

Dioscorea opposita ‘ツクネイモ’の生育中におけるアラントインおよびアルギニン含有量の変化

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Change in Allantoin and Arginine Contents in *Dioscorea opposita* 'Tsukuneimo' during the Growth

Akio Ninomiya¹, Yoshiyuki Murata², Mikiro Tada^{1*} and Yasuaki Shimoishi²

¹Graduate School of Natural Science and Technology, Okayama University, Okayama 700–8530

²Faculty of Agriculture, Okayama University, Okayama 700–8530

Summary

The ureides, allantoin and allantoic acid, are believed to play an important role in the storage and translocation of nitrogen in higher plants. Arginine is a major component in the free amino acid pool in some plants. To investigate the behavior of ureides and free amino acids, especially arginine, in developing *Dioscorea opposita* plants 'Tsukuneimo', their contents as well as those of water and nitrogen in leaves, stems and tubers were quantitatively analyzed. The content of allantoin in the leaves decreased after any fertilization, while that in the stems did not increase after the 1st fertilization, 96 days after planting; it significantly increased after the 2nd fertilization, 26 days later. Allantoin content in tubers remained constant during tuber development. Arginine content in leaves and stems during the growth of 'Tsukuneimo' were lower than 0.15 and 4.8 $\mu\text{mol} \cdot \text{g}^{-1}\text{FW}$, respectively. The time course of arginine in stems content was similar to that of allantoin. In tubers, arginine content at 170 days after planting increased to reach 14 times that at 142 days but then decreased, demonstrating that *D. opposita* transiently accumulates arginine in stems as well as allantoin in leaves and stems but only accumulates the latter in developing tubers.

Key Words: allantoin, amino acid, arginine, *Dioscorea opposita*, Tsukuneimo.

Introduction

Since *Dioscorea* contains allantoin that is considered to prevent inflammation and ulcers in the human body, the genus is used as a pharmaceutical preparation, Hachimi-Gan (Sagara et al., 1988). In general, ureides (allantoin and allantoic acid) are recognized as important compounds for the storage and transport of nitrogen in the plants. Arginine exists as a free amino acid in 'Tsukuneimo', and it could be a new pharmacological agent to inhibit a pathological hemostasis activation (Thomas et al., 2001).

Investigations on ureides in cultivated soybean (Matsumoto et al., 1977a, b; Osaki et al., 1988; Thomas and Schrader, 1981) and winged bean plants (*Psophocarpus tetragonolobus* L.) (Motior et al., 1999) have been made but only a few researches on the behavior of ureides in tubers of *Dioscorea* have been published. Allantoin is accumulated in roots and stems of developing soybean plants bearing nodules but it decreases during seed formation (Matsumoto et al., 1977a). However, it is still unclear how ureides, especially allantoin, physiologically function in plants. Ninomiya et al. (2003) analyzed for allantoin in *D. opposita* 'Tsukuneimo' (spherical shape),

'Yamatoimo' (spherical shape), 'Nagaimo' (long shape), and *D. japonica* 'Jinenjo'; they found that the levels of allantoic acid were negligible and that allantoin was undetectable in leaves and stems of 'Tsukuneimo' at harvest.

Arginine is accumulated as a major component in the forb Sourdock (*Rumex acetosa*) (Bausenwein et al., 2001), loblolly pine (King and Gifford, 1997), boreal understory plants (Nordin and Näsholm, 1997), common mistletoes (Urech, 1997). Arginine, as well as proline and aspartic acid, seems to play an important role in nitrogen storage in Australian mistletoes (Pate et al., 1991).

So far no study on the behavior of both allantoin and arginine in *D. opposita* was reported. In this study, the contents of ureides, especially allantoin and amino acids, especially arginine, in leaves, stems and tubers of *D. opposita* were determined to clarify the behavior of allantoin and arginine in these organs of *D. opposita* 'Tsukuneimo' during plant growth.

Materials and Methods

Materials

About 50 g of seed tubers of *D. opposita* 'Tsukuneimo' were planted at intervals of 32 cm in an area of 18 a in Mitsu town of Okayama Prefecture on March 30, 2002. The soil is fertile, and the distance

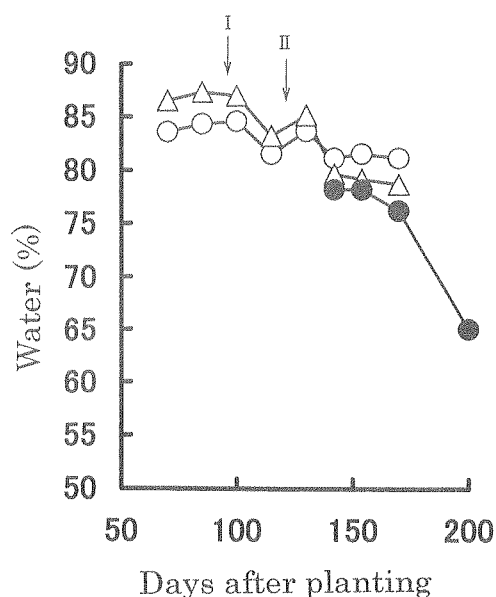


Fig. 1. Change of the water contents in the leaves (○), stems (△), and tubers (●) in *D. opposita* 'Tsukuneimo'. I: 1st fertilization, II: 2nd fertilization.

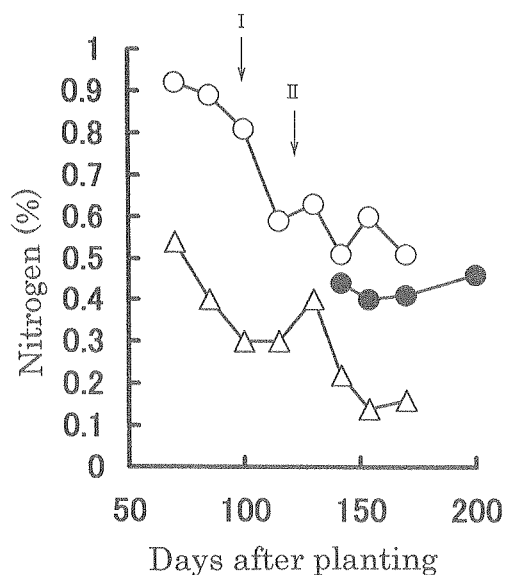


Fig. 2. Change of the nitrogen contents in the leaves (○), stems (△), tubers (●) in *D. opposita* 'Tsukuneimo'. I: 1st fertilization, II: 2nd fertilization.

between ridges was 1.5 m. Basal fertilizer was applied before planting and topdressings, $7 \text{ kg} \cdot \text{a}^{-1}$ of compound fertilizer (IBS1)(10N-10P₂O₅-10K₂O), were applied at 35 and 73 days after planting. Furthermore, $6 \text{ kg} \cdot \text{a}^{-1}$ and $4 \text{ kg} \cdot \text{a}^{-1}$ of the compound fertilizer CDU; 2-oxo-4-methyl-6-ureido-hexahydropyrimidine (16N-8P₂O₅-12K₂O) were applied at 96 days (1st fertilization) and again at 122 days after planting (2nd fertilization). The plants were watered at intervals of three days from June to August but they were not irrigated after September. Leaves and stems were

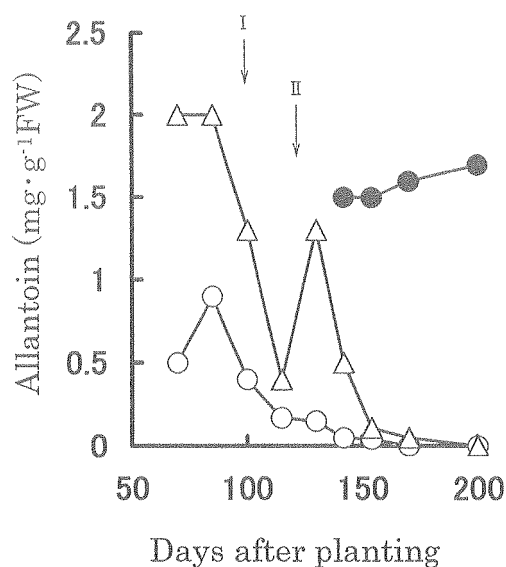


Fig. 3. Change of the allantoin contents in the leaves (○), stems (△), tubers (●) in *D. opposita* 'Tsukuneimo'. I: 1st fertilization, II: 2nd fertilization.

sampled at 70, 85, 100, 115, 130, 142, 154, and 170 days after planting, and tubers at 142, 154, 170 and 200 days. Samples of leaves, stems, and tubers were harvested from two 'Tsukuneimo' plants and mixed equally. Each data for leaves and stems was obtained from two independent sets of the mixed samples.

Determination of water and nitrogen contents

Water contents of leaves, stems, and tubers of 'Tsukuneimo' were determined as previously described (Ninomiya et al., 2003), whereas nitrogen contents in leaves, stems, and tubers were analyzed by using CHN Analyzer (model LECO '1000', LECO, Ltd. St. Joseph, USA).

Extraction of ureides and amino acids

Six to 9 g of leaves or 8 to 12 g of stems, dried at 75°C for 24 to 48 hr, were blended in 100 ml of 80% ethanol with a Polytron homogenizer. The homogenate was filtered through filter paper (QUALITATIVE2, Toyo Roshi Kaisha, Ltd) and the residue was homogenized again with 50 ml of 80% ethanol and re-filtered. The extraction and filtration procedures were repeated two more times to extract completely ureides and amino acids. The combined filtrate from four extractions was evaporated, and the concentrate was dissolved in 50 ml of water and the aliquot analyzed for ureides and amino acids according to Ninomiya et al. (2003).

Determination of contents of ureides and amino acids

The content of allantoin was determined by HPLC as described previously (Ninomiya et al., 2003), while that of allantoic acid was quantified colorimetrically at 535 nm (Vogels and Drift, 1970). Amino acids were analyzed with an amino acid analyzer (model JLC-300;

JEOL, Ltd., Tokyo), equipped with a packed column (model LC30-B609F1; JEOL, Ltd.), using lithium buffers eluents.

Results

Growth of 'Tsukuneimo'

The plant heights of 'Tsukuneimo' were approximately 1, 2, 4 m at 70, 85, and 100 days, respectively after planting. The flowering occurred between 130 and 140 days after planting. The average diameters of five tubers were approximately 5, 10, and 10 to 15 cm at 142, 154, and 200 days, respectively after planting.

Water contents in leaves, stems and tubers

In leaves and stems, the water contents decreased before the 2nd fertilization, but they increased and then remained constant between 79 and 81% (Fig. 1). The water content of tubers gradually decreased from 77% at 142 days to 65% at 200 days (harvest).

Nitrogen contents in leaves, stems and tubers

The nitrogen contents in leaves and stems decreased before the 2nd fertilization, temporarily increased after the 2nd fertilization, and then gradually decreased again (Fig. 2). In tubers, the percentage of nitrogen content was approximately 0.43% from 142 days to 200 days after planting.

The contents of ureides in leaves, stems and tubers

Allantoin content in leaves reached a maximum ($0.90 \text{ mg} \cdot \text{g}^{-1}\text{FW}$), and then decreased after the 1st fertilization (Fig. 3), to an undetectable level in leaves after the development of tubers (170 days). Its content in stems fluctuated from $2.0 \text{ mg} \cdot \text{g}^{-1}\text{FW}$ at 70 to 80 days after planting to less than $0.5 \text{ mg} \cdot \text{g}^{-1}\text{FW}$ before 2nd fertilization; it increased to $1.3 \text{ mg} \cdot \text{g}^{-1}\text{FW}$ and subsequently decreased to $0.05 \text{ mg} \cdot \text{g}^{-1}\text{FW}$ at 170 days after planting; it was undetectable at harvest, 200 days after planting. Allantoin content in stems was greater than that in leaves from 70 to 154 days after planting. Allantoin content in tubers increased slightly between 142 and 200 days after planting. At tuber harvest, allantoic acid contents in leaves, stems and tubers were undetectable (less than $0.01 \text{ mg} \cdot \text{g}^{-1}\text{FW}$).

The contents of total amino acids in leaves, stems and tubers

The content of total amino acids in stems was higher than that in leaves from 70 to 130 days (Table 1); the total in leaves and stems fluctuated from a maximum at 70 to 85 days, decreased to minimum before the 2nd fertilization 122 days after planting, and temporarily increased to reach another maximum after the 2nd fertilization and then decreased until harvest. The time course of the contents of amino acids was similar to that of allantoin in stems. The amino acid content in tubers at

170 days was 6.9 times that in tubers at 142 days.

Alanine and cysteine contents in leaves were 2.6 and $1.5 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ at 85 days after planting, respectively, whereas those of the other amino acids were less than $1.5 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ before the 1st fertilization 96 days after planting. Arginine and serine contents in stems before the 1st fertilization were 4.8 and $3.8 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$, respectively. The other amino acids in stems constituted less than $1.9 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ before the 1st fertilization. In tubers, arginine content was $3.6 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ at 170 days after planting, while those of the other amino acids were less than $1.0 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$.

Thus, arginine in stems fluctuated from $4.8 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ before the 1st fertilization, then decreased and temporarily increased $2.0 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ after 2nd fertilization and then decreased. The leaf arginine level was $0.15 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ at 70 days and gradually decreased. The content of arginine in tubers at 170 days was 14 times larger than that at 142 days.

Discussion

Water contents in tubers

Water contents in tubers at 170 days and 200 days were 76% and 65%, respectively, which is consistent with our previous result (Ninomiya et al., 2003), indicating that young tubers have a higher water content than do fully developed tubers.

Ureides and amino acid contents in leaves and stems of 'Tsukuneimo'

Our analyses of leaves and stems of 'Tsukuneimo' revealed that allantoin and amino acid contents reached a maximum at 70 to 85 days after planting, and before flowering. In winged bean plants, their levels in the xylem exudates showed maximum at flowering time (70 to 84 days after germination) (Motior et al., 1999), whereas that of allantoin in developing leaves was generally less than $1 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$ (Thomas and Schrader, 1981). In 'Tsukuneimo' leaves, allantoin content at 85 days was $5.7 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$.

The percentages of allantoin-N against total nitrogen in leaves and stems of 'Tsukuneimo', at 85 days after planting, were 3.6 and 18%, respectively. The amounts of allantoin-N against total nitrogen in stems are larger than those in leaves of 'Tsukuneimo' as reported in soybean (Osaki et al., 1988). The percentages of allantoic acid-N against total nitrogen in leaves and stems of 'Tsukuneimo' at 85 days after planting amounted to less than 0.001%. The percentages of amino acids-N against total nitrogen in leaves and stems of 'Tsukuneimo' at 85 days were 1.3 and 11%, respectively. The amount of amino acids-N against total nitrogen in 'Tsukuneimo' in stems is larger than those in leaves as reported in soybean (Osaki et al., 1988).

Table 1. The contents of amino acids in leaves, stems and tubers of 'Tsukuncimo' during the growth after planting.

Amino acid ($\mu\text{mol}\cdot\text{g}^{-1}\text{FW}$)	Days after planting ^z																										
	70			85			100			115			130			142			154			170			200		
	L	S	T	L	S	T	L	S	T	L	S	T	L	S	T	L	S	T	L	S	T	L	S	T	L	S	T
Asp	0.11	0.15	0.16	0.16	0.15	0.03	0.04	0.04	0.04	0.16	0.28	0.06	0.04	0.07	0.11	0.01	0.14	0.14	0.14	ND ^y	ND	ND	0.51	0.63			
Thr	0.40	1.20	0.43	1.10	0.39	0.62	0.03	0.06	0.03	0.33	1.20	0.14	0.21	0.14	0.13	0.04	0.90	0.90	0.90	0.13	0.04	0.98	0.33				
Ser	0.76	2.80	0.69	3.80	0.38	1.70	0.05	0.22	0.05	0.31	3.00	0.13	0.28	0.23	0.06	0.08	0.82	0.82	0.82	0.08	0.05	0.71	0.38				
Glu	0.47	0.12	0.40	0.12	0.18	0.06	0.06	0.01	0.06	0.20	0.19	0.03	0.02	0.11	ND	0.01	0.33	0.33	0.33	0.04	ND	0.95	1.00				
Gly	0.48	0.98	0.33	1.30	0.34	0.70	0.06	0.11	0.10	0.10	1.00	0.05	0.08	0.05	0.03	0.03	0.18	0.18	0.18	0.03	0.02	0.11	0.04				
Ala	2.50	2.00	2.60	1.80	2.80	1.30	0.10	0.11	0.10	2.00	1.70	1.10	0.66	0.16	1.00	0.21	0.28	0.28	1.00	0.13	0.10	0.08	0.14				
Cys	1.80	1.90	1.50	1.50	1.20	0.72	0.01	0.03	0.01	1.90	2.90	0.55	0.62	0.05	0.65	0.23	0.36	0.36	0.34	0.10	0.10	0.20	0.16				
Met	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	0.03	0.03	ND	ND	0.02	0.04				
Ile	0.26	1.10	0.24	0.62	0.31	0.22	0.01	0.01	0.01	0.32	0.63	0.18	0.12	0.05	0.17	0.07	0.30	0.30	0.30	0.19	0.06	0.30	0.13				
Leu	0.52	1.40	0.49	0.83	0.53	0.24	ND	0.01	0.01	0.50	0.63	0.28	0.14	0.03	0.25	0.08	0.15	0.15	0.26	0.07	0.07	0.08	0.09				
Tyr	0.08	0.20	0.05	0.13	0.12	0.05	ND	ND	ND	0.13	0.17	0.05	0.04	0.02	ND	ND	0.03	0.03	ND	ND	ND	0.04	0.02				
Phe	0.16	0.16	0.19	0.16	0.20	0.06	ND	ND	ND	0.18	0.12	0.13	0.05	0.01	0.13	ND	0.07	0.07	0.13	0.13	ND	0.04	0.06				
Lys	ND	0.32	ND	0.32	ND	0.05	ND	0.03	ND	ND	0.19	ND	0.03	0.07	ND	ND	0.49	0.49	ND	ND	ND	0.82	0.26				
His	0.22	0.28	0.20	0.27	0.13	0.09	0.01	ND	0.04	0.04	0.16	0.01	ND	0.01	0.01	ND	0.03	0.03	0.02	0.02	ND	0.12	0.05				
Arg	0.15	4.00	0.11	4.80	0.11	1.10	0.05	0.18	0.07	0.07	2.00	0.03	0.08	0.25	0.03	ND	1.70	1.70	0.03	0.03	0.07	3.60	2.30				
Hyp	0.10	0.14	0.13	0.10	ND	ND	ND	ND	0.07	0.07	0.28	ND	ND	0.06	ND	ND	0.13	0.13	ND	ND	ND	0.49	0.63				
Pro	0.30	0.22	0.31	0.13	0.39	0.08	ND	ND	0.40	0.40	0.37	0.17	0.10	0.01	0.19	0.04	0.08	0.08	0.15	0.15	ND	0.04	0.07				
Total	8.31	16.97	7.83	17.13	7.11	7.03	0.42	0.81	6.71	14.82	2.91	2.47	2.47	1.32	2.76	0.80	6.02	6.02	2.40	0.54	0.54	9.09	6.33				

^z L: Leaves, S: Stems, T: Tubers.^y ND: not detected.

The accumulation of allantoin and arginine in 'Tsukuneimo'

As allantoin was undetectable in leaves and stems but accumulated in tubers of 'Tsukuneimo' at 170 days after planting, it indicates that allantoin is extremely localized in 'Tsukuneimo'.

Arginine content in leaves of 'Tsukuneimo' was lower than $0.15 \mu\text{mol} \cdot \text{g}^{-1}\text{FW}$, but the weight percentages of arginine to total amino acids in stems at 85 days after planting and tubers at 200 days after planting of 'Tsukuneimo' were 38 and 42%, respectively. The time course of the content of arginine was similar to that of allantoin in stems.

Arginine is a major component of the free amino acid pool in the megagametophytes, making up approximately 28% of the free amino acids pool (King and Gifford, 1997). In the European mistletoe (*Viscum album* L.), arginine is also a solute that may be accumulated to levels exceeding $30 \text{mg} \cdot \text{g}^{-1}\text{FW}$ and as much as 90% of the total pool amino acids (Urech, 1987).

While allantoin content in tubers was constant during its development, arginine content increased 14 times between an early sample and that at harvest. These results suggest that *D. opposita* transiently accumulates arginine in stems and allantoin in leaves and stems, but allantoin is only found in developed tubers at harvest.

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Dioscorea opposita ‘ツクネイモ’ の生育中におけるアラントインおよびアルギニン含有量の変化仁宮章夫¹・村田芳行²・多田幹郎¹・下石靖昭²¹ 岡山大学大学院自然科学研究科 700-8530 岡山市津島中² 岡山大学農学部 700-8530 岡山市津島中

摘 要

窒素形態としてウレイド(アラントインとアラントイン酸)を多く含有するヤマノイモ科‘ツクネイモ’を試料として, ‘ツクネイモ’中のウレイドとアミノ酸の挙動を明らかにするために, 農家の圃場で栽培された‘ツクネイモ’を材料として, その生育過程で採取した葉, 茎および新芋中のウレイドとアミノ酸(特にアルギニン)を定量分析した. 葉のアラントイン含有量はCDUの追肥をしても経時的に減少した. 茎のアラントイン含有量は1回目のCDU施肥(植え付け後96日)後増加しなかったが, 2回目(植え付け後122日)のCDU施肥後に増加した. 新芋のアラントイン含有量は新

芋の生長過程を通してほぼ一定であった. 葉および茎のアルギニン含有量は生育過程で, それぞれ0.15および4.8 $\mu\text{mol}\cdot\text{g}^{-1}\text{FW}$ 以下であった. 茎のアルギニン含有量の経時変化はアラントイン含有量の経時変化と類似した. 新芋の植え付け170日後のアルギニン含有量は142日後の14倍に増加し, 170日後以降減少した. これらの結果から, ‘ツクネイモ’は葉と茎中にアラントインとアルギニンを一時的に貯蔵し, 最終的には生長した新芋中にアラントインの形で蓄えることが示唆された.