GENETIC DIVERSITY ANALYSIS OF CHICK PEA (*Cicer arietinum* L.) USING SEED PROTEIN PROFILING

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Abstract: From ancient time onward chickpea (*Cicer arietinum*) is most important source of nutrients in the human diet. Along with the nutritional importance it possesses many medicinal values. It is very much essential to improve the quality of chickpea, an economic important crop, hereby in this study a biochemical approach had been employed to determine taxonomic and evolutionary information of chickpea. Forty Five accession of *Cicer arietinum* were taken for the study of genetic variation within intragenic varieties based on their seed protein using SDS PAGE. A total of 20 and 16 distinct bands were detected for albumin and globulin protein respectively, from the obtained band pattern a binary matrix was created and subjected to combined cluster analysis of albumin and globulin protein using UPGMA method. Based on analysis of cluster, FLIP-90-160 showed distinct relation from other cultivars. Present study concluded both globulin and albumin protein possess a great potentiality for representing as a genetic variation which could be useful for phylogenetic studies and crop improvement.

Key words: Chickpea; UPGMA; SDS PAGE; Jaccard's similarity coefficients.

1. Introduction:

Legumes are one of the most important and a protein rich dietary source of human life. They are good source of food and fodder and play a vital role in nitrogen fixation [1]. India is the largest producer of *Cicer arietinum* which is commonly known as chickpea and Bengal gram. It is a member of family Fabaceae, and an ancient self-pollinated leguminous crop, diploid annual (2N=16 chromosomes) grown since 7000BC, in different area of the world but its cultivation is mainly concentrated in semi-arid environments. As a legume crop chickpea drives 70% nitrogen through symbiotic nitrogen fixation which makes it an acclaimed crop for crop rotation. *Cicer arietinum* is a useful crop consumed all over the world and a very good source of carbohydrate, proteins and all essential amino acid except sulphur containing amino acid, unsaturated fatty acid such as linoleic and oleic acid, vitamins such as riboflavin, niacin, thiamin, folate, vitamin A, sterols such as β-Sitosterol, campersterol and stimasterol and many macro and microelement which includes Mg, P, Ca, K etc [2]. Several countries use *Cicer arietinum* as a traditional medicine for the treatment of type II diabetes, digestive disease and carcinomas. Study of genetic diversity of plants helps to improve its efficiency which eventually enhances food production and thus the increasing demand of *Cicer arietinum* can be accomplished [3]. Extensive studies have shown that through germplasm profiling, modifications can be done in this economically important plant to enhance its nutritional value consequently crop improvement [4]. Protein finger printing is a highly efficient and informative way for characterizing diversity among cultivars. Protein markers can assist in resolving genetically diverse classes and their evolutionary relationship with wild relatives. Various results were put forward for the study of germplasm based on profiling of the seed protein to determine the taxonomic and evolutionary aspects of several crop plants [5&6]. Study of genetic variation within intragenic and intergenic species, study of genomic relationships, genetic resource conservation and crop improvement are the some objectives can be accomplished by seed protein profiling. Seed protein are not much affected by environmental factors, hence it is the most robust approach for studying germplasm [7,8,9,10]. For the present biochemical study, appropriate isolation of seed protein is the essential step for getting distinct band pattern [11]. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis is the most simple, economically and widely used technique for

protein profiling [12]. The electrophoresis of seed protein is widely employed to investigate genetic variation and classify plant varieties; presence of isozymes in protein content of the cultivars provides rapid and reliable means for producing protein fingerprints [13, 14]. The aim of the present investigation is to study the genetic diversity based on seed protein profiling.

2. Material Methods:

2.1 Isolation of protein

This study was carried out with 45 Accessions of *Cicer arietinum* (Table 1) procured from, Division of Genetics, Indian Agriculture Research Institute (IARI), Pusa, New Delhi.

For isolation of albumin 0.3 g of seeds were crushed and grinded using mortar and pestle and homogenized in 3 ml of chilled distilled water and mixed thoroughly, followed by 45 min incubation at 0 °C. This above step was repeated 4 times and further sample were centrifuged at 3500 rpm for 15 min. Now the obtained supernatant was separated which contains albumin.

For isolation of Globulin 500 µl of chilled water was added to the pellet obtained during isolation of albumin and centrifuged at 3500 rpm for 10 min. In the pellet portion, 1 ml of 0.5 ml Nacl was added and vortex followed by incubation at 0 °C for 45 min. Now the samples were subjected to centrifugation at 3500 rpm, resulting supernatant was collected in a separate centrifuge tube which contains globulin protein.

2.2 SDS PAGE

All 45 samples of *Cicer arietinum* having albumin and globulin protein were quantified using Bradford method [15] and a uniform 8 µg protein sample with 6 µl of Unstained Protein Molecular Weight Marker: Fermentas Cat No: SM0431 were loaded in the wells of SDS polyacrylamide gel comprising of 4% stacking gel and 12.5% separating gel. The running condition was optimized for better resolution in which equal concentration of proteins was loaded in each well minimizing the time lapse between loading and then a constant 30 A (Amphere) of current was applied for first 30 min followed by 35 A till the Bromophenol Blue reaches the bottom of the gel. After electrophoresis, the gels were stained with 1% commassie blue solution for three hours and destained by solution containing 10% (v/v) acetic acid, 40% (v/v) methanol and distilled water in ratio of 10:40:50 respectively.

2.3 Percent polymorphism:

Percent polymorphism study was carried out for both albumin and globulin protein for which gel was divided into four zone and occurrence of polymorphic bands were calculated with respect to total number of bands (Table 2 &3).

2.4 Construction of Dendrogram:

The data were analyzed in NTSYS-PC software (version 2.1b). Utilizing binary data generated by albumin and globulin banding pattern Jaccard's similarity coefficients [16] were calculated between genotypic pairs using NTSYS-pc 2.02 programme [17]. From the similarity coefficients matrix, thus generated, the dissimilarity coefficients (JD; Genetic distances =1- similarity coefficient) were calculated. For Clustering, UPGMA was used based on the similarity matrix generated on combined data. Using the data matrix for the presence and absence of each allele, a PCoA was performed using the same software and the two principal coordinates were used to visualize the dispersion of genotypes.

3. Results:

The present study deals with seed protein profiling through SDS PAGE and construction of Dendrogram to study the genetic diversity and their relation.

3.1 SDS PAGE Analysis:

Seeds of each accession were studied for their average molecular weight, total number of obtained bands in globulin protein and total number of bands obtained from albumin protein. It was recorded that only one accession has its average seed weight less than 0.10 gram, 16 accession of *Cicer arietinum* had shown average seed weight varying from 0.1gram to 0.20 gram , 26 accession showed their average seed weight in range of 0.20 to 0.40 gram and only 2 accession has its molecular weight more than 0.40 gram. It clearly indicates that maximum accession of chickpea has an average seed weight which falls in the range of 0.20 to 0.40 gram. It was also observed that maximum number of albumin bands i.e. 20 was found in accession no. BG 391 while minimum number of albumin band i.e. 7 was found in FLIP-90-166. In case of globulin maximum number of band are recorded in two accession BG 391 and BG2024 while minimum bands was noted for SBD 377, BGD 112, ICCV 10 (Table 1).

Based on the obtained bands (Fig: 1A-F) SDS gel was divided into four zones, demarcated as zone 1, 2, 3, 4 respectively. These zones were used for the study of percentage polymorphism. Bands which were unique or found only in few species are classified as polymorphic bands while bands which shows maximum repetitive occurrence were considered as monomorphic bands. Two different samples of protein which includes albumin and globulin were studied separately. A 20 clear distinct scorching band was obtained for albumin protein, among which zone 1 had 5 bands, zone 2 had 4 bands, zone 3 had 5 bands, zone 4 had six bands For albumin protein zone 1 has shown 40% polymorphism, zone 2 has also shown 25% polymorphism, zone 3 has shown no polymorphism and zone 4 has shown 33% polymorphism while contribution towards total polymorphism is 25 %.Sixteen clear distinct scorching bands was obtained for globulin protein out of which all four zones contain 4 clear distinct bands. 50% polymorphic bands was obtained for zone 1, 2 and 4 while 25% polymorphism was obtained for zone 3 while contribution towards total polymorphism is 43.5% (Table 2 & 3 ; Fig: 2 & 3).

3.2 Study of Genetic Diversity:

A Jaccard`s similarity coefficient was calculated from the obtained Data set to obtained the genetic relationship among accessions. Average Jaccard`s similarity coefficient was also calculated among all the genome which was found to have 0.768115 and the average genetic similarity for all 45 genotype showed a range of 0.375 to 0.964.

Results obtained from the analysis of gel were used for making similarity index matrix using NTSYS-PC software (version 2.1 b). Based on the similarity index matrix a dendrogram was obtained (Fig: 4) that's found to have two clusters among which cluster 1 having the maximum accession while only one accession in cluster 2 thereby cluster one was further subdivided into two major group among which major group 2 has only two accessions while major group1 was shown to extend in minor subgroup 1 and 2. Minor subgroup 2 have 7 accession while minor subgroup 1 have two branches, minor subgroup 1a having 10 accession while minor subgroup 1b having 25 accession which clearly indicates that all 25 accessions present minor subgroup 1b were closely related while accession in cluster two is far distinct in relation from others. Annegiri& ICC4458 and ICCV42 & ICC1882 were found to be maximum similarity as they fall on a single branch.

4. Discussion:

Cicer arietinum is a mandate crop for many organizations such as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located in India and at the International Center for Agricultural Research in the Dry Areas (ICARDA), located in Syria [18]. Development and analysis of molecular data for accession of *Cicer arietinum* would assist many breeders for identification of unique accession [19]. Study of genetic variation opens new doors and improved methodology for germplasm management, utilization, improvement, genetic finger printing and selection. Besides several DNA markers had shown a great potentiality of marker assisted selection, protein profiling is proved to be an emerging and promising methodology for the crop improvement based studies. Several past studies proved that SDS is a powerful, reliable tool for studying genetic diversity and species identification [20]. In the present study an effort was made to produce the blueprint of protein subunits of albumin and globulin protein which can be used as markers for the selection of the varieties among the various genomes. A 20 detectable band were obtained for albumin protein from which four polymorphic bands

were spotted which clearly indicates a 20% polymorphism and 16 detectable bands were obtained for globulin among which seven are polymorphic bands hence it contributed 43% towards total polymorphism. Bands obtained by SDS PAGE were analyzed through Jaccard`s Similarity Coefficient. Dendrogram was plotted from the data has shown two major clusters which are demarcated as cluster 1 and 2. cluster 1 was found to have maximum accession which indicates the close relation among accession while cluster 2 was found to have only one accession which shows distinct relation of FLIP-90-160 from others accessions. Cluster analysis clearly indicates that protein profiling of albumin and globulin can efficiently use to developed relation among genome.

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Table 1: List of accession number of *Cicer arietinum* along with their average seed weight and obtained Albumin and Globulin bands.

S.No	Accession number	Genome	Average weight in gram per seed	No. of obtained albumin bands	No. of obtained Globulin bands
1	JG 62	*Cicer arietinum*	0.14	16	10
2	BG 209	*Cicer arietinum*	0.13	15	9
3	PG 0515	*Cicer arietinum*	0.31	14	9
4	BG 261	*Cicer arietinum*	0.15	14	12
5	ICC 4458	*Cicer arietinum*	0.26	15	9
6	BG 362	*Cicer arietinum*	0.23	12	9
7	BG 2490	*Cicer arietinum*	0.16	12	11
8	SBD 377	*Cicer arietinum*	0.28	14	7
9	BGD 112	*Cicer arietinum*	0.28	10	7
10	ICCV 5	*Cicer arietinum*	0.18	18	9
11	ICC1882	*Cicer arietinum*	0.24	18	9
12	ICCV 10	*Cicer arietinum*	0.19	18	7
13	BG 1105	*Cicer arietinum*	0.26	19	12
14	PG 96006	*Cicer arietinum*	0.52	19	11
15	BG 391	*Cicer arietinum*	0.14	20	8
16	BGM 408	*Cicer arietinum*	0.18	17	12
17	BGD 72	*Cicer arietinum*	0.12	18	11
18	BG 254	*Cicer arietinum*	0.28	15	10
19	BG 212	*Cicer arietinum*	0.12	13	10
20	Pusa 5023	*Cicer arietinum*	0.33	15	10
21	WR 315	*Cicer arietinum*	0.18	15	12
22	BG 372	*Cicer arietinum*	0.09	17	11
23	BGD 72	*Cicer arietinum*	0.28	15	13

S.No	Accession number	Genome	Average weight in gram per seed	No. of obtained albumin bands	No. of obtained Globulin bands
24	JG 11	*Cicer arietinum*	0.22	16	14
25	BG 2024	*Cicer arietinum*	0.28	17	15
26	BG 1088	*Cicer arietinum*	0.19	15	12
27	GNG 469	*Cicer arietinum*	0.25	15	12
28	BG 1003	*Cicer arietinum*	0.32	15	12
29	Annegiri	*Cicer arietinum*	0.22	14	9
30	CSG 8962	*Cicer arietinum*	0.16	13	12
31	ICC 4958	*Cicer arietinum*	0.26	14	8
32	Avarodhi	*Cicer arietinum*	0.23	14	14
32	RSG 888	*Cicer arietinum*	0.17	14	10
34	KWR 108	*Cicer arietinum*	0.12	13	12
35	FLIP-90-166	*Cicer arietinum*	0.31	7	10
36	PG 0515	*Cicer arietinum*	0.56	14	11
37	BG 5028	*Cicer arietinum*	0.29	17	10
38	BG 1108	*Cicer arietinum*	0.26	17	10
39	BGM 417	*Cicer arietinum*	0.16	18	10
40	BG 1053	*Cicer arietinum*	0.28	15	10
41	Pusa 1103	*Cicer arietinum*	0.25	15	10
42	ICCV 42	*Cicer arietinum*	0.25	13	12
43	KAK2	*Cicer arietinum*	0.24	18	10
44	ICCV2	*Cicer arietinum*	0.24	18	13
45	ICCV92944	*Cicer arietinum*	0.22	16	11

Table 2: Percentage polymorphism study for albumin protein

ZONE	Total number of bands	Monomorphic band	Polymorphic band	Percentage polymorphism	Polymorphism contribution towards total bands.
ZONE 1	5	3	2	40	10
ZONE 2	4	3	1	25	5
ZONE 3	5	5	0	0	0
ZONE 4	6	4	2	33.33	10

Table 3: Percentage polymorphism study for Globulin protein

ZONE	Total number of bands	Monomorphic band	Polymorphic band	Percentage polymorphism	Polymorphism contribution towards total bands.
ZONE 1	4	2	2	50	12.5
ZONE 2	4	3	1	50	12.5
ZONE 3	4	2	2	25	6.25
ZONE 4	4	2	2	50	12.5

Figures:

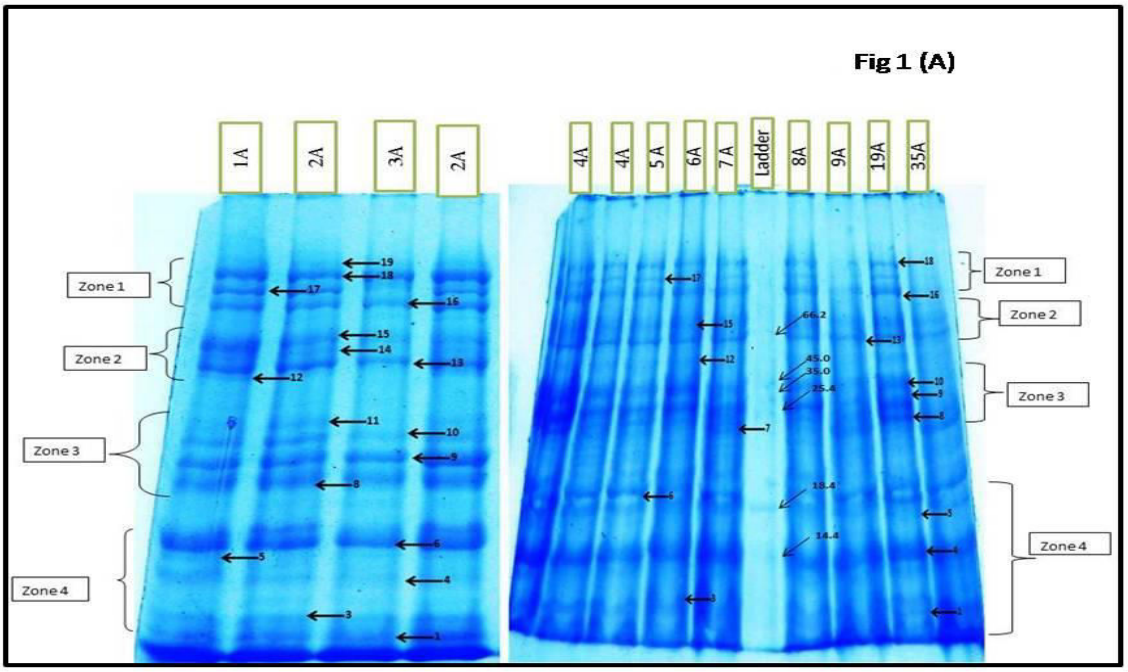

Fig1(A): **Electrophoretic banding pattern of Albumin protein of *Cicer arietinum*.** Horizontal arrow indicates presence of bands while tilted arrow indicates protein ladder (1A: JG 62; 2A: BG 209; 3A: PG: 0515; 4A: BG 261; 5A: ICC 4958; 6A: BG 362; 7A: BG 2490; 8A: SBD 377; 9A: BGD 112; 19A: BG 212; 35A: FLIP-90-166)

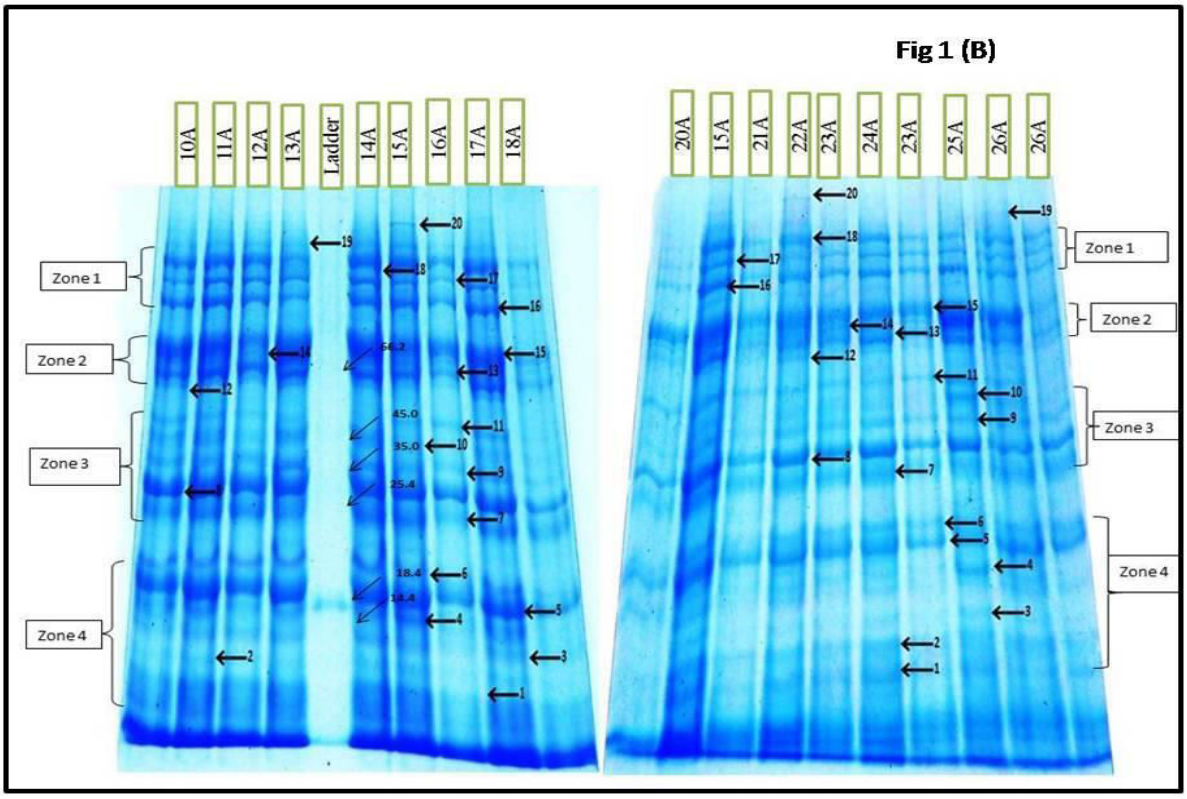

Fig1(B): **Electrophoretic banding pattern of Albumin protein of *Cicer arietinum*.** Horizontal arrow indicates presence of bands while tilted arrow indicates protein ladder (10A: 1CCV 5; 11A: ICCV 1882; 12A: ICCV10; 13A: BG 1105; 14A: PG96006; 15A: BG391; 16A: BGM 408; 17A: BGD 72; 18A: BG 254; 20A: Pusa 5023; 21A: WR 315; 22A: BG 372; 23A: BGD 72; 24A: JG 11; 25A: BG 2024; 26A: BG 1088)

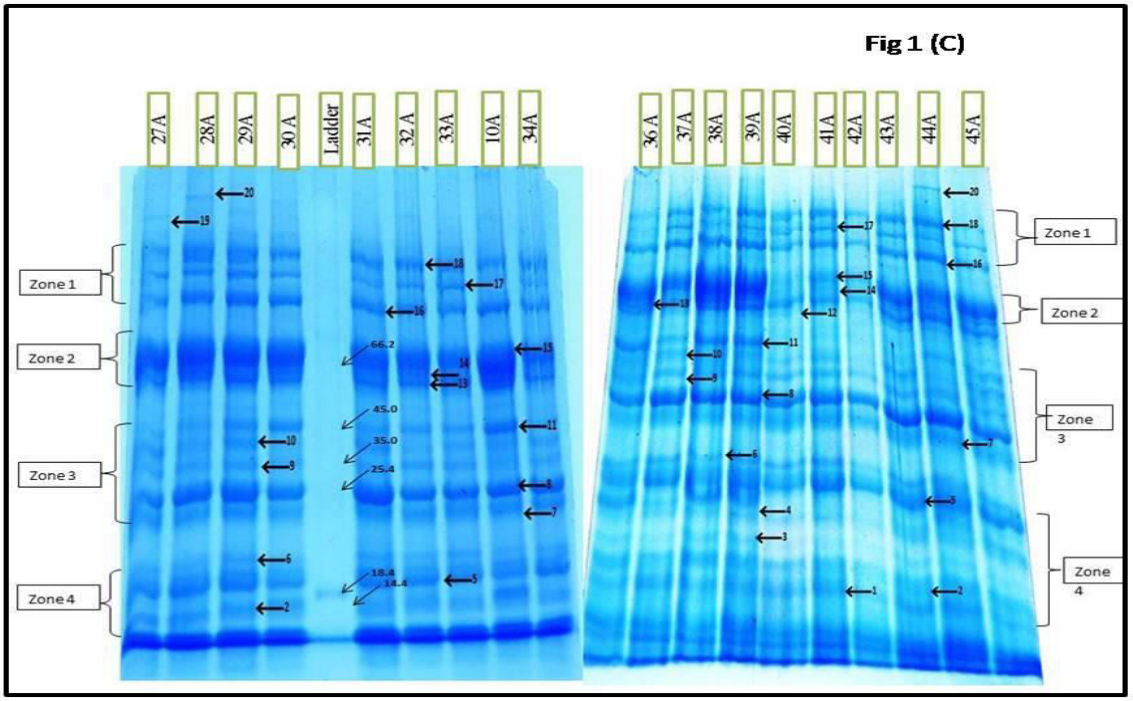

Fig 1(C): **Electrophoretic banding pattern of Albumin protein of *Cicer arietinum*.**
Horizontal arrow indicates presence of bands while tilted arrow indicates protein ladder (27A: GNG 469; 28A: BG 1003, 29A: Annegiri; 30A:CSG 8962; 31A: ICC 4958, 32A: Avarodhi, 33A: RSG 888; 34A: KWR 108; 36A: PG 0515; 37A: BG 5028; 38A: BG 1108; 39A:BGM 417; 40A:BG 1053; 41A: Pusa 1103; 42A: ICCV 43; 48A:KAK 2; 44A: ICCV 2; 45A:ICCV 92944)

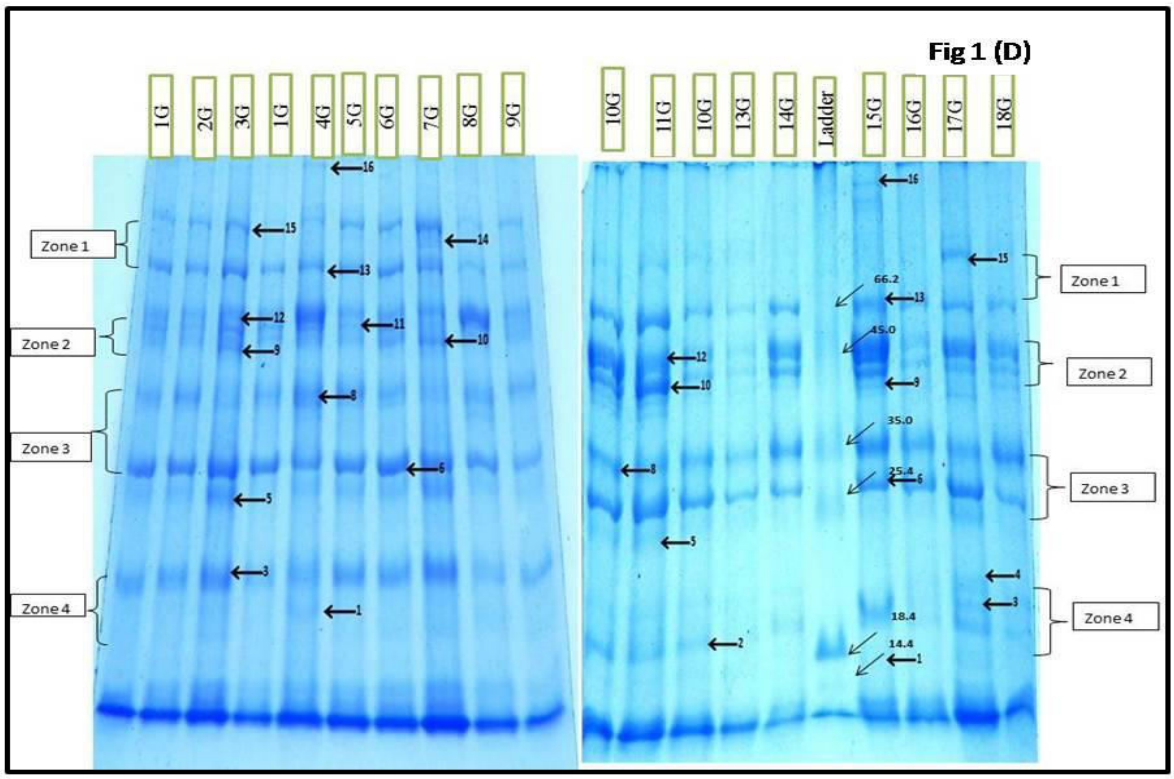

Fig 1(D): **Electrophoretic banding pattern of Globulin protein of *Cicer arietinum*.**
Horizontal arrow indicates presence of bands while tilted arrow indicates protein ladder (1G: JG 62; 2G: BG 209; 3G :PG:0515; 4G: BG 261; 5G: ICC 4958; 6G: BG 362; 7G: BG 240; 8G: SBD 377; 9G:BGD 112 ; 10G: 1CCV 5 ; 11G: ICCV 1882; 12G: ICCV10;13G: BG 1105; 14G: PG96006; 15G: BG391; 16G: BGM 408; 17G: BGD 72; 18G: BG 254)

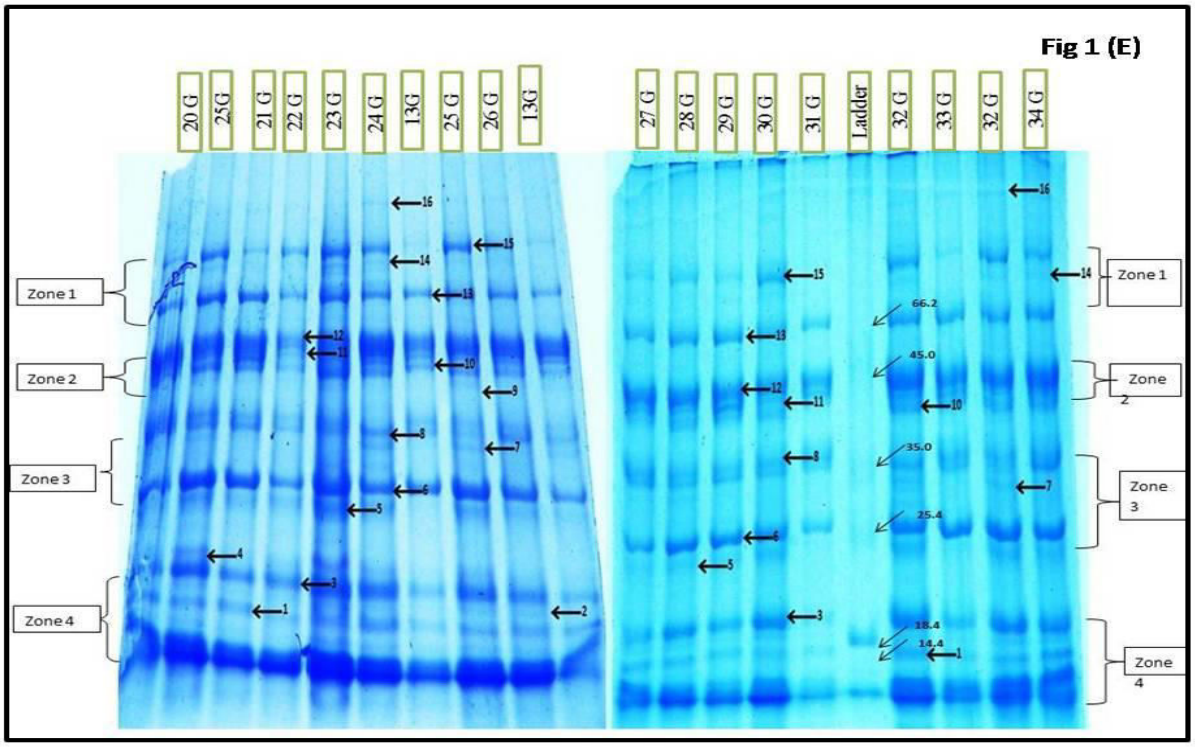

Fig 1(E): **Electrophoretic banding pattern of Albumin protein of *Cicer arietinum*.**
Horizontal arrow indicates presence of bands while tilted arrow indicates protein ladder (20G:Pusa 5023; 21G: WR 315; 22G: BG 372; 23G: BGD 72; 24G: JG 11;25G: BG 2024; 26G:BG 1088; 27G: GNG 469; 28G: BG 1003, 29G: Annegiri; 30G:CSG 8962; 31G: ICC 4958, 32G: Avarodhi, 33G: RSG 888; 34G: KWR 108)

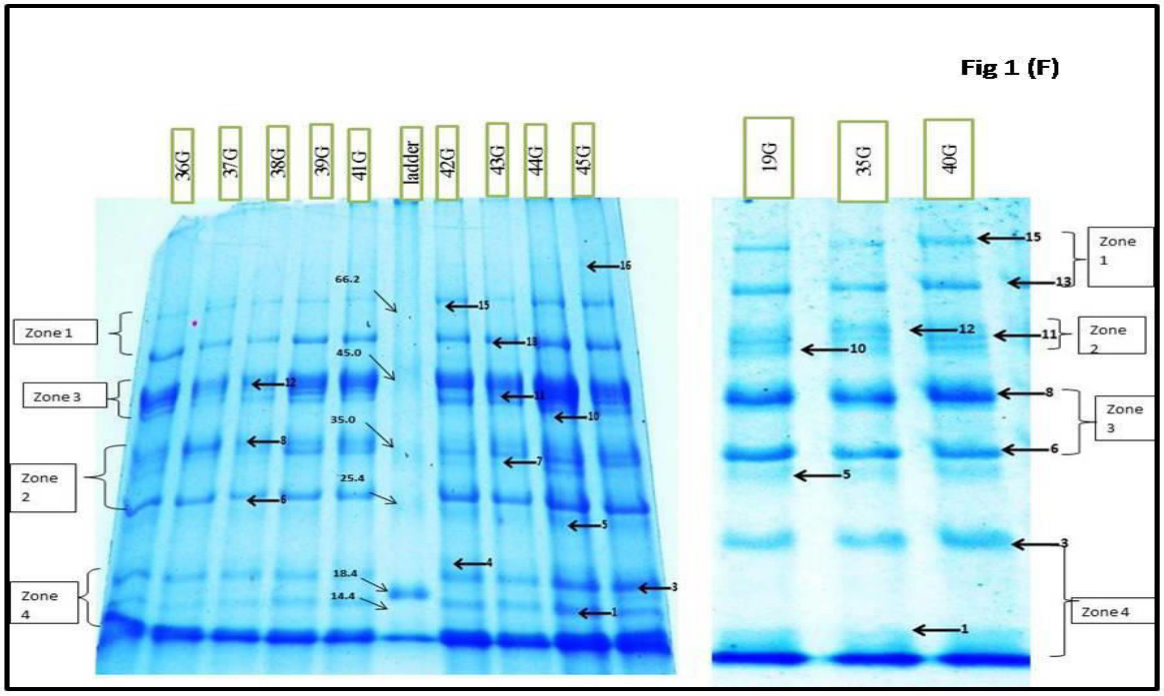

Fig1(F): **Electrophoretic banding pattern of Globulin protein of *Cicer arietinum*.**
Horizontal arrow indicates presence of bands while tilted arrow indicates protein ladder (36G: PG 0515; 37G: BG 5028; 38G: BG 1108; 39G: BGM 417; 41G: Pusa 1103; 42G: ICCV 42; 43G: KAK 2; 44G: ICCV 2; 45G: ICCV 92944; 19G: BG 212; 35G: FLIP-90-166: 40G: BG 1053)

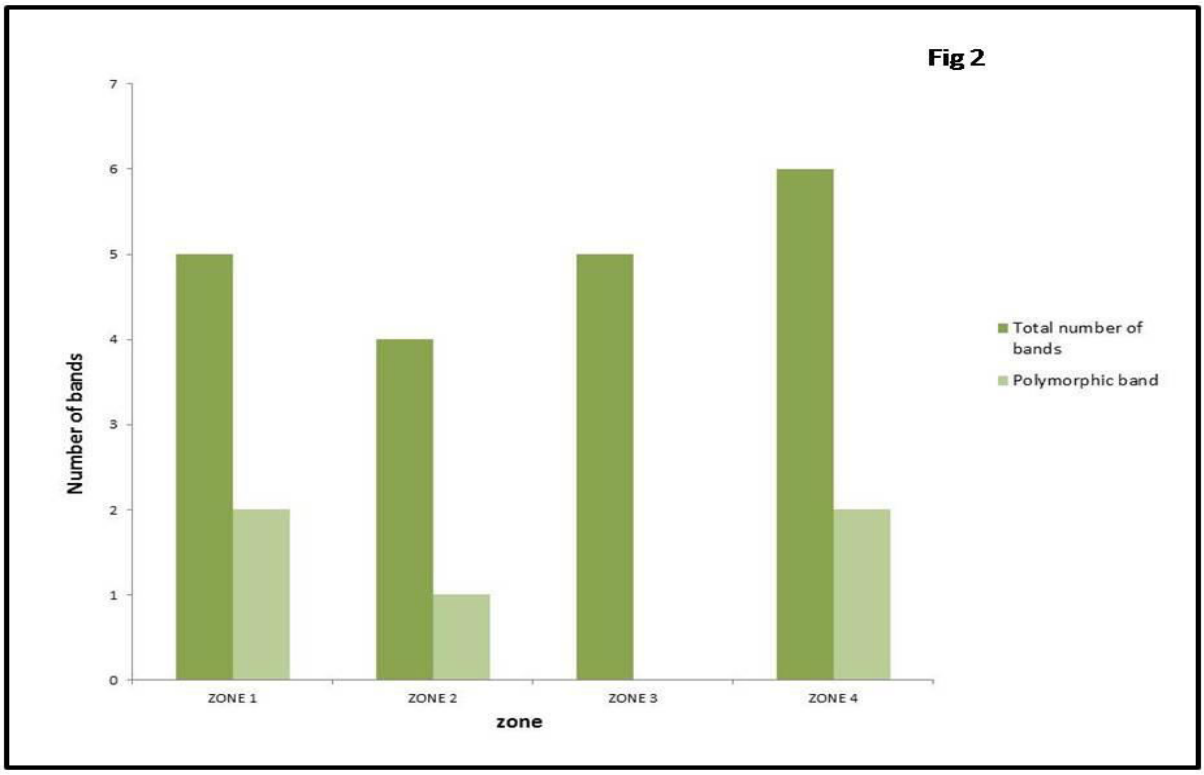

Fig 2: Graphical Representation of polymorphic bands with respect to total no. of bands obtained for Albumin protein

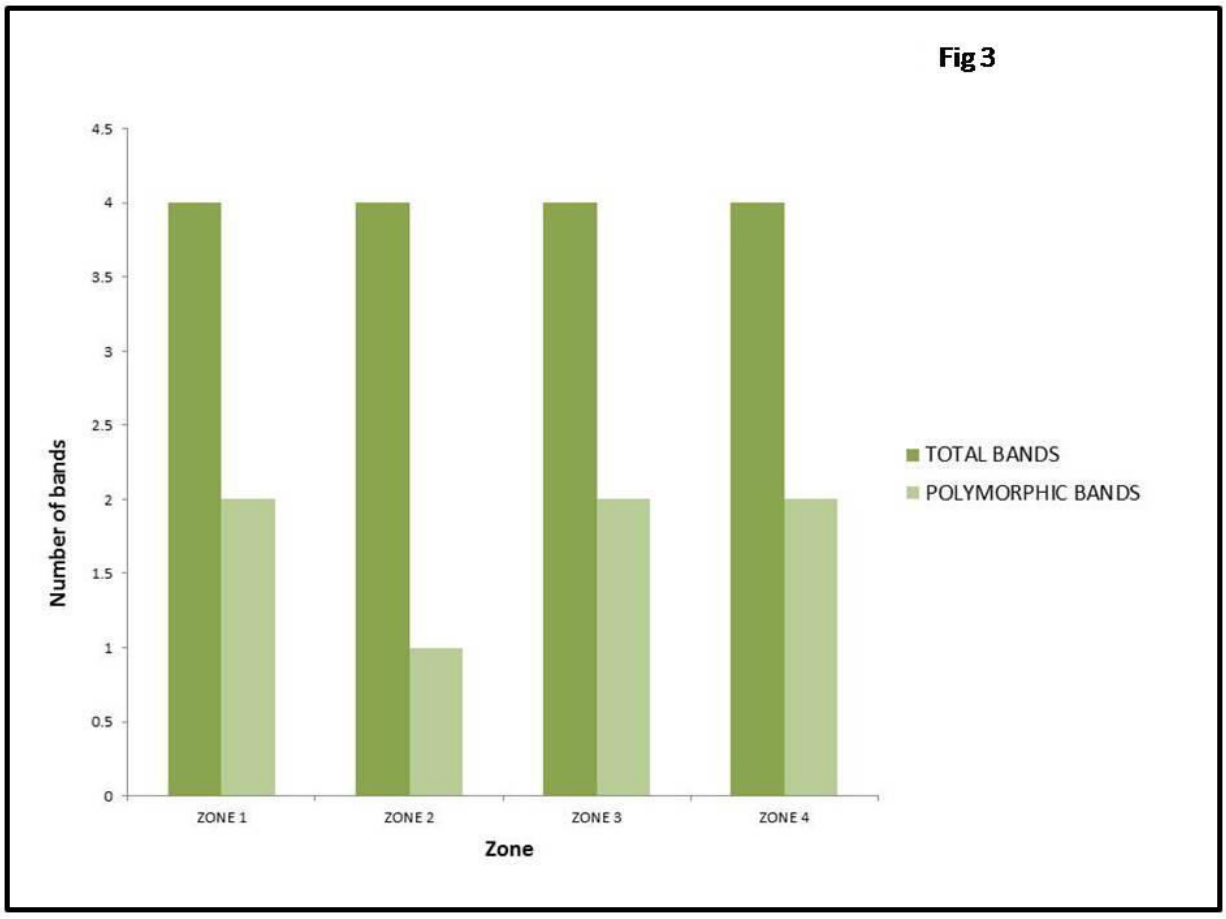

Fig 3: Graphical Representation of polymorphic bands with respect to total no. of bands obtained for Globulin protein

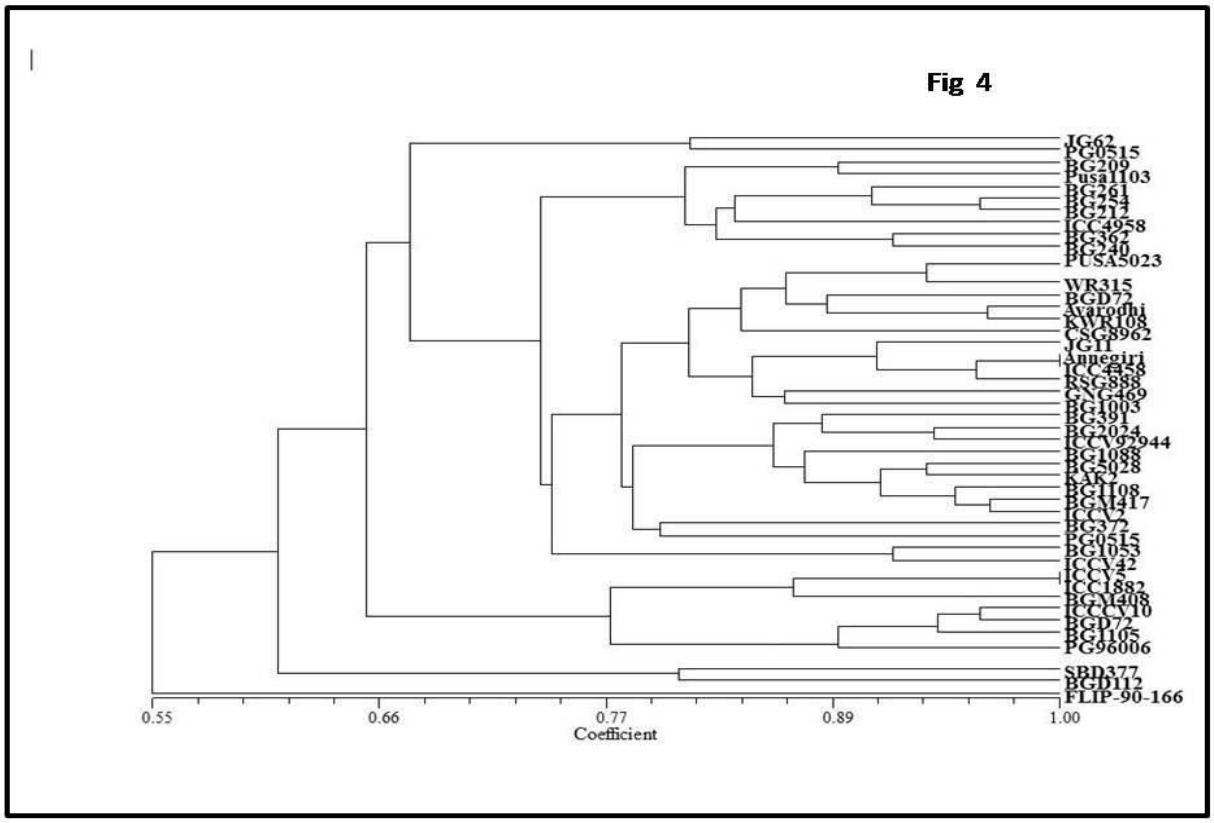

Fig 4: Phylogenetic tree of all 45 accessions of chickpea using UPGMA

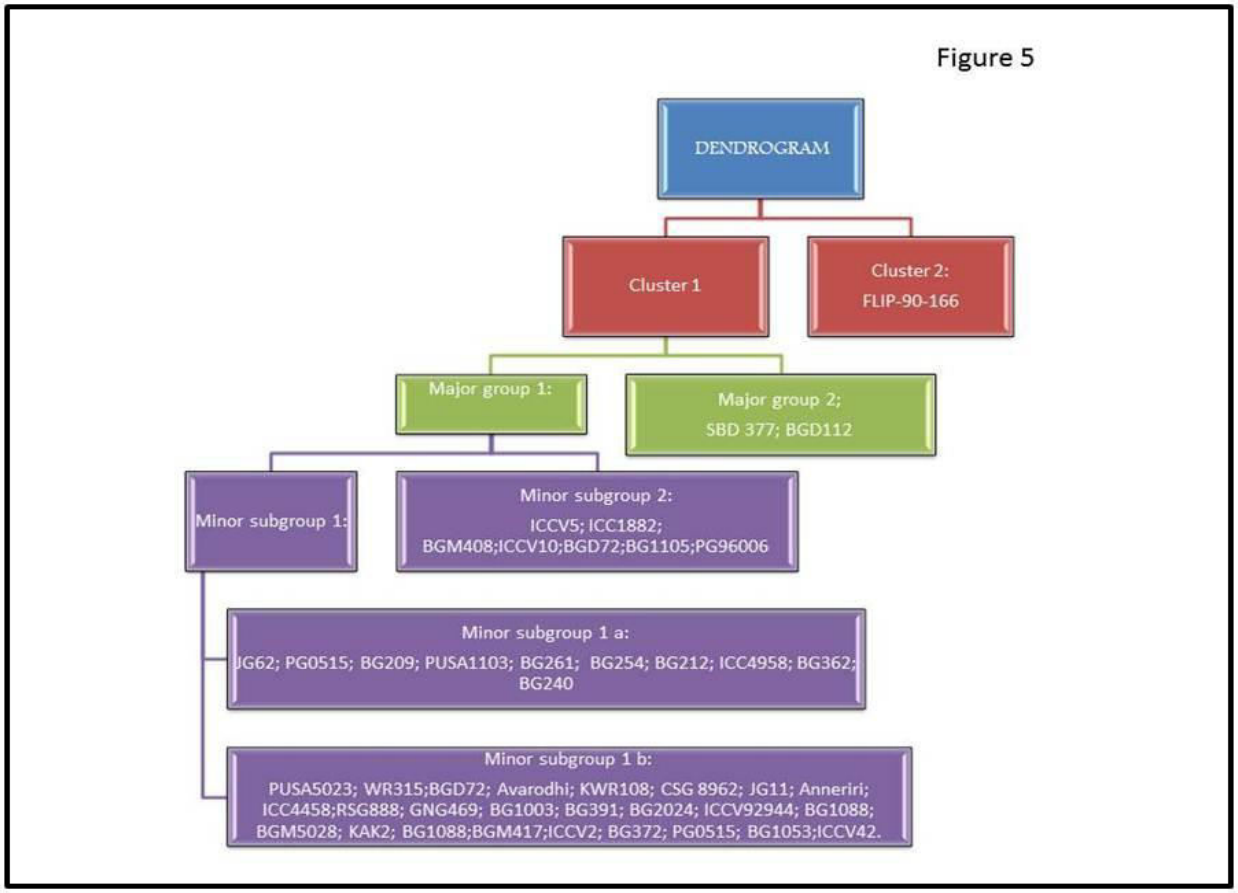

Fig 5: Schematic representation of clusters obtained from Dendrogram of all 45 accessions.

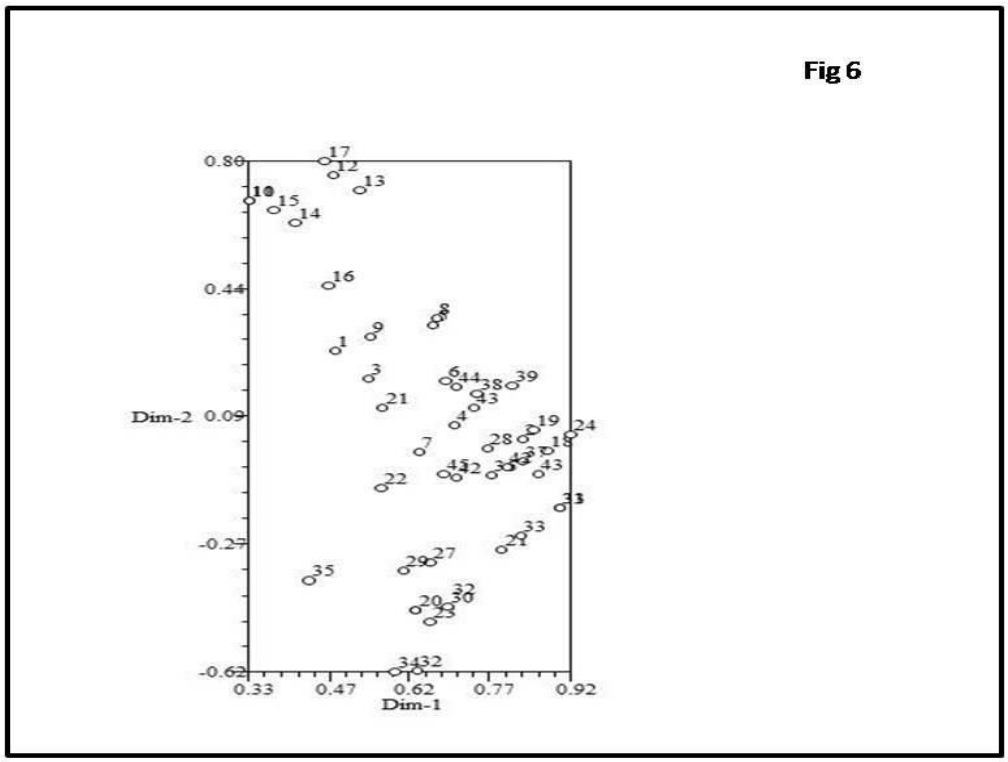

Fig 6: 2D representation of Dendrogram

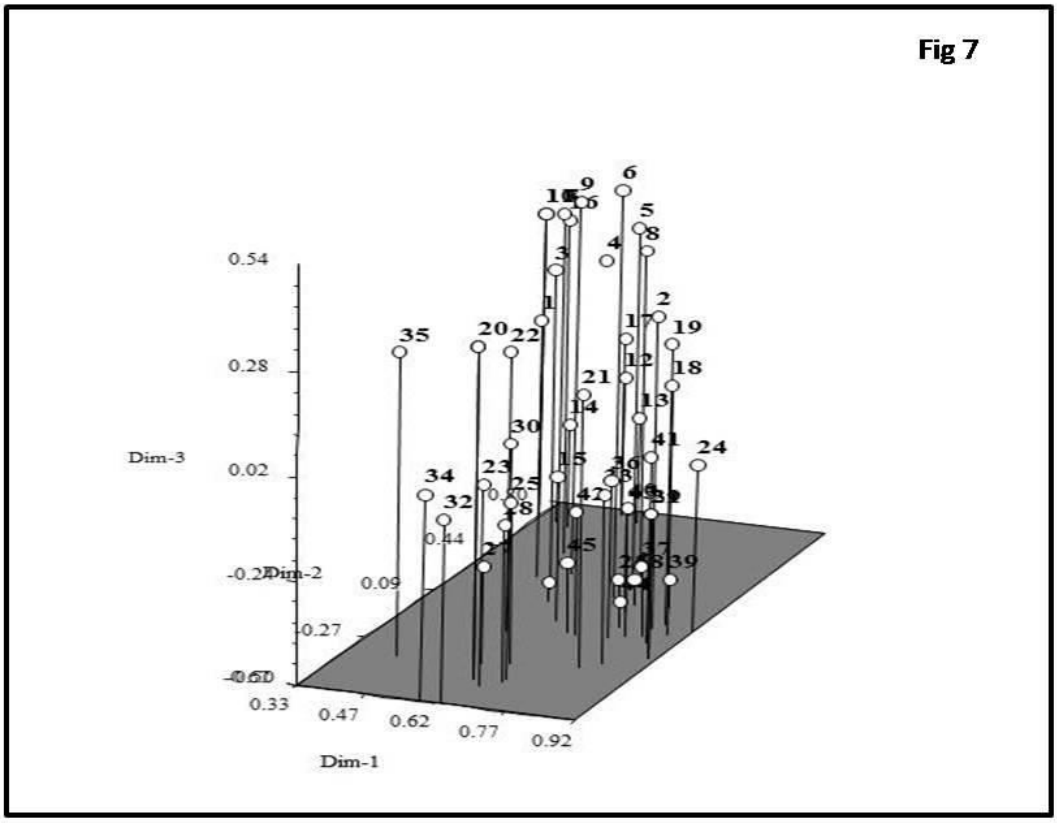

Fig 7: 3D representation of Dendrogram