Current Research Journal of Biological Sciences 2(4): 250-252, 2010
ISSN: 2041-0778

Submitted Date: May 11, 2010 Accepted Date: May 22, 2010 Published Date: July 20, 2010

Phylogeny and Genetic Diversity Studies in Capsicum Using Seed Storage Protiens

Nazia Akbar, Habib Ahmad, Sajid ul Ghafoor, Imtiaz Ahmed Khan
Department of Genetics, Hazara University, Mansehra

Abstract: Present study was undertaken to estimate genetics diversity in local and exotic genotypes of *Capsicum* (chili) using SDS-PAGE analysis. Total seed proteins were extracted and separated on 12.5% Polyacrylamide gels using standard protocols. Protein fragments of various molecular weight were separated in various *Capsicum* genotypes. Individual protein fragments were considered as allele / loci. Estimates of Genetic Distances (GD) ranged from 0 to 100%. Nineteen *Capsicum* genotypes were clustered in four groups A, B, C and D comprising 7, 5 and 7 genotypes, respectively. It was concluded that genotypes Fehsil Bibber 1 and Ajay Bibber 1 were most distantly related to each other and hence it is recommended that these two genotypes should be crossed to create a segregating population with maximum genetic diversity for the improvement of chili crop in Pakistan. .

Key words: *Capsicum annum*, dendrogram, genetic distance, phylogenetic relationship, seed storage proteins, SDS-PAGE

INTRODUCTION

Capsicum annuum L. belongs to family Solanaceae, is a dicotyledonous flowering plant commonly grown worldwide, with many general names in English, such as hot pepper, chili, chili pepper, and bell pepper etc., (Knapp *et al.,* 2004; Hunziker, 2001). Capsicum has been known as part of the human diet since the beginning of civilization (MacNeish, 1964). It's centre of diversity is believed to be in south-central South America (Gonzalez and Bosland, 1991). Presently Chillies are cultivated on more than 1.5 million hectares in numerous countries especially China, India, Indonesia, Mexico, Korea, Nigeria, Ghana and Turkey (FAO, 2007). The fruits of most *Capsicum* are pungent, because the placenta accumulates capsaicinoids (e.g., capsaicin) (Zewdie and Bosland, 2001; Thompson *et al.,* 2005). Previously chilli accessions were usually classified based on fruit characteristics including pungency, color, shape, flavor, size, and use etc (Smith *et al*., 1987; Bosland, 1992). Recently genetic diversity in *Capsicum* has been studied using morphological, cytological and biochemical marker systems (Kaur and Kapoor, 2001; Gopinath *et al*., 2006). Present research was conducted to analyze phylogeny and genetic diversity in *Capsicum* germplasm using seed storage protein analysis.

MATERIALS AND METHODS

Material used during present study included 20 accessions of *Capsicum* from Turkish origins as well as local accessions collected from Mansehra district. Exotic plant material was kindly provided by Mr. YUCEL, CEO Yucel Turk Construction Company presently located in Hazara University through courtesy of Prof. Dr. Habib Ahmad Dean faculty of science. Initially the accessions were grouped in Information regarding the morphological characters (Table 1).

For SDS-PAGE analysis, at least 10 seeds from each accession were grounded to a fine powder with mortar and pestle. Four hundred ml of protein extraction buffer (Sambrook *et al.*, 1989) was added to 0.01 g of seed flour and vortexed (using Gyro mixer vortex machine) thoroughly to homogenize. The proteins were extracted at room temperature for 20 min. In order to purify, the homogenate samples were centrifuged (using Eppendorf centrifuge model No 0021586) at 12,000 rpm for 10 min at room temperature. The extracted crude proteins were recovered as clear supernatant and were transferred to a new 1.5 mL eppendorf tubes and stored at 4ºC until they were run on the polyacrylamide gel.

The electrophoretic procedure was carried out using slab type SDS-PAGE Model: MGV-202, with 12.5% polyacrylamide gel. A 12.5% resolving gel (3.0M Tris-HCL pH9, 0.4% SDS and 4.5% stacking gel (0.4M Tris-HCL pH 7.0, 0.4% SDS) was prepared and polymerized chemically by addition of 17 ml of N, N', N', N' tetra ethylene diamine and 10% Ammonium persulphate. Electrode buffer solution was poured into the bottom pool of the apparatus. Then electrode buffer (0.025 M Tris, 1.29 M Glycine, 0.125% SDS) was added to the top pool of the apparatus. Fifteen ml of the extracted protein was

Corresponding Author: Imtiaz Ahmed Khan, Department of Genetics, University of Karachi, Karachi, Pakistan

Table 1: Accessions of *Capsicum* used during present study.

S.No.	Sample no.	Accession name	Morphological characteristics	Source
1	1-3	Feshil bibber(FB)	Fruit small, single and downward.	YUCEL, TURK/Turkey
2	4-6	Local chili(LB)	Fruit single and straight.	Mansehra.
3	7-8	Turkish genotype A(TGA)	Fruit single, straight and upward.	YUCEL, TURK/Turkey
4	9	Ajay bibber(AB)	Fruit extra small, single and upward.	YUCEL, TURK/Turkey
5	10-11	Khog bibber(KB)	Fruit large, single, and straight,	YUCEL, TURK/Turkey
6	12-13	Habib Ahmad(HA)	Fruit single, straight, and medium in size.	Donated by Prof. Dr. Habib Ahmad
7	14-15	Tomato bibber(TB)	Fruit single, round and upward.	YUCEL, TURK/Turkey
8	16-17	Dulmalik bibber(DB)	Fruit single, round and downward.	YUCEL, TURK/Turkey
9	18	Pear bibber(PB)	Fruit single and pear in shape.	YUCEL, TURK/Turkey
10	19	Sus bibber (SB)	Fruit straight upward bunch.	YUCEL, TURK/Turkey

Table 2: Genetic distance estimates among 19 genotypes of *Capsicum* using SDS-PAGE analysis

Alleles	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
2	0.7																	
3	0.7	0.0																
4	0.4	0.8	0.8															
5	0.5	1.0	1.0	0.1														
6	0.6	1.0	1.0	0.6	0.5													
7	0.6	0.7	0.7	0.6	0.7	0.6												
8	0.3	0.8	0.8	0.3	0.5	0.5	0.3											
9	0.5	0.8	0.8	0.5	0.6	0.5	0.2	0.1										
10	0.5	0.8	0.8	0.2	0.4	0.8	0.7	0.4	0.5									
11	0.2	0.8	0.8	0.2	0.4	0.7	0.5	0.1	0.3	0.3								
12	0.5	1.0	1.0	0.2	0.1	0.7	0.7	0.4	0.3	0.3	0.3							
13	0.5	1.0	1.0	0.2	0.1	0.7	0.7	0.4	0.3	0.3	0.3	0.0						
14	0.5	0.5	0.5	0.7	0.8	1.0	0.8	0.6	0.8	0.6	0.6	0.8	0.8					
15	0.7	1.0	1.0	0.8	0.8	1.0	1.0	0.8	1.0	0.8	0.8	0.8	0.8	0.5				
16	0.7	1.0	1.0	0.8	0.8	1.0	1.0	0.8	1.0	0.8	0.8	0.8	0.8	0.5	0.0			
17	0.5	0.5	0.5	0.7	0.8	1.0	0.8	0.6	0.8	0.6	0.6	0.8	0.8	0.0	0.5	0.5		
18	0.2	0.8	0.8	0.2	0.4	0.7	0.5	0.1	0.3	0.3	0.0	0.3	0.3	0.6	0.8	0.8	0.6	
19	0.4	0.7	0.7	0.4	0.5	0.6	0.4	0.3	0.2	0.5	0.2	0.5	0.5	0.8	1.0	1.0	0.8	0.2

1=FB-1, 2= FB-2, 3 = TB-1, 4 = TB-2, 5 = TB-3, 6 = AB-1, 7= LB-1, 8= LB-2, 9= LB-3, 10 =LB-4, 11= TG-A1, 12= TG-A 2, 13= TG-B1, 14 = TG-B2, 15= KB-1, 16= KB-2, 17= DB-1, 18= DB-2, 19= SB-1.

loaded with the micropipette into the wells of the gel. The apparatus was connected with constant electric supply (75 V) till the tracking dye "bromophenol blue" (BPB) reaches the bottom of the gel. After electrophoresis the gels were stained with staining solution comprising 0.2% (W/V) Comassie Brilliant Blue (CBB) R 250 dissolved in 10% (V/V) acetic acid, 40% (V/V) methanol for about an hour at room temperature. Gels were destained in a solution containing 5 % (V/V) acetic acid and 20% (V/V) methanol. Gels were shacked using Double Shaker Mixer DH –10 gently until the background of the gel became clear and protein bands were clearly visible. The excess CBB was removed by addition of piece of tissue paper Kim wipes in the distaining solution. After destaining the gels were photographed using gel documentation systems"Uvitech".

For genetic diversity analysis, every scorable band was considered as single allele/locus and was scored 1 for presence or 0 for absence. The bivariate 1-0 data were used for estimation of Genetic Distance (GD) following Unweighted Pair Group of Arithmetic Mean (UPGMA) procedures described by Nei and Li (1979). The dendogram was prepared using computer programmed Popgene32 version 1.31 (Yeh *et al*., 1999).

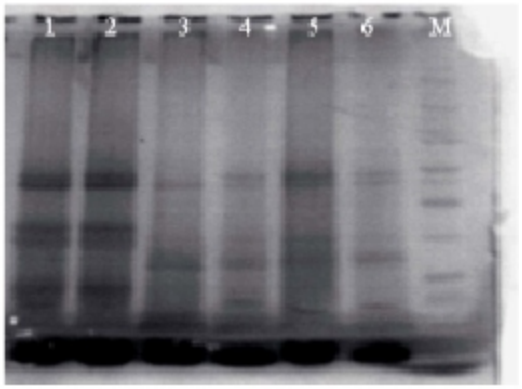

Fig. 1: Total seed storage protein profile of 6 *Capsicum* genotypes using SDS-PAGE 1=FB-1, 2= FB-2, 3 = TB-1, 4 = TB-2, 5 = TB-3, 6 = AB-1, M= Molecular weight marker

RESULTS AND DISCUSSION

Nineteen *Capsicum* genotypes were characterized on the basis of seed storage proteins using SDS-PAGE. An example of SDS-PAGE analysis are presented in Fig. 1. Every protein band was considered as a single locus /

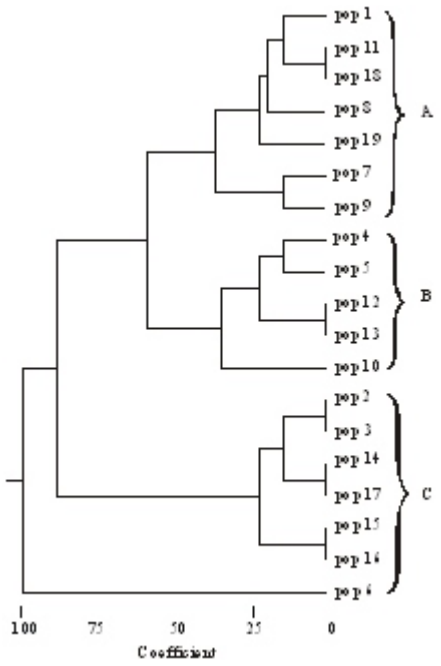

Fig. 2: Dendrogram constructed for 19 *Capsicum* genotypes using data obtained from SDS-PAGE 1= FB-1, 2 = FB-2, 3 = TB-1, 4 = TB-2, 5 = TB-3, 6 = AB-1, 7 = LB-1, 8 = LB-2, 9 = LB-3, 10 =LB-4, 11 = TG-A1, 12 = TG-A 2, 13 = TG-B1, 14 = TG-B2, 15= KB-1, 16= KB-2, 17 = DB-1, 18 = DB-2, 19 = SB-1.

allele. Alleles were scored as present (1) or absent (0). Bivariate (1-0) data matrix was generated. In total 73 alleles were observed in 19 genotypes giving an average of 3.8 alleles per genotype. Genetic variability among the genotypes was estimated using Nei and Li (1979) procedure of UPGMA (Unweighted Pair Group of Arithmetic Means). High range of genetic distances (GD = 0-100%) was estimated among various comparisons (Table 2). Five comparisons showed complete homozygosity (G.D = 0%) while twenty-two comparisons showed maximum genetic distance estimates (G.D = 100 %). Remaining comparisons showed GD ranging from 10 to 80 %. The bivariate data obtained from SDS-PAGE analysis was also used for the construction of Dendrogram using computer program "Popgene ver 32" (Fig. 2). Nineteen *Capsicum* genotypes were grouped in 3 clusters viz; A, B and C comprising 7, 5 and 7 genotypes, respectively. Genotypes Feshil bibber and Ajay bibber were most distantly related to each other. It is recommended that these two genotypes (Feshil bibber and Ajay bibber) should be crossed to create more genetic diversity in *Capsicum* breeding programs.

REFRENCES

Bosland, P.W., 1992. Chiles: A diverse crop. Hort. Technol., 2: 6-10.

FAO (Food and Agriculture Organization of the United Nations), 2007. FAO Production Yearbook. FAO, Rome, pp: 333.

Gonzalez, M. and P. Bosland, 1991. Strategies for stemming genetic erosion of *Capsicum* germplasm in the Americas. Diversity, 7(1-2): 52-53.

Gopinath, K., N.V. Radhakrishnan and J. Jayaral, 2006. Effect of propiconazole and difenoconazole on the control of anthracnose of chili fruit caused by *Colletotrichum capsici*. Crop Prot., 25: 1024-1031.

Hunziker, A.T., 2001. Genera Solanacearum: The Genera of Solanaceae Illustrated, Arranged According to a New System. Gantner Verlag, Ruggell, Liechtenstein, pp: 516.

Kaur, C. and H.C. Kapoor, 2001. Antioxidants in fruits and vegetables - the millennium's health. Int. J. Food Sci. Tech., 36: 703-725.

Knapp, S., L. Bohs, M. Nee and D.M. Spooner, 2004. Solanaceae a model for linking genomics with biodiversity. Comp. Funct. Genom., 5: 285-291.

MacNeish, R.S., 1964. Ancient mesoamerican civilization. Science, 143: 531-537.

Nei, N. and W. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., 76: 5269-5273.

Sambrook, K.J., E.F. Fritsch and T. Manantis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Harbor Laboratory Press, New York.

Smith, P.G., B. Villalon and P.L. Villa, 1987. Horticultural classification of pepper grown in the United States. Hort. Sci., 22: 11-13.

Thompson, R.Q., K.W. Phinney, M.J. Welch and E. White, 2005. Quantitative determination of capsaicinoids by liquid chromatography-electrospray mass spectrometry. Anal. Bioanal. Chem., 381: 1441-1451.

Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao, 1999. POPGENE 3.2, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada. Retrieved from: http://www.ualberta.ca./~fyeh/fyeh.

Zewdie, Y. and P.W. Bosland, 2001. Capsaicinoid profiles are not good chemotaxonomic indicators for *Capsicum* species. Biochem. Syst. Ecol., 29: 161-169.