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Phytochemical and morphological characterization of seventy-one cultivars and selections of culinary rhubarb (*Rheum* spp.)

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SUMMARY
Measurements of soluble and total oxalate, malate, anthocyanins, total sugars, dry matter as well as several petiole and leaf characters were taken for 71 genotypes of culinary rhubarbs (*Rheum* spp.). The organic acids were simultaneously analysed by ion moderated partitioning chromatography, an HPLC technique that employs multiple mechanisms for separation. Sample run time was reduced to 11 min by allowing overlapping chromatograms. The grand mean (range of genotype means) of water soluble oxalate was 3.13% (1.56–6.03%), of total oxalate 5.88% (3.21–9.17%), of malate 20.98% (12.16–29.24%) all of dry matter. The range of anthocyanins in the petiole juice was 0–154 mg/kg of fresh weight. Negative correlations were found between the anthocyanin content and some growth characteristics.

Rhubarb (*Rheum* spp.) is a minor vegetable crop adapted to a cool temperate climate. The early yield and the distinctive aroma in the juicy petioles have contributed to its popularity, especially in home gardens. Rhubarb is also well suited to ecological growing as a commercial crop, although today it is of limited economical importance. However the interest for rhubarb as a cheap filler for industrial production of marmalade, jam and syrup is slightly increasing in the Nordic countries (Rumpunen, 1996) where rhubarb is cultivated on approx. 60 ha. The cultivated area in United States and Canada is approx. 400 ha (Foust and Marshall, 1991). The potential yield is large, 40–70 tons per ha (Thuesen, 1982), still approx. 20 tons saleable petioles is an average in less intensively managed commercial production where the plants are harvested only once or twice during spring and early summer.

The content of oxalate in the petioles is a major drawback of this crop and has been the subject of several recent papers (Libert and Creed, 1985; Libert, 1986, 1987ab). Genetic and non-genetic sources of variation were studied. Most important for the accumulation of oxalate in the petioles was found to be the genotype, the petiole age and the developmental stage of the plant. This knowledge is important not only when sampling and evaluating genetic resources but also when harvesting petioles for consumption. Furthermore, the relative oxalate absorption is independent of oxalate concentration in the diet (Libert, 1986). A reduced oxalate content in the diet therefore results in a reduced total oxalate intake.

This information has contributed to an increasing public awareness of cultivar differences and contributed to a demand for low oxalate rhubarbs in Sweden and Denmark. Cultivars of interest are virus-indexed, if necessary freed from viruses and micropropagated (Rumpunen, 1990). Since 1991, 150,000 plants of cv. Elmsblitz, a German low-oxalate cultivar recommended for home gardens, have been propagated at the Swedish Elite Plant Station and sold to nurseries and garden centres. However, as cv. Elmsblitz produces lots of flower stalks which are labour intensive to remove, the variety is not recommended for commercial production.

The objective of this study was to investigate the genetic variation and to characterize a large rhubarb collection for important horticultural traits. We also wanted to select low-oxalate genotypes for further trials and future breeding aimed at cultivars for fresh market and industry. Relationships between morphological and phytochemical data were investigated. Special interest was paid to the anthocyanin content since petiole colour influences consumer preferences and determines the use in processing. Previously, only some information has been reported on anthocyanin content of rhubarb (Hetmanski and Nybom, 1968; Fuleki, 1969).

A reliable and rapid method was needed for the simultaneous analysis of the main organic acids in rhubarb, oxalic and malic acid. Many HPLC methods have been suggested for the determination of organic acids in general and oxalic acid in particular. Especially, oxalic acid analysis can cause difficulties because of the strong chelating capacity of oxalate and its low retention in a reverse-phase system. The main direct HPLC systems proposed for the analysis of oxalic acid is based either on an ion-coupling system in reverse-phase (Libert, 1981), an ion-moderated partition system (Bushway *et al.*, 1984; Picha, 1985; Holloway *et al.*, 1989) or an anion exchange column (Huang and Tanudjaja, 1992). After careful screening we chose to adopt the Aminex column system (ion-moderated partition chromatography) for analysis of rhubarb

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extract because of its simplicity, stability and successful retention of oxalate.

Since environmental factors influence the phytochemical content, concordance between the present data and those previously published by Libert and Creed (1985) was also investigated.

MATERIALS AND METHODS

Plant material

The genotypes studied have been collected since the nineteen-fifties and originate from many plant breeders, nurseries and experimental stations in Denmark and abroad (Thuesen, 1982). Until 1972 the cultivars were held at Spangsbjerg Research Station near Esbjerg, Denmark but afterwards were moved and reestablished at the Danish Institute of Agricultural Sciences, Research Centre Aarslev. The collection is part of the Nordic rhubarb collections at the Nordic Gene Bank. To keep the collection healthy and suited for further investigations the plants are dug up and re-established at a new place/experimental field approximately each tenth year. Seventy-one genotypes were investigated (Table I). Some of the cultivars have previously been evaluated for yield characteristics (Christiansen and Henriksen, 1944; Thuesen, 1982). Nineteen of the genotypes are advanced selections produced by crossings and may be considered as genetic stocks.

This experiment was planted in April 1988 with a row distance of 150 cm and a plant distance of 75 cm. There are no guard plants between the cultivars but an extra plant distance of 75 cm. Each plot consists of two plants without replication. The soil is a sandy loam with a medium content of plant nutrients. Each spring the rhubard plants are fertilized with 3–400 kg NPK 14–4–17 per ha. No pesticides have been applied.

Sampling for phytochemical analysis

Three fully developed petioles were harvested on 20 June from each of two plants of each genotype, put in plastic bags and transported to Sweden (ca. 48 h). Upon arrival the petioles were washed, the middle part of the petioles cut into ca. 0.5 cm sections and frozen. All phytochemical analyses were conducted on frozen samples.

Chemicals

Standards were prepared with l-malic acid (M = 134.09 EINECS 2026015 Purum, Fluka Chemie AG, Buchs) and oxalic acid sodium salt (M = 134.00 EINECS 2005503 Biochemika MicroSelect, Fluka Chemie AG, Buchs). Hydrochloric acid (30%, M = 36.46, Suprapur, Merck, Darmstadt) was used to resolve oxalic crystals in plant tissue. Sulphuric acid (96%, M = 98.08, 1l = 1.84 kg Suprapur, Merck, Darmstadt) was diluted and used as mobile phase for HPLC analysis.

Chemical analysis

Duplicate sub-samples of ca. 10 g for total soluble solids, ca. 5 g for dry-matter determination and ca. 20 g for anthocyanins were taken from frozen samples.

Sub-samples for soluble oxalate and malate were homogenized in 50 ml H_2O for 2 min, diluted to 200 ml and put in a shaker overnight at room temperature. The crude extract was filtered through a Whatman paper filter no. 00A, 0.5 ml extracts were then diluted with 0.5 ml HCl (to prevent precipitation during storage) and 4 ml H_2O. The diluted extract was then frozen.

Sub-samples for total oxalate and malate were homogenized in 50 ml 2 M HCl for 2 min, incubated at 100°C for 20 min, diluted to 200 ml and stored overnight at room temperature. The crude extract was filtered through a Whatman paper filter no. 00A, a 0.5 ml extract was then diluted with 4.5 ml H_2O and frozen.

A selective sample filtration and clean-up step was included before injection. An Isolute SPE MF C18 column (500 mg per 10 ml) was cleaned with 5 ml of methanol, washed and pre-equilibrated with 5 ml of water. Three ml of thawed sample was loaded, the column connected to vacuum and the first ml of filtered sample discarded. Then a ca. 1.5 ml sample was collected in an HPLC vial. Each Isolute SPE column was reused five times.

The HPLC system used was delivered by Shimadzu and consisted of an auto-injected (SIL 10A) a communication bus module (CBM 10A) two pumps (LC 10AD), a variable-wavelength UV-detector (SPD 10A) and an LC workstation for control and data processing (Class LC10 on a Scandic PC 486). An 300 × 7.8 mm Aminex HPX-87H column and a 30 × 4.6 mm guard column (Cation H cartridge) were purchased from Bio-Rad. HPLC column temperature was controlled by heating the column in an external water bath. Operating conditions were: flow rate, 0.6 ml min^{-1}; mobile phase, 0.0125 M sulphuric acid; column temperature, 30°C; wavelength for detection, 210 nm.

The injected volume was always 20 μl. External standards were injected before each set of ten samples. Four calibration levels were used and the curve fitted by the least squares method. Quantitation by area was performed automatically by the LC 10 workstation following integration of the chromatograms. The automatically quantitated results was checked by visual inspection of the resulting chromatogram. Malate and oxalate peaks were identified by comparing retention times with standards.

Dry matter was determined by weighing before and after drying at 105°C for 24 h.

Soluble solids were determined in raw juice, obtained by gently compressing ca. 10 g of thawed rhubarb slices, and analysed by a digital refractometer (ATAGO, PR-100 Palette).

For the determination of anthocyanin content a modified pH differential method was used. Buffers of pH 1.0 and pH 4.5 were prepared according to Wrolstad (1976). 20 g of frozen rhubarb petioles were thoroughly homogenized with 40 ml of a 47.5% (v/v) ethanol-water solution for 1 min. After filtration through Whatman paper filter no. 3, the solution was stored in darkness overnight at room temperature. The following day, 0.5 ml samples were diluted 1:1 with the appropriate buffer and the samples allowed to equilibrate during two hours. The absorbances were measured at 700 nm and 518 nm. Finally the difference in absorbance was calculated as follows: Absorbance = (A_{518} pH 1.0-A_{700} pH 1.0) – (A_{518} pH 4.5 – A_{700} pH 4.5). This absorbance

Table I
Phytochemical content of 71 rhubarb genotypes

No.	Variety	Sol. oxalate (%dm)	Oxalate (%dm)	Malate (%dm)	Dry matter (%)	Sol. solids (Brix)	Anthocyanins (mg/kgfw)	Logant*
1	A Côte Rouge	4.98	7.41	19.23	7.70	5.2	5	1.59
2	Amerikansk Kæmpe	2.00	6.29	25.64	6.76	4.7	14	2.55
3	Appleton's Forcing	3.10	6.56	19.75	6.87	4.3	18	2.91
4	Appleton's Red	3.28	5.14	23.71	6.43	4.0	58	4.05
5	Appleton's Triumph	3.54	3.88	24.13	5.98	3.9	10	2.12
6	Behrens Rabarber	1.56	4.57	23.23	6.76	4.6	12	2.24
7	Brown's Red	3.22	5.35	17.58	6.48	5.2	2	0.66
8	Buck's Early Red	3.48	8.80	24.74	6.76	4.2	19	2.81
9	Canada Red	4.03	5.56	18.59	6.23	4.0	60	4.09
10	Collis Seedling	4.24	6.92	29.24	4.61	3.6	5	1.59
11	Crimson Queen	2.64	8.10	19.86	6.94	3.7	23	3.04
12	Dancer's Early Red	1.56	5.97	12.16	7.69	4.5	4	1.13
13	Daw's Challenge	2.25	6.63	19.85	6.67	4.4	25	2.92
14	Daw's Champion	2.19	5.70	21.90	7.64	5.4	7	1.85
15	Donkere Bloedrode	2.55	5.27	26.51	6.29	4.6	11	2.41
16	Early Albert	3.65	6.37	22.77	6.26	4.3	9	2.20
17	Early Red Seedling	4.23	6.28	18.01	6.03	4.0	17	2.84
18	Early Red (Wisley)	2.79	5.95	23.10	5.48	3.8	21	3.03
19	Early Sunrise	3.25	5.46	20.04	7.11	4.6	29	3.34
20	Edina	2.47	4.61	23.53	5.81	4.2	10	2.25
21	Elmsfeuer	3.36	6.18	19.94	7.76	5.1	86	4.35
22	Fenton's Special	4.53	5.94	25.47	5.50	3.4	37	3.57
23	Frigo (SP.45x)	4.33	6.62	18.10	6.46	3.8	62	4.11
24	Glaskin's Perpetual	6.03	8.07	16.95	6.92	4.2	46	3.82
25	Hawkes Champagne	2.57	5.40	20.94	5.82	4.0	16	2.77
26	Hobday's Gigant	1.73	3.32	17.09	7.22	3.7	0	0.00
27	Holsteiner Blut	4.80	6.10	18.33	5.78	3.4	23	3.09
28	Laxton's nr. 1	3.71	6.47	19.69	6.22	4.5	40	3.69
29	Linnaeus	2.60	4.62	25.56	6.29	4.7	4	1.27
30	Marshall's Early Red	2.96	6.70	21.18	7.86	3.9	123	4.79
31	McDonald	2.39	4.39	15.26	7.67	4.3	90	4.50
32	Mitchell's Early Albert	2.87	5.69	21.56	6.58	4.3	6	1.48
33	Paragon	3.11	4.53	25.20	5.50	4.6	15	2.71
34	Prince Albert	2.39	4.87	24.22	6.45	4.6	36	3.56
35	Red Delikatesse	2.97	6.72	19.90	6.50	4.2	21	3.03
36	Reed's Early Superb	2.96	3.77	21.58	5.97	4.1	28	3.35
37	Riverside Gigant	4.12	5.84	22.14	6.03	4.0	11	2.37
38	Rosara (SP. nr. 123)	4.23	6.54	26.60	5.56	3.8	22	3.11
39	Rosenhagen	1.64	5.21	22.29	7.61	5.3	16	2.68
40	Ruby	3.25	6.89	14.34	8.19	4.3	42	3.72
41	R. Var. ex Sherburn Park. Glos.	3.10	5.40	16.68	7.14	4.5	26	3.22
42	Seedling nr. 1 (Merton Foremost)	3.71	3.76	23.47	4.90	3.8	3	0.95
43	Seedling nr. 3	3.47	4.99	24.84	5.52	3.8	14	2.41
44	Seedling nr. 6	1.82	3.57	19.57	6.91	4.2	23	3.14
45	Seedling nr. 7	3.77	6.51	16.09	6.42	3.6	3	1.79
46	Seedling nr. 8	2.91	4.47	19.64	4.97	3.9	18	2.89
47	Seedling nr. 9	2.05	4.31	21.19	5.95	4.0	13	2.55
48	Seedling nr. 11	2.42	6.75	24.72	5.53	3.6	6	1.77
49	Seedling nr. 12	3.24	5.81	14.42	7.47	5.2	7	1.84
50	Seedling Piggott	3.12	8.04	16.54	8.70	4.7	154	4.96
51	Spangsbjerg nr. 2x	1.89	3.67	23.07	7.58	4.8	13	2.59
52	Spangsbjerg nr. 35x	2.22	4.15	22.06	7.13	3.8	27	3.26
53	Spangsbjerg nr. 53x	3.08	7.70	23.96	7.12	4.6	50	3.90
54	Spangsbjerg nr. 140	1.81	3.21	26.72	6.50	4.3	18	2.87
55	Spangsbjerg nr. 242	1.66	6.67	18.31	7.01	5.2	4	1.23
56	Spangsbjerg nr. I9	5.60	7.86	21.12	6.13	3.9	26	3.26
57	Spangsbjerg nr. I16	5.04	7.37	15.24	8.77	4.9	44	3.77
58	Spangsbjerg nr. I19	3.29	4.38	21.60	6.45	4.2	26	3.26
59	Spangsbjerg nr. I22	1.76	5.63	14.67	9.37	5.8	24	3.14
60	Spangsbjerg nr. III5	2.51	4.41	21.04	7.24	4.1	114	4.70
61	Stott's Monarch	3.23	7.47	26.30	5.34	3.9	3	1.00
62	Strawberry	3.46	5.31	25.45	5.77	4.1	11	2.13
63	Sunrise	2.81	6.67	16.38	6.49	4.1	34	3.52
64	The Sutton	3.04	6.10	25.15	6.18	3.9	27	3.24
65	Timperley Early	2.81	5.68	24.18	6.52	4.8	10	2.30
66	Tingley Cherry	3.08	6.67	20.69	8.04	5.1	23	3.10
67	Tobolsk	4.13	6.99	19.32	5.85	3.8	2	1.39
68	Valentine	3.16	6.99	19.83	6.23	3.5	39	3.66
69	Victoria	2.05	6.02	19.15	7.42	4.1	19	2.91
70	Vinrabarber (Svendborg)	3.41	9.17	19.63	6.95	4.7	126	4.83
71	Vroege Engelse	4.67	7.22	18.51	5.93	3.4	43	3.75
Grand mean		3.13	5.88	20.98	6.62	4.3		2.83
Minimum		1.56	3.21	12.16	4.61	3.4		0.00
Maximum		6.03	9.17	29.24	9.37	5.8		4.96
Coefficient of variation		31.32	22.66	17.10	13.98	12.5		37.08
LSD (0.05)		0.60	2.40	3.67	1.35	1.0		0.91

*Logant = the negative log-transformed value of the anthocyanin content

is proportional to the anthocyanin content (Wrolstad, 1976). The corresponding approximate amount was calculated assuming that cyanidin-3-glucoside and cyanidin-3-rutinosid were present in equal amounts (Fuleki, 1969) with an average MW of 520 and a molar absorbance of 29,200.

Morphological characterization

The morphological characterization of the rhubarb genotypes was based on the UPOV guide-lines for the evaluation of distinctness, homogeneity and stability (Anon., 1978). In addition a few characteristics important to agronomy were included. Descriptors selected for evaluation were: leaf blade (size; colour; shape of apex; anthocyanin colouration of main veins), petiole (attitude; length; width; shape of cross-section; flesh colour; number of ribs at back; superimposed colour of epidermis at base, at midpoint and 75 mm below leaf blade) budburst, total number of petioles, number of flower stalks and time of flowering.

Statistical analysis

Statistical calculations were carried out with the software SYSTAT for Macintosh, Version 5.2 (SYSTAT, Inc.). Since variation in anthocyanin content was strongly dependent on the mean, log-transformed values were used. The variation of oxalic acid content (soluble and total) were only slightly dependent on the mean and were therefore not transformed.

Relationships between characters were investigated with Pearson and Spearman rank correlation procedures using Bonferroni-adjusted probabilities.

RESULTS AND DISCUSSION

The HPLC method developed proved to be very efficient and reliable. A typical chromatogram obtained is shown in Figure 1. The Aminex-column was stable throughout the investigation without noticeable loss in separation or sensitivity. The sample clean-up procedure markedly improved the resolution of the oxalate peak from the front peak. A complete run was performed within 20 min. By allowing overlapping chromatograms this time could be shortened to 11 min, which is very convenient when utilizing the method for routine analysis of breeding populations. In the same run both oxalate and malate are quantified.

The grand mean, minimum, maximum, coefficient of variation, LSD as well as genotype mean for phytochemical contents of the genotypes investigated are shown in Table I. The grand mean (and genotype mean range) for content of water soluble oxalate was 3.13% (1.56–6.03%), of total oxalate 5.88% (3.21–9.17%), and of malate 20.98% (12.16–29.24%), all on dry matter. Both the average and the range of oxalate and malate is in close agreement with previously published results (Libert and Creed, 1985). However, comparison of rank correlation coefficients for oxalate and malate of the 36 varieties that were included in both investigations, resulted in a strong correlation (0.71, $P<0.001$) only for malate content. Lack of a significant correlation for oxalate content probably reflects the strong influence of environmental factors on oxalate synthesis and accumulation (Libert, 1986). Varieties with a very low content of soluble oxalate are 'Behrens Rabarber', 'Dancer's Early Red', 'Rosenhagen' and 'Hobday's Gigant'. Varieties with a very high content of soluble oxalate are 'Glaskin's Perpetual', 'Spangsbjerg nr. I9', 'Spangsbjerg nr. I16' and 'A Côte Rouge'.

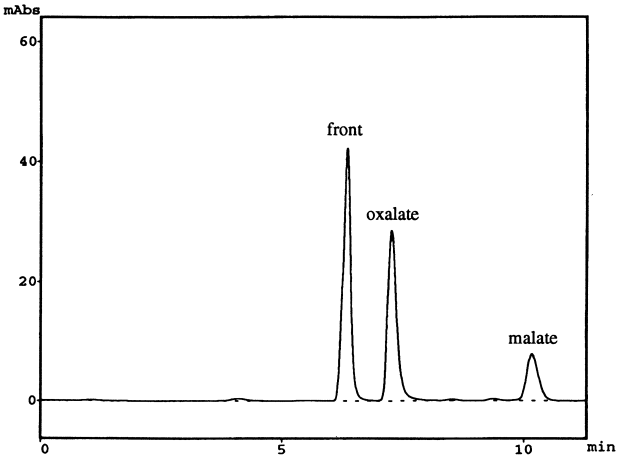

FIG. 1
Typical HPLC-chromatogram of a rhubarb sample. Selective sample filtration improves the separation of oxalate from unretained substances. Column: Aminex 87H and guardcolumn. Detection: UV.

Content of soluble oxalate correlated with total content of oxalate but to a smaller extent than might be expected (0.48, $P<0.001$). No significant correlations were found between the contents of organic acids and the morphological characteristics investigated in contrasts to results reported by Libert and Creed (1985). One reason may be that we scored the morphology into rather few classes which makes it difficult to use the characters in correlation tests.

Content of anthocyanins ranges from 0 to 154 mg kg^{-1} fresh weight (Table I). For comparing different varieties logtransformed values should be used since the distribution is skewed. A completely green cultivar

TABLE II
*Spearman rank correlation coefficients for anthocyanin content, petiole colour and some selected morphological characters. (Bonferroni probabilities, n.s. = non-significant, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$)*

	Anthocyanins		Petiole colour					
			base		middle		top	
Petiole colour, base	0.78	***	—		—		—	
Petiole colour, middle	0.76	***	0.94	***	—		—	
Petiole colour, top	0.66	***	0.82	***	0.90	***	—	
Petiole colour, flesh	0.60	***	0.66	***	0.70	***	0.66	***
Petiole width	−0.62	***	−0.41	*	−0.39	*	−0.31	n.s.
Leaf vein colour	0.52	***	0.58	***	0.72	***	0.81	***
Leaf size	−0.46	**	−0.34	n.s.	−0.29	n.s.	−0.26	n.s.

Table III
Classified data of selected morphological and physiological rhubarb characteristics important to agronomy and the market

		Flower	Petiole		Petiole colour				Petiole		
	Na Variety	stalks[1]	length[2]	width[3]	base[4]	middle[5]	top[6]	flesh[7]	yield[8]	breaking[9]	growth[10]
1	A Côte Rouge	3	4	3	3	2	1	2	7	1	8
2	Amerikansk Kæmpe	7	7	5	3	2	1	2	5	1	4
3	Appleton's Forcing	5	7	7	5	4	3	3	7	2	5
4	Appleton's Red	3	6	6	7	6	5	3	7	2	9
5	Appleton's Triumph	7	4	7	4	3	2	2	5	1	8
6	Behrens Rabarber	3	7	8	5	4	4	3	3	1	9
7	Brown's Red	7	9	7	4	3	2	2	5	3	7
8	Buck's Early Red	9	5	5	4	4	3	3	7	2	6
9	Canada Red	3	3	3	7	6	5	4	7	1	9
10	Collis Seedling	5	8	9	4	3	3	3	7	1	8
11	Crimson Queen	7	5	5	5	4	4	2	5	1	6
12	Dancer's Early Red	9	6	5	4	3	2	2	5	2	7
13	Daw's Challenge	5	6	8	7	6	6	3	7	2	6
14	Daw's Champion	5	8	8	5	4	4	2	7	1	5
15	Donkere Bloedrode	5	5	5	7	5	4	3	7	1	7
16	Early Albert	5	5	6	5	4	3	2	7	6	4
17	Early Red Seedling	5	4	5	5	4	3	2	7	5	4
18	Early Red (Wisley)	3	7	4	4	3	3	2	7	2	6
19	Early Sunrise	5	9	6	7	6	5	4	7	1	8
20	Edina	3	6	6	6	5	5	3	7	1	3
21	Elmsfeuer	2	6	5	7	6	5	4	5	2	7
22	Fenton's Special	3	4	4	6	5	5	3	7	3	7
23	Frigo (SP.45x)	3	9	5	6	5	4	4	7	3	7
24	Glaskin's Perpetual	5	2	2	7	4	3	2	5	7	7
25	Hawkes Champagne	3	6	6	5	4	4	3	5	1	3
26	Hobday's Gigant	3	9	9	3	3	3	2	5	1	6
27	Holsteiner Blut	3	8	7	6	5	5	4	5	2	9
28	Laxton's nr. 1	5	4	4	6	5	4	2	7	1	6
29	Linnaues	7	6	6	3	2	2	2	5	1	5
30	Marshall's Early Red	2	6	3	7	6	5	4	7	3	8
31	McDonald	5	4	3	7	6	5	3	5	1	6
32	Mitchell's Early Albert	5	5	6	5	4	3	2	7	7	4
33	Paragon	1	–	–	–	–	–	–	5	–	7
34	Prince Albert	7	7	7	7	5	3	3	7	2	5
35	Red Delikatesse	3	8	4	6	5	5	3	7	4	5
36	Reed's Early Superb	3	7	6	7	5	3	3	7	1	6
37	Riverside Gigant	7	8	6	5	4	4	2	7	1	4
38	Rosara (SP. nr. I23)	5	7	6	5	4	4	2	7	2	4
39	Rosenhagen	7	4	6	4	3	3	3	7	1	6
40	Ruby	5	7	4	6	5	5	3	7	3	7
41	R. Var. ex Sherburn Park, Glos	9	3	4	4	3	3	2	5	2	7
42	Seedling nr. 1 (Merton Foremost)	1	8	7	3	2	1	2	7	1	5
43	Seedling nr. 3	5	7	9	4	3	3	2	7	3	6
44	Seedling nr. 6	1	9	6	6	5	5	4	7	1	7
45	Seedling nr. 7	9	7	8	4	3	2	2	7	2	6
46	Seedling nr. 8	3	6	6	6	5	5	3	7	1	6
47	Seedling nr. 9	1	8	8	5	4	4	3	5	3	8
48	Seedling nr. 11	5	8	5	4	4	3	2	7	4	6
49	Seedling nr. 12	9	7	6	5	4	3	3	7	2	6
50	Seedling Piggott	2	3	3	7	6	5	3	5	1	7
51	Spangsbjerg nr. 2x	9	8	6	5	4	3	2	5	1	5
52	Spangsbjerg nr. 35x	5	7	6	5	4	3	3	5	2	7
53	Spangsbjerg nr. 53x	5	4	4	5	4	3	3	3	1	6
54	Spangsberg nr. 140	2	6	6	4	4	3	2	7	4	6
55	Spangsbjerg nr. 242	5	7	7	4	3	2	2	5	3	8
56	Spangsbjerg nr. I9	3	5	4	6	4	3	2	7	3	7
57	Spangsbjerg nr. I16	7	5	3	7	6	6	3	7	5	4
58	Spangsbjerg nr. I19	3	8	5	5	4	3	3	5	1	6
59	Spangsbjerg nr. I22	2	7	4	5	4	3	2	3	3	5
60	Spangsbjerg nr. III5	5	4	4	6	5	4	3	7	2	7
61	Stott's Monarch	9	9	8	3	3	2	2	5	1	9
62	Strawberry	7	6	6	3	3	2	2	5	2	5
63	Sunrise	2	6	4	7	6	5	3	7	2	6
64	The Sutton	3	7	4	6	5	4	2	7	2	5
65	Timperley Early	3	8	7	4	4	3	2	7	3	3
66	Tingley Cherry	7	8	7	5	4	3	3	7	3	7
67	Tobolsk	3	9	9	4	3	2	2	7	3	3
68	Valentine	1	8	5	7	6	5	4	7	1	8
69	Victoria	5	9	5	4	3	2	3	7	3	4
70	Vinrabarber (Svendborg)	2	9	4	7	6	5	4	7	1	8
71	Vroege Engelse	2	9	7	6	5	4	3	7	4	8

– = missing value.

Characteristics:	Scale:
[1]Inflorescence, number of stalks	absent (1), few (3), medium (5), many (7), very many (9)
[2]Petiole length	short (3), medium (5), long (7), very long (9)
[3]Petiole width	thin (3), medium (5), thick (7), very thick (9)
[4]Petiole superimposed colour of skin at base	no (1), weak (3), medium (5), strong (7)
[5]Petiole superimposed colour of skin at mid-point	no (1), weak (3), medium (5), strong (7)
[6]Petiole superimposed colour of skin at top	no (1), weak (3), medium (5), strong (7)
[7]Petiole flesh colour	white (1), green (2), pink (3), red (4)
[8]Petiole yield	low (3), medium (5), high (7)
[9]Petiole tendency to break	no (1), weak (3), medium (5), strong (7)
[10]Petiole time of first growth (budburst)	early (3), medium (5), late (7), very late (9)

with no detectable anthocyanins is 'Hobday's Gigant'. Comparatively large amounts of anthocyanins can be found in 'Seedling Piggot', 'Vinrabarber' (Svendborg), 'Marshall's Early Red' and 'Spangsbjerg nr. III5.'

Correlations between anthocyanin content and selected morphological characteristics are shown in Table II. Not surprisingly, anthocyanin content is well correlated with the colour of petiole-base, -middle and -top. Strong or moderate negative correlations were found between coloration and some growth characteristics (leaf size and petiole width). This is in agreement with common experience that varieties with red petioles have comparatively reduced yield and thinner petioles (Thuesen, 1982). It therefore seems difficult to combine red colour and high productivity in a variety.

Classified data of selected morphological and physiological rhubarb characteristics important to agronomy and market are shown in Table III. Complete morphological data for each cultivar are available on request to the Nordic Gene Bank (NGB), Alnarp, Sweden.

Organoleptic qualities and suitability for processing must also be considered in selection and plant breeding. In a preliminary investigation we noticed considerable variation among genotypes for important traits like taste of petioles and stability of products as tendency to ageing, colour, aroma and consistency. Some selected genotypes have been propagated and are now further evaluated in field trials.

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REFERENCES

Anon. (1978). Guide-lines for the conduct of tests for distinctness, homogeneity and stability, Rhubarb (*Rheum rhabarbarum* L.). UPOV.

Bushway, R. J., Bureau, J. L. and McGann, D. F. (1984). Determinations of organic acids in potatoes by high performance liquid chromatography. *Journal of Food Science*, **49,** 75–81.

Christiansen, E. and Henriksen, A. (1944). Experiments with rhubarb varieties. 1933–43. *Tidsskrift for Planteavl*, **49,** 234–51 (Danish).

Foust, C. M. and Marshall, D. E. (1991). Culinary rhubarb production in North America: History and recent statistics. *HortScience*, **26,** 1360–3.

Fuleki, T. (1969). The anthocyanins of strawberry, rhubarb, radish and onion. *Journal of Food Science*, **34,** 365–9.

Hetmanski, W. and Nybom, N. (1968). The anthocyanins of edible rhubarb. *Fruchtsaft-Industrie ver Confructa*, **12,** 256–60.

Holloway, W. D., Argall, M. E., Jealous, W. T., Lee, J. A. and Bradbury, J. H. (1989). Organic acids and calcium oxalate in tropical root crops. *Journal of Agriculture and Food Chemistry*, **37,** 337–41.

Huang, A. S. and Tanudjaja, L. S. (1992). Application of anion-exchange high-performance liquid chromatography in determining oxalates in taro (*Colocasia esculenta*) corms. *Journal of Agriculture and Food Chemistry*, **40,** 2123–6.

Libert, B. (1981). Rapid determination of oxalic acid by reversed-phase high-performance liquid chromatography. *Journal of Chromatography*, **210,** 540–3.

Libert, B. (1986). *Breeding a low-oxalate rhubarb: a genetic approach to improve the nutritional quality of oxalate-accumulating crop plants*. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Libert, B. (1987a). Genotypic and non-genetic variation of oxalate and malate content in rhubarb (*Rheum* spp. L.). *Journal of Horticultural Science*, **62,** 513–21.

Libert, B. (1987b). Breeding a low-oxalate rhubarb (*Rhubarb* spp. L.). *Journal of Horticultural Science*, **62,** 523–9.

Libert, B. and Creed, C. (1985). Oxalate content of seventy-eight rhubarb cultivars and its relation to some other characters. *Journal of Horticultural Science*, **60,** 257–61.

Picha, D. H. (1985). Organic acid determination in sweet potatoes by HPLC. *Journal of Agriculture and Food Chemistry*, **33,** 743–5.

Rumpunen, K. (1990). Micropropagation of rhubarb. Report 1988–1989. *Balsgård – Department of Horticultural Plant Breeding, Swedish University of Agricultural Sciences*, 126–33 (Swedish).

Rumpunen, K. (1996). Development of selection methods and strategies for breeding of culinary rhubarb. *Report 1992–94. Balsgård - Department of Horticultural Plant Breeding, Swedish University of Agricultural Sciences*, 90–4 (Swedish).

Thuesen, A. (1982). Evaluation of rhubarb varieties 1977–79. *Tidsskrift for Planteavl*, **86,** 177–84 (Danish).

Wrolstad, R. E. (1976). *Color and pigment analyses in fruit products*. Station Bulletin 624, Agricultural Experiment Station, Oregon State University, Corvallis.

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