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METABOLOMIC VARIATION OF *BRASSICA RAPA* VAR. *RAPA* (VAR. RAAPSTELEN) AND *RAPHANUS SATIVUS* L. AT DIFFERENT DEVELOPMENTAL STAGES

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Abstract

Brassica rapa (var. raapstelen) and *Raphanus sativus* (red radish) are being used as food and fodder while also known as model in recent plant research due to the diversity of metabolites as well as genetic resemblance to *Arabidopsis*. This study explains the change in metabolites (amino acids, organic acids, chlorophyll, carotenoids, tocopherols, ascorbic acid, sucrose, phenylpropanoids and glucosinolates) during plant development. In present study the metabolomic variation in relation to plant growth has been evaluated, for *Brassica rapa* (var. raapstelen) and red radish (*Raphanus sativus*) at three different developmental stages. A non-targeted and targeted metabolomic approach by NMR and HPLC in combination with Principal component analysis (PCA) of the data was used to identify phytochemicals being influenced by plant growth. The results lead to the better understanding of metabolic changes during plant development and show the importance of plant age with respect to the metabolomic profile of vegetables.

Introduction

Raphanis sativus (red radish) and *Brassica rapa* (var. raapstelen) are among *Brassicaceae* vegetables known for its nutritional compounds (Curtis, 2003; Zhao-liang *et al.*, 2008) and are being used as model in recent plant research, due to their short growth time, ease to grow, small size and food value (Klinger *et al.*, 1991). *Brassica* vegetables are a good source of health promoting phytochemicals, in terms of containing primary and secondary metabolites including, amino acids, glucosinolates (Rossetto *et al.*, 2013), vitamins (ascorbic acid, carotenoids, tocopherols, folate, etc.), phenolics and sugars etc. (Jahangir *et al.*, 2009). These compounds play an essential role for human and animal nutrition (Barnes, 2008; Kusznierewicz *et al.*, 2008). The health supporting role of *Brassica* vegetables is attributed to aforementioned compounds, including minerals and vegetable oil content (Huber *et al.*, 2009). While on the other hand the importance of dietary fiber is also well known (Rodriguez *et al.*, 2006). *Brassica* vegetables are also a good source of antioxidants (Soengas *et al.*, 2012).

These phytochemicals are also vital for plant survival (Zhao *et al.*, 2007), by either providing nutrition source for plant growth or protecting it from cell damage by biotic and abiotic stress including their role in plant defense signaling pathway (Williams *et al.*, 2004). These stress factors also disturb the balance in endogenous growth regulators (Zehra *et al.*, 2013). There is a substantial and significant nutritional variation, both within and between the subspecies (Singh *et al.*, 2007). Whereas, apart from different infections, a range of other conditions influence the nutritional profile of *Brassicaceae* vegetables, including biological (Kusznierewicz *et al.*, 2008) and seasonal variation (Yosr *et al.*, 2013), pre-harvest growth factors and post-harvest processing conditions (Lisiewska *et al.*, 2008). In the same way the harvest time also affects the phytochemicals related quality profile of *Brassica* vegetables (Scalzo *et al.*, 2007). Plant developmental stage is considered as detrimental crucial factor for the quality and quantity of health promoting compounds in vegetables (Vallejo *et al.*, 2003).

Metabolomics is defined as both the qualitative and quantitative analysis of a large range of metabolites present in an organism or extract (Hendriks *et al.*, 2011). By using a number of spectroscopic and chromatographic tools as a system biology approach, biological systems are visualized and queried. Among these tools the most common and well known metabolomics platforms are nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), high performance liquid chromatography – mass spectrometry (HPLC – MS), and gas chromatography – mass spectrometry (GC-MS) (Sumner *et al.*, 2003; Kim *et al.*, 2011).

A significant amount of data is available on the effect of biotic or abiotic factors on the plant metabolome. On the other hand there is little information concerning plant age dependent metabolomic variation. Such development based metabolomic alteration can be a significant variable that determine nutritional alteration, such as drop in sugar accumulation in berries (Zhaosen *et al.*, 2014). The objective of this study is to follow the metabolomic changes during plant growth and development, affecting the nutritional profile of the plants. So metabolomic profile at three different developmental stages of *Brassica rapa* (var. raapstelen) and *Raphanus sativus* (red radish) have been studied by metabolomics, using both untargeted (NMR) and targeted (HPLC) analyses approaches.

Material and Methods

Plant material: Seeds of both *Raphanus sativus* (red radish) purchased from Carl Sperling & Co. GmbH, Germany, and *Brassica rapa* (var. raapstelen, Groene Gewone) purchased from Pieterpikzonen b.v. Holland, were sown in pots containing soil and kept in cold room (4°C) for 2 days and then transferred to a green house in 16 : 8 hours, light : dark conditions. After 6 days of germination, the individual seedlings were transferred to separate pots and watered daily.

Sample preparation: Plants were harvested at 6, 8 and 10 weeks of age (starting from germination) and were washed with deionised water, dried smoothly with a tissue paper. Both the roots and leaves were separated from each other and weighed to determine fresh weight and placed immediately in separate aluminum foils to be frozen in liquid nitrogen. All the samples were grinded to a fine powder in liquid nitrogen and freeze dried in aluminum wrapped containers till a constant weight. After freeze drying the samples were weighed for dry weight and then stored at −80°C until extraction and analysis.

NMR based metabolomic assessment: A non-targeted analysis of metabolites was performed by NMR. NMR sample preparation, sample analysis and data processing was done as previously reported (Jahangir *et al.*, 2008). This method has the ability of simultaneously detecting diverse groups of metabolites in a single run with a strong reproducibility. Identification of metabolites was done by ^{1}H-NMR, along with 2D spectra including J-resolved, COSY and HMBC analysis. Quantitation of amino acids (alanine, valine, threonine), organic acids (fumaric acid, γ-amino butyric acid), sugars (sucrose, total glucose) was performed by calculating the relative ratio of the peak area for selected proton signals of the target compounds, to the known amount of TMSP (trimethyl silyl propionic acid) as internal standard.

Glucosinolates assessment: Glucosinolate extraction and desulphation was carried out as reported previously (van Dam *et al.*, 2005). For glucosinolate extraction, a 100 mg of freeze dried sample was weighed in a 15 ml glass tube and extracted with 2 ml of boiling 70% methanol solution, desulphated with arylsulphatase (Sigma, St Louis, IL) on a DEAE-Sephadex A25 column prepared by 0.5 ml of sephadex in a Pasteur pipette column, and separated on a reversed phase C-18 column (Alltima C-18, 150 × 4.6 mm, 3 μm; Alltech, Breda, The Netherlands) on HPLC with an acetonitrile–water gradient (0–65% acetonitrile from 0 to 30 min; flow 0.75 ml min−1). Detection was performed with a photodiode array detector and peaks were integrated at 229 nm. We used the response factors for detection at 229 nm by using a sinigrin standard curve for quantitative analysis.

Ascorbic acid (vitamin-C) assessment: For vitamin C, analysis was done by HPLC-PDA as reported previously (Helsper *et al.*, 2003), using 10 mg of freeze dried sample weighed in 10 ml glass tube, extraction in 2 ml of ice cold 5% meta-phosphoric acid (Sigma ACS; 35%), ultrasonication for 15 minutes and centrifugation at 2500 rpm for 10 minute, followed by filtration over a 0.2 μm PTFE filter into 1.8 ml HPLC vials.

Isoprenoid assessment: Freeze dried samples were weighed as 50 mg in a 15 ml glass tube. The tocopherols (α, β, γ, δ) and carotenoids (lutein, β-carotene, 9-cis-β-carotene, violaxanthin, neoxanthin), chlorophylls (A, B) were extracted with chloroform and analyzed by HPLC with PDA and fluorescence detection as previously reported (Helsper *et al.*, 2003; Bino *et al.*, 2005).

Data analysis: Data processing, scaling and bucketing for ^{1}H NMR is done as reported previously (Kim *et al.*, 2010). For the quantitative data processing, different metabolites were quantified by either NMR or HPLC. Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were performed with the SIMCA-P software (v. 12.0, Umetrics, Umeå, Sweden) based on a unit-variance scaling method as reported previously (Jahangir *et al.*, 2008). For better understanding of results, we used metabolomic data of leaves (Fig. 1A) and roots (Fig. 1B), separately for PCA analysis.

Results and Discussion

The amino acids (acetate, alanine, γ-amino-butyric acid (GABA), serine, threonine, valine, phenylalanine and tyrosine), organic acids (glutamate, glutamine, malate, fumarate), carbohydrates (sucrose and glucose), malate conjugated phenylpropanoids (caffeoyl malate, coumaroyl malate, feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate) were identified in *Brassica* and *Raphanus*, and quantified by NMR (Tables 1 & 2; supplementary data).

Further analysis by HPLC additionally proved the changes in ascorbic acid (vitamin C) (Table 2; supplementary data), chlorophyll (A and B) (Table 3; supplementary data), carotenoids (lutein, β-carotene, 9-cis-β-carotene, violaxanthin and neoxanthin) (Table 4; supplementary data), tocopherols (α, β, γ and δ–tocopherol) (Table 5; supplementary data) and glucosinolates (glucobrassicin, glucoerucin, gluconapin, gluconasturtiin, glucoraphanin, neoglucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, 3OH-propylglcosinolate, sinigrin) (Tables 6 & 7; supplementary data).

The data obtained by HPLC and NMR was analyzed by Principal Component Analysis (PCA), where HPLC results were found similar to the PCA from the NMR bucket data and is shown in same figure (Fig. 1). The results are presented in figure 1, separately for leaves (Fig. 1A) and roots (Fig. 1B). To further illustrate the quantitative differences between samples, bar charts are presented for carotenoids content (violaxanthin and neoxanthin) of the leaves (Fig. 2A), whereas results for ascorbic acid (Fig. 2B) and glucosinolates (Fig. 3) are presented for both the roots and leaves of *Brassica rapa* and *Raphanus sativus*.

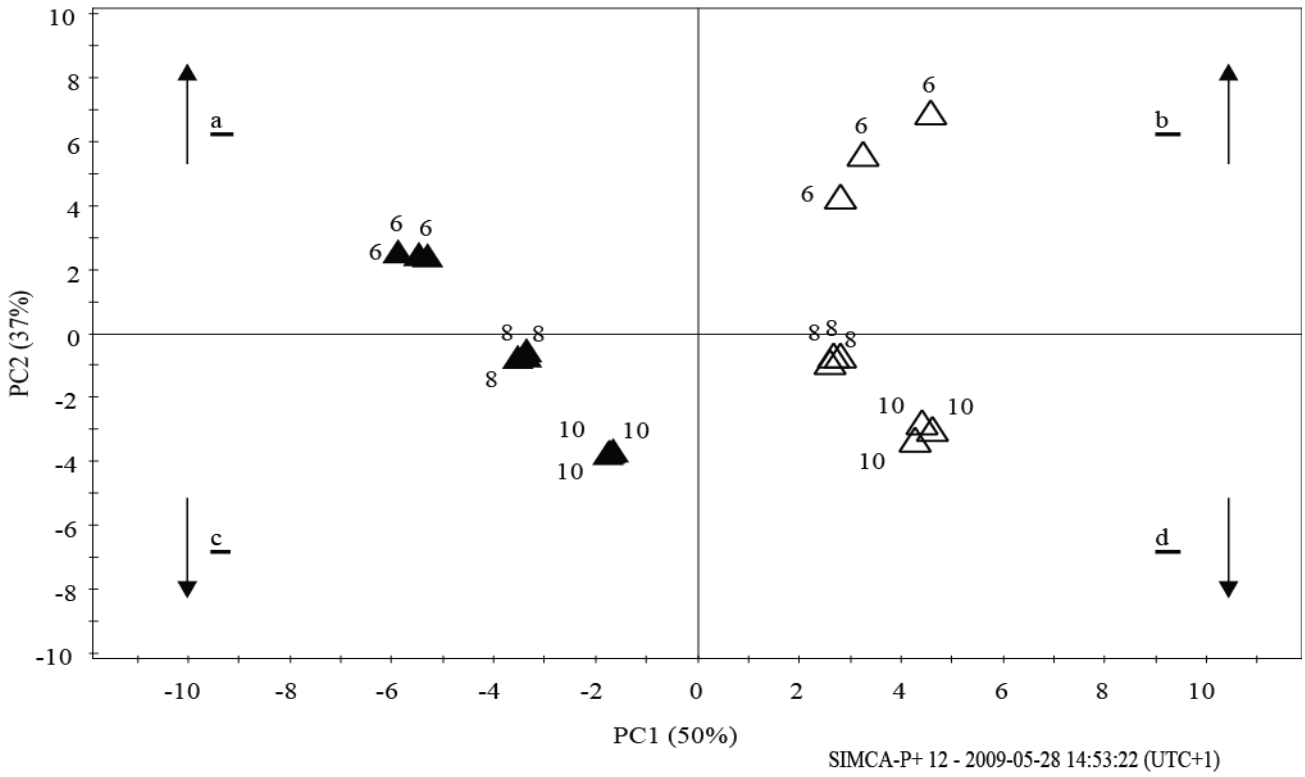

Fig. 1A. Score plot of PCA for *Brassica rapa* (var. Raapstelen) and *Raphanus sativus* (radish) leaves, based on whole range of ^{1}H-NMR signals (δ 0.3 – δ 10.0) (^{1}H NMR bucket data), ^{1}H NMR and HPLC (quantitative) data. Radish, 6 week (6▲); 8 week (8▲); 10 week (10▲) old plants. *Brassica rapa*, 6 week (6Δ); 8 week (8 Δ); 10 week (10 Δ) old plants. **a** = Glutamine, glutamate, alanine, threonine, valine, chlorophyll, leutin, *β*-carotene, 9-cis-β-crotene, neoxanthin, violaxanthin, 3OH-propylglucosiolate. **b** = Ascorbic acid, glucose, fumaric acid, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, gluconasturtiin, gluconapin, glucoraphanin, sinigrin. **c** = Phenylpropanoids, α-tocopherol, δ-tocopherol, glucobrassicin. **d** = β-tocopherol, γ-tocopherol, sucrose, dry weight, neo-glucobrassicin.

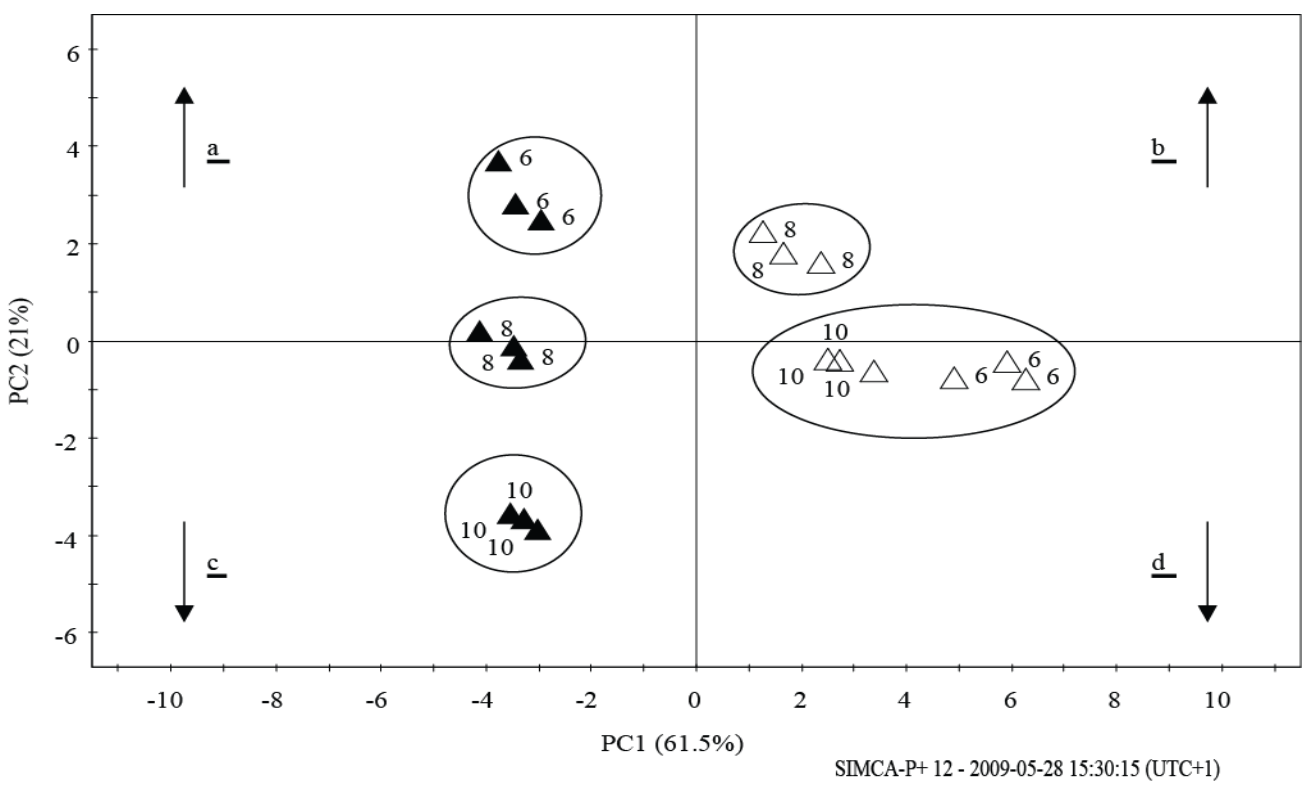

Fig. 1B. Score plot of PCA for *Brassica rapa* (var. Raapstelen) and *Raphanus sativus* (radish) roots (B), based on whole range of ^{1}H-NMR signals (δ 0.3 – δ 10.0) and HPLC data. Radish, 6 week (6▲); 8 week (8▲); 10 week (10▲) old plants. *Brassica rapa*, 6 week (6Δ); 8 week (8 Δ); 10 week (10 Δ) old plants. **a** = Fumaric acid, β-carotene, γ-amino butyric acid. **b** = threonine, glucose. **c** = Phenylpropanoids, valine, leutin, alanine, glucobrassicin. **d** = sucrose, dry weight, 3OH-propylglucosiolate, glucoraphanin, sinigrin, 4-hydroxyglucobrassicin, gluconasturtiin, gluconapin, neo-glucobrassicin.

Table 1. Table of mean and standard deviations of *Raphanus sativus* (radish) leaves (RHL), roots (RNR), *Brassica rapa* (var. Raapstelen) leaves (RNL) and roots (RHR), for sucrose, glucose and dry weight.

	Sucrose (mg/g D.W.)	Glucose (mg/g D.W.)	% Dry weight (g/100 g D.W.)
RHL-6	3.59 ± 0.21	9.44 ± 0.32	6.49
RHL-8	6.10 ± 1.38	8.19 ± 0.62	7.59
RHL-10	11.03 ± 0.04	6.47 ± 0.35	8.53
RHR-6	16.96 ± 3.66	199.71 ± 9.42	8.02
RHR-8	31.42 ± 3.25	160.84 ± 1.45	7.87
RHR10	77.30 ± 1.62	125.30 ± 2.53	9.03
RNL-6	9.94 ± 0.54	27.13 ± 0.48	11.11
RNL-8	18.25 ± 1.70	13.89 ± 0.02	12.50
RNL-10	20.29 ± 1.57	15.13 ± 0.79	15.38
RNR-6	67.56 ± 1.14	195.84 ± 2.50	13.04
RNR-8	46.17 ± 1.27	222.78 ± 2.12	13.74
RNR10	66.96 ± 1.69	175.36 ± 1.19	16.20

Table 2. Table of mean and standard deviations of *Raphanus sativus* (radish) leaves (RHL), roots (RNR), *Brassica rapa* (var. Raapstelen) leaves (RNL) and roots (RHR), for amino acids and organic acids (mg/g D.W.).

	Ascorbic acid	Fumaric acid	γ-Amino butyric acid	Valine	Threonine	Alanine
RHL-6	6.88 ± 0.20	0.06 ± 0.01	ND	0.33 ± 0.04	0.67 ± 0.05	0.41 ± 0.01
RHL-8	4.96 ± 0.15	0.02	ND	0.20 ± 0.02	0.59 ± 0.01	0.17 ± 0.01
RHL-10	4.10 ± 0.05	0.02 ± 0.01	ND	0.16 ± 0.02	0.60 ± 0.03	0.11 ± 0.01
RHR-6	3.92 ± 0.25	1.05 ± 0.12	5.02 ± 0.33	0.85 ± 0.04	0.59 ± 0.04	0.38
RHR-8	3.91 ± 0.05	0.75 ± 0.16	3.02 ± 0.48	0.77 ± 0.01	0.49 ± 0.02	0.40 ± 0.01
RHR10	4.42 ± 0.06	0.48 ± 0.14	2.19 ± 0.12	0.78 ± 0.01	0.39 ± 0.01	0.59 ± 0.01
RNL-6	8.23 ± 0.13	0.50 ± 0.02	ND	0.11 ± 0.01	0.52 ± 0.02	0.25 ± 0.01
RNL-8	6.30 ± 0.11	0.49 ± 0.02	ND	0.11 ± 0.01	0.41 ± 0.01	0.11
RNL-10	5.97 ± 0.19	0.49 ± 0.02	ND	0.09 ± 0.05	0.36 ± 0.03	0.10 ± 0.06
RNR-6	4.76 ± 0.04	0.45 ± 0.22	1.42 ± 0.34	0.18 ± 0.01	0.56 ± 0.03	0.26 ± 0.02
RNR-8	4.25 ± 0.06	0.75 ± 0.17	1.79 ± 0.13	0.24 ± 0.02	0.46 ± 0.01	0.20 ± 0.01
RNR10	4.33 ± 0.16	0.46 ± 0.09	1.37 ± 0.14	0.27 ± 0.01	0.53 ± 0.02	0.17 ± 0.01

Table 3. Table of mean and standard deviations of *Raphanus sativus* (radish) leaves (RHL), roots (RNR), *Brassica rapa* (var. Raapstelen) leaves (RNL) and roots (RHR), for chlorophyll (mg/g D.W.).

	chlorophyll-A (mg/mg)	chlorophyll-B (mg/mg)
RHL-6	8.87 ± 0.20	3.17 ± 0.08
RHL-8	6.88 ± 0.13	2.50 ± 0.04
RHL-10	4.82 ± 0.02	1.74 ± 0.01
RHR-6	ND	ND
RHR-8	ND	ND
RHR10	ND	ND
RNL-6	5.73 ± 0.08	1.99 ± 0.02
RNL-8	4.01 ± 0.10	1.54 ± 0.02
RNL-10	2.24	0.81
RNR-6	ND	ND
RNR-8	ND	ND
RNR10	ND	ND

Table 4. Table of mean and standard deviations of *Raphanus sativus* (radish) leaves (RHL), roots (RNR), *Brassica rapa* (var. Raapstelen) leaves (RNL) and roots (RHR), for carotenoids (µg/g D.W.).

	Lutein	β-carotene	9-cis-β-carotene	Violaxanthin	Nioaxanthin
RHL-6	0.06	0.04	0.01	400.35 ± 11.10	664.43 ± 20.55
RHL-8	0.05	0.03	0.01	274.62 ± 7.41	512.73 ± 10.30
RHL-10	0.04	0.02	0.00	177.10 ± 0.72	334.83 ± 5.18
RHR-6	ND	ND	ND	ND	ND
RHR-8	ND	ND	ND	ND	ND
RHR10	ND	ND	ND	ND	ND
RNL-6	0.04	0.02	0.01	264.02 ± 8.88	367.70 ± 8.12
RNL-8	0.03	0.01	0.01	204.15 ± 4.12	291.25 ± 6.10
RNL-10	0.02	0.01	0.00	118.80 ± 1.245	142.51 ± 5.32
RNR-6	ND	ND	ND	ND	ND
RNR-8	ND	ND	ND	ND	ND
RNR10	ND	ND	ND	ND	ND

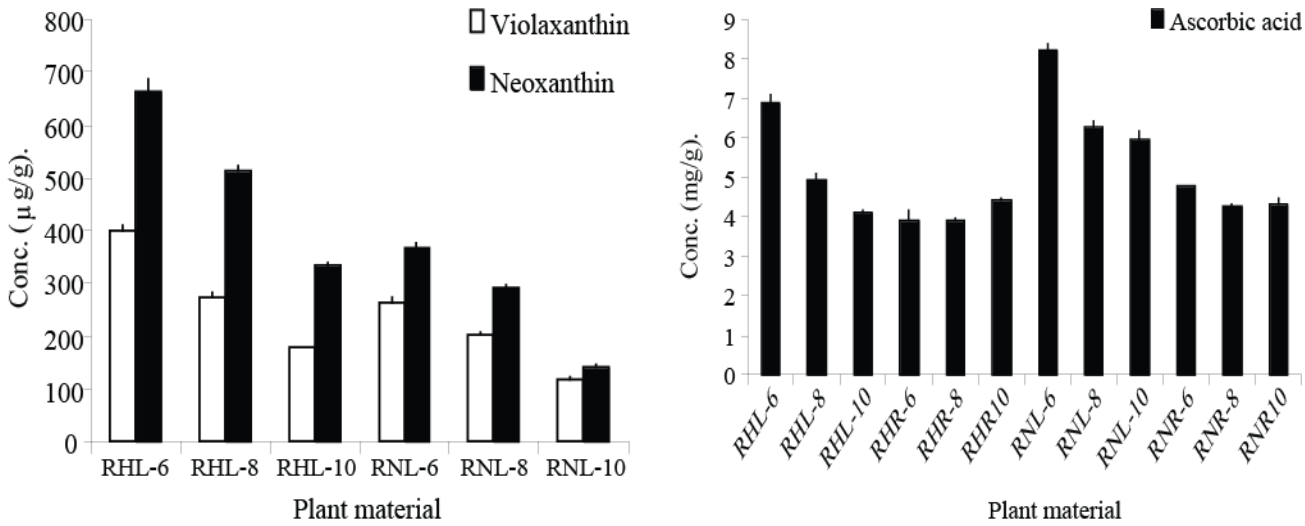

Fig. 2. Concentration of violaxanthin and neoxanthin (µg/g of dry weight) (Fig. 2 A) and concentration of ascorbic acid (mg / g of dry weight) (Fig. 2 B) at different developmental stages of *Brassica rapa* (var. raapstelen) and *Raphanus sativus* leaves. RHL = Radish leaves; RHR = Radish roots; RNL = Raapstelen leaves; RNR = Raapstelen roots; 6 week old plant (6); 8 week old plants (8); 10 week old plants (10).

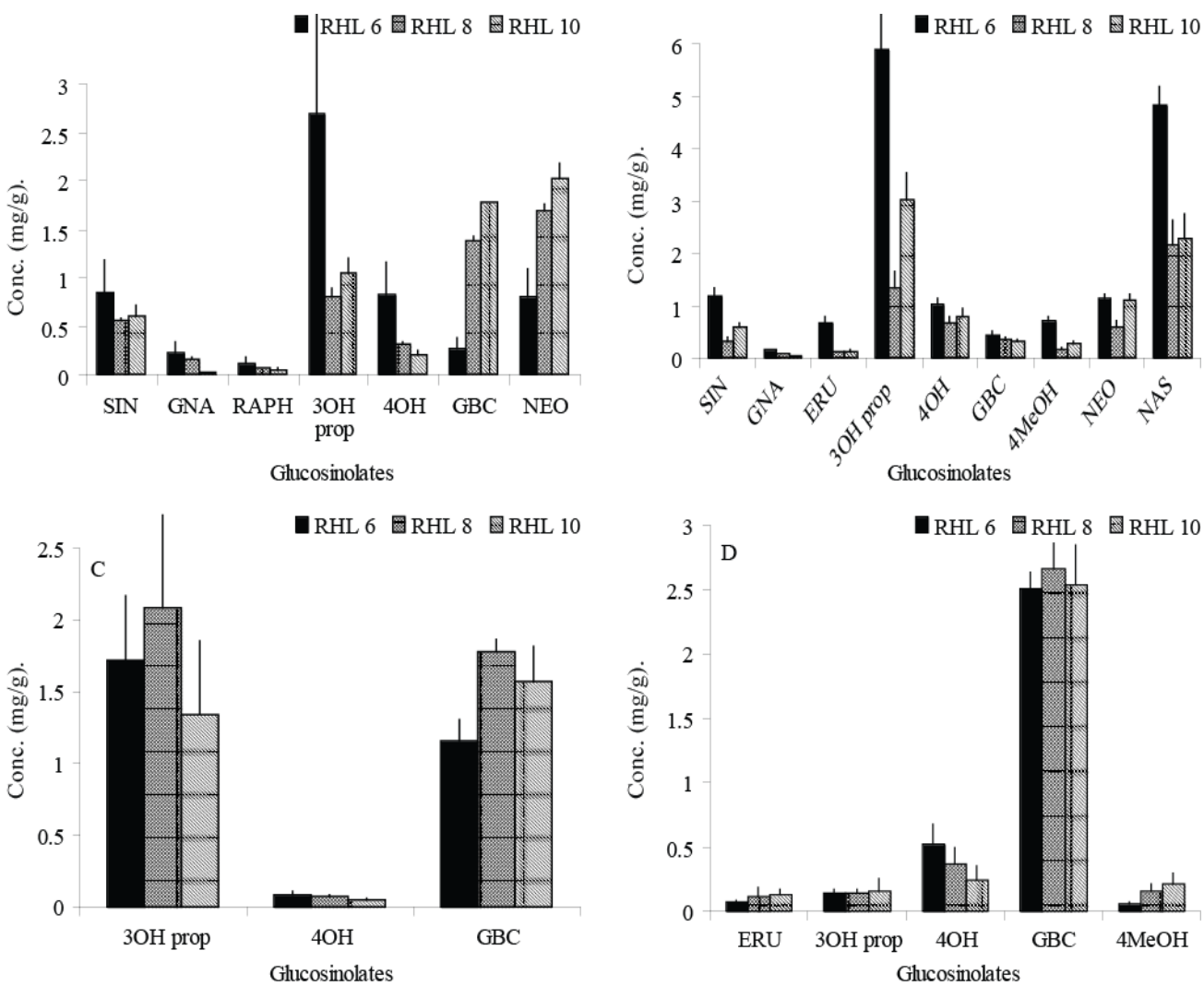

Fig. 3. Concentration of glucosinolates (mg/g of dry weight) at different developmental stages of *Brassica rapa* (var. Raapstelen) (Fig. 3 A & B) and *Raphanus sativus* (radish) (Fig. 3 C & D). RHL = Radish leaves; RHR = Radish roots; RNL = Raapstelen leaves; RNR = Raapstelen roots; 6 week old plant (6); 8 week old plants (8); 10 week old plants (10). Glucobrassicin (GBC), Glucoerucin (EU), Gluconapin (GNA), Gluconasturtiin (NAS), Glucoraphanin (RAPH), Neo-glucobrassicin (NEO), 4-hydroxyglucobrassicin (4OH), 4-methoxyglucobrassicin (4MeOH), 3OH-propylglcosinolate (3OH prop), Sinigrin (SIN).

Table 5. Table of mean and standard deviations of *Raphanus sativus* (radish) leaves (RHL), roots (RNR), *Brassica rapa* (var. Raapstelen) leaves (RNL) and roots (RHR), for tocopherols (µg/100g D.W.).

	δ-tocopherol	γ-tocopherol	β-tocopherol	α-tocopherol
RHL-6	0.0000584	0.0000880	0.0006004	0.000359237
RHL-8	0.0000712	0.0001352	0.0011351	0.000475871
RHL-10	0.0000742	0.0002176	0.0021684	0.000761418
RHR-6	ND	ND	ND	ND
RHR-8	ND	ND	ND	ND
RHR10	ND	ND	ND	ND
RNL-6	0.0000370	0.0000699	0.0008891	0.000236459
RNL-8	0.0000577	0.0001069	0.0018132	0.000419920
RNL-10	0.0000581	0.0002139	0.0021874	0.000536630
RNR-6	ND	ND	ND	ND
RNR-8	ND	ND	ND	ND
RNR10	ND	ND	ND	ND

Table 6. Table of mean and standard deviations for variation of glucosinolate contents (µg/g D.W.) with developmental stages of *Raphanus sativus* for leaves (RHL) and roots (RHR).

*	ERU	3OH prop	4OH	GBC	4MeOH	NEO
RHL 6	–	1724 ± 449	403 ± 87	1152 ± 156	2 ± 2	–
RHL 8	–	2091 ± 640	321 ± 60	1777 ± 94	10 ± 2	7 ± 3
RHL 10		1345 ± 508	236 ± 55	1576 ± 247	5 ± 3	1 ± 0
RHR 6	69 ± 21	144 ± 25	2461 ± 725	2506 ± 124	54 ± 17	–
RHR 8	114 ± 65	145 ± 23	1710 ± 569	2669 ± 188	155 ± 62	11 ± 13
RHR 10	123 ± 52	151 ± 105	1149 ± 488	2535 ± 315	206 ± 89	–

* = ERU (Glucoerucin), 3OH prop (3OH-Propylglcosinolate), 4 OH (4-Hydroxyglucobrassicin), GBC (Glucobrassicin), 4MeOH (4-Methoxyglucobrassicin), NEO (Neo-glucobrassicin)

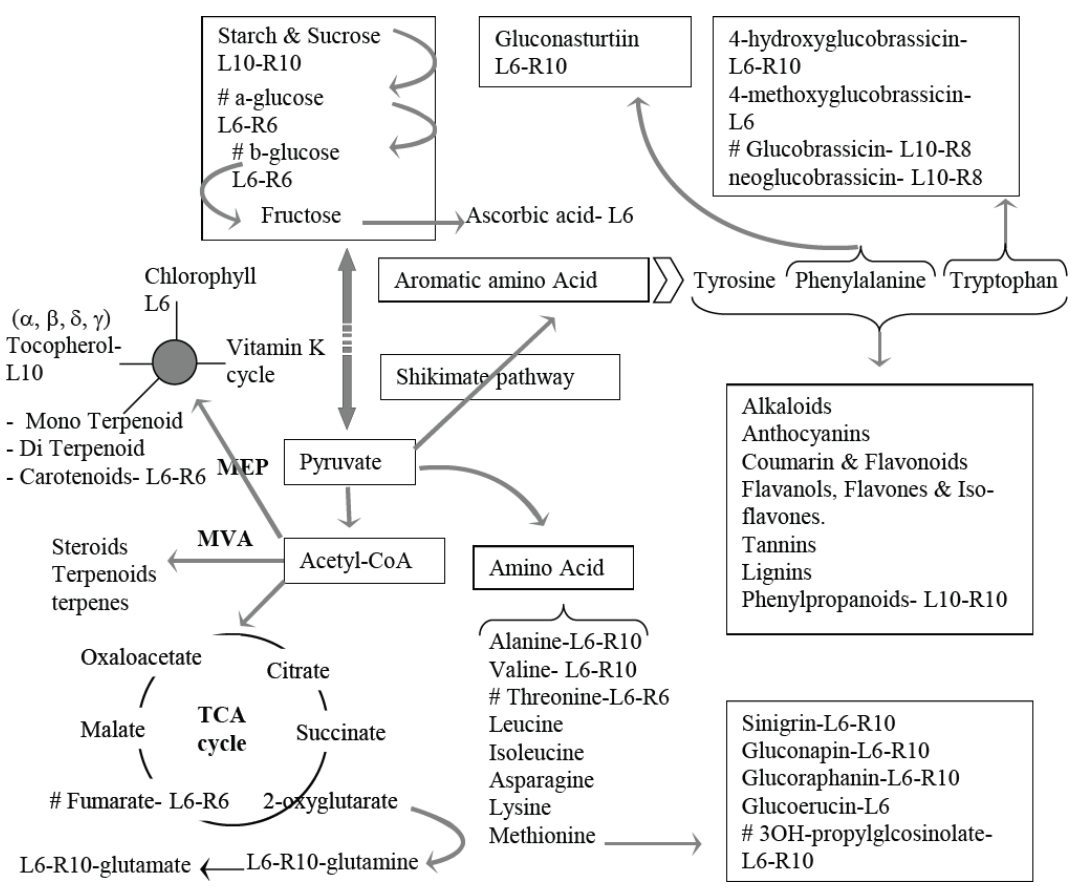

Fig. 4. Change in metabolite quantities during plant growth. L6 = high in 6 week old plants leaves; L8 = high in 8 week old plants leaves; L10 = high in 10 week old plants leaves; R6 = high in 6 week old plants roots; R8 = high in 8 week old plants roots; R10 = high in 10 week old plants roots. # = Fumarate, threonine and glucose are higher in 8 week old roots in case of Brassica rapa while glucobrassicin and 3OH-propylglcosinolate are higher in 8 week old radish leave and glucobrassicin is also higher in 8 week old radish roots. CAC = citric acid cycle; MVA= mevalonic acid pathway; MEP = 2-C-methyl-D-erythritol 4-phosphate pathway.

Table 7. Table of mean and standard deviations for variation of glucosinolate contents (µg/g D.W.) with developmental stages of among *Brassica rapa* (var. Raapstelen) for leaves (RNL) and roots (RNR).

*	RAPH	ERU	SIN	GNA	3OH prop	NAS	4OH	GBC	4MeOH	NEO
RNL 6	121 ± 52	–	845 ± 334	233 ± 90	2685 ± 1035	55 ± 41	3843 ± 1518	274 ± 112	27 ± 13	798 ± 281
RNL 8	61 ± 13	–	558 ± 28	161 ± 12	800 ± 92	–	1401 ± 146	1372 ± 53	3 ± 1	1683 ± 65
RNL 10	39 ± 18	–	605 ± 100	14 ± 11	1044 ± 154	–	938 ± 192	1770 ± 11	14 ± 4	2024 ± 144
RNR 6		677 ± 93	1187 ± 135	138 ± 16	5893 ± 644	4815 ± 344	4790 ± 548	439 ± 53	717 ± 80	1155 ± 64
RNR 8	–	99 ± 14	295 ± 91	63 ± 3	1331 ± 311	2162 ± 462	3022 ± 561	334 ± 70	165 ± 20	587 ± 125
RNR 10	–	129 ± 43	592 ± 90	45 ± 13	3004 ± 508	2267 ± 466	3674 ± 634	295 ± 64	255 ± 57	1099 ± 98

* = RAPH (Glucoraphanin), ERU (Glucoerucin), SIN (Sinigrin), GNA (Gluconapin), 3OH prop (3OH-Propylglcosinolate), NAS (Gluconasturtine), 4 OH (4-Hydroxyglucobrassicin), GBC (Glucobrassicin), 4MeOH (4-Methoxyglucobrassicin), NEO (Neo-glucobrassicin)

Young leaves have more photosynthetic activity and hence are more valuable for plants by providing more energy as compared to old leaves (Reifenrath & Muller, 2007). Similarly by analyzing plants at different developmental stages, further age-dependent changes in primary and secondary metabolites were observed. For leaves of both species (*Brassica rapa* and *Raphanus sativus*), amino acids and organic acids, glucose, ascorbic acid, chlorophyll, carotenoids and glucosinolates (3OH-propylglucosinolate, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, gluconasturtiin, gluconapin, glucoraphanin, sinigrin) are higher in 6 weeks-old plants as compared to the older plant samples, whereas further growth to 10 weeks resulted in an relative increase in sucrose, phenylpropanoids, tocopherols, two glucosinolates (glucobrassicin, neo-glucobrassicin) and dry weight of leaves (Fig. 4) as compared to young plants. Almost similar behavior was noticed in the roots (Fig. 4).

In previous reports for leaves of *B. oleracea* var. *costata*, age also proved to affect the phenolic pattern, where five kaempferol derivatives and 10 cinnamic acid derivatives were found in high amount in young leaves, while *p*-coumaroyl-*3*-*O*-quinic acid and 13 kaempferol derivatives were detected in old leaves (Sousa *et al.*, 2008).

When inter-varietal comparison of leaves of *Brassica rapa* and *Raphanus sativus* was studied, a high amount of amino acids, chlorophyll, phenylpropanoids, carotenoids, α-tocopherol, δ-tocopherol, glucobrassicin and 3-OH-propylglucosiolate was found in *Raphanus sativus*, while dry weight, carbohydrates, ascorbic acid, fumaric acid, β-tocopherol, γ-tocopherol, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, gluconasturtiin, gluconapin, glucoraphanin, sinigrin and neo-glucobrassicin were found higher in *Brassica rapa* leaves (Fig. 1; Tables 6 & 7).

Generally a decrease in glucosinolate content was observed with increasing age of plants (Yábar *et al.*, 2011) of both species, except for glucobrassicin and neo-glucobrassicin which were found to increase in *Brassica rapa* leaves (Fig. 3). For sinigrin, 3OH-propylglucosiolate, 4-hydroxyglucobrassicin and glucobrassicin a different trend was observed: these glucosinolates showed a temporarily increase or decrease in their concentration in 8-weeks old plants, followed by a decrease or increase in 10-week old plants, respectively. This behavior was observed in both leaves and roots, showing that this effect occured in the whole plant (Fig. 3). The glucosinolate profiling showed that 3OH-propylglucosinolate and glucobrassicin are the major glucosinolates in the leaves of both species (Figs. 3A & 3C), while *Brassica rapa* leaves also contained high amounts of neoglucobrassicin (Fig. 3A) as compared to other glucosinolates. Gluconasturtiin and 3OH-propylglucosinolate were found as major glucosinolates in *Raapstelen* roots (Fig. 3B), while glucobrassicin was the major compound in radish roots (Fig. 3D).

Violaxanthin and neoxanthin were the major carotenoids found in leaves of both species (Fig. 2A). A decrease in these carotenoids was observed with increasing the age of plant. The same decreasing behavior in quantity was observed for ascorbic acid in the leaves of both species, while in their roots the concentration of ascorbic acid was found almost static and no significant difference was observed (Fig. 2B). As a whole for metabolites, a similar pattern (although not so profound in case of *Brassica rapa* roots) is observed for the leaf and root parts of both species. This change in metabolomic profile during growth stage may represent the change in metabolomic fluxes in different pathways (Fig. 4).

The present study shows the importance of plant age as a factor for metabolomic variation and changes in nutritional value of plants for human consumption. It appeared that young plants are a better source of nutrients and health promoting compounds as compared to older plants.

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