Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2009 7(4), 730-736
ISSN: 1695-971-X

The effect of irrigation, soil cultivation system and nitrogen fertilizer on the vitality and content of selected sugars in *Vicia faba* seed

D. Kurasiak-Popowska[1], J. Szukała[2] and K. Gulewicz[3]*

[1] *Department of Genetics and Plant Breeding. Poznań University of Life Sciences. Wojska Polskiego Str. 71C. 60-625 Poznań. Poland*
[2] *Department of Soil and Plant Cultivation. Poznań University of Life Sciences. Mazowiecka Str. 45/46. 60-625 Poznań. Poland*
[3] *Institute of Bioorganic Chemistry PAN. Noskowskiego Str. 12/14. 61-704 60-625 Poznań. Poland*

Abstract

In this study the influence of sprinkler irrigation, various soil cultivation systems (conventional, reduced tillage, zero tillage system) and the level (0, 30, 60, 90 kg N ha^{-1}) of nitrogen (N) fertilization on the vitality and content of selected sugars in faba bean seeds (*Vicia faba* L.) of the cultivar Nadwiślański was examined. Sprinkler irrigation of faba bean improved seed energy and germination in all three years of the study (1999-2001) —on average germination energy by 8.8% and total germination by 3.2%—. Germination of faba bean seed under conventional tillage in the drier years was significantly higher than in the zero tillage system. In the wetter year, seed from both simplified systems produced seeds with higher germination than in traditional conventional tillage. Nitrogen (N) fertilizer affected germination energy, but had no effect on faba bean germination. Sprinkler irrigation and N fertilization had no effect on the content of the sugars studied in the faba bean seed. However, the stachyose content of faba bean seeds from conventional tilled plants was significantly higher than in seed of zero tilled plants (0.78 mg g^{-1} seed dm), and the galactose content of seed from zero tilled plants was significantly higher than in the other two cultivation systems — 0.34 and 0.28 mg g^{-1} seed dm in seeds from conventional and reduced tillage system, respectively.

Additional key words: agronomic treatment, faba bean seeds, RFOs sugars.

Resumen

Efectos del regadío de primavera, del manejo del suelo y de la fertilización nitrogenada sobre la vitalidad de las semillas y el contenido de azúcares en *Vicia faba*

El objetivo de este estudio fue evaluar los efectos del riego por aspersión bajo diferentes sistemas de laboreo (tradicional, reducido o no laboreo) y con diferentes dosis (0, 30, 60 y 90 kg N ha^{-1}) de fertilización de nitrógeno, sobre la vitalidad y el contenido de azúcares en semillas de haba cv. Nadwislanski. Se observó que en los tres años del estudio (1999-2001) el riego mejoró la energía y capacidad de germinación de las semillas —de media la energía de la germinación un 8,8% y la germinación total un 3,2%—. La capacidad de germinación de las semillas de habas bajo laboreo tradicional en los años más secos fue significativamente mayor que sin laboreo. En el año más húmedo, las habas bajo laboreo reducido produjeron semillas con mayor capacidad de germinación que bajo laboreo tradicional. La fertilización nitrogenada produjo diferencias en la energía de la germinación pero no tuvo efecto sobre la capacidad de germinación. El riego y la fertilización de N no tuvieron efecto sobre el contenido de los azúcares estudiados en las semillas. Sin embargo, bajo laboreo tradicional, el contenido de estaquiosa en semillas fue significativamente más alto que sin laboreo (0,78 mg g^{-1} semillas dm), y el contenido de galactosa en semillas sin laboreo fue significativamente más alto que en los otros dos sistemas de laboreo (0,34 y 0,28 mg g^{-1} semillas dm en semillas cultivadas bajo laboreo tradicional y reducido, respectivamente).

Palabras clave adicionales: azúcares, OFR, semillas de habas, tratamientos agronómicos.

* Corresponding author: krysgul@ibch.poznan.pl
Received: 10-12-08; Accepted: 02-09-09.

Abbreviations used: Fru (fructose), Gal (galactose), Glu (glucose), Raff (raffinose), RFOs (raffinose family oligosaccharides), Rha (rhamnose), Sacch (sucrose), Stach (stachyose), Verb (verbascose).

Introduction

Raffinose family oligosaccharides (RFOs), also known as α-galactosides, are galactosylic derivatives of sucrose whose biosynthesis starts during seed development (Arentoft *et al*., 1993; Piotrowicz-Cieślak, 2005). Under natural conditions, an intense accumulation of RFOs occurs near the end of seed ripening (Blackman *et al*., 1992; Górecki *et al*., 2000). During legume pod fill sucrose and *myo*-inositol levels rapidly decrease, and accumulation of RFOs and galactinol start (Horbowicz *et al.,* 1995; Frias *et al*., 1996; Górecki *et al.*, 2000; Lahuta *et al*., 2005b).

The RFOs are important carbon reserves and are readily available on germination. They are considered to be stress metabolites during water deficit and cold (Keller and Pharr, 1996; Górecki *et al*., 1997a; Peterbauer and Richter, 2001; Pinheiro *et al*., 2005). High number of hydroxyl groups and high molecular mass are important for the osmoprotective functions of carbohydrates including the RFOs. Verbascose contains seventeen hydroxyl groups, and is an osmoprotectant with high molecular mass (828 Da) (Piotrowicz-Cieślak *et al.*, 2007).

According to the literature RFOs are a reserve material which accumulate in seed and fruits (Dey, 1985, 1990; Górecki *et al.,* 1997a,b). Further, they are responsible for the transport of nutrient components in the plant. They also increase tissue tolerance to low temperatures (Larsson *et al.,* 1993; Bachmann *et al*., 1994). Oligosaccharides give more stable intracellular glasses in dry seed than in mono- and disaccharides, and they correlate with acquisition of desiccation tolerance and seed longevity (Hoekstra *et al.,* 2001). Further, the RFOs detoxicate cells by binding free galactose. The decrease in monosaccharide concentration influences a reduction in respiration intensity and impediment of metabolic processes being a source of free radicals. On the other hand, a low level of cell fructose, glucose and galactose eliminates Maillard's reaction whose products contribute to degradation of enzymatic and structural proteins (Dey, 1985, 1990).

The hypothesis of this study was that agronomic treatment such as sprinkler irrigation, soil cultivation systems and N fertilizer can change the levels of sugars in faba bean seed. The aim of the research was to determine the effect of rainfall and sprinkler irrigation of three soil cultivation systems and four levels of N fertilizer on changes in selected sugars in the seed of the faba cultivar Nadwiślański.

Material and methods

The studies were carried out on faba bean (*Vicia faba* L. *minor* Harz.) seed harvested from a field experiments grown in 1999-2001 at the Experimental Farm Złotniki near Poznań (Poland) in a randomised block design with four replicates. The first factor was water – sprinkler irrigation or no irrigation; the second factor was soil cultivation system (conventional, reduced tillage or zero tillage system); the third factor was different N fertilizer levels (with N doses of 0, 30, 60 and 90 kg N ha^{-1}).

The duration of faba bean vegetative growth, without irrigation was: in 1999, 98 d; in 2000, 113 d; and in 2001, 117 d. Total rainfall was 216 mm, 201 mm and 256 mm, in the respective years. With sprinkler irrigation the faba bean vegetative duration was 110 d in 1999, 121 d in 2000 and 117 d in 2001. The sum of natural rainfall was respectively: 220.7 mm, 250.7 mm and 256 mm. On the sprinkler irrigated plots, in 1999-2000, irrigation was applied five times at 40 mm a time giving a total of 200 mm $year^{-1}$. In 2001, irrigation was applied three times in doses of 40 mm, 40 mm and 30 mm respectively; a total of 110 mm. The irrigation was applied during the period of highest plant sensitivity to water deficit (from the end of May to the end of July or first days of August —during budding, flowering and beginning of pods setting). The irrigation maintained soil moisture, in the 0-30 cm layer at approximately 70% of field capacity.

Laboratory analyses conducted included seedling value (parameters such as percentage of germination, energy of germination, seed purity, the incidence of seed-borne diseases) and the content of oligosaccharides in the faba bean seed. Energy and germination were determined on seed samples taken from plot. The RFO sugars and their concentration were determined using a mean sample from the four replicates. Germination energy and total germination were determined on 100 seeds from each plot. They were placed in a cuvette on filter paper, at 20°C. The energy value was taken at 5 d and total germination at 14 d following ISTA rules.

The α-galactosides were isolated from the seed according to the method of Muzquiz *et al.* (1992) with some modifications. Faba bean flour (0.5 g) was homogenized in 5 mL of 50% ethanol for 1 min at room temperature in an Ultraturrax homogenizer. The mixture was centrifuged for 5 min at 3,000 g. The supernatant was decanted and the procedure was repeated twice. The sample extract was purified using C_{18} cartridges

(500 mg/6 mL) connected to a vacuum system. The effluent was evaporated to dryness, redissolved in deionized water (1 mL) and centrifuged for 8 min at 6,000 g. Analysis of the RFOs was carried out on the supernatant by high performance liquid chromatography using a Beckman HPLC with a refraction index detector. For oligosaccharide separation a Spherisorb-5-NH_2 column (250 × 4.6 mm i.d.) and acetonitrile/water 60-40 (v/v) were used in the mobile phase. Solvents were filtered through a Millipore FH (0.45 μm) membrane and degassed under a vacuum. The injection volume was 20 μL. Quantification of each sugar was accomplished by comparing peak sample areas with a standard solution over the range of 0-4 mg mL^{-1} and the coefficients of determination above 0.99.

The results were statistically analyzed, using the variation analysis in STATPAK software. Significant differences was determined using the Tukey test at $\alpha = 0.05$. Variation analysis of germination was performed after conversion to Bliss values. Relationships among seed characteristics were determined using Pearson's correlation coefficient.

Results

Irrigation had no effect on germination energy in 1999. However, in 2000 and 2001 it was significantly increased (Table 1). In all three years sprinkler irrigation improved the total germination of faba bean seed and particularly in 2000. On average, sprinkler irrigation increased germination energy by 8.8% and total germination by 3.2%.

Overall cultivation systems had no significant effect on germination energy and total germination. Soil cultivation system had no effect on germination energy in 1999. However, in 2000, conventional tillage was significantly better than the other two systems. In year 2001, both simplified systems significantly improved germination energy compared with conventional tillage (Table 1). In 1999 and 2000 total germination was significantly higher in seed from conventionally tilled plants compared with zero tillage. It was different in 2001 when seeds from both simplified cultivation systems had significantly higher total germinations than seed from conventionally tilled plants.

On average, over the three years, the highest N dose significantly decreased germination energy compared with the three lower doses. However, there was no significant difference in total germination in response to N dose both for mean values and for all analyzed years.

Among the sugars studied, in all years, the highest content in faba bean seed was sucrose which was 45% of the total sugar content (average over all treatments). Among the oligosaccharides, the highest content, 15%, was stachyose. Verbascose was 10% and raffinose 4% of the total content of the sugars studied.

Table 1. Germination energy and total germination of faba bean seed depending on year of growth, irrigation, soil cultivation system and nitrogen fertilizer (%)

Factors	Germination energy				Total germination			
	1999	2000	2001	Mean	1999	2000	2001	Mean
Irrigation								
Sprinkler irrigation	85.6^{a}	91.7^{a}	93.4^{a}	90.3^{a}	96.6^{a}	98.3^{a}	96.6^{a}	97.2^{a}
No sprinkler irrigation	86.3^{a}	75.5^{b}	82.7^{b}	81.5^{b}	94.5^{b}	93.3^{b}	94.4^{a}	94.0^{b}
Tillage systems								
Conventional	87.1^{a}	86.8^{a}	85.5^{b}	86.4^{a}	96.6^{a}	97.0^{a}	94.0^{b}	95.9^{a}
Reduced tillage	85.3^{a}	82.8^{b}	89.7^{a}	85.9^{a}	95.3^{ab}	95.7^{ab}	96.3^{a}	95.7^{a}
Zero till	85.3^{a}	81.4^{b}	88.9^{a}	85.2^{a}	94.7^{b}	94.7^{b}	96.1^{a}	95.2^{a}
Nitrogen fertilizer (kg N ha^{-1})								
0	86.8^{b}	84.0^{b}	89.4^{a}	86.7^{a}	95.0^{a}	95.6^{a}	95.7^{a}	95.5^{a}
30	89.0^{a}	82.8^{bc}	87.2^{a}	86.3^{a}	95.8^{a}	96.7^{a}	95.0^{a}	95.8^{a}
60	83.8^{c}	87.3^{a}	88.1^{a}	86.4^{a}	95.9^{a}	95.5^{a}	95.6^{a}	95.6^{a}
90	84.2^{c}	80.5^{c}	87.5^{a}	84.1^{b}	95.5^{a}	95.6^{a}	95.4^{a}	95.5^{a}

Letters a-c indicate statistical significance at $p \leq 0.05$.

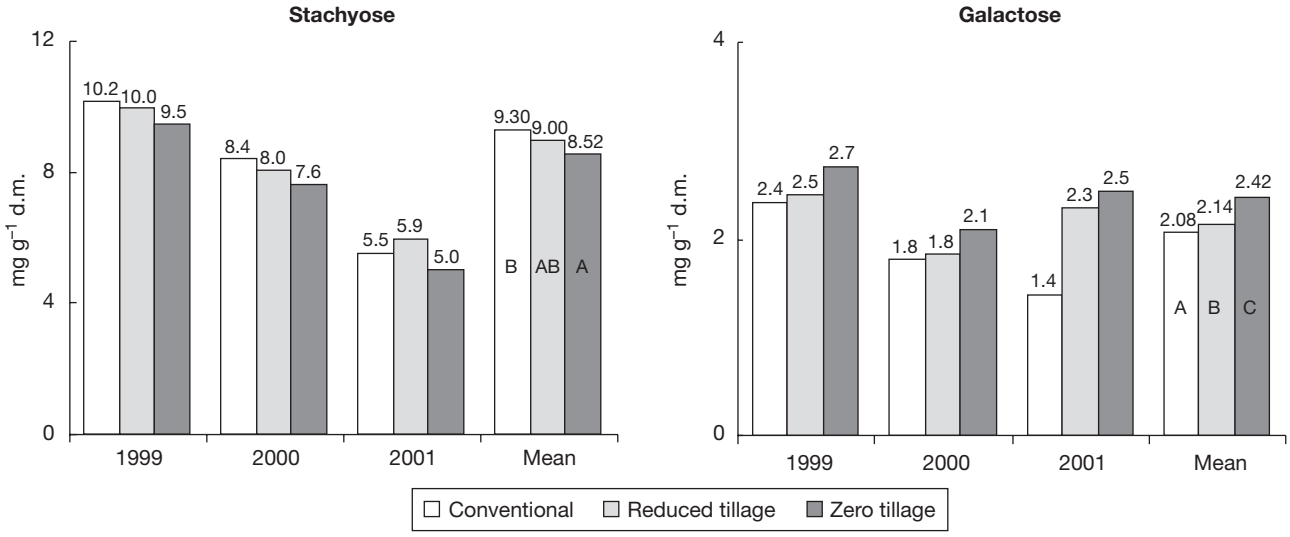

Figure 1. Effect of the tillage system on the content of stachyose and galactose in faba bean seed in 1999-2001 and the means of the 3-years.

On average, among the oligosaccharides analyzed, neither sprinkler irrigation nor N fertilizer had any effect on the content of the sugars analyzed. However, in all years, cultivation system significantly modified the galactose and stachyose content (Fig. 1). In the case of galactose, seed from zero cultivated plants contained 13% more of this sugar than seed from reduced till plants, and 16.3% more than seed from conventionally tilled plants. The reverse was observed for stachyose concentration, where seed from plants which had simple cultivation system, had a decreased stachyose content.

The fructose content of faba bean seeds depended on the interaction of water with N fertilization. With no irrigation increased N doses had no effect on fructose contents (Table 2). With irrigation, and 30 kg N ha^{-1}, compared with the control, there was a significant increase in seed fructose content. However, with 90 kg N ha^{-1} there was a significant decrease in fructose content —21.3%— in seed from irrigated plots compared with seed from unirrigated plots.

On the average, the highest seed galactose content was in seed from zero tilled plots. There were significantly lower contents in seed from traditionally and reduced till plots. Under irrigation, the lowest seed galactose content was in seed from conventionally tilled plots. There was a significantly higher galactose content in seed from reduced till plots, and the statistically highest galactose content was in seed from zero till plots (Table 2). With no irrigation, seeds from zero till plots contained significantly more galactose than seed from conventional and reduced till plots.

With conventional tillage seed from unirrigated plots had a significantly higher galactose level, 13.5%, than seed from irrigated plots. With reduced till, there was a trend for a higher galactose content in seed from unirrigated plots.

Pearson's linear correlation analyses between all analysed sugars are presented in Table 3, and one of the strongest connections is shown in Figure 2.

Table 2. Effect of the irrigation and nitrogen fertilization on fructose content and effect of the irrigation and soil cultivation system on the galactose content of faba bean seed (mg g^{-1} seed d.m.)

	Sprinkler	**No sprinkler**	**Mean**
Nitrogen fertilizer (kg N ha^{-1})/Fructose			
0	1.19^{Aa}	1.46^{Aa}	1.33^{A}
30	1.50^{Ba}	1.34^{Aa}	1.42^{A}
60	1.22^{ABa}	1.38^{Aa}	1.3^{A}
90	1.22^{ABa}	1.55^{Ab}	1.39^{A}
Tillage systems/Galactose			
Conventional	1.93^{Aa}	2.23^{Ab}	2.08^{A}
Reduced tillage	2.07^{Ba}	2.21^{Aa}	2.14^{A}
Zero till	2.41^{Ca}	2.42^{Ba}	2.42^{B}

In a column values followed by the same uppercase letter do not differ. Means in a row followed by the same lowercase letter do not differ at $p \leq 0.05$.

Table 3. Correlation coefficients between the parameters analyzed for faba bean seed

Compared indices	Total germination	Raff	Stach	Verb	Sum of RFOs	Rha	Glu	Fru	Gal	Sum of Glu+ Fru+Gal	Sacch	Inositol
Germination ability	1											
Raff	0.00^{A}*											
Stach	0.12^{A}	0.66^{C}										
Verb	0.15^{A}	0.80^{D}	0.73^{C}									
Sum of RFOs	0.17^{A}	0.84^{D}	0.92^{D}	0.93^{D}								
Rha	0.22^{B}	0.82^{D}	0.74^{C}	0.81^{D}	0.86^{D}							
Glu	0.31^{B}	$–0.13^{A}$	0.31^{B}	0.18^{A}	0.26^{B}	0.34^{B}						
Fru	$–0.16^{A}$	0.25^{B}	$–0.25^{B}$	$–0.19^{A}$	$–0.22^{B}$	$–0.05^{A}$	$–0.14^{A}$					
Gal	0.37^{B}	$–0.003^{A}$	0.34^{B}	0.21^{B}	0.31^{B}	0.56^{C}	0.42^{B}	$–0.08^{A}$				
Sum of Glu + Fru + Gal	0.32^{B}	$–0.05^{A}$	0.00^{A}	0.02^{A}	0.005^{A}	0.05^{A}	0.79^{D}	0.14^{A}	0.83^{D}			
Sacch	0.19^{A}	0.82^{D}	0.69^{C}	0.85^{D}	0.85^{D}	0.92^{D}	0.31^{B}	$–0.03^{A}$	0.36^{B}	0.03^{A}		
Inositol	0.18^{A}	0.75^{D}	0.62^{C}	0.74^{C}	0.75^{D}	0.8^{D}	0.24^{B}	0.01^{A}	0.18^{A}	$–0.05^{A}$	0.81^{D}	
Total sugars	0.05^{A}	0.83^{D}	0.79^{D}	0.90^{D}	0.92^{D}	0.94^{D}	0.45^{B}	$–0.09^{A}$	0.53^{C}	0.2^{A}	0.96^{E}	0.81^{D}

Raff: raffinose. Stach: stachyose. Verb: verbascose. RFOs: oligosaccharides family raffinose. Rha: rhamnose. Glu: glucose. Fru: fructose. Gal: galactose. Sacch: saccharose. A: $0 \le r < 0.2$, practically no connection between the features. B: $0.2 \le r < 0.5$, faint connection between the features. C: $0.5 \le r < 0.75$, medium connection. D: $0.75 \le r < 0.95$, strong connection. E: $0.95 \le r < 1$, practically functional connection.

Discussion

According to Horbowicz and Obendorf (1994), RFOs have a positive effect on the storing ability of seed generic resources. In this process, the proportion of sucrose to oligosaccharides is a significant factor. It is suggested that seed characterized by the proportion of sucrose to RFOs less than 1.0 lose 50% of their vitality after being stored for over 10 years. When the proportion is greater than 1.0, the period is significantly shorter. In this work, the proportion of sucrose to oligosaccharides was 1.6. Thus it can be suggested that these seeds may lose 50% of their vitality in storage in a period of significantly less than 10 years.

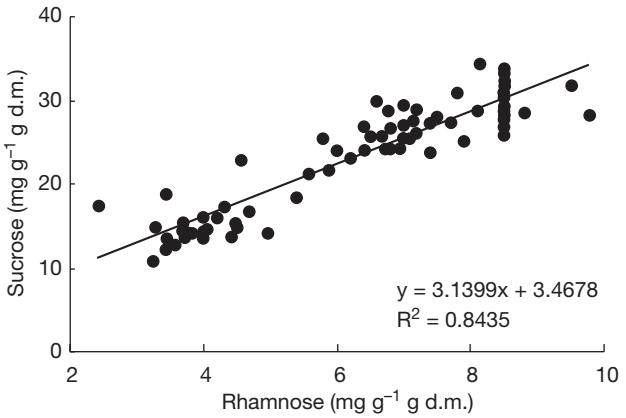

Figure 2. Relationship between the rhamnose and sucrose content of faba bean seed.

In seed of different legume species, a different content of raffinose homologues is observed. Prusinski (2001a, b) argued that in RFOs accumulation, synthesis of verbascose usually dominates, preceded by accumulation of raffinose and stachyose, which conditions the acquisition of tolerance to seed desiccation. Lahuta *et al.* (2005a) found that the dominant sugar of mature seed of some species of the genus *Vicia* genus was verbascose, while raffinose and stachyose occurred in smaller amounts. In this study, among the analyzed α-galactosides raffinose, stachyose and verbascose, the highest amount was stachyose – 8.94 mg g^{-1} (mean value for the three years), which makes it 53.2% of the RFOs studied. In this study, the total content of oligosaccharides in faba bean seed was variable over the years. It ranged from 7.98 mg g^{-1} in the moist year of 2001 to 18.33 mg g^{-1} in the dryer year of 1999.

The level of sucrose, raffinose and stachyose decreased during starch accumulation. In contrast, during seed ripening, and particularly at the beginning of desiccation there was a dynamic increase of the amount of seed verbascose. This was correlated with the formation of vigour of the ripening seeds (Lahuta *et al.,* 1993). In our study there was no correlation between verbascose content of mature faba bean seed and their vigour.

The results of this work indicate that neither sprinkler irrigation nor N fertilizer had a significant effect on the content of the sugars studied, including the oligosaccharides in faba bean seed. However, cultivation system did influence seed stachyose content. There was a significant increase of the content of this sugar with conventional tillage compared with zero tillage. There was a reverse, statistically confirmed, dependence in the case of galactose, where seed from zero tilled plants contained more galactose than those from conventionally tilled plants. Despite the fact that cultivation systems did not differentiate the germination ability of the faba bean seeds, in 1999 and 2000, conventional tillage was significantly more favourable for germination than zero tillage.

In conclusion, the data presented here show that sprinkler irrigation of faba bean over three years of study improved the germination energy and total germination. In dryer years, the total germination of faba bean seed, under conventional tillage, was significantly higher than in seed from zero tilled plants. On the other hand, in a moister year, seed from both simplified cultivation systems produced seed with a higher total germination than in from the conventional system. Nitrogen fertilizer at 0, 30, 60, 90 kg N ha^{-1} differentiated the germination energy, but had no effect on total germination. Sprinkler irrigation and N fertilizer had no effect on the content of the sugars studied sugars in the faba bean seed. The stachyose content of faba bean seed from plants grown under conventional tillage was significantly higher than in seed from direct seeded plants. On the other hand, the glucose content of seed from direct seeded plants was significantly higher than from the other two cultivation systems.

Acknowledgements

This work was financially supported by the State Committee for Scientific Research (KBN) Ministry of Science and Information Grant No. 5 PO6B 018 17.

References

ARENTOFT A.M., MICHAELSEN S., SORENSEN H., 1993. Determination of oligosaccharides by capillary zone electrophoresis. J Chromat A 652, 517-524. doi: 10.1016/0021-9673(93)83273-U.

BACHMANN M., MATILE F., KELLER F., 1994. Metabolism of the raffinose family oligosaccharides in leaves of *Ajuga reptans* L. Cold acclimation, translocation, and sink to source transition: discovery of chain elongation enzyme. Plant Physiol 105, 1335-1345.

BLACKMAN S.A., OBENDORF R.L., LEOPOLD A.C., 1992. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. Plant Physiol 100, 225-230. doi:10.1104/pp.100.1.225.

DEY P.M., 1985. D-Galactose containing oligosaccharides. In: Biochemistry of storage carbohydrates in green plants (Dey P.M., ed). Acad. Press, London. pp. 53-129.

DEY P.M., 1990. Oligosaccharides. In: Methods in plant biochemistry. Vol. 2. Carbohydrates (Dey P.M., ed). Acad. Press, New York. pp. 189-218.

FRIAS J., VIDAL-VALVERDE C., KOZLOWSKA H., GÓRECKI R., HONKE J., HANDLEY C.L., 1996. Evolution of soluble carbohydrates during the development of pea, faba bean and lupin seeds. Zlebensm Unters Forsch 203, 27-32. doi:10.1007/BF01267765

GÓRECKI R.J., PIOTROWICZ-CIEŚLAK A.I., LAHUTA L.B., OBENDORF R.L., 1997a. Soluble carbohydrates in desiccation tolerance of yellow lupin seeds during maturation and germination. Seed Sci Res 7, 107-115.

GÓRECKI R.J., PIOTROWICZ-CIEŚLAK A., OBENDORF R.L., 1997b. Soluble sugars and flatulence-producing oligosaccharides in maturing yellow lupin (*Lupinus luteus* L.) seeds. Seed Sci Res 7, 185-193.

GÓRECKI R.J., LAHUTA L.B., HEDLEY C., JONES A., 2000. Soluble sugars in maturing pea seeds of different lines in relation to desiccation tolerance. In: Seed biology: advances and applications (Black M., Bradford K.J., Vasquez-Ramos J., eds). Cab Intnl. pp. 67-74.

HOEKSTRA F.A., GOLOVINA E.A., BUITINK J., 2001. Mechanisms of plant desiccation tolerance. Trends Plant Sci 6(9), 431-438.

HORBOWICZ M., OBENDORF R.L., 1994. Seeds desiccation tolerance and storability: dependence on flatulence-producing oligosaccharides and cyclitols-review and survey. Seed Science Res 4, 385-405. doi:10.1017/S0960258500002440.

HORBOWICZ M., OBENDORF R.L., MC KERSIE B., VIANDS D.R., 1995. Soluble saccharides and cyclitols in alfalfa (*Medicago sativa* L.) somatic embryos, leaflets, and mature seeds. Plant Sci 109, 191-198. doi:10.1016/0168-9452(95)04155-N.

KELLER F., PHARR D.M., 1996. Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides. In: Photoassimilate distribution in plants and crops. Source-sink relationship (Zamski E., Schaffer A.A., eds). Marcel Dekker, Inc, NY. pp. 157-183.

LAHUTA L., REJOWSKI A., GÓRECKI R.J., 1993. Accumulation of carbohydrates during maturation of faba bean seeds [Gromadzenie węglowodanów w dojrzewających nasionach bobiku]. Konf. Nauk. Znaczenie jakości materiału siewnego w produkcji roślinnej, Warszawa. pp. 168-175. [In Polish].

LAHUTA L.B., GÓRECKI R.J., GOJŁO E., HORBOWICZ M., 2005a. Differences in accumulation in soluble α-galactosides during seed maturation of several

Vicia species. Acta Physiologiae Plantarum 27 (4A), 505-513.

LAHUTA L.B., HORBOWICZ M., GOJŁO E., GOSZCZYŃSKA J., GÓRECKI R.J., 2005b. Exogenously applied D-pinitol and D *chiro*-inositol modifies the accumulation of α-D-galactosides in developing tiny vetch [*Vicia hirsuta* (L.) S.F. Gray] seeds. Acta Soc Bot Pol 74(4), 287-296.

LARSSON S., JOHANSSON L.A., SVENNINGSSON M., 1993. Soluble sugars and membrane lipids in winter wheat (*Triticum aestivum* L.) during cold acclimation. Eur J Agron 1, 85-90.

MUZQUIZ M., REY C., CUADRADO C., FENWICK G.R., 1992**.** Effect of germination on the oligosaccharide content of lupin species. J Chromatogr 607**,** 349-352. doi:10.1016/0021-9673(92)87094-O.

PETERBAUER T., RICHTER A., 2001. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Sci Res 11, 185-197.

PINHEIRO C., RODRIGUES A.P., SARAIVA DE CARVALHO I., CHAVES M.M., RICARDO C.P., 2005. Sugar metabolism in developing lupin seeds is affected by a short-term water deficit. J Exp Bot 56(420), 2705-2712. doi:10.1093/jxb/eri263.

PIOTROWICZ-CIEŚLAK A.I., 2005. Composition of seed soluble carbohydrates and ultrastructural diversity of testa in lupins from the Mediterranean region. Acta Soc Bot Pol 74(4), 281-286.

PIOTROWICZ-CIEŚLAK A., MICHALCZYK D.J., ADOMAS B., GÓRECKI R.J., 2007. Different effects of soil drought on soluble carbohydrates of developing *Lupinus pilosus* and *Lupinus luteus* embryos. Acta Soc Bot Pol 76(2), 119-125.

PRUSIŃSKI J., 2001a. Plonowanie termoneutralnej i samokończącej odmiany łubinu żółtego (*Lupinus luteus* L.) przy zróżnicowanym terminie siewu i obsadzie roślin. [Yellow lupin (*Lupinus luteus* L.) thermoneutral and self-completing cultivar yielding for varied dates of sowing and plant density]. Fragm Agron 71, 123-138. [In Polish].

PRUSIŃSKI J., 2001b. Polowa zdolnośń wschodów roślin strączkowych. CZ. III. Właściwości biologiczne nasion. [Legume field emergence potential. Part III. Biological seed properties]. Fragm Agron 71, 139-160. [In Polish].