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Peroxidase Isozymes in Various Cultivars of Leek and Kurrat

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Introduction

Allium ampeloprasum L. is an extremely variable species, ranging as a wild plant from southern Europe and northern Africa through the Middle East into western and southern U.S.S.R.[13)] Besides the wild plants, three cultivated groups, that is, leek (synonym: *A. porrum*), kurrat (syn. *A. kurrat*) and great-headed garlic, belong to *A. ampeloprasum* and they are regarded as derivations from wild *A. ampeloprasum*[13)]. In leek, the edible part is the blanched leaf-sheath, and the green leaf blades of kurrat are the edible part[14)]. On the other hand, bulbs of great-headed garlic are the edible part like garlic. Wild *A. ampeloprasum* is called Levant garlic or perennial sweet leek[25)]. Wild *A. ampeloprasum* shows various levels of polyploidy, 2x, 3x, 4x, 5x, 6x and 7x (x=8)[4,15,16,18)]. However, tetraploid and hexaploid are predominant in wild populations[2,3,15,16)], and the three cultivated groups are also tetraploid and hexaploid. Leek and kurrat are tetraploid (4x=32), and great-headed garlic is hexaploid (6x=48)[18)].

Sometimes, the relationship between *A. ampeloprasum* and *A. sativum,* garlic, is discussed because of morphological resemblance. From a viewpoint of karyosystematics, Tarasova[24)] suggested a possibility of evolution from *A. sativum* through *A. ampeloprasum* to *A. porrum.* Leek and kurrat of tetraploid are propagated by seed, while great-headed garlic of hexaploid is propagated vegetatively by bulbs. Garlic of diploid (2n=16) is sterile and it is also propagated vegetatively by bulbs. However, fertile garlics were recently discovered in Soviet Central Asia by the present author[5−8)]. Moreover, those fertile garlic clones showed a particular isozyme band of peroxidase[7,9)]. As garlic is presumed to have evolved from the sexually-propagated type to the vegetatively-propagated type, it is reasonable to think that this particular isozyme of peroxidase might have existed in the primitive forms of garlic. When we think of the relationship between garlic and *A. ampeloprasum,* a primitive character such as this may be made use of as a tool for us to analyze this relationship.

In the present examination, various cultivars or strains of leek and kurrat were compared with fertile and sterile clones of garlic by peroxidase isozymes for discussing the relationship between leek or kurrat and garlic.

Materials and Methods

As the materials, 54 cultivars or strains of leek, five strains of kurrat and two garlic clones were used (Table 1). All the cultivars or strains of leek and kurrat were collected and offered by Ing. Q. P. van der Meer of IVT of the Netherlands. All the materials of leek or kurrat were

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Table 1. Materials used for the isozyme analysis and their native countries

No.	Cultivar	Country	No.	Cultivar	Country
	LEEK and KURRAT		34	Winterreus Vincent	Netherlands
1	Monstrueux de Carentan	France	35	Yebeer	Netherlands
2	De Gennevilliers	France	36	Enormus	Netherlands
3	Bleu de Solaise	France	37	Zwiherre reuzen Albinstar	Netherlands
4	De Liege	France	38	Herfstreus Snowstar	Netherlands
5	D'Hiver de Saint-Victor	France	39	Herfstreus Bombarde	Netherlands
6	Gros long d'été	France	40	Artico RS	Netherlands
7	Geant amélioré de Saulx	France	41	Lglo	Netherlands
8	Geant précoce	France	42	Empro.	Switzerlands
9	Monstrueux d'Elboeuf	France	43	L-Leeks	Egypt
10	Geant de Verrieres	France	44	Herfstreus SG162	Netherlands
11	Geant amélioré de Saulx	France	45	Herfstreus Otina	Netherlands
12	Kamuch	Bulgaria	46	Vr. herfst Rese	Sweden
13	Long de Mezieres	France	48	Blauwgroene Winter Bleustar	Netherlands
14	Jolant	Netherlands	49	Herfstreusen Snowstar B	Netherlands
15	A-Kurrat	Egypt	50	Castelstar	Netherlands
16	B-Kurrat	Egypt	51	Zwitserse Reuzen Albinstar	Netherlands
17	C-Kurrat	Egypt	52	Herfstreus Lawine	Netherlands
18	D-Kurrat	Egypt	53	Regius	Netherlands
19	Starozagorski	Bulgaria	54	Herfstreus Baton	Netherlands
20	E-Leeks		55	Platina	Netherlands
22	G-Leeks	Egypt	56	Alaska	Netherlands
24	I-Kurrat	Egypt	57	Winterreus Super	Netherlands
26	Winterreus Attila	Netherlands	58	Siberia	Netherlands
27	Herfstreus Kazan	Netherlands	59	SG 178	Netherlands
28	Vroege herft Titan	Sweden	60	Winterreus Triumphator	Netherlands
29	Herfst Odin	Sweden	61	Winterreus Wila	Netherlands
30	Herfst Kong Vinter	Sweden	62	Acadia	Netherlands
31	Late herft Regius	Sweden	63	Taree irani	Iran
32	Winter Siegfried	Sweden		GARLIC	
33	Winter Kopenhamns Torg	Sweden		Shanhai-wase	Japan
				No. 130	U.S.S.R.

offered as seeds. Two garlic clones were collected by the authors. One garlic clone 'Shanhai-wase' is sterile and one of the leading varieties in Japan. Another clone 'No. 130' was found to be fertile in 1983 by the present author[5,6].

The seeds of leek or kurrat were sown in the seed bed after soaking in October of 1987, and the seedlings were transplanted into the pots in January of 1988. The cloves of garlic were planted in November of 1987.

The young leaves, approximately 15 cm long from the tips, were picked as materials for electrophoresis in February of 1988, and they were frozen at $-20^{\circ}C$ immediately after picking. After weighed, one gram of the frozen leaves 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 at 5°C in a centrifuge tube for about two hours, and it 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 a manner described by Ogita *et al.*[20)], excepting for continuous buffer system. Gel buffer contained 0.21 g of NaOH and 1.8 g of H_3BO_3 in one liter of water (pH 8.52), and electrode buffer contained 3.4 g of NaOH and 18.5 g of H_3BO_3 in one liter of water (pH 8.60). Catalyst-monomer solution was prepared as follows: A (9.5 g acrylamide, 0.5 g BIS; N,N'-methylenebisacrylamide, 100 ml gel buffer), B (1 ml TEMED; N,N,N',N'-tetramethylethylenediamine, 100 ml water), C (120 mg ammonium persulfate, 100 ml water). These solutions, A, B and C, were mixed in the ratio of 2 : 1 : 1 (volume).

The thin layer gel was 14 cm long, 5 cm wide, and 0.8 mm thick, having six sample slots. The supernatant mentioned above was poured into each slot with a micropipet. Both ends of the gel plate were connected with the filter paper to the gel buffer solution. The gel buffer and the electrode buffer were also connected with the U-shaped glass tubes filled with agar-gelled electrode buffer.

Electrophoresis was carried out at 5°C by a constant current of 5 mA for 3 hours and 40 minutes. 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. This naphthol solution was filtrated, and then 1.5 ml of 3% solution of hydrogen peroxide was added. After electrophoresis, the gel plate was soaked in this staining solution. The isozyme bands of peroxidase were observed after being soaked for half an hour. The experiment was always done twice.

Results

All the peroxidase isozyme bands in leeks, kurrat, and garlic appeared on the anodic side, and they are diagrammatically illustrated in Fig. 1. The zymogram patterns revealed were six (a-f) in leek, two in kurrat(c, d), and two in garlic(g, h). In total, eight isozyme bands were observed in leek, kurrat and garlic. The respective isozyme band was named after the previous reports[7,9)]. P-4, -5, -6 and -8 bands were already observed in the garlic clones of the previous analyses. P-9 and -10 bands appeared at the same Rf value as that of large P-3 band in the previous analyses. P-11 and -12 bands were also observed in a clone of great-headed garlic, *A. ampeloprasum*, in the previous report[9)]. P-5 and -6 bands were observed only in garlic, while P-11 and -12 were observed only in leek and kurrat. P-11 band was sometimes very close to P-12, and most of P-11 bands were stained light. P-4 and -10 bands were observed in all of the materials of leek, kurrat and garlic in common. Accordingly, P-4, -10 and -11 were observed in all the cultivars or strains of leek and kurrat in common. P-4 band was always accompanied by a colorless zone, which led easy identification of p-4 in the present experiment as well as in the previous ones[7,9)]. P-10 band was frequently stained dark among the isozyme bands. P-8 and -9 bands were observed in leek and garlic. The darkness of P-8 band varied with cultivars. Some of P-9 bands were also stained light, but many of them were stained darker than P-8 bands. P-9 and -10 bands were stained very dark in two garlic clones.

P-8 band was the particular band which was observed in fertile garlic clones, though a few of sterile garlic clones showed this band[9)]. In the present examination, this P-8 band was also observed in the fertile garlic clone (zymogram-h), while the sterile clone lacked it (zymogram-g). Moreover, P-8 band was observed in various leek cultivars of four zymograms, a, b, e, and f.

Table 2 shows cultivars in each zymogram and their origin. Zymogram-f included the largest

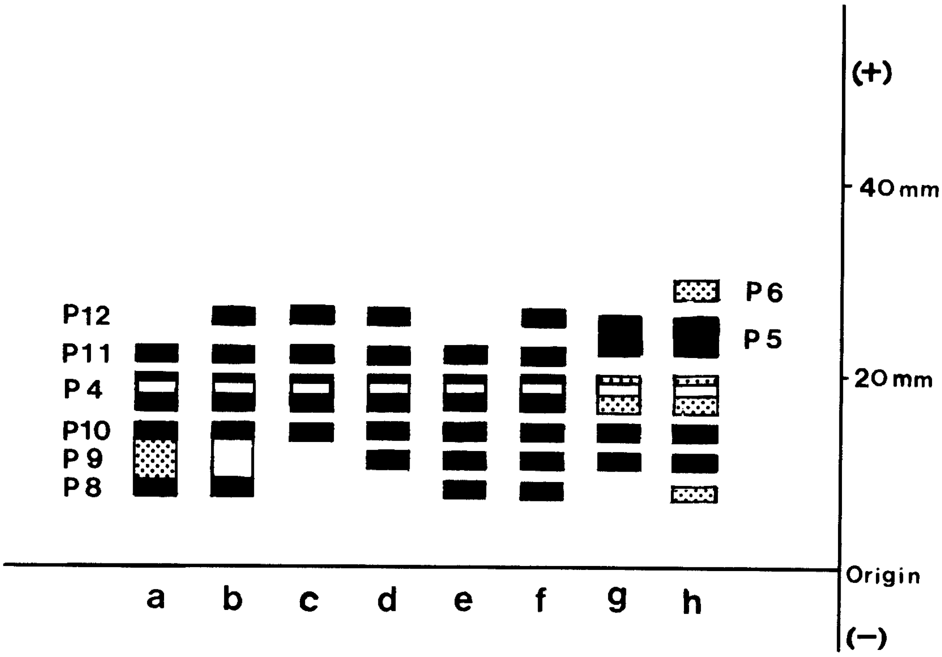

Fig. 1. Diagrammatic zymograms of the peroxidase isozymes in leek, kurrat and garlic. The leek and kurrat cultivars of the zymograms (a-f) are shown in Table 2. Zymogram-g was seen only in the sterile garlic, and zymogram-h was seen only in the fertile garlic.

Table 2. Classification of the cultivars, strains or clones of leek, kurrat and garlic according to zymogram patterns of peroxidase isozymes and the native countries

Native country	Cultivars of leek, kurrat and garlic							
	Zymograms							
	a	b	c	d	e	f	g	h
Netherlands	34	39,57 58,60 61		35,36,38, 40,45,48, 52,53,62	44,51	14,26,27, 37,41,49, 50,54,55, 56,59		
Sweden		28,30	33	29,32,46	31			
France	5	2	3,9		7,10 11	1,4,6, 8,13		
Switzerland						42		
Bulgaria						12,19		
Egypt	22		15,16* 17,18	24,43				
Iran			63					
Unknown	20							
Japan							Garlic 'Shanhai -wase'	
U.S.S.R.								Garlic 'No. 130'

* Kurrat is underlined.

number of leek cultivars among the present zymograms. This zymogram-f also included the largest number of isozyme bands among the zymograms shown in leek or kurrat. The other zymograms (a-e) lacked one or two bands of those in zymogram-f. Zymogram-a included four leek cultivars from Europe, Egypt and one unknown country. Zymogram-b included eight cultivars from three different European countries, and this zymogram showed dark P-8 band. In these two zymograms, the zone between P-8 and P-10 bands was stained light, but no band was identified there. Zymogram-c included four leek cultivars from Europe and Iran and also four kurrat strains from Egypt. As zymogram-c and -d are regarded as zymograms derived from zymogram-f, kurrat may also be regarded as one derived form of leek. Zymogram-d included the second largest number of leek cultivars among the zymograms, and those cultivars came from Europe and Egypt. The leek cultivars of zymogram-e and -f came from Europe including Bulgaria.

In conclusion, as mentioned above, it was found that P-8 band in question appeared in leek cultivars and that the zymograms of leek resembled those of garlic. It was also found that kurrat showed two zymograms which were thought to be derived from that of leek.

Discussion

In genus *Allium*, isozyme analyses have been made by several researchers for the identification of species or cultivars[11,12,19]. However, most of their materials used for analyses were seeds, and the intraspecific polymorphism of isozymes was studied only in a few species[11,12,22]. The present authors analyzed peroxidase isozymes in the leaves of various garlic clones previously[7,9,10]. In leek or kurrat, no isozyme analysis has ever been done to clarify the polymorphism of cultivars and to discuss the relationship between garlic and leek or kurrat.

In the present experiment, six isozyme bands were detected in leek and kurrat. Among those six bands, P-4, -10 and -11 appeared in all the materials of leek or kurrat. Probably, these three bands are essential ones in leek and kurrat.

Zymogram-f may be the most fundamental among those of leek and kurrat, because it showed the largest number of isozyme bands, including the largest number of leek cultivars. Accordingly, there is a possibility that the leek cultivars of zymogram-f may be more primitive than the other cultivars. It may reasonably be assumed that the rest, zymograms (a-e), lost one or two isozyme bands on the way from this original type of zymogram-f.

Garlic clone 'No. 130' is fertile, and this fertility seemed to be closely related with P-8 band[7,9]. This clone was presumed to be one of the most primitive garlic clones[7]. In the present examination, zymogram-f of leek and zymogram-h of garlic 'No. 130' showed four common isozyme bands (P-4, -8, -9, -10). Probably garlic and leek may have had a common ancestor, judging from these common isozyme bands.

Zymogram-e lacks P-12 band, and the cultivars of zymogram-e are much fewer than those of zymogram-f. It is probable that zymogram-e was derived from zymogram-f. There is also a probability that zymogram-d was derived from zymogram-f, and then zymogram-c was derived from zymogram-d. According to Tackholm and Drar[23], a difference between leek and kurrat may also be recognized in ancient texts from the Sumeric period. And yet, kurrat may rather belong to a group derived from the ancestor of the present leek even if it had been done in ancient times, because the kurrat strains showed only zymogram-c and -d. As only five strains of kurrat were used in the present examination, it is ncessary to collect and examine more materials of kurrat for making more detailed discussion on this relationship. Probably zymogram-b was derived from zymogram-f, and then zymogram-a may have been derived from zymogram-b. Consequently, there is a possibility that the cultivars of zymogram-a were derived from those of zymogram-f through -b.

A. ampeloprasum complex includes several species[1-4] besides *A. ampeloprasum* itself. Bothmer conducted broad researches concerning wild populations of this complex in Greece[2], in the Aegean area[3] and in Crete[4]. He found different populations of diploid to heptaploid in this complex, but he found only 4x, 5x, 6x and 7x in *A. ampeloprasum* itself. In his investigation, tetraploids and hexaploids were predominant in *A. ampeloprasum.* He thought that one diploid record by Renzoni[21] in Italy was probably due to misidentification[3]. Kollmann[15,16] also studied wild populations of *A. ampeloprasum* in Israel, and he found 4x, 5x and 6x populations in subsp. *ampeloprasum* and 2x, 3x and 4x populations in subsp. *truncatum.* In both subsepcies, the tetraploid was the most frequent. Diploids were rare. According to Kollmann[16], the diploids may have been replaced in arid areas by the vigorous tetra- and triploids, and hexaploids presumably originated from tetraploids, probably independently in various localities. Probably tetraploid is the most frequent or essential in both wild and cultivated *A. ampeloprasum.* Was there any possibility that in ancient times there was a relationship between garlic of diploid and leek of tetraploid ?

The mordern garden leek is not known in the wild state, but it shows a distinct affinity with the wild leek at the same ploidy level[17,18]. The idiogram drawn from the cultivated leek did not differ from that of the wild *A. ampeloprasum*[16]. Moreover, the pronounced proximal chiasma localization in bivalents in wild subsp. *ampeloprasum* was also observed in the cultivated leek[16]. Probably cultivated leek was derived from wild *A. ampeloprasum* of tetraploid. It is probable that leek originated in the eastern Mediterranean region or the Near East, where they have been cultivated for about 3000 or 4000 years[17,25].

Garlic probably originated in Central Asia[8]. However, the Caucasia and the Mediterranean area are considered to be the secondary centers of origin[25]. In the Caucasia, besides Central Asia, fertile garlic clones were discovered by the authors (unpublished). Moreover, garlic was already known in Egypt before 3000 B.C.[13] There is a possibility that the common ancestor of modern garlic and leek may have been distributed in the Near East including the Caucasia. And this may be the cause of the similarity in the zymograms between garlic and leek in the present analysis.

Kurrat is closely related to leek, and they are interfertile[14]. It is cultivated in the Nile region of Egypt[23]. Kadry and Kamel[14] hybridized these two to examine F_1 and thought that these two belonged to the same species, or two closely related ones in view of cytological studies. From their results and the present examination, it may reasonably be concluded that kurrat was derived from leek. For the detailed study, it is necessary to collect and examine more materials of both wild and cultivated leek from northern Africa, the eastern Mediterranean region and the Near East.

Summary

Peroxidase isozymes of the leaves in 59 cultivars or strains of leek and kurrat, *Allium ampeloprasum* L., were analyzed by means of horizontal thin layer polyacrylamide gel electrophoresis, and they were compared with those of strile and fertile garlic, *A. sativum* L. The gel was stained with 4-chloro-1-naphthol. In total, six and five isozyme bands were detected in leek and kurrat, respectively. In garlic, six bands were also detected, and four of them were common to those of leek. The fertile garlic clone showed one particular band which was absent in the sterile garlic clone. This band was also detected in leek cultivars. Six zymogram patterns were

observed in leek, and four of them showed this particular band. The zymograms of kurrat were supposed to be derived from that of leek. From the results obtained in the present isozyme analysis, it was concluded that garlic and leek may have a common ancestor and that kurrat may have been derived from leek.

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