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Original Article

PROTEOMIC ANALYSIS OF POLLEN AND BLOSSOM HONEY FROM RAPE SEED *BRASSICA NAPUS* L.

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Abstract

In the study, honey from oilseed rape *Brassica napus* L., and both hand-collected (winter rape Visby and Cult) and bee-collected pollen of oilseed rape were analyzed for their proteome content, in order to see if any plant proteins were present to allow the proteo-typing of the oilseed rape honey. Proteins were fractionated by two-dimensional gel electrophoresis (2DE), stained by Coomassie blue and then analyzed by mass spectrometry. All identified proteins were divided into few groups due to their biological function. In 2DE gels with separated proteins from blossom honey, only bee (*Apis mellifera*) main proteins (Major royal jelly protein 1-5 and Glucosidase) were found. So we analyzed all proteins using gel-free based analysis with the SYNAPT G2 high definition mass spectrometry. We identified proteins that were present in both oilseed rape pollen and honey (Bna, Polygalacturonase, Non-specific lipid-transfer protein, GAPDH and others). We believe that these proteins are important for the nutritional value of plant pollen-enriched honey and further research is required on honey and honeybee pollen protein.

Keywords: *Brasica napus* L., honey, honeybee pollen, proteomics, rape

INTRODUCTION

In recent years, there has been an increased demand for honeybee pollen as a healthy food supplement. The content of protein, fat, phosphorus, iron and vitamins (E, D, B_{12}) makes pollen's nutritive value comparable to dried beans or beef (Erlund, 2004). The unique properties of pollen were one of the reasons for using it as a pharmaceutical preparation. It was also important whether pollen was gathered by hand directly from the flower or by bees which is particularly rich in vitamins with a far higher concentration of pantothenic acid. The German Federal Board of Health has officially recognized the use of pollen as medicine; pollen has been helpful when administered in cases of chronic prostatitis (Cai et al., 2014; Wagenlehner et al., 2009).

Some chronic diseases such as cancer, coronary, and neurological degeneration have been reported to be a consequence of oxidative damage (Babizhayev, Vishnyakova, & Yegorov, 2014; Jiang, Sun, & Chen, 2016). The therapeutic potential of honey is almost always associated with the antioxidant capacity against reactive oxygen species (Ferreira et al., 2009). Therefore, in recent years, studies have been focused on the composition of honeys and their biological properties such as antioxidant, anti-inflammatory and antimicrobial activities in wound healing, as well as in the treatment of

skin ulcers and gastrointestinal disorders (Al-Mamary, Al-Meeri, & Al-Habori, 2002; Tonks et al., 2003; Brudzynski & Kim, 2011; Nasir et al., 2010; Erejuwa, Sulaiman, & Wahab, 2012).

Oilseed rape (*Brassica napus*) is one of the most cultivated crop plants around the world. It is an important source of oil and medicinal components. The cultivation rate of oilseed rape around the globe has been increasing over the last ten years with about 31.5 Mha of lands used to cultivate in 2010 (Gulden, Warwick,& Thomas, 2008; Islam et al., 2013).

Rape seed (*Brassica napus* L.) crops and honey are very good and commonly used bee food. Its pollen contains such amino acids as Threonine, Valine and Methionine and 23% to 24% crude protein, but the exact content is not exactly known. Honey differs in its composition due to plant contribution and environmental conditions; the honey properties depend on the nectar/pollen of the original plant, colour, flavour, moisture or sugar contents (da Silva et al., 2016). Not much is known about the protein content of honey. In recent years a great deal of information has published about proteins that could be allergens in oilseed rape pollen (Chardin et al., 2001; Focke et al., 2003; Poikonen et al., 2006). The aim of this study was to identify protein content and composition of the pollen from oilseed rape (*Brassica napus* L.) honey as well as hand-collected and bee-collected rape pollen and to classify detected proteins according their biological function.

MATERIAL AND METHODS

Collection of pollen from rape

Rape pollen grains for the study were collected either by hand from flowers or from pollen traps. All pollen samples were collected from the experiment fields of the Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture in Kėdainių district, Lithuania. Mature pollen grains were hand-collected from freshly open winter rape Cult blossoms into Eppendorf tubes and stored at −80°C until analysis. Rape pollen gathered by bees was collected in accordance with good beekeeping practices and did not interfere with normal colony growth (Gracham, 1992). Honeybee-gathered pollen was collected from a standard pollen trap mounted on the hive entrance in good weather during rape blossoming. After removal from traps, the pollen was cleaned and kept in a refrigerator at −80°C in air-tight plastic bags.

Monofloral honey samples

Monofloral rape honey was harvested from bee colonies located in the Kėdainių district, Lithuania and used in this study. Honey samples were preserved in glass bottles and refrigerated (5°C) until analysis.

Protein isolation from pollen

Proteins from mature pollen (approx. 20 mg) were isolated as described by Sheoran et al., (2007) with some modifications. Briefly, mature pollen was homogenized with acetone containing 10% trichloroacetic acid (TCA) and 1% dithiothreitol (DTT). The solution was centrifuged 20.000 × g for 20 min at 4°C. The supernatant was collected as the first extract and pellet of pollen remains was washed two times more with acetone solution containing 1% DTT. The pellet was dried in a vacuum and proteins were extracted with isoelectric focusing (IEF) lysis buffer, the second extract. After centrifugation, both extracts were combined and directly used for protein analysis or stored at −20°C until analysis.

Protein isolation from honey samples

The honey samples (0.2 g/ml) were dissolved in distilled water, centrifuged at 3000 rpm for 20 min (K-24) and filtered on glass fibre prefilter (Millipore, 5-15 µ) under vacuum. The carbohydrate was removed using a capillary dialyzer Xevonta Lo 20 (B. Braun, Avitum, Melsungen, Germany). Part of the solution was concentrated about three times in a dialysis tubing VISKING (Serva, Heidelberg, Germany), keeping onto dry polyethylene glycol (PEG). Proteins were precipitated by adding four volumes of cold 80% acetone and incubated overnight at −20°C. The pellet was then washed with 1 ml chilled 80%

acetone several times. The pellet was dried for 5 min at room temperature, suspended in IEF buffer as described above and stored at –20°C until analysis.

Electrophoretic separation of proteins by SDS/PAGE and 2DE

The proteins isolated from hand- and bee-collected pollen and rape honey were fractionated by SDS/PAGE on gradient (7.5 – 15%) polyacrylamide gel and also resolved by two-dimensional gel electrophoresis (2DE). An Immobiline DryStrip kit, pH range 3–11, and Excel gel SDS, gradient 8–18% was conducted for 2DE according to the manufacturer's instructions (Immobiline DryStrip kit for 2DE with Immobiline DryStrip and Excel gel SDS, Pharmacia Biotech, Sweden). For protein visualization, the gels were stained with Colloidal Coomassie G-250 (Bio-Rad Laboratories, USA). For 2DE fractionation of pollen proteins, three independent biological experiments were carried out.

In-gel digestion and MALDI-TOF MS

Areas of interest were cut out from the 2DE gels and subjected to overnight in-gel tryptic digestion (Shevchenko et al., 1996). For MALDI-TOF analysis, the peptides were prepared and mass spectrometry analysis performed.

Protein sample preparation and mass spectrometry analysis

Extracted proteins from hand- and bee-collected pollen as well as rape honey proteins were analysed by direct gel-free mass spectrometry analysis. For this, isolated proteins were applied on Amicon Ultra-0.5 mL 30 kDa centrifugal filter unit (Sigma-Aldrich, USA). Trypsin digestion was done according to a modified FASP protocol as described by Wisniewski et al., (2009).

Data processing, searching and analysis

Raw data files were processed and examined using ProteinLynx Global SERVER (PLGS) version 2.5.2 (Waters Corporation, UK). The following parameters were used to generate peak lists: (i) minimum intensity for precursors set to 100 counts, (ii) minimum intensity for fragment ions set to 30 counts, (iii) intensity set to 500 counts. Processed data was analyzed using trypsin as the cleavage protease, one missed cleavage was allowed and fixed modification was set to carbamidomethylation of cysteines, and variable modification was set to the oxidation of methionine. Minimum identification criteria included two fragment ions per peptide, five fragment ions per protein and a minimum of two peptides per protein. The false discovery rate (FDR) for peptide and protein identification was determined based on the search of a reversed database, which was generated automatically using PLGS when the global false discovery rate was set to 1 %. Functional protein association networks were constructed using AgBase, version 2.00 (agbase.msstate.edu).

Gel scanning and image analysis

The 2DE gels with visualized proteins were scanned using a specialized gel imaging system whose components were ImageScanner™ III (GE Healthcare Bio-Sciences, UK) and LabScan v6.0 sofware. The scanner was calibrated using the provided step tablet with the known optical density values. Gels were digitized at 16-bit pixel depth and 300 dpi resolution, and stored as TIFF™ format graphic files.

The scanned 2DE gel images were analysed using our developed software toolset which runs in the Matlab™ environment (The MathWorks, Natick MA, USA). It included custom image pre-processing, alignment, segmentation, spot pairing, successive data analysis and visualization instruments. Our workflow of gel image analysis was based on one of the common procedures where image segmentation was performed after image registration. A more detailed sequence of operations was as follows: image pre-processing, spot detection in individual images, image registration, spot detection in the set of aligned images and spot pairing, extraction of spot boundaries in the original images, spot quantitation and differential analysis.

Image pre-processing algorithms cope with intensity distortions caused by impulse noise (randomly occurring clearly brighter and darker

pixels) and non-uniform background (slowly varying background intensity level). Additionally, gel images are cropped to remove excessive areas that are not useful for the image registration. Image registration is based on feature matching strategy and has coarse and fine stages. Detected features are likely locations of protein spots, where local feature descriptors should be extracted. Paired features serve as control points for the initial rigid deformation of gels. Fine image registration detects refined correspondences between gels and performs elastic thin-plate spline transformation (Bookstein, 1989) to put matching spots into the same locations.

Gel images must be segmented (Serackis & Navakauskas, 2010) and corresponding segments be found to enable a quantitative comparison of spots from different 2DE gel images (Dowsey et al., 2010; Valledor & Jorrín, 2011). Performing spot detection on registered images allows us to achieve improved results compared to spot detection in original gels separately. Spot detection provides only probable positions of protein spots. Spot segmentation gives information on spot boundaries and is performed in original undistorted gels. Segmentation is guided by spot location information that was extracted during an earlier detection stage. Segmentation gives spot boundaries and area for the integration of the spot intensity, i.e. spot volume. Collected data on protein spot area and matchings between gels is used to perform differential analysis. Ratios of normalized spot quantities describe the differences between experimental groups.

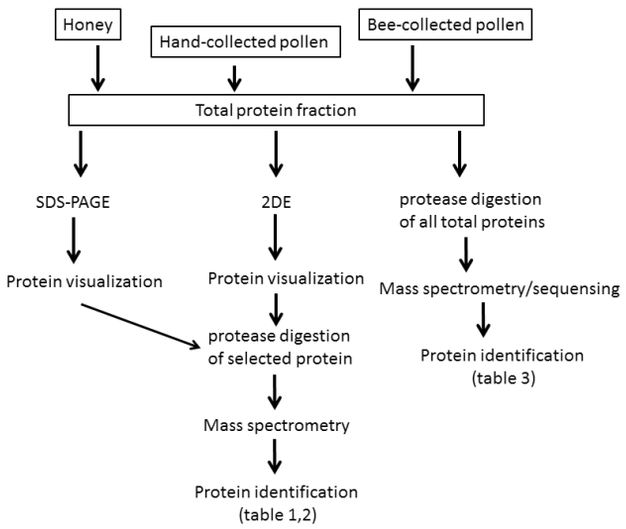

Fig. 1. Scheme of protein separation and identification from hand- or bee-collected pollen and honey samples. Samples from oilseed rape (*Brassica napus)* hand- and bee-collected pollen or honey were separated on SDS-PAGE (1DE) and 2DE system and then subjected to mass spectrometry analysis.

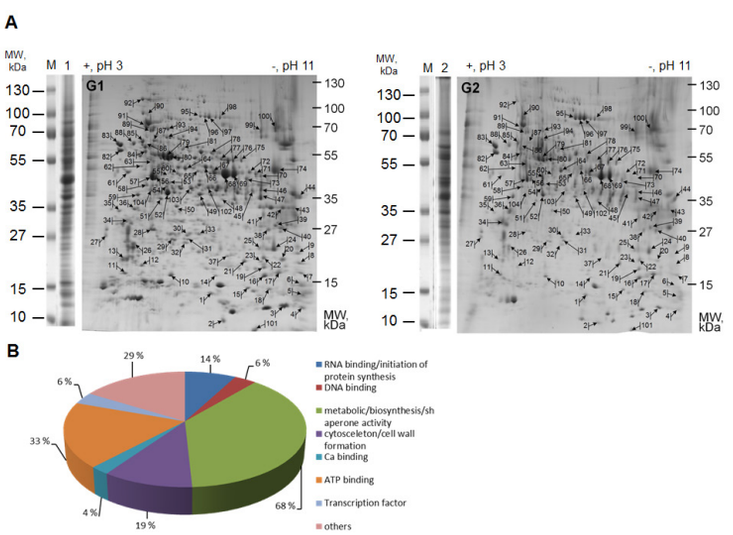

Fig. 2. Quantitative analysis of the proteins from hand- and bee- collected Brassica napus pollen. Proteins from hand- (G1, winter rape Cult) and bee- (G2) collected pollen were fractionated by ordinary SDS/PAGE (A, lines 1 and 2) and by 2DE (A, G1 and G2). Protein maps representing hand- (G1) and bee- (G2) collected *Brassica napus* pollen were overlapped and quantitative changes in protein levels were evaluated by computer-assisted analysis (Table 1). Numbers in the 2DE maps indicate the positions of proteins supplied to MALDI-224 TOF MS/MS and identified. Spot labels are the same as in Table 1. Representative images from one of the three experiments showing similar results are shown. Part B represents protein functions of *Brassica napus* pollen, fractionated by 2DE and identified by MS.

RESULTS

The soluble proteins extracted from oilseed rape blossom honey, hand-collected oilseed rape pollen and bee-collected oilseed rape pollen were analyzed in three different ways (Fig. 1); proteins were separated by either SDS-PAGE or by 2DE on pH 3-11 IPG strips and stained

Table 1

Proteins identified from hand- and bee-collected *Brassica napus* by MS.

No.	AC [accession number]	Description of protein [protein name, organism]	Sequence coverage, %	Theoretical Peptides	Digest Peptides	Theor. Mw, kDa	Theor. pI, pH	Exp. Mw, kDa	Exp. pI, pH	Fold change G1/G2
3	A1EA43	Translation initiation factor IF-1, chloroplastic OS=*Agrostis stolonifera*	7.7	26	2	12.45	9.52	12.2	8.4	3.6
8	P42794	60S ribosomal protein L11-2 OS=*Arabidopsis thaliana*	11.4	35	4	20.84	9.94	17.3	9.3	3.3
13	P43349	Translationally-controlled tumor protein homolog OS=*Solanum tuberosum*	21.1	19	4	18.83	4.58	18.1	4.5	3.0
20	P34944	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3 OS=*Marchantia polymorpha*	12.5	24	3	23.33	6.21	17.9	8.3	3.4
23	P49208	50S ribosomal protein L1, chloroplastic (Fragment) OS=*Pisum sativum*	2.6	39	1	23.48	10.2	17.6	7	4.2
28	P21216	Soluble inorganic pyrophosphatase 2 OS=*Arabidopsis thaliana*	3.4	29	1	24.65	5.72	23.9	5	5.5
33	Q38829	Auxin-responsive protein IAA11 OS=*Arabidopsis thaliana*	13.8	29	4	26.49	5.82	22.9	5.8	9.2
37	Q39056	Cyclic pyranopterin monophosphate synthase accessory protein, mitochondrial OS=*Arabidopsis thaliana*	16.7	36	6	29.49	8.22	18.6	6.3	6.3
46	F6HDM2	ATP-dependent (S)-NAD(P)H-hydrate dehydratase OS=*Vitis vinifera*	4.8	42	2	38.08	8.3	36.4	7.1	3.9
50	Q9M7I7	Chlorophyllase-2, chloroplastic OS=*Arabidopsis thaliana*	15.2	33	5	34.88	6.5	29.8	5.5	3.6
51	Q9SID0	Probable fructokinase-1 OS=*Arabidopsis thaliana*	15.8	38	6	35.25	5.31	32.9	5.2	3.7
55	Q9SRT9	UDP-arabinopyranose mutase 1 OS=*Arabidopsis thaliana*	25.6	39	10	40.60	5.61	42.6	5.3	−6.0
87	P29197	Chaperonin CPN60, mitochondrial OS=*Arabidopsis thaliana*	7.9	76	6	61.24	5.66	65.6	5.1	5.0
90	Q39043	Mediator of RNA polymerase II transcription subunit 37f OS=*Arabidopsis thaliana*	22.3	94	21	73.51	5.11	92.5	4.9	−3.8
95	O50008	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 OS=*Arabidopsis thaliana*	17.2	87	15	84.30	6.09	89.6	5.8	−3.4
96	O50008	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 OS=*Arabidopsis thaliana*	19.5	87	17	84.30	6.09	89.3	5.8	−4.1
102	Q9FHD5	Cysteine-rich repeat secretory protein 57 OS=*Arabidopsis thaliana*	22.7	22	5	31.85	5.56	37.2	5.8	3.2
103	P13911	DNA-directed RNA polymerase subunit alpha OS=*Pisum sativum*	16.3	43	7	38.90	7.2	36.9	5.7	7.4
104	Q38799	Pyruvate dehydrogenase E1 component subunit beta-1, mitochondrial OS=*Arabidopsis thaliana*	15.0	40	6	39.15	5.67	36.1	5	3.2

with CCB and then mass spectrometry were prepared, or soluble proteins were analysed by direct gel-free mass spectrometry analysis using HDMS Synapt G2 mass spectrometer.

Protein profile comparison from hand-collected and bee-collected oilseed rape pollen

We aimed to characterize the difference in proteome maps between hand-collected (Fig. 2A, G1) and bee-collected (Fig. 2A, G2) oilseed rape pollen. Over 200 spots were detected using pH 3-11 IPG strips (Fig. 2A, G1/G2) and analysed

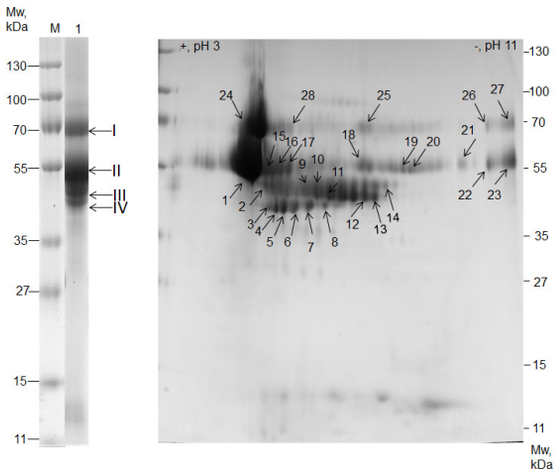

Fig. 3. Analysis of the proteins from Brassica napus honey. Proteins were fractionated by ordinary SDS/PAGE (lines 1) and by 2DE. Numbers in the SDS-PAGE and 2DE maps indicate the positions of proteins supplied to MALDI-224 TOF MS/MS and identified. Spot labels are the same as in Table 2. Representative images from one of the three experiments showing similar results are shown.

by MALDI-TOF MS, of which 107 spots were successfully analysed (Fig. 2A, G1/G2). The identified proteins, along with the protein index, MW, pI, and the fold change between G1 (hand-collected pollen), G2 (bee-collected pollen) and protein fold change between G1 and G2 are listed in Supplement 1. Some of the identified proteins were present as multiple spots on gels that could correspond to multiple isoforms of protein. Almost all proteins isolated from hand- and bee- collected oilseed rape pollen showed similar protein content, but some showed quantitative differences and are listed in Table 1. Translation initiation factor IF-1 (no. 3), 50S ribosomal protein L1 (no. 23), Soluble inorganic pyrophosphatase 2 (no. 28), Auxin-responsive protein (no. 33), Cyclic pyranopterin monophosphate synthase accessory protein (no.37), ATP-dependent (S)-NAD(P)H-hydrate dehydratase

Table 2

Oilseed rape blossom honey identified proteins: I–IV identified from SDS-PAGE and 1–28 identified from 2DE

Spot no.	AC [accession number]	Description of protein [protein name, origin]	Matching [sequence coverage %]	Theoretical		Experimental		Protein Score C.I. %
				Mw, kDa	pI, pH	Mw, kDa	pI, pH	
I	Q17058	Alpha-glucosidase OS=*Apis mellifera*	46	65.5	5.06	67–71	–	100
Ia	Q17060	Major royal jelly protein 3 OS=*Apis mellifera*	28	61.6	6.47	62–68	–	100
II	O18330	Major royal jelly protein 1 OS=*Apis mellifera*	57	48.8	5.1	50–55	–	100
III	O77061	Major royal jelly protein 2 OS=*Apis mellifera*	39	51	6.83	47–51	–	100
IV	O77061	Major royal jelly protein 2 OS=*Apis mellifera*	36	51	6.83	47–51	–	100
IVa	O97432	Major royal jelly protein 5 OS=*Apis mellifera*	17	70	5.95	47–51	–	99.96
1; 15a; 16b; 17b; 18; 19; 20; 21; 22; 23	O18330	Major royal jelly protein 1 OS=*Apis mellifera*	35–48	4.8	5.1	53.6	4.7	100
2	Q9Y823	Homocitrate synthase, mitochondrial OS=*Schizosaccharomyces pombe*	3	46.2	5.69	46.8	4.9	0
3; 4; 5a; 6a; 9a; 10c; 11b; 12b; 13b; 15b; 16a; 17a	Q17060	Major royal jelly protein 3 OS=*Apis mellifera*	23–27	61.6	6.47	39.6	5.0	100
5b; 6c; 7b; 9b; 10a; 13a; 14	O77061	Major royal jelly protein 2 OS=*Apis mellifera*	27–30	51.04	6.83	39.4	5.2	99.97
6b; 7a; 10b; 11a; 11c; 12c	O97432	Major royal jelly protein 5 OS=*Apis mellifera*	31–35	70.1	5.95	39.5	5.3	100
8	O94657	DnaJ protein homolog xdj1 OS=*Schizosaccharomyces pombe*	22	46.09	5.83	40.0	5.6	0
24; 25; 26; 27; 28	Q17058	Alpha-glucosidase OS=*Apis mellifera*	33–45	65.5	5.06	71–75	4.9–9.8	89–100

Table 3

Proteins identified from *Brassica napus* honey using gel-free mass spectrometry analysis. BRANA- *Brassica napus* origin, APIME- honeybee origin

Protein description	Entry	Hand-collected pollen		Bee-collected pollen		Honey	
		Coverage (%)	Amount (fmol)	Coverage (%)	Amount (fmol)	Coverage (%)	Amount (fmol)
Biological function: metabolic and biosynthesis process							
Triosephosphate isomerase	A0A078CJ83_ BRANA	71.2	8.05	71.6	7.5	38.5	3.9
BnaA03g06790D	A0A078CPV5_ BRANA	72.2	43.8	75.8	37.3	50.9	5.5
BnaA06g31290D	A0A078DBI5_ BRANA	56.5	35.5	56.5	35.5	16.9	2.9
Malic enzyme	A0A078DLS5_ BRANA					22.9	1.2
Glyceraldehyde 3 phosphate dehydrogenase	A0A078IJW0_ BRANA	85.1	31.9	85.1	7.9	55.6	12.3
Fructose bisphosphate aldolase	A0A078JFE6_ BRANA	88.2	26.3	88.2	26.2	10.8	1.4
Glucosylceramidase	A0A088APM5_ APIME					42.2	15.6
BnaC02g07610D	A0A078EPU3_ BRANA	67.4	25.1	69.9	24.5	45.2	4.9
BnaC02g38360D	A0A078EWU2_ BRANA	65.4	71.9	59.1	71.9	16.1	1.0
BnaC04g36920D	A0A078G4E6_ BRANA	48.9	21	48.9	21	38	1.2
BnaC01g39610D	A0A078G6P5_ BRANA	67.7	80.3	67.7	80.3	59.9	27.6
Malate dehydrogenase	A0A078GG36_ BRANA	70.8	9.1	70.8	12.2	56.4	1.5
Alcohol dehydrogenase 1	ADH1_ YEAST	46.5	25	46.5	25	34.1	25
Alpha glucosidase	Q25BT6_ APIME					78.3	17.5
Polygalacturonase	Q7Y1T6_ BRANA	68	53.2	68	53.2	53.1	13.8
Alpha amylase	Q9U8X5_ APIME					73	18.4
Nucleoside diphosphate kinase	A0A078HV31_ BRANA	74.3	18.9	74.3	15.7	13.5	2.2
Biological function: lipid/ion transport							
Non specific lipid transfer	A0A078I7R0_ BRANA	49.1	5.5	49.1	5.5	49.1	24.9
Transferrin	A0A088AFH7_ APIME					46.3	0

(no. 46), Chlorophyllase-2 (no. 50), Probable fructokinase-1 (no. 51), Chaperonin CPN60 (no. 87) and DNA-directed RNA polymerase subunit alpha (no. 103) exceeded the protein level by three times in hand-collected (Fig. 2, G1) oilseed rape pollen than in bee-collected. Only a few proteins with those quantitative changes observable and overexpressed in bee-collected oilseed rape pollen were detected, i.e. UDP-arabinopyranose mutase (no. 55), Mediator of RNA polymerase II transcription (no. 90), 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (no. 95, 96).

Detected proteins were described and divided

into a few groups according to their biological functions presented in Figure 2B. We found these transcription factors (~6% of total identified proteins): probable WRKY transcription factor (Q9SUP6), protein SHI RELATED (Q9LQZ5) and a few Auxin-responsive proteins (Q38829, P49680). Another large group of detected proteins (68% of total identified proteins) in 2DE gels were involved in metabolic processes and biosynthesis: Ferredoxin (P00221), UDP-glucuronic acid decarboxylase (Q9SN95), fructokinase-1 (Q9SID0), 3-ketoacyl-CoA thiolase 2 (Q56WD9) and others listed in Table 1. The other identified proteins were important for ATP binding (33% of total identified proteins), transcription processes (3%), cytoskeleton/cell wall formation (19%), etc.

Protein profile from oilseed rape blossom honey

Proteins from oilseed rape blossom honey were separated by SDS-PAGE or 2DE on pH 3-11 IPG strips and stained with CCB for visualization (Fig. 3). Approximately four to five protein bands were seen on SDS-PAGE gel and were all excised and subjected to in-gel tryptic digestion and identification by mass spectrometry analysis (MALDI-TOF-MS) and plant/bee protein database searching. We identified only proteins of bee origin: major royal jelly proteins and galactosidase (Tab. 2, I-IV). 2DE map (Fig. 3) showed similar results. 28 protein spots were excised and subjected to in-gel tryptic digestion. The positions of all proteins identified on 2DE gels were within the expected range of their theoretical isoelectric points and molecular sizes. From the 2DE gels we identified only proteins of bee origin: major royal jelly proteins 1-5 and galactosidase (Tab. 2, no. 1-28).

Protein profile comparison from hand- or bee-collected pollen and oilseed rape blossom honey by gel-free analysis

Since we did not identify plant origin proteins in the honey samples, further the soluble proteins extracted from oilseed rape blossom honey, hand-collected oilseed rape pollen and bee-collected oilseed rape pollen were analyzed by direct gel-free mass spectrometry analysis using HDMS Synapt G2 mass spectrometer.

All identified proteins from the gel-free samples are listed in Supplement 2. Table 3 contains the list of proteins that were identified in honey samples of *Brassica napus* origin and honeybee origin. They were divided into three groups based on their biological function: metabolic/biosynthesis, lipid/ion transport and unknown function.

DISCUSSION

The goal of this study was to characterize proteins in rape seed (*Brassica napus L.*) pollen and honey and to find proteins of plant origin that would help to characterize the honey. Few studies had dealt with the proteome of honey or pollen collected from different plants, but in 2012 F. Girolamo reported about seven proteins that were constituents in every type of honey. All seven proteins were of animal origin (*A. mellifera*) except one glyceraldehyde-3-phosphate dehydrogenase from *Mesembryanthemum crystallinum*, which was found, apparently accidentally, in only one honey variety , and no additional proteins being attributed to plants, e.g. in pollen, nectar (Girolamo, D'Amato, & Righetti, 2012).

Since hand-collected and bee-collected pollen can differ in chemical properties, e.g. vitamin content, we decided to compare proteomic 2DE maps and find the differences. All identified proteins were divided into groups depending on their function in the plant cell. A separate group of identified proteins are transcription factors. We observed that these proteins were more expressed in hand-collected pollen samples, especially Auxin-responsive protein IAA11, which were involved in many aspects of plant growth and development and long known to control diverse responses to external stimuli (Chandler, 2016). Another transcription factor, the protein SHI RELATED, bound DNA on 5'-ACTCTAC-3' and promoted auxin homeostasis-regulating gene expression, e.g. YUC genes, as well as genes affecting stamen development, cell expansion and timing of flowering (Hong et al., 2012). In

a recent study, we had performed proteomic analysis of red, berseem and white clover pollen (Treigytė et al., 2014) and detected over 30 protein spots whose quantitative levels were most divergent in investigated clover pollen.

In rape seed we also identified PHD finger protein ALFIN-LIKE 4 protein (Supplement 1) which could be involved in chromatin remodeling and protein RALF-like 16 (Supplement 1) which could be important in cell-cell signaling as was suggested by Kayum et al. (2015). In our study, we reported that SDS-PAGE or 2DE separation of rape seed blossom honey proteins could not represent all protein content. Only major royal jelly proteins and galactosidase of bee origin were detected and no plant proteins during gel-based analysis.

We carried out a gel-free analysis of rape seed pollen (hand- or bee-collected) and rape seed honey. We observed around twenty different plant (rape seed) proteins in the honey sample and all were involved in metabolic or biosynthesis processes like Tiosephosphate isomerase, Malic enzyme and Malate dehydrogenase. We also detected all major jelly proteins (1-9) from honeybees in the honey sample. The major royal jelly proteins (MRJPs) comprised 12.5% of the mass and 82-90% of the protein content of honeybee (*Apis mellifera*) royal jelly (Girolamo, D'Amato, & Righetti, 2012). Royal jelly is a substance secreted by the cephalic glands of nurse bees and is used to trigger the development of a queen bee from a bee larva. The biological function of the MRJPs is unknown, but they are believed to play a major role in nutrition due to their high essential amino acid content (Bhattacharya et al., 1999). Two royal jelly proteins, MRJP3 and MRJP5, contain a tandem repeat that results from a high genetic variability. This polymorphism may be useful for genotyping individual bees. We suggest that all identified proteins can be used for further investigation to find biological markers for honey of different origin types.

Conflict of Interest: The authors declare that they have no conflict of interest.

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SUPPLEMENT 1

Nr.	AC [accession number]	Entry	Description of protein [protein name]	Sequence coverage. %	Theoretical Peptides	Digest Peptides	Theor.		Exp.		Fold change
							Mw, kDa	pI	Mw, kDa	pI, pH	G1/G2
1	P00221	FER1_SPIOL	Ferredoxin-1, chloroplastic OS=Spinacia oleracea	27.3	11	3	15.64	4.33	13.4	5.9	2.2
2	Q0J9V6	Y4294_ORYSJ	Uncharacterized protein Os04g0629400 OS=Oryza sativa subsp. japonica	42.9	7	3	12.04	6.69	10.9	6.2	2.3
3	A1EA43	IF1C_AGRST	Translation initiation factor IF-1, chloroplastic OS=Agrostis stolonifera	7.7	26	2	12.45	9.52	12.2	8.4	3.6
4	Q0G9T9	RK20_DAUCA	50S ribosomal protein L20, chloroplastic OS=Daucus carota	9.4	32	3	15.24	11.81	12	9.5	1.7
5	P07924	RT13_WHEAT	Ribosomal protein S13, mitochondrial OS=Triticum aestivum	26.1	23	6	13.42	10.57	13.2	9.5	1.5
6	Q852K5	SAP6_ORYSJ	Zinc finger A20 and AN1 domain-containing stress-associated protein 6 OS=Oryza sativa subsp. japonica	15.4	26	4	17.52	8.9	14.4	9.4	2.9
7	Q9FZP6	MBD12_ARATH	Putative methyl-CpG-binding domain protein 12 OS=Arabidopsis thaliana	16.7	24	4	17.87	9.41	15.9	9.4	1.1
8	P42794	RL112_ARATH	60S ribosomal protein L11-2 OS=Arabidopsis thaliana	11.4	35	4	20.84	9.94	17.3	9.3	3.3
9	P51427	RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana	10.0	30	3	22.90	9.66	19.1	9.2	2.8
10	Q9C505	IF5A3_ARATH	Eukaryotic translation initiation factor 5A-3 OS=Arabidopsis thaliana	16.7	18	3	17.19	5.56	15.7	5.4	2.3
11	Q9LR33	R27A2_ARATH	60S ribosomal protein L27a-2 OS=Arabidopsis thaliana	9.4	32	3	16.28	10.5	16	4.5	1.2
12	Q40089	ATP4_IPOBA	ATP synthase subunit delta', mitochondrial OS=Ipomoea batatas	22.2	18	4	21.30	5.93	17	4.8	2.4
13	P43349	TCTP_SOLTU	Translationally-controlled tumor protein homolog OS=Solanum tuberosum	21.1	19	4	18.83	4.58	18.1	4.5	3.0
14	Q68S00	ATPE_PANGI	ATP synthase epsilon chain, chloroplastic OS=Panax ginseng	12.5	16	2	15.36	5.25	15.6	5.9	2.8
15	P81766	NDK3_SPIOL	Nucleoside diphosphate kinase 3 OS=Spinacia oleracea	13.6	22	3	17.10	8.12	14	6.8	1.6
16	P24525	CYPH_BRANA	Peptidyl-prolyl cis-trans isomerase OS=Brassica napus	30.4	23	7	18.50	8.57	15.5	7.3	1.6
17	Q04613	MI25_ARATH	ATP synthase protein MI25 OS=Arabidopsis thaliana	18.5	27	5	21.67	9.53	16.1	8.1	1.0
18	P93224	NLTP2_SOLLC	Non-specific lipid-transfer protein 2 OS=Solanum lycopersicum	36.4	11	4	11.47	8.04	13.9	7.5	1.3
19	P29110	OLEO3_BRANA	Oleosin Bn-III OS=Brassica napus	17.4	23	4	21.52	9.3	16.5	7.2	-1.0
20	P34944	NDUS3_MARPO	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3 OS=Marchantia polymorpha	12.5	24	3	23.33	6.21	17.9	8.3	3.4
21	P93000	GL23_ARATH	Germin-like protein subfamily 2 member 3 OS=Arabidopsis thaliana	5.6	18	1	23.01	8.85	17.7	6.7	-1.1
22	Q0ZIY0	RR3_VITVI	30S ribosomal protein S3, chloroplastic OS=Vitis vinifera	15.4	39	6	25.22	9.97	18.2	7.2	1.1
23	P49208	RK1_PEA	50S ribosomal protein L1, chloroplastic (Fragment) OS=Pisum sativum	2.6	39	1	23.48	10.23	17.6	7	4.2
24	Q8GXZ3	Y5102_ARATH	Serine/threonine-protein kinase At5g01020 OS=Arabidopsis thaliana	1.9	53	1	45.58	9.21	19.9	7.8	1.9
25	Q9ZU31	PP138_ARATH	Pentatricopeptide repeat-containing protein At2g01360 OS=Arabidopsis thaliana	19.0	21	4	20.32	6.07	20	7	1.3

26	P49680	IAA6_PEA	Auxin-induced protein IAA6 OS=Pisum sativum	6.3	32	2	20.31	6.74	20.7	4.7	1.0
27	O81488	ALFL4_ARATH	PHD finger protein ALFIN-LIKE 4 OS=Arabidopsis thaliana	11.1	36	4	28.76	5.01	22.4	4.2	1.3
28	P21216	IPYR2_ARATH	Soluble inorganic pyrophosphatase 2 OS=Arabidopsis thaliana	3.4	29	1	24.65	5.72	23.9	5	5.5
29	Q9ZT49	ATL45_ARATH	RING-H2 finger protein ATL45 OS=Arabidopsis thaliana	15.8	19	3	21.06	7.01	21.7	5.4	2.2
30	Q01197	E6_GOSHI	Protein E6 OS=Gossypium hirsutum	14.8	27	4	28.20	5.14	22.6	5.6	1.4
31	P42748	UBC4_ARATH	Ubiquitin-conjugating enzyme E2 4 OS=Arabidopsis thaliana	21.1	19	4	21.28	4.23	21.8	5.6	2.1
32	O04905	KCY_ARATH	UMP-CMP kinase OS=Arabidopsis thaliana	13.3	30	4	22.46	5.79	21	5.6	2.5
33	Q38829	IAA11_ARATH	Auxin-responsive protein IAA11 OS=Arabidopsis thaliana	13.8	29	4	26.49	5.82	22.9	5.8	9.2
34	Q9FI61	UBC27_ARATH	Ubiquitin-conjugating enzyme E2 27 OS=Arabidopsis thaliana	10.0	20	2	21.24	5	26.1	4.6	-1.7
35	Q75IR6	ALFL1_ORYSJ	PHD finger protein ALFIN-LIKE 1 OS=Oryza sativa subsp. japonica	13.5	37	5	28.85	5.52	29	4.4	2.9
36	Q9C5W6	14312_ARATH	14-3-3-like protein GF14 iota OS=Arabidopsis thaliana	20.6	34	7	30.52	4.83	29.4	4.7	-1.5
37	Q39056	CNX3_ARATH	Cyclic pyranopterin monophosphate synthase accessory protein, mitochondrial OS=Arabidopsis thaliana	16.7	36	6	29.49	8.22	18.6	6.3	6.3
38	Q01197	E6_GOSHI	Protein E6 OS=Gossypium hirsutum	18.5	27	5	28.20	5.14	24	7.5	1.3
39	Q10GP0	Y3198_ORYSJ	Putative B3 domain-containing protein Os03g0619850 OS=Oryza sativa subsp. japonica	12.9	31	4	28.11	7.13	24.9	7.9	1.2
40	O36052	RR4_PATSQ	30S ribosomal protein S4, chloroplastic (Fragment) OS=Patersonia sp. (strain Lejeune 1997)	10.8	37	4	22.78	10.48	24.2	8.2	2.1
41	O23609	PER41_ARATH	Peroxidase 41 OS=Arabidopsis thaliana	4.7	43	2	36.17	8.51	27.4	7.2	1.3
42	Q10GP0	Y3198_ORYSJ	Putative B3 domain-containing protein Os03g0619850 OS=Oryza sativa subsp. japonica	16.1	31	5	28.11	7.13	30	8.3	1.5
43	P83291	NB5R2_ARATH	NADH-cytochrome b5 reductase-like protein OS=Arabidopsis thaliana	13.5	37	5	35.96	8.76	30	9	1.2
44	Q84WM7	PPME1_ARATH	Pectinesterase PPME1 OS=Arabidopsis thaliana	18.9	37	7	39.11	8.74	32.2	9.3	1.3
45	Q9SN95	UXS5_ARATH	UDP-glucuronic acid decarboxylase 5 OS=Arabidopsis thaliana	11.4	44	5	38.36	7.1	36	6.9	1.0
46	F6HDM2	NNRD_VITVI	ATP-dependent (S)-NAD(P)H-hydrate dehydratase OS=Vitis vinifera	4.8	42	2	38.08	8.3	36.4	7.1	3.9
47	Q9FLA3	FBD42_ARATH	Putative FBD-associated F-box protein At5g44940 OS=Arabidopsis thaliana	12.5	48	6	44.07	8.52	34.1	7.1	1.9
48	Q9M0R4	ATL37_ARATH	Putative RING-H2 finger protein ATL37 OS=Arabidopsis thaliana	10.0	40	4	39.97	7.9	32.6	6.1	-2.0
49	P60314	RPOA_AMBTC	DNA-directed RNA polymerase subunit alpha OS=Amborella trichopoda	13.0	46	6	38.39	7.61	34.3	5.8	2.9
50	Q9M7I7	CLH2_ARATH	Chlorophyllase-2, chloroplastic OS=Arabidopsis thaliana	15.2	33	5	34.88	6.5	29.8	5.5	3.6
51	Q9SID0	SCRK1_ARATH	Probable fructokinase-1 OS=Arabidopsis thaliana	15.8	38	6	35.25	5.31	32.9	5.2	3.7
52	Q9SRT9	RGP1_ARATH	UDP-arabinopyranose mutase 1 OS=Arabidopsis thaliana	28.2	39	11	40.60	5.61	35.2	5.3	2.1
53	O82662	SUCB_ARATH	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial OS=Arabidopsis thaliana	16.1	56	9	45.31	6.3	39.5	5.4	2.6
54	Q9FHJ2	DRL34_ARATH	Probable disease resistance protein At5g45440 OS=Arabidopsis thaliana	10.0	50	5	39.42	5.2	37.7	5.3	1.7
55	Q9SRT9	RGP1_ARATH	UDP-arabinopyranose mutase 1 OS=Arabidopsis thaliana	25.6	39	10	40.60	5.61	42.6	5.3	-6.0
56	P53494	ACT4_ARATH	Actin-4 OS=Arabidopsis thaliana	28.2	39	11	41.75	5.37	41.7	5.1	1.4

57	Q9SUP6	WRK53_ ARATH	Probable WRKY transcription factor 53 OS=Arabidopsis thaliana	10.3	39	4	36.25	6.34	42.1	5	1.9
58	P42748	UBC4_ARATH	Ubiquitin-conjugating enzyme E2 4 OS=Arabidopsis thaliana	21.1	19	4	21.28	4.23	38.3	4.9	1.2
59	Q4PT02	GAOX5_ARATH	GN=GA20OX5 PE=2 SV=1	8.9	45	4	43.13	8.04	34.2	4.8	-1.0
60	Q9M6A3	ASC1_SOLLC	Protein ASC1 OS=Solanum lycopersicum	2.8	36	1	36.27	7.66	44	5.5	1.3
61	Q9SMT9	FBD9_ARATH	FBD-associated F-box protein At3g49020 OS=Arabidopsis thaliana	3.8	52	2	51.38	5.71	44.4	4.6	2.2
62	Q9SJA6	RZ22A_ARATH	Serine/arginine-rich splicing factor RSZ22A OS=Arabidopsis thaliana	6.5	46	3	21.90	11.34	47.7	4.8	1.1
63	P53496	ACT11_ARATH	Actin-11 OS=Arabidopsis thaliana	5.3	38	2	41.64	5.23	48.6	5	-1.8
64	Q9SJL8	METK3_ARATH	S-adenosylmethionine synthase 3 OS=Arabidopsis thaliana	2.2	45	1	42.47	5.76	46.2	5.6	-1.1
65	Q9M1D3	CISY5_ARATH	Citrate synthase 5, mitochondrial OS=Arabidopsis thaliana	17.0	47	8	51.69	6.2	45.1	5.7	1.4
66	Q9LQZ5	SRS5_ARATH	Protein SHI RELATED SEQUENCE 5 OS=Arabidopsis thaliana	12.5	32	4	38.53	6.67	46.9	5.8	2.4
67	P35337	PGLR_BRANA	Polygalacturonase OS=Brassica napus	22.0	41	9	42.42	6.34	45.6	5.9	-1.3
68	Q39980	VTSS3_HYOMU	Vetispiradiene synthase 3 (Fragment) OS=Hyoscyamus muticus	14.0	43	6	41.07	5.01	43.5	6.2	1.1
69	P35337	PGLR_BRANA	Polygalacturonase OS=Brassica napus	24.4	41	10	42.42	6.34	43.4	6.5	1.2
70	Q56WD9	THIK2_ARATH	3-ketoacyl-CoA thiolase 2, peroxisomal OS=Arabidopsis thaliana	11.1	54	6	48.54	8.62	42.4	7.1	2.8
71	Q56WD9	THIK2_ARATH	3-ketoacyl-CoA thiolase 2, peroxisomal OS=Arabidopsis thaliana	9.3	54	5	48.54	8.62	43.6	6.9	2.3
72	Q56WD10	THIK2_ARATH	3-ketoacyl-CoA thiolase 2, peroxisomal OS=Arabidopsis thaliana	13.0	54	7	48.54	8.62	44.6	6.8	-1.1
73	Q9LSC2	Y3589_ARATH	PTI1-like tyrosine-protein kinase At3g15890 OS=Arabidopsis thaliana	2.2	45	1	40.92	5.36	41.1	6.8	1.3
74	Q8LFV3	CDF3_ARATH	Cyclic dof factor 3 OS=Arabidopsis thaliana	13.5	52	7	49.69	6.61	46.6	8	1.1
75	O23254	GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana	15.1	53	8	51.68	6.8	51.4	6.7	-1.1
76	O23254	GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana	15.1	53	8	51.68	6.8	51.5	6.5	-1.2
77	P32290	CATA_VIGRR	Catalase OS=Vigna radiata var. radiata	1.7	59	1	56.80	6.79	52	6.1	1.4
78	P22201	ATPAM_BRANA	ATP synthase subunit alpha, mitochondrial OS=Brassica napus	12.3	57	7	55.10	6.23	56.2	5.9	1.1
80	Q9LEJ0	ENO1_HEVBR	Enolase 1 OS=Hevea brasiliensis	12.2	49	6	47.80	5.57	53.1	5.4	2.1
81	Q42290	MPPB_ARATH	Probable mitochondrial-processing peptidase subunit beta OS=Arabidopsis thaliana	12.9	62	8	59.12	6.3	59.2	5.6	1.0
82	Q38858	CALR2_ARATH	Calreticulin-2 OS=Arabidopsis thaliana	11.3	53	6	48.12	4.37	57.6	4.4	-1.0
83	O04151	CALR1_ARATH	Calreticulin-1 OS=Arabidopsis thaliana	14.0	57	8	48.49	4.46	62.3	4.4	1.0
84	Q8L6Y7	PP193_ARATH	Pentatricopeptide repeat-containing protein At2g38420, mitochondrial OS=Arabidopsis thaliana	10.5	57	6	51.56	6.3	57.2	4.9	-1.1
85	Q9SRG3	PDI12_ARATH	Protein disulfide isomerase-like 1-2 OS=Arabidopsis thaliana	8.8	57	5	56.32	4.9	65.1	4.8	1.0
86	O49048	VPS45_ARATH	Vacuolar protein sorting-associated protein 45 homolog OS=Arabidopsis thaliana	10.6	66	7	64.90	6.22	61.7	4.9	1.5
87	P29197	CH60A_ARATH	Chaperonin CPN60, mitochondrial OS=Arabidopsis thaliana	7.9	76	6	61.24	5.66	65.6	5.1	5.0
88	P29828	PDI_MEDSA	Protein disulfide-isomerase OS=Medicago sativa	1.8	56	1	57.05	4.98	62.9	4.7	1.7
89b	Q39291	VATA_BRANA	V-type proton ATPase catalytic subunit A OS=Brassica napus	22.4	67	15	68.68	5.19	74.3	5	1.9
90	Q39043	MD37F_ARATH	Mediator of RNA polymerase II transcription subunit 37f OS=Arabidopsis thaliana	22.3	94	21	73.51	5.11	92.5	4.9	-3.8

91	P11143	HSP70_MAIZE	Heat shock 70 kDa protein OS=Zea mays	10.7	84	9	70.52	5.22	78	4.9	1.6
92	Q10MJ1	CGEP_ORYSJ	Probable glutamyl endopeptidase, chloroplastic OS=Oryza sativa subsp. japonica	1.9	104	2	10.38	5.66	113.7	4.9	2.6
93	O82663	DHSA1_ARATH	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial OS=Arabidopsis thaliana	7.4	68	5	69.61	5.86	76.3	5.3	1.8
94	O04499	PMG1_ARATH	2,3-bisphosphoglycerate-independent phosphoglycerate mutase 1 OS=Arabidopsis thaliana	4.9	61	3	60.54	5.32	68.4	5.3	-1.2
95	O50008	METE1_ARATH	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 OS=Arabidopsis thaliana	17.2	87	15	84.30	6.09	89.6	5.8	-3.4
96	O50008	METE1_ARATH	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 OS=Arabidopsis thaliana	19.5	87	17	84.30	6.09	89.3	5.8	-4.1
97	O50008	METE1_ARATH	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 OS=Arabidopsis thaliana	17.2	87	15	84.30	6.09	88.6	5.8	-2.8
98	Q9SRV5	METE2_ARATH	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 2 OS=Arabidopsis thaliana	17.0	88	15	84.53	6.09	88.8	6.1	-1.4
99	P09801	VCLB_GOSHI	Vicilin C72 OS=Gossypium hirsutum	5.7	87	5	69.68	7.87	74.1	7.1	-1.9
100	Q00624	ASOL_BRANA	L-ascorbate oxidase homolog OS=Brassica napus	22.8	57	13	62.09	8.9	76.8	7.9	-2.5
101	A8MRM1	RLF16_ARATH	Protein RALF-like 16 OS=Arabidopsis thaliana	33.3	12	4	10.66	7.64	10.2	7	2.7
102	Q9FHD5	CRR57_ARATH	Cysteine-rich repeat secretory protein 57 OS=Arabidopsis thaliana	22.7	22	5	31.85	5.56	37.2	5.8	3.2
103	P13911	RPOA_PEA	DNA-directed RNA polymerase subunit alpha OS=Pisum sativum	16.3	43	7	38.90	7.2	36.9	5.7	7.4
104	Q38799	ODPB1_ARATH	Pyruvate dehydrogenase E1 component subunit beta-1, mitochondrial OS=Arabidopsis thaliana	15.0	40	6	39.15	5.67	36.1	5	3.2

SUPPLEMENT 2

		Hand-collected pollen		Bee-collected pollen		Honey	
Description	Entry	Coverage (%)	Amount (fmol)	Coverage (%)	Amount (fmol)	Coverage (%)	Amount (fmol)
BnaC04g16060D protein OS Brassica napus	A0A078C2W4_BRANA	60.1942	7.4521	63.1068	7.4521	42.7184	3.5109
BnaA03g28400D protein OS Brassica napus	A0A078CBH7_BRANA	72.4183	0	70.719	0	44.0523	1.5619
BnaA01g01570D protein OS Brassica napus	A0A078CFF0_BRANA					26.3333	8.0721
BnaA03g29240D protein OS Brassica napus	A0A078CGN6_BRANA	55.9633	36.8737	72.9358	36.8737	37.156	0
Triosephosphate isomerase OS Brassica napus	A0A078CJ83_BRANA	71.2598	8.0569	71.6535	7.5337	38.5827	3.9513
BnaA01g04240D protein OS Brassica napus	A0A078CJM4_BRANA					23.7113	1.2064
BnaA03g06790D protein OS Brassica napus	A0A078CPV5_BRANA	72.2876	43.8474	75.817	37.3449	50.9804	5.5425
BnaC08g37340D protein OS Brassica napus	A0A078CYF5_BRANA	36.3128	27.4707	62.5698	27.4707	22.905	2.7887
BnaC09g06400D protein OS Brassica napus	A0A078CZ01_BRANA					47.4227	5.249
BnaC07g28960D protein OS Brassica napus	A0A078CZP0_BRANA					0.883	11.0073
BnaC08g17860D protein OS Brassica napus	A0A078D684_BRANA	46.2857	3.2642	46.2857	3.2642	11.4286	2.7911
BnaC07g25330D protein OS Brassica napus	A0A078D7Z6_BRANA	62.234	43.9519	62.0567	43.9519	16.6667	4.5353
BnaA06g31290D protein OS Brassica napus	A0A078DBI5_BRANA	56.5141	35.5261	56.5141	35.5261	16.9014	2.9526
Malic enzyme OS Brassica napus	A0A078DLS5_BRANA					22.9592	1.232
BnaA08g18150D protein OS Brassica napus	A0A078DNT9_BRANA	63.1579	52.8471	73.0994	52.2726	35.6725	0
BnaA03g26250D protein OS Brassica napus	A0A078DNY7_BRANA	43.6709	20.3399	43.6709	20.3399	15.1899	2.2399
Malic enzyme OS Brassica napus	A0A078DRQ2_BRANA					11.0544	0.4181
BnaC03g33530D protein OS Brassica napus	A0A078DS18_BRANA	72.4183	26.6452			43.3987	0
BnaC01g11000D protein OS Brassica napus	A0A078EEH5_BRANA	79.6512	27.7614	48.8372	24.9081	33.1395	8.4917
BnaC06g28200D protein OS Brassica napus	A0A078EI76_BRANA					6.0567	0.152
BnaC02g07610D protein OS Brassica napus	A0A078EPU3_BRANA	67.451	25.1671	69.9346	24.5928	45.2288	4.9973
BnaC01g16500D protein OS Brassica napus	A0A078EQS9_BRANA	91.2568	55.3382	75.9563	55.3382	74.3169	7.171
BnaA07g14060D protein OS Brassica napus	A0A078ERR4_BRANA	63.764	0.7921	63.764	0.9641	19.9438	1.3396
BnaA07g13000D protein OS Brassica napus	A0A078EV53_BRANA	73.1544	56.1043	73.1544	56.0589	61.745	5.3308
BnaAnng00740D protein OS Brassica napus	A0A078EV77_BRANA	41.3245	14.5407	42.1192	14.0971	9.2715	1.5774
BnaC02g38360D protein OS Brassica napus	A0A078EWU2_BRANA	65.4912	71.9718	59.194	71.9718	16.1209	1.0456
BnaA02g31820D protein OS Brassica napus	A0A078EY46_BRANA					0.8282	3.4022
BnaC05g04310D protein OS Brassica napus	A0A078FDN9_BRANA	18.3673	0.8807	18.3673	0.8446	9.9125	5.1398

BnaC07g15930D protein OS Brassica napus	A0A078FEN2_BRANA					40.9211	2.0944
BnaA02g12590D protein OS Brassica napus	A0A078FHF1_BRANA					76.5306	4.0066
BnaC08g40620D protein OS Brassica napus	A0A078FSK2_BRANA					44.4444	60.6021
BnaA02g12130D protein OS Brassica napus	A0A078FTY1_BRANA					4	0.968
BnaC09g36920D protein OS Brassica napus	A0A078FUC5_BRANA					9.4309	1.4866
BnaA09g06900D protein OS Brassica napus	A0A078FW97_BRANA					48.1959	7.6627
BnaA01g26720D protein OS Brassica napus	A0A078G1R2_BRANA					9.8196	1.0149
BnaC08g13830D protein OS Brassica napus	A0A078G2D0_BRANA					3.1042	2.4954
BnaC04g36920D protein OS Brassica napus	A0A078G4E6_BRANA	48.954	21.0095	48.954	21.0095	38.0753	1.2826
Lactoylglutathione lyase OS Brassica napus	A0A078G6I2_BRANA					27.5618	3.4529
BnaC01g39610D protein OS Brassica napus	A0A078G6P5_BRANA	67.7582	80.3766	67.7582	80.3766	59.9496	27.6636
BnaC01g39620D protein OS Brassica napus	A0A078G9V9_BRANA					56.546	0
BnaA06g37080D protein OS Brassica napus	A0A078GAH2_BRANA					51.8519	4.2187
BnaC05g39930D protein OS Brassica napus	A0A078GE19_BRANA					3.4591	55.5784
Malate dehydrogenase OS Brassica napus	A0A078GG36_BRANA	70.8823	9.1554	70.8823	12.2704	56.4706	1.546
BnaA07g02960D protein OS Brassica napus	A0A078GG58_BRANA					16.4063	1.7875
BnaC03g17030D protein OS Brassica napus	A0A078GH67_BRANA					3.5294	2.3993
BnaC05g34710D protein OS Brassica napus	A0A078GIN3_BRANA					34.4196	6.1036
BnaA02g10950D protein OS Brassica napus	A0A078GKL6_BRANA					2.4938	1.0046
BnaC09g42270D protein OS Brassica napus	A0A078GM91_BRANA					1.3699	0.7787
Malate dehydrogenase OS Brassica napus	A0A078GMM3_BRANA	80.9384	25.2233	79.1789	6.162	56.305	3.4602
BnaC06g02730D protein OS Brassica napus	A0A078GSI3_BRANA	77.8846	27.3447	77.4038	27.3447	23.5577	1.807
BnaA06g25580D protein OS Brassica napus	A0A078GVL8_BRANA					7.045	2.3227
BnaA02g25430D protein OS Brassica napus	A0A078GWD0_BRANA					20.1681	1.0443
BnaA03g49470D protein OS Brassica napus	A0A078H057_BRANA					10.3704	0
BnaA09g16230D protein OS Brassica napus	A0A078H2Z2_BRANA					8.9431	1.0999
BnaC08g41860D protein OS Brassica napus	A0A078HAB6_BRANA	56.4486	5.6147	42.8037	6.0681	16.0748	1.6376
BnaA09g47550D protein OS Brassica napus	A0A078HBG6_BRANA	63.9405	16.1599	56.8773	15.7065	23.6059	2.1135
BnaC09g37570D protein OS Brassica napus	A0A078HBP0_BRANA					8.2687	0.0903
BnaA01g14010D protein OS Brassica napus	A0A078HBY7_BRANA	91.2568	36.7332	75.9563	36.7332	74.3169	10.9305
BnaA03g11410D protein OS Brassica napus	A0A078HEF2_BRANA					44.4444	83.0132
BnaC06g03590D protein OS Brassica napus	A0A078HFH3_BRANA					85.1852	0.6324

BnaA04g20520D protein OS Brassica napus	A0A078HPI7_BRANA					17.3333	5.0177
BnaA04g13950D protein OS Brassica napus	A0A078HSF0_BRANA					38.0753	4.6976
Cysteine proteinase inhibitor OS Brassica napus	A0A078HUB5_BRANA	22.0588	2.3338	22.0588	1.6099	19.1176	1.0753
BnaC02g08710D protein OS Brassica napus	A0A078HUR8_BRANA	25.5319	4.6653	25.5319	4.6653	25.5319	1.5782
Nucleoside diphosphate kinase OS Brassica napus	A0A078HV31_BRANA	74.3243	18.9886	74.3243	15.7224	13.5135	2.29
BnaA04g20210D protein OS Brassica napus	A0A078HYN8_BRANA					6.7164	1.988
BnaC05g48410D protein OS Brassica napus	A0A078I298_BRANA					56.3025	0
Non specific lipid transfer protein OS Brassica napus	A0A078I7E1_BRANA					16.0494	4.3583
Non specific lipid transfer protein OS Brassica napus	A0A078I7R0_BRANA	49.1667	5.5197	49.1667	5.5197	49.1667	24.971
BnaC02g43200D protein OS Brassica napus	A0A078I9J4_BRANA					35.4756	0.2601
BnaC02g16970D protein OS Brassica napus	A0A078IHY1_BRANA					76.5306	1.0341
BnaA06g23560D protein OS Brassica napus	A0A078IJR4_BRANA					44.8718	7.5963
Glyceraldehyde 3 phosphate dehydrogenase Fragment OS Brassica napus	A0A078IJW0_BRANA	85.119	31.9453	85.119	7.9288	55.6548	12.3664
Pectinesterase OS Brassica napus	A0A078IK58_BRANA					14.5648	3.1886
Nucleoside diphosphate kinase OS Brassica napus	A0A078J8I9_BRANA					7.8571	3.1706
Fructose bisphosphate aldolase OS Brassica napus	A0A078JFE6_BRANA	88.2682	26.359	88.2682	26.2954	10.8939	1.4896
BnaCnng50750D protein OS Brassica napus	A0A078JFP0_BRANA					12.6437	0.2728
BnaA06g37760D protein Fragment OS Brassica napus	A0A078K080_BRANA	76.9231	2.8346	75.2137	3.1489	40.1709	0.4667
Uncharacterized protein OS Apis mellifera	A0A087ZR10_APIME					31.25	5.3396
Uncharacterized protein OS Apis mellifera	A0A087ZRC8_APIME					20.2667	1.9393
Uncharacterized protein OS Apis mellifera	A0A087ZSJ0_APIME					47.4747	48.4586
Uncharacterized protein OS Apis mellifera	A0A087ZSJ1_APIME					62.766	43.1387
Uncharacterized protein OS Apis mellifera	A0A087ZVX2_APIME					65.8462	9.9438
Uncharacterized protein OS Apis mellifera	A0A087ZVX4_APIME					12.0579	2.2705
Uncharacterized protein OS Apis mellifera	A0A087ZWK4_APIME					76.1578	198.1699
Uncharacterized protein OS Apis mellifera	A0A087ZXA0_APIME					58.2237	17.6406
Uncharacterized protein OS Apis mellifera	A0A087ZXA2_APIME					43.5644	10.1937
Uncharacterized protein OS Apis mellifera	A0A087ZYX8_APIME					18.2716	1.6879
Uncharacterized protein OS Apis mellifera	A0A088A030_APIME					42.1384	18.509
Uncharacterized protein OS Apis mellifera	A0A088A031_APIME					47.6423	41.7336
Uncharacterized protein OS Apis mellifera	A0A088A3F5_APIME					65.3659	0
Uncharacterized protein OS Apis mellifera	A0A088A4K9_APIME					70.7692	6.6022

Carboxylic ester hydrolase OS Apis mellifera	A0A088A5D7_APIME					45.9313	13.188
Uncharacterized protein OS Apis mellifera	A0A088A9G7_APIME					6.0841	7.1801
Uncharacterized protein OS Apis mellifera	A0A088AC16_APIME					47.5138	22.8407
Uncharacterized protein OS Apis mellifera	A0A088ADM5_APIME					52.9745	11.8234
Transferrin OS Apis mellifera	A0A088AFH7_APIME					46.3483	0
Uncharacterized protein OS Apis mellifera	A0A088AJR6_APIME					22.547	8.0861
Uncharacterized protein OS Apis mellifera	A0A088AMK2_APIME					21.1581	2.9243
Glucosylceramidase OS Apis mellifera	A0A088APM4_APIME					41.5709	11.4653
Glucosylceramidase OS Apis mellifera	A0A088APM5_APIME					42.2701	15.6591
Uncharacterized protein OS Apis mellifera	A0A088AQK1_APIME					29.5238	1.3666
Uncharacterized protein OS Apis mellifera	A0A088ARX6_APIME					7.7236	1.3544
Uncharacterized protein OS Apis mellifera	A0A088ASF2_APIME					13.3038	1.3003
Uncharacterized protein OS Apis mellifera	A0A088AU20_APIME					51.7241	16.6315
Uncharacterized protein OS Apis mellifera	A0A088AU21_APIME					68.4211	16.5041
Uncharacterized protein OS Apis mellifera	A0A088AU22_APIME					68.0191	39.897
Uncharacterized protein OS Apis mellifera	A0A088AU26_APIME					83.8235	1.4387
Uncharacterized protein OS Apis mellifera	A0A088AU27_APIME					76.4045	10.4338
Polygalacturonase inhibitor protein 15 OS Brassica napus	A9YBZ6_BRANA	42.6513	1.5934	44.6686	1.5934	36.0231	0.6289
Major royal jelly protein 8 OS Apis mellifera	B3GM11_APIME					10.3614	1.4027
Major royal jelly protein 1 OS Apis mellifera	C6K481_APIME					86.3426	265.2684
Major royal jelly protein 2 OS Apis mellifera	C6K482_APIME					84.5133	267.9784
Major royal jelly protein 4 OS Apis mellifera	D3JXA7_APIME					52.3707	10.4773
MRJP5 OS Apis mellifera	D3JZ08_APIME					56.3545	133.1504
Hexamerin 110 OS Apis mellifera	D3KZF8_APIME					18.57	0
Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae strain ATCC 204508 S288c	ADH1_YEAST	46.5517	25	46.5517	25	34.1954	25
Alpha glucosidase OS Apis mellifera	Q25BT6_APIME					78.3069	17.5721
Major royal jelly protein 3 OS Apis mellifera carnica	Q3L632_APICA					65.1123	18.3481
Chemosensory protein 1 OS Apis mellifera	Q3LBA7_APIME					42.2414	1.8087
Major royal jelly protein 9 OS Apis mellifera	Q4ZJX1_APIME					35.6974	12.0965
Major royal jelly protein 7 OS Apis mellifera	Q6IMJ9_APIME					77.2009	131.8121
Major royal jelly protein MRJP6 OS Apis mellifera	Q6W3E3_APIME					68.4211	59.2834
Polygalacturonase OS Brassica napus	Q7Y1T6_BRANA	68.0101	53.2394	68.0101	53.2394	53.1486	13.8384

Polygalacturonase inhibitory protein Fragment OS Brassica napus	Q8LJS4_BRANA	60.3226	9.2852	62.5806	9.2852	34.1936	0.8992
Alpha amylase OS Apis mellifera mellifera	Q8N0N7_APIME					69.7769	16.5656
Alpha amylase OS Apis mellifera	Q9U8X5_APIME					73.0223	18.4879