitative and rough quantitative analyses of sugars occurring during the successive developmental phases in two poles — micropylar and chalasal — of the seeds of *Iris pseudoacorus* L. were made. Comparing the sugar dynamics of the respective developmental phases distinct differences were found in mature and immature seeds. Soluble polysaccharides being an initial form of accumulation do not occur in ripe seeds. No differences were found in the contents of insoluble sugars in chalasal and micropylar poles. The endosperm and the seed coat differ in the level of some sugars.

INTRODUCTION

Konopska (1972) confirmed the differences in the endosperm of the chalasal and micropylar poles of *Iris pseudoacorus* demonstrated previously at the cellular and subcellular levels (Mikulska et al. 1967; Ryczkowski 1966). Her research deals with nitrogen and protein fractions, phosphorus compounds, ribonucleic acids (Konopska 1972) and the enzyme activities of RNase, DNase and GPase (Gabara, Konopska & Olszewska 1972).

There is no evidence in literature concerning sugars in the *Iris* seeds. Only Horton and Wolfrom (1963) mention the glucomannans occurring in the seeds of *Iris* sp. Bidwell, Krotkov and Reed (1952) found in the leaves of *Iris* sp. 1.1 % of glucose, 0.2 % of fructose, 0.6 % of sucrose and 0.04 % of xylose per fresh weight in the alcohol soluble fraction.

This paper is concerned with qualitative and rough quantitative analyses of sugars occurring during the successive developmental phases in two poles of the seeds of *Iris pseudoacorus*.

MATERIAL AND METHODS

The seeds of *Iris pseudoacorus* collected during III, IV and V development phases (see Olszewska and Gabara 1966) were fixed in 96 % ethanol. Then they were

cut in two parts in such a manner that the endosperm from the micropylar pole constituted about 2/3 of the length of this tissue and that of the chalasal pole 1/3. The seed coats were removed and analysed separately. Up to phase IV the endosperm was analysed together with the embryo which was extremely small. The embryo was removed only from dry seeds (phase V).

The micropylar pole endosperm and separately that of the chalasal pole were analysed. Endosperm tissues were homogenized in a porcelain mortar and the alcohol soluble sugars present in them were eluted with water. A residue was dried. 20 mg samples were hydrolysed in sealed glass ampoules in 1 N H_2SO_4 (1 ml) for 10 hours. Hydrolysates were neutralized with a saturated $\text{Ba}(\text{OH})_2$ solution (in 120°C). The sugars were separated in the way of one-dimensional descending chromatography using Whatman No 1 filter paper. To identify sugars and to compare their levels at the respective developmental phases and poles 1% standard solution and equal quantity of each sample were placed on the sheets of Whatman paper. The chromatograms were run in an equilibrated mixture of ethyl acetate : water : acetic acid — 3 : 3 : 1 (v/v/v) — upper layer and in ethyl acetate : water : acetic acid — 3 : 1 : 3 (E. Lederer, H. Lederer 1959).

The sugars were analysed analogously, namely the sugars soluble in alcohol and aqueous eluates of sugars which will be described at the results.

In order to identify the reducing sugars we used the spraying procedure applying anisidine phthalate (0,1 M p-anisidine solution and 0,1 M solution of a phthalic acid in 96% ethanol). After heating at temp 80°C for several minutes coloured spots appeared. Hexoses produce green spots, pentoses — red-violet, methylpentoses — yellow-green, uronic acids however — brown ones (Medwecka-Borkowska 1957).

To detect ketoses another spray was used additionally: 20% ortho-phosphoric acid and 0.2% alcohol solution of naphthoresorcine in relation 1:10. After drying at temperature $100\text{--}105^\circ\text{C}$ for 5—10 minutes the sugars containing a ketose — for instance fructose, sucrose or raffinose produce red spots. Blue coloured spots of aldoses and uronic acids appear until several hours. In the case of aldoses this spray is less sensitive than that of anisidine phthalate. The aldose spots are visible only at considerable sugar concentrations.

RESULTS

An alcohol extract from the seeds of *Iris pseudoacorus* at III development phase contained: glucose, fructose, sucrose, raffinose, stachyose and a large amount of sugar material at the starting line (Fig. 1 and 2).

For this reason the alcohol eluate after its previous evaporation to dryness was hydrolysed with 1 N H_2SO_4 in glass ampoules for 10 hours and then neutralized with a saturated $\text{Ba}(\text{OH})_2$ solution (in 120°C). This was placed on two chromatograms which were developed simultaneously in the system ethyl acetate : water : acetic acid (3 : 3 : 1) for 42 hours. Using anisidine phthalate we detected: rhamnose,

Fig. 1 and 2. Chromatographic partition of alcohol soluble sugars from *Iris pseudoacorus* seeds at III developmental phase (ethyl acetate : acetic acid : water — 3 : 1 : 3 (v/v/v — 42 hours)

Fig. 1. Chromatogram sprayed with anisidine phthalate

Fig. 2. Chromatogram sprayed with naphthoresorcin in o-phosphoric acid

A, B — varying quantities of material per spot; S — standards

ribose, xylose, arabinose and increased amounts of fructose and glucose (Fig. 3). 3 slightly visible unidentified spots appeared above the starting line. They could not be sugar polymers because they appeared also after 12 hours lasting hydrolysis.

Comparing the chromatograms of eluates prepared before (Fig. 1 and 2) and after hydrolysis (Fig. 3) it can be seen that during hydrolysis a break down of sucrose, raffinose, stachyose and of the spot localized at the start took place. Such sugars however as rhamnose, ribose, xylose, arabinose or increased amounts of fructose and glucose appeared.

After a qualitative determination of sugars contained in the alcohol extracts of seeds being at phase III we made a rough quantitative analysis of sugars present in equal volumes of fixing alcohol (corresponding to phases III, IV and V). The alcohol extracts of respective phases were placed on chromatograms side by side (Table 1).

The analysis of alcohol soluble sugars revealed large amounts of sugars readily soluble in alcohol like glucose, fructose, sucrose, raffinose, stachyose and of polysaccharides containing rhamnose, xylose, arabinose, glucose and fructose occurring

Fig. 3. Chromatographic partition of alcohol soluble sugars from *Iris pseudoacorus* seeds at III developmental phase after hydrolysis (ethyl acetate : acetic acid : water — 3 : 1 : 3 v/v/v — 42 hours). Chromatogram sprayed with anisidine phthalate

A, B, S — see Fig. 1 and 2

Table 1

Soluble sugar contents in endosperm of *Iris pseudoacorus* seeds at III, IV and V development phases

	Development phase	Rhamnose	Ribose	Xylose	Arabinose	Fructose	Glucose	Sucrose	Raffinose	Stachyose	Start
Sugars before hydrolysis	III	—	—	—	—	++	+++	+	++	++	+++
	IV	—	—	—	—	++	+++	+	++	++	+++
	V	—	—	—	—	—	+	+	—	—	—
Sugars after hydrolysis	III	+	+	+	++	+++	++++	—	—	—	—
	IV	++	+	+	++	+++	++++	—	—	—	—
	V	—	—	—	—	+	++	—	—	—	—

A relative quantitative estimation of sugars was made on the basis of visual comparison of the spots intensity:

- absent
- + traces
- ++ pale
- +++ medium
- ++++ much

in immature seeds (phase III and IV). In ripe seeds however (phase V) the concentration of alcohol soluble sugars decreases rapidly. Only slight amounts of glucose and sucrose were revealed.

Before the rough quantitative analysis of the alcohol insoluble sugars at the respective developmental phases and poles we identified the sugars occurring in the endosperm at phase III

After soluble sugars elution we hydrolysed the endosperm with 1 N H_2SO_4 . Galactose, glucose, mannose, fructose, arabinose, xylose, ribose and rhamnose were found in this hydrolysate (Fig. 4). An unidentified spot coloured with anisidine

Fig. 4. Chromatographic partition of insoluble sugars from *Iris pseudoacorus* seeds at III developmental phase after hydrolysis (ethyl acetate : acetic acid : water — 3 : 1 : 3 v/v/v — 42 hours). Chromatogram sprayed with anisidine phthalate.

A, B, S — see Fig. 1 and 2

phthalate appeared below maltose. Incomplete separation of fructose and mannose can be seen in Fig. 4 but the reaction with naphthoresorcine proves the presence of trace amounts of fructose.

Then the rough quantitative and qualitative analyses of the endosperm at the respective phases and poles were performed. Table 2 represents a comparison of sugar contents after hydrolysis in chalasal and micropylar poles at III, IV and V phases.

There are no distinct differences in the sugar contents in chalasal and micropylar poles, but they occur between V and III, IV phases of the seed development. Rhamnose, ribose and fructose are absent from phase V; galactose occurs in lower

Table 2

Insoluble sugar contents in endosperm of *Iris pseudoacorus* seeds at III, IV and V development phases (after hydrolysis). The estimation of contents see under Table 1.

Pole	Development phase	Rhamnose	Ribose	Xylose	Arabinose	Fructose	Mannose	Glucose	Galactose	Uronic acids
Chalasal	III	+	+	+	++	+	+++	++++	+++	+
	IV	+	+	+	++	+	+++	++++	+++	+
	V	—	—	+	++	—	++++	++++	++	+
Micro-pylar	III	+	+	+	++	+	+++	++++	+++	+
	IV	+	+	+	++	+	+++	++++	+++	+
	V	—	—	+	++	—	++++	++++	++	+

concentrations than at phases III and IV, mannose however in higher ones. Uronic acids producing violet-blue spots with naphthoresorcine were also revealed. They occur in both poles at all phases.

Seed coats were analysed analogously as endosperm. Table 3 illustrates the results of the seed coat rough quantitative analysis (after hydrolysis) at the respective developmental phases.

The same sugars which occur in endosperm were found in the seed coat but their concentrations were different. The level of mannose in the seed coat is very low;

Table 3

Insoluble sugar contents in seed coats of *Iris pseudoacorus* (after hydrolysis). The estimation of contents see under Table 1.

Development phase	Rhamnose	Ribose	Xylose	Arabinose	Fructose	Mannose	Glucose	Galactose	Uronic acids
III	+	+	++	+++	+	+	++++	++++	++
IV	+	+	++	+++	+	+	++++	++++	++
V	+	—	+++	++	—	+	++	++	++

in the endosperm however — it is high. The levels of xylose and arabinose in the seed coat were higher than those in the endosperm. At phase V the xylose level in the seed coat increases, that of arabinose however decreases. In the endosperm the levels of these sugars are similar at all developmental phases. In the seed coat the level of uronic acids is also higher. Xylose, arabinose and uronic acids are the main components of hemicelluloses building cell walls of the seed coat. The level of glucose is also different in the seed coat and in the endosperm of mature seeds (phase

V). It is low in the seed coat — high in the endosperm. (Cellulose cannot be taken under consideration because it has not been hydrolysed under conditions of our experiments).

DISCUSSION

Large amounts of the soluble sugars, namely: glucose, fructose, sucrose, raffinose, stachyose and polysaccharides, particularly fructosans were found at III and IV developmental phases of the *Iris pseudoacorus* seeds. After the latter fraction hydrolysis—apart from fructose—arabinose, xylose, ribose and rhamnose were revealed. In the ripe seeds however (phase V) the concentration of soluble sugars decreases, glucose and fructose occur in trace amounts.

Grzesiuk (1961) found that greatest quantities of soluble polysaccharides occur at the first stage of the grain development. At the second one (during the embryo development), they disappear gradually, at the third however they occur in trace amounts (apart from rye grains where they are always present in available quantities). These compounds, particularly containing fructose, are an initial form of the sugars accumulation and the main (besides sucrose) transportable product.

Johri's and Maheshwari's research (1966) on changes in the carbohydrates during the seed development in opium poppy has shown that the soluble carbohydrates increase extremely rapidly after pollination contributing about 60—70% of the total dry weight of the ovule and are the highest on the 5-th day on a per ovule basis. The amount is then approximately seven times that of starch and amylose. Later when the coenocytic endosperm turns cellular the soluble as insoluble carbohydrates decrease markedly and presumably a considerable portion of it is utilized in the synthesis of cell walls. After 13 days there is a slight increase in the amount of starch and amylose but simultaneously the soluble fraction decreases.

Comparing the sugar dynamic of the respective developmental phases of *Iris pseudoacorus* seeds a distinct difference in the ripe seeds (phase V) and III and IV maturing phases is visible. The soluble polysaccharides being an initial form of accumulation and reserve sugars are absent from phase V. Raffinose, fructose and rhamnose do not occur; the concentrations of galactose and free glucose decrease.

There are no detectable differences in the content of insoluble sugars occurring in both poles.

The concentrations of some sugars are different in endosperm and the seed coat. In endosperm glucose and mannose predominate, in the seed coat — glucose and galactose; mannose however occurs in slight amounts. The concentrations of xylose, arabinose and uronic acids are also higher in the seed coat than in the endosperm. These compounds are main components of the hemicelluloses utilized in the synthesis of cell walls.

REFERENCES

- Bidwell G. S., Krotkov G., Reed G. B., 1952. Paper chromatography of sugars in plants. *Can. J. Botany* 30: 291—305.
- Gabara B., Konopska L., Olszewska M. J., 1972. Aktywność i lokalizacja niektórych enzymów hydrolitycznych w trakcie rozwoju bielma *Iris pseudoacorus* L. *Acta Bot. Pol.* 41: 223—233.
- Grzesiuk S., 1961. Cukrowce dojrzewającego ziarna zbóż. *Rocz. Nauk. Roln.* 83 (A-4): 707—733.
- Horton D., Wolfson M. L., 1963. Polysaccharides [in:] *Comprehensive Biochemistry* vol. 5. Carbohydrates: Ed. by M. Florkin and E. H. Stoltz, Elsevier Publishing Company, Amsterdam—London—N. York, pp. 222—227.
- Johri M. M., Maheshwari S. C., 1966. Changes in the carbohydrates, proteins and nucleic acids during seed development in opium poppy. *Plant and Cell Physiology* 7: 35—47.
- Konopska L., 1972. Biochemical investigations on endosperm development in *Iris pseudoacorus* L. *Acta Soc. Bot. Pol.* 41: 369—383.
- Lederer E., Lederer M., 1957. *Chromatography*. Elsevier Publishing Company, Amsterdam—London—New York—Princeton, pp. 237—266.
- Medwecka-Borkowska I., 1957. Chromatografia bibułowa węglowodanów. (Ed. Opieńska-Blauth J. et al.) „Chromatografia”, PWN. Warszawa. s. 583.
- Mikulska E., Gabara B., Olszewska M. J., 1967. Ultrastruktura bielma jądrowego i komórkowego u *Iris pseudoacorus* L. we wczesnych okresach rozwoju. *Acta Soc. Bot. Pol.*, 36: 699.
- Olszewska M. J., Gabara B., 1966. Recherches sur les cytokinèses dans l'endosperme d'*Iris pseudoacorus* et d'*Iris sibirica*. I. Les cytokinèses au cours du développement de l'endosperme. *Acta Soc. Bot. Pol.* 35: 557—573.
- Ryzkowski M., 1966. Niektóre fizyko-chemiczne i fizjologiczne problemy rozwoju zalążka i zarodka (rośliny jedno i dwuliścienne). Kraków.

Authors's address

Prof. dr Wacława Maciejewska-Potapczyk
and M. Pelczyńska:

Institute of Physiology and Cytology, Łódź University,
Nowopółdniowa 12/16; 90-237 Łódź, Poland

Dr Marian Saniewski

Department of Ornamental Plants,

Institute of Pomology,

96-100 Skierniewice, Poland

Cukry w nasionach *Iris pseudoacorus* L.

Streszczenie

Przeprowadzono jakościową i półilościową analizę cukrów występujących podczas kolejnych faz rozwoju nasion *Iris pseudoacorus* w dwu biegunach: mikropylarnym i chalazalnym.

Porównując dynamikę cukrowców w poszczególnych fazach rozwoju nasion stwierdzono wyraźne różnice między nasionami dojrzałymi i niedojrzalymi. Pierwszą formą akumulacji są rozpuszczalne cukrowce, które nie występują już w nasionach dojrzałych. Nie stwierdzono różnic w zawartości cukrów nierozpuszczalnych między biegunem chalazalnym i mikropylarnym. Inny jest poziom niektórych cukrów w bielmie i w łupinie.