# **Supporting Information**

# Examination of Endogenous Peptides in *Medicago* truncatula using Mass Spectrometry Imaging

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 Table S2: m/z Values Detected in Medicago Seedlings (DHB matrix)

 Table S3: m/z
 Values
 Detected in
 Medicago
 Mature
 Nodules
 (CHCA matrix)
 Medicago
 <th

 Table S4: m/z Values Detected in Medicago Seedlings (CHCA matrix)

Figure S1: Photographs comparing DHB and CHCA matrix application on root nodule slices



## Figure S2: Annotated MS/MS spectra from *de novo* sequencing.

MW 908.4238 LC-MS/MS ALC=82%







MW 990.5163

LC-MS/MS ALC=71%









#### MW 1008.4694

LC-MS/MS ALC=67%





#### MW 1068.5733 LC-MS/MS ALC=90%



#### MW 1068.5733

LC-MS/MS ALC=89%









#### MW 1106.5255 LC-MS/MS ALC=71%









MW 1159.6191

LC-MS/MS ALC=92%









MW 1170.6070 LC-MS/MS ALC=78%









MW 1175.5925 LC-MS/MS ALC=69%





#### MW 1186.5763 LC-MS/MS ALC=70%



#### MW 1192.5108 LC-MS/MS ALC=62%









#### MW 1308.6282 LC-MS/MS ALC=83%





MW 1376.7577 LC-MS/MS ALC=51%



MW 1728.9683

LC-MS/MS ALC=53%



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#### Figure S3: Annotated MS/MS spectra of unique peptides.



*m/z* 1162.59554, Low-temperature Inducible/ Nodulin-like Protein LC-MS/MS -10lgP=29.2

MALDI-MSI











#### m/z 1483.75454, Ribulose Biphosphate Carboxylase/ Oxygenase Large Chain Domain Protein MALDI-MSI



m/z 1976.02434, Basic 7S Globulin/ Peptidase A1, Pepsin MALDI-MSI LC-MS/MS -101gP=29.66

1170.5609 1283.6497

1382.7180

1545.7814

1646.8291

1266.6227 1365.6910 1528.7544

823.9146

1628.8186 1629.8021

1743.8818 1725.8713 1726.8549 872.4409 1856.9658 1838.9553 1839.9388 928.9829

806.4658 693.3817 594.3134

120.0655

86.0969 72.0812 136.0761 74.0605

70.0656

74.0605



403.7329 347.1909 297.6567

216.1250 165.6012

117.0748

60.5328

414.2230 313.1754 216.1226

103.0385

413.2394

102.0550

200

400

600

800

1000

0.01

-0.01 m/z

m/a

.

1200

1200

----bMax ---- yMax