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## Distribution and genetic diversity of *Cucumis sativus* var. *hardwickii* (Royle) Alef in India

By I. S. BISHT<sup>1\*</sup>, K. V. BHAT<sup>1</sup>, S. P. S. TANWAR<sup>1</sup>, D. C. BHANDARI<sup>1</sup>, KAMAL JOSHI<sup>2</sup> and A. K. SHARMA<sup>2</sup>

<sup>1</sup>National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, India

<sup>2</sup>National Bureau of Plant Genetic Resources, Regional Station, Bhowali (UA)-263132, India

(e-mail: bishtis@nbpgr.delhi.nic.in)

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### SUMMARY

*Cucumis sativus* var. *hardwickii* (Royle) Alef, a wild sympatric botanical variety of *C. sativus* L. var. *sativus* has long grown in the foothills of the western Himalayas and is believed to be either a progenitor or feral relative of the cultivated cucumber. It is a source of several valuable genes for the improvement of cultivated cucumber but is poorly represented in major genebanks world over. A detailed inventory of *C. s.* var. *hardwickii* from various parts of the country was therefore made for collecting the germplasm accessions, and assessing the genetic diversity in the assembled germplasm. The species is widely and abundantly distributed in the north-western Himalayas, followed by fair distribution in the Western Ghats, and sporadic distribution in the Eastern Ghats, Chhota Nagpur plateau and the central Plateau region at elevations from 800 to 1700 m.a.s.l. Of the 41 accessions collected from these regions, a set of 29 representative accessions (including one cultivated landrace) were used for assessing the diversity for morphological and RAPD markers. Some of the populations were relatively more vigorous in growth, late maturing and had more laterals. High diversity for morphological as well as RAPD markers was observed, although the clustering of accessions with respect to the composition of specific groups was not identical. In each region, the presence of segregating populations of natural crosses between *C. s.* var. *hardwickii* × *C. s.* var. *sativus* were observed, indicating the existence of free gene flow between these two taxa. This underlines the importance of collecting more populations of the weedy races (natural hybrids). The results indicate the presence of high genetic diversity for useful traits, hence *C. s.* var. *hardwickii* has high potential for the improvement of cultivated cucumber.

The genus *Cucumis* contains about 30 annual and perennial species distributed over two geographically distinct areas in Asia and Africa (Kroon *et al.*, 1979; Esquinas-Alcazar and Gulick, 1983). It contains two major commercially grown vegetables, cucumber (*Cucumis sativus* L. var. *sativus*; hereafter referred to as *C. s.* var. *sativus*;  $2n=2x=14$ ) and melon (*Cucumis melo* L.;  $2n=2x=24$ ) (Jeffrey, 1980; Kirkbride, 1993). The cucumber is one of the oldest cultivated vegetable crops. It has been known in history for over 5000 years and probably originated in India (Whitaker and Davis, 1962; Wehner and Robinson, 1991). From India, the plant seems to have spread eastwards to China and westwards to Asia Minor, North Africa and Southern Europe. In geographic areas that historically have been reservoirs of genetic variation for cucumber, such as India (primary centre) and Myanmar-Southern China (secondary centre), *C. s.* var. *sativus* populations are subject to genetic erosion (Tatlioglu, 1993). *C. sativus* var. *hardwickii* (Royle) Alef (hereafter referred to as *C. s.* var. *hardwickii*;  $2n=2x=14$ ) is a wild sympatric botanical variety of *C. s.* var. *sativus* long reported to grow in foothills of western Himalayas and believed to be progenitor and feral relative of the cultivated cucumber (Deakin *et al.*, 1971). This botanical variety is cross-compatible with *C. s.* var. *sativus* and possesses a multiple fruiting and branching habit not present in the

cultivated cucumber (Horst and Lower, 1978). It therefore represents an extreme in variation in *C. sativus* and has potential for increasing the genetic diversity available for breeding commercial cucumber (Staub and Meglic, 1993).

Genetic markers (morphological, biochemical and molecular) have been employed for the characterization of genetic diversity present in cucumber (Knerr *et al.*, 1989; Meglic *et al.*, 1996; Staub *et al.*, 1997a,b). Assessment of genetic diversity in *C. s.* var. *sativus* and var. *hardwickii* using isozymes, restriction fragment length polymorphisms (RFLPs), and random amplified polymorphic DNAs (RAPDs) indicates that diversity in *C. s.* var. *sativus* is relatively low (3–8%) when compared with other cross-fertilized species of *Cucumis* (10–25%) (Dane, 1976, 1983; Esquinas-Alcazar, 1977; Dijkhuizen *et al.*, 1996; Horejsi *et al.*, 1998; Knerr *et al.*, 1989). Polymorphism level in *C. s.* var. *hardwickii* (17–25%) is predictably higher than *C. s.* var. *sativus* (Dijkhuizen *et al.*, 1996; Horejsi *et al.*, 1999). Therefore, an analysis of a more diverse collection of *C. s.* var. *hardwickii* would enhance our knowledge of the available diversity in this closely related species, which can be utilized in plant improvement programmes.

The records of *ex situ* collections in the US-NPGS shows presence of only five accessions of *C. s.* var. *hardwickii* four of which are from India (Meglic *et al.*, 1996). Considering the poor representation of its

\*Author for correspondence.

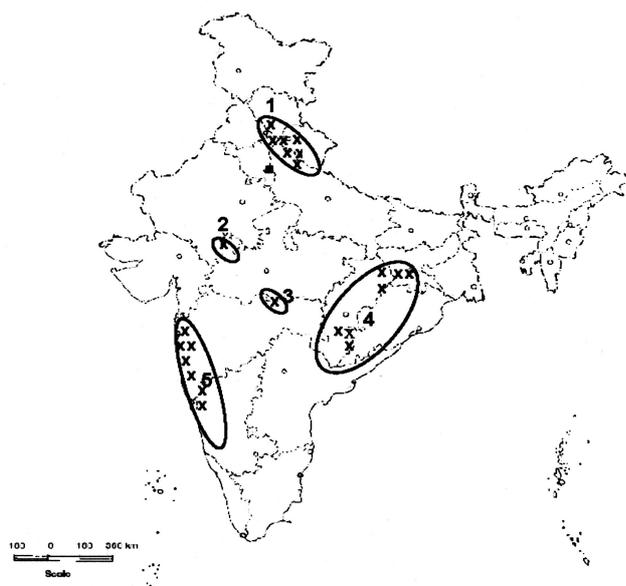


FIG. 1

Distribution of *C. s. var. hardwickii* in India ('x' indicates site of collection) 1 = Foothills of north-western Himalaya (parts of Uttaranchal and Himachal Pradesh), 2 = Mt. Abu (Rajasthan), 3 = Melghat (Maharashtra), 4 = Eastern Ghats (parts of Orissa and Chhatisgarh) and Chhota Nagpur plateau region, 5 = Western Ghats (parts of Maharashtra, Goa and Karnataka).

diversity even in the Indian national collection, this analysis was undertaken to estimate the frequency of occurrence of *C. s. var. hardwickii* in different phytogeographical regions and genetic diversity for this important wild relative of cucumber. In the present investigation therefore a detailed inventory of *C. s. var. hardwickii* from various parts of the country was conducted by collecting germplasm and assessing its genetic diversity.

## MATERIALS AND METHODS

### Survey and germplasm collecting

Germplasm exploration and collecting activities were undertaken during 1999 to 2003 from various parts of the country. The areas covered (Figure 1) by the exploration missions include the north-western Himalayas (parts of Uttaranchal and Himachal Pradesh); Chhota Nagpur Plateau region in Jharkhand state; parts of Rajasthan (Mt. Abu); central plateau region (parts of Madhya Pradesh, Chhatisgarh and Maharashtra); Western Ghats (parts of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu) and Eastern Ghats (parts of Orissa and Chhatisgarh). Explorations were conducted following a coarse grid survey. Detailed passport data on place of collection, latitude, longitude, altitude, frequency of occurrence, sample type, sampling method, associated vegetation type, habitat/ecology etc., ethnobotany for collected germplasm accessions (41 accessions) were recorded. From each location, random samples of fruits from 15 to 20 plants were bulked to constitute a population sample.

### Cytology

The collected germplasm was investigated cytologically by mitotic chromosome counts to ascertain the species status *vis-à-vis* *C. propeperum* L. (which is  $2n=24,48$ ). The mitotic chromosome spreads of root apices were prepared by the aceto-carmin squash technique.

### Morphological characterization of collected germplasm

The collected germplasm accessions (Table I), 28 populations of *C. s. var. hardwickii* and one landrace cultivar of *C. s. var. sativus*, representing different regions of the country were included in the present study. The original collections were first multiplied and then grown at the National Bureau of Plant Genetic Resources

TABLE I  
*C. s. var. hardwickii* germplasm analysed for morphological and molecular characterization, and their place of collection

S.No.	IC No.*	Place of collection	State
1.	IC-202049	Dehradun	Uttaranchal
2.	IC-202055	Dehradun	Uttaranchal
3.	IC-202058	Mussourie	Uttaranchal
4.	IC-202060	Mussourie	Uttaranchal
5.	IC-202063	Kotdwar	Uttaranchal
6.	IC-253909	Mt. Abu	Rajasthan
7.	IC-253915	Sunset Point, Mt. Abu	Rajasthan
8.	IC-253916*	Gaumukh Temple, Mt. Abu	Rajasthan
9.	IC-277000	Chikaladhara, Melghat	Maharashtra
10.	IC-277017	Khandala Ghat	Maharashtra
11.	IC-277029	Raigadh Fort	Maharashtra
12.	IC-277030	Raigadh	Maharashtra
13.	IC-277035	Ratnagiri	Maharashtra
14.	IC-277048	Ratnagiri	Maharashtra
15.	IC-277054	Panhala, Solapur	Maharashtra
16.	IC-331444	Jiva-ki-nadi, Jeypore	Orissa
17.	IC-331459	Achanakmar Reserve Forest, Bilaspur	Chhatisgarh
18.	IC-331465	Kabira, Sehdol	Madhya Pradesh
19.	IC-331609	Nagla Forest, Pantnagar	Uttaranchal
20.	IC-331616*	Rajgarh Road, Solan	Himachal Pradesh
21.	IC-331619*	Arki, Solan	Himachal Pradesh
22.	IC-331620	Arki, Solan ( <i>C. s. var. hardwickii</i> × <i>C. s. var. sativus</i> )	Himachal Pradesh
23.	IC-331626	Nahan, Sirmour	Himachal Pradesh
24.	IC-331627	Daundalakhond, Dehradun	Uttaranchal
25.	IC-331628	Rishikesh	Uttaranchal
26.	IC-331631	Kanda, Pauri Gharwal	Uttaranchal
27.	N/ASR-2092*	Bhowali	Uttaranchal
28.	IC-331443**	Koraput	Orissa
29.	IC-331445** (cv.)	Jeypore	Orissa

\* Accessions could not be included in RAPD analysis; \*\* Accessions could not be included in morphological characterization

TABLE II

List of descriptors recorded for morphological characters of *C. s. var. hardwickii* germplasm

Quantitative descriptors	
1.	Node of first flowering
2.	Days to flowering
3.	Days to maturity
4.	No. of laterals
5.	No. of fruits per plant
6.	Fruit weight (g)
7.	Fruit length (cm)
8.	Fruit width (cm)
9.	Pulp breadth (cm)
10.	No. of seeds per fruit
11.	1000-seed weight (g)
Qualitative descriptors	
12.	Early plant vigour (poor/intermediate/good)
13.	Stem pubescence (absent/present)
14.	Tendrils type (coiled/straight)
15.	Leaf shape (cordate/oblong/ovate/obovate/reniform)
16.	Leaf size (small/medium/large)
17.	Leaf pubescence density (no hairs/sparse/intermediate/dense)
18.	Sex type (monoecious/gynomonoecious/andromonoecious/gyno-andromonoecious/androecious/gynoecious)
19.	Flower colour (white/cream/yellow/orange)
20.	Fruit skin texture (plain/netted/rough)
21.	Fruit skin colour (cream/yellow/light green/dark green/orange/pink/brown)

(NBPGR) Regional Station, Bhowali (Uttaranchal) for morphological characterization during the 2002 cropping season (June to October). All the accessions were grown in a replicated trial (three replicates in randomized block design) following optimum agronomic practices. Data were recorded for 18 characters, both qualitative and quantitative (Table II) using IPGRI (International Plant Genetic Resources Institute) and NBPGR descriptors (Srivastava *et al.*, 2001). Data for quantitative traits were recorded on five randomly selected individuals per accession. Frequency distribution for qualitative characters and range, mean, and variance for quantitative traits were computed using INDOSTAT statistical package developed at the INDOSTAT Services, Hyderabad, India.

#### Multivariate analysis: Morphological characterization data

Data on means of 11 distinct quantitative characters (Table II) were subjected to multivariate analysis. Ward's Minimum Variance technique was used for cluster analysis. The data were also subjected to principal components analysis (PCA). The eigenvectors derived



FIG. 2

*C. s. var. hardwickii* population from wild habitat showing prolific fruiting.

were used to extract the first three most informative principal components. These three components were plotted in both three-dimensional and biplot mode. Only the biplots of the first two most informative components are presented here. Multivariate statistics were used to study the intra-specific diversity.

#### Molecular diversity analysis

**DNA extraction and purification:** Young actively growing leaves were harvested from the field-grown plants and used for extraction of total genomic DNA following the cetyl trimethylammonium bromide (CTAB) procedure as detailed in Saghai-Marouf *et al.* (1984). Leaves from about 10 to 15 plants per accession were bulked for constituting DNA sample of each accession. The DNA concentration (after *RNase A* treatment and phenol-chloroform extraction) was estimated with a DNA fluorimeter (Hoeffer Scientific, San Francisco, USA) using Hoechst 33258 as the DNA intercalating dye and calf thymus DNA as the standard (Brunk *et al.*, 1979).

**PCR optimization and primer survey:** The Polymerase Chain Reaction (PCR) amplification procedure was optimized by determining the most appropriate concentrations of template DNA (20 ng), Taq DNA polymerase (1 U) and  $Mg^{++}$  ion (2.5 mM) required to generate repeatable PCR amplification profiles. The random decamer primers suitable for generation of polymorphic amplification profiles among the *C. s. var. hardwickii* accessions were identified by screening the 40 primers from the commercially available OPA, OPB, OPC, OPD and OPM primers' series (Operon Technologies, Alameda, USA). Finally, a set of 10 decamer primers was selected as these primers generated polymorphic, consistent and reproducible patterns.

**PCR and agarose gel electrophoresis:** PCR reaction was carried out in a DNA Thermal Cycler (Gene Amp 9600 PCR system, Perkin-Elmer Cetus, Norwalk, CT, USA). Each 25  $\mu$ l reaction mixture contained 1  $\times$  reaction buffer (10 mM Tris-HCl, pH 8.3 and 50 mM KCl), 2.5 mM  $MgCl_2$ , 1 U of Taq DNA polymerase; 200  $\mu$ M each of dATP, dTTP, dCTP and dGTP (all reagents from



FIG. 3

Naturally occurring interspecific hybrid between *C. s. var. hardwickii*  $\times$  *C. s. var. sativus* (lane 5 in Fig. 6).

TABLE III  
Range of variation for various morphological characters

Character	Min.	Max.	Kurtosis	Skewness	Mean	CV(%)
Node of first flowering	3.0	9.0	-0.26	0.24	5.5	26.0
Days to flowering	65.0	115.0	-1.35*	-0.19	95.5	14.0
Days to maturity	88.0	130.0	10.68**	-2.40**	113.4	15.9
No. of laterals	3.0	7.0	-0.24	0.31	5.1	17.5
No. of fruits per plant	6.0	40.0	2.40**	0.95**	16.4	35.2
Fruit weight (g)	20.0	155.0	2.33**	1.22**	60.5	43.4
Fruit length (cm)	3.2	13.6	7.69**	2.27**	5.8	28.7
Fruit width (cm)	3.0	6.7	1.44**	0.93**	4.3	16.4
Pulp breadth (cm)	0.4	1.2	0.75	0.78**	0.6	31.4
No. of seeds per fruit	99.0	341.0	3.52**	-1.35**	220.9	29.2
1000-seed weight (g)	2.13	19.1	1.69**	0.67*	9.2	31.5
Early plant vigour (poor-29.6%, intermediate-11.1 %, good-59.3%)						
Stem pubescence (present-100%)						
Tendril type (coiled-100%)						
Leaf shape (cordate-100%)						
Leaf size (medium-100%)						
Leaf pubescence density (dense-100%)						
Sex type (monoecious-100%)						
Flower colour (yellow-100%)						
Fruit skin texture (plain-100%)						
Fruit skin colour (light green with yellow stripes-37.0%, dark green with yellow stripes-63%)						

Promega, USA); 0.6  $\mu$ M of decamer primer and approximately 20 ng of template DNA. The PCR amplification conditions were as follows: initial extended step of denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 32°C for 1 min and primer elongation at 72°C for 1 min, followed by an extended elongation step at 72°C for 5 min. Reaction products were mixed with 2.5  $\mu$ l of 10  $\times$  loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microfuge before gel loading (Sambrook *et al.*, 1989). The amplification products were electrophoresed in 1.8% agarose gel at 100 V followed

by staining with ethidium bromide and photographed on Polaroid 667 B/W film under ultraviolet light.

**Scoring and data analysis:** Each amplification product was considered a DNA marker. These were scored across all samples. Bands were recorded as present (1) or absent (0) across the lanes. Very faint bands were not considered for final scoring as these were inconsistent. Molecular weights of the bands were estimated by using the 100 bp DNA ladder or  $\lambda$  DNA/Hind III digest (Gibco BRL Life Technologies, New York, USA) as standard. All amplifications were repeated at least twice and only reproducible bands were considered for analyses.

TABLE IV  
Means of various quantitative characters of different *C. s. var. hardwickii* accessions

S. No.	Accession	Node of first flower	Days to 50% flowering	Days to 80% maturity	No. of laterals	No. of fruits/plant	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Pulp breadth (cm)	No. of seeds/fruits	1000-seed weight
1.	IC-202049	5.0	107.0	124.0	5.7	19.0	50.0	5.2	4.4	0.5	256.0	8.2
2.	IC-202055	5.7	113.3	126.7	5.7	11.3	56.3	5.8	4.0	0.5	192.7	10.5
3.	IC-202058	6.3	111.0	126.7	6.0	9.0	64.3	6.0	4.3	0.7	192.7	10.5
4.	IC-202060	6.0	104.7	123.0	6.0	17.7	94.2	7.0	5.1	1.0	227.7	10.9
5.	IC-202063	5.3	105.0	126.0	5.3	15.7	64.3	5.8	4.5	0.6	213.7	10.5
6.	IC-253909	6.0	113.7	113.0	4.0	17.7	66.7	6.2	4.7	0.6	221.0	10.9
7.	IC-253915	4.3	105.0	126.0	4.3	15.0	55.0	5.3	4.7	0.6	239.7	9.5
8.	IC-276991	7.0	103.0	111.7	4.3	17.0	62.6	6.4	4.2	0.7	232.0	16.2
9.	IC-277000	6.0	86.0	121.0	5.0	17.7	40.7	3.7	3.4	0.5	246.3	5.3
10.	IC-277017	6.0	95.0	124.0	4.7	19.0	39.6	4.1	3.6	0.6	206.3	3.9
11.	IC-277029	6.6	111.3	125.3	4.7	25.0	56.7	6.1	3.9	0.6	256.7	8.9
12.	IC-277030	5.3	97.7	124.7	5.3	15.0	99.7	9.9	4.8	1.0	190.7	12.4
13.	IC-277035	5.6	105.3	124.0	6.0	15.7	50.0	5.4	4.1	0.5	220.7	5.8
14.	IC-277048	6.0	103.3	126.0	6.0	23.3	37.7	4.6	3.4	0.5	196.7	6.8
15.	IC-277054	6.0	104.3	126.0	5.7	12.7	53.3	5.2	4.2	0.5	249.7	8.0
16.	IC-331444A	7.0	78.3	88.0	4.3	16.7	96.7	8.9	5.4	0.8	206.0	12.1
17.	IC-331459	5.3	84.0	106.7	4.7	15.7	37.0	4.2	3.6	0.7	204.0	8.3
18.	IC-331465	5.0	91.3	105.0	4.3	21.3	44.7	4.9	3.8	0.6	195.3	7.1
19.	IC-331609	3.3	77.7	88.7	6.0	15.3	43.3	4.9	4.4	0.5	225.3	8.1
20.	IC-331616	5.7	79.3	93.7	5.3	10.7	45.0	4.9	3.9	0.4	259.3	8.9
21.	IC-331619	3.7	78.3	93.3	5.3	17.0	58.3	5.7	4.5	0.4	241.0	9.3
22.	IC-331620	6.7	80.0	94.3	4.3	18.7	93.1	6.4	4.6	0.7	221.7	11.0
23.	IC-331626	3.3	81.7	98.3	5.3	18.7	95.3	8.3	5.4	0.8	187.3	12.3
24.	IC-331627	5.7	79.3	106.7	6.0	15.0	60.3	5.6	4.3	0.5	243.0	9.3
25.	IC-331628	3.3	82.7	106.7	4.3	14.7	56.7	4.4	3.9	0.4	257.0	8.6
26.	IC-331631	5.7	86.7	111.7	4.7	16.3	65.3	5.6	3.8	0.8	222.7	8.5
27.	N/ASR-2092	5.7	113.7	127.3	4.7	12.7	47.3	4.8	4.0	0.5	159.3	7.7
Overall Mean		5.5	95.5	113.4	5.1	16.4	60.5	5.8	4.3	0.6	220.9	9.2
C.V.(%)		21.6	4.2	11.3	14.39	33.2	24.4	18.4	12.7	18.5	18.3	16.6
C.D(5%)		1.9	6.6	20.6	1.21	8.9	22.9	1.7	0.8	0.2	62.5	2.5
Treatment MS (DF= 26)		3.3**	520.7***	658.8***	1.36**	42.8**	1495.0***	6.1***	0.9***	0.1***	8500.5***	20.8***
Error MS (DF=52)		1.4	16.3	158.6	0.54	17.9	194.6	1.1	0.3	0.01	1454.6	2.3

The two-way data matrix of varieties x amplicons was used to calculate pair-wise similarity coefficients following Jaccard (1908). This matrix of similarity coefficients was subjected to unweighted pair-group method analysis (UPGMA) to generate a dendrogram using average linkage procedure. The data matrix was used to calculate correlations among variables. These correlations were subjected to eigenvector analysis and the first three most informative principal components were extracted. Biplots of these three principal components were plotted in the three possible combinations to study the pattern of variations observed among the accessions. Only the biplot of first and second principal components is presented here. All the numerical taxonomic analyses were conducted using the computer programme NTSYS-pc, version 1.80 (Exeter Software, New York).

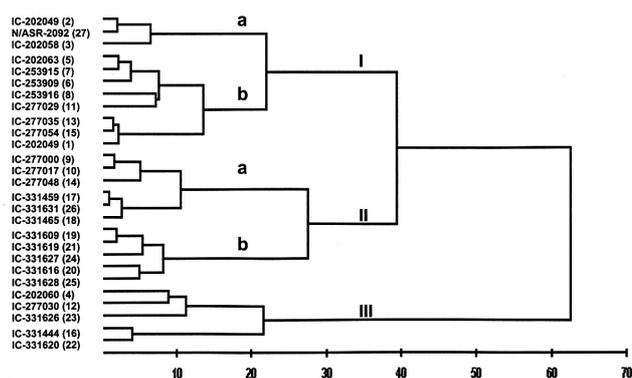


FIG. 4

Ward's minimum variance dendrogram of 27 *C. s. var. hardwickii* accessions based on morphological characterization.

## RESULTS

### *Distribution, habitat and ecology of wild C. s. var. hardwickii accessions*

Figure 1 shows the distribution of wild *C. s. var. hardwickii* species in India. Ecologically, it occurs in diverse associations and is commonly seen twining on bushes in forest scrub vegetation along water courses. The associated vegetation types are mainly sub-tropical wet hills and montane sub-tropical hill savannah. The species is widely distributed in foothills of north-western Himalayas in abundance followed by fair distribution in Western Ghats, and sporadic distribution in Eastern Ghats, Chhota Nagpur plateau and central Plateau region at elevations from 800 to 1700 masl. The accessions in pure wild habitats were less vigorous and had smaller fruits than those occurring in the proximity of cultivated fields in all geographical areas (Figure 2). The populations from the north-western Himalayas were more vigorous, in general, with more laterals, bigger fruits, bold seeds, low seed number per fruit and relatively high bitterness principle. Populations of natural interspecific hybrids between *C. s. var. sativus* and *C. s. var. hardwickii* were also collected from these areas, as weedy races, with relatively bigger and oblong fruits resembling *C. s. var. sativus* (Figure 3). The fruits are fed to animals (cows and buffaloes) for treatment of stomach disorders and other related ailments in all areas.

Cytological studies of the representative samples from all agro-ecological regions revealed similar chromosome counts of  $2n=14$  in both *C. s. var. hardwickii* and *C. s. var. sativus* accessions thereby confirming their identity.

### *Morphological characterization*

The range of variation in various morphological traits is presented in Table III. The populations did not differ much in qualitative traits except for plant vigour and fruit skin colour. A wide range of variation was observed for all the quantitative traits. A very high coefficient of variation was recorded for fruit weight, fruit number per plant, 1000-seed weight, pulp breadth, fruit length and node of first flower depicting more variation for these traits. Mean values for various characters in different accessions are shown in Table IV. Significant differences among accessions were observed for all the characters. Statistically significant variations among the populations were observed for the traits number of fruits per plant, fruit weight, fruit length and width, pulp width, seeds per fruit and 1000-seed weight.

Correlations were calculated between all the quantitative characters evaluated in this study and their coefficients are presented in Table V. Positive and significant correlations of fruit weight and 1000-seed weight were observed with fruit length, fruit width and pulp width.

Cluster analysis using Ward's Minimum Variance Technique (Figure 4) classified the accessions into three major groups. Clusters I and II comprised 11 accessions each followed by Cluster III with 5 accessions (Table VI). In general, poor association was found between geographical diversity and genetic diversity, whereas within clusters, some association was apparent, particularly in clusters I and II. Cluster I mainly comprised late flowering/maturing accessions with medium sized fruits. Cluster II was characterized by

TABLE V  
Correlation matrix for various quantitative traits of different *C. s. var. hardwickii* accessions

Characters	Node of first flower	Days to flowering	Days to maturity	No. of laterals	No. of fruits/plant	Five fruits weight	Fruit length	Fruit width	Pulp breadth	No. of seeds/fruits	1000-seed weight
Node of first flower	1.00	0.26*	0.19	-0.01	0.21*	0.09	-0.01	-0.18	0.07	0.05	0.13
Days to flowering		1.00	0.79**	0.06	-0.03	0.03	-0.01	-0.04	0.19	-0.22*	0.11
Days to maturity			1.00	0.18	-0.08	-0.08	-0.13	-0.22*	0.18	-0.23*	-0.10
No. of laterals				1.00	-0.17	-0.01	0.03	0.01	-0.04	-0.13	0.04
No. of fruits per plant					1.00	-0.09	-0.12	-0.15	-0.03	0.16	-0.10
Five fruit weight						1.00	0.82**	0.73**	0.58**	0.02	0.39**
Fruit length							1.00	0.77**	0.62**	0.02	0.33**
Fruit width								1.00	0.46**	0.14	0.39**
Pulp breadth									1.00	-0.03	0.31**
No. of seeds per fruit										1.00	0.03
1000-seed weight											1.00

Table VI

Means for various quantitative traits in different clusters, Euclidean cluster distances and number of accessions in each cluster

Characters	Cluster I	Cluster II	Cluster III
Node of first flowering	5.79	5.06	5.67
Days to flowering	108.45	85.79	88.47
Days to maturity	122.60	107.21	105.67
No. of laterals	5.12	5.12	5.07
No. of fruits per plant	15.69	17.09	16.53
Five fruit weight (g)	286.21	203.91	476.00
Fruit length (cm)	5.66	4.79	8.12
Fruit width (cm)	4.27	3.87	5.07
Pulp breadth (cm)	0.61	0.53	0.81
No. of seeds per fruit	213.15	223.15	219.07
1000-seed weight (g)	9.72	7.83	10.83
<i>Euclidean cluster distances</i>			
Cluster I	3.72	4.44	5.38
Cluster II		3.71	6.01
Cluster III			4.79
<i>Number of accessions/cluster</i>			
	11	11	5

accessions of medium maturity and relatively smaller fruits, more seeds per fruit and low seed weight. Cluster III comprised accessions with early maturity and relatively larger fruits, and include mainly the weedy races. The inter- and intra-cluster distances were the greatest for Cluster III (Table VI).

PCA performed on the standardized quantitative traits revealed that the first three most informative components accounted for 68.11% variation (Table VII). This table also shows the characters with greater weightings in each of the three principal components. Important characters with greater weightings in the PC axes I include fruit weight, fruit length, fruit width, pulp breadth and seed weight. Important characters with greater weightings in PC axes II include days to flowering and maturity, and number of seeds per fruit. Number of fruits per plant, number of laterals and node to first flower were the important characters with greater weightings in PC axes III. In general, the PCA confirmed the grouping of the accessions obtained through cluster analysis (Figure 5).

#### RAPD characterization

Of the 40 primers surveyed, ten were selected for analysis as others gave either suboptimal, indistinct or monomorphic amplification products. Table VIII gives the characteristics of the RAPD amplification products

TABLE VII

Principal components analysis using quantitative traits of different *C. s. var. hardwickii* accessions

Principal component	Eigen value	Total Variance		Eigen vectors for important traits
		%	Cumulative	
I	3.61	32.83	32.83	Fruit weight (0.94), fruit length (0.93), fruit width (0.88), pulp breadth (0.74), 1000-seed weight (0.69)
II	2.37	21.56	54.39	Days to maturity (0.92), days to flowering (0.89), number of seeds/fruit (-0.56)
III	1.51	13.72	68.11	Number of fruits/plant (0.66), no. of laterals (-0.65), node to first flowering (0.59)

2D Principal Components Plot

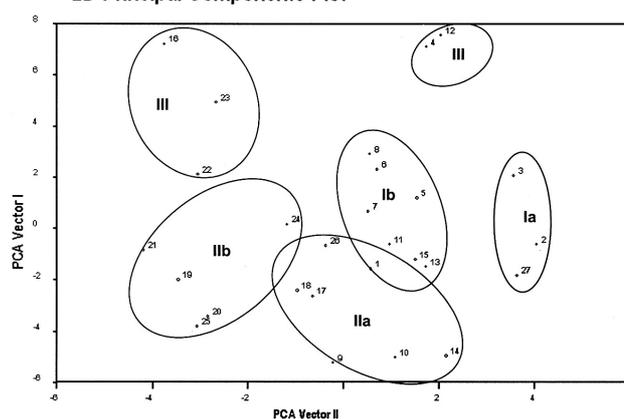


FIG. 5

Principal components ordination of 27 *C. s. var. hardwickii* accessions (groups are indicated as the pattern observed based on cluster analysis in Fig. 4).

obtained. A total of 95 amplification products were scored with the selected primers, which exhibited overall 90.50% polymorphism. The RAPD profiles obtained with two of the primers (OPA-10 and OPA-13) are presented in Figure 6, which is indicative of the extent of polymorphism observed among the *C. s. var. hardwickii* accessions. The average number of amplification products generated was 9.50 per primer with a maximum of 14 with OPN-16 and a minimum of 6 with OPA-5 and OPN-12. The size of the amplification products varied with primer used and the range was 0.25 kb to 3.0 kb. In general, the extent of polymorphism observed was high. Six out of 10 primers showed more than 90% polymorphism (OPA-08, OPA-10, OPC-13, OPC-20, OPD-02, OPD-05) and only two primers showed less than 70% polymorphism (OPA-5, OPN-12). The RAPD data obtained were subjected to UPGMA and principal components analysis to find out the relationship between the accessions analysed.

The average Jaccard's similarity coefficient between all possible comparisons of accessions was 0.638 and the clusters falling within this cut-off point were designated as distinct groups since the difference among the accessions within such groups was less than the inter-group difference. Figure 7 shows the clustering pattern obtained from the UPGMA analysis of the data. Overall, three distinct clusters were identified. Cluster I

TABLE VIII

Primers found suitable for genetic diversity analysis in *C. s. var. hardwickii* and the characteristics of the amplification products

S. No.	Primer designation*	Total no. of amplicons	No. of polymorphic bands	% polymorphism	Fragment size (kb)
1.	OPA-05	6	4	66.67	0.25-1.0
2.	OPA-08	7	7	100.00	0.40-1.0
3.	OPA-10	12	11	91.67	0.30-2.0
4.	OPA-13	9	8	88.89	0.40-1.3
5.	OPC-13	9	9	100.00	0.50-1.7
6.	OPC-20	12	11	91.67	0.40-2.0
7.	OPD-02	9	9	100.00	0.40-1.6
8.	OPD-05	11	11	100.00	0.45-3.0
9.	OPN-12	6	4	66.67	0.30-1.5
10.	OPN-16	14	12	85.71	0.40-2.5
Total		95	86	90.50	-

\*All primers were from M/S Operon Technologies, Alameda, USA.

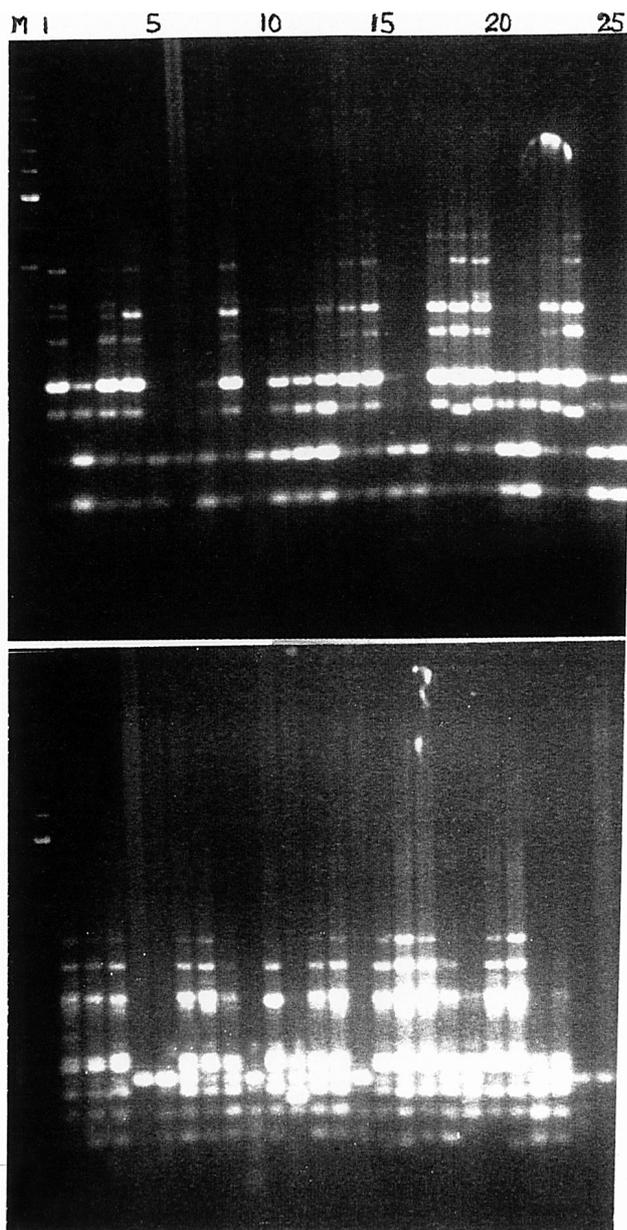


FIG. 6

RAPD profile of 25 accessions (23 *C. s. var. hardwickii*, 1 segregant of natural hybrid between *C. s. var. hardwickii* × *C. s. var. sativus* – lane 4 and 1 *C. s. var. sativus* – lane 3.) using OPA-10 (upper panel) and OPA-13 (lower panel) decamer primer (the lane marked M corresponds to the DNA molecular weight standard).

comprised 13 accessions followed by 7 accessions in cluster II and 3 accessions in cluster III. No strict association between clustering pattern and geographic origin of accessions was observed. However, cluster II, comprised mainly the accessions from Western Ghats. Two accessions, namely, IC-202060 and IC-202063 from north-western Himalayas were also distinct as they did not group with any of the three distinct clusters.

The results of principal component analysis (Figure 8) were comparable to the cluster analysis. A total of ten principal components were required to explain 100% of the total variation observed. The grouping of accessions in the biplot of principal components was comparable to the UPGMA dendrogram. The accessions were well distributed in the biplot indicating the presence of high

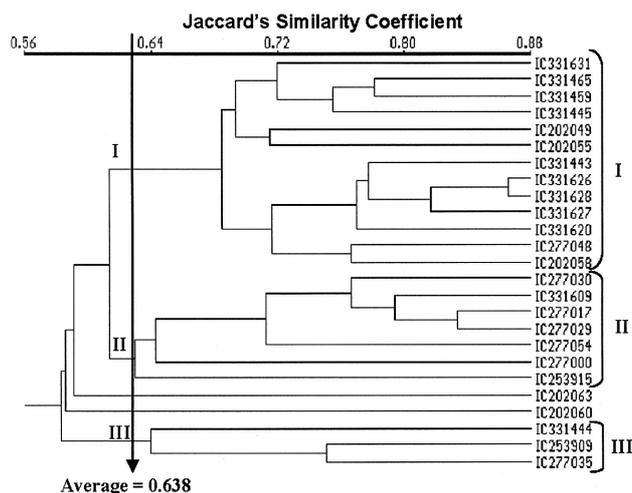


FIG. 7

UPGMA dendrogram of 25 accessions (23 *C. s. var. hardwickii*, 1 segregant of natural hybrid between *C. s. var. hardwickii* × *C. s. var. sativus* and 1 *C. s. var. sativus*) based on RAPD data.

genetic diversity for the RAPD markers studied. The first three most informative principal components explained 31.8, 23.6 and 15.6% of the total variation. Three accessions, namely, IC331444, IC253909 and IC277035 appear to be quite diverse from others in both the UPGMA-dendrogram and PCA-biplot. Further, diversity among the remaining accessions also appears to be substantially high as indicated by the similarity coefficient values (data not shown) between various accessions.

## DISCUSSION

This study has documented the widespread occurrence of *C. s. var. hardwickii* particularly in north-western India, Western Ghats, Eastern Ghats and at higher elevations in central plateau region and Chhota Nagpur plateau of the country. Its existence in parts of north-eastern region of India is yet to be explored. So far, in the existing literature, *C. s. var. hardwickii* was reported to

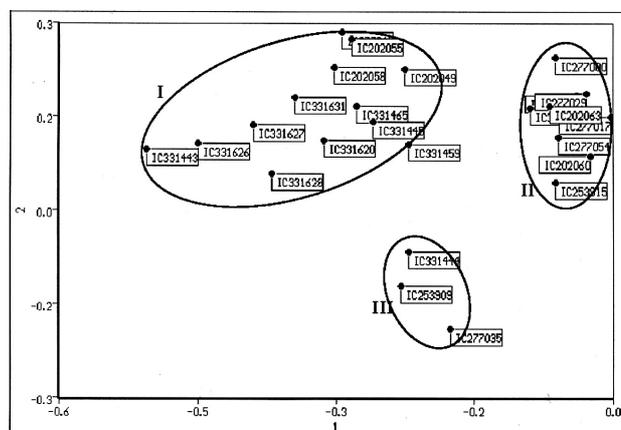


Fig. 8

Principal components ordination of 25 (23 *C. s. var. hardwickii*, 1 segregant of natural hybrid between *C. s. var. hardwickii* × *C. s. var. sativus* and 1 *C. s. var. sativus*) accessions (groups are indicated as the pattern observed based on cluster analysis in Fig. 7).

occur only in the foothills of western Himalayas (Arora and Nayar, 1984) and all four accessions of Indian origin represented in the US-NPGS collection at Ames, Iowa are from this region alone. The Indian National Genebank also had no representation of this species in its collection before this programme was initiated.

A wide range of variation was recorded in the *C. s. var. hardwickii* accessions for all the quantitative traits studied. Two populations from Western Ghats, namely, IC-277029 and IC-277048 recorded highest number of fruits per plant (Table IV). These populations were more vigorous in growth and late maturing, the latter accession also has higher laterals. Such populations could be donors of potential genes for yield enhancement in cultivated cucumbers. Three populations from north-western Himalayas (IC-202060, IC-331620 and IC-331626), one population each from Western Ghats (IC-277030) and Eastern Ghats (IC-331444), which occurred as weedy forms, had larger fruits with moderate numbers of laterals and fruits per plant. These hybrids or early generation segregates of the natural crosses between *C. s. var. hardwickii* and *C. s. var. sativus* indicate that free gene-flow occurs between the wild and cultivated forms. The presence of a high proportion of non-viable seeds and the bitterness principle in their fruits confirmed the occurrence of natural crosses between the cultivated and wild types. The segregating populations of these weedy races had longer fruits similar to cultivated cucumbers (Figure 3) indicating that *C. s. var. hardwickii* could be used effectively to increase both fruit size and fruit number.

Evaluation of the collections indicated that *C. s. var. hardwickii* possesses important and useful characters such as prolific fruit bearing, high numbers of laterals and disease resistance (data not presented here), which are of interest to breeders. Further, occasional crossing between the wild and cultivated forms leads to starting of a differentiation-hybridization cycle and release of more potential variability (Harlan, 1966). This is a remarkably elegant evolutionary process wherein barriers to gene flow maintain identity of the two types and, at the same time, limited exchange of genes occurs between the two which releases variability. Deliberate selection practiced by man from the released variability provides a new order of selection pressure making the population an array of deliberately chosen components (Harlan, 1966). In the present study, a few of the weedy races (IC-331620, IC-331626 and IC-202060) particularly from the north-western Himalayas also possessed resistance to biotic stresses (data not presented here) and have great agronomic potential for use in plant improvement programmes.

Cluster analysis and PCA of the morphological characters indicated presence of different diversity groups. The PCA ordination (Figure 5) confirms the grouping obtained through cluster analysis. Cluster I and Cluster IIa comprised accessions from all geographical areas while Cluster IIb comprised accessions from north-western Himalayas. Cluster III comprised three weedy races from north-western Himalayas and two from other parts of the country

Similar high diversity was observed for the RAPDs among the accessions. However, the RAPD clustering

pattern differed from the clustering based on morphological characteristics. The results indicated that cluster III comprising the accessions, IC-253909, IC-277035 and IC-331444 was the most diverse for RAPDs. The PCA also confirmed the UPGMA cluster analysis as these three accessions clustered distantly from others. One accession of cultivated cucumber landrace from Jeypore (Orissa), IC-331445, was found to cluster with the *C. s. var. hardwickii* accessions in cluster I which indicates a common genetic make up of these two taxonomic varieties.

Cultivated cucumber has a narrow genetic base and the *C. s. var. hardwickii* revealed higher genetic variation both in morphological and RAPD analysis. Similar high variation in *hardwickii* lines was also reported by several earlier workers (Dijkhuizen *et al.*, 1996; Meglic *et al.*, 1996). *C. s. var. hardwickii* hybridizes readily with *C. s. var. sativus*, producing fertile F<sub>1</sub> with no pronounced reduction of fertility in the F<sub>2</sub>. *C. s. var. hardwickii* sets more fruits per plant than *C. s. var. sativus* (Meglic *et al.*, 1996). *C. s. var. hardwickii* is considered useful for improving fruit yield in commercial cucumber (Deakin *et al.*, 1971; Horst, 1977; Staub and Kupper, 1985; Kupper and Staub, 1988). Selection for the fruit yield in the cultivated cucumber is a slow and costly process, as low genetic variance for fruit yield in processing cucumbers has been reported (Peterson, 1969, 1975; National Academy of Sciences, 1972; Ghaderi and Lower, 1979; Smith *et al.*, 1978; El-Shawaf and Baker, 1981). The significance of general combining ability effects, among the *C. s. var. sativus* and *C. s. var. hardwickii*, indicates that selection should be effective and that the desirable characteristics could be utilized in plant improvement programmes (Kupper and Staub, 1988). One accession of *C. s. var. hardwickii* line, LJ 90430, in the US collection has been used extensively in many breeding programmes in the USA for introgressing disease resistance in cultivated *C. s. var. sativus*. Resistance to root-knot nematode (*Meloidogyne javanica*) was also identified in this (Walters *et al.*, 1997). This line has been the only source for resistance genes and hence there is a need to identify alternate sources for utilization in breeding programmes. Cloning and expression of *fw2.2* gene for fruit weight from *Lycopersicon pennelli* to *L. esculentum* has demonstrated the utility of wild relatives as sources of useful genes for even yield related characters (Frary *et al.*, 2000). Even from this angle, the present evaluation data indicates that *C. s. var. hardwickii* is a good source of useful genes, which have failed to pass the domestication "bottle-neck".

The above account highlights the extent of diversity among populations of *C. s. var. hardwickii* in India. Pockets of diversity exhibiting both sympatric and disjunct distribution are important from an exploration standpoint, as is also their collection from ecologically variable sites within their range of distribution. The accessions from north-western Himalayas were relatively more diverse, in the present study. Since the species is distributed in this region in abundance, one is likely to get the germplasm for various adaptive traits including resistance to biotic stresses. Collecting more populations, particularly weedy races remains a priority, as these populations are important from both plant improvement and evolutionary standpoint. Areas with

sporadic occurrence also need extensive collection for build-up of more germplasm for storage in the genebank.

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