THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Patil A.S *et al*, The *Experiment, 2014., Vol. 18(4), 1297-1303*

COMPARATIVE PROTEIN PROFILING STUDY IN WILD CHICKPEA AND ITS INDUCED MUTANTS.

ABSTRACT

The genus Cicer has 9 annual species and 31 perennial species, of which only one of 9 annuals species is under cultivation- Cicer arietinum. The extremely low level of polymorphism within the cultivated chickpeas species, has been reported by many workers. The available genetic variability in chickpea has been exploited in the conventional plant breeding approaches which narrowed the genetic base for this crop. The wild chickpea and its induced mutants has been used in present study. The seed protein profile by SDS-PAGE were studied in wild chickpea treated with chemical and physical mutagens independently and in combination treatment with respect to the untreated control. The comparison was analyzed on the basis of Jaccard's similarity coefficient and UPGMA clustering. The polymorphic banding pattern was observed in the present investigation. The range of major band was observed from 7 to 12. 12 major bands were observed in the control treatment while 7 to 11 was observed in the mutants in the M_1 and M_2 generation. The polypeptide bands of different sizes ranging from 5.83 KDa to120.24KDa in M_1 generation while from 4.15 KDa to 119.08 KDa in M_2 generation were observed in all the treatments alongwith the untreated control. Genetic distance between all 15 treatments varied from 0.028 to 0.319 and 0.026 to 0.26 as reveled by Jaccard similarity coefficient. The dendrogram obtained on the basis of Jaccard similarity coefficient using UPGMA method represent one parent and 14 mutant into 3 major clusters A, B, C; cluster in M_1 generation while cluster 'A' consist of 1 parent and 5 mutant, cluster 'B' consist of 6 mutant and cluster 'C' consist of 3 mutant; cluster 'C' showed more diversity from the parent and other mutants in M_2 generation diversity decreased in M_2 generation in present investigation.

Key words: - Wild Chickpea, Jaccards similarity coefficient, UPGMA, Cluster analysis.

INTRODUCTION

Chickpea has been classified as a recalcitrant crop, as low genetic advance improvement for yield because of limited genetic variation, thus, the use of mutation breeding , inter-specific hybridization to enhance genetic variation and introduction of resistance genes in cultigens from wild species have been emphasized to broaden the genetic base of cultivated species (Van Rheenen et al.,1993).

The grain legumes play important and primary role as a sources of proteins and considered as a good substitute to animal proteins in human diet. Mutagenesis is utilized to alter the quantitative and qualitative aspects of the seed protein in many cereal crops (Amirshahi and Tavakoli, 1970). Legume seed proteins primarily increase the nutritional quality and impart a variety of functional properties, including structure, texture, flavor and colour to food products.

The many new genotypes derived through mutation breeding have been reported in self-pollinated annual diploid and allopolyploid (Sigurbjornsson and Micke, 1969; 1974). Induced mutation methods are used in plant breeding for improving varieties. Genetic variability occurred in all the mutagenic treatment has been reported in Rhododendron and gamma radiation was used to induce new variation for traits. Irradiation by physical mutagenic agent leads DNA breaks in plants (Atak et al., 2011).

The electrophoretic pattern of the protein are directly associated with the genetic background of the protein and be harnessed to certify the genetic make up (Asghar et al., 2003). The induced mutation has been reported to create the additional variability to supplement the existing germplasm (Amjad et al.,2009). SDS-PAGE (Sodium Dodecyl Sulphate -Polyacrilamide Gel Electrophoresis) is a valid technique used for species identification. Each variety of a group exhibit characteristics banding pattern of protein, accordingly, they could be identified (Asghar et al., 2003).

Legume seed proteins are composed of water-soluble albumin and salt soluble globulins and their ratio can be altered with respect to either of the two under the influence of mutated genes and such alteration improve nutritional value (Myers and Gritton, 1988).Inter and intra specific variation in seed protein have been reported in wheat, barley and their wild relatives (Masood et al.,1994).
The induced mutagenesis has been used to induce the variation in addition to natural variability (Tulmann, 1990). Ladizinsky and Adler (1975) reported that protein electrophoretic profiles of C. reticulatum resemble closely to that of C. arietinum. The electrophoresis of total seed protein has been reported as a useful and effective method to analyze genetic variation in plant genetic resources (Asghar et al., 2003). The chickpea cultivar was identified by using PAGE of seed storage protein (Singh et al., 1991).

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MATERIAL AND METHODS

The seeds of wild Cicer reticulatum L. were procured from ICRISAT, Patancheru, (AP), India. The seeds of 1st set treated with four different concentration viz. 0.1%, 0.2%, 0.3%, 0.4% of Ethyl Methane Sulphonate. The seeds of 2nd set were treated with combination treatment of EMS and gamma radiation viz. 0.1% EMS+5KR, 0.2% EMS+10KR, 0.3% EMS +15KR and 0.4% EMS+20KR. The healthy seeds were first treated with 0.1% to 0.4% EMS thereafter washed thoroughly and soaked with blotting paper to remove any residual effect of treating solution then the pre-treated seeds were irradiated with 5KR to 20 KR γ rays. The seeds of 3rd set were treated with different doses 5 KR, 10 KR, 15 KR, 20 KR, 25 KR, 30KR of gamma radiation. The treatments were encoded as, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, and T_{15} respectively. All the treated alongwith the untreated control T_1, seeds were sown to M1 and M2 generation. The M1 and M2 seed yield were collected as M1 and M2 generation for electrophoretic study.

The test seeds in T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, and T_{15} treatments were used for the estimation of protein and molecular weight determination, protein extraction, protein profile.

The seeds of M_1 and M_2 generation were powdered with the help of pestle mortar and the 25 mg of seed flour was mixed with 1ml of Protein Extraction Buffer (0.05 M Tris -HCL, 0.2% SDS, 5 M Urea and 1% β-Mercaptethanol with pH-6.8-7.00) in the eppendorf tube to extract the seed storage protein thereafter, centrifuged at 15000×g rpm for 7 Minutes at 4°C in cooling centrifuge (Remi, India). Supernatant was collected and stored in the refrigerator for protein estimation and SDS-PAGE electrophoresis (Asgar et al., 2003).

The seed protein content of mutant and control was evaluated using Bradford assay (1976) against BSA as standard at 590 nm on UV spectrophotometer (Beckmann's).

25 µl protein extracts and 25µl Laemmli buffer mixed together in eppendorff tube followed by the Laemmli protocol (1970).The 50 µg seed storage protein of each treatment along with control were loaded in the gel well after mixed with the sample buffer pH 6.8 (Laemmili Buffer). SDS- PAGE followed by Laemmli (1970) using 11.25% polyacrylamide gel. Electrophoresis was carried out at 50mA for two and half hours. In order to check reproducibility of the method two separate gels were run under similar conditions. After electrophoresis gels were stained with 0.2% (w/v) Coomassive Brilliant Blue R- 250 for 5 hours and then destained for 24 hours on a gyratory shaker.

Properly destained gels were read on gel documentation system and molecular weight of individual bands calculated with respect to molecular marker weight using ALPHA imager software. Consistency of results was ensured by analyzing a minimum of 7 seed samples from each treatment. Further electrophoresis was repeated at least twice on each protein extract. Only consistent bands were taken into account.

Data Analysis

The gets were analyzed on gel documentation system for its positions, rm (relative mobility) values, molecular weight determination. The gels were scored as presence (1) or absence (0) of bands of comparable size depicted in **Table A** for M_1 generation and **Table B** for M_2 generation (Karihaloo et al., 2002). Pair wise similarities between parents and its mutants were calculated using Jaccard's coefficient. The cluster analysis was performed on similarity matrix by UPGMA method (Sneath and Sokal, 1973; Karihaloo et al., 2002).

Co-efficient of Jaccard $Sij = n_{ij} / n_i + n_j - n_{ij}$

Where, Sij→the similarity between track or lanes i and j
nij →the number of corresponding bands for i and j
ni→total number of bands in i
nj→total number of bands in j
ni + nj - nij→ total number of bands in both track or lane

Dissimilarity=1- Sij (Simlarity). The clustering was based on Clustering Method –UPGMA (Unweighted Pair Grouping of Mean of Arithmetic Average) (Arjen Van Ooyen, 2001).

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RESULTS AND DISCUSSION

In the present study, the total 20 bands were observed in the control untreated T_1 treatment with the molecular weight ranging from 7.94 KDa to 119.08 KDa, while the change in number of bands with change in molecular weight or size of corresponding bands were observed in all other rest of the mutagenic treatment from T_2- 15, T_3 - 15, T_4 -14, T_5 -18, T_6 -14, T_7-14, T_8- 15, T_9- 14, T_{10}- 18, T_{11}- 17, T_{12}-17, T_{13}- 17, T_{14}- 18, T_{15}- 19 in M_1 generation and T_2- 18, T_3 - 16, T_4 -19, T_5 -19, T_6 -17, T_7-16, T_8- 19, T_9- 18, T_{10}- 15, T_{11}- 14, T_{12}- 14, T_{13}- 14, T_{14}- 14, T_{15}- 15 in M_2 generation. The total 18 and 22 bands ranging from 5 KDa to 70 KDa in 21 accesion of cultigen kabuli chickpea (Cicer arietinum) has been reported followed by electrophoretic study (Asgar et al., 2003). The intense band was described as 'major bands' while less intense band as 'minor band'.

The range of major band was observed from 7 to 12. 12 major bands were observed in the control treatment while 7 to 11 was observed in the mutants in the M_1 and M_2 generation while rest of the bands were minor band. The seed protein content in present study, estimated by Bradford assay was found to be increased in all the treatment as compared to the control. The higher amount of protein 36 µg was observed in 20 KR gamma rays treatment of M_1 generation and 35 µg as higher amount of protein was observed in 0.2% EMS, 0.3% EMS and 20KR gamma ray treatment of M_2 generation. The protein content has been reported increased in Phaseolus followed by the mutagenic treatment (Prasad et al., 1986). Relative increase in protein content and the highest increase have been reported in 5KR and 10KR in 2 different Phaseolus variety of bountiful and giant respectively (Prasad et al, 1986). No significant alteration in Seed protein content of mutant in chickpea has been reported (Kharkwal, 1998a, b, c; Wani and Anis, 2008). The polypeptide bands of different sizes ranging from 5.83 KDa to120.24KDa in M_1 generation while from 4.15 KDa to 119.08 KDa in M_2 generation were observed in all the treatments alongwith the untreated control. Pairwise similarity between parent and mutants derived on the basis of Jaccard's coefficient ranged between 0.021 to 1.0 with a mean of 0.168 in M_1 generation **Table A** while 0.028 to 1.0 with a mean of 0.178 in M_2 generation **Table B**. **Figure i** and **ii** represent the dendrogram obtained by UPGMA (Unweighted Pair Group Method using Arithmetic Average Method) clustering of similarity matrix, similarly it has been reported in Solanum melanogena L. and its wild relatives (Karihaloo et al., 2002). Singh and Shashtry (1977a, b) and Tallbery (1981a, b) confirmed that the alteration of protein composition is due to mutated genes. Protein and their respective pattern with regards to appearance of new bands and disappearance of old band and relative mobility and colouration of band in mutants confirm alteration in polypeptides of seed protein due to gene mutation (Gottschalk and Wolff, 1983). The seed storage protein profiling using SDS-PAGE has been reported as the potential to make a distinction between parents and mutants (Amjad et al., 2009). In the present study, Jaccards Coefficient was calculated using Unweighted Pair Group Method by Arithematic Mean (UPGMA). A polypeptide band is present in its mutants while absent in parent cultivar treatment. The mutant was polymorphic compared to other mutants in M_1 and M_2 generation. Similar observation has been reported in Chrysanthemum (Kumar et al., 2006). Genetic distance between all 15 treatments varied from 0.028 to 0.319 and 0.026 to 0.26 as reveled by Jaccard similarity coefficient. Similar observation has been reported in Chrysanthemum and its radio-mutants (Kumar et al., 2006).

The dendrogram obtained on the basis of Jaccard similarity coefficient using UPGMA method represent one parent and 14 mutant into 3 major clusters A, B, C; cluster A consists of T_1, T_6, T_7, T_8, T_9 parent cultivar T_1 and 4 mutant T_6, T_7, T_8, T_9, cluster B consists of mutants T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, cluster C consist of 4 mutant T_2, T_3, T_4, T_5. The cluster C shows more diversity from parents and other mutants in M_1 generation and depicted in the **Figure i.** Cluster 'A' consist of 1 parent and 5 mutant T_1, T_2, T_3, T_4, T_5, T_6 cluster 'B' consist of 6 mutant T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, cluster 'C' consist of 3 mutant T_7, T_8, T_9, cluster 'C' showed more diversity from the parent and other mutants in M_2 generation diversity decreased in M_2 generation in present investigation and depicted in the **Figure ii**.

Electrophoresis (SDS-PAGE) of seed storage proteins could be used to assess genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes (Amjad et al., 2009).

CONCLUSION

The chemical and physical mutagen showed the potential to cause the mutation in the wild chickpea. The SDS protein profile in M_1 and M_2 generation showed polymorphic banding pattern with respect to the untreated control treatment. The variation was found to be present between control and mutants. The electrophoregram of mutants showed the deviation from the parent on the basis of the Jaccards similarity coefficient in the present study. The morphological traits in 20 KR gamma rays treatment were found to be superior over all the treatment including control. The 0.1%, 0.2% EMS, 20 KR gamma rays treatment revealed the increase in protein in the electrophoretic study.

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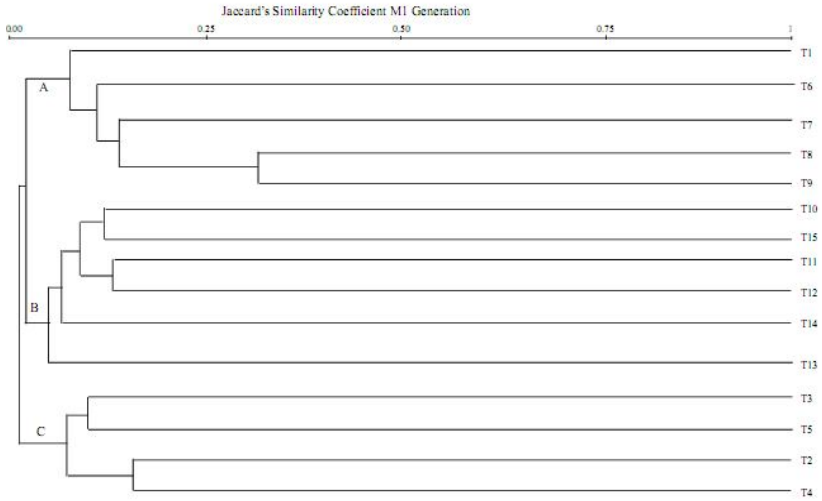

Figure i. UPGMA dendrogram based on Jaccard's Similarity of Seed Protein in Wild *Cicer reticulatum* L. Parent and its Mutants in M_1 Generation.

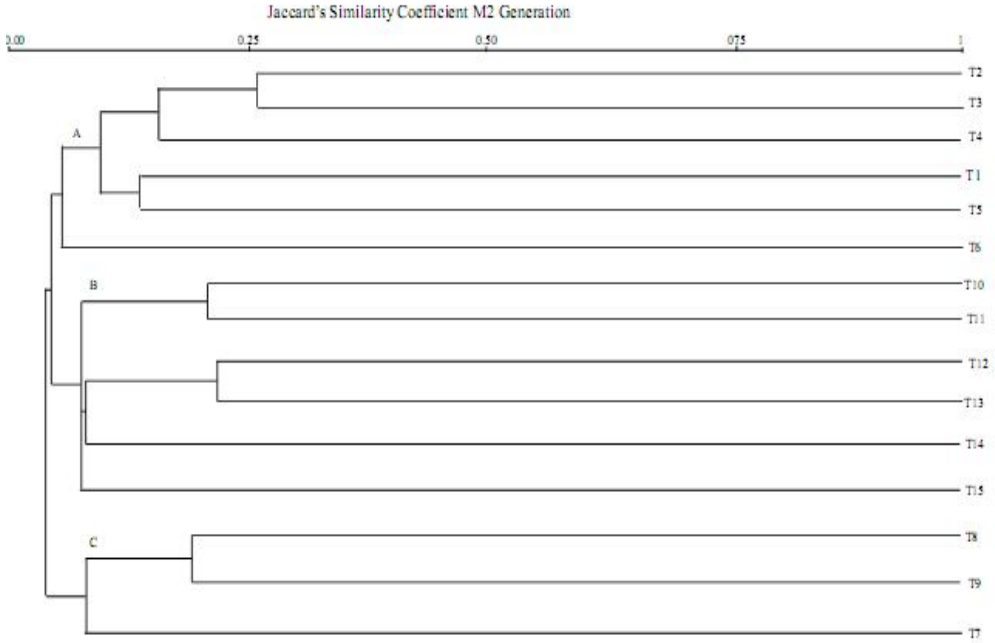

Figure ii. UPGMA dendrogram based on Jaccard's Similarity of Seed Protein in Wild *Cicer reticulatum* L. Parent and its Mutants in M_2 Generation.

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Table A Similarity Matrix in M_1 generation of wild chickpea *Cicer reticulatum* L. and its mutants

T1	1															
T2	0.03	1														
T3	0.03	0.061	1													
T4	0.031	0.16	0.075	1												
T5	0.056	0.065	0.100	0.104	1											
T6	0.097	0.000	0.000	0.000	0	1										
T7	0.097	0.075	0.036	0.038	0.067	0.120	1									
T8	0.129	0.035	0.000	0.036	0.065	0.160	0.319	1								
T9	0.097	0.000	0.036	0.000	0.000	0.077	0.077	0.075	1							
10	0.118	0.000	0.065	0.000	0.029	0.000	0.067	0.032	0.072	1						
T11	0.089	0.033	0.033	0.034	0.094	0.034	0.034	0.000	0.000	0.094	1					
T12	0.122	0.033	0.000	0.000	0.094	0.000	0.000	0.031	0.034	0.03	0.134	1				
T13	0.058	0.000	0.033	0.069	0.061	0.000	0.034	0.031	0.069	0.021	0.097	0.030	1			
T14	0.086	0.000	0.000	0.000	0.000	0.031	0.000	0.032	0.000	0.059	0.03	0.129	0.030	1		
T15	0.055	0.000	0.031	0.032	0.089	0.000	0.000	0.000	0.000	0.122	0.125	0.000	0.091	0.058	1	
	2.095	1.462	1.409	1.313	1.499	1.422	1.531	1.201	1.175	1.326	1.386	1.159	1.121	1.058	1	20.157
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	120

Table B. Similarity Matrix in M_2 generation of wild chickpea *Cicer reticulatum* L. and its mutants

T1	1															
T2	0.086	1														
T3	0.125	0.26	1													
T4	0.115	0.194	0.13	1												
T5	0.148	0.028	0.13	0.118	1											
T6	0.122	0.028	0.032	0.029	0.059	1										
T7	0.091	0.031	0.033	0.000	0.030	0.018	1									
T8	0.084	0.028	0.030	0.000	0.028	0.029	0.063	1								
T9	0.056	0.029	0.031	0.028	0.028	0.061	0.097	0.194	1							
T10	0.167	0.032	0.108	0.063	0.031	0.067	0.034	0.033	0.065	1						
T11	0.097	0.000	0.035	0.032	0.065	0.034	0.035	0.000	0.033	0.209	1					
T12	0.173	0.067	0.072	0.065	0.032	0.034	0.072	0.033	0.000	0.075	0.038	1				
T13	0.063	0.000	0.035	0.032	0.032	0.000	0.000	0.000	0.033	0.116	0.077	0.218	1			
T14	0.134	0.033	0.072	0.000	0.000	0.034	0.035	0.000	0.000	0.116	0.038	0.000	0.167	1		
T15	0.094	0.032	0.034	0.000	0.000	0.067	0.034	0.065	0.065	0.072	0.000	0.116	0.036	0.075	1	
	2.555	1.762	1.742	1.367	1.305	1.344	1.37	1.325	1.196	1.588	1.153	1.334	1.203	1.075	1	21.319
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	120

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