Hydrolytic Enzyme Activities Related to the Decomposition of Organic Nitrogen Compounds, Organic Phosphate Compounds and β-Glucosides in Tomato Field Soils

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Various organic compounds are always supplied and exist in soil. Saccharides, proteins, nucleotides are supplied from plant residues and other dead organisms. These components are decomposed in soil by biochemical processes such as enzymic actions of protease, nuclease, glucosidase etc.

Soil contains protease which hydrolyzes protein to various amino acids[6]. *L*-Glutamine, one of the basic amino acid, is hydrolyzed to glutamic acid and ammonium by glutaminase[2]. Protease-glutaminase system in soil constitutes one of the mineralization process of organic nitrogen.

Phosphodiesterase is known to hydrolyze the phosphoric diester bonds of oligonucleotides[5]. The hydrolyzed products, nucleotide monophosphate, serve as a substrate for phosphomonoesterase. These phosphatases were already detected in soils[5,9]. Phosphodiesterase-phosphomonoesterase system in soil accounts for one of the mineralization process of the organic phosphates.

β-Glucosidases constitute one of the common groups of soil enzymes[3]. They help in the hydrolysis of various β-glucosides which are frequently supplied to soil from plant residues. Among the plant β-glucosides, cellobiose is the hydrolyzed product of cellulose, the main component of plant polysaccharides, and phenolic β-glucosides are commonly found in various plants. Some of the aglycons are known to be the precursors of the toxic substance which causes soil sickness in fields where crop plants are grown in monoculture. Therefore, β-glucosidase is of interest from a phytopathological view point[1,7,8].

Outline of tomato fields and properties of the soils

An investigation was made on various green-house fields in tomato growing districts

Table 1. Outline of the green house fields of tomato examined

Field examined No.	Cropping system 1974 (Nov.-May)-(June-Oct.)-(Nov.-May)-(June-Oct.) 1975	Tomato variety	Root	Crop yield of tomato (ton/10a)
1	Tomato-rice-tomato-rice	Tanomo	Not grafted	3
2	Tomato-cucumber-tomato-cucumber	KNVF/toko K	Grafted	6
3	Tomato-rice-tomato-rice	KNVF/toko K	Grafted	10
4	Tomato-rice-tomato-rice	Tanomo	Not grafted	6
5	Tomato-cucumber-tomato-cucumber	Tanomo	Not grafted	7
6	Tomato-rice-tomato-rice	Tanomo	Not grafted	8
7	Tomato-rice-tomato-rice	Eiju	Not grafted	12
8	Tomato-rice-tomato-cucumber	KNVF/toko K	Grafted	12
9	Tomato-rice-tomato-rice	KNVF/toko K	Grafted	10

Table 2. Properties of soil samples

Sample No.	pH(H_2O)	Moisture content %	Total carbon* %	Total nitrogen* %	Clay content* %
1	5.9	23.1	1.8	0.15	19
2	5.2	17.2	2.6	0.23	26
3	5.7	20.4	2.2	0.21	21
4	6.2	16.0	2.3	0.20	12
5	6.4	18.3	2.2	0.21	18
6	6.7	18.9	1.9	0.19	20
7	6.4	29.2	3.7	0.37	24
8	6.1	27.8	3.6	0.37	19
9	6.4	11.9	2.3	0.19	4

* Per dried soil

at Oi and Fujieda in the Shizuoka Prefecture. In these districts, tomato is grown in greenhouses every year after paddy rice or cucumber. The yearly cropping of tomato is reported to bring about brown root-rot disease to the plant with the formation of corky roots. The fields which were examined were different each other in cultural condition and crop yield (Table 1). Nine soil samples were collected in May 1976 from the surface soil layer between plants in the fields. Properties of the soil samples are shown in Table 2.

Hydrolytic enzyme activities of tomato field soils

Enzyme activities of the soils were assayed and the results are given in Table 3.

For assay of soil protease activities, benzyloxy carbonyl-*L*-phenylalanyl-*L*-leucine was used as a substrate[6]. For glutaminase assay, *L*-glutamine was used[2]. For phosphodiesterase assay, bis-(*p*-nitrophenyl) phosphate was a substrate[5]. For phosphomonoesterase, *p*-nitrophenyl phosphate was used[9]. For *β*-glucosidase assay, *p*-nitrophenyl-*β*-glucoside was used as a substrate[3]. Incubation was carried for at 30°C for 1 to 2 hrs.

Average activity of soil phosphomonoesterase was 29.2×10^{-9} mole per min per g of dried soil. This value implies that soil has a potential activity to hydrolyze 462 kg of *p*-nitrophenyl phosphate or to liberate 200 kg PO_4—from the substrate per day per 10 a of tomato field.

By the same way, the potential activity of soil glutaminase was estimated as 770 kg of

Table 3. Hydrolytic enzyme activities* of tomato field soils

Soil sample No.	Protease	Glutaminase	Phosphodi-esterase	Phosphomono-esterase	*β*-Glucosidase
1	7.0	712	11.6	21.2	16.2
2	11.1	142	11.2	18.8	21.9
3	9.6	507	10.1	20.4	13.3
4	19.2	413	16.2	32.4	25.1
5	15.0	603	15.9	27.7	24.8
6	14.0	612	12.7	27.0	14.6
7	29.4	1130	25.0	47.7	22.1
8	22.6	1164	17.7	37.3	17.1
9	14.0	379	14.6	31.1	14.0

* nmole substrate hydrolyzed per min per g of dried soil

ammonium released per day per 10 a of tomato field.

Correlation between hydrolytic enzyme activities in soil and the crop yield of tomato

Correlation between the enzyme activities and the crop yields of tomato was studied to characterize the biochemical process associated with soil fertility (Table 4). Crop yield of tomato was correlated with phosphomonoesterase and protease activity in the soil but not well correlated with phosphodiesterase and glutaminase activities. Whereas β-glucosidase activity showed a low, negative correlation.

Table 4. Correlation between crop yields of tomato and soil enzyme activities

Enzyme	Correlation value r
Protease	0.673*
Glutaminase	0.542
Phosphomonoesterase	0.896**
Phosphodiesterase	0.561
β-Glucosidase	−0.229

Pattern analysis was made on these enzyme activities in tomato field soils (Table 5). The soils from the fields with high crop yields showed higher levels of the activities related to decomposition of organic nitrogen and organic phosphate compounds than that of β-glucosidase activity. Whereas, in the soils from low productive fields, the levels of protease, glutaminase, phosphodiesterase and phosphomonoesterase activities were lower than that of β-glucosidase activity.

Significance of the enzyme activity in tomato field soil under yearly cropping

Crop yield is thought to be approximately proportional to the root growth. Root affects rhizosphere micro-organisms and their biochemical activities by exuding nutrients and organic materials. Generally, root causes an increased biological activity in soil by the rhizosphere effect. Soil sample collected from the field with high crop yield contains more rhizosphere soil than that from the field with low crop yield. Therefore, the enzyme activity of the former is higher than that of the latter except β-glucosidase activity.

Vascular sap of diseased root of tomato plant is known to have β-glucosidase activity, while that of the healthy root revealed no β-glucosidase activity[1]. Injection of β-glucosidase to pea root is reported to cause necrotic

Table 5. Pattern analysis of the enzyme activities

Soil sample No.	Protease	Glutaminase	Phosphodi esterase	Phosphomono esterase	β-Glucosidase	Crop yield of tomato
1	*	**	*	*	**	low
2	*	*	*	*	**	low
3	*	**	*	*	*	high
4	***	*	**	**	***	low
5	**	**	**	**	***	low
6	**	**	**	**	*	high
7	***	***	***	***	**	high
8	***	***	**	***	**	high
9	**	*	**	**	*	high

* : Average activity × (1−0.2) >
** : Within average activity × (1±0.2)
*** : Average activity × (1+0.2) <

symptom which is similar to that brought about by innoculation of pea pathogen, *Gliocladium catemulatum*[8]. Coincidently, more phenolics were detected in vascular sap from diseased plant than in that from healthy plant on paper chromatography[8]. Considering such factors, in a series of tomato fields under yearly cropping, β-glucosidase activity in soil might indicate a measure of contamination of diseased root debris in the soil sample.

The activity of β-glucosidase extracted from soil was reported to have similar optimum pH and substrate specificity to that of fungal origin[4]. The β-glucosidase activity in field soils under yearly cropping or monoculture may be an indicator of the fungal activity releasing the toxic phenolics from β-glucosides of plant residues.

Thus, the pattern of the hydrolytic enzyme activities is thought to show some of the biochemical nature of soil related to plant productivity.

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