Collection and Electrophoretic Characterization of Genetic Diversity in Muskmelon (*Cucumis melo* L.)

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Seed storage protein profiles of muskmelon genotypes were analysed on single seed basis by Sodium Dodecyl-Polyacrylamide gel electrophoresis (SDS-PAGE) under reducing condition. The seed protein of 32 germplasm lines could be resolved into 18 bands distributed in 3 zones A, B and C zone. In general there was very high similarity between genotypes of muskmelon, apart from some minor differences in the B region while the seed proteins of different cucurbit species show polymorphism. This shows that SDS-PAGE is not very effective for distinguishing muskmelon genotypes and it will be desirable to use DNA-based markers.

Key words: Electrophoresis, Muskmelon, Protein

Muskmelon (*Cucumis melo* L. $2n=2x=14$) is one of the most important cucurbits grown as a 'desert crop' throughout the warmer regions the globe. The genus *Cucumis* comprises about 30 species. The place of origin of muskmelon is not known with certainty but as the wild species of *Cucumis* exist in Africa, it is likely that it is originated in the African continent. Secondary centres of origin are now in India, China, Persia and South Russia (Chadha and Lal, 1993).

In India, at present Akola, Ludhiana, Hissar, Modipuram, Anand, Delhi, Faizabad and Durgapur are the centres which are participating in the research programme of muskmelon. By the efforts of these stations, a number of varieties namely Hara Madhu, Punjab Sunehri, Arkajeit, Arka Rajhans, Durgapur Madhu, Pusa Sharbati, Pusa Madhuraas, Punjab Hybrid and Pusa Rasraj etc. have been released by ICAR as reported by Nandpuri (1989) and Ram (1997). Identification of cultivar by examination of morphological features sometime becomes difficult due to limited variation. The problem of cultivar identification has been simplified to a great extent by use of biochemical markers such as protein/isozyme profile (Brewbaker, 1966 and Ladizensky and Hymowitz, 1979). In recent years DNA markers are being used for the identification of plant genotypes (Dwelikat *et al.*, 1993) but there is no work on muskmelon in this direction. The aim of the present work was to investigate the extent of variation in the protein profiles of diverse cultivars of muskmelon.

Materials and Methods

Thirty two lines of muskmelon were collected from muskmelon growing areas of different parts of western Uttar Pradesh. Seed proteins of these genotypes were extracted in the sample buffer (Tris Base, SDS, glycerol and mercaptoethenol) and were subjected to SDS-PAGE in vertical slab gels (Laemmli, 1979). The cotyledon half of single seeds were crushed between folded butter paper with a hammer on metal plates. Sample were defatted with 1ml of petroleum ether in Eppendorf tubes and centrifuged for 10 min at 10000 rpm. Supernatant was discarded and whole process was repeated once again. Finally the supernatant was drained out and residue was dried completely. The defatted seed extract was transferred into a fresh Eppendorf tube and, added with 200 µl of distilled water, 200 ml of (2x) sample buffer and 8 µl of 2 mercaptoethanol in each tube to make 400 µl of 1x sample buffer containing 2% mercaptoethnol. Samples were centrifuged for 10 minute at 10,000 rpm just before loading 10 µl of supernatants in the gel slots. The run was performed at a constant current of 40mA/gel. Afterwards gel was stained in staining solution (0.25%) w/v Commassie Brilliant Blue R 250 + 6% w/v Trichloroacetic acid +18% (v/v) methanol + 60% (v/v) glacial acid. The destaning was performed in NaCl solution (3%) (Sreeramulu and Singh 1995). Gel was washed in distilled water, photographed and electrophoregrams of the seed protein profile were prepared.

Results and Discussion

The protein banding pattern of the muskmelon genotypes were characterised by three distinct zones, namely A, B and C in the increasing order of electrophoretic mobility (Fig. 1). The protein bands were stacked according to their molecular weight i.e. high molecular weight protein in upper region and low molecular weight protein in middle to lower region of the gel, respectively. The 'A'

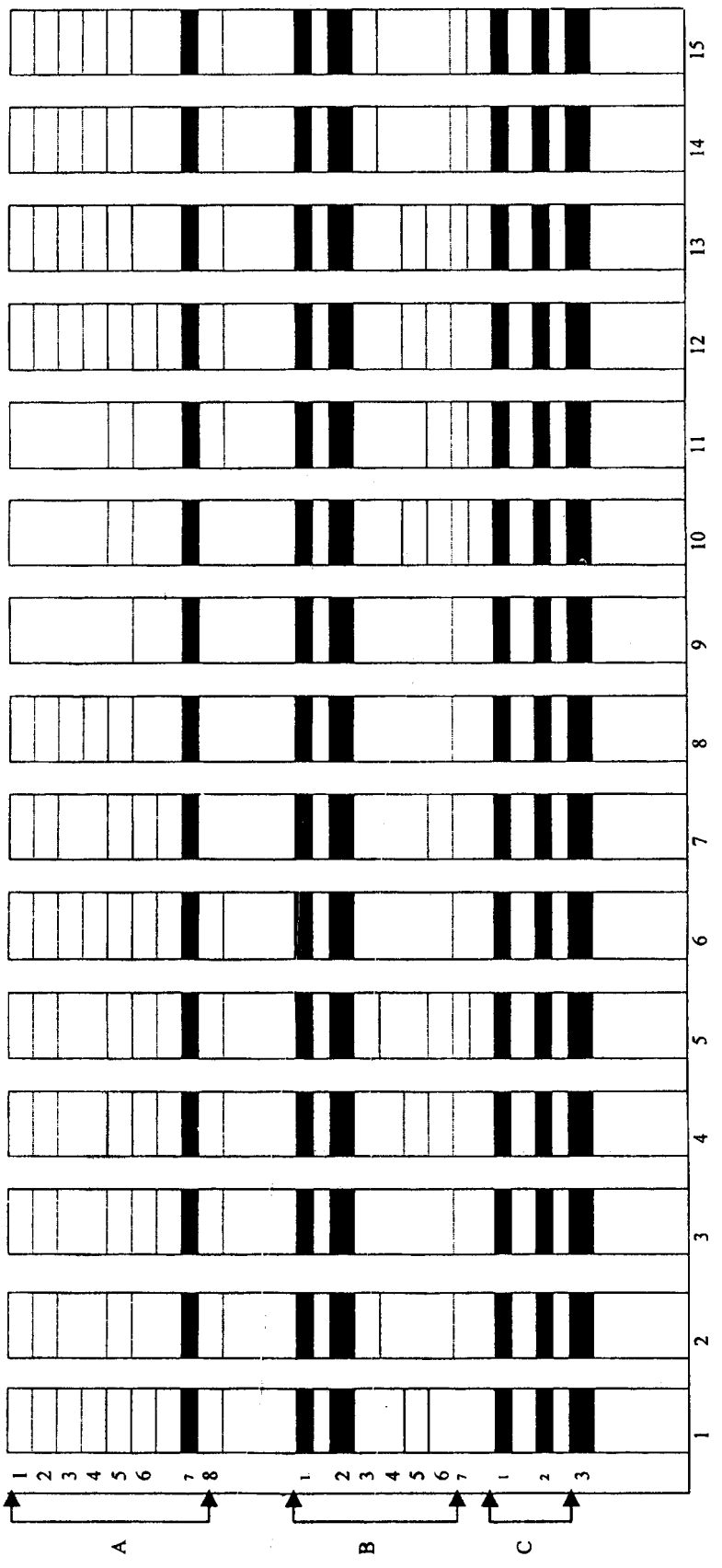

Fig. 1. Schematic diagram of the seed protein electrophoretic banding pattern in some muskmelon genotypes

region comprised of heaviest molecular weight proteins and had eight bands namely, A1-A8. Zone B represented seven bands namely B1- B7 and zone C had three bands namely C1-C3. All the 32 genotypes showed very similar banding patterns in the three zones. Only minor differences were present, particularly in zone B. The similarity index for different pairs of the genotypes is given in Table 1.

The similarity index gave an idea of genetic/ evolutionary relationship between cultivar pairs. The similarity index greater than 90 per cent indicate that two cultivars are very closely related.

Most cultivar pairs had similarity index in a narrow range of 44.0 to 99.9 percent which clearly indicated that all genotypes were closely related as far as their seed storage protein is concerned. The cultivar MM 45-B is very closely related with MM-58 having a similarity index of 94.4 percent, although morphologically they are different. MM-58 is closely related with MM-96-69 (SI.94.4 per cent) while MM-96-69 is similar to MM-96-77 (SI.94.4 per cent), and MM.96-91, MM-96-93, MM96-94, MM96-95, MM-96-96 and MM 96-97 having similarity index of 94.4%

MM-96-82 is closely related with MM96-84 (S.I. 94.4%), MM 96-85 (S.I. 99.9%) MM-96-86 (S.I. 99.9%), MM-96-88 (S.I. 94.4%), MM-96-89 (S.I. 99.9%), MM-96-91 (S.I. 94.4%) MM-96-95 (S.I.94.4%), MM-96-96) (S.I. 94.4%), MM-96-97 (S.I. 94%). MM-96-84 is similar to MM-96-85, MM-96-89, MM-96-92 and MM-96-99 having similarity index of 94.4%.

MM-96-85 is closely related with Mm-96-86 (S.I. 99.9%)s, MM-96-88 (S.I. 94.4%), MM-96-89 (S.I. 99.9%),

Table 1. Protein banding pattern in 32 germplasm of muskmelon

Genotype	Bands																	
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	B_1	B_2	B_3	B_4	B_5	B_6	B_7	C_1	C_2	C_3
MM-41	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	+	+
MM-45B	+	+	–	+	+	–	+	+	+	+	+	–	–	+	–	+	+	+
MM-58	+	+	–	+	+	–	+	+	+	+	–	–	–	+	–	+	+	+
MM-96-69	+	+	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+
MM-96-70	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
MM-96-71	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+
MM-96-72	+	+	–	+	+	+	+	–	+	+	–	–	+	+	–	+	+	+
MM-96-73	+	+	+	+	+	–	+	–	+	+	–	–	–	+	–	+	+	+
MM-96-74	–	–	–	–	–	–	+	–	+	+	–	–	–	+	–	+	+	+
MM-96-75	–	–	–	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+
MM-96-76	–	–	–	+	+	–	+	+	+	+	–	–	+	+	+	+	+	+
MM-96-77	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+
MM-96-79	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+
MM-96-80	+	+	+	+	+	–	+	+	+	+	+	–	–	+	+	+	+	+
MM-96-81	+	+	+	+	+	–	+	+	+	+	+	–	–	+	+	+	+	+
MM-96-82	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-83	–	–	–	+	+	–	+	–	+	+	–	+	+	–	–	+	+	+
MM-96-84	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+
MM-96-85	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-86	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-87	+	+	–	–	–	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-88	+	+	–	+	+	+	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-89	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-90	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-91	+	+	+	+	+	–	+	–	+	+	–	+	+	–	–	+	+	+
MM-96-92	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-93	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-94	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-95	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-96	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-97	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-98	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+	+	+
MM-96-99	+	+	+	+	+	–	+	+	+	+	–	–	–	–	–	+	+	+

+ = present; – = absent.

MM-96-91 (S.I. 94.4%), MM-96-92 (S.I. 94.4%), MM-96-93 (S.I. 94.4%) MM-96-94 (S.I. 94.4%0, MM-96-95 (S.I. 94.4%, MM-96-96 (S.I. 94.4%) and MM-96-97 (S.I. 94.4%). MM-96-86 is similar to MM-96-88 (S.I. 94.4%), MM-96-89 (S.I. 94.4%, MM-96-94 (S.I. 94.4%), MM-96-95 (S.I. 94.4%), MM-96-96 (S.I. 94.4%) and Mm-96-97 (S.I. 94.4%).

MM-96-88 is closely related to MM-96-89, MM 96-91, MM-96-92, MM-96-93, MM-96-94, MM-96-95, MM-96-97 having similarity index of 94.4%. MM-96-89 is similar to MM-96-92 MM-96-93, MM-96-94, MM-96-95, MM -96-96, MM-96-97 and MM 96-98 having similarity index of 94.4%.

MM-96-91, Mm-96-92, MM-96-93, MM-96-94, MM96-95, Mm-96-96 and Mm-96-97 are closely related to each other with a similarity index of 99.9%.

The above results with the SDS-PAGE of 32 genotypes suggest that there is very limited variation for seed storage protein among the muskmelon genotypes. In contrast, these genotypes were selected on the basis of their diverse morphological traits and origin (Table 2). This may be due to limited number of genes of a common origins for the major seed storage proteins in muskmelon. Similar SDS-PAGE analysis of seed storage protein of different cucurbit species showed extensive variation between species (Fig. 2). This clearly shows that SDS-PAGE to distinguish seeds of different varieties of the same species as similar results have been obtained with different variety of cucumber. Hence it will be desirable to make use of new DNA markers such RAPD and RFLP for the cataloguing of muskmelon germplasm.

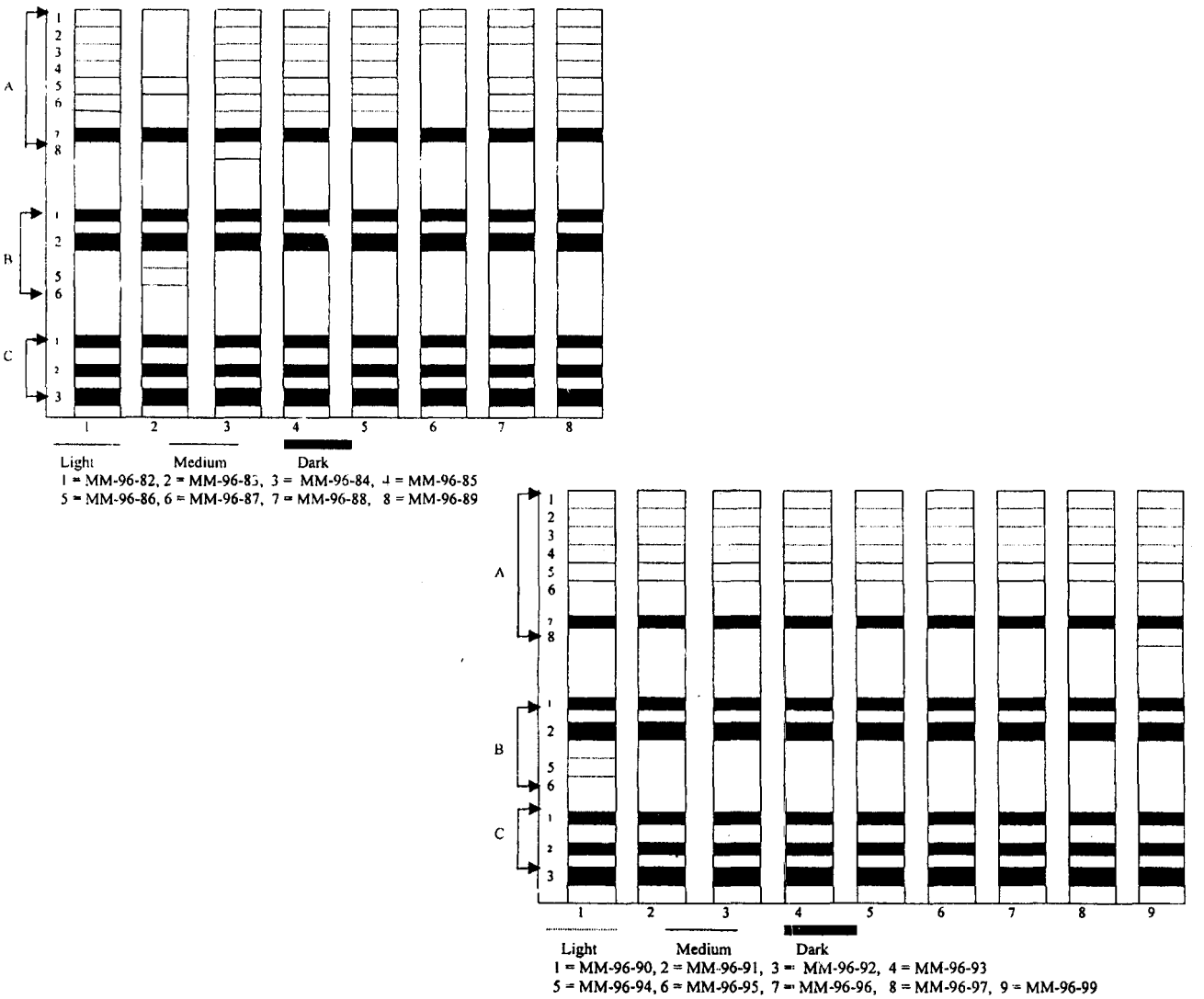

Fig. 2. Schematic diagram of the seed protein electrophoretic banding pattern in some muskmelon genotypes

Table 2. Mean values of 16 morphological characters

Geonotype	Days to Seed Germination	No of days to first male flower	No. of days to first female flower	Inter nodal length (cm)	Days to first fruit harvest	Fruit weight (gm)	No. of fruits per plant	Polar diameter (cm)	Latitudinal diameter (cm)	No of strips	Blossom and scar size (cm)	Peduncle scar size (cm)	Flesh thickness (cm)	Seed cavity size (cm)	T.S.S.	Yield per plant (cm)
MM-58	11.40	42.00	46.66	6.20	78.00	851.33	1.61	11.16	12.38	9.66	1.33	1.39	2.39	7.57	9.0	1646.40
MM-41	10.33	40.33	44.66	6.96	78.00	953.33	2.96	11.12	13.60	9.91	1.65	1.62	2.30	8.87	6.70	2705.60
MM-45 B	12.66	47.33	52.00	6.76	81.66	509.33	2.64	8.88	10.83	10.0	2.52	1.36	2.33	7.26	6.83	2186.60
MM-96-69	11.13	43.33	47.33	7.43	81.66	800.00	1.91	102.6	11.93	10.00	1.44	1.56	2.28	7.58	6.66	1832.83
MM-96-70	10.00	38.33	46.00	7.33	83.00	805.00	1.96	10.46	11.96	9.66	2.60	1.81	2.95	6.68	7.00	1685.83
MM-96-71	11.66	45.66	48.33	5.96	81.66	766.66	2.17	10.69	12.07	9.23	1.63	1.50	2.95	7.55	9.00	2429.73
MM-96-72	11.16	42.3.	42.00	6.00	79.33	1015.00	2.32	11 73	11.32	9.66	1.46	1.63	2.35	8.36	9.50	5268.00
MM-96-73	11.60	42.6	47.33	7.00	8150	1048.33	1.69	10.06	12.56	9.90	2.30	1.65	2.06	8.50	8.66	1740.93
MM-94-74	12.26	43.00	49.00	7.66	81.00	941.66	1.66	9.04	12.83	10.00	2.45	1.33	3.06	7.83	8.16	1619.16
MM-96-75	11.06	42.66	47.33	6.23	81.33	690.00	1.47	8.53	11.33	10.20	1.86	1.55	2.16	8.53	7.75	3773.16
MM-96-76	12.86	44.66	49.33	5.64	82.00	1020.00	2.85	9.57	14.16	9.06	2.05	1.52	2.56	7.30	10.00	2789.00
MM-96-77	11.00	42.66	47.33	6.13	82.66	510.00	2.06	7.30	10.50	6.43	1.91	1.90	3.03	6.60	9.66	1169.60
MM-96-78	11.33	42.66	46.33	4.53	83.00	893.33	2.53	11.23	10.90	10.00	1.88	1.60	2.51	8.53	11.66	2810.81
MM-96-79	11.66	44.00	48.33	5.26	76.33	631.66	2.36	10.05	13.16	10.16	1.93	1.44	2.35	7.37	9.43	1493.38
MM-96-80	10.80	44.33	47.66	5.56	78.33	1081.66	1.63	10.42	12.26	10.50	2.10	1.45	2.40	8.39	10.21	2362.78
MM-96-81	11.20	45.00	48.66	5.80	84.00	669.66	3.32	8.36	10.33	9.33	1.68	1.05	2.53	6.43	9.50	2159.86
MM-96-82	11.13	43.00	50.33	6.13	79.33	993.33	1.46	11.21	13.69	11.00	2.70	1.19	2.16	9.58	7.00	1831.06
MM-96-83	20.43	45.66	49.00	5.53	83.70	1155.00	2.12	16.50	13.93	10.00	2.13	1.42	3.02	9.29	10.13	2172.96
MM-96-84	11.00	44.00	45.33	5.26	80.56	578.00	1.43	8.96	10.18	10.00	1.22	1.02	1.76	7.18	10.50	841.08
MM-96-85	11.73	44.33	49.00	6.43	81.53	1007.33	1.55	10.28	12.18	9.50	2.35	1.54	2.37	8.91	9.80	1870.50
MM-96-86	11.33	43.00	48.33	5.33	78.66	787.00	2.82	11.71	12.52	9.86	2.10	1.43	2.38	8.90	6.66	2062.66
MM-96-87	12.13	45.00	49.00	6.73	82.76	703.33	2.92	10.45	9.97	9.66	1.04	1.36	1.85	6.53	8.66	1894.66
MM-96-88	10.93	44.66	49.00	5.66	80.66	3480.00	2.24	9.93	13.35	10.33	1.82	1.86	2.32	8.34	9.84	1643.98
MM-96-89	11.13	44.33	48.33	7.86	79.00	713.33	1.81	8.21	11.02	10.20	2.73	1.43	1.66	6.68	8.00	583.16
MM-96-90	11.13	44.00	49.00	6.90	78.26	623.33	3.36	9.68	11.61	12.00	2.04	1.32	2.80	7.48	9.00	5105.00
MM-96-91	11.33	48.33	52.66	5.73	82.80	690.00	1.62	9.44	11.16	10.00	1.94	1.26	2.41	1.13	10.08	580.49
MM-96-92	11.66	46.33	50.33	6.30	80.70	821.66	1.66	10.30	10.54	10.00	2.56	1.60	2.70	7.30	10.33	1503.05
MM-96-93	13.13	45.66	50.66	7.13	81.33	688.66	1.97	10.36	12.03	9.66	2.25	1.28	2.46	7.85	8.00	1564.77
MM-96-94	13.10	42.66	50.66	5.73	84.33	610.00	3.66	9.64	11.39	40.00	2.21	2.08	2.21	7.22	10.20	2876.66
MM-96-95	10.80	41.66	45.66	5.90	79.96	681.66	2.26	9.32	11.40	9.66	1.63	1.28	2.33	7.27	8.96	1208.33
MM-96-96	12.83	44.00	48.00	5.30	79.26	590.00	1.47	9.76	9.46	10.00	1.80	1.12	1.98	6.64	10.33	961.33
MM-96-97	12.83	45.33	50.33	6.00	82.33	605.00	2.36	9.37	11.20	9.66	1.58	1.32	2.39	7.32	9.53	1809.08
MM-96-99	13.00	45.33	49.00	6.23	82.33	916.66	2.38	10.53	12.56	9.66	2.18	1.76	2.68	8.48	11.00	2497.50

Table 3. Similarity Index (S.I.) for seed protein profile in different genotypes of muskmelon

	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
1.	100	88	72.2	88.8	72.2	83.3	72.2	72.2	44.4	44.4	55.3	83.3	83.3	66.6	66.6	83.3	55.5	83	83.3	83.3	61	78	72.2	89	78	83	78	78	78	78	77.7	83.3
2.			94.4	77.7	83.3	83.3	77.7	83.3	66.6	55.5	72.2	72.2	66.6	72.2	72.2	72.2	55.5	78	72.2	72.2	72	72	72.2	67	78	72	78	78	78	78	77.7	83.3
3.				94.4	77.7	88.8	77.7	88.8	72.2	61.1	77.7	77.7	77.7	83.3	83.3	77.7	66.6	83	72.2	72.2	78	83	72.2	72	83	72	83	83	83	83	83.3	83.3
4.					83.3	83.3	88.8	72.2	55.5	66.6	72.2	94.4	83.3	66.6	66.6	72.2	66.6	78	72.2	72.2	61	78	72.2	78	67	72	67	67	67	67	66.6	72.2
5.						77.7	88.8	66.6	50	61.1	77.7	77.7	77.7	83.3	83.3	66.6	55.5	72	66.6	66.6	56	67	61.1	61	61	67	61	61	61	61	61.6	66.6
6.							83.3	94.4	61.1	50	55.5	88.8	77.7	83.3	83.3	88.8	50	94	88.8	88.8	67	83	88.8	72	83	89	83	83	83	83	83.3	88.8
7.								83.3	66.6	66.6	72.2	83.3	72.2	66.6	61.1	83.3	72.2	78	83.3	83.3	72	89	83.3	78	78	83	78	78	78	78	77.7	72.2
8.									72.2	55.5	66.6	77.7	77.7	83.3	83.3	88.8	66.6	83	88.8	88.8	78	83	83.3	83	78	83	94	94	94	66	94.4	61.1
9.										55.5	72.2	50	50	55.5	55.5	61.1	72.2	56	61.1	61.1	83	61	61.1	56	94	61	67	67	67	56	66.7	50.0
10.											83.3	61.1	72.2	55.5	55.5	50	83.3	56	61.1	61.1	83	61	50.0	67	67	50	56	56	56	61	55.5	66.6
11.												66.6	77.7	72.2	72.2	55.5	72.2	61	55.5	55.5	67	61	55.5	61	56	56	61	61	61	72	61.1	77.7
12.													88.8	72.2	72.2	77.7	66.6	73	77.7	77.7	56	72	77.7	83	61	78	72	72	72	72	72.2	77.7
13.														83.3	83.3	66.6	66.6	72	66.6	66.6	56	61	66.6	67	72	67	72	72	78	78	77.7	83.3
14.															99.9	72.2	50	78	72.2	72.2	61	67	72.2	67	78	72	78	78	78	78	77.7	83.3
15.																72.2	44.4	78	72.2	72.2	61	67	72.2	67	78	72	78	78	94	94	94.4	88.8
16.																	66.6	94	99.9	99.9	78	94	99.9	83	94	94	94	94	72	72	72.2	66.6
17.																		61	44.4	44.1	67	72	66.6	83	72	67	72	72	89	89	88.8	94.4
18.																			94.4	94.4	72	89	94.4	78	89	94	89	89	94	94	94.4	88.8
19.																				99.9	78	94	99.9	83	94	99	94	94	94	94	94.4	88.8
20.																					78	94	99.9	83	94	94	94	94	83	83	83	77.7
21.																						83	77.7	72	83	78	83	83	94	94	94.4	83.8
22.																							94.4	83	94	94	94	94	94	94	94.4	88.8
23.																								83	94	94	94	94	89	89	88.8	83.3
24.																									89	83	89	89	100	100	99.9	88.8
25.																										94	95	100	94	94	94.4	88.8
26.																											94	94	100	100	99.9	94.4
27.																												100	100	100	99.9	94.4
28.																														100	99.9	94.4
29.																															99.9	94.4
30.																																94.4
31.																																94.4

1= MM-41, 2=MM-45-B,3=MM-58, 4=MM-96-69, 5=MM-96-70, 6 = MM-96-72, 8= MM-96-73-9= MM-96-74, 10= MM-96-75, 11=MM-96-76, 12=MM-96-77, 13=MM-96-79, 14=MM-96-80, 14= MM-96-80, 15= MM-96-81, 16= MM- 96- 82, 17 = MM – 96- 83, 18=MM-96-84, 19=MM-96-85, 20-MM-96-86, 21=MM-96-87, 22=MM-96, 23= MM-96-89, 24= MM-96-89, 24=MM-96-90, 25=MM-96-91, 26= MM-96-92, 27= MM-96-94, 29=MM-96-94, 29=MM-96-95, 30=MM-96-96, 31=MM-96-97, 31=MM-96-98, 32=MM-96-99.

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