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Identification of *Allium cepa* L. cultivars by means of statistical analysis of C-banded chromosomes

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Summary

The C-banded karyotypes of six *Allium cepa* cultivars, including five dry onions and one shallot, were investigated. Chromosome length and banding patterns were statistically analysed. The value of C-banding for distinguishing these *Allium cepa* cultivars was tested by the application of a discriminant analysis. It appeared that chromosomal differences between cultivars exist, but are too small to be clearly demonstrated with moderate efforts. Large variation within plants and between plants within cultivars, presumably for the greater part caused by the applied method, obscured the variation between the cultivars.

Introduction

In practice, distinction between cultivars of *Allium cepa* L. is based on some morphological and agronomic characters (Astley et al., 1982; Anonymous, 1984). Especially in the case distinction has to be made between so-called selections the above-mentioned characters prove to be insufficient. Selections of a particular cultivar may differ from each other, in e.g. earliness, yield, skin quality, although all fall within the concept of that cultivar (ICNCP, 1980).

The possibility of using chromosomal characters to make a distinction between cultivars was investigated. The chromosome number of all investigated *A. cepa* cultivars is, even combined with a karyotype, not sufficient to make any distinction ($2n = 2x = 16$; Fedorov, 1969). The standard type of karyotype analysis based on arm ratios and total chromosome lengths does not offer scope for marking chromosomes or for tracing relationships and ancestry in *Allium* (Jones, 1983). The C-banding technique can provide information about relationships between species by comparison of banding patterns (Schweizer & Ehrendorfer, 1976). Several investigations on C-banding in *Allium cepa* have been carried out (Greilhuber, 1973; Stack & Clarke, 1973; Fiskesjö, 1974, 1975; El Gadi & Elkington, 1975; Friebe, 1976; Vosa, 1976; Stack & Comings, 1979; Joshi & Ranjekar, 1980; Cortes et al., 1981; Sato, 1981). With the C-banding technique it has appeared to be possible to identify each individual chromosome of the *Allium cepa* genome. This identification is possible by observations on telomeric and intercalary bands of heterochromatin (Kalkman, 1984). The same publication describes differences in C-banding patterns between three cultivars of *Allium cepa.* Tanasch (1984) also mentions chromosomal differences between cultivars of *Allium sativum.* The C-banding pattern might therefore be used to identify cultivars.

Six cultivars of *Allium cepa,* including one shallot were investigated. The shallot is interfertile with the onion, producing viable F_1's and F_2's, and

should be considered as a form of *Allium cepa* (Astley et al., 1982). According to Stearn (1960) the shallots of present-day gardens, to which the name *A. ascalonicum* is usually applied, are cultivars of *A. cepa*. The inclusion of a shallot cultivar aimed at elucidation of its relationships with onions proper.

Material and methods

The following Dutch and German *Allium cepa* cultivars were examined:

1) 'Zeeuwsche Bruine' (ZB) (A80567).
2) 'Bommelerwaard' (BMW) (A80574).
3) 'Noord-Hollandse Strogele' (SHALLOT) (A84190).
4) 'Noord-Hollandse Strogele' (NHSG) (GB16).
5) 'Rijnsburger' (RIJNSB) (GB43).
6) 'Stuttgarter Riesen' (STUTG) (GB62).

All accessions were obtained from the Institute for Horticultural Plant Breeding, IVT, Wageningen.

The plants were grown in winter in a glasshouse at 14° C with additional light (one HPIT lamp (400W) per m^2, 1 m above the plants, for 10h/d). This resulted in a synchronized progress of the mitotic division in the root tips. Root tips were collected about six hours after the lights were switched on, when most of the dividing cells had attained the metaphase stage. The C-banding method used was basically as described by Kalkman (1984) with some modifications. The root tips were (1) pretreated in 0.05% colchicine for 26 h at 4° C; (2) fixed in 3 : 1 ethanol/glacial acetic acid for 1 to 10 days at 4° C; (3) rinsed 15 min in 0.01 M citric acid-sodium citrate buffer pH 4.5–4.8, softened in 5% pectinase (Sigma, P4625) plus 1% cellulase (Calbiochem) in the same buffer for 25 min at 40° C, rinsed in the same buffer for 15 min at 4° C; (6) incubated in 45% acetic acid for 15–30 min at 4° C; (7) squashed in 45% acetic acid. The coverslips were removed after freezing with liquid nitrogen; (8) the slides were dried and stored at least overnight; (9) rinsed in absolute ethanol, dried on hot plate; (10) treated with 45% acetic acid for 15 min at 60° C, washed in running tap water; (11) incubated in saturated barium-hydroxide for 12 min at room temperature, washed in running tap water for 45 min at least, rinsed in de-ionized water; (12) incubated in 2x SSC (0.3 M NaCl plus 0.03 M trisodium citrate, pH 7.0) for 2.5 h at 62–63° C; (13) rinsed in Sørensen buffer (pH 7.0), stained in 3–4% Giemsa (Gurr's improved R66) in the same buffer for 5 min, rinsed briefly in the buffer and de-ionized water; (14) dried on a hot plate, mounted in Euparal.

Three to five metaphase plates per plant were drawn on squared paper with the aid of a camera lucida. Besides measuring the length of both chromosome arms, the areas of the telomeric C-bands were established. The position of the intercalary bands (IB) was not taken into account because these bands could not be observed in every cell. In case corresponding IB could be observed a large variation was found between their positions, within the plants. Distinction between the arms of chromosomes was made on differences in length for the chromosomes 2C, 3C, 4C, 6C, 7C and 8C (Chromosome nomenclature after Kalkman, 1984). The chromosomes 1C and 5C were assumed to be metacentric because no distinction could be made between the long and short arm.

To allow comparison of the observations between cells of one plant the measured data were converted to standardised values:

$$\text{Relative Arm Length} = \frac{\text{arm length}}{\text{total chromosome length of cell complement}} \times 100\%$$

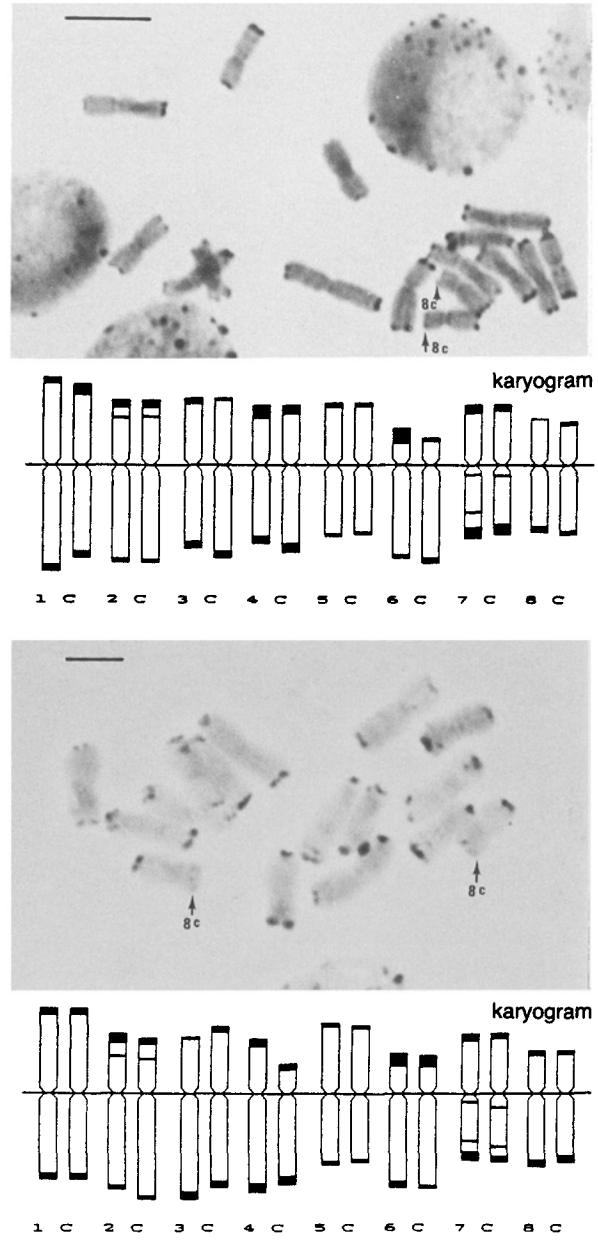
$$\text{Relative Band-Area} = \frac{\text{band-area}}{\text{total band-area of one genome}} \times 100\%$$

The karyotype as presented by Kalkman (1984) combined with the telomeric band-area made it possible to identify each individual chromosome of the *A. cepa* genome. Based on the standardised data, differences in morphology between the homologous chromosomes of one plant, can be detected. Testing (1) if the arms of one chromosome are distinguishable, is followed by testing (2) if the homologues are distinguishable. When it is known

which arms are significantly different per chromosome it can be tested if there are differences between the homologues of one cell. Homologues were considered distiguishable when the o-hypothesis was rejected with 95% reliability.

Distinction of homologous chromosomes within a plant can cause considerable problems. For instance: within some plants of for instance *Allium cepa* 'Zeeuwsche Bruine' significant differences (length, band area) between the chromosomes of one homologous pair were found. In other plants of the same cultivar, the corresponding homologous pair of chromosomes did not show these significant differences (Fig. 1–2). To compare one chromosome with the corresponding chromosome of another plant it was necessary to define the corresponding chromosome. Only the homologues of the chromosomes 3C, 6C and 7C could be distinguished with the following characters: band-area (BA) of the short arm of chromosomes 3C and 6C and BA of the long arm of chromosome 7C. These three characters achieved distinction between the chromosomes of a homologous pair for more than seven of the twenty-three plants tested. This was more than any other character did. The other measured characters of these chromosomes were looked at separately, disregarding the already obtained distinction. For the remaining chromosomes of each cell, homologues were assumed to be equal, because no reliable distinction could be made.

Discriminant analysis was carried out to test if the a-priori known groups (the cultivars) could be distinguished by chromosome measurements. The discriminant analysis consists in finding linear combinations of the discriminant descriptors, which maximize the differences between groups while minimizing the variance within each group (Legendre & Legendre, 1983). Simultaneous analysis of the character states will lead to the correct assignment of the elements and often requires measurements from a small number of characters (Dunn & Everitt, 1982). To keep the results of this multivariate analysis of variance surveyable it was necessary to pre-select those characters that showed significant differences between the populations. Therefore all characters were entered in a univariate analysis of variance (ANOVA). It was presumed that the contribution to distinguish cultivars with characters with a small F-value in the univariate ANOVA also would be small in the multivariate ANOVA. The following characters which had an F-value larger than 1.47 (the 25% point)



Figs. 1–2. Photomicrographs and karyograms of Allium cepa 'Zeeuwsche Bruine' plant 80567-4 (1) and plant 80567-10 (2). Bar equals 10 μm in all figures.

Table 1. Data (plant means and 95% confidence intervals) used for the discriminant analysis BMDP-7M.

Population	Plant number	Relative chromosome length (%) of the long (L) or short (S) arm of chromosome					Relative area of telomeric band (%) of the long (L) or short (S) arm of chromosome							
		1C (L+S)/2	2C (L)	4C (L)	4C (S)	6C (L)	1C (L+S)/2	4C (L)	4C (S)	6C (L)	8C (L)	8C (S)	3C (S)*	7C (L)**
ZB	−1	7.3±0.3	8.6±0.3	7.7±0.4	5.0±0.2	8.6±0.5	6.4±1.1	8.8±1.4	9.6±1.7	5.2±1.2	6.5±1.0	2.2±0.9	4.1±1.1	8.4±2.0
	−2	7.6±0.3	8.9±0.4	7.6±0.4	5.4±0.2	8.5±0.4	6.2±0.9	8.8±1.5	9.7±1.3	4.2±0.9	5.8±1.1	2.5±0.7	6.4±1.5	8.9±1.7
	−3	7.3±0.4	8.5±1.0	7.5±0.7	5.3±0.4	7.9±0.5	6.4±1.4	8.1±2.0	8.4±1.5	5.2±0.5	6.2±1.6	2.6±1.5	3.3±2.1	8.8±6.8
	−4	7.5±0.3	8.5±0.4	7.4±0.4	4.9±0.3	8.2±0.4	7.2±1.1	8.4±1.4	8.5±1.1	4.9±1.0	6.5±0.5	3.4±0.6	3.7±0.9	7.7±2.3
BMW	−1	7.5±0.3	8.0±0.3	7.8±0.4	4.8±0.3	8.6±0.4	3.9±0.9	9.1±1.4	5.3±1.3	5.8±1.4	8.8±1.6	2.7±2.4	7.3±3.4	10.1±1.6
	−2	7.5±0.5	8.6±0.7	8.1±1.0	4.5±0.6	8.2±0.6	4.5±1.9	11.9±2.7	4.7±0.7	6.9±2.5	6.4±1.2	2.6±1.5	5.8±2.9	8.8±4.3
	−3	7.3±0.2	8.5±0.4	7.8±0.5	5.1±0.2	8.4±0.5	5.6±1.4	8.0±3.0	7.2±2.2	5.3±1.1	7.8±1.8	4.0±1.4	8.8±1.3	7.9±3.2
SHALLOT	−1	7.3±0.3	9.0±0.6	7.6±1.0	5.4±0.2	8.7±1.1	4.3±1.0	9.9±2.0	11.1±1.9	6.8±2.1	9.4±3.7	2.9±1.7	3.5±1.1	5.1±3.3
	−2	7.5±0.2	8.8±0.6	7.8±0.4	5.2±0.3	8.7±0.5	4.1±0.9	10.3±1.5	10.7±1.5	5.8±1.2	9.6±2.3	3.3±0.7	3.4±2.2	4.7±1.2
	−3	7.3±0.5	9.0±0.9	7.7±0.6	5.2±0.4	8.7±0.4	5.0±2.6	11.5±4.0	9.9±1.4	5.5±2.4	9.3±2.4	3.3±3.2	3.9±0.7	4.1±2.5
NHSG	−1	7.5±0.3	8.7±0.4	7.9±0.3	5.0±0.2	8.2±0.3	5.7±0.5	11.3±1.4	7.0±2.0	6.2±1.2	7.1±1.0	3.7±1.2	4.3±1.2	8.4±2.0
	−2	7.5±0.3	8.6±0.6	7.7±0.5	5.2±0.7	8.3±0.5	4.5±1.4	9.6±2.0	7.7±1.6	6.0±1.5	7.6±0.8	2.3±1.4	7.3±4.3	6.8±0.8
	−3	7.5±0.3	8.2±0.6	7.2±1.3	5.4±1.0	8.3±0.4	5.8±1.5	9.7±0.9	5.0±0.6	7.2±1.6	6.8±2.0	2.5±0.9	4.1±0.6	6.9±0.6
	−4	7.6±0.3	8.4±0.5	7.7±0.3	5.3±0.3	8.4±0.5	5.4±1.1	10.3±2.0	15.2±3.5	6.0±0.9	6.9±1.5	2.6±0.9	5.0±1.4	4.6±1.6
	−5	7.5±0.3	8.6±0.1	7.8±0.5	5.2±0.1	8.7±0.4	4.6±1.2	10.1±2.2	9.5±1.7	5.7±0.9	7.1±2.4	2.2±1.4	6.8±4.6	9.3±0.8
RIJNSB	−1	7.7±0.4	8.8±0.4	7.7±0.3	5.3±0.4	8.4±0.4	5.2±1.4	9.7±1.7	8.0±2.3	5.3±1.2	9.0±1.8	1.5±0.6	9.1±2.5	10.3±4.2
	−2	7.5±0.4	8.7±0.5	7.7±0.4	5.2±0.2	8.2±0.5	5.4±1.5	11.9±1.7	6.9±1.6	6.8±1.4	6.4±1.1	2.9±1.3	6.2±0.7	7.0±1.6
	−3	7.4±0.3	8.5±0.2	7.5±0.2	5.2±0.2	8.6±0.5	4.9±0.9	11.1±1.0	8.4±2.4	5.5±0.8	6.3±2.2	1.8±0.6	8.6±0.8	10.0±2.6
	−4	7.5±0.4	8.5±0.8	7.9±0.9	5.1±0.5	8.5±0.4	4.6±0.4	9.2±3.8	5.8±3.6	5.8±2.1	5.2±1.4	2.8±1.4	4.7±7.1	8.9±3.4
STUTG	−1	7.0±0.5	8.3±0.6	8.0±0.5	5.5±0.5	8.3±0.8	5.1±1.9	10.6±1.8	12.3±3.5	6.3±1.5	10.0±2.3	1.7±1.1	3.3±3.3	7.7±1.7
	−2	7.2±0.2	8.8±0.9	7.7±0.3	5.1±0.4	8.5±0.4	5.4±0.7	9.3±3.0	11.5±3.1	5.6±0.8	7.6±2.2	2.2±0.7	4.2±1.5	4.5±4.1
	−3	7.4±0.2	8.8±0.6	7.7±0.3	5.4±0.4	8.5±0.6	4.8±0.9	6.8±1.9	11.9±2.3	6.0±1.3	5.4±0.7	1.5±0.5	3.5±2.0	7.3±4.3
	−4	7.4±0.4	8.2±0.4	7.9±0.7	5.3±0.3	8.3±0.4	5.9±1.5	10.6±1.5	9.4±2.7	5.8±0.8	8.8±1.4	2.5±1.5	4.9±0.9	6.1±1.1
CV		1.7	2.6	2.4	3.5	2.3	10.4	12.6	24.4	10.1	15.6	22.5	31.5	17.6
F-value		2.6	2.2	1.7	3.3	1.6	6.6	1.6	3.2	2.7	3.2	2.2	2.4	5.7

* = Homologue with the largest band area on the short arm, ** = Homologue with the smallest band area on the long arm, CV = Coefficient of variance.

were selected for the discriminant analysis (see also Table 1).

Character:	F-value:
1) arm length of 1C	2.6
2) length of the long arm of 2C	2.2
3) length of the long arm of 4C	1.7
4) length of the short arm of 4C	3.3
5) length of the long arm of 6C	1.6
6) total band-area of 1C	6.6
7) band-area of the long arm of 4C	1.6
8) band-area of the short arm of 4C	3.2
9) band-area of the long arm of 6C	2.7
10) band-area of the long arm of 8C	3.2
11) band-area of the short arm of 8C	2.2
12) largest band-area of the short arm of 3C	2.4
13) smallest band-area of the long arm of 7C	5.7

The variables used in computing the linear classification functions are chosen in a stepwise manner. At each step the variable that adds the most to the separation of the groups is entered into the discriminant function.

From this the canonical variables are calculated. The first canonical variable is that linear combination of selected characters which discriminates the best between the groups. The second canonical variable is the second best linear combination; orthogonal in respect of the first, etc. By plotting the first two canonical variables against each other in a graph the distinction of the groups is visualised. Group classifications are evaluated and presented in a classification table.

A disadvantage of stepwise linear discriminant analysis is that it does not take into account the possibility that two characters together have a discriminatory power.

Results

The stepwise linear discriminant analysis of computer program BMDP 7M (Dixon, 1985) selected three characters. These characters: average band-

Table 2. Summary of the reliability of the selection of the characters BA1C, BS8C and BL7C in the Discriminant Analysis. These characters distinguish the cultivars with the highest reliability.

Step number	Variable entered	F value to enter	Number of variables included	Approximate F-statistic	Degrees of freedom
1	7 BA1C	6.6277	1	6.628	17.00
2	14 BL7C	7.6456	2	6.917	32.00
3	12 BS8C	4.4439	3	6.134	41.31

Table 3. The classification based on the classification functions computed in the discriminant analysis. These functions are constructed from the characters BA1C, BS8C and BL7C.

Group	Percent correct	Number of cases classified into group					
		ZB	BMW	SHALLOT	NHSG	RIJNSB	STUTG
ZB	100.0	4	0	0	0	0	0
BMW	100.0	0	3	0	0	0	0
SHALLOT	100.0	0	0	3	0	0	0
NHSG	20.0	0	1	0	1	1	2
RIJNSB	50.0	0	1	0	1	2	0
STUTG	100.0	0	0	0	0	0	4
Total	73.9	4	5	3	2	3	6

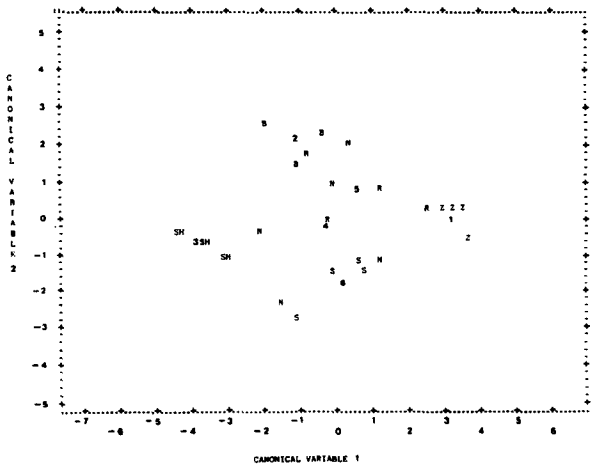


Fig. 3. Representation of the results of the discriminant analysis. SH = SHALLOT, N = NHSG, R = RIJNSB, S = STUTG, Z = ZB, B = BMW.

area of chromosome 1C (BA1C), band-area of the short arm of chromosome 8C (BS8C) and band-area of the long arm of chromosome 7C (BL7C), have the highest discriminatory power (Table 2). Table 3 shows the assignment of the cultivars based on the classification functions computed in the discriminant analysis. These classification functions are constructed from the three selected characters (Blackith & Reyment, 1971). The a posteriori identification of plants by the classification functions of the BMDP 7M program makes it possible to establish the value of the assignment of the plants to the cultivars (Legendre & Legendre, 1983). In the jackknifed classification each plant is classified into a group according to the classification functions computed from all the data with exception of the data of the plant concerned. Table 4 presents the results of the classification by this method. An impression of the distances between the plants was obtained by establishing the position of the objects (the plants) in relation to the computed canonical variables (Fig. 3). The position of the centroids of the various cultivars is also given in Fig. 3.

Discussion

As is shown in table 1 the variation of the measured values within the plants is relative large. This variation within plants is probably caused, for the greater part, while making the microscopic slides, the drawing and/or measuring of the chromosomes. Lighty & Plaisted (1960) concluded that the observed variation in the ratio of chromosomes within a plant is largely attributable to the effect of cytological manipulation rather than to variations existing in the living material. Moreover, in most cases it was impossible to make a distinction between the chromosomes of a homologous pair necessitating the use of average values of these homologues. The coefficients of variation increased enormously due to this step. In the karyogram (Figures 1 and 2) the differences between plants within cultivars are clearly demonstrated. Table 2 shows the characters selected for the highest discriminatory power. The classification matrix (Table 3) shows, even with the small number of plants

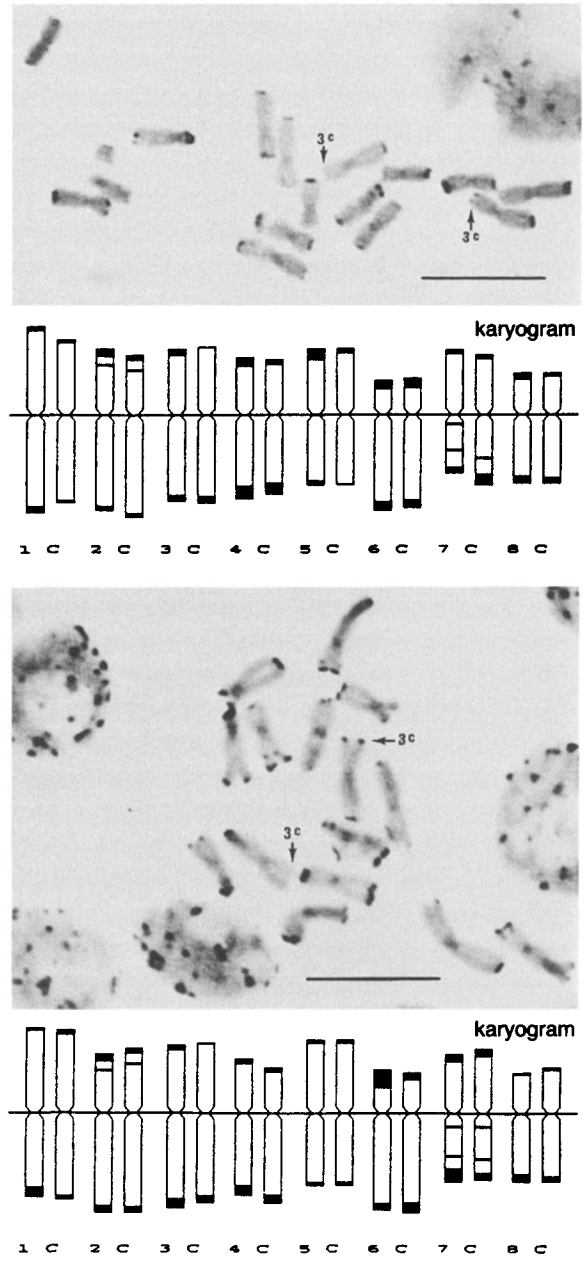
Table 4. The classification based on the jackknife classification functions as computed in the discriminant analysis.

Group	Percent correct	Number of cases classified into group					
		ZB	BMW	SHALLOT	NHSG	RIJNSB.	STUTG.
ZB	100.0	4	0	0	0	0	0
BMW	66.7	0	2	0	1	0	0
SHALLOT	100.0	0	0	3	0	0	0
NHSG	20.0	0	1	0	1	1	2
RIJNSB	25.0	1	1	0	1	1	0
STUTG	100.0	0	0	0	0	0	4
Total	65.2	5	4	3	3	2	6

analysed, that the plants from the cultivars 'Zeeuwsche Bruine', 'Bommelerwaard', 'Noord-Hollandse Strogele' (Shallot) and 'Stuttgarter Riesen' were correctly classified in all cases. Problems arose in classifying plants from the quite similar cultivars 'Rijnsburger' and 'Noord-Hollandse Strogele'. Indeed the cultivar 'Rijnsburger' is most likely developed from the cultivar 'Noord-Hollanse Strogele' (van der Meer, pers. comm.). This result can also be seen from fig. 3. In the same figure the cultivars 'Noord-Hollandse Strogele' (Shallot), 'Zeeuwsche Bruine', 'Bommelerwaard' and 'Stuttgarter Riesen' are spread as four groups. The jackknifed classification procedure (Table 4) gives a more reliable discriminant analysis. A jackknifed-validation procedure reduces the bias in the group classifications. Although there is a great resemblance in cytological characters, in all cases the shallot is distinguishable from the onion cultivars. The independent development over the years of the shallot and the onion can be put forward as explanation.

A major problem in processing data from chromosome measurements is caused by the impossibility to distinguish with sufficient reliability the chromosomes of one homologous pair. This problem can be dealt with in two ways. Firstly there is the possibility of using the mean of all homologues of all cells of the plant as value for the homologues concerned or, secondly, to select a largest and a smallest value even if there is no significant difference between the homologues. We have chosen for the second solution because the first solution either stresses any possible differences or other possible differences become blurred. Differences between the chromosomes of a homologous pair exist (Kalkman, 1984), see also figures 4 and 5. The phenomenon shown in these figures could not be used in the discriminant analyses. These photographs show a 3C chromosome without telomeric band on the short arm, which was only observed in the cultivars 'Noord-Hollandse Strogele' and 'Rijnsburger'. This again demonstrates the relation between the C-banding patterns of closely related cultivars.

If the homologues can be distinguished with full reliability the discriminant analysis is a suitable method of analysis. Differences in C-banding patterns between onion cultivars can be demonstrated, but the efforts involved are too large to assist routine screenings.



Figs. 4–5. Photomicrographs and karyograms of Allium cepa 'Noord-Hollandse Strogele' plant GB16-4 (4) and A. cepa 'Rijnsburger' plant GB43-6 (5). Bar equals 10 μm in all figures.

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References

Anonymous, 1984. 33e Beschrijvende Rassenlijst. Commissie voor de Samenstelling van de Rassenlijst voor Groentegewassen, Wageningen. pp. 182–187.

Astley, D., N.L. Innes & Q.P. van der Meer, 1982. Genetic resources of *Allium* species. IBPGR Secretariat, Rome: 38 pp.

Blackith, R.E. & R.A. Reyment, 1971. Multivariate morphometrics. London.

Cortes, F., G. Gonzalez-Gill & J.F. Lopez-Saez, 1981. Differential staining of late replicating DNA-rich regions in *Allium cepa* chromosomes. Caryologia 33: 193–202.

Dixon, W.J., 1985. (Ed.). BMDP Statistical Software Manual 1985. University of California Press, Berkeley, Los Angeles, London.

Dunn, C.G. & B.S. Everitt, 1982. An introduction to mathematical taxonomy. Cambridge University Press, Cambridge.

El Gadi, A. & T.T. Elkington, 1975. Comparison of the Giemsa C-band karyotypes of *Allium cepa, A. fistulosum* and *A. galanthum*. Chromosoma 51: 19–23.

Federov, A.A. (Ed.), 1969. Chromosome numbers of flowering plants. Reprint by O. Koeltz Science Publishers Koenigstein/ West Germany, pp. 376–378.

Fiskesjö, G., 1974. Two types of constitutive heterochromatin made visible in *Allium* by a rapid C-banding method. Hereditas 78: 153–156.

Fiskejö, G., 1975. Chromosomal relationships between three species of *Allium* as revealed by C-banding. Heriditas 81: 23–32.

Friebe, B., 1976. Differential Giemsa staining of heterochromatic regions in the chromosomes of *Vicia faba, Allium cepa* and *Paeonia tenuifolia*. Theor. Appl. Genet. 47: 275–283.

Greilhuber, J., 1973. Differential staining of plant chromosomes after hydrochloric acid treatments (Hy-bands). Öst. Bot. Z. 122: 333–351.

ICNCP, 1980. International Code of Nomenclature for Cultivated Plants. C.D. Brickell (Ed.). Bohn, Scheltema & Holkema, Utrecht. W. Junk BV Publ. The Hague. Regnum Vegetabile, volume 104.

Jones, R.N., 1983. Cytogenetic Evolution in the genus *Allium*. Cytogenetics of Crop Plants. M.S. Swaminathan, P.K. Gupta & U. Sinha (Eds.) MacMillan India Limited: 516–555.

Joshi, C.P. & P.K. Ranjekar, 1980. Technique for heterochromatin visualization and chromosome banding in plants. Nucleus 23: 169–176.

Kalkman, E.R., 1984. Analysis of the C-banded karyotype of *Allium cepa* L. Standard system of nomenclature and polymorphism. Genetica 65: 141–148.

Legendre, L. & P. Legendre, 1983. Numerical Ecology. Elsevier Scientific Publishing Company. Amsterdam, Oxford, New York.

Lighty, R.W. & R.L. Plaisted, 1960. The evaluation of the sources of variation in the preparation of a karyotype of a clone. Cytologia 25: 1–7.

Sato, S., 1981. Cytological studies on the satellited chromosomes of *Allium cepa*. Caryologia 34: 431–440.

Schweizer, D. & F. Ehrendorfer, 1976. Giemsa banded karyotypes, systematics and evolution in *Anacyclus* (Asteraceae-Anthemideae). Plant Syst. Evol. 126: 107–148.

Stack, S.M. & C.R. Clarke, 1973. Differential Giemsa staining of the telomeres of *Allium cepa* chromosomes: observations related to chromosome pairing. Can. J. Genet. Cytol. 15: 619–624.

Stack, S.M. & D.E. Commings, 1979. The chromosomes and DNA of *Allium cepa*. Chromosoma 70: 161–181.

Stearn, W.T., 1960. *Allium* and *Milula* in the central and eastern Himalaya. Bull. Brit. Mus. (Nat. Hist.), Bot. Ser. 2: 161–191.

Tanasch, L., 1984. Cytologisch-züchterische Untersuchungen an Knoblauch (*Allium sativum* L.). Aus dem Institut für Pflanzenbau und Pflanzenzüchtung: 109–125.

Vosa, C.G., 1976. Heterochromatic patterns in *Allium*. Heredity 36: 383–392.