THE METABOLISM OF AGEING SEEDS. CHANGES IN THE RAFFINOSE FAMILY OLIGOSACCHARIDES DURING STORAGE OF FIELD BEAN (*VICIA FABA* VAR. *MINOR* HARZ) SEEDS

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ABSTRACT

Seeds of field bean cv. Nadwiślański harvested in 1980, 1986 and 1992 were studied. Results of investigations showed that the four analysed sugars (saccharose, verbascose, raffinose and stachyose) made up from 60.1 mg of 1 g dry matter of seeds harvested in 1992 to 67 mg of seeds collected in 1986. After three years of storage in laboratory conditions we observed a decline of the amount of these oligosaccharides. The saccharose:raffinose family oligosaccharides ratio grows with the seed age.

KEY WORDS: oligosaccharides, seeds, ageing, field bean.

INTRODUCTION

Legume seeds contain galactosylsucrose oligosaccharides, galactomannan and starch as their carbohydrate reserves (Bailey 1971). These seeds contain considerable amounts of carbohydrates, ranging from 35% for lupines, 55-60% for field bean, to 67% in peas (Cerning-Beroard and Filiatre 1976). If present, galactomannan is found in the endosperm, starch in the cotyledons and the raffinose series oligosaccharides in both parts (Matheson and Harpal 1977). Besides the direct protective aspects of carbohydrates, their accumulation contributes to the osmotic potential of the seeds (Ooms et al. 1993). Seeds vary in their composition of oligosaccharides and some accumulate α-galactosyl derivatives of cyclitols (Horbowicz and Obendorf 1994). Some species of Leguminosae accumulate mostly stachyose series oligosaccharides, whereas seeds of other species accumulate varying levels of galactosyl derivatives of cyclitols in addition (Horbowicz and Obendorf 1994).

Stachyose, raffinose and related flatulence-producing oligosaccharides are associated with desiccation tolerance and storability of seed germplasm (Crowe et al. 1980, Koster and Leopold 1988, Kermode 1990, Leopold 1990, Skriver and Mundy 1990, Bernal-Lugo et al. 1993, Sun and Leopold 1993, Horbowicz and Obendorf 1994, Still et al. 1994). One of the suggested functions of carbohydrates is membrane protection upon dehydratation (Madden et al. 1985, Crowe et al. 1986, Ooms et al. 1993). Hoekstra and Van Roekel (1988) and Crowe et al. (1989) have shown that withdrawal of molecules from the phospholipids can lead to membrane phase transitions at physiological temperatures. When water is available, this phase coincides with membrane leakage and cell death. The soluble sugars present in the maize embryo may serve as important components of protection or may contribute to the deteriorative changes occuring during seed storage (Leopold 1990). Examination of the changes in sugars during A-A test of maize corns indicates that the decline in vigour is associated with a marked decline in monosaccharides and in raffinose. Many authors have shown that decline of raffinose correlates with loss of vigour, viability and storability of seeds (Ovcharov and Koshelev 1974, Bernal-Lugo and Leopold 1992, Bernal-Lugo et al. 1993).

MATERIAL AND METHODS

The study was carried out on field bean seeds cv. Nadwiślański. The seeds for all the experimental variants were harvested at full ripeness, to be then dried and stored in linen bags at 18-20°C and mean air humidity of 55-65% (laboratory). Seeds of the same variety harvested in 1992 served as control. All samples of seeds were analyzed twice: on February 1993 and on February 1996.

The viability of the seeds was determined using the germination method according to the international ISTA rules (Anonymous 1976). Before germination the seeds was sterilized in a 3% solution of sodium hypochlorite for 3 minutes, after which they were washed sarefully in sterile water, its surface dried and placed in sterile Petri dishes containing a triple layer of Whatman No. 1 filter paper saturated with water. The seeds were germinated for 5 (rate of germination) or 14 days (determination of germination capacity).

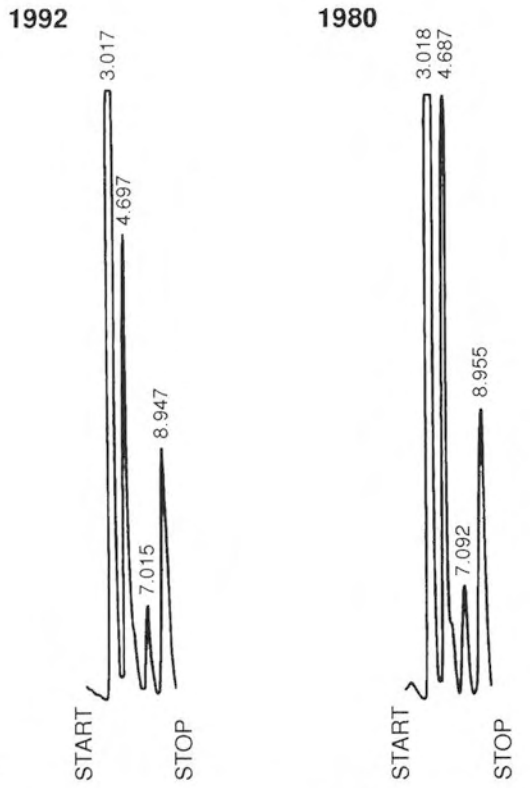

Fig. 1. Retention profile of oligosaccharides isolated from field bean seeds of different age.

Isolation and fractionation of sugars

Samples 2 g of seed were homogenized in 20 cm^3 of ethanol: water (80:20, v/v), refluxed for 30 min. at 95°C. Next the cooled homogenate was centrifuged at 12000 g. The supernatant was removed, and the pellet was used twice for additional extraction as before. The combined supernatants were evaporated to dryness at 40°C. The residue was dissolved in 1 cm^3 of deionized water and filtered through a 0.6 cm x 0.4 cm column containing Dowex 50W x 8 (H^+ form-1 cm^3, 200--400 mesh) and Dowex 2 x 8 (Cl^- form-1cm^3, 100-200 mesh, all from Flucka). The eluate was filtrated through ISO-DISC N-252 nylon membrane with 0.22 µm pore size (Supelco). Samples of 10-20 µl were injected into HPLC system consiting of Supelcosil LC-NH column (250 x 4.6 mm, Supelco), and solvent delivery module (Gc-LC system G-I, Shimadzu), comprising LC-6A pump and SCL-6B system controller. The mobile phase was acetonitryle: water (7:3, v/v, Merck) at a rate 1 cm^3/min. Soluble carbohydrates were detected by a RID-6A refractive index detector (Shimadzu). Quantities of saccharides were determined by extrapolation from standard curves (Sigma).

The total dehydrogenase activity was determined after 24 hours of imbibition of seeds in redistilled water at 20°C. The embryonic axes were isolated by hand and incubated in 0.7% tetrazolium chloride for 24 hours (29°C), after which they were homogenized in acetone in a porcelain mortar. After decantation the amount of formazane produced was determined.

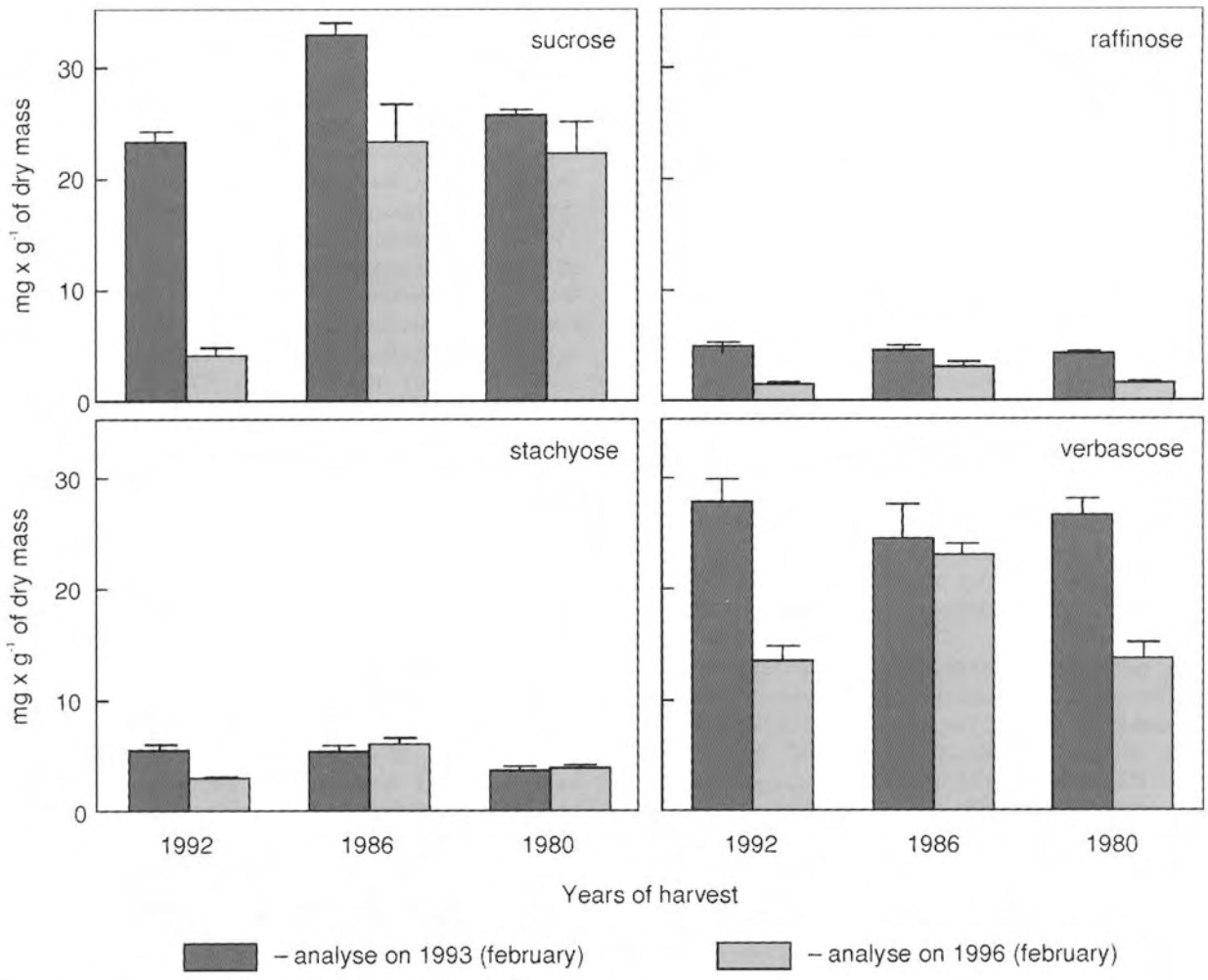

Fig. 2. Oligosaccharides composition in field bean seeds of different age.

RESULTS

Figure 1 shows sample segregation of oligosaccharides by the HPLC method. The results of the studies carried out in 1993 indicate that the four analysed sugars made up from 60.1 mg of 1 g dry matter of field bean seeds harvested in 1992 to 67 mg of seeds collected in 1986 (Fig. 2). Quantities of verbascose and sucrose in seeds were similar (25-29 mg/g), likewise the amounts of raffinose and stachyose (4-6 mg/g, Fig. 2). No relation between the levels of the oligosaccharides in seed and their age was found.

After three years of storage in a laboratory seeds were analysed by the same method as in 1993. For all the seed samples

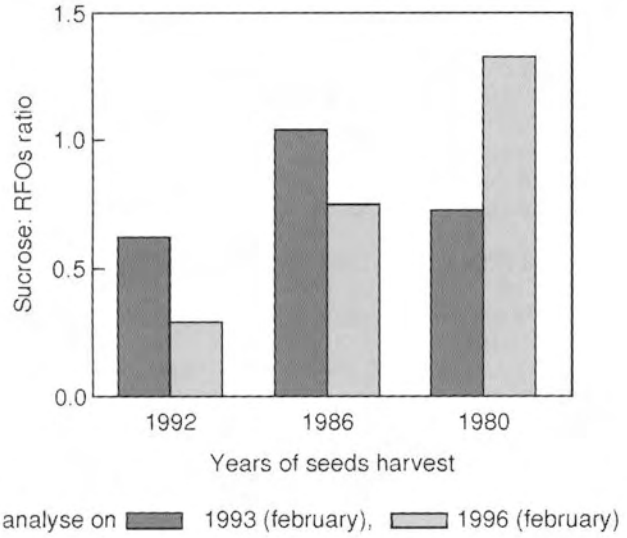

Fig. 3. The saccharose: raffinose family oligosaccharides ratio in field bean seeds of different age.

the analysis showed a decline in the amount of the oligosaccharides, the highest one for seeds collected in 1992 (60.1-22 mg), the lowest – for seeds of 1986 (67-55.6 mg). After storage the sucrose: raffinose family oligosaccharides ratio grew with the seed age (Fig. 3).

DISCUSSION

Seed vigour can be determinated by various methods. Commonly applied method include: the growth test and the analysis of dehydrogenase activity. The data present in Table 1 proves that both germination capacity in harvest year and the loss of viability by field bean seeds during storage depends primarily on biological value of seeds produced on the maternal plants. The data are in compliance with our earlier observation that germination capacity of field bean seeds stored in laboratory conditions for 5-6 years undergoes no significant modifications.

Carbohydrates are the primary storage materials in seeds of most species of cultivated plants. Seeds of leguminous plants, including field bean (Fig. 2), contain large amounts of saccharose and raffinose related oligosaccharides (raffinose, stachyose, verbascose; Górecki 1996). The compounds perform numerous/various physiological functions in plants, for instance they improve seed storability (Horbowicz and Obendorf 1994). The ratio between saccharose and other oligosaccharides, raffinose in particular, is of vital importance (Horbowicz and Obendorf 1994, Bernal-Lugo and Leopold 1995). Hydroxyl groups of saccharose and raffinose determine the glassy state of cytoplasm. The two sugars together with other oligosaccharides and cyclitols are capable of binding free radicals, which in turn protects cytomembranes from deterioration during extended storage. It can be safely concluded that the observed decrease in the quantities of oligosacharides in field bean seeds during their storage (Fig. 2) and the increase of the saccharose : raffinose ratio (Fig. 3) has an adverse effect on the physiological condition of cellular membranes. Free radicals, formed in seeds through autooxidation and enzymatic oxidation, are presently considered to be the primary cause of ageing (Wilson and McDonald 1986, Gidrol et al. 1989), as they are capable of inflicting damage to the structure of several important biopolymers, which leads to impaired structures and functions of cellular organelles and cytoplasmic membranes (Kulka 1994). It is currently assumed that this type of damage occurs mainly in protein and phospholipids which build up cell membranes. The view finds support in the findings of studies carried out on natural and artificial membranes in the recent years (Rubbo et al. 1994, Cadenas 1995, Wiśniewska and Subczynski 1995). Reduced levels of oligosaccharides, raffinose in particular, during the process of ageing, are likely to be responsible for unfavourable modifications observed in field bean seeds (Zalewski and Górecki 1993). However, it is not clear which of the two causes of ageing is the primary one, inducing other, secondary changes, adversly affecting seed viability and vigour. More and more data seem to support of the idea that carbohydrates are a primary factor contributing to seed longevity (Horbowicz and Obendorf 1994, Bernal-Lugo and Leopold 1995, Górecki et al. 1997).

TABLE 1. Viability and vigour of field bean seed of different age

Year of seed harvest	Germination capacity (%)			Rate of germination (%)	Conductivity of leachates*		Epicotile length* after 5 days of germination (mm)	Dry mass* of 25 sprouts after 5 days of germination (g)
	in harvest year	after 1-st period of storage	after 2-nd period of storage	after 2-nd period of storage	$\mu S \cdot cm\text{-}1 \cdot g^{-1}$	$S \cdot cm^{-1} \cdot 100$ seeds $^{-1}$		
1992	96.0	96.0	93.0	90.0	14.32	820	23.1	2.94
1986	98.1	97.5	92.0	84.5	16.23	687	22.6	2.36
1980	95.0	3.0	2.0	0.0	29.88	1699	–	–
LSD, p=0.01	3.55	4.92	2.17	3.26	3.10	108.4	0.011	0.246

*after 2-nd period of storage (on March 1996)

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STRESZCZENIE

Wyniki badań wykazały, że zawartość czterech analizowanych cukrów (sacharozy, werbaskozy, rafinozy i stachiozy) wynosi od 60.1 mg/g suchej masy nasion zebranych w 1992 roku do 67 mg w nasionach z roku 1986. Po trzech latach przechowywania w warunkach laboratoryjnych nastapiło obniżenie zawartości oligocukrów. Stosunek sacharoza : cukry rodziny rafinozy zwiększał się wraz z wiekiem nasion.

SŁOWA KLUCZOWE: oligocukry, nasiona, starzenie, bobik.