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SHORT REPORTS

DISTRIBUTION OF D-ALANYLGLYCINE AND RELATED COMPOUNDS IN *ORYZA* SPECIES

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Key Word Index—*Oryza* species; Gramineae; rice plants; D-alanyl-D-alanine; D-alanyl-L-alanine; D-alanylglycine.

Abstract—D-Alanine was detected abundantly in all *Oryza* species, the genome formula of which is known. In the strains containing the *AA*, *BB*, *BBCC* and *CC* genomes, D-alanine is distributed in the form of D-alanylglycine while in the strains containing the *EE* and *FF* genomes, it is distributed in the form of D-alanyl-D-alanine. In the strains containing the *CCDD* genome, D-alanylglycine or D-alanyl-D-alanine is present. Exogenously supplied D-alanine tended to be incorporated into D-alanylglycine, D-alanyl-D-alanine and D-alanyl-L-alanine in the strains of the D-alanylglycine type, and only into D-alanyl-D-alanine in those of the D-alanyl-D-alanine type.

INTRODUCTION

D-Alanine exists abundantly in the form of D-alanylglycine in the leaf blades of cultivated rice plants of the Japonica type such as Norin No. 16 and Sasanishiki [1–3]. Occurrence of alanylglycine was also found not only in the Japonica rice plant but also in several other *Oryza* species [4]. However, D-alanylglycine was not detected in the leaf blades of *O. australiensis* Domin, a wild rice species containing the *EE* genome; however, large amounts of D-alanyl-D-alanine were found in its tissues [5]. This result shows that D-alanylglycine is not necessarily present in all *Oryza* species.

In the present study, *Oryza* species, the genome formula of which had been established [6], were cultivated, and the occurrence of D-alanylglycine and related compounds in the leaf blades was studied. In addition, the effect of exogenously supplied D-alanine on the contents of D-alanylglycine and related compounds in these rice plants was investigated.

RESULTS AND DISCUSSION

The existence of D-alanylglycine and its related compounds in the 20 species of rice plants was examined. As shown in Table 1, large amounts of D-alanylglycine were detected in the cultivated plants and the wild plants containing the *AA* genome. D-Alanylglycine was also abundant in the wild plants containing the *BB*, *BBCC* and *CC* genomes and in two out of three wild plants containing the *CCDD* genome. In the remaining one wild plant containing the *CCDD* genome and in the wild plants containing the *EE* and *FF* genomes, large amounts of D-alanyl-D-alanine were detected in place of D-alanylglycine. A very small amount of D-alanyl-D-alanine was detected in the strains in which D-alanylglycine was abundant, except in the case of Sasanishiki. From the results obtained above, it is apparent that large amounts of D-alanine exist in the form of D-alanylglycine or D-alanyl-D-alanine in all *Oryza* species examined. Therefore, these plants may be classified as either the D-alanylglycine type or the D-alanyl-D-alanine type.

Strains containing the *CCDD* genome may be either the 'D-alanylglycine type' or the 'D-alanyl-D-alanine type'. To establish this point more certainly, various strains of *O. latifolia*, *O. alta* and *O. grandiglumis*, which all contain the *CCDD* genome, were analysed. As shown in Table 2, *O. grandiglumis* was the D-alanyl-D-alanine type; while among the remaining two *Oryza* species, *O. latifolia* and *O. alta*, many strains were the D-alanylglycine type. Therefore, the strains containing the *CCDD* genome may be the result of crossing of the two types.

D-Alanine was fed to various strains of *Oryza* species shown in Table 1 and the changing pattern of the contents of D-alanine-containing dipeptides in the leaf blades were examined. In the strains of the D-alanylglycine type, D-alanylglycine and D-alanyl-D-alanine contents increased and fairly large amounts of D-alanyl-L-alanine were formed following D-alanine feeding. For example, D-alanylglycine, D-alanyl-D-alanine and D-alanyl-L-alanine contents in the leaf blades of W0542 became 3.8, 4.5 and 1.5 μmol/g fr. wt respectively after D-alanine feeding (2 mM, 7 days); the D-alanylglycine and D-alanyl-D-alanine contents in the tissues before feeding were 2.6 and 0.6 μmol/g fr. wt respectively. In the strains of the D-alanyl-D-alanine type, D-alanyl-D-alanine content was increased dramatically by the D-alanine feeding. For example, its content in the leaf blades of W0613 exceeded

Table 1. Distribution of D-alanylglycine and/or D-alanyl-D-alanine in the leaf blades of various strains of *Oryza* species

	D-Alanylglycine	D-Alanyl-D-alanine
Cultivated plants		
O. sativa L.		
Sasanishiki (*AA*) Japonica type	*	—
W0108 (*AA*) Indica type	*	+
W0414 (*AA*) Indica type	*	+
O. glaberrima Steud.		
W0025 (*AA*)	*	+
W0492 (*AA*)	*	+
Wild plants		
O. perennis Moench		
W0106 (*AA*) Annual type	*	+
W0120 (*AA*) Perennial type	*	+
O. breviligulata A. Cheval et Roehr		
W0049 (*AA*)	*	+
O. punctata Kotschy		
W1593 (*BB*)	*	+
O. minuta Presl.		
W0051 (*BBCC*)	*	+
O. officinalis Wall.		
W0065 (*CC*)	*	+
W1274 (*CC*)	*	+
W1275 (*CC*)	*	+
O. latifolia Desv.		
W0542 (*CCDD*)	*	+
O. alta Swallen		
W1147 (*CCDD*)	*	+
O. grandiglumis Prod.		
W0613 (*CCDD*)	—	*
O. australiensis Domin		
W0008 (*EE*)	—	*
O. brachantha A. Cheval et Roehr		
W0654 (*FF*)	—	*
W1407 (*FF*)	—	*

*2–10 μmol/g fr. wt.; +, trace–0.5 μmol/g fr. wt.; —, not detectable.

Table 2. Distribution of D-alanylglycine and/or D-alanyl-D-alanine in the leaf blades of various strains of *Oryza* species containing the *CCDD* genome

	D-Alanylglycine	D-Alanyl-D-alanine
O. latifolia Desv.		
W0019	*	+
W0020	*	+
W0048	—	*
W0542	*	+
W1174	*	+
O. alta Swallen		
W0017	—	*
W0018	*	+
W1147	*	+
O. grandiglumis Prod.		
W0613	—	*
W1194	—	*
W1195	—	*
W1476	—	*

*2–10 μmol/g fr. wt.; +, trace–0.5 μmol/g fr. wt.; —, not detectable.

20.0 μmol/g fr. wt after the D-alanine feeding, although only 3.2 μmol/g fr. wt were present initially. D-Alanylglycine and D-alanyl-L-alanine were not detected after feeding D-alanine to strains of the D-alanyl-D-alanine type. Exogenously supplied D-alanine is well incorporated into D-alanylglycine and alanylalanine in the leaf blades of Sasanishiki ('D-alanylglycine type') [7, 8] and into D-alanyl-D-alanine in the leaf blades of W0008 ('D-alanyl-D-alanine type') [8]. Thus, on the metabolism of exogenously supplied D-alanine, the strains of the D-alanylglycine type may have the formation system of D-alanylglycine, D-alanyl-D-alanine and D-alanyl-L-alanine although those of the D-alanyl-D-alanine type may have only the D-alanyl-D-alanine-formation system.

Although D-alanylglycine is not found in higher plants other than rice, D-alanyl-D-alanine has been detected in a strain of *Nicotiana tabacum* (tobacco plant) [9] and some strains of *Phalaris tuberosa* (pasture grass) [10]. Therefore, D-alanyl-D-alanine may possibly be distributed widely in higher plants.

EXPERIMENTAL

Plant materials. Two cultivated and 20 wild species are

Table 3. List of *Oryza* species studied

Group	Distribution	Genome formula
Cultivated plants		
O. sativa L.	Asia, All the world	*AA*
O. glaberrima Steud.	West Africa	*AA*
Wild plants		
O. perennis Moench	Tropical areas of the world	*AA*
O. breviligulata A. Cheval et Roehr	West Africa	*AA*
O. punctata Kotschy	Africa	*BB*
O. minuta Presl.	South Asia	*BBCC*
O. officinalis Wall.	South Asia	
O. latifolia Desv.	Latin America	*CCDD*
O. alta Swallen	South America	*CCDD*
O. grandiglumis Prod.	South America	*CCDD*
O. australiensis Domin	North Australia	*EE*
O. brachantha A. Cheval et Roehr	West Africa	*FF*

distributed in the genus *Oryza* [11]. The genome formula of *Oryza* species having been clarified are *AA*, *BB*, *BBCC*, *CC*, *CCDD*, *EE* and *FF* [6]. Two cultivated and ten wild species were used in the present experiment, and all of the genomes described above are contained in them (Table 3). In Table 3, *O. sativa* and *O. glaberrima* are the cultivated species. Among wild species, *O. perennis* and *O. breviligulata* are said to be closely related to the cultivated species, and other wild species to be genetically distinct from the cultivated species. All strains except Sasanishiki were generously supplied from the National Institute of Genetics (Mishima, Japan).

Plant cultivation. Seeds were incubated in darkness at 45° for 4 days to break their dormancy. After being hulled, these seeds were germinated on moist filter paper in darkness at 30° for 4–8 days and cultivated in a nutrient soln in a greenhouse for 30–40 days as described in ref. [12].

D-*Alanine feeding.* Rice seedlings (30 to 40-days-old) were transferred to a basal medium [2] from which nitrogen (NH_4NO_3) had been omitted and to which 2 mM D-alanine had been added, and kept in a greenhouse for 5 more days.

Analyses. Leaf blades were cut from the seedlings (1–2 g) and immersed in hot 70% EtOH. The EtOH extract was dried below 60° and the residue was dissolved in 1–2 ml of H_2O. After centrifugation, 10 μl of the supernatant was applied to a cation exchange column, AApak (6 × 100 mm) (Japan Spectroscopic Co., Ltd.) and developed with 0.2 M citrate buffers (pH 3.25 and 4.25) and 0.2 M NaOH according to a previous paper [5]. D-Alanine-containing dipeptides such as D-alanyl-L-alanine (R_t 32 min), D-alanylglycine (33 min) and D-alanyl-D-alanine (35 min) appeared as a single peak between valine (23 min) and isoleucine (37 min) on the chromatogram. Although the D-alanylglycine and D-alanyl-D-alanine contents could be estimated by this method, the D-alanyl-L-alanine content could not necessarily be estimated clearly because the D-alanyl-L-alanine peak tended to be covered by the D-alanylglycine peak on the chromatogram. Therefore, the D-alanine-containing dipeptides were separated as a group from the leaf extract by high-voltage paper electrophoresis [5]. Subsequently, the dipeptide fraction was separated into D-alanylglycine and alanylalanine (D-L + D-D) by paper chromatography PC [13, 14]. Then, the alanylalanine was extracted and analysed by the above HPLC method. Optical configuration of these dipeptides were confirmed chromatographically [14] and enzymatically [2, 5, 14] according to previous papers.

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