

Short Communication

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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Submitted: 08 November 2014

Accepted: 02 December 2014

Published: 05 December 2014

ISSN: 2333-6668

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Keywords

- Methylated polyamine
- Methylputrescine
- Methylhomospermidine
- Tomato root
- Plant polyamine

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 *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, triamines such as spermidine, norspermidine and homospermidine, tetra-amines such as spermine, thermospermine, norspermine, canavalline 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^C)-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 *Nicotiana tabacum* and the potato

Solanum tuberosum, and is first specific metabolite on the root to some alkaloids in the *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 *Solanum* crop plants, *S. lycopersicum* (formerly *Lycopersicon esculentum*) (tomato), *S. melongena* (eggplant) and *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 ($HClO_4$) (PCA) by a mixer. After a three-time extraction by centrifugation, the supernatant of the 5% PCA extract was

subjective to a column (3cm I.D. x 1cm) 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 heptafluorobutyrylation of the concentrated polyamines [13]. Helium was used as the carrier gas. Heptafluorobutyryl (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*¹-methylputrescine, *N*¹,*N*⁴-dimethylputrescine, *N*¹-methylhomospermidine and *N*¹,*N*⁹-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*¹-methylputrescine, *N*¹,*N*⁴-dimethylputrescine, *N*¹-methylhomospermidine and *N*¹,*N*⁹-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 canavamine were not detected in the GC chromatograms.

Molar concentrations of endogenous *N*¹-methylputrescine, *N*¹,*N*⁴-dimethylputrescine, *N*¹-methylhomospermidine and *N*¹,*N*⁹-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*¹-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

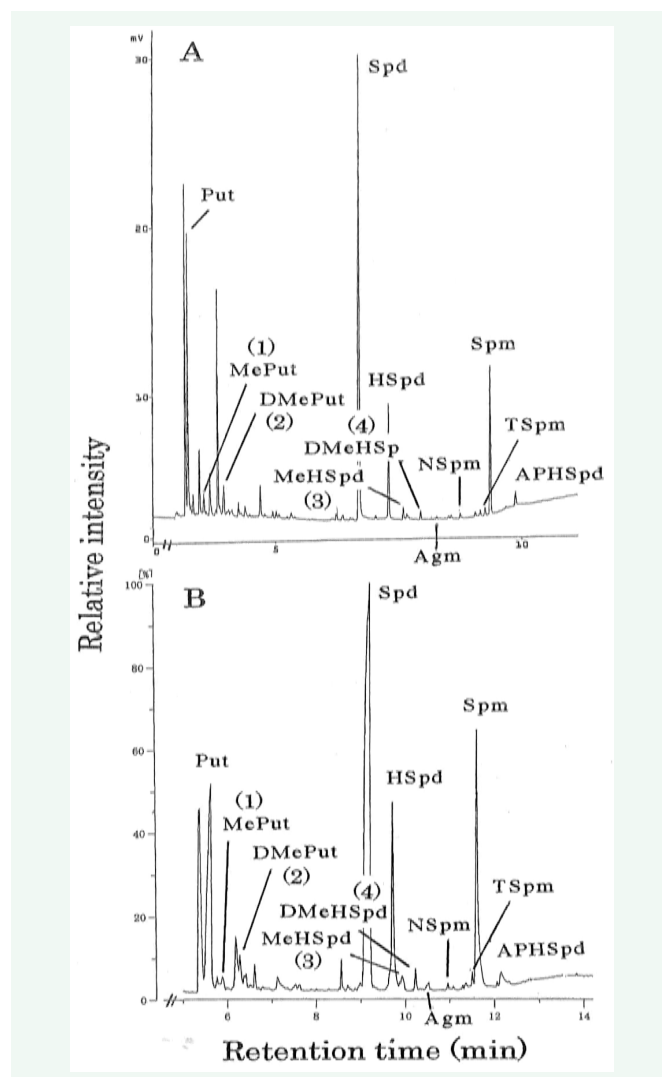


Figure 1 GC of polyamines of tomato (*Solanum lycopersicum*) root on SHIMADZU GC-17A operated at 120°C-16°C/min-280°C of column temperature (A) and JEOL JMS-700 operated at 90°C-16°C/min-280°C of column temperature (B). The resolution and retention times of peaks (1), (2), (3) and (4) correspond to the authentic heptafluorobutyryl (HFB) derivatives of MePut, DMePut, MeHSpd and DMeHSpd, respectively. Abbreviations for polyamines: Put, putrescine [NH₂(CH₂)₄NH₂]; Spd, spermidine [NH₂(CH₂)₃NH(CH₂)₄NH₂]; HSpd, homospermidine [NH₂(CH₂)₄NH(CH₂)₃NH₂]; NSpm, norspermine [NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH₂]; TSpM, thermospermine [NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₄NH₂]; Spm, spermine [NH₂(CH₂)₃NH(CH₂)₄NH(CH₂)₃NH₂]; APHSpd, aminopropylhomospermidine [NH₂(CH₂)₃NH(CH₂)₄NH(CH₂)₄NH₂]; Agm, agmatine [NH₂C(NH)NH(CH₂)₄NH₂]; MePut, *N*¹-methylputrescine; DMePut, *N*¹,*N*⁴-dimethylputrescine; MeHSpd, *N*¹-methylhomospermidine; DMeHSpd, *N*¹,*N*⁹-dimethylhomospermidine. Other unknown non-amine peaks are detected in the GC chromatograms.

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*⁶-methylagmatine is converted to *N*¹-methylputrescine, and *N*⁴-methylspermidine and *N*⁴-methylthermospermine are produced by aminopropyl-transfer to the methylated terminal

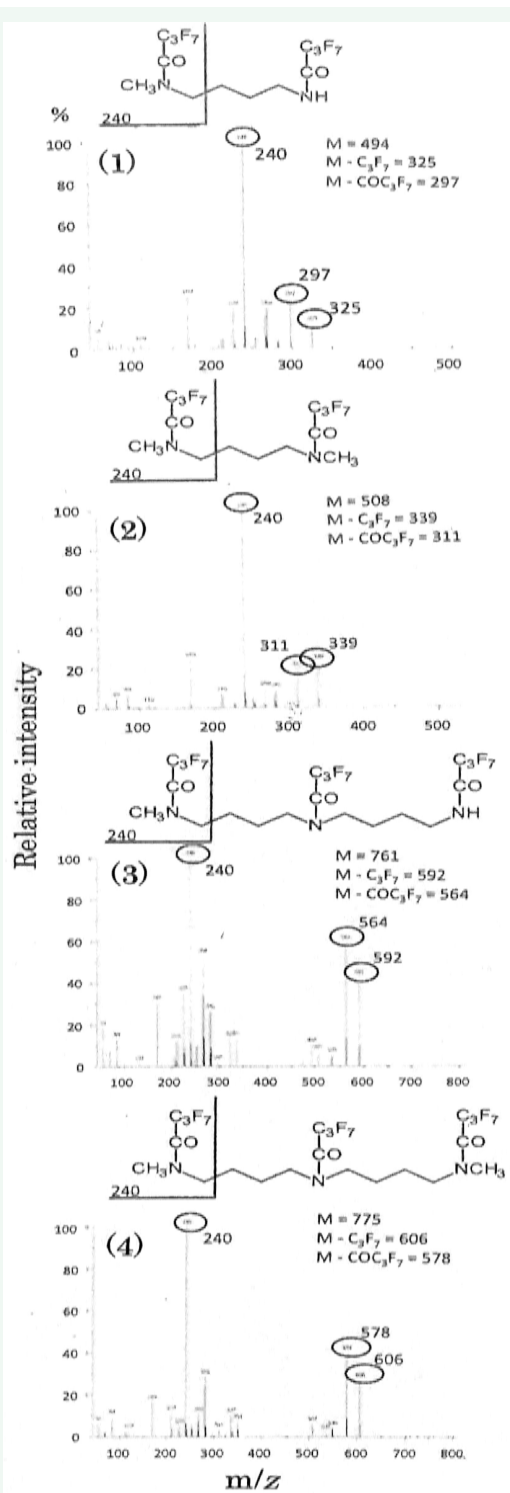


Figure 2 Mass spectra of the methylated polyamine peaks (1), (2), (3) and (4) obtained in JEOL JMS-700 (Figure 1B). The spectrum of (1), (2), (3) and (4) is identical with that of authentic HFB-MePut, HFB-DMePut, HFB-MeHSpd and HFB-DMeHSpd, respectively. JMS-700 mass spectrometer was operated in the electron impact mode at the ionization energy of 70eV. Structure, molecular weight (M) and mass number of the three fragment ions from HFB-methylpolyamines are shown. The typical mass fragment ion ($C_3F_7CON(CH_3)CH_2^+$) peak at m/z 240 is observed as a base peak in these mass spectra. Abbreviations for polyamines are shown in Figure 1.

N of N^1 -methylputrescine in the plants [4-9]. Make of N^1 -methylhomospermidine from N^1 -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^1 -methylspermidine, N^1 , N^8 -dimethylspermidine N^1,N^9 -dimethylhomospermidine and N^1,N^{12} -dimethylspermine were used as a exogenous cell growth inhibitor *in vitro* [21-23], natural occurrence of N^1,N^4 -dimethylputrescine, N^1 -methylhomospermidine and N^1,N^9 -dimethylhomospermidine has never been reported until the present study.

CONCLUSION

Four novel terminal-*N*-methylated polyamines, N^1 -methylputrescine, N^1 , N^4 -dimethylputrescine, N^1 -methylhomospermidine and N^1 , N^9 -dimethylhomospermidine were found as the endogenous minor polyamines in mature tomato root. This is the first report on the natural occurrence of N^1 , N^4 -dimethylputrescine and the two *N*-methylated homospermidine(s).

REFERENCES

- Kusano T, Berberich T, Tateda C, Takahashi Y. Polyamines: essential factors for growth and survival. *Planta*. 2008; 228: 367-381.
- Takahashi T, Takechi J. Polyamines: ubiquitous polycations with unique roles in growth and stress responses. *Ann Bot*. 2010; 105: 1-6.
- Takano A, Takechi J, Takahashi T. Thermospermine is not a minor polyamine in the plant kingdom. *Plant Cell Physiol*. 2012; 53: 606-616.
- Hamana K, Niitsu M, Samejima K, Matsuzaki S. N^4 -Methylthermospermine in leguminous seeds. *Phytochemistry*. 1992; 31: 1410-1412.
- Hamana K, Niitsu M, Samejima K. Further polyamine analyses of leguminous seeds and seedlings: the occurrence of novel linear, tertiary branched and quaternary branched pentaamines. *Can J Bot*. 1996; 74: 1776-1772.
- Matsuzaki S, Hamana K, Isobe K. Occurrence of N^6 -methylagmatine in seeds of leguminous plants. *Phytochemistry*. 1990; 29: 1313-1315.
- Hamana K, Matsuzaki S, Niitsu M, Samejima K. Distribution of unusual polyamines in aquatic plants and gramineous seeds. *Can J Bot*. 1994; 72: 1114-1120.
- Hamana K, Niitsu M, Samejima K. Unusual polyamines in aquatic plants: the occurrence of homospermidine, norspermidine, thermospermine, norspermine, aminopropylhomospermidine, bis(aminopropyl)ethanediamine, and methylspermidine. *Can J Bot*. 1998; 76: 130-133.
- Biastoff S, Brandt W, Dräger B. Putrescine *N*-methyltransferase--the start for alkaloids. *Phytochemistry*. 2009; 70: 1708-1718.
- Junker A, Fischer J, Sichart Y, Brandt W, Dräger B. Evolution of the key alkaloid enzyme putrescine *N*-methyltransferase from spermidine synthase. *Front Plant Sci*. 2013; 4: 260.
- Naconsie M, Kato K, Shoji T, Hashimoto T. Molecular evolution of *N*-methylputrescine oxidase in tobacco. *Plant Cell Physiol*. 2014; 55: 436-444.
- Stenzel O, Teuber M, Dräger B. Putrescine *N*-methyltransferase in *Solanum tuberosum* L., a calystegine-forming plant. *Planta*. 2006; 223: 200-212.

13. Niitsu M, Samejima K, Matsuzaki S, Hamana K. Systematic analysis of naturally occurring linear and branched polyamines by gas chromatography and gas chromatography-mass spectrometry. *J Chromatogr.* 1993; 641: 115-123.
14. Basu HS, Pellarin M, Feuerstein BG, Shirahata A, Samejima K, Deen DF, Marton LJ. Interaction of a polyamine analogue, 1, 19-bis-(ethylamino)-5,10,15- triazanonadecane (BE-4-4-4-4), with DNA and effect on growth, survival, and polyamine levels in seven human brain tumor cell lines. *Cancer Res.* 1993; 53: 3948-3955.
15. Bergeron RJ, Weimar WR, Wu Q, Austin JK Jr, McManis JS. Impact of polyamine analogues on the NMDA receptor. *J Med Chem.* 1995; 38: 425-428.
16. Cohen SS. *A Guide to the Polyamines.* Oxford University Press, Oxford, UK. 1998.
17. Pegg AE. Mammalian polyamine metabolism and function. *IUBMB Life.* 2009; 61: 880-894.
18. Bunsupa S, Katayama K, Ikeura E, Oikawa A, Toyooka K, Saito K, Yamazaki M. Lysine decarboxylase catalyzes the first step of quinolizidine alkaloid biosynthesis and coevolved with alkaloid production in leguminosae. *Plant Cell.* 2012; 24: 1202-1216.
19. Ober D, Hartmann T. Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase. *Proc Natl Acad Sci U S A.* 1999; 96: 14777-14782.
20. Ober D, Kaltenecker E. Pyrrolizidine alkaloid biosynthesis, evolution of a pathway in plant secondary metabolism. *Phytochemistry.* 2009; 70: 1687-1695.
21. Bergeron RJ, Feng Y, Weimar WR, McManis JS, Dimova H, Porter C, Raisler B. A comparison of structure-activity relationships between spermidine and spermine analogue antineoplastics. *J Med Chem.* 1997; 40: 1475-1494.
22. Byers TL, Lakanen JR, Coward JK, Pegg AE. The role of hypusine depletion in cytostasis induced by S-adenosyl-L-methionine decarboxylase inhibition: new evidence provided by 1-methylspermidine and 1,12-dimethylspermine. *Biochem J.* 1994; 303 : 363-368.
23. Porter CW, Cavanaugh PF Jr, Stolowich N, Ganis B, Kelly E, Bergeron RJ. Biological properties of N^4 - and N^1 , N^8 -spermidine derivatives in cultured L1210 leukemia cells. *Cancer Res.* 1985; 45: 2050-2057.

Cite this article

Niitsu M, Takao K, Kato Y, Nagase E, Furuchi T, et al. (2014) Occurrence of N^1 -Methylputrescine, N^1 , N^4 -Dimethylputrescine, N^1 -Methylhomospermidine and N^1 , N^8 -Dimethylhomospermidine in Tomato Root. *Int J Plant Biol Res* 2(4): 1022.