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Molecular, cultural, and ecological markers of genetic diversity in Mexican species of beans (Phaseolus).

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EXECUTIVE SUMMARY

Beans are the main vegetal protein source in Latin American countries. They are derived from five cultivated taxa: Phaseolus vulgaris (common bean), P. coccineus ssp. coccineus (scarlet runner bean), P.coccineus ssp. darwinianus, P. acutifolius (tepary bean), and P. lunatus (Lima bean), all native to (and probably domesticated in) Mexico. Most of the basic studies required for improving bean genetics and cultivation are based on the common bean because of its commercial importance in world markets. On the other hand, the scarlet runner bean with its different biology (e.g., outbreeder rather than inbreeder) is poorly known even though it is important staple crop to many isolated rural peoples of Mexico. Due to its importance to nourishment of rural people and to the lack of fundamental biological knowledge, detailed studies were conducted on scarlet runner bean in two regions of Mexico where indigenous peoples grow and manipulate this species and its wild relatives.

As an initial step towards a long term study of the diversification, conservation and development of scarlet runner bean, field and experimental data were analyzed in order to characterize the variation of this bean in western Chihuahua and northern Puebla. Characterization was based upon genetic (chloroplast DNA, isozyme and chromosomal features), cultural (ethnobotanical information on knowledge, cultivation and use) and ecological (pollination biology and ecophysiology) studies of selected plants by an interdisciplinary team of researchers.

Even though the two studies areas shared such features as mountainous terrain, indigenous cultures and close proximity of cultivated and assumed wild relatives, the patterns of bean diversity in the two regions were different. The analyses of the chloroplast DNA (first for P. coccineus) suggested two hypotheses for future testing which place the climbing P. coccineus ssp. darwinianus more distantly related to the typical P. coccineus than currently accepted. The subsistence Nahuatl farmers of Puebla are diversifying the former in response to changing local market demands (i.e., early and late crops which do not compete with associated maize with which it is cultivated). The wild relatives do not provide additional genetic diversity today according to pollination and genetic data. On the other hand, the Tarahumara Indians in Chihuahua have selected a early maturing form which is correlated with its distinctive (relative to other P. coccineus forms) physiology and bush form which is cultivated in monoculture (rather than as a climber over the maize). In contrast to Puebla, the wild bean of Chihuahua in the surrounding pine-oak forests crosses with the cultivated forms. This natural hybridization system is a dynamic in situ conservation strategy in a rural area where native people are subject to short (and some times catastrophic) cultivation seasons. In both cases, native farmers recognize and promote of genetic diversity and its subsequent expression in adaptive growth behavior and subsistence production under changing human demands and limiting environmental conditions.

RESEARCH OBJECTIVES

The overall aim of the project is the determination of the correlation of genetic, ethnobotanical, and ecological factors of Phaseolus diversity in two cultural-geographic areas in Mexico (Sierra Norte de Puebla and Sierra Tarahumara in Chihuahua). To accomplish this, various aspects of the beans and their relationships to the natural and cultural environment must be determined. Because of the lack of basic biological information and of its relative importance in rural (rather than urban) diets, P. coccineus (scarlet runner bean) was chosen as the central species for study. Being an outbreeder, there was a greater probability of assessing the importance of genetic transfer between cultivated and wild ancestors and hence the possibility of establishing the foundation for evaluating the necessity of in situ conservation in conjunction with indigenous farming practices. Specifically, six disciplines engaged field and laboratory studies with their own set of objectives. In order to provide the basis for comparative studies and integration of data, each research group worked with a common set of bean material. Unfortunately due to the limitations of the technology of each discipline, not all the material obtained was equitably analyzed. However, a small subset was shared among all groups. Future studies will attempt to complete the unstudied material.

Mexico (along with 2 South American areas in some cases) is the area of origin of all 5 domesticated beans: Phaseolus vulgaris L. (common bean), P. coccineus L. ssp. coccineus (scarlet runner bean), P. coccineus ssp. darwinianus Hernández X. & Miranda, P. acutifolius Gray (tepary bean), and P. lunatus L. (Lima bean). Not only are the wild relatives still growing in Mexico but also the cultural factors responsible for diversifying and domesticating these food plants are still operating. In order to understand the origin of these domesticated plants and to establish a foundation for the in situ and ex situ conservation of these genetic resources, this project was initiated. Based upon the characterization of genetic, taxonomic, ecophysiological and cultural factors, subsequent comparative studies will generate specific hypotheses for detailed studies in the future. Although not it is not the emphasis of this project, certain preliminary results are of interest to the current debate as to the origin and taxonomic position of P. coccineus ssp. darwinianus Hernández X. & Miranda which is considered to be a separate species, P. polyanthus Greenman (Schmit and Debouck 1991).

Special emphasis was placed on P. coccineus and, in some cases, the associated cultivars of P. vulgaris. The scarlet runner bean is poorly known in the commercial and scientific communities but none the less is important in local diets. Compared to the common bean, the scarlet runner bean is relatively unknown in terms of its characterization, diversity, genetics and utility. Also, the scarlet runner bean plays a

critical role in the local nourishment of the inhabitants of the mountains of northern Puebla and western Chihuahua. Socio-economic development depends, in part, upon the characterization and potential of genetic resources available for future plant breeding programs and upon the local resources with which inhabitants are familiar and have accepted. Enhancement of the value and benefits these local food assets is an alternative to introduction of new, unknown foodstuffs which may fail.

The members of the project have been working isolated on various aspects of Mexican beans for over 20 years in some cases. This project permitted the integration of their experiences and current research to focus on this collective project. Although such an innovative concept had been proposed earlier for Mexico, it was never implemented until this USAID project. New insights and more penetrating questions arose from the comparison of preliminary results. The simple correlation of early results suggested that the answer to our questions were beyond the temporal limits of this project.

The USAID support provided seed money to begin this collaborative research. Because of the preliminary success, other organizations supported related facets of the program. We received support from the principal national research funding source (Consejo Nacional de Ciencia y Tecnología or CONACyT) and from the Universidad Nacional Autónoma de México (UNAM) where most of the researchers are based.

GENERAL METHODS and DESCRIPTION OF STUDY LOCALITIES

Based upon recommendations of the preliminary evaluation of the project, the scope of investigation and the study zones were reduced. Two areas in Mexico were selected based upon the importance of Phaseolus coccineus in the diet and the agricultural systems and the presence of ethnic groups that practice traditional agriculture. The two regions included southwestern Chihuahua (located in northwestern Mexico) and northern Puebla and adjacent areas (located in east-central Mexico).

In each zone, field studies included exploration, collection and documentation of wild and cultivated beans. Experimental plots were also established in which local beans as well as beans from the reciprocal zone were cultivated. The cultivation techniques included the traditional forms of the respective region as well as experimental arrangements (e.g., equal spacing, artificial supports, etc.). Controlled pollination, ecophysiological measurements, special photographs and growth data were obtained from these cultivated populations. Living material was collected for genetic, ecophysiological and morphological analyses in the laboratories in Mexico City. Details of the methodology have presented in previous reports; the methodologies employed are briefly summarized in each section in the chapter on **Research Results.**

CHIHUAHUA: Sierra Tarahumara

The important ethnic group in this region is the Tarahumara Indians (Rarámuri) who belong to the Uto-Aztecan language family. The population size is estimated to be about 50,000 individuals. Their subsistence strategies include agriculture, animal husbandry, horticulture, hunting and gathering.

The transportation, living and communication facilities available in the area include one transcontinental railroad, one major dirt highway (Gran Visión) in construction (partially paved), limited medical, educational and commercial services, and limited agrotechnological extension.

The physiography of the region is divided into three areas. The bolson and ranges are located in east. The central sierras are the northern mountains of the Sierra Madre Occidental. To the west of the mountains are the barrancas or deep canyons. The major drainages include Rio Conchos (Rio Grande) on east and the Rios Urique, Batopilas, Chinipas, and Verde (Rio Fuerte) on west.

The vegetation types (going from NE to SW) include grassland, pinyon-juniper woodland, pine-oak forest [at the highest elevations], barrracan oak woodland, deciduous tropical forest, thorn forest, riparian forest.The soils are composed of members of mollisol, aridisol, and entisol classes.The elevation ranges from 400 m in the barrancas to over 3000 m in the mountains.The climatic classifications (going from NE to SW) are BSk, C, BSh, (A)(W).The general zone is located between 26° - 28° N latitude and 106° 30′- 109° W longitude.The growing season is about 100 days long but may by drastically shorten in certain years due to late Summer or early Fall frosts.

PUEBLA: Sierra Norte de Puebla

In this region (including adjacent Hidalgo, Tlaxcala and Veracruz) 4 important ethnic groups are found: Nahua (of the Uto-Aztecan language family), Tepehua, Totonaca (both of the Totonacan language family), and Otomi (of the Otomanguan language family).In total, they number about 800,000. The basic subsistence strategies in the zone are subsistence agriculture and limited cash crops. The transportation, living and communication services in the area include one crans-sierran road (under construction) school, medical, electricity, and drinking water facilities, and limited agrotechnological extension.

The physiographic features of the area include mountains and piedmonts as part of the southern Sierra Madre Oriental. The major river drainages in the area are Rio Tuxpan, Rio Cazones, and Rio Tecolutlan. The vegetation encountered going from NE to SE includes tropical rain forests, mountain cloud forest, pine-oak forests, oak woodland, and riparian gallery forests. The major soil classes are lithosols and ribosols. The altitude goes from 60m along the coast piedmont to 2700 m in the mountains. The climatic classifications are Am, Cm, and Cw.The general location of the area is 19° 42′ to 20° 35′ N latitude and 96° 12′- 98° 03′ W longitude.

- 98° 03′ W longitude.

METHODS AND RESULTS

The results from the project are summarized below. Preliminary results and more detailed information are available in earlier technical reports. Project data are still being analyzed and will be published in appropriate journals and books. Both discipline-specific and interdisciplinary articles are planned. As stated above, major emphasis was placed on P. coccineus with secondary emphasis on P. vulgaris when appropriate. Genetic diversity is characterized by chloroplast DNA as a molecular marker (R-1), isozyme studies (R2) and cytogenetic studies (R3). Cultural data are included in the ethnobotanical studies (R4). Ecological markers include pollination biology (as part taxonomic studies) (R5) and ecophysiology (R6).

Seeds collected during the study (547 accessions) are stored and duplicate sets distributed for storage in Mexico and at CIAT in Colombia. Selected material continues to be used for related investigations even though this AID project has been concluded. Notes, reports and photographs are on file at the Instituto de Biología de la UNAM and serve as introductory references for ongoing and future research and development projects.

Another objective of the original project was a trial period for an interdisciplinary group to study beans in Mexico. An earlier attempt (Programa de Investigación Interdisciplinaria de Frijol) in 1976 was make by the Colegio de Postgraduados of Chapingo, Mexico, which was later abandoned. Based upon the initial results of our USAID-supported project, we are planning to expand the group to include other organizations, study more profoundly certain key issues in bean diversity, and to extend the geographic and ethnic coverage of the studies.

R1 - STUDIES ON Phaseolus INTER- AND INTRASPECIFIC EVOLUTION USING cpDNA AS A MOLECULAR MARKER

Seeds from different accessions numbers of wild and cultivated subspecies of P. coccineus were used. These were germinated under laboratory conditions, the seedlings being grown out in the greenhouse. Leaves from 62 of the 65 samples were taken and maintained in an ultra-freezer (-70°C). Total DNA of 32 of those samples was isolated and digested by EcoR1 restriction endonuclease. Restriction fragments were separated by electrophoresis in 8% agarose gel. They were then transferred to Zetabind membranes to determine the RFLP patterns of each sample.

The "Southern" method was used on the transferred fragments employing DNA chloroplast fragment of Vigna radiata as a probe.

In the latter, ^{32}P is used as a marker and is intended to determine cpDNA pattern of each sample. The marked Vigna cpDNA probes have been obtained through Escherichia coli colonies with different probes. These were cloned in PBR322 plasmid, being then transferred in petri dishes. The probe was separated with Hind III endonuclease, marked with ^{32}P in the 5′ end through "Nick translation" technique, and with aid of Klenow polymerase DNA of E. coli.

The objective is to establish possible taxonomic relationships, as well evolutionary processes at the molecular level within the P. coccineus.

RESULTS and DISCUSSION

cpDNA restriction map

The cpDNA map for P. coccineus was made using a wild accession collected in Chihuahua, Mexico. For P. vulgaris, a wild accession (CIAT G12454) belonging to the P. vulgaris Mesoamerican gene pool, was employed.

The linearized P. coccineus cpDNA map is depicted in Fig. 1. Solid bars indicate the position of the inverted repeats. Arrows indicate sites that were different in P. vulgaris. Numbers to the left of each map indicate the number of sites found. The size for each map, calculated as the average of the total distance for each enzyme, was approximately 151 Kb, which is in agreement with the cpDNA sizes found in other species. The small single copy region was approximately 17.5 Kb long. Each of the repeats presented an average size of 26.9-28 Kb, whereas the large single copy region was 77.5 Kb long. Minor discrepancies among size calculations for each enzyme are probably due to small fragments (0.3-0.5 Kb) not detected under the employed conditions.

cpDNA restriction sites as molecular markers

A total of 18 restriction site differences between the P. coccineus and P. vulgaris genomes were detected. This corresponds to 5% of all the sites analyzed. Differences at all but 2 sites can be explained as simple restriction site gains or losses. No major rearrangements (large insertion, deletions, inversions or translocations) were observed. Small insertion and inversions of 2-100 bp may have been overlooked by our methods. The high conservation between the two cpDNA genomes is in agreement with that observed by other workers at the interspecific/intraspecific levels in other species (Palmer 1985).

Map location of restriction site differences are indicated in Table 1. For further analysis, all mutations, excepting those in regions 8 and 9, were considered as independent because of the lack of intermolecular recombination in cpDNA, and the lack of any major rearrangement. Mutation number 1 and 2 (Table 1) are equivalent in each repeat and were not considered as independent,

because our own results (data not shown) and those obtained in other plant species (Palmer 1985) imply possible sequence homogenization phenomena. The variable regions were screened for possible polymorphisms in several accessions belonging to wild and cultivated P. c. ssp. coccineus, P. coccineus ssp. darwinianus (= P. polyanthus), P. coccineus ssp. formosus, and P. coccineus ssp. glabellus. Regions which did not show variation between coccineus and darwinianus (as polyanthus) were also included in the analysis, representing a total of 186 restrictions sites in 30 different accessions. The 2 P. vulgaris gene pools, Mesoamerican and Andean, were compared. A tree was constructed by using the NTSYS program (Numerical Taxonomy System) using the UPGMA (Unweighted Paired-Group Method using Arithmetic averages) clustering method (Fig. 2).

We could not distinguish P. coccineus ssp. formosus and P. c. ssp. coccineus on the basis of cpDNA data. Therefore, a very close relationship among the accessions used for both subspecies is inferred. Never the less, P. coccineus ssp. formosus seems to include a heterogenous group of populations. For example, individuals with high reciprocal compatibility to P. vulgaris and lilac flowers have been described previously within P. coccineus ssp. formosus, where as red-flowered, wild plants are also included in the same group (Delgado 1989). The P. coccineus ssp. formosus accessions included in this work correspond to the second type, similar in morphology to wild P. c. ssp. coccineus. cpDNA analysis of lilac-flowered, P. vulgaris-compatible populations could indicate if P. coccineus ssp. formosus is in fact a monophyletic group or consists of a heterogeneous group of populations.

Phaseolus coccineus ssp. glabellus cpDNA, on the other hand, has diverged considerably from any of the other taxa included in this study. This divergence is supported by the lack of natural hybrids between P. coccineus ssp. glabellus and other subspecies, even in regions where they are sympatric. Recent work on the reproductive biology of P. coccineus ssp. glabellus conducted by this USAID-supported project shows clear reproductive barriers between this group and P. c. ssp. coccineus (Sousa 1992). Although P. coccineus ssp. glabellus has been included within the species P. coccineus because the presence of red flowers, cross pollination and perennial plants, the morphological differences, the presence of reproductive barriers, and the extensive divergence at molecular level suggests that it should be reclassified as a different species, and that probably does not belong to the P. vulgaris/P. coccineus complex. cpDNA analysis of other Phaseolus species would allow us to give a more precise taxonomic location of P. coccineus ssp. glabellus.

According to the results, P. coccineus ssp. darwinianus cytoplasm is considerably closer to P. vulgaris cytoplasm than to its P. coccineus counterpart. This is unexpected if we consider that previous works using nuclear markers, like isozymes (Piñero and Eguiarte 1989), antibodies against seed proteins, and our own

results using random nuclear clones, repetitive DNA and phaseolin Polymerase Chain Reaction analysis (PCR; data not shown) indicate a nuclear DNA closer to P. c. ssp. coccineus than to P. vulgaris. It is worthwhile mentioning that P. coccineus ssp. darwinianus presents similar reciprocal differences in crossability with P. vulgaris and P. c. ssp. coccineus, although in a lesser degree. On the other hand, the cross P. coccineus ssp. darwinianus x P. c. ssp. coccineus is viable in any direction, with a large percent of fertile progeny.

Two alternative pathways in the evolution of the complex have been proposed. Phaseolus coccineus ssp. darwinianus could evolve from the cross between a P. vulgaris seed parent and a P. c. ssp. coccineus pollen parent. This idea is similar to that proposed by Hernández X. and Miranda (1959), although they proposed P. c. ssp. coccineus as the seed parent. Recurrent crosses with P. c. ssp. coccineus could explain the similarity of nuclear genomes. According to the phylogenetic distances, hybridization would have occurred before the split of the two major current gene pools in P. vulgaris, Mesoamerican and Andean. The second alternative proposes that P. vulgaris and P. coccineus ssp. darwinianus derived from the same ancestor (the prevalent hypothesis nowadays). In order to discriminate between both hypothesis, additional studies on nuclear and other cytoplasmic markers (i.e. mitochondrial DNA, = mtDNA) will be made. Through the use of mapped clones from P. vulgaris, a patchy distribution of P. vulgaris and P. c. ssp. coccineus markers could indicate a reticulate origin for P. coccineus ssp. darwinianus, whereas a non-patched distribution would support the conventional idea of an origin of P. vulgaris from P. coccineus ssp. darwinianus. The use of mtDNA would provide additional evidence on the origin of the cytoplasm and therefore a more precise idea of the phylogenetic distances among groups.

R2 - GENETIC VARIATION IN POPULATIONS OF Phaseolus coccineus BASED ON ISOZYME STUDIES

The levels and patterns of genetic variation play an important role in determining the evolutionary potential of a species or a population. These patterns of genetic variation are strongly influenced by the effects of five basic evolutionary processes: 1) genetic drift, 2) migration (or gene flow), 3) breeding systems, 4) mutation, and 5) natural selection. Therefore one requirement for understanding the evolutionary biology of a species is the knowledge of its genetic variation (Brown 1979; Lewontin 1974; Slatkin 1980).

The studies of genetic variation involve two principle aspects: the first refers to the description of the levels of variation maintained within the populations and the second concerns the manner in which the variation is distributed in the population. This second point is particularly important for the conservation of natural resources. In this way, the knowledge of

the genetic structure not only provides the elements for understanding the evolutionary mechanisms that operate in organisms but also is useful for understanding the processes of domestication of cultivated plants and to provide information for management and conservation programs for natural resources (Clegg and Brown 1983; Frankel and Soulé 1981; Tanksley and Orton 1983).

The cultivated species P. c. ssp. coccineus included 7 populations from Puebla and Chihuahua. Seven populations of the other cultivated bean, P. coccineus ssp. darwinianus, were also studied. The wild relative, P. coccineus ssp. formosus, was analyzed based upon 4 populations from the south (Puebla, Tlaxcala and Morelos) and 2 from Chihuahua.

Enzymes stain recipes were modified from those of Soltis et al. (1983) and Piñero and Eguiarte (1988). For enzymes with more than one zone of activity, the last anodally migrating zone (locus) was designated as the locus; in a given locus, the alleles were named in the same way.

Enzyme systems

Genotypes for the description of the genetic variation was carried out using the youngest seedling leaf extracts of bean plantlets and standard horizontal starch gel electrophoresis. Twenty one enzymes in 5 distinct buffer systems were used. Based upon the results, 8 enzymes coding for 10 polymorphic loci were selected for subsequent analysis. The enzymes with the best resolution using 2 of the buffer systems were: MDH, SDM, PGM, PGI, ACPH using buffer system I (Piñero and Eguiarte 1988); GOT, DIA, CPX using buffer system 8 (Soltis et al. 1983) (Table 2).

Distribution of genetic variation

One commonly used measure to determine the quantity of existing variation as well as its distribution is the Index of Multilocus Genetic Diversity (Hm) and can be interpreted as the percentage of heterozygotic loci expected from an individual selected randomly from a population. Based upon the genotypic frequencies obtained by electrophoresis, this index was calculated for each population of P. coccineus.

The Index of Multilocus Fixation of Wright (Fm) represents the standard deviation of the frequency of the heterozygotes observed with respect to the expected according to the Hardy-Weinberg equilibrium model.

When it is suspected that the reproductive system is the principal determinant of the genetic structure of a population, one can obtain an estimate of the rate of outcrossing based upon Fm. This method also assumes that the population has reached equilibrium of endogamy for which the outcrossing rate obtained should be considered a preliminary approximation. The rates of outcrossing (te) assuming endogamy equilibrium are calculated from the Fm.

Genetic differentiation between populations

In order to estimate the processes that determine the genetic structure of each subspecies and of the species as a whole, one can analyze each population of the subspecies as if they were subpopulations of a structured population. This can be

done by means of Wright's Fixation Index (Fm) which measures how much the genotypic frequencies of the subdivided population diverge from the that of the expected from a random. Also, this allows for the separation of possible causes of the genetic differentiation that can be the reproductive system (Fis), genetic drift, gene flow and natural selection (Fst).

RESULTS and DISCUSSION

The patterns of the genetic variation encountered in the 10 loci analyzed are presented in the zymogram (Fig. 3). For each enzyme, the number of loci are listed as well as the number of alleles for each locus. The numbers in the lower part indicate the possible homozygous genotypes found while the banding patterns among them represent the possible heterozygotic genotypes. The shaded zones correspond to regions where genetic variation was not interpretable.

The Index of Multilocus Genetic Diversity (Hm) are summarized in Table 3. The values encountered for P. c. ssp. coccineus from Chihuahua were 0.152 and 0.313. In the case of two populations of P. coccineus ssp. darwinianus from Puebla the values were 0.221 and 0.361. Finally, P. coccineus ssp. formosus from Chihuahua was 0.238 and from Tlaxcala, 0.317.

The genetic diversity encountered are similar for other populations of the same species (e.g., 0.319, Wall and Wall 1975 in Hamrick 1983; 0.322, Coello and Escalante 1989). Also, the figures are similar to the average value reported for plants with mixed breeding systems and animal pollination (i.e., 0.29, Hamrick 1983).

Index of Multilocus Fixation of Wright (Fm) values for the populations studied are listed in Table 3. The positive values indicate a deficiency in the number of heterozygotes in the population (based upon the expected values determined by the Hardy-Weinberg model) and can be explained by the action of three processes: 1) reproductive system, 2) natural selection, and 3) genetic drift. The highest average Fixation Index was found in the wild populations of P. coccineus ssp. formosus and was 0.436. Lower values were found in the cultivated taxa P. c. ssp. coccineus and P. coccineus ssp. darwinianus with the respective values of 0.351 and 0.363. These results indicate that P. coccineus ssp. formosus is more endogamic than the cultivated relatives.

The rates of outcrossing are below the endogamy equilibrium (te) are shown in Table 3. The rate of outcrossing of P. c. ssp. coccineus is slightly greater than that obtained for the cultivated P. coccineus ssp. darwinianus and the wild P. coccineus ssp. formosus. The outcrossing rate (te) suggests that the frequency of self-fertilization of P. coccineus ssp. darwinianus and P. coccineus ssp. formosus is greater than that of P. c. ssp. coccineus. The outcrossing rates (te) encountered indicate the existence of the a mixed breeding system with a predominance of outcrossing. These results are similar to those reported in other populations of the same species (Coello and

Escalante 1989).

The F-statistics for genetic differentiation between populations are listed in Tables 4 through 10. The Fis values for P. c. ssp. coccineus, P. coccineus ssp. darwinianus, and P. coccineus ssp. formosus are high and indicate that the reproductive system is an important process involved in the determination of the genetic structure of these populations. The Fst index was higher in populations from Puebla indicating a greater differentiation among the populations within the same subspecies. None the less, later collections of P. c. ssp. coccineus, P. coccineus ssp. darwinianus from Puebla have smaller Fst values that indicate that these subpopulations are not that different and reflect the existence of gene flow between them. Also, the fact that the Fst of all the collections is greater with each subspecies suggests that there is greater differentiation between subspecies than within them. This pattern of greater genetic differentiation between subspecies than within can be seen in the dendrogram of Figs. 3 through 5. The populations of P. coccineus ssp. darwinianus group together at a genetic distance of about 0.027 while the populations of P. c. ssp. coccineus form a more heterogenous group with a distance of 0.08. A similar pattern is demostrated in the results of the genetic distances of Nei (1972) found among different populations of the 3 subspecies of P. coccineus (Tables 11 through 14).

R3 - CYTOGENETIC STUDIES OF Phaseolus coccineus AND P. vulgaris

Various populations were studied in the case of P. coccineus. For P. c. ssp. coccineus 7 cultivated populations from the Sierra Norte de Puebla were studied including 6 from Puebla and 1 from nearby Veracruz. Two cultivated populations of P. coccineus ssp. darwinianus were examined from the Sierra Norte de Puebla. In the case of P. coccineus ssp. formosus, 3 wild populations were sampled, one each from Puebla, Tlaxcala and Chihuahua. Two wild populations of P. coccineus ssp. glabellus from the Sierra Norte de Puebla were analyzed. The plants of P. vulgaris used for obtaining the chromosome number counts and karyotypes were obtained by germinating seeds collected from 6 populations located in the Sierra Norte de Puebla.

The study of the cells in the mitotic metaphase was carried out using the apical meristems of the secondary roots of the plantlets. The roots selected were between 1.5 and 2.0 cm in length and were harvested between 7 and 8 AM. Pretreatment of the root fragments consisted of immersion in an aqueous solution of 8-hydroxyquinoline (0.002 M) and maintained in the dark for five (5) hours at 18 C. The root tips were subsequently fixed in a mixture of Farmer solution, ethyl alcohol and acetic acid (3:1). The chromosomes were stained by hydrolyzing the roots with HCl (1 N) at 60°C for 10 minutes and later by placing them in a Feulgen solution. When the apical meristems acquired a

violet coloration, they were placed on a glass slide in a 1% aceto-orcein solution. Using a clear field, the tissue was observed using an optical microscope. The preparations in which cells with metaphase chromosome were observed were made permanent using the dry ice method (Conger and Fairchild 1953). Each population consisted of 10 to 12 plants in which at least 30 cells were analyzed. The 3 most representative cells of each population were photographed using a Zeiss photomicroscope and the negatives were printed.

The chromosomes of the selected cells were drawn from a projection of the negatives using a slide projectors. Based upon the drawings and the photographs, the karyotypes were made as well as the ideograms. Homologous pairs of chromosomes and their classification were made based upon the templates of Naranjo *et al*. (1986) using the methodology of Levan *et al*. (1964).

The observations of bivalent chromosomes in metaphase I of meiosis were made using pollen mother cells (PMC) from anthers obtained from young floral buds that were collected and fixed in the field from various localities in the Sierra Norte de Puebla and Chihuahua. The anthers were separated under the dissecting microscope, stained with aceto-orcein and observed in clear field of a optical microscope. The MI preparations were fixed using the same methodology listed for mitosis.

RESULTS and DISCUSSION

The diploid chromosome number of $2n=22$ was obtained for the samples of *P. vulgaris*, *P. c.* ssp. *coccineus*, ssp. *darwinianus*, ssp. *formosus*, and ssp. *glabellus*. The basic chromosome number is $n=11$ which was confirmed by the observations of 11 bivalent ($n=11$) in the meiotic preparations (Table 16).

Differences were found in the karyotypes of the four varieties of *P. vulgaris* in terms of the proportion of metacentric (m), submetacentric (sm) and telocentric (t) chromosomes as well as by the presence of satellites (double constrictions). The populations in which chromosome satellites were observed on both chromosome arms represent the first report of this characteristic in the genus *Phaseolus*. The karyotypic formula for these plants are found in Table 17.

The karyotypes of the 7 populations of *P. coccineus* ssp. *coccineus* of the Sierra Norte de Puebla and Veracruz showed no variations and consisted of 11 pairs of metacentric chromosomes with satellites on one arm of 4 chromosome pairs and with satellites on both arms of 1 chromosome pair (Figs. 6a, 8a; Table 18).

In the case of cultivated populations of *P. coccineus* ssp. *darwinianus* of the Sierra Norte de Puebla, the karyotypes were the same with 10 metacentric pairs and 1 submetacentric pair. Satellites were found on one arm of 4 metacentric chromosome pairs and double satellites on both arms of one metacentric chromosome pair (Figs. 6b, 8b; Table 18).

The 2 wild populations of *P. coccineus* ssp. *glabellus* of Puebla also expressed no variation with 9 metacentric pairs and 2

submetacentric pairs. As above, satellites were found on one arm of 4 metacentric chromosome pairs and double satellites on both arms of one metacentric chromosome pair (Figs. 6c, 8c; Table 18).

The preliminary analysis of P. coccineus ssp. formosus indicates that the mitotic count is 2n=22 (Figs. 7a, 7b).

Meiotic analysis of MI of the 3 populations of P. coccineus ("ayocote guia", "ayocote semiguia" and "tacahuaquet") and of P. coccineus ssp. formosus indicated normal behavior of the 11 bivalents with formation of 7 in ring formation and 4 in chain formation.

R4 - ETHNOBOTANY

Cultural data obtained in relation to the interaction of humans and beans include: 1) nomenclature, use and management of the different forms of P. coccineus and P. vulgaris by farmers in five communities in the regions; 2) agricultural calendars, plant phenology, description and objectives of the distinct agricultural practices along with the variations applied to the spatial and temporal structure that the farmers used to modify the agricultural cycle; 3) cultural, ritual and ceremonial practices associated with the bean and bean-maize cultures. These factors are inseparable when discussing the interrelations between beans and humans as well as in the contemporary biology of the these food plants as a basis for their diversity, evolution and conservation.

These data were obtained during periodic visits (i.e., once per 2 to 4 weeks, depending upon the agricultural cycle) through participant observation and open interviews. In each zone, indigenous peoples collaborated in the planting, maintenance and collection of field and experimental data. The ethnobotanists worked alone as well as in collaboration with members from the ecophysiology and taxonomy teams so as to ensure that cultural data and ecological data were based on comparable collections.

RESULTS and DISCUSSION

Sierra Norte de Puebla, Puebla

Ethnobotanical studies focused principally on P. coccineus although certain attention was given to P. vulgaris which is cultivated along with the former bean. One wild bean, P. coccineus ssp. glabellus grows along the margins of the forest patches near the cultivated fields. Cultivated P. coccineus is represented by two subspecies, ssp. coccineus and ssp. darwinianus. The former is also frequent as a feral plant in the area. Both taxa grow principally as vines or climbers. Phaseolus vulgaris is found as both bush and vine forms.

Beans are known generically to the Nahua and Totonaco Indians as "et" and "stapu," respectively, and include other legumes in addition to Phaseolus. Characteristics used to distinguish the various bean forms include the seed (color, form,

seed coat hardness, palatability), the pod (form and size), growth habit, duration of the life cycle, habitat where it grows and the plants with which they are associated.

The functions of beans in the local economy are to meet family food needs and for extra income through the sale of surplus beans, although the degree of importance varies considerably among the different communities. For example, in Nauzontla, P. coccineus ssp. darwinianus is a relatively important as a basis of economic interchange through the sale of green beans ("ejote ya llegado") and green bean seeds (which have a great demand in the market through All Saints Day because the tender seeds are used to make special "tamales" used as offerings on the altars of the dead). In Zoactepan and Huahuaxtla, the green pods are also cut but they are not as important as in Nauzontla. On the other hand, the green pods in Jilotzingo and Eloxochitlán are eaten exclusively in the homes and only the dry bean seeds are commercialized.

The bush form of P. vulgaris is an important crop in Huahuaxtla where it is interplanted in almost all the fields ("milpas") and orchards. The seeds are relatively important in local commerce here and less so in Jilotzingo and Eloxochitlán.

In general, the beans are used basically as human food. The dry seed, green tender seed, young tender leaves, flowers and the underground tuber ("camote") are eaten (Table 19). In addition to food use, the black seeded bush common bean is medicinal; it is drunk in a soup (after the first boiling so that it is not too cooked) in order cure "postema" or internal bruises. The crude bean soup is said to act as purgative in that it helps eliminate the 'bad blood' and thus alleviating the "postema." The foliage of cut bean plants is used as forage for sheep, goats, cattle and horses while the spontaneous sprouts and regrowth of the P. coccineus is used as forage for fowl.

In the communities studied, the agricultural systems are similar in that they distinguish between the cultivated plots in the "huertas" and the "ranchos" in terms of their distance from the house. "Huertas" are relatively close to the main dwelling while the "ranchos" are located some distance. In both sites, the polyculture of bean-maize is common. Generally, the "huertas" are managed more intensely and the density of crops is higher and may include potato, broad bean and peas along with tolerated or induced 'weeds' which are consumed by both humans and animals. Where beans are cultivated in permanent plots, some with constant use of 40 to 50 years, the swidden agricultural system is employed. The area dedicated to this practice is reduced today and is found in two forms. One form is to use area covered by primary cloud forests or oak woodlands in the bottom of hollows. The bean plantings are usually monocultures and the plots are recycled every 10 or 15 (up to 20) years. The other form is the use of secondary forests (usually dominated by alder, Alnus firmifolia) which are cut over every 5 or 6 years between cultivation. The various cultivation systems in the communities studied are listed in Table 20.

The agricultural calendar depends upon local environmental conditions as well as cultural priorities. In general, the agricultural cycle starts in January (and sometimes until April) and ends in October-November (sometimes until December-January) (Table 21) with Nauzontla and Jilotzingo being the extremes of the variations. In the five study areas, the following agricultural practices are basic: 1) ground clearing; 2) plowing or rotation; 3) planting; 4) re-planting; 5) one or two weedings; 6) hilling; 7) harvesting. The basic agricultural equipment used by the farmers include: wooden or metal plow, hoe, planting stick with or without metal point, machete, hooked pole, husker or picker. Agrochemicals are usually not used with the exception in some cases of commercial fertilizer. The cultivation of potatoes may involve the aspersion of pesticides and leaf fertilizer.

The maize-bean cultivation systems are accompanied by certain ceremonies that are similar to preHispanic practices which have since become synchronized with Catholic rituals.

The interaction of beans and humans in the Sierra Norte de Puebla viewed from the selection and management of bean diversity over time has two forms. The first is that of human action which selects plants with characteristics which meet human needs and desires. The second is that of the biology of the bean (including life forms, growth habits, reproductive systems and life cycles) upon which the domestication process functions integrating human preferences and biological potentials in a determined environment.

Specifically, the polyculture of beans in association with maize includes the management of two forms of common bean (bush and vine forms of P. vulgaris) and two subspecies of P. coccineus whose reproductive optimum is out of phase with that of the maize plant. Also the agroecosystem of maize-bean incorporates the management and use of different evolutionary phases of beans from wild to domesticated forms and including tolerated, induced and feral populations. The different evolutionary degrees of allow the farmers the liberty to select and adapt beans from a genetically diversified reservoir and integrate them into traditional agricultural practices. Two reproductive systems are available with self-fertilization in P. vulgaris and outcrossing in P. coccineus. The human maintenance different subspecies of P. coccineus and populations in varying degrees of domestication has promoted genetic exchange and selection of hybrids to meet local cultural needs and environmental limitations such as the "patlashtle pinto" bean of Jilotzingo.

The management and selection of the Phaseolus variability and it conservation in the Sierra Norte de Puebla allows a more intense use of the land (normally not amenable mechanized agriculture) with different plant products are different times of the year (Table 22). Not only does this system permit the assurance of a minimum harvest even in bad times but it also allows the farmer the possibility of experimenting with plants in order to satisfy his needs. One example is that of "exoyema abreviador" which is a precocious fruiting P. coccineus ssp.

darwinianus; this early fruiting bean meets the recent economic demand for a green beans ("ejote ya llegado") two or three weeks ahead of normal crops in the market and thus giving the farmers an economic gain two or three times greater that the normal profit during the normal season. Collaborative ecophysiological studies confirm the divergence of this population from the typical P. coccineus ssp. darwinianus with directional selection affecting more the behavioral rather than the morphological characteristics.

Sierra Tarahumara, Chihuahua

In this region of northern Mexico, three species are grown for food: P. vulgaris, P. coccineus, and P. acutifolius. The major richness in cultivated Phaseolus is found in the common bean (P. vulgaris). The wild species found in the area are: P. grayanus, P. ritensis, P. parvulus, P. acutifolius, P. coccineus ssp. formosus. Twenty sites were studied in the zone where seed collections and data on bean nomenclature, use, and management were obtained. In all but one case (Creel), the population was Tarahumara Indians (or "Rarámuri").

In the case of P. vulgaris, there are 40 varieties recognized based upon seed color, form and size as well as the growth habitat according to the farmers interviewed (Table 23). The varieties are distributed through out the sierras and the barrancas. None the less it is notable that the "frijol azufrado" is more common in the barrancas than in the sierras. The traditional form of bean cultivation is to plant the various forms in mixes. Often 3, 4 or 5 types are combined for eating. On some occasions up to 15 varieties have been seen planted and harvested together.

Phaseolus coccineus is represented by the domesticated form (ssp. coccineus) and the wild form (ssp. formosus). The cultivated form is a bush type while the latter has an indeterminant growth form. Spontaneously growing plants with intermediate characteristics (e.g., intermediate seed size, greater seed germination rate than that for the wild seed, production of fruit and viable seed in the first year of growth) along with evidence for pollen flow indicate the active genetic exchange between the domesticated and wild forms of P. coccineus.

The seeds of P. coccineus are large and are cultivated principally in the sierras and sometimes in the upper parts of the barrancas. Although there is no variation in seed form, there is considerable differences in terms of color: purple, violet, grey, dark grey, black, and white.

The Tarahumara term for bean is "muni" which is further subdivided into three groups: 1) "muni" for the common bush bean (P. vulgaris); 2) "cusímuni," "cusímari," or "busímari" for the common climbing or vine bean (P. vulgaris); and 3) "tecómari," "tecómare," "recómari," or "recómuni" for the cultivated P. coccineus. Within each group, the beans are subsequently separated and named by seed characteristics, especially color and seed coat designs.

The Tarahumara use beans principally as food in the form of dried seeds, as fresh green pods or as prepared bean pods ("ejotes pasados" which are scalded and dried for later use in the winter). On some occasions, the tender foliage is eaten as a cooked vegetable. The beans are usually grown for consumption by the farmer's family although a small amount may be used as exchange with other families or for sale. When there is an excess harvest, some Tarahumara sell a few sacks of beans in the large towns in the area, such as Creel and San Juanito.

The beans are prepared in various ways for eating. The seeds are cooked and the green fruits immersed in boiling water. The dry seed is toasted. As preparation for storage and later consumption in the winter, the tender pods are scalded and sun-dried. The dry bean can be cooked alone or with maize in order to prepare a "pozole." Phaseolus vulgaris seeds are toasted in special vessels called "esquiteras" which are also used for toasted grains of maize. Afterwards, the beans with or without the seed coat are ground on a metate and cooked with a little water. The resulting beverage is called "chowewari" which is drunk while eating tortillas.

During the winter in the barrancas, the green bean pods are harvested in small irrigated monoculture plots. These plots are almost exclusively dedicated to the production of green beans rather than mature seed.

Uses other than that for human food are known in the area. The stems and dry pods or "pajas" of bean plants are used a forage, especially for goats. The roots or "camote" of P. coccineus ssp. formosus (small seeded form) are used medicinally in order to relieve stomachic aliments.

Beans in the Sierra Tarahumara are cultivated as a monoculture or as a polyculture in association with maize. The plots may be permanent or form part of the swidden agricultural system. The bush forms of P. coccineus and P. vulgaris are usually planted as monoculture in fields adjacent to those with maize, broad bean, oat or potatoes. On some occasions, the several rows of pure beans are grown in between rows of maize. This practice is usually a replanting with beans when the originally seeded maize failed to germinate and is a form of taking advantage of an otherwise void plot. The vine forms can be planted alone using dead supports but usually are seeded with maize which serves as a live support. The bean-maize association is most common in lower areas where the growing season is longer (between frosts) because the vine beans usually mature after the maize. The bush beans usually mature earlier than the vine types, in part because the bush type is planted earlier.

Some Tarahumara farmers plant beans in permanent fields. None the less, many rotate every two or three years the seeding because the production diminishes dramatically after that time.

The swidden cultivation ("roza-tumba-quema") of beans is most common in the upper barrancan oak woodland at the interface of the barrancan and sierra vegetation. The slashing and felling of oak trees and secondary growth (e.g., "manzanita" or

Arctostaphylos pungens) is followed by a year during which the biomass dries on the site. After that, it is burnt and later planted at the start of the growing season. The soil is not plowed and the only tool used is a planting stick called "cubarra."

The bush beans (both P. coccineus and P. vulgaris) are planted "mateados" in which three or four seeds planted in each hole and with a distance of 30 to 40 (up to 50) cm between the holes. The distance between the rows is 50 to 60 (up to 70) cm. Another planting form of sowing bush beans is "chorillo" in which the seeds are spread individually along the length of the row. In the case of the vine beans associated with maize, the seeds are planted along with maize grains at a distance of 50 to 60 (up to 70) cm between the holes and 70 to 90 (up to 100) cm between the rows. In the case of the vine beans grown on dead supports, the seeds are sown at a distance of 100 to 120 cm between clumps.

Various agricultural practices are associated with bean cultivation. In general, they include: 1) preparation of the soil, either by plowing with wooden plows or by burning; 2) sowing of seeds; 3) one or two weedings, with the use of the wooden plow or hoe or by hand (depending upon the area to be cleaned and the density of herbs); and 4) harvest. Fertilization involves the application of cured goat manure. The use of chemical fertilizers is uncommon in the Tarahumara cultivations.

The agricultural calendar varies with the different sites in the Sierra Tarahumara. At sites in higher elevations, the cycle begins in early June and terminates in late September or early October, depending upon the frosts. At sites in the lower barrancas, planting occurs in June or July and the harvest in October or November. These dates usually apply to the bush beans. In the case of the vine beans, they are planted earlier along with maize during the second half of April or early May in both the sierras and the barrancas.

The differences in the agricultural calendar is due to the limiting factors on the growth period of the beans. In the sierras, the late frosts at planting and the early frosts before harvesting are the most important. In the barrancas, the availability of water (when irrigation is not available) is limited to the wet season (June-July to September).

The ceremonial role of beans among the Tarahumara Indians is limited. At the start of the harvesting period, a special "tamal" is made from young blue maize grain and filled with the earlier ripening "tecómari" beans that are cooked and ground.

One of the most important factors in the Sierra Tarahumara that has influenced the selection of diversity of beans is that of short growing season, usually about 100 days. This period is mostly defined by the climatic factors such as freezes and scarcity of rain. Among the cultural factors which influence the selection and diversity of beans, two are most striking. The Tarahumara prefer beans that are light colored and have selected and maintained many different forms based upon the intensity of color, form, size and flavor. The other factor is the eagerness

of the Tarahumara farmers to assure a minimum harvest during the short and often unpredictable growing season. This has resulted in the maintenance and management of bean mixtures which vary not only in seed shape and color but also in the duration of the life cycle and the phenological behavior which the Tarahumara are acutely aware.

R5 - TAXONOMY: POLLINATION BIOLOGY

Several aspects of the pollination biology of P. coccineus were studied. The object was to elucidate the patterns of pollen flow among the five different sympatric populations of this complex and thus decipher the relationships that may exist within it. The five populations include: P. c. ssp. coccineus "Ayocote", P. coccineus ssp. darwinianus "Gordo" (both cultivated), and P. coccineus ssp. glabellus (wild), from Puebla; and P. c. ssp. coccineus "Tecómari" (cultivated) and P. coccineus ssp. formosus (wild), from Chihuahua.

The architecture and disposition of the different flower parts were studied and described according to their attraction and coupling with pollinators. Nectar production rates were measured in the field using micropipettes and fractometers. Pollen viability was determined by standard staining techniques. Pollen/ovule ratios of each taxon were calculated. Experiments to demonstrate pollen movement by pollinators in the field sites were carried out by marking flowers with florescent powder. UV patterns on corolla were determined by photographing the flowers using various filters along with a gray scale (Kevan et al. 1973).

Flower visitors were collected (where possible) and identified in the Institute of Biology (UNAM). Birds visiting the flowers were identified in the field. True pollinators were determined by observing directly their morphology, their conduct on the flower (if they activated or not the floral mechanism that "protrudes" the stigma and pollen brush) and the number and constancy of their visits to each given subspecies. To further reinforce these observations, the distance between the nectary and the protruded stigma was measured and overlapped on the image of a bee's head comparing it to the distance between its protruded proboscis and the back part of its head, thus calculating where the stigma and brush usually touches it. A zone close to the mouth was considered less convenient than a zone further back, since the pollen may be eaten or disposed of more easily near the mouth parts.

RESULTS and DISCUSSION

The general structure of the flowers belonging to all the populations is very much the same. It follows the same pattern as the rest of the genus. The few differences refer mainly to size; the flowers of the cultivars are usually bigger, and with color (some are deep red, some are orangish, and some are white). Some

populations such as "Ayocote" and "Tecómari" display an array of almost all the colors above and all of the intermediate tones and hues. The largest flowers were the ones belonging to the cultivars: P. c. ssp. coccineus (almost all red) in Puebla displayed the largest banner (159.7 mm^2, right wing = 114.3 mm^2) and P. c. ssp. coccineus "Tecómari" (red and white) in Chihuahua the largest right wings (196.1 mm^2, banner = 127.9 mm^2). Phaseolus coccineus ssp. darwinianus (white to lilac flowers) displayed intermediate size flowers (banner = 99.84 cm^2, right wing = 141.31 mm^2), as did P. coccineus ssp. formosus (red flowers, banner = 76.6 cm^2, right wing = 97.8 mm^2). The smallest flowers belonged to P. coccineus ssp. glabellus (red banner = 47.5 mm^2, orange right wing = 38.5 mm^2), which also displayed an almost closed banner that practically concealed the wings, giving it a sort of tubular morphology, ideal for hummingbirds and insects with long probosces.

As previously noted by other workers, there exists in all five populations, a floral mechanism, without the activation of which pollination (self or crossed) is practically impossible. Therefore there must exist a mechanical activation (a pollinator) of such mechanism to: 1) perforate the stigmatic cuticle; 2) expose the pollen brush that carries the pollen grains from the flower's anthers; and 3) carry, also by means of the pollen brush, the foreign or self pollen grains to the stigmatic surface and consequently carry out the self- or cross-pollination.

Nectar production in wild bean flowers was slightly more than cultivated ones, though the cultivars generally produce nectar in a more constant manner. Phaseolus coccineus ssp. glabellus stands out in that it produces a more sugar-rich nectar than the rest of the populations. The nectar production patterns throughout the day are strikingly different from population to population, let alone between subspecies and localities. This situation leads us to believe that the daily nectar productions rates may be determined by a myriad of circumstances which must necessarily include: environmental temperature and humidity, individual genetic variation, and of course phylogenetic differences between the various taxa. On the average, P. coccineus ssp. glabellus produces more nectar (28.5 $\mu\ell$) and with a higher sugar content (28.5%) than any other studied taxon. It is also the most variable in amount of nectar produced (1.5-15 $\mu\ell$) throughout the day, but not necessarily the most variable concerning the amount of sugar (16-32%), since P. c. ssp. coccineus, with an average of 26.89% of sugar concentration (average quantity: 3.5 $\mu\ell$, 1-6.5 $\mu\ell$) varies from 12% to 32%, depending on the hour of the day. These variations mean that at different times of the day, different flowers belonging to different populations (and different subspecies at different localities) will offer different amounts of nectar with different concentrations of sugar to the visiting pollinators. This in turn, shall necessarily determine what animals will visit them throughout the day.

Pollen viability turned out to be very high in all of the populations: averages of 89% and above. The number of pollen grains per flower is also very constant among the five populations (around 5,000), except for "Tecómari," which contains an average of 4,500 pollen grains per flower.

From among the numerous visitors to P. coccineus flowers (Table 24), Bombus ephippiatus emerged as the best pollinator of "Tecómari" in Chihuahua and all of the subspecies in Puebla, rendering it and B. pullatus the probable role of "gene-vector" between P. c. ssp. coccineus and P. coccineus ssp. darwinianus. Hummingbirds of different species turned out to be very constant visitors of all the subspecies (including white "Tecómari" flowers), except P. coccineus ssp. darwinianus which was intensively visited by carpenter bees (Xylocopa). The hummingbirds of Chihuahua appear to be the important "gene-vectors" between the wild P. coccineus ssp. formosus and P. c. ssp. coccineus "Tecómari".

Many flower or flower-structure UV reflection studies are thoroughly quantified and their discussion may be deceiving, especially because UV is considered without making reference to the other basic colors visible to the insect (Kevan 1978). Time-dependant changes in coloration may occur in flowers that have not been pollinated, or have been pollinated with non-compatible pollen. Changes caused by pollinators and pollination itself may benefit the plant indicating the lack of reward to the pollinator, the change may increase its foraging efficiency, which in turn could increase the pollination efficiency of the plant itself.

This study found that the role of the keel in Phaseolus, particularly in the P. coccineus complex, is highly important in order to answer the following questions. Why does Bombus ephippiatus, an important pollinator, always "land" on the flowers' right sided wing, if it could also do so on the left one? The answer is that in this case the keel has a role of nectar guide. The keel's morphology is important, spiral-shaped and the tip pointing towards the right, as is its UV absorption, which indicates the presence of a nectary on that side to the pollinator. The keel absorbs UV, and thus contrasts with the reflecting structures around it, a feature an insect can detect at short distances.

Based upon the analysis of pollen/ovule ratios of each taxon, they all tend to be xenogamous-facultative, that is, they tend to outcross but nevertheless can self-pollinate (and consequently self-fertilize). Curiously enough, the wild subspecies (P. coccineus ssp. formosus and P. coccineus ssp. glabellus) are less variable in this respect than the cultivars in both localities.

The wild and cultivated species could apparently intercross freely, due to the facts, among others, that their flowering seasons overlap throughout the year (almost always at least 2 do so), and they share at least some of their pollinators, which appear to indiscriminately visit flowers belonging to several

subspecies on a single foraging flight. Nevertheless, in spite of one obvious hybrid in Puebla between 2 of the cultivars (P. c. ssp. coccineus × P. coccineus ssp. darwinianus), the subspecies tend to remain genetically apart: whether as a result of natural isolating mechanisms, or as a result of human intervention, the wild subspecies (P. coccineus ssp. glabellus) and the cultivars (P. c. ssp. coccineus and P. coccineus ssp. darwinianus) have remained reproductively separate in Puebla. In Chihuahua the opposite is true: the wild P. coccineus ssp. formosus populations breed in an apparent free manner with the "Tecómari" cultivar (P. c. ssp. coccineus) populations where it was seen that pollen from "Tecómari" can travel up to 200 m where the wild populations (P. coccineus ssp. formosus) are located, and further confirmed by the finding of apparent hybrid individuals in the wild populations.

There exist possible gene-flow patterns among the different subspecies populations in both localities. In Chihuahua there is active breeding between both the wild and the cultivated populations. In Puebla the wild population, although sympatric, is apparently reproductively isolated from both cultivars. These cultivars do not often interbreed, but nevertheless can produce hybrid offspring. Also, the wild subspecies from Puebla (P. coccineus ssp. glabellus) displays reproductive patterns so distinct that it should be placed apart taxonomically.

R6 - ECOPHYSIOLOGY

Ecophysiological studies of the project focused on 3 main lines: the environmental variables and limiting resources in the photosynthetic response and in the production of the different types of beans in the P. coccineus complex; 2) the kinds of morphological and physiological adaptations have developed in response to these limitations; and 3) the range of tolerance of the different bean taxa.

Members of the P. coccineus complex (P. c. ssp. coccineus, P. coccineus ssp. darwinianus, P. coccineus ssp. formosus, and P. coccineus ssp. glabellus) were cultivated in the 2 study sites (Chihuahua and Puebla) and were studied to determine phenology, growth relationships, gas exchange and water balance. These studied were designed in order to discern the possible degree of physiological differentiation between the wild and cultivated forms and the role of agricultural practices applied traditionally to them.

RESULTS and DISCUSSION

Ecophysiology of the Phaseolus coccineus complex

The species of beans are characterized by their low resistance to drought with a low (almost nil) osmotic adjustment by the cells. These measurements have been determined in the

past for P. vulgaris, P. acutifolius, and P. filiformis. We obtained similar data for varieties of P. c. ssp. coccineus and P. coccineus ssp. darwinianus (Table 25). Between May (the driest month) and July (the wettest month) in the Sierra Norte de Puebla, the difference in the osmotic adjustment was always reduced (ca. 0.5 bars). Only in the case of the bush-form P. coccineus "Tecómari" was the osmotic adjustment for the same period above 1 bar (i.e., 2.2 to 3.3 bars).

In addition to present an osmotic potential relatively high (ca. 3 bars), P. coccineus has a high relative hydrolic capacity ($C=\partial RWC/\partial \Psi$). This coincides with the presence of potential relatively high associated with the loss of turgidity (ca. -10 bars) which suggests that the cells of beans act as if they were large reservoirs of water with cell walls relatively elastic (low elastic module, eß). At similar situation exists in tomato (Jones 1983).

The photosynthetic capacity in the case of the bean taxa is only well known for the common bean (P. vulgaris) and less in the other cultivated species. In the case of the common bean, the photosynthetic capacity is different according to the variety with a range from 10 to 20 μmol $m^{-2}s^{-1}$ although on some occasions the levels exceeding 40 μmol $m^{-2}s^{-1}$ have been reported. In the case of the different Phaseolus species growing under greenhouse conditions, photosynthetic capacity is 8 μmol $m^{-2}s^{-1}$ in P. coccineus ssp. glabellus and P. c. ssp. coccineus. Within the P. coccineus complex there exist a great similarity in the photosynthetic capacity of the different species and only marginally greater in the case of P. coccineus "Tecómari" of the Sierra Tarahumara which has a semivine habit and a short vegetative cycle.

In contrast to the data reported in the literature, the low photosynthetic rates in individuals of P. coccineus come from the wild populations and the higher rates from the cultivated forms (Fig. 9). In comparative studies in the field and in the greenhouse, it was found that the response of photosynthesis to temperature in individuals of P. coccineus ssp. glabellus had an optimum of 30°C (Fig. 10), about 10°C above the medium temperatures encountered at the site of origin. This suggests that the individual plants acclimated to the conditions of the greenhouse. The acclimation capacity to temperature apparently has diminished in the variety from Chihuahua which does not naturally grow under such temperatures.

Water Balance of Phaseolus coccineus in the cultivation association of maize (Zea mays) and beans

Water relations of the cultivation associations maize-P. coccineus ssp. darwinianus and maize-P. c. ssp. coccineus had not been evaluated previously. The only factor considered prior to our study was the yield component of such agricultural associations (Lepiz 1974). The maize-bean association is a dominant cultural practice in the Sierra Norte de Puebla. Under

this condition, it is possible to determine the degree of physiological coupling developed by P. coccineus under this specific agricultural condition. This situation is a system with "simple" competition where the competitive relations can be studied from the point of view of ecophysiology and competition for resources (Tilman 1988).

The water potential in P. coccineus ssp. darwinianus alone and in P. coccineus ssp. darwinianus associated with maize presents 3 phases during the growth cycle of the bean. The period of major water stress occurs during the months of April and May while during the other 2 periods, June-July and August-September, the stress is less or intermediate, respectively, even though there is more rain in this study area during the last months of the cultivation cycle.

During the greatest drought period, P. coccineus ssp. darwinianus can present water potentials from -13 to -14 bars at noon. The major effect of the association on the potential is demonstrated during the last month of drought where P. coccineus ssp. darwinianus associated with maize has water potentials considerably less (ca. -10 bars) with the respect the bean plants cultivated alone (i.e., not associated with maize; ca. -7 bars).

The presence of statistically significant differences between the same months of the year for the data derived from 1990 and 1991 studies demonstrates that the period of major stress varies between April and May (Table 26), probably a response to the winter humidity conditions of the soil.

Using data from the period of major stress for beans in association with maize, the data indicate that even though the lowest potentials are found in individuals of P. coccineus ssp. darwinianus associated with maize, the most daily transpiration is found in leaves of individuals planted alone (Castellanos and Martínez 1992).

Ecophysiological aspects of the domestication process in the Phaseolus coccineus complex

This part of the study focused upon the architectural relationships with the physiology of two similar varieties of P. c. ssp. coccineus, both bush types but of different origin ("Tecómari" of Chihuahua and semi-vine "Ayocote" of Puebla). The detailed study of these two varieties is an attempt to clarify the physiological characteristics responsible for the adaptation of "Tecómari" to the growing conditions of the mountains of Chihuahua so that some of the events involved in the domestication process can be deduced more clearly (Smartt 1989, 1990; Plitmann and Kislev 1989).

"Tecómari" is characterized by an indeterminant bush growth form, sometimes forming a semi-vine, with a rapid vegetative and reproductive growth. "Ayocote" has a semi-vine bush growth form similar to "Tecómari" but produces greater amount of foliage and tends to be indeterminant as it has more nodes than "Tecómari."

In both varieties, the interception of solar radiation (PAR) was quite variable along the nodes of the main stem. None the

less, the peaks of high radiation were encountered in the intermediate nodes and not necessarily in the superior nodes. The semi-vine "Ayocote" maintains considerably less radiation in the foliage along the main stem due to the changes in the orientation of the leaflets of the terminal leaves and the location of the same nodes in microsites with less exposure on the inside of the foliage canopy or among other individuals.

The foliar temperatures varies with the wild P. coccineus ssp. formosus having the highest followed by the cultivated "Tecómari" and finally by the cultivated "Ayocote." The foliar temperatures of "Tecómari" along the nodes of the main axis were 3°C or more above those found in the semi-vine "Ayocote" (Fig. 11). It is possible that the "Tecómari" maintains greater temperatures due to greater opening of the foliage along the stem and by the presence of the reversible leaflet movements that permit the leaves to orient themselves perpendicular to the sun's rays.

Physiological changes are present even though both varieties respond in a similar form to temperature, foliar radiation and, consequently, vapor pressure deficit. "Tecómari" has higher rates of photosynthesis, conduction and transpiration in order to maintain temperatures and radiation greater on its leaf surface. Photosynthesis is about 2 times greater in "Tecómari" (ca. 24 μmol $m^{-2}s^{-1}$) than in "Ayocote" and even in the wild form (ca. 15 μmol $m^{-2}s^{-1}$). However, under the same microenvironment, the semi-vine "Ayocote" had photosynthetic rates similar to those of "Tecómari." This was not the case, however, for the wild P. coccineus ssp. formosus which maintains lower rates than those of both cultivated forms, even with higher radiation.

Greater rates of conductivity and transpiration in "Tecómari" were similar to those for photosynthesis. However, the water use efficiency (the quantity of CO_2 fixed per unit of water lost in transpiration) was similar or lower than that for semi-vine "Ayocote." Even less efficient in water use efficiency is the wild P. coccineus ssp. formosus.

In treatments with organic fertilizer (i.e., goat excrement), the water use efficiency in the plants was greater when no fertilization was applied. The photosynthetic rates remained the same, in particular in the case of semi-vine "Ayocote."

In both cultivated forms there exists a direct relation between the increase of water stress and the increase in transpiration. An augment in transpiration usually implies a reduction in the water potential; this indicates little control of transpiration by means of stomatic conductivity. It appears that the increase in the transpiration rate in "Tecómari" is the cost for maintaining the foliar temperature and diminishes the unfavorable conditions of low temperatures that are found in its normal cultivation zone in the mountains of Chihuahua (Guye et al. 1986, 1987).

A comparison of the differences in biomass (dry material) developed by the 2 cultivated varieties in the 4 experimental sites in the Sierra Norte de Puebla and the Sierra Tarahumara demonstrate that the differences among sites are highly significant and explains about 60% of the variation. However, it was not evident the interaction between both varieties and the experimental site; this suggests that each variety ("Tecómari" and semi-vine "Ayocote") have considerable plasticity. In the case of the sites located in the Sierra Tarahumara, the differences in total vegetative biomass was significantly different between localities and varieties. In both cases, the semi-vine "Ayocote" achieved greater vegetative biomass and contributed to a large part to the differences found for the varieties in the experimental sites.

In the case of the sites in the Sierra Norte de Puebla, highly significant differences in biomass were observed between the localities but not between the species (Table 27). Both sites affected in the same manner the variability in the total biomass found in "Tecómari" and "Ayocote." For both varieties, the vegetative biomass produced at Jilotzingo was 3 times greater than that at Nauzontla. The differences in total biomass between "Tecómari" and "Ayocote" for the 4 sites are found in Table 28.

A comparative analysis was made to study the variation in the patterns of biomass allocation for the various vegetative organs. An annual growth cycle behavior is more characteristic of "Tecómari" with the greater incorporation of biomass in the root system from the start until 100 days of age. In the case of P. c. ssp. coccineus "cimat" and the semi-vine "Ayocote," the pattern of biomass allocation to the root was similar at the start until 80-110 days of age, at which time a change related to the perennial and annual growth habit, respectively, was found.

Based upon the analysis of growth data for two cultivars of P. c. ssp. coccineus, "Tecómari" of Chihuahua and semi-vine "Ayocote" of Puebla, the great percentage of variability is explained by the differences among the sites of experimental cultivation. Similar results have been obtained for P. vulgaris, the common bean (Ghaderi et al. 1982) in which the major percentage of variability within and between groups of beans was due to the localities where they grew. It is important to emphasize that our study is based upon P. coccineus which has high rates of outcrossing and reduced self-pollination while the other studies are based upon P. vulgaris which is usually self-pollinated.

Significant differences in the growth and the yield components between sites illustrate the elevated plasticity of the varieties studied. None the less, the effect of the studied varieties, on occasion marked, with minor variation grown at sites near their regions of origin indicate that there exists limits to the plasticity and/or adaptability in alien sites.

The analysis of growth habits demonstrates an integration within the same variety growing in different sites and periods.

It is not possible to define with certainty the independent factors that control the growth habit. For example, P. c. ssp. coccineus "Tecómari" that grows as a determinant bush bean in Chihuahua can develop as an indeterminant semi-vine: 1) in Nauzontla, Puebla, where it later fruited (though in a reduced manner) and 2) in the growth chamber with reduced light (about 1/3 to 1/4 the normal daylight).

The phenological aspects during the initial growth of both the semi-vine and the vine P. c. ssp. coccineus of Puebla are generally indistinguishable. In these varieties, the major differences consist of the possibility to initiate a new stage of vegetative growth (vine habit) instead of triggering the reproductive phase. With respect to this situation, exist different hypotheses that try to explain the switch from the vegetative to the reproductive state. None the less, these changes continue to be difficult to explain. The changes observed between the second stage of vegetative growth in the vine P. c. ssp. coccineus and the reproduction of the semi-vine P. c. ssp. coccineus are similar to those produced in other species of Phaseolus and Vigna through the artificial removal of the reproductive structures (Wein et al. 1973; Ojehomon 1970; Lovett Doust et al. 1983).

Another important aspect is the architectural relations of individual plants and those induced by cultural practices in the detection of environmental signals related to reproduction. In the case of P. c. ssp. coccineus "Tecómari," the plant establishes microclimatological conditions that permit that its leaves experience more elevated temperatures than the ambient temperature so that its metabolism can increase. It is possible that the initiation of its reproduction which is earlier than the other varieties in this subspecies is related to this aspect.

The establishment of favorable microclimatic conditions inside the individual canopy is related also with the greater transpiration rate and lower water potential. Phaseolus c. ssp. coccineus "Tecómari" does not appear to be controlled by its water potential in function of the available water in the soil but in function of its ability to a maintain favorable microenvironment. We still do not know if these favorable microenvironmental conditions are oriented toward providing better metabolic rates or favorable conditions for the formation of floral buds and fruits.

In the case of the semi-vine P. c. ssp. coccineus, on the other hand, there is no direct relation between the developed biomass during the vegetable growth and the amount of reproductive biomass. The greater number of floral buds and developed fruits in the terminal portion of the individual initiate later, probably when the environmental conditions allow better formation of the same structures. This relationship to the plant architecture appears to be more important in the spatial distribution of the reproductive structures (De Moura and Foster 1986; Izquierdo and Hosfield 1983).

The analysis of more complementary data will provide a

better perspective in order to understand the relationships that exist between the physiology of the different varieties of P. coccineus and their success in certain environments and under various agricultural practices.

IMPACT, RELEVANCE and TECHNOLOGY TRANSFER

Until this project, research on beans in Mexico was limited to narrowly focused, short term investigation that benefited specific disciplines such as evaluation of selected commercial lines, taxonomy, etc. With this project, various disciplines worked together on material from the same origin so that the characterization of the beans at different levels was possible. Based upon the preliminary results, new insights on conservation strategies must be applied. Even though the two study sites shared similar features (e.g., mountains, ethnic subsistence farmers, cultivated beans and wild realties), the environment and socioeconomic conditions have promoted different responses. For example, in Puebla the diversity within the cultivated populations of P. coccineus ssp. darwinianus (possibly of ancient hybrid origin between P. coccineus and P. vulgaris) is the source of sufficient genetic variability to provide material for the traditional farmers to select divergent production forms. Hybridization (though possible in some cases) of P. coccineus ssp. darwinianus with cultivated, feral and wild forms of P. coccineus is insignificant. On the other hand, in Chihuahua the Tarahumara Indians maintain a system in which cultivated P. coccineus is grown near the pine-oak forest margins where its wild relative lives and hybridization and its products are present. This system may explain, in part, the special characters of the "Tecómari" which has phenological, architectural and physiological characteristics very different from the similar scarlet runner bush bean in Puebla and that allow it to create its own microclimate, so as to flower and mature earlier in a zone with a very short, often unpredictable growing season. The genetic diversity in the Pueblan populations of P. coccineus appear to be of infrasubspecific (rather than with its closely related cultigen and to with its assumed wild relative) origin and selected based upon cultural criteria for different harvests and types of products (e.g., pods, fresh seed, dry seed) over a longer growing season. The diversity is expressed in different growth behaviors. On the other hand, the diversity in the Chihuahuan populations could be related to extrasubspecific (in this case a wild relative) origin and selection is guided by cultural importance of early flowering and fruiting for single product in an unpredictable growing season. The diversity is expressed only in different color forms rather than growth forms.

This documentation implies that in situ conservation programs must included indigenous strategies of management of cultivated plants and their wild relatives, local needs and

criteria of indigenous inhabitants of the area, and an in depth understanding of the interactions between the biology, genetics and physiology of the plants and their cultural and natural environments. A conservation program oriented towards a single species/cultigen is inadequate. Also, the local inhabitants need to be play an active role in maintaining the dynamics of the bean populations. How should they be compensated for providing this service of diversity generation and conservation is a question that should be resolved in the near future.

Also, this documentation has pointed out the need to study more closely the local food plants that are often ignored by development programs. Because these scarlet runner beans provide different products and fill a critical niche in the cultivation (often unpredictable environments), they provide a crisis reserve when other staple foods are wanting. The enhancement of scarlet runner bean production systems and their diffusion to other parts of Mexico where local food production is subject to unpredictable calamities may help alleviate certain subsistence problems in rural areas.

Basic research in Mexico was also benefited by the project. A number of undergraduate students had an opportunity to learn and apply research skills directly to field problems (see **Project activities/outputs** for list of project personnel and human resource formation). Some of these individuals are continuing their graduate training. The principal investigators were able to know regions that they had not visited before and hence were able to compare the conditions and results which amplified their perspective on the problems of bean research in Mexico. As a consequence of periodic meetings and exchange of preliminary results throughout the project, new theoretical and practical problems arose and the original objectives were refined.

The laboratories benefited not only from the increase of personnel during the project but also from equipment. In particular, such equipment as computers, meteorological devices, special photographic lenses, and temperature and vapor measuring equipment provided various laboratories with instrumentation previous unavailable. This equipment continues to be used.

Part of the USAID funding, along with institutional resources, has aided in the updating of the isozyme section which is a major addition to the Instituto de Biología's genetic laboratory. When Dr. Llaca (whose is finishing his Ph. D. with Dr. Gepts at University of California, Davis) returns, the Instituto de Biología will amplify the genetics laboratory. Unfortunately, this USAID project did not provide funding to equip the molecular portion of the lab. In order to assure continuity of the USAID's impact of this project, we will solicit further support form USAID in the future.

The results of the project will be used in two forms. First, the analyses of preliminary data will be the basis for disciplinary and interdisciplinary publications in international and national journals. Second, the questions raised by the preliminary results are being used to formulate more specific

research projects by individuals and groups with in the original program (see **Future work** for examples). Also, the consolidation of our working group allows us to identify specific needs within the group. We have been in contact with researchers at such institutions as the Secretaría de Argicultura, Colegio de Postgraudados (Chapingo), and Instituto de Ecología with the idea of incorporating them into the program. Examples of themes to be added are: seed herbivores, ex situ conservation and germplasm banks, formal breeding programs. Hence, we feel that Mexico can establish a national working group on beans which could be based upon the upscaling of this initial USAID project.

This project has made a difference to the project personnel and the institutions involved. Not only were the students' capacitation increased (e.g., lab and field training session on pollination biology), but also they along with the researchers experience the dynamics of interdisciplinary research program which is very limited in Mexico. Certain problems arose as a consequence of the project and we all have learned from them. Most importantly, the researchers became more sensitive and appreciative of the orientation and needs of other members' research in terms of objectives, philosophy, basic assumptions and limitations. As mentioned above, selected equipment which was unavailable due to restrictions within the institutions's budget were obtained and continue to function.

Being a centralized country, politically and academically, this project also reinforced the ideas that regional studies (rather than superficial national surveys) in collaboration with local academic personnel and their institutions is critical in the realization of interdisciplinary research. When dealing with domesticated plants, it is paramount the direct involvement of indigenous peoples in the formulation, execution, and evaluation of field research. More work needs to be done on integrating native peoples into the resolution of objectives and the relevancy of research.

PROJECT ACTIVITIES/OUTPUTS

Project Personnel

Principal Investigator: Dr. Robert Bye Boettler
Co-Principal Investigators: Dr. Alejandro Castellanos
Dr. Alfonso Delgado Salinas
Dr. Paul Gepts
M. en C. Miguel Angel Martínez A.
Dra. Guadalupe Palomino Hasbach
Dr. Daniel Piñero Dalmau
Coordinators : Biol. Elia Herrera Torralba
M. en C. Luz María Mera Ovando
Research Assistants:
Jaime Aguilar Verplancken
Biol. Francisco Basurto Peña

Biol. Gerardo Coello Coutiño (Mar. - May 89)
Biol. Araceli C. Díaz Ortega (July 90 - July 91)
Biol. Ana Ma. Escalante Gonzalbo (Mar. - May 89)
Biol. Victor Llaca García
Biol. Francisco J. Ramón Martínez (Jan.90-July92)
Biol. David Martínez Moreno
Biol. Myrna Mendoza Cruz (May - July 90)
Biol. Pedro Mercado Ruaro (July 88 - Sep. 89)
Lucía Nava de Aguilar
M. en C. Nidia Peréz Nasser
Biol. Samuel Rangel Calderón (Sep.- Dec. 88)
Biol. Mario Sousa Peña
Biol. Alfredo Wong León

Published results

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Course

COURSE OF POLLINATION by Drs. PETER KEVAN and FRANCO DI-GIOVANNI
From: Department of Environmental Biology
University of Guelph
Guelph, Ontario
Canada
Place: - Jardín Botánico Exterior, Universidad Nacional Autónoma de México
- Sierra Norte de Puebla, México
Date: 6-15 September 1990.

All-project event

Reunión grupo Phaseolus
Oaxtepec, Morelos, México
3-7 February 1992

Conferences

Historia Natural de Frijoles.
Sociedad Botánica de México.
Ciclo de Conferencias: julio-octubre de 1989.

a) Delgado S., A.: Biosistemática de Phaseolus.

b) Coello, G., A. Escalante, L. Eguiarte, y D. Piñero: Estructura Genética en poblaciones de Phaseolus coccineus (frijol ayocote) silvestres y cultivados.

c) Martínez, M.A., y R. Bye: Observaciones etnobotánicas de los frijoles en México.

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Formation of Human Resources (training)

As a result of the above research, 2 undergraduate theses were completed at the Faculty of Sciences of the National Autonomus University of Mexico:
- Alfredo Wong - "Fotomacrografías de flores de Phaseolus coccineus L. (Fabaceae) en la zona de percepción de los insectos polinizadores" and
- Mario Sousa-Peña - "Polinización en el complejo Phaseolus coccineus L. (Fabaceae)".
The corresponding articles based on these theses will be published later.

The following 2 Master Degeree theses are near completion at the Faculty of Sciences of the National Autonomus University of Mexico:
- David Martínez Moreno - "Uso eficiente de agua y análisis de crecimiento en la asociación de Zea mays L. y Phaseolus coccineus L. ssp. darwinianus en el municipio de Nauzontla, Puebla" and
- Francisco Basurto Peña - "Aspectos etnobotánicos de Phaseolus coccineus L. en la Sierra Norte de Puebla".

One doctoral dissertation will be completed in 1993-1994 at the University of California (Davis) by Victor Llaca García on molecular genetics of Phaseolus coccineus.

As a consequence of their participation in this project, the following project personnel have obtained research positions within the University:
Elia Herrera Torralba (Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México)
Francisco Basurto Peña (Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México)
Luz María Mera Ovando (Sala de Agricultura y Alimentación, Museo de Las Ciencias, Universidad Nacional Autónoma de México)
David Martínez Moreno (Universidad de Sonora).

Upon completion of their Ph. D., the following project personnel will be incorporated into the University research staff in order to continue research on subjects related to this project:
Victor Llaca García (University of California, Davis; molecular genetics)
Mario Sousa Peña (University of Connecticut; pollination biology).

PROJECT PRODUCTIVITY

The project was able to complete the major objective of characterization of P. coccineus in order to provide the basis for correlation of genetic, cultural and ecological factors as related to diversity of this bean in two areas of Mexico. The unevenness of the treatment by the different disciplinary groups does not permit a full correlation evaluation at this time. None

the less certain patterns were discerned and subsequent studies were initiated (e.g., the bean-maize association combining ethnobotanical and ecophysiological preliminary results). Each disciplinary group added new knowledge to its field with respect to the scarlet runner bean. The picture was greatly clarified for the taxonomic and genetic relationships of the different members of the P. coccineus complex -- some to the point that indicates that the original assumptions (based on available data at the time) were wrong. The domestication and continuing diversification of beans in Mexico is more complicated that current models can explain. This situation opens fertile ground for theoretical research as well as for new perspectives on plant breeding and later application of new developed lines. Also, the revalorization Mexico's cultural heritage as it impacts the conservation of bean cultigens and their associated habitats requires this type of research program. Such revalorization coupled with new cultigen options derived from such a research project can productively connect basic research with improving living conditions in rural zones of Mexico.

Certain aspects of the project were not accomplished. Specific interfacing of some disciplines to resolve precise problems was unsuccessful. The response time between receiving preliminary results and reorienting field experiments was not adequate for the program time period. None the less, the long term nature of this bean working group should allow selected problems to be resolved in the near future. Also, the erratic working and living conditions in the field did not allow for us to take maximum advantage of certain trips and in some cases a whole season's study was lost due to natural or human-induced perturbation.

Only recently have our attempts to establish international contacts with other developing countries shown promise. Guatemala, Costa Rica and Colombia are of specific interest to us. Delays in communications, lost connections and infrequency of follow up contacts may be the important contributors to this situation. Perhaps special support (apart from project monies) for electronic and telephonic communications and site visits by personnel could alleviate this situation.

FUTURE WORK

Based upon the initial results of our USAID-supported project, we are planning to expand the group to include other organizations, study more profoundly certain key issues in bean diversity, and to extend the geographic and ethnic coverage of the studies. The expansion of the bean working group was outlined in the section on **Impact, relevance and technology transfer**. Preliminary steps have been taken to amplify the cultural and geographic areas of study to the Mexican states of Oaxaca, Chiapas, Durango, and Zacatecas. On the international level, preliminary field trips have been made with Colombia and next year we plan to visit Guatemala and Nicaragua.

The unusual growth patterns (architecture, phenology and physiology) of "Tecómari" merits in depth study and evaluation of this form in order to understand how the plant was domesticated to produce under severe environmental conditions and to determine how this knowledge and germplasm can be applied to resolving agricultural production in areas with limited agricultural regimes. Do these characteristics of "Tecómari" represent convergent or parallel evolution with respect to the "Ayocote" of Puebla and the "Patol" of Durango? What is the frequency and impact of hybridization between the cultigens and its wild relatives? Is there a reciprocal cycle of importance between P. coccineus and P. vulgaris that were not able to detect during this period of study? Do the populations of P. coccineus ssp. darwinianus (= P. polyanthus) of southern and central Mexico represent primary or secondary centers of diversity and origin? How did P. coccineus ssp. darwinianus arise and is it monophyletic or polyphyletic.

Great care will need to be taken with the interfacing of institutional research (such as this project) with local organizations (e.g., Centro de Estudios Agricolas de La Tarahumara, A.C.). We plan to serve as advisors to such Tarahumara associations and use there concerns and needs to guide in situ research and to seek appropriate sustainable production systems.

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#	SITE	Change	B	G12454	G35877
1	Bam17.2	0.5+3.3<->3.8	0	1	1
2	Bam16.6	0.5+3.3<->3.8	0	1	1
3	Dra7.8b	0.5+4.4<->4.9	0	1	0
4	Dra13.3	2.1+0.4<->2.5	0	1	1
5	Dra10.6	3.0+0.3<->3.3	1	0	0
6	Dra10.6b	4.0<->4.4	0	1	1
7	Dra18.8	2.2+0.2<->2.4	0	1	0
8	Dra7.8	1.5+1.4<->2.9	0	1	1
9	Dra5.6	1.5+3.1<->4.6	0	1	1
10	Eco7.8	1.4+0.2<->1.6	0	1	1
11	Eco10.6	0.7+2.4<->3.1	1	0	0
12	Eco7.5	0.7+0.8<->1.5	0	1	1
13	Eco11.1	7.2<->7.4	0	1	0
14	Eco5.6	3.1+1.4<->4.5	1	0	0
15	Hin10.6	3.1+2.1<->5.2	0	1	1
16	Pvu10.6	12.3+20.4<-> 32	1	0	0
17	Sma10.6	2.7+5.8<->8.5	1	0	0
18	Xho7.0	13.4+10.2<->24	1	0	1

Table 1. Polymorfic sites between Phaseolus coccineus accession B and Phaseolus coccineus accession G12454. Eighteen sites were polymorphic among a total of 327 sites analyzed (5% polymorphism). The polymorphic sites were analyzed in Phaseolus coccineus polyanthus (=darwinianus) accession G35877. P. coccineus polyanthus showed identical patterns to P. vulgaris in 14 cases, whereas only 4 were identical to P. coccineus coccineus.

Table 2. Ensymes used in isozyme studies of genetic variation in *Phaseolus*.

MDH	Malate dehydrogenase	EC	2.7.5.1
PGM	Phosphoglucomutase	EC	2.7.5.1
PGI	Phosphoglucose isomerase	EC	5.3.1.9
ACPH	Acid phosphate	EC	3.1.3.2
GOT	Glutamate-oxaloacetate transaminase	EC	2.6.1.1
DIA	Diaphorase	EC	1.6.4.3
CPX	Catodic peroxidase	EC	1.11.1.7

Table 3. The average size of samples used and the average number of alleles per locus of the populations of *Phaseolus coccineus* studied. The standard error is listed in ().

Population	Sample Size	no. of alleles/locus
1) Panalachi, Chih. (C)	53.0 (0)	2.8 (0.20)
2) Cusarare, Chih. (C)	52.0 (0)	3.3 (0.15)
3) Otra Pobl., Chih. (C)	31.9 (4)	2.8 (0.20)
4) Aislada, Gui., Chih. (C)	61.7 (4.4)	3.2 (0.13)
5) Nauzontla, Pue. (D)	36.4 (3.5)	3.5 (0.22)
6) Zoactepan, Pue. (D)	52.6 (7.1)	3.0 (0.26)
7) Sn. José, Pue. (F)	44.8 (4.7)	3.1 (0.23)
8) El Carmen Km 53, Tlax. (F)	26.2 (2.5)	2.9 (0.31)
9) El Carmen Km 55, Tlax. (F)	79.4 (6.9)	3.2 (0.20)
10) Cusarare, Chih. (FS)	46.5 (6.4)	2.7 (0.26)
11) Guichochi, Chih. (FS)	42.1 (3)	2.7 (0.21)
12) Nauzontla, Pue. D.Miguel (C)	47.7 (1.0)	3.3 (0.6)
13) Apulco, Pue, (C)	24.7 (0.8)	2.9 (0.3)
14) Nepopoalco, Mor. (F)	38.1 (1.7)	3.0 (0.3)
15) Nauzontla, Pue. D.José (C)	25.9 (1.9)	2.9 (0.3)

C = *Phaseolus coccineus* ssp. *coccineus*
D = *P.c.* ssp. *darwinianus*
F = *P.c.* ssp. *formosus*
FS = *P.c.* ssp. *formosus* silvestre

Table 4. The Genetic Diversity Index (Hm), the Index of Multilocus Fixation (Fm), and the Rate of Outcrossing (te) (assuming endogamic equilibrium) for the populations of *Phaseolus coccineus* studied.

coll.	subspecies	State	Hm	Fm	te
FB 15	*P.c.* ssp. *coccineus*	Chihuahua	0.152	0.041	0.921
FB 8a	*P.c.* ssp. *coccineus*	Chihuahua	0.301	0.560	0.281
Pan.	*P.c.* ssp. *coccineus*	Chihuahua	0.313	0.427	0.401
Cus.	*P.c.* ssp. *coccineus*	Chihuahua	0.282	0.332	0.500
FB 854	*P.c.* ssp. *coccineus*	Chihuahua	0.279	0.422	0.406
FB 543	*P.c.* ssp. *coccineus*	Puebla	0.227	0.375	0.454
FB 553	*P.c.* ssp. *coccineus*	Puebla	0.280	0.071	0.867
FB 601	*P.c.* ssp. *coccineus*	Puebla	0.199	0.050	0.905
FB 603	*P.c.* ssp. *coccineus*	Puebla	0.279	0.314	0.522
D.Mig.	*P.c.* ssp. *coccineus*	Puebla	0.229	0.535	0.302
D.Jos.	*P.c.* ssp. *coccineus*	Puebla	0.294	0.476	0.354
FB 775	*P.c.* ssp. *coccineus*	Puebla	0.197	0.614	0.238
FB 531	*P.c.* ssp. *darwinianus*	Puebla	0.236	0.279	0.564
FB 534	*P.c.* ssp. *darwinianus*	Puebla	0.301	0.188	0.683
FB 577	*P.c.* ssp. *darwinianus*	Puebla	0.228	0.228	0.629
FB 584	*P.c.* ssp. *darwinianus*	Puebla	0.221	0.294	0.545
FB 605	*P.c.* ssp. *darwinianus*	Puebla	0.302	0.445	0.384
FB 2	*P.c.* ssp. *darwinianus*	Puebla	0.361	0.562	0.280
Zoac.	*P.c.* ssp. *darwinianus*	Puebla	0.301	0.545	0.293
FB 937	*P.c.* ssp. *formosus*	Puebla	0.284	0.363	0.466
Nepo.	*P.c.* ssp. *formosus*	Morelos	0.304	0.432	0.396
Cus.	*P.c.* ssp. *formosus*	Chihuahua	0.238	0.547	0.292
Gui.	*P.c.* ssp. *formosus*	Chihuahua	0.297	0.694	0.181
FB 938	*P.c.* ssp. *formosus*	Tlaxcala	0.272	0.302	0.536
FB 947	*P.c.* ssp. *formosus*	Tlaxcala	0.317	0.282	0.559
	P.c. ssp. *coccineus*	Promedio	0.252	0.351	0.512
	P.c. ssp. *darwinianus*	Promedio	0.278	0.363	0.482
	P.c. ssp. *formosus*	Promedio	0.285	0.436	0.405

Table 5. F-statistics for 10 loci of four (4) populations of *Phaseolus coccineus* ssp. *coccineus* from Chihuahua.

Locus	FIS	FIT	FST
ACPH-1	0.620	0.655	0.093
CPX-1	0.313	0.376	0.092
CPX-2	0.305	0.326	0.031
GOT-1	-0.299	0.011	0.239
MDH-1	0.926	0.932	0.082
PGI-1	0.586	0.609	0.055
PGI-3	0.598	0.640	0.105
PGM-1	0.844	0.854	0.067
PGM-3	0.543	0.576	0.072
SDH-1	0.090	0.108	0.020
MEDIA	0.463	0.510	0.086

Table 6. F-statistics for 10 loci of two (2) populations of *Phaseolus coccineus* ssp. *darwinianus* from Puebla.

Locus	FIS	FIT	FST
ACPH-1	0.803	0.808	0.025
CPX-1	0.117	0.127	0.011
CPX-2	0.667	0.667	0.001
GOT-1	0.262	0.325	0.085
MDH-1	0.931	0.931	0.011
PGI-1	0.818	0.821	0.015
PGI-3	0.763	0.798	0.148
PGM-1	0.323	0.352	0.043
PGM-3	0.656	0.730	0.215
SDH-1	0.206	0.255	0.062
MEDIA	0.544	0.573	0.063

Table 7. F-statistics for 10 loci of five (5) populations of *Phaseolus coccineus* ssp. *formosus* from Chihuahua and Puebla.

Locus	FIS	FIT	FST
ACPH-1	0.335	0.404	0.104
CPX-1	0.274	0.314	0.056
CPX-2	0.521	0.659	0.289
GOT-1	0.247	0.406	0.211
MDH-1	0.617	0.671	0.141
PGI-1	0.802	0.819	0.087
PGI-3	0.471	0.559	0.168
PGM-1	0.425	0.461	0.062
PGM-3	0.341	0.358	0.026
SDH-1	0.303	0.359	0.080
MEDIA	0.420	0.491	0.123

Table 8. F-statistics for 10 loci of three (3) populations of *Phaseolus coccineus* ssp. *formosus* from Puebla and Tlaxcala.

Locus	FIS	FIT	FST
ACPH-1	0.131	0.216	0.097
CPX-1	-0.003	0.013	0.016
CPX-2	0.430	0.450	0.036
GOT-1	0.336	0.373	0.055
MDH-1	0.629	0.647	0.048
PGI-1	0.767	0.775	0.034
PGI-3	0.186	0.227	0.050
PGM-1	0.167	0.240	0.088
PGM-3	0.319	0.344	0.037
SDH-1	0.179	0.237	0.071
MEDIA	0.316	0.353	0.053

Table 9. F-statistics for 10 loci of two (2) populations of P. coccineus ssp. formosus from Chihuahua.

Locus	FIS	FIT	FST
ACPH-1	0.657	0.658	0.004
CPX-1	0.661	0.564	0.008
CPX-2	0.921	0.921	0.002
GOT-1	0.128	0.151	0.027
MDH-1	0.594	0.678	0.208
PGI-1	0.848	0.856	0.056
PGI-3	0.771	0.824	0.231
PGM-1	0.801	0.806	0.022
PGM-3	0.375	0.376	0.002
SDH-1	0.507	0.517	0.019
MEDIA	0.590	0.613	0.058

Table 10. F-statistics among populations of Phaseolus coccineus ssp. coccineus, P. coccineus ssp. darwinianus, and P. coccineus ssp. formosus from collections made in 1989 and 1990 in Chihuahua, Puebla, and Tlaxcala. The estimation is based upon 10 polymorphic loci.

Subspecies	no. of populations	FIS	FIT	FST
P.c. ssp. coccineus	4	0.463	0.510	0.086
P.c. ssp. darwinianus	2	0.544	0.573	0.063
P.c. ssp. formosus	5	0.420	0.491	0.123

Table 11. F-statistics among populations of Phaseolus coccineus ssp. coccineus and P. coccineus ssp. darwinianus from collections made in 1988 in Chihuahua, Puebla, and Chiapas. Also show is the total for the twelve (12) populations of the two subspecies. The estimation is based upon 9 polymorphic loci.

Subspecies	no. of populations	FIS	FIT	FST
P.c. ssp. coccineus	6	0.238	0.374	0.178
P.c. ssp. darwinianus	6	0.278	0.356	0.108
P.coccineus	12	0.259	0.423	0.222

Table 12. Genetic distances (determined using Nei 1972) among the populations of *Phaseolus coccineus* ssp. *coccineus* and *P. coccineus* ssp. *formosus* from Chihuahua, Puebla and Tlaxcala.

P	1	2	3	4	5	6	7	8	9	10	11
1	--	.074	.155	.181	.167	.390	.233	.315	.202	.134	.094
2		--	.122	.188	.116	.350	.230	.341	.191	.227	.156
3			--	.148	.222	.325	.152	.300	.133	.146	.103
4				--	.198	.204	.103	.143	.043	.122	.147
5					--	.178	.238	.391	.199	.276	.163
6						--	.383	.526	.240	.428	.271
7							--	.099	.052	.200	.240
8								--	.097	.262	.311
9									--	.148	.195
10										--	.101
11											--

P	Especie y Población.
1	*P.c. coccineus*, Panalachi, Chih.
2	*P.c. coccineus*, Cusarare, Chih.
3	*P.c. coccineus*, Otra Pob., Cus., Chih.
4	*P.c. coccineus*, Aislado, Gui., Chih.
5	*P.c. darwinianus*, Nauzontla, Pue.
6	*P.c. darwinianus*, Zoactepan, Pue.
7	*P.c. formosus*, Sn. José, Pue.
8	*P.c. formosus*, El Carmen km 53, Tlax.
9	*P.c. formosus*, El Carmen km 55, Tlax.
10	*P.c. formosus*, (silvestre), Cusarare, Chih.
11	*P.c. formosus*, (silvestre), Guichochi, Chih.

Table 13. Genetic distances (determined using Nei 1972) among the populations of *Phaseolus coccineus* ssp. *coccineus* from Chihuahua.

Population	1	2	3	4
1 Panalachi	----	0.074	0.115	0.181
2 Cusarare		----	0.122	0.188
3 Otra Pob., Cus.			----	0.148
4 Aislado, Gui.				----

Table 14. Genetic distances (determined using Nei 1972) among the populations of *Phaseolus coccineus* ssp. *formosus* from Chihuahua and Puebla.

Population	1	2	3	4	
1 San José	-----	0.099	0.052	0.200	0.242
2 Carmen km 53		-----	0.097	0.262	0.311
3 Carmen km 55			-----	0.148	0.195
4 Cusarare				-----	0.101
5 Guichochi					----

Table 15. Genetic distances (determined using Nei 1972) among the populations of *Phaseolus coccineus* ssp. *coccineus* and *P. coccineus* ssp. *formosus* from Puebla and Morelos.

Population	1	2	3	4
1 Nauzontla, Pue. Don Miguel	----	0.191	0.247	0.348
2 Apulco, Pue.		----	0.284	0.311
3 Nepopoalco, Mor.			----	0.192
4 Nauzontla, Pue. Don José				----

Table 16. Gametic numbers (n) in Metaphase 1 (MI) in pollen mother cells of 2 subspecies of Phaseolus coccineus.

coll.	Subspecies	locality	n	bivalents
FB865/129	P. c. ssp. coccineus	Jilotzingo, Pue	11	II_{11}
FB1075/305	P. c. ssp. coccineus	Nauzontla, Pue.	11	II_{11}
FB1080/310	P. c. ssp. coccineus	Nauzontla, Pue.	11	II_{11}
FB 878	P. c. ssp. formosus	Cusárare, Chih.	11	II_{11}

Table 17. Chromosome numbers (2n), karyotypic formula, secondary constrictions (Sec. Constr.), secondary constrictions in the 2 extremes of the chromosome (Ex.), chromosome length (Chromos. Lenght) and index of assymetry (Index Assym.) for 4 varieties of Phaseolus vulgaris.

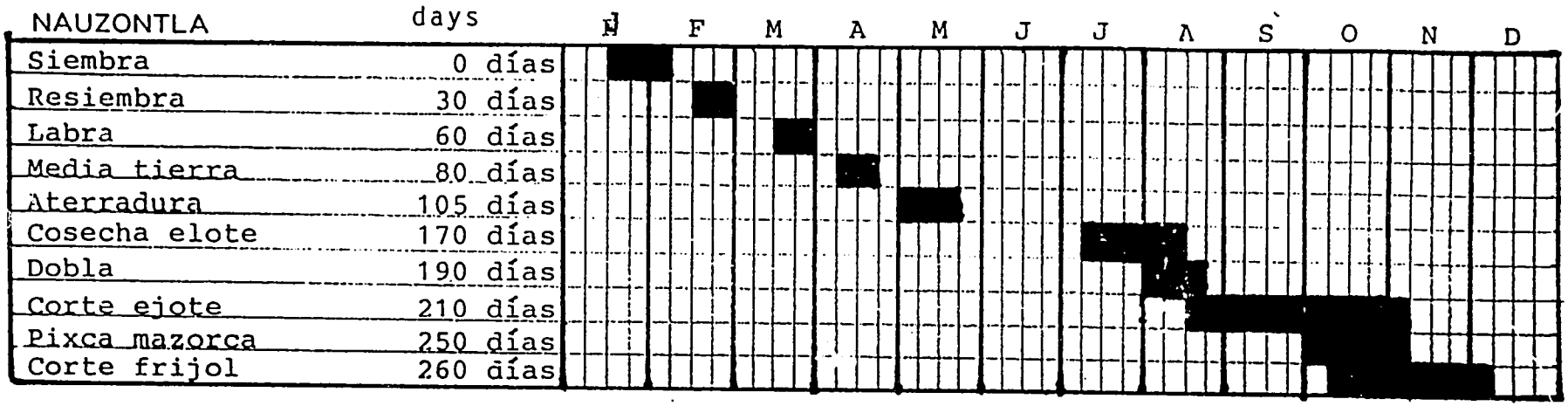
Coll.	Variety	2n	Kariotypic Formula	Sec. Constr.	Ex.	Chromos. Length (μm)	Index Assym. (%)
640	Delgado Nayarit	22	7M + 4Sm	4M 2Sm	2M	0.92-1.55	42
649	Delgado Nayarit	22	7M + 4Sm	4M 2Sm	2M	1.6-2.5	39.62
609	Negro Mateado	22	8M + 3Sm	3M 1Sm		1.29-2.09	45
750	Negro Mateado	22	4M + 5Sm + 2St	3M 2Sm 1St	1M	1-2.3	36
608	Majayan	22	8M + 3Sm	4M 1Sm	1M	1.2-2.7	46
595	Mapueleño	22	6M + 4Sm + 1St	4M 2Sm	1M 1Sm	1.2-2.35	40

Table 18. Chromosome number and kariotypes of 3 subspecies of Phaseolus coccineus.

Subspecies	2n	Range of Length of Chromosomes (μm)	Kariotype Formula	Secondary Constrictions 1 arm	2 arms
coccineus	22	1.2 - 1.8	11m	4m	1m
coccineus	22	1.1 - 1.8	11m	4m	1m
darwinianus	22	1.8 - 2.9	10m + 1sm	4m	1m
darwinianus	22	1.5 - 2.8	10m + 1sm	4m	1m
glabellus	22	1.3 - 2.8	9m + 2sm	4m	1m

Localidad	Phaseolus	Denominacion	Grano seco	Grano tierno	Follaje	Flor	Raiz	Fruto verde sin grano "ejote"	Fruto verde con grano "ejote"
Nauzontla	vulgaris(mata)	mateado	hervido. guisado	al vapor(tamal)	-----	-----	-----	hervido. guisado	hervido
	vulgaris(guia)	delgado enredador	hervido. guisado	al vapor(tamal)	cocido	-----	-----	hervido. guisado	hervido
	C. coccineus	tacuahuaket	hervido. guisado tostado	hervido. vapor. guisado	cocido	cocido. vapor(tamal)	hervido	-----	hervido
	C. darwinianus	exoyema	hervido. guisado	hervido. vapor. guisado	cocido	cocido, (poco empleado)	-----	-----	hervido
	C. glabellus	frijolillo	-----	-----	medicinal	-----	-----	-----	-----
Zoacteman	vulgaris(mata)	mateado	hervido. guisado	al vapor(tamal)	-----	-----	-----	hervido. guisado	hervido
	vulgaris(guia)	delgado enredador	hervido. guisado	al vapor(tamal)	cocido	-----	-----	hervido. guisado	hervido
	C. coccineus	tacuahuaket	hervido. guisado tostado	hervido. vapor. guisado	cocido	cocido. vapor(tamal)	hervido	-----	hervido
	C. darwinianus	exoyema	hervido. guisado	hervido. vapor. guisado	cocido	cocido. (poco empleado)	-----	-----	hervido
	C. glabellus	frijolillo	-----	-----	-----	cocido	-----	-----	-----
Huahuaxtla	vulgaris(mata)	mateado	hervido. guisado	al vapor	-----	-----	-----	hervido. guisado	hervido
	vulgaris(guia)	delgado enredador	hervido. guisado	al vapor	cocido	-----	-----	hervido. guisado	hervido
	C. coccineus	tacuahuaket	hervido. guisado tostado	hervido. vapor. guisado	cocido	cocido al vapor	hervido	-----	hervido
	C. darwinianus	exoyema	hervido. guisado	hervido. vapor. guisado	cocido	cocido (poco empleado)	-----	-----	hervido
	C. glabellus	frijolillo	-----	-----	-----	-----	-----	-----	-----
Jilotzingo	vulgaris(mata)	napual	hervido. guisado medicinal	al vapor	-----	-----	-----	hervido. guisado	hervido
	vulgaris(guia)	delgado	hervido. guisado	al vapor	cocido	-----	-----	hervido. guisado	hervido
	C. coccineus	cimat	hervido. guisado tostado	hervido. vapor. guisado	cocido	cocido al vapor	hervido	-----	hervido
	C. darwinianus	patlashtle	hervido. guisado	hervido. vapor. guisado	cocido	cocido. (poco empleado)	-----	-----	hervido
San Marcos	vulgaris(mata)	lacsinustapu	hervido. guisado	al vapor	-----	-----	-----	hervido. guisado	hervido
	vulgaris(guias)	litanpihuat	hervido. guisado	al vapor	cocido	-----	-----	hervido. guisado	hervido
	C. coccineus	tangastapu	hervido. guisado tostado	hervido. vapor. guisado	cocido	cocido al vapor	hervido	-----	hervido
	C. darwinianus	xeyonit	hervido. guisado	hervido. vapor. guisado	cocido	cocido, (poco usado)	-----	-----	hervido
	C. glabellus	stapuloua	-----	-----	-----	-----	-----	-----	-----

	P. coccineus coccineus (P.c.c.)	*P. coccineus darwinianus* (P.c.d.)	*P. vulgaris* guia (P.v.g.)	*P. vulgaris* mata (P.v.m.)
NAUZONTLA	Concomitante con maiz. P.c.d. y P.v.g. Imbricado con papa o haba intercalada con P.v.m. (poco frecuente)	Concomitante con maiz. P.c.c. y P.v.g. Imbricado con papa o haba intercalada con P.v.m. (poco frecuente)	Concomitante con maiz. P.c.c. y P.c.d. Imbricado con papa o haba intercalada con P.v.m. (poco frecuente)	Intercalado con maiz. P.c.c.. P.c.d. y P.v.g. (poco frecuente) Monocultivo en roza o parcelas permanentes (poco frecuente).
ZOACTEPAN	Concomitante con maiz. P.c.d. y P.v.g. Imbricado con papa o haba intercalada con P.v.m.	Concomitante con maiz. P.c.c. y P.v.g. Imbricado con papa o haba intercalada con P.v.m.	Concomitante con maiz. P.c.c. y P.c.d. Imbricado con papa o haba intercalada con P.v.m.	Intercalado con maiz. P.c.c.. P.c.d. y P.v.g. Monocultivo en roza o parcelas permanentes (poco frecuente).
HUAHUAXTLA	Concomitante con maiz. P.c.d. y P.v.c. Imbricado con haba o alverjon Intercalado con P.v.m.	Concomitante con maiz. P.c.c. y P.v.g. Imbricado con haba o Intercalado con P.v.m. En roza-tumba-quema de bosquetes de ilite. con maiz.	Concomitante con maiz. P.c.c. y P.c.d. Imbricado con haba o alverjon Intercalado con P.v.m.	Intercalado con maiz. P.c.c.. P.c.d. y P.v.g. (muy comun) Imbricado con haba Monocultivo de roza (poco frecuente).
JILOTZINGO	Concomitante con maiz. P.c.d. y P.v.g. Imbricado con haba Intercalado con P.v.m.	Concomitante con maiz. P.c.c. y P.v.g. Imbricado con haba Intercalado con P.v.m. Monocultivo de roza de bosque mesofilo o encinar.	Concomitante con maiz. P.c.c. y P.c.d. Imbricado con haba Intercalado con P.v.m.	Intercalado con maiz. P.c.c.. P.c.d. y P.v.g. Imbricado con haba Monocultivo de roza o en proceso permanente.
ELOXOCHITLAN	Concomitante con maiz. P.c.d. y P.v.c. Imbricado con haba Intercalado con P.v.m.	Concomitante con maiz. P.c.c. y P.v.g. Imbricado con haba o alverjon Intercalado con P.v.m.	Concomitante con maiz. P.c.c. y P.c.d. Imbricado con haba o alverjon Intercalado con P.v.m.	Intercalado con maiz. P.c.c.. P.c.d. y P.v.g. Imbricado con haba.



MONTHS

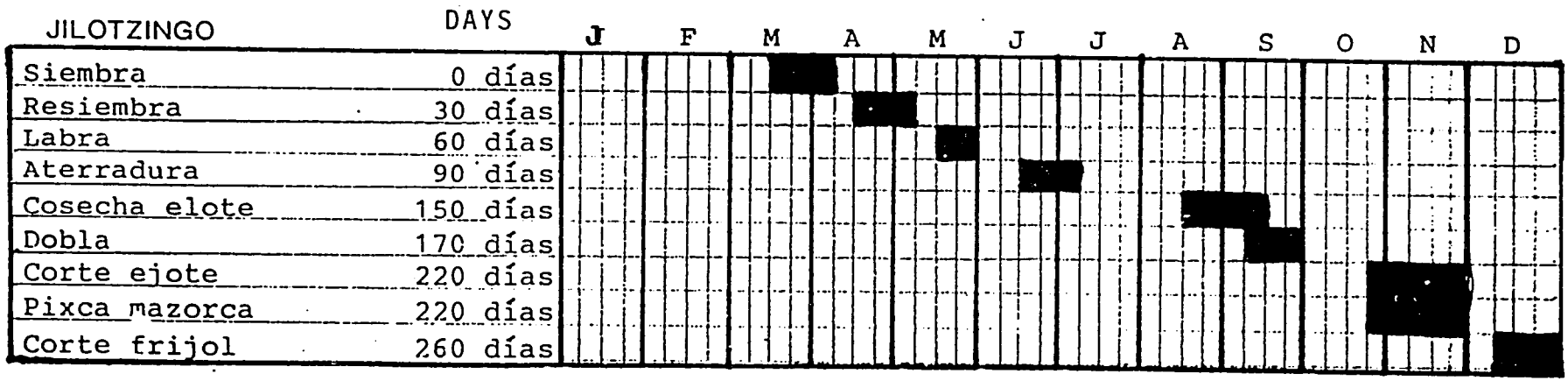


Table 21. Agricultural calendar maize/bean (Phaseolus coccineus subsp. coccineus and Phaseolus coccineus subsp. darwinianus) in Nauzontla and Jilotzingo, Puebla.

Table 22. Bean (*Phaseolus*) products from dry land cultivated field.

	ENERO	FEBRERO	MARZO	ABRIL	MAYO	JUNIO	JULIO	AGOSTO	SEPTIEMBRE	OCTUBRE	NOVIEMBRE	DICIEMBRE
P. vulgaris mata												
		quelites										
			ejote cueritos	ejote con grano tierno	grano seco	grano seco						
P. vulgaris guía												
							quelites	quelites ejotes	ejote	ejote con grano tierno	ejote con grano seco	grano seco
P. coccineus barbatulus												
	quelites (Plant.)	quelites (Plant.)				quelites cueritos ejotes	quelites cueritos ejotes	ejote con grano tierno	ejote con grano tierno	ejote con grano tierno	ejote con grano tierno grano seco	grano tierno
P. coccineus coccineus												
	canote	canote	canote									
						quelite flores	quelite flores	quelite flores	quelite flores			
										ejote con grano tierno	ejote con grano tierno grano seco	grano seco
P. coccineus escapado												
					quelites flores	quelites flores	quelites flores					
								ejote con grano tierno	ejote con grano tierno	ejote con grano tierno grano seco	grano seco	grano seco
Maíz												
							elote forraje	elote forraje	elote mazorca forraje	mazorca	mazorca	

Table 23. Phaseolus vulgaris in the sierra and barranca of western Chihuahua: seed color, local name, growth habit.

	Seed color	Common name Sierra	Common name Barranca	Grow Habit
Phaseolus vulgaris	1. Pardo oliváceo	Rosobochane, cafe, cape,	ulaname, moyachi, moyane, barroso	determinado
	2. Café con manchas oscuras o negras (ojo de cabra)	Chivabusira, ojo de cabra, shivabushira	ojo de cabra, shivabushira	determinado
	3. Crema o beige claro liso	Saguaroame, sobochame, rosobochame	barroso, napisochi frijol riata, rosacame	determinado
	4. Blanco	Tosocame, rosacame	rosacame	determinado
	5. Crema salpicado con café o pardo	Pichirome, mantequilla	mantequilla	determinado
	6. Blanco con negro	Tecomare?, comoshame		determinado
	7. Lila o morado claro, plomizo	Rosobochame, sillonome, ochocame, bayo riota	silloname	determinado
	8. Negro brillante, semilla grande	Chocame	chocame	determinado
	9. Rojizo oscurso o marrón con fondo crema	Sitacome		determinado
	10. Negro con fondo crema casi tapado	Soguepari-Soguemari		determinado
	11. Crema con manchas negras	Sipichisame, sipichiso, pinto, shipishisoame	siocho	determinado
	12. Guinda con manchas amarillas	Sitabuchame, toribushira, shivabushira-sitaka	uacamuni, sitacame pinto, sitacame, bahueame, sitacame-bahueame	determinado

Table 23 cont. Phaseolus vulgaris in the sierra and barranca of western Chihuahua: seed color, local name, growth habit.

Seed color	Common name Sierra	Common name Barranca	Grow Habit
13. Lila o morado claro con manchas negras	Chokame sipitochame	chokame sipitochame	determinado
14. Grisáceo con manchas negras	Tirosobochame, chachamuni		determinado
15. Amarillo mostaza	sarahuame, sahuaroame	sahuaroame, laname sahuaroame	determinado determinado
16. Amarillo azufrado	rohuimuni, rosobochame, sahuaroame, resoboshame	rohuimare, arohuimani, roguimani	determinado
17. Rosado con manchas crema o beige (canelo)	canecol. rojobochame, sitamurame		determinado
18. Beige amarillento arriñonado			determinado
19. Rosado o rojizo claro, liso			determinado
20. Grisáceo con manchas amarillentas			determinado
21. Colorado o rojo marrón arriñonado		virinqui	determinado
22. Crema salpicado con pardo y sombra lila			determinado
23. Guinda o rojo marrón liso	sitacame, chomegame-sitacame	napushi	determinado
24. Rojo brillante, pequeño o mediano	bacano		determinado
25. Negro opanco, semilla pequeña	huitabushari, huitabushi, rorihuitara	chocame	determinado
26. Negro con pintas crema		chocame pinto	determinado
27. Crema con manchas rojizas		mantequilla	determinado

Table 23 cont. Phaseolus vulgaris in the sierra and barranca of western Chihuahua: seed color, local name, growth habit.

Seed color	Common name Sierra	Common name Barranca	Grow Habit
28. Crema con manchado marrón		pinto	determinado
29. Amarillo claro	rosobochame	barosame	determinado
30. Crema oscuro o beige con pintas negras		uscame	determinado
31. Negro, tamaño mediano		negro, frijol negro	determinado
32. Crema con manchas rojizas, arriñonado	frijol chirrion	frijol de gringo	determinado
33. Morado o grisáceo oscuro con manchas negras	chorizcame, churihuame		determinado
34. Blanco, semilla pequeña		cerahui	determinado
35. Guinda con manchas negras		cacachila	determinado
36. Blanco con amarillo	comoshame		determinado
37. Gris con manchas negras	ojo de cabra, ojo de cabra negro	shivabushira	determinado
38. Guinda con manchas negras		rospoche	determinado
39. Pardo oliváceo, alargado	rosobochame		determinado

Table 23 cont. Phaseolus vulgaris in the sierra and barranca of western Chihuahua: seed color, local name, growth habit.

Seed color	Common name Sierra	Common name Barranca	Grow Habit
40. Crema con machas negras	cushimari		indeterminado trepador
41. Morado con manchas negras	cushimari, cusimuni	busimari	indeterminado trepador
42. Grisáceo con man-- chas negras		busimari tehueyame	indeterminado trepador
43. Crema con manchado negro (sombreado)	cushimari	busimari	indeterminado trepador
44. Blanco liso	cushimari	mushimari	indeterminado trepador
45. Guinda o marrón con mahcas negras	cushimari	cushiburi	indeterminado trepador
46. Café oscuro con manchas negras		municushiburi	indeterminado trepador
47. Amarillo mostaza liso		busimari sahua-roane, busimari	indeterminado trepador
48. Rojo o marrón liso		busimari sitacame, busimari, nusimari, sushimari	indeterminado trepador
49. Crema con manchas marrón semiesférico	cushimari		indeterminado

Table 24. Among the *Phaseolus coccineus* flower visitors, the following insects were recorded: N = Non-pollinator, O = Occasional, P = Potential, F = Frequent, ** = workers and queens.

CHIHUAHUA	*Phaseolus coccineus* Tecomari	*Phaseolus coccineus* ssp. *formosus*
HYMENOPTERA		
Megachilidae		
Megachile sp.	P	
Anthophoridae		
Anthophora sp.	P	
Deltoptila sp.	P	
Thygater sp.	F	
Apidae		
Apis mellifera Linn.	O	
Bombus ephippiatus Say **	F	
Bombus pennsylvanicus ssp. sonorus Say **	O	
LEPIDOPTERA		
Pieridae		
Colias eurytheme Boisduval	N	
Phoebis sennae eubule (L.)	N	
Hesperiidae		
Autochton cellus (Bdv. & Lec.)		N
Lycaenidae		
Leptotes marina Reak.	N	
APODIFORMES		
Trochilidae		
Calothorax lucifer	O	O
Campylopterus hemileucurus	F	F
Eugens fulgens	F	F
Hylocharis leucotis	F	F
Selasphorus rufus	F	F

Table 24 cont. Among the Phaseolus coccineus flower visitors, the following insects were recorded: N = Non-pollinator, O = Occasional, P = Potential, F = Frequent, ** = workers and queens.

PUEBLA	Pcc	Pcd	Pcg
HYMENOPTERA			
Colletidae			
Colletes sp.	P		
Halictidae			
Augochlora sp.	P		
Megachilidae			
Megachile sp.1	P		
Anthophoridae			
Centris nitida	P		
Eucerini sp.	P		
Thygater sp.2	F		
Thygater sp.3	F		
Thygater sp.4	F		
Xylocopa cyanea		F	
Xylocopa nautlana Cockerell		F	
Xylocopa tabaniformis		F	
Apidae			
Apis mellifera Linn.	F	O	
Bombus ephippiatus Say	F	F	F
Bombus medius Cresson	F		F
Bombus pullatus Franklin	F	F	F
Bombus weisi Friese	O		O
LEPIDOPTERA			
Pieridae			
Anteos clorinde (Godart)	N		
Eurema salome (Felder)	N		
Eurema mexicana (Boisduval)	N		
Leptophobia arippa (Boisduval)	N	N	
Phoebis intermedia (Butler)	N		
Hesperiidae			
Autochton cellus (Bdv. & Lec.)	N	N	
Noctuidae			
Antaplaga sp.		N	
Mocis latipes (Guenée)	N	N	

Table 24 cont. Among the Phaseolus coccineus flower visitors, the following insects were recorded: N = Non-pollinator, O = Occasional, P = Potential, F = Frequent, ** = workers and queens.

	Pcc	Pcd	Pcg
APODIFORMES			
Trochilidae			
Amazilia yucatanensis	F		F
Amazilia leucocephala	F		F
Campylopterus curvipennis	O		F
Hylocharis leucotis	F		F
Selasphorus rufus	O		O

Table 25. Osmotic adjustment in Phaseolus spp. cultivated in Puebla (data from 1989).

SPECIES	MAY	JULY
P. coccineus ssp. darwinianus	2.8	2.2
P. coccineus ssp. coccineus	1.94	2.15
P. coccineus "Tecómari"	2.2	3.3
P. vulgaris	2.85	2.3
P. glabellus	3.6	3.6*

* October

Table 26. Monthly differences in water potential of maize-bean (P. coccineus ssp. darwinianus) cultivation association and monocultures of beans in 1990 and 1991 using the Mann-Whitney test.

DATE	RESULTS
April 1990	p= 0.0495 **
May 1990	p= 0.0809 *
July 1990	p= 0.1266 *
	P= 0.6625 *
April 1991	p= 0.3827 **
May 1991	p= 0.0495 **
July 1991	p= 1 *

* = not significantly different ($p > 0.5$)
** = significanlty different ($p \leq 0.5$)

Table 27. Biomass allocation differences in the vine and semi-vine forms and "Tecómari" of Phaseolus coccineus ssp. coccineus (= PCC) (dates of 1990).

	FEB	MAR	ABR	MAY	JUN	JUL	AGO
PCC-vine							
root	23.4	424.4	740	1675	6333	11000	26000
stem	197.4	322	1960	10520	23800	27000	74000
leaves	45.6	600.4	2399	6400	17600	25000	38000
PCC-semi-vine							
root	68	394	780	2100	3740		
stem	413.8	458.8	2240	7040	11540		
leaves	87.4	708.4	3663	8500	3920		
PCC-"Tecomari"							
root	60.6	358.6	1360				
stem	402	545.2	3280				
leaves	73.8	826.8	4292				

Table 28. Biomass allocation differences in the semi-vine form and "Tecómari" of Phaseolus coccineus ssp. coccineus (= PCC).

	JUL	AUG	SEP	OCT
PCC-semi-vine				
root	164.4	468.4	2800	3200
stem	231.2	428.8	11000	12000
leaves	218.4	227.8	5800	3400
PCC-"Tecómari"				
root	299.6	690.4	1800	
stem	210	2090.8	2600	
leaves	235.4	2226	3200	

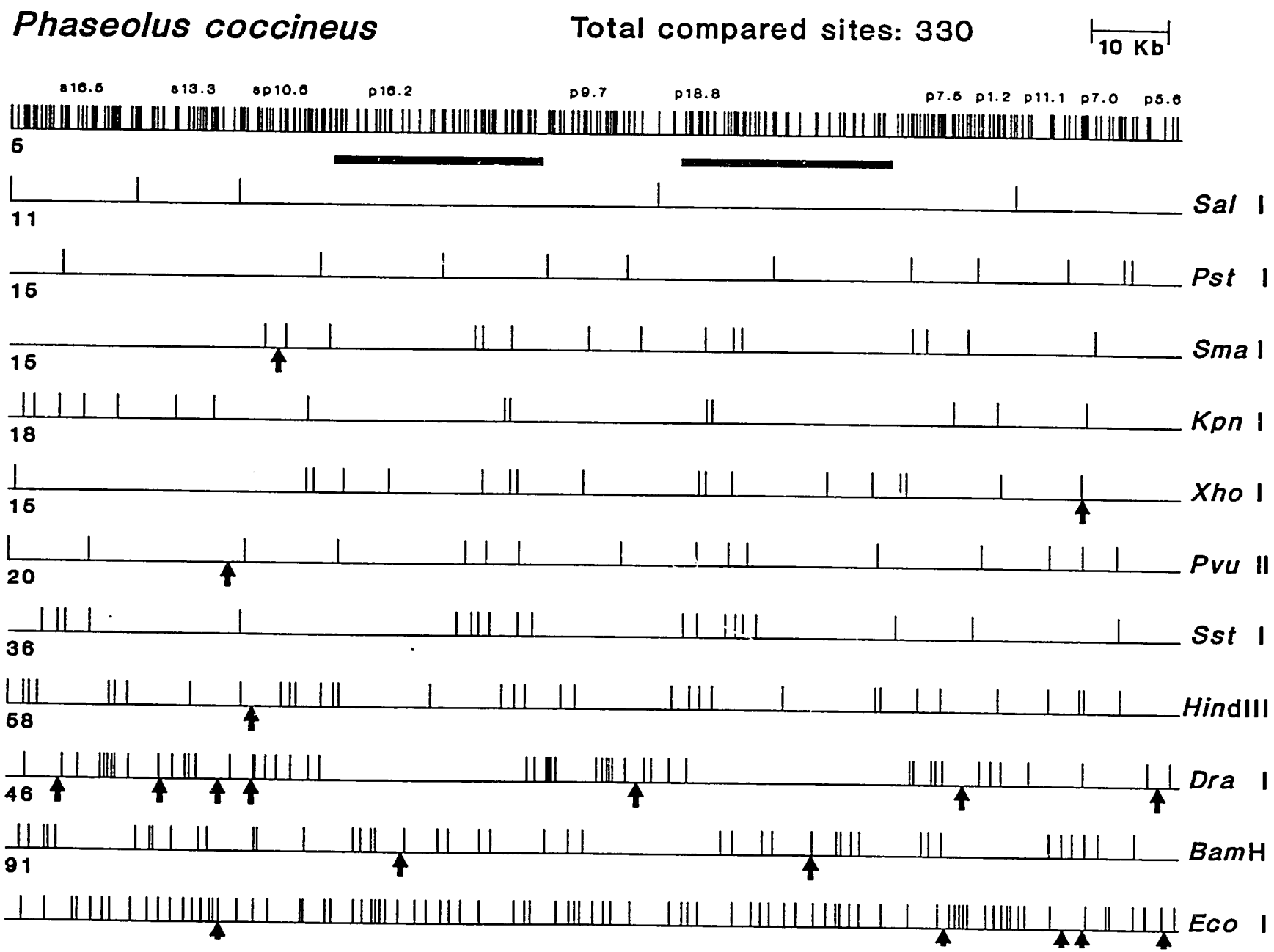


Fig. 1. Linearized cpDNA map of Phaseolus coccineus from Chihuahua, Mexico.

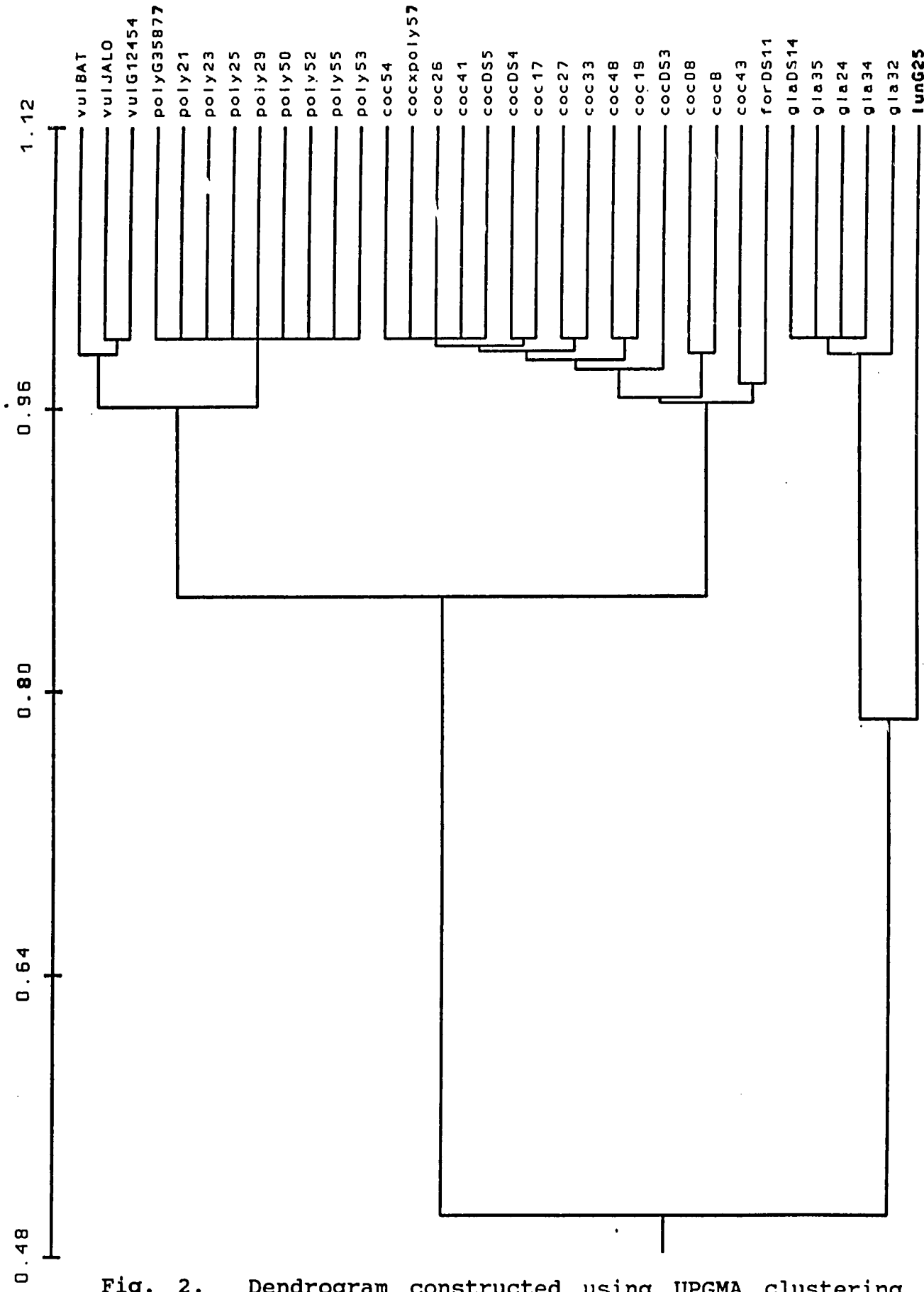


Fig. 2. Dendrogram constructed using UPGMA clustering method utilizing cpDNA restriction sites for Phaseolus coccineus subsp. coccineus (coc), P. coccineus subsp. darwinianus (poly), P. coccineus subsp. formosus (for), P. coccineus subsp. glabellus (gla), P. vulgaris (vul), and P. lunatus (lun).

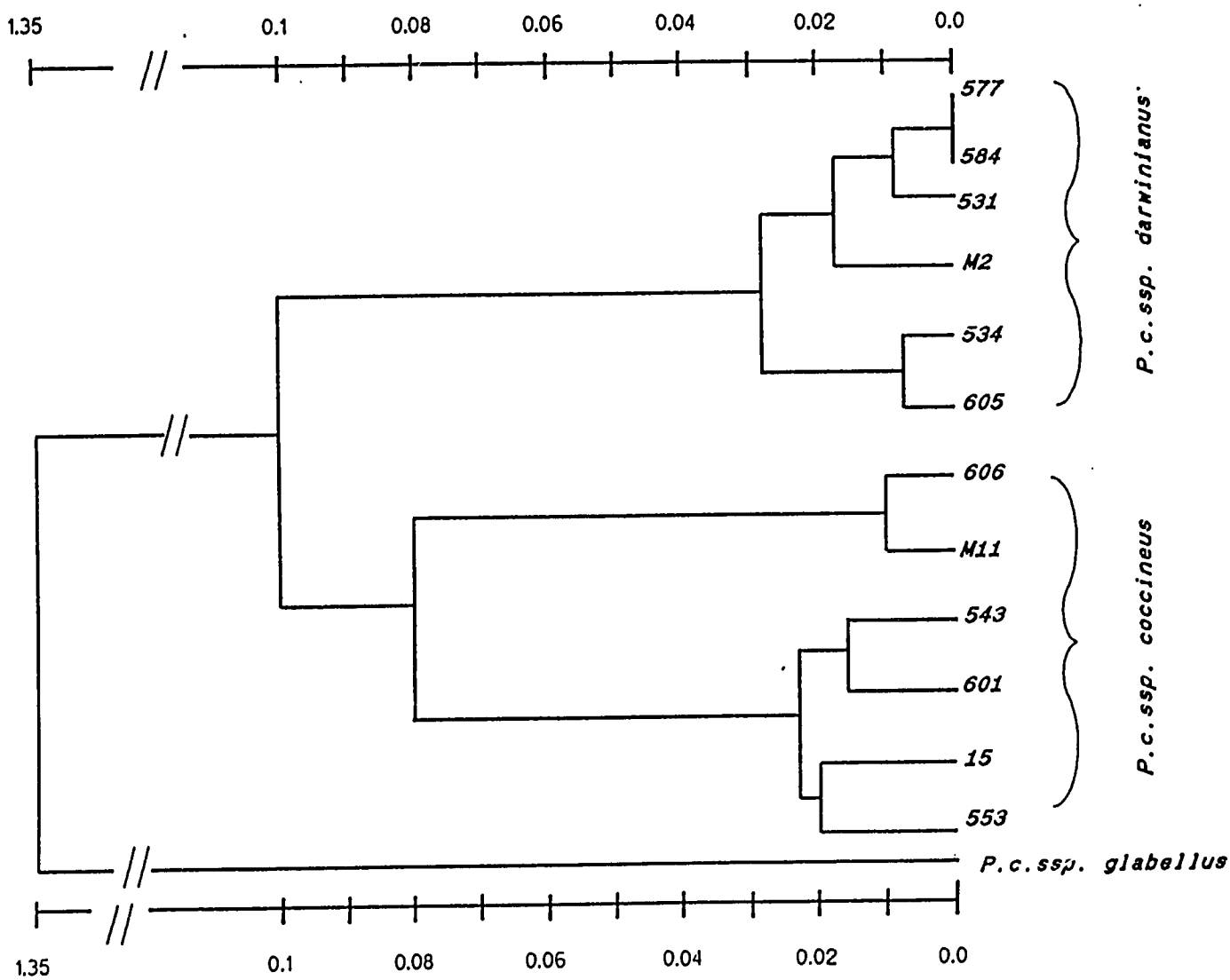


Fig. 3. Dendrogram constructed using UPGMA clustering method utilizing genetic distances (Nei 1972); Phaseolus coccineus ssp. coccineus, P. coccineus subsp. darwinianus, and P. coccineus subsp. glabellus.

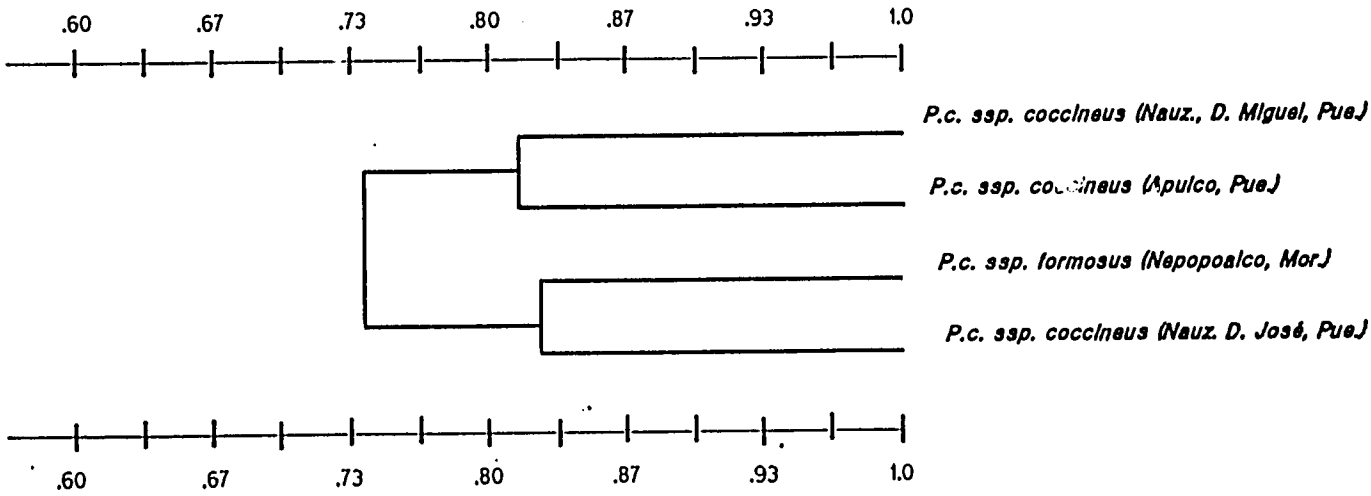


Fig. 4. Dendrogram of genetic similarity (Nei 1972) for four taxa: Phaseolus coccineus subsp. coccineus and P. coccineus subsp. formosus.

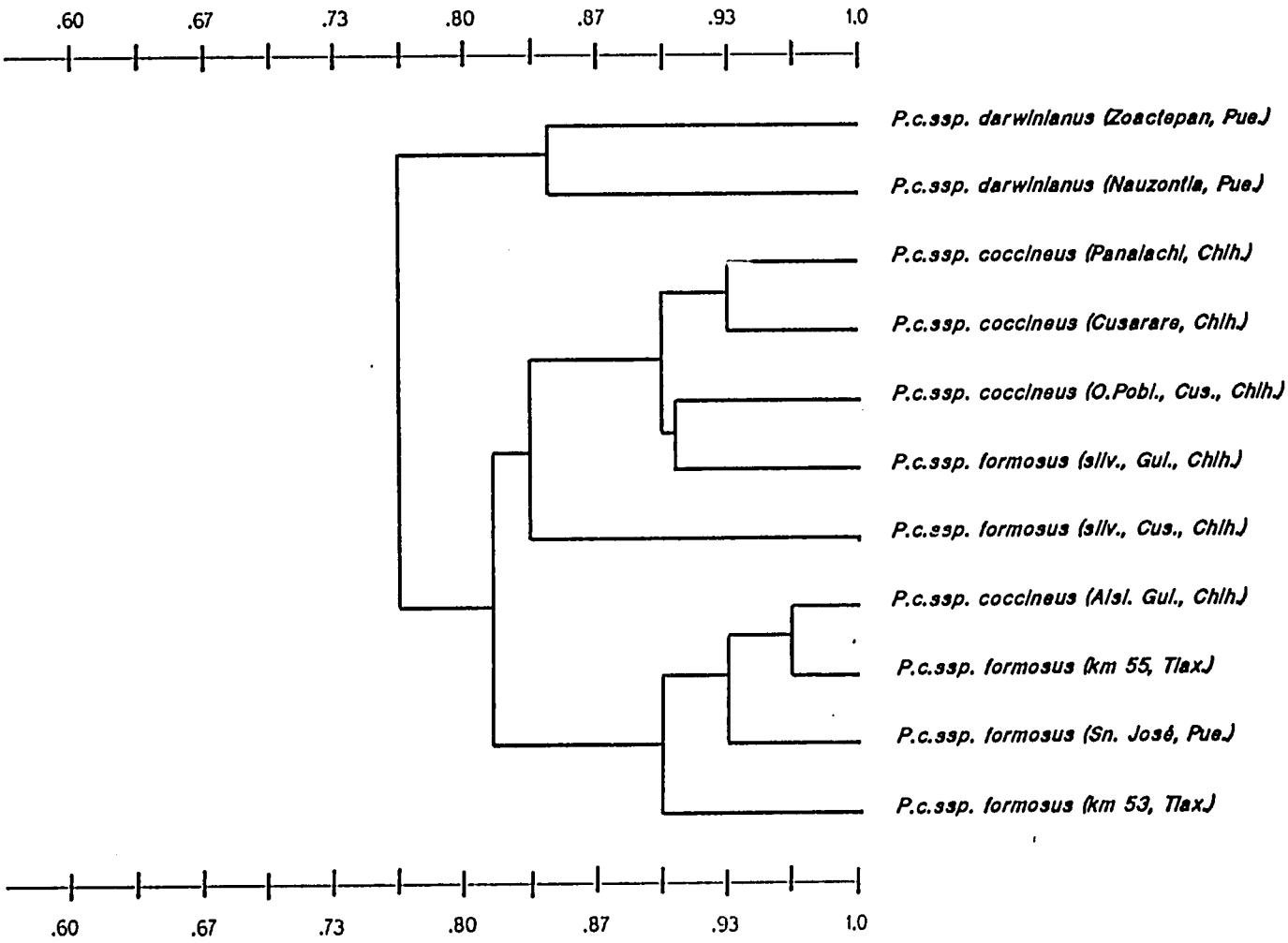


Fig. 5. Dendrogram of genetic similarity (Nei 1972) for three taxa: Phaseolus coccineus ssp. coccineus, P. coccineus ssp. darwinianus, and P. coccineus ssp. formosus.

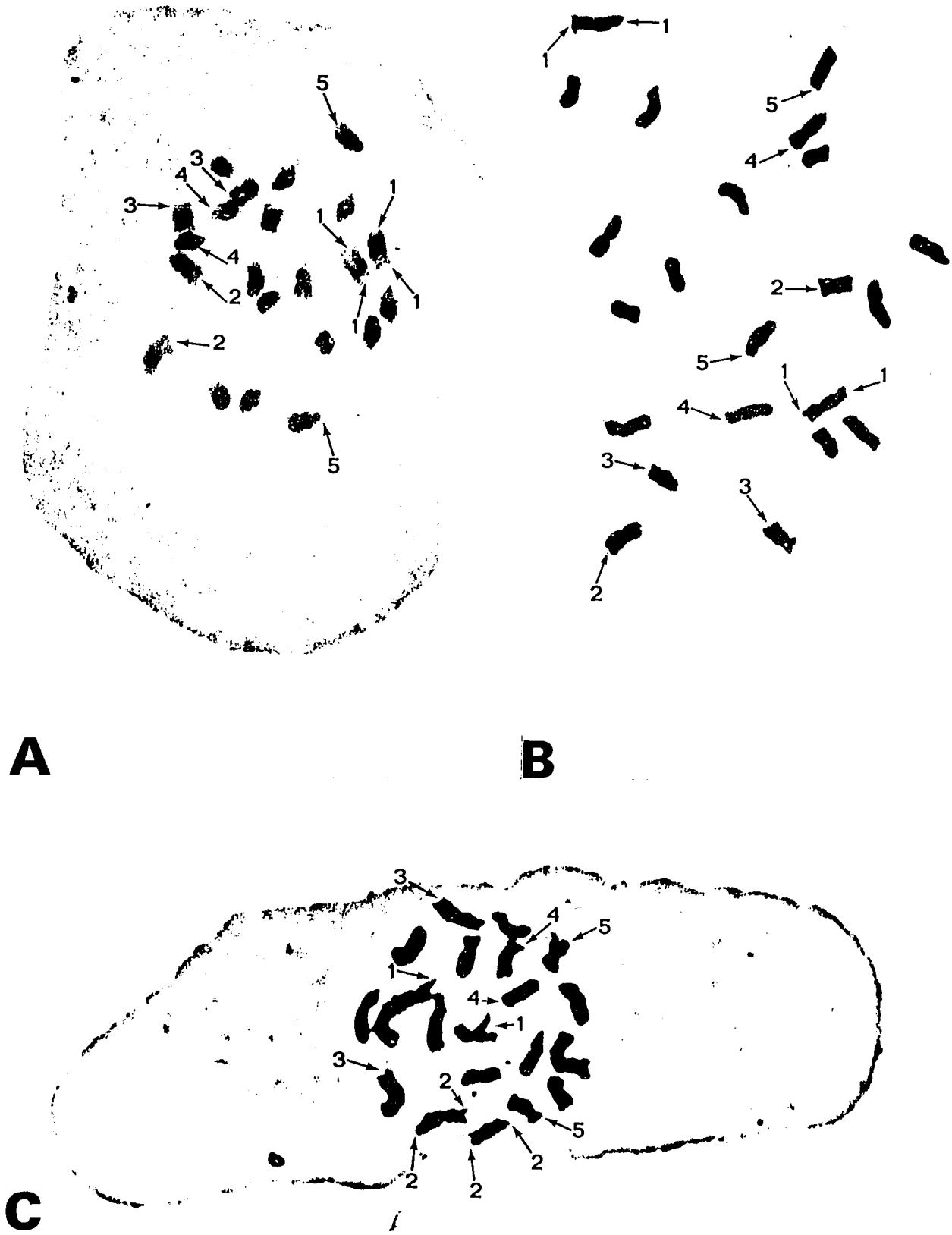


Fig. 6. Somatic cells of: A- Phaseolus coccineus subsp. coccineus from Coxquíhui, Veracruz, with 2n=22 and kariotypic formula of 11m. B- P. coccineus ssp. darwinianus (3005) from Huahuaxtla, Puebla, with 2n=22 and kariotypic formula of 10m + 1sm. C- P. coccineus subsp. glabellus (810) from Tecuicuilco, Puebla, with 2n=22 and kariotypic formula of 9m + 2sm. The numbers indicate the homologous pairs with secondary restrictions. The scale bar equals 10 μm.

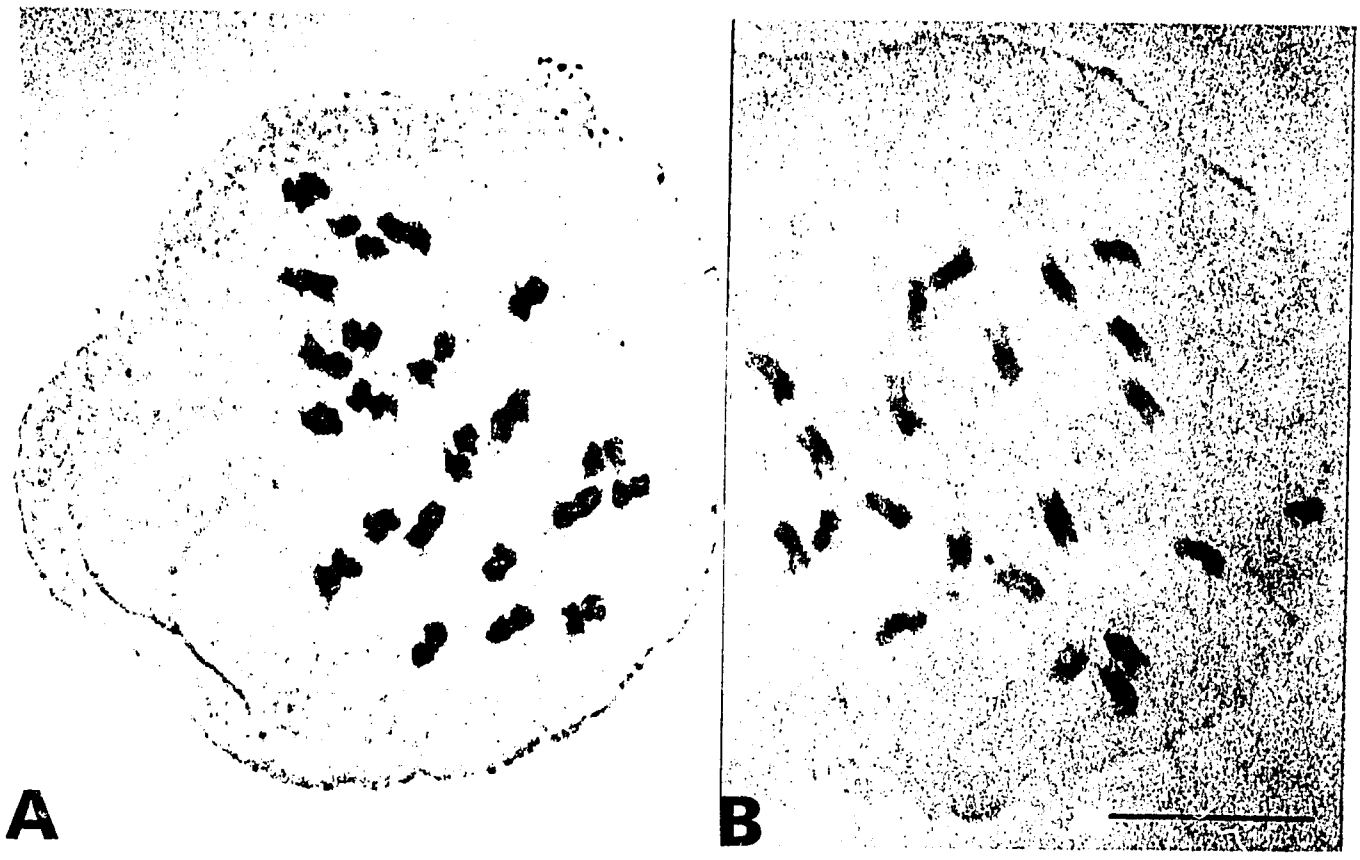


Fig. 7. Somatic cells of: A- Phaseolus coccineus subsp. formosus (936) from Nopalucan, Puebla, with 2n=22. B- P. coccineus subsp. formosus (938) from Carmen, Tlaxcala, with 2n=22. The scale bar equals 10 μm.

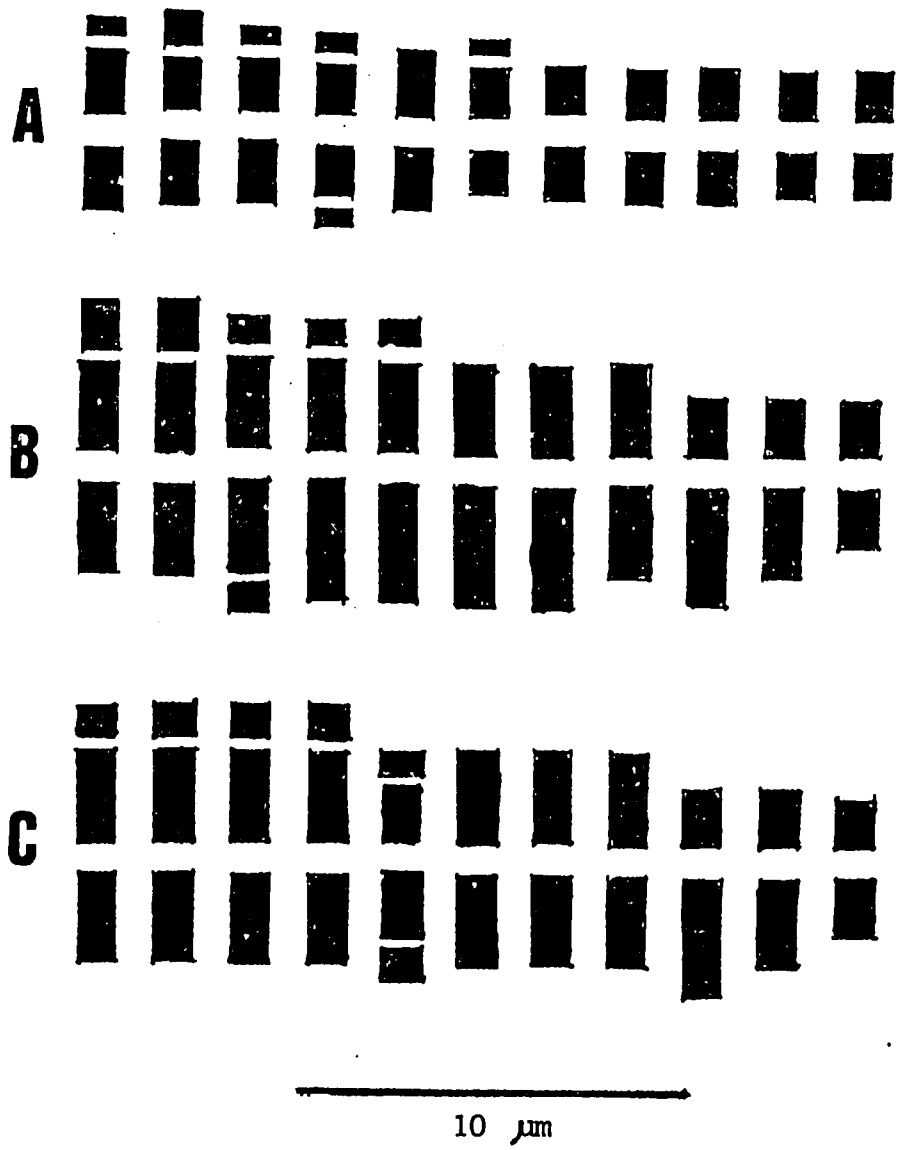


Fig. 8. Ideograms of kariotypes of: A- Phaseolus coccineus subsp. coccineus from Coxquíhui, Veracruz. B- P. coccineus ssp. darwinianus (3005) from Huahuaxtla, Puebla. C- P. coccineus subsp. glabellus (810) from Tecuicuilco, Puebla. The scale bar equals 10 µm.

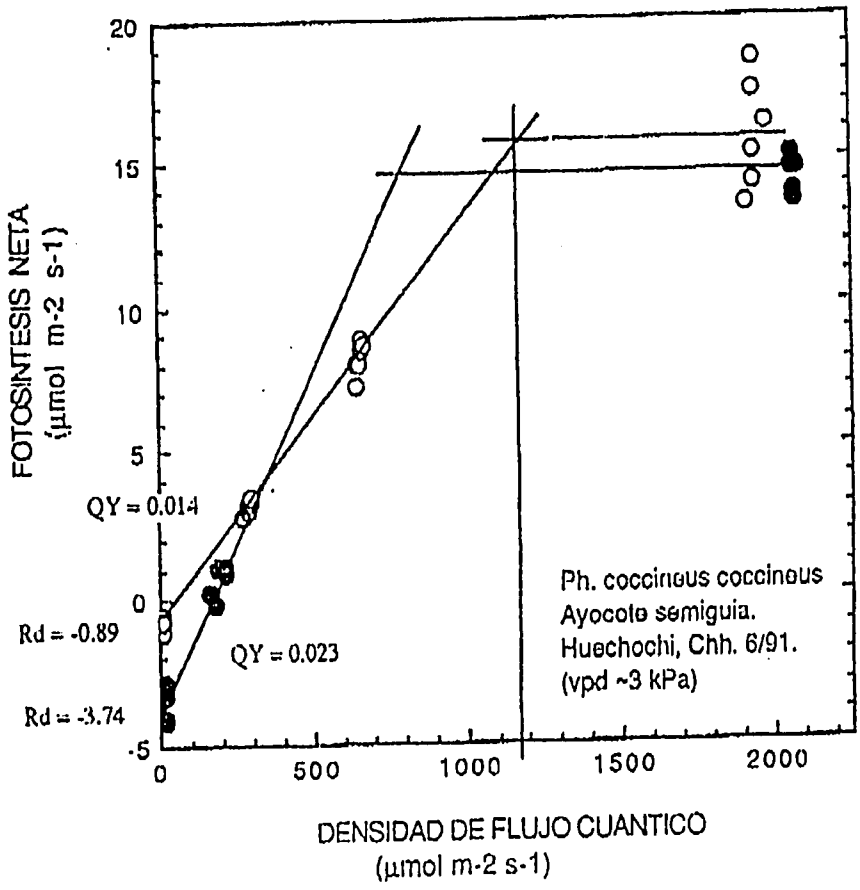


Fig. 9. Photosynthetic rates (Fotosintesis neta) of P. coccineus from wild populations (closed circles; lower rate) and from the cultivated form ("Ayocote" semi-vine) grown in Huechochi, Chihuahua.

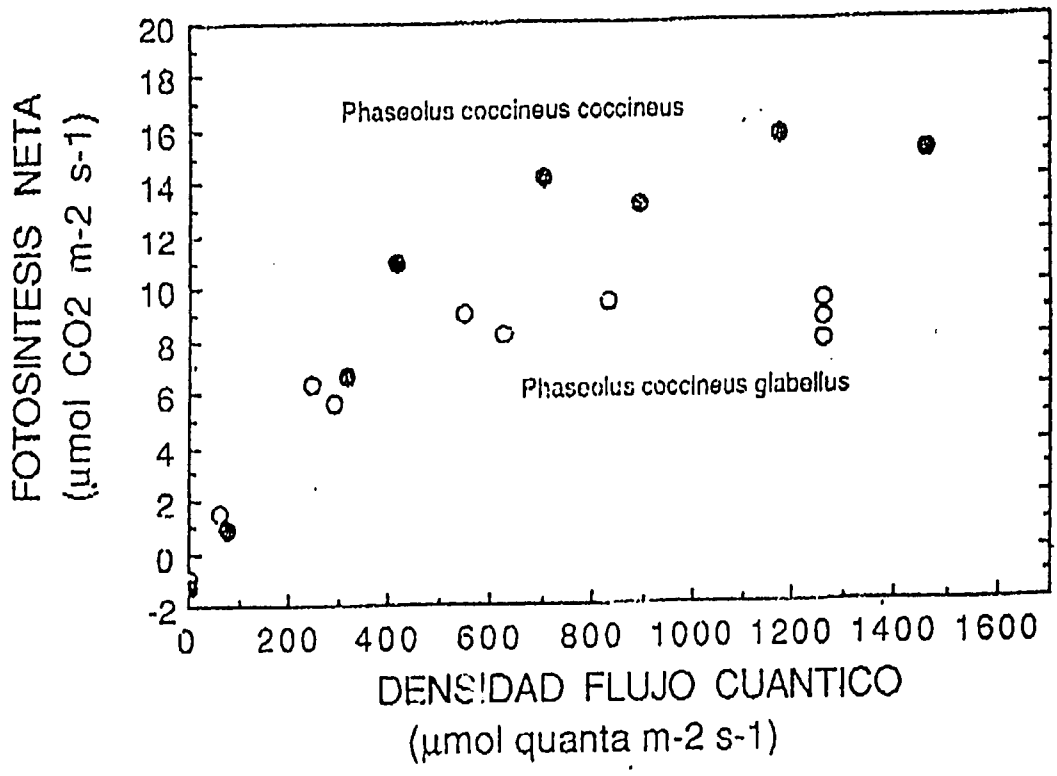


Fig. 10. Comparative study of photosynthetic rates (Fotosintesis neta) of Phaseolus coccineus subsp. coccineus and P. coccineus subsp. glabellus in the greenhouse.

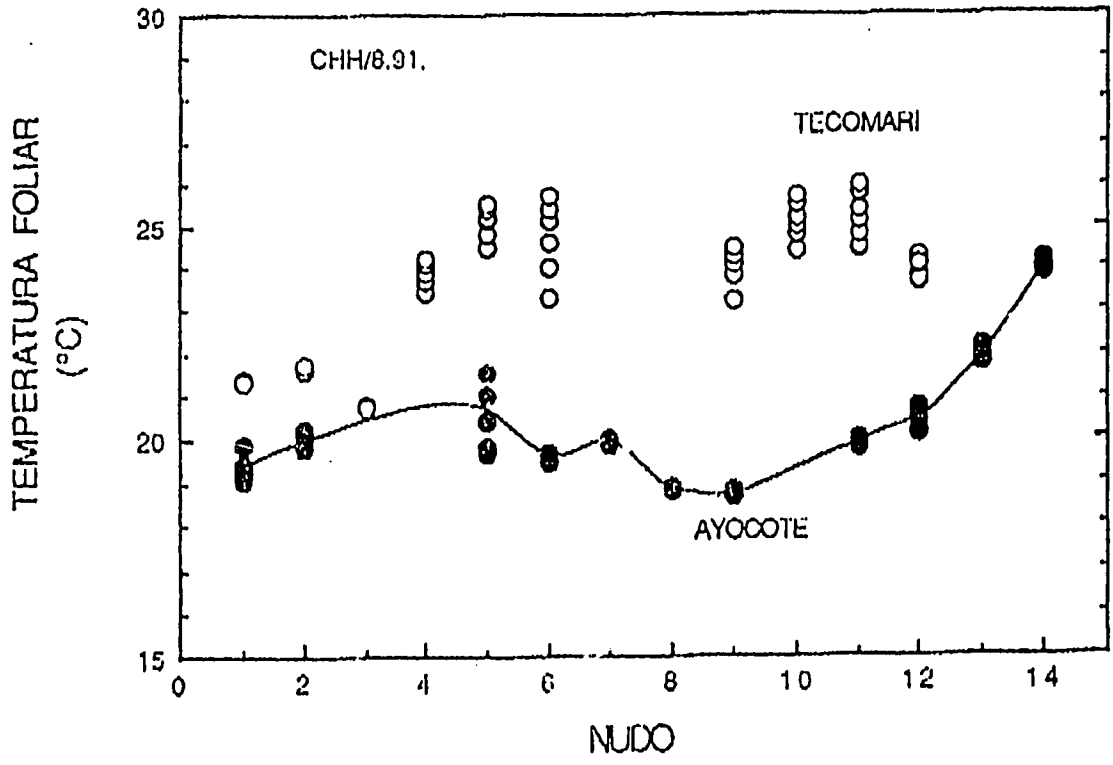


Fig. 11. Foliar temperatures of cultivated Phaseolus coccineus subsp. coccineus "Tecómari" and "Ayocote" in which the foliar temperatures of "Tecómari" along the nodes of the main axis were 3°C or more above those found in the semi-vine "Ayocote."