DIVERSITY FOR ENZYMES, FLOWERING BEHAVIOUR AND PURPLE PLANT COLOUR OF PERENNIAL KALE (*BRASSICA OLERACEA* L. VAR. *RAMOSA* DC.) IN THE NETHERLANDS

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Abstract

Perennial kale has probably been domesticated and distributed by the Romans. Some relic populations are still being grown in various parts of western Europe (Ireland, Scotland, England, the Netherlands, Belgium, France, Portugal), in Ethiopia, in Brazil and Haiti up to the present.
Most accessions of perennial kale grown in gardens in the Dutch province Limburg have lost their flowering ability. Some of them flower occasionally, others every year. No explanation can be given for this inconstant behaviour.
This predominantly diploid material is quite uniform for the enzymes acid phosphatase (ACD), esterase (EST), glutamate oxaloacetate transaminase (GOT) and shikamata dehydrogenase (SKD). The same is true for presence of anthocyanins in the leaves. The 40 fully investigated accessions could be grouped into 8 phenotypes: 26 with phenotype 1 (at least 9 are tetraploid), three with phenotype 2, six with phenotype 3, and one for each of the phenotypes 4 to 8. Accessions with phenotypes 2 and 3 had the same phenotype for the four enzymes as phenotype 1, and so have most of the 13 accessions, only investigated for enzymes.

1. Introduction

The cultivation of perennial kale in a part of the Netherlands was described by Zeven et al. (1989). They reported that this disappearing leafy vegetable and forage crop is being propagated vegetatively as the crop rarely flowers, and that due to polyploidisation at an unknown time or unknown times several autotetraploid clones have evolved. Furthermore, these authors suggested that to all appearance, the crop could be one of the first domesticants of the wild kale and that owing to human selection for high number of leaves the strong branching habit was promoted and preserved. The latter was associated with the gradual suppression of the flowering capacity.

This kale could indeed be a very old domesticant as Pliny in 70 AD refers to Tritian kale, which at that time was propagated like perennial kale nowadays in The Netherlands (von Fischer-Benzon, 1894): vegetatively with branches, which were planted in a very special way. It is probable that already at that time this kale was not able to flower anymore. This may point to a very long selection process for high number of leaves and so for high number of branches, resulting into a non-flowering, probably strongly branching kale in 70 AD or earlier.

No information about the time of introduction of this crop into the Netherlands is available.

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Also nothing is known about its former distribution in the Netherlands. It was or still is also grown (Brok & Zeven, 1988) in central and southern parts of the Dutch province Limburg. This material is also grown in England (I. Crute, pers. comm. 1994) and in Ireland, where it is known as Hungru Gap (E.Ch. Nelson, pers. comm. 1995) and Cut-and-ComeAgain (D. Astley, pers. comm. 1994). in Scotland, France (Brest, Argenteuil) (G. Dirix, personal communication), Belgium, Germany, Portugal and in some parts of Brazil (probably imported from Portugal). This kale was probably also grown on Haiti (Messiaen, 1992). Further, Astley et al (1982) found perennial kale in Ethiopia, where it was found in home gardens. Some plants reached a height of 3 m. Some accessions were non-flowering, others did flower. Maybe this patchy distribution in Europe points to an early large scale distribution in Western Europe, and that the present day growing areas being relics of this former large area. As perennial kale may have been grown for centuries in its present area in the province Limburg and as it is propagated vegetatively, it was thought that this could have resulted in the presence of only one clone or a few clones. These few clones could be related, differing at one or few mutant loci only. Furthermore, when frost killing occurred, one could have obtained new cuttings from a neighbour. This would have resulted in the spread of one clone in a certain region and another in another region. So within an area the degree of genetic variation could be very low.

2. Material and Methods

Perennial kale plants have been collected since 1984. In total 66 accessions were collected of which 12 died for various reasons. They all were discovered in home gardens and all were propagated vegetatively. Some old gardeners remembered that both their parents and themselves had 'always grown' perennial kale. In most cases only one plant type is being grown in a garden. However, in one garden three types (nos 26, 27 and 28) were observed. Plants with different morphotypes probably have a different history. But plants looking identical may also have different origins. Diploid and tetraploid accessions look identical and cannot be separated by different sizes of the leaves (Zeven et al., 1989). No regional variation was observed. These 54 clones were used for investigating the variation of four enzymes, flowering behaviour and purple leaf pigmentation.

2.1. Electrophoresis

Leaf samples were taken from the 5th or 6th leaf on 23 to 26 May 1994. These samples were stored for 30 min at -20°C and pressed in 1% 2-mercapto-ethanol in an Eppendorf tube. Electrophoresis was carried out on precast polyacrylamide gradient 8-25% PhastGels (43x50xO.45mm) as described by Anon. (1992). The buffer system in the gels consists of 0.112M tris-acetate, pH 6.4. The native buffer strips used were supplied by Pharmacia and were made of 2% agarose IEF and contained 0.25M tris and 0.88M lalanine, pH 8.8. Samples were applied on the gel using the 1M1 combs (8 wells). The program for the running condition was established on the control unit from the PhastSystem as follows:

1) 400 V 10 mA 2.5 W 30 Vh (prerun)
2) 400 V 1 mA 2.5 W 10 Vh (sample)
3) 400 V 10 mA 2.5 W 268 Vh (separation)

The temperature was kept at 15°C. The migration time was about 75 min. As control the same samples were also run according to Suurs (1987). Four enzymes were assayed: glutamate oxaloacetate transaminase (Got;E.C.2.6.1.1), esterase (Est;E.C.3.1.1.2), shikimate dehydrogenase (Sdh;E.C.1.1.1.25) and acid phosphatase (Acp;E.C.3.1.3.2). The gels were

incubated in staining solutions prepared after Vallejos (1983) for 1 hour at 30°C in the dark.

2.2. Flowering

During the years 1987, 1988 and 1994, the accessions were observed for flowering. In the first two years, several accessions received various pre-treatments (see Table 2 for details).

3. Results

Table 1 includes the results on 54 clones for variation of four enzymes, flowering behaviour and leaf pigmentation.

3.1. Variation of enzymes

Fifty-one of the 53 accessions (one accession was not included), investigated for the four enzymes, had the same phenotype for EST. Accessions nos. 24 and 25 possessed other phenotypes. No variation was found for GOT and for ACP. For SDH, 51 accessions had the same phenotype. Only two accessions (acc. 29 and 66) had other phenotypes.

3.2. Flowering behaviour

The results of our observations show that flowering in perennial kale is poor and inconstant (Table 2). It is not only inconstant for number of inflorescences and number of flowers per inflorescence, but also over the years. Most of the accessions never flower, some do flower, but then often not each year. Also flowering per plant of one clone may be inconstant. For instance, in 1993 three cuttings from one plant of accession 09 were taken at random. In 1994, only one of them flowered. Of another accession (no. 24) two of the three cuttings flowered.

In 1988 new cuttings of acc. 22 were randomly divided into two sets of 6 plants each. One set remained in the greenhouse (treatment f), and the other set survived the winter in a frost-free cold-frame (treatment g). The g-plants flowered profusely, whereas the f-plants remained vegetative. The same was done with plants from accession 33, but yet the f-plants flowered, whereas the g-plants did not.

3.3. Purple leaf pigmentation

The plants were scored for presence of purple pigmentation in the leaves. It was observed that only for three out of the 54 accessions all three Plants were free of Purple Pigment. The 51

'purple' accessions often varied in the intensity of pigmentation. This was not only true for the accessions, but also for the three plants studied per accession.

4. Discussion

The results presented in Table 1 show that, based on the characters investigated, our collection of perennial kale is composed of 8 phenotypes. Twenty-six accessions (including 9 tetraploids) of the 40 fully investigated accessions have the same phenotype. Accessions 08, 16 20, 22, 33 and 48 have flowered in 1987, 1988 or 1994, and therefore belong to phenotype 3. This information was used to prepare Table 3.

It is not yet possible to draw conclusions on the variation for flowering, because the assessment period of the second group (with unknown chromosome number) was probably

too short. It is quite likely that in a year which is favourable for flowering some of these may also flower. However, the material is less uniform than would be expected on the consideration, that most accessions of this crop are propagated vegetatively for probably many years, if not centuries, and as exchange of material between growers may be assumed. Further research of variation of more enzymes would probably reveal more phenotypes. As the position of this crop within *Brassica oleracea* probably is unique, investigation of variation chloroplast, mitochondrial and nuclear DNA would be highly desirable.

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Table 1. Phenotypes of four enzymes, flowering behaviour and plant leaf pigmentation of 54 accessions of perennial kale.

Acc.	Enzymes				F	P94	Phenotype (incl. Table 3)
	EST	GOT	SDH	ACP			
Chromosome number known (Zeven et al., 1989)							
01	a	a	a	a	-	p	1*
03	a	a	a	a	-	p	1
05	a	a	a	a	-	g	2
06	a	a	a	a	-	p	1
07	a	a	a	a	-	p	1*
08	a	a	a	a	f	p	3
09	a	a	a	a	f	p	3
12	a	a	a	a	-	p	1
13	a	a	a	a	-	p	1
15	a	a	a	a	-	p	1
16	a	a	a	a	f	p	3
17	a	a	a	a	-	p	1
18	a	a	a	a	-	p	1*
19	a	a	a	a	-	p	1*
20	a	a	a	a	f	p	3
22	a	a	a	a	f	p	3
23	a	a	a	a	-	p	1
24	b	a	a	a	f	p	4*
25	c	a	a	a	f	p	5
26	a	a	a	a	-	p	1*
27	a	a	a	a	-	p	1
28	a	a	a	a	-	p	1*
29	a	a	b	a	f	p	6
32	a	a	a	a	-	p	1
33	a	a	a	a	f	p	3
34	a	a	a	a	-	p	1
35	a	a	a	a	-	p	1
36	a	a	a	a	-	p	1
37	a	a	a	a	-	p	1
38	a	a	a	a	-	g	2
39	a	a	a	a	-	p	1
40	a	a	a	a	-	p	1*
41	a	a	a	a	-	p	1
43	a	a	a	a	-	p	1*
44	a	a	a	a	-	g	2
45	a	a	a	a	-	p	1
46	a	a	a	a	-	p	1*
48	d	a	a	a	f	p	7
49	a	a	a	a	-	p	1
50	e	a	a	a	-	p	8
51	x	x	x	x	-	g	?
Chromosome number and flowering behaviour unknown, collected since 1989							
52	a	a	a	a	-	p	?
54	a	a	a	a	-	p	?
56	a	a	a	a	-	p	?
57	a	a	a	a	-	p	?
58	a	a	a	a	-	p	?
59	a	a	a	a	-	p	?
60	a	a	a	a	-	p	?
61	a	a	a	a	-	p	?
62	a	a	a	a	-	p	?
63	a	a	a	a	-	p	?
64	a	a	a	a	-	p	?
65	a	a	a	a	-	g	?
66	a	a	c	a	-	p	?

ACP = acid phosphatase, EST = esterase, GOT = glutamate oxaloacetate-transaminase, SDH = shikamate dehydrogenase.
a to e indicate phenotypes within enzymes, F = flowering in 1987, 1988 and/or 1994 (f = flowering, - = non-flowering), P94 = leaf pigmentation in 1994 (p=purple, g= green). x = plants not investigated. * = tetraploid accession.

Table 2. History of material and year of observation for flowering.

Acc.	Year								
	1987			1988					1994
	a	b	c	d	e	f	g	h	i
08	-	f	-	f	-	f	-	-	-
09	f	f	f	f	-	f	x	x	f
10	-	-	f	-	-	-	-	-	-
14	f	f	f	-	-	f	x	x	-
16	f	f	-	-	-	f	-	-	-
20	-	-	-	-	-	f	x	x	-
22	f	-	-	-	-	-	f	-	-
24	-	-	-	-	-	-	-	-	f
25	-	-	-	-	-	f	x	x	-
29	f	f	f	-	-	f	x	x	-
31	-	-	-	-	-	f	x	x	-
33	-	f	-	f	-	f	-	-	-
48	-	f	-	-	-	-	-	-	-

f = flowering, - = non-flowering, x = no plant(s) available.
Accessions which flowered at least once have been enlisted.

cutting	winter	spring
1987		
a: new	buckets, greenhouse	greenhouse
b: new	field	field
c: new	buckets, greenhouse	field
1988		
d: one year old	cold frame,	field
e: one year old	bucket, greenhouse	field
f: new	greenhouse	greenhouse
g: new	cold frame	field
h: new	bucket, greenhouse	field
1994		
i: new	cold frame	field and cold frame

Table 3. Description of each phenotype and total number of accessions.

Code of pheno-type	No. of accession Ploidy level			Total	Flowering	Leaf pigmentation	Enzyme	Nr of accessions
	2x	4x	?					
1	17	9	0	26	no	purple	Table 1	Table 1
2	3	0	0	3	no	green	Table 1	5, 16, 38, 44, 65
3	6	0	0	6	yes	purple	Table 1	8, 9, 20, 22, 33, 48
4	0	1	0	1	yes	purple	ESTb	24
5	1	0	0	1	no	purple	ESTc	25
6	1	0	0	1	no	purple	SDPb	29
7	1	0	0	1	no	purple	ESTd	48
8	1	0	0	1	no	purple	ESTe	50
?	0	0	12	12	no	purple	Table 1	Table 1
?	0	0	2	2	no	green	Table 1	51, 65