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A critical review and a new proposal of karyotype asymmetry indices

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Abstract. In literature seven different methods of evaluating karyotype asymmetry – the TF%, the As K%, Stebbins' classification, the Rec and the Syi, the A_1 and the A_2, the DI, and the A – are used for the elucidation of phylogenetic relationships and taxonomic treatments within a particular group or taxon. The investigation of these seven methods reveals that the intervals used by Stebbins to separate the different types of karyotype asymmetry are very broad and only one quantitative parameter, the A_2 index, correctly describes the variation in chromosome length in a complement. A new asymmetry index (AI) is proposed to measure karyotype asymmetry and a new parameter, the CV_{CI}, is offered, that precisely assesses the relative variation in centromere position in a complement. The AI index, the CV_{CI} and the CV_{CL} ($= A_2 \times 100$) have the potential to display even minor karyotypic variations. Thus, these three indices together increase the precision of results in comparison with other existing methods. All this has important consequences as regards the interpretation of the results of karyological studies.

Key words: Asymmetry index, chromosomes, karyotype morphology.

Introduction

Stebbins (1971), writing about karyotype asymmetry, referred to the Russian school of comparative karyotype morphology, led by G. Levitsky (1931), who developed the concept of its symmetry vs. asymmetry. A symmetrical karyotype is characterised by the predominance of m and sm chromosomes of approximately the same size. Increasing asymmetry can occur either through the shift of centromere position from median/submedian to terminal or subterminal, or through the accumulation of differences in the relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous. These two tendencies are not correlated with one other, though they may be in some groups (Stebbins 1971).

Stebbins (1971) distinguished twelve categories with respect to karyotype asymmetry, only ten of which were known to occur in higher plants. He established these by recognising three degrees of difference (A–C) between the largest and smallest chromosome of the complement, and four degrees (1–4) with respect to the proportion of chromosomes which are metacentric with an arm ratio of less than 2:1 (Table 1).

The classification of Stebbins (1971) is the most frequently used qualitative method for assessing karyotype-symmetry conditions and describing the karyotypic relationships between different taxa (e.g. *Lathyrus*, Seijo

Table 1. The classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971)

Ratio	Proportion of chromosomes with arm ratio <2:1			
Largest/smallest	1.00 (1)	0.99–0.51 (2)	0.50–0.01 (3)	0.00 (4)
<2:1 (A)	1A	2A	3A	4A
2:1–4:1 (B)	1B	2B	3B	4B
>4:1 (C)	1C	2C	3C	4C

and Fernandez 2003; *Ophrys*, Bernardos et al. 2003; between *Davidia involucrata* and *Camptotheca acuminata*, He et al. 2004).

The total form percent (TF%), was described by Huziwara (1962) to analyse the karyotypes in the genus *Aster*. The TF% index is expressed by the ratio of the sum of the lengths of the short arms of individual chromosomes to the total haploid length of the complement:

$$\text{TF\%} = \frac{\text{Length of short arms in chromosome set}}{\text{Total chromosome length in set}} \times 100.$$

The TF% index has frequently been used to describe karyotype asymmetry and to determine the karyotypic relationships between species of genera (e.g. *Phaseolus*, Mercado-Ruaro and Delgado-Salinas 1998; *Mikania*, Ruas et al. 2000; in the Alismataceae family, Costa and Forni-Martins 2003).

Arano (1963) introduced another karyotype asymmetry index, the As K%, which was used to determine the phylogenetic relations between and within the genera *Pertya* and *Ainsliaea*. The As K% index is expressed by the ratio of the sum of the lengths of the long arms of individual chromosomes to the total haploid length of the chromosome complement:

$$\text{As K\%} = \frac{\text{Length of long arms in chromosome set}}{\text{Total chromosome length in set}} \times 100.$$

The As K% index was used subsequently to analyse chromosome evolution between species in the Umbelliferae family (Arano and Saito 1980), as well as between species of *Hypochaeris* (Weiss-Schneeweiss et al. 2003), *Armeria* (Coulaud et al. 1999), *Metagentiana* (Ho et al. 2002), *Solms-laubachia*, and two related genera (Yue et al. 2004).

Greilhuber and Speta (1976) developed two indices, the index of karyotype symmetry and the index of chromosomal size resemblance, to evaluate karyotype asymmetry. These two indices were later called by Venora et al. (2002) the Syi index and the Rec index, respectively. The Syi value indicates the ratio of the mean length of the short arms against the mean length of the long arms in a chromosome set. The Rec index expresses the mean of the ratios of the length of each chromosome (CL_i) to that of the longest one (LC):

$$\text{Syi} = \frac{\text{Mean length of the short arms}}{\text{Mean length of the long arms}} \times 100,$$

$$\text{Rec} = \frac{\sum_{i=1}^{n} \frac{CL_i}{LC}}{n} \times 100,$$

where n is the number of analysed chromosomes. Both indices have been used to estimate karyotype asymmetry and to discuss the relationships between species of *Scilla* and *Puschkinia* (Greilhuber and Speta 1976), and *Triticum* (Venora et al. 2002) only.

Romero Zarco (1986) provided an alternative method for measuring karyotype asymmetry by using quantification and graphic representation. He proposed two numerical parameters to estimate karyotype asymmetry. The first was named the intrachromosomal asymmetry index (A_1) and the second – the interchromosomal asymmetry index (A_2).

The intrachromosomal asymmetry index (A_1), ranging from 0 to 1, can be calculated for every sample using the following equation:

$$A_1 = 1 - \frac{\sum_{i=1}^{n} \frac{q_i}{p_i}}{n},$$

where q_i is the mean length for short, and p_i for long arms in every homologous chromosome pair or group; n is the number of homologous chromosome pairs or groups. The interchromosomal asymmetry index (A_2) is the ratio between the standard deviation (s_{CL}) and the mean chromosome length (x_{CL}):

$$A_2 = \frac{s_{CL}}{x_{CL}}.$$

Both Romero Zarco indices tend to be the most frequently used estimates of karyotype asymmetry between different taxa (e.g. *Lathyrus*, Seijo and Fernandez 2003; *Ligularia*, Liu 2004; Mediterranean orchids, Cozzolino et al. 2004).

Mugnier and Siljak-Yakovlev (1987) used the asymmetry index (AsI) which is probably a synonym of the As K% (Arano 1963), as both are expressed by the ratio between the sum of the lengths of the long arms of individual chromosomes and the sum of chromosome length in its set. Two years later Barghi et al. (1989), using the AsI index, referred to Arano and Saito (1980). The AsI index was used to analyse chromosome evolution among species of *Mikania* (Ruas and Aguiar-Perecin 1997).

Lavania and Srivastava (1992) introduced another chromosomal parameter they called the dispersion index (DI), which is the proportionate measure of the centromeric gradient (CG) with respect to the coefficient of variation of chromosome length (CV), calculated from the following equations:

$$CG = \frac{\text{Median length of short arm}}{\text{Median length of chromosome}} \times 100,$$

$$CV = \frac{s_{CL}}{x_{CL}} \times 100,$$

$$DI = \frac{CG \times CV}{100},$$

where s_{CL} is the standard deviation of chromosome length, and x_{CL} is the mean chromosome length. The DI index was used to discuss phylogenetic differentiation and origin in the genus *Papaver* (Lavania and Srivastava 1992, 1999).

Watanabe et al. (1999) defined degree of asymmetry of karyotype (A) as:

$$A = \frac{\sum_{i=1}^{n} \frac{p_i - q_i}{p_i + q_i}}{n},$$

where p and q are the lengths of a long arm and a short arm of chromosome i, respectively, in a cell, and n is the haploid chromosome number of an individual or taxon.

The karyotype asymmetry index is a good expression of the general morphology of plant chromosomes. It would therefore be advantageous to have a uniform system whereby the karyotypes of related genotypes and species could be compared. As shown above, scientists have developed to date, a variety of methods for assessing and analysing karyotype asymmetry in a chromosome set. The primary aim of this study was to discuss the validity and sensitivity of these methods, which are used to assess karyotype asymmetry between and within different taxa.

Materials and methods

Eight accessions of five *Calamagrostis* Adanson (Poaceae) taxa were collected (Table 2). The voucher specimens are deposited in the herbarium of the W. Szafer Institute of Botany PAS in Krakow (KRAM). The root tips were stained following the Feulgen method. No less than five cells per individual and five plants per species were examined. Idiograms were drawn using a computer program Mr. Karyo (A. Joachimiak, Institute of Botany, Jagiellonian University, Kraków).

For the numerical characterisation of the karyotypes the following parameters were calculated: (1) shortest (SC) and longest (LC) chromosome length; (2) ratio of longest to shortest chromosome (LC/SC); (3) mean long arm length (p); (4) mean and median of short (q) and of total chromosome length (CL); (5) percentage of chromosomes with an arm ratio of less than 2:1; (6) mean centromeric index (CI = 100 × length of short arm/total chromosome length); and (7) karyotype formula (Table 3).

Seven different methods were used to assess the degree of karyotype asymmetry (Table 4). The

Table 2. Taxa, accessions, chromosome numbers, ploidy level, and collection number of the examined individuals from the genus *Calamagrostis*

Taxon	Accession	Chromosome number (2n)	Ploidy level	Voucher number (in KRAM)
C. arundinacea (L.) Roth	A-55	28	4	525974-977
C. canescens (Weber) Roth	C-57	28	4	525964-968
C. epigejos (L.) Roth	E-00	28	4	525946-947
	E-01	28	4	525948-952
C. villosa (Chaix ex Villars) J. F. Gmelin	vi-58	70	10	525935-940
C. ×hartmaniana	ha-40	28	4	525953-957
[*C. arundinacea* (L.) Roth ×	ha-41	28	4	525958-963
C. canescens (Weber) Roth]	ha-56	28	4	525969-973

strength of the association between karyotype asymmetry indices (excluding the degrees of Stebbins (1971)) was tested using Pearson correlation analysis (Table 5). Coefficients of variation (CV_{CL}, CV_{CI}) were calculated by dividing the standard deviations by the means (Table 6) (Sokal and Rolf 1981). Cluster analysis using the Euclidean distance was employed to compare different data, and average linkage methods of hierarchical cluster analysis were used for clustering on standardised data. Statistical evaluation was carried out using the Statistica 6.1 software package (StatSoft 1995).

Results and discussion

This study is based on the eight accessions of *Calamagrostis* (Table 2). Karyotype formulae obtained and the parameters analysed are summarized in Table 3. The respective idiograms (Fig. 1) are based on mean values presented in Table 3. Accessions of *Calamagrostis arundinacea* (A-55) and *C. villosa* (vi-58) exhibited the most variation in chromosome length, but only *C. villosa* (vi-58) is characterised by the highest level of variation of the centromeric index (Table 3; Fig. 1).

The karyotype asymmetry was assessed, based on seven different methods (Table 4). Among these methods, one qualitative classification and eight different quantitative indices can be marked out. The analysis of index formulae and the association between quantitative indices and three karyotype characteristics (LC/SC, CL, CI) reveals three groups among them (Table 5). Five indices – the TF%, the Syi, the As K%, the A_1, and the A – have been formulated so far by different researchers to evaluate the variation in centromere position in a chromosome complement. The As K% index has a perfect negative correlation with two indices: the TF% and the Syi. None of these five indices is either a relative standard deviation (RSD) or a coefficient of variation (CV), and for that reason all of them inadequately demonstrate relationships between studied accessions.

Two indices, the Rec and the A_2, were created to assess the variation in chromosome length in a complement. The A_2 index is a relative standard deviation of chromosome length, and from the statistical point of view it is a sensible parameter, which adequately assesses the relative variation in chromosome length in a complement. The Rec index is a wrong parameter and does not reflect relationships between karyotypes too closely or at all.

The DI index was developed by Lavania and Srivastava (1992) in order to give a single value that evaluated the karyotype asymmetry. It was the first attempt to create one karyotype asymmetry index, but one of two parameters used, the centromeric gradient (CG), cannot correctly assess the variation in centromeric position. As a result, the DI index is unable to evaluate karyotype asymmetry.

Table 3. Karyotype formula according to Levan et al. 1964 and characteristics of the studied *Calamagrostis* taxa. Acc. – accession; SC – the shortest chromosome length; LC – the longest chromosome length; p – mean length of long arm; q – mean and median length of short arm; CL – mean and median length of chromosome; <2:1 – percentage of the chromosomes with an arm ratio of less than 2:1; CI – mean centromeric index; m – metacentric, sm – submetacentric, st – subtelocentric chromosomes; SD – standard deviation

Acc.	Range	Ratio	p (μm)	q (μm)		CL (μm)		<2:1	CI	Karyotype
	SC–LC (μm)	LC/SC	mean (± 1 SD)	mean (± 1 SD)	median	mean (± 1 SD)	median	(%)	mean (± 1 SD)	formula
A-55	1.27–4.19	3.30	1.67 (± 0.46)	1.26 (± 0.36)	1.19	2.93 (± 0.74)	3.10	86	43.16 (± 5.57)	11m + 3sm
C-57	1.87–3.25	1.74	1.46 (± 0.22)	1.08 (± 0.20)	1.07	2.54 (± 0.36)	2.54	100	42.44 (± 4.10)	13m + 1sm
E-00	2.35–3.90	1.66	1.66 (± 0.24)	1.30 (± 0.29)	1.26	2.96 (± 0.47)	3.01	100	43.76 (± 4.53)	12m + 2sm
E-01	2.74–4.33	1.58	1.97 (± 0.32)	1.42 (± 0.28)	1.37	3.39 (± 0.52)	3.17	100	41.79 (± 4.48)	11m + 3sm
vi-58	1.77–4.45	2.51	1.74 (± 0.37)	1.24 (± 0.42)	1.27	2.98 (± 0.71)	3.01	83	40.74 (± 7.61)	25m + 9sm + 1st
ha-40	1.94–3.53	1.82	1.59 (± 0.28)	1.19 (± 0.26)	1.19	2.78 (± 0.47)	2.78	100	42.82 (± 4.83)	11m + 3sm
ha-41	1.93–3.49	1.81	1.60 (± 0.29)	1.21 (± 0.25)	1.23	2.81 (± 0.49)	2.72	100	43.08 (± 4.58)	11m + 3sm
ha-56	1.57–3.09	1.97	1.36 (± 0.23)	0.98 (± 0.26)	0.96	2.34 (± 0.43)	2.41	93	41.46 (± 5.89)	10m + 4sm

According to Levitsky (1931) and Stebbins (1971), the karyotype asymmetry of a complement is determined by the variation in chromosome length and the variation in centromere position. Only four methods – Stebbins' classification (1971), Rec and Syi indices (Greilhuber and Speta 1976), Romero Zarco (1986) indices (A_1 and A_2), and the dispersion index (DI) (Lavania and Srivastava 1992) – use a combination of both types of variation that affect karyotype asymmetry. Relationships between *Calamagrostis* accessions based on these four methods are shown in Figs 2, 3, and 5. The remaining methods, the TF% (Huziwara 1962), the As K% (Arano 1963), and the A index (Watanabe et al. 1999), try to describe only the variation in centromere position in a chromosome complement and they have a perfect or almost perfect positive or negative correlation with the Syi index (Table 5).

The relative variation in centromere position in a chromosome set can be assessed directly on the basis of the coefficient of variation for the centromeric index (CV_{CI}). The *Calamagrostis villosa* (vi-58) is characterised by the highest value of CV_{CI}, then followed by *C.* ×*hartmaniana* (ha-56) and *C. arundinacea* (A-55), which have lower values of CV_{CI}. Remaining samples are characterised by much lower values of CV_{CI} (Table 6, Fig. 4). All these do not correspond with clusters in the dendrogram based on five indices which try to describe the variation in centromere position (the TF%, the Syi, the As K%, the A_1, and A) (Fig. 6). These five indices are parameters incapable of describing the variation in centromere position and they do not reflect the real variation in centromere position among the *Calamagrostis* accessions studied.

The CV_{CI} parameter is evaluated as the ratio between the standard deviation (s_{CI}) and the mean centromeric index (x_{CI}):

$$CV_{CI} = \frac{s_{CI}}{x_{CI}} \times 100.$$

The relative variation in chromosome length ($CV_{CI} = A_2 \times 100$) in a complement is evalu-

Table 4. Karyotypes of eight *Calamagrostis* accessions (Table 2) using different methods of evaluating karyotype asymmetry

Accession	TF% (1)	As K% (2)	Stebbins' types (3)	Rec (4)	Syi (4)	A_1 (5)	A_2 (5)	DI (6)	A (7)
A-55	42.95	57.05	2B	69.98	75.28	0.23	0.25	9.73	0.14
C-57	42.55	57.45	1A	78.04	74.07	0.25	0.14	6.03	0.15
E-00	44.00	56.00	1A	76.00	78.57	0.21	0.16	6.70	0.12
E-01	41.88	58.12	1A	78.26	72.07	0.27	0.15	6.62	0.16
vi-58	41.52	58.48	2B	66.87	70.99	0.29	0.24	10.01	0.19
Ha-40	42.94	57.06	1A	78.79	75.25	0.24	0.17	7.28	0.14
Ha-41	43.17	56.83	1A	80.41	75.95	0.23	0.17	7.83	0.14
Ha-56	41.80	58.20	2A	75.87	71.83	0.28	0.18	7.30	0.17

Table 5. Pearson correlations for asymmetry indices and three karyotype characteristics. Significant correlations ($p<0.05$) are in boldface. LC/SC: ratio of longest/shortest chromosome, CL: chromosome length, CI: centromeric index

	LC/SC	CL	CI	Rec	A_2	DI	TF%	As K%	Syi	A_1	A
LC/SC	1										
CL	0.02	1									
CI	−0.06	0.03	1								
Rec	**−0.79**	−0.14	0.41	1							
A_2	**0.93**	0.10	−0.24	**−0.88**	1						
DI	**0.87**	0.16	−0.27	**−0.83**	**0.98**	1					
TF%	−0.12	0.02	**0.97**	0.35	−0.23	−0.24	1				
As K%	0.12	−0.02	**−0.97**	−0.35	0.23	0.24	**−1.00**	1			
Syi	−0.12	0.02	**0.97**	0.35	−0.23	−0.25	**1.00**	**−1.00**	1		
A_1	0.04	−0.06	**−0.99**	−0.35	0.19	0.21	**−0.98**	**0.98**	**−0.98**	1	
A	0.18	−0.05	**−0.99**	−0.49	0.35	0.39	**−0.96**	**0.96**	**−0.96**	**0.97**	1

Table 6. Chromosome statistics for *Calamagrostis* taxa (Table 2). CV_{CL}: coefficient of variation of chromosome length; CV_{CI}: coefficient of variation of centromeric index; AI: karyotype asymmetry index

Accession	CV_{CL}	CV_{CI}	AI
C-57	14.35	9.66	1.39
E-01	15.32	10.72	1.64
E-00	15.99	10.34	1.65
ha-41	17.27	10.63	1.84
ha-40	17.04	11.28	1.92
ha-56	18.29	14.21	2.60
A-55	25.31	12.90	3.27
vi-58	23.72	18.68	4.43

ated as the ratio between the standard deviation (s_{CL}) and mean chromosome length (x_{CL}):

$$CV_{CL} = A_2 \times 100 = \frac{s_{CL}}{x_{CL}} \times 100.$$

Karyotype asymmetry depends on both the relative variation in chromosome length (CV_{CL}) and the relative variation in centromeric index (CV_{CI}). A new karyotype asymmetry index or AI is proposed as the preferable asymmetry index. It gives a measure of the heterogeneity of chromosome length and centromeric position in a given karyotype and is similar to the dispersion index (DI) (Lavania and Srivastava 1992) in the sense that the DI

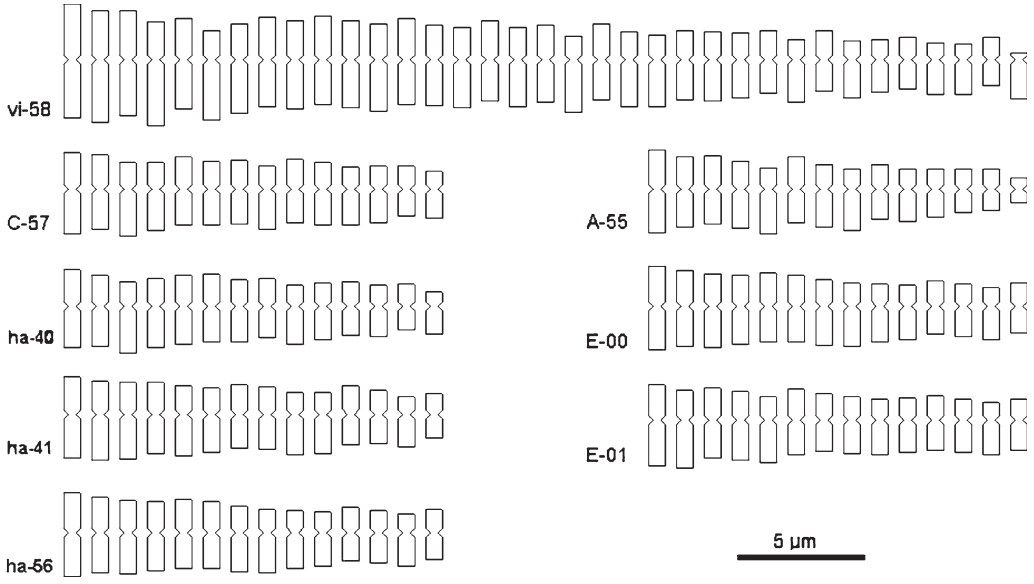


Fig. 1. Idiograms of *Calamagrostis* taxa listed in Table 2

index is a broad measure, not intended to tell about the asymmetry of a particular karyotype. The AI index can be defined as the product of a component expressing the relative variation in chromosome length (CV_{CL}) and a component expressing the relative variation in centromeric index (CV_{CI}). Relationships between these parameters are summarised by the following equation:

$$\text{Asymmetry index (AI)} = \frac{CV_{CL} \times CV_{CI}}{100}.$$

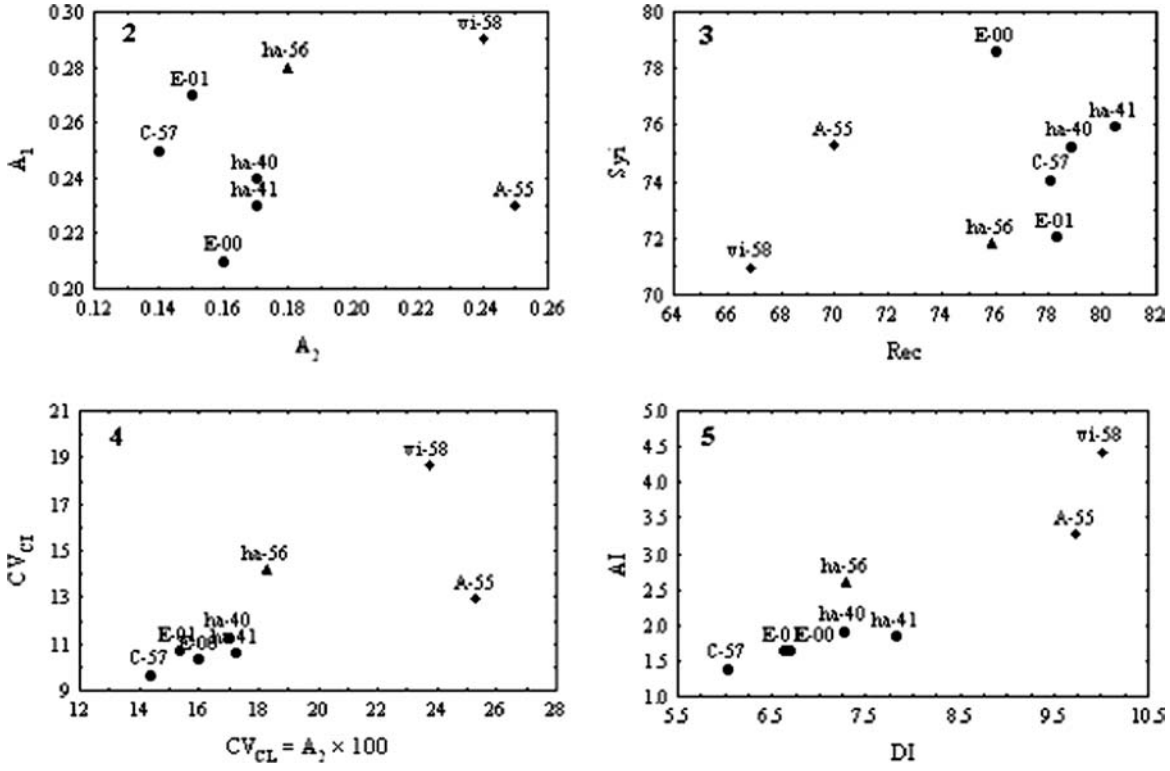
The basic interpretation of the AI value is: the higher the value, the higher the heterogeneity of chromosome length and/or centromeric index in a studied karyotype. As the AI index gets higher, so does karyotype asymmetry. As the index gets lower, it indicates greater karyotype symmetry. However, from the AI value we cannot directly assess the purpose of increasing asymmetry, because it depends on both the shift of centromere position from median/submedian to terminal or subterminal, and the accumulation of differences in the relative size between the chromosomes of the complement.

The AI value and scatter diagram based on the CV_{CL} ($=A_2 \times 100$) and the CV_{CI} seem best suited to assess overall classification strength and display relationships among *Calamagrostis* accessions with respect to karyotype asymmetry (Fig. 4, Table 6). The most symmetrical karyotype was observed in the *C. canescens* (C-57), while the *C. villosa* (vi-58) exhibited the most asymmetrical karyotype.

Stebbins' classification (1971) is a qualitative method, therefore is less powerful and less flexible in terms of the types of conclusions it can provide. This method is very original, but measurement variables are coded to become ranked variables. Stebbins' categories are nominal data. If coded numerically, the numbers chosen are arbitrary. The only allowable calculation on nominal data is to count the frequency of each value of a variable. Therefore, it is hard to say that the category 3A is higher than the category 1C (Table 1). Unfortunately, there are many statistical techniques that require greater measurement accuracy.

Conclusions

The approaches used in this study were aimed to review and improve methods, which assess karyotype asymmetry. The examination of seven methods, which try to assess karyotype asymmetry, reveal that Stebbins' qualitative



Figs. 2–5. Scatter diagrams for *Calamagrostis* accessions: **2** The A_1 parameter against the A_2 parameter, **3** The Rec index against the Syi index, **4** The CV_{CL} parameter against the CV_{CI} parameter, **5** The AI index against the DI index. Degrees of asymmetry according to Stebbins [10]: (•) – 1A, (■) – 2A, (♦) – 2B

classification is less sensible than quantitative parameters and only one parameter, Romero Zarco (1986) interchromosomal asymmetry index (A_2), correctly describes the variation in chromosome length in a complement. The

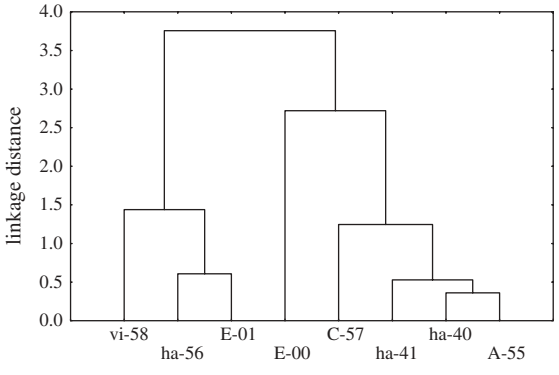


Fig. 6. Dendrogram based on the TF%, the Syi, the As K%, the A_1 and the AsI index for *Calamagrostis* accessions listed in Table 2

Rec index is an incorrect parameter for measuring the variation in chromosome length as well as five indices: the TF%, the Syi, the As K%, the A_1, and the A, have no possibility to estimate the variation in centromere position in a complement. The DI index, which tries to assess karyotype asymmetry in the chromosome set, is also an incorrect parameter.

A new asymmetry index, the AI index, was developed in order to give a single value that assesses karyotype asymmetry, and a new parameter, the CV_{CI}, was proposed as a relative measure of variation in centromeric index. The AI, the CV_{CI}, and the CV_{CL} ($=A_2 \times 100$) have the advantage of allowing a high degree of precision and sensitivity to assess karyotype asymmetry. Higher values of the AI index are considered to indicate higher levels of karyotypic heterogeneity. The scatter diagram of the CV_{CL} against the CV_{CI} seems

best suited to demonstrate relationships even between closely related taxa.

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