# Isozyme characterization of *Capsicum* accessions from the Amazonian Colombian collection

# Caracterización por isoenzimas de accesiones de *Capsicum* pertenecientes a la colección amazónica colombiana

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**ABSTRACT**

Two hundred and sixty-one accessions of the genus *Capsicum* were obtained from the Colombian Amazonian germplasm bank at Amazonian Institute of Scientific Research (Sinchi) and were evaluated with five polymorphic enzymatic systems, including esterase (EST), peroxidase (PRX), 6-phosphogluconate dehydrogenase (6-PGDH), aspartate amino transferase (GOT), and malic enzyme (ME). Using a cluster analysis (UPGMA) the genetic variability of these accessions were characterized. Grouping of the species *C. baccatum* and *C. pubescens* were observed, while the species *C. annuum*, *C. chinense* and *C. frutescens* did not group independently, a result that has been previously reported in isoenzyme analyses of this genus. Several accessions were deemed of particular interest for future ecological and evolutive studies.

**Key words:** Colombia, *Capsicum*, germplasm bank, isoenzymes, peppers.

**RESUMEN**

Doscientas sesenta y una accesiones del género *Capsicum* del banco de germoplasma del Instituto Amazónico de Investigaciones Científicas (Sinchi) se evaluaron a través de cinco sistemas enzimáticos polimórficos: esterasa (EST), peroxidasa (PRX), 6-fosfogluconato deshidrogenasa (6-PGDH), aspartato amino transferasa (GOT) y enzima málica (ME). Se utilizó un análisis de agrupamiento (Upgma) con el fin de determinar la variabilidad genética. Se observó un agrupamiento de las especies *C. baccatum* y *C. pubescens*, mientras que las especies *C. annuum*, *C. chinense* y *C. frutescens* no mostraron un agrupamiento independiente, lo cual ya ha sido reportado en estudios por isoenzimas para el género. Varias accesiones mostraron características particulares para estudios ecológicos y evolutivos.

**Palabras clave:** Colombia, *Capsicum*, banco de germoplasma, isoenzimas, ají.

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**Recibido:** abril 19 de 2005 **Aceptado:** julio 5 de 2005

## INTRODUCTION

The most common subsistence strategy in the Amazon is shifting cultivation, a technology also known as slash and burn agriculture, or "chagra". In this agricultural technique the diversity of species is maintained by means of putting them under constant selection, making the Amazon a genetic reservoir.

The genus *Capsicum* (Solanaceae) includes cultivated chili peppers and their wild relatives, and plays an important role in the "chagra", as it is one of the temporary species, essential in polyculture in the Colombian Amazon. It plays an important role both in the diet of the community, and as an essential part of its culture, since *Capsicum* is believed to possess spiritual properties that connect people to the forest and the spirits of ancestors (Garzón & Macuritofe, 1992). Indigenous communities have been selecting for important agronomical characters in species of this genus since pre-Columbian times, selection which has probably resulted in the high morphological variation observed in the modern genus.

Currently, much of this agricultural biodiversity is being lost due to the trend of some indigenous peoples to simplify and specialize their production systems towards more profitable activities, following the production model of white society.

Colombia is considered a possible center of origin for the wild complex *Capsicum annuum-chinense-frutescens* (Pickersgill, 1984). This is due to the fact that in Colombia the genus contains an exceptional amount of morphological variation. In order to assure the conservation and sustainable use of this valuable agricultural resource, a genetic assessment of the diversity of the genus *Capsicum* in Colombia is important. These species and their varieties could contain genetic resources useful for the improvement of chili pepper cultivars, providing such benefits as disease resistance and tolerance to extreme environmental conditions.

In previous work on *Capsicum* using isozymes (McLeod *et al.*, 1979; 1982; 1983a; 1983b), the phylogenetic relationships among species of the genus have been estimated. The hypothesis generated by these studies is that the five cultivated species of the genus (*C. pubescens, C. baccatum, C. annuum, C. frutescens* and *C. chinense*) have different wild progenitors, and that the last three species, known as the *annuum* group, belong to a single polytypic species that covers a wide geographical area. In this area, several local forms coexist that were independently domesticated from the same genetic pool.

Among the genetic diversity studies in *Capsicum* that have made use of isoenzymes the work of Loaiza-Figueroa *et al.* (1989) studied the genetic relationships among 186 samples of *Capsicum* from Mexico. The indices of genetic diversity suggested that these accessions of Mexican *Capsicum* were highly homozygous, pobably due to self-pollination and population bottlenecks. Significant genetic differentiation was found among populations from different geographical regions and based on the similarity of enzymatic genotypes of both the semi-domesticated and the wild varieties, two domestication centers of *C. annuum* were proposed, a primary center in the east of the country and a possible secondary center in the west.

The objective of this study is to characterize the genetic diversity of accessions from the Colombian Amazon germplasm bank of the genus *Capsicum*. We use five polymorphic isoenzyme systems to describe its variability, estimate genetic similarities and identify samples that may be important for future genetic, evolutionary and ecological studies. This characterization is a first step towards achieving an evaluation of the genetic resources of the genus *Capsicum* in the Colombian Amazon.

## MATERIALS AND METHODS

A total of 261 accessions of the *Capsicum* germplasm collection of the Amazonian Institute of Scientific Research (Sinchi) were evaluated. Seeds were collected in home orchards and in indigenous *chagras* located in the Colombian provinces of Amazonas, Caquetá, Guainía, Guaviare, Putumayo, Vaupés and Vichada (table 1, figure 1) and grown in greenhouses.

Vegetative material was taken from seedlings that were one and a half months old. For the isoenzymatic assessment proteins were extracted from leaves and young roots of the seedlings. A control sample was used for each species (table 2).

An extraction buffer solution 50mM Tris-HCl pH 8.3, 20% sucrose (w/v), 5% PVP-40 (w/v), 0.12 mM DTT, 2% Triton 100X, 14mM -Mercaptoethanol was used. The tissues were macerated in previously

**Table 1.** Samples included, species and province

| Province | *C. chinense* | *C. frutescens* | *C. annuum* | *C. baccatum* | *C. pubescens* |
|---|---|---|---|---|---|
| Amazonas | 31 | 21 | 31 | 1 | -- |
| Caquetá | 1 | 3 | 2 | -- | -- |
| Guainía | 34 | 19 | 30 | -- | -- |
| Guaviare | 3 | 1 | 2 | -- | -- |
| Putumayo | 8 | 7 | 7 | -- | 6 |
| Vaupés | 11 | 18 | 12 | -- | -- |
| Vichada | 3 | 1 | 9 | -- | -- |
| Total | 91 | 70 | 93 | 1 | 6 |

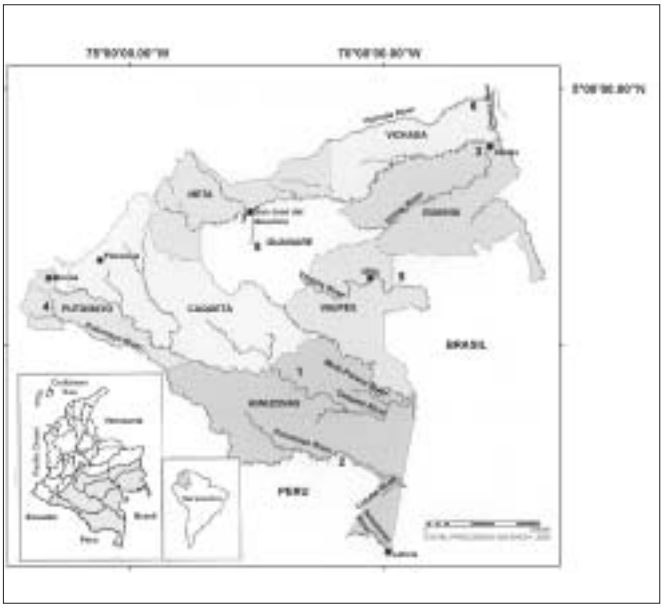


**Figure 1.** *Capsicum* collecting routes map. 1. Route: Medio Caquetá-Miritíí River; 2. Route: Bajo Putumayo-Igara Paraná River; 3. Route: Puerto Inírida; 4. Route: Alto Putumayo; 5. Route: Vaupés; 6. Route: Vichada; 7. Route: San José del Guaviare 1; 8. Route: San José del Guaviare 2.

**Table 2.** Control samples, by species

| Sample | Species | Place of origin |
|---|---|---|
| 258 | *C. frutescens* | Quindío, Colombia |
| 259 | *C. frutescens* | Quindío, Colombia |
| 260 | *C. annuum* | -- |
| 261 | *C. annuum* | Córdoba, Colombia |
| 262 | *C. chinense* | Valle, Colombia |
| 264 | *C. pubescens* | Nariño, Colombia |
| 265 | *C. baccatum* | Nariño, Colombia |
| 266 | *C. baccatum* | Nariño, Colombia |
| 267 | *C. baccatum* | Chile |

refrigerated mortars, adding the extraction buffer solution in a proportion of 1:4 (w/v) for the leaves and 1:2 (w/v) for the roots. The macerate was centrifuged for 10 minutes at 14000 r.p.m. 4ºC. The supernatant was stored at –20ºC until analysis.

A discontinuous polyacrylamide gel system was used, consisting of $T_4$ stacking gel with 0.5M Tris-HCl pH 6.8 and a $T_{10}$ separation gel with 1.5M Tris-HCl pH 8.8. The buffer system was Tris-Borate pH 9.0. The electrophoretic separation was performed between 125 and 250V, at 15mA in the beginning and 30mA at the end. The gels were run for 17 hours in refrigerated conditions, with a migration front referenced with bromophenol blue.

Preliminary tests were performed with 12 enzymatic systems. From these, five enzymes were selected for further analysis: esterase (EST), peroxidase (PRX), 6-phosphogluconate dehydrogenase (6-PGDH), aspartate amino transferase (GOT), and malic enzyme (ME) based on polymorphism, repeatability and band resolution. The protocols described by Hussain *et al*. (1986) were used for the enzyme staining. The gels were dried on hydratable cellophane paper, which was previously immersed in a drying solution (80% ethanol) for 20 minutes.

The data from zymograms were entered as a matrix of presence/absence of bands for each enzyme. A multivariate estimate of similarity was used in which symmetrical matrices summarized the similarities between all possible pairs of evaluated individuals. Similarity was calculated using the Nei-Li similarity coefficient (1945). Using these similarity matrices, phenograms were constructed using the UPGMA method with the statistical software NTSYS (Numerical Taxonomy and Multivariate Analysis System).

## RESULTS AND DISCUSSION

The five enzymatic systems showed a total of 83 bands, distributed in the whole set of samples as: PRX with 15 bands, 6-PGDH with 8 bands, GOT with 8 bands, ME with 17 bands and -EST with 37 bands. The most polymorphic enzymes were -EST and the ME, followed by PRX, 6-PGDH and GOT.

The phenogram of all the enzymes (figure 2) revealed a high variability between all of the analyzed samples, which can be noted by the numerous groups formed, which are made up of few accessions. In general, no relationship was found between the groups created in the UPGMA analysis and the geographic or ethnic origin, use, or morpho-agronomical characterization of the samples evaluated.

Five groups were identified, based on their taxonomic determination. The first group included the majority of the accessions, taxonomically determined as *C. annuum, C. chinense* and *C. frutescens*; the second group included accessions of *C. baccatum;* the third group included some materials identified as *C. annuum, C. chinense and C. frutescens*; the fourth group was made up of accessions from *C. pubescens* and the fifth group of two accessions, number 250, which was determined as *C. annuum* and accesion number 253, determined as *C. frutescens*.

Within the first group, no independent grouping of samples belonging to the same species was observed, reflecting the close genetic relationship that exists between these three species, which are commonly known as the *Capsicum annuum-chinense- frutescens* complex. This same result has been observed in previous studies that of Jensen *et al*. (1979) and McLeod *et al*. (1979, 1983b), in which they argued that isoenzymatic data alone is not sufficient to differentiate among the three species.

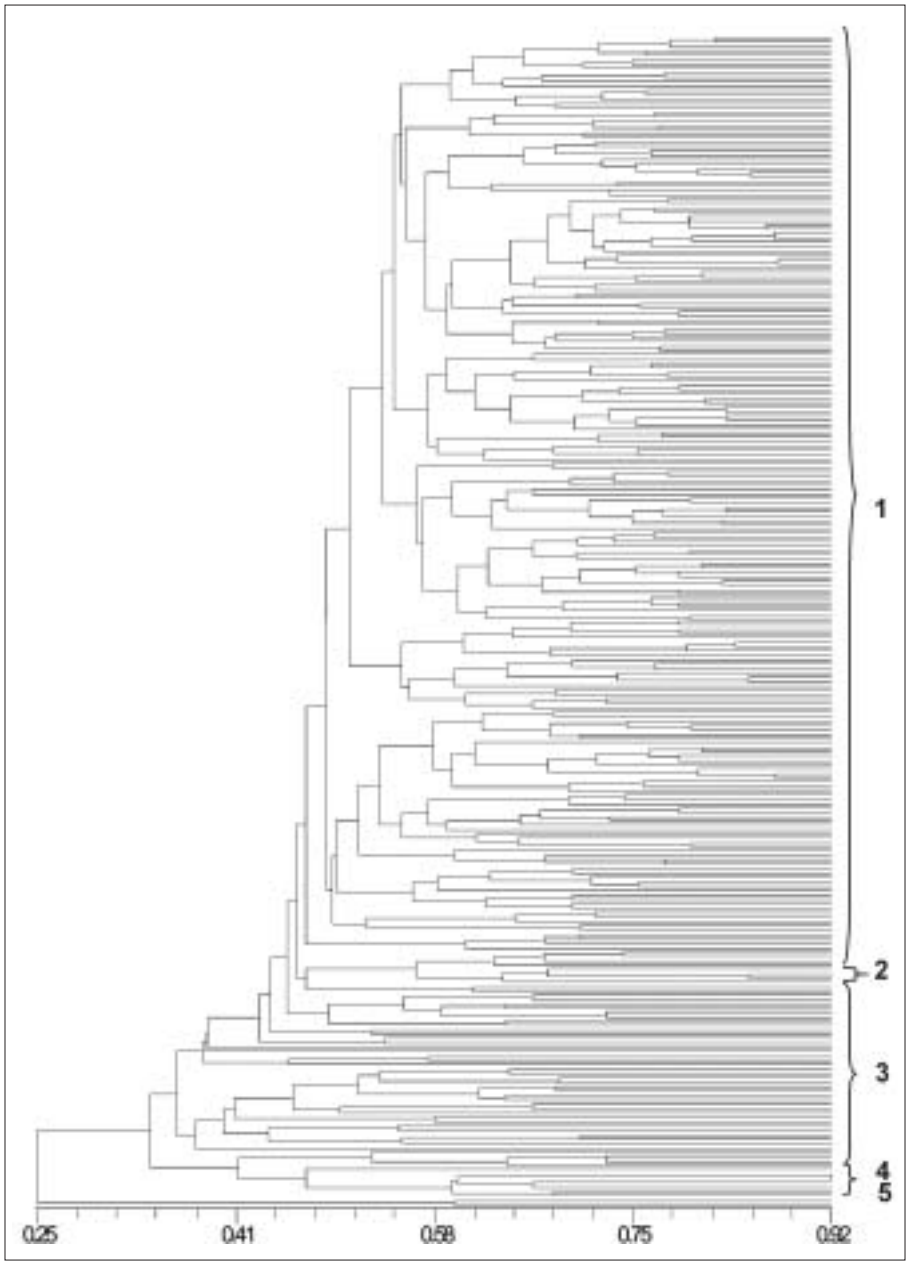


**Figure 2.** Nei Similarity phenogram of 270 *Capsicum* accessions. 1. *C. annuum*, *C. frutescens* and *C. chinense* Group; 2. *C. baccatum* Group; 3. *C. annuum*, *C. frutescens*, *C. chinense* Group; 4. *C. pubescens* Group; 5. 250 accession (*C. annuum*) and 253 accession (*C. frutescens*) Group.

In contrast, Loaiza-Figueroa *et al*. (1989) found groupings corresponding to the species *C. annuum, C. chinense* and *C. frutescens.* In our investigation, the fact that no isoenzymatic differentiation is observed between species of this complex possibly may be due to the fact that the materials for this study come from the Colombian Amazon, an area considered one of the possible centers of origin for the wild complex *Capsicum annuum-chinense-frutescens.* Hybridization between no recognized wild varieties of *C. frutescens* and cultivated *C. annuum* and *C. chinense* may also contribute to the lack of genetic structure in the complex (Pickersgill, 1984). The three wild forms should probably be considered a single genetic pool in the process of differentiation, where small differences are slowly becoming more marked as the pool colonizes new ecological niches (Debouck & Libreros, 1993).

It is also probable that the seed exchange between indigenous communities mainly by weddings between members of different ethnic groups, in which the bride takes the seeds of whatever is grown in the *chagra* or family orchard with her, may result in a continuous flow of germplasm between different communities. This lack of geographic or cultural isolation may therefore inhibit significant genetic structure between cultivated species of *Capsicum*. Given these circumstances, it is not surprising that samples belonging to *C. annuum, C. chinense* or *C. frutescens* did not form unique clusters. Alternatively, these results may support the previously mentioned hypothesis of Pickersgill (1984), in which she states that Colombia may be one of the centers of origin of the *Capsicum-annuum-chinense-frutescens* complex, and does so only through morphological and cytogenetic data.

Due the fact that this work considers only five enzymatic systems, it represents a very small proportion of the genome. Therefore, these results should be considered preliminary. It is essential that other molecular tools be used to elucidate with greater certainty the genetic structure of this complex.

The second group comprised accessions belonging to the species *C. baccatum*, including the three control samples from Nariño province and Chile, and the single Amazonian sample. Similar genetic differentiation of this species was previously reported by Jensen *et al*. (1979); McLeod *et al.* (1979) and McLeod *et al*. (1983a). The fact that only a single sample of this species was collected in the study area is probably due to the recent introduction of *C. baccatum* by white settlers, and that it has only been collected in peripheral zones of the Amazon lowlands. This species is also very rare in Central America (Pickersgill, 1984).

The grouping of *C. baccatum* individuals is probably due mainly to the frequency of bands 4 and 8 in GOT, which would indicate that this locus may be a useful marker for this species. A larger number of individuals should be evaluated with this enzyme in order to verify this possibility.

Twenty-four accessions of the species *C. annuum, C. chinense* and *C. frutescens* were included in the third group. The data of morphoagronomic characterization did not show particular characteristics, and likewise, no relationship was found with the ethnic data, geographical origin or collection site. Nonetheless, it is important to mention that of these 24 samples, 12 did not show any activity with PRX (table 3), it is probable that they do not have the same substrate affinities that are biologically important (Brewbaker *et al*., 1985) and therefore some of them are not detected.

Nonetheless, the evaluation of these materials is interesting from another perspective, namely ecological or physiological, since peroxidases seem to have a primary role in the synthesis of lignin polymers and they act in the synthesis of auxin (indol acetic acid) (Brewbaker *et al.,* 1985). It has also been noted that they act in the induction of systemic resistance as a mechanism to increase peroxidase activity in plants infected with *Phytophtora capsici.* This reaction was detected in resistant crops, even in

**Table 3.** Samples without peroxidase activity

| Accession | Specie | Province |
|---|---|---|
| 47 | *C. chinense* | Amazonas, Colombia |
| 205 | *C. frutescens* | Amazonas, Colombia |
| 276 | *C. frutescens* | Guainía, Colombia |
| 291 | *C. chinense* | Guainía, Colombia |
| 309 | *C. chinense* | Guainía, Colombia |
| 310 | *C. chinense* | Guainía, Colombia |
| 311 | *C. chinense* | Guainía, Colombia |
| 322 | *C. annuum* | Guainía, Colombia |
| 373 | *C. frutescens* | Vaupés, Colombia |
| 375 | *C. frutescens* | Vaupés, Colombia |
| 397 | *C. annuum* | Vaupés, Colombia |
| 414 | *C. annuum* | Vaupés, Colombia |

absence of progress of the infection (Alcázar *et al.,* 1995).

The fourth group is composed of the samples determined as *C. pubescens*. The separation between individuals belonging to this species has been reported in studies by Jensen *et al*. (1979) and McLeod *et al*. (1979, 1983). This separation has also been observed in flavonoid studies (Ballard *et al*., 1970 in Debouck & Libreros, 1993), morphological studies (Heiser, 1948, in Debouck & Libreros, 1993; Rick,1950, in Debouck & Libreros, 1993), and by total incompatibility in interspecific crossing (Pickersgill, 1988; Lippert *et al*., 1966 and Zijlstra *et al.,* 1991, in Debouck & Libreros, 1993).

The grouping of accessions of *C. pubescens* is due mainly to the patterns obtained with the GOT enzyme, bands 5 and 8. These bands show a characteristic pattern in individuals of *C. pubescens,* indicating that they may be good genetic markers for characterizing this species. A greater number of *C. pubescens* samples should be evaluated with this enzyme in order to corroborate this possibility.

It is important to note that the samples analyzed in this study are distinct from the control samples. This may indicate that the Amazonian materials are genetically differentiated from commercially available varieties, and that therefore they may provide a useful future source of genetic variation for crop improvement. This is also attested by the great variability within the group.

The fifth group was made up of two samples collected in the province of Guainía: accession number 250, determined as *C. annuum* and accession number 253, determined as *C. frutescens.* Sample number 250 did not show any peroxidase activity, but did not group with the materials in group 3. This accession should prove interesting for future studies, given the importance of the peroxidase enzyme which was previously mentioned.

The characterization by isozymes of the 261 accessions from the Colombian Amazonian *Capsicum* germplasm collection showed a high variability, as no identical material was found in the phenogram, except for two accessions in *C. pubescens* group that show 0.92 similarity. Based on the results of this study we recommend that all the samples should be maintained in the collection, as they are potentially a new source of genetic diversity for the improvement of commercial varieties of *Capsicum*.

## ACKNOWLEDGEMENTS

This work was carried out for the Instituto Amazónico de Investigaciones Científicas Sinchi with financial support from Sinchi and Colciencias-BID. We extend our heartfelt thanks to the indigenous communities of the Amazon region from Colombia for sharing their time and assistance during our collecting expedition. We are also grateful to the Sinchi GIS department for their help and advice. We are also grateful to doctor Daniel Debouck for his advise, to Mario García and Marcela Celis for the agronomic and taxonomic determinations respectively and to Joe Tohme of Ciat.

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