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# Genetic Diversity of a *Capsicum* Germplasm Collection from Nepal as Determined by Randomly Amplified Polymorphic DNA Markers

**J. Baral and P.W. Bosland**
*Department of Agronomy and Horticulture, MSC 3Q, New Mexico State University, Las Cruces, NM 88003-0003*

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Abstract. **Domesticated chile (*Capsicum annuum* L. var. *annuum*) is a widely cultivated spice and vegetable crop. It originated in the Western Hemisphere, but spread rapidly throughout the globe after the voyage of Columbus. However, very little is known about the genetic diversity of chile in Asia and especially in Nepal. Thus, research was conducted to document morphological as well as molecular characterization of *C. annuum* var. *annuum* landraces collected from Nepal. Genetic diversity in *C. annuum* var. *annuum* landraces from Nepal was investigated using randomly amplified polymorphic DNA (RAPD) markers and compared with that of *C. annuum* var. *annuum* landraces from the center of diversity, Mexico. RAPD marker based cluster analysis of *C. annuum* var. *annuum* clearly separated each accession. All accessions of *C. annuum* var. *annuum* from Nepal grouped into a single cluster at a similarity index value of 0.80, whereas, accessions from Mexico grouped into eight different clusters at the same similarity level indicating greater genetic diversity in Mexican accessions. RAPD analysis indicated that the Nepalese chile population went through an additional evolutionary bottleneck or founder effect probably due to intercontinental migrations. Some Nepalese accessions had unique RAPD markers suggesting that additional sources of genetic variation are available in Nepalese germplasm.**

Vavilov (1951) and subsequently Harlan (1971) conceptualized the theory of centers of diversity for crops. A center of diversity is a limited geographic area where a crop was domesticated and from which it was disbursed to other regions of the world. Mayr (1963) proposed the term "founder's principle" or "founder effect" for establishment of a new population by a few individuals, which carry only a portion of the total genetic variation of the parental population. *Capsicum* includes 25 species of which five have been domesticated (Bosland and Votava, 2000). These domesticated species differ from each other in floral morphology and furthermore in their distinct geographical distributions. The center of diversity for *C. annuum* L. is Mexico (IBPGR, 1983) from where it has become the most widely dispersed and cultivated *Capsicum* sp.

In the fifteenth century, chile (*Capsicum annuum* var. *annuum*) was introduced into Europe by Columbus, and subsequently it was spread to Asia and Africa (Bosland and Votava, 2000). In Asia, it was quickly incorporated into native cuisines. The complete acceptance of this new food crop in the region gave the erroneous impression to sixteenth century botanists that *Capsicum* originated in Asia (Fuchs, 1542). It is commonly accepted that Asia, southern central Europe, Africa, and parts of Latin America are secondary or tertiary centers for *C. annuum* (IBPGR, 1983).

Nepal has diverse geographical and ecological features. The rapid changes in elevation throughout the country play a significant role in fostering biological diversity. The existence of numerous uncharacterized chile cultivars within agricultural lands may serve as a valuable source of economically important genes. Nepalese farmers are preserving many landraces by growing them in their gardens. Systematic characterization and documentation of these landraces has not been accomplished. Therefore, it would be beneficial to know the genetic diversity of this germplasm. Gonzalez and Bosland (1991) reported the need for collection and characterization of these landraces to provide additional genetic resources. These resources could be utilized in transferring desirable traits to commercial cultivars.

*Capsicum* sp. have long been differentiated mainly on floral morphology (Hunziker, 1979). However, it is not always possible to discriminate among the different genotypes using only these traits and infer the interrelationship among accessions. Furthermore, some morphological traits are influenced by environment. Chromosome morphology (Pickersgill, 1971), electrophoresis of soluble proteins (Panda et al., 1986; Shin et al., 1989), and isozymes (Jensen et al., 1979; Loiaza et al., 1989) have been used to study diversity in the genus *Capsicum*. Recently, use of DNA based markers are being used increasingly for this purpose. Restriction fragment length polymorphism (Lefebre et al., 1993; Prince et al., 1992,1995), randomly amplified polymorphic DNA (Las Heras et al., 1996; Prince et al., 1995; Rodriguez et al., 1999), and amplified fragment length polymorphism (Nam et al., 1997; Paran et al., 1998) are the DNA based techniques being used currently to study genetic diversity of *Capsicum.*

Polymorphic markers can be generated by using a single universal 10-mer random primer. Unlike other polymerase chain reaction (PCR) based techniques, RAPD does not require target DNA sequence information for design of amplification primers. This technique is cost effective, fast, and independent of environmental variations. RAPD markers are mostly dominant. Presence or absence of one RAPD band is diagnostic of variation in the sequence within the primer binding sites in the target genome (Williams et al., 1990). Other sources of polymorphism may include deletion of a priming site, insertion that renders priming sites too distant to support amplification, or insertion that changes the size of the DNA segment without preventing its amplification (Williams et al., 1990). It has been reported that RAPD based characterizations have shown agreement with morphological trait based characterizations (Prince et al., 1995) in *Capsicum*. One problem that may arise with RAPD markers is the phenom-

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Table 1. Germplasm sources from Nepal and Mexico used to analyze genetic relationships among *Capsicum annuum* var. *annuum*.

| NMSU[z] accession no. | Synonym[y] | Source[x] |
|---|---|---|
| 99C1322 | Not available (NA) | Kathmandu, Market, Nepal |
| 99C1324 | NA | Kathmandu, Market, Nepal |
| 99C1327 | NA | Dhading Parewatar, Nepal |
| 99C1329 | NA | Kathmandu, Market, Nepal |
| 99C1330 | NA | Parewatar Dhading, Nepal |
| 99C1331 | Dhankt yellow | Hort Farm, Khumal, Nepal |
| 99C1332 | NA | Kathmandu, Market, Nepal |
| 99C1333 | NA | Kathmandu, Market, Nepal |
| 99C1334 | NA | Dhikure, Nuwakot, Nepal |
| 99C1335 | NA | Betrawati, Rasuwa, Nepal |
| 99C1336 | NA | Hort, Farm, Trishuli, Nepal |
| 99C1338 | Mariche smooth | Kalimati market, Nepal |
| 99C1342 | NA | Dhikure, Nuwakot, Nepal |
| 99C1344 | NA | Trishuli Bazar, Nepal |
| 99C1350 | NA | Jibanpur, Dhading, Nepal |
| 99C1353 | NA | Kalimati Market, Nepal |
| 99C1357 | NA | Trishuli Bazar, Nepal |
| 99C1360 | NA | Dhikure, Nuwakot, Nepal |
| 99C1361 | Dharan hot | Kalimati Bazar, , Nepal |
| 99C1362 | NA | Kalimati Bazar, Nepal |
| 99C1364 | NA | Trishuli, Bazar, Nepal |
| 99C1366 | NA | Hort Farm Trishuli, Nepal |
| 99C1373 | NA | Kalimati Bazar, Nepal |
| 99C1375 | NA | Betrawati Rasuwa, Nepal |
| 99C1377 | NA | Betrawati Rasuwa, Nepal |
| 99C1378 | Jire | Kalimati Bazar, Nepal |
| 99C1380 | NA | Trishuli Bazar, Nepal |
| 99C1383 | NA | Betrawati, Rasuwa, Nepal |
| 99C1388 | NA | Kathmandu, Market, Nepal |
| 99C1389 | NA | Kathmandu, Nepal |
| 99C1397 | NA | Hort Farm Trishuli, Nepal |
| 99C1398 | NA | Jibanpur, Dhading, Nepal |
| 99C1402 | Akbare | Pharping, Nepal |
| 99C1404 | Akbare | Pharping, Nepal |
| 99C1409 | Akase | Sundara, Nuwakot, Nepal |
| 99C1410 | Mainali local | Sundara, Nuwakot, Nepal |
| 99C1411 | Local Dhokre | Sundara, Nuwakot, Nepal |
| 99C1412 | Bagere | Nuwakot, Nepal |
| 99C1413 | NA | Nuwakot, Nepal |
| 99C1416 | Local large | Nuwakot, Nepal |
| 99C1422 | NA | Sanischare Jhapa |
| 99C1426 | CO2864 | AVRDC |
| 99C1428 | CO4202 | AVRDC |
| 99C1429 | CO4203 | AVRDC |
| 99C1430 | CO4203 | AVRDC |
| 99C1730 | Lamche | Ilam Phikal, Nepal |
| 99C1731 | Akase sopua | Ilam, Phikal, Nepal |
| 99C1732 | Sano Akase | Ilam, Phikal, Nepal |
| 99C1733 | Ghopte | Ilam, Phikal, Nepal |
| 99C1734 | Akbare | Ilam Phikal, Nepal |
| 99C1740 | BG38 | Mexico |
| 99C1741 | BG 676 | Mexico |
| 99C1742 | BG 912 | Mexico |
| 99C1743 | BG 913 | Mexico |
| 99C1744 | BG941 | Mexico |
| 99C1746 | BG 952 | Mexico |
| 99C1747 | BG 1404 | Mexico |

Table 1 (**continued**). Germplasm sources from Nepal and Mexico used to analyze genetic relationships among *Capsicum annuum* var. *annuum*.

| NMSU[z] accession no. | Synonym[y] | Source[x] |
|---|---|---|
| 99C1749 | BG 1518 | Mexico |
| 99C1750 | BG 1606 | Mexico |
| 99C1752 | BG 1649 | Mexico |
| 99C1755 | BG 1799 | Mexico |
| 99C1760 | BG 2676 | Mexico |
| 99C1764 | BG 3212 | Mexico |
| 99C1765 | BG 3302 | Mexico |
| 99C1766 | BG 3308 | Mexico |
| 99C1767 | BG 3405 | Mexico |
| 99C1768 | BG 3427 | Mexico |
| 99C1770 | Pabellon | Mexico |
| 99C1777 | Pulla chile | Mexico |
| 99C1778 | Chilhuacle | Mexico |
| 99C1779 | NA | Onza, Mexico |
| 99C1780 | NA | Costeno, Mexico |
| 99C1737 | NA | Chimayo, New Mexico |
| 99C1738 | NA | El Guique, New Mexico |
| 99C1739 | NA | Chimayo, New Mexico |
| 99C1773 | NA | Alcalde, New Mexico |
| 99C1774 | NA | Alcalde, New Mexico |
| 99C1776 | NA | Alcalde, New Mexico |
| 99C1464 | Red, rocoto (*C. pubscens*) | AVRDC |
| 99C1465 | Rocoto yellow (*C. pubescens*) | AVRDC |
| 99C1433 | PI260579 (*C. baccatum*) | AVRDC |
| 99C1436 | PBC188 (*C. chinense*) | AVRDC |
| 99C1425 | Akbare (*C. chinense*) | Nepal |

[z]New Mexico State University.
[y]Name local name or entry number of the collecting institution.
[x]NM = New Mexico and AVRDC = Asian Vegetable and Research and Development Center, Taipei, Republic of China.

enon of homoplasy, such that bands of similar electrophoretic mobility in a RAPD profile may correspond to nonhomologous DNA sequences (Stammers et al., 1994). Homology tests of the RAPD fragments conducted on soybean species (G*lycine* L. sp.) (Williams et al., 1993), cruciferous species (Thorman et al., 1994), and sunflower species (*Helianthus* L. sp.) (Rieseberg, 1996) have shown that RAPD bands of the same fragment size indeed represent homology when closely related species or populations are considered. Because of its ability to amplify DNA from dispersed polymorphic loci (Williams et al., 1990) and its power to detect small genetic differences, the RAPD method was used to characterize the genetic differences among the accessions of *Capsicum annuum* var. *annuum* from Nepal relative to accessions of *C. annuum* var. *annuum* from Mexico.

## Materials and Methods

**Plant material.** In 1997, 111 accessions of Nepalese chile were collected from various agro-climatic regions of Nepal (Baral, unpublished data). Chile fruit (>15 fruit per accession) were collected for each accession from farmers' fields as well as from various local markets. In addition to the Nepalese accessions, 28 landraces of *C. annuum* var. *annuum* collected in Mexico and New Mexico, USA were obtained from the New Mexico State University *Capsicum* Breeding and Genetics Program. These were selected to represent various geographical areas. From the Asian Vegetable Research and Development Center (AVRDC) Taipei, Republic of China, seven C. *annuum* var. *annuum* accessions collected previously in Nepal were obtained. Four accessions from three different species were included as an outgroup. The first two accessions were from *Capsicum chinense* Jacq., which also includes pod types such as habanero and scotch bonnet. The second species commonly referred to as aji was representative of *Capsicum baccatum* L. var. *pendulum*. The third species, commonly called manzano or rocoto (*Capsicum pubescens* Ruiz and Pav.) contained one accession. These outgroup accessions were necessary to confirm the monophyletic relationship among *C. annuum* var. *annuum* accessions. Each accession used in this analysis was assigned a New Mexico State University (NMSU) accession number. Their corresponding local names or entry numbers are listed in Table 1.

**Plant cultivation.** During June and July 1999, seeds were sown in 3.9 × 2.7 × 5.5 cm compartments of 12-celled bedding plant containers (Hummert International, Earth City, Mo.) containing a commercially prepared peat moss-vermiculite mixture (Peatlite, Scott-Sierra Horticultural Products Co. Marysville, Ohio). To promote seed germination the trays were placed in a greenhouse on propagation pads that maintained a soil temperature of 30 ± 2 °C. The trays were watered daily as needed. Following germination, ≈1 g of slow-release fertilizer (14N–4.2P–11.6K Osmocote, Scott's-Sierra Hort. Products) was topdressed on the surface of each cell. When the seedlings developed six to eight true leaves, they were transplanted to 6-L pots filled with a medium of 1 soil : 1 sand : 1 peat (by volume). Three plants were transplanted to each pot and a maximum of 18 plants per accession were transplanted. These plants were grown in the greenhouse. Maximum/minimum greenhouse temperatures were 29/18 °C. Plants were grown under natural

light in the greenhouse roofed by transparent polyvinyl sheets. Each pot received ≈54 g of a slow-release fertilizer (Osmocote) split among three applications, i.e., at transplanting, at active vegetative growth, and at flowering.

Data for nine morphological characters were collected from the Nepalese accessions using International Plant Genetic Resources Institute *Capsicum* descriptors (IPGRI, 1995). The morphological characters examined included number of flowers per axil, flower position, calyx constriction, filament color, anther color, stigma exertion, ripe fruit color, fruit shape, and seed color. Data were also collected for absence or presence of pungency. The purpose of morphological study was to classify the Nepalese accessions to the species level. Only those accessions belonging to *C. annuum* var. *annuum* were included for RAPD analyses.

**DNA extraction and RAPD analysis.** Leaf disks 9 mm in diameter were removed from immature leaves using the cap of a 1.5 mL sterile Eppendorf tube. A single leaf disk from each of five plants within an accession was collected and placed in an Eppendorf tube. The tubes containing the leaf disks were immersed immediately into liquid nitrogen and stored at –80 °C. DNA was isolated using a hexadecyl-tri-methyl-ammonium-bromide (CTAB) isolation method as described by Richter et al. (1991) with some modifications. Modifications involved miniaturization of the original protocol to fit into a 1.5 mL Eppendorf tube (Votava et al., 1996). The isolated DNA was quantified using a fluorometer (Dynaquant-200; Hoefer Pharmacia Biotech Inc. San Francisco). After quantification, the DNA was diluted to a concentration of 10 ng·mL$^{-1}$ using tris-EDTA buffer (10 mM Tris, 0.1 mM ethylenediamine tetra-acetic acid (EDTA), pH 8), and stored at 4 °C for further use in PCR reactions.

Random primers (10 base pairs) were obtained from Operon Technology, Alameda, Calif. To detect polymorphisms among *C. annuum* var. *annuum* accessions, 55 primers were tested. Of these, 18 informative primers that supported amplification of polymorphic loci were subsequently used in this study. The primers were OPA-04, OPA-07, OPA-09, OPA-11, OPA-16, OPA-17, OPA-20, OPAA-11, OPAK-10, OPC-05, OPC-06, OPE-02, OPE-05, OPE-12, OPM-02, OPM-04, OPM-09, and OPP-1. The RAPD reaction mixtures and PCR cycling conditions were as described by Francisco and Isabel (1997). The total volume of each PCR reaction was 20 mL consisting of 20 ng template DNA, 10 mM Tris-HCl, 10 mM KCl, 356 mM $MgCl_2$, 101 mM each DNTPs, two units Stoffel fragment DNA polymerase, and 0.24 mM primers. There was a preliminary step of 2 min at 94 °C to denature template DNA. The first 10 cycles consisted of 30 s at 94 °C for denaturation (ramp, 1.5 °C·s to reach 55 °C), 1 min at 55 °C for primer annealing (ramp, 1.5 min to reach 72 °C), and 4.5 min at 72 °C for DNA synthesis. The primer annealing temperature in the first 10 cycles was reduced by 1 °C·s$^{-1}$ in each cycle up to 46 °C. The next 25 cycles (from cycle 11 to 35) also followed the same thermal profile except the annealing temperature was fixed at 45 °C. A final step of 1 min at 72 °C for final extension was followed by holding indefinitely at 4 °C.

The PCR products were separated in 3% agarose gels with TBE buffer (89 mM Tris-borate, 2.5 mM EDTA, pH 8). The PCR product loaded in the last lane of the previous gel and first lane in the subsequent gel were duplicate samples (same template used in both reactions). The purpose for this particular duplication was to provide an internal control in scoring RAPD bands from more than one gel. The gels were stained with ethidium bromide, viewed under the ultraviolet trans-illuminator, and then photographed. The presence or absence of a RAPD band was scored as 1 or 0, respectively. To evaluate reliability and to ensure reproducibility of the reactions, 10% of the total accessions were duplicated in every run of the PCR. In each PCR run, there was a negative control containing all PCR ingredients but not the template DNA. The RAPD-PCR was repeated when it failed to produce identical bands in all pairs of duplicate samples. A matrix of the RAPD data (accession by RAPD score) was generated and similarity coefficients for all pairwise combinations of 82 accessions were calculated using the Simqual program of the software package Numerical Taxonomy and Multivariate Analysis System, NTSYS-pc ver.2.0 (Rohlf, 1998). The program calculated the similarity coefficient using Dice's formula (Dice, 1945): Similarity = 2a/2a + b + c, where a = number of bands present in both $i^{th}$ and $j^{th}$ accessions, b = number of bands present only in $i^{th}$ accession, and c = number of bands present only in $j^{th}$ accession.

The similarity value lies between 0 and 1. A similarity value of 1 indicates complete or 100% genetic similarity between two accessions. A similarity value of 0 indicates no genetic similarity between two accessions. From the Dice similarity matrix, sequential, agglomerative, hierarchical, and nested (SAHN) clustering was performed using the unweighted pair-group method with arithmetic averages (UPGMA) method by the SAHN clustering program of NTSYSpc (Rohlf, 1998) The result of clustering was plotted in the form of a dendrogram by the graphics program (tree option) of NTSYSpc (Rohlf, 1998).

## Results

**Morphological characterization.** Morphological characterization was sufficiently diagnostic to classify each Nepalese accession to a *Capsicum* spp. Among the Nepalese accessions, 78 accessions were classified as *C. annuum* var. *annuum,* 15 were *C. chinense*, and 9 were *C. frutescens* (the most familiar type of this species being tabasco pepper). A total of 11 accessions did not germinate. All accessions from Nepal were pungent.

**RAPD analysis.** All duplicate samples produced the same RAPD profile. However, in some cases a particular lane was blank or gave distorted bands indicating these errors were not associated with the RAPD analysis but due to technical problems associated with PCR or electrophoresis. A total of 146 polymorphic bands were

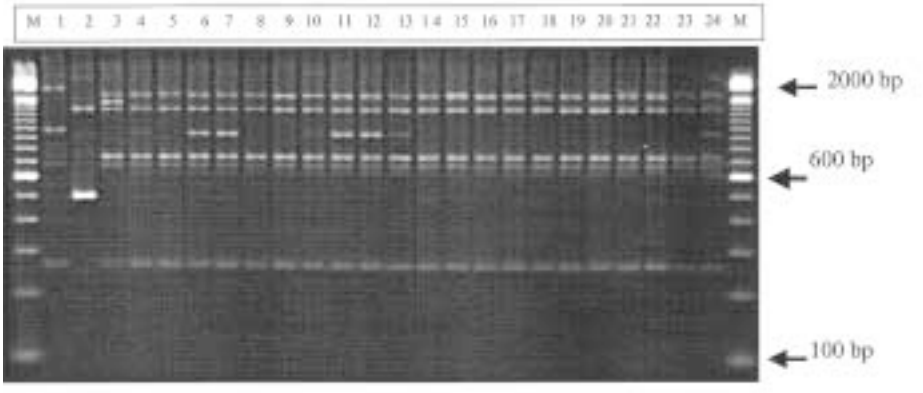


Fig. 1. DNA polymorphism detected by random primer OPA-11. M = 100 bp size marker. Lane 1 = *Capsicum pubescens*, lane 2 = *Capsicum baccatum*, lane 3 = *Capsicum chinense,* lanes 4 to 13 = *Capsicum annuum* from Mexico, lanes14 to 24 = *Capsicum annuum* from Nepal, and lanes 11 and 12 as well as lanes 19 and 20 represent duplicate samples.

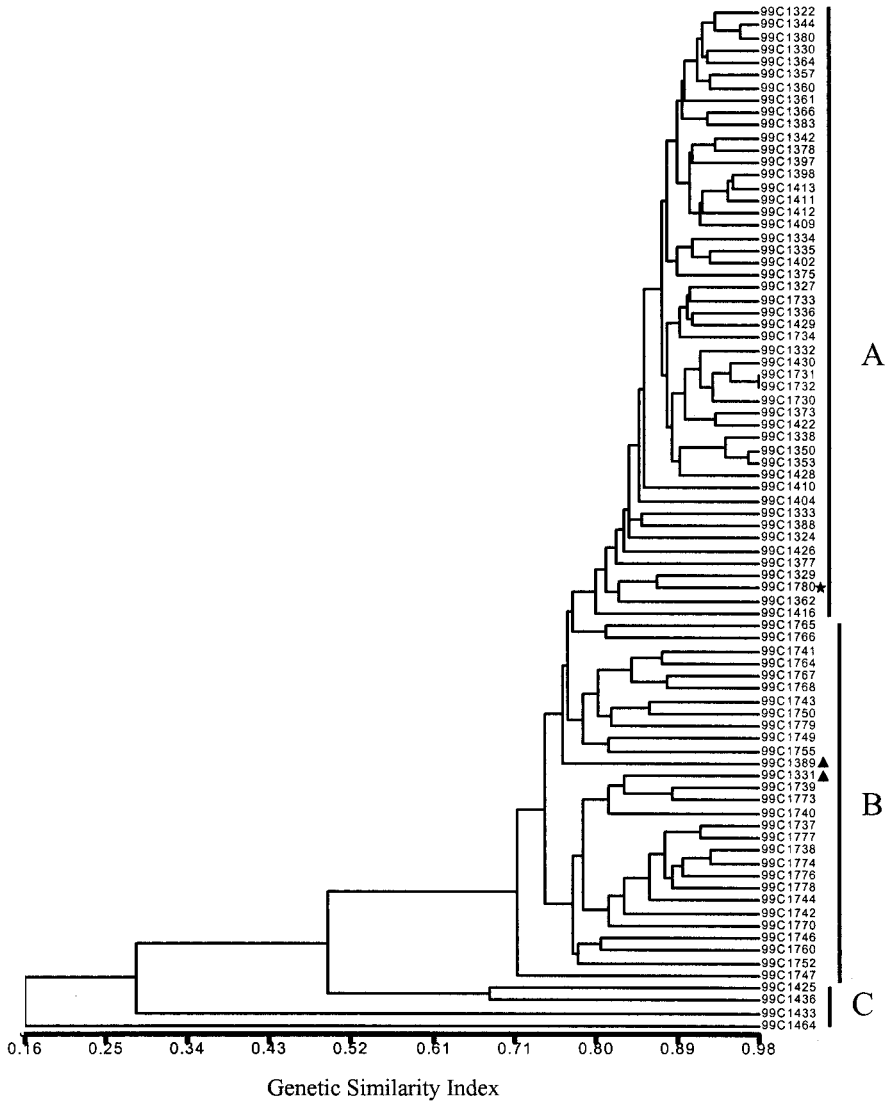


Fig. 2. Dendrogram showing genetic relationship among *Capsicum annuum* accessions. (A) Accessions from Nepal (B) accessions from Mexico and New Mexico, (C) outgroups; solid triangle = Nepalese accessions that did not cluster with other Nepalese accessions, star = Mexican accession that did not cluster with the other Mexican accessions.

scored among the *Capsicum* accessions with 18 primers. An average of 70% of the total amplification products were constantly present among all the *C. annuum* accessions (monomorphic). A total of 94 polymorphic RAPD bands were found among the *C. annuum* var. *annuum* accessions from Nepal, Mexico, and New Mexico. Some of the rare (unique) RAPD markers were present only in a few accessions from either Nepal or Mexico. No single RAPD marker was uniformly present in one subpopulation and uniformly absent in the other sub-population, or vice versa. Among the RAPD bands scored, nine were unique to Nepalese accessions and 21 were unique to the Mexico and New Mexico accessions. Only 73 RAPD bands out of 146 scored were present among the Nepalese *C. annuum* var. *annuum* accessions (50 accessions) whereas 85 were present among the accessions from Mexico and New Mexico (28 accessions). A typical electrophoretic gel showing polymorphism detected by primer OPA-11 among *Capsicum* sp. is illustrated in Fig. 1.

**Genetic similarity.** By using the NTSYS Simqual program (Rholf, 1998), the pairwise genetic similarity between all accessions was calculated. As expected, the *C. pubescens* accession (99C1464) had the lowest genetic similarity value when compared to the other accessions. The *C. baccatum* accession, 99C1433, had the next lowest similarity value. The average genetic similarity value among all *C. annuum* var. *annuum* accessions was 0.79 with a range of 0.59 to 0.98. The average genetic similarity value among the Nepalese *C. annuum* var. *annuum* accessions was 0.85. Within Nepalese accessions, the genetic similarity values ranged from 0.62 for 99C1331 and 99C1734 to 0.98 for 99C1731 and 99C1732. Likewise, the average genetic similarity value among the Mexican and New Mexican accessions of *C. annuum* was 0.75 with a range of 0.59 to 0.93.

**Cluster analysis.** The NTSYS, SAHN clustering program (Rholf, 1998) used the UPGMA method for cluster analysis and the final output is represented in the form of a dendrogram (Fig. 2). All the outgroup accessions used in this analysis grouped outside the *C. annuum* var. *annuum* cluster. All *C. annuum* var. *annuum* accessions grouped into a single cluster at a similarity index value of 0.71, providing molecular evidence for the monophyletic relationship among *C. annuum*.

All the accessions of *C. annuum* var. *annuum* from Nepal except for two, grouped into a single cluster at a similarity index value of 0.80, whereas, accessions from Mexico, grouped into eight different clusters at the same similarity level (Fig. 2). At the similarity index value of 0.76 all accessions from Nepal and 11 accessions from Mexico grouped into a single cluster whereas 16 accessions from Mexico and New Mexico formed another distinct cluster at similarity index value of 0.78. These two clusters came together at a similarity index value of 0.74. Of the two accessions from Nepal, 99C1389 formed a separate cluster at similarity index value of 0.77 whereas 99C1331 clustered with accessions from Mexico and New Mexico. Similarly, one accession from Mexico, 99C1780, clustered with accessions from Nepal. The accessions grouped exceptionally well according to the two geographic regions. Accessions from Nepal formed one group, while accessions from Mexico and New Mexico, USA formed two separate and distinct groups. Of the Nepalese accessions, 96% were found to be more similar to one another than to any of the Mexican accessions. Among the Mexican accessions, two clusters formed, one contained 57% of the accessions and the other one contained 39%. The latter cluster was more similar to the Nepalese accessions. No association was found between the Nepalese *C. annuum* var. *annuum* accessions and geographical regions of Nepal.

## Discussion

Taxonomic classification based on morphology indicated that *C. annuum* var. *annuum* was the dominant species of chile growing in Nepal. In fact, other species represent only a negligible proportion of chile production in Nepal (Baral, personal observation). The second most prevalent chile species in Nepal

was *C. chinense*. However, its production was limited to home gardens. Variation in fruit shape in this species was small. The fruit were mostly round or slightly tapering round. The last and least prevalent species found in Nepal was *C. frutescens*. Two types of *C. frutescens* were found in Nepal; one having short thick fruit pressed at the middle and the other with a very small malagueta-like fruit type (DeWitt and Bosland, 1996). The small-fruited one (malagueta-like) was popular in rural areas. An interesting point is that in Nepal, the malagueta-like accessions were not cultivated, but seem to have become naturalized, growing around farmland premises and nearby bushy areas (Baral, personal observation). The other two domesticated species, *C. baccatum* and *C. pubescens* were not found in Nepal.

When the original RAPD data were analyzed, accession 99C1425 from Nepal did not cluster with the *C. annuum* var. *annuum* cluster. This accession was obtained from AVRDC labeled as *C. annuum*. However, the accession clustered with the other known *C. chinense* accession used in the study. After studying morphological traits, this accession was determined to be *C. chinense* and not *C. annuum* (Bosland, personal observation). This suggests that the RAPD analysis method is very specific and powerful in detecting differences at the species level.

The RAPD analysis supports the fact that *C. annuum* var. *annuum* accessions from Nepal possess genetic variation. However, the level of genetic variation within *C. annuum* var. *annuum* is low as reflected by the high average genetic similarity values among Nepalese accessions. The magnitude of genetic similarity among Nepalese accessions (85%) is higher than that of the Mexican accessions (75%). Formation of a separate and distinct cluster of Nepalese accessions reveals that these are closely related genetically. The analysis showed that 11 accessions from Mexico have higher similarity with Nepalese accessions than with the other Mexican accessions. This could suggest that the area from where these Mexican accessions originated might be the source region for the *Capsicum annuum* introductions in Nepal.

Despite diverse geographical and ecological features, the clustering pattern of Nepalese *Capsicum annuum* var. *annuum* accessions was not completely according to the geographical regions of Nepal. This situation may indicate the recent movement of landraces across the country. Many accessions included in this were collected from various local markets. Thus, it was not possible to assign exact geographical locations for the origin of these samples. Some Nepalese accessions included in this study were obtained from AVRDC, and no specific location of origin is available for these accessions.

Even though great variations exist in fruit characters within the Nepalese accessions, the DNA variation was quite low. This variation might be the effect of disruptive selection on fruit characters. Pickersgill (1997) reported that the founder effect associated with domestication has restricted variation in less visible characters. Pickersgill (1997) found at low variation in karyotype among domesticated *C. annuum* var *annuum* accessions when compared with their wild counterpart (*C. annuum* var *glabrisculum)*. Founder effect associated with intercontinental migrations has reduced diversity still further in the area of introduction (Pickersgill, 1997). The grouping of all Nepalese accessions (except for two) into a single cluster at the high similarity index value of 0.80 may suggest that additional founder effect was instrumental in the introduction of chile to Nepal. Further evidence for the founder effect could be the fewer number of RAPD alleles present in the Nepalese *C. annuum* var. *annuum* population as compared to the Mexican *C. annuum* var. *annuum* population. Similarly, lack of nonpungent accessions in the Nepalese population could also be considered evidence of the founder effect.

The present study has revealed that the *C. annuum* var. *annuum* gene pool in Nepal is accumulating new variations. Occurrence of mutation followed by fixation of the mutated allele might be responsible for creation of novel RAPD alleles in Nepal. At least two accessions from Nepal had fruit types typical of other countries. Fruit of accession 99C1389 resembles a Korean fruit type and fruit of 99C1331 resembles a Hungarian wax type (Bosland, personal observation). The clustering pattern of these two accessions suggests these were neither related to each other nor to the other Nepalese accessions. The most likely explanation for this dissimilarity is that they are recent commercial seed introductions to Nepal.

Results herein demonstrate that Nepal's chile accessions are genetically diverse, albeit, at a lower level than accessions from the center of diversity, Mexico. Nevertheless, unique RAPD markers were present in Nepalese accessions. This may be an indication of mutations accumulated since their introduction into Nepal. The presence of unique RAPD markers indicates that Nepalese germplasm may also possess unique traits of economic importance. The similarity indices generated in this study provide a method to distinguish among Nepalese landraces. This information can be applied to the construction of a core collection of chile for Nepal. In fact, a core collection would be a suitable approach for Nepal to efficiently manage its genetic resources of *Capsicum* sp.

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