Occurrence of \(N^1\)-Methylputrescine, \(N^1\), \(N^4\)-Dimethylputrescine, \(N^1\)-Methylhomospermidine and \(N^1\), \(N^9\)-Dimethylhomospermidine in Tomato Root

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Abstract

Four terminal-\(N\)-methylated polyamines, \(N^1\)-methylputrescine, \(N^1\), \(N^4\)-dimethylputrescine, \(N^1\)-methylhomospermidine and \(N^1\), \(N^9\)-dimethylhomospermidine were detected and identified as an endogenous minor polyamine in the mature root of a \textit{Solanum} crop plant, tomato, by GC and GC-MS. This is the first report on the natural occurrence of \(N^1\), \(N^4\)-dimethylputrescine and the two \(N\)-methylated homospermidine(s).

INTRODUCTION

In the biogenic amines discovered to act as the endogenous polyamines in higher plants, diamines such as diaminopropane, putrescine and cadaverine, trimines such as spermine, spermidine, norspermidine and homospermidine, tetra-amines such as spermine, thermostereshmine, norspermine, canavalmine and aminopropylhomospermidine and a guanidinoamine, agmatine, are involved in plant cell proliferation and differentiation and play a defensive role against various stresses in plant cells [1-3]. Furthermore, endogenous distribution of \(N\)-methylated and \(N\)-acetylated polyamine derivatives in plants is attractive to consider intracellular polyamine metabolism in addition to its biological function in plants.

In the course of our polyamine analyses of the acid extracts from various higher plant tissues, a \(N\)-methylated tetra-amine, \(N^4\)-methylthermospermine, and a \(N\)-methylated guanidinoamine, \(N^6\) (\(N^G\))-methylagmatine, have been found in legumes [4-6], and the latter was also detected in some aquatic higher plants and gramineous plants [7]. A \(N\)-methylated triamine, \(N^4\)-methylspermidine, was discovered in an aquatic higher plant [8]. On the other hand, a \(N\)-methylated diamine, \(N^1\)-methylputrescine, has been found in the tobacco \textit{Nicotiana tabacum} and the potato \textit{Solanum tuberosum}, and is first specific metabolite on the route to some alkaloids in the \textit{Solanaceae} plants [9-12].

Although analysis of \(N\)-acetylated polyamines has never been enough in higher plants, we detected three novel \(N\)-methylated polyamines in addition to \(N^1\)-methylputrescine in mature tomato root during polyamine analyses of various tissues of the three \textit{Solanum} crop plants, \textit{S. lycopersicum} (formerly \textit{Lycopersicon esculentum}) (tomato), \textit{S. melongena} (eggplant) and \textit{S. tuberosum} (potato) in the present study.

MATERIALS AND METHODS

Young roots were harvested from young tomatoes (mini-tomato, cultivar ‘Mini-carol’) and young eggplants (long egg form-eggplant, ‘Senryo-2’) (20-30 days after sowing) purchased in May from Noguchi Nursery Co. in Maebashi, Gunma, Japan. Mature roots were harvested after the cultivation of the two young plants for four months in an outdoor farm of Maebashi. Tubers of mature potatoes (‘Kitaakari’) cultivated for four months were harvested in September from an outdoor farm in Tsumagoi, Gunma. The washed roots and tubers (100-200g) were homogenized in the same weight of 10% (1.0M) perchloric acid (HClO\(_4\)) purchased in May from Noguchi Nursery Co. in Maebashi, Gunma, Japan. The washed roots and tubers (100-200g) were homogenized in the same weight of 10% (1.0M) perchloric acid (HClO\(_4\)) by a mixer. After a three-time extraction by centrifugation, the supernatant of the 5% PCA extract was
subjective to a column (3 cm I.D. x 1 cm) of a cation-exchange resin, DOWEX 50Wx8 (Dow Chemical Co., Michigan, USA). The column was washed with 1M HCl and then polyamines were eluted with 6M HCl from the column. The concentrated polyamine fraction was evaporated, dissolved in water, and then neutralized. Gas chromatography (GC) on a SHIMADZU GC-17A (Kyoto, Japan) and gas chromatography-mass spectrometry (GC-MS) on a JEOL JMS-700 (Tokyo, Japan) equipped with a capillary column (0.32mm I.D. x 30m) of Inert Cap 1MS (GL Sciences, Tokyo, Japan), were performed after heptafluorobutyration of the concentrated polyamines [13]. Helium was used as the carrier gas. Heptafluorobutyril (HFB) derivatives of polyamines in GC were determined on a flame ionization detector. Common polyamines purchased from Sigma (St. Louis, USA), homospermidine, aminopropylhomospermidine and thermospermine synthesized as previously reported [13] and N°-methylputrescine, N°,N°-dimethylputrescine, N°-methylhomospermidine and N°,N°-dimethylhomospermidine synthesized here in our laboratory according to the previously reported methods [14,15] were used as the standard for identification of polyamine peaks on GC chromatograms and mass spectra in GC-MS.

**RESULTS AND DISCUSSION**

On the two GC chromatograms, four minor unknown peaks (1), (2), (3) and (4) corresponding to N°-methylputrescine,N°,N°-dimethylputrescine, N°-methylhomospermidine and N°,N°-dimethylhomospermidine, respectively, in addition to putrescine, spermidine, homospermidine, norspermine, thermospermine, spermine, aminopropylhomospermidine and agmatine were detected in the concentrated polyamine fraction extracted from the mature roots of the tomatoes (Figure 1). Retention times of the two N°-methylated putrescine(s) and the two N°-methylated homospermidine(s) delayed from those of putrescine and homospermidine, respectively, and are identified by the mass spectrometry of the peaks in GC-MS (Figure 2). Methylated derivatives of the two major polyamines, spermidine and spermine, furthermore, diaminopropane, cadaverine, norspermidine and canavalmine were not detected in the GC chromatograms.

Molar concentrations of endogenous N°-methylputrescine, N°,N°-dimethylputrescine, N°-methylhomospermidine and N°,N°-dimethylhomospermidine per gram of wet weight of the mature tomato roots, roughly calculated from the GC data, were 0.03 (μmol/g w.w.), 0.05 (μmol/g w.w.), 0.03 (μmol/g w.w.) and 0.05 (μmol/g w.w.), respectively. Methylated polyamines were not detected (<0.005 μmol/g w.w) in the polyamine fractions extracted from young tomato roots as well as young and mature eggplant roots, and mature potato tubers. Quantitative analysis of total endogenous polyamines including N°-methylated polyamines in the roots and other various tissues of the three Solanum crop plants will be reported elsewhere.

N°-methylated polyamines have never been found in bacterial and animal cells whereas N-acetylated putrescine, spermidine and spermine are distributed in some of them [16,17]. N°-methylputrescine has been found as the first metabolite during alkaloid synthesis in the plants tobacco and potato [9-12]. In addition, cadaverine and homospermidine are the precursor of some plant polyamine-derived alkaloids [18-20]. The four N°-methylated polyamines found together in mature tomato roots in the present study may be need for alkaloid biosynthesis in the plant.

In our previous studies, it was speculated that N°-methylagmatine is converted to N°-methylputrescine, and N°-methylspermidine and N°-methylthermospermine are produced by aminopropyl-transfer to the methylated terminal
N of \(N\)-methylputrescine in the plants [4-9]. Make of \(N\)-methylhomospermidine from \(N\)-methylputrescine is possible by homospermidine synthase in plants, however, direct mono-methylation into one or two terminal N of putrescine and homospermidine is suggested in tomato root.

Although chemically synthesized \(N\)-methylated polyamines such as \(N\)-methylspermidine, \(N\), \(N\)-dimethylspermidine \(N\),\(N\)-dimethylhomospermidine and \(N\),\(N\)-dimethylhomospermidine were used as a exogenous cell growth inhibitor in vitro [21-23], natural occurrence of \(N\),\(N\)-dimethylputrescine, \(N\)-methylhomospermidine and \(N\),\(N\)-dimethylhomospermidine has never been reported until the present study.

CONCLUSION

Four novel terminal-\(N\)-methylated polyamines, \(N\)-methylputrescine, \(N\), \(N\)-dimethylputrescine, \(N\)-methylhomospermidine and \(N\), \(N\)-dimethylhomospermidine were found as the endogenous minor polyamines in mature tomato root. This is the first report on the natural occurrence of \(N\), \(N\)-dimethylputrescine and the two \(N\)-methylated homospermidine(s).

REFERENCES


