EXPLORATION OF GENETIC DIVERSITY IN VARIOUS MOMORDICA CHARANTIA L. ACCESSIONS BY SDS-PAGE ANALYSIS

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Abstract

Momordica charantia L. (bittergourd) global is cultivation is increasing throughout the world more due to its medicinal potential and nutritional importance. Genetic diversity assessment is among the essential requirements to study the potential of any plant species. Moreover, selection of highly diverse germplasm may also lead towards improvement in its productivity. Therefore, this study was conducted to explore the genetic diversity of 31 *M. charantia* accessions from 11 different countries using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Allelic variation of 13 protein bands was observed on the gel, with molecular weights ranging from 11-120 kDa. Among them, the medicinally valued proteins with molecular weights 11 kDa (anti-hyperglycemic) and 30 kDa (anti-HIV) were found to be the most abundant, showing the importance of the selected germplasm. Similarities and dissimilarities among the accessions were explored by making correlation and cluster analyses. The greatest genetic diversity was found in accessions TOT6001 (Taiwan) and TOT4150 (Viet Nam), while the least diversity was found in accession TOT0950 (Thailand). The genetically diverse accessions were considered as the most adaptable genotypes, under various environmental comditions. These selected accessions could be further explored by advance biochemical techniques to take the advantage through future breeding programs.

Keywords: Accessions, Genetic diversity, *Momordica charantia*, SDS-PAGE

Introduction

Bitter gourd (*Momordica charantia* L.) is an important cucurbitaceous vegetable crop grown throughout the world. Every part of this plant has nutritive and medical significance (Saha and Chatterjee, 2022). Its seeds contain 41-45% of essential oil which is ten times greater than the industrially important Tung oil due to its oleostearic and stearic acid contents (Jatav *et al*., 2019). The diversity among morphological characters of this plant species provides relatively broad range of phenotypic variation (Behera *et al*., 2006). Bitter gourd contains alkaloids, insulin like peptides and a mixture of steroidal sapogenins known as charantin which are reported to be helpful in controlling diabetes (Raman and Lau, 1996). Bitter gourd seed proteins, alpha and beta momarcharin, were found to be inhibitory against AIDS (Bodeker *et al*., 2006).

Success in plant breeding depends upon the existence of genetic variability present in the breeding materials. It is proved that larger the variability, the greater is the scope for selection and improvement (Yadav *et al*., 2008). For better improvement in crop yield, it is necessary to acquire information on genetic variability and heritability. Genetic diversity assessments may increase the effectiveness of breeding programs for quality production of the crop, in terms of

greater yield (Behera *et al*., 2008). Morphological characterization is unable to provide accurate data for the estimation of genetic diversity due to multiple alleles (Rashid *et al*., 2014). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is the most widely used biochemical technique to assess the genetic variability due to its validity and simplicity for describing the genetic structure of crop germplasm (Mohamed and Aly, 2005; Rashid *et al*., 2014). Seed storage proteins are synthesized in developing seeds when cell division is complete. The proteins of this stage are more stable and largely independent of environmental fluctuations (Murphy *et al*., 1990; Ghafoor *et al*., 2002; Franzmann and Alberti, 2019). Despite the significant progress in genetic characterization of different plant species, many other nutritionally and medicinally important plant species remain genetically uncharacterized. Therefore, this investigation was aimed to explore the genetic diversity of 31 accessions of *Momordica charantia* in terms of protein profiling.

Materials and Methods

The experiments were carried out to genetically analyse 31 different accessions (Table 1) of bitter gourd (*Momordica charantia* L.) originating from 11 different countries utilizing Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Seeds of 31 bitter gourd accessions were obtained from The World Vegetable Center (Taiwan) and National Gene bank of Pakistan (National Agricultural Research Center, Islamabad).

Protein Extraction: To study the storage proteins, a few seeds from each accession were crushed in a mortar and pestle. Urea buffer was used for protein extraction (Rashid *et al*., 2014). Protein extraction buffer was prepared by adding 0.5 M tris HCl pH 8.0, 5 M urea, 0.2 % SDS and 1% 2-mercaptoethanol in distilled sterilized water. Tris HCl was dissolved in distilled water and pH was adjusted to 8.0. The solution was autoclaved, and urea and SDS were added to it. At the time of extraction, 2-mercaptoethanol and PMSF (protease inhibitor) were added. Bromophenol blue was used as tracking dye. Extraction buffer (1 ml) was added to 500 mg ground seed sample in 1.5ml eppendorf tube for centrifugation. Tubes were placed in boiling water for 5 minutes. Further centrifugation was performed at 15000 rpm to get clear supernatant, which was then transferred into new sterilized eppendorf tube.

Gel Preparation: Resolving gel of 12 % was prepared by adding 30 % acrylamide, 1.5 M tris pH 8.8, 10 % SDS, 10 % APS and TEMED to distilled water (Rashid *et al*., 2014). Casting apparatus was assembled and resolving gel was poured between gel plates. Small amount of n-butanol was poured onto the resolving gel and left for atleast 10 minutes to allow polymerization. Stacking gel was prepared by adding 30 % acrylamide, 1 M tris pH 6.8, 10 % SDS, 10 % APS and TEMED in distilled water. After the resolving gel solidified, the stacking gel was poured on it. A clean comb was inserted in stacking gel in order to make wells. The casting apparatus was left for the stacking gel to polymerize and solidify. Following gel solidification, the comb was carefully removed.

Electrophoresis: Electrophoresis was performed utilizing vertical slab gel in discontinuous buffer system. Reducing buffer was prepared by adding 0.025 M tris, 0.129 g glycine and 0.125% SDS in distilled water. Protein extract from each sample was loaded on the gel along with a standard protein marker (Xpert Pre-Stained Protein Marker with broad range; 200-10 kDa). The buffer tank was filled with reducing buffer, covered and connected to continuous power supply (100 V). After two hours of gel run, the gel was carefully separated from gel plates and dipped in staining solution. Staining solution was prepared by mixing methanol, glacial acetic acid, distilled water and Coomassie Brilliant Blue (50:10:40:0.3). The gel was kept in staining solution for two hours on an orbital shaker. After staining and appearance of bands on the gel, it was shifted in de-staining solution which was prepared by mixing methanol, glacial acetic acid and distilled water (50:10:40) and left overnight. After de-staining, the gel was analyzed in gel documentation system.

Statistical Analysis: Unknown protein values were determined from the standard curve of protein marker. Data was analysed by finding correlation among the accessions based on protein profiles. A dendrogram, showing cluster analysis of bitter gourd accessions, was created with the help of statistical software (SPSS v 13.0).

Results

Seed storage proteins of 31 accessions of *Momordica charantia* L. from eleven different countries were analysed using the SDS-PAGE technique. The best banding pattern of proteins was obtained on 12% polyacrylamide gel, representing the number and intensities of protein bands (Figure 1). The whole banding pattern was divided into three distinct sections based on band intensity, i.e., dark stained, medium stained and light stained band. In this study, the protein bands ranged from 11 to 200 kDa, were distributed in all three sections (Figure 1). A total of 13 bands was analysed, of which 4 bands were identified as dark stained ranging from 60-120 kDa, while 5 were medium stained and 4 were light stained bands ranging from 20-200 kDa and 11-50 kDa respectively.

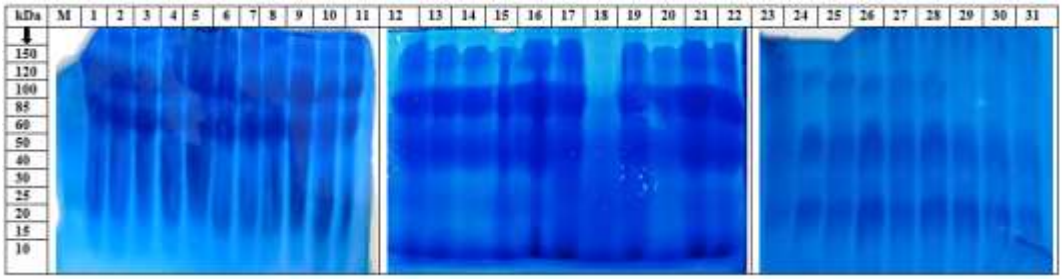

Fig 1. Polyacrylamide gel banding pattern of proteins of thirty-one accessions of *Momordica charantia*

Keys: M-Marker, 1= 32573, 2=32574, 3=32572, 4=34162, 5=32737, 6=TOT5869, 7=TOT5847, 8=TOT6001, 9=TOT5111, 10=TOT4358, 11=TOT2407, 12=TOT2533, 13=TOT6234, 14=TOT6236, 15=TOT7308, 16=TOT1843, 17=TOT1850, 18=TOT0950, 19=TOT1008, 20=TOT0899, 21=TOT4150, 22=TOT4131, 23=TOT6851, 24=TOT6831, 25=TOT6918, 26=TOT1567, 27=TOT1504, 28=TOT1568, 29=TOT7025, 30=TOT7027, 31=TOT7029

Table 1: Origin of thirty-one accessions of *Momordica charantia.*

No.	Vegetable Introduction No.	Accession No.	Country	Number of Accessions
1.	VI039826	TOT1567	Philippines	3
2.	VI039827	TOT1568	Philippines	
3.	VI041223	TOT1504	Philippines	
4.	VI057025	TOT6831	Cambodia	3
5.	VI057052	TOT6851	Cambodia	
6.	VI057150	TOT6918	Cambodia	
7.	VI039908	TOT0899	Thailand	3
8.	VI039970	TOT0950	Thailand	
9.	VI040117	TOT1008	Thailand	
10.	VI055378	TOT7308	Bangladesh	3
11.	VI050840	TOT6234	Bangladesh	
12.	VI050842	TOT6236	Bangladesh	
13.	VI047525	TOT4131	Viet Nam	2
14.	VI047550	TOT4150	Viet Nam	
15.	VI054864	TOT7025	Lao People's Democratic Republic	3
16.	VI054866	TOT7027	Lao People's Democratic Republic	
17.	VI054868	TOT7029	Lao People's Democratic Republic	
18.	VI043046	TOT1843	Indonesia	2
19.	VI043053	TOT1850	Indonesia	
20.	VI044608	TOT2407	India	2
21.	VI049940	TOT2533	India	
22.	VI049943	TOT5869	Sri Lanka	2
23.	VI049944	TOT5847	Sri Lanka	
24.	VI048307	TOT4358	Taiwan	3
25.	VI049284	TOT5111	Taiwan	
26.	VI050151	TOT6001	Taiwan	
27.	01	32572	Pakistan	5
28.	02	32573	Pakistan	
29.	03	32574	Pakistan	
30.	04	32737	Pakistan	
31.	05	34162	Pakistan	

In this study, protein bands with molecular weights of 120, 60, 50 and 20 kDa were the only bands present in all the accessions (Table 2). Accession TOT0950 from Thailand contained the least number of bands (7 bands). Protein bands of 11 and 40 kDa were the most abundant protein bands found in a total of 29 and 30 accessions respectively. Another protein with 30 kDa weight was found in 25 accessions. On the basis of protein banding pattern, accessions TOT6234, TOT6236, TOT7308 (from Bangladesh), TOT0899 (from Thailand), TOT1504 and TOT1568 (from Philippines) and TOT6851 (from Cambodia) were genetically similar to one another while accession 32737 (from Pakistan) and TOT5111 (from Taiwan) had minor differences in their banding patterns. Similarly, accessions TOT5847, TOT1843, TOT1850, TOT1008, TOT4150 and TOT4131 had similar protein bands with minor distinctions. Band of 120 kDa was missing in accessions TOT7025, TOT7027 and TOT7029, belonging to Lao People’s Democratic Republic.

Polyacrylamide gel data was analysed by multivariate analyses including correlation and cluster analysis on the basis of presence or absence of protein bands in different accessions. All the investigated accessions were correlated with a range of 0.03-1.00 (Table 3). The accessions with the same correlation value had the same protein profile. Whereas the least values showed their minimum correlation with a value of 0.03. Negative correlation was also observed in the range from -0.06 to -0.62 among different accessions.

Table 3. Correlation among protein profile of thirty-one accessions of *Momordica charantia*

	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
1																															
2	0.59																														
3	0.31	0.53																													
4	0.23	0.13	0.53																												
5	0.03	0.27	0.68	0.27																											
6	0.59	0.13	-0.16	0.13	-0.23																										
7	0.23	0.13	-0.16	0.57	-0.23	0.57																									
8	0.31	-0.16	-0.08	0.53	-0.12	0.53	0.53																								
9	-0.39	-0.23	-0.12	-0.23	0.41	-0.23	-0.23	-0.12																							
10	0.07	-0.14	-0.27	-0.14	0.03	0.23	0.23	0.31	0.46																						
11	-0.10	0.32	-0.23	-0.43	0.10	-0.06	-0.06	-0.23	0.54	0.54																					
12	0.05	0.03	-0.19	0.03	-0.28	-0.37	0.03	-0.19	-0.28	-0.28	-0.18																				
13	-0.24	-0.51	-0.27	-0.14	0.03	0.23	-0.14	0.31	0.46	0.38	0.22	-0.62																			
14	0.23	0.13	0.53	0.13	0.27	0.13	-0.30	-0.16	-0.23	-0.51	-0.43	0.03	-0.14																		
15	0.23	0.13	0.53	0.13	0.78	0.13	-0.30	-0.16	0.27	-0.14	-0.06	-0.37	0.23	0.57																	
16	0.39	0.43	0.43	0.43	0.18	0.03	0.03	0.43	-0.28	0.05	0.16	-0.08	0.05	0.03	0.03																
17	0.31	0.53	1.00	0.53	0.68	-0.16	-0.16	-0.08	-0.12	-0.27	-0.23	-0.19	-0.27	0.53	0.53	0.43															
18	-0.24	-0.14	0.31	0.23	0.46	-0.14	-0.14	-0.27	0.03	-0.24	-0.10	0.05	0.07	0.59	0.59	0.05	0.31														
19	0.46	0.27	0.68	0.27	0.41	0.27	-0.23	-0.12	-0.18	-0.39	-0.34	-0.28	0.03	0.78	0.78	0.18	0.68	0.46													
20	-0.14	0.13	0.53	0.13	0.78	-0.30	-0.30	-0.16	0.27	-0.14	-0.06	0.03	-0.14	0.57	0.57	0.03	0.53	0.59	0.27												
21	0.31	-0.16	-0.08	-0.16	-0.12	0.53	-0.16	-0.08	-0.12	-0.27	-0.23	-0.19	0.31	0.53	0.53	-0.19	-0.08	0.31	0.68	-0.16											
22	0.03	0.27	0.68	0.27	0.41	-0.23	-0.23	-0.12	-0.18	-0.39	-0.34	-0.28	-0.39	0.27	0.27	0.18	0.68	0.03	0.41	0.27	-0.12										
23	-0.28	0.03	-0.19	-0.37	0.18	0.03	0.03	-0.19	0.18	0.05	0.16	-0.44	0.05	-0.37	0.03	-0.44	-0.19	-0.28	-0.28	0.03	-0.19	0.18									
24	-0.51	-0.30	-0.16	0.13	0.27	-0.30	0.13	-0.16	0.27	-0.14	-0.06	0.03	-0.14	-0.30	0.13	-0.37	-0.16	0.23	-0.23	0.13	-0.16	0.27	0.43								
25	-0.39	-0.23	-0.12	-0.23	0.41	-0.23	-0.23	-0.12	0.41	0.03	0.10	0.18	0.03	0.27	0.27	-0.28	-0.12	0.46	-0.18	0.78	-0.12	-0.18	0.18	0.27							
26	-0.51	-0.30	-0.16	0.13	-0.23	-0.30	0.13	-0.16	-0.23	-0.51	-0.43	0.43	-0.51	0.13	-0.30	-0.37	-0.16	0.23	-0.23	0.13	-0.16	0.27	0.03	0.57	0.27						
27	0.03	-0.23	-0.12	-0.23	-0.18	0.27	-0.23	-0.12	-0.18	-0.39	-0.34	-0.28	0.03	0.27	0.27	-0.28	-0.12	0.03	0.41	-0.23	0.68	0.41	0.18	0.27	-0.18	0.27					
28	-0.14	0.13	-0.16	0.13	-0.23	-0.30	0.13	-0.16	-0.23	-0.51	-0.06	0.43	-0.51	-0.30	-0.30	0.03	-0.16	-0.14	-0.23	-0.30	-0.16	0.27	0.03	0.57	-0.23	0.57	0.27				
29	-0.51	-0.30	-0.16	0.13	-0.23	-0.30	0.13	-0.16	-0.23	-0.14	-0.06	0.03	-0.14	-0.30	-0.30	0.03	-0.16	0.23	-0.23	-0.30	-0.16	0.27	0.03	0.57	-0.23	0.57	0.27	0.57			
30	-0.51	-0.30	-0.16	-0.30	-0.23	-0.30	-0.30	-0.16	0.27	0.23	0.32	-0.37	0.23	-0.30	-0.30	0.03	-0.16	-0.14	-0.23	-0.30	-0.16	0.27	0.03	0.13	-0.23	0.13	0.27	0.13	0.57		
31	-0.41	-0.06	-0.23	-0.06	-0.34	-0.43	-0.06	-0.23	0.10	-0.10	0.35	0.16	-0.10	-0.43	-0.43	0.16	-0.23	-0.10	-0.34	-0.43	-0.23	0.10	-0.18	0.32	-0.34	0.32	0.10	0.69	0.69	0.69	1

Keys: 1= 32573, 2=32574, 3=32572, 4=34162, 5=32737, 6=TOT5869, 7=TOT5847, 8=TOT6001, 9=TOT5111, 10=TOT4358, 11=TOT2407, 12=TOT2533, 13=TOT6234, 14=TOT6236, 15=TOT7308, 16=TOT1843, 17=TOT1850, 18=TOT0950, 19=TOT1008, 20=TOT0899, 21=TOT4150, 22=TOT4131, 23=TOT6851, 24=TOT6831, 25=TOT6918, 26=TOT1567, 27=TOT1504, 28=TOT1568, 29=TOT7025, 30=TOT7027, 31=TOT7029

To identify the similarity linkage among thirty-one accessions of *Momordica charantia* L., cluster analysis was carried out by using un-weighted pair group method with arithmetic mean, based on the similarities and differences in total seed proteins. This analysis has categorized the evaluated accessions into different clusters displaying their grouping on the basis of protein profiles (Figure 2). According to the clusters formed in the present study, all accessions were divided into two major groups 1 and 2. Group 1 consisted of only one accession TOT6234 while group 2 was divided into 2I and 2II. Group 2I consisted of accession TOT4358 whereas group 2II was further divided into three groups of 2IIA, 2IIB and 2IIC. Group 2IIA and group 2IIB contained accessions 32573 and TOT0950 respectively. Group 2IIC was sub divided into four sub groups; 2IICa, 2IICb, 2IICc and 2IICd. These sub groups were further divided on the basis of minor differences in their protein profiles.

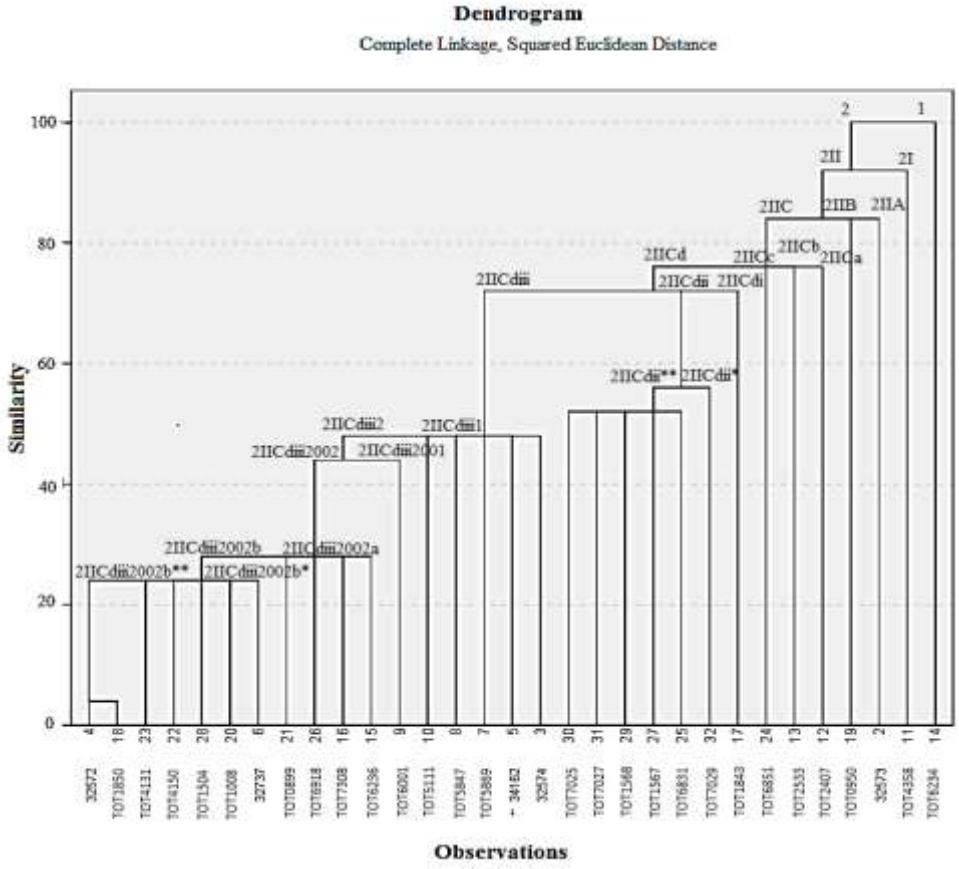

Fig 2. Dendrogram of protein profiles of thirty-one accessions of *Momordica charantia*

Discussion

In this study, the genetic diversity of 31 accessions from 11 different countries was explored utilizing the SDS-PAGE technique. Seeds were used for this purpose, as seed proteins are least affected by environmental fluctuations (Ghafoor *et al*., 2003; Rashid *et al*., 2014). This technique has been used by a number of authors, to genetically analyse the diversity among various plant species (Ghafoor *et al*., 2003; Jatoi *et al*., 2011; Masoumi *et al*., 2012; Rashid *et al*., 2014). In this study, a medicinally valued protein with 11 kDa molecular weight was found to be the most abundant protein in all accessions. According to Khanna *et al.* (1981), the protein with the same molecular weight was reported in *Momordica charantia* fruit pulp with hyperglycemic activity in gerbils, langurs and also diabetic patients.

A protein of 30 kDa was observed to be the second most abundant protein in this research. Grover and Yadav (2004) reviewed that an MAP30 protein with a 30 kDa molecular weight, plays a significant anti-HIV role. Another protein with 24 kDa molecular weight, named *momordin* by Lin *et al.* (1978), was found in 23 of the investigates accessions Similarity

among protein profiles was also observed in some of the accessions, which may be due to the same specific origin of the individuals. In a genetic study of *Spinacia oleracea*, all the accessions were found to be similar except for 3 accessions; from Lahore, Peshawar and AVDRC (Rashid *et al*., 2014). A high percentage of genetic similarity was due to the local origin of those spinach accessions. On the other hand, the accessions investigated in this study originate from different countries, with comparatively more environmental differences.

Diversity of proteins in different genotypes might distinguish them at inter and intraspecific level and to make record of genetic resources (Rahman *et al*., 2004). In this study, seed proteins of all the accessions were analysed by showing different levels of correlations among their proteins. Moreover, cluster analysis was used for the grouping of accessions under study, based on protein profiles. Similar genetic analyses were carried out by Murtaza *et al*. (2005) and Rashid *et al*. (2014) for *Gossypium* and *Spinacia* germplasms, respectively, to study the genetic variations among various accessions.

Conclusion

Results have indicated significant diversity in protein banding pattern among accessions of *Momordica charantia*. Maximum diversity was found in accession TOT6001 from Taiwan indicated by its maximum number of protein bands. Moreover, medicinally valued proteins, with 11, 24 and 30 kDa molecular weights, were also found to be among the abundant proteins in the evaluated accessions. Correlation and cluster analyses showed minor differences among these accessions. It was inferred that these minor differences may have certain concealed capabilities that should be studied in detail by using advanced molecular techniques in order to identify the most diverse genotype.

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Table 2. Polyacrylamide gel banding pattern of proteins of thirty-one accessions of *Momordica charantia*

Mol. Wt. (KDa)	Accessions of *Momordica charantia* L.																													
	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**
11	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
15	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+
24	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+
30	-	-	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+	-	-	-
40	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
60	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
85	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-
120	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-
150	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	-	-	+	-
200	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+
Total	9	10	12	10	11	11	11	13	10	9	8	9	8	10	10	11	12	7	11	10	13	12	10	11	11	11	11	11	10	10	8

Keys: 1= 32573, 2=32574, 3=32572, 4=34162, 5=32737, 6=TOT5869, 7=TOT5847, 8=TOT6001, 9=TOT5111, 10=TOT4358, 11=TOT2407, 12=TOT2533, 13=TOT6234, 14=TOT6236, 15=TOT7308, 16=TOT1843, 17=TOT1850, 18=TOT0950, 19=TOT1008, 20=TOT0899, 21=TOT4150, 22=TOT4131, 23=TOT6851, 24=TOT6831, 25=TOT6918, 26=TOT1567, 27=TOT1504, 28=TOT1568, 29=TOT7025, 30=TOT7027, 31=TOT7029