IV. RESULTS

4.1. Biological studies

According to symptomatology illustrated in **Table (6),** the collected samples from the naturally infected beet plants, exhibited virus-like symptoms in open field of El-Riad region, Kafer El-Sheikh Governorate, Egypt appear to have three viruses, i.e., BtMV, BNYVV and BCTV but the symptoms concerning BtMV was observed to be dominant.

Table 6. Symptomatology of natural infected sugar beet plants collected from Kafer, El-Sheikh Governorate, Egypt.

Symptoms	Regions	Suggested viruses	Host range	Results	Viruses
Mosaic, mottling, Malformation (40 out of 50 samples)	El-Thabet, EL-Riad	BtMV or CMV	*B. vulgaris*	Mosaic, mottling, molformation	BtMV
Stunting, malformation, rootlet (6 out of 50 samples)	Abo-Ghallab, EL-Riad	BNYVV	*Spinacia oleracea*	Necrotic lesion, chlorotic mottling, severe stunting	BNYVV
Vein clearing, leaf roll, blisters (4 out of 50 samles)	El-Hageen, EL-Riad	BCTV	*C. quinoa*	Chlorotic lesion	BCTV

4.1.1. Mechanical isolation of BtMV and symptoms on test plants

Leaf with dominant symptoms found in natural infected beet plants **(Figure 2)** were subjected to isolate the causative virus(es) by mechanical transmission on the diagnostic hosts.

Data showed that chlorotic local lesions **(Figure 3, A)** were observed on inoculated leaves of *C. quinoa* as a diagnostic host for BtMV. Single pure isolate of BtMV was obtained via single local lesion isolation on *C. quinoa.*

Results in **Table (7)** indicated that 17 out 26 plants belonging to four families were varied in their response to infect with the virus isolate. The virus isolate exhibited mosaic like-symptoms on sugar beet plants induced similar symptoms on the indicator plants when mechanically inoculated with infectious sap. These reactions included characteristic chlorotic local lesions, which spread into the veins in *Chenopodium amaranticolor* and *Ch. quinoa* (**Figure 3, A**). The viral isolate also produced systemic mosaic on *Glycin max* **(Figure 3, H),** *Spinacea oleracea*, *B. vulgaris* cv. Gazel (**Figure 3, B**), FD9402, LP12, M9651, LP13, Ras poly (**Figure 3, C**), Desprez poly (N) (**Figure 3, D**), Glorius poly (**Figure 3, E**), Pamher, , *B. patellaris* (**Figure 3, F**), *Vicia fabae* (**Figure 3, I**), and chlorotic local lesions on *B. maritime* **(Figure 3, G).** No infection was detected when the virus isolate mechanically inoculated onto plants of *Nicotiana glutinosa*, *N. tabacum* cvs. Samsun, Xanthi and White Burley, *Datura metel*, *D. stramonium, Pisum sativus* and *Cucumis sativus* cv. Atlas, after 20 days from virus mechanical inoculation even after back-inoculation was done.

Table 7. Symptomatology of BtMV isolate on tested plants.

Tested hosts	Infected plants No.	Infected plants %	Symptoms	Back inoculation to *C. quinoa*
	Family : Chenopodiaceae			
B. patellaris	18	90	M,B	+
B. maritima	15	75	M,NL	+
B. vulgaris cv. Desprezpoly (N)	08	40	M,Y	+
B. vulgaris cv.9002	10	50	SM	+
B. vulgaris cv. Pamher	11	55	M	+
B. vulgaris cv.LP12	12	60	M	+
B. vulgaris cv. Gloriuspoly	14	70	M,Mal	+
B. vulgaris cv. Raspoly	15	75	M,C,Y	+
B. vulgaris cv. M9651	16	80	M,B,C	+
B. vulgaris cv. LP13	17	85	M,B	+
B. vulgaris cv. Gazel	18	90	SM,C	+
B. vulgaris cv. FD9402	20	100	Y,M	+
C. amaranticolor	15	75	CL	+
C. quinoa	18	90	CL	+
Spinacia oleracea	08	40	NL,SS, CM	+
	Family : Fabaceae			
G. max cv. Clark	10	50	Mal,M	+
Vicia faba cv. Giza 2	07	35	M	+
Phaseolus vulgaris cv. Giza 3	00	00	-	-
Pisum sativum cv. Lincoln	00	00	-	-
	Family: Cucurbitaceae			
Cucumis sativus cv.Atlas	00	00	-	-
	Family:Solanaceae			
Datura metel	00	00	-	-
D. stramonium	00	00	-	-
Nicotiana glutinosa	00	00	-	-
N. rustica	00	00	-	-
N. tabacum cv. Samsm	00	00	-	-
N. tabacum cv. WhiteBurly	00	00	-	-

Note: A number of 20 plants were used for each tetst host.

- : no symptoms. **CL**: chlorotic lesions. **NL**: necrotic lesions.

+: positive. **CM**: chlorotic mottling. **SM:** severe mosaic.

B: blisters. **M**: mosaic. **SS**: severe stunting.

C: curling. **Mal**: malformation. **Y:** yellowing.

Figure 2. Naturally BtMV-like symptoms on *B. vulgaris* cv. Gazel.

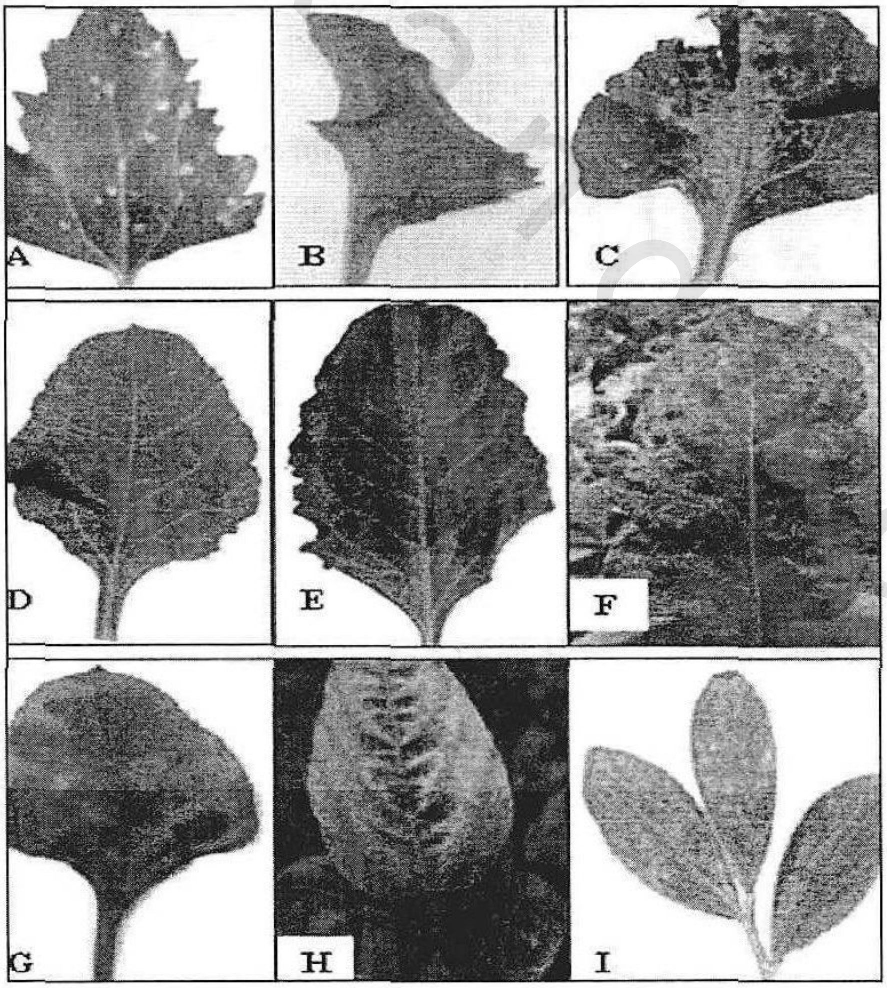

Figure 3. The external symptoms caused by BtMV. Chlorotic local lesions on *C. quinoa* (A), severe mosaic and curling on sugar beet (*B. vulgaris* cv. Gazel) (B), mosaic and yellowing on *B. vulgaris* cv. Ras poly (C), Desprez poly N (D), mosaic and malformation on cv. Glorius poly (E), mosaic, blisters and malformation on *B. patellaris* (F), necrotic

4.1.2. Aphid transmission:

Results showed that BtMV was able to transmit from infected to healthy *B. vulgaris* cv. Gazel by *M. persicae* (Sulz) and *A. fabae* (Glover) in a non persistently manner with percentage of 70 and 57.5, respectively (**Table 8**). Infected plants exposed to insects showed the characteristic symptoms of BtMV after 15 days from virus insect transmission.

Table 8. Aphid transmission of the virus isolate from infected leaves of *B. vulgaris* cv. Gazel plants by two different aphids in non-persistent manner.

Experimnets	**Infected plants inoculated with *M. persicae***		**Infected plants inoculated with *A. fabae***	
	No.	**%**	**No.**	**%**
I	15	75	12	60
II	13	65	11	55
Average	**14**	**70**	**12**	**57.5**

4.1.3. Ultrastructural of infected leaves

The electron micrographs of ultrathin sections of BtMV-infected *B. vulgaris* cv. Gazel leaves 20 days post mechanical inoculation showed CCI as PW **(Figure 4)** and laminated aggregates (LA) **(Figures 5)** in infected mesophyll cells.

4.1.4. Virus purification and negative staining

Ultraviolet absorption spectrum of the purified virus showed a typical curve of nucleoprotein with a minimum and a maximum at wave length of 245 and 260 nm, respectively **(Figure 6)**. The ratio of Amax/Amin and $A_{260/280}$ ranged from 1.6-1.7 and 1.3-1.4, respectively. According to the value of $A_{260/280}$, the percentage of BtMV-RNA was calculated to be about 6. The average yield of the purified virus was estimated to be 15-20 mg /kg infected leaf tissues based on the extinction coefficient of 2.4 for a 1 mg/L ml virus solution at 260 nm.

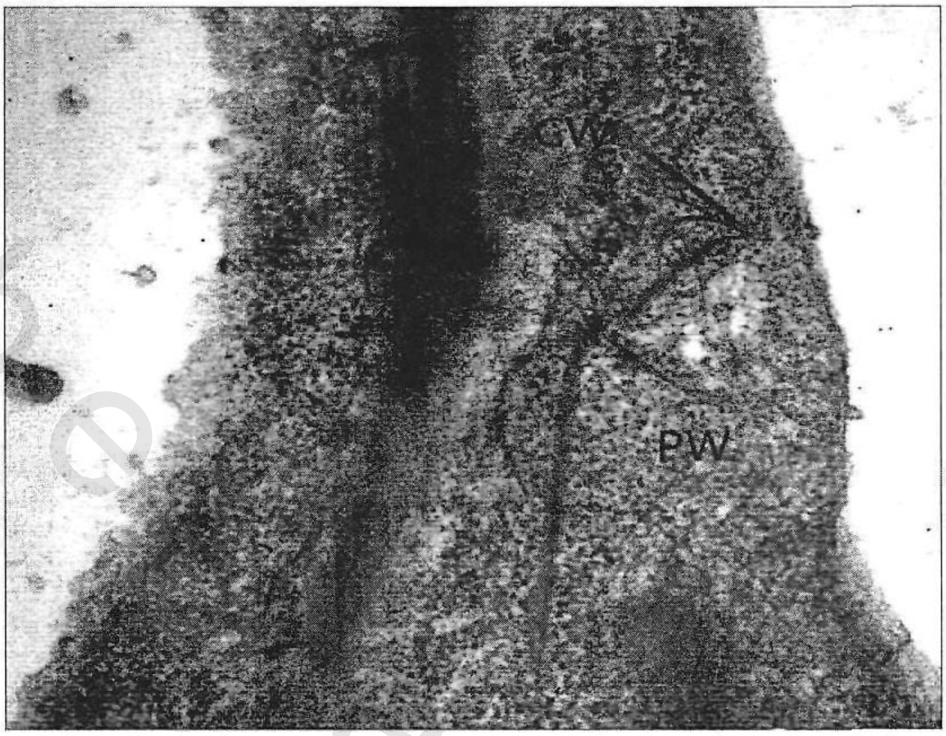

Figure 4. Electron micrographs of ultrathin section of mesopyll cells of sugar beet leaf plants, 20 days post mechanical inoculation. Note, cylindrical cytoplasmic inclusions; pinwheels (PW, Fig.4,A) and laminated aggregates (LA, Fig.4,B and C). Cell Wall(CW), Chloroplast (Ch), Vacuole (Vc).

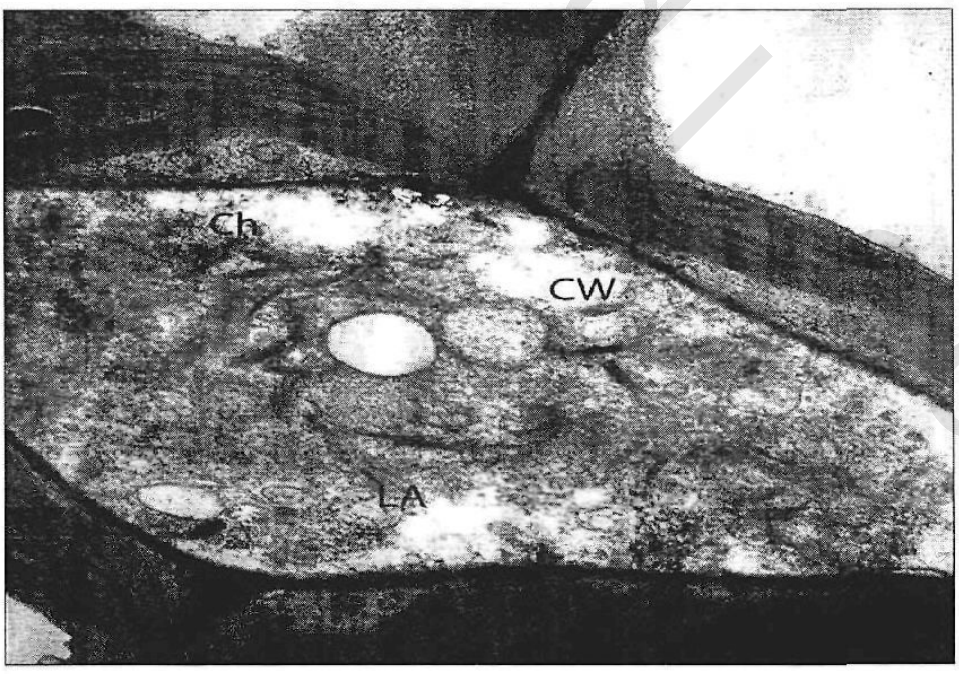

Figure 5. Electron micrographs of ultrathin section of mesopyll cells of sugar beet leaf plants, 20 days post mechanical inoculation. Note, cylindrical cytoplasmic inclusions: laminated aggregates (LA). Cell Wall (CW), Chloroplast (Ch), Vacuole (Vc).

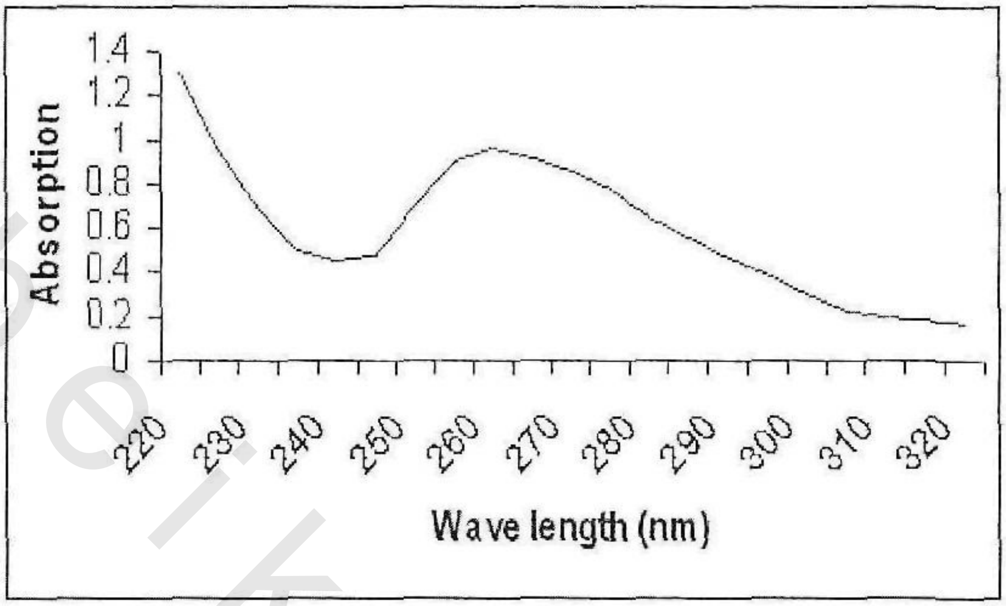

Figure 6. Ultraviolet-absorption spectrum of BtMV purified preparations.

Electron micrograph of BtMV purified preparation showed numerous unaggragated flexuous filamentous particles (**Figure 7**), ranging in length of 700-750 nm, and 13 nm in width, when negatively staining with 2 % aqueous uranyl acetate and examined in transmission electron microscope.

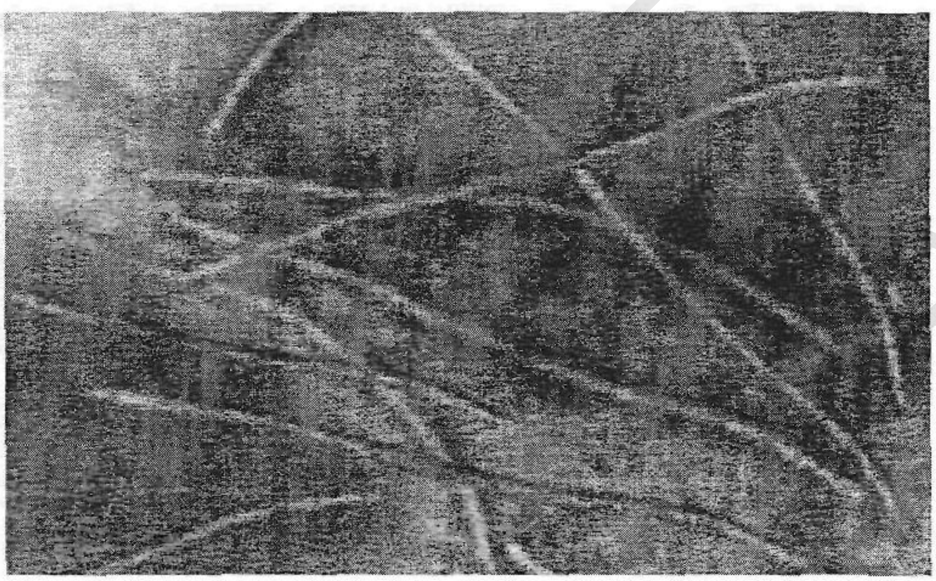

Figure 7. Electron micrograph of purified BtMV, stained with 2 % uranyl acetate.

4.2. Chemical studies

4.2.1. Determination of viral CP molecular weight

A single CP component with an estimated molecular weight of about 34 kDa was detected when the BtMV-CP electrophorsed through 12 % SDS-PAGE (**Figure 8**).

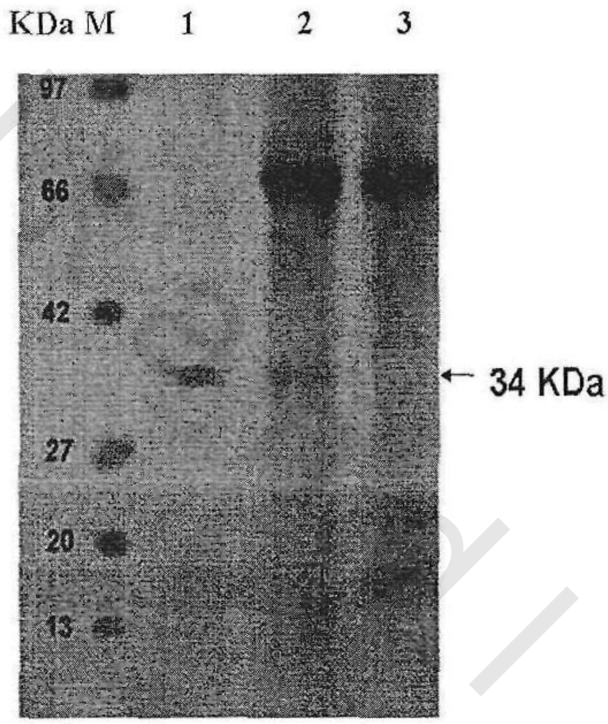

Figure 8. Electrophoresis of BtMV-CP in 12 % SDS-PAGE. Lane M, protein mol.wt marker; Lane 1, BtMV-CP from purified virus; Lane 2, protein prepared from virus-infected leaves; Lane 3, protein prepared from healthy leaves.

4.2.2. Extraction and analysis of viral RNA

Ultraviolet absorption spectrum of nucleic acid had a maximum absorbance at 260 nm and a minimum at 245 nm **(Figure 9).** The concentration of RNA was 3.2 µg / µl based on the extinction coefficient of 25 cm^{-1} mg^{-1}. Nucleic acid extracted from purified virus preparation and electrophoresed through 1 % agarose gel under denaturing condition was migrated as a single component with an estimated sizes of about 10 kb as illustrated in **Figure (10).**

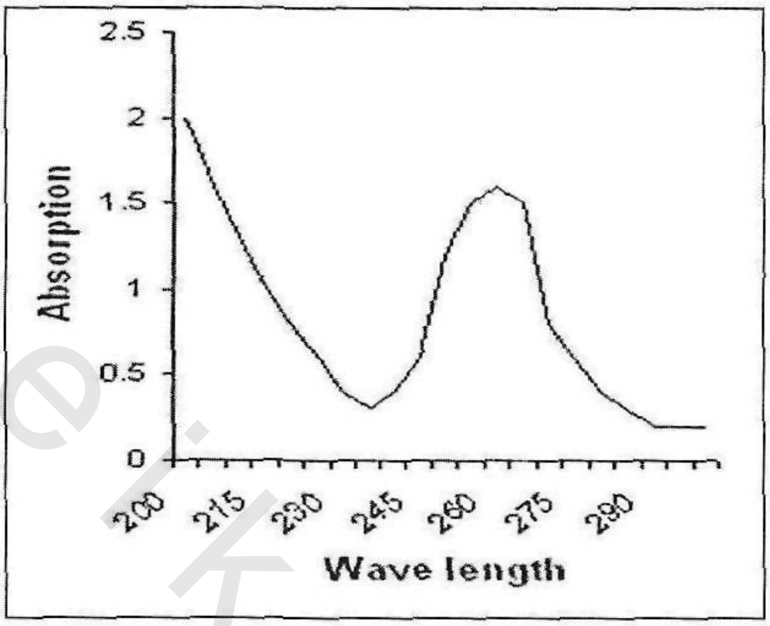

Figure 9. Ultraviolet absorption spectrum of nucleic acid of BtMV.

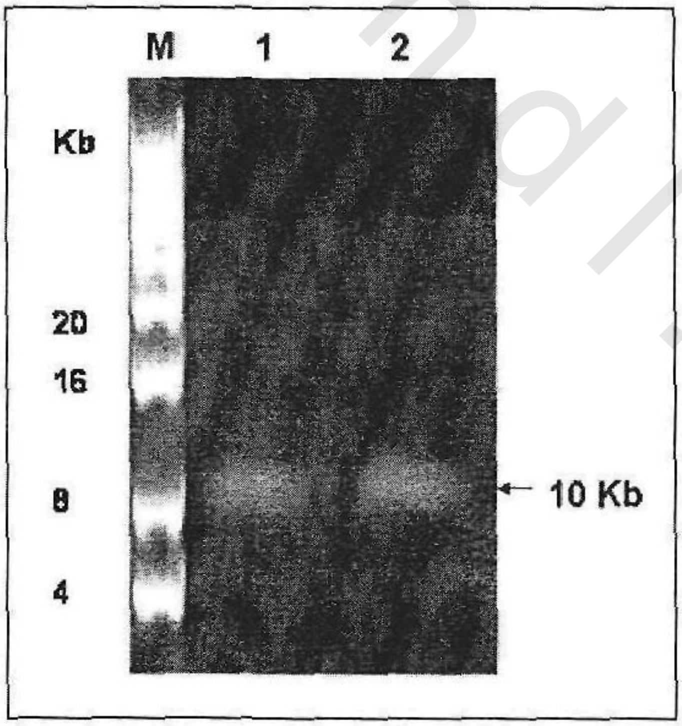

Figure 10. Agarose gel electrophoresis of BtMV-RNA extracted from viral purified particles. Lane M, RNA marker, Lane 1 and 2 , BtMV-RNA.

4.2.3. RT-PCR of BtMV- *cp* gene

After extraction and purification of the BtMV-RNA, it was used as a template in RT-PCR for synthesizing the cDNA followed by amplification of the complete *cp* gene of the viral isolate. Results in **Figure (11)** showed that a PCR fragment with a size of about 755 bp was amplified from BtMV-full genome using two oligonucleotide specific primers.

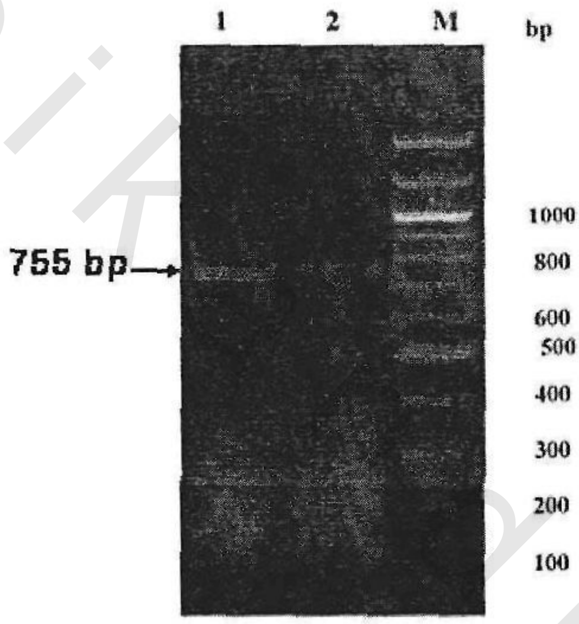

Figure 11. RT-PCR reaction in 1.0% agarose gel stained with ethidum bromide. Lane M; DNA standard marker (BioLabs). Lanes 1 and 2; BtMV-*cp* gene with a size length of about 755 bp.

4.3. Serological studies

4.3.1. Antiserum production

The DEP for the BtMV-antiserum was determined using indirect ELISA. Data presented in **Table (9)** show that the DEP of BtMV-antiserum was 1:4096, followed by 1:1024 and 1:256 after two, one and three weeks post last injection, respectively. Negative and positive ELISA values were obtained with dilutions of 10^{-1} against clarified healthy and infected sugar beet saps.

Table 9. DEP of BtMV-antiserum using indirect ELISA after one, two and three weeks post last injection.

Antisrum	**1st week**		**2nd week**		**3rd week**	
dilutions	**EV**	**R**	**EV**	**R**	**EV**	**R**
Undiluted	1.422	+	1.456	+	1.023	+
1:2	*	+	1.152	+	0.925	+
1:4	1.077	+	0.991	+	0.848	+
1:8	0.987	+	0.910	+	0.765	+
1:16	0.900	+	0.895	+	0.633	+
1:32	0.841	+	0.806	+	0.571	+
1:64	0.755	+	0.784	+	0.434	+
1:128	0.589	+	0.765	+	0.382	+
1:256	0.500	+	0.650	+	0.366	+
1:512	0.496	+	0.545	+	0.301	-
1:1024	0.400	+	0.507	+	0.215	-
1:2048	0.367	-	0.450	+	0.182	-
1:4096	0.159	-	0.329	+	0.068	-
1:8192	0.051	-	0.123	-	0.035	-
1:16384	0.047	-	0.090	-	0.021	-
	0.035					
Negative	**0.158**	-	**0.163**	-	**0.177**	-
control**	**0.921**	+	**0.959**	+	**0.850**	+
Positive						
control***						

*EV: ELISA value at 405 nm (average of 4 replicates) after 1 h from incubation at 37°C. **Negative control: Sap-extracted from healthy leaves of *B. vulgaris* cv. Gazel plants with undiluted antiserum. ***Positive control: Sap-extracted from virus-infected leaves of *B. vulgaris* cv. Gazel plants with undiluted antiserum. Note. The ELISA value that equals more than two folds of healthy control was considered as a positive (+) result.

4.3.2. Evaluation of BtMV-antiserum

4.3.2.1. Using TBIA

This method was used to evaluate BtMV-antiserum by detecting BtMV in infected beet tissues directly printed on a nitrocellulose membrane. The test gave positive results in the form of purple color development with the infected tissues, while no color was obtained with healthy ones (**Figure 12**).

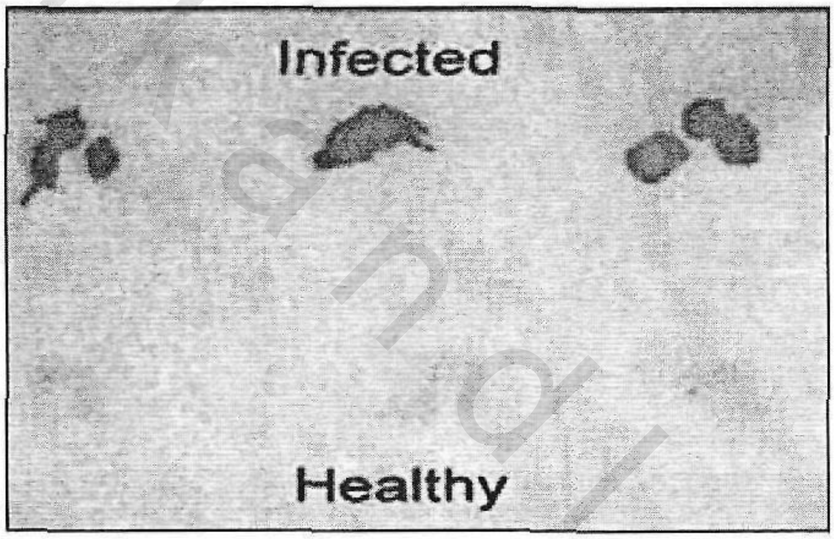

Figure 12. TBIA analysis of BtMV-detection using sugar beet infected and healthy (control) leaf tissues. Purple color was obtained with the infected tissues, while negative result was observed with control samples.

4.3.2.2. Using DBIA

Dot blot immunoassay was performed on a serial of ten fold dilutions of infected sugar beet sap. Positive results in the form of purple color dots were obtained with the purified virus preparations and with the infectious sap dilutions up to 10^{-6} (**Figure 13**). Negative result was obtained with healthy sugar beet sap.

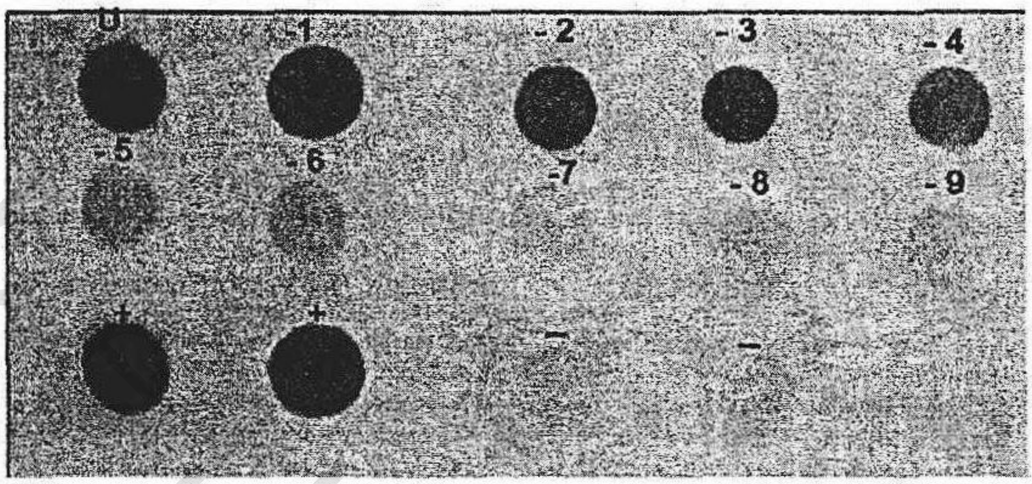

Figure 13. DBIA analysis of BtMV in a serial of ten fold dilutions of infected sugar beet sap (1-9). (-) :Negative control; Sap extracted from healthy leaves, (+): Positive control; purified virus preparations, U: undiluted sap.

4.4. Effect of BtMV on sugar beet growth and some chemical constituents

Data represented in **(Table 10)** and illustrated by **(Figure 14 and 15)** indicated that the different cultivars of sugar beet plants were affected with BtMV that reflected in the reduction in fresh and dry weight of shoots and roots which ranged from 21-51, 25-49 % in shoots and 27-57, 29-56 % in roots, respectively (significantly different than the control).

BtMV was caused significantly decrease of sucrose content in storage roots of different cultivars of sugar beet which ranged from 29-50%, whereas sodium content was significantly increased to be about 7-17 %. The amino nitrogen content in the storage roots was affected by BtMV infection with varied from significantly and non-significantly increased, whereas the potassium content was non-significantly reduced in all sugar beet cultivars **(Table 11)** and **(Figures 16-19)**.

Table 10. Effect of BtMV on fresh and dry weight of shoots and roots of some sugar beet cultivars.

Sugar beet cultivars	Shoots								Roots							
	Fresh weight (g/plant)				Dry weight (g/plant)				Fresh weight (g/plant)				Dry weight (g/plant)			
	H	I	% Reduction	S	H	I	% Reduction	S	H	I	% Reduction	S	H	I	% Reduction	S
FD9402	145*	75	48	+	31.53	16.8	46	+	150**	106	57	+	40.87	28.62	29	+
Gazel	182	93	49	+	36.4	20.46	43	+	190	98	48	+	50.23	26.48	47	+
LP13	189	102	46	+	39.65	21.31	46	+	182	79	56	+	47.41	20.50	56	+
M9651	217	105	51	+	44.41	22.50	49	+	202	100	50	+	55.16	26.42	52	+
Raspoly	120	70	41	+	25.22	15.54	38	+	161	75	53	+	43.52	21.25	51	+
Gloriuspoly	153	83	45	+	32.13	17.43	45	+	187	91	51	+	50.49	23.66	52	+
LP12	160	95	21	+	34	19.95	41	+	261	145	44	+	67.3	36.25	46	+
Pamher	122	78	26	+	25.62	16.38	36	+	178	101	43	+	47.4	26.26	44	+
9002	99	73	40	+	22.76	14.65	35	+	221	155	29	+	58.7	39.75	32	+
Dezprezpoly(N)	123	96	36	+	25.8	19.16	25	+	220	160	27	+	62	40.21	35	+

*Each number is an average of five plant replicates of shoots. ** Each number is an average of five replicates of roots. H: Roots or shoots obtained from healthy plants. I: Roots or shoots obtained from infected plants. S: Significant according to SAS analysis.

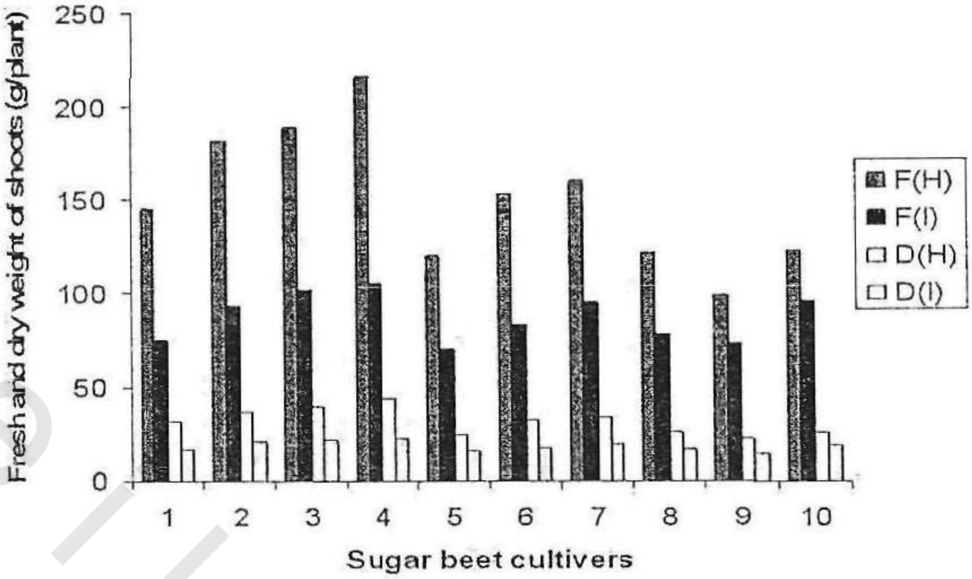

Figure 14. Effect of BtMV on fresh and dry weight of shoots of ten sugar beet cultivars: 1: FD9402, 2: Gazel, 3: LP13, 4: M9651, 5: Raspoly, 6: Gloriuspoly, 7: LP12, 8: Pamher, 9: 9002, 10: Desprezpoly (N). F(H): *fresh weight from healthy shoots* , F(I): fresh weight from infected shoots, D(H): Dry weight from healthy shoots, D(I): Dry weight from infected shoots.

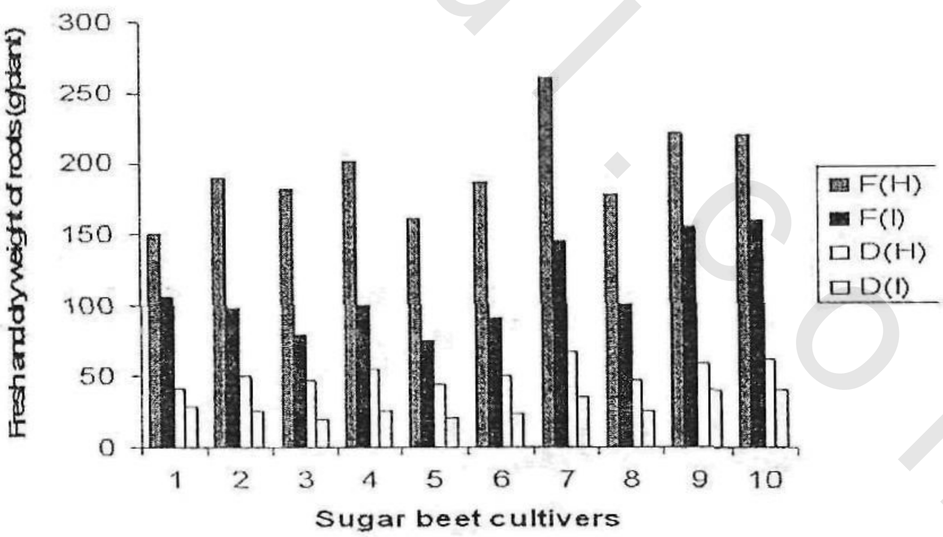

Figure 15. Effect of BtMV on fresh and dry weight of roots of ten sugar beet cultivars: 1: FD9402, 2: Gazel, 3: LP13, 4: M9651, 5: Raspoly, 6: Gloriuspoly, 7: LP12, 8: Pamher, 9: 9002, 10: Desprezpoly (N). F(H): fresh weight from healthy roots , F(I): fresh weight from infected roots, D(H): Dry weight from healthy roots, D(I): Dry weight from infected roots.

Table 11. Effect of BtMV on some chemical constituents in storage roots of some sugar beet cultivers.

Sugar beet cultivers	Sucrose content (g)				*Amino nitrogen content				*Sodium content				*Potassium content			
	H	I	% of reduction	S	H	I	% of increase	S	H	I	% of increase	S	H	I	% of reduction	S
FD9402	**25.65	14.7	42	+	**1.70	1.82	6	-	**1.84	2.04	9	+	**3.50	3.48	0.5	-
Gazel	30.12	17.44	42	+	1.75	1.85	5	-	1.82	2.17	16	+	3.39	3.37	0.5	-
LP13	29.31	16.48	43	+	1.53	1.78	14	+	1.91	2.06	7	+	3.32	3.29	0.9	-
M9651	32.54	16.2	50	+	1.84	1.92	4	-	1.71	1.94	11	+	3.47	3.44	0.8	-
Raspoly	26.23	15.8	39	+	1.60	1.88	15	+	1.75	2.13	17	+	3.40	3.37	0.8	-
Gloriuspoly	28.43	17.5	38	+	1.43	1.50	4	-	1.83	2.02	9	+	3.20	3.18	0.6	-
LP12	41.53	27.21	34	+	1.67	1.79	6	-	1.77	1.96	9	+	3.37	3.36	0.2	-
Pamher	30.20	17.43	42	+	1.75	1.83	4	-	1.80	1.98	9	+	3.49	3.47	0.5	-
9002	34.13	24.23	29	+	1.71	1.75	2	-	1.85	2.01	7	+	3.53	3.50	0.8	-
Dezprezpoly(N)	34.45	21.75	36	+	1.73	1.8	3	-	1.94	2.19	11	+	3.48	3.47	0.2	-

*Amino nitrogem, Sodium and potassium content determined per milligram / 50 g storage root. **Each number is an average of five plants replicates of roots. H: Roots obtained from healthy plants. I: Roots obtained from infected plants. S: Significant according to SAS analysis.

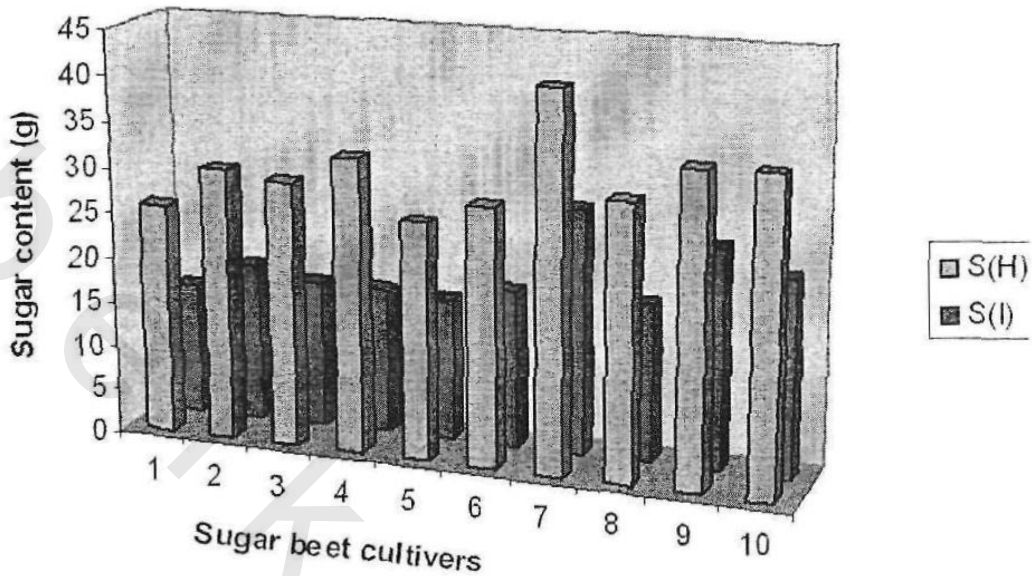

Figure 16. Effect of BtMV on sugar content in the storage roots of ten sugar beet cultivars: 1: FD9402, 2: Gazel, 3: LP13, 4: M9651, 5: Raspoly, 6: Gloriuspoly, 7: LP12, 8: Pamher, 9: 9002, 10: Desprezpoly (N). S(H): Sugar content in storage roots from healthy plants, S(I): Sugar content in storage roots from infected plants.

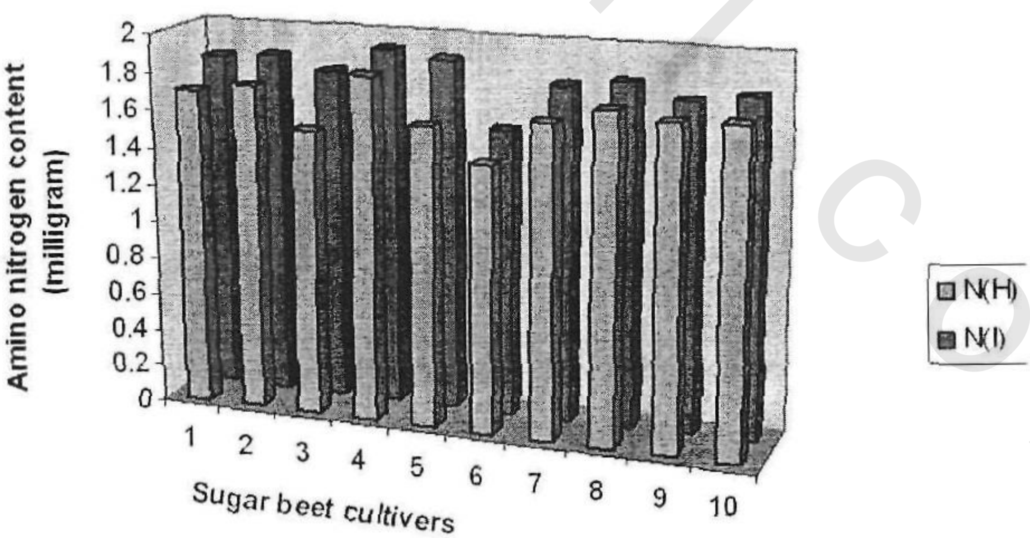

Figure 17. Effect of BtMV on amino nitrogen content of ten sugar beet cultivars: 1: FD9402, 2: Gazel, 3: LP13, 4: M9651, 5: Raspoly, 6: Gloriuspoly, 7: LP12, 8: Pamher, 9: 9002, 10: Desprezpoly (N). N(H): Amino nitrogen content in storage roots from healthy plants, N(I): Amino nitrogen content in storage roots from infected plants.

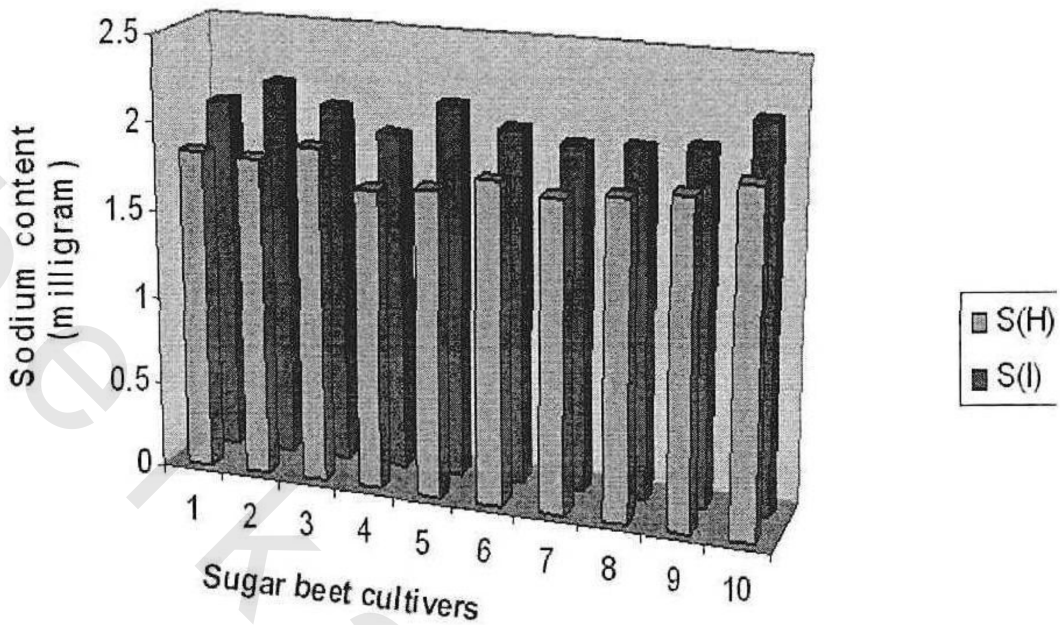

Figure 18. Effect of BtMV on sodium content of ten sugar beet cultivars: 1: FD9402, 2: Gazel, 3: LP13, 4: M9651, 5: Raspoly, 6: Gloriuspoly, 7: LP12, 8: Pamher, 9: 9002, 10: Desprezpoly (N). S(H): Sodium content in storage roots from healthy plants, S(I): Sodium content in storage roots from infected plants.

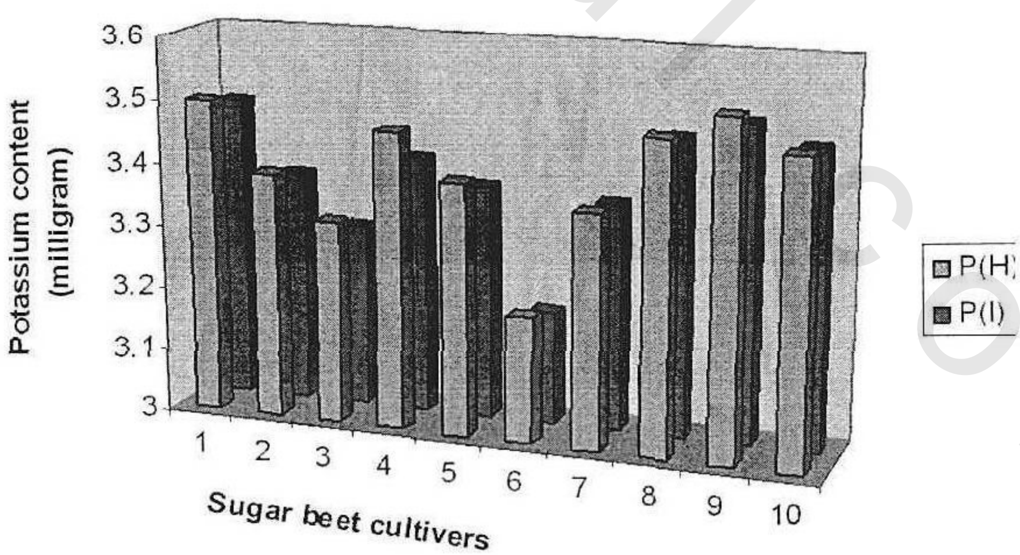

Figure 19. Effect of BtMV on potassium content of ten sugar beet cultivars: 1: FD9402, 2: Gazel, 3: LP13, 4: M9651, 5: Raspoly, 6: Gloriuspoly, 7: LP12, 8: Pamher, 9: 9002, 10: Desprezpoly (N). P(H): Potassium content in storage roots from healthy plants, P(I): Potassium content in storage roots from infected plants.

Table 12. ISSR-PCR variation of DNA genome among different cultivars of *B. vulgaris* using 6 ISSR primers.

Primer	Bands (#)	Nucleotide size (bp)	Sugar beet cultivers									
			Desprezpoly	9002	Pamher	LP12	Gloriuspoly	Raspoly	M9651	LP13	Gazel	FD9402
17899B	1	1701	-	-	+	-	-	-	-	-	-	-
	2	1342	+	+	+	+	+	+	-	+	+	+
	3	1235	-	-	-	+	+	+	+	+	-	+
	4	937	+	+	+	+	+	+	+	+	-	+
	5	848	-	-	-	-	-	+	-	+	-	-
	6	812	-	-	-	-	+	-	-	-	-	-
	7	741	+	+	+	+	+	+	+	+	+	+
	8	692	-	-	-	-	+	-	-	-	-	-
	9	616	-	+	-	+	-	-	-	-	-	+
	10	529	+	+	+	+	+	+	+	+	+	+
	11	478	-	+	-	+	-	-	-	-	-	+
	12	402	-	-	+	+	+	-	-	+	-	+
	13	341	+	+	+	+	+	+	+	+	+	-
	14	293	+	-	-	-	-	-	-	-	-	-
	15	266	-	-	-	-	-	-	-	+	-	-
17898A	1	1296	-	-	-	+	+	-	-	-	+	+
	2	1231	+	+	+	+	+	+	+	+	+	+
	3	1082	+	+	-	+	+	+	+	+	+	+
	4	1036	-	+	+	+	-	+	-	-	-	-
	5	962	+	+	+	+	+	+	+	+	+	+
	6	928	-	+	+	+	+	+	+	+	+	-
	7	869	+	+	+	+	+	+	+	+	+	+
	8	805	-	-	-	+	-	-	-	-	-	-
	9	743	+	+	+	+	+	+	+	+	+	+
	10	687	+	+	+	+	+	+	+	+	+	+
	11	647	+	+	+	+	+	+	-	+	-	-
	12	574	+	+	+	+	+	+	+	+	+	+
	13	514	+	+	+	+	+	+	+	+	+	+
	14	443	+	+	+	+	+	+	-	-	+	+

Table 12. Continue.

	15	403	+	-	+	-	+	+	+	+	+	-
	16	329	+	+	+	+	+	+	+	+	+	+
17899A	1	1434	ss+	-	+	+	+	-	-	-	+	-
	2	1283	+	+	+	+	+	+	+	+	+	+
	3	1055	+	+	+	+	+	+	-	+	+	+
	4	1007	+	+	+	+	+	+	+	+	+	+
	5	839	+	+	+	+	+	+	+	+	+	+
	6	736	+	+	+	+	+	+	+	+	+	+
	7	645	-	-	-	-	-	-	-	-	+	-
	8	610	-	-	-	-	-	-	-	-	-	+
	9	485	+	+	+	+	+	-	+	+	+	+
	10	366	+	+	+	+	+	+	+	+	+	+
	11	150	+	+	+	+	+	+	+	+	+	+
ISSR3	1	1542	-	-	-	-	+	-	-	-	-	-
	2	1358	+	-	-	+	-	+	+	+	-	-
	3	916	+	+	+	+	+	-	+	+	-	+
	4	866	+	+	+	+	+	+	+	-	+	+
	5	720	+	-	+	+	-	-	-	-	-	-
	6	666	-	-	-	-	-	-	+	+	-	-
	7	594	+	+	+	+	+	+	+	+	+	+
	8	496	-	-	-	-	-	-	+	+	-	-
	9	477	-	+	+	+	-	+	+	-	+	+
	10	465	+	-	-	-	+	-	-	+	-	-
	11	331	+	-	-	-	-	-	-	-	+	-
	12	315	-	-	+	+	+	+	+	-	-	-
	13	295	-	+	-	-	-	-	-	-	-	+
	14	290	-	-	+	+	-	-	-	-	-	-
ISSR16	1	1399	-	+	+	+	+	+	+	+	+	+
	2	1231	-	+	+	+	+	+	+	-	-	+
	3	1124	+	+	+	+	+	+	+	+	+	+
	4	1031	+	+	+	+	+	+	+	+	+	+

Table 12. Continue.

	5	950	+	+	+	+	+	+	+	+	-	+
	6	882	-	-	-	+	-	-	-	-	-	-
	7	833	-	-	-	-	-	-	+	-	+	+
	8	781	-	+	-	+	+	+	+	-	+	+
	9	711	+	+	+	-	+	-	+	+	+	+
	10	669	+	+	+	+	+	+	+	+	+	+
	11	567	+	+	+	+	+	+	+	+	+	+
	12	498	+	+	+	+	+	+	+	+	+	+
	13	431	+	-	+	+	+	+	+	+	+	+
	14	397	+	+	+	+	+	+	+	+	+	+
	15	314	+	+	+	+	-	-	-	+	+	+
	16	304	-	-	-	-	+	+	-	-	-	-
ISSR17	1	1489	+	+	-	-	+	+	+	+	+	+
	2	1290	+	+	-	-	+	+	-	-	-	+
	3	1171	+	+	+	+	+	+	+	+	+	+
	4	1070	+	+	+	+	+	+	+	+	+	+
	5	972	+	+	+	+	+	+	+	+	+	+
	6	840	-	+	-	-	+	+	+	+	+	+
	7	784	+	+	-	+	+	+	-	-	+	+
	8	717	+	+	+	-	+	+	+	+	+	+
	9	666	+	+	+	+	+	+	+	+	+	+
	10	563	+	+	+	+	+	+	+	+	+	+
	11	495	+	+	+	+	+	+	+	+	+	+
	12	431	+	+	+	+	+	+	+	+	+	+
	13	395	+	+	+	+	+	+	+	+	+	+
	14	360	-	-	-	-	+	-	-	-	-	-
	15	310	+	+	+	+	+	+	+	+	+	+

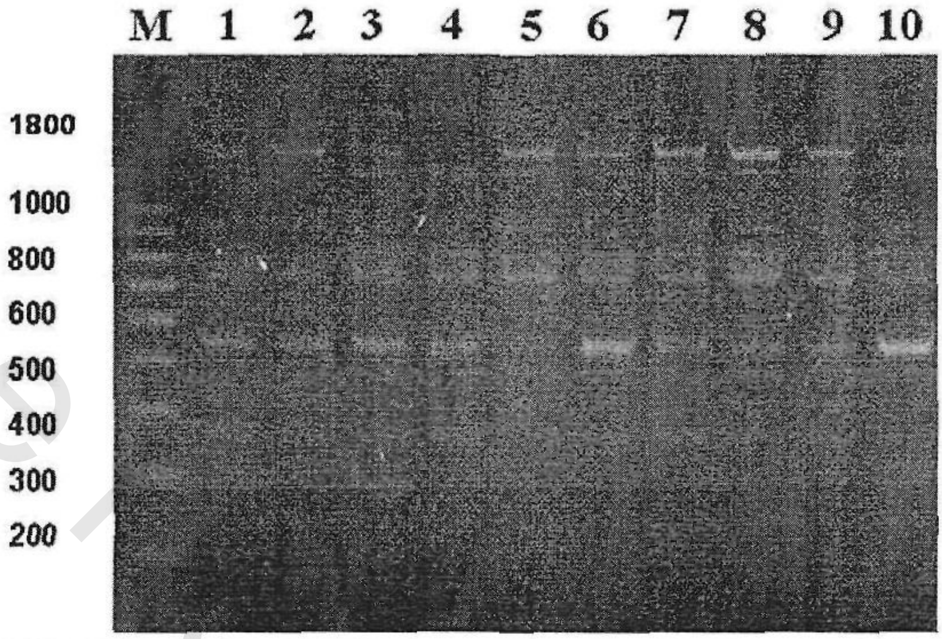

Figure 20. ISSR-PCR variation of DNA genome among *B. vulgaris* cvs Desprezpoly (N) (Lane 1), 9002 (Lane 2), Pamher (Lane 3), LP12 (Lane 4), Gloriuspoly (Lane5), Raspoly (Lane 6), M9651 (Lane 7), LP13 (Lane 8), Gazel (Lane 9) and FD9402 (Lane 10) using primer 17899B. M: DNA marker.

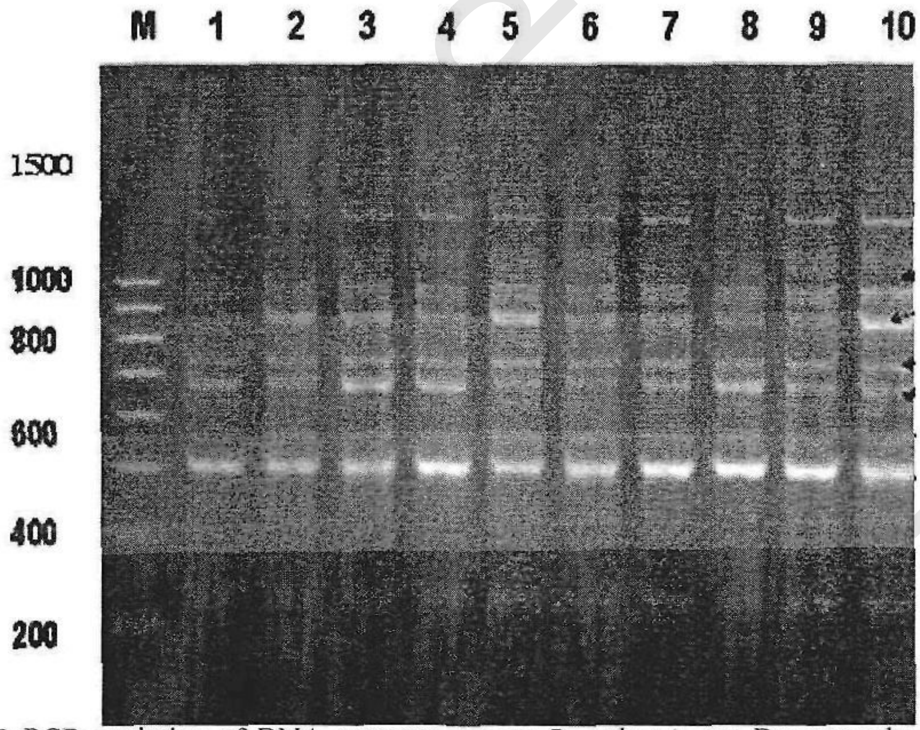

Figure 21. ISSR-PCR variation of DNA genome among *B. vulgaris* cvs Desprezpoly (N) (Lane 1), 9002 (Lane 2), Pamher (Lane 3), LP12 (Lane 4), Gloriuspoly (Lane5), Raspoly (Lane 6), M9651 (Lane 7), LP13 (Lane 8), Gazel (Lane 9) and FD9402 (Lane 10) using primer 17898A. M: DNA marker, UF: unique fragment.

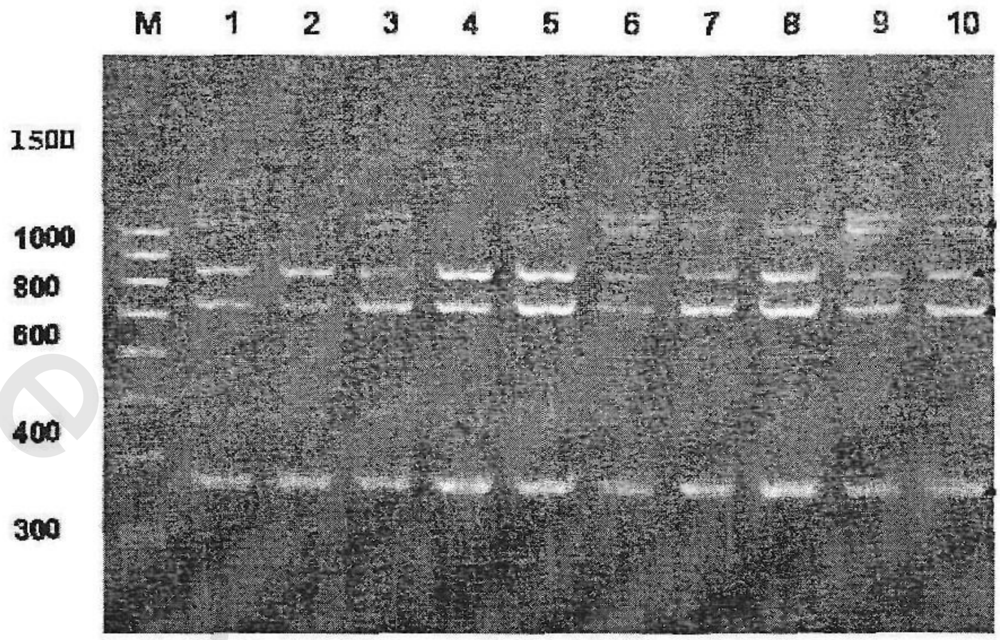

Figure 22. ISSR-PCR variation of DNA genome among *B. vulgaris* cvs Desprezpoly (N) (Lane 1), 9002 (Lane 2), Pamher (Lane 3), LP12 (Lane 4), Gloriuspoly (Lane5), Raspoly (Lane 6), M9651 (Lane 7), LP13 (Lane 8), Gazel (Lane 9) and FD9402 (Lane 10) using primer 17899A. M: DNA marker.

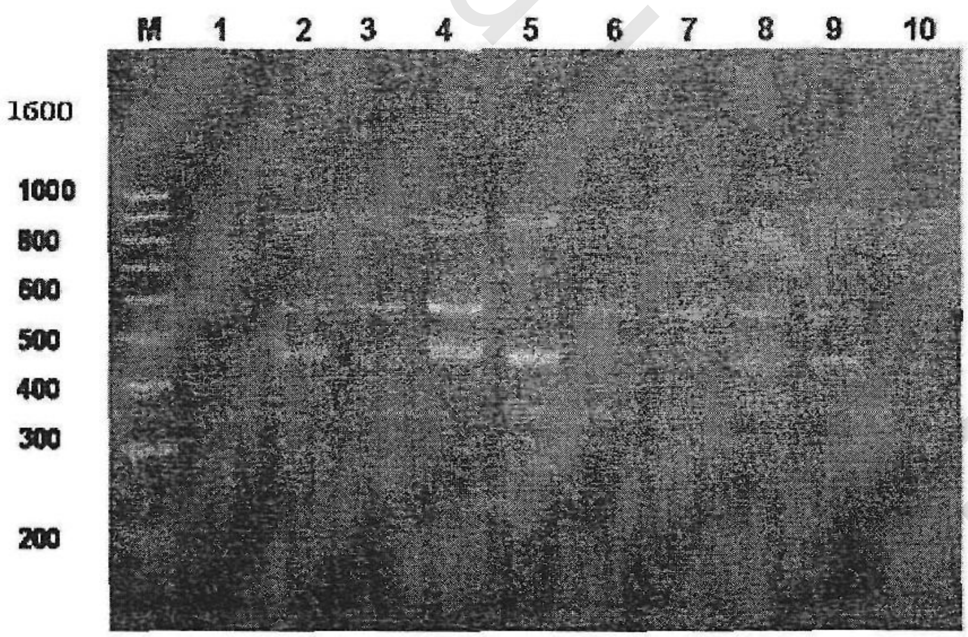

Figure 23. ISSR-PCR variation of DNA genome among *B. vulgaris* cvs Desprezpoly (N) (Lane 1), 9002 (Lane 2), Pamher (Lane 3), LP12 (Lane 4), Gloriuspoly (Lane5), Raspoly (Lane 6), M9651 (Lane 7), LP13 (Lane 8), Gazel (Lane 9) and FD9402 (Lane 10) using primer ISSR3. M: DNA marker.

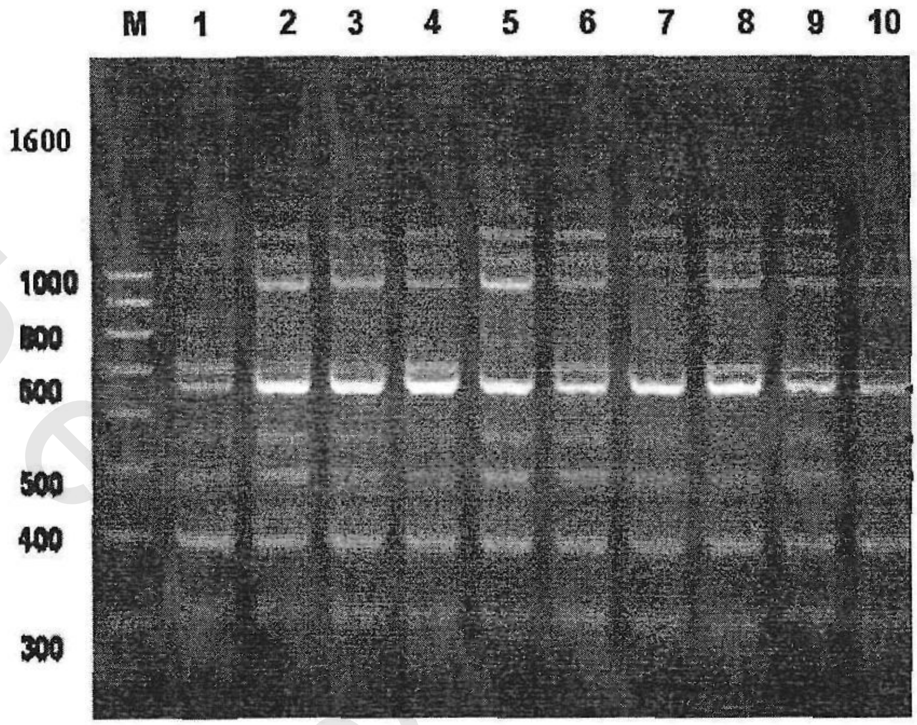

Figure 24. ISSR-PCR variation of DNA genome among *B. vulgaris* cvs Desprezpoly (N) (Lane 1), 9002 (Lane 2), Pamher (Lane 3), LP12 (Lane 4), Gloriuspoly (Lane5), Raspoly (Lane 6), M9651 (Lane 7), LP13 (Lane 8), Gazel (Lane 9) and FD9402 (Lane 10) using primer ISSR16. M: DNA marker.

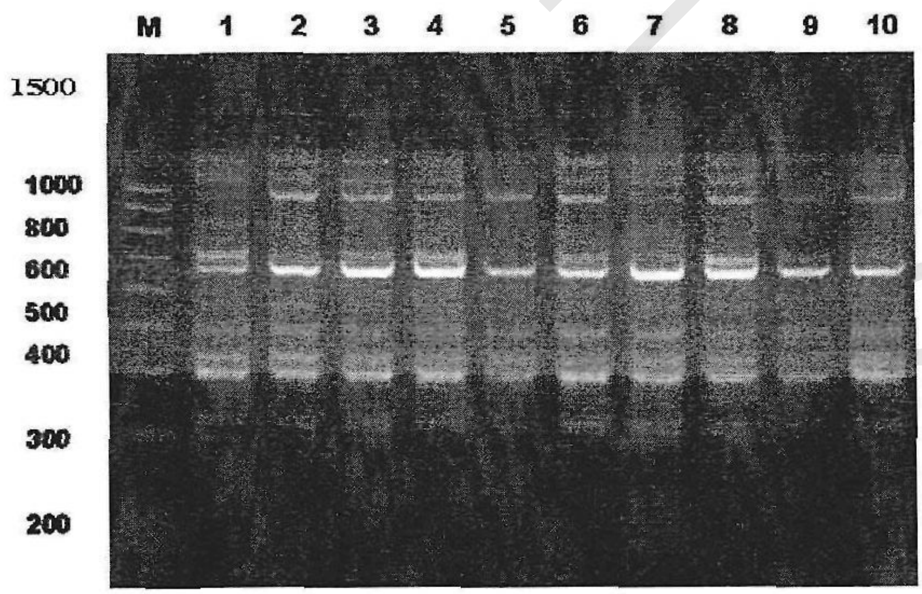

Figure 25. ISSR-PCR variation of DNA genome among *B. vulgaris* cvs Desprezpoly (N) (Lane 1), 9002 (Lane 2), Pamher (Lane 3), LP12 (Lane 4), Gloriuspoly (Lane5), Raspoly (Lane 6), M9651 (Lane 7), LP13 (Lane 8), Gazel (Lane 9) and FD9402 (Lane 10) using primer ISSR17. M: DNA marke

Data in **Table (13)** and **Figure (26)** show the similarities between the sugar beet (*B. vulgaris*) cultivars based on ISSR using 6 primers, which ranged from 79 to 90%. Also, phylogenetic tree showed the presences of three clusters: the first includes sugar beet cultivars: LP12, Pamher, 9002 & Desprez poly(N); the second includes M9651, LP13, Ras poly and Glorius poly sugar beet cultivars and the third includes Gazel and FD9402 sugar beet cultivars.

Table 13. Similarities between the sugar beet cultivars based on ISSR-PCR using six primers.

Sugarbeet cultivers	Similarities between the sugar beet cultivars									
	Gazel	FD9402	LP13	M9651	LP12	9002	Ras poly	Gloris poly	Pamher	Desprez poly (N)
Gazel	100									
FD9402	85	100								
LP13	82	82	100							
M9651	85	85	89	100						
LP12	80	85	82	81	100					
9002	83	85	84	83	86	100				
Ras poly	83	83	85	86	85	85	100			
Glorius poly	84	85	82	83	83	85	88	100		
Pamher	81	79	84	82	87	89	82	85	100	
Desprez poly(N)	85	81	85	81	81	90	83	85	87	100

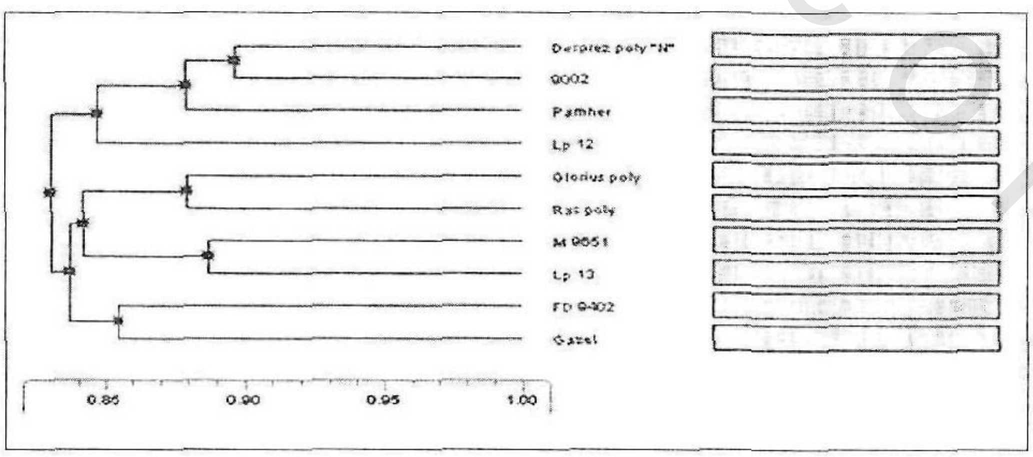

Figure 26. Phylogenetic tree of DNA genome of 10 Sugar beet (*B. vulgaris*) cultivars based on ISSR analysis using 6 primers.