Supporting information to

Modulation of Glucosinolate Composition in Brassicaceae Seeds by Germination and Fungal Elicitation by Andini, Dekker, Gruppen, Araya-Cloutier, and Vincken

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Tentative annotation of GSLs in UHPLC-PDA-ESI-MS\textsuperscript{n} analysis

1. General criteria for GSL annotation

In the MS\textsuperscript{2} fragmentation (Table S2), the deprotonated molecular ion [M-H]\textsuperscript{−} of intact GSLs produces sulfated glucosyl ion at m/z 259, which is the signature fragment ion for GSLs (Figure S1).\textsuperscript{1-3} The relative abundance of this fragment ion depends on the side chain. Frequently, this fragment ion is predominant or at a high abundance. (Methylsulfinyl)alkyl GSLs (1, 3, 6, 25) consistently have a predominant fragment ion [M-H-64]\textsuperscript{−} due to a neutral loss of CH\textsubscript{3}SOH (Figure S1).\textsuperscript{2,4}

The other fragment ions of GSLs in the MS\textsuperscript{2}, shown in Figure S1, are at m/z 195, 241, 275, and 291 which are assigned as thiogluicosyl anion, C\textsubscript{6}H\textsubscript{9}O\textsubscript{8}S\textsuperscript{−}, the sulfated thiogluicosyl anion, and C\textsubscript{6}H\textsubscript{11}O\textsubscript{9}S\textsubscript{2}\textsuperscript{−}, respectively.\textsuperscript{2,3,5} The losses of glucosyl moiety ([M-H-162]\textsuperscript{−}), thiogluicosyl moiety ([M-H-196]\textsuperscript{−}), and an intense neutral loss of sulfur trioxide and anhydroglucose ([M-H-242]\textsuperscript{−}) were also observed. This observation is in accordance with Kokkonen et al and Velasco et al.\textsuperscript{6,7}

Two indolic GSLs which exhibit identical molecular masses, i.e. 4-methoxyindol-3-ylmethyl GSL (22) and 1(or N)-methoxyindol-3-ylmethyl GSL (24), were distinguished by comparison with reported elution order during RP-LC separation.\textsuperscript{1,8} In agreement with He\textsuperscript{9}, 4-methoxyindol-3-ylmethyl GSL, which has a C-NH group, is more polar than 1-methoxyindol-3-ylmethyl GSL with its C-N-OCH\textsubscript{3}.\textsuperscript{9} Additionally, in agreement with Olsen et al. and Pfalz et al. loss of OCH\textsubscript{3} from N-methoxy was observed, but not from C-methoxy indolic GSLs (Figure S2).\textsuperscript{10,11}
2. Tentative annotation of GSLs acylated at thioglucosyl group

By extrapolation of MS fragmentation patterns from previous studies on purified acyl derivatives of other GSLs,\textsuperscript{12,13} four peaks were tentatively annotated as sinapoyl (or isomer) derivatives of \(p\)-hydroxybenzyl GSL and 3-butenyl GSL.

In MS\textsuperscript{2}, \textbf{26} \((m/z\ 630)\) was fragmented to the predominant ion \(m/z\ 424\). The fragmentation refers to a loss of 206 Da, which possibly represent a sinapoyl group or another dimethoxy-hydroxycinnamic acid. In addition, a fragment ion at \(m/z\ 223\), which refers to anion of sinapic acid or isomer, was present at a high abundance. A neutral loss of 206 and a fragment ion of 223 have been attributed to a sinapoyl (or isomer) derivative of (S)-2-hydroxy-2-phenethyl GSL (glucobarbarin) where the sinapoyl (or isomer) group was attached at thioglucosyl group.\textsuperscript{12} Furthermore, the position of the sinapoyl (or isomer) group at the thioglucosyl group and not at the side chain was confirmed in our study, as fragment ions at \(m/z\ 465, 401, 481,\) and 497 were observed (Figure S4 and Figure S5). Additionally, Bianco \textit{et al.}\textsuperscript{12} observed a fragment ion at \(m/z\ of 235\) for the purified \(6'-O\)-isoferyl-(S)-2-hydroxy-2-phenethyl GSL, which was proposed to be an ion corresponding to isoferyl attached at \(6'\)-position. Likewise, if sinapoyl (or isomer) group replaces isoferyl group, the fragment ion will have an \(m/z\ of 265\) (Figure S4-A). This fragment ion was observed for \textbf{26}. Furthermore, previous studies have indicated that acylation of GSLs at the thioglucosyl group mostly occurred at the \(6'\)-position.\textsuperscript{12-14} Taken together, \textbf{26} was tentatively annotated as \(6'-O\)-sinapoyl (or isomer)-\(p\)-hydroxybenzyl GSL (Figure S6).

Similar to peak \textbf{26}, peaks \textbf{27}, \textbf{29}, and \textbf{30} had a neutral loss of 206 Da in MS\textsuperscript{2} fragmentation, giving a fragment ion at \(m/z\ 372\), corresponding to 3-butenyl GSL. Based on this, \textbf{27}, \textbf{29}, and \textbf{30} were annotated as sinapoyl (or isomer) derivatives of 3-butenyl GSL. The annotation was also confirmed by a distinct UV absorption maximum at 300-334 nm indicating the occurrence
of an aromatic substituent, in line with Reichelt et al.\textsuperscript{13} and Lee et al.\textsuperscript{15} As 29 occurred more frequently and in a higher abundance than 27 and 30 (Table S5-6), and 29 yielded a fragment ion at m/z of 265, it is suggested that 29 corresponds to 6′-O-sinapoyl (or isomer)-3-butenyl GSL (Figure S4).

3. Tentative annotation of acylated indolic GSL

31 was tentatively annotated as a salicyloxy (or isomer) derivative of indol-3-ylmethyl GSL. Assuming that only the 1- and 4-positions can be hydroxylated in indolic GSL biosynthesis,\textsuperscript{11} the position of salicyloxy group (or isomer) was possibly at the 4-position, instead of at the 1-position. The tentative annotation was based on (i) the presence of predominant signature fragment ions at m/z 291, 275, 259, and 195 in MS\textsuperscript{2} (Figure S1) and m/z 97 in MS\textsuperscript{3} (Table S3), (ii) the m/z of the parent ion at an odd number (583, nitrogen rule), which refers to the indolic class (Table S2), (iii) the discriminating fragmentation patterns of 4- and 1-substitution of indol-3-ylmethyl GSL (Figure S2 and Figure S3), (iv) salicyloxy (or isomer) group fits to the m/z of the parent ion and fragment ions, and (v) the UV\textsubscript{max} at 275 nm (corresponding to indol-3-ylmethyl GSL) and 315 nm (corresponding to a salicyloxy group or isomer). Therefore, 31 was tentatively annotated as 4-salicyloxy (or isomer)-indol-3-ylmethyl GSL.

4. Tentative annotation of peaks 10, 12, 16, 18, 19, 23, and 28

According to the molecular weight, 10 (374 Da), 16 (388 Da), 23 (402 Da), and 28 (416 Da) could be hydroxylated alkenyl GSLs, alkyl GSLs, or oxoalkyl GSLs. The four GSLs have sequential masses by 14 Da, which is often associated to a methylene (-CH\textsubscript{2}) group.

Peak 10 and 28 demonstrated a neutral loss of 18 Da in the MS\textsuperscript{2} fragmentation which might associate to H\textsubscript{2}O. This is usually an indication of a presence of a hydroxyl group leading to a
suggestion of hydroxylated alkenyl GSLs. However, 10 eluted later than (R)-2-hydroxy-3-butenyl GSL (2), whereas 28 eluted much later than 2-hydroxy-4-pentenyl GSL (7). This raises the possibility of being oxoalkyl GSLs. 5-Oxoheptyl GSL was discovered by Kjær and Thomsen\textsuperscript{16} in another plant family, i.e. Capparidaceae. However, to date there is no fragmentation data reported for oxoalkyl GSL that would support the neutral loss of 18 Da. Therefore, the structures of 10 and 28 could not be tentatively annotated and were grouped as unclassified aliphatic GSLs (Table S3), with potential side chain formulae of C\textsubscript{4}H\textsubscript{9} or C\textsubscript{3}H\textsubscript{5}O and C\textsubscript{7}H\textsubscript{15} or C\textsubscript{6}H\textsubscript{11}O, respectively.

In contrast, 16 and 23, whose similar molecular weight to 2 and 7, respectively, did not demonstrate a neutral loss of 18 Da in the MS\textsuperscript{2} fragmentation. Meanwhile, 2 and 7, tentatively annotated as hydroxylated alkenyl GSLs (confirmed by the authentic standard 2), demonstrated a neutral loss of 18 Da. Furthermore, retention times of 16 and 23 were substantially later than 2 and 7. Therefore, 16 and 23 were possibly alkyl GSLs with C5 and C6, respectively (Table S3). However, our analytical method could not distinguish isomers (straight or branched alkyl chain).

According to the molecular weight, 12 (436 Da), 18 (438 Da), and 19 (450 Da) could be aliphatic or benzenic GSLs, but not indolic GSLs (Table S2 and Table S3). Because 12, 18, and 19 were present as trace peaks, the UV absorption around 260-270 nm shown in Figure S7 could not be used as basis for the tentative annotation. These three peaks showed similar fragmentations, e.g. a neutral loss of 18 Da.

12 and 19 have similar molecular weight to 4-(methylsulfinyl)butyl GSL (3) and 5-(methylsulfinyl)pentyl GSL (6), respectively. The possibility of being their isomers is likely (Figure 1B). If the neutral loss of 18 Da is related to the hydroxyl group substitution, 12 and 19 could be a hydroxylated 4-(methylthio)butyl GSL and a hydroxylated 5-(methylthio)pentyl
GSL, respectively. Their retention times were, as expected, earlier than 4-(methylthio)butyl GSL (15) and 5-(methylthio)pentyl GSL (21). The presence of hydroxylated (methylthio)alkyl GSLs has been reported by Kjær and Schuster\textsuperscript{17}, with hydroxylation occurred at 2-position. Olsen \textit{et al}. found the hydroxylation at 3-position.\textsuperscript{10}

According to the molecular weight and assuming that the possible subclasses of aliphatic GSLs in our samples are as mentioned in \textbf{Table S3} with possible modifications of hydroxylation, oxidation, and desaturation, 18 (438 Da) could be an aliphatic GSL, e.g. 3-(methylsulfonyl)propyl GSL. However, the retention time of 18 (9.95 min) was most likely in a disagreement as its polarity should not be largely different from 3-(methylsulfinyl)propyl GSL (1, 1.44 min). Therefore, 18 would possibly be a benzenic GSL, rather than an aliphatic GSL. The possible side chain formula for 18 would be C\textsubscript{8}H\textsubscript{9}O. The possible structure is a hydroxylated phenethyl GSL or a methoxylated benzyl GSL, where the substitution could be on the phenyl ring or on the alkyl chain. Furthermore, 18 suffered a neutral loss of 18 Da in the MS\textsuperscript{2} fragmentation. For the case of benzenic GSLs this loss is often associated to hydroxylation on the alkyl chain, rather than on the aromatic ring.\textsuperscript{2,18} In line with our study, the loss of 18 Da was not observed for the standard p-hydroxybenzyl GSL (5). Agerbirk \textit{et al}.\textsuperscript{18} also found similar observation for p- and m-hydroxyphenethyl GSLs. Furthermore, based on what has been found in nature,\textsuperscript{12,19} 2-hydroxy-2-phenethyl GSL would be more likely for GSL 18 than 1-hydroxy-2-phenethyl GSL. Unfortunately, our analysis did not include the standards of any hydroxylated phenethyl GSLs nor methoxylated benzyl GSLs, and, thus, was not able to distinguish these isomers. Therefore, 18 was suggested to be a benzenic GSL with a possible side chain formula of C\textsubscript{8}H\textsubscript{9}O.
Figure S1. Characteristic MS² fragmentation of GSL anions. The dashed red lines indicate the fragmentation sites. Fragment ion at m/z 259 was frequently predominant for most GSLs, except for (methylsulfinyl)alkyl GSLs: m/z corresponding to [M-H-64]⁻.
Figure S2. Fragmentation patterns of 4- and 1-substituted indolic GSLs. *I3M stands for indol-3-ylmethyl. †1-OH-I3M GSL is often not detectable due to its high instability."
Figure S3. MS spectra of tentatively annotated 4-hydroxy-I3M GSL (9) (A), 4-methoxy-I3M GSL (22) (B), 4-salycyloxy-I3M GSL (31) (C), and 1-methoxy-I3M GSL (24) (D), where I3M stands for indol-3-ylmethyl. The Roman numerals following the $m/z$ values refer to those in Figure S2, where the fragmentation patterns are explained.
Figure S4. MS² fragmentation of 6'-O-sinapoyl (or isomer) derivative of GSLs: proposed fragment ions extrapolated from those by Bianco et al.¹² who analyzed pure 6'-O-isofuruloyl-(S)-2-OH-2-phenethyl GSL (A) and proposed fragment ions following the diagnostic fragmentation patterns of non-acylated GSLs (B). For the sake of clarity, the acyl group is drawn as sinapoyl. According to Bianco et al.¹², the fragment ion at m/z 235 was only observed for 6'-substitution for isofuruloyl, where the analog fragment ion for sinapoyl (or isomer) has m/z of 265. This fragment ion was observed for 26 and 29. Therefore, 26 and 29 were tentatively annotated as 6'-O-sinapoyl (or isomer) derivative of p-OH-benzyl GSL and 6'-O-sinapoyl (or isomer) derivative of 3-butenyl GSL, respectively. Fragment ions, except m/z of 265, were observed for 27 and 30 too, where the position of sinapoyl (or isomer) substitution cannot be distinguished.
Figure S5. MS spectra of standard 3-butenyl GSL (8) (A), tentatively annotated unidentified sinapoyl (or isomer) derivative of 3-butenyl GSL (27) (B), and tentatively annotated 6'-O-sinapoyl (or isomer) derivative of 3-butenyl GSL (29) (C), which illustrate the different fragmentation patterns of sinapoyl (or isomer) derivatives of a GSL where the sinapoyl (or isomer) group is in different positions (6'-O vs others).
Figure S6. Sinapoyl (or isomer) derivatives of p-OH-benzyl GSL and 3-butenyl GSL, and 4-salicyloxy (or isomer)-indol-3-ylmethyl GSL. Bold face numbers refer to those in Table S3. For the sake of clarity, the acyl group is drawn as sinapoyl or salicyloxy.
Figure S7. UV absorption spectra of authentic standards of 4-(methylsulfinyl)butyl GSL (3) (A), p-OH-benzyl GSL (5) (B), benzyl GSL (14) (C), and indol-3-ylmethyl GSL (17) (D), and of tentatively annotated x-hydroxy-4-(methylthio)butyl GSL 12 (E), a benzenic GSL 18 (F), and x-hydroxy-5-(methylthio)pentyl GSL 19 (G).
**Table S1.** NI-MS-based relative response factors (RRFs) of the twelve GSL standards

| no. | GSL                  | RRF$^a$ | applied for GSL |
|-----|----------------------|---------|-----------------|
|     |                      |         |                 |
| **aliphatic** |                   |         |                 |
| 15$^b$ | 4-(methylthio)butyl | 0.92    | 15, 11, 12      |
| 21   | 5-(methylthio)pentyl | 1.22    | 21, 19          |
| 1    | 3-(methylsulfinyl)propyl | 0.50 | 1               |
| 3    | 4-(methylsulfinyl)butyl | 0.56 | 3, 6, 25        |
| 4    | allyl                | 0.62    | 4               |
| 8    | 3-butenyl            | 0.68    | 8, 27, 29, 30   |
| 13   | 4-pentenyl           | 1.00    | 13, 16, 23      |
| 2    | (R)-2-OH-3-butenyl   | 0.56    | 2, 7            |
| **benzenic** |                   |         |                 |
| 14   | benzyl               | 1.71    | 14              |
| 20   | phenethyl            | 2.18    | 20, 18          |
| 5    | p-OH-benzyl          | 1.00    | 5, 26           |
| **indolic** |                  |         |                 |
| 17   | indol-3-ylmethyl     | 1.00    | 17, 9, 22, 24, 31 |

$^a$The response factors of aliphatic GSLs, aromatic GSLs, and indolic GSL were set relatively to 4-pentenyl GSL, p-OH-benzyl GSL, and indol-3-ylmethyl GSL, respectively. The values were an average of three independent repetitions with a relative standard deviation less than 11%. The relative response factors of p-OH-benzyl GSL and indol-3-ylmethyl GSL to 4-pentenyl GSL were 0.53 and 0.74, respectively.

$^b$Bold face numbers correspond to the numbers in Table S3.
Table S2. Fast screening to identify the class of GSLs in the PDA-UHPLC-MS analysis.

| class                  | $\lambda_{\text{max}}$ | [M-H]$^-$ | fragment ion at m/z 259? |
|------------------------|-------------------------|-----------|--------------------------|
| non-acylated           |                         |           |                          |
| aliphatic              | -233                    | even number | yes                      |
| benzenic               |                         |           |                          |
| with O on phenyl       | -233, 263-275 nm        | even number | yes                      |
| without O on phenyl    | -233                    | even number | yes                      |
| indolic                | -233, 265-301 nm        | odd number | yes                      |
| aromatic-acylated      | 300-334 nm              | even or odd number | yes                      |

$^a$UV absorption spectra of representatives of each class are illustrated in Figure S7. GSLs have a characteristic UV spectrum, typically a maximum near 225-235 nm from the thiohydroxymate group. There is no $\lambda_{\text{max}}$ at 280 nm for benzenic GSLs, which is characteristic of indolic GSLs. The $\lambda_{\text{max}}$ of indolic GSLs can vary from 265-301 nm depending on the substituents. Additional $\lambda_{\text{max}}$ around 300-334 nm corresponds to aromatic acyl moiety. In the text, the aromatic acyl moiety is frequently mentioned without the word “aromatic”.

$^b$Even or odd number depends on the class.
Table S3. (Tentative) annotation of 31 GSLs found in the untreated (U), germinated (G), R. oryzae-germinated (Ro-G), F. graminearum-germinated (Fg-G), and F. oxysporum-germinated (Fo-G) S. alba (Sa), B. napus (Bn), and B. juncea var. rugosa rugosa (Bj) seeds.

| no. | t_R  | λ_max | [M-H] | MS² | MS³ | potential molecular formulae | (semi)systematic name | occurrence |
|-----|------|-------|-------|-----|-----|-------------------------------|-----------------------|------------|
|     | (min)| (nm)  |       |     |     |                               |                       |            |
| Met-derived GSLs |       |       |       |     |     |                               |                       |            |
| 11  | 6.35 | -     | 406   | 259, 326, 275 | 139, 241 (7), 199 (5), 181 (3), C₁₁H₂₀NO₉S₃ | 3-(methylthio)propyl GSL (glucoiberin) | Bj |
|     |      |       | c     | (27), 291 (6), 275 (41), 228 (23), 227 (13), 210 (8), 195 (15), 164 (8) |                     |            |
| 15  | 8.80 | -     | 420   | 259, 340, 275 | 139, 241 (7), 199 (6), 181 (2), C₁₂H₂₂NO₉S₃ | 4-(methylthio)butyl GSL (glucoerucin)* | Sa, Sa_Fg, Bn (except Bn_Ro-G), Bj |
|     |      |       |       | (24), 291 (9), 275 (38), 242 (24), 241 (3), 227 (22), 224 (8), 195 (17), 178 (15) |                     |            |
| 21  | 12.22| -     | 434   | 259, 354, 275 | 139, 241 (5), 199 (11), 97 (36), C₁₃H₂₄NO₁₀S₃ | 5-(methylthio)pentyl GSL (glucoberteroin)* | Bn (except Bn_Ro-G), Bj |
|     |      |       |       | (23), 291 (43), 256 (20), 241 (21), 238 (13), 227 (10), 195 (22), 192 (20) |                     |            |
| Hydroxy-(methylthio)alkyl GSLs |       |       |       |     |     |                               |                       |            |
| 12  | 7.57 | -     | 436   | 259, 418 | 139, 244 (2), 199 (9), 179 (5), C₁₂H₂₂NO₁₀S₃ | possible x-hydroxy-4-(methylthio)butyl GSL* | Sa (except Sa_Ro-G), Bj, Bj_Ro-G, Bj_Fg-G |
|     |      |       |       | (16), 390 (12), 368 (15), 356 (22), 346 (6), 291 (16), 275 (21), 274 (2), 258 (24), 240 (2), 195 (7), 194 (12), 129 (4) |                     |            |
| 19  | 10.23| -     | 450   | 259, 432, 370 | 139, 199 (6), 169 (7), 97 (23), C₁₃H₂₄NO₁₀S₃ | possible x-hydroxy-5-(methylthio)pentyl GSL | Bn_G, Bn_Fg-G |
|     |      |       |       | (16), 404 (18), 383 (10), 370 (23), 361 (64), 305 (22), 291 (21), 288 (2), 275 (26), 272 (19), 254 (2), 208 (16) |                     |            |
| (methylsulfinyl)alkyl GSLs |       |       |       |     |     |                               |                       |            |
| 1   | 1.44 | -     | 422   | 358, 407 | 259, 291 (2), 278 (16), 275 (38), 227 (11), 196 (11), 195 (21), 180 (21), 162 (59), 145 (6), 135 (6), 116 (9) | C₁₁H₂₀NO₁₀S₃ | 3-(methylsulfinyl)propyl GSL (glucoiberin)* | Bj (except Bj_Fo-G) |
|     |      |       |       | (4) | 259, 291 (2), 278 (16), 275 (38), 227 (11), 196 (11), 195 (21), 180 (21), 162 (59), 145 (6), 135 (6), 116 (9) |                     |            |
| 3   | 1.70 | -     | 436   | 372, 421 | 259, 292 (23), 291 (3), 275 (49), 241 (6), 227 (13), 210 (7), 195 (31), 194 (21), 176 (4), 145 (6), 130 (9) | C₁₂H₂₂NO₁₀S₃ | 4-(methylsulfinyl)butyl GSL (glucoraphanin)* | Sa, Sa_Fg-G, Bn_U, Bn_Fg-G, Bj (except Bj_Fo-G) |
|     |      |       |       | (3) | 372, 421 (3), 291 (1), 275 (1), 259 (4) |                     |            |
| 6   | 3.12 | -     | 450   | 386, 435 | 259, 354 (3), 306 (23), 291 (3), 275 (53), 241 (5), 227 (17), 224 (4), 208 (16), 195 (24), 190 (7), 145 (10), 144 (15) | C₁₃H₂₄NO₁₀S₃ | 5-(methylsulfinyl)pentyl GSL (glucoallysin) | Bn, Bj (except Bj_Fo-G) |
|     |      |       |       | (2) | 386, 435 (2), 291 (1), 259 (2) |                     |            |
| no. | \( t_r \) (min) | \( \lambda_{\text{max}} \) (nm) | [M-H] | MS² | MS³ | potential molecular formulae | (semi)systematic name (trivial name)* | occurrence |
|-----|-----------------|-----------------|--------|-----|-----|-----------------------------|-------------------------------------|-----------|
| 25  | 16.13           | -               | 520    | 456, 505 (3), 291 (2), 259 (1) | 259, 376 (33), 291 (11), 278 (38), 275 (52), 263 (23), 227 (11), 214 (28), 195 (19) | C₁₈H₃₄NO₁₅S₃ | 10-(methylsulfonyl)decyl GSL (glucocamelin) | Bn, U, Bn, G, Bn, Fg-G |
|     |                 |                 |        |     |     | allyl GSL (sinigrin)* | Alp * | Sa (except Sa, Ro-G), Bn, Bj |
| 4   | 1.94            | -               | 358    | 259, 291 (1), 278 (19), 275 (48), 241 (5), 227 (11), 195 (24), 180 (21), 165 (2), 162 (63), 145 (7), 135 (5), 116 (8) | 139, 241 (7), 199 (2), 97 (57) | C₁₀H₁₆NO₆S₂ | 3-butenyl GSL (gluconapin)* | Sa, U, Sa, Fg-G, Sa, Ro-G, Bn, Bj |
| 8   | 4.00            | -               | 372    | 259, 292 (21), 291 (3), 275 (44), 241 (4), 236 (15), 227 (8), 210 (4), 195 (26), 194 (23), 179 (3), 176 (7), 149 (3), 145 (6), 130 (9) | 139, 241 (3), 97 (36) | C₁₁H₁₈NO₆S₂ | 4-pentenyl GSL (glucobrassicanapin)* | Bn, Bj |
| 13  | 7.94            | -               | 386    | 259, 306 (22), 291 (2), 275 (47), 241 (3), 227 (11), 224 (4), 208 (18), 195 (24), 193 (6), 190 (9), 163 (19), 145 (6), 144 (12), 139 (2) | 139, 241 (6), 199 (6), 97 (28) | C₁₂H₂₀NO₆S₂ | (R)-2-hydroxy-3-butenyl GSL (progoitrin)* | Sa, Bn, Bj |
| 2   | 1.66            | -               | 388    | 332, 370 (3), 308 (24), 275 (45), 259 (85), 210 (43), 195 (30), 192 (10), 146 (11), 136 (40) | 97, 291 (4), 252 (3), 241 (3), 170 (22), 154 (17), 136 (41) | C₁₁H₁₆NO₁₅S₂ | 2-hydroxy-4-pentenyl GSL (gluconapoleiferin) | Sa, G, Sa, Fg-G, Sa, Ro-G, Bn, Bj, Fg-G, Bj, Ro-G |
| 7   | 3.13            | -               | 402    | 259, 384 (3), 332 (48), 322 (30), 291 (10), 275 (48), 241 (4), 227 (6), 224 (57), 206 (10), 195 (27), 160 (14), 139 (4), 136 (15) | 139, 241 (4), 199 (10), 149 (3), 97 (27) | C₁₂H₂₀NO₁₅S₂ | possible unidentified sinapoyl (or isomer) derivative of 3-butenyl GSL | Bj (except Bj, Ro-G) |
| 27  | 18.41           | 300             | 578    | 223, 532 (7), 510 (13), 497 (46), 481 (70), 465 (69), 447 (69), 432 (9), 401 (33), 372 (60), 325 (10), 281 (10), 253 (13), 247 (24) | 164, 208 (15), 179 (13) | C₂₂H₂₈NO₁₅S₂ | possible 6'-O-sinapoyl (or isomer)-3-butenyl GSL | Bn, U, Bn, G, Bn, Ro-G |
| 29  | 20.02           | 334             | 578    | 372, 497 (12), 481 (15), 465 (33), 447 (9), 401 (6), 368 (21), 367 (69), 354 (45), 325 (17), 265 (4), 223 (44) | 259, 356 (3), 292 (22), 275 (35), 227 (10), 210 (4), 195 (23), 194 (15), 176 (5), 145 (9) | C₂₂H₂₈NO₁₅S₂ | possible unidentified sinapoyl (or isomer) derivative of 3-butenyl GSL | Bn, U, Bn, G, Bn, Ro-G, Bj |
| 30  | 20.35           | 328             | 578    | 223, 532 (17), 510 (12), 497 (34), 481 (36), 465 (60), 447 (11), 372 (9), 401 (1), 259 (1), 247 (44), 223 (77) | 164, 208 (6), 179 (15), 149 (6) | C₂₂H₂₈NO₁₅S₂ | possible unidentified sinapoyl (or isomer) derivative of 3-butenyl GSL | Bj (except Bj, Ro-G) |
| no. | \( t_R \) (min) | \( \lambda_{max} \) (nm) | [M-H] | MS² | MS³ | potential molecular formulae | (semi)systematic name (trivial name)* | occurrence |
|-----|-----------------|-----------------|--------|------|-----|-------------------------------|--------------------------------------|-----------|
| **alkyl GSLs** | | | | | | | | |
| 16 | 9.63 | - | 388 | 343, 344 (27), 342 (47), 308 (12), 301 (39), 291 (2), 275 (36), 259 (71), 227 (10), 210 (9), 192 (4), 195 (19), 165 (6), 163 (6), 145 (6), 139 (3) | 301, 326 (2), 324 (3), 316 (3), 314 (2), 299 (13), 298 (12), 296 (3), 283 (16), 281 (3), 275 (2), 201 (2), 165 (2) | \( \text{C}_{12}\text{H}_{22}\text{NO}_{9}\text{S}_{2} \) | pentyl GSL or isomer | \( \text{Bj} \) |
| 23 | 14.24 | - | 402 | 259, 357 (22), 322 (20), 315 (11), 291 (4), 275 (47), 241 (6), 227 (12), 224 (26), 211 (3), 209 (11), 206 (12), 195 (23), 179 (5), 163 (6), 160 (10), 145 (8), 139 (3) | 139, 241 (23), 199 (5), 97 (31) | \( \text{C}_{13}\text{H}_{24}\text{NO}_{9}\text{S}_{2} \) | hexyl GSL or isomer | \( \text{Bj} \) (except \( \text{Bj}_{\text{Fo-G}} \)) |
| **unclassified aliphatic GSLs** | | | | | | | | |
| 10 | 5.99 | - | 374 | 259, 356 (33), 330 (38), 328 (78), 306 (28), 302 (23), 294 (13), 291 (6), 286 (18), 275 (45), 228 (8), 196 (19), 195 (23), 181 (7), 178 (7), 163 (8), 145 (8), 132 (7) | 139, 241 (4), 199 (13), 169 (7), 130 (9), 97 (39) | \( \text{C}_{11}\text{H}_{20}\text{NO}_{9}\text{S}_{2} \) or \( \text{C}_{10}\text{H}_{16}\text{NO}_{10}\text{S}_{2} \) | possible \( \text{C}_4\text{H}_9\text{GSL} \) or \( \text{C}_3\text{H}_5\text{OGLS} \) | \( \text{Bj} \) (except \( \text{Bj}_{\text{Fo-G}} \)) |
| 28 | 18.45 | - | 416 | 259, 398 (9), 372 (34), 370 (22), 349 (27), 348 (13), 343 (22), 336 (15), 291 (6), 275 (34), 238 (26), 227 (12), 223 (9), 220 (8), 195 (14), 174 (12), 163 (6) | 139, 199 (17), 170 (7), 125 (9), 97 (18) | \( \text{C}_{14}\text{H}_{26}\text{NO}_{9}\text{S}_{2} \) or \( \text{C}_{13}\text{H}_{22}\text{NO}_{10}\text{S}_{2} \) | possible \( \text{C}_7\text{H}_{15}\text{GSL} \) or \( \text{C}_6\text{H}_{11}\text{OGLSL} \) | \( \text{Bn}_G, \text{Bn}_Fg-G, \text{Bn}_Fo-G, \text{Bj}_G, \text{Bj}_Ro-G, \text{Bj}_Fg-G \) |
| **Tyr/Phe-derived GSLs** | | | | | | | | |
| 14 | 8.51 | - | 408 | 259, 328 (27), 291 (2), 275 (38), 246 (1), 241 (3), 230 (18), 227 (6), 217 (2), 215 (11), 212 (36), 195 (16), 166 (22), 163 (3), 145 (3), 139 (1), 129 (2) | 139, 241 (6), 199 (8), 183 (1), 161 (3), 143 (3), 97 (32) | \( \text{C}_{14}\text{H}_{18}\text{NO}_{9}\text{S}_{2} \) | benzyl GSL (glucotropaeolin)* | \( \text{Sa, Bn}_G, \text{Bn}_Fg-G, \text{Bn}_Fo-G, \text{Bj} \) (except \( \text{Bj}_U \)) |
| 18 | 9.95 | - | 438 | 259, 420 (12), 392 (11), 371 (10), 358 (31), 291 (8), 276 (3), 275 (19), 260 (32), 242 (1), 196 (14), 195 (9), 129 (7) | 139, 97 (30), 169 (10), 241 (3) | \( \text{C}_{15}\text{H}_{20}\text{NO}_{10}\text{S}_{2} \) | possible \( \text{C}_8\text{H}_9\text{GSL} \) | \( \text{Sa}_Ro-G, \text{Bn}_G, \text{Bn}_Fg-G \) |
| 20 | 11.95 | - | 422 | 259, 382 (25), 291 (5), 275 (41), 260 (6), 244 (21), 241 (5), 231 (2), 229 (12), 226 (8), 199 (2), 195 (18), 180 (21), 163 (2), 145 (4), 139 (2), 129 (2) | 139, 241 (4), 199 (19), 169 (4), 161 (3), 97 (45) | \( \text{C}_{15}\text{H}_{20}\text{NO}_{10}\text{S}_{2} \) | phenethyl GSL (gluconasturtiin)* | \( \text{Sa} \) (except \( \text{Sa}_U \)), \( \text{Bn, Bj} \) |
| no. | t_R  | λ_max | [M-H] | MS² | MS³ | potential molecular formulae | (semi)systematic name (trivial name) | occurrence |
|-----|------|-------|-------|-----|-----|-------------------------------|----------------------------------|------------|
| 5   | 2.93 | 276   | 424   | 259, 344 (29), 291 (12), 275 (48), 246 (19), 241 (4), 231 (11), 228 (13), 195 (12), 182 (30), 163 (2), 145 (4), 139 (3) | 139, 241 (3), 199 (14), 97 (36) | C₁₄H₁₈NO₁₀S₂ | p-hydroxybenzyl GSL (glucosinalbin)* | Sa, Bn (except Brassicaceae) |
| 26  | 18.25| 278   | 630   | 424, 550 (4), 497 (35), 481 (22), 466 (68), 447 (5), 401 (1) 351 (4), 303 (5), 265 (3), 231 (2), 228 (3), 223 (38) | 259, 344 (33), 275 (69), 228 (33), 195 (28), 182 (39) | C₂₂H₂₈NO₁₄S₂ | possible 6’-O-sinapoyl (or isomer)-p-hydroxybenzyl GSL | Sa |

**Trp-derived GSLs**

| no. | t_R  | λ_max | [M-H] | MS² | MS³ | potential molecular formulae | (semi)systematic name (trivial name) | occurrence |
|-----|------|-------|-------|-----|-----|-------------------------------|----------------------------------|------------|
| 17  | 9.67 | 279   | 447   | 259, 367 (24), 291 (32), 275 (42), 269 (18), 256 (4), 254 (12), 251 (10), 241 (3), 227 (9), 205 (29), 195 (14), 163 (3), 139 (3) | 139, 241 (5), 199 (13), 169 (4), 97 (39), 81 (5) | C₁₆H₁₉NsO₁₅S₂ | indol-3-ylmethyl (I3M) GSL (glucobrassicin)* | Sa, Bn, Bj |
| 9   | 5.42 | 265   | 463   | 285, 383 (7), 291 (1), 275 (3), 267 (83), 259 (7), 240 (13), 221 (4), 205 (2), 195 (1), 160 (8) | 97, 268 (2), 205 (1), 187 (1), 112 (3) | C₁₆H₁₉N₂O₁₅S₂ | 4-hydroxy-I3M GSL | Sa_G, Sa_Fg, Sa_Fo, Bn, Bj |
| 22  | 13.15| 266   | 477   | 259, 397 (17), 299 (25), 291 (58), 284 (8), 281 (16), 275 (70), 254 (3), 241 (7), 235 (19), 227 (17), 204 (3), 195 (14), 163 (4) | 139, 241 (5), 223 (2), 199 (16), 131 (2), 97 (30) | C₁₇H₂₂N₂O₁₅S₂ | 4-methoxy-I3M GSL | Sa (except Sa_U), Bn, Bj |
| 24  | 15.89| 280   | 477   | 446, 285 (1), 259 (1) | 383, 416 (36), 413 (11), 365 (22), 348 (5), 341 (14), 313 (4), 291 (9), 284 (97), 275 (6), 259 (49), 224 (43), 205 (6), 188 (6), 160 (4), 154 (19), 144 (10) | C₁₇H₂₃N₂O₁₅S₂ | 1-methoxy-I3M GSL | Sa, Bn, Bj |
| 31  | 20.58| 275   | 583   | 291, 565 (8), 553 (7), 539 (6), 503 (27), 405 (26), 390 (26), 387 (16), 341 (80), 308 (10), 305 (24), 275 (42), 259 (76), 227 (6), 195 (8) | 97, 275 (4), 263 (7), 262 (7) | C₂₂H₂₃NO₁₅S₂ | possible 4-salicyloxy (or isomer)-I3M GSL | Sa_Ro-G |

*The annotation of 12 GSLs marked with (*) was confirmed by the authentic standards. The word “possible” indicates that the suggested GSLs have never been identified in Brassicaceae plants by conclusive methods.

*The apparent λ_max was only ~233 nm.

*The m/z of the molecular anion of a GSL, [M-H], is the same as its molecular weight. Mostly 5 isotopes were detected in MS spectra for [M-H]: M, M+1, M+2, M+3, M+4 with relative abundance of 100%, 15-23%, 11-17%, 2-4%, and 1-2%, respectively.

*The most abundant fragment ion is mentioned at first without any brackets following afterwards. The most abundant ion in MS² fragmentation was further fragmented in MS³.

*The position of hydroxylation (x) could be at 2 or 3,10,17

*The side chain formula of C₆H₆ and C₆H₇ can refer to alkyl GSLs, whereas C₆H₄O and C₆H₃O can refer to x-oxoalkyl GSLs or hydroxylated alkyl GSLs. However, the tentative annotation for peaks 10 and 28 remains as unclassified GSLs (further explanation can be found in section 4).

*Because peak 18 was present in trace amounts, its UV spectrum (Figure S7F) could not be used for tentative peak annotation. Multiple isomers for 18 could be possible, i.e. a non-phenolic or phenolic hydroxylated phenethyl GSL or a methoxy/ated benzyl GSL.
| No. | GSL² | content (µmol/g DW) | \(S_a\_U\) | \(S_a\_G\) | \(S_a\_Ro-G\) | \(S_a\_Fg-G\) | \(S_a\_Fo-G\) |
|-----|------|---------------------|-------|-------|-------|-------|-------|
| 11  | 3-(methylthio)propyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 15  | 4-(methylthio)butyl | n.d. ⁶ | n.d. ⁶ | 0.00±0.00 | n.d. ⁶ | 0.01±0.00 | 0.00±0.00 |
| 21  | 5-(methylthio)pentyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| sub-total MTalkyl | n.d. ⁶ | 0.00±0.00 | n.d. ⁶ | 0.01±0.00 | 0.00±0.00 |
| 12  | possible 3-OH-4-MTbutyl | 0.16±0.04 | 0.78±0.15 | n.d. ⁶ | 3.66±0.22 | 6.23±0.78 |
| 19  | possible 3-OH-5-MTpentyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| sub-total OH-MTalkyl | 0.16±0.04 | 0.78±0.15 | n.d. ⁶ | 3.66±0.22 | 6.23±0.78 |
| 1   | 3-(methylsulfanyl)propyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 3   | 4-(methylsulfanyl)butyl | n.d. ⁶ | 0.00±0.00 | n.d. ⁶ | 0.00±0.00 | 0.00±0.00 |
| 6   | 5-(methylsulfanyl)pentyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 25  | 10-(methylsulfanyl)decyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| sub-total MSalkyl | n.d. ⁶ | 0.00±0.00 | n.d. ⁶ | 0.00±0.00 | 0.00±0.00 |
| 4   | allyl | 0.07±0.07 | 0.02±0.00 | n.d. ⁶ | 0.03±0.00 | 0.01±0.00 |
| 8   | 3-butenyl | 0.60±0.60 | 0.00±0.00 | n.d. ⁶ | 0.05±0.00 | 0.02±0.00 |
| 13  | 4-pentenyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| sub-total alkenyl | 0.66±0.66 | 0.02±0.00 | n.d. ⁶ | 0.08±0.01 | 0.03±0.00 |
| 2   | (R)-2-OH-3-butenyl | 3.19±0.99 | 2.55±0.30 | 1.55±0.51 | 0.34±0.01 | 0.12±0.01 |
| 7   | 2-OH-4-pentenyl | n.d. ⁶ | 0.02±0.00 | n.d. ⁶ | 0.01±0.00 | 0.00±0.00 |
| sub-total OH-alkenyl | 3.19±0.99 | 2.57±0.30 | 1.55±0.51 | 0.35±0.01 | 0.13±0.01 |
| 27  | sinapoyl-3-butenyl or isomer | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 29  | sinapoyl-3-butenyl or isomer | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 30  | sinapoyl-3-butenyl or isomer | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| sub-total sinapoyl alkenyl or isomer | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 16  | pentyl or isomer | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| 23  | hexyl or isomer | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| sub-total alkyl | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ | n.d. ⁶ |
| total aliphatic | 4.01±1.64 | 3.39±0.24 | 1.55±0.51 | 4.10±0.22 | 6.39±0.78 |
| 14  | benzyl | 0.02±0.01 | 0.39±0.01 | 0.07±0.01 | 0.30±0.04 | 0.12±0.01 |
| 20  | phenethyl | n.d. ⁶ | 0.02±0.01 | 0.28±0.02 | 0.02±0.00 | 0.00±0.00 |
| 5   | p-OH-benzyl | 72.29±43.83 | 212.92±18.10 | 176.17±12.32 | 160.18±12.48 | 74.04±9.07 |
| 26  | sinapoyl-p-OH-benzyl or isomer | 0.04±0.04 | 1.79±0.43 | 0.34±0.02 | 0.40±0.27 | 0.61±0.03 |
| 18  | possible \(C_4H_9O\) | n.d. ⁶ | n.d. ⁶ | 0.06±0.00 | n.d. ⁶ | n.d. ⁶ |
| total benzenic | 72.35±43.88 | 215.12±17.90 | 176.91±12.29 | 160.89±12.66 | 74.77±9.04 |
| 17  | indol-3-ylmethyl (I3M) | 0.12±0.07 | 0.31±0.06 | 1.27±0.22 | 0.26±0.02 | 0.05±0.00 |
| 9   | 4-OH-I3M | n.d. ⁶ | 0.02±0.00 | n.d. ⁶ | 0.01±0.00 | 0.00±0.00 |
| 24  | 1-OCH₃-I3M | 0.02±0.02 | 0.95±0.18 | 2.64±0.36 | 0.47±0.04 | 0.03±0.00 |
| 22  | 4-OCH₃-I3M | n.d. ⁶ | 0.66±0.09 | 0.37±0.08 | 0.72±0.06 | 0.06±0.01 |
| 31  | salicyloxy (or isomer)-I3M | n.d. ⁶ | n.d. ⁶ | 0.27±0.01 | n.d. ⁶ | n.d. ⁶ |
| total indolic | 0.14±0.08 | 1.95±0.31 | 4.55±0.51 | 1.46±0.11 | 0.15±0.01 |
| total GSLs | 76.50±42.70 | 220.45±17.93 | 183.01±12.28 | 166.44±12.76 | 81.31±8.65 |

²Details of the tentative annotation of the peaks follow Table S3.

⁶n.d. peak not detected.
Table S5. Content of GSLs in the untreated (U), germinated (G), *R. oryzae*-germinated (Ro-G), *F. graminearum*-germinated (Fg-G), and *F. oxysporum*-germinated (Fo-G) *B. napus* (Bn) seed.

| no. | GSL* | content (μmol/g DW) |
|-----|------|---------------------|
|     |      | **Bn_U** | **Bn_G** | **Bn_Ro-G** | **Bn_Fg-G** | **Bn_Fo-G** |
| 11  | 3-(methylthio)propyl | n.d. | n.d. | n.d. | n.d. | n.d. |
| 15  | 4-(methylthio)butyl | 0.07±0.05 | 0.01±0.02 | n.d. | 0.03±0.01 | 0.02±0.00 |
| 21  | 5-(methylthio)pentyl | 0.01±0.01 | 0.03±0.04 | n.d. | 0.05±0.05 | 0.03±0.01 |
| sub-total MTalkyl | 0.08±0.05 | 0.04±0.06 | n.d. | 0.08±0.06 | 0.04±0.01 |
| 12  | possible x-OH-4-MTbutyl | n.d. | n.d. | n.d. | n.d. | n.d. |
| 19  | possible x-OH-5-MTpentyl | n.d. | 0.01±0.01 | n.d. | 0.01±0.02 | n.d. |
| sub-total OH-MTalkyl | n.d. | 0.01±0.01 | n.d. | 0.01±0.02 | n.d. |
| 1   | 3-(methylsulfinyl)propyl | n.d. | n.d. | n.d. | n.d. | n.d. |
| 3   | 4-(methylsulfinyl)butyl | 0.21±0.22 | n.d. | n.d. | 0.00±0.00 | 0.00±0.00 |
| 6   | 5-(methylsulfinyl)pentyl | 0.18±0.13 | 0.03±0.02 | 0.03±0.03 | 0.02±0.02 | 0.02±0.00 |
| 25  | 10-(methylsulfinyl)decyl | 0.02±0.03 | 0.02±0.03 | n.d. | 0.04±0.07 | n.d. |
| sub-total MSalkyl | 0.41±0.27 | 0.05±0.01 | 0.03±0.03 | 0.06±0.05 | 0.03±0.00 |
| 4   | allyl | 0.07±0.14 | 0.04±0.03 | 0.23±0.01 | 0.08±0.05 | 0.02±0.00 |
| 8   | 3-butenyl | 2.51±0.27 | 0.77±0.11 | 0.27±0.04 | 1.12±0.58 | 0.63±0.05 |
| 13  | 4-pentenyl | 0.21±0.02 | 0.24±0.03 | 0.09±0.01 | 0.22±0.02 | 0.12±0.01 |
| sub-total alkenyl | 2.79±0.38 | 1.04±0.10 | 0.59±0.03 | 1.42±0.65 | 0.77±0.07 |
| 2   | (R)-2-OH-3-butenyl | 4.97±0.41 | 1.70±1.56 | 2.53±0.30 | 1.25±1.22 | 0.40±0.03 |
| 7   | 2-OH-4-pentenyl | 0.06±0.02 | 0.08±0.08 | 0.06±0.03 | 0.08±0.06 | 0.03±0.00 |
| sub-total OH-alkenyl | 5.02±0.39 | 1.79±1.64 | 2.60±0.29 | 1.33±1.28 | 0.43±0.04 |
| 27  | sinapoyl-3-buteryl or isomer | n.d. | n.d. | n.d. | n.d. | n.d. |
| 29  | sinapoyl-3-buteryl or isomer | 0.02±0.03 | 0.71±0.45 | n.d. | 0.32±0.64 | 1.03±0.13 |
| 30  | sinapoyl-3-buteryl or isomer | n.d. | n.d. | n.d. | n.d. | n.d. |
| sub-total sinapoyl alkenyl or isomer | 0.02±0.03 | 0.71±0.45 | n.d. | 0.32±0.64 | 1.03±0.13 |
| 16  | pentyl or isomer | n.d. | n.d. | n.d. | n.d. | n.d. |
| 23  | hexyl or isomer | n.d. | n.d. | n.d. | n.d. | n.d. |
| sub-total alkyl | n.d. | n.d. | n.d. | n.d. | n.d. |
| total aliphatic | 8.33±0.42 | 3.63±1.21 | 3.21±0.28 | 3.22±0.82 | 2.30±0.07 |
| 14  | benzyll | n.d. | 0.01±0.01 | n.d. | 0.00±0.00 | 0.01±0.01 |
| 20  | phenethyl | 0.06±0.04 | 0.13±0.03 | 0.10±0.01 | 0.13±0.02 | 0.06±0.01 |
| 5   | p-OH-benzyl | 0.02±0.04 | 0.19±0.12 | n.d. | 0.09±0.06 | 4.36±0.56 |
| 26  | sinapoyl-p-OH-benzyl or isomer | n.d. | n.d. | n.d. | n.d. | n.d. |
| 18  | C,H,O | n.d. | 0.12±0.06 | n.d. | 0.03±0.04 | n.d. |
| total benzenic | 0.08±0.08 | 0.45±0.10 | 0.10±0.01 | 0.25±0.05 | 4.43±0.57 |
| 17  | indol-3-ylmethyl (I3M) | 0.07±0.02 | 0.43±0.16 | 0.97±0.12 | 0.30±0.01 | 0.04±0.00 |
| 9   | 4-OH-3M | 1.04±0.38 | 0.53±0.60 | 0.34±0.00 | 0.17±0.14 | 0.08±0.01 |
| 24  | 1-OCH3-3M | 0.02±0.00 | 2.04±0.43 | 2.84±0.26 | 2.49±0.86 | 0.04±0.00 |
| 22  | 4-OCH3-3M | 0.02±0.00 | 1.13±0.22 | 1.26±0.09 | 1.60±0.61 | 0.05±0.01 |
| 31  | salicyloxy (or isomer)-I3M | n.d. | n.d. | n.d. | n.d. | n.d. |
| total indolic | 1.16±0.37 | 4.13±0.99 | 5.41±0.22 | 4.56±1.62 | 0.21±0.02 |
| total GSLs | 9.56±0.67 | 8.22±2.10 | 8.72±0.23 | 8.03±2.26 | 6.94±0.58 |

*Details of the tentative annotation of the peaks follow Table S3.

*n.d. peak not detected.*
Table S6. Content of GSLs in the untreated (U), germinated (G), *R. oryzae*-germinated (Ro-G), *F. graminearum*-germinated (Fg-G), and *F. oxysporum*-germinated (Fo-G) *B. juncea* var. rugosa rugosa (Bj) seed.

| no. | GSLa | content (μmol/g DW) |
|-----|------|---------------------|
|     |      | Bj_U               | Bj_G               | Bj_Ro-G             | Bj_Fg-G             | Bj_Fo-G             |
| 11  | 3-(methylthio)propyl | 0.05±0.02          | 0.58±0.87          | 0.64±0.04           | 0.86±1.09           | 0.03±0.00           |
| 15  | 4-(methylthio)butyl  | 1.00±1.55          | 1.89±2.27          | 3.57±0.05           | 2.21±2.36           | 0.20±0.03           |
| 21  | 5-(methylthio)pentyl | 0.03±0.01          | 0.10±0.06          | 0.08±0.00           | 0.10±0.07           | 0.02±0.00           |
|     | sub-total MTalkyl    | 1.08±1.26          | 2.57±3.20          | 4.29±0.02           | 3.17±3.51           | 0.25±0.04           |
| 12  | possible x-OH-4-MTbutyl | n.d.              | 0.03±0.07          | 0.07±0.01           | 0.07±0.09           | n.d.                |
| 19  | possible x-OH-5-Mpentyl | n.d.          | n.d.              | n.d.                | n.d.                | n.d.                |
|     | sub-total OH-MTalkyl | n.d.              | 0.03±0.07          | 0.07±0.01           | 0.07±0.09           | n.d.                |
| 1   | 3-(methylsulfonyl)propyl | 0.75±0.05          | 0.16±0.32          | 0.24±0.03           | 0.31±0.43           | n.d.                |
| 3   | 4-(methylsulfonyl)butyl | 2.13±1.23          | 0.21±0.33          | 2.35±0.28           | 1.54±1.98           | 0.00±0.00           |
| 5   | 5-(methylsulfonyl)pentyl | 0.15±0.06          | 0.08±0.06          | 0.07±0.00           | 0.09±0.07           | 0.01±0.00           |
| 25  | 10-(methylsulfonyl)decy1 | n.d.             | n.d.              | n.d.                | n.d.                | n.d.                |
|     | sub-total MSalkyl    | 3.03±1.01          | 0.45±0.70          | 2.67±0.20           | 1.93±2.46           | 0.01±0.00           |
| 4   | allyl                | 9.26±0.82          | 5.55±4.55          | 8.18±0.56           | 7.17±6.04           | 0.53±0.02           |
| 8   | 3-butenyl            | 61.60±26.64        | 46.83±13.98        | 33.04±3.10          | 46.84±25.18         | 8.03±0.54           |
| 13  | 4-pentenyl           | 0.05±0.01          | 0.10±0.02          | 0.05±0.00           | 0.08±0.04           | 0.01±0.00           |
|     | sub-total alkenyl    | 70.91±22.44        | 52.47±18.48        | 41.27±2.99          | 54.09±31.13         | 8.57±0.45           |
| 2   | (R)-2-OH-3-butenyl   | 3.20±0.34          | 2.81±3.94          | 7.81±0.66           | 6.78±7.68           | 0.04±0.00           |
| 7   | 2-OH-4-pentenyl      | n.d.               | 0.62±0.42          | n.d.                | 0.27±0.30           | 0.15±0.01           |
|     | sub-total OH-alkenyl | 3.20±2.97          | 3.43±3.54          | 7.81±0.54           | 7.06±7.41           | 0.19±0.01           |
| 27  | sinapoyl-3-butenyl or isomer | 0.05±0.04          | 1.93±1.42          | n.d.                | 0.94±0.88           | 1.79±0.23           |
| 29  | sinapoyl-3-butenyl or isomer | 0.95±0.40          | 34.06±24.43        | 0.41±0.04           | 14.12±13.60         | 24.95±2.44           |
| 30  | sinapoyl-3-butenyl or isomer | 0.07±0.06          | 1.26±0.88          | n.d.                | 0.79±1.01           | 1.52±0.34           |
|     | sub-total sinapoyl alkenyl or isomer | 1.06±0.41          | 37.26±26.73        | 0.41±0.03           | 15.85±15.42         | 28.26±1.90           |
| 16  | pentyl or isomer     | 0.14±0.01          | 0.44±0.21          | 0.03±0.01           | 0.20±0.09           | 0.08±0.00           |
| 23  | hexyl or isomer      | 0.27±0.04          | 0.29±0.57          | 0.41±0.02           | 0.50±0.58           | n.d.                |
|     | sub-total alky1      | 0.41±0.03          | 0.72±0.40          | 0.44±0.01           | 0.70±0.54           | 0.08±0.00           |
|     | total aliphatic      | 79.77±24.05        | 96.99±14.87        | 56.96±4.06          | 82.90±29.31         | 37.38±2.49           |
| 14  | benzyl               | 0.01±0.00          | 0.33±0.19          | 0.03±0.01           | 0.17±0.14           | 0.07±0.01           |
| 20  | phenethyl            | 0.04±0.04          | 0.06±0.02          | 0.27±0.05           | 0.17±0.17           | 0.01±0.00           |
| 5   | p-OH-benzyl          | n.d.               | 0.08±0.05          | n.d.                | n.d.                | 0.02±0.01           |
| 26  | sinapoyl-p-OH-benzyl or isomer | n.d.             | n.d.              | n.d.                | n.d.                | n.d.                |
| 18  | C6H12O               | n.d.               | n.d.              | n.d.                | n.d.                | n.d.                |
|     | total benzenic       | 0.06±0.04          | 0.47±0.26          | 0.30±0.04           | 0.3±0.13            | 0.10±0.01           |
| 17  | indol-3-ymethyl (I3M) | 0.09±0.02          | 0.90±0.33          | 1.50±0.14           | 1.34±1.59           | 0.17±0.01           |
| 9   | 4-OH-I3M             | 0.25±0.13          | 0.48±0.67          | 0.40±0.06           | 0.40±0.55           | 0.04±0.01           |
| 24  | 1-OCH2-I3M           | 0.17±0.10          | 2.51±0.46          | 3.57±0.41           | 4.09±1.90           | 0.25±0.02           |
| 22  | 4-OCH2-I3M           | 0.13±0.06          | 2.78±1.10          | 3.63±0.51           | 4.90±2.43           | 1.19±0.09           |
| 31  | salicyloxy (or isomer)-I3M | n.d.             | n.d.              | n.d.                | n.d.                | n.d.                |
|     | total indolic        | 0.64±0.18          | 6.68±1.81          | 9.11±0.99           | 10.75±5.67          | 1.65±0.11           |
|     | total GSLs           | 80.47±24.19        | 104.18±15.64       | 66.45±5.00          | 94.06±33.20         | 39.13±2.54           |

aDetails of the tentative annotation of the peaks follow Table S3.
b n.d. peak not detected.
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