Biotechnological Protein Profiling Study in Wild Chickpea Treated with Ethyl Methane Sulphonate

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ABSRACT

The genus Cicer belongs to the family Papilionaceae (Leguminosae) with 9 annual species and 31 perennial species, of which only one of 9 annuals species is under cultivation- Cicer arietinum. The level of polymorphism is very low within the cultigen as has been reported by many workers. The available genetic variation has been utilized in the breeding programmes which culminate into narrowing of the genetic base for this crop. The seed storage protein profile by SDS-PAGE Electrophoresis has been studied in wild chickpea treated with chemical mutagens EMS in M_3 generation. The comparative analysis on the basis of Jaccard's similarity coefficient matrix and UPGMA clustering has been studied. The banding pattern revealed polymorphism in the present investigation with 7 to 12 major band while other minors in control and EMS-mutants. The polypeptide bands of various sizes ranging from 6.13 KDa to120.24 KDa were observed in all the treatments alongwith the control parent. The genetic distance between all 5 treatments varied from 0.2 to 0.6 as reveled by Jaccard similarity coefficient matrix. The Cluster analysis separated control and EMS-mutants into different group and genetic distance observed between them low.

Key words: - Wild Chickpea, Jaccards similarity matrix, EMS, EMS-mutant, UPGMA.

Introduction

The grain legumes play important primary role as a sources of proteins and as well as a good substitute to animal proteins in human diet. The Chickpea is one of the important grain legume cereal crops and has been classified as a recalcitrant crop due to low level of genetic variation in cultigens therefore; the genetic advance improvement is constrained in the crop. It has been reported that there is no any significant alteration in the protein content followed by physical and chemical mutagenic treatment in cultigens chickpea (Kharkwal, 1980 a, b). The mutation breeding is one of the useful techniques to increase the genetic variability and to change the quantitative and qualitative characters of the seed protein in many crops (Amrishahi and Tavakoli, 1970). The desired genes from wild species could be introgressed into the cultigens to maximize and broaden the genetic base of the crop (Van Rheenen et al., 1993). The protein electrophoretic pattern is directly related with the genetic background and could be used for certification of the genetic makeup (Asghar et al., 2003). The banding pattern of protein of each variety is species specific and particular (Asghar et al., 2003). The protein electrophoregram of Cicer reticulatum shows the close resemblance with that of the cultigens (Ladizinsky and Adler,

1975). The electrophoresis of the seed protein can be used as a significant and effective tool to analyze the genetic variation in plant genetic resources (Asghar et al., 2003).The induced mutation can be used to create the additional variability into the existing germplasm (Amjad et al., 2009). Inter and intra specific variation in seed protein has been reported in various cereal crops such as wheat, barley and their wild relatives (Masood et al., 1994). Ladizinsky and Adler (1975) reported that protein electrophoretic profiles of Cicer reticulatum resemble closely to that of Cicer arietinum. The SDS-PAGE electrophoresis of total seed storage protein has been reported as a useful and effective method to analyze genetic variation in plant genetic resources (Asghar et al., 2003).

Material and Method

The seeds of wild Cicer reticulatum L. were obtained from ICRISAT, Patancheru, (AP), India. The seeds were treated with the Ethyl Methane Sulphonate (EMS) solution of four different concentration viz. 0.1%, 0.2%, 0.3%, 0.4% of Ethyl Methane Sulphonate. The treatments were encoded as, E_2, E_3, E_4, and E_5 respectively while untreated control was encoded as C_1. All the treated seeds alongwith the untreated control were sown to raise M_3 generation. The seed yield of M_3 generation were collected and subjected to the electrophoretic study. The test seeds of all the treatments were used for the estimation of molecular weight determination and protein profile.

The seeds were powdered 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) thereafter, centrifuged at 15000×g rpm for 7 Minutes at 4°C in cooling centrifuge. The supernatant was collected and used for SDS-PAGE electrophoresis (Asgar et al., 2003). The quantitation of protein was carried out by Protein-Dye binding assay against bovine serum albumin (BSA) as standard (Bradford, 1976).

The 25 µl protein extracts and 25µl Laemmli buffer mixed together in eppendorff tube followed by the Laemmli protocol (1970).The 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). The SDS- PAGE electrophoresis was followed as per 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) Coomassie Brilliant Blue R- 250 for 5 hours and then distained for 24 hours. The properly destained gels were read on gel documentation system and molecular weight of individual bands calculated with respect to molecular marker weight. Only the consistent bands were taken into account.

The gels were analyzed as (1) for presence of particular band and as (0) for absence of bands tabulated in Table 2. The Pair wise similarities were calculated using Jaccard Similarity Coefficient between the control and its EMS-mutants represented in Table 1. The cluster analysis has been performed by mean of similarity matrix using UPGMA (Sneath and Sokal, 1973; Karihaloo et al., 2002; Kamble and Petkar, 2015).

Jaccards Coefficient Sij = nij / ni + nj – nij---------- Equation 1

Where, Sij- the similarity between lanes i and j, nij-the number of the similar corresponding bands for i and j, ni-total number of bands present in the lane i, nj-total number of bands present in the lane j, nij- total number of bands present in both lane. The dissimilarity can be calculated by means of subtraction of the similarity from one i.e. 1- Sij (Similarity). The clustering was performed using UPGMA (Unweighted Pair Grouping of Mean of Arithmetic Average) Clustering Method (Arjen Van Ooyen, 2001).

Result and Discussion

The total 20 bands in the C_1 treatment were observed representing molecular weight range between 7.94 KDa to 119.08 KDa which is in the conformity with the previous report (Kamble and Patil, 2014; Kamble and Petkar, 2015), whereas the qualitative and quantitative variation

were observed in all other treatments E_2 to E_5. The total number of bands observed in the untreated and induced mutants in the present study are C_1=20, E_2=16, E_3=16, E_4=16, E_5=17 represented in Figure 1. The total 18 and 22 bands has been reported in 21 accession of kabuli chickpea (Cicer arietinum), showing range between 5KDa to 70 KDa by using electrophoretic study (Asgar et al., 2003). The polypeptide bands of different sizes ranging from 6.13 KDa to120.24 KDa were observed in all the treatments alongwith the untreated control in present study. The pairwise similarity between parent and EMS-mutants has been obtained on the basis of Jaccard's coefficient and ranged between 0.2 to 1.0 with a mean of 0.592 represented in Table 1. The genetic distance between all the five treatments varied from 0.288 to 0.6 as revealed by Jaccard similarity coefficient matrix (Table 1).

Table 1: Similarity Matrix in M_3 generation of wild chickpea and EMS- mutants. (Cicer reticulatum L.)

C_1	1					
E_2	0.333333	1				
E_3	0.333333	0.6	1			
E_4	0.285714	0.454545	0.52381	1		
E_5	0.2	0.269231	0.375	0.5	1	
	2.152381	2.323776	1.89881	1.5	1	8.874967
	C_1	E_2	E_3	E_4	E_5	

Cluster analysis separated control and EMS-mutants into groups and genetic distance observed between them low. The observation is in accordance with the similarity indices and has been reported in chrysanthemum (Scott et al., 1994). Figure 2 represents the dendrogram obtained by UPGMA (Unweighted Pair Group Method using Arithmetic Average Method) clustering of similarity matrix; similar observation has been reported in Solanum melanogena L. and its wild species (Karihaloo et al., 2002).

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 in control and it's induced EMS-mutants. The increase in the protein content has been reported in Phaseolus by using the mutagenic treatment and mutation breeding (Prasad et al., 1986). There has been no any significant change in the seed protein content of mutant in the cultivated chickpea (Kharkwal, 1980 a, b; Wani and Anis, 2008).

It has been confirmed that the change in protein composition is due to the mutated genes (Tallbery, 1981a, b). The Protein and corresponding pattern regarding the appearance of new bands and disappearance of old band, relative mobility and colouration of band in mutants reflects the alteration in polypeptides of seed protein due to gene mutation (Gottschalk and Wolff, 1983). The seed storage protein profile has been reported as one of the potential methods to distinguish the parents and mutants (Amjad et al., 2009). Jaccard Coefficient was computed on the basis of Unweighted Pair Group Method by Arithematic Mean (UPGMA). Some polypeptide band is present in EMS- mutants while absent in control. The mutants revealed polymorphic banding pattern as compared to the control in M_3 generation.

The genetic distance between control and induced EMS-mutants varied from 0.2 to 0.6 as reveled by Jaccard similarity coefficient. The similar observation has been reported in Chrysanthemum and its radio-mutants (Kumar et al., 2006), in wild chickpea and its induced mutants(Kamble and Patil, 2014; Kamble and Petkar, 2015). The polymorphism was observed in the banding pattern in the present investigation. The dendrogram derived with the help of Jaccard similarity coefficient by using UPGMA method and EMS-mutants show the deviation from the control parent. The first cluster consists of C_1 and four induced mutants E2, E3, E4, E5, form the second cluster The second cluster shows protein diversity from parents as shown in the Figure 2. The SDS-PAGE Electrophoresis of seed storage proteins could be employed for the assessment and to distinguish the mutants from their parent genotypes.

Conclusion

The chemical mutagenic agent EMS is potent to cause the mutation in the wild chickpea. The SDS-PAGE electrophoregram of seed storage protein in M3 generation revealed the polymorphic banding pattern as compared to the control. The SDS-PAGE electrophoretic pattern of the induced EMS-mutants represented the deviation from the control with low genetic distance on the basis of Jaccards similarity coefficient matrix in present study. The cluster including all the EMS-mutants shows more protein diversity from parents.

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Table 2: Polypeptide bands observed in control wild Cicer reticulatum L. and its mutants treated with EMS in M_3 generation

Sr. No.	Molecular Wt. in KD	C_1	E_2	E_3	E_4	E_5
1.	120.24	0	1	1	0	1
2.	119.08	1	1	1	1	1
3.	116.29	0	0	0	1	1
4.	109.12	1	1	1	1	1
5.	105.93	0	1	0	0	0
6.	103.41	0	0	0	1	1
7.	101.4	0	0	1	0	0
8.	98.46	0	0	0	0	1
9.	96.56	1	1	0	0	0
10.	83.97	1	1	1	1	0
11.	73.01	1	0	0	0	1
12.	70.93	0	1	0	1	1
13.	69.16	0	1	1	1	0
14.	67.84	0	0	0	0	1
15.	65.75	1	1	1	1	0
16.	58.18	1	0	0	0	0
17.	52.39	1	0	0	0	0
18.	51.34	0	1	1	1	1
19.	44.77	1	0	0	0	0
20.	41.84	0	1	1	1	1
21.	38.26	1	1	1	1	1
22.	34.45	1	1	1	0	0
23.	31.02	1	0	0	1	1
24.	28.43	1	0	0	0	0
25.	26.44	0	1	1	0	0
26.	24.72	1	0	0	0	0
27.	24.44	0	0	0	1	0
28.	19.7	1	0	1	0	1

29.	16.54	1	0	0	0	0
30.	14.13	1	1	1	1	0
31.	11.87	1	0	0	0	0
32.	9.62	1	0	1	1	1
33.	8.96	0	0	0	0	1
34.	7.94	1	1	0	0	0
35.	6.13	0	0	1	1	1
36.	**Total**	**20**	**16**	**16**	**16**	**17**

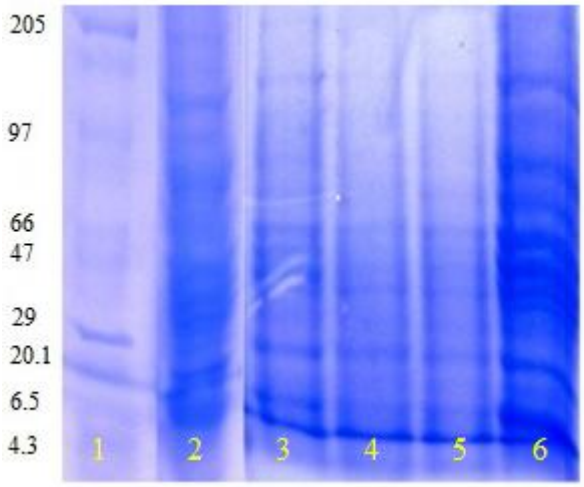

Figure 1: Gel Electrophoresis of the wild chickpea and EMS-mutants showing polypeptide banding pattern.Lane 1=Molecular Weight, Lane 2=Control C_1, Lane3= E_2, Lane 4= E_3, Lane 5= E_4, Lane 6= E_5

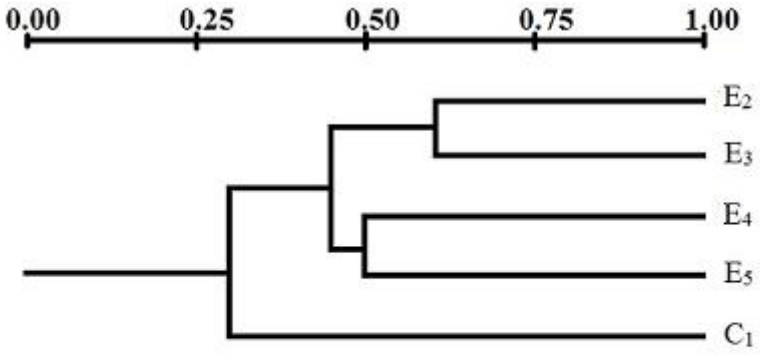

Figure 2. Dendrogram based on Jaccards Similarity Coefficient of Seed Storage Protein in Wild Chickpea and Induced Mutants using UPGMA in M_3 Generation.