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Taxonomic Relationships of A Rare *Cucumis* Species (*C. hystrix* Chakr.) and Its Interspecific Hybrid with Cucumber

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Abstract. The current *Cucumis* taxonomic classification places *C. hystrix* Chakr. in subgen. *Cucumis* based on its morphological similarities to cucumber (*C. sativus* L., 2n = 14). However, the chromosome number of *C. hystrix* was identified as 2n = 24, the same number as in subgen. *Melo*. *Cucumis hystrix* is therefore considered the first wild *Cucumis* species of Asiatic origin possessing 12 basic chromosomes. Thus, any research regarding its biosystematics would challenge the basic chromosome number and geographic location theories that govern the current taxonomic system. The production of the amphidiploid species (*Cucumis* ×*hytivus* Chen and Kirkbride, 2n = 38) obtained from the cross between *C. hystrix* and *C. sativus* and subsequent chromosome doubling would provide an effective means of investigating the relationship between *Cucumis* species with two different basic chromosome numbers. Thus, RAPD markers were used to study the taxonomic placement of *C. hystrix* and its interspecific hybrid with cucumber. Of the 220 arbitrary primers screened, 31 were used for analysis where 402 (96.3%) fragments were polymorphic among the germplasm examined. A UPGMA-based cluster analysis partitioned 31 accessions into two main groups [*C. sativus* (CS) and *C. melo* (CM)]. Under the similarity coefficient threshold of 0.23, these two groups can be further divided into five clusters with *C. hystrix*, *C.* ×*hytivus*, and *C. sativus* as separate clusters in the CS group. A modified taxonomic system is proposed based on these results and findings of a previous chloroplast DNA analysis with the genus *Cucumis* containing subgen. *Cucumis* with three species and subgen. *Melo* with six series.

It has been more than two and a half centuries since Linnaeus defined the genus *Cucumis* in the Cucurbitaceae (Jeffrey, 1980). Currently, *Cucumis* is partitioned into two subgenera according to species origins and basic chromosome numbers (Kirkbride, 1993) (Fig. 1). The subgen. *Melo*, comprised mainly of African species (2n =2x = 24), includes 30 type species grouped into six series: *Humifructuosi*, *Melo*, *Hirsuti*, *Metuliferi*, *Angurioidei*, and *Myriocarpi*. Subgen. *Cucumis* contains two species of Asian origin: *C. sativus* L. (2n = 2x = 14) with three botanical varieties (var. *sativus*, var. *hardwickii* (Royle) Gabaev, and var. *xishuangbannesis* Qi Chun Zhang and Yuan Zhen Zhen, nom. nud.), and *C. hystrix* Chakr. (2n = 2x = 24).

Previous biosystematic studies indicated that cucumber (*C. sativus* var. *sativus*) is the species that is most distantly related to others *Cucumis* species (Danin-Poleg et al., 2001; Garcia-Mas et al., 2000; Perl-Treves et al., 1985). Unequivocal taxonomic and evolutionary relationships among *Cucumis* species are difficult to ascribe due to different chromosome numbers, geographic origins, morphological characteristics, and strong cross incompatibility that exist between melon (*C. melo* L.) and cucumber (Pangalo, 1950; Ramachandran and Seshadri, 1986; Sujatha and Seshadri, 1989). The investigation of a novel, wild *Cucumis* species (e.g., 2n = 24) that provides a fertile bridge between cucumber and melon would be useful for characterizing *Cucumis* species relationships and taxonomy (Kirkbride, 1993; Parthasarathy and Sambandam, 1980; Trivedi and Roy, 1970).

The wild Asian *C. hystrix* was mentioned in some taxonomic and systematic studies (den Nijs and Visser, 1985; Staub et al., 1987; Dane, 1991). However, its morphology was never completely described until its rediscovery by J. F. Chen as reported by Chen et al. (1994). *Cucumis hystrix* was initially thought to be genetically similar to *C. sativus* var. *sativus*, and in fact was classified as a distinct species in the subgen. *Cucumis*, based on its morphological similarity with cucumber (Kirkbride, 1993). With the recent rediscovery of *C. hystrix* in isolated forests of Southern China (Chen et al., 1994), however, investigations have revealed that *C. hystrix* possesses a chromosome number of 2n = 24 (Chen et al., 1997a). Thus, *C. hystrix* is the first strictly Asian *Cucumis* species identified that possesses greater genetic affinities with *C. sativus* var. *sativus* in biochemistry than with *C. melo*, even though *C. hystrix* and *C. melo* possess the same number of chromosomes (Chen et al., 1995).

A cross between *C. hystrix* and *C. sativus* var. *sativus* has been successfully made (Chen et al., 1997a). The F_1 hybrid plants had a chromosome number of 2n = 19 (12 from *C. hystrix* and 7 from *C. sativus* var. *sativus*), and were both male and female sterile. Chromosome doubling of the F_1 hybrid plants restored partial or near-full fertility to progeny (Chen et al., 1997b), and further fertility selection resulted in the production of a primary amphidiploid (2n = 38) that regularly produced fertile flowers and set fruit with viable seeds (Chen and Kirkbride, 2000). Repeated production of representative amphidiploid plants resulted in their specific classification as *C. hytivus* by Chen and Kirkbride (Chen and Kirkbride, 2000).

The taxonomic placement of *C. hystrix* is of special interest because it bears a morphological

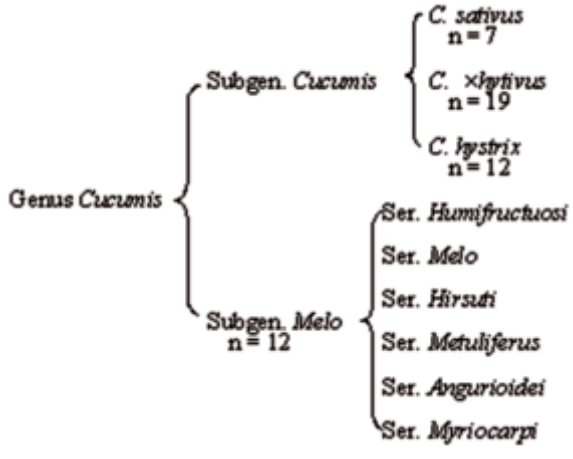

Fig. 1. The proposed *Cucumis* classitication system based on the present study and Kirkbride's (1993).

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Table 1. *Cucumis* species and varieties in the study.

Assigned code	Name	Chromosome no.	Source[z]
AC1	*C. sativus* var. *sativus* 'WI 1701'	2n = 2x = 14	UW
AC2	*C. sativus* var. *sativus* 'GY-14'	2n = 2x = 14	UW
AC3	*C. sativus* var. *sativus* 'GY-421'	2n = 2x = 14	UW
CC1	*C. sativus* var. *sativus* 'Erzhaozi'	2n = 2x = 14	CAAS
CC2	*C. sativus* var. *sativus* 'Baisitiao'	2n = 2x = 14	CAAS
CC3	*C. sativus* var. *sativus* 'Beijingjietou'	2n = 2x = 14	CAAS
CC4	*C. sativus* var. *sativus* 'Jinyan No. 4'	2n = 2x = 14	CAAS
CC5	*C. sativus* var. *xishuangbannesis* line 1	2n = 2x = 14	NJAU
CC6	*C. sativus* var. *xishuangbannesis* line 2	2n = 2x = 14	NJAU
CC7	*C. sativus* var. *xishuangbannesis* line 3	2n = 2x = 14	NJAU
EC1	*C. sativus* var .*sativus* 'Deltastar'	2n = 2x = 14	RZ
EC2	*C. sativus* var. *sativus* 'Harmonie'	2n = 2x = 14	RZ
EC3	*C. sativus* var. *sativus* 'Condesa'	2n = 2x = 14	RZ
WC2	*C. sativus* var. *hardwickii*	2n = 2x = 14	CU
H1-2	*C.* ×*hytivus* lines1	2n = 4x = 38	NJAU
H3-4	*C.* ×*hytivus* lines2	2n = 4x = 38	NJAU
H6	*C.* ×*hytivus* lines3	2n = 4x = 38	NJAU
C1-1-14	*C.* ×*hytivus* × C. sativus and self-pollinated lines1	2n = 2x = 14	NJAU
C1-3-15	*C.* ×*hytivus* × *C. sativus* and self-pollinated lines2	2n = 2x = 14	NJAU
C1-1-17	*C.* ×*hytivus* × *C. sativus* and self-pollinated lines3	2n = 2x = 14	NJAU
S1	*C. hystrix*	2n = 2x = 24	NJAU
M1	*C. Melo* var. *conomon* 'denggua'	2n = 2x = 24	NJAU
M3	*C. Melo* var. *melo* 'Vir'	2n = 2x = 24	H
M5	*C. Melo* var. *melo* 'Ogen'	2n = 2x = 24	H
M6	*C. Melo* var. *melo* 'GI'	2n = 2x = 24	H
M7	*C. Melo* var. *melo* 'huanghemi'	2n = 2x = 24	CAAS
M8	*C. Melo* var. *melo* 'GI × A_7'	2n = 2x = 24	CU
M9	*C. Melo* var. *melo* 'A_7'	2n = 2x = 24	CU
M14	*C. Melo* var. *melo* 'A_{14}'	2n = 2x = 24	CU
WM1	*C. agrestis*	2n = 2x = 24	CU
WM3	*C. metuliferus*	2n = 2x = 24	CU

[z]UW = Department of Horticulture, University of Wisconsin; CAAS = Chinese Academy of Agricultural Sciences; NJAU = Nanjing Agricultural University; RZ = Rijk Zwaan Seeds De Lier, The Netherlands; CU = Clemson University; H = Hollar Seeds, Rocky Ford, Colo.

and biochemical affinity to cucumber while its chromosome number is the same as in melon. The new synthetic species itself may be useful not only as a bridge species to transfer desirable characters into cucumber, but also as a breakthrough to deepen the study of the genetic relationship between the two basic chromosome numbers (n = 7 and n = 12) in *Cucumis* (Walters and Wehner, 2002). Chung et al. (2005) hypothesize that *C. hystrix* is a progenitor species of *C. sativus*, or that they at least share a common ancestral lineage. To investigate the phylogenetic relationships in Cucumis species, associations among 31 Cucumis accessions were investigated using random amplified polymorphism DNA (RAPD) markers. These RAPD results combined with the findings by Chung et al. (2005) were used to propose an appropriate reassignment of *C. hystrix* and *C. hytivus* in an adjusted *Cucumis* taxonomic system.

Materials and Methods

Plant materials. Table 1 lists the cultivated and wild *Cucumis* taxa used in this study. H1-2, H3-4, and H6 were *C.* ×*hytivus* lines. C1-1-14, C1-3-15, and C1-1-17 were progenies of self-pollinated amphidiploid lines from *C.* ×*hytivus* × cucumber. All accessions were grown in the middle of March 2001 on a farm of Nanjing Agricultural University, China. In late May, 15 plants of each accession were selected for sampling.

RAPD procedure. DNA from young leaf tissues of 15 plants of each accession was isolated by the modified CTAB method (Murray and Thompson, 1980) while only three plants of *C. hytivus* were used. RNA was removed by incubating with final concentration 100 μg·mL^{-1} RNaseA (Sangon Company, Shanghai, China) for 30 min at 37 °C. The DNA concentration was determined with ethidium bromide staining after electrophoresis.

PCR amplification was performed in a final volume of 20-μl containing 50 mM KCl, 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM dNTPs (Sangon Company, Shanghai, China), 0.4 μM 10-mer primer, 40 ng of DNA, and 1 unit of Taq DNA polymerase (Sangon Company, Shanghai, China) in a MJ Research PTC-100 thermocycler (MJ Resarch Inc., Chatham, New Jersey), with the protocol involving one cycle at 94 °C/4 min; three cycles at 94 °C/15 s, 35 °C/15 s, 72 °C/75 s; 40 cycles at 94 °C/15 s, 40 °C/15 s, 72 °C/75 s; one cycle at 72 °C/7 min; followed by 4 °C soak (Staub et al., 2000).

To select primers with both higher level of polymorphism and reproducible patterns, 220 decamer primers (Sangon Company, Shanghai, China; eleven sets of A, B, C, D, E, F, G, H, I, K, and N) were initially evaluated with 4 samples. Thirty one primers were used to detect polymorphisms in 31 accessions. The products were analyzed by electrophoresis in 1.2% agarose gel in 1×TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH 8.0) under 95 V for 1.5 h. The gels were stained with 5 μg·mL^{-1} ethidium bromide following the standard method (Sambrook et al., 1989) and immediately photographed with a Seagull camera (Shanghai, China) under UV light. The molecular weight of fragments was measured according to the standard marker DL2000 (Takara company, Daliang, China) with the Four-Star SX-100 gel image system (Shanghai, China).

Data analysis. Data were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence of a band. A pairwise similarity matrix was generated with SPSS 10.0 for Windows software using Jaccard's coefficient as follows: $J_{ij} = a/(a + b)$, where a = the number of bands in common to both accessions and b = the number of bands missing in one accession, but present in the other (Staub et al., 2000). The genetic distance (D) between two samples was calculated as: $D = 1 - J_{ij}$. Cluster analysis and multimensional scaling were performed on D estimates using the computer program NTSYSpc version 2.1 using the unweighted pair-group method using the arithmetic average (UPGMA) method.

Results

RAPD polymorphisms. Thirty-one primers that produced polymorphisms were used to analyze the genetic relationships of the 31 accessions. A total of 402 fragments could be scored with high confidence. The molecular weights of bands ranged from 200 to 3200 bp, and 96.3% of the markers were polymorphic. Only 15 bands were shared by all accessions.

From the amplified products, it was found that *C. hystrix* possessed 12 specific fragments, *C. hytivus* only 3, *C. sativus* var *hardwickii* 12, and *C. metuliferus* 18. An example of the amplification products using primer E20 is shown in Fig. 2. Marker E20-1750 bp (band A) distinguished *C. hystrix* from the other accessions.

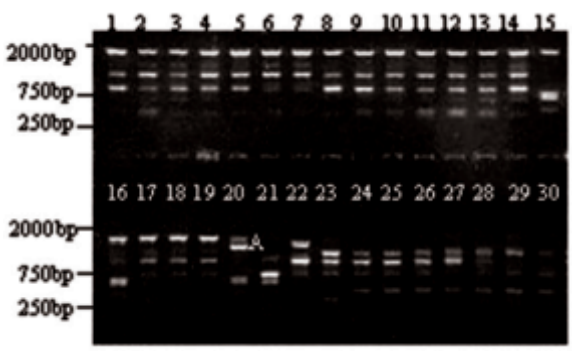

Fig. 2. RAPD profiles generated by primer E20 in 30 *Cucumis* accessions: 1 AC1, 2 AC2, 3 AC3, 4 CC1, 5 CC2, 6 CC3, 7 CC4, 8 CC5, 9 CC6, 10 CC7, 11 EC1, 12 EC2, 13 EC3, 14 WC1, 15 H1-2, 16 H6, 17 C1-1-14, 18 C1-3-15, 19 C1-1-17, 20 S1, 21 M1, 22 M3, 23 M5, 24 M6, 25 M7, 26 M8, 27 M9, 28 M14, 29 WM1, 30 WM3. Band A was the specific fragment of *C. hystrix.*

Species relationship analysis. Genetic distance (D) among 31 *Cucumis* accessions ranged from 0.06 to 0.78 (Fig. 2). *Cucumis hystrix* was closer to *C. sativus* (average D value of 0.51; range: 0.40 to 0.59) than *C. melo* (average D value of 0.70; range: 0.67 to 0.73). This result is consistent with morphological observations (Kirkbride, 1993) and isozyme analyses (Chen et al., 1995, 1997b). Average D value within cucumbers was 0.18 (range from 0.06 to 0.29). In the amphidiploids (*C. hytivus*), although half of its genome came from cucumber, the D value between them and *C. sativus* was 0.35 (range from 0.30 to 0.40), significantly larger than the D value within cucumbers. The average D value between progenies of *C. ×hytivus* (C1-1-14, C1-3-15, and C1-1-17) and *C. sativus* varieties was 0.28, similar to the D value between *C. sativus* var. *hardwickii* and var. *sativus*, var. *xishuangbannesis* (0.25).

Two main major accession groupings (designated CS and CM) were identified after cluster analysis and multidimensional scaling (Figs. 3 and 4). The *C. hystrix*, C. *sativus* var. *sativus*, and *C. ×hytivus* accessions examined grouped together in the CS cluster, which is a direct reflection of their above genetic distances. At a coefficient threshold of 0.23, these two main clusters groups were further partitioned into five smaller subcluster groupings. The first cluster consisted of different cucumber ecotypes (AC1-3, CC1-7, EC1-3 and WC2) and the progenies from backcrossing *C. ×hytivus* to cultivated cucumbers. While the lines of *C. ×hytivus* make the second cluster, *C. hystrix* alone makes the third cluster. The fourth cluster is one containing six var. *melo* cultigens, *C. agrestis* and var. *conomon*. *Cucumis metuliferus* was grouped into the fifth cluster.

European cultigens ('Deltastar', 'Harmonie', and 'Condesa') and American cultigens ('WI 1701', 'GY-14', and 'GY-421') were clustered together, indicating their similarity of origin. The three var. *xishuangbannesis* accessions (CC5~7) were clustered with the other Chinese cultivated cucumber ecotypes (CC1-4), which agrees with their geographical origin. The var. *hardwickii* and three lines of progenies of *C. ×hytivus* formed a branch outside the other cucumber entries.

C. metuliferus did not form a cluster with any other *C.* subgen. *Melo* species. The D value between *C. metuliferus* and *C. melo* was as large as 0.66 (range: 0.64 to 0.68), which is in agreement with a previous study by Staub et al. (Staub et al., 1997).

Discussion

Our previous studies revealed a phylogenetic relationships among *C. hystrix*, *C. sativus* and *C. melo*, based on morphology and isozyme variation (Chen et al., 1997a) and cross compatibility (Chen et al., 1997b). Chung et al. (2005) used nine chloroplast SSR (ccSSRs) markers to investigate the phylogenetic relationships among African *Cucumis* species (x = 12) accessions, *C melo* accessions, *C. sativus* accessions, and *Cucumis hystrix* accessions. Sequence variation analysis identified a group of African *Cucumis* species and a group composed of *C. melo*, *C. sativus*, and *C. hystrix* species leading to the conclusion that *C. hystrix* is a progenitor species of *C. sativus*, or that they at least share a common ancestral lineage. Based on the dendrogram produced from UPGMA clustering (Fig. 3) and the results of Chung et al. (2005), we propose that *C. hystrix* should remain in subgen. *Cucumis*, although it has a chromosome number different from that of *C. sativus*. With the interspecific hybrids as the third species, subgen. *Cucumis* is thus made up by three species. This proposed systematic system is shown in Fig. 1.

Cucumis hystrix and its interspecific hybrids (*C. ×hytivus*) are classified in subgen. *Cucumis*. Although the basic chromosome number and geographic location theories (Jeffery, 1980; Raamsdonk et al., 1989; Kirkbride, 1993) are challenged by the proposed system, the combined data presented herein along with those of Chung et al. (2005) provide strong support for it. The use of this revised system will likely assist in the exploitation of the wild *Cucumis* species in Asia. In fact, the new synthetic species itself (*C. ×hytivus*) may be useful not only as a bridge species to transfer desirable characters into cucumber, but also as a breakthrough to deepen the study of the genetic relationship between the two basic chromosome numbers (n = 7 and n = 12) in *Cucumis* (Walters and Wehner, 2002). Thus, it is important not only to improve the taxonomy of *Cucumis*, but for use in cucumber and melon breeding programs.

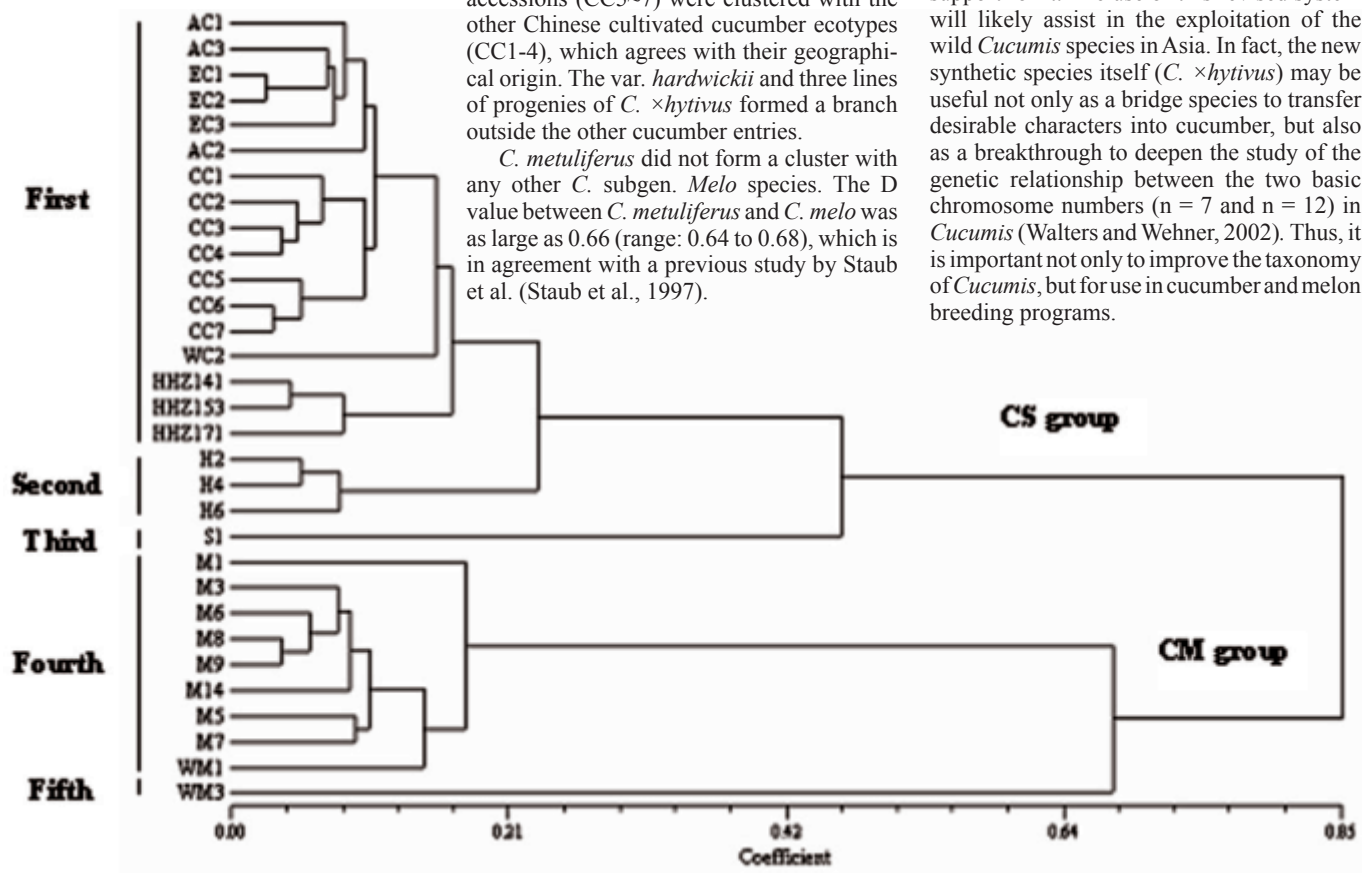

Fig. 3. Dendrogram derived from analysis of 31 *Cucumis* accessions based on RAPD data with UPGMA cluster analysis.

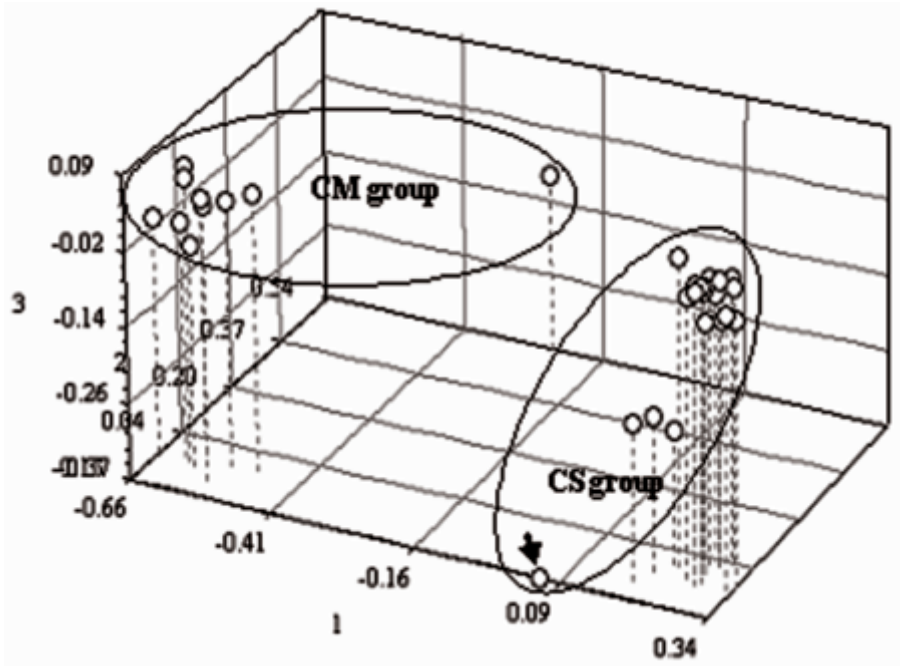

Fig. 4. Genetic relationships among 31 *Cucumis* accessions as depicted by mutimensional scaling of variation observed with 31 RAPD primers. Arrow indicates *C. hystrix*.

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