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Peroxidase Isozymes in the Leaves of Various Clones of Garlic, *Allium sativum* L.

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Introduction

Garlic, *Allium sativum* L., is an ancient crop of central Asian origin[9] and has been cultivated widely in the world. A large number of cultivars have been propagated vegetatively as clones, because garlic is completely sterile. The morphological variation among the garlic cultivars is great[4]. The karyotypic and meiotic variations of the clomosomes were also observed among the clones of garlic[1,2,3]. Some of the garlic clones may belong to other species. For example, Konvicka and Levan[8] reported a clone which might belong to *A. longicuspis* Regel, and Khoshoo *et al.*[5,6] reported a clone which ought to have belonged to *A. ampeloprasum* L. There are some authors assuming *A. longicuspis* Regel to be the wild ancestor of *A. sativum* L.[9]. One of the cultivated forms of *A. ampeloprasum* L. is called great-headed garlic.

In order to identify the garlic clones, adding to the morphological classification, chemical classification of the clones may be useful. The isozyme analysis has often been attempted to identify the clones with special cares paid to the limitations of the technique[10]. Enzyme polymorphism has reported in many plants[13]. Some attempts were made to analyze the relationship between zymograph patterns and habitats of cultivars in rice[11] and in sweet potato[7]. In garlic, however, no attempt had been made to clarify the isozymes, and the present investigation was conducted to reveal the peroxidase isozymes of the leaves in various clones of garlic.

Materials and Methods

Materials used here were ninety-three clones of garlic, *A. sativum* L., as shown in Table 1.

The cloves of all the clones were planted in the field at Kagoshima University in October 1979. The newly expanding leaves, approximately 10 cm long from the tips, were collected as the materials for electrophoresis in February 1980. Those materials, just after sampling, were stored in the freezer at about −20°C. One gram of the leaves stored in the freezer were ground in a mortar with one ml of 0.2% $NaNO_3$-0.8% NaCl (1: 1) solution. Paste-like crude extract obtained was kept for about two hours at 5°C in a centrifuge tube, and then was centrifuged at 12,000 rpm for 20 minutes at 5°C. The supernatant, just after centrifugation, was used for horizontal polyacrylamide gel electrophoresis to separate peroxidase isozymes. The thin layer electrophoresis was carried out without a cellophane sheet in the manner described by Ogita *et al.*[12], except for a continuous buffer system. Gel buffer contained 0.2 g of NaOH and 1.8 g of boric acid in one liter of water (pH 8.52), and electrode buffer contained 3.4 g of NaOH and 18.5 g of boric acid in one liter of water (pH 8.60). Catalyst-monomer solution was as follows: A (9.5 g acrylamide, 0.5 g

Table 1. Garlic clones examined and their clone Nos.

No.	Clone	No.	Clone	No.	Clone
1	Hokkaidō	36	Wase-ninniku	74	Ambon (Indonesia)
2	Howaito-Roppen (Miyagi)	37	Okute-B	75	Kushikino-wase
3	Tochigi-Roppen	38	Red Salmona	80	Okinoerabu
4	Niigata	40	Kōkotsu	81	Morioka-A
5	Howaito-Roppen (Niigata-Kubiki)	41	Roppen-shu	82	Morioka-B
		42	Taiwan-ooninniku-B	83	Nyū-Howaito-Roppen (Iwate)
6	Niigata-Sado	43	Taiwan		
7	Sado-zairai	44	Taiwan-daikyu-pinku	84	Ichinoseki
8	Ibaraki	45	Taiwan-shōkyu-pinku	85	Sendai-A
9	Chiba-A	46	Taiwan-pinku	86	Sendai-B
10	Chiba-B	47	Hong Kong	87	Iwate-Howaito (Fukushima)
11	Howaito-Roppen (Ishikawa)	50	Kankō (Hankow, China)		
		51	Taichū-Nankotsu	88	Tōkyō
12	Matsumoto	53	Kōka (Hinghwa, China)	89	Sweden
13	Shizuoka-zairai	54	Fukushū (Foochow, China)	90	Kasari-Sani (Amami)
14	Shizuoka-wase	55	Egypt	93	Mito-A
15	Hamamatsu	56	California Early	94	Mito-B
16	Wakayama-Roppen	57	California Late	95	Mito-C
17	Howaito-Roppen (Tottori)	58	Thailand	96	Utsunomiya-A
18	Shimane-Tsunozu	60	Chili	97	Utsunomiya-B
19	Kōchi-shōkyū	61	Italy	98	Takasaki-A
20	Kōchi	62	U.S.A.	99	Takasaki-B
21	Kōchi-daikyū	63	Saga-zairai	100	Takasaki-C
22	Kōchi-zairai	64	Shanhai-wase	101	Kōriyama
23	Saga-Ariura	65	Iki-shu	102	Yonezawa
24	Saga-ooninniku	66	Tokushima-zairai	103	Yamagata-A
25	Kagoshima-A	67	Amami-A	104	Yamagata-B
26	Kagoshima-B	68	Amami-B	105	Shinjō
27	Kagoshima-zairai	69	Colombia	106	Sakata
30	Amami-2	70	Taiwan-A	108	Akita
31	Iki-No. 1	71	Iki-wase (Tokushima)		
32	Iki-No. 3	72	Kagawa-howaito-Roppen (Tokushima)		
34	Howaito-Napori				
35	Toroku-kuroba-Kōkotsu	73	Shanhai-wase (Tokushima)		

BIS, 100 ml gel buffer), B (1 ml TEMED, 100 ml water), C (120 mg ammonium persulfate, 100 ml water). Three kinds of the solution, that is, A, B and C, were mixed in the ratio 2: 1: 1 in volume.

The thin layer gel was 14 cm long, 5 cm wide and 0.8 mm thick, and had six specimen slots. Five μl of the supernatant mentioned above was poured into each slot. Both ends of the gel plate were connected with the filter paper to the gel buffer solution.

Electrophoresis was carried out at a constant current of 0.5 mA per cm for three hours and forty minutes at 5°C. To make a staining solution, 0.3 g of 4-chloro-1-naphthol was stirred for thirty minutes in 150 ml of the buffer solution (pH 4.0) which contained 2.4 g of sodium acetate and 4.5 ml of acetic acid. To the naphthol solution, 1.5 ml of 3% solution of hydrogen peroxide was

added. After electrophoresis, the plate was dipped and stained in the staining solution, that is, the naphthol solution containing hydrogen peroxide. The isozyme bands of peroxidase were observed after the staining executed for half an hour.

Results

The peroxidase isozyme bands in the garlic leaves observed in the present experiment are diagrammatically represented in Fig. 1. Seven bands in total were observed; one (P1-band) in the cathodic side and six (P2–P7) in the anodic side.

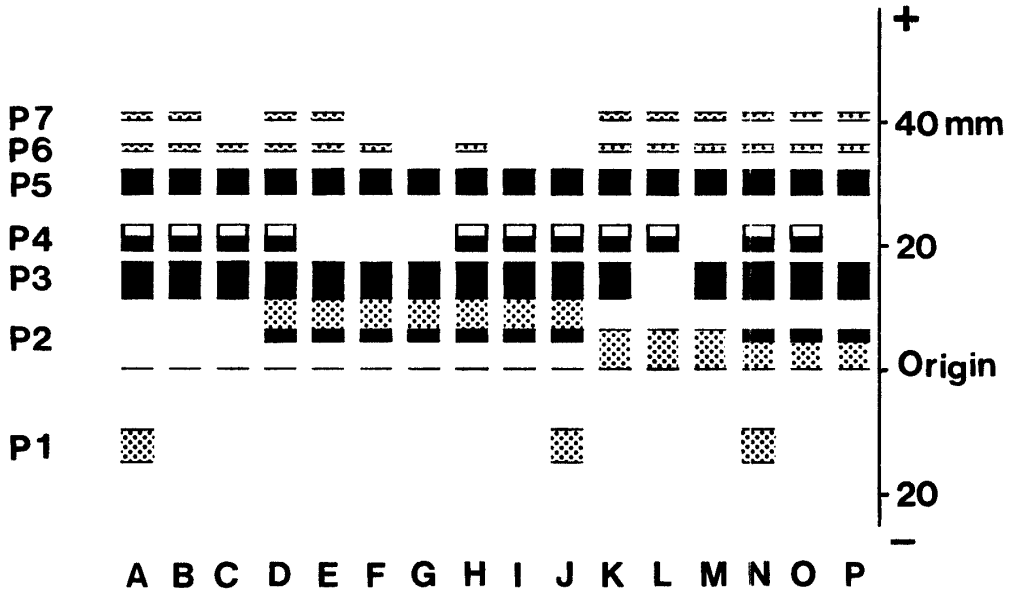

Fig. 1. Diagrammatic zymograms of the peroxidase isozymes in the leaves of various garlic clones.

The zymograms of all the clones examined were classified into sixteen types (A–P).

The zymograms observed here were classified into sixteen types (A–P), as shown in Fig. 1. P1-band was observed only in several clones. P2-band accompanied a light-stained zone in either side of itself, namely, between P2- and P3-bands in the types D–J and between P2-band and origin in the types N–P. There was another light-stained zone to be observed in the anodic side of origin also in the types K–M, but these types lacked P2-band.

There might be a possibility that P3-band in this experiment was composed of some different bands to be separated, because Rf-value of P3-band varied a little with the clones. However, all of the P3-bands were assumed here to belong to the same P3-band group, since they were wide and situated in almost the same region of the zymograms. P3-bands were always observed in each zymogram with only one exception of clone No. 106. Table 2 represents the types of zymograms in each clone examined and the distance from origin to P3-bands. P3-bands were stained dark in many clones, as shown in Table 3, though they were faint in some other clones.

P4-band was clearly observed, and there was a colorless zone in contact with P4-band which was usually observed in the anodic side (Fig. 1). However, the following clones of those having P4-band did not show the colorless zone; Nos. 4, 5, 14, 18, 22, 24, 26, 31, 42, 82, 84, 86, 89 and 101. P5-band was only the one band common to all the types of zymograms, in other words,

Table 2. Types of zymograms and clone Nos.

Types of zymograms	Clone No.				
	Distance from Origin to P3-band (mm)				Without P3-band
	10	11	12	13	
A			34, 57	56	
B	89	7, 38, 61		55	
C		70			
D		6			
E	46				
F	44, 53, 68, 75	67			
G		74			
H		54, 90			
I		47, 80			
J			58		
K		94	5, 9, 22, 25, 42, 50, 64, 71, 73	43, 51, 97, 100, 103, 104, 105, 108	
L					106
M			62	1, 11, 99, 102	
N		95	63, 85		
O		93	2, 3, 14, 18, 19, 20, 21, 30, 31, 35, 40, 65, 72, 81, 82, 83, 84, 86, 98	4, 10, 13, 23, 24, 26, 36, 69, 96, 101	
P		15	8, 12, 16, 17, 32, 37, 41, 45, 66, 88	27, 60, 87	

Table 3. Darkness of P3- and P5-bands

Clone No.	Band P3	P5	Clone No.	Band P3	P5	Clone No.	Band P3	P5	Clone No.	Band P3	P5	Clone No.	Band P3	P5	Clone No.	Band P3	P5
1	−	−	17	+	−	36	+	−	56	++	+	73	−	−	95	+	+
2	+	−	18	−	−	37	+	−	57	++	+	74	++	−	96	+	−
3	+	−	19	++	+	38	++	+	58	++	−	75	++	−	97	−	−
4	−	−	20	−	−	40	++	−	60	+	−	80	++	−	98	−	−
5	+	−	21	+	−	41	+	−	61	++	+	81	++	−	99	−	−
6	++	−	22	+	−	42	+	−	62	+	−	82	++	−	100	−	−
7	++	+	23	+	−	43	+	−	63	+	+	83	++	−	101	+	−
8	−	−	24	++	−	44	++	−	64	−	−	84	+	+	102	−	−
9	+	−	25	+	−	45	−	−	65	+	−	85	+	−	103	+	+
10	−	−	26	−	−	46	++	−	66	+	−	86	+	−	104	+	+
11	++	−	27	+	−	47	++	−	67	++	−	87	+	−	105	+	+
12	++	+	30	+	−	50	+	−	68	++	−	88	+	−	106		+
13	−	−	31	+	−	51	+	−	69	−	−	89	++	+	108	+	−
14	+	−	32	++	−	53	++	−	70	++	+	90	++	−			
15	+	−	34	++	+	54	++	−	71	++	−	93	+	+			
16	++	−	35	+	−	55	++	+	72	++	−	94	+	−			

++: Very dark, +: Dark, −: Light

common to all the clones. P5-band was stained not so dark in case of P3-bands, but it was stained darker than the other bands except P3-band in some clones (Table 3). P6- and P7-bands were always faint, and P7-band was never observed without P6-band.

Among the clones examined in this experiment, the clones included in the zymogram types (A–J) without the light-stained zone between origin and P2-band were much fewer than those in the types (K–P) with the light-stained zone. Furthermore, P6- or P7-band, or both, were absent only in some of the types A–J. P3-bands were always stained darker in the types A–J as compared in the types K–P (Table 3). In the present experiment concerned, most of the clones were included into one of the three types of O, K and P, all of which contained, without exception, P3-, P5-, P6- and P7-bands and were devoid of P1-band. Zymogram type O which included the largest number of clones showed also P2- and P4-bands, but the other two types showed either P2- or P4-band.

Discussion

In ninety-three clones examined, the peroxidase isozymes of garlic leaves represented various types of zymograms, though their bands were not so many as those in sweet potato[7]. On the other hand, the morphological variation among cultivars is great, and differences can be found in several characteristics such as plant size, yield, content of solids, time of maturity, ease of bolting, storability, and the number, size, shape and color of the cloves[4]. Almost all the clones have local names, because of their being reproduced vegetatively, but only a few attempts have been made to collect and compare the clones of many countries, especially those of main garlic-producing countries.

Table 4 shows two characteristics in the clones examined; time of maturity and ease of bolting. The late cultivars examined here came from the cool region, or the northern part, of Japan. Consequently, almost all the plants of these late cultivars have neither fully matured nor developed long

Table 4. Time of maturity and ease of bolting in the clones examined

Time of maturity	Clone No.		
	Long scapes with flower buds	A few or short scapes without flower buds*	No scape
Extremely early		47	
Early	14, 15, 30, 35, 37, 40, 42, 51, 69	6, 38, 44, 45, 46, 53, 54, 55, 58, 61, 67, 68, 70, 74, 75, 80, 90	34, 56, 57
Medium	1, 3, 4, 5, 8, 9, 10, 11, 13, 17, 18, 19, 20, 21, 22, 23, 25, 31, 32, 36, 41, 43, 50, 60, 62, 63, 64, 65, 66, 71, 72, 73, 85, 94, 95, 99, 100	12, 16, 24, 26, 27, 97	2, 7
Late	101, 106	93, 102, 103, 104, 105, 108	81, 82, 83, 84, 86, 87, 88, 89, 96, 98

* Most of the clones occasionally develop the scapes, though very short, bearing a small number of bulblets in the inflorescences, but have never developed flower buds in Kagoshima. Some of the clones have developed a few scapes bearing a small number of flower buds after hard vernalization.

scapes bearing flower buds in Kagoshima, that is, the southern part of Japan. Besides these clones, Nos. 1, 2, 7, 12, 16 and 85 of the medium cultivars also showed some of the characteristics of the clones from the cool region, for instance, interruption of growth in winter. Most of these late cultivars or clones examined would bolt in the northern part of Japan. The clones from subtropics which vigorously grow at Kagoshima even in winter are Nos. 6, 44–47, 53, 54, 58, 67, 68, 70, 74, 75, 80 and 90. Clone Nos. 34, 55–57, and 61 were supposed to belong to the same one group, that is, to the A-type garlic described by Konvicka and Levan[8]. A few plants of clone No. 34 had occationally bolted in Kagoshima, whereas no plant bolted in 1979–1980 growing season. Clone No. 38 is supposed to belong to the A-type garlic. However, bolting was often observed in clone No. 38, though the inflorescences were composed of only red bulblets and were devoid of flower buds.

All of the clones without P6- or P7-band, or both, of the zymograms came from the subtropics and had never developed long scapes bearing flower buds. The zymogram types D–J, all of which had P2-band and the light-stained zone between P2- and P3-band, were observed in the subtropical cultivars. The clones showing the zymogram types A–J whose P3-bands were always stained very dark did not develop any flower buds in 1979–1980 growing season. The distance between P3-bands and origin was shorter in the zymogram types A–J than that in the zymogram types K–P. It is interesting that the early cultivars which grow vigorously in winter, that is, time of sampling in this experiment, have the dark-stained P3-bands generally situated nearer to origin than those of the late or the medium cultivars. On the other hand, the late or the medium cultivars examined here never lacked P6- or P7-band.

In the previous experiment[2], the chromosome pairings at meiosis were observed in most of the bolting cultivars examined in this experiment. Besides these, some preliminary observation for the chromosome pairings at meiosis was made here in some other bolting cultivars and occasionally bolting cultivars, and revealed that these cultivars showed multivalent chromosomes such as those in the previous experiment[2]. Namely, clone Nos. 24, 26, 85, 94, 95, 99 and 101 mostly represented $1_{VI}+5_{II}$, while clone Nos. 30, 100 and 103 mostly represented $1_{VIII}+4_{II}$. However, clone Nos. 12 and 97 showed both of PMCs having $1_{VI}+5_{II}$ and PMCs having $1_{VIII}+4_{II}$, though there were merely several pollen mother cells observed. It seemed that there was no correlation between the chromosome configuration at meiosis and the patterns of peroxidase isozymes in the leaves of garlic clones examined.

Summary

Peroxidase isozymes of the leaves in 93 clones of garlic, *A. sativum* L., were investigated by means of horizontal thin layer polyacrylamide gel electrophoresis. The gel plates were stained with 4-chrolo-1-naphthol. Seven isozyme bands were observed in total. There were sixteen types of zymograms to be observed. Distance from origin to P3-bands varied a little with the clones. P3- or P5-band, or both, were stained dark in many clones. The early cultivars, or clones, examined here had no scapes or scapes without flower buds, and showed not only the zymogram types A–J but also dark stained P3-bands. On the other hand, the medium or the late clones lacked neither P6- nor P7-band. P3-bands were nearer to origin in the early cultivars than those in the others. It seemed that there was no correlation between meiotic variation of the chromosome pairing and zymographic variation in the clones examined.

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