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# ISOZYME DIVERSITY IN RELATION TO DOMESTICATION OF GAUR (*Cyamopsis tetragonoloba* (L.) TAUB.)

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**A survey of isozyme systems in a relatively little known legume, the guar (clusterbean), *Cyamopsis tetragonoloba* L.) Taub., has been reported for the first time. The plant material included seven landrace accessions from India, six released cultivars from the United States, and two wild species populations of *C. senegalensis* and *C. serrata*. Starch gel electrophoresis protocols for seven isozyme systems, viz., MDH, IDH, PGD, ADH, PGI, GOT and ME were largely developed. The combination of cotyledons as the plant tissue, and Tris-HCl with ascorbic acid, PVP, PVPP and 2-mercaptoethanol as the extraction buffer provided the best resolution of isozymes. Out of the four gel-electrode buffer systems attempted, the Histidine system was selected to study the patterns of diversity. All the cultivated species accessions were uniformly monomorphic. Two isozyme phenotypes were recorded for ADH in *C. senegalensis*, and for GOT in *C. serrata*. A preliminary interpretation of the banding patterns in relation to domestication of guar has been made.**

**Key words :** Guar, isozymes, diversity, genetic distance

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) has been cultivated in India, Pakistan, Indonesia and other parts of southern and south-eastern Asia as a vegetable and forage crop for a long time (Whyte *et al.*, 1953; ICAR, 1970). Guar gum is used extensively by the paper, mining, explosive, food, pharmaceutical, cosmetic, textile and oil industries around the world (Hymowitz and Maltock, 1963). Guar seed protein also has great industrial potential, both for human diet and cattle feed.

The genus *Cyamopsis* is (tribe Indigofereae, Family : Leguminosae, Sub-family; Papilionoideae) consists of three species viz., *C. tetragonoloba, C. senegalensis* and *C. serrata* (Gillett, 1958; 1971). The forms intermediate between the latter two wild species and found in south-western Africa alongwith *C. serrata* were named as *C. dentata* by Torre (1960). Whistler and Hymowitz (1979) followed Gillett's taxonomic treatment of *Cyamopsis*. In cytological studies, Senn (1938) had suggested close relationship between the Genus *Cyamopsis* and *Indigofera*, but Gillett (1958) preferred to retain *Cyamopsis* as a distinct genus. *Cyamopsis* is a uniformly diploid genus with 2n = 14.

The relationship between the guar cultigen, *C. tetragonoloba*, grown in the Indian sub-continent and its allied wild species has not been studied in depth experimentally. Linguistic evidence to ascribe antiquity is also very poor (Hymowitz, 1972). On morphological grounds, however, the cultigen appears to be closest to the wild species, *C. senegalensis*, found from north-east and north-west Africa and as far north as Arabia. The other related species, *C. serrata* and *C. dentata*,

have been found in south-western Africa, a region distant from the distribution of *C. senegalensis*. No conspecific wild forms of *C. tetragonoloba* have been found, nor any remains in any Neolithic site in the Indo-Pakistan sub-continent. Gillett (1958) was also not sure of the cultigen's presence of Asia east of Arabia and did not record its presence in Arabia. Linnaeus (1767) indicated that the distribution of *C. tetragonoloba* was Arabia. Hymowitz (1972) has reviewed the history of domestication of guar, and suggested that the focus of its domestication should be concentrated towards the drier regions in north-western India and adjacent lands of Pakistan, where hot dry climates allow it to flourish as a field crop. The possibility of its domestication in other parts of the sub-continent has been ruled out, other regions having high soil moisture conditions or drizzling weather with high humidity in which the pathogens cause appreciable damage to the cultigen. The paradox of an apparent domestication of guar in the Indian sub-continent far removed from the African continent can be explained by the trans-domestication concept of Hymowitz (1972). His thesis takes support from the history of Arab-Indian trade in early times. Horses were a major item of import to Indian ports and the Arabs must have taken on board their ships huge quantities of fodder to feed horses while on the seas, and perhaps sold any remaining fodder to the Indians. Seed of *C. senegalensis* probably came with the fodder, and since the climatic conditions in the dry regions of Indo-Pakistan sub-continent are similar and quite favourable, any seed brought along by the Arabs, and which subsequently germinated, could have easily formed the basis for development through selection of the present day *C. tetragonoloba*. The arrival of *C. senegalensis* in the sub-continent is thought to have occurred between the 9th and 13th centuries A.D. when extensive trade took place between the Arabs and Indians. Thus, the African wild species, *C. senegalensis*, appears to be the ancestor of the west Asian domesticate, *C. tetragonoloba*. The domestication process took place in not very distant times in dry areas of the north-western region of the Indo-Pakistan sub-continent.

Despite the morpho-agronomic diversity in guar germplasm collections (Dabas *et al.*, 1989), variation in isozymes in germplasm accession populations have not been studied to provide additional set of data to interpret genetic diversity in a still greater depth. Research work reported here is one small attempt to understand isozyme diversity in this relatively little known legume, the guar (clusterbean); *Cyamopsis tetragonoloba*. Isozyme survey work has not been reported in this crop, and was considered appropriate in view of the economic importance of crop. The objectives of the research work were to (1) develop starch gel electrophoresis protocols for analysis of seven enzyme systems; and (2) compare isozyme diversity in the primitive landrace accessions from India alongside the released cultivars from the United States, and the two wild relatives.

## MATERIALS AND METHODS

### (i) Plant materials

Seven landrace accessions, IC Nos. 40034(1), 40052(2), 40057(3), 40284(4), 40348(5), 40378(6) and 41110(7) from guar growing Rajasthan state of India, obtained from NBPGR, New Delhi, India; six US cultivars viz., Brooks (8), Esser (9), Hall (10), Kinman (11), Lewis (12) and Santacruz (13) and samples of the two wild relatives viz., *C. senegalensis* (14) and *C. serrata* (15) and US cultivars were obtained from USDA, Beltsville, Maryland, USA.

### (ii) Seed germination

Seeds were scarified with dilute sulphuric acid (1:8) for 10 minutes for the cultivated types and for 30 minutes for the wild species. Seeds were then thoroughly washed with distilled water, and sown in 'Professional All-Purpose Compost' from the Sinclair Horticulture and Leisure Co.

(SHL), UK in 6-inch size pots in a controlled environment chamber maintained at a temperature of 28-30°C. Germination was rapid (2 days), and seedlings suitable for isozyme analysis could be obtained in 7 days.

**(iii) Selection of plant tissue and extraction buffer**

Four plant tissues representing different organs of seedlings viz., cotyledons, hypocotyls, roots and primary leaves were evaluated for the extraction of enzymes using five different extraction buffers from Catty and Jackson (1989) viz., i) Tris- HC1, ii) Tris-HC1 with 2-mercaptoethanol, iii) Tris-HC1 with ascorbic acid, PVP, PVPP and 2-mercaptoethanol; iv) Tris-HC1 with $NaNO_3$ and NaCl, and v) distilled water. The performance of separation of isozymes was evaluated using three criteria viz., level of enzyme activity expressed on the starch gel, clarity in the separation of bands, and the intensity of arcing. Thus, coyledons were selected for study of the patterns of isozyme variation using 0.1M Tris-HC1 extraction buffer (pH 7.2) containing ascorbic acid (0.03%), polyvinylpyrrolidone-40(4%), polyvinyl polypyrrolidone (2%) and 2-mercaptoethanol (1 µ/ml) per 1 $cm^2$ cotyledonous tissue in 2ml of extraction buffer to extract the enzymes. Since deep freezing (–40°C) of extracted homogenate did not yield better results, fresh plant material was used every time a gel was run.

**(iv) Selection of buffer systems with differential gel and electrode buffers**

Four gel and electrode buffer systems, first three from Catty and Jackson (1989) and fourth from Chen (1985), viz., i) Histidine system: Electrode buffer (E.b.) = 0.2M trisodium citrate $2H_2O$, pH 7.0; Gel buffer (G.b.) = 0.005 L-histidine monohydrochloride, pH 7.0 ii) Buffer System 1 : E.b. = 0.135 M tris, 0.043 M citric acid, pH 7.0; G.b. = diluting 20 ml of E.b. to 300 ml; iii) Buffer system F : E.b. = 0.3M sodium borate (boric acid), pH 8.3; G.b. = 0.0036M citric acid, 0.152 M tris, pH 7.6; and iv) Tris/Citrate and Lithium Borate System; Buffer (A) E.b.) = 0.2 M Lithium borate, Buffer (B) = 0.05 M tris citrate; G.b. = 9B:1A; were evaluated.

The concentration of starch (Sigma, USA) in the gel was maintained at 38g in 300 ml of gel buffer. On the basis of three criteria viz., separation of bands, quality of staining and duration of the run, Histidine system was selected for seven selected enzyme systems viz., alcohol dehydrogenase (ADH), phospho glucoisomerase (PGI), glutamate-oxaloacetate transminase (GOT), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME) and 6-phosphogluconate dehydrogenase (PGD) using the staining recipes described in Catty and Jackson (1989). The electric current was maintained at 30 mA at the start of electrophoresis, and 25 mA after the removal of wicks. The electrophoresis was run for 5-5.5 hrs at 4°C in a refrigerator. To keep the electrophoretic conditions as cool as possible, the gel was covered with a polythene cling film sheet after the removal of the wicks, and some ice was placed is a small tray on the top of the cling film. Buffer system 'F' gave additional resolution for ME for the two wild species.

**(v) Data analysis**

The isozyme profiles obtained for the seven polymorphic enzyme systems were scored for "allelic" differences within and among the three species. Although this scoring was based on the analysis of individual seedlings from each germplasm accession, a genetic confirmation of the interpretation is required for more accurate analyses. The species X allele datamatrix for 26 allelic variants from seven enzyme systems was used to calculate genetic distances among the three species (Nei, 1972). The genetic distances were used to construct a UPGMA-based dendrogram to depict inter-relationships among

the species. All the statistical calculations were performed using NTSYS-pc, version 1.80 software package (Rohlf, 1992).

## RESULTS AND DISCUSSION

All the accessions of the cultivated species, *C. tetragonoloba*, whether landrace accessions from Rajasthan (India) or US cultivars, were uniformly monomorphic for all the severn enzyme systems. The population of *C. senegalensis* indicated two isozyme phenotypes for ADH. The population of *C. serrata* expressed two phenotypes for GOT. The diagrammatic representation in the forms of zymograms and representative photographs are given in Fig. 1 and 2 respectively.

### Malate drhydrogenase (MDH)

There were indications of two zones of activity. The fast migrating zone consistently displayed well-resolved equally- spaced three bands at Rf = 0.311, 0.338 and 0.365. However, in just a few cases of individual plants, there was an indication of a fourth faint band at Rf = 0.233 (e.g. in IC 40034, IC 40052, IC40057) or at Rf = 0.455 (e.g. IC 40284, IC 40438). It was difficult to determine whether these were extra bands or just artefacts. Likewise, the slow migrating zone, from Rf = 0 to 0.085, was very faint and was not observed in all the gels screened.

### Isocitrate dehydrogenase (IDH)

The cultivated accessions consistently displayed a single band at Rf = 0.263. However, in the populations of *C. senegalensis* and *C. serrata*, the single band was fast migrating compared to the cultivated accessions, at Rf = 0.337.

### 6-Phosphogluconate dehydrogenase (PGD)

Two isozyme bands at Rf = 0.549 and 0.396 were common in both the cultivated and wild accessions. There was a third slow migrating band at Rf = 0.212 in the cultivated accessions, and at Rf = 0.321 in the two wild accessions.

### Alcohol dehydrogenase (ADH)

Two isozyme bands at Rf = 0.309 and 0.480 were uniformly expressed in all the cultivated accessions. The slow migrating band was generally faint in expression. In *C. senegalensis*, two isozyme phenotypes were noticed. One phenotype (freq. = 0.03) was observed to be the same as in the cultivated accessions. However, the other phenotype was throughout consistent (freq. 0.97). This phenotype consisted of two bands at Rf = 0.412 and 0.516 i.e. the isozyme forms were fast migrating compared to the forms in the cultivated

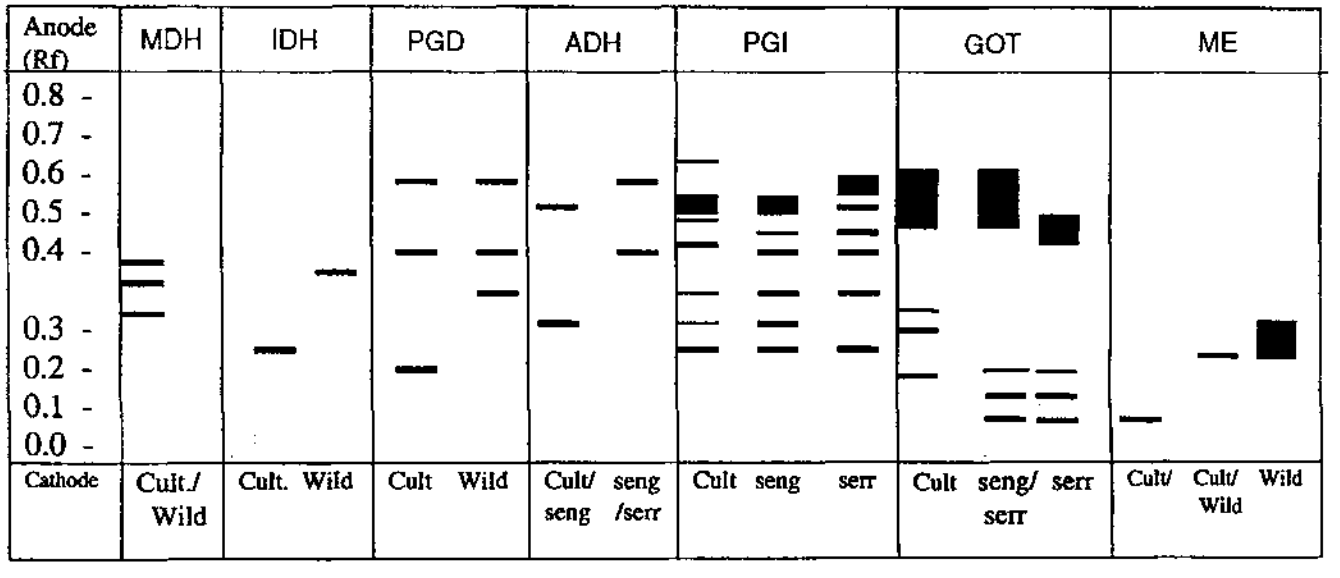


Fig. 1. Representative zymograms of the seven enzyme profiles analysed in *C. tetragonoloba, C. serrata* and *C. senegalensis*

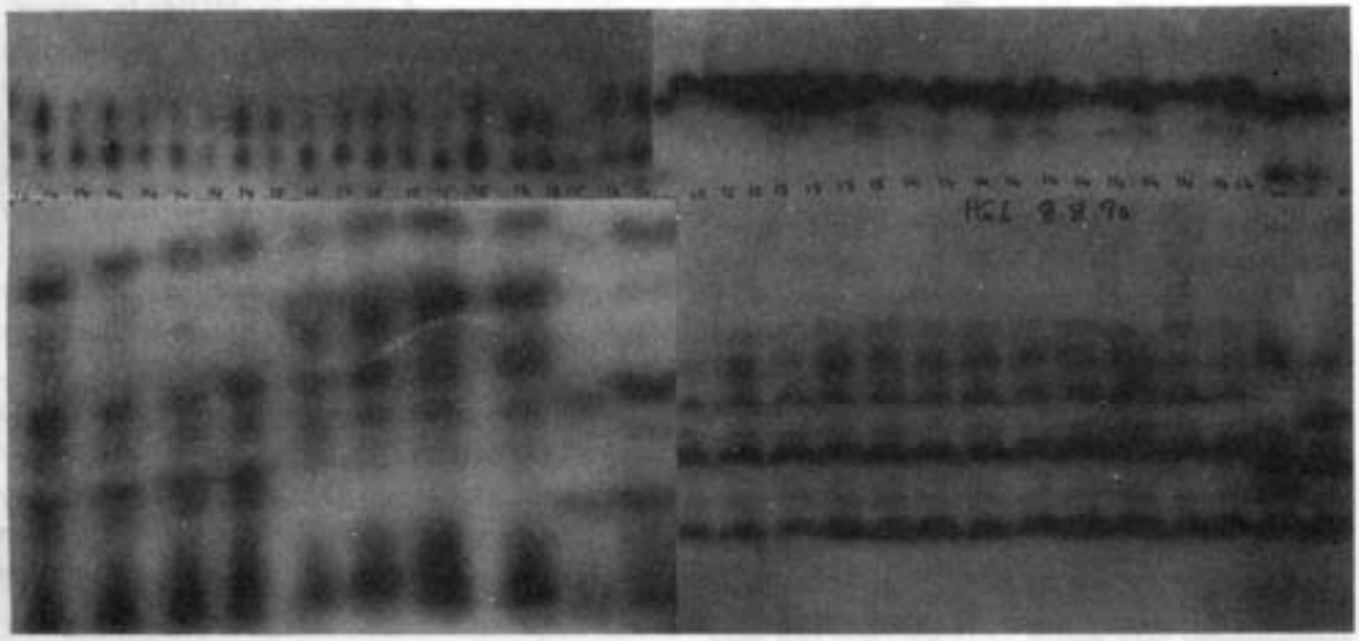

**Fig. 2. Representative isozyme profiles for phosphoglucoisomerase (top panel), 6-phosphogluconate dehydrogenase (left half of lower panel) and alcohol dehydrogenase (right half of lower panel) in *C. tetragonoloba*, *C. serrata* and *C. senegalensis***

accessions. The population of *C. serrata* also uniformly expressed the common phenotype of *C. senegalensis* population.

**Phosphoglucoisomerase (PGI)**

Apparently, there were two zones of activity in the cultivated accessions. The slow migrating Zone I expressed two strongly staining bands at Rf = 0.225 and 0.371, and three faint bands at Rf = 0.281, 0.303 and 0.404. The fast migrating Zone II displayed two strongly staining bands at Rf = 0.467 and 0.528, and a faint band at Rf = 0.607. The two strongly staining bands of Zone II expressed diffused activity between these bands in all the individuals of cultivated accessions and did not indicate the presence of any faintly staining bands as in Zone I. The two zones together were same in all the individuals of the cultivated accessions.

The isozyme phenotype was uniformly different in the two wild species. In *C. senegalensis*, the isozyme phenotype corresponding to Zone 1 of the cultivated accessions, expressed strong enzyme activity at Rf = 0,225, 0.281, 0.303 and 0.371, and faint acticity at Rf = 0.404. Corresponding to Zone V, it expressed a strong but diffused zone of activity between Rf = 0.482 and 0.528. In *C. serrata*, the isozyme phenotype, corresponding to Zone I of the cultivated accessions, expressed strong activity at Rf = 0.225, 0.303 and 0.371. Corresponding to Zone II, it expressed two additional strongly staining bands at Rf = 0.428 and 0.459 and a strong but diffused zone of activity between Rf = 0.482 and 0.528, as observed in the population of *C. senegalensis*.

**Glutamate-oxalo-acetate transaminase (GOT)**

Two zones of activity could be visualised. The slow migrating Zone I expressed two strongly staining bands at Rf = 0.195 and 0.253, and a faint band at Rf = 0.310. The fast migrating Zone II did not separate a strong but diffused zone of activity between Rf = 0.460 and 0.575. In *C. senegalensis*, the enzyme activity corresponding to Zone I of the cultivated accessions, was comparatively slow migrating. In this case, the Zone I expressed two strongly staining bands at Rf = 0.136 and 0.146, and a

faint band at Rf = 0.195. The enzyme activity corresponding to Zone II of the cultivated accessions was similar to cultivated accessions. In *C. serrata*, the enzyme activity in Zone I was similar to that in *C. senegalensis*. Corresponding to Zone II, two phenotypes were observed. One phenotype (freq. 0.86) was the same as in *C. senegalensis*. The other rare phenotype (freq. 0.14) expressed a comparatively slow migrating Zone II between Rf = 0.432 and 0.465. In one gel, where two individuals each of the seven Indian accessions and three US cultivars were screened, the pH of the gel buffer was modified to 7.9. This modification could resolve the Zone II into three clear strongly staining bands of Rf = 0.453, 0.558 and 0.604. In this case, the bands of Zone I were weak in expression, and were observed at Rf = 0.244, 0.291 and 0.337.

### Malic enzyme (ME)

This enzyme system was screened in both the Histidine ad F gel-electrode buffer systems. In the Histidine system, the cultivated accessions and also *C. senegalensis* consistently expressed a single band at Rf = 0.122. In *C. serrata*, the expression was very faint, and some enzyme activity was expressed near the origin with streaking upto R. = 0.122. In the F buffer system, the isozyme phenotype of the cultivated accessions was different compared to the two wild populations. The cultivated accessions expressed a single strongly staining band at Rf = 0.270. The two wild species expressed faint bands at Rf = 0.191 and 0.270 with diffused activity between these bands.

### Development of starch gel electrophoresis protocols

Some limitations observed in the developed protocols are as follows. The problem of arcing in isozyme bands in MDH, IDH, ADH, PGI and ME is still present to a certain extent. The isozymes of PGD could only be expressed as 'blobs', and not as discrete and tight bands. It was not possible to resolve adequately the slow migrating zone of activity in MDH. In general, the two wild species have expressed weak enzyme activity, and the arcing of bands was relatively acute in some of the gels run. These observations suggest the need for attempting further modification particularly in the extraction buffer used for releasing enzymes from the plant material. Several workers have also noted that the choice of extraction buffer was one of the most important factors affecting enzyme resolution (Kephart, 1990). The selected extraction buffer was chosen based on its generally better performance on the cultivated accessions. Thus, the wild species may require some further modification in the extraction buffer to provide better resolution of isozyme bands.

The cotyledons were selected as the organ for extraction of enzymes based on greater enzyme activity. However, it becomes a destructive approach and poses limitations on the seedling to mature into a full-grown plant. Rhoades and Cates (1976) have indicated that the concentration of phenolics and other secondary compounds can vary inversely with age. It may be possible that if more research is conducted in the modifications of the extraction buffer, the primary leaf, which gives slightly less enzyme activity with the identified extraction buffer, may become the ideal plant part of better resolution of isozymes. In a survey through a questionnaire to scientists using starch gel electrophoresis, Kephart (1990) found that the respondents rated pH, chemical composition and molarity as the most important factors in selecting the gel-electrode buffer systems. It is quite possible that some further modification in any of these factors may help in better resolution of isozymes from the primary leaf.

The isozyme phenotypes have been determined using one set of electrophoretic conditions (except in ME) used. Coyne *et al.* (1978) have found that the additional variation is proportional to the variation detected by one

condition. Thus, the general picture of variation for an enzyme is not changed after additional analysis, and the preponderance of evidence in the literature also suggests the same (Gottlieb, 1981). The study of Coyne *et al.* (1979) is particularly significant because a number of different electrophoretic techniques were utilised to investigate 6-glycerophosphate dehydrogenase heterogeneity within and between species of *Drosophila.* No additional variation was detected within species but additional variation among species was documented. Gottleib (1979) also did not find any additional variation in *Stephanomeria matheurensis* with additional electrophoretic analyses, using several different conditions of pH and pore size, in acrylamide gels as compared to that detected with starch gels. The evidence to date also suggests that in most instances the increased variation detected with different electrophoretic conditions will cause little if any change in systematic and phylogenetic inferences made from original surveys using only one condition (Ayala, 1982; Gottleib, 1981).

**Interpretation of zymogram patterns**

The zymograms for MDH, IDH, PGD, ADH and ME expressed simple banding patterns. As such, a preliminary interpretation is made of the number of gene loci involved, and their nature, whether homozygous or heterozygous.

MDH - This is a dimeric enzyme. The presence of three equally- spaced bands throughout the cultivated and wild accessions is perhaps indicative of 'fixed heterozygosity' for this locus.

IDH - The single band detected for this enzyme indicated that this IDH locus is uniformly homozygous. However, the nature of this gene locus is different in the two wild species populations as it yields a comparatively fast migrating form of the isozyme.

PGD - The three bands at different Rf positions could perhaps be interpreted as products of three different homozygus gene loci. The two fast migrating isozymes occupy the same position in both the cultivated and wild accessions, and this may represent two homologous gene loci. The third slow migrating isozyme occupies different positions in the cultivated accessions vis-a-vis the two wild species populations. Thus, the nature of this gene locus is apparently different in the two cases.

ADH - The two bands consistently observed in all the cultivated accessions and the wild species could also be interpreted as two homozygous loci. Two gene loci for ADH have also been reported in many other diploid plant species (Gottlieb, 1981). The isozyme forms of the two homozygous gene loci observed in *C. serrata* were comparatively fast migrating. In *C. senegalensis,* the two homozygous gene loci were either similar to the cultivated accessions or to the population of *C. serrata.*

ME - The single band observed in the Histidine system throughout the cultivated accessions and *C. senegalensis* also apparently represents a homozygous gene lcus. However, the gene locus expressed in the F system was observed as heterozygous in the two wild species populations, but homozygous in the cultivated accessions.

It was difficult to interpret the zymogram patterns for PGI and GOT in terms of the number of gene loci involved. For PGI, there appears at least three gene loci (two for Zone I, andone for Zone II) for the cultivated accessions. In the two wild species, the number of loci appears more than the cultivated accessions. The banding pattern perhaps further suggests that the number of gene loci involved is more in *C. serrata* than in *C. senegalensis.* In GOT, there appears at least two gene loci for Zone I and one locus for Zone II, for both the cultivated accessions and the two wild species populations.

**Isozyme phenotypes in cultivated accessions vs. wild species**

The multiple forms of enzymes recorded in the seven enzyme systems studied arise due to the phenomenon of gene duplication. This is more intensity depicted in the case of PGI and GOT resulting in complex banding patterns. The excessive number of strongly staining bands for PGI, particularly in *C. serrata*, is indicative of more gene duplication in this primitive species. The greater number of bands makes the interpretation more difficult. In the presence of faint as well as strongly staining bands, it is difficult to know whether each product represents a different locus or is a result of intergenic interactions, or is just a 'ghost' band. The interpretation of such complex patterns is perhaps possible only after formal genetic analysis or progeny tests. Only an appropriate genetic analysis could allow the distinction to be made between different forms of enzymes that are allozymes or isozymes. This is particularly difficult in this case where these phenotypes are monomorphic.

Essentially, the cultivated accessions have been found uniformly monomorphic for all the gene loci involved in the seven isozyme systems studied. The gene loci for MDH and ME, and two fo the lci in PGD are apparently same in the cultivated species and its supposed progenitor *C. senegalensis*. The two gene loci of ADH in the cultivated species have also been found the same in *C. senegalensis* in the rare phenotype. In GOT, the cultivated species and *C. senegalensis* appear the same for the activity Zone II. In PGI, all the bands in activity Zone I occupy the same position in both the cultivated species and *C. senegalensis* although there are differences in the intensity of enzyme activity. The enzyme activity corresponding to Zone II in PGI is also partly similar in the cultivated species and *C. senegalensis*. On the other hand, the two wild species together express the same phenotype for the gene loci involved in MDH, IDH, PGD, ADH and ME. There is some similarity in the banding patterns between the two wild species for PGI and GOT also.

Thus, the overall picture indicates that while there have been some changes in the isozyme phenotypes during the process of domestication from the progenitor species, *C. senegalensis*, to its derivative domesticate, *C. tetragonoloba*, the indicated similarities in the two wild species suggest that the geographical separation of *C. serrata* in south-western Africa from the *C. senegalensis* of northern Africa has perhaps not brought about many differences at the level of isozymes.

The electrophoretic results in other diploid annual plant species related as progenitor and derivative have suggested that the genome of each derivative species was extracted from the repertoire of genetic polymorphisms already present in its progenitor (Gottlieb, 1981). Thus, the evolution of the cultivated *C. tetragonoloba* from *C. senegalensis* as a result of domestivation, and the differentiation between *C. senegalensis* and *C. serrata* as a result of geographical separation, is supported from the evidence of similarities in isozyme banding patterns. Minimal genetic divergence between two tomato species (Rick *et al.*, 1976) and between various animal species (Sene and Carson, 1977; Nevo and Cleve, 1978) in the electrophoretic data has also been reported. Gottlieb (1981) has indicated that it may be that differences between species do not involve electrophoretically detectable differences at large number of single genes, and Wilson *et al.* (1974) have postulated that the differences between species are rather the changes in regulatory sequences. Also the genus *Cyamopsis* is a self-compatible annual, and autogamy has often been associated with accelerated species formation in many annual groups (Grant, 1963). In at least one instance (*Tetramolopium*), speciation apparently has been rapid, with morphological divergence

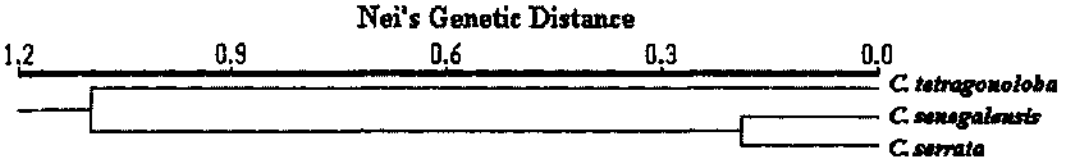


**Fig. 3. UPGMA dendrogram based on allozyme variations depicting the relationships among *C. tetragonoloba, C. serrata* and *C. senegalensis***

accompanying radiation into different habitats yet with little divergence at isozyme loci (Crawford, 1983). The case of *Cymaposis* appears the same.

**Relationships among *Cyamopsis* species**

The allozyme variation observed within and among the three *Cyamopsis* species as well as the UPGMA-dendrogram (Fig. 3) supports the earlier view that *C. tetragonoloba* is closer to *C. senegalensis* than to *C. serrata*. The genetic distance between *C. tetragonoloba* and *C. senegalensis* was 0.63 as against a distance of 1.56 between *C. tetragonoloba* and *C. serrata*. The zymogram presented in Fig 1 also substantiates this evidence. These findings are comparable to the earlier views (Hymowitz, 1972) based on the patterns of variations observed for morphological traits in *C. tetragonoloba, C. senegalensis* and *C. serrata*. However, in the present study, at these ten isozyme loci the cultivated species appear to have diverged from its putative ancestor species.

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