

POLYAMINE ANALYSIS OF LEGUMINOUS SEED, SPROUT, LEAF, FLOWER, POD, ROOT AND ROOT NODULE: DISTRIBUTION OF DIAMINOHEXANE, AMINOBUTYLCADAVERINE, METHYLPOLYAMINES AND ALKANOLPOLYAMINES

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ABSTRACT

To determine organ-specific and species-specific polyamine distribution profiles in legumes (leguminous plants), polyamines acid-extracted from a total 68 samples of the seeds, sprouts, leaves, flowers, pods, roots and/or root nodules from 27 leguminous species belonging to the family *Fabaceae* (formerly *Leguminosae*) were analyzed by high-performance liquid chromatography and high-performance gas chromatography-mass spectrometry. Unusual tetraamines such as thermospermine, aminopropylhomospermidine, canavamine and homospermine were detected in the mature seeds but not in the immature seeds and other organs. An unusual long aliphatic diamine, 1,6-diaminohexane, was found in the roots of ten species and the seeds of two species. In the sprout (seedling), the root region increased in the levels of putrescine and cadaverine, and the stem and seed parts increased in cadaverine level. 1,3-Diaminopropane, norspermidine, norspermine and/or caldopentamine were distributed in some roots and leaves. In addition to the high levels of cadaverine and homospermidine, both polyamines and aminobutylcadaverine were found selectively in the root nodules of *Phaseolus*, *Pueraria* and *Vigna* species. *N*¹-Methylputrescine, *N*¹,*N*⁴-dimethylputrescine, *N*⁸-methylspermidine, *N*⁴-methylspermidine, *N*¹-methylhomospermidine and/or *N*⁵-methylhomospermidine were distributed within the root and root nodule of *Glycine*, *Phaseolus*, *Pueraria* and *Vigna* species. A new *N*-methylated diamine, *N*¹-methylcadaverine, was detected in the root of *Canavalia gladiata* rich in cadaverine. New types of alkanolpolyamine (polyaminoalkylalcohol), *N*-(4-aminobutyl)aminoethanol and/or *N*-(4-aminobutyl)aminopropanol, were found in the root nodules of *Glycine soja*, *Phaseolus vulgaris* and in three *Vigna* species. Leguminous crop plants

important for agriculture and foods are good sources for plant polyamine analyses.

Keywords: alkanolpolyamine, aminobutylcadaverine, diaminohexane, legume, methylpolyamine, root nodule

INTRODUCTION

Diamines (e.g. 1,3-diaminopropane, putrescine and cadaverine), triamines (e.g. spermidine, norspermidine and homospermidine), tetra-amines (e.g. spermine, norspermine, thermospermine, aminopropylhomospermidine and canavalmine), and guanidinoamines (e.g. agmatine) have been found as endogenous cellular polyamines in the higher land plants, angiosperms and gymnosperms (Fuell et al., 2010; Hamana and Matsuzaki, 1985; Hamana et al., 1992a, 1993, 1994, 1996, 2015, 2017; Minguet et al., 2008; Shaw et al., 2010; Takano et al., 2012), however, were detected within some organs of taxonomically sporadic plant species. By the transgenic studies on polyamine synthetic and catabolic enzymes, using a model plant, *Arabidopsis thaliana*, it has been proven that plant polyamines are involved in plant cell proliferation and differentiation, and play a defensive role against various stresses in plant cells (Kusano et al., 2008; Takahashi and Kakehi, 2010; Naka et al., 2010; Minocha et al., 2014). Therefore, we systematically examined endogenous polyamine levels in the roots, stems, leaves, flower buds, flower and seeds of eight *Brassica* crop plants (Hamana et al., 2015) and five *Solanales* crop plants (Hamana et al., 2017), in addition to our previous polyamine analyses of osmotic-resistant aquatic plants (Hamana et al., 1994, 1998, 2000) and the seeds and seedlings of the major crop plants belonging to the families *Fabaceae* (formerly *Leguminosae*) (Hamana and Matsuzaki, 1985b; Hamana et al., 1992a, 1993, 1996; Otsuka et al., 2005) and *Poaceae* (formerly *Gramineae*) (Hamana et al., 1994).

Polyamines are considered to play a role in the longevity of living animals and in prolonging mammalian lifespan (Yue et al., 2017; Handa et al., 2018), so that ingestion of polyamines from foods have been proposed to combat the decrease in mammalian cellular polyamine levels caused by aging (Nishimura et al., 2006) and to increase blood polyamine levels to prevent aging-associated diseases (Eisenberg et al., 2017; Madeo et al., 2018; Soda et al., 2009a, 2009b, 2013). Analyses of crop plant polyamines contribute as some nutritional information on polyamine ingestion, in addition to a phylogenetic polyamine distribution catalogue in higher land plants and a function of the polyamine profile in various differentiated plant organs (Tiburcio and Alcazar, 2018).

On the other hand, to know the symbiotic or parasitic effects of polyamine profiles in host crop plant roots, we have analyzed polyamines in *Brassica* clubroots caused by the infection of a

cercospora, *Plasmodiophora brassicae*, root-knot galls caused by the infection of a root-knot nematode, *Meloidogyne incognita*, in the root of tomato and eggplant, and powdery scab, a disease of the tuber of the potato, caused by the infection of another cercospora, *Spongospora subterranean* (Hamana et al, 2015, 2017). Legumes (leguminous plants) have been cultivated as a major crop and notable in that most of them have the symbiotic nitrogen-fixing bacteria *Rhizobium* or *Bradyrhizobium* in the structures called root nodules (Fujihara, 2009).

In the present study by our new techniques of high-performance liquid chromatography (HPLC) and high-performance gas chromatography (HPGC), we attempted to conduct a polyamine analysis of roots and root nodules in comparison with seeds, sprouts, leaves and flowers in 27 leguminous plant species and to determine the cellular polyamines after the symbiosis of root nodule bacteria into leguminous plant roots. Leguminous plants consist of 650-745 genera (12,000-19,500 species) belonging to a large taxon, family *Fabaceae* (formerly *Leguminosae*) of the order *Fabales* in angiosperms and are important for agriculture and food usage (Kondo et al., 1989).

MATERIALS AND METHODS

Legume organs

Glycine max, *Vigna angularis*, *Vigna unguiculata*, *Vigna umbellata*, *Phaseolus coccineus* and *Phaseolus vulgaris* were cultivated at Yokozawa farm, Tsumagoi, Gunma, Japan. Their roots, roots containing nodules and root nodules were harvested in August and their seeds were harvested in October. Pods including immature seeds, roots and/or root nodules of *Amphicarpaea edgeworthii*, *Glycine soja*, *Medicago sativa*, *Trifolium repens*, *Pueraria montana* and *Wistaria floribunda* were collected in Koei Hamana's home forest, Tsumagoi, in October. *Pisum sativum*, *Vicia faba*, *Senna obtusifolia*, *Cyamopsis tetragonoloba*, *Arachis hypogaea*, *Canavalia gladiata* were cultivated in the home garden of Koei Hamana, Maebashi, Gunma. Their leaves, pods, roots and/or seeds were harvested in August through October. Pods, seeds and roots of *Vicia hirsuta* and *Vicia sativa* were collected in August in Maebashi. Mature seeds of *Vicia villosa*, *Parkia speciosa* (bitter bean), "Kaimame" (in Japanese) and lima bean, sprouts of *Pisum sativum* ("Toumyo" in Japanese, is used as a food), seedlings of *Glycine max* ("Daizu-Moyashi" in Japanese) and *Vigna mungo* (black matpe), and dried leaves of *Aspalathus linearis* (rooibos, red bush) (using as "Rooibos tea", a product of South Africa) were purchased from a market in Maebashi. Edible tuberous roots of *Apios americana* were purchased in Tsumagoi. Flowers of the locus tree were collected in Maebashi. Samples No. 1, No. 2 and No. 3 were harvested in different seasons and analyzed in the present study.

Polyamine analysis

Legume organs (10-100g) were homogenized in the same weight of 10% (1.0M) perchloric acid (PCA) by a mixer. After extraction with 5% PCA three times, the supernatant of the PCA extract was applied to a column (3cm I.D. x 1cm) of a cation-exchange resin, DOWEX W50x8 (Dow Chemical Co., Michigan, USA), and then polyamines were eluted with 6M HCl from the column. The concentrated polyamines were analyzed on a Hitachi HPLC L6000 (Tokyo, Japan) using a column of cation-exchange resin, Hitachi 2619F (=Hitachi 2720) (4mm I.D. x 50mm) at 70°C and determined by post-labeled fluorometry after heating with *o*-phthalaldehyde (Hamana et al., 2015). HPGC on a SHIMADZU GC-17A (Kyoto, Japan) and HPGC-mass spectrometry (HPGC-MS) on a JEOL JMS-700 (Tokyo, Japan), equipped with a long capillary column (0.32mm I.D. x 30m) of Inert Cap 1MS (GL Sciences, Tokyo, Japan) were performed after heptafluorobutyrylation of the concentrated polyamines (Niitsu et al., 2014; Hamana et al., 2015). Heptafluorobutyryl derivatives of polyamines were determined by a flame ionization detector. Typical GC chromatograms in HPGC-MS are shown in Fig. 1. Polyamines, methylpolyamines (*N*-methylated polyamines) and alkanolpolyamines (polyaminoalkylalcohols) synthesized in our laboratory (Hamana et al., 1992b; Niitsu et al., 2014) were used as the standard for identification and determination of polyamine peaks on HPLC and HPGC chromatograms and mass spectra in HPGC-MS. Molar concentrations of endogenous polyamines per gram of wet weight ($\mu\text{mol/g}$ wet weigh) of 68 samples estimated from the HPLC and HPGC analyses of the polyamine extracts are shown without statistical analysis in Table 1. Our previous polyamine data of the 36 leguminous samples related to the 68 present polyamine data are cited in Table 1. Alphabetical abbreviations and numeric codes (number of methylene (CH_2) groups between amino (NH_2) or imino (NH) groups) of polyamines are used in Table 1 and Fig. 1.

RESULTS AND DISCUSSION

1. Polyamines of the seed and pod

Legume crops are rich sources of polyamines, especially in their mature seeds. When we analyzed the mature seeds of 46 leguminous plant species, unusual tetra-amines norspermine (333), thermospermine (334), aminopropylhomospermidine (344), canavalmine (434) and homospermine (aminobutylhomospermidine) (444), and penta-amines caldopentamine (3333), aminopropylcanavalmine (3434), aminobutylcanavalmine (4434) and homopentamine (4444) were sporadically distributed (Hamana et al., 1991, 1992a, 1996; Matsuzaki et al., 1990b). Tertiary branched N^5 -aminobutylhomospermine (4(4)44) was detected in the seeds of *Vicia villosa*, *Vicia sativa* and *Phaseolus coccineus* (Hamana et al., 1991, 1992a, 1996). Quaternary

branched N^4 -bis(aminopropyl)spermidine (3(3)(3)4) was detected in the seeds of *Crotalaria spectabilis* (Hamana et al., 1996).

Penta-amines were not detected in the mature seeds of four leguminous plant species which are also analyzed in the present study. Polyamines of mature seeds, immature seeds and/or pods in the nine legumes *Phaseolus coccineus*, *Phaseolus lunatus*, *Phaseolus vulgaris*, *Pisum sativum*, *Senna obtusifolia*, *Vicia villosa*, *Vigna angularis*, *Vigna umbrellata* and *Vigna unguiculata* were newly or further analyzed in the present study (Table 1). The tetra-amines, thermospermine (334), aminopropylhomospermidine (344), canavamine (434) and homospermine (444) were detected in mature seeds but not in immature seeds or their pods (Table 1), indicating that tetra-amines and penta-amines are synthesized during maturation in the leguminous seeds. Without statistical analysis, distribution profiles of minor unusual polyamines in the mature seed of *Phaseolus vulgaris* (kidney bean) were varied in the five cultivars (Table 1). Polyamine levels of soybean seeds and seedlings were higher in the cultivar “Kurodaizu” and lower in the cultivar “Shirodaizu” (Table 1). Since polyamine components of soybean seeds increased during fermentation using *Bacillus subtilis* to produce “Natto” (Otsuka and Hamana, 2006), comparison of soybean cultivars for enhancement of the polyamine content in soybean “Natto” has been reported (Kobayashi et al., 2017).

2. Polyamines of the sprout, seedling, leaf and flower

We had analyzed polyamines of the whole seedlings (sprouts) after the germination of the seeds of 31 leguminous species in our laboratory (Hamana et al., 1993, 1996, 2005). The levels of the diamines putrescine (1,4-diaminobutane) (4) and cadaverine (1,5-diaminopentane) (5), and/or a guamininoamine, agmatine (Agm), increased in the legume seedlings. We purchased the seedlings “Daizu-Moyashi” of soybean and black matpe (black gram) from a market and analyzed to confirm the increased cadaverine (5) and putrescine (4) in the present study (Table 1). In the sprouts of garden pea “Tomyou”, the root region increased in putrescine and cadaverine levels, and the stem and seed parts increased in cadaverine level (Table 1). These results show different induction of ornithine decarboxylase (ODC) activity to produce putrescine and lysine decarboxylase (LDC) activity to produce cadaverine in the different tissue regions of the leguminous sprout.

Polyamines of whole leaves and flowers (inflorescences) of various leguminous plants have been analyzed. Usual and common polyamines were detected in them (Hamana et al., 1992a, 1996). The flower of the locust tree (*Robina pseudoacacia*) is a sweet edible flower dish and contained usual polyamines (Table 1). The dried leaf of rooibos (*Aspalathus linearis*) used for “Rooibos tea” contained the two penta-amines caldopentamine (3333) and homocaldopentamine (3334) in

addition to the tetra-amines norspermine (333) and thermospermine (334) in the present study (Table 1). The four unusual polyamines were not distributed in many other leguminous leaves and flowers (Hamana et al., 1992a, 1996), and the latter penta-amine has never been found in the leguminous mature seeds and roots containing other various penta-amines. In spite of the major occurrence of spermine (343), thermopentamine (3343) was not detected in the rooibos leaf. Although it was indicated that the penta-amines caldopentamine and homocaldopentamine were produced by an aminopropyl-transfer into the aminopropyl terminal NH₂ of the tetra-amines norspermine and thermospermine, respectively, in rooibos leaves, further polyamine analyses of other rooibos samples are being planned.

3. Polyamines of the root and root nodule

We have analyzed some leguminous roots and root nodules and they exhibited a high homospermidine level (Hamana et al., 1992a, 1996). On the other hand, Fujihara et al. (1995) and Lopez-Gomez et al. (2014) reported the occurrence of a novel triamine, aminobutylcadaverine (4-aminobutylcadaverine) (45) in the root nodules of *Vigna angularis* and *Phaseolus vulgaris*, respectively. Therefore, we cultivated or collected 17 leguminous plants and harvested roots, roots containing nodules and/or root nodules. Aminobutylcadaverine (45) was found in the root nodules of *Phaseolus coccineus*, *Phaseolus vulgaris*, *Vigna angularis* and *Vigna umbellata*, and roots containing nodules of *Pueraria montana* (Fig. 1 and Table 1). It was suggested that aminobutylcadaverine is produced by an aminobutylation of cadaverine (5) under the occurrence of homospermidine (44) produced by an aminobutylation of putrescine (4) and cadaverine. In fact, the root nodules of the five leguminous species were observed to be under these polyamine-distributing conditions. A peak corresponding to cadaverine (5) was found in the HPGC of *Glycine soja* polyamines (Fig. 1-B) but not identified as cadaverine by MS analysis. Aminobutylcadaverine was not detected in the root nodules of soybean (*Glycine max*) as well as wild soybean (*Glycine soja*). Occurrence of aminobutylcadaverine was limited species-specifically in leguminous root nodules. Polyamine synthetic abilities of the leguminous root and the symbiotic bacteria are possible to combine in the root nodules.

N₂-Fixing root nodule bacteria belonging to *Rhizobium* and *Bradyrhizobium* have homospermidine as the major polyamine, however, the production of aminobutylcadaverine from cadaverine has never been confirmed in their pure cultures (Fujihara, 2009; Fujihara and Harada, 1989; Fujihara et al., 1995; Hamana et al., 1988). On the other hand, homospermine (aminobutylhomospermidine) (444) was produced from a pure culture of some fast-growing root nodule bacteria including *Bradyrhizobium* and *Rhizobium* species (Fujihara, 2009; Fujihara and Harada, 1989).

When polyamines of the tuberous root of *Apios americana* were analyzed, a low level of homospermidine (44) was detected (Table 1), suggesting a low polyamine synthetic activity of the tuberous root. Homospermine (444) was detected in the root of *Wistaria floribunda* rich in homospermidine but not in the many root nodules analyzed in the present study (Table 1), indicating that the production of homospermine from homospermidine is not enhanced in the root nodules.

On the other hand, the coralloid root of a fern palm, *Cycas revolute*, belonging to gymnosperms (the phylum Gymnospermae) contains a symbiotic cyanobacterium, *Anabaena cycadea*. The closed leaf pocket of the ferns *Azolla imbricate* and *Azolla japonica* belonging to the phylum Pteridophyta contains a symbiotic cyanobacterium, *Anabaena azollae*. A significant amount of homospermidine (44), however, aminobutylcadaverine (45) and homospermine (444) have never been detected in the N_2 -fixing *Anabaena*-symbiotic organs (Hamana and Mastuzaki, 1985a; Hamana et al., 1988).

In the roots of ten leguminous species and the seeds of two leguminous species, a novel long aliphatic diamine, 1,6-diaminohexane (6), was detected and identified by HPGC and HPGC-MS (Fig. 1 and Table 1). This study is the first report on the occurrence of 1,6-diaminohexane in higher land plants. Although biological synthetic process of the diamine has never been investigated, the diamine was rich in the roots containing a high level of agmatine (Agm), a guamidinoamine as a polyamine component, as shown in Table 1. Biological decarboxylation of L-homolysine (L-2,7-diaminoheptanoic acid), a non-natural amino acid, to produce 1,6-diaminohexane is unknown. 2-Phenylethylamine (Pea), an aromatic amine, analyzed in HPLC and HPGC as a polyamine component, was distributed in the root nodules of *Glycine max* and two *Vigna* species (Table 1). Agmatine produced from L-arginine by decarboxylation and 2-phenylethylamine produced from L-phenylalanine by decarboxylation did not co-exist in the leguminous roots and root nodules analyzed in the present study.

1,3-Diaminopropane (3), norspermidine (33), norspermine (333) and/or caldopentamine (3333) are not common polyamine in higher land plants, nevertheless, were distributed sporadically in some leguminous roots and leaves (Table 1) as well as mature seeds previously analyzed (Hamana et al., 1991, 1992a, 1996; Matsuzaki et al., 1990b), indicating continued aminopropyl-transfers into their amino-terminals in the leguminous organs. However, 1,3-diaminopropane as a starting diamine is possible to produce from L-2,4-diaminobutylic acid by decarboxylase and also from endogenous norspermidine (33), spermidine (34), norspermine (333), spermine (343) or thermospermie (334) by polyamine oxidases in plants (Fuell et al., 2010; Takahashi et al., 2010).

4. Distribution of methylpolyamines (N-methylated polyamines)

N^4 -methylthermospermine (3(M)34) and N^6 -methylagmatine (MAgm) were first found in several leguminous mature seeds (Hamana et al., 1992a, 1993, 1996; Matsuzaki et al., 1990a). N^6 -methylagmatine has been found in several gramineous plant seed and seedlings (Hamana et al., 1994). N^4 -methylspermidine (3(M)4) was discovered in an aquatic plant (Hamana et al., 1998). The occurrences of N^1 -methylputrescine (M4), N^1,N^4 -dimethylputrescine (M4M), N^1 -methylhomospermidine (M44) and N^1,N^9 -dimethylhomospermidine (M44M) were identified in *Solanales* plant roots (Hamana et al., 2017; Niitsu et al., 2014). Endogenous distribution of N-methylated polyamine derivatives in plants is attractive for considering polyamine metabolism and its function in plants.

The present study shows the occurrence of N^1 -methylcadaverine (M5) in the root of *Canavalia gladiata* rich in cadaverine (5). N^1 -Methylputrescine (M4), N^1,N^4 -dimethylputrescine (M4M), N^8 -methylspermidine (34M), N^4 -methylspermidine (3(M)4), N^1 -methylhomospermidine (M44) and/or N^5 -methylhomospermidine (4(M)4) in the roots, roots containing nodules or root nodules of *Glycine max*, *Glycine soja*, *Phaseolus vulgaris*, *Pueraria montana*, *Vigna angularis*, *Vigna umbellata* and *Vigna unguiculata* (Table 1). N^4 -methylspermidine was found in the seedling of *Vigna mungo* (Table 1). N^6 -methylagmatine was not detected in the present leguminous organs. These results indicate a wide distribution of various N-methylated diamines and triamines in higher land plants, furthermore, the first detection of the two new types of methylated polyamines, N^1 -methylcadaverine and N^8 -methylspermidine, in legume root nodules.

5. Distribution of alkanolpolyamines (polyaminoalkylalcohols)

The two aminopropylaminoalcohols N-(3-aminopropyl) aminoethanol (32OH) and N-(3-aminopropyl) aminopropanol (33OH) were first found in mature seeds of a leguminous plant, *Dolichos lablab* (Hamana et al., 1992b). The latter was also detected in the seeds of leguminous *Cassia obtusifolia*, *Sesbania rostrata* and *Gleditschia japonica* (Hamana et al., 1996). In addition to the former two, N,N-(3-aminopropyl)aminopropanol (333OH) had been found in the seeds of leguminous *Vigna mungo* (*Rudua mungo*) (Otsuka et al., 2005) as cited in Table 1.

N-(3-Aminopropyl) aminopropanol have been found in gramineous sorghum (*Sorghum bicolor*) and timothy (*Phleum pratense*) seeds (Hamana et al., 1994).

In the present study, two new types of alkanolpolyamine (polyaminoalkylalcohol), N-(4-aminobutyl)aminoethanol (42OH) and N-(4-aminobutyl)aminopropanol (43OH), were found in the root (containing nodules) of *Glycine soja* (Fig. 1-B and Table 1). N-(4-Aminobutyl)aminoethanol (42OH) was detected in the root nodule of *Phaseolus vulgaris*, *Vigna*

angularis, *Vigna umbellate* and *Vigna unguiculata* (Fig. 1-C, D, F, H and Table 1). Although the biological function of alkanolpolyamines is not clear, occurrence of the alcohol-derivatives of polyamines in leguminous roots is interesting.

The hydroxy derivatives of polyamines, 2-hydroxyputrescine (abbreviated as H-4 or H-Put) and γ (3)-hydroxyagmatine (H-Agm) found in some leguminous seeds and seedlings (Hamana et al., 1993), were not detected in the leguminous organs analyzed in the present study. *N*-acetylated putrescine, spermidine and spermine distributed in bacterial and animal cells have never been found in plants. However, since acetyl moiety of *N*-acetylpolyamines is acid-labile, their detection by HPLC and HPGC after the heat-treatment with 6M HCl of concentrated polyamine fraction is difficult in our plant polyamine analyses. The two guanidinoamines homoagmatine (HAgm) produced from L-homoarginine and γ -guanidinooxypropylamine produced from L-canavanine in some leguminous seeds (Hamana and Matsuzaki, 1985; Hamana et al., 1993), were not detected in the legume organs analyzed in the present study.

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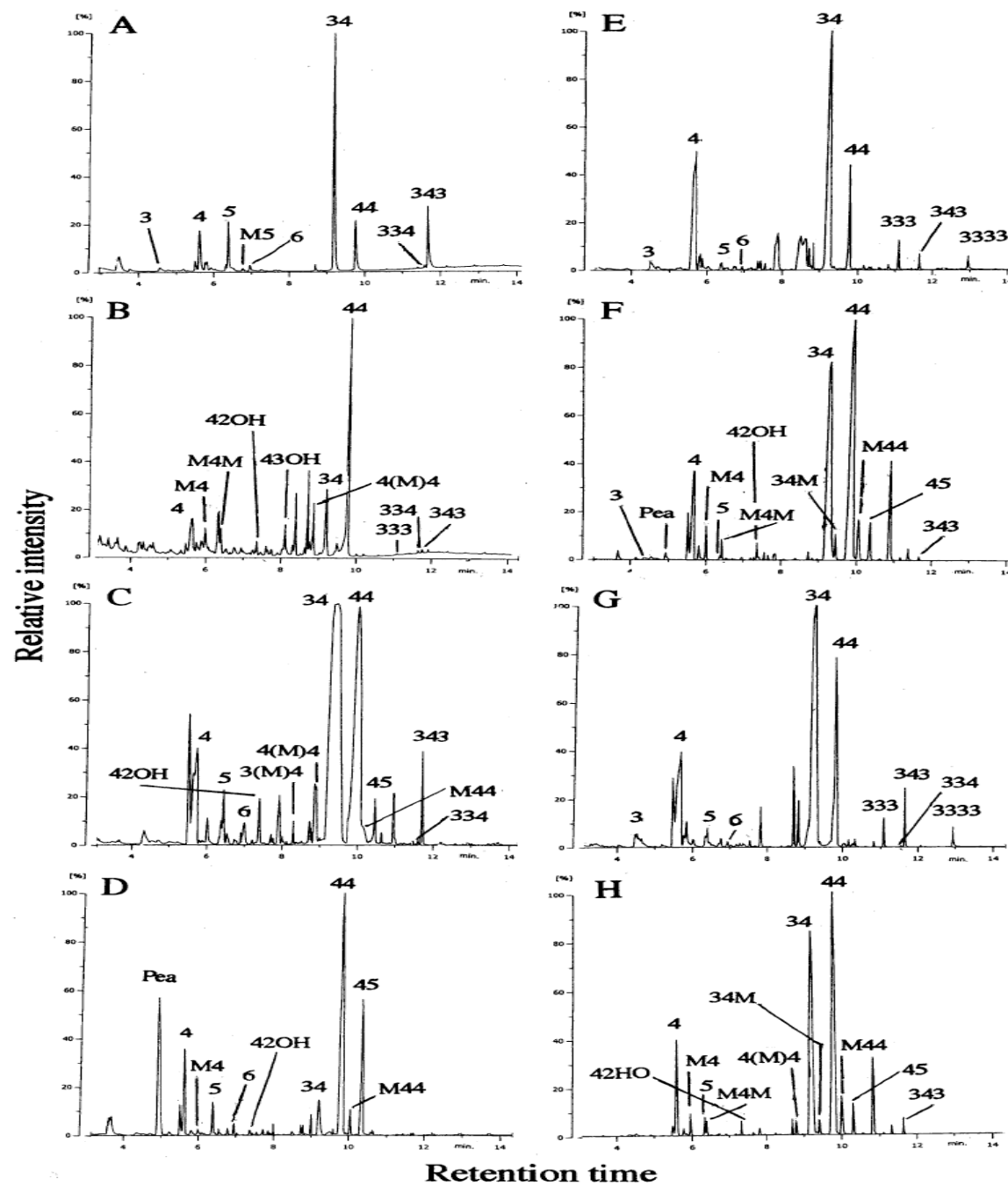


Fig. 1. HPGC analysis of polyamines extracted from the roots of *Canavalia gladiata* (sword bean) "Natamame" (A), the roots (containing nodules) of *Glycine soja* (wild soybean) "Tsurumame" (B), the root nodules of *Phaseolus vulgaris* (kidney bean) "Kintoki-mame" (C), the root nodules of *Vigna angularis* (azuki bean) "Dainagon" No.2 (D), the roots (E) and the root nodules (F) of *Vigna umbellata* (rice bean) "Tsuru-azuki", and the roots (G) and the root nodules (H) of *Vigna unguiculata* (cow pea) "Sasage" on a JEOL JMS-700 at the column temperature 90°C-(16°C/min)-280°C. Abbreviations for polyamines are shown in Table 1. Agmatine (Agm) is destroyed during HPGC analysis. Other non-amine peaks judged by retention time and MS data were detected on the HPGC charts.

Alphabetical legume species and organs Ref.			Polyamines (μmol/g wet weight)																																
			Dap	Put	Cad	Dah	NSpd	Spd	HSpd	ABCad	NSpm	TSpm	Spm	AHSpd	Can	HSpm	CPen	HCPen	Agm	Pea	M4	M5	M4M	34M	3(M)4	M44	4(M)4	3(M)34	42OH	43OH	32OH	33OH	333OH		
			3	4	5	6	33	34	44	45	333	334	343	344	434	444	3333	3334																	
<i>Albizia julibrissin</i> (Silk tree)	root	1996	-	0.14	-	-	-	0.45	0.35	-	-	-	0.35	-	-	-	-	-	0.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amphicarpaea edgeworthii</i> "Yabumame"	seed	1996	-	0.02	-	-	-	0.16	-	-	-	-	1.06	-	-	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	root		-	0.15	0.01	-	-	1.05	0.55	-	-	0.02	0.50	-	-	-	-	-	0.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	pod (including seeds)		-	0.13	-	-	-	1.07	0.03	-	-	0.02	0.53	0.01	-	-	-	-	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Apion americana</i> (Apion)	tubrous root		-	0.05	0.04	-	-	1.06	0.01	-	-	0.01	0.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Arachis hypogaea</i> (Peanut) "Rakkusei"	seed	1992a	-	0.18	-	-	-	1.80	-	-	-	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	root (containing nodules)		-	0.10	0.05	-	-	0.72	0.30	-	-	0.02	0.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	pod (including seeds)		-	0.05	-	-	-	0.64	-	-	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Aspalathus linearis</i> (Rooibos)	leaf		-	0.04	0.02	-	-	1.05	0.01	-	0.13	0.06	0.75	-	-	-	0.01	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Canavalia gladiata</i> (Sword bean) "Natamame"	seed	1992a	-	0.01	-	-	-	0.13	0.01	-	-	0.01	0.17	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	pod (including seeds)		-	0.62	-	-	-	1.05	0.01	-	-	0.02	0.87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	root		0.03	0.15	0.20	0.03	-	1.07	0.45	-	-	0.02	0.40	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cyamopsis tetragonoloba</i> (Cluster bean, Guar)	root		-	0.09	0.01	0.02	-	1.06	0.16	-	-	0.03	0.55	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	pod (including seeds)		-	0.04	-	-	-	1.02	-	-	-	0.02	0.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Glycine max</i> (Soybean) "Shiro-daizu"	root	1992a	-	0.01	-	-	-	0.12	0.01	-	-	-	0.03	-	-	-	-	-	-	-	*	*	*	*	*	*	*	-	-	-	-	-	-	-	
	root No.1		0.03	0.40	-	-	0.01	0.85	1.22	-	0.01	0.01	0.06	-	-	-	-	-	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	No.2		0.04	0.17	-	-	-	0.83	1.04	-	-	0.01	0.05	-	-	-	-	-	0.25	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-		
	No.3		-	0.15	-	-	-	0.65	0.40	-	-	0.01	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	root nodule	1992a	-	0.27	-	-	-	0.66	0.44	-	-	-	0.04	-	-	-	-	-	-	-	*	*	*	*	*	*	*	-	-	-	-	-	-	-	
	root nodule No.1		0.03	0.50	0.02	-	-	0.63	1.20	-	-	-	0.01	-	-	-	-	-	0.10	-	-	-	-	-	0.05	-	0.10	-	-	-	-	-	-		
	No.2		0.10	0.55	-	-	-	0.72	1.25	-	-	-	-	-	-	-	-	-	0.57	0.02	-	0.12	0.02	0.03	0.03	0.02	-	-	-	-	-	-	-		
	No.3		-	0.15	-	-	-	1.25	1.00	-	-	-	-	-	-	-	-	-	-	0.05	-	0.05	0.11	0.32	0.01	0.33	-	-	-	-	-	-	-		
	"Daizu-Moyashi" seedling	1993	-	0.05	0.60	-	-	0.15	-	-	-	-	0.03	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			-	0.37	0.77	-	-	1.08	-	-	-	0.02	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	seed (immature)	1992a	-	0.10	0.01	-	-	0.38	0.01	-	-	-	0.02	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	seed (mature)	1992a	-	0.04	0.01	-	-	1.40	0.01	-	-	-	0.03	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
"Kuro-daizu"	seedling	2005	-	0.60	1.80	-	-	1.40	-	-	-	0.20	-	-	-	-	-	-	0.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	seed (mature)	2005	-	0.80	0.10	-	-	1.56	0.03	-	-	-	0.29	0.01	-	-	-	-	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Glycine soja</i> (Wild soybean)	root (containing nodules)		-	0.16	-	-	-	0.29	1.07	-	-	0.01	0.01	-	-	-	-	-	-	0.07	-	0.07	-	-	-	-	0.22	-	0.04	0.13	-	-	-	-	
	"Tsurumame" pod (including seeds)		-	0.04	0.06	-	-	1.08	0.02	-	-	-	0.73	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Lespedeza cytobotrya</i> (Bush clover)	root	1996	-	0.25	0.49	-	-	0.47	0.32	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Medicago sativa</i> (Alfalfa) "Umagoyashi"	seed (mature)	1992a	0.07	0.07	-	-	-	1.25	-	-	0.02	-	0.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	root (containing nodules)		-	0.10	0.30	0.06	-	0.77	0.30	-	-	0.04	0.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Purkia speciosa</i> (Bitter bean, Petia)	seed (mature)		-	0.21	-	0.01	-	1.07	-	-	0.01	0.02	0.94	-	-	-	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Phaseolus coccineus</i> (Runner bean) "Hasamame"	root No.1		-	0.30	-	-	-	1.18	0.05	-	-	0.01	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	No.2		-	0.33	-	-	-	1.16	0.52	-	-	0.01	0.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	No.3		-	0.15	-	-	-	1.07	0.04	-	-	0.01	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	root (containing nodules)	0.01	0.40	0.03	0.03	-	-	0.59	1.09	-	-	-	0.25	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	root nodule		-	0.31	0.26	-	-	0.26	1.07	0.02	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	seed (mature)	1992a	0.01	0.15	-	-	-	1.20	0.04	-	-	-	1.20	0.01	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			-	0.03	-	-	-	1.06	0.01	-	-	-	0.01	0.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	seed (immature)		-	0.17	-	-	-	1.07	-	-	0.01	0.03	0.87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Phaseolus lunatus</i> (Lima bean)	seed (mature)		-	0.40	-	-	-	1.05	0.04	-	-	-	0.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Phaseolus trilobatus</i> (Wildgram)	seed (mature)	1996	-	0.02	-	-	-	0.54	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Phaseolus vulgaris</i> (Kidney bean) "Morocco-ingen"	root		-	0.45	0.03	-	-	1.17	1.10	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	"Kaimame" seed (mature)		-	0.02	-	-	-	0.50	-	-	-	-	1.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	"Kuro-ingen" seed (mature)	2005	-	0.30	-	-	-	1.40	0.02	-	-	-	0.70	0.01	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	"Kintokimame" seed (mature)																																		

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**Table 1. Polyamine concentrations in the organs of leguminous plants belonging to the family
Fabaceae (formerly *Leguminosae*)**

Alphabetical abbreviations and numeric codes (number of methylene (CH₂) groups between amino (NH₂) or imino (NH) groups) of polyamines are used. Dap (3), 1,3-diaminopropane [NH₂(CH₂)₃NH₂]; Put (4), putrescine [NH₂(CH₂)₄NH₂]; Cad (5), cadaverine [NH₂(CH₂)₅NH₂]; Dah (6), 1,6-diaminohexane; NSpd (33), norspermidine [NH₂(CH₂)₃NH(CH₂)₃NH₂]; Spd (34), spermidine [NH₂(CH₂)₃NH(CH₂)₄NH₂]; HSpd (44), homospermidine [NH₂(CH₂)₄NH(CH₂)₄NH₂]; ABCad (45), aminobutylcadaverine [NH₂(CH₂)₄NH(CH₂)₅NH₂]; NSpm (333), norspermine [NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH₂]; TSpm (334), thermospermine [NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₄NH₂]; Spm (343), spermine [NH₂(CH₂)₃NH(CH₂)₄NH(CH₂)₃NH₂]; AHSpd (344), aminopropylhomospermidine [NH₂(CH₂)₃NH(CH₂)₄NH(CH₂)₄NH₂]; Can (434), canavamine [NH₂(CH₂)₄NH(CH₂)₃NH(CH₂)₄NH₂]; HSpm (444), homospermine (aminobutylhomospermidine) [NH₂(CH₂)₄NH(CH₂)₄NH(CH₂)₄NH₂]; CPen (3333), caldopentamine [NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH₂]; HCPen (3334), homocaldopentamine [NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH(CH₂)₄NH₂]; Agm, agmatine [NH₂C(NH)NH(CH₂)₄NH₂]; Pea, 2-phenylethylamine [C₆H₅(CH₂)₂NH₂]; M4, N¹-methylputrescine; M5, N¹-methylcadaverine; M4M, N¹,N⁴-dimethylputrescine; 34M, N⁸-methylspermidine; 3(M)4, N⁴-methylspermidine; M44, N¹-methylhomospermidine; 4(M)4, N⁵-methylhomospermidine; 3(M)34, N⁴-methylthermospermine; 42OH, N-(4-aminobutyl)aminoethanol; 43OH, N-(4-aminobutyl)aminopropanol; 32OH, N-(3-aminopropyl)aminoethanol; 33OH, N-(3-aminopropyl)aminopropanol; 333OH, N,N-(3-aminopropyl)aminopropanol. -, not detected (<0.005 μmol/g wet weight). ★, analysis of N-methylated polyamines by HPGC was not performed. " ", species names or cultivar names in Japanese. (), popular name. Citations of our previous data from the References (Ref.) are shown as the year of publication (68 blanks are the new data of this study in 2019). Standard GC with a packed column and old HPLC had been used in our previous studies in 1992-2005.