Studii şi Cercetări	Martie 2018	Biologie	27/1	120-123	Universitatea"Vasile Alecsandri" din Bacău

THE STUDY OF COMPOSITION IN SOLUBLE SUGARS AT HAPLOID GAMETOCLONES WITH GYNOGENETIC ORIGIN

Maria Prisecaru, Ionuț Stoica, Gabriel Alin Iosob, Maria Călin

Key words: *in vitro gynogenesis, haploid plants, soluble sugars, chromatographic and spectrophotometer analysis, quantitative dosing*

INTRODUCTION

Long time haploids available to higher plants were spontaneous, rare occurrences of "abnormalities" that may occur during sexual reproduction, or the results of the application of specific experimental procedures, almost all of which originated in the egg cell, or in male gametophyte cells. Androgenetic and experimental ginogenesis has made it possible to obtain haploids in many plant species that are used for the creation of new genotypes.

A common characteristics of all haploids appeared at superior plant species is represented by the low vitality and slow development due to the so called "haploid depression", state originated in the hemizigotic structure of haploids. Thus, in generally, on observe a diminution of some chemical components quantity, like alkaloids and volatile acids, diminish that is not always proportional with the ploidy degree.

Taking in consideration the fact that in the literature there are few citations for researches regarding the organic chemical composition of haploid plants obtained in "in vitro" conditions, comparatively with the origin forms and no citations regarding the soluble sugars compositions we aimedtoward these objectives at wheat (*Triticum aestivum* L.), sugar beet (*Beta vulgaris* L.), sunflower (*Helianthus annuus* L.) and potato (*Solanum tuberosum* L.), obtained from unfertilised ovaries and ovules through experimental gynogenesis.

The purpose of this study is a better understanding and knowledge of the biochemical transformations that occur during the development of haploids and a better integration of these plants in the breeding processes.

MATERIAL AND METHODS

The qualitative and quantitative chemical analyses were accomplished in fully developed plants, with an average length of 10-12 cm, obtained in culture "in vitro" directly from ovules or from callus with gynogenetic origin, after the estimation of chromosome number in the root meristems. As control we utilised 2n plants with the same length obtained through the germination of corresponding donor plant seeds. The plant material was first weight and dried in oven at 50^{o}C for 24 hours, and then reweighted for the determination of dry substance content and the drying efficiency.

The probs thus prepared were subjected to the qualitative chemical analysis. The quantitative analyses were accomplished in fresh material. The fresh plant material was first weighted and than subjected to extraction with methanol 70%.

The plant material, which represents the probes (haploid plants and diploid plants as control) in different quantities, varying between 0.850 and 3.907 g, was extracted with dichloromethane through repeated reflux.

The dichloromethane extracts were filtrated, keeping only the vegetal residue. After depletion with dichloromethane, the vegetal residues were subjected to extraction with methanol, at reflux, until the methanolic solutions became colourless. The methanolic extracts were concentrated through distillation and than processed for chromatographic analysis on thin layer. In order to accomplish this, the stationary stage – silica gel with binder *plaster of Paris*, was laid in thin batch on glass plate TCL 20 x 5cm.

Chromatography on paper. The hydrolyzed extract was chromatographed on Whatman 1 paper, the eluent being composed of the mixture: n-butyl-acetone-water alcohol (4: 1: 5) or n-butyl-pyridine-water alcohol (6: 4: 3). The discovery was performed with a solution of the periodic mixture of benzidine and diphenylamine-aniline, followed by heating to 105 ° C. The standards used were: galactose (GAL), glucose (GLU), fructose (FRU), arabinose (ARA), xylose (XIL), mannose (MAN) and sucrose (ZAH).

Thin layer chromatography. The aqueous extracts were subjected to the hydrolysis reaction and then chromatographed. As a support Kieselgel 60, Fertigplatten (10x20cm) was used; the n-butanol-acetone-acetic acid-water mixture (35: 35: 10: 20) was used as the eluent. The discovery was carried out by spraying with the periodic mixture of benzidine

and diphenylamine-aniline followed by heating at 105 ° C. The standards used were the same as in the case of paper chromatography.

Quantitative dosing. In its initial form, the quantitative analysis is represented through an appreciation of intensity of coloration in spot comparative with a scale of colours obtained based on spots with known sugar content (method Souchon and Granau, 1976).

The visual appreciation was compared with the spectrophotometer method. The etalon curves obtained through the photometring of sugars etalons at specific length wave, were utilised for the quantitative determinations by applying the low of Lambert- Beer:

A = ε bc, where:

A = absorbance;

ε = molar absorbtivity;

b = the thickness of absorbing layer;

c = concentration.

Each solution of sugars has a certain molar absorbtivity (epsilon) whose value was taken from the specialty tables. The curve thickness in which the measurement was accomplished was 1cm. The probes concentration was graphically determined, after the absorbance was read, according with the formula: **c = A / ε b (mol/ml).**

RESULTS AND DISCUSSIONS

From the aposs extract they determined the free oases resulting from hydrolysis. The aspects of the chromatograms are shown in Figures 1 and 2.

On **wheat** has been identified 5 sugars in the diploid plant at Rf corresponding to arabinose (0.64), mannose (0.63), fructose (0.63), galactose (0.48) and sucrose (0.46) were identified in wheat. In the haploid plant there are 3 spots corresponding to arabinose (0.64), mannose (0.63) and fructose (0.63).

All sugar used as a standard in the diploid plant, except for galactose, has been identified in **sugar beet**.

There is also a different spot from the standards used. Only 3 spots in the haploid plant are present, at Rf = 0.65 corresponding to xylose, Rf = 0.54 corresponding to glucose and Rf = 0.48 corresponding to galactose. And in this sample there is a different spot from the standards and is not present in the donor plant.

In the extract obtained from the **sunflower** diploid plant, 4 sugars were also found as standard: xylose (Rf = 0.65), arabinose (0.64), mannose (0.63), fructose (0.63). In the haploid plant, only 3 sugars present in the donor plant were identified with lower intensity spots at Rf = 0.65, corresponding to xylose, Rf = 0.63 mannose and Rf = 0.63 fructose.

The diploid plant **potato** has 5 spots corresponding to arabinox, mannose, fructose, galactose and sucrose on the chromatogram, while the haploid plant has only 4 less well-known spots corresponding to arabinoze, mannose, fructose, and sucrose.

Qualitative analyzes on samples of plant extracts from in vitro haploid plants and donor diploid plants were supplemented with quantitative analysis performed on thin layer chromatography and confirmed by spectral data. The results of the quantitative analysis are shown in Table 1, fig. 1.

It is found that in haploid plants, in all studied species, the sugar values are low, compared to the corresponding diploid plants. Interestingly, some sugar beet haploids have unidentified sugars in 2n donor plants.

Table 1 shows that in some haploid haploid chromosome sets and small cell size, so with low biosynthesis, the amount of sugar in the plant extract is almost equal to or even slightly exceeds the value of the donor plant. This is the case for fructose in wheat, sunflower and potato and sucrose wheat haploid.

Table 1. Comparative content of soluble sugars in haploid and diploid donor plants, expressed in values evaluated at the integrator for the corresponding spot

No	Sugars	Rf	Wheat		Sugar beet		Sunflower		Potato	
			2n	n	2n	n	2n	n	2n	n
1	Xylose	65	-	-	(14)	(9)	(11)	(4)	-	-
2	Arabinose	64	(31)	(21)	(22)	-	(9)	-	(14)	(7)
3	Mannose	63	(3)	(traces)	(8)	-	(2)	-	(10)	(4)
4	Fructose	63	(57)	(36)	(76)	-	(51)	(traces)	(64)	(36)
5	Glucose	54	-	-	(84)	(40)	-	(31)	(141)	-
6	Galactose	48	(41)	-	-	(12)	-	-	-	-
7	Sucrose	46	(62)	(41)	(93)	-	-	-	(72)	(36)
8	Other sugars	-	-	-	+	+	-	-	-	-

Legend: + = present; - = absent; ( ) = quantitative

Obs. = Rf values are valid for the elution systems: acetone-n-butanol-acetic acid-water.

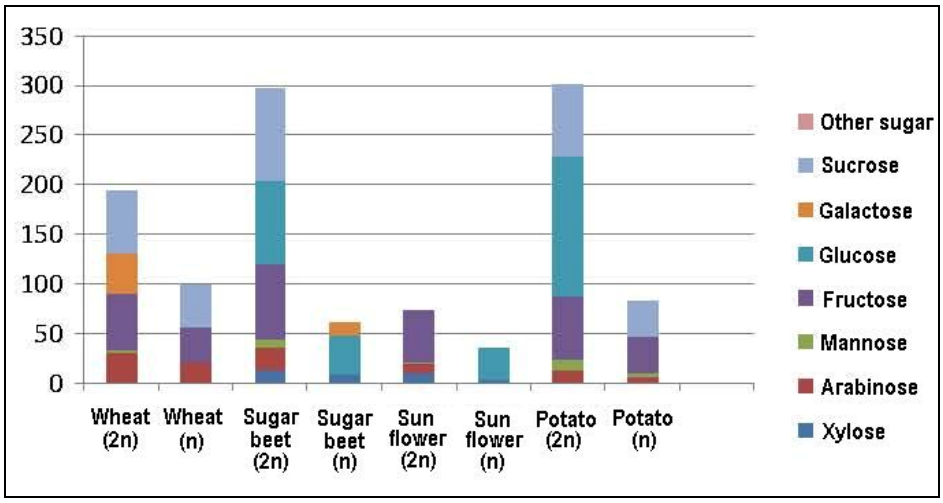

Fig. 1. Graphic representation of sugar content in haploid (n) plants and control plants (2n)

CONCLUSIONS

The results of the chromatographic examination and UV-VIS spectral analysis of the oases showed diminished proportions of the substances synthesized in haploid plants obtained in vitro compared to the donor diploid plants.

On chromatograms of haploid plants there is a lack of sugars identified in the donor diploid plants, but also the presence of new or synthesized sugars in a similar proportion or slightly exceeding the value recorded in the diploid plant.

It is possible that by doubling the number of chromosomes, some dihaploids may show improved performance in the production of sugars.

ABSTRACT

The soluble sugars that are present in the haploid plants of 10-12 cm length obtained by gynogenesis „in vitro" via the culture of nonfertilized ovaries and ovules have been identified and quantitatively measured in wheat (*Triticum aestivum* L.), sugarbeet (*Beta vulgaris* L.), sunflower (*Helianthus annuus* L.) and potato (*Solanum tuberosum* L.) by using the chromatographic method upon thin larger and UV-VIS spectrophotometry.

Diploid plants of the same length where used for control. They were obtained by germination of the donor plants seeds from where ovaries and ovules for cultivation in vitro were sampled. Thus, it is found that in the haploids obtained in vitro, for all studied species, the values of sugars are in general clearly low, as compared to the control diploid plants.

The haploid plants chromatograms show also the lack of some sugars identified in the control diploid plants. It is interesting to note that in some haploids new sugars appear, but they are not identified in the 2n plants, or in almost equal quantitative values, or higher that the 2n donor plant value (case of sugar beet).

REFERENCES

1. PRISECARU, M., CRISTEA, T.O., 2007 - Comparative study to induction of haploid plants by in vitro anther and ovule culture of tomato (*Lycopersicon esculentum* Mill.), Scientific St. and Researches. Biology, 12:41-43;
2. PRISECARU, M., CRISTEA, T.O., 2009 – In vitro gynogenesis in *Solanum tuberosum* L., Scientific St. and Researches. Biology ,16: 26-30;
3. PRISECARU, M., CRISTEA, T.O., 2009 - Induction of haploid plants from the female gametophyte of *Triticum aestivum* L., Scientific St. and Researches. Biology,16: 63-68;
4. PRISECARU, M., CRISTEA, T.O., 2011, Observations regarding the outturn of experimental gynogenesis at wheat (*Triticum aestivum* L.) and sugarbeet (*Beta vulgais* L.). Scientific St. and Researches. Biology, Vol. 1, ISSN: 1224 919 X ;
5. PRISECARU, M., CRISTEA, T.O., 2011, Observations regarding the outturn of experimental gynogenesis at sunflower (*Helianthus annuus* L.) and potato (*Solanum tuberosum* L.) Scientific St. and Researches. Biology, Vol. 1,ISSN: 1224 919 X;
6. PRISECARU MARIA, CRISTEA OANA TINA, 2014 - In vitro culture of unfertilized ovules in *Brassica oleracea* L., Scientific St. and Researches. Biology, 2014, Vol. 23, no. 1, ISSN: 1224 919 X, pg. 48-51;
7. PRISECARU, MARIA, CRISTEA. OANA TINA, 2012. Spectral analysis of major organic

compounds in wheat plants (*Triticum aestivum* L.), sugarbeet (*Beta vulgaris* L.), sunflower (*Helianthus anuus* L.) and potato (*Solanum tuberosum* L.) of gynogenetic origin. Scientific St. and Researches. Biology, Vol. 1, ISSN: 1224 919 X, pg.76-82;

8. PRISECARU, M., CRISTEA, T.O., CALIN, M., 2012. The study of composition in free aminoacids at haploid gametoclones with gynogenetic origin at *Ttriticum aestivum* L., *Beta vulgaris* L., *Helianthus annuus* L. and *Solanum tuberosum* L. Scientific St. and Researches. Biology Vol. 1, ISSN: 1224 919 X, pg.47-51;
9. PRISECARU M., GHIORGHITA G., 2002 – Haploidia experimentală în contextual biotehnologiilor modern (Experimental haploidy in context of modern biotechnology), Editura Tehnica, Bucurest (In Romanian).

AUTHORS' ADDRESS

PRISECARU MARIA, STOICA IONUȚ - „Vasile Alecsandri" University of Bacau, Faculty of Science, Department of Biology, Ecology and Environment Protection, Marasesti Street, no. 157, Romania, e-mail: prisecaru_maria@yahoo.com; ionut_stoica23@yahoo.com

IOSOB GABRIEL ALIN - Doctoral School - „Vasile Alecsandri" University of Bacau, Marasesti Street, No 157, Bacau, Romania, e-mail: iosob.gabriel@gmail.com;

CALIN MARIA - Vegetable Research and Development Station Bacau, Calea Barladului, No. 220, Bacau, code: 600388.