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1. Introduction

The combination of chromatographic methods and mass spectrometry (MS) techniques is very useful in analysis of metabolites or components of complex biological samples. Chromatography has become the most important technique for separation and analyses, whereas MS is one of the most effective analytical methods used today for the determination of element concentrations, especially in the trace range; for the structural studies of organic and bioorganic compounds as well as isotopic analysis, due to its very high sensitivity, low detection limits and very small sample volumes needed\(^1\,^2\). Using chromatography and MS to separate and measure the concentration of amino acids has been well documented. Bengtsson\(^3\) was the first to introduce a micro-method for the analysis of free amino acids in natural waters by gas chromatography (GC); the technique includes removal of interfering organic substances by chloroform extraction and purification of amino acids by cation exchange. Later, the procedure for analysis of amino acids from protein acid hydrolyzates as their tert-butyldimethylsilyl(tBDMS)-derivatized amino acids levels by gas chromatography and mass spectrometry has been developed\(^4\,^9\) and applied in biological matrix analysis\(^10\,^13\). Johansen et al.\(^12\) and Rolin et al.\(^13\) studied the nitrogen metabolism of external hyphae of the AM fungus using measurement of tert-butylidemethylsilyl(tBDMS)-derivatized amino acids levels by gas chromatography and mass spectrometry. Arbuscular mycorrhizal (AM) fungus is the oldest obligate symbiont,\(^14\) benefiting the host plants by taking up N, P and other macronutrients, trace elements, and water from the soil. Among many components important for plant nutrition, nitrogen is often the most limiting but the AM fungi can improve the nitrogen (N) levels of their hosts.\(^15\,^17\) The extraradical hyphae of the fungi effectively acquire nitrate (NO\(_3^-\))\(^18\,^20\), ammonium (NH\(_4^+\))\(^22\,^21\,^23\), and amino acids\(^24\,^27\) from the external medium. However, for a long time, it has been unclear in what form nitrogen is translocated along the...
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hyphae (extraradical mycelium, ERM) of the fungus to the fungal structures within roots (intraradical mycelium, IRM), and how it is transferred across the mycorrhizal interface to the plant. To follow the uptake, assimilation and transfer of nitrogen in the arbuscular mycorrhizal symbiosis, we added isotopically labeled substrates to in vitro arbuscular mycorrhizal cultures of carrot (Daucus carota L.) roots colonized by G. intraradices.\textsuperscript{[28-30]} When grown in divided Petri plates, this model mycorrhiza excludes other microorganisms and prevents diffusion of nonvolatile solutes between the compartments. This model system shows normal life cycle and development of fungal morphology.

Following N uptake, its incorporation into amino acids via the glutamine synthetase/glutamine 2-oxoglutarate amidotransferase (GS/GOGAT) cycle has been observed in AM fungi. Using chromatographic separation and mass spectrometry analysis of amino acids of the hyphae, Johnsen et al.\textsuperscript{[12]} have proved that G. intraradices grown in a medium containing \textsuperscript{15}NH\textsubscript{4}\+ generated abundant free AAs in the ERM. Among these amino acids, \textsuperscript{15}N- labeled glutamate (Glu), glutamine (Gln), asparagine (Asn), aspartate, and alanine were predominant. Jin et al.\textsuperscript{[28]} have confirmed the mechanism of N transport to the host plant via the AM fungi proposed by Bago et al.\textsuperscript{[31]} (Fig. 1). When mycorrhizae of G. intraradices and Ri T-DNA-transformed carrot roots were grown in two-compartment Petri dishes, containing \textsuperscript{15}NH\textsubscript{4}Cl synthetic medium in the fungal compartment, the measurement of amino acids N-Methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) derivatives with GC-MS revealed that all free amino acids of the ERM were \textsuperscript{15}N-labeled to a high level. Among these amino acids, arginine (Arg) was the most abundant (over 90% of the total \textsuperscript{15}N in the free amino acids). This was confirmed by analysis of phenyl isothiocyanate (PTC)-derivatized amino acids with high performance liquid chromatography (HPLC). When the [U-\textsuperscript{13}C] Arg (MTBSTFA derivative with an m/z of 448) was used as the labeled substrate in the fungal compartment, the isotopomer analysis with GC-MS confirmed that this amino acid was intact both in the mycorrhizal root tissue (m/z of 448) and in the ERM. This result suggests that Arg is either taken up or synthesized by the ERM and transported intact to the IRM; the conclusion is also consistent with the outcome of other [U-\textsuperscript{13}C/U-\textsuperscript{15}N] Arg labeling experiments.\textsuperscript{[30]} This finding indicates that the AM fungi take up N and incorporate it into Arg, and this organic form of N is then transported to IRM (Fig. 1).

When either [U-\textsuperscript{13}C] or [U-\textsuperscript{15}N/U-\textsuperscript{13}C]-labeled Arg was added to the fungal compartment to follow up Arg catabolism in AM fungi (Fig. 1), GC-MS\textsuperscript{[30]} analysis of Arg isotopomer found either [U-\textsuperscript{13}C] or [U-\textsuperscript{15}N/U-\textsuperscript{13}C] ornithine(Orn) in the mycorrhizal root tissues. This result demonstrates that once Arg is translocated to the potential N-limited sites in the mycelium of AM fungi, it is degraded into Orn and urea. Although N released from Arg degradation is transferred to the host plant, it has been shown (using \textsuperscript{13}C\textsubscript{1,2}-acetate labeling in fungal compartment and subsequent mass spectrometry analysis of amino acids of mycorrhizal root) that the Arg-originated C is not incorporated into the host C pool and remains in the IRM\textsuperscript{[29]}. Analysis of \textsuperscript{15}N-labeled Glu and Gln isotopomers using GC-MS has demonstrated that, following Arg degradation, Orn is recycled to Glu and Gln, which serve as C donors. Small amounts of \textsuperscript{15}N label have been found in Glu and Gln (in spite of their low levels) after [guanido-\textsuperscript{15}N\textsubscript{2}] Arg translocation from the ERM to the mycorrhizal roots, whereas other
amino acids have displayed only negligible $^{15}$N labeling. Thus, the Orn cycle seems to be an efficient path for Arg biosynthesis, nitrogen transfer to the host, and carbon recycling in AM symbiosis.

**Figure 1. A model of N uptake, translocation, degradation and transfer in the AM fungal symbiotic system.** Various forms of N sources ($\text{NO}_3^-$, $\text{NH}_4^+$, amino acids, peptides, and proteins) are taken up, assimilated, and incorporated into arginine (Arg) by the extraradical mycelium (ERM). The accumulated Arg as well as polyphosphate (PolyP) is then bi-directionally translocated along the coenocytic fungal hyphae from the ERM to the intraradical mycelium (IRM) or from the mycorrhizal compartment tissue to the ERM. Arg is catabolized through the catabolic arm of the urea cycle in the IRM, releasing $\text{NH}_3$/$\text{NH}_4^+$ in the arbuscules. The $\text{NH}_4^+$ ion is deprotonated prior to its transport across the plant membrane by AMT protein and released in its uncharged $\text{NH}_3$ form into the plant cytoplasm. Figure modified from Govindarajulul et al. (2005).

In addition, the results of MS analysis of $^{15}$N-labeled amino acids isotopomers have indicated that germinating spores break down stored N (Arg or proteins) during pre-symbiotic growth. HPLC analysis of amino acids derivatives using PITC revealed that the germinating AM spores combine the C skeletons originating from the degradation of the internally stored lipids with the N released from the stored N compounds (Arg or proteins) for de novo biosynthesis of free amino acids, mostly producing serine and glycine. This is consistent with a large flux in the glyoxylate cycle and the utilization of lipids as a C source for carbohydrate and amino acid biosynthesis reported by Lammers at al. Although exogenous N is not required for pre-symbiotic growth, it can be used for de novo biosynthesis of amino acids. MS analysis of $^{15}$N enrichment of amino acids after $^{15}$N labeling
The experiment showed that NH$_4^+$ and urea are assimilated more rapidly than NO$_3^-$ and exogenous amino acids. *De novo* biosynthesis of free amino acids in the AM spores was increased greatly after the uptake of exogenous NH$_4^+$, urea, and NO$_3^-$. In cases of a low C:N ratio (no exogenous glucose), the measurements of PITC-derivatized AAs with HPLC showed that Asn was the predominant amino acid in the AM spores. These results suggest that during spore germination, the main carbon source for amino acids biosynthesis is derived mostly from the degradation of stored lipids and the glyoxylate cycle. In contrast, HPLC analysis of PITC-amino acids derivatives has revealed that at a high C:N ratio (available exogenous glucose) Arg is the main amino acid produced and incorporated into the proteins of germinating AM spores\[^{[33]}\]. This is consistent with the report of Tisserant et al.\[^{[35]}\] showing that the transcripts coding for the enzymes of Arg biosynthesis are highly expressed in germinating spores of AM fungus *G. intraradices*.

To summarize, chromatographic separation and analysis of $^{15}\text{N}/^{13}\text{C}$-labeled amino acids have determined in what form nitrogen is taken up and assimilated, and clarified the mechanisms of Arg transport and degradation in AM fungal symbiosis. In the following sections, we will discuss in some detail the application of chromatographic methods in the studies of nitrogen metabolism and transport in AM fungal system.

### 2. Cleanup and separation of amino acids using ion-exchange chromatography

Sample preparation is often required to improve the analysis, eliminate interference and increase sensitivity.\[^{[1]}\] This is necessary when a sample cannot be directly analyzed or when the analysis generates poor results. Most cleanup and concentration techniques are based on separations. Separation techniques have a number of characteristics such as fractionation capacity, load capacity, adaptability to analyte volatility, type of selectivity, speed and convenience, static and dynamic procedures. The type of selectivity depends on physicochemical properties of the components in the sample. For example, differences in boiling point allow separation by distillation and are important in gas chromatographic separation, and acid/base dissociation constant is important in ion-exchange chromatographic analysis. Chromatographic separations are used both for sample preparation and core analytical separation and measurements. To check nitrogen utilization and amino acids metabolism in AM fungal symbiotic system, free amino acids from cultured tissues can be extracted with a mixture of methanol/chloroform/water, then separated on a cation exchange column (DOWEX 50 *4-200, hydrogen form) and recovered after freeze-drying\[^{[28-30]}\].

In the method published by Jin et al., after 3-week culture of mycorrhizal roots,\[^{[28]}\] the extraradical mycelium (ERM) and mycorrhizal root tissues were recovered on a 38-$\mu$m sieve, rinsed with deionized water and lyophilized. The lyophilized mycorrhizal roots and ERM were ground in a mortar with a pinch of acid-washed sand and extracted with a mixture of methanol/chloroform/water (12 : 5 : 3, v/v/v), which recovered 30–35% more amino acids than extraction with NH$_4$HCO$_3$ buffer (pH 8 with 0.2% NaN$_3$) or 80% ethanol.\[^{[13]}\]
Methylene chloride and water were added to the extraction solution to facilitate the separation of chloroform and the methanol–water phases. The methanol–water phase containing the amino acids was collected and evaporated in a rotary evaporator at 50°C. Bengtsson & Odham have pointed out that losses of amino acids during evaporation prior to derivatization are negligible, and, using a radioactive amino acid tracer, demonstrated low losses of amino acids co-precipitated with carbonates and hydroxides. Losses from nutrient rich samples were further reduced by acidifying the sample before evaporation. During evaporation, Maillard reaction can be avoided by keeping the temperature at 50°C and reducing evaporation pressure. A direct cation exchange has been shown to be inadequate in obtaining a sufficiently pure solution for derivative formation. However, it has been demonstrated that extraction of the aqueous sample with chloroform prior to ion exchange efficiently removes interfering organic substances without detectable losses of amino acids.

Most amino acids are not soluble in nonpolar solvents and are soluble in water; they display amphoteric properties (caused by COOH and NH₂ groups), and many exist as zwitterions in the form R-CH(NH₃⁺)-COO⁻. In acidic solutions, the amino groups are at least partly protonated whereas the ionization of the carboxyl group is very low. For the cation exchange, strong acidification is therefore necessary to convert the monoamino-acids completely to the univalent cation form. For example, at pH 2.5, 35% of Phe, 66% of Thr, and 100% of the diamino-acids are in the cationic form. In the micro-method procedure published by Bengtsson, the residues containing the amino acids were dissolved in 2 ml of 0.01 M HCl and loaded onto a cation exchange column, previously washed with 2 M NH₄OH, deionized H₂O and 2 M HCl, and followed by a wash with deionized H₂O until the effluent was neutral. The neutral compounds, principally carbohydrates, were washed off the column with 5 ml of water, and the free amino acids (except cysteine(Cys) and methionine(Met), whose recoveries were low), were eluted with 2 ml of 1 M NH₄OH. Sulfur-containing amino acids are partly oxidized during the ion-exchange procedure or derivatization, therefore, this method is not suitable for recovery and purification of Cys and cystine. Nevertheless, Myung et al. have developed a method employing SPME (solid-phase micro-extraction) technique and GC–MS to determine homocysteine (Hcy), Cys and Met levels in aqueous samples. This method provides a new approach to the studies of S uptake and transfer in AM symbiosis.

3. Determination of amino acid concentrations with high performance liquid chromatography (HPLC)

Since amino acids are non-volatile compounds and most of them show low UV absorbance, they have been commonly analyzed by liquid chromatography (LC) methods with pre-column or post-column derivatization using UV chromophore or fluorophore reagents. The use of HPLC analysis is extremely common because this technique has no specific analyte volatility or thermal stability restrictions. Derivatization can make the analysis more sensitive, gives a linear detection response and avoids specific interference. The common
approach to the preparation of derivatized samples for HPLC and GC analysis is to replace the active hydrogens to form a desired physical property. However, in HPLC, the elimination of all active hydrogens from the analyte is not usually necessary. Some derivatizations requiring only the attachment of a chromophore or fluorophore group to the analyte use one of the functional groups (such as phenyl isothiocyanate, O-phthalaldehyde or 9-fluorenylmethyl chloroformate) which react only at the amino group. Other derivatizations of amino acids involve both NH$_2$ and COOH groups; for example, when isothiocyanates are used to form thiolydantoin. The quantification of derivatized amino acids, such as phenylthiocarbamoyl (PTC) derivatives, is commonly used prior to their analysis by HPLC. Phenyl isothiocyanate (PITC) reacts both with the primary and secondary amino-groups, at room temperature, within 5-20 min; PITC-amino acids are very stable in dried samples, and their elution and detection only requires a binary gradient pump and a UV detector. The disadvantages of the method are its limited sensitivity (because of the lack of fluorogenic derivatives) and the need for removal of excess reagent. Therefore, in our experiments to determine the levels of free amino acids in the ERM and mycorrhizal root tissues, we used a Waters Pico-Tag amino acid analyzer (HPLC), employing the Pico-Tag method. As in the study by Endres & Mercier\cite{40}, the amount of each amino acid was measured by high-performance liquid chromatography (HPLC) of the PITC derivatives. The extracted amino acids were dissolved in 0.1 M HCl and vacuum-dried in a Pico-Tag workstation, then ethanol/ water/triethylamine mixture was added and evaporated by vacuum-drying. 20 ml of ethanol/ water/triethylamine/ phenylisothiocyanate (7 : 1 : 1 : 1) was added to derivatize the amino acids at 23°C for 20 min. The samples were then dried under vacuum and re-dissolved in 100 μl of Pico-Tag sample diluent. 20 μl of each sample was loaded onto a reverse-phase C18 column (3.9 mm ID X 150 mm long) using a Waters 510 autosampler. An eluent gradient consisting of 38 ml Pico-Tag Eluent A (0.05 M sodium acetate) and Eluent B (0.1 M sodium acetate/acetonitrile/methanol (46:44:10) was used as mobile phase. The flow rate was 1.0 ml min$^{-1}$, with the proportion of Eluent B rising from 0–100%. The elution was monitored at 254 nm with a Waters 486 tunable absorbance detector. The concentrations of the amino acids were calculated by comparing the integrated peak area with those for standard amino acids at known concentrations using Waters MILLENIUM software (Waters Chromatography Division). The threshold for detection of amino acids in standard solutions was 30 pM of each amino acid per assay, corresponding to <10 nmol g$^{-1}$ of dry weight of tissue.

As shown in Table1, our HPLC analysis of free amino acid levels reveals that Arg is by far the most abundant fungal amino acid (between 50 and 200mM depending on developmental stage), representing c. 90% of the total free amino acids in the ERM. Arg levels are also substantially higher in colonized than in un-colonized roots (54.2 ± 19.3% versus 10.9± 4.8% of free amino acids). Johansen et al.\cite{12} have observed, without reporting absolute levels, that Arg is the dominant free amino acid in extraradical mycelium of Glomus claroideum. However, they have not measured Arg levels in G. intraradices because of the problems with derivatization and decomposition of the silated product.
Table 1. Concentration of free amino acids, established using HPLC, in the root compartment tissue and extraradical mycelium of AM fungus G. intraradices after culturing for 1 or 3 weeks in two-compartment Petri dishes, with $^{15}$NH$_4$Cl labeling in fungal compartment.*

4. Identification of derivatized amino acids and their isotopomer analysis with gas chromatography-mass spectrometry

Derivatization involves reactions with one or more reagents to change the chemical nature of the analyte to make it more suitable for analysis. As chemical reactions,[41] derivatizations are efficient chemical processes between the analyte and the reagent, such as reactions forming acyl, alkyl or aryl derivatives, silylation reactions, adding to carbon-hetero multiple bonds, formation of cyclic compounds, etc. These reactions result in a replacement of active hydrogens in an analyte in functional groups such as OH, COOH, SH, NH, CONH.

The purpose of derivatization varies depending on the analyte, the matrix of the sample, and the analytical method to be applied.[41] Some derivatizations are used in the sample cleanup or concentration process. Much more frequently, they are done to change the analyte properties for the chromatographic separation, to achieve better thermal stability, better detectability and improve separation in GC analysis. In GC-MS analytical technique,
derivatization may help in spectra identification. For HPLC using liquid mobile phase, derivatization is performed mainly to increase detectability and improve the separation.

The trimethylsilyl (TMS) derivatives are obtained in one-step derivatization procedure, whereas almost all other derivatives are formed in two or more reaction steps. The derivatization with the formation of silylated derivatives is applied to replace the active hydrogens in an analyte in groups such as OH, SH, NH, CONH, POH, and SOH. The purpose of silylation is to reduce the polarity of the analyte, increase its stability and improve detectability. Although the TMS derivatives are by far the most commonly used for analytic purposes, TBDMS is used when compounds more resistant to hydrolysis are required. The thermal stability of TBDMS derivatives is better than that of TMS derivatives. The TBDMS derivatives give reproducible results in amino acid analysis. Gehrke\cite{36} pointed out that the best foundation for a successful amino acid analysis by GC is (a) reproducible and quantitative conversion of amino acids to suitable derivatives; and (b) separation and quantitative elution of the derivatives from the chromatographic column. For satisfactory analysis of amino acids by GC, a complete derivatization is essential. In my own experiments, free amino acids samples were derivatized with MTBSTFA containing 1% N-methyl-N-t-butyldimethylchlorosilane; such derivatized Arg and ornithine (Orn) are shown in Fig.2.\cite{30}

![MTBSTFA-derivatized ion of arginine, m/z 442 (M-188)](image)

![MTBSTFA-derivatized ion of ornithine, m/z 474](image)

**Figure 2. Molecular structure of ions of N-methyl-N-(t-butyldimethylsilyl)trifluoroacetamide (MTBSTFA)-derivatized Arg and Orn.** This figure gives the structure of the ion remaining after a 70 eV impact on the MTBSTFA derivative of Arg and Orn. It demonstrates that derivatized Arg has an m/z of 442, which is the M-188 (molecular ion minus 188) fragment arising from losing the guanido Ns and a t-butyl group. This fragmentation is different from that of Orn, which only loses a t-butyl group (M-57) in its fully derivatized form.\cite{30}

MTBSTFA has been reported as a very powerful tBDMS silyl donor capable of tert-butyldimethylsilylating active protic functions (hydroxyl, amino, carboxylic and thiol
moieties),[42] and has been employed in the derivatization of Arg and Gln for analysis by gas liquid chromatography (GLC).[43] Mawhinney et al.[4] reported an analytic method which employs the tBDMS derivatives of amino acids for their separation and quantification in a single GLC analysis. The hydrochloride salts of the amino acids, dissolved in dimethylformamide, are derivatized in a single step using MTBSTFA. As the tBDMS-amino acid derivatives, the neutral and acidic amino acids are stable for over 24 h and the basic amino acids are stable for 6 h. Mass spectroscopy is probably the most powerful tool used for compound identification purposes. The mass spectrum for each tBDMS-amino acid is relatively simple, being dominated by a unique and unambiguous mass minus 57 \([M - 57]\) fragment ion which for many of the amino acids serves as the base fragment ion. Employing amino acid standards, a linear response curve in the range 1-100 nmol was obtained for each neutral and acidic amino acid using a flame ionization detector. The basic amino acids lysine and arginine demonstrated a linear response curve in the range 2-150 nmol. Histidine (His) displayed a linear response curve in the range of 5-150 nmol. In contrast with the results of my experiments (data not published) employing amino acid standards, a linear response curve in the range of 10-30 nmol can be obtained only for Leu, Ser, Asp, Cys; Met; Thr, and Tyr, but not for His, Arg, Gln and Pro. These last four amino acids produced a non-linear curve with GC-MS in a Trace 2000 gas chromatograph (Thermo Electron).

Figure 3. The GC-MS total ion chromatogram (TIC) of amino acid mixture in the ERM of AM fungi.

In our experiments, identities of amino acids were confirmed by comparison with mass spectra of authentic standards (Fig.3). The mass isomer distribution for each derivatized
amino acid was determined by measuring the M-57 ions which result from the loss of a tert-butyl group (i.e., [M - C(CH₃)₃]) from the molecules of MTBSTFA derivatives, except for Arg whose m-188 ion was used. The Arg ion examined had a m/z of 442, which corresponds to M-188 (molecular ion minus 188) fragment arising from the loss of one guanido nitrogen together with a tBDMS and DMS group from the tetra-substituted tert-butyldimethylsilylimethylsilylimethylsilyl(tBDMS)-derivatized Arg (Fig. 2). When using [guanido-2-¹⁵N] Arg, we observed an ion at an m/z of 443 (M-189, molecular ion minus 189). This isotopomer corresponds to the derivatized [guanido-2-¹⁵N]Arg because the ion loses one of the guanido nitrogens by fragmentation at 70 eV.

5. Analysis of ¹⁵N-labeled amino acid isotopomers with gas chromatography-mass spectrometry

Unlabeled, derivatized Arg yields an ion at an m/z of 442, which corresponds to a molecular fragment containing three ¹⁴N atoms. Thus, the maximum number of ¹⁵N atoms detected is three, resulting in a mass isomer distributions of M, M+1, M+2 and M+3. These were used to calculate the isotopic enrichment in each amino acid after correction for natural isotopic contents by comparison with the mass isomer distributions measured for unlabeled standards. As shown in Fig. 1, to test whether Arg is translocated from the ERM to the IRM,
13C6 arginine was added to the ERM. After 6 weeks, MS analysis of MTBSTFA-derivatized Arg isotopomer revealed that 34% of the free Arg in the ERM and 33% of the free Arg in the colonized roots showed 13C6 labeling (M+6, Fig. 4). The mass spectra showed that the free Arg molecules in the colonized roots are either completely unlabeled (natural abundance mass isomer distribution) or labeled in all six carbon positions, thus indicating that Arg is transported intact from ERM to IRM.

6. Conclusion
Chromatographic and mass spectrometry analysis of amino acids, in combination with isotopic tracing, shows that various forms of N sources (NO\textsubscript{3}, NH\textsubscript{4}, amino acids) are taken up, assimilated, and incorporated into Arg by the ERM. The accumulated Arg as well as polyP is then bidirectionally translocated along the coenocytic fungal hyphae from the ERM to the IRM or from the mycorrhizal compartment tissue to the ERM. Arg is catabolized through catabolic arm of the urea cycle (utilizing arginase and urease activities) in the IRM, releasing NH\textsubscript{3}/NH\textsubscript{4} in the arbuscules. The NH\textsubscript{3}/NH\textsubscript{4} acquired by the plant is either transported into adjacent cells or immediately incorporated into AAs, as shown in Fig.1 (modified from Govindarajulu et al.\textsuperscript{[29]}).

However, although Ala, Gly, Val, Leu, Ileu, Pro, Ser, Thr, Phe, Asn, Asp, Glu, Orn, Gln, Arg and tyrosine(Tyr) are detected, GC-MS of samples from AM fungal tissues performed after MTBSTFA-derivatized amino acids cleaned up on a cation exchange column (DOWEX 50 *4-200, hydrogen form) does not detect some S-containing amino acids and basic amino acids lysine (Lys) and His. Some amino acids cannot be quantified with chromatographic MS due to non-linear response. Nevertheless, analysis of PITC-derivatized amino acids with HPLC shows excellent linear relationship between the molar concentrations of amino acids and peak areas in the chromatogram, and thereby can be used for effective quantification. Although many techniques are already in use in this field, we will need some novel methods, yet to be developed, to achieve a simultaneous measurement and identification of all free amino acids in biological tissues.

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Abbreviations
GC: gas chromatography; GLC: gas liquid chromatography; MS: mass spectrometry; HPLC: high performance liquid chromatography; MTBSTFA: N-methyl-N-(t-butyldimethylsilyl) trifluoroacetamide; tBDMS: tert-butyldimethylsilyl; TMS: trimethylsilyl; PTC: phenylthiocarbamoyl; PITC: phenyl isothiocyanate; SPME: solid-phase microextraction.

Ala: alanine; Gly : glycine; Val: valine; Leu: leucine; Ile: isoleucine; Pro: proline; Ser: serine; Thr: threonine; Phe: phenylalanine; Asp: aspartate; Glu: glutamate; Orn: ornithine; Asn:
asparagine; Gln: glutamine; Arg: arginine; Tyr: tyrosine; Lys: lysine; Cys: cysteine; Met: methionine, His: histidine; Hcy: homocysteine.

AM: arbuscular mycorrhizae; ERM: extraradical mycelium; IRM: intraradical mycelium; GS/GOGAT: glutamine synthetase/glutamine 2-oxoglutarate amidotransferase.

7. References


