Studies on volatile compounds of different varieties in *Brassica napus* L.

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Abstract

To compare volatile components of different varieties, volatile compounds were extracted by water distillation approach from entire plants 25 days before maturation in *Brassica napus* L and assayed by capillary gas Chromatography-mass spectrometry (GC-MS). A total of 107 different components were identified. Volatile profile of four varieties Tapidor, Ningyou No.7, NJ5412 and Heza No.7 included 57,51,45 and 43 compounds, respectively. 15 compounds were common among the 4 varieties, including high-content phytol and oleic acid, also including β-damascenone,β-ionone and myristic acid, etc. Volatile profiles were quantitatively and qualitatively different among the varieties. In Tapidor, there were 2 kinds of isothiocynate with relative content 0.36%, but there were 7 kinds of isothiocynate with relative content 2.43% in Ningyou No.7. As to linoleic acid content, it was 18.55% in Ningyou No.7, but less than 2% in the others. As an exception, NJ5412 did not contain insect attractant cis-3-hexenol. Totally, different varieties had different volatile profiles that probably are basis of genotype selection.

Key words: *Brassica napus L*; volatile compounds; GC-MS

Introduction

The volatile compounds play crucial roles in whole growing period of plants. Plants release small amounts of diverse volatile blends including terpenoids, benzenoids, and fatty-acid derivatives, etc, accounting for about 1% of the plant secondary metabolites [1], but that little materials released function in many important aspects such as pollinator attraction, defense and communication [2]. Interestingly, by releasing volatiles, signaling plant can reduce number of herbivores more than 90% [3], and can also warn neighboring plants about the pathogen attack [4]. Recently, great progress has been made in biochemical and molecular characterization on mechanism of plant volatile formation and release. Research achievements imply that controlling release of special volatile compounds benefits well variety development through bioengineering or conventional approach.

In limited reports on *Brassica* volatiles, glucosinolate–myrosinase system (GLS-MYR), which produces special chemical mixtures in defense systems, plays very important roles in antagonizing biotic and abiotic stresses [5]. The main aim of this paper is to provide a preliminary screening of volatiles from different rapeseed varieties for revealing variety differences.

Materials and methods

Plant materials

Four varieties (line) of rapeseed, Tapidor, Heza No.7, Ningyou No.7 and NJ5412, from germplasm bank in Nanjing Agricultural University, were used for evaluation.

Essential oil

500 grams of fresh plants were harvested from jiangpu experiment field about 25 days before maturation, washed, cut into small pieces for volatile extraction. After water distillation in Clevenger apparatus along with 1200ml pure water for 6h, essential oil were extracted by aether, then the extracts were dried in vacuum to eliminate aether. Further drying of the oils were by anhydrous sodium sulphate for overnight and stored at -20°C for determination. Samples diluted with methylformate were injected into GC/MS for volatile molecule recognition.

Instrument analysis

The analysis were carried out with a Finnigan TRACE GC gas chromatograph coupled with a Finnigan TRACE DSQ mass spectrometer equipped with a DB-5 fused silica capillary column(30m × 0.25mm ID × 0.25μm film thickness).The oven temperature programmed from 60°C to 180°C at 6°C,then from 180°C to 220°C at 2°C,further from 220°C to 260°C at 8°C(30min isothermal). Carrier gas was high purity helium(flow rate 1ml/min).Injector temperature was 280°C and the split ratio was 1:30.The transfer-line temperature was 250°C.The mass spectra was acquired with a source temperature of 200°C under a 70eV ionization potential. Full-scan analyses were performed in the mass rang 30-400m/z.

Data was evaluated by Xcalibur 1.3 system software. Identification of the compounds was done by comparing the retention times and by the use of mass spectra database search (NIST MS search 2.0) and retention indices from accessible scientific literature as well as comparison of mass spectra from relevant literature.

Results and discussion

Volatile profiles identified by GC-MS (Fig.1), were quantitatively and qualitatively different among the varieties in *B.*

napus (table 1). The four varieties (Tapidor, Heza No.7, Ningyou No.7 and NJ5412) contained 55, 45, 51, and 44 kinds of compounds, respectively, accounting for 89.03%, 93%, 88.67% and 85.14% in their total volatiles, respectively. To sum up, there were a total of 107 different compounds identified, which may be classified as hydrocarbon, acid, aldehyde, ketone, alcohol, ester, isothiocyanate (ITC), nitrile (CN), sulfide and others. 15 compounds were common to four varieties, including high-content phytol and oleic acid, also including some flavor compounds such as β-damascenone, β-ionone and myristic acid,etc.

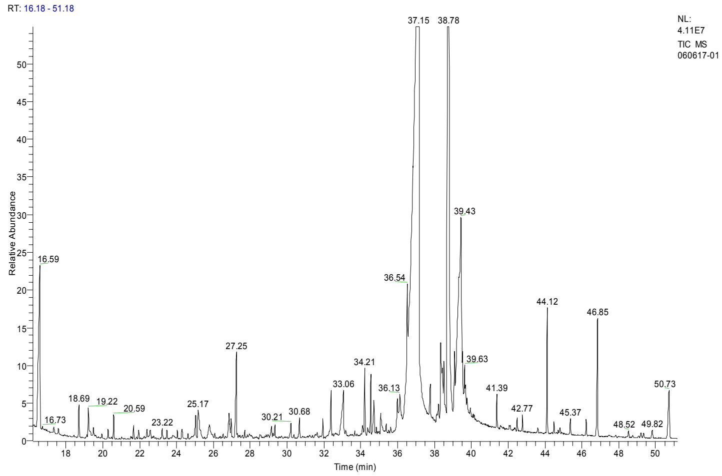

Fig.1 Typical chromatographic profile of the volatile fraction isolated from variety Tapidor

Acid fraction accounted for 63.98%, 73.97%, 68.91% and 62.52% in variety volatiles of Tapidor, Heza No.7, Ningyou No.7 and NJ5412, respectively. Acids with 18-carbon were main parts in acid fraction of volatiles, including hexadecanoic acid, oleic acid, linoleic acid, but for different varieties, main components and constitutions were different. Main parts were hexadecanoic acid (30.6%) and oleic acid (29.1%) for Tapidor, n-tridecanoic acid (25.6%) and oleic acid (40.9%) for Heza No.7, n-tridecanoic (32.1%) acid and linoleic acid (18.5%) for Ningyou No.7, n-tridecanoic acid (10.8%) and oleic acid (47%) for NJ5412. This phenomena that fatty acid may be high content in one variety volatile but low in another, may imply that different varieties have different metabolism mechanism or defense system. In addition, a interesting result may be that odd-numbered fatty acids unusual in plants, of high-content in three varieties of *B. napus*, were discovered for the first time.

Relatively nonreactive GLS in *B. napus* are easy to be hydrolyzed by myrosinase and converted to unstable aglycones. These intermediates then undergo a spontaneous rearrangement to produce a variety of toxic metabolites such as isothiocyanates (ITC), nitriles (CN), and thiocyanates [6]. By GC-MS technique that can be used as a way to volatile molecular identification, decomposed products like volatile ITCs and CNs can be identified. Present reports revealed 7 ITC, and 1 CN. From literatures, among the ITCs in this research, 3-butenyl ITC and 2-phenylethyl ITC were known to be involved in plant-insect communication [7]. GLS of ITC with cyclopentane side-chain structure was discovered firstly in *B. napus*, other GLS were in accordance with earlier reported in *brassicaceae* [8].

Agreeing with known information of varieties, this results revealed that GLS believed to be involved in plant-insect interactions as antibiotic effectors [9] and as antinutritional compounds to animals [10], was quantitatively and qualitatively different between double-high rapeseed and canola varieties, and for Ningyou No.7, containing 7 ITC and 1 CN implied its better stress tolerance. Except ITC, other 6 kinds of sulfur-containing volatiles characteristic of flavour and odour, were confirmed in our study. These sulfur-containing materials were also observed in earlier reports on biosynthetic induction experiments of sulfides regarded as effectors of insect herbivory [11].

Cis-3-hexenol as an insect attractant is the precursor for the straight-chain esters [12]. There was a remarkable increase in catches of the boll weevil when traps were baited with cis-3-hexenol combining with the boll weevil aggregation pheromone [13]. In addition, field baits of cis-3-hexenol and the pheromone enhanced the number of female *Plutella xylostella* in *B. oleracea subsp. capitata* caught in traps several fold over those baited with natural attractant alone [14]. Comparing varieties, we found that NJ5412 did not have cis-3-hexenol, but other three varieties had it with high content. This was a fact noteworthy, revealed that mechanism why canola line NJ5412 usually does not have insect damage.

In addition, other sense compounds such as thujopsene, safranal, β-linalol and 4-terpineol, were also identified, which are antibacterial molecules. Many other materials listed in table 1, are not discussed here. In total, different varieties had different volatile profiles that probably are basis of genotype selection.

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Table 1.The Relative contents and classes of compounds from Tapidor (Td), Heza No.7 (Hz), Ningyou No.7 (Ny) and NJ5412 (NJ)

Compounds	Td	Hz	Ny	NJ	Compounds	Td	Hz	Ny	NJ
Hydrocarbon					trans-Geranylacetone	0.18	0.05	0.06	0.13
cis-3-Nonene	-	-	-	0.75	2-Tridecanone	-	-	0.03	-
n-Dodecane	0.02	-	-	-	2-Hexadecanone	0.46	-	-	-
1,4,6-Trimethyl-1,2-dihydronaphthalene	-	-	-	0.38	Farnesyl acetone	0.37	-	-	-
2,5,8-Trimethyl-1,2-dihydronaphthalene	0.23	0.02	0.09	-	(2E)-1-(2,6,6-Trimethyl-1-Cyclohexen-1-yl)-2-Buten-1-one	0.18	-	-	0.11
Tridecane	0.02	-	-	-	Methyl 14-methylpentade canoate	0.16	-	-	-
Thujopsene	0.24	-	-	-	Hexahydeofamesyl acetone	0.36	0.12	0.42	0.22
2,6,10-Trimethyltetradecae	0.24	0.36	1	-	Methylheptadecyl ketone	-	0.34	0.42	0.3
Nonadecane	-	1.46	0.43	-	15-Nonacosanone	1.09	-	-	-
Heneicosame	5.58	-	-	-	**Alcohol**				
Heptacosane	-	0.45	0.42	1.53	cis-3-Hexenol	2.05	1.41	1.04	-
Acid					trans-2-Methylcyclopentanol	0.28	-	-	-
Hexadecanoic acid	30.6	0.69	7.63	0.59	cis-5-Octen-1-ol	-	-	-	0.59
Oleic acid	29.1	40.9	1.91	47	β-Linalol	-	-	0.01	-
n-Tridecanoic acid	-	25.6	32.1	10.8	4-Terpineol	0.03	-	0.04	-
Linoleic acid	0.75	1.25	18.5	0.4	β-Citrylideneethanol	-	0.12	-	-
d-(+)-Glyceric acid	0.02	-	-	-	1-Tridecanol	0.18		0.73	
Glutaconic acid	-	-	0.07	-	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl) but-2-en-1-ol	-	0.1	0.13	-
Decanoic acid	-	-	-	0.24	Phenol, 2,5-di-tert-butyl-	-	0.1	-	-
Lauric acid	-	0.52	0.48	0.56	Farnesol	-	0.19	-	0.56
Myristic acid	1.54	2.83	3.29	1.69	(2Z, 5Z) -2, 5-Pentadecadien-1-ol	-	-	-	0.05
(Z) -9-Tetradecenal	-	-	-	0.29	Hexa-hydro-farnesol	0.12	0.05	0.12	0.11
Pentadecylic acid	-	0.57	1.42	-	13-Heptadecyn-1-ol	-	-	0.21	-
9-Hexadecenoic acid	0.4	0.35	-	-	2-Methylhexadecan-1-ol	0.02	-	-	-
7-Methyl-Z-tetradecen-1-ol acetate	0.12	0.28	-	-	1,2-Methyl-E,E-2,13-octadecadien-1-ol	0.24	0.17	-	-
Z-10-Methyl-11-tetradecen-1-ol propionate	-	-	1.31	-	Phytol	5.43	8.93	7.42	4.94
α-Linolenic acid	0.12	-	2.03	-	**Ester**				
18-Nonadecenoic acid	-	-	-	0.89	Isovalericacid cis-3-hexenyl ester	0.05	-	-	-
cis-8,11,14-Eicosatrienoic acid	1.25	0.35	0.07	-	cis-3-Hexenyl benzoate	0.05	-	-	-
Z-14-Octadecen-1-olacetae	-	0.56	-	-	n-Hexyl salicylate	0.1	-	-	-
Aldehyde					Acetic acid, phenyl-, 3-hexenyl ester	0.03	-	-	-
Safranal	0.06	-	-	-	Phthalic, diisobutyl ester	0.63	-	-	-
β-Cyclocitral	0.12	0.05	0.12	-	Methyl (9E, 12E) 9,12-hexadecadienoate	-	-	-	0.19
2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	0.07	-	0.03	-	Octyl-10-undecenoate	-	0.45	-	-
(4E) -2-Methyl-4-octenal	-	-	-	0.05	Phthalic scid,butyl ctyl ester	-	0.49	0.86	0.13
n-Nonaldehyde	0.1	0.13	0.09	0.51	**Isothiocyanate(ITC)**				
(E) -2-Nonenal	-	-	-	0.08	3-Butenyl ITC	-	0.14	0.46	-
n-Decadehyde	0.13	0.09	0.07	0.11	2-Phenethylester ITC	0.33	1.46	1.21	0.48
(E,E)-2,4-Decadienal	-	-	0.03	0.24	Cyclopentane ITC	-	0.14	0.46	0.24
trans-2-Decenal	-	-	-	1.88	Butyl ITC	-	-	0.07	-
n-Undecanal	0.13	-	0.09	-	4-Methylpentyl ITC	-	0.02	0.09	-
2-Undecenal	-	-	-	0.33	Hexyl ITC	-	-	0.06	-
Dodecanal	-	-	0.15	-	n-Heptyl ITC	0.03	0.05	0.07	-
Tridecylic aldehyde	1.78	-	-	-	Nitrile(CN)				
Cinnamaldehyde,β-hexyl	-	-	0.03	-	3-Phenylpropionitrile	-	0.02	0.03	-
Myristaldehyde	1.27	0.9	2.44	0.94	**Sulfide**				
(11E)-11,13-Tetradecadienal	-	-	-	0.08	Trisulfide,dimethyl	0.06	0.1	0.06	1.45
(Z) -7-Tetradecenal	-	0.04	-	-	Dimethyl tetrasulphide	-	-	-	0.24
(Z)-7-Hexadecenal	-	-	-	0.35	Methyl methylthiomethyl disulfide	0.05	-	-	-
(3E)-13-Octadecenal	0.93	-	-	-	Methane,(methylsufinyl)methylthio-	-	-	-	0.03
14-Octadecenal	0.13	-	-	-	Sulfide isopentyl methyl	-	-	0.02	-
E-11-Octadecenal	-	-	-	0.5	Sulfide,heptylmethyl	-	0.03	-	-
Ketone					**Others**				
4,5-Dihydro-2 (1H)-pentalenone	0.15	-	-	-	D-Mannose	-	0.02	0.02	-
(2E) -2-Nonen-4-one	-	-	-	0.11	1-Methoxy-1H-indole	-	-	0.04	-
2-Methyl-1-none-3-one	0.05	-	-	-	3-(1-Cyclopentenyl) furan	0.02	-	-	-
β-Damascenone	0.19	0.12	0.18	0.13	Guaia-1 (5),7(11)-diene	-	-	-	0.48
β-Ionone	0.9	0.45	0.51	0.11	8β,13β-Kaur-16-ene	-	0.46	-	4.29
β-Damascone	0.21	-	-	-					

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