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Seed proteometrics studies on *capsicum*

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ABSTRACT

The present study was aimed to characterize the fourteen *Capsicum* species using SDS- PAGE seed protein profile. The SDS- PAGE profile revealed the nine region of activity in the electrophoresis system. Each and every region showed genetic similarity and variations between the selected fourteen species and cultivars of *Capsicim*. A total of 153 bands were obtained in nine active regions of the SDS-PAGE profile.

INTRODUCTION

Nature has myriads of life forms on this planet among which variation are of ubiquitous occurrence. In the wilderness of the tropics, plants grow in extreme situations along longitudinal, latitudinal and temperature gradients and therefore variations with in and between populations of a species are not uncommon. Plant biologists use morphological characters of plants which can be compared, measured counted and described to assess the differences or similarities in plant taxa, and use these characters for plant identification, classification and descriptions. When characters are used in descriptions or for identification they are called diagnostic or key characters which can be either qualitative or quantitative. Plants exhibit natural variation in their form and structure. While all organisms vary from individual to individual, Plants exhibit an additional of variation. With in a single individual, parts are repeated which may differ in form and structure from other similar parts. Traditionally, genetic diversity is assessed based on morphological features such as plant height, reproductive features, day length sensitivity, local adaptation etc, though such, characters exhibit enormous variation for the particular use of the crop. Plants exhibit natural variation in their form and structure. While all organisms vary from individual to individual, Plants exhibit an additional of variation. With in a single individual, parts are repeated which may differ in form and structure from other similar parts.

A wide spectrum of simple and overlapping variations is now documented in plants [1,2,3,4,5,6]. Genetic variation is a prerequisite for any crop improvement programme. Assessment of the extent and distribution of genetic variation in a crop species and its relatives is essential in understanding pattern of diversity and evolutionary relationships between accessions that help to sample genetic resources in a more systematic fashion for conservation and plant improvement.

In recent years, limitations of numerical taxonomy viz. morphology, anatomy and cytology have been over come by biochemical and molecular markers. Among the different modern biochemical and molecular markers, some are relatively cheaper (Protein (SDS-PAGE) and Isozymes (PAGE) are simple to use in a variety of applications in plant research. The information on polymorphism using protein and isozyme profiles in a set of genotypes is useful in tagging genes of interest and genetic mapping in long run to facilitate marker assisted selection. The genus *Capsicum* is a member of the solanaceae family. The genus *Capsicum* consists of approximately 22 wild species and five domesticated species[7]. The present study was intended to characterize the chellies using the seed protein profiles and isozyme profiles as a marker. In addition the present study may be useful to find out the evolutionary lineages, genetic similarity between the species and cultivars.

MATERIALS AND METHODS

Plants of *Capsicum frutescens* var. Fasciculatum (L_1), *Capsicum breviatum* (L_2), *Capsicum pubescens* (L_3), *Capsicum frutescens longum* var. Conides (L_4), *Capsicum baccatum* (L_5), *Capsicum frutescens longum* var. cerasiforme (L_6), *Capsicum frutescens longum* var. baccatum (L_7), *Capsicum frutescens longum* Var abbreviatum (L_8), *Capsicum chinense* (L_9), *Capsicum frutescens longum* (L_{10}), *Capsicum baccatum* var. Pendulum (L_{11}) , *Capsicum baccatum* var. melegueta (L_{12}), *Capsicum baccatum* var. microcarpum (L_{13}) and *Capsicum annuum* (L_{14}), were collected from the Athmanilayam nursery garden, Marthandam, Kanayakumari District. For protein isoenzyme analysis, the fresh young leaves were harvested from the mother plants and washed once in de-ionized water and mashed in a pre-chilled mortar with 500µl of phosphate buffer (pH 7.0). The resultant slurry was centrifuged at 10000 rpm for 10min at 4^0C in a Mikro 22 R centrifuge and the supernatant was stored at -70^0C before use. SDS - PAGE was carried out for proteins and PAGE was carried out for isoperoxidase analysis. Both electrophoresis and staining were followed by Manickam and Sadasivam[8] methods. After electrophoresis (PAGE), the gel was observed using a Vilber Loubermat gel documentation system (Germany) and banding profiles of protein and isoenzyme of *Capsicum* cultivars compared by Biogene software analysis (Germany). The similarity and variation between the cultivars were estimated by Biogene software analysis and the dendrograms were documented.

RESULTS AND DISCUSSION

Multiple regions of activity were obtained for protein electrophoretic system P_1 to P_9. Region 1 contained twelve bands (**Figure** 1) P_1^5 (0.044) was showed by L_1-Capsicum frutescent var. fasciculatum and (L_2)-Capsicum frutescens abbreviatum; P_1^8 (0.069) was shared their presence commonly in L_4- Capsicum frutescens longum var.conides, (L_6) - Capsicum frutescens longum var. cerasiforme, and Capsicum chinense (L_9). P_1^9 was showed its presence and similarity in Capsicum frutescens abbreviatum, Capsicum pubescens (L_3) and Capsicum frutescens longum var. conides. P_1^1 (0.016) was showed its unique present in Capsicum baccatum var. microcarpum (L13). P_1^2 (0.019) was restricted to Capsicum frutescens longum var. conides (L_4); P_1^3 (0.034) was showed its presence only in Capsicum pubescens (L_3). P_1^4 (0.041) was unique to Capsicum frutescens longum var. cerasiforme

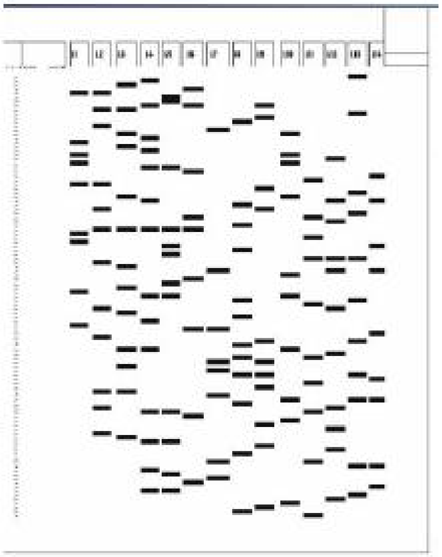

Figure 1: SDS-page protein profiles of fourteen cultivars of *Capsicum*: L_1 - *Capsicum frutescens* var. Fasciculatum ; L_2 - *Capsicum breviatum* ; L_3 - *Capsicum pubescens*; L_4 - *Capsicum frutescens longum* var. Conides; L_5 - *Capsicum baccatum*; L_6 - *Capsicum frutescens longum* var. Cerasiforme; L_7 - *Capsicum frutescens longum* var. Baccatum; L_8 - *Capsicum frutescens longum* Var abbreviatum; L_9 - *Capsicum chinense*; L_{10} - *Capsicum frutescens longum*; L_{11} - *Capsicum baccatum* var. Pendulum; L_{12} - *Capsicum baccatum* var. Melegueta; L_{13} - *Capsicum baccatum* var. Microcarpum; L_{14} - *Capsicum annuum*

(L_6); P_1^6 (0.047) and P_1^7 (0.066) were restricted to Capsicum baccatum.(L_5); P_1^{10} was present only in Capsicum baccatum.Var. microcarpum (L_{13}); P_1^{11} showed its expression only in Capsicum chinense (L_9) and P_1^{12} (0.100) was restricted to Capsicum frutescens longum var. abbreviatum (L_8).

Region 2 showed 15 bands (Figure 1) in different positions P_2^3 was showed its presence jointly in *Capsicum, pubescens* (L_3) and *Capsicum frutescens longum* (L_{10}); P_2^8 (0.140) and P_2^{10}(0.165) were showed their presence and similarity between *Capsicum frutescens var. fasciculatum* (L_1) and *Capsicum frutescens longum* (L_{10}); P_2^{11} (0.l72) was showed by *Capsicum frutescens longum var. conides* (L_4) and *Capsicum baccatum (*L_5) and P_2^{15} was showed its presence and similarity between *Capsicum frutescens var. fasciculatum* (L_1) and *Capsicum frutescens abbreviatum* (L_2).Band P_2^5(0.125) was restricted to *Capsicum frutescens var. fasciculatum* (L_1); P_2^1 (0.110) was showed its presence only in *Capsicum frutescens abbreviatum* (L2); P_2^6 (0.135) was unique to *Capsicum pubescens* (L_3). P_2^4 (0.122) and P_2^7 (0.138) for *Capsicum frutescens longum var. conides* (L_4). P_2^{12} (0.176) was restricted to *Capsicum frutescens longum var. cerasiforme* (L_6); P_2^{13} (0.190) was showed its unique presence in *Capsicum annum*; P_2^{14} (0.190) was showed its expression only in *Capsicum* baccatum var. pendulam (L_{11}). *Capsicum frutescens longum var. abbreviatum* (L_8) and *Capsicum chinense* (L_9) were failed to express in this region.

Region 3 illustrated sixteen different positions in the banding profile (Figure1). P_3^4 (0.216) observed in *Capsicum frutescens longum var. conides* (L4), *Capicum baccatum var. melegneta* (L_{12}) and *Capsicum annuum* (L_{14}) P_3^{11} (0.251) was showed its presence in *Capsicum frutescens abbreviatum* (L_2), *Capsicum pubescens* (L_3), *Capsicum frutescens longum var. conides* (L_4), *Capsicum baccatum* (L_5) and *Capsicum frutescens longum var. cerasiforme* (L_6) and expressed the similarity linkage between the cultivars P_3^3 (0.212) was shared by *Capsicum* pubescens (L_3) and *Capsicum frutescens longum* (L_{10}). P_3^6 (0.224) was obtained in *Capsicum frutescens abbreviatum* (L_2) and *Capsicum chinense* (L9); P_3^8 (0.234) was expressed jointly in *Capsicum frutescens longum var. cerasiforme* (L_6) and *Capsicum baccatum var. pendulum* (L_{11}) and P_3^{15}(0.292) was showed its presence in *Capsicum baccatum* (L_5) and *Capsicum annuum* (L_{14}). P_3^1 (0.202) was showed its expression only in *Capsicum chinense*, P_3^2 (0.209) and P_3^7 (0.227) were restricted to *Capsicum baccatum* var. microcarpum (L_{13}). P_3^6 (0.224), P_3^{10} (0.249) and P_3^{16} (0.299) were present only in *Capsicum frutescens longum var. abbreviatum* (L_8); P_3 (0.240) was obtained only in *Capsicum baccatum var. melegnueta* (L_{12}). *Capsicum frutescens var. fasciculatum* (L_1) showed its unique expression in P_3^{12} and P_3^{14} and their Rf values respectively 0.266 and 0.285. P_3^{13} was restricted to *Capsicum baccatum var. pendulum* (L_{11}).

Region 4 contained eighteen bands in different position. P_4^2 (0.305) showed its presence jointly in *Capsicum baccatum var.pendulum* (L_{11}), *Capsicum baccatum var. melegneta* (L_{12}) and *Capsicum baccatum var. microcarpum* (L_{13}). Similar to that P_4^5 (0.320) was shared by *Capsicum frutescens longum var. baccatum* (L_7*), Capsicum baccatum. var. melegueta* (L_{12}) and *Capsicum annuum* (L_{14}). Band P_4^{11} (0.368) was observed in *Capsicum frutescens longun var. conides* (L_4), *Capsicum baccatum and Capsicum frutescens longum* (L_{10}) P_4^{12} (0.377) was showed by *Capsicum frutescens longum var-abbreviatum* (L_8) and *Capsicum baccatum var. microcarpum* (L_{13}); P_4^{14} was showed its presence commonly in *Capsicum frutescens abbreviatum* (L_2). *Capsicum baccatum. var.melegueta* (L_{12}).B and P_4^1 (0.301) and P_4^8 (0.348) were present only in *Capsicum baccatum* (L_5); P_4^3 (0.307) was restricted to *Capsicum frutescens abbreviatum* (L_2); P_4^4 (0.310), P_4^9 (0.351) and P_4^{15}(0.386) were showed their presence and expressed the identity for *Capsicum pubescens* (L_3). P_4^6(0.327) was its unique presence in *Capsicum frutencens longum* (L_{10}); P_4^7 (0.329) was restricted to *Capsicum frutescens longum var. cerasiforme* (L_6); P_4^{10} (0.357) and P_4^{18} (0.398) were showed their presence only in *Capsicum frutescens var. fasciculatum* (L_1). P_4^{13} was present only in *Capsicum baccatum var. pendulum* (L_{11}); P_4^{16} (0.389) showed its unique presence in *Capsicum frutescens longum var. abbreviatum* (L_8) and P_4^{17} was demonstrated its expression in *Capsicum frutescens longum var. conides* (L_4).

Region 5 obtained 12 bands in different positions (Figure1). P_5^6 (0.436) was showed its presence and expressed the similarity between *Capsicum pubescens* (L_3) *Capsicum frutescens longum var. conides*(L_4) and *Capicum frutescens longum* (L_{10}). P_5^{12} (0.490) also showed the similarity between *Capsicum frutescens longum var abbreviatum* (L_8). *Capsicum chinense* (L_9) and *Capsicum baccatume var. microcarpum* (L_{13}); P_5^1 (0.404) was showed by *Capsicum frutescens longum var. cerasiforme* (L_6) and *Capsicum frutescens longum var. baccatum* (L_7); P_5^4 (0.427) was showed its jointly presence in *Capsicum chinense* (L_9) and *Capsicum baccatum var. microcarpum* (L_{13}); P_5^8 (0.449) was expressed commonly in *Capsicum frutescens longum var. abbreviatum* (L_8) and *Capsicum baccatum var. pendulum* (L_{11}) and P_5^9 (0.452) was obtained in *Capsicum frutescens longum var. baccatum* (L7) and *Capsicum chinense* (L_9). P_5^2 (0.408) was restricted to *Capsicum annuum* (L_{14}). P_5^3 (0.417) was present only in *Capsicum frutescens abbreviatum* (L_2). P_5^5 (0.433) was expressed only in *Capsicum frutescens longum var. abbreviatum*. P_5^7 (0.442) was showed its unique presence in *Capsicum baccatum var. Melegueta*. P_5^{10} (0.464) was obtained only in *Capsicum pubescens* (L_3) and P_5^{11} (0.470) was restricted to *Capsicum frutescens longum var. baccatum* (L_7).

Region 6 showed 18 bands in different positions (Figure1). P_6^6 (0.536) was illustrated in *Capsicum frutescens longum* (L_{10}), *Capsicum baccatum var. microcarpum* (L_{13}) and *Capsicum annuum* (L_{14}) and P_6^9 (0.548) was expressed in *Capsicum frutescens longum var. conides* (L_4), *Capsicum baccatum* (L_5) and *Capsicum baccatum var. pendulum* (L_{11}) and demonstrated the similarity and relationships between cultivars. P_6^4 (0.520) showed the similarity between *Capsicum frutescens abbreviatum* (L_2) and *Capsicum frutescens longum* (L_3); P_6^8 (0.542) was obtained in *Capsicum* frutescens abbreviatum (L_2) and *Capsicum baccatum var. melegueta* (L_{12}) and P_6^{16} (0.571) showed its jointly presence in *Capsicum frutescens longum var. conides* (L_4) and *Capsicum baccatum*. P_6^1 (0.502) was restricted its expression only in *Capsicum annuum* (L_{14}); P_6^2 (0.505) was showed its unique presence in *Capsicum baccatum var. pendulum* (L_{11});P_6^3 (0.514) was present only in *Capsicum chinense* (L_9). P_6^5 (0.527) was obtained only in *Capsicum frutescens longum var. baccatum* (L_7); P_6^7 (0.539) was illustrated only in *Capsicum frutescens longum var. abbreviatum* (L_8); P_6^{10} (0.552) was restricted to *Capsicum frutescens longum var. cerasiforme* (L_6); P_6^{11}(0.555) was showed unique presence in *Capsicum frutescens longum* (L_{10}); P_6^{12} (0.558) and $P6^{17}$(0.589) present only in *Capsicum chinense* (L_9). Similar to that P_6^{13} (0.561) and P_6^{18} (0.592) showed their presence only in *Capsicum pubescens* (L_3).

Region 7 contains only four bands (Figure 1) P_7^2 (0.617) was showed by *Capsicum frutescens longum* (L_{10}) and *Capsicum baccatum var. microcarpum* (L_{13}); P_7^3 (0.621) was showed its presence in *Capsicum frutescens longum var. baccatum* (L_7) and *Capsicum baccatum var. pendulum* (L_{11}); P_7^4 (0.673) was present in *Capsicum baccatum var. microcarpum* L_{13}) *and Capsicum annum* (L_{14}). And P_7^1 (0.614) was restricted to *Capsicum frutescens longum var. abbreviatum* (L_8). Region 7 reveated the relationship between cultivars *Capsicum frutescens longum* (L_{10}) and *Capsicum baccatum var. microcarpum* (L_{13}); *Capsicum frutescens longum var baccatum* (L_7) and *Capsicum baccatum var. pendulum*(L_{11}) and *Capsicum baccatum var. microcarpum* (L_{13}) *Capsicum annuum* (L_{14}).

Region 8 showed Nine bands in different positions (Figure1), P_8^6 (0.771) was expressed in *Capsicum frutescans longum var. conides* (L_4) and *Capsicum baccatum*(L_5). P_8^1 (0.721) was specific to *Capsicum frutescens longum var. conides* (L_4); P_8^2 (0.724) was unique to *Capsicum baccatum* (L_5); P_8^3 (0.740) for *Capsicum frutescens longum var. baccatum* (L_2) P_8^4 *(0.746) for Capsicum frutescens longum var. cerasiforme* (L_6); P_8^5 (0.757) for *Capsicum annuum* (L_{14}); P_8^7 (0.788) for *Capsicum baccatum var. microcarpum* (L_{13}) and P_8^9 (0.791) for *Capsicum baccatum var. melegneta* ($_{L12)}$. Other cultivars to failed to express in this regions.

Region 9 illustrated with four bands in different positions (Figure1). They failed to express the similarity between the cultivars. P_9^1 (0.801) was showed its restricted expression in *Capsicum frutescens var. fasciculatum* (L_1); P_9^2 (0.804) demonstrated its unique presence in *Capsicum chinense* (L_9). P_9^3 (0.819) was present only in *Capsicum* frutescens longum var. abbreviatum (L_8) and p_9^4 (0.822) was showed only in *Capsicum* baccatum var. pendulum (L_{11}).

Isozymes

A zone of activity was observed in isoperoxidase enzyme system. Region one contained a single band (PRX1^1) whose position did not vary in any of the cultivars used in this system (Figure 2). With reference to the morphological characters, protein profile and isozyme analysis the variability among the fourteen cultivars of *Capsicum* (Figure 2). The present study revealed that, the selected fourteen cultivars were easily separable / distinguishable by SDS-PAGE protein pattern. Protein markers are practical, useful genetic and biochemical markers as well as good estimators of genetic variability in plant populations (Hamrick et al., 1997). The present study also coincided with this, the presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. Protein and isozymes (esterase, peroxidase) has been utilized to find the genetic line age of different plants and crops[9,10,11,12,13,14]. The present study clearly indicated that protein markers could be effectively used for genetic diversity studies among *Capsicum* cultivars. The results obtained suggested that by using protein markers the newly evolved *Capsicum* cultivar can be easily differentiated from the other varieties. Protein diversity among these variants in terms of similarity indicates may be useful in identifying diverse cross combinations for deriving hybrids of *Capsicum*. The cladogram of *Capsicum* revealed the genetic similarity and variation among the selected cultivars (Figure 3). The cladogram placed few cultivars close to each other depicting their genetic relatedness, since they were developed from a single parent, *Capsicum frutescent* var. *fasciculatum*(L_1), *Capsicum frutescens abbreviatum* (L_2) and *Capsicum frutescens longum* (L_3) and *Capsicum frutescens longum* (L_{10})) were confirmed to one group of cluster (C1). The other cultivars *Capsicum frutescans longum var. conides* (L_4), *Capsicum baccatum* (L_5), *Capsicum frutescens longum var. cerasiforme* (L_6), *Capsicum frutescens longum var baccatum* (L_7), *Capsicum frutescens longum* var. abbreviatum (L_8), *Capsicum chinense* (L_9), *Capsicum* baccatum var. pendulum (L_{11}), *Capsicum baccatum var. melegneta* (L_{12}), *Capsicum baccatum var. microcarpum* (L_{13}) and *Capsicum annuum* (L_{14}) were confirmed to Cluster 2 (C2). In cluster 2, some cultivars were diverged to two major branches. Both branches of cluster 2 further diverged from their origin

ISOZYME protein profile of selected taxa of *Capsisum* (seed)

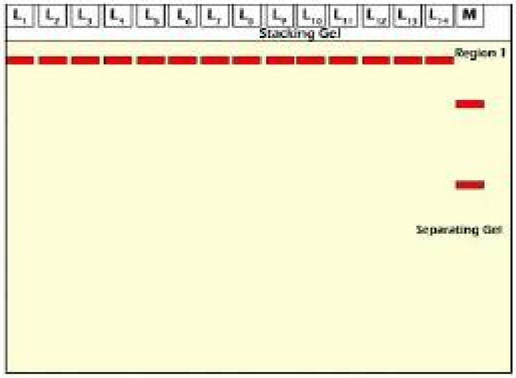

Figure 2: Isoperoxidase profiles of fourteen cultivars of *Capsicum*: L_1 - *Capsicum frutescens* var. Fasciculatum ;L_2 - *Capsicum breviatum*; L_3 - *Capsicum pubescens*; L_4 - *Capsicum frutescens longum* var. Conides; L_5 - *Capsicum baccatum*; L_6 - *Capsicum frutescens longum* var. Cerasiforme; L_7 - *Capsicum frutescens longum* var. Baccatum; L_8 - *Capsicum frutescens longum* Var abbreviatum; L_9 - *Capsicum chinense*; L_{10} - *Capsicum frutescens longum*; L_{11} - *Capsicum baccatum* var. Pendulum; L_{12} - *Capsicum baccatum* var. Melegueta; L_{13} - *Capsicum baccatum* var. microcarpum; L_{14} - *Capsicum annuum*

Dendrogram Homology Coefficient %: 0.0(UPGMA)

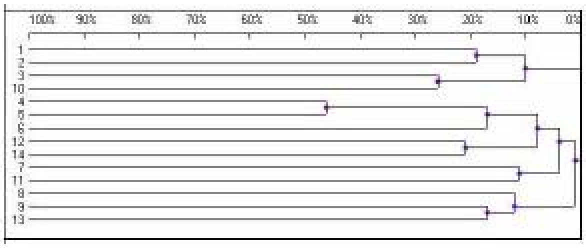

Figure 3: SDS-page cardiograms of fourteen cultivars of *Capsicum*: L_1 - *Capsicum frutescens* var. Fasciculatum; L_2 - *Capsicum breviatum* ; L_3 - *Capsicum pubescens*; L_4 - *Capsicum frutescens longum* var. Conides; L_5 - *Capsicum baccatum* ; L_6 - *Capsicum frutescens longum* var. Cerasiforme; L_7 - *Capsicum frutescens longum* var. Baccatum; L_8 - *Capsicum frutescens longum* Var abbreviatum; L_9 - *Capsicum chinense* ; L_{10} - *Capsicum frutescens longum*; L_{11} - *Capsicum baccatum* var. Pendulum; L_{12} - *Capsicum baccatum* var. Melegueta; L_{13} - *Capsicum baccatum* var. Microcarpum; L_{14} - *Capsicum annuum*

node, which may be due to induced chromosomal aberrations under stress conditions. The small genetic variation between the cultivars could be attributed to polyploidy and the highly heterozygous nature of *Capsicum*. The protein polymorphism in *Capsicum* cultivars shows that this gene pool is still a good resource for breeding. The protein profiling of *Capsicum* has the utility in *Capsicum* breeding programme for selecting the desirable cultivars. This can identify the cultivar variation which can be used for identifying diverse lines for use as parents in further studies. These results also support the classification which is based on seed colour, shape, size and morphological characters of the selected cultivars of *Capsicum*.

REFERENCES

[1] S.Sen, Sharma; Nucleus, **333**, 4-10 **(1990)**.

[2] A.G.Connolly, I.D.Godwin, M.Cooper, I.H.Delacy; Theor.App.Genet., **88**, 332-336 **(1994)**.

[3] C.N.Stewart (Jr.), D.M.Porter; Biological Conservation, **74**, 135-142 **(1994)**.

[4] T.Demeke, D.R.Lynch, L.M.Kawchuk, G.C.Kozub, J.D.Armstrong; Plant Cell Reports, **15**, 662-667 **(1996)**.

[5] G.Sonnante, A.Spinosa, A.Marangi, D.Pignone; Annals of Botany, **80**, 741-746 **(1997)**.

[6] E.A.James, G.R.Ashburner; Biological Conservation, **82**, 253-261 **(1997)**.

[7] P.W.G.Bosland; 'Chillies: History, Cultivation, Anduses', Elsevier Publ., New York, 347-366 **(1994)**.

[8] Manickam, Sadassivam, J.L.Ha,rick, M.J.W.Godt, D.A.Murawski, M.D.Loveless, D.A.Falk, K.E. Hosinger; 'Genetics and Conservation of Rare Plants', Oxford University Press, New York, 75-86 **(1997)**.

[9] W.J.Yu; Zuower Pinshong Ziyuan, **1**, 35-36 **(1987)**.

[10] H.Yanunong, S.Xinili, W.Xiangkun; Agric.Arch., **24**, 2-8 **(1984)**.

[11] H.S.Suh, Y.I.Sata, H.Morishma; Thero.Appl.Genet., **94**, 316-321 **(1997)**.

[12] K.N.Srivasatava, M.Rai, R.S.Tyagi, G.Kaur; Ind.J. Plant Physiol., **7**, 227-233 **(2002)**.

[13] W.Taderse, E.Bekele; Lathyrus Lathyrism Newsletter, **2**, 43-46 **(2001)**.

[14] A.N.Onus, B.Pickergill; Turk.J.Bot., **24**, 311-318 **(2000)**.