

# Protein Electrophoretic Study for Isolation Distances Detection for Egyptian Colver Cultivars

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## ABSTRACT

The Present study was carried out to employ the polyacrylamide gel electrophoresis (SDS-PAGE) characterization of Serw 1, as a parent, Gemmiza 1 as a mother varieties and their first generations polycrosses a long 50,100, 150 and 200 meter distances from the parent variety (Serw 1).

To determine isolation distance (50m) between cultivated Egyptian clover, polyacrylamide gel electrophoresis (SDS - PAGE) was employed to detect variation in total soluble protein content technique among five distances of Egyptian clover namely, 200m 150, 100, 50m and Gemmiza 1 distance. After detection of isolation distance with 50m, different protein fragmentation was recorded for the cultivars which ranged from sixteen to eleven for distance 100, 200m and Gemmiza 1; respectively, with molecular weight ranged from 207 to 46 KDa. Finding of similarity and dissimilarity for protein patterns of cultivated Egyptian clover showed that, distances No. Serw 1 and 50m showed same protein patterns with almost protein loci and molecular weights with fourteen protein bands. According to electrophoretic study, phylogenetic tree was constructed and indicated clear genetic base for distances Serw 1 and 50m which belongs to the same subgroup. Our previous findings detected isolation distance with 50m could cause genetical mixing probability between Serw 1 and 50m cultivars which represented as male and females; respectively.

A chart of minimum recommended isolation distances for Egyptian clover, the chart includes three minimum distances recommendations:

- Isolation distance of 200m for foundation seed.
- Isolation distance of 150m for registered seed.
- Isolation distance of 100m for certified seed.

**Keywords:** Egyptian clover, isolation distance, SDS-PAGE technique, genetical mixing.

## INTRODUCTION

Isolation distance might be defined as minimum separation required between two or more varieties of the same species for the purpose of keeping seed pure. Species in the same genus or family often have similar minimum isolation distance requirement, but occasionally certain varieties within a species may require longer isolation distance. In addition, many environmental factors can affect how far and how effectively pollen can be transferred by wind or by insects (Rakita and McCormack, 2004). Isolation

distance and plot size are criteria established to ensure genetic purity of pedigreed red clover (*Trifolium pratense L.*) seed in Canada (Can. Seed Growers Assoc, 1973).

A wide range of genetic mechanisms lie at the cause of sexual incompatibilities among populations, leading to speciation. Among others, ploidal variations, chromosomal changes and genetic incompatibilities. In plants, genetic speciation mechanisms can be active anywhere from pre-pollination to endosperm or embryo failure and/or hybrid sterility (Williams et al., 2011). White clover is one of the most important pasture legumes in global temperate regions. It is an out crossing; insect-pollinated species with gene flow occurring naturally between plants. Paternity was confirmed using simple sequence repeat markers. A leptokurtic pattern of gene flow was observed under conditions designed to measure maximized gene flow with the majority of pollination occurring in the first 50 m from the donor pollen source. The combined use of simple sequence repeat and visual markers confirmed that there was also a white clover pollen source in addition to the donor plants. This research confirms the difficulty in ensuring absolute containment of gene flow in an out crossing species grown in an environment when endemic populations are known to exist. (De Lucas et al., 2011).

In USA, general clover standards for field of less than 2 ha require an isolation distance of 274 m for foundation seed (maximum 0.1 % contamination with other varieties permitted), 137 m for registered seed (maximum 1.0% contamination with other varieties permitted) and 50 m for certified seed (maximum 1.0% contamination with other varieties permitted) (Association of Official Seed Certifying Agencies 2003). Otherwise, in Europe, an isolation distance of 200 m is required (for fields of 2 ha or less) basic legume seed. Furthermore, OECD standards for legumes are consistent with South Australian White Clover Standards (Smith and Baxter 2002) and New Zeland seed standards for legume (MAF Biosecurity 2007).

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## MATERIALS AND METHODS

### Cultivars And Cultivation Conditions

#### SDS PAGE electrophoresis

Total protein content was determined in grounded fine powder seeds of each sample by the method described by Bradford (1976) using bovine serum albumin (96%, Sigma Chemical Co, St. Louis, MO, USA), as standard. Then, total soluble proteins was extracted with extraction buffer. Fifty  $\mu$ l of the extract were mixed with 50  $\mu$ l of SDS-sample buffer (0.15 M TRIS-HCl, pH 6.8, 3% w/v SDS, 5% v/v  $\beta$ -mercaptoethanol, 7% v/v glycerol and 0.03% Bromphenol Blue) and boiled for 7 min in a boiling water bath. 14 $\mu$ l of the sample was loaded onto each well. Electrophoresis SDS-PAGE was carried out according to the procedures of Laemmli (Laemmli, 1970) in 1.5 mm thick gels with 14 % (w/v) separating gel and 4% (w/v) stacking gel in a vertical electrophoresis unit (Cleaver Scientific, England). SDS-PAGE was carried out at 75 volt for 3 hours. After electrophoresis, the gels were overnight stained using 0.1% (w/v) Coomassie Brilliant Blue R-250. Then, destained using a 10% (v/v) acetic acid solution until a clear background was achieved. A Page ruler pertained protein ladder (Thermo-Fisher Scientific) was used as protein molecular weight marker. Gel documentation system (GelDoc-It<sup>e</sup> Imaging System, UVP, England), was applied for data scoring and documentation. Total lab analysis software (TotalLab TL120, v2008) was employed for constructing binary matrix for SDS PAGE data according to presence or absence of a band of each sample which remarked as 1 or 0.

### RESULTS AND DISCUSSIONS

As data in Table (1) show, total soluble protein studying via SDS-PAGE technique, all *Egyptian clover* samples reflected variable distinguishable protein fragments. On one hand, 100 meter isolation distance was superior in protein band number with sixteen fragments. On the other hand, 200 meter isolation distance and Gemmiza 1 expressed lowest protein patterns with eleven fragments. Based on [previous finding which cleared highly similarity patterns for

cultivar serw 1 and 50 meter isolation distance, it could explain mixing probability between each other.

However, second and seventh cultivars showed identical protein patterns with almost same protein fragments loci and molecular weights with fourteen protein bands. At 10 % of genetic similarity, all *Egyptian clover* cultivars divided into two main clusters. First cluster excised at 46 % of genetic similarity and composed of *Egyptian clover* 100 meter isolation distance and Gemmiza 1 which indicate high similarity value for protein fractionated patterns. An identical protein pattern for cultivars Serw 1 and 50 meter isolation distance was reflected on genetic relationship between them. High closely genetic similarity was representing in existence of Serw 1 and 50 meter isolation distance in the same sub cluster. Clear difference for 200 meter isolation distance protein patterns resulting in its presence in isolated sub cluster. Based on phylogenetic tree which evaluate genetic similarity among *Egyptian clover* cultivars under study, second and seventh cultivars clearly belongs to the same subgroup.

Three unique protein fragments were recorded only for serw 1 and 50 meter isolation distance samples with 195, 86 and 51KDa which summons further studies for getting clear understanding relation between fractionated protein bands and genetic mixing mechanism.

The isolation distance with 50m was minimal of which detected by Pankiw (1974) , present isolation distance of 183 m, with a minimum of contaminant bloom in the area, be retained to the smaller acreages of foundation seed, but that for larger fields as occurs with certified seed production and effect on clover seed production. On the other hand, 50m isolation distance which used in our investigation was in agreement with Georg (2011), who found that in red clover is cross-pollinated and diploid cultivars don't set seeds when pollinated by tetraploids , or the reciprocal of these two types. The two types should have an isolation distance at least 50m to avoid a reduction of potential seed yield.

**Table 1. Total Soluble protein studying Via SDS – PAGE**

200 meter isolation distance	100 meter isolation distance	Serw 1	50 meter isolation distance	Gemmiza 1
KDa 204	KDa 204	-	-	-
-	KDa 196	KDa 196	-	-
-	KDa 194	KDa 194	KDa 194	KDa 194
KDa 165	-	KDa 165	KDa 165	KDa 165
KDa 130	-	KDa 130	KDa 130	KDa 130
KDa 127	KDa 127	-	-	-
KDa 80	KDa 80	KDa 80	KDa 80	KDa 80

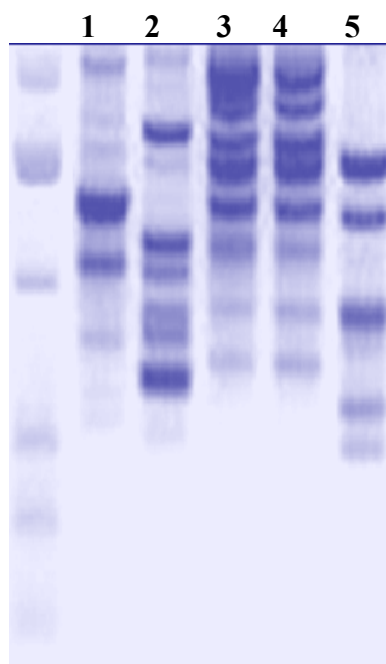
SDS-PAGE technique as accurate indicator methodology was added more support for diagnostic different microflora in clover by Liu et al., (2007) PCR-amplified 16S rDNA RFLP, numerical taxonomy, SDS-PAGE of whole cell proteins, sequencing of 16S rDNA and DNA-DNA hybridization were applied to analyze the diversity and relationships of rhizobia in the subtropical and tropical zones of China. Nevertheless, the use of wild relatives in breeding programmes is of importance in allopolyploid species with complex systematic like alfalfa or white clover, and wild relatives have been used successfully to introgress specific characters into the cultivated species, such as the profuse flowering trait from *Trifolium nigrescens* into white clover (Marshall et al. 2008).

### CONCLUSION

SDS Polyacrylamide gel electrophoresis (SDS-PAGE) gave a huge help for isolation distance evaluation among five cultivated *Egyptian clover* namely, 200 meter, 100 meter, Serw 1, 50 meter isolation distance and Gemmiza 1 cultivar.

According to electrophoretic study, Serw 1 and 50 meter isolation distance has almost same genetic base and belongs to the same subgroup in constructed phylogenetic tree. Based on our obtaining data, genetical mixing probability occurs between second and seventh cultivars which represented as male and female, respectively. Additional studies should be carried out to clearly relation between fractionated protein bands and genetic mixing mechanism.

Based on SDS-PAGE technique, genetic relation could be cleared as follow: all samples composed of two main clusters. First cluster divided into two sub clusters. *Egyptian clover* 200 meter isolation distance represent the first. Highly genetic relationship between *Egyptian clover* serw 100 and *Egyptian clover* 50 meter isolation distance composed them in the same second sub cluster. *Egyptian clover* meter isolation distance represent the second cluster. According to previous data it could be detected mixing between *Egyptian clover* serw 1 and 50 meter isolation distance which resulted in highly similarity between them.

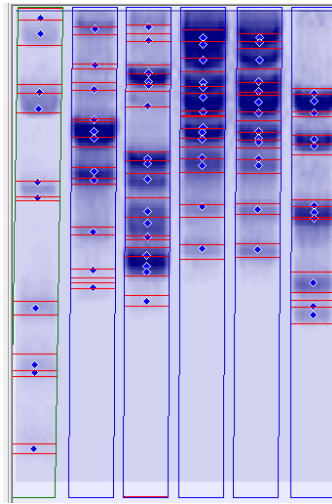


**Figure 1. Protein fingerprinting patterns of five *Egyptian clover* cultivars**

Where:

Protein marker  
 -*Egyptian clover* cultivar 9  
 (100 meter isolation distance).  
 4- *Egyptian clover* cultivar 7  
 (50 meter isolation distance).

-*Egyptian clover* cultivar 10  
 (200 meter isolation distance).  
 3- *Egyptian clover* cultivar 2 (*Serw 1*).  
 5- *Egyptian clover* cultivar 1 (Gemmiza 1).



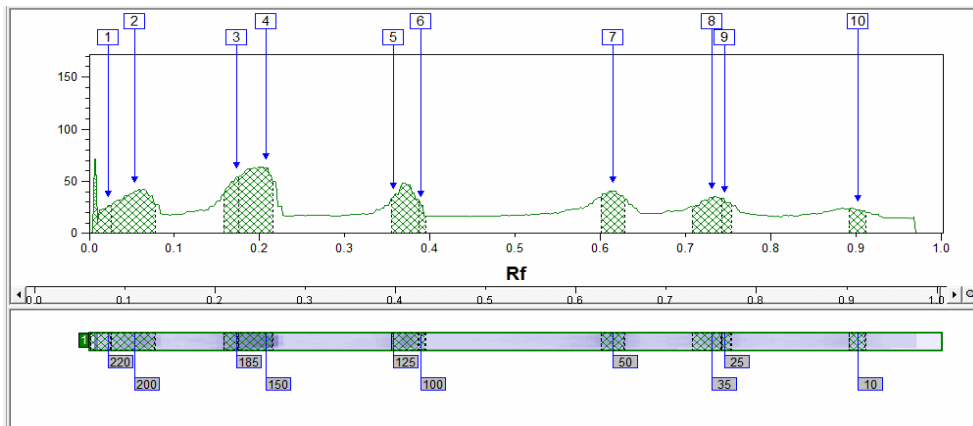
**Figure 3. Computerized detection of five *Egyptian clover* cultivars.**

Where:

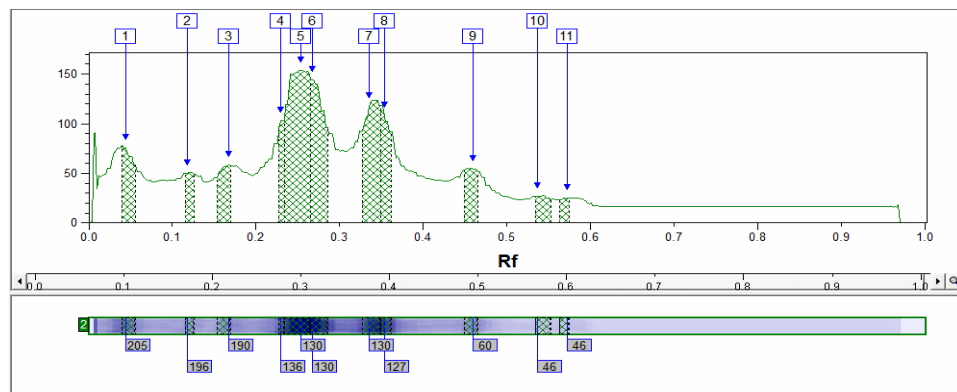
Protein marker

- 3- *Egyptian clover* cultivar 9  
(100 meter isolation distance).
- 5- *Egyptian clover* cultivar 7  
(50 meter isolation distance).

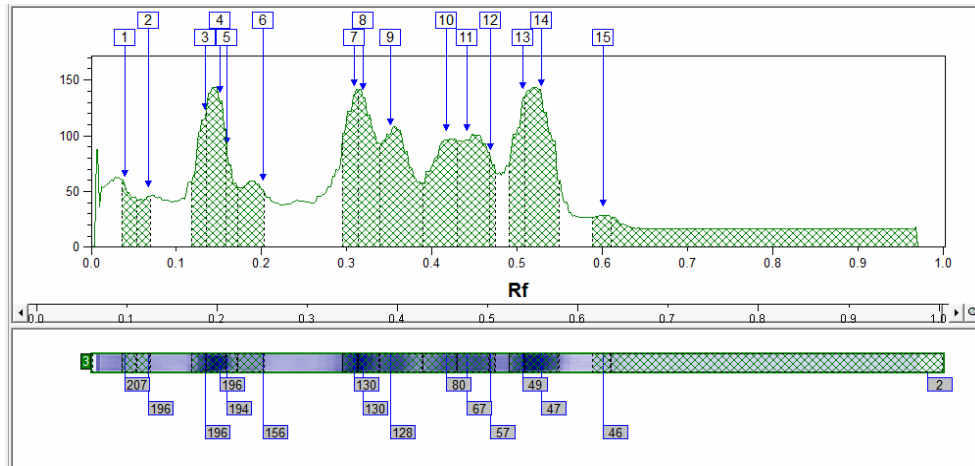
- 2- *Egyptian clover* cultivar 10  
(200 meter isolation distance).
- 4- *Egyptian clover* cultivar 2 (Serw 1).
- 6- *Egyptian clover* cultivar 1 (Gemmiza 1).



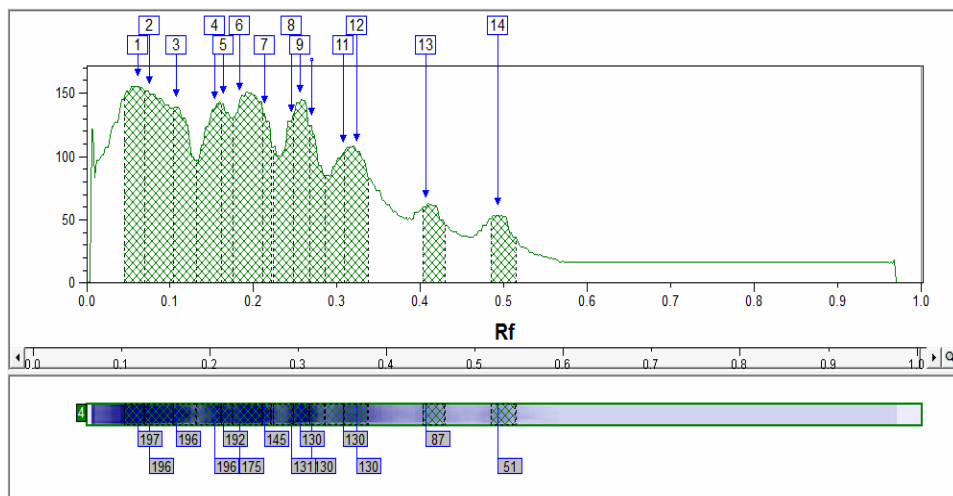
**Figure 4. Dendrogram for protein pattern of protein marker**



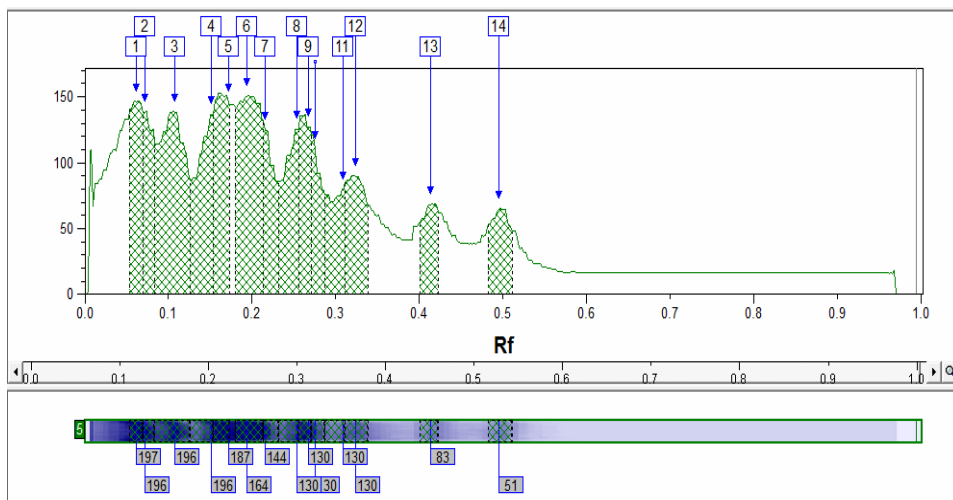
**Figure 5. Dendrogram for protein pattern of 200 meter isolation distance**



**Figure 6. Dendrogram for protein pattern of 100meter isolation distance**



**Figure 7. Dendrogram for protein pattern of Serw 1 parent variety**



**Figure 8. Dendrogram for protein pattern of 50 meter isolation distance**

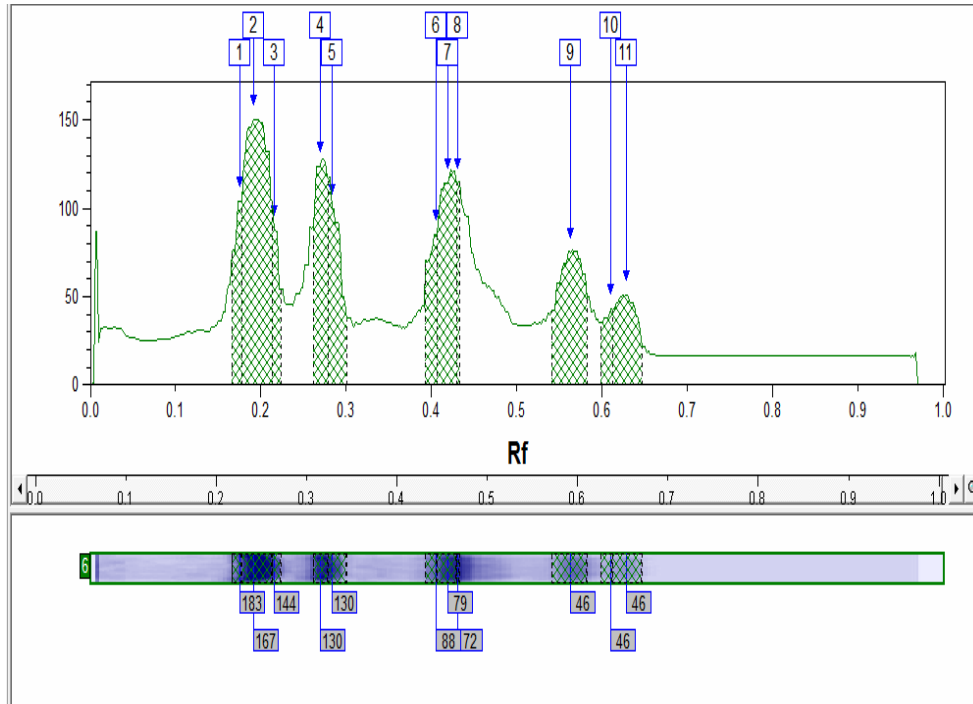


Figure 9. Dendrogram for protein pattern of Gemmiza 1 mother variety

Table 2. Data analysis of protein patterns parameters for protein marker

Band No	position	volume	Peak height	Area	Band%	Lane%	Mw	Rf
1	11	14968.00	26.57	552.00	6.32	2.68	220.000	0.022
2	27	43303.00	40.70	1196.00	18.30	7.75	200.000	0.054
3	87	19508.00	54.35	414.00	8.24	3.49	185.000	0.174
4	104	55757.00	62.83	920.00	23.56	9.98	150.000	0.208
5	179	30093.00	34.26	736.00	12.72	5.39	125.000	0.357
6	195	4712.00	26.93	184.00	1.99	0.84	100.000	0.389
7	308	24664.00	40.72	644.00	10.42	4.41	50.000	0.615
8	366	24577.00	34.63	782.00	10.38	4.40	35.000	0.731
9	374	8603.00	31.57	276.00	3.64	1.54	25.000	0.747
10	452	10475.00	22.35	460.00	4.43	1.87	10.000	0.902

Table 3. Data analysis of protein patterns parameters for 200 meter isolation distance

Band No	position	volume	Peak height	Area	Band%	Lane%	Mw	Rf
1	22	25806.00	75.63	368.00	7.15	2.65	204.939	0.044
2	59	11601.00	49.85	230.00	3.21	1.19	195.569	0.118
3	84	20005.00	58.48	368.00	5.54	2.06	189.588	0.168
4	115	13888.00	103.22	138.00	3.85	1.43	135.699	0.230
5	127	105870.00	153.70	736.00	29.32	10.88	130.218	0.253
6	134	58683.00	143.74	460.00	16.25	6.03	130.013	0.267
7	168	57900.00	118.04	506.00	16.04	5.95	130.013	0.335
8	177	29466.00	108.30	276.00	8.16	3.03	127.215	0.353
9	230	20023.00	54.17	368.00	5.55	2.06	60.118	0.459
10	269	11043.00	27.33	414.00	3.06	1.14	46.386	0.537
11	287	6747.00	25.11	276.00	1.87	0.69	46.202	0.573

**Table 4. Data analysis of protein patterns parameters for 100 meter isolation distance**

Band No	position	volume	Peak height	Area	Band%	Lane%	Mw	Rf
1	20	20930.00	55.00	414.00	2.28	1.80	207.360	0.040
2	34	16026.00	46.20	386.00	1.75	1.38	196.300	0.068
3	67	37644.00	114.76	414.00	4.10	3.23	195.569	0.134
4	76	67705.00	130.63	506.00	7.38	5.82	195.569	0.152
5	80	23480.00	84.00	322.00	2.56	2.02	194.256	0.160
6	101	41079.00	51.63	736.00	4.48	3.53	155.825	0.202
7	155	50481.00	136.22	414.00	5.50	4.34	130.013	0.309
8	160	70998.00	133.15	598.00	7.73	6.10	130.013	0.319
9	176	100454.00	101.26	1150.00	10.94	8.63	128.201	0.351
10	209	76450.00	96.41	920.00	8.33	6.57	80.148	0.417
11	221	84116.00	96.07	874.00	9.16	7.23	67.393	0.441
12	235	14323.00	79.04	184.00	1.56	1.23	56.846	0.469
13	254	42971.00	134.22	414.00	4.68	4.68	48.765	0.507
14	265	113344.00	136.28	920.00	12.35	12.35	46.733	0.529
15	301	14173.00	28.43	506.00	1.54	1.22	46.202	0.601
16	492	143746.00	0.00	598.00	15.66	12.35	2.308	0.982

**Table 5. Data analysis of protein patterns parameters for Serw 1 Parent variety**

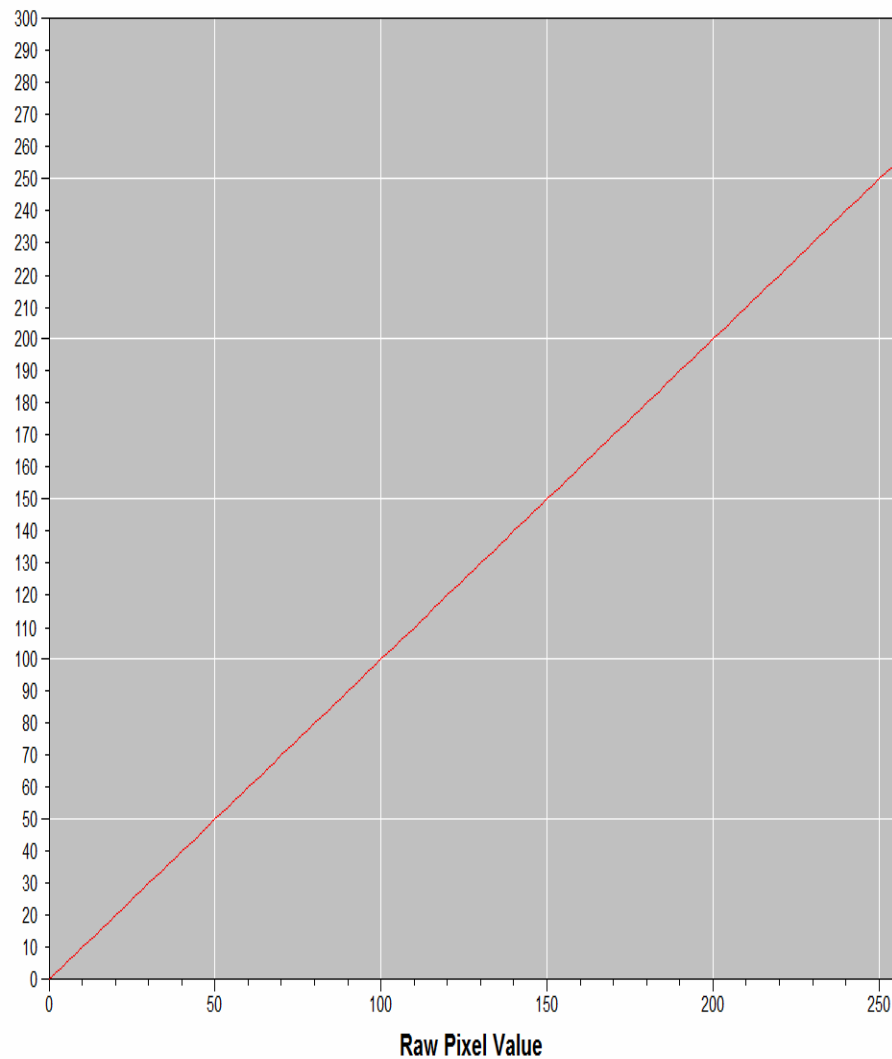
Band No	position	volume	Peak height	Area	Band%	Lane%	Mw	Rf
1	31	84755.00	155.22	552.00	9.31	4.16	197.451	0.062
2	38	113938.00	149.65	782.00	12.51	3.30	195.612	0.076
3	54	80091.00	139.30	644.00	8.79	9.16	195.569	0.108
4	77	84996.00	136.98	690.00	9.33	5.39	195.563	0.154
5	82	43593.00	141.61	322.00	4.79	5.33	192.118	0.164
6	92	119370.00	143.87	828.00	13.11	8.91	175.349	0.184
7	107	29905.00	134.00	230.00	3.28	3.49	145.020	0.214
8	123	60906.00	127.35	552.00	6.69	4.41	131.124	0.246
9	128	63970.00	142.20	460.00	7.02	3.77	130.109	0.255
10	135	43828.00	124.09	414.00	4.81	2.82	130.13	0.269
11	154	509924.00	103.43	552.00	5.59	3.20	130.13	0.307
12	162	66366.00	104.35	644.00	7.29	4.27	130.13	0.323
13	204	34724.00	59.67	598.00	3.81	2.51	86.590	0.407
14	247	33369.00	53.67	690.00	3.66	2.98	51.003	0.493

**Table 6. Data analysis of protein patterns parameters for 50 meter isolation distance**

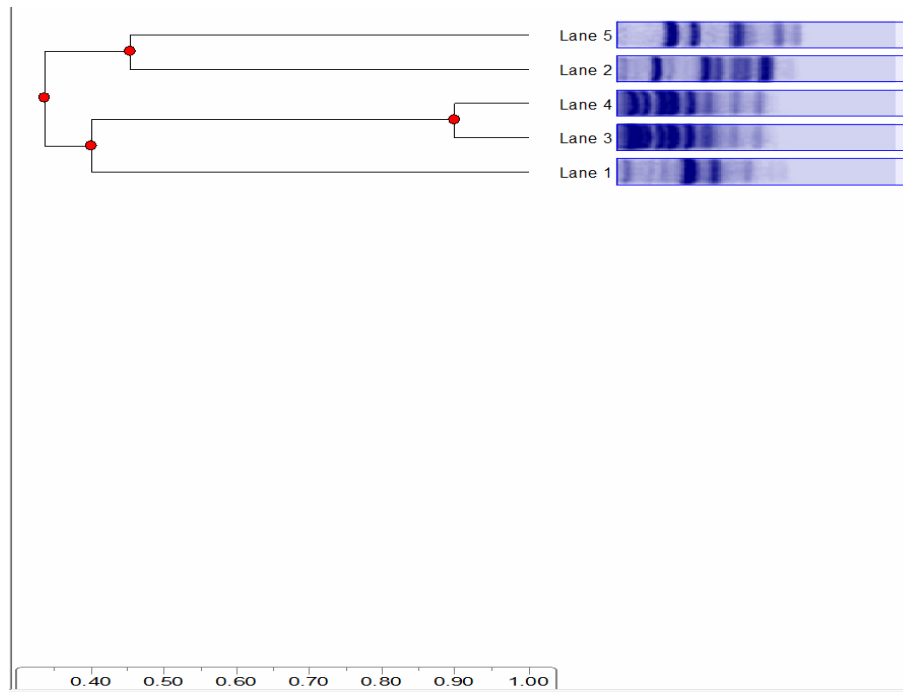
Band No	position	volume	Peak height	Area	Band%	Lane%	Mw	Rf
1	31	53336.00	146.24	368.00	6.53	4.16	197.451	0.062
2	36	42363.00	138.50	322.00	5.19	3.30	195.847	0.072
3	54	117434.00	138.33	966.00	14.38	9.16	195.569	0.108
4	76	69123.00	136.70	644.00	8.46	5.39	195.563	0.152
5	86	68377.00	145.83	460.00	8.37	5.33	186.640	0.172
6	97	114255.00	150.52	782.0	13.99	8.91	164.420	0.194
7	108	44753.00	124.37	414.00	5.48	3.49	143.552	0.216
8	127	56513.00	125.43	552.00	6.92	4.41	130.218	0.253
9	134	48353.00	127.09	368.00	5.92	3.77	130.013	0.267
10	138	36195.00	109.83	368.00	4.43	2.82	130.013	0.275
11	155	41080.00	78.74	552.00	5.03	3.20	130.013	0.309
12	162	54694.00	89.17	644.00	6.70	4.27	130.013	0.323
13	207	32237.00	67.80	506.00	3.95	2.51	82.642	0.413
14	248	38153.00	64.59	644.00	4.67	2.98	50.634	0.495

**Table 7. Data analysis of protein patterns parameters for Gemmiza 1 mother variety.**

Band No	position	volume	Peak height	Area	Band%	Lane%	Mw	Rf
1	88	24256.00	104.15	276.00	5.38	2.51	183.248	0.176
2	96	114332.00	149.80	828.00	25.34	11.82	166.631	0.192
3	108	19323.00	87.65	230.00	4.28	2.00	143.552	0.216
4	135	49167.00	123.61	414.00	10.90	5.08	130.013	0.269
5	142	42373.00	99.96	506.00	9.39	4.38	130.13	0.283
6	203	24494.00	85.41	322.00	5.43	2.53	87.963	0.405
7	210	56576.00	114.35	506.00	12.54	5.85	78.942	0.419
8	216	10511.00	114.09	92.00	2.33	1.09	72.262	0.431
9	282	62438.00	75.09	966.00	13.84	6.46	46.202	0.563
10	306	12390.00	43.04	322.00	2.75	1.28	46.202	0.611
11	315	35348.00	50.61	782.00	7.83	3.66	46.202	0.629

**Figure10. Calculation method for protein patterns profile.**





**Figure 11. Phylogenetic tree for ten samples based on SDS-PAGE profile**

- Egyptian clover cultivar 10 (200 meter isolation distance).
- Egyptian clover cultivar 2 (Serw 1).
- Egyptian clover cultivar 1 (Gemmiza 1).
- Egyptian clover cultivar 9 (100 meter isolation distance).
- Egyptian clover cultivar 7 (50 meter isolation distance).

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