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Faculty of Graduate Studies

Detection of Cauliflower Mosaic Virus on Brassica Plant Family in West Bank-Palestine

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Dedication

This thesis is dedicated to my mother and father without their endless love and encouragement I would never have been able to complete my studies.

To my husband who was encourage and support me to complete my high study and he still increasing my ambition for a PhD.

To my beloved sisters.

To my lovely brothers.

Thanks all for your love, support and lasting respect for my work on a Master thesis

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Great thanks for the Palestinian Ministry of Agriculture engineers

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All thanks for miss Naela

I also thanks my family for standing with me in each step of my life.

الاقرار

أنا الموقع أدناه، مقدّم الرسالة التي تحمل العنوان:

Detection of Cauliflower Mosaic Virus on Brassica Plant Family in West Bank-Palestine

أقر بأن ما اشتملت عليه هذه الأطروحة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة إليه حيثما ورد. وأن هذه الرسالة كاملة، أو اي جزء منها لم يقدم من قبل لنيل اي درجة او لقب علمي او بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالبة:** رحيق اعبه ابو عمر

Signature: **التوقيع:**

Date: **التاريخ:** ١١ - ١١ - ٢٠٢١

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List of Abbreviations

dsDNA	Double stranded DNA
CTAB	Cetyltrimethylammonium bromide
Gm	Grams
Kda	Kilo Dalton
Ml	Mill mole
µl	Microliter
MW	Molecular Weight
Nm	Nanomolar
PCR	Polymerase Chain Reaction
ºC	Degree Celsius
DAS-ELISA	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
ddH2O	double-distilled water
SDS	Sodium dodecyl sulfate
Rpm	revolutions per minute
Min	Minute
dNTPs	Deoxy nucleotide triphosphates

Detection of Cauliflower Mosaic Virus on Brassica Plant Family in West Bank-Palestine

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Abstract

Cauliflower mosaic virus (CaMV) belongs to the genus Caulimovirus; that specifically infect plant family Brassicaceae as broccoli, cabbage, cauliflower and turnips. This study was aimed to verify the presence of CaMV in northern Palestine. Fields were surveyed to report any viral symptoms on brassica plants in An-Nassaria; Qabatyeh; Aqqaba; Tubas and Tulkarem, to determine the occurrence and distribution of cauliflower mosaic virus in several cruciferous crops in Palestine. The results of field surveys revealed viral symptoms on cabbage and cauliflowers ranged from yellow leaves; mottling; leaf deformation; dwarfism and stunt growth. A total of 200 samples were collected randomly from different fields and tested by using molecular tool (PCR). DNA extractions were applied on these collected samples after several methods of extraction were verified. Modified dellaporta protocol was found the best for DNA extraction. Using PCR test, the viral incidence was reported in 12.5% of tested samples. We believe that this value is alarming since the virus can be easily transmitted by aphid (Myzus persicae). Besides, this research was the first to confirm the existence of CaMV virus in Palestinian fields.

Chapter one
Introduction and Literature Review

1.1 Introduction

Brassica oleracea is a genus of plants that are infected with the cauliflower mosaic virus (CaMV). The scientific name for numerous plant species is *Brassica oleracea* (broccoli, cabbage, cauliflower, kale, and turnips). *Brassica oleracea* is a popular crop in Palestineand considered one of the most economic vegetable crops, where it is used to treat and prevent a variety of metabolic, degenerative, and cardiovascular disorders (Jaradat et al., 2017). Due to its broad variety of forms and importance as a cultivated vegetable crop, cultivated Brassica oleracea has piqued the interest of scholars for generations. (Makenzie et al., 2021), and (Hunter et al., 2002). Brassica vegetables are high in glucosinolate, which is a sulfur-containing chemical. These vegetables are high in vitamins, low in fat, and antioxidants due to the presence of catalytic enzymes, carotenoids, vitamin C, and vitamin E. (Bischoff, 2016).

The relevance of these plants and their historical existence in Palestine were reported in research released in 2021. *Brassica* oleracea is believed to have originated in the Eastern Mediterranean region, which includes Cyprus, Greece, Palestine, Lebanon, Syria, Jordan, Turkey, Egypt, and Israel, according to the findings of this study. (Mabry et al., 2021).

According to the (Palestinian Central Bureau of Statistics; personal communication) Brassicacea family was expanded on an area of 7490

dunum in Jenin, Tulkarem, Nablus, and Tubas in 2019-2020. See Table (1). According to agriculture department white cabbage and cauliflower plants were the most plants that were cultivated in the mentioned areas.

Table 1: This table represent the areas that planted with brassica plants.

Governorate	The name of area	Brassica plants area in dunum					
		Cauliflower	White Cabbage	Red cabbage	Rocked salad	radish	Turnip
Jenin	Qabatia	800	600	-	-	15	100
Tubas	Tubas	-	100	-	-	-	-
Tubas	All Governorate	810	1210	130	-	-	-
Tulkarem	Tulkarem	395	120	26	18	25	9
Tulkarem	All Governorate	2185	390	92	59	60	19
Nablus	Al Nassaria	260	165	-	2	-	-

CaMV is the most common virus that infects brassica plants and has an economic impact (Spence et al., 2007). This research aimed to test the existence of this important virus on brassica plants in northern Palestine. The outcome of this study will be the first-ever report of the virus on Palestinian territories.

1.2. Literature Review

1.2.1. Brassica plant infecting viruses

Using PCR, ELISA, electron microscopy, and biological testing, earlier research on CaMV that causes infection in the brassica family revealed that the most dominant viruses infecting the brassica family were cucumber mosaic virus and cauliflower mosaic virus. (Ayyaz et al., 2018)

Brassicacea family may be infected by a number of virus see Table (2).

Table 2. The common virus that infect brassica plants. (Raybould et al., 1999).

Viruses Name		**Genus**
Cauliflower mosaic virus	CaMV	Caulimovirus
Cucumber Mosaic Virus	CMV	Cytomegalovirus
Beet Western Yellows Virus	BWYV	Polerovirus
Radish Mosaic Virus	RaMV	Comovirus
Turnip Mosaic Virus	TuMV	Potyvirus
Turnip Yellow Mosaic Virus	TYMV	Tymovirus
Turnip Yellows Virus	TuYV	Polerovirus

CaMV is a virus that infects members of the Brassicaceae family and has a significant economic impact (Moreno et al., 2004). The highest prevalence of CaMV infection was frequently connected with cauliflower and turnip plantings, according to several research (Farzadfar et al., 2005). According

to a study published in Iran in 2019, seven samples out of 25 were infected with three primary viruses that infect brassica plants.

TuMV, CaMV, and CMV infections were found in 3%, 2%, and 2% of infected samples, respectively (Sevik, 2019).

According to a virus survey (Tabrestani et al., 2010), TuMV, CaMV, and BWYV infection rates in the field were 4%, 2%, and 6 %, respectively. This is a unique study that aims to discover the global distribution of the viruses that most typically infect Brassica. Turnip seed, sometimes known as canola in recent years, is the world's third most important source of edible oil, after soybeans and cotton seeds. (Rimmer et al., 1995), (Kolte, 1985). TuMV is the most common and harmful virus that infects farmed Brassicas around the world (Nguyen et al., 2013).

1.2.2. Cauliflower mosaic virus ecology and pathology

Cauliflower mosaic virus is a virus that infects cauliflower. Pathogenicity is a multifaceted phenomenon influenced by both the CaMV and the host genomes. Mechanical transmission is used to transmit the plant virus to its host. This is due to the fact that cell walls are too thick, and some viruses enter the host via shattered cells, animal vectors, or their own seeds. Cauliflower Mosaic Virus (CMV) Aphids are the carriers of Myzus persicae, the disease spread by aphids. Aphids have a needle-like tongue that helps them to penetrate plants. Aphids then consume the plant's nutrients and sap, leaving saliva behind. If the aphid harbors a virus that

was consumed previously, this saliva can infect the plant (Sutic et al., 1999). CaMV is spread in a non-circulatory manner (Palacios et al.,2002). CaMV is not transmitted through seeds or pollen. (Blance et al., 2001).

Figure 1: Myzus persicae (aphids)

1.2.3. Cauliflower mosaic virus replication

Cauliflower mosaic virus belongs to the Caulimovirus genus, which is one of six genera in the Caulimoviridae family of plant-infecting pararetroviruses. Pararetroviruses, like retroviruses, proliferate through reverse transcription. (Haas et al., 2002). However, the viral particles contain DNA rather than RNA. CaMV is a 52-nanometer-diameter icosahedron made up of 420 capsid protein subunits. CaMV includes an 8.0-kilobase circular double-stranded DNA molecule. Shepherd et al., 1970). Cauliflower mosaic virus belongs to the V11 viral category. Aphids are the most common way for CaMV to spread from plant to plant. (Kennedy et al., 1962).

The virus's dsDNA genome is inserted by an aphid bite. The viral genome is unencapsulated after being injected into the host, and the DNA is transcribed by the host RNA polymerase to generate a copy of viral RNA.

The reverse transcriptase in the host then transcribes viral RNA to DNA. In addition to assembling progeny virions, viral RNA is employed to make gene products such as new capsid proteins and motility proteins in tandem with viral DNA. Capsid proteins protect viruses by encasing their genetic material. Movement proteins interact with plasmodesmata to facilitate transport that would otherwise be impossible. Through plasmodesmata, movement proteins assist the passage of offspring virions into uninfected cells of the host plant. (Shepherd, 1981) and (Spence et al., 2007). After entering the cytoplasm of the host cell, the virus particles release their DNA (Figure 2). Breaks exist in the double-stranded DNA genome. After entering the nucleus of the plant cell, where it interacts with histones and takes on the shape of chromatin fibers, these are repaired. The 19 S and 35 S RNA transcripts are produced by the viral minichromosome. The cytoplasm is where the 19 S and some of the 35 S RNA transcripts are converted into viral proteins. The remaining 35 S RNA enters the cytoplasm as well, but it is used as a substrate by reverse transcriptases. The minus strand complementary to the mRNA is created initially by reverse transcriptase. The extra DNA strand is then created using this combination. Virus particles are made up of two strands of the viral DNA. (Zhang et al., 2019).

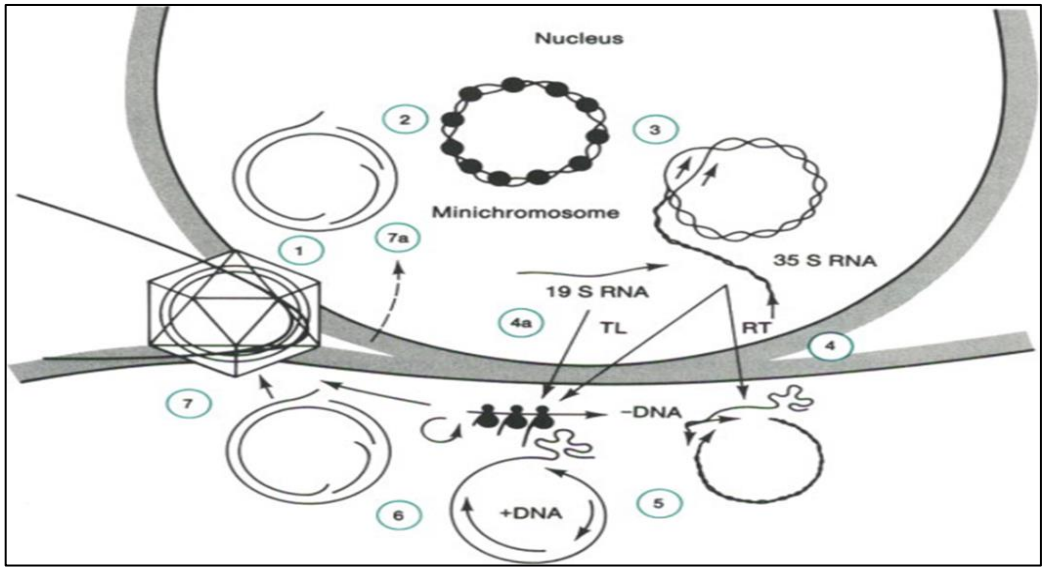

Figure 2: The replication of dsDNA of the CaMV

1.2.4. Cauliflower Mosaic Virus used in Biotechnology

The Cauliflower mosaic virus (CaMV) was initially discovered in Chinese cabbage in 1921, and similar mosaic-like lesions were discovered on cauliflower in California at the same time (Schultz, 1921). Plant biologists soon identified the potential utility of CaMV as a cloning vector for plant transformation and expression of their genes of interest in the plant after learning that CaMV inserted its DNA into plant cells and that this DNA was then expressed at high levels (Covey and Hull, 1981), (Hull, 1987), and (Hohnand Hohn, 1982). The promoter of the cauliflower mosaic virus (CaMV) can be transcribed in a variety of plant tissues and organs. The 35'S promoter transcribes all 8000 nucleotides of the CaMV genome into an RNA transcript, which is then used as a template by reverse transcriptase to reproduce the viral genome (Berges et al.,2018).

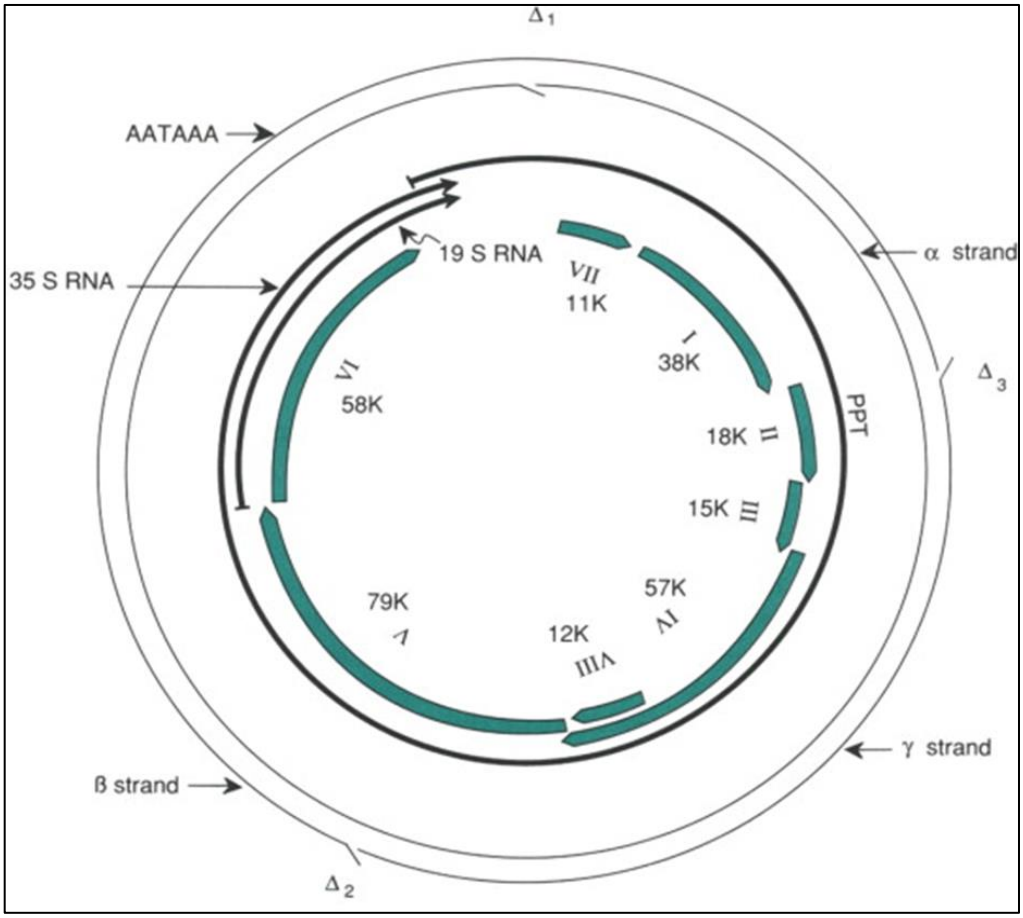

Figure 3: The CaMV genome is a circular DNA molecule with 3 gaps.

The transcription elicited from the 35'S promoter is controlled by the plant, with transcription being initiated by plant RNA polymerase II and regulated by the interaction of plant transcription factors with regulatory sequences in the 35'S promoter that are identical or very similar to regulatory signals found in many other plant genes (Somssich, 2018).

1.2.5. CaMV in Agriculture and management practices to reduce CaMV infection

CaMV is a virus that has spread throughout the world and can cause severe losses in Brassicaceae crops, particularly when co-infected with other viruses. (Shepherd, 1981; Sutic et al., 1999; Spence et al., 2007; Li et al., 2019; Farzadfar et al., 2005).

Figure 4: Show the geographic map for distribution of CaMV in Iran. (Farzadfar et al., 2005). CaMV prevalence can easily surpass 70%, and subsequent yields can be lowered by up to 20%–50%. (Shepherd, 1981; Sutic et al., 1999). Chlorosis (loss of green leaf color), mosaic (patches of light and dark green on leaves), vein clearing (abnormal transparent or translucent tint of veins), and/or stunting are some of the systemic symptoms that the virus can cause. CaMV can be found in Brassicaceae crop and weed hosts such as wild radish, turnip weed, canola, mustard, cauliflower, broccoli, and cabbage, and weed hosts are recognized virus reservoirs outside of the growing season. (Farzadfar et al., 2005; Bergès et al., 2018).

Figure 4: Map of Iran showing the location of CaMV (1 to 10) where cruciferous crops were surveyed during the growing seasons 2004 and 2005; 1: Azerbaijan, 2: Zanjan, 3: Qazvin, 4: Tehran, 5: Khorasan, 6: Markazi, 7: Esfahan, 8: Khuzestan, 9: Yazd and 10 Fars.

1.2.6. Cauliflower mosaic virus meets plant biotechnology

It is vital to demonstrate that in the late 1970s and early 1980s, molecular biology and genetics were still in their infancy, with Arabidopsis thaliana serving as a paradigm in plant genetic research. It has a tiny genome that has been guessed at, a short life cycle, and is simple to mutagenize (Somssich, 2018).

Plant biologists identified the potential use of CaMV as a cloning vector for plant transformation and for expressing the interest gene in the plant after the CaMV inserted its DNA into plant cells and this DNA was then expressed at high levels (Hohn et al.,1982) as shown in Figure 5.

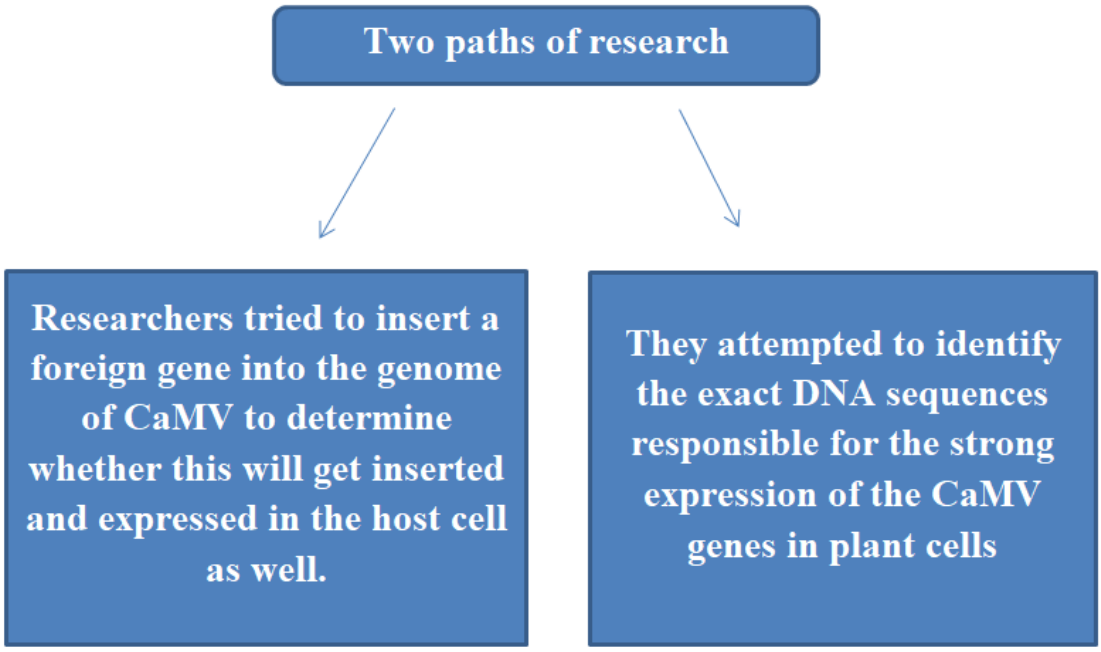

Figure 5: The path of research in which plant biologists recognized the potential use of CaMV as a cloning vector.

1.3. Methods for determination of plant virus infection

1.3.1. Symptomology

Mottling, discoloration of leaves, leaf malformation, smaller plants, and reduced growth are all symptoms of CaMV infection in Brassicaceae plants. (Farazadfar et al.,2005). Figure 5: show the symptoms which induced on the host).

When brassica plants become infected with the virus, they underwent a variety of changes, as previously stated. By employing the PCR approach, certain samples with symptoms such as yellow, mosaic, or leaf deformation were identified as uninfected to CaMV, indicating that these symptoms in brassica plants are not caused by CaMV. Other diseases, such as

environmental conditions, mineral deficiencies, and genetic disorders, might induce symptoms that are similar to viral infection (Ghomi, 2014).

Figure 6: the symptoms of CaMV infection in Brassicaceae plants.

1.3.2. Molecular method

A-PCR was used to detect CaMV, and several techniques were utilized to extract CaMV DNA. This procedure was both effective and precise.

The in vitro amplification of a specific nucleic acid sequence is known as PCR. DNA template, two primers, Taq polymerase, dNTPs, buffer solution, Divalent cations, potassium ions, and mineral oil are among the components and reagents required for PCR. The PCR cycle includes (Denaturation, in which dsDNA is heated to 94 degrees to convert to ssDNA, Annealing, in which the temperature is reduced, and this temperature is dependent on primers to facilitate base pairing with complementary primers. The third cycle called Extension during this step Taq DNA polymerase extends the primers mediating to synthesis of DNA.

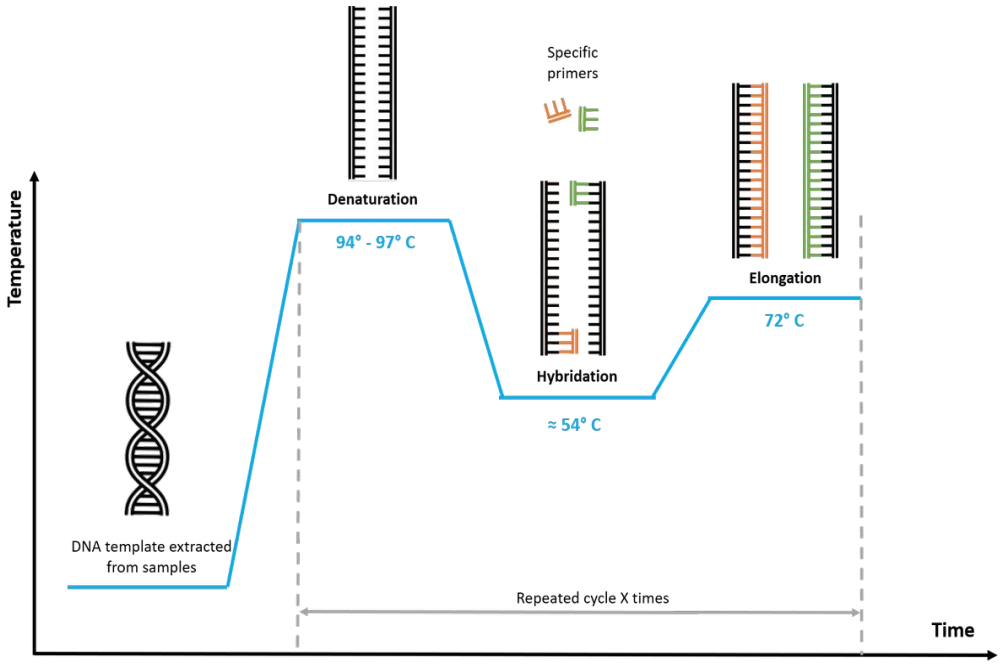

Figure 7: Show the PCR cycle temperatures.

1.4. Objectives

The main objectives of this study were as following:

- Determine the existence of viral syndromes and symptoms on brassica plant in northern region of Palestine (Nablus, Tulkarem, Jenin, and Tubas districts) by applying field surveys to detect visually any of viral symptoms on Brassica plants.
- Assessing the viral incidence by applying molecular tool (PCR) for detecting the CaMV in the randomly collected samples.

Chapter Two
Materials and Methods

2.1. Field Survey and Sample Collections

Field searching and sample collection was carried out during 2020-2021 from five Governorates. According to the previous studies in which samples were selected randomly. Each sample was collected randomly and from separate areas of the same field to avoid bias in the selection of plants. Besides plants which carry known symptoms such as mosaic, deformation of leaves; mottling, discoloration, of leaves, leaf deformity, smaller plants and stunted growth and dwarfing plants were collected (Shepherd, 1981; Daubert et al., 1985). Samples were collected from different areas in Palestine before PCR will be done.

2.2. Location of samples and sampling in the field

Brassica field of four district were studied in the northern region of the west bank (Nassaria, Tulkarem, Tubas, and Jenin). About 240 samples of brassica fields were collected randomly. Only 200 samples of which were used in this research.

Table 3: The number of brassica plants samples and the number of the samples collected at each city.

City	Sampling area	Number of samples	Total
Nassaria	Field A	10	50
	Field B	10	
	Field C	10	
	Field D	10	
	Field E	10	
Tulkarim	Shweke	15	40
	Irtah	15	
	North Tulkarim Plains	10	
Tubas	Al-Fara'a and al Jadedah	40	40
Jenin	Qabatya	20	20
Nassaria	Wadi Al Fara	30	50
TOTAL			200

Brassica fields from five districts were studied in the northern regions of the West Bank (Figure 8) (Jenin, Tubas, Tulkarem, Nablus).

Figure 8: The sampling areas in Jenin, Tubas, Tulkarem, and Nablus districts.

Most brassica plants were collected during 13/Oct /2020-27/JUNE/2021. By using sterile gloves and scissors, the samples were collected from leaves and stem and stored in plastic bags then transfer to the cold room 4˚C degree.

2.3. Optimization of DNA extraction methods

Dellaporta nucleic acid extraction method (Dellaporta et al., 1983) was followed:

50 mg of plant tissues were added to 500 µL of extraction buffer (500 mMNaCl, 100 mMTrisHCl pH 8. 0, 50 mM, and 10 mM 2-mercaptoethanol) and ground with a mortar and pestle. After that, 35 µL of (20%) SDS was added, and the slurries were incubated at 65-70 C for 10 minutes with shaking. Then 160 µL of potassium acetate were added. Then vortex and chill for 10 minutes before centrifuging at 13,000 rpm for 10 minutes. 700µL of supernatant should be transferred to a fresh tube. Add 700 µL of (phenol/chloroform/isoamyl alcohol) to filthy pellets, then centrifuge for 5 minutes at 10,000. After that, Chloroform/Isoamyl alcohol were added to the aqueous phase. Centrifuge at 13,000 rpm for 5 minutes. 500µL of the aqueous phase before adding 0.5 volume ice-cold isopropanol to the mixture and incubation at -20 C, for 20 minutes. Supernatant was removed after centrifuging for 15 minutes at 13,000 rpm. The pellet was then rinsed in 500 liters of 70% ethanol, spin for 5 minutes at 13,000 rpm, and carefully poured away as much supernatant as possible. Allowing 1 hour for air drying, then the pellet. In 50 µL of sterile water, ddH2O, or TE,

the particle was resuspended before immediate use or storing at -20 ℃for later use.

Modified dellaporta nucleic acid extraction

To make 50mL of Dellaporta Extraction Buffer in total. 5 ml 1M Tris pH 8. 0, 5 ml 0. 5M EDTA pH 8. 0, 150µl -mercaptoethanol, 5 ml 5M NaCl, and finally 50 ml H2O. The tissue was then ground using a pestle and mortar. 50 mg of plant tissue was mixed with 500µl of Dellaporta Extraction Buffer. Allowed then to vortex for 10 minutes at 65C. A1/5 volume of potassium acetate was added. Allowed then10 minutes for the tube to incubate on ice. After that, centrifuge at 4 ℃ for 20 minutes at 13,000 rpm. After removing 500µlof supernatant from the tube, an equal volume of isopropanol was added. Incubation for 10 minutes at -20℃ then centrifugation at 13,000 rpm for 10 minutes and discard the supernatant.Phenol, chloroform, and isoamyl alcohol were used to treat the pellet. Then centrifuge for 10 minutes at 13,000 rpm. The aqueous phase was taken next, followed by the addition of chloroform/isoamyl alcohol. Then spin for 10 minutes at 13,000 rpm. Threevolumes of 100% ethanol were added to 150 µl of aqueous phase. The pellet was collected by centrifuge for 10 minutes at 13,000rpm after being kept at -20 C for 30 minutes. The pellet was cleaned with 30 µl of 70% ethanol. Dry the pellet after centrifuging for 10 minutes at 13,000rpm. In 50 µl of sterile water, ddH2O, or TE, the particle was resuspended and stored at -20℃.

CTAB Nucleic acids extraction:

The extraction buffer composed of 4 %CTAB; 4 %PVP 40000; 100mM TrisHCl pH 8. 0, 20mM EDTA, and 1. 5M NaCl, were prepared andadded to100 mg of plant tissues in 1 ml of extraction buffer. Mortar and pestle were used to grind the ingredients. 700µl of the slurry was taken and incubated at 65-70 ℃ for 30 minutes with shaking. After that, a total of 160 µl of potassium acetate were added. For 40 minutes, vortex and incubate on ice. before centrifuge at 4 ℃ for 10 minutes at 13,000 rpm. 700 µl of supernatant were transferred to a fresh tube. Optional step for filthy pellets: 700 µl of (phenol/Chloroform/Isoamyl alcohol, mixed for 5 minutes, then centrifuge at 10,000 rpm for 5 minutes.500µl of the aqueous phase was taken, and 0.7 volumes of cooled isopropanol was added. At -20 ℃, incubate for 20 minutes. Remove the supernatant after centrifuging for 15 minutes at 13,000 rpm. The pellet was rinsed in 500µl of 70% ethanol, spun for 5 minutes at 13,000 rpm, and as much supernatant as possible was carefully poured away. Allowing 1 hour for air drying or use a speed vac for 5 minutes. The pellet was resuspended in 100 µl of sterile water, ddH 2 O, or TE. Storedfor later use at -20 ℃.

2.4. PCR for DNA virus

Specific primers were selected to detect the CaMV: Forward primer [5' GGTAACAGTGCTTCATCCTC 3'] and Reverse primer [5' CTTAGAAGCCGTTGCAGCG 3'].

For preparation of 25µl volume of reaction mix, the PCR mixture was to be under sterilized conditions (hood). PCR mixture contained (2µl of DNA, 0.5µl (0.2 µM) of Primer1 (10µM), 0.5µl (0.2 µM) of Primer2(10µM), 2.5µL (*1x*) of 10X Taq pol. Buffer, 1.5µl (for Taq buffer without Mg++) of $MgCl_2$ 25 mM, 1µl of dNTPs 10mM, 0.2 µl (1Unit/reaction) of Taq DNA pol.(5U/µl), and 16.8 µl of H2O to reach 25 µl of PCR reaction mix. After that were put the PCR mix in PCR machine and set the following program:

Hot start (Denaturation) 94°C/ 5 min (the first cycle was denaturation at 94°C / 30 sec, the second cycle was called Annealing at 56°C / 30 sec, and the last one called Extension, and it was occurred at 72°C / 45 sec. these cycles were repeated for 35 cycles, after that the PCR product stored at 16°C/overnight, or can be stored at 4°C for quick use or at -20°C for long storage.

After obtained the PCR product the results were viewed through the Gel Electrophoresis Analysis. 1.2% Agarose gel in TAE buffer was prepared and the put 0.7µl of GelRed stock as a nucleic acid stain in the gel, after that 8µl of PCR was mixed with 1.7µl of 6X Loading Dye. Then Load the mix (~10µl) in the well. After that 5µl of DNA ladder (i.e 100bp DNA ladder) was loaded. then allow to run for 30-40 min at 100V or 100mA, and let to view the gel under UV-light detector (at 254nm for the one in ANU-lab; wear eye protector).

Chapter Three
Results and Discussion

The work presented in this paper describes the occurrence, prevalence and distribution of CaMV infecting brassica crops in four producing vegetable fields in regions of Palestine. CaMV is among the economically important virus infecting brassica family worldwide (Shahraeen, 2012) and (Sevik, 2016). The most prevalent symptoms were discoloration of leaves, dwarfism, and change the shape of flowers.

3.1. Field surveys and symptoms observation

More than four surveys were carried out during the growing seasons of the year 2020-2021. These surveys were carried in northern part of Palestine (Table 3). Viral symptoms were observed in Nassaria field and Qabatieh ones. Some samples were with symptoms such as yellow, mosaic or leaf deformation the symptoms were pictured as seen in the figure 9 which this samples were found in Qabatiya and Al-Nassaria regions.

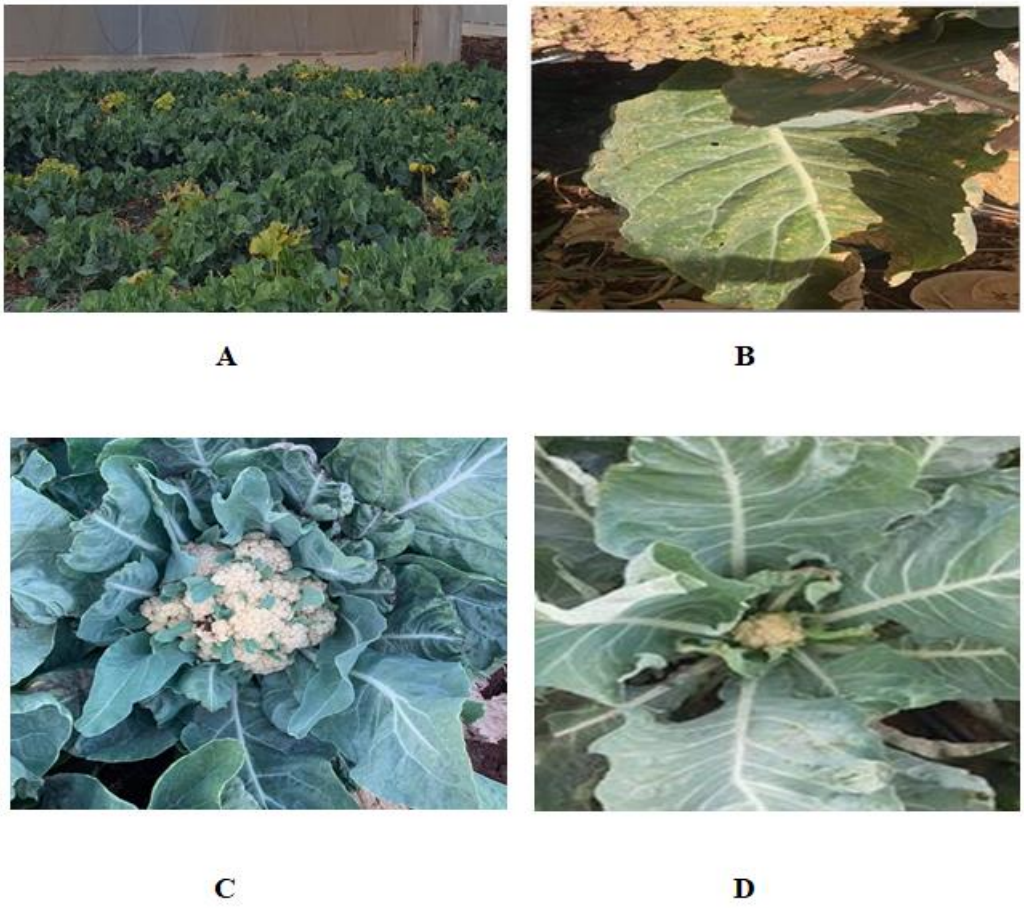

Figure 9: The observed symptoms through the field surveys. (A) the Yellowing and leaf deformation, (B) Mosaic and necrotic regions, (C) leaf deforming and change in shape of the flower, and (D) dwarfism, stunted, and smaller plants.

The symptoms of infecting plants with CaMV were similar (mosaic, mottling, necrotic spots, malformation, dwarfism, vein banding, vein clearing, yellowing, and chlorosis symptoms were common among the samples collected, (Sevik, 2019; Farzadfar et al., 2007; Ayyaz et al., 2019).

Samples were collected with symptomatic and asymptomatic ones from different brassica varieties to be molecularly tested for the CaMV in the lab.

3.2. Molecular detection of the CaMV

PCR was the molecular techniques that were chosen for detecting the virus due to its sensitivity and specificity. According to the previous study accepted in 2014 specific PCR results show more accuracy of ELISA method (Ghomi, 2014).

DNA extraction were verified to choose the best method. A comparative study had been achieved to measure the best product of DNA that can be obtained using three different extraction methods: Dellaporta procedure; Modified dellaporta nad CTAB extraction procedure). The results were visualized on 1.2% TAE agarose gel (Figure 10).

The gel showed the purity for obtaining DNA in which the RNA appeared in the gel due to as a result of not using RNAase. This gel confirms the purity of the DNA because we do not use the RNAase that destroys RNA.The best visualized nucleic acids were noticed in modified dellaporta procedure.

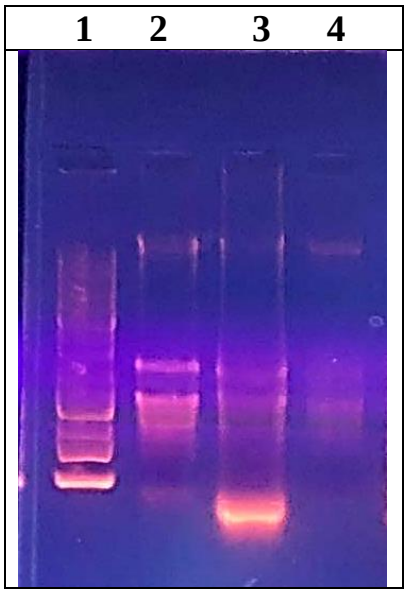

Figure 10: optimization of DNA extraction method from brassica plants. Lane 1;is 1 kb DNA Ladder™ GeneRuler; Lane 2: modified Dellaporta; Lane3: Dellaporta; lane 4 CTAB extraction method. Gels were visualized and photographed with UV-illumination.

PCR tests were able to detect the virus in 25 samples out of 200 collected ones.

The positively reported samples were mainly from An-Nassaria and Qabatia fields. It was the first records about this virus in Palestine.

Nanodrop (JENWAY, Genova Nano) quantification was used to compare the quantity of DNA in the samples of three extraction methods (Table 4).

Table 4: This table shows that the quantity of purified DNA (ng/µl).

Sample	Dellaporta	ModifiedDellaporta	CTAB
1	103	534.5	204
2	151	307.2	119.6
3	308.5	165.6	458.4
4	90	283.8	162.6
AV	163.125	**322.775**	236.15
SD	86.95284	133.5038	131.7405

The best results were obtained by modified Dellaporta method. Statistically and according to SPSS (Statistical Package for the Social Sciences) which is one of the most widely used programs for analyzing statistical information the preference among the three methods could not been determined (Table 5)

Table 5: ANOVA test was applied, where the statistical significance was calculated

ANOVA					
	Sum of Squares	**df**	**Mean Square**	***F***	**Significance**
Between Groups	51099.552	2	25549.776	1.345	0.308
Within Groups	170958.465	9	18995.385		
Total	222058.017	11			

By ANOVA test, the statistical significance was calculated to be as 0.308, which is greater than the value 0.05, and this means that there is no statistically significant relationship between the three methods.

Besides, multiple comparisons table was constructed and showed that all the methods did not statistically indicate the preference of each of them, as the statistical significance in all methods was greater than 0.05 and the reason was statistically that the sample size was not sufficient.

Table 6: The multiple comparisons showed the statistical significance in all methods.

Multiple Comparison						
(I) Test type	(J) Test type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Della porta	Modified	-159.65000	97.45611	0.280	-431.7480	112.4480
	CTAP	-73.02500	97.45611	0.742	-345.1230	199.0730
Modified	Della porta	159.65000	97.45611	0.280	-112.4480	431.7480
	CTAP	86.62500	97.45611	0.660	-185.4730	358.7230
CTAP	Della porta	73.02500	97.45611	0.742	-199.0730	345.1230
	Modified	-86.62500	97.45611	0.660	-358.7230	185.4730

PCR tests were able to detect the virus in 25 samples out of 200 collected ones.

It was the first records about this virus in Palestine.

The positively reported samples were mainly from An-Nassaria and Qabatia fields. The most likely appearance of the virus in the Nassaria and Qabatiya area is due to the climatic conditions in those areas where high temperature helps the Aphids to transmit rapidly to different regions and that increase the emergency of these virus and encourage us to do a lot of survey to another region in Palestine.

The soil where it is used in this regions in which different type of crops growing in a short period of time.

Chapter Four
Conclusion and Recommendations

This is the first report of the existence of CaMV in planted Brassica in Palestine. Further research work is recommended to cover all Palestinian regions. This research is also recommended to search for virus resistant brassica verities to be recommended to the farmers. Molecular characterization of Palestinian virus isolate is also recommended. Thus, future research will be focused on epidemiology, yield loss assessment, and management strategies of these virus.

Finally, depending on this study researcher may be used the result as a base to do a lot of another molecular test, and to make more research about this virus.

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الملخص

تعتبر نباتات الزهره والملفوف والبروكلي واللفت من اشهر المحاصيل في فلسطين. وهذه المحاصيل تصاب بعدد كبير من الفيروسات الممرضه من اشهرها فيروس الفسيفسائي(CaMV) الذي يصيب عائله نبات البراسيكا ويدمر محاصيل شاسعه من هذه النباتات وحدوث اضرار كبيره وخسائر اقتصاديه تقع على عاتق المزارع تهدف هذه الدراسة الى الكشف عن وجود هذا الفيروس ومدى انتشاره في مناطق مختلفه من اراضي الضفه الغربيه_فلسطين لاول مره تم جمع ما يقارب 240 عينه من مناطق فلسطينيه مختلفه وذلك خلال الموسم الزراعي 2021/2020. من خلال المسوح الميدانيه التي يتم من خلالها جمع العينات المتوقع انها تحمل هذا الفيروس وذلك بالاعتماد على الاعراض المعروفه عن الفيروس والتي يسببها. من اشهر تلك الاعراض التي يسببها الفيروس هي اعراض اصفرار وتقزم والتبرقش في النبات المصاب، حيث لوحظ وجود اعراض على عده محاصيل في حقول جنين وطوباس وطولكرم ونابلس. بواسطه تفاعل البوليميريز المتسلسل (PCR) الذي تم اجراؤه في مختبرات جامعه النجاح اثبت وجود هذا الفيروس في فلسطين، والتي اظهرت ان نسبه انتشار الفيروس تصل ال 12.5%، حيث ان هذه النسبه تنذر بوجود خطر وذلك لان الفيروس يستطيع التنقل والانتشار على نطاق واسع من خلال الناقل الخاص به والذي يسمى (*Myzus persicae*). يعتبر هذا البحث اول دراسهلهذا الفيروس في فلسطين.