ACTA AGROBOTANICA
Vol. 48, z. 1 – 1995
75-81

Galactooligosaccharides of seeds during growth of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. beans

RYSZARD KOSSON

Research Institute of Vegetable Crops,
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

(Received: April 05, 1995)

Abstract

The quantitative changes of raffinose and stachyose in seeds of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. during their growth and maturation in a two year experiment were investigated. Trace amounts of raffinose were found in *Ph. vulgaris* seeds during their vegetative growth in 1990. Time of raffinose accumulation in seeds in 1991 began not earlier than on 33rd day since inflorescence. Stachyose started to accumulate in seeds between 33rd and 47th day after inflorescence of all tested cultivars. It was noticed that stachyose and raffinose contents in seeds of most tested cultivars after ten years of storage did not differ significantly when compared to not stored ones.

INTRODUCTION

The raffinose family saccharides are important from a nutritional point of view as they are known to be indigestible and hence flatulent. It was demonstrated by Shallenberger and Moyer (1961) for peas that the level of galactooligosaccharides increases during maturation of seeds. Tanusi (1972) found similar results for soybeans and remarked that in the cotyledons the oligosaccharides are gradually detected during growth in sequence: sucrose-raffinose-stachyose--verbascose. According to current knowledge on biosynthesis of these sugars the most important role is played by galactose, myoinositol, sucrose and galactinol (Dey, 1985). Castillo et al. (1990) showed that galactinol synthase and raffinose synthase are the key enzymes in the biosynthesis of raffinose family oligosaccharides. In young developing legume seeds of *Phaseolus vulgaris* (Gould and Greenshields, 1964), *Phaseolus lunatus* (Korytnyk and Metzler, 1962) it was observed that raffinose family of oligosaccharides do not appear in the tissue

until the onset of ripening. K a n d l e r and H o p f (1980) confirmed that in *Phaseolus vulgaris* seeds, raffinose oligosaccharides are formed de novo in the developing seeds and are broken down very rapidly during germination. They accumulate at the late stage of seed maturation as the seed desiccates towards dormancy and have been proposed to play roles in response of plants to cold and in seed desiccation (O v c h a r o v and K o s h e l e v, 1974; K a n d l e r and H o p f, 1980; S a r a v i t z et al., 1987).

It is important to find the beginning of galactosugar accumulation, specially in seeds of snap bean which is usually harvested after three weeks since inflorescence (W o y k e and G a b r y l, 1982) when the seeds are physiologically immature. If pods are harvested before beginning of galactosugar accumulation the bean does not cause flatulence after its ingestion.

The aim of the study reported here was the quantitation of raffinose and stachyose accumulation in developing seeds of *Phaseolus coccineus* and *Phaseolus vulgaris*. The effect of ten years of bean storage on raffinose and stachyose content in seeds was evaluated too.

MATERIAL AND METHODS

One cultivar of *Phaseolus coccineus* L., Piękny Jaś and two cultivars of *Phaseolus vulgaris* L., Justynka and Złota Saxa, were used in a 2 year experiment. Piękny Jaś and Justynka are known as cultivars grown for dry seeds and Złota Saxa cultivated for snap beans. Piękny Jaś is the only cultivar of *Phaseolus coccineus* grown in Poland. All three cultivars were grown on sandy-loam soil. No fertilization was applied. Seeds were sown on May 26, 1990 and May 8, 1991. Pods with seeds were harvested after 19, 33, 47, 64 days from inflorescence. Final harvest of pods of Piękny Jaś cultivar was done after 77 days. The weather conditions during growth period, namely, air temperature, rainfall and insolation are given in Table 1.

T a b l e 1

Some meteorological observations from the district of Skierniewice during vegetation season 1990 and 1991

Month	Air temperature (C degree)		Rainfall (mm)		Insolation (nr of sunny hours)	
	1990	1991	1990	1991	1990	1991
May	13.9	10.6	22.9	60.6	230.8	n.r.*
June	16.4	15.8	67.1	57.1	250.4	n.r.
July	18.2	19.4	83.3	59.7	240.3	n.r.
August	17.3	18.5	73.4	51.1	217.2	n.r.
September	13.4	14.9	46.8	29.8	165.5	n.r.

*n.r. – not recorded

The galactooligosaccharides of seeds – raffinose and stachyose – were quantitatively analyzed by high performance liquid chromatography according to early described method (K o s s o n, 1990). The only modification of the method applied was the use of Lichrosorb-NH_2 column in HPLC instead of Lichrosorb-NH_2 HCD column. In consequence of that the mobile phase had to be changed.

Solution acetonitrile:water, 55 : 45 v/v was replaced by a solution of acetonitrile and water of ratio 80 : 20 v/v.

Results were statistically analyzed by Student's „t" test and LSD were calculated at p = 0.05. D e a n and D i x o n (1951) test was also used in some statistical calculations.

RESULTS AND DISCUSSION

Results of accumulation of raffinose and stachyose in seeds during maturation are shown in Figures 1-4. Values of sugars contents are the averages of two to four replicates. The final harvest of mature pods with seeds of Justynka and Złota Saxa cultivars was done after 65 days, and for Piękny Jaś after 77 days from inflorescence. The trace amounts of raffinose (< 0.05 %) were found in *Phaseolus vulgaris* seeds of Justynka and Złota Saxa cultivars after 33, 47 and 65 days from inflorescence during growth in 1990 year (Fig. 1). In 1991 the *Phaseolus vulgaris* and *Phaseolus coccineus* seeds began to accumulate raffinose between 33rd and 47th day after inflorescence (Fig. 2). The highest concentration of raffinose in seeds of final harvest in 1990 and 1991 was found in *Phaseolus coccineus* bean. The further accumulation of raffinose in seeds during 4 months of storage (Fig. 1) was observed. In the stored seeds of snap bean Złota Saxa the trace amounts of raffinose were found.

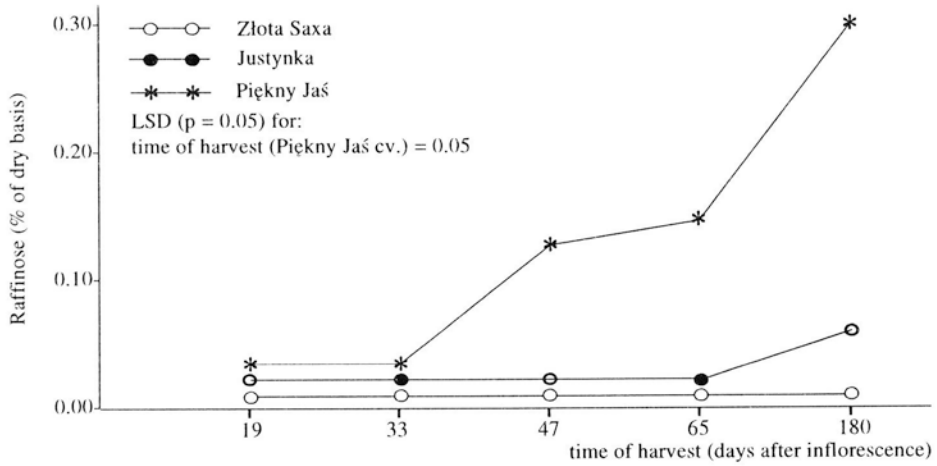

Fig. 1. Changes of raffinose content in seeds of *Phaseolus vulgaris* and *Phaseolus coccineus* during bean maturation (1990)

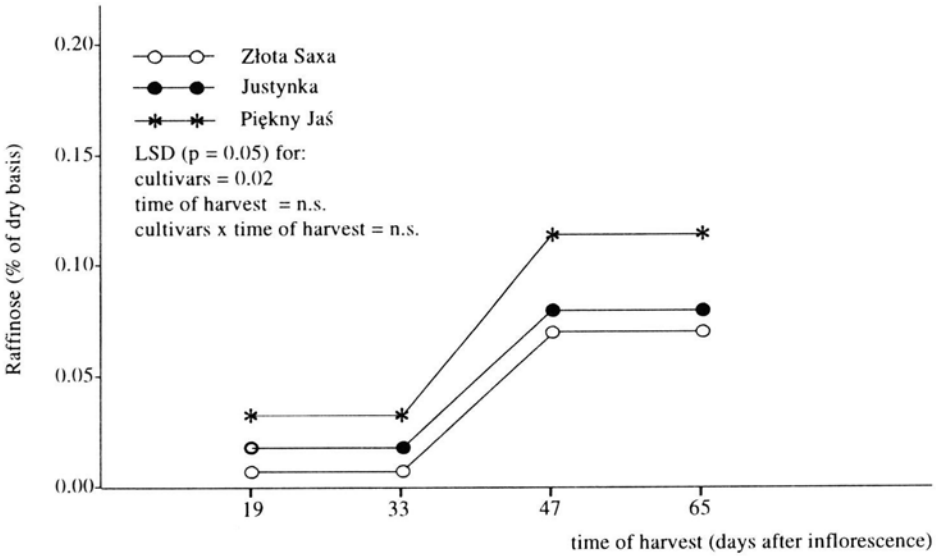

Fig. 2. Changes of raffinose content in seeds of *Phaseolus vulgaris* and *Phaseolus coccineus* during bean maturation (1991)

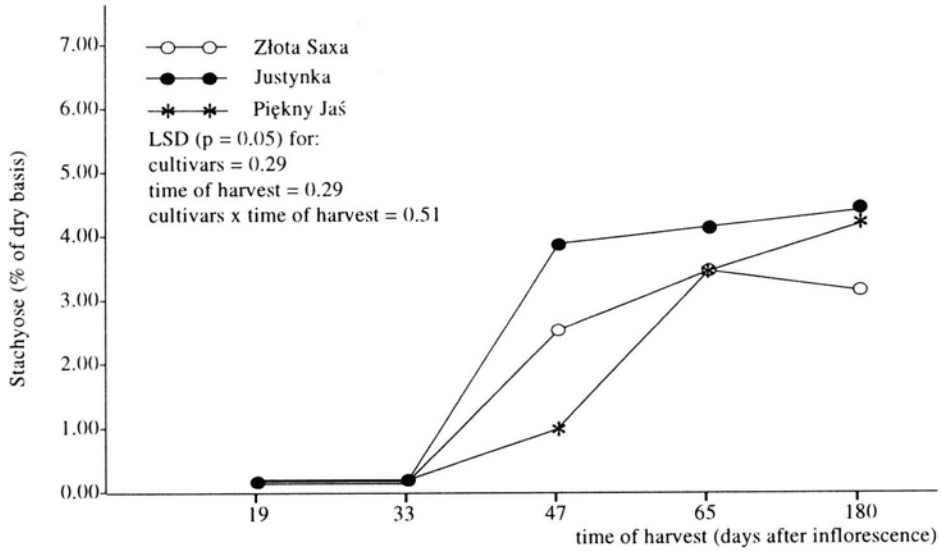

Fig. 3. Changes of stachyose content in seeds of *Phaseolus vulgaris* and *Phaseolus coccineus* during bean maturation (1990)

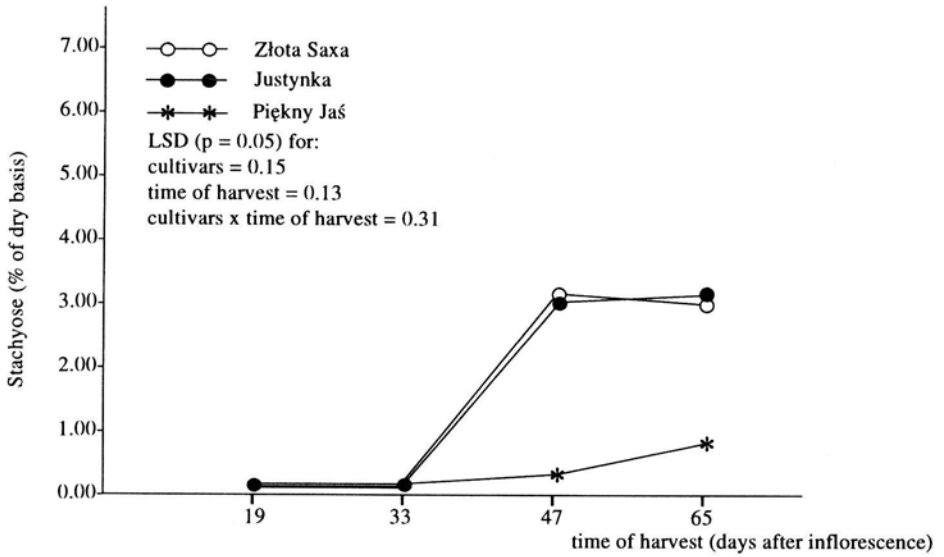

Fig. 4. Changes of stachyose content in seeds of *Phaseolus vulgaris* and *Phaseolus coccineus* during bean maturation (1991)

Accumulation of stachyose began in seeds of all cultivars in 1990 and 1991 between 33rd and 47th day from inflorescence (Fig. 3 and 4). In the case of *Phaseolus vulgaris* (Złota Saxa and Justynka) this increase was more rapid than in *Phaseolus coccineus* (Piękny Jaś). It can be caused by the fact that Piękny Jaś is a late cultivar (W o y k e and G a b r y l, 1982) and the accumulation of sugars is slower. During the final pods harvest (65th day) the stachyose content in seeds of *Phaseolus vulgaris* cultivars reached the level of about 3 % dry weight (Fig. 3 and 4). The pods of Złota Saxa cultivar are usually harvested between 15th and 25th day after inflorescence (W o y k e and G a b r y l, 1982), if cultivated for snap bean. Analysis of beans harvested at that time has shown the absence of raffinose and stachyose in seeds. The differences in dynamics of raffinose and stachyose accumulation in seeds grown in 1990 and 1991 could be affected by weather conditions, namely, higher temperatures and lower rainfall during vegetation in 1991 as compared to 1990 (Tab. 1).

G o u l d and G r e e n s h i e l d s (1964) have analyzed semi-quantitatively, by paper chromatography, the raffinose and stachyose formation in seeds of *Ph. vulgaris* beans. They found raffinose and stachyose in seeds after 50th day from inflorescence. K o r y t n y k and M e t z l e r (1962) have studied the raffinose and stachyose accumulation in lima beans (*Phaseolus lunatus*). These sugars were found in seeds in the third stage of maturity, namely, when the moisture content in seeds reached 74.35 % and weight of one seed – 1.07 g. No comparative publications for *Phaseolus coccineus* species were found in available literature.

The effect of long time storage (10 years) on raffinose and stachyose content in seeds of *Phaseolus vulgaris* is presented in Table 2.

Table 2

Effect of ten years storage on galactooligosaccharides content in bean seeds (in % of dry seeds)

Cultivar	Raffinose		Stachyose	
	not stored	stored	not stored	stored
Igołomska	0.15 ± 0.00	0.14 ± 0.00	3.17 ± 0.22	3.36 ± 0.01
Biała Wyborowa	0.42 ± 0.04	0.39 ± 0.14	2.04 ± 0.15	2.58 ± 0.10
Bomba	0.17 ± 0.04	0.23 ± 0.12	2.79 ± 0.53	2.78 ± 0.10
Słowianka	0.40 ± 0.03	0.30 ± 0.07	2.07 ± 0.23	2.39 ± 0.00
Wiejska	0.19± 0.01	0.08± 0.00	1.94 ± 0.08	3.05 ± 0.08

Data in Table 2 represents mean value + confidence limit at p = 0.95, according to Dean and Dixon test (1951).

Seeds of five Polish cultivars of bean were analyzed after harvest (as not stored) and after 10 years of storage. Seeds were stored in closed glass jars in temperature 0-5°C. The stored and not stored beans originated from different batches, and therefore results in Table 2 can be considered only as indication. Nevertheless, it was observed that the stachyose content in stored seeds of most bean cultivars did not differ significantly when compared to not stored ones. The raffinose content in seeds was found to be on the same level in stored and not stored bean – excluding Wiejska cultivar. The quantitative changes of contents of both galactosugars – raffinose and stachyose – were noticed in stored seeds of Wiejska cultivar when compared to not stored seeds.

REFERENCES

Castillo E. M., de Lumen B. O., Reyes P. S., Lumen H. Z., 1990. Raffinose synthase and galactinol synthase in developing seeds, and leaves of legumes. J. Agric. Food Chem. 38: 351-355.

Dean R. B., Dixon W. J., 1951. Simplified statistics for small numbers of observations. Anal. Chem. 23: 636-638.

Dey P. M., 1985. D-galactose containing oligosaccharides. [In:] Biochemistry of storage carbohydrates in green plants: Dey P. M., Dixon R. P. (eds.); Academic Press, New York, pp. 53-130.

Gould M. F., Greenshields R. N., 1964. Distribution and changes in the galactose-containing oligosaccharides in ripening and germinating bean seeds. Nature 202: 108-109.

Kandler O., Hopf H., 1980. Metabolism and function of oligosaccharides. [In:] Biochemistry of Plants Vol. 3. Carbohydrates; Preis J. (ed), Academic Press, New York, 231-270.

Korytnyk W., Metzler E., 1962. Formation of raffinose and stachyose in lima beans (*Phaseolus lunatus*). Nature 195: 616-617.

Kosson R., 1988. Flatulence-causing galactooligosaccharides of *Phaseolus coccineus* and *Phaseolus vulgaris*. Acta Soc. Bot. Pol. 57: 493-497.

Kosson R., 1992. Oznaczanie cukrów typu rafinozy w nasionach roślin strączkowych metodą wysokociśnieniowej chromatografii cieczowej-HPLC. Roczn. PZH, XLIII, 2: 179-185.

O v c h a r o v K. G., K o s h e l e v Y. P., 1974. Sugar content in corn seeds with different viability. Fiziol. Rast. 21: 969-974.
S a r a v i t z D. M., P h a r r D. M., C a r t e r T. E., 1987. Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes. Plant Physiol. 83: 185-189.
S h a l l e n b e r g e r R. S., M o y e r J. C., 1961. Relation between changes in glucose, fructose, galactose, sucrose and stachyose, and the formation of starch in peas. J. Agric. Food Chem. 9: 137-140.
T a n u s i S., 1972. Changes of carbohydrate contents of the soybean seed during growth (cotyledon, hull and hypocotyl). J. Jap. Soc. Food Nutr. 25: 89-93.
W o y k e H., G a b r y l J., 1982. Warzywa strączkowe. PWRiL, Warszawa.

Galaktooligosacharydy nasion w czasie wzrostu fasoli *Phaseolus coccineus* L. i *Phaseolus vulgaris* L.

S t r e s z c z e n i e

Badano ilościowe zmiany rafinozy i stachiozy w rozwijających się nasionach fasoli wielokwiatowej *Phaseolus coccineus* L. i fasoli zwykłej *Phaseolus vulgaris* L. W czasie wzrostu wegetacyjnego fasoli *Ph. vulgaris* w 1990 roku rafinozę wykrywano w nasionach tylko w śladowych ilościach. Początek gromadzenia się rafinozy w nasionach w 1991 roku przypadał na okres nie wcześniejszy niż przed 33-im dniem od momentu kwitnienia. Stachioza zaczęła się gromadzić w nasionach wszystkich badanych odmian fasoli pomiędzy 33 a 47 dniem od momentu kwitnienia. Nie stwierdzono statystycznie istotnych różnic w zawartości stachiozy i rafinozy w nasionach większości analizowanych odmian pomiędzy fasolą przechowywaną przez 10 lat a fasolą nie przechowywaną.