



## RESEARCH ARTICLE

# Raffinose family oligosaccharides in seeds of *Pisum* wild taxa, type lines for seed genes, domesticated and advanced breeding materials

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**Abstract** The content of raffinose family oligosaccharides (RFOs) in pea seeds constrains their usage in feeding humans and animals. In our research, the content of soluble carbohydrates—particularly  $\alpha$ -D-galactosides of sucrose (RFOs)—was analyzed. The materials were as follows: 248 accessions from the Polish *Pisum* Genebank including representatives of taxa (from species to convarietas), type lines for genes controlling seed characters, and breeding materials and cultivars. Accessions were divided into homogeneous groups considering content of total soluble carbohydrates, total RFOs and individual RFOs: raffinose, stachyose and verbascose. The highest content of total soluble carbohydrates and total RFOs were stated for accessions with wrinkled seeds (*r* and *rb* genes) and the lowest content for seeds of the wild species *P. fulvum*

Sibth. et Sm. Accessions valuable for breeding (for further decreasing of anti-nutritional compounds) were found among domesticated taxa (*P. sativum* subsp. *sativum* convar. *vulgare* Alef. and *speciosum* (Dierb.) Alef.), breeding lines, and some wild taxa. Accessions with decreased content of a total RFOs and verbascose are particularly valuable. It was found that the content of total RFOs was the most highly, frequently, and positively correlated with a stachyose and verbascose content. However, in *P. fulvum* seeds with the lowest content of RFOs and verbascose, a high correlation between the content of total RFOs and stachyose was revealed. Contents of all oligosaccharides were substantially lower in lines with dominant alleles of main pea seed genes (*R*, *A*, and *I*). It can be assumed that wild, primitive peas were characterized by not-all-to-high (rather not high) content of oligosaccharides; then recessive mutations in mentioned genes resulted in an increased content of RFOs. It seems to be an interesting observation from an evolutionary point of view.

**Keywords** Anti-nutritional compounds · Genetic resources valorization · Pea seed genes · *Pisum sativum* L. · Raffinose family oligosaccharides

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## Introduction

The pea (*Pisum sativum* L. sens. lat. Gov.) is the model plant in genetics (Świącicki et al. 2000). The

taxonomic system for a very rich natural variation was elaborated (Lehmann 1954). It covers following species: *P. formosum* (Stev.) Boiss. (now accepted as a separate genus *Vavilovia formosa* (Stev.) Boiss.—see Lock and Maxted 2005), *P. fulvum* Sibth. et Sm., *P. elatius* (M.B.) Stev., *P. abyssinicum* A. Braun, *P. syriacum* (Berger) Lehm. (*P. humile* Boiss. et Noe 1856 non Mill. 1768), *P. sativum* L. sens. lat. Gov. with subspecies *asiaticum* Gov., *trascasicum* Gov. and *sativum* (Clav.) Gov. Based on own studies Makasheva (1983) suggested that the genus *Pisum* L. consisted of the following species: *P. formosum* (Stev.) Alef., *P. fulvum* Sibth. et Sm., *P. syriacum* (Berger) Lehm. and *P. sativum* L. sensu amplissimo Gov. with the following subspecies: subsp. *trascasicum* Gov., subsp. *elatius* Schmalh, subsp. *asiaticum* Gov., subsp. *abyssinicum* Gov. and subsp. *sativum* Gov. Following a progressive research on the pea genetics, there is also an elaboration on characteristics of genes controlling a lot of plant characters—their expression and chromosome localization. A large coincidence of both systems of a variation classification was stated (Lehmann and Blixt 1984). A number of characters dividing the *Pisum* genus into lower taxa (from species to varietas) appeared to be controlled by single genes. A substantial contribution in this area had research results of Lamprecht (1974), Blixt (1972), and the *Pisum* Genetics Association activity. The achieved results enabled a precise description of resources gathered in gene banks, for example, in John Innes Institute, United Kingdom or in Poznan Plant Breeders Ltd. at Wiatrowo (PPB Ltd.). The pea is an important crop; fresh seeds are used as a vegetable and dry seeds as food for men and animals. So, better usage of genetic resources as initial breeding material depends on a valorization of qualitative characters. Collection databases cover most frequently passport and taxonomic data, sometimes monogenic characters and yielding structure. Qualitative characters are presented rather rarely (Kamel et al. 2015).

The use of pea seeds in human and animal nutrition is limited by the presence of anti-nutritive substances, and among them are  $\alpha$ -D-galactosides of sucrose (raffinose family of oligosaccharides, RFO). In the digestive tract of humans and monogastric animals,  $\alpha$ -D-galactosidase, an enzyme hydrolyzing the  $\alpha$ -D-(1 → 6)-glycosidic bonds between molecules of galactose moieties present in the oligosaccharides, is missing. Therefore, RFOs are digested with the

involvement of the bacterial microflora homing further sections of the intestine, especially the colon (Cummings and Englyst 1995; Southgate 1995). As a result of sugar decomposition and fermentation of releasing monosaccharides, excessive amounts of carbon dioxide and hydrogen are produced, causing flatulence and discomfort associated with this (Coon et al. 1990; Suarez et al. 1999). The increased content of RFOs in the feed which is used to feed monogastric livestock can cause diarrhea and reduce the use of energy contained in the feed (Liyang et al. 2003; Baker et al. 2010). Kuriyama and Mendel (1917) reported that 3–5 g of raffinose given to rats caused diarrhea and the presence of raffinose in stool. Inclusion of 4–8 % of the oligosaccharide extract of lupine seeds to a 24-h rat diets resulted in decreased absorption of glucose, methionine, and water (Pilar et al. 2001). In the feed containing a standard amount of soy (16 %), the content of oligosaccharides is approx. 8 g/kg. In the feed, in which soya bean was replaced by seeds of pea and lupine, the content of RFOs increased to 16–20 g/kg (Pilar et al. 2001). The benefits of consuming diet containing oligosaccharides were also noted. It is believed that oligosaccharides may act as prebiotics (Muzquiz et al. 2012); they can promote the growth of bifidobacteria populations, which in turn reduce diarrhea, stimulate the immune system, and increase the resistance to infection (Gibson et al. 2004).

A variation in content and qualitative composition of oligosaccharides in seeds of 70 pea accessions from John Innes *Pisum* germplasm collection was described (Jones et al. 1999). According to authors, a stachyose content was from 0.7 to 4.1 % of seed dry weight (g/100 g DW), but a verbascose content was from 3.1 % to a very low amount. Then Vidal-Valverde et al. (2003) analyzed a variation in 18 pea accessions originating from a Valladolid (Spain) germplasm collection. They described the following results: total  $\alpha$ -D-galactosides, 22.6–63.4 g/kg DW; stachyose, 10.7–26.7 g/kg DW; verbascose, 0.0–26.7 g/kg DW; raffinose, 4.1–10.3 g/kg DW; and sucrose, 11.6–25.4 g/kg DW. They also stated correlations between a brown color of seed coat and the lowest content of verbascose and sucrose and between seed size and a content of verbascose and total content of oligosaccharides.

The aim of our study was to analyze the content of total soluble carbohydrates, RFOs in particular (raffinose, stachyose, and verbascose), in accessions of Polish *Pisum* Genebank and advanced breeding materials.

## Materials and methods

About 248 accessions from the Polish *Pisum* Genebank at Wiatrowo and two breeding companies, Danko Ltd. and Poznan Plant Breeders Ltd. (PPB Ltd.), were selected. Accessions were grouped into 20 classes (Table 1). The important part was accessions representing main *Pisum* taxa according to Lehmann (1954)—species, subspecies, and convarietas. *P. abyssinicum* A. Braun., *P. elatius* (M.B.) Stev., *P. fulvum* Sibth. et Sm., *P. syriacum* (Berger) Lehm., and *P. sativum* subsp. *asiaticum* Gov. and subsp. *transcaucasicum* Gov. are wild and primitive (classes 2–7). *P. sativum* subsp. *sativum* convar. *axiphium* Alef. (edible pods), convar. *medullasaccharatum* (Körn.) Lehm. and *medullare* Alef. (gene *a*—without anthocyanin, gene *r*—wrinkled seeds, fresh seeds used as vegetable)—classes 8–10, convar. *vulgare* Alef. (without anthocyanin—gene *a*, smooth seeds—gene *R*, dry seeds used for food and feed)—class 11 and convar. *speciosum* (Dierb.) Alef. (fodder peas with anthocyanin, gene *A*)—class 12, all accessions are domesticated lines and cultivars. Classes 15–20 cover accessions with monogenic seed characters, that is, type lines for seed genes—size, seed coat,

cotyledons, hilum, and color (acc. to Blixt 1972 the typeline usually means the line in which the gene in question was first found). For comparison, advanced breeding lines and cultivars from two pea breeding companies (different breeding gene pools)—Danko Ltd., breeding station at Szelejewo (class 1: without anthocyanin, gene *a*), and PPB Ltd., breeding station at Wiatrowo (class 13: without anthocyanin, gene *a*; class 14: with anthocyanin, gene *A*)—were investigated.

The material was multiplied in a field experiment at Wiatrowo. The experiment was conducted in a completely randomized design in two replications, plot size of 1 m<sup>2</sup>, 100 seeds per plot. Whole plots were harvested in full maturity of each plot. Seed samples for analyses were collected: 100 g from each plot of both replications.

Soluble carbohydrates were extracted and analyzed using a gas chromatography method, described previously (Lahuta 2006). Seeds were homogenized in a mixer mill and soluble carbohydrates were extracted from 40 to 45 mg of meal with 800 µL of ethanol and water mixture (1:1, v/v, at 90 °C for 30 min), containing 100 µg of xylitol (Sigma, St. Louis, USA) as an internal standard. During extraction, samples were vigorously mixed (two times on vortex

**Table 1** Classes of investigated *Pisum* accessions and their number

Class no.	Name of class	Number of accessions
1.	Breeding lines from DANKO Ltd. (no anthocyanin, gene <i>a</i> )	49
2.	<i>P. abyssinicum</i>	3
3.	<i>P. elatius</i>	3
4.	<i>P. fulvum</i>	3
5.	<i>P. syriacum</i>	3
6.	<i>P. sativum</i> subsp. <i>asiaticum</i>	3
7.	<i>P. sativum</i> subsp. <i>transcaucasicum</i>	3
8.	<i>P. sativum</i> subsp. <i>sativum</i> convar. <i>axiphium</i>	2
9.	<i>P. sativum</i> subsp. <i>sativum</i> convar. <i>medullasaccharatum</i>	2
10.	<i>P. sativum</i> subsp. <i>sativum</i> convar. <i>medullare</i>	20
11.	<i>P. sativum</i> subsp. <i>sativum</i> convar. <i>vulgare</i>	23
12.	<i>P. sativum</i> subsp. <i>sativum</i> convar. <i>speciosum</i>	28
13.	Breeding lines from PPB Ltd. (no anthocyanin, gene <i>a</i> )	37
14.	Breeding lines from PPB Ltd. (with anthocyanin, gene <i>A</i> )	13
15.	Accessions with seed coat genes	10
16.	Accessions with seed size genes	4
17.	Accessions with seed coat and hilum genes	5
18.	Accessions with seed coat color genes	30
19.	Accessions with cotyledon color genes	4
20.	Accessions with cotyledon structure genes	3

at 3000 rpm for 20 s). After cooling to room temperature, samples were centrifuged (21 000 g for 20 min at 4 °C), and 400 µL of aliquots were deionized with a mixture (300 µL) of cation and anion exchange resins (1:1, v/v, both type Dowex, Sigma) for 45 min at 1200 rpm (vortex Genie 2, Scientific Industries, USA). After centrifugation (as mentioned above, for 10 min) 200 µL of clear aliquot was transferred to a gas chromatography 2 mL vials containing shell style inserts and dried in a speed vacuum rotary evaporator to dryness. Dry residues were derivatized with 200 µL of TMSI and pyridine mixture (Sigma) at 70 °C for 45 min. The TMS-derivatives of soluble carbohydrates were analyzed by high-resolution gas chromatography on a ZEBRON ZB-1 capillary column (15 m length, 0.25 mm diameter, and 0.1 µm film thickness, Phenomenex, Torrance, USA), using a gas chromatograph GC210 (Shimadzu, Japan). Carbohydrates were quantified by using standards: glucose, fructose, *myo*-inositol, sucrose, raffinose, stachyose (purchased from Sigma), verbascose (Megazyme, Australia), and galactinol (Research Industries, New Zealand). Carbohydrate content was calculated from standard curves of the appropriate component. Results of analyses presented as mg g<sup>-1</sup> of dry weight (DW) were means of three independent replicates ± standard error (SE). A single factor analysis of variance (Gomez and Gomez 1984) was applied to test hypotheses concerning the differences among the lines for studied oligosaccharides. Moreover, for particular oligosaccharides, the Gabriel procedure (Gabriel 1964) was used for the division of the sets of 20 classes of accessions into homogeneous groups. As a measure of the linear dependence between the content of total RFO and its three components separately, the correlation coefficients were calculated.

## Result and discussion

A very broad differentiation of the investigated 248 *Pisum* accessions was stated considering the total content of soluble carbohydrates—from 58.4 up to 241.3 mg/g DW (Table 2). Statistical analysis separated seven homogenous groups. The highest content was stated for accessions with genes *r* and *rb* causing wrinkled seeds (class 20) and for accessions belonging to convar. *medullosaccharatum* and *medullare*,

grouping wrinkled seed accessions also (classes 10 and 9). The *r* and *rb* loci of the pea are known to affect the starch content and composition of the seed (Wang and Hedley 1991; Wang et al. 1998). Both loci encode enzymes of the starch biosynthetic pathway (*R*—starch branching enzyme and *Rb*—ADP-glucose pyrophosphorylase, respectively) and are called “*rugosus*” (wrinkled) because seeds of homozygous recessive plants for either of these loci are wrinkled at maturity (Smith et al. 1989; Hedley et al. 1994). In addition, different mutations leading to disturbances in starch synthesis also affect starch composition—amylose to amylopectin ratio (Harrison et al. 1998; Hedley et al. 2002) and physical properties of the seed (Lyll et al. 2003). The mutations leading to disturbances in starch accumulation exert pleiotropic effects on the accumulation of other reserves. Seeds of homozygous alleles in *r* and *rb* loci (*rr rbrb*) accumulate higher amounts of lipids (Lyll et al. 2003), proteins (Hughes et al. 2001), and RFOs (Jones et al. 1999; Górecki et al. 2000). The increasing accumulation of soluble carbohydrate, including sucrose and RFOs in such mutants, is not surprisingly in regard to the fact that biosynthesis of RFOs occurs in the later stages of the seed development. Thus, the decrease in the accumulation of starch leads to a higher concentration of sucrose in cotyledon tissues (Borisjuk et al. 2003) and accumulation of higher amounts of RFOs (Karner et al. 2004). The role of sucrose in biosynthesis of RFO can be explained by at least in two ways. First, sucrose plays the role of the first galactosyl residue acceptor during synthesis of raffinose (Peterbauer et al. 2002). Second, sucrose is also a source of UDP-glucose, which after epimerization to UDP-galactose is used for synthesis of galactinol—mainly donor of galactosyl residues in RFOs biosynthetic pathway (Peterbauer and Richter 2001). In effect, elevated concentration of sucrose can stimulate the RFOs pathway via increasing the effectiveness of metabolic activity of appropriate enzymes. In seeds of pea cv. Kelvedon Wonder (with a mutation in starch branching enzyme, *rr* loci), synthesis of galactinol, is also increased by elevated concentration of *myo*-inositol (Karner et al. 2004).

The lowest content of soluble carbohydrates were stated in seeds of wild species *P. fulvum* (class 4). The content in remaining *Pisum* taxa appeared to be as follows. Wild taxa—*P. elatius*, *P. abyssinicum*, and *P. sativum* subsp. *asiaticum* and *transcaucasicum*

**Table 2** Homogeneous groups for the concentration of total soluble carbohydrates (TSCs), total RFOs, raffinose, stachyose, and verbascose, separately ( $\text{mg g}^{-1}$  DW) in *Pisum* seeds

Trait	Homogeneous group	Concentration			Classes
		Min	Max	Mean	
TSCs	1	163.5	241.3	209.7	20
	2	102.9	173.1	144.5	10, 9
	3	100.5	195.6	131.0	19
	4	69.0	134.2	96.2	18, 5, 16
	5	65.5	135.1	88.0	11, 12, 8, 15, 13
	6	58.4	100.2	83.0	3, 2, 6, 1, 14, 17, 7
	7	62.5	67.5	65.3	4
Total RFOs	1	121.6	177.6	157.4	20
	2	68.4	112.0	100.7	10
	3	60.0	140.0	90.0	9, 19
	4	63.7	103.3	79.0	16
	5	45.2	85.0	64.0	8, 18, 13, 5
	6	37.7	89.3	58.0	2, 6, 7, 17, 1, 11, 15, 3, 12, 14
	7	40.9	42.3	41.4	4
Raffinose	1	15.8	19.5	17.4	9, 20
	2	9.4	20.4	13.8	10
	3	8.0	14.9	10.2	13, 16
	4	6.3	12.6	8.8	5, 11, 14,
	5	3.6	15.4	7.9	18, 12, 8, 19, 2, 6, 3
	6	2.7	11.0	7.2	15, 1
	7	3.1	8.6	5.9	7, 17, 4
Stachyose	1	47.8	68.1	60.8	20
	2	24.9	47.7	37.8	10, 6, 5
	3	22.4	42.5	32.0	9, 13, 16
	4	17.4	47.2	28.0	14, 2, 19
	5	15.6	40.7	25.3	18, 4, 3
	6	11.8	35.2	22.3	15, 17, 1, 7, 8, 12, 11
Verbascope	1	57.5	91.6	78.5	20
	2	29.5	87.2	50.0	10, 19
	3	28.0	50.9	36.7	16, 9
	4	12.8	43.5	30.0	12, 17, 15, 8, 18
	5	8.3	46.3	27.2	11, 7, 1
	6	2.9	37.1	22.0	2, 5, 13, 14, 3
	7	3.2	14.1	9.3	6, 4

(classes 2, 3, 6, and 7, respectively) had a low content (the homogeneous group 6). Only *P. syriacum* (class 5) shows somewhat increased content (the group 4). Taxa covering domesticated accessions and cultivars (*P. sativum* subsp. *sativum* convar. *axiphium*—class 8, convar. *vulgare*—class 11 and convar. *speciosum*—class 12) with decreased content are present in homogeneous group 5. Decreased content (groups 5 and 6) has also breeding materials and cultivars—with

(gene *A*) and without (gene *a*) anthocyanin, originating from both, different breeding gene pools (classes 1, 13, and 14).

A broad differentiation in the RFO content, from 37.7 to 177.6  $\text{mg/g}^{-1}$  DW was stated. A division of seven classes was similar for a content of total soluble carbohydrates (TSCs) as well as for a content of total RFO (Table 2). Accessions in homogeneous groups 1 and 2 (classes 20 and 10 with wrinkled seeds) are

characterized by a highest RFO content. The lowest content of RFOs was stated in wild and primitive taxa—*P. fulvum*, but also *P. abyssinicum*, *P. elatius*, *P. sativum* subsp. *asiaticum* and *transcaucasicum*. To the homogeneous group 5 with somewhat higher content belong *P. syriacum* and *P. sativum* subsp. *sativum* convar. *aexiphium* accessions. Domesticated accessions (class 11: convar. *vulgare*; class 12: convar. *speciosum*) and cultivars from both breeding gene pools (classes 1, 13, and 14) have clearly lower content of RFOs (groups 6 and 5). The content of RFOs as % of total content of soluble carbohydrates oscillates from 59.6 to 75.3; mean, 68.9 (Table 3).

The results for individual RFOs (raffinose, stachyose, and verbascose) appeared to be similar but not identical. Domesticated taxa and cultivars presented most often a decreased content of all three, raffinose, stachyose, and verbascose. For example, breeding materials from class 1 are distinguished by low content of raffinose and stachyose (homogeneous groups 6 and

7), while those from classes 13 and 14 by low content of verbascose (homogenous group 6). The content of individual RFOs in wild taxa appeared to be as follows. The lowest content of raffinose was found in *P. fulvum* and *P. sativum* subsp. *transcaucasicum* accessions (homogenous group 7). The lowest content of stachyose (homogeneous group 6) was stated in *P. transcaucasicum* and *P. fulvum* + *P. elatius* (5 homogenous group). All wild taxa (excluding *P. sativum* subsp. *transcaucasicum*, 5 homogenous group) are found in 6 and 7 homogenous group with the lowest content of verbascose.

Classes 15–19 group accessions distinguished by monogenic seed characters (type lines for genes controlling seed shape, size, and color, hilum and seed coat color—Table 1). No correlations were found between lines and donors of seed genes and a content of analyzed oligosaccharides. But beneficial for consumers, a low content of total RFOs and verbascose was stated in type lines for genes *s* (49.3 mg g<sup>-1</sup> DW

**Table 3** Share of raffinose, stachyose, and verbascose in total RFOs (as %)

Class no.	Name of class	RFOs content		as % of total RFO		
		% of TSCs	Total (mg g <sup>-1</sup> DW)	Raffinose	Stachyose	Verbascope
1.	Danko Ltd., gene <i>a</i>	69.1	57.4	12.7	38.6	48.7
2.	<i>P. abyssinicum</i>	69.5	54.6	15.3	49.5	35.2
3.	<i>P. elatius</i>	75.0	58.0	14.6	45.7	39.7
4.	<i>P. fulvum</i>	63.5	41.4	15.0	61.6	23.4
5.	<i>P. syriacum</i>	68.7	67.5	12.7	56.8	30.5
6.	<i>P. sativum</i> subsp. <i>asiaticum</i>	66.4	55.0	15.1	67.6	17.3
7.	<i>P. sativum</i> subsp. <i>transcaucasicum</i>	65.8	55.7	10.5	40.5	48.9
8.	<i>P. sativum</i> subsp. <i>sativum</i> convar. <i>axiphium</i>	70.0	61.2	13.2	37.3	49.6
9.	convar. <i>medullasaccharatum</i>	59.6	87.3	19.2	36.5	44.3
10.	convar. <i>medullare</i>	69.9	100.7	13.7	37.6	48.7
11.	convar. <i>vulgare</i>	66.9	57.9	15.3	40.5	44.2
12.	convar. <i>speciosum</i>	68.4	59.6	13.2	38.1	48.7
13.	PPB Ltd., gene <i>a</i>	72.3	64.2	16.7	50.2	33.2
14.	PPB Ltd., gene <i>A</i>	71.3	59.8	15.0	45.9	39.1
15.	Accessions with seed coat genes	66.9	58.0	11.9	35.5	52.7
16.	Accessions with seed size genes	75.3	79.0	13.3	42.1	44.6
17.	Accessions with seed coat and hilum genes	67.9	57.3	10.2	36.8	53.0
18.	Accessions with seed coat color genes	67.4	63.6	12.3	39.8	47.8
19.	Accessions with cotyledon color genes	69.1	91.3	9.0	32.7	58.3
20.	Accessions with cotyledon structure genes	75.0	157.4	11.7	38.7	48.6

TSCs total soluble carbohydrates

and 15.0 mg g<sup>-1</sup> DW, for RFOs and verbascose, respectively), *oh* (45.2 and 13.8 mg mg g<sup>-1</sup> DW, respectively), and *Umb* (47.1 and 16.9 mg g<sup>-1</sup> DW, respectively). Obviously, an exception is a large number of accessions with wrinkled seeds (*r* and *rb* genes, classes 9, 10, and 20) which—as presented earlier—were characterized by a higher content of analyzed oligosaccharides in comparison to smooth-seeded accessions. Therefore, an additional, statistical analysis (t-Student test) was conducted to compare mean contents of analyzed oligosaccharides in groups of accessions differed by main three monogenic seed characters controlled by dominant and recessive alleles, respectively. Compared were lines with smooth seeds (*R* gene) to wrinkled seeds (*r* gene), lines with colored seed coat (*A* gene) to uncolored seed coat (*a* gene), and lines with yellow cotyledons (*I* gene) to green cotyledons (*i* gene) (Table 4). These divisions were done for accessions classified according to the *Pisum* taxonomy (classes 2–12). It appeared that contents of all oligosaccharides were substantially lower in lines with dominant alleles (*R*, *A*, and *I*, respectively). It can be assumed that wild, primitive

peas were characterized by not-all-to-high (rather not high) content of oligosaccharides; then recessive mutations in mentioned genes resulted in an increased content. It seems to be an interesting observation from an evolutionary point of view.

An estimation of a qualitative content of the RFO group allows for following remarks (Table 3). In accessions belonging to domesticated taxa, an average share of raffinose, stachyose, and verbascose in the total RFOs was 14.9, 38.0, and 47.1 %, respectively. Similar was a qualitative content in breeding gene pool of the class 1. But in materials from classes 13 and 14, a superiority of stachyose over verbascose was stated. It shows that the usage of different gene pools can result in qualitative differences. Similarly, in wild taxa, a stachyose predominates over verbascose, with an exception of *P. sativum* subsp. *transcaucasicum*.

In addition, the influence of the content of raffinose, stachyose, and verbascose on the content of RFOs in seed DW was investigated in seven homogenous groups. For individual pairs, “content of individual sugar—total RFOs—the Pearson correlation coefficient were estimated. Table 5 gives the mean content of

**Table 4** Comparison of the mean contents of soluble carbohydrates (TSCs), total RFOs and individual oligosaccharides (mg g<sup>-1</sup> DW) in groups differentiated by main pea seed characters

Oligo-saccharides	Mean	Variation coefficient (%)	Mean	Variation coefficient (%)	Difference	Least significant difference (0.01)
	Smooth seeds, gene <i>R</i>		Wrinkled seeds, gene <i>r</i>			
TSCs	85.13	14.65	146.25	24.75	61.12**	5.30
Total RFOs	57.87	16.49	101.98	28.01	44.11**	4.15
Raffinose	8.03	26.12	14.14	23.85	6.11**	0.61
Stachyose	24.76	29.57	38.49	29.41	13.72**	2.10
Verbascose	25.10	36.15	49.35	31.55	24.25**	2.74
	Colored seedcoat, gene <i>A</i>		Colorless seedcoat, gene <i>a</i>			
TSCs	84.64	12.83	95.78	26.26	11.14**	3.59
Total RFOs	58.36	14.27	66.37	26.37	8.01**	2.52
Raffinose	8.03	25.97	9.48	32.64	1.45**	0.46
Stachyose	25.93	29.94	27.58	27.91	1.65**	1.27
Verbascose	24.43	35.95	29.29	37.61	4.86**	1.71
	Yellow cotyledon, gene <i>I</i>		Green cotyledon, gene <i>i</i>			
TSCs	93.73	27.66	117.35	25.71	23.62**	7.18
Total RFOs	63.66	27.99	80.95	28.71	17.29**	5.09
Raffinose	8.92	35.88	11.16	32.53	2.25**	0.88
Stachyose	26.29	32.21	32.08	24.66	5.79**	2.23
Verbascose	28.48	42.82	37.70	35.13	9.22**	3.32

\*\* significant at  $P = 0.01$

**Table 5** Mean content of carbohydrates (mg g<sup>-1</sup> DW) in homogeneous groups and significant correlations between individual carbohydrate and sum of RFO

Homogeneous groups and (classes)	1 (20)	2 (10)	3 (9,19)	4 (16)	5 (8,18,13,5)	6 (2,6,7,17,1, 11,15,3,12,14)	7 (4)
<b>Concentration</b>							
Raffinose	18.2	13.8	10.8	10.2	9.1	7.8	6.3
Stachyose	60.8	37.7	30.0	33.4	29.2	23.3	25.5
Verbascope	78.4	49.2	49.1	35.4	25.8	26.6	9.6
<b>Correlations</b>							
Raffinose	0.76**	0.63**	0.38*	0.30	0.41**	0.06**	0.49*
Stachyose	0.94**	0.88**	0.94**	0.91**	0.44**	0.07*	0.94**
Verbascope	0.96**	0.87**	0.95**	0.80**	0.38**	0.08*	0.37

\* significant at  $P = 0.05$

\*\* significant at  $P = 0.01$

individual oligosaccharide (the mean content of total RFO is given in Table 2) and the correlation coefficients of those sugars with the biggest positive (substantial at the level 0.01) influence on the total RFOs.

Data show that of total RFOs is highly, positively, and substantially correlated (in six groups, excluding the homogenous group 6) with stachyose and verbascope. It underlines an importance of an accumulation of these oligosaccharides on a total RFOs content. In the homogenous group 7 (*P. fulvum*: class 4) with the lowest content of total RFOs and verbascope, a high correlation was revealed between a total RFOs and stachyose content and no correlation with verbascope content. It can be assumed that mutations leading to a decrease of verbascope content can also cause a decrease of total RFOs content.

Above data show a decreased content of the raffinose family of oligosaccharides, RFOs in domesticated taxa, and advanced breeding material. In the homogenous groups 6 and 7 with the lowest RFOs content, accessions useful for further decreasing of a level of these anti-nutritional compounds can be found. These are not only domesticated genotypes, but also wild taxa, excluding *P. fulvum*. This species confirms own distinctness of being almost always in the homogenous group with the lowest content of a sum of RFO and individual oligosaccharide. Unfortunately, its usage in *P. sativum* breeding is not recommended because of different chromosome structure and partial sterility of segregating hybrid progenies.

Particularly valuable, in terms of lowering flatulence, seems to be accessions with reduced levels of RFO. RFOs, with a linear molecular structure, are much better used by a number of probiotic bacteria than other prebiotic oligo- and polysaccharides, containing glucose and fructose bonded via  $\beta$ -linkages and/or branched molecules (Mei et al. 2011). In addition, the rate of gas formation due to fermentation of RFO is correlated to the rate of release of monosaccharides from RFO (Rackis 1981). Therefore, the decrease in the content of RFOs in seeds, especially by weakening the accumulation verbascope (containing more linearly connected galactose residues than in stachyose and raffinose), can contribute to the reduction of anti-nutritive properties of peas. A valuable initial material for breeding are lines with the lowest content of RFOs (below 50 mg g<sup>-1</sup> DW). They are among wild accessions (class 2—Wt 2, class 6—Wt 502, class 7—Wt 810) as well as breeding gene pool (class 1—SZD 175, class 14—cv. Sokolik and class 17—Wt 2902, Wt 2915). Also valuable are lines with decreased content of RFOs (below 60 mg g<sup>-1</sup> DW) and particularly low content of verbascope (below 10 mg g<sup>-1</sup> DW)—class 2—Wt 10, class 3—Wt 102, class 6—Wt 506 and Wt 507.

An interesting result of conducted analyses was that groups of accessions with wild/dominant alleles of three main *Pisum* seed genes (*I*, *A*, *R*) were characterized by lower content of all analyzed oligosaccharides in comparison to accessions with recessive alleles of these genes.



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