Indian J. Pl. Genet. Resources 4(2): 50-56, 1991

ISOZYME POLYMORPHISM AND GENETIC DIVERSITY IN GARLIC (*ALLIUM SATIVUM* L.) GERMPLASM

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*Twenty-three accessions of garlic (*Allium sativum *L.) collected from five diverse agro-climatic regions of India differing for various agro-morphological traits were analysed for isozyme polymorphism. Esterase, superoxide dismutase and catalase were the enzymes studied. Esterase and superoxide dismutase showed polymorphism to some extent. However, catalase was observed to be monomorphic. The study indicated that there was less genetic diversity among the garlic germplasm analysed. Broader groupings of the accessions was possible even though the enzyme systems studied were not ideal for 'fingerprinting' of germplasm. The implications of the findings with reference to genetic diversity in garlic germplasm and the usefulness of the isozyme technique for germplasm characterisation has been discussed.*

Garlic (*Allium sativum* L.) is an important spice and is widely cultivated in India and China. It is an essentially vegetatively propagating species. The source of genetic variation is obviously through somatic mutations, which may include both natural and induced types. Evolution of new cultivars in garlic has mainly been through selection and introduction from other agro-climatic regions. Therefore, the new cultivars are expected to differ from one another for few genes only. The present study was undertaken to evaluate the genetic diversity prevalent in the garlic accessions and to characterise the germplasm using the technique of electrophoresis of isozymes.

Isozyme electrophoresis has been applied in a number of crop species to evaluate the genetic diversity and to characterise the cultivars. Systematic and evolutionary studies were made in 29 genera using estimates of isozyme variation. Further, in 40 different genera, isozymes were used for cultivar identification (Simpson and Withers, 1986). Such

studies in *A. sativum* are very scanty, however studies on alcohol dehydrogenase, isocitrate dehydrogenase, phosphoglucomutase and phosphoglucoisomerase in 188 accessions in *A. cepa* and 29 in *A. fistulosum* have been reported. This underlines the need to survey isozyme variation in the germplasm collections so that the exact nature of cultivar diversification can be understood.

MATERIALS AND METHODS

Twenty-three accessions of garlic (*A. sativum* L.) from 750 germplasm collections held at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India were chosen for the study (Table 1). All

Table 1. Source of *A. sativum* accessions analysed

Accession Numbers	Place of Collection
IC 35280, IC42870, IC 48628, IC 48649, IC 48661, IC 49410	Maharashtra
IC 49343, IC 49350, IC 49351, IC 49356, IC 49358, IC 49359, IC 49371, IC 49373 IC 49400, IC 49405	Himachal Pradesh
IC 35231, IC 35334, IC 35341	Rajasthan
IC 19084	Orissa
IC 35315	Madhya Pradesh
IC 2053, IC 12370	Not known

accessions were being maintained by clonal multiplication and no heterogeneity within a collection was apparent. This was further evident from the analysis of individual plants within a collection. Hence, young leaves from a single plant per accession were considered for the present analysis.

The cloves of each accession were planted in pots filled with 1:1 mixture of compost and soil. First half emerged leaf at 30 days of sprouting was collected over ice and used for isozyme analysis. The leaves were first powdered after freezing in liquid nitrogen. The powdered sample before thawing was homogenised with acetone to remove chlorophyll and other secondary metabolites. The homogenate was filtered through Whatman No. 1 filter paper. The residue on the filter paper was air-dried and stored at -10°C till use. The samples were homogenised in prechilled pestle and mortar over ice in an extraction buffer comprising 0.1 M tris-Cl pH 7.4, 17 per cent sucrose and 0.1 per

cent each of ascorbic acid and cysteine-Cl. The sample to buffer ratio was maintained at 1:5 for all the samples. The homogenates were centrifuged at 12,000x g for 15 min. at 4°C in a refrigerated centrifuge and the clear supernatant was collected. The protein content of each sample was estimated by Folin-Ciocalteau reagent method (Lowry *et al.*, 1951). The protein content of the extracts was equalised by the addition of extraction buffer. The tracking dye, bromophenol blue (to a final concentration of 30% per cent) and sucrose (to a final concentration of 0.002%) were added to the extract and stored frozen till use. Anodic vertical slabs gel electrophoresis was performed on polyacrylamide gels according to Davis (1964). For each sample 60 µg protein equivalent was loaded on the gels. On completion of electrophoresis, the gels were incubated in the respective reaction mixtures of different enzymes. The enzyme localisation procedures were followed according to Brewbaker *et al.*, (1968) for esterase, Thorup *et al.* (1961) for catalase and Beauchamp and Fridovich (1971) for superoxide dismutase. The gels on optimum staining were fixed in 7 per cent acetic acid. The relative migration velocity (Rf) was calculated for each band and zymograms were constructed from the Rf values.

RESULTS AND DISCUSSION

The investigations compared a total of three gene loci (one for each enzyme). Since the total genome size in *Alliums* is very large, it is necessary to study more enzyme systems so as to compare the maximum possible amount of hereditary material. The present study adds to the genetic information available about the genus *Allium* in general and *A. sativum* specifically. The zymograms for esterase, catalase and superoxide dismutase drawn to scale with Rf values are depicted in figure 1.

Isozyme polymorphism

Garlic is an essentially vegetatively propagated species. Only one report was sited on collection of fertile flowering garlic types from the former Soviet Union (Kotlinska *et al.*, 1990). Therefore, the main source of genetic variation available in garlic has so far been by accumulation of natural mutations. The rate of somatic mutations in nature is less than 0.01 per cent. The propagation of mutations expressing in the form of changed morphological traits and/ or biochemical parameters would further be reduced. Hence, the proportion of detectable variations in such obligate vegetatively propagated crops like garlic, where the chances of diversification through natural/artificial hybridization and recombination is less, would naturally be limited.

The lack of substantial polymorphism for allo/iso-enzymes of esterase, catalase and superoxide dismutase among the twenty-three acces-

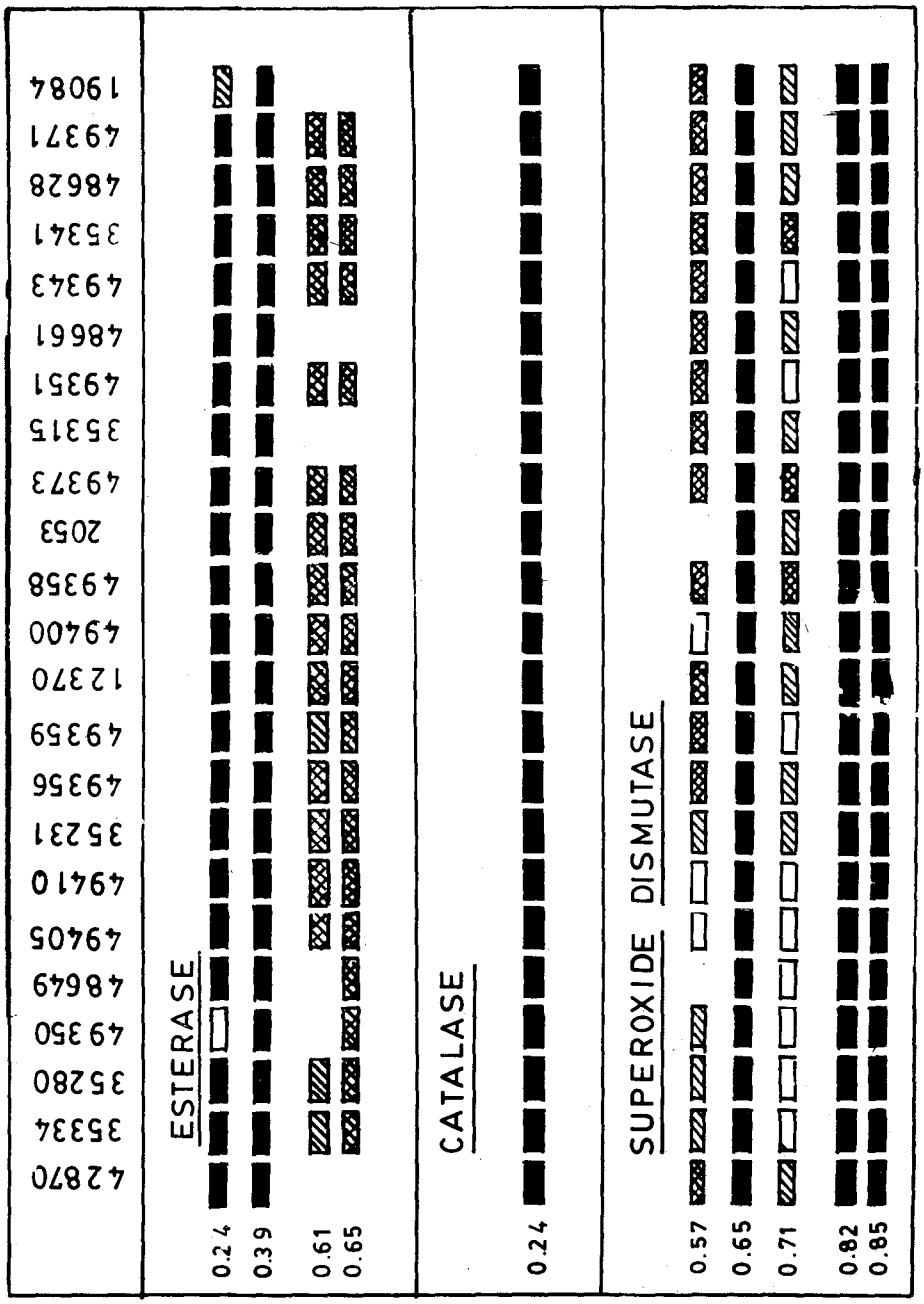

Fig. 1. Profiles of Isozymes in the garlic accessions.

sions of garlic are evident from Fig.1. Catalase was found to be monomorphic as only one band was observed in all the accessions. On the contrary, polymorphism was observed for the other two enzymes esterase and superoxide dismutase.

For esterases, differences were noticed for the fast migrating bands with Rfs 0.61 and 0.65. The slow moving bands at Rfs 0.24 and 0.39 were monomorphic and are probably two different isozymic forms. But the two fast migrating bands possibly represent the allelic forms of the third isozymic form of esterase. In the present study, a total of three isozyme phenotypes for esterases were observed. However, there were pronounced differences in the intensity of fast moving bands. These might be useful for germplasm characterisation as there was consistency in the results. In case of superoxide dismutase, polymorphism was prevalent only for the slowest migrating band (Rf 0.57). Further, differences among the accessions in the intensity were noticed for the band at Rf 0.71. The remaining three bands (Rfs 0.65, 0.82 and 0.85) did not differ even for intensity of staining in any of the accessions.

The present results are interesting considering the lack of variation for IDH and PGI among 1293 plants of *A. cepa* and *A. fistulosum* (Peffley and Orozco-Castillo, 1987). Further, in *A.cepa*, an open pollinating seed propagated species, no variation was observed for PGM by these workers in contrast to *A. fistulosum* where polymorphism for PGM was reported. This investigation stresses the fact that lack of variations for two or more enzyme systems do not necessarily mean lack of genetic differences between the accessions studied. On the other hand, it calls fo studying different sets of isozymes so that extent of similarities and differences between the genotypes compared, can be estimated. Germplasm characterisation is, therefore, a continuous process whereby it is essential to utilize all the available techniques so that maximum amount of total genomic DNA can be sampled.

Study of isozyme polymorphism has been used as a technique to determine genetic diversity in crop plants such as barley (Clegg *et al.*, 1984; Jana and Pietrzak, 1988), pearl millet (Gepts and Clegg, 1989) and common beans (*Phaseolus vulgaris* L.) (Singh *et al.*, 1991). Extensive studies of polymorphism in isozymes give reliable estimates of genetic relationship among the genotypes compared. Another important application of isozyme studies is in cultivar identification. For unambiguous identification of cultivars, it is essential to use as many enzyme systems as possible. The data on enzyme systems which are monomorphic in a given set of cultivars is also essential. Considering these factors as requirements for identification of cultivars, a system has been developed which helps in classifying the cultivars in case the extent of isozyme limited polymorphism prevalent is lesser. This study indicated that

polymorphism prevalent for the isozymes analysed. Although it is not possible to differentiate cultivars with the prevalence of limited polymorphism, the study would be useful for broader classification of the collections based on the similarities. It was not possible to clearly identify any of the 23 accessions of garlic analysed based on only one isozyme pattern. However, when both esterase and SOD profiles were considered, three collections, namely, IC 49350, IC 48649 and IC 2053 could be distinguished from others. For SOD, the accessions IC 48649 and IC 2053 had similar isozyme profiles but for IC 49350 the pattern was different. On the other hand for esterases, the banding pattern was different for IC 48649 and IC 2053 while IC 49350 and IC 48649 had same pattern. This combination of enzyme bands was not found in any of the other accessions. In case of SOD, polymorphism was noticed only for slowest migrating band (Rf 0.57). A total of two isozyme phenotypes were present for SOD among the 23 accessions. The other four bands (Rfs 0.65, 0.71, 0.82 and 0.85) were monomorphic in the materials studied. However, differences in the intensity of staining were present for the bands at Rf 0.57 and 0.71.

The remaining 20 accessions could be classified into two groups. The first group included four accessions, namely, IC42870, IC 35315, IC 48661 and IC 19084 and 16 remaining accessions were in the second group as they had similar patterns for esterase and SOD. However, when differences in the intensity of bands were considered, the second group could be subdivided into four groups (Table 2). The accession IC 494000 of the first sub group was seen to be different from others as the first SOD band at Rf 0.57 was very faint. The number of accessions in other sub groups are mentioned in Table 2.

A comparison of the accessions under each group with their place of collection indicated that all the ten accessions from Himachal Pradesh

Table 2. Classification of 23 garlic accessions based on the banding pattern for esterases and superoxide dismutases

Group 1	**Group 5**	IC49343
IC49350	Sub group 1	Sub group 4
Group 2	IC 49400	IC 12370
IC 48649	Sub group 2	IC 35231
Group 3	IC 49405	IC 35341
IC 2053	IC 49410	IC 48628
Group 4	Sub group 3	IC 49356
IC 42870	IC 35334	IC 49358
IC 35315	IC 35280	IC 49371
IC 48661	IC 49359	IC 49373
IC 19084	IC 49351	

and three from Rajasthan were in group V. Even though, further classification of these collections based on the intensities of each band was attempted, barring in few cases like IC 49400 and IC 49405 where substantial differences existed in stainability of one or more bands no unambiguous identification of accessions was possible. It is evident that the accession from Maharashtra were more diverse compared to accessions from other states. These results probably indicate large number of duplicates in germplasm collections at NBPGR.

ACKNOWLEDGEMENTS

The authors acknowledge Sh. T.A. Thomas, ex Principal Scientist, National Bureau of Plant Genetic Resources, New Delhi, for the garlic accessions supplied and Dr.R.S. Rana, Director, NBPGR, New Delhi for the facilities provided. This project was financed by the Department of Biotechnology, Government of India.

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