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MOLECULAR DIVERSITY BASED CLUSTERING OF CHICKPEA (*CICER ARIETINUM* L.) GERMPLASM

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ABSTRACT

The present investigation undertaken at National Research Centre Plant Biotechnology, Pusa, New Delhi in 2005 with the aim of studying inter-relationship existing between the chickpea genotypes. 46 genotypes from chickpea and its wild relatives were collected that include 45 from Cicer arietinum and one from interspecific hybrid (Cicer arietinum x Cicer reticulatum). A total of 11 STMS loci were analyzed, covering various bin locations on different linkage groups. All the 11 STMS loci, in the genetic material under study were found to be highly polymorphic except TA-72. The STMS data was utilized for preparing genetic similarity matrix and further analyzed using UPGMA clustering algorithm. The dendrogram clearly showed 4 large clusters which were divided in sub clusters. Pant G114 and BG 1107 showed remarkable genetic similarity (0.77) followed by II, III and IV. The cluster 1 to 4 comprised of 8, 25,7 and 6 genotypes respectively. The study support greater resolving power of Sequence Tagged Microsatellite Sites molecular markers for chickpea germplasm where very little morphological distinction can be found between the different cultivars.

Key Words: Chickpea, Molecular Markers, STMS, Diversity, Clustering

INTRODUCTION

Chickpea is a self-pollinated crop with 16 diploid chromosome number. It is third most important grain legume in the world after dried beans and dry pea. Its cultivation is mainly confined to Asia with 90 per cent of the global area and production. Chickpea is cultivated on about 10.4 m ha area adding 8.57 m tones of grains to the global food basket with the average productivity of 825 Kg per ha. As many as 42 countries grow chickpea but a dozen countries namely India, Pakistan, turkey, Canada, Mexico, Iran, Ethiopia, Myanmar, Syria, Bangladesh, and Spain contribute 97% of the global production (FAOSTAT 2004).

India is the largest producer and consumer of chickpea, presently it is grown in 6.5 million hectare area in India with 5.77 million ton production at the productivity of 887.7 Kg/ha, which represent 32 and 42% of the national pulse acreage and production respectively. Chickpea production has gone up from 3.65 to 5.77 million tons between 1950-51 and 2003-04, registering a modest growth of 0.1% annually during the period, while the area has declined (FAOSTAT, 2004).

In recent years plant breeding has benefited from DNA marker technologies that were used to establish saturated genetic maps in major crop species, accessions genotyping and diversity analysis. Molecular technology can complement the conventional breeding efforts in effective characterization and utilization of the genetic resources. The DNA based markers are being increasingly utilized in plant breeding because of their phenotypic stability, high polymorphism, ease of development and diverse applications in breeding. Chickpea mapping is severely hampered by extraordinary little genetic polymorphisms in cultivated genotypes, and earlier used molecular markers such as isozymes, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) failed to reveal intraspecific variation (Kazan and Muehlbauer 1993; Udupa and Baum 2001; Simon and Muehlbauer 1997). Among a range of DNA-based markers, Sequence Tagged Microsatellite Site (STMS) markers are widely preferred in various crop plants, including chickpea due to their abundance, genomic coverage, genetically codominant nature and high polymorphism (Powell, 1996). STMS molecular markers first time developed

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by Huttel *et al* in 1999 in chickpea, revealed polymorphism up to desirable extent in Chickpea accessions.

Recent experiments on a STMS in the pulse family revealed that some of the STMS identified in one species are also capable of revealing polymorphism in other pulse species, it means once developed in chickpea they can also be used in other chickpea related species (Choumane *et al,* 2000). Such studies are required to ascertain the potential utility of these markers in analyzing molecular polymorphism in wild relatives of chickpea particularly *Cicer reticulatum*.

Keeping the above information in background the present study has been proposed with the following objective of fingerprinting based grouping of elite germplasm or cultivars in terms of genetic relatedness.

MATERIALS AND METHODS

The present investigation undertaken at Genetics division of IARI, Pusa, New Delhi in 2005. 46 genotypes from chickpea and its wild relatives were collected that include 45 from *Cicer arietinum* and one from derivatives of interspecific hybrid of *Cicer arietinum* x *Cicer reticulatum* from the Pulse Block of the division. As chickpea is highly self-pollinated crop therefore samples can be bulked. DNA from the germplasm was extracted with the help of CTAB method of Doyle and Doyle, 1987 with minor modifications. The STMS primers were synthesized by the Gene Script Corporation under the DBT funded project on molecular mapping of chickpea genome. The most polymorphic primer sequence were selected from the what available in the public domain (Winter *et al*, 1999 and Huttel *et al* 1999). STMS marker analysis was completed in the following steps, 1). Genomic DNA isolation and quantification. 2). DNA amplification in PCR with help of STMS primers. 3). Gel electrophoresis of the amplified products. 4). SSR data entry and verification. STMS markers used in the study was CaSTMS 25, TA 2, TA 8, TA 21, TA 43, TA 72, TA 80, TA 125, TR 58 and TS 45.

RESULT

In the present study, a total of 11 STMS loci were analyzed, covering various bin locations on different linkage group (Table 2). All the 11 STMS loci, in the genetic material under study were found to be highly polymorphic except *TA*-72. Excellent polymorphism was revealed by rest of the STMS markers employed for this analysis (Fig. 1). Data from 9 STMS loci were only utilized for further statistical analysis due to missing data (more than 30%) in 2 STMS loci. Similarly data of two lines EC 90039 and FCI-105 were not included in the analysis as these entries showed more than 30% missing data across the STMS loci analyzed.

A total of 40 alleles were found for the 9 STMS loci with an average of 9.4 per locus. The highest number of alleles were observed in TA21 (six alleles) followed by *TA-2* (5 alleles),*TA-8* (5 alleles), TA-64 (5 alleles), *TA80* (5 alleles), *TA125* (5 alleles), CaSTMS (4 alleles)and *TA72* (2 alleles). Out of these 2 alleles of *TA72* a single allele was present in most of the entries i.e. a_2 allele in 45 entries out of the selected 46 entries without any null allele.

Similarity verses Dissimilarity Analysis

The STMS data was utilized for estimating pair wise genetic similarities among various entries using Jaccard's coefficient (1908) method. The genetic similarity matrix was further analyzed using UPGMA clustering algorithm by software programme NTSYS pc version 2.11. The dendrogram derived from this analysis was depicted as Fig. 2. The dendrogram clearly showed 4 large clusters which were divided in sub clusters. Pant G114 and BG 1107 showed remarkable genetic similarity (0.77) followed by II, III and IV and 6ILC 3279 and Mexico local. The cluster I comprised of 8, 25,7 and 6 genotypes respectively as depicted in Fig.3. (Table 1)

DISCUSSION

Molecular markers have been utilized for variety of purposes including construction of linkage maps examining genetic relationships between individuals and identification of crop cultivars. These are considered as valuable tools for plant breeding programmers as well as for studies related to evolution and biodiversity conservation. Among the various DNA based markers, the microsatellite or STMS markers have become highly popular and the 'The markers system of choice in diverse crop plants owing to their abundance in the genome, robustness, reproducibility, hypervariability and codominance (Powell *et al.,* 1996). Application of STMS markers in genetic analysis of chickpea, started with an initial study of Huttel *et al.* (1999). Since then, the power and potential of Simple Sequence Repeat markers for a wide range of applications in genetic and breeding of chickpea has been well demonstrated by several researchers (e.g. Huttel 1999, Winter *et al*, 1999 and Flandez-galvez, 2003, W Choumane 2000 etc.), but still substantial number of chickpea microsatellites are not available in public domain. Microsatellite genotypic data from a number of loci have potential to provide unique allelic profiles or DNA fingerprints for establishing genotypes identity. While carrying out STMS profiling, due consideration was given to stratified sampling of polymorphic STMS loci covering bin location on various chromosomes. The STMS polymorphism were assayed using a DNA pooling strategy, although it is not supposed to do as all the genotypes under study are supposed to be pure lines.

When genetic relationship pattern obtained by STMS matrix data was composed with the pedigree information available for 46 genotypes/ varieties of genus *Cicer*, only few generalizations could be made. Of course, BG1103 a derivative from Pusa256 X Pusa362 *Cicerreticulatum* belonged to cluster I exhibiting closer relation with *Cicer reticulatum*. Apart from this no clear cut pattern, especially for different clustering (*i.e.* genetic dissimilarities) and source population diversity could be found one of the reason for apparent non-conformity of genetic relationship and pedigree information might be attributed to the base of source population; from which the genotypes were derived. Pedigree information might not be a clear cut indicator of diversity and large variation between the genotypes derived from the same source may be expected.

Cluster analysis revealed erratic pattern of clustering in aforesaid mentioned example of BG 1103 as three parents involved were grouped many genotypes of different in one cluster. This could be possible due to: (1) No trait observed (2) less number of STMS markers (3) possibility of negative / erroneous scoring. In addition, DNA marker may be affected by selection, drift and mutation, factors that are ignored when estimating association among genotype based on pedigree or origin. It may represent alleles identical in state which may not be identical by descent (Senior e*t al*, 1998). Finally, it can also be outcome from the clustering process whatever clusters are non-overlapping. Because of the latter, a genotype which is related to other genotypes from separate cluster wise only be grouped with one it is most closely related.

The STMS (Sequence Tagged Microsatellite Sites) molecular marker proved to be highly polymorphic for chickpea germplasm and can be reliably used for resolving differences existing at DNA level between two genotypes including the extent of their similarity with each other.

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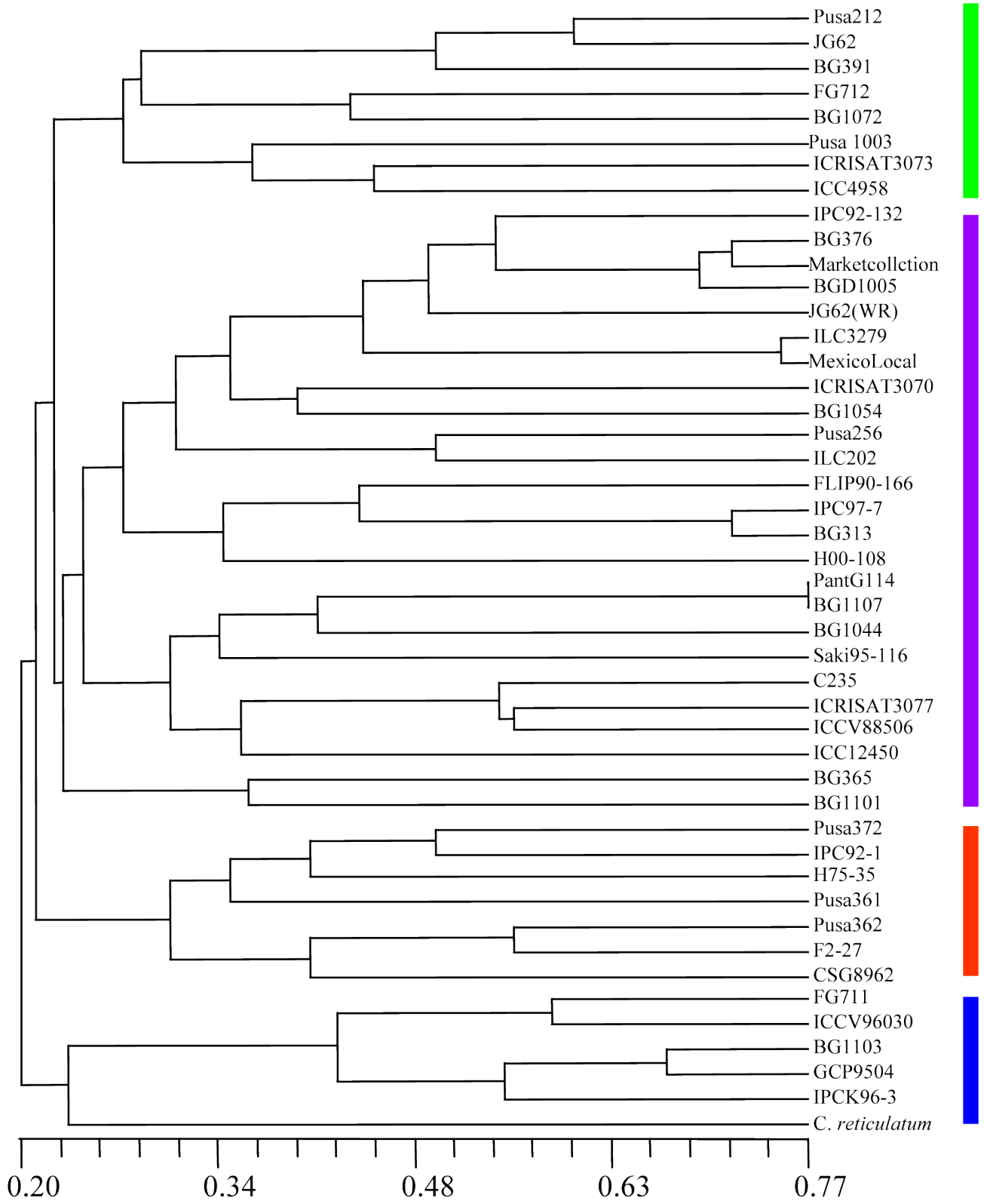

Figure 1: Dendrogram for genotypes of *Cicerarietinum* L. including one from *C. reticulatum*

Table : Jaccard's Similarity Coefficients

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
1	1.00																																													
2	0.30	1.00																																												
3	0.33	0.41	1.00																																										
4	0.27	0.30	0.30	1.00																																									
5	0.27	0.30	0.16	0.16	1.00																																							
6	0.35	0.46	0.26	0.26	0.38	1.00																																					
7	0.60	0.35	0.33	0.14	0.60	0.50	1.00																																				
8	0.14	0.17	0.21	0.23	0.45	0.40	0.38	1.00																																			
9	0.36	0.20	0.25	0.14	0.40	0.25	0.30	0.21	1.00																																		
10	0.41	0.33	0.30	0.21	0.41	0.37	0.58	0.26	0.20	1.00																																	
11	0.16	0.50	0.40	0.44	0.20	0.21	0.16	0.16	0.30	0.25	1.00																																
12	0.16	0.28	0.36	0.15	0.36	0.18	0.33	0.28	0.36	0.21	0.33	1.00																															
13	0.26	0.09	0.05	0.16	0.18	0.19	0.15	0.23	0.31	0.21	0.13	0.26	1.00																														
14	0.13	0.11	0.13	0.21	0.00	0.10	0.05	0.05	0.12	0.17	0.15	0.13	0.21	1.00																													
15	0.15	0.11	0.15	0.21	0.14	0.16	0.12	0.12	0.13	0.26	0.18	0.14	0.29	0.58	1.00																												
16	0.26	0.22	0.18	0.28	0.28	0.33	0.31	0.40	0.05	0.29	0.13	0.11	0.25	0.15	0.23	1.00																											
17	0.14	0.35	0.14	0.21	0.45	0.40	0.28	0.28	0.21	0.46	0.40	0.23	0.22	0.11	0.28	0.31	1.00																										
18	0.30	0.18	0.18	0.40	0.20	0.25	0.30	0.18	0.07	0.44	0.20	0.20	0.28	0.40	0.40	0.45	0.27	1.00																									
19	0.20	0.16	0.12	0.28	0.20	0.27	0.25	0.25	0.05	0.40	0.14	0.12	0.20	0.40	0.42	0.43	0.42	0.66	1.00																								
20	0.14	0.21	0.23	0.36	0.07	0.11	0.14	0.14	0.07	0.30	0.27	0.27	0.26	0.30	0.36	0.18	0.23	0.44	0.50	1.00																							
21	0.14	0.30	0.23	0.30	0.18	0.46	0.23	0.54	0.15	0.33	0.18	0.25	0.28	0.14	0.16	0.28	0.36	0.22	0.30	0.25	1.00																						
22	0.15	0.27	0.15	0.40	0.37	0.33	0.27	0.27	0.16	0.40	0.30	0.20	0.13	0.07	0.08	0.21	0.40	0.30	0.33	0.27	0.30	1.00																					
23	0.25	0.16	0.25	0.44	0.09	0.23	0.16	0.27	0.16	0.27	0.30	0.20	0.21	0.50	0.44	0.30	0.27	0.50	0.60	0.40	0.30	0.16	1.00																				
24	0.13	0.17	0.30	0.21	0.21	0.29	0.26	0.46	0.12	0.25	0.25	0.21	0.09	0.33	0.35	0.37	0.26	0.25	0.31	0.21	0.23	0.15	0.50	1.00																			
25	0.07	0.12	0.25	0.36	0.16	0.25	0.13	0.54	0.23	0.20	0.30	0.25	0.23	0.20	0.13	0.33	0.30	0.27	0.26	0.25	0.55	0.40	0.40	0.38	1.00																		
26	0.20	0.31	0.12	0.18	0.41	0.76	0.42	0.42	0.18	0.31	0.14	0.20	0.20	0.05	0.11	0.27	0.42	0.25	0.37	0.20	0.38	0.50	0.15	0.23	0.26	1.00																	
27	0.45	0.30	0.14	0.27	0.33	0.35	0.33	0.21	0.15	0.41	0.07	0.07	0.35	0.06	0.07	0.35	0.23	0.20	0.20	0.14	0.33	0.27	0.07	0.00	0.15	0.28	1.00															
28	0.33	0.18	0.14	0.30	0.15	0.23	0.12	0.20	0.30	0.26	0.27	0.14	0.37	0.26	0.20	0.16	0.20	0.16	0.25	0.33	0.25	0.40	0.27	0.11	0.30	0.25	0.45	1.00													
29	0.16	0.20	0.27	0.23	0.16	0.17	0.13	0.12	0.23	0.12	0.33	0.23	0.16	0.12	0.13	0.17	0.13	0.07	0.18	0.27	0.07	0.18	0.18	0.12	0.14	0.18	0.16	0.30	1.00												
30	0.23	0.30	0.33	0.27	0.08	0.18	0.14	0.06	0.15	0.13	0.27	0.16	0.05	0.06	0.07	0.11	0.06	0.08	0.12	0.23	0.15	0.15	0.15	0.06	0.07	0.12	0.14	0.23	0.55	1.00											
31	0.20	0.08	0.09	0.22	0.25	0.36	0.18	0.30	0.33	0.07	0.10	0.22	0.23	0.00	0.09	0.14	0.30	0.10	0.15	0.08	0.30	0.20	0.20	0.07	0.30	0.36	0.18	0.18	0.22	0.18	1.00										
32	0.08	0.07	0.08	0.20	0.10	0.14	0.07	0.07	0.08	0.15	0.20	0.09	0.21	0.07	0.08	0.13	0.16	0.33	0.23	0.27	0.08	0.44	0.18	0.07	0.16	0.20	0.07	0.27	0.33	0.40	0.18	1.00									
33	0.09	0.07	0.09	0.09	0.09	0.13	0.07	0.14	0.16	0.06	0.10	0.16	0.11	0.23	0.07	0.20	0.15	0.22	0.30	0.08	0.18	0.09	0.33	0.06	0.25	0.13	0.08	0.15	0.27	0.30	0.33	0.30	1.00								
34	0.28	0.10	0.12	0.13	0.30	0.15	0.25	0.23	0.11	0.31	0.06	0.05	0.20	0.10	0.11	0.43	0.17	0.25	0.29	0.05	0.05	0.14	0.14	0.10	0.17	0.15	0.38	0.17	0.26	0.12	0.15	0.14	0.30	1.00							
35	0.15	0.28	0.36	0.36	0.07	0.11	0.06	0.13	0.14	0.12	0.44	0.15	0.16	0.12	0.13	0.25	0.13	0.16	0.11	0.25	0.07	0.16	0.27	0.20	0.23	0.05	0.15	0.21	0.45	0.36	0.20	0.30	0.16	0.26	1.00						
36	0.14	0.05	0.14	0.33	0.07	0.10	0.05	0.28	0.06	0.11	0.16	0.14	0.22	0.05	0.05	0.23	0.12	0.15	0.11	0.14	0.25	0.36	0.15	0.11	0.30	0.11	0.23	0.28	0.13	0.14	0.30	0.40	0.15	0.25	0.30	1.00					
37	0.07	0.15	0.16	0.18	0.33	0.21	0.15	0.23	0.27	0.14	0.18	0.27	0.38	0.06	0.16	0.20	0.25	0.30	0.13	0.15	0.25	0.27	0.16	0.14	0.25	0.21	0.15	0.07	0.30	0.15	0.27	0.36	0.16	0.21	0.27	0.25	1.00				
38	0.25	0.20	0.25	0.33	0.16	0.25	0.21	0.21	0.33	0.12	0.30	0.15	0.16	0.12	0.13	0.11	0.13	0.16	0.11	0.15	0.25	0.27	0.27	0.20	0.23	0.18	0.07	0.13	0.14	0.25	0.20	0.30	0.16	0.05	0.14	0.21	0.40	1.00			
39	0.16	0.13	0.16	0.15	0.44	0.18	0.33	0.33	0.25	0.41	0.33	0.16	0.25	0.06	0.14	0.26	0.33	0.18	0.28	0.27	0.18	0.44	0.18	0.21	0.25	0.28	0.27	0.33	0.36	0.16	0.09	0.30	0.07	0.28	0.25	0.14	0.27	0.15	1.00		
40	0.14	0.11	0.14	0.21	0.55	0.16	0.28	0.38	0.30	0.35	0.27	0.23	0.37	0.11	0.28	0.31	0.38	0.25	0.25	0.23	0.25	0.25	0.25	0.26	0.30	0.17	0.23	0.20	0.21	0.14	0.16	0.25	0.14	0.25	0.21	0.12	0.45	0.30	0.60	1.00	
41	0.23	0.21	0.23	0.36	0.30	0.18	0.23	0.33	0.36	0.30	0.40	0.16	0.35	0.21	0.25	0.26	0.23	0.27	0.28	0.33	0.25	0.36	0.36	0.30	0.36	0.20	0.23	0.45	0.40	0.23	0.08	0.27	0.08	0.20	0.36	0.14	0.36	0.36	0.75	0.60	1.00
42	0.22	0.27	0.37	0.33	0.50	0.33	0.30	0.44	0.50	0.27	0.50	0.33	0.21	0.16	0.44	0.23	0.44	0.20	0.25	0.22	0.37	0.25	0.42	0.40	0.33	0.25	0.11	0.18	0.33	0.22	0.33	0.12	0.11	0.15	0.20	0.08	0.66	0.50	0.37	0.71	0.57	1.00
43	0.09	0.18	0.20	0.22	0.57	0.25	0.27	0.44	0.27	0.30	0.25	0.33	0.21	0.16	0.30	0.23	0.44	0.20	0.36	0.22	0.37	0.22	0.37	0.27	0.27	0.25	0.09	0.08	0.16	0.30	0.20	0.20	0.27	0.23	0.08	0.07	0.40	0.40	0.30	0.66	0.33	0.71	1.00
44	0.08	0.20	0.18	0.20	0.28	0.16	0.20	0.33	0.20	0.20	0.22	0.22	0.15	0.08	0.10	0.15	0.20	0.18	0.27	0.50	0.33	0.33	0.20	0.18	0.33	0.30	0.09	0.20	0.22	0.30	0.11	0.37	0.25	0.07	0.09	0.08	0.33	0.50	0.37	0.44	0.44	0.42	0.50	1.00
45	0.20	0.16	0.20	0.30	0.50	0.23	0.36	0.36	0.25	0.50	0.37	0.18	0.18	0.14	0.15	0.20	0.36	0.20	0.30	0.20	0.22	0.33	0.33	0.23	0.23	0.23	0.20	0.25	0.15	0.18	0.09	0.30	0.25	0.30	0.15	0.23	0.16	0.36	0.50	0.54	0.44	0.37	0.55	0.42	1.00	
46	0.27	0.16	0.16	0.27	0.22	0.14	0.16	0.18	0.27	0.27	0.33	0.09	0.21	0.16	0.18	0.15	0.16	0.25	0.25	0.30	0.10	0.44	0.30	0.16	0.18	0.15	0.18	0.55	0.27	0.40	0.10	0.71	0.22	0.16	0.27	0.27	0.22	0.44	0.44	0.40	0.62	0.33	0.28	0.50	0.44	1.00

Cultivars used in the below mentioned sequence as in the similarity table based on the Jaccard's similarity coefficient

1. PUSA 212, 2.PUSA 372, 3.H 75-35, 4.PUSA 361,5.IPC 92-132, 6.PANT G 114, 7.JG 62, 8.C 235, 9.BG 365, 10.G 391, 11.IPC 92-1, 12.BG 1101, 13.. BG 1103, 14.FG 711, 15.ICCV 96030, 16.FG 712, 17.BG1044, 18.PUSA 1003, 19.GCP 9504, 20.PUSA 256, 21.ICRISAT 3077, 22.FLIP 90-166, 23.C.reticulatum derivative, 24.ICC 12450, 25.ICCV 88506, 26.BG 1107, 27.ICRISAT 3073, 28.IPC 927, 29.PUSA 362, 30.F 2-27, 31.SAKI 95-116, 32.IPC 97-7, 33.IPCK 96-3, 34.BG 1072, 35.CSG 8962, 36.HOO 108, 37.ICRISAT 3070, 38.ICC 4958, 39.ILC 3269, 40.BG 376, 41.Mexico Local, 42.MCD 3, 43.BGD 1005, 44.ILC 202, 45.JG 62(WR), 46.BG 313.