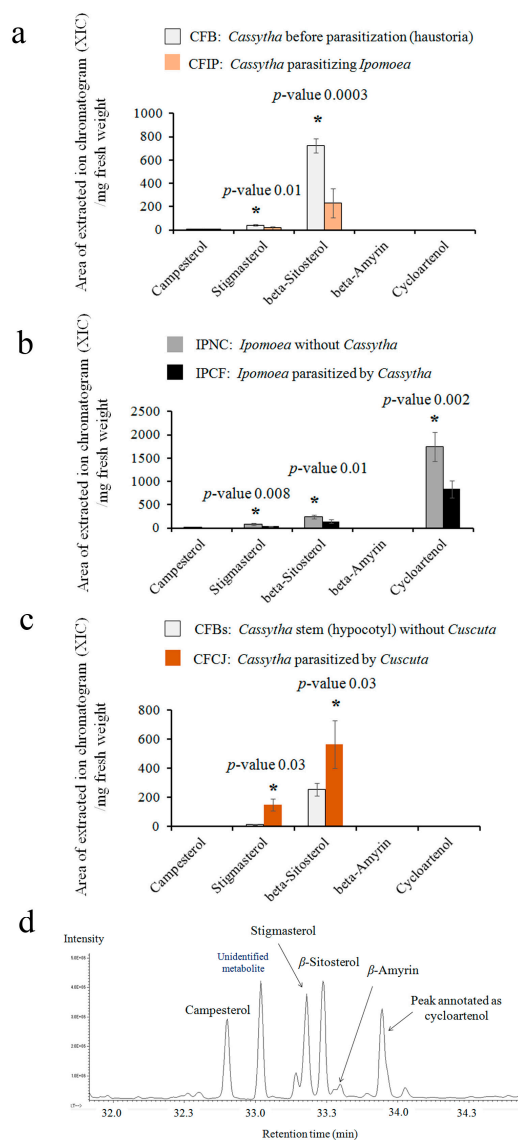
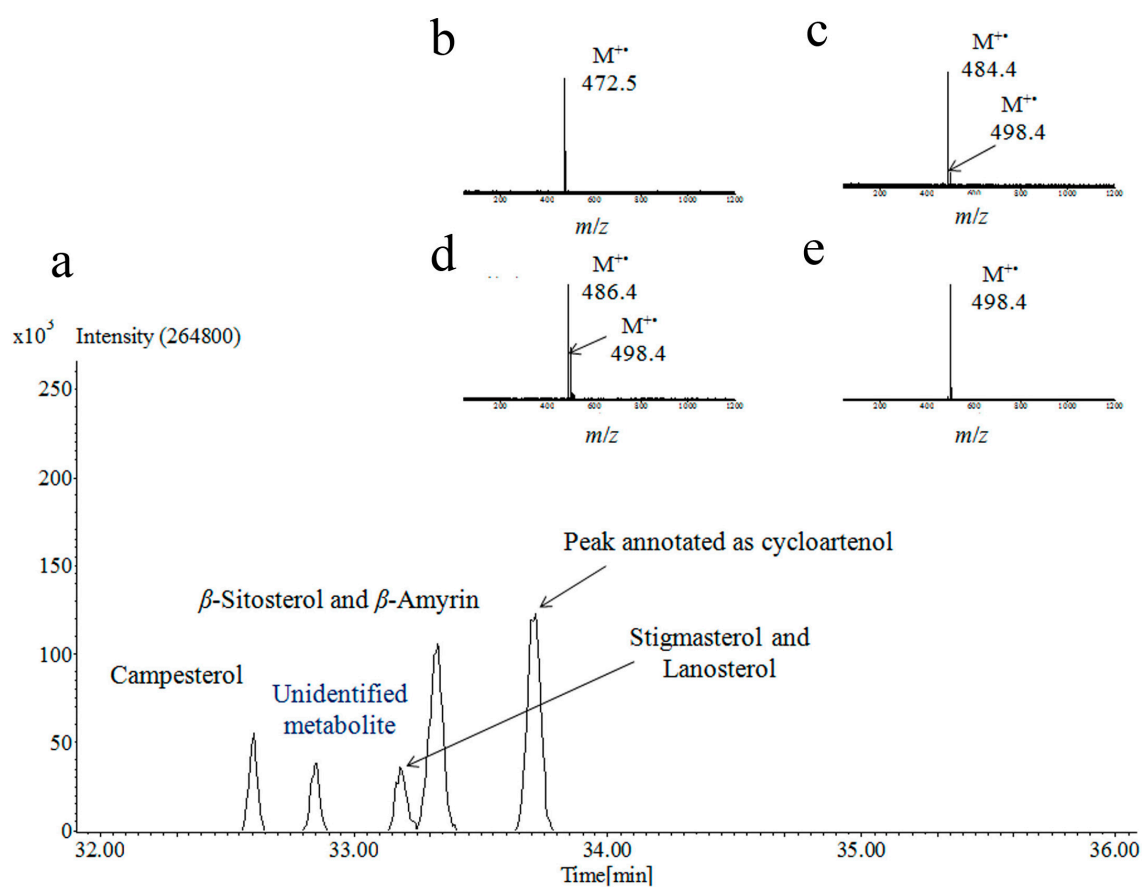


# Supplementary Materials: Analysis of Metabolites in Stem Parasitic Plant Interactions: Interaction of *Cuscuta-Momordica* versus *Cassytha-Ipomoea*

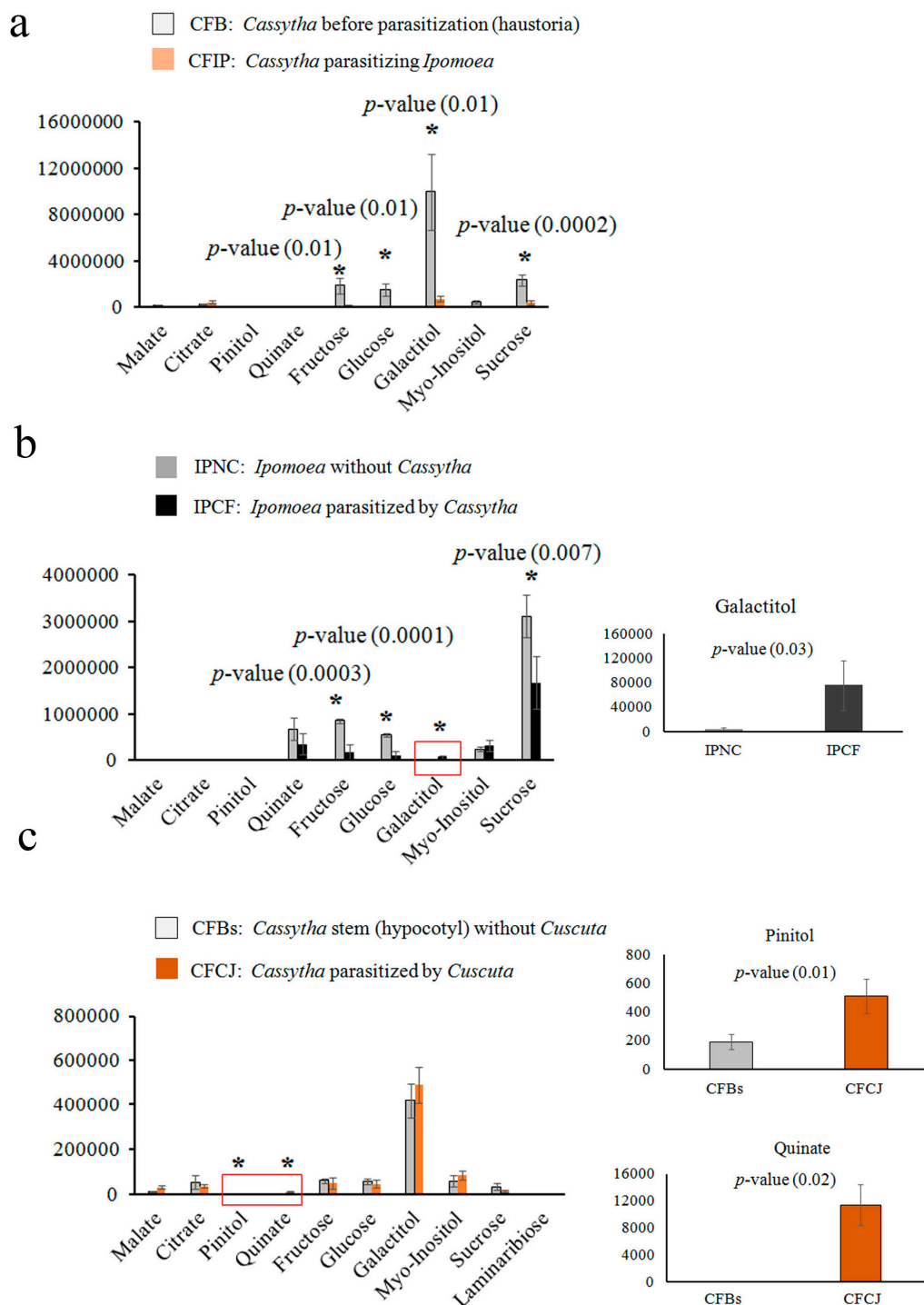
Takeshi Furuhashi, Takemichi Nakamura and Koji Iwase



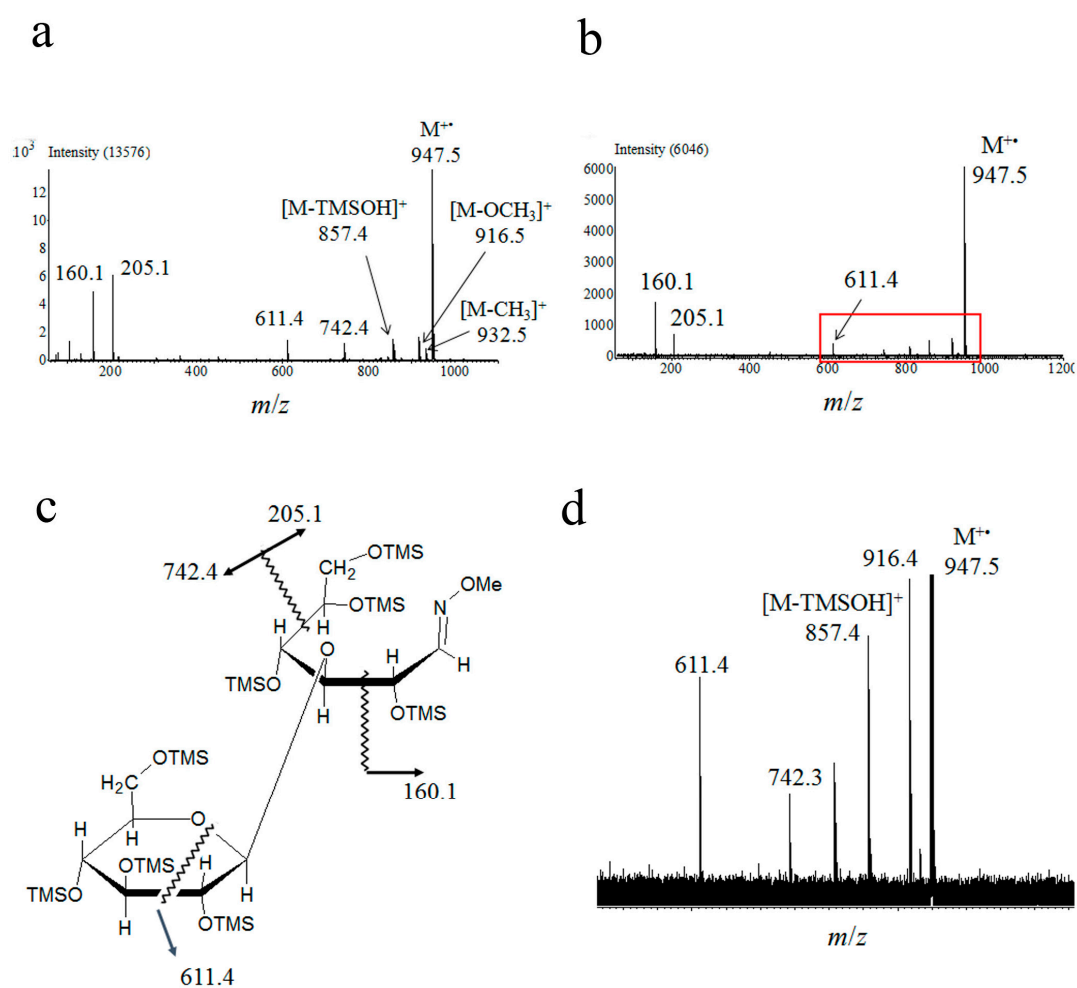
**Figure S1.** (a–c) Relative quantification of steroid. (a) *Cassytha* (epicotyl as haustoria-forming part) before and after parasitizing *Ipomoea* (stem as parasitized part); (b) *Ipomoea* with and without *Cassytha* parasitization; (c) *Cassytha* stem (hypocotyl) with and without *Cuscuta* parasitization: steroid quantification data were obtained with GC-EI-MS. Value at y axis indicates peak area of extracted ion chromatogram (XIC)/mg fresh weight ( $n = 3$  as biological replicates). CFB, *Cassytha* before parasitization (haustoria-forming part); CFIP, *Cassytha* parasitizing *Ipomoea*; IPNC, *Ipomoea* without *Cassytha*; IPCF, *Ipomoea* parasitized by *Cassytha*; CFBs, *Cassytha* stem (hypocotyl) without *Cuscuta*; CFCJ, *Cassytha* parasitized by *Cuscuta*. \* Indicates statistical significance compared with negative control.  $p$ -Value ( $t$ -test) showed statistical significance between samples. (d) GC chromatogram of steroid profiling of *Cuscuta* haustoria-forming part, parasitizing *Cassytha* stem (hypocotyl).  $\beta$ -Amyrin and a peak annotated as cycloartenol was observed. Values at x and y axis indicate retention time and relative intensity, respectively. Error bar indicates standard deviation (SD).



**Figure S2.** GC chromatogram and MS spectra of *Cuscuta* parasitizing *Momordica* at stage 3. In GC-FI, exclusively molecular ions of steroids (all 1TMS forms) were observed. (a) GC-FI-MS chromatogram of *Cuscuta*; (b–e) MS spectra of each peak; (b) campesterol; (c) stigmasterol and lanosterol; (d) sitosterol and  $\beta$ -Amyrin; (e) annotated as cycloartenol.



**Figure S3.** Relative quantification of polar metabolites: (a) *Cassytha* (epicotyl as haustoria-forming part) before and after parasitizing *Ipomoea* (stem as parasitized part); (b) *Ipomoea* with and without *Cassytha* parasitization; Magnified graph of galactitol is on right side; (c) *Cassytha* stem (hypocotyl) with and without *Cuscuta* parasitization. Magnified graph of pinitol and quinate are on right side. Polar metabolites quantification data were obtained with GC-EI-MS. Value at y axis indicates peak area of extracted ion chromatogram (XIC)/mg fresh weight ( $n = 3$  as biological replicates). CFB, *Cassytha* before parasitization (haustoria-forming part); CFIP, *Cassytha* parasitizing *Ipomoea*; IPNC, *Ipomoea* without *Cassytha*; IPCF, *Ipomoea* parasitized by *Cassytha*. CFBs, *Cassytha* stem (hypocotyl) without *Cuscuta*; CFCJ, *Cassytha* parasitized by *Cuscuta*. \* Indicates statistical significance compared with negative control.  $p$ -Value ( $t$ -test) showed statistical significance between samples. Error bar indicates standard deviation (SD).



**Figure S4.** FI-MS spectra of laminaribiose standard and *Cuscuta* parasitizing *Momordica* at stage 3. (a) FI-MS spectra of laminaribiose standard; (b) FI-MS spectra of peak annotated as laminaribiose in *Cuscuta* sample; (c) Fragment assignment of laminaribiose FI-MS spectra; (d) Magnified MS spectra of red square of (b). In GC-FI-MS, molecular ions of laminaribiose (8TMS forms with 1Methyloxime) as well as characteristic fragment ( $m/z$  611.4) were observed as  $m/z$  947.5. No characteristic fragments of other potentially co-eluting isomeric disaccharides (i.e., trehalose, sophorose, maltose), which are  $m/z$  903.4, 409.2, 787.4, were detected.